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Lipase-catalyzed condensation of erythritol and medium-chain fatty acids in acetonitrile with low water content

Shuji Adachi *, Kayoko Nagae, Ryuichi Matsuno

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

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Abstract

Erythritol could be esterified with medium-chain fatty acids with carbon numbers of 7 to 12 by immobilized lipase from *Candida antarctica* in acetonitrile with low water content at 50°C. The highest equilibrium conversion, 50%, of erythritol–lauric acid ester was achieved at a water content of about 1% (v/v). It was shown that the activity coefficients of the substrates, product and water would play an important role in determination of the equilibrium conversion. Continuous production of erythritol–lauric acid ester at a conversion of ca. 70% was realized using a packed-bed reactor without significant lowering of the conversion for at least 10 days.

Keywords: Erythritol; Erythritol–fatty acid ester; Lipase; Surfactant; Condensation

1. Introduction

Food includes both hydrophillic and hydrophobic substances such as saccharides and fatty acids. If they can be chemically bound, the products would be edible surfactants. Enzymatic preparation of surfactants has extensively been investigated using mainly lipase because an enzymatic method is superior in regioselectivity and the absence of complicated protection and deprotection steps. In previous investigations, fatty acid esters of glycerol [1,2], sugar alcohols such as sorbitol [3–5] and mono- or disaccharides [6–10] were usually used as hydrophillic substrates.

Erythritol is a sugar alcohol with a carbon number of 4 and is a naturally occurring sweetener [11]. Erythritol-fatty acid esters also seem to be surface-active. However, only a report on their enzymatic preparation has been published [4], where a saturated aqueous solution of erythritol wad emulsified with oleic acid and condensed to produce erythritol–oleic acid ester using lipase AY Amano from *Candida* sp. under reduced pressure.

In this study, we investigated the preparation of erythritol–fatty acid esters through lipase-catalyzed condensation of erythritol and fatty acids with carbon numbers of 7 to 12 in acetonitrile with low water content. Some factors affecting the conversion of the desired product were examined using mainly lauric acid as a fatty acid. Because water is one of the products in the condensation reaction, the effect of water content in the reaction medium on the equilibrium conversion was examined in detail. Fur-

* Corresponding author.

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thermore, the continuous production of erythritol–lauric acid ester was also performed using a reactor packed with immobilized lipase.

2. Materials and methods

2.1. Materials

Immobilized lipase (Novozym® 435, from *Candida antarctica*) was a gift from Novo Nordisk Bioindustry Japan, Chiba, Japan. *meso*-Erythritol and fatty acids with chain lengths of 7 to 12 were purchased from Nacalai Tesque, Kyoto. Acetonitrile, methanol and molecular sieves 5A 1/16 were purchased from Wako, Osaka, Japan. *n*-Octyl β-D-glucoside, which was used as an internal standard in HPLC analysis, was obtained from Dojin Chemicals, Kumamoto, Japan. Octadecyl silica gels (DM1020T) for conventional chromatography were supplied from Fuji Silysia Chemical, Nagoya, Japan.

2.2. Condensation reaction

Reaction methods reported by Ljunger et al. [8] have been modified. Acetonitrile was dehydrated over molecular sieves. The water content of the dehydrated acetonitrile, which was determined by Karl Fischer titration using a Kyoto Electronics MKS-1s, was ca. 0.03% (v/v). Unless otherwise specified, the dehydrated acetonitrile was used as a reaction medium. A typical condensation reaction was carried out as follows. A total of 1 mmol of erythritol, 5 mmol of fatty acid and 20 ml of dehydrated acetonitrile were put into a glass vial with a screw-cap. A total of 1 g of the immobilized lipase was then added. The vial was screw-capped tightly and immersed into a thermo-regulated water bath at 50°C. The condensation reaction was carried out under reciprocal shaking at ca. 110 rpm. Under these conditions, some of the erythritol added remained a solid due to its low solubility. At appropriate intervals, a portion of the reaction mixture was sampled and mixed with an equal volume of 20 mmol/l octyl β-D-glucoside, which was an internal standard in the HPLC analysis. The mixture was used for analysis.

To examine the effect of water content in acetonitrile, an adequate volume of water was added to dehydrated acetonitrile. The initial water content of the reaction medium was measured by the Karl–Fischer titration.

2.3. Purification and identification of product

After the condensation of erythritol and lauric acid reached equilibrium, the reaction mixture was taken out with care not to contaminate the immobilized lipase particles. The mixture was cooled in a refrigerator to remove a part of the lauric acid by solidifying it. The solvent in the supernatant was removed using a rotary evaporator. The residue was dissolved with a small amount of methanol.

The solution was applied to a bed packed with ODS resin (DM1020T, 2.0 cm $\phi \times 50$ cm) and eluted with a mixture of acetonitrile, methanol and water (70:15:15 in volume) at a flow rate of 4.0 ml/min. The elution profile was monitored by a refractometer (YRU-880, Shimamura Keiki Seisakusho, Tokyo, Japan). The effluent corresponding to the peak of the erythritol–lauric acid ester was collected. By evaporating the effluent, we obtained the ester as a powder. The product was analyzed by ^{1}H NMR and was confirmed to be 1-*O*-lauroyl-*meso*-erythritol (erythritol–lauric acid ester). ^{1}H NMR (500 MHz, CD_3OD, 27°C): δ 0.70 (t, $J = 6.9$ Hz, 3H), 1.30 (m, 16H), 1.62 (m, 2H), 2.36 (d, $J = 7.5$ Hz, 2H), 3.59 (m, 2H), 3.74 (m, 2H), 4.13 (dd, $J = 11.5$, 6.5 Hz, 1H), 4.30 (dd, $J = 11.5$, 3.0 Hz, 1H).

2.4. Solubility of erythritol in acetonitrile with various water contents

Acetonitrile with different water contents was prepared by adding water to dehydrated acetonitrile. Erythritol of 0.25 g or more was added to 10 ml of acetonitrile with a given water content and allowed to stand at 50°C with shak-

ing for 24 h. The concentration of erythritol was determined by HPLC.

2.5. Continuous production of erythritol–lauric acid ester

Novozym 435 was packed into a cylindrical glass column of 2.0 cm i.d. The bed height was 7.0 cm. Erythritol and lauric acid were dissolved in acetonitrile with 1.0% (v/v) water at the concentrations of 15 and 75 mmol/l, respectively. The substrate solution was continuously fed to the bed at a flow rate of 0.14 ml/min, which corresponded to a superficial residence time of 157 min, with a peristaltic pump. The column, pump, feed reservoir, and product reservoir were all installed into a chamber where the temperature was regulated at 50°C.

2.6. Analysis

The erythritol–fatty acid ester was analyzed using HPLC (LC-6A, Shimadzu Seisakusho, Kyoto) with an ODS column (Nucleosil 5C18, 4.6 mm $\phi \times 300$ mm, Chemco Scientific, Osaka) and a refractometer. The eluent used was a mixture of acetonitrile, methanol and water (75:15:15 in volume). The concentration of the ester was determined by comparing its peak area with that of octyl β-D-glucoside. The calibration curve was prepared using the ester prepared above.

In measurement of the solubility of erythritol in acetonitrile, an St/6DVB-15(N) column packed with cation-exchange resin in sodium-ion form (0.75 cm $\phi \times 300$ mm, Japan Organo, Tokyo) was used. The eluent used was distilled water.

3. Results and discussion

3.1. Effect of reaction temperature

Fig. 1 shows the time courses in conversion during condensation of erythritol and lauric acid,

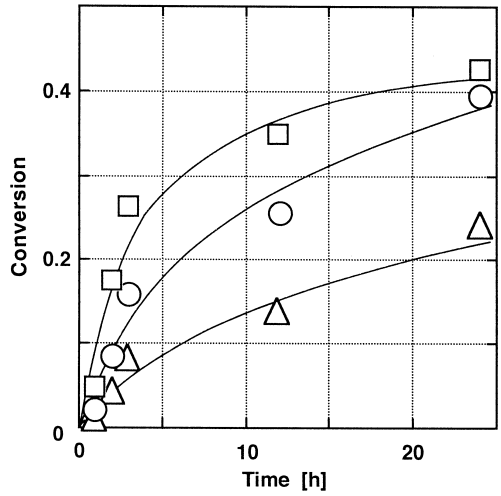

Fig. 1. Condensation of erythritol and lauric acid at 40°C (△), 50°C (○) and 60°C (□). The overall concentrations of erythritol, lauric acid and immobilized lipase were 50 mmol/l, 250 mmol/l, and 50 g/l, respectively. Acetonitrile dehydrated over molecular sieves was used as a reaction medium.

the overall concentrations of which were 50 and 250 mmol/l, respectively, at 40, 50 and 60°C. Although erythritol did not fully dissolve in the acetonitrile, the conversion was calculated based on the amount of erythritol added. The condensation proceeded faster at higher temperature. Because the conversion after 24 h was almost the same from condensations at 50°C and at 60°C, subsequent experiments were carried out at 50°C to lessen the enzyme denaturation during the condensation.

3.2. Effects of concentrations of erythritol and lauric acid on conversion

The effect of the molar ratio of erythritol to lauric acid was examined at a fixed erythritol concentration of 50 mmol/l. The enzyme concentration was fixed at 50 g/l. As shown in Fig. 2, both the initial reaction rate and the conversion at 48 h increased as the ratio increased. However, the time courses were similar at the ratios of 5 and 10. Based on these results, the molar ratio of 5 seemed to be adequate.

At the fixed molar ratio of 5, the condensation reactions were carried out at different initial

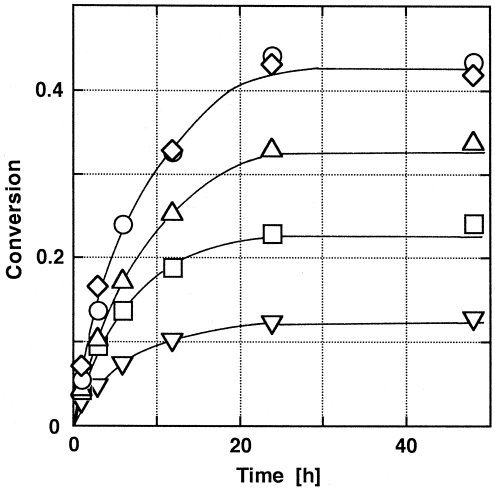

Fig. 2. Production of erythritol–lauric acid ester at different molar ratios of erythritol and lauric acid at 50°C. The initial concentration of erythritol was fixed at 50 mmol/l, and the concentration of lauric acid was varied: (▽) 50 mmol/l, (□) 100 mmol/l, (△) 150 mmol/l, (○) 250 mmol/l, and (◇) 500 mmol/l. The concentration of immobilized lipase and the reaction medium were the same as in Fig. 1.

erythritol concentrations of 25 to 125 mmol/l (Fig. 3). In these experiments, the ratio of substrate to enzyme was inevitably changed because the enzyme concentration was fixed at 50 g/l. The time courses at the initial erythritol concentrations of 25 and 50 mmol/l were almost the same, but the conversion was lower at higher initial erythritol concentrations. The higher the initial erythritol concentration, the higher the product concentration. To achieve the highest conversion, we adopted 50 mmol/l erythritol in subsequent experiments.

3.3. Condensation of erythritol with fatty acids with various chain lengths

Fig. 4 shows the conversion at 24 h for condensations of erythritol and various fatty acids with carbon numbers of 7 to 12. The concentrations of erythritol and fatty acid were 50 and 250 mmol/l, respectively, in all condensations. We could not obtain powdery samples of erythritol–fatty acid esters other than erythritol–lauric acid ester. Therefore, we calculated the conversions for the esters using the molecular mass of each product and the calibration curve for erythritol–lauric acid ester which was prepared based on the concentration in units of gram/liter. The conversion at 24 h, when equi-

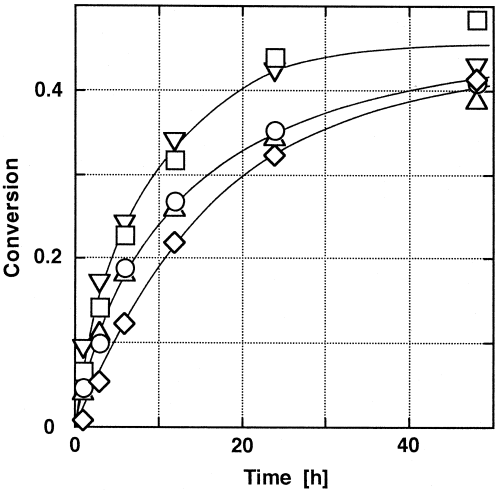

Fig. 3. Condensation of erythritol and lauric acid at different concentrations with a fixed molar ratio of 5 and at 50°C. The initial concentrations of erythritol and lauric acid were (▽) 25 and 125 mmol/l, (□) 50 and 250 mmol/l, (△) 75 and 375 mmol/l, (○) 100 and 500 mmol/l, and (◇) 125 and 625 mmol/l. Other conditions were the same as in Fig. 1.

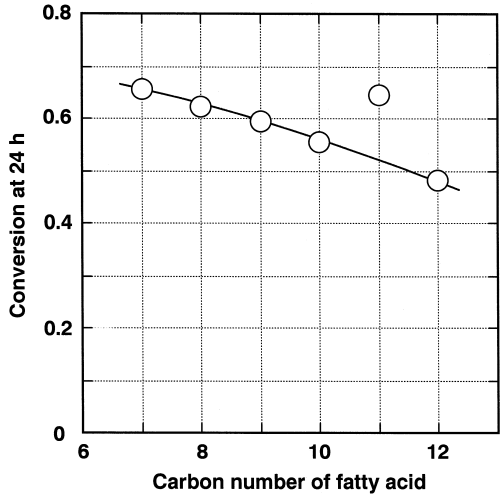

Fig. 4. Dependence of the conversion at 24 h on carbon number of fatty acid for condensation of erythritol and fatty acid at 50°C. The initial concentrations of erythritol and fatty acid were 50 and 250 mmol/l, respectively. Other conditions were the same as in Fig. 1.

librium seemed to be achieved, was higher for fatty acid with smaller carbon number, although the plot for undecanoic acid (C11) did not lie on the curve.

3.4. Effect of water content in acetonitrile on conversion

Because water is one of the products in the condensation, its content in the reaction medium would affect the equilibrium conversion. The condensation of erythritol and lauric acid was carried out at various initial water contents. As shown in Fig. 5, the equilibrium conversion (that at 24 h) largely depended on the initial water content, and it was the highest at the initial water content of ca. 1% (v/v).

The equilibrium conversion would be a function of the concentrations of erythritol, fatty acid, product and water. The equilibrium constant K_C for the condensation, based on their concentrations at equilibrium, is given by

$$K_C = C_{Pe}C_{We}/C_{Ee}C_{Fe} \quad (1)$$

where C is the concentration, the subscripts E, F, P, and W represent erythritol, fatty acid, erythritol–fatty acid ester, and water, and the subscript e indicates equilibrium. If the K_C is truly a constant, the plots of $C_{Pe}C_{We}$ against $C_{Ee}C_{Fe}$ should give a straight line, passing through the origin, with a slope of K_C.

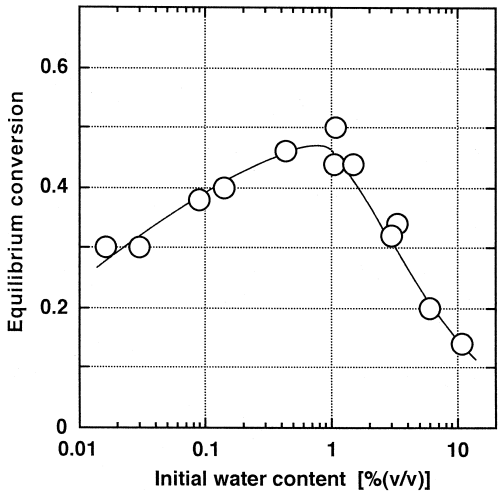

Fig. 5. Effect of the initial water content of acetonitrile on the equilibrium conversion of erythritol–lauric acid ester at 50°C. The conditions were the same as in Fig. 1 except for the initial water content of acetonitrile. The conversions at 24 h were regarded as the equilibrium conversions.

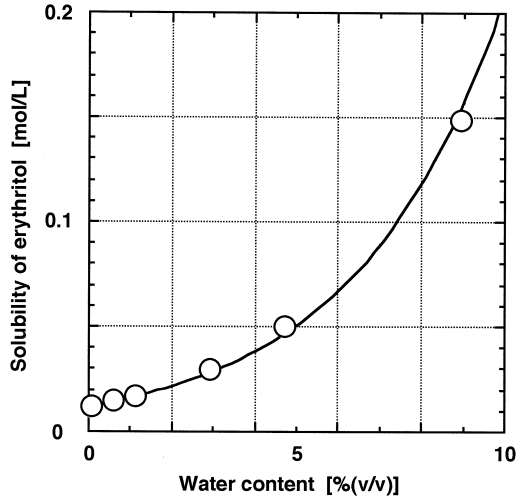

Fig. 6. Solubility of erythritol in acetonitrile with different initial water contents at 50°C.

To make the plot, we measured the solubility of erythritol in acetonitrile with various initial water contents (Fig. 6). The saturated concentration of erythritol C_E could be expressed empirically as a function of initial water content w:

$$C_E = 0.0123e^{0.283w} \quad (2)$$

where C_E and w are expressed in units of mol/l and %(v/v), respectively.

Fig. 7 shows the plots of $C_{Pe}C_{We}$ against $C_{Ee}C_{Fe}$. The initial water contents in the condensation reaction w were converted to the initial water concentrations C_{W0} using the density and molecular mass of water. The C_{Fe} and C_{We} values were estimated from their initial concentrations and the experimentally observed C_{Pe}. The C_{Ee} value was calculated by substituting the C_{We} into Eq. (2), which was converted in units of %(v/v). The plots did not give a straight line. This would indicate that use of the equilibrium constant based on the concentrations is not adequate for the present condensation reaction system.

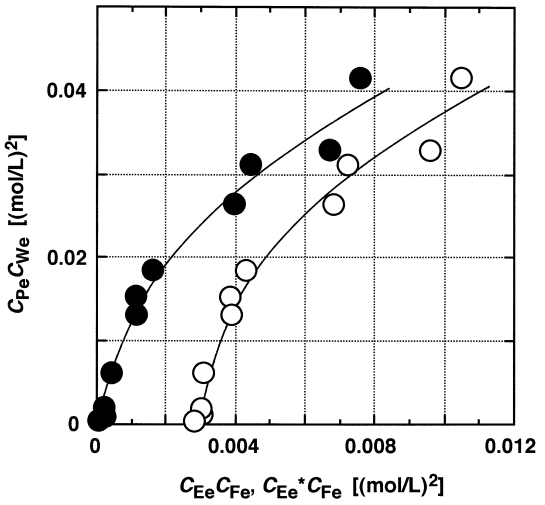

Fig. 7. Plots of $C_{Pe}C_{We}$ against $C_{Ee}C_{Fe}$ (○) or $C_{Ee}{}^{*}C_{Fe}$ (●). C_{Ee}, C_{Fe}, C_{Pe}, and C_{We} are the equilibrium concentrations of erythritol, lauric acid, erythritol–lauric acid ester, and water, respectively. $C_{Ee}{}^{*}$ is the hypothetical effective concentration of erythritol and its definition is described in the text.

The equilibrium constant should be, in principle, defined based on the activities of the substrates and products as follows:

$$K_a = \gamma_P x_{Pe} \gamma_W x_{We} / \gamma_E x_{Ee} \gamma_F x_{Fe} \quad (3)$$

where γ and x represent the activity coefficient and the molar fraction. Unfortunately, we could not estimate all the activity coefficients of the substrates and products. Accordingly, we evaluated only the activity coefficient of water γ_W according to Wilson's method [12] assuming a water–acetonitrile binary system, then plotted $\gamma_W x_{We} x_{Pe}$ against $x_{Ee} x_{Fe}$ (data not shown). The plots also did not give a straight line. This would indicate that either or all of the activity coefficients of erythritol, fatty acid, and erythritol–fatty acid depend on the composition of the reaction system.

The equilibrium constant based on the concentrations would be convenient for practical use. Thus, we calculated the apparent equilibrium constants $K_{C,app}$ and plotted them against the equilibrium concentrations of water C_{We}. As shown in Fig. 8, the $K_{C,app}$ value largely depended on the concentration of water when the concentration was less than ca. 0.6 mol/l, while it was almost constant at higher concentrations of water. The lower $K_{C,app}$ values at lower water concentrations would be the reason why the equilibrium conversions of erythritol–lauric acid ester were low at low water concentrations. At higher concentrations of water, the $K_{C,app}$ value was constant. The hydrolysis reaction predominated and resulted in low conversions at those water concentrations. These facts would be the reason why the equilibrium conversion of erythritol–lauric acid ester became the highest at the water content of ca. 1% (v/v).

The curve connecting the open symbols in Fig. 7 intersected the *x*-axis not at zero but at a positive value. This indicates that no erythritol–fatty acid ester is produced even if certain concentrations of erythritol and fatty acid exist in the reaction system. As shown in Fig. 6 or by Eq. (2), a certain amount of erythritol could dissolve in acetonitrile with no water. We assumed that erythritol dissolving in fully dehydrated acetonitrile could not be utilized as a substrate and introduced an effective erythritol concentration C_E^* which was defined as

$$C_E^* = C_E - C_E|_{C_W = 0} \quad (4)$$

The plots of $C_{Pe}C_{We}$ against $C_{Ee}^{*}C_{Fe}$ are shown by the closed symbols in Fig. 7. The

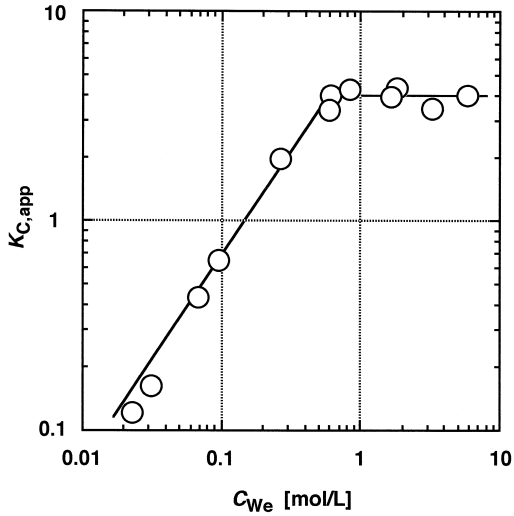

Fig. 8. Dependence of the apparent equilibrium constant $K_{C,app}$ for erythritol–lauric acid formation on the equilibrium concentration of water C_{We}.

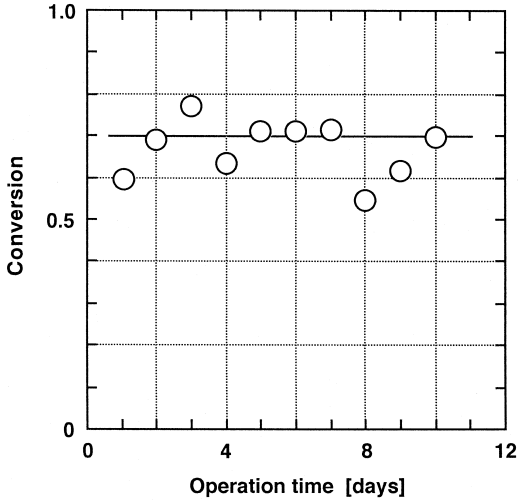

Fig. 9. Continuous production of erythritol–lauric acid ester at 50°C using a packed-bed reactor of immobilized lipase. Details of operating conditions are shown in the text.

curve connecting the plots intersects the origin although it is not a straight line. This might indicate that the activity coefficient of erythritol is extremely small at very low water concentrations.

3.5. Continuous production using a packed-bed reactor

As shown in Fig. 6, erythritol dissolved in acetonitrile with low water content although its concentration was low. The solubility of erythritol in acetonitrile with 1% (v/v) water content was about 15 mmol/l. Accordingly, we tried a continuous production of erythritol–lauric acid ester using a column packed with Novozym 435 at erythritol and lauric acid concentrations of 15 and 75 mmol/l. Fig. 9 shows the conversion of erythritol–lauric acid ester in the effluent as a function of operation time. A high conversion of ca. 70% was achieved without any significant reduction for 10 days. The conversion was much higher than those observed in batchwise reactions. In this experiment, erythritol was fully dissolved in the reaction medium. On the other hand, a part of the erythritol remained in the state of powder in the batchwise experiments. The conversion was calculated, in this study, based on the amount of erythritol added to the reaction system, and the amount of erythrytol in the powder state lowered the overall conversion. These factors would provide a reason for the higher conversion realized in the packed-bed reactor.

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