LABORATORY AND PILOT SCALE REMEDIATION EXPERIMENTS OF PAH CONTAMINATED CONSTRUCTION RUBBLE

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Abstract
For the microbiological remediation of construction rubble usually organic substrate amendments were applied. Thus alkalinity and lack of nutrients were tackled by adding organic or neutralising compounds. Though, these approaches have only limited success or transfer the problem by increasing waste volumes or decreasing recycling possibilities due to the then increased organic content. In former investigations indigenous species were isolated, selected and adapted to metabolise organic contaminants (petroleum hydrocarbons, PAH, herbicides) directly in the alkaline milieu. The found specialised alkaliphilic consortia were capable to degrade the single contaminants directly on the debris matrix with reduction rates from 50 % to 80 % in about 100 days. For scale-up and the transfer into an applicable remediation technology further laboratory column and pilot scale tests with polycyclic aromatic hydrocarbon (PAH) contaminations on rubble were performed. The contaminant reduction rates were usually lower. As main reason a severe inhibition of added selected bacteria (e.g. *Dietzia sp.*) was found. This inhibition (by either toxic metabolic compounds or other constituents of the complex contamination) is currently investigated. Nevertheless, a remarkable 30 % to 60 % reduction of PAH contamination could be achieved. Though, this might not be sufficient and economic for a successful decontamination in a number of cases. Further studies shall address the problem of metabolic performance and separation of inhibiting agents.

Introduction

Polycyclic aromatic hydrocarbons (PAH) represent a critical and persistent environmental concern. Some of the PAH congeners are extremely cancerogenic, e.g. benzo(a)pyrene or benzo(b)fluoranthene. They are components of a number of products generated by natural or human-related thermal processes (fire, explosions, incineration, gasification, pyrolysis) on the basis of carbon, usually under reducing or oxygen limited conditions. Thus, the technical items and objects with potential PAH contamination are coke, tar, tar oils and related or derived products as well as the respective production or processing equipment and sites. Likewise, these are the subjects for remediation approaches.

Compared with other contaminants, PAH are generally relatively resistant, both against chemical and biochemical reagents. Therefore, microbiological degradation of different PAH species is very much restricted or, for some congeners, even strongly inhibited. This is especially due to their low bioavailability; which is a result of their generally low water solubility, between 0.26 µg/L for benzo(ghi)perylene and 30 mg/L for naphthalene, and their apolarity and adsorption tendency, expressed by a large octanol-water partitioning coefficient log K_{OW}, between 3.37 for naphthalene and 7.66 for indeno(1,2,3-cd)pyrene.

Hence, often incineration or disposal have been and are the sole remediation options. However, under certain conditions also bioremediation or (possibly assisted or supported) “natural attenuation” might be successful approaches. In several scientific studies the ability of different species (bacteria, fungi, algae, yeasts) to utilise or to co-metabolically degrade PAH was shown [e.g. 1 - 6]. Though, most of these studies are (at least to some extent) restricted on model (i.e. not direct in-vivo) systems. For technical bioremediation applications often different amendments are added; like organic or inorganic substrates (wood, bark chops, peat, ashes, clay [7]; food industry, crop and animal residues and faeces, compost [8]), nutrients (protein [7], mineral salts, N and P sources [9]), enzymes (laccase [10]) or neutralising and buffering agents. Also a combination of a thermal [8, 11] or physical-chemical pre-treatment [9, 11] and subsequent bio-treatment is proposed and technically applied.

These amendment and/or combined techniques were also applied for construction rubble – usually only with limited success. The problems of alkalinity and the scarcity of nutrients of these residues were either neglected or “camouflaged” by the added organic or other neutralising substrates and amendments. Former investigations in this direction led to partly successful results [12]. For that, a strict processing scheme and parameter (moisture, temperature, pH value, particle size, limited nutrient amendment) control are absolutely necessary for the cultivation and adaptation of degrading species. Furthermore another strategy was pursued to improve remediation results and to further restrict amendments dosage as well as prior homogenisation and processing efforts: the isolation and selection of indigenous (autochthonous) alkaliphilic or alkali-tolerant species. Those might tolerate the alkaline milieu (up to pH 12) and (co-)metabolise contaminants even with strongly reduced or totally without amendments. However, these species or consortia will only readily metabolise specific contaminants. Therefore the isolation strategy has to be carried out with the respective contaminant and substrate or matrix. Perfect successes and even technical decontamination have been achieved for the remediation of herbicide contaminated building rubble – with a total degradation of up to 95 % [e.g. 13]; also, but to a minor extent, of petroleum hydrocarbons (PHC) on gas plant construction rubble – with a PHC reduction of 50 to 80 % [12].

Though, the isolated and cultivated (e.g. *Gordonia* or *Ochrobactrum anthropi*) species possess the ability to use PHC as the sole organic source on construction rubble, the accompanying PAH contamination (generally also prevailing and even dominating on gas plant sites) could not be remarkably affected. Following the approach discussed above (and as main focus of this study), isolation and cultivation experiments for alkaliphilic and/or alkali-tolerant PAH degrading species were performed. The found species were then studied in further laboratory column and pilot scale tests with PAH contaminations on rubble. With these results a scale-up and transfer into an applicable remediation technology is prepared.

For that, so far no communications are known from literature. Only a few papers are approaching the problem in model systems [14]. Therefore the problem was studied in a joint enterprise – research institute – university project of the above given partners. Here the first set of laboratory and pilot scale investigations are presented. Detailed summaries are e.g. given in [15, 16].

Materials and Methods

Bacteria isolation and taxonomy

From different PAH contaminated sites (e.g. a 1998 dismantled gas plant in Riesa, Germany) construction debris material was retrieved and crushed to a particle size less than 10 mm. For further experiments the fraction of particle size of 2...4 mm was used. To enrich PAH degrading bacteria strains, building rubble (50 g) was mixed with 150 mL mineral salt medium (MSM), pH value 8.5, and incubated at 30 °C at shaker machine. To achieve a better bacteria recovery, glass percolation columns were used for enrichment at ambient temperature of about 23 °C. There the rubble was percolated 4 weeks, with a MSM at pH 9.8 and 10.3, enriched with different PAH. Stirring the percolation fluid collection flask guaranteed sufficient oxygen supply [15, 17].

Microorganisms isolations were performed on peptone - yeast extract - fructose (PYE) and PAH sprayed agar after 72 (to 120) hours breeding at 30 °C. The isolation to pure cultures was done on agar plates. Often a special technique with perforated and PAH saturated filter stripes as PAH carrier was applied [15, 17]. Further cultivation and augmentation of selected cultures were performed in PHC and known PAH degradation intermediates, e.g. salicylic, 1-hydroxy-naphthoic and hydroxyl-2-naphthoic acid at various pH values.

The colony morphology was studied after growth on PYE. The individual colonies were classified upon colour, contour, profile, margin, surface, consistency, smell and size as well as classical gram stain. Commercial test stripes were used for the qualitative determination of enzyme activity of the pure cultures. The strain diagnostics was performed with the BIOLOG® system and r-DNA analysis. Complementary for the strain diagnostics the fatty acid spectrum was recorded. Also the oxygen consumption under different substrate conditions was investigated in an oxygraph unit (50 µL of bacterial suspension in 2 mL MSM with pH 8.5 at 30 °C), and compared with the colony forming units (CFU) counting to assess viability of the species under (PAH and substrate limiting) stress conditions.

Degradation tests

The degradation tests with certain PAH concentrations were performed in shaker flasks (500 mL), to some extent by the mentioned filter stripes technique, and in columns. For the tests with suspensions MSM and for the filter stripes test MSM agar were used with respective pH values. Parallel blind tests with PAH, MSM and deadened biomass permitted the exclusion of random interference. Whereas in the tests in columns the organic contamination of the construction rubble was a nutrition source. For the 55 days long column tests, glass columns (length 850 mm, inner diameter 55 mm) were filled each with about 1500 g of PAH contaminated rubble (see Figure 1). At test start all columns were supplied with MSM (pH 9.5), nutrients (with ratios C:N:P = 100:7:1, with C derived from PHC and PAH contaminations), inoculated with bacteria. During the tests, only the moisture content was readjusted at sampling days. A parallel blind test was also carried out as reference. For aeration, water saturated air was flowing with a controlled flow rate of about 5 L/h from column top to bottom. The first pilot scale experiments were performed in 1 m^3 boxes (see Figure 2) filled with about 550 to 900 kg of rubble, depending on the experimental objectives and parameters [18].

Figure 1: Photograph of the column test unit

Figure 2: Photograph of one of the pilot scale box test units

Analytics

The biomass concentration was determined by the optical density of the suspension at a wave length λ = 700 nm (OD 700) with a Spectrophotometer U-2000 (Hitachi, Lorsch). For the CFU counting, 200 µL of each dilution step were applied on PYE agar. All dilution steps were done parallel and bred at 30 °C for 72 hours.

The PAH analysis was carried out with a Shimadzu HPLC system (10AV series) with UV and fluorescence detection, according to the adapted German standard DIN 13877 [16]. Ultrasonic extraction (1 hour) of PAH was carried out for the column samples (about 5 g each) with acetonitrile. Extracts were cleaned by SPE technique using benzosulfonic acid modified silica gel columns. Similarly the filter stripes were completely extracted, however by shaker extraction. Usually double determinations were performed. Furthermore were measured: PHC by IR method after ultrasonic extraction with 1,1,2 – trichlorotrifluoroethane, conductivity (WTW TetraCon 325) and pH value of eluats (by WTW SenTix 41) with WTW MultiLab 540 as well as dry mass (moisture content).

Results and Discussion

Isolation and enrichment of PAH degrading bacteria

The different methods of inoculation and enrichment on PAH plates coated with the substrate as well as in percolation columns were successful for PAH utilising strains. After some (14 to 28) days visible clear zones indicated PAH degradation. In a number of experiments the total substrate on the plate was degraded during 28 to 42 days. Direct incubation of building rubble in shaker flasks and plating percolation liquid onto PYE plates proved to be ineffective for enrichment of PAH degrading strains. Though, also with that method a variety of bacteria cultures could be isolated – but, only a minority of those showed PAH degrading abilities [17].

Colonies with PAH utilisation were inoculated on MSM plates coated with different PAH, after passing them via PYE medium to control purity of individual. Table 1 gives an overview on some of the isolated bacteria and their degradation spectra as well as morphology and taxonomy. Figure 3 shows a microscopic view of the SK 3 strain. Because of its degradation spectra as well as behaviour and viability on PAH plates, that SK 3 strain was selected for the first set of column and pilot scale remediation tests with contaminated rubble from the mentioned gas plant site.

Figure 3: Microscopic view of selected SK 3 strain [17]

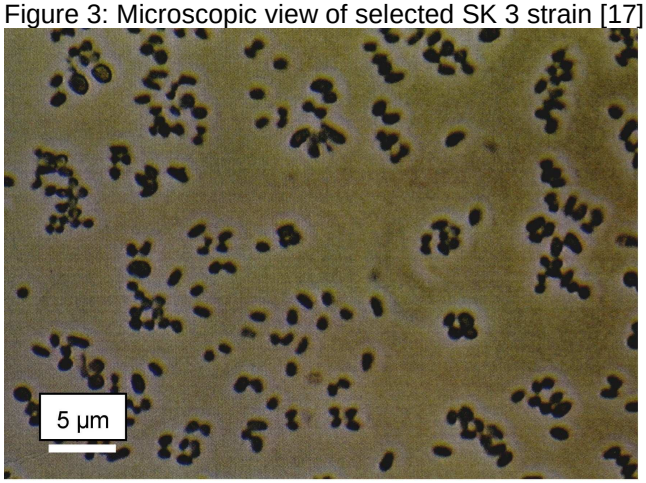


Table 1: Degradation spectra, morphology and taxonomy of selected isolated bacteria (n.d. = not determined)

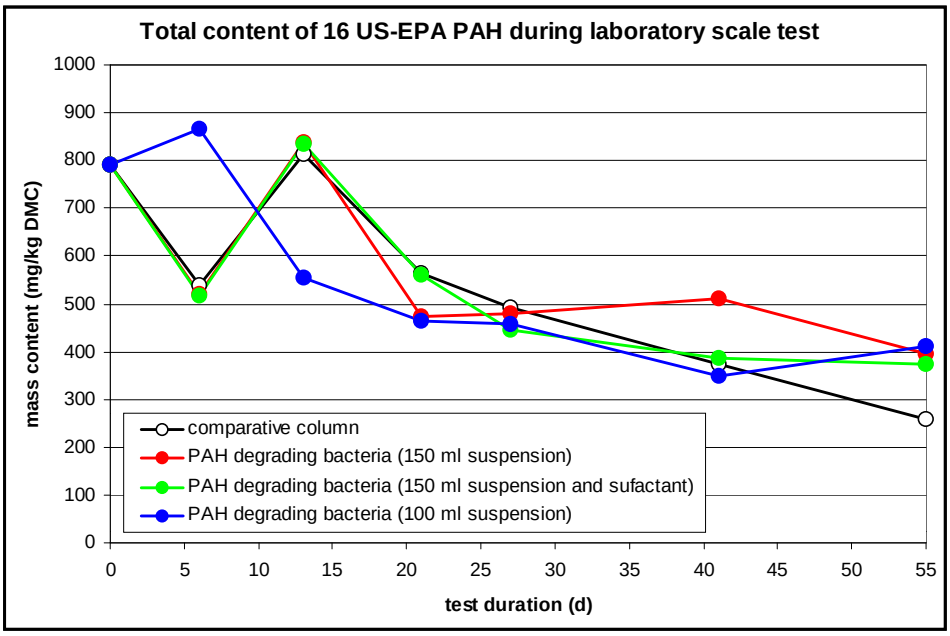
identification of bacterial strain		SK 3	SK 6.1	SK 11.11	SK 12.2	SK 16
morphology	colour	red	dark pink	yellowish	yellow	orange
	surface	smooth	smooth	smooth	rough	smooth
	outline	round	round	round	irregular	round
	kind	cocci	n.d.	rods	rods	n.d.
gram stain		positive	positive	positive	negative	negative
16S rDNA analysis		*Dietzia* sp.	n.d.	n.d.	*Pseudomonas denitrificans*	*Pseudomonas straminea*
degradation of PAH (qualitative assessment)	fluorene	+++	+++	+++	+++	-
	phenanthrene	+	++	+	+++	+++
	anthracene	+++	+	++	-	-
	fluoranthene	-	n.d.	n.d.	+++	+++
	pyrene	+	+	+	+++	++

At higher pH values (> 9.4) degradation spectra of almost all the described bacteria are remarkably limited. In addition with increasing molecular weight of substrate, a qualitative reduction of degrading capabilities could be determined.

Laboratory column tests

Figure 4 depicts the time graphs of the total content of the 16 US-EPA PAH for the column tests of 3 SK 3 experiments and the (blind) comparative column. All columns show a similar trend of slightly decreasing PAH contents from a starting value of 790 mg PAH/kg dry mass of rubble (DMC) to about 260 to 410 mg PAH/kg DMC after 55 days. There was no clear dependence from the addition and amount of bacteria and surfactant. The significant data scattering between sampling dates have to be attributed to the main sampling errors due to the inhomogeneity and large particle sizes of the rubble. That fact is well known for these matrices [12, 16]. Detailed investigations showed an increasing deviation of double analyses and determinations with increasing particle size. They totalled to less than 10 % for particle sizes < 1 mm; but increased drastically, the larger the particle size. For the taken average samples with particle sizes up to 6.3 mm, the range of mentioned deviations of double determinations can amount to 30...40 %. 2 and 3 ring PAH were reduced by about 90 % to 50 %, respectively; some 4 ring PAH (fluoranthene and pyrene) by less than 50 %; other 4 to 6 ring PAH much less – for the strongly cancerogenic benzo(b)- and benzo(k)fluoranthene by under 10 % [16, 18]. A further finding – not so obvious from Figure 4 – was made: the viability of SK 3 (and thus the determinable degradation effect) dropped drastically after about 10 days [18]. More intrinsic microbiological tests (with oxygraph and under stress and limiting conditions) proved that fact, which might be attributed to the accumulation of toxic intermediates of PAH decay and/or the permanent effect of stress conditions on the bacteria [15].

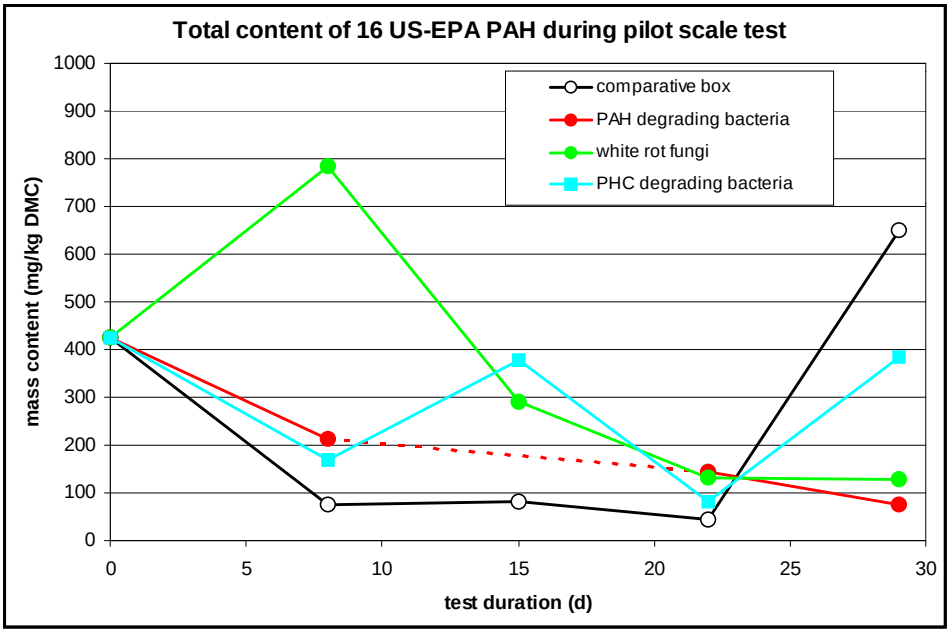
Figure 4: Total content of 16 US-EPA PAH during column tests (comparative and 3 SK 3 columns)



Pilot scale tests

In Figure 5 represents the time graphs of the total content of the 16 US-EPA PAH during pilot scale tests for 4 selected boxes, i.e. comparative, SK 3 (*Dietzia* sp.) as PAH degrader, *Gordonia* sp. as PHC degrader [12] and white rot fungi (*Pleurotis ostreatus*). Here the data scattering as well as analytical and determination deviations were much greater. Even PAH contents much larger then the starting value of about 420 mg PAH/kg DMC were determined, making the analytical deviations larger than 80 % - obviously due to the much larger particle size of up to 56 mm. This was proved by total recovery determination tests of some PAH depending on the particle size. Thus, a definite statement and clear conclusions could not be derived. Especially, because the comparative box results were similarly low as those for the PAH degraders for a number of sampling dates during the tests. Nevertheless, a tendency towards decreasing PAH (by about 60 to 80 %) for SK 3 (PAH degrader box) could be assumed – though not experimentally reassured. With respect to the degradation of PAH with different ring numbers and the viability reduction, comparable findings resulted as for the column tests [18].

Figure 5: Total content of 16 US-EPA PAH during pilot scale tests (comparative, SK 3, PHC degrader and fungi box)



Summary and Conclusions

In an experimental study bacterial strains were isolated and selected from construction rubble contaminated with PAH from a former gas plant site. The bacteria are able to degrade PAH in model systems and on real contaminated rubble material in alkaline milieu. Thus, the main representatives should have alkaliphilic or alkali-tolerant growth behaviour. The preliminary taxonomic screening and the degradation tests indicate e.g. *Dietzia* sp. (SK 3) as one of the potential performance activists. Unfortunately, their viability was drastically reduced after about 10 days. Therefore their characteristics and those of other isolates have to be further studied. SK 3 behaviour under stress and intermediates accumulation conditions should be the focus for further studies. However, also the role and importance of other strains have to be understood.

The determined degradation rates of less than 10 % for some 4 to 6 ring PAH up to 90 % for 2 to some 3 ring PAH in laboratory and pilot scale tests are yet to be confirmed, and then transferred into large scale applications.

Lower degradation rates and cross-over effects in larger scale tests might be due to the more complex contaminant spectrum in large scale remediation of “old” contaminations. For that reason, a further study should also investigate the influence of certain other contaminants (especially persistent PAH, intermediates and heavy metals), matrix effects as well as material origin and age of the rubble material. E.g. certain contaminant and matrix properties are important to consider (water solubility, bioavailability, penetration, permeability). The investigations were aimed on testing a simplified microbiological remediation technology for PAH contamination of alkaline building rubble. So far no viable, technically feasible and efficient bioremediation technology could be proposed. Further studies have to find the causes for the mentioned bacteria viability reduction and should focus on other isolated bacteria and/or consortia.

Acknowledgements

The investigations were supported by a German industrial fund AiF grant (KF 0013206 KUL1 and KF 0249601 KUL1). For support, discussions and project management we want to express gratitude the project attendant, Mrs. U. Liebing. Special thanks for laboratory and field work to the co-workers at UFZ – S. Krauße, M. Neytschev and C. Schumann, at P-D – C. Höse and P. Schulz and from Zwickau – H. Stemmler, K. Maurer, S. Lürtzing, S. Schedewy, K. Zwiener and F. Gränitz.

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