UNIVERSITY OF CAMPINAS

School of Chemical Engineering

Alcoholic Fermentation using Immobilized Cells

July 22 th to 25 th, 2014

Introduction

Biomass & Bioenergy

Ethanol

- *Less polluting*
- *Got from renewable source*
- *Environmentally friendly*

Brazil: cane sugar

USA: corn

Germany: wheat

ANDRIETTA, S.R.; STECKELBERG, C. & ANDRIETTA, M.G.S., Bioetanol – Brasil, 30 anos na vanguarda. *Multiciência*, Universidade de Campinas, 2006
FURTADO, T.A. & SCANDIFFIO, M.G., Álcool no Brasil – Uma longa história. *Scientific American Brazil*, n. 53, p.66-71, Outubro, 2006

Introduction

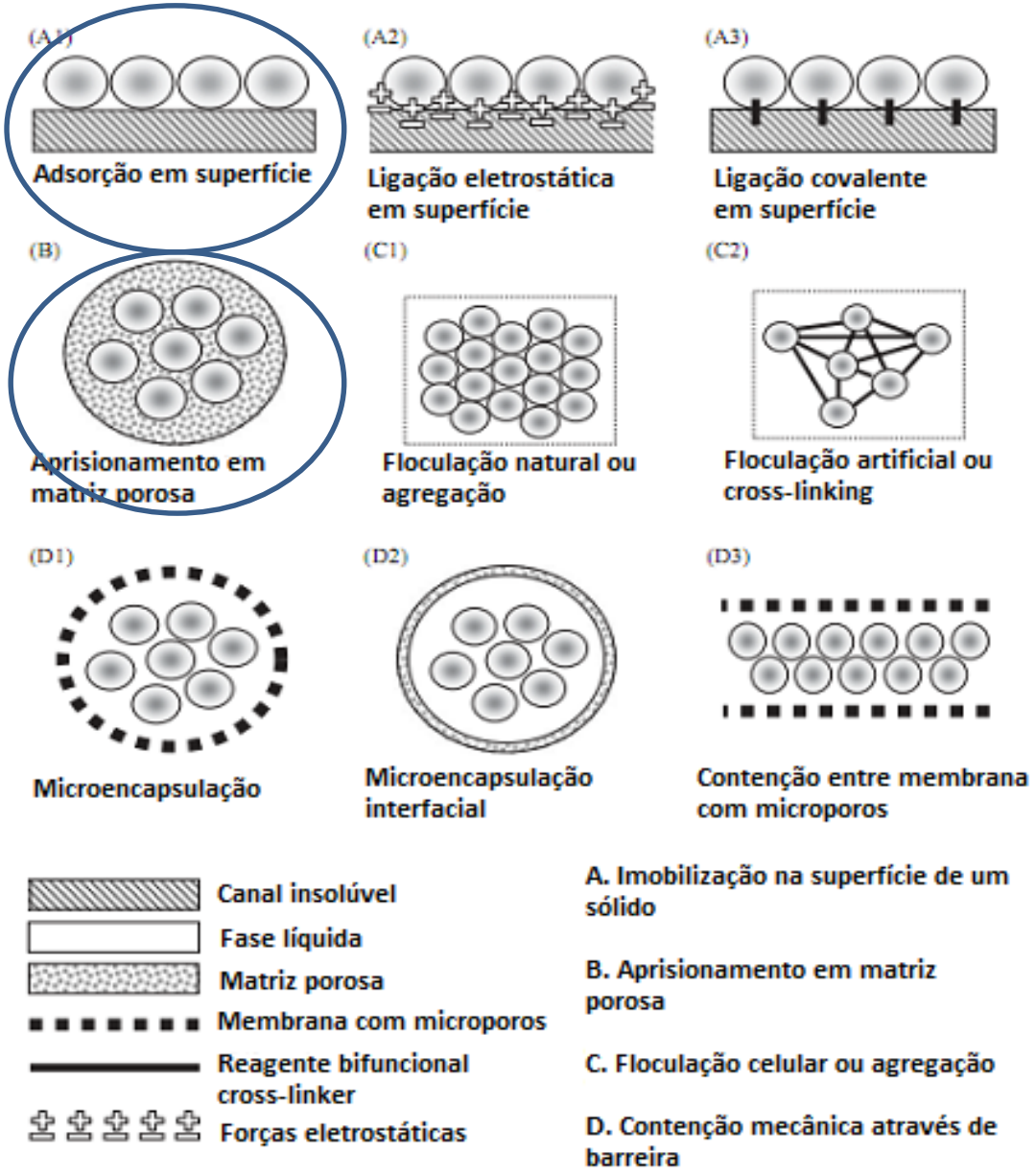
R. Wendhausen, A. Fregonesi, P. J. S. Moran, I. Joekes, J. A. R. Rodrigues, E. Tonella, and K. Althoff. Continuous fermantation of sugar cane syrup using immobilized yeast cells. *J. of Biosc. and Bioeng.*, 91(1):48–52, 2001.

Glucose (%)	Theoretical yield based on CO_2 measured (%)	
	Immobilized cells [a]	Free cells [b]
10	87.0	90.0
20	92.0	88.6
30	96.0	77.8
40	72.4	54.6
50	57.8	31.6

[a] – 0,60 g of yeast in o,6 g of Chrysotile

[b] – 0,60 g of yeast

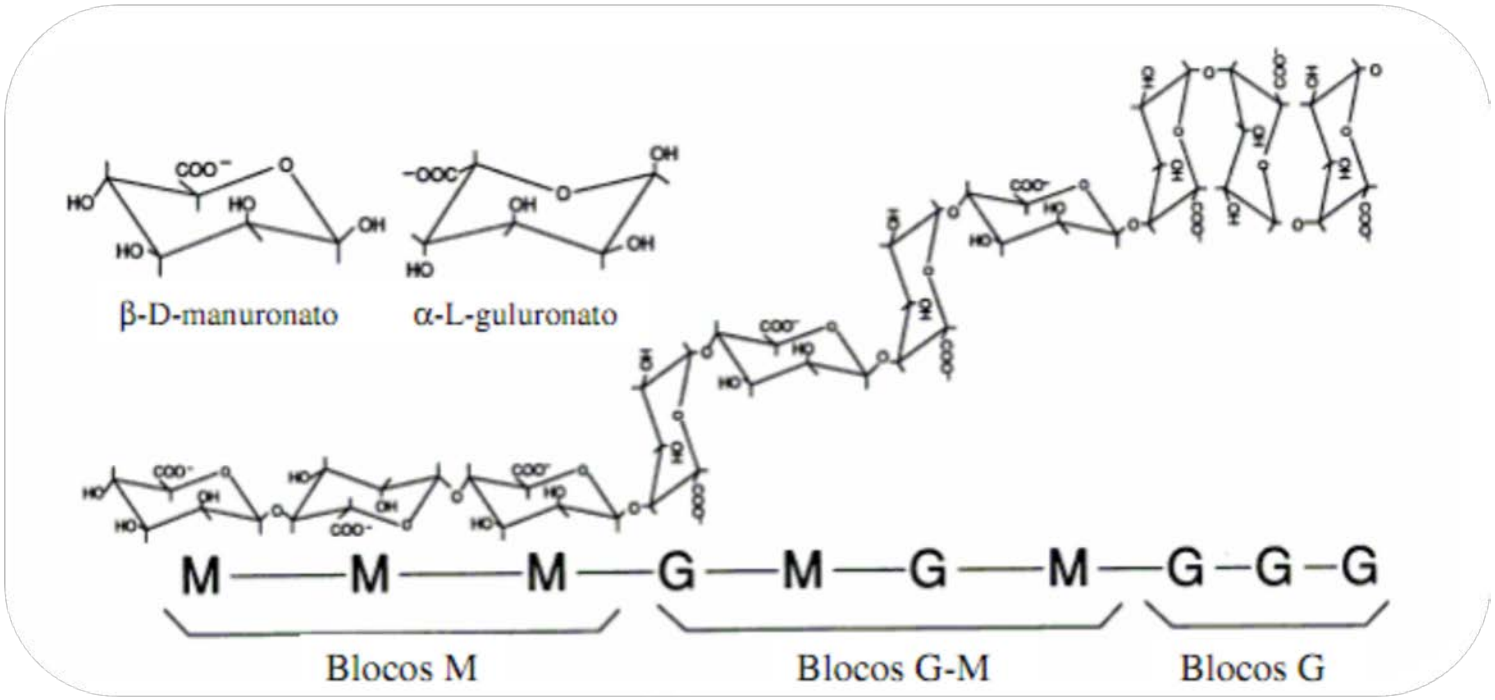
Cell immobilization methods



FONTE: Figura modificada KOURKOUTAS, Y., *et al.* Immobilization technologies and support materials suitable in alcohol beverages production: a review. *Food Microbiology.* V.21, p.377-397, 2004.

Methods

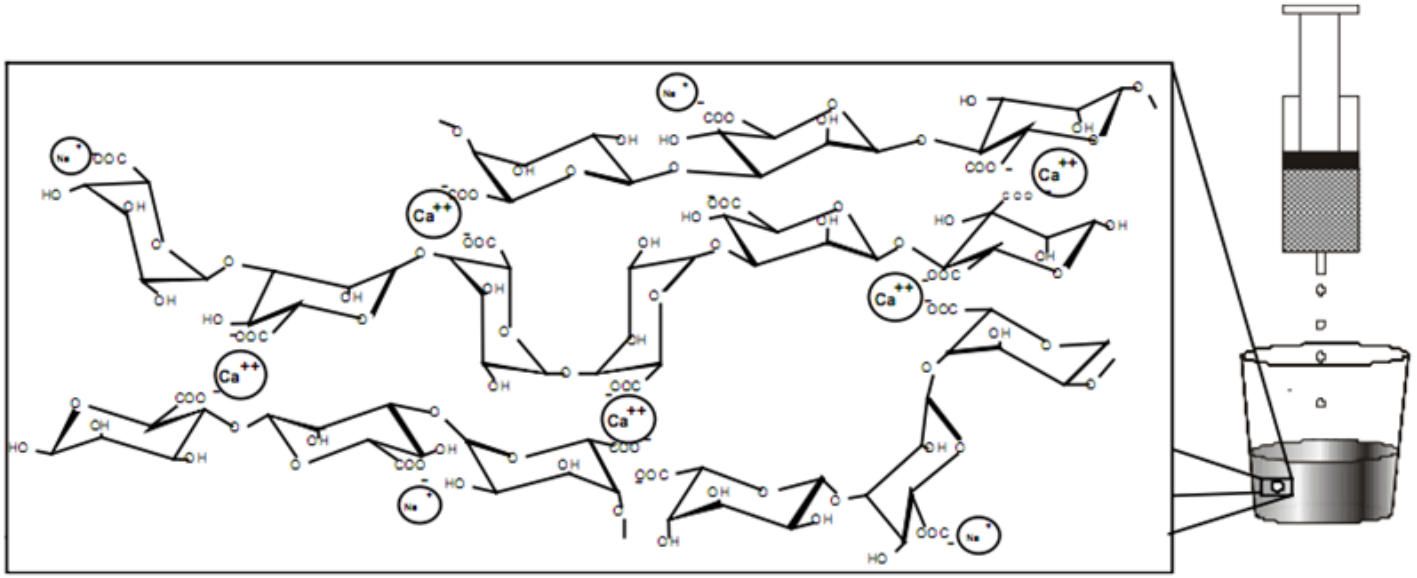
Sodium alginate



RODRIGUES, A.P., Preparação e caracterização de membranas de quitosana e alginato para aplicação na terapia de lesões. 2008. Tese (Doutorado em Engenharia Química) – Universidade Estadual de Campinas, Campinas, SP.

Methods

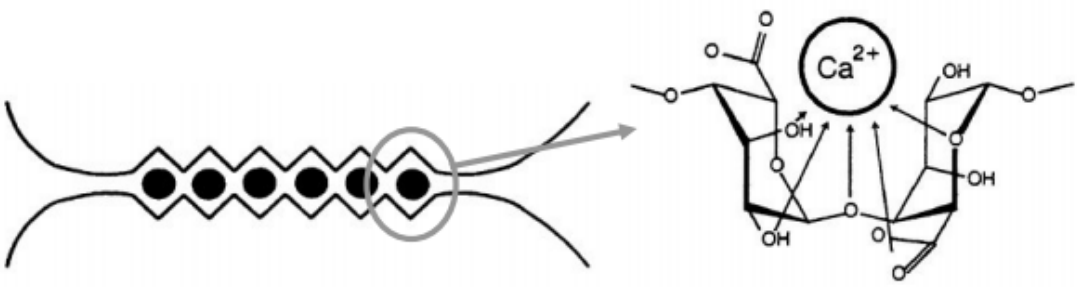
Calcium alginate preparation



Sodium alginate and *Saccharomyces cerevisiae*

$CaCl_2$

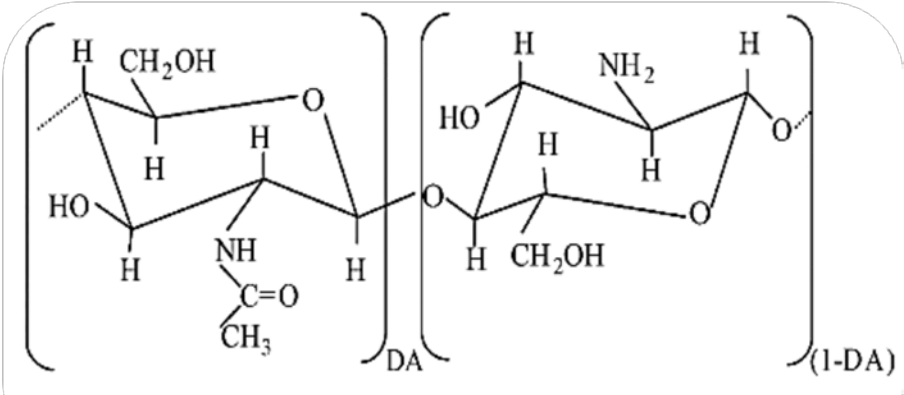
COVIZZI, L.G., et al., Semina: Ciências Exatas e Tecnológicas, Londrina, v.28, n.2, p.143-160, jul./dez., 2007.



RODRIGUES, A.P., Preparação e caracterização de membranas de quitosana e alginato para aplicação na terapia de lesões. 2008. Tese (Doutorado em Engenharia Química) – Universidade Estadual de Campinas, Campinas, SP.

Methods

Chitosan

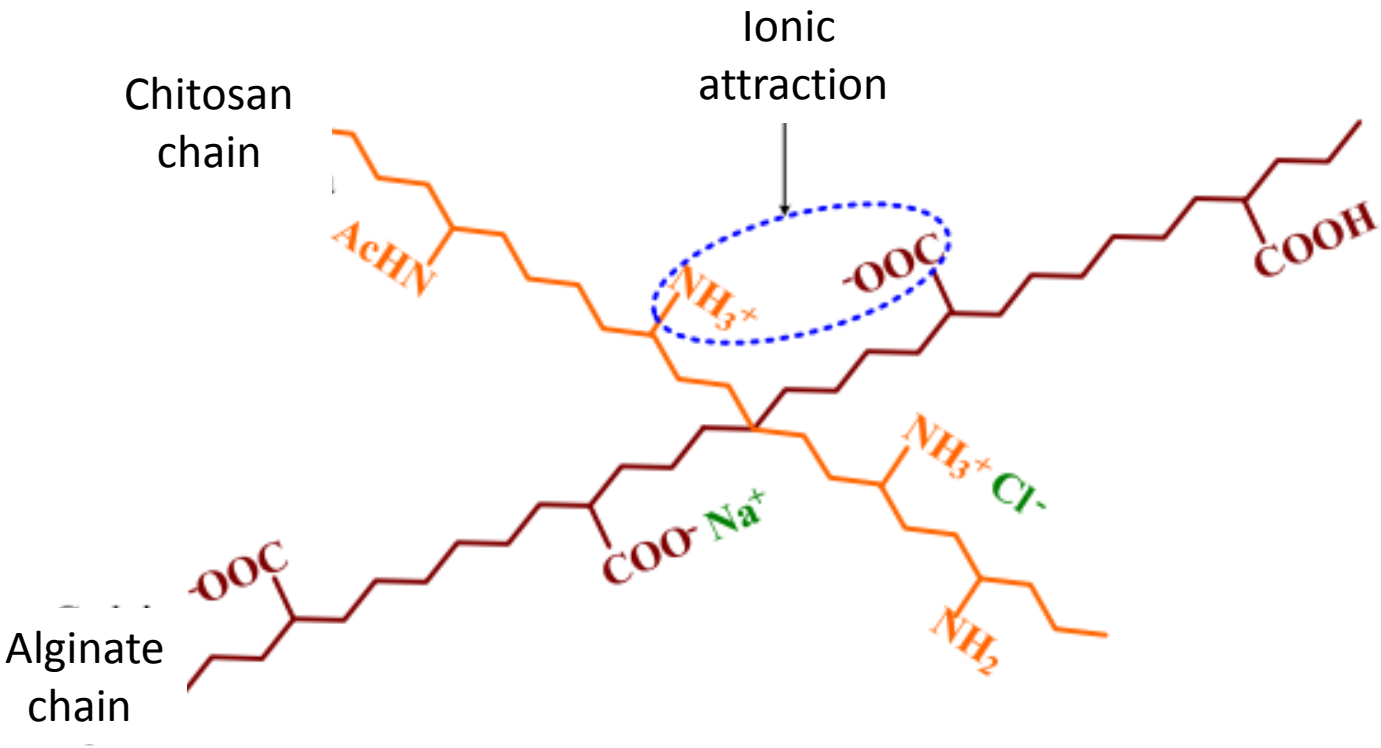


acetylglucosamine unit glucosamine unit

Modificada de DASH, M.; CHIELLINI, F.; OTTENBRITE, R.M.; CHIELLINI, E., Chitosan—A versatile semi-synthetic polymer in biomedical applications, *Progress in Polymer Science*, v. 36, p. 981–1014, 2011.

Methods

Calcium alginate and Chitosan interaction



RODRIGUES, A.P., Preparação e caracterização de membranas de quitosana e alginato para aplicação na terapia de lesões. 2008. Tese (Doutorado em Engenharia Química) – Universidade Estadual de Campinas, Campinas, SP.

Experimental

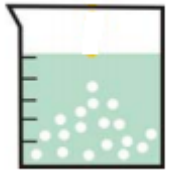
Alcoholic fermentation using immobilized cells

Calcium alginate beads

~6 g wet cells → water 100mL →  → agitation → 

alginate 3% (100 mL)

Cell + alginate 3%

1h →  → Filtering washing → Fermentation

$CaCl_2$ 2%

Experimental

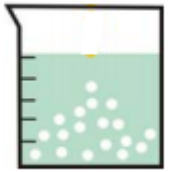
Alcoholic fermentation using immobilized cells

chitosan-covered calcium alginate beads

~6 g wet cells → water 100mL →  → agitation → 

alginate 3% (100 mL)

Cell + alginate 3%

1h → 

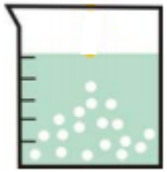
$CaCl_2$ 2%

Experimental

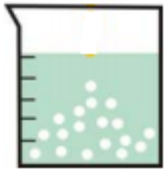
Alcoholic fermentation using immobilized cells

chitosan-covered calcium alginate beads

After 1h in $CaCl_2$ solution:



Filtering
washing



Chitosan solution 0,25% in acetic acid 5% (30 min).

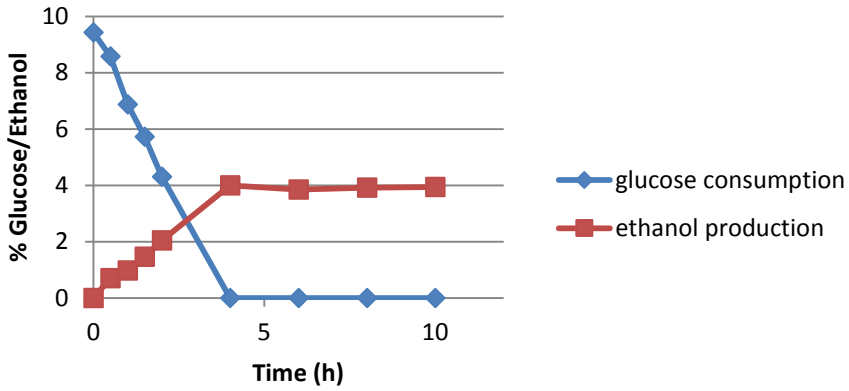
Filtering
washing

Fermentation

Free cells fermentation

Results and discussion

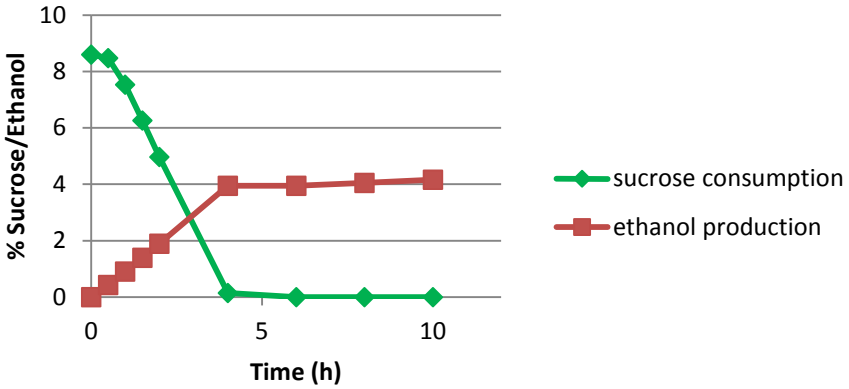
Glucose consumption and ethanol production



Carbon source: Glucose

Final ethanol concentration: 40 g/L
yield: 78%

Sucrose consumption and ethanol production



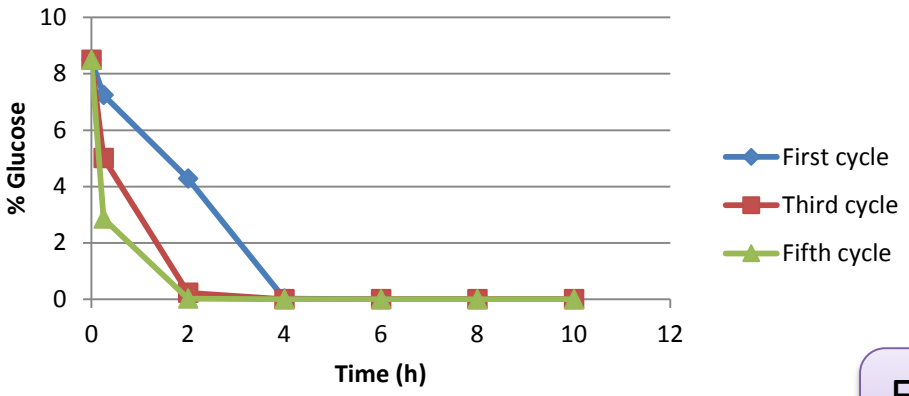
Carbon source: Sucrose

Final ethanol concentration: 40 g/L
yield: 74,3%

Immobilized cells fermentation

Results and discussion

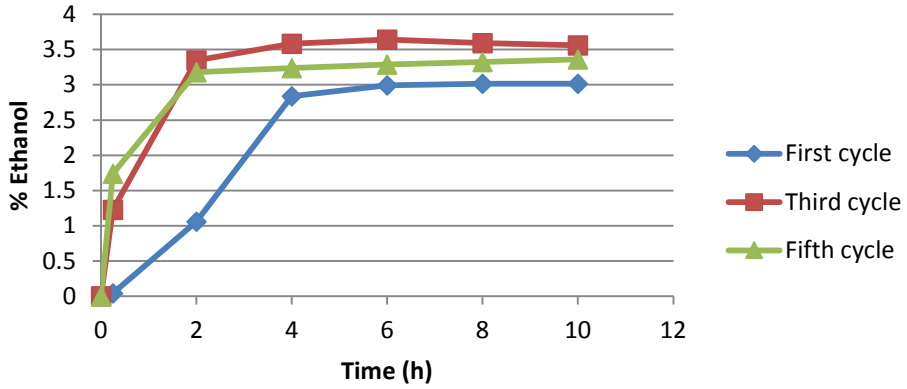
Glucose consumption - immobilized cells in calcium alginate beads



Carbon source
Glucose

Final ethanol concentration: 33 g/L
yield: 64,6%

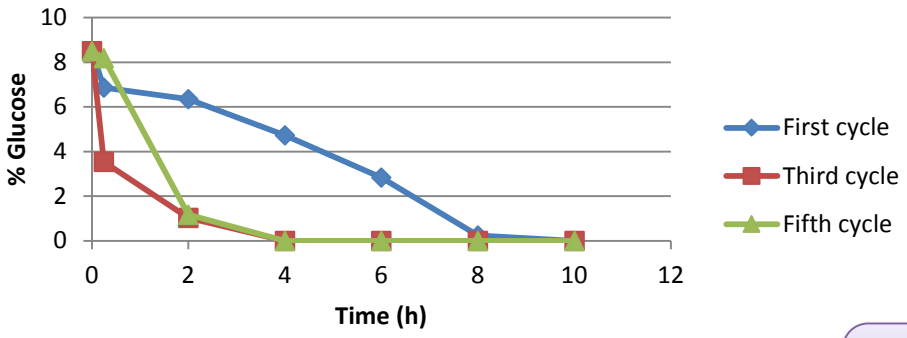
Ethanol production - immobilized cells in calcium alginate beads



Immobilized cells fermentation

Results and discussion

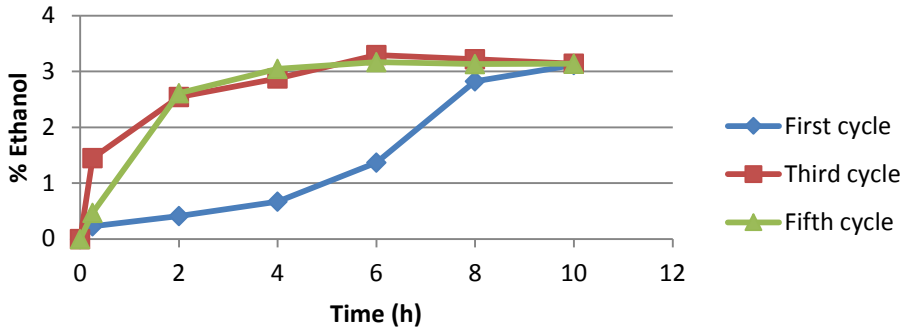
Glucose consumption - immobilized cells in chitosan covered calcium alginate beads



Carbon source
Glucose

Final ethanol concentration: 31 g/L
yield: 60,7%

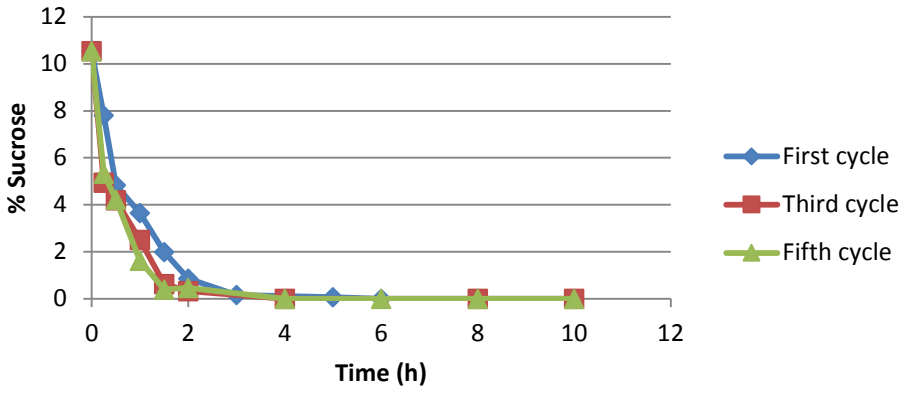
Ethanol production - immobilized cells in chitosan covered calcium alginate beads



Immobilized cells fermentation

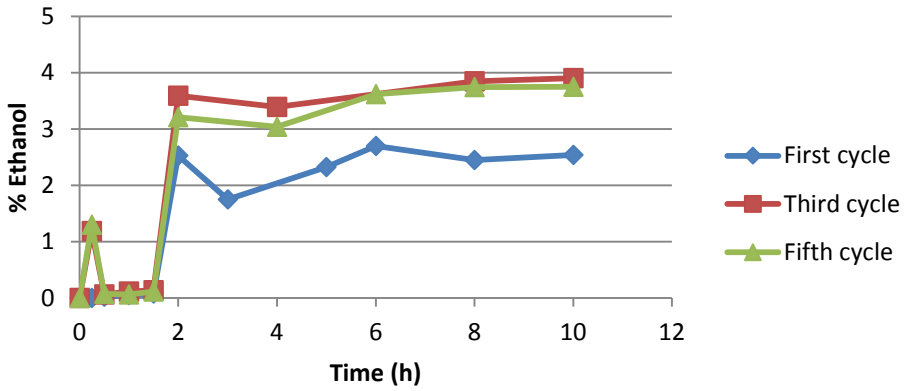
Results and discussion

Sucrose consumption - immobilized cells in calcium alginate beads



Carbon source
Sucrose

Ethanol production - immobilized cells in calcium alginate beads

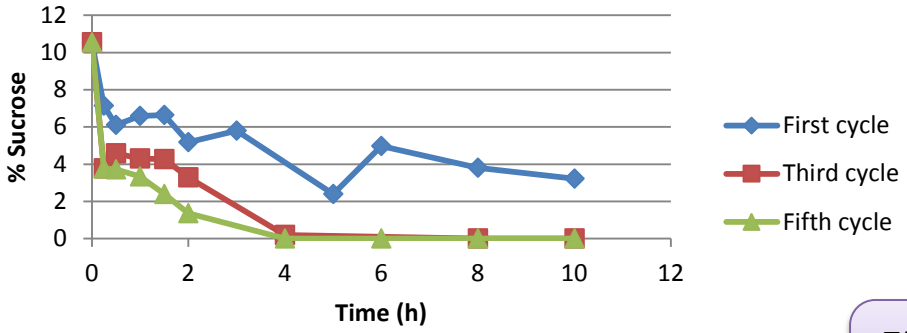


Final ethanol concentration: 33 g/L
yield: 61,3%

Results and discussion

Immobilized cells fermentation

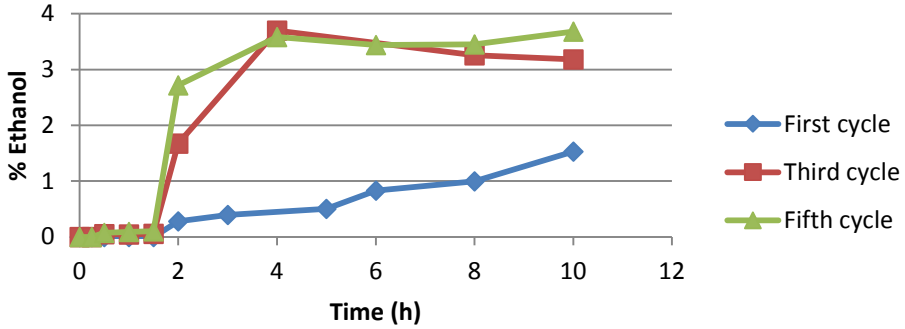
Sucrose consumption - immobilized cells in chitosan covered calcium alginate beads



Carbon source
Sucrose

Final ethanol concentration: 32 g/L
yield: 59,5%

Ethanol production - immobilized cells in chitosan covered calcium alginate beads



Results and discussion

	Free cells		Immobilized cells CAB		Immobilized cells CCCAB	
	glucose	sucrose	glucose	sucrose	glucose	sucrose
Ethanol production (g/L)	40	40	33	33	31	32
Yield (%)	78	74,3	64,6	61,3	60,7	59,5

Legend:
CAB: Calcium alginate beads
CCCAB: Chitosan covered calcium alginate beads

Conclusions

1. Immobilized cells in calcium alginate and in chitosan-covered calcium alginate beads:

a. Substrate consumption and product production were faster after the first cycle: it suggests **adaptation**

b. After free substrate consumption there was ethanol production: it suggests **difusion** process

c. Using **glucose** as carbon source, maximum ethanol production was reached in 4 and 6 h to CAB and CCCAB, respectively

d. Using **sucrose** as carbon source, maximum ethanol production was reached in 2 and 4 h to CAB and CCCAB, respectively

e. Calcium alginate and chitosan covered calcium alginate beads allowed beads reuse during 8 cycles of approximately 10 hours of fermentation each

Conclusions

f. Ethanol production at the end of each cycle were somewhat similar

g. Chitosan covered calcium alginate beads were less brittle and broke in a smaller amount than calcium alginate beads

h. Chitosan exerted a physical barrier, so the consumption of substrate and product production was slower

Thanks

UNICAMP

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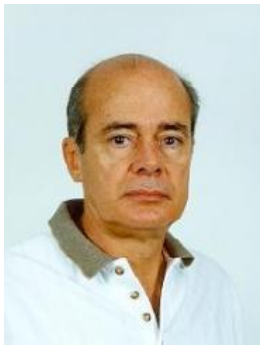
PAN-AMERICAN BIOFUELS & BIOENERGY SUSTAINABILITY

AN NSF RESEARCH COORDINATION NETWORK

Prof. Paulo J. S. Moran



Prof. J. Augusto R. Rodrigues