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HYDROCARBON BIOREMEDIATION STUDIES IN PORTUGUESE SOIL SAMPLES

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ABSTRACT

Experimental studies were carried out, referring to the verification of the existence and characterization of hydrocarbonoclastic organisms in soil samples obtained in two different areas, one located on the outskirts of the city (Praia da Alburrica), where various anthropocentric activities contribute to the increase in fuel pollutants and the other in a protected wood area, 7 km from the same city, where only leisure activities and environmental education take place (Mata da Machada). Hydrocarbon's biodegradability studies, colonies morphologically and biochemically characterization of the microorganisms were performed. The values of the bioremediation rate of hexane, toluene and gasoline showed higher bioremediation rates when using consortium cultures which highlight functional potential of the soil microbial community.

Keywords: Bioremediation, biodegradability, hydrocarbonoclastic

Background information

Despite all changes that are happening in the world and concerns to decrease the carbon footprint, fossil fuels are still the driver of economic growth. The low polarity of these compounds leads to high rates of persistence and ecotoxicity, being the hydrocarbons considered one of the main pollutants of the planet, causing adverse effects on the abiotic and biotic components of the Earth ecosystems. The environment contaminated with these nonpolar pollutants, both aquatic and terrestrial, also naturally contain the organisms capable of resisting their presence and able to metabolize them into harmless or non-toxic substances. Among the different treatment methods that involve the decontamination of environments, technologies based on bioremediation processes are one of the most promising, gaining flow globally due to their cost effective and green approach that are on the path to sustainability (Sarkar *et al.*, 2020). There is a multiplicity of organisms with bioremediation potential, which can metabolize and not suffer harmful effects due to the presence of the contaminating agent in their habitat. These organisms or their enzymes, define the bioremediation types and the technological approach needed to implement the process (Badieyan *et al.*, 2018).

Methods

The experimental studies were carried out to the verification of the existence and characterization of hydrocarbonoclastic organisms in soil samples obtained during the month of October 2020 in Mata da Machada and Praia da Alburrica, municipality of Barreiro according to the sampling procedure (Carter and Gregorich, 2008).

Laboratory studies continued with the determination of the rate of bioremediation of hexane, gasoline, toluene, by single or mixed cultures of selected colonies. The biodegradability determination of hydrocarbons resorted to the use of the redox indicator 2,6- dichlorophenolindophenol through the spectrophotometric method using cell suspension and using two different concentrations of each one of the hydrocarbons (adding 100 ml 0.2% (v/v) and adding 600 ml 1.0% (v/v)).

Colonies were morphologically and biochemically characterized by direct observation and performing the Gram Test. The identification of *Pseudomonas aeruginosa* was done using a selective medium Pseudomonas CN Agar Base (Dehydrated Culture Media) for microbiology - ITW Reagents, 2020).

Main results

Cell growth was monitored based on the growth curve using spectrophotometric analysis of the optical density at 590 nm, during 30 hours at batch culture. Initial growth studies were performed using the microorganism's population, obtained from soil samples collect at Mata da Machada and at Praia da Alburrica, in Bushnell-Haas (BH) medium plus hydrocarbons as a carbon source (figure 1).

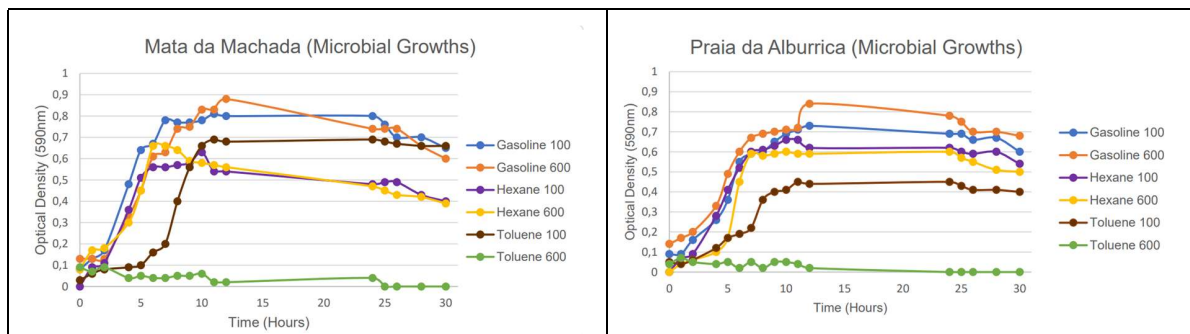


Figure 1: Microbial growths observed in the samples obtained at Mata da Machada and at Praia da Alburrica with two different concentrations of gasoline, hexane and toluene. (Note: 100 refers the 0.2 % v/v and 600 to 1.0 % v/v).

In both analysed locals, toluene, a polyaromatic hydrocarbon, was the hydrocarbon that most limited the growth of microorganisms, especially at a concentration of 1.0 %.

Regarding the characterization of the colonised microorganisms, it was possible to identify 7 distinct colonies in the Mata da Machada sample, (one Gram -, two Gram + and four containing Gram + and Gram - strains) and five different colonies in the Praia da Alburrica (two Gram + and three with Gram + and Gram - strains). The growth on selective medium of *Pseudomonas* CN Agar Base allowed the identification of *Pseudomonas aeruginosa* in both studied locals.

The biodegradability of the hydrocarbons was achieved after five days; however, and the values obtained were similar regardless of the place of origin. With hexane, it was obtained an average bioremediation value of 26%, without showing a significant increase when using the culture of the total microorganisms existing. With gasoline, the average value was 35%, and increased to 41% in the mixture of microorganisms, and with toluene, the bioremediation rate was significantly lower, only 8% having increased to 15% with the microorganism mixture.

Conclusions

The results showed that in the Mata da Machada soil sample, despite being a protected area and a natural reserve, there are hydrocarbonoclastic bacteria, including *Pseudomonas aeruginosa*, which manage to degrade the studied hydrocarbons. In the samples from Praia da Alburrica, being a river area, the existence of these bacteria that manage to degrade hydrocarbons was more likely, and this development occurred through the use of the studied hydrocarbons. The values of the bioremediation showed that gasoline a complex hydrocarbon was the one with higher bioremediation rate. The increased of bioremediation when using the sample of microorganisms selected from the soil, seems to indicate that this process is more effective using mixed cultures and may reveal positive interactions between microorganisms.

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