Spring 6-13-2013

Producing hydrocarbon drop-in biofuelsfrom cellulosic materials

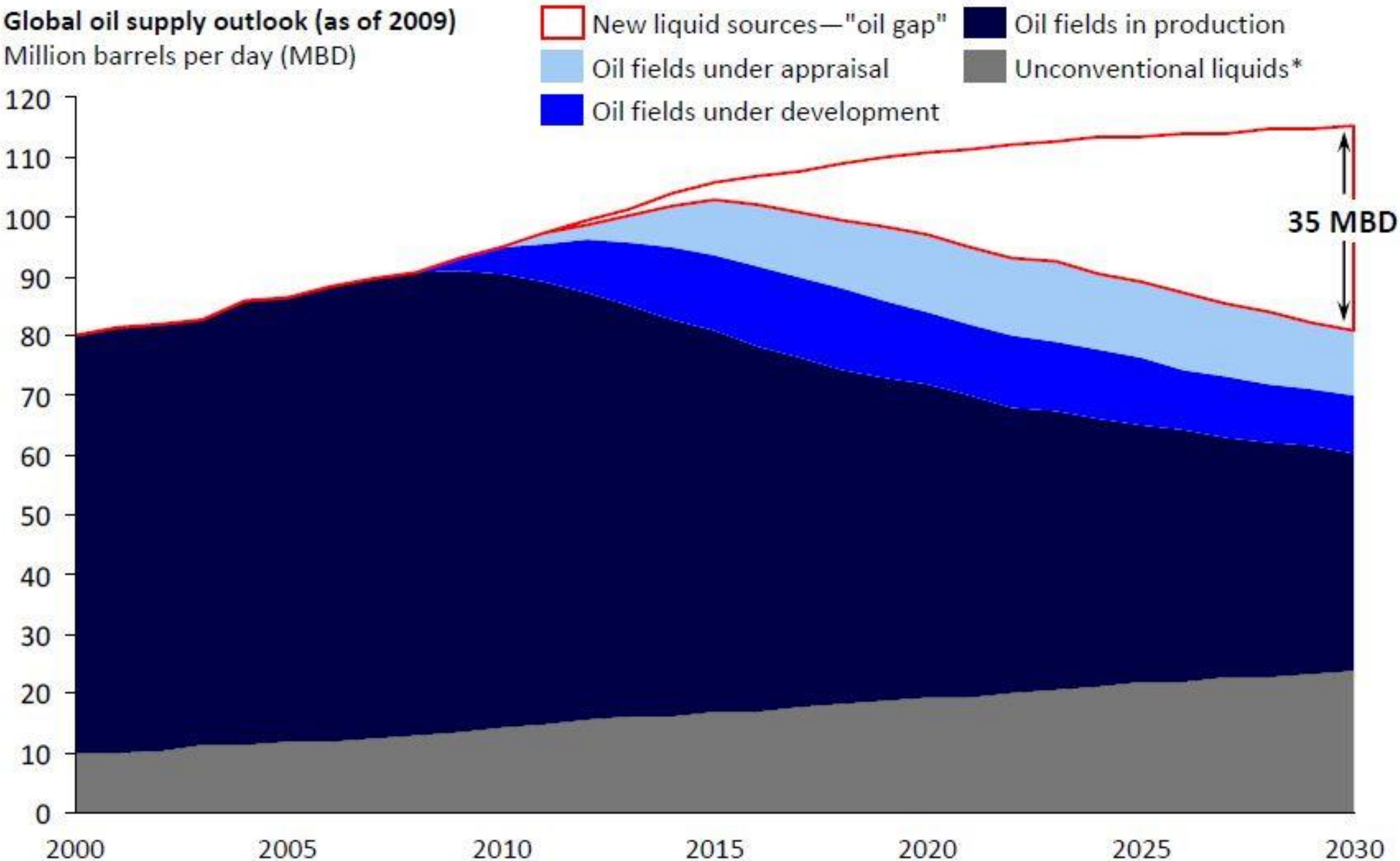
Birgitte Ahring
Washington State University

Producing hydrocarbon drop-in biofuels from cellulosic materials

Birgitte K. Ahring,
Center for Bioproducts and Bioenergy
Washington state University

WASHINGTON STATE UNIVERSITY
World Class. Face to Face.

CERA estimates that by 2030, the world will demand 35 million barrels per day of liquids from unidentified sources—an "oil gap" that must be filled



* Includes extra heavy oil, coal-to-liquids, gas-to-liquids, natural gas liquids, ethanol, biodiesel

Source: Cambridge Energy Research Associates "The Future of Global Oil Supply", 2009

All sources of non-fossil transportation fuel will be required, but some are better than others

Crude replacements

- Drop-in replacements, compatible with existing infrastructure
- Derived from either food or non-food photosynthetic feedstocks

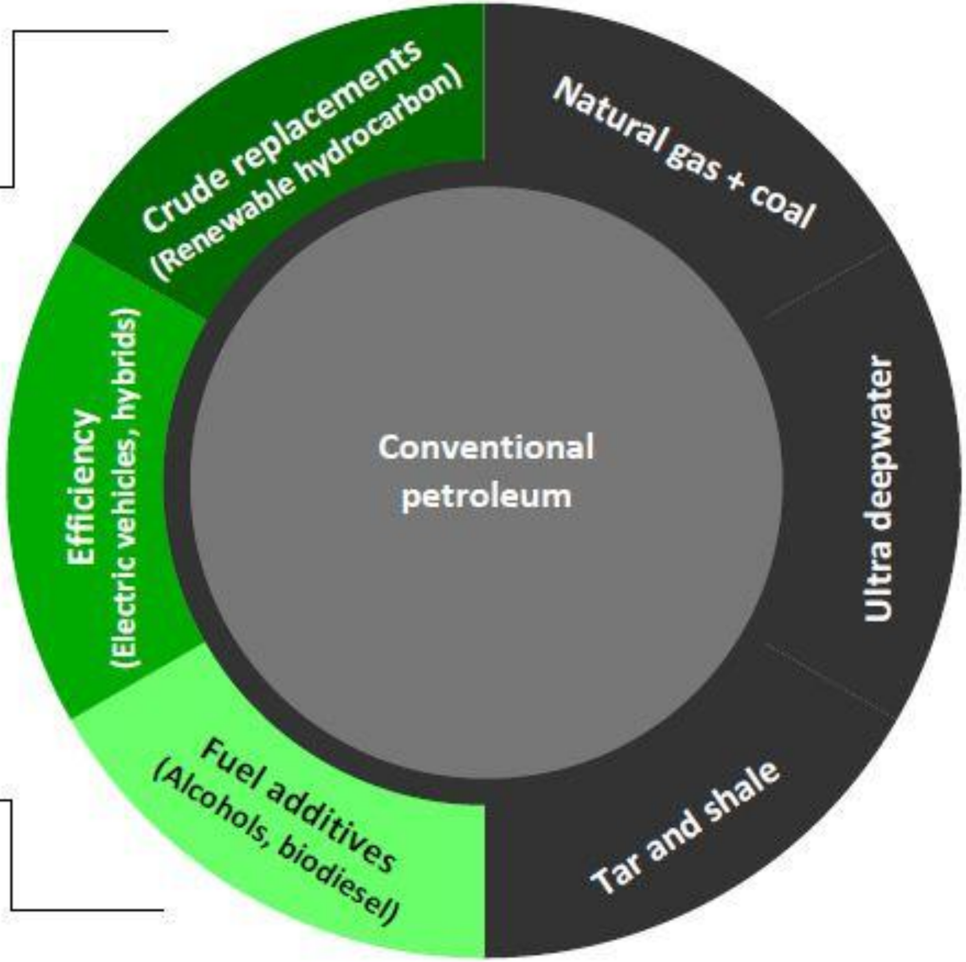
Efficiency

- Appropriate for limited light-duty vehicle and urban applications
- Cannot replace petrochemicals
- Infrastructure investment required

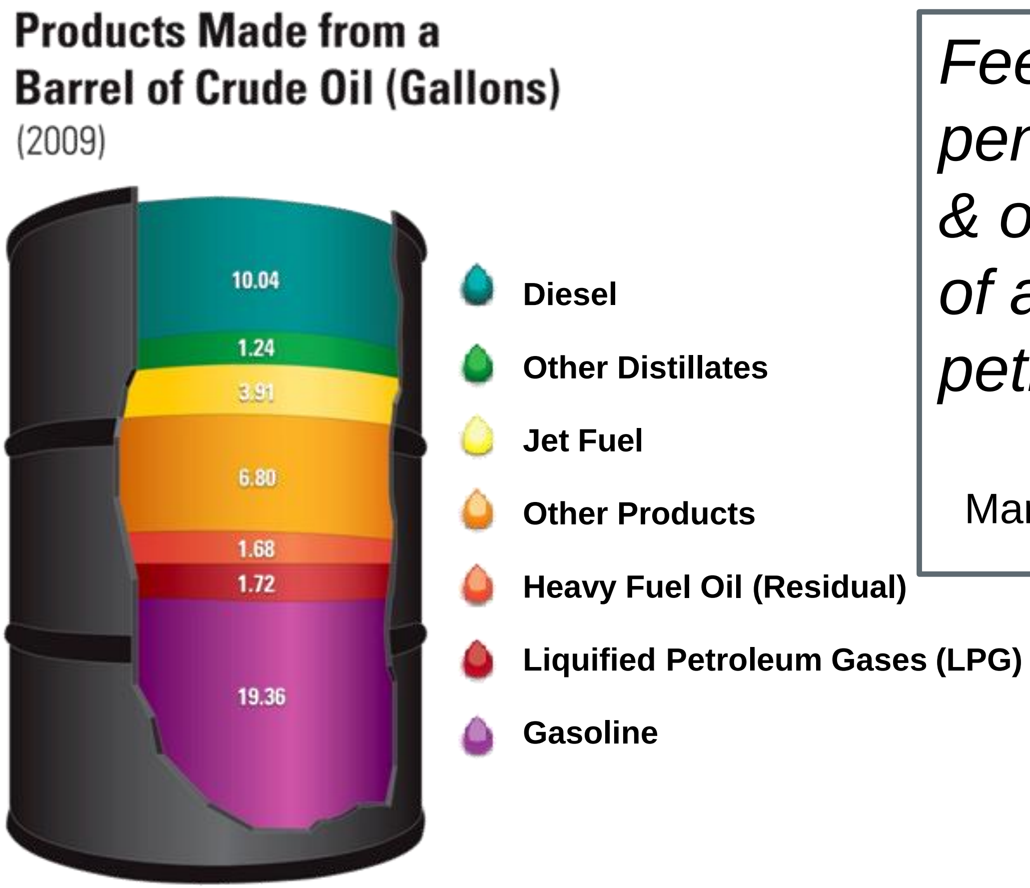
Fuel additives

- Most feedstocks compete for agricultural resources; not massively scalable
- Infrastructure investment required because they are not drop-in fuels

Future fuel sources



Current Uses of Petroleum

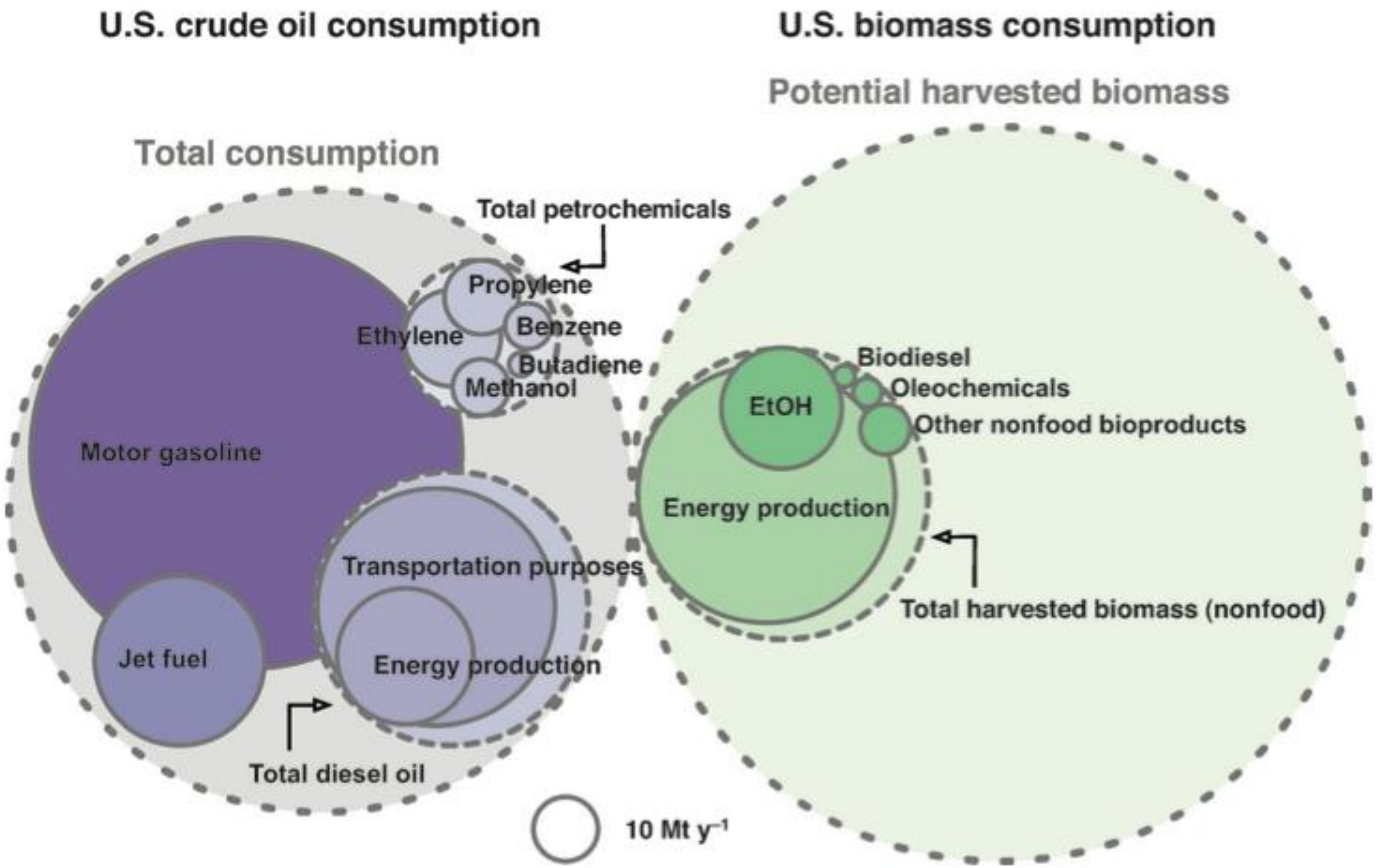


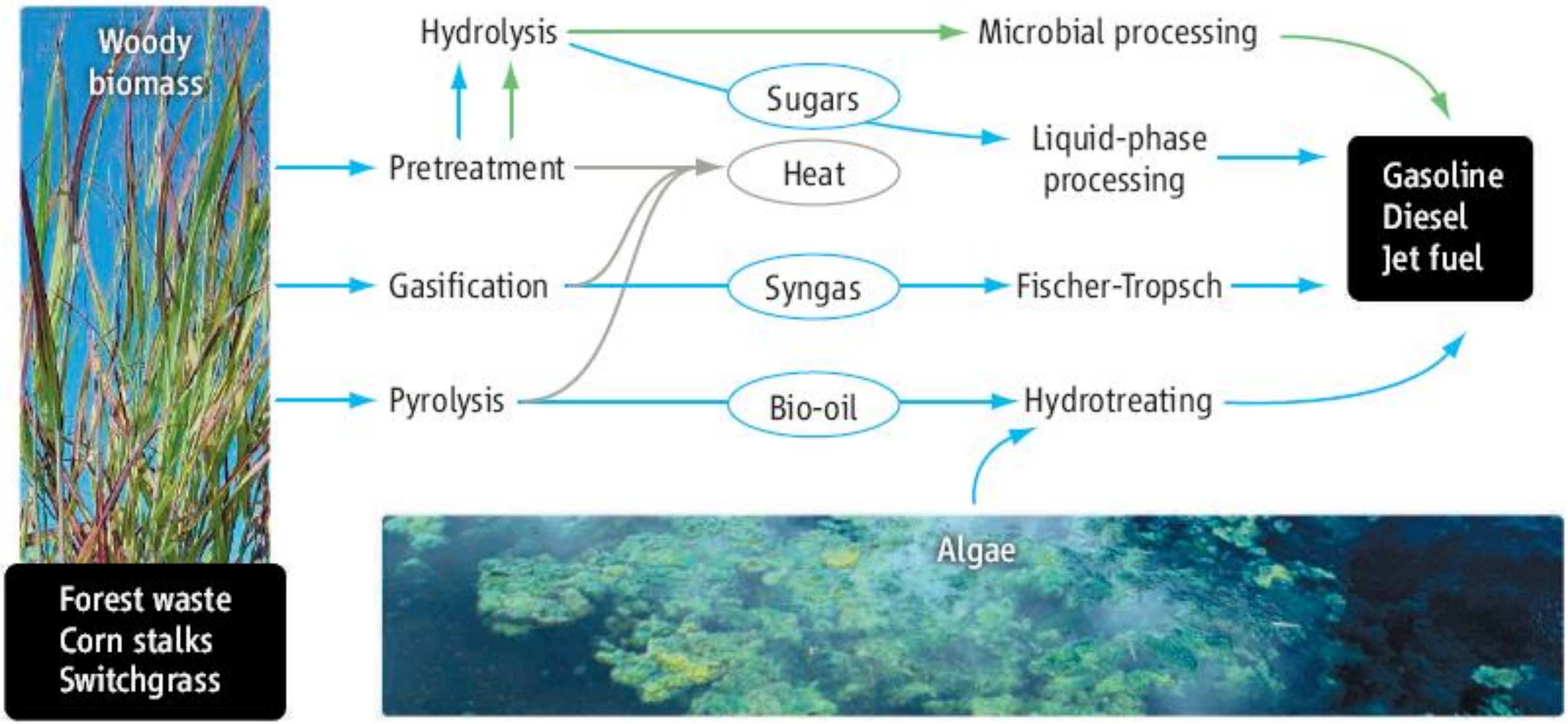
Source: Energy Information Administration, "Oil: Crude Oil and Petroleum Products Explained" and AEO2009, Updated February 2010, Reference Case.

Feedstocks like naphtha, pen-hex, BTX, light paraffins & olefins help form the basis of a ~$375 billion petrochemical industry.

Marshall *New Scientist*, **2007**, 28-31

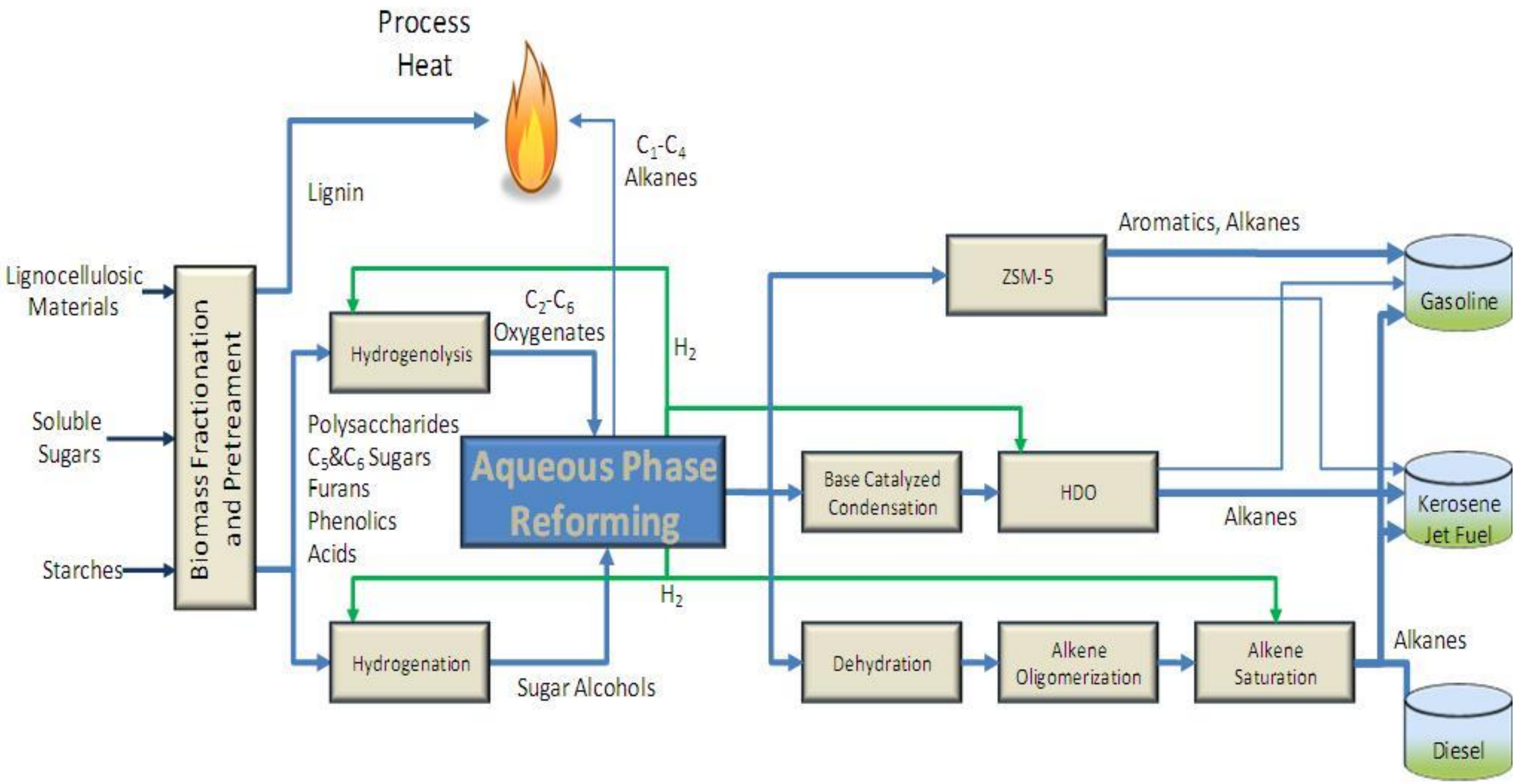
Value from Fuels & Products





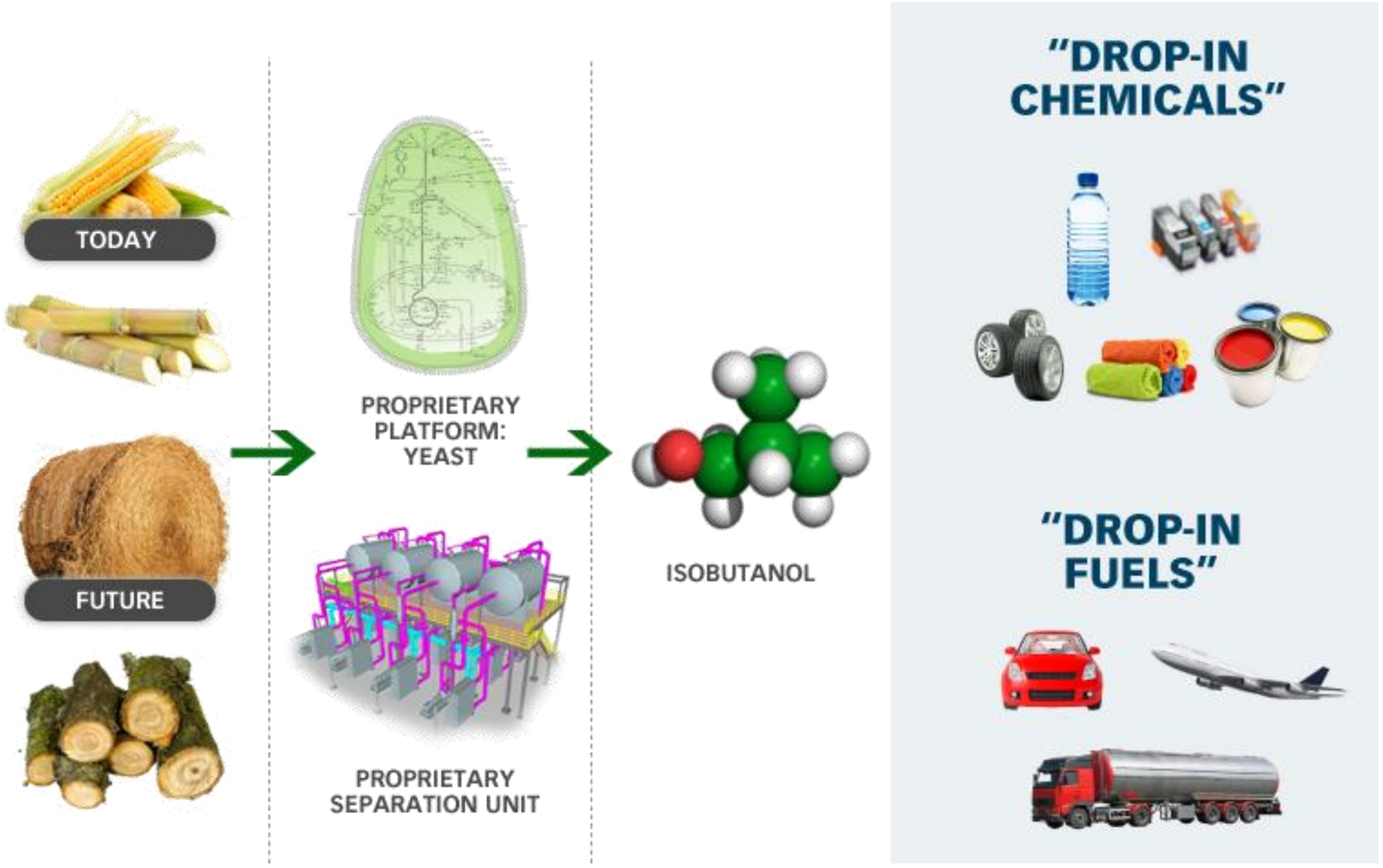
Woody biomass
Forest waste
Corn stalks
Switchgrass
Hydrolysis
Pretreatment
Gasification
Pyrolysis
Microbial processing
Sugars
Heat
Syngas
Bio-oil
Liquid-phase processing
Fischer-Tropsch
Hydrotreating
Gasoline
Diesel
Jet fuel
Algae

Catalysis of Sugars



BIOFORMING PROCESS

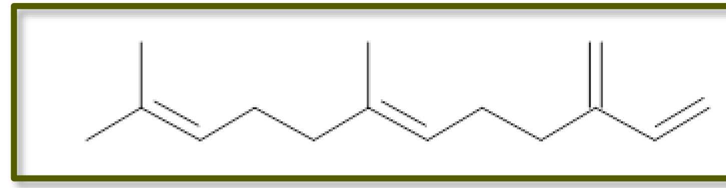
Gevo Yeast Fermentation



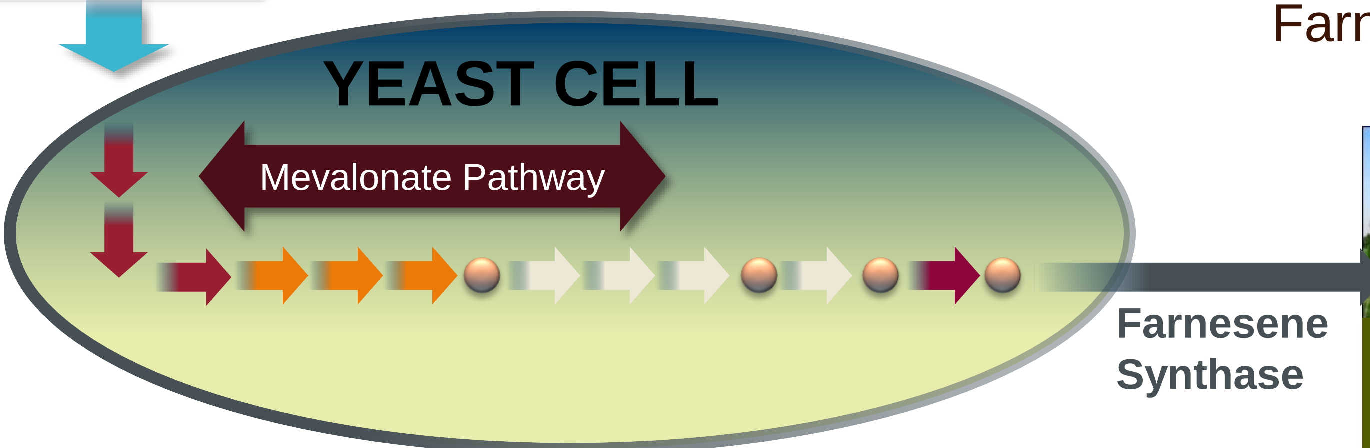
Website message: we make drop in chemicals too! (not just fuels)

Farnesene Building Block (Fermentation Tech)

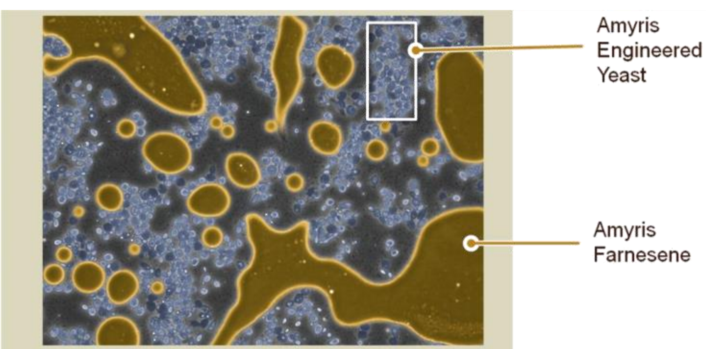
hydrolysate



Farnesene



Diesel & Chemical Precursor

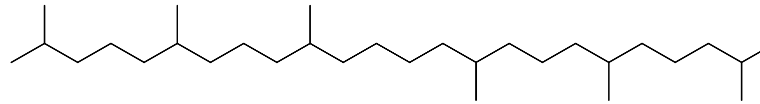


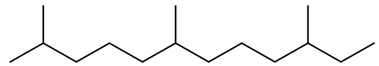
Phase-Contrast Micrograph of Amyris Engineered Microbes Producing Farnesene

[1] Cane juice
[2] Fermentation broth
[3] Separations
[4] Purification

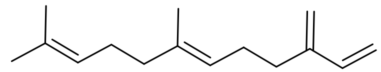
Farnesene Biosynthesis Technology Provides opportunities for many applications

Fuels
- Diesel
- Jet





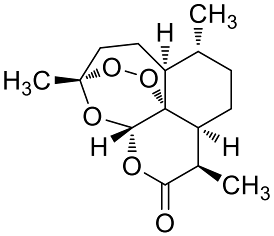
Lubricants (Novvi SA)
- Hydraulic fluids
- Compressor/turbine oils
- Food grade lubricants
- Gear Lubricants
- Greases
- Transmission Fluids
- 2-Cycle Engine oils
- Engine Oils

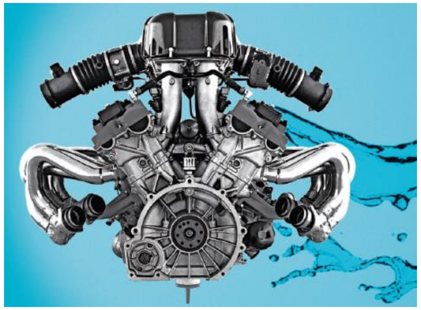


Farnesene

Personal Care
- Squalane (Soliance)
- Flavors
- Fragrances

Therapeutic
- Artemisinin (from artemisinic acid)
- (antimalarial)





Polymer Applications (M&G)
- Ingredients for PET
- Plasticizers
- Adhesives
- Plastics
- Packaging

BioChemCat- a DOE funded project

Principal Investigator: Birgitte K. Ahring, PhD
Organization: Washington State University

A partnership between the Port of Benton, WSU, CleanVantage, LLC and PNNL

PORT OF BENTON   WASHINGTON STATE UNIVERSITY TRI-CITIES World Class. Face to Face.   CleanVantage   Pacific Northwest NATIONAL LABORATORY

Role of Partners

Project holder, Delivery of biomass feedstocks, Public Education & Outreach

IP Holder, Pretreatment, Fermentation, Low/Moderate Severity Lignin Conversion

Research Lead, Pilot plant operations, Analytical Testing & Public Education & Outreach

Sub-contractor, Catalytic upgrading into fuels

Goal Statement

- **To develop an integrated thermochemical/biochemical conversion process that can efficiently and cost-effectively process agricultural residues and other biomass wastes into infrastructure compatible biofuels and bioproducts.**

Full Biomass Utilization & Feedstock Flexibility

Integrated biorefinery process concept maximizes the utilization of the biomass resource (i.e., converting the biomass available into a number of high energy products). We call it the:

"THE CARBON SLAUGHTERHOUSE"

Wheat Straw

Grape Pommace

Corn Stover

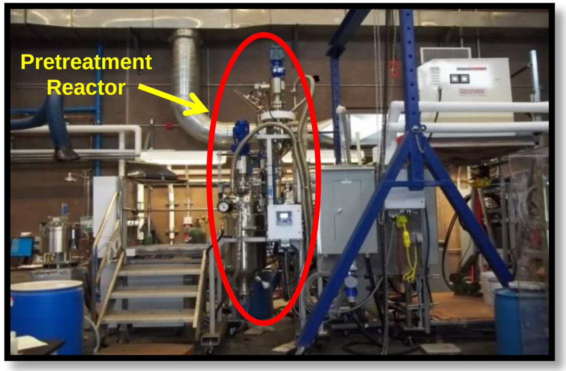
Hop Wastes

Paper and Food Waste

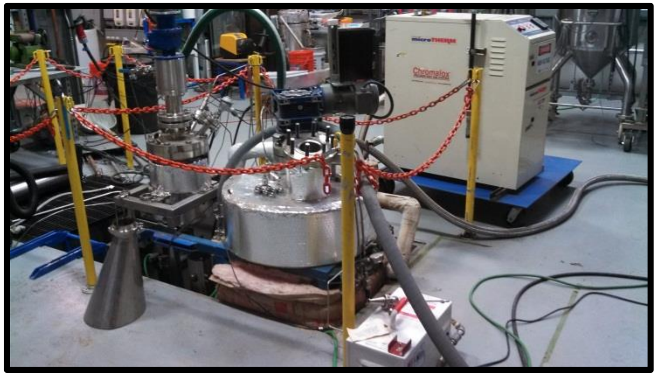
Yard Wastes

The Integrated Biorefinery concept can use many different types of biomass materials

WSU Biomass Pilot Plant

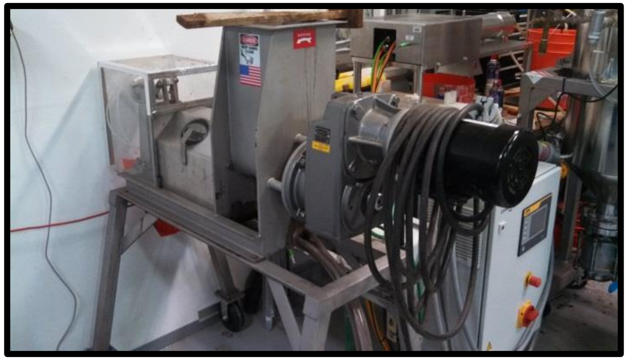


10 Liter Pretreatment Reactor



100 Liter Pretreatment Reactor (NEW)

400 Liter Fermentation Vessels (NEW)

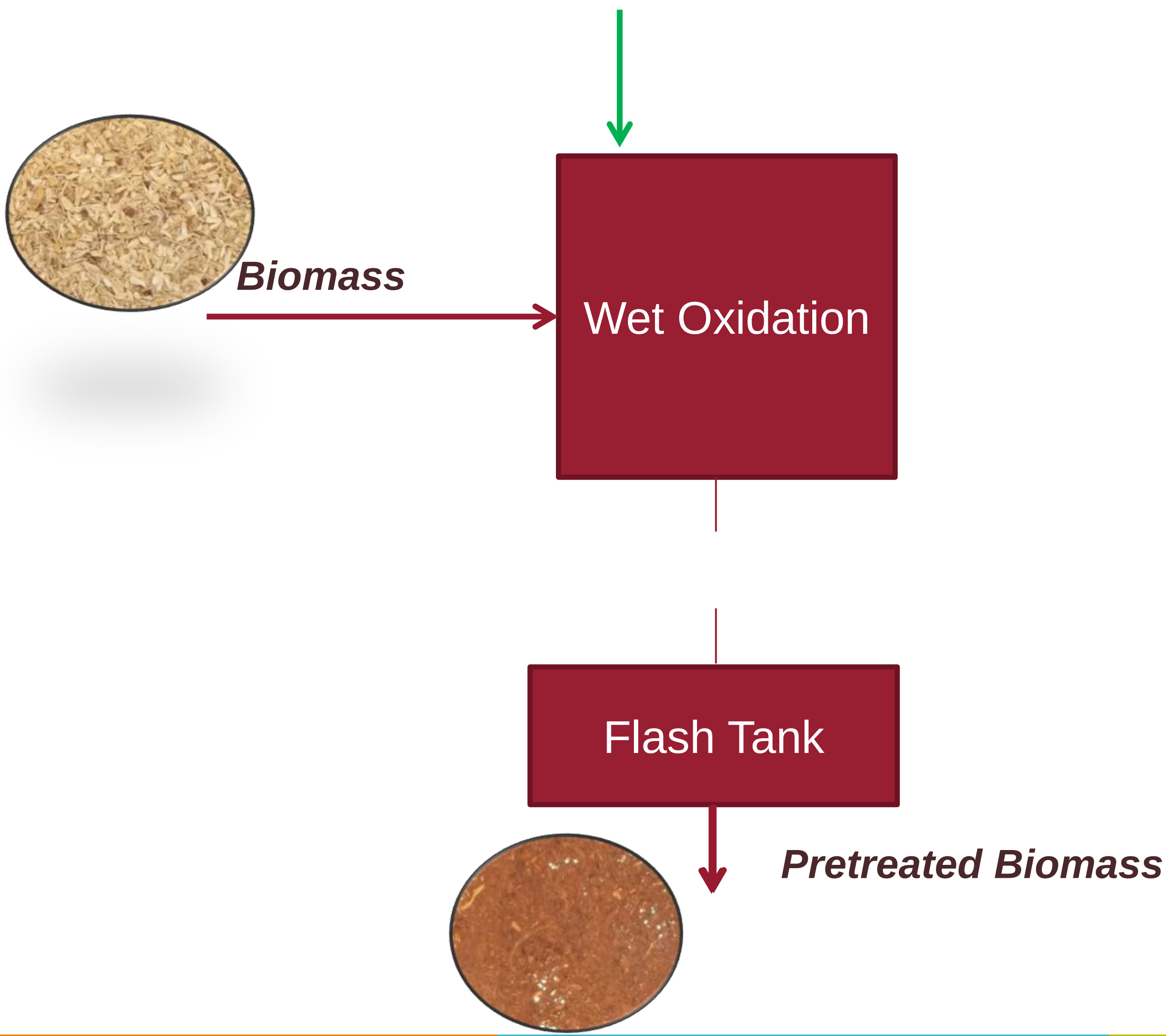


Screw Press Liquid/Solid Separation (NEW)

High Speed Centrifuge Liquid/Solid Separation (NEW)

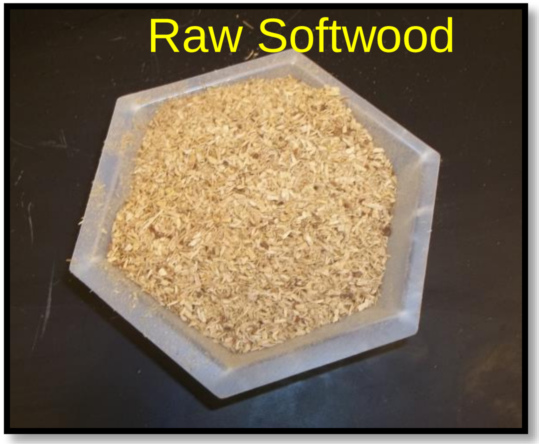
Focus on Softwood
Start with focus on pretreatment

WET EXPLOSION PRETREATMENT



Softwood to hydrolysate and sugars

Milling



Pretreatment

Enzymatic

Hydrolysis

WEx Pretreatment - Material Balance - Douglas Fir - FS-01

Douglas Fir, Water & Oxygen (1)

20% DM

100 kg of Slurry

Glucan	8.94	kg
Xylan	0.50	kg
Arabinan	0.42	kg
Galactan	0.40	kg
Mannan	2.36	kg
Lignin	5.92	kg

WEx Pretreatment
10 L Pilot Plant
(185 ºC, 25 min, 5 bar Oxygen)

Slurry →

Enzymatic Hydrolysis
250 mL Shaker
Ctec2: 40 mg protein/ g glucan
Htec2: 10% of Ctec2
(90 Hrs, pH 5.0, 50ºC)

Enzymes

4M KOH

→ Separation

(3) Solids

Solids Yield	19.75	kg	Normalized data
Glucan	7.87	kg	88%
Xylan	0.10	kg	21%
Arabinan	0.15	kg	36%
Galactan	0.11	kg	28%
Mannan	1.33	kg	56%
Lignin	5.76	kg	97%

(2) Liquid

Liquid Yield	80.25	kg	Normalized data
Glucose	0.77	kg	8%
Xylose	0.42	kg	74%
Arabinose	0.30	kg	62%
Galactose	0.33	kg	74%
Mannose	1.20	kg	46%
Lignin	0.07	kg	1%
HMF	0.01	kg	-
Furfural	0.07	kg	-
Acetic Acid	0.14	kg	-

	Monomers	Oligomers	
Glucose	0.34	0.43	kg
Xylose	0.22	0.20	kg
Arabinose	0.07	0.23	kg
Galactose	0.18	0.15	kg
Mannose	0.46	0.74	kg

(5) Solids

Solids Yield	10.08	kg	Normalized data
Glucan	1.09	kg	12%
Xylan	0.02	kg	3%
Arabinan	0.00	kg	0%
Galactan	0.02	kg	5%
Mannan	0.10	kg	4%
Lignin	5.65	kg	95%

(4) Liquid

Liquid Yield	89.92	kg	Normalized data
Glucose	8.79	kg	89%
Xylose	0.54	kg	96%
Arabinose	0.48	kg	100%
Galactose	0.43	kg	96%
Mannose	2.46	kg	94%
Lignin	0.17	kg	3%
HMF	0.04	kg	-
Furfural	0.27	kg	-
Acetic Acid	0.38	kg	-

	Monomers	Oligomers	
Glucose	8.60	0.19	kg
Xylose	0.53	0.01	kg
Arabinose	0.43	0.05	kg
Galactose	0.42	0.01	kg
Mannose	2.04	0.42	kg

Conversion

	Overall
Glucose	88.5%
Xylose	95.9%
Arabinose	99.8%
Galactose	96.0%
Mannose	93.6%
Lignin	2.9%

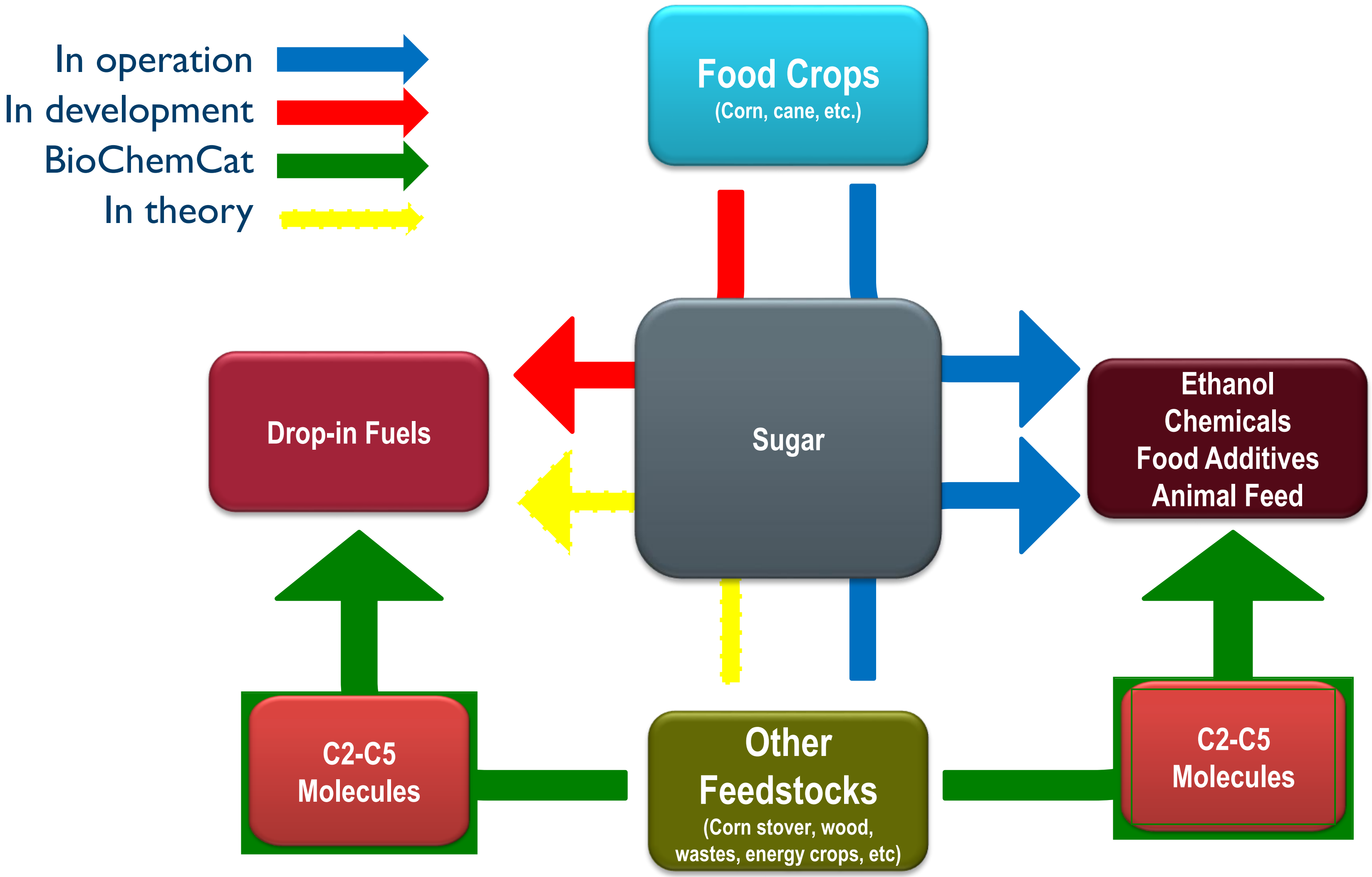
Recovery

	Overall
Glucose	101%
Xylose	99%
Arabinose	100%
Galactose	101%
Mannose	98%
Lignin	98%

Sugars Yields from Softwood

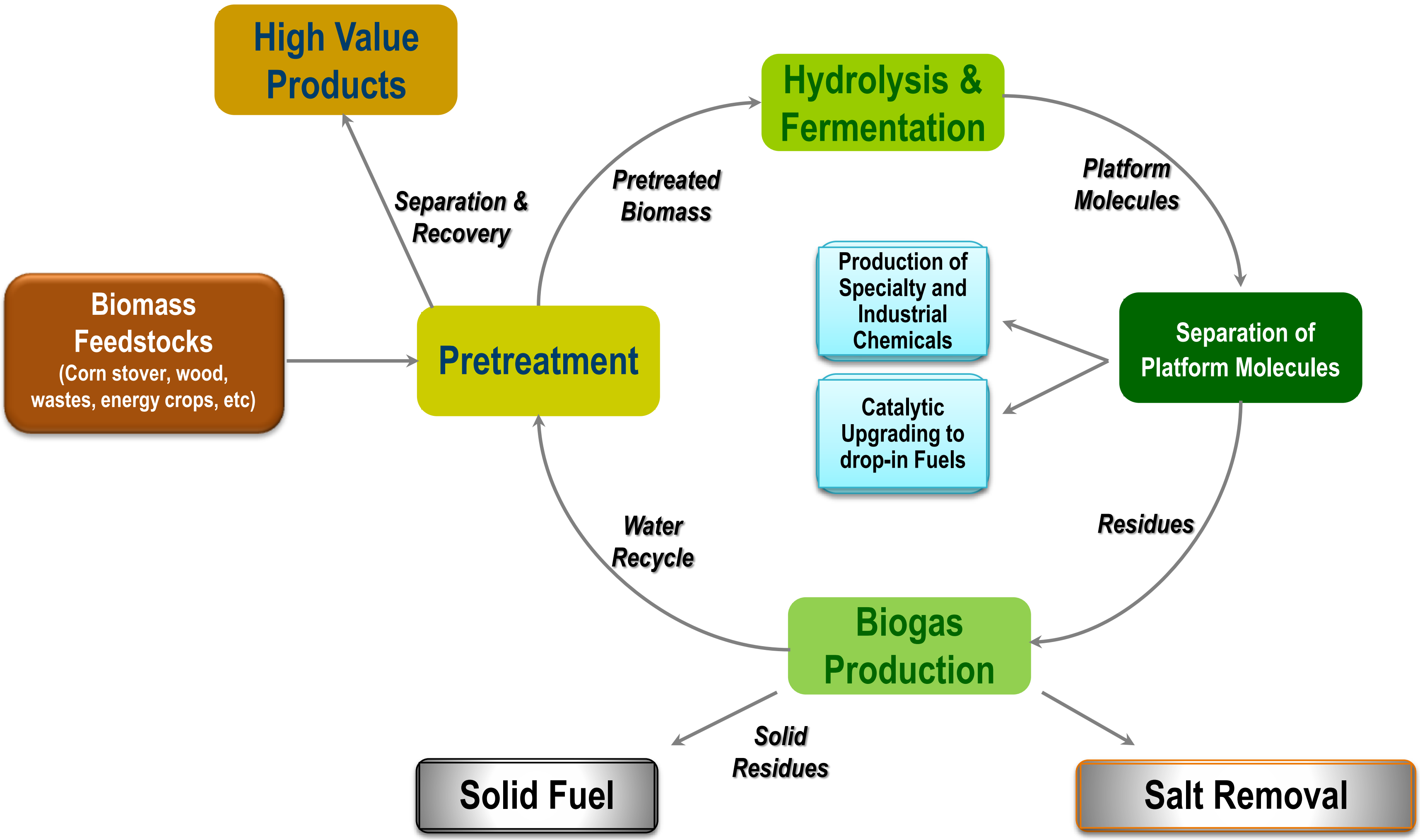
Type of Biomass	Type of Pretreatment	Pretreatment Temperature (^{0}C)-Time (min)	Enzymatic Hydrolysis	Theoretical Yield (Total Sugars)	Reference
Softwood	Two- step Steam Pretreatment	Stage 1: 190-2, 3% **SO_2** Stage 2: 220-5, 3% **SO_2**	2% DM	80%	Söderström J. et al. (2002)
Pinus rigida	Organosolv	210-10, 1% **$MgCl_2$**	1% DM	75.88%	Park N. et al. (2010)
Bettle Killed Lodgepole	One step Steam Pretreatment	200-5, 4% **SO_2**	2% DM	75%	Ewanick S. et al. (2007)
Loblolly pine	Wet Explosion	180-20, 6 bar **O_2**	25% DM	**96.00%**	Rana D. et al. (2012)

Today's Biorefineries & BioChemCat



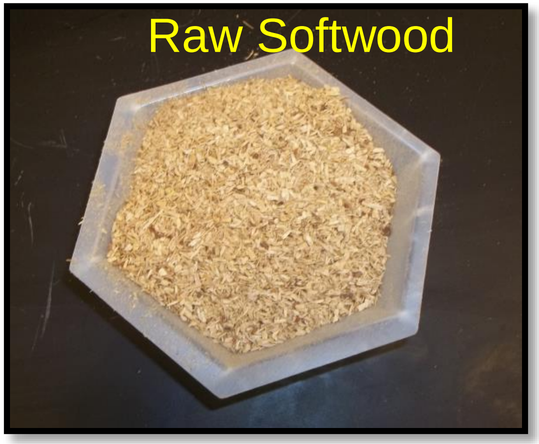
The BioChemCat Process

A hydrolysate platform



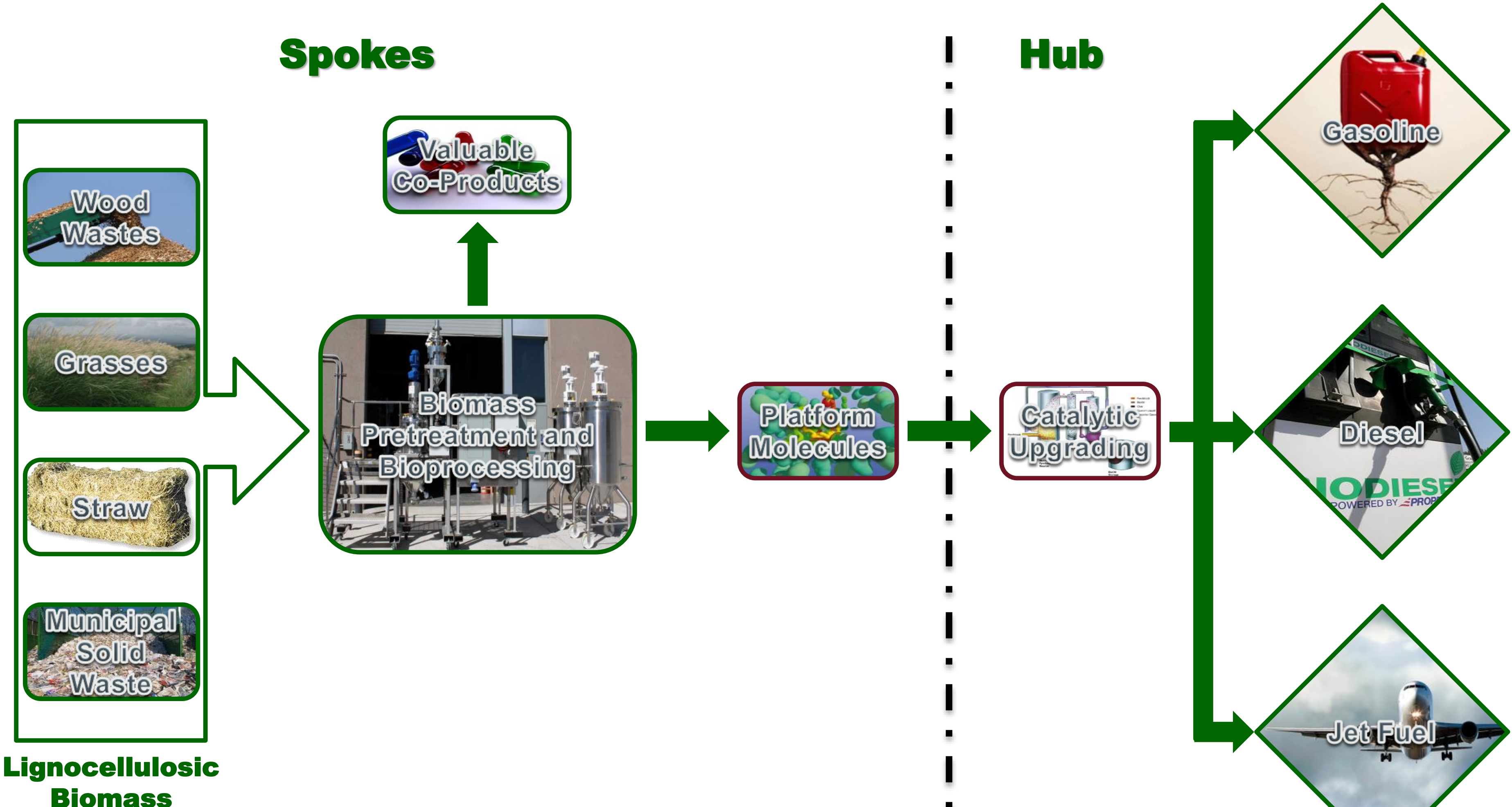
Softwood to Hydrolysate

Milling

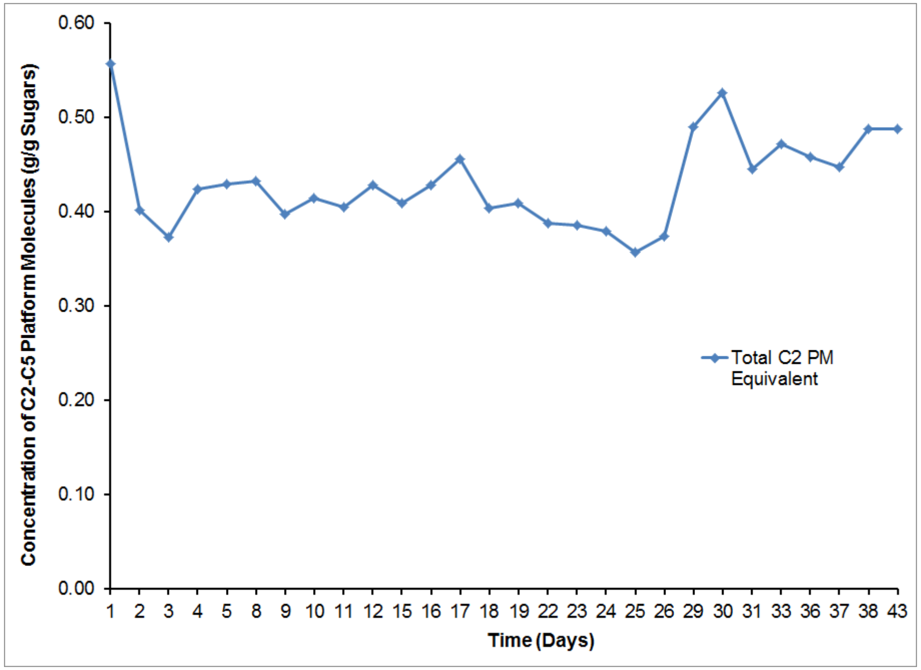


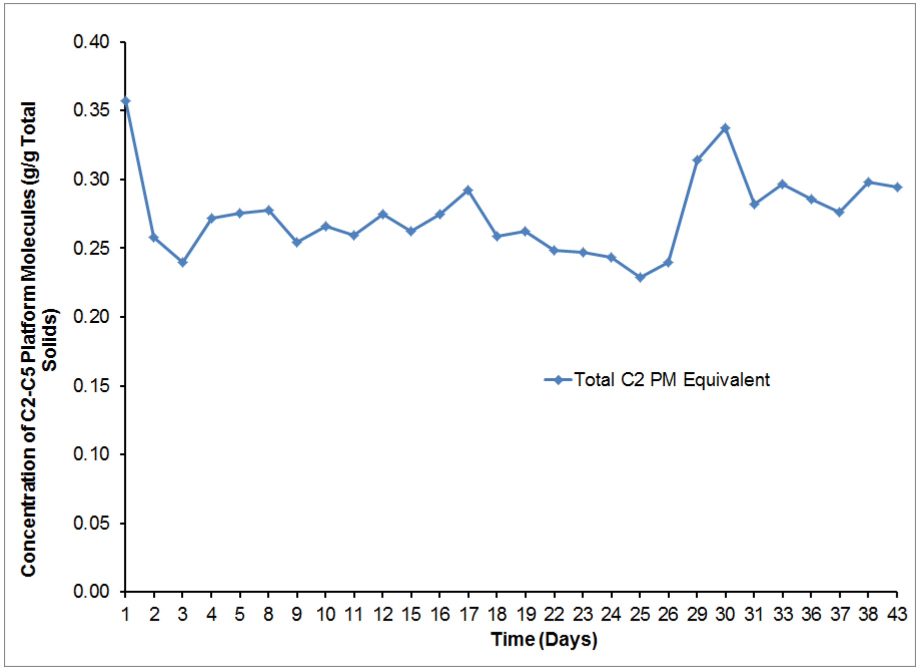
Pretreatment

Non-enzymatic Hydrolysate Platform BioChemCat Process



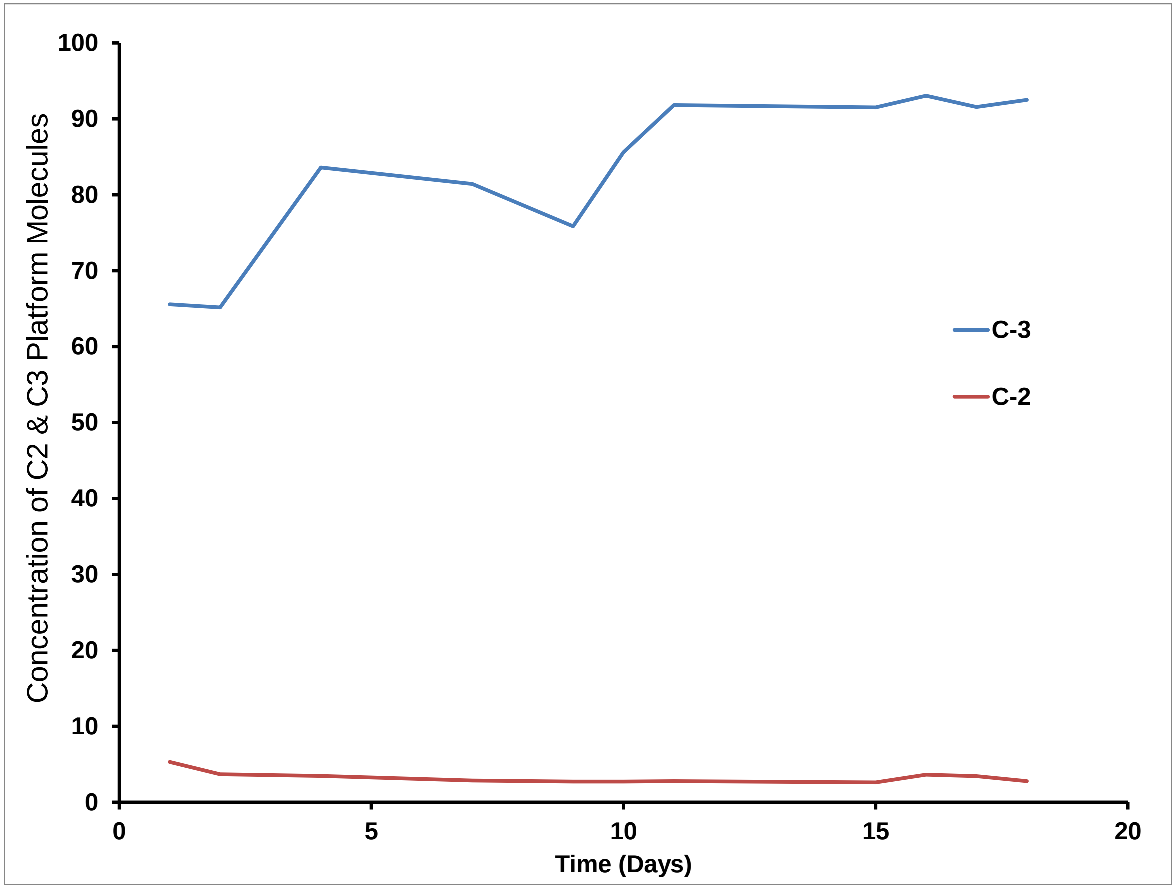
Platform Molecules (PM) Current Fermentation Results





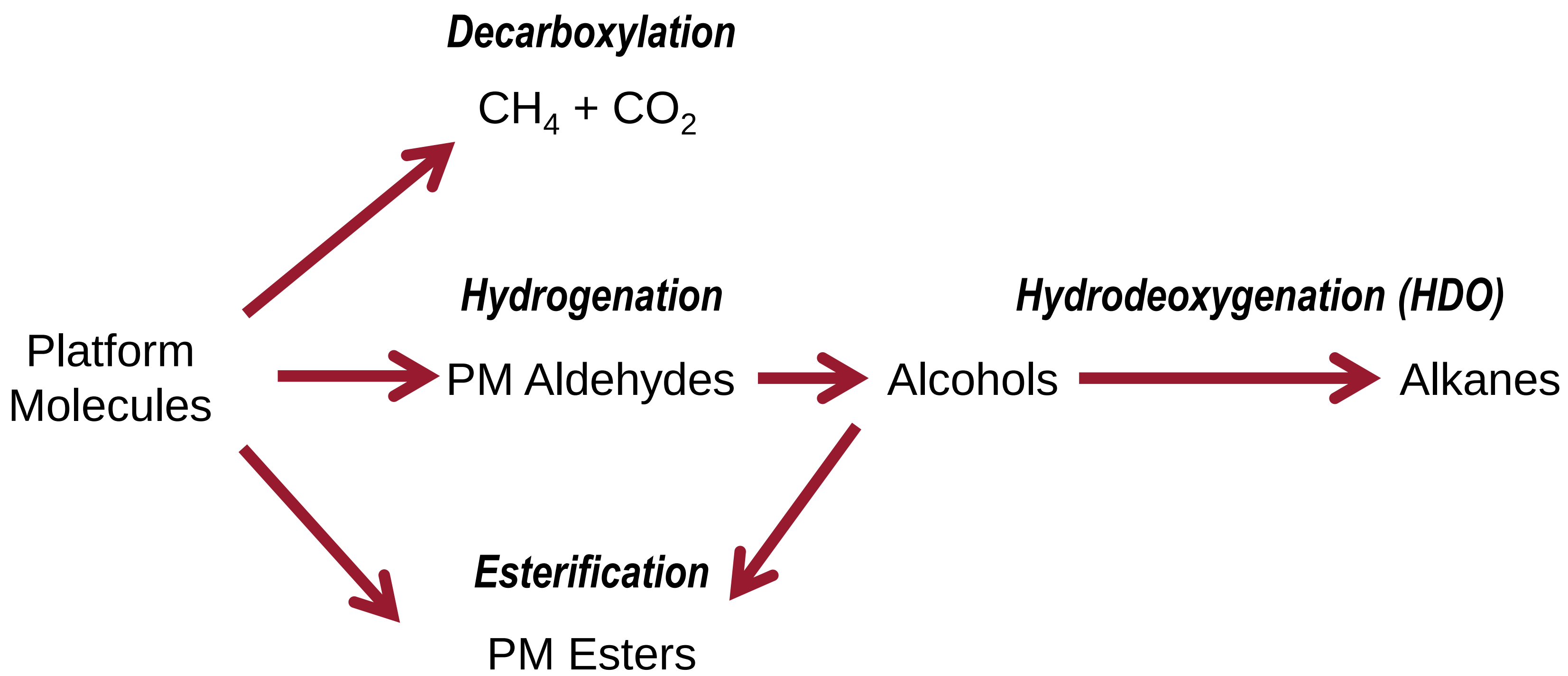
Productivity: 0.4 g/L/h

Using a defined coculture



C3 yield of 91% and productivity of 1.2 g/L/h

Catalysis Process



Reaction Pathway for Conversion of Platform Molecules over Pd-Re/C Catalyst at 180-240°C
(>95% Conversion to Alcohol @ Optimal Temperature)

Technoeconomic Data

Equipment Costs (2010$) (Biochemical)	Current Costs	Intermediate Target Costs	Final Target Costs
	Capital Cost (MM$)	Capital Cost (MM$)	Capital Cost (MM$)
Feedstock Handling	$ 6.31	$ 6.17	$ 5.83
Pretreatment	$ 40.04	$ 37.63	$ 35.87
Separation of PMs and Lignin	$ 74.55	$ 71.74	$ 69.38
Fermentation Organism Production	$ 65.85	$ 65.86	$ 65.86
Biogas	$ 21.75	$ 21.76	$ 21.76
Catalytic conversion & product recovery	$ 90.55	$ 83.50	$ 78.79
Wastewater Treatment	$ 38.57	$ 38.57	$ 38.57
Storage	$ 14.82	$ 14.82	$ 14.82
Civil Infrastructure (Bldgs., HVAC, etc.)	$ 24.46	$ 24.46	$ 24.46
Utilities	$ 48.10	$ 48.10	$ 40.90
Total Installed Capital	$ 425.00	$ 412.60	$ 396.24
Total Installed Capital per Annual Gallon	$8.50	$8.25	$7.92
Operating Costs (2010$)	MM$/yr	MM$/yr	MM$/yr
Feedstock	$ 42.86	$ 40.00	$ 37.50
Organism Production Nutrients	$ 1.50	$ 1.35	$ 1.25
Fermentation Nutrients	$ 3.00	$ 2.85	$ 2.70
Enzymes (Cellulase)	$ 0.00	$ 0.00	$ 0.00
Fermentation Organism (include licensing fees)	$ 10.00	$ 10.00	$ 10.00
Conversion Catalyst	$ 55.00	$ 28.00	$ 14.00
Other Raw Materials	$ 1.25	$ 1.20	$ 1.15
Waste Disposal	$ 5.00	$ 5.00	$ 5.00
Steam	$ 2.00	$ 2.00	$ 2.00
Electricity	$ 20.50	$ 19.50	$ 18.75
Labor and Maintenance	$ 26.45	$ 25.00	$ 23.75
Total Operating Costs	$ 182.56	$ 144.90	$ 121.10
Co-product Credits	$ 11.36	$ 10.60	$ 9.94
Net Operating Costs	$ 171.20	$ 134.30	$ 111.16
Net Fuel Production Costs ($/gal)	**$ 3.12**	**$ 2.49**	**$ 2.12**

BioChemCat: A Game Changing Technology

- It uses a stable consortia of bacteria and has no need for enzymes or sterility ***reducing the operational and capital cost significant*** (at least 15% reduction of OPEX compared to sugar platform biofuels)
- The stable consortia allows for changing between different biomass feed stocks for instance on a seasonal basis
- The process can be operated in a spoke and hub manner allowing for distributed production of PM close to the biomass raw materials- and upgrading in a centralized hub
- The process allows for simultaneous production of chemicals and drop-in biofuels buying down the cost of biofuels production
- Bolt-on to a corn ethanol plant is possible sending the C6 sugar to the corn ethanol facility and using all other fractions as input to the BioChemCat process

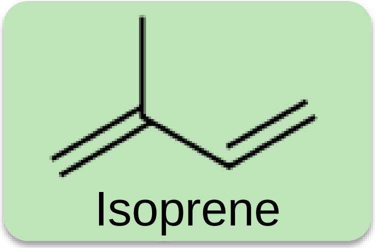
Summary

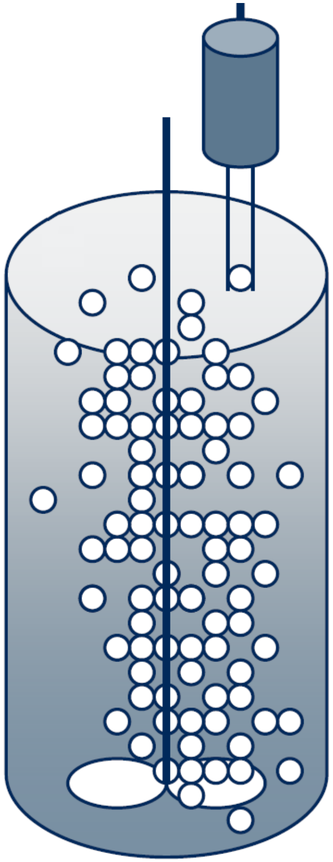
1) **Approach:** BioChemCat is an innovative new approach for making biofuels/bioproducts
2) **Technical accomplishments:** The project with its extended timespan is fully on track and has already proven that the concept is viable
3) **Relevance:** The project has direct relevance fore DOE's mission of decreasing US's dependence of foreign oil. It further shows ways for better use of biomass and organic waste in general. All in all- the BioChemCat could allow for a new successful US based business
4) **Critical Success factors and challenges:** With the results obtain until now the technical challenged all seems possible to overcome. The dry investor environment could be the most critical success factor and challenge.
5) **Future Work**: In the coming period the fermentation process will further upscale to pilot scale along with the selected separation method. After finalizing the techno-economics the process is ready for further up scaling.
6) **Technology transfer**: Different outreach activities are planned for the coming period around the BioChemCat project. CleanVantage will further work for setting up licensing agreements around their IP.

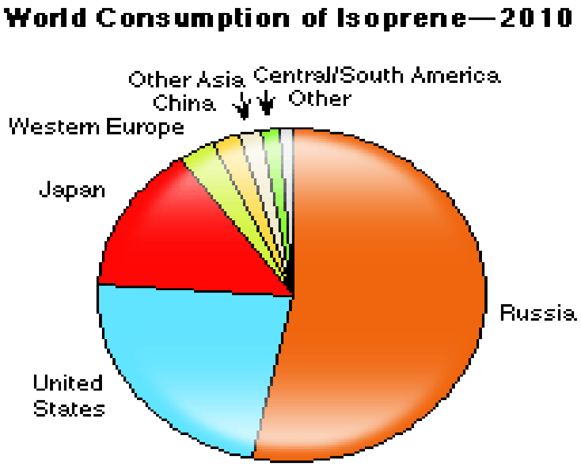
Making isoprene from lignocellulosic biomass using *Bacillus* species

Isoprene (2-methyl-1, 3-butadiene)

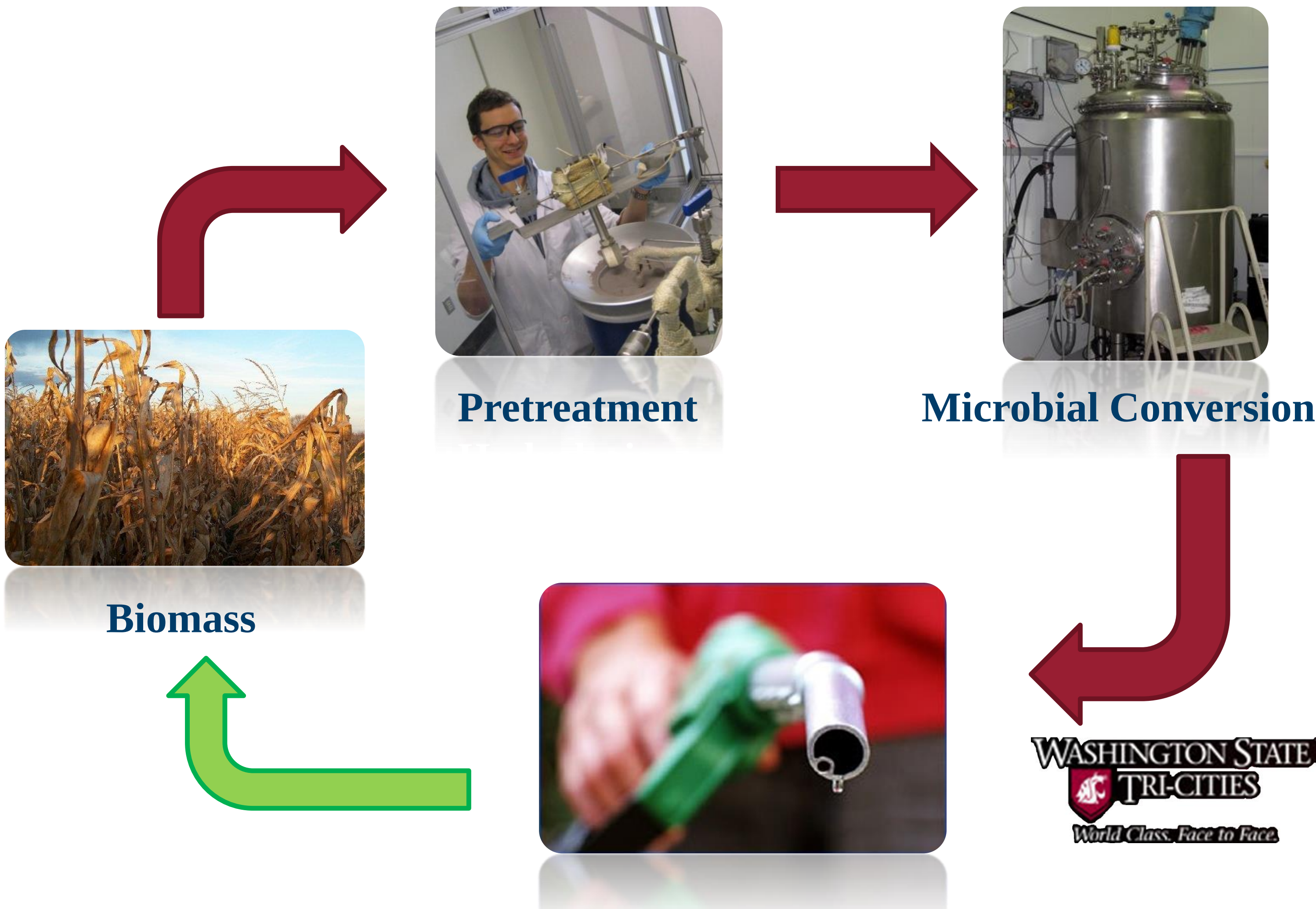
- Pure hydrocarbon
- Volatile
- Versatile







Strategy and Environmental impact



WASHINGTON STATE UNIVERSITY
TRI-CITIES
World Class. Face to Face.

Applications

- Drop in fuel
- Rubber
- Pharmaceuticals

WASHINGTON STATE UNIVERSITY
TRI-CITIES
World Class. Face to Face.

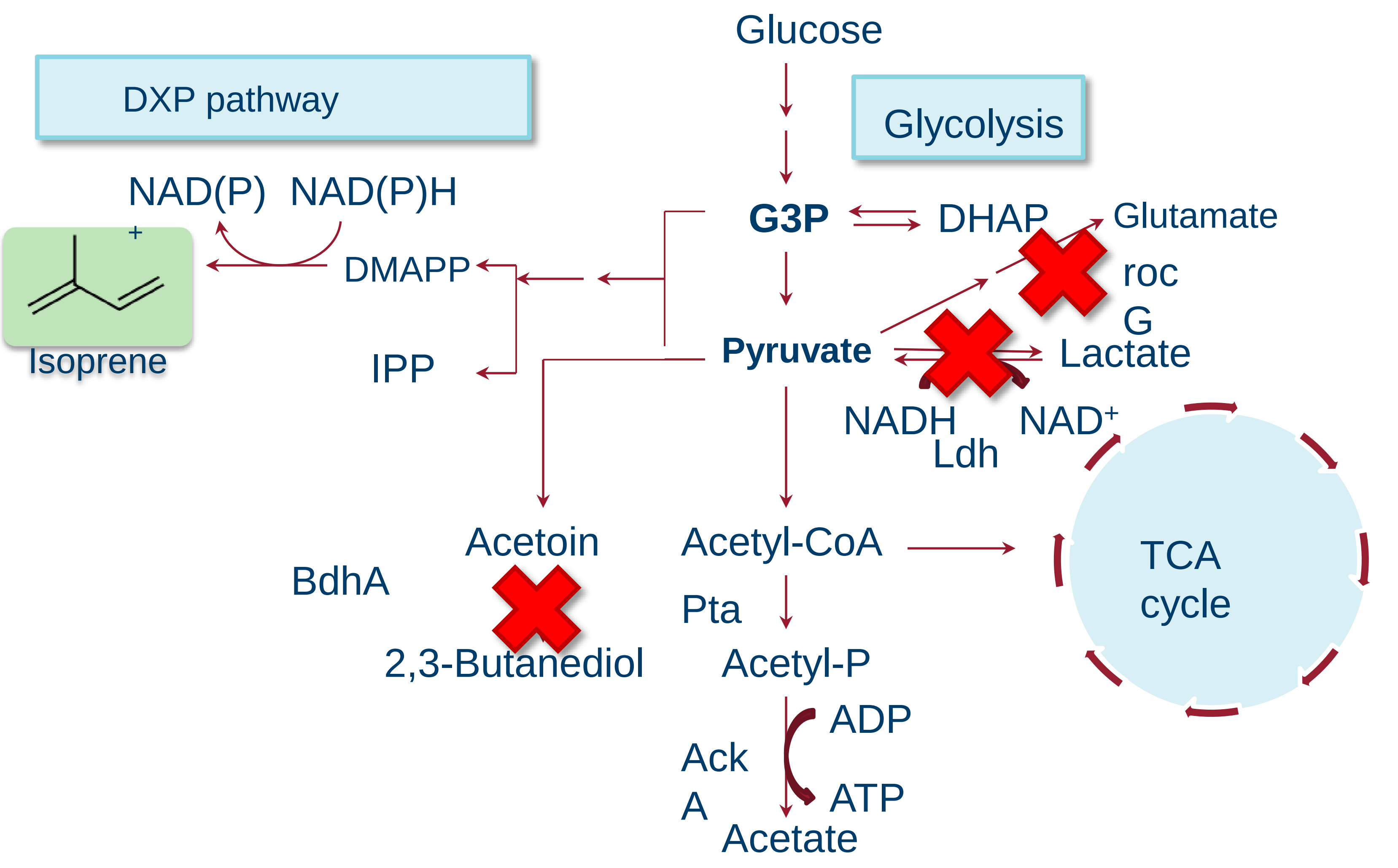
APPROACH

Multi-omica analysis and methodology development

- Transcriptomics
- Proteomics
- Metabolomics

Funding to EMSL (DOE User Facility) came as part of a Research Campaign

The DXP pathway and the central carbon metabolism



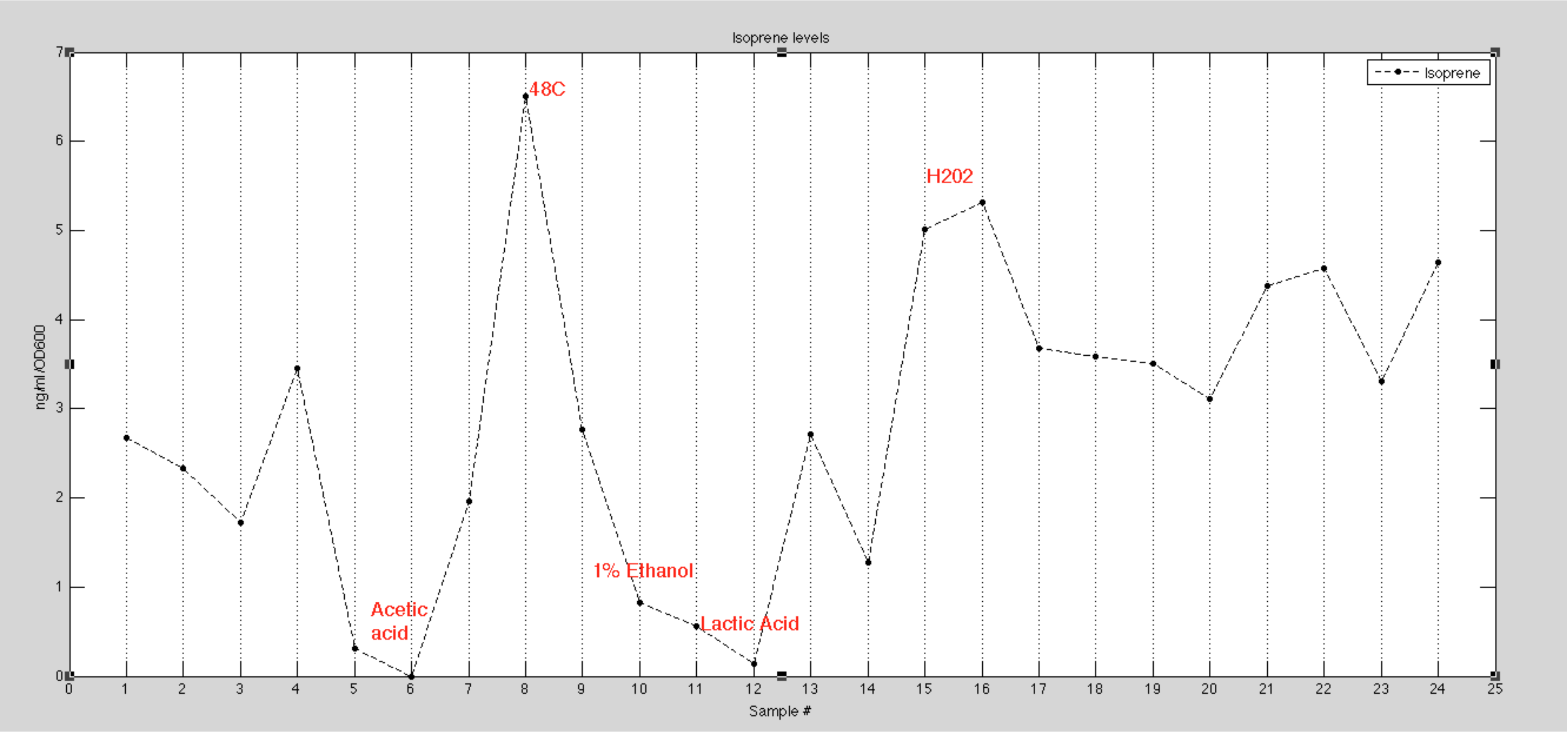
Selective over expression of the DXP pathway genes is a viable strategy to overproduce isoprene

Growth phase	DSM10	DSM10/pHT dxs	DSM10/pHT dxr	DSM10/pHT dxsr
Early log phase	2.67±0.16	3.73±0.36	2.61±0.22	3.45±0.37
Mid-log phase	2.33±0.24	3.29±0.15	2.47±0.16	3.27±0.53
Late log phase	1.72±0.09	1.92±0.09	1.62±0.01	2.31±0.15

Values are expressed as average concentrations (ng/ml/OD_{600} ± standard deviations (n=3).

J. Xue and B.K. Ahring. 2011. AEM

Production of isoprene could be induced and repressed under environmental and experimental perturbations in *B. subtilis*

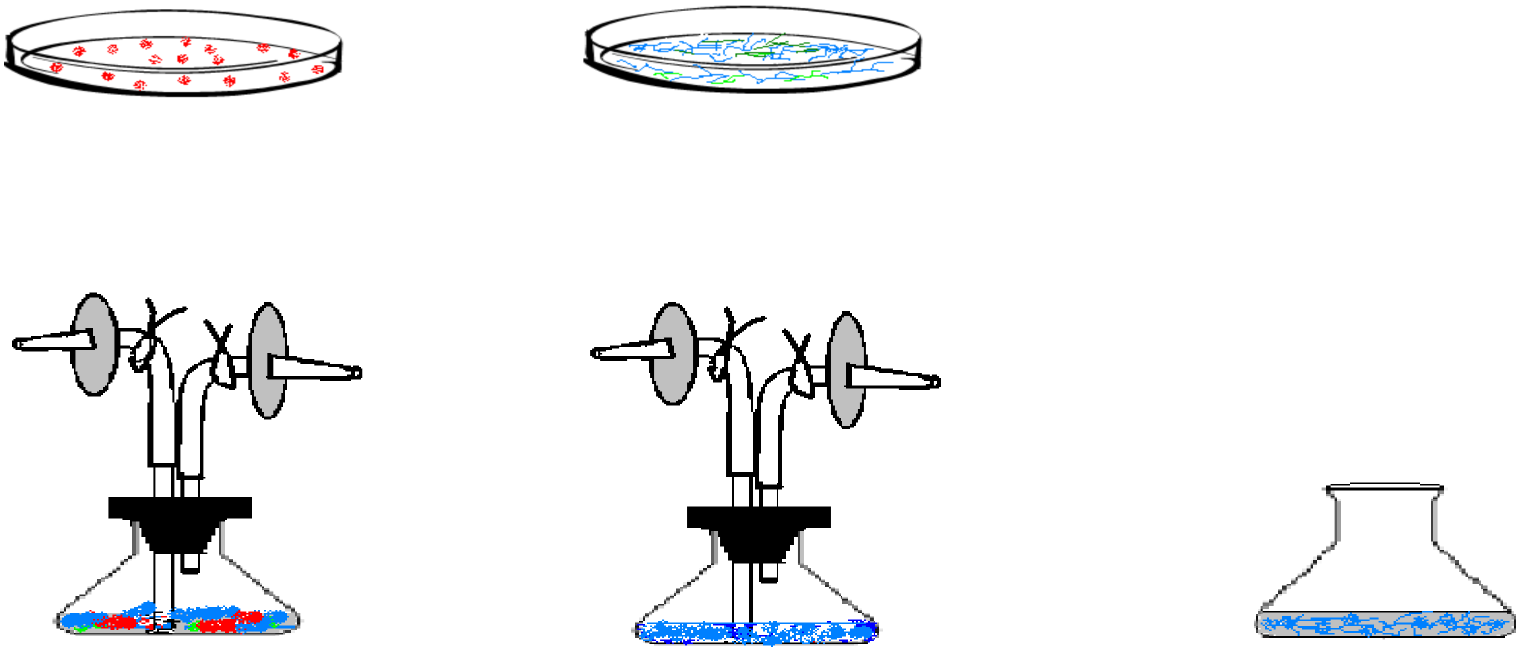


Information we have collected

- General Statistics
  - **24 samples**
    - Isoprene levels 0-6.5 ng/ml/OD600 (mean 2.85)
  - **Metabolomics**
    - 47 metabolites from each cell lysate and supernatant (94 total)
  - **Transcriptomics**
    - 4184 gene transcript levels between 1-75122 RPKM (99% of all)
  - **Proteomics**
    - 927 proteins measured

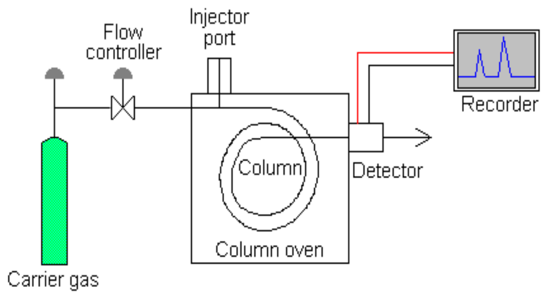
Production of infra-structure ready biofuels by filamentous fungi grown on cellulose derived from agricultural and forest waste products.

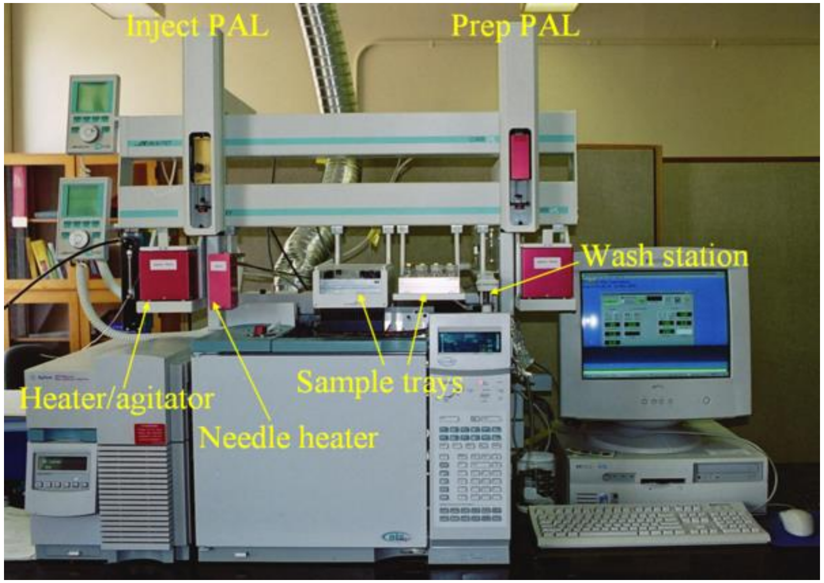
Schematic overview of hydrocarbon production in filamentous fungi grown on cellulosic biomass

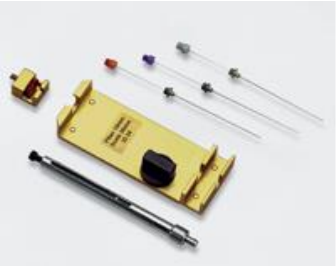


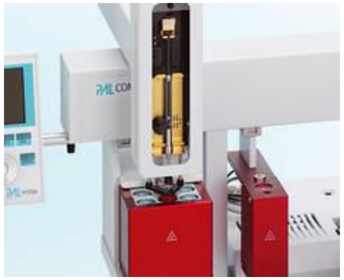
Micro-aerophilic conditions (low oxygen) Co-culture (F+B)	Micro-aerophilic conditions (low oxygen) Mono-culture (F)	Aerobic conditions (High oxygen)
High hydrocarbon production	**Low hydrocarbon production**	**No hydrocarbon production**

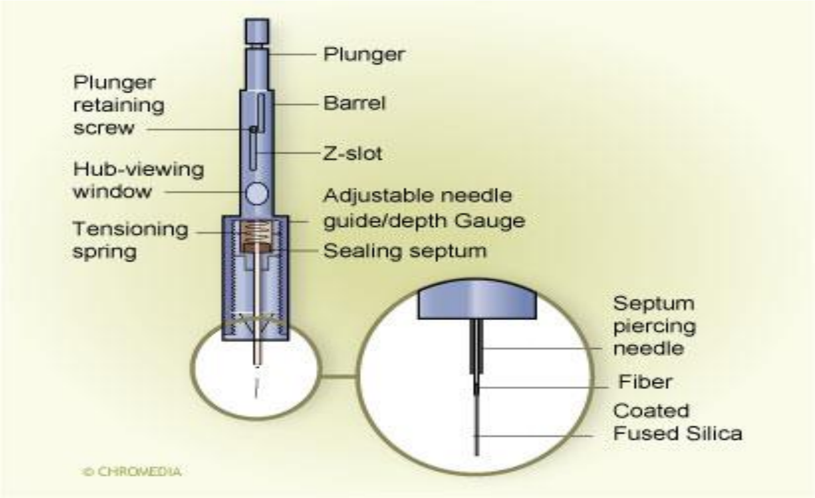
GC/MS-SPME technique used to analyze head space gases for hydrocarbon production from filamentous fungi

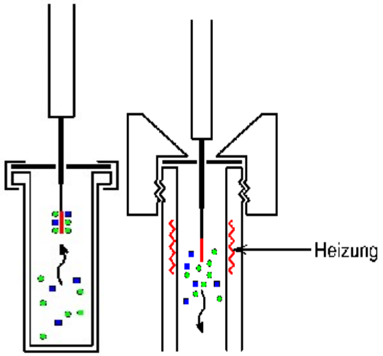












Washington State University
Tri-Cities
World Class. Face to Face.

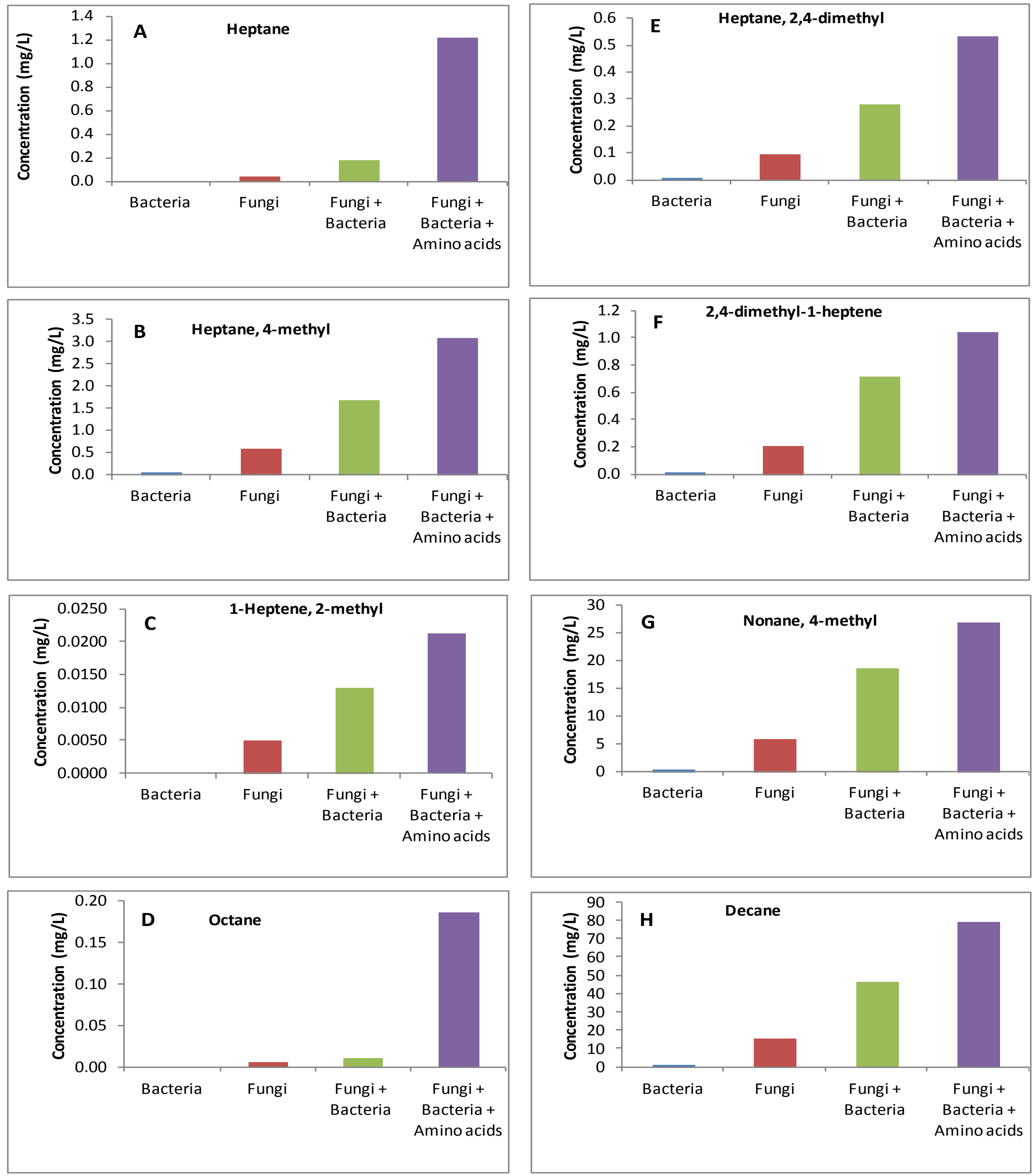
Quantitative production of hydrocarbons from *Gliocladium spp.* grown on oatmeal.

Compound name	Retention time (min)	62724		1165		62726	
		Mono-culture mg/ml	Co-culture mg/ml	Mono-culture mg/ml	Co-culture mg/ml	Mono-culture mg/ml	Co-culture mg/ml
Hexane	2	0.0637604	0.00174	0.00615	0.01078	0	0.00065
Benzene	2.569	0	0	0.0017	0	0.00677	0
Heptane	2.97	0.0041906	0.00241	0.009195	0.00794	0.01103	0.00585
Hexane, 3,4-dimethyl-	4.01	1.89626	0	1.13659	2.61875	0.26003	0.04224
1-Octene	4.37	0.04362	0	0.02424	0.0463	0.00653	0.00051
Octane	4.5	0.013502	0.0001429	0.0094	0.02048	0	0.00084
Xylene	5.576	16.89644	1.76236	6.24379	12.206	1.80799	0.08831
Nonane	6.079	2.42824	0.1197	2.29448	8.0016	0.11047	0.27269
Nonane, 3-methyl-	7.27	0.06031	0	0.36836	0.2931	0	0
Decane	7.756	0.33016	0	4.19179	5.3093	1.86465	5.64353
Undecane	9.358	1.21564	0	2.55508	7.51772	0.60195	0.56261
Dodecane	10.869	0.08378	0	1.81978	2.96351	1.64062	2.53186
Tridecane	12.288	0.05759	0	0.3966	0.77747	0.22954	1.36965
Hexadecane	16.081	0.01268	0.00434	0.01077	0.04591	0.00966	0.02876
Nonadecane	19.314	0.06714	0.04561	0.12288	0.04919	0.07728	0.03758

Increase in hydrocarbon production in filamentous fungi by amino acids induction

1. L-leucine
2. L-phenylalanine
3. L-tryptophane
4. L-tyrosine
5. Benzoic acid
6. L-phenylglycine
7. L-phenylpyruvic acid
8. L-cysteine

Quantitative production of hydrocarbons by amino acids induction in *G. roseum*



Compound name	Molar Mass	Retention time (Min)	Hydrocarbons in Endophytic fungi	
			Oatmeal media (mg/L)	Oatmeal with Amino acids (mg/L)
Heptane	100	2.970	0.0392	1.2134
Hepatane, 4-methyl-	114.2	3.974	0.5786	3.0765
Hexane,3,4-dimethyl-	114.2	4.010	2.6188	
1-Octene	112	4.370	0.0463	
Octane	114	4.500	0.0205	0.1849
m-Xylene	106	5.576	16.8964	
Nonane	128	6.079	8.0016	
Nonane,3-methyl-	142.2	7.270	0.3684	26.8922
Decane	142.2	7.756	5.6435	79.0509
Undecane	156	9.358	7.5177	2.6911
Dodecane	170	10.869	2.9635	27.591
Tridecane	184	12.288	1.3697	

Conclusion

- Focus in the future will be on drop-in biofuels
- Platforms molecules using sugars for production of drop-in biofuels are getting closer to the market
- New biocatalysts are under developent both for sugar based platforms as well as for consolidated processes
- New concept will emerge such as the BioChemCat process having no need for enzymes
- Future biorefineries will be capable of producing biofuels or bioproducts from the whole biomass raw material including lignin

Special thanks to my group and collegues at BSEL, the Port of Benton, CleanVantage and PNNL.

Dr. Birgitte K. Ahring
Director and Battelle Distinguished Professor

Washington State University
Bioproducts Sciences and Engineering Laboratory

Phone:	**509.372.7683**
Email:	**cbb@tricity.wsu.edu**
Web:	**www.tricity.wsu.edu/bsel**

QUESTIONS?