The use of immobilized enzyme in starch bioconversion: an update review

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Abstract. Starch bioconversion enzymes play an important role in the food industry, raising up a vast research space. Immobilization of alpha-amylase, amyloglucosidase and glucose isomerase is a promising topic for ongoing research. In this review, we provide an updated overview of various carriers for carbohydrase immobilization, with the primary focus on the food industry. The method used in this review is the literature study method. The immobilization methods of carbohydrase enzymes are encapsulation by Ca-alginate, covalent and ionic bonding by chitosan, adsorption by ion exchange resin and cross-linking by glutaraldehyde and Bovine serum albumin as protein feeder, and mix of them. The research shows the ability of enzymes that can be used repeatedly while maintaining their activity. Immobilization increases the enzymes stability towards pH, temperature, and type of substrate. Through this method, various types of sugar such as maltose, glucose and fructose can be produced with reduced production costs. In future, immobilized enzymes are going to play a vital role in various industries not only in food, but including pharmaceuticals, chemicals, and fuel.

1 Introduction

The use of enzymes in food processing technology has seen rapid development in the 21st century. Enzymes have become an important agent that actively contributes to the downstream processing and development of food products to create functional foods that are not only rich in nutrition, but also have an excellent taste and wide variety.

Enzymes are substantially extracted from plant, animal, and microorganism tissues. This makes enzymes more environmentally friendly and does not produce side reactions that can contaminate the product. However, behind these advantages and benefits, there are obstacles such as the cost being relatively expensive when compared to other chemicals in the market. Several solutions have been attempted to maximize the use of enzymes and one of them is by using immobilization techniques. The enzyme will be linked to a certain matrix so the enzyme can be recovered after the reaction. This allows the enzymes to be used repeatedly depending on the immobilization technique.

This paper aims to review the latest research results on the use of immobilized enzymes in starch bioconversion process.

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The starch bioconversion by enzyme is a process commonly carried out to produce food products with varying tastes and nutritional content. Starch is broken down into its monomers, namely simple sugars. Such as in the making of glucose syrup, fructose syrup, and similar sweeteners. This product is produced through two main processes, called liquefaction and saccharification. The isomerization process can be carried out as an additional process to alter the kind of simple sugars to different forms to increase their sweetness.

Three types of enzymes play a role in the process of liquefaction, saccharification, and isomerization. They are alpha-amylase, amyloglucosidase (AMG), and fructose isomerase.

Initially, the enzyme could only be used once per cycle. However, with the development of research, the enzyme can be modified by methods that are suitable for the enzyme and substrate until it is possible to recover the enzyme after the cycle and use it again for the same cycle on a different substrate.

2 Methods and Literature Research

The method used in this journal review is the literature study method. This method is carried out by collecting literature sources in the form of books, current research results, and references that are relevant to the topics discussed. Search engines such as ScienceDirect, Google Scholar, Google, and Researchgate are also used in the literature search process using keywords "enzyme immobilization" and "starch bioconversion." The data obtained is then analyzed and classified according to the sub-sections in the discussion.

3 Results and Discussion

3.1 Immobilized Alpha-amylase

Amylase works endogenously in hydrolyzing the α-1,4 bond on the glycosyl residue of the polysaccharide chain. Research on Alpha-amylase immobilization is mostly performed by encapsulation method using sodium alginate dissolved in water. After that, enzyme concentrations of 2%, 3%, and 4% were added. The beads are formed when the solution is dripped using a syringe into 100 ml of CaCl₂ solution while stirring continuously. This method produced immobilized Alpha-amylase that worked optimally at a temperature of 60℃, incubation time of 20 minutes, and amylase activity of 2522 U/ml. The number of repetitions was 6 times and 12 times. Meanwhile, amylase beads from Aspergillus sclerotiorum with a size of 0.3 mm, weight of 0.5 g, and enzyme concentration of 3%.

The repetition was 7 times and the enzyme decreased in activity by 35% at the end of the cycle. Bentonite matrix became the object of research to trap Alpha-amylase which was purified from Aspergillus fumigatus at an optimum temperature of 70℃, 6 repetitions, and the activity remained 42% at the end of the repetition.

Apart from the encapsulation method, it is also possible to use a combination method of covalent bonding to the chitosan matrix. A glutaraldehyde solution was used to precipitate the chitosan before mixing it with amylase isolated from Rhizoctonia solani AG-4. That immobilized enzyme can be used for 7 repetitions with a 20% reduction in activity in the last repetition. Amylase linked to acrylic amidrazone fabric matrix can maintain its activity until the 15th use with up to 53% reduction in activity at the end of the cycle. This is a combination method between crosslinking and covalent binding.
The large number of cycles obtained proves that the method has the opportunity to be applied not only in the food industry but also in pharmacies and fuel. The broad space for the Alpha-amylase immobilization method caused researchers to combine the two methods. Immobilization of Thermamyl 2X Alpha-amylase with a combined method of adsorption and crosslinking on duolite A-568 resin exchange matrix. The optimum immobilized Alpha-amylase was obtained at pH 7, used 3 times, and could be stored for 25 days.

3.2 Immobilized Amiloglucosidase (AMG)

Amiloglucosidase (AMG) is an exogenous enzyme that hydrolyzes starch from the non-reducing end of the α-1,4 and 1,6 bonds. The product of this enzyme is free glucose molecules. The effectiveness of this enzyme is utilized in the starch saccharification process. A large group of microorganisms such as fungi, yeast, and bacteria have the ability to produce AMG.

AMG has been immobilized using several methods such as cross-linking. AMG Cross-Linked Enzyme Aggregates (CLEAs) prepared with the addition of a magnetic nanoparticles matrix yielded 93% activity compared to the free enzyme. There is increased stability, and more resistance to high temperatures, and the enzyme is more sensitive to substrates. The ease of re-separation allows the enzyme to be used up to 43 times. Immobilized AMG on Chitosa-SDS capsules with a diameter of 0.3 mm and 83% activity compared to free enzyme have been tried on corn starch substrate.

Magnetic CLEAs of AMG from Aspergillus niger were studied with the addition of Bovine serum albumin (BSA) as a protein feeder. This method resulted in AMG CLEAs that could be used 6 times and could produce glucose syrup with a high Dextrose Equivalent (DE) of 95% at pH 4.5. The large number of lysine amino acid residues in the enzyme affects the effectiveness of crosslinking, which can be overcome by adding a protein source such as BSA, which is rich in amino acids. This enhances the bond formation performance, size, flexibility and stability of AMG CLEAs. Furthermore, AMG immobilization can be performed using a covalent bonding technique on the carrageenan matrix. Activation of the matrix can be done by using Polyethylene amine (PEI) and then followed by the addition of glutaraldehyde up to a concentration of 20mM. The results show 11 times usage, and the enzyme activity was maintained at 100%. In addition, immobilized AMG is more resistant to acidic conditions than free AMG enzyme.

3.3 Immobilized Glucose Isomerase (GI)

Glucose isomerase (GI) is an enzyme that catalyzes the reversible isomerization reaction of glucose to fructose. This enzyme plays an important role in the production of High fructose corn syrup (HFCS). GI works by catalyzing aldose to ketose. This enzyme can be isolated from microorganisms such as Steptomyces sp., S. rubiginosus, A. missouriensis, Thermus neapolitana, Caldicrobacter algeriensis, and Anoxybacillus gonenis. GI is optimal at 60°C and pH 7.5. This enzyme requires activators such as Mg2+ ions in MgSO4.7H2O compound.

GI can be immobilized using several techniques to improve their stability and reusability. Some of the matrices that can be used include DEAE-sepharose, chitosan, alginate, agar and graphene oxide (GO). Immobilized GI from Caldicrobacter algeriensis by adsorption method maintained the enzyme activity as much as 89% at pH 7 and temperature 90°C. In addition, GI from Anoxybacillus gonenis G2 can also be effectively immobilized using the DEAE-sepharose matrix by utilizing a combination of covalent and ionic bonds. The model is promising to be applied to the sweetener industry such as the manufacture of HCFS.
not much different compared to free GI enzymes, enzymes in the immobilized state are more resistant to acidic pH, heat resistance, and can be stored longer [46, 47, 48]. Overall, the use of immobilized carbohydrases is summarized in Table 1.

<table>
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4 Conclusion

Conclusio

The use of immobilized enzyme in starch bioconversion process has a good prospect to be studied. The research summarized in this paper shows several successful immobilization techniques such as crosslinking method, entrapment method, encapsulation method, covalent and ionic bonding, combining two methods and adding some agents that improve the ability of enzyme to bind with its matrix. The encapsulation method of starch hydrolyzing enzyme is the most frequently developed topic. All methods show...
improvement in enzyme stability, temperature resistance, resistance to high and low pH, and maintenance of enzyme activity during repeated use.

References


