Consolidated Bioprocessing

An Alternate Method for Lignocellulose Processing

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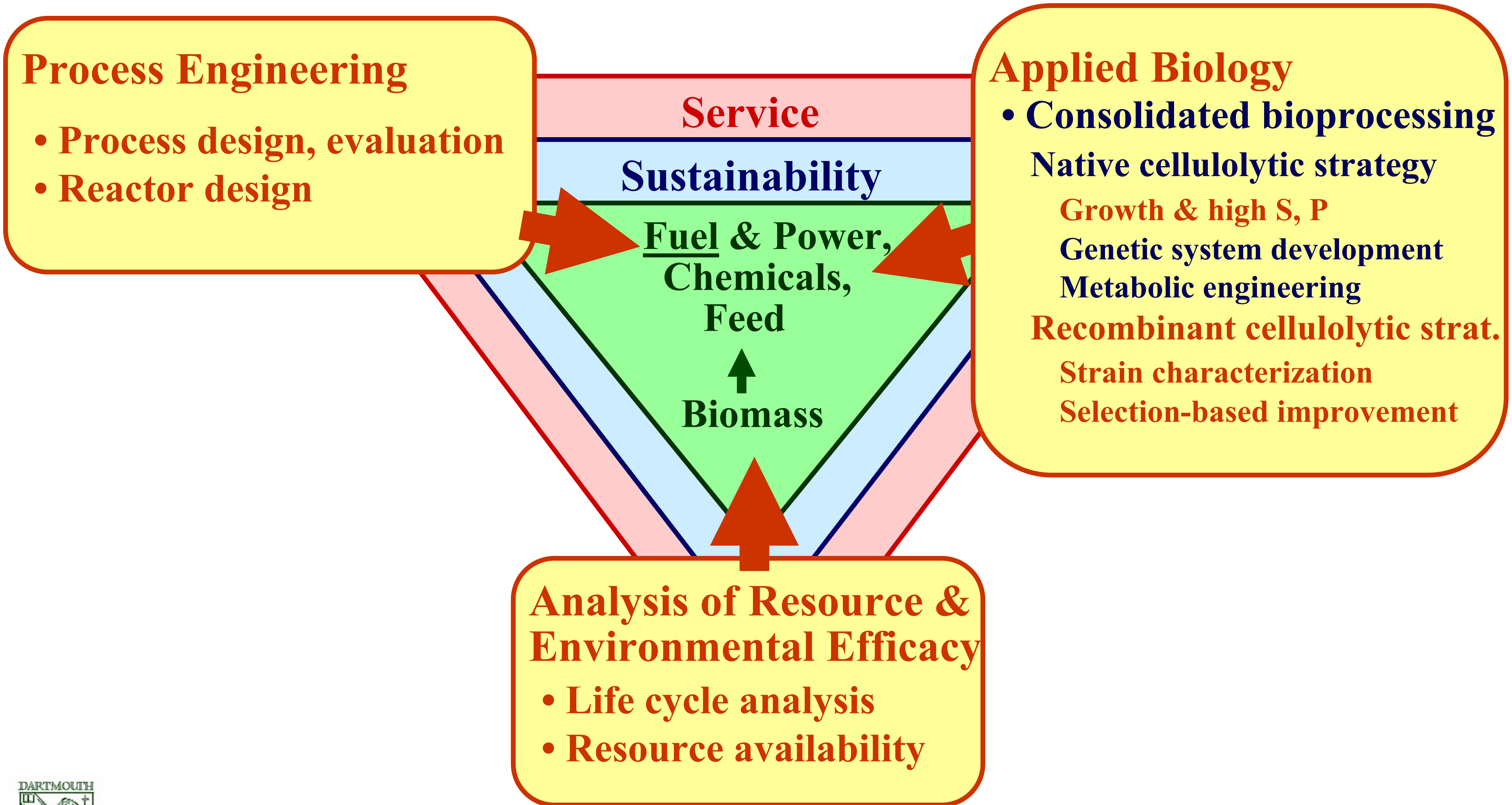
Lynd Group Activity and Motivation Map



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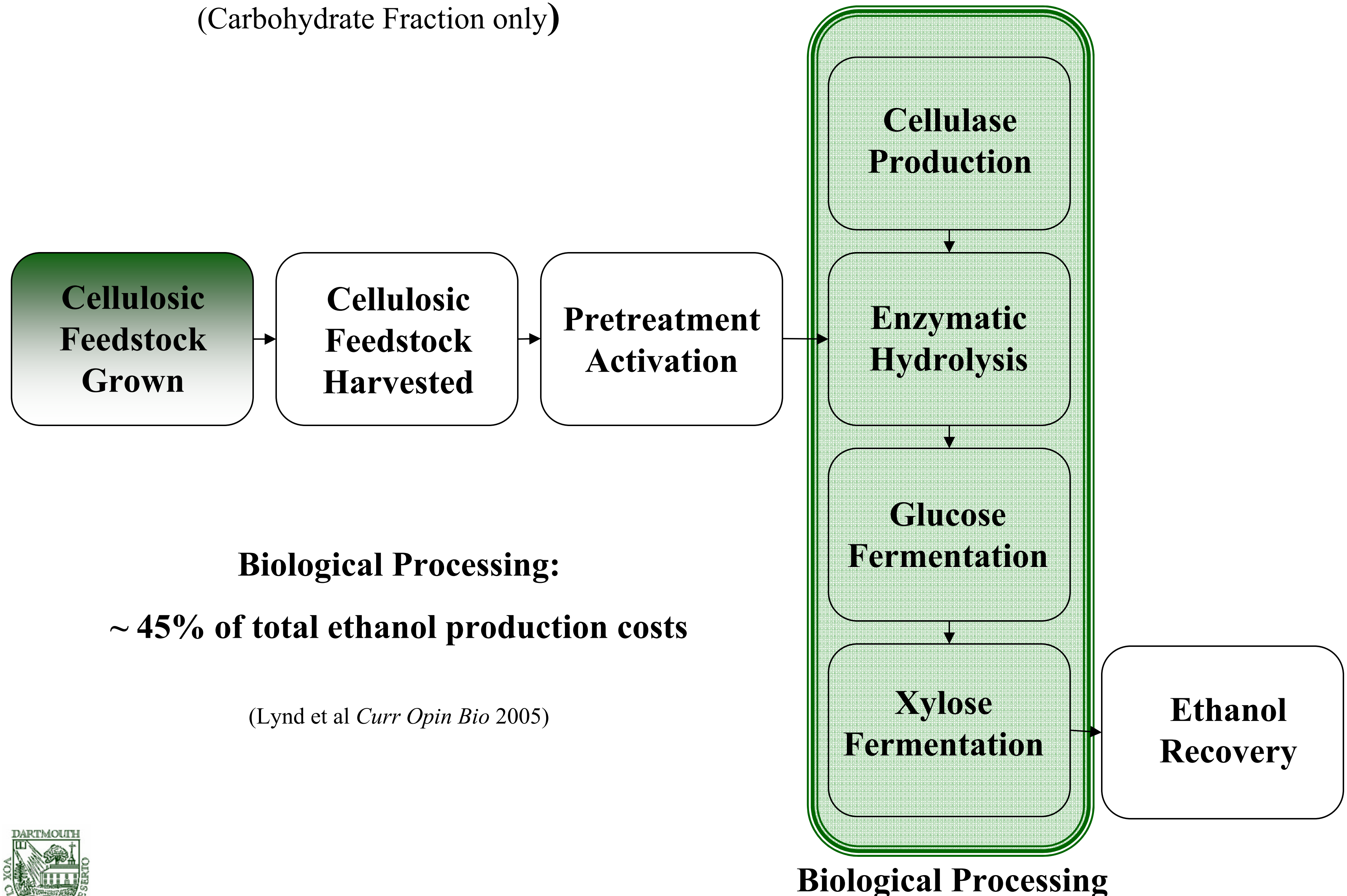
Cellulosic Biomass Conversion to Ethanol

(Carbohydrate Fraction only)



Biological Processing:

~ 45% of total ethanol production costs

(Lynd et al *Curr Opin Bio* 2005)

Evolution of Biomass Processing Featuring Enzymatic Hydrolysis

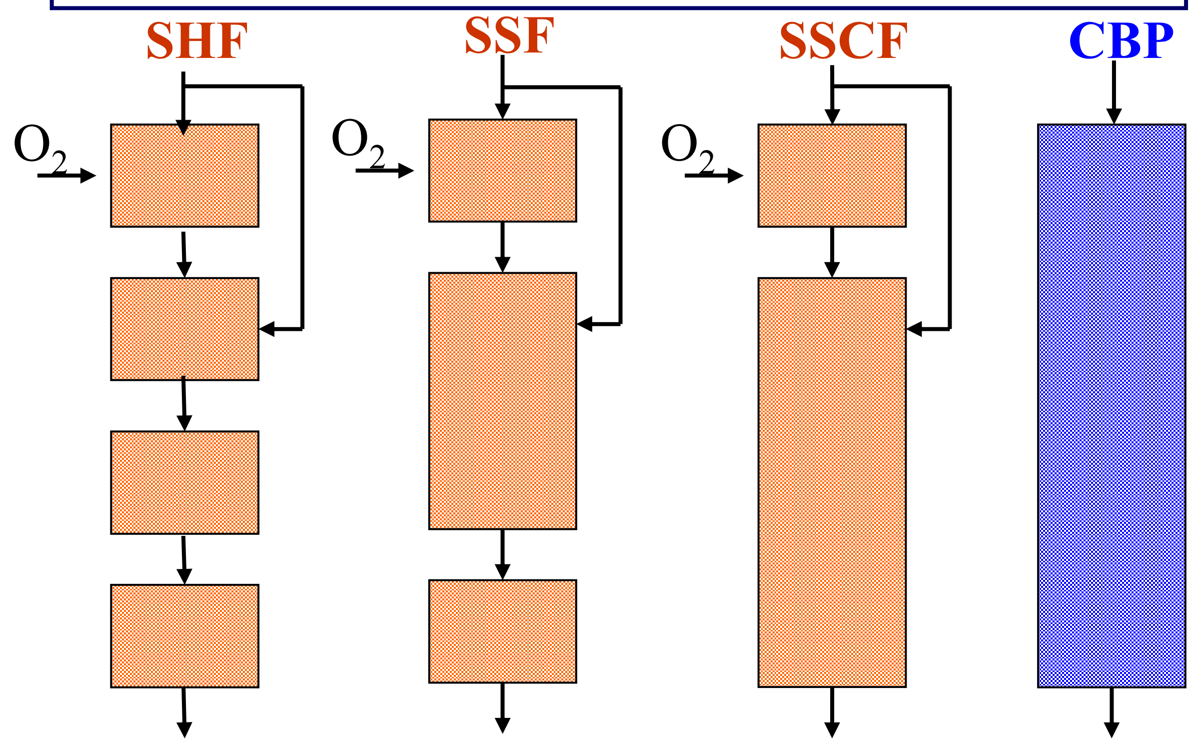
Biologically-Mediated Event	Processing Strategy (each box represents a bioreactor - not to scale)

Cellulase production

Cellulose hydrolysis

Glucose fermentation

Xylose fermentation



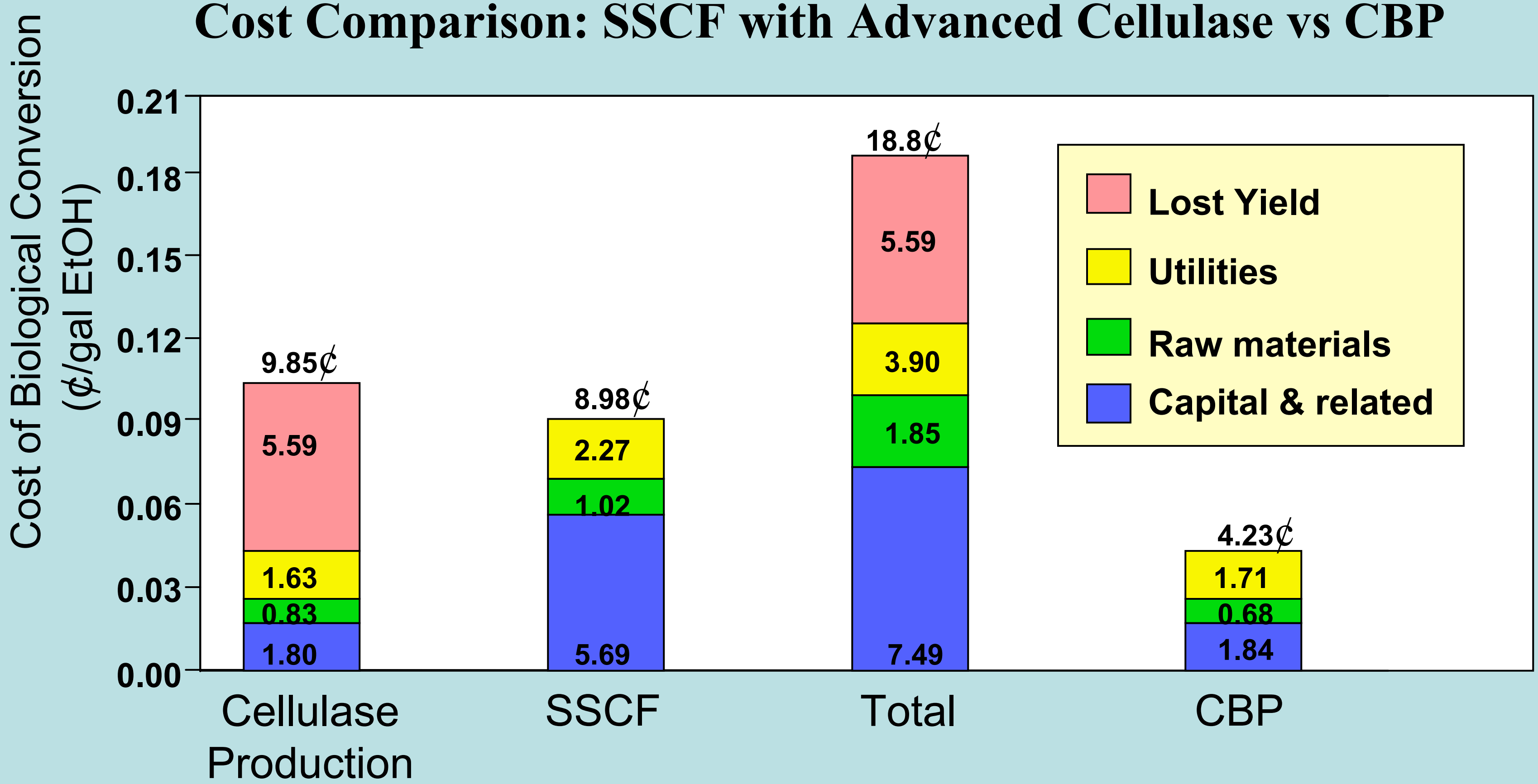
SHF: Separate hydrolysis & fermentation

SSF: Simultaneous saccharification & fermentation

SSCF: Simultaneous saccharification & co-fermentation

CBP: Consolidated bioprocessing

Cost Comparison: SSCF with Advanced Cellulase vs CBP



Plant scale, 5,000 tpd; Hydrolysis conversion, 95%; Fermentation yield, 95%; Ethanol concentration, 50 g/L; Temp, 37°C

Cellulase costs based on Wooley et al., 1999. SSCF costs from RBAEF process models, 7 day reaction time

Lynd et. al., Curr. Opin. Biotechnol., 2005

Substituting CBP for SSCF with advanced cellulase:

> 4-fold reduction in cost of biological processing

> 2-fold reduction in the cost of processing overall

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CBP: A Widely-Applicable Breakthrough in Low-Cost Cellulose Processing

Strategies to Create a CBP Organism

I. Engineer cellulase enzymes into a native ethanol producer

(yeast)

II. Engineer a native cellulose user to produce ethanol

(thermophilic bacteria)

I. Yeast (*Saccharomyces cerevisiae*)

Advantages

- Very good ethanol producer from soluble sugars
- Strain has been engineered to use xylose efficiently
- Most industrial experience with this strain

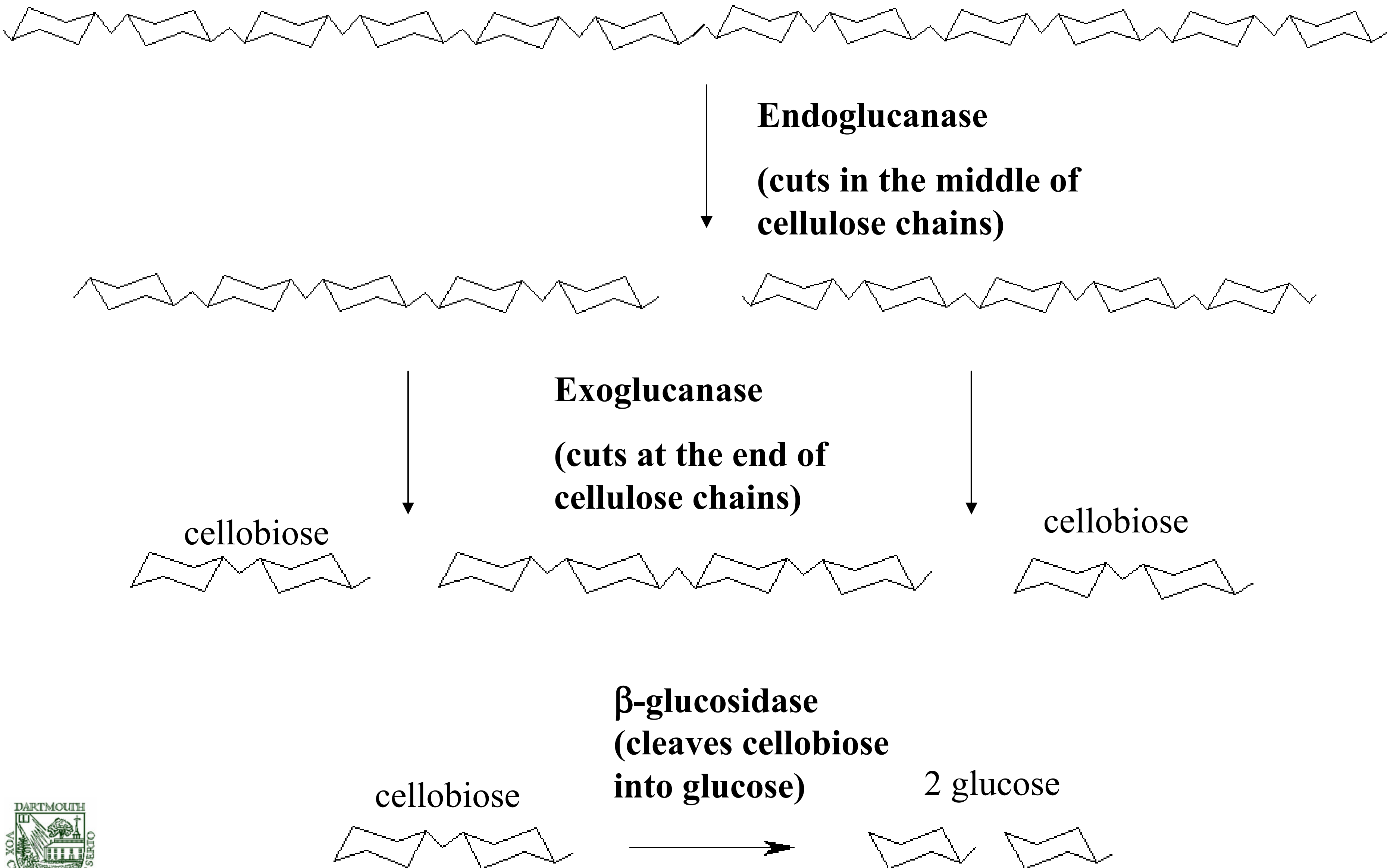
Challenges

- Cellulose degradation is one of the most difficult biochemical reactions to catalyze
- Multiple cellulase enzymes expressed at high levels

Goal

- Create a yeast strain that is able to grow on cellulose

Basic Mechanism of Cellulose Hydrolysis

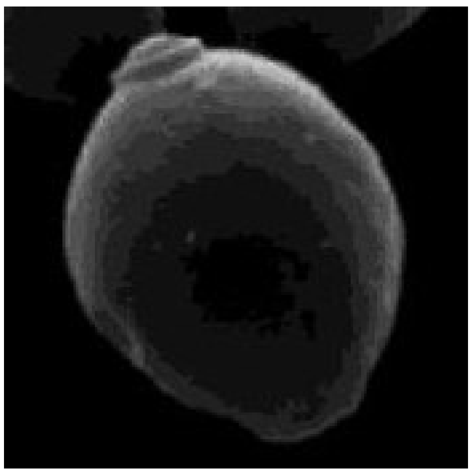


What will it take?

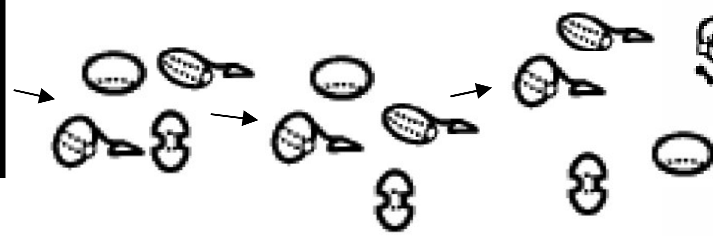
How much cellulase expression is enough?

Demand of usable substrate for growth:

q = grams of usable sugar needed/ gram cell/hour = $\mu / Y_{X/S}$

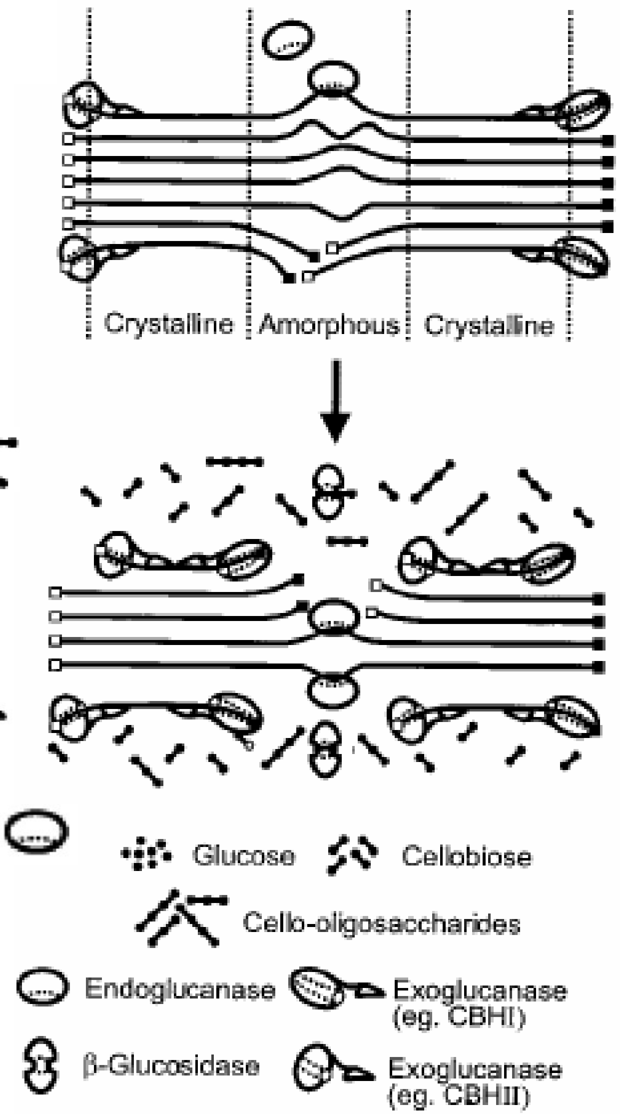


Sufficiency = a/q_{max}



Supply: Secretion of cellulase with a cell-specific activity:

a = grams of usable sugar produced/ gram cell/hour

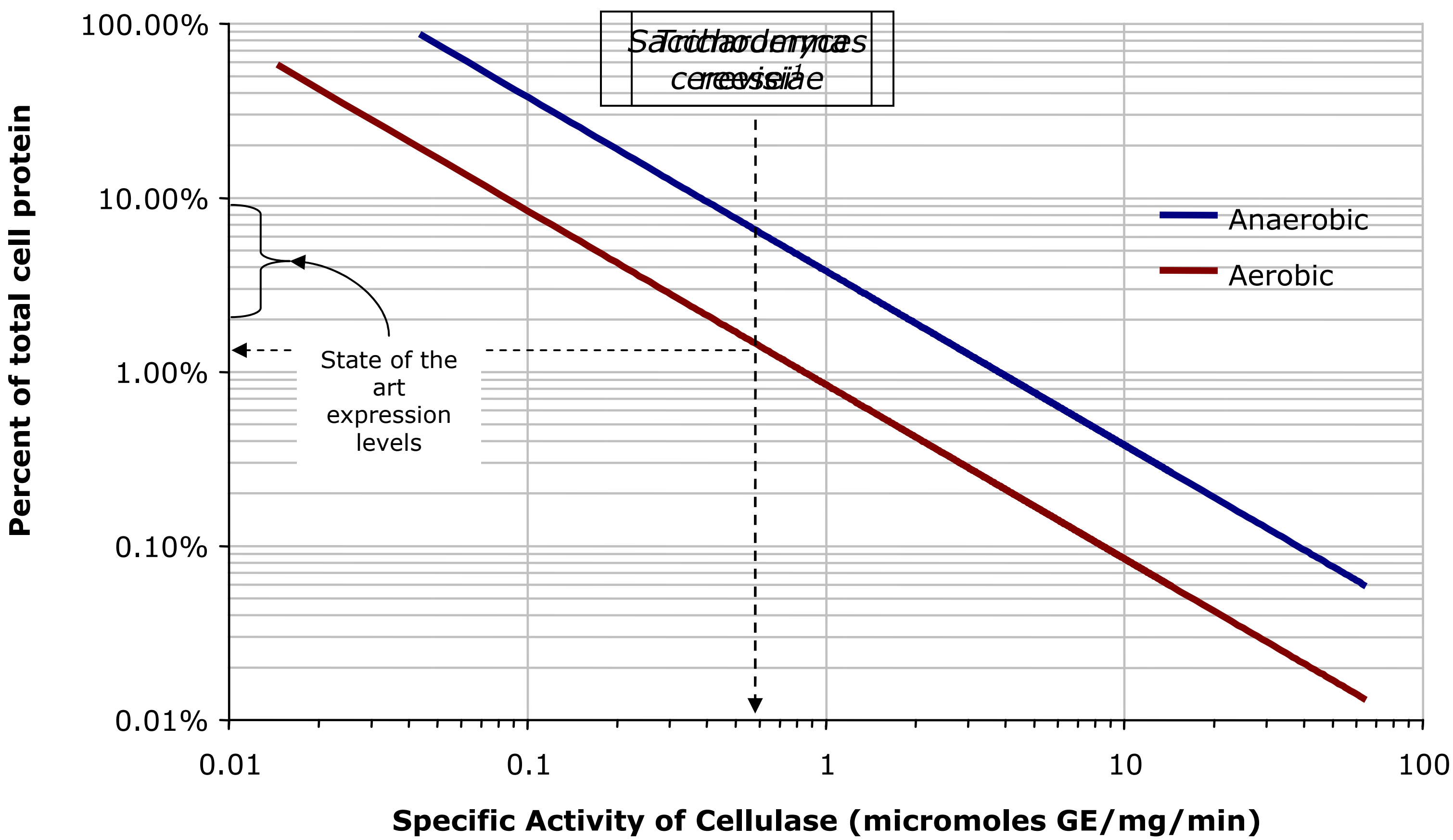


Extension of analysis: feasibility

Demand: $q=(\mu/Y_{X/S})$ (g GE/g cell/hr)

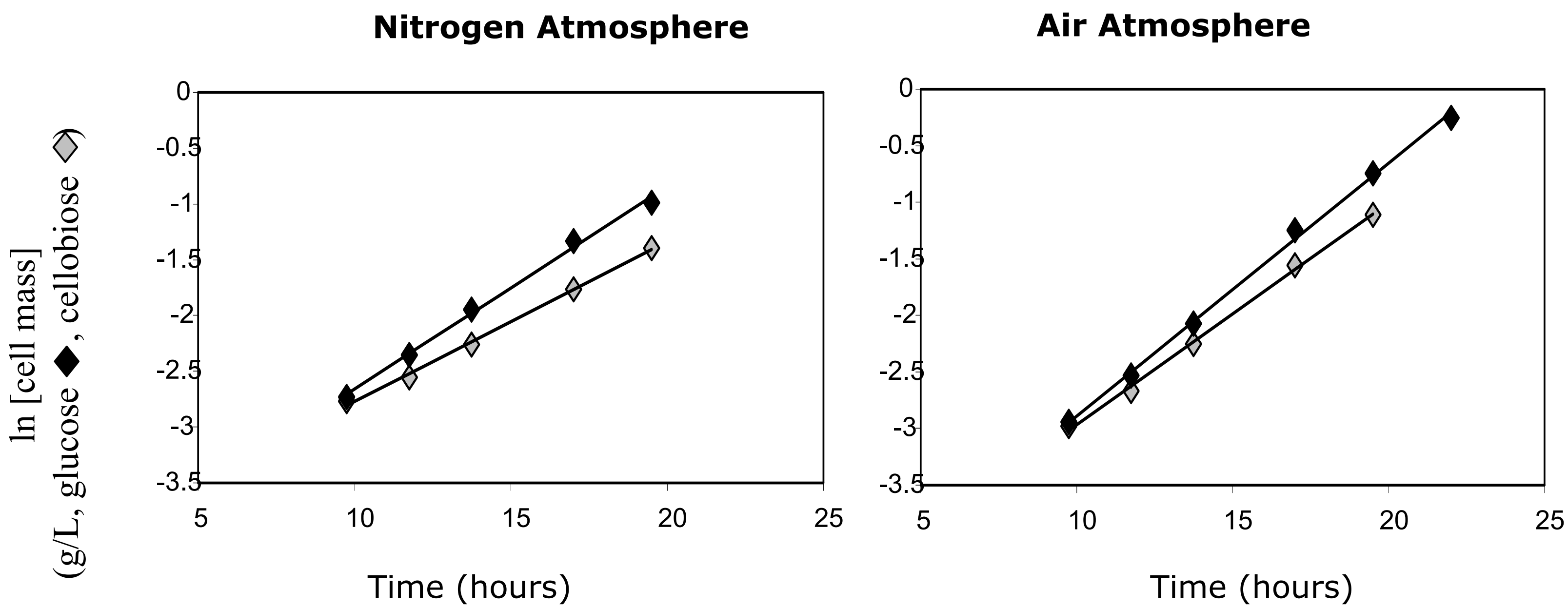
q/SA = (mg cellulase/g cell) -> **% Cell Protein**

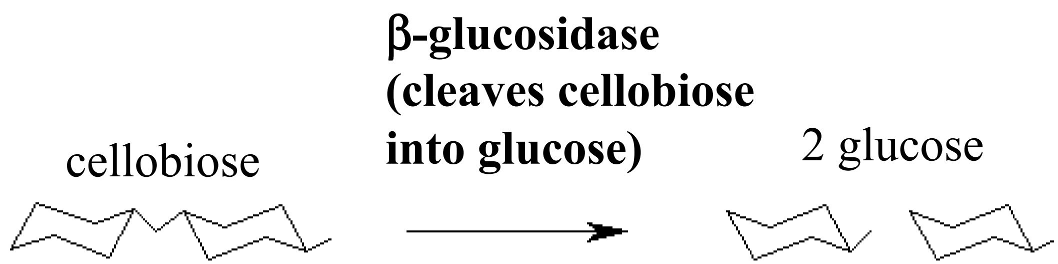
Supply: **Specific Activity (SA)**
(μmol/mg/min) ->
(g GE/mg/hr)



1) Esterbrauer et al. (1991); 2) Zhang & Lynd (2003)

Yeast strain that can grow almost as well on cellobiose as glucose (β-glucosidase expression)





Challenge is to express enough of the remaining cellulase enzymes (endoglucanase, exoglucanase)

Two Additional Positive Results

Exoglucanase (CBHI) has been actively expressed in yeast by our group and a group based in Japan; although at levels much lower than necessary to support growth

Our collaborators at the University of Stellenbosch created a yeast strain expressing β-glucosidase and endoglucanase which is able to grow on phosphoric acid swollen cellulose (semi-soluble cellulose)

Summary of progress towards CBP with *S. cerevisiae*

- Growth and ethanol production from xylose and arabinose have been established -- Kuyper (2005); Pronk (presentation at Met. Eng. VI)
- Growth on cellobiose at the same rate as glucose
- Expression of numerous cellulase components
- Growth on phosphoric acid swollen cellulose

Future Work:

Increase expression of key cellulase components

Use selection based methods to improve cellulose hydrolysis

II. Thermophilic bacteria (*Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum*)

Advantages

• Thermophililc bacteria are among the best cellulose degraders isolated to date

• Co-culture of *C. thermocellum* and *T. saccharolyticum* can completely use all carbohydrates found in biomass

• More economical heat management, process requirements with fermentation at 50-60°C

Challenges for thermophiles

• Branched Pathway Fermentation lowers ethanol yield to ~ 60% of theoretical

• Low final ethanol concentration (due to toxicity of organic acids produced)

• Extremely difficult to genetically engineer (Introducing foreign DNA limiting step)

Goal

• Engineer a thermophilic strain for high ethanol yield, and see if it can reach high ethanol concentrations

Clostridium thermocellum (ATCC 27405)

One of the highest growth rates on cellulose among described microbes

Similar growth rates on model substrates (Avicel) & pretreated lignocellulose

Does not ferment pentoses, grows poorly on glucose

Cellulose hydrolysis

Mediated by a complex, the “cellulosome”, with over 20 distinct proteins

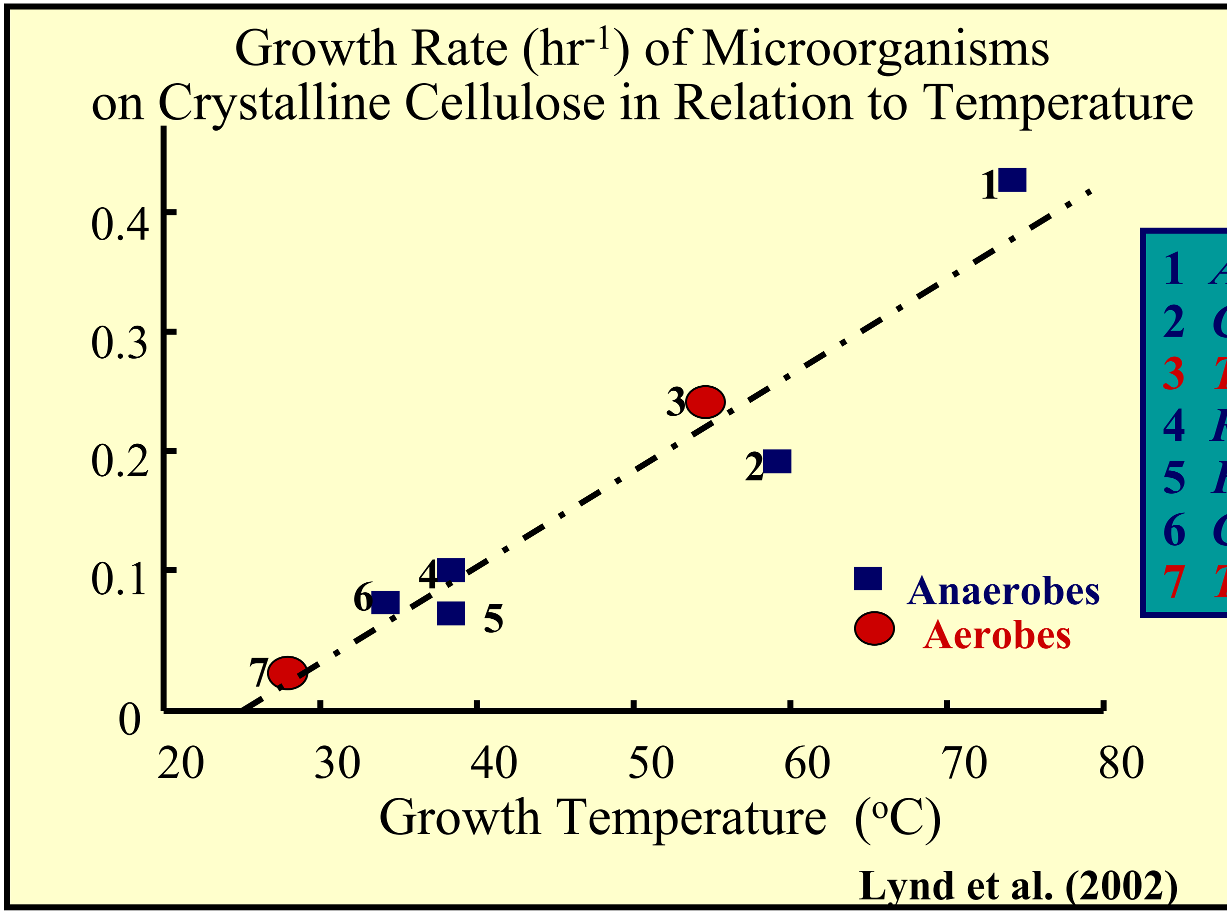
Cells adhered to fibers in cellulose-enzyme-microbe (CEM) complexes

Thermoanaerobacterium saccharolyticum (ATCC 8691)

Ferments xylan & almost all soluble biomass sugars
Produces xylanase enzymes

Does not ferment cellulose

Thermophiles are able to get the job done quicker



1 *Anaerocellum thermophilum*
2 *Clostridium thermocellum*
3 *Thermomonospora* sp N-35
4 *Ruminococcus flavefaciens*
5 *Fibrobacter succinogenes*
6 *Clostridium cellulolyticum*
7 *Trichoderma reesei*

Theoretical savings in process design (more efficient reactors) as well as savings in cellulase production costs

T. saccharolyticum JW-SL YS485

Isolated from a hot spring in Yellowstone National Park
(Liu. et al 1993)

- 30 - 66°C, T_{opt} 60°C
- pH 3.85 – 6.5
- strict anaerobe

(Shao et. al. 1994)

Substrates Supporting Growth

Monosaccharides

- glucose
- xylose
- mannose
- arabinose
- galactose
- fructose

Disaccharides

- cellobiose
- sucrose
- maltose

Polysaccharides

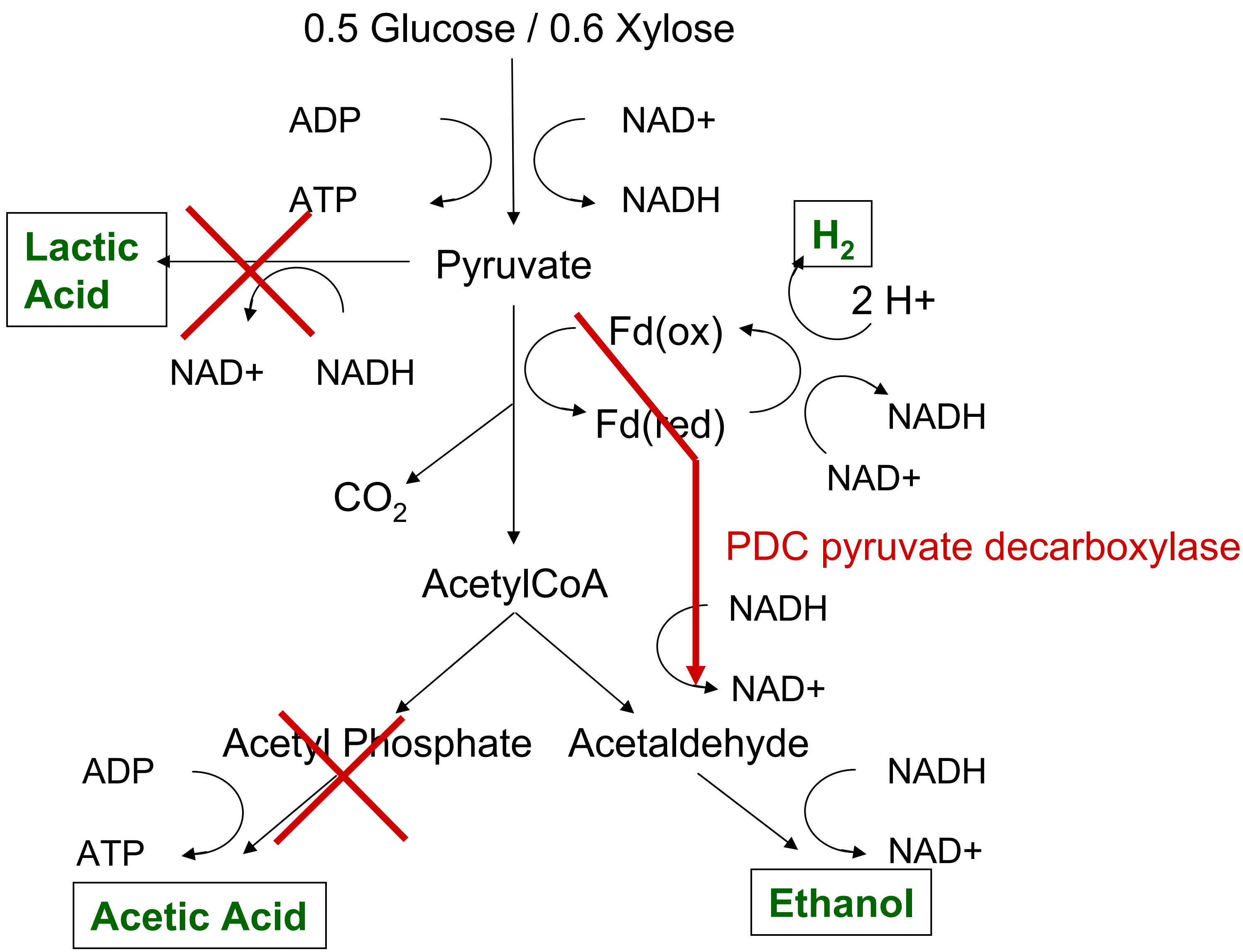
- starch (soluble potato)
- xylan (birch wood)
- mannan
- *not cellulose*

Main Products

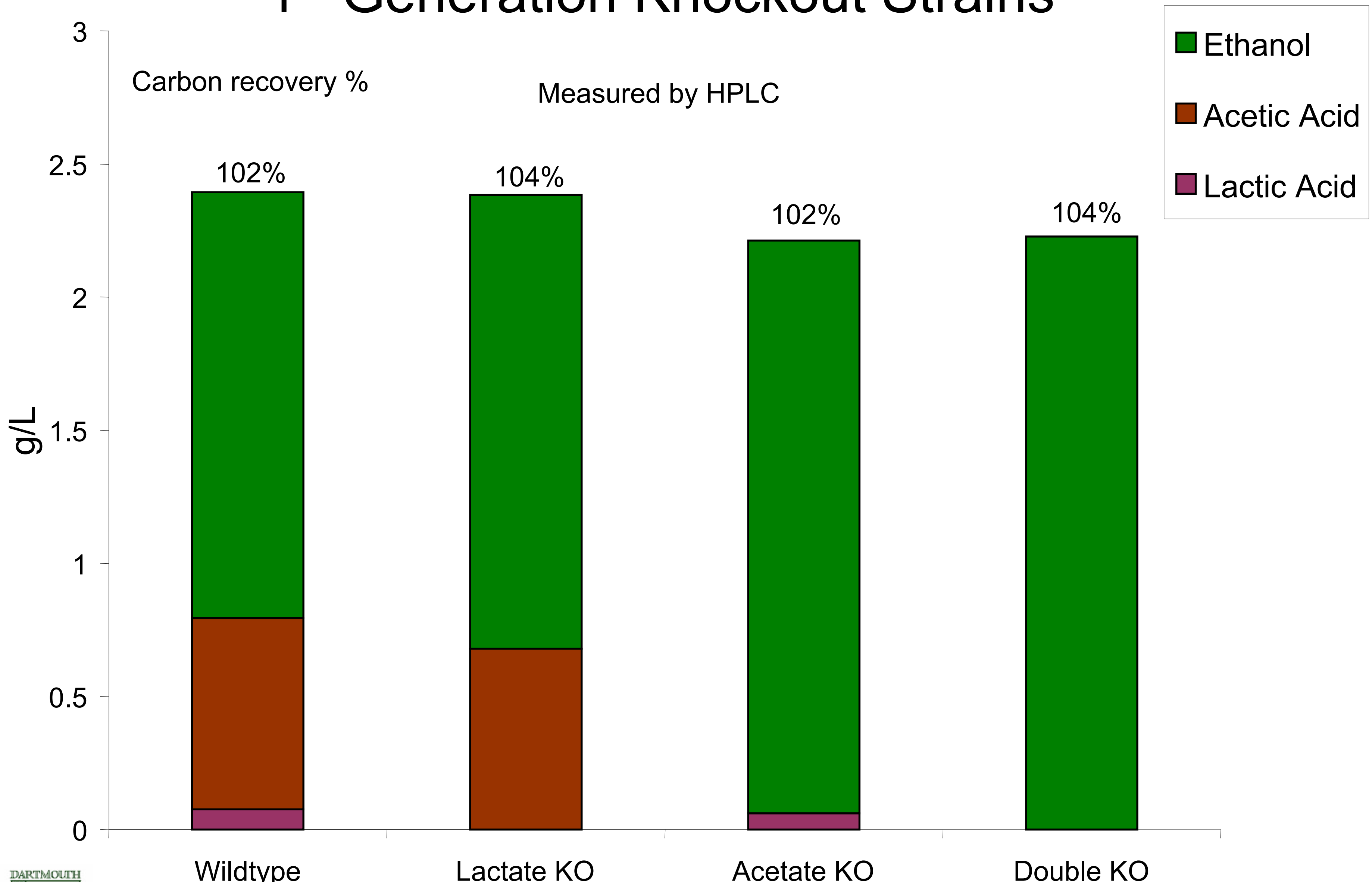
- Ethanol
- Lactic Acid
- Acetic Acid
- CO_2
- H_2

(Shaw et al, unpublished)

Branched Catabolic Pathway

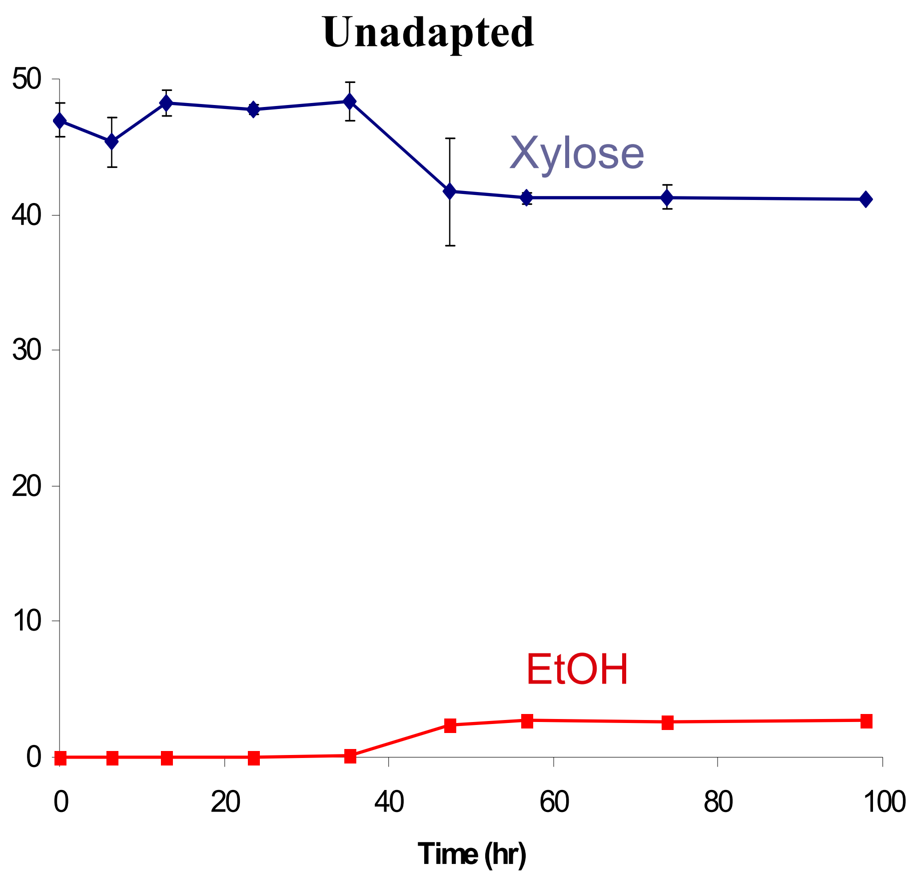


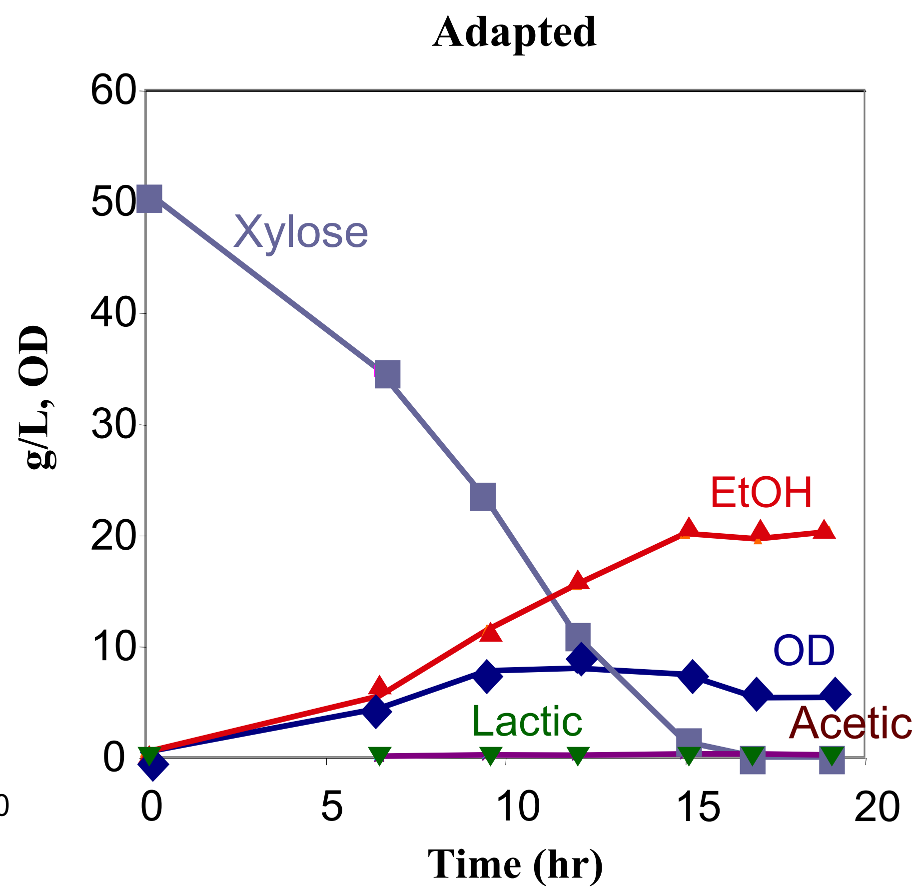
1st Generation Knockout Strains



Initial 4.2 g/L Xylose, 2.5 g/L YE, 56^{o}C

Adapted knockout fermentation





Consolidated Bioprocessing (CBP)

- Offers a very large advantage (4x) over current biological processing

- No organism available currently, engineered strains required

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Yeast

- Shown to grow rapidly on cellobiose

- Growth on PAS cellulose

- Framework in place to create strain that can grow on crystalline cellulose

Thermophilic Bacteria

- *T. saccharolyticum* producing ethanol at high yield

- Adaped to high ethanol concentration

- Promising for engineering of *C. thermocellum*

Conclusion

- Significant progress made on both strategies

- Potential to revolutionize cost of cellulose processing

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