Kinetic Study on Transfructosylation by β-Fructofuranosidase from Aspergillus niger ATCC 20611 and Availability of a Membrane Reactor for Fructooligosaccharide Production

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Transfructosylation catalyzed by fructooligosaccharide-producing β-fructofuranosidase from Aspergillus niger ATCC 20611 was kinetically studied and a reaction model of transfructosylation was proposed. Kinetic parameters (Vmax, Km, and Ki) were determined from experimental data on the transfructosylation rates at various substrate concentrations with and without addition of glucose. Transfructosylation reaction was found to be inhibited non-competitively (Ki=0.12 mol·L−1) by glucose. Simulation results in sugar composition due to the action of a β-fructofuranosidase were in good agreement with the experimental data. In order to get higher reaction conversion with a simultaneous removal of glucose, a membrane reactor system was developed using nano-filtration membrane, through which glucose permeated but sucrose and fructooligosaccharides did not permeate. Fructooligosaccharide percentage of the reaction product was increased to above 90%, which was much higher than that of the batch reaction product (55–60%) and comparable to that of the chromatography processed product. The membrane reactor system will be applicable for production of fructooligosaccharides.

Keywords: fructooligosaccharides, transfructosylation, membrane reactor, kinetics

Fructooligosaccharides (FOS) are short-chain fructans with a terminal or in-chain glucose moiety and are found in many plant species, such as onion, edible burdock, asparagus and others (Becker et al., 1977; Shiomi & Izawa, 1986; Darbyshire & Henry, 1981). A mixture of FOS, namely 1-kestose (GF2), nystose (GF3) and 1F-fructofuranosylnystose (GF4), is commercially produced from sucrose through the transfructosylation of an enzyme from Aspergillus niger ATCC 20611 by Meiji Seika Kai- sha, Ltd., Tokyo (Neosugar G and Neosugar P).

FOS possess useful physico-chemical and physiological properties, which make them widely applicable to food and feed stuffs. They are stable at neutral pH and at temperatures up to 140°C, and have a good quality sweetness. Because FOS are non-digestible, after ingestion they pass through the small intestine, where they are selectively utilized by bifidobacteria of the intestinal microflora (Hidaka et al., 1986). Many studies have shown that FOS relieve constipation, improve blood lipid composition in hyperlipidaemia (Yamashita et al., 1984), enhance calcium and magnesium absorption (Ohta et al., 1995), and suppress the production of intestinal putrefactive substances in both animals and humans (Hidaka et al., 1991). These indicate the usefulness of FOS as a healthy ingredient of foods and feeds.

Hidaka et al. (1988) reported that Aspergillus niger ATCC 20611 was selected as the most suitable strain for FOS production. This strain showed high productivity of β-fructofuranosidase, of which transfructosylation activity was much higher than hydrolysis activity. Hirayama et al. (1989) reported purification and properties of β-fructofuranosidase from this strain. The molecular weight of the enzyme was 3.4×105 by gel filtration. The optimum pH was 5.0–6.0 and the optimum temperature was 50–60°C. The enzyme catalyzed almost exclusively transfructosylation reaction in 50 wt% sucrose solution to produce a mixture of FOS and glucose.

FOS have been produced commercially using a conventional batch system as follows. The enzyme is added to 50–60 wt% sucrose solution at pH 5.5–6.0. After stirring for 4–20 h at 50–60°C, the reaction mixture containing FOS, glucose and residual sucrose is heated to 90°C for 30 min in order to deactivate the enzyme. The reaction mixture is cooled below 50°C, clarified by filtration and deionized by ion-exchange resin column. The purified reaction mixture is concentrated to 75 wt% by evaporation. The product is called Neosugar G, and is a liquid sweetener. Percentages of FOS and residual sucrose in saccharide composition of Neosugar G are 55–60% and 10–12%, respectively. Neosugar P is produced from Neosugar G by removing glucose and residual sucrose using a simulated moving-bed chromatographic separator. FOS percentage of Neosugar P is above 95%. Considering the high cost of chromatographic separation for Neosugar P investigation of other feasible production methods such as a membrane reactor system is required.

In this paper, first kinetics of transfructosylation reaction by β-fructofuranosidase from Aspergillus niger ATCC 20611 were studied. Purified β-fructofuranosidase was used for determining kinetic parameters. Computer simulation of saccharide composi-

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tion changes through a batch reaction was done and compared with the experimental results. Finding that transfructosylation was inhibited by glucose, a membrane reactor system for FOS production was investigated using nanofiltration membrane, which would reject sucrose and fructooligosaccharides.

Materials and Methods

Chemicals The mixture of FOS used in this study was Neosugar G (Meiji Seika Kaisha, Ltd., Tokyo). The saccharide composition of Neosugar G was as follows; glucose (30.1%), sucrose (10.7%), GF2 (26.7%), GF3 (27.4%), and GF4 (5.1%). GF2 and GF3 were further purified from a mixture of FOS by preparative liquid chromatography and recrystallization. Each purity was as follows; GF2 (99.1%) and GF3 (98.0%).

Enzyme purification β-Fructofuranosidase was purified from an A. niger ATCC 20611 culture by the following modified method (Hirayama et al., 1989). The culture was centrifuged at 5°C and the supernatant containing extracellular β-fructofuranosidase was concentrated through a hollow fiber ultrafiltration (UF) membrane (molecular weight cut off, 104; poly-sulfone, Asahi Chemical Industry Co. Ltd., Tokyo). Solid calcium acetate was added to a crude concentrate up to 4% saturation. After standing overnight, precipitates were removed by centrifugation. The supernatant was concentrated to twice the original concentration by UF, which was further purified by gel filtration column (TOYOPEARL HW-65S, Tosoh Co. Ltd., Tokyo).

Analytical method Enzyme activity and protein were measured by the method of Hirayama et al. (1989). One unit was defined as the amount of enzyme required to produce 1 mmol of 1-kestose per min from 10% (w/v) sucrose at 40°C in 40 mmol-l−1 McIlvaine buffer (pH 5.0). The initial rate of transfructosylation was determined from the first reaction data up to 5% conversion at 55°C in 40 mmol-l−1 McIlvaine buffer (pH 6.0) for initial substrate (sucrose, GF2, and GF3) concentrations varying 0.05–1.0 mol-l−1. The inhibitory constant Ki was also determined under the above conditions with and without addition of glucose.

Saccharide weight composition was measured by high performance liquid chromatography (HPLC) with a finely packed SIL-NH2 column (Kanto Kagaku Co. Ltd., Tokyo) and a detector RID-300 (Nihon Bunko Co. Ltd., Tokyo). A typical HPLC chromatogram for a mixture of glucose, sucrose and FOS (GF2, GF3, and GF4) is shown in Fig. 1.

Membrane screening test Membranes used in this study are shown in Table 1. Membranes of NTR-7410, NTR-7450, NTR-7250 and NTR-729HF were obtained from Nitto Denko Co. Ltd., Tokyo and membrane of NF-45 was obtained from Sanko Shokai Co. Ltd., Tokyo. The screening test was as follows. Several nanofiltration membranes were cut into circular discs (diameter, 75 mm; effective membrane area, 32 cm2), and fitted into a membrane cell (Model: C40-B, Nitto Denko Co. Ltd). The cell was placed on a magnetic stirrer and the magnetic spin bar fitted into the cell provided the agitation. The cell and magnetic stirrer were placed in a thermostatically controlled incubator. The pressure, temperature and stirrer spin bar speed were maintained at 4 MPa, 40°C and 400 rpm, respectively. The unit was operated in batch mode by charging the cell with 100 g of Neosugar G, which was diluted to 7.5 wt% in 40 mmol-l−1.

Fig. 1. HPLC chromatogram for a mixture of glucose, sucrose and FOS. A mixture of glucose and FOS was Neosugar G (Meiji Seika Kaisha, Ltd., Tokyo). Peak (1), glucose; (2), sucrose; (3), 1-kestose (GF 2); (4), nystose (GF 3); (5), 1-Fructofuranosyl-nystose (GF 4). HPLC condition: column, SIL-NH2; mobile phase, 72% (v/v) acetonitrile, 28% (v/v) acetonitrile, 28% (v/v) H2O; flow rate, 1.0 ml/min; detector, RI; temperature, 40°C.

Membrane screening test Membranes used in this study are shown in Table 1. Membranes of NTR-7410, NTR-7450, NTR-7250 and NTR-729HF were obtained from Nitto Denko Co. Ltd., Tokyo and membrane of NF-45 was obtained from Sanko Shokai Co. Ltd., Tokyo. The screening test was as follows. Several nanofiltration membranes were cut into circular discs (diameter, 75 mm; effective membrane area, 32 cm2), and fitted into a membrane cell (Model: C40-B, Nitto Denko Co. Ltd). The cell was placed on a magnetic stirrer and the magnetic spin bar fitted into the cell provided the agitation. The cell and magnetic stirrer were placed in a thermostatically controlled incubator. The pressure, temperature and stirrer spin bar speed were maintained at 4 MPa, 40°C and 400 rpm, respectively. The unit was operated in batch mode by charging the cell with 100 g of Neosugar G, which was diluted to 7.5 wt% in 40 mmol-l−1.

Table 1. Rejections of glucose, sucrose and fructooligosaccharides on various membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Material</th>
<th>Rejection of NaCl (%)</th>
<th>Permeate flux (10−6 m3·m−2·s−1)</th>
<th>Rejection of saccharides (%)</th>
<th>glucose</th>
<th>sucrose</th>
<th>GF2</th>
<th>GF3</th>
<th>GF4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-450</td>
<td>PA</td>
<td>45</td>
<td>65</td>
<td>74 98 &gt;99 &gt;99 &gt;99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR-7410</td>
<td>SPES</td>
<td>15</td>
<td>23</td>
<td>1 3 5 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR-7450</td>
<td>SPES</td>
<td>51</td>
<td>23</td>
<td>1 2 14 12 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR-7250</td>
<td>PVA</td>
<td>60</td>
<td>9</td>
<td>75 97 &gt;99 &gt;99 &gt;99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR-729HF</td>
<td>PVA</td>
<td>92</td>
<td>7</td>
<td>91 93 &gt;99 &gt;99 &gt;99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aPA, polyamide; SPES, sulfonic polyether sulfone; PVA, polyvinyl alcohol.
bSanko Shokai Co. Ltd., Tokyo.
cNitto Denko Co. Ltd., Tokyo.
dRejection of NaCl is catalogue value.
ePermeate flux was measured using 7.5 wt% of diluted Neosugar G at pressure of 4MPa, 55°C.
zyme purification. The specific activity of crude enzyme was 71
outlet pressures of the module, and
membrane pressure was determined as
calculation pump (RO/UF D10, Sanko Shokai Co., Ltd., Tokyo).
re-circulated from the tank to the membrane module using a cir-
fructosylation reaction occurred in the tank. The reactants were
of 2.5–25 units per gram of substrate were employed. The trans-
membrane pressure of 4.0 MPa. For enzyme addition, activities
nosidase were supplied to a reaction tank (volume, 10
contrast, GF3 and GF4 percentages increased gradually. Thus,
maximum of 41–43% after 1.5 h, then decreased below 25%, in
FOS percentage increased to 55–60%. Glucose percentage also
Results and Discussion
of the reactants and the permeates were measured by HPLC.

Substrate solution (30 wt% sucrose, pH 6.0) and β-fructofuranosidase were supplied to a reaction tank (volume, 10 l) at trans-
membrane pressure of 4.0 MPa. For enzyme addition, activities
were re-circulated from the tank to the membrane module using a cir-
culation pump (RO/UF D10, Sanko Shokai Co., Ltd., Tokyo).
The membrane system was controlled at 55°C. The trans-
membrane pressure was determined as
\[ P_t = \frac{(P_i + P_o)/2 - P_p}{P_i + P_o} \]
where \( P_t \) is the trans-membrane pressure, \( P_i \) and \( P_o \) inlet and outlet pressures of the module, and \( P_p \) the permeate pressure (almost equal to atmospheric pressure). Saccharide compositions of the reactants and the permeates were measured by HPLC.

Table 2. Purification of β-fructofuranosidase from Aspergillus niger ATCC 20611.

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Total activity (U)</th>
<th>Total protein (mg)</th>
<th>Specific activity (^{a}) (U/mg protein)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>1600</td>
<td>1.87×10^5</td>
<td>26400</td>
<td>71±3</td>
<td>100</td>
</tr>
<tr>
<td>Calcium acetate</td>
<td>895</td>
<td>1.56×10^5</td>
<td>8440</td>
<td>185±12</td>
<td>83</td>
</tr>
<tr>
<td>Anion exchange column(^{b})</td>
<td>800</td>
<td>1.45×10^5</td>
<td>640</td>
<td>2269±49</td>
<td>76</td>
</tr>
<tr>
<td>Gel filtration column(^{c})</td>
<td>1358</td>
<td>1.45×10^5</td>
<td>548</td>
<td>2646±66</td>
<td>76</td>
</tr>
</tbody>
</table>

\(^{a}\)Values are means±SE, \( n=3 \).
\(^{b}\)DEAE-TOYOPEARL, Tosoh Co. Ltd., Tokyo.
\(^{c}\)TOYOPEARL HW-65s, Tosoh Co. Ltd., Tokyo.

Increased gradually, and reached 30%. Fructose percentage was negligible because hydrolysis reactions of sucrose and FOS did not occur under these conditions. For production of Neosugar G', the reaction is stopped when the concentration of GF2 becomes equal to that of GF3. In the present batch reaction, the required time was about 4 h.

Experiments for kinetics The concentration effects of sucrose, GF2, and GF3 on the initial rate of transfructosylation are shown in Fig. 4. The rates increased with the increase of sub-

Fig. 3. Simulation of changes of saccharide composition by action of β-
fructofuranosidase. Curves are simulation results. Conditions: enzyme activity, 12.5 U·g\(^{-1}\) sucrose; initial sucrose concentration, 50 wt%; temperature, 50°C; pH, 6.0. Symbols: ○, glucose; △, sucrose; ×, GF2; ▼, GF3; ▲, FOS.

Fig. 4. Initial transfructosylation rates as a function of substrate concentration. Curves are simulation results. Symbols: ○, sucrose; △, GF2; ▼, GF3.
substrate concentration. Sucrose gave the highest transfructosylation rate among three substrates, and GF₃ gave the lowest.

The effects of substrate concentration (0.05–0.75 mol·l⁻¹) and glucose addition (0–0.3 mol·l⁻¹) on the initial transfructosylation rate are shown in Fig. 5 (substrate, sucrose) and Fig. 6 (substrate, GF₃). Initial transfructosylation rates in both cases were significantly affected by glucose addition. For example, the initial transfructosylation rate, at initial glucose concentration of 0.3 mol·l⁻¹, decreased to less than half that without glucose addition.

Mathematical model for transfructosylation reaction

A reaction model for transfructosylation is proposed:

\[
\begin{align*}
\text{GF} + \text{GF} & \rightarrow \text{GF}₂ + \text{G}, \\
\text{GF}₂ + \text{GF}₂ & \rightarrow \text{GF}₃ + \text{GF}_2, \\
\text{GF}₃ + \text{GF} & \rightarrow \text{GF}₄ + \text{GF}_2, \\
\text{GF} + \text{GF}₂ & \rightarrow \text{GF}₃ + \text{G}, \\
\text{GF}₂ + \text{GF}₂ & \rightarrow \text{GF}₃ + \text{G}, \\
\end{align*}
\]

where GF is sucrose, G glucose, GF₂ 1-kestose, GF₃ nystose, GF₄ 1-fructofuranosyl nystose, and r₁, r₂, r₃, r₄, r₅ and r₆ respective transfructosylation rates. This model assumes that hydrolysis reactions of GF, GF₂, GF₃, and GF₄ do not occur, and formation of GF₅ is negligible (Hirayama et al., 1989).

Mass balance equations for transfructosylation reaction of each component derived from Eqs. (1)–(6) can be as follows:

\[
\begin{align*}
\frac{d[G]}{dt} &= r₁ + r₅ - r₄, \\
\frac{d[GF]}{dt} &= r₆ - r₄ - r₃ - r₂, \\
\frac{d[GF₂]}{dt} &= r₅ - r₃ - r₂, \\
\frac{d[GF₃]}{dt} &= r₄ - r₃ - r₁, \\
\frac{d[GF₄]}{dt} &= r₆ - r₅ - r₄, \\
\end{align*}
\]

where [G], [GF], [GF₂], [GF₃] and [GF₄] are the concentration of glucose, sucrose, GF₂, GF₃, and GF₄, respectively.

Assuming a two-substrate random bi-bi model (Price & Stevens, 1989), rate equations for Eqs. (1)–(3) are obtained as:

\[
\begin{align*}
V_m &= \frac{V_{m₁}[GF] \cdot [GF₂] \cdot [GF]}{K_m₁ + [GF] + [GF₂] + [GF]²}, \\
V_m &= \frac{V_{m₂}[GF] \cdot [GF₂] \cdot [GF₃] \cdot [GF]}{K_m₂ + [GF] + [GF₂] + [GF]²}, \\
V_m &= \frac{V_{m₃}[GF] \cdot [GF₃] \cdot [GF₄] \cdot [GF]}{K_m₃ + [GF] + [GF₃] + [GF]²}, \\
\end{align*}
\]

where [E], [E-GF], [E-GF₂-GF], [E-GF₃-GF₂], [E-GF₄-GF₃] and [E-GF₂-GF₂] are concentrations of enzyme-sucrose complex, enzyme-sucrose-1-kestose complex, enzyme-sucrose-1-fructofuranosyl nystose complex, enzyme-sucrose-1-fructofuranosyl sucrose complex, enzyme-sucrose-1-fructofuranosyl sucrose complex, respectively. Vₘ and Kₘ are maximum transfructosylation rate and dissociation constants for Eqs. (12)–(20), respectively. Kₘ denotes dissociation constant for enzyme-substrate complex and Kₙ denotes dissociation constant for enzyme-substrate-substrate complex.

Rate equations for Eqs. (4)–(6) are also obtained by two-substrates random bi-bi model as follows:

\[
\begin{align*}
V_m &= \frac{V_{m₁}[GF] \cdot [GF] \cdot [GF₃] \cdot [GF₄] \cdot [GF₅]}{K_m₁ + [GF] + [GF₂] + [GF]²}, \\
V_m &= \frac{V_{m₂}[GF] \cdot [GF₂] \cdot [GF₃] \cdot [GF₄] \cdot [GF₅]}{K_m₂ + [GF] + [GF₂] + [GF]²}, \\
V_m &= \frac{V_{m₃}[GF] \cdot [GF₃] \cdot [GF₄] \cdot [GF₅]}{K_m₃ + [GF] + [GF₃] + [GF]²}, \\
\end{align*}
\]

where [E-GF₂-GF], [E-GF₃-GF₂], [E-GF₄-GF₃] and [E-GF₄-GF₃] are concentrations of enzyme-sucrose-GF₂ complex, enzyme-GF₂-sucrose complex, enzyme-sucrose-GF₃ complex, enzyme-GF₃-sucrose complex, enzyme-GF₂-GF₂ complex and enzyme-GF₂-GF₂ complex, respectively.
Estimation of kinetic parameters. The effect of substrate concentration on the initial rate of transfructosylation given in Eqs. (1)-(3) is shown in Fig. 4. Based on the experimental data and Eqs. (12)-(20), the maximum transfructosylation rates \( V_m \) and dissociation constants \( K_m \) were estimated using the Gauss-Jordan method as shown in Table 3. As shown in Fig. 4, simulation and experimental data are in good agreement. The case in GF1 gave the smallest \( V_m \), which was almost half those of sucrose and GF2. The case in sucrose gave the smallest \( K_m \) and \( K_{i,m} \). As the number of fructose units in the substrate increased, \( V_m \) decreased and \( K_m \) increased. These indicate that interaction between enzyme and substrate may decrease with the increase in number of fructose units. These results agreed with those of Duan and Sheu (1994), although a simple Michaelis-Menten model was applied to the transfructosylation.

Inhibitory effect of glucose. Transfructosylation reaction was found to be inhibited by glucose as shown in Fig. 5. The inhibition mechanism was investigated by comparing the experimental results with competitive or non-competitive inhibition model.

\[
\begin{align*}
\text{(Competitive inhibition)} & \\
E+GF & \rightarrow E\cdot GF \\
E\cdot GF+GF & \rightarrow E\cdot GF\cdot GF+G \\
E+G & \rightarrow E\cdot G
\end{align*}
\]

\[
\begin{align*}
K_{i,m}\cdot GF \cdot G & \rightarrow K_{i,m}\cdot GF\cdot GF \\
K_{i,n}\cdot GF \cdot G & \rightarrow K_{i,n}\cdot GF\cdot GF
\end{align*}
\]

Where \( K_{i,m} \) and \( K_{i,n} \) are competitive inhibitory and non-competitive inhibitory constants, respectively.

The experimental data were analyzed by two kinetic models with the competitive (Eq. (41)) and non-competitive (Eq. (48)) inhibitory effects of glucose. The curve obtained from Eq. (48), the non-competitive inhibitory model, showed good agreement with the experimental data; however, the curve obtained from Eq. (41), the competitive inhibitory model did not agree well with the experimental data. Non-competitive inhibitory constant \( K_{i,n} \) could be estimated from the experimental data using \( V_m \), \( K_{i,m} \) and \( K_{i,n} \) obtained from initial transfructosylation rates without addition of glucose as given in Table 3. \( K_{i,n} \) was determined to be 0.12 mol⁻¹ as a non-competitive inhibitory constant.

Computer simulation of transfructosylation reaction. Prediction of concentration time course by the mathematical model for transfructosylation reaction is shown in Fig. 3. Best fitting kinetic parameters given in Eqs. (21)-(32) are shown in Table 4. The simulated curve showed good agreement with the experimental data, which demonstrated that the present model given in Eqs. (1)-(6) was reasonable and appropriate for transfructosylation by β-fructofuranosidase.

Membrane screening. Rejections of glucose, sucrose and FOS (GF2, GF3 and GF4) for several nanofiltration membranes are summarized in Table 1. Rejection of NaCl and permeate flux are also shown. Little rejection of glucose was observed in NTR-7410 and NTR-7450 membranes, and permeation of sucrose and FOS took place. On the other hand, almost complete rejection of FOS was observed in the membranes of NF-45, NTR-7250 and NTR-729HF; and permeate flux values for the membrane of NTR-7250 and NTR-720HF were below 10×10⁻⁶ m²·m⁻²·s⁻¹. The highest rejection of sucrose and the highest permeate flux of 65×10⁻⁶ m²·m⁻²·s⁻¹ were obtained in the NF-45 membrane (pure water permeate flux was 2.1×10⁻⁴ m²·m⁻²·s⁻¹ of 4 MPa transmembrane pressure at 25°C). From these, NF-45 membrane was found to be the best one for the membrane reactor of FOS production. HPLC chromatograms of feed and permeate solution for

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**Table 3.** Estimated rate constants of transfructosylation for Eqs. (12)-(20).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( V_m ) (µmol·l⁻¹·min⁻¹)</th>
<th>( K_{m,GF} ) (µmol·l⁻¹)</th>
<th>( K_{i,c,GF} ) (µmol·l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>508</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>GF1</td>
<td>499</td>
<td>0.15</td>
<td>0.95</td>
</tr>
<tr>
<td>GF2</td>
<td>265</td>
<td>0.35</td>
<td>1.35</td>
</tr>
</tbody>
</table>

**Table 4.** Estimated rate constants of transfructosylation for Eqs. (21)-(32).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( V_m ) (µmol·l⁻¹·min⁻¹)</th>
<th>( K_{m,GF} ) (µmol·l⁻¹)</th>
<th>( K_{i,c,GF} ) (µmol·l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>GF1</td>
<td>100</td>
<td>0.40</td>
</tr>
<tr>
<td>GF1</td>
<td>4</td>
<td>0.70</td>
<td>0.01</td>
</tr>
<tr>
<td>GF2</td>
<td>GF3</td>
<td>208</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**Table 5.** Saccharide weight compositions of Neosugar G, Neosugar P and membrane reactor product.

<table>
<thead>
<tr>
<th>Saccharide weight composition (%)</th>
<th>glucose</th>
<th>sucrose</th>
<th>GF2</th>
<th>GF3</th>
<th>GF4</th>
<th>FOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neosugar G</td>
<td>30</td>
<td>10</td>
<td>27</td>
<td>27</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Neosugar P</td>
<td>2</td>
<td>3</td>
<td>36</td>
<td>36</td>
<td>50</td>
<td>95</td>
</tr>
</tbody>
</table>

**Fig. 7.** HPLC chromatograms of feed and permeate solution.
NF-45 membrane are shown in Fig. 7, indicating that glucose permeated selectively through the membrane.

Production of FOS using the membrane reactor

Operation parameters of the membrane reactor were substrate concentration, temperature, pH, enzyme concentration, membrane area, volume of reaction tank, trans-membrane pressure and flow rate along the membrane surface. Transfructosylation rate and permeation rate of glucose were the most important factors for the membrane reactor, because transfructosylation was non-competitively inhibited by glucose. In this study, efficient removal of glucose required volume of reaction tank and area of membrane to be 10 l and 0.36 m², respectively.

Results in saccharide weight composition of Neosugar G of batch reactor product, Neosugar P and membrane reactor product are summarized in Table 5. FOS percentage in saccharide composition during transfructosylation by a tooligosaccharide-producing enzyme from Aspergillus niger was partly supported by a grant from the Bio-oriented Technology Research Advancement Institution (BRAIN), Japan.

Production of FOS using the membrane reactor

Operation parameters of the membrane reactor were substrate concentration, temperature, pH, enzyme concentration, membrane area, volume of reaction tank, trans-membrane pressure and flow rate along the membrane surface. Transfructosylation rate and permeation rate of glucose were the most important factors for the membrane reactor, because transfructosylation was non-competitively inhibited by glucose. In this study, efficient removal of glucose required volume of reaction tank and area of membrane to be 10 l and 0.36 m², respectively.

Results in saccharide weight composition of Neosugar G of batch reactor product, Neosugar P and membrane reactor product are summarized in Table 5. FOS percentage in saccharide composition during transfructosylation by a tooligosaccharide-producing enzyme from Aspergillus niger was partly supported by a grant from the Bio-oriented Technology Research Advancement Institution (BRAIN), Japan.

In summary, transfructosylation reactions catalyzed by tooligosaccharide-producing β-fructofuranosidase from Aspergillus niger ATCC 20611 were kinetically studied and a model of transfructosylation reaction was proposed. Kinetic parameters ($V_m$, $K_{mi}$, and $K_i$) were determined from the experimental data at various substrate concentrations with and without glucose. Transfructosylation was found to be inhibited non-competitively ($K_{i}=0.12$ mol·l$^{-1}$) by glucose. A simulated curve of change in saccharide composition during transfructosylation by a β-fructofuranosidase was in good agreement with the experimental data.

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