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Enzyme, β-galactosidase immobilized on membrane surface for galacto-oligosaccharides formation from lactose: Kinetic study with feed flow under recirculation loop

Tapas Palai, Avaneesh K. Singh, Prashant K. Bhattacharya *

Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India

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ABSTRACT

The work focuses on producing galacto-oligosaccharides (GOS) through an enzymatic reaction with lactose under a partial recirculation loop by utilizing membrane-immobilized β-galactosidase. Cross-linking through covalent bonding, using gluteraldehyde, was employed to immobilize enzyme on a microporous polyvinylidene fluoride membrane. GOS synthesis was carried out in a laboratory fabricated reaction cell, whereby three immobilized membranes were housed in series. The reaction was conducted at varying initial lactose concentrations (ILCs) and feed flow rates at pH 6 and 40 °C. A maximum GOS of 30% (dry basis) was obtained after 60 h of reaction time, 50 g/L ILC, 241 U of enzyme (specific loading of 600 U/g-membrane), and 0.5 mL/min of feed flow rate at 56% lactose conversion. The GOS yield increased with increased ILC and decreased feed flow rate. The selectivity of GOS formation increased by increasing both the ILC and the feed flow rate, whereas the reverse was true for mono-saccharides. The immobilized enzyme retained ~50% of its initial activity after 30 days of storage at 20 °C, while the native enzyme lost 100% of its activity within 21 days. Furthermore, a five-step, nine-parameter model was developed, and simulated results showed excellent agreement with the experimental data.

1. Introduction

The immobilization of enzymes is expected to be an alternative route to overcome some of the drawbacks that are associated with conventional batch reactors with enzymes under free-enzyme conditions. Immobilization provides several benefits such as enzyme reusability, formation of products in continuous mode [1], increased enzyme stability and productivity, improved product purity and quality [2], reduced processing costs [3], and finally eliminating the step to separate the enzyme from the reaction mixture [4]. Furthermore, continuous product formation is considerably helpful for high productivity and alleviates product inhibition compared to batch processes. The formation of galacto-oligosaccharides (GOS) under continuous mode has been reported by few investigators [5–8] who employed different bioreactor configurations at the laboratory scale. For its commercialization in developing countries like India, further studies are required. By introducing the concept of immobilized enzymatic membrane reactor (IEMR), products may be produced under continuous mode and enzymes may be simultaneously separated from the reaction mixture [5].

A study by Axelsson et al. [9] showed that enzyme immobilization on some supports is economically feasible irrespective of the type and configuration of the reactor. Among all the conventional immobilization techniques that are available, such as physical adsorption, entrapment, gelification, ionic binding, covalent binding, and cross-linking [3,10], covalent binding, which is an irreversible approach, is most suitable [11]. However, immobilization has certain limitations such as support fouling, decrease in enzyme activity, and diffusion problems [1]. Extensive studies have been reported in the literature for the immobilization of enzymes on several supports such as ion-exchange resins, merckogel [12], chitosan beads [5], agarose beads [13,14], graphite [15], and cotton cloth [2]. Membrane-supported enzyme immobilization has also been reported in the literature [16,17].

Enzyme-assisted conversion processes are of increasing use in the fields of food engineering, pharmaceutical, and fine chemicals

Abbreviations: BCA, bicinchoninic acid; FESEM, field-emission scanning electron microscopy; GOS, galacto-oligosaccharides; HFMC, hollow fiber membrane contactor; IEMR, immobilized enzymatic membrane reactor; ILC, initial lactose concentration; ONP, *o*-nitro phenol; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; PVDF, polyvinylidene fluoride.

* Corresponding author. Tel.: +91 512 2597093; fax: +91 512 2590104.
E-mail address: pkbhatta@iitk.ac.in (P.K. Bhattacharya).

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Notations

E	enzyme
$f(k)$	objective function of rate constants
Glu	glucose
Gal	galactose
K_I	equilibrium constant of inhibition
K_m	Michaelis–Menten constant
k	apparent rate constants for step reactions
L	lactose
$\tilde{y}$ and y	experimental and simulated data
U	unit of enzyme
V_m	maximum reaction rate

syntheses, and for environmental purposes [1]. Dairy, which is the oldest industry, produces huge quantities of effluent that contains dissolved sugars, proteins, and fats. Lactose is one such major component and accounts for around 5–6% of whey [17], which is a dairy waste that is produced during cheese separation. This may be utilized more effectively by separating it via membrane methodologies, and converting it into marketable products such as GOS by employing enzymatic conversion techniques [17,18]. GOS is a non-digestible, short-chain oligo-saccharide that is also recognized as a nutraceutical and prebiotic food ingredient. It is composed of galactose units and a terminal glucose unit and has three to nine degrees of polymerization [8]. It acts as an alternative food ingredient for lactose-intolerant people [19]. GOS finds applications in fermented milk products, breads, jams, and beverages [20].

The enzymatic formation of GOS from lactose by using β-galactosidase (EC 3.2.1.23) is well established and has been reported by several investigators [21–24]. Two major reactions occur in the reaction media simultaneously. Lactose polymerizes to tri-, tetra-, and higher oligosaccharides via trans-galactosylation reaction. The same enzyme also catalyzes the hydrolysis of lactose, whereby glycosidic bonds split to form glucose and galactose [25]. Hence, the final reaction mixture contains considerable amounts of mono-saccharides (glucose and galactose) and unreacted lactose, which must be separated [26,27] from GOS for its application as a nutraceutical. In most cases, the reaction has been carried out under free-enzyme conditions in a classical batch reactor, whereby the enzyme needs to be deactivated after each reaction, which is often undesirable from an industrial point of view.

The mechanism of GOS synthesis is very complex because several reactions occur simultaneously [28]. Modeling such a system is useful to understand its reaction pathway. Several authors [29–31] have proposed models to quantify the reaction progress. These models are either mechanistic (for understanding reaction kinetics), or empirical (for optimizing operating parameters) or a combination of both. Most mechanistic models are based on Michaelis–Menten kinetics with inhibition and most are not able to predict GOS formation at lower lactose concentrations. In an earlier investigation [32], a simplified four-step, six-parameter kinetic model as well as an empirical relationship were developed for GOS formation under free-enzyme conditions. Furthermore, a six-step, eleven-parameter model that considered enzyme inhibition and the hydrolysis of GOS was proposed for an immobilized batch reaction system [4], The present work, however, further develops this six-step, eleven-parameter model by lowering the number of reaction steps and kinetic parameters.

In a previous study [4] hydrophobic polyvinylidene fluoride (PVDF) membrane was utilized for enzyme immobilization and GOS formation was carried out with feed under non-flowing condition. Though, there have been ample studies with feed under stagnant mode, there is little or negligible attempt for feed under continuous mode. It is understandable that immobilization of enzymes as well as any momentum given to any of the streams of the reactor would change the kinetics from intrinsic to apparent. Furthermore, in order to scale-up enzymatic reactor, it is necessary to have feed and other streams under continuous modes. Scale-up may bring the possibility of enzyme immobilization on a hollow fiber membrane contactor (HFMC) for the continuous formation of GOS. HFMC is expected to provide higher enzyme loading capabilities, as it provides a higher surface area per unit volume of the module [33]. This work is an attempt in this direction by designing the experimental set-up with feed under recirculation loop. Enzymatic kinetics of GOS formation out of lactose solution with feed under stagnant mode is well established. However, little attempts have been made to study the kinetics with feed under flowing mode. Such a study would help understand designing a bio-reactor. In this study, the objective was to investigate the formation of GOS with the feed under partial recirculation loop by utilizing commercial grade β-galactosidase that was immobilized on a microporous PVDF membrane. Studies were performed to observe the effect of the lactose concentration and the feed flow rate on GOS formation. The work was attempted to ascertain the possibility of utilizing HFMC for GOS formation, whereby the reaction could be carried out under continuous mode. Furthermore, a kinetic model was developed to understand the reaction pathway. The model includes enzyme inhibition by glucose and the hydrolysis of GOS. Simulation of the model was carried out using COPASI and the results were compared with the experimental data.

Table 1
Specification of membrane as obtained from the supplier.

Membrane material	PVDF
Nature	Hydrophobic
Pore size (m)	2.20×10^{-7}
Thickness (m)	1.25×10^{-4}
Diameter (m)	0.047
Mass (g)	0.134
Porosity (%)	75
Water flow rate (L/m^2 h)	9000
Protein binding (g/m^2)	1.50

2. Materials and methods

2.1. Materials and chemicals

Commercial grade β-galactosidase (EC 3.2.1.23; commercial name: Biolacta FN5) that was derived from *Bacillus circulans* was provided by Daiwa Kasei K.K., Japan. Lactose (monohydrate), D-galactose (minimum assay 99%), and glutaraldehyde (25% aqueous solution) were procured from Loba Chemie Pvt. Ltd., India. D-Glucose (anhydrous) was purchased from Qualigens Fine Chemicals, India. *o*-Nitrophenyl-β-D-galactopyranoside (ONPG) (assay ≥ 98%) was obtained from Sigma-Aldrich, India. Bicinchoninic acid (BCA) protein reagent kit was supplied by Novagen, USA. Milli-Q (Millipore Corporation, India) water was used for the preparation of all solutions. All of the chemicals were used in the state that they were received in from their suppliers. Hydrophobic PVDF membrane of 0.047 m diameter (pore size 2.2×10^{-7} m) was supplied by Millipore Corporation, India. The specifications of the membrane are shown in Table 1.

2.2. Enzyme assay and protein estimation

The activity of β-galactosidase was measured as described earlier [32,34] by using ONPG as a substrate. The formation of *o*-nitrophenol (ONP) during the reaction was measured in an UV–vis spectrophotometer (Hitachi, U2900) at 410 nm. BCA method [35]