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BIOLOGICAL ACTIVITY OF SOIL CONTAMINATED BY BIODIESEL

The aim of the research was to evaluate the influence of biodiesel on the microorganisms biomass and dehydrogenase activity. Biodiesel was introduced into the soil at a concentration of 1.5 and 10%. The influence of biofuel on the amount of biomass of living microorganisms was various and depended on the dose which was used. Stimulation of dehydrogenases activity was observed in 1% dose of biodiesel (about 60–300% higher than the values in non-contaminated soil). Higher doses, within a longer period of time, had a negative influence on microorganisms (reduction of their activity up to 35–92% compared to the control).

1. INTRODUCTION

The danger connected with the depletion of petroleum resources as well as ecological threat caused by the infiltration of petroleum into environment during its extraction, transportation, processing, distribution and use urge the production of ecological fuels. Biofuels that come from natural components are considered to be renewable energy sources and at the same time they are environmentally friendly.

Biodiesel, which is obtained mainly from rapeseed oil during transesterification process, is of particular interest [1]. Similar properties of diesel fuel and biodiesel as well as the requirements of the fuel strategy of the European Union (Biofuels Directive 2003/30/EC) have resulted in the emergence of mixtures of both types of fuels on the markets of many countries, however this means new threats to the environment. Although biodiesel is considered to be easily biodegradable, it is a foreign compound in natural environment, like conventional diesel fuel. Few studies referring to the effect of biodiesel on living organisms point to their ambiguous response to its environmental

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presence. Experiments evaluating biodiesel toxicity carried out by PETERSON and REECE [2] showed that it was 15 times less harmful to *Daphnia magna* than diesel fuel. In experiments prepared by von WEDEL [3], LC_{50} for the early developmental stage of *Menidia beryllina* was 578 ppm for biodiesel compared to 27 ppm for diesel fuel. According to LAPINSKIENE et al. [4], biodiesel is non-toxic up to a concentration of 12%, whereas diesel fuel shows toxic properties just above 3% of soil content. BÜNGER et al. [5] presented different data in their paper. After comparing the toxicity of exhaust gases from biodiesel-fuelled engine and a diesel fuel-fuelled one for mice, they found biodiesel exhaust gas four-times more toxic than the other one, they explained this by a larger quantity of not combusted fuel absorbed on carbon black particles.

The present study aimed at determining how the presence of biodiesel affected the activity of soil microbiocenosis on the basis of the measurement of live organism biomass and dehydrogenases activity.

2. MATERIALS AND METHODS

Biodiesel used in the study was made available by its manufacturer, i.e. one of the Polish petroleum refineries.

Clayey soil (light loam) used in the present study was collected from a 0–15 cm horizon. The content of total carbon, determined with the Tiurin method, was 19 $g \cdot kg^{-1}$, whereas that of nitrogen, determined by the Kiejdahl method, was 1.5 $g \cdot kg^{-1}$ of clayey soil. Soil material was divided into samples, 1 kg each, and then brought to 50% maximum water capacity. Such humidity was maintained for the whole experiment and possible losses (measurement of current moisture*)* were completed with distilled water. Biodiesel was introduced into the soil at a concentration of 1.5 and 10% (w/w per d.w. soil); three test objects set up in this way were respectively marked I, II and III. The soil sample without biodiesel was a control sample (treatment K). The experiment was carried out in laboratory conditions at 20 °C for 112 days. Analyses were performed on experiment set-up day and then after 7, 14, 28, 56 and 112 incubation days. During the examination microbiological analyses were made, i.e. the determination of the biomass of viable microorganism by the SIR method according to ANDERSON and DOMSCH [6], as well as biochemical ones, i.e. the determination of dehydrogenases activity using the modified method of THALMANN [7].

All measurements were done in three repetitions. The findings were analysed statistically using the two-way analysis of variance.

3. RESULTS AND DISCUSSION

Biodiesel is a relatively new product, thus there are almost no data on its effect on microflora. However, numerous studies have been carried out on the behaviour of

conventional diesel fuel, the results of these studies may serve as a comparative material due to certain similar properties of diesel fuel and biodiesel as well as the planned bringing of their mixtures onto the market.

The biomass of viable microorganisms and dehydrogenases activity belong to the basic indicators of the biological activity of contaminated soils [8], [9]. The statistical analysis, carried out during the experiment, showed a significant effect of the treatments and incubation days as well as the influence of both examined factors on the biomass of viable microorganisms and dehydrogenase activity (table 1).

Table 1

Results of the statistical analysis of the amount of microbial biomass and dehydrogenases activity of soil contaminated with biodiesel

Factor No.	Number of independent variables	Mean square sum	Number of independent variables for error	Mean square sum for error	Value *F*	Value *P* (≤ 0.05)
Biomass of viable microorganisms						
1	5	6100612	48	392245,4	15.55305	0.00
2	3	14952314	48	392245,4	38.11979	0.00
1·2	15	1993281	48	392245,4	5.08172	0.00
Dehydrogenases activity						
1	5	450,889	48	3.697859	121.933	0.00
2	3	6240,982	48	3.697859	1687.728	0.00
1·2	15	333,985	48	3.697859	90.319	0.00

Factors: 1 – incubation days, 2 – treatments.

Table 2

Microbial biomass of soil contaminated by biodiesel [mg C·100 g^{-1}]

Treatments	Incubation days						Mean
	0	7	14	28	56	112	
K	2660	3098	2882	3313	1980	1455	2565
I (1%)	2653	4000	3165	2842	3273	1495	2905
II (5%)	687	4929	3057	1441	2007	1024	2191
III (10%)	1906	849	755	755	499	196	826
Mean	1977	3219	2465	2088	1940	1042	–

$LSD_{0.05}$
Incubation days (ID) 767.38
Treatments (T) 560.84
Interaction (ID)·(T) 2015.70

Table 2 shows the evolution of soil microbial biomass during the experimental period. The effect of 1% biodiesel contamination was not simple. Both biomass weight

reduction (86% of the control values) after 28 days of incubation and stimulation (maximum 165% in relation to the values recorded in control object) were observed (figure 1). In this treatment, the content of biomass decreased below control values only on the 14th incubation day. Maximum biomass content in the treatment with a 5% biodiesel dose was observed after 7 incubation days (almost 60% more than in the control treatment). A clearly negative response of microorganisms was observed after the contamination of soil by biofuel at a dose of 10%, i.e. the content of biomass during the whole experiment was lower than the values observed in the control treatment. As far as conventional fuels are concerned, the available literature does not expressly determine the effect of soil contamination on the activity of soil microorganisms. HAWROT and NOWAK [10] found in their study on the effect of diesel fuel on microflora activity at 5% contamination that the biomass of viable microorganisms was higher in relation to the non-contaminated treatment and reached a maximum after 112 days of incubation (almost 200% more than in control treatment). Stimulation of microflora activity in the soil contaminated by hydrocarbons, which is most likely connected with microorganism capability to decompose a new source of carbon, is also confirmed by other authors [11], [12]. Different results were obtained by MICHALCEWICZ [13] in her study on the biomass of viable microorganisms in the soil contaminated by diesel fuel at 1%, 2% and 5% concentrations. This author showed that the mean biomass level during the whole experiment (150 days) ranged from 20 to 30% below the control values. Also MEGHARAJ et al. [14] observed that biomass weight decreased with the increase of hydrocarbon contamination.

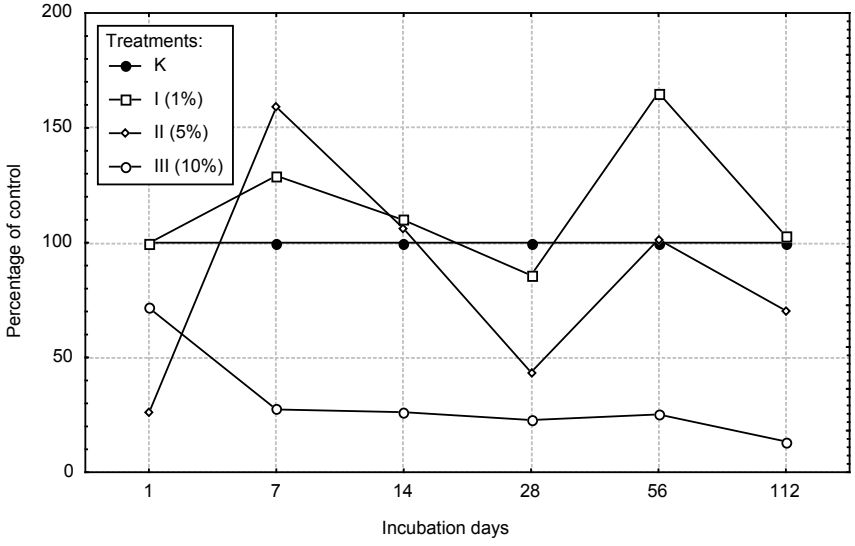

Fig. 1. Microbial biomass (percentage in relation to the control soil). *K* – control soil, I – soil+1% of biodiesel, II – soil+5% of biodiesel, III – soil+10% of biodiesel

Dehydrogenases activity, as the basic enzymatic system responsible for the oxidation of petroleum derivative hydrocarbons, is considered – in the case of conventional fuels – to be the indicator of the capability of microorganisms to quickly degrade biodiesel [15]. In the present study, dehydrogenases activity in the control object (*K*) on the first incubation day was 16 mg TPF·kg^{-1} d.w. soil and remained on a similar level over the whole time of experiment (table 3). Dehydrogenases activity was maintained above the control values only in the soil contaminated by biodiesel at a dose of 1% (figure 2). After the introduction of 5% biodiesel addition into soil (treatment II), a decrease in dehydrogenases activity was observed as early as seven days after the beginning of incubation, while inhibition was maintained over the whole time of incubation. In treatment III, with a 10% biodiesel dose, less than 30% of the control values were determined as early as 14 days after the beginning of incubation, while dehydrogenases activity remained on a similarly low level until the end of experiment. The results observed by LAPINSKIENE et al. [4] were quite different. Together with an increase of the applied biodiesel doses (1, 3, 6, 9 and 12% w/w), dehydrogenases activity increased, too.

Table 3

Dehydrogenases activity of soil contaminated by biodiesel (mg TPF·kg^{-1})

Treatments	Incubation days						Mean
	0	7	14	28	56	112	
K	16	18	15	22	18	17	18
I (1%)	31	72	48	36	70	35	49
II (5%)	23	12	9	4	6	5	10
III (10%)	20	25	4	2	3	3	9
Mean	22	31	19	16	24	15	–

$LSD_{0.05}$
Incubation days (ID) 2.36
Treatments (T) 1.72
Interaction (ID)·(T) 6.19

Conventional fuels did not clearly affect enzymatic activity either. In the study of HAWROT et al. [16], with a 5 percent level of diesel fuel contamination, dehydrogenases activity was higher than that in the control object by 95–108% for 60 days of incubation. On the following days, this activity decreased quite significantly, with the determined values being by 25–60% lower than those in the non-contaminated treatment. Similar, negative response to the presence of petroleum derivative substances was observed by DAWSON et al. [17]. The study of KUCHARSKI and JASTRZĘBSKA [18] showed that dehydrogenases activity was inhibited by increasing doses of petroleum derivative substances. Different results were obtained by MAŁACHOWSKA-JUTSZ et al. [19], also SMOLIK and NOWAK [20], who showed a stimulating effect of diesel fuel on dehydrogenases activity. The study of SMOLIK et al. [21], carried out on the black-earth with a 10% diesel fuel

contamination, showed an activating effect of contamination on dehydrogenases. As early as incubation day 1, their activity was higher than in the non-contaminated object about over 320% and remained on a very high level until the end of experiment.

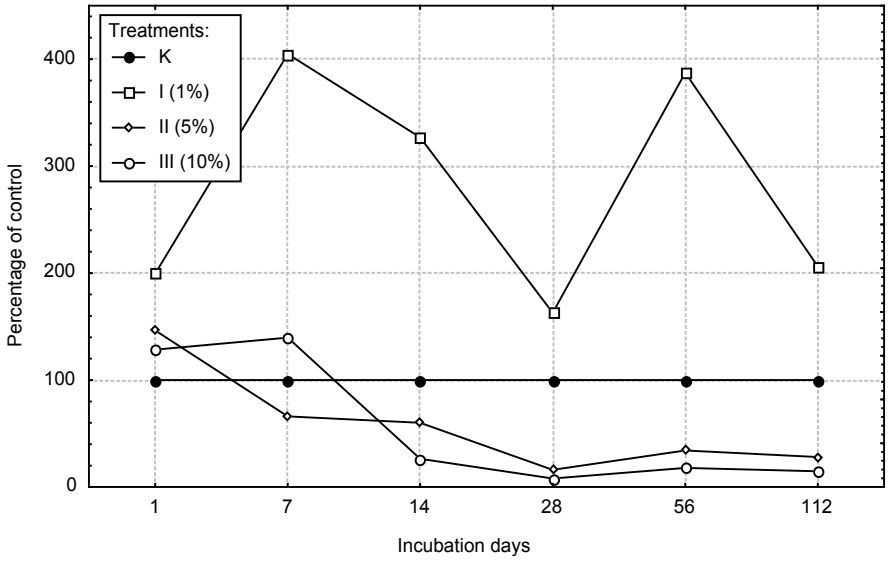

Fig. 2. The activity of dehydrogenases (percentage in relation to the control soil). *K* – control soil, I – soil+1% of biodiesel, II – soil+5% of biodiesel, III – soil+10% of biodiesel

4. CONCLUSIONS

1. Contamination of soil by biodiesel had diverse influence on soil activity depending on the applied dose of biofuel.

2. The addition of biodiesel caused, on average, an increase of soil microbial biomass (+13%) at a concentration of 1%, decrease of 5% (–15%) and 10% (–68%) contamination.

3. Dehydrogenases activity was also connected with the dose of contamination – stimulation was observed at 1% concentration, whereas higher doses had negative effect after a longer period of time (the maximum reduction of activity – 84% and 92%, respectively, at 5 and 10% concentration of biodiesel).

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