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Monitoring fructooligossacharides production using *Aspergillus aculeatus* by HPLC-ELSD

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INTRODUCTION

- ✓ Fructooligosaccharides (FOS) are dietary sugars quite used as food ingredients being incorporated as dietary fibers in food products.
- ✓ FOS are present in plants and fruits at low concentrations and with varying individual relative proportions.
- ✓ FOS extraction from natural sources may not be economically viable and so, FOS production by fermentation using fungi can be an alternative.
- ✓ FOS may have a positive health impact, the beneficial health effects depend on the relative FOS composition (nystose-rich diet promotes a higher anti-hydroxyl radical activity compared to a kestose-rich preparation).

AIMS

- ✓ To monitor FOS production by HPLC-ELSD.
- ✓ To evaluate the influence of **initial sucrose concentration** and/or **fermentation temperature** on the **FOS yield** and **FOS production profiles**.
- ✓ To identify the experimental conditions that may enhance the production rate of specific FOS.
- ✓ In this work, it was intended to produce FOS using *Aspergillus aculeatus* at different sucrose initial concentrations (88 to 265 g/L) and at temperatures from 22 to 27ºC.

Fermentation assays

Cuture medium: 400 ml Czapek liquid medium (modified) OXOIDE CM0095 (sodium nitrate 2.0 g/L, potassium chloride 0.5 g/L, magnesium glycerophosphate, 0.5 g/L, ferrous sulfate 0.01 g/L, potassium sulfate 0.35 g/L and sucrose 30 g/L)
Inocolum: 10^5 spores/mL of *A. aculeatus*
Fermentations: rotary shaker at 22 or 27°C for 96 h under agitation (100 rpm). Samples taken at 0, 24, 48, 72, 96 h

HPLC-ELSD (sugar quantification)

Conditions:

Eluent

→ Acetonitrile/H_2O (70:30, v/v)

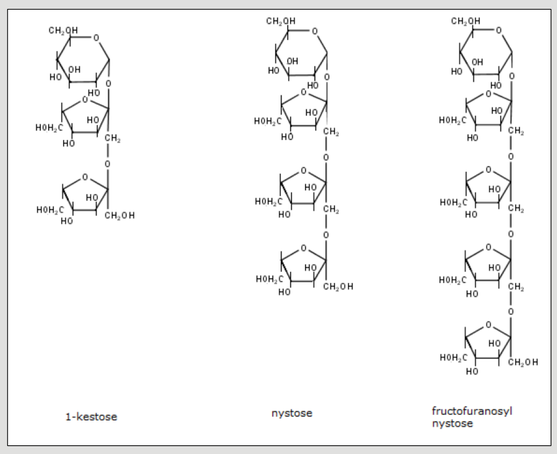
Flow

→ 0.9 mL/min

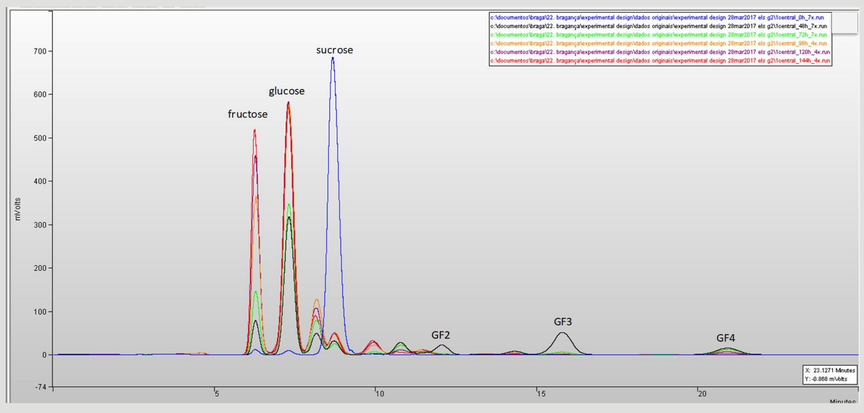
Detector

→ ELS (Gain 2; 50ºC; 3.3 bar)

FOS



Typical chromatographic profile of a fermentation broth with *A. aculeatus* with sucrose as carbon source:



RESULTS

BIOMASS (*A. aculeatus*: 2^2-full factorial design)

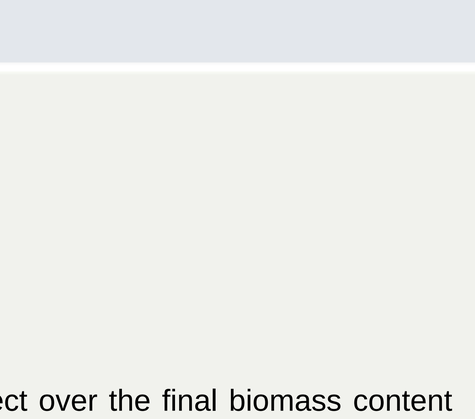
- ❖ Initial sucrose concentration had a significant and positive statistical effect over the final biomass content (*P*-value = 0.0214, two-way ANOVA)
- ❖ Temperature and the interaction [sucrose concentration] × [temperature] not statistically significant at a 5% significance level (*P*-value ≥ 0.6550, two-way ANOVA).

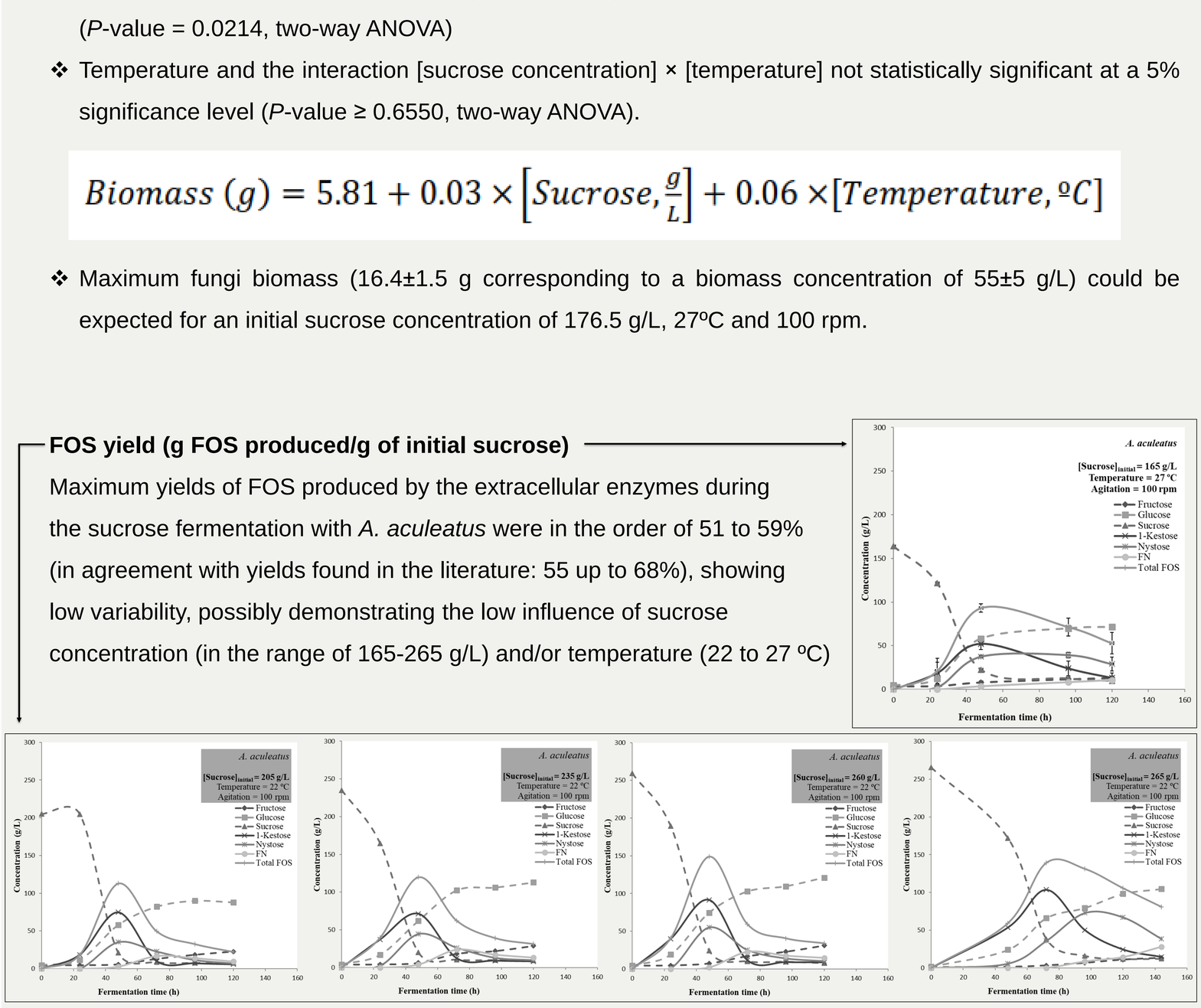
$$Biomass\ (g) = 5.81 + 0.03 \times \left[Sucrose, \frac{g}{L}\right] + 0.06 \times [Temperature, ºC]$$

- ❖ Maximum fungi biomass (16.4±1.5 g corresponding to a biomass concentration of 55±5 g/L) could be expected for an initial sucrose concentration of 176.5 g/L, 27ºC and 100 rpm.

FOS yield (g FOS produced/g of initial sucrose)

Maximum yields of FOS produced by the extracellular enzymes during the sucrose fermentation with *A. aculeatus* were in the order of 51 to 59% (in agreement with yields found in the literature: 55 up to 68%), showing low variability, possibly demonstrating the low influence of sucrose concentration (in the range of 165-265 g/L) and/or temperature (22 to 27 ºC)





CONCLUSIONS

- Fermentation temperature did not significantly affect neither the *A. aculeatus* biomass growth neither the FOS yield.
- The initial sucrose concentration significantly influenced biomass growth.
- The initial sucrose concentration did not significantly affect the maximum FOS yield obtained.
- The preliminary results pointed out that fermentation conditions may lead to different FOS production profiles, which could be further studied to increase.

Acknowledgements

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