Use of Biosurfactants in Bioremediation of Petroleum-Contaminated Soil

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Abstract

The objective of this research was to use biosurfactant-producing bacteria in bioremediation of oil-contaminated soil. Two approaches were studied. Biostimulation with the addition of crude biosurfactants extracted from *Rhodococcus fascians,* strain already isolated from hydrocarbon contaminated soils, and bioaugmentation with the addition of bacterial cells suspensions of the same strain. The bacterial quantification results revealed significant microbial abundance in both treatments. The results of soil hydrocarbon analysis revealed an efficiency of hydrocarbons elimination in both treatments (>30%). Metals analysis showed significant results of metals removal using both treatments (>75% for chromium). The ecotoxicity test showed an important decrease of oil-contaminated soil toxicity using the two treatments (>55%). Both approaches used showed an efficient elimination of hydrocarbons and metals and represented an environmentally friendly technology.

Keywords

Biosurfactants • Bioremediation • Hydrocarbons • Metals • Petroleum • Bacterial cells

1 Introduction

The decontamination of polluted soils has become an urgent need today, especially in the presence of petroleum activities, such as oil extraction, transporting, storage processing in oil landfill, which lead to environmental pollution risks (Belhouchet et al. 2019; Ben Said et al. 2019). Bioremediation applications were known as a cost-effective clean-up technology to treat petroleum-polluted soils and sediments (Ebadi et al. 2017). In recent years, researchers have focused on biosurfactants application in bioremediation as ecological alternative technologies for the removal of these contaminants (Pradeep et al. 2012). Biosurfactants are amphiphilic molecules produced by microorganisms to help them to use hydrocarbons as carbon source, either by making the hydrocarbon accessible by releasing biosurfactant into the environment or by changing its cell surface so that the contaminant can be absorbed (Ben Said et al. 2015). Moreover, they are ecofriendly biomolecules, due to their unique properties like low toxicity and emulsification (Pradeep et al. 2012). Our purpose was to use biosurfactant-producing bacteria in bioremediation of hydrocarbons contaminated-soils of the STIR refining company using two approaches, biostimulation with crude biosurfactant of *Rhodococcus fascians* and bioaugmentation with addition of bacterial cells of the same strain.

2 Materials and Methods

2.1 Biosurfactant-Producing Bacterial Strain

Rhodococcus fascians strain used in this study was obtained from the strain collection of our Environment Biomonitoring Laboratory LBE LR 01 ES 14. The bacterial strain is already isolated from oil-contaminated soil of STIR refinery. *Rhodococcus fascians* selection was based on its ability to degrade hydrocarbons and resist to metals as well as its biosurfactant production potential.

2.2 Bioremediation Trials

The studied soil was sampled in oil landfills of STIR refining company. Three types of mesocosms were set up in

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triplicates, one control and two treatments. Each mesocosm contained 400 g of soil. All mesocosms were incubated in room temperature for 41 days (Ben Said et al. 2015). During the experimental period, the soil was mixed weekly until the end, and the soil water evaporation was adjusted by the addition of sterile distilled water. The hydrocarbons contaminated soil was bioaugmented in the first test with 4% of bacterial cells and biostimulated in the second test with 4% of biosurfactants (Pradeep et al. 2012).

2.2.1 Quantification of Oil-Degrading Bacteria

Microorganisms counting were estimated using the “Most Probable Number” (MPN) method (Quadros et al. 2016). The growth medium was mineral salt medium (MSM) supplemented with 0.2 μL of crude oil and 0.1 μL aliquot resazurin was added to each tube. A serial dilution was performed from a suspension of 1 g of soil in 9 mL physiological water, and each tube was inoculated with 10^{-3}–10^{-5} serial dilutions. The tubes were incubated at 30 °C for 7 days (Ebadi et al. 2017).

2.2.2 Monitoring of Total Petroleum Hydrocarbons (TPH) and Metal Analyses

The TPHs analysis was performed, after their extraction, by Gas Chromatography coupled to a Flame Ionization Detector (GC-FID Model no Agilent 78908B) (Pradeep et al. 2012). The metal analysis was performed, after calcination and extraction, by Inductively Coupled Plasma—Atomic Emission Spectroscopy (ICP-AES Model no iCAP 6300) (Ben Said et al. 2019).

2.2.3 Germination Test

Soil phytotoxicity was assessed using a corn seeds germination test (*Zea mays* L.). Seeds were placed in sterile Petri dishes containing oil-contaminated soil and then placed in an incubator at 25 ± 1 °C in dark, for 7 days. The germination rate (G) was presented as follows (Ebadi et al. 2017).

$$G\% = \frac{\text{number of sprouted seeds}}{\text{total number of seeds sown}} \times 100$$

3 Results

3.1 Abundance of Oil-Degrading Bacteria in Oil-Contaminated Soil

After 41 days of incubation, the results revealed a significant abundance of oil-degrading bacteria in both treatments. Thus, for the treatment with bacterial suspensions, the abundance reached was from $1.6 \times 10^3 \pm 0.2 \times 10^3$ bacteria/g of soil up to $4.8 \times 10^5 \pm 0$ bacteria/g of soil. In the case of addition of biosurfactants, the abundance reached from $1.6 \times 10^3 \pm 0.2 \times 10^3$ bacteria/g of soil up to $7.2 \times 10^5 \pm 3.6 \times 10^5$ bacteria/g of soil (Fig. 1).

3.2 Total Petroleum Hydrocarbons TPHs Biodegradation in Oil-Contaminated Soil

The treatment with bacterial suspensions of *R. fascians* showed a TPH degradation percentage of 32.25 ± 9.15%. While the treatment with biosurfactants indicated 37.38 ± 58.52%.

3.3 Metal Removal in Oil-Contaminated Soil

Both treatments showed a high percentage of metal removal (>70%) from oil-contaminated soil mainly for copper (87.14%) and chromium (86.08%) using bacterial cells and nickel (75.71%) and zinc (73.42%) using crude biosurfactants.

3.4 Germination Test

The treatment using crude biosurfactant favored the germination of about 60 ± 10% of the seeds, while the germination percentage recorded in the treatment using bacterial suspensions was about 55 ± 10%.

4 Discussion

After 41 days of mesocosms incubation, we observed a significant increase in abundance of oil-degrading bacteria in both used treatments. The bacterial abundance was higher in the bioaugmentation treatment than in untreated contaminated soil (control), indicating that the bioaugmentation increased the initial bacterial abundance presented in the oil-contaminated soil. The TPH analysis revealed an

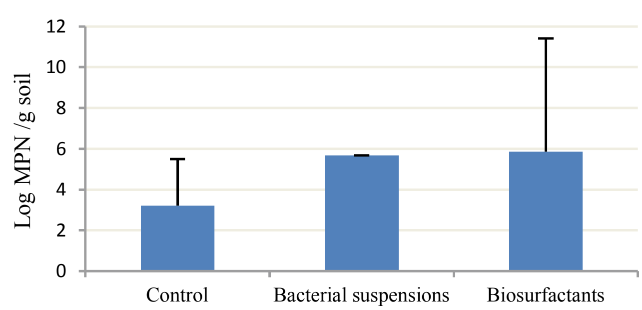

Fig. 1 Abundance of oil-degrading bacteria in oil-contaminated soil with different treatments

efficiency of TPHs elimination in the two treatments. Many studies reported similar results of TPH removal from the soil (Quadros et al. 2016). Both treatments, effectively, reduced metal contents in soil. The percentage of reduction, using bacterial suspensions cells of *R. fascians* strains, reached more than 80%, mainly for copper and chromium. Also using biosurfactants, the percentage of reduction exceeded 70% for nickel and zinc. In a previous study, Ben Said et al. (2019) reported similar results of reducing metals in bioremediation treatment of oil-contaminated soil. The germination test showed an important decrease of oil-contaminated soil toxicity using the two treatments. Both treatments improve soil quality by supporting plant growth.

5 Conclusion

The two approaches used for bioremediation treatment in oil-contaminated soil showed a significant effect on increasing the abundance of oil-degrading bacteria, the decreasing of TPH and metal content and the improvement of soil quality. This biological treatment represents an environmentally friendly technology. Further studies will be needed to improve the efficiency of the bioremediation techniques as well as to investigate the potential of biosurfactants with other approaches.

References

Belhouchet, N., Hamdi, B., Chenchouni, H., Bessekhouad, Y.: Photocatalytic degradation of tetracycline antibiotic using new calcite/titania nanocomposites. J. Photochem. Photobiol. A: Chem **372**, 196–205 (2019). https://doi.org/10.1016/j.jphotochem.2018.12.016

Ben Said, O., Louati, H., Soltani, A., Preud'homme, H., Cravo-Laureau, C., Got, P., Prin-gault, O., Aissa, P., Duran, R.: Changes of benthic bacteria and meiofauna assemblages during bio-treatments of anthracene-contaminated sediments from Bizerta lagoon (Tunisia). Environ. Sci. Pollut. Res. **22**(20), 15319–15331 (2015). https://doi.org/10.1007/s11356-015-4105-7

Ben Said, O., Belgacem, R., Ben Gaffar, B., Beyrem, H., Kahn, J.R.: Remediation treatments and economic assessment of oil residual sludge from the bottom of tunisian refinery crude oil storage tanks. In: Recent Advances in Geo-Environmental Engineering, Geomechanics and Geotechnics, and Geohazards, pp. 81–83. Springer, Cham (2019). https://doi.org/10.1007/978-3-030-01665-4_19

De Quadros, P.D., Cerqueira, V.S., Cazarolli, J.C., Maria do Carmo, R. P., Camargo, F.A., Giongo, A., Bento, F.M.: Oily sludge stimulates microbial activity and changes microbial structure in a landfarming soil. Int. Biodeterioration Biodegradation **115**, 90–101 (2016). https://doi.org/10.1016/j.ibiod.2016.07.018

Ebadi, A., Sima, N.A.K., Olamaee, M., Hashemi, M., Nasrabadi, R.G.: Effective biore-mediation of a petroleum-polluted saline soil by a surfactant-producing *Pseudomonas aeruginosa* consortium. J. Adv. Res. **8**(6), 627–633 (2017)

Pradeep, N.V., Anupama, S., Anitha, G., Renukamma, A.S., Afreen, S. S.: Bio-remediation of oil contaminated soil using biosurfactant produced by *Pseudomonas aeruginosa*. J. Res. Biol. **2**(4), 281–286 (2012)