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The enzymatic degradation of technical lignins into monolignols for fuel production

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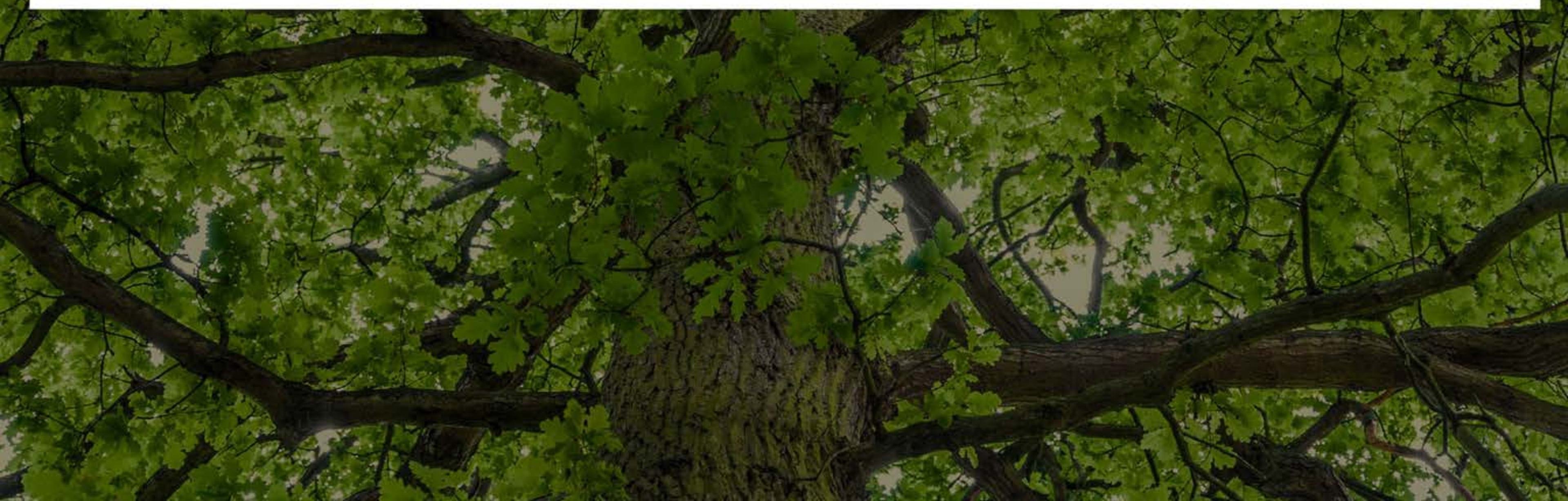
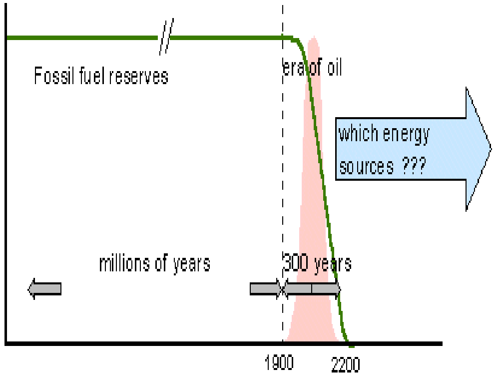


Why biofuels?

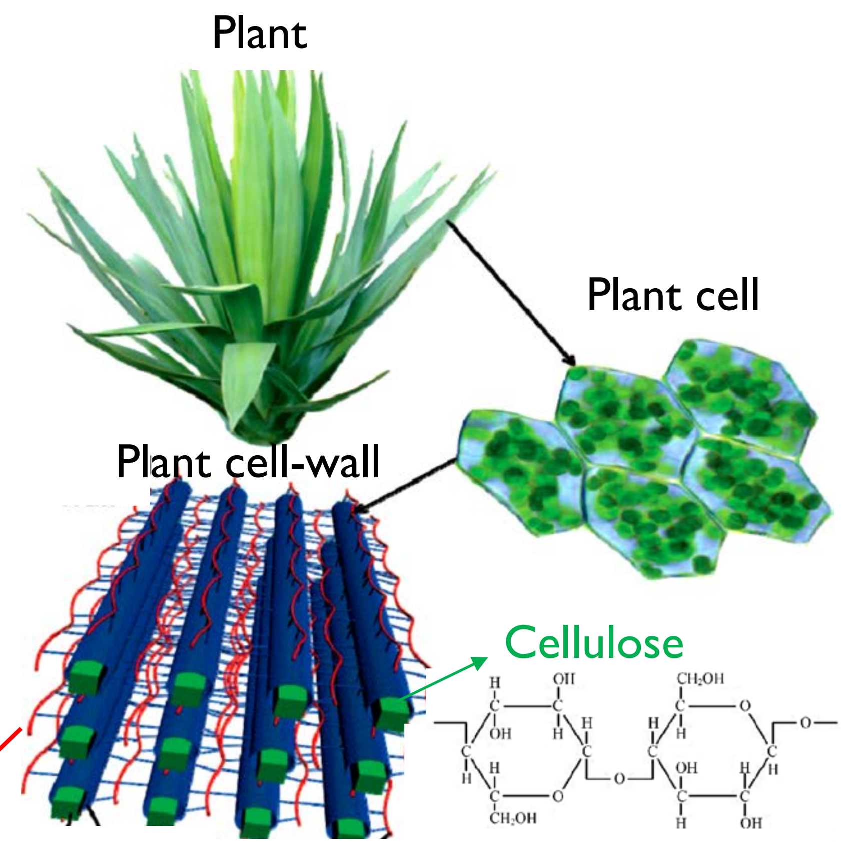
- Increasing energy requirements
- Depletion of fossil fuel reserves
- Global warming

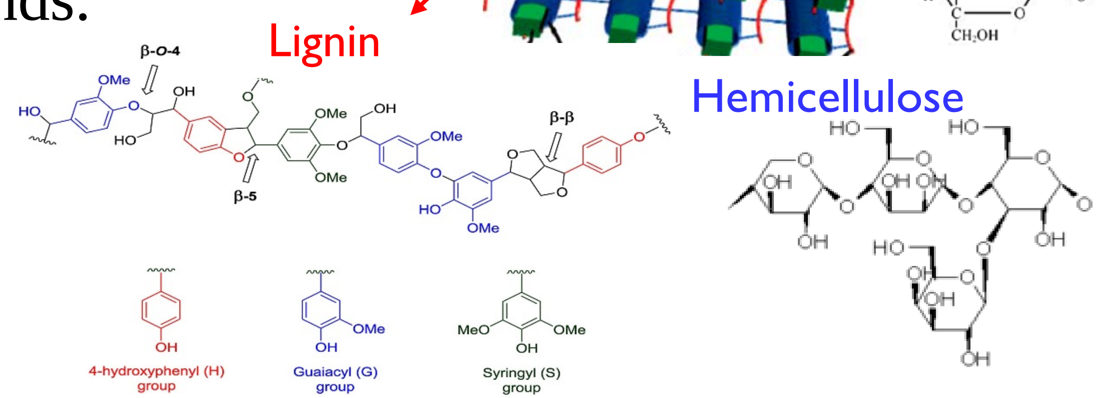


References: Constant *et al.*, 2016; Wang *et al.*, 2017

What is lignin?

- Cell-wall constituent of vascular plants (and some algae).
- Composed of aromatic residues (H:G:S)
- Lignin monomers are linked by β-O-4, β-β, β-5 bonds.





Reference: Georgina *et al.*, 2016

Sources and potential uses of lignin

- Industrial processes producing lignin (technical lignin)

1. Soda
2. Kraft
3. Sulphite
4. Steam explosion (Cellulosic ethanol process)

- Potential uses of lignin

1. Direct use e.g., cement additive (especially sulphite lignin)
2. Degraded lignin e.g., **fuel, fine chemicals**

References: Gordobil *et al*., 2016; Hussin *et al*., 2013

Lignin depolymerisation methods

- Thermal methods

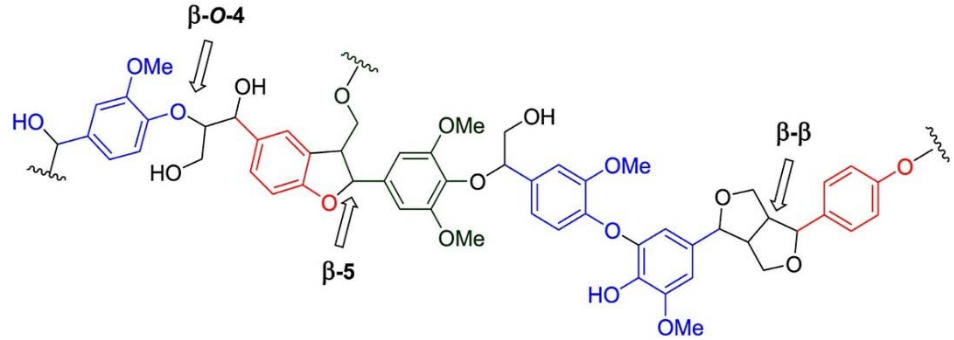
1. Pyrolysis
2. Combustion

- Chemical methods

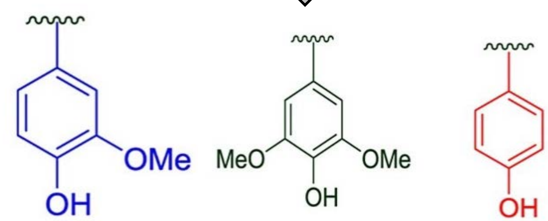
1. Alkaline (e.g., NaOH)
2. Acid (e.g., formic acid)

- Biological methods

1. Whole cells (fungi and/ or bacteria)
2. **Enzymes**



Lignin polymer



Lignin monomers

Fuel

Reference: Azadi *et al.*, 2013; Naron *et al.*, 2019

Enzymes suitable for lignin degradation and their limitations

- Enzymes capable of degrading lignin

1. Laccase
2. Lignin peroxidase
3. Manganese peroxidase

- Accessory enzymes involved in lignin degradation

1. Cellobiose dehydrogenase
2. Quinone reductase, etc.

- Problems with the utilisation of enzymes

1. Difficult to produce
2. Difficult to implement due to:

    I. Heterogeneity of the lignin polymer
    II. Poor accessibility to the lignin polymer by the enzymes
    III. Polymerisation vs depolymerisation

Aim and Objectives

- Aim

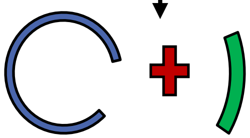
1. To develop an enzymatic approach for effective lignin degradation into low molecular weight compound for fuel production

- Main Objectives

1. To investigate the thermal stability of the lignins after enzymatic treatment
2. To identify low molecular weight aromatic compounds by GC-MS
3. To use GPC and 13C-NMR to explain the difference in the lignin structures

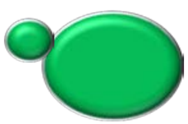
Methods

Enzyme production



Gene

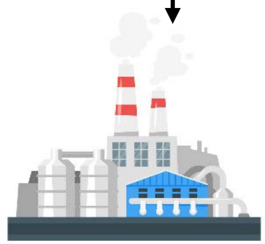
Plasmid



Recombinant yeast (*Pichia pastoris*)

Fed-batch in 15 L bioreactor

Lignin collection and preparation



Pulp industry

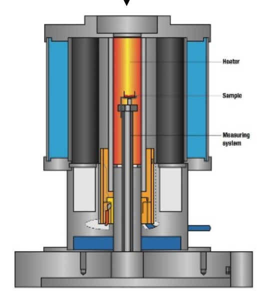
Black liquor

Extraction and purification

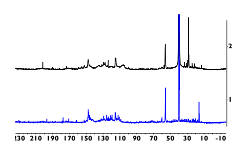
Lignin

Lignin degradation and product analysis

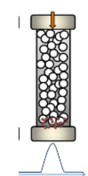
Biodegradation
Enzymes :LiP alone
LiP with CDH and QR
Conditions:
30 °C; pH=3.0; 6 h;
200 rpm



Thermal gravimetric analysis



13-C NMR

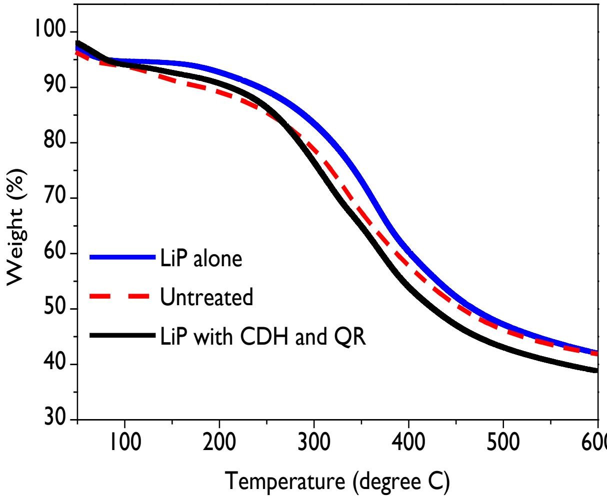


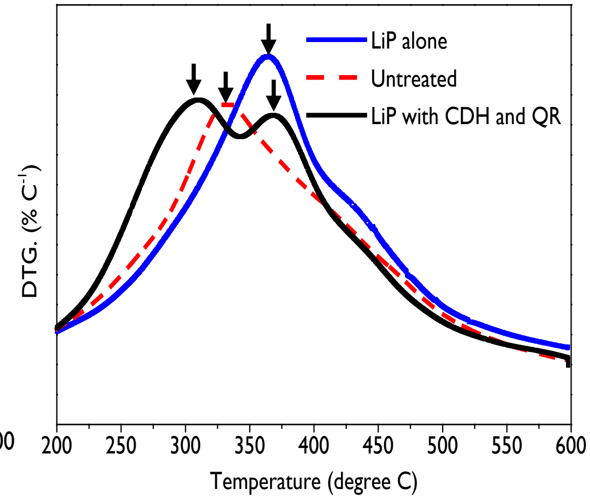
GPC

Structural analysis

Results: Thermogravimetric analysis

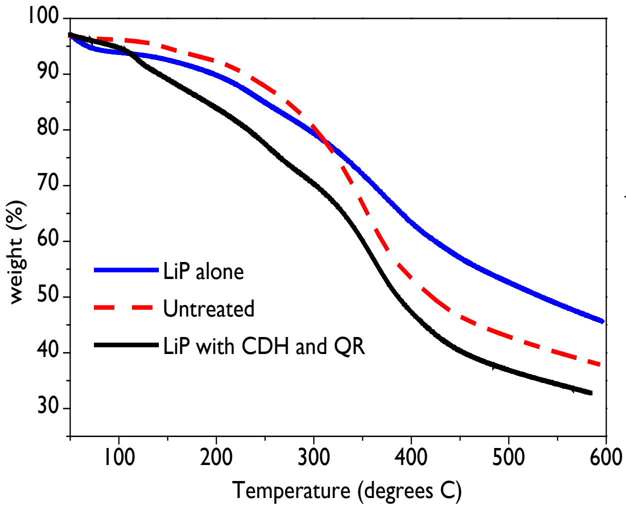
STEAM EXPLOSION lignin

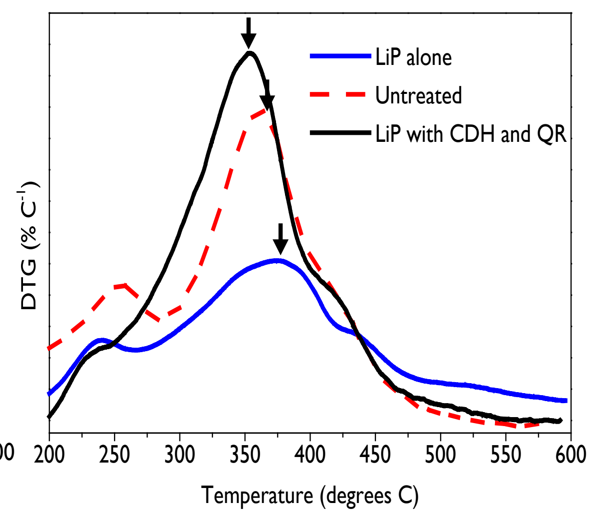




	Char yield (wt%)	Maximum DTG (°C)
Untreated	41.9	340.5
LiP alone	42.1	370.1
LiP with CDH and QR	38.8	312.8 and 372.1

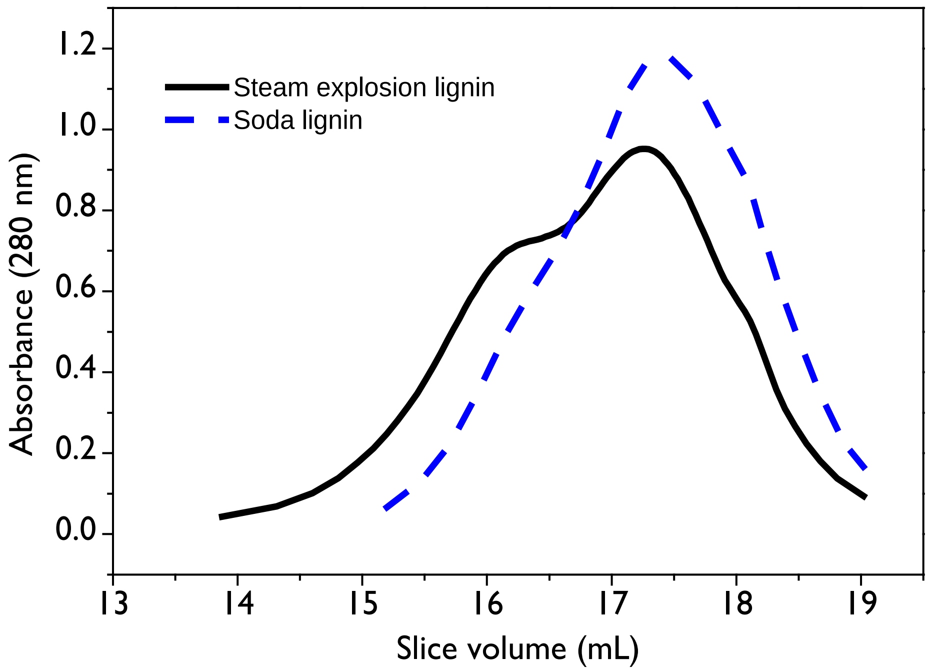
SODA lignin





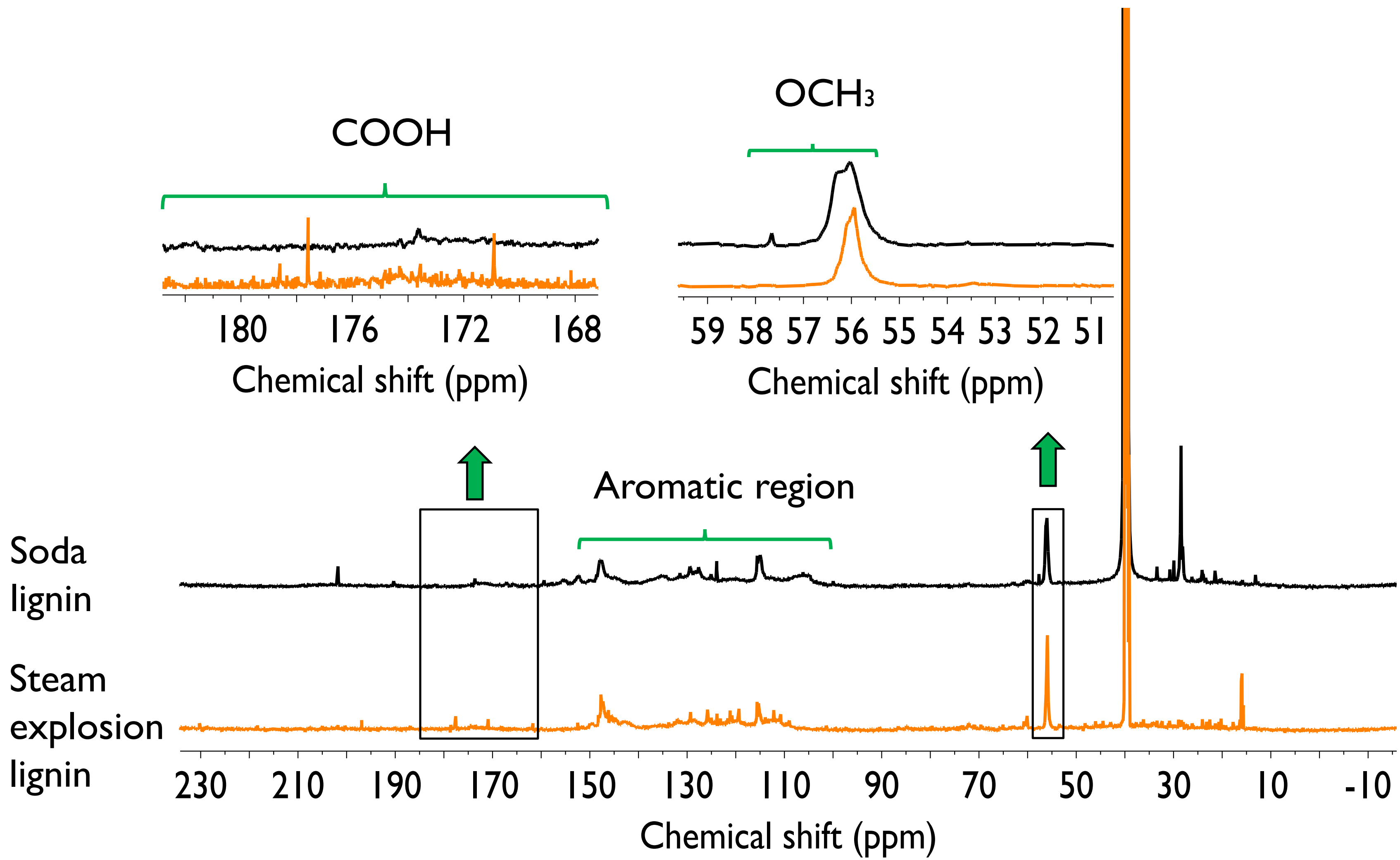
	Char yield (wt%)	Maximum DTG (°C)
Untreated	37.9	367.2
LiP alone	45.7	381.7
LiP with CDH and QR	32.7	355.3

Results: Molecular weight properties of lignins determined by GPC



	Mw (g/mol)	Mn (g/mol)	Polydispersity index(Mw/Mn)
Steam explosion lignin	6335	2263	2.8
Soda lignin	3253	1721	1.9

Results: Structural properties of lignin samples determined using 13C NMR



Conclusions and direction for future work

- The study demonstrated effective degradation of lignin though the use of a combined enzyme cocktail (LiP:CDH:QR).

- Knowledge of the lignin structure helped in understanding lignin:enzyme interactions.

Future work

- Analysis of the degradation products using GC-MS to elucidate the most viable lignin residues for fuel production.

Acknowledgments

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DR Luvuyo Tyhoda

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