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Polycyclic aromatic hydrocarbons (PAHs) biodegradation in the soil of a former gasworks site: selection and study of PAHs-degrading microorganisms

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Abstract

Natural PAHs biodegradation was shown in the soil of a former gasworks site and the influence on biodegradation efficiency of sawdust addition studied. Cultures enriched by serial transfer through a mineral medium containing either fluorene, phenanthrene, anthracene, fluoranthene, or pyrene were used to obtain several bacterial communities. The obtained consortia were able to grow on different individual PAHs as their sole carbon source and consisted of a mixing of gram-negative and gram-positive bacterial strains. The reintroduction of the bacterial communities into sterilized polluted soil microcosms was investigated by following the PAHs and the microbial levels in the soil.

Keywords: Polycyclic aromatic hydrocarbons; Biodegradation; Bacterial communities; Gasworks site

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are molecules of great environmental interest because of their carcinogenic, teratogenic and toxic properties [1,2]. They are ubiquitous products of the combustion of carbon-based materials. Particularly, they are products of the incomplete combustion of fossil fuels and thus, often contaminate the gasworks surroundings [3]. Microbial degradation [4–7] is the best known way to remove PAHs from contaminated environments.

The natural biodegradation of a soil collected from a former gasworks site towards the 16 EPA-targetted PAHs was investigated for a possible biological remediation of the site along with the opportunity to amend the soil with sawdust to enhance biodegradation. Then, bacterial communities that were able to grow on some individual PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene) were selected to study their microbial composition, their substrate versatility and their biodegradation performances towards the 16 EPA-targetted PAHs

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after reintroduction into the original soil. Results on phenanthrene are given as example.

2. Results and discussion

The work has been conducted with a PAHs-contaminated soil from a former gasworks site provided by ICF Kaiser Environnement (Gennevilliers, France).

2.1. Naturally occurring biodegradation

A rapid decrease in total PAHs and non-carcinogenic PAHs contents occurs (Fig. 1) in the first 100 days of incubation, in the presence of 30% (w/w) sawdust or not. It corresponds with an increase in microbial population of the soil until day 50 and a stagnation after. This could then be attributed to the biological activity.

The sawdust addition slightly enhances biodegradation: 29% initial total PAHs remain at day 100 in the presence of sawdust, and 37% in its absence. For non-carcinogenic PAHs, the remaining percentages are 18% and 27%, respectively while 5- and 6-rings PAHs are not or little degraded in the presence of sawdust or not. Sawdust addition does not increase co-metabolism mechanisms for this class of PAHs. It seems to act mainly on soil aeration and thus, on natural microbial activity rather than on microbial population: microbial levels are similar in the presence of sawdust or not.

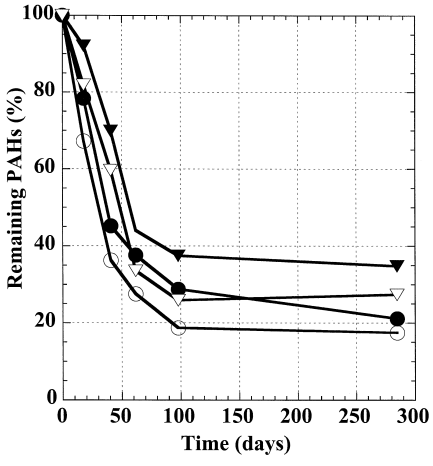

Fig. 1. Percentage of remaining PAHs in the polluted soil. ● Total PAHs, soil added of 30% sawdust (w/w); ▼ Total PAHs, no additive; ○ Non-carcinogenic PAHs, soil added of 30% sawdust (w/w); ▽ Non-carcinogenic PAHs, no additive. Incubation conditions: 25°C, 20% moisture, weekly manual aeration. Soil analysis was conducted as follows: soxhlet extraction (EPA 3540 method), extract purification (EPA 3630 method) and HPLC analysis (EPA 8310 method). Remaining PAHs (%) = (PAHs content at time t)/(initial PAHs content) × 100.

2.2. Selection of PAH-degrading microorganisms

As no significant diminution in the 5- and 6-rings PAHs contents was observed in this soil sample, there was little chance to select PAH-degrading microbial communities (consortia). Consequently, consortia were selected on smaller PAHs by serial transfer through a mineral medium with fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and acenaphtene as individual carbon source using the PAHs polluted soil as primary inoculum.

Selection was effective on fluorene, phenanthrene, anthracene, fluoranthene, and pyrene because an increase in the 600 nm-optical density of the culture medium was measurable (Fig. 2). No growth occurred on acenaphtene, which is not surprising because its content was undetectable in the studied soil, whereas the other PAHs used were present at high levels and biodegradated in the original soil.

The consortia selected on fluorene, phenanthrene, anthracene, fluoranthene, and pyrene are designed by the abbreviations FN, PT, AT, FT, and PY, respectively in the following text.

2.3. Identification

Gram-stains of the selected consortia showed that a broad variety of microorganisms were present and the isolated bacterial strains involved were identified with different identification kits as API20NE (bioMérieux), BBL Crystal E/NF (Becton-Dickinson), Microscan Neg

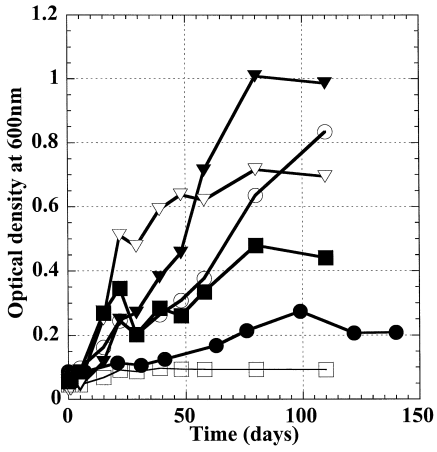

Fig. 2. Selection of microorganisms able to grow on different PAHs as sole carbon source. Growth is followed in terms of increase of the culture medium turbidity by measuring the optical density at 600 nm with a UV-visible spectrophotometer after obtention of cultures by serial transfer through a mineral medium containing ● fluoranthene (FT), ○ phenanthrene (PT), ▼ pyrene (PY), ▽ anthracene (AT), ■ fluorene (FN), □ acenaphtene as sole carbon source. Conditions: 25°C, 150 mg PAH (crystals)/150 ml mineral medium pH = 7.0 in a 500 ml erlenmeyer flask stirred at 110 rpm on a rotary shaker.

Combo 4I (Baxter), Microscan Pos Combo 4I (Baxter). For AT, 8 gram-negative and 1 gram-positive strains were isolated, 8 gram-negative and 1 gram-positive strains for FT, 10 gram-negative and 1 gram-positive strains for PT, 16 gram-negative and 1 gram-positive strains for PY. In the gram-negative strains, the *Pseudomonas* and *Flavobacterium* genera were the most representative, and in each consortium, only one gram-positive strain was isolated. For FT, PT, AT and PY, these gram-positive strains are respectively *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Micrococcus kristinae*, *Staphylococcus capitis*.

Culture of the consortia on Sabouraud-chloramphenicol-gentamicine agar showed no presence of yeasts or fungi in the consortia.

2.4. Substrate versatility

The ability of the selected consortia to use several PAHs (different from the PAH of selection) as sole carbon source was studied. In a natural polluted soil, different PAHs are present and bacterial communities with a wide range of substrates are more interesting to introduce to enhance biodegradation since they act on different PAHs simultaneously.

Table 1 resumes the results of cross-experiments using fluoranthene, phenanthrene, pyrene, anthracene, and fluorene as carbon sources for the different consortia. FN only grew on phenanthrene and fluorene and no changes in UV-visible absorbance spectra on fluoranthene, pyrene or anthracene was observed. FT, PT, PY and AT all grew on fluoranthene, phenanthrene, pyrene, and anthracene, but not on fluorene. On fluorene, culture medium of FT and PT became bright yellow, a reaction already observed in the literature [7], whereas no changes in UV-visible absorbance spectra for PY and AT occurred.

FT and PT have the highest substrate versatility towards the tested PAHs, followed by AT and PY, and at last FN.

Gram-stains of cross-cultures show that for AT, PT, PY, and FT, mainly gram-positive bacteria grow in the presence of fluoranthene, pyrene, and anthracene, whereas only gram-negative bacteria are observable when growth is made on phenanthrene.

2.5. Introduction into the original soil

The microbial population levels in the non-inoculated and inoculated microcosms during

Table 1
Results of growth of consortia FT, PT, PY, AT and FN on individual PAHs as carbon source

	FT	PT	PY	AT	FN	Medium colorization[a]
Fluoranthene	+	+	+	+	−	Brown
Phenanthrene	+	+	+	+	+	Transient pink
Pyrene	+	+	+	+	−	Pink-orange
Anthracene	+	+	+	+	−	Pink-orange
Fluorene	−	−	−	−	+	Yellow (FT, PT)

Conditions: 150 mg PAHs (crystals)/150 ml mineral medium in a 500 ml Erlenmeyer flask stirred at 110 rpm on a rotary shaker, at 25°C.

[a]No changes in medium colorization were observed concerning growth of FN on fluoranthene, anthracene and pyrene, of PY and AT on fluorene.

incubation were measured. In the non-inoculated microcosm, no significant population was found before day 20, but it raises to 150×10^6 UFC/g dried soil at day 50 and finally to 200×10^6 UFC/g at day 80 (Fig. 3): autoclaving has not completely destroyed the indigenous soil microflora, which needs between 20 and 50 days to be able to repopulate the soil, and when this level is high enough, PAHs-biodegradation occurs again as shown for phenanthrene (Fig. 4). The behaviours of the consortia used for inoculation are different in terms of microbial population although inoculation levels were initially similar: FT and PY levels increase to 250×10^6 UFC/g dried soil at day 20, whereas AT increases to 300×10^6 UFC/g dried soil at day 20, and PY increases to 450×10^6 UFC/g dried soil at day 50, and they all decrease to 50×10^6 UFC/g dried soil at day 80 (Fig. 3). Their final microbial population level is lower than in the non-inoculated control certainly because no growth substrate is available at day 80 in FT-, PT-, PY- and AT-inoculated microcosms, whereas there is still enough carbon sources in the non-inoculated control because microbial activity has begun between day 20 and day 50. For phenanthrene (Fig. 4), fluorene and anthracene degradation by FT, PT, PY, and AT is effective and almost complete after 20–25 days incubation, whereas fluoranthene and pyrene degradation is not much enhanced. The observed decrease in phenanthrene in the inoculated microcosms is attributed to the used consortia because of the comparisons between microflora and PAHs levels (Figs. 3 and 4) in the inoculated and non-inoculated microcosms. No degradation of higher molecular weight PAHs by the inoculated consortia was observed during the experiment.

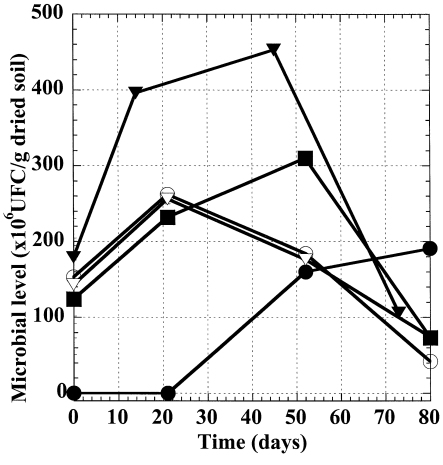

Fig. 3. Microbial evolution after reintroduction of consortia FT, PT, PY, and AT into original PAHs-polluted soil microcosms. ● Non-inoculated, ○ FT, ▼ PT, ▽ PY, ■ AT. Conditions: Original soil autoclaved 3 times at 121°C, 1.5 bar and growth of the consortia overnight on nutrient broth prior to inoculation. Incubation at 25°C, 20% moisture, manual aeration every week.

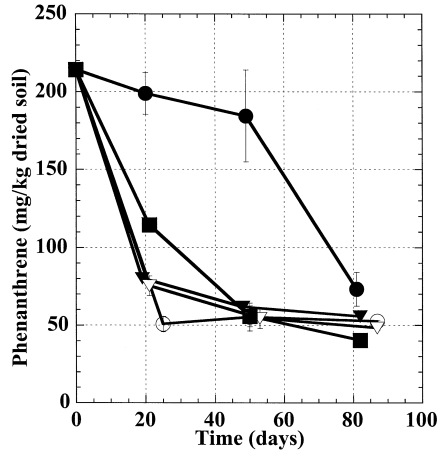

Fig. 4. Phenanthrene degradation after reintroduction of consortia FT, PT, PY, and AT into original PAHs-polluted soil microcosms. ● Non-inoculated, ○ FT, ▼ PT, ▽ PY, ■ AT. Conditions: Original soil autoclaved 3 times at 121°C, 1.5 bar and growth of the consortia overnight on nutrient broth prior to inoculation. Incubation at 25°C, 20% moisture, manual aeration every week.

3. Conclusions

The batch selection of different bacterial communities able to grow on 3-and 4-rings PAHs conducted on the PAHs-contaminated soil from a former gasworks site was successful: one, FN, selected on fluorene is of limited interest due to its high substrate specificity, the four others are much more interesting because they are non-specific. FT, PT, AT, and PY selected respectively on fluoranthene, phenan-

threne, anthracene, and pyrene can effectively use those compounds as growth substrates and furthermore, with FT and PT also acting on fluorene. Reintroduction of these four consortia into microcosms of the original soil after autoclaving shows the ability of FT, PT, PY, and AT to degrade fluorene, phenanthrene and anthracene in a soil matrix. Further study is to be conducted on their biodegradation abilities in a non-sterile environment.

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References

[1] A.W. Andrews, L.H. Thibault, W. Lijinsky, Mutation Res. 51 (1978) 311.
[2] K. Fujikawa, F.L. Fort, K. Samejuna, Y. Sakamoto, Mutation Res. 290 (1993) 175.
[3] Problems arising from the redevelopment of gasworks and similar sites, Environmental Resources, 2nd edn., HMSO, London, 1987.
[4] A.O. Thomas, J.N. Lester, Environ. Technol. 14 (1993) 1.
[5] B. Boldrin, A. Thiem, C. Fritzche, Appl. Environ. Microbiol. 59 (6) (1993) 1927.
[6] Y.E. Dingyi, M.A. Siddiqi, A.E. Maccubin, S. Kumar, H.C. Sikka, Environ. Sci. Technol. 30 (1996) 136.
[7] M. Grifoll, S.A. Selifonov, C.V. Gatlin, P. Chapman, Appl. Environ. Microbiol. 61 (10) (1995) 3711.