Products from Thermochemical/Biochemical Hybrid Processes

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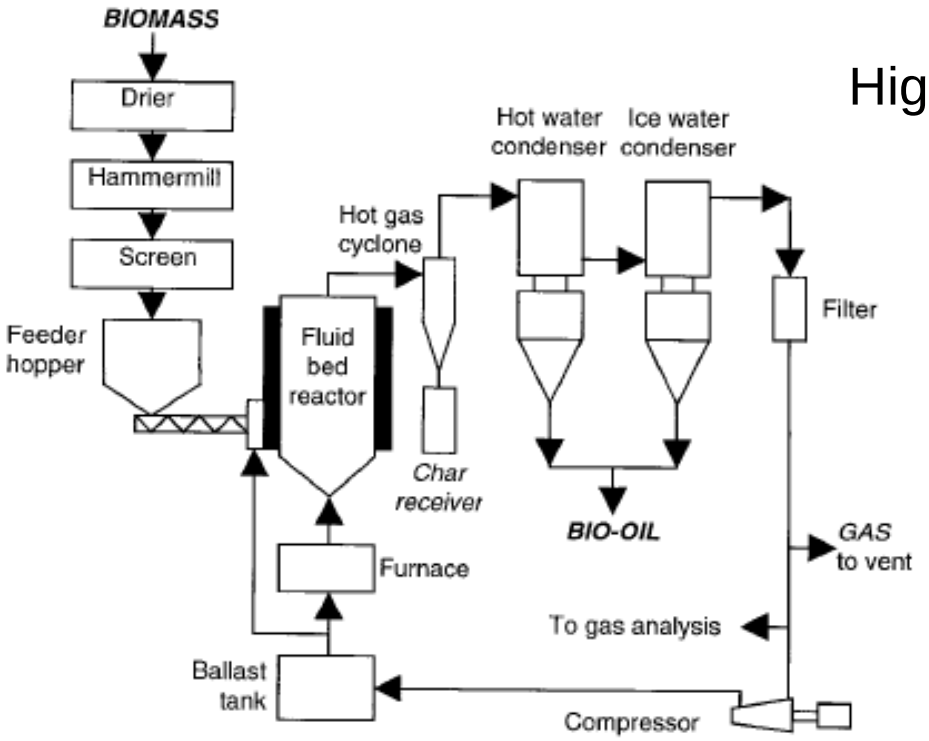
Outline

- 1. Introduction
- 2. Enhancing the Production of Levoglucosan
- 3. Conversion of Levoglucosan into Bio-fuel precursors (Lipids, Ethanol, Butanol)
- 4. Conclusions

Introduction

Fast Pyrolysis

High temperature process (300 - 600 ^{o}C) in which biomass is rapidly heated in the absence of oxygen to produce high yields of oil (***up to 70 mass %***).



High yields of liquids are obtained when:

(1) Very small particle (less than 2 mm) is used

(2) The residence time of the pyrolysis vapors is less than 2 s to minimize secondary reactions.

Introduction

Problem 1

Existing pyrolysis technologies *convert more than 50 mass % of Lignin into precursors of transportation fuels (mono-lignols and Lignin Oligomers)*, however only a relatively small fraction of *cellulose (around 10 %) is converted into hydrolysable anhydro-sugars (levoglucosan)*, the rest is converted into C1-C4 molecules, gases or poorly known cross-linked oligomeric anhydrosugars with limited markets.

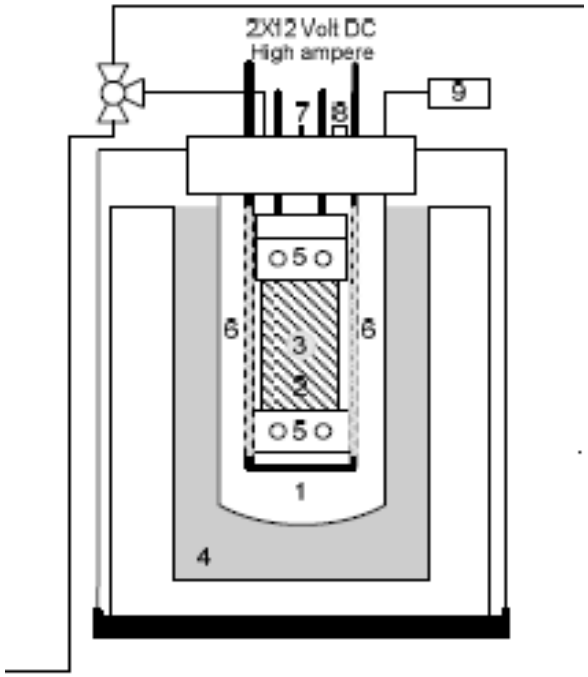
Problem 2

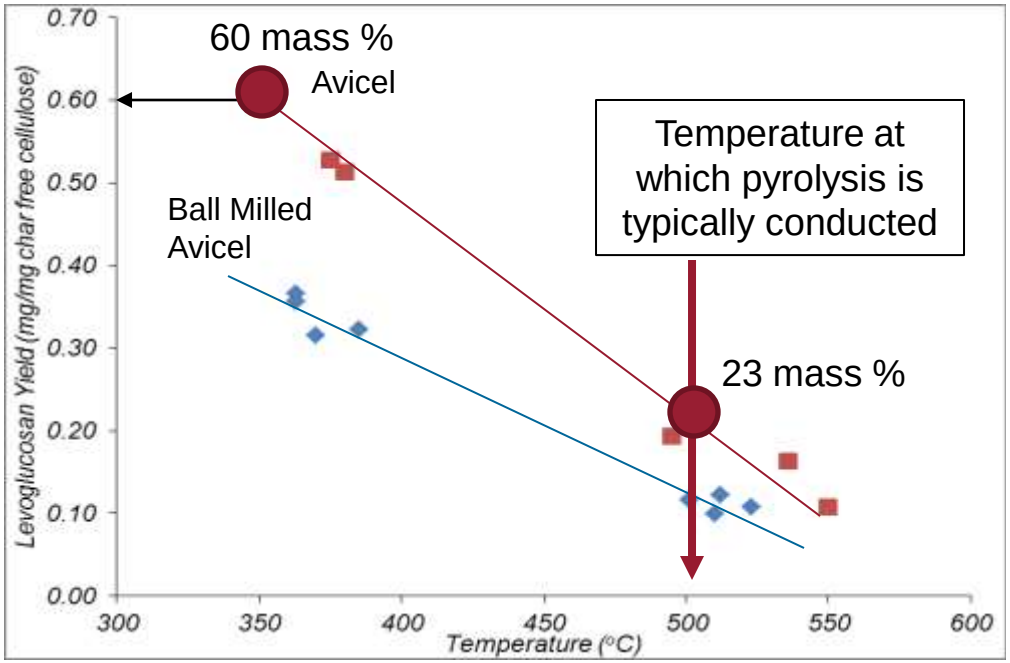
Hydrotreatment strategies *are not well suited to convert cellulose products into transportation fuels and chemicals*. Biological Conversion strategies for levoglucosan could offer new opportunities.

Introduction

Pyrolysis of Cellulose

Vacuum Mesh Reactor





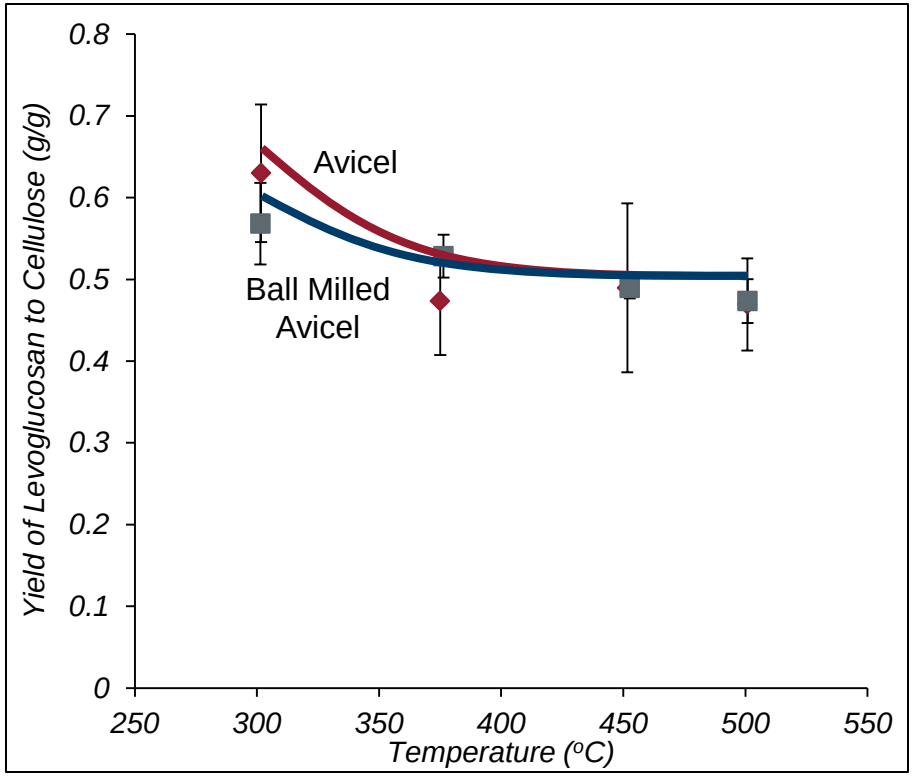
Low yields of Levoglucosan achieved in existing technologies are due to: (**1**) Pyrolysis temperature optimized to maximize oil yield (**2**) Catalytic effect of alkalines

In Collaboration with the University of Twente (Z. Wang, R. Westerhof and S. Kersten)

Introduction

Pyrolysis of Cellulose

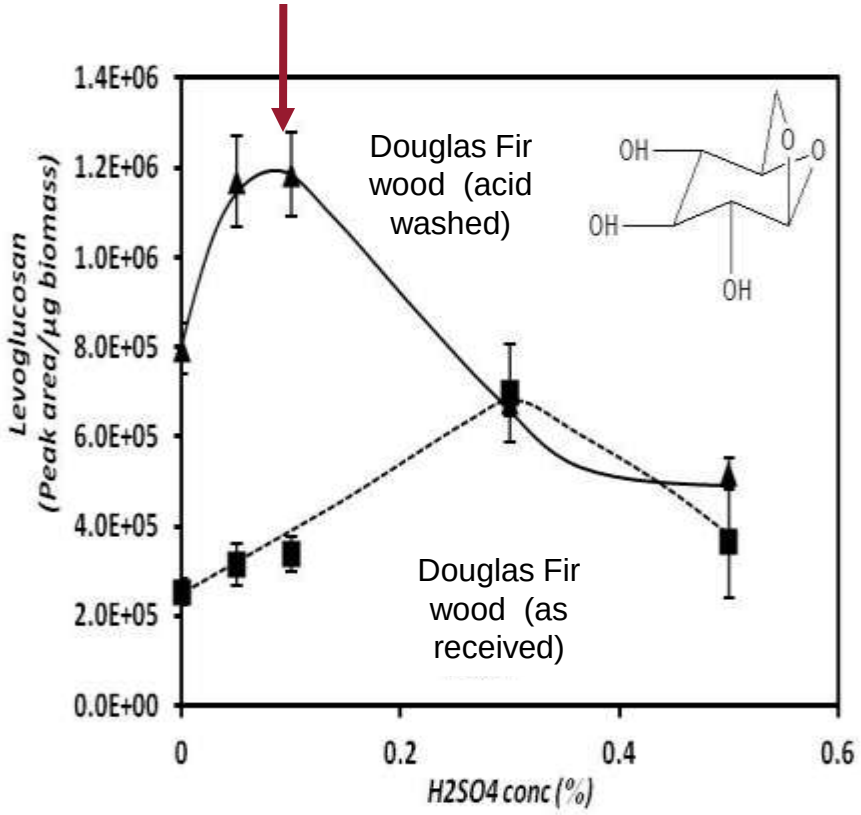
Atmospheric Mesh Reactor at Washington State University

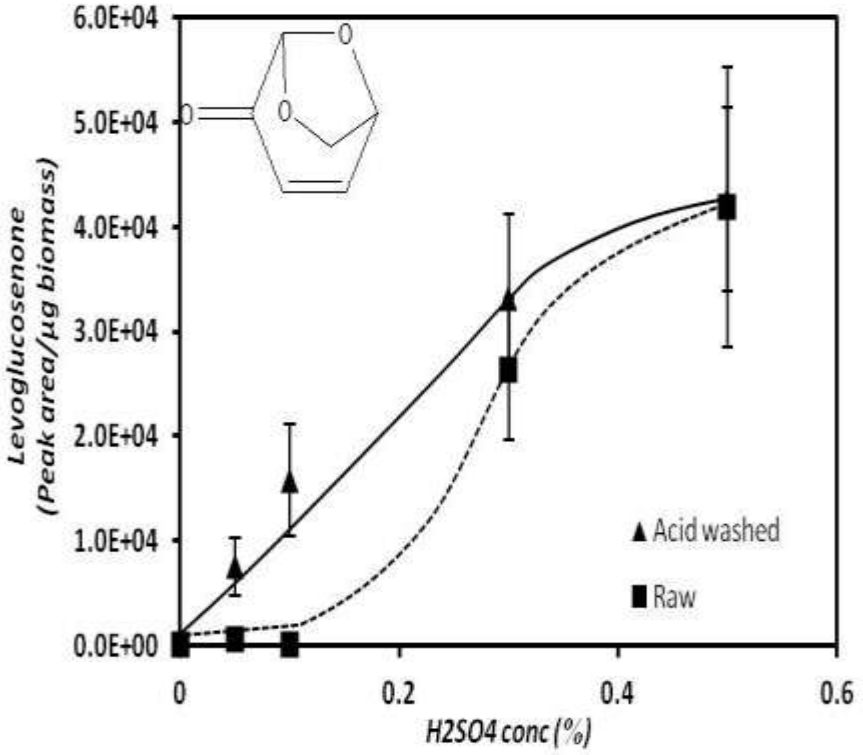


Low yields of Levoglucosan achieved in existing technologies are due to: (**1**) Pyrolysis temperature optimized to maximize oil yield (**2**) Catalytic effect of alkalines

Enhancing the Production of Levoglucosan

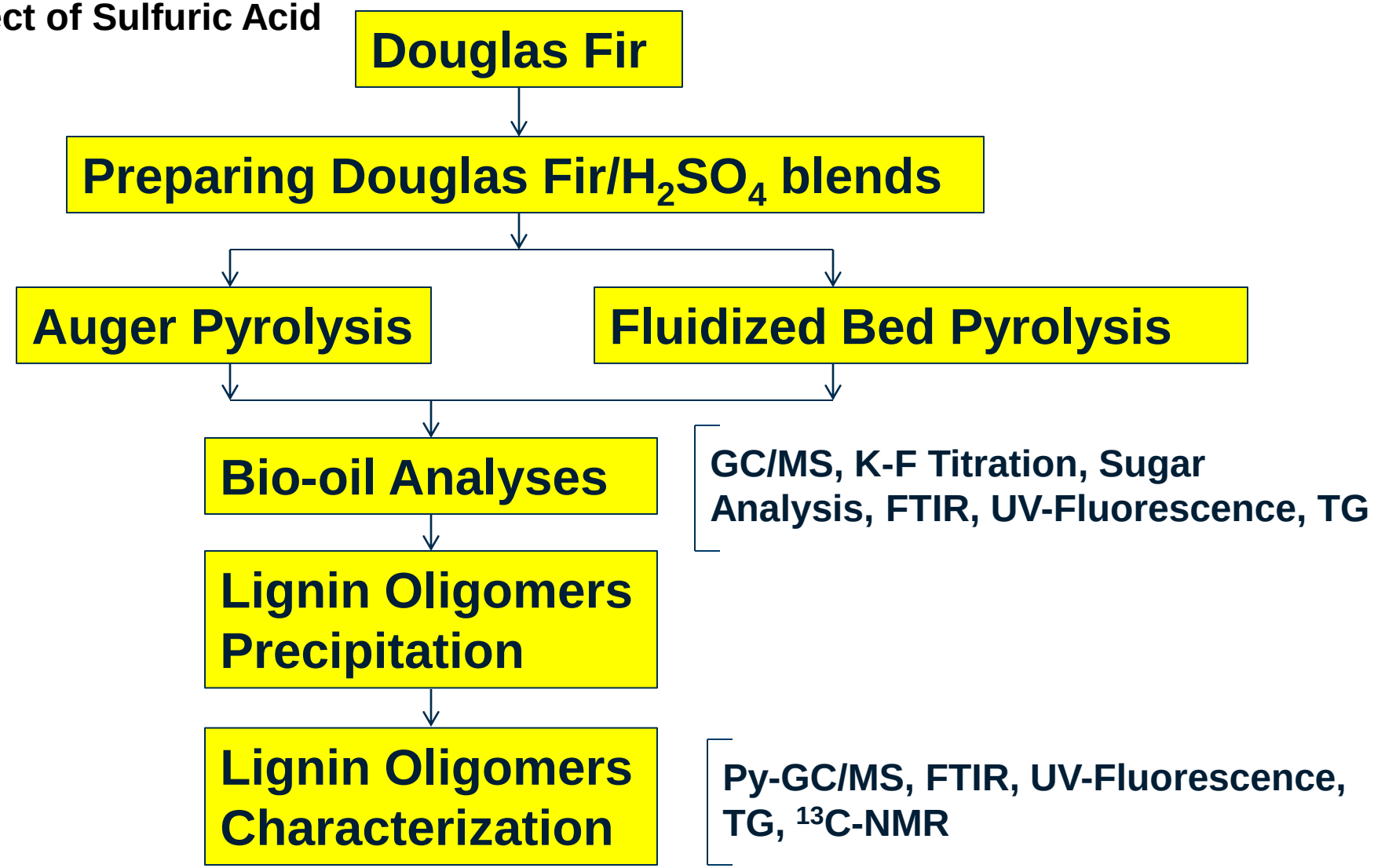
Effect of Sulfuric Acid Addition (Py-GC/MS) (Screening)





Enhancing the Production of Levoglucosan

Effect of Sulfuric Acid



Enhancing the Production of Levoglucosan

Auger Pyrolysis Reactor

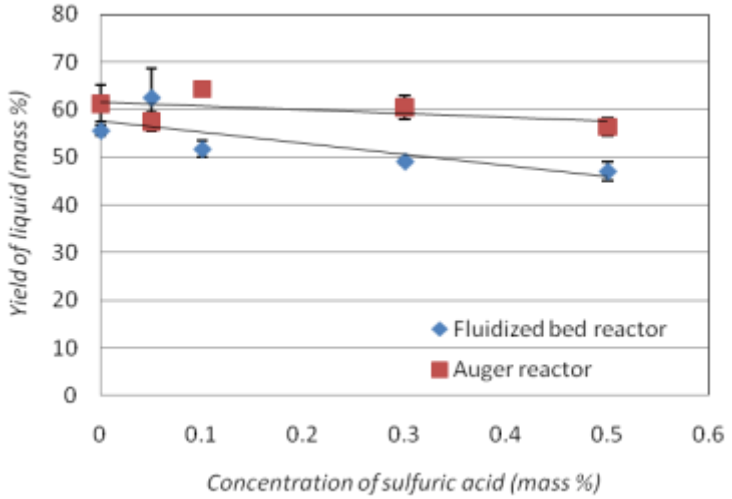
Washington State University

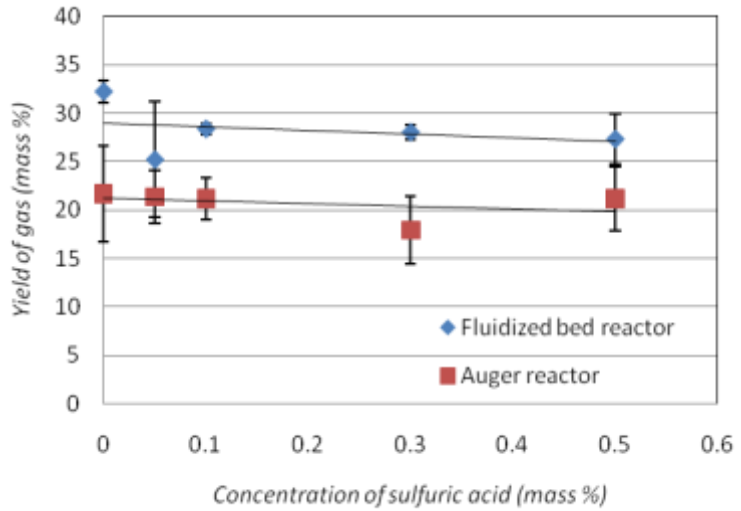
Fluidized Bed Pyrolysis Reactor

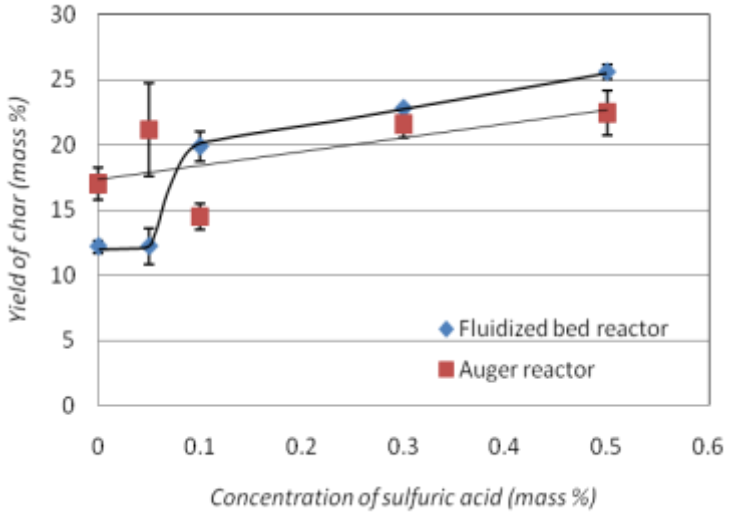
Curtin University (Australia)

Enhancing the Production of Levoglucosan

Product Yields







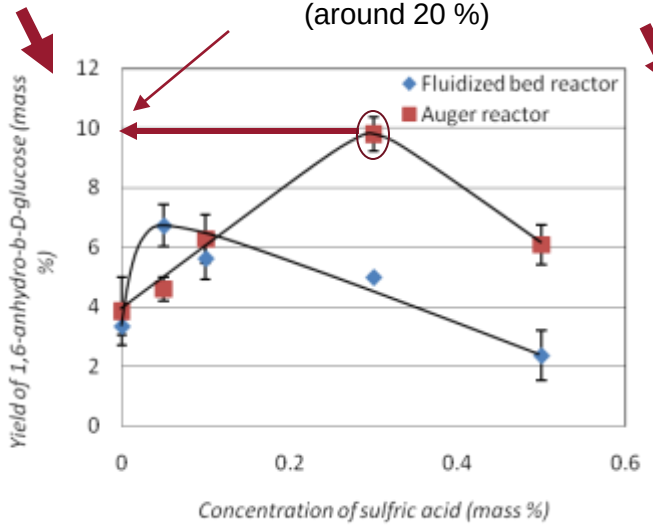
The **yield of products** obtained with an Auger reactor and with a Fluidized bed reactor **were comparable.**

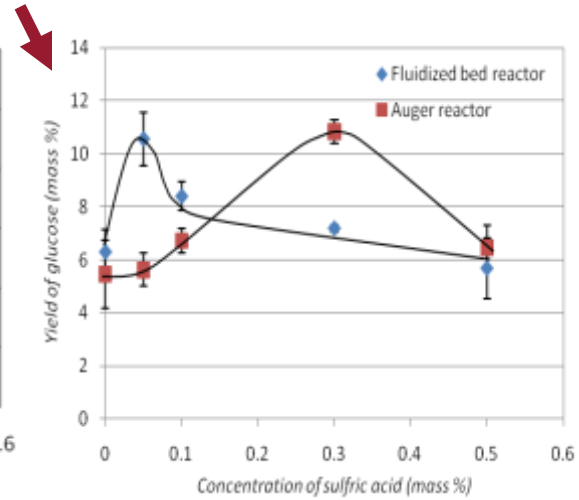
Bio-oil yield decreased slightly and bio-char yield increased as sulfuric acid concentration increased.

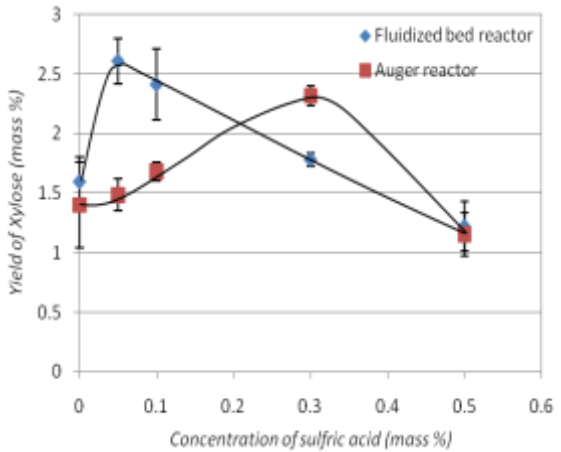
Enhancing the Production of Levoglucosan

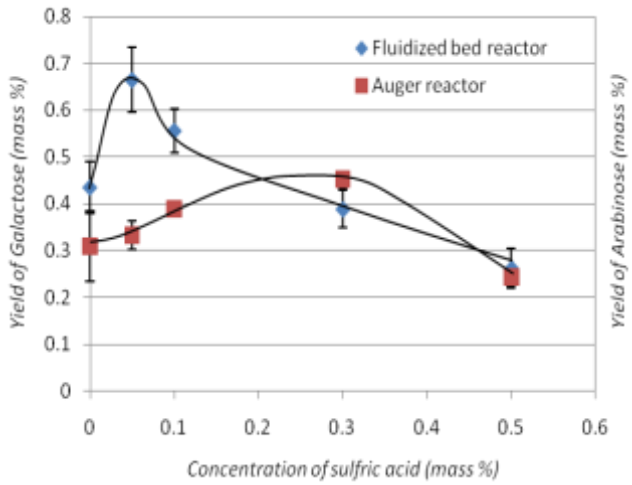
Yields of Sugars

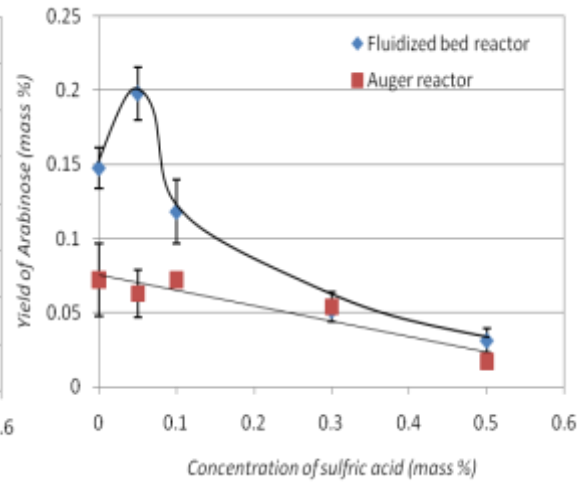
Expected maximum
(around 20 %)

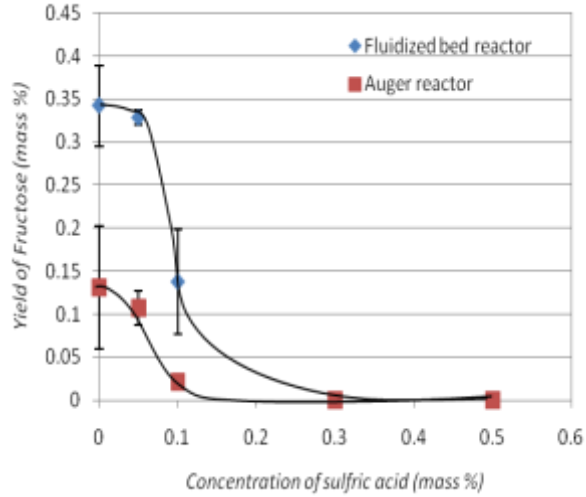








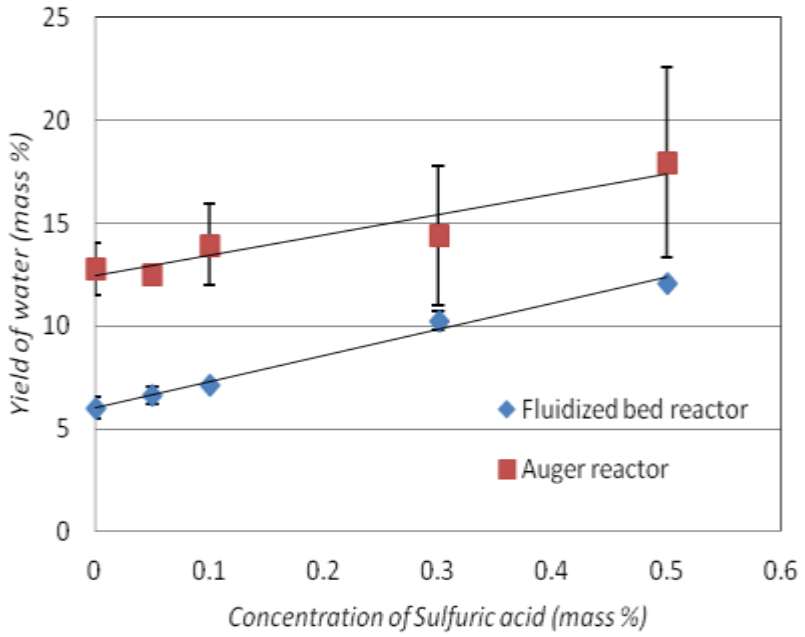


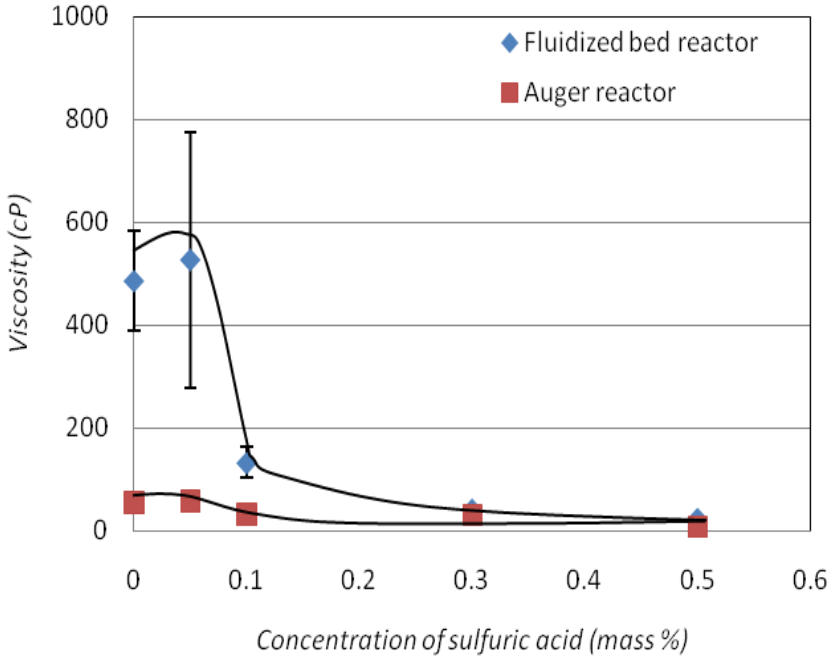


The maximum yield of these sugars was obtained at sulfuric acid concentrations of 0.05 mass % for the fluidized bed reactor and of 0.3 mass % for the auger Pyrolysis reactor.

Enhancing the Production of Levoglucosan

Yield of Water and Viscosity

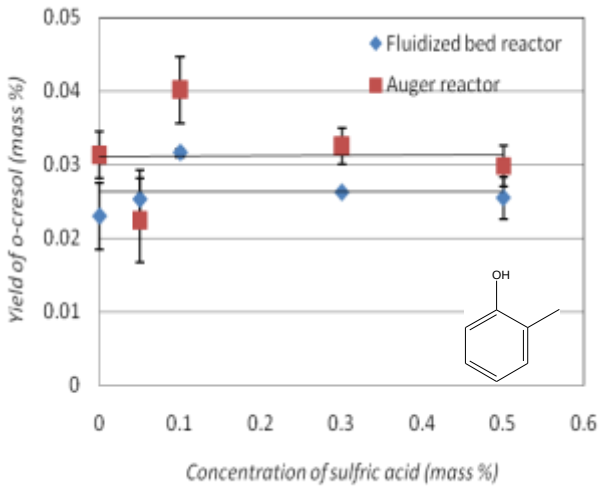


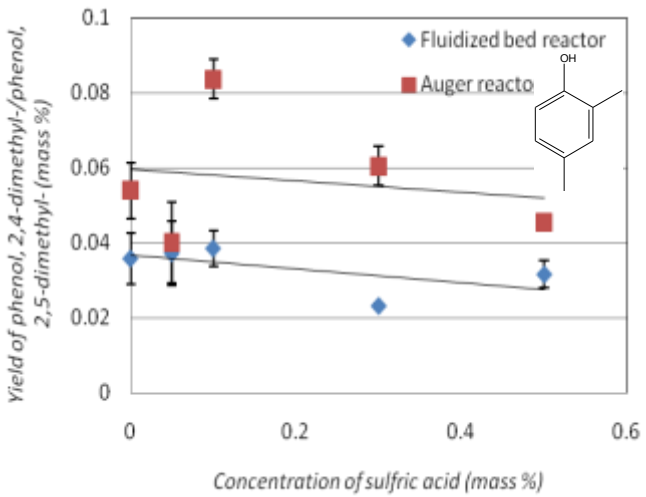


In both reactors the ***water yield increased linearly*** with the concentration of sulfuric acid indicating ***acceleration of dehydratation reactions***. Bio-oil viscosity decreases as sulfuric acid concentration increases.

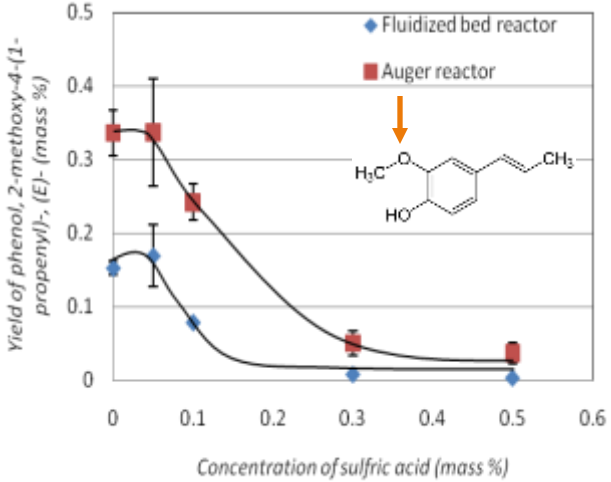
Enhancing the Production of Levoglucosan

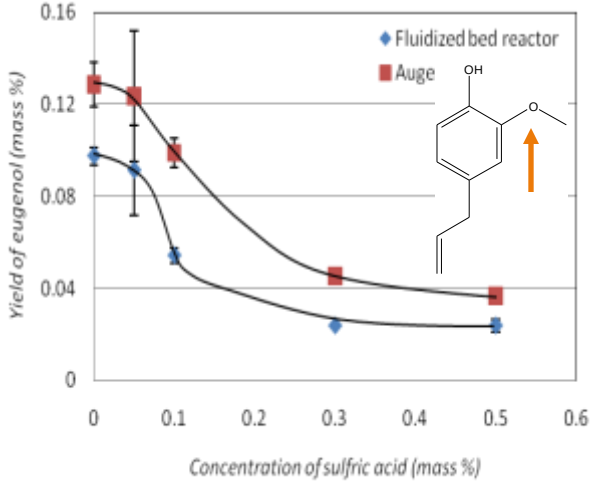
Yields of Mono-Lignols: GC/MS

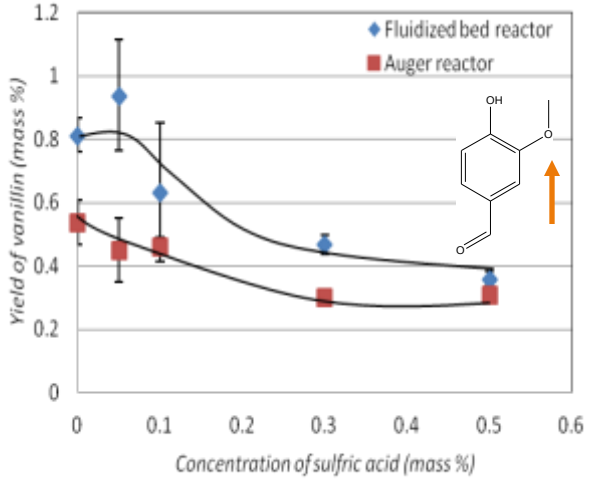




The production of vanillin, phenol - 2 - methoxy - 4 - (1 -propenyl)- and eugeol was drastically reduced as the concentration of sulfuric acid increased. **These compounds have methoxy groups in their structures** which make them very reactive.

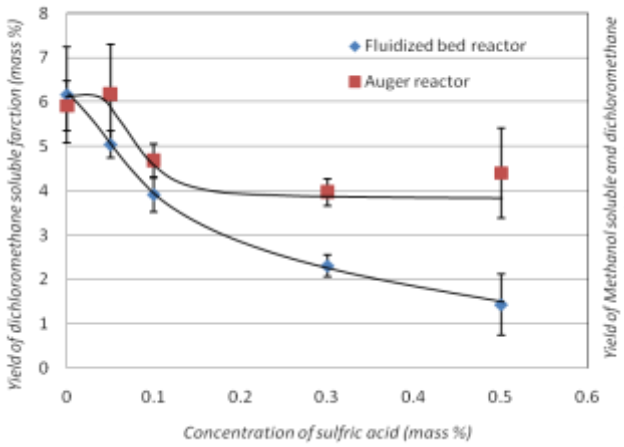


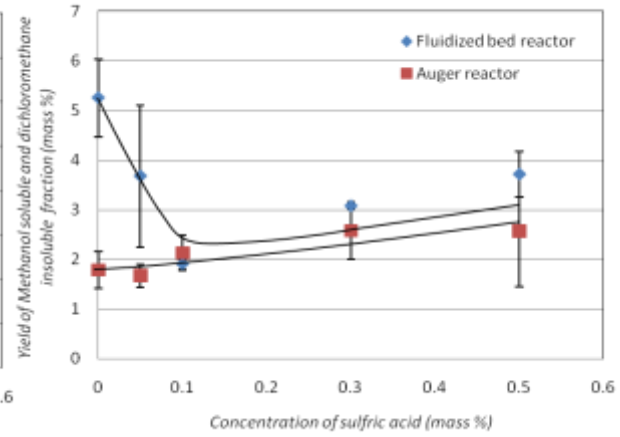


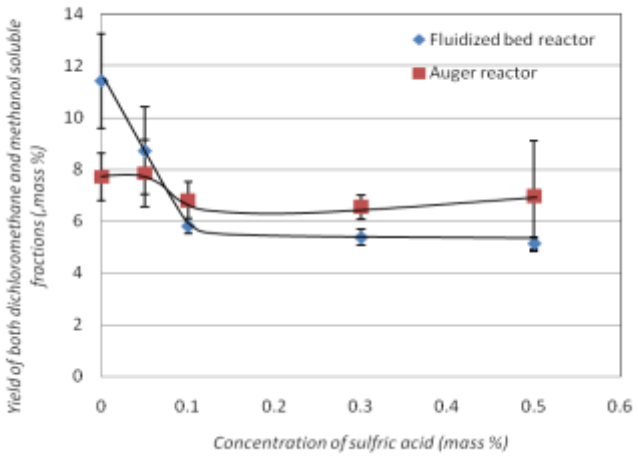


Enhancing the Production of Levoglucosan

Yield of Lignin Oligomers



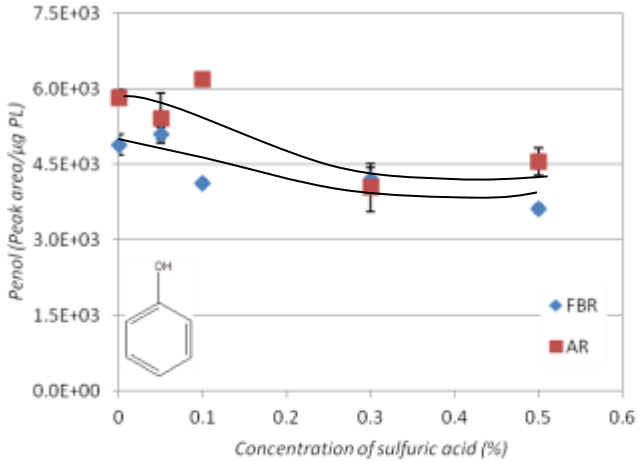


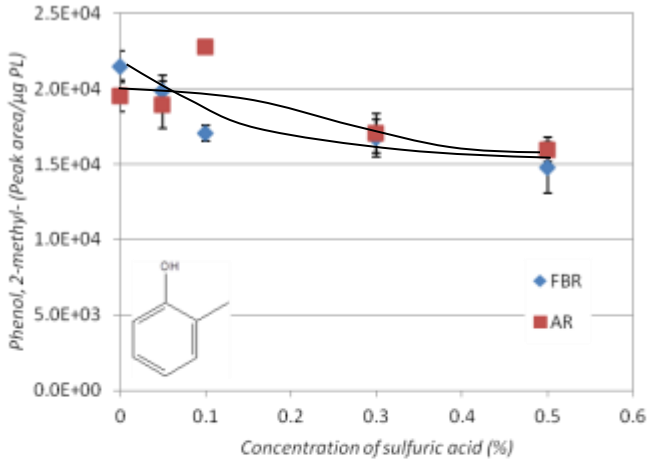


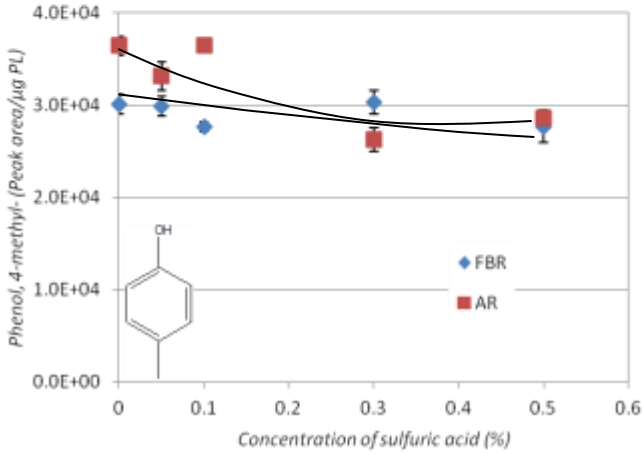
The oils produced in the auger reactor showed higher yields of water insoluble-CH_2Cl_2 soluble fraction (low molecular weight oligomers) but lower yields of the water-CH_2Cl_2 insoluble-methanol soluble fraction (high molecular weight oligomers). ***The addition of sulfuric acid reduces the yield of all lignin oligomeric fractions for both the auger and the fluidized bed pyrolysis reactor***.

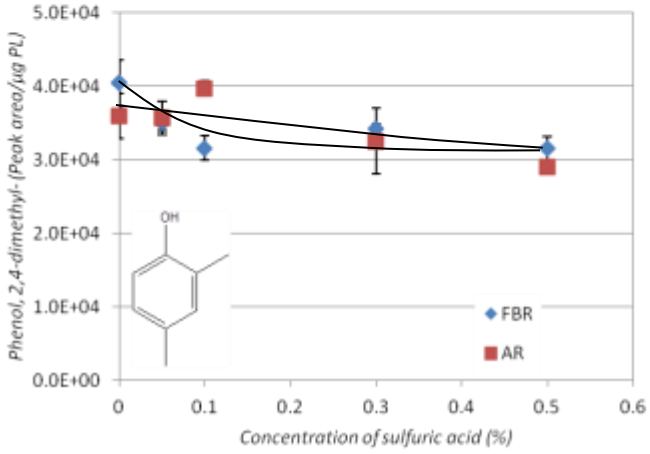
Enhancing the Production of Levoglucosan

Analysis of Lignin Oligomers: Py-GC/MS





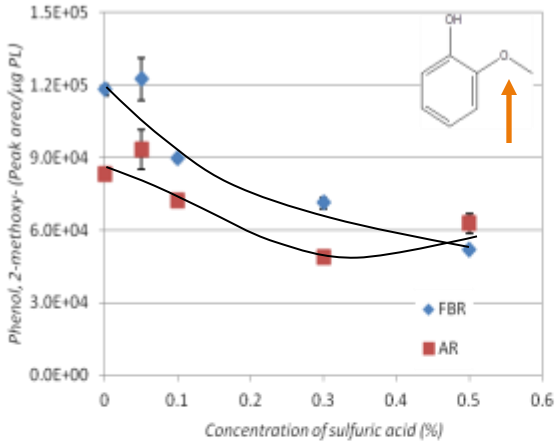


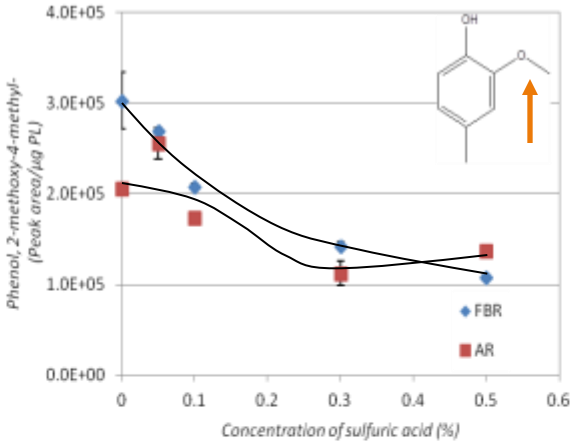


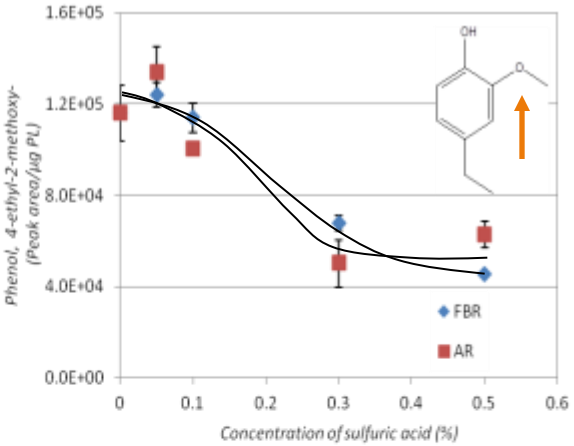
The **yield of alkylated phenols were reduced as the acid increased**. These compounds are mainly generated from the H unit in lignin or are products of secondary reactions.

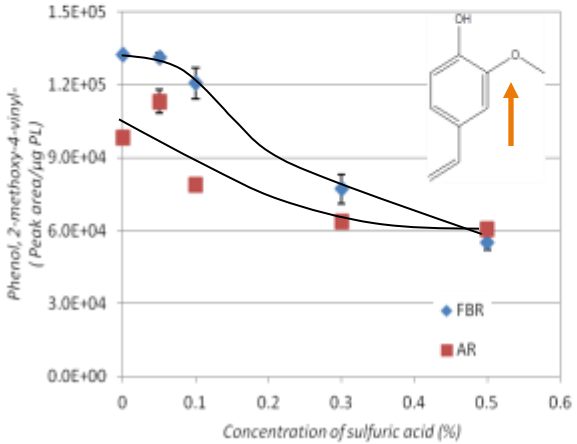
Enhancing the Production of Levoglucosan

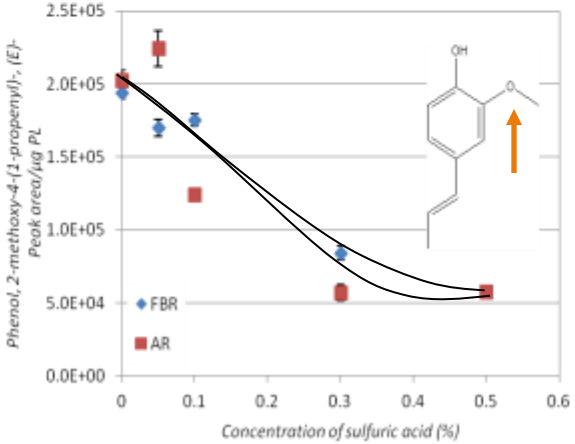
Analysis of Lignin Oligomers: Py-GC/MS

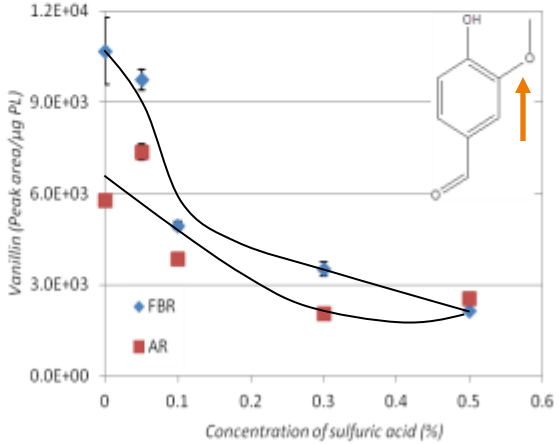








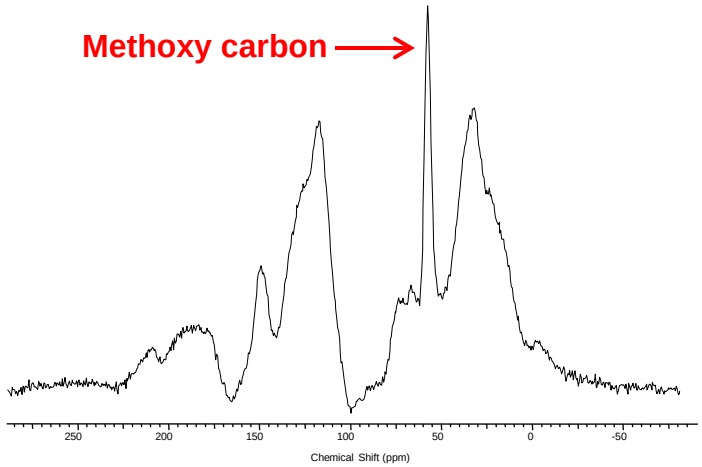




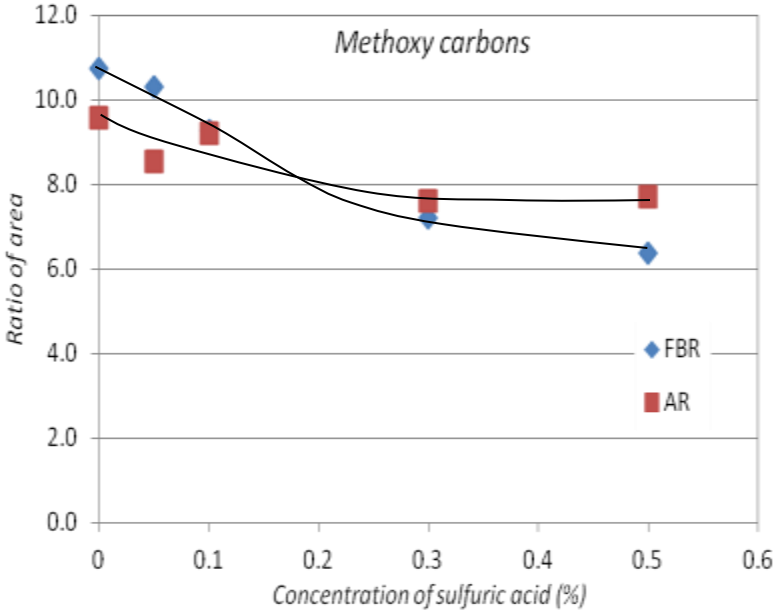
The use of sulfuric acid **significantly reduces the yield of phenolic compounds with methoxy group**. The methoxy groups are known to be electron donors.

Enhancing the Production of Levoglucosan

Analysis of Lignin Oligomers: ^{13}C Solid State NMR



Solid state ^{13}C NMR spectra of lignin oligomer



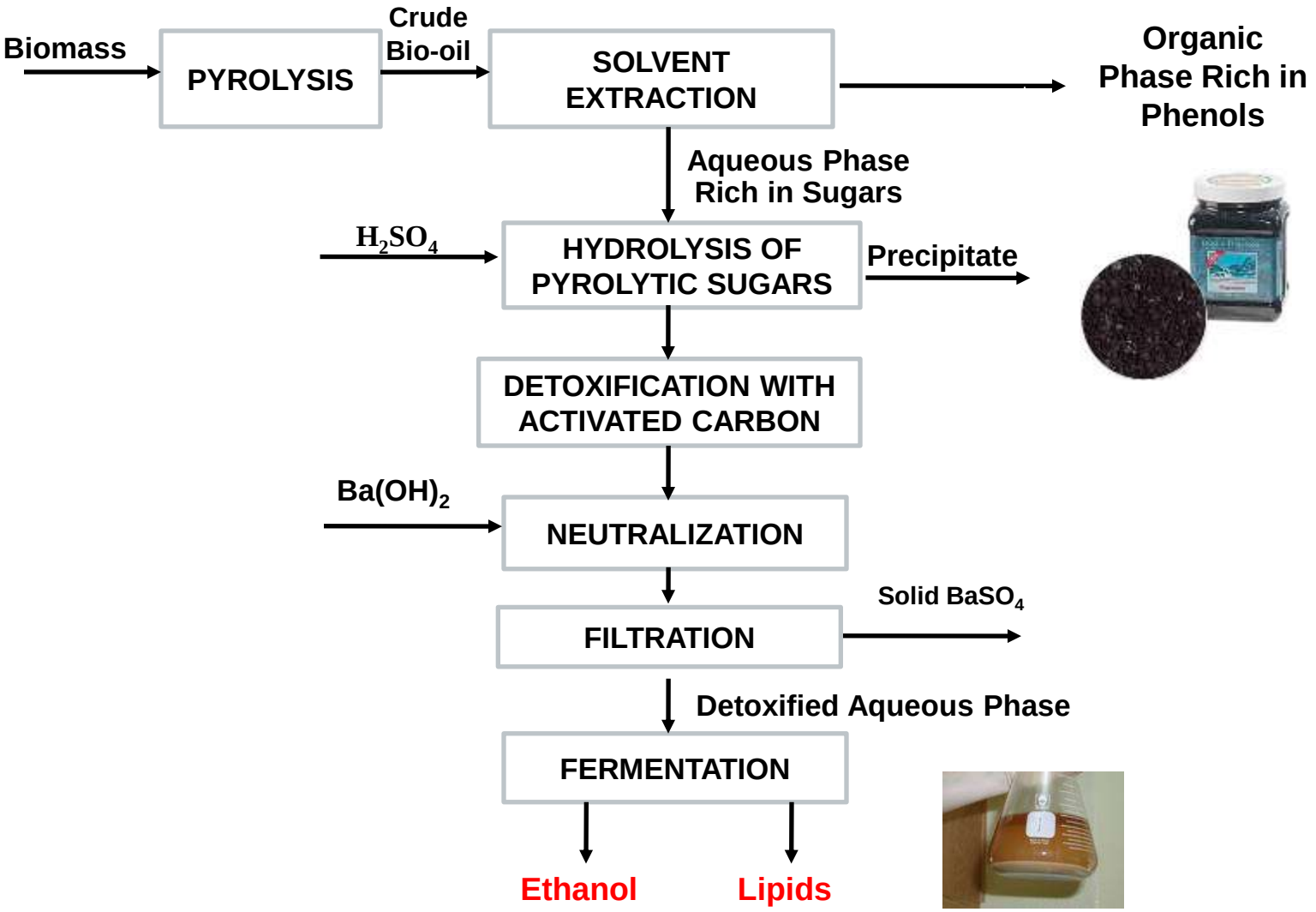
Peak assignment

Chemical shift / ppm	Functional groups
0-50	Aliphatic carbons
50-60	Methoxyl carbons
60-80	Aliphatic C-O carbons
100-140	Aromatic carbons
140-165	Oxygenated aromatic carbons
165-230	Carbonyl carbons

A dramatic decrease in the content of methoxy groups confirms the Py-GC/MS findings and clearly suggest that the presence of this functional group activate the ring and accelerate the formation of polyaromatic structures in the bio-char produced.

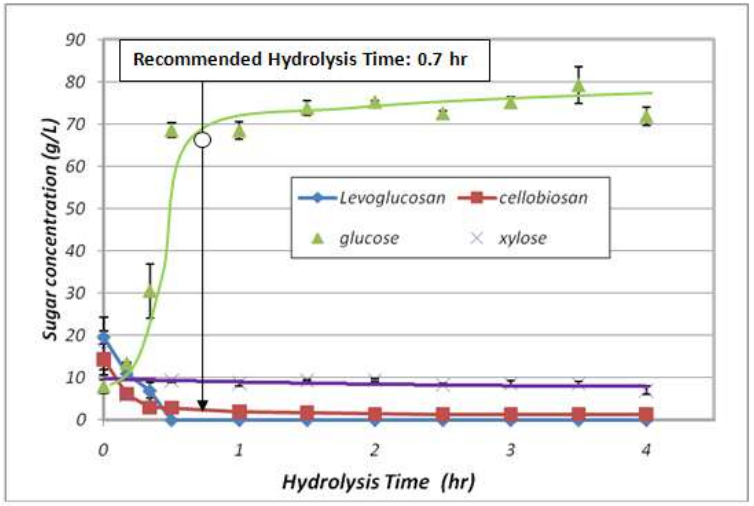
Conversion of Levoglucosan into Transportation Fuel Precursors

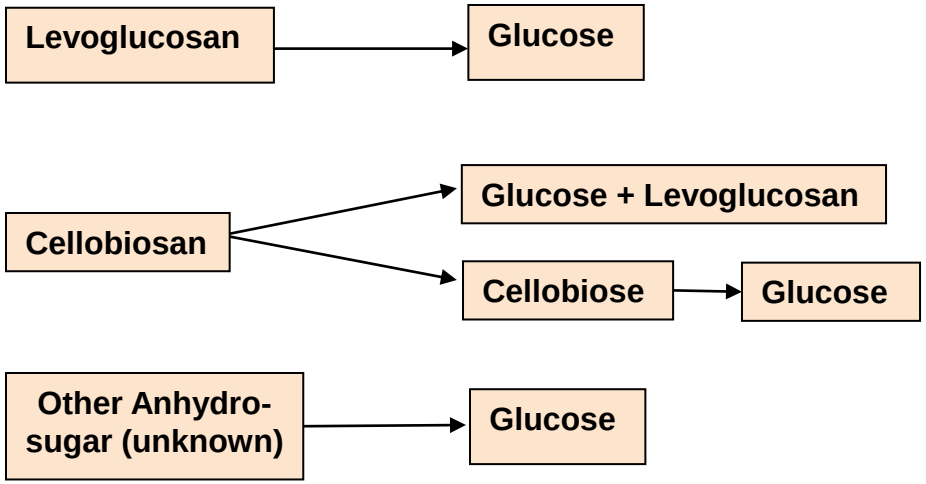
Process to convert Pyrolytic Sugars into Ethanol or Lipids



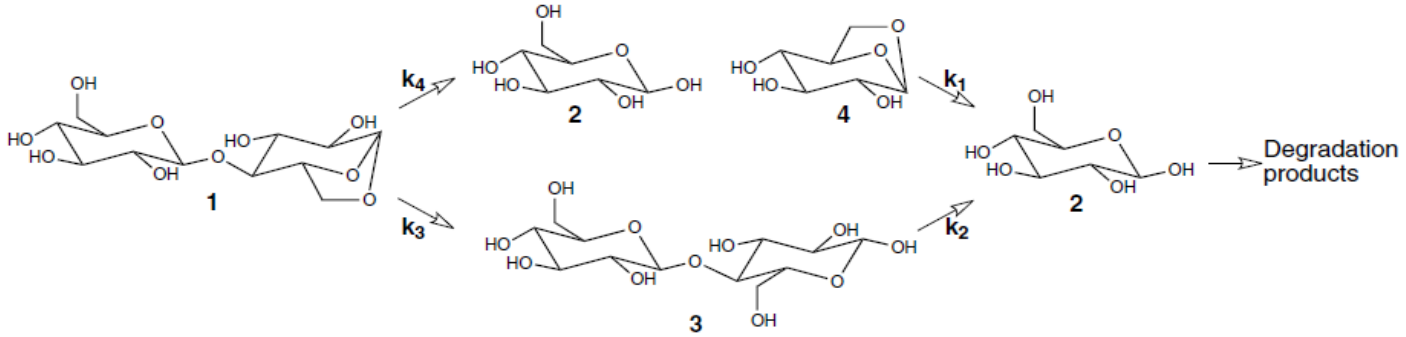
Conversion of Levoglucosan into Transportation Fuel Precursors

Pyrolytic Sugars Production and Hydrolysis to Produce Glucose





Cellobiosan hydrolysis scheme: Cellobiosan (1), Glucose (2), Cellobiose(3), and Levoglucosan (4)

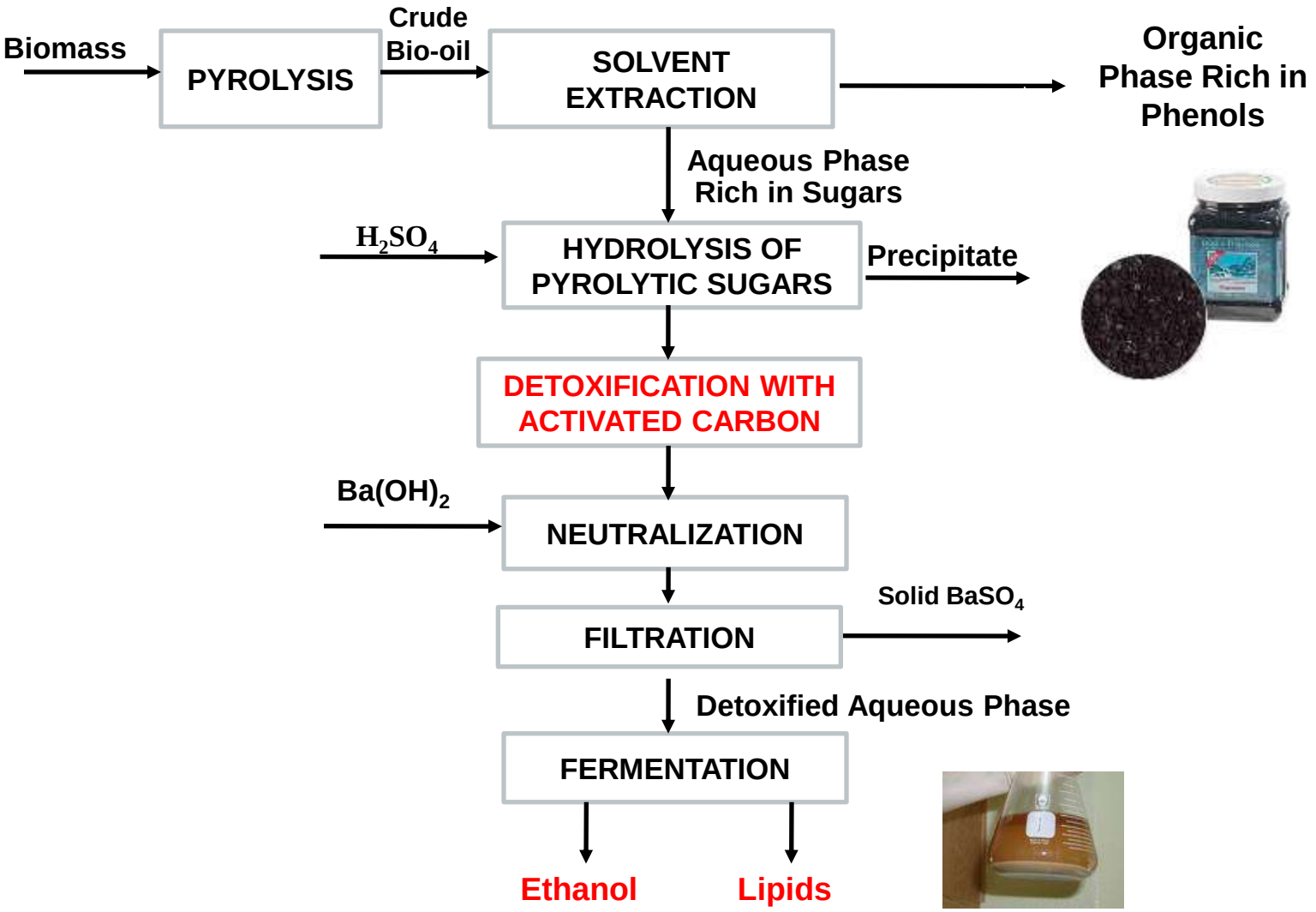


Helle, S., Bennett, N.M., Lau, K., Matsui, J.H., Duff, S.J.B., 2007. A kinetic model for the production of glucose by hydrolysis of Levoglucosan and Cellobiosan from pyrolysis oils. Carbohydrate Research 342, 2365–2370.

Lian J, Chen S, Zhou S, Wang Z, O'Fallon J, Li C-Z: Separation, hydrolysis and fermentation of pyrolytic sugars to produce ethanol and lipids. Bioresource Technology 101 (2010) 9688-9699

Conversion of Levoglucosan into Transportation Fuel Precursors

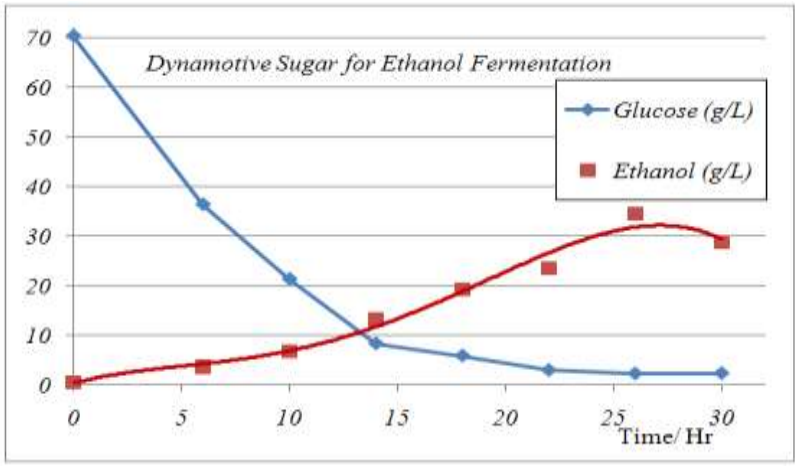
Process to convert Pyrolytic Sugars into Ethanol or Lipids



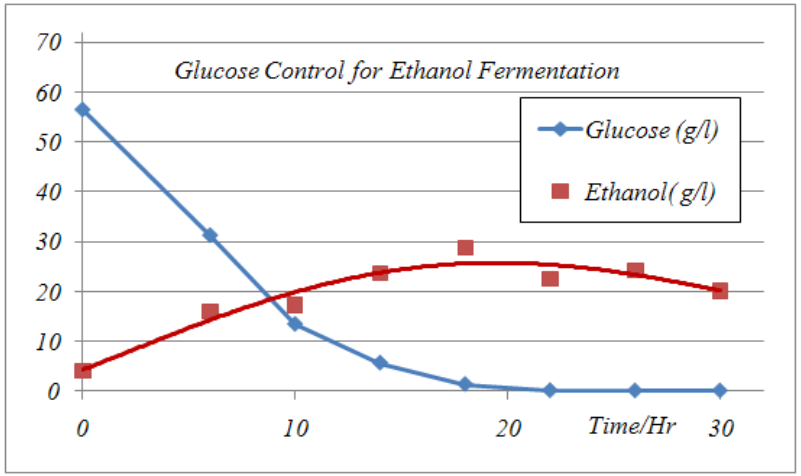
Conversion of Levoglucosan into Transportation Fuel Precursors

Saccharomyces cerevisiae for Ethanol Fermentation

Pyrolytic Sugars Fermentation



Glucose Control



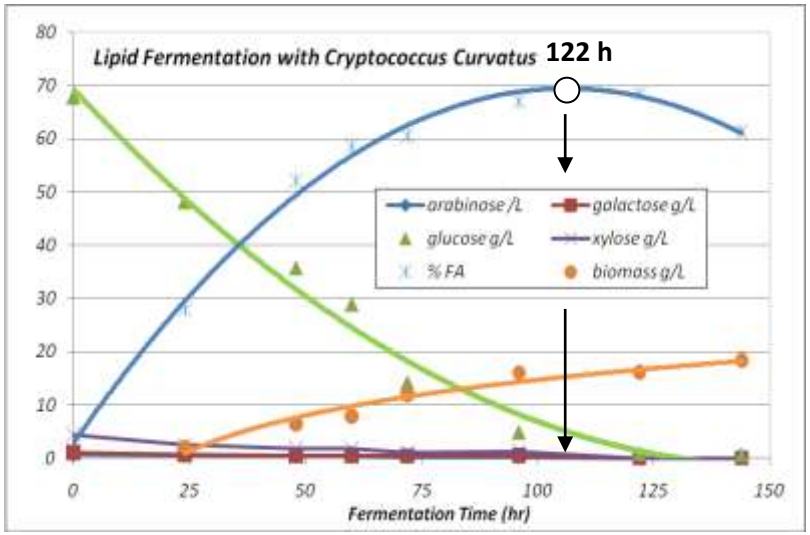
In both cases, ethanol yields were very close to 0.5 g of ethanol per gram of glucose.

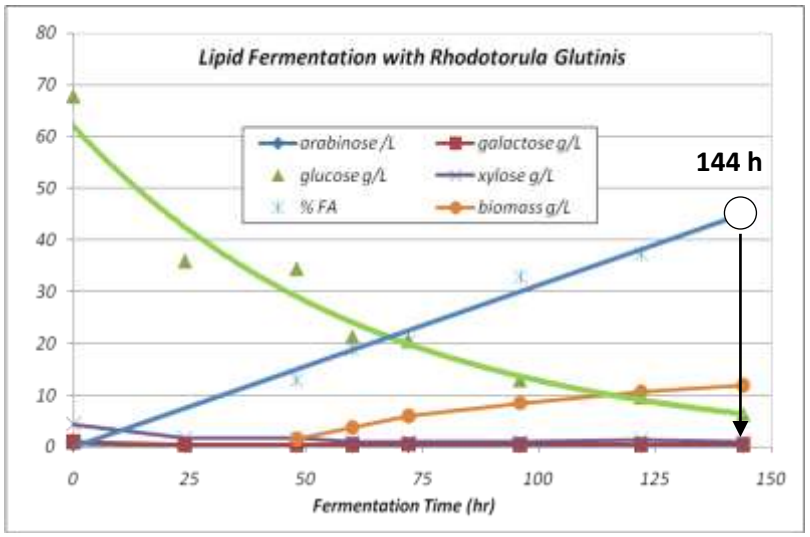
Toxic compounds could be the main reason for slow consumption rate of pyrolytic sugar.

Lian J, Chen S, Zhou S, Wang Z, O'Fallon J, Li C-Z: Separation, hydrolysis and fermentation of pyrolytic sugars to produce ethanol and lipids. Bioresource Technology 101 (2010) 9688-9699

Conversion of Levoglucosan into Transportation Fuel Precursors

Cryptococcus curvatus and *Rhodotorula glutinis* for Lipid Fermentation





Cryptococcus curvatus could produce up to 68 % lipid mass/cell mass in 122 hr and 16 g lipid / 100 g glucose conversion in144 hr.

Rhodotorula glutinis could produce up to 46 % lipid mass/cell mass and 8.9 g lipid / 100 g glucose conversion in144 hr.

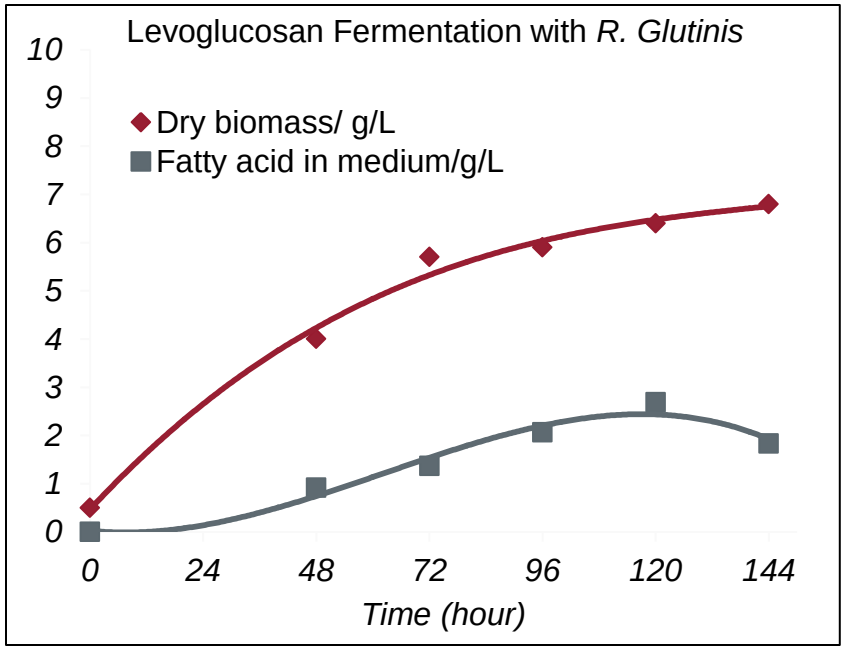
Conversion of Levoglucosan into Transportation Fuel Precursors

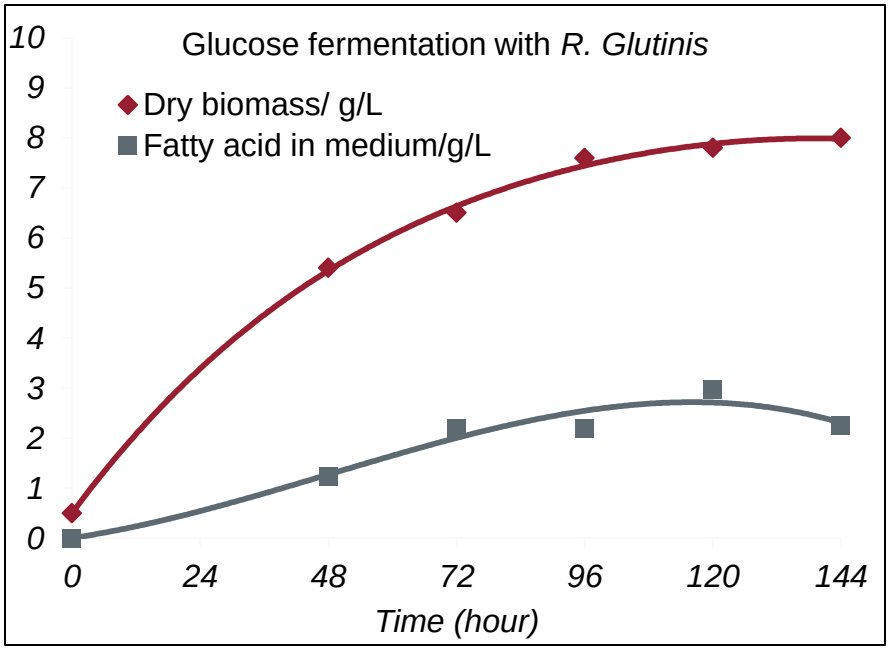
Oleaginous Yeasts Strain Selection for Levoglucosan Fermentation

Strains	Growth
Lipomyces starkeyi ATCC12659	-
Cryptococcus curvatus ATCC20509	+
Yarrowia lipolytica ATCC20460	-
***Rhodosporidium toruloides* ATCC10788**	++
***Rhodotorula glutinis* ATCC204091**	++

Conversion of Levoglucosan into Transportation Fuel Precursors

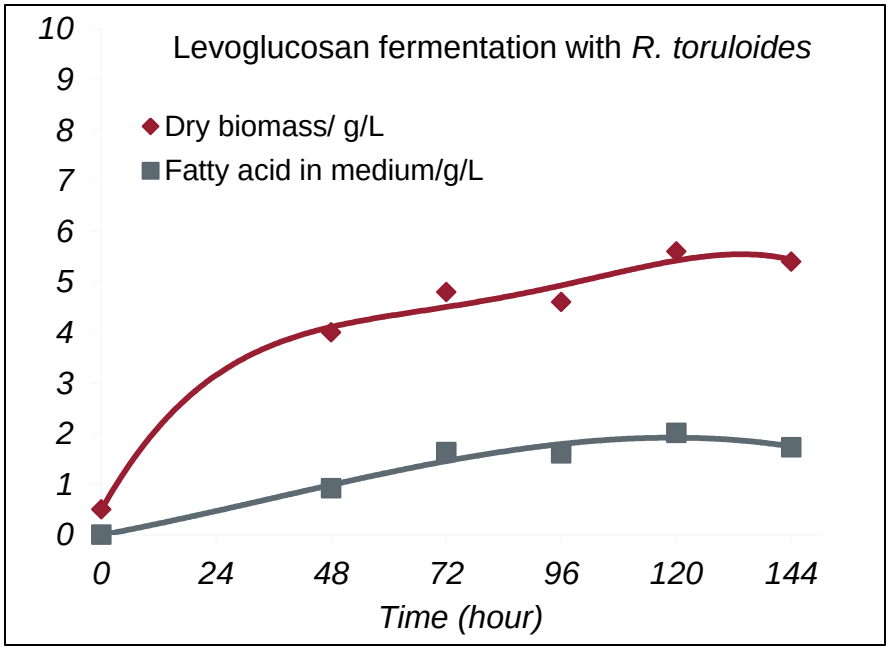
Levoglucosan and glucose fermentation with oleaginous yeast *R. glutinis*

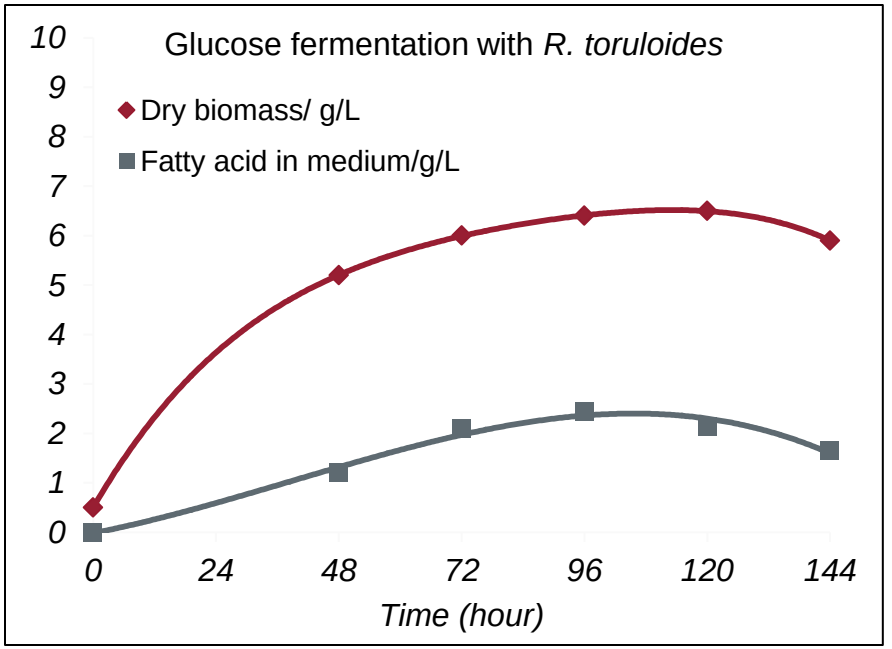




Conversion of Levoglucosan into Transportation Fuel Precursors

Levoglucosan and Glucose Fermentation with Oleaginous yeast *R. toruloides*





Conversion of Levoglucosan into Transportation Fuel Precursors

Levoglucosan Fermentation with fungi

Advantage of levoglucosan fermentation with fungi

Low harvesting cost

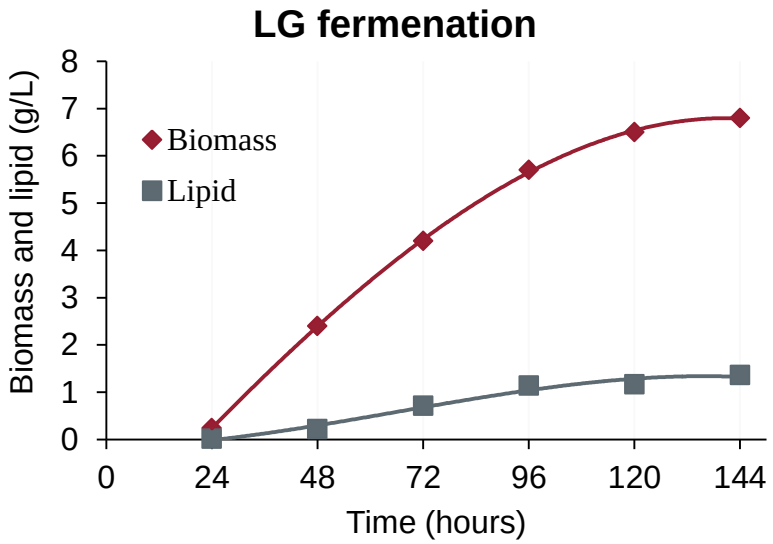
Variety of substrate utilization

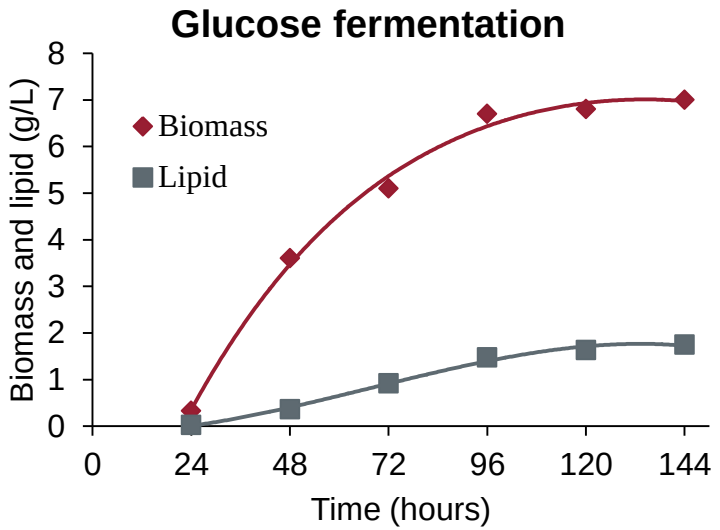
Fungi Screening

Fungi	Mortierella isabellina NRRL 1757	-
	Umbelopsis vinacea ATCC 20034	-
	Mucor janssenii NRRL 3628	-
	Cunninghamella elegans NRRL 2310	-
	Aspergillus terreus NRRL 1960	**++**

Conversion of Levoglucosan into Transportation Fuel Precursors

Total lipid and biomass of oleaginous fungi 1960

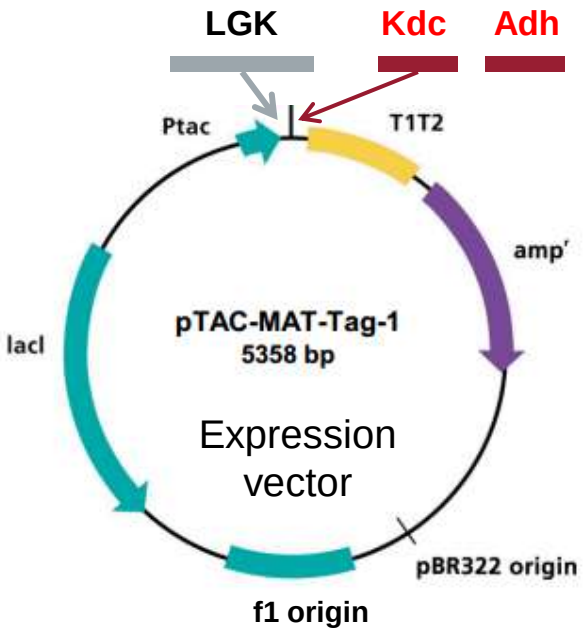


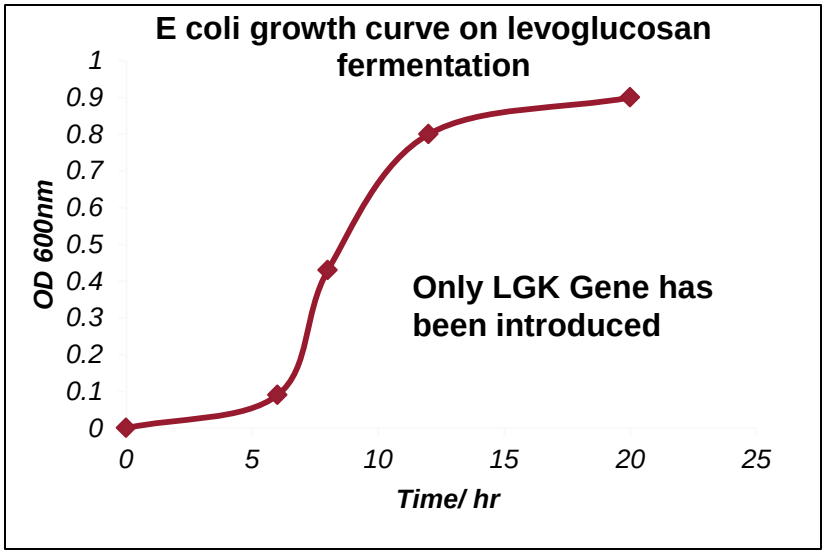


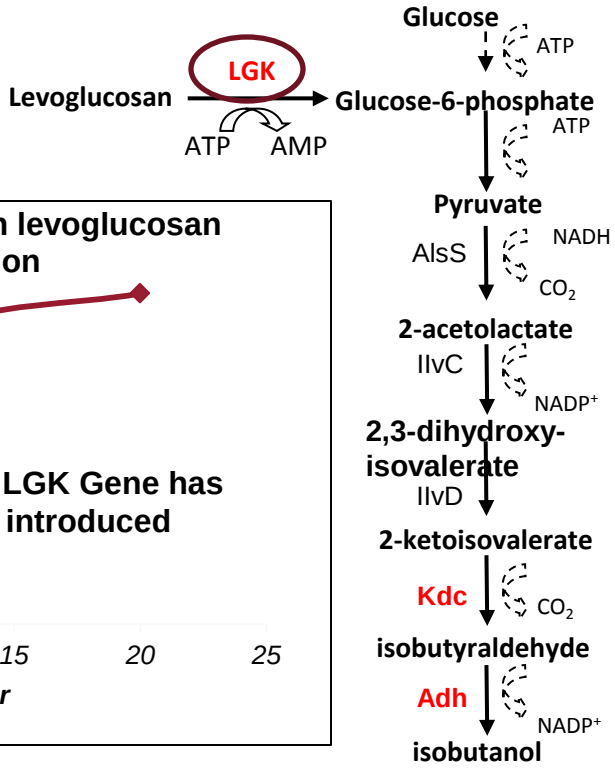
LG fermentation with oleaginous fungi 1960 reach 6.8 g/L of biomass, compared with 7.0 g/L for glucose fermentation. **However, lipid content was low.**

Conversion of Levoglucosan into Transportation Fuel Precursors

Genetic modified *E. coli* for levoglucosan utilization to produce iso-butanol







Isobutanol has been produced by *E. coli* with heterologous expression two genes of Kdc (2-ketoisovalerate decarboxylase) and Adh (aldehyde reductase) amplified from *Lactococcus lactis.* E. coli was obtained from *Washington University in St. Louis*.

LGK gene was synthesized based on the LGK gene from *Lipomyces starkeyi* YZ-215 in Gene wiz.

Conclusions

- Levoglucosan yields as high as 60 mass % can be obtained from cellulose. We are very hopeful similar yields could be produced from lignocellulosic materials.

- The presence of sulfuric acid mitigates the catalytic effect of alkalines enhancing the production of levoglucosan but reduces drastically the production of lignin derived precursors of transportation fuels (mono-lignols and oligomers).

- The methoxy substituted aromatic rings are more likely to be polycondensed to form extra-char in the presence of sulfuric acid.

Conclusions

- The pyrolytic sugars can be hydrolysed and fermented to produce ethanol and lipids
- Yeasts and fungi containing Levoglucosan Kinase (LK) can directly convert Levoglucosan into Lipids.
- New genetically modified micro-organisms able to directly use levoglucosan for ethanol, lipid and butanol production are being developed at Washington State University

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QUESTIONS ?

Conversion of Levoglucosan into Transportation Fuel Precursors

Toxicity of Bio-oil Chemicals to *Saccharomyces cerevisiae* ethanol Fermentation

Phenols and the carboxylic acids are the main family of toxic compounds limiting the production of ethanol and inhibiting yeast growth.

Furans are also inhibitors, but their inhibition rate is much lower.

		Reduction of Yeast Growth				*Inhibition ratio of Ethanol*			
Nº	Compound	Chemicals Concentration Found in the Bio-oil							
		25%	50%	75%	100%	25%	50%	75%	100%
1	Acetic acid	97.8	97.8	98.0	98.2	78.9	91.4	93.9	89.7
2	Propanoic acid	97.0	97.5	97.8	98.0	51.2	77.8	82.7	94.0
3	Cyclopentanone	-22.1	-5.3	-4.4	21.7	16.1	-4.3	8.30	15.2
4	2-Furaldehyde	7.8	8.0	11.0	95.9	-3.8	-12.3	3.09	35.6
5	Furfuryl alcohol	5.9	6.9	7.8	7.8	-0.1	16.0	21.4	29.8
6	Phenol	77.0	96.9	97.4	97.7	11.8	91.2	96.3	97.9
7	Eugenol	97.0	97.2	96.6	97.0	87.4	93.7	89.4	89.0
8	Acetol	44.2	77.8	78.9	80.1	98.8	99.2	98.7	99.1
9	2-(5H)-Furanone	3.1	5.9	8.8	10.4	11.2	17.0	6.5	18.4
10	Stilbene	9.2	32.6	37. 9	45.1	-7.2	-11.2	-45.0	-15.3
11	Vanillin	81.5	82.4	81.8	81.9	98.2	98.7	99.1	99.2
12	Syringaldehyde	71.7	79.9	81.5	82.0	78.8	98.3	98.7	98.4
13	o-xylol	5.0	5.2	5.3	5.4	13.7	4.1	-3.3	-4.2
14	Pyrocatechol	84.5	90.5	91. 7	92.1	90.5	98.4	99.0	99.2
15	Palmitic acid	2.6	0.4	-2.0	-4.2	2.1	5.2	4.6	4.2
16	Toluene	-0.7	1.0	1.7	2.1	10.6	1.2	-12.3	4.2
17	Tetradecane	1.1	4.7	10.5	10.6	8.3	3.9	-11.9	43.5
18	Petadecane	4.7	7.4	8.9	9.8	3.7	-15.8	7.6	-9.4

Conversion of Levoglucosan into Transportation Fuel Precursors

Fatty acid profiles of *R. glutinis* and *R. toruloides* obtained at 120 hr culture with levoglucosan (LG) and Glucose medium.

		R. glutinis		*R. toruloides*	
		LG	Glucose	LG	Glucose
Fatty acid	Structure	mass % FA	mass % FA	mass % FA	mass % FA
Myristic	C14:0	0.4	0.5	0.2	0.6
Palmitic	C16:0	**24.3**	18.1	**25.1**	19.7
Palmitoleic	C16:1n7	0.2	1.2	0.8	0.9
Heptadecanoic	C17:0	0.2	0.1	0.1	0.4
Stearic	C18:0	10.1	**24.4**	9.2	**17.3**
Oleic	C18:1n9	**53.2**	44.6	**50.8**	43.5
Linoleic	C18:2n6	6.8	5.6	7.2	9.8
Gamma- Linolenic	C18:3n6	1	0.7	1.1	0.6
Linolenic	C18:3n3	0.1	0.9	0.7	0.2
Arachidic	C20:0	0.3	1.6	0.4	1.2
Behenic	C22:0	0.7	0.6	0.7	0.8
Lignoceric	C24:0	2.4	1.7	3.5	4.7
Saturated		39.1	46.1	39.9	44.1
MUFA		**53.4**	45.8	**51.6**	44.4
PUFA		7.2	**8.1**	8.3	**11.2**
Omega-3		0.3	1.6	0.4	1.2
Omega-6		6.9	6.5	7.9	10
Identified		99.7	100	99.8	99.7
Unknown		0.3	0	0.2	0.3
		mass % in dry biomass	mass % in dry biomass	mass % in dry biomass	mass % in dry biomass
Total Fat		42	45	36	38