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 microbial 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 overcome inhibition.

Introduction
Polycyclic aromatic hydrocarbons (PAH) are of critical environmental concern due to distribution, persistence and toxicity. 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 (e.g. coke, tar) 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. PAH are generally relatively resistant, both against chemical and biochemical attack. Therefore, microbiological degradation of different PAH species is rather restricted or, for some congeners, even strongly inhibited. This is especially due to their low bioavailability, apolarity and adsorption tendency. Under certain conditions 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 - 3]. Though, most of these studies are at least to some extent restricted to model systems. For technical bioremediation applications often various amendments were added, like organic or inorganic substrates [4], nutrients [5], enzymes [6] or neutralising and buffering agents.
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. This strategy was successfully applied in a semi-technical

scale for the remediation of herbicide [7] as well as petroleum hydrocarbon (PHC) [8] contaminated building rubble. Following that approach, isolation and cultivation experiments for alkaliphilic and/or alkali-tolerant PAH degrading species were performed. The found species were then studied in laboratory column and pilot scale tests with PAH contaminated rubble. With these results a scale-up and transfer into an applicable remediation technology is prepared. Here the first set of laboratory and pilot scale investigations are presented.
So far, no essential communications to that approach are known from literature as to PAH contaminated construction rubble. Only a few papers are approaching this problem in model systems [9].

Materials and Methods

Isolation and taxonomy of bacteria
From different PAH contaminated sites (e.g. a 1998 dismantled gas plant in Riesa, Germany) construction debris material was retrieved and crushed to particle sizes less than 10 mm. For further experiments the fraction 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 on a rotary shaker. Alternatively, glass percolation columns were used for enrichment at ambient temperature of about 23 °C. The rubble was percolated for 4 weeks with MSM at pH 9.8 and 10.3, applying various PAH coated on sinter glass plates in addition to the contaminants as carbon and energy sources. Isolation of microorganisms was performed on peptone - yeast extract - fructose medium (PYE) and PAH sprayed on MSM agar after breeding at 30 °C. Growth in liquid medium was performed on PYE or, for reason of certain selectivity, on PHC or known intermediates of PAH degradation, e.g. salicylic, 1-hydroxy-naphthoic or hydroxyl-2-naphthoic acid at various pH values [10].
The colony morphology was studied after growth on PYE. Commercial test stripes were used for the qualitative determination of enzyme activities. The strain diagnostics was performed with the BIOLOG® system and by 16S r-DNA analysis. Complementary, the fatty acid spectrum was recorded. Viability of strains under various conditions was derived from measuring oxygen consumption in an oxygraph and estimating colony forming units (CFU).

Degradation tests
The degradation test was performed in shaking flasks (500 mL), coated with PAH. Alternatively, filter stripes penetrated with different PAH were placed on agar plates on which respective strains were equally streaked. Finally percolation columns were used with the contaminants as potential carbon and energy sources. MSM as the inorganic basis was adjusted for the pH; at pH values above 9.5 the medium was buffered with carbonate. Parallel blind tests with PAH, MSM and autoclaved biomass permitted the exclusion of random interference. 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). The columns were run with MSM (pH 9.5), adjusted to a nutrient ratio C:N:P of 100:7:1 (with C derived from PHC and PAH contaminations). During the tests, only the moisture content was readjusted at times of sampling. A parallel blind test was also carried out as reference. For aeration, water saturated air was flowing with a 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 [11].

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, Lorch). For CFU counting, 200 µL of each dilution step were plated on PYE agar. All dilution steps were done in 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 shaking 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).

Figure 1: Photograph of the column test unit

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

Results and Discussion

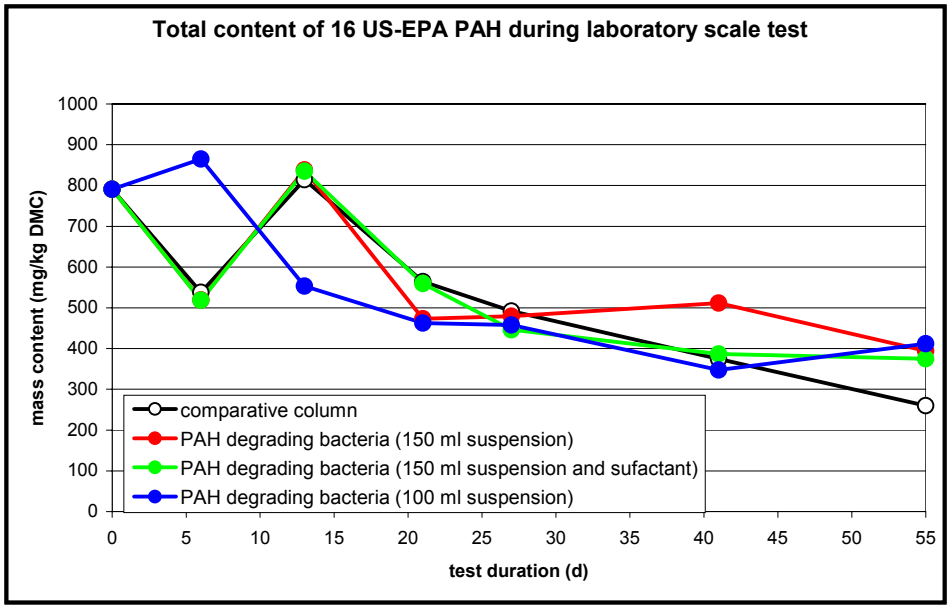
Isolation and enrichment of PAH degrading bacteria

Percolation of contaminated construction rubble proved a suitable method of isolating PAH-degrading microorganisms. Plating samples on agar coated with PAH revealed visible clear zones after 14 to 28 days of incubation indicated PAH degradation. In a number of experiments the substrate on the plates was even completely degraded during 28 to 42 days [10]. Colonies showing PAH utilisation were inoculated on MSM plates coated with various PAH, after passing them via PYE medium to control purity of individual strains. Because of its degradation spectra as well as its growth behaviour and viability on PAH plates, strain SK 3 was selected for a first set of column and pilot scale remediation tests.

Laboratory column tests

Figure 4 depicts the course of the total content of the 16 US-EPA PAH for three columns inoculated with strain SK 3; an uninoculated column served as blind experiment (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 of treatment. The effect of adding bacteria or surfactants was not clearly evident. The reason for the significant data scattering has to be attributed to sampling errors due to the inhomogeneity and large particle sizes of the rubble. That fact is well known for such matrices [8, 12]. Detailed investigations showed increasing deviation of doublate samples with increasing particle size. They totalled to less than 10 % for particle sizes < 1 mm. For the taken composite samples with particle sizes up to 6.3. mm, the deviations of double determinations can amounted to 30...40 %. Individual analyses showed that 2- and 3-ring PAH were reduced by about 90 % and 50 %, respectively, some 4-ring PAH (fluoranthene and pyrene) were degraded by less than 50 %; other 4- to 6-ring PAH – e.g. the strongly cancerogenic benzo(b)- and benzo(k)fluoranthene – were reduced by less than 10 % [11, 12]. More detailed microbiological tests (with oxygraph and under stress and limiting conditions) showed, that the viability of SK 3 dropped drastically after about 10 days. This might be attributed to the accumulation of toxic intermediates of PAH decay and/or the permanent stress conditions exerted on the bacteria in this inhospitable milieu.

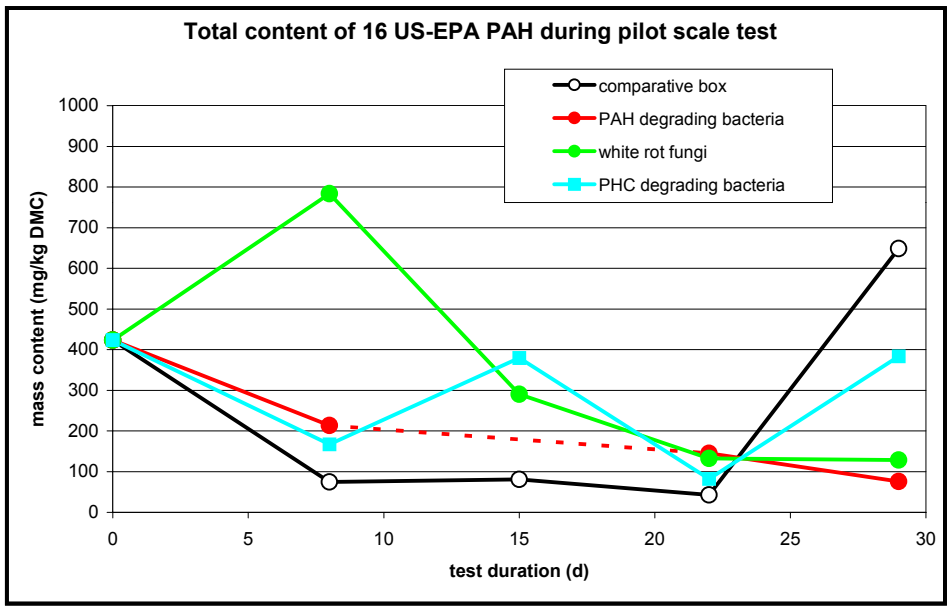
Figure 4: Total content of 16 US-EPA PAH during column tests (comparative and 3 SK 3 columns)



Pilot scale tests

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, *Dietzia* sp. SK 3 as PAH degrader, *Gordonia* sp. TH4 as PHC degrader [8] and white rot fungi (*Pleurotis ostreatus*). Under these conditions the data scattering was 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 of 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, similar restrictions applied as for the column tests [11].

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 strains. Unfortunately, its viability was drastically reduced after about 10 days. Therefore its characteristics and those of other isolates have to be further studied. Influence of milieu conditions or accumulation of toxic intermediates might be likely explanations. However, the contribution and effect of other strains inhabitant at this matrix have to be considered.

The degradation rates determined to be less than 10 % for some 4- to 6-ring PAH and amounting up to 90 % for 2- to some 3-ring PAH in laboratory and pilot scale tests are yet to be confirmed, and later on tested in 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 study should follow for investigating 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 (water solubility, bioavailability, penetration, permeability) are important to consider. The investigations were aimed at 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 limitations and constraints and should imply further bacteria and/or consortia.

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