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Biodegradation of creosote oil in soil bioaugmented with the selected bacterial strains

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Abstract: *Biodegradation of creosote oil in soil bioaugmented with the selected bacterial strains.* Creosote oil is widely used as wood preservation agent to protect railway sleepers, picket fences and tele-technical poles from the biodeterioration caused by fungi, bacteria, algae and insects. Due to its toxic properties, mainly caused by the presence of polycyclic aromatic hydrocarbons (PAHs) in its chemical composition, it can be dangerous to animals and humans. The contamination of soil with creosote oil is especially common in timber preserving plants, where large volumes of creosote are used. In this study, the biodegradation efficiency of creosote oil (either type B or C) were examined with respect to two types of soil (i.e. sterile or non-sterile) and two bacterial strains (*Pseudomonas putida* DA1, *Pseudomonas sp.* OS4). Biodegradation of PAHs was higher in non-sterile soil with respect to creosote type B containing lower concentration of PAHs with higher number of aromatic rings (biodegradation extent exceeded 80% in 28-day test). Although both bacterial strains were similarly efficient in degradation of creosote oil type B, *Pseudomonas putida* DA1 was more effective towards creosote type C (in non-sterile soil system).

Keywords: biodegradation, creosote oil, *Pseudomonas*

INTRODUCTION

Biodeterioration of wood caused by fungi, bacteria, algae and insects can be inhibited by the application of effective wood preservatives. Creosote oil is one of the most common and cheap wood preservation agents. It is widely used to protect wooden railway sleepers, picket fences and tele-technical poles. From the chemical point of view, creosote oil is a mixture of numerous compounds obtained during distillation of coal tar in the range of ca. 200 do 360°C. Polycyclic aromatic hydrocarbons (PAHs) make up ca. 40-80% of creosote oil and are considered as its most dangerous (both for animals and humans) components.

The production of impregnated wood in the form of railway sleepers and tele-technical poles, according to available data, is about 1.3 million m^3 per year in Europe, and the production of wood impregnated with creosote of about 0.7 million m^3 (Fojutowski *et al.* 2015), which with an annual production at the level of approx. 10-15 thousand m^3 is produced in approx. 45-70 production plants. This indicates a potentially large market of companies interested in solving problems with the contamination of soil by the creosote oil.

In connection with the tightening of EU environmental legislation, biological methods have become the preferred techniques for the treatment of soils contaminated with organic contaminants. The cleaning of the soil can be carried out using specified microbial species that are characterized by the ability to degrade toxic pollutants such as polycyclic aromatic hydrocarbons (Kołwzan *et al.* 2005). Currently, the most effective bioremediation technique is considered to be bioaugmentation, i.e. the use of specially selected microbial strains with high biodegradation potential, which are cultivated in laboratory conditions, and then introduced into a contaminated environment, where they support the decomposition of hardly degradable compounds (Fernández-Luqueño*et al.*2011). The biodegradation of PAHs contained in creosote oil may include bacteria from the genera *Acinetobacter*, *Alcaligenes, Mycobacterium*, *Nocardia* or *Pseudomonas*, and fungi from species *Phanerochaete chrysosporium, Bjerkandera adusta*, *Irpex lacteus*, *Trametes versicolor* and *Pleurotus ostreatus* (Borràs Camps *et al.* 2012, Eggen and Majcherczyk, 1998; Gali *et al.* 2006, Zabielska-Matejuk *et al.* 2017).

The aim of the study was to evaluate the biodegradation potential of two *Pseudomonas* bacterial species with respect to soil contaminated with creosote oil (either type B or C).

MATERIALS

Creosote oil type B and C was obtained directly from the producer – Centrala Obrotu Towarami Masowymi DAW-BYTOM (Poland). Creosote oil type C contains lower concentration of naphthalene, which is usually a dominant component among all PAHs in creosote oil. On the other hand, it contains more PAHs with higher number of aromatic rings. *Pseudomonas* sp. OS4, and *Pseudomonas putida* DA1 bacterial speciesisolated from areas contaminated with petroleum hydrocarbons were used :in biological tests. Bacterial cultures were pre-cultivated in 250 mL Erlenmeyer flasks for 72 hours on a mineral medium (50mL) enriched with creosote oil (conc. 0.1 v/v %) in order to improve the adaptation of microorganisms to this particular carbon source. After 72 hours, 1 mL of the biomass suspension was introduced into new Erlenmeyer flask with fresh medium enriched with creosote oil and cultivated for 72 hours. This procedure was repeated three times in total, and then microbial biomass was next used in bioaugmentation of the studied soil variants.

In each variant, 60g of garden soil and 60g of sand was mixed and placed inside the 1L glass container. Half of the containers was sterilized in autoclavein 121^{o}C (sterile variants), while the second half was used as non-sterile variants. Next, each soil variant was contaminated with 0.6 mL of creosote oil (either type B or C, to obtain ca. 0.5% of creosote concentration in soil) and vigorously mixed. Finally, 10 mL of bacterial suspension (adjusted to OD_{600} = 0.2) was added to the soil variants and vigorously mixed. Eventually, the containers were tightly closed and left for 28 days in 25^{o}C. The studied variants included: 1) clean, sterile soil without addition of microorganisms; 2) clean, non-sterile soil without addition of microorganisms; 3) contaminated, sterile soil without addition of microorganisms (creosote type B); 4) contaminated, non-sterile soil without addition of microorganisms (creosote type B); 5) contaminated, sterile soil without addition of microorganisms (creosote type C); 6) contaminated, non-sterile soil without addition of microorganisms (creosote type C); 7) contaminated, sterile soil with addition of OS4 species (creosote type B); 8) contaminated, non-sterile soil with addition of OS4 species (creosote type B); 9) contaminated, sterile soil with addition of OS4 species (creosote type C); 10) contaminated, non-sterile soil with addition of OS4 species (creosote type C); 11) contaminated, sterile soil with addition of DA1 species (creosote type B); 12) contaminated, non-sterile soil with addition of DA1 species (creosote type B); 13) contaminated, sterile soil with addition of DA1 species (creosote type C); 14) contaminated, non-sterile soil with addition of DA1 species (creosote type C). Each variant was done in triplicates.

After 28 days, 3g of soil from each container belonging to the same variant was taken and mixed (to obtain 9g soil sample in total). The soil samples were then subjected to a 3-stage ultrasound assisted extraction with toluene (15 mL for each stage). The extracts of each extraction stage were then combined and quantitatively analyzedusing high-performance liquid chromatography with FLD detector. The mobile phase was a gradient methanol/water system. The stationary phase was the specialized Thermo Fisher Scientific column dedicated to the determination of PAHs.

RESULTS

The obtained results suggest that biodegradation efficiency of creosote oil is significantly higher in non-sterile soil. No significant biodegradation was observed in steriled soil variants contaminated with creosote oil type C. This may suggest that non-sterile soil is preferred system for the studied microorganisms that enhances their metabolic activity. This is, most likely, caused by the fact, that soil during sterilization is subjected to extreme conditions (121^{o}C) that can damage its chemical structure and decompose the organic matter,

which is especially is important for living organisms. Previous studies showed that sterilization of the soil may release dissolved organic matter (DOC) into soil solution and increase in the soil surface area (Berns et al. 2008). This may, however, influence the sorption of organic xenobiotics in the soil and their bioavailability to microorganisms.

Regardless of sterilization of the soil, the biodegradation of PAHs in creosote oil type B was higher compared to the creosote type C. The maximal biodegradation of sum of PAHs of creosote type C reached 57% in non-sterile variant augmented with DA1 *Pseudomonasputida* strain. On the other hand, the maximal biodegradation of sum of PAHs in creosote type B reached 90% and was observed in non-sterile variant augmented with OS4 *Pseudomonas* sp. strain. The differences in biodegradation rate of sum of PAHs are most likely caused by the differences in chemical composition between the two types of creosote oil. The analysis showed that the concentration of PAHs with higher number of rings (such as benzo(a)pyrene) was higher in creosote type C. This group of compounds is commonly considered as more toxic to microorganisms compared to the PAHs with lower molecular mass.

When comparing both bacterial strains, it can be observed that *Pseudomonas putida* strain DA1 is more efficient in biodegradation of creosote oil type C (in non-sterile soil only) as the biodegradation was three times higher than for OS4 *Pseudomonas* sp. strain. When creosote oil type B is considered, both bacterial strains are efficient PAHs degraders, as in non-sterile soil biodegradation extent exceeded 80%.

In general, both bacterial species seem as promising microbial agents that can be used in bioremediation of the soil contaminated with creosote oil. In this case, however, *Pseudomonas putida* DA1 strain seems more resistant and robust against more toxic PAHs with higher number of aromatic rings.

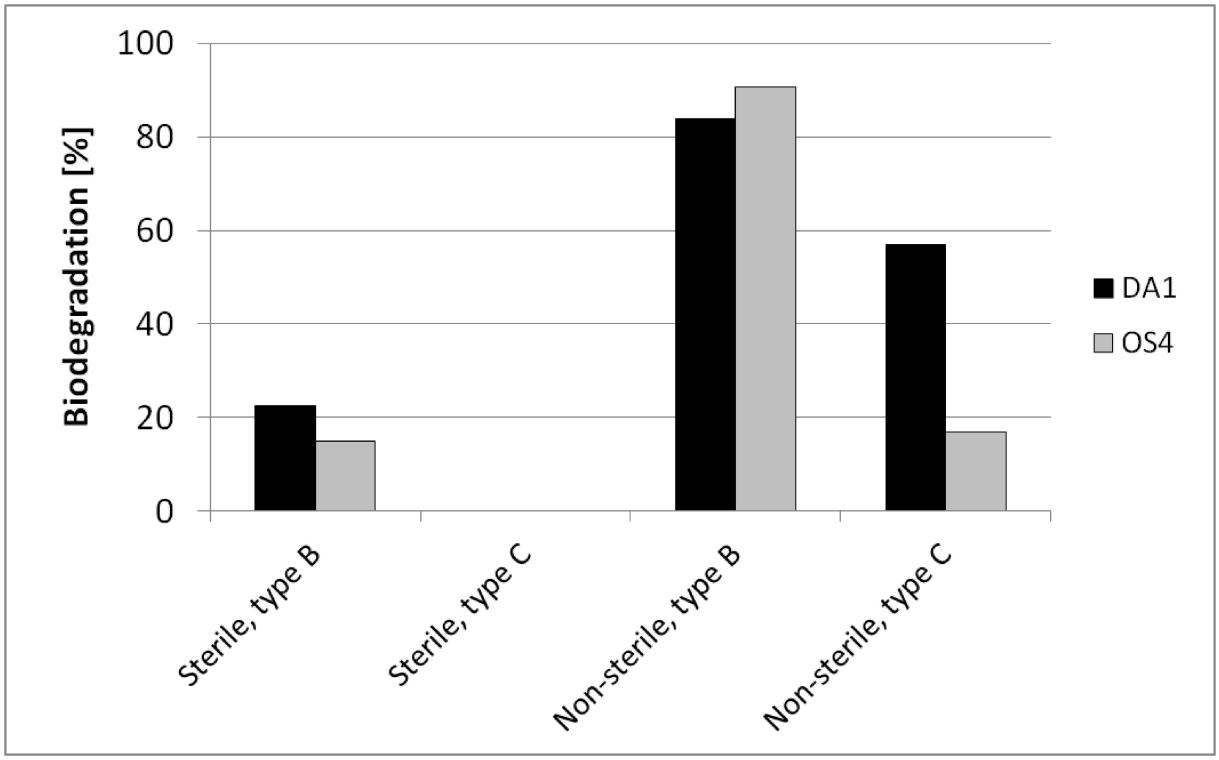

Figure 1. The biodegradation of sum of PAHs after the 28-day test using two types of creosote oil (B and C) and two *Pseudomonas* bacterial strains (DA1 and OS4).

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Streszczenie: *Biodegradacja oleju kreozotowego w glebie przy użyciu wyselekcjonowanych szczepów bakteryjnych*. Olej kreozotowy jest szeroko stosowany do impregnowania podkładów kolejowych, palików farmerskich i słupów teletechnicznych jako środek zabezpieczający drewno przed działaniem grzybów, bakterii, alg i owadów. Ze względu na swoje toksyczne właściwości, głównie spowodowane obecnością w swoim składzie wielopierścieniowych węglowodorów aromatycznych (WWA), może być niebezpieczny dla zwierząt oraz ludzi. Zanieczyszczenie gleby olejem kreozotowym występuje powszechnie zwłaszcza na terenach nasycalni drewna, gdzie zużywane są duże objętości tego środka. W tej pracy określono skuteczność biodegradacji oleju kreozotowego (zarówno typu B, jak i C) w dwóch typach gleb modelowych (sterylnej oraz niesterylnej) zaszczepionych dwoma różnymi szczepami bakteryjnymi (*Pseudomonas putida* DA1 oraz *Pseudomonas* sp. OS4). Efektywność biodegradacji WWA była wyższa w glebie niesterylnej w odniesieniu do oleju kreozotowego typu B zawierającego mniejsze ilości WWA o większej liczbie pierścieni (efektywność biodegradacji przekroczyła 80% w 28-dniowym teście). Pomimo tego, że oba szczepy bakterii były podobnie skuteczne w rozkładzie oleju kreozotowego typu B, *Pseudomonas putida* DA1 był bardziej wydajny w biodegradacji oleju kreozotowego typu C (w niesterylnym wariancie glebowym).

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