COMPARATIVE STUDY OF ENCAPSULATED AND ENTRAPPED YEAST FOR BIOETHANOL PRODUCTION

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Introduction

Yeast Immobilization is a technology used more and more in bioethanol production. In the present study four (4) methods of yeast entrapment (single alginate beads, carrageenan beads coated with chitosan, double alginate beads and double alginate beads coated with chitosan and alginate) and two (2) methods of yeast encapsulation (polyethylene glycol hollow alginate beads and xanthan hollow alginate beads) have been comparatively studied. Synthetic medium with 25 g/L, 50 g/L and 100g/L of glucose were use for the fermentations. Yeast viability, cell leakage, mechanical and chemical strength of the beads were compared, as well as fermentation kinetics, sugar consumption, ethanol, glycerol and acetic acid production.

Immobilized Yeast Bead Formation

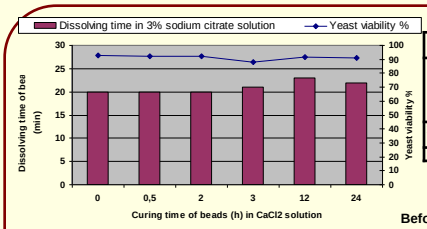

Diagram 1. Effect of curing time of the beads on cell viability and bead dissolving time

Method	Alginate	Carrageenan	Hollow Alginate Polyethylene Glycol	Hollow Alginate Xanthal Gum	Alginate-Alginate (Double Layer)	Alginate-Alginate Chitosan (Double Layer)
Materials	2.5% Alginate (w/v) + yeast	2.0% Carrageenan (w/v) + yeast, 0.25% Chitosan (w/v) (protective layer)	1.92% Alginate (w/v) (membrane), 20% PEG (MW=7500) + 3% CaCl2 (w/v) + yeast	1.92% Alginate (w/v) (membrane), 0.3% Xanthan Gum + 3% CaCl2 + yeast	2.5% Alginate (w/v) + yeast (inner layer), 2.0% Alginate (w/v) (outer layer)	2.5% Alginate (w/v) + yeast (inner layer), 2.0% Alginate (w/v) (outer layer), 0.5% (w/v) Chitosan (protective layer), 0.1% Alginate (w/v)
Bead diameter (mm)	2,80	2,96	3,81	4,06	3,78	3,85
Symbol	Alg bead	Car bead	Alg(PEG) capsule	Alg(Xan) capsule	Alg(Alg) bead	ACA bead

Before fermentation

After fermentation

Photo 1. Detail of broken hollow Alg(Xan) bead after fermentation

Results & Discussion

Breakage % of beads & capsules

Table 1. Percentage of broken beads. Mean value (triplicate) of the ruptured beads after fermentation in different glucose concentration solutions.

Initial Sugars / Method	25g/l	50g/l	100g/l
Alg	10%	47%	93%
Alg(Alg)	3%	30%	60%
Alg(PEG)	20%	37%	20%
Alg(Xan)	10%	20%	20%
ACA	3%	43%	70%
Car	27%	33%	70%

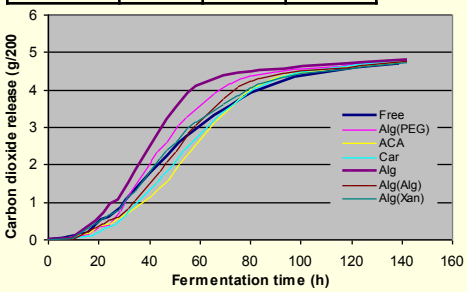

Diagram 2. Comparison of the CO2 release kinetics in fermentation medium with 100g/l glucose.

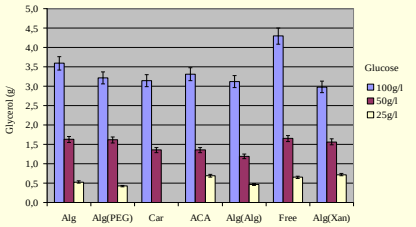

Diagram 5: Glycerol production of the different immobilization method compared to free cells.

Chemical instability and cell leakage

Table 2. Chemical instability and Bead cell leakage in mild solutions of chelating agents after 12 days and on. Mean value of triplicate.

	Single immobilization Entrapment		Double immobolization Entrapment	Encapsulation in hollow beads
	Alg	Car	Alg/Alg & ACA	Alg(PEG) & Alg(Xan)
Citric acid 300 mg/L	High	Medium	Null	Null
Lactic acid 500 mg/L	Null	Low	Null	Null
Phosphate buffer 0,01M	Null	Null	Null	Null

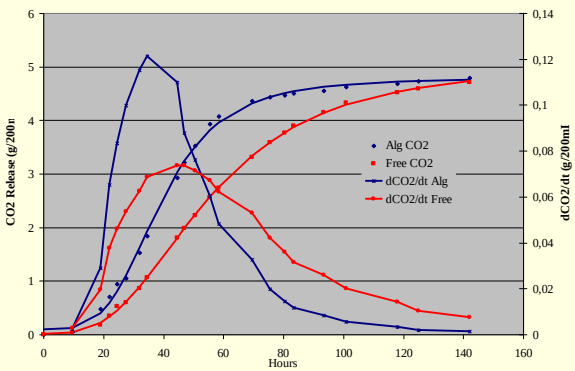

Diagram 3. Comparison of the CO2 release kinetic and dCO2/dt (CO2 release rate) of the Alg beads and free cells in 100g/l glucose concentration

Results

- The fermentation time for Alg beads was shorter in all sugar concentrations from all other immobilization methods as well as free cell fermentation.
- Immobilized yeast cells produce significantly lower amounts of glycerol and secondary metabolites, when compared with free cells, in any method of immobilization.
- Cell release (leakage) was observed for carrageenan beads as they exhibited peeling of the chitosan layer after 2 days in lactic acid and citric acid solutions. Cell leakage was also observed for single layer alginate beads but only after one (1) week. Phosphate buffer solutions did not affect cell leakage.
- There was no significant differences in the production of ethanol and acetic acid, nor in final pH for the immobilized cells and free cells. However some immobilized yeast system had higher ethanol production yield and productivity

Conclusions

Yeast immobilization for ethanol production has several advantages over free cell fermentation. Immobilized cells produced lower amounts of glycerol and secondary metabolites and in some cases higher ethanol production yield and productivity.
Cell leakage was observed in all cases except when beads (double alginate beads) were used for the second fermentation in sparkling wine production in wine with 11%vol ethanol, 25 g/L sugars and CO_2 pressure where no cell release was observed.
Immobilized cells for bioethanol production can be used in continuous fermentation were cell wash out will be minimized. Cell growth will be lower and thus secondary metabolites minimized.
In continuous fermentation higher fermentation rates and ethanol yield can be obtained minimising the cost for ethanol production.

P52:Comparative Study of Encapsulated and Entrapped Yeast for Bioethanol Production

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Yeast Immobilization is a technique used more and more in bioethanol production. In the present study four (4) methods of yeast entrapment (single alginate beads, carrageenan beads coated with chitosan, double alginate beads and double alginate beads coated with chitosan and alginate) and two (2) methods of yeast encapsulation (polyethylene glycol hollow alginate beads and xanthan hollow alginate beads) have been comparatively studied.
Yeast viability was studied after dissolving beads in sodium citrate. Sodium citrate did not have any significant effect on viability, although in some cases a 5% drop in viability was observed. Carrageenan beads could not be dissolved in sodium citrate in a few hours time unless heat was used.
Protocols were optimized in order to improve gel formation and bead size. Chemical stability was studied in solutions containing lactic acid, citric acid, phosphate buffer and synthetic model medium.
Cell release (leakage) was observed for carrageenan beads as they exhibited peeling of the chitosan layer after 2 days in lactic acid and citric acid solutions. Cell leakage was also observed for single layer alginate beads but only after one (1) week. Phosphate buffer solutions did not affect cell leakage.
Fermentation kinetics in synthetic model medium with 25g/L, 50g/L, 100g/L and 200g/L of glucose showed that single alginate beads had higher fermentation rates and completed fermentation in shorter time even in comparison with free cells. The same results were observed for polyethylene glycol-alginate and xanthan-alginate hollow beads in 50 g/L of glucose. Cell multiplication during fermentation increased accordingly with the initial sugar content of the medium except for carrageenan beads.
Bead stability was very good in media with 25g/L of glucose, with less than 3% of broken beads. Entrapped yeast beads (polyethylene glycol-alginate and xanthan-alginate) increased in size due to CO_2 production and 20 % of the beads in 50g/L and 100g/L. All other beads exhibited high percentage of broken beads from 60% up to 93% in 100 g/L of glucose.
Cell leakage was observed in all cases except when beads (double alginate beads) were used for the second fermentation in sparkling wine production in wine with 11%vol ethanol, 25 g/L sugars and CO_2 pressure where no cell release was observed.
Ethanol productivity and yield vary between different techniques and sugar concentration levels.

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