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Enzyme Synergies in the Hydrolysis of AFEX Pretreated Biomass

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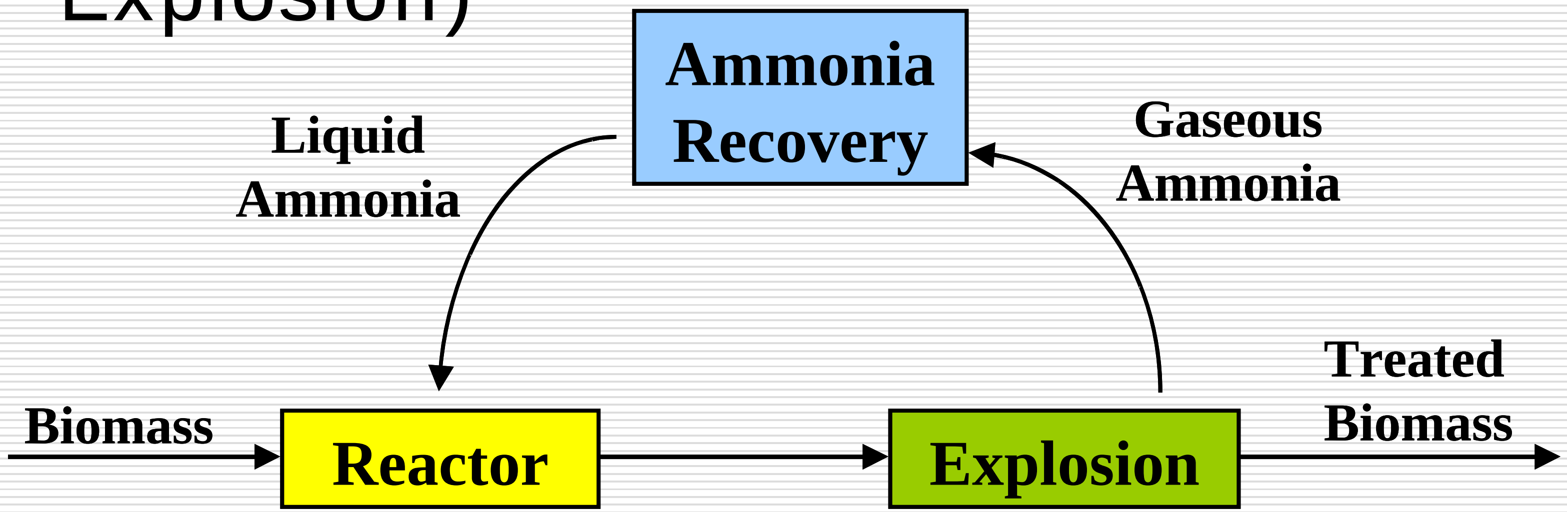
Scope of Presentation

- Effect of AFEX (Ammonia Fiber Explosion)
- Understanding mechanism of AFEX & effect on enzyme synergy
  - ESCA (Electron Spectroscopy for Chemical Analysis)
  - Prussian Blue Characterization
  - Effect of particle size on AFEX
  - Washed vs. Unwashed Biomass Hydrolysis
- Enzyme Synergy for Lignocellulosics
- Micro-plate Experiments for “Enzyme Cocktail” Optimization

PART 1

- EFFECT OF AFEX (Ammonia Fiber Explosion)

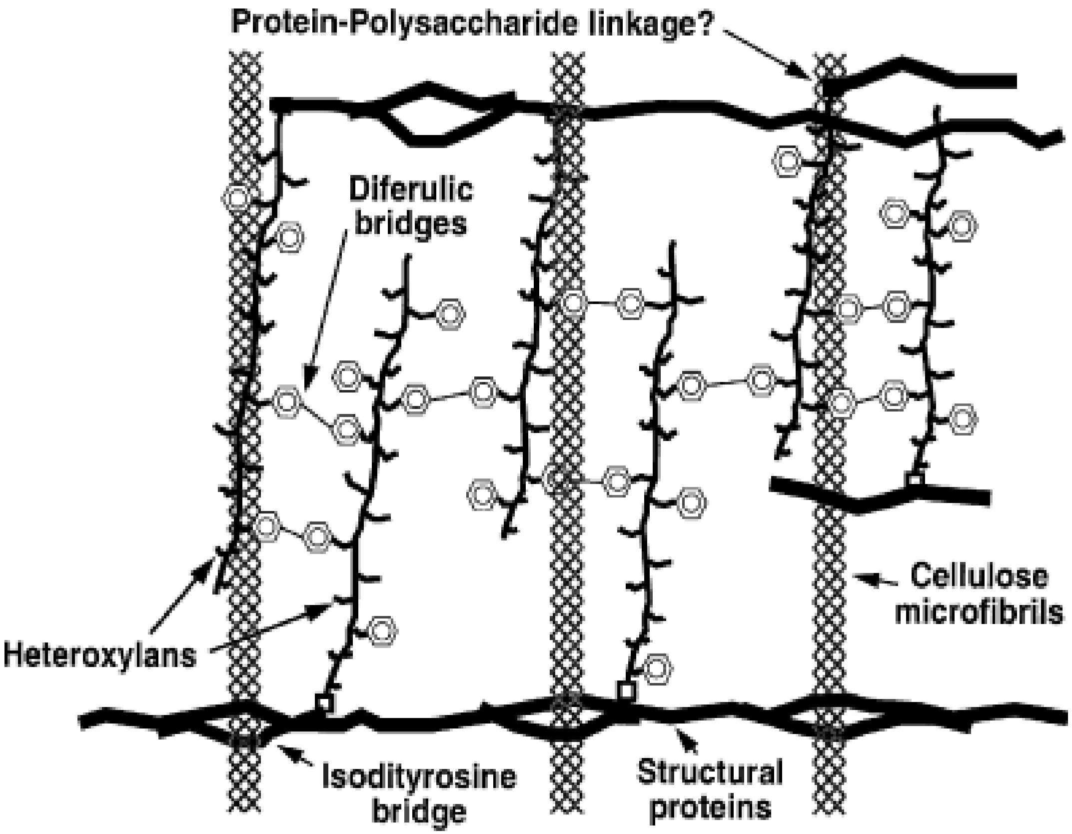


Typical AFEX Conditions

Pressure (20-30 atm),
Temperature (60-120 ^{o}C),

Time (5-15 mins)

Removal of Alkali Solubles by AFEX



Corn Fiber Cell Wall Model

❑ **AFEX Effect on**

- Acetyl Groups
- Hemicellulose
- Cellulose
- LCC Bonds
- Lignin
- Proteins
- Ca as binding agent for Uronic acids/ Pectins etc.

Source: Saulnier, 1999

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AFEX Integrated Biorefinery

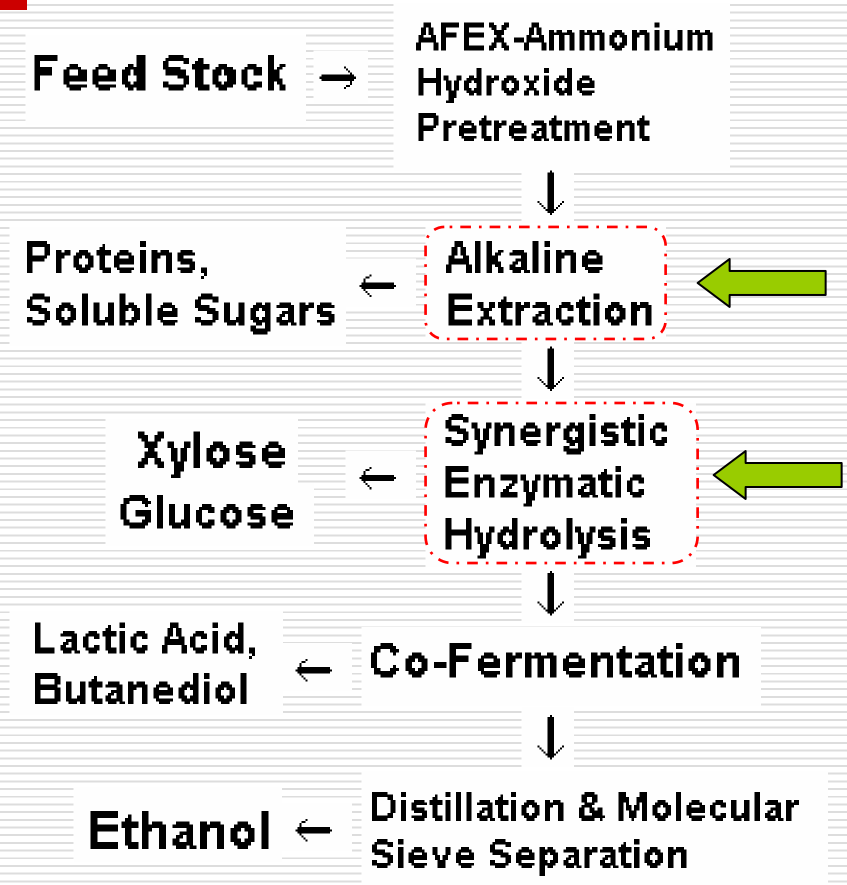
Key Points:

- **Relevance of washing / alkali extraction on the synergistic hydrolysis**
- **<u>Water Washing Protocol</u>**

  10% Biomass in water

  Stir for 15 mins

  Drain / Squeeze till dry



Integrated Biorefinery Unit Operations

everything biomass

Types of Biomass Studied

1. Biomass Samples (Corn Stover, Poplar, DDG)
2. Corn Stover Mesh Cut Notation & Composition *

Sample Notation	Size (microns)	Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin	Protein	Water Solubles	Alcohol Solubles
uncut CS	> 850	32.58	23.44	1.37	2.74	0.26	12.27	3.13	3.89	3.53
# 20	850	33.45	27.52	1.02	2.35	0.69	12.46	1.75	0.14	2.28
# 35	500	34.35	24.73	1.31	2.68	0.38	12.37	2.01	2.68	3.15
# 40	425	34.42	23.54	1.27	2.52	0.30	12.63	2.25	4.31	3.24
# 70	212	33.99	22.58	1.40	2.62	0.21	12.59	2.71	5.98	3.62
# 80	180	33.99	21.85	1.51	2.66	0.07	12.94	2.89	7.91	3.85
# 100	150	33.33	21.92	1.66	2.89	0.05	12.82	3.27	8.51	3.90
# 120	<150	32.29	20.06	1.75	2.93	0.00	12.01	4.27	11.28	4.37

* CS Compositional Analysis – Courtesy Dr Bonnie Hames, NREL

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PART 2

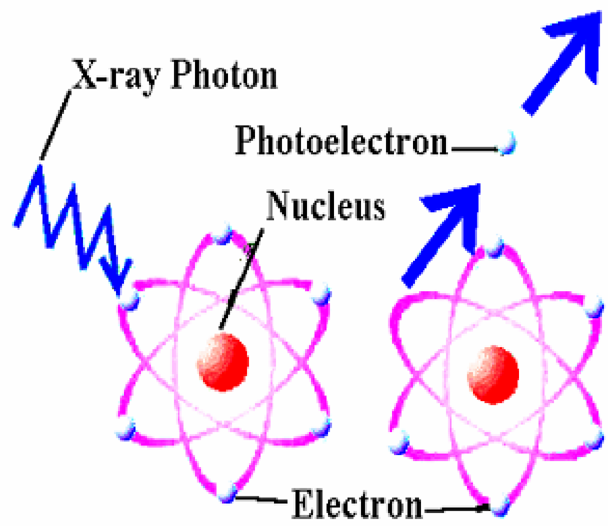
- UNDERSTANDING EFFECT OF AFEX VIA VARIOUS TECHNIQUES
  - ESCA
  - PRUSSIAN BLUE
  - PARTICLE SIZE
  - WATER WASHING

ESCA as a tool to study effect of AFEX

- ESCA or XPS (X-ray Photoelectron Spectroscopy) Principles
  - Surface Analysis (depth upto 1-20 nm)
  - Types of Chemical Bonding (C,O,N,Ca)
  - O/C ratio
    - Eg. (Gustafsson, 2003)

      Cellulose O/C = 0.8

      Lignin O/C = 0.33



Kinetic Energy of emitted e- = (Incident Energy of X-ray) - (Binding Energy)

ESCA O/C ratio for Corn Stover Mesh Cuts

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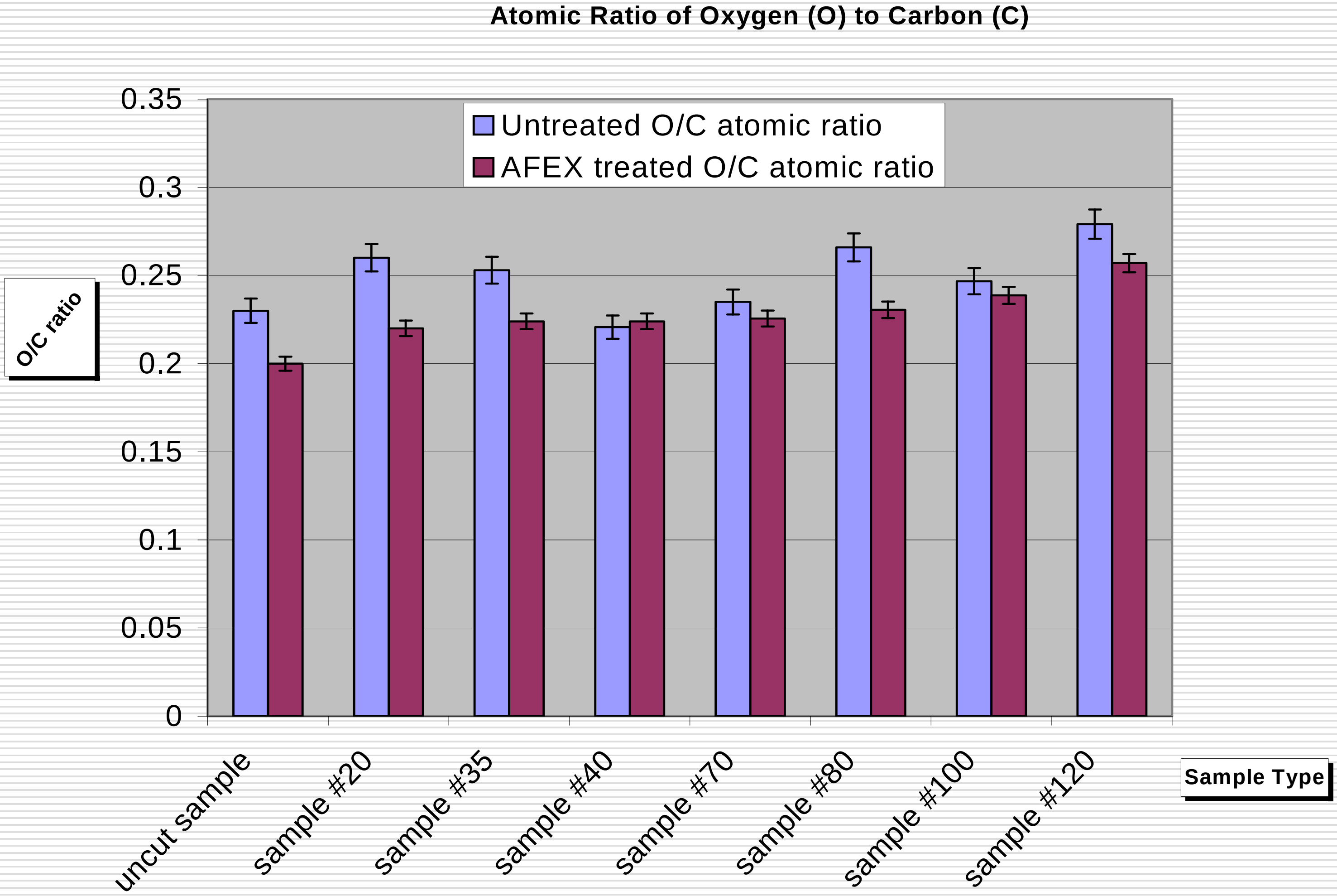
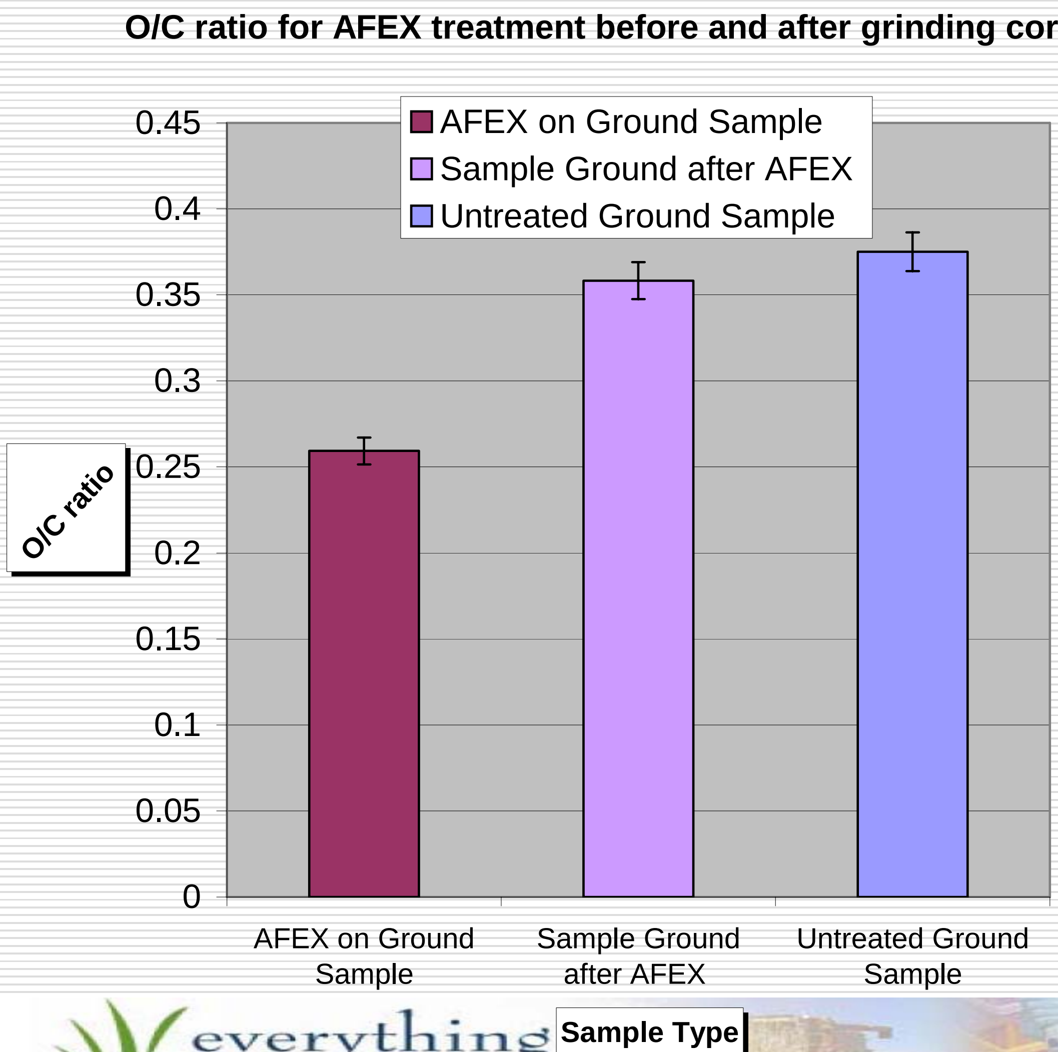


everything biomass

Effect of Size on AFEX



Reducing particle size aids in extraction of lignin cleavage fragments & other alkali extractives to the surface

ESCA Results Summary

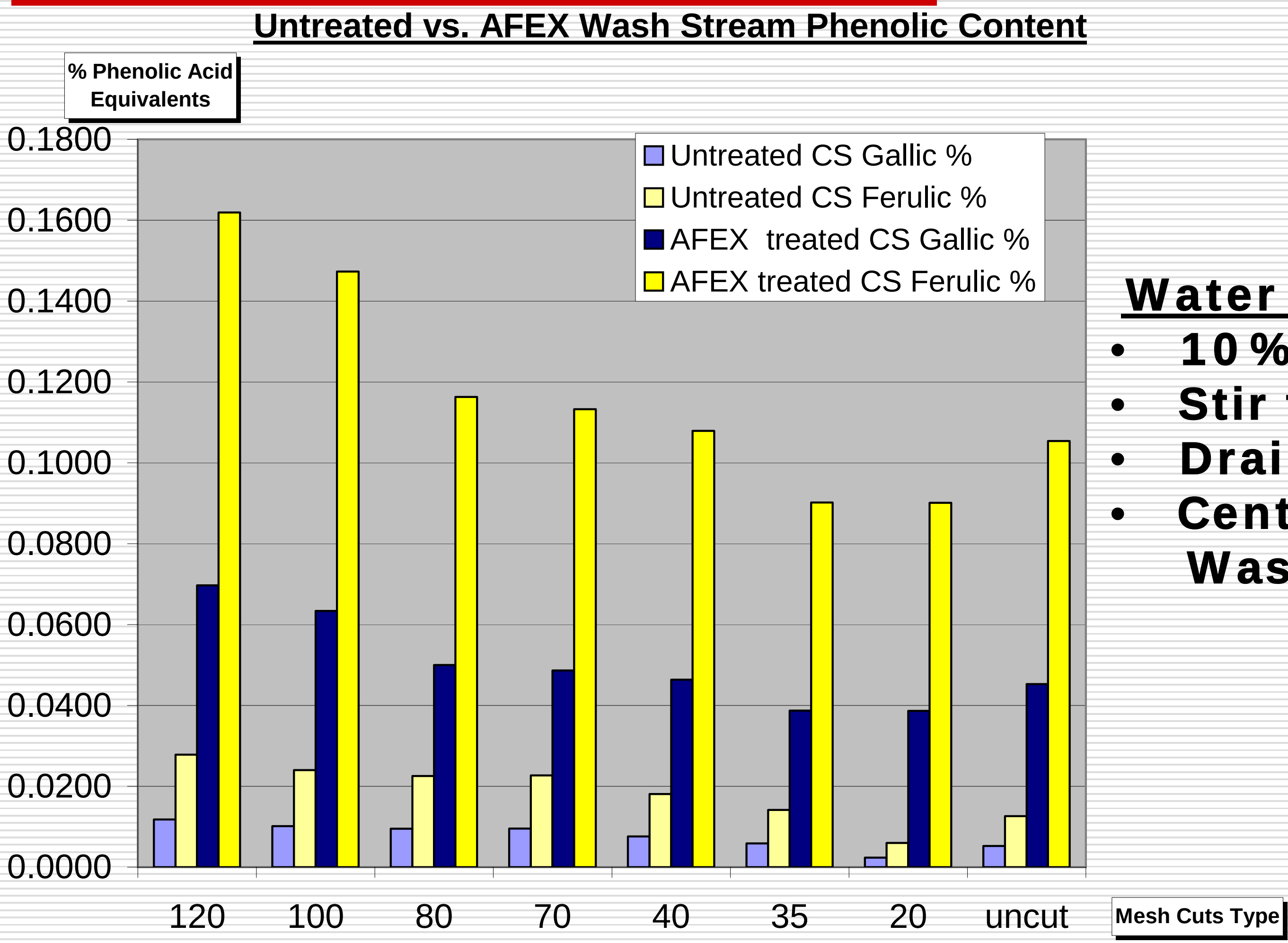
- Nitrogen & Calcium signal increased substantially after the AFEX for Corn Stover
- 5-15 % drop in O/C ratio after AFEX for Corn Stover
- 30 % drop in O/C ratio after AFEX on Ground Corn Stover

Modified Prussian Blue Test

- Oxidation of phenolic groups…coupled to the reduction of reagent
- Quantitative Colorimetric Method
- Standard Phenolic Compounds
  - Gallic Acid & Ferulic Acid
- Stability of Color
- High Reproducibility

Reference: Graham, 1992

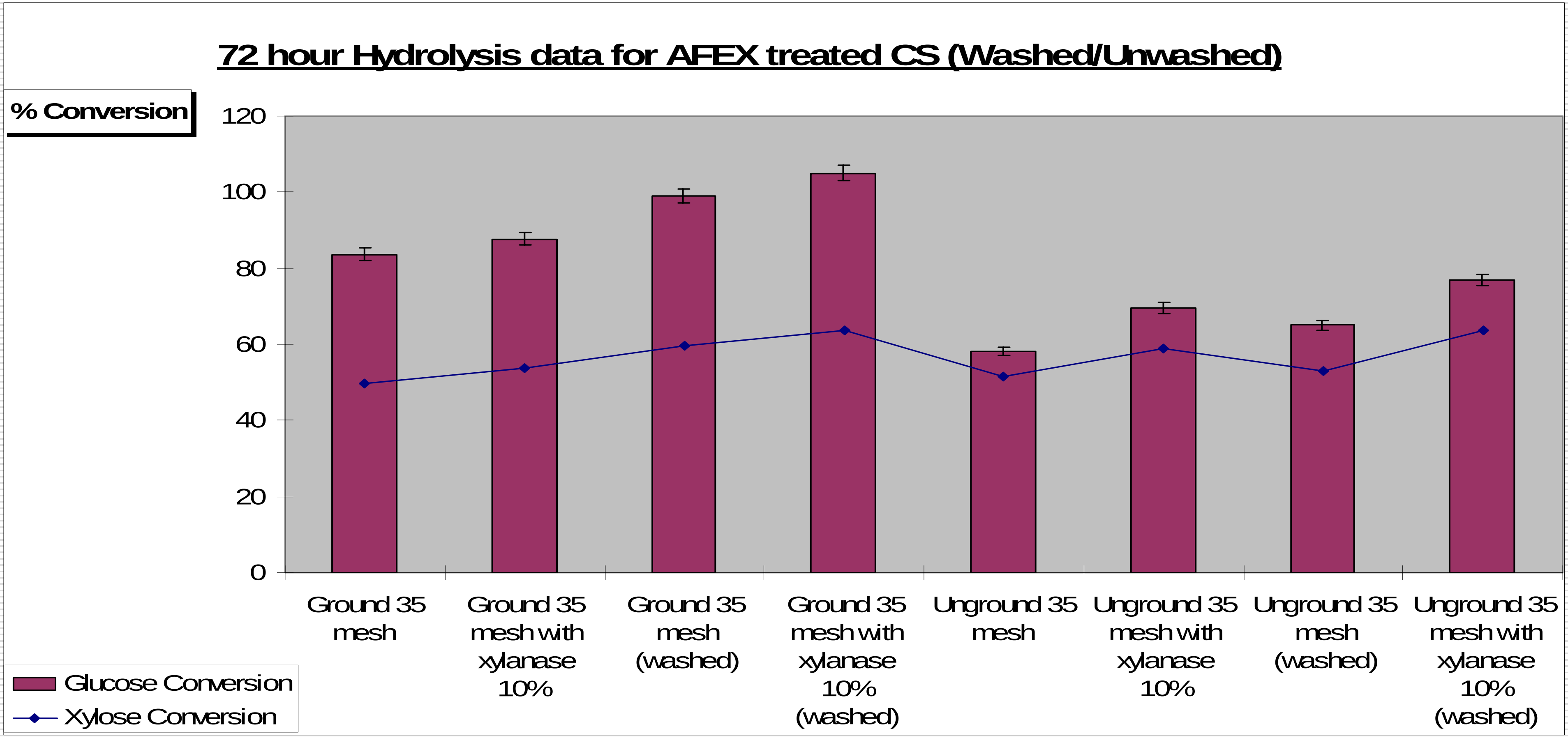
Phenolic Content of Wash Streams



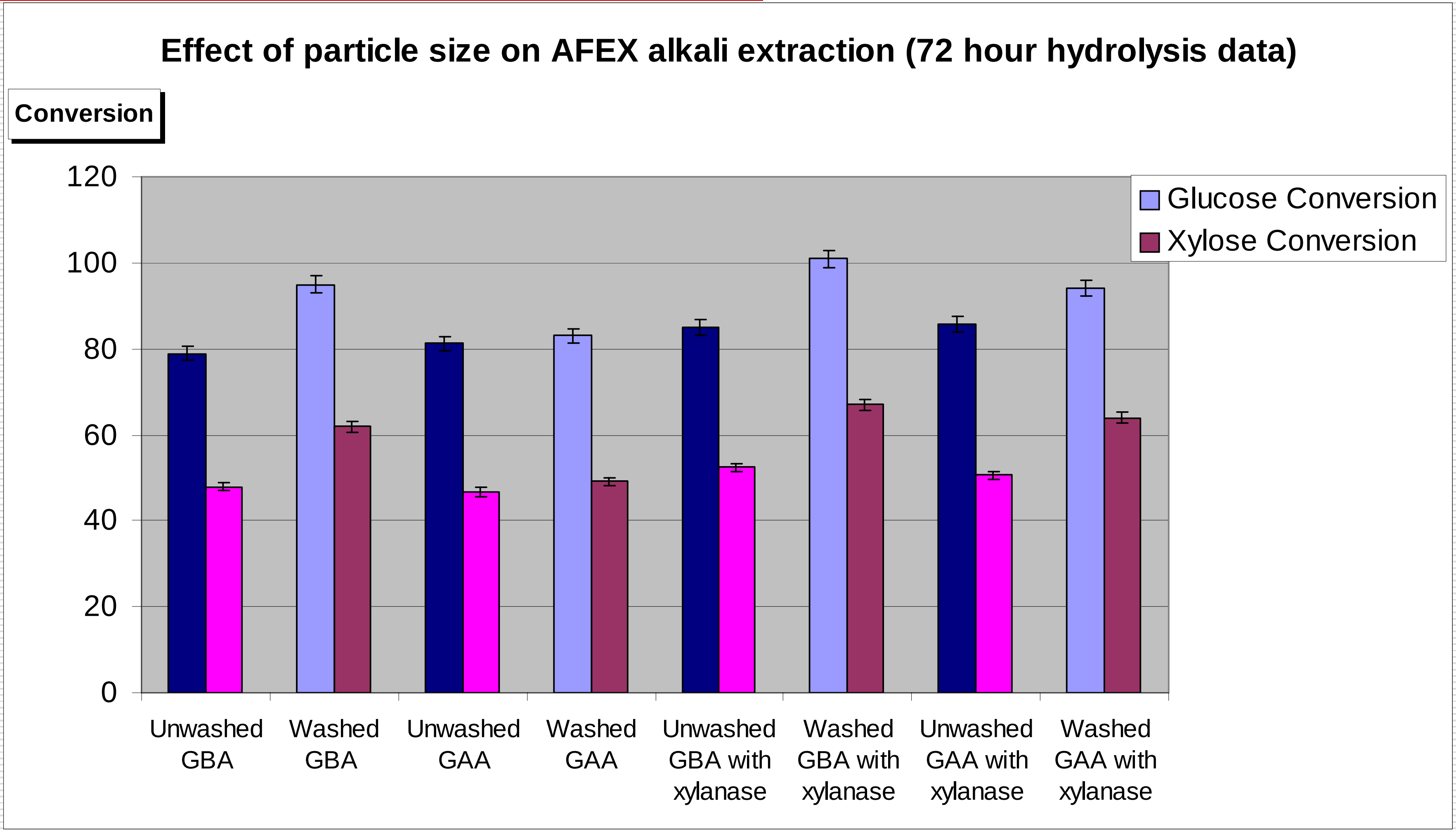
Water Washing Protocol

- 10% Biomass in water
- Stir for 15 mins
- Drain/Squeeze till dry
- Centrifuge & Filter Wash Liquid

Hydrolysis Data for Washed vs. Unwashed 35 Mesh Cut



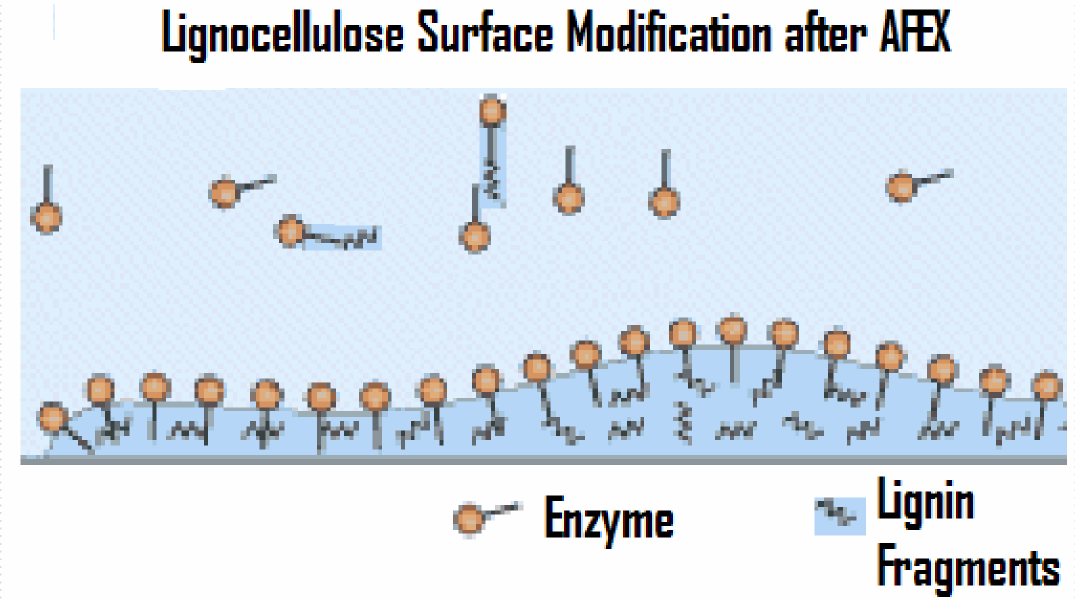
NOTE. Unground 35 Mesh Cut (500 microns)
Ground 35 Mesh Cut (<100 microns)



GBA- Ground Before AFEX

GAA- Ground After AFEX

Possible Effect of Lignin Fragments extracted during AFEX



- This might explain why washing aids in the hydrolysis of biomass, as some of these phenolic fragments are removed.

- What would be the next step is to couple alkali extracted biomass (for protein removal) with the AFEX process.

Source: Modified from www.chemsoc.org/.../ 2003/hargreaves_jul03.htm

PART 3

- SYNERGISTIC HYDROLYSIS FOR LIGNOCELLULOSIC BIOMASS

Synergistic Hydrolysis

- **Need to study synergy for lignocellullosic hydrolysis**
  - Role of Lignin and influence of alkali extraction
  - Optimizing multi-enzyme systems could aid the enzyme companies in preparing tailor made enzymes
    - lack of hemicellulase activity,
    - specific cocktails for different pretreatments,
    - cocktail optimization for various biomass compositions,
    - Multiple enzyme cocktails for SSF/SSCF systems
  - Reducing net amount of enzymes (synergy, additives)
  - Reducing hydrolysis time (alkali/water extraction, size reduction)

PART 4

- DEVELOPMENT OF MICROPLATE TECHNIQUE FOR “ENZYME COCKTAIL SCREENING”

Advantages of Microplate Experiments

Ultimate aim – analyze hydrolysis profile for multi enzyme systems rapidly over a ***larger gamut of input variables***, much ***faster*** than the conventional protocol in a ***reproducible manner***.

- AFEX conditions (better screening of AFEX conditions)
- Enzyme cocktails & concentrations
- Substrate concentration
- Additives
- Protein Extraction (Integrated Step)

Process Flowchart for Microplate Enzymatic Digestion

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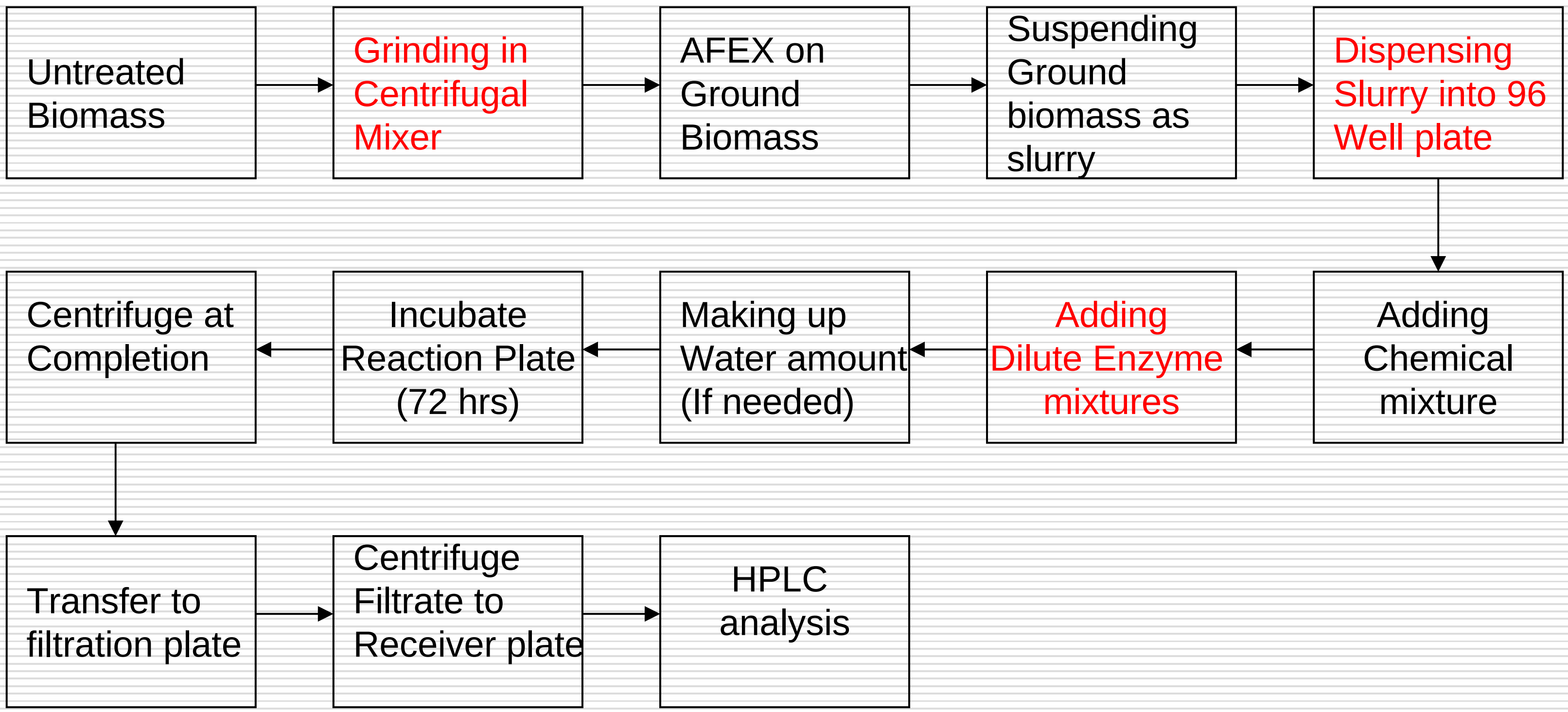

Preliminary Microplate Results

- Measure enzymatic hydrolysis of cellulose and hemicellulose as a function of cellulase and xylanase and beta glucosidase loadings for Corn Stover
- Determine key trends and synergistic interactions between enzymes

Continuing Work

- AFEX achieved slightly higher yields from enzymes even though no lignin was removed in comparison to other pretreatments and hence need to study the lignin fragmentation due to AFEX
- Analysis of wash stream sugar/xylooligosaccharides/lignin
- Fermentability of washed/unwashed biomass streams
- Inhibition of enymes for washed/unwashed biomass
- Microplate Experiments for various enzyme combinations

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Questions

Biomass Conversion Research Group at MSU

References

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