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STUDY OF A BACTERIAL DEFINED CONSORTIUM ADAPTED TO LOW PHENANTHRENE BIOAVAILABILITY

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A general problem of bioremediation of polycyclic aromatic hydrocarbons (PAH) contaminated soil is that the degradation appears rather slow, due to the propensity of these nonpolar contaminants to adsorb strongly to organic matter.

The establishment of microniches of PAH degrading bacteria in an aged PAH contaminated soil represents a process strongly influenced by the manner in which the PAH are exposure in the soil and bacterial capability to develop physiological strategies to adapt to the PAH bioavailability. Due to the reduced bioavailability, the bioaugmentation strategy with cultures obtained by enrichment in solid phase systems, could be a promising strategy to apply in aged contaminated soils.

Applying an enrichment method with the phenanthrene provided in a sorbed state, a degrading consortia was previously obtained. The predominant strains from the consortia were isolated and identified as belonging to *Sphingobium, Acidovorax*, *Rhodococcus* and *Arthrobacter* genera (strains C1, C2, C3 and C4, respectively) suggesting that the strains could develop different strategies to integrate the degrader consortium.

Because the successful of survival and establishment of a consortium in soil is probably higher than those obtained with the inoculation of only one strain, we focussed our study in understanding how a defined consortium built by these four strains could be more efficient in reducing sorbed phenanthene than pure cultures.

The Amberlite® XAD-2 preloaded with phenanthrene (10.8mg of phenanthrene g-1 XAD2) was used as solid model in batch system with mineral medium (10% p/v). Each strain and the defined consortium were inoculated in the solid model in batch system with phenanthrene as only carbon source. Systems without phenanthere were inoculated to evaluate the inespecific adsortion to the resin beads.

The biomass developed on the beads was monitoring by R2 agar count and FTIR spectroscopy (evaluating the increase in Amide I band at 1650cm^{-1}) after 10 and 30 days of incubation at 28C.

Only the *Arthrobacter sp.* C4 strain growing as pure culture exceeded the 10^6 ufc/g of XAD2-Phen after 10d. Both by plate count and FTIR we could determine that, C1 and C4 biomass obtained in pure cultures were more abundant on XAD2 beads than XAD2-Phen, suggesting the strains could degrade the resin.

When the four strains were inoculated together in XAD2-Phen, each strain rose to 10^7 CFU/g and remained this value after 30d. Both the high amount of carbohydrates observed for *Acidovorax* sp. C2 strain (1200-900cm^{-1} and 3500cm^{-1}) and the significant production of lipids for *Rhodococcus sp.* C3 strain (3000-2800 cm^{-1}) could be detected in the defined consortium. These results demonstrated that at least these two strains could establish and express their phenotypes during the consortium development.

Both FTIR spectroscopy and HPLC techniques allow the detection of phenanthrene by products accumulation along the pure cultures, which were reduced in the consortium.