High Gravity and High Cell Density Enhance the Fermentation of Hexose Sugars Present in Softwood Hydrolysates

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Abstract

Biomass-based ethanol has the potential to provide substantial environmental, energy security and geopolitical benefits in comparison to fossil based transportation fuels. However, fermentation of softwood derived sugars to ethanol is problematic due to the generally low sugar concentrations that can be supplied and due to the naturally occurring and process derived inhibitors that are typically present. In the present study, the hexose rich, water soluble fraction obtained after steam treatment of Douglas fir chips was supplemented with glucose up to 22% (v/v) to simulate high solids, un-detoxified substrate, to see if a high sugar concentration/high cell density approach could better cope with known inhibitory materials. The addition of supplemental glucose enabled the faster and quantitatively higher removal of hydroxymethylfurfural (HMF) and this observed boosting effect was more pronounced with three superior strains. It appears that high cell density can provide effective fermentation at high sugar concentrations while enhancing inhibitor reduction. A 77% ethanol yield could be achieved with strain Lallemand 4 after 48 hours at high cell density with some nutritional supplementation.

1. Introduction

In contrast to sugar and starch based ethanol, the sugars in lignocellulosic feedstocks are present as complex polymers (1,2). Thus, lignocellulosic ethanol faces four primary challenges:

- ✓ need for complex pretreatment and enzymatic hydrolysis steps to obtain fermentable sugars
- ✓ prevalence of both pentose and hexose sugars
- ✓ hydrolysates with low sugar concentrations
- ✓ high levels of fermentation inhibitors.

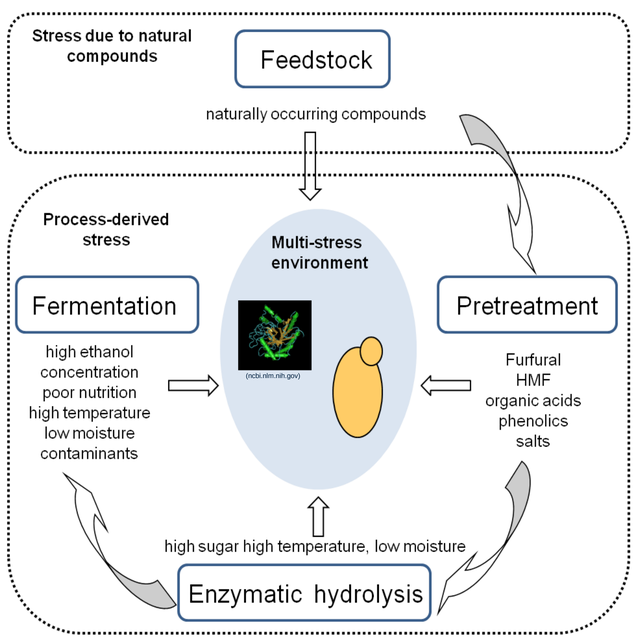

Figure 1. Multi-stress environment encountered by the yeast in lignocellulosic ethanol production.

2. Fermentation of lignocellulosic feedstock: challenges

- ➢ Low sugar concentration in the liquor after pretreatment and hydrolysis steps leading to low ethanol titer.
- ➢ Presence of naturally occurring and process derived (pretreatment through fermentation) inhibitors.

3. Objectives

To determine whether a combined high cell density-high sugar concentration approach can be used successfully to overcome the effect of fermentation inhibitors on yeast metabolism to improve ethanol yield and productivity from softwood hydrolysates.

4. Fermentation strategy

Substrate

✓ *Low sugar concentration*:
water soluble fraction (WSF) generated by steam pretreatment of Douglas-fir with 3.17% total monomeric sugars content

✓ *High sugar concentration*:
simulated with glucose supplementation of the original WSF to reach 22% total monomeric sugars

✓ *Inhibitors concentration*:

hydroxymethylfurfural (HMF) 2.4 g/L
furfural 0.5 g/L
acetic acid 3.0 g/L
total phenolics 1.7 g/L

Cell density

✓ Low cell density: $6x10^6$ cells/ml
✓ High cell density: $150x10^6$ cells/ml

Yeast strains

✓ Lallemand: L1, L2, L3, L4
✓ Tembec: T1, T2

5. Key Results

High cell density improved the fermentation performance

- **Without glucose supplementation**

✓ While low cells density resulted in poor fermentation performance, for most strains, high cell density resulted in > 70% ethanol yield in 48 hours (Fig. 2).

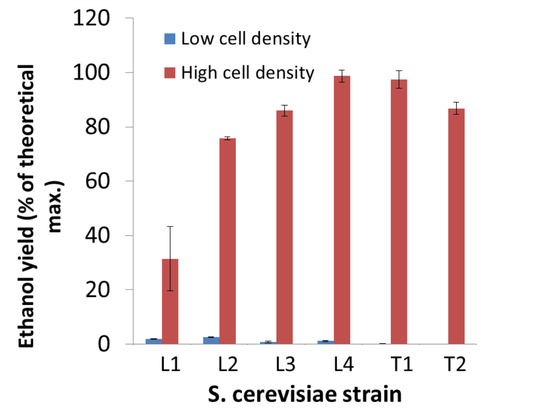

Figure 2. Ethanol production by the studied strains in glucose supplemented WSF (G-WSF) at low and high cell density.

✓ With high cell density, the best performing strains L4 and T1 resulted in ~98% yield with complete glucose and mannose consumption after 8 h and 4 h, respectively (Fig. 3).

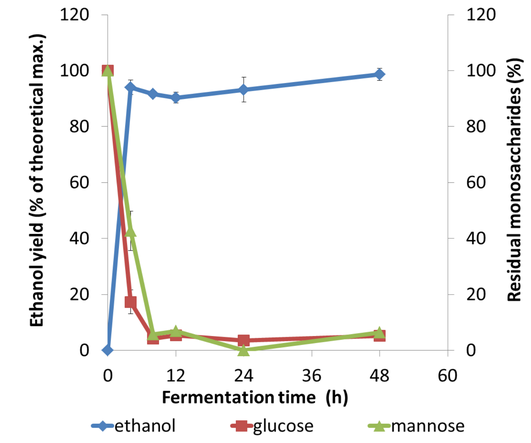

Figure 3. Fermentation profile of strain L4 at high cell density.

- **With glucose supplementation**

✓ With added glucose (G-WSF), strain L4 at high cell density performed the best, resulting in 65% ethanol yield in 48 hours.

✓ Further nutrient supplementation for strain L4 increased the yeast growth rate by 25% and achieved an ethanol yield of 77% (% of theoretical maximum).

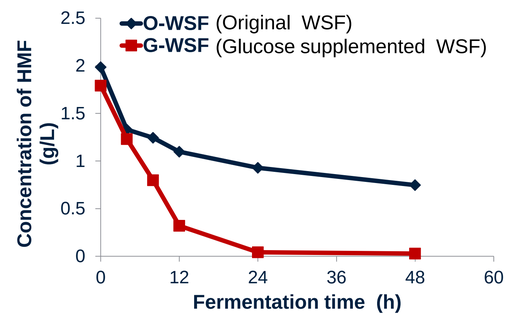

Figure 4. HMF removal by strain L4 at high cell density from the original and glucose supplemented WSF.

Glucose supplementation improved inhibitor removal

✓ At high cell density, more then 98% of HMF in the glucose supplemented fermentations were metabolized within 24 hours by strain L4 (Fig. 4).

✓ At high cell density, furfural was nearly completely converted for both the glucose supplemented and the original WSFs.

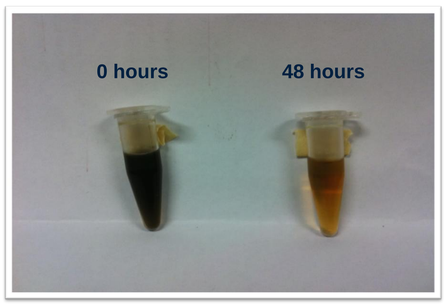

Figure 5. Colour change potentially due to inhibitor uptake by strain L4 after 48 fermentation hours at high cell density and high sugar concentration.

6. Conclusions

✓ High cell density is an effective strategy to ferment steam-pretreated Douglas-fir hydrolysates to improve sugar consumption and obtain high ethanol yields.

✓ Nutrient supplementation in addition to high sugar concentration and high cell density further improved the ethanol yield.

✓ Higher initial glucose concentration together with high cell density enables faster and quantitatively higher removal of hydroxymethylfurfural (HMF) and furfural.

References:

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[2] Humbird et al., 2011. Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol. *Technical report* NREL/TP-5100-47764