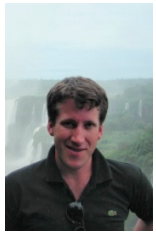
P-037	**Together we are strong! Inhibitor tolerance conferred by good neighbors?**	

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INTRODUCTION AND OBJECTIVES

Bioethanol from lignocellulosic materials is one of the desired alternatives to meet the increased demand of renewable fuels. However, there are challenges in several steps of lignocellulose processing, including pretreatment, hydrolysis and fermentation – especially due to compounds inhibitory to the yeast, formed during the pretreatment. Results from our lab have however shown that there are ways of reaching a high inhibitor tolerance in low tolerance yeast strains – namely encapsulation of the yeast (Talebnia 2005). This also gives a number of other advantages, such as easier product recovery and higher possible biomass in the reactor. Using flocculating strains in the fermentation shows many similar advantages and is in many ways a similar system. In this work, a flocculating yeast strain has thus been used as a model system for encapsulated yeast, in order to understand the mechanisms leading to increased inhibitor tolerance.

MATERIALS AND METHODS

Hydrolyzate

The carbon and energy source used in the hydrolyzate experiments in this work was hydrolyzate made by dilute acid hydrolysis of spruce chips.

Encapsulation

The yeast strain CBS 8066 was encapsulated in liquid core capsules by mixing the cells with $CaCl_2$, carboxymethylcelluose and Tween20 and subsequent dripping of this solution into a stirred alginate solution. The formed capsules were thereafter hardened in a $CaCl_2$ solution.

Fermentations

Batch fermentations with the flocculating yeast and the encapsulated yeast were performed in shake flasks in a water bath at 30°C. The flocculating and the non flocculating yeast strains were cultivated in defined medium containing different inhibitors or in hydrolyzate medium. The encapsulated yeast, CBS 8066, was cultivated in hydrolyzate medium.

RESULTS AND DISCUSSION

A flocculating yeast strain (Figure 1) was isolated from a Swedish ethanol plant (Domsjö Fabriker AB) fermenting sulphite liquor, and registered at Culture Collection University of Gothenburg as CCUG 53310. It has previously been shown in our laboratory that this strain can successfully ferment lignocellulosic hydrolyzates, where a freely suspended strain, CBS 8066, failed to assimilate any sugar (Purwadi 2007). However, upon encapsulation in Ca-alginate capsules (Figure 2), the strain CBS 8066 was able to successfully withstand the effect of the inhibitors and ferment lignocellulosic hydrolyzate in both batch (Talebnia 2005) and continuous mode (Talebnia 2006).

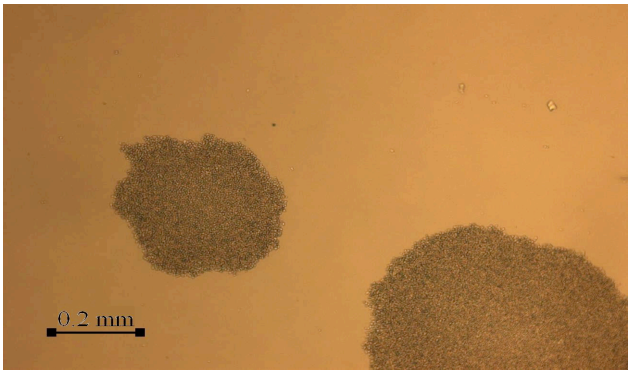

Figure 1 : Yeast flocs of the strain CCUG 53310 seen in a light microscope.

Figure 2 : *S. cerevisiae* encapsulated in Ca-alginate capsules.

The batch cultivations showed a significantly greater tolerance of the flocculating yeast to the carboxylic acids, the furan aldehydes and the hydrolyzate medium, whereas, surprisingly, the non flocculating strain could better tolerate the phenolic compounds medium (Figure 3). The non flocculating strain was however able to ferment the toxic hydrolyzate when encapsulated (Talebnia 2005).

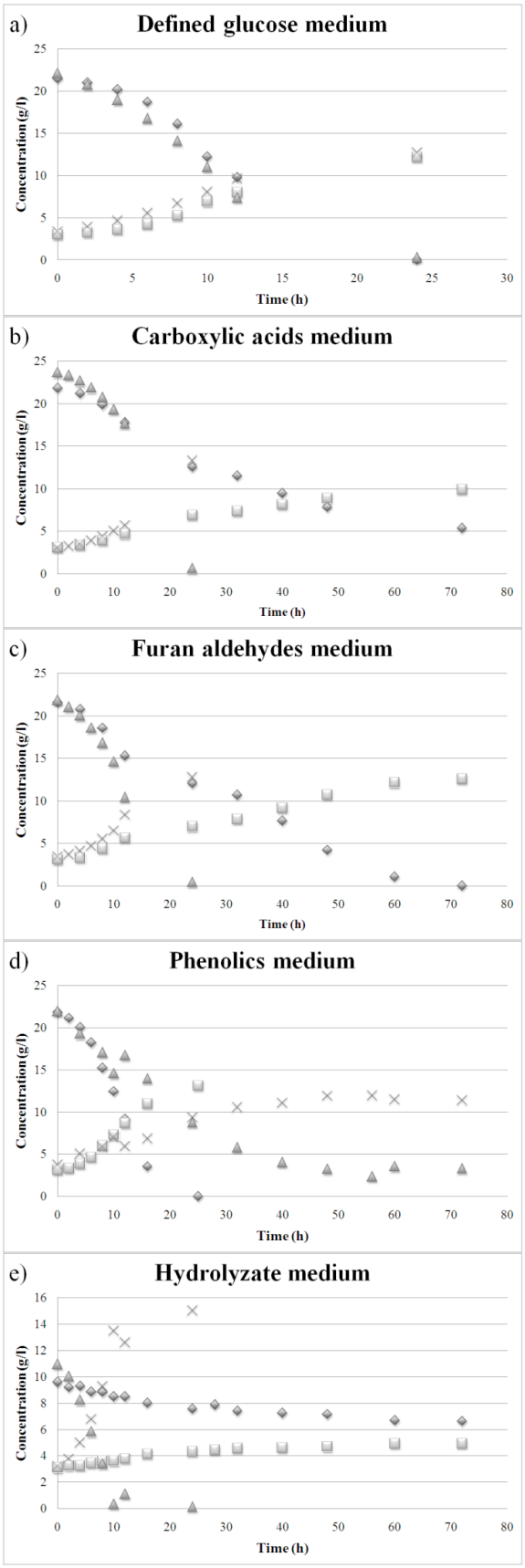

Figure 3 : Glucose consumption and ethanol production curves for the flocculating yeast strain CCUG 53310 and the strain CBS 8066. Diamonds and triangles – glucose consumption for CBS 8066 and CCUG 53310 respectively; squares and crosses – ethanol production for CBS 8066 and CCUG 53310 respectively in different media.

There are similarities between yeast cells living in large flocs and yeast living inside a capsule, such as a high local cell concentration. The encapsulation of yeast cells can almost be seen as an artificial way of producing flocs, by keeping cells tight together. We hypothesize that this greatly enhanced local biomass concentration strongly contributes to this increased tolerance, since more cells will be able to survive. That high biomass concentration leads to a greater number of living cells have also been known for a long time (Chung 1985). Further we think that the cells toward the outside of the floc, as well as in the outer layer inside the capsule, convert most of the inhibitors and partly die, protecting the inner lying cells from toxic levels of the inhibitory compounds.

For a better understanding of the resistance mechanism a study of the inhibitor tolerance against different classes of inhibitors for the encapsulated CBS 8066 will be performed. Proteomic and genomic studies of encapsulated yeast in comparison with the same strain in suspension will also be done to show changes in the yeast physiology due to the encapsulation, giving further clues to why there is an increased resistance.

CONCLUSIONS

The effect of different inhibitor classes present in the hydrolyzate, (furan aldehydes, organic acids and phenolic compounds,) on the flocculating strain as well as the freely suspended strain was investigated, showing that the flocculating strain was indeed more tolerant against furan aldehydes and carboxylic acids. Interestingly though, the CBS 8066 strain, that could only tolerate hydrolyzate when encapsulated, could tolerate the presence of phenolic compounds in the growth medium. This shows that higher local cell concentration does not help against all inhibitors, but there must also be other underlying reasons, i.e. differences between the strains.

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