Evaluation of Agro-industrial, Synthetic and Mineral Carriers for Immobilization of *Aureobasidium Pullulans* Cells for Fructooligosaccharides Production

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INTRODUCTION

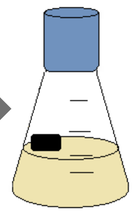
Fructooligosaccharides (FOS) are prebiotic oligosaccharides with increased commercial interest [1]. FOS can be produced from sucrose by free or immobilized microorganisms with transfructosylating enzymes [2]. Cells immobilization can be applied to improve FOS yields and percentage in the medium as it increases cells stability and concentration in the medium [3]. Nevertheless, a good performance of the system depends on the right selection of the suitable carrier to adhere cells [4].

In this work, selected agro-industrial carriers to immobilize ***Aureobasidium pullulans*** fungi cells were compared to synthetic and mineral materials. Agro-industrial carriers include mandarine and banana peels, and nut, almond, pistachio and chestnut shells; synthetic carriers include foams, vegetal and synthetic fibers, porous clay and glass wool; and mineral carriers include pumice and porous stone. Cells were immobilized in situ by adhesion, through direct contact to the carrier at the beginning of the batch fermentations. The cells immobilization capacity was evaluated based on the biomass amount free and adhered to the carrier and by the water absorption index (WAI) and the critical humidity point (CHP) values determined for each carrier.

BATCH FERMENTATIONS

Aureobasidium pullulans $9x10^7$ spores/ml

Dried Carrier (± 1g)



- ✓ **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

SELECTED CARRIERS FOR CELLS IMMOBILIZATION

Organic carriers

O1: Mandarine peel

O2: Banana peel

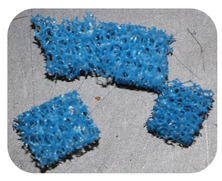
O3: Nutshell

O4: Pistachio shell

O5: Almond shell

O6: Chestnut shell

Synthetic and mineral carriers



S1: Polyurethane foam ϕ=1.5mm

S2: Polyurethane foam ϕ=1mm

S3: Vegetal foam

S4: Vegetal fiber mop

S5: Synthetic fiber mop

S6: Porous clay

S7: Glass wool

S8: Polyester staple fiber

M1: Pumice stone

M2: Porous stone

RESULTS AND DISCUSSION

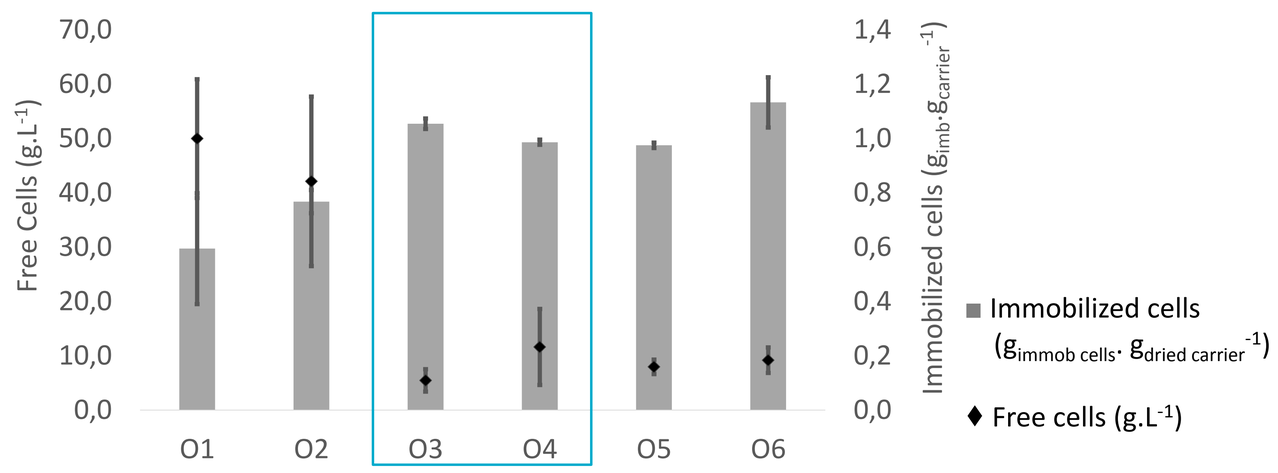
ORGANIC CARRIERS

Water absorption index (WAI) and Critical humidity point (CHP)

Code	WAI ($g.g_{dried\ matter}^{-1}$)	CHP (%)
O1	5.9	50
O2	12.5	40
O3	12.9	25
O4	9.9	24
O5	7.6	43
O6	2.0	50

- ✓ Nutshell (O3) and pistachio shell (O4) presented decreased CHP values, that is, lower quantity of water linked to the support that is not used by the microorganisms;

A. pullulans cells immobilization capability of the selected carriers

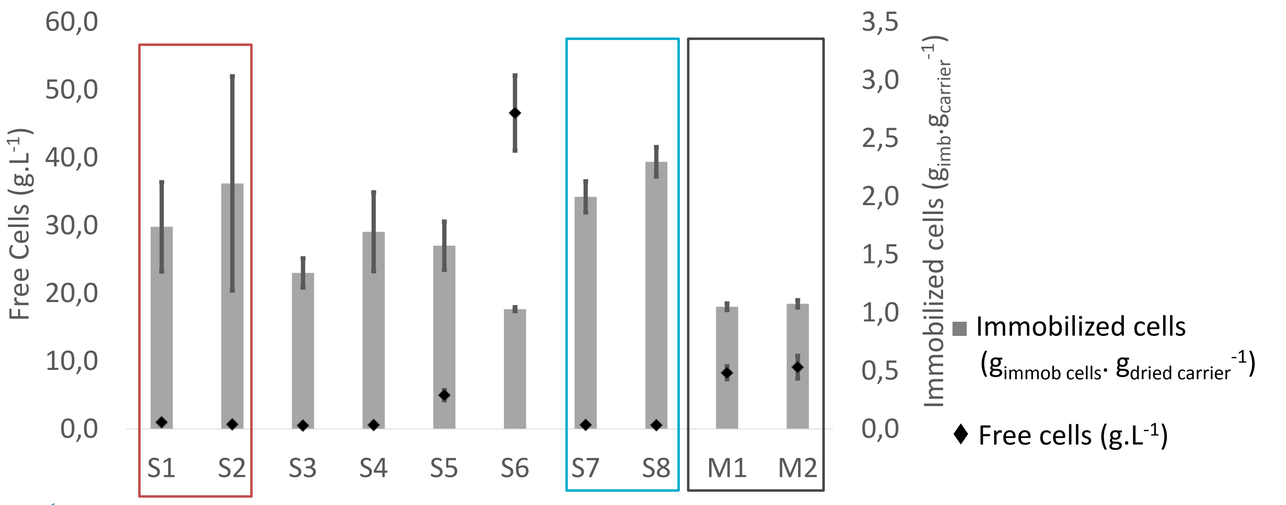


- ✓ Organic products shells structures (O3, O4, O5, O6) were able to better adsorb cells;
- ✓ Mandarine (O1) and banana (O2) peels seem to have a positive effect in free cells growing.

SYNTHETIC/MINERAL CARRIERS

Code	WAI ($g.g_{dried\ matter}^{-1}$)	CHP (%)
S1	5.9	50
S2	12.5	40
S3	12.9	25
S4	9.9	24
S5	7.6	43
S6	2.0	50
S7	26.2	13
S8	26.1	40
M1	1.8	50
M2	1.8	50

- ✓ Glass wool (S7) and polyester staple fiber (S8) presented higher WAI values and lower values of CHP, suggesting increased capability to adsorb *A. pullulans* cells.
- ✓ Low WAI and high CHP values of mineral materials, M1 and M2 suggest lower immobilization capacity.



- ✓ Polyurethane foams (S1, S2), glass wool (S7) and polyester staple fiber (S8) immobilized higher amount of cells;
- ✓ Mineral materials, pumice stone (S7) and porous stone (S8), presented lower ability to immobilize cells, and obtained higher free cells in the medium.

CONCLUSIONS

- ➢ The selected materials were able to immobilize *A. pullulans* cells, although with different capacities;
- ➢ The immobilization ability can be related to the physical-chemical properties, WAI and CHP, of the materials;
- ➢ Synthetic materials present in general lower WAI and thus lower amounts of immobilized cells than organic carriers;
- ➢ It is crucial to understand the impact of the immobilization ability of the carriers on FOS production.

REFERENCES

[1] De Preter, V *et al* (2011) Molecular Nutrition & Food Research 55 (1): 46-57.
[2] Sangeetha, PT *et al* (2004) Process Biochemistry 39 (6): 753-758.
[3] Fenice, M et al (1998) Journal of Biotechnology 62: 119-131.
[4] Mussatto, S et al (2009) Carbohydrate Research 344: 795-800.

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