ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 11 2011

Małgorzata HAWROT-PAW[1], Beata SMOLIK
and Agnieszka KAMIENIECKA

PRELIMINARY STUDY ON THE EFFICIENCY OF BIODIESEL BIOLOGICAL DECOMPOSITION WITH AUTOCHTHONOUS SOIL MICROFLORA

WSTĘPNE BADANIA NAD EFEKTYWNOŚCIĄ BIOLOGICZNEGO ROZKŁADU BIODIESLA PRZY UDZIALE AUTOCHTONICZNEJ MIKROFLORY GLEBOWEJ

Abstract: The presented study referred to biodegradation of biodiesel with natural soil microflora and its effect on biofuel decomposing microorganisms and heterotrophic microorganisms. It was found, basing on the obtained results, that microorganisms decomposed biodiesel in 51–52 % at 1 and 5 % soil contaminations and only in 34 % at 10 % biodiesel dose in soil. The stimulating effect of biodiesel fuel on the number of microorganisms participating in biodegradation and of heterotrophs was mainly observed for 1 % contamination.

Keywords: biodiesel, biodegradation, soil, microorganisms

Biofuels that come from natural components are considered to be sources of renewable energy and the same of environmental-friendly one. It has been found after many studies that the best alternative for conventional diesel fuel is rapeseed oil methyl or ethyl ester [1, 2].

Biodiesel is obtained in the first place in the process of transesterification [3], mainly from rapeseed oil but can be also obtained from soybean, palm, cottonseed, sunflower, coconut oil as well as from waste oil and animal fat [4]. In the transesterification process, esters (biodiesel) are produced in the reaction of vegetable or animal oil with alcohol (methyl or ethyl ones), as well as glycerol as a by-product. Usually, sodium hydroxide is a catalyst [3] and the reaction proceeds according to the diagram presented below (Fig. 1).

[1] Department of Microbiology and Environmental Biotechnology, West Pomeranian University of Technology, ul. J. Słowackiego 17, 71–434 Szczecin, Poland, phone: +48 91 44 96 423, email: Malgorzata.Hawrot-Paw@zut.edu.pl

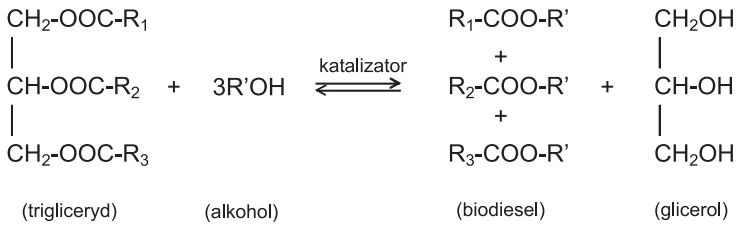

Fig. 1. The diagram of transestrification process [3]

Similar properties of both fuels as well as requirements of the fuel policy of the European Union (2003/30/EC) have led to the appearance of mixtures of diesel fuel with biodiesel on the markets of many countries, which bring new threats for environment. Biodiesel, although considered to be easily degraded in environment like conventional diesel fuel, is a foreign substance in soil. Similar properties of both fuels open possibilities of using the same methods of bioremediation to lands contaminated both with diesel fuel and biodiesel. Easy biodegradability of biodiesel is commonly pointed to, which is confirmed by the study of Peterson and Moller [5]. Some authors propose to use biodiesel as a biological solvent in the process of cleaning sand from crude diesel oil contamination [6, 7].

The present study aimed at broadening the knowledge referring to biodiesel biodegradation in natural soil environment. The aspect of autochthonous soil flora participation in this process was of particular importance. Also the effect of biofuel on the number of soil microorganisms was determined in this study.

Materials and methods

In the study, the soil was used from a plough land in the locality of Ostoja near Szczecin. Soil material was collected from a 0–15 cm horizon. It was a black earth (light loam) with the content of carbon at 19 g · kg^{-1} d.m. soil and of nitrogen at 1.5 g · kg^{-1} d.m. soil. Biodiesel used in the study was obtained directly from manufacturer, ie one of the Polish petroleum refineries. The soil under study was divided into 1 kg samples and brought to 50 % maximum water capacity, thereafter introducing biodiesel at the following concentrations: 1 % (object I), 5 % (object II) and 10 % (object III) (w/w – converted to d.m. soil). The soil sample without biodiesel was a control sample (K). The experiment was carried out at room temperature for 112 days. Analyses were made on experiment set-up day and then after 7, 14, 28, 56 and 112 incubation days.

Biodiesel content changes during incubation were analysed with the weight method according to PN-75/C-04573/10 using the Soxhlet apparatus with a 100 cm^3 extraction flask. During examinations, also the number of heterotrophic microorganisms as well as of those decomposing biodiesel was determined by soil dilution culture method. All measurements were made in three repetitions. For determination of heterotrophic microorganisms, meat peptone agar MPA (Difco) was used. Determination of the number of microorganisms decomposing biodiesel was made using Bushnell-Haas

culture medium with the following composition [g · dm^{-3}]: $MgSO_4 \cdot 7H_2O$ – 0.2, K_2HPO_4 – 1.0, KH_2PO_4 – 1.0, $FeCl_3$ – 0.05, NH_4NO_3 – 1.0, $CaCl_2$ – 0.02, and NaCl – 2.0 %; a 2 % biodiesel addition was introduced into it. Culture incubation was carried out at 28 ^{o}C. The number of heterotrophic microorganisms was determined after 3 days, while that of biodiesel decomposing microorganisms after 14 days. The number of microorganisms was converted to 1 g dry matter of soil.

Results and discussion

There is relatively little information in the available literature on biodegradation of biodiesel and its effect on microflora. However, numerous studies have been performed on the behaviour of conventional diesel fuel, the results of which can be used as comparative material due to certain similar properties of both fuels and planned introduction of their mixtures onto market.

In object I (with 1 % contamination dose), biodegradation of biodiesel after 14 days reached the level of 24 %, after 56 days – 44 %, while 51 % of this biofuel was decomposed after 112 days (Fig. 2). In the object with 5 % biodiesel dose (object II), a loss of 17 % of this biofuel was observed after 14 days and 40 % after 56 days; reduction of its quantity increased gradually up to 52 %. In object III (with 10 % biodiesel addition), 18 % of biodiesel contamination was decomposed after 14 days and 30 % after 56 days. Maximum contamination reduction level after incubation was 34 %. Peterson and Moller [5] examined decomposition of 0.001 % biodiesel in the water environment using bacterial inoculum and obtained an 84 % biodegradation after 28 incubation days. They also carried out the analysis of biodegradation of that contamination in soil. After 28 days, biodiesel introduced into soil at a concentration of 1 %

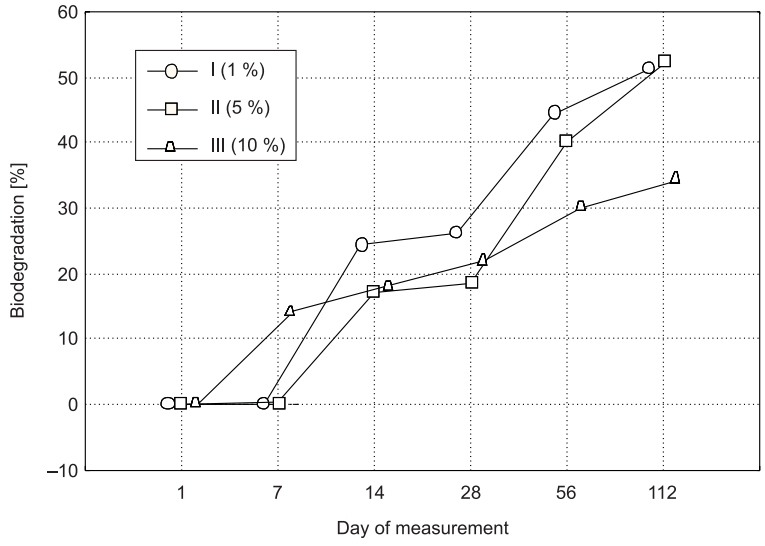

Fig. 2. Level of biodiesel biodegradation

was decomposed in 88 %. In the study of Pasqualino et al [7] referring to biofuel decomposition in water, its biodegradation reached almost 100 % at substrate initial concentration being equal to 1.5 %. Its decomposition proceeded with the use of specialised microorganism strains. Zhang et al [8] obtained a 60 % decomposition of contamination (after 14 days) in their study on decomposition of 1 % biodiesel addition in the water environment. In comparison with these results, biodiesel decomposition in the soil environment observed in the present study was considerably lower – a 26 % reduction was obtained for 1 % dose after 28 incubation days, 18 % for 5 % dose and 22 % for the highest concentration. In the study on conventional diesel fuel, Hawrot and Nowak [9] obtained a 73 % decomposition of substrate for 5 % contamination dose in non-modified soil, ie with the participation of autochthonous microflora. Their experiment was carried out for 150 days and they obtained considerably better result when compared with biodiesel decomposition observed in the present study (52 % decomposition after 112 days), despite the same dose applied. High biodegradation values were also obtained by Boszczyk-Maleszak et al [10], who reported a decomposition of 5 % contamination reaching 44 % after 21 days; biodiesel decomposition in the same time period of own examination reached 17 %. The analyses carried out by Nowak and Hawrot [11] on the biodegradation activity of bacterial strains demonstrated large efficiency of using bacterial inoculates in the processes of cleaning soils from petroleum derivative substances. Hawrot and Nowak [9] found that inoculation of soil increased diesel fuel biodegradation by 46 % when compared with the soil not modified by any treatment. The study of Bieszkiewicz et al [12] carried out on liquid culture at diesel fuel concentration of 0.1 % showed that the loss of diesel fuel fraction in single strain cultures amounted to 80–90 % after 12 days, whereas in strain mixture culture even to 98 %. The study of Nowak and Hawrot [13] on 5 % and 15 % soil contamination with diesel fuel also showed a positive effect of applying inoculation. These authors found that basic agrotechnical measures increased biodegradation only by 5 %, whereas bioaugmentation in light soil allowed reduction of 83 % of contamination at 5 % diesel fuel dose and of 39 % at substrate concentration of 15 %. In medium-heavy soil, these values were respectively 52 % at 5 % addition of diesel fuel and 17 % at a dose of 15 %.

Such numerous findings pointing to favourable effect of bacterial inoculates on diesel fuel decomposition suggest application of similar methods in case of biodiesel. Considering the unsatisfactory effect of its biodegradation observed in the present study, research works on decomposition of this fuel should be continued using the modifications applied in remediation of soils contaminated with conventional fuels.

Changes in the number of microorganisms participating in biodiesel decomposition are presented in Fig. 3A. As reported by Kolwzan et al [14], the number of bacteria required for carrying out biodegradation process should be within a range of 10^5–10^7 cells in 1 g d.m. soil. In the own study, the number of biodiesel decomposing microorganisms at 1 and 5 % contaminations reached values of 10^5 in the whole experimental period (ie minimum values required for effective biodegradation). At 10 % biofuel addition, the number of bacteria after 14 days reached values near or equal to zero. Increase in the number of microorganisms for which this product is a source of

carbon and energy was observed by Hawrot and Nowak [9] in the object contaminated with 5 % addition of diesel fuel. In the study of Boszczyk-Maleszak et al [10] on conventional fuel, the number of bacteria amounted to 10^7 cells in 1 g d.m. soil after 21 days at 1 % contamination, whereas 10^6 cells in 1 g d.m. soil at 5 % contamination. Increase in the number of microorganisms decomposing diesel fuel was also confirmed by Ruberto et al [15]. In their study on biodegradation of 1.5 % diesel fuel contamination, the number of microorganisms decomposing it in non-contaminated soil had a value of 10^4 cells in 1 g d.m. soil, whereas their number after contamination increased to 10^6 cells after 28 days.

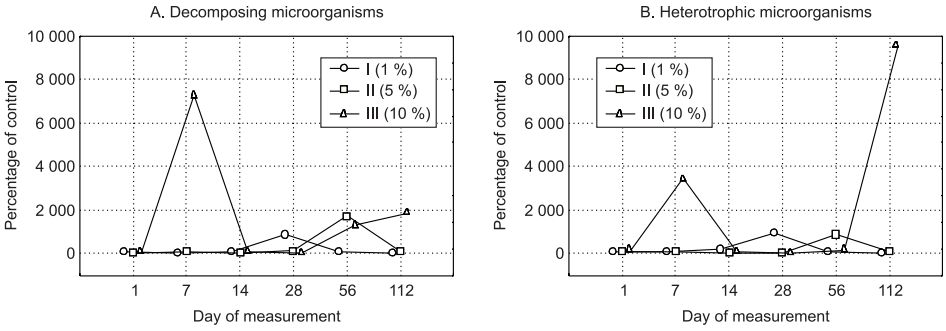

Fig. 3. Number of decomposing (A) and heterotrophic (B) microorganisms in soil as percentage in relation to control sample

Examination of the number of heterotrophic microorganisms showed a strong effect of biodiesel stimulating their growth at its 1 % contamination of soil (Fig. 3B). The largest values, amounting to 10^6 cells in 1 g d.m. soil, were recorded on experimental day 14. Similar results were obtained Ruberto et al [15]. At 1.5 % contamination of soil with diesel fuel, they observed a considerable increase in the number of microorganisms in contaminated soil (10^6–10^7 cells in 1 g d.m. soil) when compared with the control. The number of heterotrophs in object II (5 % biodiesel) was maintained at a level of 10^4–10^5 cells in 1 g d.m. soil. In the object contaminated with 10 % biodiesel addition, this number gradually decreased within a range of 10^5–10^2 cells, until it reached values approximating zero. In the study of Bento et al [16], the number of heterotrophic microorganisms and that of microorganisms decomposing contamination were found to be similar at 1 % contamination with diesel fuel. On the other hand, Margesin et al [17] found that contamination with diesel fuel at a dose of 0.5 % did not cause larger changes in the number of heterotrophs.

Conclusions

The natural origin of biofuel used in experiments suggested a possibility of its quick and effective biodegradation in soil. However, the study showed that biodiesel is relatively resistant to the activity of natural microflora and is decomposed more slowly

than conventional diesel fuel. The obtained results suggest a need to continue experiments concerning biodegradation of this fuel, in particular in the context of regulations which impose the necessity of using it as a biocomponent to conventional fuels.

References

[1] Graboski M.S. and McCormick R.L.: Progr. Energy. Combust. Scr. 1998, **24**, 125–164.
[2] Bünger J., Krahl J., Baum K., Schröder O., Müller M., Westphal G., Ruhnau P., Schulz T.G. and Hallier E.: Arch. Toxicol. 2000, **74**, 490–498.
[3] Leung D.Y.C., Koo B.C.P. and Guo Y.: Biores. Technol. 2006, **97**, 250–256.
[4] Knothe G.: The biodiesel handbook. AOCS Press, Champaign, Illinois 2005.
[5] Peterson CH.L. and Möller G.: Biodegradability BOD_5, COD and toxicity of biodiesel fuels, University of Idaho, Moscow 1998.
[6] Mudge S. and Pereira G.: Spill Sci. Technol. Bull. 1999, **5**, 353–355.
[7] Pasqualino J., Montane D. and Salvado J.: Biomass Bioenergy 2006, **30**, 874–879.
[8] Zhang X., Peterson CH.L., Reece D., Möller G. and Haws R.: Trans. ASAE 1998, **41**(5), 1423–1430.
[9] Hawrot M. and Nowak A.: Zesz. Probl. Post. Nauk Roln. 2004, **501**, 151–157.
[10] Boszczyk-Maleszak H., Bieszkiewicz E., Lelas A., Dukielska A. and Kacieszczenko J.: *Wpływ wybranych czynników biotycznych i abiotycznych na przebieg biodegradacji produktów naftowych w glebie*, [in:] Materiały II Ogólnopol. Symp. Nauk.-Techn. „Bioremediacja gruntów", Wisła–Jarzębata 2000, 109–118.
[11] Nowak A. and Hawrot M.: Zesz. Probl. Post. Nauk Roln. 2003, **492**, 211–216.
[12] Bieszkiewicz E., Mycielski R., Boszczyk-Maleszak H. and Wyszkowska B.: Biotechnologia 1997, **1**, 70–77.
[13] Nowak A. and Hawrot M.: *Izolacja bakterii zdolnych do biodegradacji substancji ropopochodnych ze środowisk naturalnych oraz metodyka oceny ich aktywności*, [in:] Materiały VI Ogólnopol. Symp. Nauk.-Techn. „Biotechnologia Środowiska", Wrocław 1999, 237–242.
[14] Kołwzan B., Traczewska T., Piekarska K. and Juchniewicz M.: *Mikrobiologiczna ocena możliwości bioremediacji gruntów skażonych produktami naftowymi*, [in:] Materiały V Ogólnopol. Symp. Nauk.-Techn. „Biotechnologia środowiska", Ustroń–Jaszowiec 1997, 11–17.
[15] Ruberto L., Vazquez S. and Mac Cormak W.: Int. Biodeter. Biodegr. 2003, **52**(2), 115–125.
[16] Bento F., Camargo F., Okeke B. and Frankenberger W.: Biores. Technol. 2005, **96**, 1049–1055.
[17] Margesin R., Zimmerbauer A. and Schinner F.: Chemosphere 1999, **40**, 339–346.

WSTĘPNE BADANIA NAD EFEKTYWNOŚCIĄ BIOLOGICZNEGO ROZKŁADU BIODIESLA PRZY UDZIALE AUTOCHTONICZNEJ MIKROFLORY GLEBOWEJ

Zakład Mikrobiologii i Biotechnologii Środowiska
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Przedstawiono badania dotyczące biodegradacji biodiesla przy udziale naturalnej mikroflory glebowej oraz jego wpływu na organizmy rozkładające biopaliwo i mikroorganizmy heterotroficzne. Na podstawie otrzymanych wyników stwierdzono, że mikroorganizmy rozkładały biodiesel na poziomie 51–52 % przy skażeniu 1 i 5 % oraz zaledwie 34 % przy 10 % dawce biopaliwa w glebie. Stymulujacy wpływ biodiesla na liczebność organizmów uczestniczących w biodegradacji oraz heterotrofów obserwowano przede wszystkim przy zanieczyszczeniu na poziomie 1 %.

Słowa kluczowe: biodiesel, biodegradacja, gleba, mikroorganizmy