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Synthesis and separation of galacto-oligosaccharides using membrane bioreactor

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HIGHLIGHTS

- Highest β-galactosidase loading on membrane surface was 85% at pH 8.2.
- Optimum ratio of PEI to β-galactosidase was 1:25.
- Optimum substrate concentration was 0.045 M.
- Optimum feed flow rate was 25 mL·hr^{-1}.
- Optimum conditions: 45 °C temperature, 4.0 kgf cm^{-2} TMP, 80 r.p.m. stirrer speed.

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ABSTRACT

In the present investigation, a dead-end membrane module, equipped with β-galactosidase immobilized flat-sheet ultrafiltration (UF) membrane, has been considered as a membrane bioreactor (MBR) for galacto-oligosaccharide (GOS) synthesis and separation. β-galactosidase was immobilized on the membrane surface by the combination of a series of mechanisms, such as adsorption of polyethyleneimine (PEI) on membrane matrix, formation of PEI and β-galactosidase aggregates, and finally cross-linking of PEI and β-galactosidase complex on the membrane surface by glutaraldehyde. The optimum pH for PEI-enzyme aggregation was found to be 8.2 and the optimum pH for crosslinking of PEI-enzyme aggregates on the membrane surface was found as 7.0 when the ratio of PEI to β-galactosidase was 1:25. Stable immobilization could be achieved over broad temperature range (4 °C to 65 °C). It was found that at optimum condition β-galactosidase loading on the membrane surface was 85% at multilayer form. GOS was synthesized by MBR with retentate recirculation, using simulated lactose solution, as well as de-proteinated whey as a substrate. Optimum operating conditions for the GOS synthesis were found at 0.045 M initial substrate concentration, 25 mL·h^{-1} feed flow rate, 45 °C temperature, 3.0 kgf cm^{-2} trans-membrane pressure (TMP), 80 r.p.m. stirrer speed.

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1. Introduction

Over the past few decades, rapid developments in the field of biotechnology provide a set of tools that can be applied for the sustainable development in agriculture, natural resources, health, pharmaceuticals, food and beverage industries. Development of upstream and downstream processing of any bioprocess, particularly towards synthesis and purification of biopharmaceuticals originated from biological sources are responsible for prolifications of the field of biotechnology [1].

The lactose hydrolyzing enzyme, β-galactosidase (*β-galactosidase galacto hydrolysase*, *trivially lactase*) has long been accepted as an important ingredient in food processing industries. β-galactosidase catalyzes the hydrolysis of lactose to produce glucose and galactose, and in some cases it takes part in transgalactosylation reaction, that produces galacto-oligosaccharide (GOS) (for example, Gal (β1→3) Gal (β1→4) Gal (β1→6)) [2]. The schematic diagram of lactose hydrolysis as well as transgalactosylation reaction is described in Fig. 1. In dairy industry, β-galactosidase has been used to prevent crystallization of lactose, to improve sweetness, to increase the solubility of milk product, to prepare low lactose containing food products for low lactose tolerant people and for the utilization of cheese whey, which would otherwise be an environmental pollutant [3–7].

In last two decades there has been an increasing interest in 'functional foods', which impart a health benefit above and beyond the nutritional value expected from regular food stuff. Recognized functional food ingredients, namely, oligosaccharides are carbohydrates that contain three to ten sugar units, joined by glycosidic bonds [8]. There are several classes of oligosaccharides but GOS has attracted particular attention because of their presence in fermented milk [9]. The biochemical mechanism for β-galactosidase activity as well as the synthesis of GOS offers insight into some of the important parameters, such as the structure of enzymes, substrate concentration, types and concentration of buffer, pH and temperature of catalysis system [10–14].

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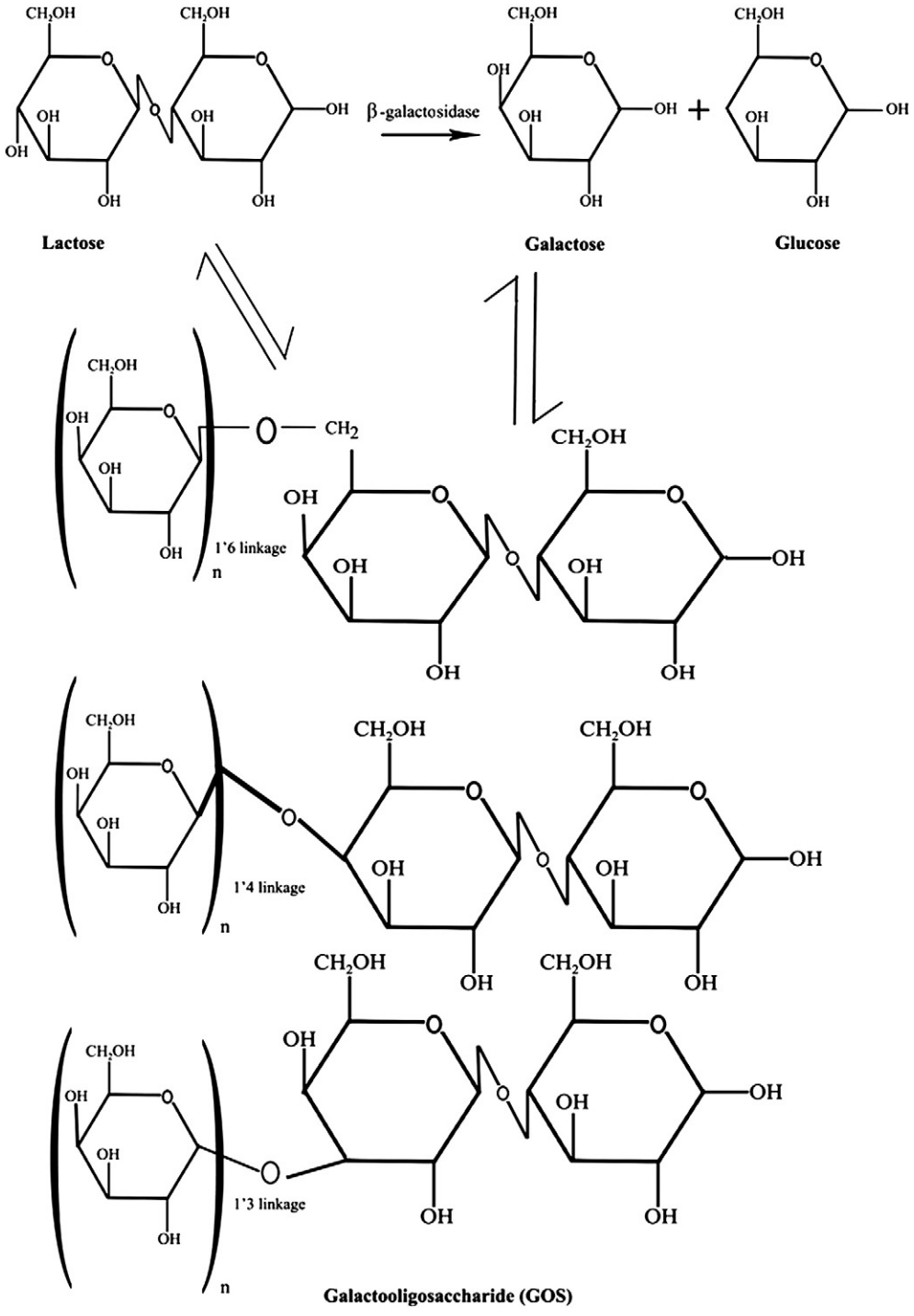

Fig. 1. Schematic diagram of lactose hydrolysis and transgalactosylation reaction.

Integrating the properties of membranes with biological catalyst, such as enzymes, called membrane bioreactor (MBR), is advancing rapidly around the world both in research and large scale commercial practices [15–19]. Simultaneous reaction and separation of product molecules in MBR promote the desire reaction and reduce the processing time. Generally, enzymes are immobilized on membrane matrix where substrates have direct contact with enzymes, and product molecules are transported through the porous channels of membrane. Although a short disadvantage of MBR is concentration polarization and low conversion efficiency, advantages of MBR over packed bed and free-enzyme biochemical reaction are better product recovery together with heterogeneous reactions and reuse of enzymes. Further, due to possible separation of products and reactant molecules, reversible reactions are always facilitated in this type of reactor. The performances of MBR are considerably influenced by the operating conditions (trans-membrane pressure (TMP), feed flow rate, stirrer speed), the physical properties of membrane (porosity, membrane thickness and membrane material), membrane and enzyme coupling (pH, cross-linking chemical) and catalysis reactions (optimum pH and temperature) [20]. In the case of lactose

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