

Zymomonas mobilis

Special Topics Session Microbial Pentose Metabolism

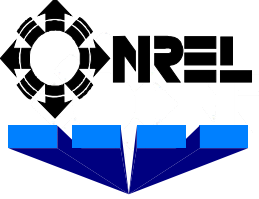
25th Symposium on Biotechnology for Fuels and Chemicals

May 5, 2003

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National Renewable Energy Laboratory

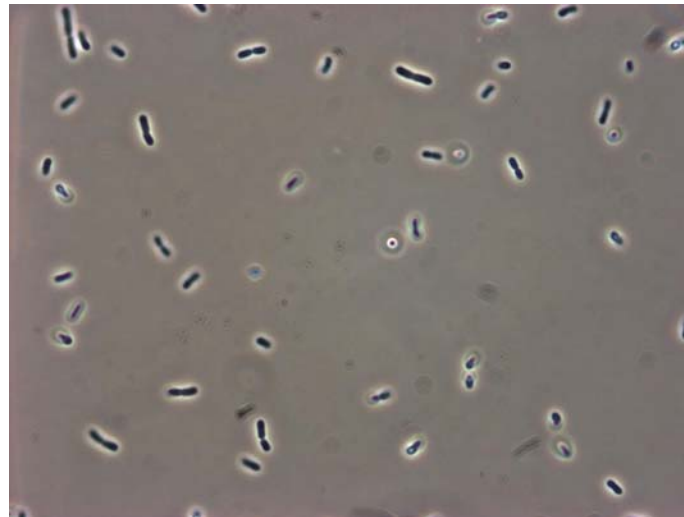




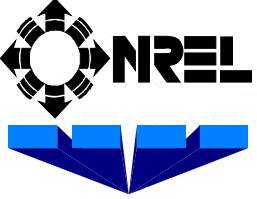
Zymomonas mobilis



Glucose →
Fructose →
Sucrose →
D-Xylose →
L-Arabinose →

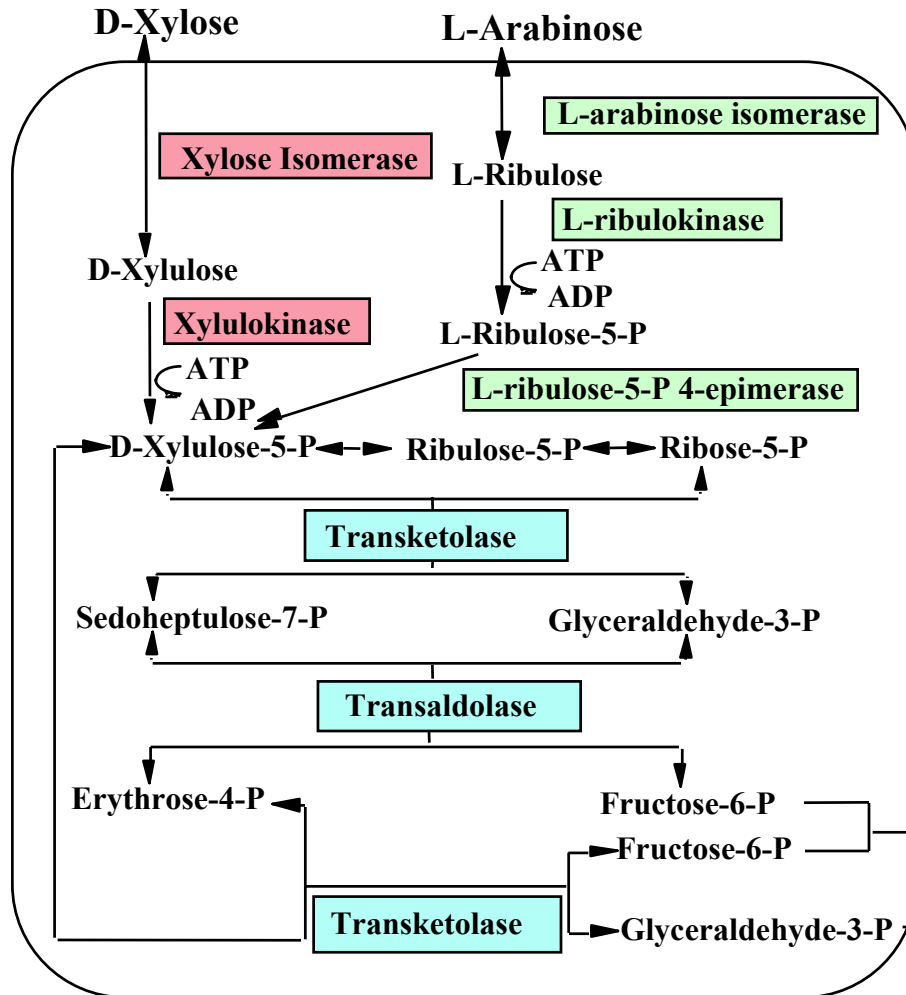


Ethanol

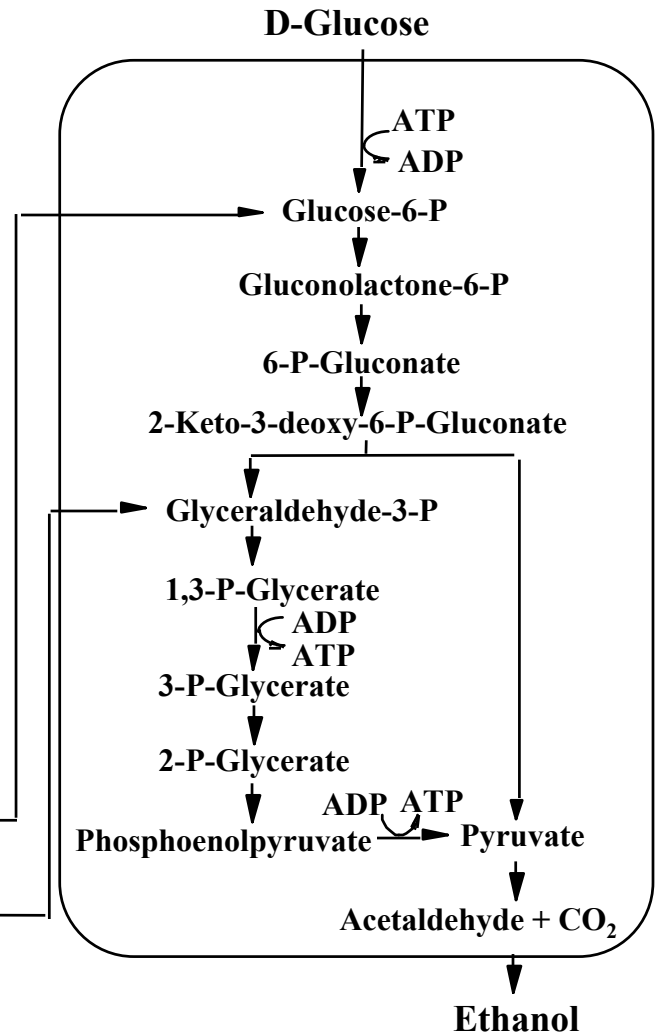


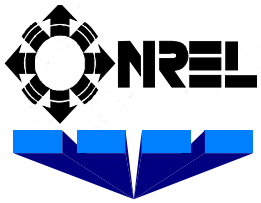
Metabolic Engineering for Pentose Fermentation

Pentose Metabolism Pathway



Entner-Doudoroff Pathway





rZymomonas Strains Developed

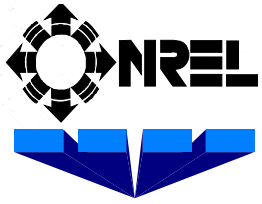
▪ **Xylose-Fermenting Strains**

- Host CP4: CP4(pZB4) and CP4(pZB5) etc.
- Host 39676: 39676(pZB4), 39676(pZB5) and 39676(pZB4L)
 - Hydrolysate-adapted 39676(pZB4L)
 - **C25 (genomic integrated)**
- Host 31821/ZM4: ZM4(pZB5)
 - **321(5), 2032 and 8b (genomic integrated)**

▪ **Xylose and Arabinose-Fermenting Strains**

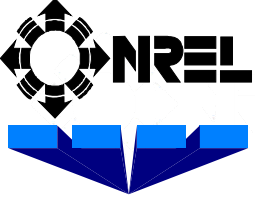
- Host 39676: 206C(pZB301) and 206C(pZB401)
 - BC1(pZB301)
 - **AX strains (genomic integrated)**

▪ **Xylose, Arabinose and Mannose-Fermenting Strains**



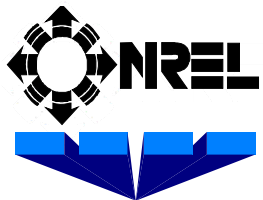
Highlights of *Zymomonas mobilis*

- **Natural fermentative microorganism (GRAS)**
- **High ethanol yield from glucose (95-98% or 0.49-0.50 g/g)**
- **Low cell mass formation**
- **No oxygen requirement**
- **High ethanol tolerance (13% ethanol from 30% glucose)**
- **High specific productivity (2-6 g ethanol g dcw⁻¹ . hr⁻¹)**
- **High sugar uptake rate (up to 10 g glucose g dcw⁻¹ . hr⁻¹)**

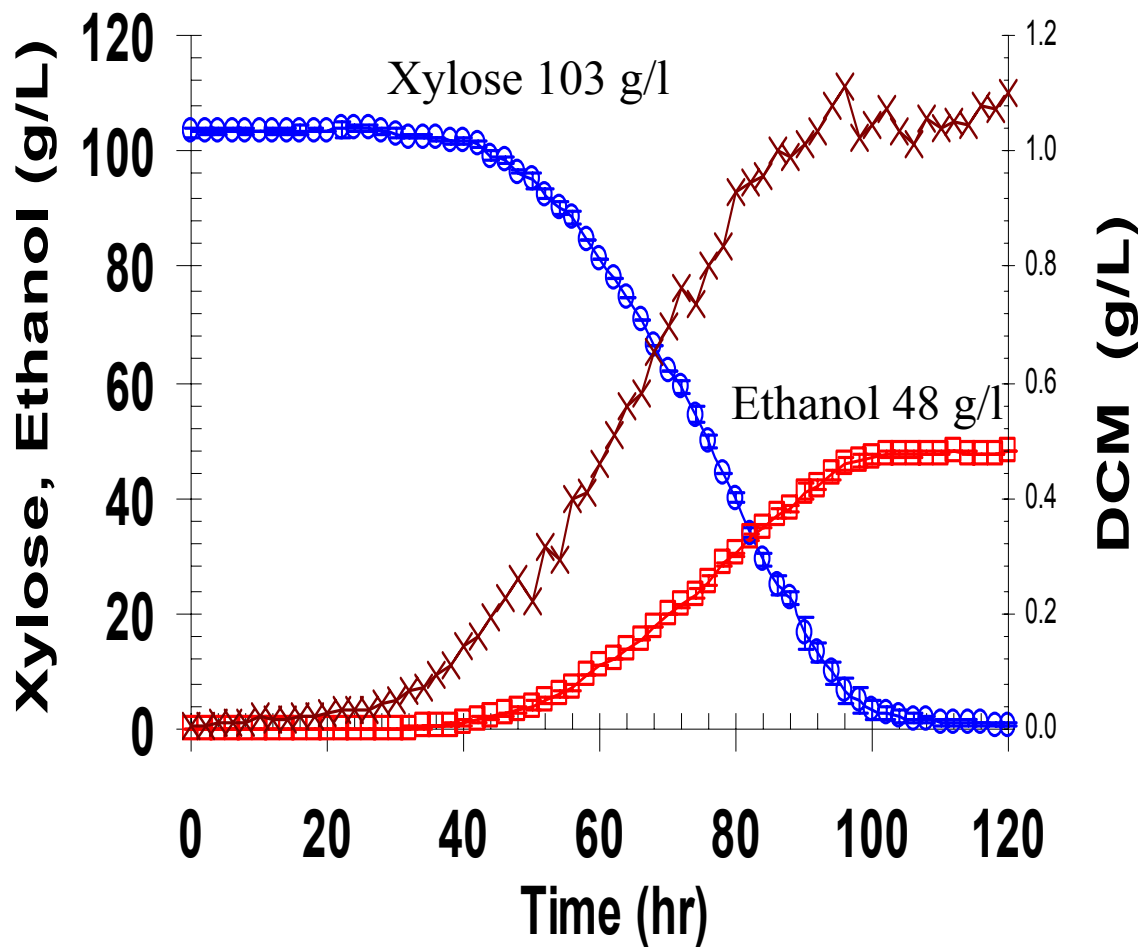


Cultivation Conditions

- Media:
 - RM: 10 g/L YE, 2 g/L KH_2PO_4 , 2% glucose (desired sugars)
or
Clarified CSL (cCSL) (1%) + sugars
 - Fermentation Media using hydrolysate : cCSL (1%) + Hydrolysate, overlimed or neutralized supplemented with extra glucose.
- Inoculum Size: 0.03-0.25 OD @600 nm (0.01 - 0.085 g cell/L)
- Fermentor: pH 5 or pH 6 controlled with KOH (2N)
- Temperature at 30°C or 37°C
- No aeration



Z. mobilis 8b on RM 10% xylose

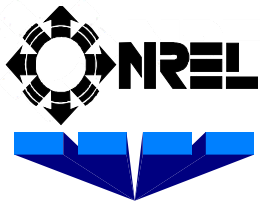


Y_m : 0.47 g/g

Y_p : 0.46 g/g

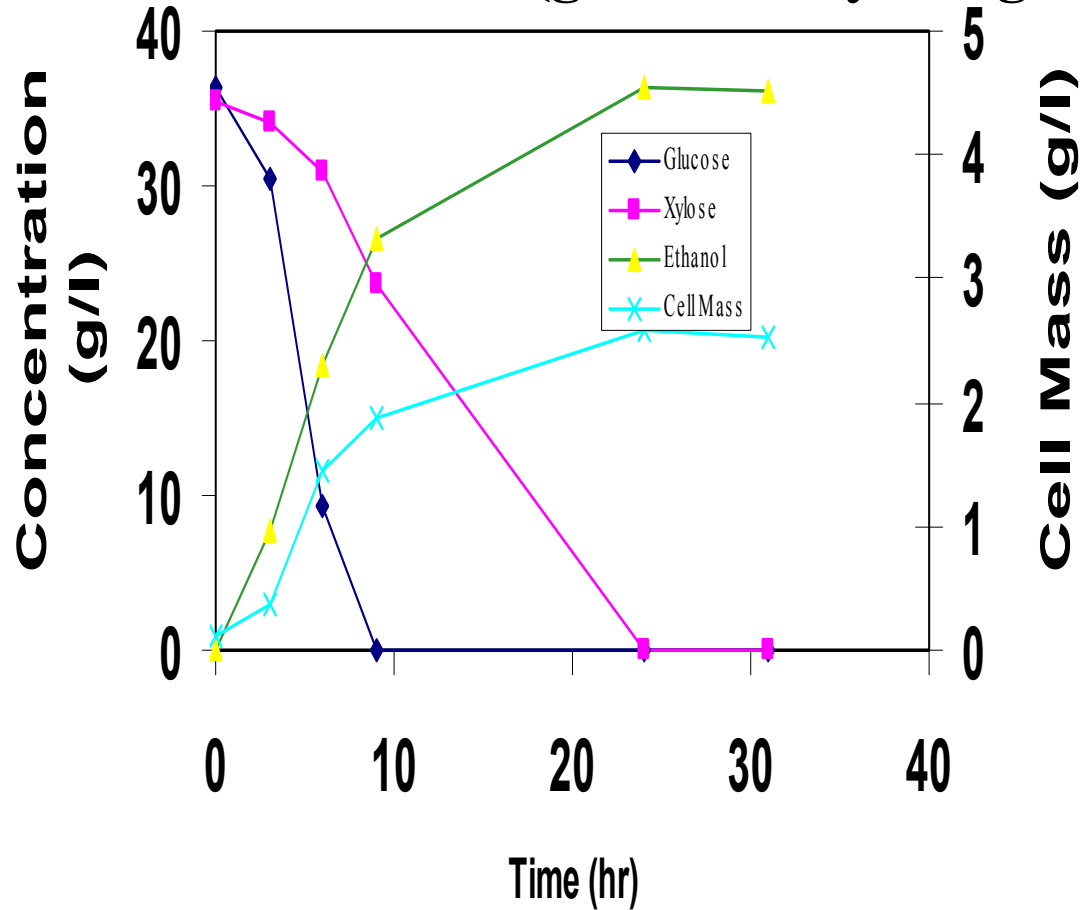
q_E : 1.3 g/g/h

Vol. Prod.: 0.4 g/l/h



Fermentation of Xylose-Fermenting *Z. mobilis* Strain

Z. mobilis 8b (genomically integrated)



Y_m : 0.51 g/g

Y_p : 0.51 g/g

q_E : 1.13 g/g/h

Vol. Prod.: 1.5 g/l/h

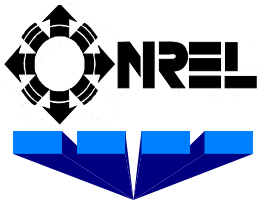
RM media, pH=5.5, T=30°C



Corn Stover Hydrolysates

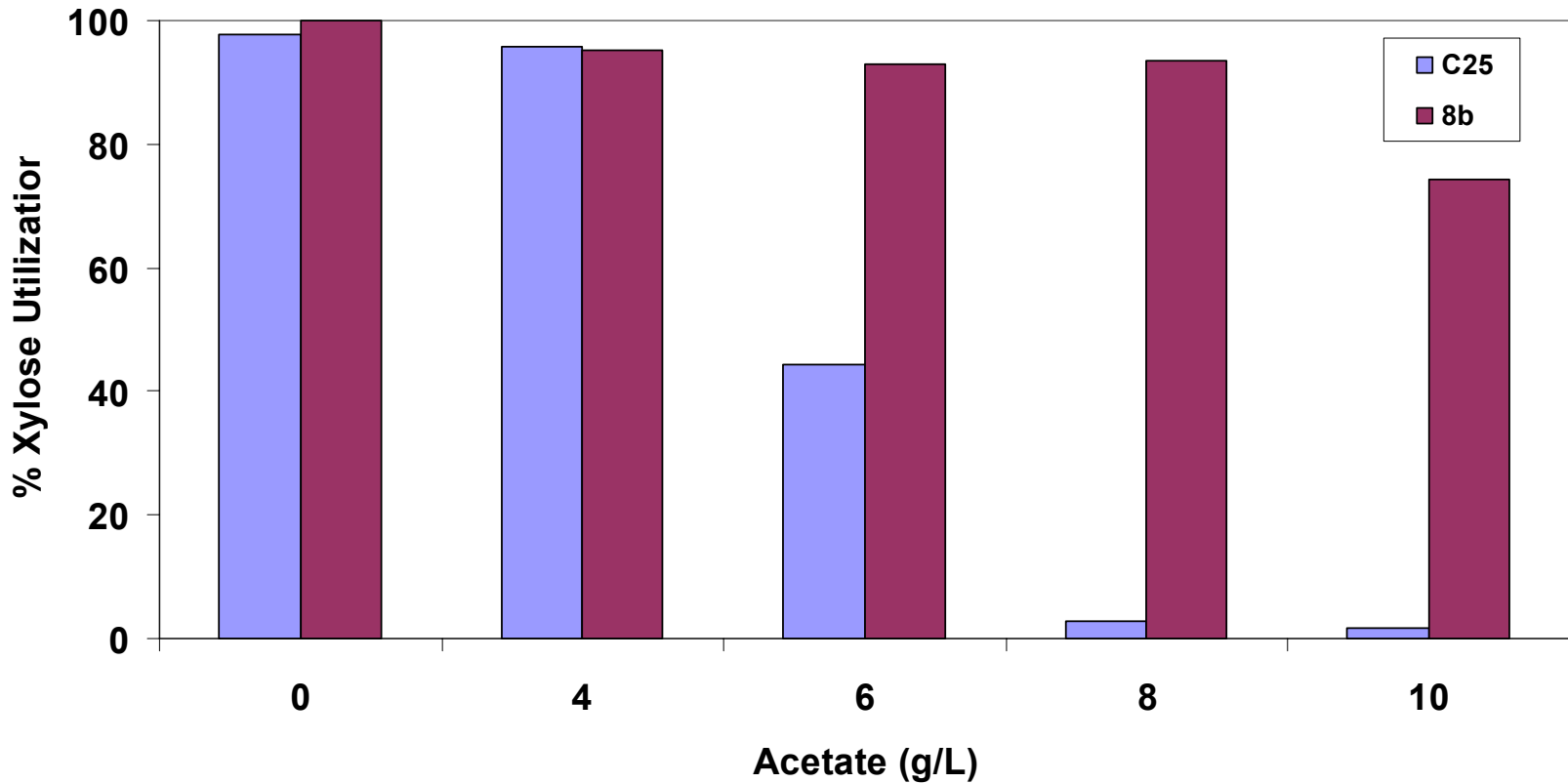
Compound	Concentration (g/L)	Total Sugar Conc. (g/L)
Cellobiose	1.91	110
Glucose	15.65	
Xylose	69.20	
Arabinose	11.87	
Galactose	6.63	
Mannose	5.05	
Acetic acid	10.98	
Lactic acid	2.15	
HMF	0.67	
Furfural	1.14	
Acid soluble lignin	10.77	

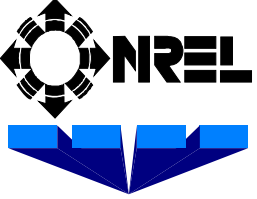
Pretreatment: using the flow-through mode of operation and at 25% solids, 190°C and 0.048 g acid/g dry biomass (Schell et al. 2002).



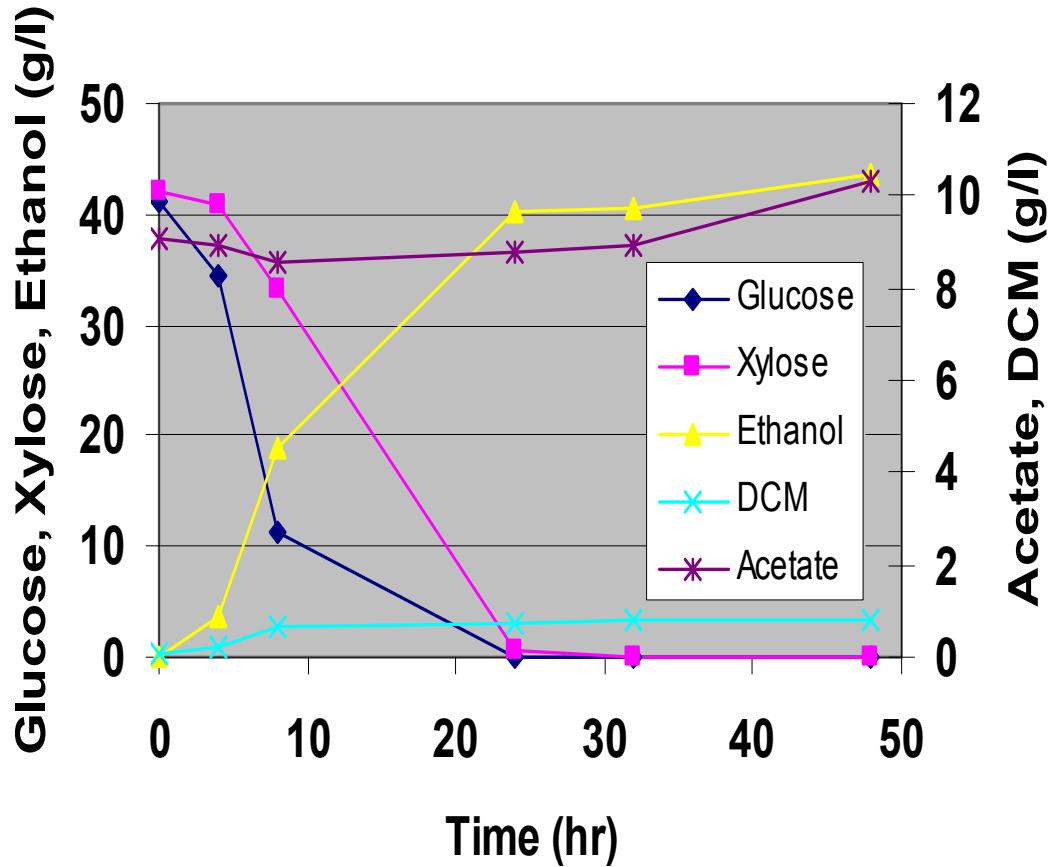
Increased Acetate Tolerance

Comparison of %Xylose Utilization by Strains C25 and 8b at T 37°C, Grown on RMGX (2%:2%) in Baffled Shake Flask





Fermentation of Strain *Z. mobilis* 8b in cCSL+4% glu + 4 % xyl in the presence of 8 g/l Acetate at 37 C at pH 6

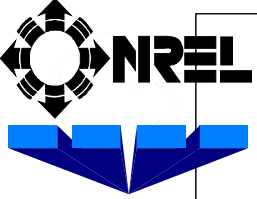


$Y_m:$ 0.52 g/g

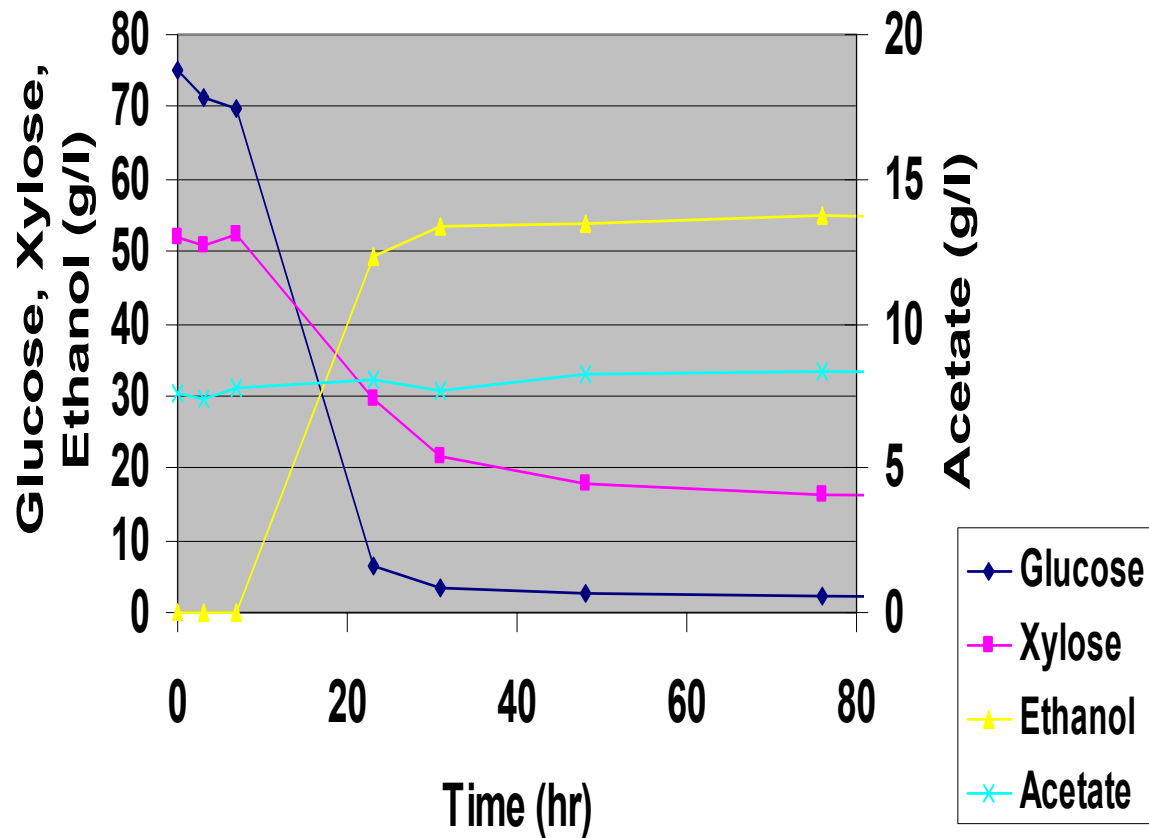
$Y_p:$ 0.52 g/g

$q_E:$ 4.1 g/g/h

Vol. Prod.: 1.68 g/l/h



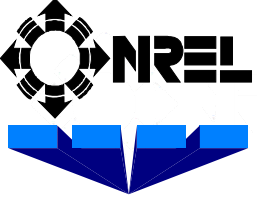
Fermentation profile of *Z. mobilis* 8b in 80% OL-Corn Stover Hydrolysate spiked with glucose at pH6 and 37C



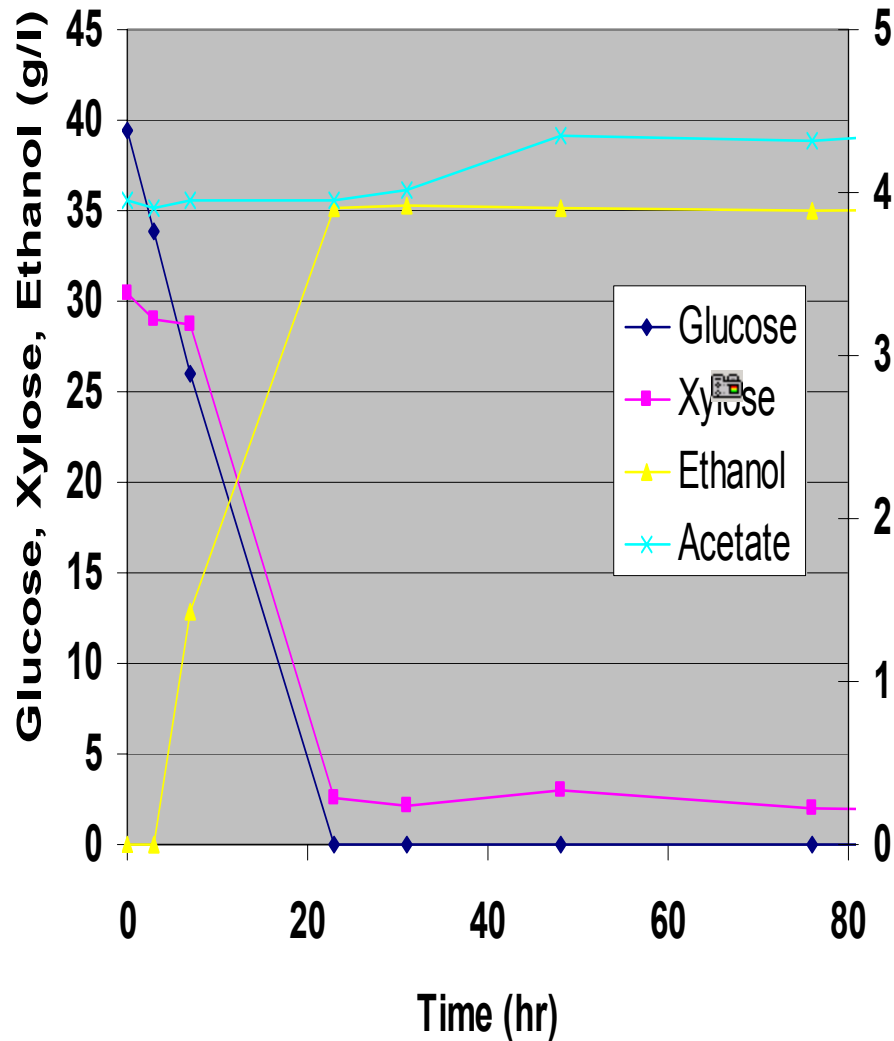
Y_m : 0.52 g/g

Y_p : 0.42 g/g

Vol. Prod.: 2.1 g/l/h



Fermentation of *Z. mobilis* 8b in 40% Neutralized CSH spiked with glucose at 37 C pH6

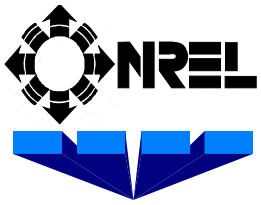


Y_m : 0.52 g/g

Y_p : 0.51 g/g

Vol. Prod.: 1.45 g/l/h

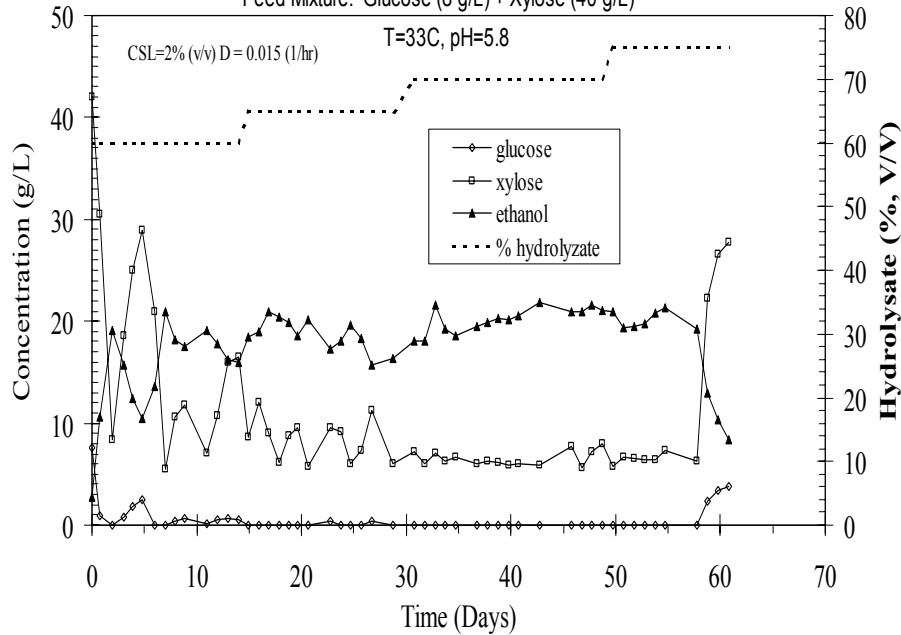
Acetate (g/l)



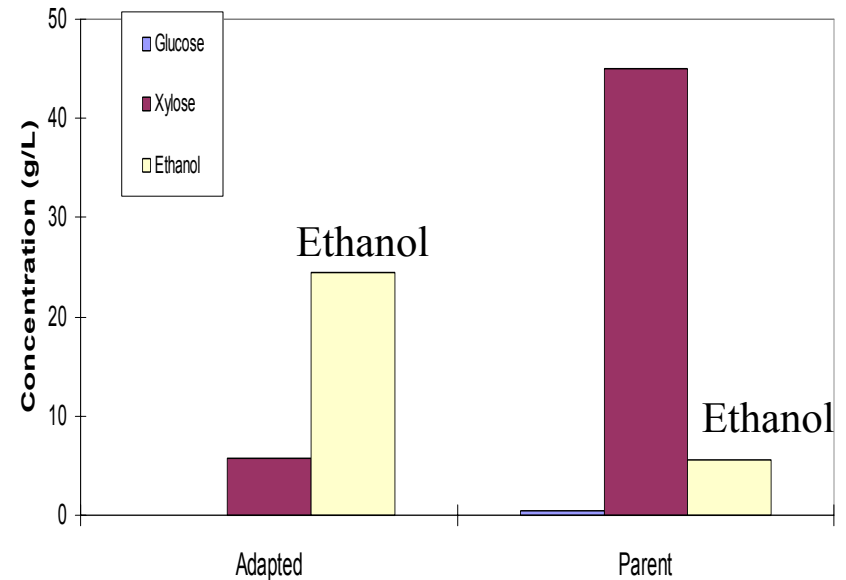
Strain Improvement through Adaptation

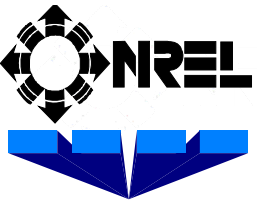
Adaptation of *Zymomonas* 39676(pZB4L) to above 60% (v/v) OL Hydrolyzate

Feed Mixture: Glucose (8 g/L) + Xylose (40 g/L)



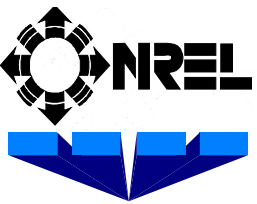
Comparison of Adapted and Parent Strain 39676(pZB4L) Grown on cCSL (2%), 60% Sawdust Hydrolyzate at pH 5.8, T 30°C, Initial Sugar Conc. (G:X 1%:5%)





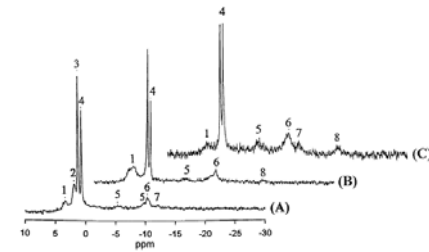
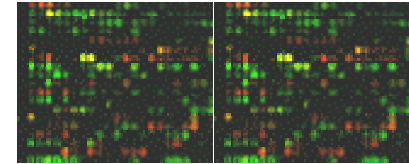
Future Improvements

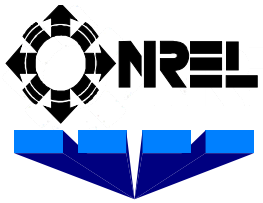
- **Ferment all biomass sugars (including mannose and galactose)**
- **Improve pentose fermentation yield and productivity**
- **Improve tolerance to hydrolysate toxicity**



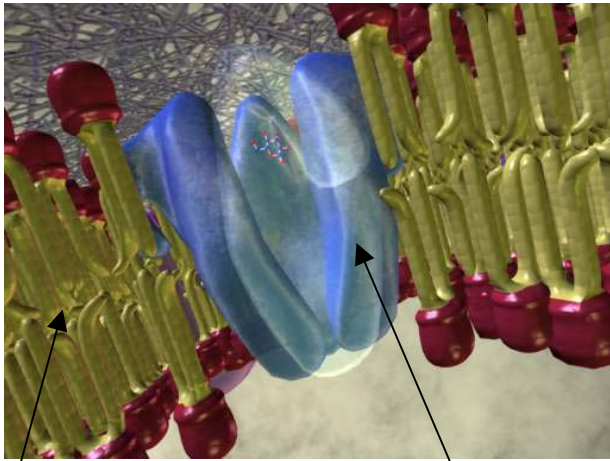
Metabolic Engineering Tools

- Transcriptomics
 - Examine gene expression at global level
- Metabolomics
 - Examine metabolites at global level
- High-throughput Enzyme Assays
 - Compare strains
 - Compare activities at different stages of growth
 - Compare activities in different growth conditions
 - Develop improved enzymes
- Mathematical approaches
 - Metabolic flux analysis
 - Kinetic modeling



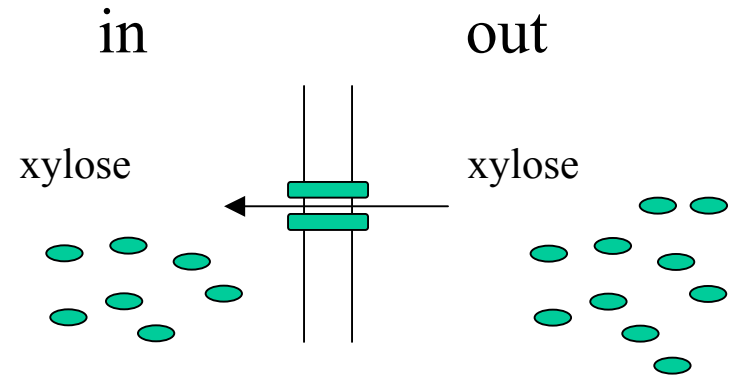


Is Sugar Transport limiting?



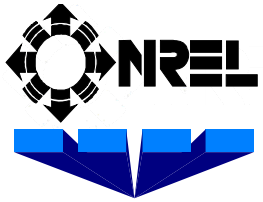
Transporter protein

Cytoplasmic membrane



Facilitated Diffusion Transport

No energy required



Acknowledgements

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of the US Department of Energy**