PREPARATION OF POROUS-CROSS LINKED ENZYME AGGREGATES USING SUCROSE AS POROUS AGENT

Noor Namirah Nawawi[1], Boon Cheng Kai[1], Zanariah Hashim[1], Nardiah Rizwana Jaafar[1], Rosli Md Illias[1,2]

[1]School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia
[2]Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia

Abstract: The use of maltogenic amylase (MA) for maltooligosaccharides (MOS) synthesis offers various advantages. However, lack of enzyme stability and high solubility brings major barriers for its industrial application. The exploitation of cross linked enzyme aggregates (CLEAs) method for enzyme stabilization has been studied for many years. Though, the compact structure of CLEAs leads to the substrate diffusion problem. Therefore, to create porosity and improve substrate accessibility of CLEAs, preparation of porous-CLEAs of MA (MA-p-CLEAs) was performed with the addition of sucrose as a porous agent. The MA solution was mix with different concentration of sucrose and the MA-p-CLEAs was incubated at different incubation time and temperature in order to remove sucrose. The MA-p-CLEAs prepared at 5% (w/v) sucrose yielded a 1.06-fold increase in activity compared to MA-CLEAs. In summary, the addition of sucrose for CLEAs preparation of MA improves the activity of CLEAs by creates porosity for better substrate diffusion.

Keywords: Cross Linked Enzyme Aggregates; Maltogenic Amylase; Porous Agent; Sucrose; Sucrose

1. Introduction

Maltogenic amylase (EC 3.2.1.133) (MA) is a biocatalyst that able to produce various lengths of MOS through the process of hydrolysis of various substrates [1]. Nonetheless, the use of the free enzyme for the synthesis of MOS is hampered due to the lack of its stability and reusability in which will increase its production cost. Cross linked enzyme aggregates (CLEAs) method offer various advantages such as involve simple procedures, enhance storage and operational stability of the enzyme as well as provides good reusability for the enzyme [2,3]. However, due to some undesired shortcoming of this method which is a substrate diffusion problem, a further modification of CLEAs needs to be carried out by the formation of porous-CLEAs (p-CLEAs) [4]. In this study, the development of p-CLEAs of maltogenic amylase (MA-p-CLEAs) using sucrose as a porous agent was performed to solve the problem of substrate diffusion limitation. The optimum preparation conditions for MA-p-CLEAs preparation were investigated. Then, its activity was compared with non-porous CLEAs of maltogenic amylase (MA-CLEAs).

2. Materials and Methods

2.1 Preparation of MA-p-CLEAs and MA-CLEAs

In a 50ml falcon tube, MA solution with and without sucrose was added into ammonium sulphate to generate MA-p-CLEAs and MA-CLEAs, respectively and was incubated at 4°C under continuous shaking of 200rpm. Next, the cross linking operation was performed for 1.5 hours using chitosan. Then, the mixture was centrifuged and the supernatant was discarded. The insoluble form of CLEAs were washed 3 times using 50mM potassium phosphate buffer (pH 7) and were re-suspended with potassium phosphate buffer and stored at 4°C for further use. As for MA-p-CLEAs, different amount of sucrase was added into MA-p-CLEAs solution. The different incubation times and incubation temperatures were applied to remove sucrose from MA-p-CLEAs. Lastly, the insoluble MA-p-CLEAs has washed again for 3 times using 50mM potassium phosphate buffer (pH 7), re-suspended in the same buffer and stored at 4°C for further use.

2.2 Enzyme activity

The enzyme activity of MA-CLEAs and MA-p-CLEAs were measured using dinitrosalicylic acid (DNS) method [5] with beta-cyclodextrin (β-CD) as a substrate. The assay was performed for 10 minutes at 40°C. The activity recovery of MA-CLEAs and MA-p-CLEAs was calculated using Equation 1:

Activity recovery =
[Total activity of CLEAs (U) / MA activity used for CLEAs preparation (U)] X 100 (1)

3. Results and Discussions

The first investigated factor when preparing MA-p-CLEAs is the concentration of sucrose. It has been noted that the concentration of porous agent will affect the size of pores of p-CLEAs. Wang [4] found that the activity of papain-p-CLEAs was increased with the addition of a high concentration of starch (porous agent). However, as mentioned by another investigator, an excessive amounts of porous agent will leads to the formation of bigger and irregular pores structure which can cause rupture to the CLEAs structures and consequently leads to enzyme leakage and affects the activity of CLEAs [6]. Other factors that need to be considered during p-CLEAs preparation are the incubation time and temperature to remove sucrose. In fact, these factors can also affect the activity of MA-p-CLEAs. Shorter incubation time and low temperature can cause incomplete removal of sucrose and can prevent the accessibility of the substrate to the active site of MA-p-CLEAs. In comparison, at longer incubation time and higher temperature, the reduction of MA-p-CLEAs activity could be due to denaturation for MA-p-CLEAs. It has been noted that exposure of the enzyme at a higher temperature in a longer incubation period could cause changes in the conformation of the enzyme and leads to enzyme denaturation [7]. Hence, this study suggested that incubation for 15 minutes at 30°C is the best condition to remove most of the sucrose of MA-p-CLEAs to form pores and yet least denaturation of MA-p-CLEAs. The optimized conditions for the preparation of MA-p-CLEAs are presented in Table 1.

Then, the activity recovery of both MA-p-CLEAs and MA-CLEAs was compared. MA-p-CLEAs exhibited a 1.06-fold increase of activity than MA-CLEAs. Therefore, in this study, the

enhancement of MA-p-CLEAs activity indicated that sucrose can be used as a porous agent for the preparation of p-CLEAs. The addition of sucrose during CLEAs preparation and its removal after the cross linking step leaving a pore structure on the CLEAs particles which enhances substrate accessibility to the active site of the Mag1 and subsequently increases the catalytic activity of CLEAs.

Table 1: Optimized conditions for preparation of MA-p-CLEAs

Factors	Optimized conditions
Sucrose concentration	5% (w/v)
Incubation time	15 minutes
Incubation temperature	30 minutes

4. Conclusion

The porous-cross linked enzyme aggregates of maltogenic amylase (MA-p-CLEAs) with improved activity as compared to MA-CLEAs has been developed in this study. This developed MA-p-CLEAs is a potential biocatalyst that can use for the production of MOS which can be applied in various applications such as for prebiotics synthesis.

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References

[1] N.H.A. Manas, S. Pachelles, N.M. Mahadi, R.M. Illias, The characterisation of an alkali-stable maltogenic amylase from Bacillus lehensis G1 and improved malto-oligosaccharide production by hydrolysis suppression, PloS one 9(9) (2014) e106481.

[2] R. Sheldon, R. Schoevaart, L. Van Langen, Cross-linked enzyme aggregates (CLEAs): A novel and versatile method for enzyme immobilization (a review), Biocatalysis and Biotransformation 23(3-4) (2005) 141-147.

[3] J.D. Cui, S.R. Jia, Optimization protocols and improved strategies of cross-linked enzyme aggregates technology: current development and future challenges, Critical reviews in biotechnology 35(1) (2015) 15-28.

[4] M. Wang, C. Jia, W. Qi, Q. Yu, X. Peng, R. Su, Z. He, Porous-CLEAs of papain: application to enzymatic hydrolysis of macromolecules, Bioresource technology 102(3) (2011) 3541-3545.

[5] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analytical chemistry 31(3) (1959) 426-428.

[6] J. Cui, S. Jia, L. Liang, Y. Zhao, Y. Feng, Mesoporous CLEAs-silica composite microparticles with high activity and enhanced stability, Scientific reports 5 (2015) 14203.

[7] R. Vishwakarma, R. Banerjee, Process Optimization for Enhancement of Fermentable Sugar from Cyperus sp. through Enzymatic Saccharification, Journal of Biofuels 10(1) (2019) 1-11.