303a Quantifying Growth and Maintenance Atp Requirements for Cofermentation of Glucose and Xylose by Metabolically Engineered *Zymomonas Mobilis*

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An intracellular adenosine-5'-triphosphate (ATP) balance-based method is described for quantifying glucose- and xylose-associated growth and non-growth (maintenance) energy requirements. *Zymomonas mobilis*, a gram-negative bacterium that distinctively uses the Entner-Doudoroff pathway (net yield of only one mole ATP per mole of glucose or –putatively- xylose consumed), is capable of efficiently converting both glucose and xylose to ethanol as well as moderately tolerating acetic acid and other inhibitory components typically present in biomass hydrolyzates. We have measured the concentrations of sugars (glucose and xylose), ethanol, byproducts (e.g., xylitol and acetic acid), as well as intracellular ATP concentrations, across a 2-factor, 3-level response surface design using the methods of analysis, techniques, and calculations described previously [*AIChE 2004 Annual Meeting Proceedings*, paper 29a]. Batch and various continuous fermentations of mixtures of 5% glucose/5% xylose (w/v) were conducted at a temperature of 30 °C and at pH 5.0, 5.3, or 6.0 in the presence of varying amounts of acetic acid (0, 4, and 8 g/L) in the input fermentation media.

There are large differences in *Z. mobilis*' fermentation kinetics across the experimental design space but only modest changes in intracellular ATP levels (1.5-3.8 mg ATP/g DCM). Fermentation performance results for ethanol process yields, extents of xylose utilization, maximum specific growth rates, and ATP production and accumulation levels by the cells quantitatively demonstrate the strong effect pH has on modulating the inhibitory effect of acetic acid on fermentation kinetics, specifically the ability of *Z. mobilis* to grow and produce ethanol. Despite the substantial performance differences observed, however, specific ATP accumulation rates (0-1 mg ATP/g DCM-h) across the design were roughly three orders of magnitude lower than the specific rates of ATP production calculated based on sugar uptake (7-26 g ATP/g DCM-h). This means that the accumulation and dilution terms can be neglected in the ATP balance, such that the specific rates of ATP production and consumption (i.e., the sum of the ATP consumption rates for cell growth and maintenance) become equivalent quantities.

ATP requirements for cell growth and maintenance were calculated to quantify the interaction effects between acetic acid (undissociated and dissociated forms) and pH (hydrogen ion concentration). A significant dependence upon pH is observed, with the concentration of undissociated acetic acid exerting the strongest effect on maintenance requirements. For example, in the presence of 4 g/L of initial acetic acid, the calculated rate of ATP consumption for maintenance doubles from 6 g ATP/g DCM-h at pH 6 to 12 g ATP/g DCM-h at pH 5.3. A mathematical model describing how the rate of ATP consumption for cell growth and maintenance varies as a function of pH and acetic acid concentration is under development. Preliminary modeling results and their implications will be discussed.