



Enzymatic Hydrolysis of Poplar Pretreated by Ammonia Fiber Explosion

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Hybrid Poplar advantages

- Relatively high productivity (18-26 dry Mg/(ha·year))
- Fast growing
- Widely available
- Pest-resistant
- Drought-resistant
- High density reduces cost of transportation

**Populus nigra x
Populus maximowiczii
(Hardwood)**



Comparing Biomasses

| | Glucan | Xylan | Lignin |
|------------------------------------|--------|-------|--------|
| Poplar-II (High lignin) | 43.8% | 14.9% | 29.1% |
| Poplar-I (Low Lignin) | 45.1% | 17.8% | 21.3% |
| Corn Stover | 37.7% | 21.6% | 18.6% |
| Switch-grass | 33.5% | 26.5% | 18.1% |



AFEX process Conditions:

Soaking/un soaking

Moisture in biomass ~50 to 70%

Temperature range 80-200 °C

BM : Ammonia loading 1: 0.6 -1: 2.0

Residence Time: 10 min.

Hydrolysis Conditions

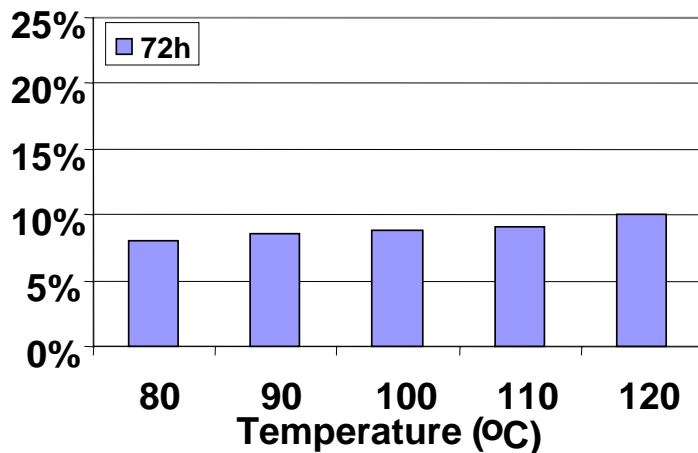
LAP009 (NREL Protocol)

15ml reaction volume, 50 °C, 1% BM

Enzymes: Spezyme CP and
Multifect Xylanase 720 (Genencore)
 β -Glucosidase (Novozyme)

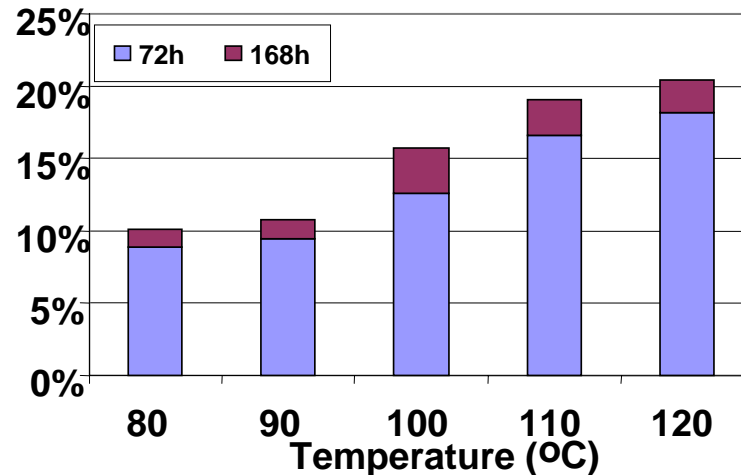


No Soak - Glucan conversion



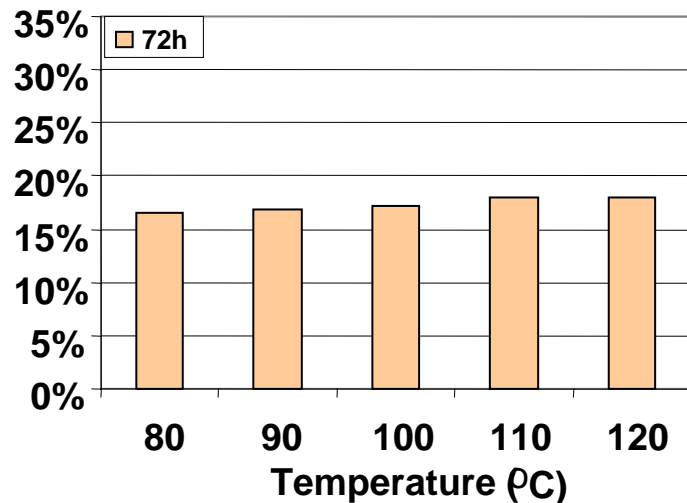
AFEX:1:1.2 biomass to NH₃, 5 min.

Water soak - Glucan conversion



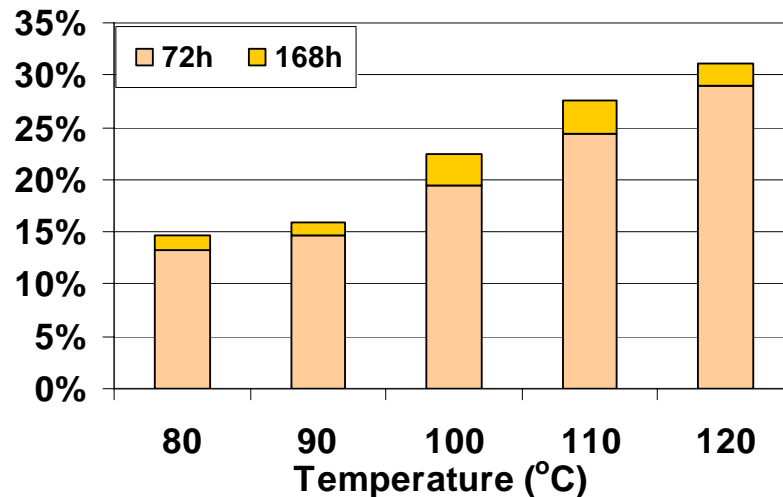
AFEX: 1:1.2 biomass to NH₃, 10 min.

No soak - Xylan conversion



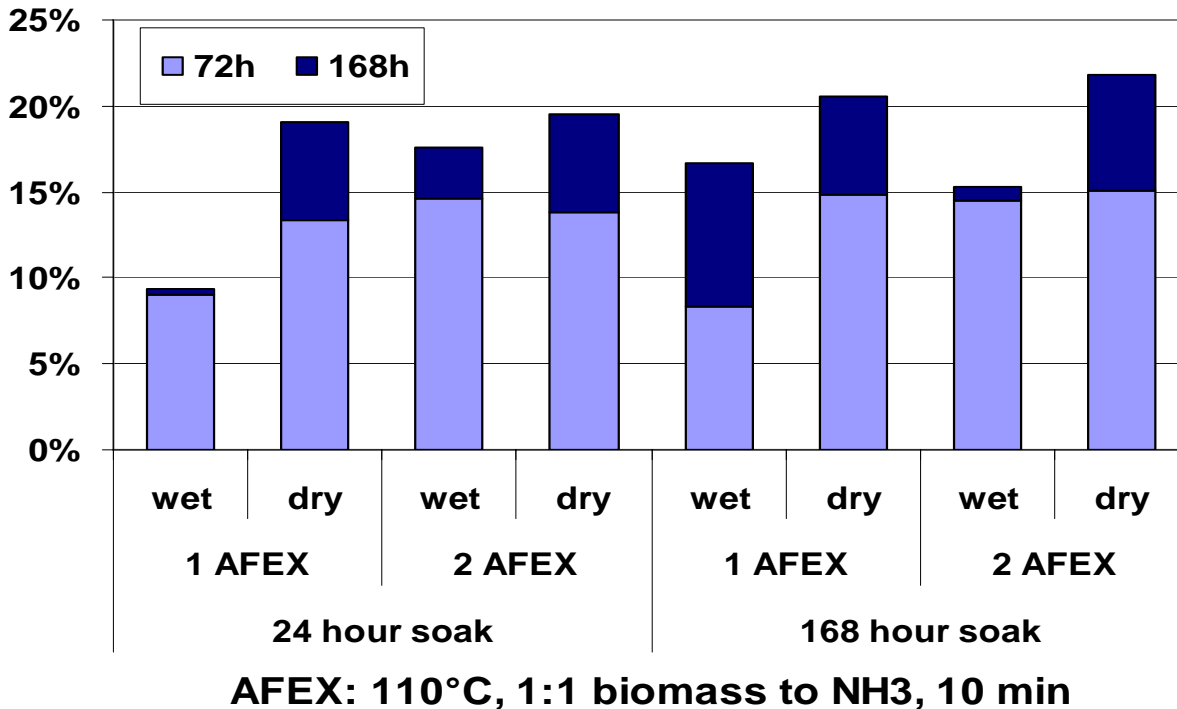
AFEX:1:1.2 biomass to NH₃, 5 min.

Water soak - Xylan conversion



AFEX: 1:1.2 biomass to NH₃, 10 min.

Water/Ammonium Hydroxide Soak- Glucan Conversion



Wet: Sample used immediately after AFEX, adjusted the pH to 4.8 by 1N HCl, followed by hydrolysis

Dry: Sampled dried in hood after AFEX overnight, adjusted the pH to 4.8 by 1N HCl, followed by hydrolysis

1AFEX: Doing AFEX reaction once

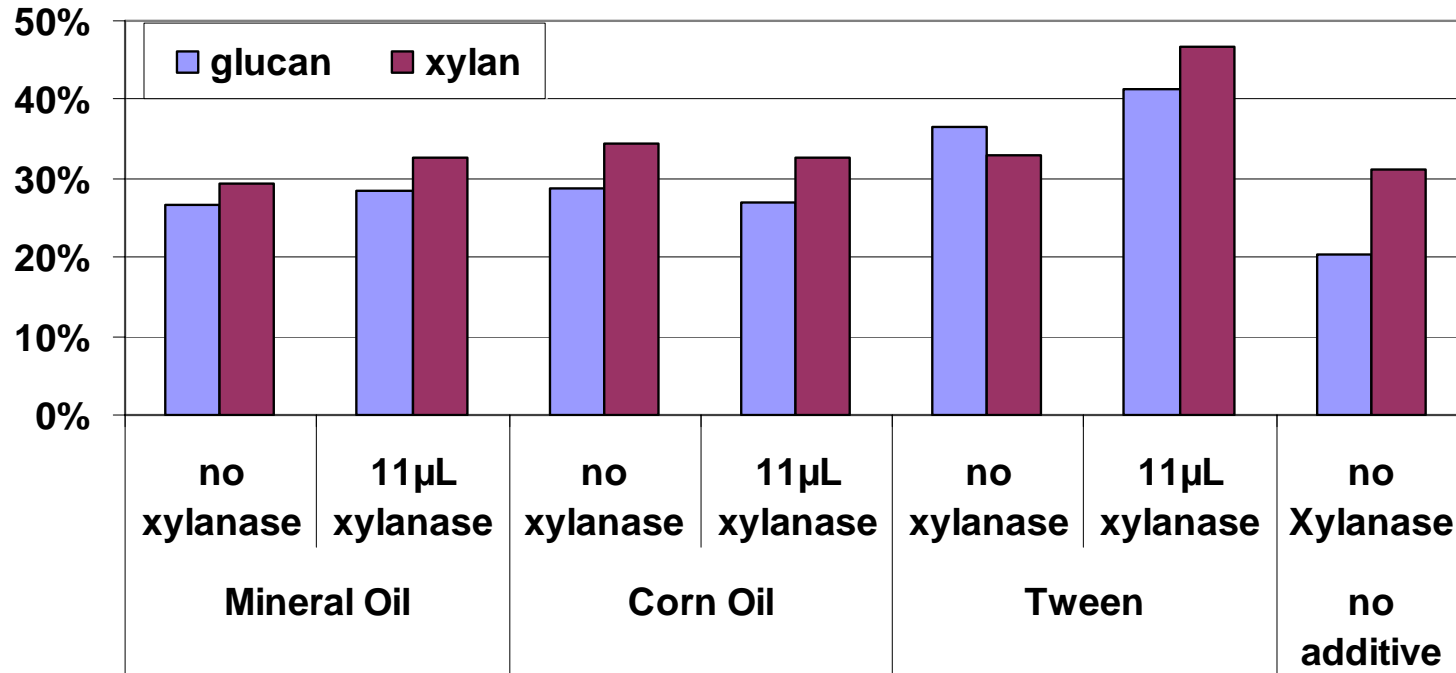
2AFEX: Doing AFEX reaction twice (consecutively)

- Drying the sample AFEX results in high glucan Conversion
- Multiple AFEX does not improve the conversions

Other Strategy:

- Adding xylanase to increase xylose conversion and in turn to help glucan conversion
- To look for additives which can increase both glucan and xylan conversions
- Effect of oil addition during enzyme hydrolysis

Effect of Xylanase and additive after 168 hours of Hydrolysis



AFEX: 24h water soak, 120°C, 1:1.2 biomass to ammonia

Surfactant (Tween 80) 0.15g/g of dry biomass basis

AFEX Conditions

Mild conditions

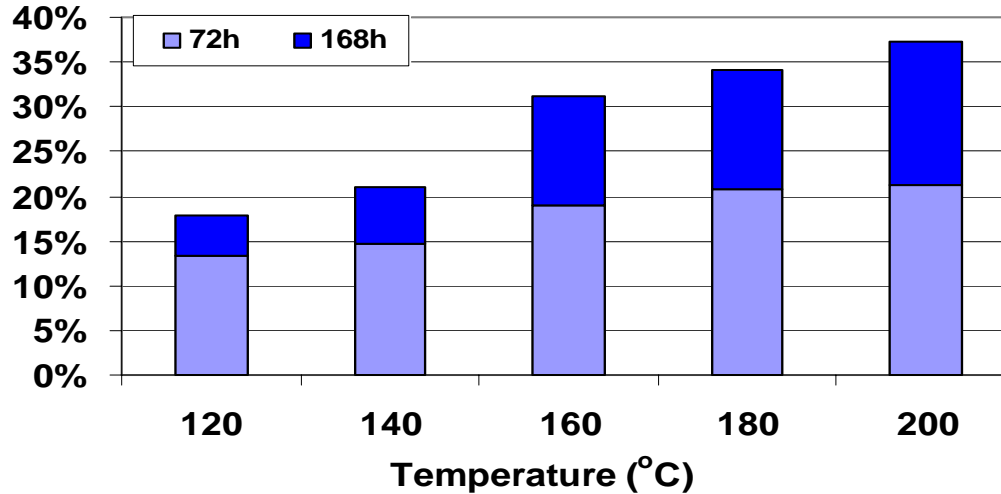
Upto 120 °C, 1.0 : 1.2 Biomass to ammonia loading
(pressure raise up to 300 psi)

Tough conditions

Upto 200 °C, 1.0 : 2.0 Biomass to ammonia loading
(pressure raise up to 600 psi)

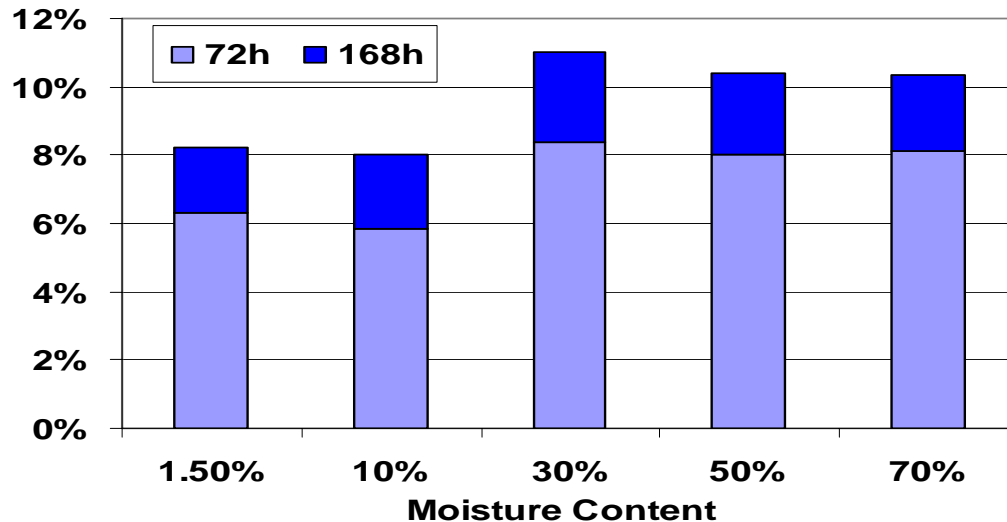
Temperature and moisture conditions during AFEX

Water soak - Glucan Conversion



High temperature
During AFEX
Improves the
pretreatment

AFEX: 1:1 biomass to NH₃, rinsed Oven Dried - Glucan Conversion

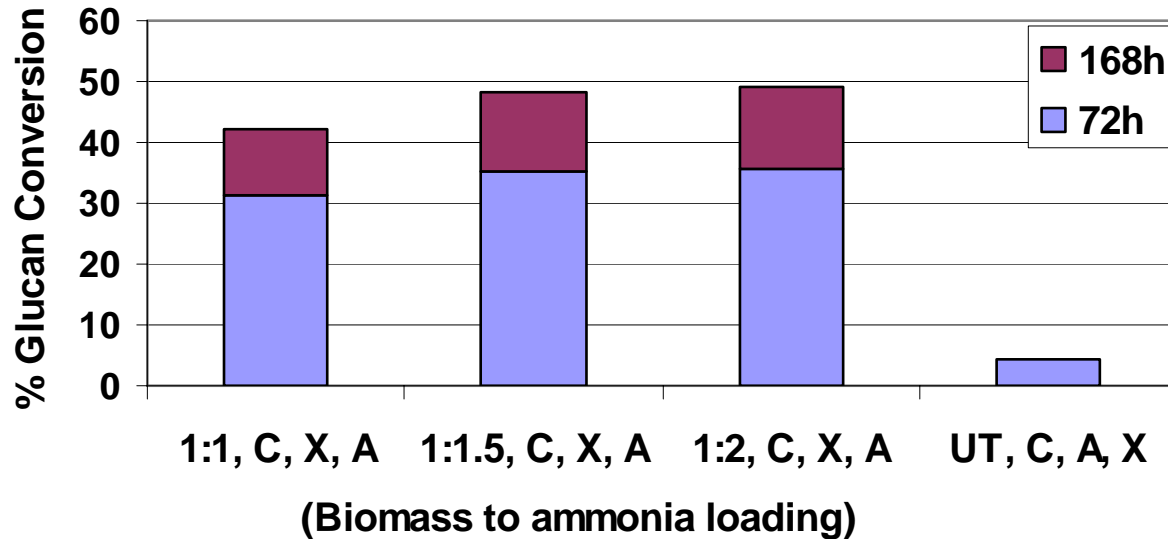


Moisture has no
Effect during
AFEX pretreatment

AFEX: 1:0.8 biomass to NH₃, 100 °C

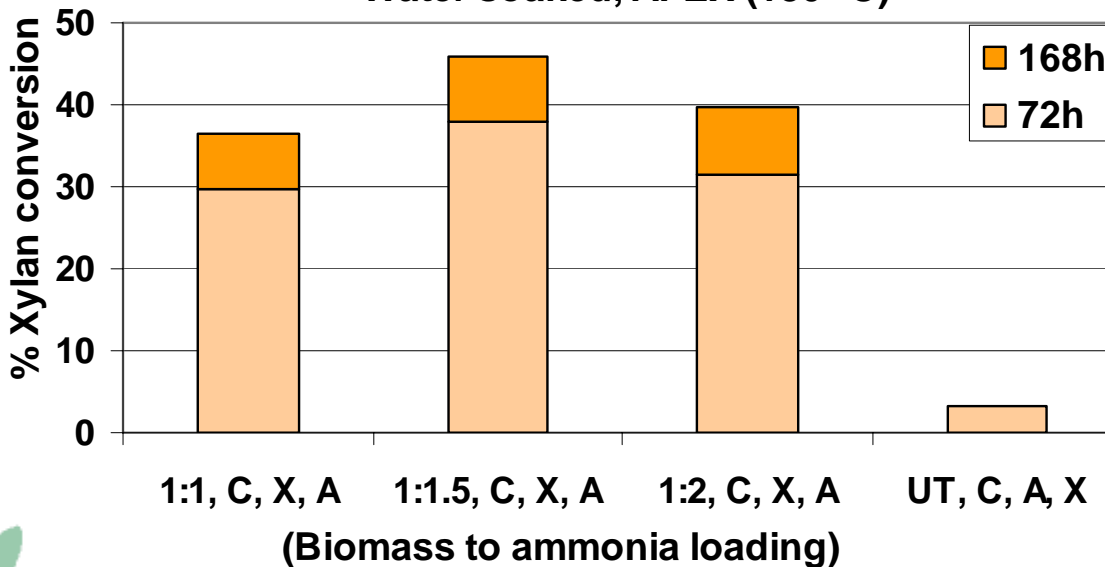
Effect of Ammonia loading

Water soaked, AFEX (180 °C)



Increasing ammonia Loading has some influence on pretreatment

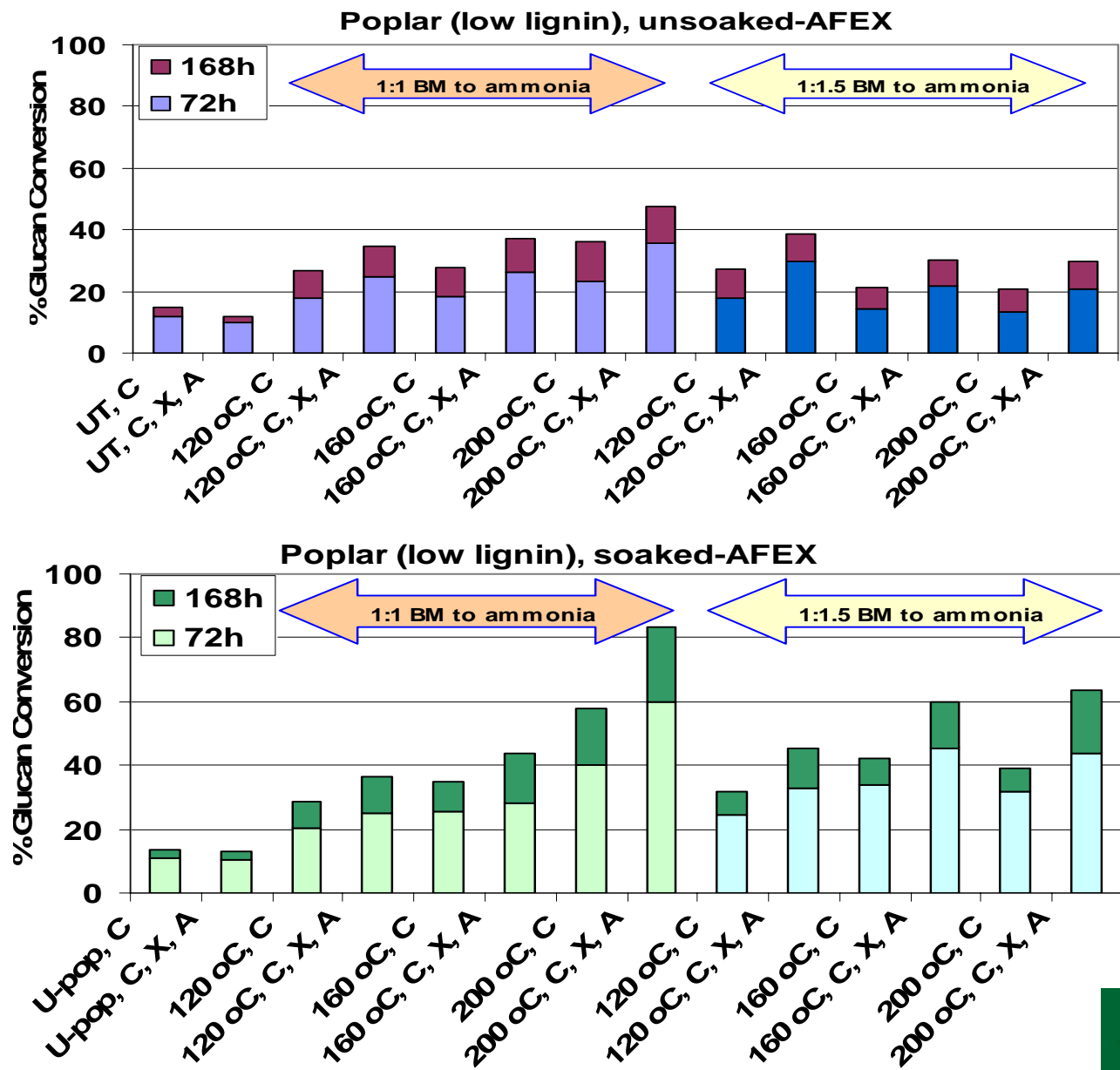
Water soaked, AFEX (180 °C)

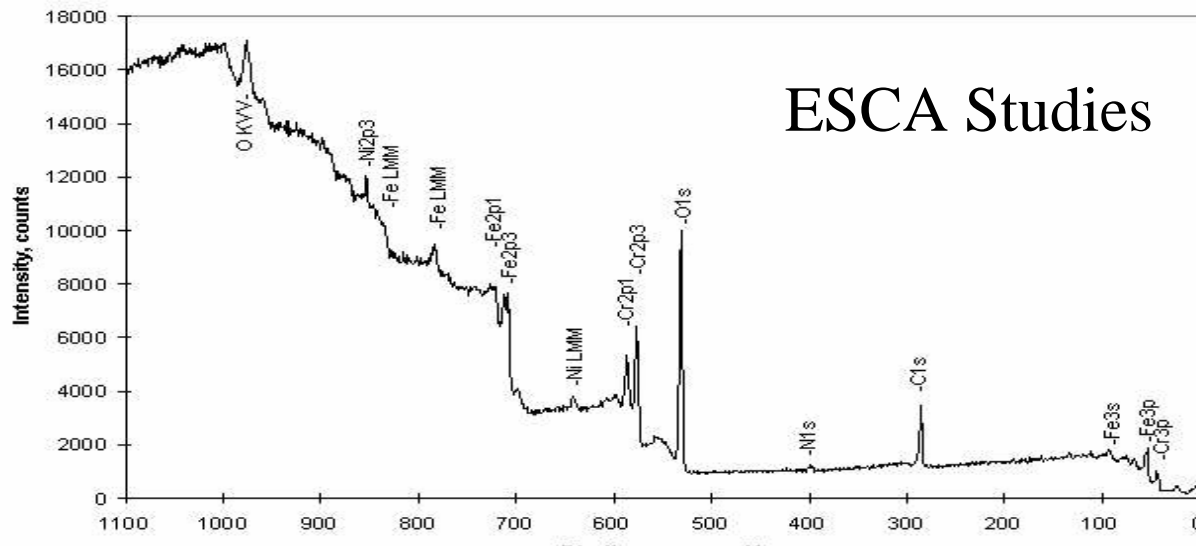


To Brief:

- Poplar I (high lignin) shows resistance to hydrolysis
- Soaking followed by AFEX does help to increase hydrolysis
- Varying moisture during AFEX does not influence hydrolysis but, increasing temperature during AFEX has some influence
- Increasing the ammonia loading shows some improvement in pretreatment
- Addition of Xylanase and additives like tween 80 along with cellulase does increase the hydrolysis

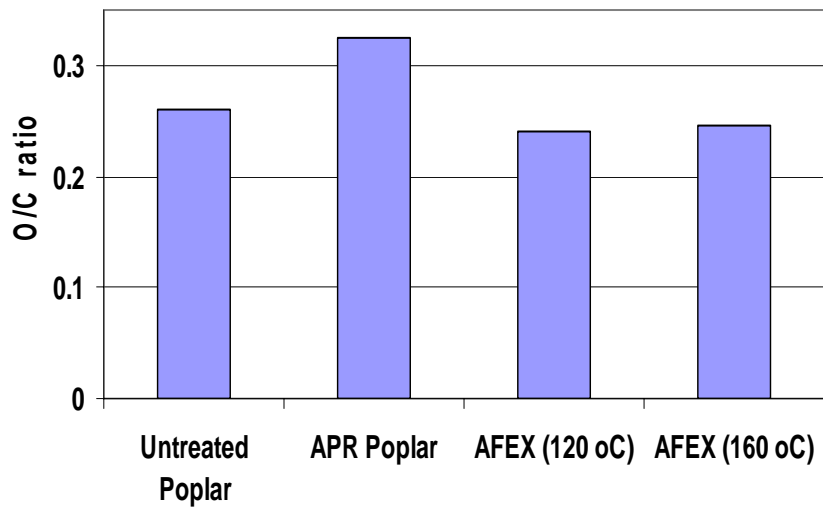
Some results on Poplar I (low lignin)



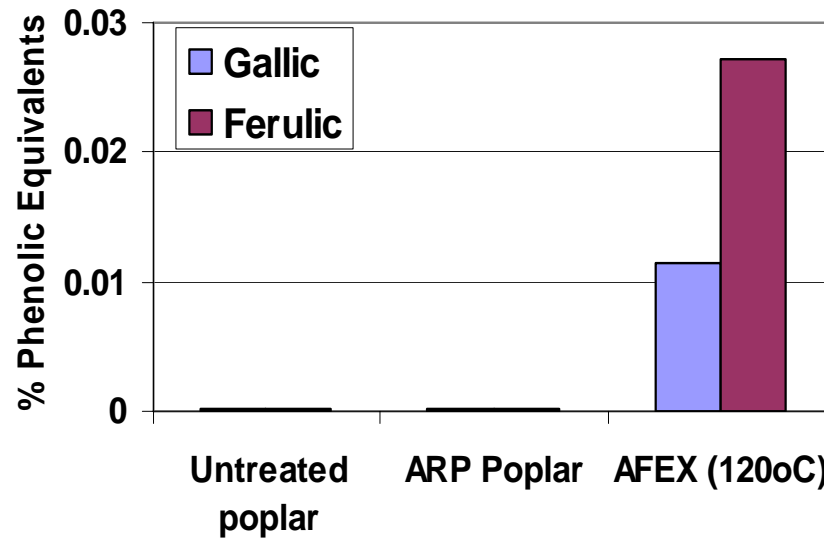


Higher the O/C ratio
 Lesser the lignin on
 the surface of poplar

ESCA Results on Poplar



Prussian Blue method



Conclusion:

Best AFEX conditions so for....

| | AFEX condition | Glucan conversion | Xylan conversion |
|------------------------------------|--------------------------------|-------------------|------------------|
| Poplar-II (High lignin) | 1:5, 200 °C, 50% MC | 50% | 44% |
| Poplar-I (Low Lignin) | 1:1, 200 °C, 50% MC | 83% | 57% |

Wash stream after AFEX and before hydrolysis – to be analyzed

Acknowledgement

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