GENETICALLY MODIFIED BIOMASS: EFFECT OF SINGLE GENE MODIFICATIONS ON THE COMPOSITION OF FAST PYROLYSIS BIO-OILS

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Introduction

It has been premised that lignocellulosic biomass is the main renewable energy resource available here on earth and that it can be considered as one of few sources that can provide renewable liquid, gaseous and solid fuels[1]. In contrast to fossil fuels, the use of biomass for energy and chemicals renders significant environmental advantages. Plant growth needed to generate biomass feedstocks removes atmospheric carbon dioxide, which offsets the increase in atmospheric carbon dioxide that results from biomass fuel combustion.

One promising and clean way to acquire bio-oil is by the fast pyrolysis of lignocellulosic biomass, which is considered as a network of hemicellulose and cellulose bound by lignin[2]. Pyrolysis is a thermal decomposition process carried out in the absence of oxygen, or when significantly less oxygen is present than required for complete combustion, yielding gaseous products, liquids (bio-oil and water) and solid charcoal. During the heating in the fast pyrolysis process the biomass constituents are decomposed due to a complicated set of primary and secondary radical reactions. This leads to a complex mixture of water and several hundred organic compounds, with molecular weights ranging from 18 to over 10000 g/mol. These organic compounds present in pyrolysis oil belong to acids, aldehydes, ketones, alcohols, esters, anhydrosugars, furans, phenols, guiacols, syringols, nitrogen compounds as well as large molecular oligomers (holocellulose-derived anhydro-oligosaccharides and lignin-derived oligomers). Pyrolysis oil also contains negligible amounts of ash, and have a volumetric energetic density 5 to 20 times higher than the original biomass.[3] Therefore they offer the potential to be used as a liquid energy carrier or as a renewable raw material for the chemical industry in the production of high-value chemicals and liquid biofuels[4]. Its usage as a substitute for a fuel oil in any stationary heating or electricity generation application and to produce a range of specialty and commodity chemicals has recently gained a lot of interest [5]. However, realizing those potential applications requires having the control over the chemical composition of the bio-oil. Thus the right type of biomass feed and the pyrolysis conditions should be selected. In short, to understand and to be able to predict the pyrolysis behavior, it is essential to understand the kinetics of the thermal reactions that are involved in biomass pyrolysis and relate that to detailed information about the starting material.

Up to now, the main goal of most fast pyrolysis studies was limited to convert as much biomass as possible to liquid bio-oil, neglecting the effect(s) of the biomass composition and/or the process

conditions on the bio-oil composition[6]. Therefore in the present contribution the role of small differences in feed composition on the composition of the formed products is investigated. Both pyrolysis GC as well as pilot plant data have been used and advanced characterization techniques such as comprehensive 2D GC (GC×GC) [7] are employed for analysis purpose. Single gene modifications of poplar have been evaluated to assess the effect of these modifications in biomass composition on the produced bio-oil and bio-char.

Experimental

Transgenic lines

The genetically engineered poplar lines are all modified to alter their formation of lignin. The amount of lignin strongly depends on the wood type, ranging from 16% to 33% of the mass of dry wood [8, 9]. Lignin genetic engineering and the lignin biosynthetic pathway are receiving considerable interest. Significant progress in this field has been made by genetic, bioinformatics and biochemical approaches, providing the plant material to research the monolignol biosynthetic pathway [10]. Lignins are complex aromatic heteropolymers derived mainly from three hydroxycinnamyl alcohol monomers, *p*-coumaryl, coniferyl, and sinapyl alcohols, which differ in their degree of methoxylation:. These monolignols result in *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) phenylpropanoid units respectively, when incorporated into the lignin polymer [10]. Hardwood lignins are mainly composed of S and/or G units linked by a series of ether and carbon-carbon bonds, while softwood lignins are composed mostly of G units with low levels of H units [10, 11].

Starting from the metabolic grid, which is represented in Figure 1, the monolignol biosynthesis can be easily understood. It starts with the deamination of phenylalanine and involves successive hydroxylation reactions of the aromatic ring, followed by phenolic *O*-methylation and conversion of the side-chain carboxyl to an alcohol group [10]. Note that the route that is given in Figure 1 is the one that is expected to be the most favored with hardwood types, based on lignin compositional analyses, enzymatic assays and transgenic plants. [10]

Table 1: List of the 15 poplar samples

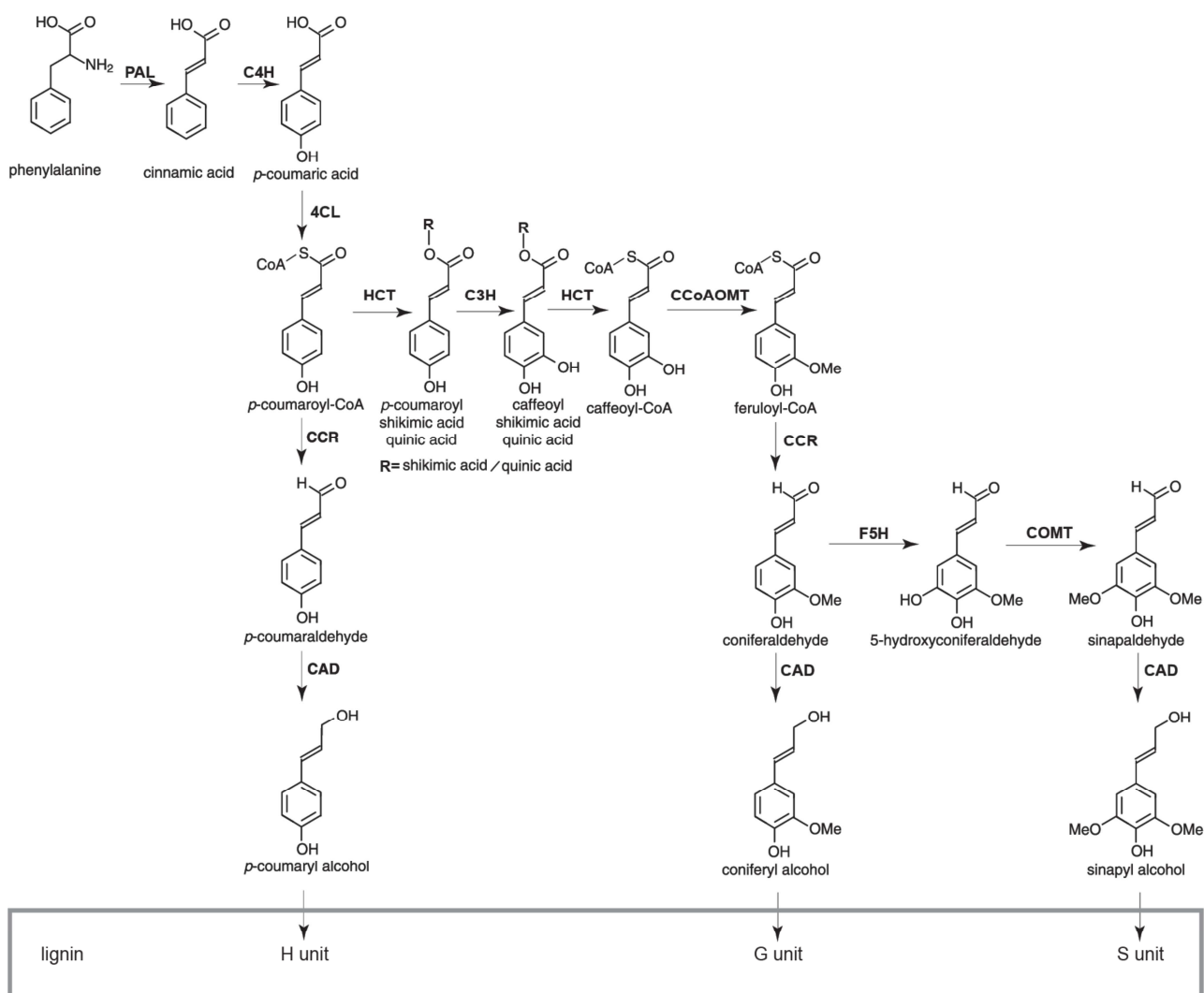| Sample nr.	Specific type	Branches
1	COMT - ASB10B	1,3,5
2	COMT - ASB10B	2.0,2.1,2.2
3	WT – Biological	1-3
4	WT – Biological	4-5
5	CAD T21	1-3
6	CAD T21	4-6
7	COMT - ASB2B	1-3
8	COMT - ASB2B	4-6
9	CCoAOMT-416	1-3
10	CCoAOMT-416	4-6
11	CCoAOMT-429	1-3
12	CCoAOMT-429	4-6
13	WT – Technical	7-15
14	WT – Technical	7-15
15	WT – Technical	7-15

Figure 1: The monolignol biosynthetic pathway.

The transgenic lines were down-regulated in either cinnamyl alcohol dehydrogenase (*CAD*), caffeic acid *O*-methyltransferase (*COMT*) or caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*). Down-regulation of these genes has the consequence that the corresponding steps in the lignin pathway (see Figure 1) are (partly) interrupted, leading to an altered lignin amount and/or composition. CCoAOMT assures the conversion from caffeoyl-CoA to feruloyl-CoA. Down-regulation of this enzyme leads to a decrease of the formation of the G and S units, which is equal to the decrease in the formation of lignin itself. Down-regulation of the multifunctional CAD-enzyme hinders the final reduction of coniferaldehyde and sinapaldehyde to the corresponding alcohols. As a consequence, more aldehydes and fewer alcohols are formed. Previous research [10, 12, 13] indicates that *CAD* down-regulation increases the presence of the free phenolic groups in lignin. *COMT* down-regulation hinders the reaction of 5-

hydroxyconiferaldehyde to sinapaldehyde. As a result the amount of G units increases, while the amount S units remarkably decreases. The used plant materials were one-year-old greenhouse-grown poplars (*Populus tremula* x *Populus alba*). Two lines with reduced COMT activity were used (COMT-ASB10B and COMT-ASB2B-2)[14], two with reduced CCoAOMT activity (CCoAOMT-416 and CCoAOMT-429)[15] and one with reduced CAD activity (CAD T21)[16]. From each genetically engineered line, two biological replicates were made. For the wild type on the other hand, two biological and three technical (i.e. material from the same plants) replicates were made. Note that for each sample three different plants of the same line were used. In total 15 samples were prepared for the experiments, as shown below in Table 1. The material consisted of dried one year old stems that were debarked.

Pilot plant experiments

A schematic overview of the experimental apparatus is shown in Figure 2. The unit can be divided in three main sections, i.e., the feed section, the reactor section and the condensation/collection section. The pyrolysis of a single sample, including collection of the liquid and char fractions, as well as the preheating of the oven, took approximately 3 to 4 hours. For each run, approximately 10 grams of raw material is used. The stem material was chopped into pieces with a length and an internal diameter of max. 10 mm and 5 mm, respectively. The pyrolysis reactions are carried out in a vertical, tubular, stainless steel reactor (d x L = 3,8 cm × 30 cm), which is heated by means of an electric furnace.
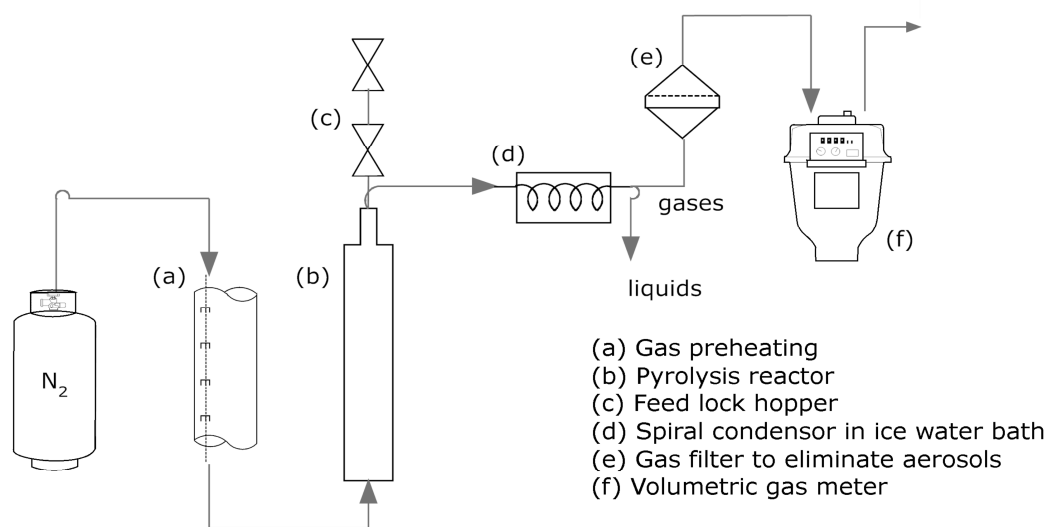

(a) Gas preheating
(b) Pyrolysis reactor
(c) Feed lock hopper
(d) Spiral condensor in ice water bath
(e) Gas filter to eliminate aerosols
(f) Volumetric gas meter

Figure 2: Flow diagram of the batch pyrolysis reactor. (1) nitrogen gas supply, (2) flow control, (3) gas preheater, (4) electric tube furnace, (5) pyrolysis reactor, (6) sintered base plate, (7) fluidised sand/ biomass bed, (8) biomass lock hopper, (9) condenser, (10) condensate/gas separator, (11) cotton filter , (12) diaphragm gas flow meter, (13) gas vent and (14) bio-oil recovery.

In the reactor section itself, the biomass gets into contact with the heat carrier. In this case the heat carrier is pre-heated silica sand with a mean diameter of 250 µm, particle density of 2600 kg/m^3 and a bulk density of 1600 kg/m^3. The pyrolysis reactions take place at 500°C with a residence time of 10

minutes. To acquire the liquid fraction, the spiral condenser is detached after the experiment. Tetrahydrofuran (THF) is injected with a syringe into the spiral to relinquish the bio-oil. After the experiment, the char can be removed from the inner tube of the reactor. A detailed description of the analysis of bio-oils using GC×GC can be found in Djokic et al. [7] A chromatogram of a crude bio-oil is shown in Figure 3. For each sample 100 to 110 components were identified and quantified. The biochar is analyzed using an elemental analyzer of the Thermo Scientific FLASH 2000 Series. Each sample was run at least three times, if the results showed a too large deviation additional runs were carried out.
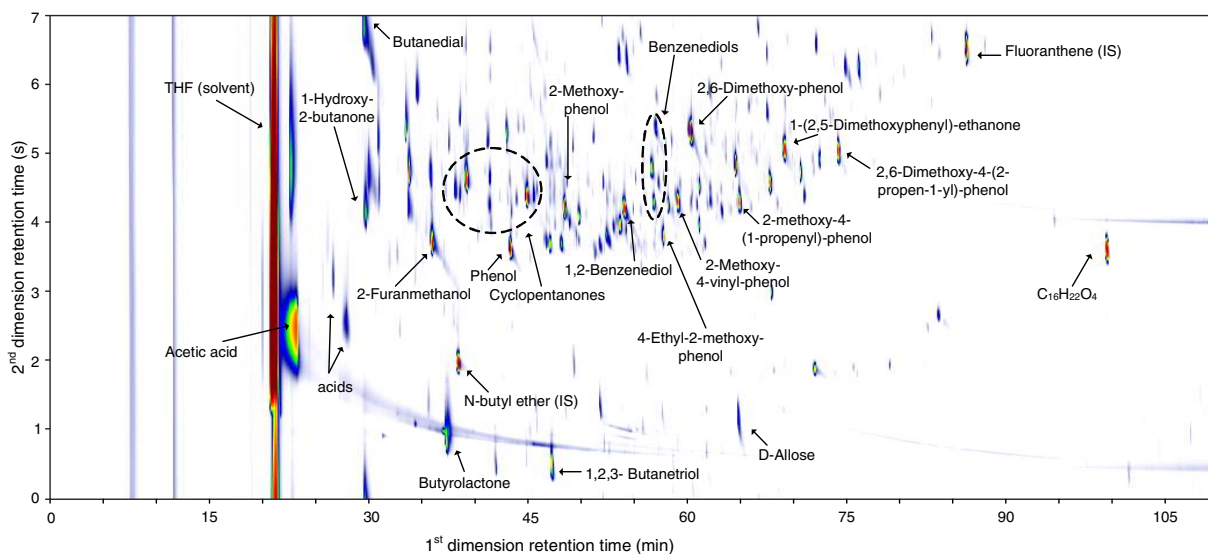

Figure 3: GC×GC-FID colour plot of crude poplar wood bio-oil sample, S13-WT.

Micro-pyrolysis set-up

A schematic overview of the micro-pyrolysis set-up is given in Figure 4. The residence time of the vapours in the micro-pyrolyser is limited to 15-20 ms. The micro-pyrolysis unit (FrontierLab Multi-shot pyrolyzer EGA/PY-3030D) consists of a sampler, a quartz pyrolysis tube that can be preheated to a desired temperature with a furnace, interface and a deactivated needle (inserted into the gas chromatograph (GC) injector).
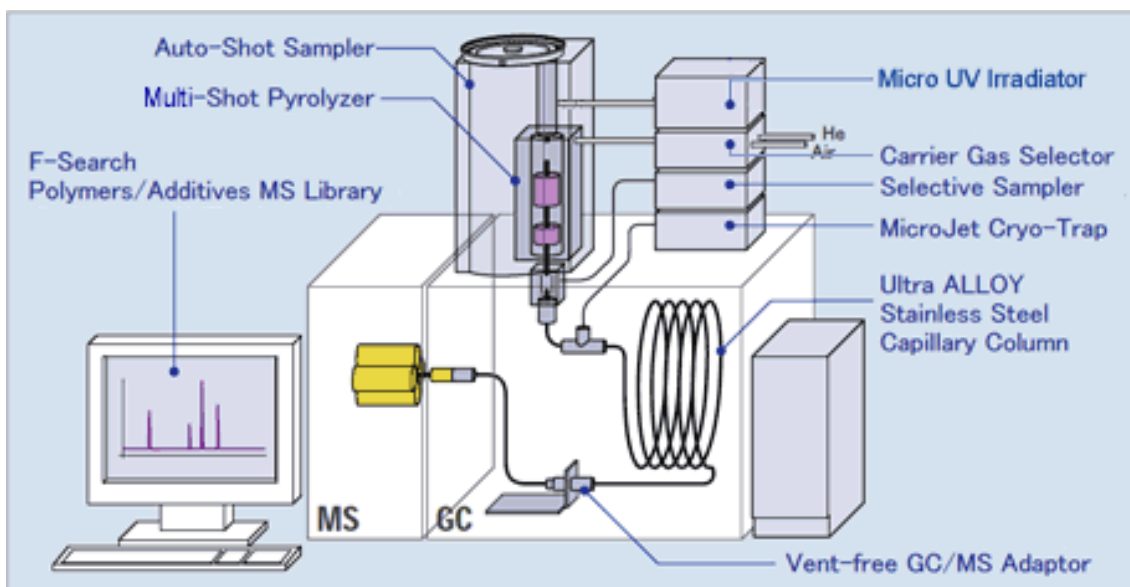
Figure 4: Schematic overview of the pyrolysis-GC/MS set-up

The loaded sample cup (constructed of deactivated stainless steel) is dropped into a furnace, which is preheated to 500°C. The cup contains about 300 micrograms of finely ground biomass. The loaded cup falls freely into the preheated furnace by gravity in a very short time period. During this period the sample is heated to the pyrolysis temperature, ensuring rapid pyrolysis (>2000 °C/s). The pyrolysis vapors are directly swept into the GC using helium as the carrier gas. The constituents of the pyrolysis vapor are separated in the GC column and identified using a mass spectrometer (MS) (Thermo Trace GC Ultra / ISQ MS. The chromatographic separation of pyrolysis products is performed using a Restek capillary column with a stationary phase consisting of 14% cyanopropylphenyl and 86% dimethyl polysiloxane and with a carrier gas flow velocity of 1ml/min.

Results and discussion

Bio-Char and Original Biomass

For the process conditions specified above on average 25.0 ± 0.5 wt% of char was obtained for all samples. The results of the elemental analysis of the biochars and the studied biomass feeds are summarized in Figure 5.

Figure 5 shows that for both the original material and the bio-char the differences in the elemental composition are very subtle. Moreover, the variation in the H and N composition for the COMT lines compared to the others are of the range as the variation in H and N composition for the wild type mutually. Hence, our results indicate that for present study there are no statistically significant differences in elemental composition between the different poplar types. This suggests that even though the feedstock differs in its genetic structure, it is neither traceable in the elemental composition of the original material nor in the produced bio-char.
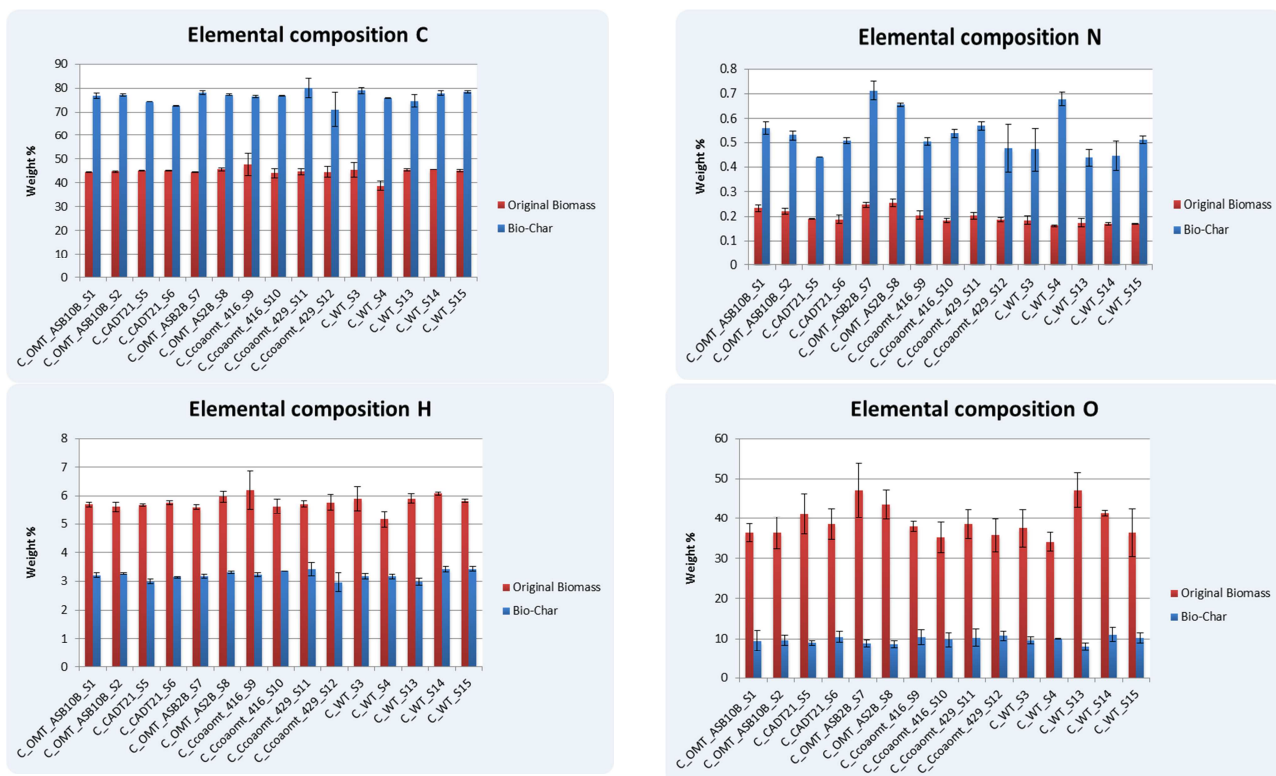
Figure 5: Effect of single gene modifications on the elemental analysis results of original biomass material and formed biochars

Bio-Oil

To assess the differences in composition for the bio-oils (pilot plant set-up) and the pyrolysis vapors (micro-pyrolyser) principal component analysis (PCA) was applied. This is a powerful and facile technique that allows extracting significant information from the detailed compositional information. It also allows to visualize these results and to reveal differences in composition of the bio-oils derived from the different biomass feeds. Both score plots and loading plots were evaluated to interpret the results. Figure 6 shows such a loading plot based on the detailed analysis of the bio-oils analyzed by GC×GC and reveals that indeed the produced differ statistically in composition from each other.

The first three principal components of the PCA explain 65.7% of the variation in the data, with PC1 describing 27.6%, PC2 explaining 25.2% and PC3 explaining 12.9%. The resulting score plot of PC2 versus PC1 (see Figure 6) shows that there are two groups of transgenic lines separated from the wild type, the COMT lines and the CAD lines. The wild type and the CCoAOMT lines are both located around the centre, apart from Sample 12. The separation between COMT lines and wild type is caused by both PC1 and PC2, while the separation between CAD lines and wild type is only caused by PC2. The loading plot reveals that the COMT lines have a significant lower amount of S units compared to the wild type. On the other hand the two S-aldehydes (syringaldehyde and sinapaldehyde) are significantly higher in the CAD samples compared to the wild type and explain why these fractions are statistically different from each other.

The fact that the bio-oils from CCoAOMT lines are not significantly different from the wild type needs to be further studied, considering that down regulation of this enzyme affects mainly the amount of lignin differences between CCoAOMT and the wild type material are expected.

Taking into account the results obtained for the elemental composition of the biomass feeds, it can be concluded that even though the elemental composition of the biomass feedstock is almost identical, the produced bio-oils can have different compositions for a given fixed set of process conditions.
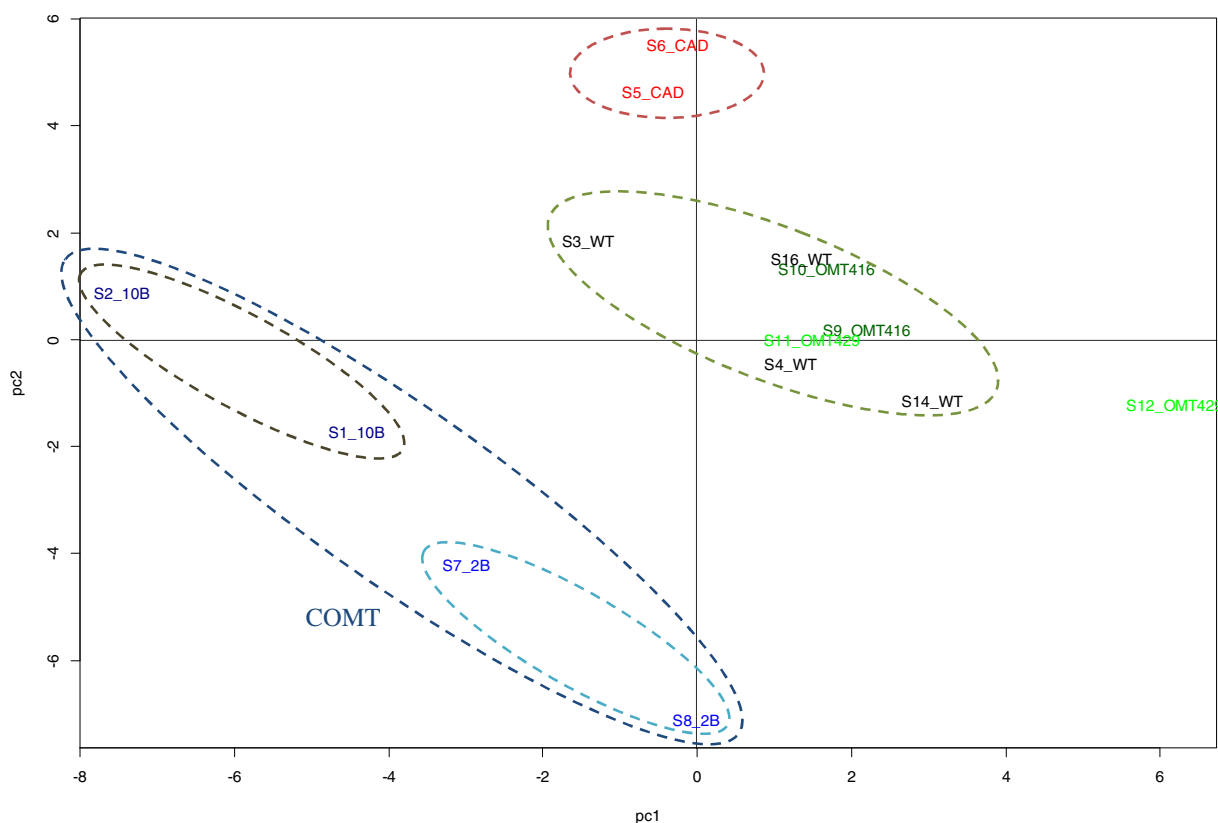

Figure 6: Loading plot for PCA analysis of bio-oils derived from wild and genetically modified poplar

Conclusions

The elemental composition of both the original materials and the obtained bio-chars show that for both the original materials and the bio-chars the differences in the elemental composition are very subtle. There are no noticeable shifts between the different genetic poplar types. This proves that even though the feedstock differs in its genetic structure, it is neither traceable in the elemental composition of the original material nor in the produced bio-char.

The analysis of the bio-oils produced in the pilot plant set-up and the results obtained on the micro-pyrolysis set-up show clear differences between the COMT lines and the wild type, which could be attributed to a lower amount of syringyl (S) units in the lignin of the COMT lines. Subtle shifts

between the CAD lines and the wild type were also detected. However, there were no clear shifts traceable for the CCoAOMT lines.

Finally, our results also prove that even though the elemental composition of the biomass feedstock is statistically not different it does not imply that the produced bio-oils have the same composition for identical process conditions. Hence more structural information then elemental analysis is needed to predict bio-oil compositions for a given process condition.

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