P-091 **Increasing the termotolerance of *Saccharomyces cerevisiare* by encapsulation**

Ylitervo P. [1,2#], Franzén CJ. [2*] and Taherzadeh MJ. [1*]
[1] University of Borås - Borås, Sweden
[2] Chalmers University of Technology – Gothenburg, Sweden
* Supervisor # paivi.ylitervo@hb.se

INTRODUCTION AND OBJECTIVES

Encapsulated yeast has several advantages for ethanol production from lignocellulosic materials such as enhanced inhibitor tolerance and cell stability, higher biomass concentration inside the reactor, easier cell recovery and shortened fermentation time (Talebnia 2005).

During encapsulation, cells are captured inside a spherical capsule composed of an outer semipermeable membrane and an inner liquid core. Compared to entrapment in a porous gel bead, the diffusion resistance is therefore much lower trough the capsule membrane (Talebnia 2005).

Encapsulation has in several studies shown to stabilize cells and improve the tolerance for inhibitors (Talebnia 2005, Pourbafrani 2008). The main goal of the present work was to investigate if encapsulation can also improve the termotolerance characteristics of *S. cerevisiae* in order to produce ethanol at high temperatures. In the experiments glucose conversion and ethanol production was recorded during 24 h in encapsulated and suspended yeast at high temperatures.

MATERIALS AND METHODS

Yeast strain and medium

Saccharomyces cerevisiae CBS 8066 was used in all the experiments. The cultivations were performed in a defined synthetic medium (Taherzadeh 1996). The initial glucose concentration was 30 g/L.

Encapsulation method

Cell-seeded capsules were prepared by first growing yeast in shake flasks in synthetic media for 24 h. The cell suspension was centrifuged to collect the yeast cells and thereafter mixed with a sterile solution containing 1.3% (w/v) $CaCl_2$ and 1.3% carboxymethylcellulose. To produce alginate capsules this solution was added drop-wise into a 0.6% (w/v) sodium alginate solution with 0.1% (w/v) Tween 20. After gelling (10 min), capsules were washed with water and hardened in 1.3% $CaCl_2$.

The alginate capsules were thereafter immersed in a 0.2% (w/v) chitosan solution with 300 mM $CaCl_2$ for 24 h to increase the strength of the capsule membrane. Low molecular weight chitosan was used and dissolved in a 0.040 M acetate buffer solution with pH 4.5. The diameter of the capsules measured prior to cultivation was 3.7-3.8 mm.

Batch cultivations

Prior to anaerobic cultivations, biomass was accumulated aerobically for 48 h to ensure a high biomass concentration inside the capsules. Five times more synthetic medium was used for each volume of capsules. During the biomass accumulation the medium was changed after 24 h. Then, 10 ml cell-seeded capsules were transferred to each 250 ml conical flask with 100 ml growth medium.

The suspended yeast was prepared by growing it in a similar manner as in the capsule biomass accumulation cultivation for 48 h. Suspended cells were separated by centrifugation before adding new medium. In order to provide the same yeast concentration in both encapsulated and suspended yeast cultivations some capsules were crushed and the OD was measured at 600 nm. Based on this, suspended yeast was added to achieve the same total concentration as in 10 ml capsules.

For anaerobic cultivations, each flask was equipped with a silicon rubber stopper, with stainless steel capillaries for sampling and a glass tube with a loop trap (Taherzadeh 1997). Four different temperatures were tested namely 45, 47, 50 and 55°C. Duplicate cultivations were used for each temperature.

RESULTS AND DISCUSSION

The encapsulated yeast had successfully fermented all glucose after 24 h at 45°C (Fig. 1). At 47°C the glucose conversion decreased to 76±7%. When the temperature was to 55°C glucose consumption became very limited and only occurred in the initial cultivation phase. Even at 55°C the glucose concentration decreased. However, this is probably due to the fact that glucose will diffuse into the capsules, hence lowering the glucose level in the medium.

The highest ethanol concentration (12 g/L) was produced by the encapsulated yeast at 45°C (Fig. 2). In neither of the suspended yeast cultivations, ethanol level increased above 2 g/L. At 55°C no ethanol was produced by either encapsulated or suspended yeast (Table 1).

At the end of the batch cultivation, the capsules were filled with yeast and were completely yellow (Fig. 3). The fact that the yeast is forced to grow in close contact of each other can be a reason to the improved thermotol-

erance. Encapsulated yeast has a different cellular composition compared to suspended yeast. Prolonged growth of encapsulated yeast for example results in higher accumulation of carbohydrates such as glycogen and trehalose (Talebnia 2007). It is known that trehalose often accumulates during stress conditions.

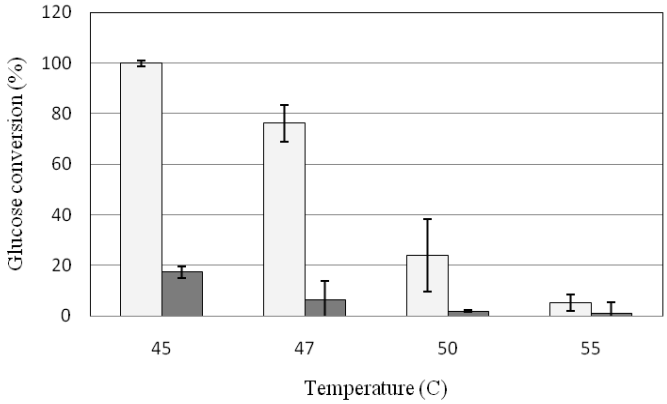

Figure 1 : Glucose consumption after 24 h anaerobic cultivation of encapsulated (white bars) and suspended (gray bars) *S. cerevisiae*, with a 95 % confidence interval

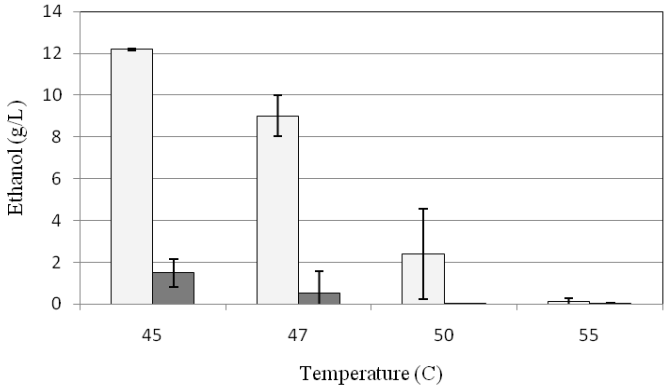

Figure 2 : Ethanol concentration after 24 h anaerobic cultivation of encapsulated (white bars) and suspended (gray bars) *S. cerevisiae*, with a 95 % confidence interval

Figure 3 : Chitosan/alginate capsules, 3.7 -3.8 mm in diameter, containing *Saccharomyces cerevisiae*

Table 1 : Ethanol yield after 24 h

	Capsules	Susp. yeast
Temp. (C)	**Y_{ES}**	**Y_{ES}**
45	0.441 ± 0.020	0.321 ± 0.187
47	0.441 ± 0.038	0.316 ± 0.243
50	0.401 ± 0.146	0.028 ± 0.008
55	0.08 ± 0.07	0.10 ± 0.28

Yields are in g/g consumed glucose after 24 h with a 95 % confidence interval.
Y_{SE} – Ethanol yield

CONCLUSIONS

There was a major difference in the glucose conversion capacity between encapsulated and suspended yeast at higher temperatures. Yeast grown in suspension showed a very poor glucose conversion at all the examined temperatures, whereas, encapsulated yeast succeeded to ferment all sugars at 45 °C. Therefore, encapsulation can be a promising method to increase the thermotolerance of yeast. However, the long term stability of the encapsulated yeast has to be examined to evaluate the robustness of the system.

REFERENCES

• Alosta H. (2008) *Cell Encapsulation Technology: The Effect of Nonionic Surfactants on the Diffusional Properties of Hydrogel Capsules*, VDM Verlag Dr. Müller Aktiengesellschaft & Co. KG, Dudweiler Landstr., Saarbrücken, Germany

• Pourbafrani M. et al. (2007) *Protective Effect of Encapsulation in Fermentation of Limonene-containing Media and Orange Peel Hydrolyzate*, International Journal of Molecular Sciences 8(8), 777-787

• Taherzadeh MJ. et al. (1996) *The effect of pantothenate deficiency and acetate addition on anaerobc batch fermentation of glucose by Saccharomyces cerevisiae.* Applied Microbiology and Biotechnology 95:45-57

• Taherzadeh MJ. et al. (1997) *Characterization and fermentation of dilute-acid hydrolyzate from wood.* Industrial & Engineering Chemistry Research 36:4659-4665

• Talebnia F. et al. (2005) *Ethanol Production From Glucose and Dilute-Acid Hydrolyzates by Encapsulated S. cerevisiae*, Biotechnology and Bioengineering Vol. 90, No. 3 p 345-353

• Talebnia F. et al. (2007) *Physiological and morphological study of encapsulated Saccharomyces cerevisiae*, Enzyme and Microbial Technology 41:683-688