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Performance of Rhizomucor Miehei in Amidation Reaction for Fatty Monoethanolamide Synthesis Based on Distillated Palm Fatty Acid

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Abstract. Lipase is a type of biocatalyst that can work with substrates of lipid classes. Rhizomucor meihei is of lipase enzymes classes, that can react specifically with Palm Fatty Acid Distillate (PFAD) forming fatty monoethanolamide. Monoethanolamide surfactants are compounds which are widely used in the cosmetics industry as emulsifiers. Monoethanolamide having OH- groups, so it is easily soluble in water. Monoethanolamide can be produced from fatty acids with monoethanolamide using alkaline catalyst or a biocatalyst. Production using chemical catalysts will involve high reaction temperature reaches 120°C-150°C, while the reactions involving biocatalysts are like enzyme can be done at a relatively low temperature. This research was conducted by using amidation reaction with 3 independent variables i.e. amidation temperature at the level of 30°C-70°C, the mole ratio of PFAD: Monoethanolamine at (1:1) - (1:20) and ratio of enzyme at 0.05%-0.5% (w/w) and reaction time of 4 hours. The research results show that the Rhizomucor meihei give the best performance at the level of temperature 60°C, ratio mole (1:4) and the ratio of enzyme 0.2% (w/w) with a conversion product of 86.25%.

1. The first section in your paper

Alkanolamine compounds are first obtained from the reaction between fatty acids and alkanolamines. The fatty acids used are mainly fatty acids with C12-C18 carbon chains, thus providing an opportunity for palm distillate fatty acids as raw materials. Usually in industrial area, alkanolamide is produced chemically using oil or fat with alkanolamines at a certain temperature. Conventional chemical techniques have weaknesses related to energy use and the formation of unwanted by-products. Alkanolamide synthesis in conventional chemistry was carried out using a base catalyst at a temperature of 120°C-150°C. At the end of the product, amine soap will be obtained which will increase the pH, so further purification steps are needed. Therefore, an enzymatic process is developed to produce diethanolamide from fatty acids and vegetable oils using a biocatalyst. The biocatalyst used can be a lipase enzyme. The lipase enzyme used must have specific properties on the substrate containing palmitic acid. Specific properties are the ability of an enzyme to act on a particular type of substrate [1].

Rhizomucor meihei, Candida antarctica Lipase sp is a type of lipase enzyme commonly used in esterification reactions of fatty acids for the production of fatty acid methyl esters (FAME), cosmetics



(palm oil emulsifiers) which contain high palmitic acid, food industry (cocoa butter), oil industry vegetable like corn oil, sunflower oil, olive oil. The use of enzymatic reactions in fatty acids and vegetable oils has several advantages, namely (i) the specific nature of high lipase so that pure products will be produced (ii) relatively low temperature and pressure use, (iii) relatively cheaper waste management costs and (iv) the products produced are safer compared to conventional chemical processes [2].

Monoethanolamide is a group of fatty amide and is a non-ionic surfactant which is widely used as a material for making shampoos, bath foam, latex stabilizers, rust inhibitors, household cleaning products and liquid detergents. Surfactants are compounds that have two groups, namely hydrophobic (lipophilic) and hydrophilic (lipophobic) in one molecule, therefore they are called amphiphilic compounds.

Indonesia's surfactant imports amounted to 44,500 tons and it is predicted that these imports will continue to grow annually in line with the growth of the cosmetics, food, pharmaceutical, textile and leather tanning industries [3]. Indonesia is one of the largest palm oil producers in the world. A portion of the palm oil production is exported in the form of crude palm oil and the rest is used as raw material for domestic industrial purposes. Consumption of palm oil in the country is only used as raw material for industrial cooking oil, margarine, soap, and oleochemical industries that produce palm fatty acids, methyl esters and fatty alcohols. In the cooking oil industry, there is a refining stage which aims to improve the quality of the oil produced. At this stage, in addition to the main product in the form of cooking oil, byproducts were also produced, namely palm distillate fatty acid (PFAD) which has a high palmitic acid content, around 56.55%. This indicates that the availability of raw materials for continuation for the surfactant industry involves enzymatic reactions. The function of an enzyme is as a catalyst for biochemical processes that occur in cells or outside cells. An enzyme can accelerate the reaction 10⁸ to 10¹¹ times faster than if the reaction was carried out without a catalyst. So, enzymes can function as catalysts that are very efficient, besides having high (specific) characteristics. Like other catalysts, enzymes can reduce the activation energy of a chemical reaction. There are chemical reactions that require energy (endergonic reactions) and some that produce energy or release energy (exergonic). The specific nature (specificity) of enzymes causes enzymes to only work in one reaction [1]. To be able to work on a substance or substrate there must be a connection or contact between the enzyme and the substrate. An enzyme has a size larger than a substrate. Therefore, not all parts of the enzyme can be directly related to the substrate. The relationship between substrates and enzymes only occurs in certain parts or places. The place or part of the enzyme that has a relationship or contact with the substrate is named the active site. The relationship is only possible if the active part has the right space to accommodate the substrate. If the substrate has another shape or conformation, it cannot be accommodated in the active part of an enzyme. In this case the enzyme cannot function against the substrate. This is an explanation of why each enzyme has specific properties to certain substrates. The relationship or contact between the enzyme and the substrate causes the formation of a substrate enzyme complex. This complex is an active complex, which is temporary and will break down again if the desired reaction has occurred. Simply put the decomposition of a compound or substrate by an enzyme can be described as follows:

2. Material and Method

This study consists of two stages, namely enzyme screening (enzyme selection) followed by synthesis of fatty monoethanolamide compounds involving enzymatic reactions.

2.1. Material

Palm Fatty Acid Distillate (PFAD), monoethanolamine, n-Hexane, Rhizomucor meihei (Novozyme), Candida antarctica (Lipozyme) and Lipase sp.

2.2. Biocatalyst Screening

Enzyme screening is done to select the type of biocatalyst that has the highest conversion to the amidation reaction through the acquisition of yield amount. Enzyme screening was carried out at temperature conditions of 60°C, mole ratio of monoethanolamine/PFAD (1:4) and biocatalyst ratio of 0.2% (w/w) in 3 (three) types of lipase enzymes, namely Rhizomucor Meihei, Candida Antarctica and Lipase Sp. Yield the fatty monoethanolamide products formed are expressed by the percentage of the decrease in free fatty acid conversion (FFA).

2.3. Synthesis of Monoethanolamide Fatty

The type of enzyme that provides the highest yield at the screening stage was chosen as the type of enzyme in subsequent fatty monoethanolamide synthesis. In this synthesis, there is an enzymatic amination reaction based on palm oil fatty acid distillation as a limiting reactor and excess monoethanolamine. The use of excess monoethanolamine aims to increase the strength of the peptide bonds formed. The following is the reaction of the formation of monoethanolamide



3. Results and Discussion

3.1. Biocatalyst Screening

From the result of Biocatalyst screening that have been carried out on three types of biocatalysts, namely Rhizomucor meihei (Novozyme), Candida antarctica (Lipozyme), and Lipase Sp. Based on the results of the study it was found that Rhizomucor meihei gave the highest conversion compared to other types of enzymes. Conversion is calculated through changes in the free fatty acid (FFA) content of raw materials and products. A high percentage of FFA reduction indicates that many palm fatty acids have initially been free (not bound to the second substrate) to bind to the second substrate, in this case ethanolamine forms an enzyme-product complex.

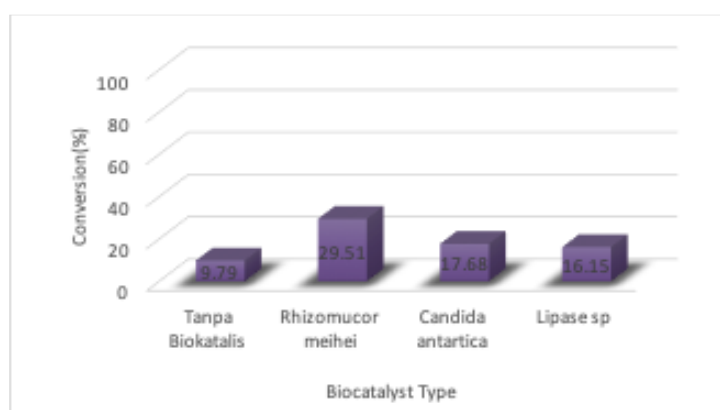


Figure 1. Effect of Biocatalyst Type on Monoethanolamide Conversion

Rhizomucor meihei has a high specificity for the amination reaction of palm distillate fatty acid (PFAD) based on a conversion of 29.155%. Rhizomucor meihei is able to work on PFAD substrates with the largest constituent components being medium to long chain fatty acids (C14-C18). This is consistent with previous studies conducted by Kurniasih (2013)[5] and Elisabeth (1998)[2] regarding the use of Rhizomucor meihei on substrates with medium to long chain fatty acid content, which has a positive influence on biocatalyst variables.

Based on research observations, it was concluded that Candida antica was only able to work on the medium chain fatty acid content substrate, so that the conversion was not real, whereas Lipase sp was

known to not be able to withstand the amination reaction throughout the reaction time because its characteristics were more sensitive to changes in temperature and reaction conditions. As a control of the reaction, an enzyme-free amination reaction is performed to see the specificity of each biocatalyst. For control amination reaction obtained a decrease in FFA of 9.79% which showed a significant effect for variable enzymes on the continuity of enzymatic amination reactions. For the next research phase, *Rhizomucor meihei* is used as a fixed variable for biocatalyst types.

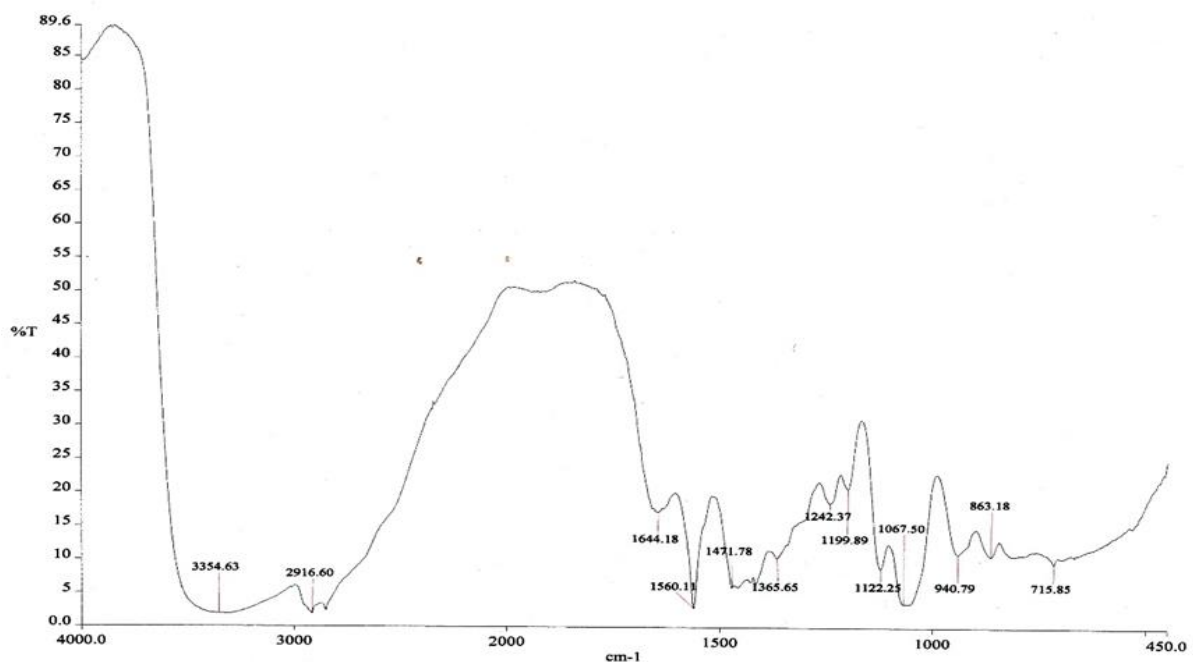
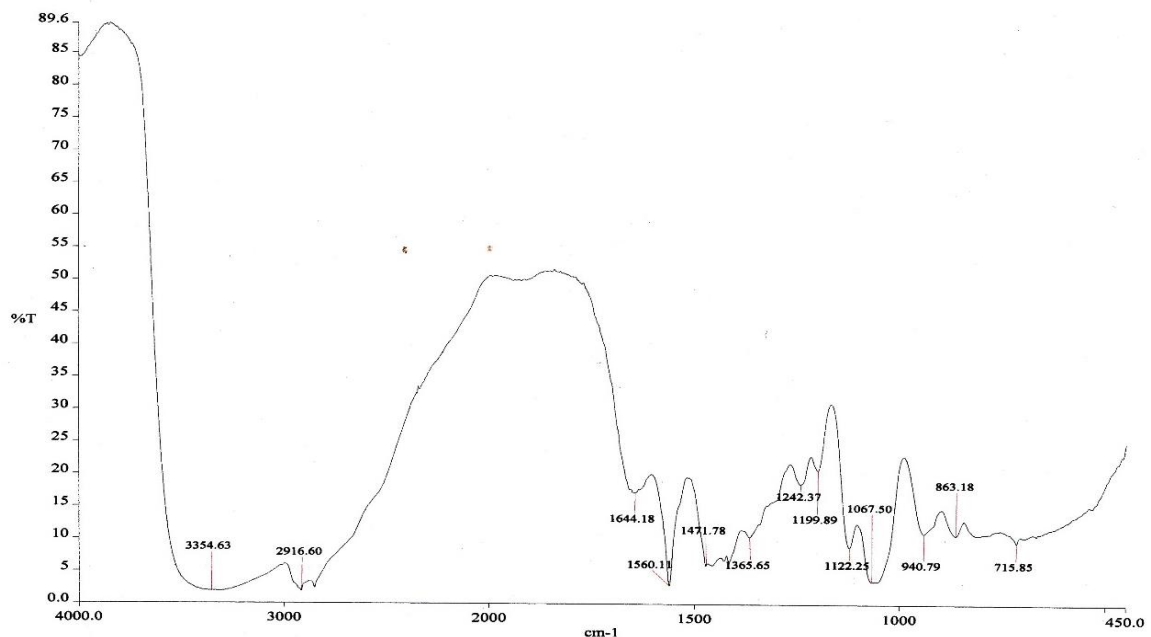


Figure 2. Infra-Red Spectrum (FT-IR) (a) Non-biocatalyst, (b) *Rhizomucor meihei*

Table 1. Non-Enzyme Monoethanolamide Fatty Wave Numbers [4]

| Wave Numbers (Cm ⁻¹) | Chemical Formula* |
|----------------------------------|-----------------------|
| 3374,09 | - OH |
| 2916,47 | - C-H |
| 1774,24 | - C - O (Amina ester) |
| 1645,95 | - C = O (Amide) |
| 1466,60 | - C-N |
| 715,77 | - C-H ₂ |

* Source : Fessenden, Ralph J (1999)

Table 2. Wave numbers of Fatty Monoethanolamide (*Rhizomucor meihei*) [4]

| Wave Numbers (Cm ⁻¹) | Chemical Formula* |
|----------------------------------|--------------------|
| 3364,77 | - OH |
| 2916,95 | - C-H |
| 1644,18 | - C = O (Amida) |
| 1455,60 | - C-N |
| 715, 85 | - C-H ₂ |

* Source : Fessenden, Ralph J (1999)

3.2. Synthesis of Monoethanolamide Fatty

Monoethanolamide is a type of non-ionic surfactant that acts as a foaming booster in cosmetics. Fatty monoethanolamide is produced using ethanolamine and fatty acids. In this study PFAD was used as a source of excess fatty acids and ethanolamine. More ethanolamine is used, so that PFAD functions as a limiting reactant to be observed.

The use of excess ethanolamine also has the opportunity to increase the strength of the peptides formed [5]. But the use of excess ethanolamine gives the possibility of forming more H₂O molecules, so it is necessary to determine the right level of mole ratio in this amidation reaction.

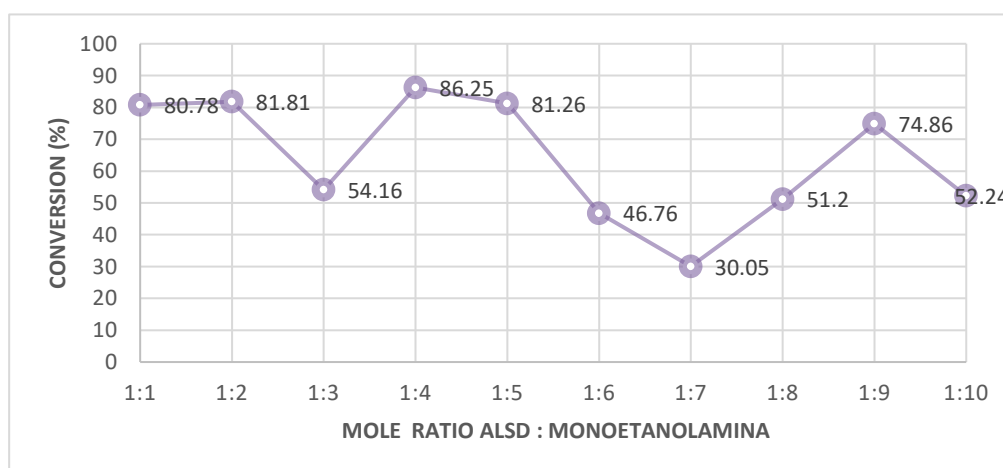


Figure 2. Effect of Mol ratio of PFAD: Ethanolamine to Monoethanolamide Conversion

In this study, the level of the PFAD mole ratio: Ethanolamine was tested from 1: 1 to 1:10 to determine the optimum mole ratio level. Selection of level of PFAD mole ratio: Ethanolamine is carried out at temperature reaction conditions of 60°C, biocatalyst ratio of 0.2% (b/b).

Based on the results of the study, it is known that the level of the PFAD mole ratio: Ethanolamine (1:4) provides optimum conversion, as evidenced by the increase in ethanolamine, there is no increase in conversion but a sharp decrease in product conversion. This is due to the substrate resistance in the reaction system, because the substrate has fully bind to the biocatalyst to form the substrate enzyme complex. This is consistent with Rahan's (2003) study, that increasing the ratio of ethanolamine to 1:10 gives the reality of decreasing product conversion.

4. Conclusion

From the results of the research and study, it was found that *Rhizomucor meihei* gave a positive effect on the acquisition of fatty monoethanolamide products. This shows that *Rhizomucor meihei* is selective in medium to long chain fatty acids and is able to withstand enzymatic amination reactions. By varying the amount of excess monoethanolamine, it is expected to form peptide bonds. From the reaction results it is known that the mole ratio of PFAD/ethanolamine (1: 4) gives the best conversion, while the increase in ethanolamine ratio shows the occurrence of substrate resistance in the reaction of the substrate enzyme complex, because ALSD has been reacted with monoethanolamine.

5. Acknowledgement

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