Production of high-content fructo-oligosaccharides by different fermentation approaches

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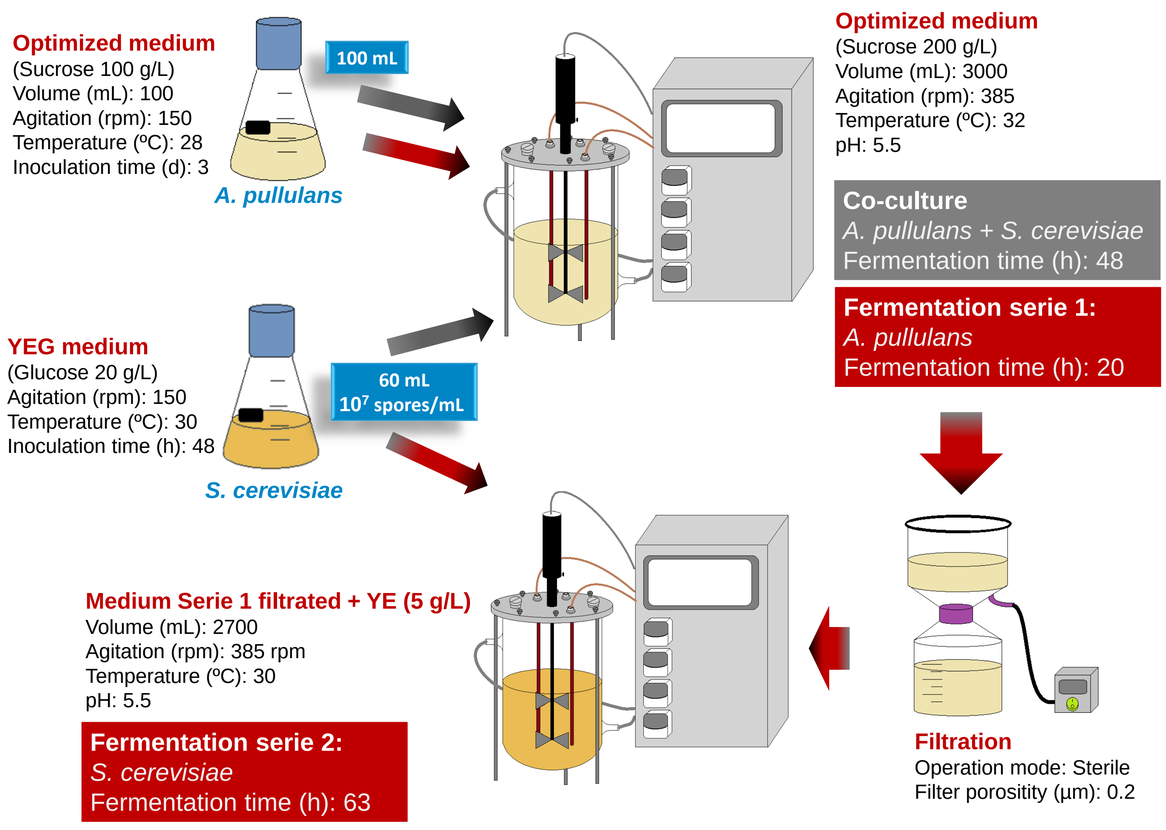
Introduction

Fructo-oligossacharides (FOS), or prebiotic sugars, have an important commercial interest in the food market due to their health beneficial properties [1]. They are known to prevent and treat a large number of intestinal disorders, and improve the quality of food products, in which they are introduced [2].

Industrially, FOS are produced by microbial enzymes from sucrose through transfructosylation using a two-stage process, where enzymes are first obtained by the microorganism and further extracted for the enzymatic synthesis of FOS [3]. Sucrose is converted to FOS by *Aureobasidium pullulans* in yields between 55-60%. To increase the percentage of FOS in these mixtures, the non-prebiotic sugars (fructose, glucose and sucrose) present have to be removed.

Here we proposed, firstly, the reduction of salts concentration in the fermentative broth, that will be further purified in the simulated moving bed chromatography (SMB) [4], and FOS production using an one-stage process fermentation with the whole cells of *A. pullulans* [5]; secondly, the use of *Saccharomyces cervisiae* strain to consume the small sugars before purification with SMB, using two different approaches, one step fermentation (co-culture), and two-steps fermentation (series).

Strategy



Results and Discussion

A Experimental design for optimization of the fermentation broth in shaken flasks

	$NaNO_3$ (g/L)	KH_2PO_4 (g/L)	Fermentation time (h)	Maximum FOS (g/L)	% FOS (w/w)	$Yield_{P/S}$ (% w/w)	Productivity (g/L.h)
A1	5.0	8.0	47.8	101.3	48.9	54.0	2.1
A2	20.0	4.0	53.5	91.5	43.5	46.0	1.7
A3*	12.5	6.0	53.5	103.7	48.1	53.0	1.9
A4	5.0	4.0	47.8	95.4	50.8	50.0	2.0
A5	5.0	6.0	53.5	95.1	49.6	48.0	1.8
A6	12.5	8.0	53.5	101.8	49.8	51.0	1.9
A7*	12.5	6.0	47.8	104.3	50.6	53.0	2.2
A8	20.0	6.0	47.8	105.7	49.3	53.0	2.2
A9	12.5	4.0	53.5	105.6	49.7	53.0	2.0
A10	20.0	8.0	53.5	103.0	47.0	52.0	1.9
A11*	12.5	6.0	53.5	99.9	49.0	50.0	1.9

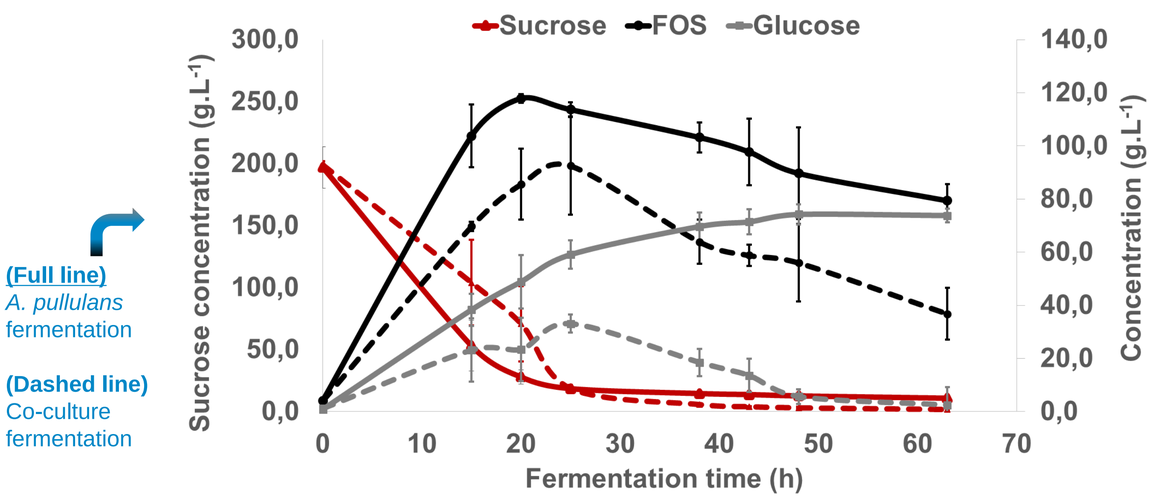
- ✓ No statistical differences were found in the shaken flasks fermentations results;
- ✓ The reduction of the concentration of the salts in the medium was encouraged;
- ✓ The concentrations of $NaNO_3$ and KH_2PO_4 selected were 5.0 and 4.0 $g.L^{-1}$.

B FOS production by *A. pullulans* at low salt concentration in bioreactor

	Average	IC (95% confidence)	Reference [5]
Time (h)	20		43
FOS (%)	54.0	1.6	
Yield (% $w_{FOS}/w_{Sucrose}$)	63.0	3.2	64.1
Productivity (g/L.h)	4.8	1.4	2.9
FOS (g/L)	118.6	1.6	123.0
Yield ($g_{1\text{-}kestose}/g_{Sucrose}$)	37.8	7.9	43.6
Yield ($g_{nystose}/g_{Sucrose}$)	23.1	5.3	20.6

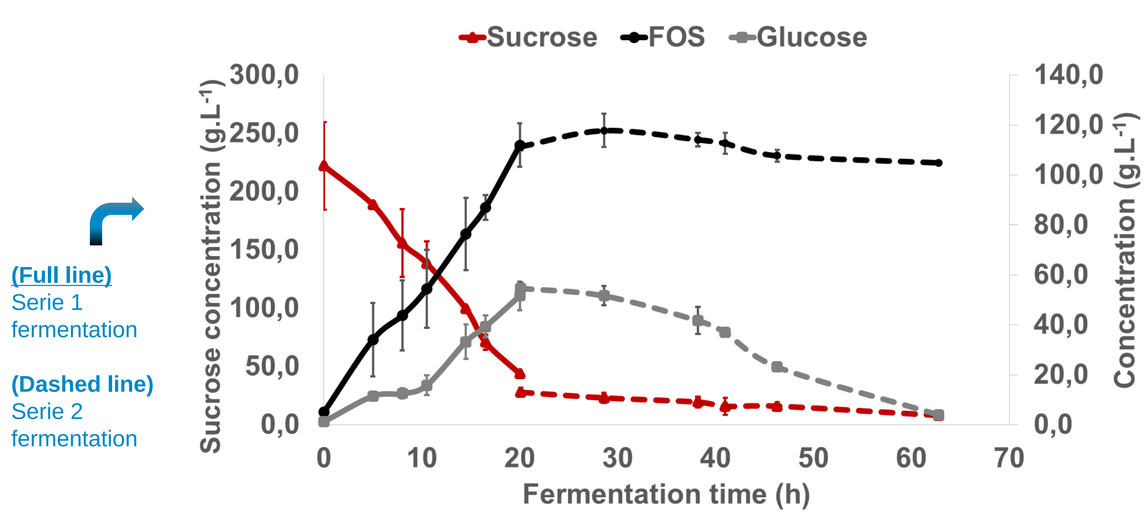
- ✓ The reduction of salts in the bioreactor fermentations did not influence the yield and the percentage of FOS;
- ✓ The optimal fermentation time decreased and thus the productivity increased.

C FOS production by *A. pullulans* in co-culture with *S. cerevisiae* in bio-reactor



- ✓ The presence of *S. cerevisiae* and *A. pullulans* in the same fermenter decreases the concentration of FOS produced;
- ✓ *S. cerevisiae* decreases continuously the small saccharides in the medium.

D FOS production by *A. pullulans* in serie with *S. cerevisiae* in bio-reactor



- ✓ *S. cerevisiae* was able to remove small saccharides in the medium;
- ✓ An increased percentage of FOS was achieved in this process, up to 81% (w/w).

Conclusions

- ❑ The minimization of the salts quantity in the fermentation broth does not influence the amount of FOS produced and increases the productivity of the process.
- ❑ Higher productivity and lower concentration of salts needed reduce the process cost.
- ❑ The co-culture fermentation process is less efficient than the fermentation in series.
- ❑ Fermentation in series is an advantageous and efficient approach for FOS production and purification.

References

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