Oyster mushroom's lipase enzyme entrapment on calcium alginate as biocatalyst in the synthesis of lauryl diethanolamide

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Abstract. Lipase is an enzyme with large biotechnology applications, such as hydrolysis in the food industry, applications in chemical industry, synthesis of polymers and surfactants. Lipase was isolated from oyster mushroom with activity 0,93 U/mg and protein content 1,1234 mg/mL. Lipase was immobilized by entrapment method in a matrix of Ca-alginate. This report describes that we have developed for the synthesis of lauryl diethanolamide The result showed that the optimum condition of lipase immobilization was achieved on 3% Na-alginate solution with protein content 0,84 mg/mL and the activity 3,33 U/mg. An amide (22.911%) formed from the amidation of lauric acid and diethanolamine.

1. Introduction

Surfactants are widely used in various industrial detergent, softener, paints, inks, emulsifiers (emulsifier), insecticides and others. In general the surfactant synthesis using renewable and non-renewable raw materials. Surfactants are generally synthesized from petroleum derivatives, such as linear alkyl benzene sulphonate (LAS) and alkyl sulphonate (AS) [1]. However, these surfactants can cause pollution to the environment as waste after use will be difficult degraded and can not be updated (non-renewable). At this time with the limited non renewable materials, there should be efforts to replace non-renewable raw materials.

The potential of Indonesia as a producer of surfactants which are synthesized from fatty acids of coconut oil is huge, considering Indonesia's palm oil production has increased from year to year. High lauric acid content which is 50.50% pure coconut oil has the potential to make a diethanolamide surfactant. Lauril nonionic surfactant derived from alkanolamides. These surfactants derived from lauric acid and diethanolamine through enzymatic amidation. diethanolamide surfactants are more effective both as a foam stabilizer, thickener and foam booster [2]. Diethanolamide can be found in the manufacture of detergents, emulsifiers agents, and cosmetics.

An important alternative route for the production of diethanolamide involves the use of a biocatalyst in organic media. The use of enzymes as biocatalysts have a very important role in the chemical and pharmaceutical industries. Lipase (EC 3.1.1.3) is an enzyme with biotechnology applications is very broad, such as hydrolysis of the milk fat in the food industry, the oleochemical industry applications, the synthesis of triglyceride structure and the synthesis of polymers and surfactants. Mushrooms was a source of enzymes of interest in biotechnology due to the availability and high stability. However, the use of lipase as a biocatalyst has several weaknesses, which are only

applicable to one reaction. One way to overcome this drawback is to apply the enzyme immobilization techniques to be used. Enzyme immobilization aims to improve the stability and productivity of these enzymes so that the lipase can be reused [3-5].

Methods of immobilizing enzymes entrapment in calcium alginate is one important method of immobilization. One commercially available alginate is sodium alginate has been used for more than 65 years in the food and pharmaceutical industries as coatings, emulsifying and film-forming agent. Entrapment using calcium alginate gel that does not dissolve quickly known, non-toxic, inexpensive, and versatile method of immobilization of an enzyme [6].

Surfactant synthesis of renewable materials and environmentally friendly is very important as to minimize the impact of pollution. Lauryl diethanolamide is one surfactant made from natural easily degraded and contaminant. Free lipase is an environmentally friendly biocatalyst which catalyzes the synthesis of surfactant. This report describes that we have developed for the synthesis of lauryl diethanolamide. The optimum concentration of Na alginate in the process of entrapment lipase oyster mushrooms and know entrapped lipase stability for repeated use in the synthesis of lauryl diethanolamide.

2. Methods

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2.1. Material

Oyster mushrooms, n-hexane, diethanolamine (*E Merck*, 99%), NaOH (*E-Merck)*, KOH (*E-Merck))*, Phenolphthalein (*E-Merck)*, KH_2PO_4 (*E-Merck)*, $KH_2PO_4.2H_2O$, lauric acid, H_2SO_4 (*E Merck*, 96%*)*, VCO, distilled water, ethanol (Brataco Chem), sodium alginate (Shandong Jiejing Group Corporation), bovine serum albumin (*E-Merck)*.

2.2. Isolation of Lipase

Isolation lipase of oyster mushrooms was done by washing 200 g of oyster mushrooms until completely clean and sterile. Oysters mushrooms and 100 mL aquabidest were blended until crushed oyster mushrooms and the slurry obtained centrifuged at 1000 rpm for 20 min.

2.3. Protein assay

Determination of protein content was carried by Biuret method. A total of 1 mL sample, four mL Biuret reagent and five mL of distilled water, then shaken and allowed to stand for 10 minutes until a purple solution. The wavelength of maximum absorbance at Bovine Serum Albumin (λmax = 540 nm). The protein content was known by converting the absorbance value of the standard curve regression equation Bovine Serum Albumin.

2.4. Lipase Activity

Lipase activity test with a volumetric method using the VCO as a substrate. A total of 1 mL of lipase was added to 4 ml of n-hexane and incubated at 50^{o}C for 30 minutes. The results formed extracted using ethanol solution for 5 minutes. Followed by titration of the extract using a 0.05 M NaOH solution by adding 2-3 drops of PP indicator.

2.5. Immobilization Lipase

Immobilization of lipase in Ca-alginate is done by adding 30 mL of lipase in 20 mL Na-alginate with the weight ratio between the enzyme and the Na-alginate (2:3). The mixture is stirred with a magnetic stirrer at a room temperature. Then, Na-alginate solution mixture and lipase put in 10 mL of 0.1 M $CaCl_2$ little by a little drop of 2 mL using a pipette while stirring slowly with a magnetic stirrer to form an immobile enzyme granules. A gel containing the enzyme removed from the $CaCl_2$ solution by filtration. Then the gel is stored at 4°C until to be used. To obtain optimum process conditions

immobilized lipase was determined of the percentage concentration of the immobilized enzyme with Na-alginate concentration of 1%, 2%, 3% and 4% (w/v).

2.6. Synthesis of Lauryl Diethanolamide

Diethanolamine was mixed with lauric acid in the ratio 2/1 and entrapped lipase at a concentration of 12.5% (w/w of lauric acid), then dissolved in n-hexane with a ratio of 2/1 (v/b lauric acid). The mixture was stirred using a shaker with a speed of 150 rpm at 50°C for 24 h.

3. Result and Discussion

3.1. Immobilization of Lipase from oyster mushrooms

The initial protein content was obtained from the isolation of 1.12 mg / mL and a specific activity of 4.03 U mg. Lipase enzyme immobilization process was carried out by the method of oyster mushrooms entrapment. Alginate entrapment conducted by dripping a suspension of lipase and Na-alginate into a solution of $CaCl_2$ that causes intermolecular crosslinking of alginate with Ca^{2+}. The crosslinking causes the formation of alginate gel in less than 1 minute. The gel is formed shortly after hatch is soft. After 20 minutes, alginate gel becomes harder allegedly due to crosslinking between the alginate and Ca^{2+} has been going perfectly. Calcium alginate formed is not soluble in water. That's because the calcium to form a bi-cations Ca^{2+} and forming a chelate with oxygen guluronic [3].
The initial steps to immobilize it entrapment reaction was mixed the enzyme into each Na-alginate solution of 1%, 2%, 3%, and 4% in comparison with the lipase alginate is 2: 3. Beads obtained is not the same at each concentration of Na-alginate, alginate beads were obtained small and fragile (Figure 1).

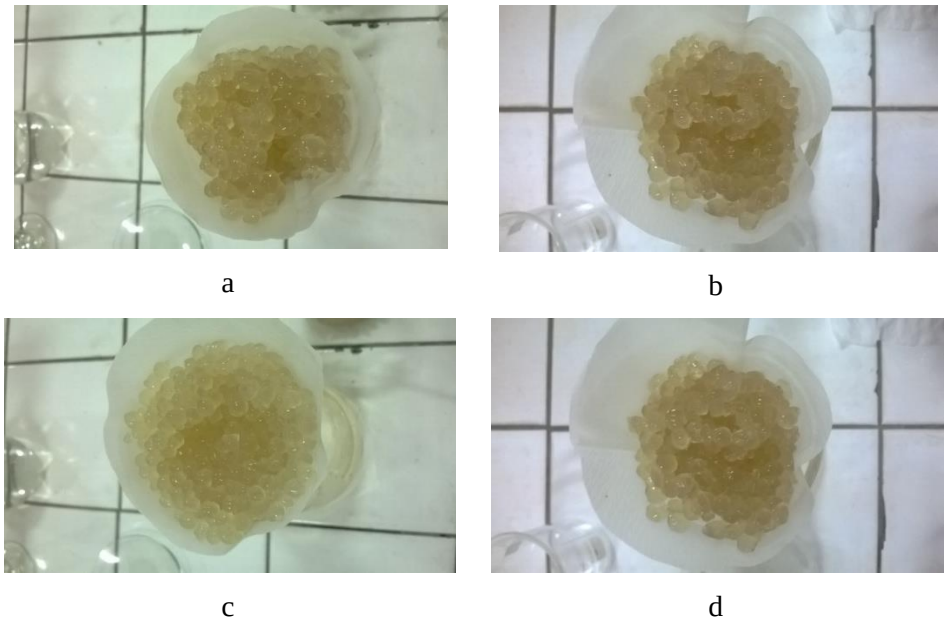

Figure 1. Bead Lipase entrapped resulting from a concentration of Na alginate. 1%, b. 2%, c. 3%, and d. 4%.

Entrapment method using Ca-alginate gel pore density is affected by the alginate. The greater the concentration of Na-alginate crosslinks has formed pores closer. The pore density can prevent lipase which has been stuck for detaching thereby increasing the amount of lipase trapped in Ca-alginate beads. Lipase activity entrapped in Ca-alginate increased to a concentration of 3% and at a concentration of 4% Na-alginate decreased activity. This is because the pores are formed more tightly

so that the process of diffusion substrate passes Ca-alginate to be blocked so that products are more difficult to form lead to decreased activity. The optimum concentration of Na-alginate obtained at concentrations of Na-alginate 3% of total lipase trapped by 0.84 mg / mL (Figure 2) and activities at 3:33 U/mL (Figure 3).

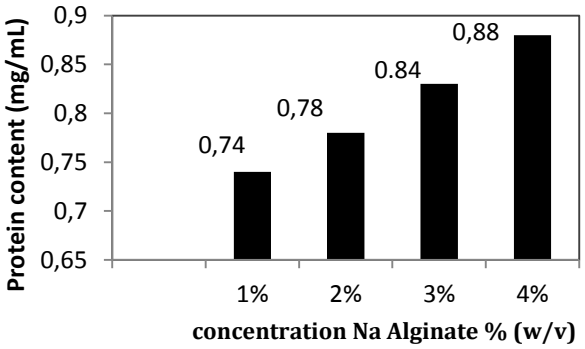

Figure 2. The relationship between the concentration of Na-alginate Protein levels Trapped

Specific enzyme activity is expressed as the number/unit of enzyme activity per mg protein enzymes. Determination of enzyme activity performed by titrimetric methods.

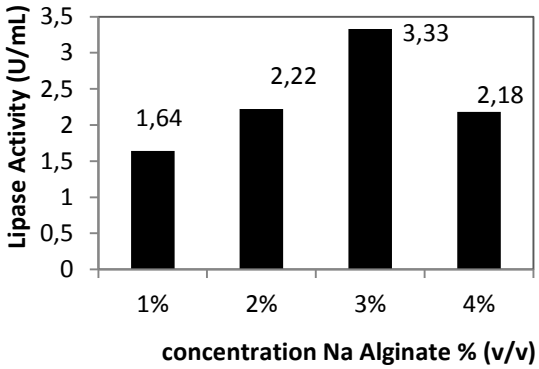

Figure 3. The relationship between the concentration of Na-alginate Protein levels Trapped

3.2. Activity of Lipase entrapped in Synthesis lauryl diethanolamine

Lauryl diethanolamide obtained from amidation reaction between lauric acid and diethanolamine. Numbers ester synthesis are relevant to the biosurfactant alkanolamide and fatty acid esters [7].

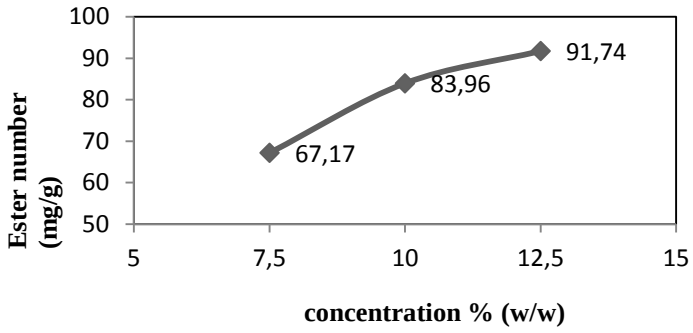

Figure 4. Relations concentration variation entrapped lipase% (w / w lauric acid) with numbers ester

The addition of lipase entrapped in the synthesis of ester lauryl diethanolamide then the numbers are increasing. The ester number was obtained on the addition of lipase entrapped concentration of 12.5% (w / w of lauric acid). The addition of concentration is then used as the optimum concentration in the stability test repeated use of lipase entrapped (Figure 4).

3.3. Stability and Reusability of Lipase immobilization

One enzyme immobilization goal was to determine whether the immobilized enzyme can be reused after being used in a reaction. Lipase has been immobilized by entrapment used as a biocatalyst in the amidation reaction between lauric acid and diethanolamine to produce lauryl diethanolamide. In this research note that entrapped lipase can still be used up to three times a reaction cycle (Figure. 5).

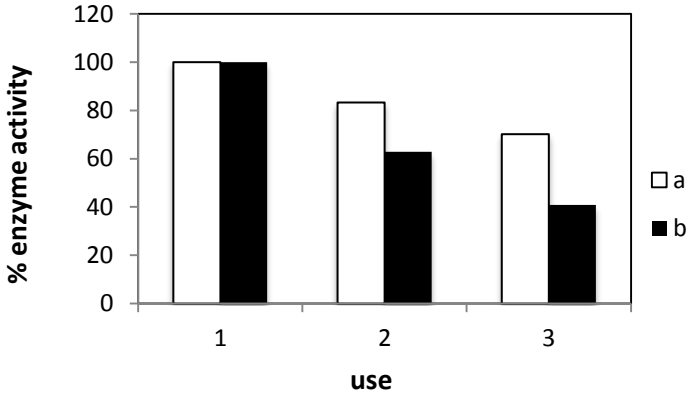

Figure 5. Relations with the repeated use of % of enzyme activity a) recovery using NaCl, b). recovery without NaCl

Entrapped lipase activity was tested for three times to determine the stability of the repeated use in the synthesis of lauryl diethanolamide. Figure 5a shows 83.25% activity during second reuse and 70.11% activity on its third use. While in Figure. 5b shows 62.86% activity during second reuse and 40.90% activity on its third use.

3.4. Characterization of product Synthesis

The product of a reaction between acid and lauric diethanolamide was then characterized using FTIR and HPLC.

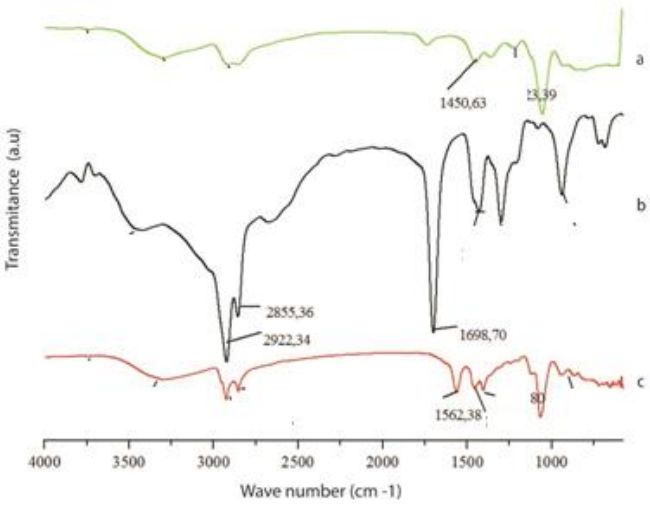

Figure 6. IR spectra a. diethanolamine, b. lauric acid, and c. product of synthesis

Analysis of a reaction between lauric acid and diethanolamine using FT-IR (Figure. 6) was suspected there is some absorption, the peak of 3748 cm^{-1} indicate the presence of strain NH, 3302 cm^{-1} indicate the presence of OH groups. The peak of 2924 cm^{-1} and 2853 cm^{-1} indicates an aliphatic CH group, the peak of 1562 cm^{-1} declared an amide group CO-N, 1456 cm^{-1} indicate the presence of CN bond stretching vibration, 1403 cm^{-1} shows the CH_2 stretching vibration, 942 cm^{-1} indicate the presence of CO stretching vibration.

Amidation diethanolamine with lauric acid was analyzed using HPLC to determine the percentage of reaction products formed. HPLC chromatogram of the results can be seen in Fig 7 and Table 1.

Table 1. The product of amidation reaction using HPLC analysis

No.	Retention Time (min)	Consentration (%)
1	2,43	24,59
2	2,63	49,01
3	5,85	0,29
4	9,22	22,91
5	12,52	1,59
6	16,29	1,22
7	19,26	0,39

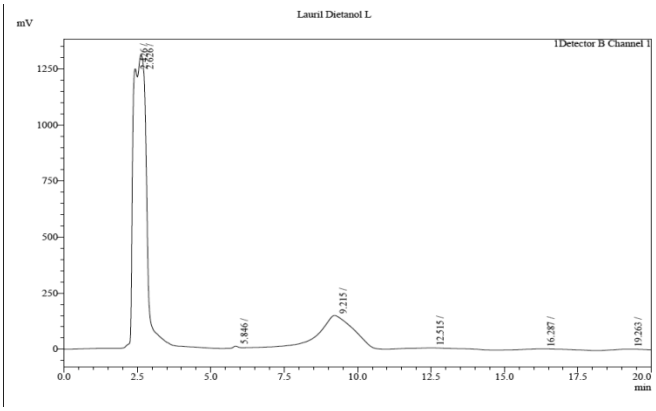

Figure 7. HPLC Chromatogram of product synthesis

The product of the amidation reaction between lauric acid and diethanolamine was characterized using HPLC [8]. The peak of lauryl diethanolamide appears at a retention time of 9.22 min with a concentration 22.91%. The peak may be suspected as an amide formed from the reaction of lauric acid amidation and diethanolamida (Figure. 8).

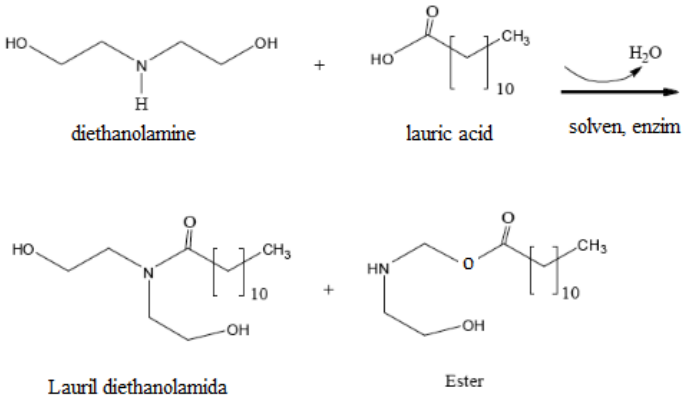

Figure 8. Amidation Reaction of Lauryl acid and diethanolamine

4. Conclusion

The result showed that the optimum condition of immobilization lipase were 3% of Na-alginate solution with protein content 0,84 mg/mL and the activity is 3,33 U/mL. Stability of entrapped lipase for the synthesis of lauryl diethanolamide showed that entrapped lipase could be used for three cycles. An amide (22.91%) was formed from the reaction amidation of lauric acid and diethanolamine.

5. References

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