Biofuels production from renewable biomass

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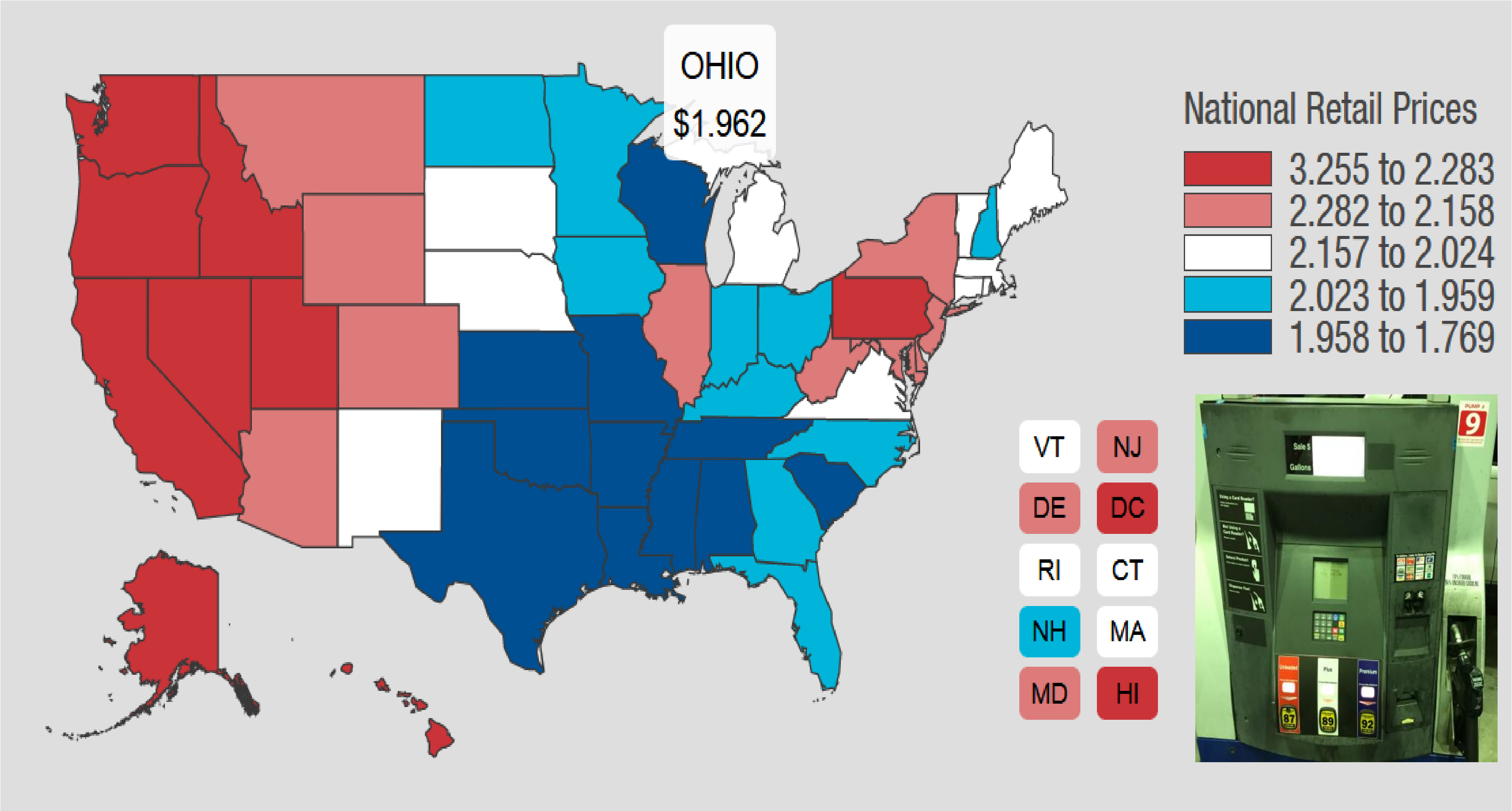
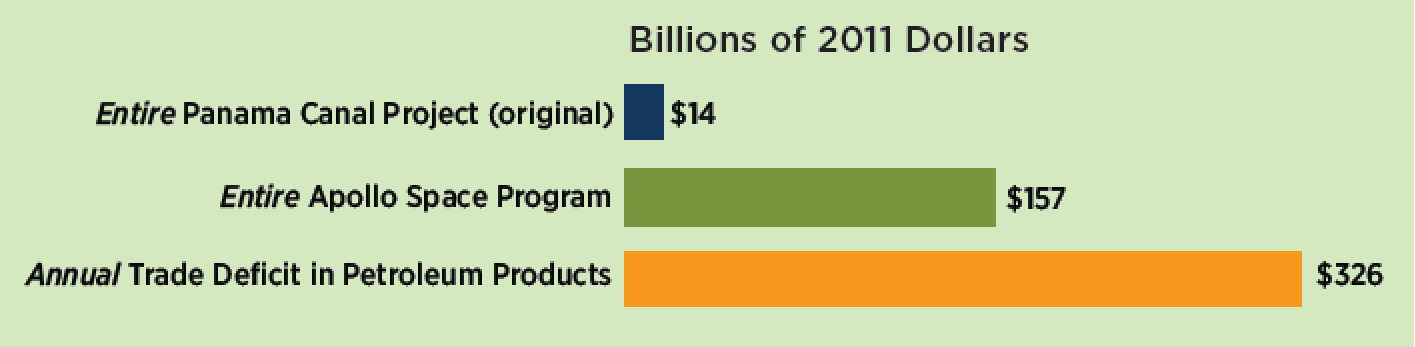
Gasoline and ethanol



Background

- U.S. spending $1 billion per day on foreign oil
- Facing critical disruptions in oil supply
- Generating economic uncertainties
- Influencing national security



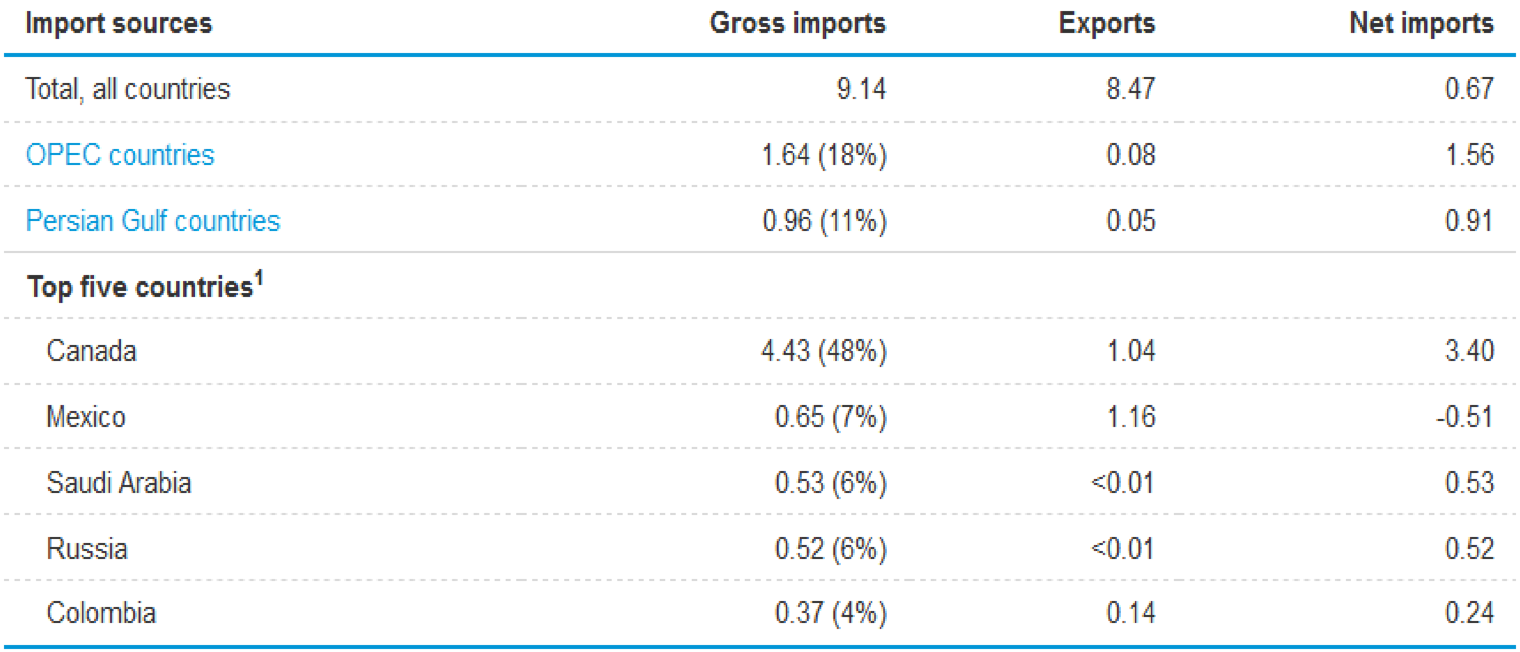
Drivers-why biofuels and bioproducts

- Increase energy security and reduce the nation's dependence on foreign oil
- Reduce greenhouse gas (GHG) emission
- Enhance sustainability of liquid fuels
- Create new economic opportunities and jobs
- Utilize 1 billon tons of renewable biomass (U.S.)

http://www.nrel.gov/biomass/biorefinery.html

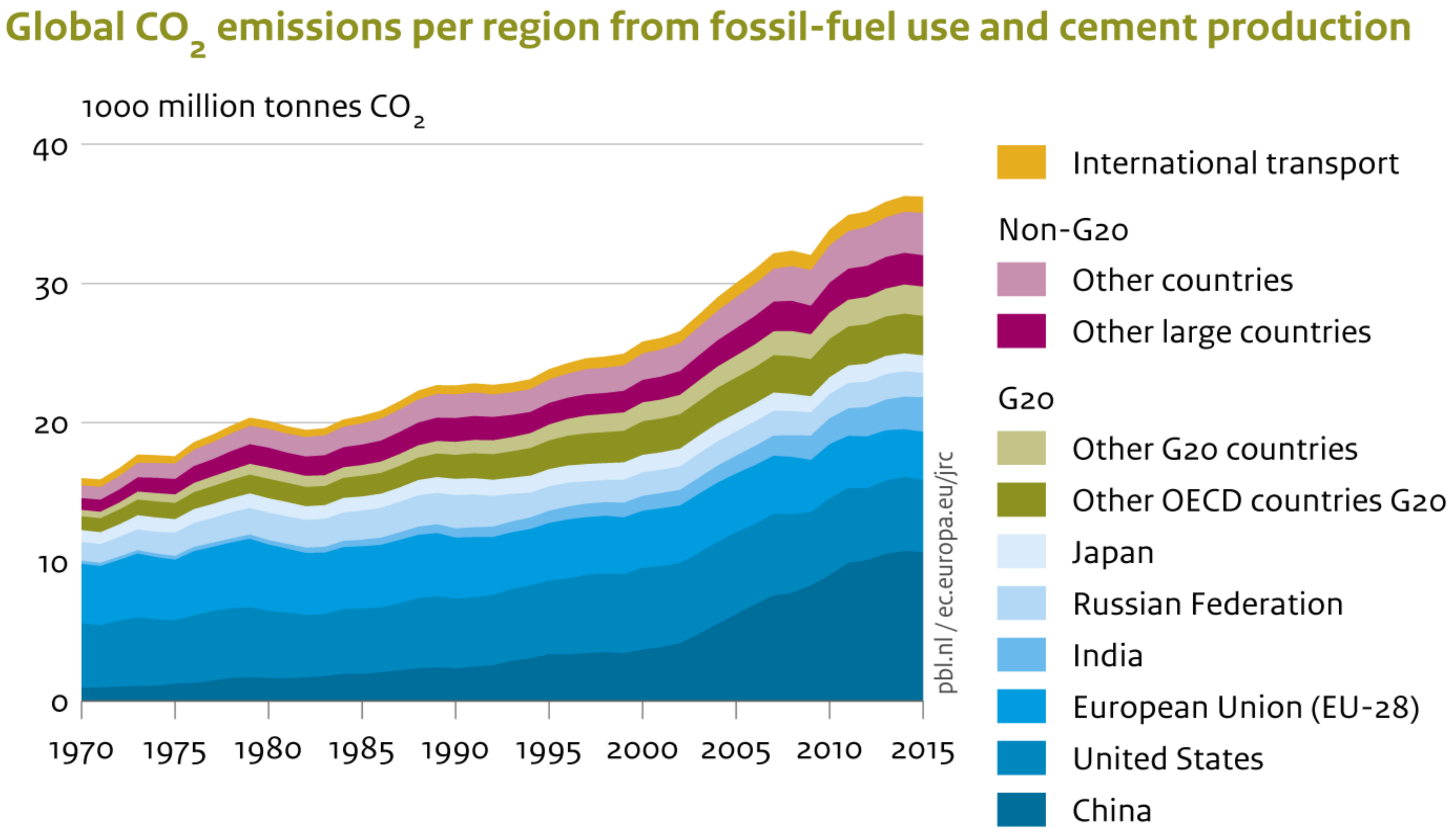
U.S. petroleum imports and exports (2019)

- U.S. petroleum imports and exports million barrels per day



Import sources	Gross imports	Exports	Net imports
Total, all countries	9.14	8.47	0.67
OPEC countries	1.64 (18%)	0.08	1.56
Persian Gulf countries	0.96 (11%)	0.05	0.91
Top five countries[1]			
Canada	4.43 (48%)	1.04	3.40
Mexico	0.65 (7%)	1.16	-0.51
Saudi Arabia	0.53 (6%)	<0.01	0.53
Russia	0.52 (6%)	<0.01	0.52
Colombia	0.37 (4%)	0.14	0.24

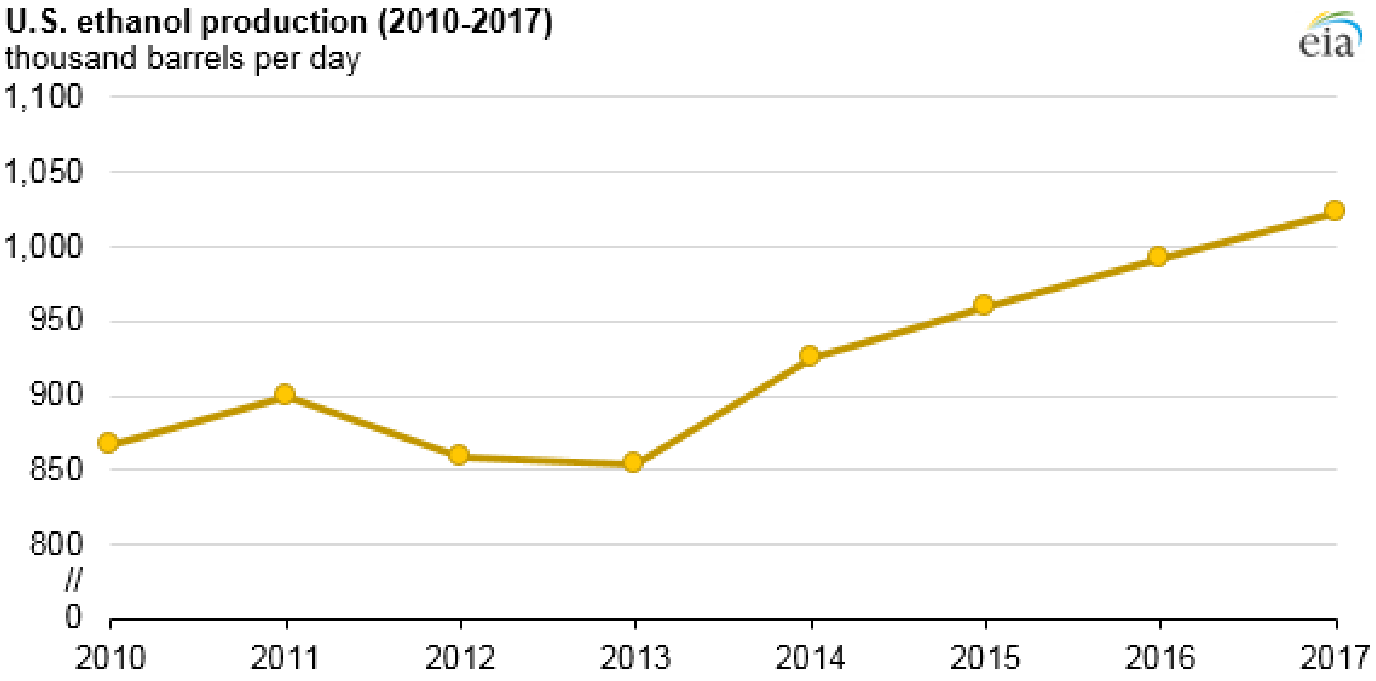
U.S. CO2 emission



Global CO_2 emissions per region from fossil-fuel use and cement production

Source: EDGAR v4.3.2 FT2015 (JRC/PBL 2016: IEA 2014 (suppl. with IEA 2016 for China, BP 2016, NBS 2016, USGS 2016, WSA 2016, NOAA 2016)

U.S. fuel ethanol production



- Corn is the primary feedstock of ethanol in U.S.
- Ethanol is blended with gasoline (10%)

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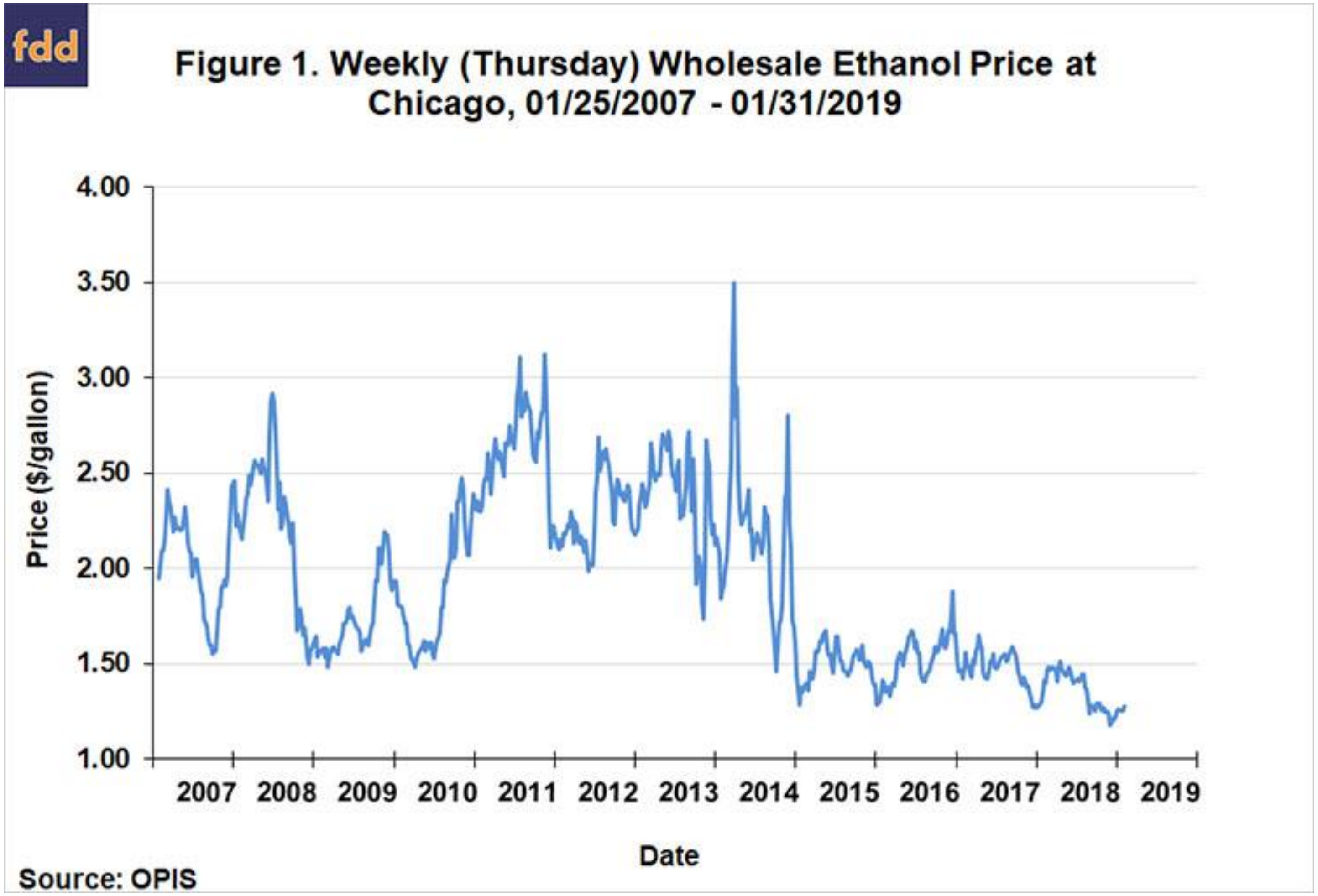
Ethanol price



Energy content of ethanol

Fuel	MJ/L	MJ/Kg
Ethanol	23.5	31.1
Gasoline	34.8	44.4
Diesel	38.6	45.4
Dry Wood	-	19.5
E85	25.2	33.2
Liq. Natural Gas	25.3	55
Methanol	17.9	19.9

Sources: wikipedia.org

Why lignocellulosic biomass?

- Biomass is carbon-based organic material, including forest residues/waste, agricultural residues, energy crops (switchgrass) and algae
- Biomass clean renewable source of energy
- Biomass absorbs carbon during growth

Federal initiative on bioenergy

- President Bush-2007
  - “Twenty-in-Ten” initiative, reduce gasoline 20% in 10 y
  - Energy Independence & Security Act mandates 36 b gallons of renewable fuels by 2022
  - Bioenergy Research Centers
- President Obama-2013
  - $2-Billion Energy Security Trust
  - Natural gas fuel & Hydrogen fuel
  - Advanced batteries
  - Cleaner biofuels

Bioenergy research centers

- DOE Joint BioEnergy Institute
- DOE Great Lakes Bioenergy Research Center
- DOE BioEnergy Science Center
  - Receive $25 million per year
  - Innovative biofuel research for another fiver years
- DOE Bioenergy Research Centers (BRCs) 2017
  - $40 million per year

Integrated biorefinery projects funded (DOE)

- INEOS first commercial biorefinery (8MG)
- POET-DSM & Abengoa produce ethanol (20/25MG)
- Myriant produces biobased succinic acid (30 MP)

http://www1.eere.energy.gov/bioenergy/pdfs/replacing_barrel_overview.pdf

Integrated biorefinery

- Abengoa
- Bluefire LLC
- Flambeau
- Mascoma
- POET
- Enerkem
- INEOS New Planet Bioenergy LLC
- Myriant
- RSA
- Sapphire Energy Inc.
- Algenol Biofuels Inc

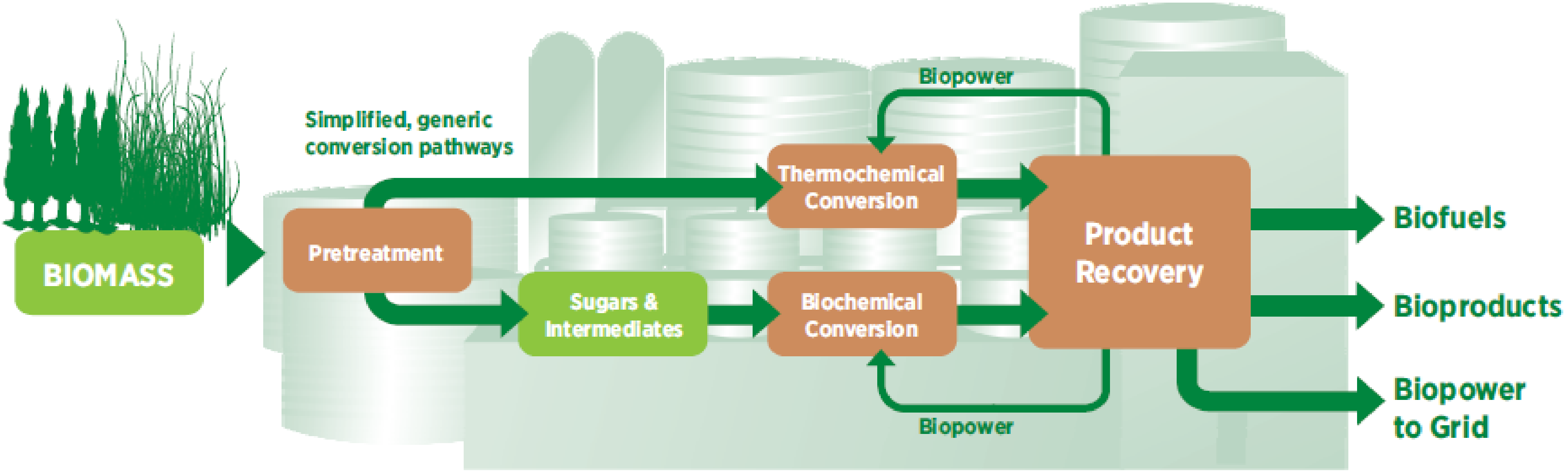
Project	Location	Scale	Conversion Technology
American Process Inc.	Alpena, MI	Pilot	Biochemical
Amyris Biotechnologies Inc.	Emeryville, CA	Pilot	Biochemical
Archer Daniels Midland	Decatur, IL	Pilot	Biochemical
Haldor Topsoe Inc.	Des Plaines, IL	Pilot	Thermo - Gasification
ICM Inc.	St. Joseph, MO	Pilot	Biochemical
Logos/EdenIQ Technologies	Visalia, CA	Pilot	Biochemical
Renewable Energy Institute International	Toledo, OH	Pilot	Thermo - Gasification
Rentech ClearFuels	Commerce City, CO	Pilot	Thermo - Gasification
Solazyme Inc.	Peoria, IL	Pilot	Algae/Sugar
UOP LLC	Kapolei, HI	Pilot	Thermo - Pyrolysis
ZeaChem Inc.	Boardman, OR	Pilot	Hybrid
Gas Technology Institute	Des Plaines, IL	Design Only	Thermo - Pyrolysis

Integrated Biorefinery Projects Funded by DOE

http://www1.eere.energy.gov/biomass/pdfs/ibr_portfolio_overview.pdf

Biorefinery pathways

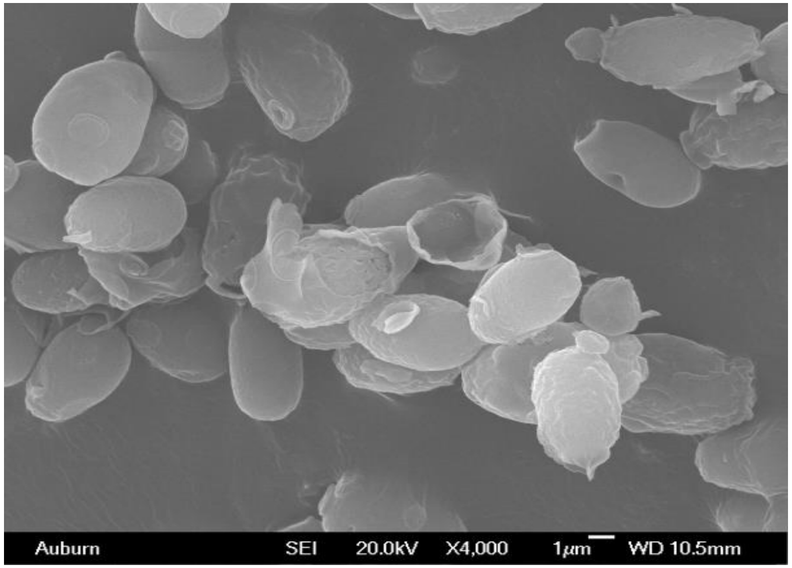
- Thermochemical conversion (gasification/pyrolysis)
- Biochemical conversion (enzymes/microbes)



https://www1.eere.energy.gov/bioenergy

Background

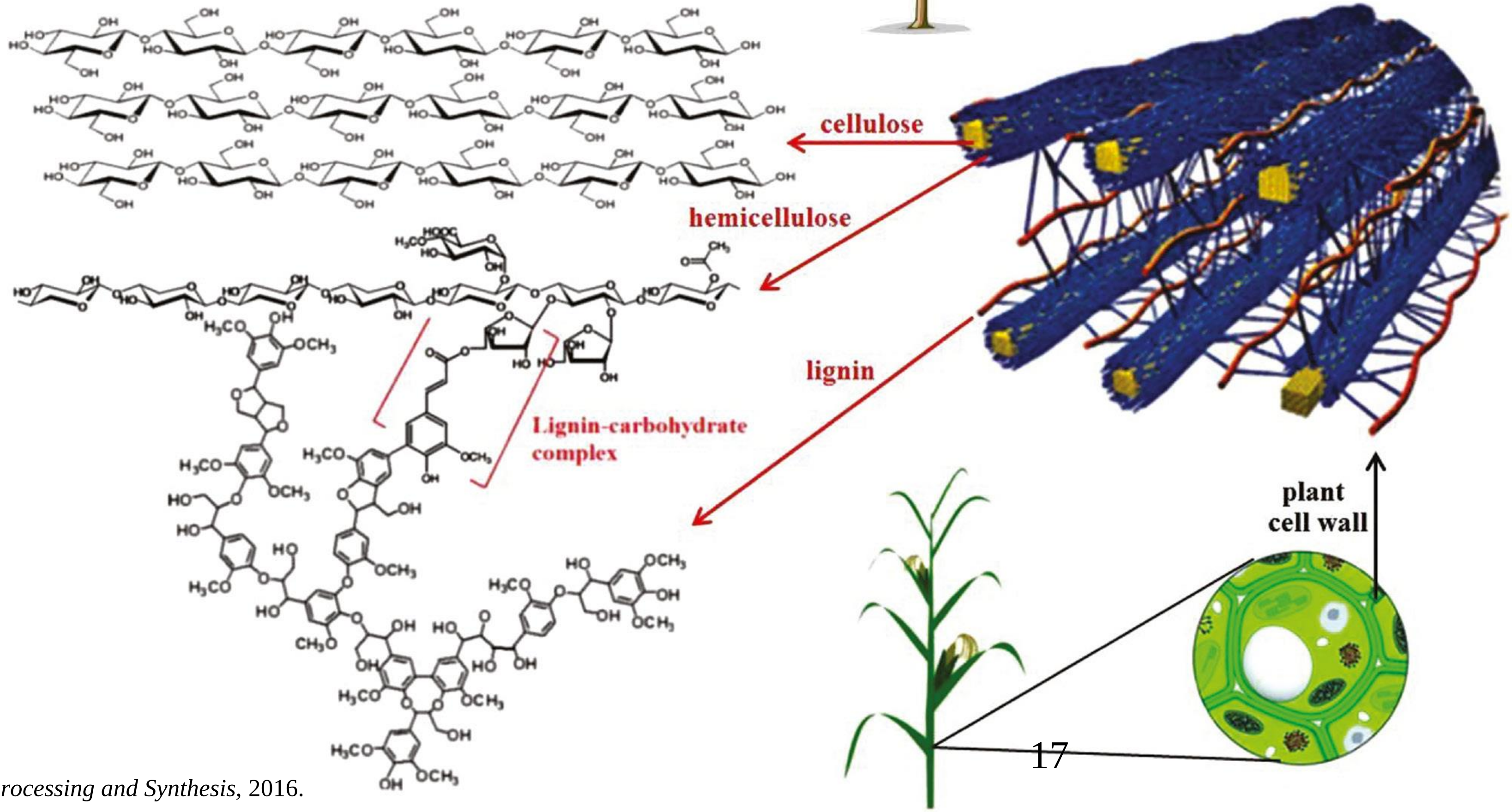
- Biomass pretreatment is needed in biorefinery
  - Break down the recalcitrant structure of cell walls
  - Subsequent enzymatic hydrolysis and fermentation
- Pretreatment undesirably generates inhibitors
  - Degradation of cellulose, hemicellulose, lignin and extractives
- Fermentation inhibition
  - Reduce microbial growth
  - Decrease fermentation rate and yield



Structure of lignocellulosic biomass

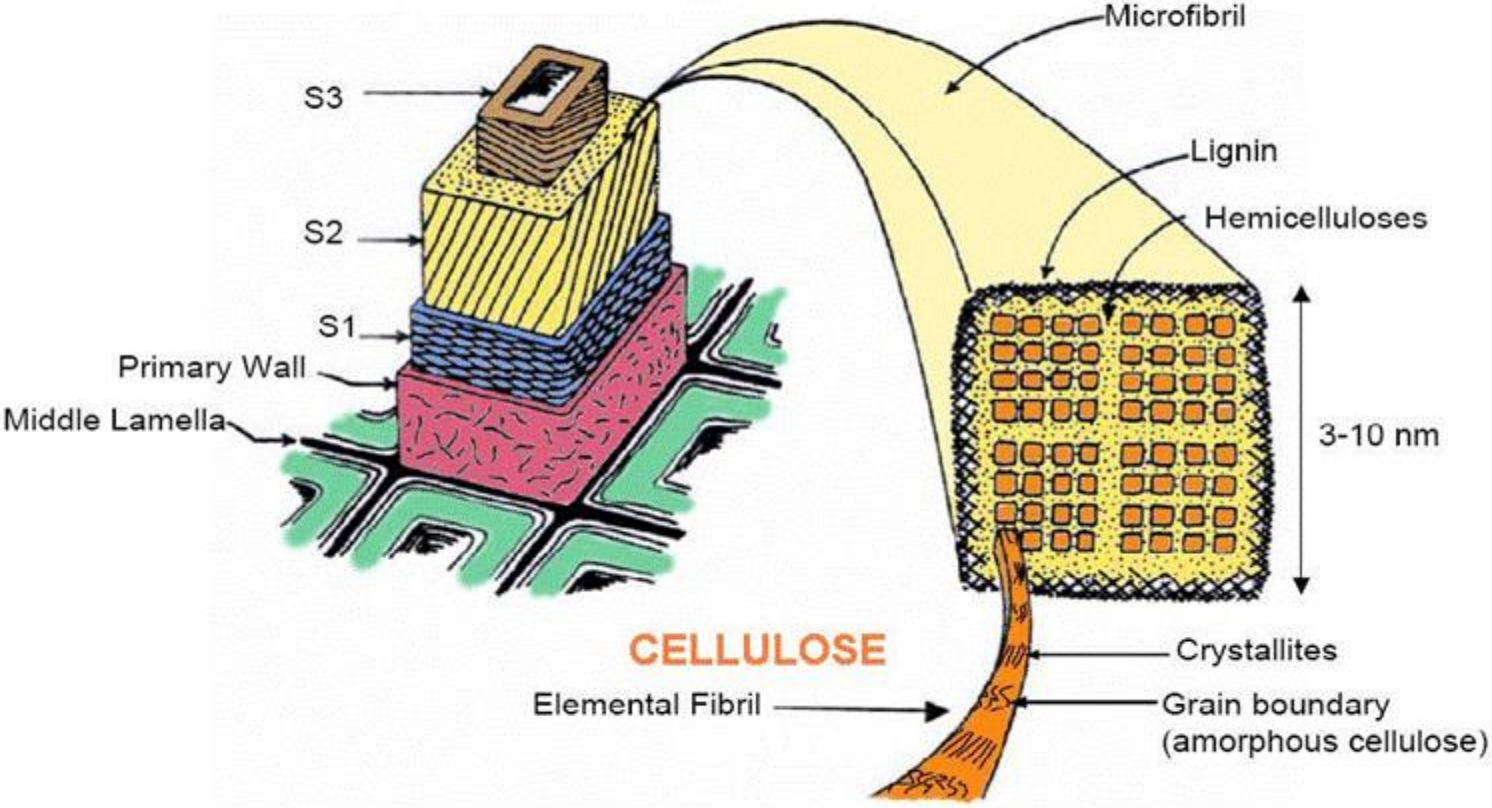
Lignocellulosic biomass

- Abundance
- Sustainability
- Not competing with food supply



[1] Volynets B. *Green Processing and Synthesis*, 2016.

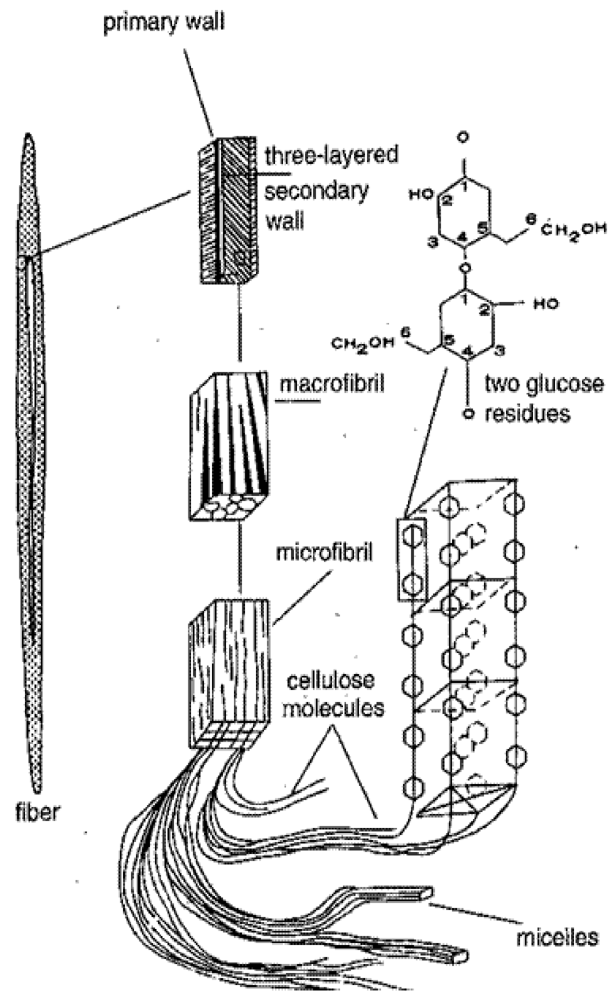
Ultrastructure of plant cell wall



Industry Canada Nov 2005

Lignocellulosic biomass chemistry

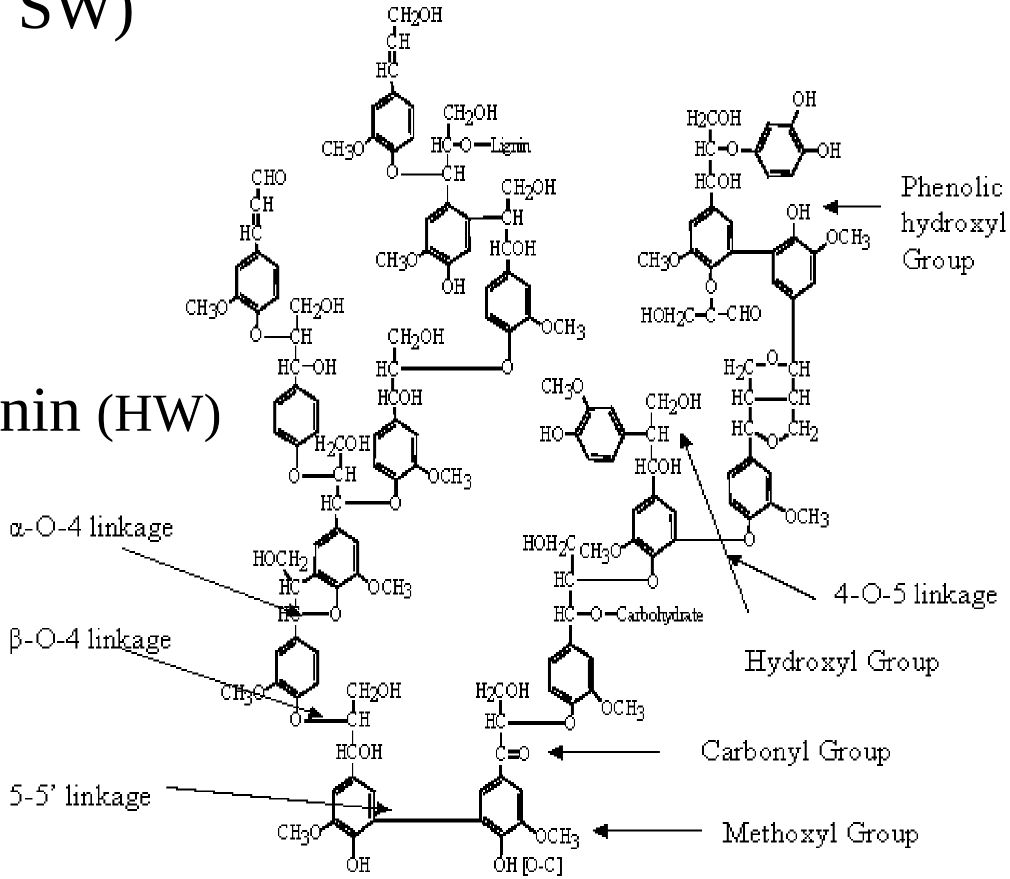
- **Cellulose** (45% HW/SW)
  - Linear polymer of β-1,4 linked glucose
  - Degree polymerization (DP), 10,000
  - Crystalline and amorphous
- **Hemicellulose** (35% HW, 25% SW)
  - Branched polymer of glucose, mannose, galactose, xylose, and arabinose
  - DP 150-200
  - Easily degraded and dissolved



From Bruley

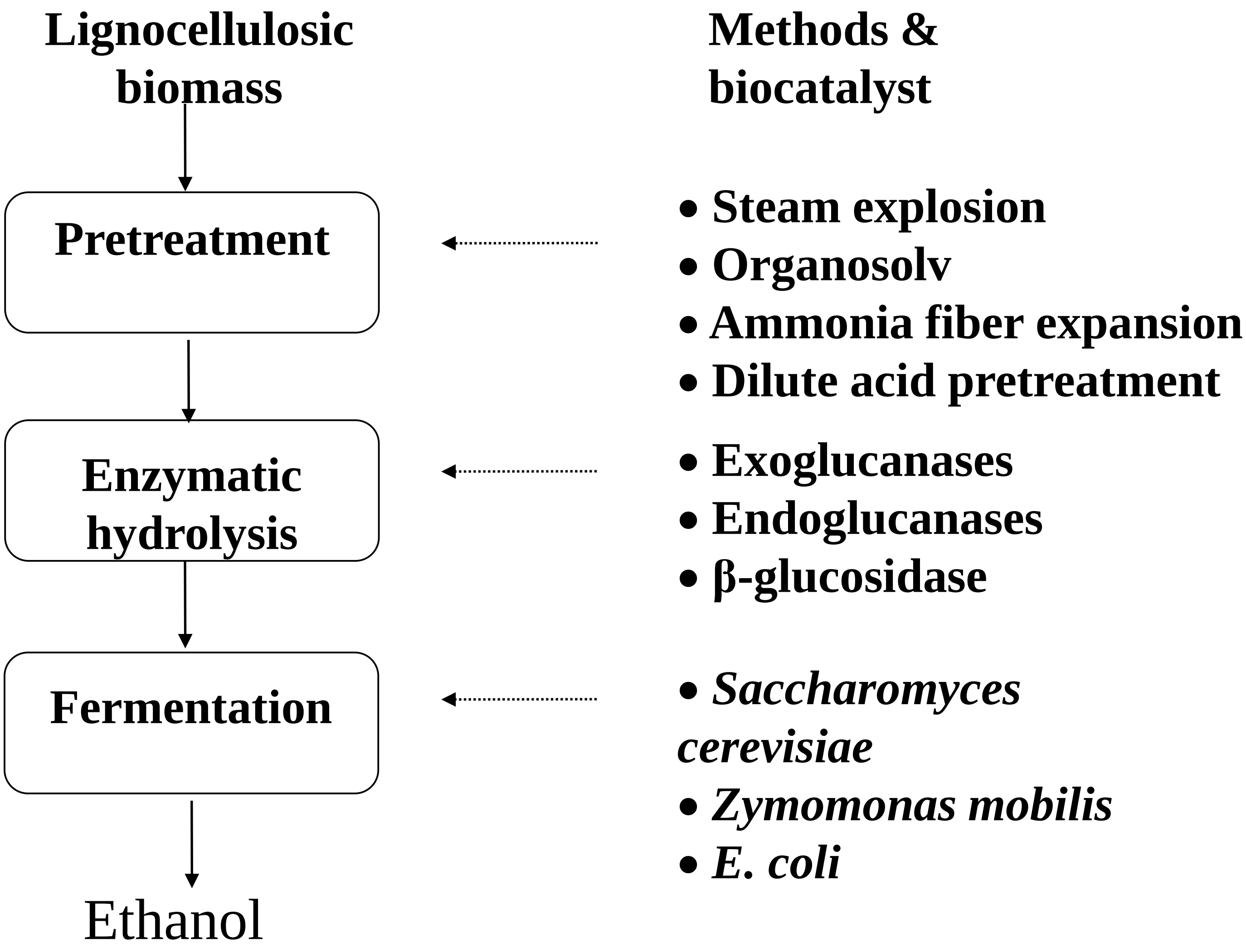
Lignocellulosic biomass chemistry

- **Lignin** (21% HW, 25% SW)
  - 3-dimension
  - Amorphous polymer
  - Phenylpropane
  - Guaiacyl-syringyl lignin (HW)
  - Guaiacyl lignin (SW)
  - Complex structure



The structure of softwood lignin (Akler)

Bioconversion process



Lignocellulosic biomass

Pretreatment

Enzymatic hydrolysis

Fermentation

Ethanol

Methods & biocatalyst

- **Steam explosion**
- **Organosolv**
- **Ammonia fiber expansion**
- **Dilute acid pretreatment**

- **Exoglucanases**
- **Endoglucanases**
- **β-glucosidase**

- ***Saccharomyces cerevisiae***
- ***Zymomonas mobilis***
- ***E. coli***

Pretreatment process

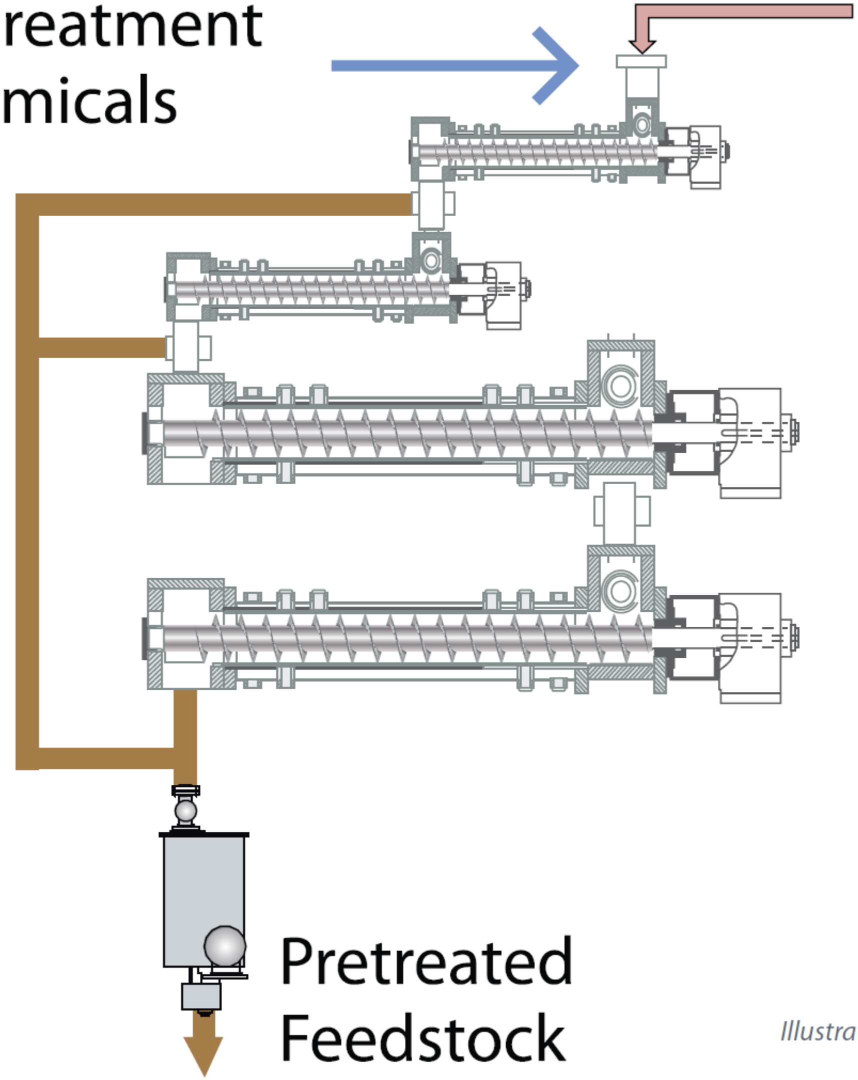
- **Steam explosion**
  - High yield of cellulose
  - High lignin content
- **Organosolv pretreatment**
  - Hydrolyzing of hemicellulose
  - Solubilization of lignin
- **Ammonia fiber explosion (AFEX)**
  - degrading crystalline cellulose, preserving hemicellulose
  - 10-20% solubilization of lignin
- **Dilute acid pretreatment**
  - Extensive hemicellulose hydrolysis
  - Furfural and other degradation products

Biomass deconstruction and pretreatment

- Chemical an mechanical deconstruction
  - Deacetylation and mechanical refining process
  - Low toxicity, high concentration sugar stream
  - Native lignin

Photo by Dennis Schroeder, NREL/PIX 17684

Biomass deconstruction



Pretreatment Chemicals

Feedstock

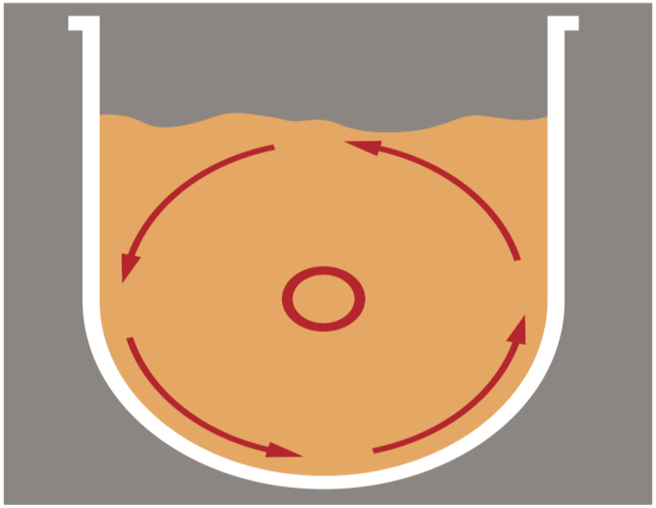
Pretreated Feedstock

- Multiple horizontal-tube reactors
- Steam heated to 150-210 °C
- Changing the auger speed to move biomass

Illustration created by Josh Bauer, NREL

Produce highly concentrated sugar streams

- Solid loadings >20% w/w
- Operated in batch mode (36h)
- Vigorous mixing at temperature
  - 40-50°C
- Biomass slurry is liquefied 24 h
- Complete **enzymatic hydrolysis**
  - in another reactor



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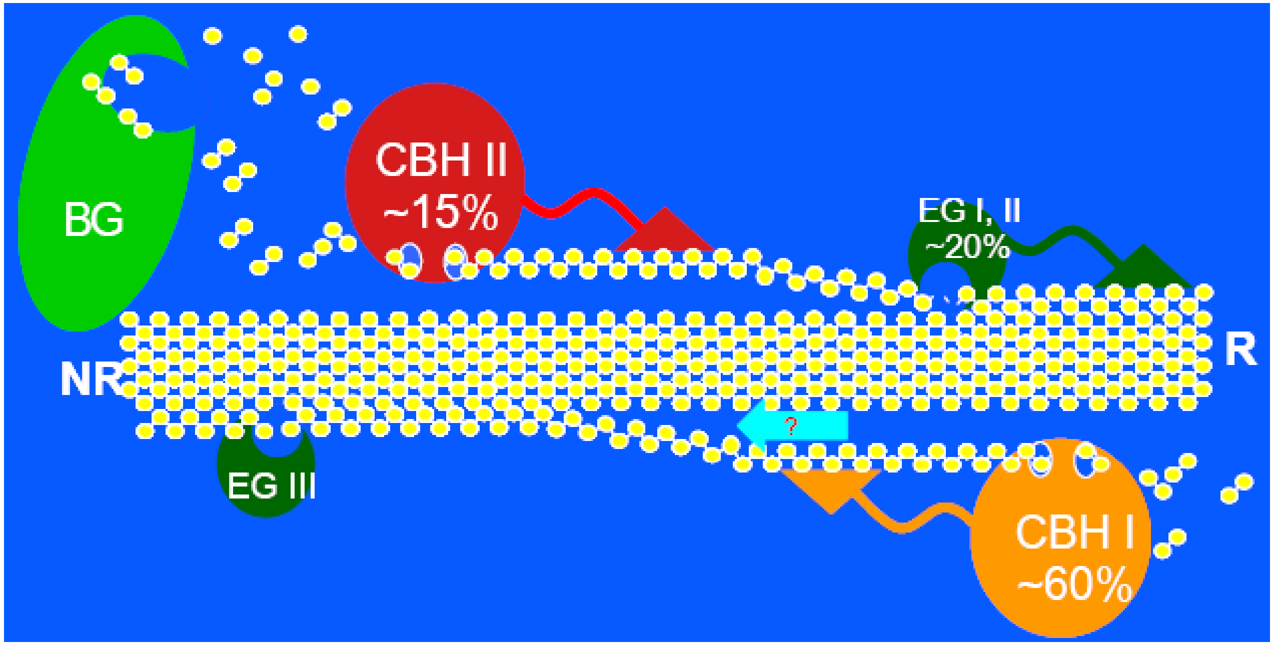
Enzymatic hydrolysis of cellulose

Trichoderma reesei Cellulases



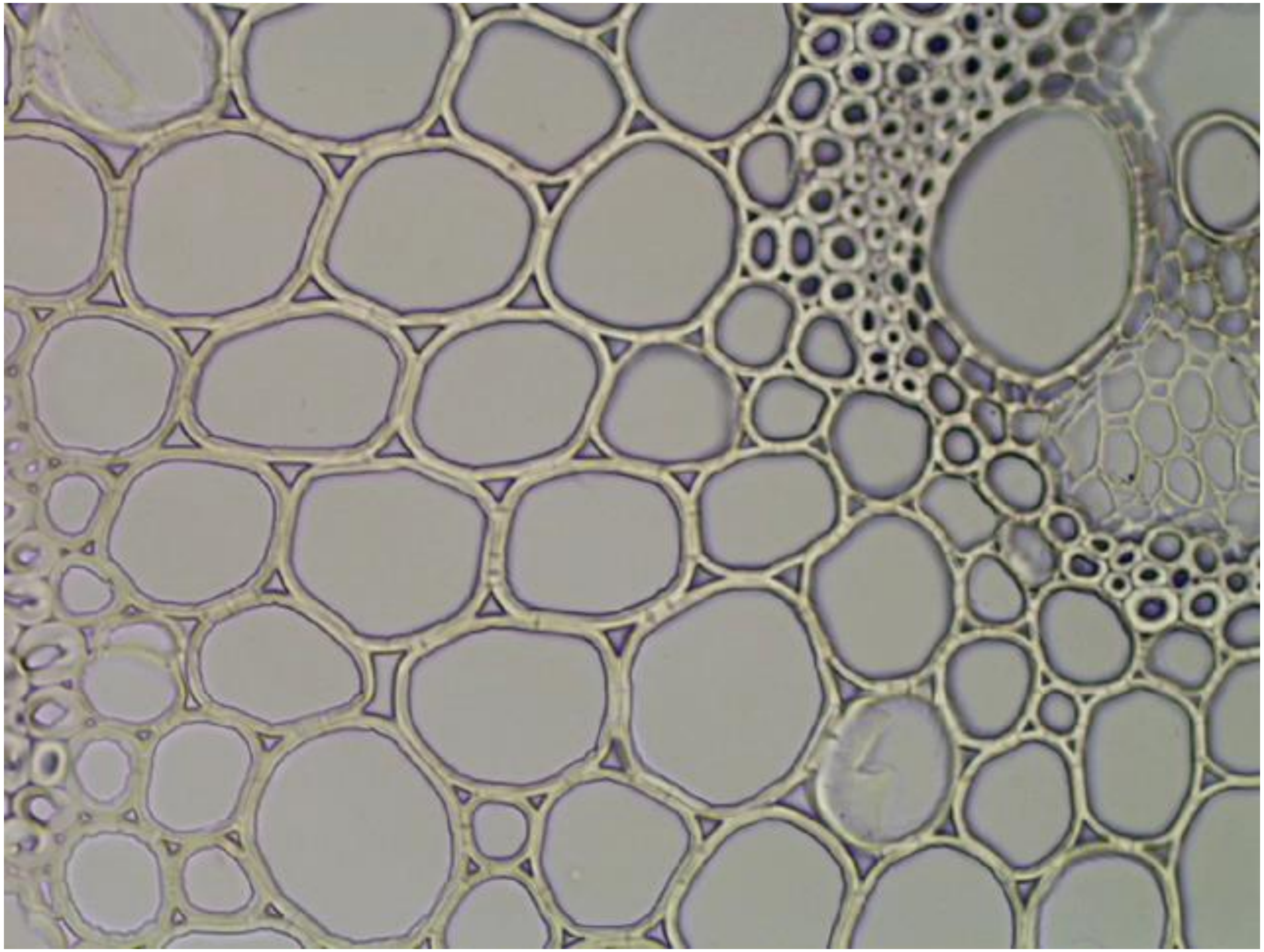
EG: endo-glucanase

CBH: cellobiohydrolase

BG: β-glucosidase

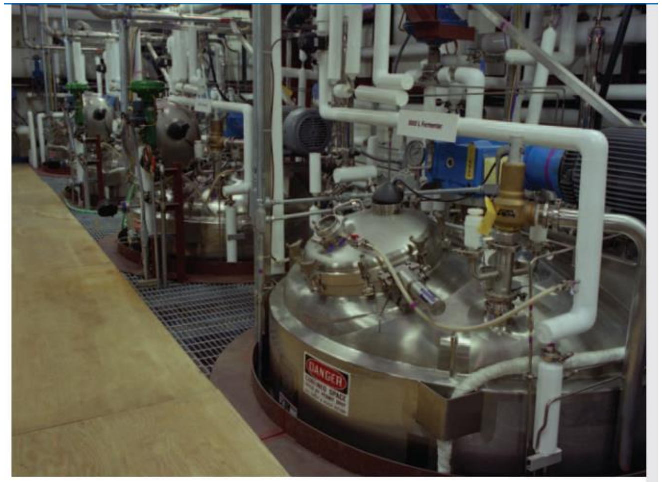
Source: novozymes

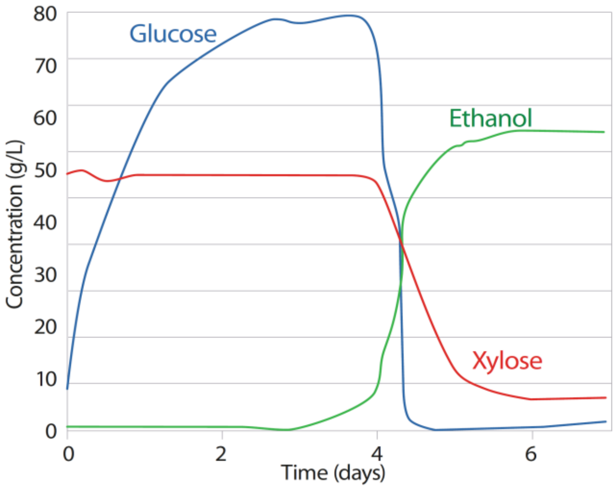
Plant cell walls digested by fungal cellulases (10 h)



Microbial fermentation

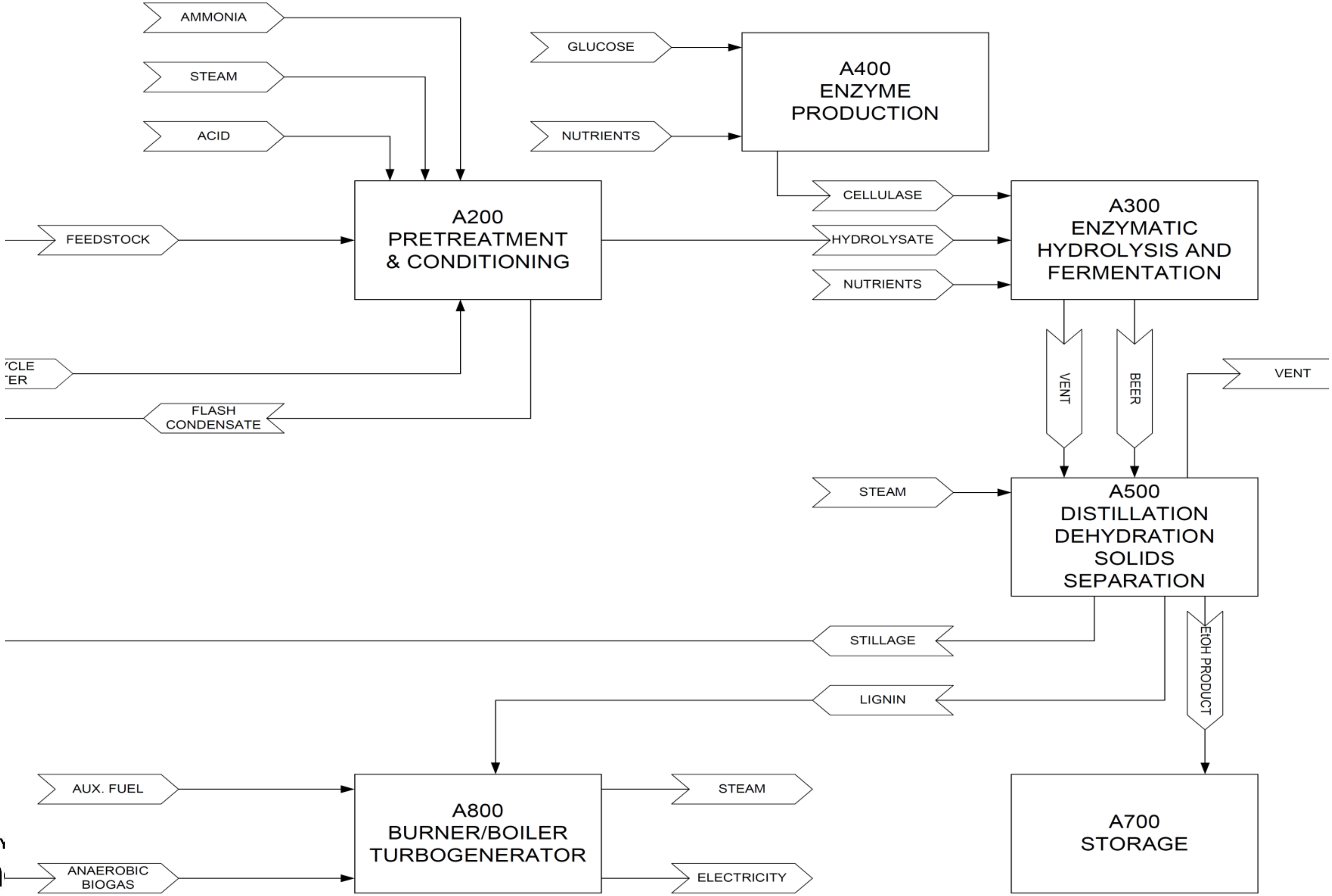
- Fermentation systems with pH,
  - Temperature, dissolved oxygen control
- Monitoring glucose and xylose
  - Consumption and ethanol production



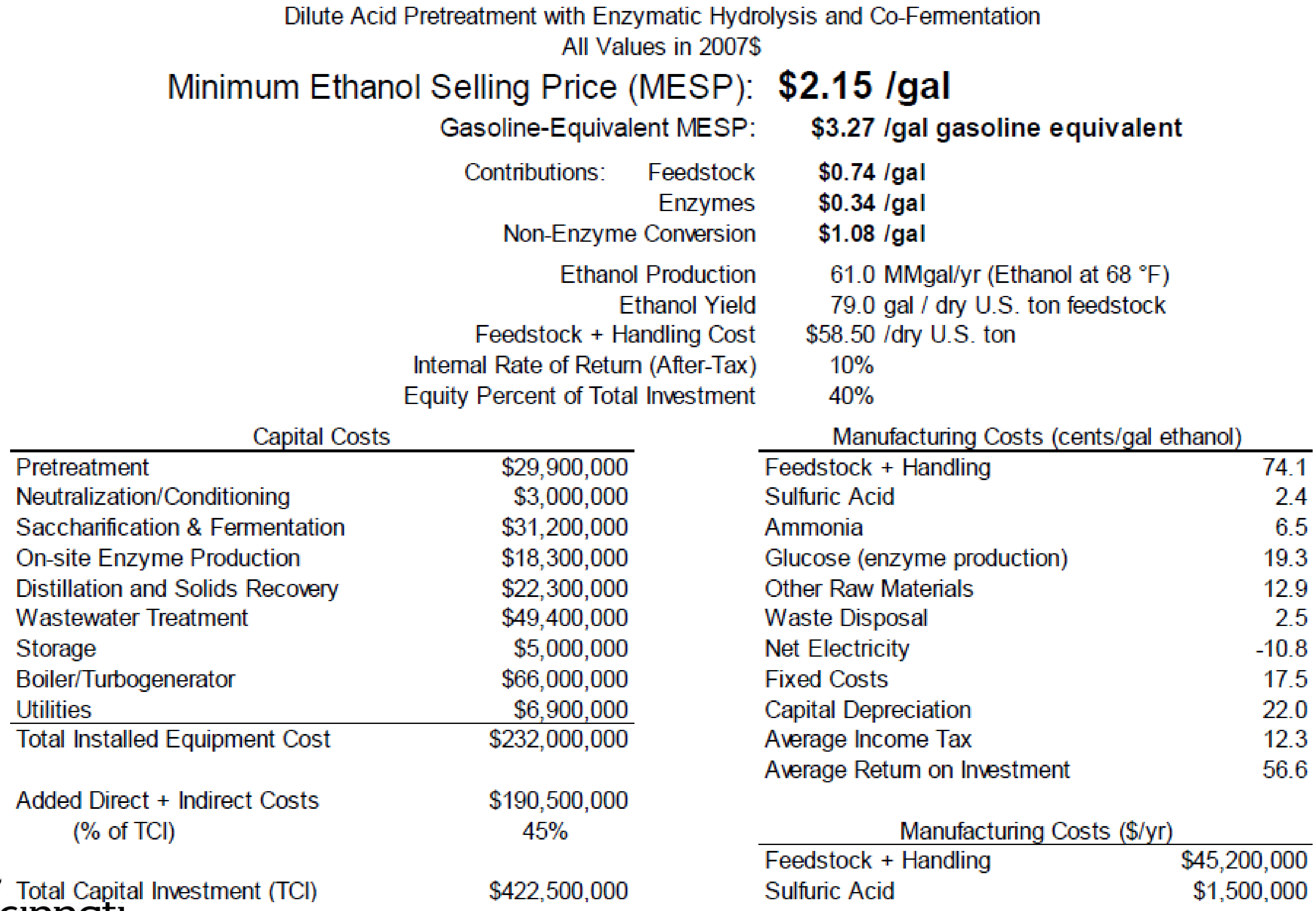


Enzymatic hydrolysis and fermentation of pretreated corn stover

Process design and economic analysis



Ethanol production engineering analsyis



Dilute Acid Pretreatment with Enzymatic Hydrolysis and Co-Fermentation
All Values in 2007$

Minimum Ethanol Selling Price (MESP):	**$2.15 /gal**
Gasoline-Equivalent MESP:	**$3.27 /gal gasoline equivalent**
Contributions: Feedstock	**$0.74 /gal**
Enzymes	**$0.34 /gal**
Non-Enzyme Conversion	**$1.08 /gal**
Ethanol Production	61.0 MMgal/yr (Ethanol at 68 °F)
Ethanol Yield	79.0 gal / dry U.S. ton feedstock
Feedstock + Handling Cost	$58.50 /dry U.S. ton
Internal Rate of Return (After-Tax)	10%
Equity Percent of Total Investment	40%

Capital Costs	
Pretreatment	$29,900,000
Neutralization/Conditioning	$3,000,000
Saccharification & Fermentation	$31,200,000
On-site Enzyme Production	$18,300,000
Distillation and Solids Recovery	$22,300,000
Wastewater Treatment	$49,400,000
Storage	$5,000,000
Boiler/Turbogenerator	$66,000,000
Utilities	$6,900,000
Total Installed Equipment Cost	$232,000,000
Added Direct + Indirect Costs	$190,500,000
(% of TCI)	45%
Total Capital Investment (TCI)	$422,500,000

Manufacturing Costs (cents/gal ethanol)	
Feedstock + Handling	74.1
Sulfuric Acid	2.4
Ammonia	6.5
Glucose (enzyme production)	19.3
Other Raw Materials	12.9
Waste Disposal	2.5
Net Electricity	-10.8
Fixed Costs	17.5
Capital Depreciation	22.0
Average Income Tax	12.3
Average Return on Investment	56.6

Manufacturing Costs ($/yr)	
Feedstock + Handling	$45,200,000
Sulfuric Acid	$1,500,000

Biomass composition in process design

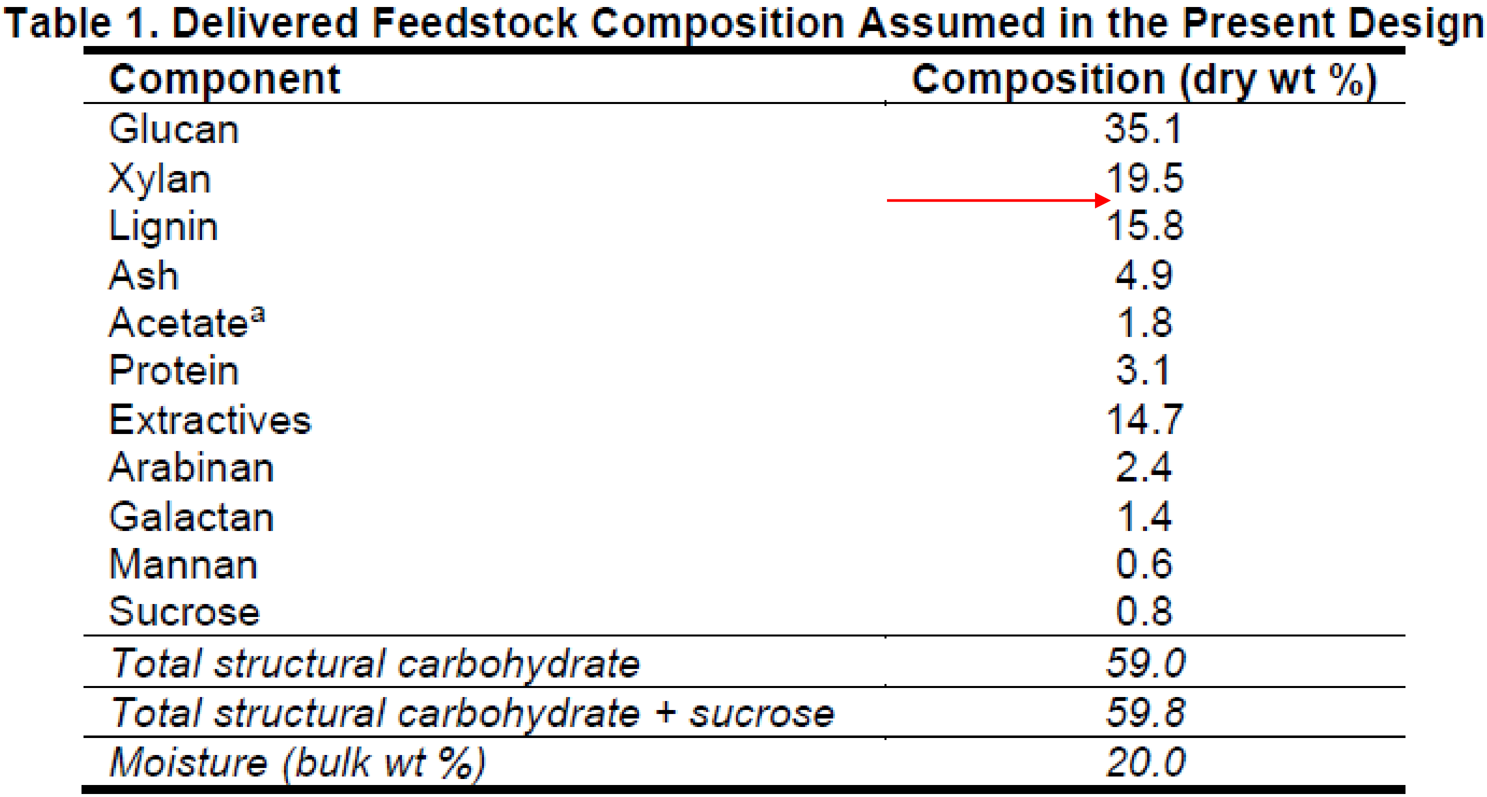


Table 1. Delivered Feedstock Composition Assumed in the Present Design

Component	Composition (dry wt %)
Glucan	35.1
Xylan	19.5
Lignin	15.8
Ash	4.9
Acetate[a]	1.8
Protein	3.1
Extractives	14.7
Arabinan	2.4
Galactan	1.4
Mannan	0.6
Sucrose	0.8
Total structural carbohydrate	*59.0*
Total structural carbohydrate + sucrose	*59.8*
Moisture (bulk wt %)	*20.0*

- Biomass cost is \$58.5/dry ton (2007\$)

Biomass pretreatment targets

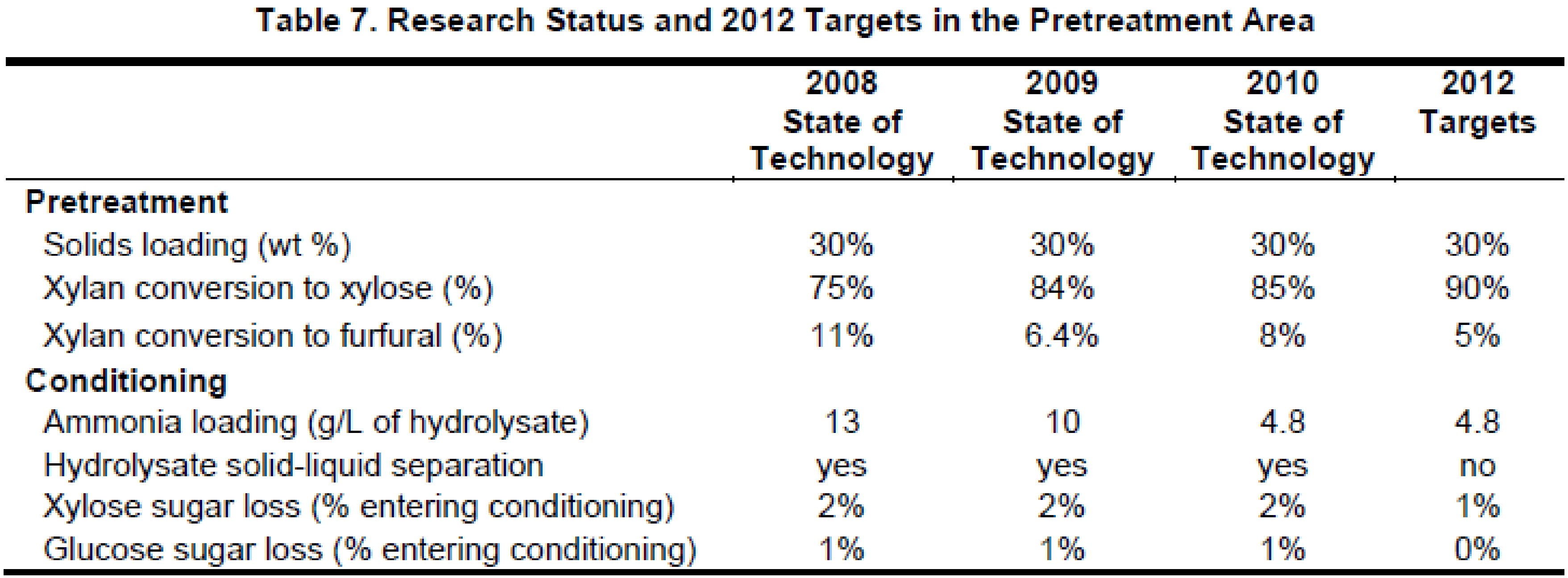
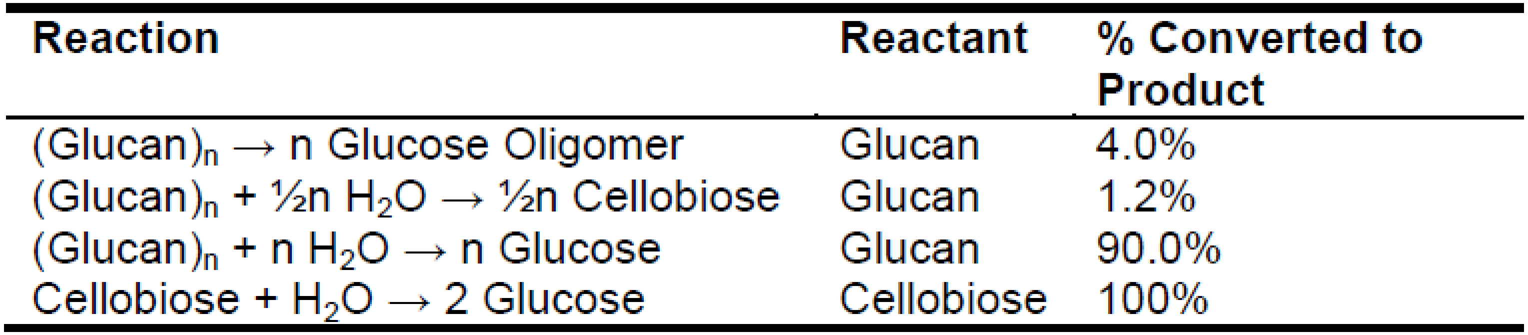


Table 7. Research Status and 2012 Targets in the Pretreatment Area

	2008 State of Technology	2009 State of Technology	2010 State of Technology	2012 Targets
Pretreatment				
Solids loading (wt %)	30%	30%	30%	30%
Xylan conversion to xylose (%)	75%	84%	85%	90%
Xylan conversion to furfural (%)	11%	6.4%	8%	5%
Conditioning				
Ammonia loading (g/L of hydrolysate)	13	10	4.8	4.8
Hydrolysate solid-liquid separation	yes	yes	yes	no
Xylose sugar loss (% entering conditioning)	2%	2%	2%	1%
Glucose sugar loss (% entering conditioning)	1%	1%	1%	0%

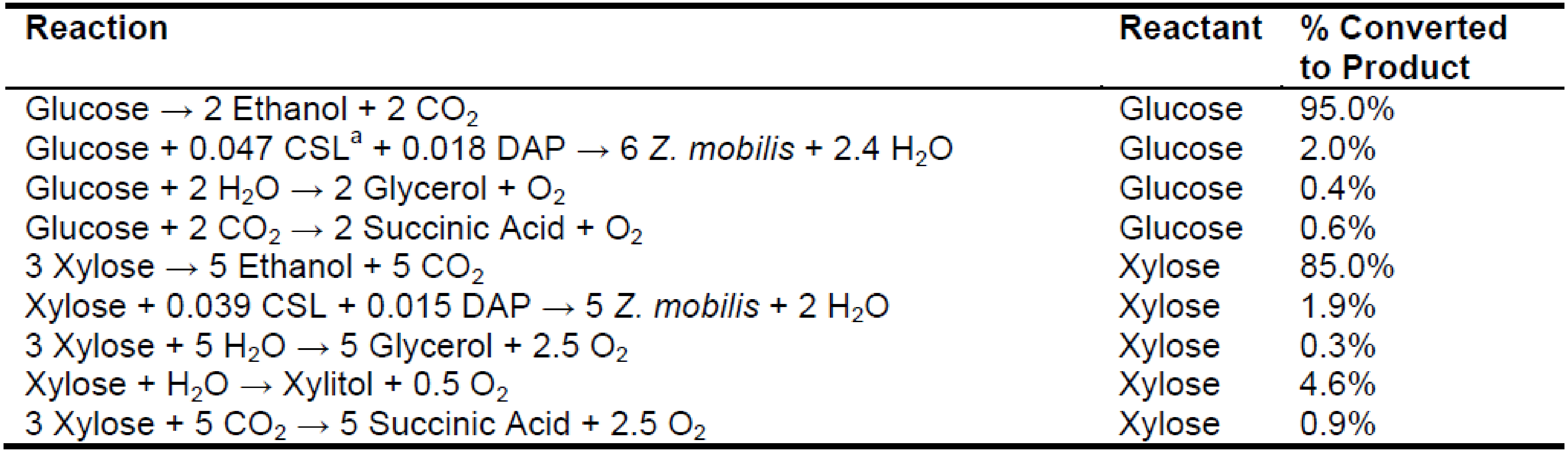
Enzymatic hydrolysis and assumed conversions



Reaction	Reactant	% Converted to Product
$(Glucan)_n \rightarrow$ n Glucose Oligomer	Glucan	4.0%
$(Glucan)_n + ½n\ H_2O \rightarrow ½n$ Cellobiose	Glucan	1.2%
$(Glucan)_n + n\ H_2O \rightarrow n$ Glucose	Glucan	90.0%
Cellobiose + $H_2O \rightarrow 2$ Glucose	Cellobiose	100%

- Temperature 48 ℃ and Initial solids loading 20 wt % total solids
- Residence time 84 h
- Number and size of continuous vessels 8 @ 950 m3 (250,000 gal) each
- Number and size of batch vessels 12 @ 3,600 m3 (950,000 gal) each
- Cellulase loading 20 mg protein/g cellulose

Co-fermentation of glucose and xylose

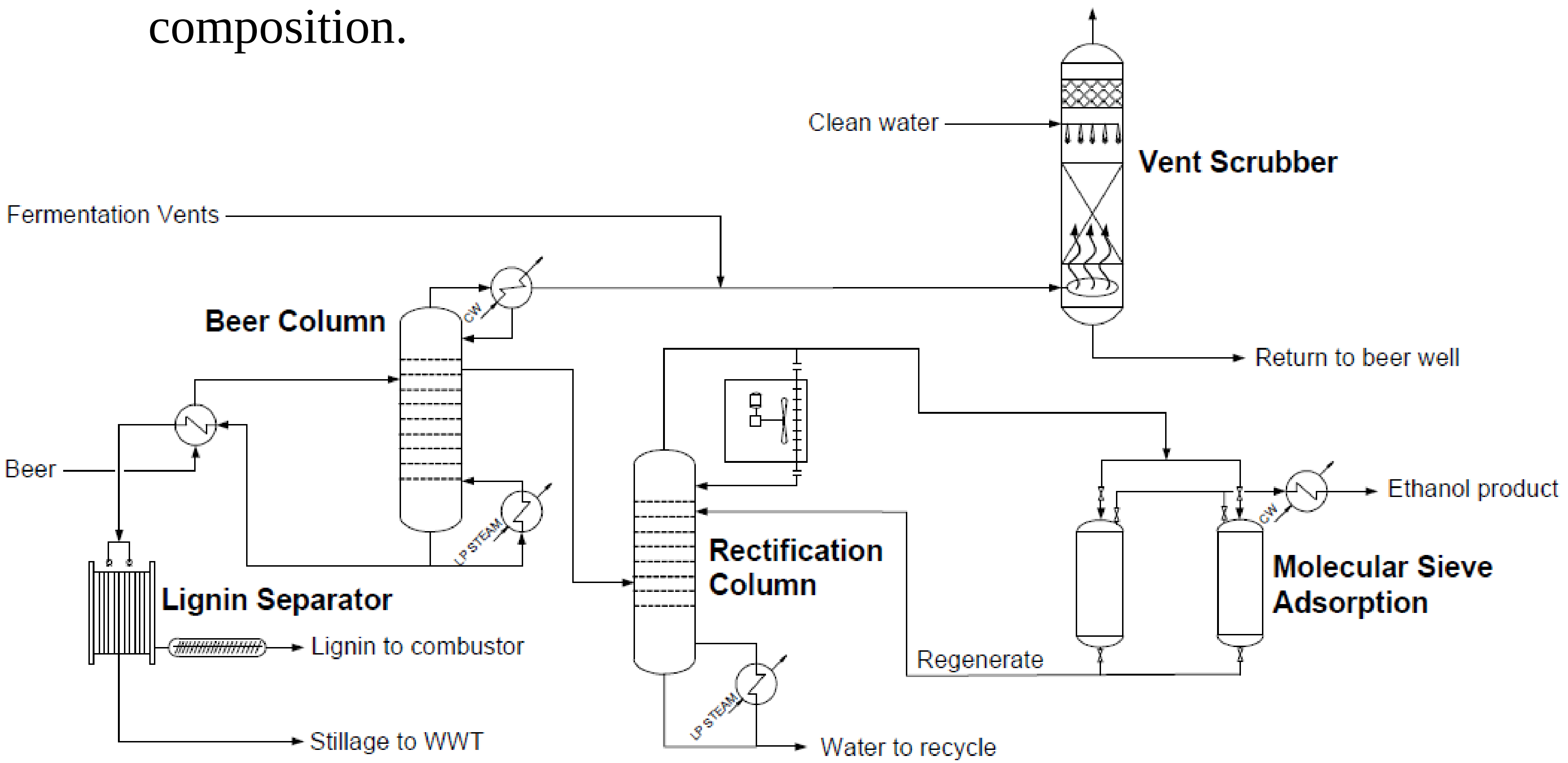


Reaction	Reactant	% Converted to Product
Glucose → 2 Ethanol + 2 CO_2	Glucose	95.0%
Glucose + 0.047 CSL[a] + 0.018 DAP → 6 *Z. mobilis* + 2.4 H_2O	Glucose	2.0%
Glucose + 2 H_2O → 2 Glycerol + O_2	Glucose	0.4%
Glucose + 2 CO_2 → 2 Succinic Acid + O_2	Glucose	0.6%
3 Xylose → 5 Ethanol + 5 CO_2	Xylose	85.0%
Xylose + 0.039 CSL + 0.015 DAP → 5 *Z. mobilis* + 2 H_2O	Xylose	1.9%
3 Xylose + 5 H_2O → 5 Glycerol + 2.5 O_2	Xylose	0.3%
Xylose + H_2O → Xylitol + 0.5 O_2	Xylose	4.6%
3 Xylose + 5 CO_2 → 5 Succinic Acid + 2.5 O_2	Xylose	0.9%

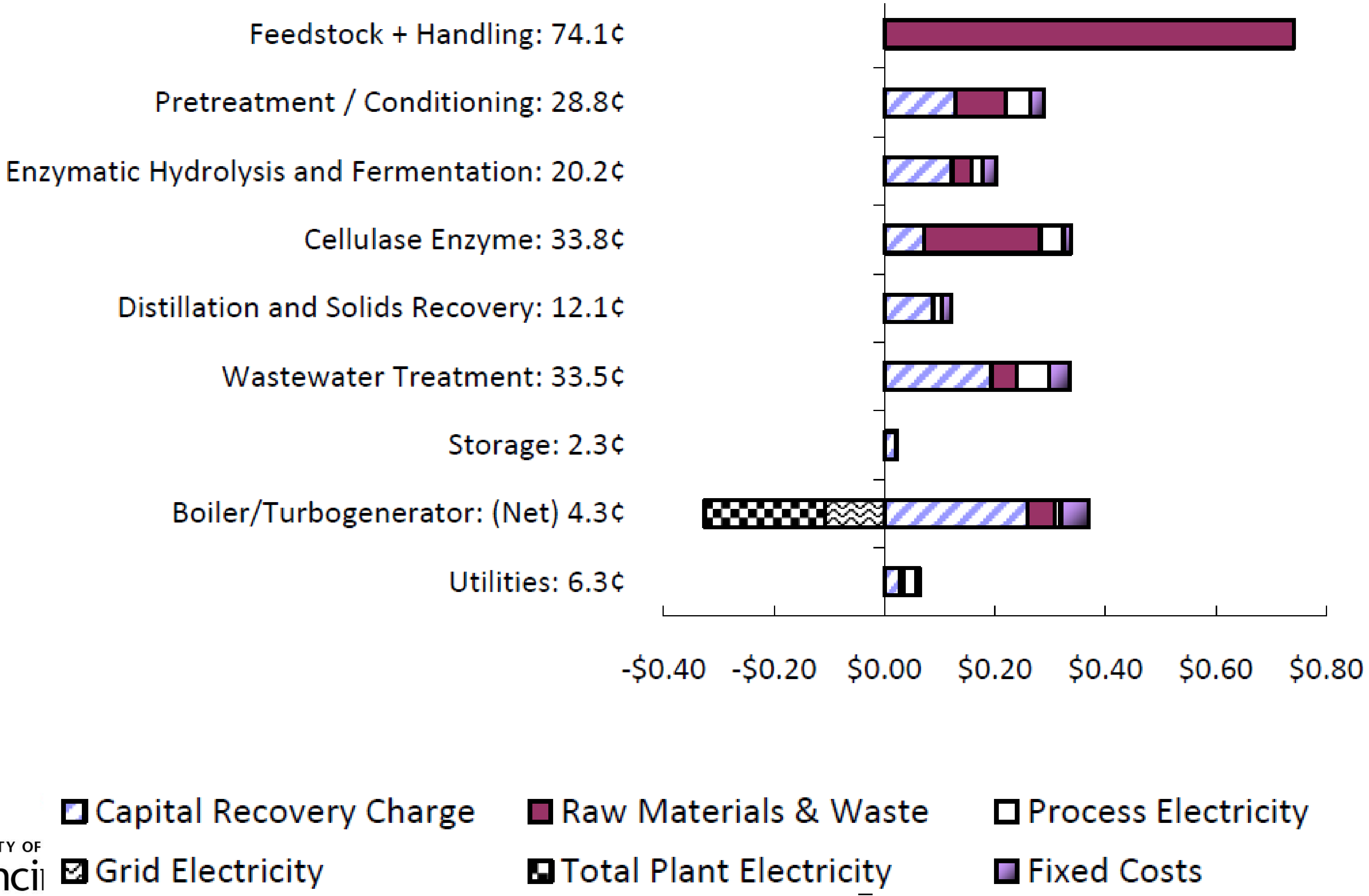
- Organism Recombinant *Zymomonas mobilis*
- Temperature 32 ℃
- Initial fermentation solids level 19.8% total solids
- Residence time 1.5 days (36 h)
- Inoculum level 10 vol %

Ethanol distillation and separation

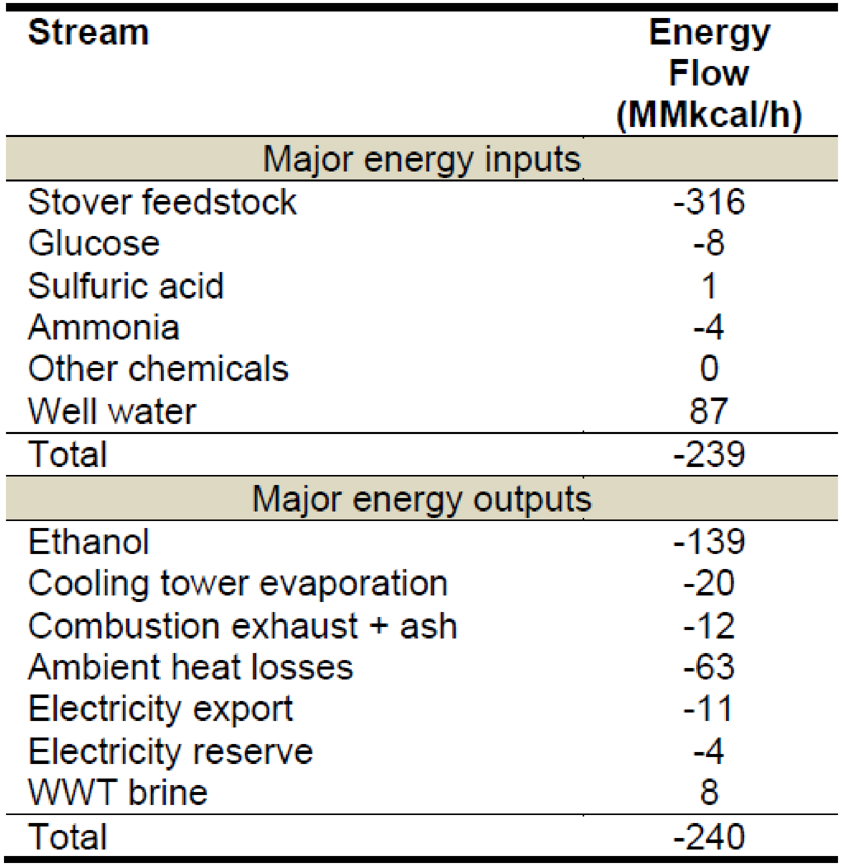
- Distillation and molecular sieve adsorption to recover ethanol
- Distillation is accomplished in two columns:
  - Beer column, removes the dissolved CO2 and most of the water.
  - Rectification column to concentrates ethanol to a near azeotropic composition.



Cost contribution from each process area



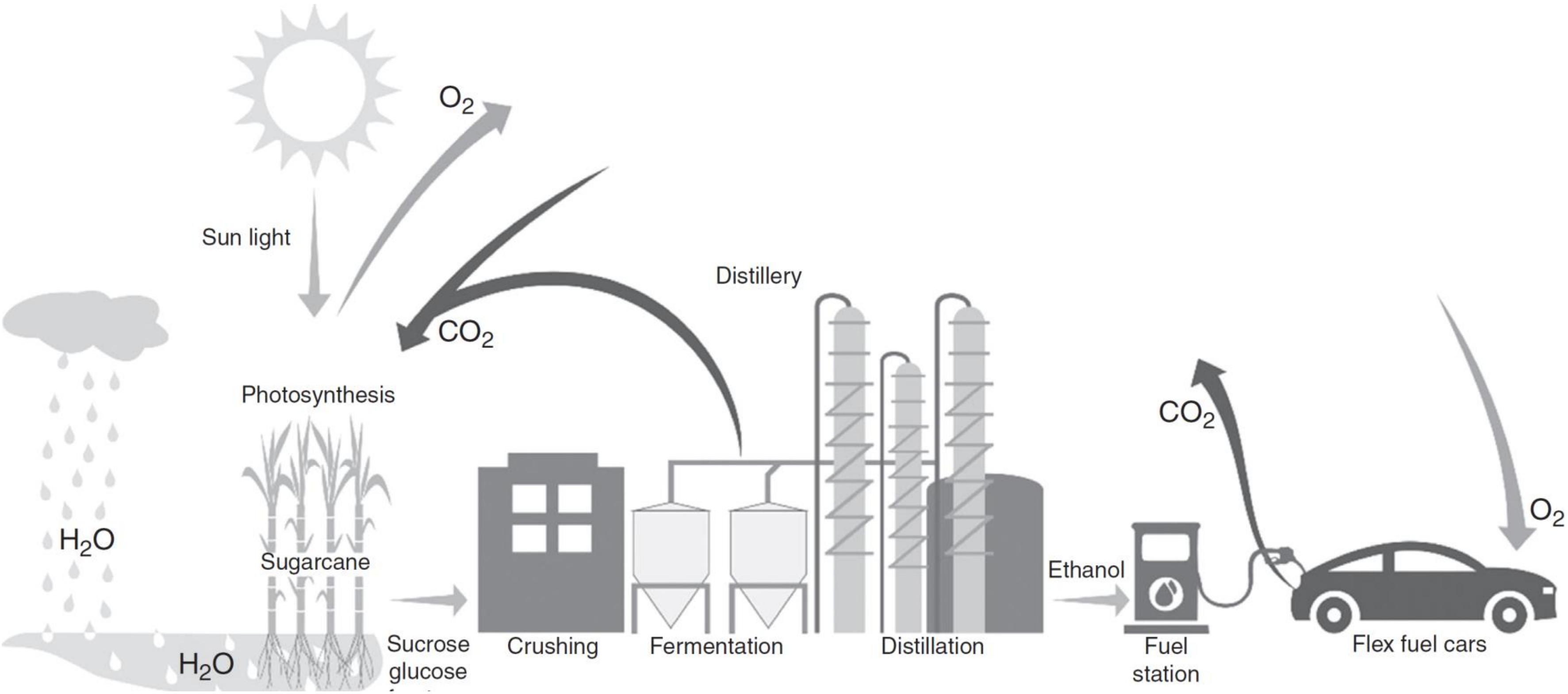
Ethanol plant energy balance



Stream	Energy Flow (MMkcal/h)
Major energy inputs	
Stover feedstock	-316
Glucose	-8
Sulfuric acid	1
Ammonia	-4
Other chemicals	0
Well water	87
Total	-239
Major energy outputs	
Ethanol	-139
Cooling tower evaporation	-20
Combustion exhaust + ash	-12
Ambient heat losses	-63
Electricity export	-11
Electricity reserve	-4
WWT brine	8
Total	-240

- Energy balance performed to compare the energy products from the process (ethanol & electricity) to the potential energy in the biomass feed.
- The percent of carbohydrate energy in the feed recovered as ethanol is about 72%

Sugarcane ethanol in brazil

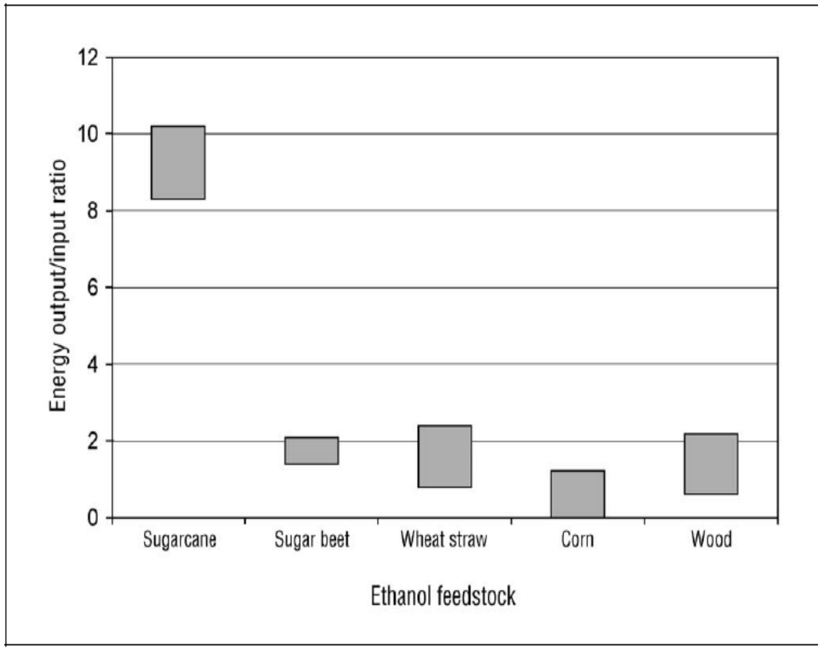


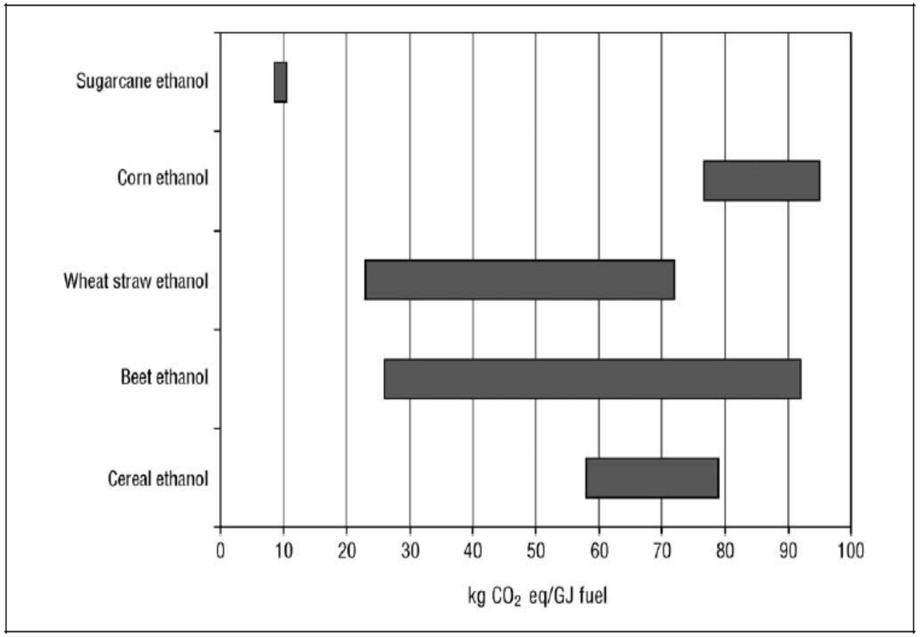
- CO_2 and H_2O absorbed and converted to sugars, which are fermented by yeasts to ethanol.

Sugar cane to ethanol process

Harvest-milling-fermentation-distillation (self sufficient)

Energy input and output





- Energy output and input from different feedstocks
- Greenhouse gas emissions from different fuels

Coelho, 2006

Comparison of ethanol from corn & sugarcane

Cost item	US corn wet milling	US corn dry milling	US sugarcane	Brazil sugarcane
Feedstock cost	0.40	0.53	1.48	0.30
Processing cost	0.63	0.52	0.92	0.51
Total cost	1.03	1.05	2.04	0.81

- Estimated ethanol production costs ($ per gallon)
  - Excludes capital costs
  - Feedstock costs for U.S. corn wet and dry milling are net feedstock costs
  - USDA report (2006)

Bioproducts from biorefinery

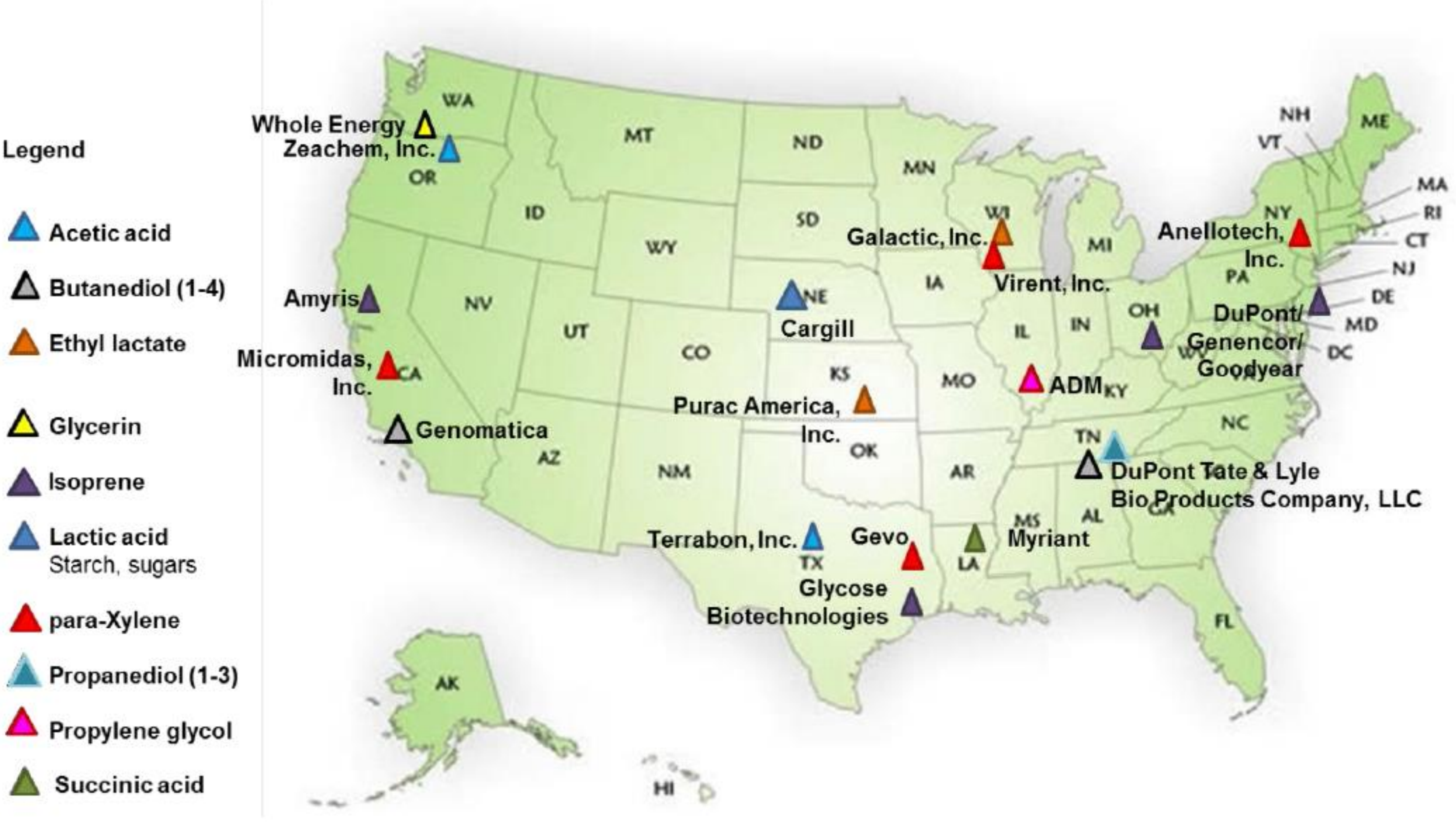
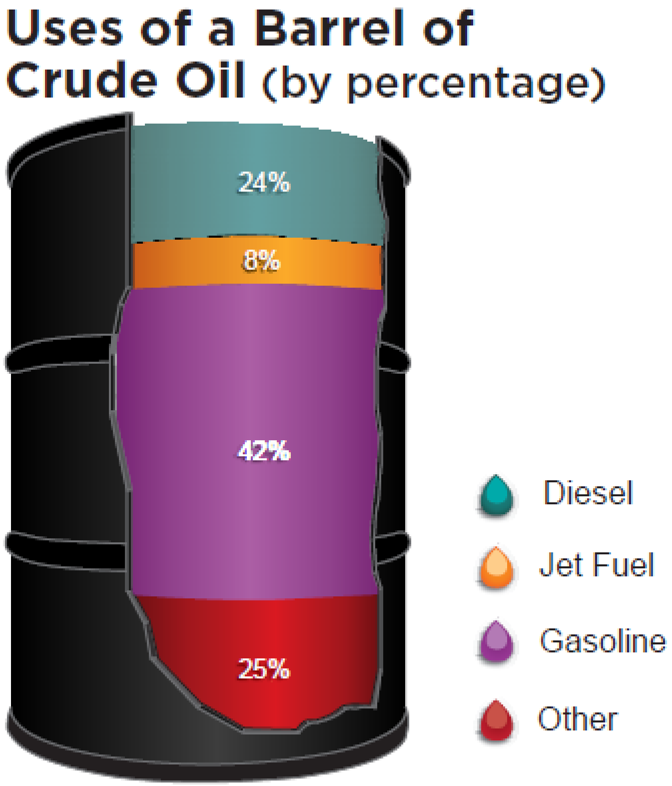


Figure 4. Overview of current and planned bioproduct facilities in the United States

Source: Cameron Cargill

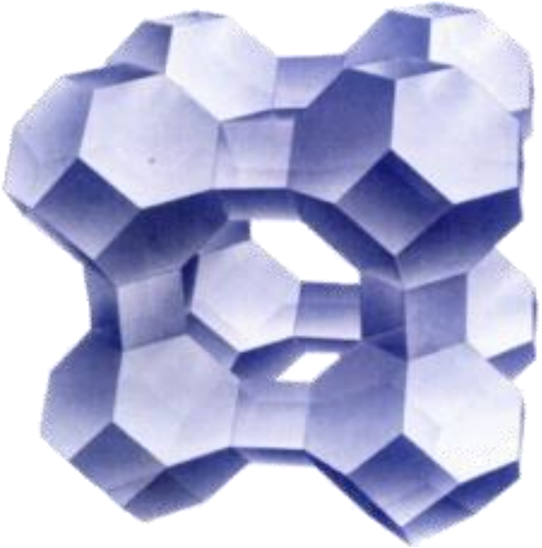
Replacing the whole barrel

- Cellulosic ethanol can
  - displace only 42% of a barrel
  - Of crude oil)gasoline)
- Hydrocarbon biofuels
  - “drop-in” fuels to replace
  - Diesel, jet fuel and others
- 7% of barrel used to make
  - glues, solvents and plastics

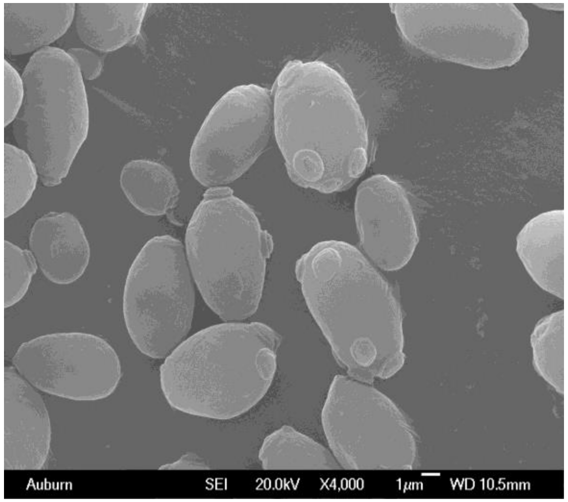

Uses of a Barrel of Crude Oil (by percentage)

Source: Energy Information Administration; data

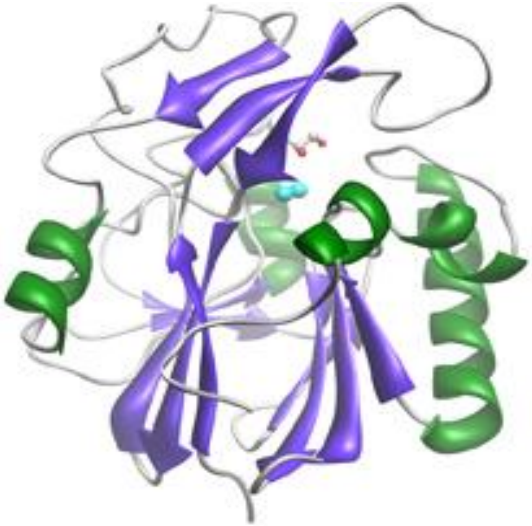
Catalyst and biocatalyst



Zeolite



Yeast

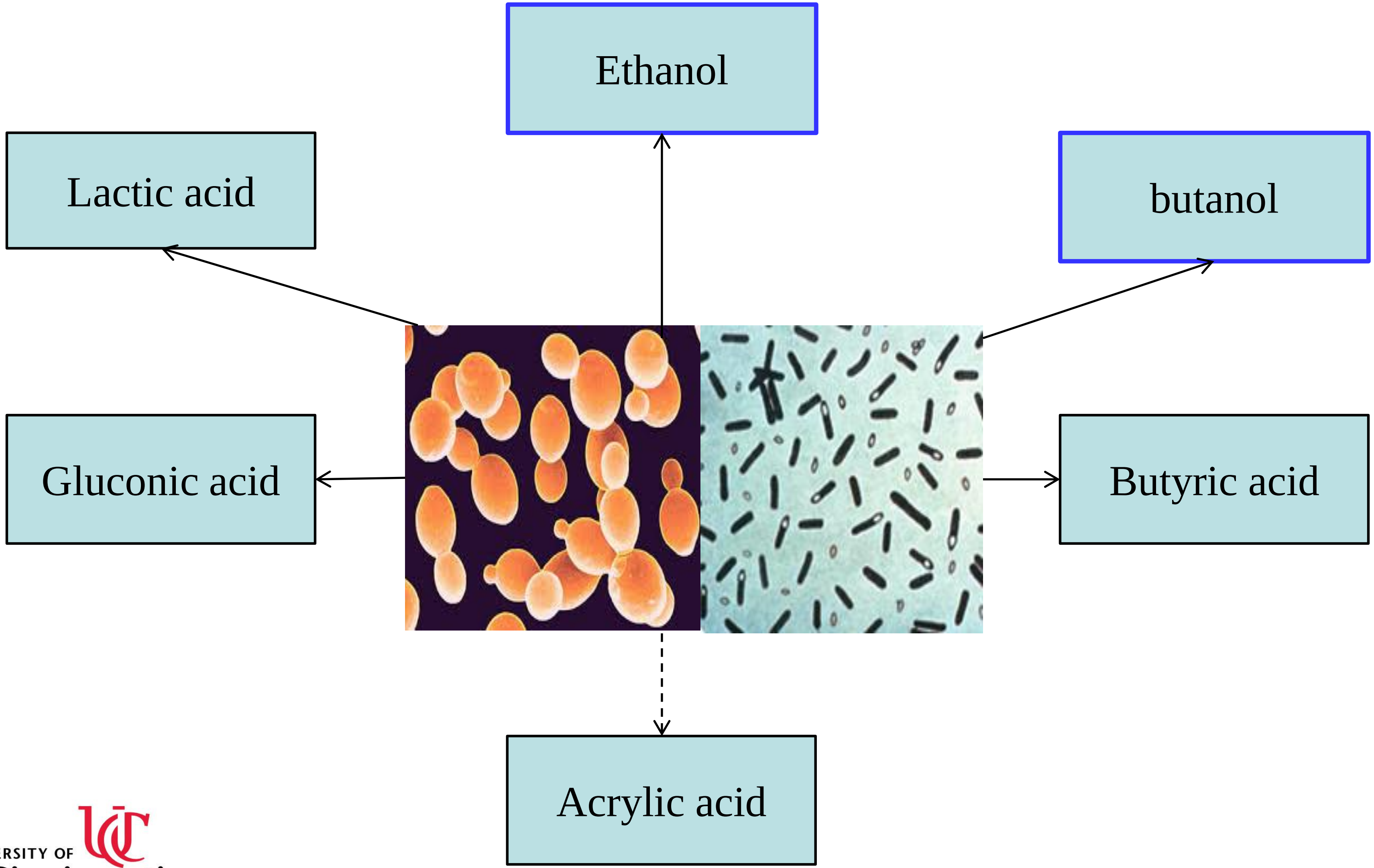


Enzyme

Catalyst and process engineering

- Engineers turn molecules into money
  - develop and operate processes to convert raw materials into valuable products
  - Reactor design, process control, reaction kinetics, mass and heat transfer and separation
  - **Catalyst plays essential role** in many of these processes

Tu research: fuels and chemicals



Ethanol

Lactic acid

butanol

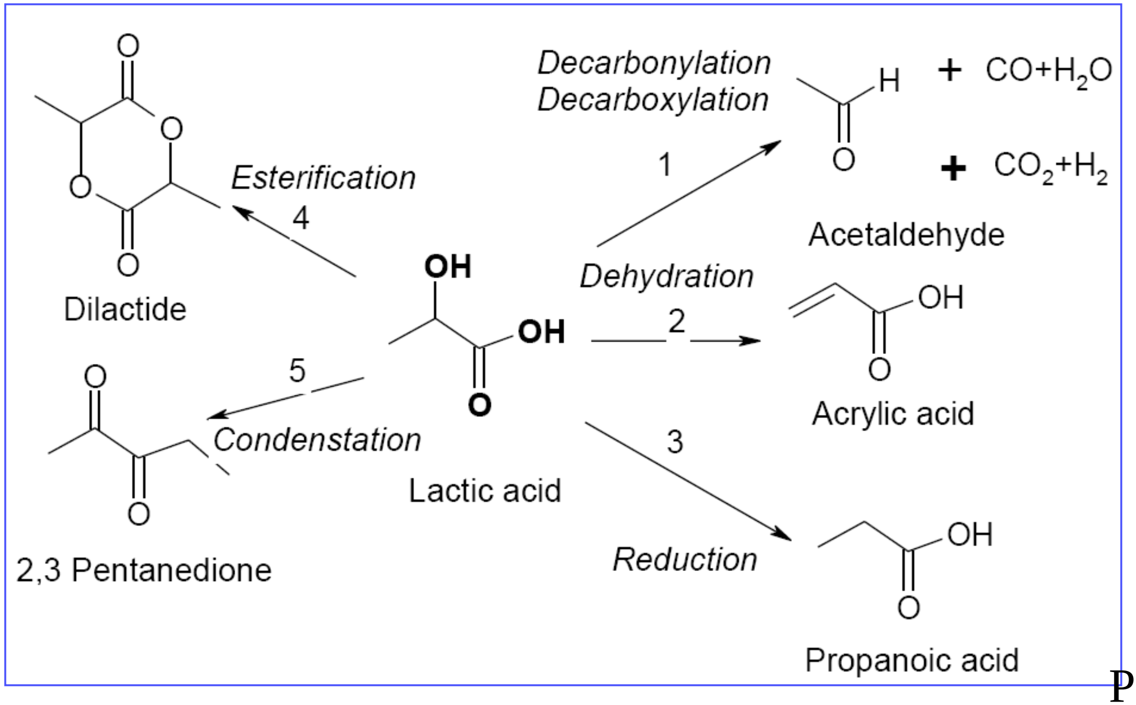
Gluconic acid

Butyric acid

Acrylic acid

Our research on acrylic acid

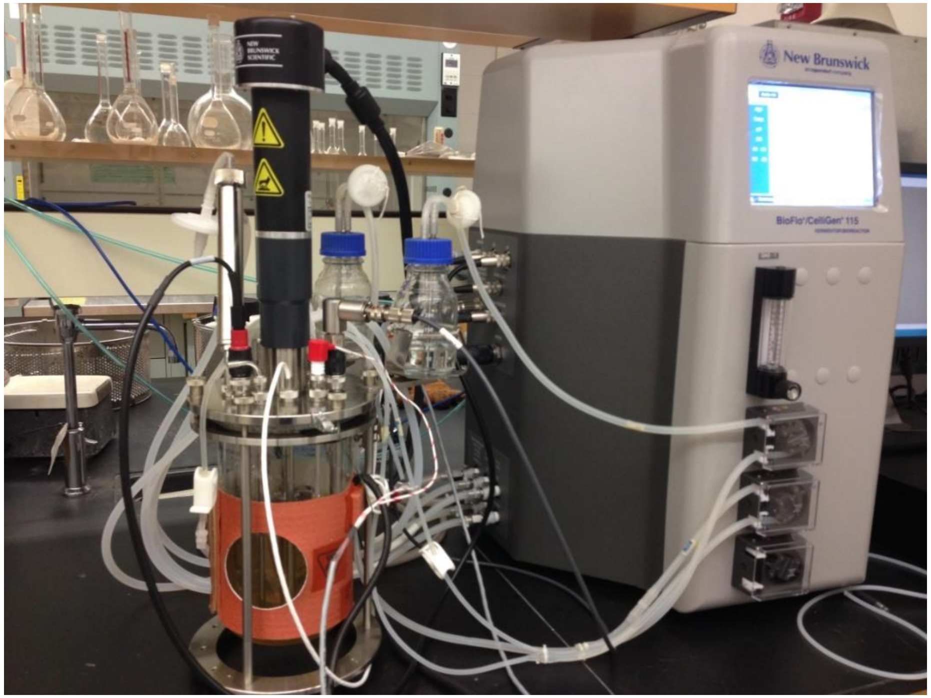
- Integrating biochemical conversion and chemical catalysis to produce new chemicals
  - Biomass to lactic acid by fermentation
  - Catalytic conversion of lactic acid to acrylic acid



Procter & Gamble

Our research on butanol

- Biofuels and bioproducts manufacturing
  - Butanol production from renewable biomass by *Clostridium acetobutylicum*
  - Carbonyl inhibition of biofuels production



New approach

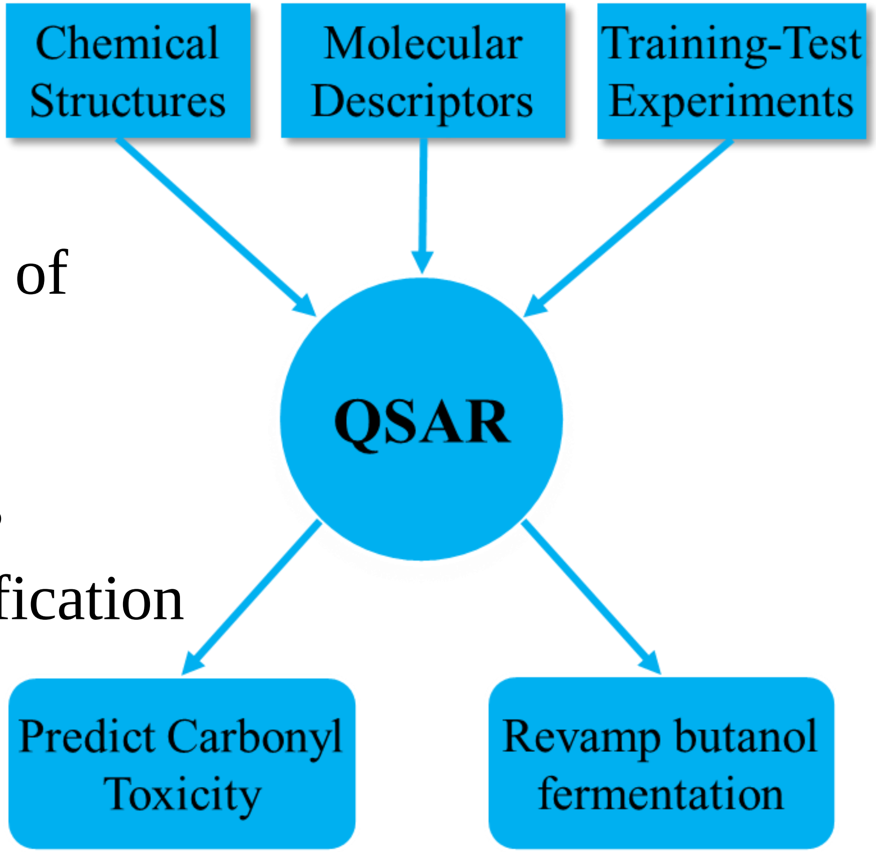
- Quantitative structure-activity relationship (QSAR) approach
  - Computational study
  - Experimental determination

❖Predict the inhibitory effects of

  ❖degradation compounds

❖Identify the potent inhibitors

❖Design new selective detoxification



Chemical Structures

Molecular Descriptors

Training-Test Experiments

QSAR

Predict Carbonyl Toxicity

Revamp butanol fermentation

Objectives and Hypothesis

- Objectives:
  - Establish QSAR between molecular descriptors and inhibitory effects
    - of carbonyl compounds on microbial fermentation
  - Design carbonyl-based selective chemical reactions
    - For detoxifying biomass hydrolysates
- Hypothesis:
  - *Inhibition of carbonyl compounds is governed by their electrophilic reactivity to biological nucleophiles, the reactivity is further dominated by physicochemical properties*

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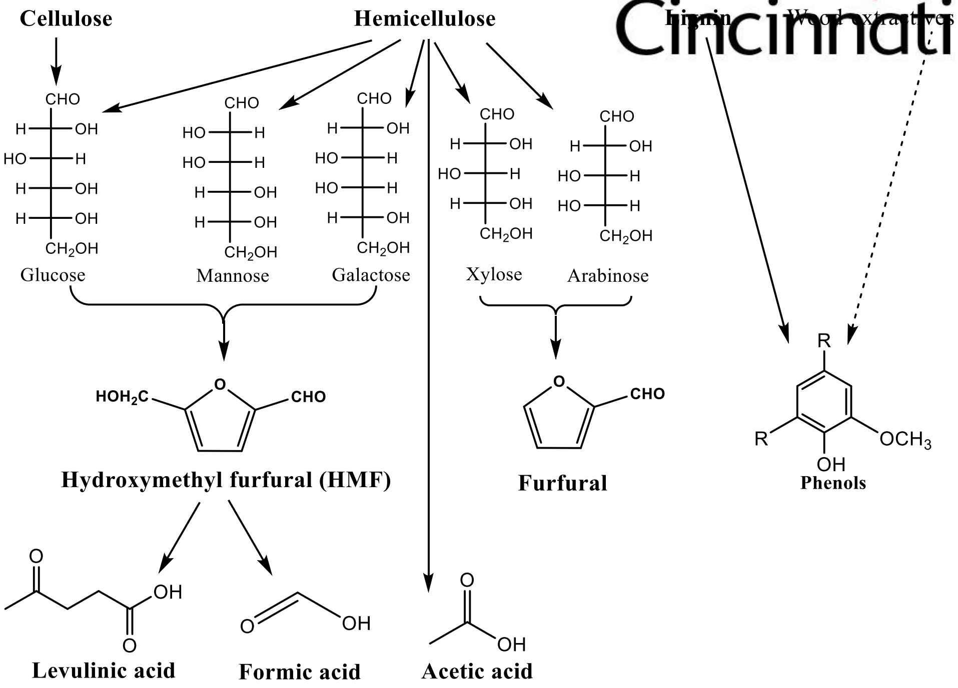
Formation of carbonyl inhibitors

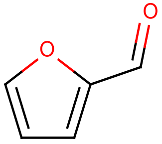
- **Furans**
  - From sugars (Furfural and HMF)
  - Toxicity low
- **Carboxylic acids**
  - Aliphatic acid from sugar
  - Phenolic acid from lignin
- **Phenolic compounds**
  - Mostly from lignin and extractives
  - Low concentration
  - Higher toxicity



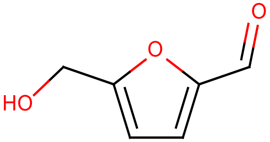
Questions: What are the most potent inhibitors?

[1] Jönsson J. *Biotechnology for Biofuels*, 2013

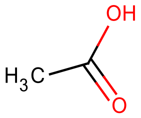
Fermentation inhibitors: carbonyl compounds



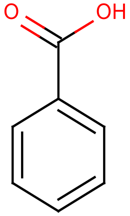
Furfural



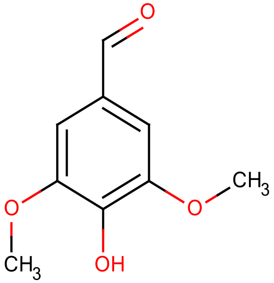
HMF



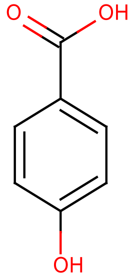
Acetic acid



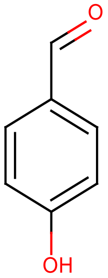
Benzoic acid



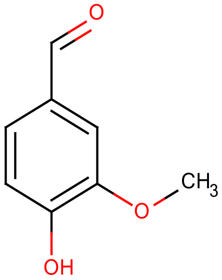
Syringaldehyde



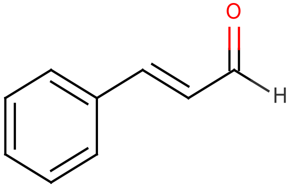
4-Hydroxybenzoic acid



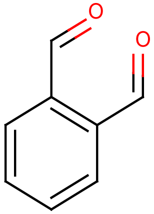
4-Hydroxybenzaldehyde



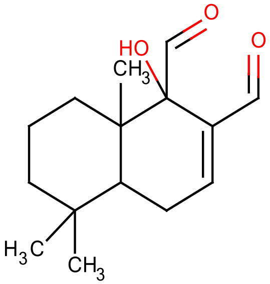
Vanillin



Cinnamaldehyde



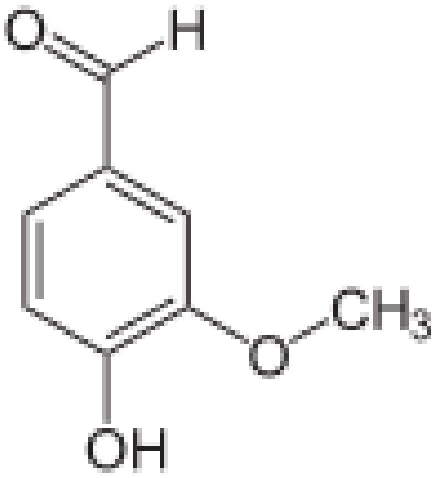
Ortho-phthalaldehyde



Warburganal

Critical issues and questions

- What are the ***most potent inhibitors***?
- How can they be ***selectively removed***?
- Which *functional groups* are responsible for their inhibition?



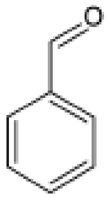
Methods

- Calculate the physiochemical properties of model compounds
  - The E_{LUMO}, E_{HOMO}, dipole moment (u), molar refractivity (MR) calculated by semi-empirical methods using Gaussian 09. The electrophilicity index (ω) calculated by the equation :

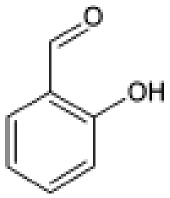
$$\omega = \frac{\mu^2}{2\eta} = \frac{E_{HOMO}{}^2 + 2E_{HOMO}E_{LUMO} + E_{LUMO}{}^2}{4(E_{LUMO} - E_{HOMO})}$$

- Determine the inhibitory effects of model carbonyl compounds on yeast fermentation
  - Glucose consumption rate
  - Final ethanol yield

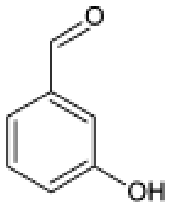
Phenolic model compounds



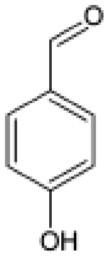
benzaldehyde



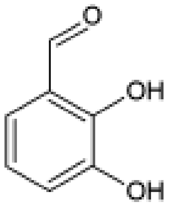
2-hydroxybenzaldehyde



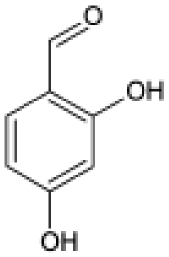
3-hydroxybenzaldehyde



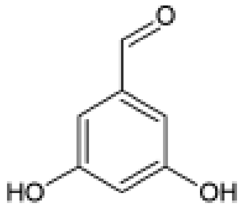
4-hydroxybenzaldehyde



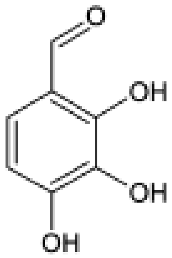
2,3-dihydroxybenzaldehyde



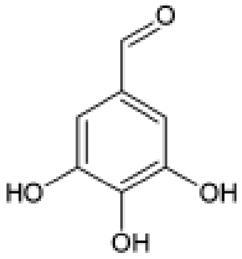
2,4-dihydroxybenzaldehyde



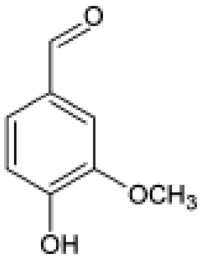
3,5-dihydroxybenzaldehyde



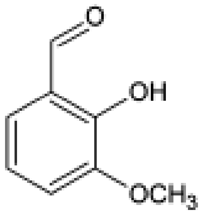
2,3,4-trihydroxybenzaldehyde



3,4,5-trihydroxybenzaldehyde

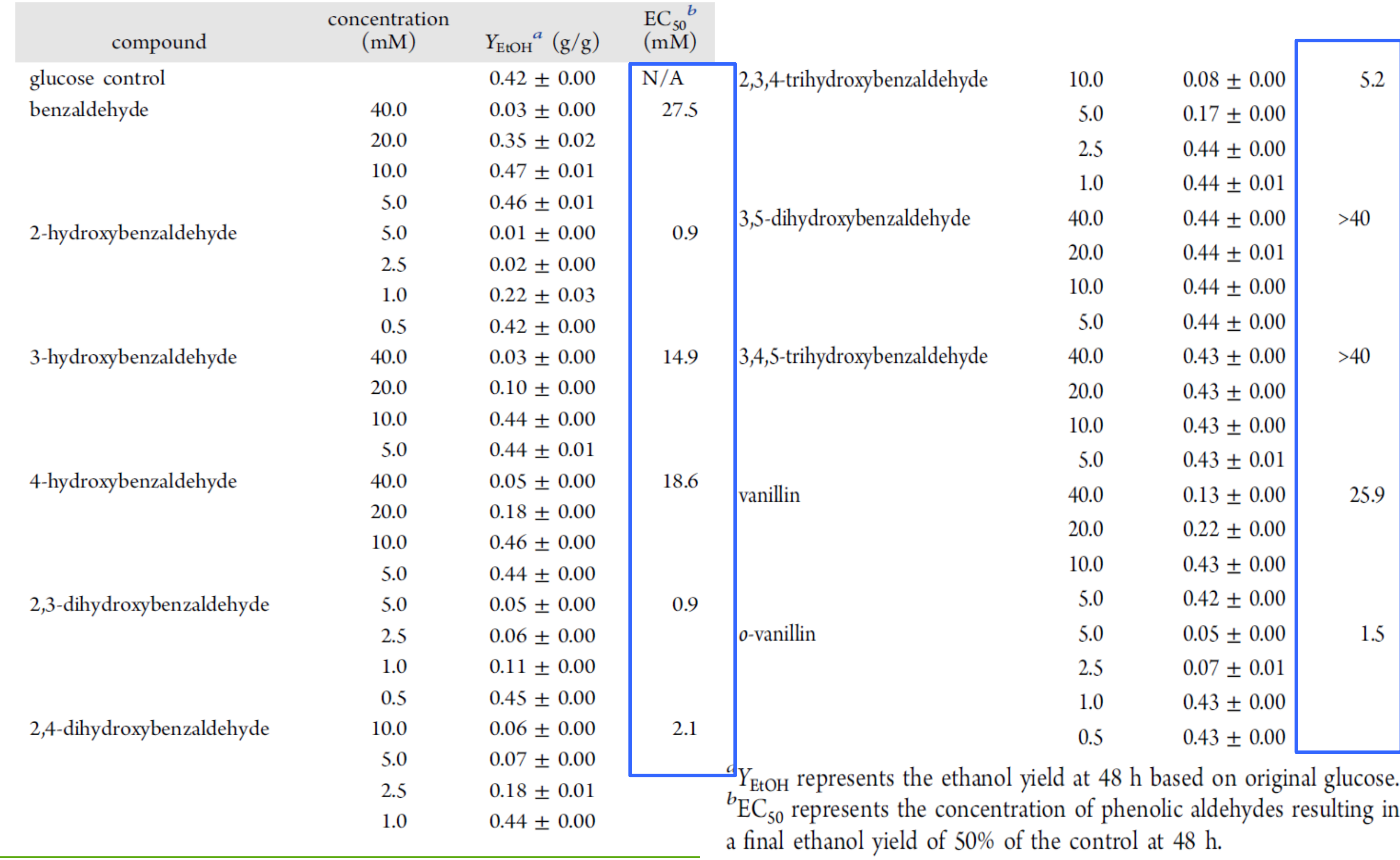


vanillin



o-vanillin

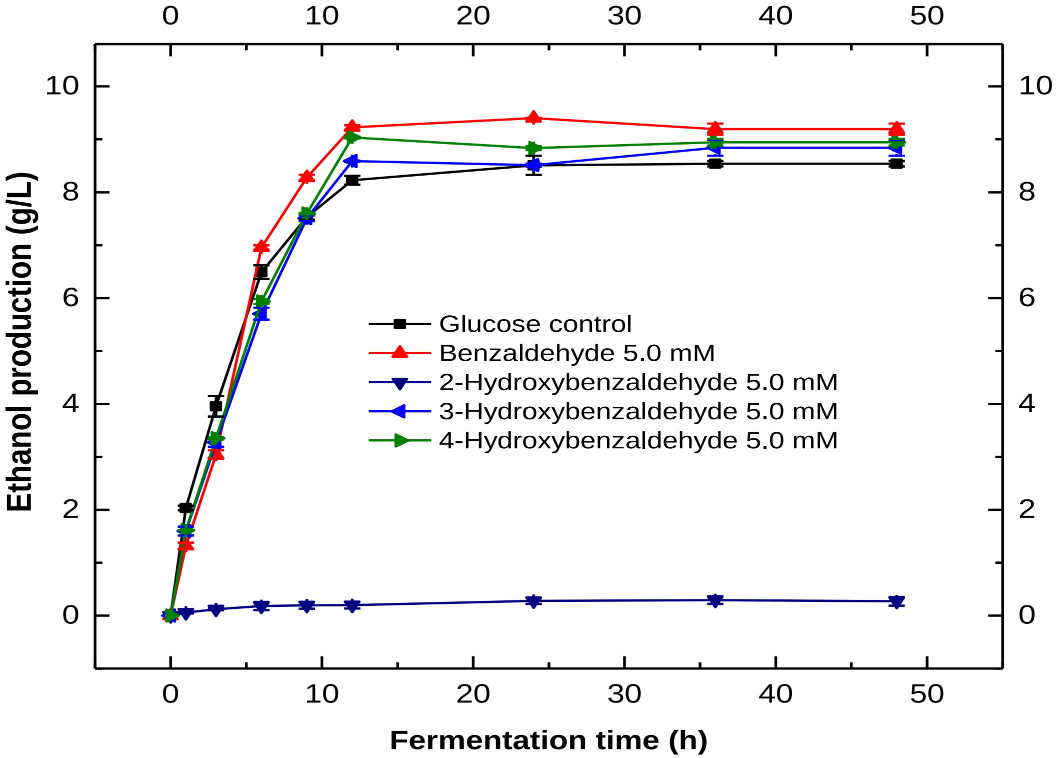
Substitution Effects of Phenolic Aldehyde Inhibition on fermentation



compound	concentration (mM)	Y_{EtOH} [a] (g/g)	EC_{50} [b] (mM)
glucose control		0.42 ± 0.00	N/A
benzaldehyde	40.0	0.03 ± 0.00	27.5
	20.0	0.35 ± 0.02	
	10.0	0.47 ± 0.01	
	5.0	0.46 ± 0.01	
2-hydroxybenzaldehyde	5.0	0.01 ± 0.00	0.9
	2.5	0.02 ± 0.00	
	1.0	0.22 ± 0.03	
	0.5	0.42 ± 0.00	
3-hydroxybenzaldehyde	40.0	0.03 ± 0.00	14.9
	20.0	0.10 ± 0.00	
	10.0	0.44 ± 0.00	
	5.0	0.44 ± 0.01	
4-hydroxybenzaldehyde	40.0	0.05 ± 0.00	18.6
	20.0	0.18 ± 0.00	
	10.0	0.46 ± 0.00	
	5.0	0.44 ± 0.00	
2,3-dihydroxybenzaldehyde	5.0	0.05 ± 0.00	0.9
	2.5	0.06 ± 0.00	
	1.0	0.11 ± 0.00	
	0.5	0.45 ± 0.00	
2,4-dihydroxybenzaldehyde	10.0	0.06 ± 0.00	2.1
	5.0	0.07 ± 0.00	
	2.5	0.18 ± 0.01	
	1.0	0.44 ± 0.00	
2,3,4-trihydroxybenzaldehyde	10.0	0.08 ± 0.00	5.2
	5.0	0.17 ± 0.00	
	2.5	0.44 ± 0.00	
	1.0	0.44 ± 0.01	
3,5-dihydroxybenzaldehyde	40.0	0.44 ± 0.00	>40
	20.0	0.44 ± 0.01	
	10.0	0.44 ± 0.00	
	5.0	0.44 ± 0.00	
3,4,5-trihydroxybenzaldehyde	40.0	0.43 ± 0.00	>40
	20.0	0.43 ± 0.00	
	10.0	0.43 ± 0.00	
	5.0	0.43 ± 0.01	
vanillin	40.0	0.13 ± 0.00	25.9
	20.0	0.22 ± 0.00	
	10.0	0.43 ± 0.00	
	5.0	0.42 ± 0.00	
o-vanillin	5.0	0.05 ± 0.00	1.5
	2.5	0.07 ± 0.01	
	1.0	0.43 ± 0.00	
	0.5	0.43 ± 0.00	

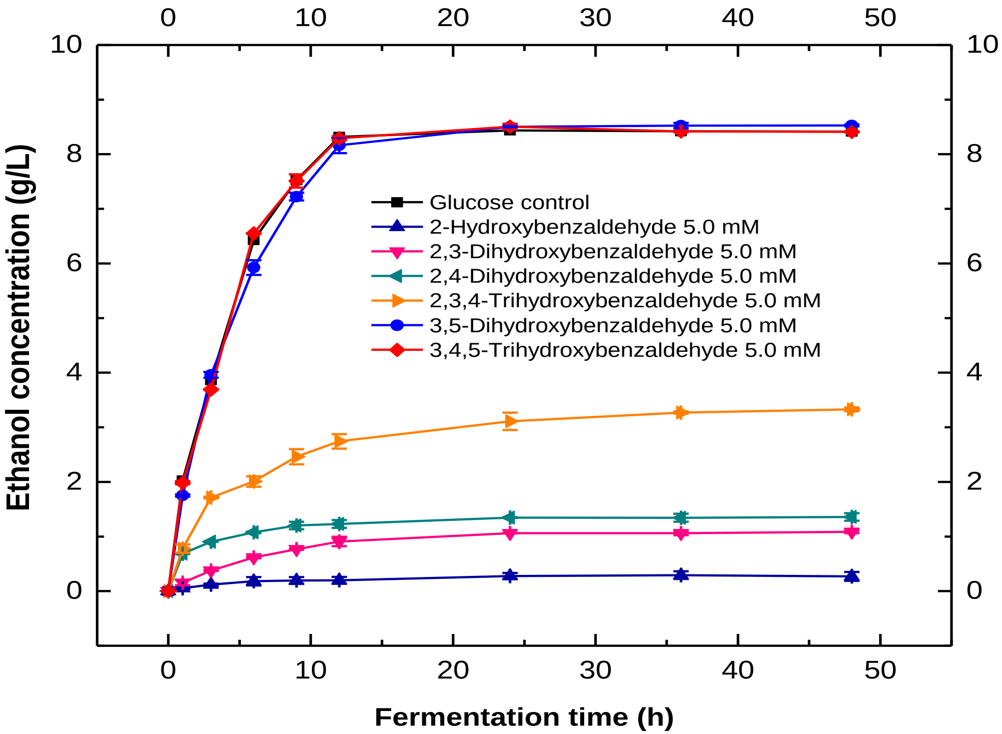
[a] Y_{EtOH} represents the ethanol yield at 48 h based on original glucose.
[b] EC_{50} represents the concentration of phenolic aldehydes resulting in a final ethanol yield of 50% of the control at 48 h.

Effect of 2-, 3- and 4-hydroxybenzaldehydes on fermentation



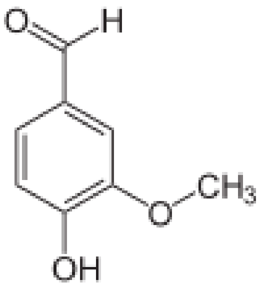
- 2-hydroxybenzaldehyde showed 30-fold higher inhibition activity than benzaldehyde
- *Ortho*-substituted 2-hydroxybenzaldehyde resulted in 15-20 fold higher inhibition than the meta- or para-substituted analogues of 3- and 4-hydroxybenzaldehydes

Effects of di- and trihydroxybenzaldehydes on yeast fermentation

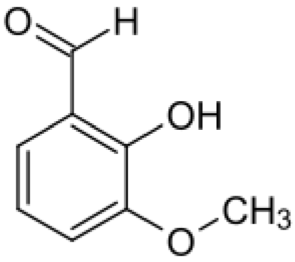


- 3,5-dihydroxybenzaldehyde was much less inhibitory (EC50, > 40 mM) than 2,3- and 2,4-dihydroxybenzaldehydes (EC50, 0.9-2.1 mM)
- *Ortho* -OH group can influence the inhibition significantly.

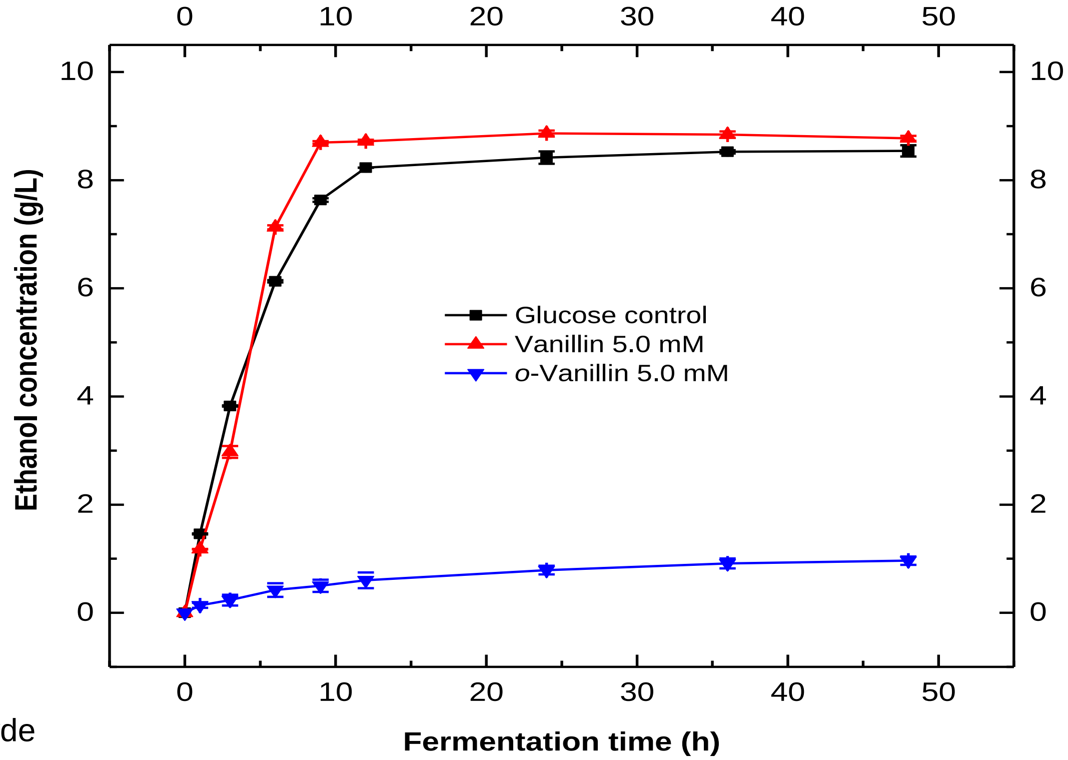
Effect of vanillin and o-vanillin on fermentation



4-Hydroxy-3-methoxybenzaldehyde



2-Hydroxy-3-methoxybenzaldehyde



- Methoxyl group not important in benzaldehyde inhibition
- the position of –OH group contributed to the higher inhibitory activity of o-vanillin

Physicochemical descriptors and inhibitory activity

Compound	C_{carb}	O_{carb}	C_1	Log P	E_{HOMO} (a.u)	E_{LUMO} (a.u)	Dipole (Debye)	ω	Log EC_{50}' [a]
Benzaldehyde	0.435	0.529	0.177	1.69	-0.3255	-0.0394	3.380	0.116	4.439
2-Hydroxybenzaldehyde	0.427	0.510	0.224	2.03	-0.2990	-0.0322	4.331	0.103	2.954
3-Hydroxybenzaldehyde	0.436	0.525	0.154	1.38	-0.3011	-0.0407	4.372	0.112	4.173
4-Hydroxybenzaldehyde	0.433	0.539	0.209	1.38	-0.3015	-0.0305	4.481	0.102	4.270
2,3-Dihydroxybenzaldehyde	0.428	0.508	0.213	1.73	-0.2872	-0.0318	5.711	0.100	2.954
2,4-Dihydroxybenzaldehyde	0.425	0.519	0.252	1.73	-0.2950	-0.0220	4.959	0.092	3.322
3,5-Dihydroxybenzaldehyde	0.437	0.520	0.134	1.08	-0.2940	-0.0410	4.138	0.111	4.602
2,3,4-Trihydroxybenzaldehyde	0.426	0.515	0.235	1.43	-0.2888	-0.0218	6.474	0.090	3.716
3,4,5-Trihydroxybenzaldehyde	0.434	0.531	0.163	0.78	-0.2911	-0.0323	5.630	0.101	4.602
Vanillin	0.433	0.547	0.190	1.22	-0.2860	-0.0299	2.286	0.097	4.413
o-Vanillin	0.428	0.511	0.218	1.87	-0.2820	-0.0276	5.939	0.094	3.114

[a] Log EC_{50}' represents Log (EC_{50}*1000), in which the concentration unit of EC_{50} was changed from mM to µM.

Quantitative structure-inhibition relationship

Regression	*n*	r^2	*S*	*F*	*P*
Log $EC'_{50} = -53.19 + 132.36\ C_{\text{carb}}$	10	0.73	0.37	24.70	<0.001
Log $EC'_{50} = -17.97 - 41.75\ O_{\text{carb}}$	10	0.60	0.45	13.69	0.005
Log $EC'_{50} = 6.57 + 13.79\ C_1$	10	0.57	2.56	11.79	0.007
Log $EC'_{50} = 6.10 - 132.36 \log P$	10	0.69	0.40	19.58	0.002
Log $EC'_{50} = 2.01 - 19.89\ E_{\text{HOMO}}$	10	0.12	0.66	1.25	0.293
Log $EC'_{50} = 2.26 + 50.55\ E_{\text{LUMO}}$	10	0.25	0.61	2.99	0.118
Log $EC'_{50} = 5.11 - 0.265$ Dipole	10	0.23	0.62	2.68	0.136
Log $EC'_{50} = -0.17 - 39.79\ \omega$	10	0.25	0.61	3.01	0.117
Log $EC'_{50} = -31.71 + 85.50\ C_{\text{carb}} - 0.86 \log P$	10	0.87	0.27	25.88	<0.001

- Strong association was observed between **log P and EC50 value**.
- Good correlation observed between the partial charge on carbonyl carbon (Ccarb) and the EC50 value of aldehydes

Summary

- *Ortho*-substituted 2-hydroxybenzaldehyde resulted in 15-20 fold higher inhibition than the *meta*- or *para*-substituted analogues of 3- and 4-hydroxybenzaldehydes.
- Strong relationship between log *P* (octanol/water partition coefficient) of aldehydes and EC50.
- ***Ortho* –OH group** capable of forming an intramolecular hydrogen bond, which can potentially increase the cell membrane permeability and their toxicity.