Ph.D. Thesis

Abstract

Effectiveness of Techniques Enhancing the Biodegradation of Petroleum Hydrocarbon Contaminants in Soil from a Waste Pit Area

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The management of drilling waste has become one of the major environmental challenges facing the oil and gas industry. For many years, drilling waste was systematically deposited in so-called waste pits, resulting in the long-term contamination of soils in these areas and creating the need for remediation measures. Bioremediation is currently one of the most widely applied methods for the remediation of sites historically contaminated with petroleum hydrocarbons due to its high efficiency, relatively low environmental impact, and comparatively low operating costs. However, it has been observed that conventional bioremediation techniques may exhibit limited effectiveness in heavily degraded soils. Therefore, this doctoral thesis aimed to modify existing bioremediation techniques and develop integrated bioremediation strategies to achieve more thorough remediation of soils from waste pit areas.

This doctoral thesis is based on seven thematically coherent scientific publications presenting various approaches to the modification of existing bioremediation techniques, including both the modification of soil inoculation using a biopreparation developed on the basis of autochthonous bacteria and the application of integrated remediation strategies.

The literature review discusses issues related to environmental contamination with petroleum-derived compounds, the characteristics of petroleum hydrocarbons, and methods for the remediation of soils contaminated with these substances, with particular emphasis on biodegradation and techniques supporting the biodegradation of petroleum contaminants. Particular attention was paid to the specific characteristics of soils originating from waste pit areas, which constituted the research material used in the studies included in this doctoral thesis.

The experimental section presents the research material and methodologies used to evaluate the effectiveness of the proposed modifications to the bioremediation of waste pit soils and provides a detailed discussion of the results presented in Articles I–VII, which constitute the basis of this doctoral thesis. An important aspect of this work is the comprehensive nature of the research presented in the included scientific publications, encompassing respirometric, chromatographic, microbiological, and toxicological analyses. This multidisciplinary approach enabled a comprehensive assessment of the effectiveness of the proposed modifications to bioremediation processes and demonstrated the potential applicability of the obtained results under future industrial conditions.

The individual articles included in this doctoral thesis present novel bioaugmentation-assisted bioremediation approaches aimed at improving the biodegradation efficiency of petroleum-derived contaminants in waste pit soils. Article I presents a modification of the biopreparation inoculation technique based on autochthonous bacteria through the application of γ-PGA. The results of this study were published in the journal *Materials* (Wojtowicz et al., 2022).

The findings reported in Article I provided the basis for the further development of the biopreparation inoculation strategy by enriching it with additional compounds enhancing contaminant bioavailability, such as biosurfactants. Comprehensive laboratory biodegradation studies employing the modified inoculation technique supplemented with biosurfactants enabled the assessment of their effects on the biodegradation of aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) in waste pit soil. The results of these studies were published in the journal *Sustainability* (Wojtowicz et al., 2025).

The next stage of the research involved modifying the biopreparation based on

autochthonous bacteria by incorporating selected strains of autochthonous fungi. In addition, phytoremediation using the crop plant *Zea mays* and the ornamental plant *Echinacea purpurea* was introduced to achieve more thorough remediation of waste pit soils. Based on the results obtained in Articles I and II, the possibility of combining bioaugmentation, phytoremediation, and γ-PGA was also investigated, demonstrating the superiority of integrated approaches over conventional methods in enhancing the remediation efficiency of soils historically contaminated with petroleum hydrocarbons. The results of these studies were published in the journals *Molecules* (Wojtowicz et al., 2023b) and *Applied Sciences* (Wojtowicz et al., 2023a).

Article V further developed the concept of bioaugmentation-assisted phytoremediation by employing wild plant species, namely *Scirpus sylvaticus* and *Cirsium oleraceum*, collected from crude oil-contaminated areas located in the vicinity of the waste pit from which the soil samples were obtained. The use of wild vegetation exhibiting high tolerance to elevated concentrations of petroleum-derived contaminants significantly improved the efficiency of waste pit soil remediation. Furthermore, the bacterial and ciliate communities present in the soil after completion of the experiment were characterised, thereby extending the scope of the investigations beyond those presented in the remaining articles. The results of these studies were published in the journal *International Biodeterioration & Biodegradation* (Wojtowicz et al., 2026a).

Article VI proposed a novel approach to enhancing the biodegradation efficiency of petroleum-derived contaminants by combining bioaugmentation with soil amendment using selected nanomaterials. Laboratory-scale biodegradation experiments demonstrated the high effectiveness of this modification of the bioremediation process applied to waste pit soil. Consequently, the concept was further developed by incorporating phytoremediation using *Lolium perenne*, and the results of these studies were published in the journal *Molecules* (Wojtowicz et al., 2026b).

The final article included in this doctoral thesis investigated the influence of co-occurring contaminants, such as polychlorinated biphenyls (PCBs), on the biodegradation efficiency of petroleum-derived contaminants in waste pit soil. The study demonstrated the effectiveness of biopreparations developed on the basis of several bacterial strains with different metabolic capabilities in improving the remediation efficiency of soils contaminated with multiple classes of pollutants. The results of this research were published in the journal *Molecules* (Steliga et al., 2020).

The comprehensive laboratory-scale and pilot-scale investigations of the proposed modifications to bioremediation processes presented in Articles I–VII enabled the successful achievement of all objectives of this doctoral thesis and the formulation of its principal conclusions. The results demonstrated that the proposed modifications of existing bioremediation techniques, together with the application of integrated remediation strategies, significantly enhanced the efficiency of the bioremediation of waste pit soils and exhibit considerable potential for future application under field conditions.

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