혼성 SiO_2-chitosan bead를 이용한 단백질 분해 효소의 고정화와 연속적인 고정화 효소 막반응기의 설계

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Immobilization of Protease into the hybrid silica-chitosan beads and a design of continuous immobilized enzyme membrane reactor

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1.Introduction

An enzyme is a biological catalyst, which has high efficiency under mild conditions and is highly selective. In the concept of utilizing the natural catalysts for the manufacture of various products, such as foods or pharmaceuticals, the technique for immobilizing enzyme was born. Immobilized enzymes are currently the object of considerable interest. This is due to the expected benefits over soluble enzymes or alternative technologies. There are several reasons for the preparation and use of immobilized enzymes. The two main targeted benefits are easy separation of the enzyme from the product, continuous operation of the reactor and reuse of the enzyme. The number of applications of immobilized enzymes is increasing steadily. So, numerous methods of immobilization on a variety of different materials have been developed.[1-2]

The objective is to prepare the hybrid silica chitosan beads and investigate the continuous immobilized enzyme membrane reactor system. Furthermore,

trypsin was selected as a model enzyme to assess its potential application for the enzyme immobilization on the organic-inorganic hybrid beads. The membranes were used for the separation of oligopeptide in immobilized enzyme reactor.[3]

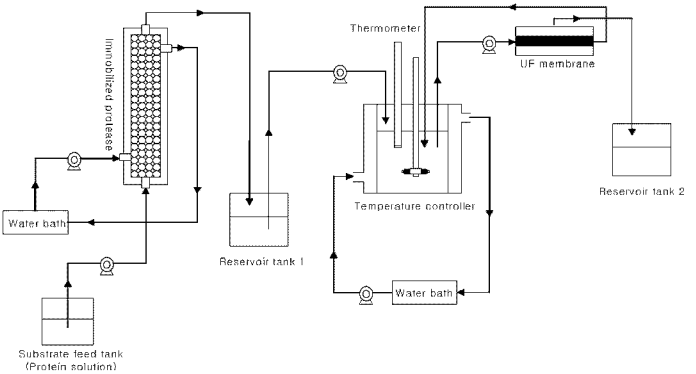


Fig. 1. Schematic diagram of the immobilized enzyme reactor system.

2.Experimental

2.1. Absorption of protease in TMOS-based silica.

Tetramethylorthosilicate (TMOS) was prehydroyzed by addition of a diluted ammonia solution and sonicated. Trypsin(1wt%) buffered at pH 7.8 were then added and mixtures stirred for 1min. The mixture was condensed quickly after the addition of the surfactant and resulting particles were left to stand for 10min at room temperature. The resulted particles were washed with phosphate buffer (pH 7.8) and stored at 4℃

2.2. Preparation of chitosan solution

Low molecular chitosan flakes were added into distilled water and suspended by magnetic stirring for 10min. Acetic acid was then added and

mixing continued for 3hr at room temperature and filtered through a non-woven fabric.

2.3. Immobilization of adsorbed protease into chitosan beads

The stored particles containing trypsin were suspended in chitosan solution. This suspension was injected into the crosslinking solution. The beads were cured for 30min and washed with phosphate buffer (pH 7.8). The obtained beads were left to dry overnight at 4℃ and then used. [4]

2.4. The activity test of immobilization protease

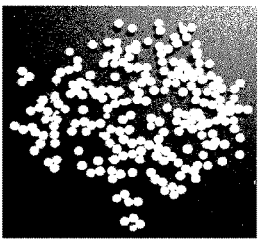
The activity of trypsin was measured at 25℃ by following the increase in absorbance at 405nm with N-benzoyl-DL-arginin-p- nitroanilid hydrochlorid (BAPNA) as a substrate. A bovine serine albumin as a model protein was chosen to reveal the possibility of immobilized enzyme reactor. [2]

3.Results and Discussion

Table 1. The activity of trypsin immobilized to different methods. (The Activity test was measured BAPNA substrate for 30min at 25℃)

soluble trypsin	immobilization of trypsin into chitosan	absorbtion of trypsin onto TMOS -based silica	immobilization of absorbed trypsin into chitosan beads
0.714	0.154	0.702	0.359

The directly immobilized trypsin showed the lowest activity. But, absorbed trypsin in TMOS-based silica was similar in activity to the free trypsin(Table 1).



Fig, 2. The photograph of the immobilized trypsin into the hybrid silica-chitosan beads.

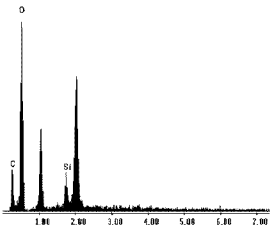


Fig. 3. EDS peaks of the immobilized trypsin into the hybrid silica-chitosan beads.

Table 2. Effect of ratio of TMOS to chitosan and condition of hydrolysis.

chitosan(g)	TMOS(g)	hydrolysis	activity
8g	0.3g	HCl	0.103
8g	0.3g	NH_3	0.194
8g	0.6g	HCl	0.187
8g	0.6g	NH_3	0.359

The physical and chemical properties of the immobilized trypsin in comparison to the free enzyme were studied using various stability tests. The results have shown that the trypsin immobilized into silica-chitosan beads

exhibits an improved chemical resistance and storage stability.

4. Conclusion

It has been shown that enzyme like trypsin can be immobilized successfully to silica-chitosan beads is actually improved compared with the free enzyme. Also, our preliminary investigations indicate that several other enzyme can be immobilized using the hybrid silica chitosan beads immobilized method.

5. reference

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