

Tomasz CIESIELCZUK<sup>1</sup> and Teresa KRZYŚKO-ŁUPICKA<sup>2</sup>

**KINETICS OF DEGRADATION OF MINERAL OIL  
AND DIESEL FUEL IN SOIL CONTAMINATED  
WITH PETROLEUM SUBSTANCES AFTER STIMULATION  
WITH FYRE-ZYME ENZYME REAGENT  
AND HYDROGEN PEROXIDE**

**KINETYKA ROZKŁADU OLEJU MINERALNEGO I NAPĘDOWEGO  
W GLEBIE SKAŻONEJ SUBSTANCJAMI ROPOPOCHODNYMI  
PO STYMULACJI PREPARATEM ENZYMATYCZNYM FYRE-ZYME  
I NADTLENKIEM WODORU**

**Abstract:** Petroleum substances are widely used in many industries and are used as lubricants and fuels in motor vehicles. Due to the pipeline failure, lost of substances on the loading stations area and traffic crashes, petroleum contamination of soils are very common. High concentration of petroleum pollutants in the soil, leads to reduced activity of the indigenous microflora and extends their fate in the environment. The high costs of removal of oil spills to the soil and ground, forces to search for low-cost and effective methods of soils decontamination by „in situ” methods. The aim of this study was to compare the effectiveness of biodegradation of diesel fuel and mineral oil in the soil contaminated with petroleum substances, after “Fyre-Zyme” enzyme reagent stimulation and/or hydrogen peroxide. Obtained results indicate on stimulation of degradation process of diesel fuel and mineral oil, either by used enzyme and hydrogen peroxide compared with the control samples in 60 days period. This indicates the possibility of use of tested additives for soils bioremediation processes.

**Keywords:** oil, bioremediation, soil, enzymes, hydrogen peroxide

Petroleum substances are widely used in many industries and are used as lubricants and fuels in motor vehicles. Due to the pipeline failure, lost of substances on the loading stations area and traffic crashes, petroleum contamination of soils are very common. High concentration of petroleum pollutants in the soil, leads to reduced activity of the indigenous microflora and extends their fate in the environment [1-3]. In case of low amount of hydrocarbons contamination, it is possible use of compost and lime for improve natural degradation processes and plants growth conditions [4]. But high load of diesel fuel or lubricants lead to very long time of natural bioremediation. The high costs of removal of oil spills to the soil and ground, forces to search for low-cost and effective methods of soils decontamination by “in situ” methods [3, 5, 6].

The aim of this study was to compare the effectiveness of biodegradation of diesel fuel and mineral oil in the soil heavily contaminated with petroleum substances, after “Fyre-Zyme” enzyme reagent stimulation and/or hydrogen peroxide.

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<sup>1</sup> Department of Land Protection, University of Opole, ul. Oleska 22, 45-052 Opole, phone 77 401 60 20, email: tciesielczuk@uni.opole.pl

<sup>2</sup> Department of Biotechnology and Molecular Biology, University of Opole, ul. kard. B. Kominka 6a, 45-035 Opole, phone 77 401 60 57, email: teresak@uni.opole.pl

## Material and methods

Determination of the degree of degradation of diesel fuel and mineral oil in contaminated soil was conducted in the laboratory conditions by use:

- enzyme preparation Fyre-Zyme (S + FZ)
- hydrogen peroxide (S + H)
- preparation Fyre-Zyme enzyme and hydrogen peroxide (S + FZ + H)

Each experimental pot was fed by 1 kg of contaminated soil, and then the enzyme reagent Fyre-Zyme was added in an amount of 10% (m/m). Hydrogen peroxide was added in an amount of 0.5 g O<sub>2</sub>/dm<sup>3</sup>. Control samples (C) without the addition was incubated. Humidity was maintained at 60% and at 2 days periods weight loss of water was replenished. The prepared soil in pots were incubated at room temperature 22 (±2°C). The duration of the experiment was 60 days. Pot experiments established in 3 replications.

The kinetics of the chemical changes were monitored by taking 1 g soil samples from each pots, and then averaged and at 0, 30 and 60 days was determined changes in the concentration of oil products (diesel fuel and mineral oil) in the soil. Also only aliphatic hydrocarbons were determined. These compounds were divided on two group (C8-C21 and C22-C40) connected to boiling temperatures characteristic for diesel and mineral oil.

Aliphatic hydrocarbons, diesel fuel and mineral oil were determined by GC-FID method on capillary column VF1-ms (30 m x 0.53 mm x 1.50 µm). All samples were extracted in fexIKA extractor after drying with anhydrous sodium sulphate [7]. Diesel fuel and mineral oil were calculated as summary peaks of all detected organic compounds in specific boiling temperature ranges.

## Results and discussion

Obtained results indicate on stimulation effect of used additions on petroleum products in investigated soil. On the end of experiment (60 day indicated by grey bars in Fig. 1) diesel fuel concentration was lower in all tested samples. The highest effectivity was noted in pot with hydrogen peroxide addition (S + H). Over 44.1% of diesel fuel was degraded in 60 days period (Fig. 1). Also in control sample degradation process was effective (29.8% of decomposition). Similar situation was noted in degradation of mineral oil. Best results were obtained in case of S + H sample (43.9%). Biodegradation results in case of stimulation with Fyre Zyme reagent with or without hydrogen peroxide was equal (37.8% in both samples). Worst results were obtained in control sample - only 21.7% (Fig. 2). This shows that long-chain hydrocarbons are more resistant on microbial degradation than compounds with short carbon chain.

Degradation of n-alkanes was different in all tested samples. In case of "short-chain" compounds, the best results were obtained in sample with hydrogen peroxide addition 54.0% (similar to total diesel fuel content). Worst results for these compounds (only 18.1%) were observed in samples with enzyme reagent. In case of "long-chain" n-alkanes (C22-C40), the highest degradation intensity (46.5%) was observed in samples with enzymes and with enzyme Fyre-Zyme. Similar to diesel fuel in samples with enzymes and H<sub>2</sub>O<sub>2</sub> addition and only with hydrogen peroxide was observed good results (41.2 and 40.3% respectively) (Figs. 3 and 4).

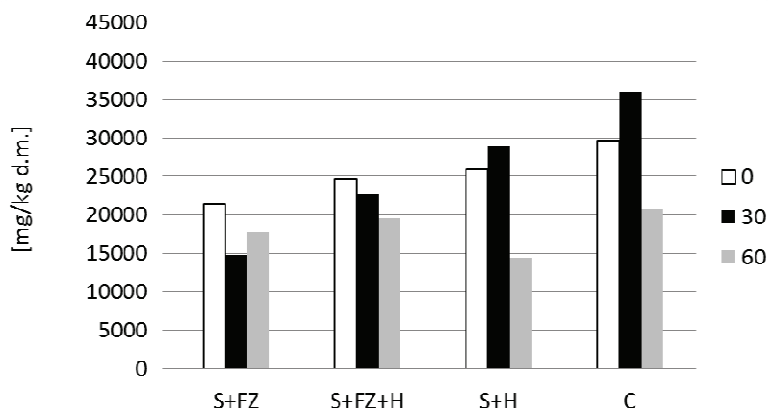


Fig. 1. Diesel fuel content in soil samples with different additions

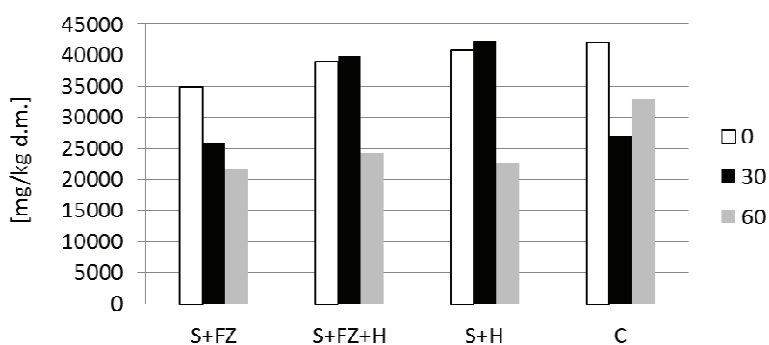


Fig. 2. Mineral oil content in soil samples with different additions

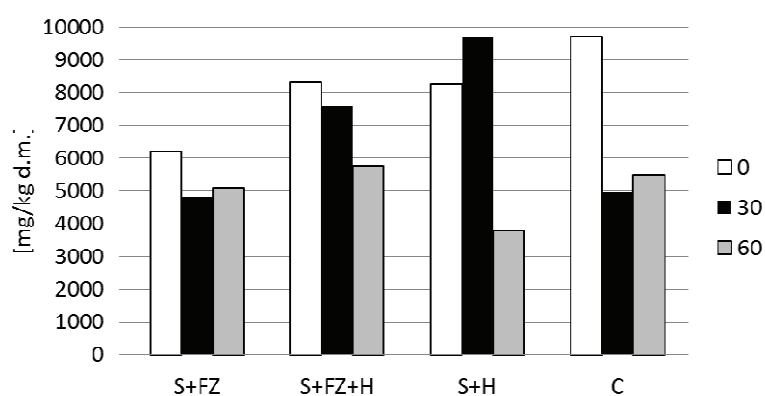


Fig. 3. Aliphatic hydrocarbons (C-8-C21) content in soil samples with different additions

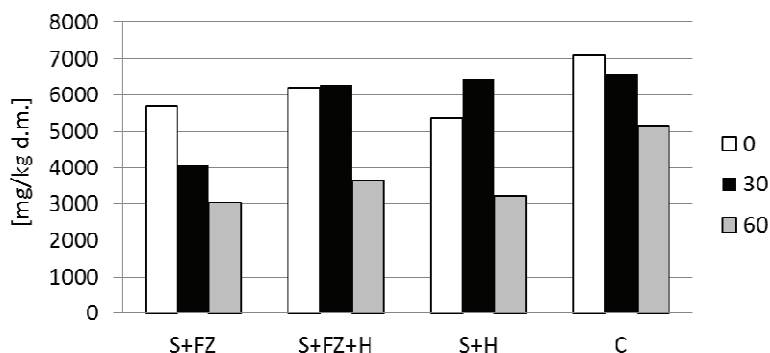


Fig. 4. Aliphatic hydrocarbons (C-22-C40) content in soil samples with different additions

### Conclusion

Obtained results indicate on stimulation of degradation process of diesel fuel and mineral oil in heavily contaminated soil. The best results were obtained either for samples with Fyre-Zyme enzyme or hydrogen peroxide compared with the control samples in 60 days period. Compilation of enzyme reagent and hydrogen peroxide also was effective but especially for heavy compounds (*eg* mineral oil fraction). This indicates the possibility of use of tested additives for environmentally friendly, highly effective soils bioremediation processes.

### References

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<sup>1</sup> Samodzielna Katedra Ochrony Powierzchni Ziemi, Uniwersytet Opolski

<sup>2</sup> Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

**Abstrakt:** Substancje ropopochodne znajdują szerokie zastosowanie w wielu gałęziach przemysłu, a także są stosowane jako środki smarne i pędne w pojazdach mechanicznych. Z uwagi na awarie sieci przesyłowych, obrót tymi substancjami na stacjach przeładunkowych, a także katastrofy w ruchu lądowym zanieczyszczenia ropopochodne gleb są częstym zjawiskiem. Duża koncentracja zanieczyszczeń naftopochodnych w glebie prowadzi do zmniejszenia aktywności mikroflory autochtonicznej i wydłuża czas ich zalegania w środowisku. Wysokie koszty likwidacji skutków wycieków ropopochodnych do gleb i gruntu zmuszają do poszukiwania tanich i efektywnych metod usuwania zanieczyszczeń metodami „in situ”. W pracy porównano efektywności biodegradacji oleju napędowego i mineralnego w glebie skażonej substancjami ropopochodnymi po stymulacji preparatem enzymatycznym Fyre-Zyme lub/i nadtlenkiem wodoru. Zastosowane preparat enzymatyczny oraz nadtlenek wodoru stymulują rozkład oleju napędowego oraz mineralnego w glebie w porównaniu do grupy kontrolnej w okresie 60 dni. Wskazuje to na potencjalne możliwości zastosowania badanych dodatków do bioremediacji gleb.

**Słowa kluczowe:** ropopochodne, bioremediacja, gleba, enzymy, nadtlenek wodoru