Robust liquid core APTES-alginate-chitosan-alginate capsules for 2nd generation bioethanol production

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INTRODUCTION

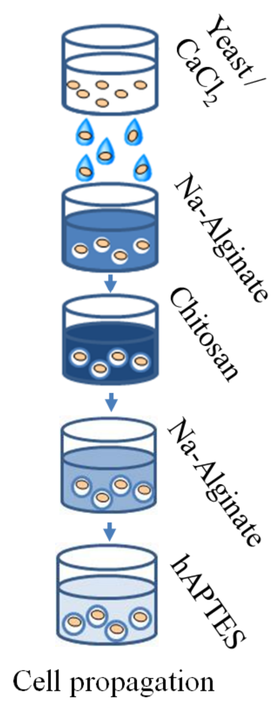
Encapsulation of yeast in liquid core gel membrane capsules has been shown to improve the inhibitor tolerance and even thermo-tolerance of yeast[(1,2)]. Encapsulation is therefore a very promising bioprocessing methodology for second generation bioethanol production using lignocellulose hydrolyzates. However, for industrial applications, capsules must be made robust enough to endure long periods and numerous cultivations without breakage due to chemical degradation or mechanical attrition.

In this study, liquid core Alginate-Chitosan-Alginate (ACA) capsules containing *Saccharomyces cerevisiae* were produced by the liquid-droplet-forming method and treated with 3-aminopropyltrietoxysilane (APTES) to coat the increase the mechanical durability of the capsules. The integrity of these membranes is not compromized by a lack of Ca^{2+} ions.

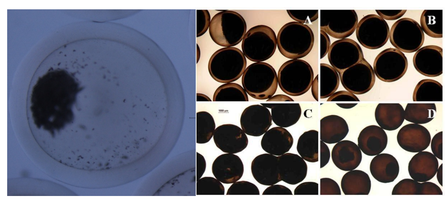
RESULTS

Encapsulation procedure

The yeast *Saccharomyces cerevisiae* CBS8066 was encapsulated by dripping a suspension of yeast with $CaCl_2$ and CMC into a Na-alginate solution. The resulting capsules were treated with chitosan dissolved in acetate buffer. A second coating of alginate was added, after which the alginate - chitosan - alginate (ACA) capsules were treated with different concentrations of 3-aminopropyl-trietoxysilane, hydrolyzed over-night in water. This results in poly-(3-aminopropylsiloxane) - alginate - chitosan - alginate capsules.

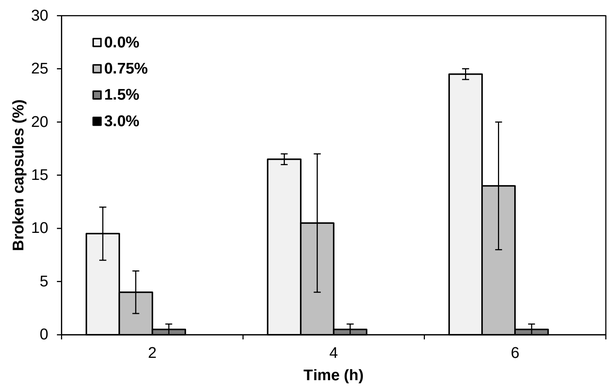


The encapsulated yeast was propagated aerobically 24 h in defined glucose medium, before being used in hydrolysate fermentations.



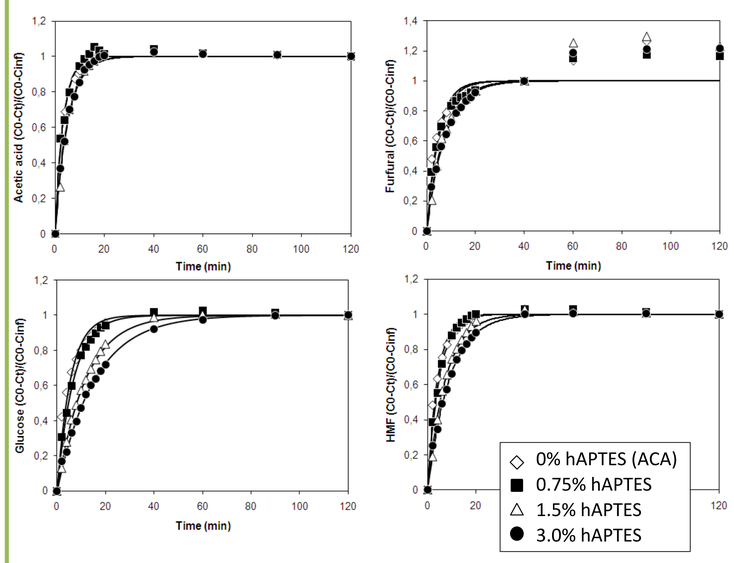
Robustness test

The robustness was examined by mixing 100 capsules with 35 ml 0.9% NaCl in a baffled 200 ml SARA-fermentor (custom made by Belach Bioteknik, Stockholm, Sweden). The contents were mixed vigorously at 600 rpm at room temperature.



ACA capsules had poor mechanical robustness, since 25% of the capsules ruptured within 6 h when subjected to intense agitation. Capsules treated with a 1.5% hAPTES solution were more robust, and only 0-2% of these capsules broke in the shear test.

Permeability of the capsules



Treatment with hAPTES caused a decrease in the permeability of the capsule membrane. The decrease was dependent on the hAPTES concentration.

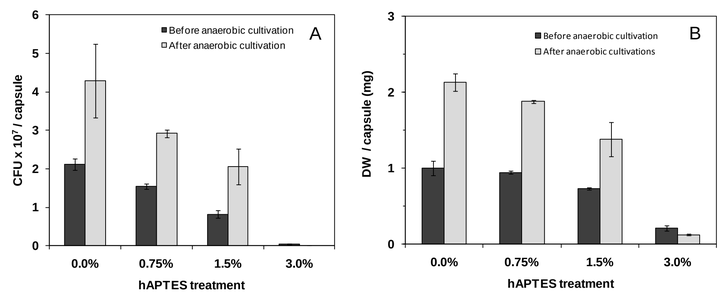
Capsules were immersed in solutions containg 50 mM of the indicated compounds, and the concentration was monitored. The volumetric overall mass transfer coefficient *K* was estimated from the decrease in the solute concentration in the liquid surrounding the capsules.

Table I. Calculated volumetric mass transfer coefficient (K) of components into empty untreated or hAPTES treated ACA capsules. Calculated based on the average of two samples.

	K (cm^3/min)			
hAPTES conc.	**0%**	**0.75%**	**1.5%**	**3.0%**
Glucose	5.2	4.2	2.4	1.9
Acetic acid	8.6	8.6	5.5	5.7
HMF	7.0	6.0	3.9	3.2
Furfural	6.1[a]	5.6[a]	4.0[a]	3.8[a]

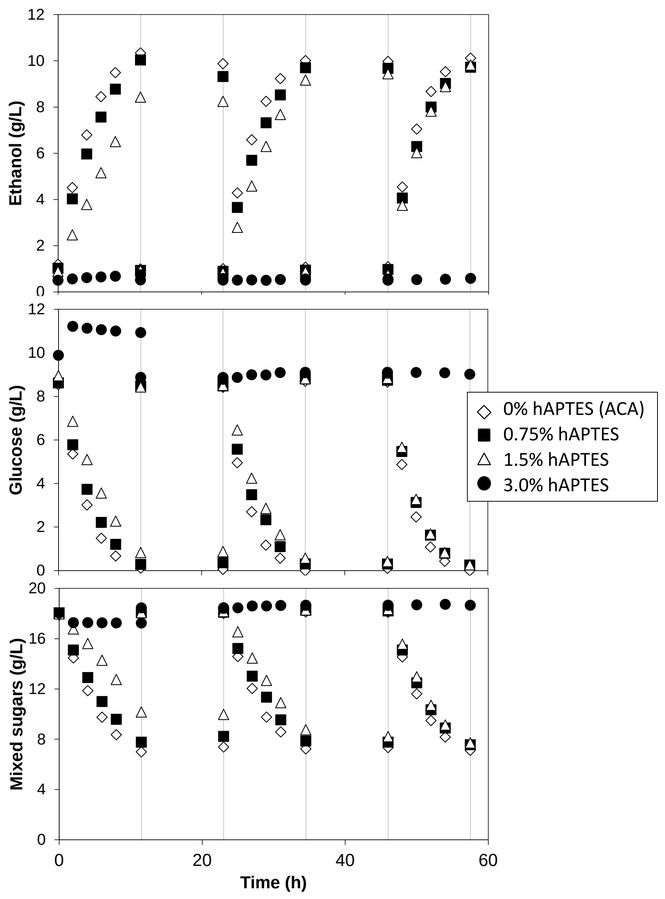
[a] Calculated based on data points from 0-40 min

Viability after 5 batch fermentations



The cell content in the capsules decreased with increasing hAPTES concentration. This was clear both after the aerobic propagation culture and after the five repeated anaerobic hydrolyzate fermentations. However, because the size of the capsules decreased during hAPTES treatment, the concentration of cells inside the capsules was approximately equal, 13-14 g/l, up to 1.5% hAPTES. In the 3% hATPES treatment, cell viability was very low, likely due to a negative effect of the hAPTES or the low permeability of these capsules.

Fermentation efficiency



During pretreatment and / or hydrolysis of ligno-cellulosic raw materials, inhibitors are generated as degradation products from both the lignin and hemicellulose fractions. Encapsulation enables fermentation of such inhibitory hydrolyzates[(1,3)]. The fermentation rate in the hAPTES-treated capsules was lower than in the ACA capsules in the first batches. However in the fifth repeated batch, the ethanol production was nearly the same as for untreated ACA capsules

CONCLUSIONS

The mechanical robustness of ACA capsules can be easily improved by treating the capsules with hAPTES. Capsules treated with 1.5% hAPTES showed excellent mechanical robustness and similar ethanol production profile as untreated ACA capsules in the fifth consecutive batch cultivation. The obtained poly(3-aminopropylsiloxane) - alginate - chitosan - alginate capsules are likely to be appropriate for industrial application.

References:

[(1)]Talebnia, F., *et al.*, (2005) *Biotechnol and Bioeng*, **90**, 345-353.

[(2)] Ylitervo, P., *et al.*, (2011) *J Biotechnol*, **156**, 22-29.

[(3)] Talebnia, F. and Taherzadeh, M.J., (2007) *Enzyme Microb Technol*, **41**, 683-688.

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