STUDENT #1

SYNCHRONIZATION AND ISOLATION OF SWITCHGRASS FOR INTERECOTYPIC HYBRID DEVELOPMENT

Laxman Adhikari and Yanqi Wu

Department of Plant and Soil Sciences,

Oklahoma State University

Objectives

- To know the flowering behavior of upland and lowland switchgrass.
- To determine the effects of synchronization and isolation in interecotypic hybrid development.
- To identify male sterile genotypes in interecotypic hybrid.

Materials and Methods

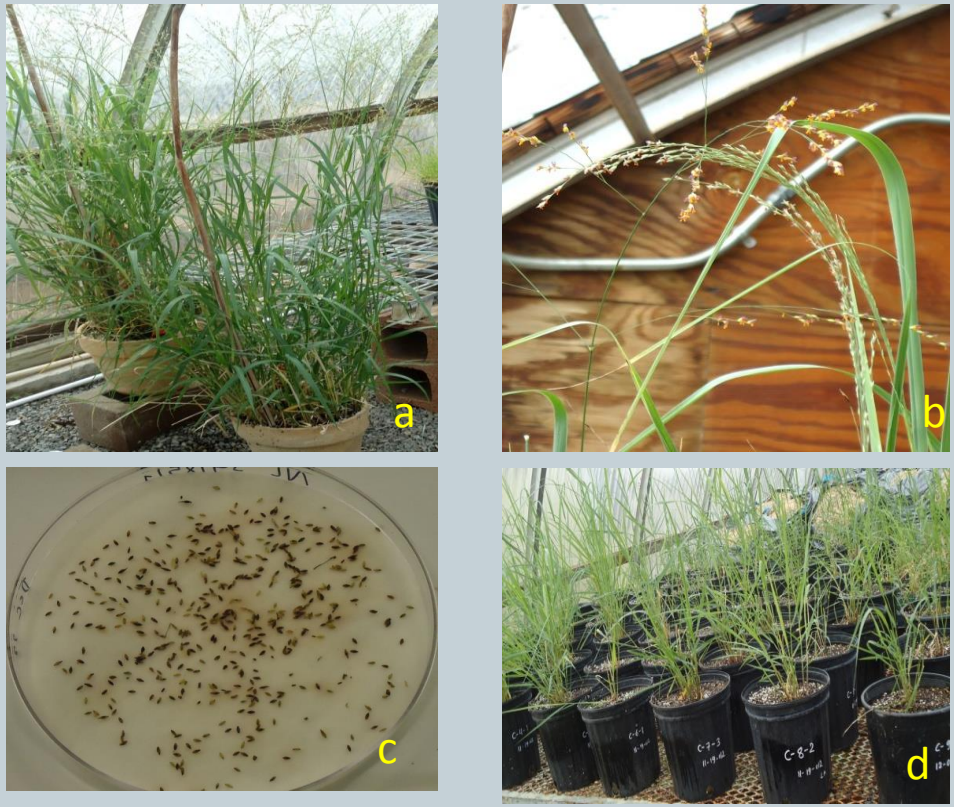
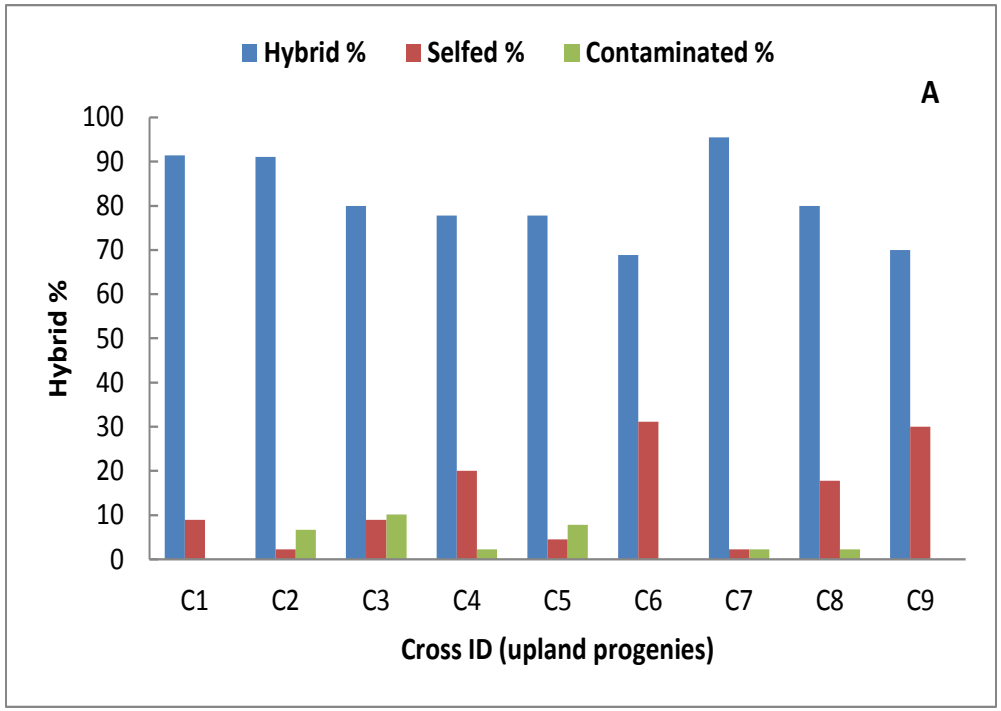


Fig 1. (a) Isolated synchronized upland and lowland plants, (b) Panicles from two ecotypes crossing each other, (c) hybrid seeds, and (d) hybrid plants.

1. Earlier flowering upland plants were trimmed.
2. Crossing pairs were isolated.
3. Hybrid seeds were collected.
4. Hybrid seeds' genetic origins were identified using SSR markers.
5. Male sterile lines were identified using pollen stainability and pollen germination.

Results



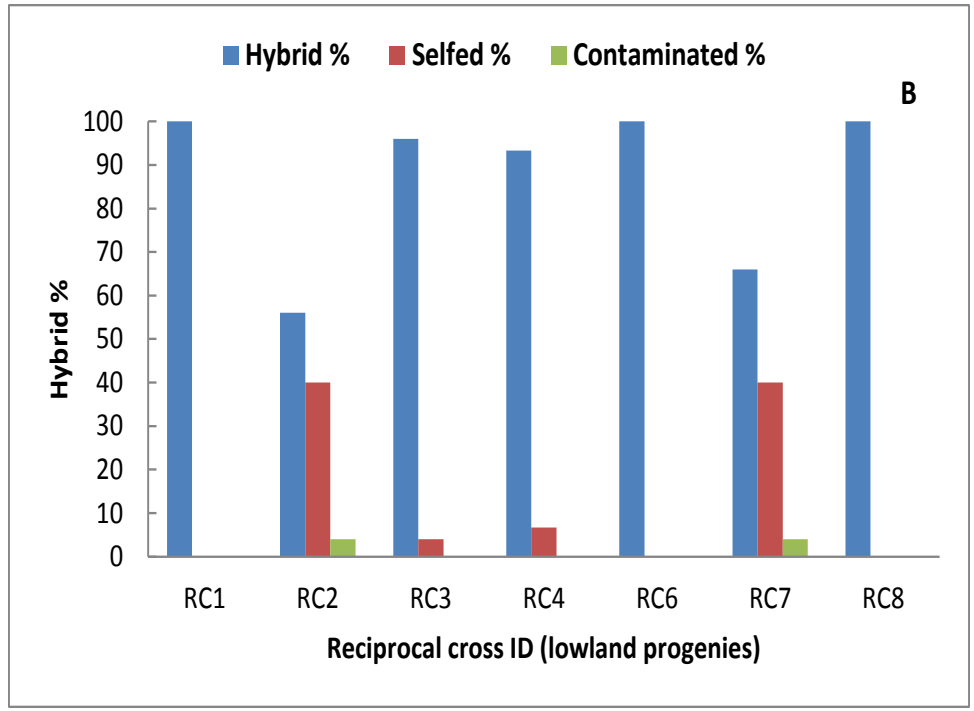


Fig 2. (A) F1 upland progenies and their genetic origin, and (B) lowland F1 progenies and their genetic origin.

Conclusions

- **Proper synchronization of reciprocal parents yielded 58 to 100 % interecotypic hybrids.**
- **Improper synchronization yielded relatively higher selfed seeds of reciprocal parents.**
- **The F1 genotypes C-8-17 and RC-3-3 were detected as possible male sterile line, no pollen germination.**
- **The information from this study will be valuable in the development of hybrid switchgrass.**

Oklahoma NSF EPSCoR Bioenergy Research and Education

STUDENT #2

An equilibrium based process modeling of a packed bed scrubbing system for the removal of model tar compounds

Prakash Bhoi, Research Engineer

Dr. Krushna Patil, Assistant Researcher

Dr. Ajay Kumar, Assistant Professor

Dr. Raymond Huhnke, Professor

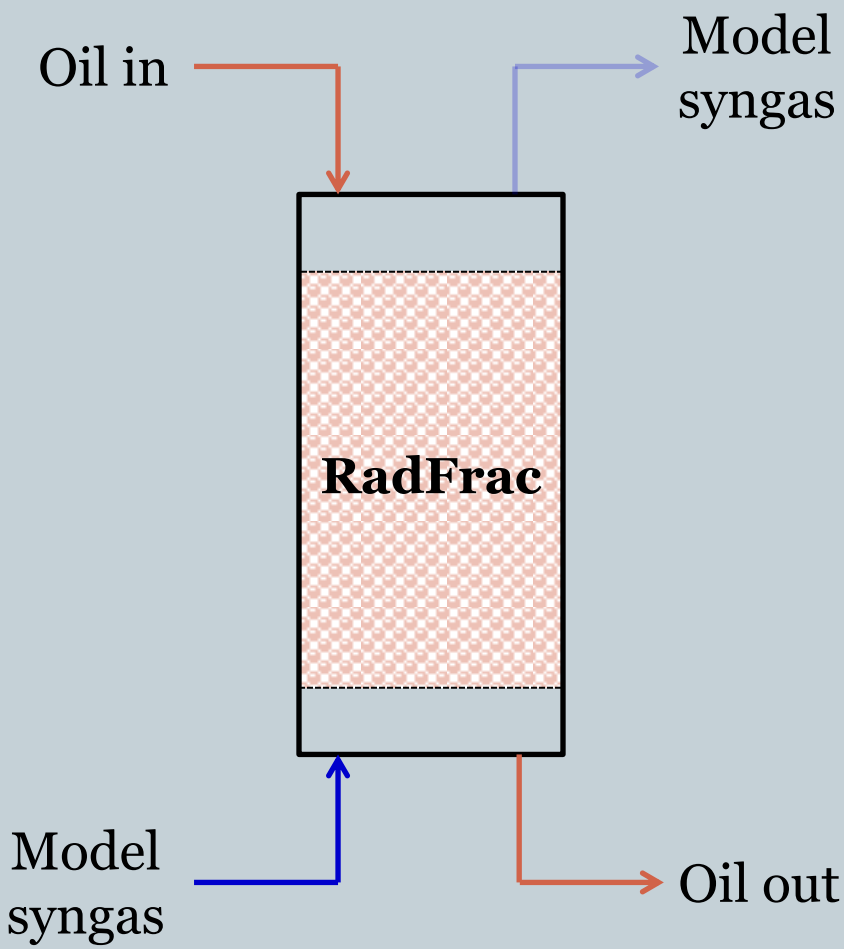
Biosystems & Agricultural Engineering Department

Oklahoma State University, Stillwater, OK 74078

Objectives

- To develop an equilibrium based process model of a wet packed bed scrubber for the removal of model tar compounds.
  - Equation of state (EOS) models
  - Activity coefficient models
- To study the effect of important variables on the removal efficiency of model tar compounds
  - Packing bed height
  - Solvent temperature
  - Liquid-to-gas (L/G) ratio

Methods



Thermodynamic property methods:

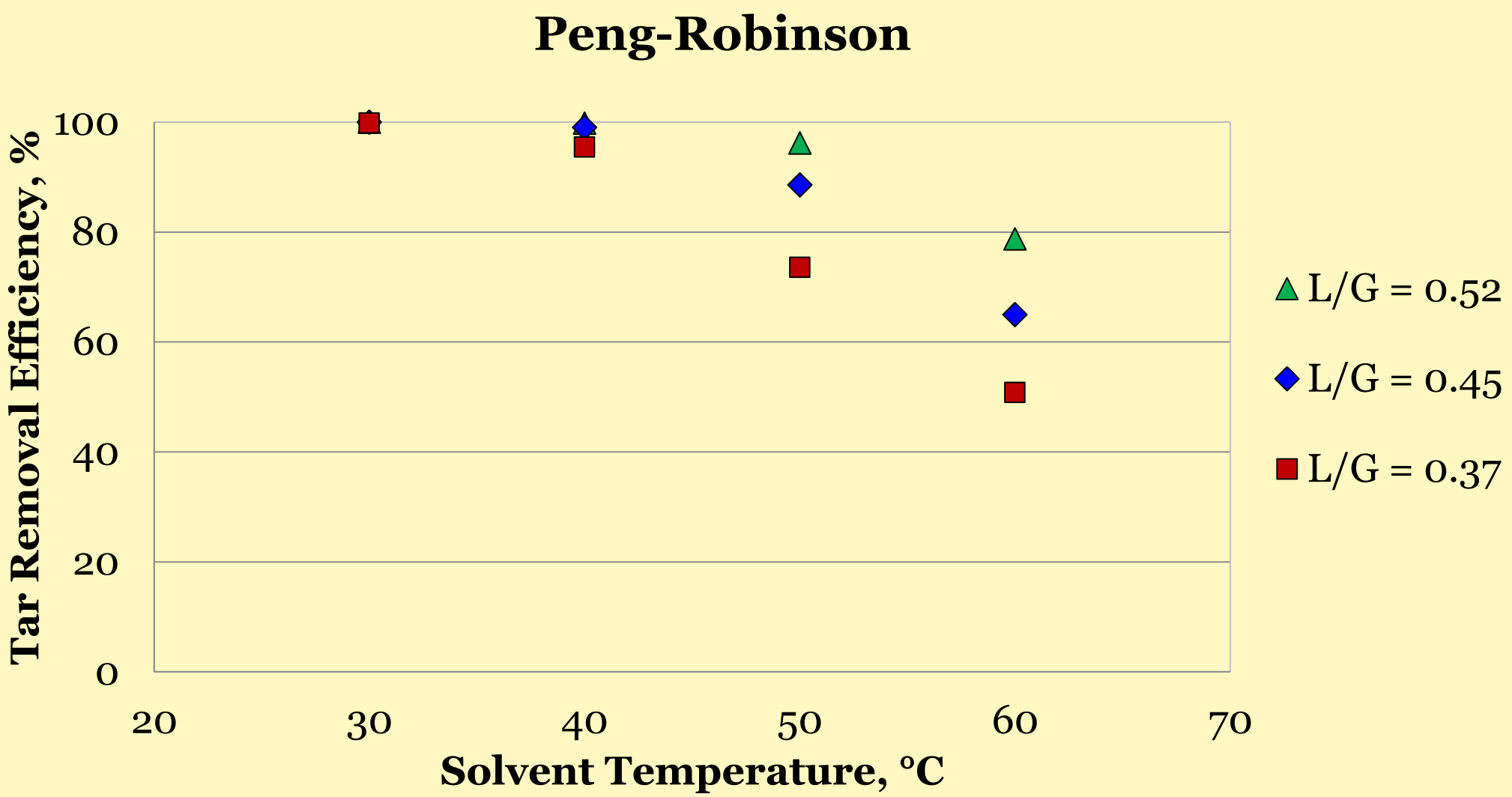
- Peng-Robinson
- RK-Soave

Solvent: Soybean oil

Packing media: 6 mm Raschig ring

Raschig Ring Characteristics	Values
Density, Kg/m^3	900
Surface are, m^2/m^3	900
Packing factor, 1/m	2300
Void fraction, %	89

Results


Peng-Robinson
Tar Removal Efficiency, %
100
80
60
40
20
0
20
30
40
50
60
70
Solvent Temperature, °C
L/G = 0.52
L/G = 0.45
L/G = 0.37

Conclusions

- Both property models (Peng-Robinson and RK-Soave) lead to comparable results.
- Packed bed height significantly increases tar removal efficiency.
- Solvent temperatures above 40°C significantly reduce tar removal efficiency.
- An increase in liquid-to-gas (L/G) ratio substantially increases tar removal efficiencies for solvent temperatures above 40°C.

STUDENT #3

Genome Sequence of the Anaerobic Gut Fungi Orpinomyces sp. strain C1A

MB Couger, Noha H. Youssef, Audra S. Liggenstoffer, and Mostafa Elshahed

Oklahoma State University Stillwater, Oklahoma.

Objectives

- Establish a high quality, well annotated, genome sequence from a member of the anaerobic fungal genera Neocallamastix
- Identify the unique salient features of the genome and conduct comparative analysis to other microbial genomes
- Identify enzymatic components of the genome that allows it to have the ability to thrive in the Rumen.

Methods

Sequence Generation

Genome

illumina

PACIFIC BIOSCIENCES

10x Coverage

100x Coverage

100MB 3.5KB n50

PacBioToCA

Celera Assembler

Transcriptome

35GB

22,000 Models N50 1080

Gene Calling

GLIMMERHMM Eukaryotic Gene-Finding System

PASA

Augustus [gene prediction]

EVidenceModeler (EVM)

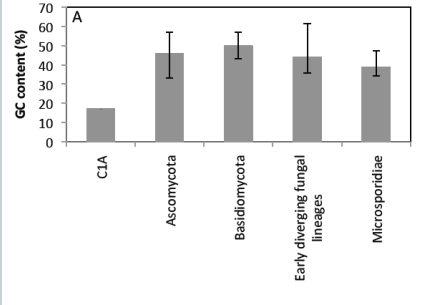
EVM

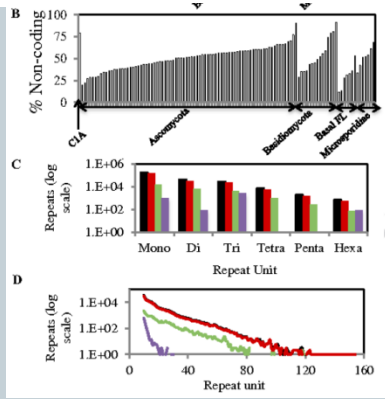
Final Gene Models

16,347 Models Average Gene Length 1.6KB

Results

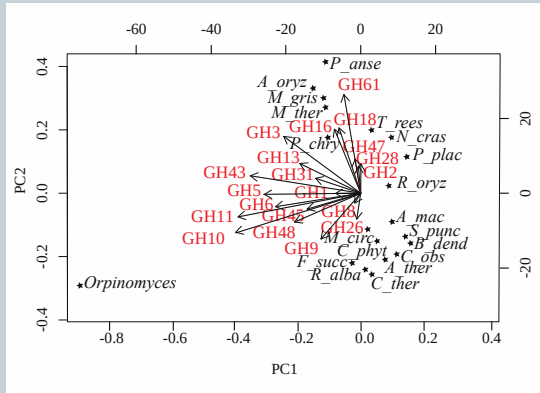
Unique Genome Features





Phylum	Species Name	GH	CE	PL
Neocallimastigomycota	*Orpinomyces* sp. strain C1A	358	92	24

Bacterial Homolog	247
Rumen Homolog	141
Eukaryotic Homolog	110



Genome Statistics

Genome size	100.95 MB
Number of Contigs	32,574
Protein Coding	20.60%
Non- coding intergenic	73.60%
Non-coding introns	5.10%
rRNA	0.67%
5.8S	183 (30,763 bp)
18S	272 (168,110 bp)
28S	366 (457,301 bp)
tRNA	0.06%
	770 (58,292 bp)
Number of Genes	16,347
Number of Genes with transcripts	14,009
Average Gene Length	1623
Number of Intron	35,697
Introns/gene	2.18
Average Intronlength	163
GC content	17.00%
Protein Coding	26.80%
Intergenic	14.80%
Intron	8.10%
SSR Repeats	4.90%
TE repeats	3.31%

Conclusions

- Analysis of the Genome of Orpinomyces C1A reveals a distinct genome structure from other members of Mycota.
- Anaerobic fungi contain a uniquely evolved enzymatic system for plant cell wall degradation, many members of which where obtained from horizontal gene transfer from other prokaryotic members residing in the rumen. C1A contains the capacity to degrade all major chemical moieties found in hemicellulose.
- This unique system combined with the invasiveness of fungi make this organism a very promising agent for consolidated bioprocessing.

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STUDENT #4

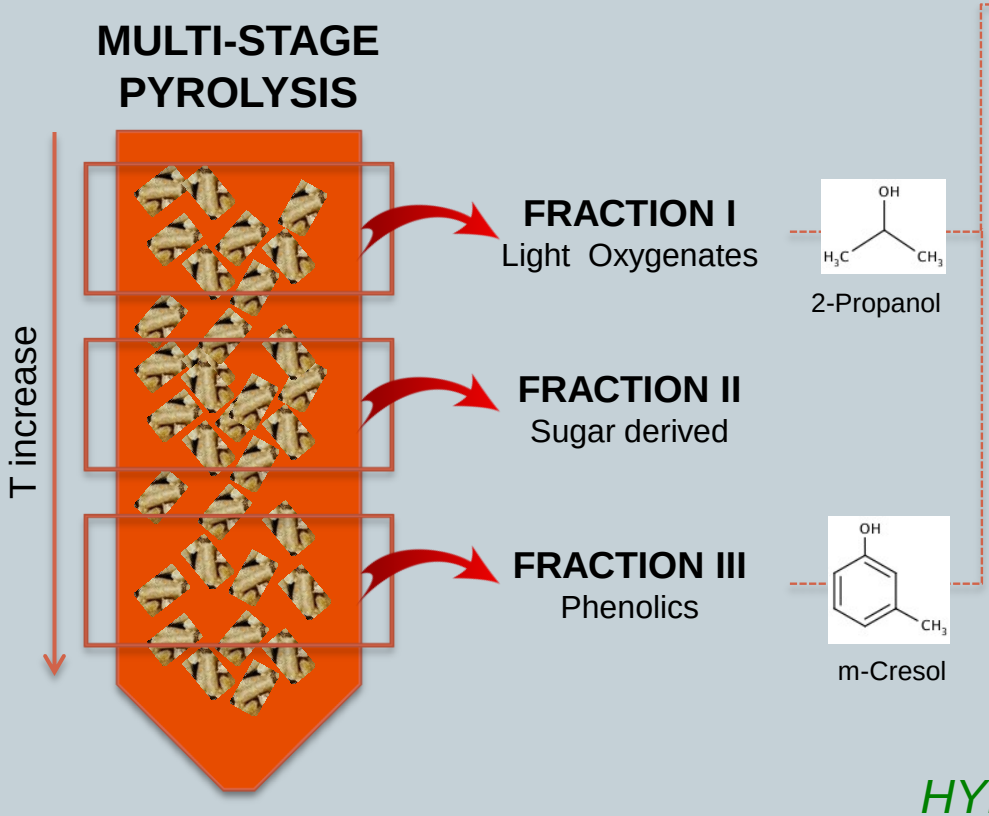
Alkylation Reactions for the Upgrading of Bio-oil in the Presence of Liquid Water Using Hydrophobic Zeolites

Miguel A. Gonzalez Borja, Daniel E. Resasco
School of Chemical, Biological & Materials Engineering
University of Oklahoma

Objectives

- To develop bio-oil upgrading strategies that maximize the yield of liquid products.
  - To evaluate the performance of water-resistance catalyst for alkylation reactions in aqueous media.
  - To understand the reaction pathways for the alkylation of phenolic compounds with 2-isopropanol.

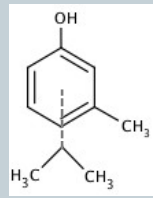
Methods



2-Propanol

m-Cresol

ALKYLATION



CHALLENGES

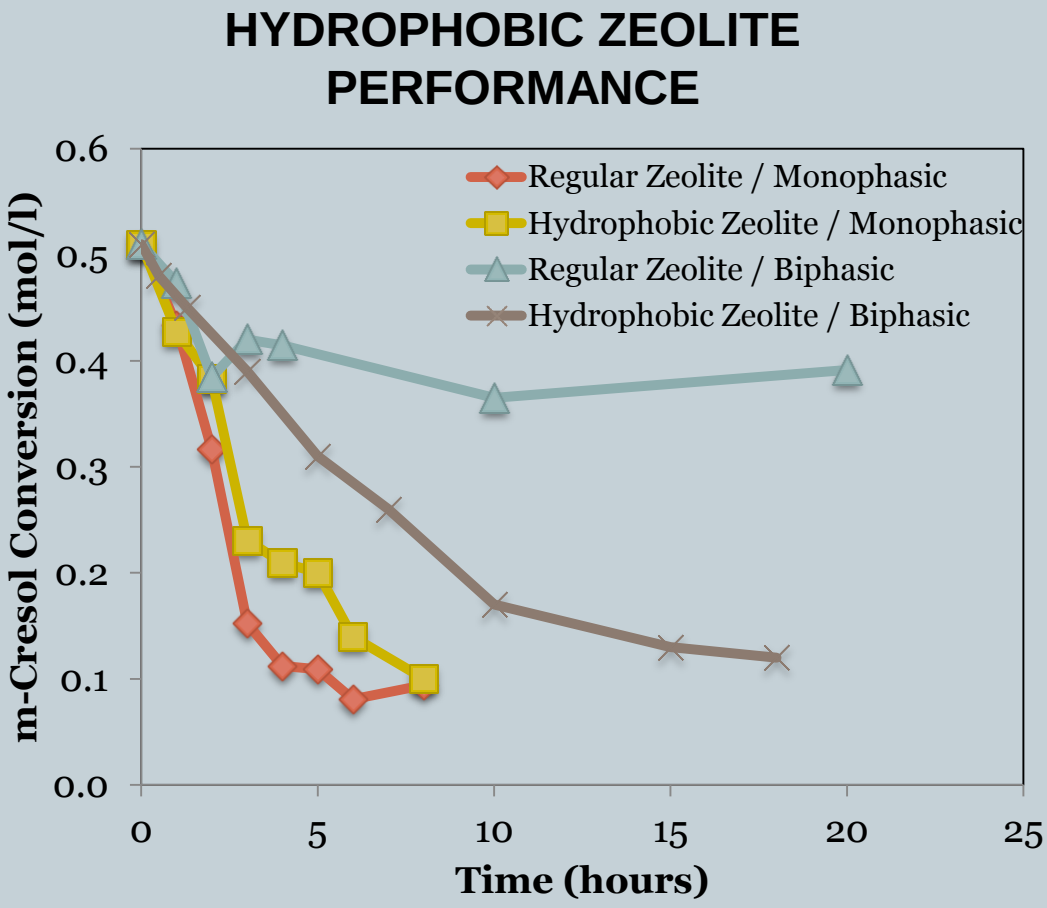
- Bio-oil unstability upon heating
- Phase separation
- Deactivation of catalyst by water

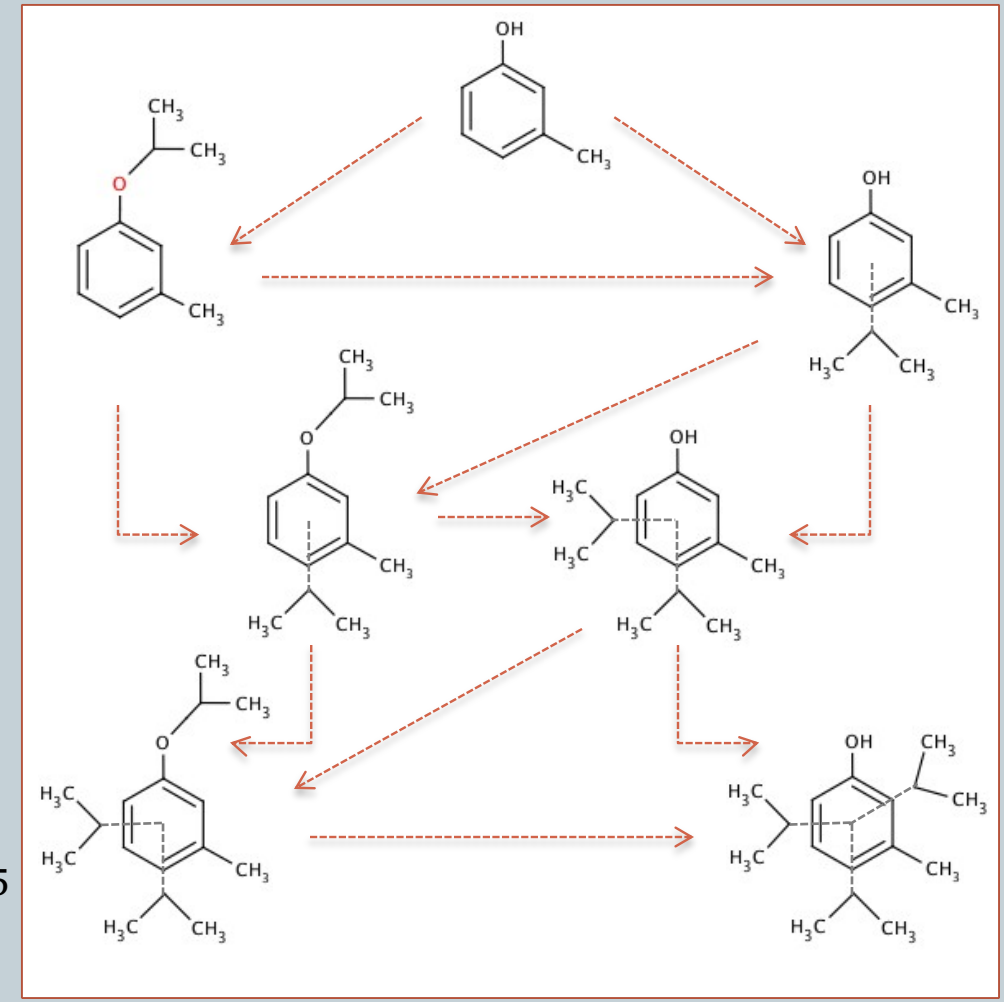
APPROACH

- Work in liquid phase
- Use catalyst that remains at liquid-liquid interphase
- Use catalyst that is stable in the presence of water.

HYDROPHOBIC ZEOLITE IN A BIPHASIC LIQUID PHASE REACTOR

Results


HYDROPHOBIC ZEOLITE PERFORMANCE
m-Cresol Conversion (mol/l)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25
Time (hours)
Regular Zeolite / Monophasic
Hydrophobic Zeolite / Monophasic
Regular Zeolite / Biphasic
Hydrophobic Zeolite / Biphasic


OH
CH3
O
H3C

Conclusions

- Alkylation reactions between light oxygenates and phenolics appear to be an effective strategy for bio-oil upgrading while maximizing the yield of liquid products.
  - Hydrophobic zeolites that remain at the oil-water interphase posses improved stability for alkylation reactions in the presence of liquid water.
  - 2-Propanol can be incorporated into the aromatic ring of phenolics via alkylation or via etherification. Ethers can in turn convert to the alkylated product via trans-alkylation.

STUDENT #5

Identification of Grass Cell Wall Synthesis Genes by Correlation Analysis between Gene Expression and Cell Wall Composition

F. Lin[a], C. Manisseri[b], A. Fagerstrom[d], B. Williams[c], D. M. Chiniquy[b,c], M. L. Peck[a], P. Saha[a], M. Vega-Sanchez[b,c], J. U. Fangel[d], W. T. Willats[d], H. V. Scheller[b], P. C. Ronald[b,c], L. E. Bartley[a,b,c]

[a] Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019
[b] Joint BioEnergy Institute, Emeryville, CA 94608 and Lawrence Berkeley National Laboratory, Berkeley, CA 94720
[c] Department of Plant Pathology and The Genome Center, University of California, Davis, CA 95616
[d] Department of Plant and Environmental Sciences , University of Copenhagen, Denmark

Objectives

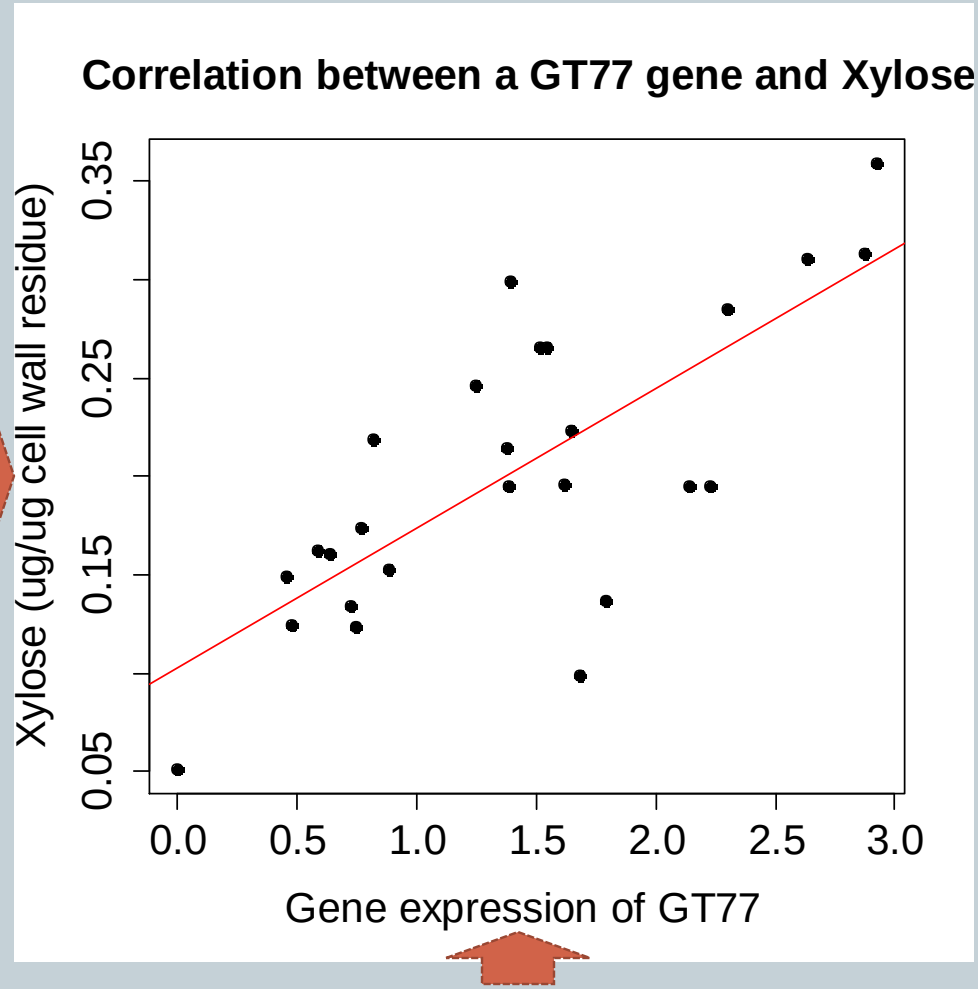
- Focus on grass-specific cell wall biosynthesis.
- Develop a correlation based method to identify cell wall synthesis genes
- Improve grass cell walls as a feedstock for biofuel production

Developmental Time Course Based Correlation Analysis

30 samples from different developmental stages

...

16 cell wall components and 18 cell wall epitopes



Quantitative PCR results of 74 grass diverged genes

Putative Cell Wall Synthesis Genes Identified by Correlation Analysis

Gene	Cell Wall Component	Gini correlation coefficient	Pearson correlation coefficient	Reference
AT4-OsPMT	*p*-coumarate	0.776	0.669	Withers, 2012
GT8-GAUT8-1	L5-galactan	0.716	0.633	
GT8-GAUT8-1	GalA	0.787	0.742	
GT8-GAUT1-1	L5-galactan	0.746	0.760	
GT17-C-1	coumarate	0.699	0.609	
GT17-C-1	Xyl	0.709	0.660	
GT17-C-1	L10-xylan	0.732	0.680	
GT77-4	Xyl	0.705	0.742	
GT77-4	L10-xylan	0.740	0.733	

The cutoff is set as FDR<0.05 and |correlation coefficient|>0.6

Conclusions

- It is feasible to identify cell wall synthesis genes using the correlations between gene expression and cell wall composition.
- This analysis reveals the putative functions in cell wall synthesis of 18 genes, which can now be tested by experiments.

STUDENT #6

Improving Carbon Retention in Bio-oil Upgrading by Hydrogenation and Alkylation

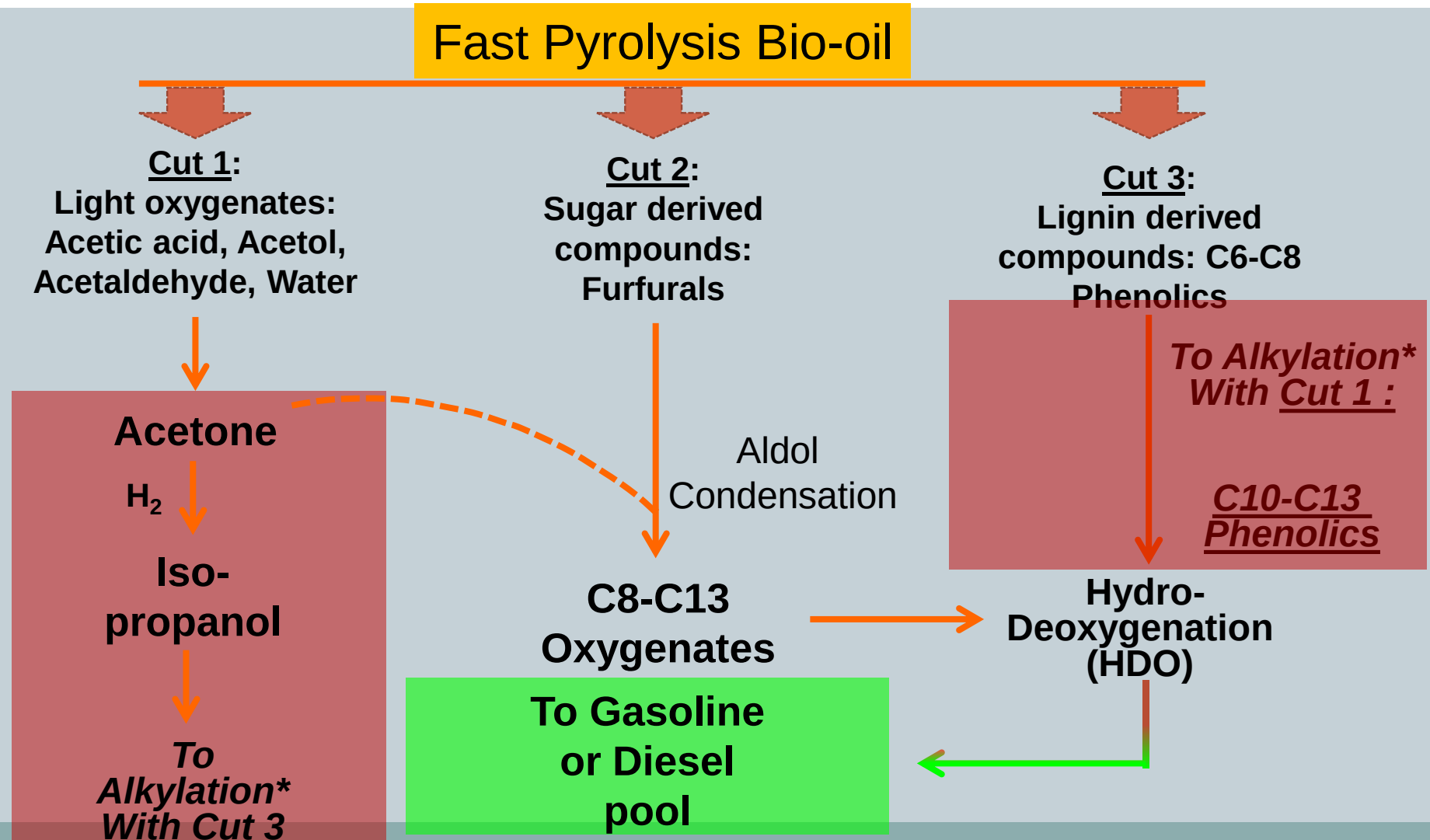
Lei Nie, Daniel E Resasco*

University of Oklahoma

Objectives

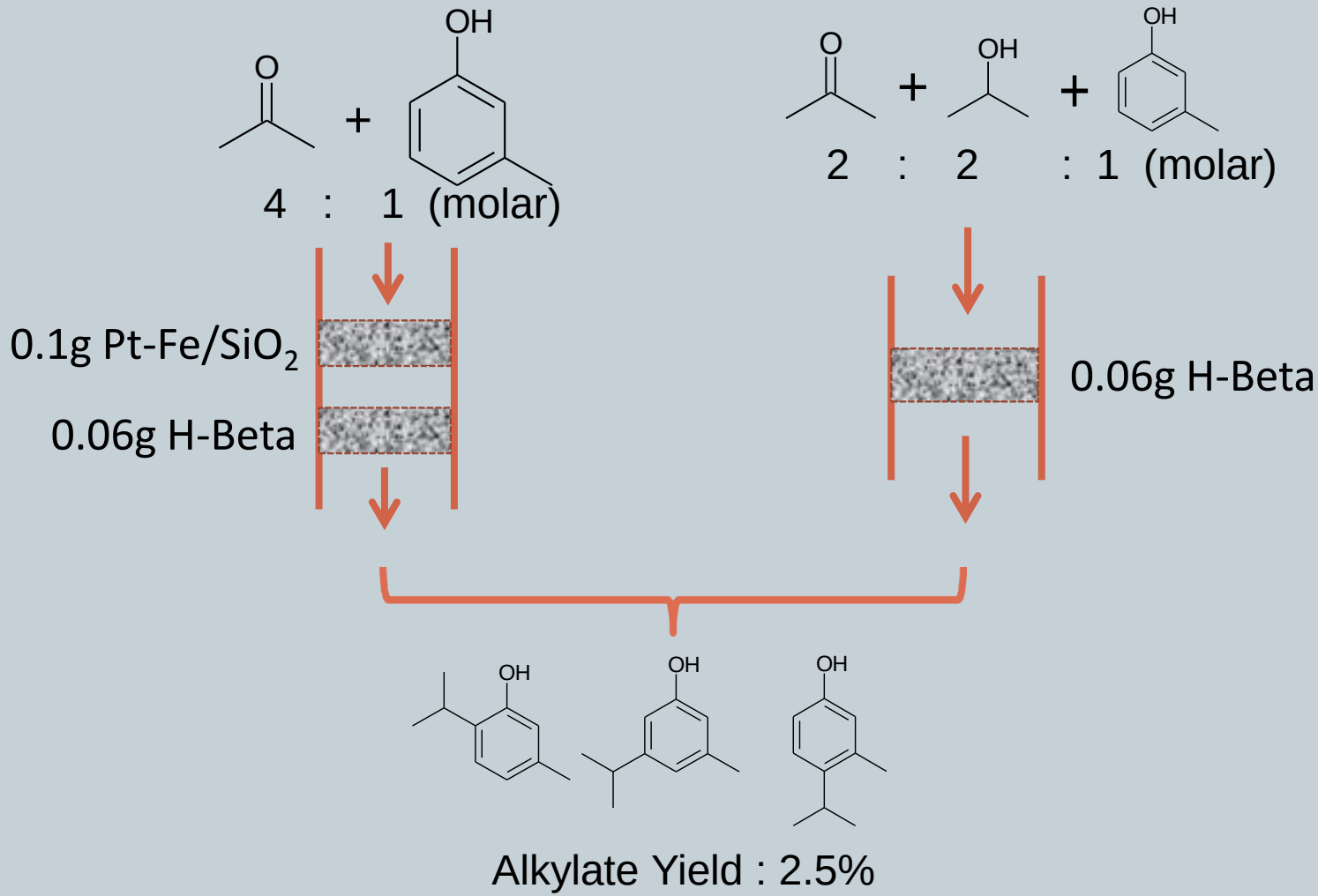
- Fast Pyrolysis Bio-oil Upgrading :
- Oxygen Removal
- Improving Carbon Retention

Methods



L. Nie, D.E. Resasco / Applied Catalysis A: General *447–448 (2012) 14 – 21*

Results



Alkylate Yield : 2.5%

Conclusions

- An alternative bio-oil upgrading straegy is proposed: combination of hydrogenation and alkylation.
- Relative low reaction temperature needed.
- The dehydration product, propylene, is the true alkylating agent.
- Pt-Fe/SiO_2 was found to be a selective hydrogenation catalyst.

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STUDENT #7

MECHANISM OF METHOXYBENZENE CONVERSION ON RUTHENIUM TITANIA CATALYSTS

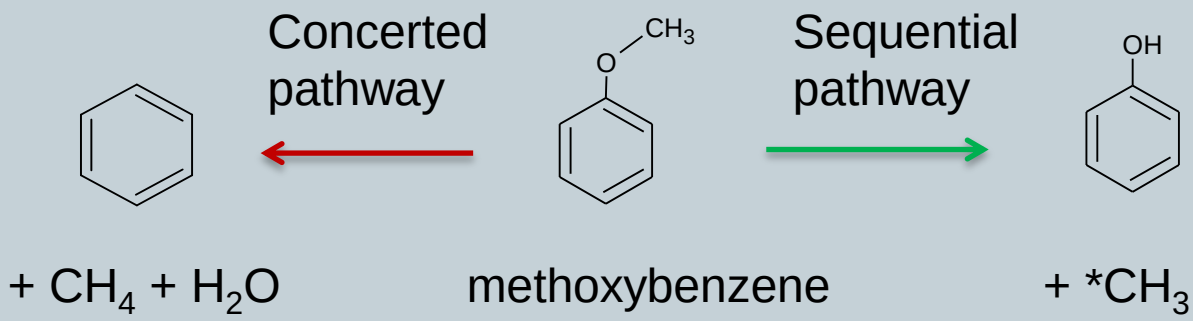
Taiwo Omotoso

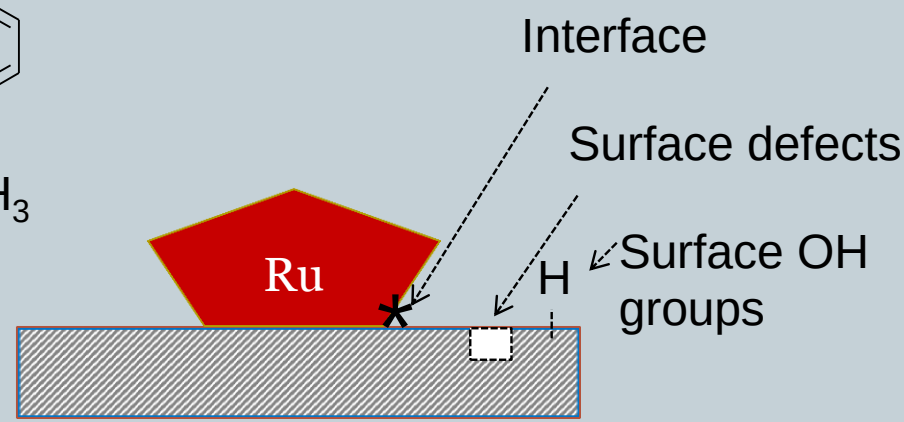
Steven Crossley

School of Chemical, Biological and Materials Engineering
University of Oklahoma

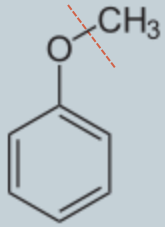
Objectives

- Investigate mechanism of methoxy group conversion
- Minimize catalyst deactivation
- Catalytically remove oxygen from liquid bio oil





Methods



Model compound, Methoxybenzene

= Ru

Ru/TiO_2

PRODUCT DISTRIBUTION

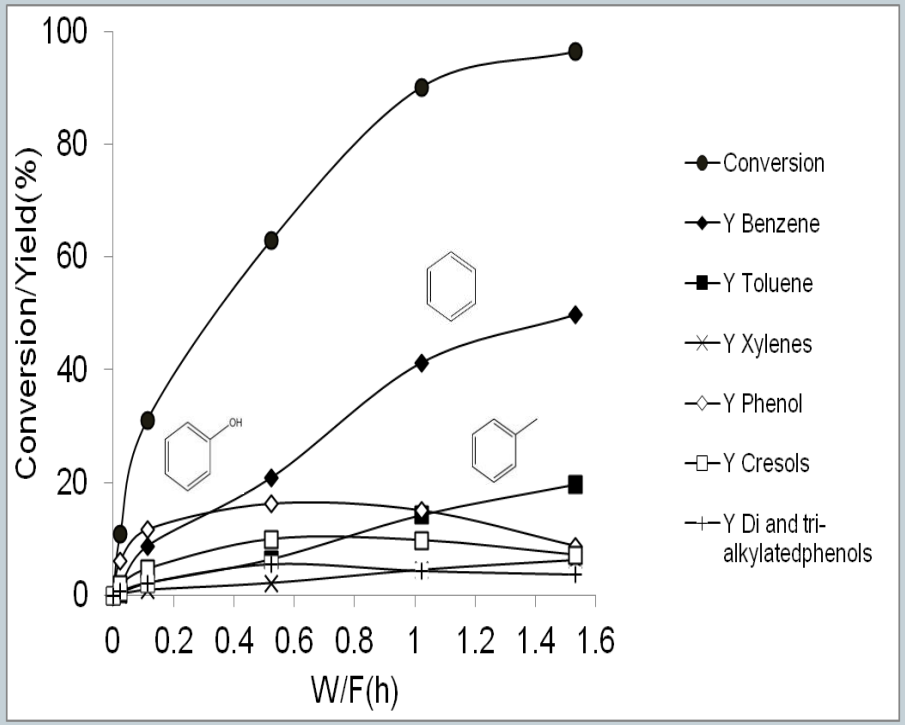
- Primary products
- Secondary products

CALCINATION PRETREATMENT

- Calcine under air at 400 °C
- Calcine under air at 500 °C

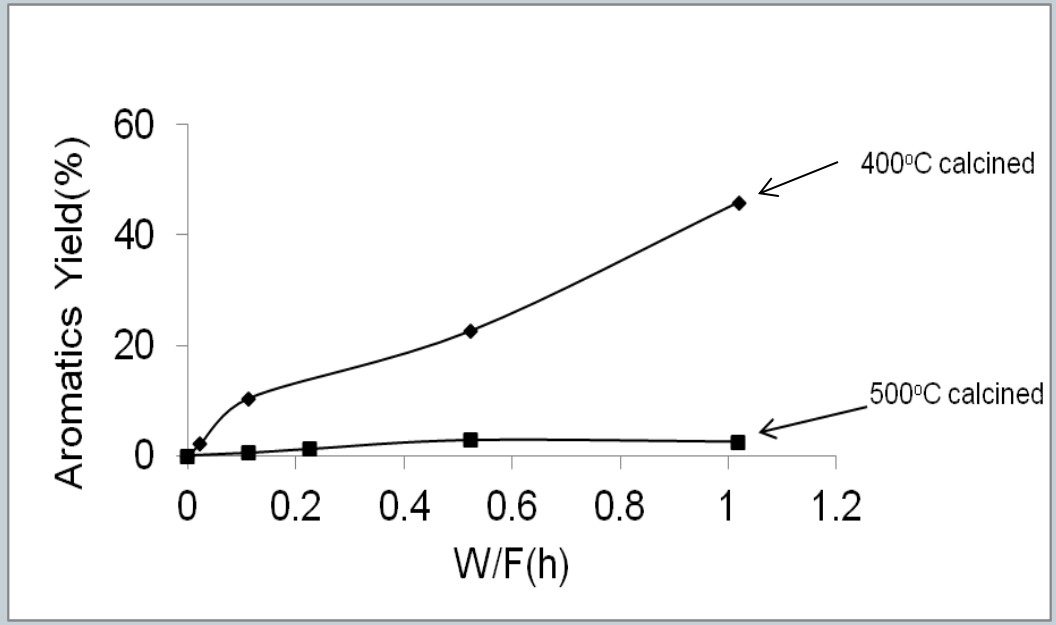
Results

Product Distribution



Calcination Temperature Effects

Catalyst	Ru wt%	Particle size(nm)
$Ru/TiO_2(400)$	3.66	6.9
$Ru/TiO_2(500)$	3.86	>10



Conclusions

- Ru/TiO_2 is an active catalyst for deoxygenation of model phenolic compounds under atmospheric pressure of hydrogen.
- A sequential mechanism for the conversion of methoxybenzene via formation of phenol as a primary product is proposed.
- Calcination temperature plays an important role in the activity and selectivity of the Ru/TiO_2 catalyst for catalytic upgrading.

STUDENT #9

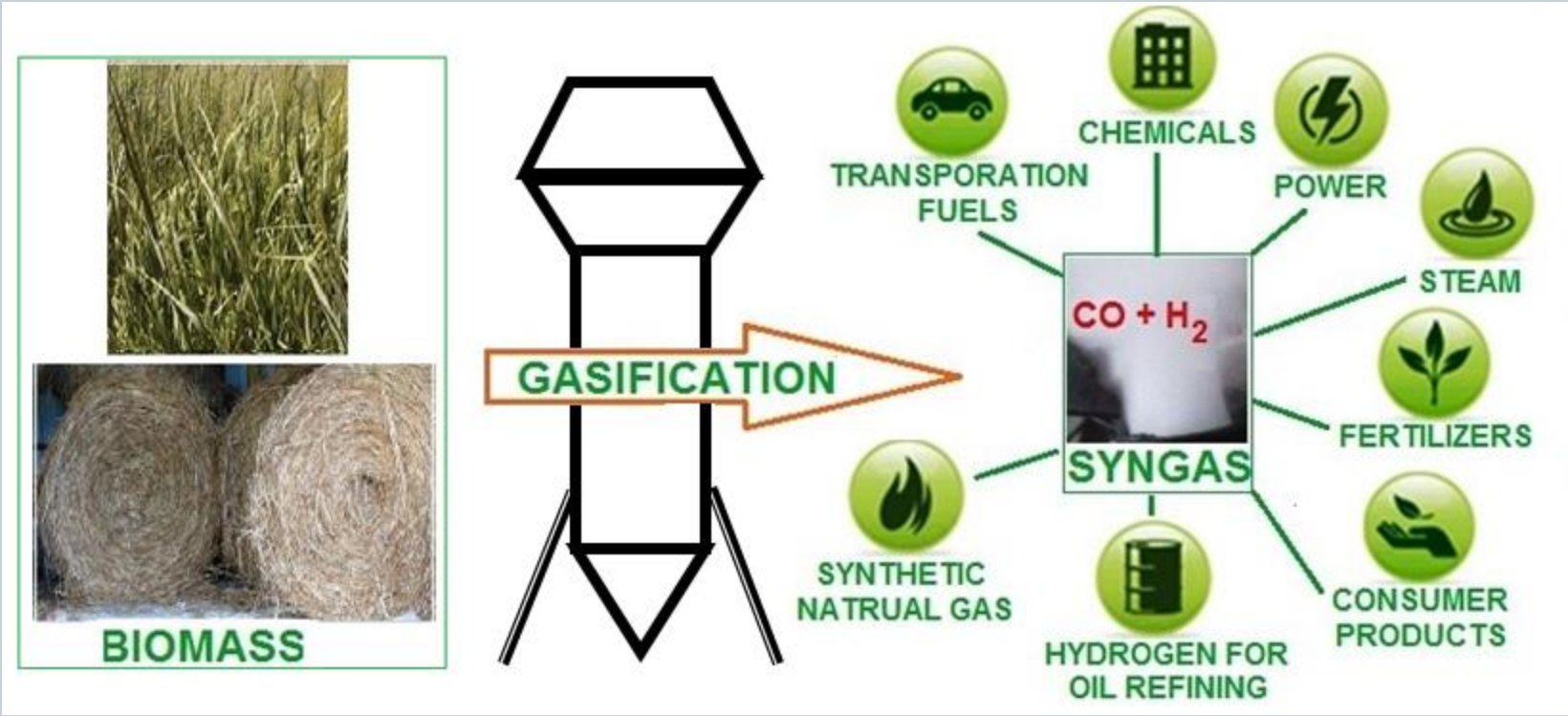
Reaction kinetics-based biomass gasification model to predict syngas quality suitable for biofuel production

Ashokkumar M. Sharma[a], Ajay Kumar[a], Sundar Madihally[b], Rob Whiteley[b], Raymond L. Huhnke[a]

[a] Biosystems and Agricultural Engineering Department

[b] Chemical Engineering Department
Oklahoma State University, Stillwater, OK 74078 USA

Objective

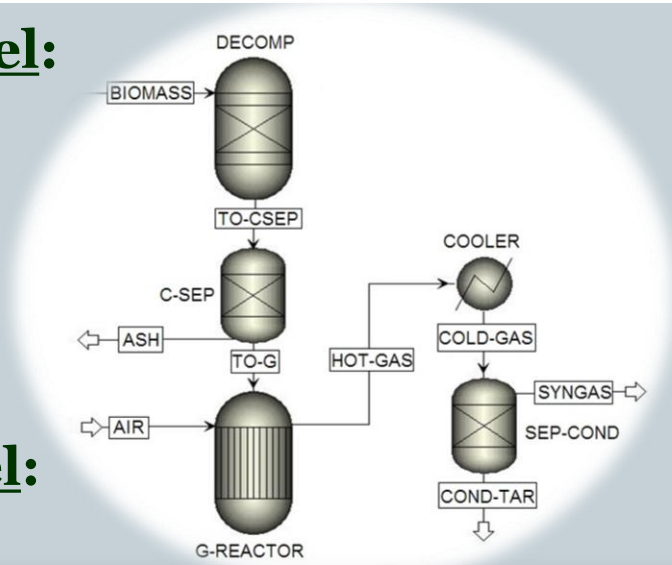


- To develop a reaction kinetics-based gasification model using a continuous stirred-tank reactor (CSTR) to predict syngas composition and yield.

Methods

1) Gibbs Equilibrium Model:

Inputs
- Biomass flowrate
- Air flowrate
- Gasifier temperature
- Gasifier pressure



Outputs
- Gas composition
- Gas yield

2) Reaction-Kinetics Model:

Inputs
- Biomass flowrate
- Air flowrate
- Gasifier temperature
- Gasifier pressure
- Gasification reactions
- Reaction rates (**r** values)
- Rate constants (**k** values)
- Residence time (**τ**)

Gasification reactions			ΔH (kJ mol^{-1})	Extent of reaction
$C(s) + H_2O$	↔	$CO + H_2$	+ 131	ξ_1
$C(s) + CO_2$	↔	$2CO$	+ 172.6	ξ_2
$CO + H_2O$	↔	$CO_2 + H_2$	- 41.2	ξ_3
$CH_4 + 2\ O_2$	→	$CO_2 + 2H_2O$	- 76	ξ_4
$CH_4 + H_2O$	↔	$CO + 3H_2$	+ 206	ξ_5
$C(s) + O_2$	=	CO_2	- 394.4	ξ_6
$C(s) + ½\ O_2$	=	CO	- 111	ξ_7

CSTR design equation:

$$F_{Ao}\,(in) - F_A\,(out) + r_A V_R = 0$$

Outputs
- Gas composition
- Gas yield

Results

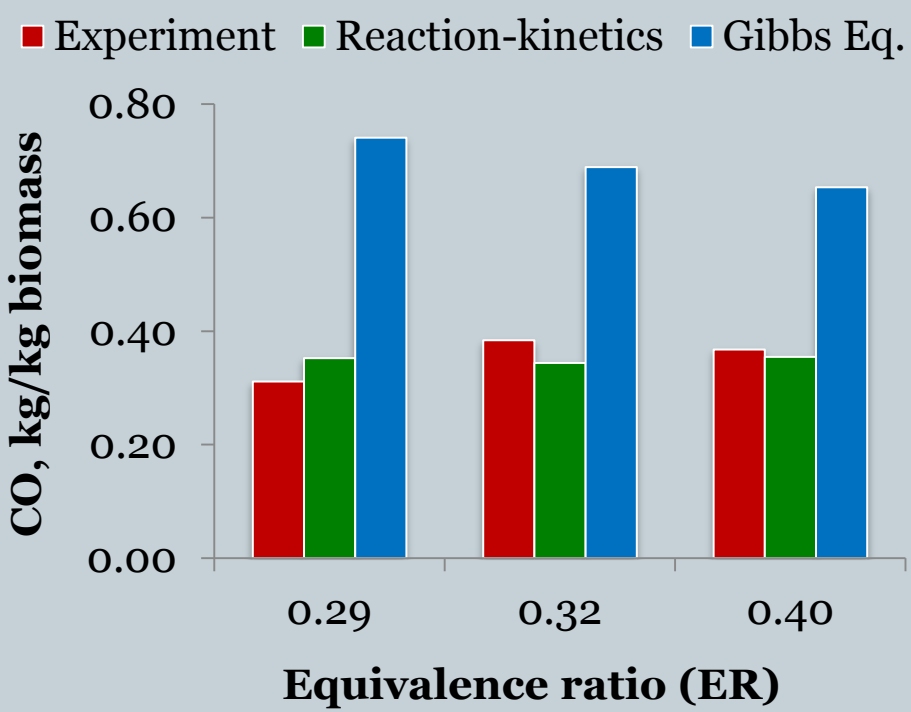


Fig.1 – Experimental and predicted CO yield with varying ER

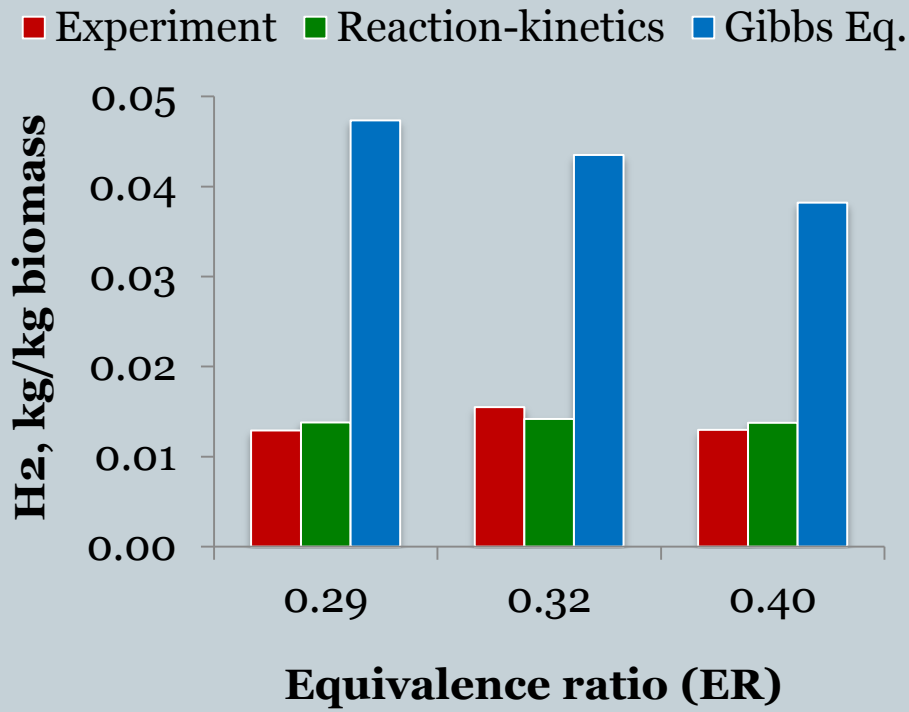


Fig.2 - Experimental and predicted H2 yield with varying ER

Conclusions

- As compared to experimental results:
  - Gibbs equilibrium-based gasification model predicted CO and H_2 yields 78% and 180% higher, respectively.
  - Reaction kinetics based gasification model predicted CO and H_2 yields within 13% and 9%, respectively.

STUDENT #10

Deactivation of Zeolite Catalysts During Upgrading of Pyrolysis Vapors

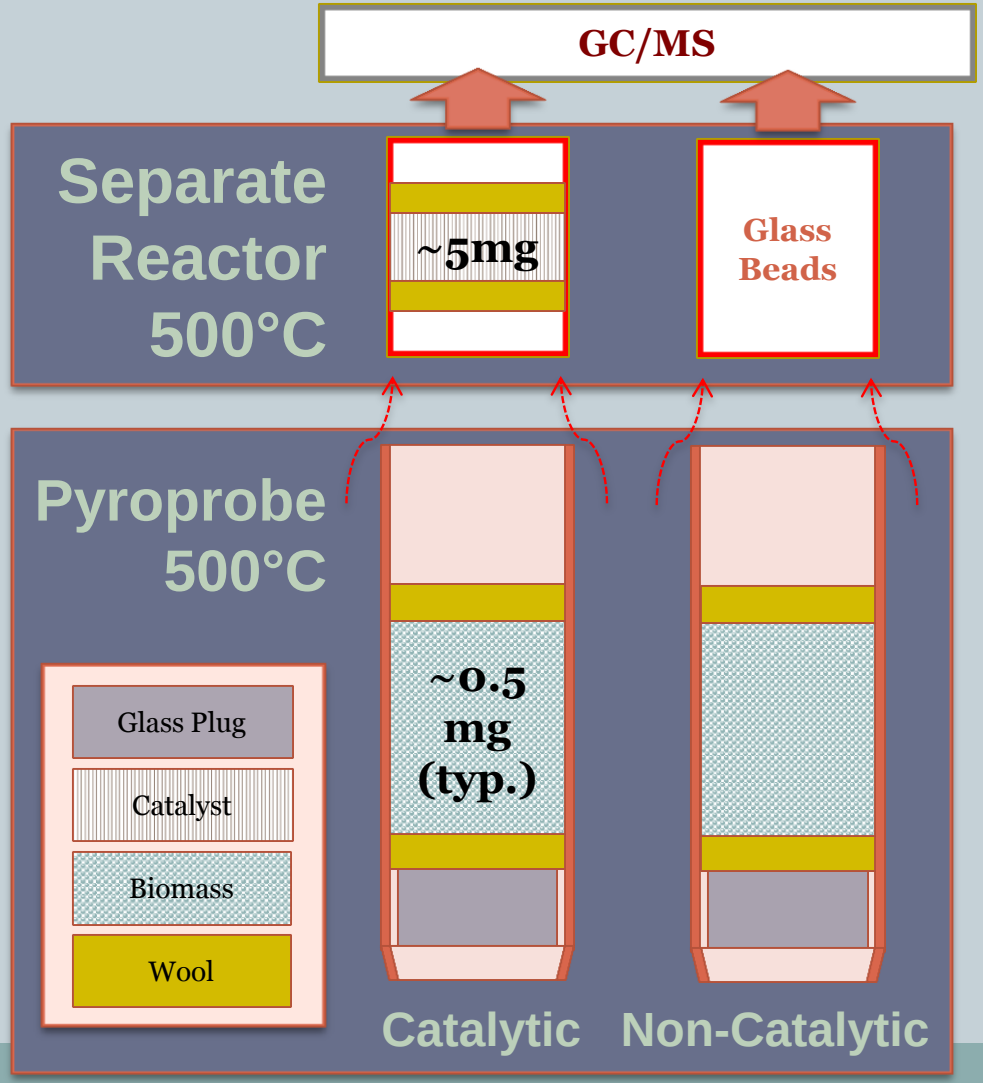
Shaolong Wan, Christopher Waters, Rolf Jentoft, Steven Crossley, Lance Lobban, Daniel Resasco, Richard Mallinson

University of Oklahoma

Objectives

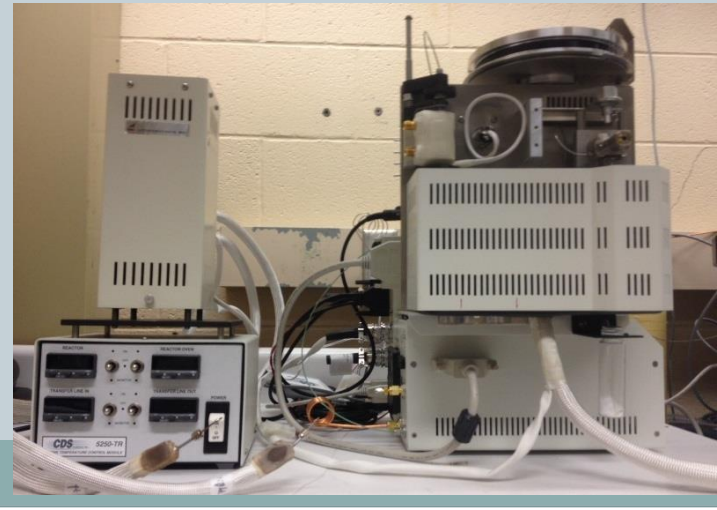
- Understand zeolite performance characteristics (specifically deactivation) over various reaction conditions with non-model biomass feedstock
- Apply known model zeolite chemistries to vapor-phase pyrolysis oil upgrading
- Increase total carbon retention in liquid product, decrease hydrogen consumption
- Develop more robust upgrade strategies and pathways for thermochemical biomass conversion to hydrocarbon fuels (gasoline, diesel)

Methods



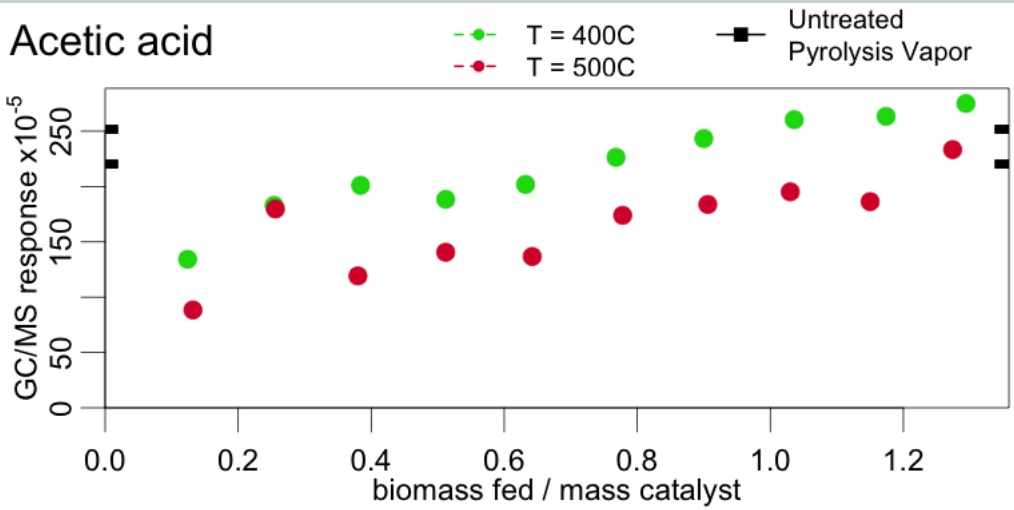
Separate reactor allows us to:

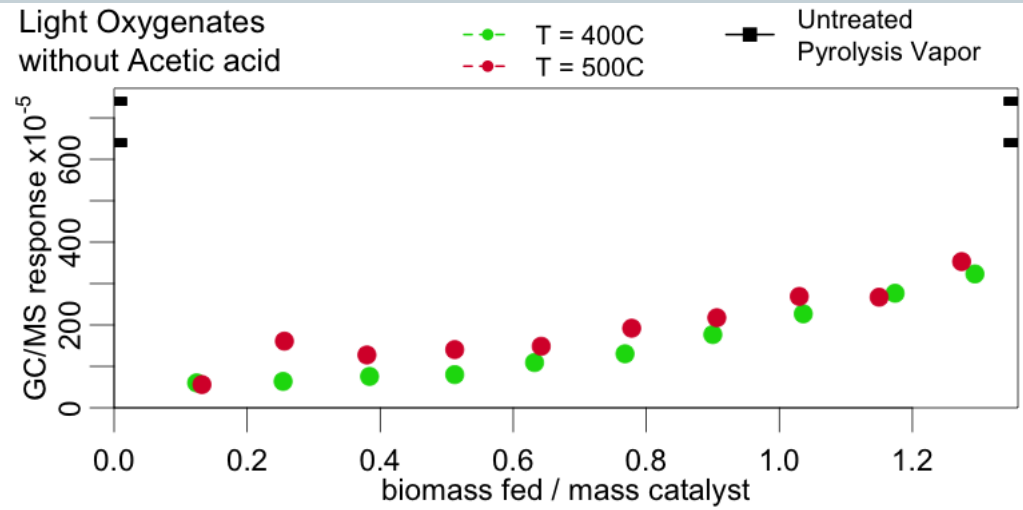
- Vary reactor conditions (temperature, residence time) independent of pyrolysis conditions
- Measure catalyst deactivation with multiple pulses
- Autosampler: very high throughput (15 pulses/day)

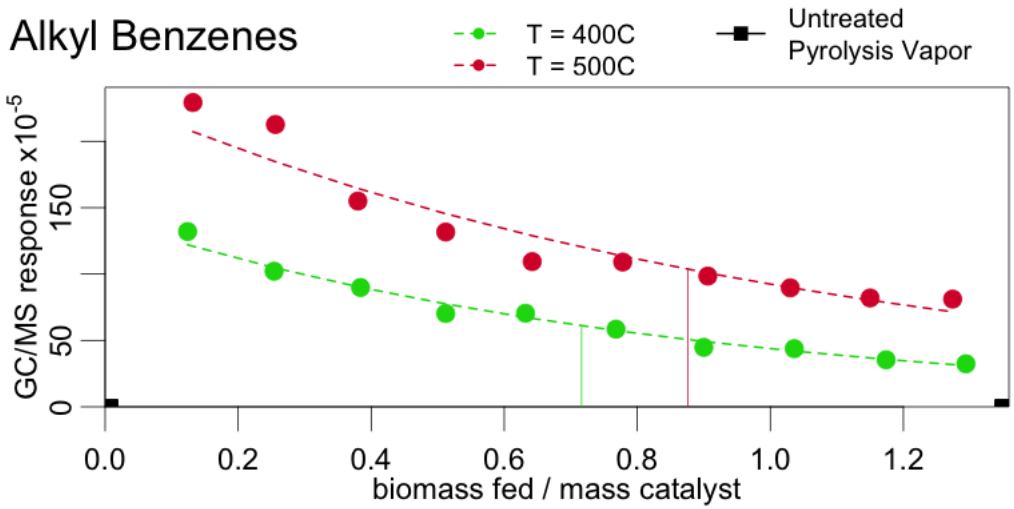


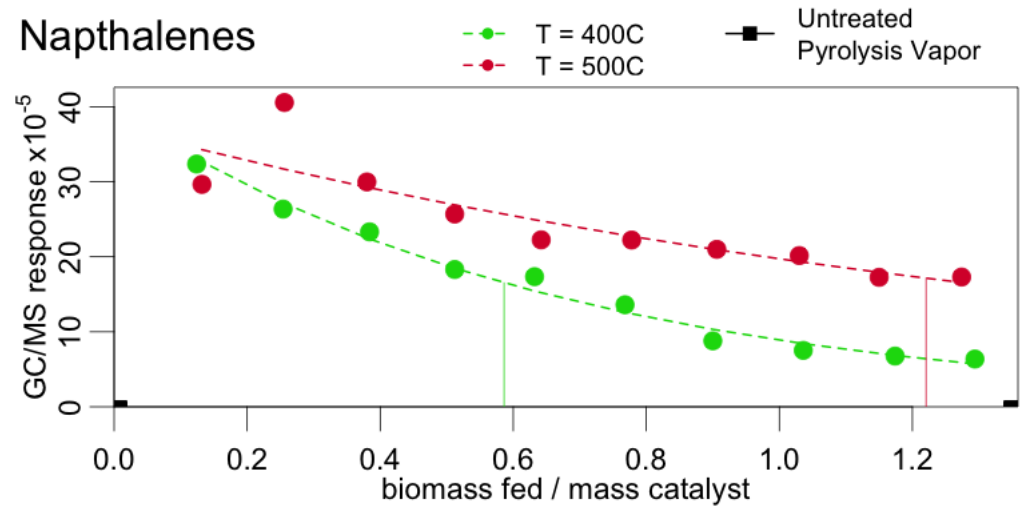
Results

Feedstock: Oak sawdust
Pyrolysis temp: 500C
Catalyst: HZSM5, 5mg









Conclusions

- Increased temperature slows catalyst deactivation
- Zeolite chemistry produces aromatic hydrocarbons from biomass pyrolysis vapors
- Separating the catalysis from the pyrolysis decouples variables and allows us to better evaluate the catalytic performance (temperature, residence time)
- Rapid screening allows for evaluation of performance of catalyst modification (crystal size, acid density, mesoporosity, additives, etc)

STUDENT #11

LigPred: A Comprehensive Prediction System for the Identification and Classification of Enzymes Related to the Synthesis and Degradation of Lignin

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Objectives

- Better understand processes related to lignin.
- Improve machine learning techniques for protein functional classification.
- Discover novel lignin-related enzymes.

Methods

- Obtain high quality datasets
- Reduce datasets to 40% sequence similarity
- Split datasets into independent and training datasets.
- Do 5-fold testing on training datasets to find optimum kernel and parameters.
- Generate models with training datasets and to independent testing with independent dataset.
- Classify all Swiss-Prot proteins not in dataset to further test specificity.
- Classify true datasets; metagenomes, NCBI unknowns, etc.

Results

- 50 classes identified, 37 suitable for SVM classification.
- 5-Fold testing excellent.
  - Maximum Matthews Correlation Coefficient (MCC): 1.0
- Independent Training acceptable.
  - Max MCC ~0.7
- Negative Set testing poor (maybe).
- Sequences predicted from metagenome and NCBI unknowns.

Conclusions

- High chance of correctly predicting lignin related enzymes.
- False positives likely very high.
- False positives can likely be reduced with modifications to experimental procedure.

STUDENT #12

Cellulosomal protease inhibitor protects key cellulosomal cellulases in *Clostridium cellulolyticum*

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Objectives

- Characterize the enzymatic activity of a cellulosomal protease inhibitor in *Clostridium cellulolyticum*
- Examine the physiological function of this inhibitor
- Examine the role of this inhibitor in regulating cellulosomal composition

Methods

- Enzymatic activity test
  Ni affinity chromatography + *In vitro* inhibitor assay

- Physiological characterization
  Mutagenesis+ Growth determination+
  Cellulose hydrolysis test

- Analysis to cellulosomal composition
  SDS-PAGE + Mass spectrometry+ Densitometry analysis

Results

- The cellulosomal protease inhibitor is an effective inhibitor of cysteine protease.
- Lack of the inhibitor reduced cell growth and decreased cellulose utilization.
- Lack of the inhibitor greatly reduced the protein abundance of several cellulosomal components.
- Two major cellulosomal components, Cel48F and Cel9E, are pivotal cellulases for cellulose hydrolysis.

Conclusions

- This is the first study to uncover the physiological importance of a cellulosomal protease inhibitor in protecting key cellulosomal cellulases in cellulose-degrading *Clostridia*.

- The presence of this protease inhibitor allows *C. cellulolyticum* to maintain a higher efficiency in metabolizing lignocellulosic biomass.