Bio-ethanol Production from Corn Stover using Low Moisture Anhydrous Ammonia (LMAA) Pretreatment and SSCF

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Biorenewable Engineering Lab at ISU

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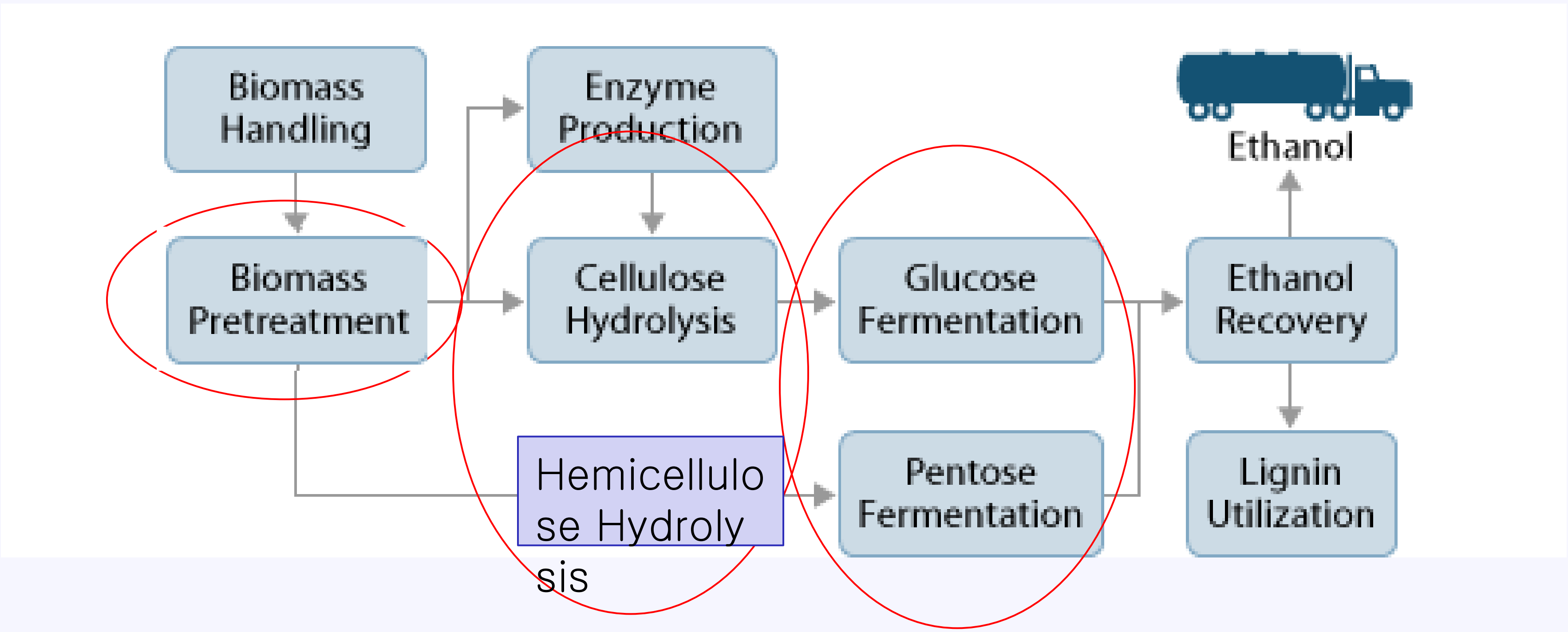
Schematic of a biochemical cellulosic ethanol production process



(Source: NREL web site, accessed in March 2011)

Pretreatment?

Pretreatment

Purpose:

- It is required for efficient enzymatic hydrolysis of biomass because of the physical and chemical barriers that inhibit the accessibility of enzyme to the cellulose substrate.
- Open up the rigid structure.
- Pretreatment is one of the key elements in the bioconversion of lignocellulosic materials.

Common Effects:

- Decrease of lignin, hemicellulose, and extraneous components.
- Increase of surface area, porosity, and pore size.
- Reduce the crystallinity of cellulose
- Enhance the accessibility of enzyme to the cellulosic substrate

(Source: Kim, T.H. Book Chapter: Pretreatment of lignocellulosic biomass, In-press, Book title- Bioprocessing Technology~, John Wiley&Sons)

Ideal biomass pretreatment

- **H**igh enzymatic hydrolysis rate and high yields of ethanol/butanol (products).
- **M**inimal degradation of the carbohydrate fractions.
- **N**o production of compounds that are inhibitory to microorganisms used in the fermentation step.
- **I**nexpensive materials of construction.
- **M**ild process conditions to reduce the capital costs.
- **R**ecycle of chemicals to reduce the operating costs.
- **M**inimal wastes.

(Source: Drapcho, **Nghiem**, Walker, 2008. Biofuels Engineering Process Technology, McGraw Hill)

Problems of conventional pretreatment methods

- High liquid input: high energy cost
  - High chemical loading
  - Washing step is required
- Can "**low-liquid pretreatment**" be possible?

- Pretreatment which uses no-additional water and low
- **Low moisture anhydrous ammonia (LMAA) pretreatment**

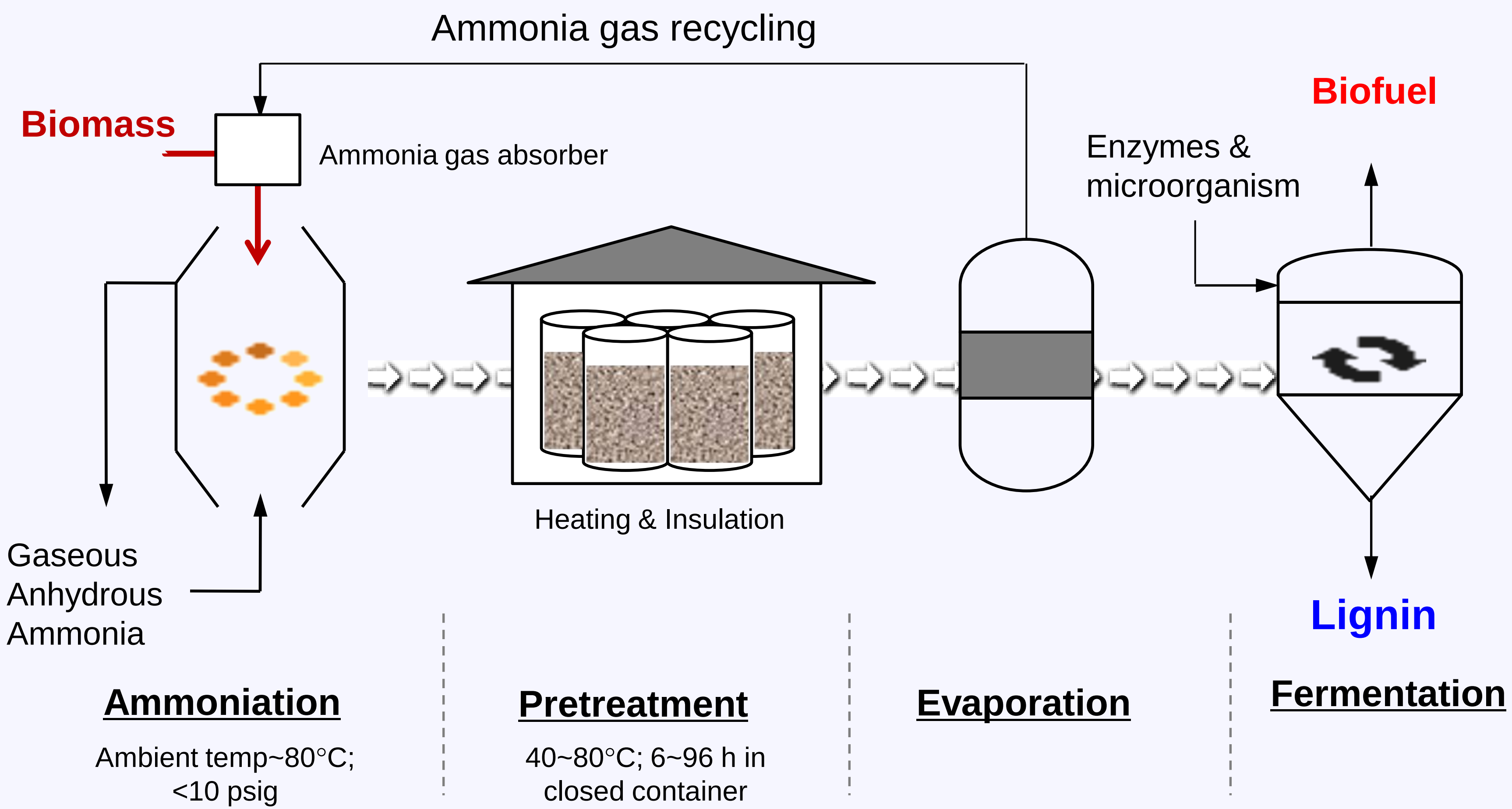
Chemical and water inputs in various pretreatment methods and their ethanol yields

Water input does not include washing water

Methods	Reaction conditions	Chemical input [g/g-biomass]	Water input[1] [g/g-biomass]	Max. theoretical EtOH yield & conc.[2] [%] ([g/l])
Dilute acid[3]	190 °C, 2.0 min, **2.6 g**-liquid/g biomass, 1.9 wt.% H_2SO_4	0.05	**2.5**	**?**
ARP[4]	170 °C, 10 min, **3.3 g**-liquid/g-solid, 15 wt.% NH_3	0.5	**2.8**	**?**
SAA[5].	60 °C, 12 h, **6.0 g**-liquid/g-solid, 15 wt.% NH_3	0.9	**5.1**	**?**
LMAA[6]	80 °C, 84-96 h, **1.1 g**-liquid/g biomass, 50-70% MC	0.1	**1.0~2.3**	**?**

<u>Note:</u> 1. **Water input does not include washing water**; 2. Maximum ethanol yield and concentration are based on the optimal reaction conditions; ethanol yields are calculated based on total glucan and xylan in untreated corn stover; 3. Dilute acid (Kazi et al., 2010, NREL Report); 4. ARP: Ammonia recycle percolation (Kim et al., 2006); 5. SAA: Soaking in aqueous ammonia (Kim and Lee, 2005; Kim and Lee, 2007); 6. LMAA: Low moisture anhydrous ammonia (Yoo et al., 2011)

Schematic of corn stover-to-ethanol conversion using LMAA and SSCF



LMAA process conditions

Substrate

Corn stover (central Iowa, 2009)

Ammoniation

30 – 70 % MC, 10 min, ~10 psi

Bench-scale reactor (2.85 in. ID×6.5 in. L, 690 ml of internal volume)

Pretreatment

20-140 °C, 24-144 h

Batch reactor (0.93 in. ID×6 in. L, 67 ml of internal volume)

Evaporation

Ambient condition, 12 h

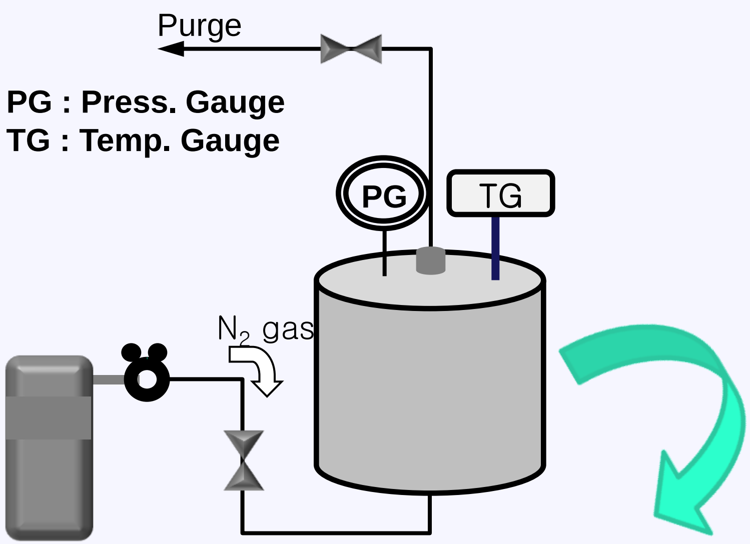
Ammonia as a pretreatment reagent

- Highly selective and effective in **delignifying** the biomass.
- Minimal interaction with cellulose and hemicellulose
- Prevention of fungal growth
- Easy to recover and reuse because of **high volatility**.
- One of the most widely used commodity chemicals (one-fourth the cost of sulfuric acid).
- **Non-polluting** and **non-corrosive** chemical.

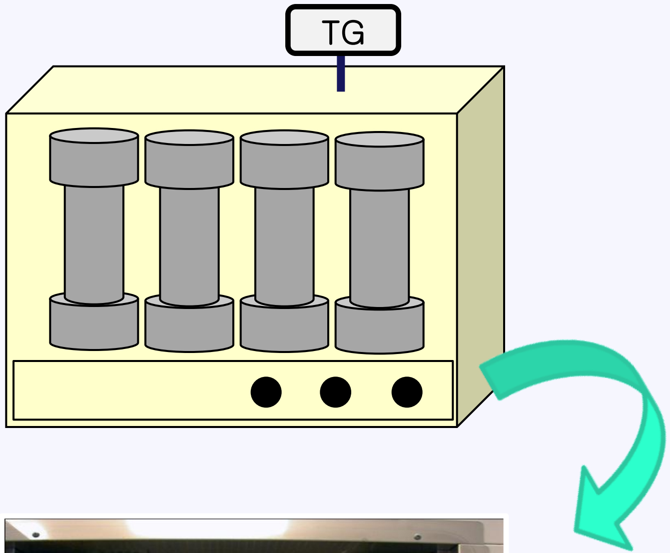
Lab-scale LMAA experiments setup

Ammoniation

PG : Press. Gauge
TG : Temp. Gauge

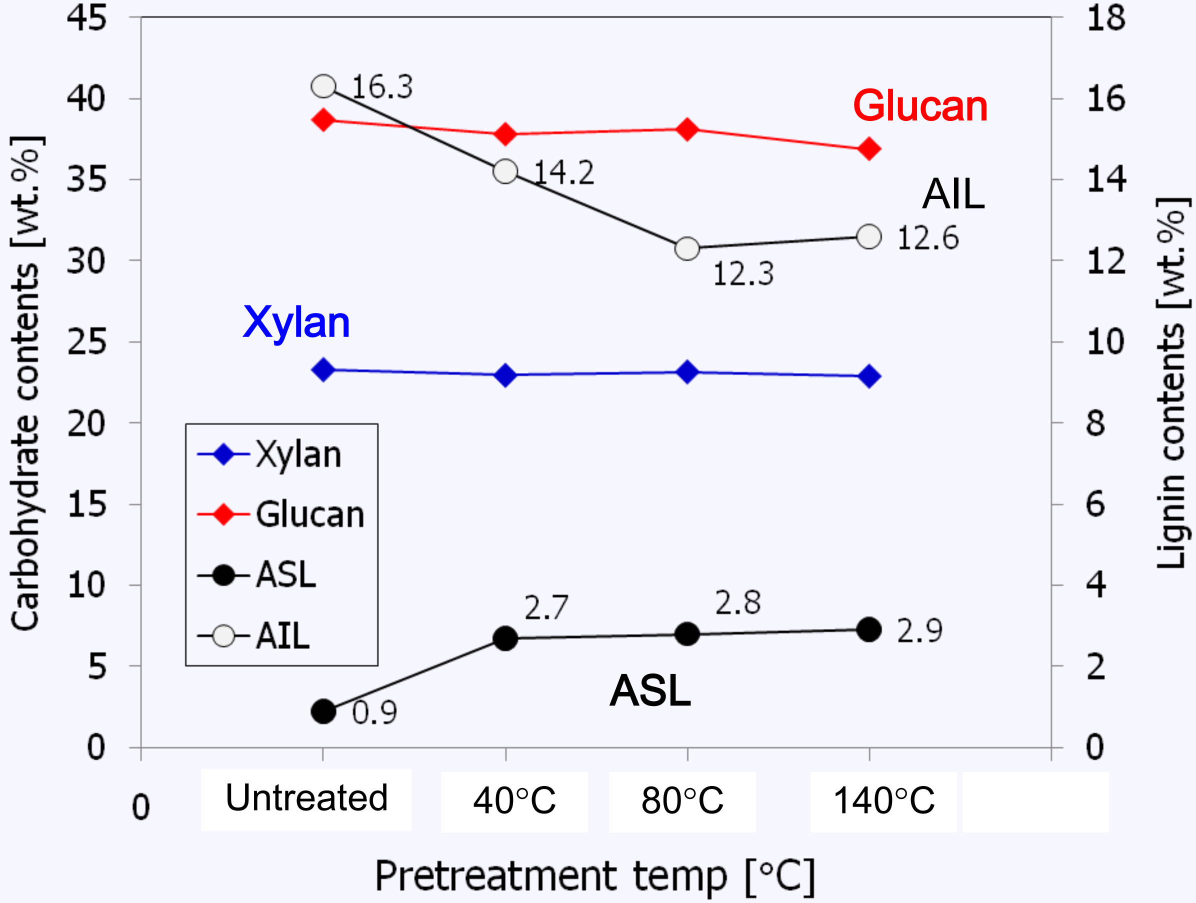


Pretreatment



1. Effects of LMAA Pretreatment on Ethanol Fermentation

Effects of LMAA pretreatment temperature on corn stover composition
(96 hour pretreated corn stover)



Note. Conditions: Anhydrous Ammonia treatment; reaction conditions: 70% MC, 40 – 140 ˚ C, 96 hours; All numbers are based on dried untreated corn stover
ASL: Acid soluble lignin / AIL: Acid insoluble lignin

SSCF test using "recombinant *E. coli* ATCC ® 55124 (KO11)"

Microorganism

Recombinant *Escherichia coli KO11 ATCC® 55124*

Medium

LB medium (0.5% of Yeast extract, 1% of Tryptone)

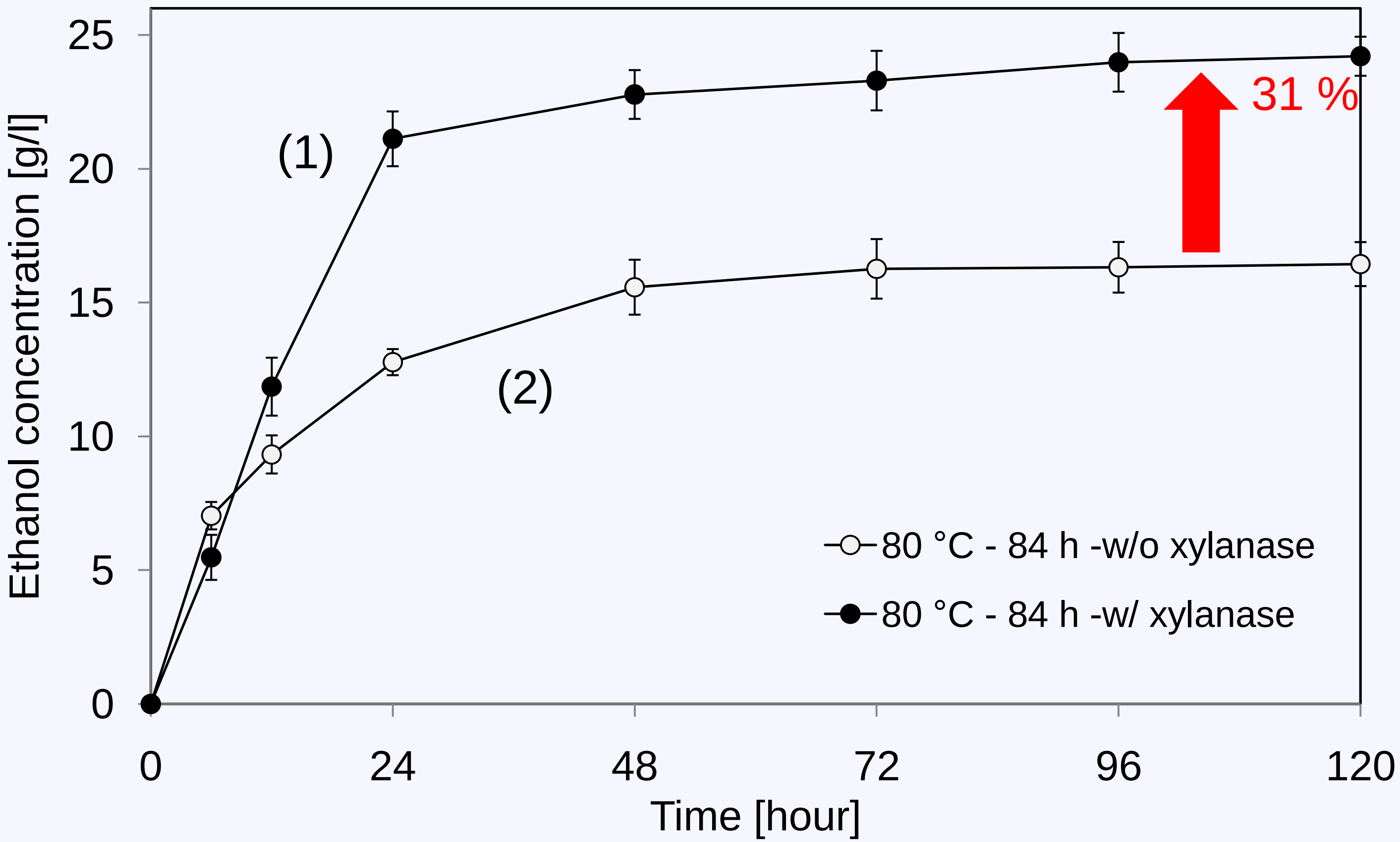
Substrate

LMAA pretreated corn stover (50-70 % MC, 40-120° C, 24-144 h)
3% w/v glucan loading

Enzyme loading

15 FPU of GC220 + 30 CBU of Novozyme188 + 1,000 GXU of Multifect xylanase (5 mg protein) per g-glucan

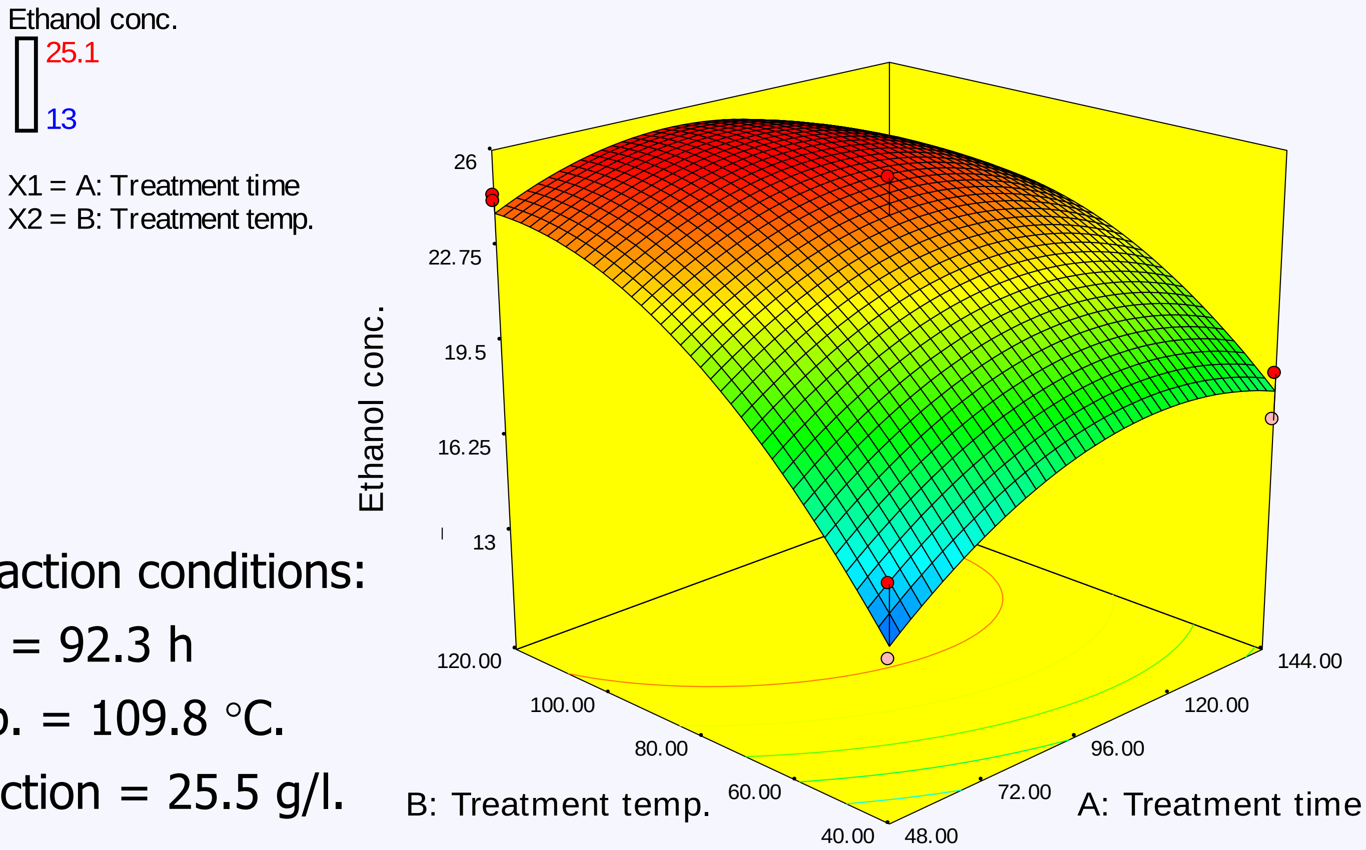
Effects of xylanase on SSCF of LMAA-treated solid



Note. Conditions: (1) Anhydrous Ammonia treatment; reaction conditions: 50% MC, 80˚C, 84 hours; (2) Anhydrous Ammonia treatment; reaction conditions: 70% MC, 80˚C, 84 hours ; SSF/SSCF condition: 3% glucan loading; 15 FPU GC220, 30 CBU Novo188; *E.Coli* KO11, 37˚C, 150 rpm, pH 7.0 (1) with Multifect xylanase 5mg protein/g-glucan; (2) without xylanase

Effects of pretreatment conditions on ethanol fermentation

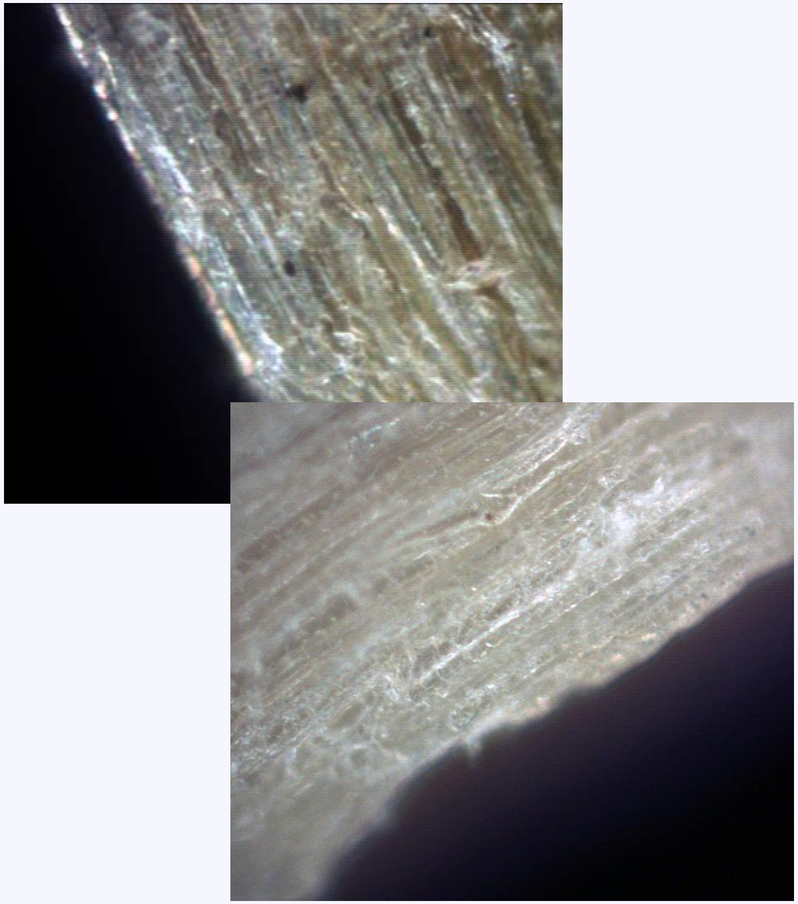
Ethanol conc. = -6.92969 +0.24235 * Treatment time +0.38824 * Treatment temp. -6.38021E-004 * Treatment time * Treatment temp. -9.41277E-004 * Treatment time^2 -1.49433E-003 * Treatment temp.^{2}



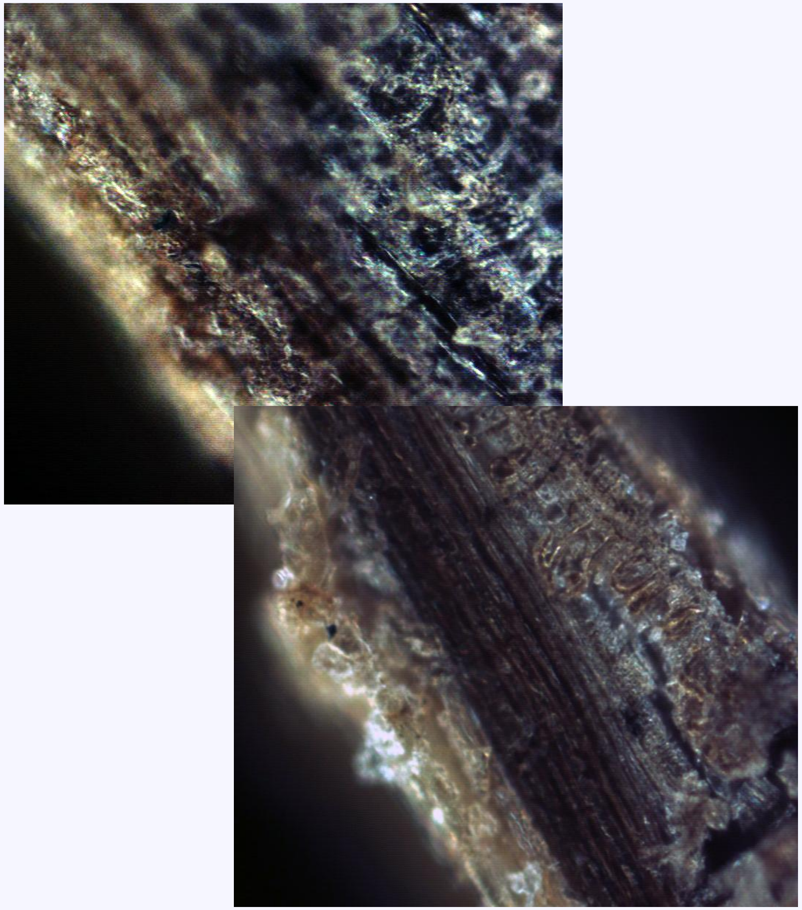
The optimum reaction conditions:

- Reaction time = 92.3 h
- Reaction temp. = 109.8 °C.
- ethanol production = 25.5 g/l.

Images of biomass structure



Untreated Corn Stover
(20X objectives with dark-field)

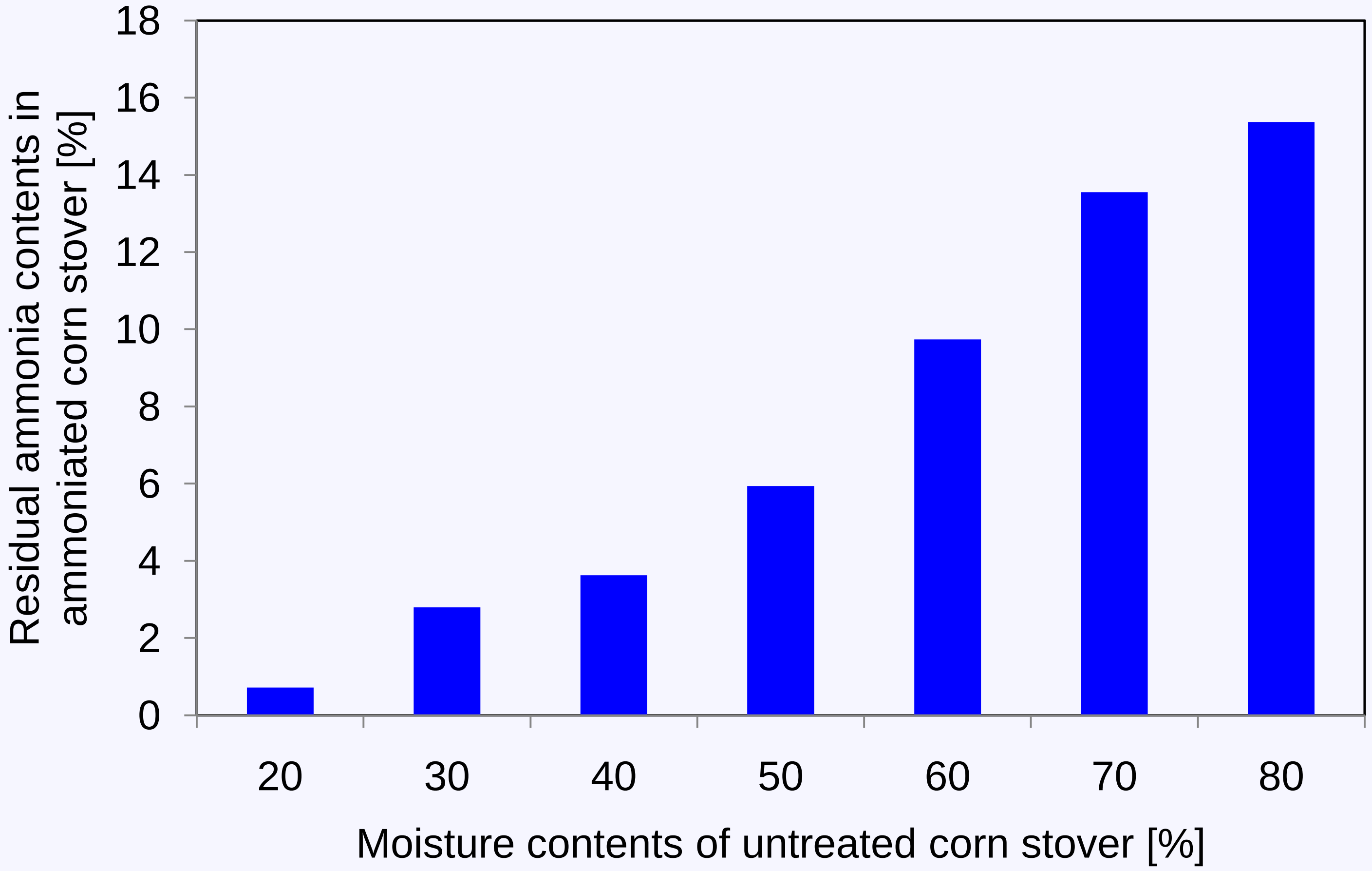


LMAA treated Corn Stover
(20X objectives with dark-field)

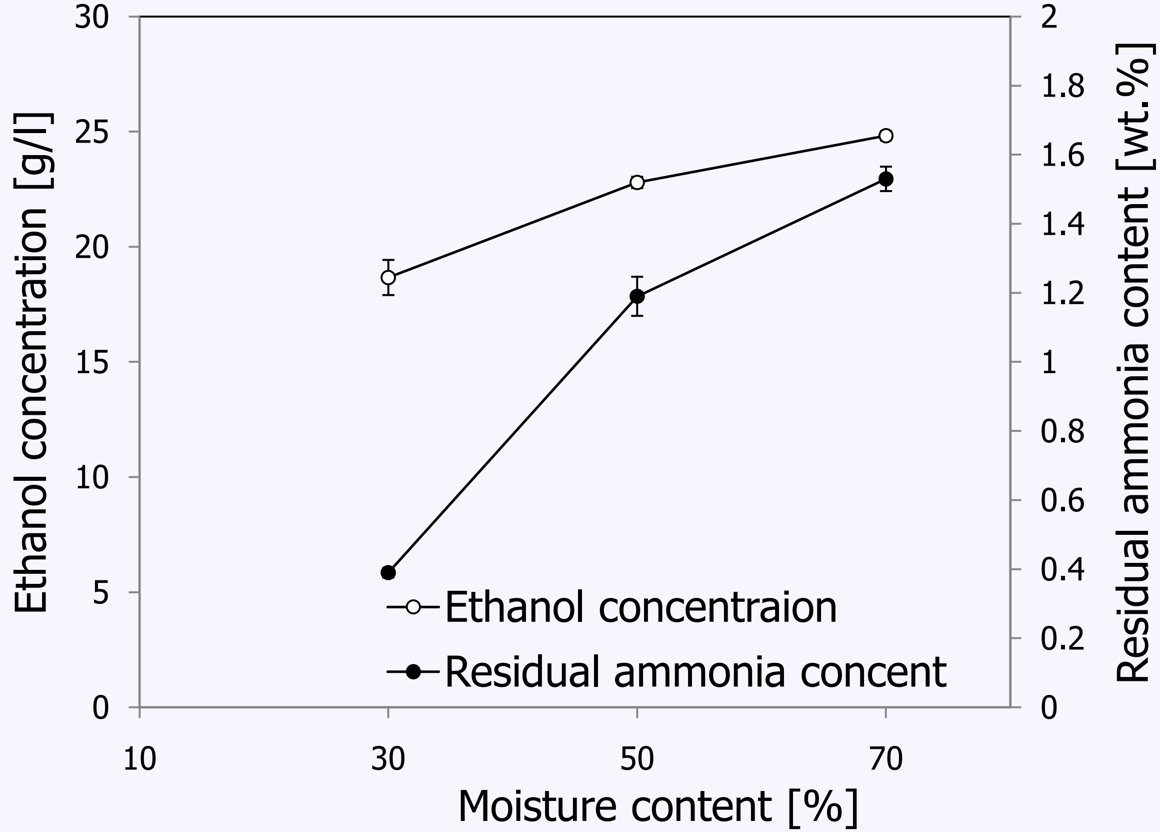
Note. Conditions: Anhydrous Ammonia treatment; reaction conditions: 70% MC, 110° C, 92 hours
Images are taken by DXR Raman Microscope (Thermo scientific, IA, USA)

2. Effect of Residual Ammonia Content on Ethanol Fermentation

Moisture contents vs. Residual ammonia in the Ammoniated Corn Stover

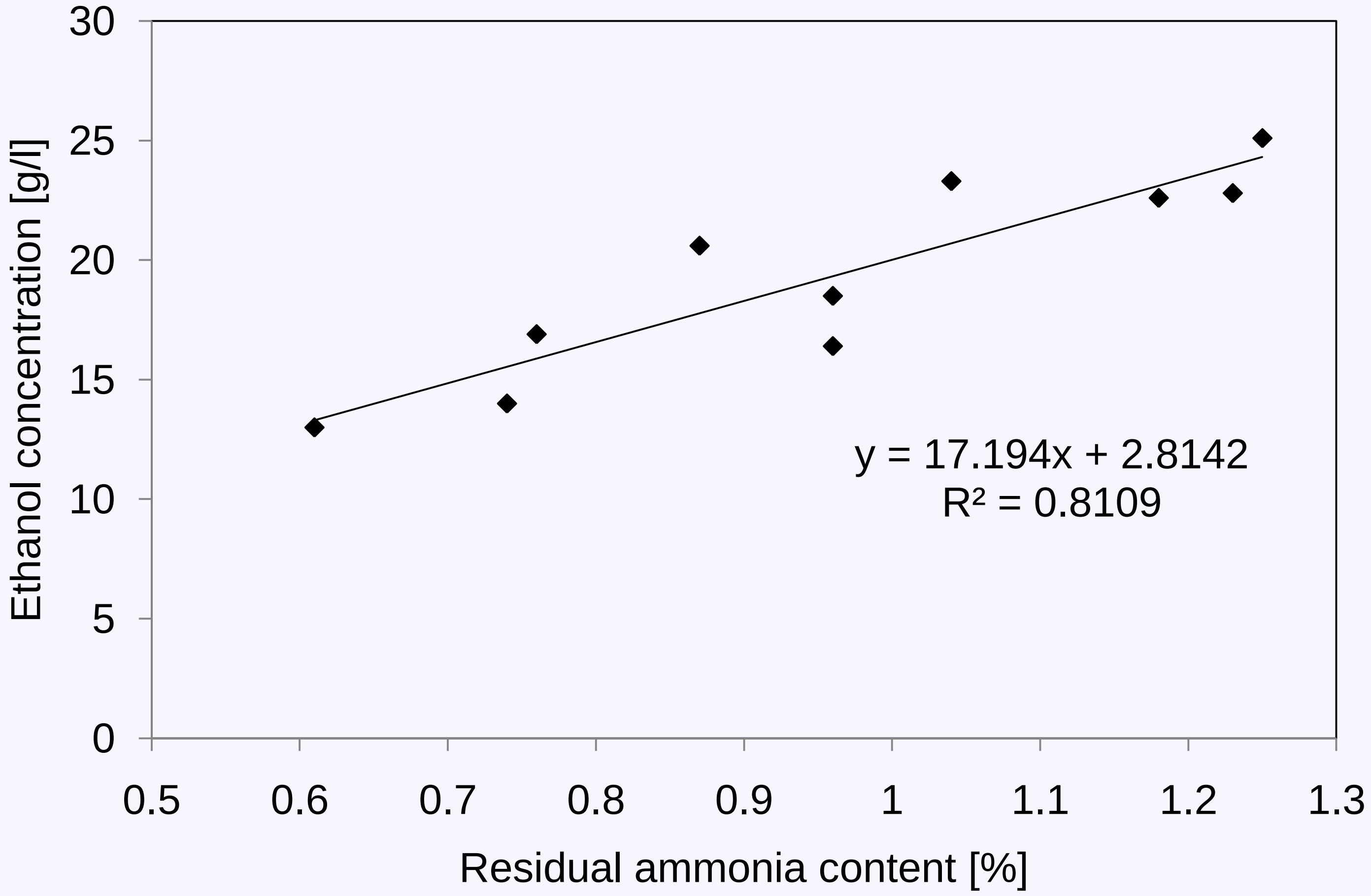


Effect of moisture content in untreated C.S. on 120-h fermentation and residual ammonia content of LMAA-treated biomass

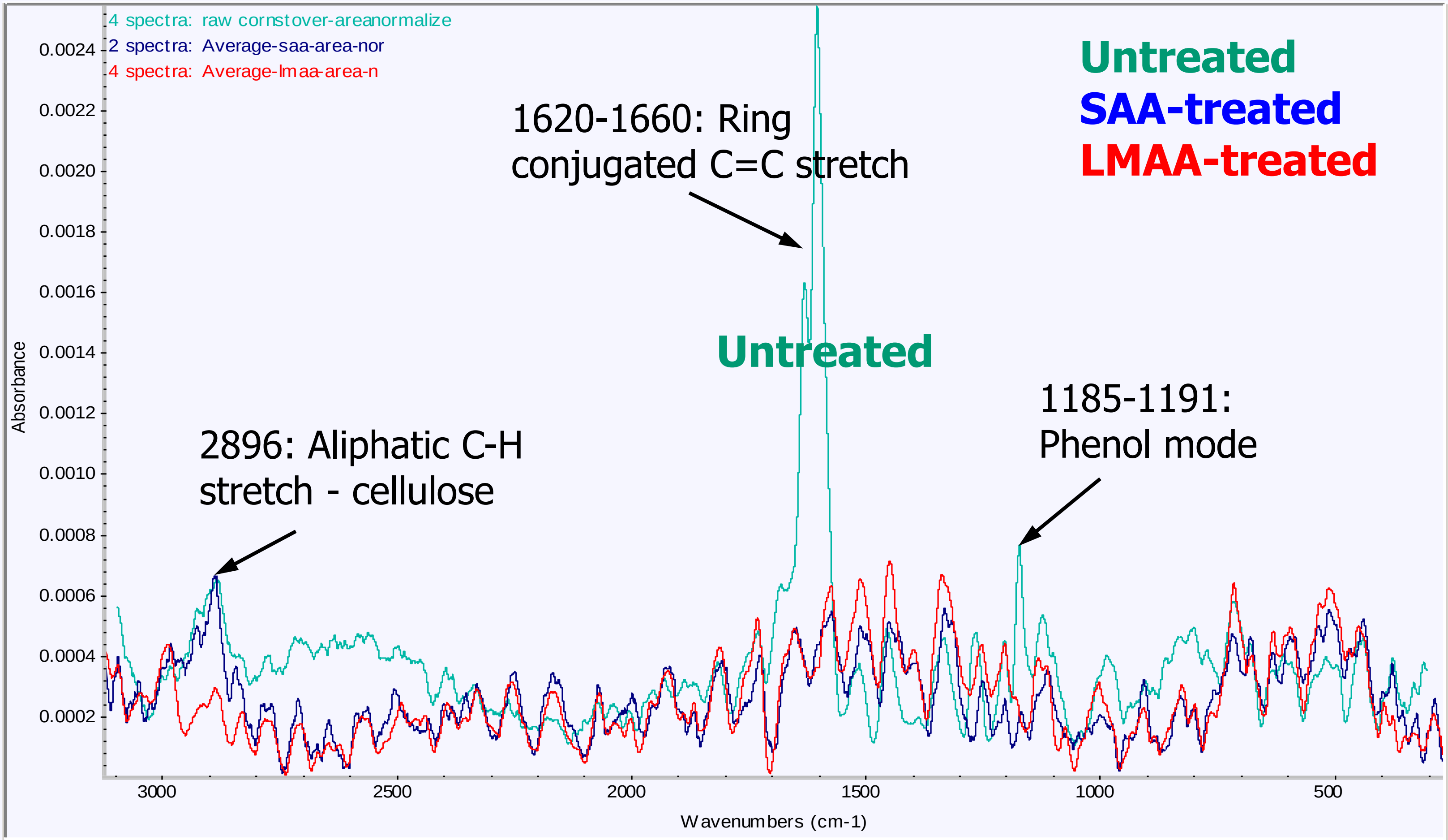


Note: Pretreatment condition: 30 - 70 % of MC, 110 °C, 92 h, SSCF condition: 3 % w/v glucan loading/50 ml working volume; recombinant *Escherichia. coli* KO11 (ATCC® 55124); 15 FPU of GC 220/g-glucan, 30 CBU of Novozyme 188/g-glucan, 1,000 GXU of Multifect xylanase /g-glucan; LB medium; anaerobic condition; 37°C, 150 rpm. Ammonia content is based on dry biomass weight.

Effects of residual ammonia on ethanol fermentation



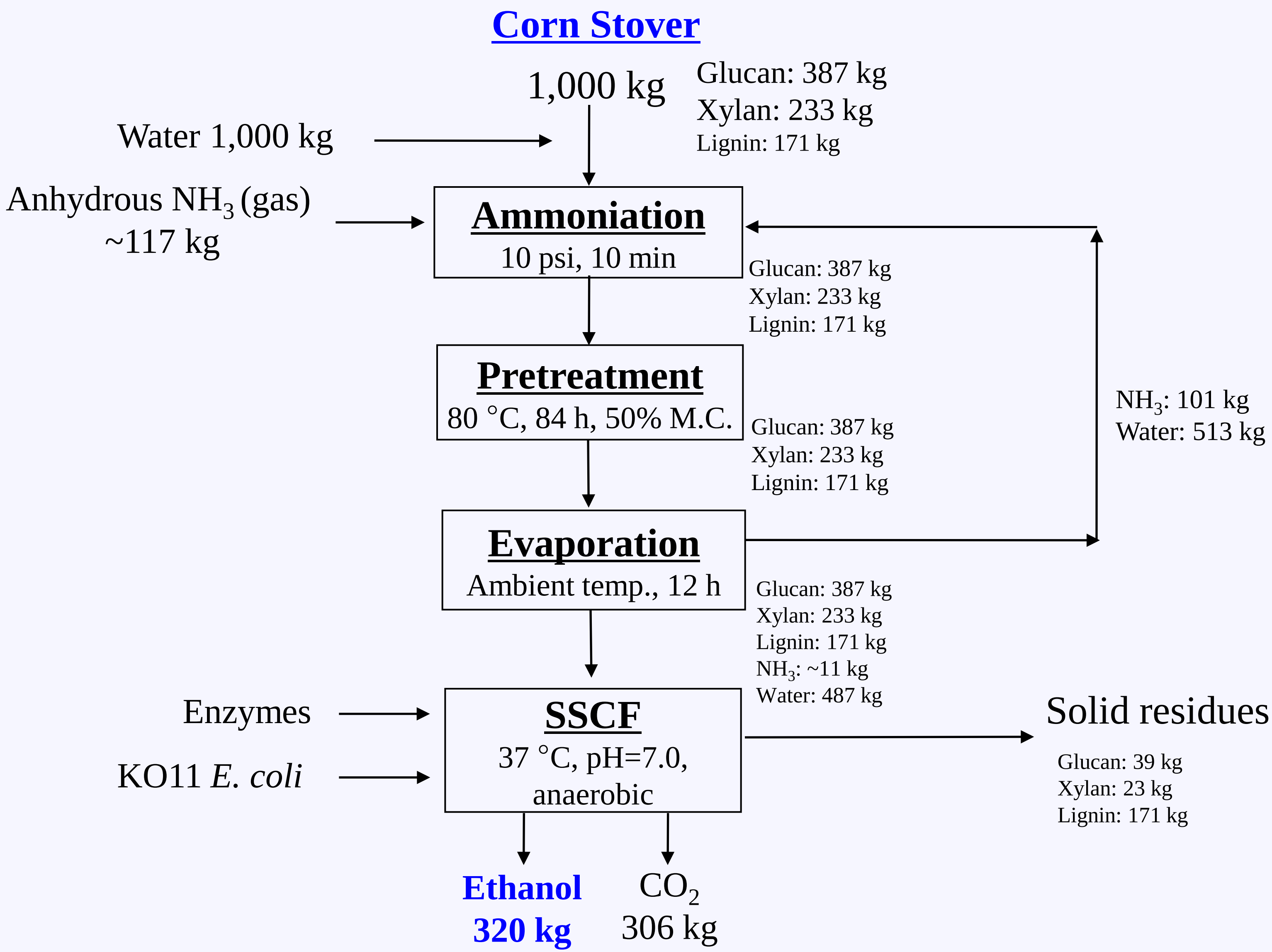
Raman spectroscopic image of the untreated, LMAA, and SAA pretreated corn stover



Note. Pretreatment Conditions: (1) Anhydrous Ammonia treatment; reaction conditions: 70% MC, 110˚ C, 92 hours; (2) Soaking in Aqueous Ammonia; reaction conditions: 15 wt.% aqueous ammonia, 60˚ C, 24 hours, solid : liquid=1:8

DXR Raman microscope (Thermo Scientific, Barrington, IL)

Overall mass balance of LMAA process
(50% moisture content case)



Chemical and water inputs in various pretreatment methods and their ethanol yields

Water input does not include washing water

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Dilute acid[3]	190 °C, 2.0 min, **2.6 g-liquid/g biomass**, 1.9 wt.% H_2SO_4	0.05	2.5	**67** (18.5)
ARP[4]	170 °C, 10 min, 3.3 g-liquid/g-solid, 15 wt.% NH_3	0.5	2.8	71 (19.4)
SAA[5].	60 °C, 12 h, **6.0 g-liquid/g-solid**, 15 wt.% NH_3	0.9	5.1	**70** (19.2)
LMAA[6]	80 °C, 84-96 h, **1.1 g-liquid/g biomass**, 50-70% MC	0.1	1.0	89 (24.9)

Note: 1. **Water input does not include washing water**; 2. Maximum ethanol yield and concentration are based on the optimal reaction conditions; ethanol yields are calculated based on total glucan and xylan in untreated corn stover; 3. Dilute acid (Kazi et al., 2010, NREL Report); 4. ARP: Ammonia recycle percolation (Kim et al., 2006); 5. SAA: Soaking in aqueous ammonia (Kim and Lee, 2005; Kim and Lee, 2007); 6. LMAA: Low moisture anhydrous ammonia (Yoo et al., 2011)

"Very rough" Estimated ethanol production cost (2,000 ton corn stover/day basis)

	Unit	Dilute Acid	SAA	LMAA
Estimated EtOH Cost	**[$/gal EtOH]**	**3.39**	**3.31**	**2.54**
Corn stover Input	[ton/day]	2,000	2,000	2,000
EtOH production	[MMGal/year]	73	66	81
Operating cost	**[$/gal EtOH]**	**2.78**	**2.68**	**2.07**
- Pretreatment	[$/year]	34,870,659	15,641,239	7,566,725
- Chemical & Water recovery + WWT	[$/year]	13,347,286	14,252,993	2,592,294
Capital cost	**[$/gal EtOH]**	**0.61**	**0.63**	**0.47**
- Pretreatment	[$/year]	36,200,000	17,244,807	3,975,780
- Chemical & Water recovery + WWT	[$/year]	3,500,000	10,137,892*	10,137,892*

* same numbers are applied.

(by C.G. Yoo and T.H. Kim, 2011)

Conclusions

- **Ammonia** and **water inputs** were significantly reduced using LMAA pretreatment (~ 1:1 of biomass : liquid).
- LMAA pretreatment process resulted in high fermentation yield (**89-**91% theoretical maximum ethanol yield based on glucan + xylan in corn stover) with enzymes (15 FPU + 30 CBU + 1,000 GXU/g-glucan) and *E. coli* KO11 strain.
- There is a strong relationship between residual ammonia contents and ethanol fermentation yield - Ammoniation can supply assimilable nitrogen for microbial growth in the fermentor.
- **Moisture content, residual ammonia content, pretreatment temperature, and pretreatment time** are important factors affecting ethanol fermentation.

Acknowledgements

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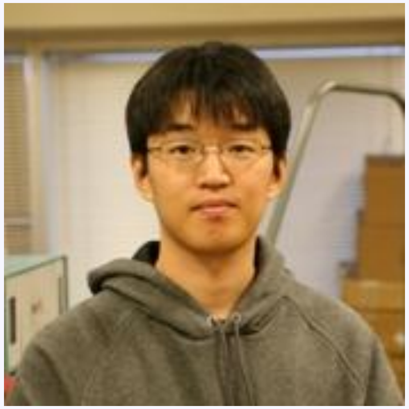

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Pretreatment of corn stover using low-moisture anhydrous ammonia (LMAA) process

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Thank you!


CORN STOVER


RICE STRAW


WOOD CHIP


CORN STOVER


BAGASSE


SAWDUST