포괄법에 의해 고정화한 전분 가수분해 효소의 반응 경향성 연구

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Study on the reaction behavior of starch hydrolysis by encapsulated enzyme

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Introduction

Starch is major storage product of many economically important crops such as wheat, rice, potato. Beside the use of the starch-containing plant parts directly as a food source, starch is harvested and used as such or chemically or enzymatically processed into a variety of different products such as starch hydrolysates, glucose syrups, fructose, starch or maltodextrin derivatives. Moreover, starch has been used as a thickening agent in textile industries. the chemical methods to remove starch from textiles, however, are harmful for fabrics and environment.

In order to figure out these problems, enzymatic approaches including α-amylase family have been extensively studied in recent years. α-amylase and amyloglucosidase are widely used in food and textile industry, and hydrolyzed from starch to glucose.

Conventionally, enzyme reactions have been carried out in batch processes by incubating a mixture of substrate and soluble enzyme. It is technically very difficult to recover active enzyme from the reaction mixture after the reaction for reuse. Accordingly, the enzyme and other contaminating proteins are generally removed by denaturation by pH or heat treatment during procedures to isolate the product from the reaction mixture. This is uneconomical, as active enzyme is lost after each batch.

In this study, to eliminated the disadvantages such as stability of enzyme, cost, recycle of enzyme and diffusion limitation of substrates in the conventional process, amyloglucosidase was covalently immobilized on the surface of silica gel 60 and DEAE-cellulose to reduce the substrate transfer limitation. In order to enhance the formation of substrate-enzyme complex, the surface characteristics of support materials were modified by the silanization and polyethyleneimine coating.

Experimental

1. Material

   Soluble starch was purchased from SHOWA (Japan). Amyloglucosidase, silica gel 60, 3-aminopropyltriethoxysilane, sodium alginate, DEAE-cellulose were obtained from Sigma Chemicals Co (St,Louis,MO). Glutaraldehyde, polyethyleneimine were purchased from Merck (Darmstadt, Germany).

2. Immobilization of DEAE-cellulose of non-controlled surface

   2g DEAE-cellulose were dispersed in 144ml pH 7.0 phosphate buffer. Then 16ml

glutaraldehyde was added with magnetic stirring for 2 hour. Then mixture was washed and filtered with pH 7.0 phosphate buffer. The solid material was dissolved in 20ml amyloglucosidase solution(400u/ml). The immobilization process was performed for 3 hour at 5℃.

3. Immobilization of Hydrophilic modulation on DEAE-cellulose

1.32ml Polyethyleneimine and 2g DEAE-cellulose were dissolved in 36.68ml pH 7.0 phosphate buffer. The mixture was stirred at RT for 1 hour, then washed and filtered with pH 7.0 phosphate buffer. And 2g DEAE-cellulose were dispersed in 144ml pH 7.0 phosphate buffer. Then 16ml glutaraldehyde was added with magnetic stirring for 2 hour. Then mixture was washed and filtered with pH 7.0 phosphate buffer. The solid material was dissolved in 20ml amyloglucosidase solution(400u/ml). The immobilization process was performed for 3 hour at 5℃.

4. Encapulation of Immobilized enzyme on DEAE-Cellulose

1.2g sodium alginate was dissolved in 100ml pH 5.5 acetate buffer for 3h. Then 2g immobilized enzymes were added in sodium alginate solution. And alginate-enzyme mixture was degassed under reduced pressure. The alginate-enzyme mixture was carried by microtubing pump and dropped into 0.1mol calcium chloride solution (100ml) by dropping device (Fig 3).

Result and Conclusion

The surface characteristic of carriers was adjusted to hydrophobicity by silanization. The profiles of glucose concentration with time intervals were investigated by immobilizaed amyloglucosidase at 60℃ and pH 5.5 for 16 hour. As shown in Fig 1, the formation of glucose increased as more amounts of immobilized amyloglucosidase were used in reaction system, then the reaction reached an equilibrium. Then, the produced glucose was shown the low value in all range of enzyme concentration.

In general, starch has a high hydrophilic properti in aqueous solution, whereas silanized silica gel has a hydrophobic surface characteristic. Therefore these repulsive traits between starch and immobilized enzyme led to the limited formation of enzyme-substrate complex as well as low concentration of glucose.

And, the surface characteristic of carriers was adjusted to hydrophilicity by polyethyleneimine coupling. the profiles of glucose concentration with time intervals were investigated by immobilized amyloglucosidase at 60℃ and pH 5.5 for 16 hour. As shown in Fig 2, the hydrophilic surface characteristic of PEI-couplied silica gel was more favorable to the hydrolysis of starch than the hydrophobic surface characteristic of silanized silica gel. The hydrophilic treatment of carriers by PEI coupling functioned as a driving force to form complexes between amyloglucosidase and starch.

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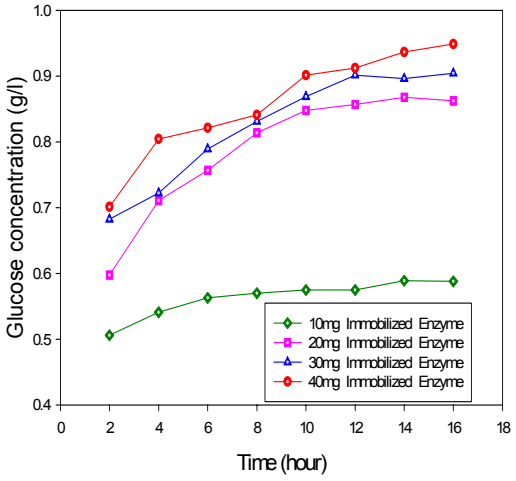

Figure 1. Efficiency of immobilized enzyme by silica gel 60 (Hydrophobicity)

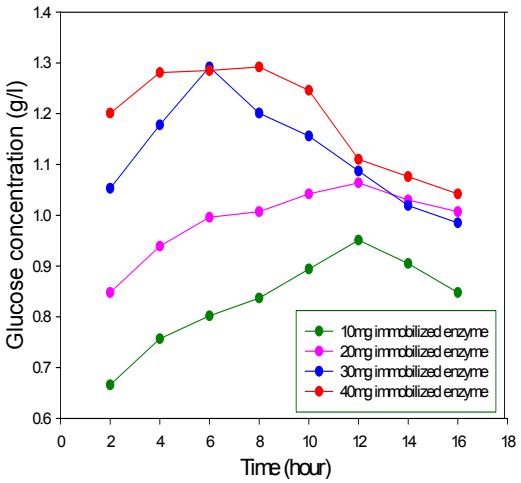

Figure 2. Efficiency of immobilized enzyme by silica gel 60 (Hydrophilicity)

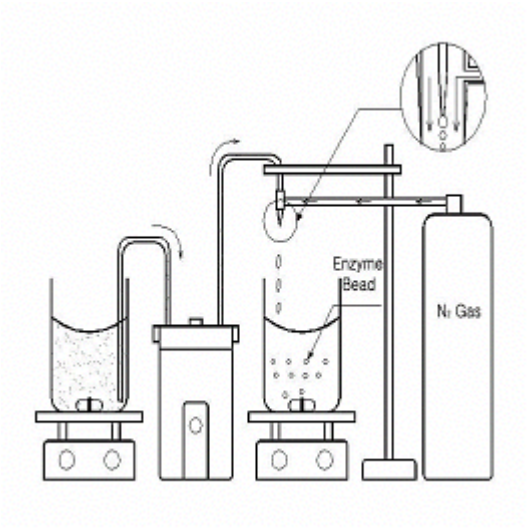

Figure 3. Experimental apparatus for bead formation