Willow Biomass Delignification, Characterization, and Pretreatment

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GVSU GREEN CHEMISTRY

Anthropocene => new geologic age

- Contaminating ecosystems
  - New world meet Old world (e.g. tomatoes in Italy, potatoes in Ireland, wheat in Americas)

- Agriculture
  - The Haber-Bosch process has created the greatest disturbance in the nitrogen cycle since microbial equilibrium was establish 2.5 billion years ago [Canfield, Glazer, and Falkowski, 2010].

- Industrial pollution
  - Plastic, nuclear, PFAS, ethylene oxide, et cetera

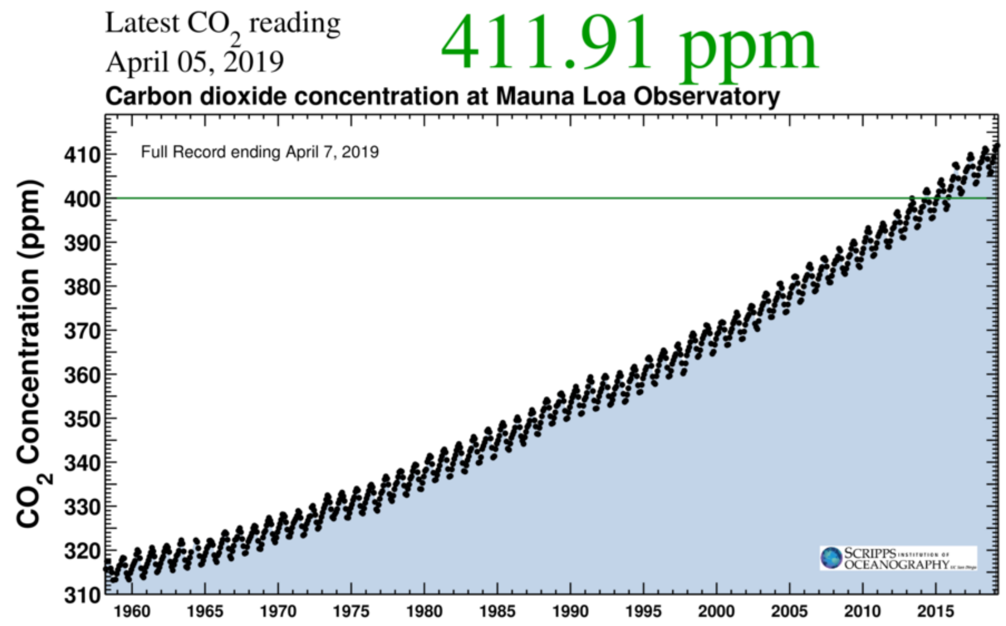
Simon L. Lewis and Mark A. Maslin. Defining the Anthropocene. *Nature*, **2015**, 519, 171-180.
Donald E. Canfield, Alexander N. Glazer, Paul G. Falkowski. The evolution and future of Earth's nitrogen cycle. *Science*. **2010**, 330, 192-196

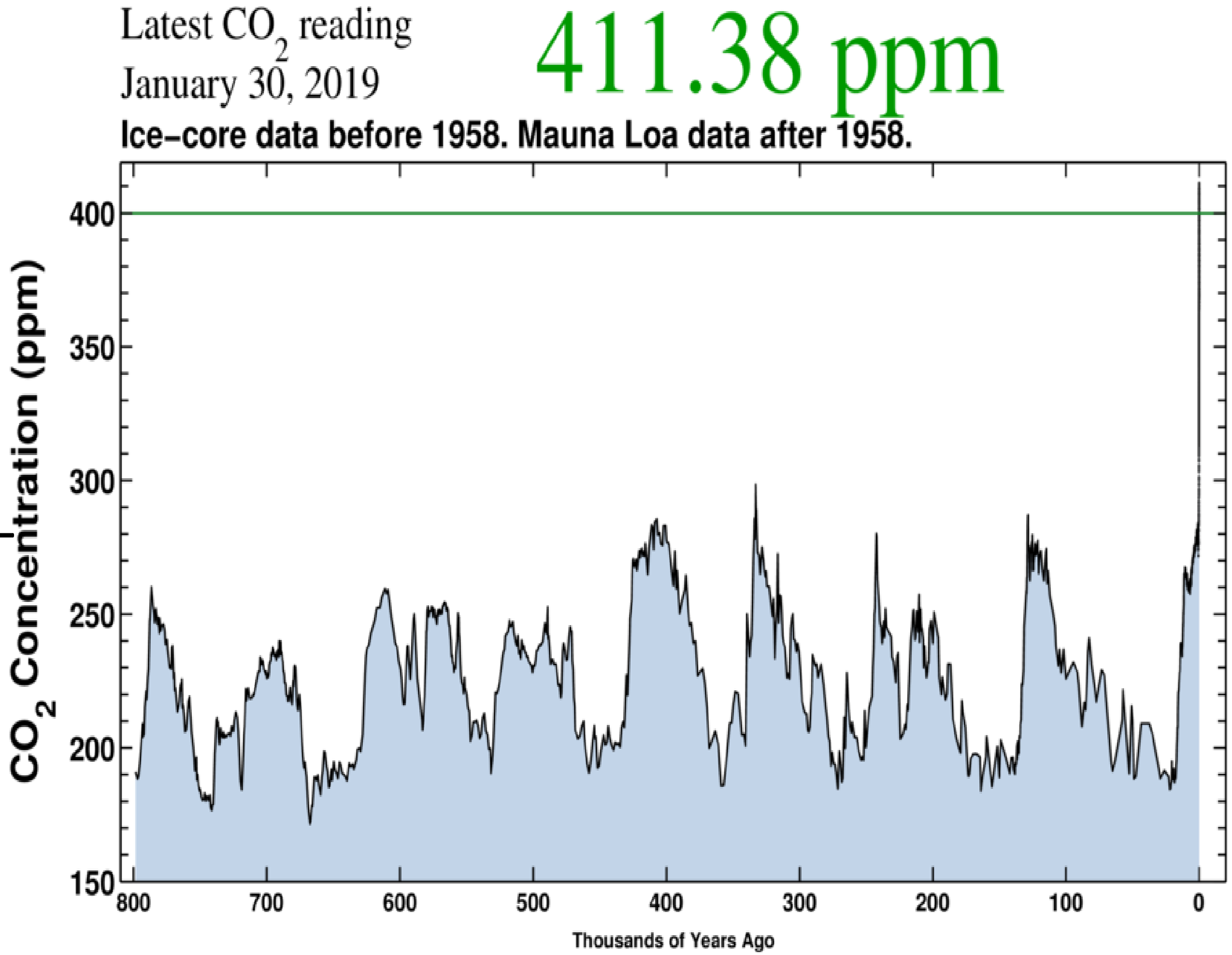
Global climate change

“High confidence” of reaching 1.5°C above pre-industrial levels between 2030 and 2052 at the current rate [IPCC, 2018].

Intergovernmental Panel on Climate Change (IPCC). Global Warming of 1.5°C. **2018**

Lower photosynthetic rate —> lower food security





https://scripps.ucsd.edu/programs/keelingcurve

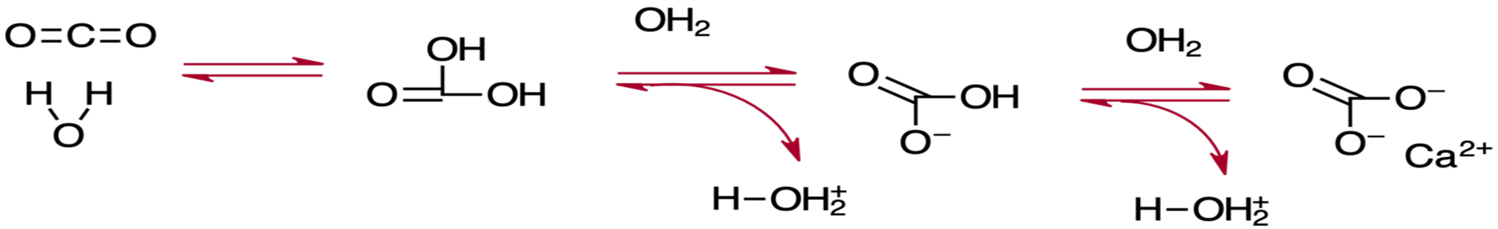
Miocene

Paul N. Pearson and Martin R. Palmer. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature*. **2000**, 406, 695-699.

Mass extinction (6th)

- 1000x to 10,000x preindustrial [De Vos et al., 2014].
  - Greatest in 65 million years
- Coral reefs – home to ~32% of all marine species [Costello, 2015] – may be extinct by 2100 [Carpenter et al., 2008]; >99% extinction @ 2°C warming [IPCC, 2018].

Acropora, https://en.wikipedia.org/wiki/Coral_bleaching#/media/File:Bleachedcoral.jpg

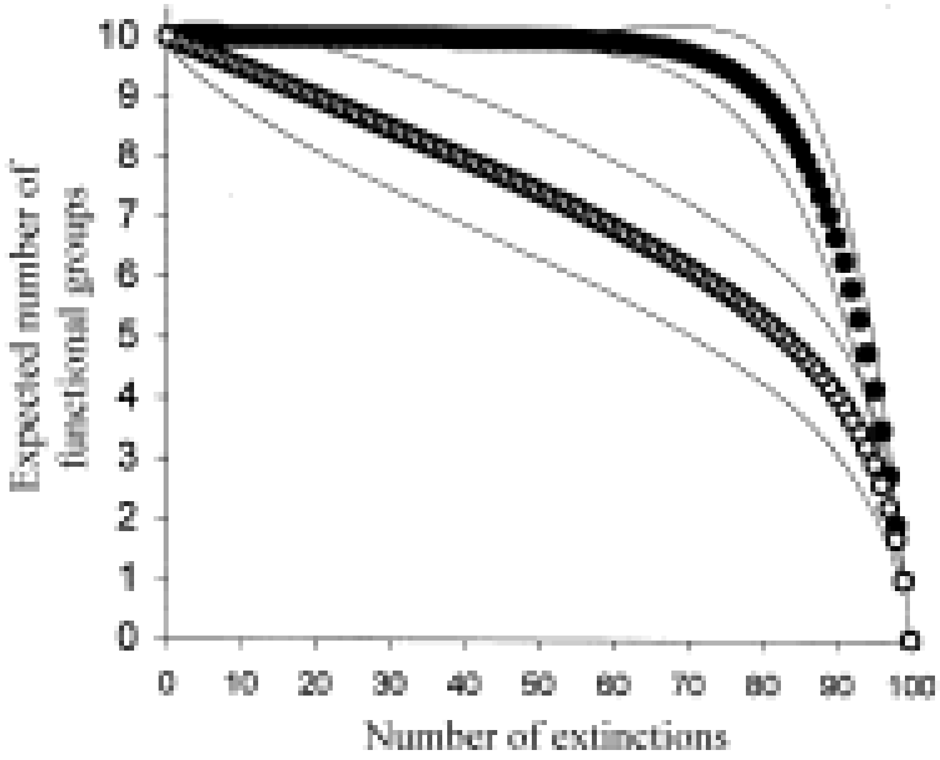


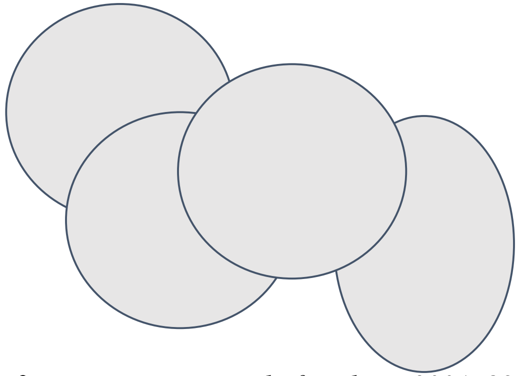
Gerardo Ceballos, Paul R. Elrlich, Anthony D. Bamosky, Andrés García, Robert M. Pringle, Todd M. Palmer. Accelerating modern human-induced species losses: Entering the sixth mass extinction. *Environmental Sciences*, **2015**.
Jurriaan M. De Vos, Lucas N. Joppa, John L. Gittleman, Patrick R. Stephens, Staurt L. Pimm. Estimating the normal background rate of species extinction. *Conservation Biology*. **2014**, 29(2), 452-462
Rodolfo Dirzo, Hillary S. Young, Mauro Galetti, Gerardo Ceballos, Nick J. B. Isaac, Ben Collen. Defaunation in the Anthropocene. *Science*. **2014**, 345 (6195), 401-406
Mark J. Costello. Biodiversity: the known, unknown, and rates of extinction. *Current Biology*.**2015**, 25, 368-371.
Kent E. Carpenter, Muhammad Abrar, Greta Aeby, Richard D. Aronson, Stuart Banks, Andrew Bruckner, Anglet Chiriboga, Jorge Cortés, J. Charles Delbeek, Lyndon DeVantier, Graham J. Edgar, Alasdair J. Edwards, Douglas Fenner, Héctor M. Guzmán, Bert W. Hoeksema, Gregor Hodgeson, Ofri Johan, Wilfredo Y. Licuanan, Suzanne R. Livingstone, Edward R. Lovell, Jennifer A. Moore, David O. Obura, Domingo Ochavilla, Beth A. Polidoro, William F. Precht, Miledel C. Quibilan, Clarissa Reboton, Zoe T. Richards, Alex D. Rogers, Jonnell Sanciangco, Anne Sheppard, Charles Sheppard, Jennifer Smith, Simon Stuart, Emre Turak, John E. N. Veron, Carden Wallace, Ernesto Weil, Elizabeth Wood. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science*. **2008**, 321, 560-563

Functional redundancy hypothesis

By Narong Khueankaew, royalty-free

- Analogy to janga: each block is a species
  - Loss in biological diversity transpires an accelerating loss in ecological function





Carlos Roberto Fonseca and Gislene Ganade. Species functional redundancy, random extinctions, and the stability of ecosystems. *Journal of ecology*. **2001**, 89, 118-125.

Personal contributions => consumer choices

- Reducing Carbon footprint [Jones and Kammen, 2011]
  - Transportation
  - Diet
    - 30% of European greenhouse gas emissions are agricultural [Petrovic, **2015**]
  - Electricity

Jones, Christopher M. and Kammen, Daniel M. Quantifying carbon footprint reduction opportunities for U.S. households and communities. *Environmental Science Technology*. **2011**, 45, 4088-4095

Petrovic, Zoran; Vesna Djordjevic; Dragan Milicevic; Ivan Nastasijevic; Nenad Parunovic. Meat production and consumption: environmental consequences. **2015**. *Procedia Food Science*. 5, 235-238.

Industrial contributions => Green Chemistry

1. Prevent waste: Design chemical syntheses to prevent waste. Leave no waste to treat or clean up.
2. Maximize atom economy : Design syntheses so that the final product contains the maximum proportion of the starting materials. Waste few or no atoms .
3. Design less hazardous chemical syntheses : Design syntheses to use and generate substances with little or no toxicity to either humans or the environment.
4. Design safer chemicals and products : Design chemical products that are fully effective yet have little or no toxicity.
5. Use safer solvents and reaction conditions : Avoid using solvents, separation agents, or other auxiliary chemicals. If you must use these chemicals, use safer ones.
6. Increase energy efficiency : Run chemical reactions at room temperature and pressure whenever possible.
7. Use renewable feedstocks: Use starting materials (also known as feedstocks) that are renewable rather than depletable. The source of renewable feedstocks is often agricultural products or the wastes of other processes; the source of depletable feedstocks is often foss il fuels (petroleum, natural gas, or coal) or mining operations.
8. Avoid chemical derivatives : Avoid using blocking or protecting groups or any temporary modifications if possible. Derivatives use additional reagents and generate waste.
9. Use catalysts, not stoichiometric reagents : Minimize waste by using catalytic reactions. Catalysts are effective in small amounts and can carry out a single reaction many times. They are preferable to stoichiometric reagents, which are used in excess and carry out a reaction only once.
10. Design chemicals and products to degrade after use : Design chemical products to break down to innocuous substances after use so that they do not accumulate in the environment.
11. Analyze in real time to prevent pollution : Include in-process, real-time monitoring and control during syntheses to minimize or eliminate the formation of byproducts.
12. Minimize the potential for accidents : Design chemicals and their physical forms (solid, liquid, or gas) to minimize the potential for chemical accidents including explosions, fires, and releases to the environment.

https://www.epa.gov/greenchemistry/basics-green-chemistry

The evolution of research goal

- Fall 2016 undergraduate research fair
- “Biofuels from willows at the SAP”

Andrew Freiburger <freibura@mail.gvsu.edu> Thu, Oct 6, 2016, 7:12 AM
to nordmane

Hi Dr. Nordman,
I glanced over your renewable energy research at the Undergraduate Research Fair this past week, and I am interested to know more about it and any opportunities that exist for undergraduates. I was told by Youseff at the SAP that some willow trees are apart of your research into biomass energy, however, this is the extent of my knowledge about your research. Let me know if there is anything that I can do or if there is someplace that I should go to learn about your research.
Thanks!
Andrew

- Dr. Witucki —> Dr. Kovacs <– –> Professor Krikke

- Starting from scratch
  - Develop methods for an undergraduate lab
  - Investigate and characterize willow biomass

Fast growing!

GVSU's SAP May 31, 2017

September, 2017

November 3, 2018

15-25ft growth in each 3 year harvesting cycle

- Fishcreek – *Salix purpurea*, US plant patent 17,710
- Millbrook – *Salix purpurea* x *Salix miyabeana*, US plant patent 17,646
- SX64 – *Salix sachalinensis,* Developed at the University of Toronto
- Fabius – *Salix viminalis* x *Salix miyabeana*

Doubleavineyards.com

Woody Biomass > foody biomass

Table 1: The annual requirements of first generation biomass sources (food crops) and second generation biomass sources (woody crops).

Biomass source	Water requirements ($\frac{m^3 \text{ of water}}{L \text{ of biofuel} \cdot year}$)	Land requirements ($\frac{m^2 \text{ of land}}{L \text{ of biofuel}}$)	Fertilizer requirements ($\frac{kg \text{ of fertilizers}}{Hectare \text{ of plot} \cdot year}$)	Crop yield ($\frac{Kg \text{ of crop}}{Hectare \text{ of plot} \cdot year}$)	Growth cycles (annual or perennial)	Direct-effect greenhouse gas emission ($\frac{\text{Grams of } CO_2 \text{ equivalents}}{\text{Megajoules of energy produced}}$)
Corn	2.01[1]	4.75[1]	338[2]	5001[1]	Annual[3]	30.6[7]
Soybeans	15.63[1]	28.40[1]	——	1720[1]	Annual[3]	——
Shrub willow	——	——	100[5]	7,700[5]	Perennial[4], harvested in 3 year cycles for ~10 cycles	0.68[6]

[1]Sourced from Yang et al., **2009**

[2]Nitrogen contributes 162kg, Phosophate contributes 68kg, Potash contributes 90kg, and Sulfer contributes 18kg. Sourced from *USDA*,**2016**.

[3]Sourced from *USDA*, **1985**.

[4]Sourced from Heller et al., **2003**.

[5]Exclusively nitrogen fertilization; the addition of potassium and phosphorous fertilizers were not associated with increased growth rates. Sourced from Hytönen, **1995**.

[6]Sourced from Heller et al., **2003**

[7]Compared with for coal [Liska et al., **2009**]. Sourced from [Liska et al., **2009**].

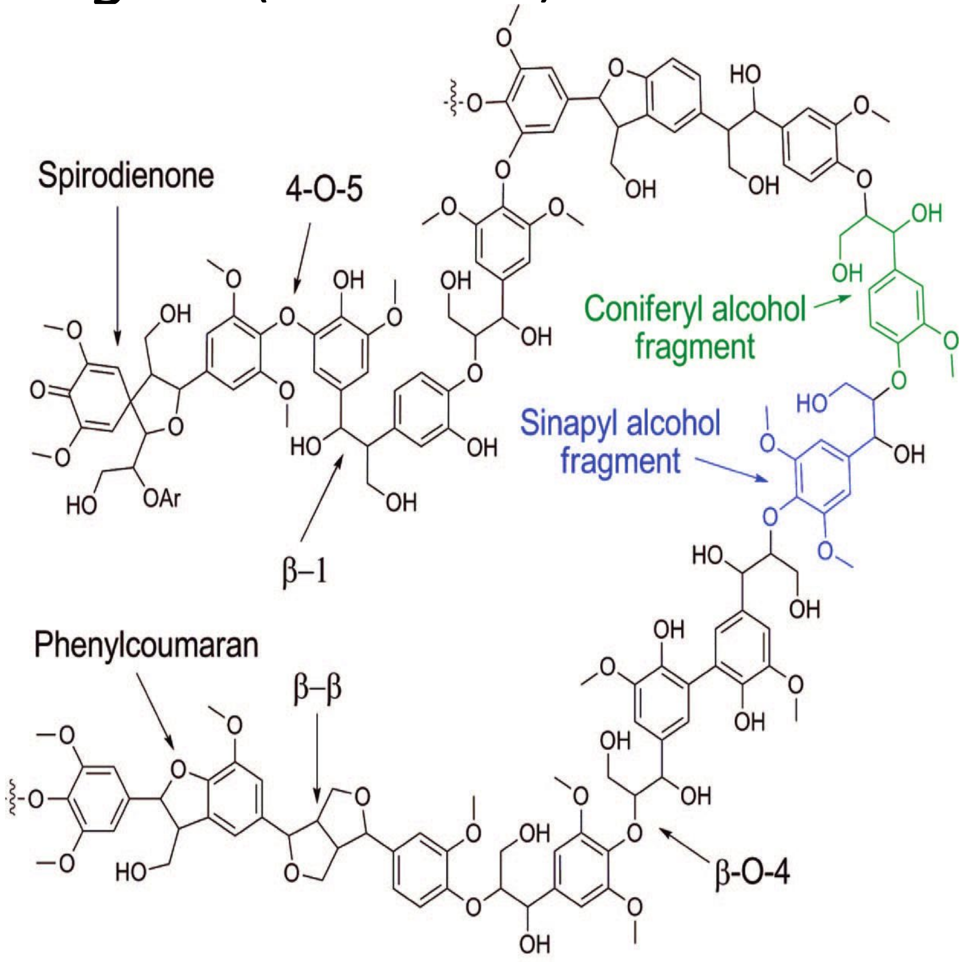
Biomass = **Bio**logical **mass**. Woody biomass

Substance	**Percent of fresh mass (variable)**		**Biological function**
Water	50		Solvent and reactant
Cellulose	20		Structure, cell wall
Hemicellulose	12	**Lignocellulose**	Structure, cell wall
Lignin	11		Binder and rigidity, middle lamella
Metabolites	6		Immune, hormonal, and metabolic function
Minerals	1		Catalyts and enzyme complexes

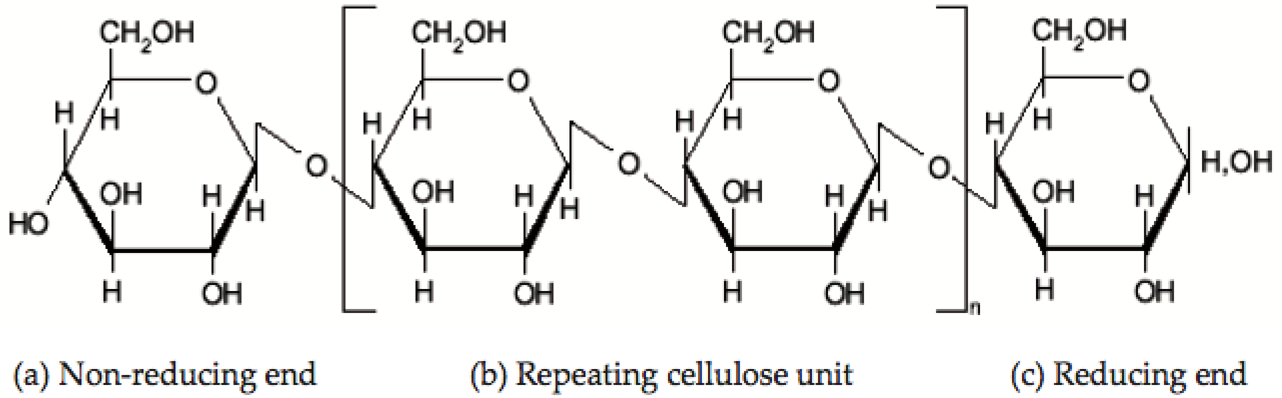
*Ligno*cellulose

- Lignocellulose is the most abundant material on the plant. 1.5 x 10^{12} tons of cellulose exclusively is produced per year [Van de Ven and Godbout, 2013].

Lignin (hardwood)

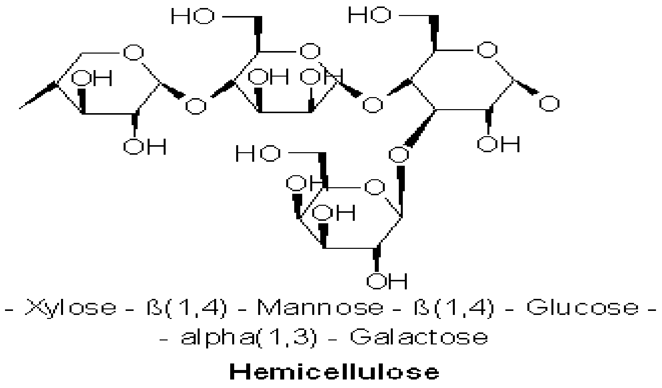


Cellulose



Theo van de Ven and Louis Godbout. Cellulose – fundamental aspects. *InTech*. **2013**.

BerserkerBen, https://en.wikipedia.org/wiki/Hemicellulose#/media/File:Hemicellulose.png



Hemi-Cellulose

Joseph Zakzeski, Pieter C. A. Bruijnincx, Anna L. Jongerius, and Bert M .Weckhuysen. The catalytic valorization of lignin for the production of renewable chemicals. *Chemical Reviews*.**2010**. 110, 3552-3599.

Valorization; the Biorefinery model

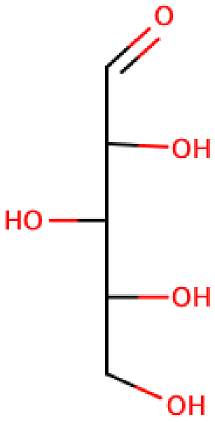
GVSU's SAP 11/3/2018

hv

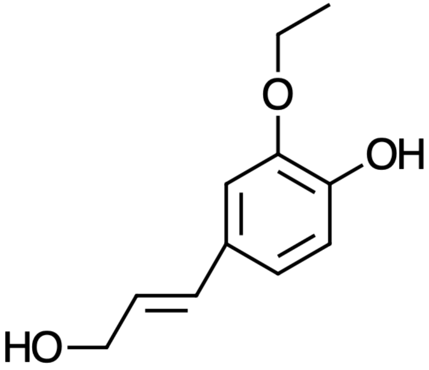
CO_2
H_20

Biorefinery

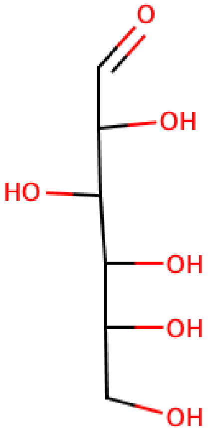
Fractionate



Hemicellulose



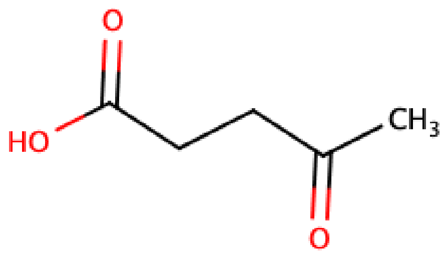
Lignin



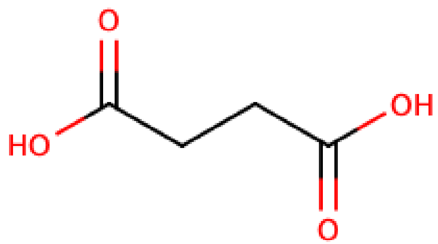
Cellulose

Derivatize

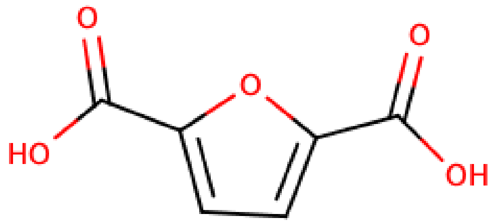
Top 12 value-added chemicals from biomass. *USDE,* **2004.**



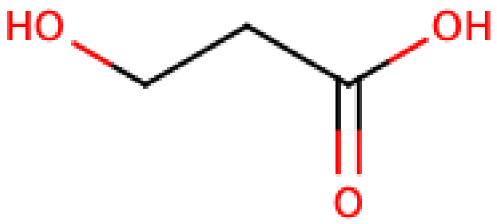
Levulinic Acid



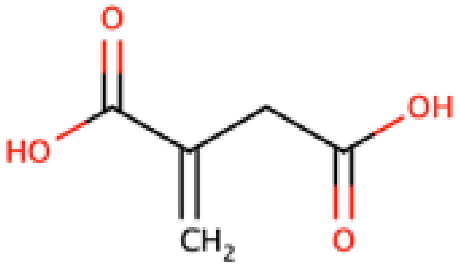
1,4 – diacids (e.g. Succinic Acid)



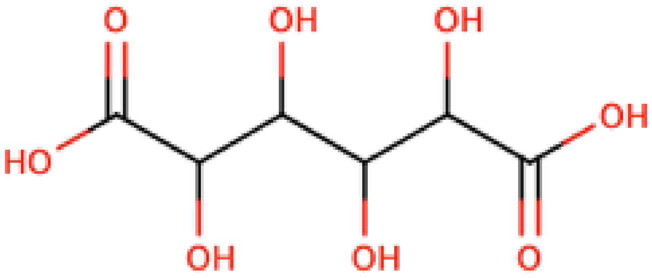
2,5 – Furandicarboxylic acid



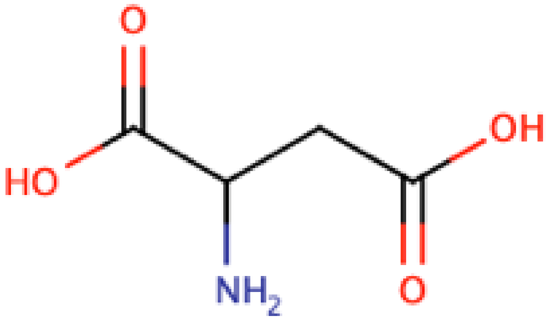
3 – hydroxypropionic acid



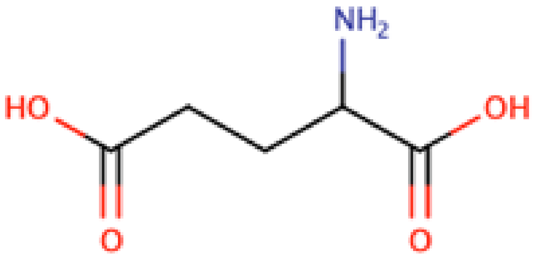
Itaconic Acid



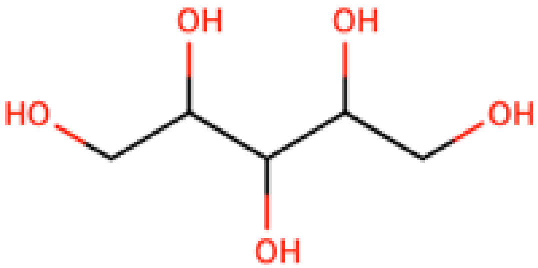
Glucaric Acid



Aspartic Acid

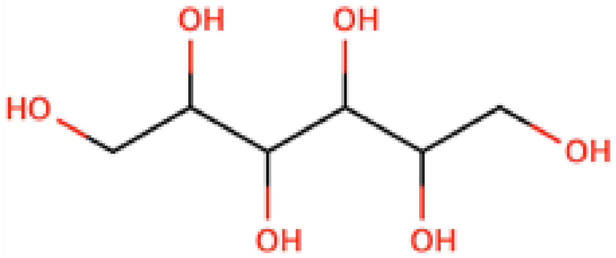


Glutamic Acid

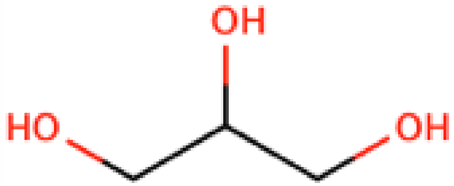


Xylitol

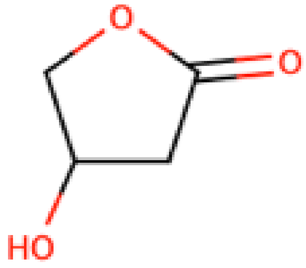
Carbohydrates



Sorbitol

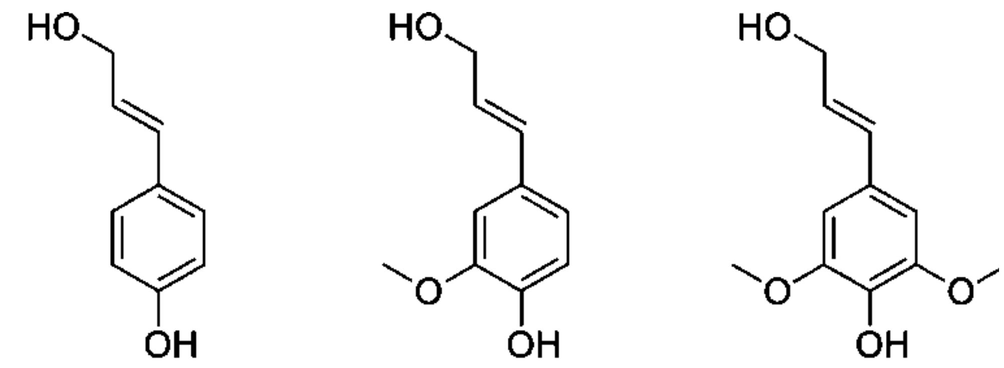


Glycerol



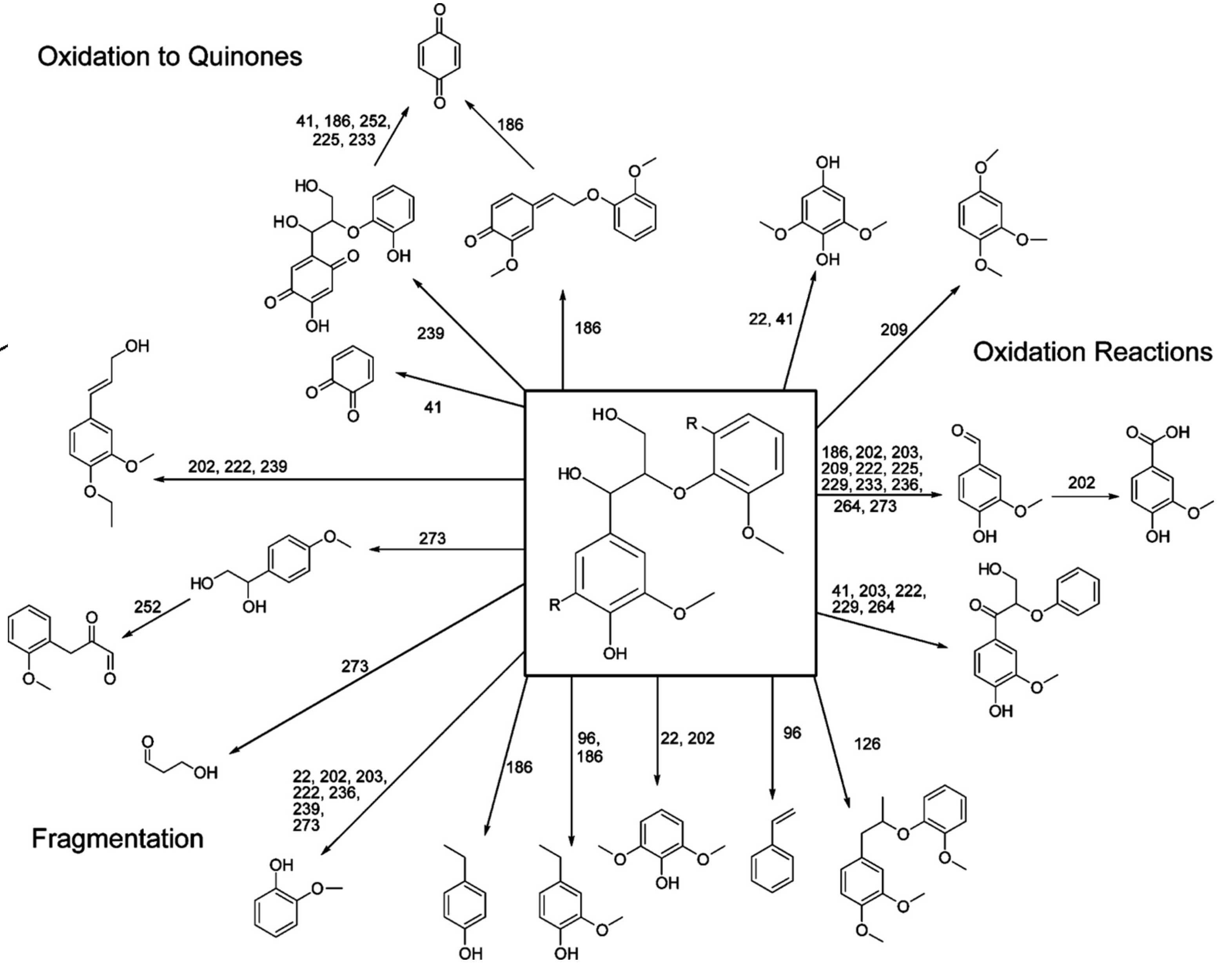
3-Hydroxybuyrolactone

Lignin derivatives



p-coumaryl alcohol   coniferyl alcohol   sinapyl alcohol

Joseph Zakzeski, Pieter C. A. Bruijnincx, Anna L. Jongerius, and Bert M .Weckhuysen. The catalytic valorization of lignin for the production of renewable chemicals. *Chemical Reviews*.**2010**. 110, 3552-3599.



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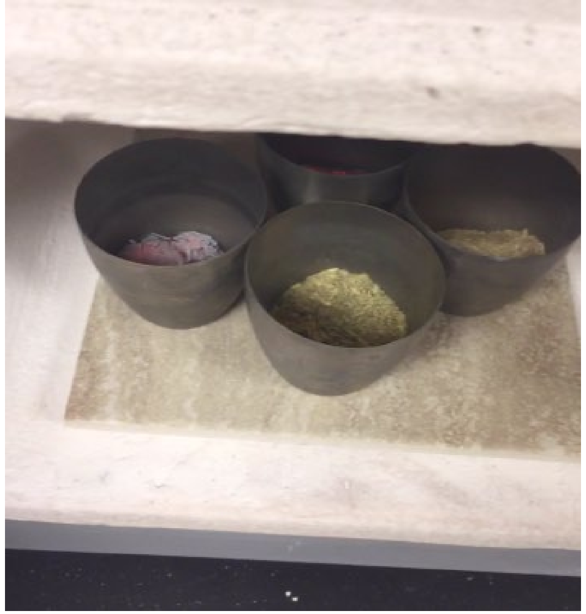
Problems (2 primarily)



Ash

- Damage machinery (especially in gasificiation) [Dave Prouty, Heat Transfer International]
- Deposits must be filtered [Livingston, 2006].
  Bill Livingston. Ash related issues in biomass combustion. *ThermalNet* Workshop. **2006.**
- Catalyze side reactions

Lignin

- Different for each plant
- Ether bonds are resistant to chemical treatment
- **Carboni**ferous period (~360 million – 300 mya) and coal forests

Pretreatment

- Degrade lignin from lignocellulosic material
- Separate each monomer in sequential steps

Mechanical

- Physical
  - Drying or freezing
  - Cutting/chipping/grinding
- Gasification
  - Pyrolysis
- High energy cathode rays

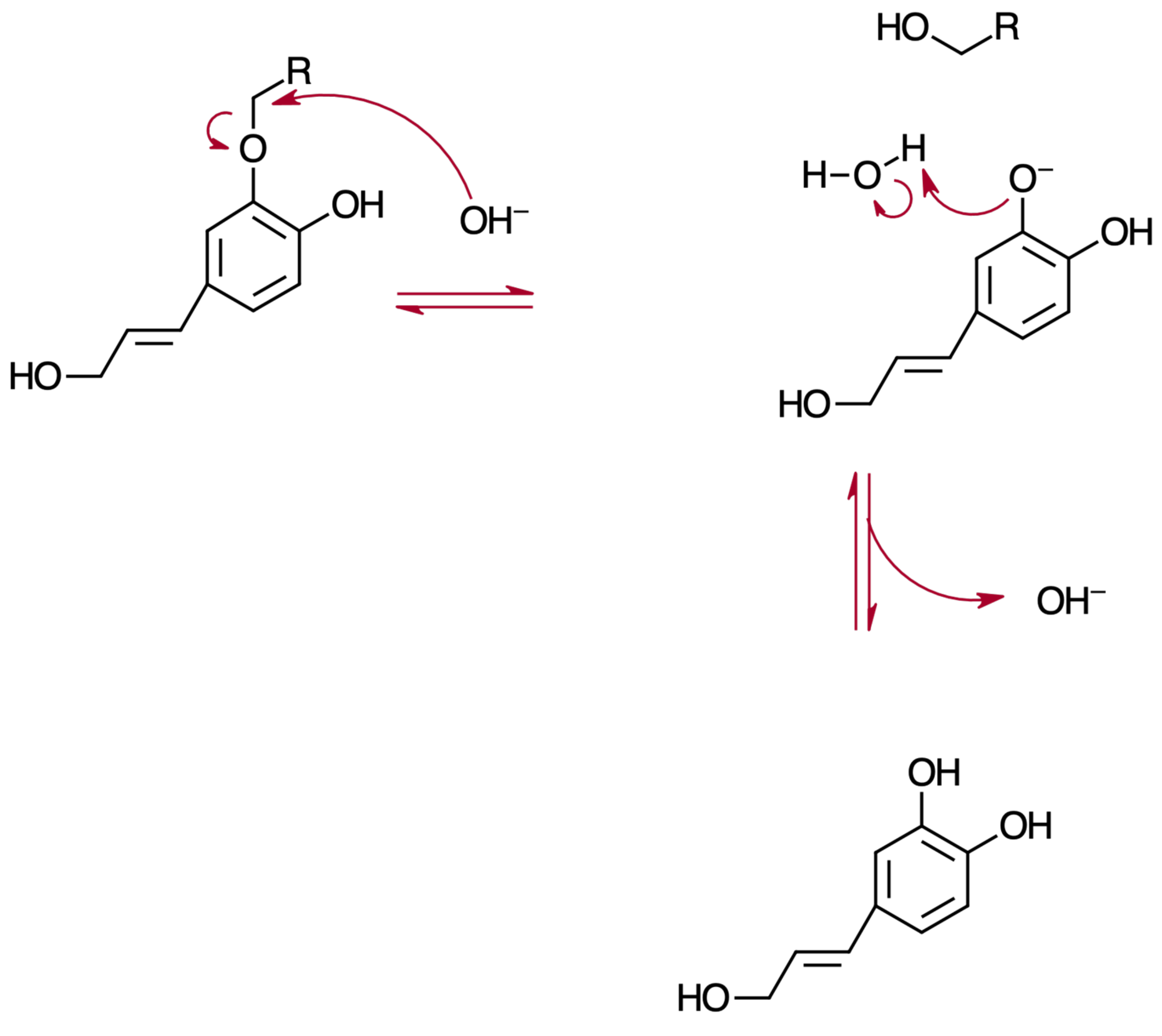
Chemical

- Solvent extraction
  - Deep Eutectic Solvents
  - Aqueous
    - Acid or Base
  - Organic extraction
    - e.g. ethanol, acetone, DCM
- Enzymatic depolymerization
  - Cellulases
  - Lignin peroxidases

Basic pretreatment (Kraft Process)

- >50 million tons of lignin are derived annually from the pulping industry, however, 98% is burned [Zakzeski et al., **2010**].

Selectively depolymerizes lignin and hemicellulose from the cellulosic fibers [Gottumukkala et al., 2016].



Joseph Zakzeski, Pieter C. A. Bruijnincx, Anna L. Jongerius, and Bert M .Weckhuysen. The catalytic valorization of lignin for the production of renewable chemicals. *Chemical Reviews*.**2010**. 110, 3552-3599.

Lalitha Devi Gottumukkala, Kate Haigh, François-Xavier Collard, Eugéne van Rensburg, Johann Görgens. Opportunities and prospects of biorefinery-based valorization of pulp and paper sludge. *Bioresource Technology*. **2016**, 215, 37-49.

Mechanical pretreatment

Shrub willows @ GVSU Sustainable Agriculture Project , 09/ 2017

Hand garden loopers

Doubleavineyards.com/millbrook

Thermo Scientific Oven

Kitchen aid coffee grinder

Physical characterization

% moisture

Thermo Scientific Oven

Kitchen aid coffee grinder

% ash

Enthalpy of combustion

% moisture

- Oven dried
  - 3 days at 80°C
- Lyophilized
  - ~24 hours @ -50°C
- Devised a procedure for homogenizing chip length/width with scissors.

Sample	**Average % moisture oven**	**SD**	**Lyophilization % moisture**
Old	19.76	0.26	
New	50.05	0.71	
Fishcreek	43.6	1.6	42.3
Fabius	52.2	2.0	53.6
Millbrook	51.6	1.2	51.1
SX64	50.1	2.2	50.4

No apparent difference between oven-drying and lyophilization

% ash

- Platinum crucibles
- Heated for 1.5 hours at 575°C to clean
- Samples were heated at 575°C for 2.5 hours.
- Samples were massed before and after ashing.

Sample	**Average % ash**	**SD**
Old	2.44	0.27
New	2.86	0.37
Fishcreek	1.51	0.24
Fabius	2.39	0.37
Millbrook	2.72	0.53
SX64	2.53	0.37

Fishcreek possesses a significantly lower ash concentration

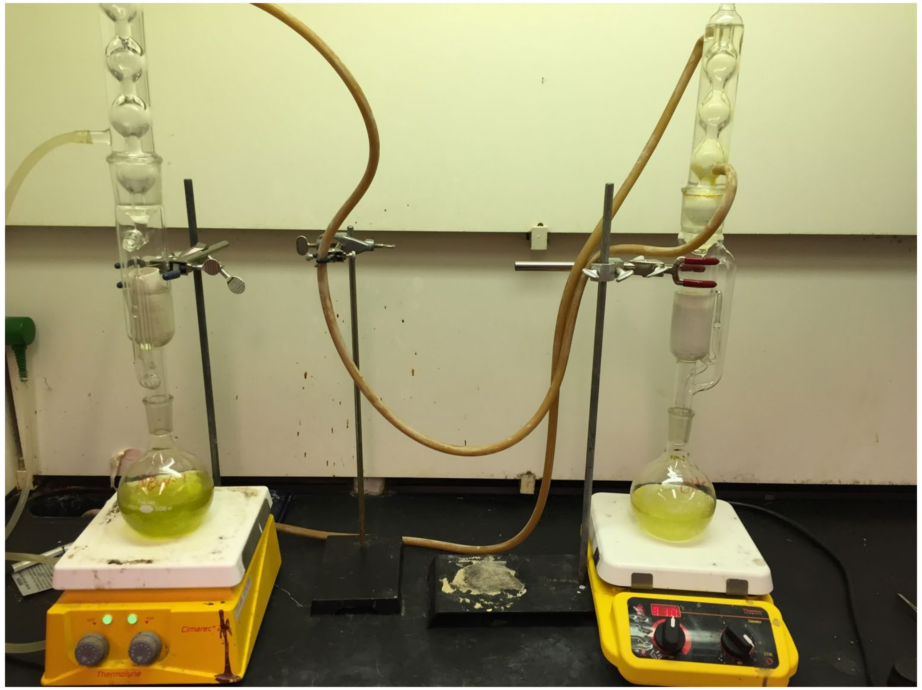
Enthalpy of combustion

Millbrook has a significantly greater enthalpy of combustion

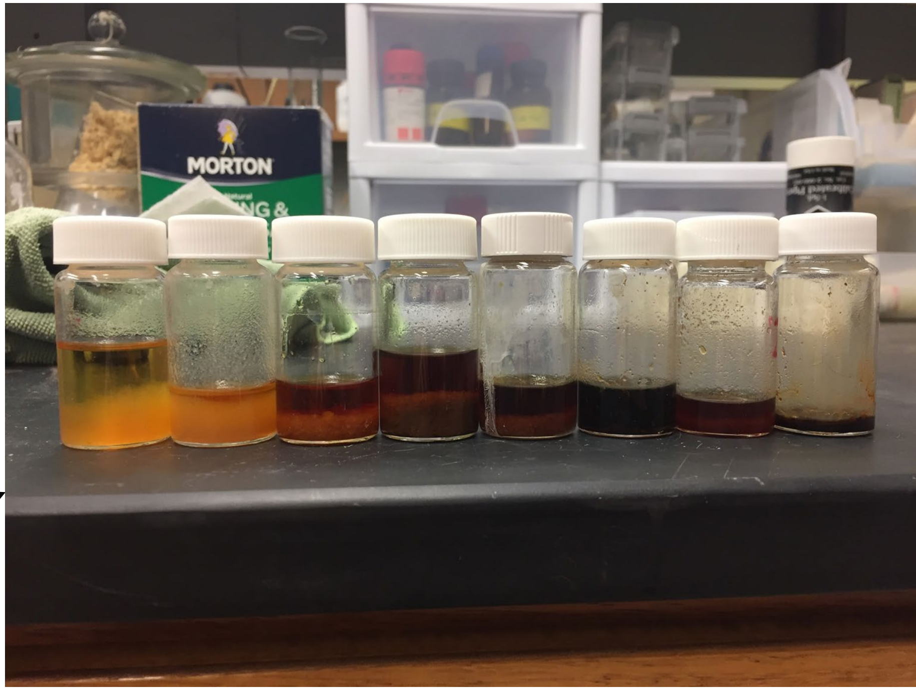
- ~~Pellets~~
- ~0.5 grams:0.5 grams ground dry sample:vegetable oil
- Purged twice with 20atm of oxygen, and analyzed with 25atm oxygen
- Stable starting and ending temperatures (~7 total minutes)

	Average Qcal (joules/gram)	**SD Qcal (joules/gram)**
Vegetable oil	43400	270
3-Fabius	14300	450
3-SX64	15600	540
3-Millbrook	17600	260
3-Fishcreek	15500	196

Chemical pretreatment and characterization

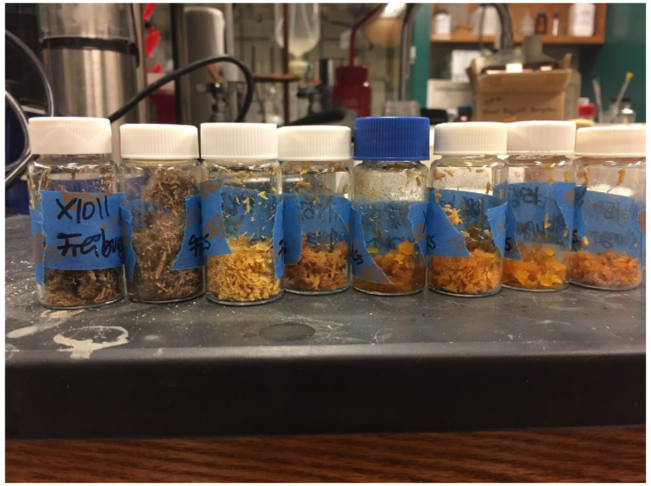


Soxhlet extraction



Steam distillation

Kraft pretreatment



Soxhlet procedure

- Extractions conducted in series

- 6.5 hour extraction period
  - 50-60 reflux cycles

- ~275 mL of extraction solvent
  - Hexanes, DCM, Acetone, and Ethanol

- % extraction determined by massing dried post-extraction sample relative to its pre-extraction mass

Soxhlet extraction

Sample	Acetone % extraction	Hexanes % extraction	Ethanol % extraction	DCM % extraction
old-1	10.46	3.87	12.31	1.99
new-1	——	3.55	11.05	1.69

SX64 has the greatest extractable concentration

Sample	Average % extraction with ethanol	SD
Old	12.31	
New	11.05	
Fishcreek	9.84	1.07
Fabius	11.22	0.90
Millbrook	10.72	0.70
SX64	12.42	0.40

- % extraction
  - Ethanol, DCM, Hexanes, Acetone
- UV-Vis
- Dried vs. undried

Organic extraction Braun's lignin

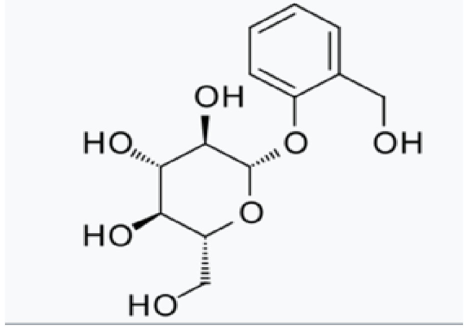
- Depolymerization of lignin during hot ethanol extraction [Braun, **1939**].

Resembles protolignin

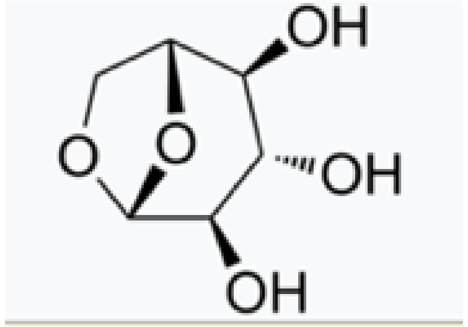
F. E. Brauns. Native Lignin I: Its isolation and methylation. *Journal of American Chemical Society*. **1939**. 61(8), 2120-2127.

- Precipitation

Soxhlet extraction GC-MS quantified compounds

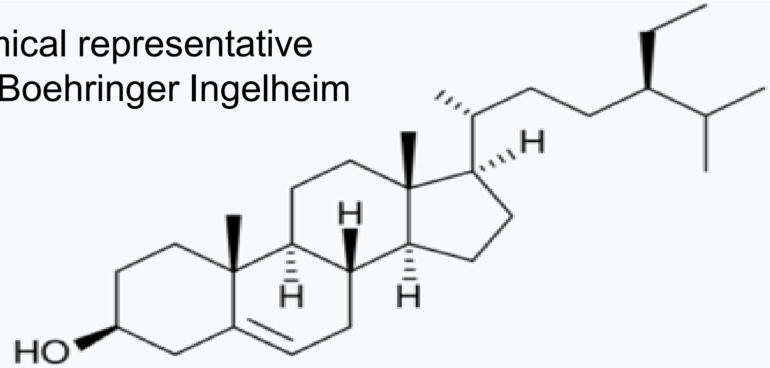


Salicin

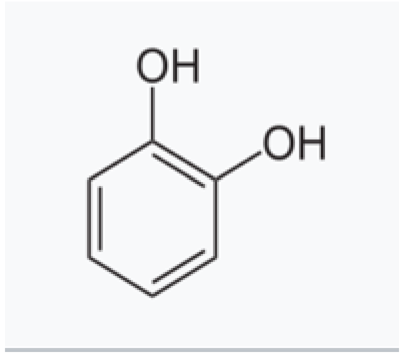


Levoglucosan

Chemical representative from Boehringer Ingelheim



Beta-Sitosterol

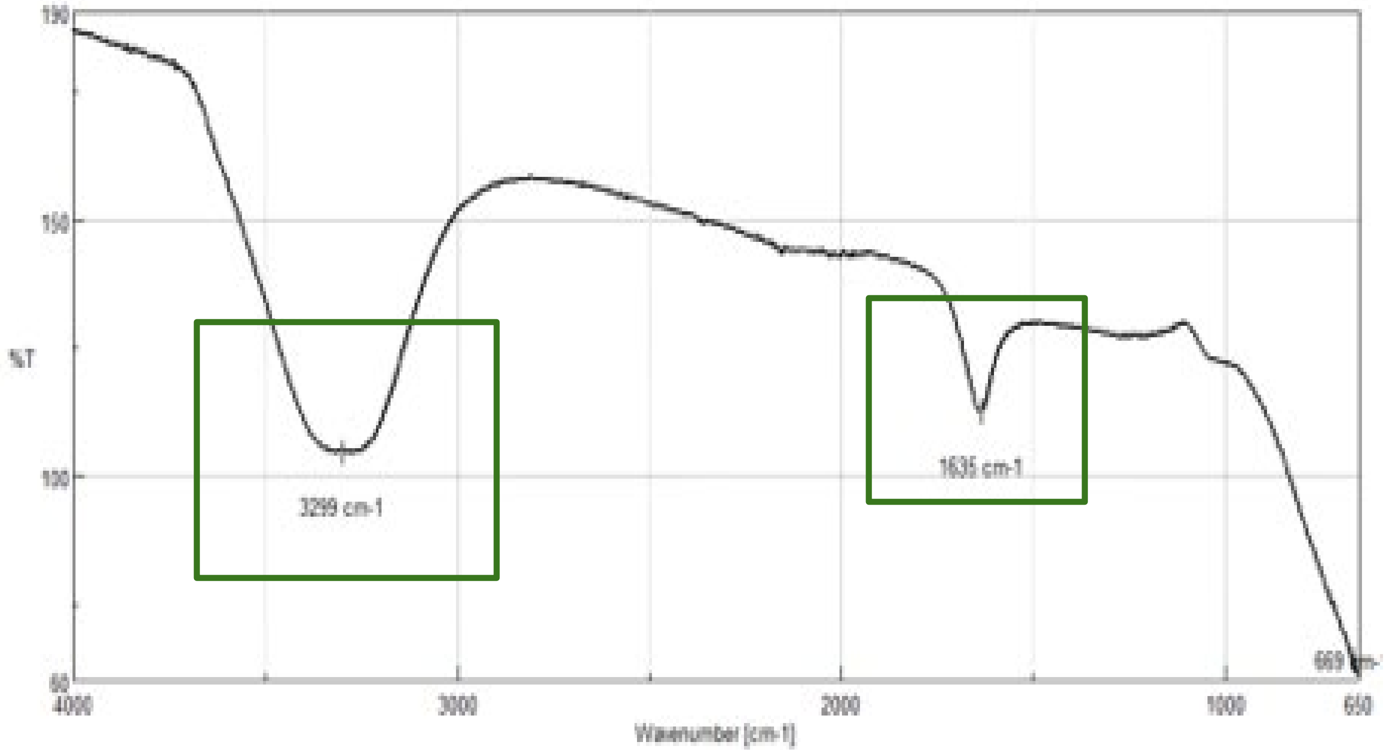


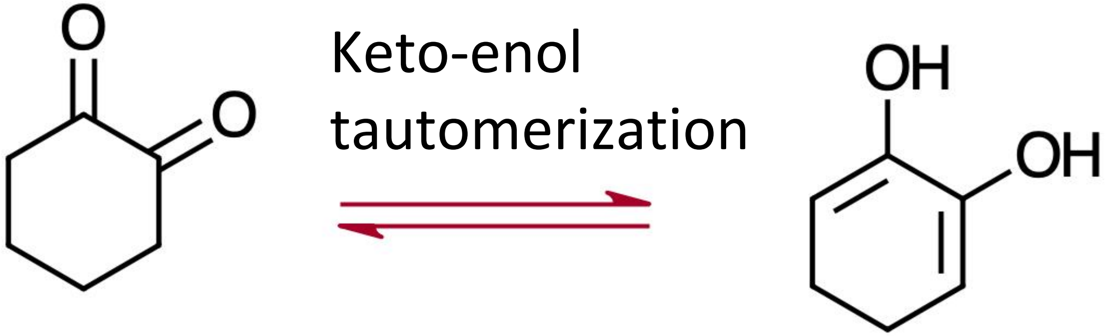
Catechol

Compound / Sample	Catechol	Salicylic alcohol	2-hydroxy-Aceto-phenone	Levoglucosan	(E)-Coniferol	Palmitic acid	Salicin	b-Sitosterol	a-Amyrin	Astaxanthin
SX-64	7.33	2.95	8.99	7.37	3.61		19.99	9.53		
Fish creek	2.74	1.85		7.15			**43.29**	10.2		2.46
Millbrook	4.83	2.75	3.11	5.19		**11.06**	27.17	7.87	3.28	3.02
Fabius	**22.26**			**13.15**	4.95	2.22	11.56	**13.24**	2.23	2.29

Steam distillation

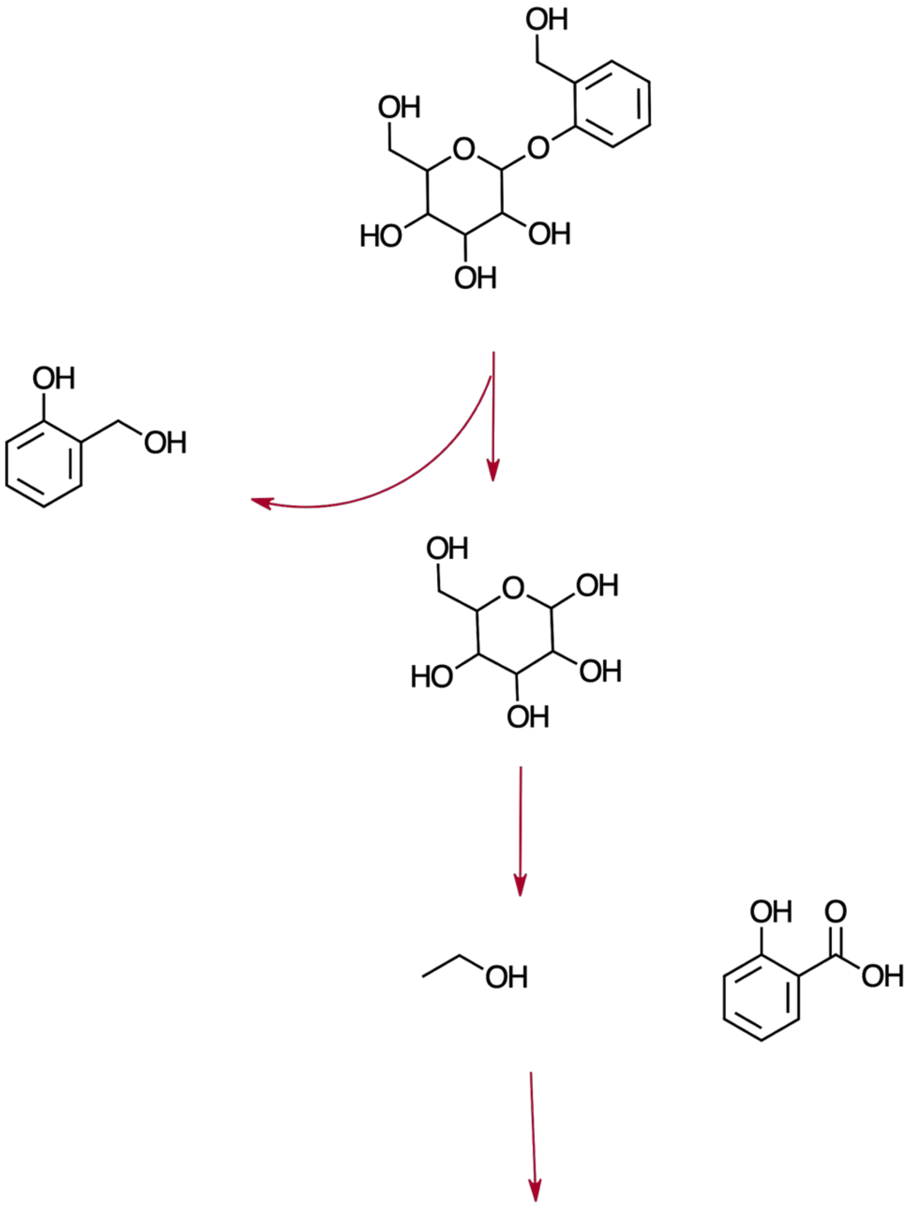
- Liquid-liquid extraction
  - Hexanes and ethyl acetate; only ethyl acetate produced identified compounds via GC-MS.





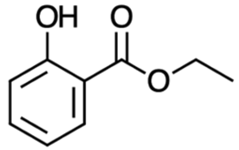
Fun times with fungis

Accidental creation of ethyl salicylate via fermentation of old steam distillate sludge



Microbiology professor, boiling resistant spores

Wintergreen agent

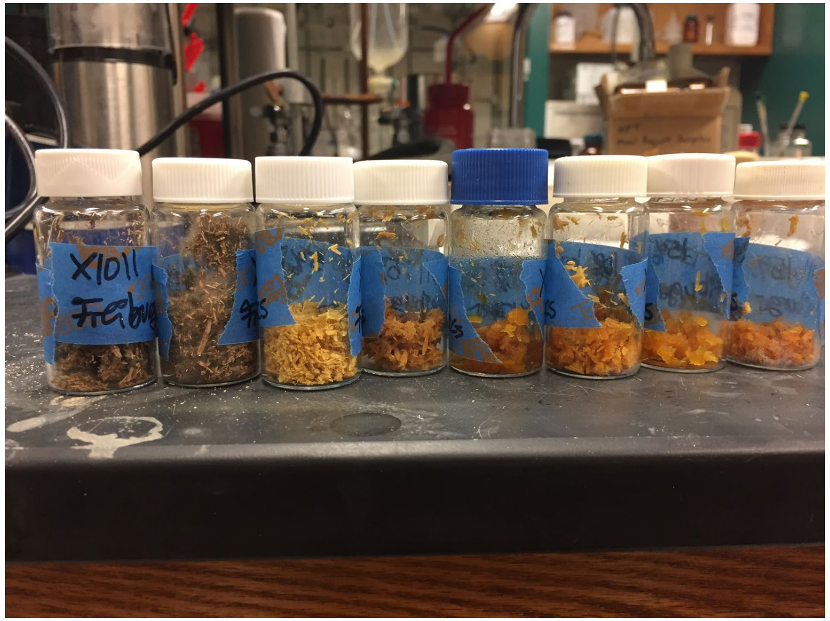


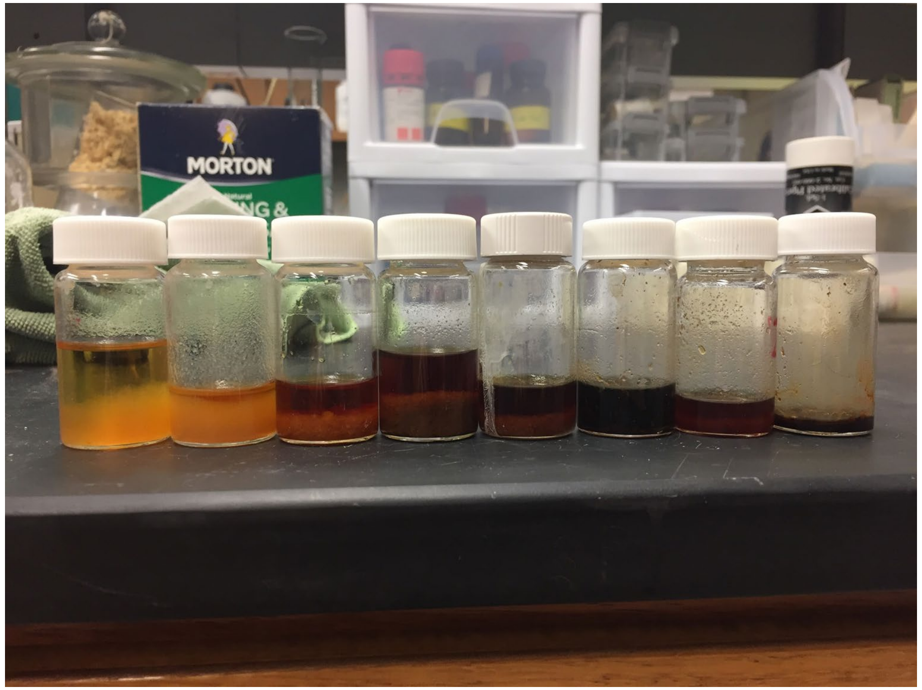
Kraft processing

- 0%, 5%, 10%, and 15% sodium hydroxide solutions

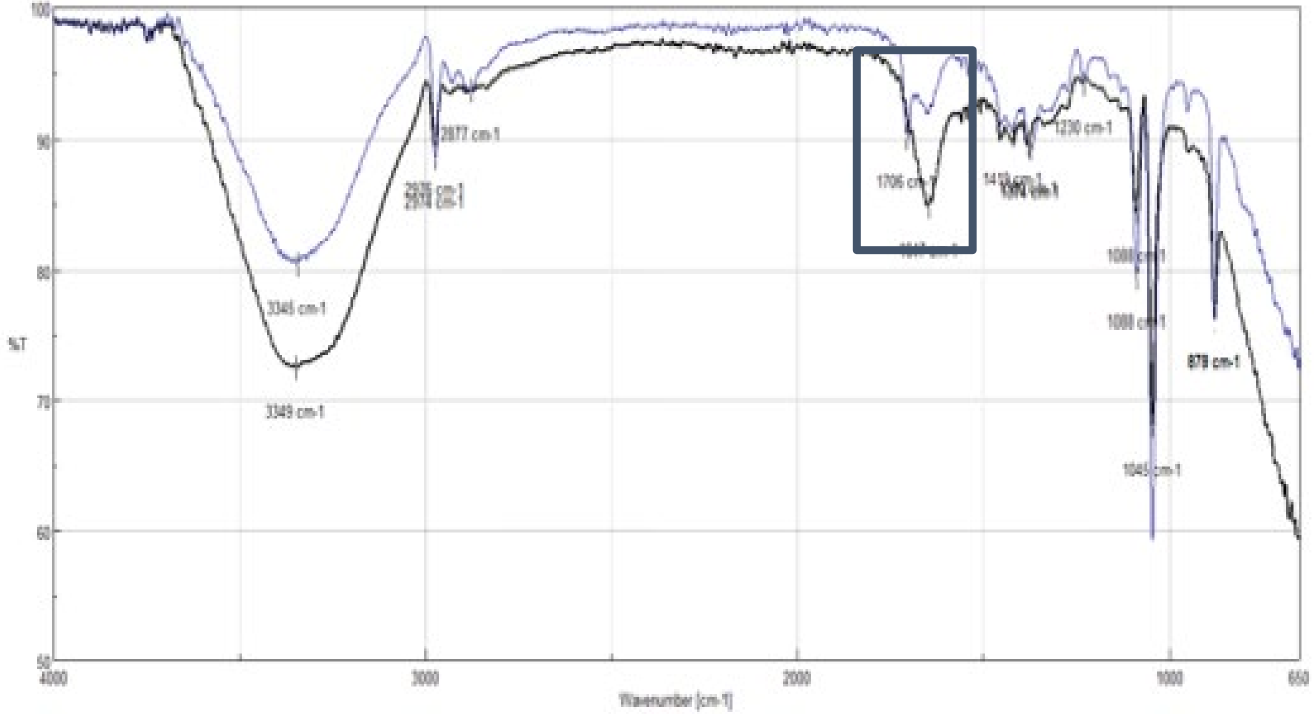
- Heated to boiling and applied to ~5g of sample while being stirred.
  - Half were heated below boiling for additional time and stirred while the other half were stirred at room temperature.

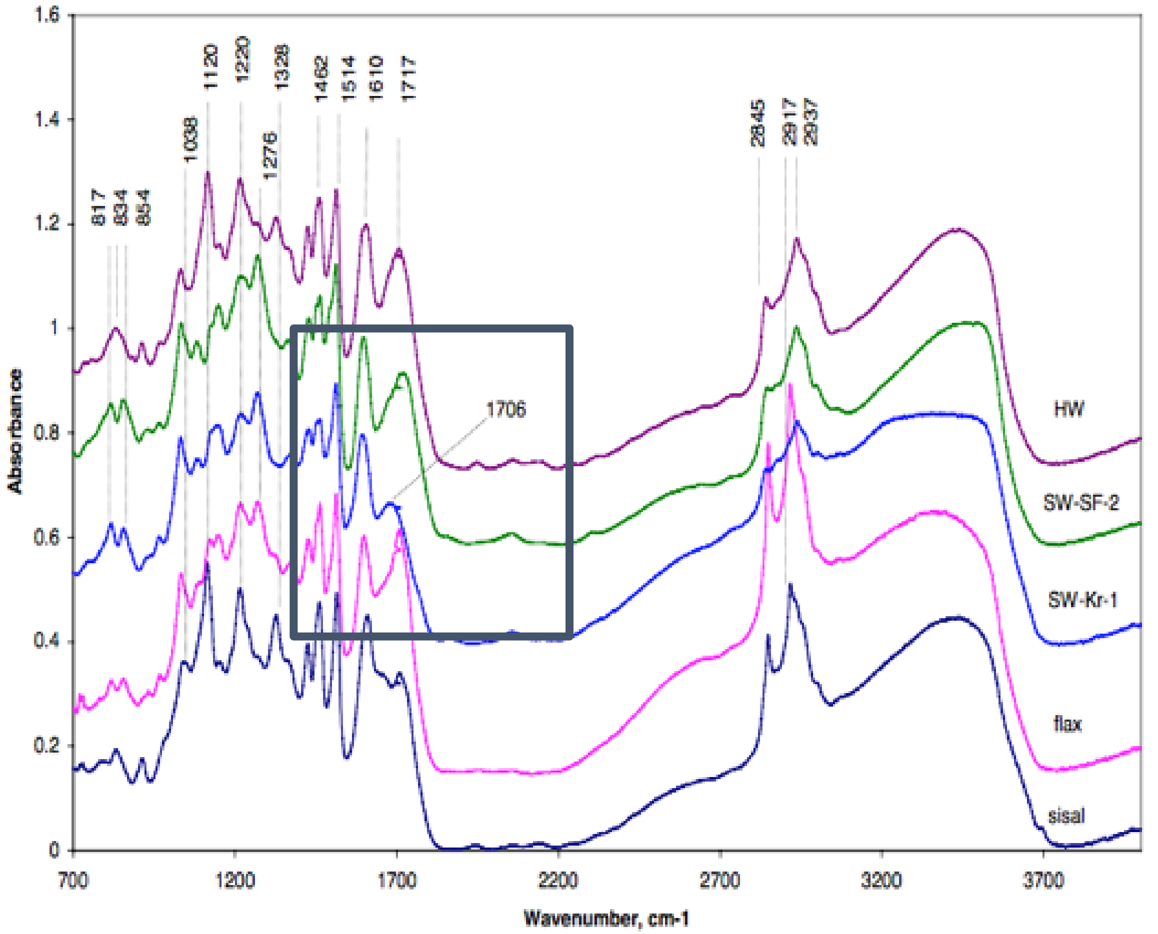
- The undigested wood was filtered from the digested wood (lignin and hemicelluloses) after a few days of continuous agitation.





Kraft depolymerization





- X906 10% sample
- Hot = top and RT = bottom.

- Literature source contains the same peak for kraft-treated wood (SW-Kr-1 = soft wood Kraft 1) [Boeriu et al., 2004].

Carmen G. Boeriu, Dominique Bravo, Richard J. A., Jan E. G. van Dam. Characterization of structure-dependent functional properties of lignin with infrared spectroscopy. *Industrial Crops and Products*, **2004.** 20, 205-218.

Conclusions

- Feasible undergraduate experimental procedures were devised

- Fishcreek appears to be preferable to other hybrids in terms of ash, moisture, and salicin content.
- SX64 possesses the greatest concentration of extractable content
- Millbrook appears to be preferable in terms of enthalpy of combustion

- Hot ≥10% alkaline solution are supported to delignify willow lignocellulose

- Air-drying sample can reduce % moisture without affecting % extractable

Willow biomass appears to be a promising alternative feedstock

The rest is still unwritten

Natasha bedingfield

- ICP-MS of ash
  - Bioremediator?
- Derivatization of high-value chemicals
- Depolymerization of cellulose
- Pyrolysis (Dave Prouty of Heat Transfer International)

Acknowledgements

- Dalila Kovacs
- Jim Krikke
- Erik Nordman
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- Laurie Witucki
- George McBane
- Diane Laughlin

Appendix

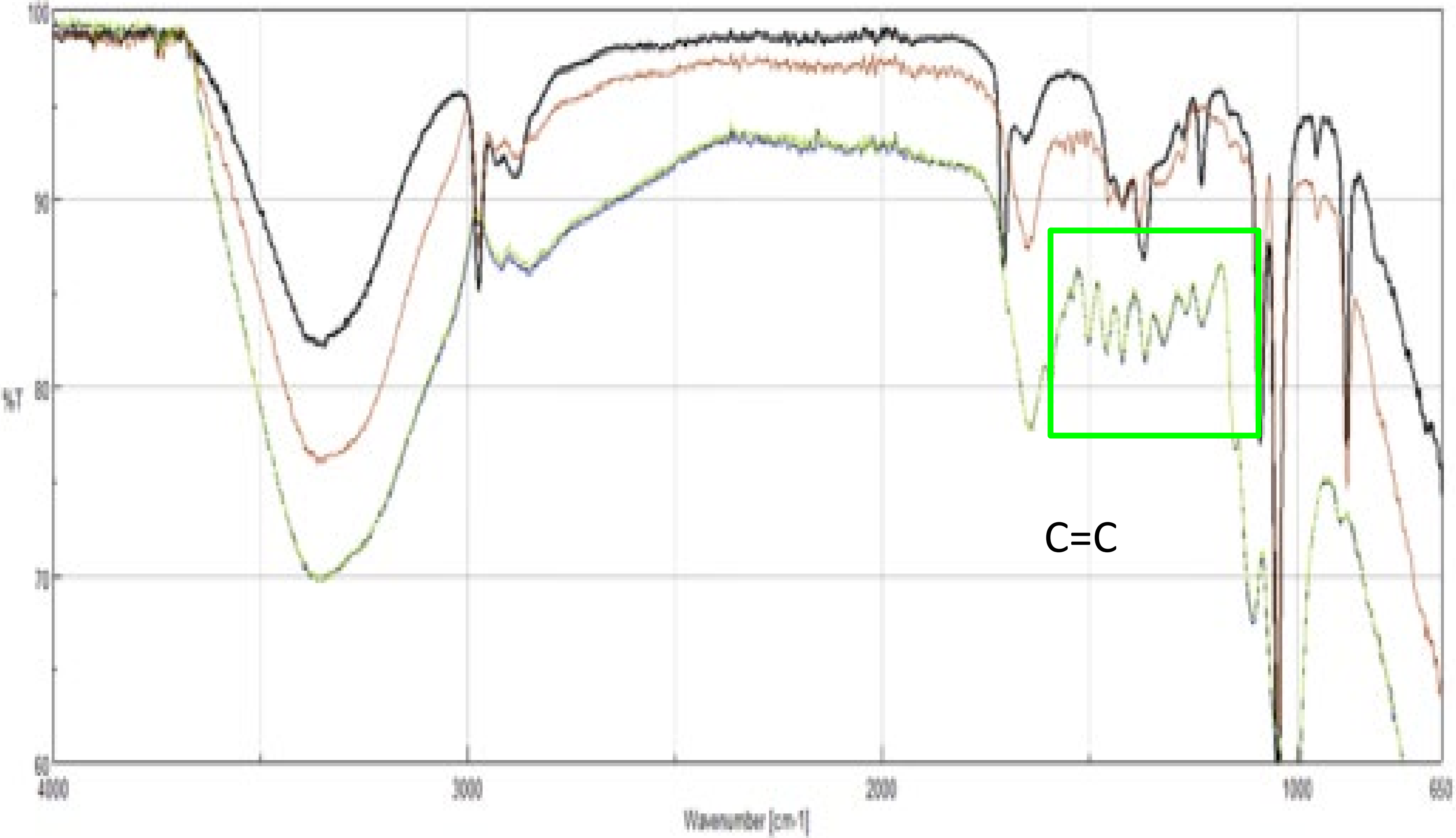
Why high value chemicals in lieu of biofuels?

By Professional Sun Clouds Blue Sky background stock photos, license under CC0 Public Domain

Single step collection of solar energy

By AleSPA, https://en.wikipedia.org/wiki/Solar_panel#/media/File:Photovoltaik_Dachanlage_Hannover_-_Schwarze_Heide_-_1_MW.jpg, is licensed under CC BY-SA

Kraft depolymerization



- X906 15%
- black = hot
- red = RT
- Green = RT solid
- Blue = hot solid

Lignin enzymes

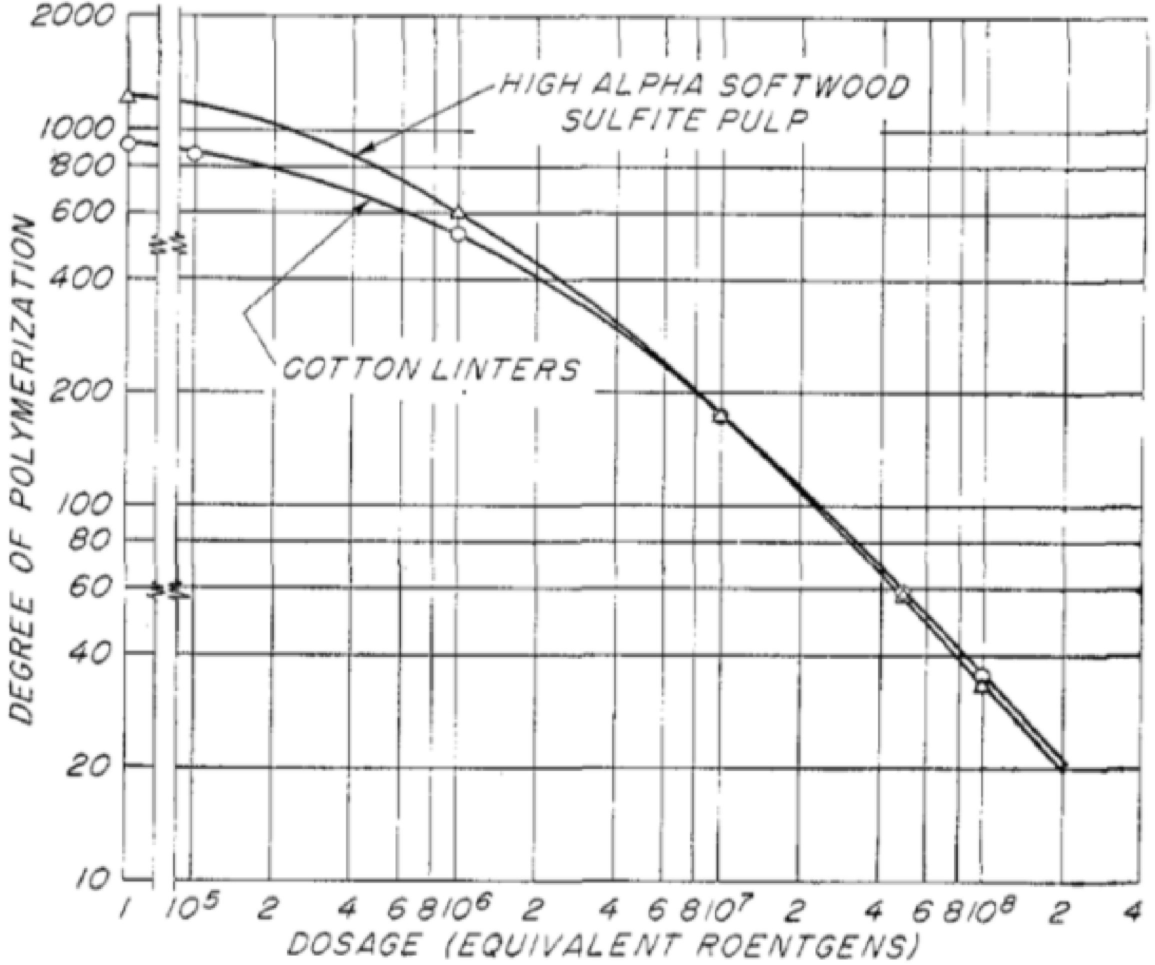
- Lignolytic microbes degrade via lignin peroxidases (heme peroxidase) [Martinez and Ruiz-Duenas, 2009]

Francisco J. Ruiz-Dueñas and Ángel T. Martínez. Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. *Microbial Biotechnology*, **2009**, 2(2), 164-177.

Cathode ray

- 800 kv peak voltage and 143,000 roentgens (1 R = 2.58E-4 C/kg) per second @ a distance of 10 cm.
- 5E8 roentgens produced water soluble derivatives of cellulose (~400 gray – chemo treatments are ~20-60 gray).

  - Wood pulp hydrolyzed quicker than cotton
  - >70% glucose yield after acid hydrolysis



Xyleco

Jerome F. Saeman, Merrill A. Millett, Elliott J. Lawton. Effect of high energy cathode rays on cellulose. *Industrial Engineering Chemistry*. **1952**, 44(12), 2848-2852.

Gasification

- Between combustion (100% oxygenating environment) and pyrolysis (0% oxygenating environment).
  - Water, volatiles (oil if cooled), and fixed carbon (i.e. coal).

- Thermally degrades
  - Distills separately hemicellulose, cellulose, and lignin at different temperatures

- Syn-gas, town gas, imbert cars in WWII

Bundesarchiv, Bild 183-V00670A
Foto: o.Ang. | 1946

https://www.lowtechmagazine.com/2010/01/wood-gas-cars.html

Enzymes

- Cellulases – Cellulose
- Lignin peroxidases – Lignin
- Optimum selectivity

- Inefficient because of high dilution
- Generally slow reactivity
- Costly to produce

Organic extraction

- Ideal for isolating metabolites
- Minor depolymerization of lignin – Braun's lignin [Braun, 1939] – and hemicellulose with hot alcohol extraction

F. E. Brauns. Native Lignin I: Its isolation and methylation. *Journal of American Chemical Society*. **1939**. 61(8), 2120-2127.

Deep Eutectic Solvents (DES)

- Ionic liquid (liquid salts at room temperature)

- ~90% pure lignin with >70% yields from woody biomass [Alvarez-Vasco, 2016].

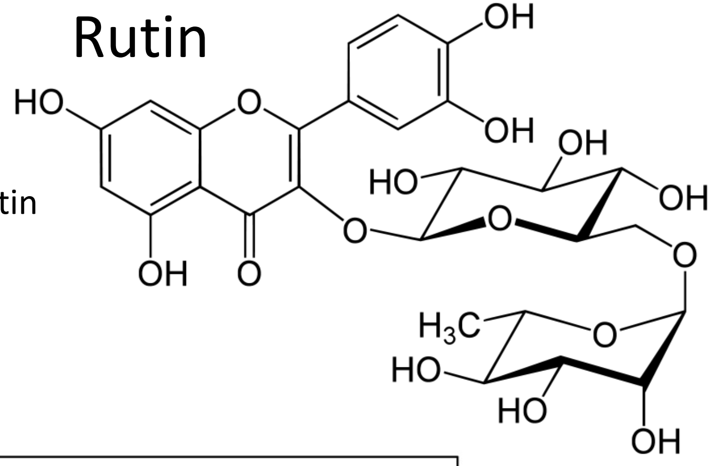
- DES lewis/bronsted acids and bases that create a eutectic system
  - Choline chloride (MP = 303°C) and Urea (MP = 134°C) 1:2 creates DES (MP = 12°C) [Smith et al., 2014].

Emma L. Smith, Andrew P. Abbott, and Karl S. Ryder. Deep Eutectic Solvents (DESs) and their applications. *Chemical Reviews*. **2014.** 114, 11060-11082.

Carlos Alvarez-Vasco; Ruoshui Ma; Melissa Quintero; Mond Guo; Scott Geleynse; Karthikeyan K. Ramasamy; Michael Wolcott; Xiao Zhang. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization. *Green Chem*, **2016**, 18, 5133-5144.

Natural Deep Eutectic Solvents

Flavonoid
Rutin

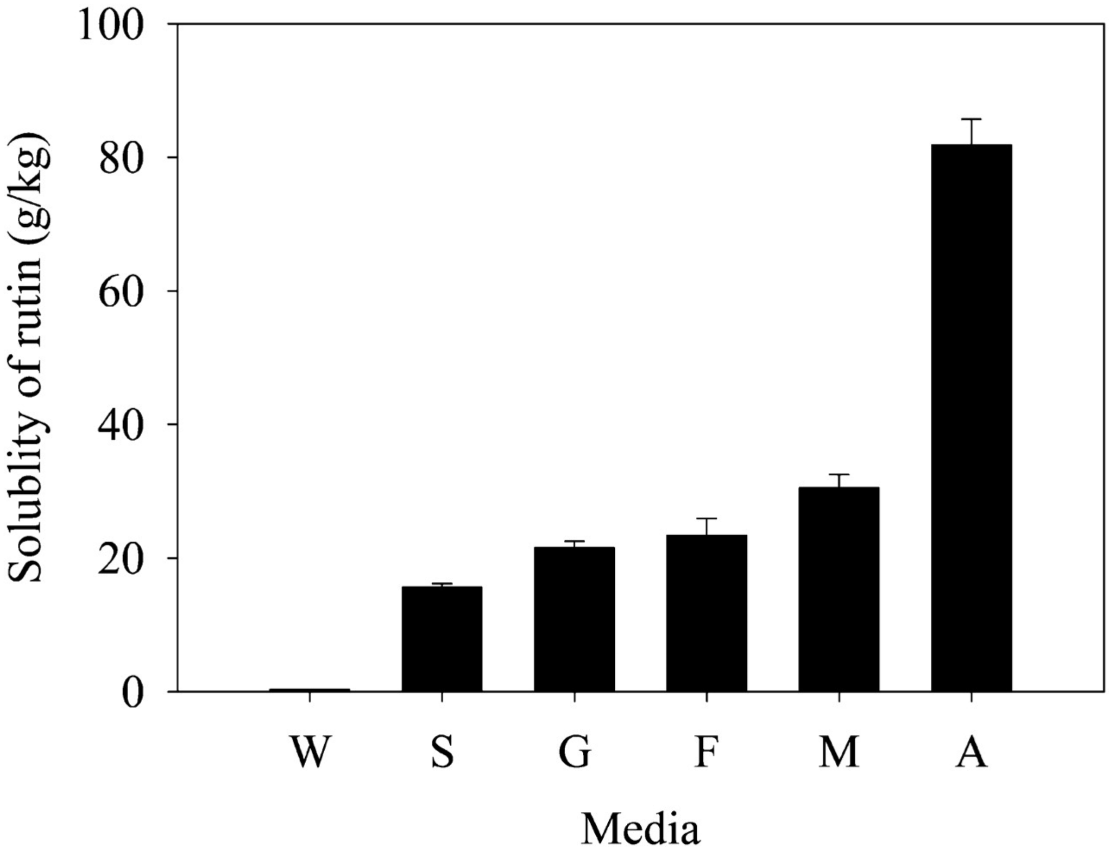


Yikrazuul,
https://en.wikipedia.org/wiki/Rutin

- Proposed third phase of life: water, lipids, and "natural" deep eutectic solvents [Choi et al., 2011].

W = water
S = Sucrose-choline chloride
G = Glucose-choline chloride
F = Fructose-choline chloride
M = Malic acid-choline chloride
A = Aconitic acid-choline chloride

[Choi et al., 2011]

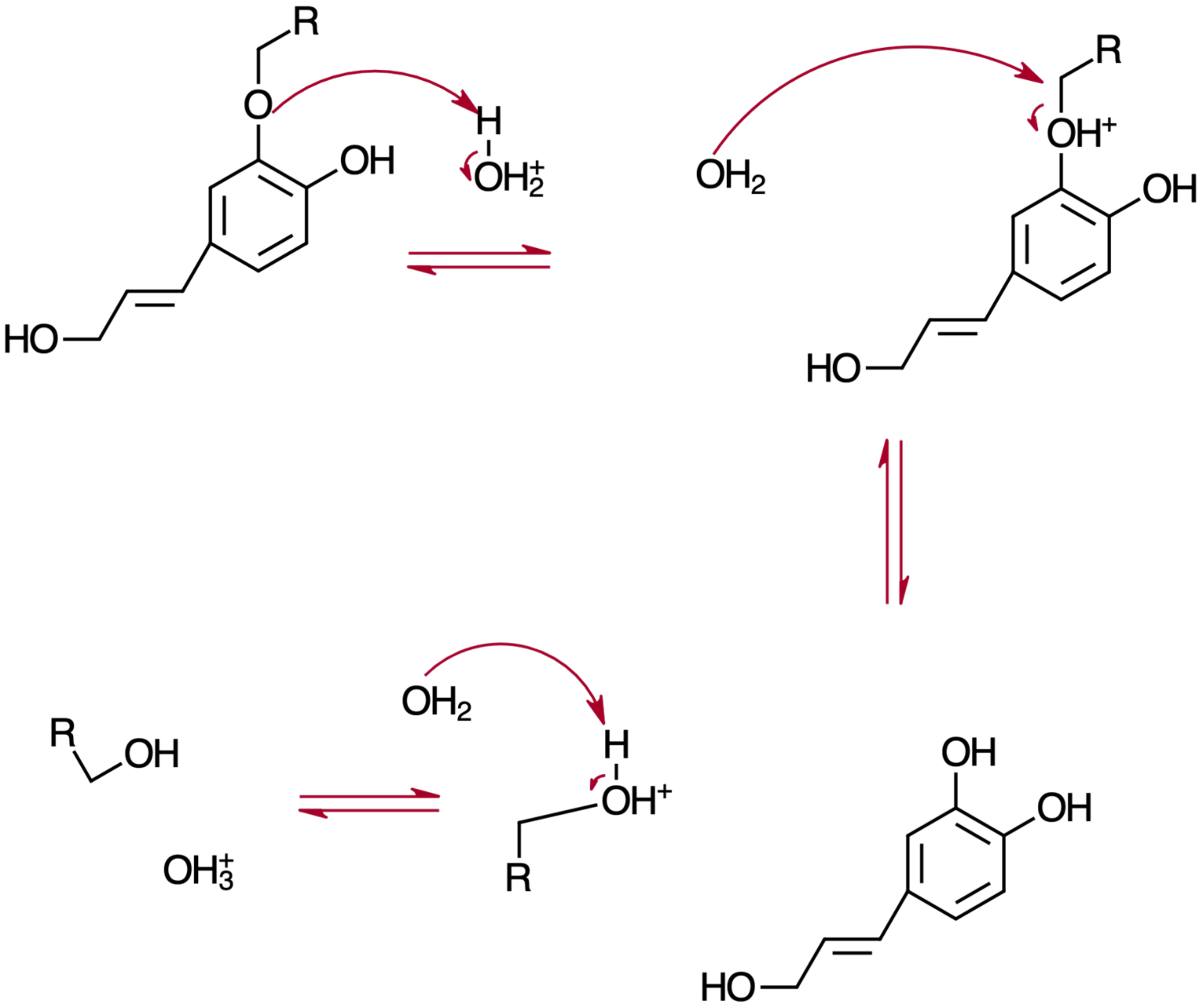


Young Hae Choi, Jaap van Spronsen, Yuntao Dai, Marianna Verberne, Frank Hollmann, Isabel W.C.E. Arends, Geert-Jan Witkamp, and Robert Verpoorte. Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology?.*Plant Physiology.***2011**. 156, 1701-1705.

Klason (acid) processing

- Common pulping method [Gottumukkala et al., 2016].
- Mechanism
  - Ether cleavage

corrosive to machinery!



Lalitha Devi Gottumukkala, Kate Haigh, François-Xavier Collard, Eugéne van Rensburg, Johann Görgens. Opportunities and prospects of biorefinery-based valorization of pulp and paper sludge. *Bioresource Technology*. **2016**, 215, 37-49.

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Liska, Adam J.; Haishun S. Yang; Birgil R. Bremer; Terry J. Klopfenstein; Daniel T. Walters; Galen E. Erickson; Kenneth G. Cassman. Improvements in life cycle energy efficiency and greenhouse gas emmisions of corn-ethanol. **2009**. *Journal of Industrial Ecology*. 13(1), 58-74.

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