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RESEARCH

Enzymatic Synthesis of Prebiotic Carbohydrates from Lactose: Integration of Osmotic Membrane Distillation with Batch Reactor System

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Abstract
This study aimed to enhance galactooligosaccharide (GOS) yield by maintaining high lactose concentration levels using osmotic membrane distillation (OMD) throughout the synthesis. The main problem influencing GOS yield during transgalactosylation is the decrease of lactose concentration over extended reaction periods. By integrating OMD with a continuous stirred batch reactor, water was progressively removed from the reaction medium. Compared to a standard batch reactor, the OMD-integrated system not only increased GOS yield by a maximum of 20.1% but also reduced the time needed for equivalent lactose conversion about 15–90 min. This integration particularly boosted the formation of longer-chain GOS. A consistent GOS yield of 67% was achieved, with up to 28% lactose conversion, surpassing the non-integrated reactor's performance. The proposed reactor design shows promise for enhancing GOS production efficiency while concurrently concentrating the reaction medium in a single step.

Keywords Galactooligosaccharide · *Aspergillus oryzae* · β-galactosidase · Osmotic membrane distillation · Reactor design · Yield

Introduction

Galactooligosaccharides (GOS), recognized for their prebiotic properties, are gaining prominence as functional food ingredients, expanding their market share due to health benefits (Yeung et al., 2024). Their applications span a diverse array of products, including baby foods, yogurts, fermented milk products, biscuits, and fruit juices (Igbal et al., 2023). However, GOS's limited natural scarcity and the inefficiencies in enzymatic production processes make them costly. Consequently, developing innovative processes for high-throughput GOS synthesis is crucial for economically meeting the growing demand for prebiotics.

GOS is produced from lactose through transgalactosylation reactions facilitated by the enzyme β-galactosidase (Bodnar et al., 2023). Nonetheless, the yield of GOS synthesis remains limited due to the enzyme concurrently catalyzing transgalactosylation and hydrolysis reactions during the production process of GOS (Osman, 2016). The yield influenced by factors like the enzyme source, water activity, substrate and enzyme concentration, temperature, pH, and the presence of inhibitors or activators (Nath et al., 2016). The enzyme source not only affects yield but also the carbohydrate unit linkages, thereby influencing the final product's prebiotic quality. While a few studies have reported GOS yields above 40% (w GOS/w lactose), most, particularly those using β-galactosidase from *Aspergillus oryzae (A. oryzae),* have noted yields of 20–25% or less (Córdova et al., 2023). Although mesophilic enzymes are industrially preferred (Vera et al., 2014), their poor thermal stability limits the use of high temperatures despite favoring GOS synthesis more. Water activity crucially impacts the reaction route (Illanes et al., 2016), with a decrease favoring transgalactosylation in thermodynamically controlled synthesis. Several attempts such as using high initial concentrations of lactose (Singh et al., 2022) and organic solvents (del-Val and Otero, 2003) have been conducted to maximize GOS synthesis.

In kinetically controlled synthesis, where enzyme characteristics dominate, methods like immobilization (Klein et al., 2013) and combining synthesis with membrane separation

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have been explored for enhanced GOS conversion. Extensive research has focused on improving GOS yield and lactose conversion via membrane processing, such as ultrafiltration (Córdova et al., 2016) and nanofiltration (Altmann et al., 2016). The primary goal of pressure-driven membrane studies incorporated into production processes is to remove glucose and galactose from the reaction medium, which exhibits inhibitory effects on the enzyme (Nath et al., 2013). However, challenges in membrane processes during GOS synthesis include lactose loss, aligning enzymatic reaction rates with permeate flux, and the hydrolysis of saccharides as synthesis progresses (Matella et al., 2006).

Recent advancements in membrane technology, specifically osmotic membrane distillation (OMD), offer a new strategy to boost GOS synthesis by concentrating the reaction medium and reducing substrate losses, a common challenge in traditional processes. By increasing the medium's lactose concentration without losing lactose, this approach steers the enzyme towards transferase activity rather than hydrolysis, thereby boosting yield. In OMD, a microporous hydrophobic membrane separates a feed solution on one side from a hypertonic salt solution on the other side. The vapor pressure difference resulting from the concentration gradient between these two solutions, and consequently the difference in water activity, drives the transfer of water vapor from the lower concentration solution to the salt solution. The driving force is both the temperature gradient across the membranes and the concentration difference between the feed solution and the hypertonic salt solution (Onsekizoglu Bagci, 2015). One of the significant advantages of the OMD process is that due to the highly hydrophobic nature of the membrane, only water vapor is allowed to pass through, preventing the transport of small molecule-weight particles such as water-soluble saccharides through the membrane pores. In addition, unlike pressure-driven membrane processes such as nanofiltration and reverse osmosis, the formation of a gel layer, which typically hampers system performance, is observed only after prolonged use in the OMD (Curcio & Drioli, 2005). This delay in gel formation is attributed to the non-transport of small molecule-weight particles with water at the membrane interface. The requirement for prolonged use to observe a decrease in permeate flux is foreseen as a significant advantage in the integrated use of enzymatic production processes with the OMD system. Although pressure-driven membrane-assisted enzymatic GOS synthesis has been previously researched, to our knowledge, this is the first study to integrate OMD into enzymatic GOS synthesis.

In the current study, the integration of OMD to the enzymatic GOS synthesis process was studied by aiming to enhance GOS yield. The OMD is used to separate water from the enzymatic reaction medium with a highly hydrophobic micro-porous membrane. In this way, the reaction medium transformed into a more concentrated form based on the lactose concentration during enzymatic processing. In this context, the effect of temperature (T), initial lactose concentration (LC), and enzyme concentration (EC) on the enzyme kinetics, GOS productivity, and GOS yield were investigated. In order to compare and to evaluate the efficiency of the OMD integrated system (reactor-2), the experiments were also conducted in a batch-type tank reactor using a free form of β-galactosidase from *A. oryzae* (reactor-1).

Incorporating OMD into GOS production holds promise for enhancing efficiency and increasing yields. Lactose, abundant in milk and often processed into whey, is a valuable industrial by-product. While current practices involve drying and using whey-derived lactose as a food additive, this study proposes an innovative approach for valorizing whey.

Materials and Methods

Materials

β-Galactosidase sourced from *A. oryzae* with a fungal lactase activity of 100,000 U g^{-1}, was procured from ENZECO® (Enzyme Development Corporation, New York, USA). High-purity lactose (99.9%) and calcium chloride ($CaCl_2$) were acquired from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). For determining retention times in HPLC, lactose (Merck, Darmstadt, Germany), glucose (Merck, Darmstadt, Germany), and galactose (Merck, Darmstadt, Germany) were utilized as analytical standards.

Production of GOS from Lactose in a Batch Reactor (Reactor-1)

Ultrapure water was utilized to create lactose solutions (LS) at concentrations of 4.5, 18.25, and 32 °Brix, with the °Brix values confirmed using an Atago PAL-3 refractometer (Tokyo, Japan). β-Galactosidase from *A. oryzae* was introduced into the LS at temperatures of 35 °C, 45 °C, and 55 °C, achieving final enzyme activities of 6, 10, and 14 U g LS^{-1}, respectively. The enzymatic GOS production was conducted by weighing 50 g of lactose solution (LS) into a beaker and then stirring it at 100 rpm in a temperature-controlled magnetic stirrer (MTops MS300HS, South Korea). The beaker was covered with a lid made of foam material, and temperature and pH were monitored throughout the enzymatic process. The pH of each reaction medium was adjusted to 4.5 with either 0.1 N HCl or 0.1 N NaOH prior to initiating the enzymatic reactions. Samples of 100 µL were taken from the reaction vessel at specific intervals—every 5 min for the first 90 min and then every 30 min—and quenched by adding 900 µL of ultrapure water at 95 °C. These samples were then filtered through a 0.45 µm nylon filter and stored at − 18 °C until sugar analysis could be performed using high-performance liquid chromatography (HPLC).

Production of GOS from Lactose in a Batch Reactor Integrated with OMD (Reactor-2)

Figure 1 presents a schematic overview of reactor-2. This setup integrated the enzymatic synthesis from reactor-1, under consistent experimental conditions (LC, T, and EC), with a membrane separation system. The system utilized a CF042A cross-flow acrylic membrane module (Sterlitech Corporation, WA, USA) equipped with a Durapore 0.22 µm hydrophobic polyvinylidene fluoride membrane (Filter code: GVHP, Millipore, Ireland), offering an active membrane area of 0.0042 m^2. The membrane's characteristics included a thickness of 125 µm and a porosity of 75%. The process involved the feed solution (3) and a hypertonic $CaCl_2$ solution (4) at 64°Brix circulating in opposite directions on either side of the membrane, creating a vapor pressure gradient due to the solute concentration and temperature differences. The temperatures at the inlet and outlet of both solutions were monitored with thermometers integrated into the system. To maintain the substrate solution's temperature, the setup included a glass heat exchanger (6), a Masterflex® L/S series peristaltic pump (Cole-Parmer Instrument Co., IL, USA) (2), and a WiseBath WB-06 water bath (Daihan Scientific, Seoul, South Korea) (7). Similarly, the temperature of the hypertonic salt solution was regulated at 18 °C using a glass heat exchanger (6) and an SMC HRS012-AF-20 cooler (Tokyo, Japan) (8). The removal of water from the enzymatic reaction medium over time (m_{water}, g) was quantified using a computer-controlled balance (OHAUS Explorer, Nänikon, Switzerland) (5), with the flux calculated as per Eq. 1:

$$J = \frac{\Delta m_{permeate}}{A\Delta t} \quad (1)$$

where J is evaporation flux (kg m^{-2} h^{-1}), $\Delta m_{permeate}/\Delta t$ is the mass flow rate of collected permeate (kg h^{-1}) and A is the effective membrane area (m^2).

The concentrations in both the reaction medium and the hypertonic salt solution were periodically assessed by measuring their °Brix values using a refractometer (Atago PAL-3, Tokyo, Japan). The concentration gradient (ΔC) was defined by the difference in °Brix values between the hypertonic salt solution and the reaction medium. Additionally, the temperature gradient (ΔT) was determined by the difference between the temperatures of the reaction medium (35 °C, 45 °C, 55 °C) and the hypertonic salt solution (18 °C).

HPLC Analysis

HPLC analysis was performed using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA). The system included an autosampler (Agilent G1329B), a column temperature controller (Agilent G1316A), a pump (Agilent G1311C), a refractive index detector (SpectraSystem RI-150), and was operated with Agilent ChemStation software. Chromatographic separation was achieved

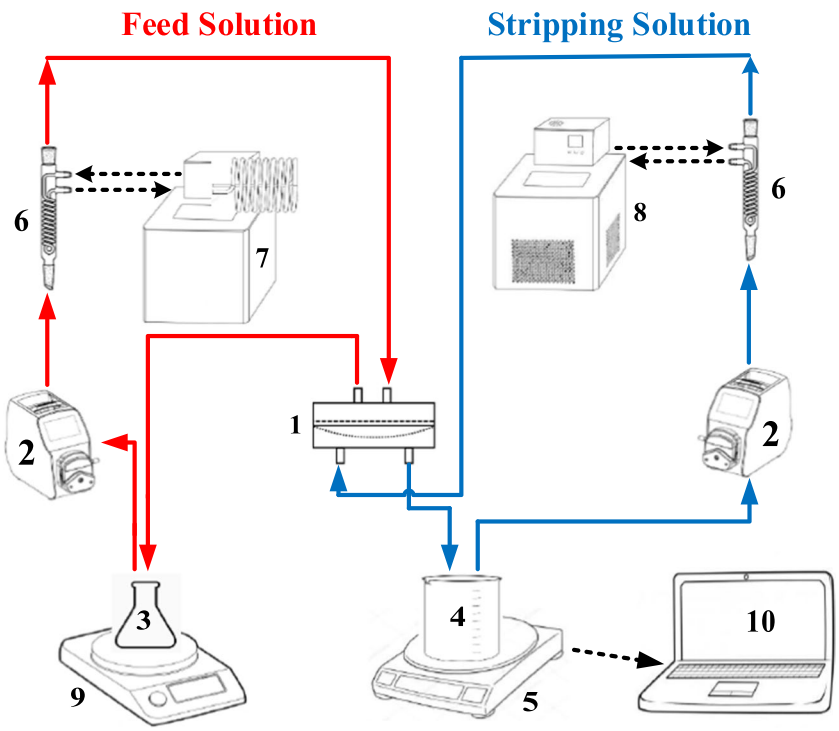


Fig. 1 Schematic representation of osmotic membrane distillation integrated batch reactor (reactor-2); (1) membrane module, (2) peristaltic pump, (3) feed, (4) concentrated salt solution (5) weighing machine, (6) heat exchangers, (7) heating system, (8) cooling system, (9) magnetic stirrer, (10) computer

using a Metacarb 87P column (300 × 7.8 mm), which was maintained at 80 °C. Ultra-pure water served as the mobile phase, with a flow rate of 0.5 mL min^{-1} and an injection volume of 20 µL.

In this analysis, performed without GOS standards, the concentrations of sugars (lactose, glucose, galactose, GOS-3, GOS-4, and GOS yield) were estimated based on their peak areas, expressed as weight percentages (w/w) of the total sugars (Boon et al., 2000). Initially, the reaction medium contains only lactose, which, upon initiation of the enzymatic process, is converted into various sugars. The peak areas for lactose, glucose, galactose, tri-saccharides (GOS-3), and tetra-saccharides (GOS-4) were denoted as LP, GLP, GAP, GOS3P, and GOS4P, respectively. The following equations were used to calculate the composition of these components (Eqs. 2–6):

$$\text{Lactose (\%)} = \frac{\text{LP}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (2)$$

$$\text{Glucose (\%)} = \frac{\text{GLP}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (3)$$

$$\text{Galactose (\%)} = \frac{\text{GAP}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (4)$$

$$\text{GOS} - 3\,(\%) = \frac{\text{GOS3P}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (5)$$

$$\text{GOS} - 4\,(\%) = \frac{\text{GOS4P}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (6)$$

Lactose conversion (%) was calculated as the percentage of lactose transformed into GOS and monosaccharides (Eq. 7), with Lac_0 and Lac_t representing the initial and any subsequent lactose concentration in the reaction medium, respectively.

$$\text{Lactose Conversion (LCON) (\%)} = \frac{Lac_0 - Lac_t}{Lac_0} \times 100 \quad (7)$$

The GOS yield (GY), GOS productivity (GOSP), and specific GOS productivity (SPGOSP) were determined using the following equations (Eqs. 8–10). GY denotes the mass percentage of total GOS-3 and GOS-4 produced relative to the initial lactose mass. SPGOSP is defined as the mass of GOS produced per milligram of enzyme per hour. GOSP represents the ratio of GOS mass to the mass of lactose transformed, expressed as a percentage.

$$\text{GY (\%)} = \frac{\text{GOS3P} + \text{GOS4P}}{\text{LP} + \text{GLP} + \text{GAP} + \text{GOS3P} + \text{GOS4P}} \times 100 \quad (8)$$

$$\text{GOSP (\%)} = \frac{\text{GOS3P} + \text{GOS4P}}{\text{LCON}} \times 100 \quad (9)$$

$$\text{SPGOSP} = \frac{\text{g GOS}}{\text{hour} \times \text{mg enzyme}} \quad (10)$$

Experimental Design and Statistical Analysis

In reactor-1, the Box-Behnken method of the Response Surface Methodology (RSM) with a 3-factor, 3-level design was employed (Cinar et al., 2020). The independent variables selected in reactor-1 included substrate solution temperature (T: 35–45-55 °C), initial lactose concentration (LC: 4.5–18.25–32 °Brix), and enzyme concentration (EC: 6–10-14 U gLS^{-1}). The 15-run experimental design was carried out in duplicate. For experiments conducted in reactor-2, a factorial design was utilized with the flow rate of feed and hypertonic salt solution maintained at 24 L h^{-1}, and the same independent variables as reactor-1, except for LC: 4.5°Brix, were chosen. The 18-run experimental design was carried out in duplicate. The outcomes measured as the process response in both reactors were the percentages of GOS-4 (Eq. 6), GOS-3 (Eq. 5), and GOS yield (GY) (Eq. 8). The results from the experimental studies were analyzed using the Design Expert 11 statistical software by Stat-Ease, Inc., Minneapolis, USA, through variance analysis.

Results and Discussion

Evaluation of Osmotic Membrane Distillation Performance

Figure 2 illustrates the relationship between the permeate flux (J) and the concentration gradient (ΔC). Initially, the lactose solution (LS) and the hypertonic salt solution had concentrations of 32°Brix and 64°Brix, respectively, establishing an initial ΔC of 32°Brix. In addition to ΔC, the temperature gradient (ΔT) served as another significant driving force for water permeation, particularly noticeable when ΔC exceeded 30°Brix. Incremental 10 °C rises in ΔT notably enhanced the initial permeate flux, especially evident at the onset of operation. Elevating the feed temperature significantly boosted permeate fluxes, with this effect being more pronounced above a ΔC of 30°Brix during operations at 55°C compared to 45°C. At a ΔC of 32°Brix, increasing the temperature from 45 to 55 °C resulted in a 2.0-fold increase in initial permeate flux, whereas a rise from 35 to 45 °C led to a 1.6-fold increase. These observations underscore the influential roles of both ΔC and ΔT on permeate flux dynamics.

In OMD, heating the feed to match or slightly exceed the brine's temperature augments the driving force, enhancing process efficiency over traditional osmotic distillation (OD) (Gryta et al., 2013). The reaction temperature increment

positively impacted the initial permeate flux across all initial LCs tested, particularly noticeable at lower ΔCs (Figs. 2 and 3b). This pattern indicates that operations at lower temperatures (35 °C) for high initial LCs tend to achieve lower permeate flux, likely due to lactose's decreased solubility at cooler membrane surfaces. This solubility challenge, potentially leading to crystal formation due to temperature polarization, could be mitigated by increasing permeate temperature, thus balancing temperature across the membrane and the bulk solution (Hausmann et al., 2011). Lactose solubility, as Portnoy et al. (2021) noted, increases by 33.3% with a 20 °C temperature rise, with Roos (2009) highlighting that mutarotation enhances lactose's overall solubility by favoring α-lactose, especially at higher temperatures. Furthermore, Anvari et al. (2020) indicated that temperature polarization becomes more pronounced at lower ΔC (high initial LC) in OMD processes. Such polarization reduces the driving force by diminishing concentration differences, further exacerbated by concentration polarization, which creates a solute concentration disparity across the membrane, weakening the driving force (Wang & Min, 2011). Additionally, rapid dilution of the hypertonic salt solution at 45 and 55 °C led to quicker flux declines compared to operations at 35 °C, suggesting that more stable permeate fluxes are achievable at 35 °C, especially below a ΔC of 30°Brix (Fig. 2).

Figure 3 depicts how the reaction medium's concentration evolves over time at different initial LCs (18.25 and 32.00°Brix). Initially, the reaction medium solely contained lactose, but as the enzymatic reaction progressed, it diversified to include lactose, glucose, galactose, and GOS, with water permeation leading to an increased concentration. The

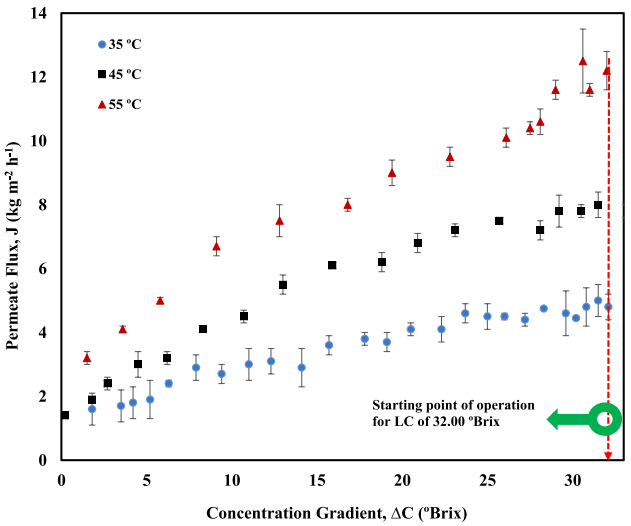


Fig. 2 Temperature-dependent change in permeate flux against concentration gradient during enzymatic GOS synthesis in the osmotic membrane distillation integrated reactor (Reaction conditions; LC: 32°Brix, EC: 6 U gLS^{-1})

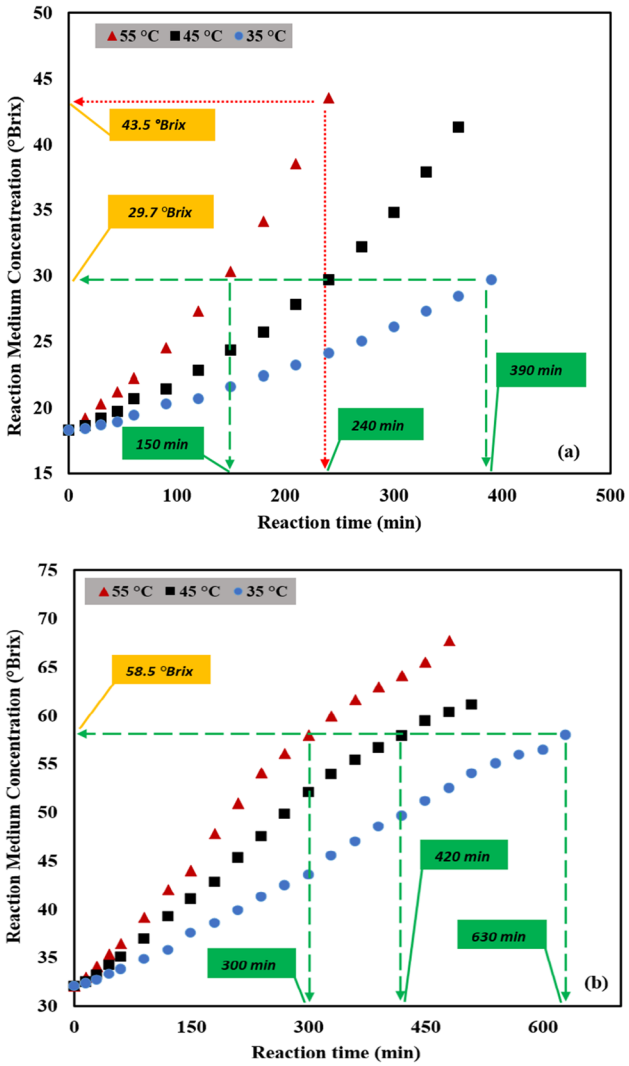


Fig. 3 The change in reaction medium concentration with time **a** for initial lactose concentration of 18.25 °Brix and **b** for initial lactose concentration of 32°Brix

duration of operations varied with the initial LC and reaction temperature, noting the longest operation times at the lowest temperatures for both initial LCs and the highest °Brix values at the highest temperatures. At 35 °C, the concentration reached 29.7°Brix in 390 min (Fig. 3a) due to the lowest permeate flux throughout the operation. Increasing the temperature by 10 °C reduced the reaction time by 150 min to achieve the same concentration, with an additional 10 °C increase (to 55 °C) saving another 90 min. These results confirm that higher operation temperatures facilitate greater initial permeate fluxes. For operations at 32°Brix initial LC (Fig. 3b), the reaction times to reach 58°Brix were 210 and 330 min shorter at 45 °C and 55 °C, respectively.

In reactor-2, the synthesis of GOS and selective water separation were achieved, leading to variable process durations based on transgalactosylation activity and water

permeation rates. The process continued until flux values neared zero permeation. The highest reaction medium °Brix values and shortest processing times were recorded at 55 °C for each initial lactose concentration due to enhanced water permeation at a significant temperature gradient (ΔT: 37 °C). When the comparison was made for the shortest process duration (240 min) for the operation at 55 °C (ΔT: 37 °C) where the highest permeate flux was detected (Fig. 3a), each 10 °C increase in reaction temperature between 35 and 55 °C caused to 1.23-fold and 1.46-fold increases in reaction medium concentration, respectively. A similar analysis for Fig. 3b showed 1.15-fold and 1.12-fold concentration increases for operations at 45 °C and 55 °C, respectively, for each 10 °C rise in temperature from 35 °C.

Comparative Evaluation of GOSP and SPGOSP in Reactor-1 and Reactor-2

Effect of Enzyme Concentration

Figure 4a shows that GOSP values in reactor-1 exhibit the same trend across different enzyme concentrations (EC). In other words, GOSP values exhibited similar trends in varying lactose conversions regardless of EC. In reactor-1, at a constant LC, different EC values resulted in similar outcomes in the enzyme's transgalactosylation activity at varying lactose conversions. Despite changes in the enzyme/substrate ratio, a similar amount of synthesized GOS was observed at identical lactose conversions. Moreover, the high initial SPGOSP at the lowest enzyme level (6 U/g LS, EC1) declined relative to higher enzyme concentrations as lactose conversion progressed. The advantage of high initial SPGOSP at lower enzyme concentrations decreased as lactose conversion increased, indicating that the initial productivity benefit at low enzyme concentrations fades with further lactose conversion as stated by Misson et al. (2017).

In contrast to reactor-1, reactor-2 demonstrated a significant increase in initial SPGOSP with higher EC (Fig. 4b). Initial SPGOSP values for EC1, EC2, and EC3 were 2.72, 5.02, and 5.22 g GOS h^{-1} mg $enzyme^{-1}$, corresponding to lactose conversions of 5.94%, 18.54%, and 19.19%, respectively. SPGOSP for EC2 exceeded that of EC1 and EC3 up to 39% and 35% lactose conversions, respectively. Beyond these points, EC had no impact on SPGOSP. The observed phenomenon may be attributed to the change in EC, which affects the ratio of hydrolysis to transgalactosylation. Indeed, as the EC increases, the hydrolysis activity has suppressed the transgalactosylation activity at earlier stages of the reaction (Osman, 2016). Unlike in reactor-1, EC influenced GOSP values in reactor-2, with EC1 showing higher GOSP values throughout the process. Botelho-Cunha et al. (2010) also stated that increased EC resulted in less GOS, likely due to accelerated hydrolysis of produced GOS rather than an enhancement in synthesis. Further increasing EC to EC3 made hydrolysis the dominant activity, failing to achieve the GOSP value seen with EC1. The GOSP values for EC3 in the initial stages of reaction, particularly at low lactose conversions, performed satisfactorily. This can be attributed to the higher EC levels at constant LC, which have enhanced both lactose hydrolysis and transgalactosylation. The increased enzyme availability facilitates the formation of enzyme–substrate complexes, leading to a higher yield of product (GOS) over time. However, at higher lactose conversions after reaching maximum GOS yield in the EC3 reactors, the degradation of GOS into glucose and galactose accelerates, likely due to the inhibitory effects of monosaccharides. Furthermore, it has been observed that as EC increases, the enzyme continues to demonstrate activity at higher lactose conversions, primarily in the direction of hydrolysis. It was concluded that reactor-2 offers an opportunity to minimize enzyme usage in GOS synthesis, potentially lowering production costs. Similar to our result, Rustom et al. (1998) reported that using a lower percentage (less than 0.1%) of β-galactosidase from *A. oryzae* is more cost-effective which can be attributed to higher ECs may raise inhibitor levels in the reaction mix.

Effect of Temperature

In both reactors, as depicted in Fig. 5a and b, an increase in temperature corresponded with a rise in initial SPGOSP, with the highest SPGOSP of 4.7 g GOS h^{-1} mg $enzyme^{-1}$ at 14.5% lactose conversion observed at 55 °C. This observation aligns with findings by Vera et al. (2012), who also reported a temperature-dependent increase in SPGOSP. In reactor-2, SPGOSP values for temperatures T1 and T2 converged at a 30% lactose conversion, while SPGOSP across all three temperatures became comparable after approximately 50% lactose conversion. Notably, SPGOSP values for T1 and T2 in reactor-2 aligned at earlier lactose conversions (around 15%) compared to reactor-1 (around 35%), likely due to the accelerated increase in monosaccharide concentration within the reaction medium, which inhibits the enzyme. Increased temperature in reactor-2 enhanced both enzyme activity and permeate flux (Fig. 2). As lactose hydrolyzed into glucose and galactose, these monosaccharides potentially reached critical inhibitory concentrations more rapidly at T2 (Vera et al., 2011), suggesting a quicker decline in transgalactosylation activity at T2 compared to T1. The lesser effect observed at T3 compared to T2 (Fig. 5a) could be attributed to an additional increase in enzyme activity at this higher temperature.

For operations in both reactors, SPGOSP values eventually leveled off after 50% lactose conversion. In reactor-1, GOSP values were lower across temperatures, with a more

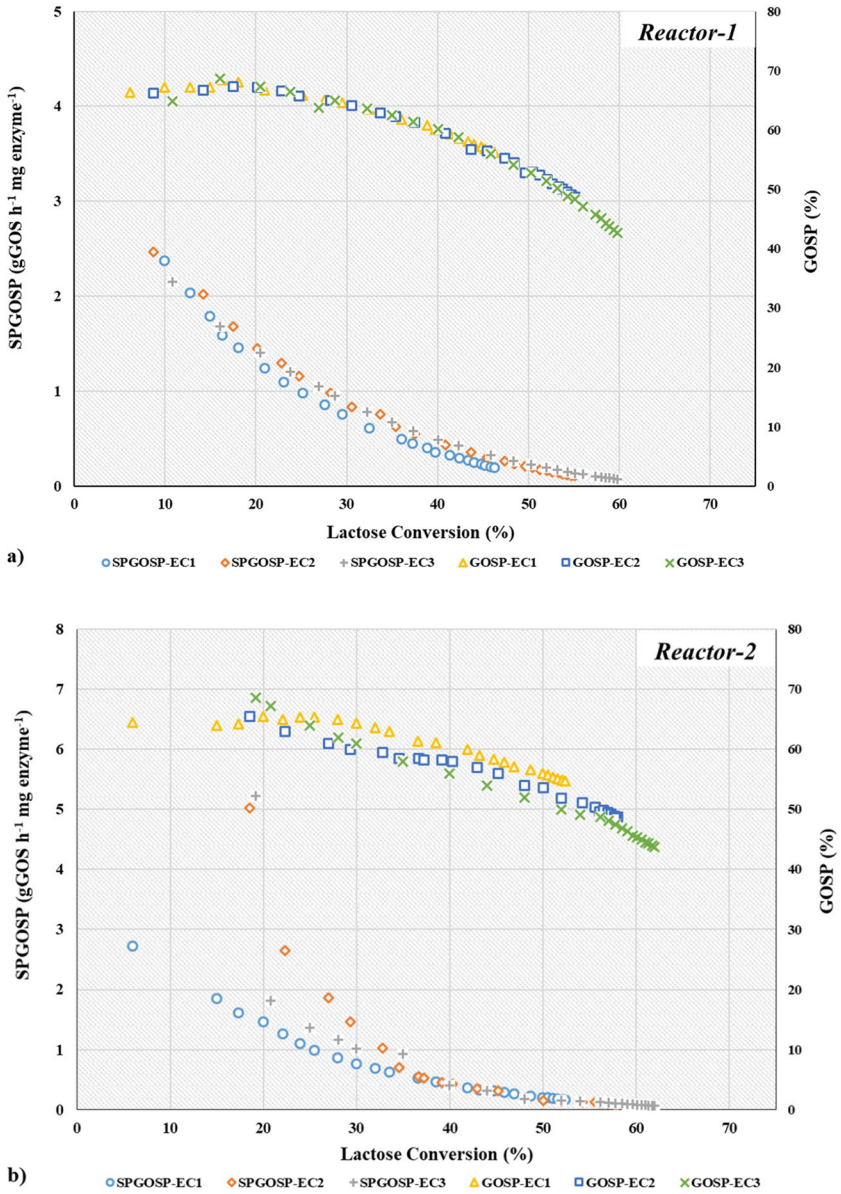


Fig. 4 Effect of enzyme concentration (EC) on galactooligosaccharide productivity (GOSP) and specific galactooligosaccharide productivity (SPGOSP) **a** for the continuous stirred batch reactor (reactor-1) and **b** for the osmotic membrane distillation integrated batch reactor (reactor-2) (Reaction conditions: temperature: 35°C, initial lactose concentration: 32°Brix, EC1: 6 gLS^{-1}, EC2: 10 gLS^{-1}, EC3: 14 gLS^{-1})

pronounced decrease after 50% lactose conversion, especially notable at T3. Conversely, in reactor-2, a slower decline in transgalactosylation activity allowed for a lower but more stable GOSP at T1 up to 40% lactose conversion (Fig. 5b). A similar GOSP trend was observed at T2 and T3, with a more rapid decline in GOSP after 40% lactose conversion at T3, suggesting sustained enzyme activity favoring hydrolysis. Given that T3 represents the enzyme's optimum operating temperature, increased enzyme activity adversely impacted the transgalactosylation/hydrolysis balance, as the enzyme tended toward hydrolysis after achieving maximum GOS synthesis. Higher temperatures may boost enzyme activity and speed up lactose conversion by providing the thermal energy needed to overcome the activation energy barrier, as stated by Sen et al. (2014).

Effect of Initial Lactose Concentration

In reactor-1, the lowest lactose concentration (LC1) not only yielded a significantly low SPGOSP but also experienced a sharp decline in the GOSP as depicted in Fig. 6a. This phenomenon is likely due to an accelerated hydrolysis rate of GOS, causing GOSP to decrease at higher lactose conversions as LC levels dropped. Elevating the initial LC from 4.5°Brix (LC1) to 18.25°Brix (LC2) markedly improved SPGOSP, a trend not mirrored at LC3 (Fig. 6a). At LC3, the enzyme's transgalactosylation activity heightened, maintaining a nearly constant GOSP rate (67%) up to 20% lactose conversion. Initially, while the enzyme exhibited high SPGOSP, this metric fell sharply as the reaction proceeded. A reduction in LC suggested that, especially in the reaction's

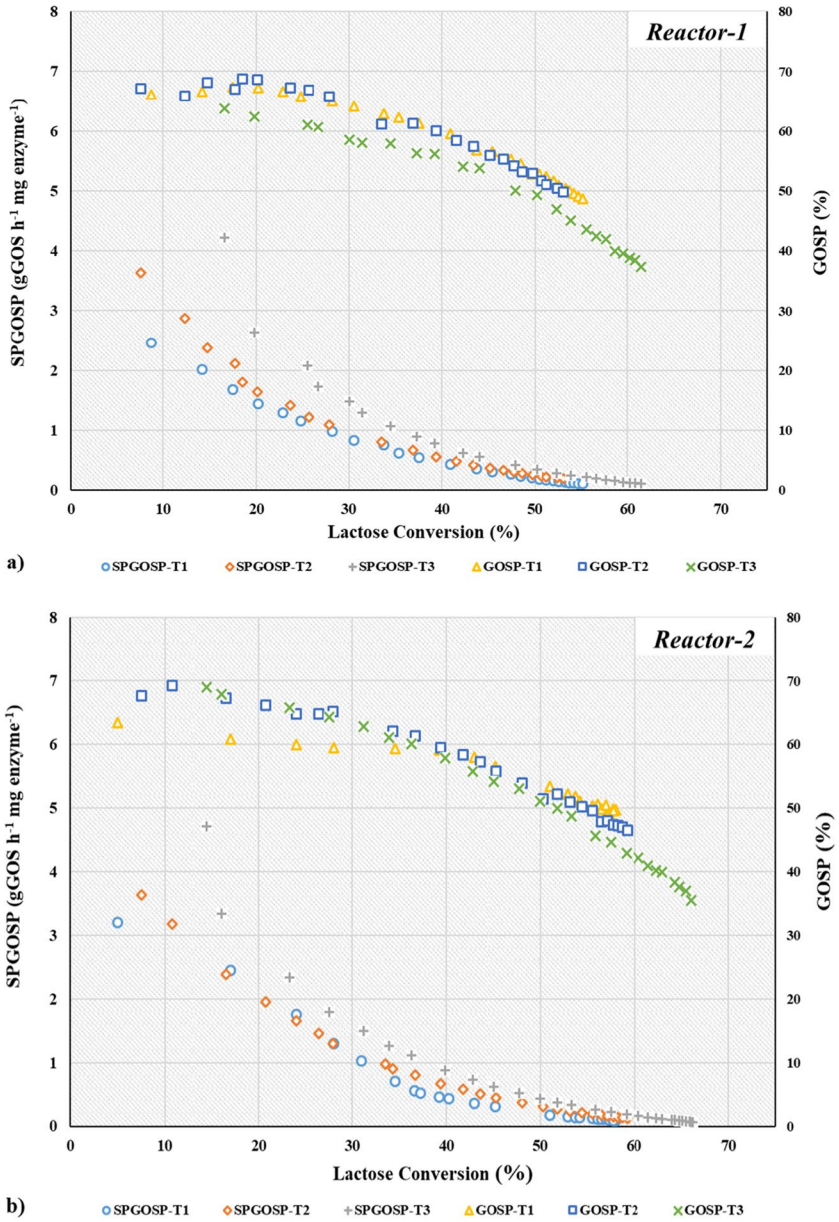


Fig. 5 Effect of temperature (T) on galactooligosaccharide productivity (GOSP) and specific galactooligosaccharide productivity (SPGOSP) **a** for the continuous stirred batch reactor (reactor-1) and **b** for the osmotic membrane distillation integrated batch reactor (reactor-2) (Reaction conditions: enzyme concentration: 10 gLS^{-1}, initial lactose concentration: 32°Brix, T1: 35°C, T2: 45°C, T3: 55°C)

initial phases, the enzyme's hydrolysis activity overwhelmed its transgalactosylation activity (Fig. 6a, LC1), leading to a swift GOSP reduction. Similar to our results, a preference for the transgalactosylation reaction over lactose hydrolysis with an increase in LC was noted by Sen et al. (2014). Furthermore, it was reported by Jovanovic-Malinovska et al. (2012) that at low LC, the hydrolysis/transgalactosylation rate escalates due to a diminished presence of glycosyl acceptors, resulting in higher levels of glucose and galactose but lower levels of GOS.

Only LC2 and LC3 were further investigated in reactor-2 due to the minimum SPGOSP level and the dramatic decrease in GOSP observed at LC1. In reactor-2, raising the initial LC from 18.25°Brix (LC2) to 32°Brix (LC3) significantly enhanced SPGOSP, an effect not observed in reactor-1. This outcome underscores the reactor-2's advantageous impact, particularly at higher initial LC levels, an effect consistent throughout the enzymatic process. As shown in Fig. 6-b, a nearly linear decline in GOSP at LC2 was noted, whereas LC3 demonstrated a more gradual GOSP decrease, maintaining stability up to 28% lactose conversion, averaging 67% GOSP.

Figure 7 reveals that in both reactors, LC diminished within the first 60 min of the reaction, albeit more slowly in reactor-2 due to the OMD process facilitating water removal. Beyond 60 min, LC continued to fall in reactor-1, whereas it

gradually increased in reactor-2, benefiting from the cumulative concentration effect of the OMD process. Hence, the OMD-induced progressive LC increase at higher lactose conversions significantly elevated GOSP values in reactor-2.

Comparative Evaluation of GOS yield in Reactor-1 and Reactor-2

In comparing the overall GOS synthesis performance of reactor-1 and reactor-2 at equivalent lactose conversion levels (Table 1), both increased enzyme concentration and reaction temperature were found to reduce reaction time due to enhanced enzyme activity. However, in reactor-1, heightened enzyme activity did not positively influence GY at the LC2. These findings suggest a shift in activity balance towards hydrolysis over transgalactosylation. Chockchaisawasdee et al. (2005) highlighted that GY is closely linked to the initial LC, with transgalactosylation being favored at higher initial LCs due to the kinetics of the reaction. Similarly, Botelho-Cunha et al. (2010), employing initial LCs of 150 g L^{-1} and 300 g L^{-1} in a bioreactor supplemented with nanofiltration, found that GY remained unchanged at lower initial LCs. Our study, however, shows that integrating a batch reactor at EC2 with the OMD process slightly improved GY, especially compared to operations at EC3 (Table 1). Moreover, enzymatic synthesis in reactor-2 required less time to reach the same lactose conversion than in reactor-1, except for the experimental set at LC2, T3, and EC3. Córdova et al. (2016) reported that lactose conversion was expedited in a membrane-assisted system, achieving

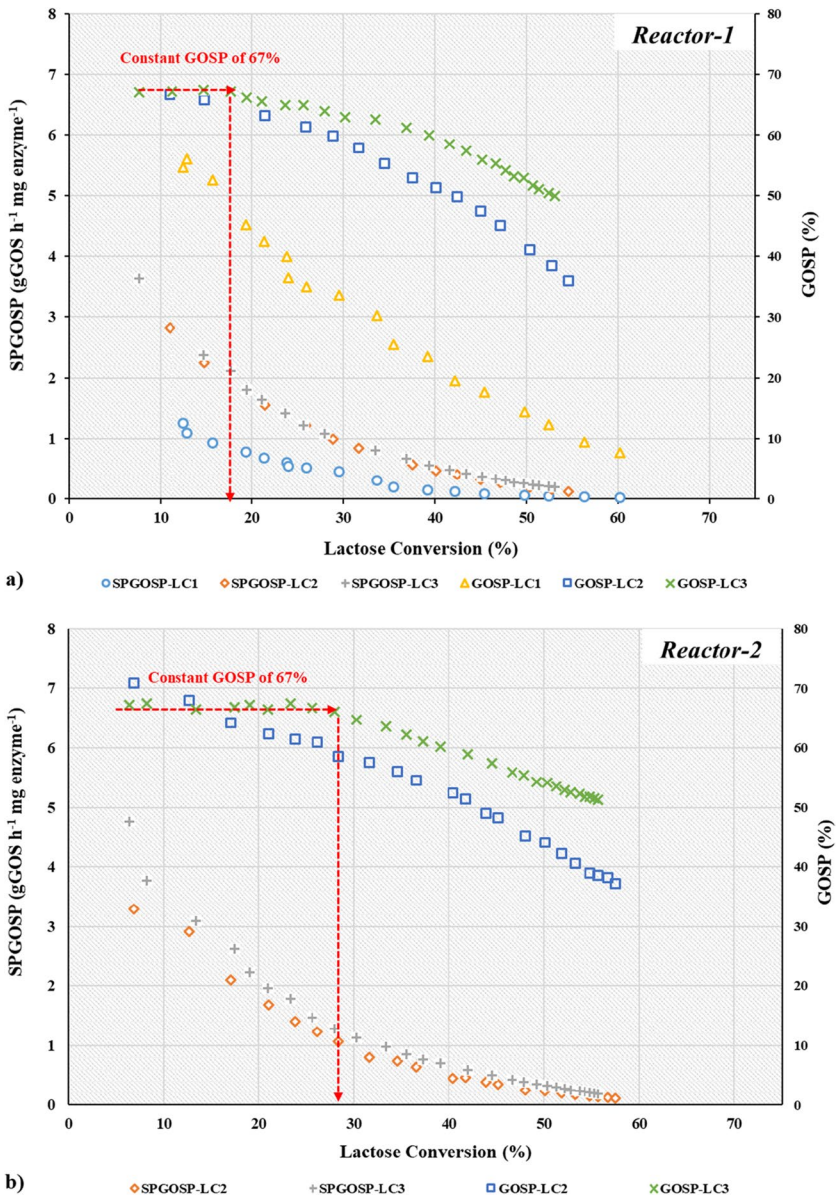


Fig. 6 Effect of initial lactose concentration (LC) on galactooligosaccharide productivity (GOSP) and specific galactooligosaccharide productivity (SPGOSP) **a** for the continuous stirred batch reactor (reactor-1) and **b** for the osmotic membrane distillation integrated batch reactor (reactor-2) (Reaction conditions: temperature: 45 °C, enzyme concentration: 6 $gLS^{-}.^{1}$, LC1: 4.5°Brix, LC2: 18.25°Brix, LC3: 32°Brix)

maximum GY more rapidly due to reduced inhibition from galactose removal. However, in this study, only water was selectively removed from the reaction medium in reactor-2, yet GY still increased despite the accumulation of galactose and glucose to critical levels. This suggests that concentrating the reaction medium through OMD likely enhanced enzyme–substrate interactions. When EC3 and T3 were used together in reactor-2, enzyme activity decreased, necessitating an additional 15 min compared with reactor-1 (Table 1). This might be related with the reaching inhibitory levels of monosaccharide concentrations. It has been reported that while glucose and galactose act as an activator of transgalactosylation activity at moderate concentrations, it act as an inhibitor at concentrations exceeding 200 mM (Vera et al., 2011). It's also noted that GY values, catalyzed by β-galactosidase from *A. oryzae*, did not exceed 28% (w/w) in literature, with most studies reporting maximum GYs around 25% (Illanes et al., 2016). Nonetheless, the reactor-2 slightly enhanced GOS efficiency, showcasing the beneficial impact of increasing lactose concentration via OMD, particularly at higher EC and temperature levels, which likely limited hydrolysis through gradual substrate concentration increases.

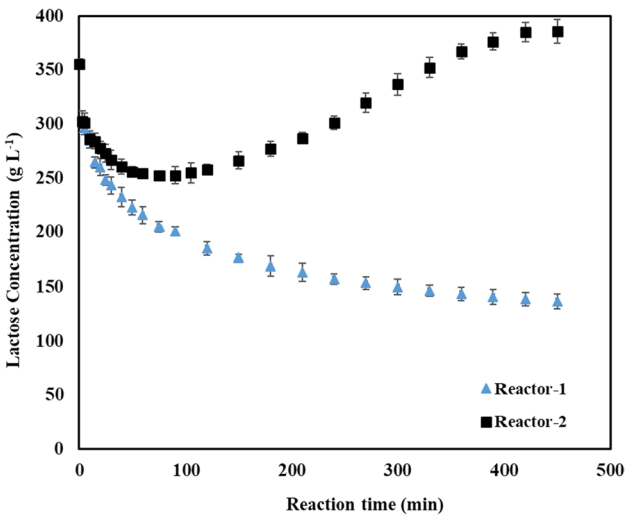


Fig. 7 The time-dependent lactose concentration during enzymatic GOS synthesis in the continuous stirred batch reactor (reactor-1) and in the osmotic membrane distillation integrated batch reactor (reactor-2)

Figure 8a highlights the remarkable impact of integrating enzymatic GOS synthesis with the OMD process, particularly on GOS-4 production, though the effect on GOS-3 was limited. Despite this, reactor-2 consistently produced higher amounts of GOS-3 than reactor-1 throughout the process. The gradual decline of produced GOS-3 in both reactors after 120 min of reaction is attributed to its use as substrate material by the enzyme for GOS-4 formation. This trend persisted up to 300 min of reaction; however, further time did not enhance GOS-4 production in reactor-1, as both GOS-4 and GOS-3 diminished due to predominant hydrolysis. In contrast, GOS-4 in reactor-2 continued to rise with increasing reaction time, indicating that gradual water removal by OMD predominantly favored long-chain oligosaccharide production. The use of GOS-3 as galactosyl acceptors in reactor-2 facilitated more GOS-4 formation, confirming a more selective transgalactosylation activity by the enzyme compared with reactor-1. Boon et al. (2000) noted the influence of reaction parameters on the polymerization degree of produced GOS, finding that increased LC led to longer GOS formations beyond GOS-3 with β-galactosidases from *B. circulans* and *A. oryzae*. Given that prebiotic activity increases with chain length

Table 1 Effect of reactor types and reaction conditions on galactooligosaccharide yield (GY) based on the same lactose conversion (LCON)

T (°C)	LC (°Brix)	EC (U/g LS)	R1 GY (%)	R2 GY (%)	R1/R2 LCON (%)	R1/R2 Time (min)	Change in GY (%)	*p* value Prob > F
35	18.25	6	22.03 ± 0.04	23.34 ± 0.01	49/49	300/240	5.9	0.0003
55	18.25	6	19.16 ± 0.16	20.59 ± 0.19	55/55	270/180	7.5	0.015
35	18.25	10	20.75 ± 0.13	22.20 ± 0.14	55/55	240/210	7.0	0.009
45	18.25	10	19.90 ± 0.13	20.66 ± 0.30	55/55	180/120	3.8	0.082
55	18.25	14	17.95 ± 0.16	18.78 ± 0.33	56/56	90/105	4.6	0.083
35	32	6	25.96 ± 0.09	28.40 ± 0.33	53/53	480/420	9.4	0.009
35	32	10	26.88 ± 0.06	27.92 ± 0.11	55/55	450/420	3.9	0.008
35	32	14	26.60 ± 0.14	27.28 ± 0.90	53/53	240/210	2.6	0.030
45	32	14	22.98 ± 0.59	26.11 ± 0.05	61/61	450/360	13.6	0.018
55	32	10	22.34 ± 0.79	26.82 ± 0.11	62/62	450/420	20.1	0.016
45	32	6	26.15 ± 0.42	27.64 ± 0.13	53/53	450/390	5.7	0.041

LC initial lactose concentration, *T* temperature, *EC* enzyme concentration, *R1* the batch reactor (reactor-1), *R2* the osmotic membrane distillation integrated reactor (reactor-2)

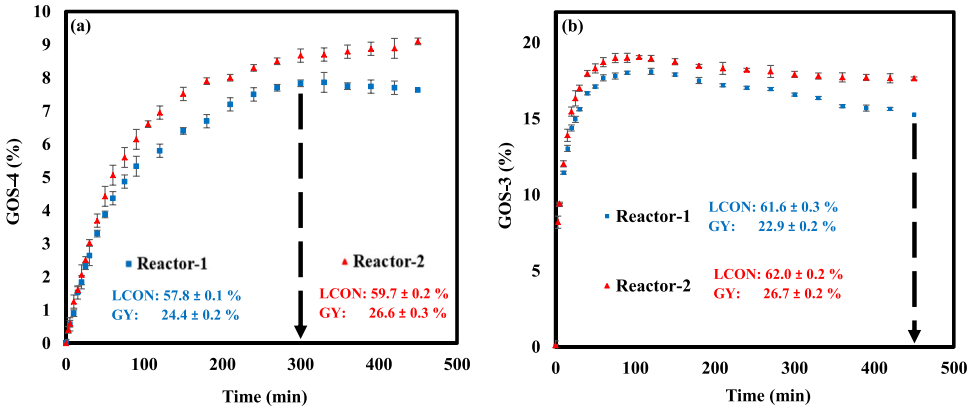


Fig. 8 The time dependent composition (%) of **a** GOS-4 and **b** GOS-3 during the enzymatic synthesis in the continuous stirred batch reactor (reactor-1) and in the osmotic membrane distillation integrated batch reactor (reactor-2)

(Rastall et al., 2016), the outcomes from reactor-2 are of significant interest from a prebiotic perspective.

The influence of OMD separation on GOS formation became more pronounced after 90 min, attributed to the halting reduction in LC (Fig. 7). This effect became increasingly evident as the reaction progressed. Although the overall GY difference between the two reactors was marginal at 300 min (Fig. 8a), it became more pronounced in favor of reactor-2 at 450 min (Fig. 8b), highlighting the cumulative concentration effect of OMD on LC and its role in preventing the enzyme from favoring hydrolysis, as seen in reactor-1. The maximum yields for GOS of varying chain lengths are typically achieved at different stages of the synthesis process. The significant impact of reaction time on the polymerization degree of GOS mixtures, where trisaccharides form first, followed by tetrasaccharides and higher oligosaccharides, due to trisaccharides serving as galactosyl receptors for subsequent oligosaccharide formation (Osman et al., 2016).

Conclusion

Osmotic membrane distillation was successfully integrated into enzymatic GOS synthesis within a continuously stirred batch reactor. This innovative reactor design (reactor-2) not only achieved higher GY than the non-integrated system (reactor-1) but also reduced the reaction times required to reach the same lactose conversion levels. From an industrial standpoint, the integrated reactor shows significant promise for enhancing the concentration of synthesized reaction products and achieving high GOS yields. Particularly noteworthy is the reactor's ability to maintain initial lactose concentrations at elevated levels, substantially benefiting the formation rate of GOS-4, especially over extended reaction durations. The evaporation flux in the OMD process, influenced by both concentration gradient and temperature, highlighted the feasibility of tailoring GOS synthesis through precise control over reaction conditions and enzyme activity to match the desired composition of the reaction medium. Consequently, the integrated reactor system outperformed its non-integrated counterpart in terms of enzymatic GOS synthesis efficiency and the concentration of the reaction medium in a single step. To fully understand the integrated system's beneficial impact on GOS productivity, further analysis of its influence on enzyme kinetics is warranted. A deeper kinetic evaluation could pave the way for innovative strategies, such as employing immobilized enzymes and mitigating monosaccharide inhibition, to enhance the outcomes of this study.

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Author Contribution Kadir Cinar: conceptualization, investigation, experimentation, documentation, data analysis, writing, review and editing; Haci Ali Gulec: resources, experimentation, technical assistance, writing, manuscript review; Pelin Onsekizoglu Bagci: experimentation, manuscript review; Ufuk Bagci: review and editing, drawing, technical assistance; Gurbuz Gunes: supervision; manuscript review.

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Data Availability Data will be made available on request.

Declarations

Competing Interests The authors declare no competing interests.

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