Journal of Engineering Science and Technology
Special Issue on SOMCHE 2014 & RSCE 2014 Conference, January (2015) 78 - 87

REACTION SYNTHESIS AND KINETIC MODELING OF ISOAMYL ACETATE VIA ENZYMATIC ESTERIFICATION IN SOLVENT-FREE SYSTEM

Y. A. NURHAZWANI, M. D. MASHITAH, R. A. S. SYAMSUL*

School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, 14300 NibongTebal, SeberangPrai Selatan, Penang, Malaysia
*Corresponding Author: chsyamrizal@usm.my

Abstract

The aim of this study is to determine the reactions involved in esterification reaction of isoamyl acetate, in solvent-free system (SFS), between acetic anhydride and isoamyl alcohol in the presence of enzyme *Candida antarctica* Lipase B (CALB) as catalyst. This study was done in a batch system using stopped rubber conical shake flask. Results show that there is two main reactions took place, which are: (i) between acetic anhydride and isoamyl alcohol, and (ii) between acetic acid and isoamyl alcohol. Kinetic modeling was also done using First Principle model and found that the kinetic constant for k_1 and k_2 value equals to -0.0135 and 0.2530 respectively.

Keywords: Esterification,Isoamyl acetate, Solvent-free system, Kinetic modeling.

1. Introduction

Esters are one of the most extensive of all naturally occurring organic compounds which contain functional group, -COOR. Esters are widely used for various purposes in chemical industry. For instance, ethyl acetate is commonly used as solvent in extraction process [1], and dialkyl phthalates are used in plastic industry to keep polymer from becoming brittle [2]. Many simple esters are pleasant-smelling liquids and mainly used as fragrant odors of fruits and flowers. For example, methyl butanoate is an element found in pineapple oil whereas isoamyl acetate is an element of banana oil [3]. These esters are also naturally present in animal fats and oil [4] and in many biologically important molecules. Esters are ubiquitous and contain 'nature-identical' substance that can be used to substitute natural flavors and fragrances.

Esterification is a process of combining an organic acid (R-COOH) with an

Nomenclatures

C	Concentration
i, j	substrates
k	Reaction rate constant
n	Order of reaction
r	Reaction rate

Greek Symbols

β_1	Temperature
β_2	Mass of enzyme
β_3	Reaction time
β_4	Reciprocal of substrates concentration

Abbreviations

CALB	*Candida antarctica* Lipase-B
SFS	Solvent-Free System

alcohol (R-OH) to form an ester (R-COO-R) and water (H_2O).Esters have great application which primarily used in food and cosmetic industries [5-7]and biodiesel production[8]. Only minimum amount of esters are used as lubricant[7], confectionary [9], and in pharmaceutical product [6]. Traditionally, esters can be directly extracted from plant material or produced by chemical synthesis [10, 11]. However, the high handling cost and low product quantity makes it inappropriate to be implemented for industrial application [12].

Researchers have come out with chemical route in the presence of a strong acid catalyst at high temperature to produce esters at a very low cost. However, there are many by-products and the removal of the catalyst from reaction medium is difficult. In addition, the final product is not natural [10] as has been defined by U.S. Food and Drug Administration in 'Code of Federal Regulation' [13]. Then, biotechnological route of ester production was introduced by using free and immobilized lipase from various sources in organic solvent to encounter the problem in the chemical route[14]. Although higher conversion yields, solvent toxicity is a problem for many applications. On top of that, some organic solvents used are too expensive to allow profitable production in industrial scale [15, 16]. Additionally, a solvent-free system increased isoamyl acetate volumetric production thus making the system preferred and attractive for industrial application [17]. Synthesis of isoamyl acetate in this study has been investigated by using acetic anhydride and isoamyl alcohol catalysed by CALB in a solvent-free system.

2. Material and Methods

2.1. Equipment and materials

The experiments were carried out in a 100ml stopped rubber shake flask, which was incubated in Incu-Shaker Mini (Benchmark Scientific, New Jersey) at 150rpm. Temperature was set at 30°C, 40°C and 50°C. Working pressure was at ambient pressure condition.

Isoamyl alcohol used was supplied by Merck Co. (Malaysia), while acetic anhydride (reagent grade, ≥98%) was supplied by Sigma Aldrich (Malaysia). All

substrates were used without any pre-treatment. Immobilized enzyme from *Candida Antarctica*, Novozyme 435 (specific activity≥ $10000\ U/g$, recombinant, expressed in *Aspergillus Niger)* supplied by Sigma Aldrich (Malaysia).

2.2. Synthesis procedure

Isoamyl acetate synthesis was carried out without any organic solvent in 100ml stopped rubber shake flask with working volume of 15ml. Acetic anhydride and isoamyl alcohol was added into the flask until alcohol to acid ratio equals to 0.1. Then 1wt% of immobilized enzyme from *Candida antarctica* was inserted into the mixture. The reaction mixture was then incubated in an Incu-shaker mini (Benchmark) at 150 rpm for 360 minutes. Samples were withdrawn Every 10 min and analysed with gas chromatograph until 6hr of reaction time. Experimental procedures were repeated for different amount of enzyme and conditions as in Table 1.

2.3. Analysis

0.5ml of the reaction mixture was withdrawn every 10 min. Samples were analysed by Agilent 7820A Gas Chromatograph supplied by Agilent Technologies, equipped with a hydrogen flame ionization detector and a SGE BP21 (FFAP) column (60m x 0.32mm x 0.25 μm). Helium was used as carrier gas at flow rate of 5ml/min. After injection of samples, the temperature of oven was kept at 100°C and linearly increased to 140°C. The rate of temperature increase was set at 70°C/min, and was kept at 140°C for the remaining time of analysis. Injector and detector temperatures were set at 200°C and 250°C, respectively. Quantification of data was done by calibration with standards samples. The peaks of the retention time are as follows: isoamyl acetate, 2.26min; isoamyl alcohol, 2.38 min; acetic anhydride, 2.48min; and acetic acid, 3.21 min [18].

2.4. Experimental design with Response Surface Methodology (RSM)

Experimental design was conducted using Design of Experiment (DoE) software (Design Expert 6.0.6), Start-Ease. A three-level and four-factor design was used to determine the enzyme kinetic constants. Four experimental parameters (operating temperature, T; mass of enzyme, m; reaction time, t; and reciprocal of substrates concentration,$1/[\mathrm{A}]$) were used in this study and 3-level indicated the level of each range (-1, 0, +1).

Table 1. Experimental ranges and levels of variables.

Variables	Coding	Unit	Levels		
			-1	**0**	**+1**
Temperature	β_1	°C	30	40	50
Mass enzyme	β_2	wt%	4	8	12
Reaction time	β_3	h	2	4	6
Reciprocal of substrates concentration	β_4	l/mol	0.12	0.20	1.18

The list of parameters and levels studied in this experiment are shown in Table 1. Preliminary studies showed that all of the variables are statistically significant and ignoring these variables may affect the design of experiment. Central Composite Design (CCD) was chosen because it provides information on experimental variable effects and overall experimental error in a minimum number of runs. CCD also can be used under different experimental regions of interest and operability. This CCD design consists of 2 parts: factorial points (-1, +1), and center points (0, 0). Six replicate runs at the center point (0, 0) of the design were performed to allow the estimation of pure error. All of the experiments were carried out in the randomized order to minimize the unexplained variability in the observed responses due to irrelevant factor.

A second order polynomial model was predicted by a multiple regression procedure. This resulted in an empirical model related to the response by the equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i{}^2 + \sum_{j=1}^{k} \beta_{ji} x_i x_{ij} + \varepsilon, \quad (1)$$

where $x_1, x_2, \ldots, x_k$ are the input variables, which influence the response Y; $\beta_i (i = 1,2, \ldots k), \beta_{ij} (i = 1,2, \ldots, k; j = 1,2, \ldots, k)$ are unknown parameters; and ε is random error.

2.5. Validation of RSM model and determination of kinetic constant

RSM model developed needs to be validated in order to evaluate its efficiency. The validation tests were done based on the conditions factor suggested by RSM and the result is compared with the experimental values.

The obtained reaction rate model equation from RSM is then fitted to the modified Michaelis-Menten equation using POLYMATH® software. By using nonlinear equation solver, kinetic constants were determined.

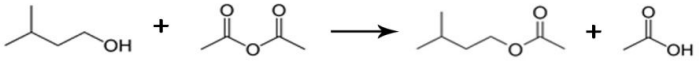
3. Result and Discussion

3.1. Isoamyl acetate reaction synthesis

Theoretically, acetic anhydride possesses two acyl groups. In reaction with isoamyl alcohol, one of the acyl from acetic anhydride will bind with isoamyl alcohol and discharge one H^+ to form isoamyl acetate and acetic acid. Then, excess acyl (from acetic acid) will react with excess alcohol to form another isoamyl acetate and water. The details of reaction scheme are as shown in Fig. 1:

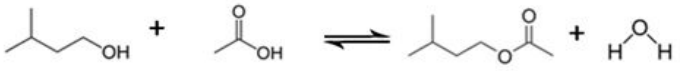
Based on the chemical reaction scheme shown, esterification of isoamyl acetate undergone two reactions, first is between the acetic anhydride and isoamyl alcohol, producing acetic acid and isoamyl acetate, and the second reaction is between the acetic acid and excess isoamyl alcohol, producing another isoamyl acetate and water as by-product. The existence of water in the mixture by the secondary reaction makes the overall esterification reactions more favorable towards the hydrolysis reaction. This fits with the Le Chatelier's Principle, where the dynamic equilibrium of the reaction will be shifted towards the left-hand side of the reversible reaction as the by-product increases. This condition was in agreement with the researches done by Ghamgui et al. (2006) and Luhong et al. (2001).

(a) Main reaction



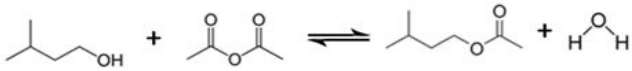
(Isoamyl alcohol) (Acetic anhydride) (Isoamyl acetate) (Acetic acid)

(b) Secondary reaction



(isoamyl alcohol) (acetic acid) (isoamyl acetate) (water)

(c) Overall reaction



(isoamyl alcohol) (acetic anhydride) (isoamyl alcohol) (water)

Fig. 1. The details of reaction scheme.

3.2. Response surface analysis

The effect of temperature, mass of enzyme, reaction time and reciprocal of substrates concentration on the enzymatic reaction rate were investigated using response surface methodology. The results of the developed CCD are given in Table 2. Multiple regression coefficients obtained from a least squares analysis used to predict quadratic polynomial model for the reciprocal enzymatic reaction rate are summarized in Table 3.

All coefficients obtained from the full quadratic polynomial model were evaluated by regression analysis and tested for their significance. The insignificant coefficients were eliminated on the basis of p values. It is well known that smaller p values (<0.001) indicated significant value of model or factor. From the result, it was found that coefficient for β_4^2 , β_{13} and β_{23} were highly insignificant, hence the predicted polynomialmodel was rearranged by eliminate terms which consist of β_4^2 , β_{13} and β_{23} in full quadratic model. The coefficient of the modified quadratic model can be seen in Table 3. The coefficient of determination for the modified quadratic model ($R^2 = 0.98$) is better than the full quadratic model ($R^2 = 0.97$) implies that the model was satisfactory.

The final model for reciprocal of enzymatic reaction rate obtained from the CCD analysis is:

$$\frac{1}{r} = 7.0784 - 0.1867\,(T) - 0.3193(M) - 0.8795(t) + 10.0771\left(\frac{1}{[A]_0}\right) + 0.0028(T^2) + 0.0239(M^2) + 0.1060(t^2) - 0.0024(TM) - 0.1341\left(T\left(\frac{1}{[A]_0}\right)\right) - 0.0732\left(M\left(\frac{1}{[A]_0}\right)\right) - 0.1623\left(t\left(\frac{1}{[A]_0}\right)\right) \quad (2)$$

Table 2. Experimental results of the CCD.

Exp No.	Parameter				Response			
	Temp (°C)	M_{enz} (%)	Time (h)	1/[A](l/mol)	[ester] (mol/l)	[alc] (mol/l)	[anh] (mol/l)	Reaction rate (mol/l.min)
1	50	4	2	0.1490	1.8970	0.4000	2.6890	0.4310
2	40	8	4	0.2022	2.6480	1.4390	0.1350	0.6917
3	50	4	6	0.1490	2.3710	0.0860	2.3600	0.4882
4	50	4	2	1.1794	0.9700	5.6190	0.0060	0.1819
5	30	4	2	1.1794	0.9550	5.3800	0.0080	0.1256
6	30	12	2	0.1490	2.2320	0.1090	2.3590	0.5600
7	40	8	4	1.1794	1.0540	5.2330	0.0290	0.2493
8	30	12	6	0.1490	1.8240	0.0650	1.9150	0.6184
9	50	8	4	0.2022	2.6680	0.4530	0.3890	0.7016
10	30	4	6	0.1490	1.9270	0.1260	2.5430	0.4872
11	30	4	6	1.1794	1.0880	5.1200	0.0060	0.1449
12	30	12	2	1.1794	1.0390	5.0510	0.0070	0.1319
13	40	12	4	0.2022	2.7000	0.0680	1.9000	0.6920
14	30	12	6	1.1794	1.0980	5.2590	0.0070	0.1609
15	40	8	4	0.2022	2.6500	0.4360	0.1400	0.6920
16	50	12	6	0.1490	2.1600	0.0380	2.2980	0.5805
17	50	12	2	0.1490	2.0290	0.1710	2.6780	0.5224
18	40	4	4	0.2022	2.5530	0.7550	0.6610	0.6525
19	40	8	6	0.2022	2.8480	0.3540	0.0910	0.6940
20	30	8	4	0.2022	3.0180	0.3570	0.1000	0.7372
21	50	12	2	1.1794	1.1040	5.3940	0.0310	0.2560
22	50	4	6	1.1794	0.9850	0.9850	0.0060	0.2103
23	40	8	4	0.1490	3.0720	0.5800	0.2340	0.7339
24	40	8	2	0.2022	2.6040	0.6190	0.2580	0.6169
25	30	4	2	0.1490	1.8770	0.2690	2.9230	0.4350
26	40	8	4	0.2022	2.7000	0.4350	0.1370	0.6922
27	50	12	6	1.1794	0.9760	4.7230	0.0270	0.2856

Table 3. Regression coefficients of the quadratic model for CCD.

Factor	Full quadratic model		Modified quadratic model	
	Prob> F	Std. Error	Prob> F	Std. Error
Model	< 0.0001	0.581429	< 0.0001	0.117647
β_1	0.1083	0.120609	0.0807	0.111738
β_2	0.2676	0.120609	0.2287	0.111738
β_3	0.6098	0.120609	0.5800	0.111738
β_4	0.3903	3.431322	< 0.0001	0.131503
β_1^2	0.2107	0.209439	0.1492	0.186699
β_2^2	0.0987	0.209439	0.0588	0.186699
β_3^2	0.0697	0.209439	0.0383	0.186699
β_4^2	0.9051	2.61164	-	-
β_{12}	0.2677	0.083963	0.2288	0.077787
β_{13}	0.3775	0.083963	-	-
β_{14}	< 0.0001	0.152103	< 0.0001	0.140916
β_{23}	0.8902	0.083963	-	-
β_{24}	0.0781	0.152103	0.0552	0.140916
β_{34}	0.0542	0.152103	0.0360	0.140916
R^2	0.99		0.99	
adjusted R^2	0.97		0.98	

3.3. Validation of RSM model

Model developed in the above part has been validated by experimental data. The model validation experiment was conducted at 40°C at 8wt% of mass enzyme for 6hrs. Figure 2 shows the comparison of isoamyl acetate reaction rate between model developed by RSM and from experimental data.

The continuous line indicates the actual result from the experimental data and the dashed line indicates the result from model developed by RSM. The model and experimental result shows a good agreement for reaction rate prediction with R^2value of 0.98. This indicates that the model can be used to find the kinetic constant for this study.

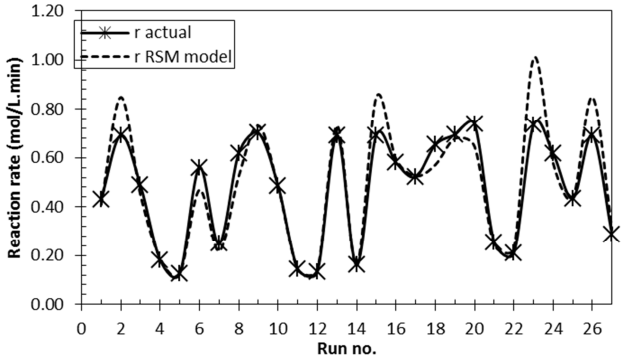


Fig. 2. Reaction rate curve for Isoamyl Acetate production for developed model and experimental data calculation.

3.4. Kinetic constant

Based on the general reaction rate equation,

$$r_j = kC_j^n \tag{3}$$

where r_j is the reaction rate for product j, k is the reaction rate constant, C_j is the concentration of product j, and n is the order of reaction.

Isoamyl acetate enzymatic synthesis between acetic anhydride and isoamyl alcohol having forward and backward reaction, hence Equation 3 becomes;

$$r_j = k_1 C_j^n - k_2 C_i^n \tag{4}$$

where $k_1$andk_2 are rate constants for forward and backward reaction respectively, $C_i and C_j$ are concentration for substrates i and product j respectively, and n is the order number of the reaction.

Overall reaction for isoamyl acetate esterification from acetic anhydride and isoamyl alcohol is:

$$2CH_3CH(CH_3)CH_2CH_2OH + (CH_3CO)_2O \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2CH_3COOCH_2(CH_3)CHCH_3 + H_2O \tag{5}$$

Based on Eq. (4), there were few reaction rate equations that are possible for this isoamyl acetate enzymatic esterification reaction, therefore all of the reaction rate constants for all equation developed were find out using nonlinear equation solver in POLYMATH and the regression of each equation were compared. All of the possible reaction rate equation, constant developed and regression analysis were tabulated in Table 4.

Table 4 shown that the significant reaction rate equation was for the third reaction rate with $R^2 = 0.9385$ and $adj.R^2 = 0.9360$. The kinetic constant for $k_1$andk_2 equals to -0.0135 and 0.2530 respectively with order number of 1.

Table 4. Possible reaction rate equation, constant developed and regression analysis.

No.	Reaction rate equation	k_1	k_2	R^2	$Adj.R^2$
1	$r = (k_1 C_a) + (k_2 C_c)$	0.0461	0.3984	0.5705	0.5533
2	$r = (k_1 C_a) + (k_2 C_p)$	0.0078	0.2426	0.9121	0.9086
3	$r = (k_1 C_b) + (k_2 C_p)$	-0.0135	0.2530	0.9385	0.9360
4	$r = (k_1 C_a^2) + (k_2 C_p)$	0.0013	0.2447	0.9109	0.9073
5	$r = (k_1 C_a^2) + (k_2 C_p^2)$	0.0187	0.0912	0.8111	0.8035
6	$r = (k_1 C_b^2) + (k_2 C_p^2)$	0.0032	0.0961	0.7864	0.7778
7	$r = (k_1 C_b) + (k_2 C_p^2)$	0.0172	0.0951	0.7890	0.7806
8	$r = (k_1 C_a C_b) + (k_2 C_p^2)$	0.1912	0.0909	0.7936	0.7853
9	$r = (k_1 C_a C_b) + (k_2 C_p)$	-0.0147	0.2474	0.9109	0.9073

3.5. Validation of kinetic model

To certify the validity of kinetic constant developed by POLYMATH in above section, comparison of reaction rate from developed kinetic constant and actual reaction rate from experimental data was done. Figure 3 shows the comparison of isoamyl acetate reaction rate calculated by using kinetic constant developed by POLYMATH and reaction rate from experimental data.

The continuous line indicates the actual result from the experimental data and the dashed line indicates the result from model developed by RSM. The model and experimental data shows a good agreement with R^2 value of 0.94. This indicates that the developed kinetic constants are valid to be use in the study of enzymatic esterification of isoamyl acetate from acetic anhydride in solvent free system.

Therefore, the final reaction rate equation for enzymatic esterification reaction from acetic anhydride and isoamyl acetate is;

$$r = -0.0135 C_b + 0.2530 C_p \quad (6)$$

where C_b is the concentration of isoamyl alcohol, and C_p is the concentration of ester.

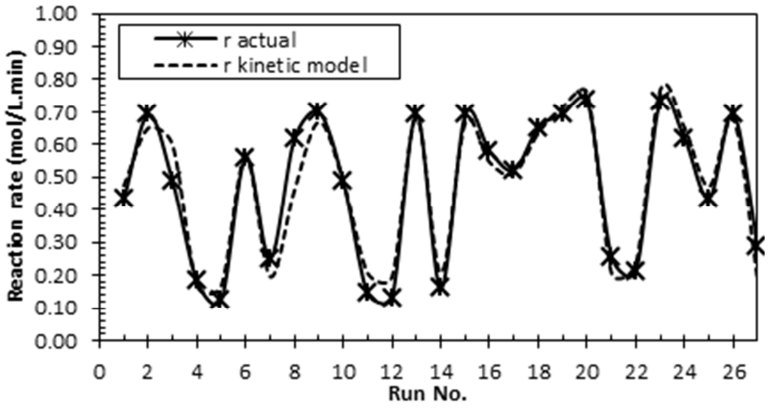


Fig. 3. Reaction rate curve for Isoamyl Acetate production from developed kinetic constant and experimental data calculation.

4. Conclusion

Synthesis of isoamyl acetate was carried out by reacting acetic anhydride with isoamyl alcohol in a solvent-free system. *Candida Antarctica* lipase-B (CALB) was used to enhance the production rate of ester. There are two chemical reactions involved in the esterification of isoamyl acetate from acetic anhydride and isoamyl alcohol. The main reaction is between acetic anhydride and isoamyl alcohol, and the second reaction is between produced acetic acid with the excess isoamyl alcohol. The modelling and optimization process of this enzymatic esterification process were done using RSM based model. A reciprocal of reaction rate model was developed and the ANOVA test implied that the model was satisfactorily representing the real relationship of the main reaction parameters with R^2 (0.98). Kinetic constants of the kinetic equation were obtained from POLYMATH software by using the experimental data and reaction rate model developed before by RSM. The final model resulted k_1 and k_2 value equals to$-0.0135 min^{-1}$and $0.2350\, min^{-1}$ respectively. Validation test were done by doing graphical comparison between the kinetic model developed and experimental data, it shows that the kinetic model developed is having a good agreement with the actual experimental data by R^2 value of 0.94.

Acknowledgement

This study has been supported by a Fundamental Research Grant Scheme (FRGS) (PJKIMIA/6071255).

References

1. Siek, T.J. (1978). Efective use of organic solvents to remove drugs from biologic speciments. *Clinical Toxicology*, 13(2), 205-230.
2. Lowell Center for Sustainable Production. (2011). *Phthalates and their alternatives: health and environmental concerns*. University of Massachusetts Lowell, U.o.M.L., MA, USA.
3. Mc Murry, J.; and Kiselica, S. (2008). *Organic chemistry*. *Carboxylic acid derivatives: Nucleophilic Acyl substitution reaction*. 7th ed., USA: Thomson Learning, Inc. 808.
4. Shaw, J.-F.; Wu, H.-Z.; and Shieh, C.-J. (2003). Optimized enzymatic synthesis of propylene glycol monolaurate by direct esterification. *Food Chemistry*, 81(1), 91-96.
5. Romero, M.D.; Calvo, L.; Alba, C.; Daneshfar, A.; and Ghaziaskar, H.S. (2005). Enzymatic synthesis of isoamyl acetate with immobilized Candida antarctica lipase in n-hexane. *Enzyme Microbial Technology*, 37(1), 42-48.
6. Romero, M.D.; Calvo, L.; Alba, C.; Habulin, M.; Primozic, M.; and Knez, Z. (2005). Enzymatic synthesis of isoamyl acetate with immobilized Candida antarctica lipase in supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 33(1), 77-84.
7. Garcia, T.; Coteron, A.; Martinez, M.; and Aracil, J. (2000). Kinetic model for the esterification of oleic acid and cetyl alcohol using an immobilized lipase as catalyst. *Chemical Engineering Science*, 55(8), 1411-1423.

8. Bollin, P.M.; and Viamajala, S. (2012). Reactive extraction of Triglycerides as fatty acid Methyl Esters using Lewis Acidic Chloroaluminate ionic liquids. *Energy & Fuels*, 26(10), 6411-6418.
9. Shintre, M.S.; Ghadge, R.S.; and Sawant, S.B. (2002). Lipolase catalyzed synthesis of benzyl esters of fatty acids. *Biochemical Engineering Journal*, 12(2),131-141.
10. Guvenc, A.; Nurcan, K.; Emine, B.; and Ülkü Mehmeto, ĞLU. Optimization of the enzymatic production of Isoamyl Acetate with Novozym 435 from Candida Actarctica. (2010). *Chemical Engineering Communications*, 5-8, 948-961.
11. He, H.Y.; Guo, X.; and Zhu, S.L. (2006). Comparison of membrane extraction with traditional extraction methods for biodiesel production. *Journal of the American Oil Chemists' Society*, 83(5), 457-460.
12. Romero, M.D.; Calvo, L.; Alba,C.; Daneshfar, A. (2007). A kinetic study of isoamyl acetate synthesis by immobilized lipase-catalyzed acetylation in n-hexane. *Journal of Biotechnology*, 127(2), 269-277.
13. Code of Federal Regulation. (2012). Food and Drugs. Retrieved December 20,2012from:http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFR Search.cfm?fr=501.22.
14. Romero, M.D.; Gomez, J.M.; Beatriz Diaz, S.; and Alicia Garcia, S. (2011). Study of the influence of alcohols in the synthesis of short chain esters. *Chemical Engineering Transaction*, 24, 37-42.
15. Yahya, A.R.M.; Anderson, W.A.; and Moo-Young, M. (1998). Ester synthesis in lipase-catalyzed reactions. *Enzyme and Microbial Technology*, 23(7-8), 438-450.
16. Guvenc, A.; Kapucu, N.; and Mehmetoğlu, Ü. (2002). The production of isoamyl acetate using immobilized lipases in a solvent-free system. *Process Biochemistry*, 38(3), 379-386.
17. Sandoval, G.; Condoret, J.S.; Monsan, P.; and Marty, A. (2002). Esterification by immobilized lipase in solvent-free media: kinetic and thermidynamic arguments. *Biotechnology and Bioengineering*, 78(3), 313-320.
18. Nurhazwani, Y.A. (2014). *Modeling and optimization of solvent-free Isoamyl Acetate production via enzymatic esterification*. School of Chemical Engineering, Universiti Sains Malaysia, Penang, Malaysia.