

1 Different enzymatic conditions and methods for producing maltoheptaose

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4 Outline

- 5 1. Introduction
- 6 2. One-pot production of maltoheptaose (DP7) from starch by sequential addition of
7 cyclodextrin glucotransferase and cyclomaltodextrinase
- 8 3. Efficient biotransformation and synergetic mechanism of dual-enzyme cascade reaction
9 in nonreducing maltoheptaose synthesis
- 10 4. Conclusion

11 Abstract

12 Maltoheptaose is a maltooligosaccharide containing seven glucose units, characterized by
13 lower osmotic pressure, higher viscosity, better moisturizing effect, and more robust film-
14 forming properties, it has been widely used in fields such as food, medicine and cosmetics
15 industries. Cyclomaltodextrinase (CDase) is an enzyme, which hydrolyzes cyclodextrins (CDs)
16 efficiently. Using CDs as a substrate, maltodextrin is formed via CDase and subsequently
17 hydrolyzed to prepare maltose heptasaccharide. Therefore, this study aims to explore the
18 characteristics and synergetic mechanism between enzymes for the preparation of
19 maltoheptaose. The cyclodextrin glucotransferase (*GaCGT*) from *Gracilibacillus alcaliphilus*
20 and cyclomaltodextrinase (*BsCD*) from *Bacillus sphaericus* catalyzed the one-pot cascade
21 reaction to produce maltoheptaose, the results showed that the optimal conditions were at pH
22 7.0 and temperature 30°C, in the presence of Ca²⁺. Furthermore, the optimum enzyme units for
23 the reaction were 80 U/g of *GaCGT* and 1 U/g of *BsCD*, and sequential addition of the enzymes
24 exhibited a 5-fold higher conversion rate over simultaneous addition. On the other hand,
25 cyclodextrinase (CDase) from *Bacillus sphaericus* E-244 and maltooligosyltrehalose synthase
26 (MTSase) from *Arthrobacter* sp. Q36 exhibits optimal conditions at pH 7.0 and 40°C for one-
27 pot synthesis of nonreducing maltoheptaose (N-G7). Simultaneously adding CDase and
28 MTSase at 40°C for 4 hours have maximum yield of N-G7 at 77.3%, the conversion efficiency
29 of the sequential treatment is lower than that of the simultaneous method, which further proves
30 that the simultaneous reaction of the two enzymes contributes to the improvement of
31 conversion efficiency. In summary, the method of dual-enzyme cascade reaction treatment has
32 great potential for synthesizing N-G7 and application in the food industry.