Cooperation of bacteria and fungus on polycyclic aromatic hydrocarbons degradation

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Abstract
Polycyclic aromatic hydrocarbons are diverse family of hydrophobic organic pollutants. They can be removed from environment by bacterial degradation. One of the process which could limit biodegradation is transfer of hydrophobic contaminants in to aqueous phase were degrading microorganisms live. In this work we would like to evaluate possible role of fungi in increasing transport of hydrophobic pollutants such as polycyclic aromatic hydrocarbons in to the aqueous phase. Increased transport should result in increased degradation of contaminants in our case polycyclic aromatic hydrocarbons.
Keywords: bacterial degradation, mycelia, transport, PAH

1. Introduction

Degradation of hydrophobic contaminants is limited by theirs bioavailability. Mycelial microorganisms can increase degradation of hydrophobic contaminants by two ways. First is active transport of contaminants to active degraders and second is possibility for degrading microorganism to use hyphal net as a transporting system.

Active transport is possible because mycelial microorganisms have developed highly polarized internal cellular organization with interconnected vacuolar organelles which could be used as a intercellular pathways for active and diffusive transport (Furuno et al. 2012). This network could be used for nutrients, water or pollutants transportation.

Degrading bacteria could use hyphal network for enhancing their mobility. For this, can be used liquid film which is situated around hydrophilic fungal hyphae (Furuno et al. 2010).

Next to this transporting functions mycelial microorganisms can also degrade polycyclic aromatic hydrocarbons (PAHs) by deferent degradation pathways than bacteria (Cerniglia 1997).

This work is concerned on microbial degradation of mixture of PAHs. Degradation ability of selected bacteria and fungus was determined. Finally degradation potential of consortia consisting of bacteria and fungi was determined. PAHs were in mineral oil to simulate conditions of low availability of PAHs in two phase system. Assumption was that the presence of filamentous fungus could lead to an increase in degradation.

2. Materials and Methods

Polycyclic aromatic hydrocarbons were dissolved in sterile mineral oil in final concentration 2 mM from each. Microbial strain 20a3 (*Comamonas testoteroni*) previously isolated from PAH contaminated sediment (Wald et al. 2015) was cultivated in minimal media ($(NH_4)_2SO_4$ 1 g/l; KH_2PO_4 2.7 g/l; Na_2HPO_4 4,34 g/l; $Ca(NO_3)_2$ 0,03 g/l; $FeSO_4$ 0,01 g/l a $MgSO_4$ 0,2 g/l) with phenanthrene three days at 28°C and 130 RPM. After three days culture was filtered and centrifuged, two times washed with fresh media and dissolved in minimal media. *Aspergillus* sp. was cultivated on petri dishes with potato dextrose agar after 7 days of cultivation spores were harvested in to minimal media. Degradation mixtures were prepared in triplicates in 12 ml glass bottles with screw cup with PTFE septum, volume of degradation mixture was 4 ml, final OD_{600} of bacteria was 0,4 and OD_{600} of *Aspergillus* sp. spores was 0,01 if used. For degradation test minimal media with 10 ml of Luria-Bertani broth in 1 liter was used. Finally 60 µl of mineral oil with PAH mixture were added. Mixtures were static cultivated at 24°C 35 days. Concentration of PAH were determined after 7, 14, 21, 28 and 35 days. Samples were extracted by 2 ml of diethyl ether and concentration of PAHs was measured by HPLC with DAD.

3. Results

In the beginning was proved that bacteria and fungus are able to grow as a consortium. Than degradation ability of bacteria (fig. 1) and fungi (fig. 2) alone was determined on PAH mixture.

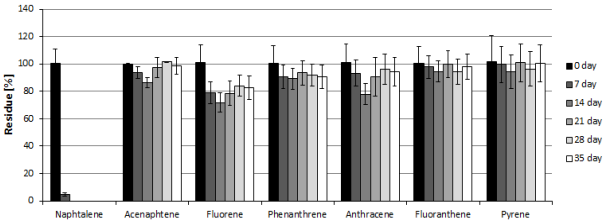

Figure 1. Degradation of PAH mixture by strain 20a3 (*Comamonas testoteroni*)

Both microorganisms were able to degrade naphthalene from the mixture within first two weeks. Next to naphthalene bacteria also degraded fluorene. Fungus degraded next to naphthalene acenaphtene and anthracene from PAHs mixture.

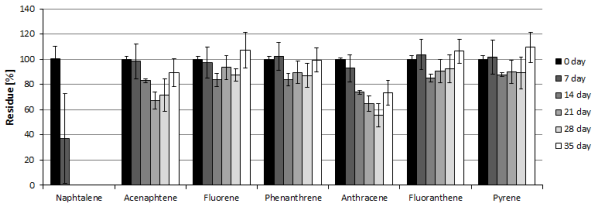

Figure 2. Degradation of PAH mixture by *Aspergillus* sp.

Degradation test done with consortium of bacteria and fungus (fig. 3) showed faster degradation of naphthalene. Acenaphtene and anthracene were also degraded if both organisms were present.

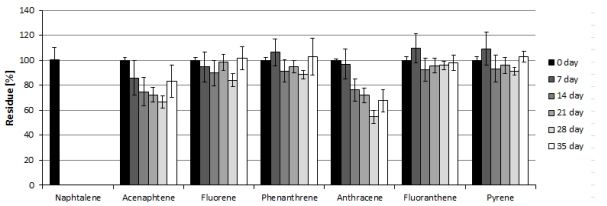

Figure 3. Degradation of PAH mixture by consortium of strain 20a3 (*Comamonas testoteroni*) and *Aspergillus* sp.

Beneficial impact of fungus on bacterial degradation is not visible from data obtained from degradation experiment with consortium. This could be due to high concentration of PAHs in oil phase or the over-complex PAHs mixture used. The results could be different if either lower initial concentration of PAHs mixture or mixture consisting; for example, only from three PAHs was used.

4. Conclusions

The increased PAHs degradation observed when a consortium was used is not conclusive enough to confirm assumption that the presence of filamentous fungus is beneficial for PAHs degrading bacteria.

Acknowledgement

Authors would like to thank FV 20666 for financial support.

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