



INCREASED THE SYNTHESIS OF ALKANOLAMIDE THROUGH RECOVERY ENZYMES AND THE USE OF METAL CATALYSTS

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ABSTRACT

Increased the synthesis of alkanolamide surfactant namely lauroyl-diethanolamide, oleoyl-diethanolamide, lauroyl n-methyl glucamide and coco fatty acid amide were observed in this study. At this stage, immobilized lipase recoveries were observed and the $ZrCl_4$ metal catalysts were used. Alkanolamides obtained from fatty acids and fatty acid methyl esters (FAME) from coconut oil are reacted with diethanolamine, monoethanolamine (MEA) and n-methyl glucamine assisted with two types of catalysts, immobile lipases, and $ZrCl_4$. The results of the study show that in the application of enzyme recovery, immobilized lipases can be reused up to 4 times with only a slight reduction in reactivity. While observing the use of metal catalysts it was found that the reaction time could be reduced to three hours, with a substrate ratio of 4:1 (MEA: FAME) and the acquisition of fatty acid conversion reaching 90%.

Keywords: enzyme recovery, metal catalyst, alkyl-diethanolamide, alkyl n-methyl glucamide, coco fatty acid amide.

INTRODUCTION

The production of alkanolamide surfactants has been widely developed lately [1-4]. Alkanolamide synthesis is generally through the reaction of alkanolamine with carbonyl compounds and the method used resembles the method of forming esters [5]. The amide is a derivative of the carboxylic acid where the -OH of the acid has been replaced by the -NR₂ group, which can be alkyl, R=H, etc. [6].

Alkanolamide is a non-ionic surfactant where there is no ionization of molecules [7]. The methyl amide group contained in alkanolamide is useful for increasing the solubility of surfactants. Alkanolamides from vegetable oils are biodegradable, soft and non-irritating, both for the skin and eyes and can be used over a wide pH range. These surfactants also have low toxicity, good and stable foaming, and produce a large surface tension reduction [8, 9]. Alkanolamide surfactants are also very compatible with the other three types of surfactants namely anionic, cationic and amphoteric surfactants [10]. As with other nonionic surfactants, alkanolamides show good performance such as high solubility, are also stable against various enzymes and alkaline media [11]. Because of these properties, this surfactant can be used as food, medicine, cosmetics, and industrial applications and can be used in the range of use of anionic surfactants.

Products that use alkanolamide surfactants include non-irritating shampoo, liquid bath soap, hair care products, lotions, creams, cleaning products and cosmetics, pharmaceutical, biochemical and biomedical products [2-4]. Synthesis of alkanolamide surfactant can be done using chemical catalysts, biocatalysts, or metal catalysts. Several studies have been carried out on alkanolamide synthesis using biocatalysts [12]. The sources of fat/fatty acids used include fatty acid methyl esters, fatty acid ethyl esters, oleic acid, lauric acid, and triglycerides from palm oil, palm kernel and various other oil sources.

The solvents commonly used in the synthesis of alkanolamides are hexane, isopropanol and tert-amyl

alcohol [3]. If the stoichiometric mixture between oleic acid and N-methyl-glucamine is react using lipase at 55°C and atmospheric pressure, the presence of fatty acids will dissolve n-methyl-glucamine by forming ion pairs as shown in Figure-1.

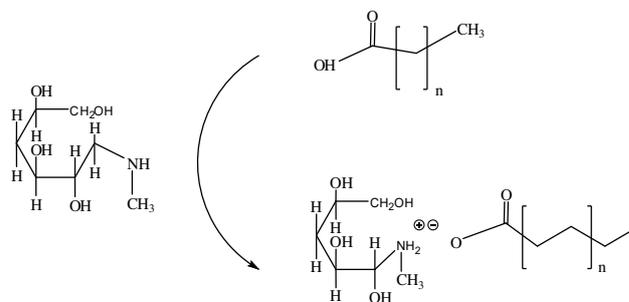


Figure-1. Fatty acids will dissolve n-methyl-glucamine by forming ion pairs [13].

Chemoselectivity of the reaction in the use of immobilized lipases from *Rhizomucor miehei* (Lipozym) in the synthesis of fatty acids with N-methyl-glucamine will vary depending on the ratio of acid to amine. For the ratio of acid to an amine of 8 (excess acid), then most will form esters. All n-methyl glucamine will transform into 6-O-oleoyl-N-methylglucamine. If the ratio is smaller than 1 (excess amine), oleoyl-n-methyl glucamide will be formed. These results indicate that the importance of maintaining acid-base conditions especially if the substrate molecule contains an ionic group because acid-base conditions determine the ionic location of both the enzyme substrate and catalyst and then also the efficiency and chemo-selectivity of the synthesis [13-14].

The effect of lipase was also observed in the synthesis of alkanolamides from oleic acid and monoethanolamine, and alkanolamides were obtained with a yield of 73.5% at 6 hours reaction time [9]. While sodium methoxide is used as a chemical catalyst, the yield of 70.3% obtained with a reaction time of 3 hours [15].



In order to obtain a better yield and an efficient process, it is necessary to examine the efforts that can be made to increase the yield of alkanolamide. Among them is by observing the effect of enzyme recovery. For this reason, commercial lipase enzymes are chosen for use because they are suitable for alkanolamide synthesis, easily separated from the product mixture and the main one can be reused repeatedly. This repeated use will save the cost of using enzymes, given that the biocatalysts, both self-synthesized and commercial, require a high cost to obtain.

In addition to enzyme recovery, increasing the process efficiency can be done by using more economical and efficient catalysts such as zirconium tetrachloride. Zirconium tetrachloride ($ZrCl_4$) is a metal catalyst with abundant availability in the earth crust. This catalyst is easily soluble in various solvents, can be recycled, and is an efficient catalyst for the amidification of carboxylic acids with primary and secondary amines [1, 16].

MATERIALS AND METHODS

Materials

The materials used in this study included, monoethanolamine (MEA), diethanolamine (DEA), n-methyl glucamine (NMGL), lauric acid (LA), oleic acid (OA), fatty acid methyl ester (FAME) from coconut oil, zirconium tetrachloride ($ZrCl_4$), tert-amyl alcohol, n-hexane, methanol (CH_3OH), isopropanol (C_3H_8O), sodium hydroxide (NaOH), potassium hydroxide (KOH), phenolphthalein ($C_{20}H_{14}O_4$), ethanol (CH_3CH_2OH) and hydrochloric acid (HCl). The materials used are obtained from E Merck, Darmstadt Germany.

Immobilized lipase from *Candida antarctica* supported on an acrylic resin, obtained from Sigma Aldrich, United States.

Methods

Enzyme recovery method

Alkanolamide synthesis was performed in a 100 ml stoppered flasks. Immobilized lipase was used as a catalyst in amidification two types of fatty acids (oleic acid and lauric acid) with two types of amines (diethanolamine and n-methyl glucamine). Fatty acids, tert amyl alcohol, amines, and immobile lipase are reacted at $70^\circ C$ for 24h with a 150 and 250 rpm stirring rate. The range of values for enzyme amounts (10-12%) and substrate molar ratio (1:1 to 5:1 amine to fatty acid) are determined from preliminary studies [4].

The product purification is carried out by decantation where the reaction mixture is washed with acetone. Alkanolamide and diethanolamine are obtained as a bottom product while the top products are fatty acids and esters which mix with acetone. Then, an acid number and saponification number were analyzed to determine the number of fatty acids converted.

Method of using metal catalyst

Monoethanolamine with methyl esters dissolved in tert-amyl alcohol with the solvent ratio of tert-amyl alcohol to methyl ester of 4:1 (v: wt). The substrate molar ratio used is 1:1 to 5:1 (MEA: FAME). $ZrCl_4$ catalyst was added to the solution with a concentration of 5% by weight of the total solution.

The solution is stirred with a stirring speed of 250 rpm with a time variation of 1 to 5 hours. The mixture is separated from the catalyst by filtration, the solvent and ethanol are evaporated. Determined the acid numbers, saponification number, surface tension analysis, and Hydrophilic-Lipophilic Balance (HLB) value for all samples.

RESULTS AND DISCUSSIONS

Enzyme recovery

One of the most recent studies of enzymatic synthesis in organic solvents is the synthesis of compounds using immobilized lipases [17, 18]. The main advantage of the use of lipase in the synthesis of alkanolamide is that lipase is chemoselective and less energy so that it can protect the amine group from being carbonized with CO_2 [19, 20]. Besides that, the lipase enzyme, especially the immobilized one, can be recovered up to more than 15 times so that it saves the cost of using the catalyst [6]. Recovery enzyme aims to maximize the use of enzymes. Recovery enzymes were observed in the synthesis of lauroyl-diethanolamide, lauroyl-n-methyl glucamide and oleoyl-diethanolamide.

The results of observing enzyme recoveries for the synthesis of lauroyl-diethanolamide are shown in Figure-2. It was observed that overall the enzyme can be used up to 4 times. It's just that the percent conversion of fatty acids obtained has decreased. In all observations, a marked decrease was observed in the use of enzymes that were 3 and 4 times in which enzymes lost their activity to percent fatty acid conversion decreased by almost 40%.

It is possible that the enzyme is not so able to maintain its activity, because before reuse the enzyme is stored temporarily at $10^\circ C$. Temporary enzyme storage is carried out because research is not continuously carried out from one test to the fourth. Low conversion of fatty acids in enzymatic reactions in organic solvents is also caused by competition between synthesis and amide hydrolysis in the reaction, where both amide synthesis and amide hydrolysis can be use by the same enzyme [12, 18].

The results of the observations of enzyme recoveries in the synthesis of lauroyl-n-methyl glucamide are obtained in Figure-3. It was observed that overall the enzyme can be used up to 4 times. The decrease in percent lauric acid conversion in the use of repeated enzymes is not so obvious in the synthesis of lauroyl-n-methyl-glucamide. After using up to four times, percent fatty acid conversion is still quite high, which is in the range of 40-50%.

This shows that for the synthesis of lauroyl-n-methyl-glucamide, the enzyme still allows it to be reused more than 4 times. One synthesis period is 2 days (48



hours), the use of 4 times is done for 12 days of reaction because there is an interval of two days the enzyme that has been used is temporarily stored in the refrigerator. This means that the enzyme can still be used for more than two weeks, considering that after 12 days the ability of the enzyme to convert fatty acids is still high.

The results of observations of enzyme recoveries in the synthesis of oleoyl-diethanolamide are obtained in Figure-4. It was observed that overall the enzyme can also be used up to 4 times. The decrease in conversion was observed in the use of enzymes after two times.

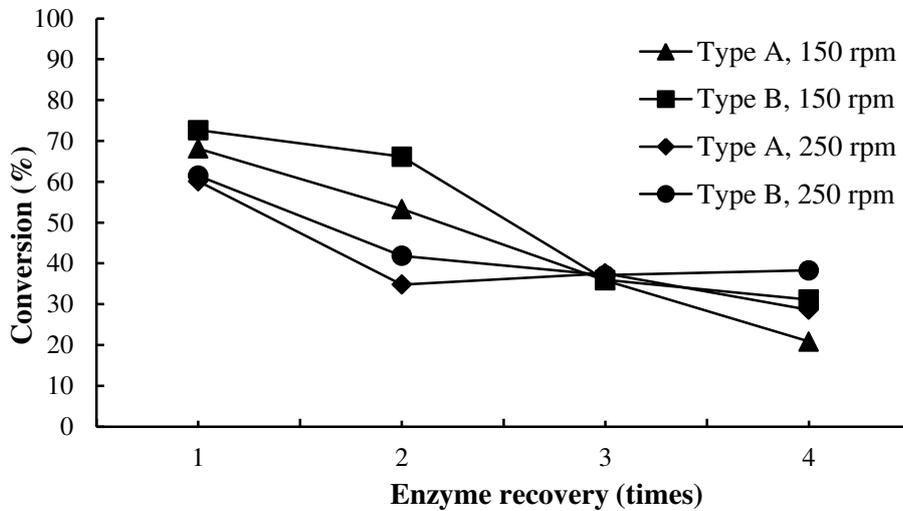


Figure-2. The enzyme recoveries for the synthesis of lauroyl-diethanolamide.

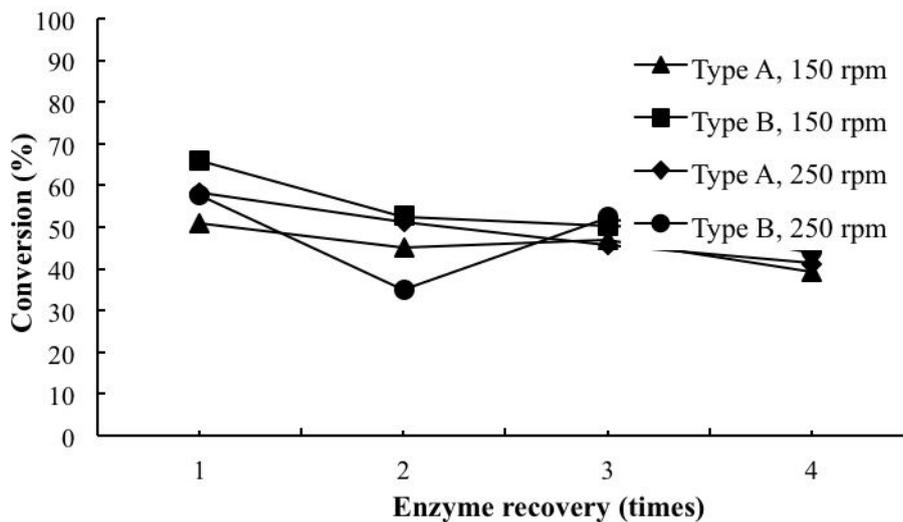


Figure-3. The enzyme recoveries for the synthesis of lauroyl-n-methyl glucamide.

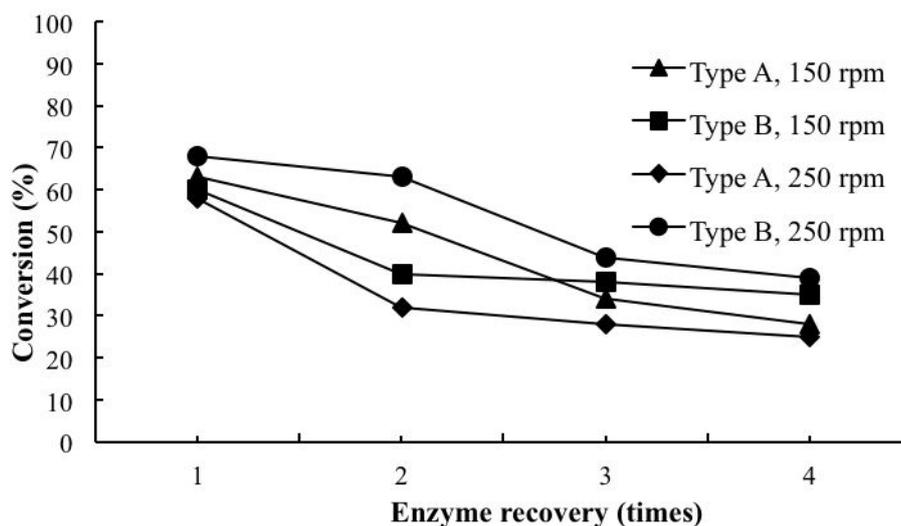


Figure-4. The enzyme recoveries for the synthesis of oleoyl-diethanolamide.

However, this decrease was greater than the decrease in conversion in the synthesis of lauroyl-n-methyl glucamide and lauroyl-diethanolamide. The lipase enzyme is very stable in the reaction conditions selected for about 14 days of reaction at 90°C if the water is evaporated and 7 days if the water remains in the system [6].

Use of metal catalyst

The effect of the use of metal catalysts was observed by studying changes in reaction conversion in each change in reaction time and substrate molar ratio. The effect of reaction time on the synthesis of coco fatty acid amide is shown in Figure-5. The reaction time used in this study consisted of 5 levels, namely, 1, 2, 3, 4 and 5 hours. While the substrate mole ratio used also has 2 levels, namely 2:1 and 4:1. The synthesis of coco fatty acid amide was carried out using $ZrCl_4$ with a concentration of 5%, reaction temperature 80°C and stirring speed of 250 rpm.

It is seen that the highest percentage conversion is at a reaction time of 3 hours, and will gradually decrease

after over 3 hours. This is because, when the reaction has passed the equilibrium stage, the increase in reaction time will further cause the reaction to shift to the transesterification of the methyl ester, and the conversion will decrease [8]. The results of the research in Figure-5 show that the maximum conversion can be obtained at a reaction time of 3 hours. In this reaction condition, it was found that the conversion of coco fatty acid amide reached 90%.

The effect of the substrate mole ratio on the conversion of methyl esters to coco fatty acid amide is shown in Figure-6. The mole ratio of the substrate used in this study consisted of 5 levels, namely, 1:1; 2:1; 3:1; 4:1 and 5:1. While the reaction time used has 3 levels, namely 1, 3 and 5 hours.

The mole ratio of the substrate is one of the important variables in alkanolamide synthesis [2, 3]. Figure-6 shows the effect of the substrate mole ratio on conversion.

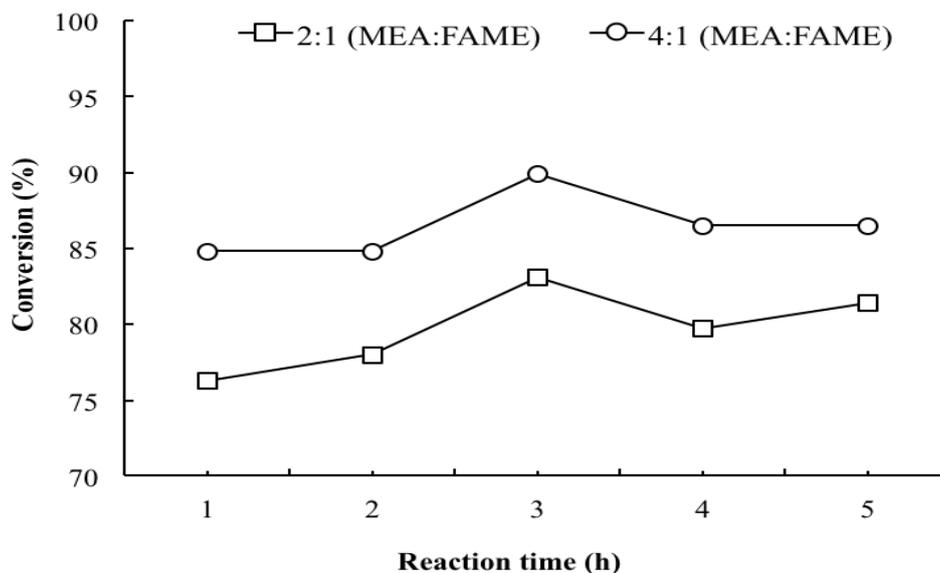


Figure-5. The effect of reaction time on the synthesis of coco fatty acid.

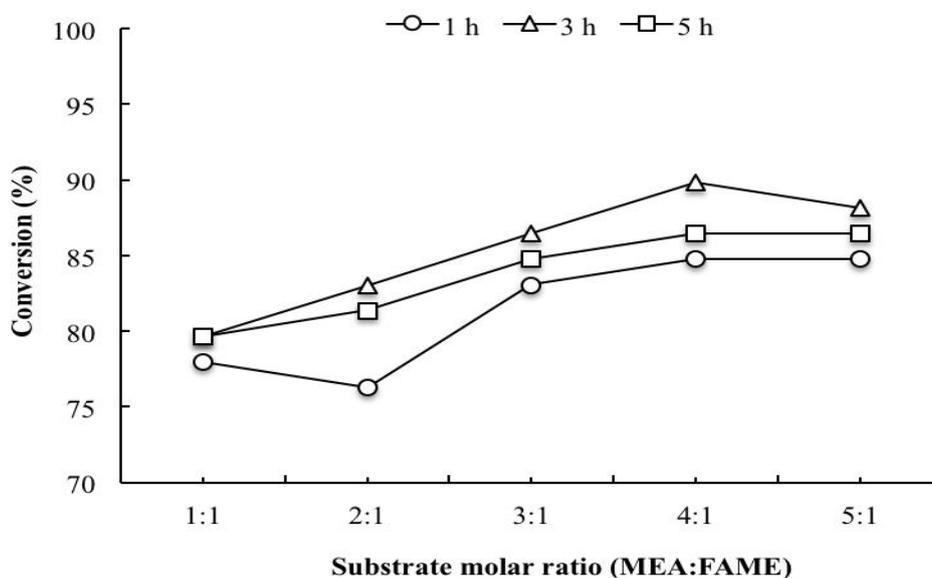


Figure-6. The effect of the substrate mole ratio on conversion.

It can be seen that if the substrate ratio is increased, there will be a change in the percent conversion which will increase with increasing substrate ratio and conversion will decrease at one particular point. The results showed that the maximum conversion can be obtained if the ratio of substrate mole ratio is 4:1 with a reaction time of 3 hours.

Apart from the amidation reaction, the reaction between fatty acids and OH groups from an alkanolamine will be an ester. This esterification reaction is reversible, wherein the presence of excess water the ester will tend to become a carboxylic acid again [18]. The esterification reaction is not expected in this synthesis so that the yield of the resulting ester must be as small as possible.

One way to overcome this is to use lauric acid on a limited basis. In addition, if the expected result is an ester with a high yield, it is better to esterify between alcohol and an acid anhydride or an acid chloride which will react irreversibly. The esterification reaction between an alcohol and a carboxylic acid is less reactive than alcohol with acid anhydride [18]. Thus, setting the optimum substrate molar ratio between fatty acids and alkanolamine is expected to increase the resulting alkanolamide and minimize the formed esters [1, 16, 21].

CONCLUSION

Amide bonds on alkanolamide surfactants are known to be very potential to interact with hydrogen bonds. Besides that, the polarity of amide bonds will



increase the solubility in water from alkanolamide surfactants. The use of immobilized lipases and $ZrCl_4$ metal catalysts was able to improve the efficiency of alkanolamide synthesis because immobilized lipases could be recovered up to 4 times and the $ZrCl_4$ metal catalyst was able to reduce synthesis time to 3 hours. So that overall alkanolamide synthesis as one of the potential surfactants can be considered.

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