Optimization of Fermentation Conditions for Fructooligosaccharides Productivity by *Aureobasidium*

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The BIOLIFE project involves the development of novel biotechnological processes, enzymatic and fermentative, for the production of prebiotics, namely, galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) from food industry by-products. Under this scope, a team of scientists, from both university and company, are building strategies for the development of an industrial plant to produce oligosaccharides that will be incorporated in liquid and solid food formulations, in order to obtain functional foods targeted towards gut function. Therefore, this study was done in the BIOLIFE project context aiming the process optimization of FOS production.

FOS have received particular attention recently because of their excellent biological and functional properties, namely, as prebiotic compounds. A prebiotic is a nondigestible food ingredient whose beneficial effects on the host result from the selective stimulation of growth and/or activity of members of the bacterial community that inhabits the gut microbiota. Additionally, FOS are low calorie non-carcinogenic sweeteners with numerous suggested health benefits. These include immune system activation, resistance to infections, synthesis of B-complex vitamins and calcium absorption. They can also be used as a treatment for breast cancer, diarrhoea, and constipation.

FOS can be produced by the action of enzymes with transfructosylation activity (i.e. fructosyltransferase (EC 2.4.1.9) and/or fructofuranosidase (EC 3.2.1.26)) from plants and microorganisms. Nevertheless, microbial FOS production by fungi in bioreactors is more realistic, as several fungal strains are known to produce extracellular or intracellular enzymes with transfructosylation activity, namely *Aspergillus* sp., *Penicillium* sp. and *Aureobasidium* sp.

The aim of this work was the optimization of operation parameters for FOS productivity using experimental design tools. The experiments were conducted in a stirred batch reactor, using sucrose (200 g/l) as substrate, and an *Aureobasidium* sp as a FOS producer. In order to identify which operation parameters had a significant effect on FOS productivity a factorial design was developed. In this type of designs the influences of all experimental variables, factors, and interaction effects on the responses variables are investigated. Therefore, three main operation parameters (temperature, pH and agitation) to be set as factors and three response variables (FOS maximum concentration, specific enzymatic activity and time for FOS maximum production) were selected. According to the factorial design, 2^3 experiments plus 3 central points were performed. For each response variable, a first-order model was then fitted

to the data obtained from the factorial design experiments using statistic software. The quality of the fit of the model equation was expressed by the coefficient of determination R^2, and its statistical significance checked by a F-test. The significance of the regression coefficient was tested by a t-test. The level of significance was given as values of Prob>F less than 0.1. A differential calculation was then employed for predicting the optimum point.

From the results of the experimental design performed, FOS concentration varied markedly from 4.12 g/l to 164.64 g/l and the temperature was found to the most significant factor for the FOS productivity. The model established for FOS concentration was found to be significant (F-value = 100.46) and explains 99.5% of the variability in the data ensuring a good adjustment of the first order model to the experimental data. Moreover, the lack of fit F-value of 5.91 is not significant relative to the pure error and the adequate precision value that measures signal to noise ratio is greater than 4 (30.72) which is desirable. Thus, this model can be used to navigate the design space.

Concluding, with the experimental design optimization a 80% production yield of FOS was achieved in a 32 h experiment using the following operation parameters: 25ºC, pH 5.0 and 55 rpm.