Journal of Molecular Catalysis B: Enzymatic 123 (2016) 147–153

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Synthesis of galacto-oligosaccharides from lactose using immobilized cells of *Kluyveromyces marxianus* NCIM 3551

A. Srivastava*, S. Mishra, S. Chand

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz-Khas, New-Delhi 110016, India

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form 2 November 2015
Accepted 12 November 2015
Available online 1 December 2015

Keywords:
K. marxianus NCIM 3551
galacto-oligosaccharides
permeabilized cells
sodium alginate
packed bed reactor

ABSTRACT

Sodium alginate immobilized cells of *Kluyveromyces marxianus* NCIM 3551 cells were used for synthesis of galacto-oligosaccharides (GOS) using lactose as a substrate through transgalactosylation approach. The immobilized cells retained 84.4% of the original β-galactosidase activity displayed by free cells. An increase in thermo-stability and a slight shift in pH optimum was observed for β-galactosidase in immobilized cells. Maximum GOS concentration of 72.0 g/L and specific GOS productivity 60.0 g/L/U was obtained using free cells as opposed to 42.6 g/L and productivity of 35.6 g/L/U for immobilized cells. A repeated batch reaction was carried out using free and immobilized cells for 10 cycles, of 3 h each, using 20% lactose (w/v) with a nearly similar productivity of 24.0 g/L/h and 21.3 g/L/h respectively. Based on the stability of the immobilized cells, continuous synthesis of GOS was carried out for 10 days in packed bed bioreactor using immobilized cells at a loading of 28.8 units/reactor volume. A yield of 64.0 g/L of GOS and a productivity of 21.3 g/L/h was achieved at an optimized flow rate of 1.0 mL/min. The system here demonstrated the feasibility of using whole cells for production of GOS in a packed bed reactor.

1. Introduction

Galacto-oligosaccharides (GOS) are non-digestible carbohydrates formed by the transgalactosylation activity of β-galactosidase (EC.3.2.1.23). GOS are metabolized by the host intestinal bacteria thereby promoting their growth in the colon [1] and preventing proliferation of harmful bacteria such as *Escherichia coli* and Clostridia [2–4]. The transgalactosylation process is normally performed in the presence of high concentrated lactose solution where the galactosyl residues are transferred to lactose, forming products with different degrees of polymerization. The nature of the glycosidic linkages could be $\beta(1\rightarrow 2)$, $\beta(1\rightarrow 4)$ or $\beta(1\rightarrow 6)$. The major bottleneck in GOS synthesis lies in the availability of the biocatalyst (i.e., the enzyme), its stability and source of the enzyme. Immobilization has been reported to be a suitable process that improves the stability of the enzyme. Reusability provided by immobilized enzyme preparations also lowers the overall cost of production of GOS. Recently [5,6] whole cells are increasingly preferred over purified enzymes as the tedious process of enzyme purification can be avoided. Immobilization of whole cells is an added advantage if associated enzymes are more stable which is often the case, as enzymes are in their natural environment [7,8].

Different methods are reported for immobilization of enzymes including adsorption, covalent binding, entrapment and membrane confinement. Many of these have been exploited for the synthesis of GOS using β-galactosidase enzyme. For instance, calcium alginate beads have been employed for entrapment of β-galactosidase from *Penicillium expansum* F3 [9]. Covalent binding/attachment has been reported for β-galactosidase of *Kluyveromyces lactis* on cellulose acetate [10] and of *Aspergillus oryzae* on glyoxal agarose beads [11]. Non covalent binding of β-galactosidase from *Bulleria singularis* ATCC 24193 has also been reported [12]. Cross-linking with glutaraldehyde has been reported to work effectively for immobilization of *A. oryzae* β-galactosidase on magnetized polysiloxane coated with polyaniline [13]. The major advantage presented in these cases was the repeated use of the biocatalyst in a closed system, like a bioreactor, preventing contamination. Enzyme stability and reusability were achieved leading to increase in process productivity.

Among all immobilization techniques, entrapment is one of the most commonly employed techniques and uses polyacrylamide or sodium and calcium alginate. Alginate is a natural polysaccharide extracted from seaweed and is safe for use in food industry. When suspended in ionic solution, it forms gel particles that exhibit good physical strength. Due to its porous nature, continuous sub-

* Corresponding author.
E-mail addresses: anitasri27@gmail.com, anita_sri@rediffmail.com (A. Srivastava).

http://dx.doi.org/10.1016/j.molcatb.2015.11.017
1381-1177/

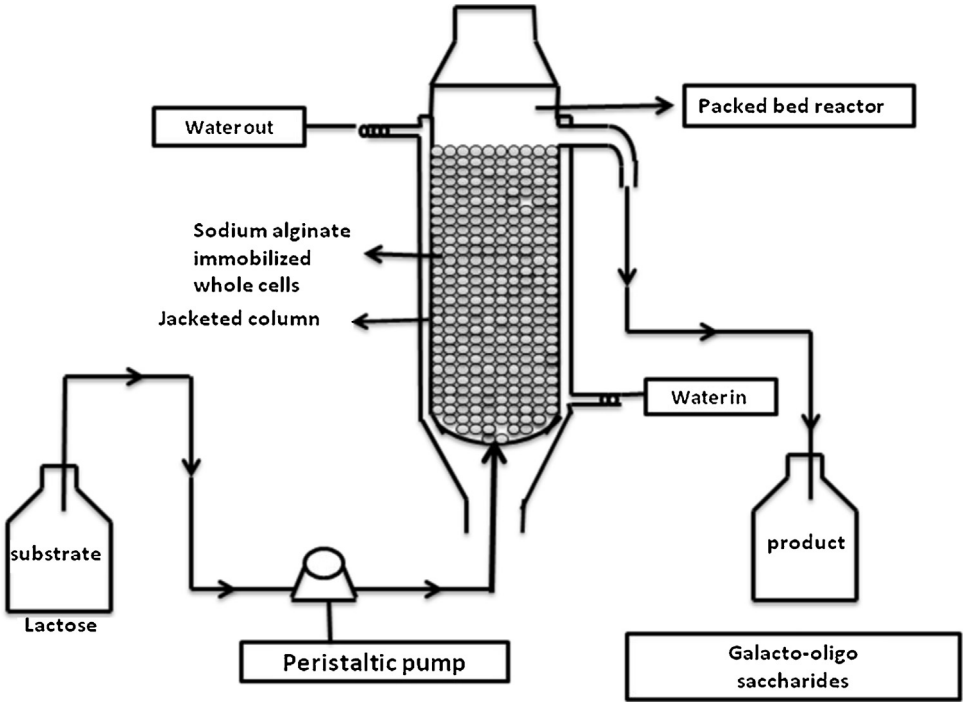

Fig. 1. A schematic diagram of the packed bed reactor for galacto-oligosaccharides synthesis using immobilized cells.

strate hydrolysis and product synthesis can occur. Use of alginate beads in packed bed reactors would be of considerable advantage as packed bed reactors allow continuous usability of the biocatalyst for sustained production of oligosaccharides [14,15]. The present work reports on application of sodium alginate entrapment method using whole cells of *Kluyveromyces marxianus* NCIM 3551 for GOS synthesis. This cell bound enzyme system was previously reported to result in 80% conversion of lactose leading to synthesis of a mixture of galacto-triose (GOS-3) and galacto-tetraose (GOS-4) [16]. The immobilized system was investigated for operational stability, reusability of the cell bound β-galactosidase in a continuous packed bed reactor.

2. Materials and methods

2.1. Materials

Chemicals used for cultivation of cells were of highest purity from Himedia Labs, India. Standard GOS-4, GOS-3 and allolactose were from Carbosynth, U.K. The microbial culture of *K. marxianus* NCIM 3551 was procured from National Chemical Laboratory, Pune.

2.2. Growth and transgalactosylating activity of microbial strain

The cells were grown in a medium consisting of (g/L): yeast extract 15.0; ammonium sulfate 9.0; potassium dihydrogen phosphate 5.0; magnesium sulfate 0.4; lactose 30.0 in 50 mM sodium phosphate buffer pH 6.5. The culture was incubated at 28 °C in an orbital shaker at 200 rpm for 24 h. The production medium was inoculated with 10% (v/v) inoculum and incubated overnight. Lactose, used as the carbon source and inducer of β-galactosidase activity, was filter sterilized using 0.22 μ syringe filter and added to the growth medium. Cells were analyzed for cell bound β-galactosidase activity using o-nitrophenyl-β-D galactopyranoside (oNPGal) as the substrate. For transgalactosylation reactions, whole cells (free or immobilized) were suspended in 30% (w/v) lactose (in sodium phosphate buffer, pH 6.5) in a total volume of 3.0 mL contained in 15 mL flat bottomed vials. The vials were incubated shaken at 50 °C in an orbital shaker at 150 rpm for 8 h. Samples were removed every hour to analyze residual lactose and GOS concentration.

2.3. Permeabilization of cells

Transgalactosylation reactions were performed with free or immobilized cells. For this, the cells were permeabilized in 50% (v/v) ethanol and sodium phosphate buffer, pH 6.5 for 15 min at 25 °C. The treated cells were separated by centrifugation at 5000 rpm for 5 min followed by washing twice with buffer. The cells were stored at 4 °C as described [17] until further use.

2.4. Immobilization of K. marxianus NCIM 3551cells

The permeabilized cells were immobilized on/in different matrices (activated bagasse, alginate, agar and gelatin) as described below.

2.4.1. Immobilization in activated sugar cane bagasse

The sugarcane bagasse was activated by treatment with 1 N NaOH, 0.25 N epichlorohydrin, 0.6 M hexamethylenediamine, 0.1 M $NaHCO_3$ and 20% (v/v) acetone. Two grams of the carrier was suspended in 100 mL of 1 N sodium hydroxide. To this suspension, 2.5 mL of epichlorohydrin was added and the mixture was vigorously stirred at 60 °C for 30 min. The activated matrix was collected and washed with distilled water thoroughly until no epichlorohydrin was detected. The resulting matrix was then suspended in 30 mL of 0.6 M solution of hexamethylenediamine. The suspension was gently shaken at 60 °C for 2 h. The aminohexyl treated carrier was collected by filtration and washed with 200 mL of water followed by washing with 200 mL of 0.1 M $NaHCO_3$ solution. This carrier was then suspended in 50.0 mL of 0.25 N NaOH solution and 5.0 mL of epichlorohydrin and shaken at 60 °C for 30 min. After the reaction, the epichlorohydrin activated aminohexyl bagasse was