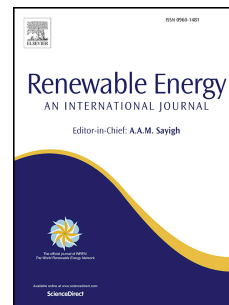


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Mohadese Babaki, Maryam Yousefi, Zohreh Habibi, Mehdi Mohammadi



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1 **Process optimization for biodiesel production from waste cooking oil using**
2 **multi-enzyme systems through response surface methodology**

3 Mohadese Babaki ^a, Maryam Yousefi ^{1,b,*}, Zohreh Habibi ^{a,**}, Mehdi Mohammadi ^c

4 ^aDepartment of Pure Chemistry, Faculty of Chemistry, Shahid Beheshti University, G.C., Tehran, Iran

5 ^bNanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

6 ^c Bioprocess Engineering Department , Institute of Industrial and Environmental Biotechnology, National
7 Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

8

9 **Abstract:**

10 Lipase from *Rhizomucor miehei* (RML) and lipase B from *Candida antarctica* (CALB) were
11 covalently immobilized onto epoxy-functionalized silica. In this study, we developed a multi-
12 enzyme system to produce biodiesel with waste cooking oil and methanol. To increase the
13 biodiesel production yield, a mixture of 1,3-specific lipase (RML) and nonspecific lipase
14 (CALB) was used. Response Surface Methodology (RSM) and a central composite rotatable
15 design (CCRD) was used to study the effects of four factors, CALB:RML ratio, ratio of *t*-butanol
16 to oil (wt.%), water adsorbent content (wt.%) and reaction time on the fatty acid methyl esters
17 (FAME) yield. A quadratic polynomial equation was obtained for methanolysis reaction by
18 multiple regression analysis. The optimum combinations for the reaction were CALB:RML ratio
19 (3:1), *t*-butanol to oil (10 wt.%), water adsorbent content (22.5 wt.%) at the reaction time of 10
20 h. FAME yield of 91.5%, which was very close to the predicted value of 95.6%, was obtained.
21 Verification experiment confirmed the validity of the predicted model.

22 **Keywords:** Biodiesel, Lipase, Immobilization, Waste cooking oil, Response surface
23 methodology

* Corresponding author. Tel.:+982 122 432 020; fax: +982 122 432 021.

** Corresponding author. Tel.:+982 129 903 110; fax: +982 122 431 663.

E-mail addresses: M.yousefi@avicenna.ac.ir (M. Yousefi), Z_habibi@sbu.ac.ir (Z. Habibi)

¹ These authors contributed equally to this work.

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