Biofuel Production via Cellulose Fermentation

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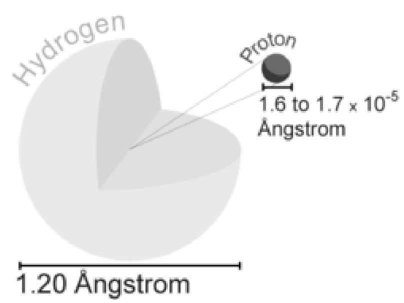


Cellulosic Biofuels

2nd Generation Biofuels

3rd Generation Biofuels

① Feedstock Pretreatment

Simpler feedstock processing

Extensive pretreatment
- Steam explosion
- Acid hydrolysis

② Saccharification

Endogenous Cellulase production

Exogenous Cellulase production

③ Fermentation

④ Biofuel recovery
- Ethanol
- Hydrogen

1st & 2nd Generation Biofuels

SSF & SSCF

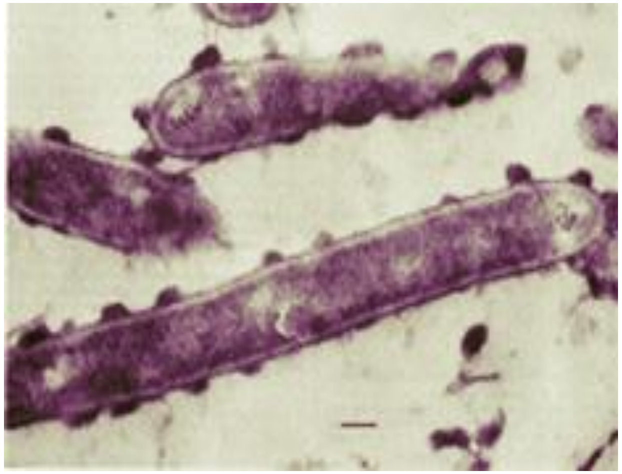
- **Simultaneous**
  - **Cellulase production**
  - **Substrate hydrolysis**
  - **Fermentation**

Lower energy inputs

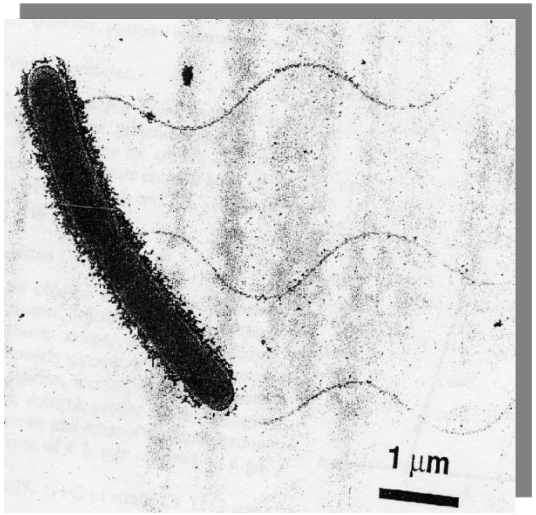
Higher conversion efficiencies

Biofuels from Direct Cellulose Fermentation

- ***Clostridium thermocellum***: thermophilic, cellulolytic, gram +ve, anaerobic
- ***Clostridium termitidis***: mesophilic, cellulolytic, gram +ve, anaerobic
- **Degrade cellulose and synthesize: Ethanol, H_2 and CO_2, VFAs - acetate (formate, lactate)**
- ***C. thermocellum* possesses the highest rate of cellulose-degradation of all known microorganisms**
- ***C. termitidis* cellulose hydrolysis comparable to *C. thermocellum***



Clostridium thermocellum

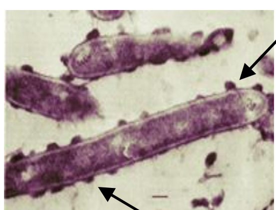


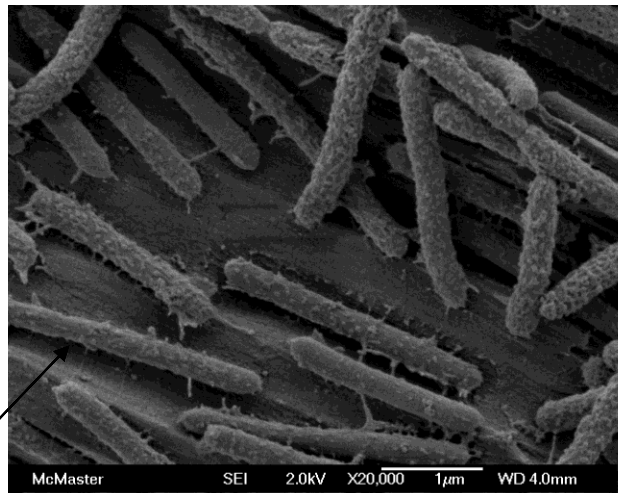
Clostridium termitidis

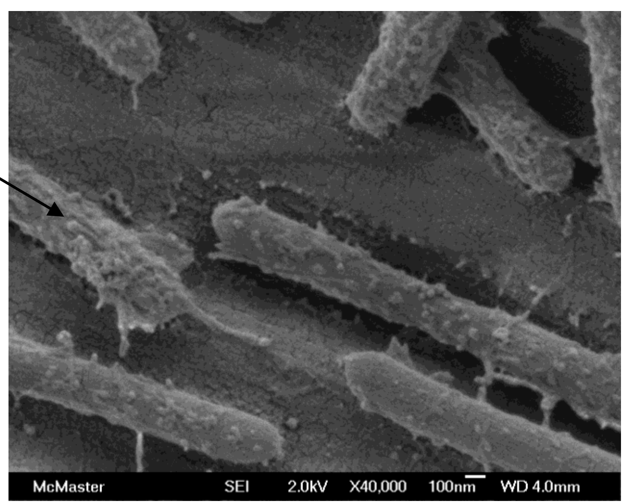
Biofuels from Direct Cellulose Fermentation

C. thermocellum and *C. termitidis* cultured on α-cellulose

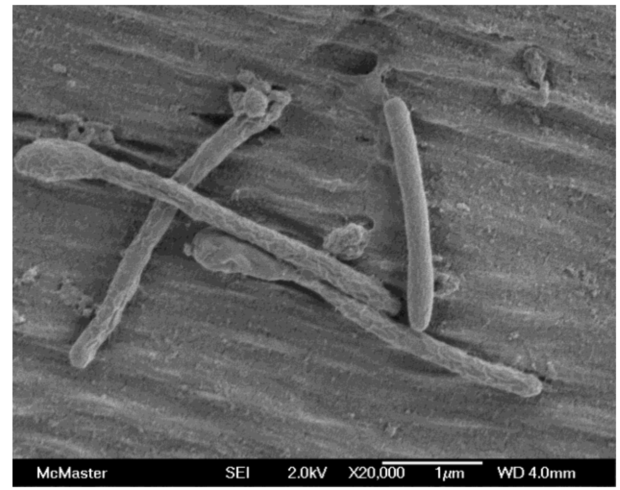
Clostridium thermocellum

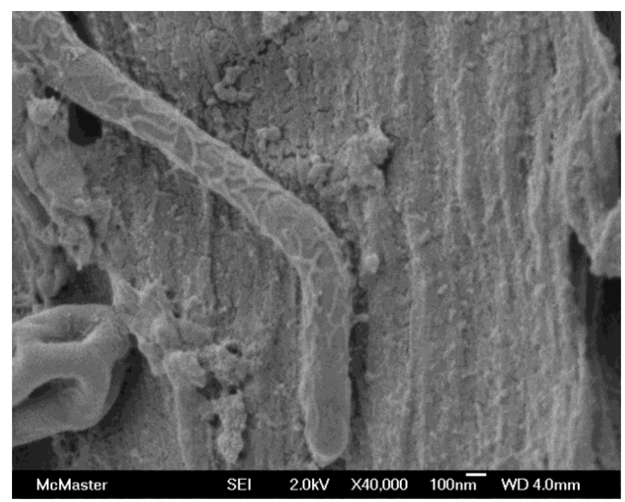






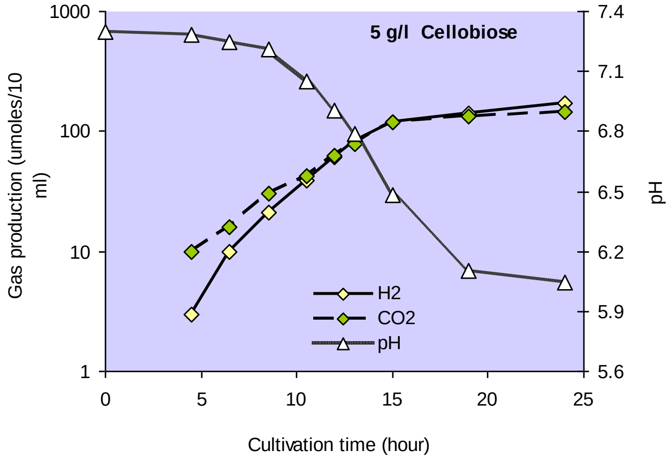
Clostridium termitidis



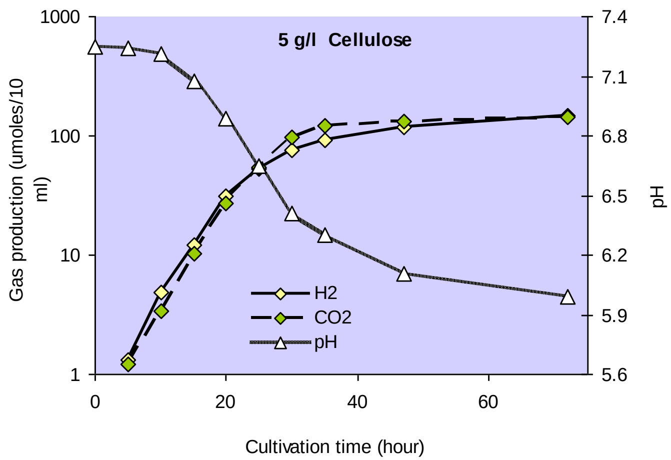


Gas production by *C. thermocellum*: Batch Cultures

A) H_2, CO_2, & pH change on cellobiose

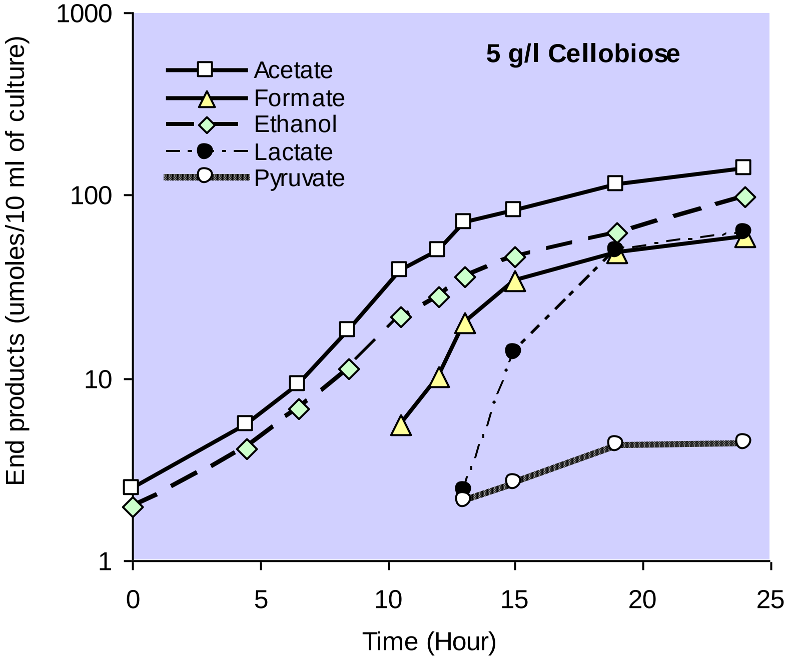


B) H_2, CO_2, & pH change on α-cellulose

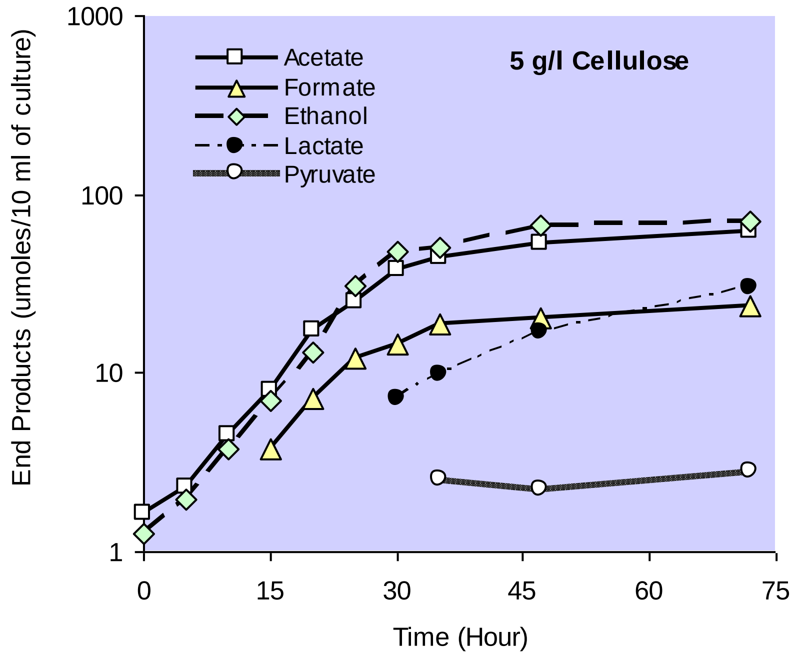


Solubl End-product Synthesis Patterns: Batch Cultures

A) VFAs & Ethanol on cellobiose



B) VFAs & Ethanol on α-cellulose



End-product Synthesis Patterns: Batch Cultures

- Rates of end-product synthesis by *C. thermocellum* are much slower when grown on α-cellulose compared with cellobiose

Production Rates of Fermentation End-products

mmoles/L/hr

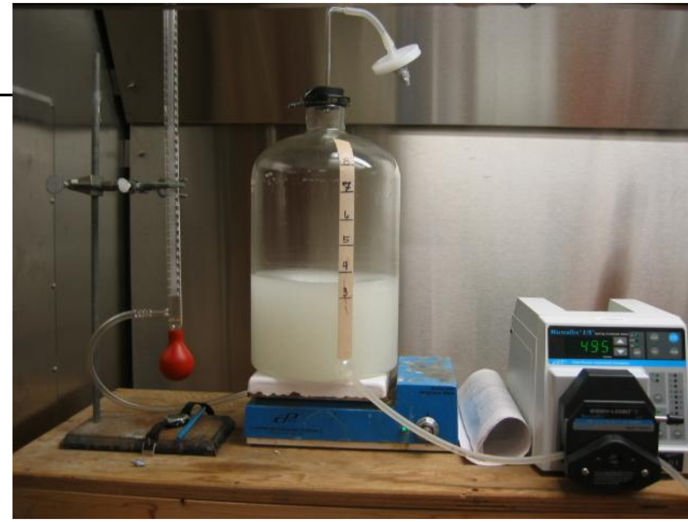
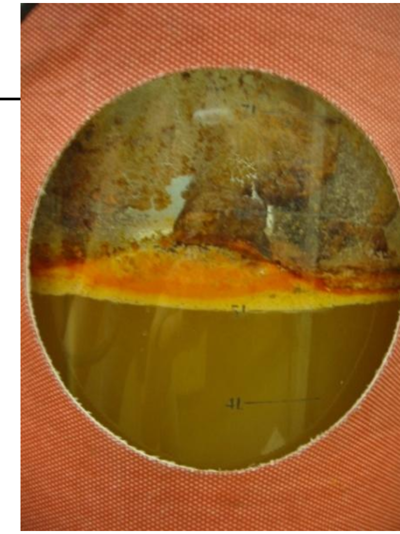
Substrate	H_2	CO_2	Acetate	Formate	EtOH	Carbon balance	O/R ratio
Cellobiose	2.1	1.5	2.00	0.98	0.80	84%	0.93
α-cellulose	0.45	0.54	0.25	0.10	0.33	79%	1.12

mmoles/g protein/hr

Substrate	H_2	CO_2	Acetate	Formate	EtOH	Carbon balance	O/R ratio
Cellobiose	29.6	24.36	50.79	13.60	14.19	84%	0.93
α-cellulose	9.84	8.65	4.79	2.76	6.49	79%	1.12

Reported specific rates are the highest rates obtained within samples collected during exponential phase: Cellobiose, 12 hr post-inoculation; α-cellobiose, 20 hrs post-inoculation.

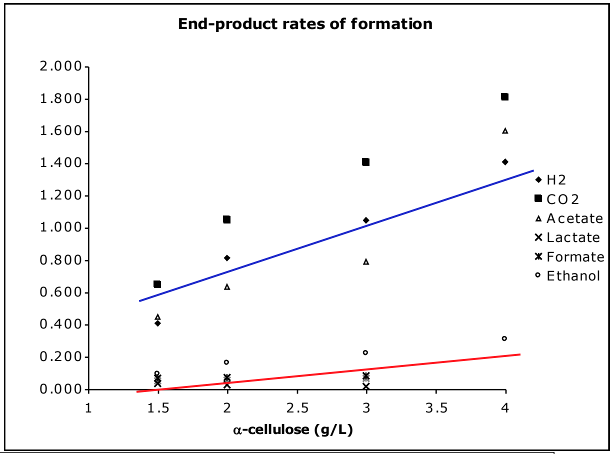
Continuous Culture of *C. thermocellum* on α-cellulose

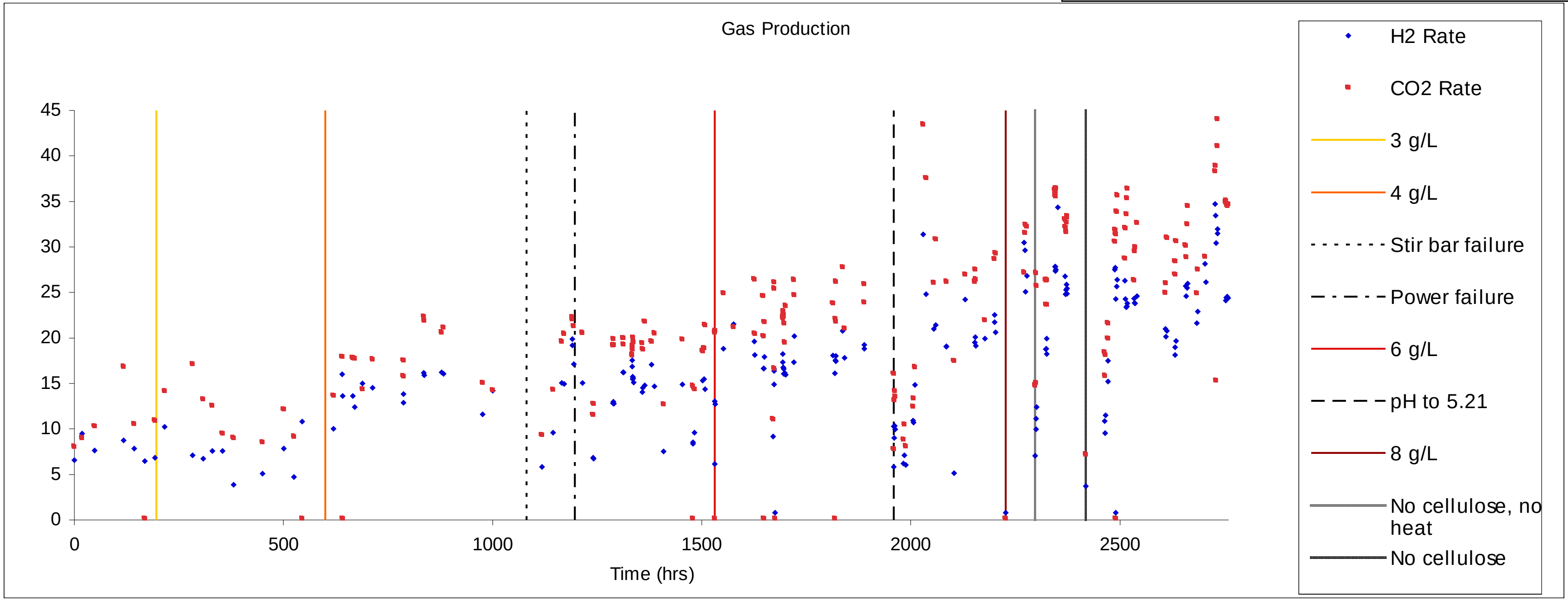




- **Continuous culture of *C. thermocellum* for over 3 months**
- **Tested α-cellulose concentrations from: 1.5 - 4 g/L (0.015 to 0.4%)**
- **pH maintained at 7.0**
- **Temperature maintained at 60 ^{o}C**

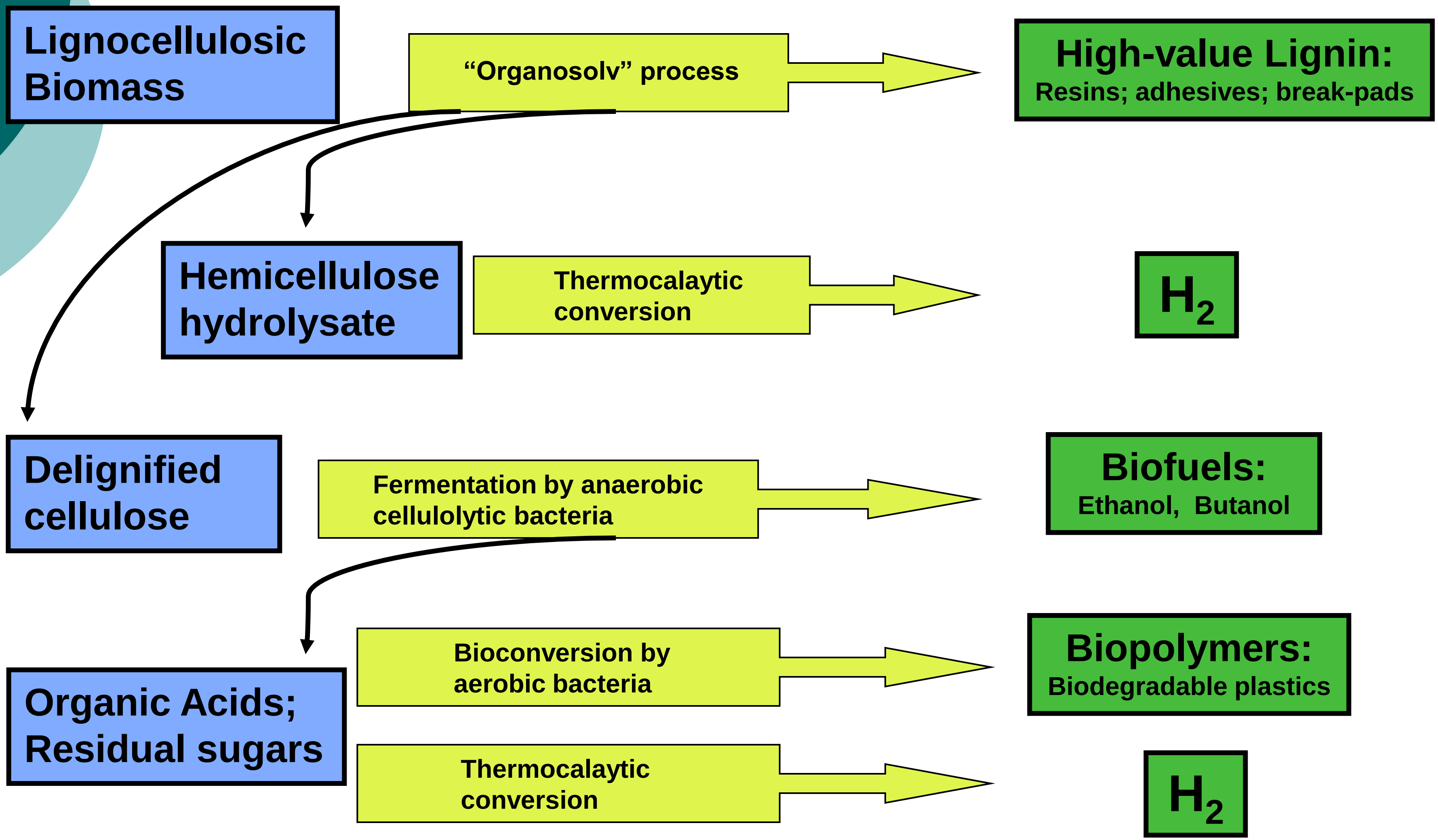
Continuous Culture of *C. thermocellum* on α-cellulose

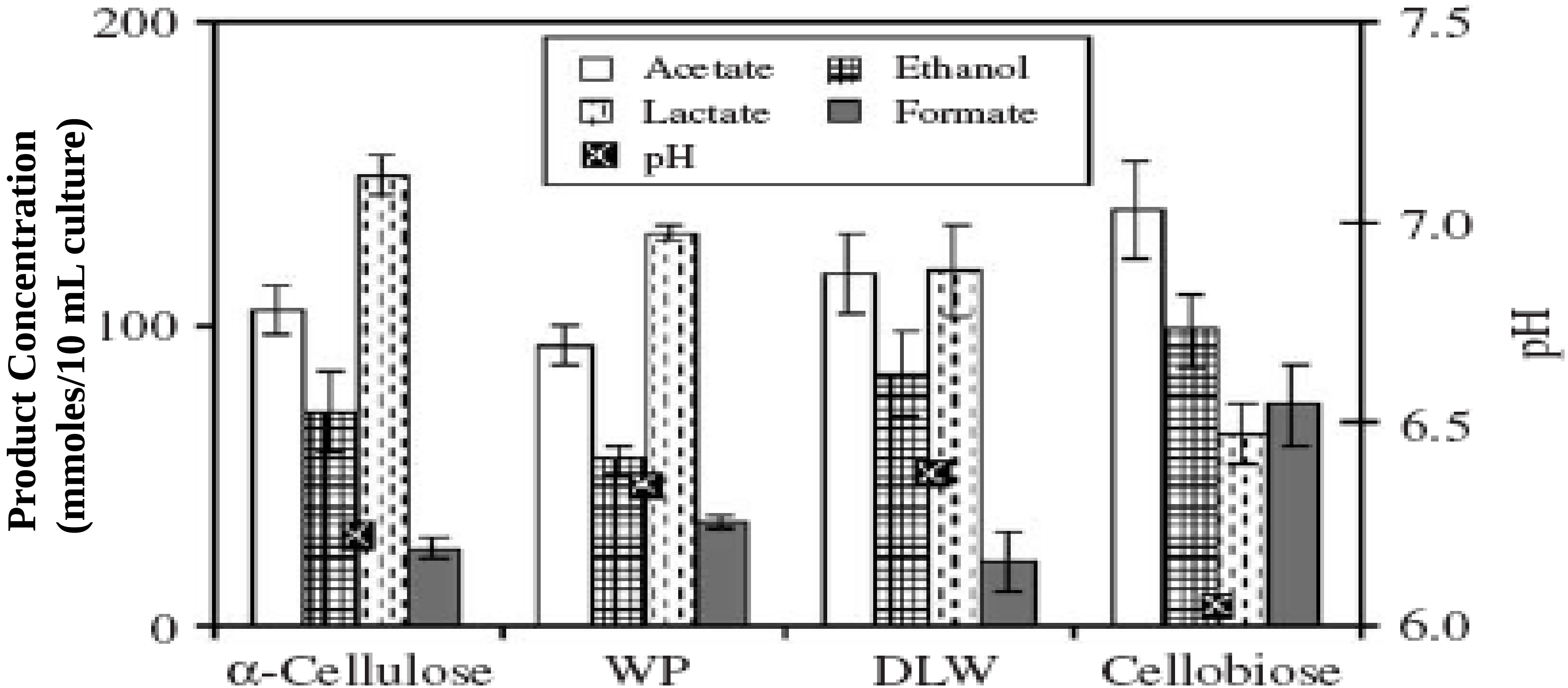
- **Gas production profile over 125 days (3000 hrs)**
- **Fermentation recovered rapidly from system failures**





Biorefining Concept

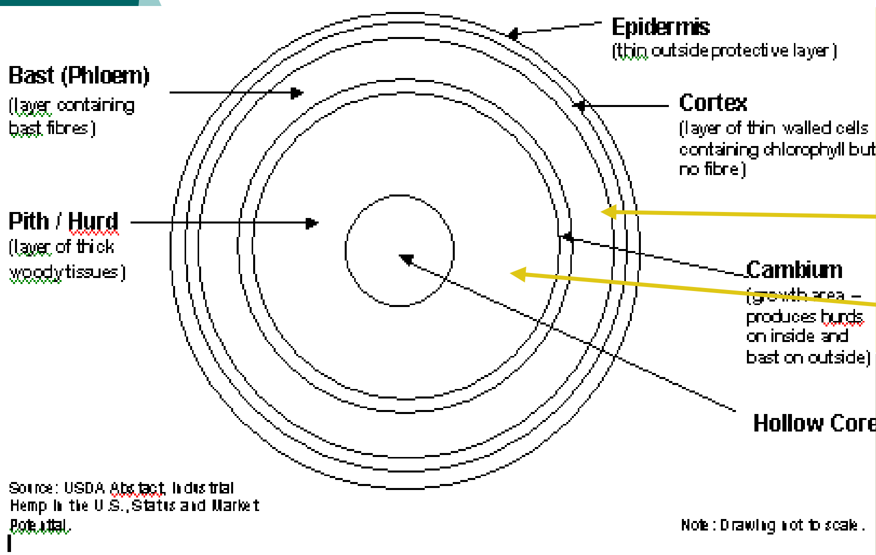




- **Fermentation end-products of *C. thermocellum* and corresponding pH values at the end of growth for α-cellulose, Whatmann paper (WP), delignified softwood (DLW,) and cellobiose, at 4.5 g/L. Product amounts are expressed in total μmoles produced per 10 mL culture. From: Levin *et al*., 2006. IJHE 31(11), 1496-1503**

Potential Feedstocks

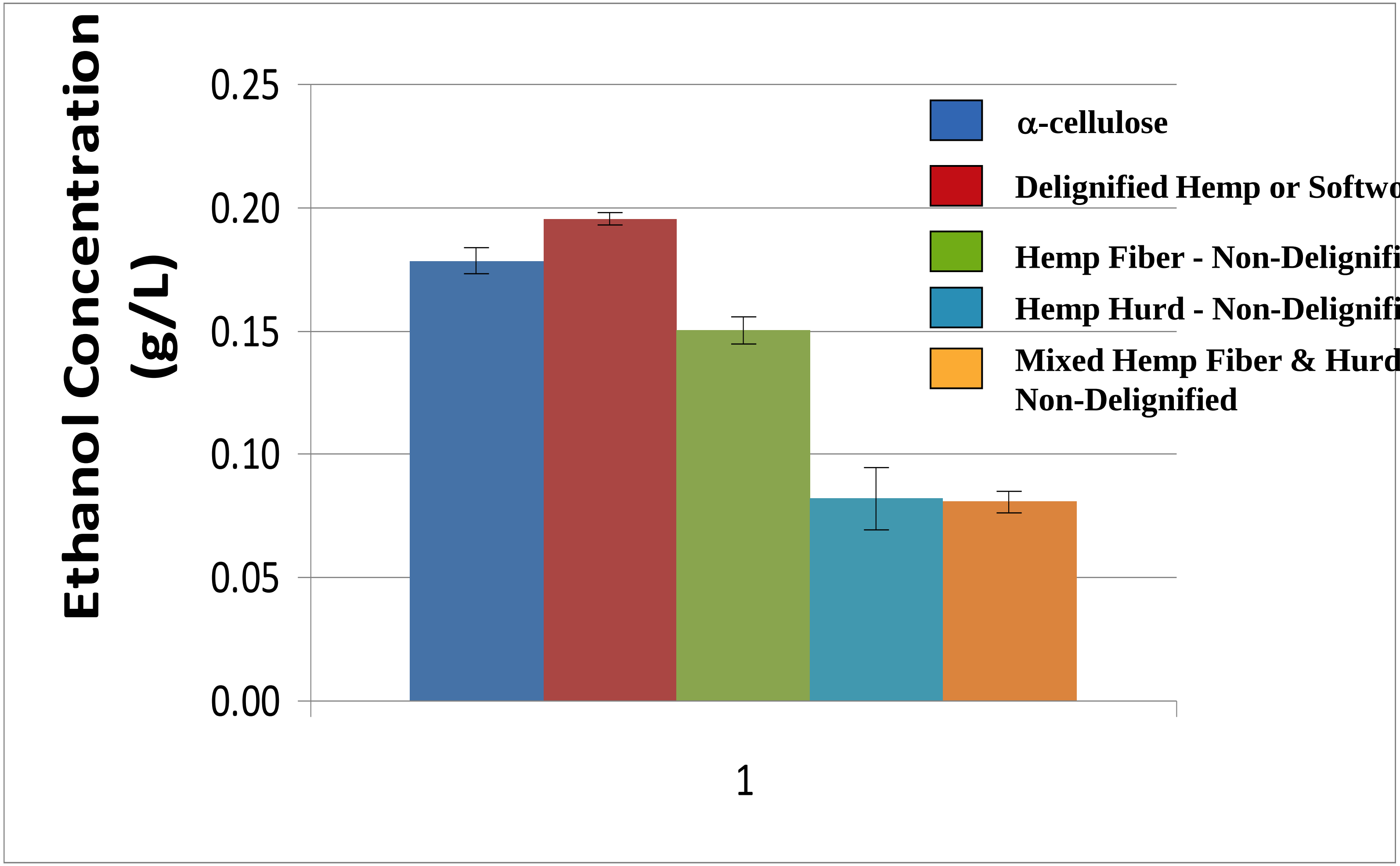
- Want to use abundant, inexpensive cellulose residues for fermentation substrate
- Initial experiments are focused on hemp and softwoods



Hemp	Cellulose	Hemicellulose	Lignin
Bast	64.8 %	7.7%	4.3 %
Core (Hurd)	34.5 %	17.8%	20.8 %
Soft Pine	44%	26%	27.8%
Spruce	42%	27%	28.6%
Wheat Straw	34%	27.6%	18%
Rice Straw	32.1%	24.0%	12.5%
Corn Stover	28%	28%	11%
Switchgrass	32.5%	26.4%	17.8%

Chemical composition of Industrial Hemp as compared to other plant matter

Ethanol Production by *C. thermocellum* on Various Substrates at 60 ^oC

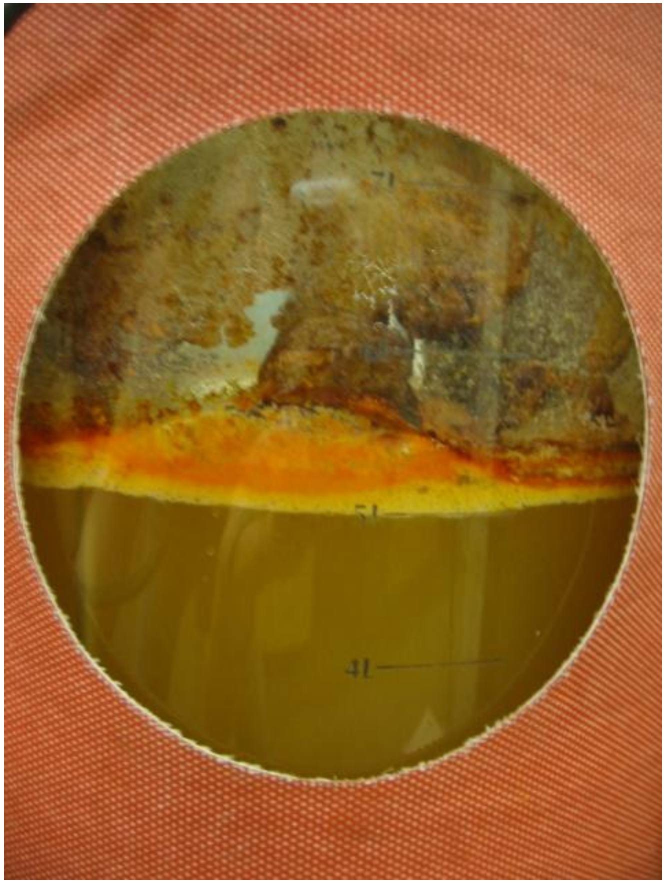


Potential for Ethanol Production by Cellulose Fermentation with *C. thermocellum*

- **Husky Energy grain-based ethanol production: 15% = 150 g/L**
- **Best ethanol production by *C. thermocellum* with delignified wood or hemp @ 2g/L: 0.17 g/L**
- **Possible ethanol production if substrate concentration is increased to 200 g/L: 17% = 170 g/L**
- **Can we get there? How do we get there?**

BioProcess Engineering: High Solids Fermentation

- Initiating fermentation studies with delignified wood fibers with the goal of achieving substrate concentrations of 200 to 250 g/L cellulose



BioProcess Engineering: High Solids Fermentation

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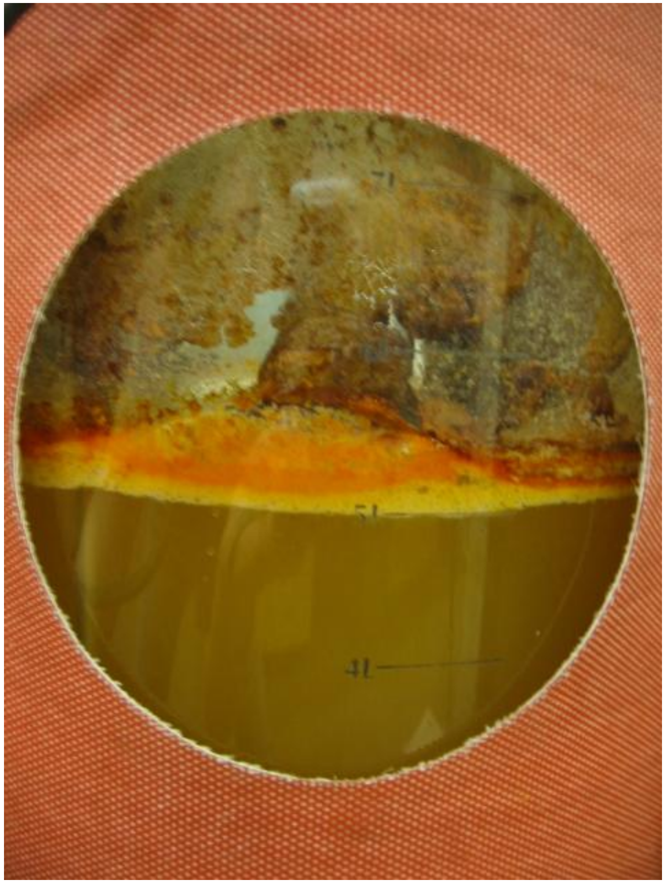
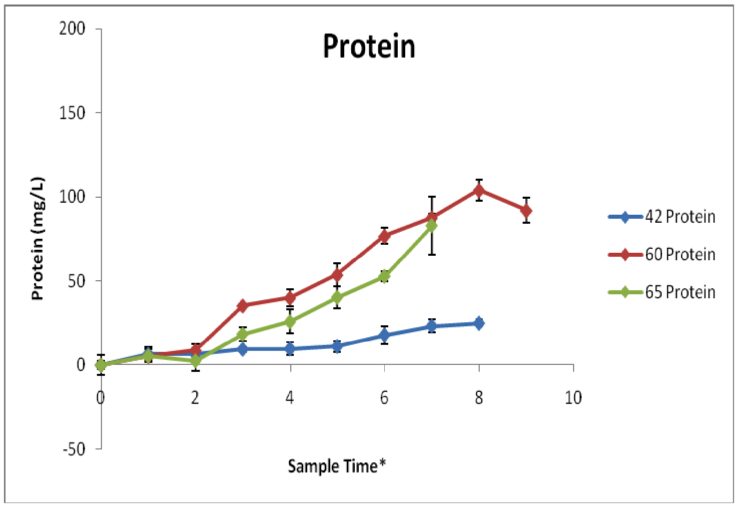
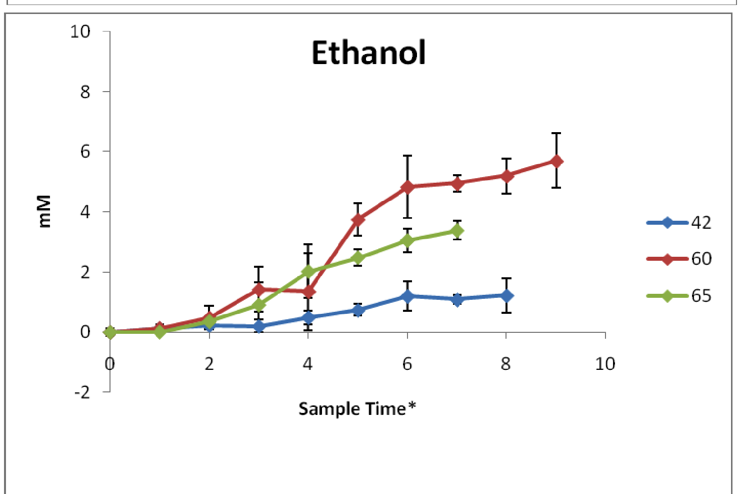


BioProcess Engineering:
Higher Temperature Fermentation

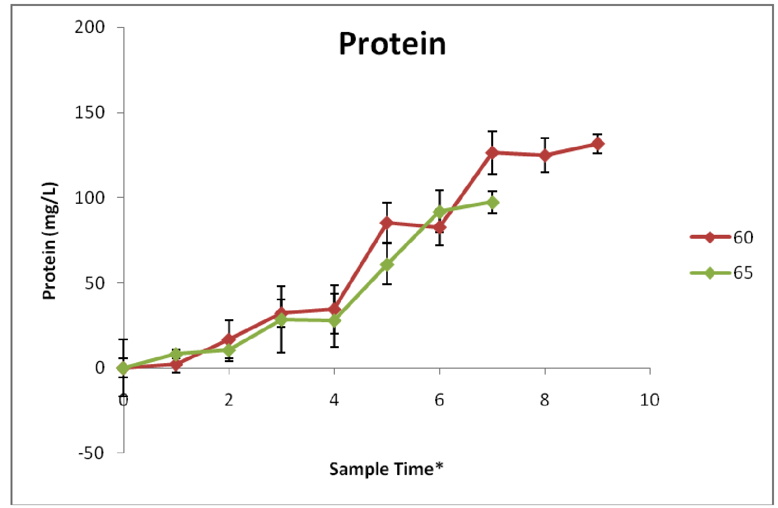
- *C. thermocellum* produces more ethanol at 65 ºC than at 60 ºC
- More ethanol in the vapor phase at higher temperature

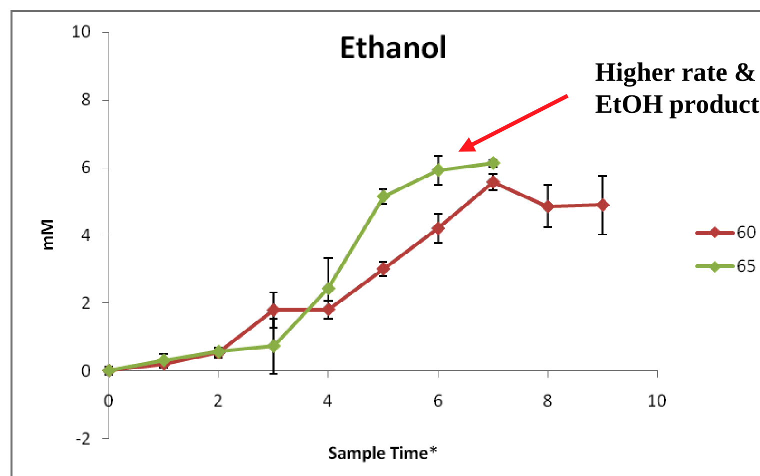
α-cellulose





Delignified Wood

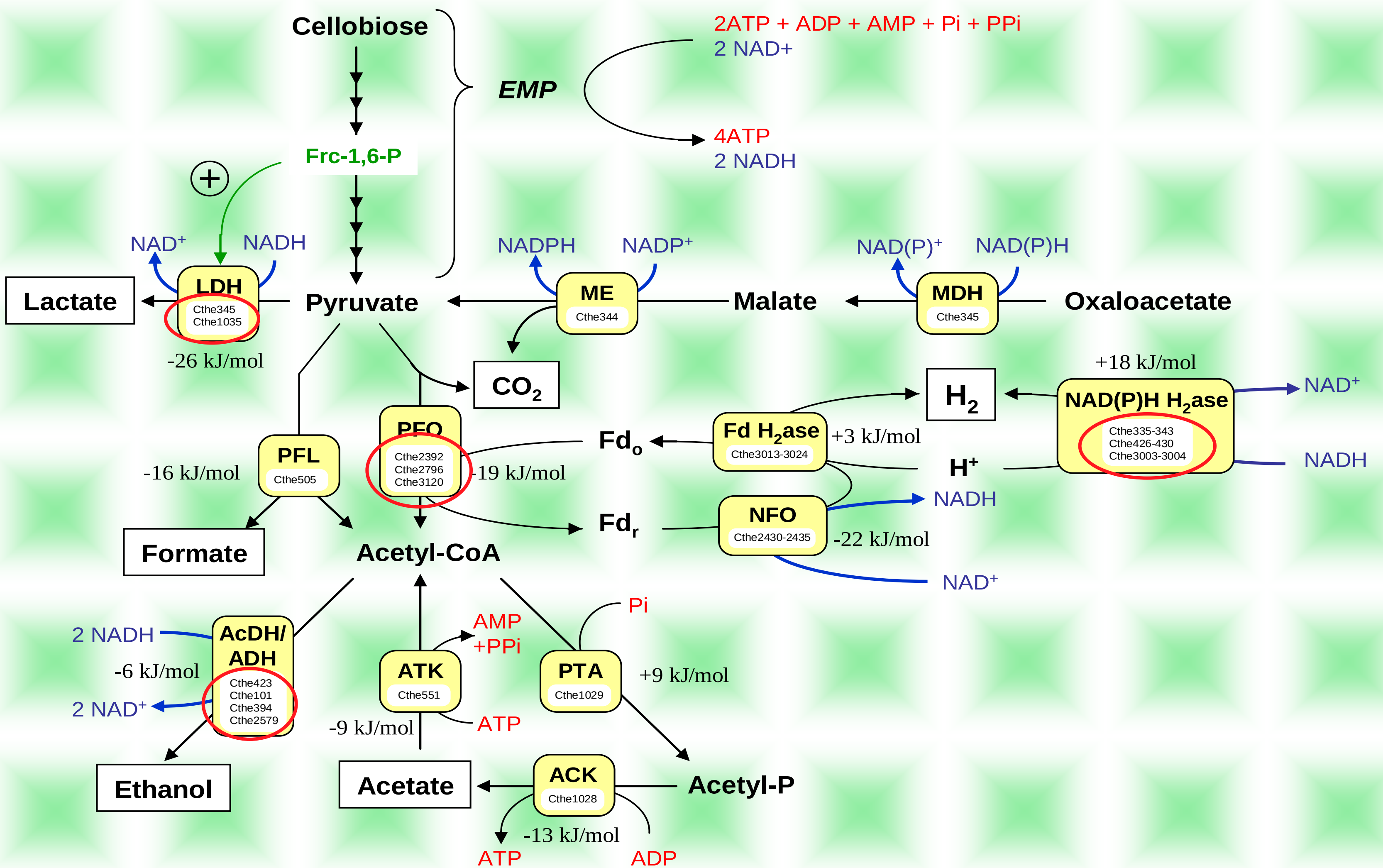




Higher rate & specific EtOH production @ 65 ºC

Manipulating Metabolism

Improved production requires understanding of microbial metabolism:
***C. thermocellum* - mixed product fermentation**
- multiple homologues of key genes



Manipulating Metabolism: Metabolic shifts

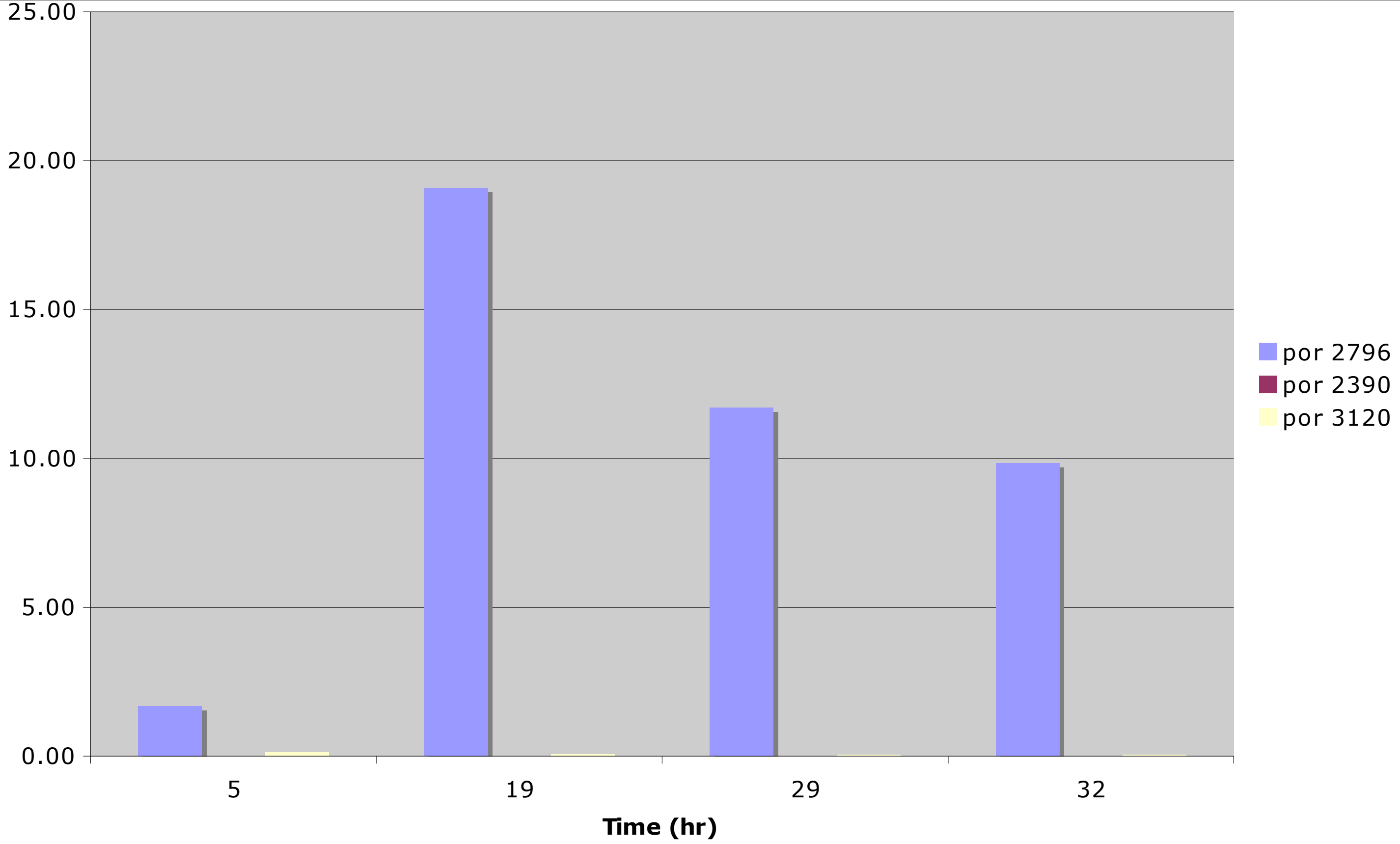
- Addition of acetate to the headspace at start of fermentation increased ethanol production by 28%

l Product (mM)	End Product Added					
	None	H_2	Acetate	Ethanol	Formate	Lactate
Biomass	2.4 (0.2)	2.4 (0.2)	2.4 (0.1)	2.1 (0.1)	2.6 (0.2)	2.2 (0.2)
H2	4.1 (0.1)	—	3.8 (0.1)	6.3 (0.3)	4.7 (0.3)	5.7 (0.1)
CO2	6.2 (0.2)	4.6 (0.1)	6.4 (0.6)	5.8 (0.2)	6.2 (0.2)	7.0 (0.1)
Acetate	3.2 (0.4)	2.9 (0.2)	—	3.9 (0.1)	2.8 (0.2)	4.6 (1.1)
Ethanol	4.3 (0.3)	4.8 (0.1)	5.5 (0.2)	—	4.7 (0.9)	5.1 (0.1)
Formate	2.2 (0.4)	3.1 (0.3)	1.7 (0.6)	1.9 (0.1)	—	0.5 (0.1)
Lactate	0.1 (0.1)	0.1 (0.0)	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	—

Multiple Homolgues of Key Genes:

Which genes are transcribed and at what level?

Transcription of *por* genes in *C. thermocellum* grown on α-cellulose

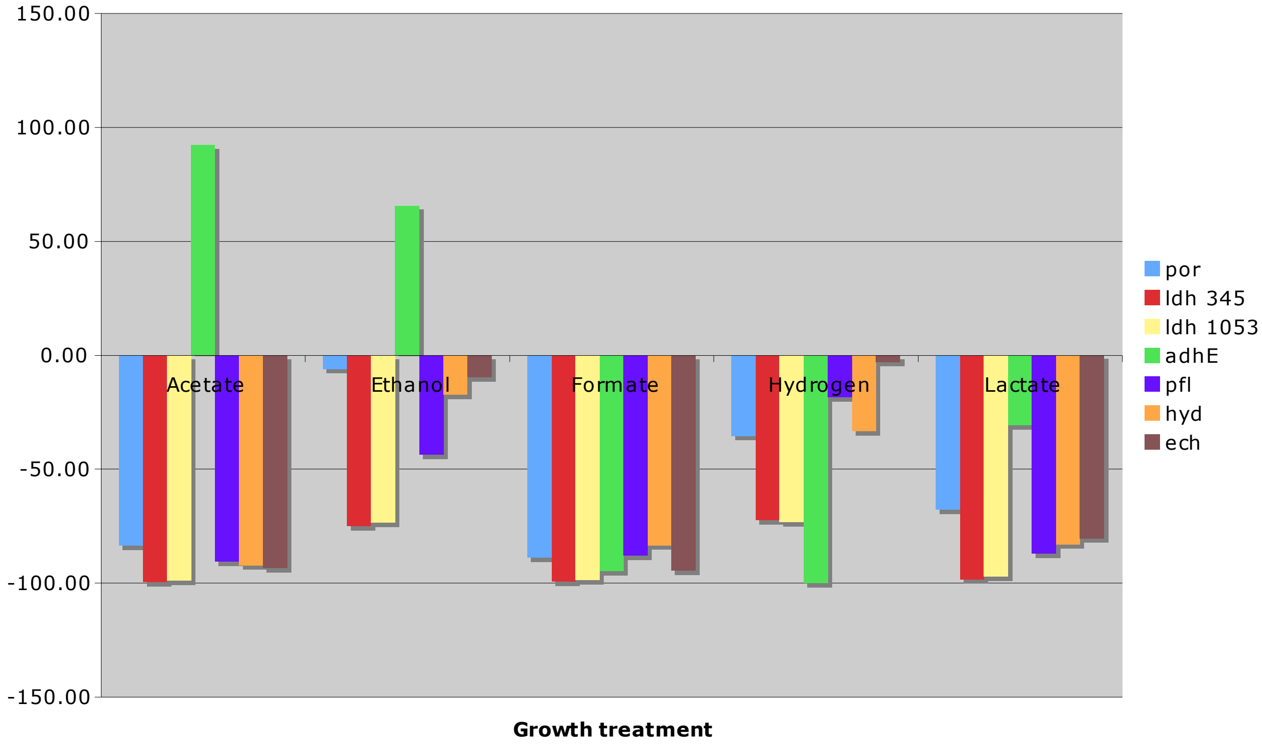


- ***por*** **2796 is most activity transcribed in *C. thermocellum* grown on α-cellulose**
- **transcription of *por* 2930 was orders of magnitude below *por* 2796**
- **transcription of *por* 3120 was below detectable levels**

Multiple Homolgues of Key Genes:

Which genes are transcribed and at what level?

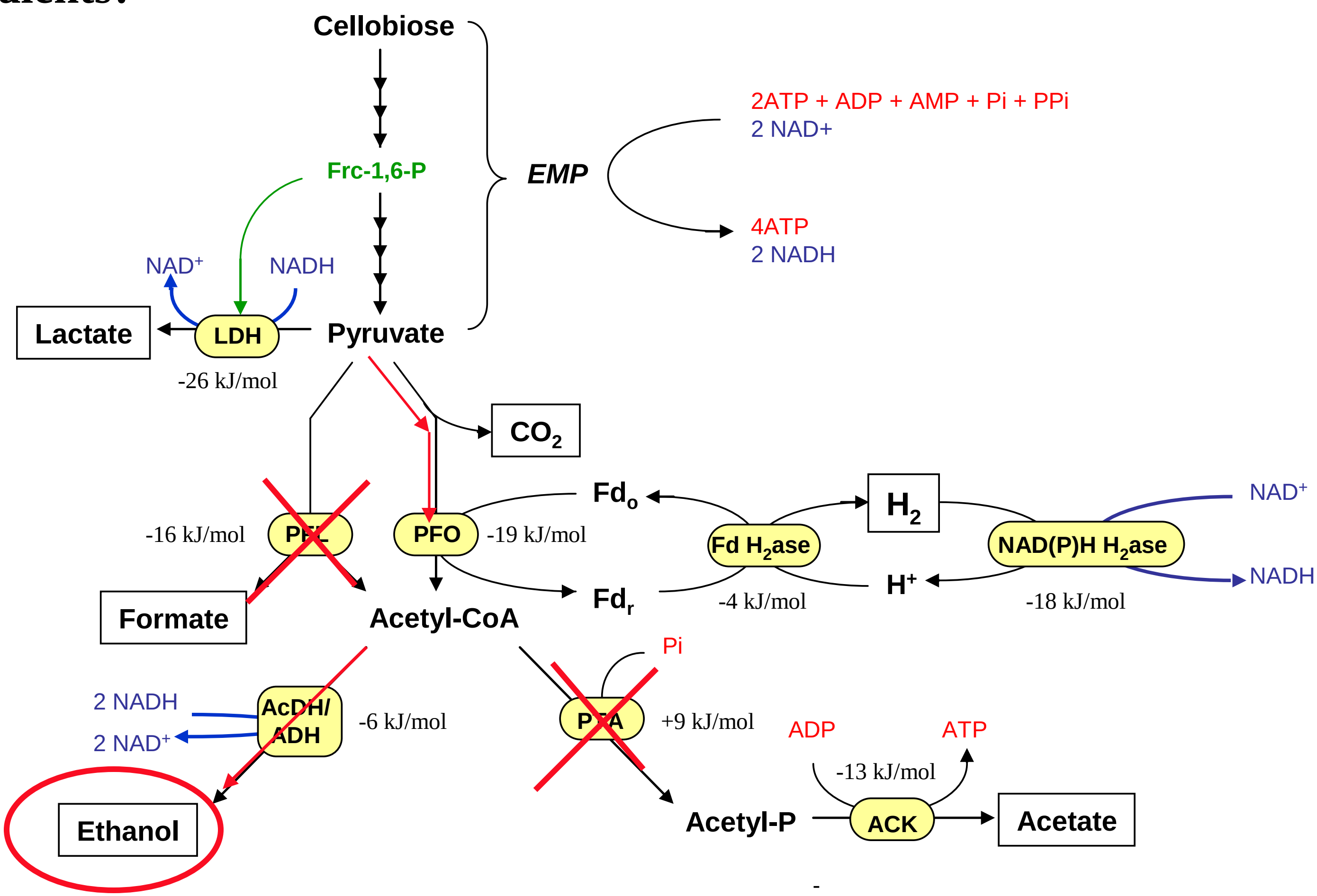
Transcription levels in presence of 10-fold excess end-products in *C. thermocellum* grown on cellulobiose



Gene	Annotated number	Growth treatment				
		Acetate	Ethanol	Formate	Hydrogen	Lactate
por (α)	2796	-83.68	-6.09	-88.80	-35.37	-67.64
ldh	345	-99.52	-74.95	-99.32	-72.43	-98.40
ldh	1053	-98.96	-73.48	-98.79	-73.24	-97.06
adhE	423	$\boxed{92.20}$	$\boxed{65.56}$	-94.68	-100.00	-30.70
pfl	505	-90.52	-43.60	-87.82	-18.46	-87.18
hyd	430	-92.20	-17.19	-83.51	-33.31	-83.05
echA	3013	-93.42	-9.61	-94.36	-2.87	-80.46

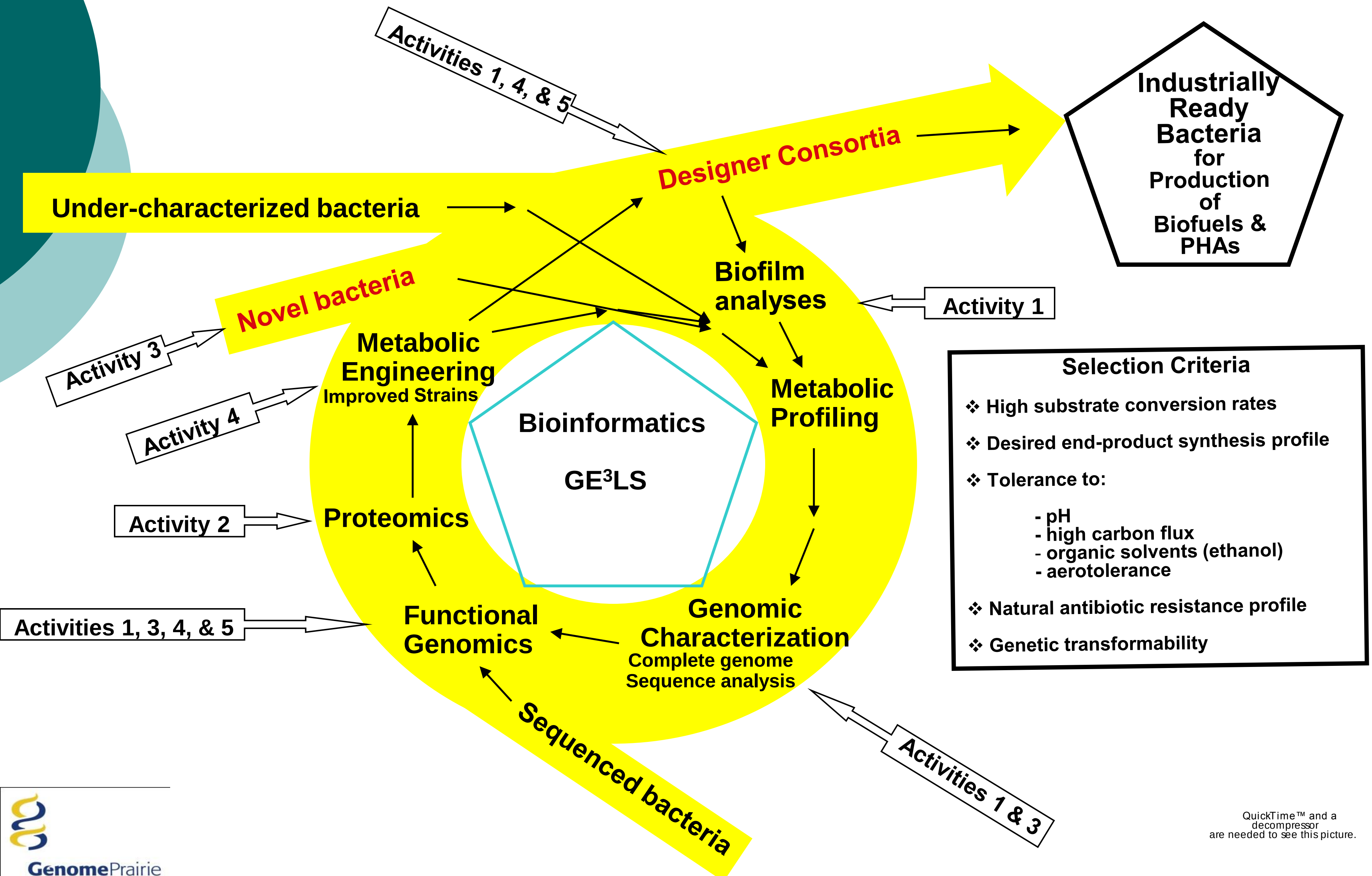
Manipulating Metabolism: Metabolic Engineering

- **Can we redirect carbon and electron flow toward H_2 or ethanol synthesis pathway, and away from pathways that compete for carbon and reducing equivalents?**



Genome Canada Project

“Microbial Genomic for Biofuels & CoProducts



GenomePrairie


Thank-you
Questions?
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