Journal of Molecular Catalysis B: Enzymatic 91 (2013) 81–86

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Bioconversion of sucralose-6-acetate to sucralose using immobilized microbial cells☆

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ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form 6 February 2013
Accepted 25 February 2013
Available online 5 March 2013

Keywords:
Sucralose
Sucralose-6-ester
Biotransformation
Packed bed reactor
Immobilization

ABSTRACT

Bioconversion of sucralose-6-acetate to sucralose, an artificial sweetener has been carried out using *Arthrobacter* sp. (ABL) and *Bacillus subtilis* (RRL-1789) strains isolated at IIIM, Jammu, India. Biotransformation of sucralose-6-acetate to sucralose involves use of microbial whole cells, immobilized whole cells and immobilized whole cell bioreactor. Immobilized whole cells packed bed reactor has shown much superior biotransformation process in aqueous system using green technology, where purification of the final product is not required. The final sucralose bioproduct was directly concentrated under vacuum to get white crystalline powder. The immobilized whole cell bioreactor was used for more than three cycles continuously, thus provided much cheaper, less time consuming and easy down streaming process. Moreover, the method does not require any purification steps, which is otherwise requisite for presently available methods for sucralose production, resulting in even lower cost of overall process.

1. Introduction

Sucralose, trichlorogalactosucrose or 4,1′,6′-trichlorogalactosucrose is known as an artificial sweetener having 600 times sweetening ability than sucrose. It is used in place of sugar to eliminate or reduce calories in a wide variety of products, including beverages, baked goods, desserts, dairy products, canned fruits and syrups. Sucralose is chemically known as 1,6-dichloro-1,6-dideoxy-beta-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside and has been derived from sucrose by replacing the hydroxyl groups in the 4,1′, and 6′ positions with chlorine.

First report on sucralose appeared in 1987 by Queens Elizabeth College in London and Tate & Lyle, a private company where, chemical modification of sucrose by selective replacement of three hydroxy groups with chlorine atoms was reported. Canada was the first country to approve use of sucralose in foods in 1991 followed by the United States in 1998. It is now being used in at least 28 countries as sweetening agent. Sucralose is being sold under the brand name Splenda by McNeil Specialty Products Company, New Brunswick, New Jersey. About 120 products using sucralose as a sweetener are in the U.S. market.

Sucralose is generally synthesized by chemical methods involving five-step process by selective substitution of three chlorine atoms for three hydroxyl groups in the sucrose molecule. These methods for synthesis of sucralose (4,1′,6′-trichlorogalactosucrose; TGS) involve the chlorination through multistep protection and deprotection strategy to obtain final sucralose. The reagents used in the reaction are hazardous and involve the formation of different byproducts, which need to be removed [1–7]. Involvement of several purification steps, reduce the overall yield of the process for production of sucralose.

The enzymatic methods of sucralose synthesis involve removal of 6-chloro-6-deoxygalactosyl moiety from the 6-position of chlorinated sugar tetrachlororaffinose (TCR) [8]. Luo et al. [9] described improved synthesis of sucralose from sucrose by chemical synthesis, which again needs purification to obtain pure product. Ratnam et al. [10] reported enzymatic process for sucralose production wherein they could achieve maximum 95% deacetylation using free or immobilized enzyme. Such process essentially requires several downstream processing and product purification steps. Thus, the process is quite expensive and time consuming. Alternative methods involve deacetylation of sucrose-6-ester followed by chlorination and purification to get pure sucralose [11]. To the best of our knowledge, this is only one report of enzymatic deacetylation of sucralose-6-acetate, however *Arthrobacter* sp. or *Bacillus sublilis* strains were not used. We have therefore, used these two indigenous strains for a single step hydrolysis of sucralose-6-acetate to obtain the sucralose (TGS) with 100% purity without any purification. The method is green and does not involve any hazardous reagents.

☆ IIIM Communication number: IIIM/1515/2012.

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http://dx.doi.org/10.1016/j.molcatb.2013.02.007

Present report demonstrates the enzymatic hydrolysis of sucralose-6-acetate to sucralose in a single step using acyl hydrolase enzyme from IIIM Jammu, India microbial isolates; *Arthrobacter* sp. (ABL) and *Bacillus subtilis* (RRL 1789). The bioconversion of sucralose-6-ester has been carried out in aqueous system without pH adjustment or use of any buffering salts using free enzyme/whole cells/immobilized enzyme/immobilized whole cells. The final bioproduct does not require any purification and can be directly concentrated to get crystalline powder.

2. Experimental

2.1. Materials and general experimental conditions

The reagents and solvents used in the present study were mostly LR grade. Substrate sucralose-6-acetate was a kind gift from Dr. V. Arosker, Mumbai. Silica gel coated aluminum plates from M/s Merck were used for TLC. Commercial enzymes were purchased from Sigma. 1H NMR spectra in CD_3OD were recorded on Bruker 500 MHz spectrometers with TMS as the internal standard. Chemical shifts were expressed in parts per million (δ ppm). MS were recorded on LC MS Agilent 1100 series. IR was recorded on a FT-IR Hitachi (270-30) spectrophotometer. Optical rotations were measured on Perkin-Elmer 241 polarimeter at 25 °C using sodium D light.

2.2. Enzymes

Commercial enzymes such as *Candida rugosa* lipase (CRL), *Candida cylindracea* lipase (CCL), *Candida antarctica* lipase (CAL), *Pseudomonas* sp. lipase (PSL), *Mucor miehei* lipase (MM), *Porcine pancreas* lipase (PPL), *Mucor javanicus* lipase (MJL) were procured from Sigma. Indigenous lipase such as *Arthrobacter* sp. lipase (ABL), *B. subtilis* lipase (RRL-1789) and *Trichosporon beigelii* lipase (Y-15) were grown in specified media and conditions followed by enzyme isolation by ultrasonication as described previously [12–14].

2.3. Production of biomass

Biomass from *Arthrobacter* sp. was grown in 1% peptone, 0.5% beef extract and 0.5% NaCl, pH 7.0 as described previously [12]. *B. subtilis* biomass was grown in 1% peptone, 0.1% yeast extract, 0.5% NaCl, and 0.5% sucrose at pH 7.2 for 30–36 h [13].

2.4. Immobilization of microbial cells

2.4.1. Entrapment in sol–gel supports

Arthrobacter sp./*B. subtilis* microbial cells were immobilized in sol–gel supports prepared from tetraethylorthosilicate precursor as previously reported [15]. Entrapment was carried out by adding ABL cells suspension/free enzyme to the homogenized sol during polymerization process. Gelation was carried out further for 24 h. The gel was filtered and washed several times with buffer to remove any adhered whole cells/free enzyme. The entrapped ABL was then further used for biotransformation studies.

2.4.2. Entrapment of whole cells in calcium alginate

Freshly harvested *B. subtilis*/*Arthrobacter* sp. cells were mixed with sodium alginate (sodium alginate:cell biomass used was 1:1, 1:2, 1:3) and passed through syringe into 2% calcium chloride solution as previously described [16]. The entrapped cells thus obtained were washed with water and further used for hydrolysis of sucralose-6-acetate at different substrate concentrations.

Table 1
Screening of various indigenous and commercial enzymes for bioconversion of sucralose-6-acetate to sucralose.

Enzyme	Time (h)	Conversion (%)
Arthrobacter sp. lipase (ABL)	96	100
Bacillus subtilis (RRL-1789)	48	100
Trichosporon beigelii (Y-15)	24	5
Pig Liver Esterase (PLE)	50	100
Candida antarctica (CAL-B)	96	85
Pseudomonas fluorescence (PSF)	24	ND
Pseudomonas sp. lipase (PSL)	24	5
Mucor miehei lipase (MM)	24	ND
Mucor javanicus (MJ)	24	5
Amano AS	5	90% + 10% side product
Porcine pancreas lipase (PPL)	24	20
Candida rugosa lipase (CRL)	24	30
Candida cylindracea (CCL)	24	25

2.5. Biotransformation of sucralose-6-ester to sucralose using free isolated enzyme

Biotransformation of sucralose-6-ester to sucralose was performed using several commercial lipases as shown in Table 1 and indigenous lipase/esterase enzymes *B. subtilis* (RRL-1789)/*Arthrobacter* sp. (ABL), *T. beigelii* (Y-15) for comparative performance (Table 1). Enzymatic reaction was performed by suspending sucralose-6-ester and enzymes (purified lyophilized powder) in 1:1 ratio in water/buffer under shaking. Biotransformation reaction was monitored by TLC at different time intervals till complete biotransformation is achieved. On completion of biotransformation, reaction was terminated by centrifugation to remove insoluble impurities and the product was extracted with chloroform, dried and purified by column chromatography in 5:95%, methanol:DCM solvent system. Bioproduct was finally analyzed by HPLC, optical rotation and NMR for its purity.

2.6. Biotransformation of sucralose-6-ester to sucralose using microbial cells

Biotransformation of sucralose-6-ester to sucralose (Scheme 1) was carried out using freshly harvested microbial cells from indigenous strains *B. subtilis*/*Arthrobacter* sp. Reaction mixture was prepared by suspending sucralose-6-ester in requisite amount of water to obtain 10–50 g/L substrate concentration. Enzymatic reaction was initiated by addition of enzyme/cell biomass/immobilized enzyme in equal amount of substrate. Biotransformation was monitored on TLC at different time intervals. On completion of biotransformation, the reaction was terminated by centrifugation and concentrated under vacuum, purified by column chromatography followed by crystallization. The product purity was tested by HPLC, optical rotation and NMR.

2.7. Biotransformation of sucralose-6-acetate to sucralose in immobilized whole cells/immobilized whole cell reactor

Reaction mixture was prepared by dissolving 1.0 g of sucralose-6-ester in water to get 10–50 g/L concentration. The reaction was initiated by addition of immobilized whole cells (beads) equivalent to requisite amount of immobilized/entrapped whole cells beads so as to make S:E ratio 1:1. To carry out reaction with 1.0 g of substrate, 30.0 g of entrapped whole cell beads (equivalent to 1.0 g of whole cells) were used under shaking at 50 rpm. Biotransformation was monitored on TLC at different time intervals till complete biotransformation was achieved. Aqueous product was separated by filtration, beads were washed with water and concentrated under vacuum to obtain white crystalline powder.