

**MESOPHILIC VS. THERMOPHILIC COUPLING SYSTEMS OF  
HYDROGENASE WITH OXIDATIVE PENTOSE PHOSPHATE CYCLE  
ENZYMES FOR HYDROGEN EVOLUTION**

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To be presented as an oral presentation  
at the  
23<sup>rd</sup> Symposium on Biotechnology  
for Fuels and Chemicals  
to be held in  
Breckenridge, Colorado  
May 6 - 9, 2001

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## MESOPHILIC VS. THERMOPHILIC COUPLING SYSTEMS OF HYDROGENASE WITH OXIDATIVE PENTOSE PHOSPHATE CYCLE ENZYMES FOR HYDROGEN EVOLUTION

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To increase the production of hydrogen from biomass-derived glucose and achieve the maximum molar yield of H<sub>2</sub> by employing the enzymes of the pentose phosphate cycle in conjunction with the hydrogenase from *Pyrococcus furiosus*. This process centers on three NADP<sup>+</sup> dependent enzymes, glucose-6 phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH) and hydrogenase from *Pyrococcus furiosus*. The dehydrogenases are currently obtained from mesophilic sources. However, in order to increase the rates of hydrogen production, work is being carried out to isolate the genes for the glucose-6 phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase from the Archaeon *Sulfolobus solfataricus*. 6-Phosphogluconate dehydrogenase has been partially purified and determined to have the following N-terminal sequence :

MKIGLIGLGIMGYRIAANLAKANKLNLVYDRTQE?IE(R). Degenerate primers were designed within the N-terminal sequence and a highly conserved region downstream to produce a 150-200bp PCR fragment for use in hybridization experiments. Sequencing of the hybridized clone is also hoped to reveal a glucose 6-phosphate dehydrogenase in its flanking regions.

The maximum yield of hydrogen from glucose using only the oxidative portion of the pentose phosphate pathway (two moles per mole of glucose 6-phosphate) has been achieved using mesophilic sources of these enzymes in conjunction with a hyperthermophilic hydrogenase at 40°C. Additional monitoring of CO<sub>2</sub> evolution confirmed the stoichiometry of H<sub>2</sub> to CO<sub>2</sub> to be 2:1. Preliminary results indicate that the kinetic properties of the pathway are different compared to the properties of the individual components