Improved synthesis of isomaltooligosaccharides using immobilized α -glucosidase in organic–aqueous media

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Abstract α -Glucosidase was immobilized onto an epoxy-activated resin (Eupergit C) to catalyze maltose into isomaltooligosaccharides (IMO), and then the effects of organic–aqueous media on the enzymatic properties of immobilized α -glucosidase were examined. An immobilization efficiency of 79.61% was obtained under the condition of pH 6.0, ionic strength of 2.0 M, and 30 mg of protein/g of resin. The butyl acetate–aqueous biphasic system was found to significantly improve the catalytic activity of the immobilized enzyme and the yield of IMO. The highest yield of IMO (50.83%, w/w) was obtained at pH 4.5 and 55 °C in a butyl acetate/buffer system (25:75, v/v). In addition, the immobilized enzyme particles were distributed into the organic phase after the completion of transglycosylation, which facilitates the separation and recycling use of the immobilized enzyme. Immobilized α -glucosidase retains a robust reusability in this continuous

operation model. The present findings are of potential in improving the IMO manufacturing process.

Keywords α -Glucosidase · Eupergit C · Immobilization · Isomaltooligosaccharides · Organic–aqueous media

Introduction

Oligosaccharides have become increasingly attractive as new food ingredients because of their potential to improve the quality and nutritional properties of food [1]. As a main category of oligosaccharides, isomaltooligosaccharides (IMO) contain 2–5 glucose units with one or more α -(1,6) linkages. IMO are attracting considerable interest in the food industry because of their bifidus-stimulating activity, low caloric value, and low cariogenic properties [2, 3]. At present, IMO are manufactured through enzymatic processes, wherein an α -glucosidase from *Aspergillus niger* catalyzes transglycosylation to the 6-OH of the accepting glucose unit, resulting in formation of new (1,6)-oligosaccharides such as isomaltose, panose, and isomaltotriose [4].

Immobilized enzymes have been used in the food, pharmaceutical, and other industrial bioprocesses to not only eliminate potential protein contamination from the product but also to reuse the enzyme and hence reduce operational costs [4, 5]. Various immobilization techniques have been reported in the literatures, including adsorption, entrapment, and chemical interactions (either covalent or non-covalent) between the enzyme and the support [5]. Epoxy (oxirane)-activated resins, such as Eupergit C or Sepabeads, are among the most widely used supports for enzyme covalent binding [5]. In a recent study, transglucosidase immobilized onto Amberzyme oxirane resin

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improved the catalytic activity and the stability toward pH and temperature [4].

Enzymes have been applied for the catalytic processes in organic–aqueous media due to their industrially attractive advantages, which include elimination of microbial contamination in the reaction mixtures, shifting of thermodynamic equilibrium to favor synthesis over hydrolysis, reduction in undesirable side reactions, and variations of specificity of some enzymes [6, 7]. The production processes of oligosaccharides in organic–aqueous systems have been extensively studied. In this context, the yield of galactooligosaccharides (GOS) catalyzed by β -galactosidase was improved when the buffer medium was replaced with an organic–aqueous biphasic system [8, 9]. The use of transfructosylase or inulinase for the biosynthesis of fructooligosaccharides (FOS) in organic–aqueous systems was investigated and the results showed that the addition of an organic solvent shifted the equilibrium of a reversible reaction between hydrolysis and synthesis to favor synthesis [7, 10, 11]. Although the use of an organic solvent in enzymatic reactions has been shown to improve the yield of oligosaccharides [7, 10, 11], the utility of immobilized α -glucosidase in organic–aqueous media for improving the efficiency of IMO synthesis remains to be investigated.

In this study, biochemical characterization of immobilized α -glucosidase in aqueous and organic–aqueous media was studied and the potential of using immobilized α -glucosidase in organic–aqueous media for the synthesis of IMO was examined. The optimum condition for enzyme immobilization was also exploited.

Materials and methods

Enzyme and reagents

α -Glucosidase (EC3.2.1.20) from *A. niger* was purchased from Jiangsu Ruiyang Biotech Co., Ltd. (Wuxi, Jiangsu, China). The enzyme had a specific activity of approximately 450,000 units per gram. The Eupergit C support (Tianjin Orui Biotech Co., Ltd, Tianjin, China) was used as a carrier for enzyme immobilization. Glucose, maltose, maltotriose, isomaltose, isomaltotriose, and panose were used as references and purchased from Jiangsu Ruiyang Biotech Co., Ltd. (Wuxi, Jiangsu, China). All other reagents were of analytical grade and used without further purification.

Immobilization of α -glucosidase

The immobilization trials were performed in a thermostatic reactor containing Eupergit C (1 g), phosphate buffer, and enzyme in a total volume of 10 mL at 25 °C with shaking

(150 rpm) for 48 h. After immobilization, the solution was filtered and washed with deionized water on a sintered glass filter.

Optimum immobilization conditions were determined by individually varying the conditions: buffer pH from 5.6 to 7.0; buffer concentration from 1.5 to 2.5 M; and concentration of enzyme from 10 to 50 mg of protein/g of resin. During optimization, the parameters not being tested were kept constant at conditions stated above in the immobilization procedure. The immobilization efficiency was calculated using following formula:

$$\begin{aligned} \text{Immobilization efficiency (\%)} \\ = \frac{\text{total activity of immobilized enzyme}}{\text{total enzyme activity}} \times 100. \end{aligned}$$

Enzyme assay

The hydrolytic activity of α -glucosidase was determined by measuring the release of glucose at 37 °C for 1 h with maltose solution (200 g/L) in sodium acetate buffer (20 mM, pH 5.0). The samples (0.1 mL) were withdrawn and boiled for 10 min to inactivate the enzyme prior to HPLC analysis. One unit of activity was defined as the amount of enzyme required to produce 1 μ mol of glucose per minute under trial conditions. The transglycosylation activity was performed in the same conditions as described above and was monitored by the contents of transglycosylation products (isomaltose, panose, and isomaltotriose) in the reaction mixture, taking standards sugar as a reference.

Enzymatic synthesis of IMO

The synthesis of IMO was conducted using maltose as the substrate. The reaction mixture (10 mL) contained maltose (200 g/L) complemented with 1500 units of immobilized α -glucosidase in 20 mM sodium acetate buffer (pH 5.0). The reaction was performed at 55 °C for up to 12 h with magnetic stirring (600 rpm). After the reaction, the supernatant of the aqueous phase (0.5 mL) was withdrawn and boiled for 10 min to terminate the reaction for sugar analysis.

The effect of organic solvents with different log *P* values [N,N-dimethylformamide (DMF, −1.0), ethanol (−0.3), acetone (−0.24), ethyl acetate (0.73), n-butanol (0.84), isoamyl alcohol (1.16), methyl tert-butyl ether (1.19), butyl acetate (1.78), cyclohexane (3.44), and n-heptane (4.66)] on the synthesis of IMO was investigated under the same condition as that of the aqueous phase synthetic reaction as described above, except that the solvent was modified with organic–aqueous media (20:80, v/v). To investigate the effect of butyl acetate concentration on transglycosylation, different concentrations of butyl

acetate, ranging from 10 to 80% (v/v), were added into the aqueous phase synthetic reaction as described above.

IMO yield were calculated using the following formula:

$$\text{IMO yield (\%, w/w)} = \frac{\text{IMO formed (isomaltose, panose, and isomaltotriose)}}{\text{total sugars}} \times 100.$$

Characterization of immobilized α -glucosidase in aqueous and organic–aqueous media

The effect of pH on immobilized α -glucosidase activity in aqueous and organic–aqueous media was investigated at 37 °C using 200 g/L maltose as the substrate at pH 3.0–6.0 (20 mM citrate buffer) or pH 6.0–7.0 (20 mM phosphate buffer). The effect of temperature was studied at 35–70 °C and pH 4.5. The enzyme activity was determined by the same method described above in the enzyme assay. The percentage of relative enzyme activity was determined by comparing with the highest enzyme activity within the same experiment series.

The kinetic parameters (K_m and V_{max}) of enzyme reaction were determined by assaying the total activity (sum of hydrolysis and transglycosylation) and were calculated by Michaelis–Menten equation. Different concentrations of maltose (5–500 mM) were incubated with immobilized α -glucosidase at optimal pH and temperature in the absence and presence of the butyl acetate (25:75, v/v), respectively. The reaction products were analyzed by HPLC.

IMO formation kinetic analysis

Variations in the reaction time on IMO synthesis were determined in optimum operating conditions (pH 4.5 at 55 °C) in the absence and presence of butyl acetate. The concentration of maltose was 200 g/L and the ratios of butyl acetate/buffer were 25:75 (v/v). The ratio of transglycosylation to hydrolysis was determined by comparing the content of transglycosylation products (isomaltose, panose, and isomaltotriose) with the glucose content.

Reusability of immobilized α -glucosidase in butyl acetate/buffer biphasic system

The repeated batch reaction of immobilized enzyme in butyl acetate/buffer media (25:75, v/v) was assessed by conducting IMO synthesis for five consecutive batches under the optimal synthetic condition described above. Each cycle of reaction was performed with magnetic stirring (600 rpm) for up to 10 h. After each cycle of operation, the aqueous phase was taken out and then new maltose solution was added to restart the reaction. The

reaction products were analyzed by HPLC and the IMO yield of each batch was determined.

Carbohydrates analysis

Quantitative analysis of individual carbohydrates was performed using HPLC (Agilent Technologies, Palo Alto, CA, USA) with an ELSD detector 2000s (Grace Davison Discovery Sciences, Deerfield, IL, USA). The column used was a PrevailTM Carbohydrate ES 5u (250 mm $\times$ 4.6 mm, Grace Davison Discovery Sciences, Deerfield, IL, USA). A mixture of water/acetonitrile (25:75, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 10 μ L. Quantitative measurement of each peak was performed using standard calibration curves of sugar standards as references.

Results and discussion

Optimization of immobilization procedure

The optimum buffer conditions for immobilization were determined by varying the pH of the 1.5–2.5 M buffers. As shown in Fig. 1(A), the highest immobilization efficiency in 2.0 M phosphate buffer was achieved at pH 6.0 and the increase in pH from 6.0 to 7.0 led to an inverse effect, consistent with previous reports that Eupergit C showed low reactivity at neutral pH values in immobilization of β -galactosidase, transfructosylase, and glucansucrases [12–14]. The proteins can bind Eupergit C at slightly acidic pH with the carboxyl groups, at neutral or slightly alkaline pH with the thiol groups, and at pH > 9 with the amino groups [15], suggesting that Eupergit C may react with the carboxyl groups of α -glucosidase.

On the other hand, the immobilization efficiency was higher in the 2.0 and 2.5 M buffer than in the 1.5 M buffer (Fig. 1A), suggesting that ionic strength significantly affected the enzyme immobilization efficiency. Several previous studies [14, 16–18] reported that the highest immobilization efficiency of glycosidase onto Eupergit C was achieved with the 1.0–1.5 M buffers and the use of Eupergit C was favored at high ionic strength due to the increase in the strength of hydrophobic adsorption of proteins, reducing the mobility of the enzyme molecule and, therefore, the possibility of multipoint covalent reaction [19]. However, in this study, immobilization of α -glucosidase was shown to require a higher ionic strength (2.0 M) to increase hydrophobic adsorption of the proteins. As shown in Fig. 1(A), the optimal buffer system for immobilization of α -glucosidase was ionic strength of 2.0 M and pH of 6.0.

The effect of α -glucosidase concentrations on its immobilization is shown in Fig. 1(B). With the increase of

Fig. 1 Optimal conditions for immobilization of α -glucosidase onto Eupergit C at 25 °C for 48 h. (A) buffer pH and concentrations and (B) enzyme concentrations

enzyme concentrations from 10 to 30 mg/g of resin, the immobilization efficiency did not significantly decrease ($p > 0.05$). However, further increase of the enzyme concentrations resulted in the reduction of the immobilization efficiency ($p < 0.05$). The obvious decrease in immobilization efficiency for higher enzyme concentrations indicated that the capacity of resin to bind enzyme reached saturation levels. Thus, the optimal enzyme/support ratio was approximately 30:1 (mg of protein: g of resin) and the immobilization efficiency was 79.61%.

Butyl acetate/buffer system improved enzymatic synthesis of IMO

To evaluate the effect of different organic phases on transglycosylations of immobilized α -glucosidase, organic solvents with different polarity, with log P (defined as the

Fig. 2 Enzymatic synthesis of IMO in organic aqueous media. Reaction conditions: 200 g/L maltose solution, 150 U/mL, 20 mM sodium acetate buffer (pH 5.0), 55 °C and 12 h. (A) the ratios for all organic solvent/buffer media were 20:80 (v/v). (B) the ratios of butyl acetate/buffer media were 10:90–80:20 (v/v)

partition coefficient of a given compound in a two-phase n -octanol and water system) from -1.0 to 4.66 , were chosen and sodium acetate buffer (20 mM, pH 4.5) was used as the control. As shown in Fig. 2(A), compared to the control (46.38%, w/w), the maximum IMO yield (48.81%, w/w) was obtained in a butyl acetate/buffer system (20:80, v/v) and the other tested organic solvents showed decreased IMO yields, particularly cyclohexane and n -heptane (log $p > 2$). The different effects on IMO synthesis in different non-aqueous media can be attributed to the direct effect of each solvent on the operational performance of the enzyme because solvents can facilitate unfolding by stripping

essential water from the hydration shell of the proteins, and directly influence the activity of enzymes by altering hydrogen bonds and hydrophobic interactions [20]. In a previous study [11], butyl acetate/buffer system was also observed to increase FOS synthesis by the immobilized inulinase. This effect could be accounted for by the fact that butyl acetate decreased the polarity of the aqueous phase and the hydrogen bonding of the external protein molecules, indicating that hydrophobic interaction in the active center of the protein was reduced, and part of the protein molecules was unfolded. This unfolding of the immobilized α -glucosidase, to some extent, counteracted diffusional limitations and favored substrate access to individual enzyme molecules [9, 21]. This altered microenvironment caused a change of thermodynamic equilibrium and improved the IMO yield [9, 21, 22]. However, other levels of unfolding and changed microenvironment caused by other organic solvents decreased the activity of enzymes and the IMO yield. In particular, for the ethyl acetate/buffer system, our result was different from what was reported wherein increased yield of galactooligosaccharides (GOS) was observed using immobilized β -galactosidase [9]. Consequently, although log *P* has been most commonly used to describe solvent polarity and provides the best correlation with enzyme activity, no clear linear correlation was observed between the polarity of organic solvents and the yields of the transglycosylation product in this study (Fig. 2A).

To determine the optimal butyl acetate concentration for IMO synthesis, transglycosylation reactions were performed in different butyl acetate concentrations by immobilized α -glucosidase. As shown in Fig. 2(B), the highest IMO yield was obtained with addition of 25% (v/v) butyl acetate, and further increase of the butyl acetate concentration decreased the IMO yield. When the concentration of butyl acetate was higher than 40%, the IMO yield was lower than that of the control. These phenomena indicated that high butyl acetate concentration deprives essential water required for maintaining the enzyme activity, thereby causing enzyme inactivation. In this study, the maximum IMO yield was obtained in a butyl acetate/buffer system (25:75, v/v) and thus this system was used for further study.

Biochemical characterization of immobilized α -glucosidase in aqueous and butyl acetate/buffer media

The immobilized α -glucosidase in aqueous and butyl acetate/buffer media (25:75, v/v) was characterized based on the relative activity of the enzyme, with the free enzyme in aqueous media as the control. The results showed that optimum reaction temperature (55 °C) was not affected by

immobilization and the optimal temperature of immobilized α -glucosidase in the biphasic medium was the same as that in the aqueous medium (Fig. 3A). Moreover, in the biphasic medium, the immobilized α -glucosidase exhibited a broader temperature optimum range, indicating that stabilization of immobilized enzyme occurred by the addition of butyl acetate.

In terms of pH, the optimum pH shift between immobilized and free enzyme was more pronounced. The optimum pH of the free enzyme was found to be around 5.5, whereas for the immobilized α -glucosidase, it shifted toward more acidic pH (4.5) (Fig. 3B). This shift is possibly due to the secondary interactions (e.g., ionic and polar interactions, hydrogen bonding, and hydrophobic interaction) between

Fig. 3 Effects of temperature (A) and pH (B) on the activity of free and immobilized α -glucosidase in aqueous and butyl acetate/buffer biphasic media (25:75, v/v) using maltose (200 g/L) in 20 mM sodium acetate buffer

Table 1 Kinetic parameters of the total activity for immobilized α -glucosidase in aqueous and butyl acetate/buffer (25:75, v/v) media. Values are presented as mean $\pm$ SD ($n = 3$)

Reaction systems	K_m (μ M)	V_{max} (μ mol/min/mg immobilized enzyme)	V_{max}/K_m (1/min/mg immobilized enzyme)
Aqueous media	280.41 $\pm$ 1.57	87.11 $\pm$ 0.89	0.31 $\pm$ 0.01
Butyl acetate/buffer media	307.99 $\pm$ 2.11	116.22 $\pm$ 0.77	0.37 $\pm$ 0.03

Table 2 Carbohydrate composition (% w/w) of the reaction mixture and the transglycosylation/hydrolysis ratio catalyzed by immobilized α -glucosidase in aqueous and butyl acetate/buffer (25:75, v/v) media

Reaction time (h)	G (%)	G ₂ (%)	IG ₂ (%)	G ₃ (%)	P (%)	IG ₃ (%)	IMO (%)	T/H
Aqueous media								
0	0	100	0	0	0	0	0	0
1	7.50 $\pm$ 0.85	79.37 $\pm$ 2.15	0	3.60 $\pm$ 0.83	9.53 $\pm$ 1.23	0	9.53 $\pm$ 0.59	1.27 $\pm$ 0.09
3	10.74 $\pm$ 1.18	58.70 $\pm$ 1.61	5.09 $\pm$ 0.71	5.47 $\pm$ 0.88	15.43 $\pm$ 1.22	4.57 $\pm$ 0.54	25.09 $\pm$ 1.24	2.34 $\pm$ 0.17
6	20.88 $\pm$ 0.73	35.46 $\pm$ 1.82	7.75 $\pm$ 0.70	5.37 $\pm$ 0.87	25.14 $\pm$ 1.39	5.40 $\pm$ 0.44	38.29 $\pm$ 0.89	1.83 $\pm$ 0.10
7	25.30 $\pm$ 0.96	27.31 $\pm$ 1.20	9.77 $\pm$ 1.11	5.77 $\pm$ 0.57	26.20 $\pm$ 1.23	5.66 $\pm$ 0.13	41.63 $\pm$ 0.33	1.65 $\pm$ 0.05
8	26.26 $\pm$ 1.17	24.87 $\pm$ 1.35	11.06 $\pm$ 0.96	4.82 $\pm$ 0.46	27.38 $\pm$ 0.96	5.62 $\pm$ 0.57	44.05 $\pm$ 0.68	1.68 $\pm$ 0.07
9	28.08 $\pm$ 1.28	21.39 $\pm$ 1.74	12.16 $\pm$ 0.59	4.74 $\pm$ 0.61	25.81 $\pm$ 1.17	7.82 $\pm$ 0.81	45.79 $\pm$ 0.71	1.63 $\pm$ 0.04
10	28.88 $\pm$ 1.24	19.83 $\pm$ 1.17	12.93 $\pm$ 0.31	5.22 $\pm$ 0.33	25.50 $\pm$ 0.78	7.64 $\pm$ 0.93	46.07 $\pm$ 1.05	1.60 $\pm$ 0.04
11	29.61 $\pm$ 0.99	18.80 $\pm$ 1.21	13.33 $\pm$ 0.88	5.12 $\pm$ 0.73	25.66 $\pm$ 1.49	7.49 $\pm$ 0.79	46.47 $\pm$ 0.83	1.57 $\pm$ 0.01
12	32.41 $\pm$ 1.27	16.25 $\pm$ 0.73	15.30 $\pm$ 1.19	5.60 $\pm$ 0.88	23.84 $\pm$ 1.21	6.60 $\pm$ 0.55	45.74 $\pm$ 0.84	1.41 $\pm$ 0.10
13	34.76 $\pm$ 1.39	15.56 $\pm$ 1.21	15.63 $\pm$ 1.03	5.44 $\pm$ 0.91	22.31 $\pm$ 1.34	6.30 $\pm$ 0.53	44.24 $\pm$ 1.08	1.27 $\pm$ 0.09
Butyl acetate/buffer media (25:75, v/v)								
0	0	100	0	0	0	0	0	0
1	8.62 $\pm$ 1.13	75.84 $\pm$ 2.50	0	4.09 $\pm$ 1.17	11.44 $\pm$ 1.90	0	11.44 $\pm$ 1.76	1.33 $\pm$ 0.07
3	12.10 $\pm$ 1.87	55.64 $\pm$ 1.63	5.17 $\pm$ 1.22	5.25 $\pm$ 0.73	16.82 $\pm$ 1.19	5.02 $\pm$ 0.76	27.01 $\pm$ 1.39	2.23 $\pm$ 0.15
6	22.18 $\pm$ 1.19	31.44 $\pm$ 1.31	8.19 $\pm$ 0.99	5.10 $\pm$ 0.66	27.64 $\pm$ 0.98	5.44 $\pm$ 0.24	41.27 $\pm$ 0.58	1.86 $\pm$ 0.12
7	27.07 $\pm$ 0.80	21.84 $\pm$ 1.29	10.71 $\pm$ 1.15	6.33 $\pm$ 0.87	27.95 $\pm$ 0.77	6.10 $\pm$ 0.41	44.76 $\pm$ 1.61	1.65 $\pm$ 0.07
8	28.98 $\pm$ 0.92	18.40 $\pm$ 0.97	13.47 $\pm$ 0.67	5.01 $\pm$ 0.93	26.81 $\pm$ 1.33	7.33 $\pm$ 0.60	47.60 $\pm$ 1.19	1.64 $\pm$ 0.05
9	30.42 $\pm$ 0.91	15.16 $\pm$ 0.75	14.84 $\pm$ 1.10	5.29 $\pm$ 0.88	26.05 $\pm$ 1.31	8.25 $\pm$ 0.44	49.13 $\pm$ 0.77	1.62 $\pm$ 0.04
10	31.20 $\pm$ 1.19	13.25 $\pm$ 0.61	16.36 $\pm$ 1.09	4.71 $\pm$ 0.53	25.74 $\pm$ 0.99	8.73 $\pm$ 0.59	50.83 $\pm$ 0.91	1.63 $\pm$ 0.02
11	32.12 $\pm$ 1.18	11.98 $\pm$ 0.90	17.24 $\pm$ 1.21	5.78 $\pm$ 0.69	24.51 $\pm$ 1.19	8.37 $\pm$ 0.78	50.12 $\pm$ 0.89	1.56 $\pm$ 0.02
12	33.59 $\pm$ 1.26	11.91 $\pm$ 1.23	17.24 $\pm$ 1.28	4.97 $\pm$ 0.38	24.03 $\pm$ 1.44	8.25 $\pm$ 0.86	49.52 $\pm$ 1.01	1.47 $\pm$ 0.06
13	34.07 $\pm$ 1.48	12.06 $\pm$ 0.75	17.28 $\pm$ 1.21	5.29 $\pm$ 0.80	22.58 $\pm$ 1.19	8.72 $\pm$ 0.30	48.58 $\pm$ 0.75	1.43 $\pm$ 0.07

G glucose, G₂ maltose, IG₂ isomaltose, P panose, G₃ maltotriose, IG₃ isomaltotriose, T/H transglycosylation/hydrolysis ratio

Values are presented as mean $\pm$ SD ($n = 3$)

the enzyme and Eupergit C [15]. Lowering of the optimum pH for immobilized α -glucosidase could be valuable because it allows better contamination control, which is of tremendous importance in immobilized enzyme reactors operating at high sugar contents. On the other hand, with the addition of butyl acetate, the optimum pH of immobilized α -glucosidase was 4.5, which was not different from the reactions in the aqueous medium, suggesting that butyl acetate did not affect the enzyme and support surface charge (Fig. 3B). Compared with the reaction in the aqueous medium, the immobilized α -glucosidase in a biphasic medium showed an improvement of relative enzymatic

activity at pH 3.0–6.0, whereas there was a reduced enzyme activity at pH 6.5–7.0.

The kinetic parameters K_m , V_{max} , and catalytic efficiency (V_{max}/K_m) of the immobilized α -glucosidase in two reaction media were calculated and are summarized in Table 1. Addition of butyl acetate resulted in the increase of K_m from 280.41 to 307.99 μ M, indicating decreased affinity between the enzyme and substrate. In accordance with our result, Risso reported that the K_m of immobilized inulinase was increased by the addition of butyl acetate [11]. On the other hand, compared to the aqueous medium, both the V_{max} and the catalytic efficiency (V_{max}/K_m) of

immobilized α -glucosidase was improved in the biphasic medium.

IMO formation kinetics in optimum conditions

For the kinetics of IMO formation catalyzed by immobilized α -glucosidase in aqueous and butyl acetate/buffer (25:75, v/v) media, the carbohydrate composition of the reaction mixture and the ratio of transglycosylation to hydrolysis are presented in Table 2. Addition of butyl acetate advanced 1 h to reach the maximum IMO yield (from 11 to 10 h) and this result could be due to the increase of $V_{\max}$ and catalytic efficiency ($V_{\max}/K_m$) described above.

On the other hand, Table 2 shows a significant increase in the maximum IMO yield from 46.64% to 50.83% (w/w) after the addition of butyl acetate ($p < 0.05$). In general, a high rate of initial panose formation was accompanied by a rapid decrease in maltose concentration. As the reactions continued, panose formation leveled off and then decreased, whereas isomaltose and isomaltotriose level increased continuously. On the other hand, glucose levels continued to increase during the entire reaction process. Therefore, the ratio of transglycosylation to hydrolysis first increases and then decreases. It was observed that the IMO yield depends on the relative rates of transglycosylation and hydrolysis reactions [4, 23]. In this study, when the IMO yield reached the peak value, the percentage of isomaltose and isomaltotriose was 16.36 and 8.73% (w/w), respectively, in the biphasic medium, which showed higher levels than that in the aqueous medium (13.33 and 7.49%, w/w) (Table 2). In addition, when the IMO yield reached the maximum value, the ratio of transglycosylation to hydrolysis also increased from 1.57 to 1.63 after the addition of butyl acetate. These results may explain why the IMO yield was improved in a butyl acetate/buffer biphasic system.

Reusability of immobilized α -glucosidase in the butyl acetate/buffer biphasic system

Currently, the column, membrane reactor, and other devices are generally used for the separation and recycling of the immobilized enzyme in industrial production settings [24]. These operations require complex equipment, high standard for operability, and high cost. Instead, in our study, after the completion of IMO synthesis, the immobilized α -glucosidase was distributed into the organic phase (the upper layer). This phenomenon can be attributed to the gradual decrease in the interfacial tension of the liquid–liquid and liquid–solid interface with the temperature increased to the optimum reaction temperature, and the epoxy support was adsorbed into the organic phase because of the smaller interfacial tension between the butyl acetate to epoxy support [25].

Fig. 4 Reusability of immobilized α -glucosidase in butyl acetate/buffer biphasic media (25:75, v/v) was evaluated for five cycles with 200 g/L maltose at optimal reaction conditions (pH 4.5, 55 °C), after which the IMO yield of each batch was determined

With the completion of the reaction and direct removal of products from the bottom, continuous production can be implemented by adding new substrate at the upper part of the reactor. Based on this continuous operation model, the reusability of immobilized α -glucosidase in butyl acetate/buffer biphasic media (25:75, v/v) was evaluated through IMO synthesis for successive batches. As shown in Fig. 4, the IMO yield decreased gradually with the increasing number of reaction cycles, and the IMO yield of the fifth batch was 45.8% (w/w), which was decreased by 9.1% than the that of first batch (50.4%, w/w). This result demonstrated that immobilized α -glucosidase by this novel continuous operation model demonstrated robust reusability and good application potential for IMO production.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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