Proceedings of BALTTRIB'2015 edited by prof. J. Padgurskas
eISSN 2424-5089/eISBN 978-609-449-093-4

DOI: 10.15544/balttrib.2015.08

ENZYMATIC SYNTHESIS OF α-PROPYLENE GLYCOL WITH (9Z)-OCTADECENOIC ACID BY LIPOLYTIC ENZYME

M.Gumbyte*†, R. Kreivaitis*, T. Balezentis**
* Laboratory of Chemical and Biochemical Research for Environmental Technology, Aleksandras Stulginskis University, Lithuania
** Lithuanian Institute of Agrarian Economics, Lithuania

Abstract: Enzymatic synthesis of bio-lubricant from (9Z)-octadecenoic acid with α-propylene glycol has been investigated in this article and the purpose of this study has been to find out the optimum reaction conditions of esterification. Lipolytic enzyme (Lipolase ®100L) has been used to catalyze esterification reaction in solvent-free systems. The optimum reaction conditions of esterification process were achieved. The assessment of the bio-lubricant production options was studied. Tribological properties of α-propylene glycol esters of (9Z)-octadecenoic acids as bio-lubricants were assessed.

Keywords: bio-lubricant, enzymatic synthesis, TOPSIS, tribological

1. INTRODUCTION

Since end of 1960 scientists began to take interest in physical, environmental and lubricating properties of lubricants produced from bio-based raw materials. Rising crude oil prices and decreasing its reserves environmentally friendly lubricating oil demand increases. The increase is also affected by legislations [1].

Vegetable oils are the main raw material for biodegradable lubricants. Bio-lubricants can be produced, for instance, by esterification of (9Z)-octadecenoic acid, which is the core of the various vegetable and animal fats. The variety of alcohols having long alkyl groups or a complex hydrocarbon structure with more than one hydroxyl group is used for esterification. Bio-lubricants can be produced directly by transesterification of vegetable oils by such alcohols [2, 3].

The relatively new and promising bio-lubricants production way is enzymatic esterification using biocatalyst lipases. Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) - hydrolysis class enzymes, catalyst in the reaction where hydrolytic degradation of chemical bonding takes place inside the molecules. Depending on the reaction conditions they are able to catalyze not only the hydrolysis of lipids, various fatty acid esters can be sintered as well [3, 2].

Production of the bio-lubricant involves consumption of certain inputs and results in respective outputs. Both inputs and outputs can be identified by considering certain indicators. Indeed, the production process should satisfy certain objectives, namely to minimize the input consumption simultaneously securing the best possible level of outputs. In our case, the production process of the bio-lubricant was described by choosing the four indicators of temperature (°C), time (h), substrate molar ratio (mole/mole), and enzyme content (%) as the input indicators describing the costs of production. On the other hand, (9Z)-octadecenoic acid content (%) was considered as an output indicator describing the quality of the bio-lubricant. Whereas the former four indicators should be minimized, the latter one needs to be maximized. Therefore, the assessment of the bio-lubricant production options can be facilitated as a multi-criteria decision making (MCDM) problem.

The MCDM have been employed for material selection problems [4, 5]. This paper employs the TOPSIS method to rank the options of the bio-lubricant production. We will therefore employ the ordinary and the Mahalanobis TOPSIS methods to rank the options of the bio-lubricant production.

† Author for contacts: Dr. Milda Gumbyte
E-mail: Milda.Gumbyte@asu.lt

The aim of current study is to investigate the α-propylene glycol and (9Z)-octadecenoic acid usability in the biotechnological esterification process.

2. METHODS

2.1. Materials

Lipolytic enzyme (Lipolase ®100L), was kindly donated by an M/s Novozym, Denmark representative from Lithuania JSC "Biopolis" (Lithuania). The enzyme belongs to the class of triacylglycerol acylhydrolases (EC 3.1.1.3) with activity of 122.000 KLU/g (K = Kilo, LU = Lipase unit, 1 LU liberates 1 μmole of butyric acid). All other used reagents were of the purest analytical grade available and were purchased from Sigma-Aldrich.

2.2. Esterification reactions

The experiments were performed in a thermostatically controlled three-necked flask with a connected condenser, thermometer, and leak-tight mixer (with a constant rotation speed of 250 min^{-1}). The required amount of (9Z)-octadecenoic acid was poured into the flask, and the acid was continuously stirred and thermostatically controlled. When the acid was heated up to the required temperature, α-propylene glycol was poured into the flask and the biocatalyst was added. The quantity of reacted acids was periodically evaluated according to the reduction of acidity in the reaction medium. The reaction conditions were optimized by carrying out different sets of experiments with varying α-propylene glycol to (9Z)-octadecenoic acid molar ratio, dosage of enzyme and reaction period. At the end of reaction, process temperature was increased to 80 °C, in order to denature the enzyme and the enzyme was separated out by filtration.

2.3. Determination of Acid Value

Formation of the products was determined by a decrease in acidity of the reaction media. Acidity analysis was performed following standard EN ISO 660:2009 Animal and vegetable fats and oils. Determination of acid value and acidity.

2.4. TOPSIS method

Behzadian et al. (2012) presented a comprehensive survey on applications of the TOPSIS method[6].

2.5. TOPSIS extended with the Mahalanobis distance

Criteria used in MCDM are often correlated. Therefore, the use of multivariate methods accounting for suchlike interrelationships is rather convincing in the framework of MCDM. The TOPSIS method has been extended with the Mahalanobis distance [7, 8] to tackle the issue. The latter method will be referred to as the TOPSIS-M.

2.6. Tribological test

Tribological properties were evaluated with a four-ball tribometer which is in the line with standard method DIN 51 350, part 3. The balls (12.7 mm diameter), were made of 100Cr6 bearing steel. The load of 150 N was used. Test duration was 1 hour. At least 3 repetitions were made to determine wear and friction.

The lubricity evaluation parameters were wear scar diameter (WSD), mean friction, and wear surface morphology. WSD were measured using an optical microscope (MBI-6) - 0.007 mm accuracy. Wear surfaces were analysed using 160× magnification.

3. RESULTS AND DISCUSSION

3.1. The effect of the (9Z)-octadecenoic acid to α-propylene glycol molar ratio

In order to choose the effect of (9Z)-octadecenoic acid to α-propylene glycol molar ratio in the solvent-free system, the esterification reaction was carried out with various molar ratios of α-propylene glycol to (9Z)-octadecenoic acid (ranging from 1 to 7) at a fixed enzyme concentration of 2

% (w/w of (9Z)-octadecenoic acid), temperature at 30 °C, duration at 2 h and with magnetic stirring rate of 250 rpm. The results are demonstrated in Fig. 1.

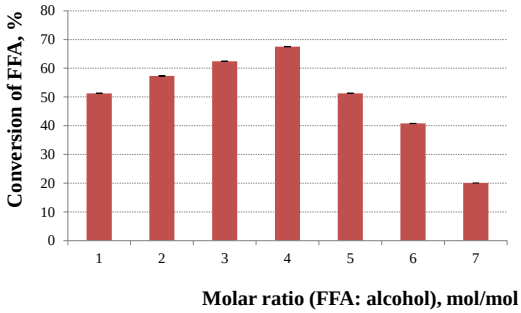


Figure 1. Effect of (9Z)-octadecenoic acid to α-propylene glycol molar ratio on the conversion of free fatty acid catalyzed by Lipolase ®100L (2 % based on (9Z)-octadecenoic acid weight) at 30 °C and 250 rpm for 2 h.

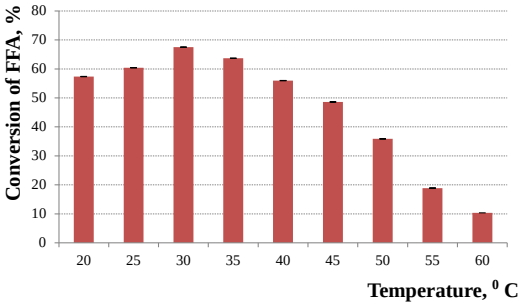


Figure 2. Effect of reaction temperature on the conversion of (9Z)-octadecenoic acid (FFA) catalyzed by Lipolase ®100L (2 % based on (9Z)-octadecenoic acid weight), α-propylene glycol to (9Z)-octadecenoic acid molar ratio at 1:4 and 250 rpm for 2 h.

The presented data show (Fig. 1), that α-propylene glycol to the (9Z)-octadecenoic acid molar ratio increased from 1:1 to 1:4. The highest α-propylene glycol esters of (9Z)-octadecenoic acids (1.2-PO) conversion of 67.51 % was reached at the molar ratio of 1:4. The increase of (9Z)-octadecenoic acid to the molar ratios of 1:5 to 1:7 caused slightly decreasing of the conversion. Excess of (9Z)-octadecenoic acid was required in order to shift the esterification in forward direction. Shifting the substrate molar ratio above or below the optimum value decreased the α-propylene glycol esters of (9Z)-octadecenoic acids yield with all the acids.

3.2. Effect of reaction temperature

Changes in the reaction temperature can affect the activity and stability of the enzyme and thus the rate of reaction. Effect of temperature also can be apportioned to its effect on substrate solubility as well as its direct influences on the esterification reaction and the enzyme [9]. Figure 2 shows the influence of temperature on the esterification reaction within temperature range between 20 °C – 60 °C.

The presented data show (Fig. 2), that the highest conversion of (9Z)-octadecenoic acid was observed at 30 °C. Lipase lost its activity above 30 °C due to thermal deactivation therefore the percentage conversion declined after 30 °C.

3.3. Effect of reaction time and of dosage of enzyme

The time course is a good indicator of enzyme performance and reaction progress. It can pinpoint the shortest or adequate time necessary to obtain a good yield and minimize the process cost. The different amount of enzyme over time induced the percentage of conversion of (9Z)-octadecenoic acid shown in Figure 3. It was found that FFA remained in the reaction mixture only 0.01 % and it could reach with the use of Lipolase ®100L at 2 % (w/w of (9Z)-octadecenoic) at 4 h.

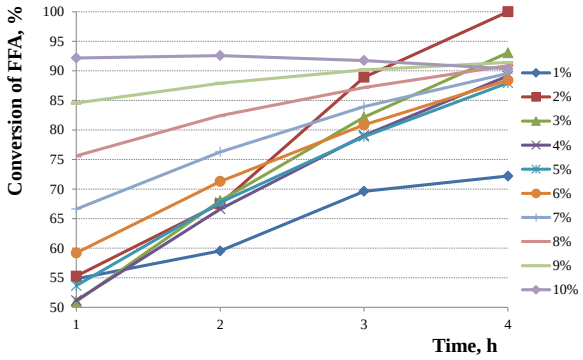


Figure 3. Effect of reaction time and dosage of enzyme (% based on (9Z)-octadecenoic acid weight) on the conversion of (9Z)-octadecenoic acid (FFA). Reaction conditions: α-propylene glycol to (9Z)-octadecenoic acid molar ratio at 1:4 and 250 rpm.

3.4. Analysis of TOPSIS method

The two groups of indicators (criteria) were employed for the analysis, namely the input indicators and a single output indicator. Input indicator group comprised temperature (°C), time (h), substrate molar ratio (mole/mole), and enzyme content (%). As for the output indicator, (9Z)-octadecenoic acid content (%) was considered. These two groups gave rise to a pattern of criteria weights. Indeed, these weights enabled to test the robustness and sensitivity of the results.

Table 1 presents the four weight patterns used for ranking of the bio-lubricant production options. Scenario I put equal weights of 0.2 on all the criteria. Scenarios II to IV gradually increased the importance of the output indicator and, thus, put lower weights on the input indicators.

Table 1. The patterns of weights used in the analysis of the bio-lubricant production options.

Scenario	**Temperature, °C**	**Time, h**	**Substrate molar ratio, mole/mole**	**Enzyme content, %**	**(9Z)-octadecenoic content, %**
	Min	Min	Min	Min	Max
I	0.2	0.2	0.2	0.2	0.2
II	0.1875	0.1875	0.1875	0.1875	0.25
III	0.125	0.125	0.125	0.125	0.5
IV	0.0625	0.0625	0.0625	0.0625	0.75

The ranking was carried out by employing both the ordinary TOPSIS and the TOPSIS-M methods. The TOPSIS-M proceeded by establishing the ideal alternatives of the original data. Subsequently, the Mahalanobis distances between an alternative and the ideal alternatives were obtained for each alternative.

The results indicated that option 44 was among the ten most attractive options of the bio-lubricant production under each weighting scheme and MCDM method. However, its rank shrunk for some instances of the TOPSIS-M method. Options 17 and 28 were attractive for most of rankings save those of TOPSIS-M with lower importance of the output indicator. Similarly, options 34 and 6 were preferable in case relatively lower values of oleic acid content were acceptable (cf. scenarios I-II). At the other end of spectrum, option 14 was attractive in case restrictions on the inputs required were relaxed to a certain extent (as it was the case under scenarios III-IV).

Meanwhile, options 4 and 12 can be those most undesirable ones, for they were placed among the least referable alternatives across MCDM methods and weighting schemes. Option 24 was also among mostly undesirable alternatives according to most of the rankings. Options 2, 31, and 39 were least attractive in terms of the inputs consumption.

3.5. Tribological test

The prepared base oil α-propylene glycol esters of (9Z)-octadecenoic acids (1.2-PO) are a low viscosity 25 cSt (at 40 °C) synthetic oil, having high viscosity index - 152. Low viscosity base oils

with high viscosity index are desirable in present day applications. The low viscosity ensures lower energy consumption, while high viscosity index ensures its properties in wide temperature range. Despite the low viscosity 1.2-PO has a high flash point reaching 230 °C which means - low volatility.

Tribological properties of prepared 1.2-PO ester were compared with that of conventional synthetic base oil PAO 4 (Fig. 4). It was observed that wear reduction properties of both base esters were pour. Despite that pure PAO 4 has better wear reduction it was still in the high wear range. The antiwear additive improved both base oils. Additive loaded 1.2-PO ester has almost 1.8 times lower WSD then that observed in the pure oil. As it was the case for pure base oils the additive loaded PAO 4 has better wear reduction properties then that of 1.2-PO. The additive was effective in both, low and high, load conditions. In the higher load conditions the difference between wear reduction properties of 1.2-PO and PAO 4 decreases, however PAO 4 is still better then 1.2-PO.

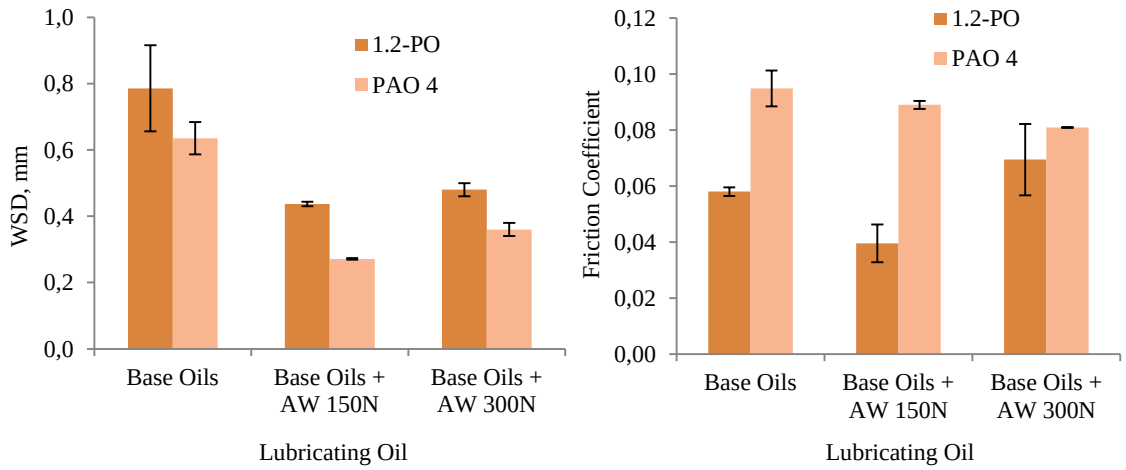


Figure 4. Wear reduction and friction coefficient observed in pure and additive loaded base oils in both load conditions.

Analysing friction reduction data, presented in Fig. 4, it is clear that pure and additive loaded 1.2-PO base oil is superior to PAO 4. The lower friction of 1.2-PO can be explained by mono- and diglycerides presented in this base oil. Such molecules are polar and even small amount of them can form physically adsorbed monolayer on the metal surface. Physically adsorbed layers are generally known as friction reduction barrier between contacting surfaces. Therefore despite that 1.2-PO has worse wear reduction properties it has significantly lower friction. Lower effect of antiwear additive on wear reduction properties of 1.2-PO can be explained by polarity of base oil. The polar molecules compete with molecules of additive for the metal surface seats. Therefore molecules of additive cannot make successful surface protection, which leads to higher wear. Due to weak physical bonding adsorbed layers are not effective in the higher load conditions, where molecules of additive are still active enough and make their work well protecting the surface against wear.

The mean friction is compatible with friction variation data which is presented in Fig. 5. The friction coefficient of 1.2-PO was more stabile during the test than that of PAO 4. The antiwear additive improved stability of PAO 4, but it still has increasing tendency. The additive loaded 1.2-PO friction in both loads distinguish by stabile friction. Undoubtedly, physically adsorbed polar layers, have a great influence on this.

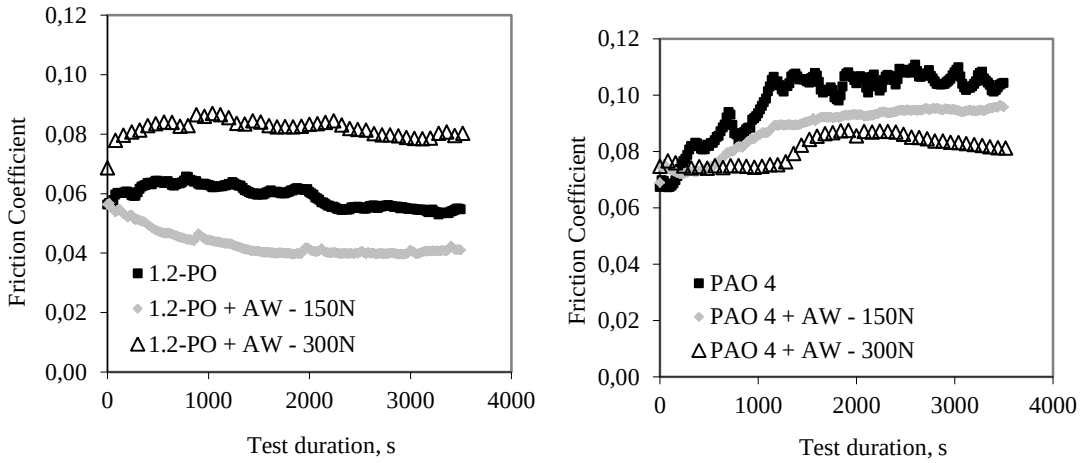
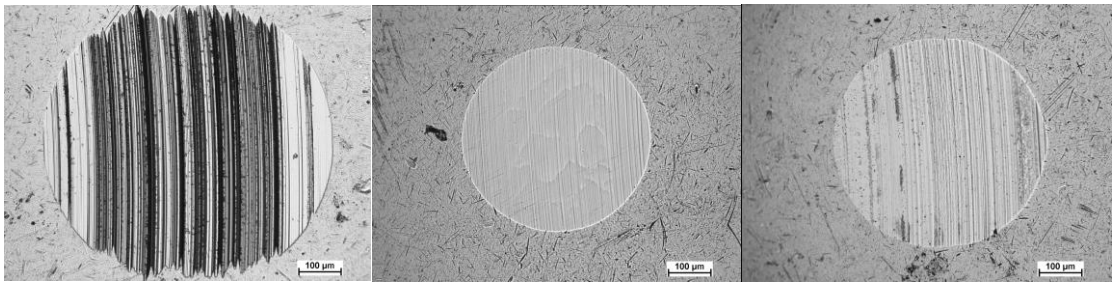


Figure 5. Friction coefficient variation during the test in both base oils.

The wear surfaces lubricated with pure and additive loaded 1.2-PO show great influence of antiwear additive (Fig. 6). Not only the size of wear scars decrease, the surface wear mechanism changes as well. Deep scratches and abrasion can be observed in wear scars observed after lubrication with pure base oil. The additive changed the situation dramatically. In low and high loads the surfaces have only shallow scratches with some residues of antiwear coating.



(a) (b) (c)

Figure 6. Wear scars on lubricated surfaces after tribological test: a - pure 1.2-PO base oil, 150 N; b - additive loaded 1.2-PO, 150 N; c - additive loaded 1.2-PO, 300 N.

The even better results can be expected if special additives for polar base oils will be used. In this case both the wear and friction reduction properties could be improved.

4. CONCLUSION

The esterification of (9Z)-octadecenoic acid with α-propylene glycol to obtain α-propylene glycol esters of (9Z)-octadecenoic acids could be achieved effectively using lipolytic enzyme (Lipolase ®100L). The conversion of (9Z)-octadecenoic acid into ester was significantly influenced by operating parameters; the most suitable reaction conditions for maximization of product were achieved when enzyme amounts of 2 % w/w, reaction temperature 30 °C, reaction time 4 h, α-propylene glycol to (9Z)-octadecenoic acid molar ratio of 1:4. The ester showed good friction reduction properties in comparison to conventional synthetic oil. However its wear reduction properties, even with antiverat additive, are pure. Special additives for polar base oils can improve these drawbacks.

ACKNOWLEDGMENT

The authors acknowledge funding from FP7 program IRSES Project - "Oil and Sugar" (GA -2011-295202).

REFERENCES

[1] P. Nagendramma, S. Kaul, Development of ecofriendly/biodegradable lubricants: An overview, Renewable and Sustainable Energy Reviews 16, 2012, p. 764-774.
[2] J. Madarász, D. Némethb, J. Bakosa, L. Gubiczab, P. Bakonyi, Solvent-free enzymatic process for biolubricant production in continuous microfluidic reactor, Journal of Cleaner Production 93, 2015, p. 140-144.
[3] J. S. Mirandaa, N. C. A. Silvaa, J. J. Bassib, M. C. C. Corradinib, F. A. P. Lageb, D. B. Hiratab, A. A. Mendes, Immobilization of Thermomyces lanuginosus lipase on mesoporous poly-hydroxybutyrate particles and application in alkyl esters synthesis: Isotherm, thermodynamic and mass transfer studies, Chemical Engineering Journal 251, 2014, p. 392-403.
[4] S. Chakraborty, Applications of the MOORA method for decision making in manufacturing environment, International Journal of Advanced Manufacturing Technology 54, 2011, p.1155-1166.
[5] P. Karande, S. Chakraborty, Application of multiobjective optimization on the basis of ratio analysis (MOORA) method for materials selection, Materials and Design 37, 2012, p. 317-324.
[6] M. Behzadian, S. Khanmohammadi Otaghsara, M. Yazdani, J. Ignatius, A state-of the-art survey of TOPSIS applications, Expert Systems with Applications 39 (17), 2012, p. 13051-13069.
[7] J. Antuchevičienė, A. Zakarevičius, E. K. Zavadskas, Multiple criteria construction management decisions considering relations between criteria,Technological and Economic Development of Economy, 16(1), 2010, p. 109-125.
[8] Z. X. Wang, Y. Y. Wang, Evaluation of the provincial competitiveness of the Chinese high-tech industry using an improved TOPSIS method, Expert Systems with Applications, 41 (6), 2014, p. 2824-2831.
[9] K. M. W. Syamsul, M. R. Salina, S. O. Siti, M. N. Hanina, M. A. R. Basyaruddin, K. Jusoff, Green Synthesis of Lauryl Palmitate via Lipase-Catalyzed Reaction, World Applied Sciences Journal 11 (4), 2010, pp. 401-407.