Recycling Technology - A Cost Effective Approach for the Synthesis of Alternative Fuel

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ABSTRACT

Recycling is an effective technology for minimization of process cost. Recycling of biocatalyst along with recycling of used oil is a new technique for the preparation of alternative fuel. Preparation of alternative fuel through cost minimization is supposed to be the most challenging job in the present academicians and researchers. Biodiesel is one of the most important alternative fuels in the near future and it attracts considerable attention as environment friendly, renewable and non-toxic fuel. In the present research investigation, waste cooking oil (WCO) is utilized as cheap raw materials for this purpose and enzyme recycling technology has been adopted to prepare biodiesel. Recycling of enzyme is a novel technology which can reduce the process cost. In our study, nonspecific enzyme Novozyme 435 (*Candida antarctica*) is utilized and recycled ten times for the transesterification reaction of WCO and methanol maintaining definite reaction parameters like alcohol to oil molar ratio, reaction temperature, mixing intensity and biocatalyst concentration. The physical properties of WCO methyl ester and diesel fuel have been compared and it shows significant results. So recycling of enzyme for the production of alternative fuel from recycled oil can be utilized to mitigate scarcity of non-renewable fuel in the future world.

Keywords: Waste cooking oil, Biodiesel, *Candida antarctica*, Biocatalyst, Recycling.

1. Introduction

Alternative fuel is one of the important issues in the present researchers and academicians throughout the world. To prepare it from cheap sources through cost effective methodology is another prime thinking nowadays which spreads in scientists and technologists of different areas of like chemistry, biology, mathematics, engineering fields and many more. Technological development is only useful to the whole of mankind whenever the product or production cost is minimum. Preparation of alternative fuel or biodiesel, its use and emission analysis is studied by many researchers using various techniques [1-9]. One of the cost effective methodologies can be obtained by selecting cheap raw materials. In this regard, recycled oil or WCO plays an important role for the production of alternative fuel. Many researchers use WCO for biodiesel production which ensures cost effective fuel. Present author also identified WCO for production of alternative fuel using bioprocess technology [10].

Karmee et. al [11] also identified WCO as cost effective feedstock for alternative fuel in Hong Cong. WCO has also been utilized for biofuel production by many researchers and scientists using different chemical and biochemical catalyst [12-18]. Another method of minimizing cost for biodiesel production is recycling of catalyst. Chemical catalyst is difficult to reuse due to complex separation and isolation procedure. Biocatalyst can easily be recycled for this purpose. Reuse of biocatalyst for the synthesis of biodiesel has been applied by the present author by taking rice bran oil fatty acid distillate as raw material [19]. But rice bran oil fatty acid distillate has been used as soap making process and some other purposes.

Babaki et. al [20] used 20 cycles of immobilized enzyme from *Thermomyces lanuginosus* and studied the effect of water, t-butanol and blue silica gel content for biodiesel production. Aguiéiras et. al [21] investigated the reuse of immobilized lipases in biodiesel synthesis and identified the influence of different solvents in lipase activity. Azócar et. al [22] reutilized lipase in an anhydrous medium with enzyme reutilization to produce biodiesel with low

acid value. Chen et. al [23] studied the optimization of continuous biodiesel production in packed bed reactor by reusing enzyme. Manurung et. al [24] recycled Lipozyme RM IM to produced biodiesel from Crude Palm Oil (CPO) as feedstock. But very few studies have been made regarding the production of biodiesel using recycled raw materials with recycling of enzyme. In the present research investigation, WCO have been identified as recycled raw material for biodiesel production with the reuse of enzyme Novozyme 435 (*Candida antarctica*) after optimization of reaction conditions. Results showed that ten times of recycling of enzyme has been successfully done by applying 6:1 molar ratio of MeOH and WCO at 60⁰C temperature for 8 hrs with 8% biocatalyst.

2. Experimental

2.1 Materials

WCO was collected from different cafeterias, fast-food centres and restaurants of Madhyamgram, Kolkata, West Bengal. The enzymes used in the present study was Novozyme 435, an immobilized non specific lipase based on immobilization via interfacial activation of lipase B from *Candida antarctica* on a resin, Lewatit VP OC 1600 with ester synthesis activity of 10000 propyl laurate unit/g and it was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India. The chemicals monoglycerides and diglycerides were purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

2.2 Optimization of Reaction

For optimization of transesterification reaction, initially 250 ml of WCO was filtered and taken in an Erlenmeyer flask and heated up to 80⁰C to drive off moisture by continuous stirring for about 1 h. After that, reaction was carried out by adding methanol in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer for 8 hours maintaining other reaction conditions in the presence of 8% (w/w) immobilized enzyme Novozyme 435 (w/w). Stepwise addition of methanol was allowed to minimize the deactivation of enzyme.

2.3 Product Analysis

For the analysis of the products, definite amount of samples were withdrawn into a capped vial at suitable intervals, immediately immersed in boiling water for at least 5 minutes to denature the enzyme and stop the reaction. After that the samples were centrifuged for 15 min to remove immobilized lipase. The supernatant part was taken in hexane and no leaching of enzyme was observed in this part. It was then evaporated to dryness and the products were isolated and their amounts were determined by thin layer chromatographic (TLC) method. TLC was done by spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent. The lipid spots were identified by iodine absorption with triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG) and BD as standard. The composition of WCO esters was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexane-diethyl ether: 99:1 as eluting solvent. The enzyme was washed with hexane, dried and reused for the next experiment. During recycling of enzyme, same procedure has been followed for identification and determination of WCO esters (biodiesel). Biodiesel characterization after each batch of experiment was done according to the American Standard Testing Method.

Values are reported as mean $\pm$ s.d., where $n=3$ (n =no of observations). Enzyme recycling was done ten times in laboratory scale for preparation of biodiesel. After each esterification process, the enzyme was collected through filtration. It was then washed with solvent hexane and vacuum dried. Used enzyme was then reused further for biodiesel synthesis maintaining identical parameters and process conditions.

3. Results and Discussions

3.1 Analysis of WCO

The physicochemical properties of WCOs for ten batches are shown in Table 1. Ten batches of WCO were identified as Batch no. 1, 2, 3, 4, 5 and so on. The characteristics of each batch was determined based on free fatty acids (FFAs), iodine values, neutral glycerides, kinematic viscosity, moisture and peroxide value.

Table 1: Physicochemical properties of WCO

Batch No.	Neutral glycerides (wt%)	Iodine value ($I_2/100g$)	FFA (%)	Moisture (%)	Kinematic Viscosity at 40°C (mm^2/s)	Peroxide value (meq/kg)
1	88.78 $\pm$ 0.108	87.57 $\pm$ 0.139	6.21 $\pm$ 0.027	0.2456	28.67 $\pm$ 0.135	20.49 $\pm$ 0.166
2	89.23 $\pm$ 0.111	101.56 $\pm$ 0.167	5.57 $\pm$ 0.017	0.2011	32.46 $\pm$ 0.179	8.23 $\pm$ 0.175
3	89.72 $\pm$ 0.154	89.91 $\pm$ 0.177	5.06 $\pm$ 0.011	0.2032	30.71 $\pm$ 0.131	12.59 $\pm$ 0.123
4	90.57 $\pm$ 0.187	88.98 $\pm$ 0.167	4.30 $\pm$ 0.017	0.1837	31.67 $\pm$ 0.123	12.34 $\pm$ 0.183
5	91.11 $\pm$ 0.109	89.99 $\pm$ 0.172	4.37 $\pm$ 0.012	0.1934	27.38 $\pm$ 0.157	12.61 $\pm$ 0.113
6	90.23 $\pm$ 0.186	102.45 $\pm$ 0.163	4.58 $\pm$ 0.027	0.2148	28.40 $\pm$ 0.159	8.23 $\pm$ 0.127
7	89.45 $\pm$ 0.138	99.67 $\pm$ 0.167	5.29 $\pm$ 0.012	0.2025	23.90 $\pm$ 0.198	8.59 $\pm$ 0.129
8	91.88 $\pm$ 0.184	103.78 $\pm$ 0.191	3.86 $\pm$ 0.016	0.1639	29.98 $\pm$ 0.185	8.23 $\pm$ 0.135
9	92.05 $\pm$ 0.195	96.26 $\pm$ 0.168	3.47 $\pm$ 0.011	0.1612	32.78 $\pm$ 0.164	11.34 $\pm$ 0.108
10	91.34 $\pm$ 0.188	90.69 $\pm$ 0.111	4.71 $\pm$ 0.032	0.1954	29.89 $\pm$ 0.191	12.39 $\pm$ 0.123

It has been found from Table 1 that the neutral glycerides of ten samples are in the range of 89.23 $\pm$ 0.111 to 92.05 $\pm$ 0.195%. This value indicates hydrolysis of oils during prolonged cooking. Iodine value of WCO samples are in the range of 87.57 $\pm$ 0.139 to 103.78 $\pm$ 0.191 $I_2/100g$. The upper limit of iodine value is defined as 120 by EN14214 due to polymerization tendency of high iodine value fuels. In addition, the strong relationship between the oxidative instability with high iodine number is observed for the biodiesels. In our study, iodine values of all WCO samples are within the maximum limit. The properties like kinematic viscosity (23.90 $\pm$ 0.198 to 32.78 $\pm$ 0.164 mm^2/s at 40°C) and FFA content (3.47 $\pm$ 0.011 to 6.21 $\pm$ 0.027%) are found to vary significantly. For WCO

samples, it is found that kinematic viscosity and FFA content increase with the darkness of oil colour. This may be due to the fact that during cooking process at high temperature, some additional volatile and non-volatile compounds are generated in the oil causing darker the oil colour and the higher the oil viscosity and FFA content. Peroxide value of WCO samples are found to be in the range of 8.23 ± 0.127 – 20.49 ± 0.166 meq/kg. Higher value of peroxide value affects the activity of catalyst specifically biocatalyst. So before processing, peroxide value should be low for WCO samples.

3.2 Optimization of Reaction Parameters

Initially, biodiesel preparation from WCO has been optimized and the optimized parameters have been applied for ten batches during recycling. Optimization of the transesterification reaction in laboratory scale between WCO and methanol has been carried out by using enzyme Novozyme 435. The optimized parameters identified are 6:1 molar ratio of MeOH and WCO, 60°C temperature, 8% enzyme for 8 hrs of reaction duration.

FIG. 1: ANALYSIS OF MOLAR RATIO OF MEOH TO WCO FOR BIODIESEL PRODUCTION
[TEMPERATURE: 60°C , TIME: 8 HRS AND ENZYME NS 435: 8% (W/W)]

FIG. 2: ANALYSIS OF TEMPERATURE FOR BIODIESEL PRODUCTION
[MOLAR RATIO: 6:1 (MEOH:WCO), TIME: 8 HRS AND ENZYME NS 435: 8% (W/W)]

Fig. 1 and Fig. 2 show the optimization of molar ratio of methanol: WCO and temperature of reaction respectively for biodiesel production. It has been observed from Fig. 1 that 6:1 molar ratio is the optimum ratio of methanol and WCO for maximum conversion of biodiesel. Enhancement of amount of alcohol did not increase the rate of production due to the decrement of possible no of collisions between the molecules. 60⁰C temperature is the ideal temperature after which rate of conversion decreases probably due to the inactivation of enzyme at higher temperature.

FIG. 3: ANALYSIS OF ENZYME CONCENTRATION FOR BIODIESEL PRODUCTION

[MOLAR RATIO: 6:1 (MEOH:WCO), TIME: 8 HRS AND TEMPERATURE: 60⁰C]

FIG. 4: ANALYSIS OF REACTION TIME FOR BIODIESEL PRODUCTION

[MOLAR RATIO: 6:1 (MEOH:WCO), ENZYME NS 435: 8% (W/W) AND TEMPERATURE: 60⁰C,
CONVERSION MEASURED AT 2, 4, 6, 8 AND 10 HRS]

Optimization of concentration of enzyme and duration of transesterification reaction were identified as shown in Fig. 3 and Fig. 4 respectively. Increasing concentration of enzyme enhances the conversion of reaction and optimization was observed at 8% concentration of enzyme as shown in Fig 3. Further enhancement of enzyme

concentration does not increase the conversion of biodiesel and it may be due to the agglomeration of enzyme at its higher concentration which ultimately decreases the availability of active sites of enzymes. Production conversion vs time has been analysed in Fig. 4 through a pie chart diagram. It has been observed from the Fig. that optimization of reaction time goes for 8 hrs where 92.37% conversion was achieved. Further continuation of reaction has a very minor impact for production efficiency.

3.3 Recycling of Enzyme

After optimization of reaction parameters, recycling of enzyme has been done for ten times with ten new batches of WCO and methanol. Time study analysis w.r.t percent conversion of biodiesel is shown in Table 2.

Table 2: Time study analysis w.r.t. percent conversion of biodiesel

Batch no	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs
1	42.64±0.102	59.38±0.127	80.49±0.162	92.35 ±0.209	92.45±0.235
2	42.60±0.094	59.34±0.143	80.33±0.173	92.24 ±0.193	92.27±0.217
3	42.44±0.115	59.22±0.149	80.21±0.155	92.17 ±0.222	92.18±0.202
4	42.37±0.136	59.02±0.173	80.17±0.168	92.01 ±0.194	92.03±0.194
5	42.05±0.129	58.91±0.142	80.13±0.151	91.89 ±0.188	91.91±0.176
6	41.93±0.119	58.83±0.162	80.03±0.172	91.81 ±0.173	91.83±0.206
7	41.88±0.107	58.76±0.147	79.91±0.169	91.73 ±0.210	91.77±0.213
8	41.72±0.111	58.66±0.145	79.82±0.166	91.66 ±0.179	91.62±0.201
9	41.54±0.137	58.47±0.152	79.77±0.158	91.58 ±0.180	91.49±0.212
10	41.33±0.128	58.38±0.163	79.69±0.173	91.39 ±0.204	91.32±0.216

Here, the percent conversion of biodiesel was observed from the amount of methyl ester through chromatographic method at definite time interval i.e. 2 hrs, 4 hrs, 6 hrs, 8 hrs and 10 hrs. The optimized parameters were maintained in all ten batches for esterification reaction. It has been observed from Table 2 that reusing of enzyme as catalyst for the esterification reaction is very much effective. The same enzyme may also be used even after ten batches which may help to decrease the process cost.

Table 2 shows that percent conversion of WCO to biodiesel in Batch 1 was 92.35 ±0.209% after 8 hrs of reaction while after using 10 times, the same reused enzyme can convert 91.39 ±0.204%. So conversion efficiency of enzyme is still active after ten batches and it can be recycled many more times which helps a lot to minimize the process cost. Initial percentage of FFA is different for different batches as the WCO was collected from different sources. The characterization of biodiesel was done for all ten batches and these are compared with diesel fuel and biodiesel standard. Table 3 shows the different comparative characteristics of ten batches of biodiesel. It has been

observed from the table that preparation of biodiesel can be successfully done using recycled oil and recycled enzyme and properties of biodiesel for each batch has close proximity with biodiesel standard and diesel fuel. Cetane number and flash point are comparatively better in case of biodiesel than diesel fuel as observed from the Table. So use of biodiesel as fuel is safer than diesel fuel. Other properties like specific gravity, kinematic viscosity, density and cloud point are quite similar. Calorific value of biodiesel is somewhat less than diesel fuel though it is in the range of biodiesel standard. So biodiesel prepared from WCO and methanol using recycled enzyme has a good conformity with diesel fuel and is in good agreement with biodiesel standard. This work also proves that the efficacy of enzyme remains almost equal even after using many times which helps to reduce the process cost.

Table 3: Comparative characteristics of biodiesel with diesel fuel

Material	Specific gravity (15°C)	Kinematic Viscosity (mm ² /s)	Density at 150C, (kg/m ³)	Calorific value (MJ/kg)	Cloud point (°C)	Flash point (°C)	Cetane number
BD Std.	0.86 - 0.90	1.96 - 6.0	865 - 900	33-40	5	>120	>40
Diesel	0.82 - 0.95	1.3 – 4.1	820 - 860	45	-----	60 - 80	50
Batch 1	0.84±0.001	4.96±0.007	880.6±0.312	37.46±0.117	5.3±0.003	129±0.138	51±0.134
Batch 2	0.83±0.002	4.91±0.002	881.6±0.273	37.33±0.121	5.4±0.001	130±0.133	50±0.132
Batch 3	0.84±0.001	4.98±0.010	883.6±0.310	37.01±0.132	5.3±0.002	126±0.124	51±0.129
Batch 4	0.82±0.006	4.96±0.002	885.6±0.276	37.51±0.114	5.3±0.004	126±0.151	56±0.145
Batch 5	0.87±0.002	4.92±0.011	884.6±0.317	37.80±0.109	5.4±0.003	5.4±0.003	53±0.185
Batch 6	0.81±0.001	4.93±0.007	882.6±0.257	36.89±0.099	5.2±0.002	128±0.137	55±0.149
Batch 7	0.82±0.002	4.94±0.008	885.6±0.311	36.97±0.128	5.3±0.004	128±0.129	52±0.174
Batch 8	0.84±0.004	4.90±0.009	883.6±0.311	37.07±0.125	5.1±0.001	129±0.135	50±0.128
Batch 9	0.87±0.001	4.97±0.009	881.6±0.276	37.27±0.103	5.5±0.003	127±0.144	51±0.152
Batch 10	0.88±0.004	4.93±0.004	883.6±0.307	36.88±0.118	5.6±0.002	122±0.128	53±0.148
Test Method	ASTM D 6751-02	ASTMD 445	ASTMD- 4052-96	ASTM- 6751	ASTM D2500	ASTMD 93	ASTMD 675

4. CONCLUSION

Recycling of enzyme using recycling oil for the preparation of alternative fuel is a novel idea which reduces the process cost as well as contributes a clean process technology. Waste cooking oil is used as cheap raw material in this methodology which also helps to produce low-priced biodiesel. Activity of enzyme is slightly reduced for

recycling of biocatalyst though it does not hamper the productivity of the process. Initial process parameters have been optimized which is applied in recycling technology. In our study, ten times recycling have been successfully completed with desired conversion of biodiesel. This recycling technology for enzymatic production of biodiesel from recycled raw materials is useful to mitigate the scarcity of fossil fuels in near future. Our process technology contributes a new ray of knowledge to the future researchers to think differently for the production of environment friendly alternative fuel.

REFERENCES

- 1) K. A. Zahan and M. Kano, Biodiesel production from palm oil, its by-products, and mill effluent: A review, *Energies*, 11, 2132; doi:10.3390/en11082132, 2018..
- 2) B. Q. Qiao, D. Zhou, G. Li, J. Z. Yin, S. Xue and J. Liu, Process enhancement of supercritical methanol biodiesel production by packing beds, *Bioresour. Technol.*, 228, 298–304, 2017.
- 3) R. S. Nursal, Z. Zali, H. Haizri, C. Amat, S. Amear, S. Ariffin and A. Khalid, Comparative study of the performance and exhaust gas emissions of biodiesels derived from three different feedstocks with diesel on marine auxiliary diesel engine, *ARPN J. Eng. Appl. Sci.*, 12, 2017–2028, 2017.
- 4) M. A. Vieira da Silva, B. L. Gil Ferreira, L. G. da Costa Marques, A. L. Soares Murta and M. A. V. de Freitas, Comparative study of NO_x emissions of biodiesel-diesel blends from soybean, palm and waste frying oils using methyl and ethyl transesterification routes, *Fuel*, 194, 144–156, 2017.
- 5) S. Nandi and S. Ganguly, Enzymatic packed bed reactor for biodiesel production from *Jatropha Curcas* oil, *Journal of Chemical, Biological and Physical Sciences, Section A*, 6 (4), pp. 1419-1428, 2016.
- 6) S. Nandi, R. Bhattacharyya and G. Misra, Investigation of biodiesel from Canola oil deodorizer distillate using dual biocatalyst, *Asian Journal of Applied Science and Technology*, 4 (1), 131-138, January-March, 2020.
- 7) S. Nandi, R. Bhattacharyya and T. K. Ghosh, Process optimization and kinetics of biodiesel production from renewable raw materials, *Saudi Journal of Engineering and Technology*, 4(6), 248-252, 2019.
- 8) P. K. Roy, S. Datta, S. Nandi and F. A. Basir, Effect of mass transfer kinetics for the production of biodiesel from *Jatropha Curcas* oil: A mathematical approach, *Fuel*, 134, 39-44, 2014.
- 9) S. Nandi and R. Bhattacharyya, Studies on enzymatic production of biodiesel from *Jatropha Curcas* Oil by varying alcohols, *Journal of Chemical, Biological and Physical Sciences*, 6(1), 311-320, 2016.
- 10) S. Nandi, R. Bhattacharyya, D. Marik and T. K. Ghosh, Utilisation of Waste Cooking Oil as Biodiesel through Bioprocess Technology, *Int. J. for Research in Applied Science & Engineering Technology*, 8(IV), 133-137, 2020.
- 11) S. K. Karmee, R. D. Patria and C. S. K. Lin, Techno-Economic Evaluation of Biodiesel Production from Waste Cooking Oil, *International Journal of Molecular Sciences*, 16, 4362-4371; doi:10.3390/ijms16034362, 2015.
- 12) M. Raqeeb and R. Bhargavi, Biodiesel production from Waste cooking oil, *Journal of Chemical and Pharmaceutical Research*, 7 (12), 670-681, 2015.

- 13) M. C. Bobadilla, R. L. Lorza, R. E. García, F. S. Gómez and E. P. V. González, An Improvement in Biodiesel Production from Waste Cooking Oil by Applying Thought Multi-Response Surface Methodology Using Desirability Functions, *energies*, 10 (130), 1-20, 2017.
- 14) R. Alarcón., D. M. Romero and A. Ladino, Biodiesel production from waste frying oil and palm oil mixtures, *Chemical Engineering Transactions*, 57, 571–576, 2017.
- 15) K. Balakrishnan, M. A. Olutoye and B. H. Hameed, Synthesis of methyl esters from waste cooking oil using construction waste material as solid base catalyst, *Bioresour. Technol.*, 128, 788–791, 2013.
- 16) Z. Yaakob, M. Mohammad, M. Alherbawi, Z. Alam and K. Sopian, Overview of the production of biodiesel from Waste cooking oil, *Renew. Sustain. Energy Rev.*, 18, 184–193, 2013.
- 17) M. Atapour, H. R. Kariminia and P. M. Moslehabadi, Optimization of biodiesel production by alkali-catalyzed transesterification of used frying oil, *Process Saf. Environ. Prot.*, 92, 179–185, 2014.
- 18) M. D. Solikhah., I. Paryanto and B. R. Barus, Efek Kualitas Minyak Jelantah terhadap Harga Proses Produksi dan Kualitas Biodiesel, *Prosiding Seminar Nasional Teknik Kimia Indonesia- SNTKI*, Bandung, 2009.
- 19) Y. Wang., S. Qou., P. Liu and Z. Zhang, Preparation of Biodiesel from Waste Cooking Oil via Two-Step Catalyzed Process, *Energy Conversion & Management*, 48 (1), 184-188, 2007.
- 20) S. Nandi, R. Bhattacharyya, D. Marik and T. K. Ghosh, “Recycling of enzyme – A novel technology for cost effective synthesis of biodiesel”, *J. of Chemical, Biological and Physical Sciences, Section A*, 10 (1), 1-11, 2019.
- 21) M. Babaki, M. Yousefi, Z. Habibi, M. Mohammadi, P. Yousefi and J. Brask, Enzymatic production of biodiesel using lipases immobilized on silica nanoparticles as highly reusable biocatalysts: effect of water, t-butanol and blue silica gel contents, *Renewable Energy*, 91, 196-206, 2016.
- 22) E. C. G. Agueiras, D. S. Ribeiro, P. P. Couteiro, et. al, Investigation of the Reuse of Immobilized Lipases in Biodiesel Synthesis: Influence of Different Solvents in Lipase Activity, *Appl. Biochem. Biotechnol.*, 179, 485–496, <https://doi.org/10.1007/s12010-016-2008-9>, 2016.
- 23) L. Azócar, G. Ciudad, H. J. Heipieper, R. Muñoz, and R. Navia, Lipase-catalyzed process in an anhydrous medium with enzyme reutilization to produce biodiesel with low acid value, *Journal of Bioscience and Bioengineering*, 112, 583–589, 2011.
- 24) H. C. Chen, H. Y. Ju, T. T. Wu, Y. C. Liu, C. C. Lee, C. Chang, Y. L. Chung, and C. J. Shieh, Continuous Production of Lipase-Catalyzed Biodiesel in a Packed-Bed Reactor: Optimization and Enzyme Reuse Study, *BioMed Research International*, Volume 2011 |Article ID 950725 | 6 pages.
- 25) R. Manurung, R. Afrianto and M. Widyawati, Recyclable of lipozyme rm im in biodiesel synthesis from CPO through interesterification process, *Journal of Engineering Science and Technology special issue on SOMCHE 2014 & RSCE 2014 conference*, 27-35, January, 2015.