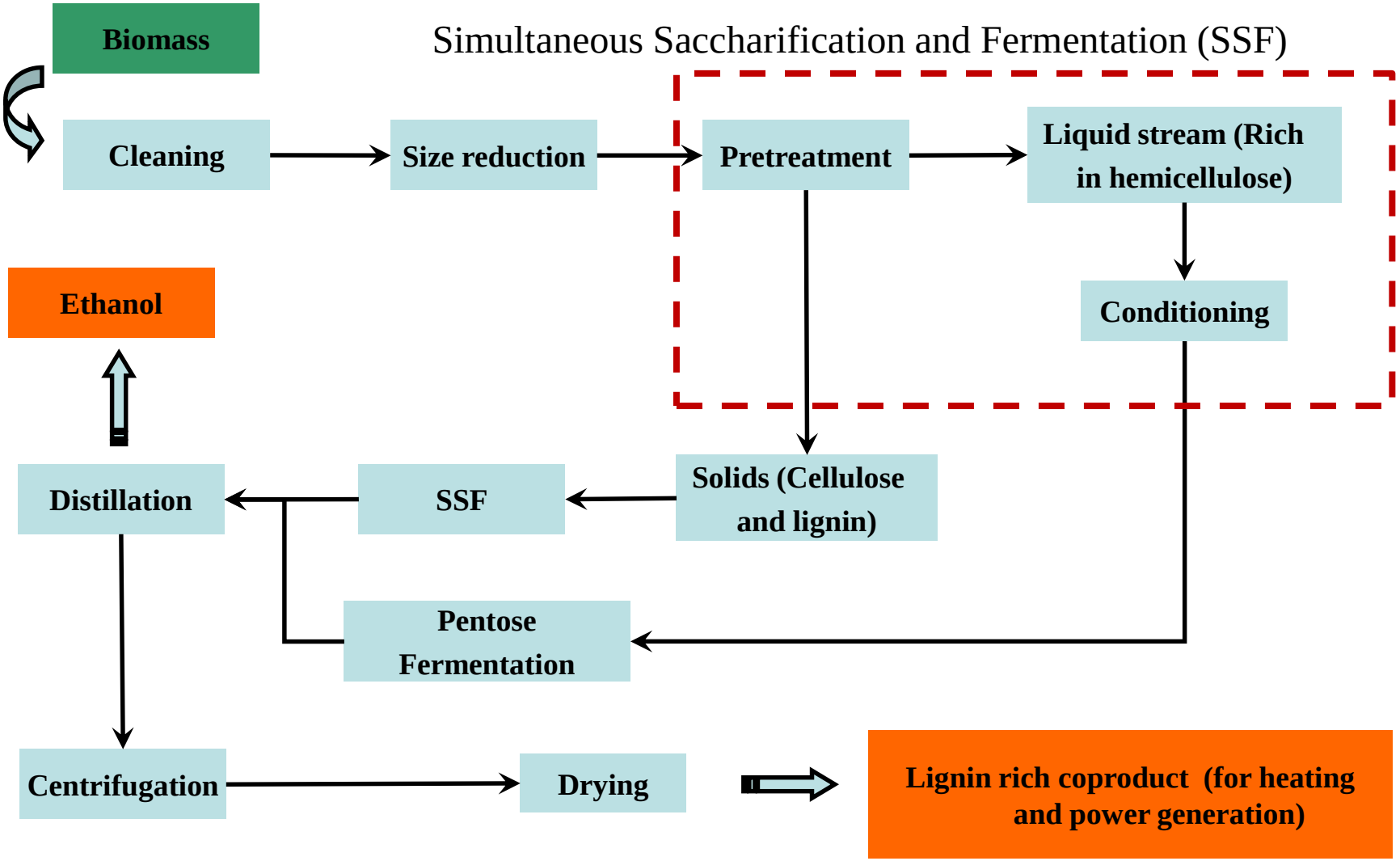
# Topic Seven

# Cellulosic Ethanol Production

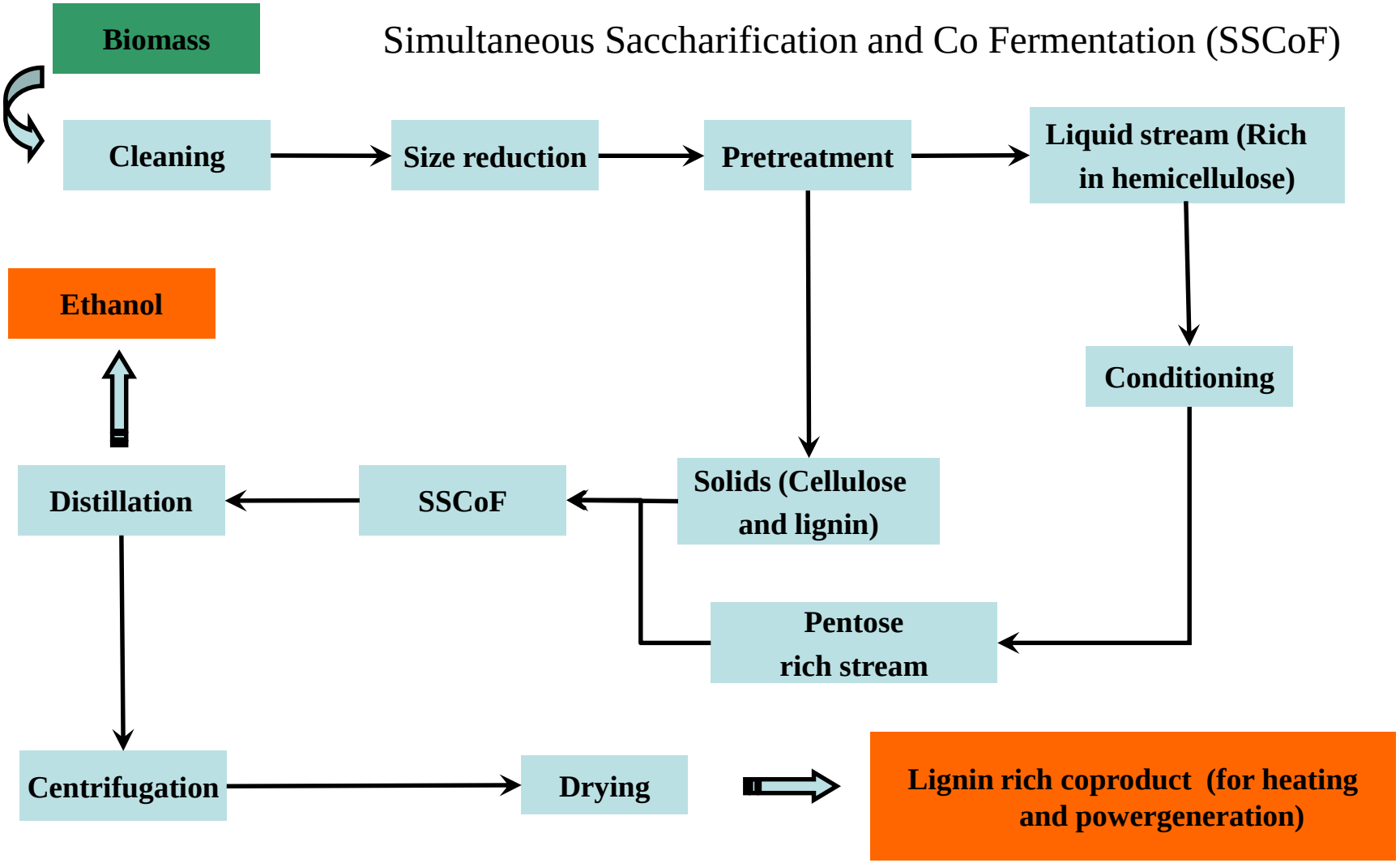
# Lecture Fourteen

# Cellulosic Ethanol Production: Pretreatment

# Generic Cellulosic Ethanol Process

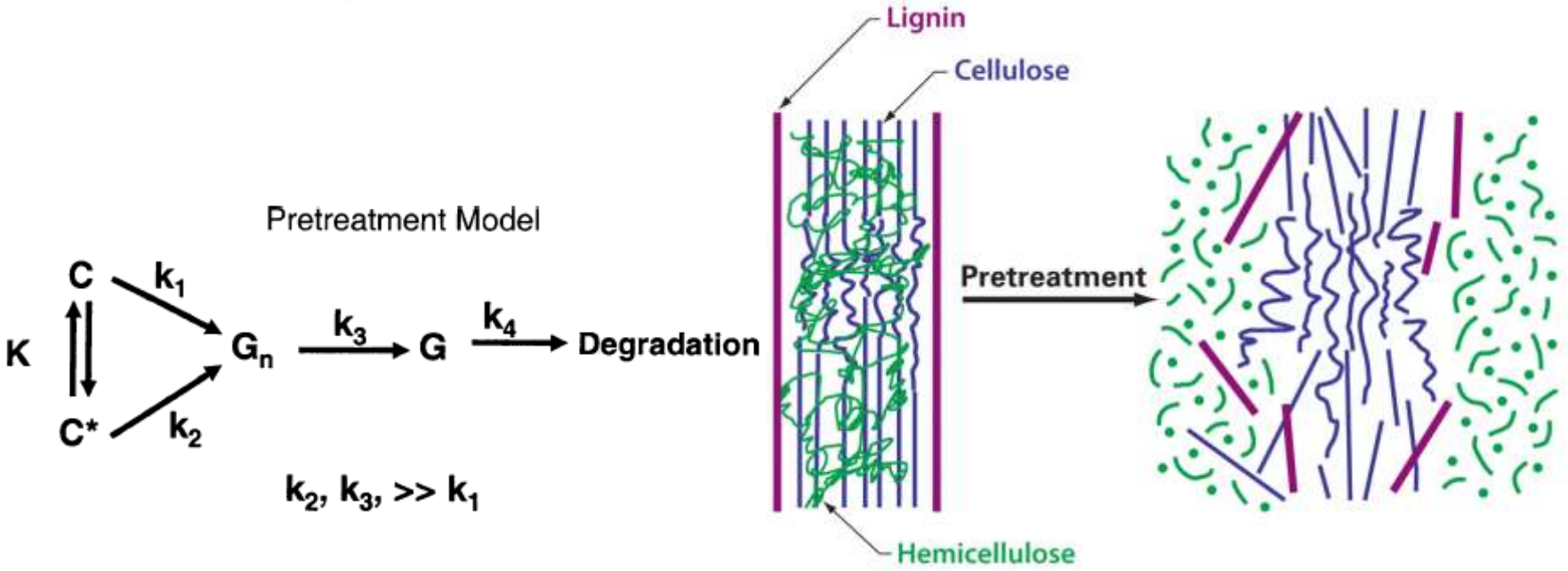


# Generic Cellulosic Ethanol Process



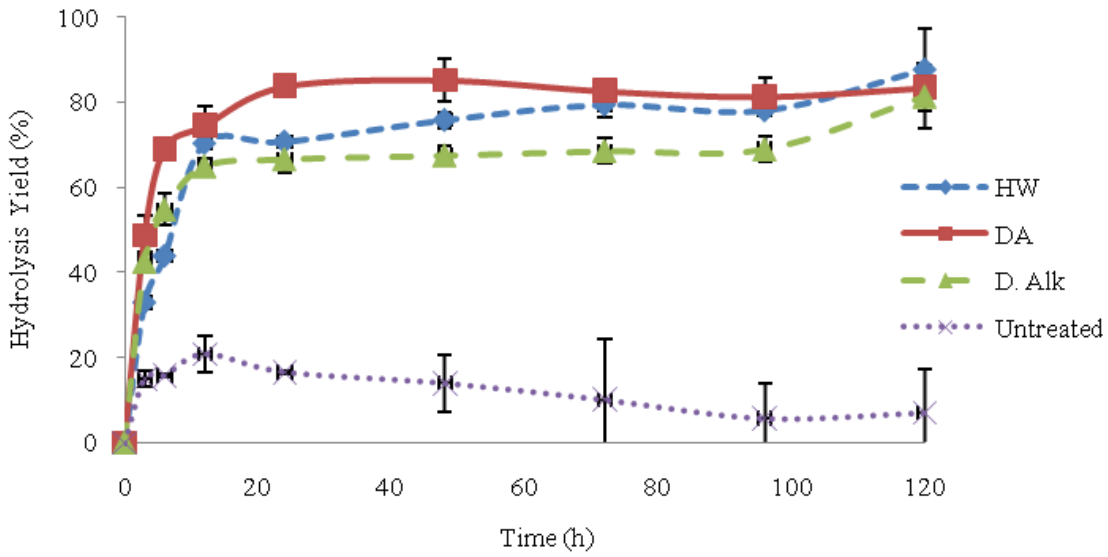
# Pretreatment

Primary goal of any pretreatment process is to facilitate enzyme action by making the substrate available. Pretreatment processes are critically dependent on type of feedstock




Ref: Sanchez and Cardona (2008) Mosier et. al (2005). POSTED ON COURSE WEBSITE REQUIRED READING !

# Effect of Pretreatments on Biomass: Bent Grass

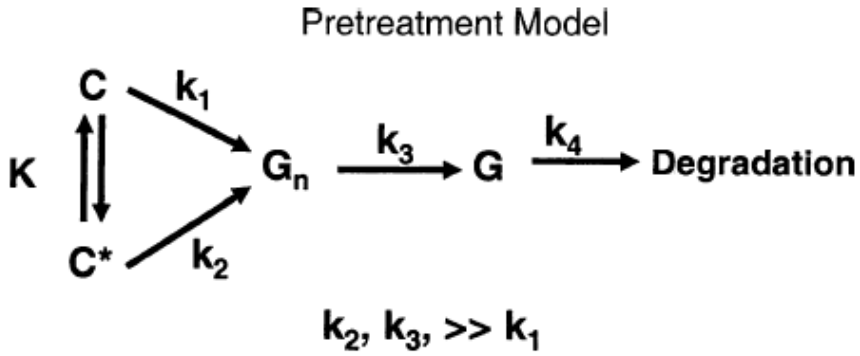



Hydrolysis yield with time for bentgrass (HW = hot water, DA = dilute acid, and D.Alk = dilute alkali).

# Pretreatment

Characteristics of an ideal pretreatment process are

- Removes the barriers to effective hydrolysis of cellulose
- Preserves sugars
- Does not result in degradation of sugars or formation of inhibitors.
- Does not depend on particle size
- Minimizes energy and resource consumption
- Does not produce any waste streams
- Cheap
- Safe




Ref: Mosier et. al (2005). POSTED ON COURSE WEBSITE REQUIRED READING !

# Pretreatment of Biomass

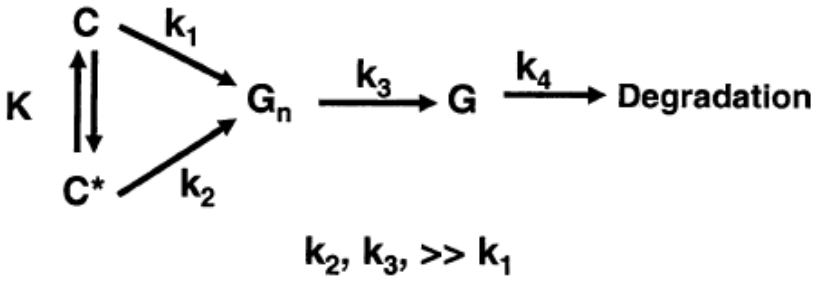
Physio-Chemical Methods

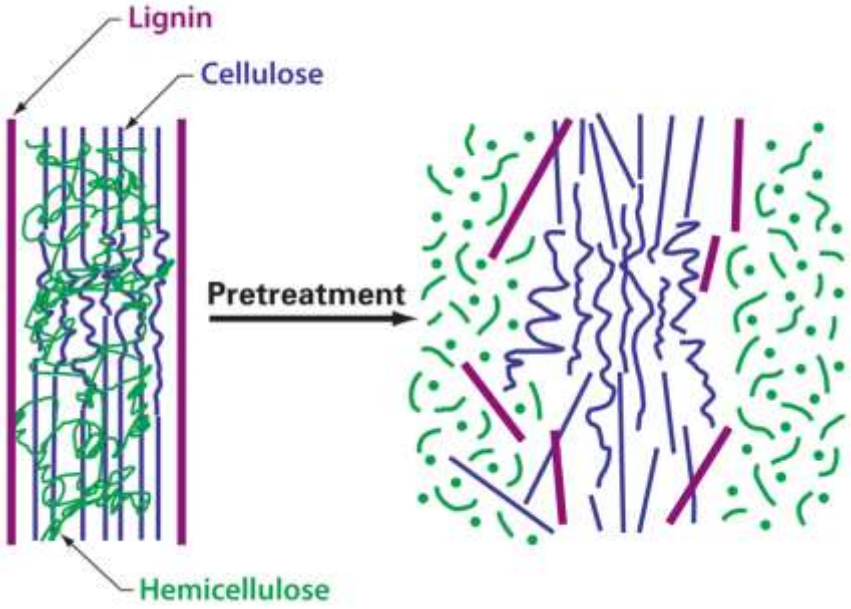
- *Steam Explosion*
- *Liquid Hot Water*
- $CO_2$ Explosion
- *Ammonia Fiber Expansion (AFEX)*
- Wet Oxidation
- Ionic Liquids

Chemical Methods

- *Acid Hydrolysis*
- *Alkali Hydrolysis*
- Ozonolysis
- *Organosolv Process*

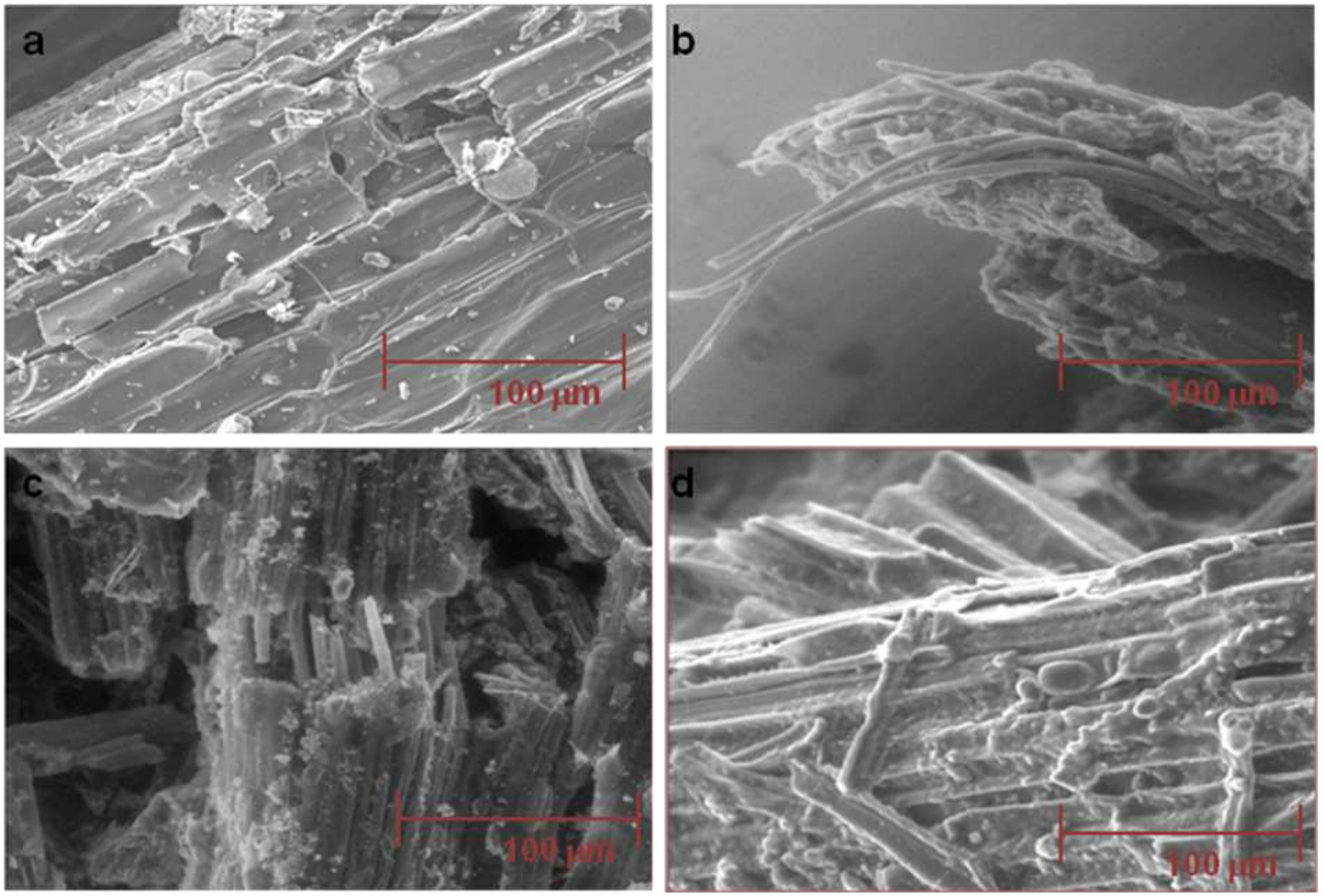
Pretreatment Model




Ref: Sun and Cheng (2002)

# Effect of Pretreatments on Biomass: Bent Grass



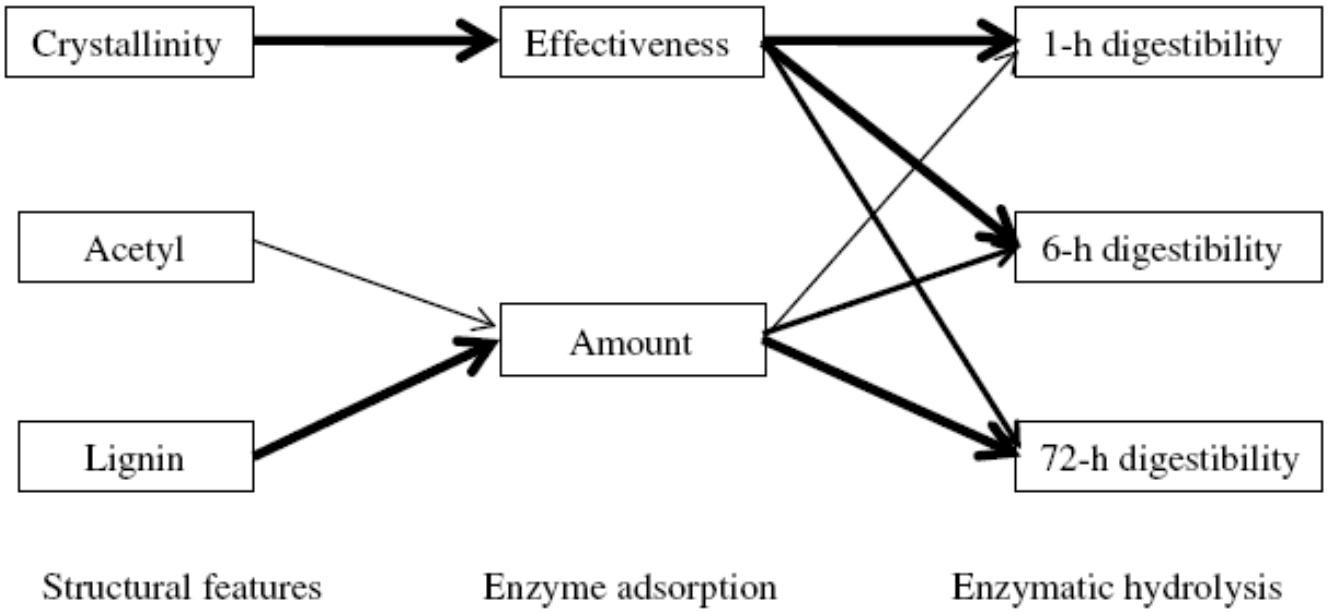

SEM images of bentgrass at 1000× magnification: (a) untreated, (b) hot water pretreated, (c) dilute acid pretreated, and (d) dilute alkali pretreated.

# Effect of Composition on Processing of Biomass

| Properties | Effect on Digestibility |
|---|---|
| Surface area | Positive |
| Crystallinity | Negative /no correlation |
| Pore Volume | Positive |
| Particle size | No correlation? |
| Degree of polymerization | Negative /no correlation |
| Lignin | Negative |
| Hemicellulose | Negative |
| Acetyl groups | Negative |

Ref: Zhu et al. (2008)

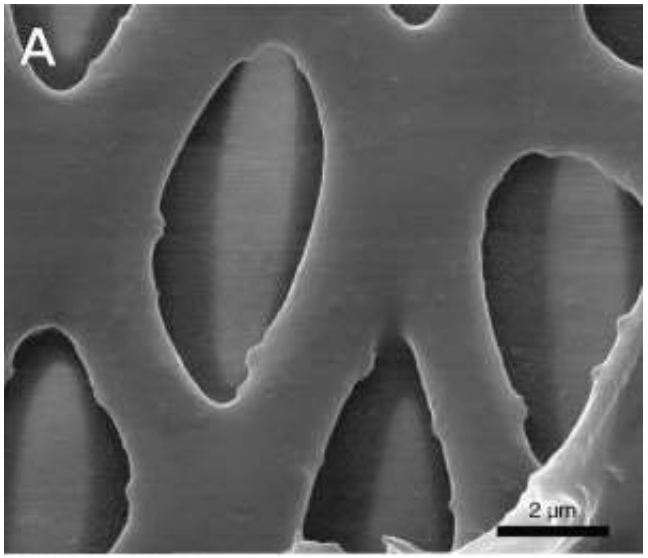
# Effect of Biomass Properties on Enzymatic Digestibility

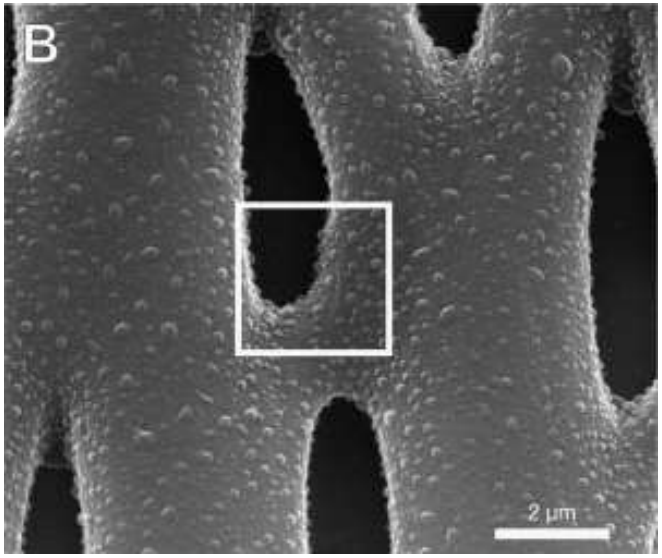


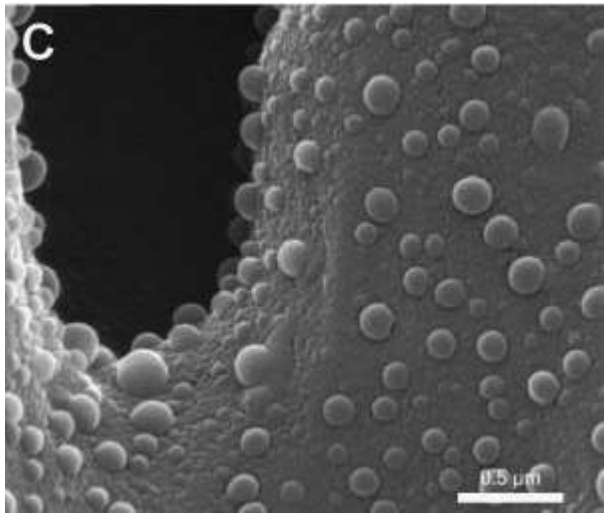

Pretreatment processes are critically dependent on type of feedstock.
Pretreatment processes change some of these properties of the feedstock.

Ref: Zhu et al. (2008)

# Lignin Migration During Pretreatment



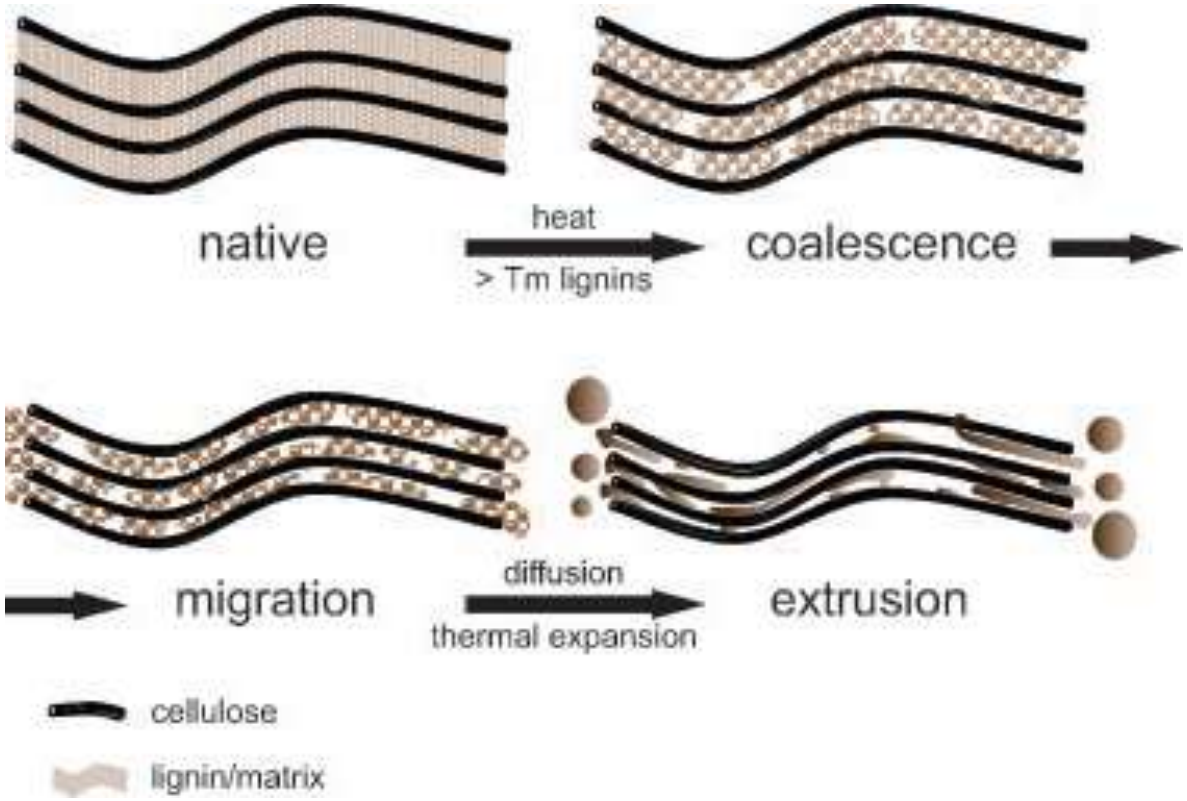




SEM Micrographs of corn stover A) before B) after C) after (higher magnification) pretreatment using 0.8% $H_2SO_4$.

Ref: Donohoe et al. (2008)

# A Model of Lignin Migration During Pretreatment




A recent paper (Brunecky et al. 2008) also postulates that the xylans rapidly hydrolyze and migrate towards the cell surfaces where they react with lignin and coalesce.

Ref: Donohoe et al. (2008)

# Pretreatment of Biomass

Steam explosion: Saturated steam at 160-290°C up to 1 min followed by decompression.

80-100% hemicellulose hydrolysis; destruction of a portion of xylans, 45-65% xylose recovery, formation of inhibitors

Mode of action: Removal of hemicellulose from the lignocellulose matrix.

Efficiency of the subsequent hydrolysis process improves upon addition of sulfuric acid, sulfur dioxide or carbon dioxide. Energy efficient size reduction compared to grinding or comminuation

Feedstocks: Poplar, aspen, eucalyptus, Douglas fir (softwood)
Bagasse, corn Stover, wheat, rice and barley straw, timothy grass, alfa alfa, reed canary grass

Ref: Mosier et al. (2005) Sanchez and Cardona (2008)

# Pretreatment of Biomass

Liquid Hot water:

Pressurized hot water and temperature (170-230°C) for up to 46 min; Solids content <20%

Mode of action: water acts as an acid at high temperatures, hemiacetyl linkages, acids released (acetic, glucuranic acids) help in breakdown of ether linkages, dissolves lignin,

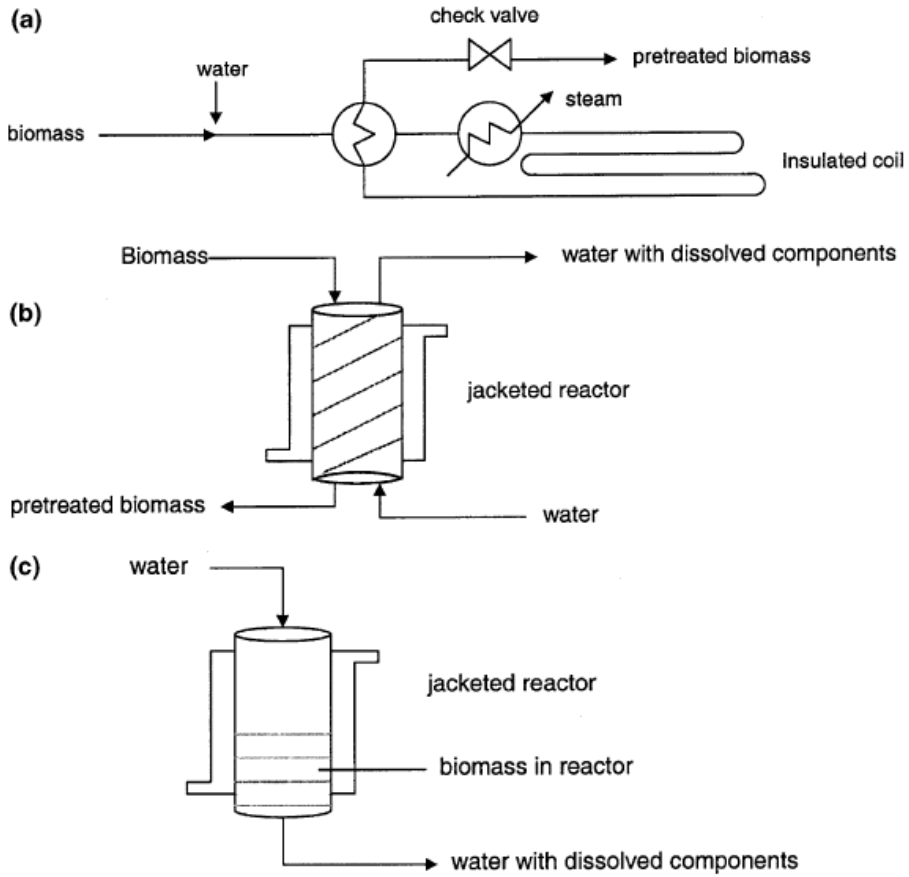
Lignin is not solubilized; 80-100% hemicellulose hydrolysis; 88-98% xylose recovery, low or no formation of inhibitors. Further cellulose conversion >90%

Feedstocks pretreated: Bagasse, corn stover,olive pulp, alfaalfa fiber

Ref: Sanchez and Cardona (2008)

# Pretreatment of Biomass

Configurations of liquid hot water preprocessing




Ref: Mosier et al. (2005)

# Pretreatment of Biomass

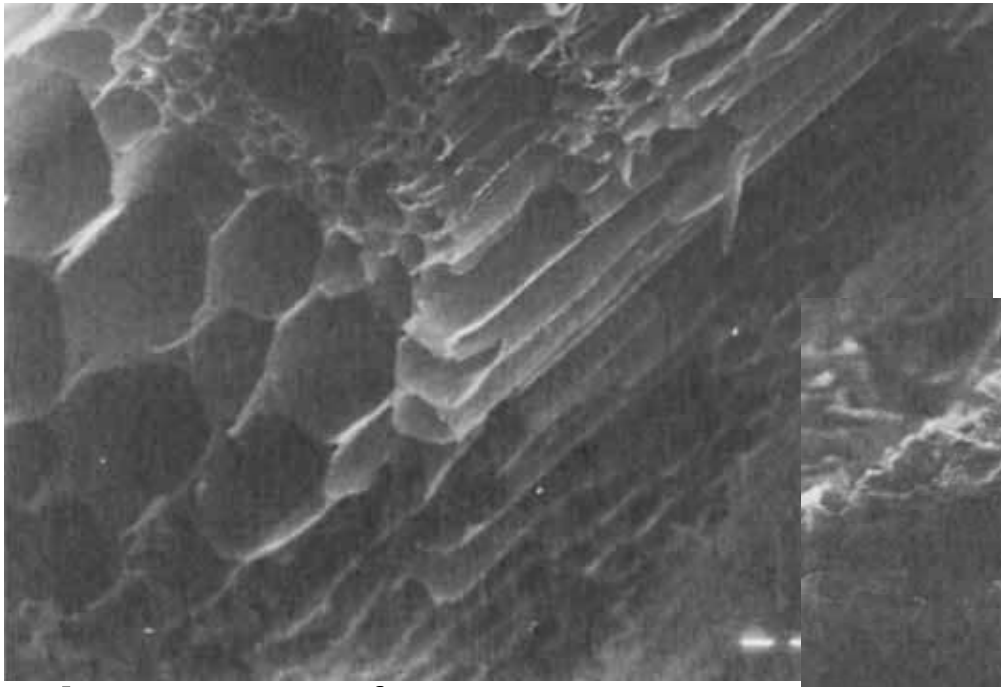
Dilute acid hydrolysis: 0.75-5% mineral acids, temperature (160-200°C), variable solids loading

80-100% hemicellulose hydrolysis; 75-90% xylose recovery, Lignin not solubilized but redistributed

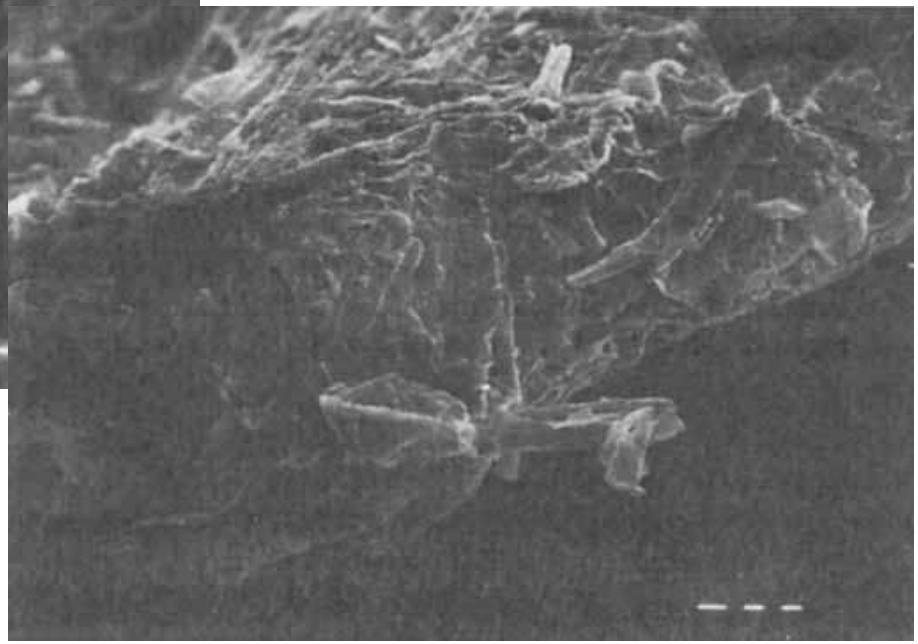
Feedstocks: Bagasse, corn stover, wheat, barley and rye straw, switch grass, bermuda grass

Ref: Sanchez and Cardona (2008)

# Pretreatment of Biomass



Wheat straw: Before pretreatment



Wheat straw: After pretreatment with 1% $H_2SO_4$

Ref: Gonzalez et al (1985)

# Pretreatment of Biomass

Dilute alkali hydrolysis: Dilute NaOH at 60°C for 2 hr; $Ca(OH)_2$, 4 hr at 120°C

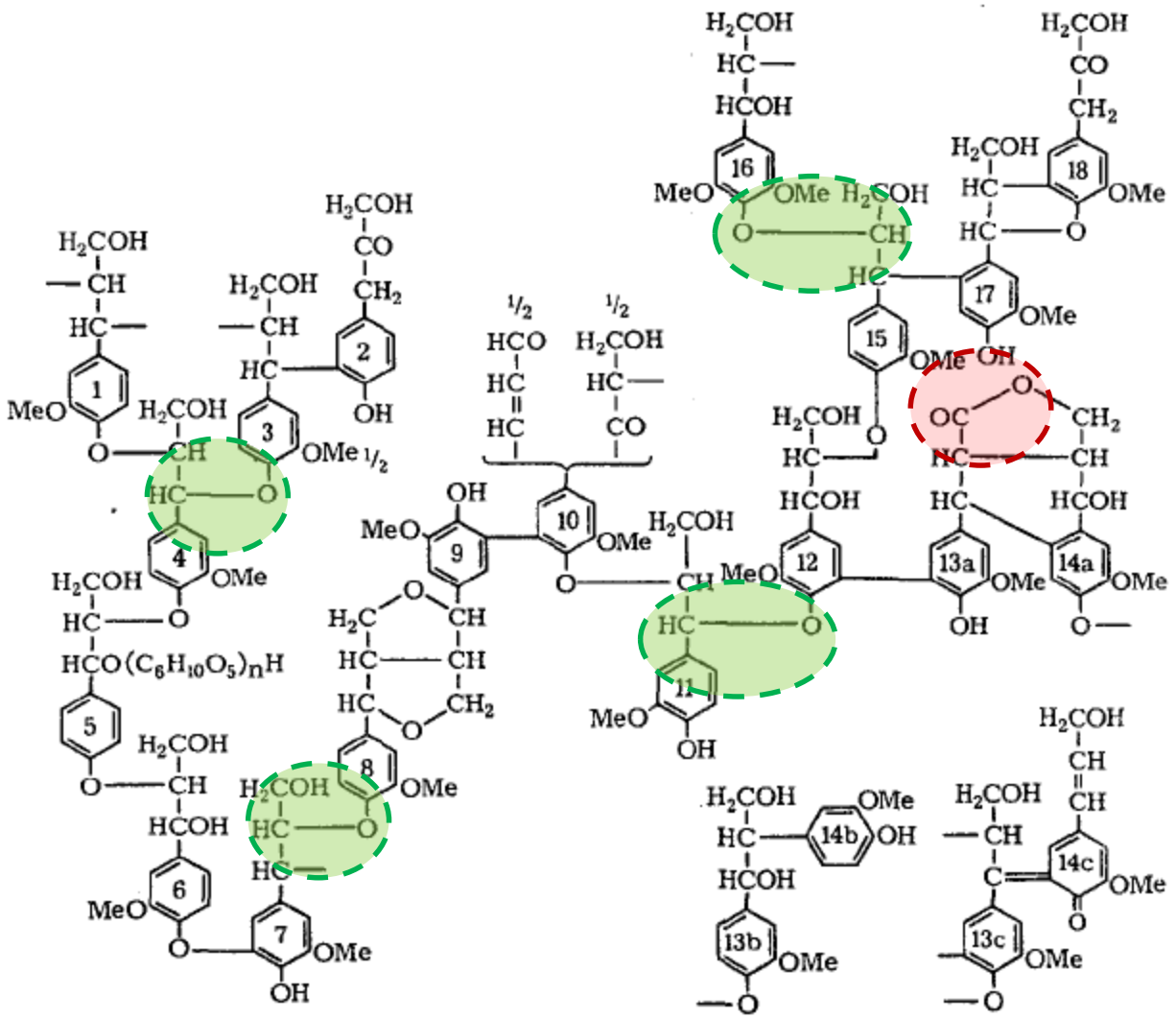
>50% hemicellulose hydrolysis; 60-75% xylose recovery, Lignin removal (up to 55%), low inhibitor formation; cellulose swelling

Mode of action: Dissolve lignin, cleave acetyl linkages in hemicellulose, cellulose swells increasing surface area and pore volume.

Feedstocks: Hardwood, bagasse, corn stover, cane leaves

Ref: Sun and Cheng (2002)

# Pretreatment of Biomass



Ref: Eggeling (1983)

# Pretreatment of Biomass

Ammonia Fiber Expansion (AFEX): 1-2 kg ammonia/kg dry biomass temperature 90°C, up to 30 min

0-60% hemicellulose hydrolysis depending on moisture; no formation of inhibitors; cellulose depolymerization to some degree. Subsequent cellulose hydrolysis >90%; ~10-20% lignin solubilization for high lignin biomass

Mode of action: reduces lignin, hemicellulose is solubilized, decrystallizes cellulose, Cellulose I→ Cellulose III.

Feedstocks: Aspen wood chips, bagasse, wheat, barley and rice straw, corn stover, switch grass, bermuda grass, alfaalfa, newsprint

Ref: Sun and Cheng (2002)

# Pretreatment of Biomass

Ammonia Recycle Percolation (ARP): 10-15% aqueous ammonia/kg dry biomass temperature 150-170°C, 10-20 min.

0-60% hemicellulose hydrolysis depending on moisture; no formation of inhibitors; cellulose depolymerization to some degree. Subsequent cellulose hydrolysis >90%; significant lignin solubilization for high lignin biomass

Mode of action: reduces lignin, hemicellulose is solubilized, decrystallizes cellulose, Cellulose I→ Cellulose III.

Feedstocks: Aspen wood chips, bagasse, wheat, barley and rice straw, corn stover, switch grass, bermuda grass, alfaalfa, newsprint

Ref: Kim and Lee (2005)

# Pretreatment of Biomass

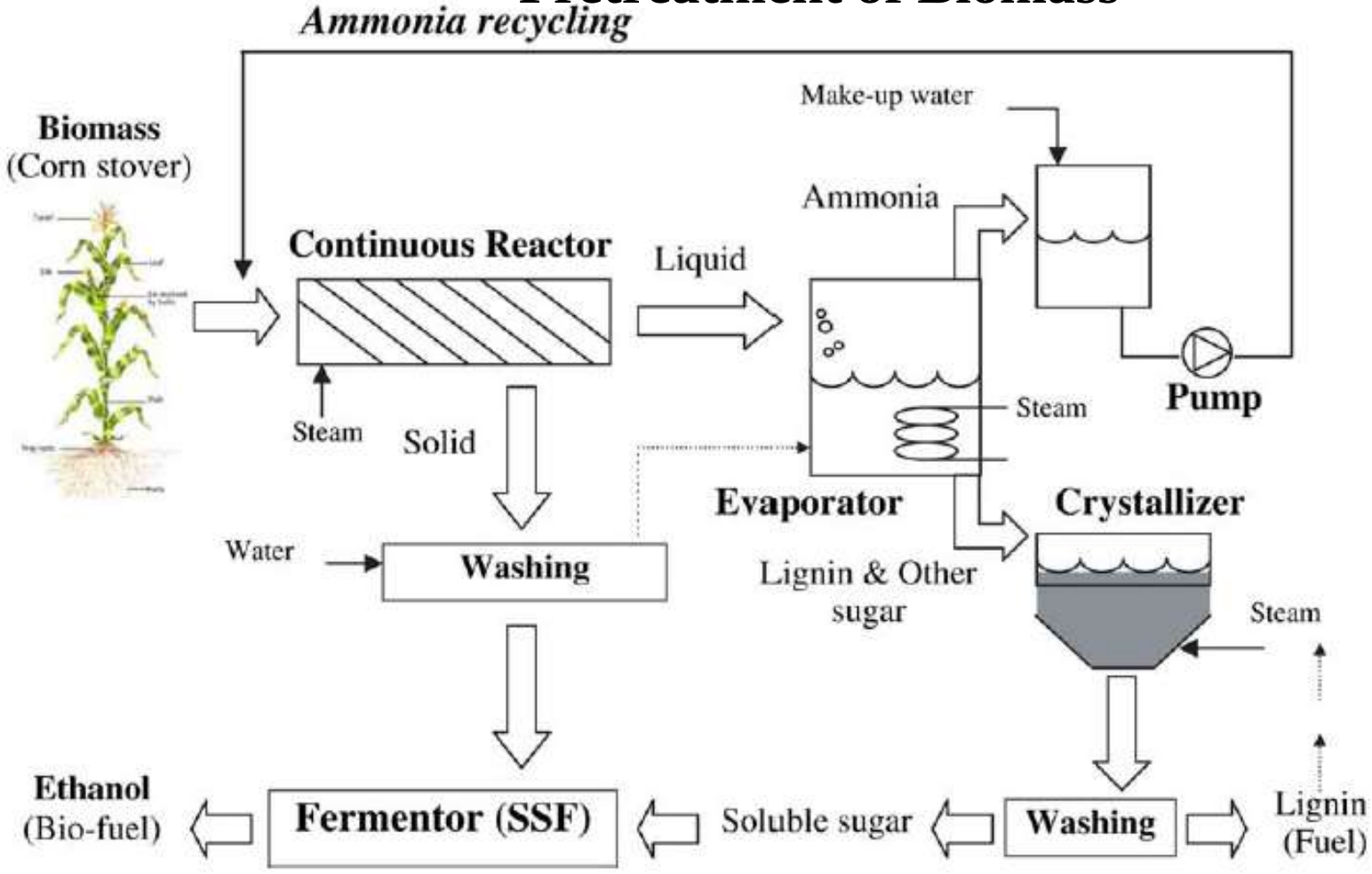
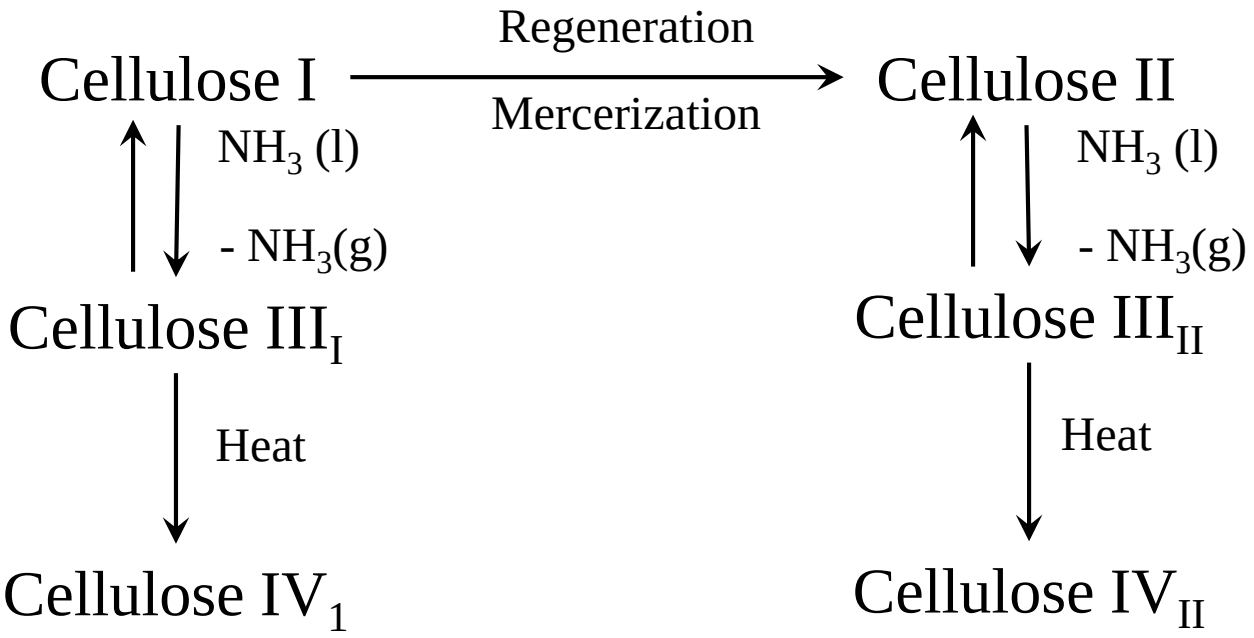



Fig. 1. ARP (Ammonia Recycle Percolation) process diagram.

Ref: Kim and Lee (2005)

# Effect of Pretreatment Conditions on Cellulose




Ref: O'Sullivan (1997)

# Pretreatment of Biomass

Organosolv process: Organic solvents (methanol, ethanol, acetone, ethylene glycol, triethylene glycol) or their mixture with 1% $H_2SO_4$ or HCl; 185-198°C, 30-60 min, pH 2.0-3.4

Almost total hemicellulose hydrolysis; high xylose recovery, almost total lignin solubilization, solvent recovery required

Feedstocks: Poplar and mixed soft wood

Ref: Sun and Cheng (2002)

# Example of Different Pretreatment Process Conditions

| Pretreatment | Chemicals | Temperature (C) | Pressure (bar) | Time (min) |
|---|---|---|---|---|
| Dil. Acid | 0.5-3.0 % $H_2SO_4$ | 130-200 | 3-15 | 2-30 |
| Flowthrough | 0.0-0.1 % $H_2SO_4$ | 190-200 | 20-24 | 12-24 |
| Controlled pH | Water or stillage | 160-190 | 6-14 | 10-30 |
| AFEX | 100% Anhydrous Ammonia | 70-90 | 15-20 | <5 |
| ARP | 10-15% Ammonia | 150-170 | 9-17 | 10-20 |
| Lime | 5-15% (w/w) $Ca(OH)_2$ | 70-130 | 1-6 | 1-6h |
| Lime+Air | 5-15% (w/w) $Ca(OH)_2$ | 25-60 | 1 | 2 weeks-2 months |

Ref: Wyman et al (2005)

# Example of Different Pretreatment Process Conditions

| Pretreatment | Xylose Yield (%) | Glucose Yield (%) | Total Sugars (%) |
|---|---|---|---|
| Dil. Acid | 34.5 | 57.2 | 91.7 |
| Flowthrough | 2.4 | 61.4 | 63.8 |
| Partial Flow | - | - | - |
| Controlled pH | 30.7 | 58.2 | 88.9 |
| AFEX | 30.2 | 61.8 | 92 |
| ARP | 17.0 | 59.4 | 76.4 |
| Lime | 20.5 | 59.8 | 80.3 |

Ref: Wyman et al (2005)

# Optimum Pretreatment Conditions for Corn Stover

| Pretreatment | Chemicals | Temperature (C) | Time (min) |
|---|---|---|---|
| Dil. Acid | 0.49 % $H_2SO_4$ | 160 | 20 |
| Flowthrough | 0.0 | 200 | 24 |
| Controlled pH | 0.0 | 200 | 24 |
| AFEX | 100% Anhydrous Ammonia | 90 | 5 |
| ARP | 15% Ammonia | 170 | 10 |
| Lime+air | 8% (w/w) $Ca(OH)_2$ | 55 | 4 weeks |

Ref: Wyman et al. (2005b)

# Effect of Pretreatments on Biomass

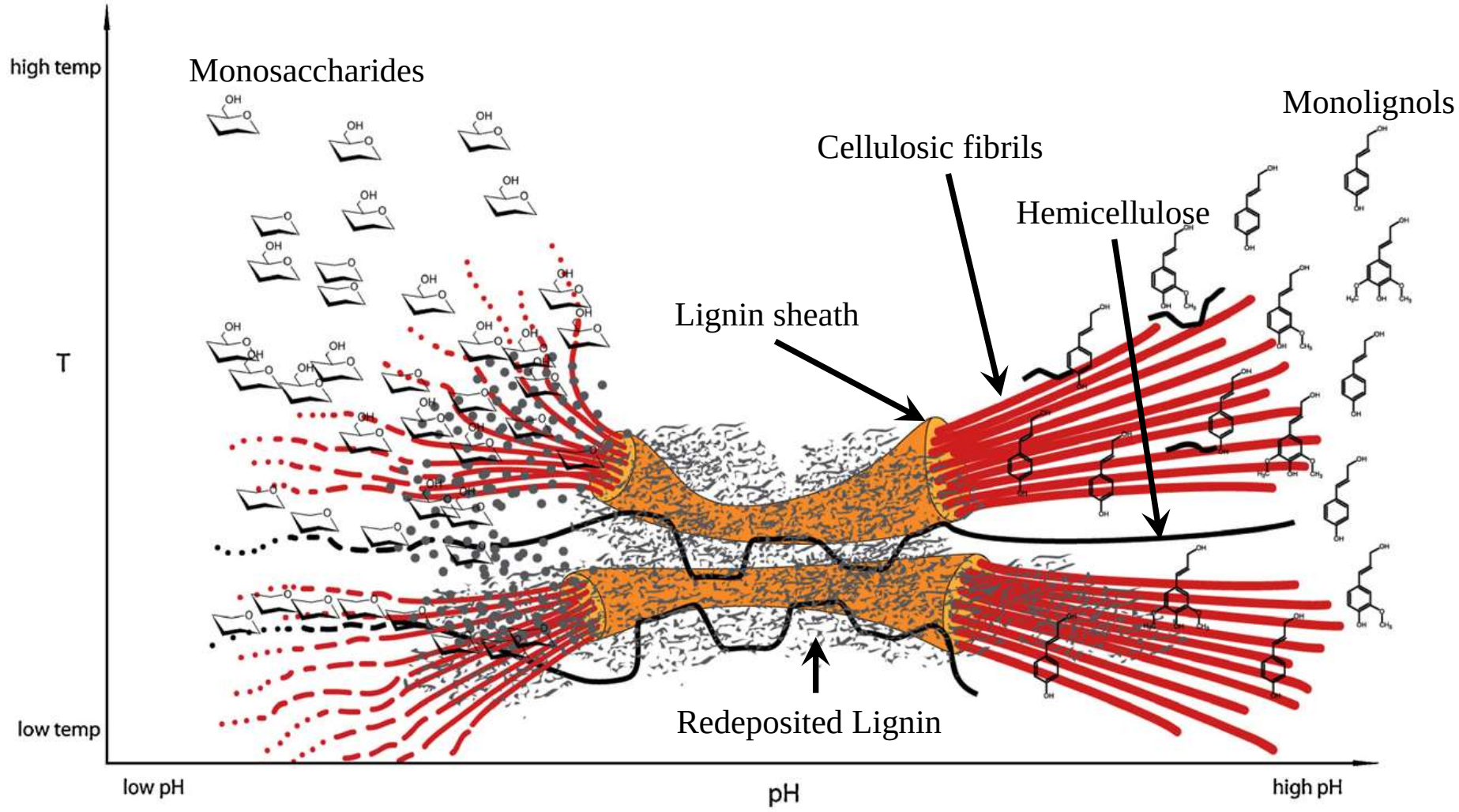
Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass

| | Increases accessible surface area | Decrystalizes cellulose | Removes hemicellulose | Removes lignin | Alters lignin structure |
|---|---|---|---|---|---|
| Uncatalyzed steam explosion | ■ | | ■ | | ▪ |
| Liquid hot water | ■ | ND | ■ | | ▪ |
| pH controlled hot water | ■ | ND | ■ | | ND |
| Flow-through liquid hot water | ■ | ND | ■ | ▪ | ▪ |
| Dilute acid | ■ | | ■ | | ■ |
| Flow-through acid | ■ | | ■ | ▪ | ■ |
| AFEX | ■ | ■ | ▪ | ■ | ■ |
| ARP | ■ | ■ | ▪ | ■ | ■ |
| Lime | ■ | ND | ▪ | ■ | ■ |

■: Major effect.
▪: Minor effect.
ND: Not determined.

Ref: Mosier et al (2005)

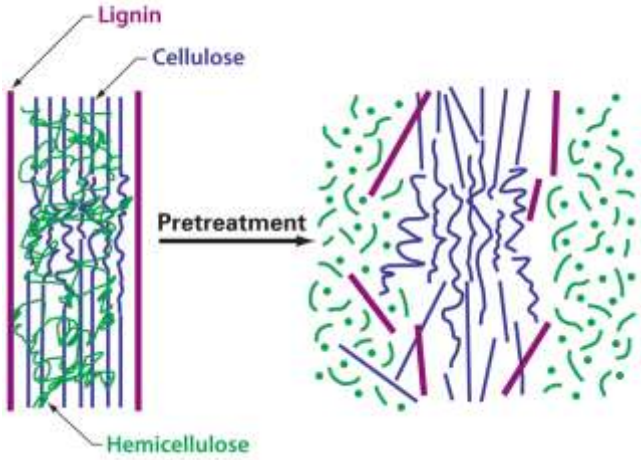
# Effect of Temperature and pH on Pretreatments on Biomass




Ref: Pendersen Meyer (2010)

# Severity Index of Pretreatment Process

Severity factor is an indicator of the combined effect of temperature (T, °C), reaction time (t, min). This is specific for a particular feedstock and specific pretreatment method.




$$R_o = \int_a^b \exp\left(\frac{T(t) - 100}{14.75}\right) dt = t \cdot \exp\left(\frac{T(t) - 100}{14.75}\right)$$

$$M_o = t \cdot C^n \cdot \exp\left(\frac{T(t) - 100}{14.75}\right)$$

C is the concentration of the chemical catalyst

n=0.849 for $H_2SO_4$ and 3.90 for NaOH

$$R'_o = R_o \cdot [\mathrm{H}^+]$$

$$\log(R'_o) = \log(R_o \cdot [\mathrm{H}^+]) = \log(R_o) - \mathrm{pH}$$

$$\boxed{\log(R''_o) = \log(R_o) + |\mathrm{pH} - 7|}$$

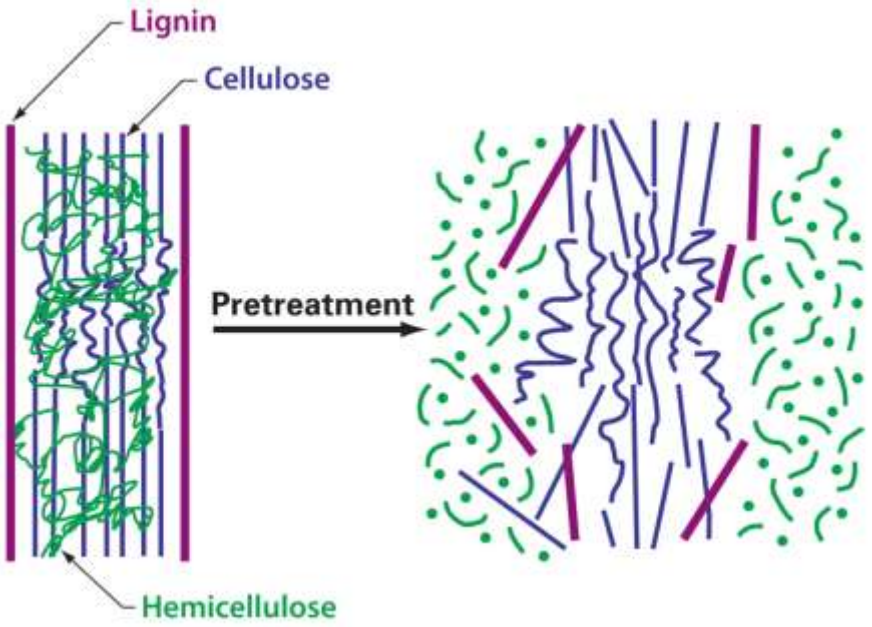
Ref: Palmqvist et al. (2000)

# Severity Index of Pretreatment Process

Combined severity factor is an indicator of the combined effect of temperature (T, °C), reaction time (t, min) and pH. This is specific for a particular feedstock and specific pretreatment method.

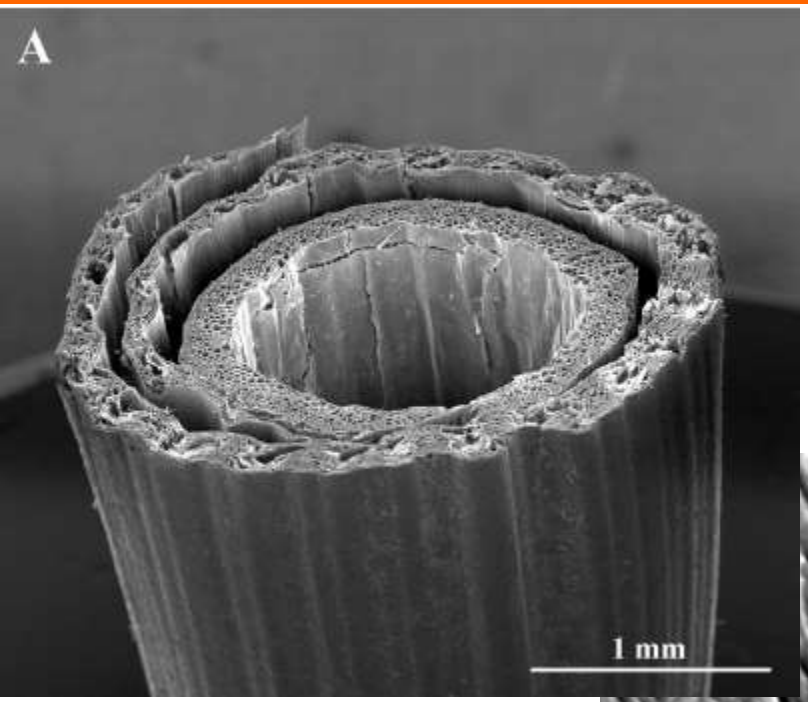
$$CS = \log (t\, e^{((T-100)/14.75)}) - |pH-7|$$

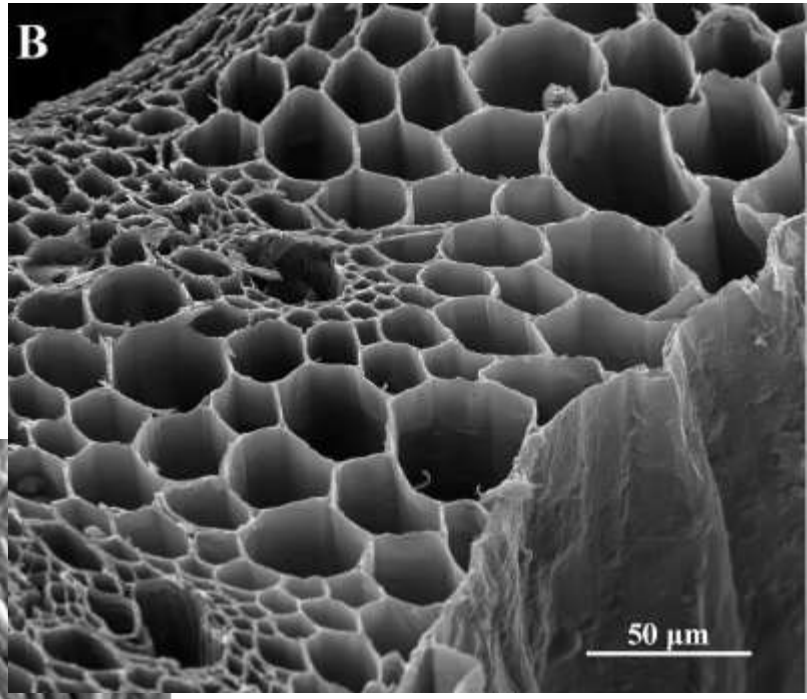
Increasing CS beyond the value for maximum sugar release results in formation of inhibitors.

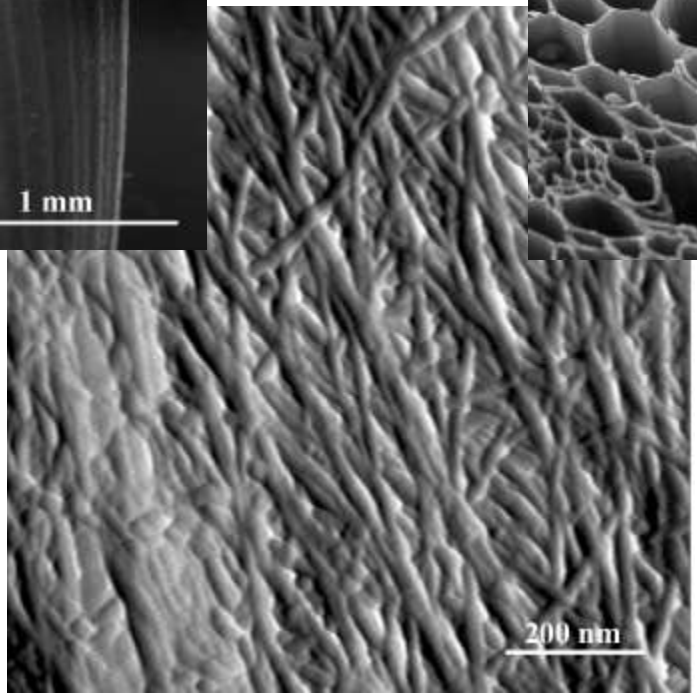



Ref: Palmqvist et al. (2000); Pendesen Meyer (2010)

# Effect of Pretreatments on Biomass: Wheat straw

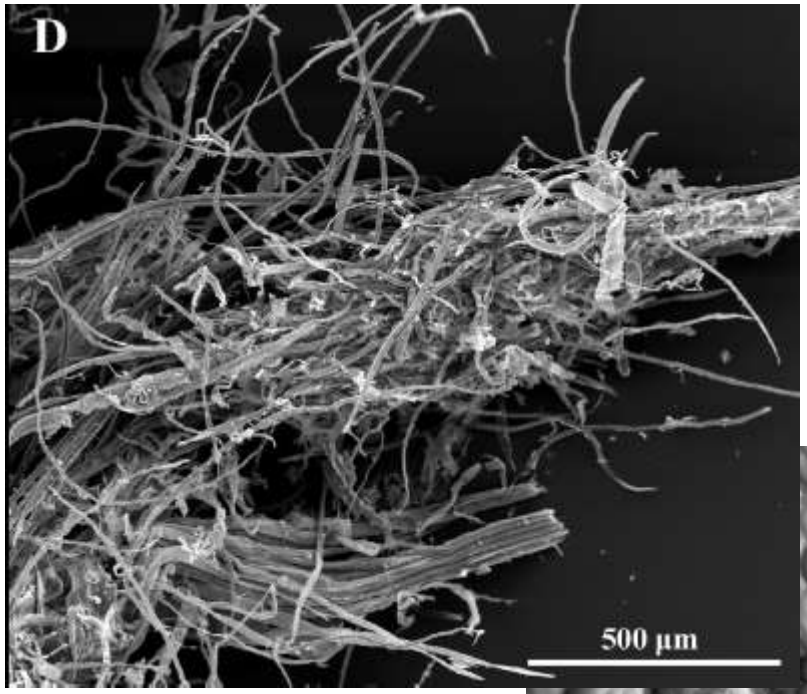


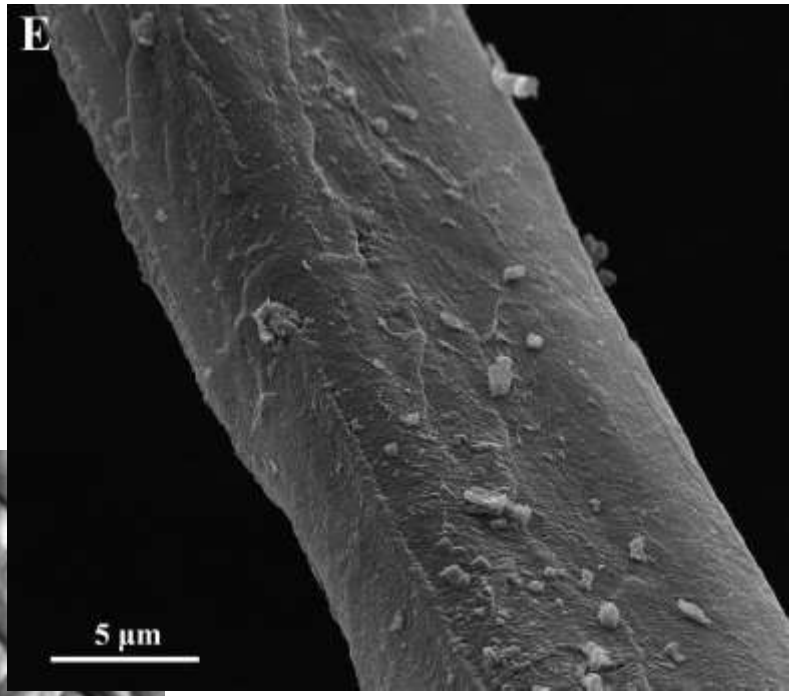



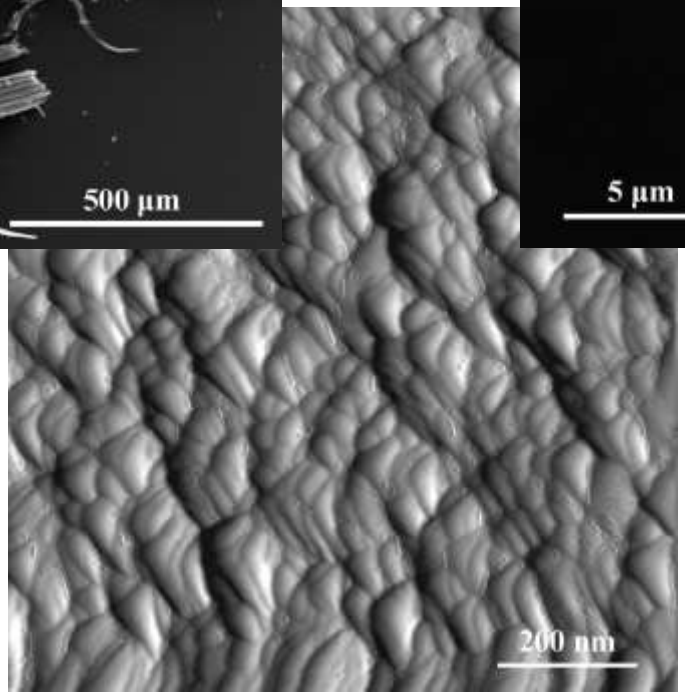




Ref: Kristensen et al (2008)

# Effect of Pretreatments on Biomass: Hydrothermal pretreatment

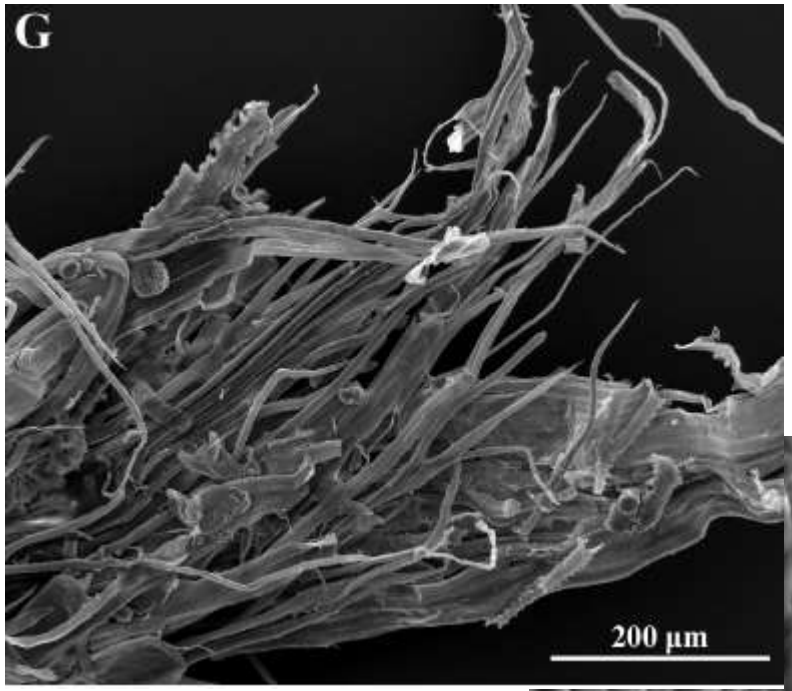


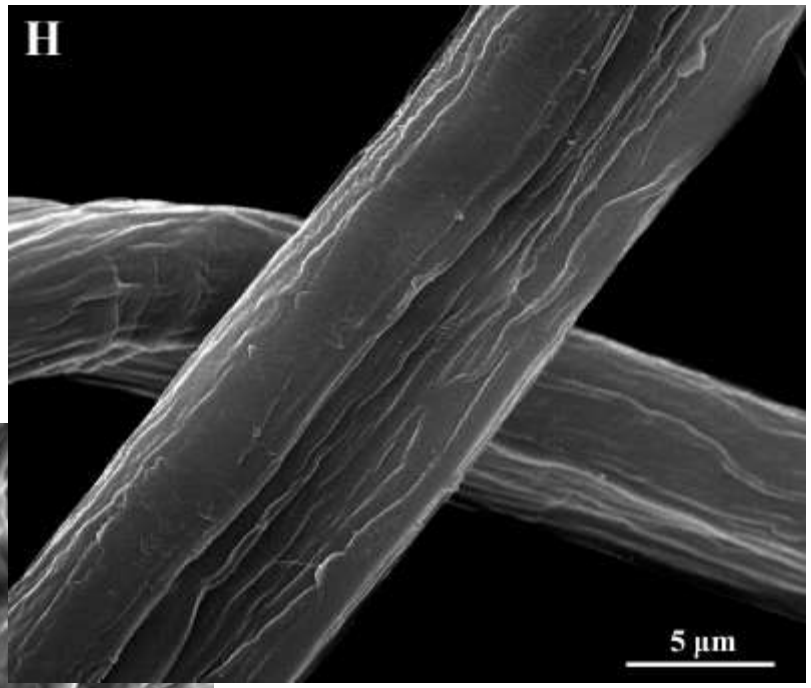



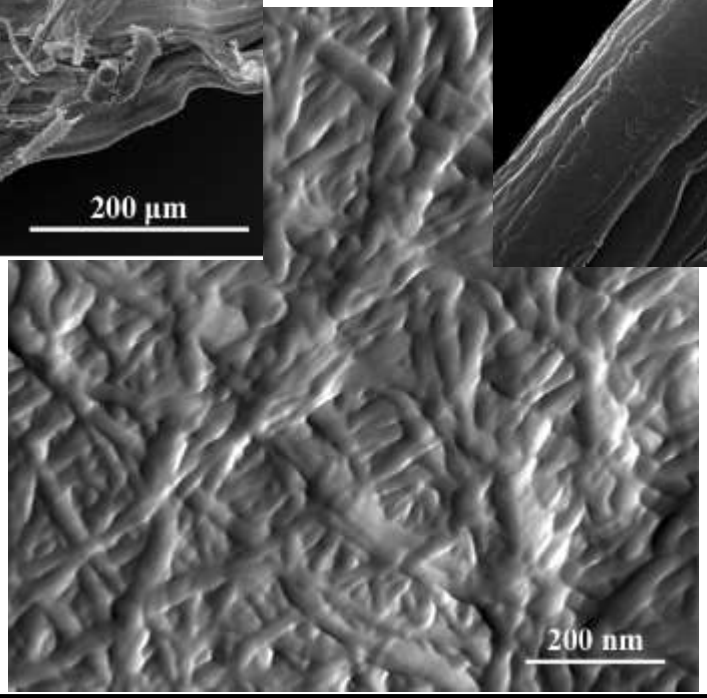




Ref: Kristensen et al (2008)

# Effect of Pretreatments on Biomass: Hydrothermal, delignification

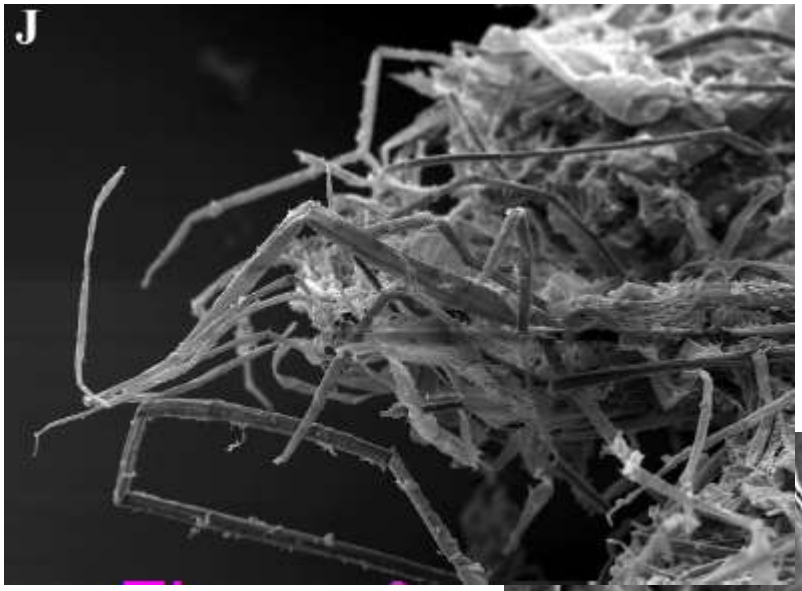


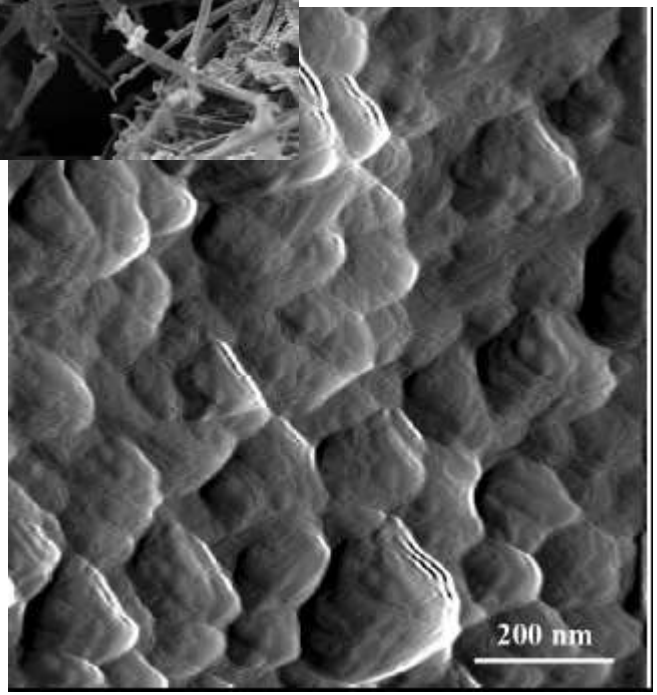



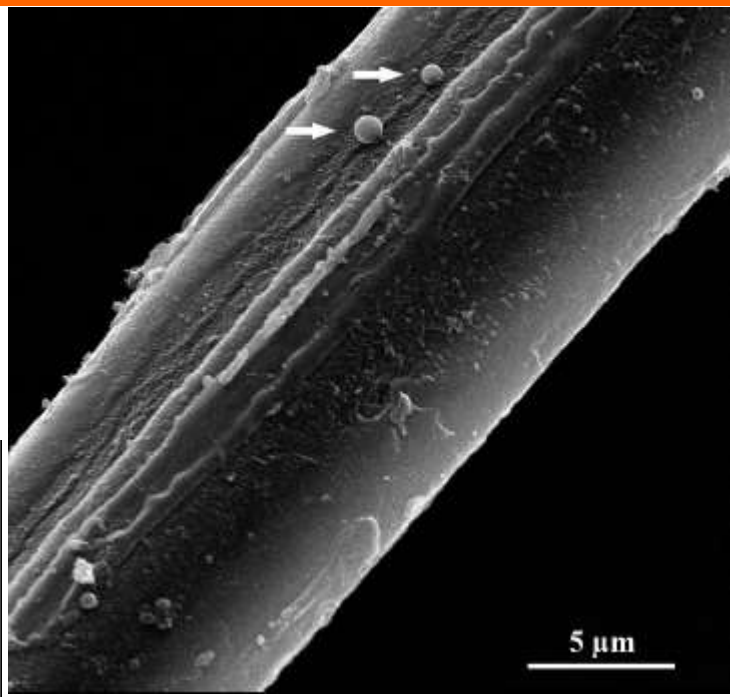




Ref: Kristensen et al (2008)

# Effect of Pretreatments on Biomass: Steam explosion



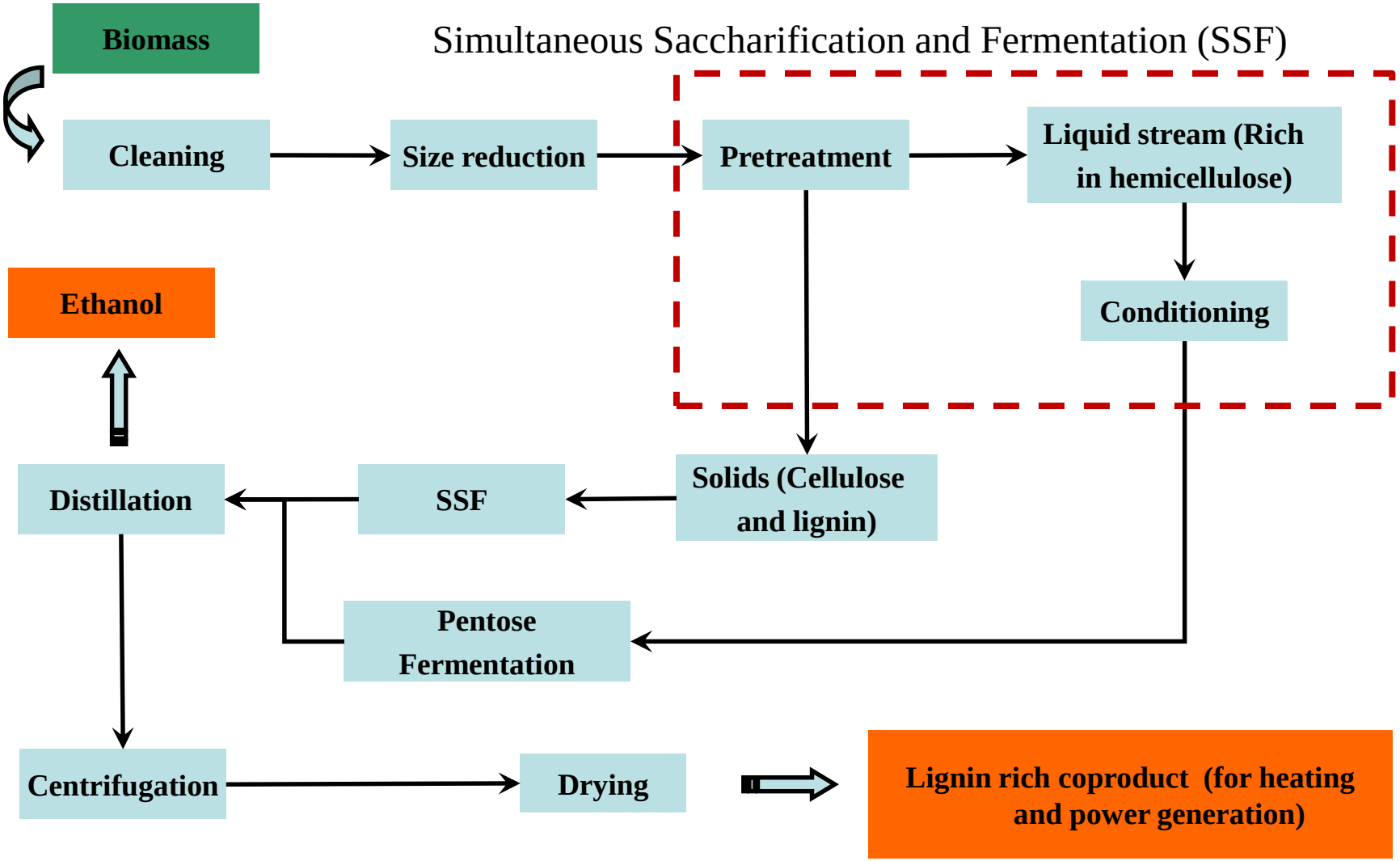

Ref: Kristensen et al (2008)

Thank you

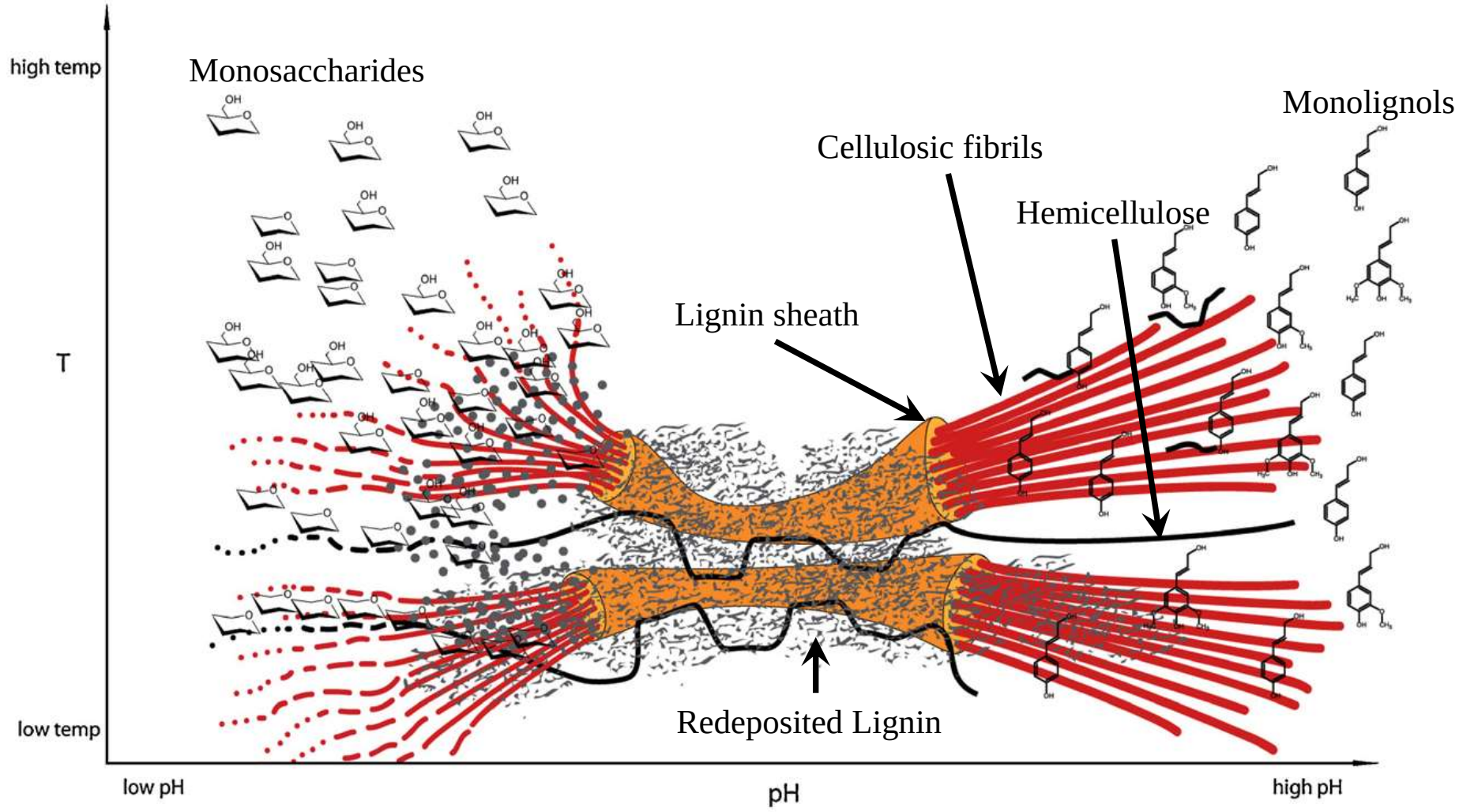
# Lecture Fifteen

# Cellulosic Ethanol Production: Conditioning

# Summary of Lecture Fourteen



# Effect of Temperature and pH on Pretreatments on Biomass



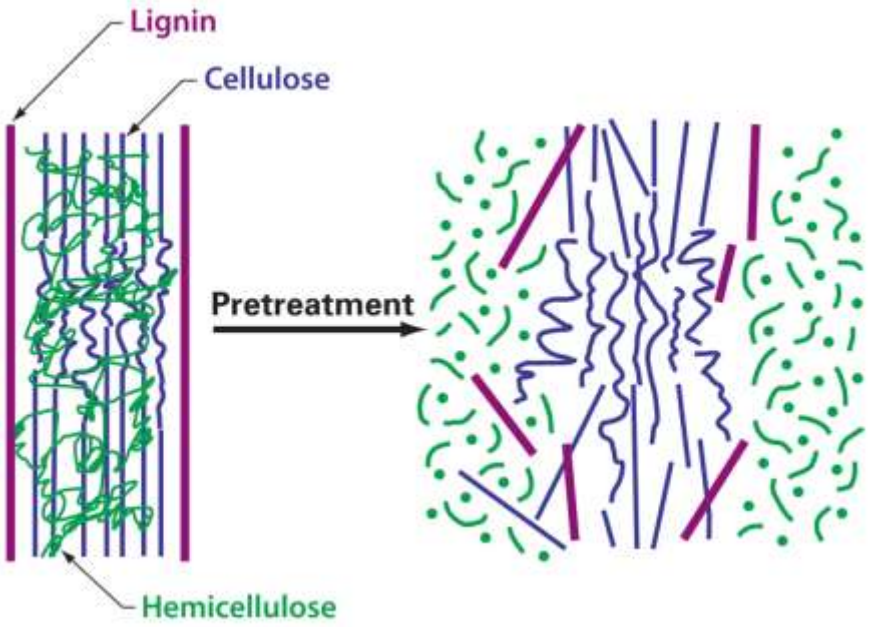

Ref: Pendersen Meyer (2010)

# Severity Index of Pretreatment Process

Combined severity factor is an indicator of the combined effect of temperature (T, °C), reaction time (t, min) and pH. This is specific for a particular feedstock and specific pretreatment method.

$$CS = \log (t\, e^{((T-100)/14.75)}) - |pH-7|$$

Increasing CS beyond the value for maximum sugar release results in formation of inhibitors.



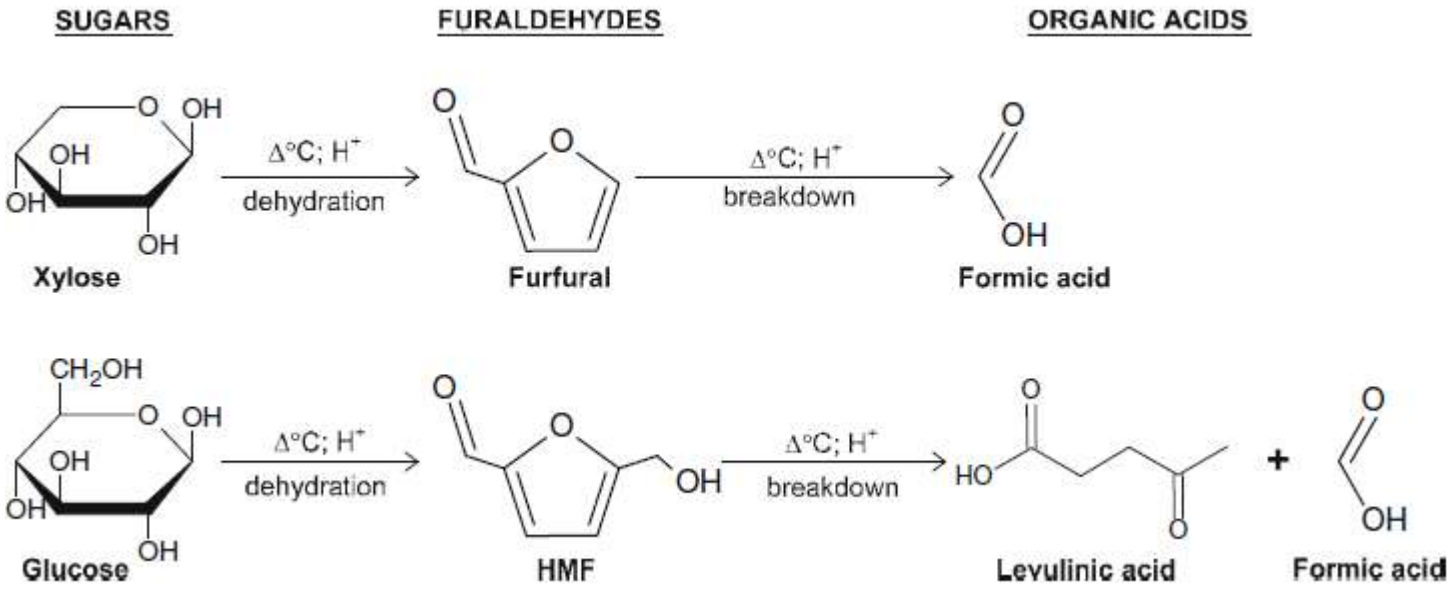

$$CS = \log (t\, C^{n}\, e^{((T-100)/14.75)}) - |pH-7|$$

Ref: Palmqvist et al. (2000); Pendesen Meyer (2010)

# Inhibitors Formation During Pretreatment

Inhibitors produced during pretreatment:

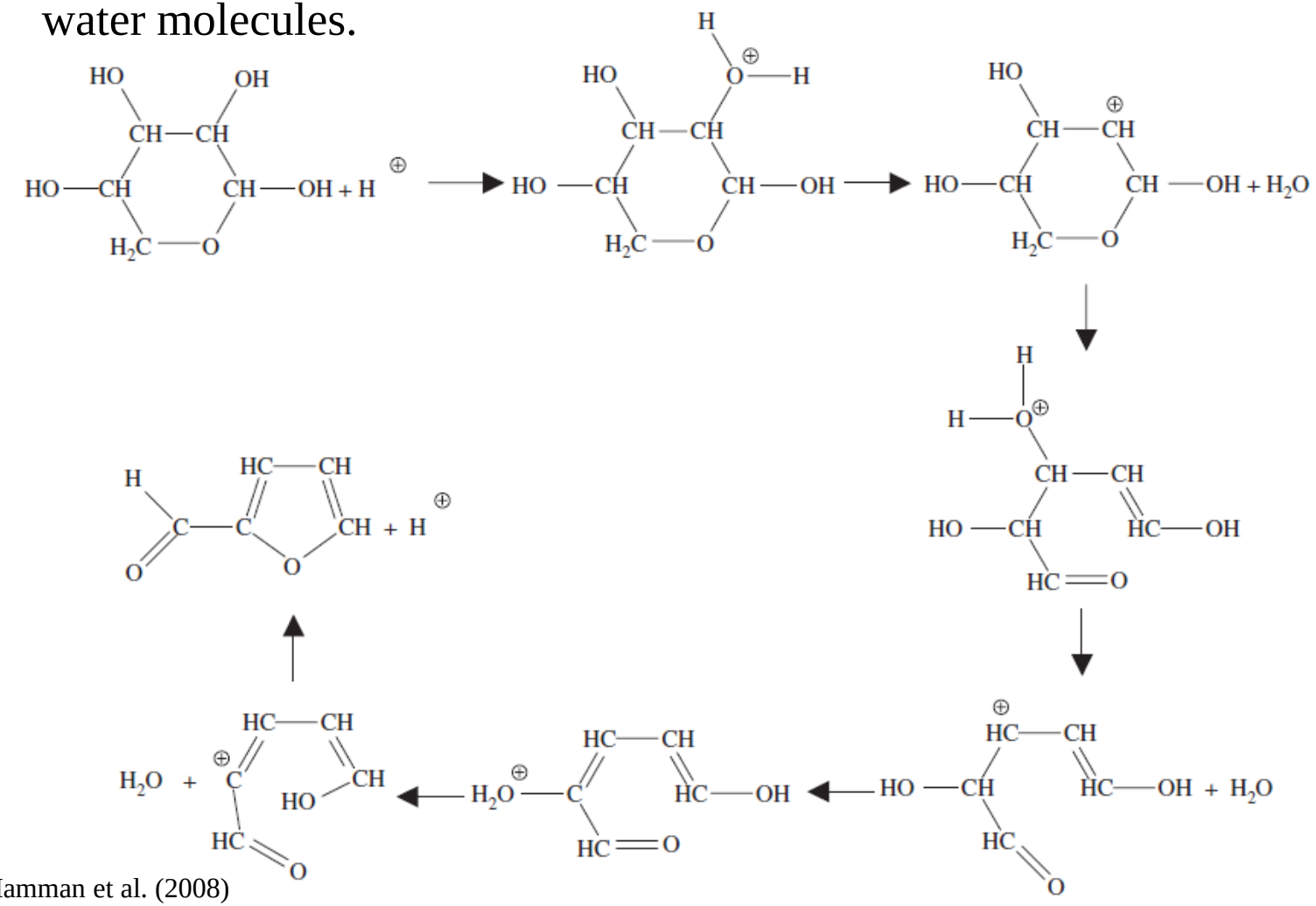
- Sugars: Hydroxy Methyl Furfural (HMF) , Furfural
- Lignin: Cinnamaldehyde, p-hydroxybenzaldehyde and syringaldehyde
- Hemicellulose degradation products: acetate, formic, glucuronic and galacturonic acids)




Ref: Sanchez and Cardona (2008) Almeida et al. (2009)

# Inhibitors Formation During Pretreatment

Mechanism of furfural formation by pentose dehydration and elimination of 3 water molecules.



Ref: Mamman et al. (2008)

# Types of Inhibitors

More than 70 different inhibitors have been identified in lignocellulose hydrolyzate.

Inhibitors belong to three principal classes

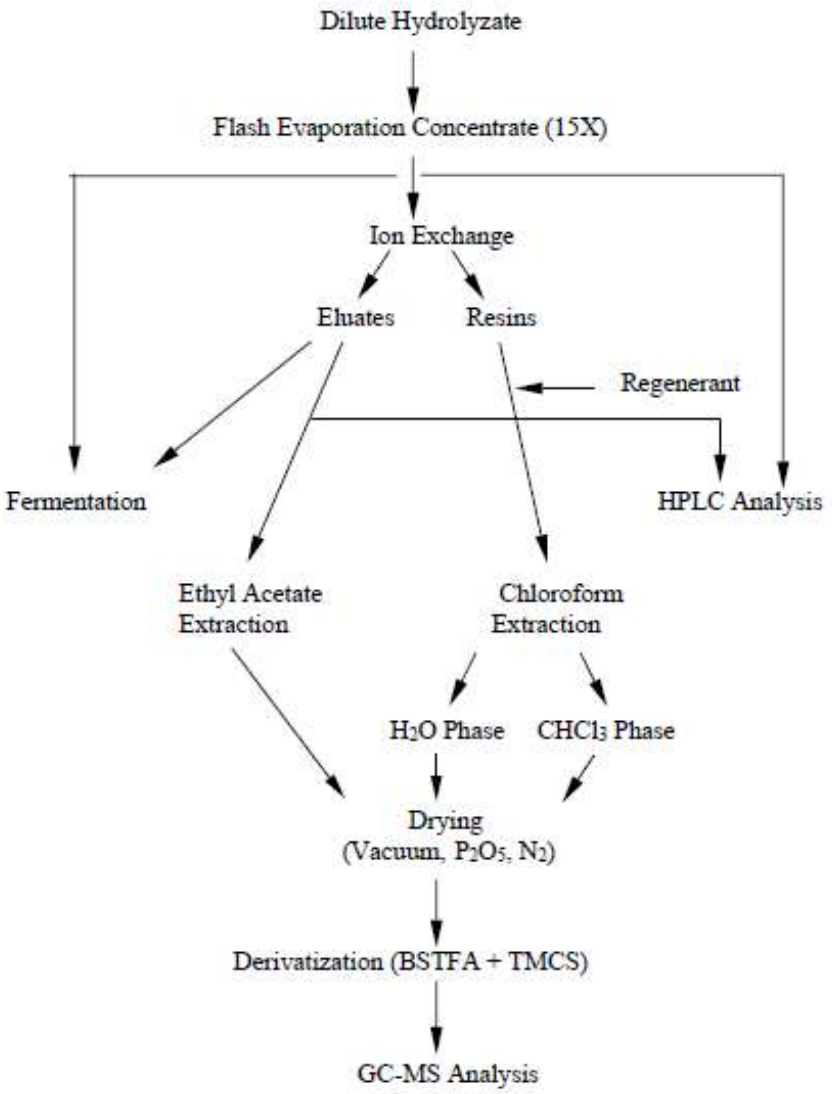
- Aliphatic acids
- Furan compounds
- Aromatic aldehydes

Degree of toxicity of aromatic compounds can be determined by the functional groups attached to the benzene ring. (Ando's toxicity rule)

- CH=CH : +3.0  COOH : +0.5
- CHO : +1.5  m-OH : 0.0
- p-OH : +1.0  $OCH_3$ :-1.0

Ref: Luo et al. (2002)

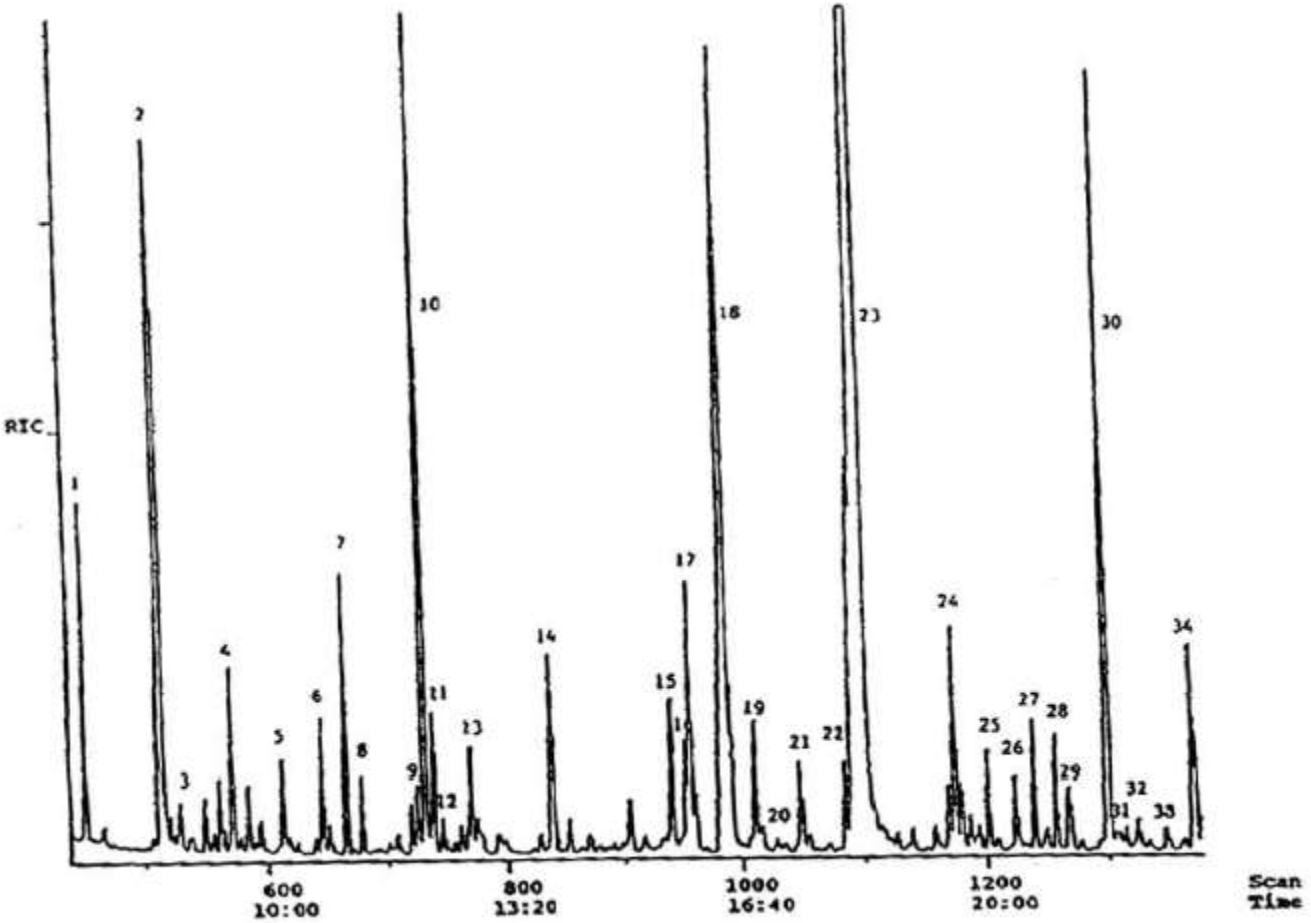
# Types of Inhibitors: Analysis




Ref: Luo et al. (2002)

# Types of Inhibitors: Analysis

Dilute acid hydrolyzate of hybrid poplar (partial list).




Ref: Luo et al. (2002)

# Compounds in Hydrolyzate : Aliphatic acids

| Compounds |
|---|
| Acetic acid |
| 4-oxo-pentanoic acid |
| 2-methyl-2-hydroxybutanoic acid |
| 3-hydroxy-propanoic acid |
| Methyl propanedioic acid |
| Methyl butanedioic acid |
| 2-butanedioic acid |
| Hydroxybutanedioic acid |
| Hexanedioic acid |
| 2-hydroxypentanedioic acid |
| 2-hydroxy-2-pentenedioic acid |
| Hexadecanoic acid (palmitic acid) |
| Ethanedioic acid |
| 9,12-octadecadienoic acid |
| 9-octadecenoic acid (oleic acid) |
| Octadecanoic acid (stearic acid) |
| 2,3-dihydroxypropandioic acid |

Levulinic acid

Ref: Luo et al. (2002)

# Compounds in Hydrolyzate : Aromatic compounds

| Compounds |
|---|
| 3-methoxy-4-hydroxy-benzaldehyde (vanillin) |
| 4-hydroxybenzoic acid ← *Indicates cleavage of ester and ether bonds* |
| 3,5-dimethoxy-4-hydroxy-benzaldehyde acid (syringaldehyde) |
| 2,5-dihydroxy-benzoic acid |
| 3-methoxy-4-hydroxy-benzoic acid (vanillic acid) |
| 3,4-dihydroxy-benzoic acid |
| 3,5-dimethoxy-4-hydroxy-benzoic acid (syringic acid) |
| 4-methoxy-3-hydroxy-cinnamic acid (isoferulic acid) |
| G-CO-CH(OH)-$CH_3$ [a] |
| G-$CH_2$-COOH [a] |
| 3-methoxy-4-hydroxy-cinnamic acid (ferulic acid) |
| 4-methoxy-α-hydroxy-benzenacetic acid |
| G-CH(OH)-CO-$CH_3$ [a] |
| 5-methoxy-2-hydroxy-benzoic acid |
| 2-methoxy-α-hydroxy-benzenacetic acid |

Ref: Luo et al. (2002)

# Types of Inhibitors

More than 70 different inhibitors have been identified in lignocellulose hydrolyzate.

Inhibitors belong to three principal classes

- Aliphatic acids
- Furan compounds
- Aromatic aldehydes

Degree of toxicity of aromatic compounds can be determined by the functional groups attached to the benzene ring. (Ando's toxicity rule)

- CH=CH : +3.0  COOH : +0.5
- CHO : +1.5  m-OH : 0.0
- p-OH : +1.0  $OCH_3$ :-1.0

Ref: Luo et al. (2002)

# Inhibitor Action Mechanism

Inhibitors action can be classified into three broad categories:

- Chemical interference with cell maintenance functions
- Inhibition on ethanol production pathways.
- Osmotic effect on cells.

Ref: Luo et al. (2002)

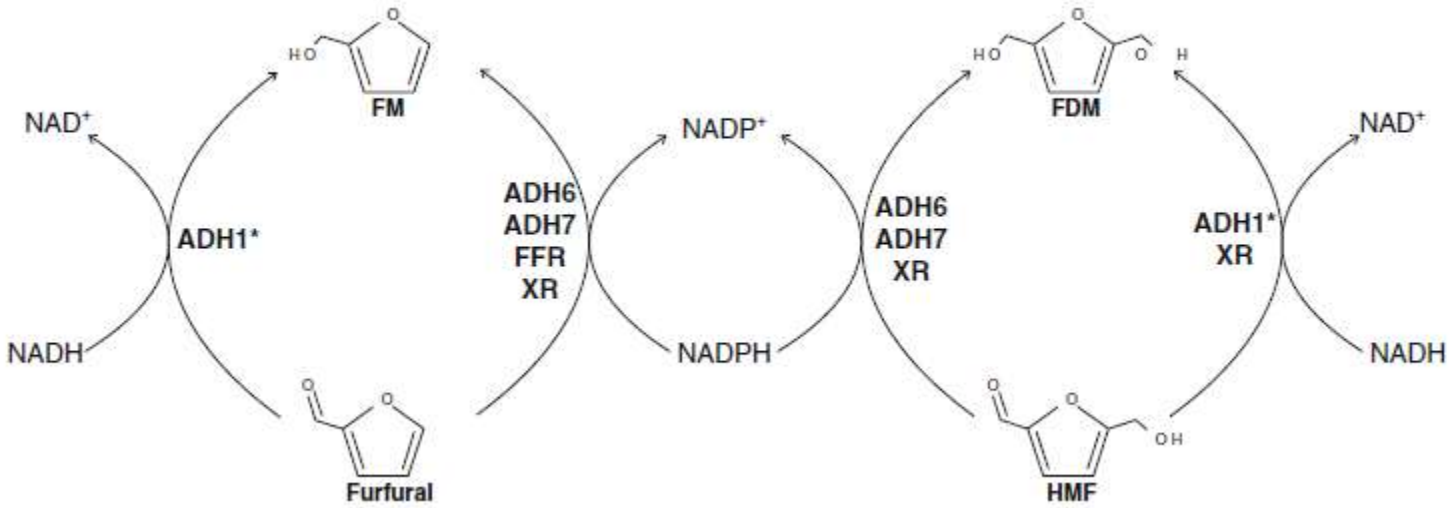
# Inhibitor Action Mechanism

- Weak acids
  - Concentration of undissociated acid is a function of pH and pKa. Weak acids have high pKa values (acetic acid : 4.75; formic acid: 3.75; levulinic acid: 4.66).
  - Dilute acid hydrolyzates of spruce has buffering capacity up to pH 5.5
  - Undissociated weak acids are liposoluble. But anionic forms are lipophobic.
  - Two theories: Uncoupling and intracellular anion accumulation.

Ref: Palmqvist et al. (2000) Pienkos and Zhang(2009)

# Inhibitor Action Mechanism

- Furfural and HMF:
  - Furfural and HMF (with a lower efficiency due to lower membrane permeability) is metabolized by *S. cerevisiae* in aerobic, oxygen limited and anaerobic conditions to corresponding alcohols.
  - Pejo et al (2008) observed reduction in xylitol and glycerol production in steam exploded wheat straw hydrolyzate.




Ref: Palmqvist et al. (2000) Almeida et al. (2009) Pejo et al (2008)

# Inhibitor Action Mechanism

- Phenolic compounds
  - Low molecular weight phenolic compounds are most inhibitory.
  - 4-hydroxybenzoic acid is the most inhibitory compounds.
  - Water solubility of the phenolic compounds is low.
  - Effect membrane integrity

Ref: Palmqvist et al. (2000) Luo et al. (2002)

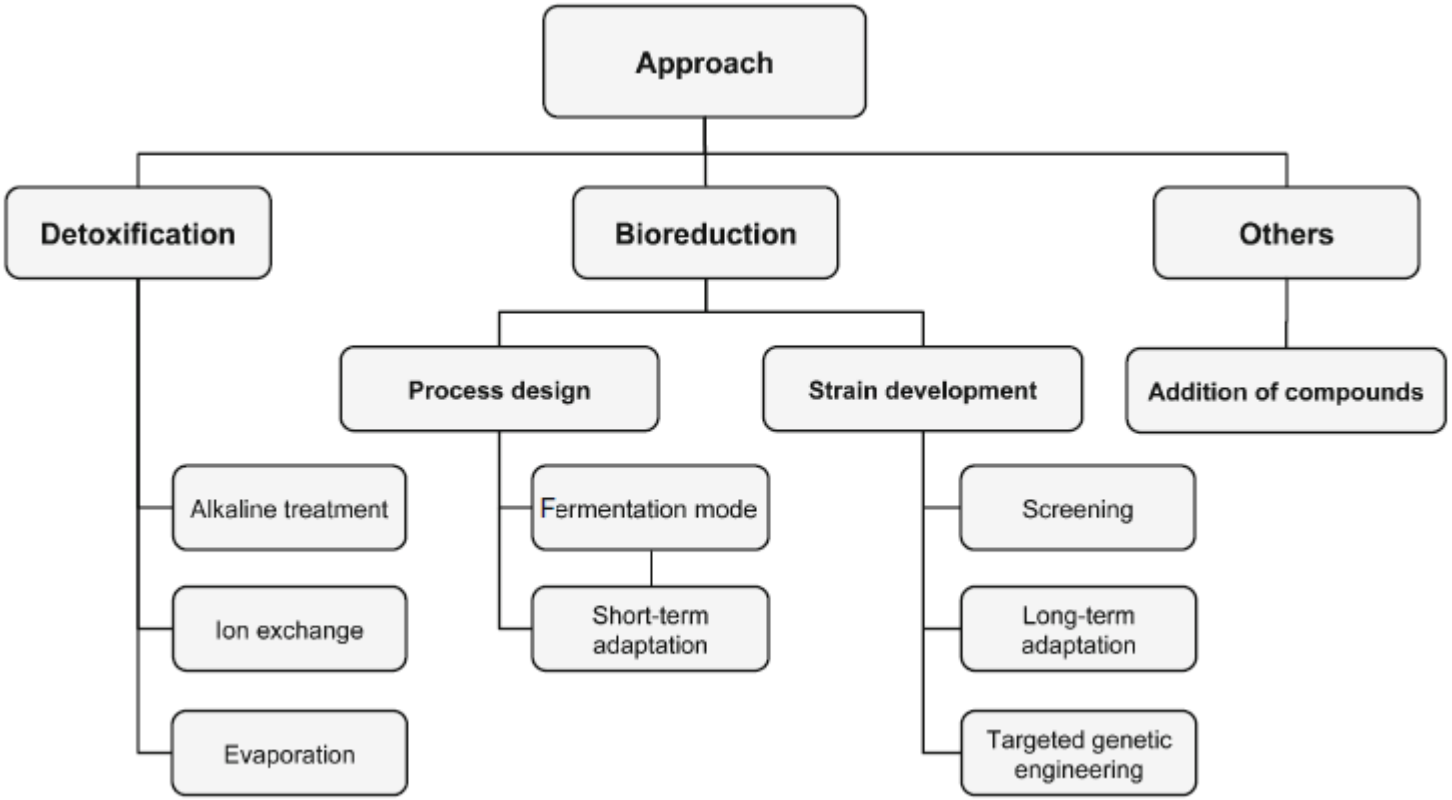
# Inhibitor Action Mechanism

- Furfural and HMF: Glycolysis especially dehydrogenases.
- Phenolics: loss of membrane integrity, interfere with sugar transport and cell growth.
- Weak acids: Proton gradients and increased cell maintenance.
- Aldehydes: Hydrophobicity, NADH and NADPH requirement and other unknown effects. Do not disrupt membrane integrity or effect proton gradients.
- Alcohols: membrane structure. Less toxic than weak acids and aldehydes.

Ref: Pienkos and Zhang (2009)

# Inhibitor Effect Suppression Strategies

Strategies for removing the inhibitory effect




Ref: Almeida et al. (2009)

# Pretreated Biomass Conditioning/Detoxification

Removal of inhibitors and toxic compounds is an important downstream step in cellulosic biomass processing. Some of the methods are:

- Evaporation:
  - Acetic acid, furfural and vanillin are the more volatile compounds that are removed.
  - HMF has lower volatility and therefore is not removed to the same extent as furfural.
- Extraction with organic solvents:
  - Diethyl ether at pH 2.0 extracts acetic, formic and levulinic acid, furfural, HMF and phenolic compounds.
  - Extracted inhibitory compounds were not alkali stable.

Ref: Sanchez and Cardona (2008), Palmqvist et al. (2000)

# Pretreated Biomass Conditioning/Detoxification

- Ionic exchange resins
  - Weak base anion exchange resins remove almost all of HMF, furfural, acetic acid and color.
  - Strong anion exchangers remove HMF, furfural and color significantly, Acetic acid and xylose are not removed to a significant extent.
  - More expensive treatment. Resins must be regenerated for economic performance.
  - Fouling, deposit formation are other potential issues in the use of ion exchange resins.

Ref: Sanchez and Cardona (2008), Palmqvist et al. (2000)

# Pretreated Biomass Conditioning/Detoxification

• Adsorption on activated charcoal, molecular sieves: Reduced furfurals, acetic acid and phenolics.

- Low pH is favorable for adsorption of weak acids and phenols.
- Higher pH is favorable for adsorption of weak basic compounds.
- Organic acids are best adsorbed from acidic solutions and organic bases are adsorbed from basic solutions.
- Increasing the charcoal : sugar ratios beyond a certain ratio results in loss of sugars.
- Higher temperatures favors higher adsorption rates.

Ref: Mussatto et al. (2004), Palmqvist et al. (2000)

# Pretreated Biomass Conditioning/Detoxification

- *Alkaline Detoxification (Over liming):*
  - Adjustment solution pH to 10-12.0.
  - $Ca(OH)_2$ is more effective than NaOH. Although treatment with NaOH and $Ca(OH)_2$ resulted in similar removal rates for furfurals and HMF, $Ca(OH)_2$ treated samples had much higher ethanol productivities. ➔ unknown inhibitors in hydrolyzate.
  - $NH_4OH$ and $Ca(OH)_2$ treatments to pH 10 reduce HMF, furfural, phenols compounds, vanillin, coniferyl alcohol and cinnamic acid.
  - Neutralization to 5.5 pH with any base has little effect on inhibitor concentration.
  - Higher pH and temperature result in conversion of inhibitory compounds to formic, acetic and levulinic acids.

Ref: Pienkos and Zhang (2009), Palmqvist et al. (2000)

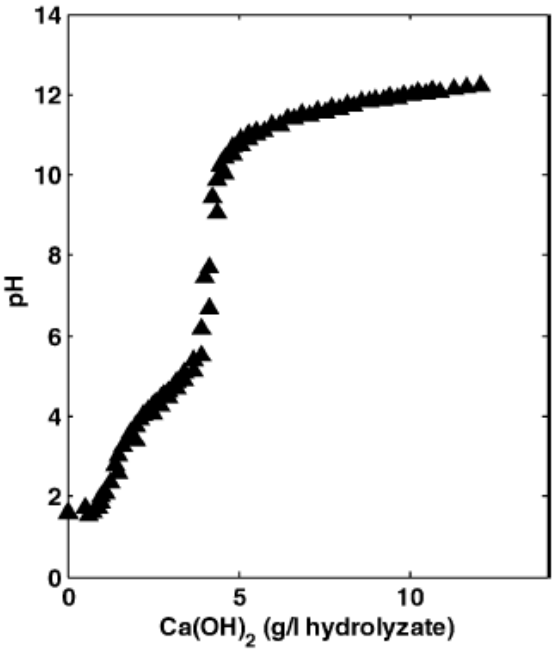
# Pretreated Biomass Conditioning/Detoxification

- Combined alkaline and sulfite detoxification
  - Although $Ca(OH)_2$ is sufficient to detoxify hydrolyzate, sulfite treatment increases the fermentation rates.
  - Hibbert ketones and volatile compounds were removed at higher temperatures.

Hibbert Ketones

G*, guaiacyl = 4-hydroxy-3-methoxyphenyl

$G^*\text{-}CHOHCOCH_3$
$G^*\text{-}COCOCH_3$
$G^*\text{-}CH_2COCH_2OH$
$G^*\text{-}COCHOHCH_3$
$G^*\text{-}CH_2COCH_3$




Ref: Sanchez and Cardona (2008), Palmqvist et al. (2000)

# Pretreated Biomass Conditioning/Detoxification

- Enzymatic detoxification
    - Laccase, lignin peroxidases, Mn peroxidase (white rot fungi): Heme peroxidase : blue copper oxidases result in oxidation of phenolics, non phenolic lignin units, $Mn^{3+}$ (oxidizer) acts on phenolic and non-phenolic lignin units.
- Microbial detoxification
    - Lignolytic fungus *Trametes versicolor (laccase)*
        - Oxidative polymerization of low molecular weight phenolic compounds and phenolic acids.
    - White rot fungus
        - Remove a wide variety of chemicals including chlorinated aromatic compounds, heterocyclic aromatic hydrocarbons, dyes, and synthetic polymers.
        - Strong oxidative activity and low substrate specificity of the lignolytic enzymes. Oxidative process (Phenol oxidases)

Ref: Sanchez and Cardona (2008)

# Pretreated Biomass Conditioning/Detoxification

- Brown rot fungus
- Soft-rot fungus *Trichoderma reesei*
  - Mostly act by removing acetic acid, furfural and benzoic acid derivatives.
  - Can degrade secondary cell wall of plants. Active on higher moisture, lower lignin biomass.

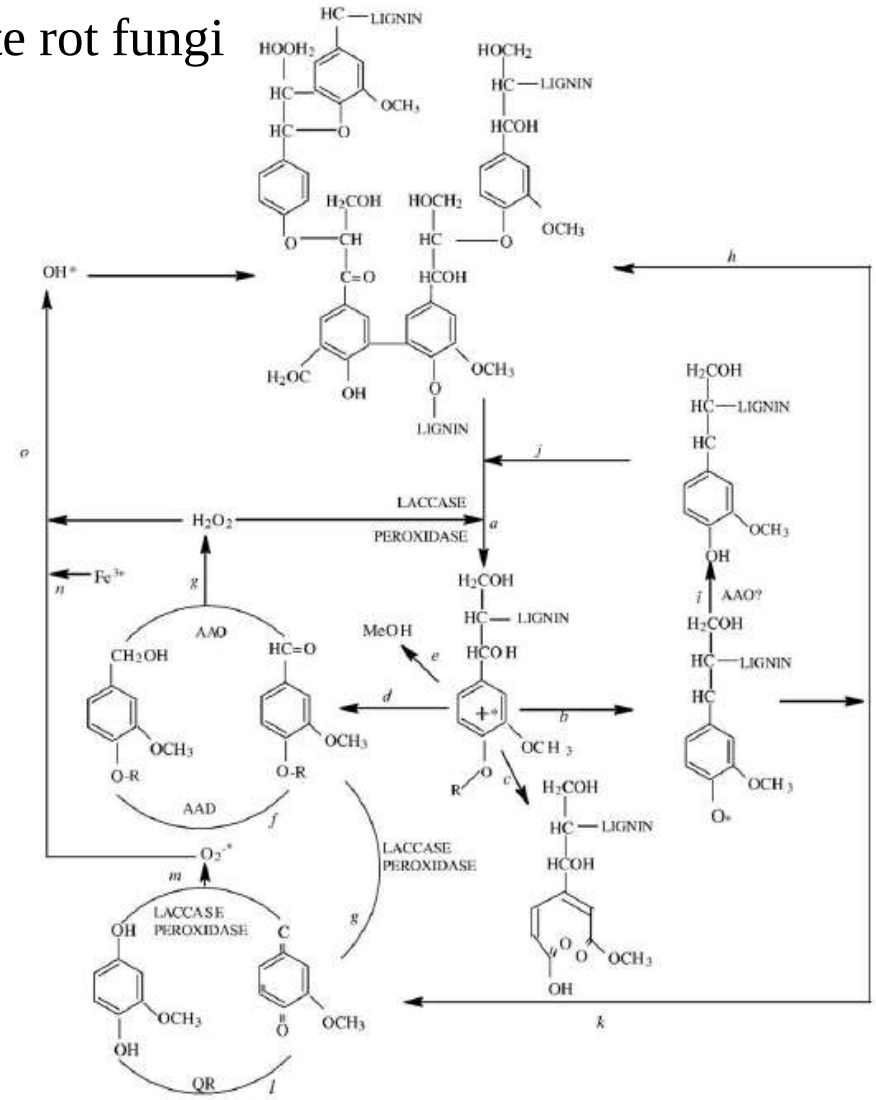
Fungi with high specific lignase activities.

| Organism | Enzyme | Substrates | Specific activity (μmol $min^{-1}$ $mg^{-1}$) | Opt. *T* (°C) | Opt. pH |
|---|---|---|---|---|---|
| *Phanerochaete chrysosporium* | Diarylpropane peroxidase (ligninase) | 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol+$H_2O_2$/1-(3,4-diethoxyphenyl)-1,3-dihydroxy-2-(4-methoxy-phenyl)propane+$O_2$+$H_2O_2$/1-(4-ethoxy-3-methoxyphenyl)-1,2-propane+$O_2$+$H_2O_2$/1-(4-ethoxy-3-methoxyphenyl)-1,2-propene+$O_2$+$H_2O_2$/2-Keto-4-thiomethylbutyric acid+$H_2O_2$/3,4-dimethoxybenzyl alcohol+$H_2O_2$ | 28 | 23/37 | 3/4.5 |
| *Botrytis cinerea* | Laccase | 1,2,4-benzenetriol+$O_2$/1-naphthol+$O_2$/2-naphthol+$O_2$/3,5-dimethoxy-hydroxy-benzaldazine+$O_2$/4,5-dimethyl-o-phenylenediamine $O_2$/4-amino-N,N`-dimethylaniline+$O_2$/4-methylcatechol+$O_2$/ascorbate+$O_2$/caffeic acid+$O_2$/catechol+$O_2$/ferrocyanide+$O_2$/gallic acid+$O_2$/guaiacol+$O_2$ | 5778 | 55 | 4 |
| *Stropharia coronilla* | manganese peroxidase | $Mn^{2+}$+$H^+$+$H_2O_2$ | 692 | 25 | NA |

Ref: Sanchez (2009)

# Pretreated Biomass Conditioning/Detoxification

Lignin biodegradation by white rot fungi




Ref: Sanchez (2009)

# Comparison of Inhibitor Suppression Strategies

Dilute acid pretreated spruce

| Group of compounds | Compounds[b] | Concentration (g/L) |
|---|---|---|
| Sugars | Glucose | 25.7 |
| | Mannose | 6.5 |
| | Galactose | 3.7 |
| | Xylose | 3.5 |
| | Cellobiose | 0.7 |
| | Arabinose | 0.6 |
| Furan derivatives | 5-hydroxy-methyl-furfural | 5.9 |
| | Furfural | 1.0 |
| Aliphatic acids | Levulinic acid | 2.6 |
| | Acetic acid | 2.4 |
| | Formic acid | 1.6 |
| Phenolic compounds | Vanillin | 0.12 |
| | Dihydroconiferylalcohol | 0.098 |
| | Coniferyl aldehyde | 0.035 |
| | Vanillic acid | 0.034 |
| | Hydroquinone | 0.017 |
| | Catechol | 0.009 |
| | Acetoguaiacone | 0.007 |
| | Homovanillic acid | 0.005 |
| | 4-Hydroxy-benzoic acid | 0.005 |

Ref: Larsson

# Comparison of Inhibitor Suppression Strategies

Dilute acid pretreated spruce

| Group of compounds | Compounds[b] | Concentration (g/L) |
|---|---|---|
| Hibbert's ketones | G*-$CHOHCOCH_3$ | 0.048 |
| | G*-$COCOCH_3$ | 0.029 |
| | G*-$CH_2COCH_2OH$ | 0.028 |
| | G*-$COCHOHCH_3$ | 0.025 |
| | G*-$CH_2COCH_3$ | 0.016 |

Ref: Larsson et al. (1999)

# Comparison of Inhibitor Suppression Strategies

Dilute acid pretreated spruce

| Method | Fermentable sugars (glucose + mannose) | Levulinic acid | Acetic acid | Formic acid | Furfural | 5-HMF | Total phenolic content (GC-MS) | Total phenolic content (Prussian blue)[b] |
|---|---|---|---|---|---|---|---|---|
| None | 97 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| pH 10 (NaOH) | 96 | 100 | 100 | 100 | 81 | 84 | 82 | 96 |
| pH 10 ($Ca(OH)_2$) | 96 | 100 | 100 | 100 | 80 | 78 | 81 | 81 |
| pH 5.5, 0.1% sulfite | 96 | 100 | 100 | 100 | 100 | 100 | 92 | ND |
| pH 10, 0.1% sulfite | 95 | 100 | 100 | 100 | 88 | 89 | 96 | ND |
| pH 5.5, 1% sulfite | 96 | 100 | 100 | 100 | 61 | 63 | 92 | ND |
| pH 10, 1% sulfite | 96 | 100 | 100 | 100 | 47 | 48 | 81 | ND |
| Evaporation of 10% | 97 | 100 | 100 | 100 | 63 | 100 | 92 | 99 |
| Evaporation of 90% | 97 | 100 | 35 | 26 | 0 | 96 | 98 | 85 |
| pH 5.5, anion exchange | 92 | 14 | 11 | 8 | 69 | 74 | 31 | 29 |
| pH 10, anion exchange | 74 | 7 | 4 | 3 | 27 | 30 | 9 | 9 |
| Laccase | 96 | 100 | 100 | 100 | 100 | 100 | 20 | 7 |
| Laccase control | 96 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *T. reesei* | 65 | 100 | 100 | 100 | 15 | 75 | 94 | 95 |
| *T. reesei* control | 94 | 100 | 100 | 100 | 24 | 81 | 100 | 100 |

Ref: Larsson et al. (1999)

# Comparison of Inhibitor Suppression Strategies

Dilute acid pretreated spruce

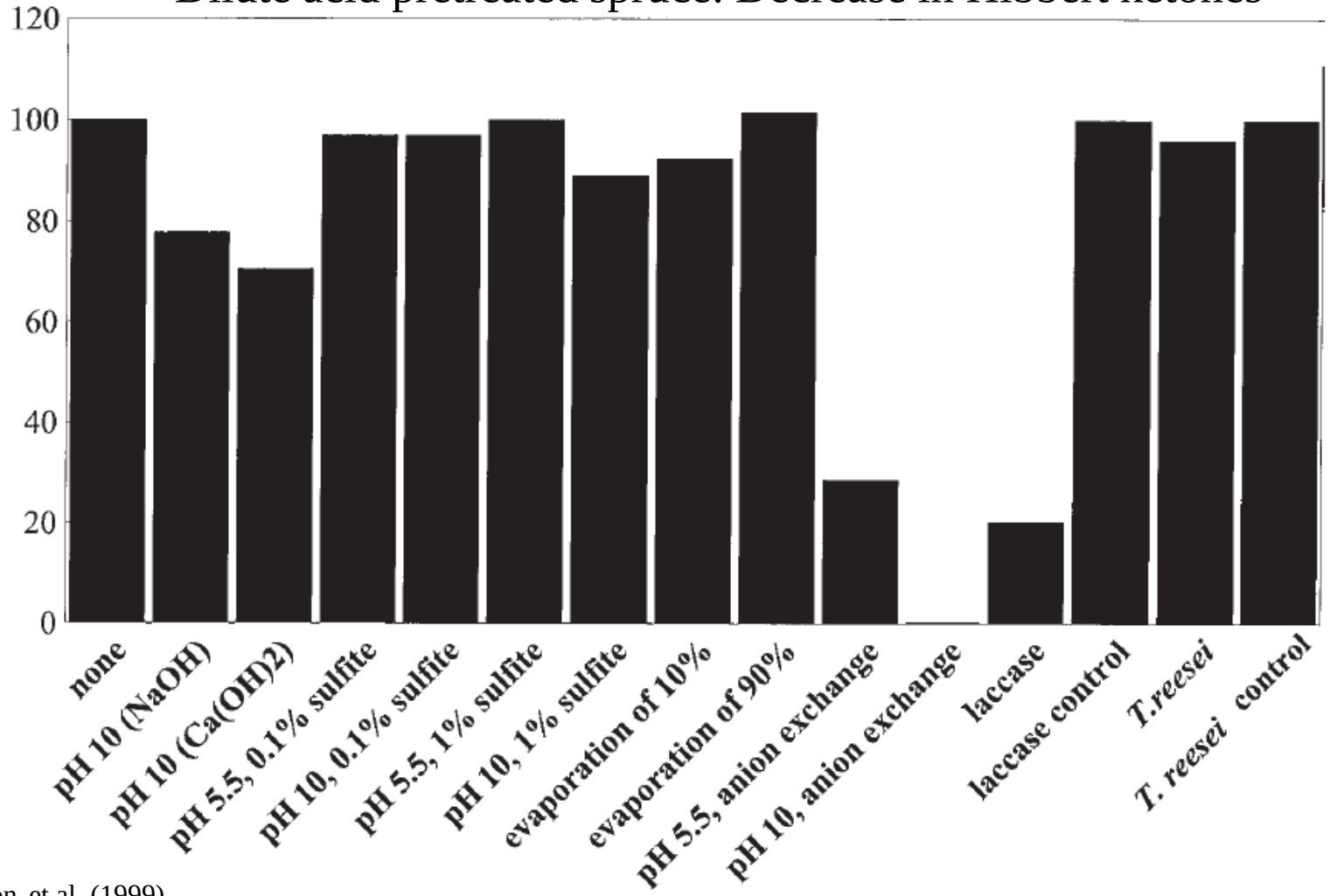
| No | Detoxification method | Y EtOH (g/g) | Q 6 h (g/L/h) | Yx (g/g) |
|---|---|---|---|---|
| 1 | Reference fermentation | 0.45 ± 0.004 | 1.46 ± 0.021 | 0.090 ± 0.001 |
| 2 | None[a] | 0.32 ± 0.004 | 0.04 ± 0.015 | 0.010 ± 0.001 |
| 3 | pH 10 (sodium hydroxide) | 0.42 ± 0.005 | 0.46 ± 0.019 | 0.015 ± 0.001 |
| 4 | pH 10 (calcium hydroxide) | 0.44 ± 0.007 | 1.21 ± 0.065 | [b] |
| 5 | pH 5.5, 0.1% sulfite | 0.34 ± 0.006 | 0.09 ± 0.035 | 0.020 ± 0.001 |
| 6 | pH 10, 0.1% sulfite | 0.42 ± 0.004 | 0.45 ± 0.015 | 0.030 ± 0.001 |
| 7 | pH 5.5, 1% sulfite | 0.42 ± 0.005 | 0.45 ± 0.017 | 0.020 ± 0.001 |
| 8 | pH 10, 1% sulfite | 0.43 ± 0.004 | 0.47 ± 0.016 | 0.040 ± 0.002 |
| 9 | Evaporation of 10% | 0.34 ± 0.006 | 0.06 ± 0.017 | 0.015 ± 0.001 |
| 10 | Evaporation of 90% | 0.42 ± 0.010 | 0.33 ± 0.026 | 0.030 ± 0.002 |
| 11 | pH 5.5, anion exchange | 0.45 ± 0.008 | 0.66 ± 0.052 | 0.060 ± 0.010 |
| 12 | pH 10, anion exchange | 0.49 ± 0.010 | 1.42 ± 0.040 | 0.080 ± 0.005 |
| 13 | Laccase | 0.47 ± 0.004 | 0.68 ± 0.007 | 0.055 ± 0.003 |
| 14 | Laccase control | 0.32 ± 0.006 | 0.05 ± 0.032 | 0.012 ± 0.001 |
| 15 | *T. reesei* | 0.43 ± 0.005 | 0.55 ± 0.070 | 0.055 ± 0.005 |
| 16 | *T. reesei* control | 0.42 ± 0.006 | 0.17 ± 0.035 | 0.030 ± 0.003 |

[a]pH was adjusted to 5.5 with NaOH prior to the fermentation.
[b]Not determined owing to the precipitation.

Ref: Larsson et al. (1999)

# Comparison of Inhibitor Suppression Strategies

Dilute acid pretreated spruce: Decrease in Hibbert ketones




Ref: Larsson et al. (1999)

Thank you

# Lecture Sixteen

# Cellulosic Ethanol Production: Cellulases and Product Recovery

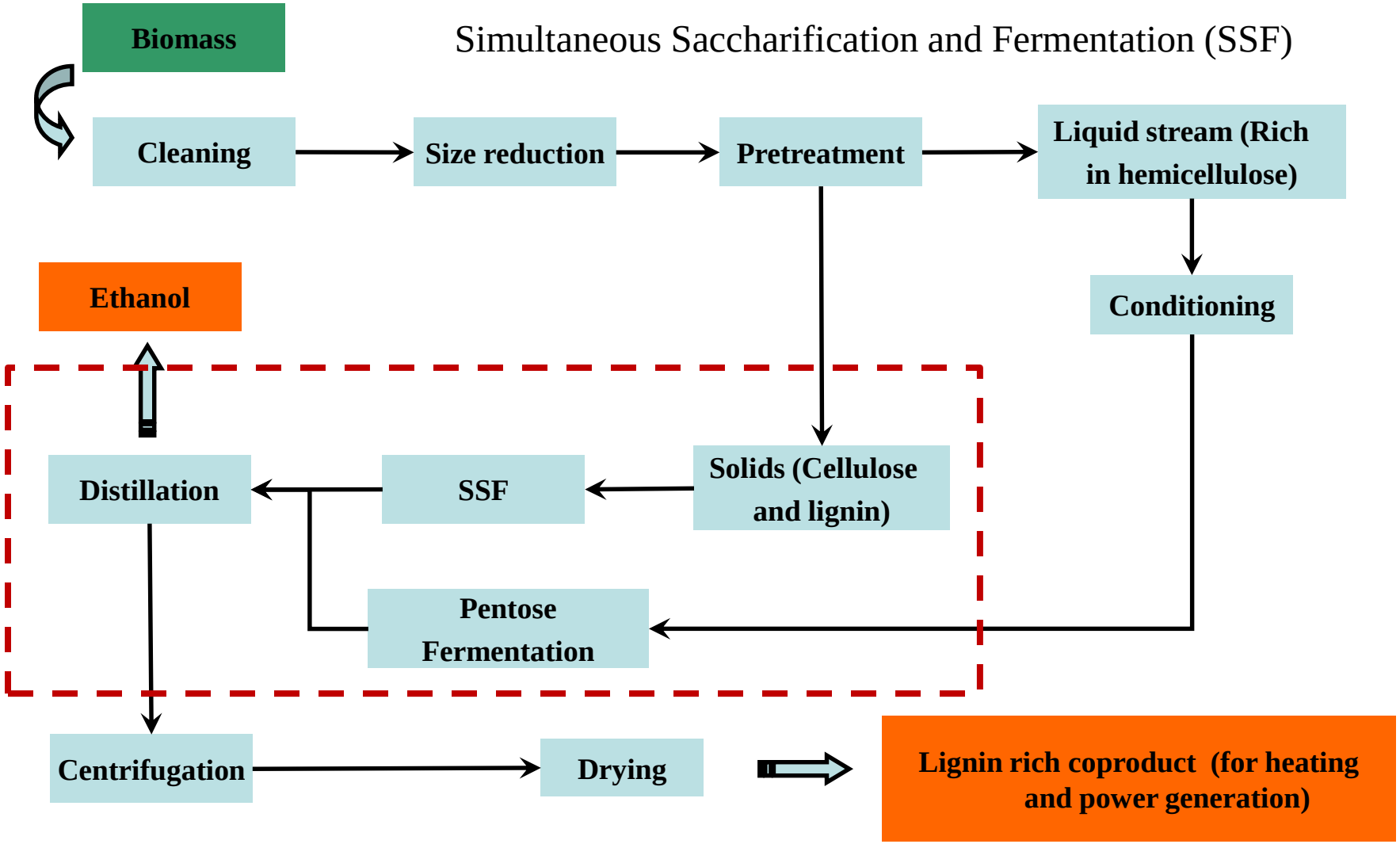
# Pretreated Biomass Conditioning/Detoxification

Removal of inhibitors and toxic compounds is an important downstream step in cellulosic biomass processing. Some of the methods are:

- Evaporation:
- Extraction with organic solvents
- Ionic exchange resins
- Adsorption on activated charcoal, molecular sieves
- *Alkaline Detoxification (Over liming)*
- Combined alkaline and sulfite detoxification
- Enzymatic detoxification
- Microbial detoxification

Ref: Sanchez and Cardona (2008), Palmqvist et al. (2000)

# Cellulosic Ethanol Production

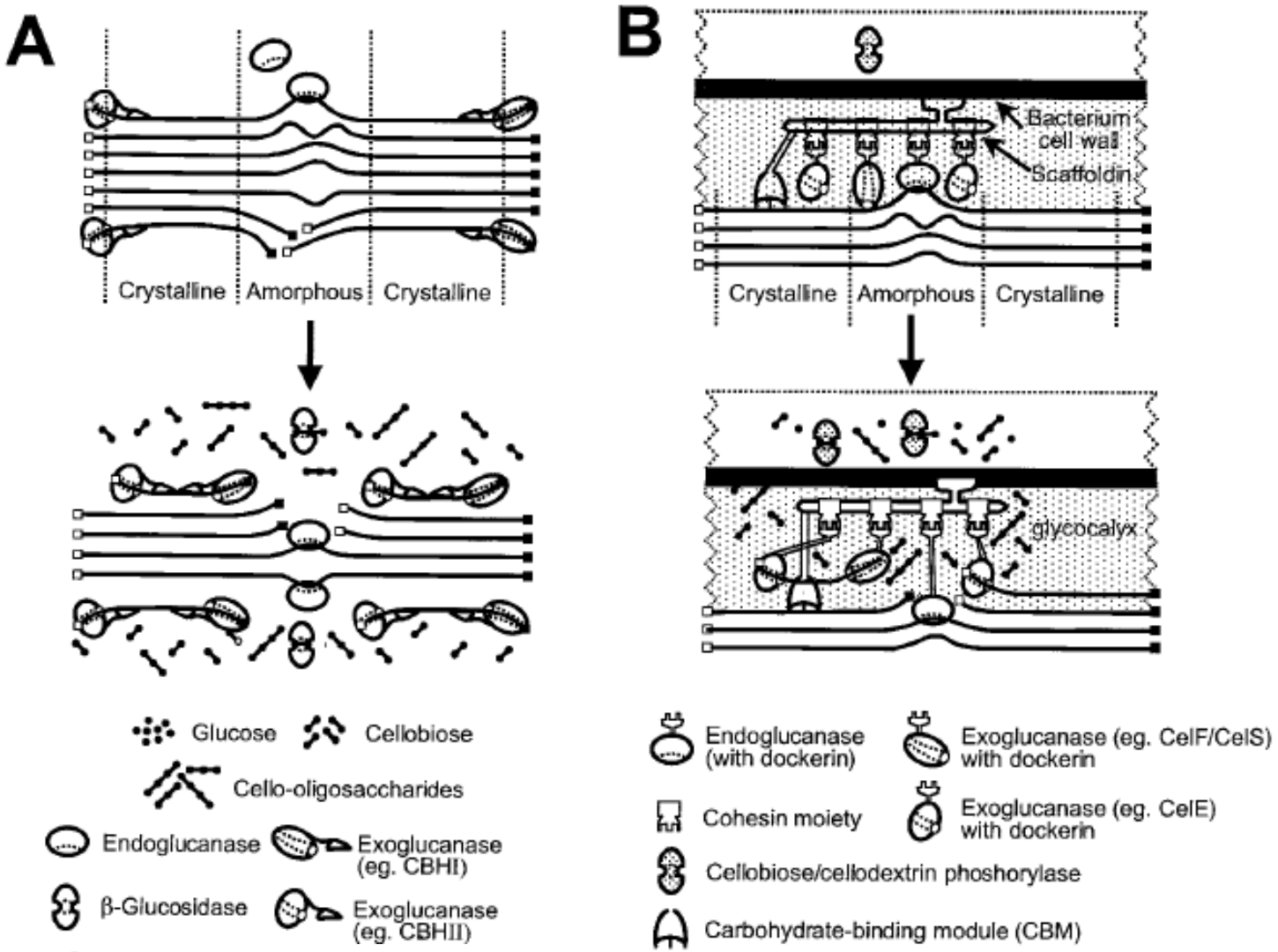


# Cellulase Enzymes

- Endo Cellulases: Facilitate hydrolysis by exposing cellulose chains and disrupting the crystalline structure
- Exo cellulases (Cellobiohydrolases or CBH I and II): They further hydrolyze cellulose and yield cellobiose (a disaccharide)
- Cellobiase: These enzymes hydrolyze cellobiose to glucose.
- Oxidative cellulases: “Depolymerize cellulose by radical reactions”
- Cellulose phosphorylases: “Depolymerize cellulose using phosphates instead of water”
- Processive and non-processive cellulases

Ref: Wilson, D.B. (2009, 2011); Gowen and Fong (2010)

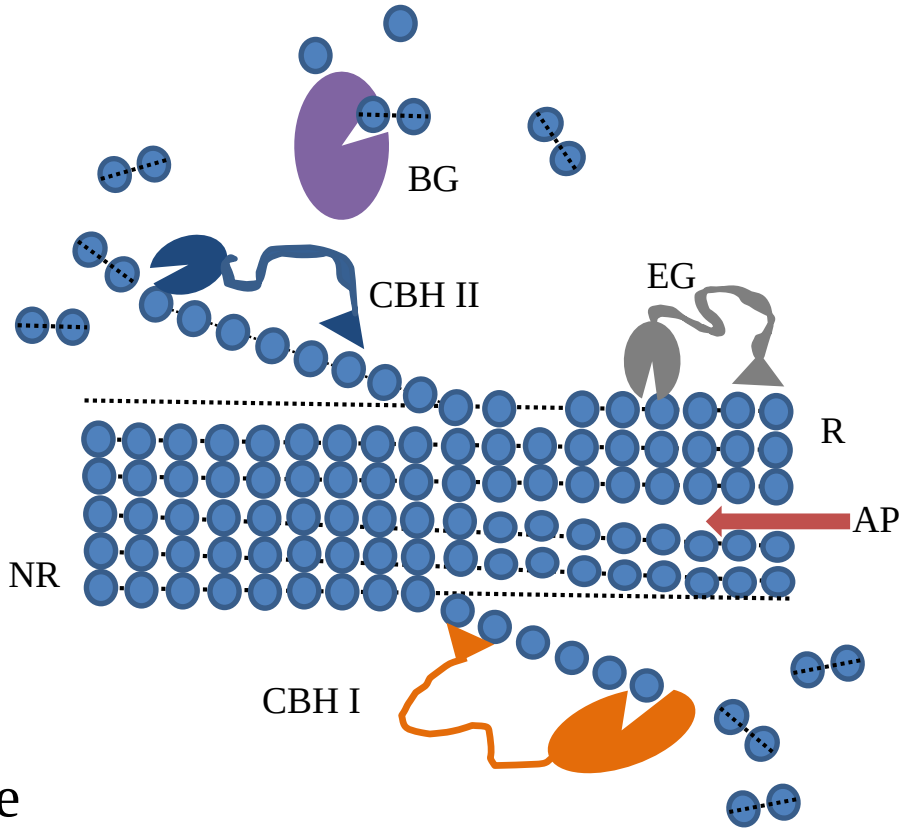
# Cellulase Enzymes




Ref: Lynd et al. 2002

# Cellulase Enzymes

- Endoglucanases (EG) act on internal chains to create additional chains.
- Exoglucanase: CBH I act from the reducing ends (R)
- Exoglucanase: CBH II act from the non-reducing ends (NR).
- Betaglucosidase (BG) acts of the cellobiose/cellodextrins to produce glucose.
- Accessory proteins (AP) facilitate the hydrolysis through a currently unknown mechanism.



# Cellulase Enzymes Classification

- All cellulolytic enzymes have two enzyme nomenclatures : EC 3.2.1.4 and 3.2.1.9
- Cellulases are further classified based on their catalytic domain (CD) and carbohydrate binding module/domain(CBM/D).
- About 70 structural families of glycosyl hydrolases (EC 3.2.1)
- CD classification (represented using Arabic numerals).
  - Cellulases and hemicellulases are assigned to families: 5,6,7,8,9,10,11,12,26,44,45,48,51,60 and 61.

    *ex. Trichoderma reesei* CBHI is *T. reesei Cel7A*

Ref: Rabinovich et al. (2002); Wilson (2008;2010)

# Cellulase Enzymes Classification

- CBM classification (represented using Roman numerals).
  - There are as many as 45 structural families of CBM
- CBM I: about 30 amino acids. Found in fungal cellulases
- CBM II: about 120 amino acids. Found in aerobic bacteria cellulases
- CBM III: These are cellulosomal scaffoldins
- Many cellulases contain multiple CBMs.

Ref: Rabinovich et al. (2002); Wilson (2008;2010)

# Enzymes

## Cellulase producing fungi in nature

| Fungi | Fungi |
|---|---|
| *Acremonium cellulolyticus* | *Talaromyces emersonii* |
| *Aspergillus acculeatus* | *Thielavia terrestris* |
| *Penicillium funmiculosum* | *Trichoderma koningii* |
| *Phanerochaete* | *Trichoderma reesei* |
| *chrysosporium* | *Trichoderma viride* |
| *Schizophyllum commune* | *Aspergillus fumigatus* |
| *Sclerotium rolfsii* | *Aspergillus niger* |
| *Sporotrichum cellulophilum* | *Fusarium solani* |

Ref:http://www.fao.org/docrep/w7241e/w7241e08.htm

# Cellulase Enzymes in *Trichoderma reesei*

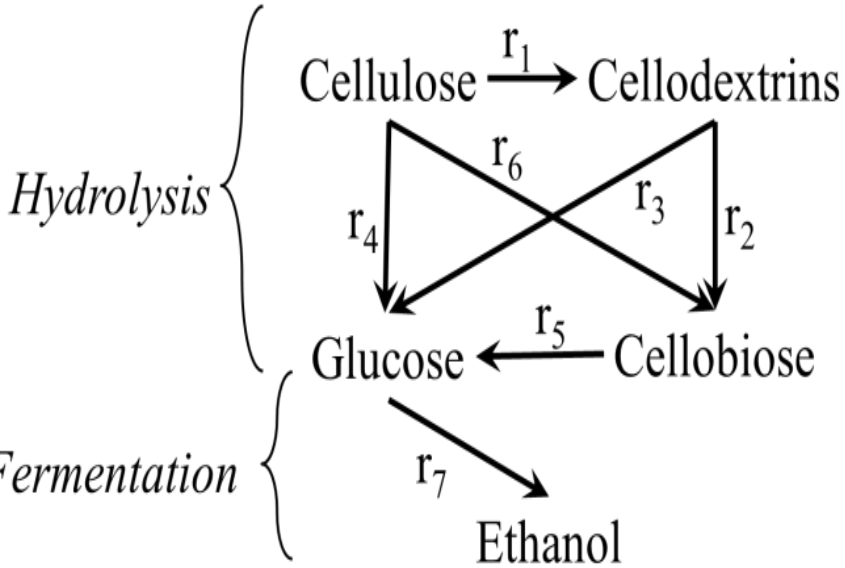
- Highly glycosylated.
- Cel7A (CBH I) : about 70% of the cellular protein secreted.
- Cel6A (CBH II) : about 10% of the cellular protein secreted.
- Seven endoglucanases (Cel7B (EGL I), Cel5A (EGL II), Cel12A (EGL III), Cel61A (EGL IV), cel45A (EGLV), Cel5B and Cel61B
  - All except Cel12A, Cel5B and *Cel61B* have Family I CBM.
  - Cel12A has also shown expansin activity.
  - Swollenin is also secreted.

Ref: Wilson (2008;2010)

# Cellulose Hydrolysis by Cellulase Enzymes

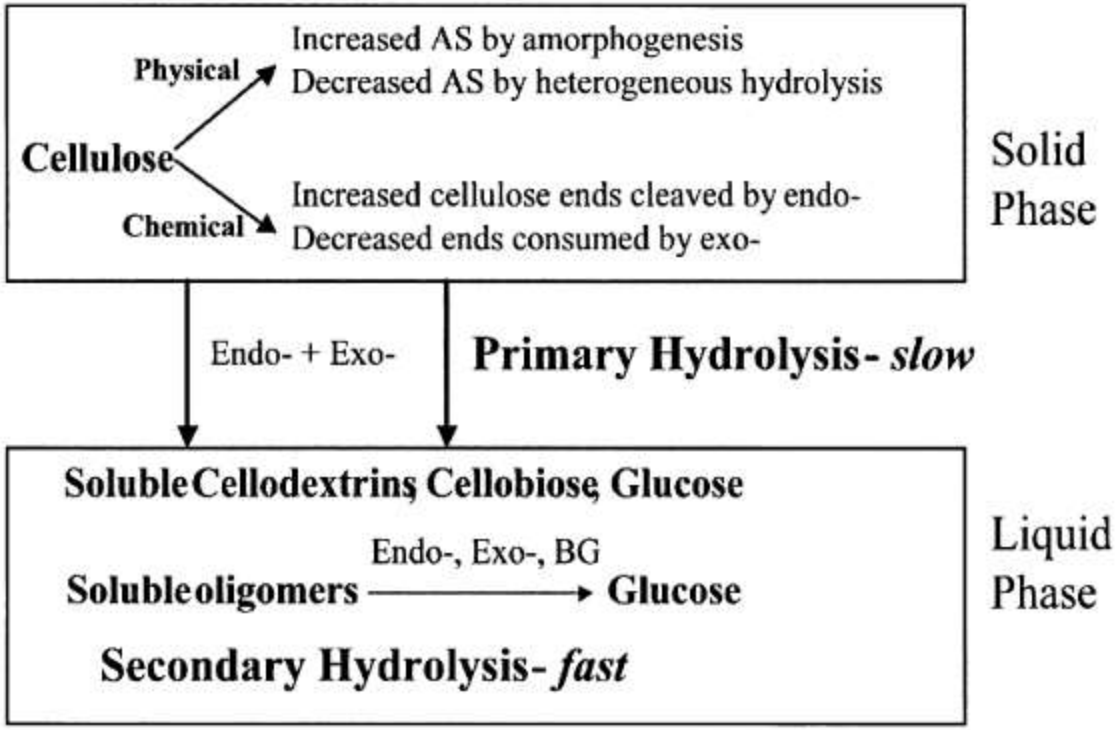
Reactions catalyzed by enzyme classes:

1. Processive EG: $r_1$
2. Non-processive EG: $r_1$
3. Processive CBH I : $r_2$, $r_3$, $r_4$, $r_6$
4. Non-processive CBH I : $r_2$, $r_3$, $r_4$, $r_6$
5. Processive CBH II: $r_2$, $r_3$, $r_4$, $r_6$
6. Non-processive CBH II: $r_2$, $r_3$, $r_4$, $r_6$
7. BG: $r_5$
8. Fermentation: $r_7$



# Cellulase Enzymes

Enzymatic hydrolysis mechanism hypothesis



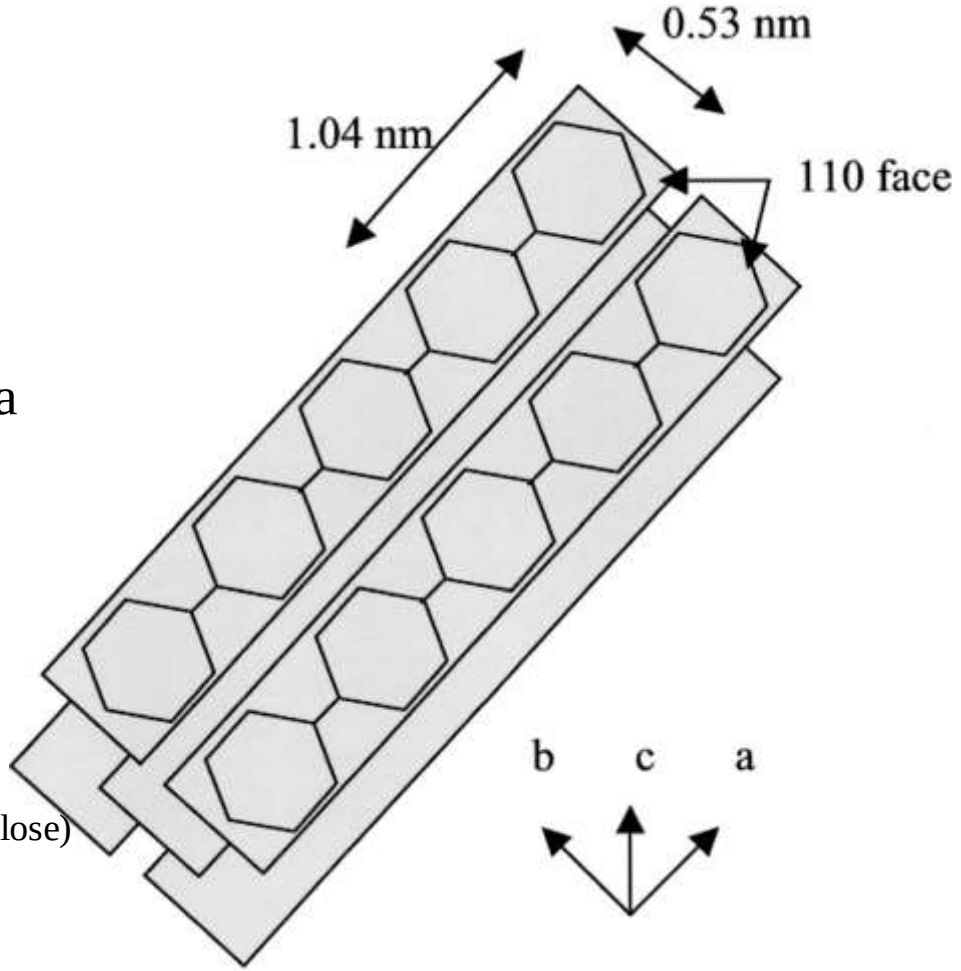

Ref: Zhang and Lynd (2004)

# Cellulase Enzymes

Factors effecting cellulose hydrolysis:

- Crystallinity Index
- Degree of polymerization
- Accessibility
  - Internal and external surface area
- Cellulase adsorption

$$E_a = \frac{A_{max} S K_p E_f}{1 + K_p E_f}$$




$E_a$ = Adsorbed cellulose (mg cellulase/ L)

$A_{max}$ = Maximum cellulose adsorption (mg cellulase/ g cellulose)

$S$ = Cellulose concentration (g/L)

$E_f$ = Free cellulase (mg cellulase/ L)

$K_p$ = Dissociation constant (L/g cellulose)

Ref: Zhang and Lynd (2004)

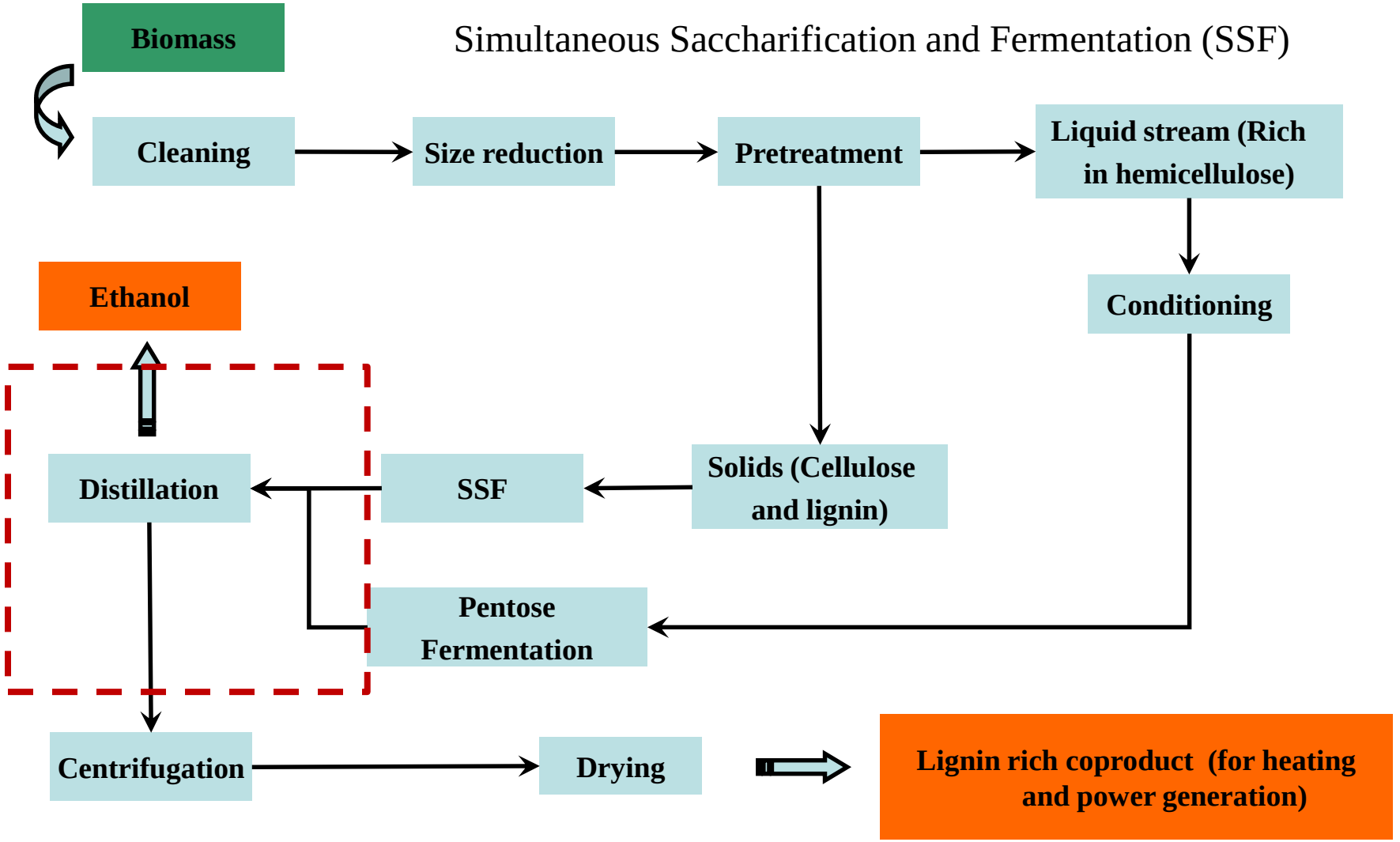
# Cellulase Enzymes

Factors effecting cellulose hydrolysis:

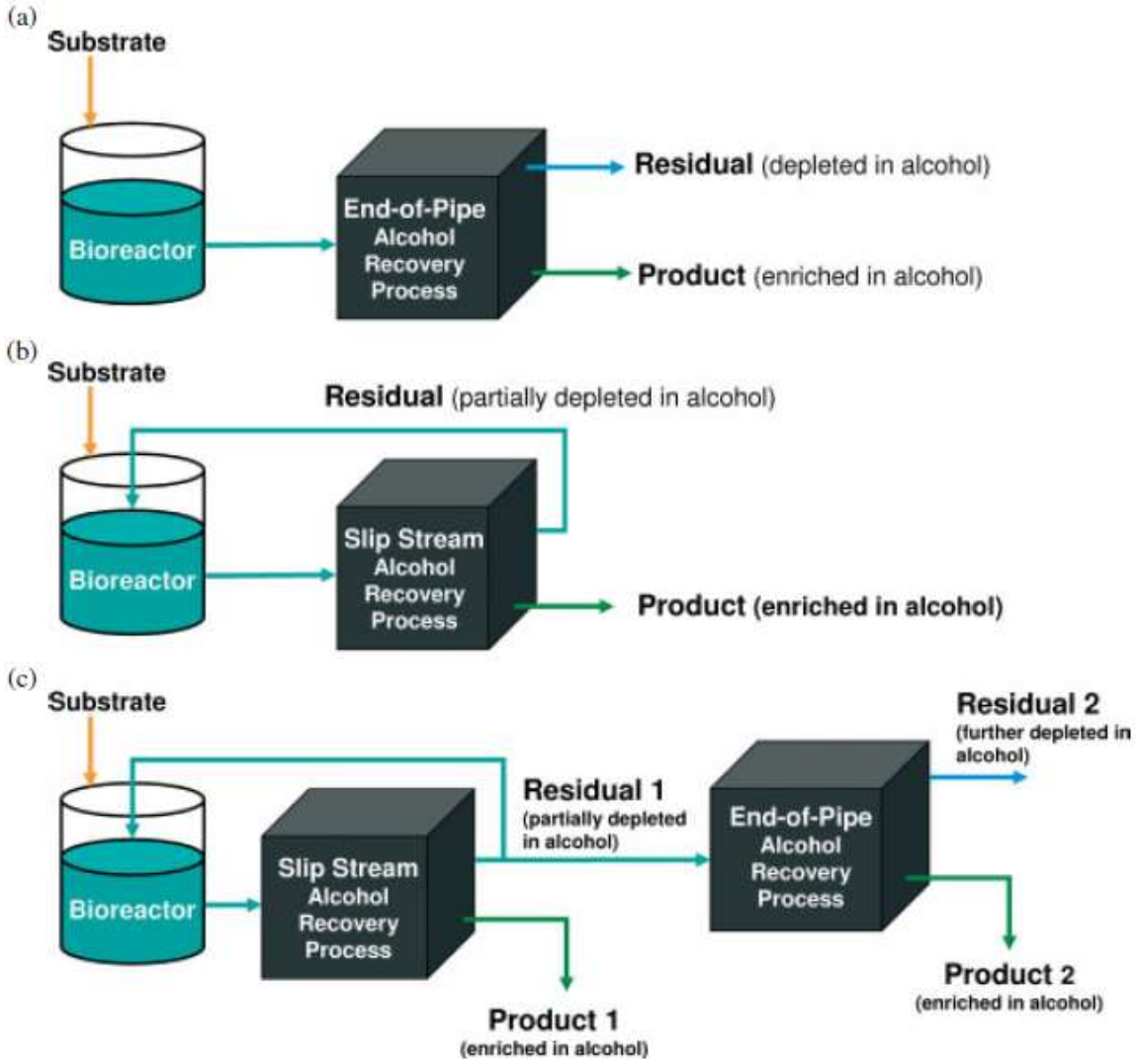
- Langmuir Adsorption model inconsistencies:
  - Partially reversible cellulase adsorption.
  - Interaction among adsorbing cellulase components.
  - Multiple adsorption sites.
  - Cellulase entrapment by pores in cellulose.
  - Multicomponent cellulase adsorptions.
  - Interference with lignin
  - Lignin adsorption is inversely proportional whereas cellulase adsorption is proportional to pretreatment temperature.
- Intensity of agitation has little effect on cellulose hydrolysis.
- Area occupied is by cellulase is larger than area of repeating cellobiose lattice.

Ref: Zhang and Lynd (2004)

# Cellulosic Ethanol Production



# Ethanol Recovery Technologies

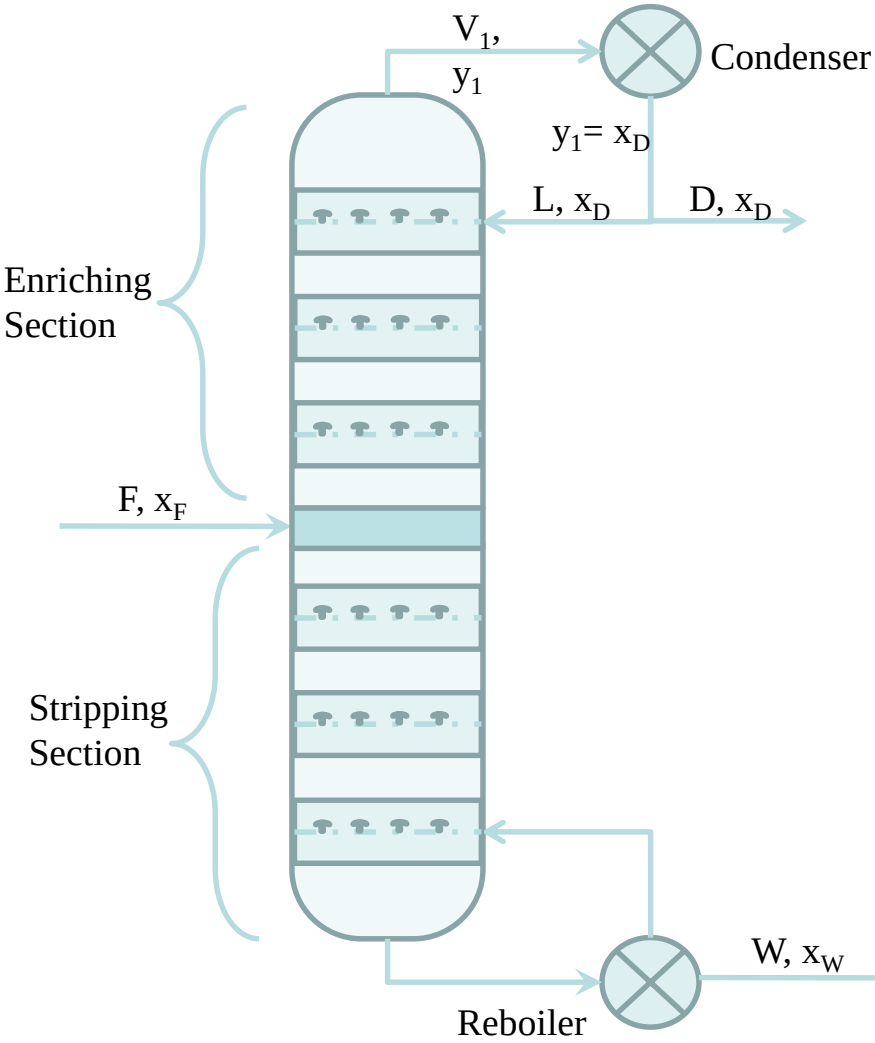


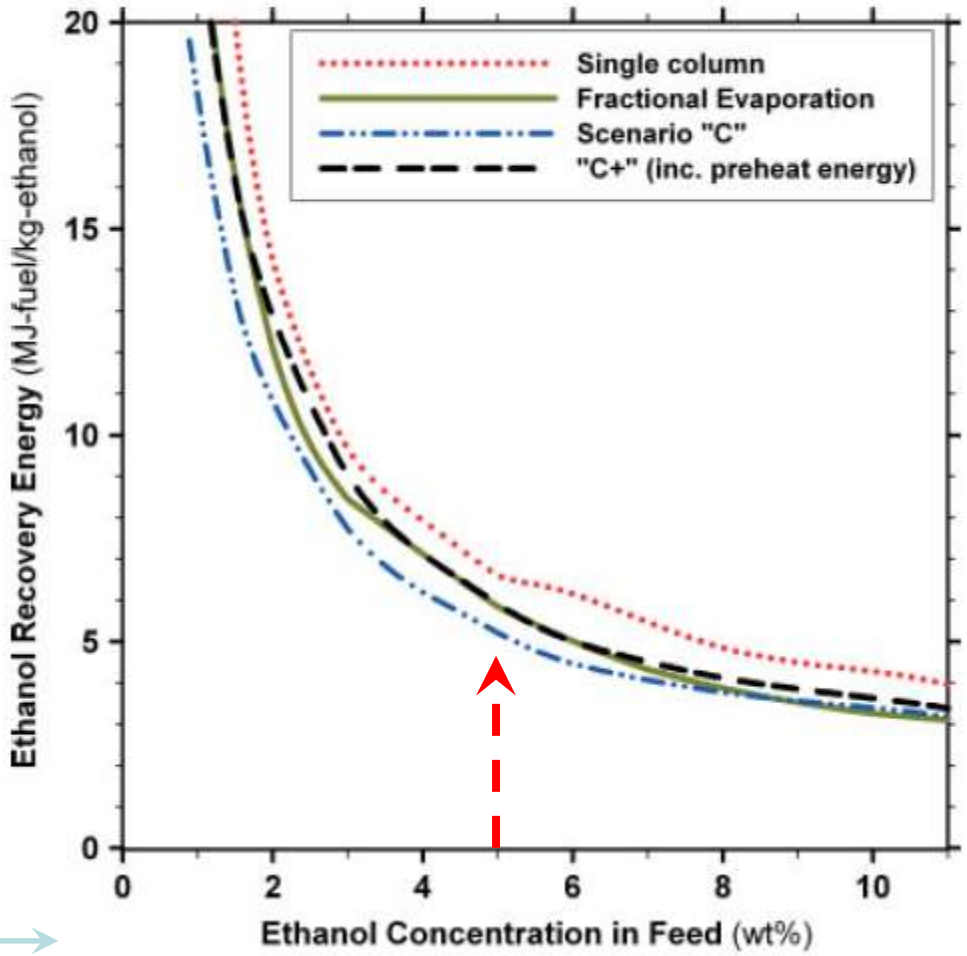

Ref: Vane (2008)

# Ethanol Recovery Technologies

- Distillation
- Gas/steam stripping
- Liquid Liquid extraction
- Adsorption
- Pervaporation

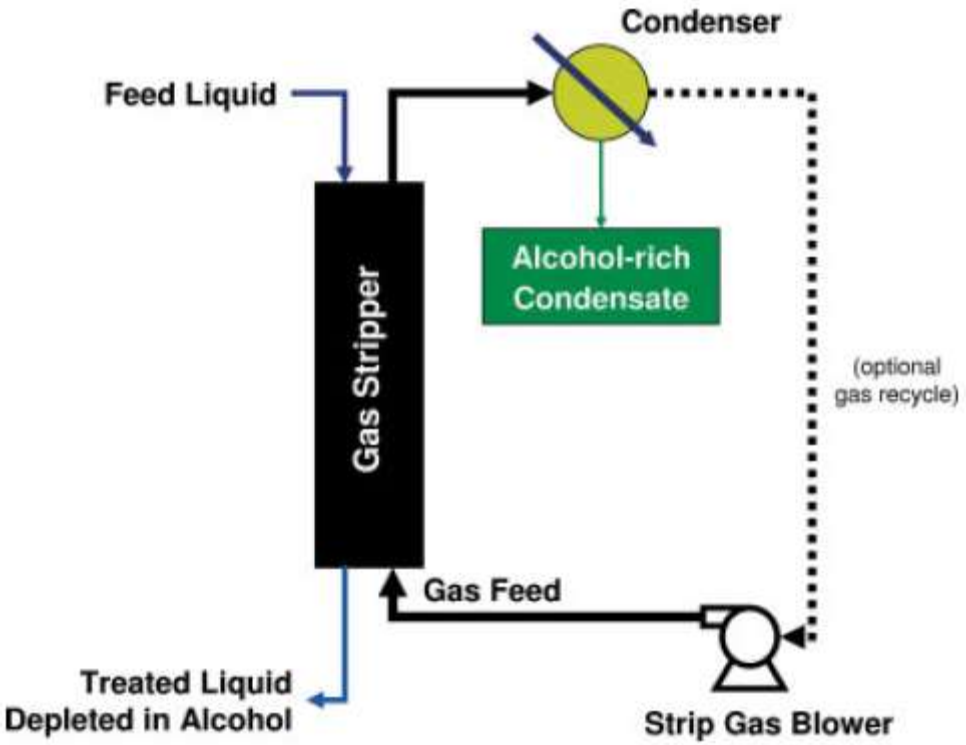
# Ethanol Recovery Technologies: Distillation



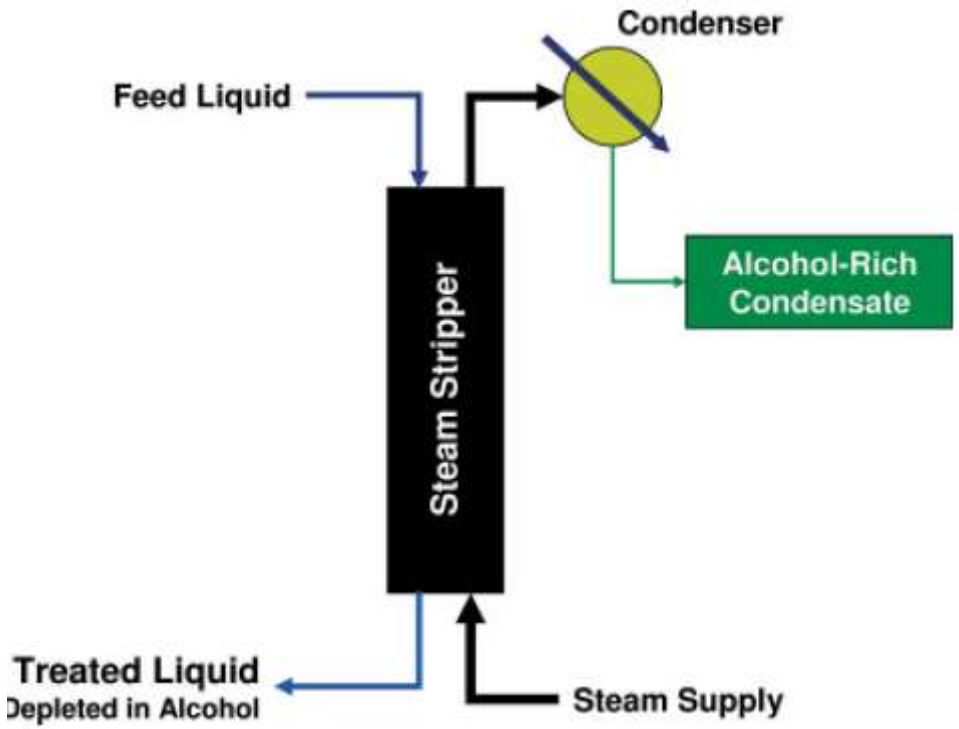

Ref: Vane (2008)

# Ethanol Recovery Technologies: Gas stripping




Ref: Vane (2008)

# Ethanol Recovery Technologies: Steam stripping

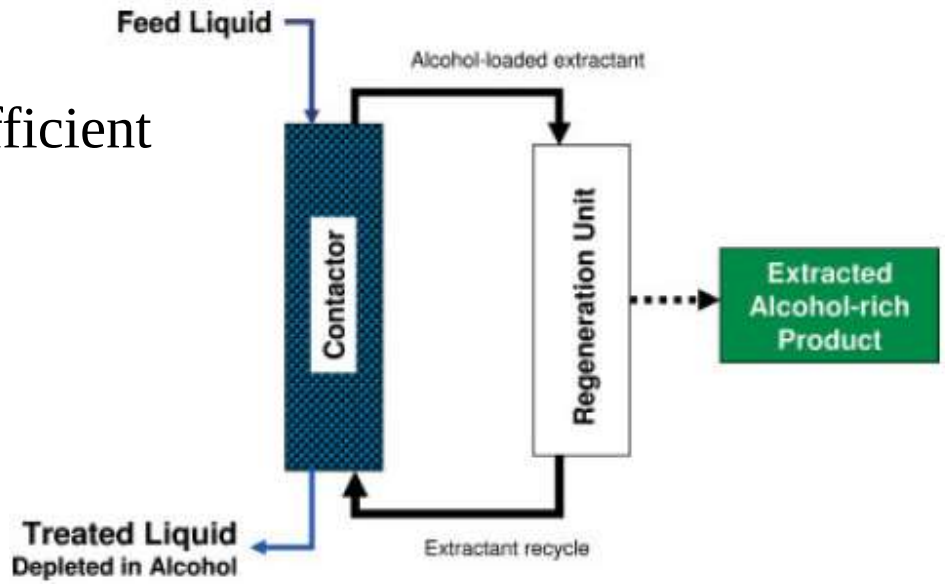



Ref: Vane (2008)

# Ethanol Recovery Technologies: Liquid-Liquid extraction

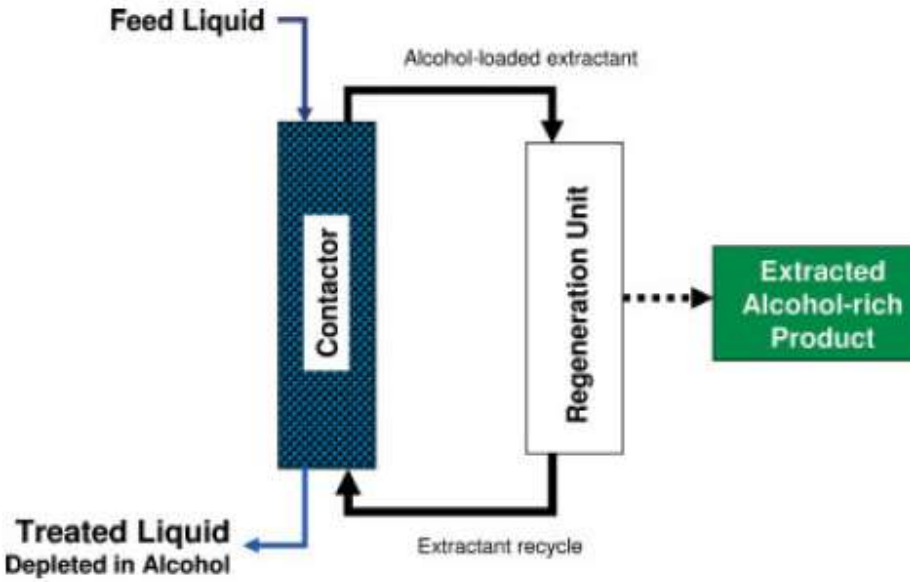
Factors influencing the liquid-liquid separation

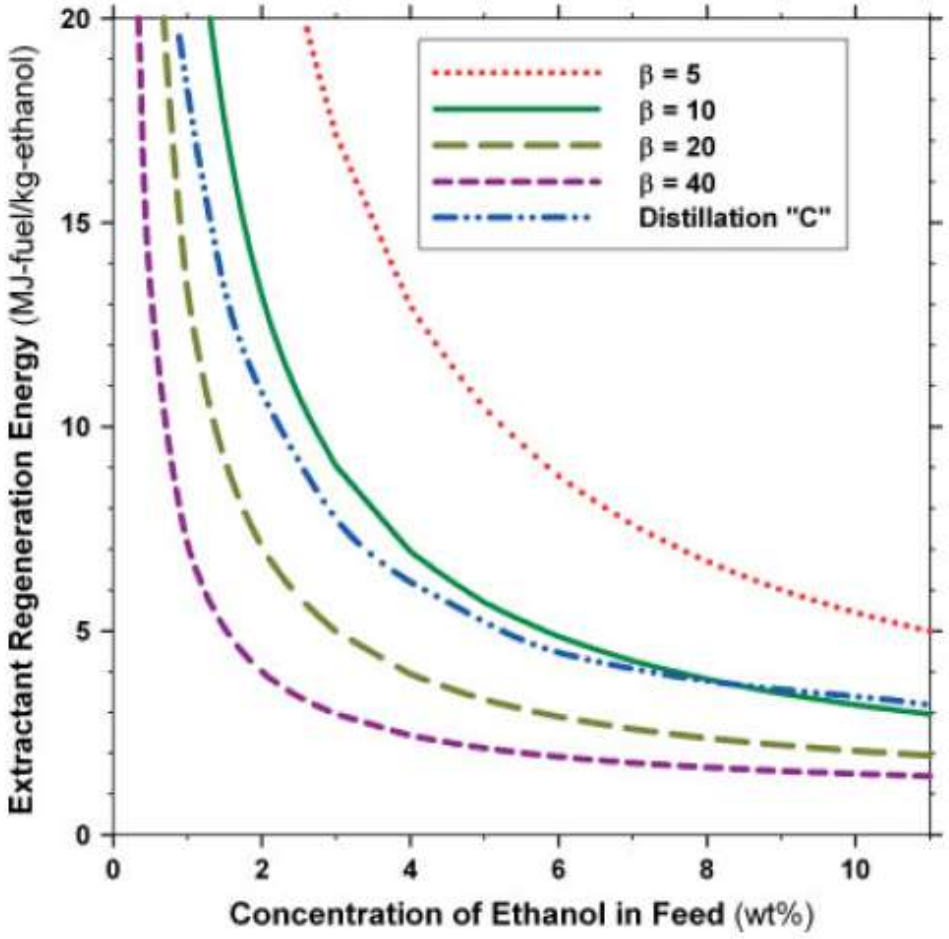
- Selectivity
- Equilibrium distribution coefficient
- Mutual solubility of solvents
- Interfacial tension
- Extractant viscosity
- Toxicity and safety issues
- Reactivity
- Volatility
- Cost




Ref: Vane (2008)

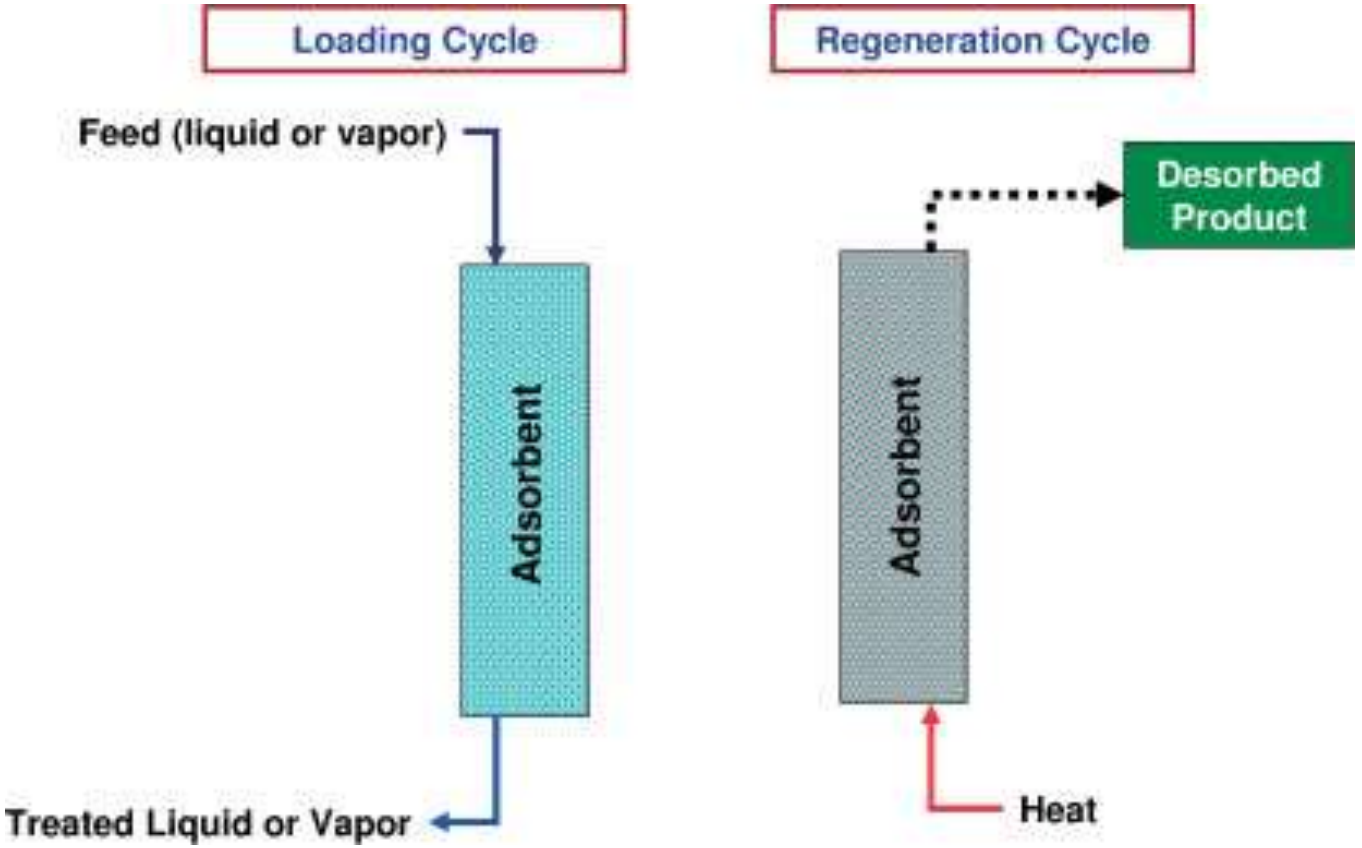
# Ethanol Recovery Technologies: Liquid-Liquid extraction




Ref: Vane (2008)

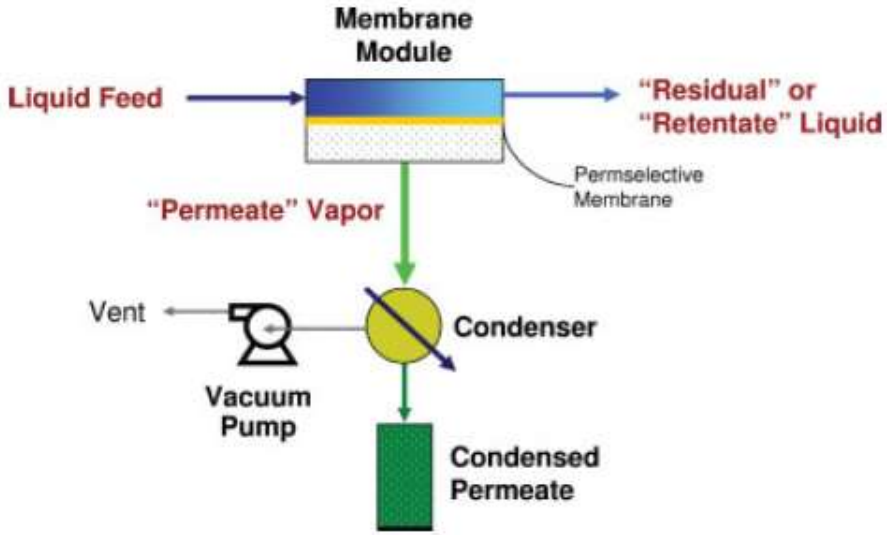
# Ethanol Recovery Technologies: Adsorption

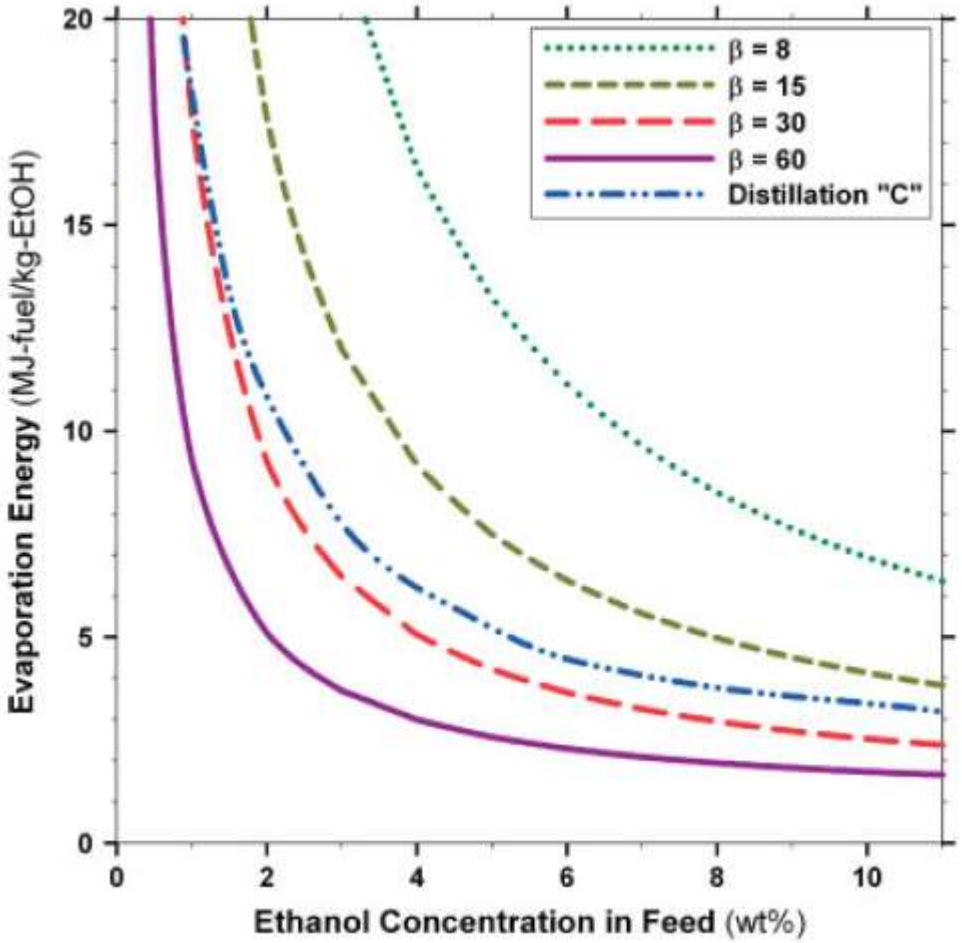



Ref: Vane (2008)

# Ethanol Recovery Technologies: Pervaporation

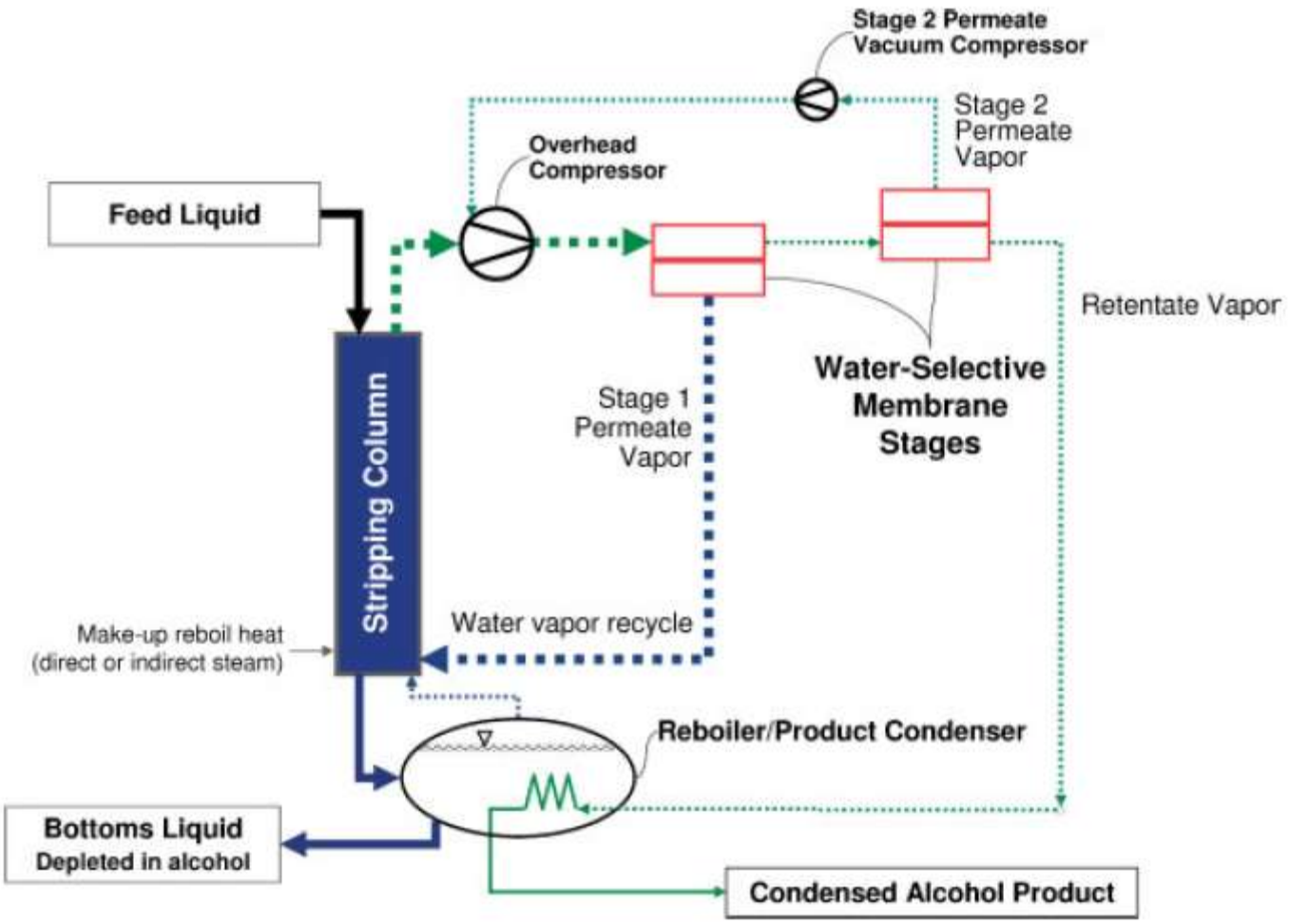
- Hydrophobic porous membranes are used.




Ref: Vane (2008)

# Ethanol Recovery Technologies: MAVS Hybrid Process



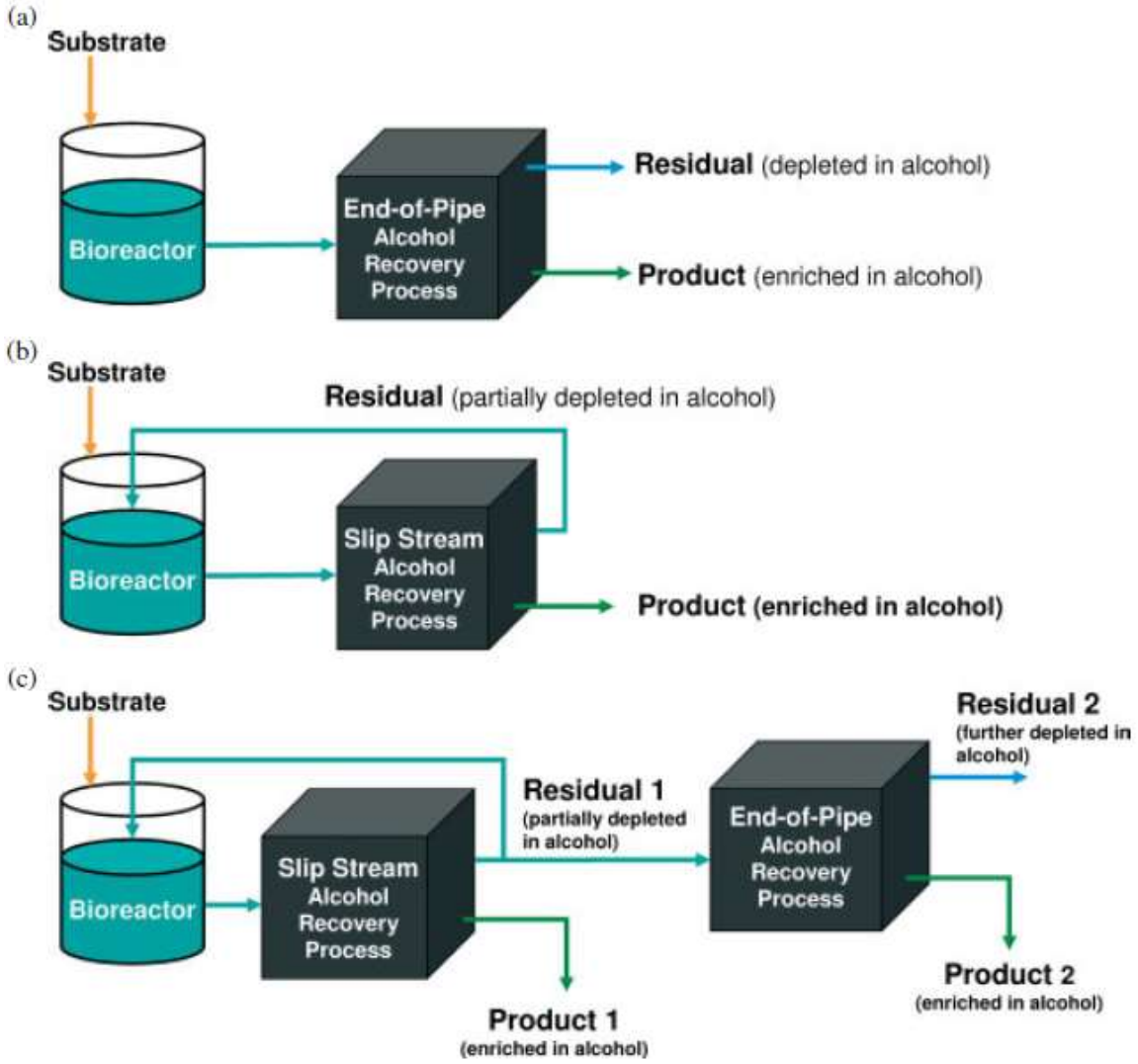

Ref: Vane (2008)

Thank you

# Lecture Seventeen

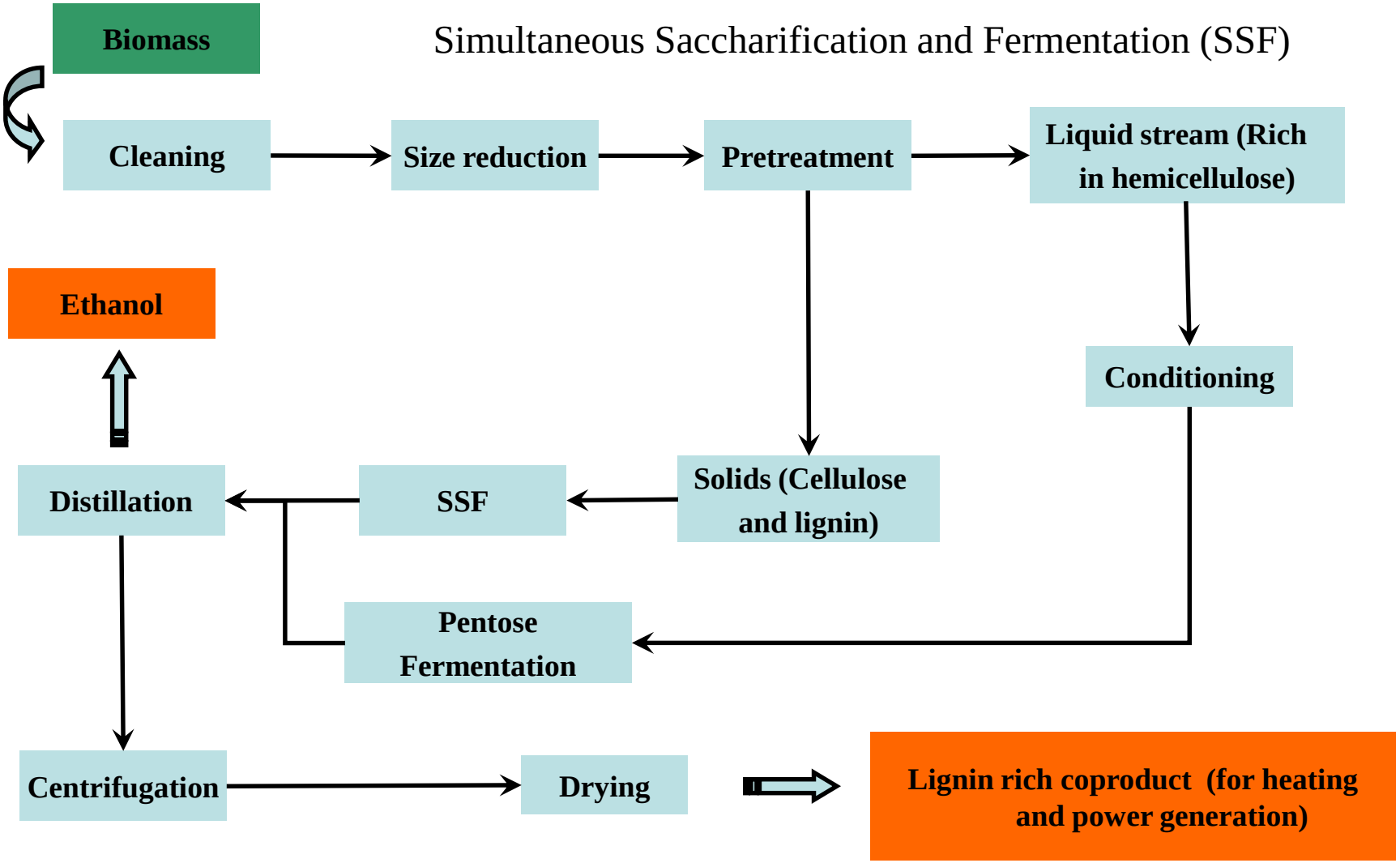
# Cellulosic Ethanol Yield Calculations and Ethanol Process Modeling

# Summary of Lecture Sixteen

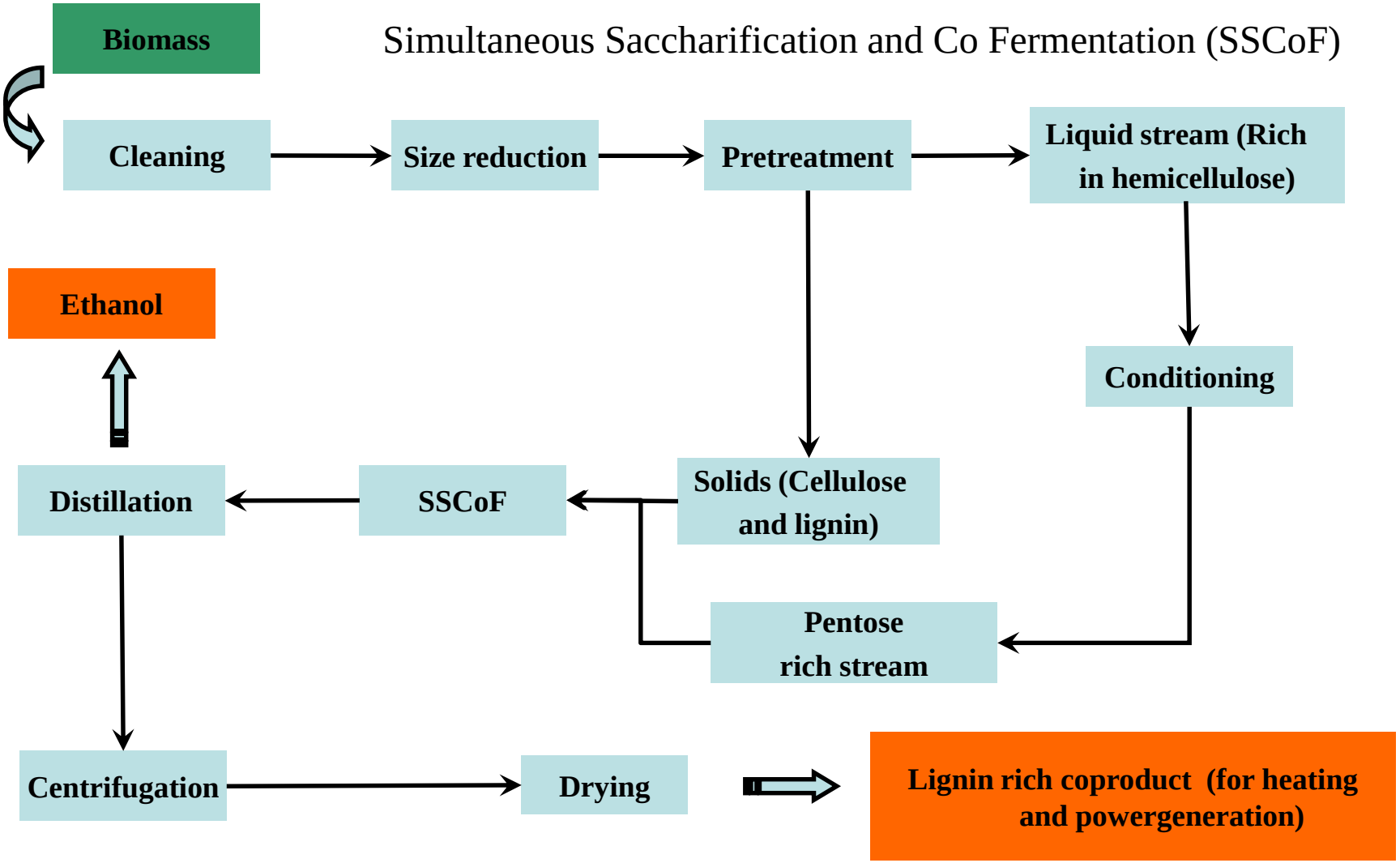



Ref: Vane (2008)

# Generic Cellulosic Ethanol Process



# Generic Cellulosic Ethanol Process



# Ethanol Production: Process Calculations

Hexose hydrolysis

$[C_6H_{10}O_5]_n + (n\text{-}1)\ H_2O \rightarrow n\ [C_6H_{12}O_6]$

(162 g) + (18 g) → (180 g) Hydrolytic gain: 1.11

Pentose hydrolysis

$[C_5H_8O_4]_n + (n\text{-}1)\ H_2O \rightarrow n\ [C_5H_{10}O_5]$

(132 g) + (18 g) → (150 g) Hydrolytic gain: 1.136

Hexose Fermentation

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{Heat}$

(180: 1 g) → (2 x 46: 0.511 g) + (2 x 44: 0.489 g)

Pentose Fermentation

$3C_5H_{10}O_5 \rightarrow 5\ C_2H_5OH + 5CO_2 + \text{Heat}$

(3 x 150: 1 g) → (5 x 46: 0.511 g) + (5 x 44: 0.489 g)

# Ethanol Production: Process Calculations

Feedstock: Wheat straw

Composition:

Glucomannans: 33.7 Xylans: 21.57

Lignin: 16.85 Ash: 10.22 Extractives: 15.19

- Basis : 1 dry ton.
- Hydrolysis efficiency: 80
- Inhibitors generation: 1% from hexoses and 2% from pentoses
- Fermentation efficiency
  - Hexose fermentation: 98
  - Pentose fermentation: 60
- Distillation efficiency: 99

# Ethanol Production: Process Calculations

- Hydrolysis

Hexoses = Biomass* Glucan* $\eta_{hydrolysis}$*Hydrolytic gain*(1-inhibitor production)

Hexoses = 1000* 0.337*0.8*1.11*(1-0.01)=296.26 kg

Inhibitors (HMF) =1000* 0.337*0.8*1.11*(0.01)=2.99 kg

Pentoses=Biomass* Xylans* $\eta_{hydrolysis}$*Hydrolytic gain*(1-inhibitor production)

Pentoses = 1000* 0.2157*0.8*1.136*(1-0.02)= 192.11kg

Inhibitors (F) =1000* 0.2157*0.8*1.136*(0.02)= 3.92kg

- Fermentation

Ethanol = Hexoses * ethanol yield * hexose fermentation efficiency + Pentoses * ethanol yield * pentose fermentation efficiency

Ethanol = 296.26*0.511*.98+192.11*0.511*0.6=207.26 Kg.

# Ethanol Production: Process Calculations

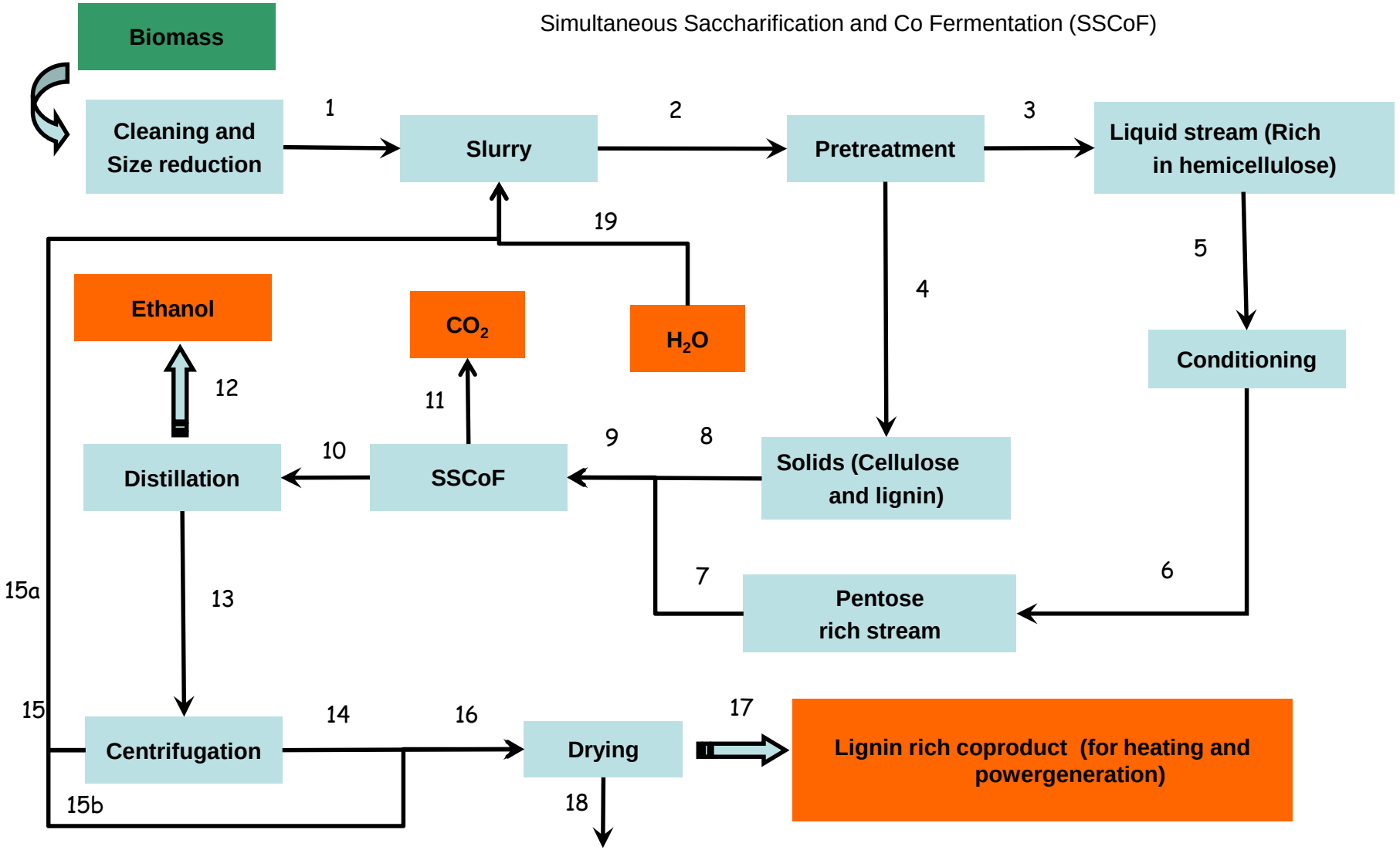
- Distillation

Ethanol (L) = Ethanol (Kg) * distillation efficiency / ethanol density (kg/L)

= 207.26 *0.99 /0.789 = 260 L

- Overall Ethanol yield= 260 L/1000 kg = 0.26 L/Kg = 68 gal/ton
- Total inhibitors= 2.99+3.92=6.91 kg
- Residual lignin coproduct: 1000*0.4226 =422.6 Kg

# Ethanol Production: Process Calculations Using MS Excel



# Ethanol Production: Process Calculations Using MS Excel

Feedstock: Wheat straw

Composition: Glucomannans: 33.7 Xylans: 21.57

Lignin: 16.85 Ash: 10.22 Extractives: 15.19

- Basis : 1 dry ton.
- Pretreatment efficiency: 80
- Hydrolysis efficiency: 80
- Inhibitors generation:
  - From hexoses: 1%
  - From pentoses: 2%
- Fermentation efficiency
  - Hexose fermentation: 98
  - Pentose fermentation: 60
- Distillation efficiency: 99

Thank you