CROSS-LINKED ENZYME AGGREGATES AND ENTRAPMENT IMMOBILIZATION OF MALTOGENIC AMYLASE FROM *Bacillus lehensis* G1 FOR MALTOOLIGOSACCHARIDES SYNTHESIS

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ABSTRACT

Maltooligosaccharides (MOS) are potential oligosaccharides in food-based applications and can be synthesized through the enzymatic synthesis of maltogenic amylase from *Bacillus lehensis* G1 (Mag1). Although MOS can be synthesized by using free enzymes, this process is hampered by poor enzyme recovery and lack of enzyme stability, which makes it unrealistic for applications. To overcome these drawbacks, several optimization methods, including enzyme immobilization approach, could be applied. A carrier-free immobilization technique that uses cross-linked enzyme aggregates (CLEAs) enhances the stability of the enzymes. Indeed, a decrease in substrate accessibility of CLEAs may hinder CLEAs applications. The substrate accessibility problem of CLEAs formation was overcome by the addition of porous agents to generate porous CLEAs (p-CLEAs). However, p-CLEAs have particles that are small in size, soft and mechanically unstable, which can cause enzyme leaching and reduce the activity, as well as the performance of the enzyme. To address these problems, p-CLEAs were entrapped in calcium alginate beads (CA). In this study, a formation of cross-linked enzyme aggregates of Mag1 (Mag1-CLEAs) were carried out to improve the stability and reusability of free Mag1. The substrate accessibility problem of Mag1-CLEAs was solved by the formation of porous CLEAs of Mag1 (Mag1-p-CLEAs). All factors affecting the formation of CLEAs were investigated. The highest activity recovery of Mag1-CLEAs 58.14 % (18.6 U/mg) was obtained at 80 % (w/v) ammonium sulphate (precipitant), 0.25 % (w/v) chitosan (cross linker) with cross-linking time of 1.5 h. In comparison, Mag1-p-CLEAs prepared with 0.8 % (w/v) citrus pectin (porous agent) exhibited 91.20 % (29.2 U/mg) activity. This developed porous material exhibited larger particles size (1.60 µm) and pore size distribution of 8 – 1000 nm. Mag1-p-CLEAs noticeably retained 80 % of their activity after 30 min of incubation at 40 °C and showed longer half-life compared to free Mag1 and Mag1-CLEAs. The 1.68-fold increase in V_{max} value in comparison to Mag1-CLEAs showed that the presence of pores of Mag1-p-CLEAs enhanced the beta-cyclodextrin (β-CD) accessibility. Next, Mag1-p-CLEAs were entrapped into calcium alginate beads. Mag1-p-CLEAs-CA prepared with 2.5 % (w/v) sodium alginate and 0.6 % (w/v) calcium chloride yielded 53.16 % (17.0 U/mg) activity and showed a lower deactivation rate and longer half-life than those of entrapped free Mag1 (Mag1-CA) and entrapped non-porous Mag1-CLEAs (Mag1-CLEAs-CA). Moreover, Mag1-p-CLEAs-CA exhibited low enzyme leaching and high tolerance in various solvents compared to Mag1-p-CLEAs. A kinetic study revealed that Mag1-p-CLEAs-CA exhibited relatively high affinity towards β-CD (K_m = 0.62 mM). MOS (261.9 mg/g) were synthesized by Mag1-p-CLEAs-CA at 50 °C through hydrolysis reaction of β-CD. Although, Mag1-p-CLEAs-CA have low transglycosylation activity, they have superior reusability and can maintain their activity for up to 11 cycles. In conclusion, the combination of CLEAs technology with entrapment approach, has proven to be a promising tool to develop stable enzymes. The developed Mag1-p-CLEAs-CA are potential biocatalyst for the continuous production of MOS.

ABSTRAK

Maltooligosakarida (MOS) adalah oligosakarida yang berpotensi dalam aplikasi berasaskan makanan dan boleh disintesis melalui sintesis enzimatik menggunakan amilase maltogenik dari *Bacillus lehensis* G1 (Mag1). Walaupun MOS boleh disintesis dengan menggunakan enzim bebas, proses tersebut terhad disebabkan oleh perolehan enzim yang lemah dan kekurangan kestabilan enzim yang menjadikan proses ini tidak realistik untuk digunakan. Untuk mengatasi kekurangan ini, beberapa kaedah pengoptimuman, termasuk pendekatan imobilisasi enzim, boleh di aplikasikan. Teknik imobilisasi bebas pembawa yang menggunakan agregat enzim terpaut silang (CLEAs) mampu meningkatkan kestabilan enzim. Namun, penurunan capaian substrat terhadap CLEAs boleh menghalang aplikasinya. Masalah kebolehcapaian substrat oleh CLEAs boleh diatasi dengan penambahan agen berliang untuk menghasilkan CLEAs berliang (p-CLEAs). Walaubagaimanapun, p-CLEAs mempunyai saiz yang kecil dan lembut, dan tidak stabil secara mekanikal yang boleh menyebabkan enzim terbebas dan mengurangkan aktiviti serta prestasi enzim. Bagi mengatasi masalah ini, p-CLEAs dikepung di dalam manik kalsium alginat (CA). Dalam kajian ini, agregat enzim terpaut silang Mag1 (Mag1-CLEAs) dibentuk untuk meningkatkan kestabilan dan kebolehgunaan semula Mag1 bebas. Masalah kebolehcapaian substrat oleh Mag1-CLEAs dapat diselesaikan dengan pembentukan CLEAs berliang Mag1 (Mag1-p-CLEAs). Semua faktor yang mempengaruhi pembentukan CLEAs dikaji. Pemulihan aktiviti tertinggi Mag1-CLEAs 58.14 % (18.6 U/mg) diperoleh pada 80 % (w/v) ammonium sulfat (pemendak), 0.25 % (w/v) kitosan (agen pemaut silang) dan 1.5 jam masa pemautsilang. Sebagai perbandingan, Mag1-p-CLEAs yang dibentuk dengan 0.8 % (w/v) sitrus pektin (agen berliang) menghasilkan aktiviti 91.20 % (29.2 U/mg). Bahan berliang ini menunjukkan saiz yang besar (1.60 µm) dan penyebaran saiz liang 8 – 1000 nm. Mag1-p-CLEAs mengekalkan 80 % aktiviti selepas pengeraman selama 30 minit pada 40 °C dan menunjukkan separuh hayat yang lebih lama berbanding dengan Mag1 bebas dan Mag1-CLEA. Peningkatan 1.68 kali ganda nilai V_{max} berbanding dengan Mag1-CLEAs menunjukkan bahawa kehadiran liang pada Mag1-p-CLEA meningkatkan kebolehcapaian beta-siklodekstrin (β-CD). Seterusnya, Mag1-p-CLEAs dikepung ke dalam manik kalsium alginat. Mag1-p-CLEAs-CA yang dibentuk dengan natrium alginat 2.5 % (w/v) dan 0.6 % (w/v) kalsium klorida menghasilkan aktiviti 53.16 % (17.0 U/mg) dan menunjukkan kadar penyahaktifan yang lebih rendah dan separuh hayat yang lebih lama daripada Mag1 bebas yang terkepung (Mag1-CA) dan Mag1-CLEAs yang terkepung (Mag1-CLEAs-CA). Selain itu, Mag1-p-CLEAs-CA mempamerkan enzim terbebas yang rendah dan toleransi yang tinggi terhadap pelarut organik berbanding Mag1-p-CLEAs. Kajian kinetik mendedahkan Mag1-p-CLEAs-CA mempunyai tarikan yang tinggi terhadap β-CD (K_m = 0.62 mM). MOS (261.9 mg/g) telah disintesis oleh Mag1-p-CLEAs-CA pada 50 °C melalui tindak balas hidrolisis β-CD. Walaupun Mag1-p-CLEAs-CA mempunyai aktiviti pentransglikosilan yang rendah, mereka mempunyai kebolehkitaran yang lebih baik dan boleh mengekalkan aktiviti sehingga 11 kitaran. Kesimpulannya, gabungan teknologi CLEAs dan teknik kepungan telah terbukti menjadi kaedah yang baik untuk menghasilkan enzim yang stabil. Mag1-p-CLEAs-CA menjadi biomangkin yang berpotensi untuk penghasilan MOS secara berterusan.

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LIST OF ABBREVIATIONS

3D	-	Three-dimentional
A295	-	Absorbance at optical density of 295 nm
A275	-	Absorbance at optical density of 275 nm
A600	-	Absorbance at optical density of 600 nm
B. lehensis	-	*Bacillus lehensis*
B. licheniformis	-	*Bacillus licheniformis*
B. stearothermophilus	-	*Bacillus stearothermophilus*
B. subtilis	-	*Bacillus subtilis*
BSA	-	Bovine serum albumin
β-CD	-	Beta-cyclodextrin
CV	-	Column volume
Da	-	Dalton
dH_2O	-	Distilled water
DMSO	-	Dimethyl sulphoxide
DNS	-	3, 5-Dinitrosalicylic acid
E. coli	-	*Escherichia coli*
EDTA	-	Ethylenediamine tetraacetic acid
EG-NHS	-	Ethylene glycol-bis (succinic acid *N*-hydroxysuccinimide)
E_d	-	Activation energy for thermal denaturation
g	-	Gram
h	-	Hour
HCL	-	Hydrochloric acid
HPLC	-	High-performance liquid chromatography
IPTG	-	Isopropyl β-D-1-thiogalactopyranoside
J	-	Joule
K	-	Kelvin
kDa	-	Kilodalton
kJ	-	Kilo joule
L	-	Liter

L. gasseri	-	*Lactobacillus gasseri*
LB	-	Luria-Bertani
mm	-	Milimeter
M	-	Molar
Mag1	-	Maltogenic amylase from *Bacillus lehensis* G1
mg	-	Milligram
min	-	Minute
ml	-	Mililiter
mM	-	Milimolar
MOS	-	Maltooligosaccharides
MW	-	Molecular weight
Ni^{2+}	-	Nickel ion
NaOH	-	Sodium hydroxide
nm	-	Nanometer
OD	-	Optical density
OFAT	-	One factor at a time
PMSF	-	Phenylmethylsulfonyl fluoride
PEG	-	Polyethylene glycol
rpm	-	Revolutions per minute
s	-	Second
SDS-PAGE	-	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	-	Scanning electron microscopy
™	-	Trademark symbol
T. pendens	-	*Thermofilum pendens*
U	-	Unit
U/ml	-	Unit per mililiter
USA	-	United State of America
UV	-	Ultraviolet
UV-VIS	-	Ultraviolet-visible spectrophotometry
V	-	Volt
V	-	Velocity
w/v	-	Weight per volume

LIST OF SYMBOLS

α	-	Alpha
β	-	Beta
°C	-	Degree Celsius
γ	-	Gamma
µm	-	Micro meter
µl	-	Micro liter
µmol	-	Micromol
%	-	Percent
-	-	Minus
+	-	Plus
1 X	-	One time
10 X	-	Ten times

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Galactooligosaccharides (GOS), fructooligosaccharides (FOS) and lactulose are well established functional oligosaccharides and their prebiotics role has been recognized in Europe, Japan and the United States (Tuohy *et al.*, 2005). Other emerging prebiotics such as xylooligosaccharides (XOS), soybean oligosaccharides (SOS) and maltooligosaccharides (MOS) also provide a potential for various applications. Maltooligosaccharides are sugar oligomers that are composed of 3 – 10 of glucose monomers that are linked together by α-1,2 and α-1,4 glycosidic bonds. It have been demonstrated as potential prebiotics in many studies (Prapulla *et al.*, 2000; Rastall, 2010). MOS that act as prebiotics can beneficially affect the host health by stimulating the growth and activity of bacteria in the colon. In fact, the prebiotics act as a nutrient for the probiotics in the host colon, especially *Bifidobacterium* and *Lactobacillus* spp. Besides, it have been used as sugar substitute, food flavour enhancer and widely used as additives in food and beverage products to control the bacterial contamination and extending the shelf life of the food. Since MOS are one of the candidates for prebiotics and is applied in food industrial areas, it is important to increase its production to meet its demand. Oligosaccharides are acquired from natural sources such as from fruits and vegetables as well as whole grains. Unfortunately, their consumption through natural elements might not be sufficient. Hence, a better way to consume oligosaccharides is by their supplementation in food products. As mentioned before, oligosaccharides can be acquired and extracted from natural sources. However, this approach is not suitable for large scale application because only a limited number of plants which are chicory root and Jerusalem artichoke are suitable sources for oligosaccharides in industrial applications (Van Loo *et al.*, 1995). Thus, chemical and enzymatic syntheses are other alternative methods to obtain oligosaccharides. Yet, enzymatic synthesis is preferable in

comparison to chemical synthesis because this method involves milder reaction processes and the uses of enzymes are feasible for industrial processes. This is because, enzymes are robust, easy to handle and utilizes inexpensive and simple substrate for oligosaccharides production. Furthermore, oligosaccharides synthesis by enzymatic approaches has attracted growing interest as an alternative to old-fashioned chemical synthesis method (Koeller and Wong, 2001).

Enzymes are the natural biocatalysts that are produced by living organisms to increase the rate of chemical reactions for life sustaining. The uses of microbial enzymes, approximately 200 types are applied in various sectors including food, agriculture, chemicals and medicine industries. Nevertheless, only 20 enzymes have been produced in large scale industry (Li *et al.*, 2012). An enzymes offer various beneficial properties, for example, it provides efficient reaction rate even at low concentration, the reaction processes takes place under milder conditions (temperature and pH value) and the process involves low toxicity along with ease of activity termination (Choi *et al.*, 2015; Li *et al.*, 2012). Moreover, the enzyme with upgraded properties becomes an important tool for recognized practical applications and is rapidly gaining interest in many studies. Thus, the advancement in enzyme technology needs to be performed to increase the number of industrial enzymes that are useful in different industrial sectors. In addition, the increasing demand for consumer goods, the need for low production cost and the decrease in the use of natural resources are the driving factors that trigger for enzyme development in processing industry, specifically in food manufacturing (Choi *et al.*, 2015).

Commercially available enzymes in most of industrial areas are amylases, protease, lipase, cellulase, xylanase and catalases, with the most versatile are amylases. Amylases can convert starch to sugar syrups and MOS. Enzymes that can produce maltooligosaccharide are mainly from family 13 (GH 13) and considered as maltooligosaccharide-forming amylase (MFAses). Glucan-1,4-α-maltotetraohydrolase or known as maltotetraose-forming amylase, glucan-1,4-α-maltohexaohydrolase or maltohexaose-forming amylase, maltotriose-forming amylase also known as (glucan-1,4-α-maltotriohydrolase) and glucan-1,4- α-maltohydrolase or usually called maltogenic amylase produce maltotetraose, maltohexaose, maltotriose, and maltose, respectively, as a main products from the hydrolysis reaction of starch.

In recent years, the importance of amylases as industrial catalysts has grown steadily. Many researches are focusing on developing amylases with improved properties to fit the needs in industrial level (Li *et al.*, 2012). It is worth mentioning that the use of enzymes in the industrial applications are challenging. This is because the enzymes are biological molecules that are difficult to work in the conventional industrial environments, such as at elevated pH and temperature and in the presence of mechanical stress due to harsh handling. Enzymes also cannot be reuse due to their solubility (Sheldon, 2010). These extreme conditions ultimately induce the conformation changes of the enzymes and leads to the reduction or loss of their activity. Thus, the improvements for the enzymes are needed to create a route for the success of enzymes applications. Moreover, most of the enzymes dissolve in an aqueous solution and it is impossible for their recovery and reuse. This condition leads to the increase of the production cost of the enzymes because of their relatively high price (Sheldon, 2007a).

In order to tackle these problems, several tools and techniques have been explored to develop a stable and efficient enzymes with an excellent properties (Silva *et al.*, 2018). Exploitation of methods to stabilize the enzymes, such as their isolation from extremophiles bacterium or through protein engineering, chemical modification, medium engineering and enzyme immobilization have been studied in many years. Although each approach has their own advantages and disadvantages, enzyme immobilization strategies have been broadly investigated on a diverse type of enzymes. The advantages of this technique include the possibility for re-utilization of the enzyme for the continuous-flow processes and modify the final properties of the enzyme by enhancing the stability, activity, selectivity, purity, specificity toward the different substrate and prevention of enzyme inhibition (Rodrigues *et al.*, 2013). Generally, immobilization techniques were applied to increase enzymes stability and recyclability while maintaining their catalytic activity. In fact, for the commercialization of the enzymes, their reusability becomes more important and failing to provide this would cause the enzymes to be uneconomic (Datta *et al.*, 2013). Thus, enzyme immobilization techniques are used as an alternative method to enhance the properties and reproducibility of enzyme for their relevant applications. In addition, immobilized enzymes could be able to retain most of their activity and

offer similar or better performance compared to soluble enzyme, if the right immobilization approach was performed (Cao *et al.*, 2003).

Various applications of immobilized enzyme can be found in industry. Immobilized β-galactosidase is used for production of lactose-free milk, immobilized invertase for synthesis of glucose and fructose mixture and immobilized nitrile hydratase for the production of nicotamide (Homaei *et al.*, 2013). In any commercialization of immobilized enzyme, the choice of immobilization techniques must be based on a specific compromise about the advantages and disadvantages between free and immobilized enzymes. There are numerous strategies of enzyme immobilization techniques that were comprehensively studied. This is includes adsorption, entrapment, affinity binding, covalent attachment and cross-linking or combination of several methods. Cross-linked enzyme aggregates (CLEAs) which are carrier free enzyme immobilization protocol have attracted increasing attention in many studies. This method was invented by Sheldon (Sheldon *et al.*, 2005). This technique display outstanding advantages, such as providing high catalytic activity, high stability, facile enzyme recovery and reusability and the procedure require low cost due to exclusion of carrier usage and does not require extensive enzyme purification for its preparation. Although CLEAs preparation is a straightforward procedure, CLEA formation might be ineffective and problematic if the enzyme contains low external lysine residues and the CLEAs have substrate accessibility problem. In addition, the CLEAs particles are normally small in sizes which are less than 10 µm. Indeed, CLEAs particles are also too soft which are not suitable to be applied in reactors. Also, they are not mechanically stable, susceptible to enzyme leaching under harsh condition and has low solvent tolerance, which can reduce the activity, as well as the performance of the enzyme (Garcia Galan *et al.*, 2011).

The rapid developments in combination of carrier and carrier-free immobilization techniques have stimulated strong interest to improve the quality of the CLEAs. CLEAs may be require physical support to increase their rigidity and stability (Garcia Galan *et al.*, 2011). The accelerated development of entrapment approach using polymers to improve the quality of the enzymes have been established in many studies (Jadhav and Singhal, 2014; Larosa *et al.*, 2018). To

exploit the advantages of entrapment using calcium alginate beads, it is essential to investigate the potential of this approach in stabilizing the CLEAs particles. In this work, cross-linking and entrapment immobilization techniques of maltogenic amylase from *Bacillus lehensis* G1 (Mag1) was performed to enhance its stability. Since Mag1 is an enzyme candidate for MOS synthesis, it was selected for further investigation.

1.2 Problem Statement

Maltooligosaccharides (MOS) offer various health and industrial benefits. The enzymatic synthesis approach is preferable and a popular method to obtain MOS. This method exhibits high product yield and involved low production cost compared to when the natural extraction method is employed. Maltogenic amylase from *Bacillus lehensis* G1 (Mag1) is a potential biocatalyst for MOS synthesis. It catalyzes both hydrolysis and transglycosylation reactions for the formation of MOS with various lengths. Although Mag1 has great potential in the formation of MOS, the use of free enzymes remains challenging due to their poor stability and cannot be reused, and this resulted in higher production cost. Due to the importance of Mag1 in the food, beverages and pharmaceutical industries, many strategies were applied to increase their operational stability. Cross-linked enzyme aggregates (CLEAs), a growing technique has been practiced for many years as a method for enzyme stabilization. The chemical linkages that were formed between enzyme molecules allows the formation of a rigid and stabilized but active enzymes. CLEAs also allow easy separation of the enzyme for subsequent process. Indeed, CLEA formation might be ineffective and problematic if (i) the enzyme contains low external lysine residues, which could be solved by co-aggregation of the enzyme with polymers or proteic feeders, such as polyethyleneimine (PEI) and bovine serum albumin. (ii) A decrease in substrate accessibility of CLEAs may hinder CLEA applications. In this study, preparation of porous CLEAs (p-CLEAs) was performed in order to solve substrate accessibility problem of CLEAs. Although p-CLEAs provide high stability for Mag1 and solves substrate accessibility problem of CLEAs technology, enzyme leaching occurs due to their small size and soft particle, which will restrict their

application. Besides, p-CLEAs might have low tolerance toward non-aqueous solvents. Thus, p-CLEAs of Mag1 are entrapped into calcium alginate beads to prevent enzyme leaching, enhance their solvent tolerance and allow ease of enzyme separation. In this study, cross-linking and entrapment approaches were applied to improve the properties of Mag1 to allow its use as biocatalyst for MOS synthesis.

1.3 Objectives of Study

There are three main objectives in this study. The objectives are:

a) To improve the stability of free Mag1 by CLEAs method and enhance substrate accessibility of CLEAs by the preparation of porous CLEAs.

b) To investigate the effects of entrapment of CLEAs into calcium alginate beads.

c) To examine the performance of immobilized Mag1 in the hydrolysis and transglycosylation reactions for MOS synthesis.

1.4 Scopes of Study

This study focuses on the improvement of Mag1 stability by the cross-linking and entrapment immobilization techniques and the application of immobilized Mag1 for MOS synthesis. Hence, the following scopes are outlined:

a) Expression and purification of recombinant maltogenic amylase from *Bacillus lehensis* G1 (Mag1).

b) Investigate the factors for the preparation of CLEAs; type and concentration of precipitant, type and concentration of cross linker, cross-linking time, type and concentration of additives and type and concentration of porous agent.

c) Characterization of CLEAs (Mag1-CLEAs, Mag1-CLEAs-Tween 20 and Mag1-p-CLEAs).

d) Screening of the factors for entrapment of CLEAs into calcium alginate beads; concentration of sodium alginate, concentration of calcium chloride and curing time.

e) Study the effect of entrapment of CLEAs into calcium alginate beads.

f) Determine the factors that affect the hydrolysis and transglycosylation reactions of immobilized Mag1 for MOS synthesis.

1.5 Rational and Novelty of the Study

The potential of Mag1 for the synthesis of MOS has been recognized by many researchers. The synthesis of MOS requires the enzyme to work in industrial operating conditions, including at elevated temperature, at the acidic and basic environment, under the harsh operating conditions and in the presence of an organic solvent. However, a soluble enzyme has moderate stability and inefficient for recyclability which hampers their application. Enzyme immobilization is one of the promising approaches to tackle those limitations. It is worth mentioning that exploring robust and renewable enzymes are important for various applications. Moreover, a continuous offering of data regarding the potential enzymes could be beneficial for the industrial organization and provide more knowledge to the scientific community in understanding the effect of enzyme immobilization in the performance of the enzyme. In the past decades, CLEAs, a simple carrier-free immobilization technique has been widely explored and this technique displays superior advantages such as provides high operational stability and high enzyme recovery. However, CLEAs formation might be problematic for the enzymes that contain low number of external lysine residue. Therefore, the addition of additive during cross-linking process is known to improve cross-linking efficiency and produce stable CLEAs. In addition, a compact structure of CLEAs will cause

substrate accessibility problem. This report is the first study in demonstrating the formation of p-CLEAs of Mag1 using citrus pectin as porous agent. Previously, only starch was applied as porous agent in the preparation of p-CLEAs. Exploring other porous agents for the preparation of p-CLEAs is crucial because not all enzymes will exhibit a higher activity if starch is used. In addition, the application of chitosan as a cross linker is also needed to be further considered. Although glutaraldehyde has been employed for CLEAs preparation for many years, its adverse effects to human and its deactivation effect for the enzyme have motivated the researcher to search for safe, biodegradable, environment-friendly and macromolecule cross linker. The findings demonstrated that the use of chitosan as a macromolecule cross linker and the addition of additive or porous agent during CLEAs preparation is a potential element that could be exploited for the production of stable and reusable CLEAs.

Moreover, because of some of the problems associated with CLEAs such as their small particle size, which might be too soft and mechanically unstable as well as their low solvent tolerance, immobilization of CLEAs on the matrices is proposed to enhance their properties and to overcome above-mentioned limitations of CLEAs, especially p-CLEAs. Limited studies were available to observe the effect of CLEAs that was entrapped into calcium alginate beads. To the best of our knowledge, this report is the first study that demonstrates the use of entrapped p-CLEAs for MOS synthesis. Also, the use of immobilized Mag1 for transglycosylation reaction remains elusive. In this study, the hydrolysis and transglycosylation reaction of immobilized Mag1 was investigated to gain a better understanding of the effect of immobilization on the reaction process and product formation by immobilized Mag1.

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