Measurement of Xylose Transport in Xylose-fermenting *Zymomonas mobilis*

Christina Eddy[1]*, Mete Altintas[2], Dhinakar S. Kompala[2], James D. McMillan[1], and Min Zhang[1]

National Bioenergy Center[1]
Biotechnology for Fuels and Chemicals Division
NREL, Golden, CO 80401

Chemical Engineering Department[2]
University of Colorado
Boulder, CO 80309

Abstract

Recombinant strains of *Zymomonas mobilis* efficiently convert glucose, xylose and arabinose to ethanol, however glucose is fermented more quickly and completely than the pentose sugars. Conversion of pentose sugars to ethanol requires transport into the cell followed by metabolism through the pentose phosphate pathway. In *Z. mobilis* all sugars enter the cell by facilitated diffusion through a single transport protein called the glucose facilitator, Glf. Rapid filtration assays measuring uptake for 3 seconds indicate that glucose is transported with a V_{max} of 200-300 nmol/min/mg protein and a K_m of 15 to 20 mM. Improving pentose fermentation requires knowledge of which step is rate limiting. We are examining the kinetics of glucose and xylose transport in a variety of strains of *Z. mobilis* with different genetic backgrounds and fermentation profiles to determine whether transport limits ethanol production.

Introduction

- Recombinant *Zymomonas mobilis* engineered to use pentose sugars is potentially important for industrial production of ethanol and chemicals from lignocellulosic biomass (7).
- Sugars transport into *Z. mobilis* by facilitated diffusion catalyzed by the glucose facilitator protein (Glf)(2-5, 7). Glf is reported to transport a variety of sugars including glucose, fructose, xylose, arabinose and mannose(6).
- The goal of this investigation was to develop a method to measure the initial uptake rates of glucose and xylose by *Z. mobilis* at 30°C.

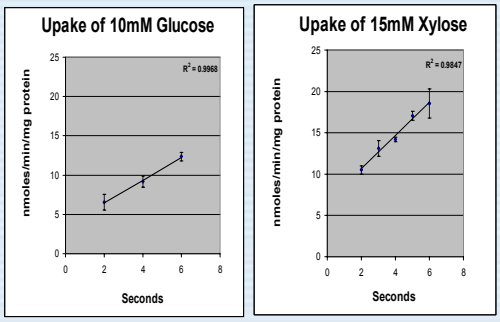
Materials and Methods

- **Growth and Preparation of Cells for Transport Assays**
  - Strains C25 and 206c, derived from strain 39676 (1) were inoculated from glycerol stocks stored at –70°C and grown at 30°C in rich medium (RM) containing 10 g/l yeast extract, 2 g/l KH_2PO_4, and 50 g/l glucose for at least 10 generations until they reached 0.8 and 1.1 OD_{600nm}.
  - Cells were collected by centrifugation at 4°C and washed three times in ice-cold transport buffer (0.05M potassium phosphate with 0.1g/l $MgSO_4$-$7H_2O$, pH 6.5). Cells were suspended to a final OD_{600nm} of 30 to 45.
- **Transport Assays**
  - Assays were performed in 15 ml Falcon tubes pre-warmed to 30°C for at least 5 minutes.
  - 32.5 ul of cells were transferred from ice to the bottom of the 30°C Falcon tube and incubated for 1.5 minutes.
  - 32.5 ul of radiolabeled sugar with specific activity of at least 0.4 µCi/µMol was added to the side of the tube with the cells. The reaction was started by vortexing.
  - The reaction was precisely timed with a timer set to beep every 2 or 3 seconds and stopped by rapid addition of 5 ml of transport buffer with 500 mM sugar at –3 °C which was immediately filtered through a 45 mm diameter, 0.8 micron polysulfone filter from Gelman.
  - Reactions were washed on the filter with an additional 20 ml of –3 °C transport buffer with 500 mM sugar.
  - Filters were immediately transferred to 10 ml of scintillation fluid.
  - Isotope was counted in a Beckman scintillation counter after 12-18 hours of incubation at room temperature.

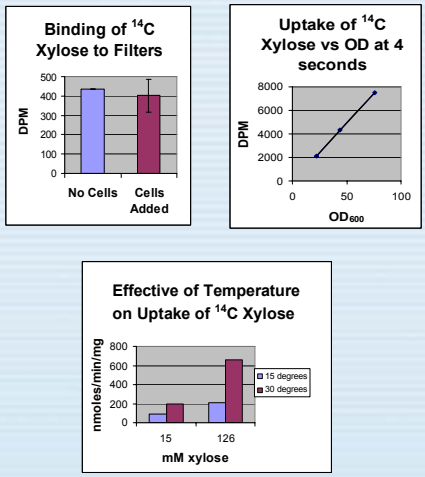
Acknowledgement

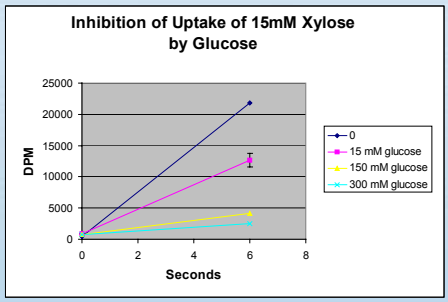
This work supported by the Office of the Biomass Program of the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy

Linear Uptake of Glucose and Xylose

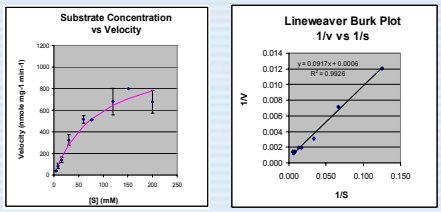


Factors Influencing the Assay





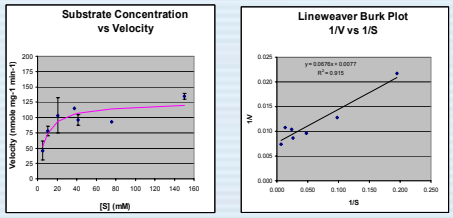
Kinetics of Xylose Uptake



Km: 92 mM
Vmax: 1130 nmole mg^{-1} min^{-1}

Km: 148 mM
Vmax: 1540 nmole mg^{-1} min^{-1}

Kinetics of Glucose Uptake



Km: 10 mM
Vmax: 130 nmole mg^{-1} min^{-1}

Km: 7 mM
Vmax: 125 nmole mg^{-1} min^{-1}

Summary

1. A method to measure the uptake of glucose and xylose in at 30°C has been established for *Z.mobilis*.
2. Mixing cells, sugar and quench buffer simultaneously gave background binding to the filter that did not reflect any possible non-specific binding to the cells. Therefore, initial uptake rates were measured between 2 and 4 seconds.
3. Glucose is taken up with an apparent K_m of 7-10 mM and V_{max} of 125-130 nm/min/mg protein.
4. Xylose is taken up with an apparent K_m of 92-148mM and V_{max} of 1130-1540 nM/min/mg protein.
5. Xylose appears to be taken up by the glucose transporter since xylose uptake is inhibited by glucose.
6. The assay will be used to explore whether xylose uptake limits xylose metabolism in *Z. mobilis*.

References

1. **DiMarco, A. A., and A. H. Romano.** 1985. D-Glucose Transport System of *Zymomonas mobilis*. Appl Environ Microbiol **49:**151-157.
2. **Gao, Q., M. Zhang, J. D. McMillan, and D. S. Kompala.** 2002. Characterization of heterologous and native enzyme activity profiles in metabolically engineered *Zymomonas mobilis* strains during batch fermentation of glucose and xylose mixtures. Appl Biochem Biotechnol **98-100:**341-55.
3. **Parker, C., W. O. Barnell, J. L. Snoep, L. O. Ingram, and T. Conway.** 1995. Characterization of the *Zymomonas mobilis* glucose facilitator gene product (glf) in recombinant *Escherichia coli:* examination of transport mechanism, kinetics and the role of glucokinase in glucose transport. Mol Microbiol **15:**795-802.
4. **Snoep, J. L., N. Arfman, L. P. Yomano, R. K. Fliege, T. Conway, and L. O. Ingram.** 1994. Reconstruction of glucose uptake and phosphorylation in a glucose-negative mutant of Escherichia coli by using *Zymomonas mobilis* genes encoding the glucose facilitator protein and glucokinase. J Bacteriol **176:**2133-5.
5. **Weisser, P., R. Kramer, H. Sahm, and G. A. Sprenger.** 1995. Functional expression of the glucose transporter of *Zymomonas mobilis* leads to restoration of glucose and fructose uptake in *Escherichia coli* mutants and provides evidence for its facilitator action. J Bacteriol **177:**3351-4.
6. **Weisser, P., R. Kramer, and G. A. Sprenger.** 1996. Expression of the *Escherichia coli pmi* gene, encoding phosphomannose-isomerase in *Zymomonas* mobilis, leads to utilization of mannose as a novel growth substrate, which can be used as a selective marker. Applied and Environmental Microbiology **62:**4155-4161.
7. **Zaldivar, J., J. Nielsen, and L. Olsson.** 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Applied Microbiology and Biotechnology **56:**17-34.