Performance Screening of Chemostat Adapted Recombinant *Zymomonas mobilis* Strains

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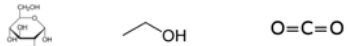
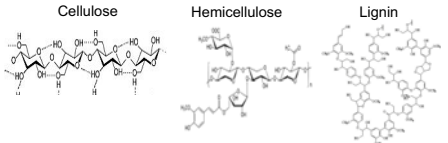
Objectives

To determine which strains of *Zymomonas mobilis* that were isolated from a continuous fermentation have become more fit than the current strain 8b in the presence of inhibitors and to determine whether or not the continuous fermentation method is an effective strategy to adapt *Zymomonas mobilis* to inhibitors such as furfural and acetic acid.

Background



Alcoholic Fermentation: Sugar **2 Ethanol + 2 Carbon Dioxide**



- **Corn Stover biomass** is comprised mainly of: 33.9% cellulose (C6 sugar chains), 24.1% hemicellulose (C5 sugar chains), and 11.4% Lignin.
- For maximum ethanol yield, all sugars including **C5 sugars must be metabolized**.
- Pretreatment conditions such as the use of dilute acid and high temperatures break down hemicellulose into soluble C5 sugars and produce inhibitors to fermentations such as **acetic acid** and **furfural.** The ethanol produced from the fermentation also acts as an inhibitor.

- NREL developed a recombinant glucose-xylose co-fermenting ***Zymomonas mobilis*** to ferment ethanol because of its high ethanol yield, its ability to metabolize in a low pH environment, and for its high tolerance to inhibitors.
- Continuous fermentation method was used with selective pressures to adapt *Zymomonas mobilis* to stressful environments over a long period of time.
- Samples of the culture at various time points in continuous fermentation were frozen back in glycerol to be analyzed for performance in the presence of inhibitors.

Materials and Methods

Small Scale Fermentations

- 15 sub strains of *Z. mobilis* 8b revived from various time points in continuous fermentation RMG10%X2% revival media, ~5.8 pH, 33 degrees Celsius incubating shaker box
- 5 varying concentrations of two main inhibitors: Acetic acid and Furfural

Figure 1

- Analysis of Glucose, Xylose, Acetic Acid, Ethanol, and pH at 0 and 24 hours using HPLC
- Determined glucose utilization, xylose utilization, ethanol production, ethanol product yield

Figure 1. shows that cell density decreases from left to right as the concentration of furfural increases.

Bioscreen C

- *Bioscreen C was used to plot cell growth via optical density every 10 minutes over a 24 hour time interval*
- *Each sample compared to a "blank" solution identical to the sample without any cells*
- *Growth points were taken from an average of four wells per sample with 300 microliters per sample*
- *Each sample contained exact media from small scale fermentations*

Figure 2

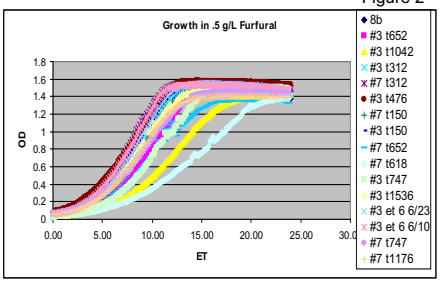


Figure 2. shows a growth curve derived from the Bioscreen C of all 16 strains over a 24 hour time period

Results

Maximum Growth Rates

Figure 3

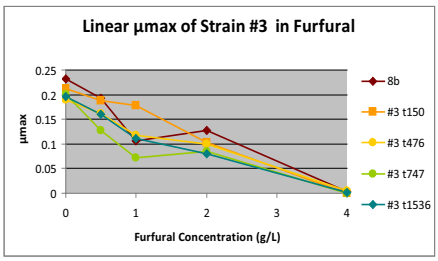


Figure 3. *shows a comparison of the maximum growth rates of sub-strain #3 in different concentrations of furfural. The graph was derived from the maximum linear growth on a growth curve very similar to one shown in Figure 2.*

- *Growth rates are comparable 8b. There is no correlation between an increase in growth rate and time spent in the chemostat .*

Ethanol Production Yield

Ethanol Product Yield=

$$\frac{\text{(Ethanol Produced/ Initial Glucose+Xylose)}}{0.51} \times 100$$

Figure 4. *shows a plot of ethanol product yields of different sub-strains of #7. We would expect to see a higher ethanol product yield in the sub-strains chronologically over time compared to 8b.*

- *There was no increase in ethanol product yield compared to 8b. Similar results are seen with #3 as well as the two strains in acetic acid.*

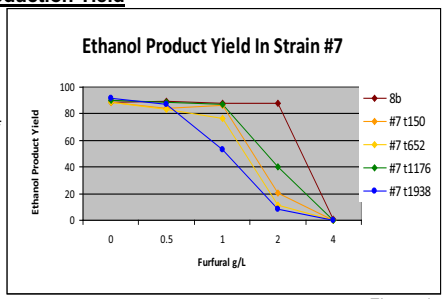


Figure 4

Inhibition and Ethanol Product Yield Profiles

Figure 5. *shows both the maximum growth for strain #7 in acetic acid and furfural as well as the ethanol product yields in both inhibitors. Concentrations of 10 g/L acetate and 2 g/L furfural were chosen because those are the concentrations in the corn stover hydrolyzate .*

- *There is no increase in growth rate or ethanol product yield over the duration of chemostat.*

Figure 5

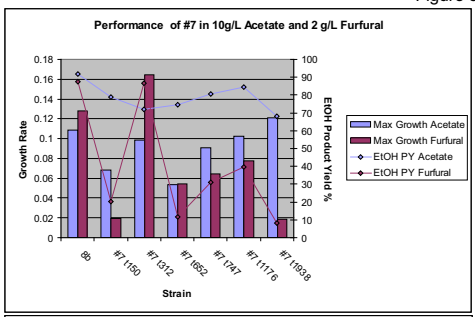


Figure 6. *is a similar representation as Figure 5. for strain #3 results.*

- *There is no trend in max growth rate or ethanol product yield over the duration of the continuous fermentation method.*

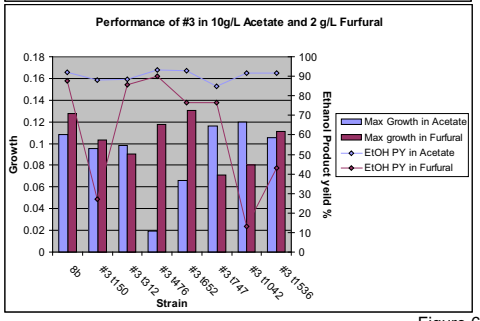


Figure 6

Conclusions

Evaluation of Sub-strains

- Neither sub-strain #3 nor #7 seemed to perform better than 8b in the presence of furfural or acetic acid.
- Further screening of the two strains in hydrolyzate is needed to confirm results

Evaluation of Chemostat Method

- Continuous fermentation method may have been ineffective
- Further screening of strains adapted via other methods such as daily transfer may show better results

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