VALORISATION POTENTIAL OF NATURAL LOW HCT POPLAR MUTANTS IN CATALYTIC FAST PYROLYSIS

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ABSTRACT: Biomass feedstocks having altered lignin concentration and/or lignin chemical composition might be attractive with respect to improving the yield of valuable chemicals in fast pyrolysis. pyGC-MS was used to to investigate the correlation between conversion products and biomass, being low HCT poplar mutants (*Populus nigra*), which are characterised by lignin having an abnormally high H-unit content. Regarding the production of phenolic compounds, increases in non-methoxylated phenols were observed, at the expense of mono-methoxylated phenols. Catalytic fast pyrolysis with zeolite Y was also performed on the mutant and wild type poplar samples. Although the use of the catalyst failed to increase the production of phenols, differences in the phenolic spectrum of the fast pyrolysis vapours indicated differences in the thermal decomposition behaviour of the H-rich lignin in the low HCT mutant versus wild type poplar.

1 INTRODUCTION

The rising market price of fossil fuels, the depletion of fossil resources and their contribution to rising atmospheric levels of CO_2 which is associated with global climate change, has prompted the development of biomass for renewable energy and resource production. Biomass fast pyrolysis is a thermochemical conversion process in which the biomass feedstock is converted into bio-oil, non-condensable gases and solid char [1-3]. Fast pyrolysis is carried out in the absence of oxygen and at elevated temperatures. Depending on the heating rate and temperature, different distributions of the aforementioned products can be obtained [4, 5]. In fast pyrolysis, process conditions are selected in order to maximize the bio-oil yield.

Chemically, the major biomass constituents (cellulose, hemicellulose and lignin) are decomposed through a complex set of primary and secondary decomposition reactions during fast pyrolysis. Consequently bio-oils are complex mixtures of water and several hundreds of organic compounds, with molecular weights ranging from 18 to over 10000 g/mol and belonging to the chemical classes of acids, aldehydes, ketones, alcohols, esters, anhydrosugars, furans, phenols as well as large molecular oligomers [6]. The chemical species distribution depends on the feedstock type and pyrolysis process conditions.

Having a HHV ranging of 21 MJ/kg, crude bio-oil can be used as a substitute for fossil fuel oil in static combustion units, including cogeneration plants. However, crude bio-oil suffers from a number of quality and stability issues, requiring its further upgrading if the transformation into transportation biofuels is pursued. Another application for bio-oil is the extraction of high-value, bio-based chemicals. However, realizing bio-oil derived chemicals production requires having control over the chemical composition of the bio-oil.

Up to now, the goal of the fast pyrolysis is to convert as much biomass as possible to liquid bio-oil, often neglecting the effect(s) of the biomass composition and/or the process conditions on the bio-oil composition. Most of the efforts up to now trying to overcome the negative properties of current pyrolysis oil have focused heavily on the upgrading of the produced oil [5]. However, large potential exist in the control of the processes upstream of the pyrolysis process, i.e. the biomass production, its composition and eventual pretreatment. With respect to the biomass feedstock composition, it has become clear that lignin is a major factor in lignocellulosic biomass recalcitrance and efficient processing. Consequently, research focus on lignin biosynthesis pathways and modifications thereof have become a primary focus [7].

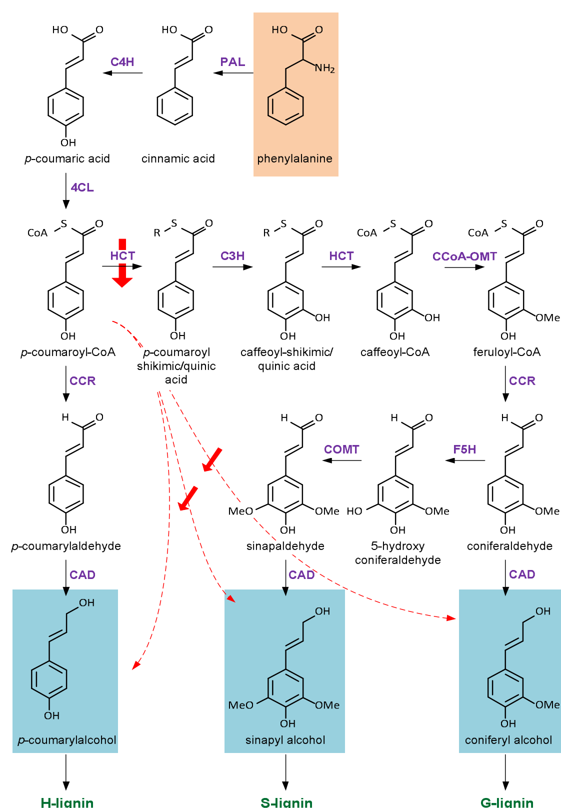

Figure 1: Biosynthesis pathway of lignin and the role of the HCT enzyme in the production of G and S units. The defective allele coding for the HCT enzyme leads to higher H-units in the lignin of the HCT low mutant [10].

One way to cultivate biomass with altered cell wall (i.e. lignin) composition, is to identify genotypes containing mutations with respect to the genes encoding for enzymes in the lignin biosynthesis pathway using

high-throughput screening of large populations of individuals (i.e. plants, trees,…). As most of these infrequent occurring mutations are recessive, they are not expressed in the phenotype of random, naturally mating populations [8]. However, purposefully cross-breeding of identified mutants allows these mutatations to be expressed in phenotypes with altered plant cell wall composition.

In this study, low HCT mutant poplar (*Populus nigra*) was used as biomass feedstock. The naturally occurring poplar mutants were identified from high-throughput sequencing by Marroni *et al.* [9] and are characterized by having a defective allele which codes for the HCT enzyme (hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase 1). Lignin is a polymer built from three different subunits: *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. The HCT enzyme is essential for the synthesis of the G and S units in lignin, as shown in the lignin biosynthesis pathway in Figure 1 [10]. Consequently, low HCT mutants have an altered lignin composition characterized by a 10- to 18-fold increase in H units.

Lignocellulosic biomass containing H-unit enriched biomass is hypothesized to yield multiple benefits with respect to its valorization potential in fast pyrolysis: First, higher yields in phenol and other non-methoxylated aromatic species in the bio-oil are expected and second, as H-unit rich lignin polymers have lower molecular weight than their regular lignin counterparts (i.e. wild type G and S concentrations) [11], thermochemical decomposition should be facilitated yield lower amounts of char and higher amounts of condensable pyrolysis vapours. Furthermore, prelimanry test were conducted in applying catalysis to enhance cracking of higher molecular lignin-derived oligomers from these low HCT mutants and compared against wild type poplar.

In this study, micropyrolysis or pyGC-MS will be used to analyse the pyrolytic vapours. Py-GC/MS has proven to be a useful tool for analysing the chemical reactions in the vapour phase during pyrolysis and was employed to study the catalytic fast pyrolysis of lignocellulosic biomass samples comprising oak, corn cob, corn stover, and switchgrass, as well as the fractional components of biomass, i.e. cellulose, hemicellulose and lignin. [12, 13].

2 MATERIALS AND METHODS

2.1 Biomass samples

Poplar (*Populus nigra*) samples as identified by Marroni *et al.* [9] and cross-bred by Vanholme *et al.* [8] were used. In total, the 7 genotype samples consisted of 1 low HCT homozygous mutant (genotype 71030, 4 biological replicates), of 3 low HCT heterozygous mutants (genotypes BSL39, 3 biological replicates; VDL47, 2 biological replicates; BSL01, 2 biological replicates) and of 3 wild type samples (genotypes VDL06; 2 biological replicates; BSL12, 2 biological replicates; LOW17, 2 biological samples). In total there were 17 phenotypes. The lignin composition – in terms of G, H and S units – of these different poplar lines is shown in Table 1.

Table 1: Lignin composition of the Poplar samples. H-units accumulate in the homozygote line compared to wild type and heterozygote samples. Wild types: VDL06 (2 reps), BSL12 (2 reps), LOW17 (2 reps); Heterozygotes: BSL39 (3 reps), VDL47 (2 reps), BSL01 (2 reps); Homozygotes: 71030 (4 reps).

	% H	% G	% S
WT	0.39 ± 0.09	35.23 ± 3.17	63.20 ± 3.18
Heterozygous	0.56 ± 0.10	35.03 ± 2.86	64.41 ± 2.93
Homozygous	6.98 ± 0.95	26.98 ± 0.42	66.04 ± 1.37

2.2 Micropyrolysis experiments (pyGC-MS)

Fast pyrolysis experiments were performed on a micro-pyrolysis unit (FrontierLab Multi-shot pyrolyser EGA/PY-3030D) coupled to a gas chromatograph and mass spectrometer (Thermo Fisher Scientific Trace GC Ultra and Thermo ISQ MS) for detection of the pyrolysis products. Identification and integration was performed in Xcalibur. The micro-pyrolysis unit consists of a sampler, a quartz pyrolysis tube that can be furnace heated to the desired temperature, a heated interface and deactivated needle which is directly inserted into the GC injector. The loaded sample cup (constructed of deactivated stainless steel) is dropped into the quartz pyrolysis tube situated inside the furnace preheated at desired temperature (500°C). The cup contains about 500 µg of the finely ground biomass sample. Sample cups were weighed before pyrolysis using a Mettler Toledo microbalance with a sensitivity of 0.001 mg. Each sample was run in triplicate.

The loaded cup falls freely into the preheated furnace by gravity in a very short time period of 15-20 ms. As such the sample is heated to the pyrolysis temperature, ensuring rapid pyrolysis. The pyrolysis vapours are directly swept into the GC using helium as the carrier gas (gas flow 100 ml/min). Interface temperature was 350°C. The pyrolysis vapours are directly injected into the GC using a split/splitless injection port (split ratio 1:100) at 300°C. The chromatographic separation of pyrolysis products is performed using a Restek capillary column (Rtx-1707, 60m L x 0.25 mm I.D. x 0.25 µm df) with a stationary phase consisting of a crossbond 14% cyanopropylphenyl and 86% dimethyl polysiloxane and a constant helium carrier gas flow of 1ml/min. the GC oven temperature program started with a 3 min hold at 40°C followed by heating to 280°C at 5°C/min. The final temperature was held constant for 1 min.

2.3 Catalytic pyrolysis experiments (pyGC-MS)

Catalytic experiments were performed in micropyrolysis as well, both in in-bed and ex-bed mode. In-bed mode refers to the mixing of biomass with catalyst prior to loading the sample in the micropyrolyzer, while ex-bed refers to contacting the vapours (i.e. from a regular pyrolysis reaction) with catalyst (see Figure 2). Hence, ex-bed mode is also known as vapour phase upgrading.

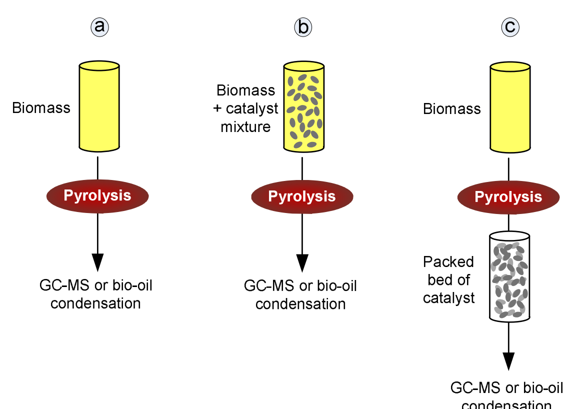

Figure 2: Regular uncatalyzed pyrolysis (a), in-bed mode catalytic pyrolysis (b) and ex-bed catalytic pyrolysis or vapour phase upgrading (c).

The pyGC/MS is equipped with Frontier Tandem Pyrolyzer, a secondary miniature tube furnace (5 mm inner diameter), mounted downstream of the pyrolysis reactor and independently controlled (i.e. temperature control, separate from the one used in the primary pyrolysis reactor). This reactor tube is packed with catalysts in powder form allowing to test ex-bed catalysis as well.

The catalyst used in both in-bed and ex-bed experiments was a commercial FCC catalyst, zeolite Y. For in-bed mode, a catalyst-to-biomass ratio was maintained between 10 ~ 12. For ex-bed catalysis, 40.3 mg of zeolite Y catalyst was packed in the Tandem Pyrolyzer and held at 500°C. Catalytic experiments were run in duplicate.

2.4 GC-MS data processing

Peak areas were obtained from the total ion current (TIC) chromatogram. Individual compounds in the spectra were identified using the National Institute of Standards and Technology (NIST) MS library. Component concentrations were expressed in relative abundance (component peak area divided by total peak area). For further analysis, only phenolic compounds – known to be degradation products from lignin – were taken into consideration. A full list of these 25 compounds is given in Table 2. Furthermore, compounds were grouped according to non-, mono and dimethoxylated phenolic species. Statistical analysis, to highlight component differences among the homozygous, heterozygous and wild type *P. nigra* samples, was performed by means of one-way ANOVA (MS Excel).

In the catalytic pyrolysis experiments, all components (having a relative abundance of > 0.05%) were identified and quantified. These different compounds were then grouped according to chemical functionality (CO_2, aliphatic hydrocarbons, aromatic hydrocarbons, alcohols, ketones, aldehydes, ethers, carboxylic acids, furans, phenolics and sugars) to allow elucidating catalyst chemical activity.

3 RESULTS AND DISCUSSION

3.1 Non-catalyzed micropyrolysis

The different phenolic compounds in the pyrolysis vapours where grouped according to the number of methoxy functional groups ($H_3C–O–$) on the aromatic moiety. The rationale of this grouping is based on the hypothesis that H, G and S-units will mainly yield non-, mono- and dimethoxylated phenolic compounds, respectively. In Figure 3, the results of this phenolic species quantification in pyGC-MS is shown, according to the homozygous, heterozygous and wild type genotypes of the analysed poplar samples. The total phenolic derivatives ranged between 29.48% and 40.68% of the total peak area of all detected and quantified pyrolysis compounds for each individual sample. The remainder of the peak area on the pyGC-MS chromatogram is explained by the presence of (hemi)cellulose derived compounds, other minor lignin-based compounds and gases including CO_2.

Despite the difference in lignin composition, only minor differences were observed: The homozygous mutants yielded slightly more non-methoxylated phenolics, but not statistically significant ($p > 0.01$). However, a significant difference was observed ($p > 0.01$) in the production of monomethoxylated phenolics, compared against the heterozygous mutants and wild type poplar samples. As shown in Table 1, the lower G-unit content in the homozygous mutants supports this observation. No significant differences were found between the heterozygous mutants and wild type poplar samples, which corroborates the fact that the mutation causing the defective HCT enzyme is recessive and consequently, cell wall composition in terms of lignin is not different (see also Table 1).

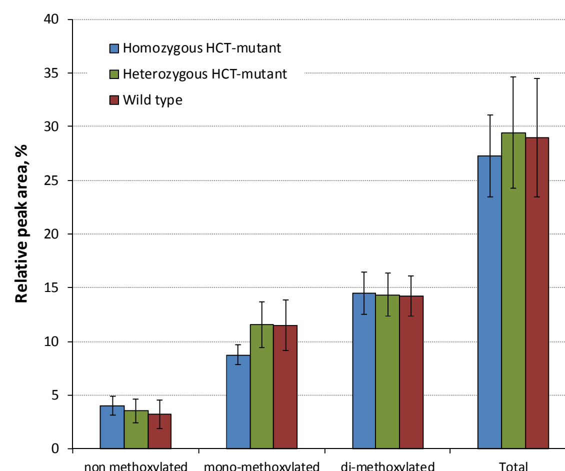

Figure 3: Relative abundance of phenolic species (grouped in non, mono and dimethoxylated) of the homozygous, heterozygous poplar mutants and wild type in pyGC-MS.

When considering individual pyrolysis compounds (Table 2), no significant differences were found between wild type and heterozygous mutant poplar lines. However, for the homozygous mutants, significant ($p > 0.01$) differences were found among both the non and monomethoxylated phenolic compounds. Most noticeable, homozygotes had small quantities of p-cresol and p-coumarylalcohol in the pyrolysis vapours, which were undetectable in both heterozygotes and wild types. These compounds are clearly derived from the H-units of the lignin in the plant cell wall of the homozygotes.

However, the yield of these compounds is rather small (< 0.5 wt%) and the production of p-cresol and p-coumarylalcohol is offset by significant reduction in the production of compound coniferylalcohol, and to a lesser extent vinylguaiacol and isoeugenol - linked to a decrease in the number of G-units of the homozygotes.

Table 2: All 25 quantified compounds in micropyrolysis of the different poplar samples. Group identification: n (non-methoxylated), m (monomethoxylated), d (dimethoxylated). Significant differences ($p > 0.01$) marked by 'a' between wild type and homozygous mutant samples, 'b' between heterozygous and homozygous mutant samples.

Compound	Group	Homo-zygous	Hetero-zygous	Wild Type	
Phenol	n	1.89	1.89	1.56	
Guaiacol	m	1.33	1.53	1.59	
o-Cresol	n	0.10	0.19	0.15	
p-Cresol	n	0.41	0.05	0.12	a, b
m-Cresol	n	0	0.03	0.03	
Creosol	m	0.84	0.99	1.01	a, b
4-Ethylguaiacol	m	0	0.10	0.18	a
p-Vinylguaiacol	m	1.63	2.00	1.96	a, b
Eugenol	m	0.41	0.49	0.51	a, b
Syringol	d	2.78	2.77	2.72	
Isoeugenol E	m	0.34	0.43	0.40	a
p-Allylphenol	n	0.12	0	0	
Isoeugenol Z	m	1.04	1.40	1.36	a, b
1,2,4-Trimethoxybenzene	-	1.95	1.92	1.93	
Vanilin	m	0.42	0.52	0.52	a, b
1,2,3-Trimethoxy-5-methylbenzene	-	0.43	0.59	0.53	
acetovanillone	m	0.31	0.47	0.43	
3',5'-Dimethoxy-acetophenone	m	3.16	3.25	3.16	
Methoxyeugenol	m	3.99	3.93	3.92	
Syringaldehyde	m	1.49	1.52	1.48	
p-Coumarylalcohol	n	0.36	0	0	a
Acetosyringone	d	0.69	0.64	0.65	
Coniferylalcohol	m	2.44	3.61	3.56	a, b
1-(2,4,6-Trihydroxyphenyl)-2-Pentanone	n	1.15	1.39	1.38	a, b
2,5-Dimethoxybenzyl acetate	d	2.56	2.39	2.47	
sinapaldehyde	d	1.87	1.72	1.77	

3.2. Catalytic fast pyrolysis

The pyrolysis vapour constituents grouped among different chemical functionalities is shown in Figure 4 for both in-bed, ex-bed catalytic and non-catalyzed modes. Only the two most distinctive genotypes were selected (the homozygous 71030 and the wild type VDL06) for these preliminary catalytic tests.

Zeolite Y catalysed pyrolysis in both in- and ex-bed mode resulted in a larger production of CO_2 compared to other detectable compounds. The concentration more than doubled for in-bed mode compared to the base case (non-catalyzed) and was significantly larger (up to 4 times the base case) for ex-bed mode. This indicates a more thorough decomposition of pyrolysis vapours due to catalysis. This trend is further confirmed as the amount of lighter oxygenates (aldehydes, carboxylic acids, esters and furans) increases to a significant extent in the pyrolysis vapours, while heavier phenolic and sugar compounds decrease in abundance (respectively 31-34 to 13-18% and 5-8 to 0-1%). Alcohols and ketones remain largely unaffected whereas mono-aromatics seem to have the largest abundance for in-bed catalysis.

Also, there appears to be a significant difference between non-catalytic and catalytic pyrolysis experiments for both poplar samples (Figure 6). When considering the differences in catalytic pyrolysis behaviour between the homozygous mutant (71030) and wild type poplar (VDL06), they appear to be small but noticeable regarding the production of phenolics. However, these differences cannot be considered statistically because the current number of analyses in this preliminary study was rather small to highlight intricate differences in composition.

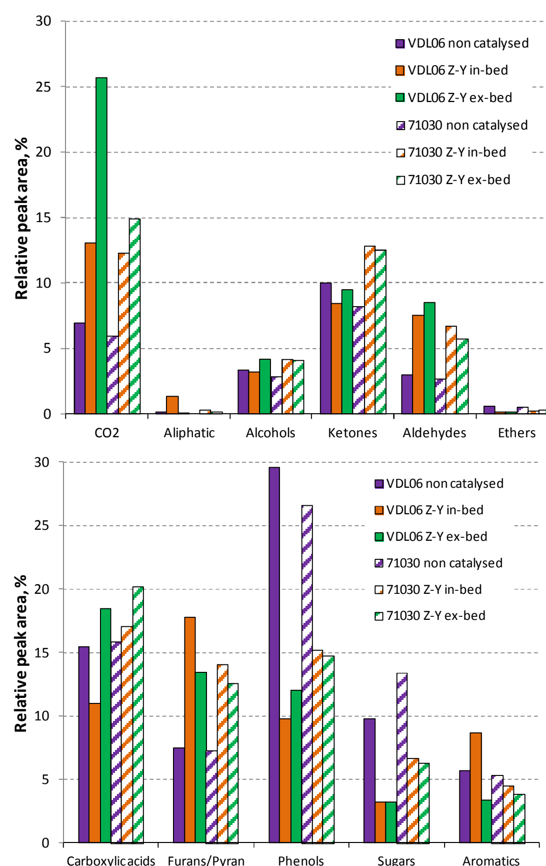

Figure 4: Relative abundance of chemical species in pyrolysis vapours (grouped according to chemical functionality) of the homozygous (71030) and wild type (VDL06) poplar in pyGC-MS, for both non-catalysed, in-bed and ex-bed catalytic pyrolysis.

Furthermore the composition of the phenolics in catalytic pyrolysis was compared (Figure 5), according to their methoxylation degree, for the 3 pyrolysis modes. Remarkably, both wild type and homozygous mutant poplar yield similar levels of non and monomethoxulated phenolic compounds in zeolite Y catalyzed pyrolysis, even tough the concentration in H and G units is different in their lignin (Table 1). The levels of dimethoxylated phenolic species doubled in the homozygous mutant compared to the wild type poplar in both ex and in-bed catalytic pyrolysis, although both biomass sample have comparable S unit levels. Regardless of the fact that catalysis with zeolite Y failed to increase the levels of produced phenolics, these differences in the phenolic compounds spectrum are a clear indication of different lignin cracking behaviour of H-unit enriched lignin of

homozygous low HCT mutants.

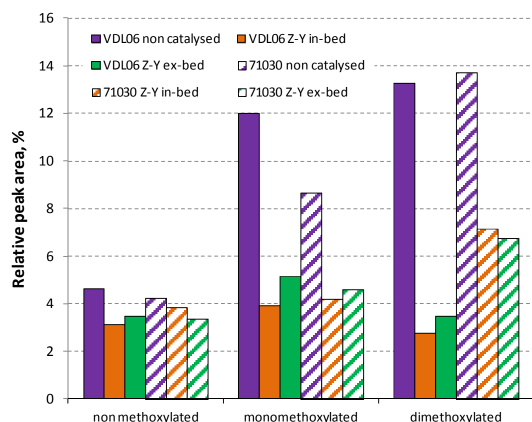

Figure 5: Relative abundance of phenolic species in pyrolysis vapours (grouped according to degree of methoxylation) of the homozygous (71030) and wild type (VDL06) poplar in pyGC-MS, for both non-catalysed, in-bed and ex-bed catalytic pyrolysis.

5 CONCLUSIONS

In this study, non-catalytic and catalytic pyrolysis was performed on poplar mutants having an altered lignin composition. Non-catalytic results showed 4-(3-hydroxy-1-propenyl)-phenol, (p-coumarylalcohol) and p-cresol being reported for all homozygote samples while not for the heterozygote and wild type samples, apparently at the expense of mono-methoxylated phenolics (G-unit derived degradation compounds). Concerning catalytic pyrolysis, the use of zeolite Y as both in-bed and ex-bed catalyst yielded less detectable pyrolysis compounds, suggesting that zeolite Y catalyses the pyrolysis reaction of poplar towards heavy tars, coke and non-condensable gases. Nonetheless differences in phenolic compound production could be observed in catalytic pyrolysis of homozygous and wild type poplar samples, suggesting differences in catalytic thermal decomposition of the H-unit rich lignin of the low HCT mutant poplars. Future research with more appropriate catalysts should further validate the valorisation potential of lignocellulosic biomass with altered lignin biosynthesis pathways for catalytic fast pyrolysis applications

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