

Optimization of Enzymatic Transesterification of Beef Tallow Using *Candida Antarctica* Novozyme 435 with 2-Butanol and Hexane

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ABSTRACT

The effectiveness of enzymatic transesterification of animal tallow using the enzyme *Candida antarctica* Novozyme 435 was studied. The effects of oil : alcohol molar ratio (1:1, 1:2, 1:3, 1:4 and 1:5), reaction temperature (35, 40, 45 and 50°C) and reaction time (4, 8, 12 and 16 h) on the biodiesel conversion yield were evaluated in a solvent system using n-hexane. The highest conversion yield of biodiesel was obtained at the 1:4 oil : alcohol molar ratio, 16 h reaction time and 45°C reaction temperature. Increasing the oil : alcohol molar ratio from 1:1 to 1:4 increased the conversion yield of biodiesel by 21.24-82.11%, depending on the reaction temperature and time. The rate of conversion of fatty acid esters to biodiesel increased with increases in reaction time. The reaction proceeds slowly at the beginning and then rapidly due to the initial mixing and dispersion of alcohol into the oil substrate and the activation of enzyme. Increasing the reaction time from 4 to 16 h increased the conversion yield of biodiesel by 13.51-34.07%, depending on the oil : alcohol molar ratio and reaction temperature. The interactions between enzyme polymer surface and substrate appears to be dependent on reaction temperature due to hydrogen bonding and ionic interactions which play important roles in maintaining the thermostability of lipase in the system. The high temperature of 50°C denatured the specific structure of enzymes and resulted in a decrease in methyl esters formation. Increasing the reaction temperature from 40 to 45°C increased the biodiesel conversion yield by 8.48-33.95%. Using n-hexane in the reaction helped to stabilize the enzyme and minimize the toxicity of alcohol. The activity of the enzyme catalyst *Candida antarctica* Novozyme 435 in the presence of 2-butanol and n-hexane remained relatively stable for 10 cycles and then decreased rapidly reaching 11% after 50 cycles.

Keywords: Biodiesel, Enzyme Transesterification, Novozyme 435, Solvent, Oil : Alcohol Molar Ratios, Reaction Time, Reaction Temperature.

INTRODUCTION

The high demand for fossil fuels, their limited and unsecure supply and high cost have prompted the search for alternative energy sources such as biofuels, solar, wind, wave, hydro, geothermal and nuclear. However, renewable biofuels such as biodiesel, bioethanol and biogas from biomass materials are more economical, and environmentally friendly [1-3]. Biodiesel has several qualities over diesel including: being sulfur free, non-toxic, biodegradable and non-carcinogenic [4]. These characteristics make it greener and more eco-friendly than diesel [5-9]. In addition, biodiesel can be used in compression-ignition engines instead of petroleum diesel [10-11].

Biodiesel can be produced from many raw materials including: plant oils, animal fats, microbial mass and waste materials [6]. Popular plants used as a feedstock are jatropa, canola, coconut, cottonseed, groundnut, karanj, olive, palm, peanut, rapeseed, safflower, soybean and sunflower [6,9-10].

The most popular animal sources used as a feedstock are beef tallow, chicken fat, lamb fat, lard, yellow grease and hemp oil [10.12-14]. The waste materials include: waste cooking oil, greasy by-product from omega-3 fatty acid production and fish waste [6,9].

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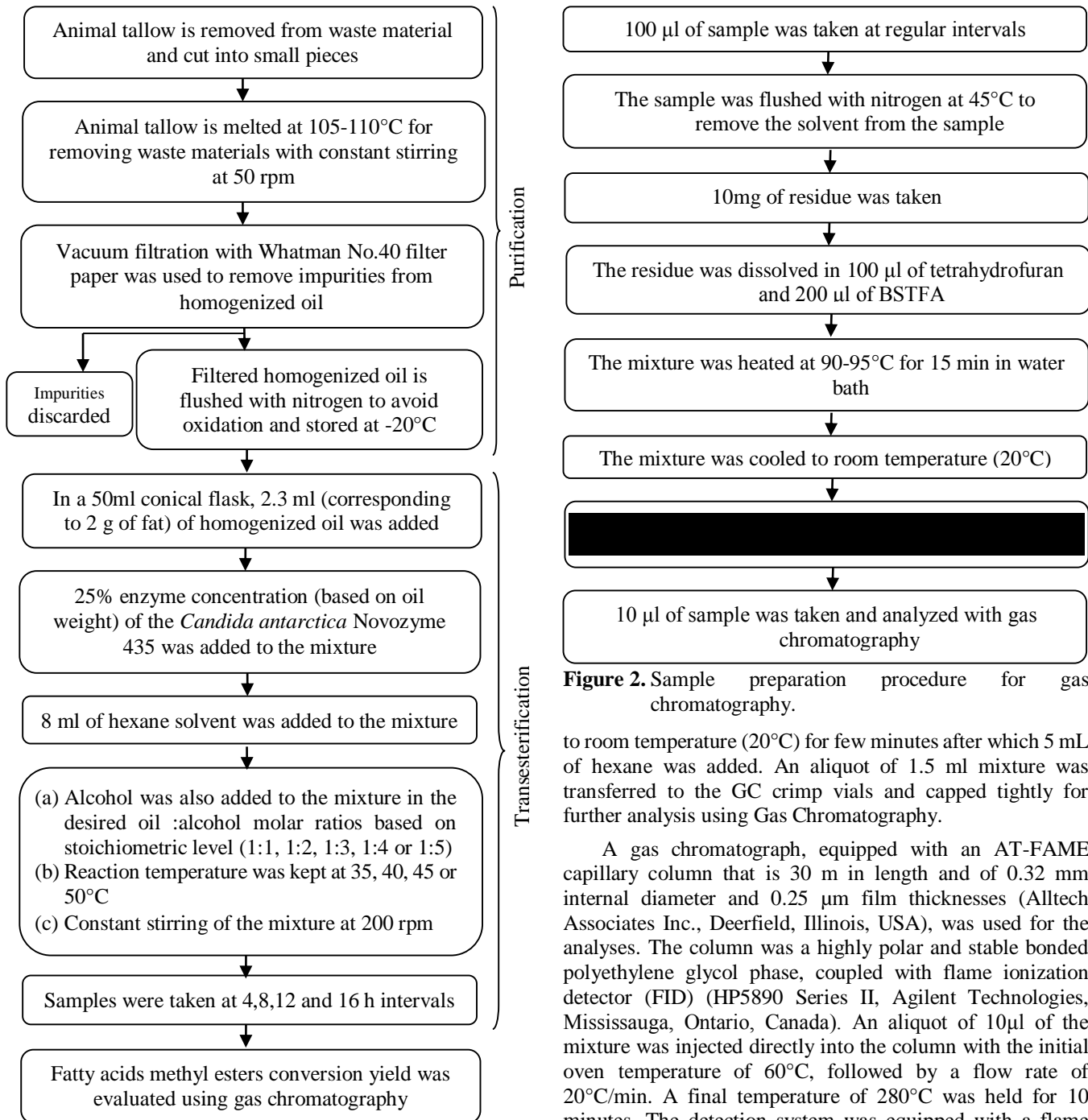


Figure 1. Purification and enzymatic transesterification steps for the animal tallow.

aliquot was taken from the transesterification process and flushed with nitrogen in a microprocessor-controlled water bath (280 series, Fisher Scientific, Toronto, Ontario, Canada) at 45°C in order to evaporate the hexane. A 10 mg portion of the residue was dissolved in 100 µL of tetrahydrofuran and 200 µL of BSTFA. Then, the mixture was heated in the water bath at 90-95°C for 15 minutes. The sample was then cooled

Figure 2. Sample preparation procedure for gas chromatography.

to room temperature (20°C) for few minutes after which 5 mL of hexane was added. An aliquot of 1.5 ml mixture was transferred to the GC crimp vials and capped tightly for further analysis using Gas Chromatography.

A gas chromatograph, equipped with an AT-FAME capillary column that is 30 m in length and of 0.32 mm internal diameter and 0.25 µm film thicknesses (Alltech Associates Inc., Deerfield, Illinois, USA), was used for the analyses. The column was a highly polar and stable bonded polyethylene glycol phase, coupled with flame ionization detector (FID) (HP5890 Series II, Agilent Technologies, Mississauga, Ontario, Canada). An aliquot of 10µl of the mixture was injected directly into the column with the initial oven temperature of 60°C, followed by a flow rate of 20°C/min. A final temperature of 280°C was held for 10 minutes. The detection system was equipped with a flame ionization detector (FID) operating at 275°C with helium as a carrier gas at a flow rate of 0.6 mL/min. The total run time was 40 minutes. The yield was calculated as follows:

$$\text{Conversion yield of Peak A (wt\%)} = \frac{\text{Peak area A} \times 100}{\sum(\text{Peak area A} + \text{Peak area B} + \dots + \text{Peak area N})} \quad (2)$$

Where:

Peak area A = Methyl Oleate

Peak area B = Methyl Palmitate

Peak area N = no. of unknown peaks

Thermomyces lanuginosus, immobilized Lipozyme *Rhizomucor miehei*, immobilized *Rhizopus oryzae* and experimental enzyme NS88001, respectively.

Several researchers [45,50,52] stated that repeated use of enzyme in the reaction without removing glycerol from the system might inhibit the interaction between the substrate and lipase. Xu *et al.* [46] reported that while using methyl acetate as acyl acceptor, no glycerol was produced in the reaction with no loss of enzyme activity for 10 cycles in the reaction. The byproduct from the reaction was triacetyl glycerol instead of glycerol which did not affect the product quality.

CONCLUSIONS

The effectiveness of enzymatic transesterification of animal tallow using the *Candida antarctica* Novozyme 435 was studied. The effects of oil: alcohol molar ratio (1:1, 1:2, 1:3, 1:4 and 1:5), reaction temperature (35, 40, 45 and 50°C) and reaction time (4, 8, 12 and 16 h) on the biodiesel conversion yield were evaluated using n-hexane as a solvent. The highest conversion yield of biodiesel was obtained at the 1:4 oil:alcohol molar ratio, 16 h reaction time and 45°C reaction temperature. Increasing the oil : alcohol molar ratio from 1:1 to 1:4 increased the conversion yield of biodiesel by 21.24%. The rate of conversion of fatty acid esters increased with increases in reaction time. The reaction proceeds slowly at the beginning and then rapidly due to the initial mixing and dispersion of alcohol into the oil substrate and activation of enzyme. Increasing the reaction time from 4 to 16 h increased the conversion yield of biodiesel by 13.51%. The interactions between enzyme and substrate appears to be dependent on reaction temperature due to hydrogen bonding and ionic interactions which play important roles in maintaining the thermostability of lipase in the system. The higher temperature denatured the specific structure of enzymes and resulted in a decrease in methyl esters formation. Increasing the reaction temperature from 40 to 45°C increased the biodiesel conversion yield by 8.48%. Using n-hexane in the reaction helped to stabilize the enzyme and minimize the toxicity of alcohol. The activity of experimental enzyme catalyst in the presence n-hexane was slightly reduced after 10 cycles, it then decreased rapidly and stopped after 50 cycles.

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REFERENCES

- Saka, S and D. Kusidana, 2001. Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. *Fuel*, 80: 225-231. DOI: 10.1016/S0016-236(00)00083-1
- Xie, W and H. Li, 2006. Alumina-supported potassium iodide as a heterogeneous catalyst for biodiesel production from soybean oil. *Journal of Molecular Catalysis A: Chemical*, 225: 1-9. DOI: 10.1016/j.molcata.2006.03.061
- Kumar, S., Ghaly, A. E., M. S. Brook and S. M. Budge, 2014. Effectiveness of enzymatic transesterification of beef tallow using experimental enzyme NS88001 with methanol and hexane. *Enzyme Engineering*, 2(2):1-10. DOI: 10.4172/2329-6674.1000116
- Venkataraman, C., G. Negi, S. B. Sardar and R. Rastogi, 2002. Size distributions of polycyclic aromatic hydrocarbons in aerosol emissions from biofuel combustion. *Journal of Aerosol Science*, 33: 503-518. DOI: 10.1016/S0021-8502(01)00185-9
- Bondioli, P., A. Gasparoli, A. Lanzani, E. Fedeli, S. Veronese and M. Sala, 1995. Storage stability of biodiesel. *Journal of the American Oil Chemists' Society*, 72: 699-702. DOI: 10.1007/BF0263658
- Akoh, C. C., S. Chang, G. Lee and J. Shaw, 2007. Enzymatic approach to biodiesel production. *Journal of Agricultural and Food Chemistry*, 55: 8995-9005. DOI: 10.1021/jf071724y
- Basha, S. A., K. R. Gopal and S. Jebaraj, 2009. A review on biodiesel production, combustion, emissions and performance. *Renewable and Sustainable Energy Reviews*, 13:1628-1634. DOI: 10.1016/j.rser.2008.09.031
- Shafiee, S. and E. Topal, 2008. When will fossil fuel reserves be diminished? *Energy Policy*, 37: 181-189. DOI: 10.1016/j.enpol.2008.08.016
- Robles-Medina, A., P. A. Gonzalez-Moreno, L. Esteban-Cerdán and E. Molina-Grima, 2009. Biocatalysis: Towards ever greener biodiesel production. *Biotechnology Advances*, 27: 398-408. DOI: 10.1016/j.biotechadv.2008.10.008
- Demirbas, A., 2003. Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods, a survey. *Energy Conversion and Management*, 44: 2093-2109. DOI: 10.1016/j.fuel.2007.08.007
- Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technology*, 86: 1059-1070. DOI: 10.1016/j.fuproc.2004.11.002
- Marchetti, J. M., V. U. Miguel and A. F. Errazu, 2008. Techno-economic study of different alternatives for biodiesel production. *Fuel Process Technology*, 89: 740-748. DOI: 10.1016/j.fuproc-2008-01-007
- Ranganathan, S. V., S. L. Narasimhan and K. Muthukumar, 2008. An overview of enzymatic production of biodiesel. *Bioresource Technology*, 99: 3975-3981. DOI:10.1016/j.biortech.2007.04.060
- Antczak, M. S., A. Kubiak, T. Antczak and S. Bielecki, 2009. Enzymatic biodiesel synthesis-key factors affecting efficiency of the process. *Renewable Energy*, 34:1185-1194. DOI: 10.1016/j.renene.2008.11.013
- Pinto, A. C., L. N. Lilian, L. L. N. Guarieiroa, M. J. C. Rezendea and N. M. Ribeiro., 2005. Biodiesel: An overview. *Journal of the Brazilian Chemical Society*, 16: 1313-1330. DOI: 10.1590/S0103-50532005000800003

16. Demirbas, A., 2008. Relationships derived from physical properties of vegetable oil and biodiesel fuels. *Fuel*, 87: 1743-1748. DOI: 10.1080/15567030701268401
17. Fukuda, H., A. Kondo and H. Noda. 2001. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering*, 92: 405-416. DOI:10.1016/S1389-1723(01)80288-7
18. Schuchardt, U. L. F., R. Sercheli and R. M. Vargas, 1998. Transesterification of vegetable Oils: A review. *Journal of the American Oil Chemists' Society*, 9: 199-210. DOI: 10.1590/S0103-505311998000300002
19. Wang, Z. X., J. Zhuge, H. Fang and B. A. Prior, 2001. Glycerol production by microbial fermentation : A review. *Biotechnology Advances*, 19: 201-223. doi:10.1016/S0734-9750(01)00060-X
20. Watanabe, Y., Y. Shimada, Y., Sugihara, A. and Y. Tominaga, 2002. Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antarctica* lipase. *Journal of Molecular Catalysis B: Enzymatic*, 17: 151-155. DOI: 10.1016/S1381-1177(02)00022-X
21. Dorado, M. P., E. Ballesteros, M. Mittelbach and F. J. Lopez, 2004. Kinetics parameters affecting the alkali-catalyzed transesterification process of used olive oil. *Energy and Fuels*, 18:1457-1462. DOI: 10.1021/ef034088o
22. Kulkarni, M. G and A. K. Dalai, 2006. Waste cooking oil- An economic source for biodiesel: A-review. *Industrial and Engineering Chemistry Research*, 45: 2901-2913. DOI: 10.1021/ie0510526
23. Nelson, L. A., T. A. Foglia and W. N. Marmer, 1996. Lipase-catalyzed production of biodiesel. *Journal of the American Oil Chemists' Society*, 73: 1991-1994. DOI: 10.1007/BF02523383
24. Kumari, V., S. Shah and M. N. Gupta, 2007. Preparation of biodiesel by lipase catalyzed transesterification of high free fatty acid containing oil from *Madhuca indica*. *Energy and Fuels*, 21: 368-372. DOI: 10.1021/ef0602168
25. Kumari, A., P. Mahapatra, V. K. Garlapati and R. Banerjee, 2009. Enzymatic transesterification of *Jatropha* oil. *Biotechnology and Biofuels*, 2: 1-7. DOI: 0.1186/1754-6834-2-1
26. Chen, G., M. Ying and W. Li, 2006. Enzymatic conversion of waste cooking oils into alternative fuel-biodiesel. *Applied Biochemistry and Biotechnology*, 129: 911-921. DOI: 10.1385/ABAB132:i:911
27. Tamalampudi, S., R. M. Talukder, S. Hamad, T. Numatab and A. Kondo., 2008. Enzymatic production of biodiesel from *Jatropha* oil: A comparative study of immobilized-whole cell and commercial lipases as a biocatalyst. *Biochemical Engineering Journal*, 39: 185-189. DOI:10.1016/j.bej.2007.09.002
28. Dizge, N. and B. Keskinler, 2008. Enzymatic production of biodiesel from canola oil using immobilized lipase. *Biomass and Bioenergy*, 32: 1274-1278. DOI: 10.1016/j.biombioe.2008.03.005
29. Bernardes, O.L., J. V. Bevilacqua, M. C. M. Leal, D. M. G. Freire and M. A. P. Langone, 2007. Biodiesel fuel production by the transesterification reaction of soybean oil using immobilized lipase. *Applied Biochemistry and Biotechnology*, 137: 105-114. DOI: 10.1007/s12010-007-9043-5
30. Andre, L. F., I. N. Correa, C. O. Veloso and M. A. Langone, 2008. Enzymatic synthesis of biodiesel via alcoholysis of palm oil. In: *The 30th Symposium on Biotechnology for Fuels and Chemicals*, New Orleans, Louisiana, May 4-7.
31. Chen, J. W. and W. T. Wu, 2003. Regeneration of immobilized *Candida antarctica* lipase or transesterification. *Journal of Bioscience and Bioengineering*, 95: 466-469. DOI: 10.1016/S1389-1723(03)80046-4
32. Samukawa, T., M. Kaieda, T. Matsumoto, K. Ban and A. Kondo, 2000. Pretreatment of immobilized *Candida antarctica* lipase for biodiesel fuel production from plant oil. *Journal of Bioscience and Bioengineering*, 90: 180-183. DOI: 10.1263/jbb.90.180
33. Salis, A., M. Pinna, M. Monduzzi and V. Solinas, 2005. Biodiesel production from triolein and short chain alcohols through biocatalysis. *Journal of Biotechnology*, 119: 291-299. DOI: 10.1016/j.biotech.2005.04.009
34. Al-Zuhair, S., F. W. Ling and L. M. Jun, 2007. Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase. *Process Biochemistry*, 42: 951-960. DOI: 10.1016/j.procbio.2007.03.002
35. Freedman, B., E. H. Pryde and T. L. Mounts, 1984. Variables affecting the yields of fatty esters from transesterified vegetable oils. *Journal of the American Oil Chemists' Society*, 61: 1638-1643. DOI: 10.1007/BF02541649
36. Nouredini, H. and D. Zhu, 1997. Kinetics of transesterification of soybean oil. *Journal of the American Oil Chemists' Society*, 74: 1457-1463. DOI: 10.1007/s11746-997-0254-2
37. Modi, M. K., J. R. C. Reddy, B. V. S. Rao and R. B. N. Prasad, 2006. Lipase-mediated transformation of vegetable oils into biodiesel using propan-2-ol as acyl acceptor. *Biotechnology Letters*, 28: 637-640. DOI: 10.1007/s10529-006-0027-2
38. Ma, F., L. D. Clements and M. A. Hanna, 1998. Biodiesel fuel from animal fat. Ancillary studies on transesterification of beef tallow. *Industrial and Engineering Chemistry Research*, 37: 3768-3771. DOI: 10.1021/ie980162s
39. Leung, D. Y. C and Y. Guo, 2006. Transesterification of neat and used frying oil: optimization for biodiesel production. *Fuel Process Technology*, 87: 883-890. DOI: 10.1016/j.fuproc.2006.06.003
40. Meter, F., C. Zarcu and C. Kiss, 2007. Enhancement of lipases enantioselectivity by entrapment in hydrophobic sol-gel materials: Influence of silane precursors and

- immobilization parameters. *Journal of Biotechnology*, 131: S109. DOI: 10.1016/j.jbiotec.2007.07.187
41. Eevera, T., K. Rajendran and S. Saradha, 2009. Biodiesel production process optimization and characterization to access the suitability of the product for varied environmental conditions. *Renewable Energy*. 34(3): 762-765. doi:10.1016/j.renene.2008.04.006
42. Rodrigues, R. C., G. Volpato, K. Wada and M. A. Z. Ayub, 2008. Enzymatic synthesis of biodiesel from transesterification reactions of vegetable oils and short chain alcohols. *Journal of the American Oil Chemists' Society*, 85: 925-930. DOI: 10.1007/s11746-008-1284-0
43. Nie, K., F. Xie, F. Wong and T. Tan, 2006. Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production. *Journal of Molecular Catalysis B: Enzymatic*, 43: 142-147. DOI: 10.1016/j.molcatb.2006.07.016
44. Mittelbach, M., 1990. Lipase catalyzed alcoholysis of sunflower oil. *Journal of the American Oil Chemists' Society*, 67: 168-170. DOI: 10.1007/BF02539619
45. Soumanou, M. M. and U. T. Bornscheuer, 2003. Improvement in lipase-catalyzed synthesis of fatty acid methyl esters from sunflower oil. *Enzyme and Microbial Technology*, 33: 97-103. DOI: 10.1016/S0141-0229(03)00090-5
46. Xu, Y., W. Du, D. Liu and J. Zeng, 2003. A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium. *Biotechnology Letters*, 25: 1239-1241. DOI: 10.1023/A:1025065209983
47. Shimada, Y., Y. Watanabe, A. Sugihara and Y. Tominaga, 2002. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *Journal of Molecular Catalysis B: Enzymatic*, 17: 133-42. DOI: 10.1016/S1381-1177(0200020-6)
48. Fjerback, N., K. V. Christensen and B. Norddahl. 2009. A review of the current state of biodiesel production using enzymatic transesterification. *Biotechnology and Bioengineering*, 102(5):198-315, doi:10.1002/bit.22256.
49. Lu, J., Y. Chen, F. Wang and T. Tan, 2009. Effect of water on methanolysis of glycerol trioleate catalyzed by immobilized lipase *Candida sp.* 99-125 in organic solvent system. *Journal of Molecular Catalysis B: Enzymatic*, 56: 122-125. DOI: 10.1016/j.molcatb.2008.05.00
50. Ghamgui, H. M. Karra-Chaabouni and Y. Gargouri, 2004. 1-Butyl oleate synthesis by immobilized lipase from *Rhizopus oryzae*: a comparative study between n-hexane and solvent-free system. *Enzyme and Microbial Technology*, 35: 355-363. doi:10.1016/j.enzmictec.2004.06.002
51. Xu, Y., M. Nordblad, P. M. Nielsen, J. Brask and J. M. Woodley, 2011. *In situ* visualization and effect of glycerol in lipase-catalyzed ethanolysis of rapeseed oil. *Journal of Molecular Catalysis B: Enzymatic*, 72: 213-219. doi:10.1016/j.molcatb.2011.06.008
52. Dossat, V., D. Combes and A. Marty, 1999. Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: influence of the glycerol production. *Enzyme and Microbial Technology*, 25: 194-200. doi:10.1016/S0141-0229(99)00026