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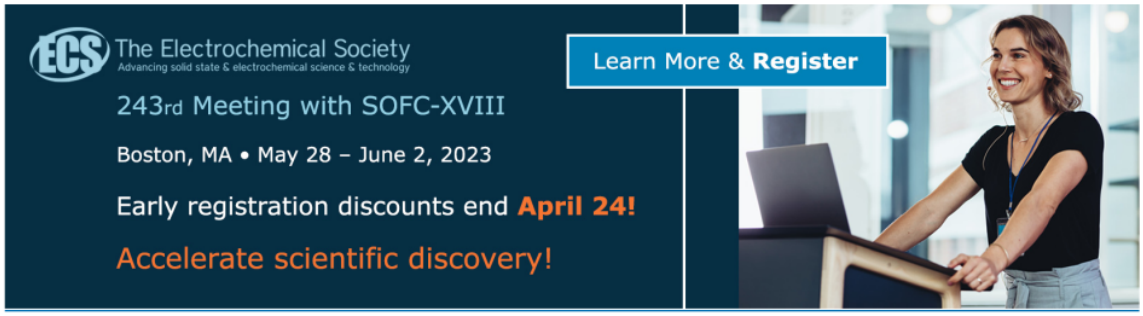

Thermal and Reused Stability of Immobilized Lipase in Carrageenan

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Abstract. Lipase is one kind of enzyme that is widely used as a catalyst in industrial and medical. Lipase has a great catalytic ability, but is easily affected by temperature, and is also difficult to separate at the end of the reaction. This causes most of lipase used only in one reaction cycle. Therefore an enzyme immobilization is needed, so that lipase can maintenance it's activity in high temperatures, and can also be separated at the end of the reaction so that it can be re-used. This study aimed to determine the thermal stability and re-used stability of the immobilized lipase in carrageenan. The method used for immobilizing lipase is through entrapment method. The effectiveness of immobilized lipase tested through the hydrolysis reaction of palm oil. The result was found that the immobilized lipase in carrageenan was able to maintain its catalytic activity up to 50 °C and also up to five reaction cycles.

Keywords. Lipase, carrageenan, immobilization, thermal stability, reused stability

1. Introduction

Enzymes are functional protein units that play a role in catalyzing reactions in cell metabolism and other reactions in the body. Spesifications of enzyme in substrate are very high in accelerating chemical reactions.

Lipase is one kind of enzyme that have potential properties to be utilized, including large catalytic power and specificity to the substrate of the reaction catalyzed[1]. Lipase carry out their catalytic activities by reducing activation energy and accelerating the achievement of fidelity but not changing the equilibrium point of the reaction[2].

Although it has a very large catalytic ability, activity of lipase is highly dependent on environmental conditions. It takes an optimum temperature condition so that the enzyme can work optimally. Conditions like too high temperatures can cause the enzyme to become damaged so that its catalytic activity decreases. Besides being easily affected by changes in temperature, enzymes also difficult to separate at the end of the reaction making it difficult to reuse[3].

To overcome the weakness of the enzyme, a method is needed to modify the enzyme, so that the enzyme is not easily damaged due to temperature and is also easy to separate at the end of the reaction. Enzyme immobilization is one kind of the enzyme modifications by binding or attaching enzymes physically and chemically to the supporting solids (immobilizing matrix) like chitosan, carrageenan, zeolite, etc which are insoluble in water. By attaching enzymes to the solid support, it is expected that the enzyme will become stronger, resistant to changes in reaction conditions so that it can be used repeatedly and can improve the reaction results[3].

Carrageenan is a group of galactose polysaccharides extracted from seaweed. Carrageenan has the ability to form a gel thermoreversible which is melted if heated and forms a gel again if cooled[4]. Based on the carrageenan ability, it is expected that carrageenan is able to bind or hold lipase enzymes in the cavity formed when carrageenan is heated, so it is interesting to learn about immobilization of lipase enzymes through entrapment method using carrageenan. The effectiveness of immobilization was observed by the hydrolysis reaction of palm oil.

2. Materials and Method

2.1. Materials and equipment

The materials used in this study were palm oil, carrageenan, pancreatic lipase enzyme, Whatman 41 filter paper, pH indicator paper, aquadest, n-hexane, ethanol, sodium hydroxide (NaOH), sodium monohydrogen phosphate ($Na_2HPO_4.2H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$), biuret reagent, indicator phenolphthalein, serum albumin bovine (BSA). The equipment used in this research is a set of glassware (Pyrex), shakers, ELISA reader, 50 mL Falcon bottles, pH meters, magnetic stir bar, glass funnel, burette, micropipette, analytical balance, oven, refrigerator.

2.2. Standardization of NaOH

Standardization is carried out by means of 0.05 grams of oxalic acid inserted in Erlenmeyer then added 25 mL of distilled water. The next solution is added 2-3 drops of phenolphthalein indicator and titrated with NaOH. Normality of NaOH is determined by the formula:

$$N = \frac{W \; x \; 2}{0{,}126 \; x \; V\,NaOH} \qquad (1)$$

W	: weight of oxalic acid (gram)
V	: Volume of NaOH (mL)
0,126	: Molecular weight of oxalic acid (g/mmol)
N	: Normality of NaOH

2.3. Measurement of protein concentration

The standard solution is made by dissolving 100 mg of Bovin Serum Albumin (BSA) in a 10.0 mL measuring flask with the addition of distilled water to the boundary mark, so that the BSA standard solution is 10 mg / mL. From a standard solution of 10 mg / mL BSA, multilevel dilution was carried out with distilled water so that standard solutions were obtained with concentrations of 8, 6, 4, 2 mg/mL. Furthermore, each concentration of standard solution was taken 100 µL and added 160 µL of reagent biuret, mixing in ELISA plate. The mixture was then allowed to stand for 10 minutes then read the absorbance at λ 630 nm with a mixture of 100 µL aquadest and 160 µL biuret. The results of absorbance of standard solutions are used to make a standard curve (absorbance vs. concentration). Measurement of protein concentration was carried out using 100 µL of a protein solution plus 160 µL of a biuret reagent then carried out in the same manner as making a standard curve. The sample protein concentration is obtained by entering the sample absorbance data into the standard curve equation[5].

2.4. Lipase activity assay

As much as 1 gram of palm oil is put into a 10 mL volumetric flask and 10 µL of aquadest are added and then added n-hexane to the boundary mark. The solution was then transferred into a 50 mL falcon

bottle and added 150 mg of free lipase enzyme. The solution is stirred in a shaker incubator for 5 hours at 37°C. To control the reaction, palm oil is used without the addition of lipase enzymes with the same procedure. The reaction results are filtered then the filtrate plus 10 mL ethanol and 2-3 drops of the phenolphthalein indicator. Lipase enzyme activity was measured by determining the free fatty acids formed using a standardized 0.05 M NaOH solution. Calculation of activity units and specific activities uses the following formula[6]:

$$\% \text{ FFA} = \frac{\text{MW palm oil } x \text{ N NaOH } x \text{ V NaOH}}{\text{W palm oil}} \quad (2)$$

$$\text{Activity Unit (U)} = \frac{\text{V NaOH } x \text{ N NaOH}}{\text{time reaction}} \quad (3)$$

$$\text{Specific Activity} = \frac{\text{U}}{\text{W enzyme}} \quad (4)$$

MW : molecular weight (g/mmol)
W : weight (gram)
V : Volume of NaOH (mL)
N : Normality of NaOH

2.5. Lipase immobilization

The general immobilization procedure is by means of 500 mg carrageenan dissolved with 15 mL physiological NaCl then heated to a temperature of 70°C then cooled to 37 °C. Then 5 mL of 1% lipase enzyme solution was heated to 37 °C. After the carrageenan solution and enzyme solution reach the same temperature, the two solutions are mixed and stirred until smooth and then cooled. From this result, the enzyme is immobilized in carrageenan and the rest of the enzyme solution [6]. The resulting filtrate measured the protein content in accordance with the procedure for measuring protein concentration. The amount of immobilized lipase enzyme is the result of reducing the amount of the initial enzyme minus the amount of residual enzyme [7].

$$\% \text{ of immobilized enzymes} = \frac{\text{number of immobilized enzymes (mg)}}{\text{number of initial enzymes (mg)}} \; x \; 100\% \quad (5)$$

2.6. Activity assay of immobilized lipase

Immobilized lipase enzyme activity test was carried out through hydrolysis reaction. The method used is the same as the free lipase enzyme activity test method. However, free lipase enzymes are replaced with immobilized lipase enzymes [6].

2.7. Stability assay of immobilized lipase

The thermal stability test was carried out by heating the free lipase enzyme and the lipase enzyme immobilized at 35, 40, 45, and 50 °C for 20 minutes. After the enzyme is heated, it is then used in the oil hydrolysis reaction. In the repeated use stability test, lipase enzymes and free lipase enzymes that have been used in hydrolysis reactions are separated from the substrate, then reused for subsequent hydrolysis reactions [6].

3. Result and Discussion

3.1. Protein standard curve

Protein standard curve has been made by using Bovin Serum Albumin with various concentration, then added with a biuret reagent and measured the absorbance using ELISA reader at a wavelength of 630 nm. From the results of the concentration vs absorbance plot, the line equation y = 0.0484x + 0.0371 with R^2 = 0.9903. This equation is then used to calculate the levels of the initial protein and the remaining protein after enzyme immobilization.

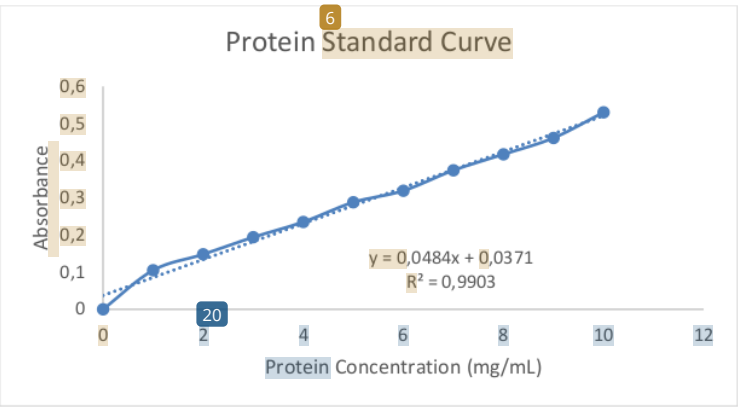

Figure 1. Protein standard curve

3.2. Thermal stability of immobilized lipase

Lipase has optimum stability at certain temperatures. The thermal stability assay of lipase aims to determine how the temperature affects lipase activity in hydrolyzing palm oil. The reaction rate will increase as the temperature rises to its optimum limit because the enzyme will deactivate at a high temperature [8].

The initial stage of the thermal stability test of lipase was carried out by heating lipase and immobilized lipase with temperature variations and then observed the effect on the hydrolysis activity. The actual thermal stability assay can also be done through reaction temperature variations because the increasing temperature can affect the hydrolysis reaction of palm oil. Temperature variations were carried out at normal temperatures (at 27 °C, without preheating), 35 °C, 40 °C, 45 °C, and 50 °C. The results of thermal stability assay of lipase are presented in Figure 2 and 3.

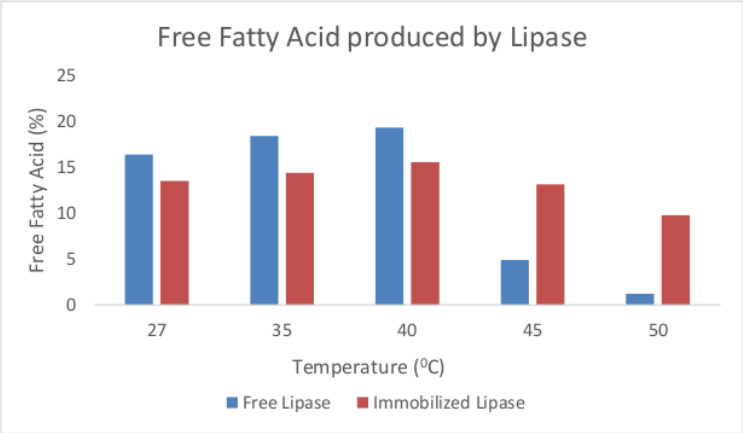

Figure 2. Free fatty acid (FFA) produced by lipase (thermal stability)

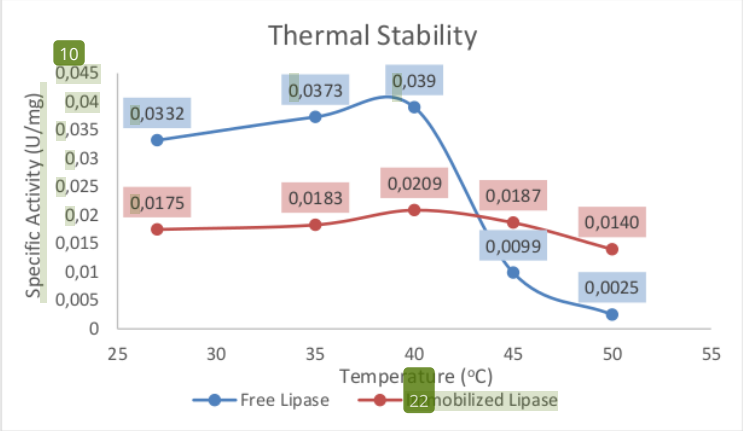

Figure 3. Thermal stability

Thermal stability of can represents the stability of immobilized enzymes. Enzymes with better thermal stability allow enzymes to be used in high temperatures[6]. From the picture above can be seen that lipase has the highest specific activity at 40 °C This is because the temperature is closest to the optimum temperature of the enzyme (37 °C). Heating treatment at 40 degrees showed the highest activity of free lipase and immobilized lipase. This shows that at this temperature the lipase structure has better interaction with the substrate[6]. At temperatures below the optimum temperatures, enzyme conformation is still not ready to catalyze the hydrolysis reaction of palm oil, so the specific activity becomes lower. At temperatures above the optimum temperature, the enzyme will get denaturation because the confirmation is damaged at high temperatures, so the activity is specific also decrease[10].

Immobilized lipase in carrageenan can maintenance its specific activity at high temperatures. This is consistent with the research conducted by Wardoyo[10]. Temperature treatment can cause the opening of protein structures and loss of enzyme activity[9]. The enzyme immobilized in supporting solids will increase its thermal stability because the supporting solids will protect the enzyme from

denaturation due to heat [11]. Immobilization causes lipase to be trapped in carrageenan which protects enzymes from temperature denaturation, so that enzyme conformation is maintained [9]. Therefore the enzyme is able to maintain its activity at high temperatures.

3.3. Reused stability of immobilized lipase

One purpose of enzyme immobilization is to find out whether the immobilized enzyme can be reused after being used in a reaction. The higher the rate of repeated use means the better the immobilization method used [5]. The results of reuse stability asssay of lipase are presented in Figure 4 and 5.

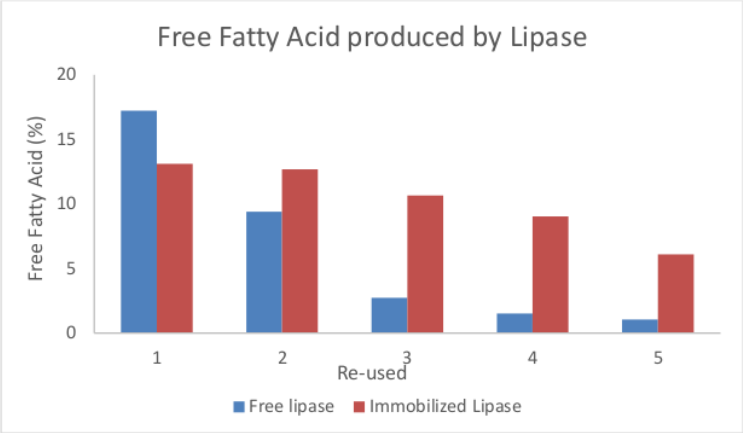

Figure 4. Free fatty acid (FFA) produced by lipase (reused stability)

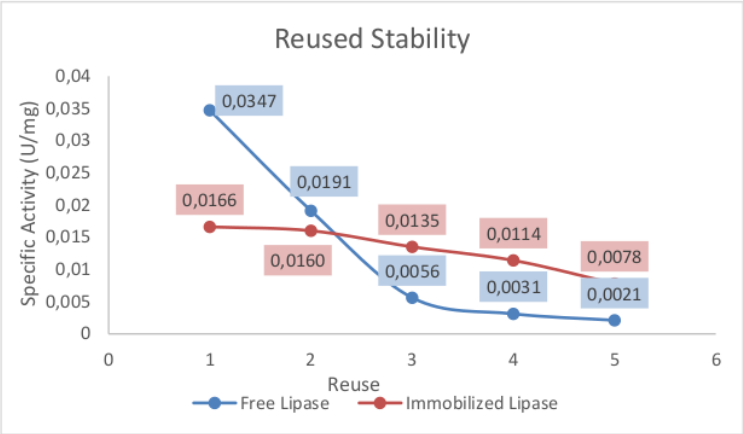

Figure 5. Reused stability

Based on figure 3, can be seen that immobilized lipase on carrageenan has better-reused stability if it compared with free lipase. Immobilized lipase on carrageenan is able to maintain its activity up to five times the reaction cycle. The results obtained are as expected, that immobilized lipase enzymes can be reused even with decreased activity.

4. Conclusion

Immobilized lipase in carrageenan has better stability than free lipase. Immobilized lipase in carrageenan improves the thermal stability up to 40 ^{0}C. Immobilized lipase in carrageenan also can maintain its activity up to five times reaction cycles, which is much better than free lipase.

Acknowledgments

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