Biofuels production from renewable biomass

Maobing Tu
Chemical and Environmental Engineering
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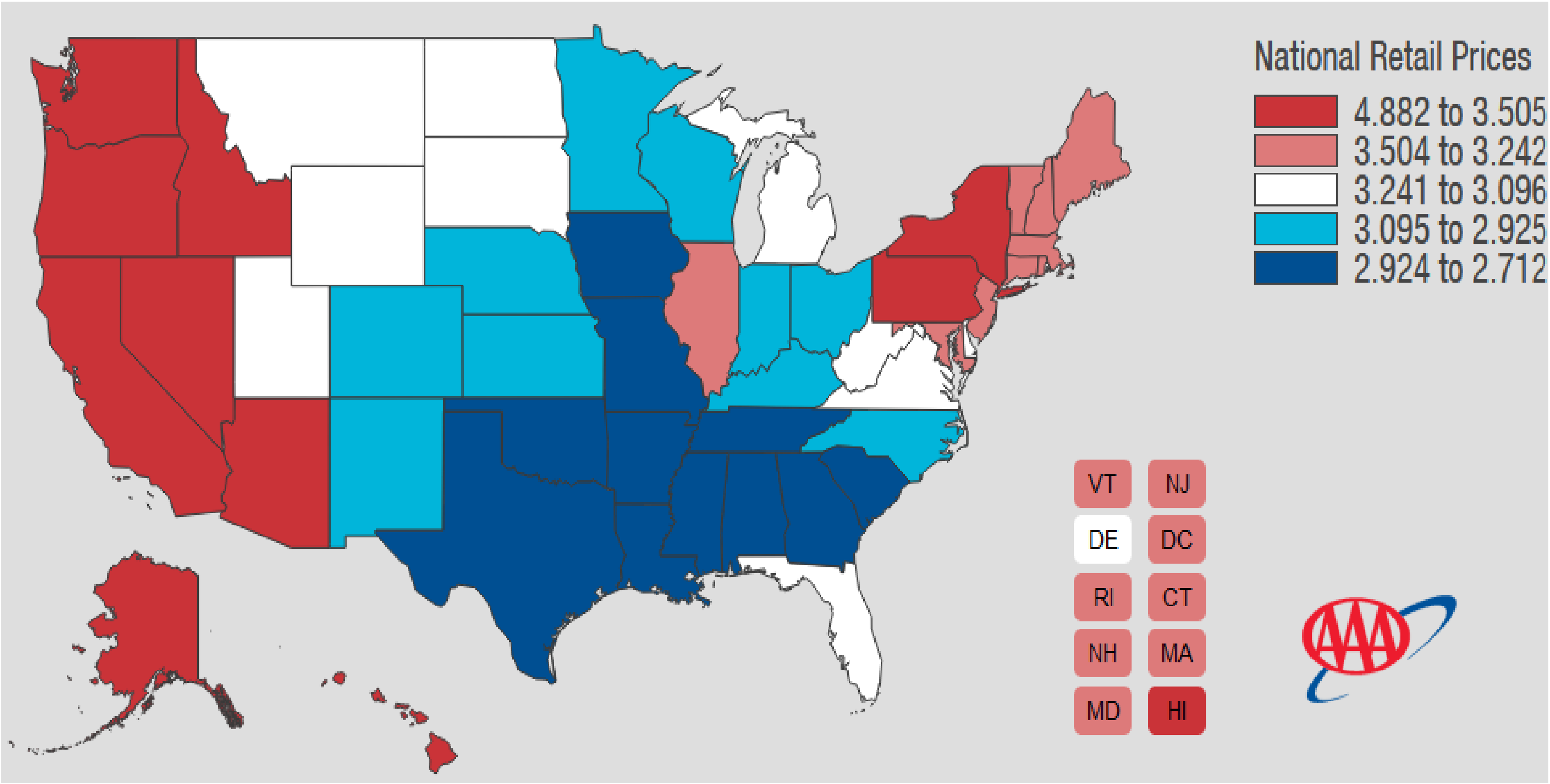
Gasoline and ethanol



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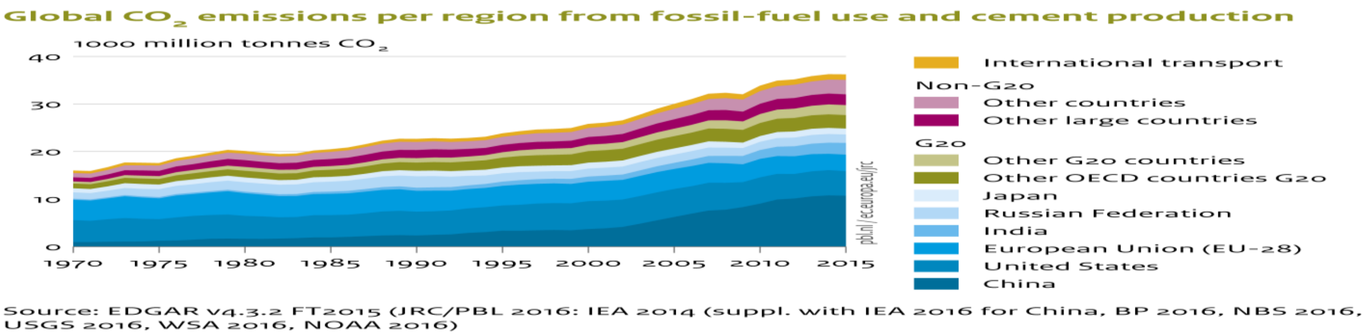
$2.984, Price as of, 11/28/23

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

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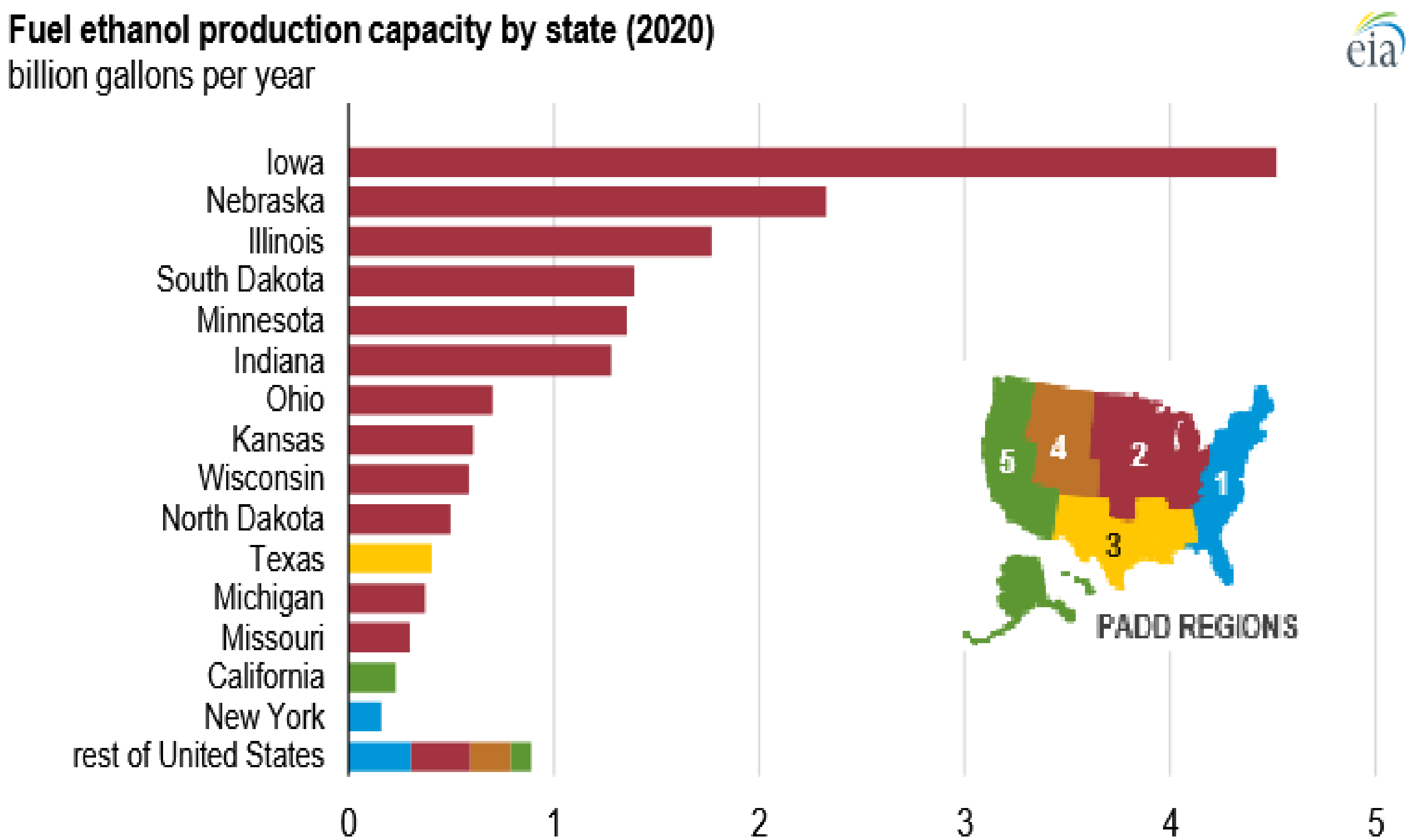
Drivers-why biofuels and bioproducts

- 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)



Source: U.S. Energy Information Administration, *U.S. Fuel Ethanol Plant Production Capacity Report*
Note: PADD=Petroleum Administration for Defense District.

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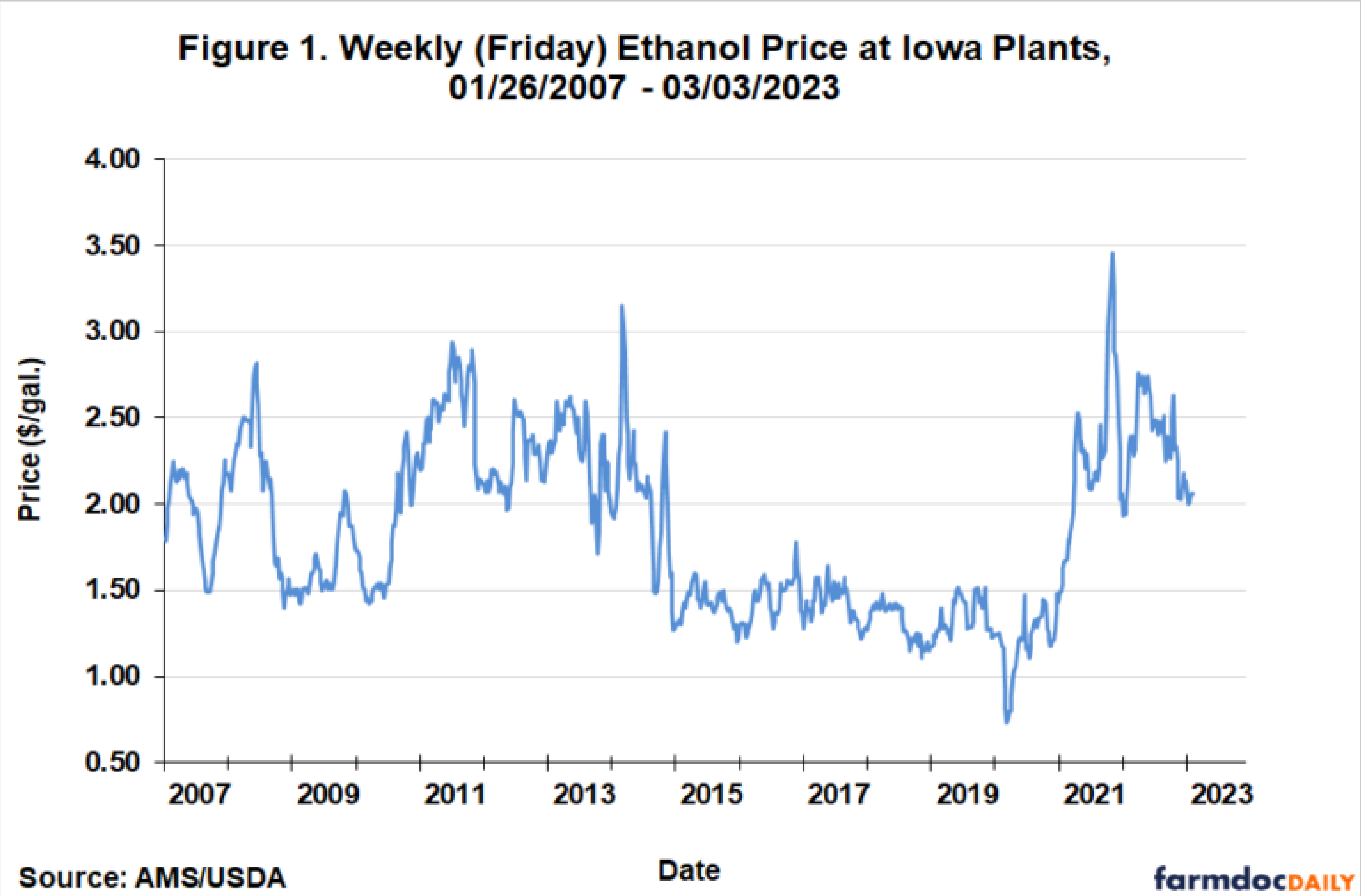
U.S. CO2 emission



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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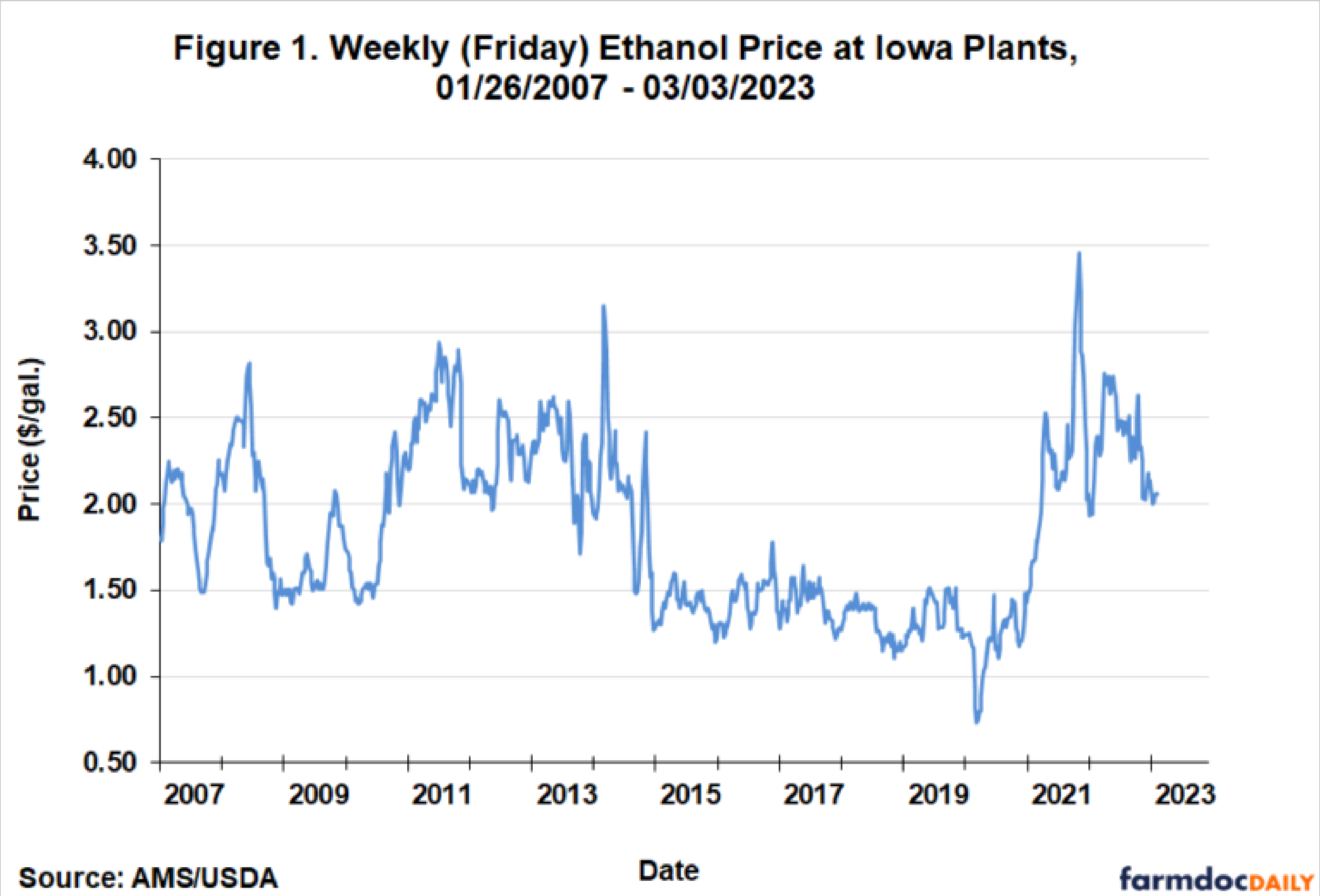
Ethanol price



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

Project	Location	Scale	Conversion Technology
Abengoa	Hugoton, KS	Commercial	Biochemical
Bluefire LLC	Fulton, MS	Commercial	Biochemical
Flambeau	Park Falls, WI	Commercial	Thermo - Gasification
Mascoma	Kinross, MI	Commercial	Biochemical
POET	Emmetsburg, IA	Commercial	Biochemical
Enerkem	Pontotoc, MS	Demo	Thermo - Gasification
INEOS New Planet Bioenergy LLC	Vero Beach, FL	Demo	Hybrid
Myriant	Lake Providence, LA	Demo	Biochemical
RSA	Old Town, ME	Demo	Biochemical
Sapphire Energy Inc.	Columbus, NM	Demo	Algae/CO_2
Algenol Biofuels Inc	Fort Myers, FL	Pilot	Algae/CO_2

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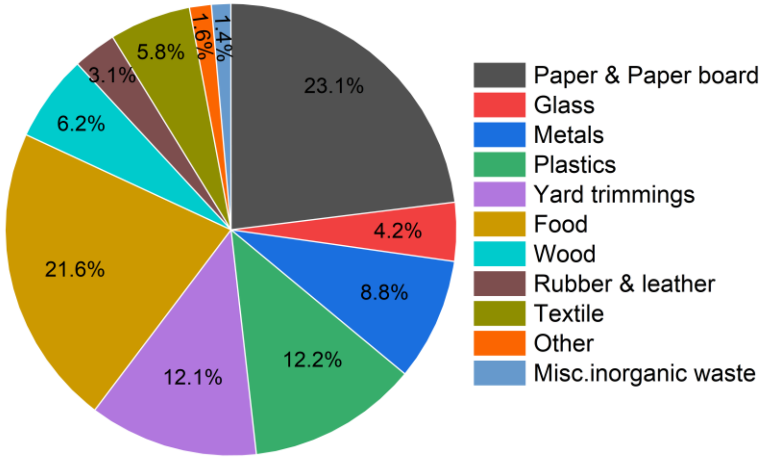
Project Overview

- Total annual MSW generation in the U.S. has increased by 93% since 1980, to 292 million tons/year in 2018
- 50% of the generated MSW was disposed of in 1,278 landfills
- Landfills were the third largest source of U.S. anthropogenic CH_4 emissions in 2020
- MSW represents a valuable source of low-cost feedstock for the development of biofuels and bioproducts



Total US MSW generation by category in 2018

- Heterogeneity and variability of MSW components are major bottlenecks for MSW use as bioenergy feedstocks
- Sorting and removing plastics produces a high-purity organic stream for MSW use as conversion-ready feedstocks

https://www.epa.gov/

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Project Overview

- **Goal:** Develop an advanced sorting and fractionation technology that can separate the organic fraction waste from municipal solid waste (MSW) to achieve 95% purity, and to blend and formulate the sorted organic waste (95% purity) with lignocellulosic biomass for biochemical conversion.
- **Objectives:**
  1. **Design and test** 1st stage pre-screening devices to separate 95% of ferrous metals and 80% of plastics from MSW (by magnetic separator and dynamic disc screen);
  2. **Conduct** mechanical milling (<50 mm) and evaluate 2nd stage screening devices (>4 mm) to obtain uniform feedstocks;
  3. **Blend and formulate** screened organic fraction MSW (OFMSW) with lignocellulosic biomass for conversion testing;
  4. **Conduct** techno-economic analysis (TEA) and life cycle assessment (LCA) of the proposed sorting and fractionation process.

Awarded through FY20 BETO FOA subtopic 2a: Advanced fractionation and decontamination of MSW for improved conversion efficiency

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Initial verification of MSW sorting by vibratory screening

- Establish the baseline of traditional screen
- Organic fraction of sorted MSW (fines) with a purity of 50-70%
- Contamination reduction percentage (plastic removal) reached ~50%.

Green Separation System with Vibratory Screen in Cartersville, GA

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Pre-screening equipment procurement, installation and initial test

- This subtask is to complete the procurement, installation, commissioning, and start-up of dynamic dis screen and conveyor. Ecostar disc screen has been ordered and shipped from Italy

Ecostar HEXACT

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Progress and Outcomes-disc screen

Pre-screening equipment procurement, installation and initial test

- Ecostar disc screen has been installed at UC research facility

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Approach and impacts

Approach

- Integration of dynamic disc screening, mechanical milling and ballistic screening
- Blending of the sorted OFMSW with cellulosic biomass to reduce MSW variability

Progress & Outcomes

- Performance of conventional vibratory & trommel screen to handle heterogenous MSW has been evaluated
- Procurement & Installation of the DDS and conveyor belt system at the project site

Potential Impacts

- High purity (>95%) organic fraction of MSW for biochemical conversions
- Address MSW heterogeneity & variability issues

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Federal initiative on bioenergy

- DOE-2013
  - $2-Billion Energy Security Trust
  - Natural gas fuel & Hydrogen fuel
  - Advanced batteries
  - Cleaner biofuels

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

- DOE -2023
  - $590 M to increase bioenergy research
  - Four Bioenergy Research Centers
  - Net-zero emissions economy by 2050

Bioenergy research centers

- DOE Joint BioEnergy Institute
- DOE Great Lakes Bioenergy Research Center
- DOE Center for Bioenergy Innovation (CBI)
- Center For Advanced Bioenergy and Bioproducts Innovation (CABBI)
  - Receive $110 million per year (2023)
  - Innovative biofuel research for another fiver years
- DOE Bioenergy Research Centers (BRCs) 2017
  - $40 million per year

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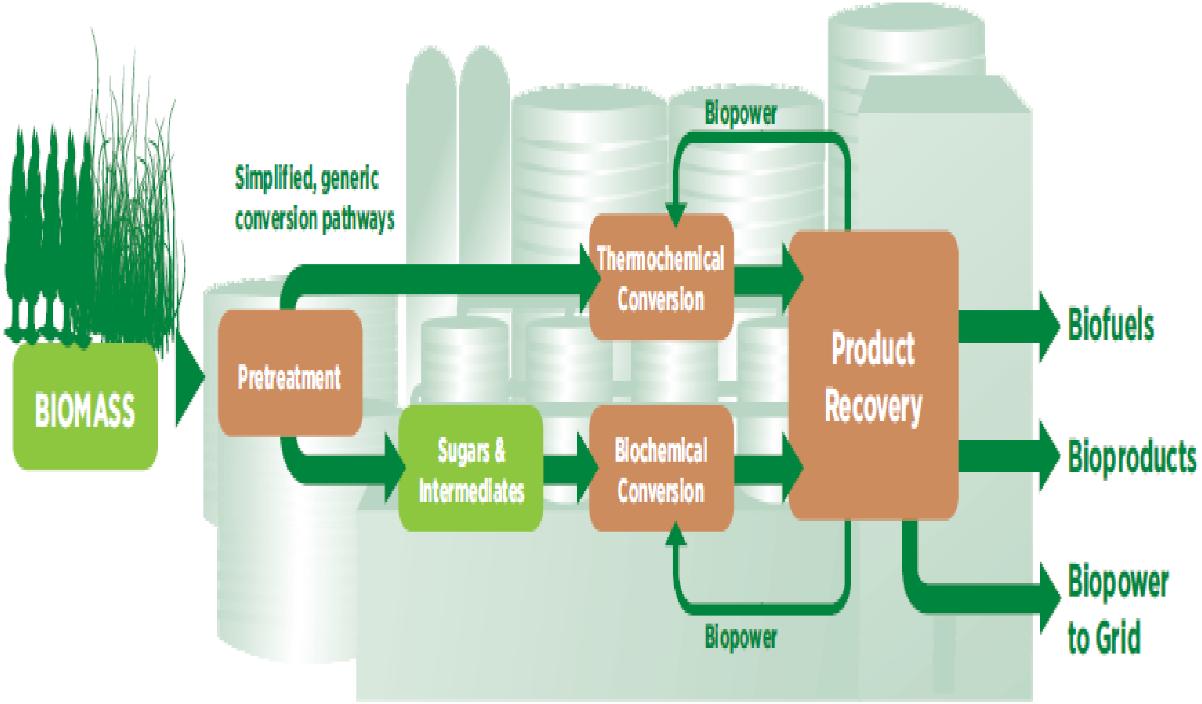
Integrated biorefinery projects funded (DOE)

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



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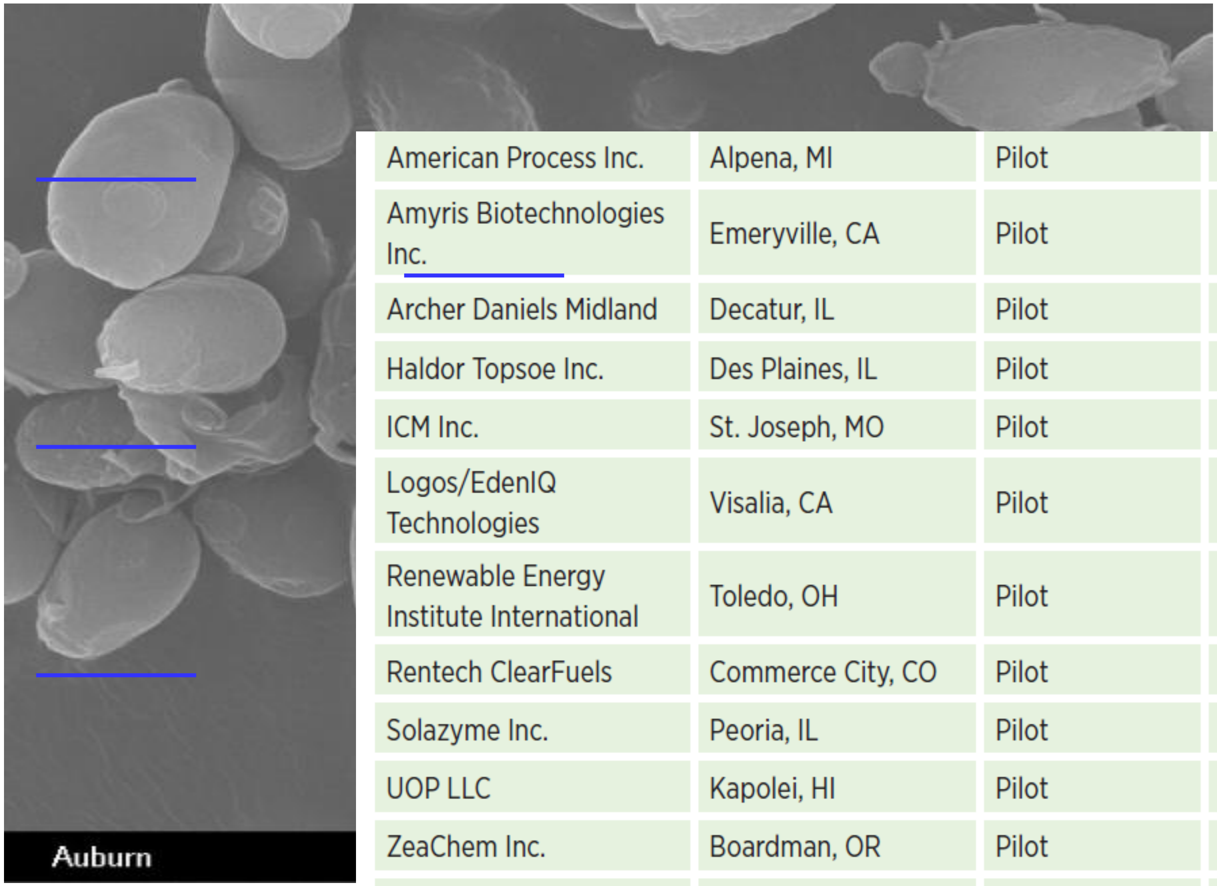
Integrated biorefinery



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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

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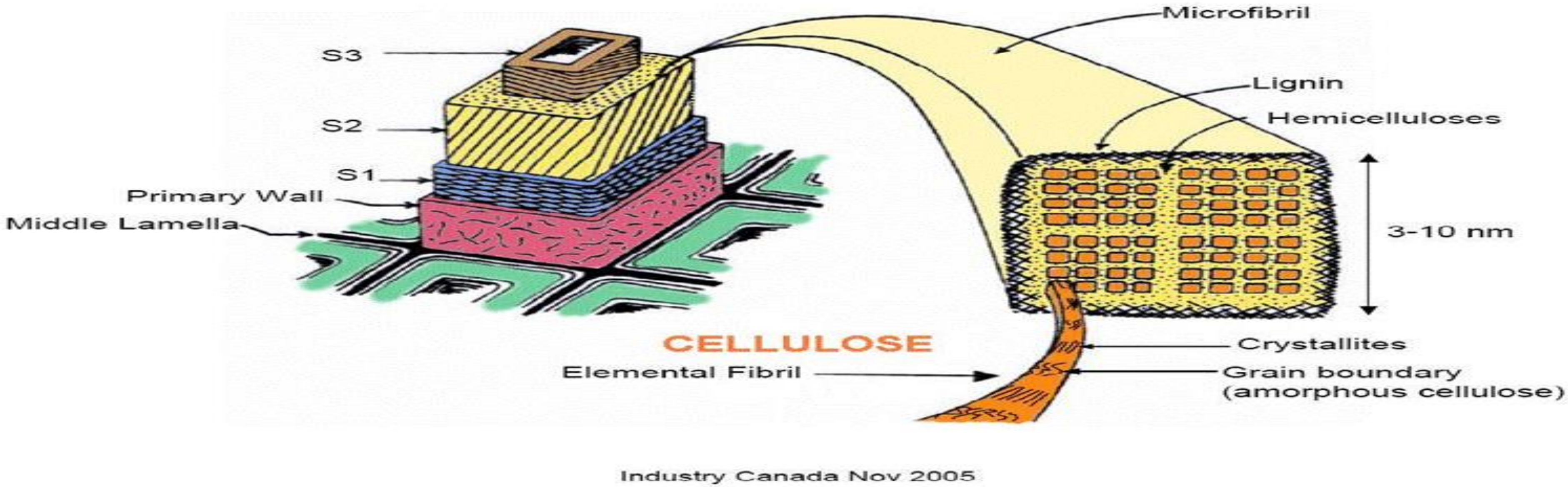
Integrated Biorefinery Projects Funded by DOE

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

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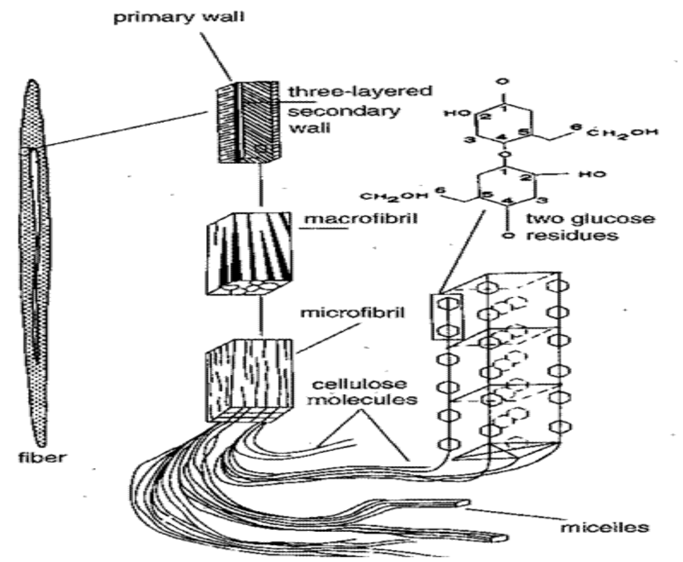
Background

- 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



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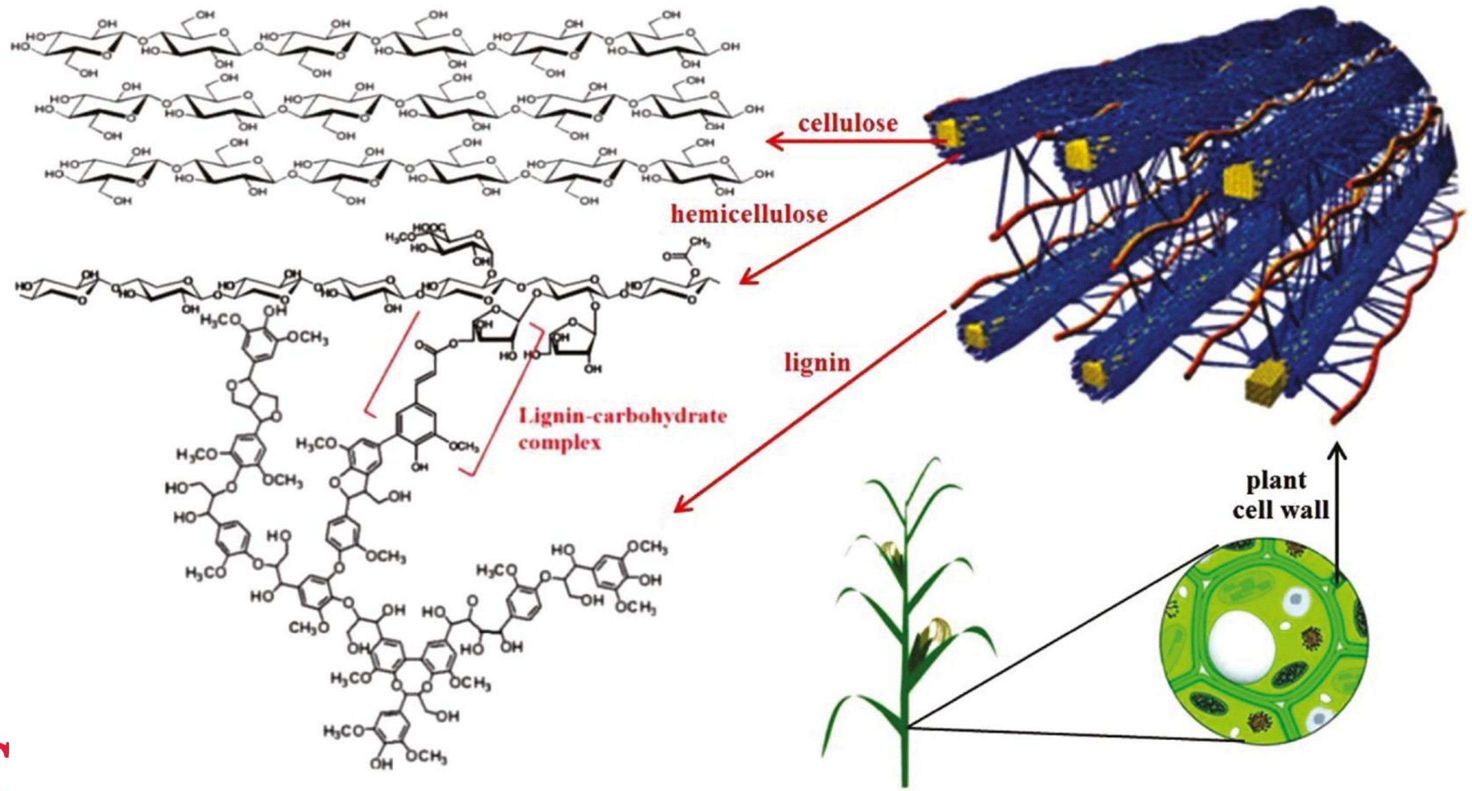
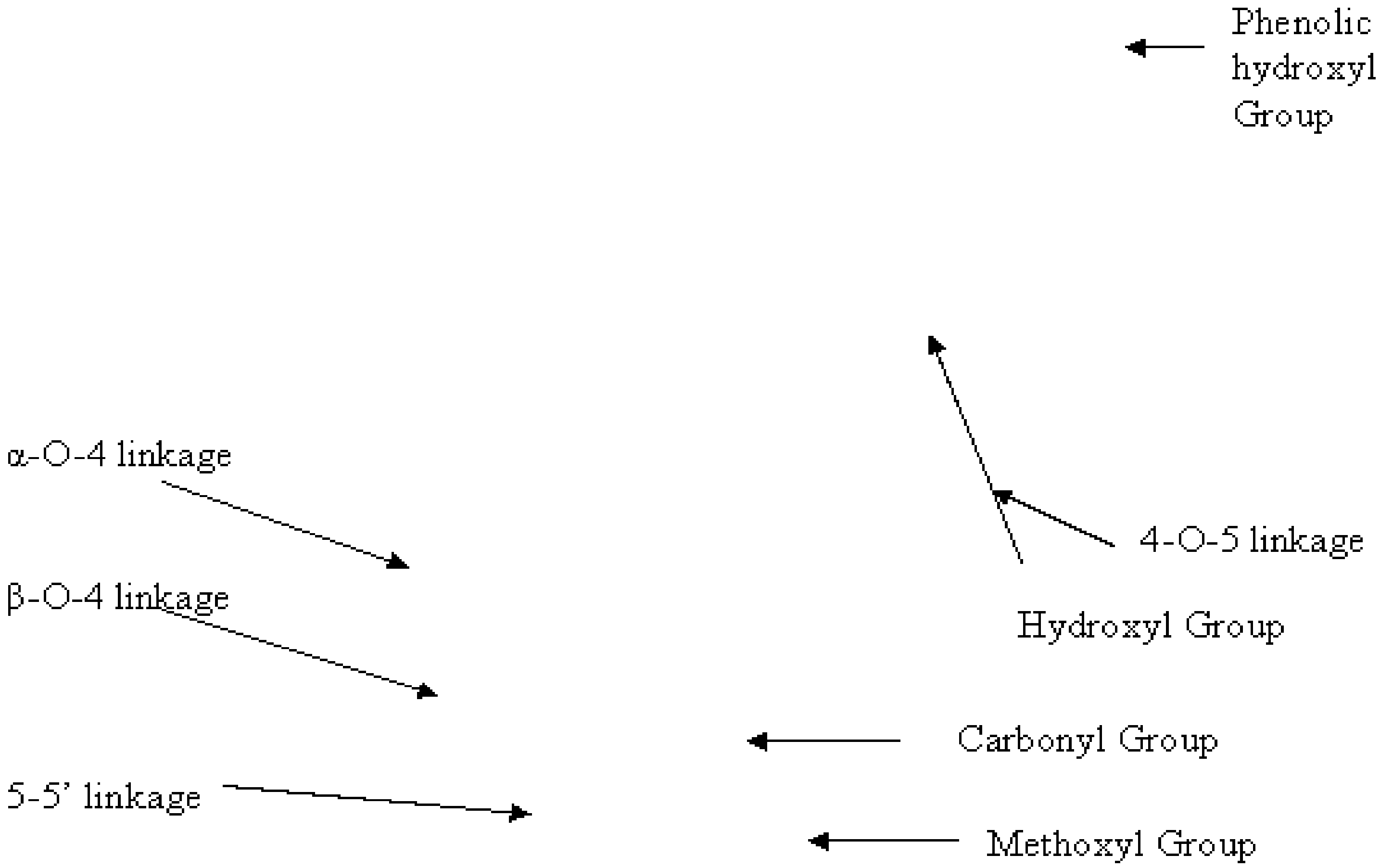
Lignocellulosic biomass

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



Volynets B. *Green Processing and Synthesis*, 2016.

Ultrastructure of plant cell wall



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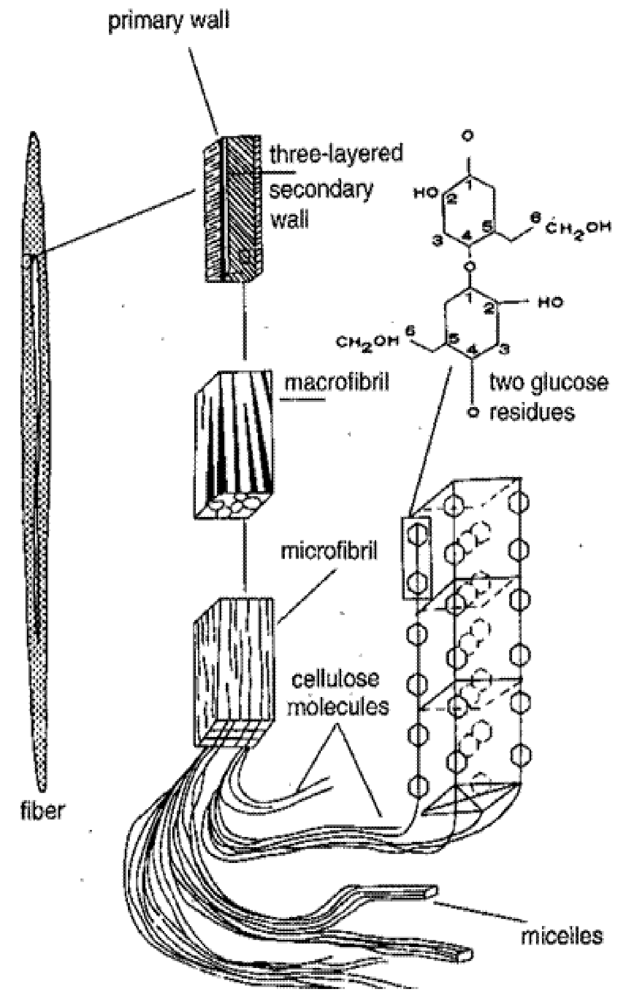
Lignocellulosic biomass chemistry

- **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

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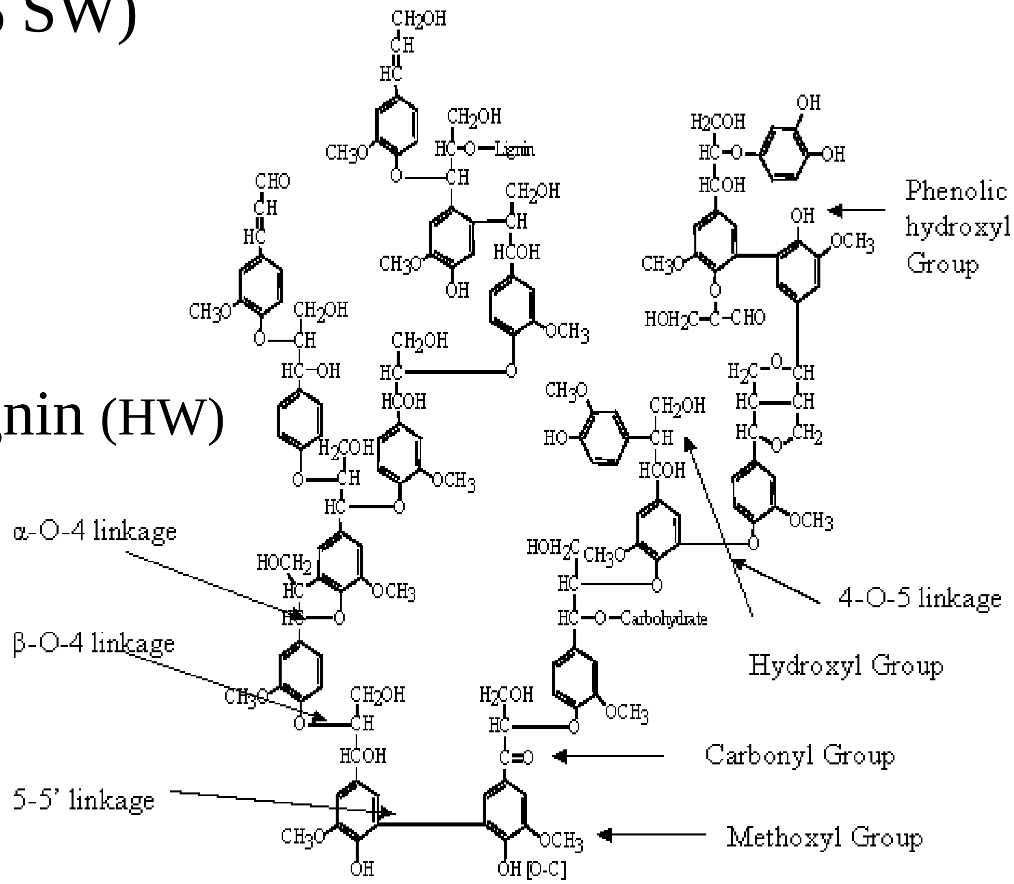
Lignocellulosic biomass chemistry

- **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)

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Bioconversion process

Lignocellulosic biomass → **Pretreatment** → **Enzymatic hydrolysis** → **Fermentation** → Ethanol

Methods & biocatalyst

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

Enzymatic hydrolysis:
- **Exoglucanases**
- **Endoglucanases**
- **β-glucosidase**

Fermentation:
- ***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 a mechanical deconstruction
  - Deacetylation and mechanical refining process
  - Low toxicity, high concentration sugar stream
  - Native lignin

Photo by Dennis Schroeder, NREL/PIX 17684

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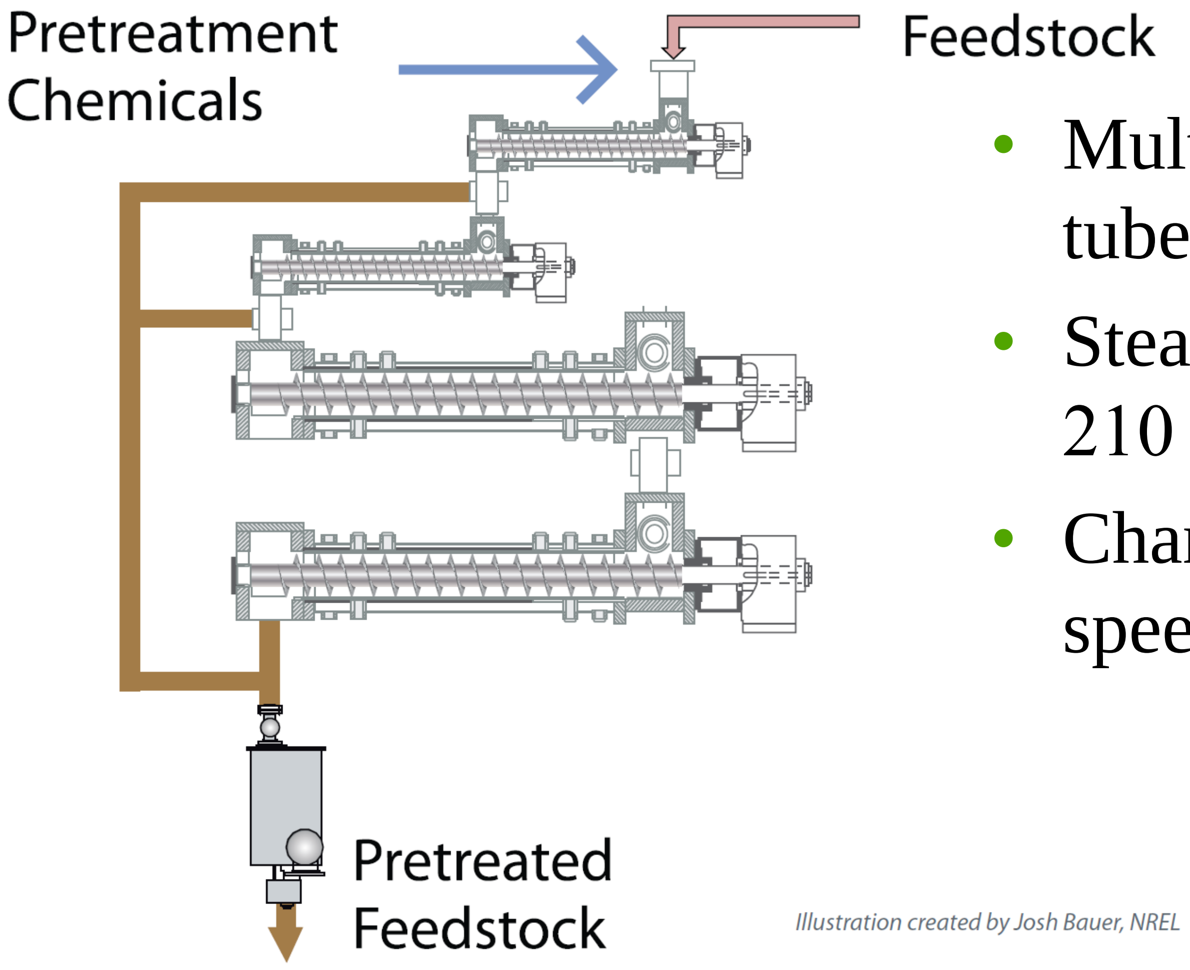
Biomass deconstruction



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

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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

https://www.nrel.gov/

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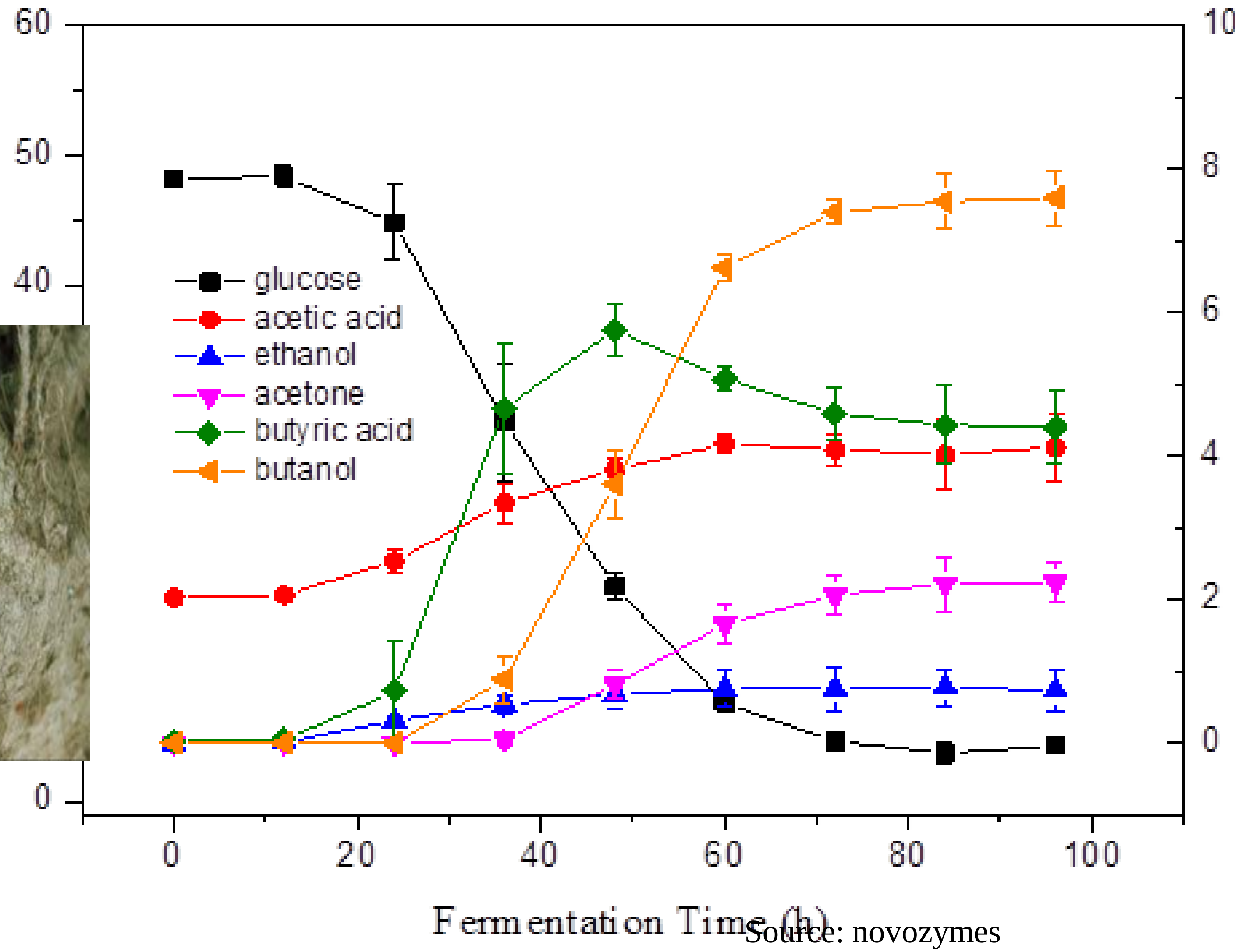
Enzymatic hydrolysis of cellulose



Source: novozymes

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Plant cell walls digested by fungal cellulases (10 h)

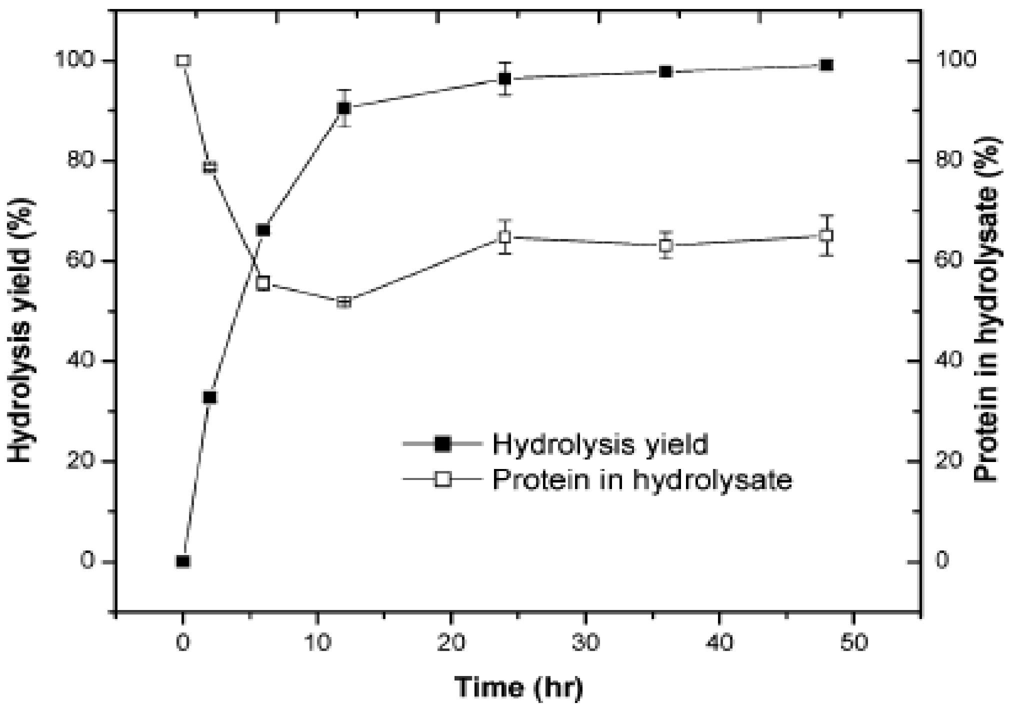
Table 2 Chemical composition of untreated and ethanol organosolv-pretreated loblolly pine

	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

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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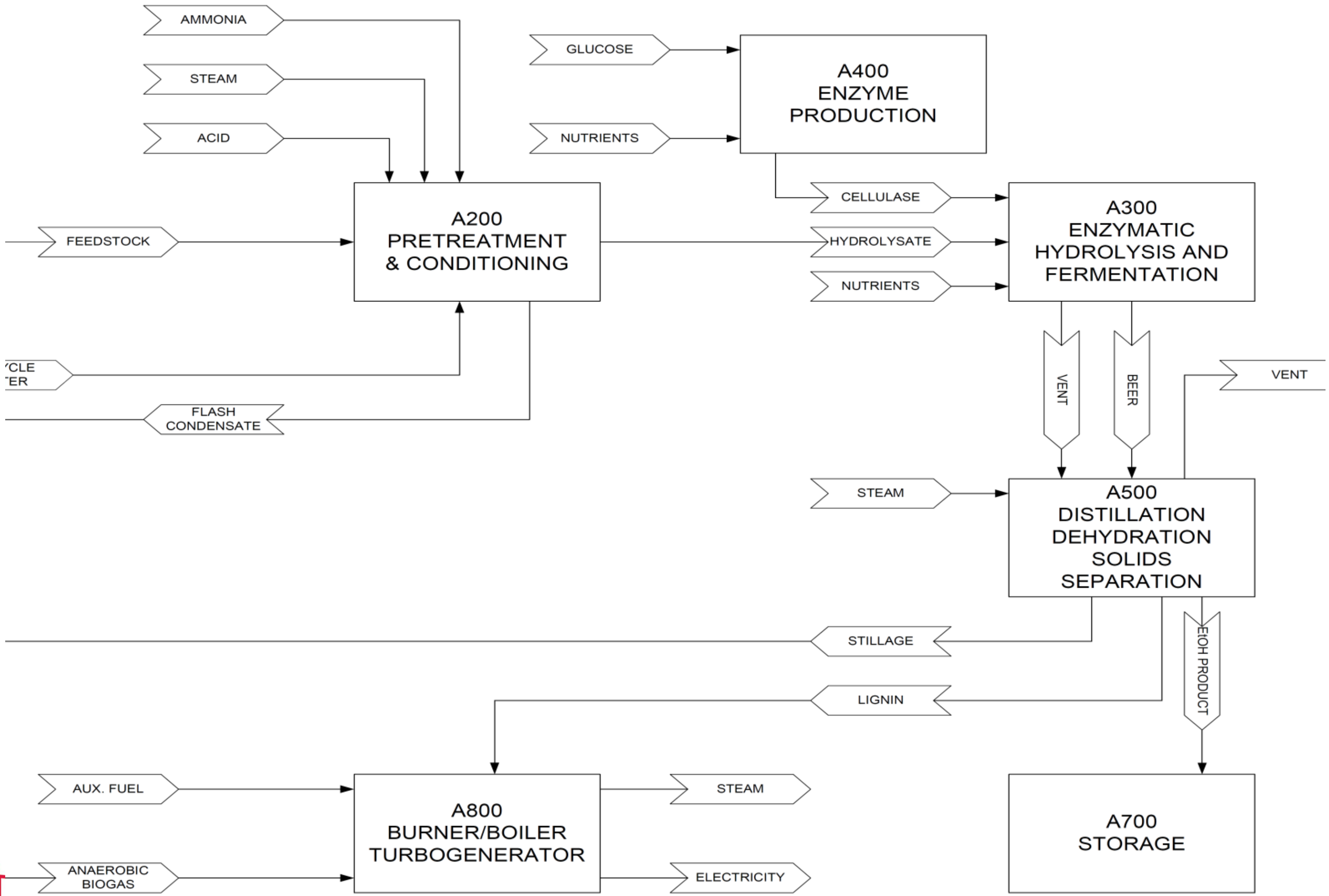
Process design and economic analysis



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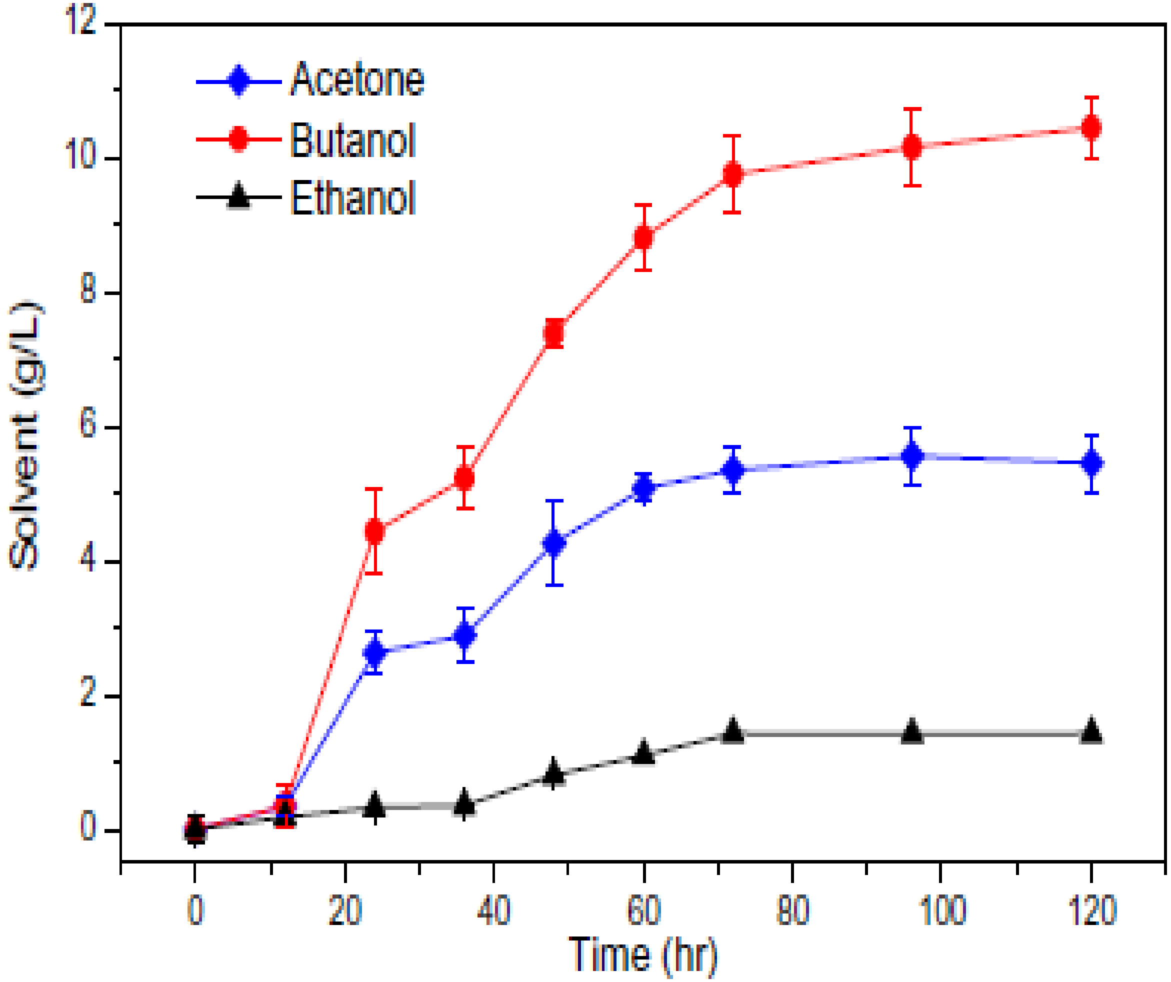
Humbird, 2011, https://www.nrel.gov/

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

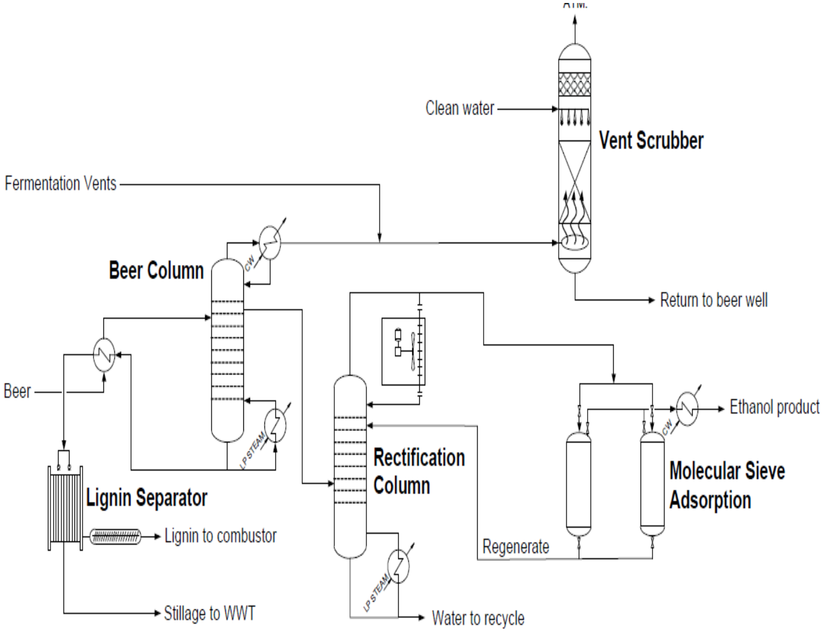


Biomass cost is $58.5/dry ton (2007$)

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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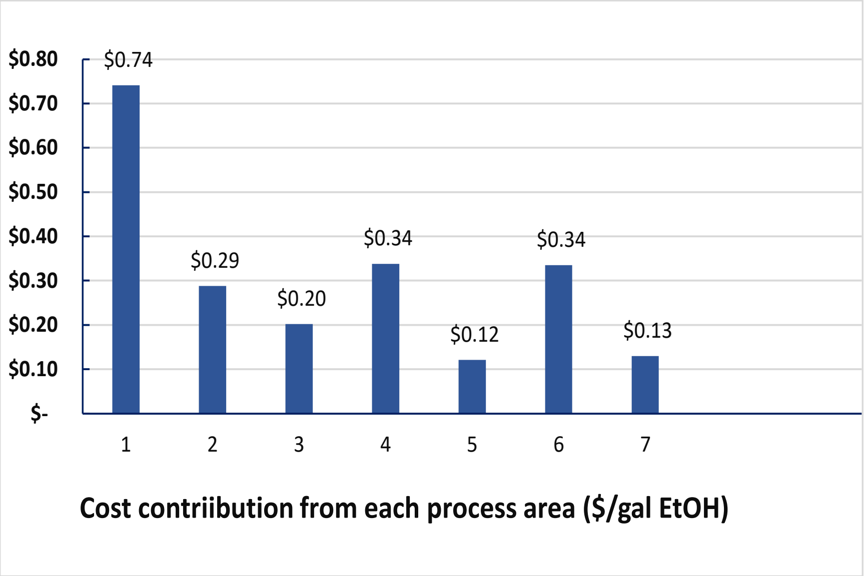
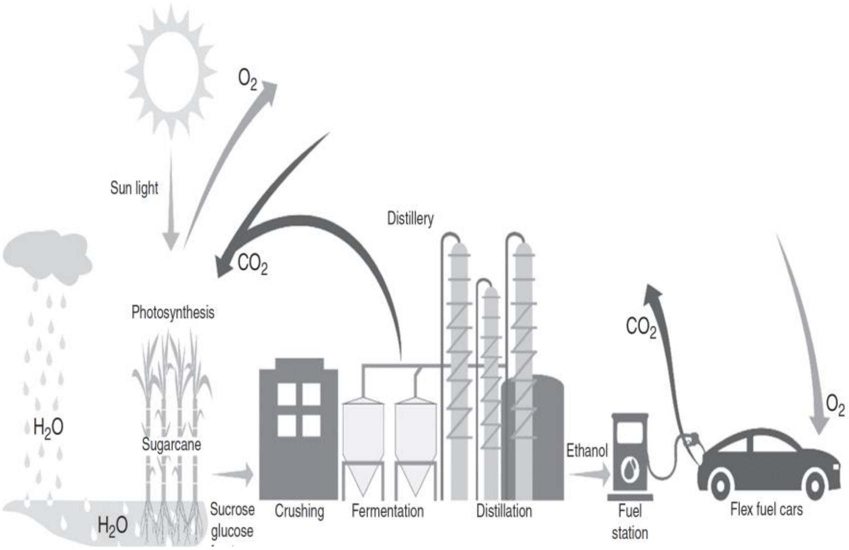
Co-fermentation of glucose and xylose

- ABE fermentation of mixed glucose and xylose

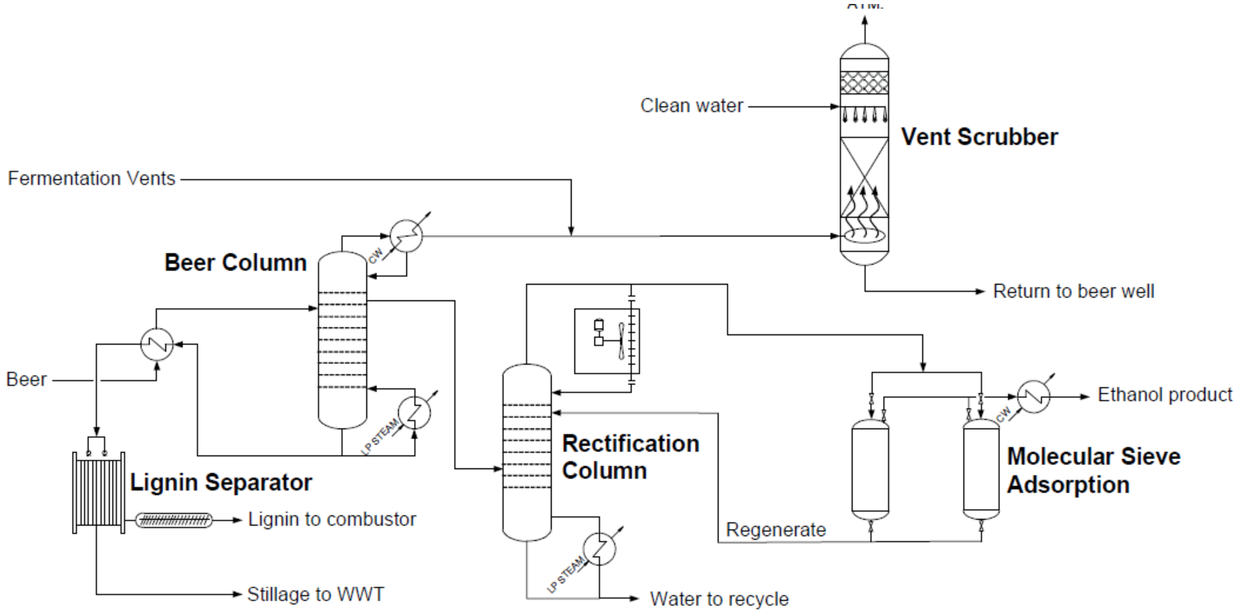




W. Guan et al. / Bioresource Technology 200 (2016) 713–721

Ethanol distillation and separation

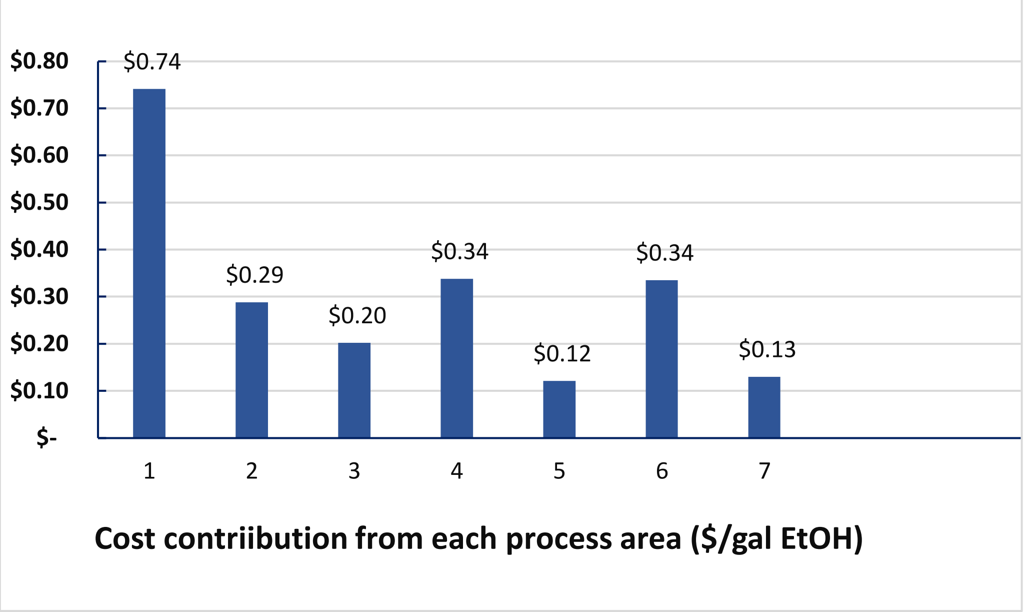
- Distillation and molecular sieve adsorption to recover ethanol
- Distillation is accomplished in two columns:
  - Beer column, removes the dissolved CO2 and most of the water.
  - Rectification column to concentrates ethanol to a near azeotropic composition.



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/

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Sugarcane ethanol in brazil

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

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

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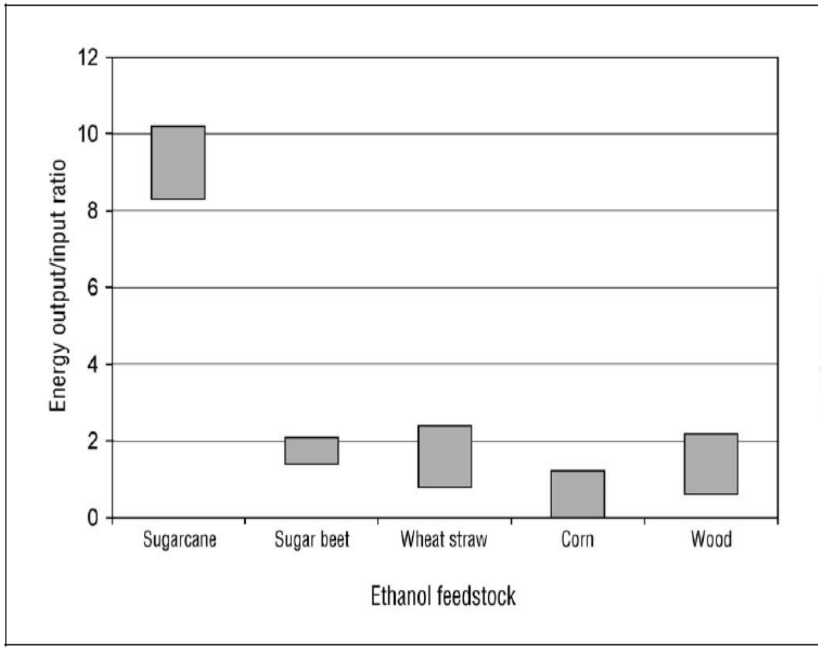
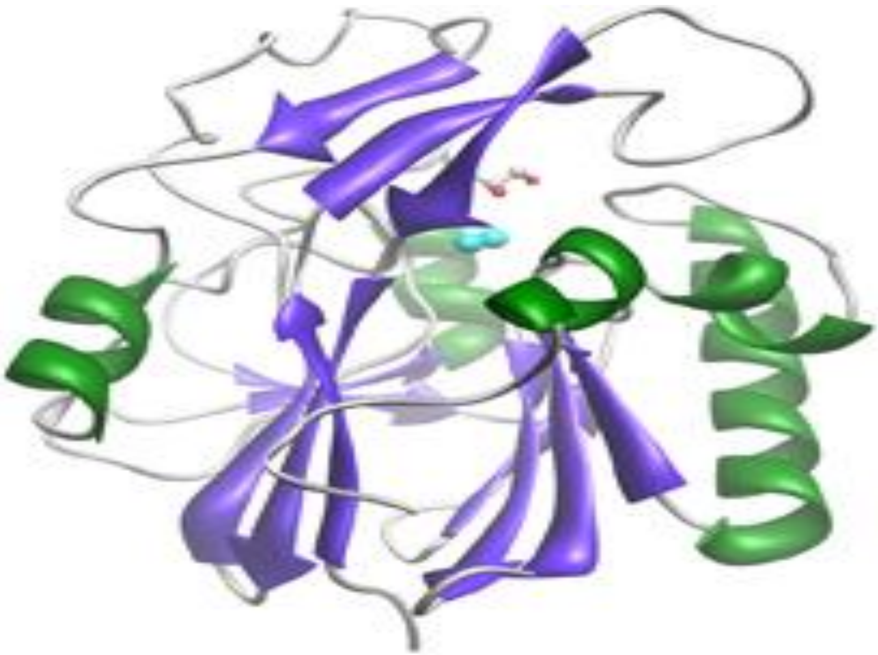
Energy input and output





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

Coelho, 2006

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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)

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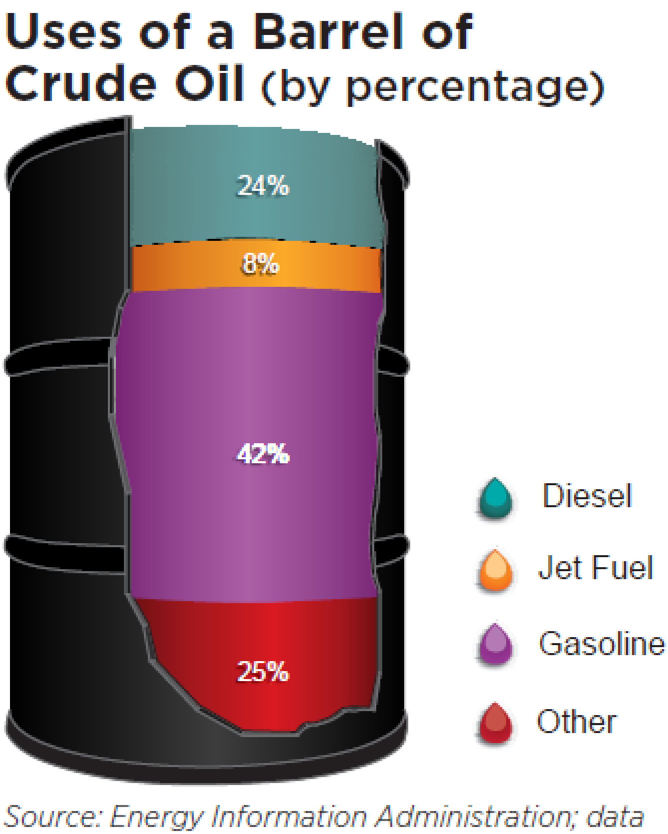
Bioproducts from biorefinery

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



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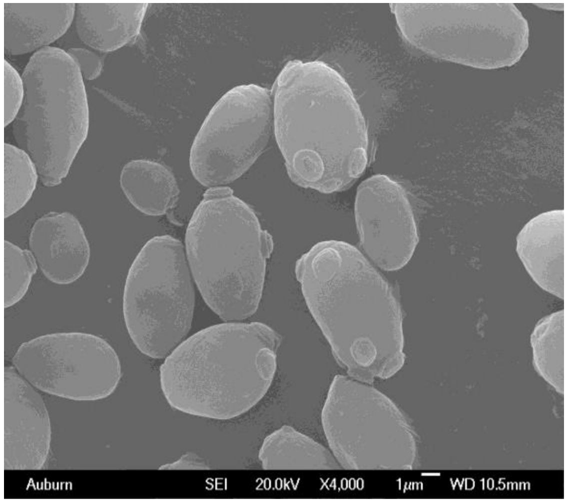
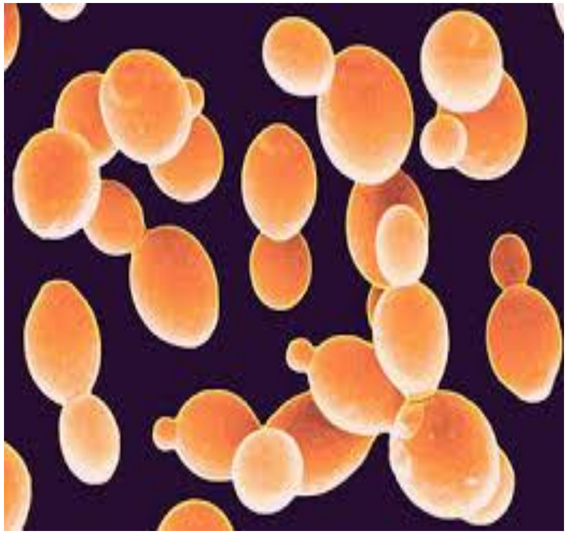
Catalyst and biocatalyst

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

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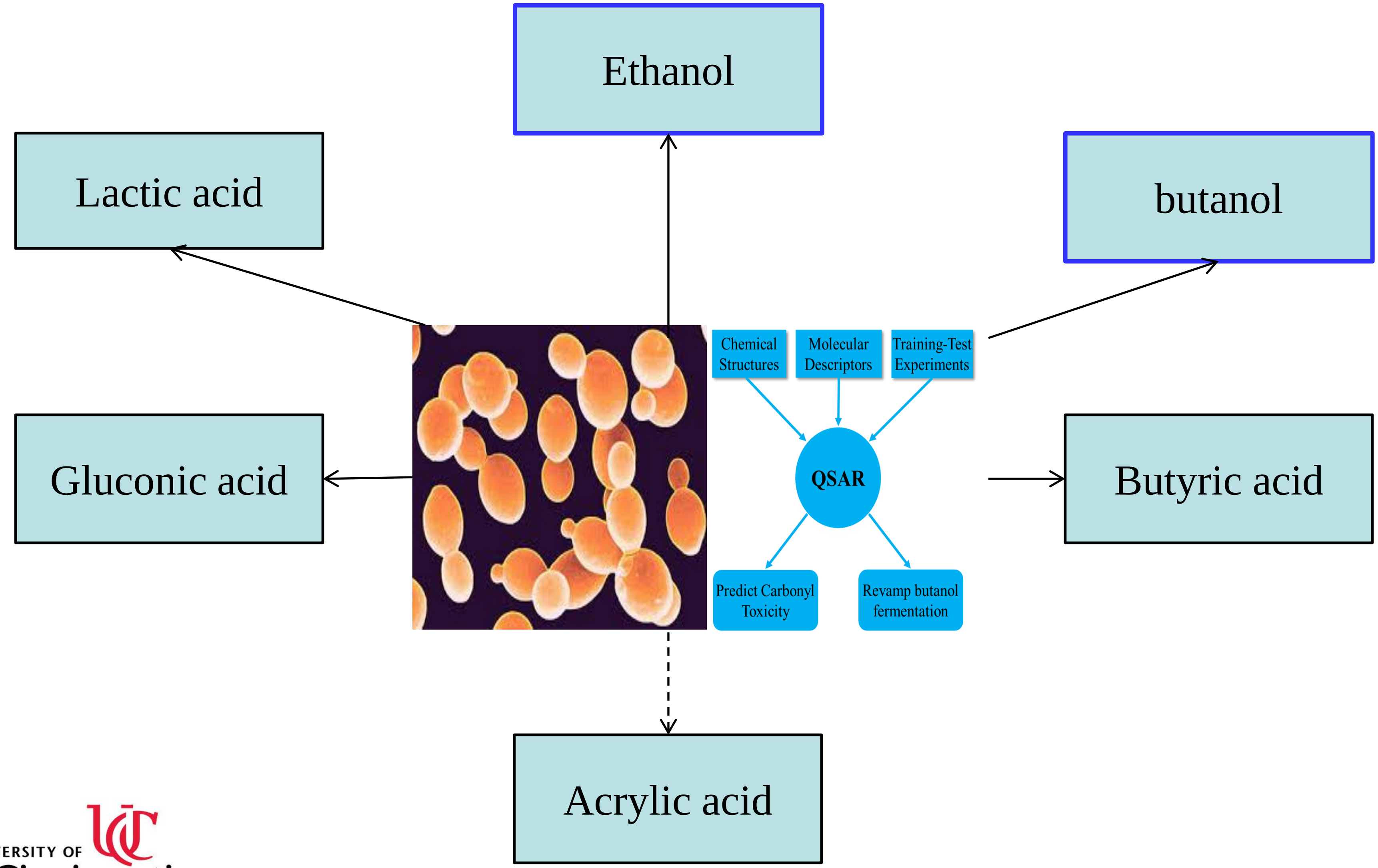
Tu research: fuels and chemicals



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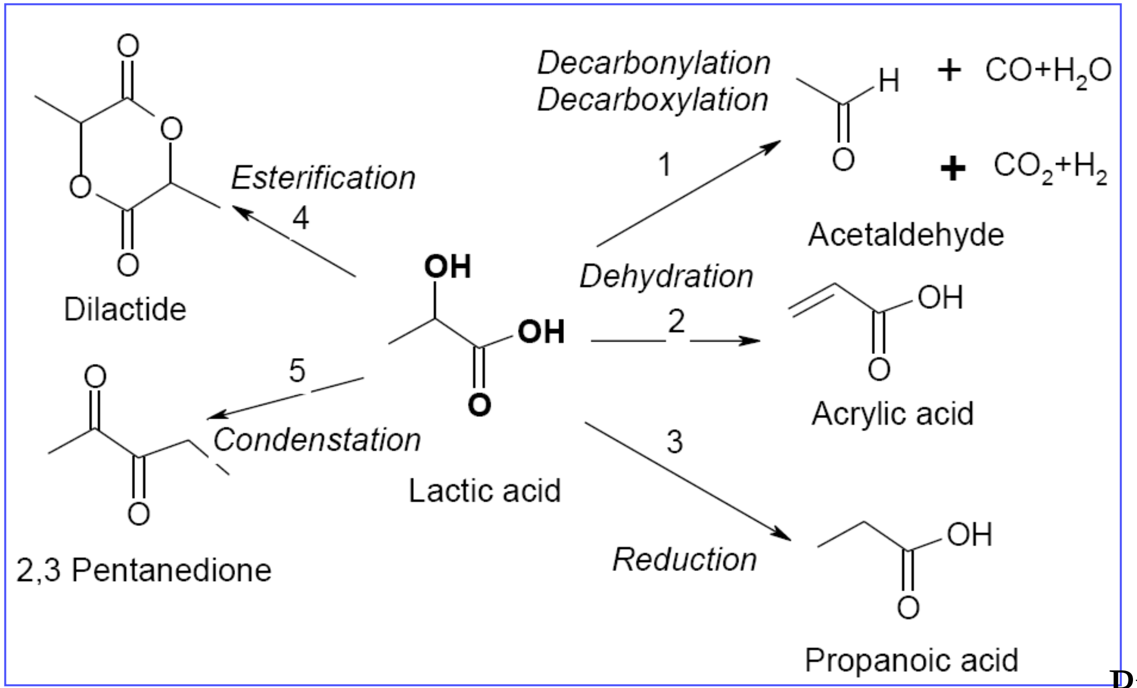
Our research on acrylic acid

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



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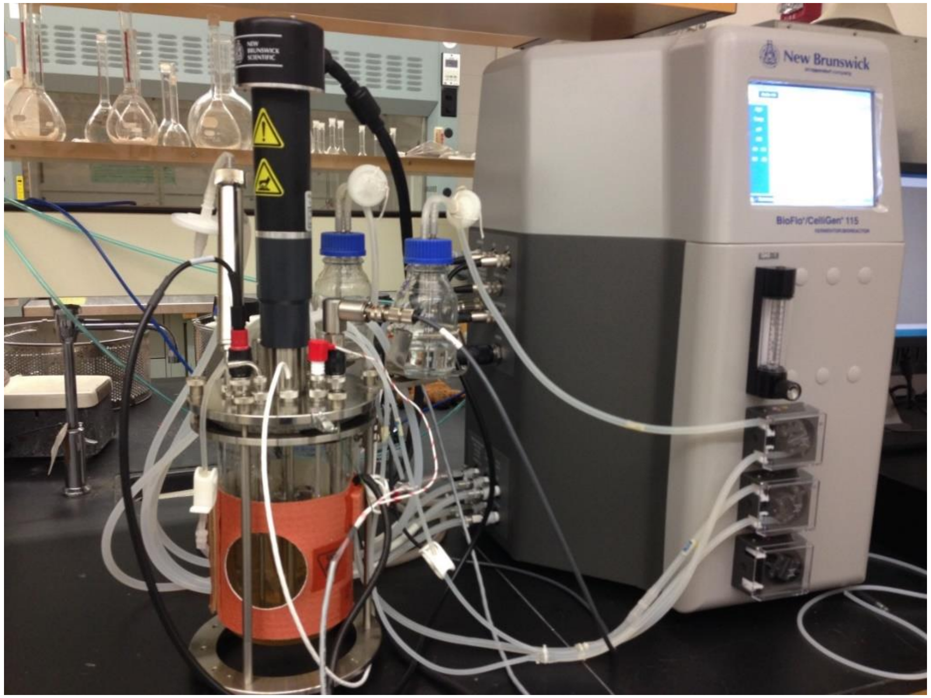
Procter & Gamble

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

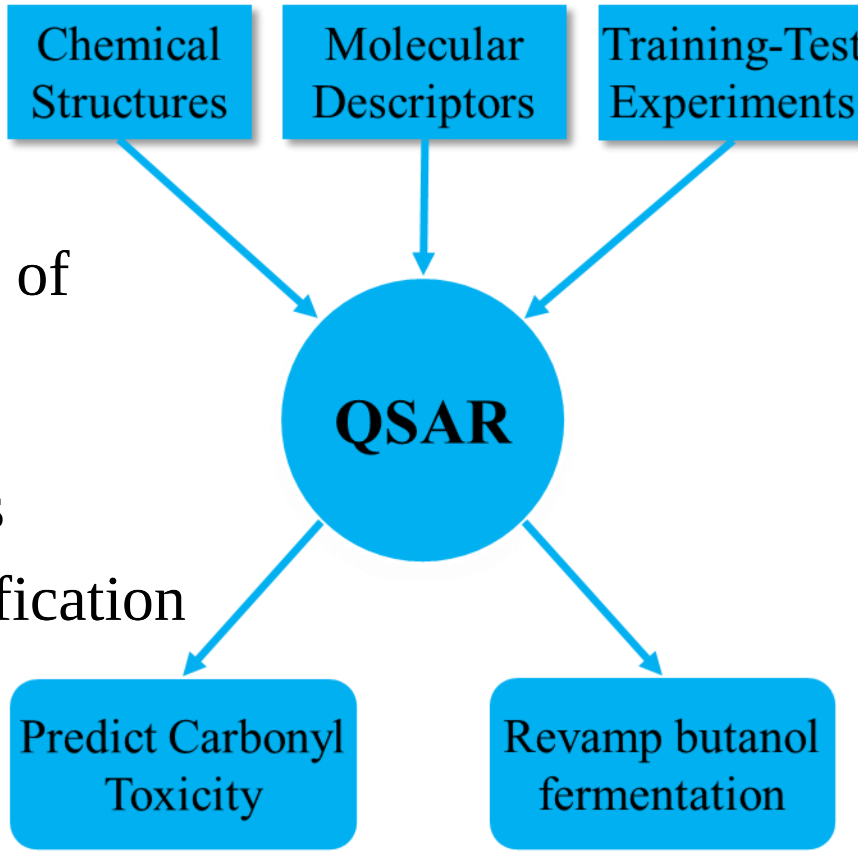
- Quantitative structure-activity relationship (QSAR) approach
  - Computational study
  - Experimental determination

❖Predict the inhibitory effects of

❖degradation compounds

❖Identify the potent inhibitors

❖Design new selective detoxification



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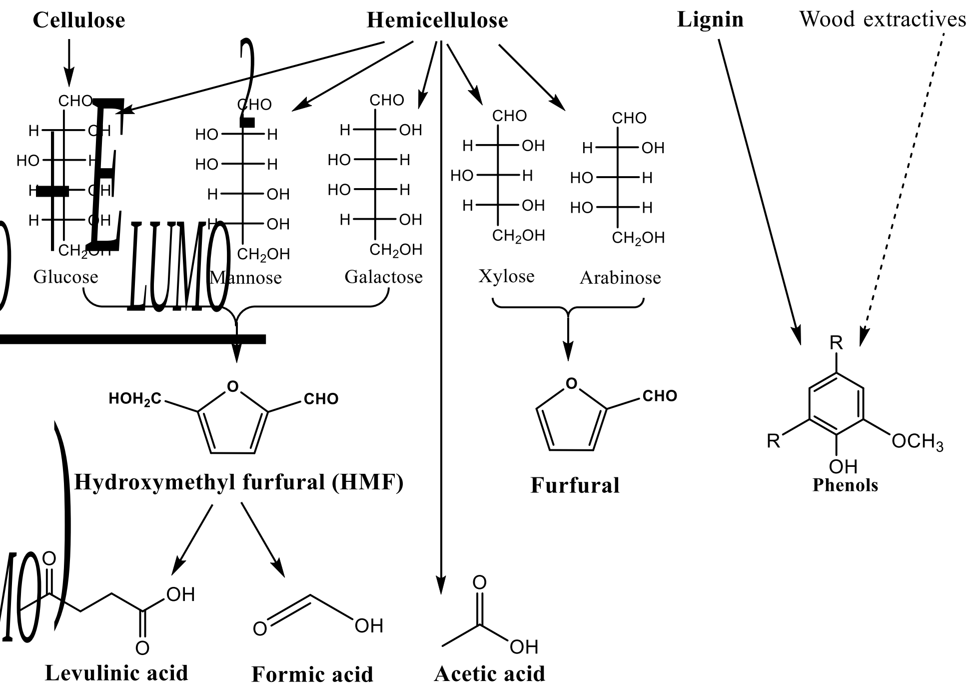
Objectives and Hypothesis

- 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*

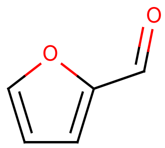
Formation of carbonyl inhibitors

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

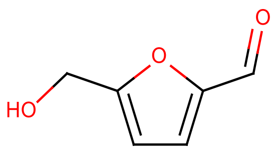


Questions: What are the most potent inhibitors?

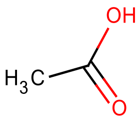
Fermentation inhibitors: carbonyl compounds



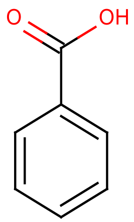
Furfural



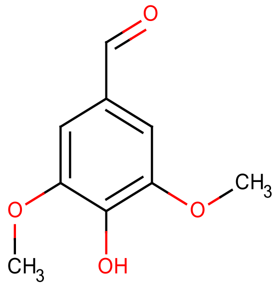
HMF



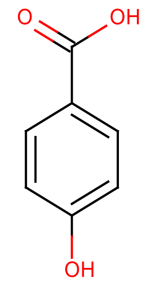
Acetic acid



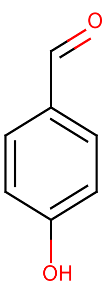
Benzoic acid



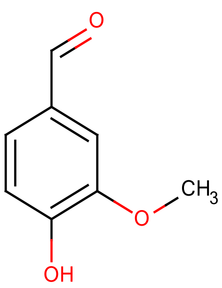
Syringaldehyde



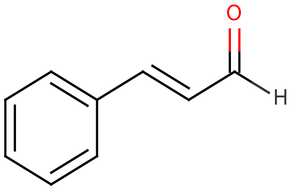
4-Hydroxybenzoic acid



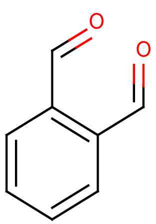
4-Hydroxybenzaldehyde



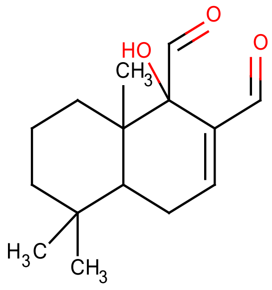
Vanillin



Cinnamaldehyde



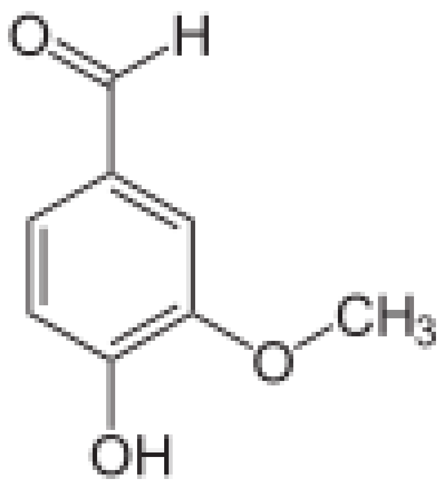
Ortho-phthalaldehyde



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

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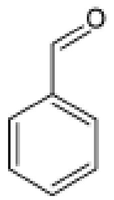
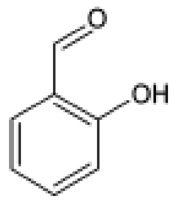
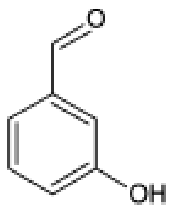
Phenolic model compounds



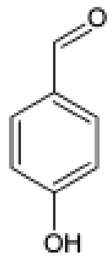
benzaldehyde



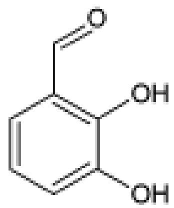
2-hydroxybenzaldehyde



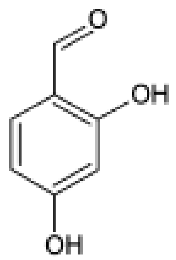
3-hydroxybenzaldehyde



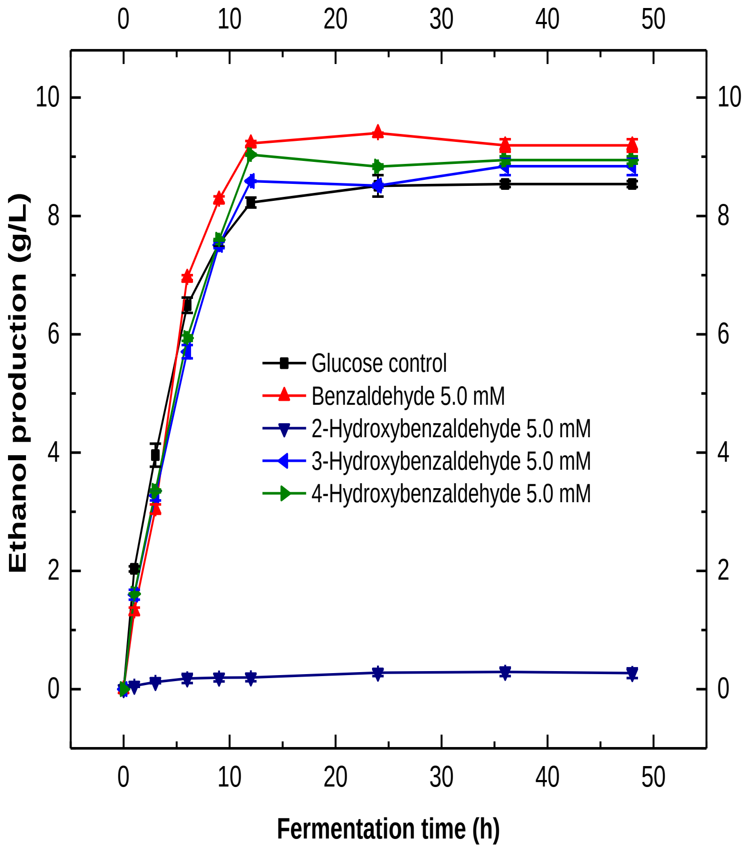
4-hydroxybenzaldehyde



2,3-dihydroxybenzaldehyde



2,4-dihydroxybenzaldehyde

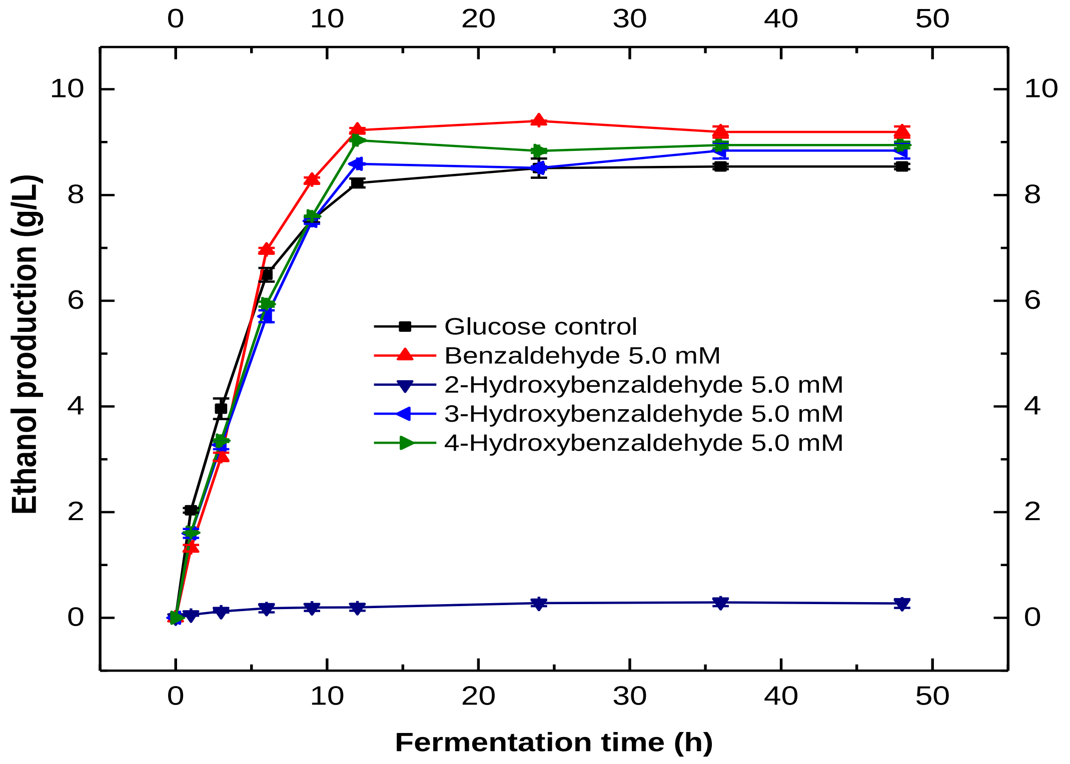


Substitution Effects of Phenolic Aldehyde Inhibition on fermentation

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

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Effects of di- and trihydroxybenzaldehydes on yeast fermentation

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.

- 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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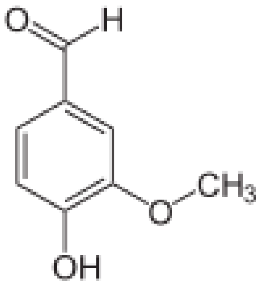
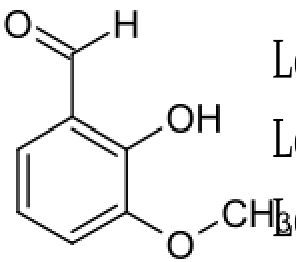
Effect of vanillin and o-vanillin on fermentation



4-Hydroxy-3-methoxybenzaldehyde



2-Hydroxy-3-methoxybenzaldehyde

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

- 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
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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

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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.

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