Biodegradation of diesel D2 by indigenous *Rhodococcus sp*. recovered from petroleum contaminated soil

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INTRODUCTION

Diesel has traditionally served as a primary fuel source for transportation and small-scale energy generation, particularly for backup or emergency power purposes (de Witt et al., 2021). Despite the global trend towards reducing fossil fuel use, petroleum derivatives can cause long-lasting environmental contamination (Marigómez, 2014). As a result, there is an ongoing need for innovative technologies to address contaminated sites (Ossai et al., 2020).

Seeking new bacterial strains for the biodegradation of diesel fuel is of high importance due to the environmental pollution and health hazards associated with diesel spills and leaks. These bacteria possess the ability to metabolize and detoxify hydrocarbons, converting them into less harmful substances, thereby accelerating the cleanup process and diminishing the ecological footprint. Furthermore, exploring diverse microbial ecosystems may reveal singular metabolic pathways that can be harnessed for industrial applications, promoting sustainable environmental practices and driving advancements in biotechnology.

This paper presents a GC/MS study on the biodegradation of hydrocarbon fractions in diesel gasoil (D2), utilizing an indigenous *Rhodococcus* species isolated from petroleum-contaminated waste soil. GC-MS analysis was employed to determine the abundance of diesel D2 fuel during 30 day of biodegradation by *Rhodococcus* sp.

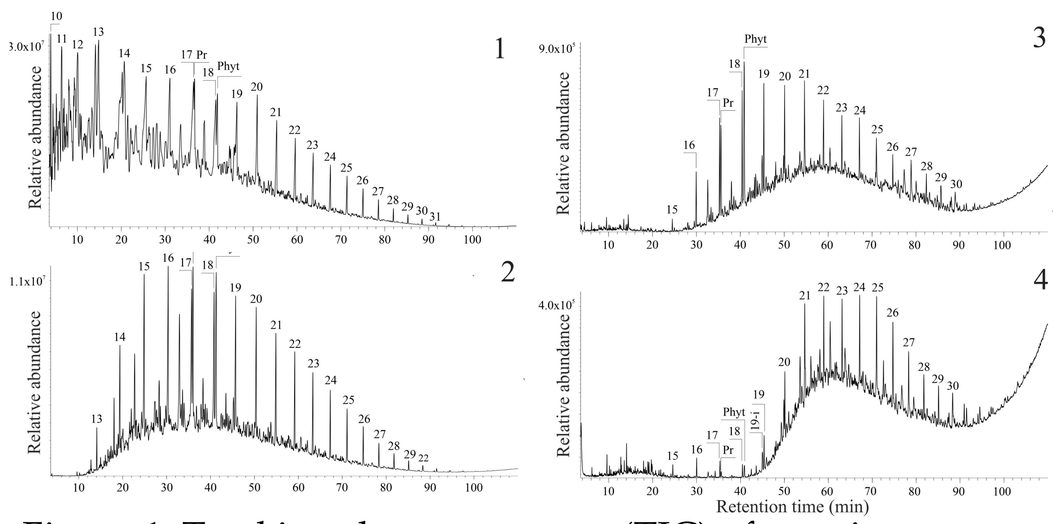

Figure 1: Total ion chromatograms (TIC) of: starting diesel oil sample 1); of diesel oil samples after degradation by *Rhodococcus* sp. RNP05 after 10, 20 and 30 days 2), 3) and 4), respectably

MATERIALS & METHODS

Bacterial strain has been isolated from waste soil heavily contaminated by petroleum (total petroleum hydrocarbon 29.99%) and its derivates taken from Pančevo Oil Rafinery (Serbia). The genomic DNA of bacteria has been amplified, and taxonomic analysis was conducted by GenBank BLAST program, 16S rRNA gene sequences of analyzed strain were deposited in NCBI GenBank under accession number JQ065876 (*Rhodococcus sp*. RNP05). The biodegradation substrate used in this study was a Diesel Gasoil (D2), which was a conventional diesel fuel oil with a specification according to the EN 590:2004. Biodegradation of diesel D2 by *Rhodococcus sp.* RNP05 was tested in laboratory conditions, in Erlenmeyer flasks with microbiological medium and diesel D2 (2000 ppm) as only source of carbons. Erlenmeyer flasks were incubated for 30 days at 28oC and 200 rpm. Dynamics of diesel fuel biodegradation was monitored by gas chromatographic-mass spectrometric (GC-MS) analysis of diesel D2.

RESULTS & DISSCUSION

n-Alkanes lower than C_{14} were completely removed in the first 20 days of the experiment. The effect of biodegradation was visible by the reduced intensity of the peaks of *n*-alkanes, with an increase in the relative content of isoprenoid biomarkers, pristane, and phytane. TIC chromatograms indicated a significant reduction in *n*-alkanes and isoprenoids over 30 days by both bacterial strains used in this study. At the end of the experiment with *Rhodococcus sp*. RNP05 a complete absence of the peaks of *n*-alkanes and isoprenoids lower than C_{14} was noticeable in the chromatogram (Figure 1.4), indicating their possible complete degradation. Furthermore, the baseline in the chromatogram in the area between *n*-alkanes C_{17} - C_{30} was elevated and deformed. This transformation of the chromatogram base line during biodegradation of crude oils and their derivatives is called unresolved complex mixture (UCM) or "hump", and it is indicative of severe biodegradation of *n*-alkanes with concomitant accumulation of less biodegradable cyclic and highly branched hydrocarbons.

CONCLUSIONS

These results highlight the potential application value of these microbial strains in the degradation diesel fuel compounds in environmental oil pollution cleanup. Based on our results, *Rhodococcus* sp. RNP05 isolated from waste soil has demonstrated a high capacity for biodegrading diesel fuel. These findings suggest that *Rhodococcus* sp. RNP05 could play a significant role in mitigating diesel pollution, offering a sustainable solution for environmental restoration. Further research and field trials will be essential to optimize its application in large-scale bioremediation projects.

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