Biofuels production from renewable biomass

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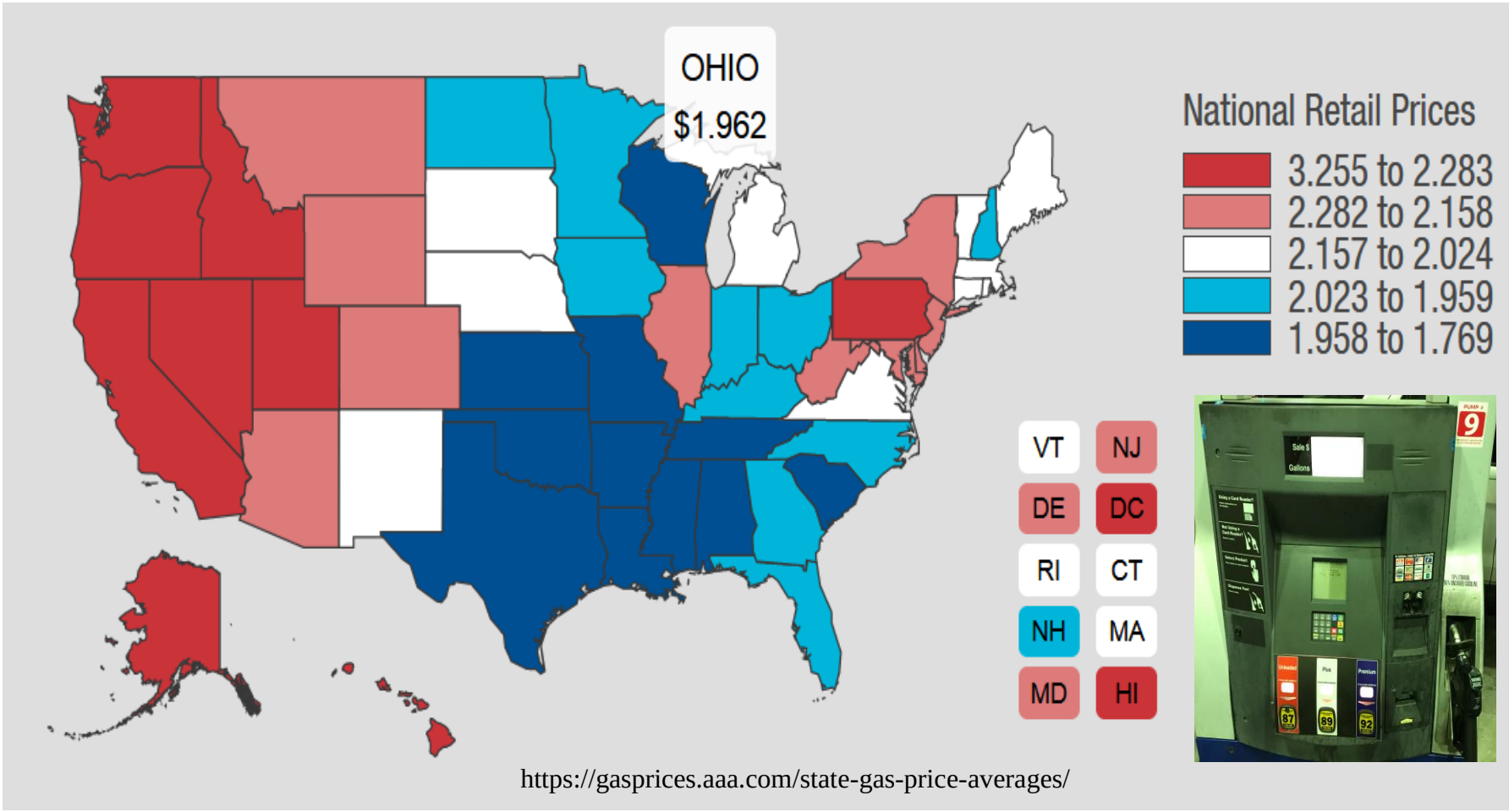
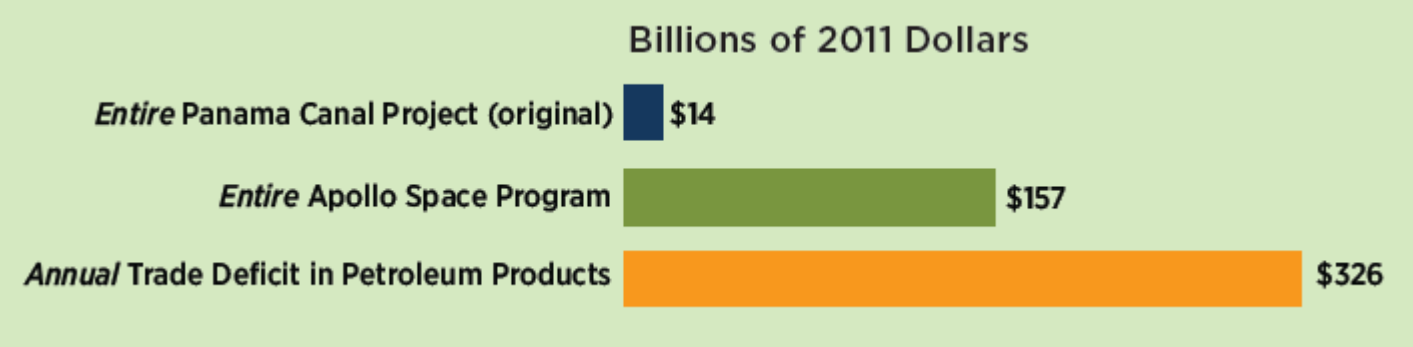
Gasoline and ethanol



https://gasprices.aaa.com/state-gas-price-averages/

Background

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



https://www1.eere.energy.gov/bioenergy/pdfs/replacing_barrel_overview_2012.pdf

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

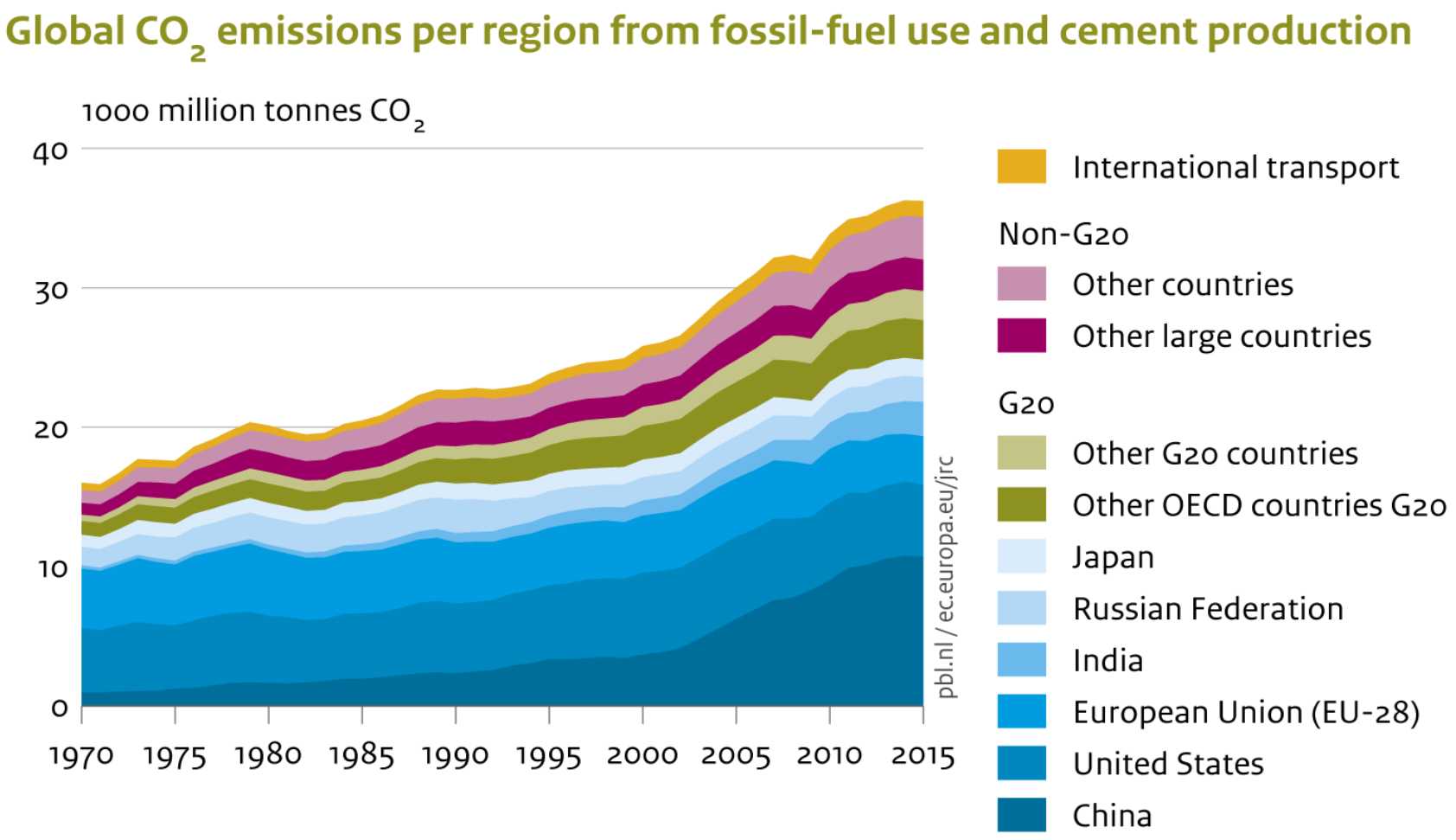
U.S. petroleum imports and exports (2019)

- 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

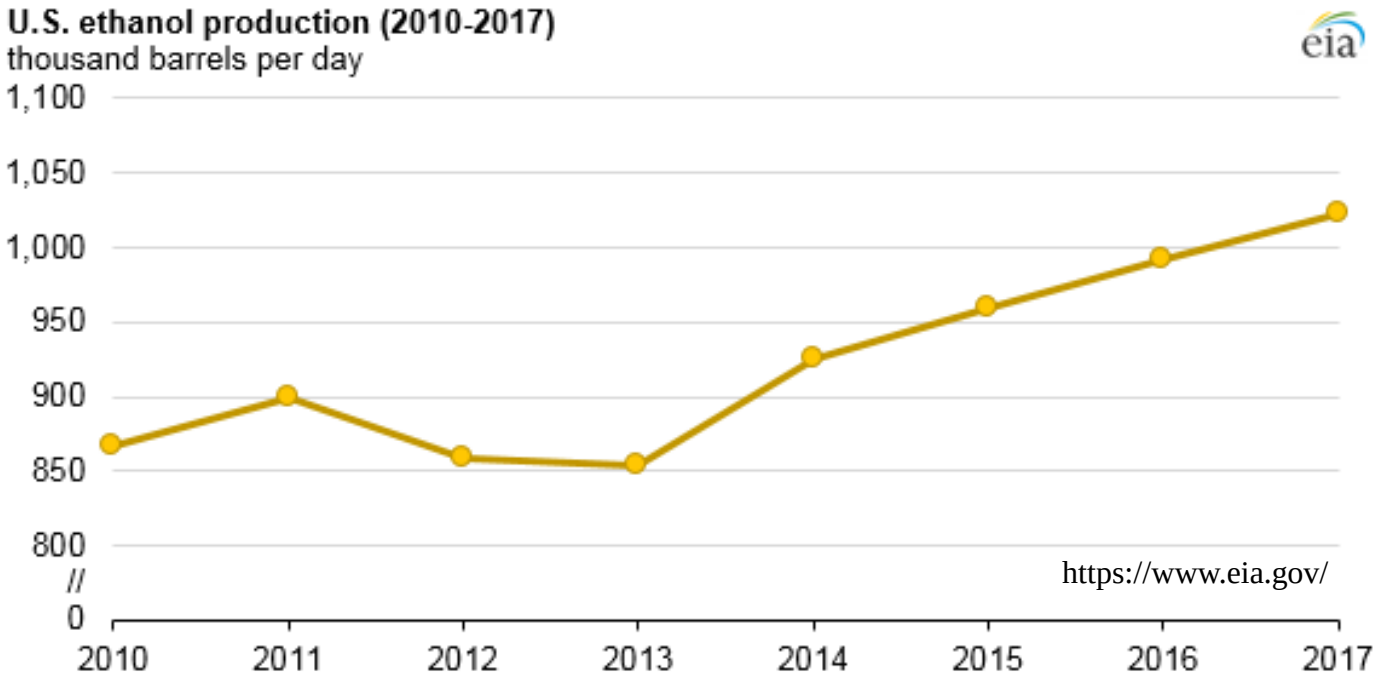
https://www.eia.gov/

U.S. CO2 emission



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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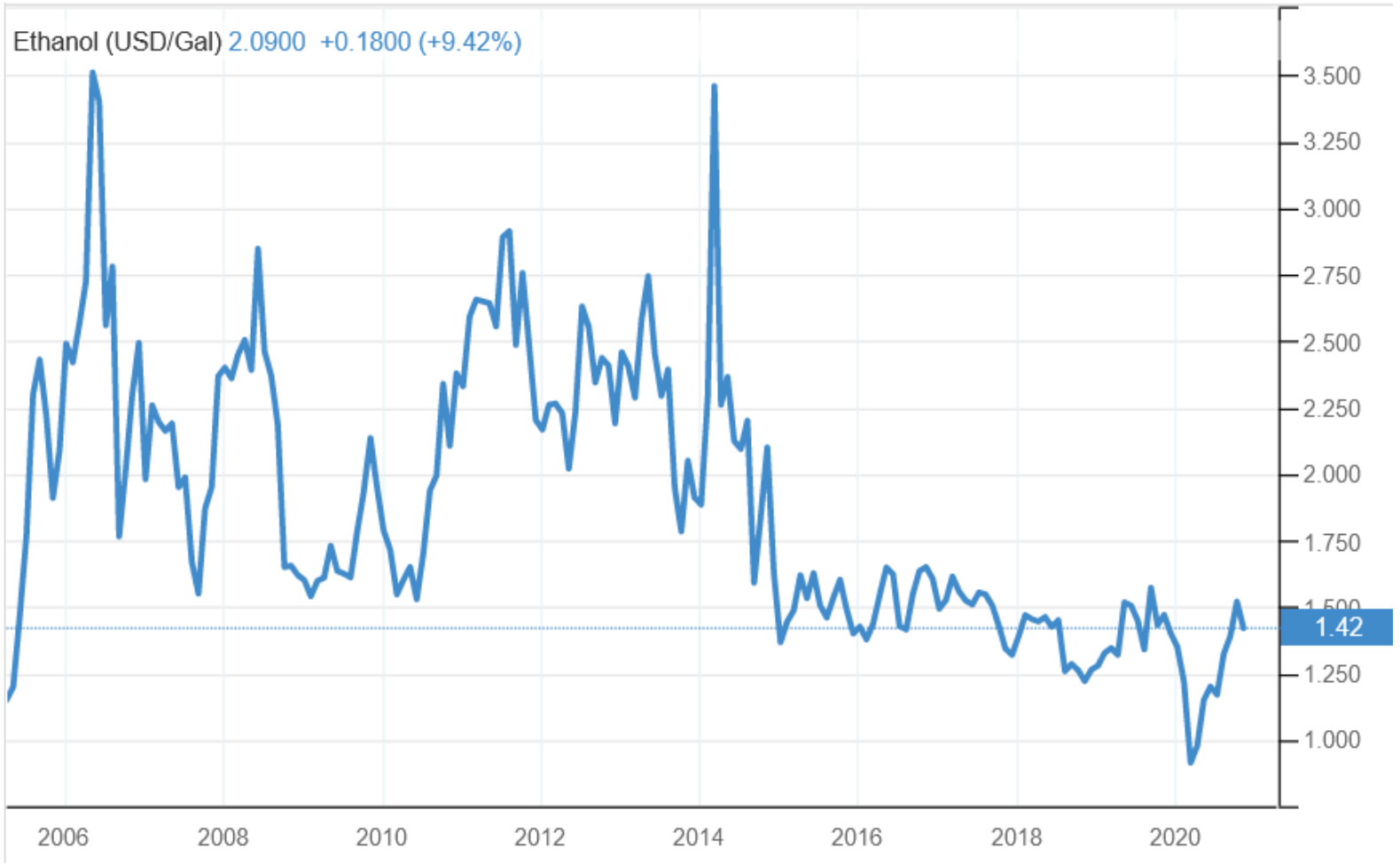
Ethanol price



https://tradingeconomics.com/commodity/ethanol

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

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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)

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

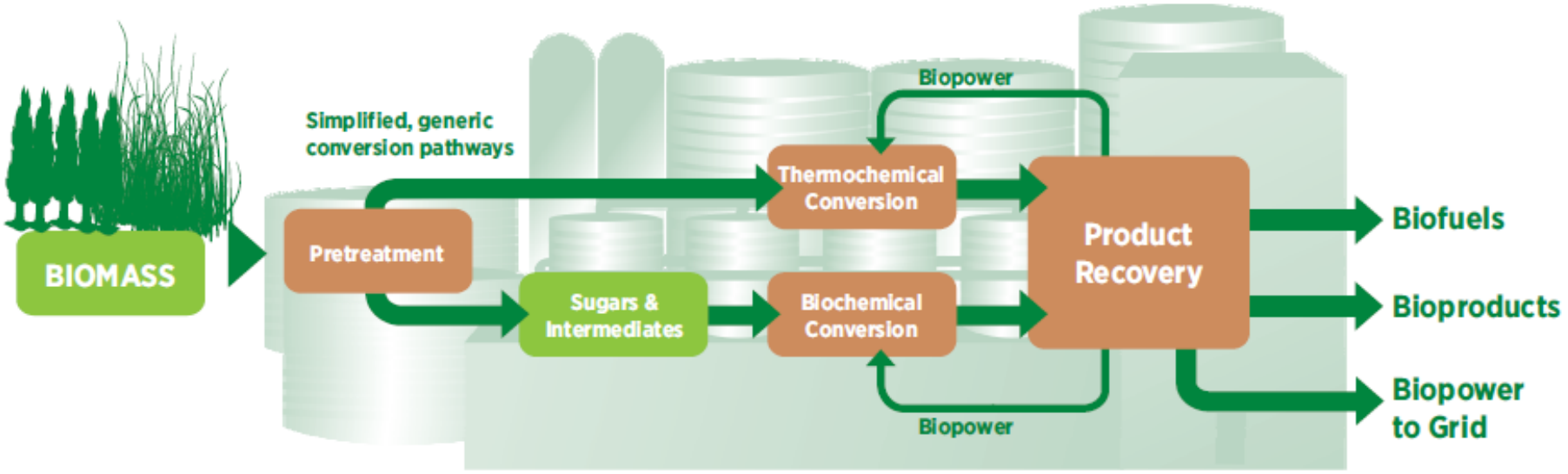
Integrated biorefinery

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

Integrated Biorefinery Projects Funded by DOE

Biorefinery pathways

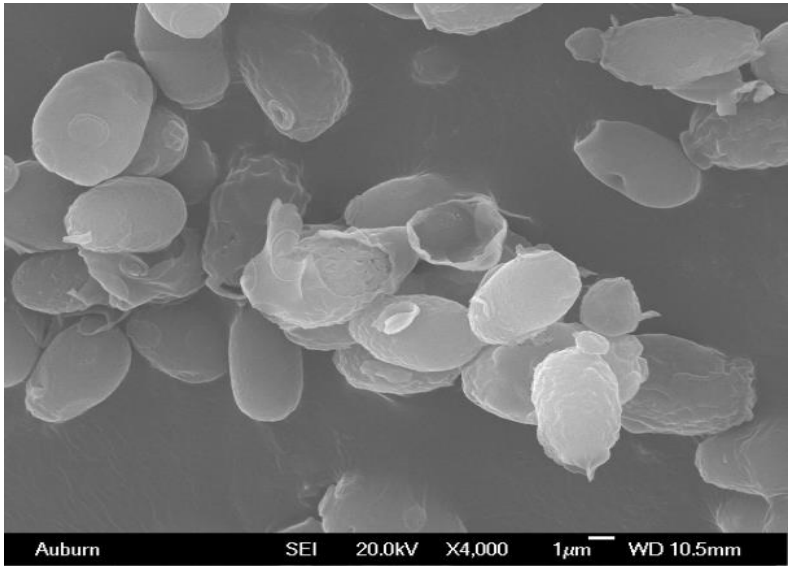
- Thermochemical conversion (gasification/pyrolysis)
- Biochemical conversion (enzymes/microbes)



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

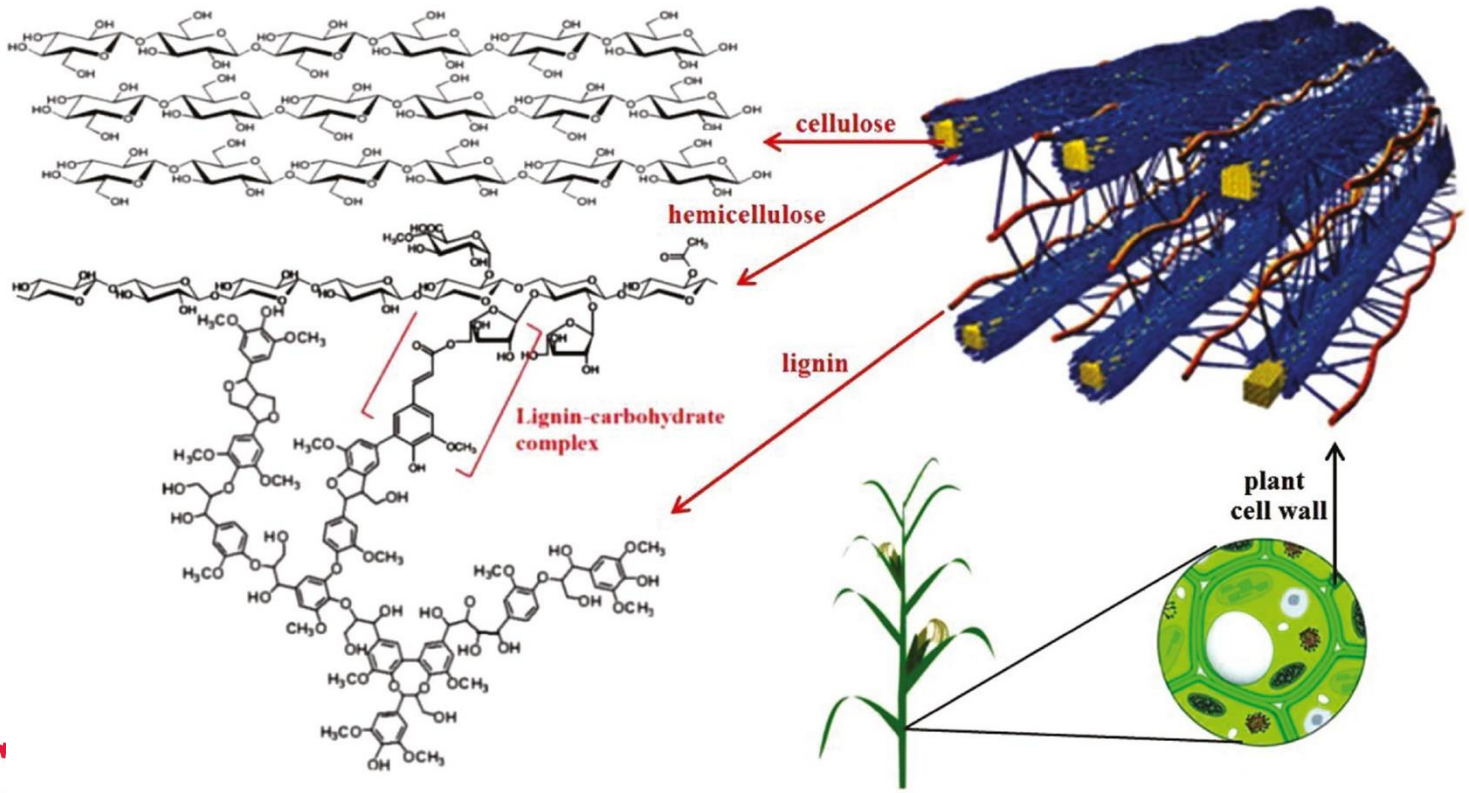
Background

- 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

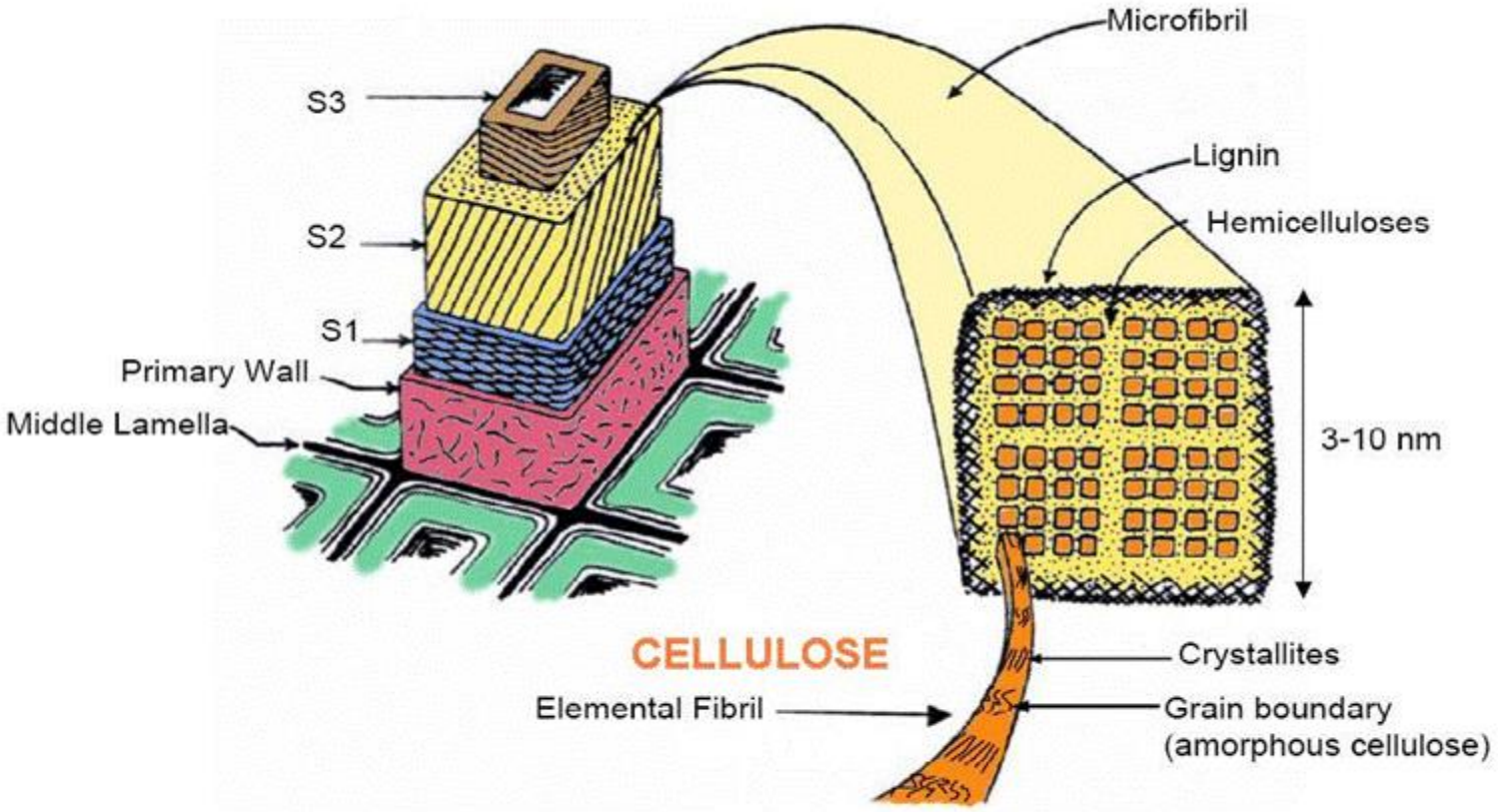


Lignocellulosic biomass

- Renewable feedstock
- Most abundant
- Cellulose, hemicellulose and lignin



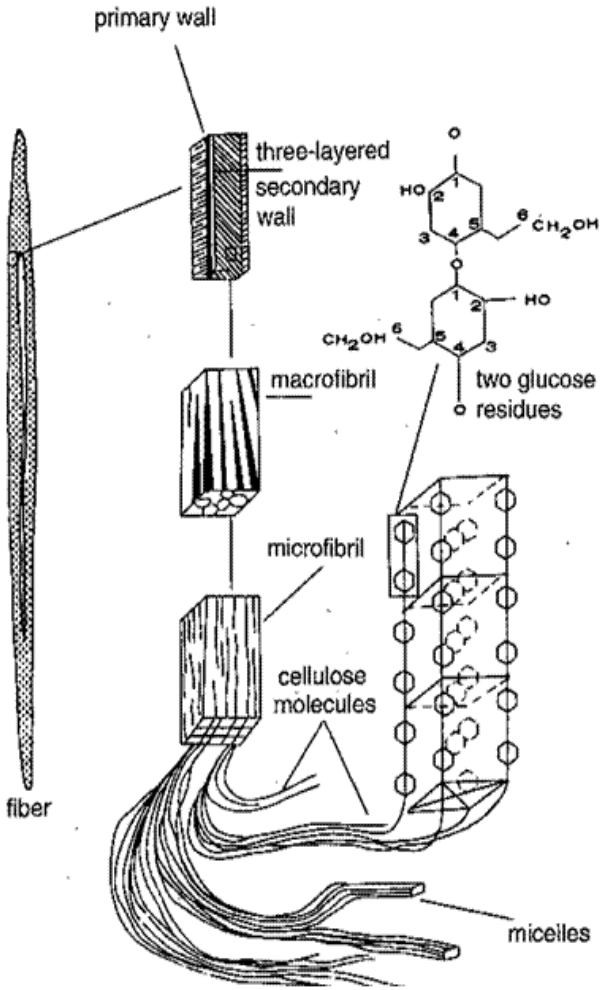
Ultrastructure of plant cell wall



Industry Canada Nov 2005

Lignocellulosic biomass chemistry

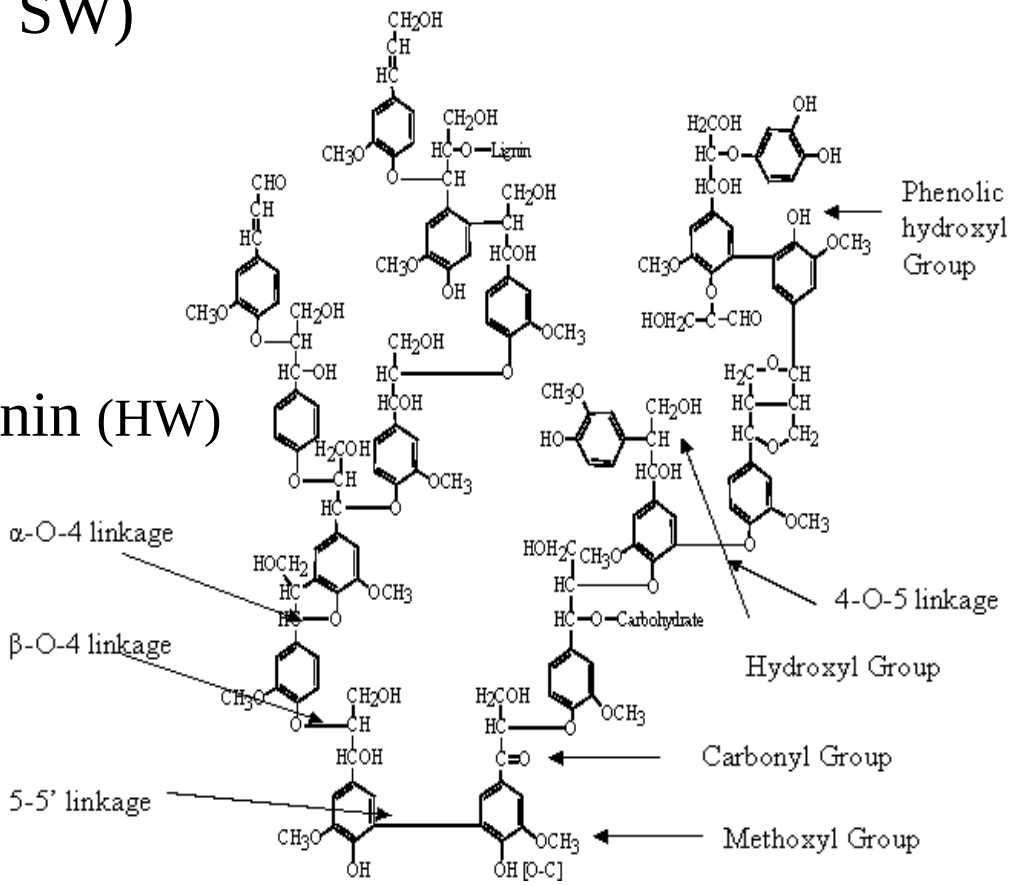
- **Cellulose** (45% HW/SW)
  - Linear polymer of β-1,4 linked glucose
  - Degree polymerization (DP), 10,000
  - Crystalline and amorphous
- **Hemicellulose** (35% HW, 25% SW)
  - Branched polymer of glucose, mannose, galactose, xylose, and arabinose
  - DP 150-200
  - Easily degraded and dissolved



From Bruley

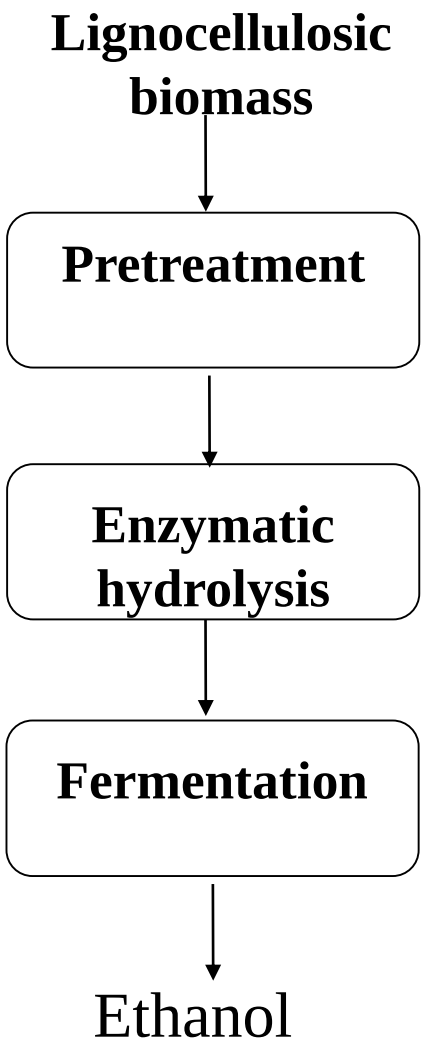
Lignocellulosic biomass chemistry

- **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



Methods & biocatalyst

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

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

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

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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

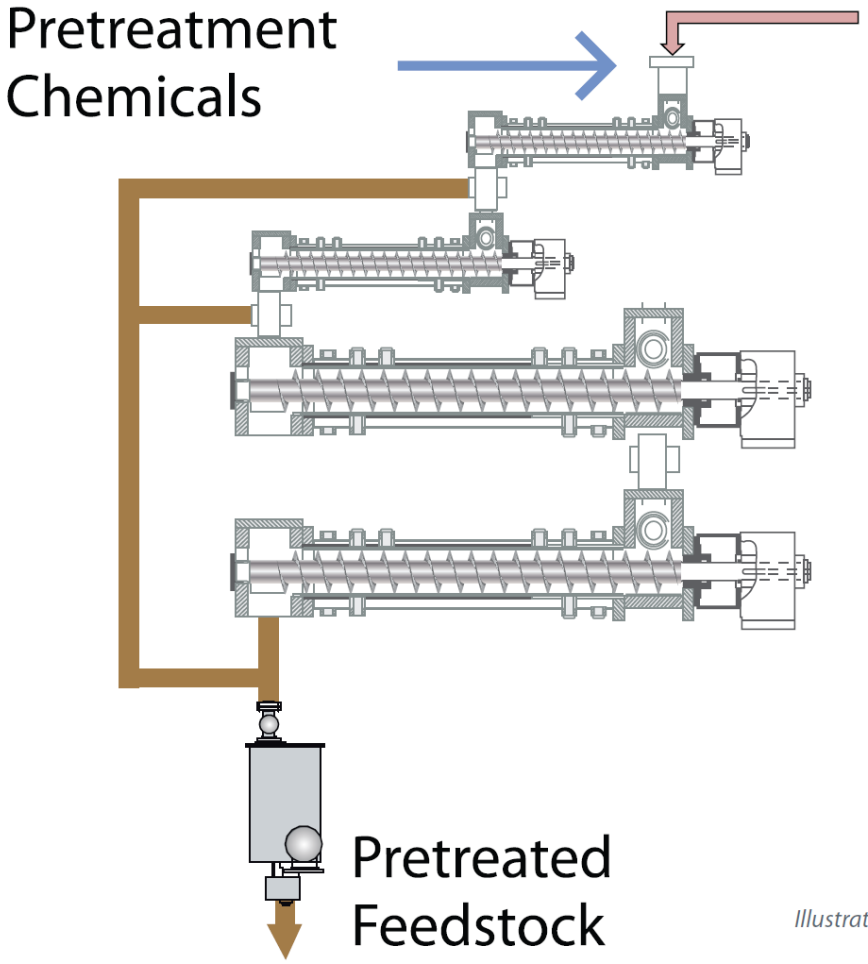


Illustration created by Josh Bauer, NREL

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

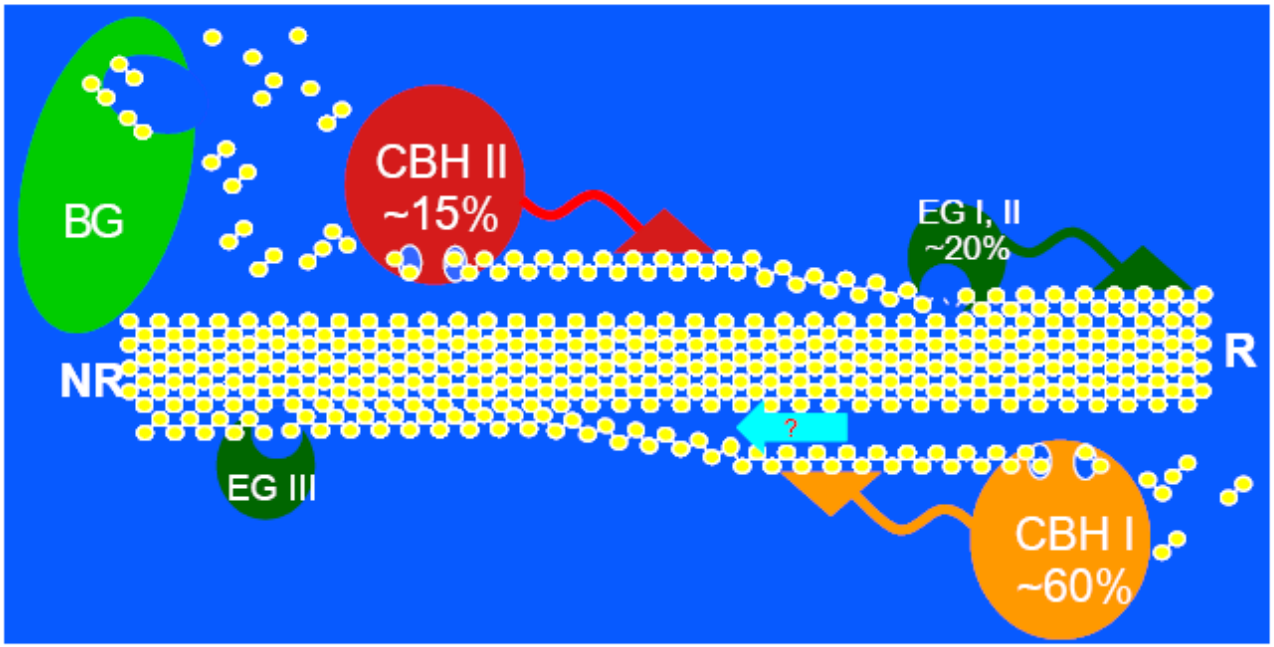
Produce highly concentrated sugar streams

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

https://www.nrel.gov/

Enzymatic hydrolysis of cellulose

Trichoderma reesei Cellulases



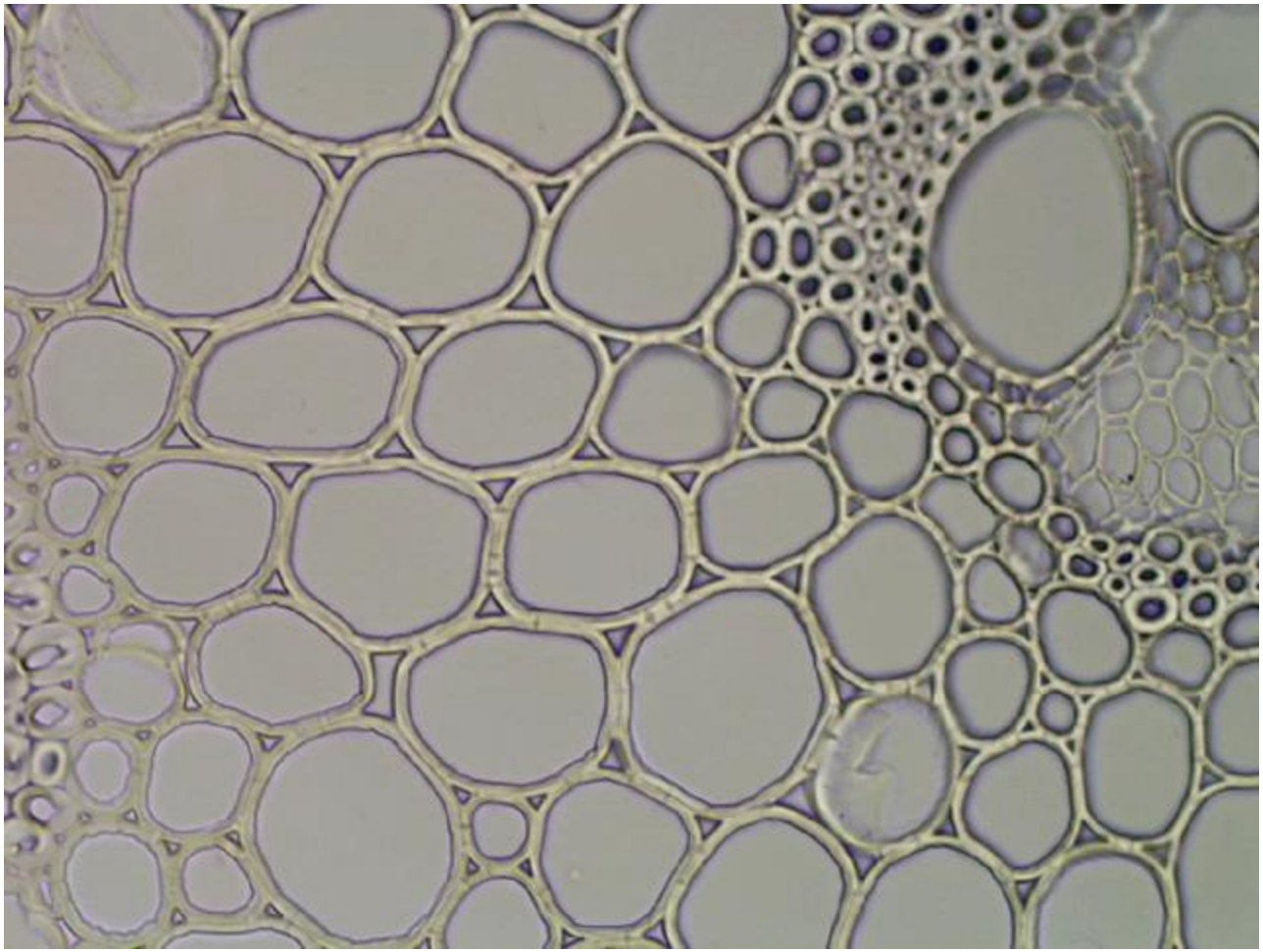
EG: endo-glucanase

CBH: cellobiohydrolase

BG: β-glucosidase

Source: novozymes

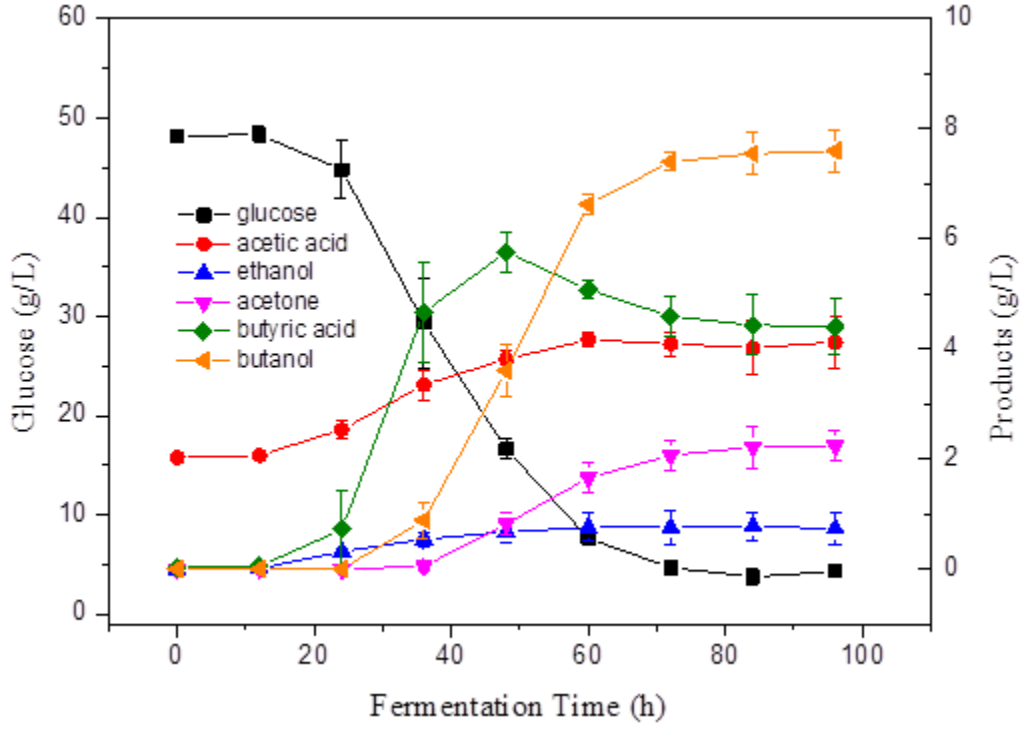
Plant cell walls digested by fungal cellulases (10 h)



Science 23 Nov 2012: Vol. 338, Issue 6110

Microbial fermentation

- Fermentation systems with pH,
  - Temperature, oxygen control
- Monitoring glucose and acetic acid
  - Consumption and butanol production



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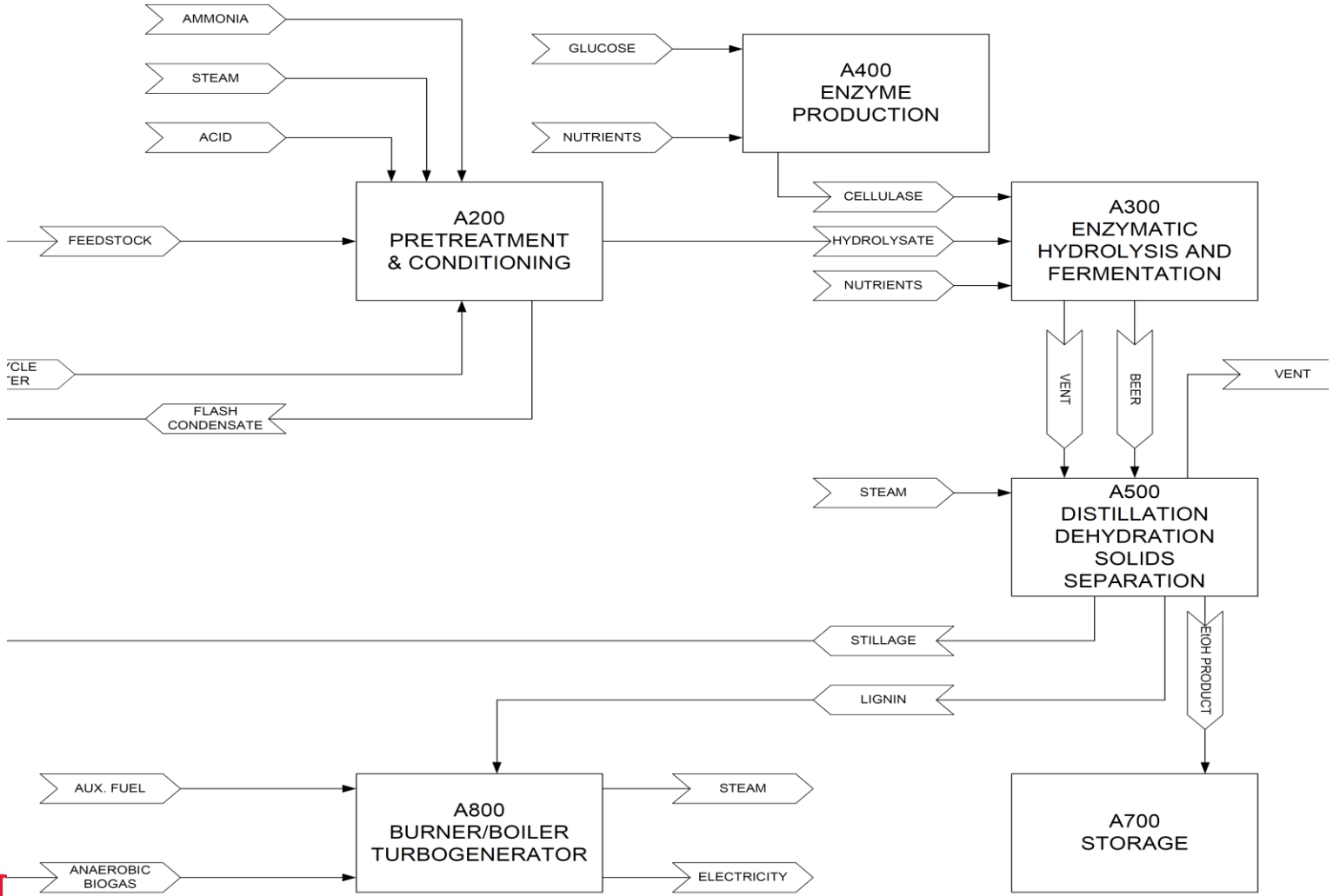
Process design and economic analysis



Humbird, 2011, https://www.nrel.gov/

Ethanol production engineering analysis

- NREL Technical report (2011)
- Dilute acid pretreatment with enzymatic hydrolysis and co-fermentation
- Minimum ethanol selling price: $2.15/gal
- Enzymes cost: $0.34/gal

Humbird, 2012, https://www.nrel.gov/

Biomass composition in process design

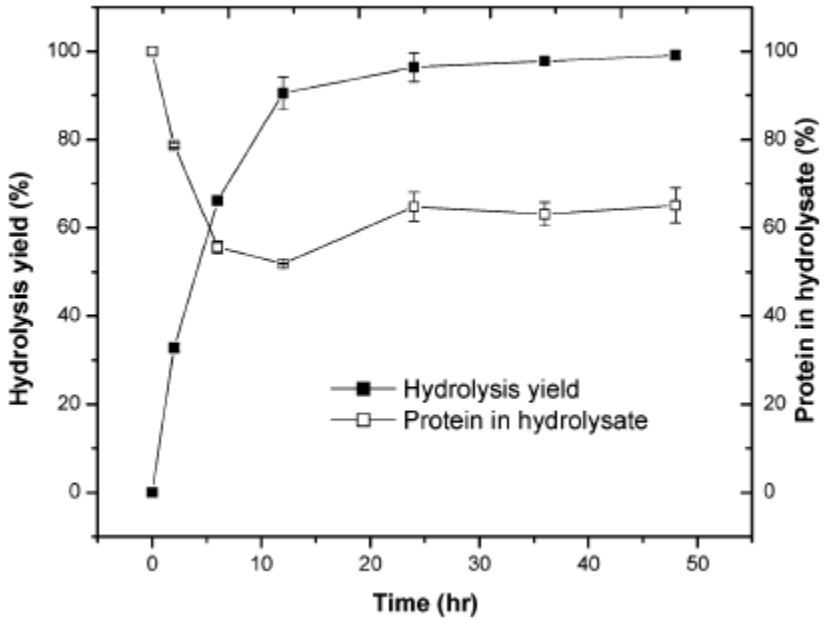
Table 2 Chemical composition of untreated and ethanol organosolv-pretreated loblolly pine

	Untreated (%)	Organosolv treated	
		OPLP-UW (%)	OPLP-W (%)
Glucan	42.30 ± 0.38	72.74 ± 0.20	82.14 ± 0.03
Xylan	7.51 ± 0.05	2.17 ± 0.01	1.69 ± 0.08
Galactan	2.96 ± 0.05	0.36 ± 0.03	0.40 ± 0.02
Arabinan	1.78 ± 0.03	0.63 ± .02	0.69 ± 0.05
Mannan	11.17 ± 0.08	1.36 ± 0.00	0.99 ± 0.02
Ethanol extractives	1.18 ± 0.05	9.64 ± 0.12	0.79 ± 0.04
Acid insoluble lignin (AIL)	29.45 ± 0.27	12.11 ± 0.15	13.52 ± 0.10
Acid-soluble lignin (ASL)	0.56 ± 0.05	0.28 ± 0.00	0.35 ± 0.01
Ash	0.36 ± 0.02	0.03 ± 0.00	0.04 ± 0.00
Total	97.27	99.31	100.61

- Biomass cost is $58.5/dry ton (2007$) Li et al. *Biotechnol Biofuels* (2020) 13:67

Enzymatic hydrolysis and assumed conversions

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



Tu, *Biotechnol. Prog.*, 2007, Vol. 23, No. 2

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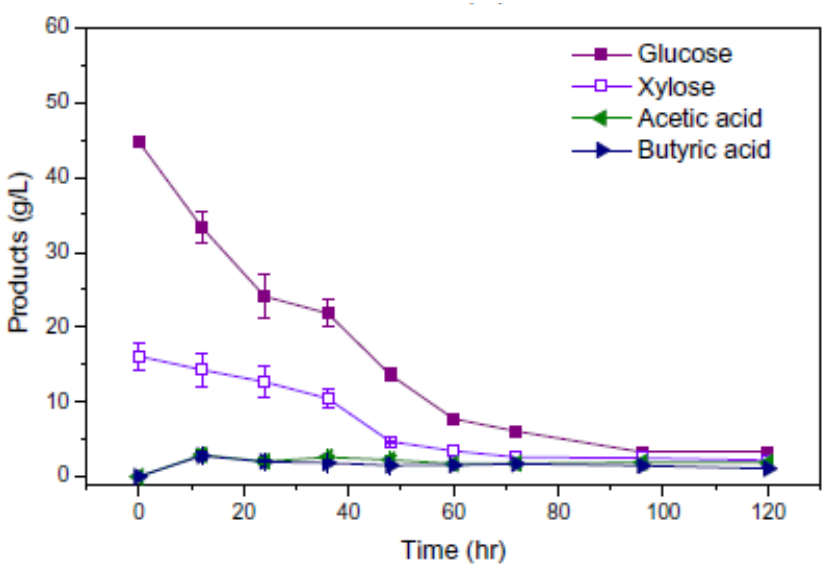
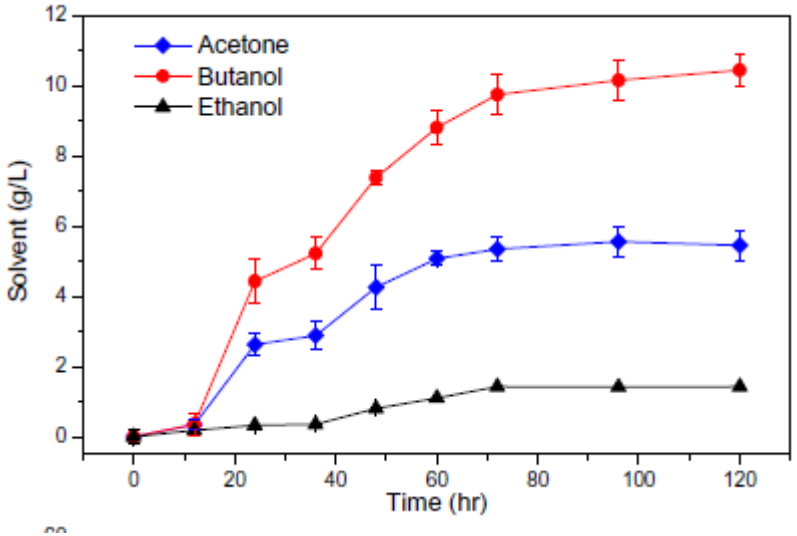
Co-fermentation of glucose and xylose

- ABE fermentation of mixed glucose and xylose

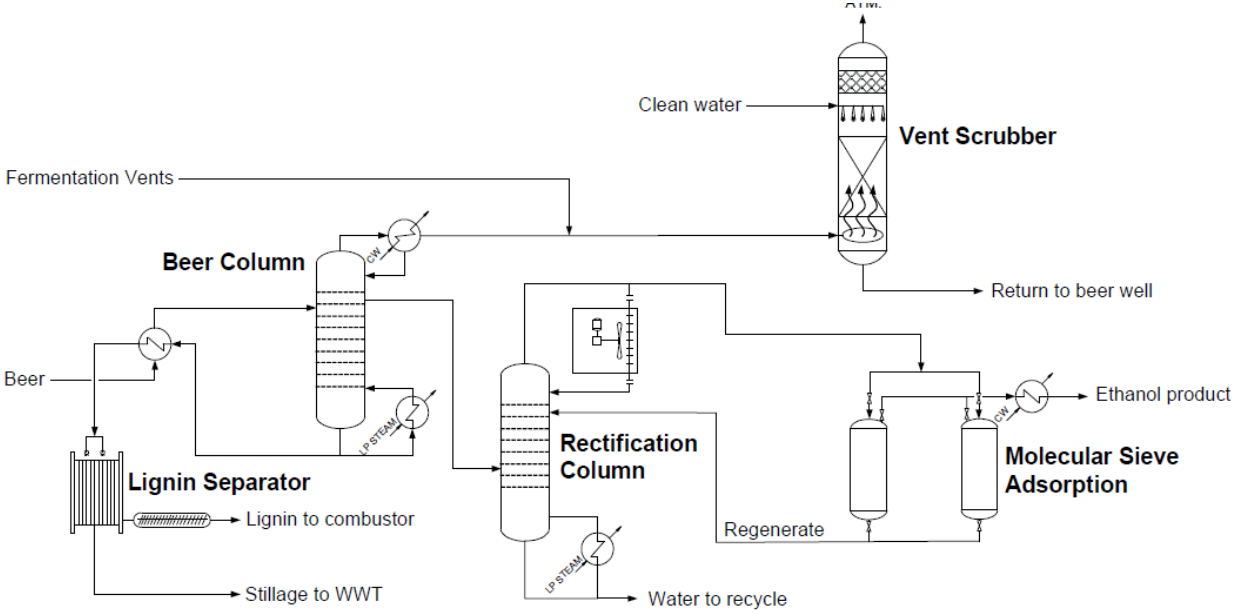




W. Guan et al. / Bioresource Technology 200 (2016) 713–721

Ethanol distillation and separation

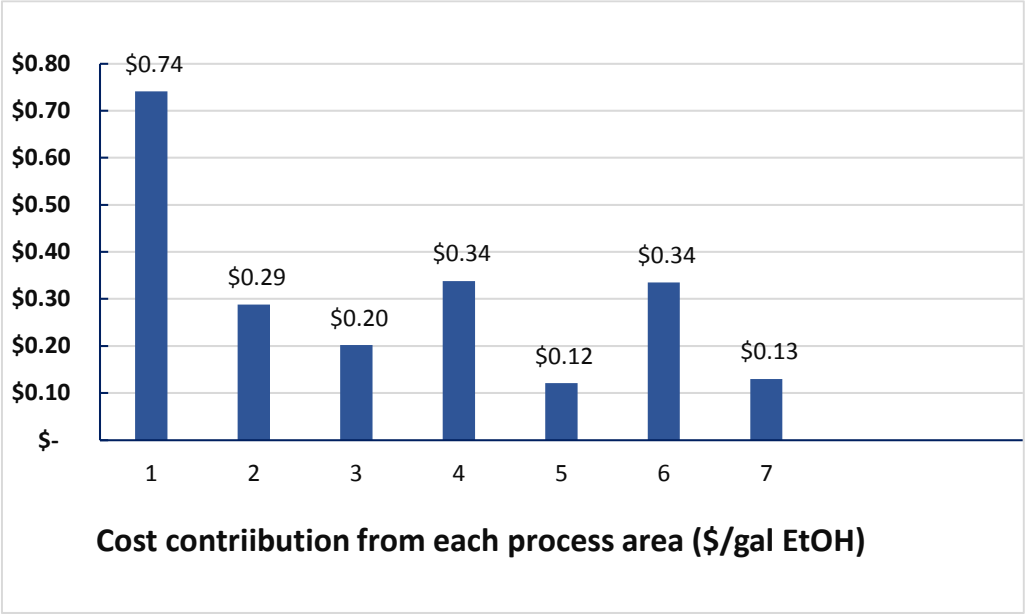
- Distillation and molecular sieve adsorption to recover ethanol
- Distillation is accomplished in two columns:
  - Beer column, removes the dissolved CO2 and most of the water.
  - Rectification column to concentrates ethanol to a near azeotropic composition.



Humbird, 2011, https://www.nrel.gov/

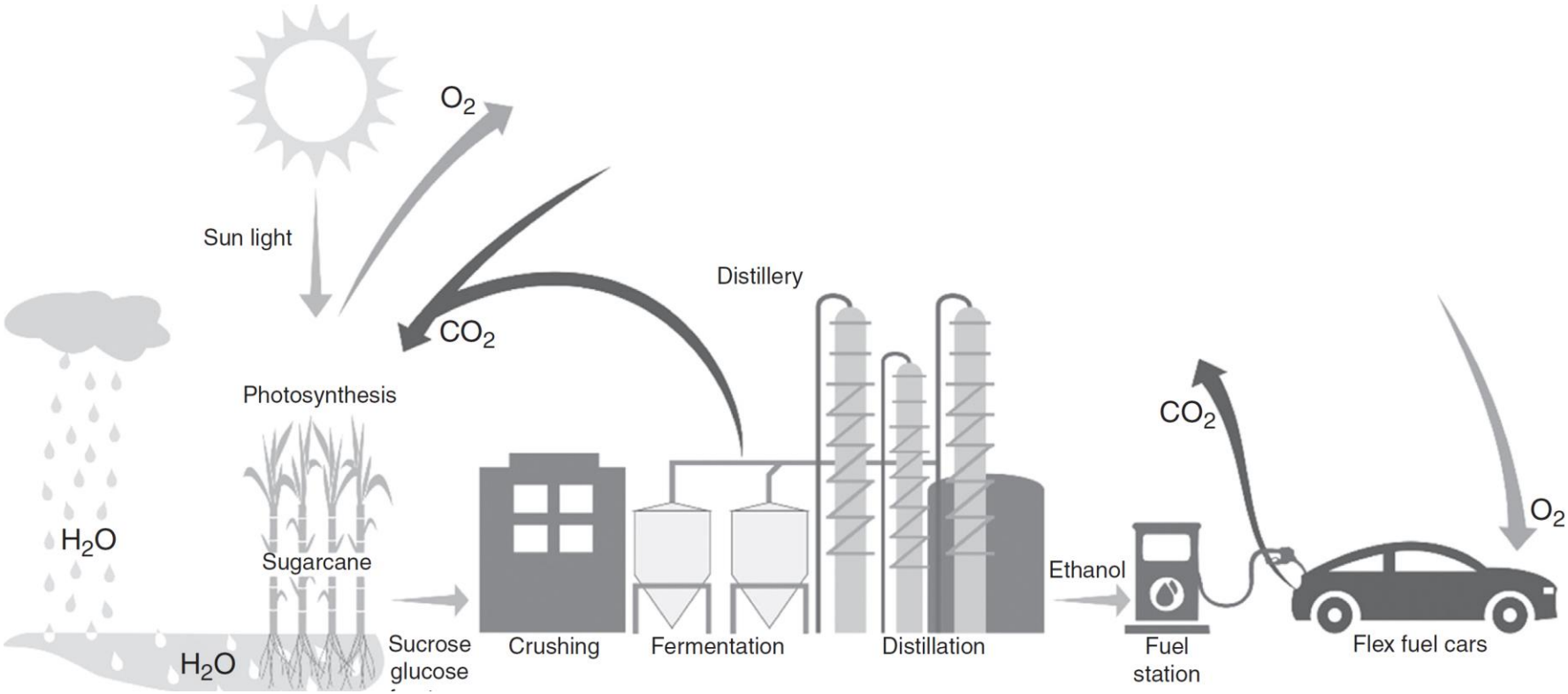
Cost contribution from each process area

1. Feedstock + handling
2. Pretreatment and conditioning
3. Enzymatic hydrolysis & fermentation
4. Cellulase enzyme
5. Distillation and solids recovery
6. Wastewater treatment
7. Storage, boiler and utilizes



Humbird, 2011, https://www.nrel.gov/

Sugarcane ethanol in brazil



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

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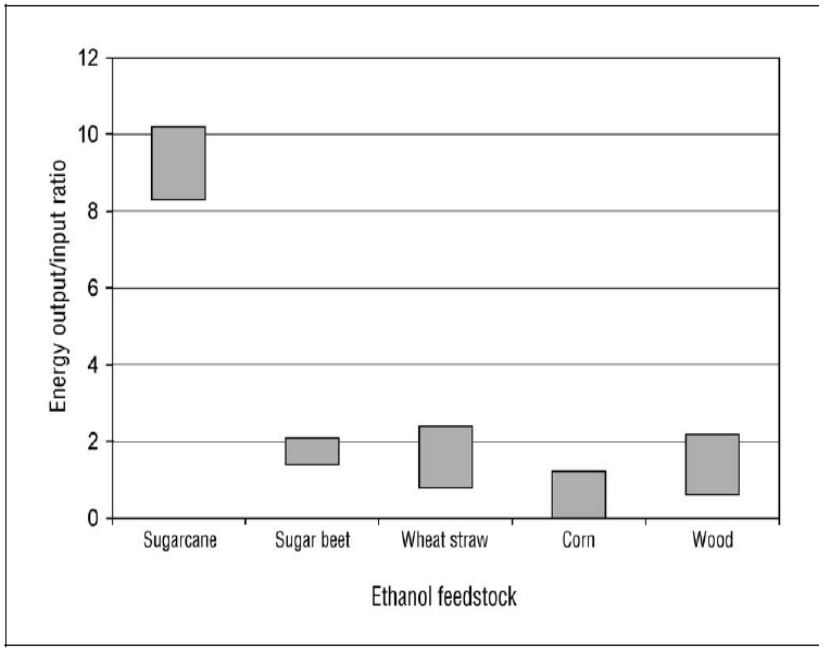
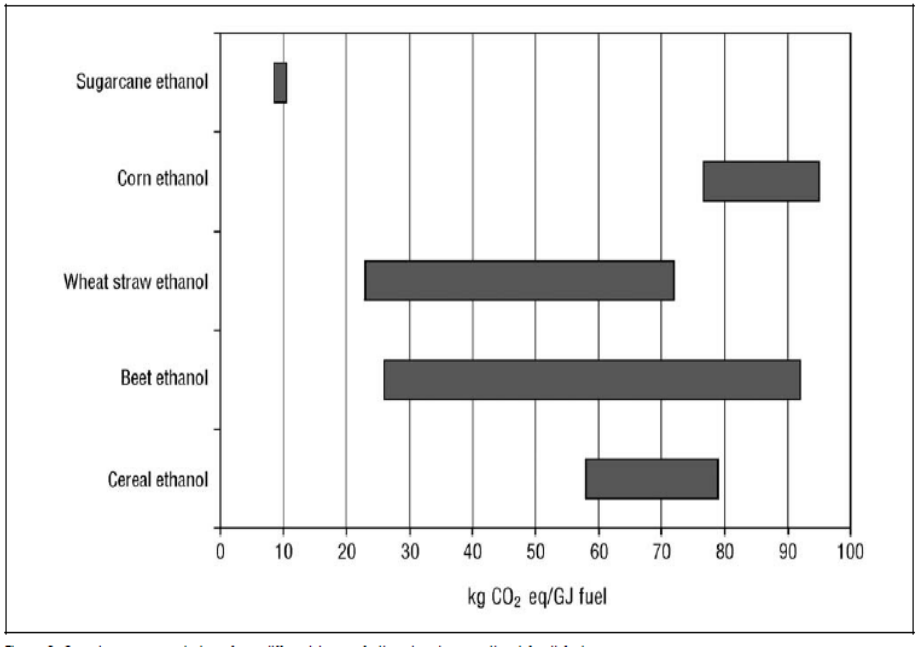
https://doi.org/10.1016/j.bjm.2016.10.003

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)

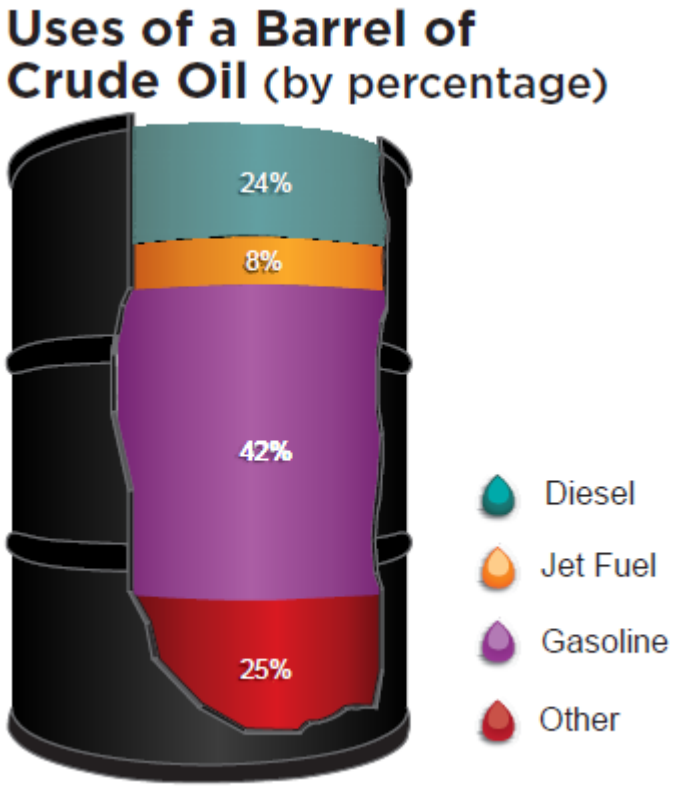
Bioproducts from biorefinery

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

Source: Cameron Cargill

Replacing the whole barrel

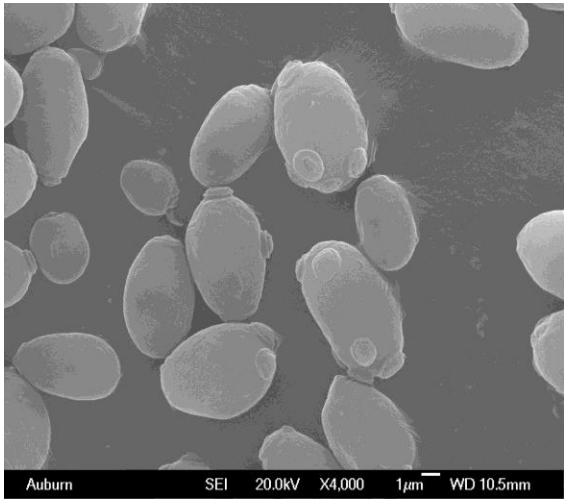
- Cellulosic ethanol can
  - displace only 42% of a barrel
  - Of crude oil)gasoline)
- Hydrocarbon biofuels
  - “drop-in” fuels to replace
  - Diesel, jet fuel and others
- 7% of barrel used to make
  - glues, solvents and plastics



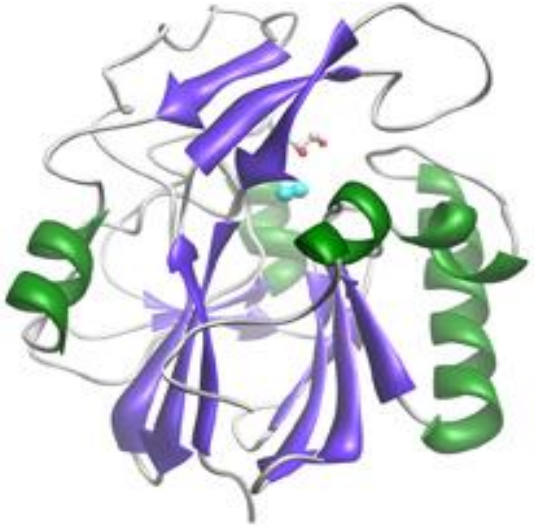
Source: Energy Information Administration; data

Catalyst and biocatalyst

Zeolite



Yeast

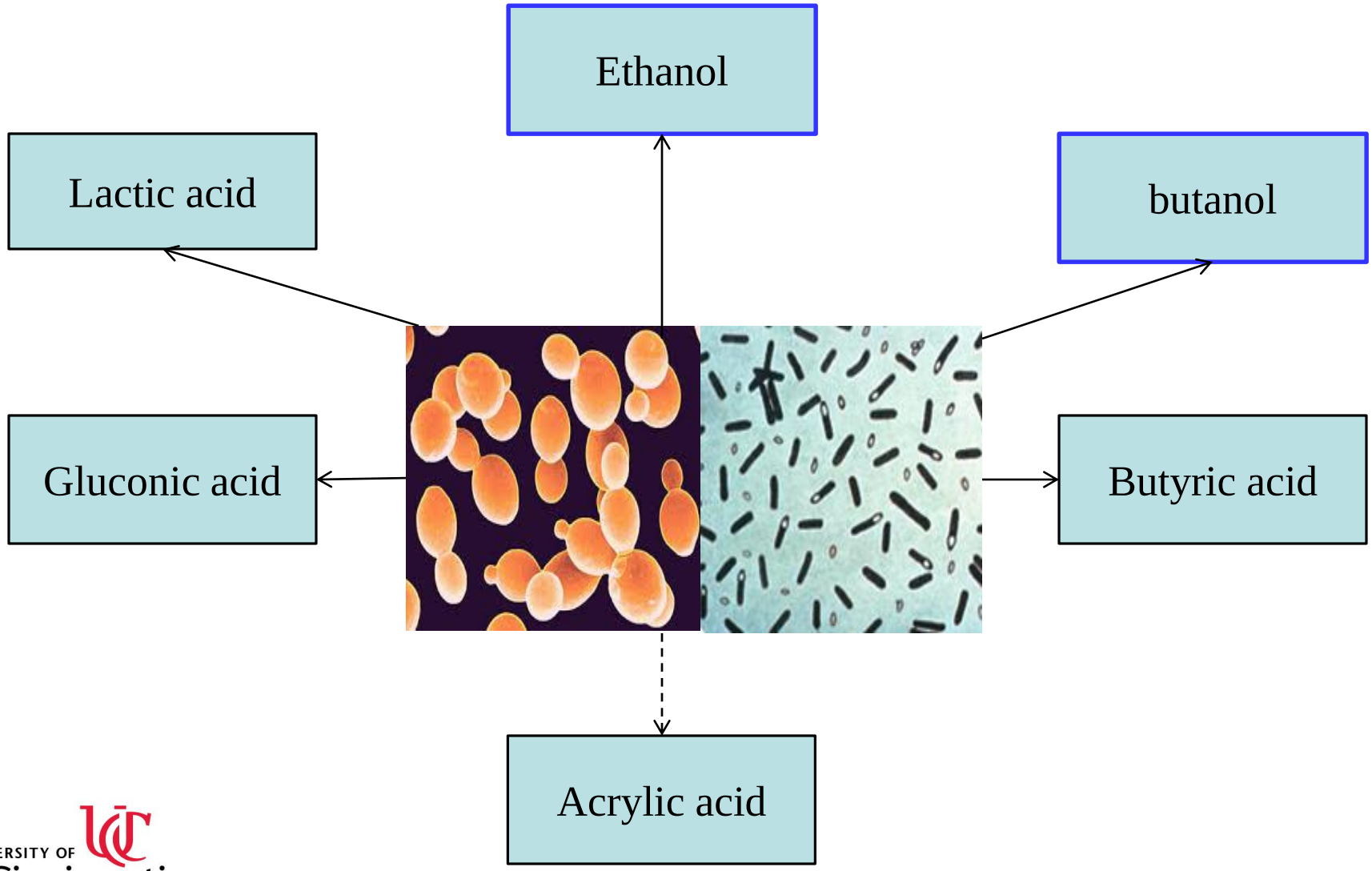


Enzyme

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



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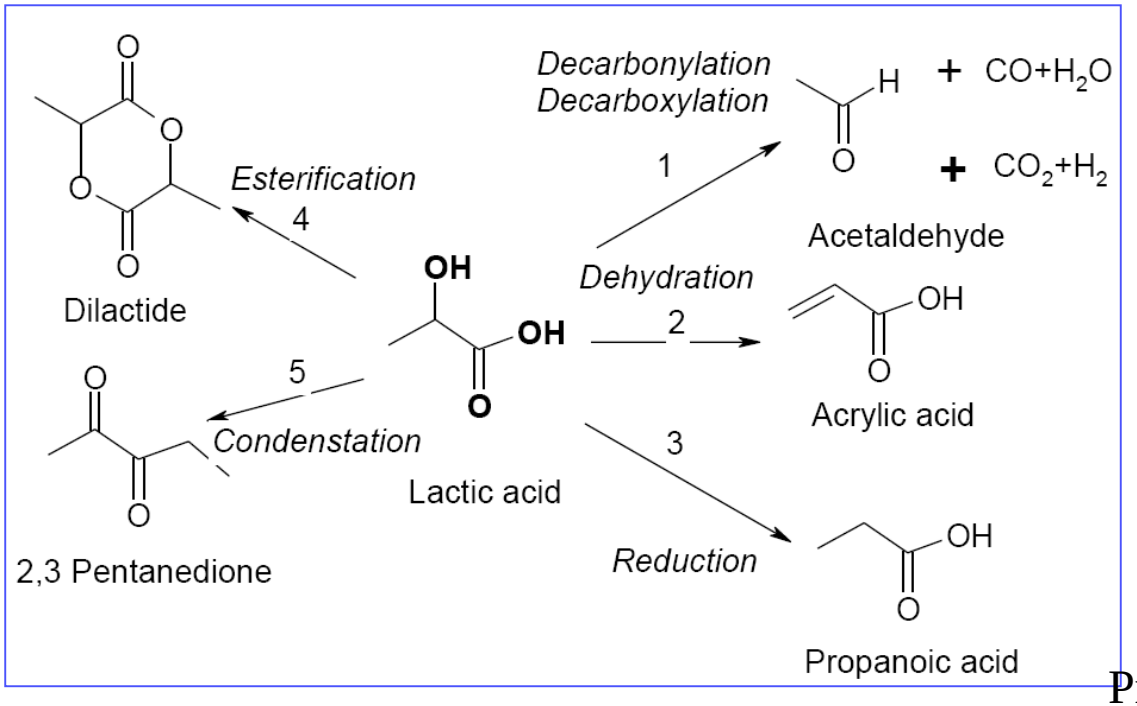
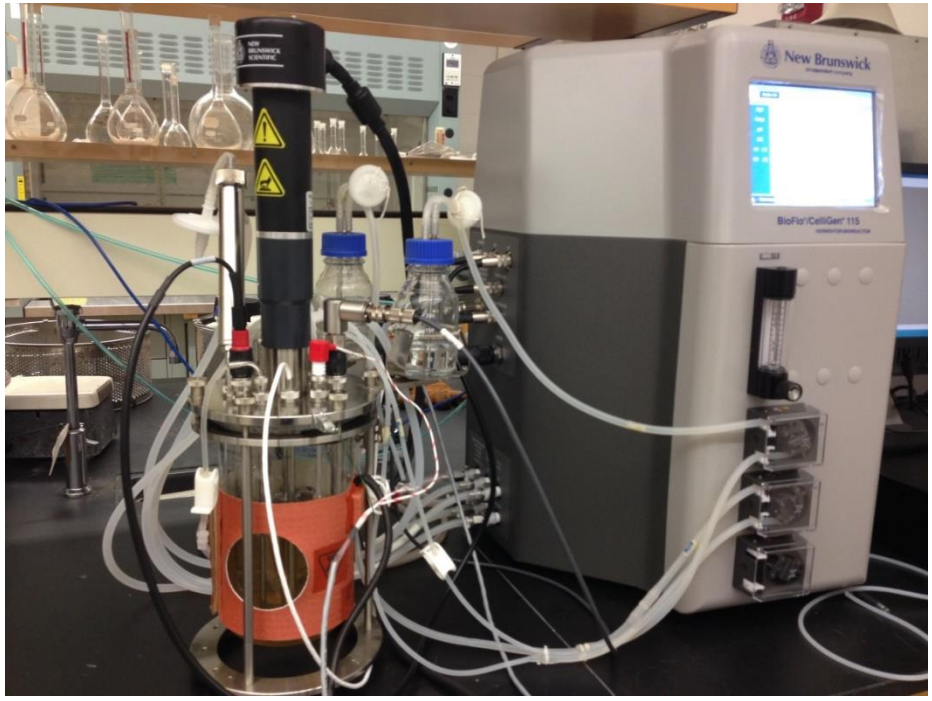
Our research on acrylic acid

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



Our research on butanol

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



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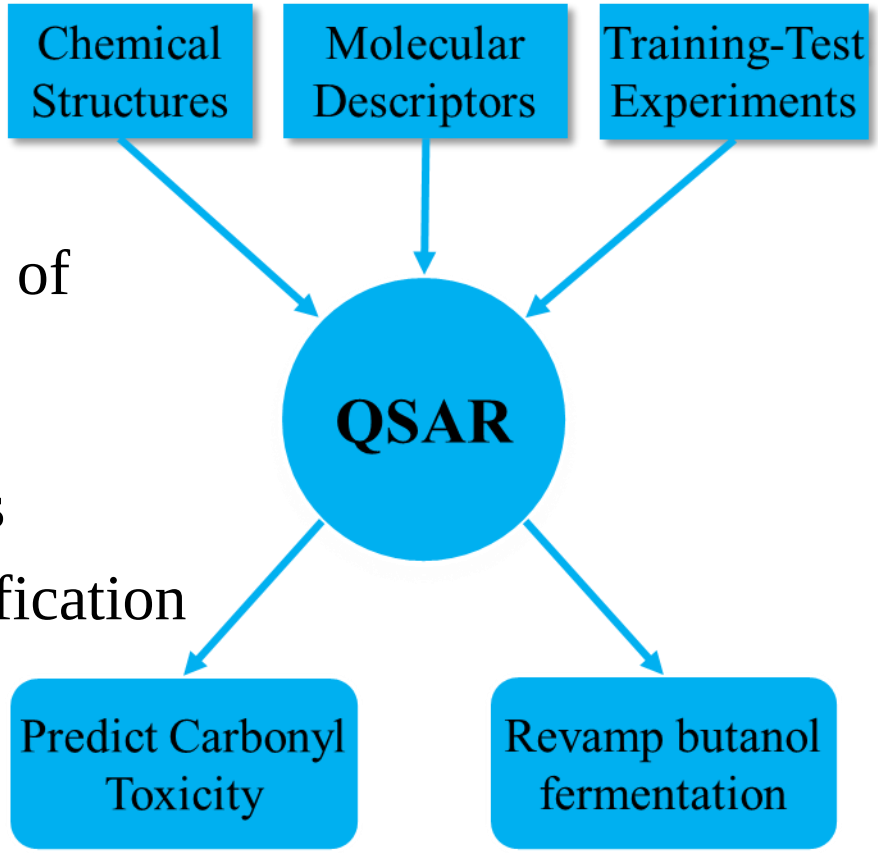
New approach

- 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

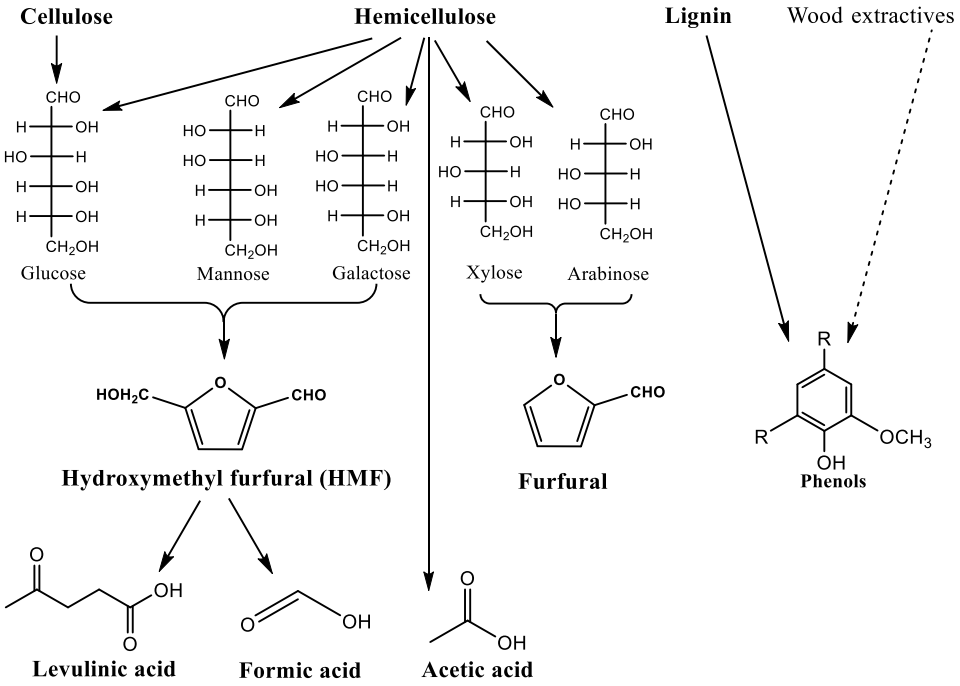


Objectives and Hypothesis

- 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*

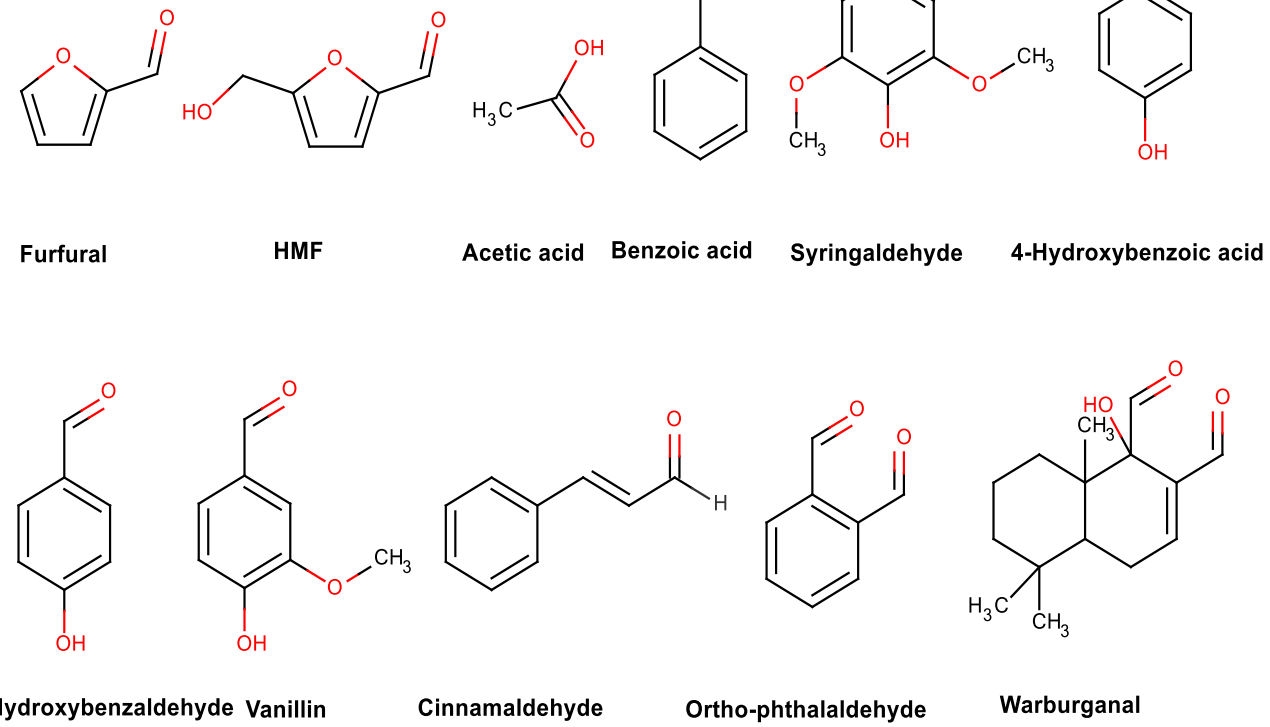
Formation of carbonyl inhibitors

- **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



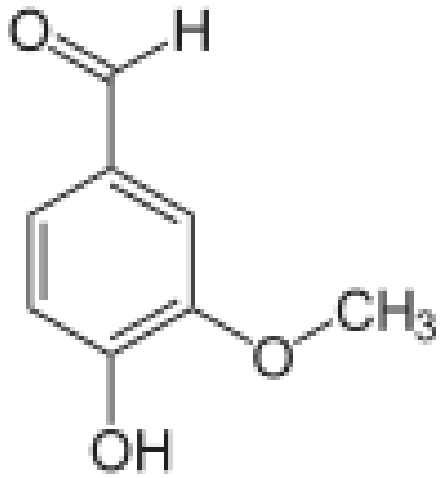
Questions: What are the most potent inhibitors?

Fermentation inhibitors: carbonyl compounds



Critical issues and questions

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



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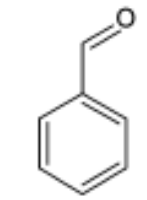
Methods

- 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 :

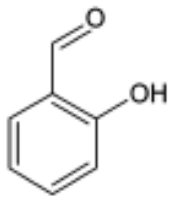
$$\omega = \frac{\mu^2}{2\eta} = \frac{E_{HOMO}{}^2 + 2E_{HOMO}E_{LUMO} + E_{LUMO}{}^2}{4(E_{LUMO} - E_{HOMO})}$$

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

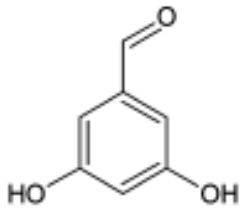
Phenolic model compounds



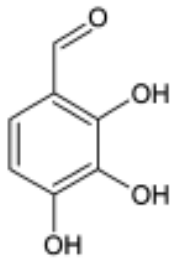
benzaldehyde



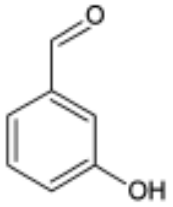
2-hydroxybenzaldehyde



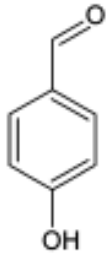
3,5-dihydroxybenzaldehyde



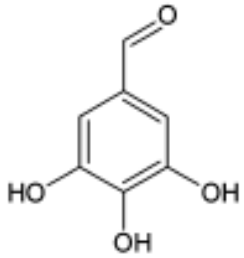
2,3,4-trihydroxybenzaldehyde



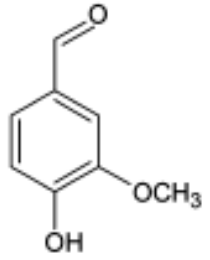
3-hydroxybenzaldehyde



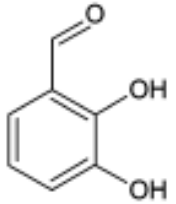
4-hydroxybenzaldehyde



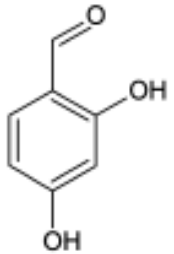
3,4,5-trihydroxybenzaldehyde



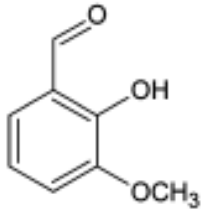
vanillin



2,3-dihydroxybenzaldehyde



2,4-dihydroxybenzaldehyde



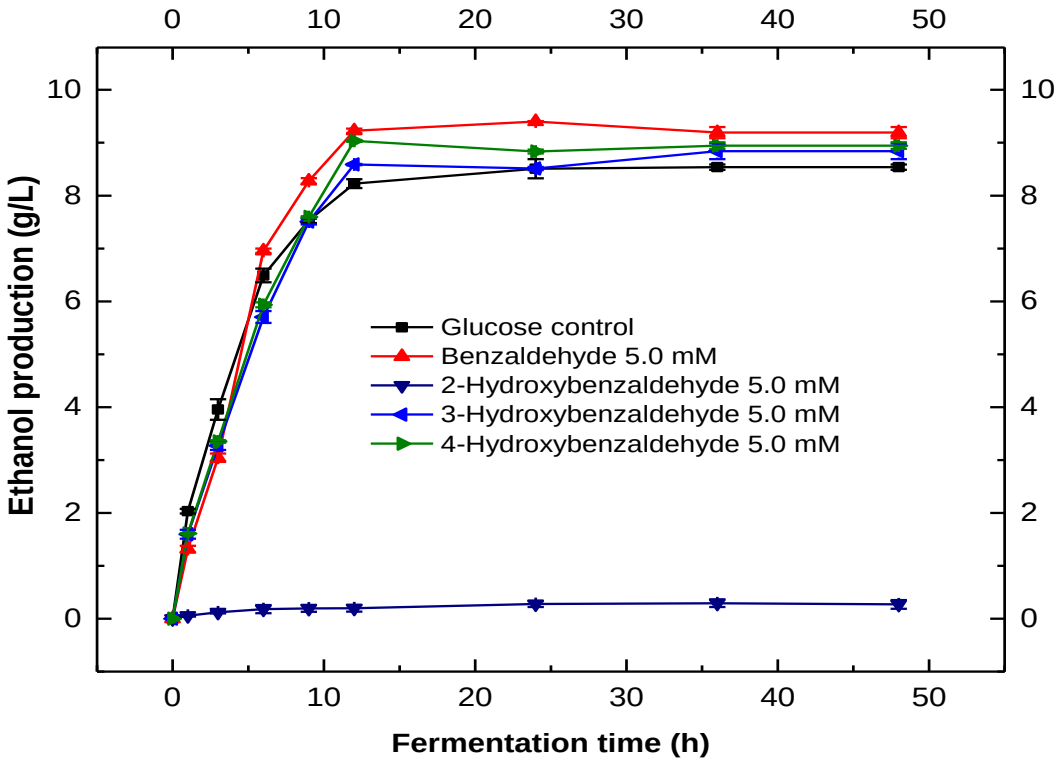
o-vanillin

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	

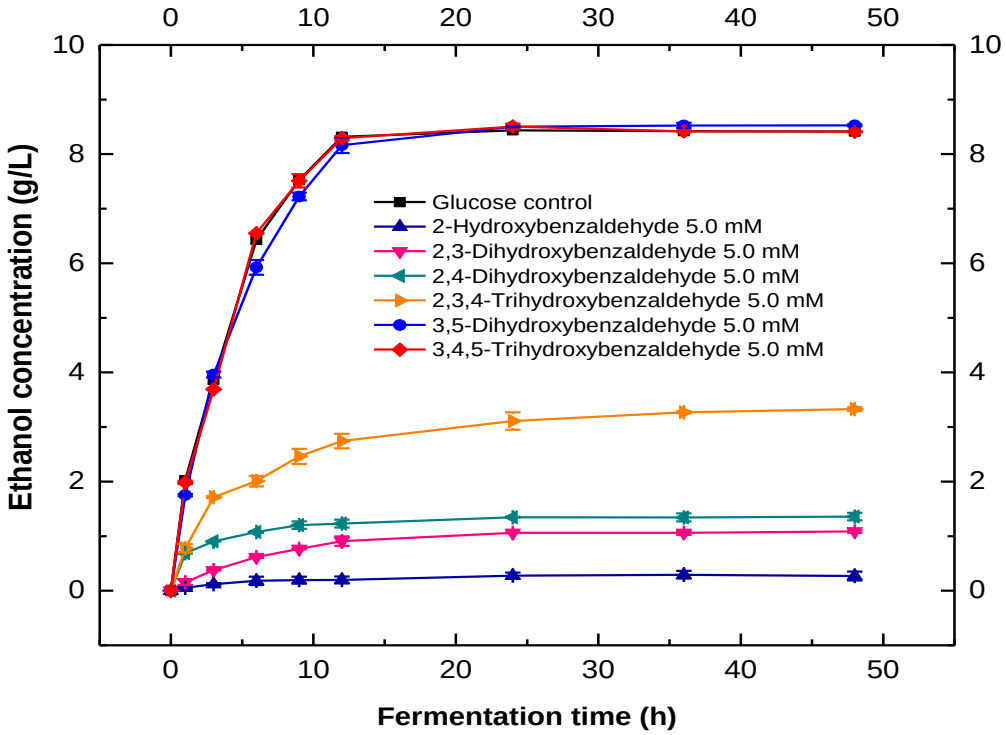
[a] Y_{EtOH} represents the ethanol yield at 48 h based on original glucose.
[b] EC_{50} represents the concentration of phenolic aldehydes resulting in a final ethanol yield of 50% of the control at 48 h.

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.

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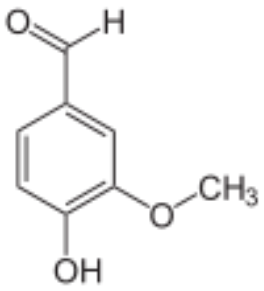
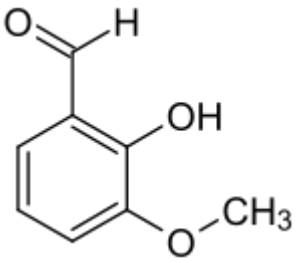
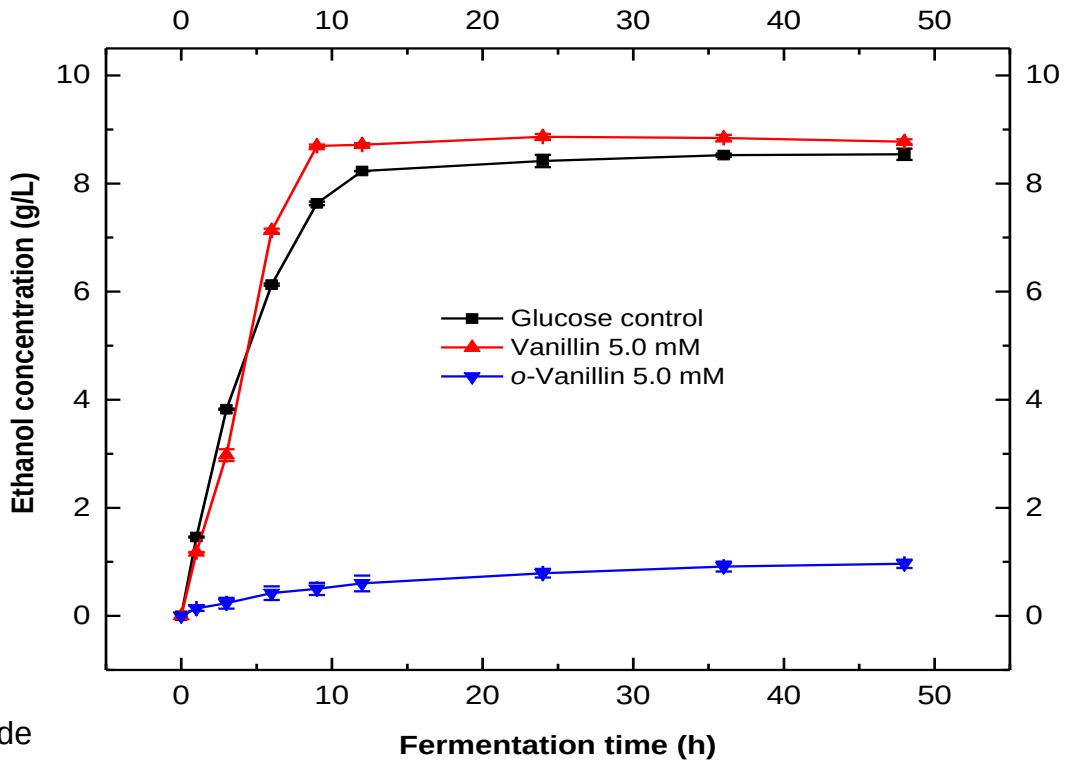
Effect of vanillin and o-vanillin on fermentation



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

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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_{carb}	10	0.73	0.37	24.70	<0.001
Log EC'_{50} = -17.97 - 41.75 O_{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_{HOMO}	10	0.12	0.66	1.25	0.293
Log EC'_{50} = 2.26 + 50.55 E_{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 ω	10	0.25	0.61	3.01	0.117
Log EC'_{50} = -31.71+ 85.50 C_{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.