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Development of a bioremediation test model strategy for oil recovery in contaminated soil:
case study of an Immobilized Mixt Bacterial Consortium

By

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Abstract

Oil is still up to date the prime source of energy for anthropogenic activities and will remain in the near future. Events of oil release in the different terrestrial ecosystems have led to the development of different practices to mitigate the toxic impacts of petroleum hydrocarbons on the environment and human health. At the forefront of remediation approaches, bioremediation exploiting the natural abilities of microorganisms to metabolize pollutants has emerged as an effective route for a sustainable response to oil contamination. Specifically regarding soil contamination, bioaugmentation has been developed as an *in-situ* remediation technique allowing the improvement of the autochthonous microbial community activity via the introduction of known hydrocarbons degraders. Especially, mixtures of microorganisms offer greater advantages for hydrocarbons degradation compared to single strains owing to the presence of a large number of diverse metabolic functionalities. However, previous studies are not unanimous on the validity of using this approach due to inconsistent results. Indeed, most of the studies in literature report effective bioaugmentation when applying indigenous microorganisms. However, attempts to apply cross bioaugmentation using microorganisms from remote to foreign regions resulted in inconsistent to no improvement of oil removal. Even commercial formulations developed to overcome this issue have not taken up the challenge. Hence, there is still a gap in the application of a microbial formulation that would be effective in different foreign locations for oil recovery. In this context, the dissertation addresses the topic oil recovery from the environment by means of microbial consortia declined as associations of bacteria. Dual goals can be defined. First of all, the study sought to establish a methodological framework allowing to implement a bioaugmentation strategy including strains with different geographical origins for the reclamation of oil contaminated soils. This framework included the definition of inclusive and exclusive criteria for the selection of bacterial strains as well as monitoring tools to assess the efficiency of the applied approach. Then, the study aimed to develop mixt bacterial consortia blending indigenous and exogenous bacteria to enable the implementation of a test model bioaugmentation targeting the reclamation of a freshly oil contaminated soil based on the developed framework. The limitations linked to the use of exogenous bacteria have been anticipated by the adoption of immobilization technique which consisted in the entrapment of the bacterial consortia on a biodegradable support. The aim was to provide a protective niche ensuring long lasting survival and activity of the immobilized strains. The expected outcome was an enhanced oil recovery by the entrapped bacterial consortia. The entrapped consortium was also lyophilized. As a result, the research activities concluded with the successful mesocosm scale application of the bacterial consortium COBIONA which included strains originating from Equatorial and Mediterranean climates, for the reclamation of a freshly contaminated soil characterized by typical aliphatic hydrocarbons belonging to diesel. The consortium was entrapped in alginate beads and strategically aided by the addition of calcium peroxide as oxygen supplier in the soil. To meet the selective chosen criteria, the bacterial strains employed in the bioaugmentation strategy exhibited biosurfactants production and/or the presence of the *alkB* gene. Biosurfactants are surface active agents from biological origin that reduce the surface tension at the interface between immiscible liquids. This property is used in bioremediation due to the formation of tiny micelles facilitating the uptake of hydrocarbons molecules by microorganisms, which results in enhanced oil recovery. On the other hand, *alkB* is a gene involved in the primary step of alkanes degradation. The overall research

activities concluded by the successful application of COBIONA consortium comprising bacterial strains originating Equatorial and Mediterranean climates for the reclamation of a freshly oil contaminated soil. Metagenomics analysis revealed that the entrapped consortium aided by calcium peroxide induced an increase of the bacterial community holding the *alkB* gene, concomitantly to observed enhanced hydrocarbons degradation. The community was dominated by Actinomycetales, and its size increased from 2.63×10^{9} /g to 1.77×10^{10} and 1.05×10^{11} /g respectively for the fresh and lyophilized alginate beads after 3 months. Similarly, the *alkB* gene concentration increased in the two treatments from 1.30×10^{7} to 2.60×10^{7} and 2.23×10^{7} copies of the gene per gram of soil respectively for the fresh and lyophilized entrapped consortium. These changes resulted in 44% total petroleum depletion by the fresh entrapped consortium, which represented 10% higher degradation compared to natural attenuation. Although the lyophilized treatment performed similarly to the latter with 34% hydrocarbons depletion, lyophilization was found to be more advantageous on the long run. Overall, these results showed the efficiency of the strategy employed. Especially COBIONA consortium containing *Achromobacter*, *Pseudomonas, Rhodococcus* and *Shinella* species, was effective in oil recovery within the developed framework. As a whole, the data presented in this dissertation pave the way for the large application of exogenous bioaugmentation. The strategy developed here is flexible enough to be adapted and used in different geographical regions.