

Study on Optimization of Enzymatic Synthesis of Biodiesel By Response Surface Methodology

李晉嘉、謝淳仁

E-mail: 9300260@mail.dyu.edu.tw

ABSTRACT

Biodiesel, a diesel substitute, has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. Although chemical synthetic methods have been commercialized at the present day, chemical synthetic methods have by-products under the condition of high temperature and high pressure. In contrast, the reacting condition of enzymatic synthetic methods are mild, cheap, none by-products and due to natural. In hence, the biosynthesis of such esters by lipase-catalyzed chemical reactions under mild conditions became much current commercial interest. The ability for immobilized lipase from *Rhizomucor miehei* (Lipozyme IM-77) or *Candida antarctica* (Novozym 435) to catalyze the transesterification of soybean oil or canola oil and methanol was investigated in this study. Response surface methodology (RSM) and 5-level-5-factor central composite rotatable design (CCRD) were employed to evaluate the effects of synthesis parameters, such as reaction time (2-10 h and 4-20 h), temperature (25-65 °C), enzyme amount (0.2-1.0 BAUN; Batch acidolysis unit of novo and 0.1-0.5 g), substrate molar ratio of methanol to soybean oil and canola oil (1:2-1:4), and added water content (0-20%) on percentage weight conversion of soybean oil methyl ester or canola oil methyl esters by transesterification. The results showed that temperature and enzyme amount were effects on percent molar conversion of soybean oil methyl ester. Based on ridge max analysis, the optimum synthesis conditions with 92.2 and 99.4% weight conversion were: reaction time 6.3 and 12.4 h, temperature 36.5 and 38.1 °C, enzyme amount 0.9 BAUN and 0.42 g, substrate molar ratio of methanol to soybean oil and canola oil 3.4:1 and 3.5:1, and added water 5.8 and 7.2%.

Keywords : Lipase; Biodiesel; Immobilization; Optimization; Response surface methodology; Transesterification

Table of Contents

第一章 緒論	1	第二章 文獻回顧	6	2.1 生化柴油之發展	6
.....	6	2.1.1 生化柴油之簡介	7	2.1.2 生化柴油之物性	7
.....	7	2.1.3 生化柴油的合成方式	8	2.1.4 轉酯化反應	10
.....	10	2.1.5 世界趨勢	12	2.1.6 國內外相關研究	13
.....	13	2.2 脂解酵素之重要性	18	2.2.1 脂解素	18
.....	18	2.2.2 酵素催化反應之優點	18	2.3 反應曲面法之應用	19
.....	19	2.3.1 反應曲面法之原理	20	2.3.2 二水準因子設計	21
.....	21	2.3.3 徒升路徑法	22	2.3.4 中心混層設計	23
.....	23	2.4 回應曲面模式適切性之統計檢驗	24	2.5 正則分析	24
.....	24	第三章 以反應曲面法研究生化柴油之最優化酵素合成	33	3.1 前言	33
.....	33	3.2 實驗材料	35	3.2.1 儀器設備	35
.....	35	3.2.2 藥品	35	3.3 實驗設計與方法	36
.....	36	3.3.1 實驗設計	36	3.3.2 生化柴油之合成方法	36
.....	36	3.3.3 萃取與分析	37	3.3.4 統計分析	37
.....	37	3.4 結果與討論	38	3.4.1 時間對莫耳轉換率之影響	40
.....	40	3.4.2 溫度對莫耳轉換率之影響	41	3.4.3 酵素用量對莫耳轉換率之影響	42
.....	42	3.4.4 水分對莫耳轉換率之影響	43	3.4.5 基質莫耳數比對重量轉換率之影響	44
.....	44	3.4.6 最優化合成之研究	44	3.4.7 相關研究之綜合討論	46
.....	46	第四章 結論	68	參考文獻	70
附錄一 Gas chromatography操作流程及注意事項	76	附錄二 pH-stat操作流程及注意事項	78	附錄三 脂解酵素之活性測定	81
.....	78	附錄四 生化柴油之相關研究	84	84

REFERENCES

1. 李昌憲、洪哲穎及熊光濱。1992。利用反應曲面法進行以 *Streptococcus faecalis* 生產酪胺酸脫羧酶之培養基最適化研究。中國農業化

學會誌。30:264-272。 2. 李根永和李孟修。1998。Corynebacterium glutamicum 在高濃度鹽份培養基脯胺酸發酵之研究。中國農業化學會誌。36:57-64。 3. 張淑微。2002。以反應曲面法研究酵素合成己醇酯類之最優化。大葉大學食品工程研究所碩士論文。 4. 張曉莉及黃世佑。1997。生物轉換法 - 有機溶劑中維持酵素活性之研究。化工。44:71-84。 5. 陳志威。2001。將廢油變黃金 - 淺談生化柴油的發展與應用。生物資源 生物技術。3:10-16。 6. Abigor, R. D., Uadia, P. O., Foglia, T. A., Hass, M. J., Jones, K. C., Okpefa, E., Obibuzor, J. U. and Bafor, M. E. 2000. Lipase-catalysed production of biodiesel fuel from some Nigerian lauric oils. Biochem. Soc. Trans. 28: 979-981. 7. Alcantara, R., Amores, J., Canoira, L., Fidalgo, E., Franco, M.J., Navarro, A. 2000. Catalytic production of biodiesel from soy-bean oil, used frying oil and tallow. Biomass Bienergy. 18: 515-527. 8. Ban, K., Hama, S., Nishizuka, K., Kaieda, M., Matsumoto, T., Kondo, A., Noda, H., Fukuda, H. 2002. Repeated use of whole-cell biocatalysis immobilized within biomass support particles for biodiesel fuel production. J. Mol. Catal. B: Enzymatic. 17: 157-165. 9. Bowman, L. and Geiger, E. 1984. Optimization of fermentation conditions of alcohol production. Biotechnol. Bioeng. 26: 1492-1497. 10. Box, G.E.P. and Wilson, K.B. 1951. On the experimental attainment optimum conditions. J. Roy. Statist. Soc., B13: 1-45. 11. Box, G.E.P., Hunter, W. and Hunter, J.S. 1978. Statistics for experimenters. John Wiley and Sons, New York. 12. Chen, S.L. 1981. Optimization of batch alcoholic fermentation of glucose syrup substrate. Biotechnol. Bioeng. 23: 1827-1836. 13. Cheynier, V., Feinberg, M., Chararas, C. and Ducauze, C. 1983. Application of response surface methodology to evaluation of bioconversion experimental conditions. Appl. Environ. Microbiol. 45: 634-639. 14. Faber, K. 1992. Biotransformations in organic chemistry. Springer-Verlag, Germany. 2-4. 15. Galas, E., Bielecki, S., Antezak, T., Weiczorek, A. and Blaszczyk. 1981. Optimization of cultivation medium composition for lytic enzyme biosynthesis. In Moo-Young, M., Vezina, C. and Singh, K. (Eds) Advances in Biotechnology-Proceedings 6th International Fermentation Symposium, Vol. 3, Pergamon Press, Canada. p. 301-306. 16. Goodrum, J. H., Eiteman, M. A., 1996. Physical properties of low molecular weight triglycerides for the development of bio-diesel fuel models. Bioresour. Technol. 56: 55-60. 17. Himmelblau, D.M. 1970. Process analysis by statistical methods. John Wiley and Sons, New York. 230-292. 18. Huang, A. and Moreau, R. 1978. Lipase in the storage tissue of peanut and other oilseeds during germination. Planta. 141: 111-116. 19. Iso, M., Chen, B., Eguchi, M., Kudo, T., Shrestha, S. 2001. Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase. J. Mol. Catal. B: Enzymatic. 17: 151-155. 20. Jogiekar, A. M. and May, A. T. 1987. Product excellence through design of experiments. Cereal Food World. 32: 857-868. 21. Kaieda, M., Samukawa, T., Matsumoto, T., Ban, K., Kondo, A., Shimada, Y., Noda, H., Nomoto, F., Ohtsuka, K., Izumoto, E., Fukuda, H. 1999. Biodiesel fuel production from plant oil catalyzed by Rhizopus oryzae lipase in a water-containing system without an organic solvent. J. Biosci. Bioeng. 88: 627-631. 22. Kaieda, M., Samukawa, T., Kondo, A., Fukuda, H. 2001. Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system. J. Biosci. Bioeng. 91: 12-15. 23. Kose, O., Tuter, M., Aksoy, H. A. 2002. Immobilized Candida antarctica lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium. Bioresour. Technol. 83: 125-129. 24. Kusdiana, D. and Saka, S. 2001. Kinetics of transesterification in rapeseed oil to biodiesel fuel as treated in supercritical methanol. Fuel. 80: 693-698. 25. Laforgia, D. and Ardito, V. 1995. Biodiesel fueled IDI engines performances emissions and heat release investigation. Bioresour. Technol. 51: 53-59. 26. Ma, F., Clements, L. D., Hanna, M. A. 1999. The effect of mixing on transesterification of beef tallow. Bioresour. Technol. 69: 689-693. 27. Ma, F. and Hanna, M. A. 1999. Biodiesel(review). Bioresour. Technol. 70: 1-15. 28. Maddox, I.S. and Richert, S.H. 1977. Use of response surface methodology for the rapid optimization of microbiological media. J. Appl. Bacteriol. 43: 17-204. 29. Mittelbach, M. 1990. Lipase catalyzed alcoholysis of sunflower oil. J. Am. Oil Chem. Soc. 67: 168-170. 30. Montgomery, D.C. 1984. Design and analysis of experiments. John Wiley and Sons, New York. 31. Moresi, M., Colicchio, A. and Sansovini, F. 1980. Optimization of whey fermentation in a jar fermenter. Eur. J. Appl. Microbiol. Biotechnol. 9: 173-183. 32. Nelson, L., Foglia, T. A., Marmar, W. N. 1996. Lipase-catalyzed production of biodiesel. J. Am. Oil Chem. Soc. 73: 1191-1195. 33. Peterson, C. L., Auld, D. L., Korus, R. A. 1983. Winter rape oil fuel for diesel engines: Recovery and utilization. J. Am. Oil Chem. Soc. 60: 1579-1587. 34. Pryde, E. H. 1983. Vegetable oil as biodiesel fuel: Overview. J. Am. Oil Chem. Soc. 60: 1557-1558. 35. Samukawa, T., Kaieda, M., Matsumoto, T., Ban, K., Kondo, A., Shimada, Y., Noda, H., Fukuda, H. 2000. Pretreatment of immobilized Candida antarctica lipase for biodiesel fuel production from plant oil. J. Biosci. Bioeng. 90: 180-183. 36. SAS. 1990. SAS User Guide, SAS Institute, Inc., Cary, NC. 37. Shieh, C. J., Akoh, C. C., and Lee, L. N. 1996. Optimized enzymatic synthesis of genranyl butyrate with lipase from Candida rugosa. Biotechnol. Bioeng. 51: 371-374. 38. Shieh, C.J., Liao, H.F., Lee, C.C. 2003. Optimization of lipase-catalyzed biodiesel by response surface methodology. Bioresour. Technol. 88: 103-106. 39. Shimada, Y., Watanabe, Y., Sugihara, A., Tominaga, Y. 2002. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. J. Mol. Catal. B: Enzymatic. 17: 133-142. 40. Thomson, D. 1982. Response surface experimentation. J. Food Process. Pres. 6: 155-188. 41. Vicente, G., Coteron, A., Martinez, M., Aracil, J. 1998. Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. Ind. Crops. Prod. 8: 29-35. 42. Wandrey, C. and wichmann, R. 1987. Production of L-amino acids in the mermbrane reactor. Biotechnol. 1: 85-92. 43. Wandrey, C. and wichmann, R. 1987. Production of L-amino acids in the mermbrane reactor. Biotechnol. 1: 85-92. 44. Watanabe, Y., Shimada, Y., Sugihara, A., Tominaga, Y. 2002. Conversion of degummed soybean oil to biodiesel fuel with immobilized candida antarctica lipase. J. Mol. Catal. B: Enzymatic. 17: 151-155. 45. Yates, F. 1970. Experimental design: selected papers of frank Yates. Griffin, London. 46. Zertuche, L. and Zall, R.R. 1985. Optimizing alcohol production from whey using computer technology. Biotechnol. Bioeng. 27: 547-554.