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POSSIBILITY OF COMBINATION INORGANIC (CELITE) AND ORGANIC (ALGINATE) MATERIALS TO IMMOBILIZE GLUCOAMYLASE ENZYME

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ABSTRACT

Glucoamylase (AMG) is an enzyme that is widespreadly used in food industry. Celite and alginate are two polymers that can immobilize enzyme rather well. With celite, under the optimal immobilization conditions:AMG/ celite was 2/1 (ml/g), performance of immobilized enzyme (%) was 51,49%. But it was easy to cause the constraint in the use fo immobilized AMG on celite in reaction column so using celite-alginate complex was observed. The result demonstrate that AMG stability was increased, Performance of immobilized enzyme was 42,75%. Otherwise, after 22th use, the retained activity for immobilized AMG was found as 53% compared to initial time at 50^{o}C. Therefore, celite-alginate complex has potential application in immobilization of AMG.

Key words. enzyme AMG- glucoamylase, immobilize.

1. INTRODUCTION

Biotechnology is one of industries that provide most important application to industry and life today. In particular, enzyme technology is applied frequently in variety of areas such as health care, environment, and food. However, in the market, the enzyme price is relatively high, so enzyme immobilization is focused to increase the ability to reuse enzyme. Therefore, we can control the enzyme process and reduce the product price.

Alginate and celite are commonly used as carriers in immobilized enzyme technology, but they often have their disadvantages [3, 4, 7]. To solve these problems, we tend to create the copolymer carrier. We initially study the possibilities of combining inorganic and organic materials to form copolymer carriers with higher efficiency [9].

Based on the results of previous research in last year with our study of the ability of AMG immobilized enzyme on celite, we continue conducting our research with following topic:

"Study of the ability of enzyme immobilization AMG by celite- alginate complex". Contents of study consist: Factors affect the immobilized enzyme process and reaction. The ability to reuse immobilized enzyme complex and conditions for preserving immobilized enzyme.

2. MATERIALS AND METHODS

2.1. Materials

AMG 300L (commercial glucoseamylase produced from genetically modified strains of Aspergillus), AMG liquid, optimum operating temperature approaches 50°C. Celite 545 with 0. 1-1 mm size is produced from Merck Company, Germany. Sodium alginate is produced from Kanto Company, Japan.

2.2. Methods

Determination activity of glucoamylase by DNS method (Miller,1959) [6]. Protein content is determined by Lowry method [5]. Method of enzyme immobilization [2, 8].

Step1: Active celite by alcohol, nitric acid.

Step2: Immobilize enzyme on celite.

Step 3: Immobilized enzyme on celite (step 2) entrapped by alginate.

**Method of calculation*

Total immobilized proteins = Total initial proteins - Total remained mobilize proteins.

Performance of immobilized proteins (%) = Total immobilized proteins /total initial proteins.

Performance of immobilized enzyme (%) = Total unit immobilized enzyme activity / Total initial unit enzyme activity.

Relative activity rate (%) = unit immobilized enzyme activity/ unit free enzyme activity.

3. RESULTS AND DISCUSSION

3.1. AMG immobilization on celite

AMG Immobilization on celite (step 1 and 2).

3.1.1. Factors affect to AMG immobilization on celite

Enzyme Immobilization on celite was reacted more effectively with alcohol and HNO_3. And Ratio AMG/ celite was 2/1. (Note: the amount of AMG must be diluted to 10 ml/ 1 gam celite). Increasing shaking speed will facilitate enzymes contact with celite and absorb deeply on the surface of celite. If the shaking speed was too high, it would make enzymes that attach on the surface push out from the celite particles.

Table 1. Factors affect to AMG immobilization on celite

Active substances					
Active substances	Alcohol	Alcohol + HNO_3	-	-	-
Performance of immobilized protein (%)	23,59	26,62			
Performance of immobilized enzyme (%)	15,17	17,43			
Ratio enzyme : celite					
Enzyme / celite (v/w)	6 / 1	3 / 1	2 / 1	1 / 1	0,6 / 1
Performance of immobilized protein (%)	60,10	72,63	76,33	78,37	81,60
Performance of immobilized enzyme (%)	37,07	46,28	49,59	40,16	37,13
Shaking speed					
Speed (round/minute)	Let stand	60	80	120	160
Performance of immobilized protein (%)	66,53	74,39	78,93	70,16	61,99
Performance of immobilized enzyme (%)	41,60	47,82	51,49	45,17	38,88

3.1.2. Factors affect on activity rate of AMG immobilized on celite

Table 2. Effects of pH and temperature on AMG immobilized on celite

pH factor							
pH	3,0	3,5	4,0	4,5	5,0	6,0	7,0
Immobilized enzyme activity rate (UI/g)	2019	2214	2357	2356	2223	1349	477
Relative activity rate (%)	85,66	93,95	100	99,96	94,30	57,22	20,24
Temperature Factor							
Temperature (^{0}C)	40	50	55	60	65	70	80
Immobilized enzyme activity rate (UI/g)	999	2223	2688	3291	3037	3782	2600
Relative activity rate (%)	26,42	58,80	71,09	87,03	80,31	100	68,75

PH range of immobilized enzyme activity was extensive from 3.0 to 5.0. When pH was greater than 5; the immobilized enzyme activity began to decline rapidly because enzyme activity became low in neutral environment or alkaline environment.

Immobilized AMG worked well at temperature ranging from 55°C - 65°C, and activity reached maximum value at 60°C.

3.1.3. The reuse of AMG immobilization on celie

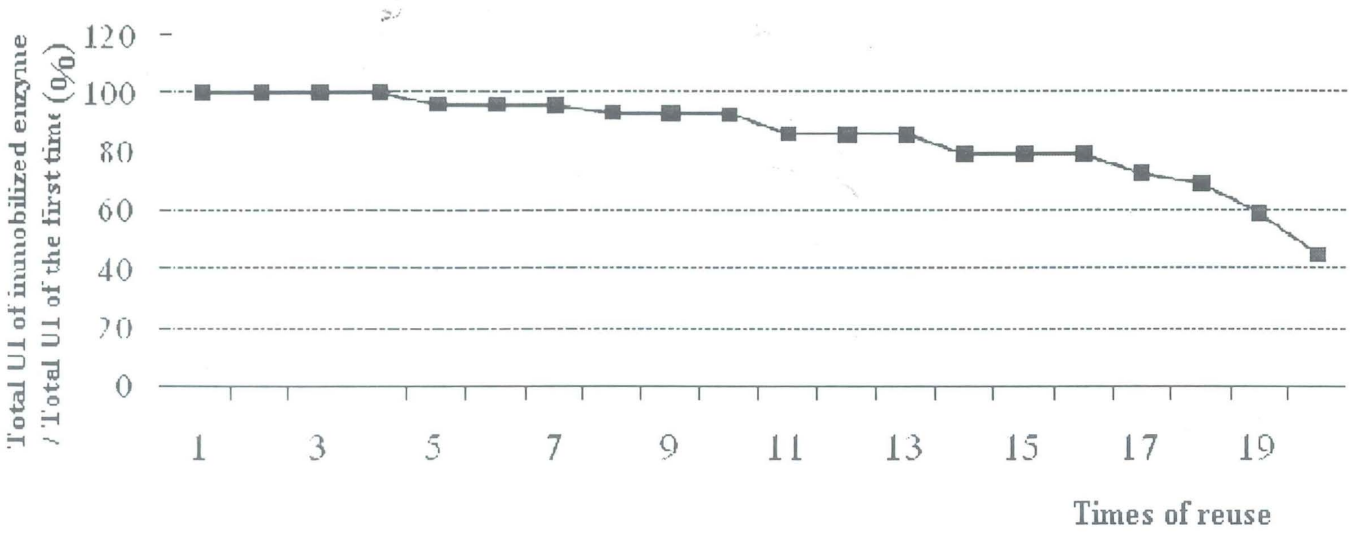

Figure 1. The ability to reuse of immobilized AMG on celite

The results shown in figure 1 demonstrated that through 10 times of reuse, the immobilized enzyme remained 93% activity compared to initial activity. At the 15th of reuse, activity remained 79% and 45% at the 20th time of reuse.

Other studies [1] also suggested that the link between glucoamylase and inorganic carriers was stable. Glucoamylase immobilized on inorganic carriers can be used in constant reaction to produce glucose.

3.2. AMG Immobilization on celite-alginate

3.2.1. Factors affect to enzyme immobilization

Due to the constraint in the use of immobilized AMG on celite in reaction column, we initially used coated alginate (step 3).

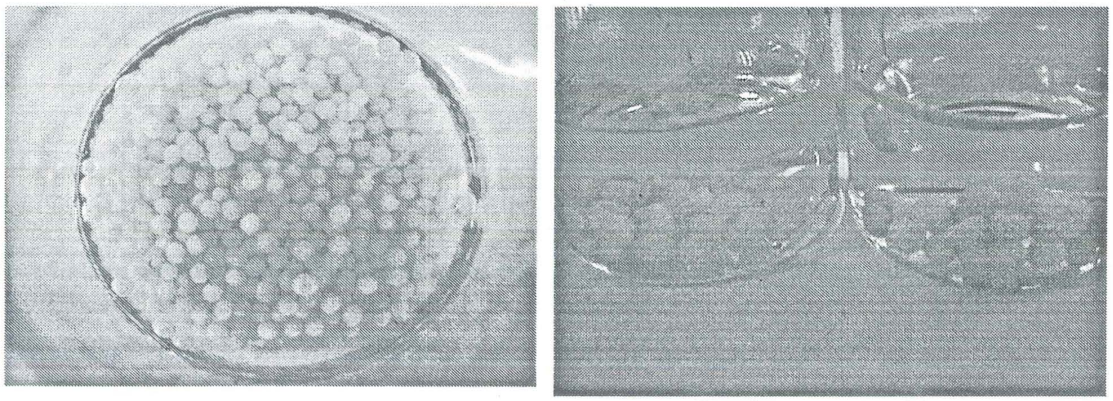

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Figure 2. AMG gel particles are immobilized on celite-alginate

Table 3. Alginate concentrate and immobilized time affect performance of AMG on celite-alginate

Alginate concentration					
Alginate concentration (%)	1	2	3	4	
Performance of immobilized protein (%)	49,40	50,23	50,00	49,68	
Performance of immobilized enzyme (%)	4,00	8,80	14,50	6,86	
Immobilization time					
Immobilization time (minute)	15	30	60	90	120
Performance of immobilized protein (%)	50,51	51,89	52,44	53,55	54,38
Performance of immobilized enzyme (%)	18,64	21,00	42,75	20,45	15,58

Based on the above results, we chose alginate with 3% concentration and immobilization time was I hour for optimal immobilization performance

3.2.2. Effects of temperature and pH on AMG immobilized activity on celite-alginate.

Table 4. Effects of temperature and pH on the AMG immobilized activity on celite-alginate

Temperature factor					
Reaction temperature (^{0}C)	40	50	60	70	80
The immobilized enzyme activity rate (UI/g)	235	261	272	245	109
Relative activity rate (%)	86,34	96,09	100	90,01	40,21
pH factor					
pH reaction	3	4	5	6	7
The immobilized enzyme activity rate (UI/g)	167	285	261	174	76
Relative activity rate (%)	58,82	100	91,69	60,98	26,76

Based on the results of our study of the effects of temperature and pH on AMG immobilized activity on celite-alginate, we obtained following findings:

Compared to the optimal temperature of free enzymes range from 45°C - 55°C, the highest temperature of immobilized enzymes reached to 70°C. It indicated that combination of organic materials and alginate as immobilizing enzyme in alginate gel increased more the ability of heat resistance of immobilized enzymes compared to free enzyme.

The immobilized enzyme activity rate reached highest level at pH = 4.

3.2.3 Application and reuse of AMG immobilized on celite-alginate

Using continuously reaction column was very effective not only for the enzyme inhibited by last product to produce glucoamylase, but also for the use of immobilized enzyme.

Study of the application of AMG immobilization on celite-alginate, we achieved following results.

With dextrin as a substance, immobilized AMG split up effectively and created more glucose than starch substance.

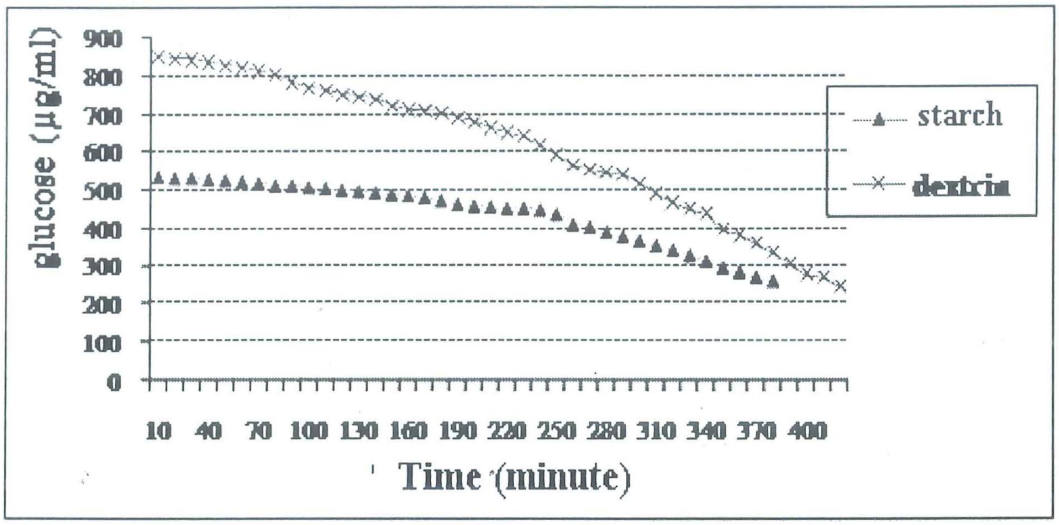

Figure 3: The capacity of starch and dextrin hydrolysis by AMG immobilized on celite alginate

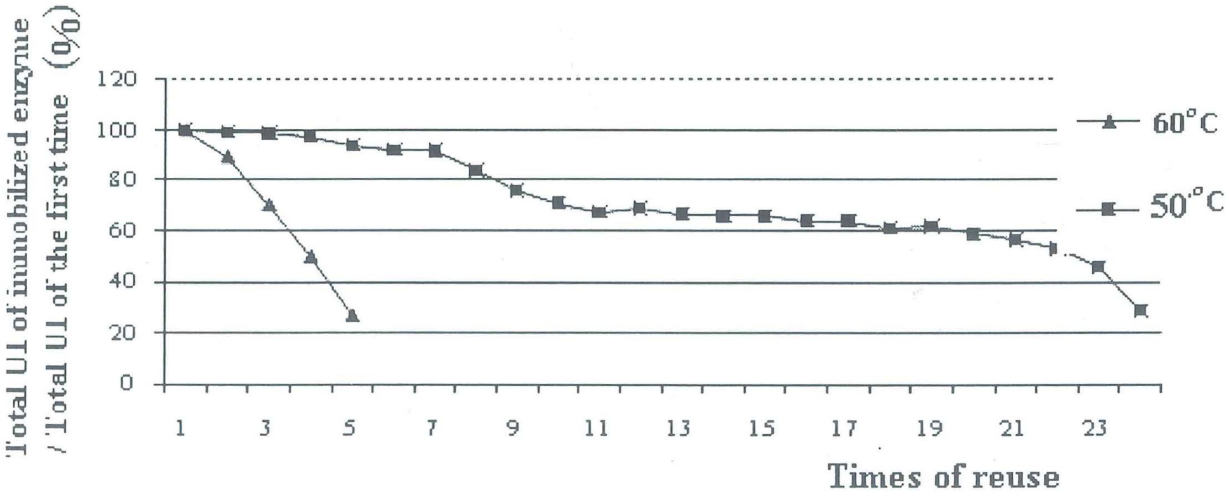

Figure 4. Ability of reuse of immobilized AMG on celite –alginate at different temperatures

Considering reusability, immobilized AMG was only reused from 3 - 5 times at the temperature 60°C. Nevertheless, AMG activity was not stable at the optimal temperature 70°C in part because gel particles enlarged that caused the loss of enzymes. After 22 times of reuse, the

enzyme activity rate remained 53% compared to initial time at temperature 50°C. Thus, the temperature 50°C was suitable for the reuse of immobilized AMG. Application of AMG immobilization is potential in applied economic to produce glucose products that have great biological values.

4. CONCLUSION

Immobilized AMG on celite-alginate avoided the constraint in the column because celite are very tiny. These were immobilization conditions: 2 ml solution AMG (dilute to 10 ml)/1 gram celite which was coated by alginate 3%, and immobilization time was 60 minutes.

Reaction conditions for enzyme activity reached the highest activity when reaction time was 20 minutes, the optimum reaction temperature was 50°C, and the optimum pH = 4.

With the temperature 50°C, enzyme can be reused up to 22 times while its activity was over 50% compared to initial activity. The study indicated that the combination of inorganic and organic materials to immobilize enzyme was effective to enhance the stability and easy – using in the reaction column.

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