Journal of Biotechnology 210 (2015) 44–51

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Modeling and simulation of fructooligosaccharides synthesis in a batch basket reactor

Maiki Roberto Detofol[a], Elizama Aguiar-Oliveira[b,*], Cindy Elena Bustamante-Vargas[c], Alexandre Batista de Jesus Soares[d], Mónica Beatriz Alvarado Soares[c], Francisco Maugeri[e]

[a] Department of Exact and Land Sciences, Integrated Regional University of the Alto Uruguai and Missões (URI), Av Sete de Setembro 1621, Postal Code: 99700-000, Erechim, Rio Grande do Sul, Brazil
[b] Department of Bioscience, Multidisciplinary Institute on Health (IMS), Federal University of Bahia campus Anísio Teixeira (CAT/UFBA), R. Rio de Contas, 80, Candeias, Postal Code: 45.029-094, Vitória da Conquista, Bahia, Brazil
[c] Department of Engineering and Computer Science, Integrated Regional University of the Alto Uruguai and Missões (URI), Av Sete de Setembro 1621, Postal Code: 99700-000, Erechim, Rio Grande do Sul, Brazil
[d] Department of Agricultural Science, Integrated Regional University of the Alto Uruguai and Missões (URI), Av Sete de Setembro 1621, Postal Code: 99.700-000, Erechim, Rio Grande do Sul, Brazil
[e] Department of Food Engineer, Faculty of Food Engineer (FEA), University of Campinas (UNICAMP), R. Monteiro Lobato, 80, Cidade Universitária, Postal Code: 13083-862. Campinas, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 21 March 2015
Received in revised form 18 June 2015
Accepted 22 June 2015
Available online 27 June 2015

Keywords:
Basket Reactor
FOS Production
Fructosyltransferase
Kinetic
Rhodotorula

ABSTRACT

Fructooligosaccharides (FOS) production was carried out in a batch basket reactor with immobilized fructosyltransferase from *Rhodotorula* sp. from 500×10^3 g m^{-3} of sucrose in 50 mM sodium acetate buffer at pH 6.0, 48 °C at 85 rpm and with an activity of 22.44×10^3 U m^{-3}. The experimental data were well adjusted to the mathematical model for FOS production using SIMULINK® (MATLAB®). The highest regression coefficient ($R^2 > 90\%$) and the lowest percentual residual standard deviation (%RSD < 4.0) and *chi*-square ($\chi^2 < 1.0$) were obtained for sucrose (GF), kestose (GF_2) and total FOS. The mass transfer coefficient (k_L) was determined as 5.6×10^{-5} m h^{-1} and the diffusivity (D_S) was 2.11×10^{-11} m^2 s^{-1}. The best predicted FOS yield (after 96 h) was 60.62%, with an equivalent productivity of 3.16×10^3 g m^{-3} h^{-1}. These results reaffirm the good potential of this enzyme for industrial application and, in addition, are in conformation to other studies conducted with the same enzyme from the same and different microbial sources.

1. Introduction

Fructooligosaccharides (FOS) are polymers frequently used as a functional food ingredient, as a dietary fiber or as a prebiotic, due to its health benefits such as the improvement in gastrointestinal health, amongst other effects (Gibson et al., 2004; Gibson and Roberfroid, 1995; Goldberg, 1994; Passos and Park, 2003). They are polymers of 1–3 fructosyl units (F) bounded to the β-2,1 position of sucrose (GF) and they are generally represented by: GF_n, in which "*n*" is the polymerization degree or the number of fructosyl units linked in chain. FOS can be obtained from several plants and vegetables (Benkeblia, 2013) but their production by enzymes is far more studied, even applied, since it can avoid the use of food substrates to obtain purified FOS. The most common enzymatically produced FOS are: 1-kestose (GF_2), nystose (GF_3) and 1^F-fructofuranosyl-nystose (GF_4) called short-chain FOS (sc-FOS); the ideal composition of each, in order to optimize its health effects, is still debatable. According to Stewart et al. (2008), longer FOS (like GF_4) result in a longer permanence inside the gut and, consequently, can cause better effects; on the other hand, Suzuki et al. (2006) affirmed that the shorter molecules (like GF_2) are more available to the probiotics and so, more effective. Regardless the perfect composition, it is a common sense that FOS consumption is beneficial in many aspects.

β-fructofuranosidases (EC 3.2.1.26) and fructosyltransferases (EC 2.4.1.9) are enzymes which catalyze the production of FOS from sucrose and they can be obtained from different vegetable and microbial sources; the yield for the conversion of sucrose into FOS is variable for each source but in general, fungi like *Aspergillus*, are responsible for the highest industrial yields, around 60%

* Corresponding author.
E-mail address: elizamaguiar@yahoo.com.br (E. Aguiar-Oliveira).

http://dx.doi.org/10.1016/j.jbiotec.2015.06.410
0168-1656/

Nomenclature

D_b	Basket diameter (m)
D_i	Impeller diameter (m)
d_p	Biocatalyst particle diameter (m)
D_S	Diffusivity coefficient of the solute in solvent ($m^2\ h^{-1}$)
D_T	Reactor diameter (m)
F	Fructose concentration ($g\ m^{-3}$)
G	Glucose concentration ($g\ m^{-3}$)
GF	Sucrose concentration ($g\ m^{-3}$)
GF_o	Sucrose feed concentration ($g\ m^{-3}$)
GF_2	1-Kestose concentration ($g\ m^{-3}$)
GF_3	Nystose concentration ($g\ m^{-3}$)
GF_4	1^F-Fructosylnystose concentration ($g\ m^{-3}$)
h_b	Basket height (m)
H	Liquid height (m)
k_B	Boltzmann constant ($g\ m^2\ K^{-1}\ h^{-2}$)
k_{cat}	Catalytic constant (h^{-1})
K_{gk}	Competitive inhibition constant by glucose for 1-kestose uptake ($g\ m^{-3}$)
K_{gs}	Competitive inhibition constant by glucose for sucrose uptake ($g\ m^{-3}$)
K_{gn}	Competitive inhibition constant by glucose for nystose uptake ($g\ m^{-3}$)
k_L	Mass transfer coefficient ($m\ h^{-1}$)
K_m	Saturation constant ($g\ m^{-3}$)
K_{mk}	Saturation constant for 1-kestose ($g\ m^{-3}$)
K_{mnh}	Saturation constant for nystose for the hydrolyzing reaction ($g\ m^{-3}$)
K_{ms}	Saturation constant for sucrose ($g\ m^{-3}$)
K_{mn}	Saturation constant for nystose ($g\ m^{-3}$)
K_{snh}	Inhibition constant for the hydrolyzing reaction for nystose uptake ($g\ m^{-3}$)
K_{ss}	Inhibition constant for sucrose uptake ($g\ m^{-3}$)
N	Agitation speed (rpm)
n_e	Number of experimental data
R_o	Solute radius (m)
P	Power number (N)
Q	Flow rate ($m^3\ s^{-1}$)
R^2	Correlation coefficient
T	Reaction temperature (°C, K)
V	Reactor volume (m^3)
V_{in}	Input variable
V_L	Liquid volume (m^3)
V_{mk}	Maximum transfructosylating rate for 1-kestose uptake ($g\ m^{-3}\ h^{-1}$)
V_{mn}	Maximum transfructosylating rate for nystose uptake ($g\ m^{-3}\ h^{-1}$)
V_{mnh}	Maximum hydrolyzing rate for nystose uptake ($g\ m^{-3}\ h^{-1}$)
V_{ms}	Maximum sucrose uptake rate ($g\ m^{-3}\ h^{-1}$)
V_{out}	Output variable
V_R	Reactor total liquid volume (m^3)
V_S	Solid volume (m^3)
SF	Sensitivity factor (%)
y_e	Experimental value
$\bar{y}_e$	Average experimental values
y_m	Predicted value

Greek letters

α	Liquid fraction of the reactor
ϵ	Energy dissipation rate per mass unit ($m^2\ h^{-1}$)
μ	Sucrose viscosity ($g\ m^{-1}\ h^{-1}$)
ν	Sucrose cinematic viscosity ($g\ m^{-1}\ h^{-1}$)
ρ	Sucrose density ($g\ m^{-3}$)
χ^2	Chi-square

Subscript letters

c	Altered condition
m	Bulk phase
s	Surface of the biocatalyst particle; standard condition

(Yun, 1996; Yun, 1996). The complex kinetic mechanism of FOS production from sucrose by fructosyltransferases was firstly proposed by Jung et al. (1989) and Duan et al. (1994) and it fits perfectly for the most fructosyltransferases, although, other mechanisms has been proposed (Vega and Zuniga-Hansena, 2014), since the same enzyme, from different sources, can perfectly present different mechanisms.

Based on previous studies regarding the application of a partially purified extracellular fructosyltransferase from *Rhodotorula* sp. immobilized by adsorption in a solid–acid support, composed mainly by niobium, it is possible to affirm that this enzyme has a good potential for industrial application (Aguiar-Oliveira and Maugeri, 2010–2013; Aguiar-Oliveira et al., 2012). For that reason, and to complement some data obtained so far about FOS production with the free and immobilized enzyme in different bioreactors (Alvarado-Huallanco and Maugeri-Filho, 2014, 2012, 2011, 2010), this work was conducted aiming to present the mathematical modeling for FOS production, from sucrose, by the immobilized fructosyltransferase from *Rhodotorula* sp. in a batch basket reactor, operated under optimized conditions for FOS synthesis.

2. Material and methods

2.1. Microorganism and immobilized enzyme

Rhodotorula sp. (LEB-V10, Laboratory of Bioengineering Process, UNICAMP, Campinas, São Paulo, Brazil) was cultivated in a sugar cane molasses and corn steep liquor based medium, its extracellular fructosyltransferase was obtained by ethanol precipitation. Aguiar-Oliveira and Maugeri (2010) described all these methodologies in details. The same authors described the methodology for enzymatic immobilization by adsorption and crosslinking onto a solid–acid support (niobium ore); the randomly adsorbed biocatalyst obtained was submitted to a sequential step – crosslinking with glutaraldehyde and sucrose (oriented adsorption) – in order to strengthen the enzymatic immobilization.

2.2. Determination of the enzymatic activity

According to the fundamentals presented by Chen and Liu (1996) for the free enzyme and later adapted by Aguiar-Oliveira and Maugeri (2010) for the immobilized enzyme, one transfructosylation activity unit (U) was defined as 1 μmol of transferred fructose per minute.

2.3. FOS production

The optimization of FOS production by the immobilized (randomly adsorbed) fructosyltransferase from *Rhodotorula* sp. was conducted previously by Aguiar-Oliveira et al. (2012). Based on that study, the FOS synthesis were carried out in a batch basket reactor, described below. The reaction conditions of 48 °C, $500 \times 10^3\ g\ m^{-3}$ of sucrose in 50 mM sodium acetate buffer at pH 6.0 were applied,