Bioresource Technology 132 (2013) 432–435

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Short Communication

Production of high-purity neofructooligosaccharides by culture of *Xanthophyllomyces dendrorhous*

Dey-Chyi Sheu *, Jan-Yi Chang, Yan-Jen Chen, Cheng-Wei Lee

Department of Bioengineering, Tatung University, 104 Taipei, Taiwan

ARTICLE INFO

Article history:
Received 13 October 2012
Received in revised form 9 January 2013
Accepted 10 January 2013
Available online 4 February 2013

Keywords:
Fermentation
Neofructooligosaccharides
Xanthophyllomyces dendrorhous

ABSTRACT

Neofructooligosaccharides (neo-FOS) were produced in submerged cultures of *Xanthophyllomyces dendrorhous*. Among the various strains of *X. dendrorhous* that have intracellular ^{6}G-fructofuranosidase (^{6}G-FFase), BCRC 21346 with high enzyme activity (3.60 U/mL) and BCRC 22367 with low enzyme activity (0.59 U/mL) were investigated in this work. Neo-FOS were generated in a 5-L jar fermenter at 20 °C, 100 rpm and 2 vvm with the pH controlled at 6.9 ± 0.1, using 250 g/L of sucrose as the substrate. Through the catalytic action of *X. dendrorhous* ^{6}G-FFase on sucrose, monosaccharides as well as neo-FOS were produced. A portion of these monosaccharides was consumed by the yeast cells. However, the production of monosaccharides was low in concentration in culture with low ^{6}G-FFase activity, indicating they might be used up concurrently during the fermentation. Consequently, neo-FOS at a purity of up to 87.4% could be obtained.

1. Introduction

^{1}F-Fructooligosaccharides (^{1}F-FOS), produced from sucrose by the catalytic action of ^{1}F-fructofuranosidase (^{1}F-FFase), are currently available prebiotics that can stimulate the proliferation of bifidobacteria in the intestine and thus improve human health (Steed and Macfarlane, 2009). Recently, there has been increasing interest in neo-FOS due to its superior bifido-stimulating effect and chemical and thermal stability compared to ^{1}F-FOS (Kilian et al., 2002; Lim et al., 2007). ^{1}F-FOS comprises 1-kestose, nystose and ^{1}F-fructosyl nystose, whereas neo-FOS consists of neokestose and neonystose (Linde et al., 2012). Mass production of neo-FOS has been carried out using a free-whole-cell biotransformation (Ning et al., 2010) or culture of *Xanthophyllomyces dendrorhous* (Ning et al., 2012). During the fermentation of sucrose with *X. dendrorhous*, sucrose is converted into neo-FOS through the transfructosylation of intracellular ^{6}G-FFase, and large amounts of glucose and fructose are produced as well (Chen et al., 2011). However, the production of high-purity neo-FOS has not yet been investigated. In recent years, a great deal of work has been devoted to the production of high-purity ^{1}F-FOS because it is non-cariogenic, low in calories and may be ingested by diabetic patients. Generally, FOS produced from sucrose through the catalytic action of ^{1}F-FFase consists of 50–60% ^{1}F-FOS, 30–40% glucose and 10–20% sucrose. During the catalytic reaction, the byproduct glucose is a competitive inhibitor of the fructosyl-transferring reaction catalyzed by ^{1}F-FFase so that considerable amounts of sucrose remain unreacted (Duan et al., 1994). High-purity ^{1}F-FOS could be obtained from sucrose by a mixed-enzyme system (Yun and Song, 1993; Sheu et al., 2001) or by a mixed-cell system (Sheu et al., 2002), in which glucose was converted into gluconic acid by the catalytic action of glucose oxidase or intracellular glucose dehydrogenase of *Gluconobacter oxydans*, respectively. During the reaction the gluconic acid generated was rendered insoluble by mixing with a calcium carbonate suspension. Consequently, high-purity ^{1}F-FOS consisting mainly of 1-kestose, nystose and ^{1}F-fructosyl nystose was obtained. Furthermore, high-purity ^{1}F-FOS could be obtained alternatively by adding a yeast fermentation step following the catalytic reaction of ^{1}F-FFase on sucrose (Yang et al., 2008).

Generally, yeast cannot directly metabolize sucrose. Invertase located in the cell wall hydrolyze sucrose into glucose and fructose, then the glucose and fructose are diffused into medium or imported into the cell by hexose transporters (Koschwanez et al., 2011). In the case of *X. dendrorhous* ^{6}G-FFase plays a similar role in the breakdown of sucrose. However, ^{6}G-FFase has both fructosyl-transferring and hydrolyzing activities on sucrose (Chen et al., 2011; Linde et al., 2012). Transfructosylation yields neo-FOS, 1-kestose and glucose, whereas hydrolysis yields glucose and fructose. During the fermentation of sucrose with *X. dendrorhous* having ^{6}G-FFase activity, the liberated glucose and fructose are consumed by the yeast cells and converted mainly into glycerol, ethanol, organic acid, biomass, carbon dioxide and energy for sustaining life. In culture of *X. dendrorhous* with high activity of ^{6}G-FFase, large amounts of glucose and fructose are accumulated,

* Corresponding author. Tel./fax: +886 2 25926846.
E-mail address: dcsheu@ttu.edu.tw (D.-C. Sheu).

0960-8524/$ - see front matter Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.061

because production rate of these monosaccharides is much higher than consumption rate. However, in culture with low activity of ^{6}G-FFase, the production rates of glucose and fructose are low. If most of the glucose and fructose were concurrently consumed by the *X. dendrorhous* cells, high-purity neo-FOS would be produced. Compared with the above-mentioned processes for producing high-purity ^{1}F-FOS, the present method offers an attractive way to produce high-purity neo-FOS.

In this work, batch-wise production of neo-FOS from sucrose was carried out using a 5-L stirred tank bioreactor. Fermentations involving *X. dendrorhous* BCRC 21346 with high ^{6}G-FFase activity and *X. dendrorhous* BCRC 22367 with low enzyme activity were investigated. The yields and compositions of neo-FOS were compared.

2. Methods

2.1. Microorganisms and chemicals

X. dendrorhous BCRC 21346 (ATCC 24202) and BCRC 22367 (ATCC 24203) were obtained from Bioresource Collection and Research Center, Taiwan. The yeasts were cultured on GYP agar plates at 20 °C and transferred every two weeks. 1-Kestose was purchased from Wako. Neokestose and neonystose were purified from the culture broth of *X. dendrorhous* BCRC 22367 by HPLC on a semi-preparative ODS-AQ column (10 × 250 mm, YMC, Japan). The moble phase was water at a flow rate of 2.5 mL/min. The detector was a Wato 410 differential refractometer (Waters, USA) with the sensitivity set at four. After passage through a syringe filter with a pore size of 0.2 μm, 200 μL of culture broth were injected. The retention times for monosaccharides (glucose and fructose), glycerol, sucrose, 1-kestose, neokestose and neonystose were 5.6, 5.9, 7.2, 9.4, 10.7 and 23.6 min, respectively. Related peak fractions were collected and concentrated by rotary evaporation. After lyophilization, the purified saccharides were weighed and used as the neokestose and neonystose standards for the HPLC analysis.

2.2. HPLC analysis of carbohydrates

The products in the culture broth were analyzed by HPLC on a Hydrosphere C18 column (4.6 × 250 mm, YMC, Japan) using a refractive index detector. The mobile phase was water and the elution rate was 0.8 mL/min. After an adequate dilution, each sample was passed through a syringe filter with a pore size of 0.2 μm. Twenty microliters of sample were injected for analysis. The retention times for monosaccharides (glucose and fructose), glycerol, sucrose, ethanol, 1-kestose, neokestose and neonystose were 4.1, 4.4, 5.6, 7.4, 8.5, 12.1 and 25.7 min, respectively.

2.3. Determination of ^{6}G-FFase activity

^{6}G-FFase activity of the yeast cells was determined based on the formation of neokestose. One milliliter of culture suspension was centrifuged in a 1.7-mL Eppendorf tube for 5 min. The supernatant was discarded. Yeast cells were suspended with 1 mL water by vortexing for 2 min and then were harvested by centrifugation again. The enzyme reaction was carried out in a 1.7-mL Eppendorf tube containing 0.5 mL of adequately diluted cell suspension and 1 mL of 60% (w/v) sucrose in 0.1 M potassium phosphate buffer (pH 7.0). After incubation at 20 °C for 20 min, the reaction mixture was heated in an 85 °C water bath for 10 min to inactivate ^{6}G-FFase and thus the enzyme reaction was terminated. Neokestose in the reaction mixture was determined by HPLC. One unit of ^{6}G-FFase activity was defined as the amount of enzyme required to produce 1 μmole of neokestose per minute under the above-described conditions.

2.4. Production of neo-FOS by submerged culture of X. dendrorhous

Three loops of *X. dendrorhous* cells, which had been grown on GYP agar plates at 20 °C for 3–5 days, were inoculated to 300 mL of culture medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% sucrose) in a 500-mL Erlenmeyer flask. After incubation in a shaker at 160 rpm and 20 °C for 72 h, 300 mL of the shake-flask culture were inoculated to a 5-L jar fermenter with a working volume of 3 L operated at 20 °C, 100 rpm and 2 vvm. The fermentation medium was composed of 25% (w/v) sucrose, 0.3% yeast extract and 0.3% peptone. During the fermentation antifoamer was added intermittently in order to prevent foaming in the bioreactor. The pH of the culture broth was controlled at 6.9 ± 0.1 by automatic addition of NaOH solution. Batch fermentation was completed when more than 97% of the initial sucrose had been consumed.

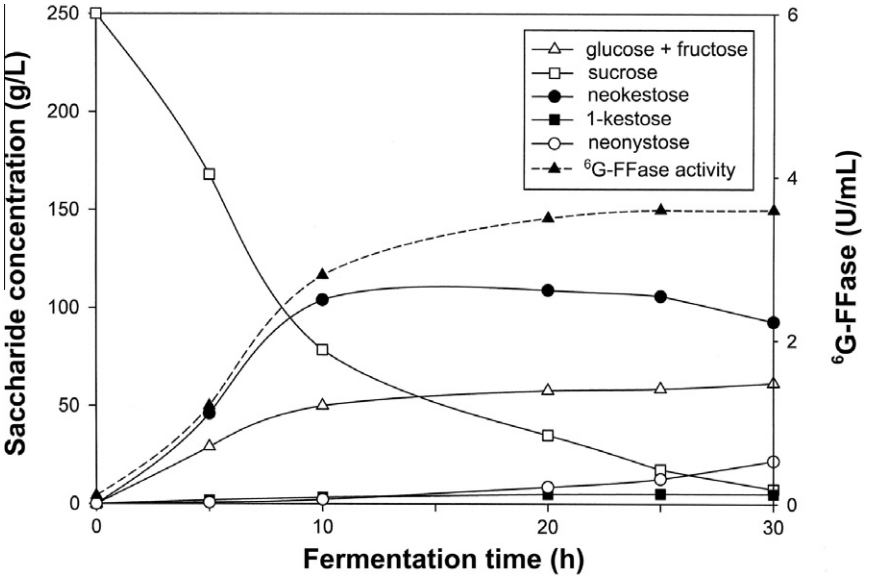

Fig. 1. Time courses of saccharide concentration and ^{6}G-FFase activity during the fermentation of 250 g/L sucrose with *X. dendrorhous* BCRC 21346 in a 5-L fermenter.