Effect of cations and anions on glucose/xylose co-fermentation and the distribution of intracellular metabolites of recombinant *S. cerevisiae* 424A(LNH-ST)

Elizabeth Casey,1,5 Nathan S. Mosier,1,5 Zachary Stockdale3, Nancy Ho,1,2 Jiri Adamec,4, and Miroslav Sedlak1,5

1. Laboratory of Renewable Resources Engineering, Purdue University  2. Chemical Engineering, Purdue University  3. Biochemistry, University of Nebraska  4. Agricultural and Biological Engineering, Purdue University

**INTRODUCTION**

The commercialization of cellulosic ethanol has faced a number of different technical hurdles. One major challenge is the engineering of robust industrial microbes that are capable of mixed sugar fermentation with tolerance to inhibitors. Significant progress has been made on the development of organisms capable of mixed sugar fermentation; our group has succeeded in developing a genetically engineered Saccharomyces industrial yeast strain capable of both glucose and xylose fermentation to ethanol. However, limited progress has been made on developing organisms tolerant of the inhibitors expected during the conversion of cellulosic biomass to ethanol.

An understanding of how inhibitors affect yeast fermentation performance is required for further organism development. Most inhibition studies have focused on furan derivatives and weak acids; however, potential fermentation inhibitors also include cations and anions. Cations and anions can originate from cellulosic biomass, chemicals added for pretreatment, and process stream conditioning. In this poster, we have investigated the effect of cations (potassium, sodium, ammonium) and anions (chloride, sulfate) on the co-fermentation of glucose and xylose by *S. cerevisiae* 424A(LNH-ST).

**RESULTS**

**Figure 1.** Profiles of the cofermentation of G/X by *S. cerevisiae* 424A(LNH-ST) in the presence of 0 (left) or 0.5M (right) NaCl. Dashed lines represent metabolite extraction time points.

**Figure 2.** Specific xylose consumption rates in the presence of 0.1 – 0.5M chloride salts (left) and sulfate salts (right).

**Figure 3.** Effect of salts on glycerol (left) and xylitol (right) metabolic yields during the co-fermentation of glucose and xylose by *S. cerevisiae* 424A(LNH-ST).

**RESULTS (CONT.)**

**Figure 4.** Metabolic time course profiles for representative glycolytic metabolites during the co-fermentation of glucose and xylose by *S. cerevisiae* 424A(LNH-ST) with 0 and 0.5M NaCl added.

**CONCLUSIONS**

Salts have a negative impact on the fermentation performance of yeast, regardless of salt type:

- Xylose fermentation more sensitive to salts than glucose fermentation
  - With 0.5M NaCl, glucose consumption rates decrease by 20%, while xylose consumption rates decrease by 65% when compared to the control
  - Approximate linear relationship found between xylose consumption rate and salt concentration
  - Overall ethanol yield (at 48 hours) decreased with increasing salt concentration
  - No consistent trend observed with metabolic ethanol yields
  - Byproduct glycerol formation increased with increasing salt concentration
  - With 0.5M NaCl, glycerol yield increased by 35% as compared to control
  - Byproduct xylitol formation decreased with increasing salt concentration
  - With 0.5M NaCl, xylitol yield was reduced by 65% as compared to control
  - The concentration of 20 intracellular metabolites (including 8/10 major glycolytic metabolites) was measured
  - Slight concentration decreases were observed for most glycolytic metabolites in the presence of 0.5M NaCl
  - Results suggest a change in flux through the glycolytic pathway, while the flux through the rest of the glycolytic pathway appears unchanged in the presence of 0.5M NaCl
  - Of the anions and cations tested, there was little difference in toxicity between the two anions; therefore, the cations are most important in determining the inhibitory effect
  - Observations showed potassium to be the least inhibitory cation

**REFERENCES**


**MATERIALS AND METHODS**

**Strain:** *Saccharomyces cerevisiae* 424A(LNH-ST)

**Fermentation:** A series of microaerobic batch fermentations were completed in 300 ml baffled sidearm Erlenmeyer flasks containing 100 ml YEP, glucose, xylose, and varying concentrations of inhibitors. Chloride and sulfate salts were used as the inhibitors at concentrations ranging from 0.1 to 0.5M. The media was inoculated to 4.7 g dry cell/L and the fermentation was monitored.

**Metabolomic Sample Preparation and Analysis:** Samples were collected at five different time points throughout the fermentation time course for metabolomic analysis. Figure 1. Sampling and metabolite extraction was performed as outlined in Gonzales et al.1 and Lange et al.2 Simultaneous quantification of glycolytic and pentose phosphate pathway metabolites was done using reversed-phase liquid chromatography-mass spectrometry and in vitro 13C labeling as described in Yang et al.3

**FERMENTATION MODELING**

**Data Analysis:** Substrate consumption and product formation was modeled using a system of ordinary differential equations (see below). Kinetic parameters were estimated by minimizing the SSE between experimental and predicted values.

- Cell growth - $X$
  \[
  \frac{d[X]}{dt} = \mu [X] \left(1 - \frac{[X]}{X_{max}}\right)
  \]

- Glucose consumption - G
  \[
  \frac{d[G]}{dt} = -v_s [G] [X]
  \]

- Xylose consumption - S
  \[
  \frac{d[S]}{dt} = \frac{\left[\frac{[S]}{[X]} \frac{1}{1 + \frac{[G]}{[S]}}\right]}{[K_M_1 + [G] + [S]}
  \]

- Ethanol production - P
  \[
  \frac{d[P]}{dt} = -Y_{P/G} \frac{d[G]}{dt} - Y_{P/S} \frac{d[S]}{dt}
  \]

- Glycerol production - Gly
  \[
  \frac{d[Gly]}{dt} = -S_{gly} \frac{d[G]}{dt} - S_{gly} \frac{d[S]}{dt}
  \]

- Xylitol production - Xyl
  \[
  \frac{d[Xyl]}{dt} = -S_{xyl} \frac{d[G]}{dt} - S_{xyl} \frac{d[S]}{dt}
  \]

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