Microbial Treatment Approaches for High-Purity Fructo-Oligosaccharides Production

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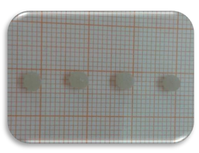
INTRODUCTION

The production of high-purity fructo-oligosaccharides (FOS), known as prebiotics, by sucrose fermentation using whole microbial cells has been recently explored. At the end of the fermentation process, FOS are present in mixture with small saccharides that are known to have an inhibitory effect of transfructosylating enzymes and to decrease the prebiotic activity of the mixture [1]. This issue can be overcome by reducing the small saccharides present in FOS broth, which can be done using a combined microbial treatment, among others, improving as well the further purification of FOS by Simulated Moving Bed (SMB) chromatography [2, 3, 4].

The main goal of this work was the use of combined microbial treatment approaches to improve FOS production and enhance a high purity FOS content.

Aureobasidium pullulans and *Saccharomyces cerevisiae* were used combined to produce FOS and reduce the concentration of the small sugars in the culture, respectively. FOS-producing microorganism was used free (APf), immobilized to a non-conventional carrier (APi) or encapsulated in Ca-alginate beads (APe), in mixture with the non-oligosaccharides consuming microorganism, free (SCf) or encapsulated in Ca-alginate beads (SCe), inoculated after 0 (IT0), 10 (IT10) and 20 h (IT20) of fermentation.

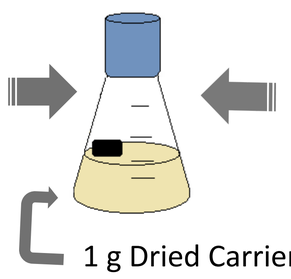
A factorial design, considering three different variables, was performed to select the microbial treatment approach through which increased FOS levels and yields can be obtained. The 36 assays were performed in shaken-flasks and the most suitable one , able to increase FOS concentration, percentage, yield and productivity was scaled-up to a 3L bioreactor. All fermentations were done in duplicate.

IMMOBILIZATION	ENCAPSULATION
	
Reticulated polyurethane foam ϕ=1mm	Calcium-alginate beads

FULL FACTORIAL DESIGN

Assays	*A. pullulans* cells	*S. Cerevisiae* cells	Inoculation time (h)
A1 / A13	Immobilized	Encapsulated	20
A2 / A4	Encapsulated	Free	0
A3 / A8	Immobilized	Free	10
A5 / A30	Free	Free	20
A6 / A10	Free	Encapsulated	0
A7 / A23	Immobilized	Free	20
A9 / A11	Free	Encapsulated	10
A12 / A20	Encapsulated	Free	20
A14 / A27	Immobilized	Free	0
A15 / A29	Free	Free	10
A16 / A36	Encapsulated	Encapsulated	0
A17 / A33	Immobilized	Encapsulated	10
A18 / A21	Encapsulated	Free	10
A19 / A34	Free	Free	0
A22 / A35	Encapsulated	Encapsulated	10
A24 / A31	Immobilized	Encapsulated	0
A25 / A28	Free	Encapsulated	20
A26 / A32	Encapsulated	Encapsulated	20

A. pullulans
9x10[7] spores/mL
- Free
- Encapsulated
- Immobilized



1 g Dried Carrier
(Immobilized *A. pullulans* assays)

S. cerevisiae
Optical density = 1
- Free
- Encapsulated

FERMENTATION MEDIUM
- ✓ **Optimized medium:** [Sucrose]: 200 $g.L^{-1}$; [$NaNO_3$]: 5 $g.L^{-1}$; [K_2SO_4]: 0.35 $g.L^{-1}$; [$MgSO_4$]: 0.5 $g.L^{-1}$; [KCl]: 0.5 $g.L^{-1}$; [KH_2PO_4]: 4 $g.L^{-1}$; [$FeSO_4$]: 4 $g.L^{-1}$
- ✓ **Volume:** 100 mL
- ✓ **Agitation:** 150 rpm
- ✓ **Temperature:** 32 ºC

RESULTS AND DISCUSSION

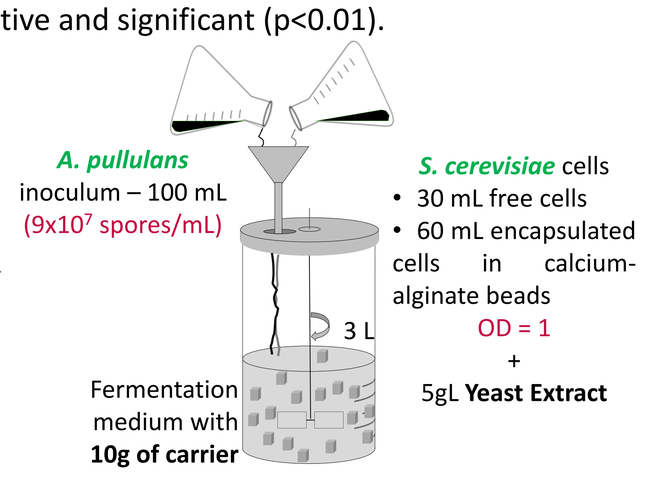
Statistical analysis of the factorial design - MANOVA

	Term	Coefficient	Standard error	t-value	p-value
FOS CONCENTRATION	**APf**	0.7753	4.5420	0.1700	0.8656
	APe	-14.2514	4.5420	-3.1400	0.0038*
	APi	13.4761	4.5420	2.9700	0.0059*
	SCf	-3.6722	3.2117	-1.1400	0.2619
	SCe	3.6722	3.2117	1.1400	0.2619
	IT0	-44.1589	4.5420	-9.7200	< 0.0001*
	IT10	4.4511	4.5420	0.9800	0.3349
	IT20	39.7078	4.5420	8.7400	< 0.0001*

- ✓ Experimental and predicted results were very similar for the responses studied, FOS concentration, percentage, yield and productivity, with high regression values;
- ✓ The use of APi shows a significant positive effect on FOS production ($p<0.01$);
- ✓ The use of APe showed a significant negative effect on FOS production ($p<0.01$);
- ✓ The introduction of SCf or SCe showed a positive effect on FOS production, although with no statistical significance;
- ✓ The inoculation of SC at IT0 showed a significant negative impact ($p<0.001$), while at IT20 the effect was positive and significant ($p<0.01$).

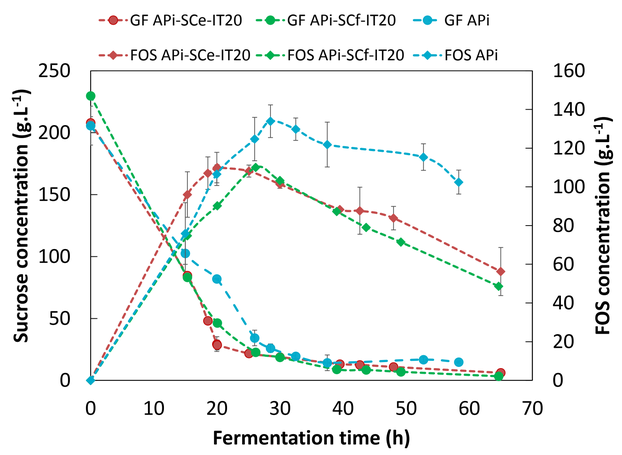
Selected conditions

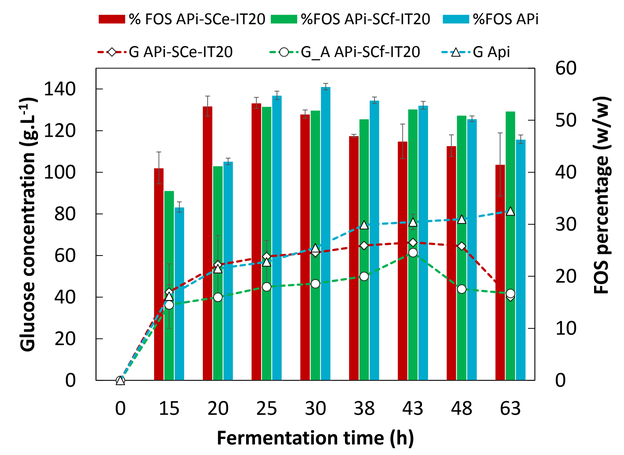
- ✓ **APi – SCf – IT20**
- ✓ **APi – SCe - IT20**
- ✓ **CONTROL: APi**



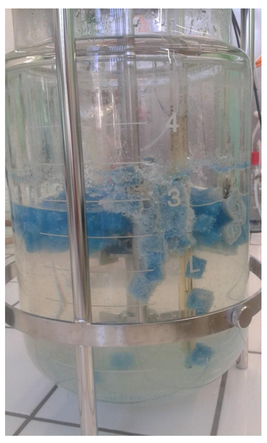
Bioreactor fermentations

Culture	Microorganisms	Time (h)	Max FOS ($g.L^{-1}$)	% FOS (w/w)	Yield ($g_{FOS}.g_{Sucrose}^{-1}$)	Q_p ($g_{FOS}.L^{-1}.h^{-1}$)
Mono-culture	APi	30 **	133.91 ± 8.42	56.46 ± 0.62	0.65 ± 0.06	4.46 ± 0.86
Co-culture fermentation	APi-SCf	25 **	110.06 ± 1.00	52.64 ± 1.00	0.48 ± 0.02	4.40 ± 0.07
with yeast extract	APi-SCe	20 **	109.97 ± 1.49	53.33 ± 1.06	0.53 ± 0.02	5.50 ± 0.07





- ✓ Mono-culture APi obtained higher concentration of FOS than the co-cultures APi-SCf or APi-SCe inoculated at IT20, mainly due to the presence of the yeast extract;
- ✓ The faster decrease of sucrose in the mixed-cultures suggests the competition for the substrate by both microorganisms;
- ✓ The behavior of APi-SCf or APi-SCe is similar (no statistical significance);
- ✓ The time needed to achieve the maximal concentration of FOS is smaller for APi-SCf or APi-SCe and so the productivities are higher;
- ✓ The variation of the percentage of FOS is similar between the three assays, and depends on the glucose present in the medium;
- ✓ The immobilization of the *A. pullulans* cells is a very attractive strategy, compared to previous studies [5] to produce high levels of FOS, providing a pre-filtrated medium at the end of the fermentation process.



CONCLUSIONS

- ➢ The immobilization of A. pullulans cells can reduce the inhibitory effect of glucose in the medium and increase the production of FOS, providing a pre-filtered medium at the end of the process;
- ➢ The presence of *A. pullulans* and *S. cerevisiae* in the same culture decreases the FOS production either by the competition for the substrate (sucrose) or by the presence of the yeast extract;
- ➢ In future works it will be important to find new alternatives to replace the yeast extract added to the fermentation and eliminate the negative impact on FOS production.

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The work presented had the financial support of FNRS 2012 (PDR14599891)
C. Nobre thanks the Fundação para a Ciência e Tecnologia the strategic funding of UID/BIO/04469/2013 unit.