Biofuels: Ethanol for The Future

BioProcess
Center at
Gateway Park

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Biofuels: Issues and Roadblocks

Change Brings Opportunity

- Biomass Source - Choose based upon region, soil, and impacts
- Production Efficiency and Yield/Acre
- Market Impact and Synergistic Effects
- Energy Output - Relative to fossil fuels and direct pyrolysis
- Carbon Balance - A move towards neutrality
- Politics: We need a consistent approach, independent of politics

Why Use Ethanol?

- Ethanol is a relatively simple product to make.
- The technology for large scale production is already available.
- Blending with gasoline increases oxygenation and boosts octane rating.
- Can be used directly in fuel cells.
- Enables independence from fossil fuel.

Relevant Equations: A Review

Glucose is created in the plant by photosynthesis.

$CO_2 + 6H_2O$ + light = $C_6H_{12}O_6 + 6O_2$

During fermentation, glucose is converted to ethanol and carbon dioxide

$C_6H_{12}O_6 + 3O_2 = 2C_2H_6O + 2CO_2$ + heat

And finally during combustion, ethanol and oxygen react to produce heat, CO2 and water

$2C_2H_6O + 6O_2$ = heat + $4CO_2 + 6H_2O$

The sum of these equations (complete reaction) results in no net change in CO2 or oxygen

light = heat

- Ethanol yield is critical to overall cost of production and economic viability of the process.

Where to get the biomass?

- Initial development used food-starch crops since the processing was easiest (corn in the US and sugar cane in Brazil, eg.)
- In the US, everyone looked to corn:
  - *largest crop produced, native species*
  - *always a surplus - farmers saw a way of getting better prices for product*
  - *harvesting and processing infrastructure already present; corn is a fungible commodity*
- Most of the sugar in corn is in the form of starch.

Yield Efficiency: Strike one for corn-starch

Ethanol from fermentable sugars

Sugar Source	Gallons Ethanol/Acre
corn [1]	320 - 420 (‘07)
sugar cane [2]	420 (‘05)
sugar cane [3]	720 - 870 (‘07)
sugar beets [4]	750

There is not enough cropland available to use corn-starch ethanol as the solution to our energy problems in and of itself.

1 Goettemoeller, Jeffrey; Adrian Goettemoeller (2007), *Sustainable Ethanol: Biofuels, Biorefineries, Cellulosic Biomass, Flex-Fuel Vehicles, and Sustainable Farming for Energy Independence*, Praire Oak Publishing, Maryville, Missouri, pp. 42
2 da Rosa, A, Fundamentals of Renewable Energy Processes, 2005, Elsevier, ISBN-13: 978-0-12-088510-7, page 13.8-9
3 Daniel Budny and Paulo Sotero, editor (2007-04). "Brazil Institute Special Report: The Global Dynamics of Biofuels" (PDF). Brazil Institute of the Woodrow Wilson Center. Retrieved on 2008-05-03.
4 Salassi, Michael E., Louisianna Agricultuere Magazine, 2007. “Economic Feasibility of Ethanol Production from Sugar Crops”, http://www.lsuagcenter.com/agmag/archive/2007/winter/the+economic+feasibility+of+ethanol+production+from+sugar+crops.htm

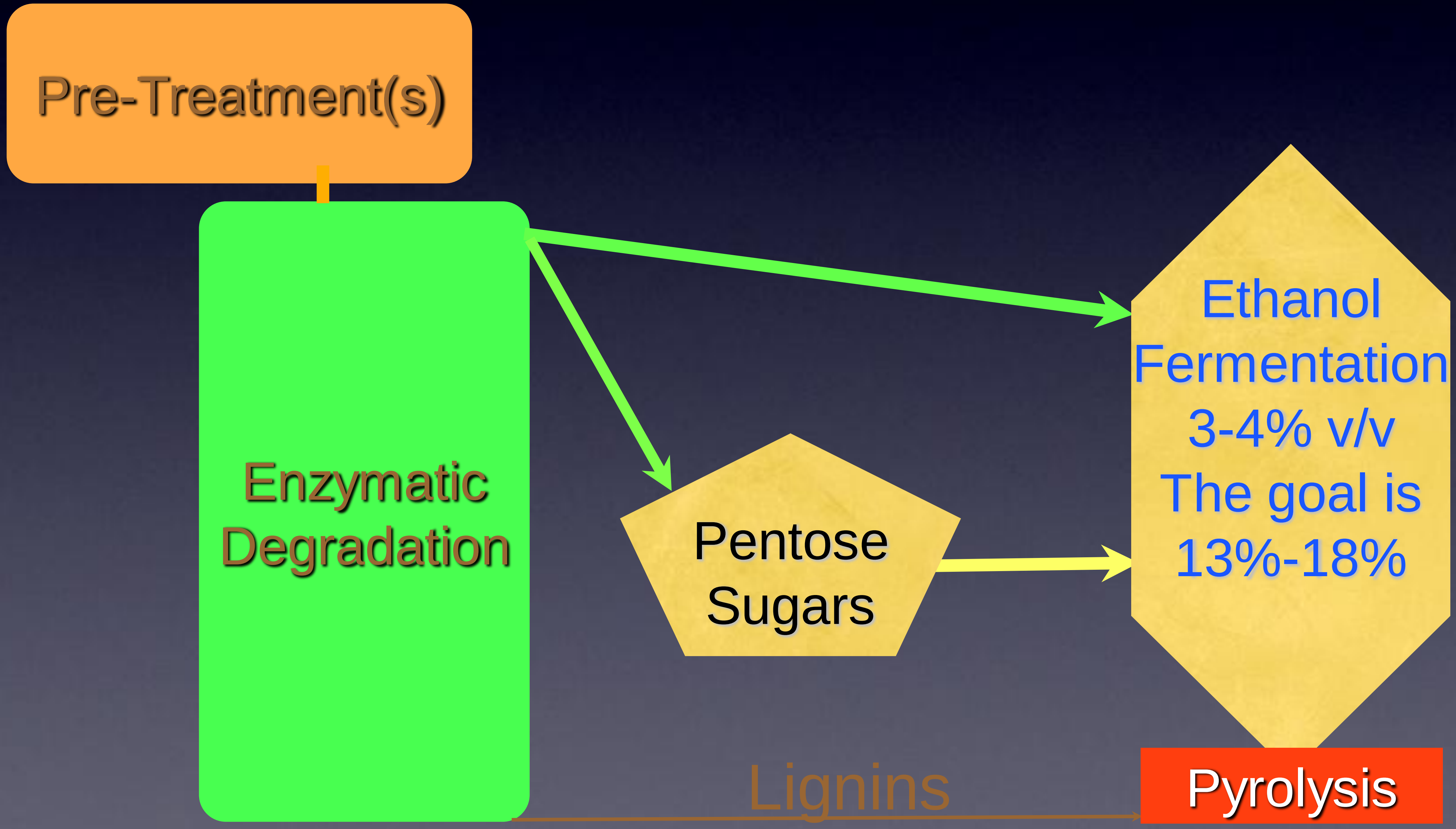
Potential Advantages of Cellulosic Ethanol

- Low impact on food crops and agricultural land.
- Higher yields and greater efficiency than corn.
- Independence from foreign supplies of energy.
- Utilization of biomass that otherwise goes unused.
- Modern selective breeding and crop improvement techniques can be applied for higher quality feedstocks.

Some Candidate Cellulosic Crops:

- Poplar - particularly reduced lignin hybrids
- Sugar Cane - current yield efficiency leader
- Switchgrass - low impact and maintenance
- Miscanthus - perennial grass (considered in Europe)
- Corn - use of entire plant is the goal
- Bagasse - remains after sugar cane processing
- Practically ANY crop residue could be used.

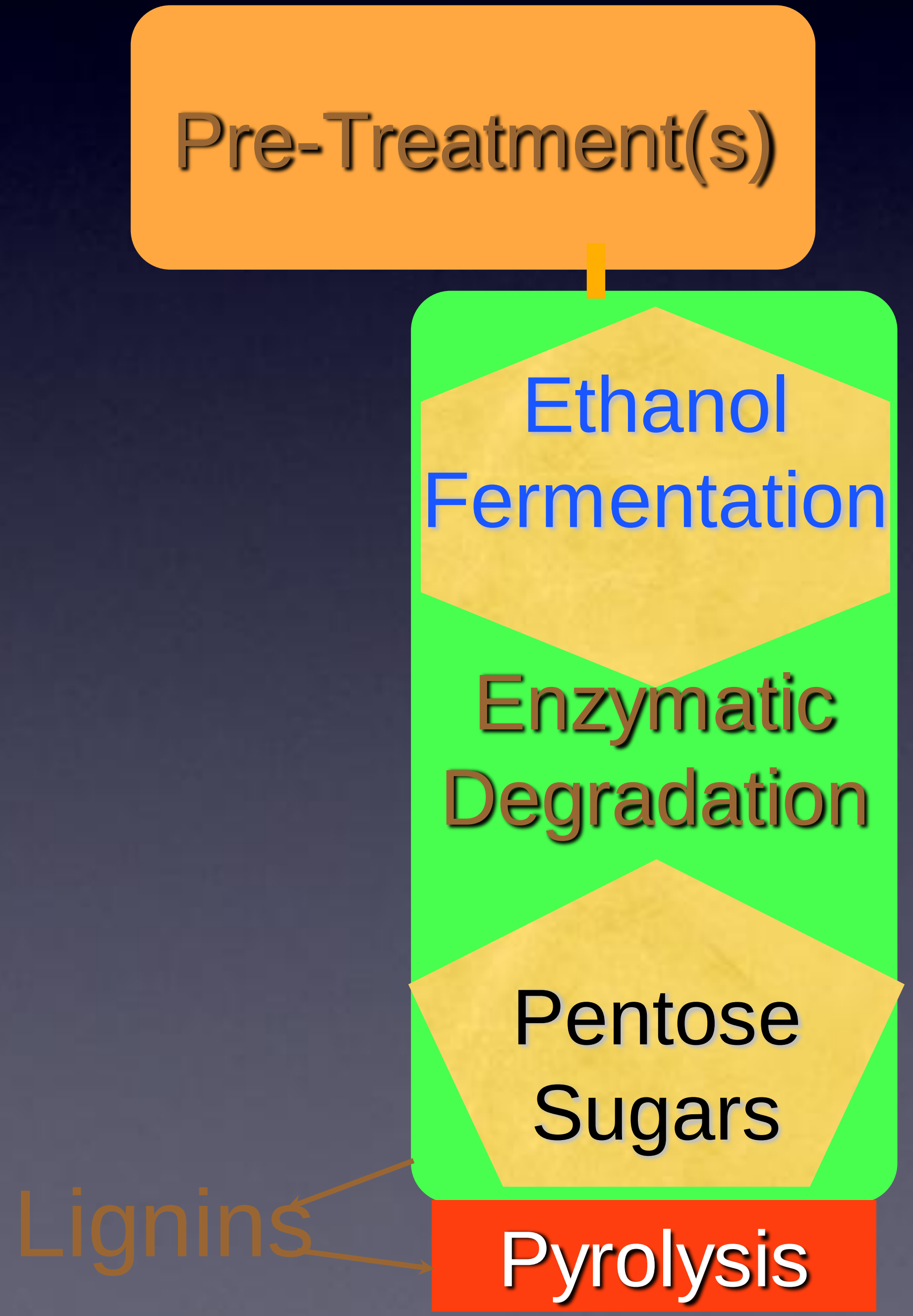
Typical Two-Step Process



Development Issues:

- Complete breakdown of ligno/cellulose matrix.
- Added costs of multiple enzyme “cocktails”.
- Typical two-stage system is time consuming and costly.
- Biomass source(s) with minimal environmental impact.
- The solution may be to choose the biomass based on soil, climate, etc.
- There is no silver bullet!

Idealized One-Step Process



Enzymatic Digestion and Ethanol Fermentation occur simultaneously in the same BioReactor!

Biomining/ Strain Improvement

- Isolate, identify and screen organisms from the environment with cellulolytic potential.
- Characterize activity of enzymes involved.
- Attempt to improve enzymatic output and enhance function through random mutation and screening.
- Clone out improved versions of enzymes or carry the organisms into process development.

Process Development

- In some cases the organisms show potential for use in a bioreactor for enzyme production.
- Starting with positive selectees on solid media screening, we take the organisms through shake-flask culturing.
- The IUPAC standard DNS(Di-Nitro-Salicylic) assay is used to determine amount of sugar produced from filter paper strips(6 cm x 1 cm) and other substrates.
- We also use a Glucose OxiDase assay.
- The best performers after these hoops go to 5-10L scale-up with analysis of growth parameters.

Hybrid Poplar

- Can grow to 90 feet in 6 years on fallow farm land

Purdue university Poplar testing facility

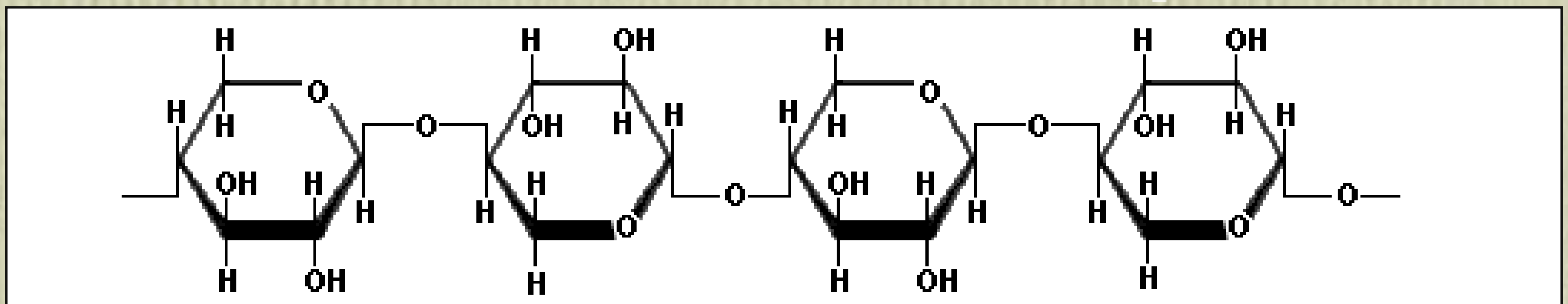
Switchgrass

University of Tennessee: http://www.agriculture.utk.edu/news/images/BrownMilanSwitchgrass.jpg

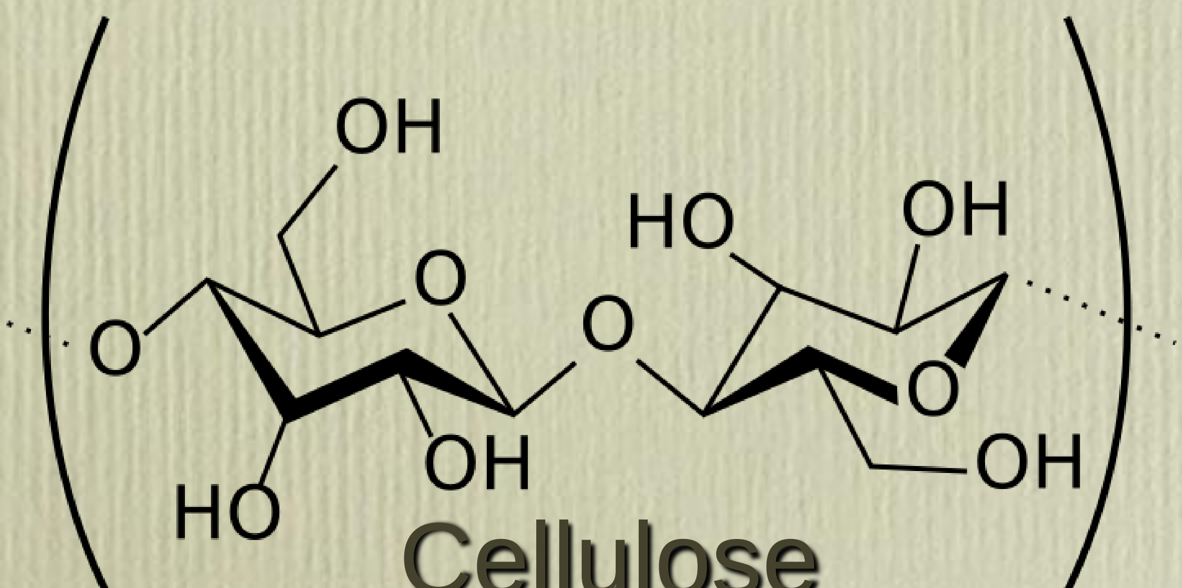
Corn Stover

http://tinyfarmblog.com/wp-content/uploads/2007/08/sum2007_earlivee_corn.jpg

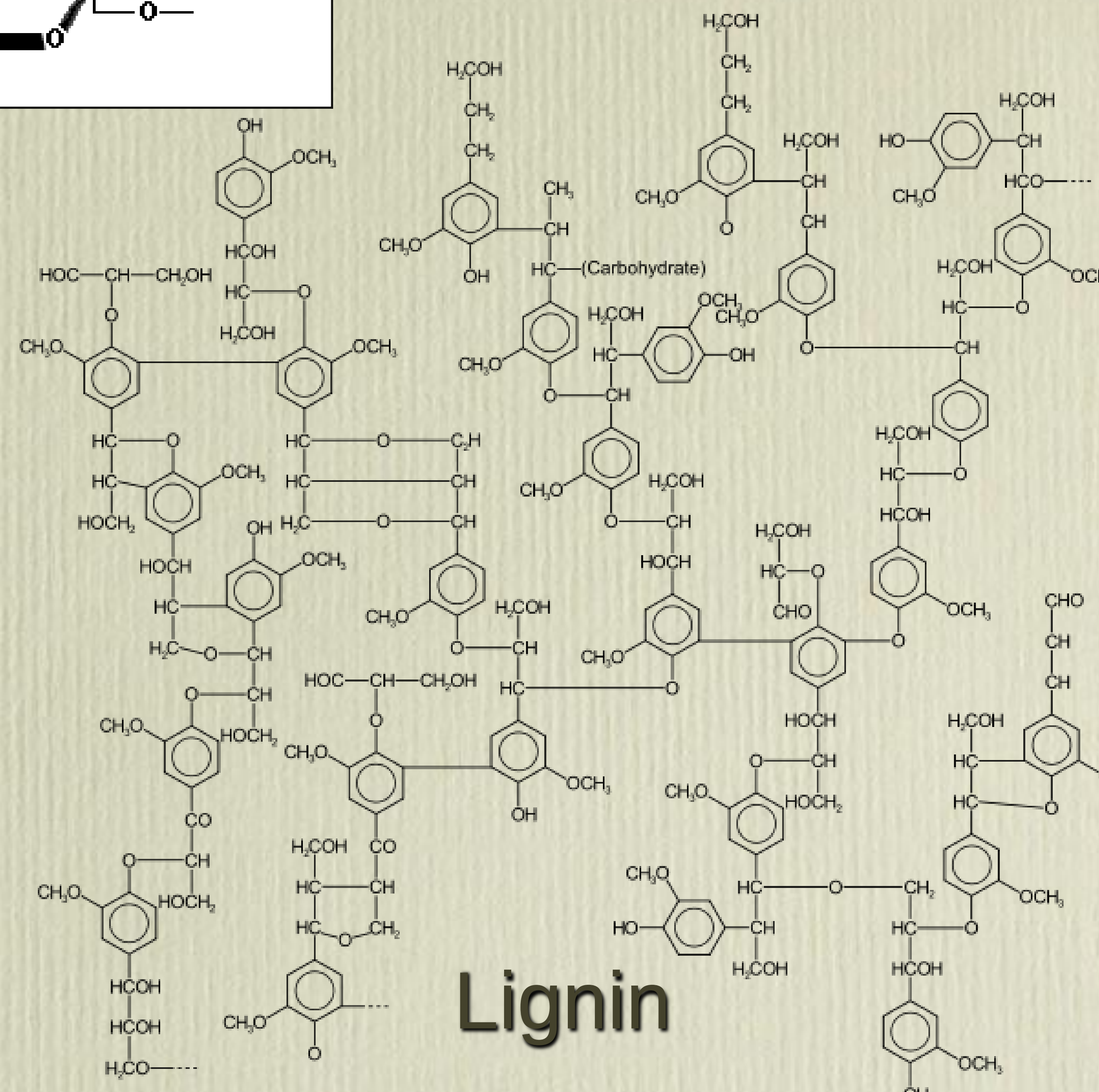
Plant Material: Polysaccharide Composition



Xylan (hemi-cellulose)



Cellulose



Lignin

Pre-treatments used at BP the Center:

- Mechanical Disruption
- Phosphoric Acid
- Flush to Neutrality (pH~7)
- Alkaline Treatment(Sodium Bicarbonate) at 25º C

Other Pre-treatments (not used at BP center)

- Windhexe
- Steam Explosion
- Ammonia Fiber Explosion (AMFE)
- Dilute Acid Treatment
- Organic Solvents

Biomining

- Anywhere vegetation is degrading is a good place to start

Nature’s Slow Degradation Process...

- Many organisms use wood for energy.
- What about termites?

They get their energy from cellulose with help. help

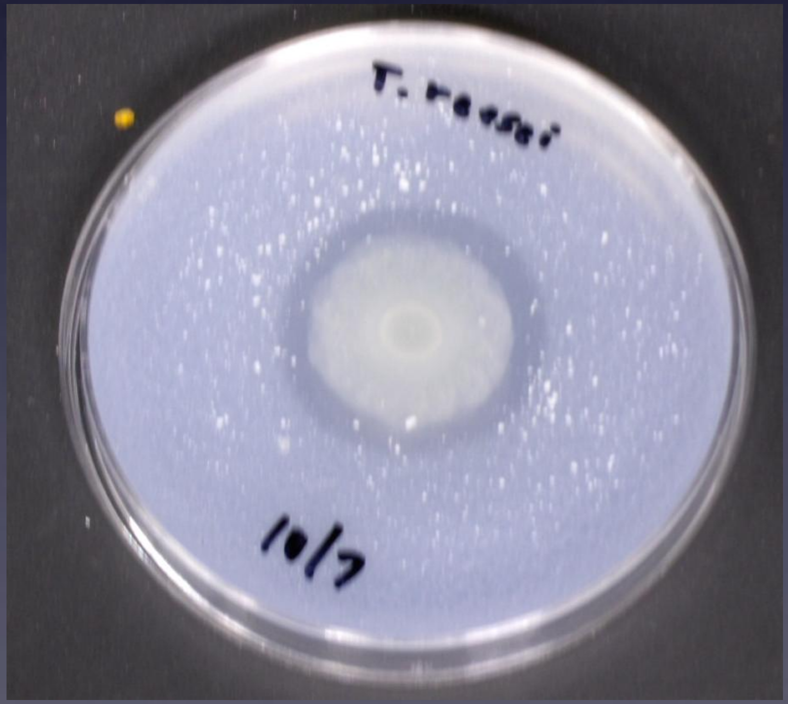
http://termiguardplan.com/db5/00466/termiguardplan.com/_uimages/bigstockphoto_Termites_1432393.jpg

Termites: cellulose degrading machines

- Primitive species have proven to be one source of cellulolytic micro-organisms
- We dissected gut tract and culture for enzyme producing microbes (pH, temp, nutrients)
- After squashing many termites, BP Center is currently working with several termite microbes with proven potential for development.

Screening for Enhanced Cellulase

- *T. reesei* (ATCC 26921) is a naturally occurring fungus which has been successfully mutated to over-express cellulases
- BF-85 is of a fungal genus which usually exhibits low cellulase production
- Acid washed solka-floc (cellulose) is used in the plates to form a haze which when digested by cellulase leaves a zone of clearing



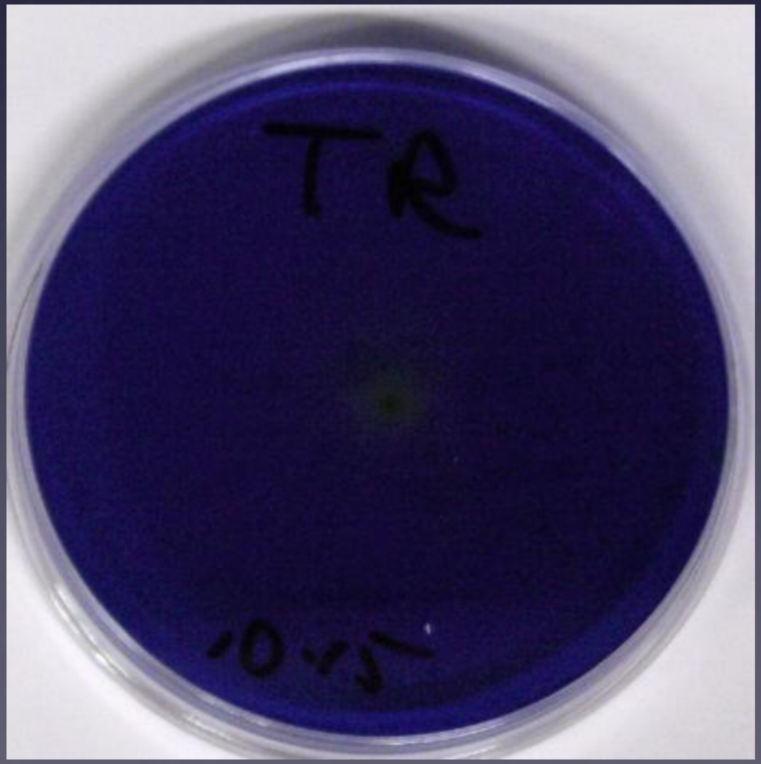
T. reesei
ATCC 26921

BF-85 parent

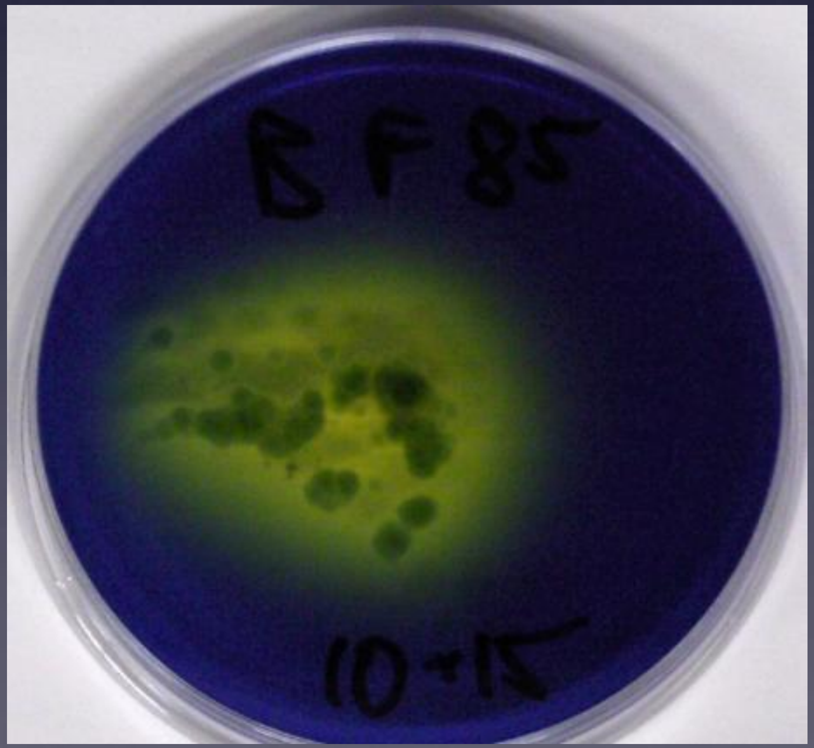
BF-85 UV mutant

Screening for Enhanced β-glucosidase

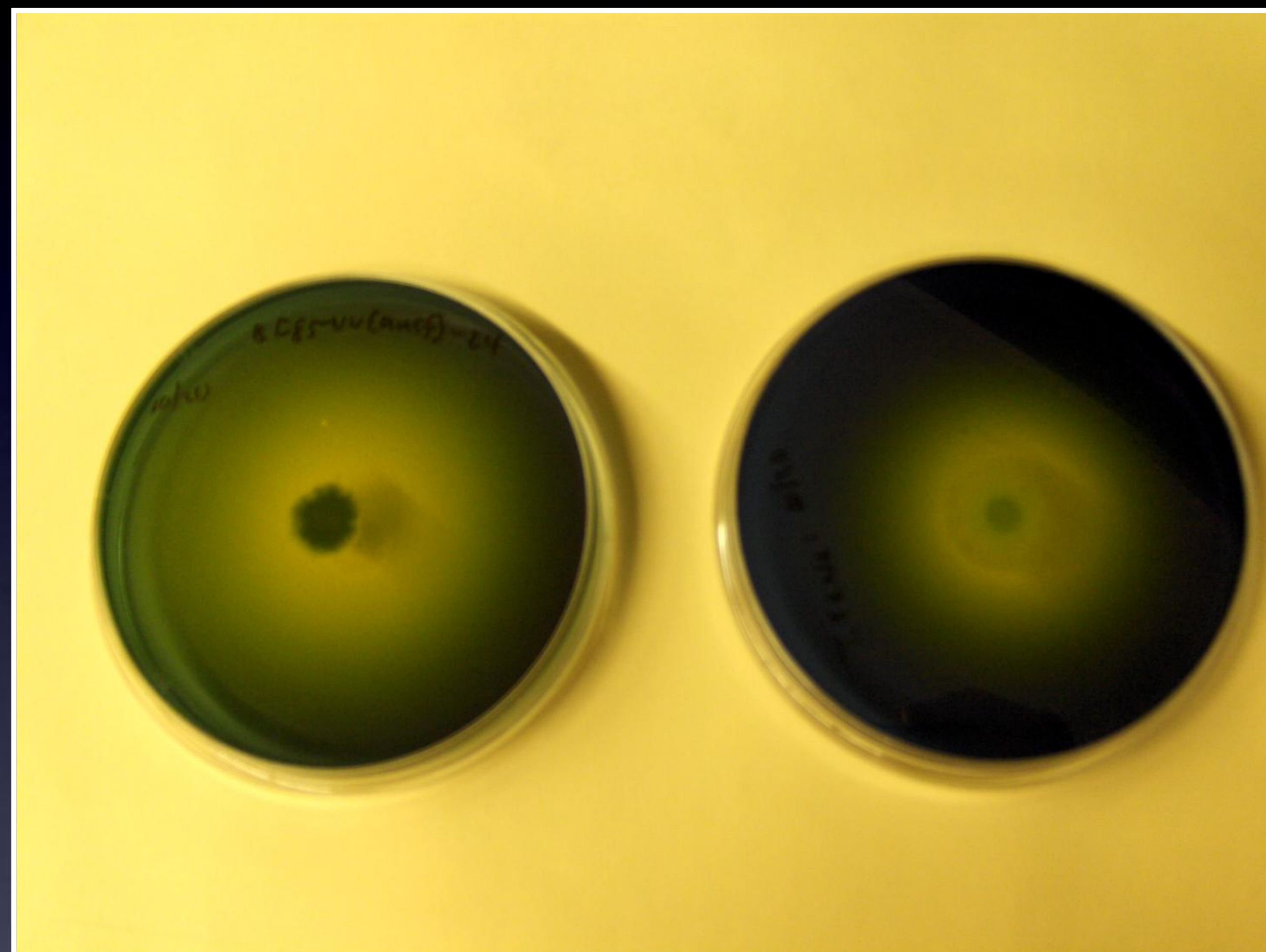
- Cellobiose/cellotetrose(CB) are the breakdown products after exo/endo-cellulase degradation.
- CB must be broken down to liberate glucose and has a competitive inhibitory effect on the cellulases.
- CB degradation can be indirectly detected by bromphenol blue. DCPIP is used for direct detection of β-glucosidase activity (photo not shown)



T. reesei *ATCC 26921*



BF-85 parent



After a few more days.

Screening for Enhanced Producers of Other Enzymes

- In addition to endo/exo-cellulases and cellobiose enzymes(CBH, CBL) rapid screening techniques are available and have been developed for other enzymes involved in degradation of plant material.
- Xylanases are enzymes capable of degrading hemi-cellulose into free xylose (pentose sugar).
- Rapid screening procedures are critical for successful mutation and strain enhancement strategies.

Induction Agents

- Sophorose is recognized as the best induction agent for cellulases, though it is very expensive.
- Substrates hydrolyze spontaneously to form small amounts of arabinose, galactose, and other free sugars.
- We are using small amounts of target substrates to induce the system towards enzyme production of these subtrates.

Efficiency of Cellulosic Degradation

- A typical “cocktail” uses varying enzyme proportions (endo- and exo-gluconase and β-glucosidase) to accommodate each cellulosic source.

Source	Cellulose	Hemicellulose	Lignin	Protein
Solka-Floc[1]	88.6%	7.7%	2.5%	---
DG[2]	16%	8%	4%	30%
Switchgrass[3]	37%	29%	19%	12%

Reducing Sugars Evolved After Enzyme Treatment (mg/ml)

Source	Solka-Floc	DG	Switchgrass
T. reesei ATCC 26921	5.6	0.96	0.16*
BF-85 Parent	4.4	1.4	0.66*

1 Finkelstein, M., 2002. 23rd Symposium on Biotechnology for Fuels and Chemicals, Appl. Biochem. and Biotech, vols 98-100, pp. 514 -515.
2 Analysis of DG sample from Edeniq by Western Milling, Inc.
3 South Dakota State University http://ncsungrant.sdstate.org/uploads/publications/SGINC1-07.pdf

* shake flask sample vs all other samples which were taken from fermentation supernatants

Shake Flask Verification

FPU assay glucose evolution

Sample	Abs 540	Glucose evolved
T. Reesei	0.507	2.21 mg/ml
BF5447UV(awsf)-8	0.332	1.45 mg/ml
BF85	0.292	1.27 mg/ml
BF85UV(awsf)-24	0.396	1.72 mg/ml

Glucose oxidase assay (for cellobiase)

Sample	Abs 540
T. Reesei	0.352
ATCC 52007	1.282
ATCC 13156	1.055
ATCC 13157	1.255
ATCC 15556	1.046
XDM7	**1.335**
BF85	**1.257**

Summary and Conclusions

- Biofuels have made significant progress in the last decade
- Corn-starch ethanol, the first large scale biofuel in the US is insufficient to handle our energy needs
- Cellulosic biomass appears to have advantages for the fuel ethanol market
- The choice of crop: probably will be specific to a given region
- Technology advances in crop yields, enzymatic digestion and overall production are necessary for an economically feasible process
- No alternate energy strategy will succeed without a healthy dose of conservation!

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