# Biocatalytic Transesterification of Oleic Acid Triglyceride in Supercritical Carbon Dioxide

**N. V. Lakina[a], ***, E. M. Sulman[a], V. Yu. Doluda[a], and V. G. Matveeva[a, b]

[a]*Tver State Technical University, Tver, 170026 Russia*
[b]*Tver State University, Tver, 170100 Russia*
***e-mail: lakina@yandex.ru**
Received September 6, 2018; revised September 23, 2018; accepted September 23, 2018
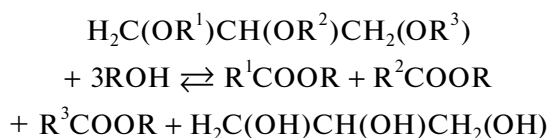
**Abstract**—The prospects of using catalysts based on immobilized lipase in the transesterification of triglyceride of oleic acid (TOA) with methanol in supercritical (SC) carbon dioxide are shown. At the optimum temperature of 40°C, $CO_2$ pressure of 15.0 MPa, and a TOA : methanol molar ratio of 1 : 3, the yield of oleic acid methyl ester is significantly higher than that obtained in methanol at atmospheric pressure.

## INTRODUCTION

Currently, special attention is being paid to alternative methods of fuel recovery from renewable vegetable raw materials [1−4]. The process for the preparation of esters of fatty acids (EFAs) (biodiesel) by the transesterification reaction of vegetable oils (triglycerides of fatty acids (TFAs)) with lower alcohols (methanol or ethanol) [5] by the reaction

$$H_2C(OR^1)CH(OR^2)CH_2(OR^3)$$
$$+\ 3ROH \rightleftarrows R^1COOR + R^2COOR$$
$$+\ R^3COOR + H_2C(OH)CH(OH)CH_2(OH)$$

has been widely applied.

It is possible to carry out transesterification of TFAs with lower alcohols in addition to homogeneous [1, 2] acidic or basic catalysts, which ensure the process of transesterification under mild conditions (30−65°C) at a high speed. In the case of heterogeneous catalysts such as alkaline earth metal oxides, zirconium oxide, zeolites, and others, the process is carried out under more severe conditions (up to 200°C) [1, 2, 6]. The difficulty of separating and purifying the product from the catalyst is a disadvantage of homogenous and heterogeneous processes [1, 2, 6].

It is proposed to carry out the transesterification of TFAs in the presence of an enzyme-lipase of microbiological, plant, and animal origin (lipoprotein lipase, pancreatic lipase, endotepialny lipase, etc. [2, 6, 7].

It is noted in the literature that heterogeneous catalysts reduce activity in the presence of water, and alkalis are sensitive to the presence of water and free fatty acids in the raw materials. Moreover, it is known that enzymes are able to maintain activity and exhibit high selectivity in the presence of water in the raw material [6]. Nevertheless, work is underway to create sustainable biocatalysts based on lipase immobilized on various carriers [8, 9].

The current focus is on the development of noncatalytic processes for the transesterification of TFAs by lower alcohols under sub- and supercritical (SC) conditions [10−16]. Lower alcohols such as methanol and ethanol can mix only in a limited way with TFAs in normal conditions due to the high polarity and the presence of hydrogen bonds. When carried out under SC conditions (critical points: 512.6 K and 8.09 MPa for methanol and 513.9 K and 6.14 MPa for ethanol), the solubility of triglycerides increases and they form homogeneous systems with alcohols. By optimizing the conditions of the process (pressure, temperature, solvent composition, solvent-to-feed ratio, etc.), a yield above 90% of (EFAs) can be achieved. SC-$CO_2$, which is actively used to extract TFAs from plant raw materials [6, 17], has been used in transesterification only as a cosolvent of SC lower alcohols [6, 18].

This work presents the results of the studying the biocatalytic transesterification of triglyceride of oleic acid (TOA) with methanol in an SC-$CO_2$ medium. Both native lipase and lipase immobilized on magnetic particles of $Fe_3O_4$ were used to carry out the process. The magnetic particles chosen as the enzyme carrier are selected based on the ease of separation from the reaction product.
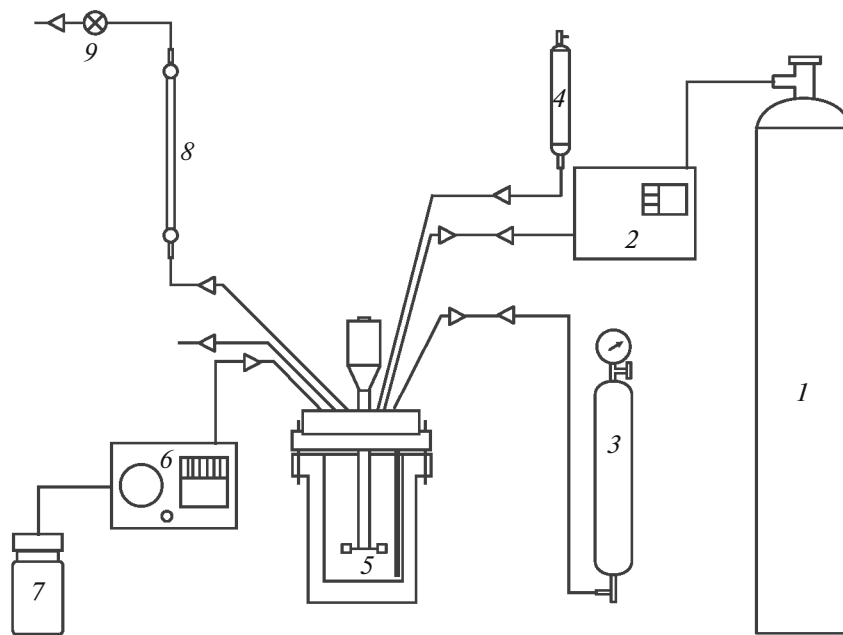
**Fig. 1.** Installation diagram for transesterification in SC-CO$_2$ medium: (*1*) cylinder with CO$_2$; (*2*) CO$_2$ supply pump; (*3*) gas-burette; (*4*) pipette for liquid; (*5*) high pressure reactor; (*6*) reagent pump; (*7*) bin for reagent; (*8*) reflux condenser; (*9*) return valve.

## EXPERIMENTAL

The following reagents and solvents are used in the study: iron chloride (II) six-state FeCI$_3 \cdot$ 6H$_2$O (pure, Reachem); iron sulfate (II) seven-water Fe$_2$(SO$_4$)$_3 \cdot$ 7H$_2$O(Reachem); phosphate buffer of composition Na$_2$HPO$_4 \cdot$ 2H$_2$O + KH$_2$PO$_4$ (pH 6.86, Uralkhimin-vest); NaOH sodium hydroxide, 98% (analytically pure, Neva-reactiv); methanol(C, Reachem); etha-nol(chemically pure, Reachem); TOA (99%, Sigma-Aldrich); serum albumin standardized lipase L3126 Type II (100−400 un./mg, Sigma-Aldrich); 3-amino-propiltrietoksisilan NH$_2$(CH$_2$)$_3$Si(OC$_2$H$_5$)$_3$ (98%, Sigma-Aldrich); glutaric dialdehyde(25%, Fluka); diphenylamine C$_6$H$_5$)$_2$NH (analytically pure, Neva-reactiv); and carbon dioxide(99.8%, Tver-gazservis).

To prepare magnetic nanoparticles (MNPs), an aqueous solution (25 mL) of a mixture of iron salts in equimolar amount (2.8 g FeSO$_4 \cdot$ 7H$_2$O and 2.7 g FeCI$_3 \cdot$ 6H$_2$O) was added dropwise to the NaOH solu-tion (1.5 M, 250 mL) with constant stirring. The resulting Fe$_3$O$_4$ black precipitate was separated from the reaction medium with a neodymium magnet, washed with water to a neutral pH, and then placed in 50 mL of 95% ethanol. Next, 0.3 mL of a solution of 3-(aminopropyl)-triethoxysilane was added to the ethanol suspension of the resulting 2 g MNPs to mod-ify their surface with amino groups, stirred for 7 h, then washed to a neutral pH. For enzyme covalent crosslinking (formation of an azomethine bond on the surface of the biocatalyst), 25 mL of a 1% glutaric dial-dehyde solution was added to the modified MNPs,

stirred for 2 h, and then washed with a five-fold excess of distilled water. The resulting modified MNPs were stirred for 6 h with a 50 mL lipase buffer solution (1 g lipase per 50 mL phosphate buffer). All operations to separate the MNPs of the lipase biocatalyst/Fe$_3$O$_4$ from the solution were carried out with a neodymium magnet.

A Parr Instruments 4307 high-pressure reactor (United States) with the total flask volume of 250 cm$^3$ and the maximum working pressure of 60 MPa was used (Fig. 1) to obtain oleic acid methyl ester (OAME) in an SC-CO$_2$ medium. Plunger pump Supercritical 24 Pump CP (SSI, United States) was used to pump the carbonic acid. The standard experiment was con-ducted as follows.

A charge of the biocatalyst (1.0 g of lipase/Fe$_3$O$_4$ or 0.5 g of free lipase), 50 mL of TOA (density 0.915 g/mL), and 6.3 mL of methanol (molar ratio TOA : methanol = 1 : 3) were added to the Teflon flask of the reactor. The reactor was purged thrice with carbon dioxide at 20 MPa; after the pressure was stabilized, more liquid CO$_2$ was pumped until the reactor was filled. The reactor was heated to a predetermined temperature and the reac-tion was started. The test was carried out for 3 h. The CO$_2$ pressure ranged from 10 to 30 MPa.

Comparative tests for the transesterification of TOA were carried out in the same reactor at the atmo-spheric pressure of nitrogen in the temperature range 30−60°C with the same molar ratio of TOA to metha-nol (1 : 3).
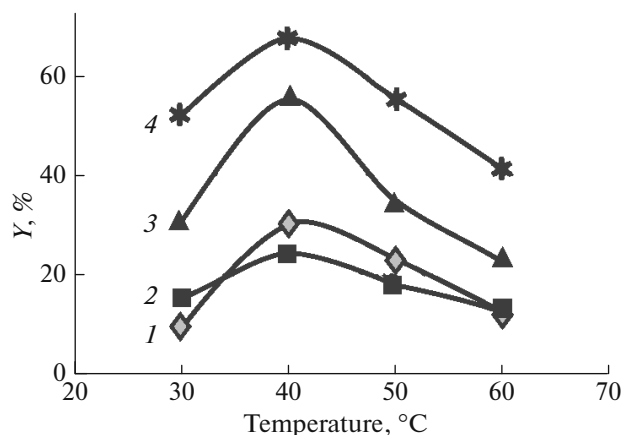
**Fig. 2.** The dependence of OAME ($Y$) yield on temperature during TOA transesterification: ($1, 3$) lipase; ($2, 4$) lipase/Fe$_3$O$_4$; ($1, 2$,) in methanol medium (at atmospheric pressure); ($3, 4$) in CO$_2$ medium (15 MPa); reaction time 180 min.



**Fig. 3.** The dependence of OAME ($Y$) yield on CO$_2$ pressure during TOA transesterification (40°C, reaction time 180 min).

The reaction mixture was analyzed by gas chromatography with the mass spectroscopic detection of substances on a GS-2010 gas chromatograph (Shimadzu, Japan) equipped with a 30 m × 0.25 mm × 0.25 μm HP-1MS capillary column and a GSMS-QP 2010S quadrupole mass spectrometer (Shimadzu, Japan). The analysis was carried out in the thermoprogramming mode: exposure for 5 min at 80°C, linear heating from 80 to 105°C (10°C/min), linear heating from 105 to 250°C (25°C/min), and exposure at 250°C for 3 min. Ultrahigh purity helium 6.0 was used as the gas carrier (54.5 mL/min, linear speed in a cm column, 36 cm/s), and injector temperature 260°C. Diphenylamine was used as the internal standard.

The effectiveness of biocatalytic transesterification was estimated by the yield of OAME ($Y$, %) achieved in 180 minutes in the presence of a free and immobilized enzyme, the amount of which was calculated by the formula $Y = 100C_i/C_o$, where $C_o$ is the theoretically achievable concentration of OAME in the reaction mixture and $C_i$ is the practically achieved concentration of OAME in 180 min.

## RESULTS AND DISCUSSION

Figure 2 shows the dependence of the OAME yield on temperature in the presence of different biocatalysts. The presented dependence shows that the effectiveness of native lipase during the transesterification of TOA in methanol at atmospheric pressure is higher ($Y = 30\%$) than immobilized lipase ($Y = 24\%$). When carried out in an SC-CO$_2$ medium, on the contrary, immobilized lipase is more effective than native lipase ($Y = 67$ and 55%, respectively).

As shown in Fig. 3, the maximum yield in both native and immobilized lipase is achieved at CO$_2$ 15 MPa (55 and 67%, respectively). There was a decrease in
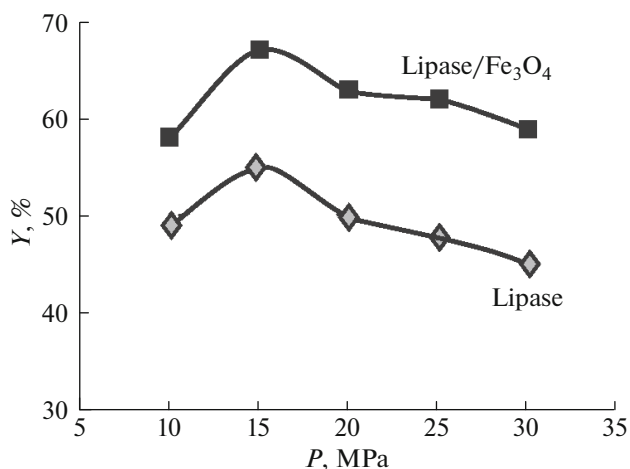
the product yield with a further increase in the CO$_2$ pressure.

Figure 4 shows a diagram of the maximum product yields during the transesterification of TOA in methanol (atmospheric pressure) and in an SC-CO$_2$ medium. The presented data show that in the SC-CO$_2$ medium the effectiveness of native and immobilized lipase is 1.8 and 2.8 times higher, respectively.

For both biocatalysts, the maximum efficiency is achieved at 40°C, which may be related to the inactivation of the enzyme at a higher temperature due to the subsequent denaturation.

In the presence of the lipase/Fe$_3$O$_4$ biocatalyst, the total conversion of the TOA in an SC-CO$_2$ medium at
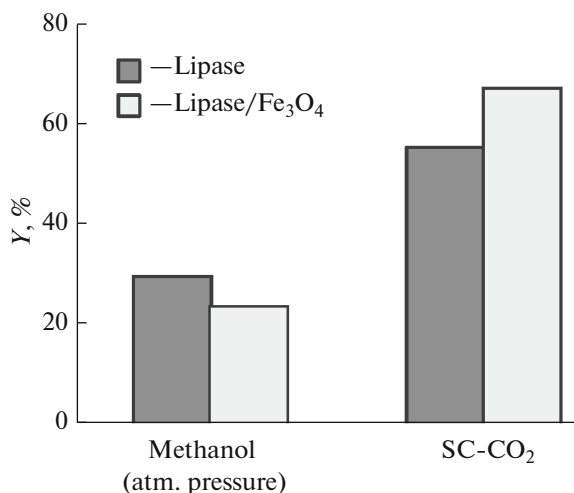


**Fig. 4.** The diagram of OAME yields in the presence of biocatalyst during the transesterification of TOA in methanol (at atmospheric pressure) and SC-CO$_2$.
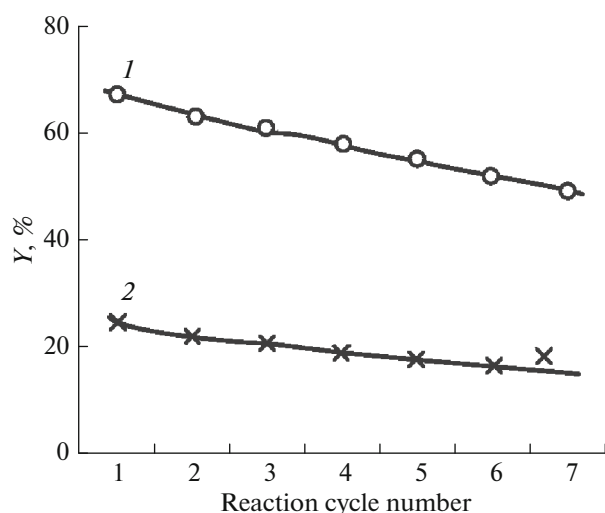
REFERENCES

1. N. G. Shemelis and M. M. Jorge, AIMS Energy **5**, 425 (2017).

2. N. Saifuddin, A. Samiuddin, and P. Kumaran, Trends Appl. Sci. Res. **10**, 1 (2015).

3. R. Gray, *Biofuels Annual, Russian Federation* (U. S. Dep. of Agriculture, 2016), p. 1.

4. A. Dhar and A. K. Agarwal, Fuel **119**, 70 (2014).

5. R. Lokanatham and K. Ravindranath, Int. J. Eng. Res. Developm. **6**, 35 (2013).

6. K. T. Lee, S. Lim, Y. L. Pang, H. C. Ong, and W. T. Chong, Prog. Energy Combust. Sci. **45**, 54 (2014).

7. A. E. Rogozhin, Cand. Sci. (Chem.) Dissertation (Dzerzhinsk, 2017).

8. A. D. Q. Melo, F. F. M. Silva, J. C. S. dos Santos, R. Fernández-Lafuente, T. L. G. Lemos, and F. A. D. Filho, Molecules **22**, 2165 (2017).

9. N. B. Carvalho, B. T. Vidal, A. S. Barbosa, M. M. Pereira, S. Mattedi, L. S. Freitas, A. S. Lima, and C. M. F. Soares, Int. J. Mol. Sci. **19**, 1829 (2018).

10. K. G. Bogolitsyn, A. A. Krasikova, and I. A. Gusakova, Russ. J. Phys. Chem. B **10**, 1048 (2016).

11. S. V. Mazanov, A. R. Gabitova, L. H. Miftahova, R. A. Usmanov, F. M. Gumerov, Z. I. Zaripov, V. A. Vasil'ev, and E. A. Karalyn, Russ. J. Phys. Chem. B **10**, 1099 (2016).

12. V. I. Anikeev, D. A. Stepanov, and A. B. Ermakova, Russ. J. Phys. Chem. A **85**, 1336 (2011).

13. H. J. Navarro-Díaz, S. L. Gonzalez, B. Irigaray, I. Vieitez, I. Jachmanián, H. Hense, and J. V. Oliveira, J. Supercrit. Fluids **93**, 130 (2014).

14. K. Bunyakiat, S. Makmee, R. Sawangkeaw, and S. Ngamprasertsith, Energy Fuels **20**, 812 (2006).

15. J. Cheng, T. Li, N. Peng, R. Huang, J. H. Zhou, and K. F. Cen, Fuel Proces. Technol. **131**, 409 (2015).

16. D. Zhou, L. Qi, B. Q. Qiao, Q. Q. Xu, and J. Zh. Yin, J. Supercrit. Fluids **120**, 395 (2017).

17. V. I. Bogdan, A. E. Koklin, V. G. Krasovsky, V. V. Lunin, Ya. E. Sergeeva, A. A. Ivashechkin, and E. P. Feofilova, Russ. J. Phys. Chem. B **8**, 1004 (2014).

18. C. Bertoldi, C. da Silva, J. P. Bernardon, M. L. Corazza, L. C. Filho, J. V. Oliveira, and F. C. Corazza, Energy Fuels **23**, 5165 (2009).



**Fig. 5.** The yield of OAME ($Y$) in the presence of lipase/$Fe_3O_4$ biocatalyst at 40°C in seven consecutive OATG transesterification cycles: (*1*) in SC-$CO_2$ at 15 atm; (*2*) in methanol at atmospheric pressure.

40°C and 15 MPa is achieved in 340 min, while in a methanol medium, at atmospheric pressure, this requires 610 min.

For immobilized enzymes, the stability of the lipase/$Fe_3O_4$ biocatalyst in seven consecutive TOA transesterification cycles in an SC-$CO_2$ medium and methanol is of greater importance. The data obtained (see Fig. 5) show that this biocatalyst can be reused in both media. At the same time, its stability in SC-$CO_2$ is higher than in methanol: after being used seven times in these media it loses 28 and 37% of its efficiency, respectively.

Thus, in an SC-$CO_2$ medium, the lipase enzyme is more efficient in the transesterification reaction of TOA up to its methyl ester than in a methanol medium. The increase in the product's yield may be due to the fact that a faster mass transfer takes place in the SC-$CO_2$ medium. The use of $Fe_3O_4$ magnetic particles to immobilize lipase allows the biocatalyst to be separated easily from the product.

*Translated by M. Drozdova*