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Efficient Continuous Production of Lactulose Syrup by Alkaline Isomerization Using an Organogermanium Compound

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Abstract: Lactulose, a keto-type disaccharide widely used in pharmaceuticals and functional foods, is produced by the isomerization of lactose. The organogermanium compound poly-*trans*-[(2-carboxyethyl) germasesquioxane] (Ge-132) is an effective reaction promoter for the conversion of lactose to lactulose because of its high affinity to ketoses. Herein, an effective method for the continuous production of lactulose syrup was developed using Ge-132 through the alkaline isomerization of lactose in a bench-scale plant. This plant carried out a continuous isomerization process using Ge-132, continuous two-step separation process for separating the sugar and Ge-132, a continuous purification and concentration processes for the lactulose syrup, and separation and purification processes for the recovery of Ge-132. In this bench-scale plant, lactulose-containing syrup (350 g/L lactulose, 92 g/L lactose, and 31 g/L galactose) was prepared. The syrup was produced at a rate of 37.7 mL/h, and the content of residual Ge-132 in the syrup was 2 mg/L. The separation process was a two-step separation system requiring an ordinary electrodialyzer and an electro deionizer, which allowed the separation of more than 99.6 % Ge-132 from the reaction mixture. Moreover, the majority of Ge-132 and sodium hydroxide were recovered through electrodialysis using a bipolar membrane. The proposed system is the first to represent the novel development of an effective continuous production system for lactulose-containing syrup on the basis of the use of organogermanium compounds and incorporation of the electrodialysis technology.

Key words: organogermanium compound, poly-*trans*-[(2-carboxyethyl)germasesquioxane], lactulose, continuous production, alkaline isomerization, electrodialysis

INTRODUCTION

Lactulose, or 4-*O*-β-D-galactopyranosyl-D-fructose, is a disaccharide that is synthesized by the isomerization of lactose (4-*O*-β-D-galactopyranosyl-D-glucose), a widely available natural disaccharide. Lactulose, which has been used for the treatment of hepatic encephalopathy[1)] and constipation,[2)] is on the World Health Organization Model List of Essential Medicines,[3)] *i.e.*, a list of the most important medications required in a basic health system. Lactulose has also attracted attention from the viewpoint of prebiotics, such as in the context of improving the intestinal flora.[4)] Lactulose is currently produced by the alkaline isomerization of lactose, but the yield of this process is low (25 %) and its production efficiency is often poor due to the batch nature of the system. For this reason, the development of an efficient continuous system is necessary.[5)]

We previously reported[6)7)] that the organogermanium compound poly-*trans*-[(2-carboxyethyl)germasesquioxane] (commonly known as Ge-132) significantly promoted the isomerization of an aldose to a ketose, such as glucose to fructose, or lactose to lactulose, with an isomerization ratio of approximately 80 %. Ge-132 is a water-soluble polymer having the formula $[(GeCH_2CH_2COOH)_2O_3]_n$ and is hydrolyzed to 3-(trihydroxygermyl)propanoic acid (THGP) monomers in aqueous solution. Ge-132 remains stable under various chemical conditions and has been shown to be safe in a variety of toxicity studies.[8)9)10)11)12)13)14)15)16)17)] In addition, the co-ingestion of Ge-132 with the bifidus activating factor has been reported to stimulate intestinal immunity and increase IgA production.[18)] In terms of the isomerization mechanism, we found that the *cis*-diol moiety of ketose and the oxyanion of THGP promote the reaction on the ketose side by forming a stable complex with a molar ratio of 1:1.[6)7)] In addition, various compounds can form a complex with the *cis*-diol moiety of a sugar, including boric acid,

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Abbreviations: Ge-132, poly-*trans*-[(2-carboxyethyl)germasesquioxane]; THGP, 3-(trihydroxygermyl)propanoic acid; ED, electrodialyzer; EDI, electro deionizer; BPM, bipolar membrane; ED-BP, electrodialyzer with bipolar membrane; HPLC, high-performance liquid chromatography; AAS, atomic absorption spectrometry; CEM, cation exchange membrane; AEM, anion exchange membrane.

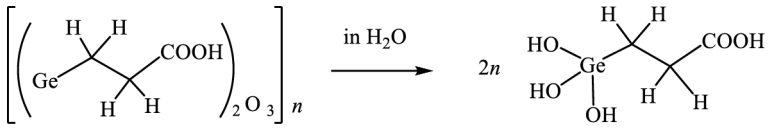

Fig. 1. Organogermanium compound Ge-132 employed herein.
Poly-*trans*-[(2-carboxyethyl)germasesquioxane] (Ge-132) was hydrolyzed to 3-(trihydroxygermyl)propanoic acid (THGP) in aqueous solution.

germanium dioxide, or sodium aluminate, and the formation of such complexes can promote the isomerization of lactose to lactulose.[19)20)21)] However, the application of these compounds in food production is considered difficult from the viewpoint of safety. Thus, we herein investigate a novel lactulose continuous production system based on the use of the reaction promotor Ge-132 due to its documented safety and chemical stability. However, as mentioned above, the batch type isomerization reaction is not practical from the viewpoint of efficiency; therefore, we examine a novel efficient continuous production method using a bench-scale plant for the production of lactulose using Ge-132.

Initially, the mixture of lactose solution and Ge-132 was heated in a reactor to promote the isomerization. Then, to separate the sugar and Ge-132 from the isomerization solution, we devised a two-step electrodialysis system involving the simultaneous use of an ordinary electrodialysis system and another one[22)23)] used for the production of ultrapure water. We then introduced a system for purifying Ge-132 using an electrodialyzer equipped with a bipolar membrane (ED-BP). As the separation of organogermanium compounds by electrodialysis has not yet been reported; therefore, we herein developed a bench-scale system for continuous lactulose syrup production based on membrane electrodialysis as the key technology. We expect this method to be an efficient and practical method for the production of lactulose, which is attracting attention as a functional disaccharide. We also aim to develop an advanced recovery technology system for rare organogermanium compounds, which will include not only Ge-132 but also other rare species.

MATERIALS AND METHODS

Reagents. The structure of the organogermanium compound Ge-132 (THGP in an aqueous solution) used in this study is shown in Fig. 1. This Ge-132 was synthesized at Asai Germanium Research Institute Co., Ltd. (Kanagawa, Japan). Lactose monohydrate (guaranteed reagent grade), sodium hydroxide (guaranteed reagent grade), and palladium matrix modifier were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). Lactulose (approximately 98 %) was purchased from Sigma-Aldrich Co., LLC (Tokyo, Japan). D-Galactose (guaranteed reagent grade) was purchased from Merck KGaA (Darmstadt, Germany). Sodium nitrate (guaranteed reagent grade) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Distilled water was prepared using an Advantec fully automatic distilled water manufacturing instrument (GSR-200; Advantec Toyo Kaisha, Ltd., Tokyo, Japan).

Alkaline isomerization reaction. Ge-132 was dissolved in distilled water to a concentration of 297 g/L and the pH was adjusted to 13 using 5 M sodium hydroxide. Lactose was dissolved in distilled water to a concentration of 300 g/L. For preparing the reaction mixture (pH 12), the lactose and Ge-132 solutions (pH 13) were mixed in a volume ratio of 2:1, to give final concentrations of 200 and 99.1 g/L, respectively, which constituted equimolar concentrations. The control solution was prepared using distilled water instead of the Ge-132 solution. All preparation steps were performed under ice-cold conditions (*i.e.*, 0 °C). The reaction mixtures (1 mL each) were incubated at 60 °C with shaking (120 rpm) for 5–60 min, during which time they were quickly placed in ice water every 5 min.

Germanium determination by atomic absorption spectrometry (AAS). The content of Ge-132 was determined by AAS using a Shimadzu AA-640-13 system equipped with a deuterium lamp background corrector, a hollow cathode lamp, and an acetylene-nitrous oxide burner. The instrumental parameters were as follows: wavelength, 265.2 nm; lamp current, 7 mA; slit width, 3.8 Å; and frame length, 10 cm. The low content of Ge-132 (< 2 ppm) was determined by Zeeman furnace AAS using a PerkinElmer 4100ZL system (PerkinElmer Japan Co., Ltd., Kanagawa, Japan) equipped with a furnace atomizer, an electrodeless discharge lamp, a Zeeman background corrector, and argon gas. The instrumental parameters were as follows: wavelength, 265.1 nm; lamp current, 280 mA; sample injection, 10 µL; and 250 mg/L palladium matrix modifier, 4 µL.

Quantification of saccharides by high-performance liquid chromatography (HPLC). The contents of lactose, lactulose, and galactose were determined by HPLC using a Shimadzu LC-10A system equipped with an LC-10AD pump (Shimadzu Corporation Co., Ltd., Kyoto, Japan), an RID-6A refractive index detector (Shimadzu Corporation), and a C-R7A integrator (Shimadzu Corporation) under the following conditions: column, Polyspher CHPB column (7.9 mm φ × 300 mm; Merck KGaA, Darmstadt, Germany); guard column, Polyspher CHPB column (4 mm φ × 50 mm; Merck KGaA); column temperature, 80 °C; mobile phase, distilled water; and flow rate, 0.4 mL/min. The sample volume applied was 10 µL. Degradation products formed in the reaction were analyzed by HPLC using a JASCO LC system equipped with an 880-PU pump (JASCO Corporation Co., Ltd., Tokyo, Japan), a UV-970 ultraviolet/visible detector, an RID-6A refractive index detector (Shimadzu Corporation), and an 807-IT integrator (JASCO) under the following conditions: column, Polyspher OAKC (7.8 mm φ × 300 mm; Merck KGaA, Darmstadt, Germany); guard column, Polyspher OAKC column (4 mm φ × 50 mm; Merck KGaA); column temperature, 40 °C; mobile phase, 1 mM phosphoric acid; flow rate, 0.4 mL/

min; and UV wavelength, 210 nm. The sample volume applied was 50 μL.

Primary separations of sugar and Ge-132 (NaOH) in the reaction mixture by electrodialysis. The sugar and Ge-132 (NaOH) present in the reacted isomerization solution were initially separated by electrodialysis. The electrodialyzer (ED) (DS-0, AGC., Ltd., Tokyo, Japan) employed for this purpose consisted of 14 sheets of cation exchange membranes (CEMs) and 10 sheets of anion exchange membranes (AEMs) (each having an effective membrane area of 0.0172 m^2/piece), 10 deionization compartment cells, and 11 concentration compartment cells. The electrode solution consisted of a 5 % sodium nitrate solution (two 500 mL tanks) and was circulated at a flow rate of 20 L/h using a magnet pump (MD-30 RX, Iwaki Co., Ltd., Tokyo, Japan). As initial solutions (0 h) of the deionized solution and the concentrated solution, 1 L of each solution prepared in the preliminary test was added to the tank (2 L each). Electrodialysis was performed at a constant current of 3 A or a constant voltage of 150 V upon magnet pump-induced circulation at a flow rate of 100 L/h. Each tank was placed in a water-circulation bath to prevent any rise in temperature.

Secondary separations of sugar and Ge-132 (NaOH) by electrodialysis. The sugar and the low concentration Ge-132 (NaOH) in the primarily separated solution were further separated by another electrodialysis. The electro deionizer (EDI) (GDI®, Ebara corporation Co., Ltd., Tokyo, Japan) employed consisted of 9 sheets of CEMs and 5 sheets of AEMs (each having an effective membrane area of 0.0432 m^2/piece), 5 sheets of cation exchange nonwoven fabric, 5 sheets of anion exchange nonwoven fabric, 10 sheets of ion conducting spacer, 5 deionization compartment cells, and 6 concentration compartment cells. The electrode solution consisted of a 5 % sodium nitrate solution (two 500 mL tanks), and was circulated at a flow rate of 20 L/h using a magnet pump. As initial solutions (0 h) of the deionized solution and the concentrated solution, 1 L of each solution prepared in the preliminary test was added to the tank (2 L each). Electrodialysis was performed at a constant voltage of 100 or 120 V upon magnet pump-induced circulation at a flow rate of 25 L/h. Each tank was placed in a water-circulation bath to prevent any rise in temperature.

Purification and concentration of the sugar solution. The deionized sugar solution was purified by passing through two resin columns (16 mm φ × 400 mm each) connected in series. Initially, it was passed through a strongly acidic cation exchange resin (Amberlite IR-120B, Organo Co., Ltd., Tokyo, Japan) to remove remaining cations. Subsequently, it was passed through a chelating resin (Diaion CRB-03, Mitsubishi Chemical Co., Ltd., Tokyo, Japan) to remove any remaining Ge-132. The purified sugar solution was then concentrated under reduced pressure (60 °C, 30 mmHg) at 800 mL/h using a thin film evaporator (MF-10A, Tokyo Rikakikai Co., Ltd., Tokyo, Japan).

Separation of Ge-132 and NaOH by electrodialysis with BPM. The Ge-132 and NaOH present in the recovered solution were separated by electrodialysis with BPM. The ED-BP (ME-0, AGC Co., Ltd.) employed consisted of 1 sheet of BPM and 2 sheets of CEMs (each having an effective membrane area of 0.005 m^2/piece), 1 acid compartment cell, and 1 alkaline compartment cell. The concentration solution and the electrode solution consisted of a 0.05 M solution of sodium hydroxide. The deionized solution (tank volume 2 L), concentrated solution (tank volume 2 L), and electrode solution (tank volume 2 L) were decationized at a constant current of 2.5 A upon tube pump-induced circulation at a flow rate of 36 L/h (PST-1000, AGC Techno Glass Co., Ltd., Shizuoka, Japan).

Process for the continuous production of lactulose syrup and reuse of Ge-132 in a bench-scale plant. Figure 2 shows a process diagram of the bench-scale plant employed for the continuous production of lactulose-containing syrup and the reuse of Ge-132. The bench-scale plant was designed based on the following: 1) A continuous alkaline isomerization process to give lactulose from lactose using Ge-132, 2) a continuous two-step separation process of the sugar and Ge-132 (NaOH) combining ED and EDI, 3) a continuous purification process for the sugar solution, 4) a continuous concentration process for the purified sugar solution, 5) a separation process for Ge-132 and NaOH based on the use of ED-BP, and 6) a purification process for Ge-132. Syrups were continuously produced by operating processes 1)–4). Processes 5) and 6) were incorporated for the reuse of Ge-132 and sodium hydroxide as the reaction catalysts. In the main processes 1) to 3), continuous operation was carried out for 6 h three times. The solution in each tank was allowed to stand for 18 h until the start of the next continuous operation. Process 4 was operated after storing the processing solution supplied from process 3. During each process, the concentration of the saccharide, concentration of Ge-132, pH value, and conductivity were measured.

Continuous production of lactulose syrup using Ge-132 in the bench-scale plant. The bench-scale plant of lactulose syrup production is illustrated in Fig. 2. Process 1 (Fig. 2) shows the continuous alkaline isomerization process employed, which consists of reagent dissolution, mixing, heating, and cooling. For preparation of the lactose solution, lactose monohydrate (631.6 g) was dissolved in distilled water (2 L) with stirring in a 5 L-jacketed stirring tank (MBF-500MC, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) while circulating hot water at 40 °C for 3 h, prior to returning to 25 °C. For preparation of the Ge-132 solution (pH 13), Ge-132 (297.4 g) was dissolved in a 5 M solution of sodium hydroxide (710 mL), and distilled water was added to the 8 L-jacketed stirring tank (MBF-800MC) at < 40 °C to give a final volume of 1 L. After preparation, the continuous operation was allowed to commence. The lactose solution and the Ge-132 solution (pH 13) were fed into the static mixer (with PTFE particles filled in a 7.5 mm φ × 30 cm stainless steel column tube) at flow rates of 100 and 50 mL/h, respectively. The resulting mixture was reacted by passing through an in-line tubular heater (6 mm φ × 265 cm) with circulating warm water at 60 °C, and the reaction was stopped by passing continuously through a tubular cooler (6 mm φ × 40 cm) with circulating cold water at 5 °C. The tubular reactors were prepared using SUS 304 tubes stored in a jacket with an inlet and an outlet for the

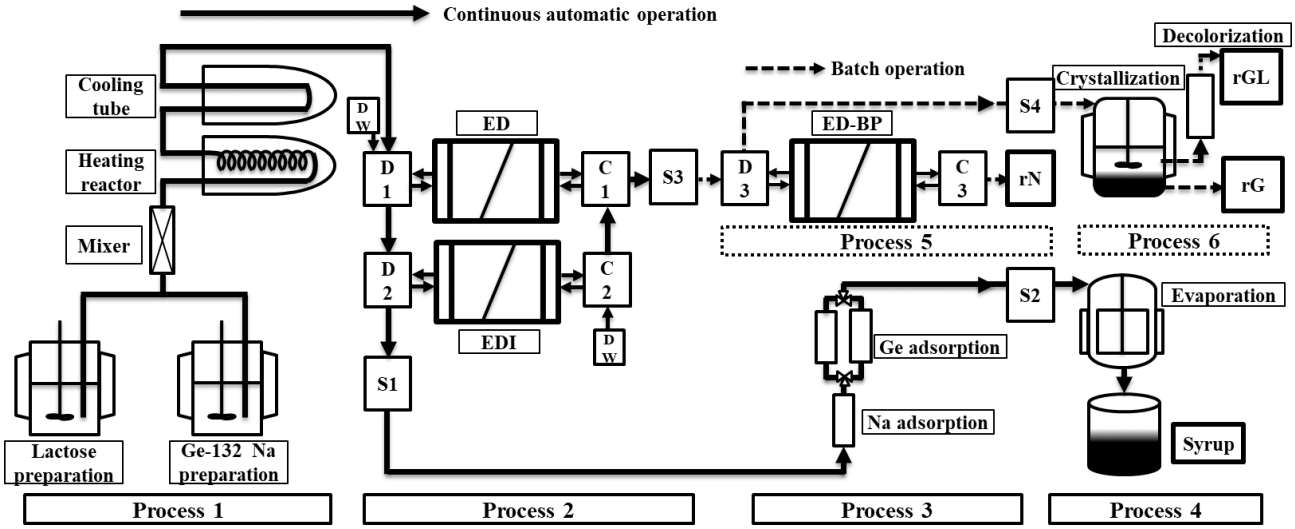

Fig. 2. Process diagram of the continuous system for the production of lactulose syrup and the reuse for Ge-132.

The bench-scale plant consists of four main processes and two reusable Ge-132 processes: Process 1) a continuous alkaline isomerization process involving reagent dissolution, mixing, and reaction; Process 2) a continuous two-step electrodialysis separation process for sugar and Ge-132 (NaOH) based on the combination of ED and EDI; Process 3) a continuous purification process for the sugar solution using an ion exchange resin; Process 4) a continuous concentration process for the purified sugar solution using a thin film evaporator; Process 5) a separation process for Ge-132 and NaOH using ED-BP; and Process 6) a purification process for Ge-132 involving cooling crystallization and decolorization by an adsorbent. D1, deionization tank of ED; D2, deionization tank of EDI; D3, deionization tank of ED-BP; C1, concentration tank of ED; C2, concentration tank of EDI; C3, concentration tank of ED-BP; S1, storage tank for the sugar solution; S2, storage tank for the purified sugar solution; S3, storage tank for the recovered solution; S4, storage tank for the de-NaOH solution; DW, distilled water; rN, recovered NaOH; rG, recovered Ge-132 crystals; rGL, recovered solution containing Ge-132 and lactulose.

heat medium. The reaction mixture was continuously fed into the ED-deionization tank (D1) at a rate of 150 mL/h.

Process 2 (Fig. 2) shows the continuous two-step electrodialysis separation process for the sugar and Ge-132 (NaOH) combining ED and EDI. In advance, as initial solutions (0 h) of the deionized solution and the concentrated solution, 1 L of each solution prepared in the preliminary test was added to the tank (D1, C1, D2, and C2). The isomerization reaction mixture supplied (150 mL/h) to the ED-deionization tank (D1) was deionized continuously by electrodialysis using ED. Distilled water was constantly supplied (330 mL/h) to D1 to reduce the viscosity of the solution. In conjunction with electrodialysis, the ED-deionized sugar solution was continuously supplied (300 mL/h) from D1 to the EDI-deionization tank (D2) and further deionized by electrodialysis using EDI. Distilled water was constantly supplied (120 mL/h) to the EDI-concentration tank (C2) to reduce the ion concentration of the solution. To facilitate dilution and collect the concentrated Ge-132, the EDI-recovery solution in C2 was continuously fed (120 mL/h) to the ED-concentration tank (C1), and the recovery solution in C1 was fed (300 mL/h) to the 10 L storage tank (S3) during electrodialysis. The deionized sugar solution in D2 was fed (300 mL/h) to the 100 mL storage tank (S1) during electrodialysis. Approximately 50 mL of the solution was stored in S1, and the feed (300 mL/h) was then started to process 3.

Process 3 (Fig. 2) shows the continuous purification process employed for the sugar solution based on the use of an ion exchange resin. The deionized sugar solution (in S1) was purified by passing successively through one sodium adsorption column and two switchable Ge-132 adsorption columns and then fed (300 mL/h) to the 5 L storage tank (S2).

Process 4 (Fig. 2) shows the continuous concentration process employed for the purified sugar solution based on the use of a thin film evaporator. A total of 4 L of purified sugar solution (in S2) was supplied (800 mL/h) to a thin film evaporator, and then concentrated approximately eight times to produce the desired lactulose-containing syrup.

Reuse of Ge-132 and NaOH in the bench-scale plant. Process 5 (Fig. 2) shows the electrodialysis separation process employed for the reuse of Ge-132 and NaOH based on the ED-BP process. The recovered solution containing Ge-132 and NaOH supplied to S3 was fed to the deionization tank (D3) in 500 mL portions and was subjected to electrodialysis by ED-BP for 160 min for separation of the NaOH. The NaOH solution separated in the concentration tank (C3) was fed to the 2 L recovery tank (rN) to obtain the recovered NaOH. The de-NaOH solution was then fed to the 5 L storage tank (S4).

Process 6 (Fig. 2) shows the Ge-132 purification process consisting of cooling crystallization and decolorization by an adsorbent to recover Ge-132 in the de-NaOH solution. More specifically, the de-NaOH solution was stirred overnight (8 °C) in 500 mL portions to precipitate Ge-132 in the crystallizer using a mixer. The resulting precipitate was separated by suction filtration and washed by stirring (8 °C) overnight in distilled water (40 mL). Following subsequent suction filtration, the obtained solid was dried under vacuum (115 °C, 5 mmHg, 5 h) to obtain the purified Ge-132 crystals in the 1 L tank (rG). Finally, the filtrate was decolorized using a column (10 mm φ × 200 mm) packed with an aromatic synthetic adsorbent (Sepabeads™ SP850, Mitsubishi Chemical Co., Ltd.). The decolorized solution was stored in the 5 L tank (rGL).

RESULTS

Optimization of the isomerization reaction time for bench-scale production.

Figure 3 shows the time courses for the isomerization of lactose to lactulose under normal alkaline conditions (Fig. 3A) and under alkaline conditions in the presence of Ge-132 (Fig. 3B). For the alkaline isomerization in the presence of Ge-132 at pH 12 and 60 °C, a reaction time of 20–30 min was optimal, giving a high yield (130–150 g/L) of lactulose and a low galactose production ratio (< 10 g/L), which originated from alkaline degradation.

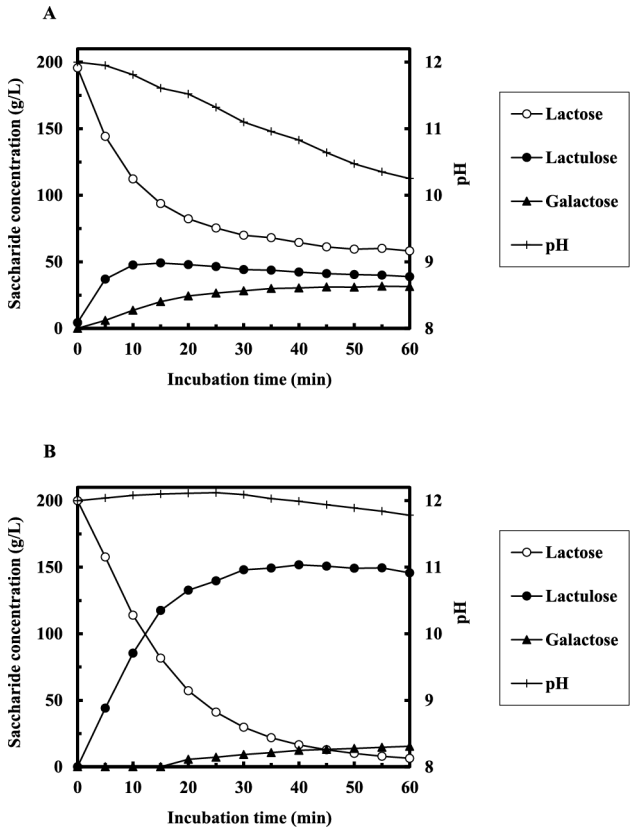

Fig. 3. Isomerization of lactose to lactulose with time (initial lactose concentration: 200 g/L; initial pH 12; reaction temperature: 60 °C).

(A) Control (normal conditions). (B) Ge-132 (in the presence of 99.1 g/L Ge-132). The concentration of each saccharide was determined by HPLC.

Table 1. Concentrations of saccharides and Ge-132, and pH values for the mixing and reaction steps of the continuous isomerization process.

	Mixture* (g/L)	Reaction mixture** (g/L)
Lactose	180 ± 8	26 ± 6
Lactulose	4.1 ± 0.8	130 ± 4
Galactose	0	11 ± 1
Ge-132	99 ± 2	93 ± 3
pH	11.9 ± 0.1	11.9 ± 0.1

Results shown are the mean ± standard deviations of independent measurements (n = 9). *Solution from the mixture sampling port (Mixer in Fig. 2) at 0.1, 3.5, and 6 h after operation start. **Solution from the reaction sampling port (Cooling tube in Fig. 2) at 0.5, 3.5, and 6 h after operation start.

Continuous alkaline isomerization in a bench-scale plant.

Table 1 lists the concentrations of lactose, lactulose, galactose, and Ge-132, along with the pH values for the mixing and reaction steps of the continuous alkaline isomerization process. When the conditions for the continuous isomerization process were set such that the isomerization reaction time was 21 min and the cooling time was 5 min, the conversion to lactulose was as expected, *i.e.*, 70–77 %, which was consistent with a result obtained from the batch experiment.

Continuous two-step separation of sugar and Ge-132 (NaOH) in the bench-scale plant.

Table 2 and Fig. 4 show the concentrations of lactose, lactulose, galactose, and Ge-132 in each tank in the continuous two-step electrodialysis separation process. Table 3 summarizes the concentration ratio of each saccharide and Ge-132.

The Ge-132 concentration in the continuously fed reaction mixture decreased to 21 % in the continuous primary separation and further to 0.34 % in the continuous secondary separation. In this process, 99.6 % of the Ge-132 could be separated. However, the flow of the sugar was also observed along with that of the Ge-132.

Continuous purification and concentration of lactulose syrup in the bench-scale plant.

Table 4 lists the concentrations of lactose, lactulose, galactose, and Ge-132 in the continuous purification process of the sugar solution. Purification was possible without any

Table 2. Concentrations of saccharides and Ge-132, and pH values in each tank in the continuous two-step electrodialysis separation process.

	Supply solution* (g/L)	Deionized sugar solution		Recovery ion solution	
		D1 (g/L)	D2 (g/L)	C1 (g/L)	C2 (g/L)
Lactose	13 ± 3	13 ± 1	12 ± 1	1.1 ± 0.2	0.39 ± 0.11
Lactose	65 ± 2	60 ± 7	45 ± 6	12 ± 2	11 ± 4
Galactose	5.5 ± 0.5	4.9 ± 0.8	3.9 ± 0.8	3.0 ± 0.5	0.20 ± 0.26
Ge-132	47 ± 1.5	9.8 ± 4.7	0.16 ± 0.09	42 ± 3	19 ± 7.1
pH		3.0 ± 0.5			

Results shown are the mean ± standard deviations of independent measurements (n = 9). *Saccharides and Ge-132 contents are calculated from the average of the reaction mixture (Table 1) multiplied by 150/300 (inflow rate/outflow rate).

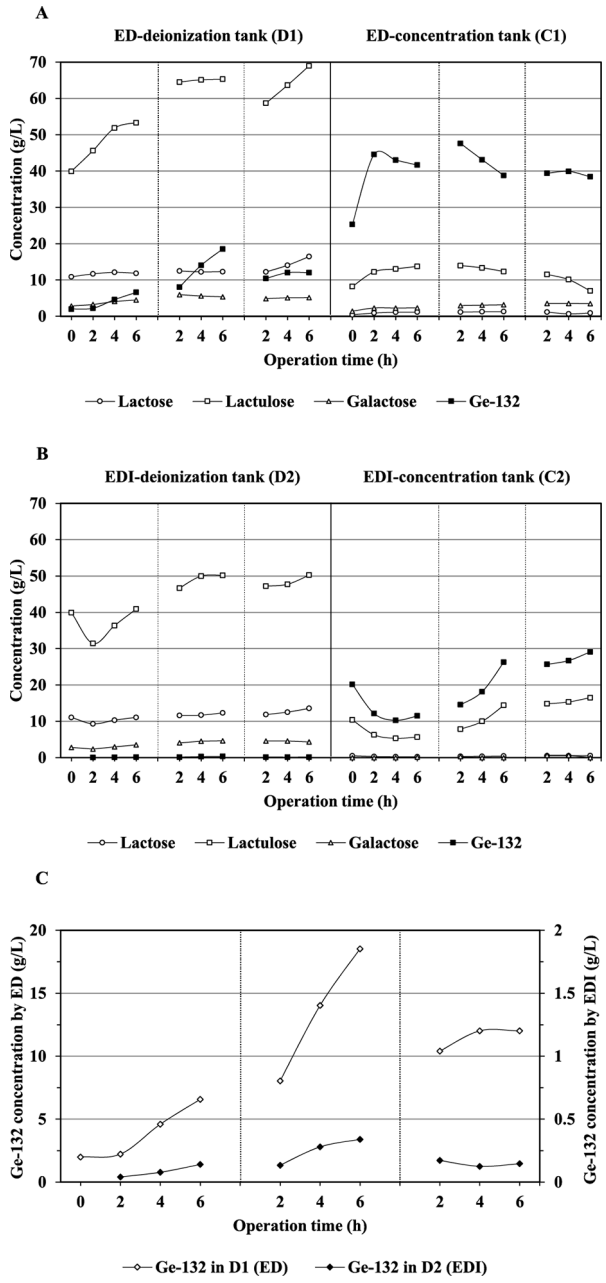

Fig. 4. Concentrations of saccharides and Ge-132 in each tank in the continuous two-step separation process using ED and EDI.

(A) Concentrations of the solutions in D1 and C1. (B) Concentrations of the solutions in D2 and C2. (C) Concentrations of Ge-132 in D1 and D2 (enlarged figure). The solution was sampled 0, 2, 4, and 6 h after the start of the operation. The operation was carried out for 6 h three times.

significant loss of the sugar. Thus, nearly 100 % of the Ge-132 added was removed from the sugar solution.

The purified sugar solution was continuously concentrated 7.95 times using a thin film evaporator to obtain 500 mL of syrup over 5 h. Table 5 lists the concentrations and yields or remaining ratios of lactose, lactulose, galactose, and Ge-132 in the final syrup. The bench-scale plant allowed lactulose-containing syrup to be produced at a rate of 37.7 mL/h.

Separation and purification of Ge-132 in the bench-scale plant.

Figure 5 shows the time course of the conductivity and pH changes when the recovered solution in the concentrated solution storage tank (S3) separated by the two-step separation process was dialyzed (500 mL per sample) by electrodialysis using ED-BP (n = 3). The content of Ge-132 in the de-NaOH tank (D3) was initially 18.4–18.8 g (total volume = 500 mL), and finally, 18.0–18.8 g remained in a

Table 3. Concentration ratios of saccharides and Ge-132 in the continuous two-step electrodialysis separation process.

	Supply solution (%)	Deionized sugar solution		Recovery ion solution	
		D1 (%)	D2 (%)	C1 (%)	C2 (%)
Lactose	100	100	92	8.5	3.0
Lactulose	100	92	69	18	17
Galactose	100	89	71	55	3.6
Ge-132	100	21	0.34	90	41

The values represent concentration ratios of saccharides or Ge-132 to the supply solution (Table 2).

Table 5. Concentrations and yields or remaining ratios of saccharides and Ge-132 in the final syrup.

	Mixture (g/L)	Syrup (g/L)	Yield or remaining ratio (%)
Lactose	180	92	13
Lactulose	4.1	350	48
Galactose	0	31	4.2
Ge-132	99	0.002	0.0005

Yields of saccharide is given as the ratio of the concentration of individual saccharides in syrup to the total saccharide content in mixture, multiplied by 300/150 (outflow rate/inflow rate) and 1/7.95 (concentration ratio). The remaining ratio of Ge-132 is given by the ratio of Ge-132 content in syrup to Ge-132 content in mixture, multiplied by 300/150 (outflow rate/inflow rate) and 1/7.95 (concentration ratio).

Table 4. Concentrations of saccharides and Ge-132 in the purification process.

	Deionized sugar solution (D2) (g/L)	Purified sugar solution* (g/L)	After purification** (%)	After separation and purification *** (%)
Lactose	12 ± 1	12 ± 1	100	92
Lactulose	45 ± 6	44 ± 7	98	68
Galactose	3.9 ± 0.8	3.9 ± 0.7	100	71
Ge-132	0.16 ± 0.09	0.00019 ± 0.00017	0.12	0.00041

Results shown are the mean ± standard deviations of independent measurements (n = 9). *Solution from the sampling port (Ge adsorption in Fig. 2) at 2, 4, and 6 h after operation start. **The values represent concentration ratios of saccharides or Ge-132 in purified sugar solution to D2. ***The values represent concentration ratios of saccharides or Ge-132 in purified sugar solution to the supply solution (Table 2).

total volume of 475–485 mL after electrodialysis for 160 min. A significant amount of Ge-132 thus remained in D3. The pH of the de-NaOH solution dropped from pH 12.3 to 2.3 upon the removal of NaOH. In contrast, the solution present in the concentration tank (C3) was a colorless alkaline solution, and no Ge-132 was detected.

Table 6 shows the results of Ge-132 precipitation through cooling crystallization of the de-NaOH solution obtained by electrodialysis using ED-BP (n = 3). The precipitated Ge-132 crystals were found to be pure by AAS analysis, and no impurities were detected by HPLC. The proportion of crystals purified was 59 %. Table 7 shows the recovery ratio of Ge-132 and the degree of browning when the filtrate was decolorized. The filtrate could be successfully decolorized without considerable loss of Ge-132. The sugar contained lactulose in the de-NaOH solution (S4), which was mostly recovered as a decolorization solution (rGL).

DISCUSSION

A continuous bench-scale plant was constructed to produce lactulose syrup using organogermanium compound Ge-132, which can effectively promote the sugar isomerization reaction as a reusable reaction promoter. In this bench-scale plant, syrup containing 350 g/L lactulose, 92 g/L lactose, and 31 g/L galactose was produced. The two-step electrodialysis separation system devised herein successfully separated 99.6 % of the Ge-132 present in the reacted isomerization solution.

The separated Ge-132 (Na) solution can be subjected to the separation of NaOH and crystallization purification to afford purified Ge-132 crystals, which, together with the concomitantly produced colorless NaOH solution, can be reused in the isomerization reaction. The remaining Ge-132 solution contained sugar that was transported with the flow of Ge-132 in electrodialysis, which was attributed to the fact that the complex of THGP (monomer of Ge-132) with the sugar is negatively charged and behaves as an electrolyte in aqueous solution. This is clear from the fact that the flow of Ge-132 and lactulose show the same behavior (Fig. 4B). The remaining ratios of each saccharide in the final syrup (relative to the corresponding saccharide in the isomerization reaction mixture) were 92 % for lactose, 67 % for lactulose, and 70 % for galactose. For the raw materials, the yields of lactose, lactulose, and galactose were 13, 48, and 4.2 %, respectively. However, sugar recovery could be increased by returning to the separation step of process 2 followed by repeated separation.

In this series of processes, decomposition of Ge-132 was not observed, thereby indicating the stability of this species. In addition, the ion exchange membrane and resin used for the separation and purification processes were regenerated by washing with 50 % ethanol after continuous operation for 3 days. Upon conducting the durability test of the electrodialysis apparatus using the Ge-132-containing recovery solution, the continuous operation time was estimated to be up to 3,110 h, indicating excellent membrane durability. Thus, based on these points, the system shown here is also likely to be applicable to an actual production scale.

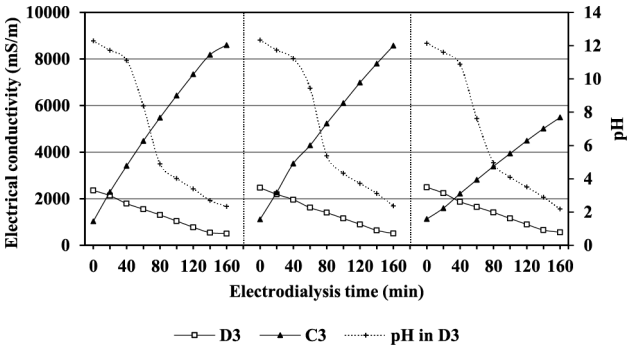

Fig. 5. Variation in conductivity and pH with time during the separation of Ge-132 and NaOH by electrodialysis using ED-BP.

The initial solution volume in C3 was set to 500 mL each on the first and second days and to 1,000 mL on the third day. The conductivity (Cyberscan CON100, Eutech Instruments Pte Ltd, Singapore) and pH value (HM-14P, TOA Co., Ltd., Tokyo, Japan) were monitored during the operation.

Table 6. Recovery and purification ratios of Ge-132 following cooling crystallization of the de-NaOH solution obtained from electrodialysis using ED-BP.

	Content of Ge-132 (g)	Recovery ratio (%)	Purification ratio* (%)
Before crystallization	18.34 ± 0.10		
Filtrate	7.15 ± 0.21	39.0 ± 1.3	
Crystal	11.18 ± 0.30	61.0 ± 1.3	
Purified Ge-132 crystal	10.81 ± 0.25		58.9 ± 1.1

Results shown are the mean ± standard deviations of independent measurements (n = 3). *Content of purified Ge-132 crystals (g)/mass before crystallization (g) × 100.

Table 7. Recovery ratio of Ge-132 and the degree of browning upon treatment with an aromatic synthetic adsorbent.

	Concentration of Ge-132 (%)	Recovery ratio (%)	Absorbance at 420 nm
Before treatment*	1.53 ± 0.04		0.865
After treatment	1.53 ± 0.04	100	0.142

Results shown are the mean ± standard deviations of independent measurements (n = 3). *Including washing solution.

As an alternative recyclable isomerization promoter, the use of sodium aluminate for lactulose production has been reported.[24] In this case, the aluminate is removed via precipitation by pH adjustment, and subsequent use of an ion exchange resin and nanofiltration membrane gives high purity lactulose.

In our Ge-132-based system, lactulose syrup production is continuous and automatic due to the implementation of a two-step electrodialysis separation method. The residual amount of Ge-132 in the prototyped syrup can be suppressed to 2 mg/L, while the separated Ge-132 can be recycled and reused with a high efficiency following electrodialysis. To the best of our knowledge, this study provides the first example of a highly efficient recovery system for such an isomerization promoter.

As described above, under optimal conditions, the described production system is expected to be well suited for the development of high value-added sugar products. For the application of this system in an actual production plant, it is essential to optimize the separation process to reduce sugar loss and further upgrade the purification process of Ge-132 from a batch system to continuous system to improve its efficiency and simplicity. The on-going research is focused on these topics.

CONFLICTS OF INTEREST

The authors, TN and KS, are employees of Asai Germanium Research Institute Co., Ltd., a manufacturing and sales company of Ge-132.

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