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FACULTY OF ENGINEERING AND ARCHITECTURE

Biomass reaction engineering driving genetic modification

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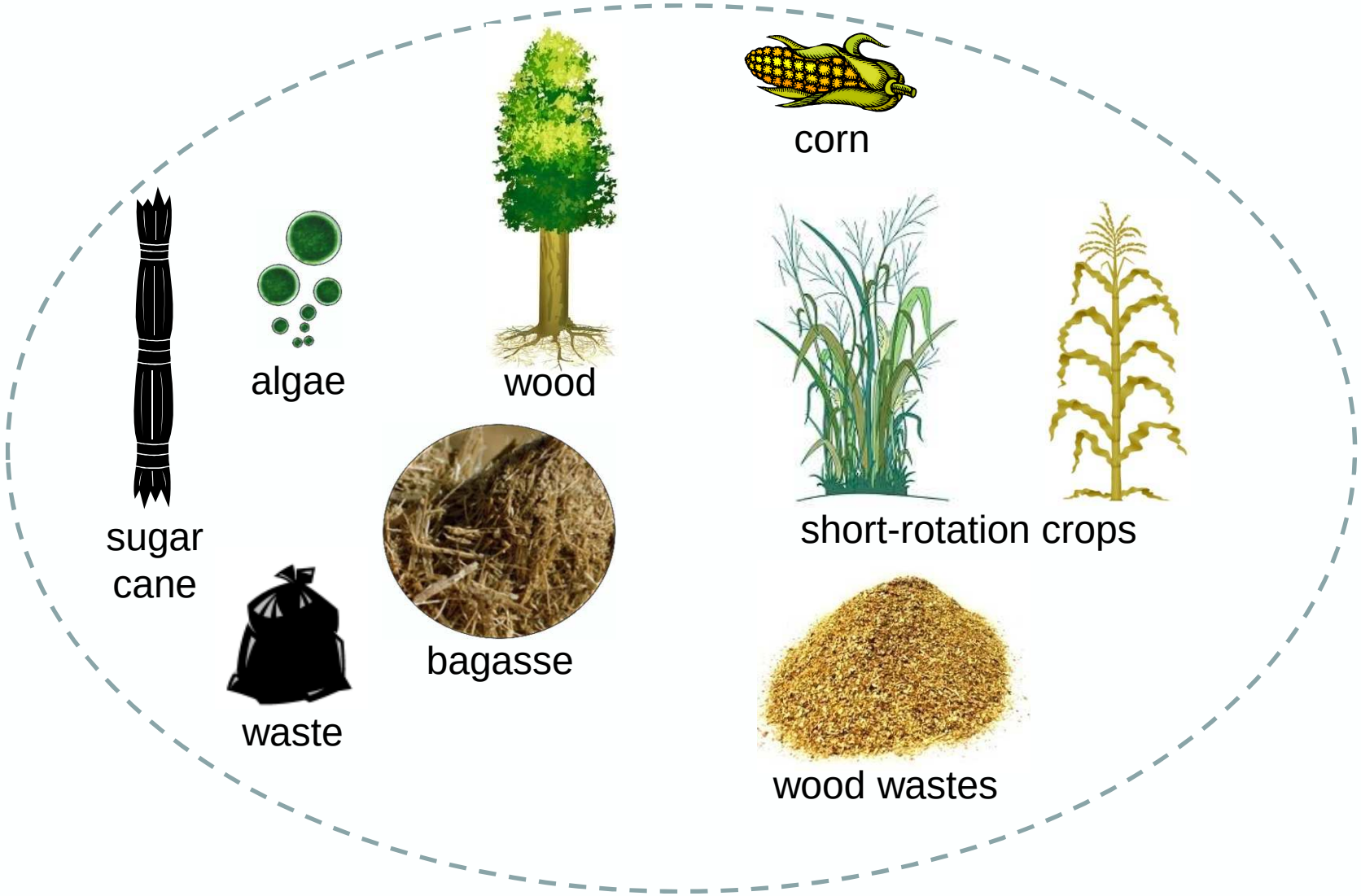
Outline

- *Global introduction*
- *Feedstock and lignin pathway*
- Pyrolysis experiments
  - Micro pyrolysis
  - Sand bed pyrolysis reactor
- Conclusions

Biomass

all organic materials that come from plants, trees, crops, and algae



Fast pyrolysis process

- Lignin
- Hemicellulose
- Cellulose

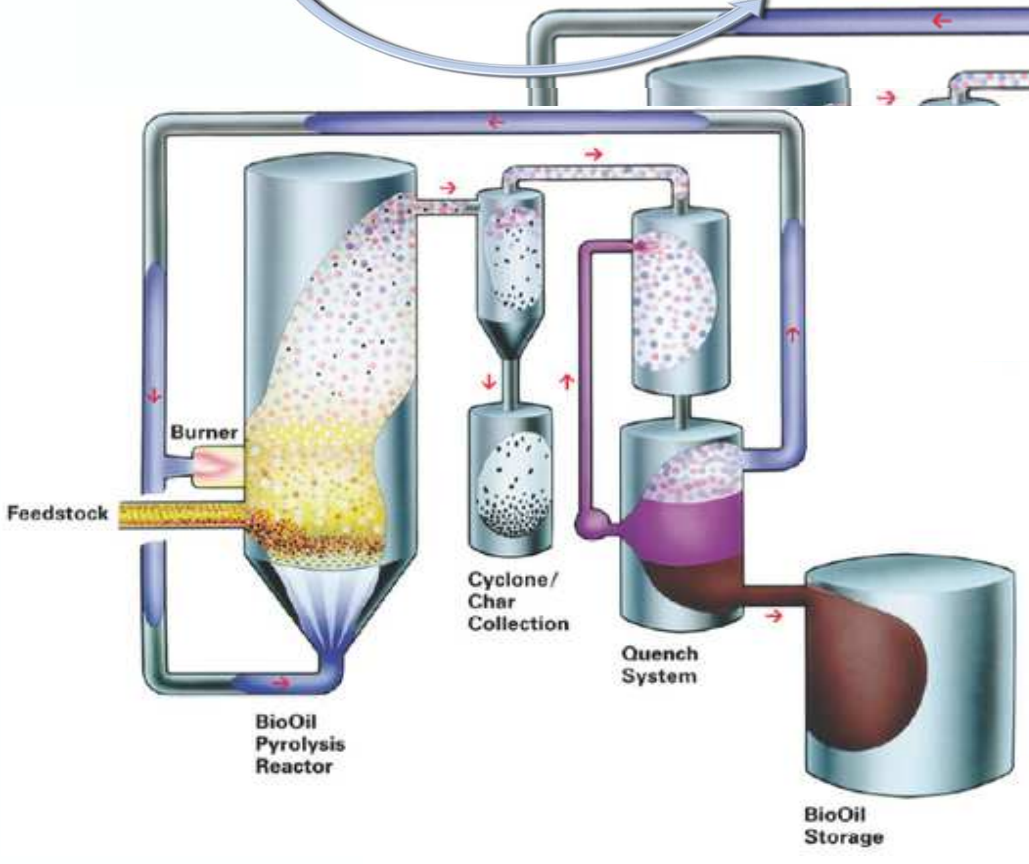
Lignocellulosic biomass

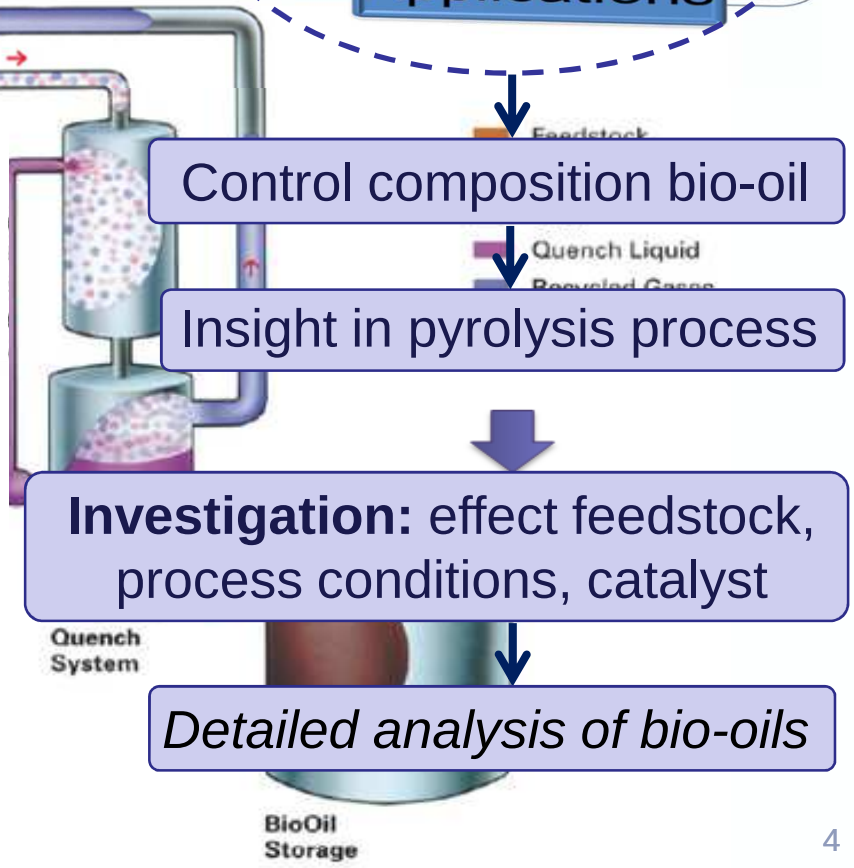
Fast pyrolysis products

- 13% Gas
- 75% Bio-oil
- 12% Char

- Energy carriers
- High-value chemicals
- HDO-> Biofuels

Applications





Control composition bio-oil

Insight in pyrolysis process

Investigation: effect feedstock, process conditions, catalyst

Detailed analysis of bio-oils

Bio-oil characteristics and upgrading

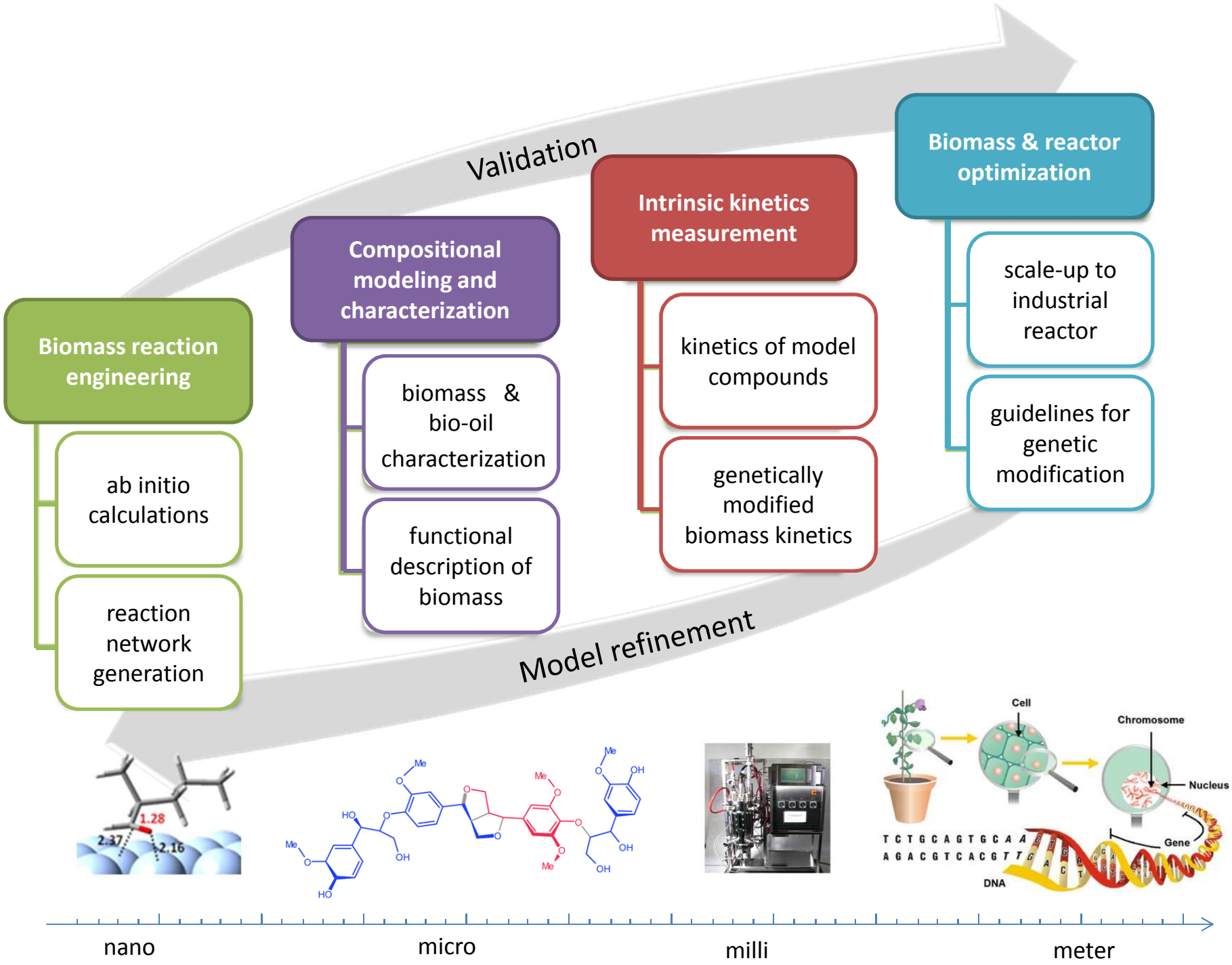
Characteristics

- Complex mixture of several hundred compounds
- Not miscible with conventional petroleum fractions
- Chemically unstable; instability increases with heating
- Ageing of the liquid, causes unusual time-dependent behaviour
- Viscosity increases with time

→ Improvement of these characteristics? → Upgrading

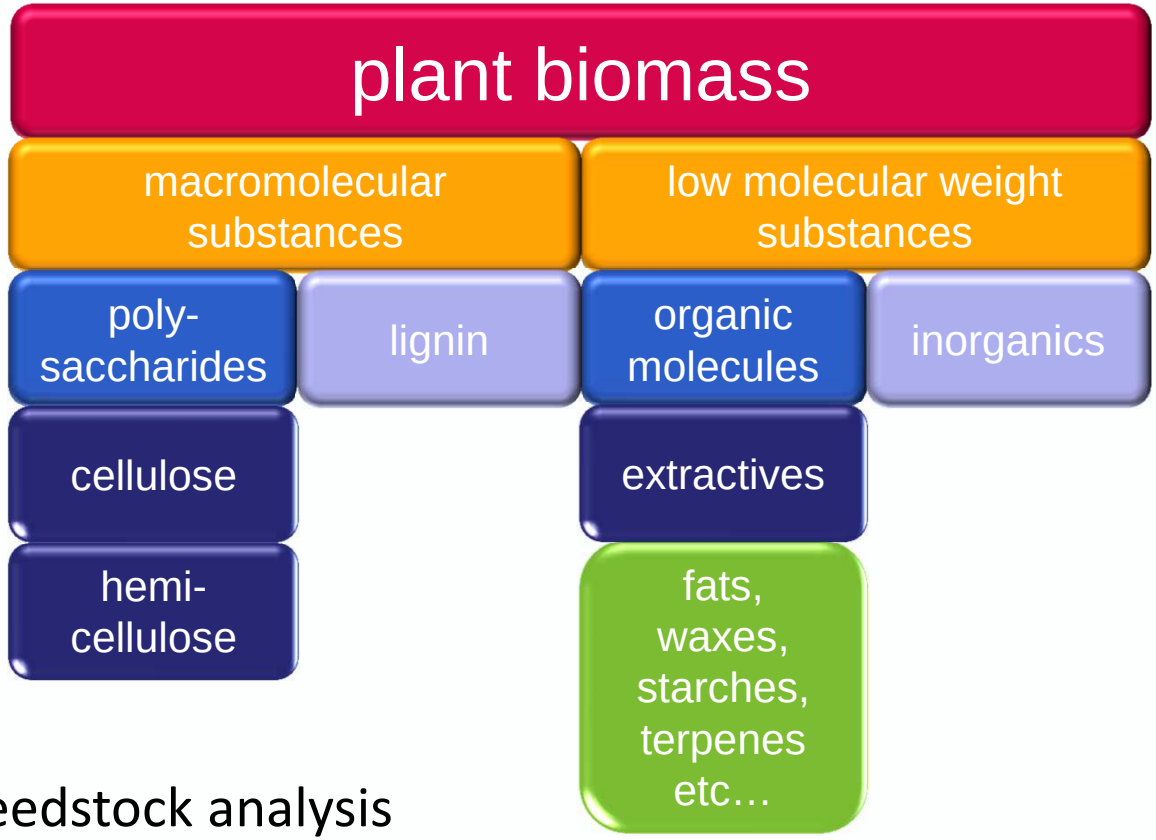
Hydrodeoxygenation (HDO)

- Oxygen containing components are converted into aliphatic and aromatic components
- **Consumption of H_2**
- Heterogeneous catalyst
- Instability is mainly caused by presence of reactive **ketones and aldehydes** (Venderbosch, 2012)
- Alcohols are much more stable and have good combustion properties


Validation
Model refinement
Biomass reaction engineering
ab initio calculations
reaction network generation
Compositional modeling and characterization
biomass & bio-oil characterization
functional description of biomass
Intrinsic kinetics measurement
kinetics of model compounds
genetically modified biomass kinetics
Biomass & reactor optimization
scale-up to industrial reactor
guidelines for genetic modification
1.28
2.37
2.16
Me
HO
OH
Cell
Chromosome
Nucleus
Gene
DNA
T C T G C A G T G C A A
A G A C G T C A C G T T
nano
micro
milli
meter

Major hurdles for biomass fast pyrolysis

Composition strongly depends on origin biomass



- feedstock analysis
- kinetic study: set-up and product analysis
- chemistry: C/H/O different from and more complex than C/H
- Mechanistic modeling: “molecular” representation impossible

Proof of concept: single gene modification

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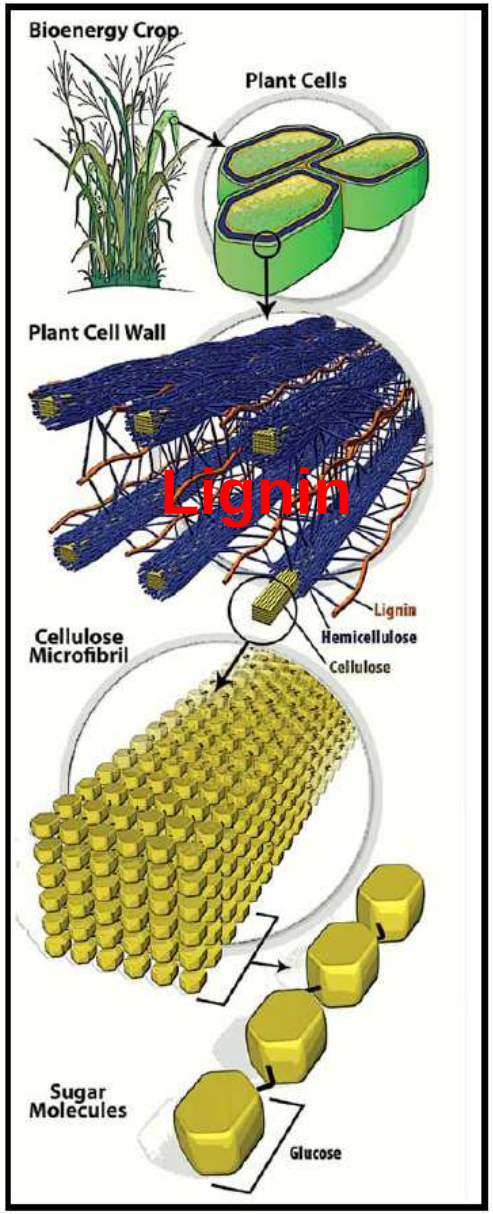
Biomass feedstock



Wood

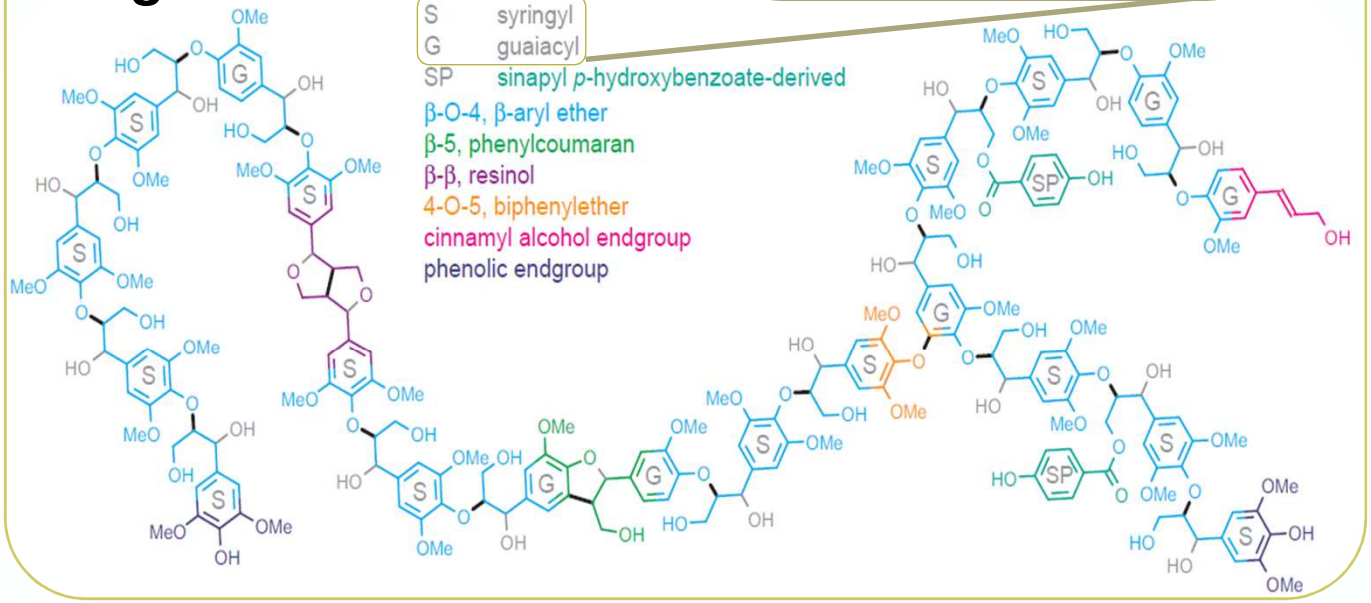
~50% cellulose

~25% hemicelluloses

~25% lignin

S syringyl
G guaiacyl

Lignin



Lignin pathway

- Less lignin
- More aldehydes
- No S, more G-units

Biomass feedstocks

- 16 samples

1	10B	COMT-ASB10B
2	10B	COMT-ASB10B
3	WT	WT-Biological
4	WT	WT-Biological
5	CAD21	CAD T21
6	CAD21	CAD T21
7	2B	COMT ASB2B-2
8	2B	COMT ASB2B-2
9	2CoA-416	CCoAOMT-416
10	2CoA-416	CCoAOMT-416
11	CCOA-429	CCoAOMT-429
12	CCoA-429	CCoAOMT-429
13	WT	WT-Technical
14	WT	WT-Technical
15	WT	WT-Technical
16	WT	WT-Biological

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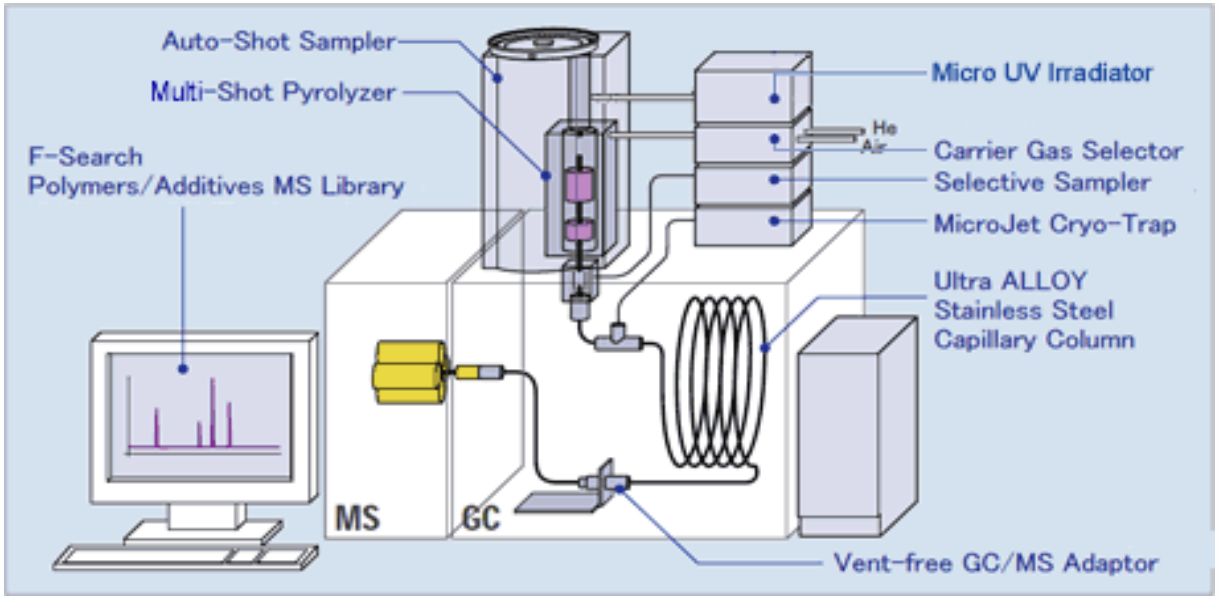
Micropyrolysis: set-up & methodology

Set-up



Methodology

1. Identification of the 41 most abundant components
2. Comparison of applying the normalised or the non-normalised data for PCA
3. Statistical analysis of the data: each includes the comparison of one of the different transgenic lines with WT

Samples and raw pyrolysis data

Identification of 45 components for each of the **15 samples**

1	10B	COMT-ASB10B
2	10B	COMT-ASB10B
3	WT	WT-Biological
4	WT	WT-Biological
5	CAD21	CAD T21
6	CAD21	CAD T21
7	2B	COMT ASB2B-2
8	2B	COMT ASB2B-2
9	2CoA-416	CCoAOMT-416
10	2CoA-416	CCoAOMT-416
11	CCOA-429	CCoAOMT-429
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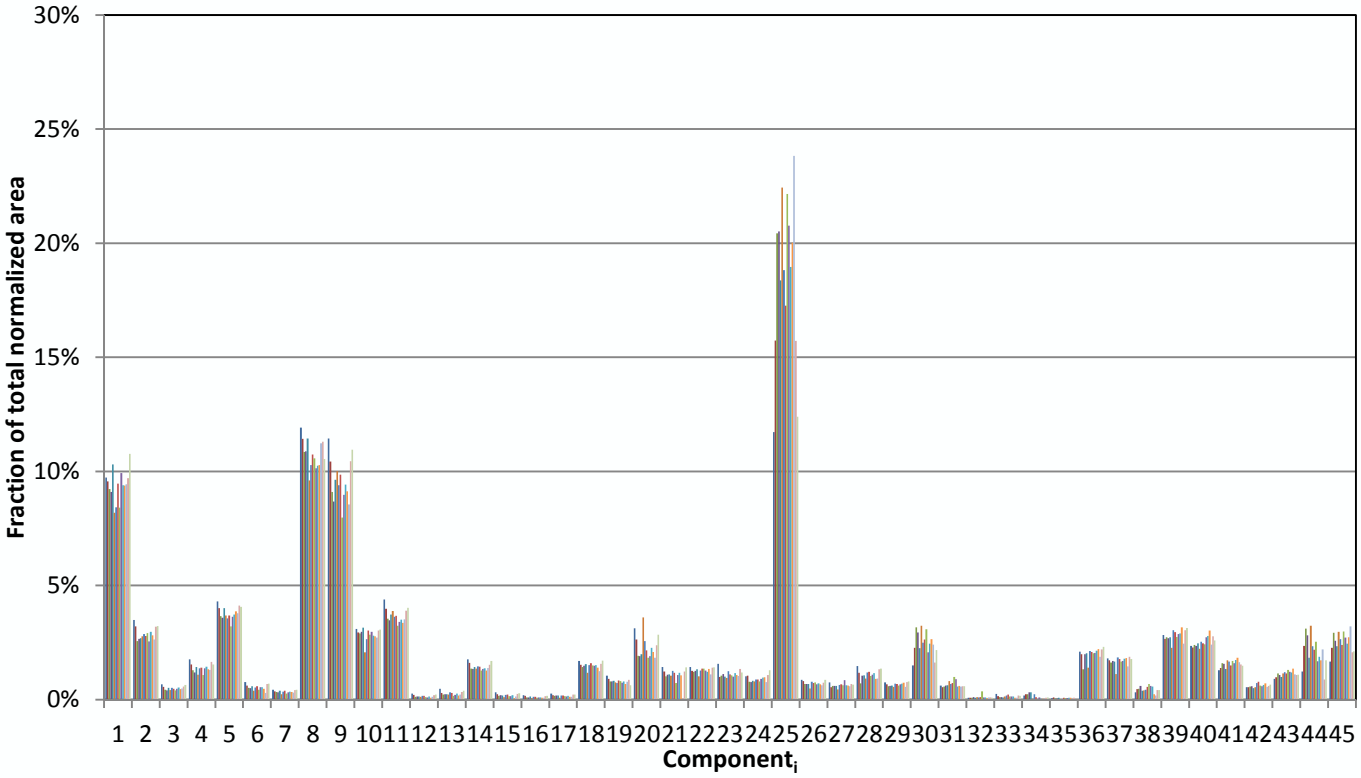


Is there a **difference** between the samples?
Is this difference **statistically significant?**

PCA + K-Means clustering

PCA & K-Means clustering

Principal Component Analysis

1) **Subtract** the normalized data with the **mean** and divide with the **standard deviation**
2) Determine the covariance matrix (C)
3) Determine the **eigenvectors** (Loadings V) and **eigenvalues (L)**
4) Select the PC that contain the **most variance** in the data set
5) Calculate the **scores** of each PC

→ Construct **new variables** which contain **most variance** present in the dataset

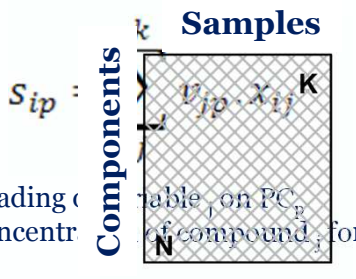
→ Observe paterns more easily

$$p_{i,j} = \frac{p_{i,j} - \mu_j}{s_j}$$

$$C = \frac{1}{n-1}.\tilde{P}^T\tilde{P}$$

$$V^T.C.V = L$$

$$S = \tilde{P}.V$$

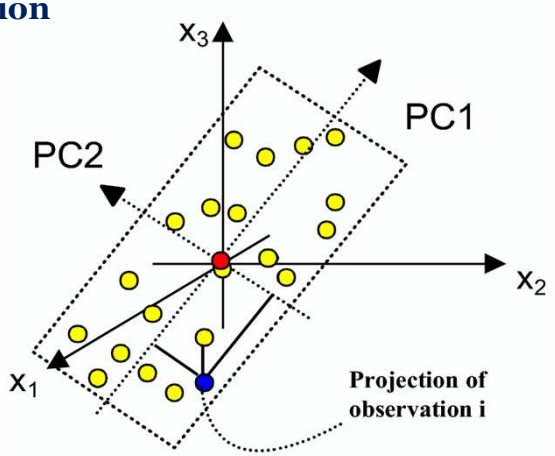


S_{ip} = $v_{jp}.x_{ij}$

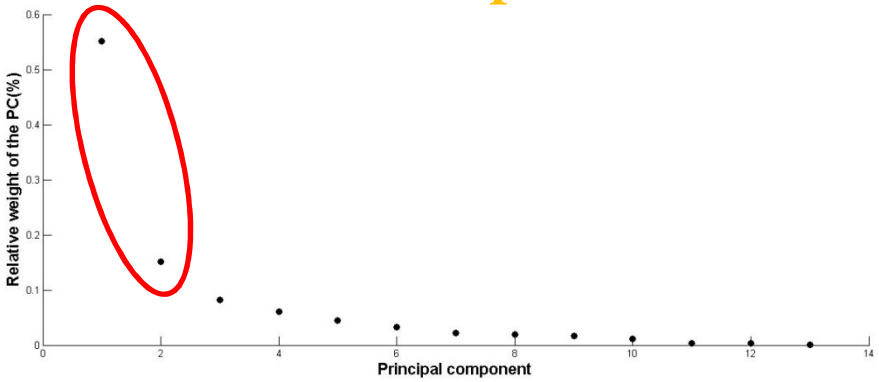
v_{jp} is the loading of variable $_j$ on PC_p

x_{ij} is the concentration of compound $_j$ for the i^{th} sample

Score plot



Scree plot



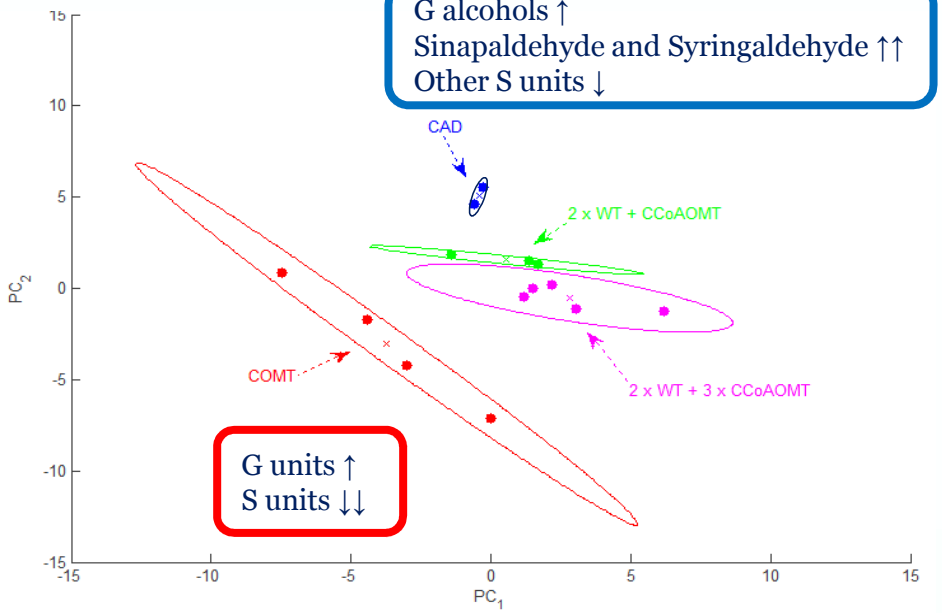
K-Means clustering

1) Select initial **amount of clusters**
2) Chose the initial cluster **centroids**
3) Calculate the **Mahalanobis Distance** (MD) for each sample with **respect to each centroid** $MD_{ik} = \sqrt{(x_i - \mu_k)^T.C^{-1}.(x_i - \mu_k)}$
4) Assign each sample to a cluster and recalculate cluster centroid
5) Iterate till **converged**
6) Draw the **confidence interval** (95%) that contains all data **similar** to the cluster **centroid**

$$T^2 = \frac{p.(n-1)}{n-p}.F_{p,n-p;0.05} = \mathrm{MD}^2$$

GC-MS results

Score plot



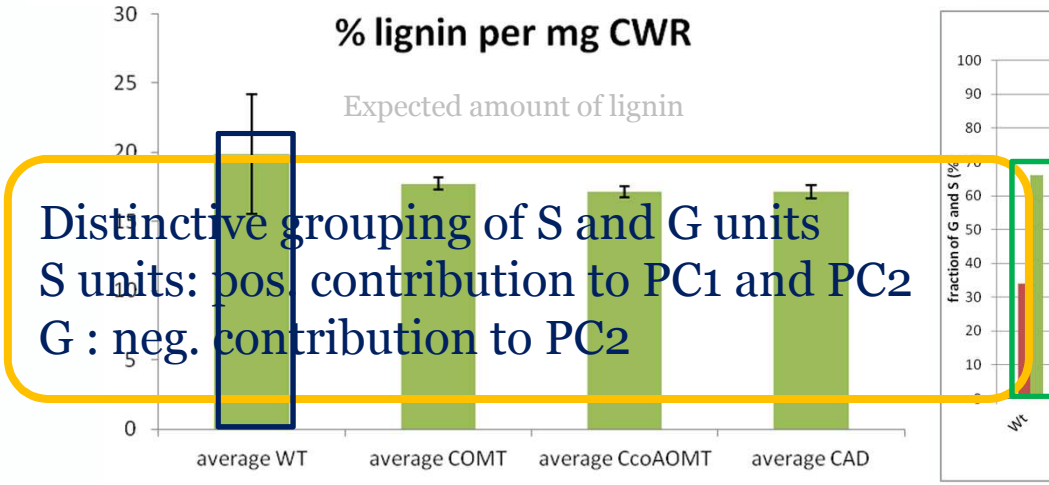
CAD
G alcohols ↑
Sinapaldehyde and Syringaldehyde ↑↑
Other S units ↓

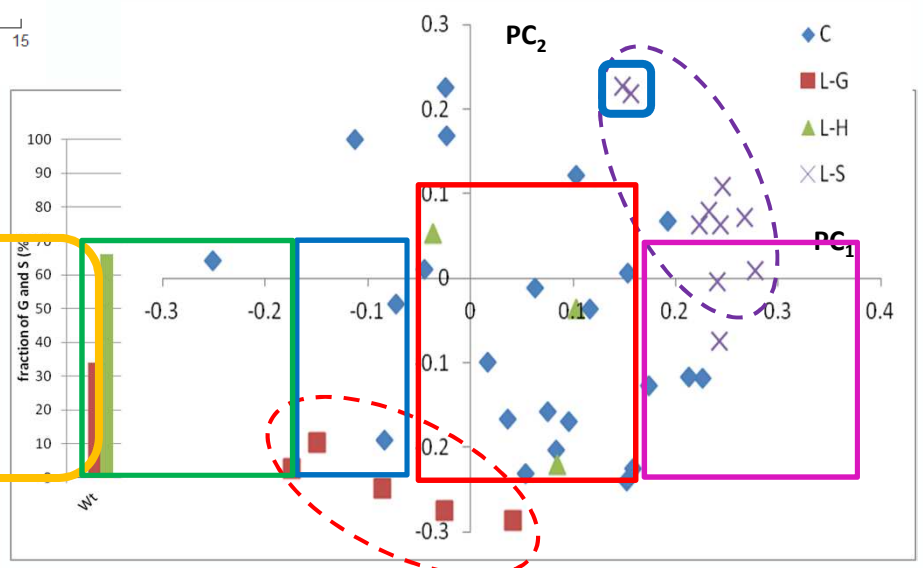
COMT
G units ↑
S units ↓↓

Distinct CAD and COMT cluster
Separated by PC2, PC1 + PC2
No separation between CCoAOMT and WT

Loading plot

Wet chemical analysis





Distinctive grouping of S and G units
S units: pos. contribution to PC1 and PC2
G : neg. contribution to PC2

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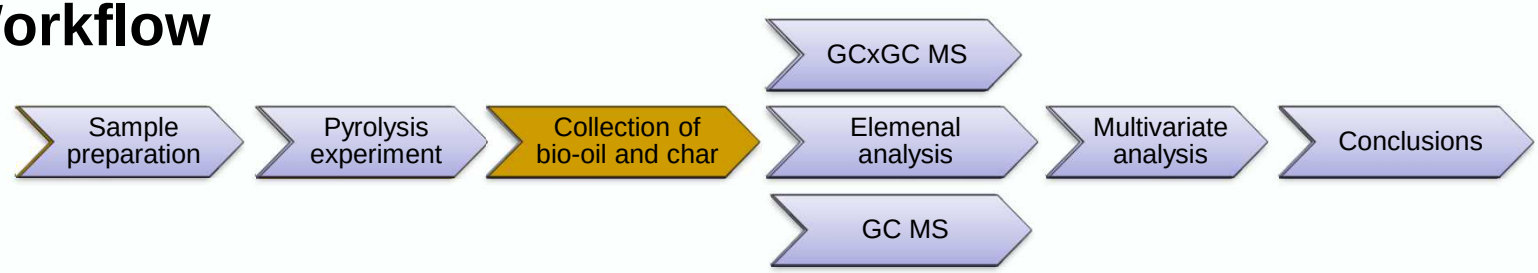
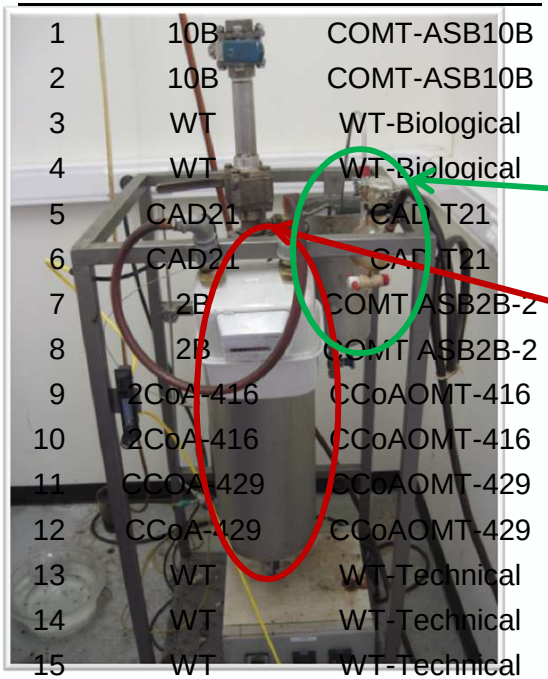
Pyrolysis experiments on the tube reactor

Workflow



16 samples



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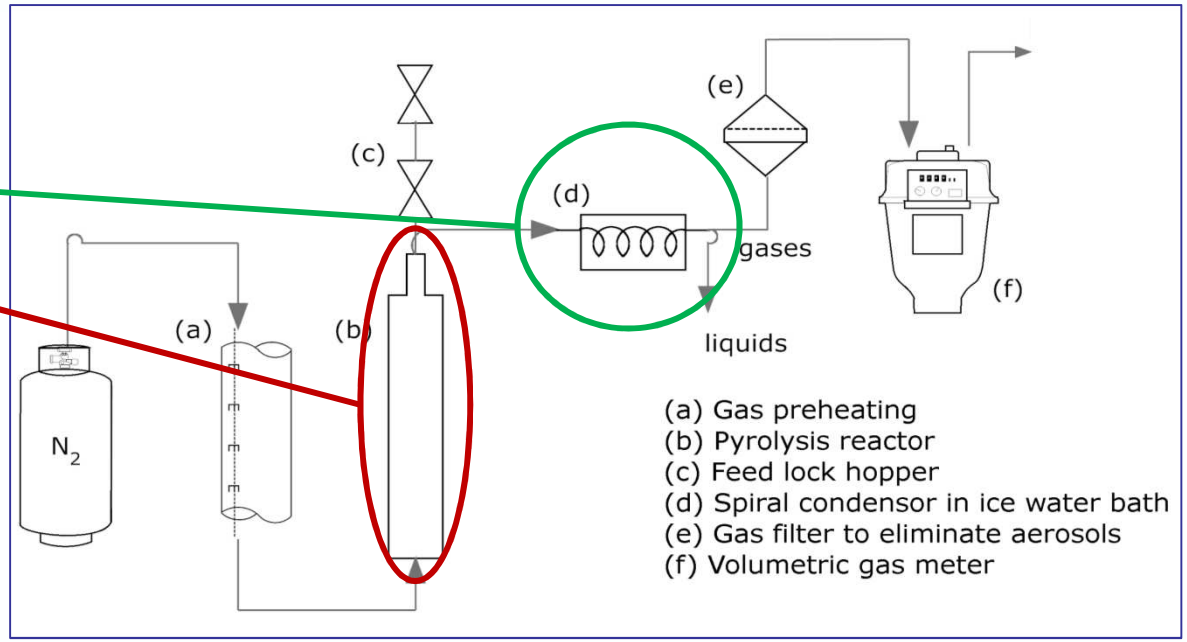
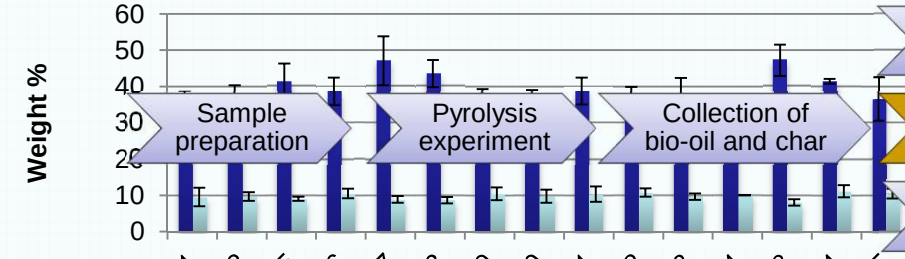


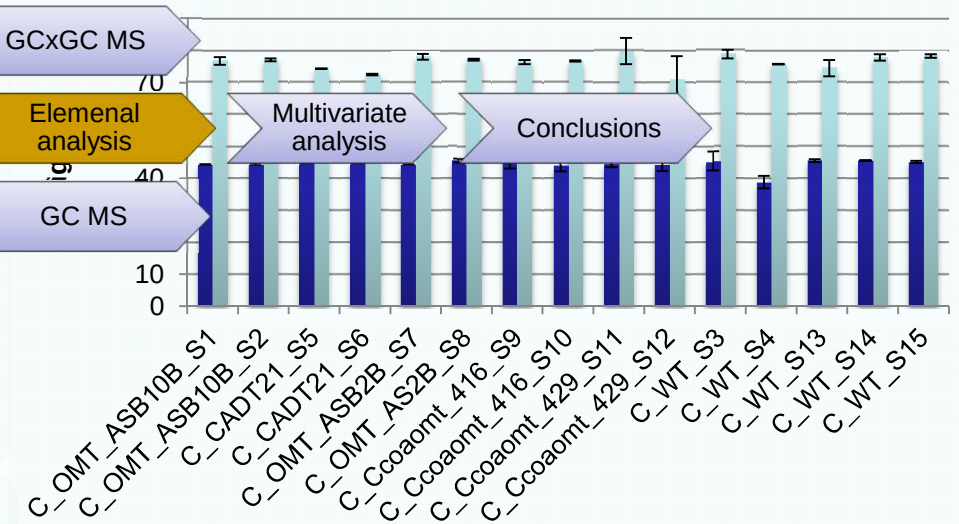
Elemental analysis: results

Workflow

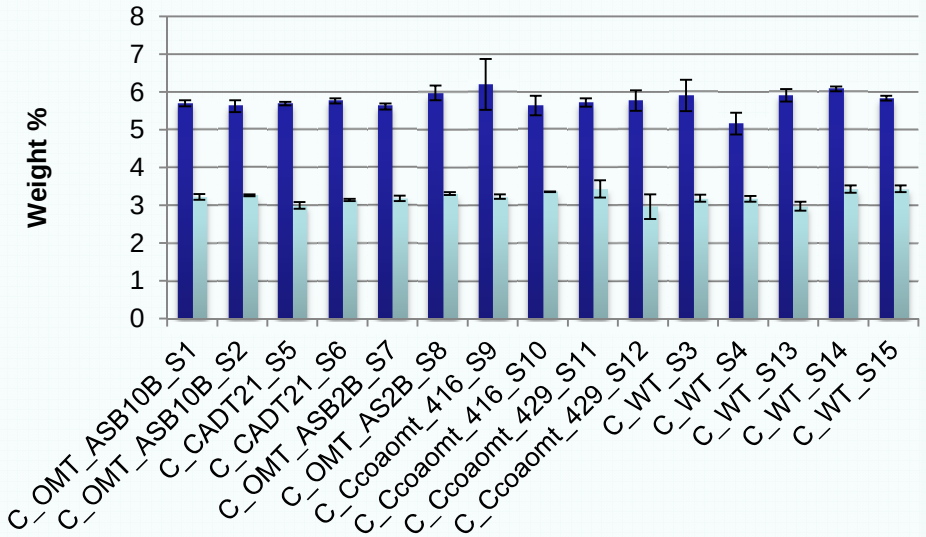


Elemental composition O

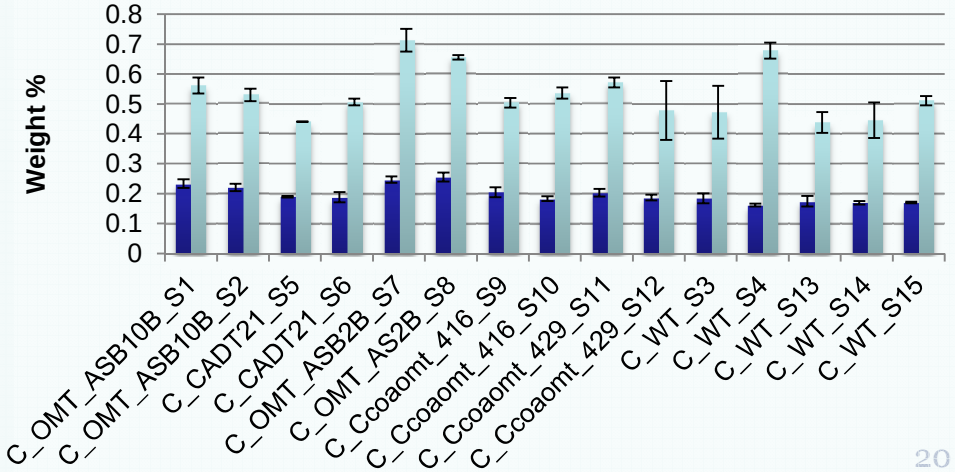
Elemental composition C



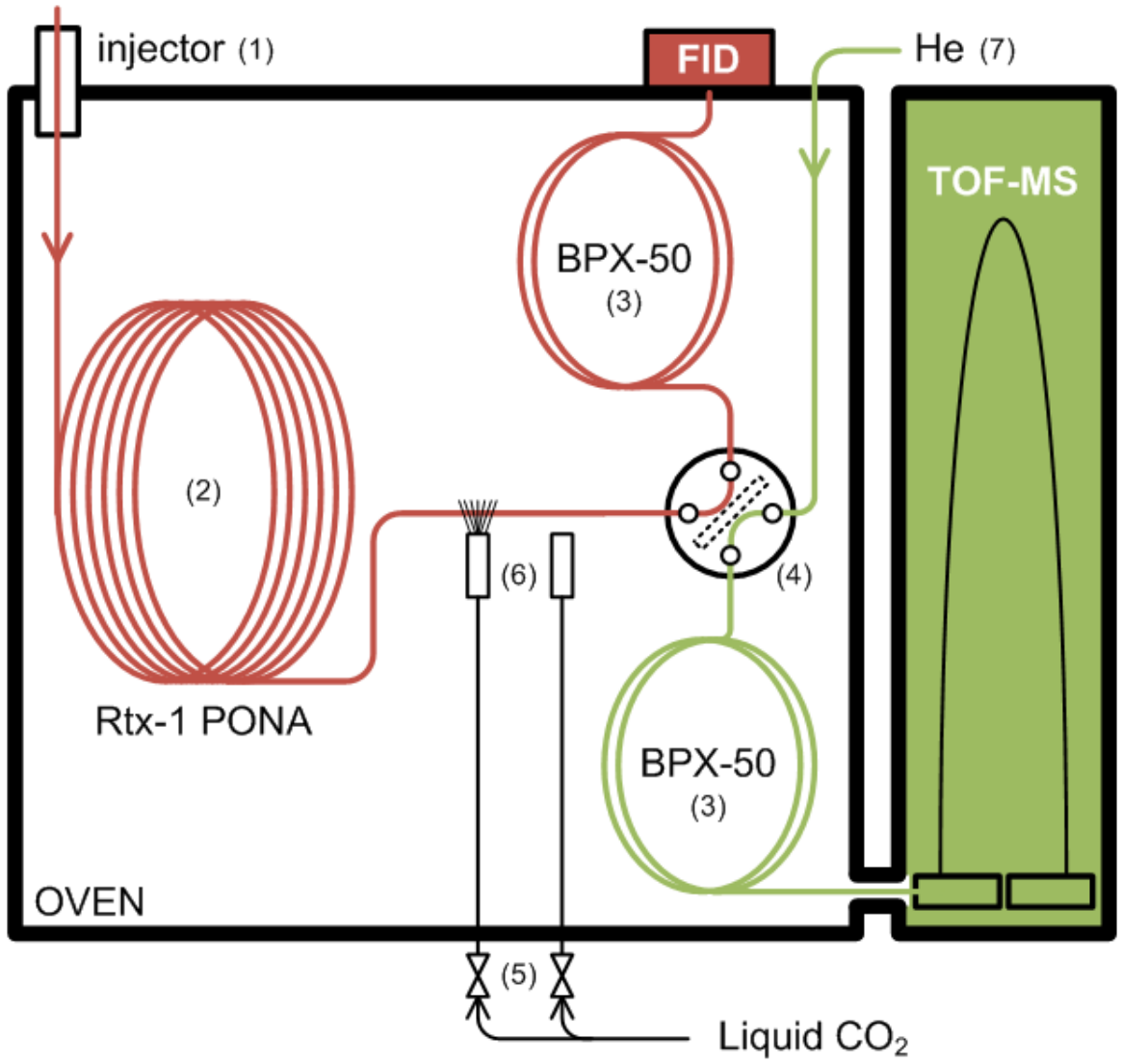
Elemental composition H



Elemental composition N



2D Gas chromatography for bio-oils



Van Geem, Pyl, et al. *J. Chrom. A.* 2010

Two independent separation mechanisms (based on BP and polarity)

Enhanced resolution compared to 1D-GC

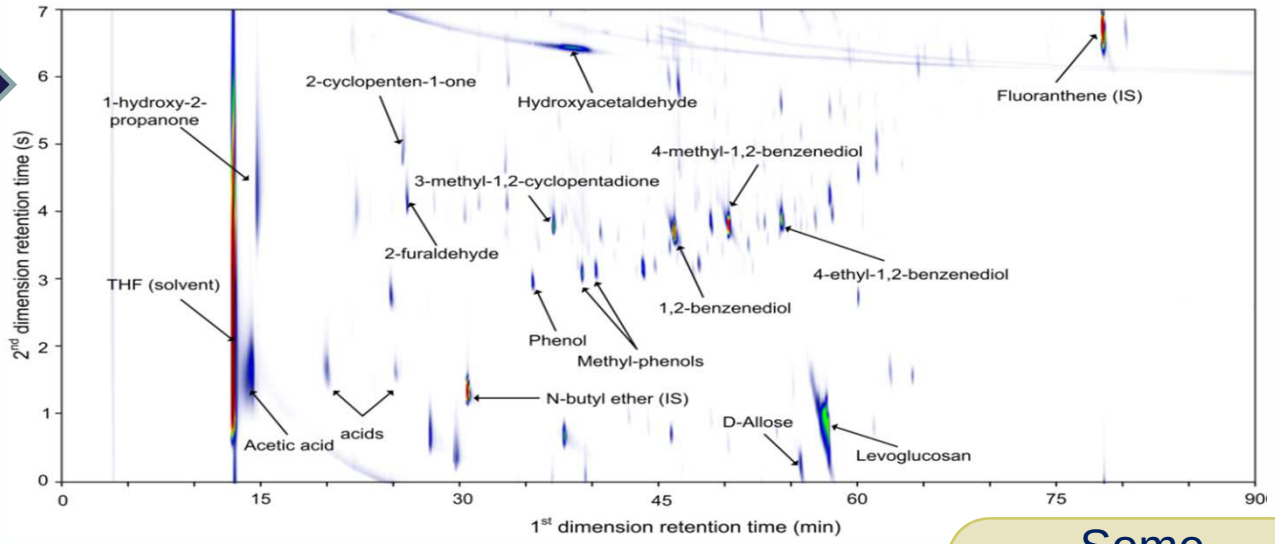
Two detectors

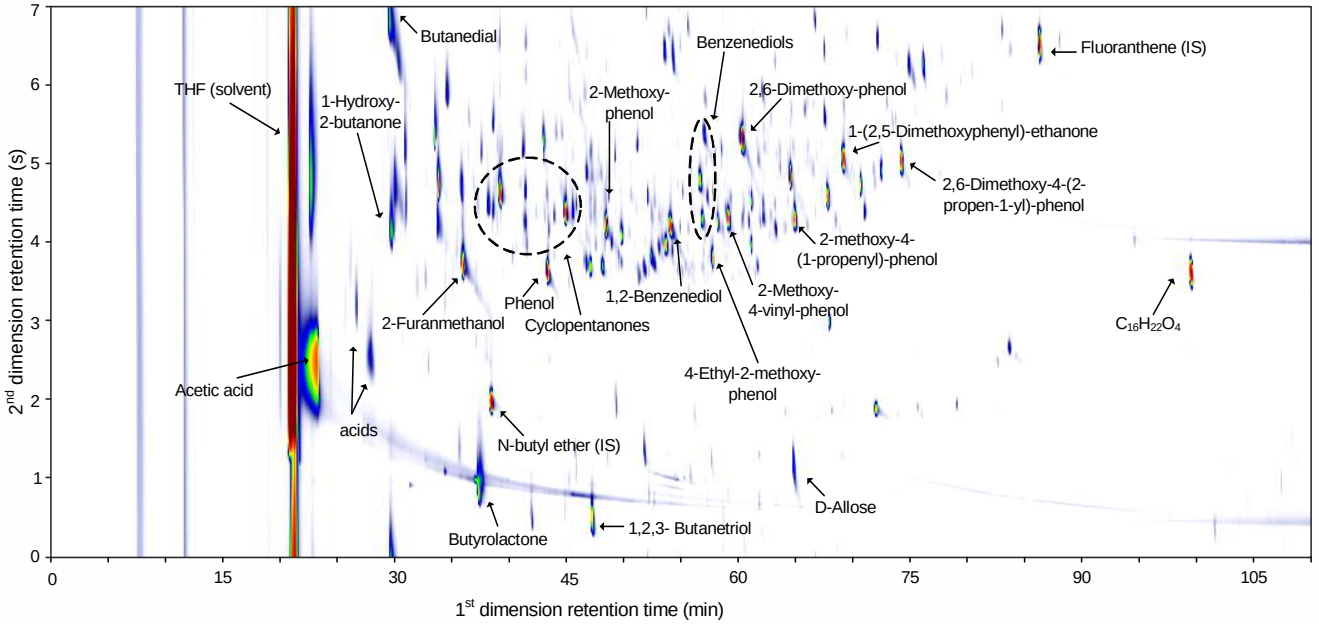
TOF-MS: peak identification (qualitative results)
FID: Quantitative results

Results: comparison crude oils

Derived from pine wood

Derived from poplar wood





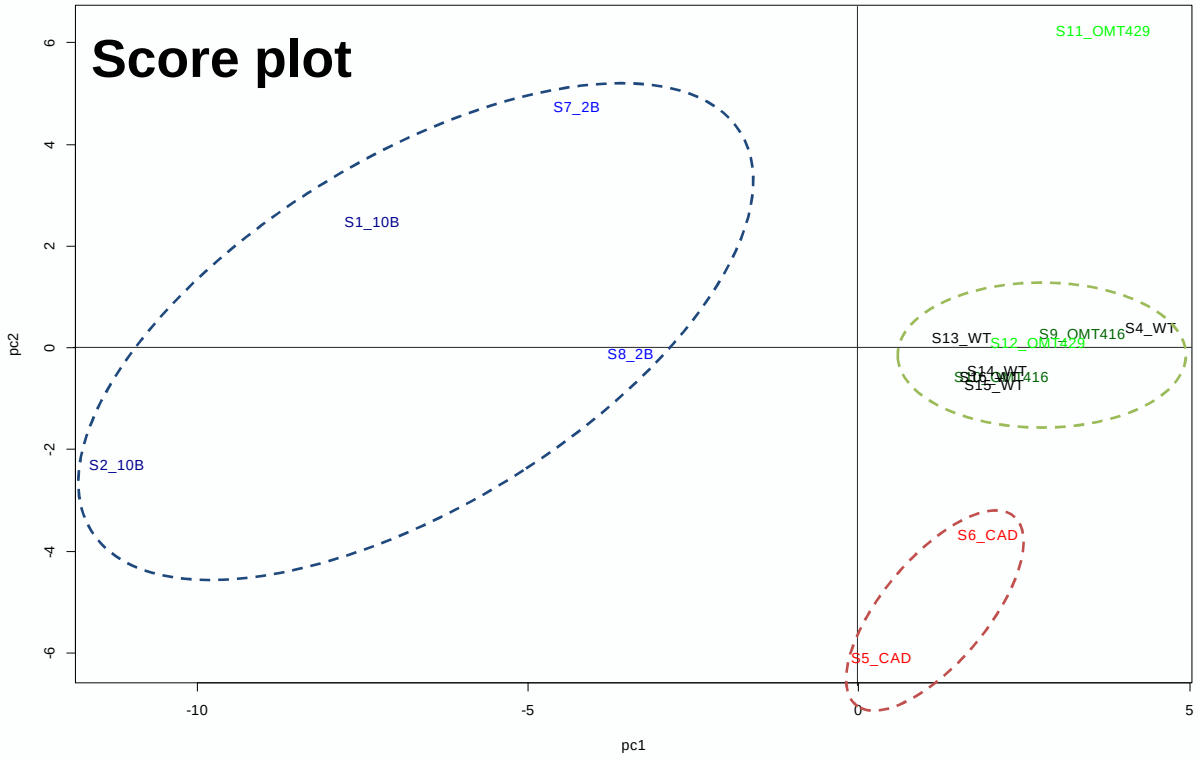
Some abundant components in common

Different feedstock leads to different components

GC×GC-MS/FID: methodology and results

Methodology

- Identification and quantification of over 100 components
- Input data for the PCA: 1) Calculated weight percentages
  2a) All identified components
  2b) Exclusion of non-lignin components
- Comparison between WT and each transgenic group with only the lignin derived components



Results

- Clear shift of COMT vs. WT
- Subtle shift of CAD vs. WT
- No shift of CCoAOMT vs. WT

Reason(s): CCoAOMT modification

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Conclusions

- ✓ Fast pyrolysis of biomass is a promising process
  - Crucial to gain insight in the inherent process and kinetics
  - Not all oxygen containing compounds in bio-oil are bad
- ✓ Detailed analysis of complex bio-oils can be obtained with GC×GC-FID/TOF-MS
  - Effect feedstock and/or catalytic treatment can be detected
  - 2D separation is crucial
- ✓ Hypotheses of COMT and CAD transgenic groups are validated
  - COMT differs the most of WT; S units lowered, G units higher. (More pronounced with 2B than 10B)
  - CAD contain more S aldehydes compared to the WT
  - No distinctive difference observed between CCoAOMT compared to WT

Questions