Successful treatment of low PAH-contaminated sewage sludge in aerobic bioreactors

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Successful Treatment of Low PAH-Contaminated Sewage Sludge in Aerobic Bioreactors

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Background, Aims and Scope. Polycyclic Aromatic Hydrocarbons (PAHs) are known for their adverse and cumulative effects at low concentration. In particular, the PAHs accumulate in sewage sludge during wastewater treatment, and may thereafter contaminate agricultural soils by spreading sludge on land. Therefore, sludge treatment processes constitute the unique opportunity of PAH removal before their release in the environment. In this study, the ability of aerobic microorganisms to degrade light and heavy PAHs was investigated in continuous bioreactors treating trace-level PAH-contaminated sludge.

Methods. Several aerobic reactors were operated under continuous and perfectly mixed conditions to simulate actual aerobic sludge digesters. Three sterile control reactors were performed at 35°C, 45°C or 55°C to assess PAH abiotic losses under mesophilic and thermophilic conditions. Three biological reactors were also operated at 35°C, 45°C or 55°C. Furthermore, 250 mM methanol were added in an additional mesophilic reactor (35°C). All reactors were fed with long-term PAH-contaminated sewage sludge, and PAH removal was assessed by inlet/outlet mass balance. In this study, PAH compounds ranged from 2 to 5-unsubstituted aromatic rings, i.e. respectively from Fluorene to Indeno(123cd)pyrene.

Results and Discussion. Significant abiotic losses were observed for the lightest PAHs (fluorene, phenanthrene and anthracene), while biodegradation occurred for all PAHs. More than 80% of the lightest PAHs were removed. Biodegradation rates inversely correlated with the increasing molecular weight, and seemed limited by the low bioavailability of the heaviest PAHs (only 50% of removal). The enhancement of PAH bioavailability by increasing the process temperature or adding methanol was tested. A temperature increase from 35°C to 45°C and then to 55°C significantly enhanced the biodegradation of the heaviest PAHs from 50% to 80%. However, high abiotic losses were observed for all PAHs at 55°C, which was attributed to volatilization. Optimal conditions were found at 45°C considering the low abiotic losses and the high PAH biodegradation rates. Similar performances were achieved by addition of methanol in the sludge. It was concluded that increasing temperatures or addition of methanol favored PAH diffusion from solids to an aqueous compartment, and enhanced their bioavailability to PAH-degrading microorganisms.

Conclusion. In this study, the use of long-term acclimated aerobic ecosystems showed the high potential of aerobic microorganisms to degrade a wide range of PAHs at trace levels. However, PAH biodegradation was likely controlled by their low bioavailability. Two aerobic processes have been finally proposed to achieve efficient decontamination of sewage sludge, at 45°C or in the presence of methanol. The PAH concentrations in reactor outlet were lower than the French requirements, and allow the treated sludge to be spread on agricultural land.

Recommendations and Outlook. The two proposed aerobic processes used physical or chemical diffusing agents. The global ecological impact of using the latter agents for treating trace level contamination must be considered. Since methanol was completely removed during the process, no additional harm is expected after treatment. However, an increase of temperature to 45°C could drastically increase the energy demand in full-scale plants, and therefore the ecological impact of the process. Moreover, since bioavailability controls PAH biodegradation, efficiency of the processes could also be influenced by the hydraulic parameters, such as mixing and aeration rates. Further experimentations in a pilot scale are therefore recommended, as well as a final assessment of the global environmental benefit of using such aerobic processes in the bioremediation of trace level compounds.

Keywords: Aerobic bioprocesses; bioavailability; biodegradation; polycyclic aromatic hydrocarbons (PAHs); sludge; temperature

Abbreviations (PAHs): Ant – anthracene; **B(a)A** – benzo(a)anthracene ; **B(b)F** – benzo(b)fluoranthene; **B(k)F** – benzo(k)fluoranthene; **B(ghi)P** – benzo(g,h,i)perylene; **B(a)P** – benzo(a)pyrene; **Chrys** – chrysene; **DB** – dibenzo(a,h)anthracene; **Fluor** – fluoranthene; **Fluo** - fluorene; **Ind** – indeno(1,2,3-c,d)pyrene; **Phe** - phenanthrene; **Pyr** – pyrene

Introduction

The amount of sewage sludge produced in Europe has sharply increased over the last ten years to reach around 8 millions of tons of dry solids in 2005 [1]. The treatment and land disposal of sewage sludge represents one of the major issues for better and long-term preservation of the environment. Because of its high organic and mineral contents, sewage sludge can partially substitute synthetic fertilizers in agriculture, and sludge recycling on land has become over the last decade the most common practice for sludge disposal throughout the European Union. However, the use of sewage sludge may lead to the accumulation of unwanted products in soils, such as trace organic pollutants. In this context, the Polycyclic Aromatic Hydrocarbons (PAHs) are particularly surveyed because of their adverse and cumulative effects at trace level concentrations. Since PAH half lives in soils vary from several months to tens of years, soils will strongly act as a successful end-point. Therefore, it is important to assess and improve their degradation before their spreading. In wastewater treatment plants, the PAHs readily adsorb into sludge particles because of their high hydrophobic properties. The sewage sludge constitutes a point of convergence for all PAHs, and represents the unique opportunity for removal before their release in the environment.

Even though the PAHs are known for their recalcitrance to biological degradation, several papers have reported PAH biodegradation under aerobic and anaerobic conditions [2-7]. Some aerobic bacteria [8-9] and fungi [10-11] have been isolated, and potential metabolic pathways have been proposed [8, 12]. However, PAH biodegradation has mainly been observed in highly contaminated or spiked environments (sediments, soils). In the latter case, it is commonly assumed that biological degradation is favored and does not represent the reality. Indeed, spiked PAHs are more easily biodegraded since they have not been in contact with organic matter for a long period, and are therefore more bioavailable [13]. Only few papers reported aerobic PAH degradation in low contaminated environments [14-16]. In these studies, PAH removal was strongly limited by their bioavailability. In addition, some authors reported that the lightest PAHs are more easily biodegraded than the heaviest ones [17-19]. Indeed, the lightest PAHs are more soluble and more bioavailable to PAH-degrading microorganisms and, consequently, more biodegradable [20]. Abiotic phenomena, such as volatilization, can also occur and be predominant under aerated conditions, especially for light PAHs [21]. Therefore, it is essential to consider the bioavailability and mass transfer limitations when studying the PAH biodegradation in low contaminated environment, especially in environments with high organic matter content, such as sewage sludge [22-23].

In this paper, the ability of aerobic microorganisms to degrade PAHs during sludge treatment was investigated in continuous bioreactors. The reactors were fed with a long term and non-spiked PAH-contaminated sewage sludge. The impact of the increase of the process temperature, or the addition of a solvent, on PAH biodegradation was then investigated.

1 Materials and Methods

1.1 Reactor design

The aerobic reactors were operated under continuous and perfectly mixed conditions by mechanic stirring at 250 rpm and continuous feeding/sampling (four times a day). The hydraulic retention time was of 20 ±1 days, and the daily organic load of 1.2 kg $COD.m^{-3}.d^{-1}$ (COD: chemical oxygen demand). In order to avoid water losses, the air supplied into the system was saturated by passing through a water column. The amount of air was equivalent to 0.3 $L_{air}.L_{reactor}^{-1}.min^{-1}$. The biogas outlet and the inlet storage flask were cooled to avoid water losses during the process, and initial biodegradation before feeding, respectively. pH was not regulated, but remained constant around 7.2 ±0.2. The temperature was regulated in the bioreactors at 35°C, 45°C or 55°C using a heating resistance combined with a temperature probe. Three control reactors were performed to assess PAH abiotic losses under mesophilic and thermophilic conditions at 35°C, 45°C or 55°C. These reactors were chemically sterilized by addition of 100 mM sodium azide (NaN_3-Riedel de Haën), and operated under the same conditions as the bioreactors. In one additional mesophilic reactor (35°C), methanol was added at a concentration of 250 mM. Since previous batch tests showed no influence of methanol addition on PAH abiotic losses, no specific control reactor was implemented.

1.2 Source of sewage sludge

The reactors were inoculated with 4 L of homogeneous activated sludge. The latter was sampled from a French urban wastewater treatment plant (WWTP) which has been contaminated by PAHs for more than 10 years. The reactors were continuously fed with a homogeneous mixture of primary and secondary sludge (50:50, v:v) sampled from the same WWTP. PAH concentrations in the feeding sludge ranged as follows: (average in $mg.kg_{dryweight}^{-1}$) Fluo (0.50 ±0.01), Phe (3.29 ±0.04), Anth (0.71 ±0.03), Fluor (8.79 ±0.22), Pyr (6.54 ±0.1), B(a)A (3.70 ±0.1), Chrys (4.43 ±0.15), B(b)F (3.45 ±0.06), B(k)F (2.34 ±0.06), B(a)P (4.06 ±0.1), DB (0.59 ±0.02), B(ghi)P (2.76 ±0.05) and Ind (2.94 ±0.07). These levels were around twice the French maximum concentrations for sludge to be spread on agricultural soils. The current French limits are of 5, 2.5 and 2 $mg.kg_{dw}^{-1}$ for Fluor , B(b)F and B(a)P, respectively.

1.3 PAH analysis

PAH analysis was carried out according to Trably et al. [24]. This method was especially implemented to monitor trace levels of PAHs in bioreactors treating long-term contaminated sludge. In the present study, total reproducibility and repeatability errors were lower than 5% for all analysis made in triplicates. The analytical method consisted of the following steps: (i) sludge drying, (ii) PAH extraction by Accelerated Solvent Extraction – ASE, and (iii) extract analysis by RP-HPLC coupled to a fluorimetric detector. Each step is detailed here below. (i) First, a sample of 350 ml

was taken from the outlet or the inlet of the reactors, and was centrifuged at 20,000g for 25 min. The aqueous phase was stored at –20°C for further solid phase extraction on C_{18} column (SPE Supelco - Supelclean ENVI18). The solid pellet was ground with glass beads and was dried in a ventilated oven at 40°C for 60 hours. The sample was then sieved on grid (diameter 2 mm), and stored at –20°C before Accelerated Solvent Extraction. Dry weight (DW) was determined by drying 20 ml of the sludge sample in an oven at 110°C for 24 hours. (ii) ASE extraction was performed in an ASE-200 system (DIONEX). The extraction solvent consisted of a mixture of hexane:acetone (50:50). The ASE cells were filled with a filter of glass fiber (Diameter 19 mm, Whatmann), 1 g of alumina (Sigma), 1.5 g of hydromatrix (Varian) and 0.5 g of dried sample. After extraction, the sample was evaporated under nitrogen flow to dryness. The residue was then dissolved in 5mL of acetonitrile and was immediately analyzed (no storage). (iii) The extracts were analyzed by RP-HPLC coupled to fluorescence detection, according to Trably et al. [24]. The analytical system was composed of a sampler injector (Waters 717plus Autosampler), a solvent degasser (Waters Inline Degasser), a peristaltic pump system (Waters 600 Controller) and a programmable fluorimetric detector (JASCO FP-1520). The excitation and emission wavelengths were changed as follows: 0 min, 280/330; 13 min, 266/312; 17 min, 250/370; 20 min, 250/400; 24 min, 280/430; 27.5 min, 260/410; 32 min, 280/430; 40.2 min, 268/384; 46 min, 234/420; 50.5 min, 270/400; 56 min, 300/407; 60 min, 300/500. The temperature of the C_{18} column (PAH16-Plus Bakerbond) was regulated at 25°C. The elution sequence followed a sequence of 5 min of isocratic elution (acetonitrile:water, 60:40, v:v), 30 min of linear gradient from 60 to 100% acetonitrile, 30 min of isocratic elution (acetonitrile 100%) and 30 min of isocratic rinsing of the column by a mixture of acetonitrile:water (60:40 v:v) (flow rate of 0.3 $mL \cdot min^{-1}$).

1.4 PAH removal and efficiency factor calculation

PAH removal (in %) was calculated at steady state by using an inlet-outlet mass balance model. PAH removal was worked out by comparing the total PAH concentration in sludge (in $mg_{PAH} \cdot L^{-1}$) in inlet and outlet, i.e. respectively, before and after treatment. The total PAH concentration (in $mg_{PAH} \cdot L^{-1}$) corresponded to an average of 5 to 7 samples analyzed in triplicate. During analysis, PAH contents were determined in both aqueous and solid phases after centrifugation. Whatever the conditions, the amount of PAH in the aqueous phase was not significant with a liquid/solid repartition ratio lower than 1% for all PAHs, as shown elsewhere [24]. Therefore, only the amount of PAH per unit of dry weight (in $mg_{PAH} \cdot g_{DW-1}$) and the dry weight content of the sludge sample (in $g_{DW} \cdot L^{-1}$) were considered for the calculation of the PAH concentration in sludge sample (in $mg_{PAH} \cdot L^{-1}$). On other hand, in order to limit the influence of the dry weight variation between the different tests, a biological efficiency factor (ε_f) was assessed by dividing the PAH biodegradation rate by the dry weight reduction efficiency of the process.

2 Results

2.1 Impact of process temperature

Aerobic digestion of low PAH-contaminated sludge was performed in continuous bioreactors working under mesophilic (35°C), intermediary (45°C) and thermophilic (55°C) conditions. Main biological activity of the aerobic microorganisms was evaluated through the consumption of organic matter during the process. Similar activity was observed in all reactors with dry weight reduction rates of 38 ±3.3% at 35°C, 41 ±2.8% at 45°C, and 36 ±3.2% at 55°C. In contrast, no significant biological activity was observed in the control reactors with reduction rates of 0.3 ±4.3% at 35°C, 1.6 ±3.6% at 45°C, and 2.9 ±2.1% at 55°C. Control reactors were therefore considered as mainly sterile, and were used to estimate the PAH abiotic losses due to the process. The results are presented in **Fig. 1**. At 35 and 45°C, PAH abiotic losses were only significant for the lightest PAHs -fluorene, phenanthrene, and anthracene, while all PAHs were significantly lost at 55°C. Surprisingly, larger amounts of heavy PAHs – from fluoranthene to indeno(1,2,3-c,d)pyrene – were recovered in the outlet of the 35°C and 45°C reactors (up to 20%). Since no water losses were measured, it was concluded that PAH diffusion occurred from the non-extractable to the extractable fraction of the solids that led to higher apparent concentrations in the outlet of the control reactors.

Fig. 1

Biological PAH removal was also assessed in the biological reactors (**Fig. 2**). At 35°C, more than 80% of PAH removal was achieved for the lightest PAHs. PAH removal decreased according to increasing molecular weights to reach around 50% for the heaviest PAHs, e.g. indeno(1,2,3-c,d)pyrene. Furthermore, PAH removal did not correlate with their initial concentration in sludge (**Table 1**). Considering that the PAHs in trace levels were strongly adsorbed into the sludge (negligible amounts of PAHs in the aqueous phase), it was concluded that PAH biodegradation was likely limited by their low bioavailability. By increasing the temperature from 35°C to 45°C, performances were significantly enhanced with more than 90% of removal for 11 PAHs out of 13. The results showed a significant but lower influence of the temperature on the dibenzo(a,h)anthracene and benzo(g,h,i)perylene removal, with an increase from 50% to 82%, and from 44% to 60%, respectively. At 55°C, PAH removal were aversely affected by the increase of temperature, and similar values to those obtained at 35°C were found, except for the three heaviest PAHs - dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene.

Fig. 2, Table 1

In order to evaluate the actual removal to be attributed to the biological activity, the part of PAHs biodegraded was assessed by substracting the abiotic losses from the removal rates measured in the biological reactors. The results are presented in **Fig. 3**. It was shown that the high removal rates previously observed for the three lightest PAHs under mesophilic and intermediary conditions were actually partly due to abiotic losses, and that the biodegraded part drastically decreased at 55°C. In contrast, biodegradation of heavier PAHs was greatly enhanced by increasing the

temperature from 35°C to 45°C. As well, thermophilic conditions (55°C) negatively affected the biodegradation of the heavier PAHs (only 25% to 50%).

Fig. 3

In order to accurately compare the different tests and reduce the influence of the dry weight reduction rate, a biological efficiency factor (ε_f) was worked out (**Fig. 4**). This factor represents the ability of the microorganisms to degrade the PAHs among the main biological activity. As previously suggested, biodegradation of the PAHs was likely limited by their low concentration and their strong adsorption into solids, especially for the heaviest PAHs. Therefore, ε_f factor may be used as an indicator of the apparent PAH bioavailability. The dry weight reduction rates did not significantly change between the assays, and therefore similar conclusions were formulated. A significant and positive impact of the temperature increase to 45°C was observed on the efficiency factors, especially for PAHs heavier than the fluoranthene. The low efficiency factors observed at 55°C reflected the high impact of the high abiotic losses on total PAH removal.

Fig. 4

2.2 Impact of methanol addition

A continuous bioreactor was operated under mesophilic conditions by addition of 250 mM methanol in the inlet. The dry weight reduction measured during the process was of 25.35 ±2.4%. This rate was lower than those obtained at 35°C in the absence of methanol. This might result from the consumption of methanol as a new carbon source instead of low biodegradable organic matter. No methanol was found in the outlet of the reactor, suggesting a complete degradation. Volatilization could also have occurred. PAH removal measured in the reactor is presented in Fig. 2. Removals were significantly higher for all PAHs with around 90% of losses for the PAHs up to the chrysene, and a benefit of 20% to 25% for the heaviest PAHs compared to the other reactors. Previous batch tests showed no influence of methanol addition on PAH abiotic losses. Therefore, the mesophilic control reactor was used to assess PAH biodegradation. The results are presented in Fig. 3. It was shown that the addition of methanol significantly enhanced biodegradation of all PAHs at 35°C. Nevertheless, global performances remained lower than the reactor at 45°C. Considering the lower dry weight reduction in the presence of methanol, the efficiency factor showed a high impact of the methanol on PAH degradation. Especially the efficiency factor reached the highest values for a group of PAHs from the fluoranthene to chrysene, suggesting a maximal effect of the methanol on the dissolution of these compounds. Biodegradation of heavier PAHs was also enhanced, but at a lower level.

2.3 PAH concentration in solids

Since PAH removal rates were higher than dry weight reduction in the reactors, PAH concentrations in solids significantly decreased (see Table 1). PAH concentrations in sludge are only concerned by the current French regulation focusing on the three following compounds: fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene (see Table 1). Because of their high efficiency on PAH biodegradation, the bioreactors exhibited lower concentrations in the outlet than in the untreated sludge. At 35°C and 55°C, concentrations in benzo(b)fluoranthene and benzo(a)pyrene were either close to the limit value or higher. At 45°C or in the presence of methanol, the concentrations in the treated sludge were significantly lower than the required values to spread sludge on land. In addition, the efficiency of total PAH degradation within sludge particles was assessed by comparing the sum of the 13 PAHs considered in this study. With an initial concentration of 44.1 ±0.4 $mg_{PAHs} \cdot kg_{DW}^{-1}$in the untreated sludge, around 56%, 86%, 46% and 77% of the total PAHs were degraded in the biological reactors, with 19.21 ±0.2 $mg_{PAHs} \cdot kg_{DW}^{-1}$ at 35°C, 6.17 ±0.3 $mg_{PAHs} \cdot kg_{DW}^{-1}$ at 45°C, 23.6 ±0.9 $mg_{PAHs} \cdot kg_{DW}^{-1}$ and 10.33 ±0.4 $mg_{PAHs} \cdot kg_{DW}^{-1}$, respectively, in the presence of methanol.

3 Discussion

The ability of aerobic bacteria to use PAHs as a sole carbon source or as a co-metabolite has been extensively studied over the last decade [2; 8; 25-26]. Many aerobic microorganisms have been isolated from heavily contaminated environments, such as soils or sediments, with concentrations about several $g \cdot kg_{DW}^{-1}$ [8; 26-27]. Mainly, it is assumed that: (i) abiotic losses significantly contribute to the removal of light PAHs, i.e. less than three aromatic rings, and (ii) low-molecular-weight PAHs are readily degraded, whereas high-molecular-weight PAHs are more persistent. Their resistance to biodegradation is partly caused by the low bioavailability of the heaviest PAHs resulting from their strong adsorption onto soil or sediment organic particles [17, 20]. In the present study, the aerobic mesophilic ecosystem showed a strong potential to degrade the PAHs at trace-level concentrations ($mg \cdot kg_{DW}^{-1}$), and in high organic matter environment (60-70% in sludge). While more than 80% of the lightest PAHs were removed, the mesophilic aerobic process surprisingly exhibited high removal rates for the heaviest PAHs (>50%). Such performances illustrate the high potential of aerobic microorganisms to degrade the light and heavy PAHs in regard to anaerobic reactors that are commonly used for sewage sludge digestion, but where PAH degradation rarely exceeds 50% under the same conditions [7, 28]. Although some authors specifically demonstrated the degradation of heavy PAHs under aerobic conditions, such as benzo(a)pyrene [9, 27], the efficient and simultaneous biodegradation of such wide range of PAHs has not yet been reported. In addition, abiotic losses were strictly restricted to the lightest PAHs under mesophilic conditions, such as the fluorene, the phenanthrene and the anthracene. The actual sterility of the control reactors may be discussed, since several microorganisms such as fungi are not sensitive to sodium azide. However, no dry weight reduction was measured in the biological reactors, and abiotic losses decreased according to PAH molecular weight,

suggesting a possible volatilization rather than a threshold biodegradation. Similar results were observed in aerated soils where PAHs with less than four aromatic rings were mainly lost abiotically [21, 29].

A strong influence of the increasing PAH molecular weight on the reduction of the biodegradation rates was shown. Since PAH degradation did not correlate to their initial concentration and seemed rather linked to dry weight consumption, especially for the heaviest PAHs, it was concluded that biodegradation was likely limited by their low bioavailability. Previous studies on PAH biodegradation reported that persistence of heavy PAHs is often associated with their low bioavailability in soils, sediments or sludge [20, 29-31]. Strong binding of the PAHs to organic substances has been demonstrated in low contaminated soils [32-33], and this might explain the low amounts of PAH found in the aqueous fraction of the sludge. Moreover, the strong binding of the PAHs into sludge particles was illustrated by the release of higher amounts of PAHs when increasing the temperature to 45°C in the control reactor that led to higher amounts of PAHs measured after treatment. In this case, PAH diffusion occurred from the non-extractable to the extractable fraction of the solids. A similar impact of the temperature on PAH sorption was previously observed in contaminated soils [34]. Therefore, assessment of PAH biodegradation in bioreactors was partly biased by the efficiency of the analytical procedure, implying that more PAHs could be diffused and degraded. The apparent PAH biodegradation could have been even more underestimated since dry weight consumption occurred in the biological reactors, and more PAHs than those measured in the abiotic control could have been released and potentially biodegraded.

In order to increase PAH biodegradation in sludge, the enhancement of their bioavailability was first considered by increasing the process temperature or by adding methanol. The use of the temperature as a diffusion enhancer was previously recommended by Bonten et al. [34] who reported the benefit of a short-term heating treatment on PAH diffusion in soils. Moreover, the temperature plays a central role in diffusion phenomena since solubility, diffusion coefficient, and the K_{ow},octanol-water partition coefficient of pure PAHs are all closely linked to the temperature [34-35]. In the present study, it was shown that a temperature higher than 35°C positively affected the PAH removal. The temperature enhanced the diffusion of the heaviest PAHs as well as the volatilization for the lightest. The increase of temperature also improved the part of PAHs biodegraded that may result from the enhancement of their bioavailability. Unfortunately, thermophilic temperature (55°C) drastically increased the abiotic losses by either volatilization, or unspecific chemical reaction with organic compounds. Because of the sensitivity of the PAHs to the temperature, thermophilic conditions are consequently not recommended for bioremediation of contaminated sludge. In addition, the thermophilic temperature reduced the part of biodegraded PAHs, suggesting a possible disturbance of the degrading microbial communities. Optimal conditions were therefore found at 45°C considering the low abiotic losses and the high PAH biodegradation rates. On the other hand, the addition of methanol to enhance bioavailability showed a positive impact on PAH removal. Since no significant methanol amounts were measured in the outlet of the reactor, it was concluded that methanol did not reach toxic concentrations to inhibit aerobic bacteria. Indeed, the methanol was either totally degraded or evaporated during the process. While no significant increase of the abiotic losses were previously observed, the enhancement of PAH biodegradation was attributed to an increase of PAH bioavailability. Indeed, lower consumption of the organic matter led to a higher efficiency factor suggesting that PAH diffuses from solids to a more bioavailable fraction. Such reduction of the dry weight may be due to a change in microbial population or the use of methanol preferably rather than low biodegradable organic matter. Since less organic matter was degraded and PAH removal remained constant or higher, the methanol may locally have reacted by increasing the solubility of the PAHs when dry weight is consumed. Furthermore, the efficiency factors on biological degradation were even higher here than at 45C, proving the potential of adding a solvent as a bioavailability enhancer, as previously suggested by Lee et al. [36].

The increase of PAH removal implies that higher amounts of PAH were degraded than dry weight consumed. Consequently, the raw PAH concentration in the outlet of the reactor was very low as shown in Table 1. The untreated sludge showed PAH concentrations twice above the French allowed values in sludge. After mesophilic and thermophilic treatment, the treated sludge exhibited PAH concentrations close to the limited values and may not be suitable for long-term use in full-scale plants. In contrast, the amounts of PAHs recovered under intermediary (45°C) or after the addition of methanol were significantly lower than the requirements and could be recommended for further use. Nevertheless, intermediary temperature processes will be preferred since dry weight reduction was higher during the process. In our knowledge, no successful biological process for bioremediation of low PAH-contaminated sludge has been reported in the literature.

4 Conclusions, Recommendations and Outlook

It is commonly assumed that PAH biodegradation is strongly limited by low bioavailability, especially in high organic matter environments such as sewage sludge. In this study, the use of long-term acclimated aerobic ecosystems showed the high potential of aerobic microorganisms to degrade a wide range of PAHs at trace levels. Confirming previous results, the biodegradation of these compounds was likely to be controlled by their low bioavailability. Therefore, the optimization of the bioremediation process focused on the improvement of PAH diffusion from the solids to the aqueous fraction of the sludge. The impact of the temperature or addition of a solvent were tested and could be extended to other diffusion enhancers, such as surfactants. However, the global ecological benefit of using the latter agents for treating trace level contamination must be considered. Since methanol was completely removed during the process, no additional harm could be expected after treatment. In conclusion, two aerobic processes have been proposed to achieve the efficient decontamination of sewage sludge. The PAH concentrations in reactor outlet were lower than the French

requirements, and allow the treated sludge to be spread on agricultural land. Since PAH diffusion controlled their degradation, upscale of the process, however, would be influenced by the hydraulic parameters, such as mixing and aeration rates. Further experimentations at a pilot scale are therefore recommended, as well as the assessment of the global environmental benefit of using aerobic processes in bioremediation of trace level compounds.

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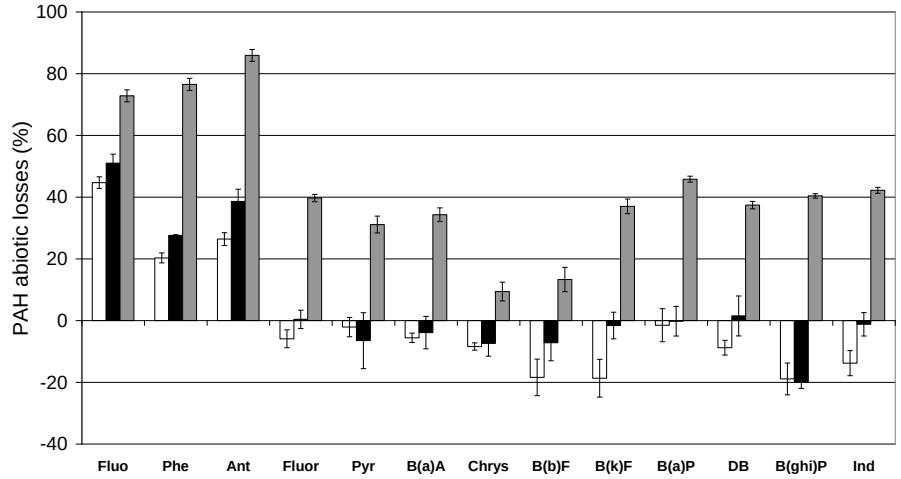

Fig.1: PAH abiotic losses in control reactors at 35°C (white), 45°C (black) and 55°C (gray). Error bars correspond to 95% confidence intervals

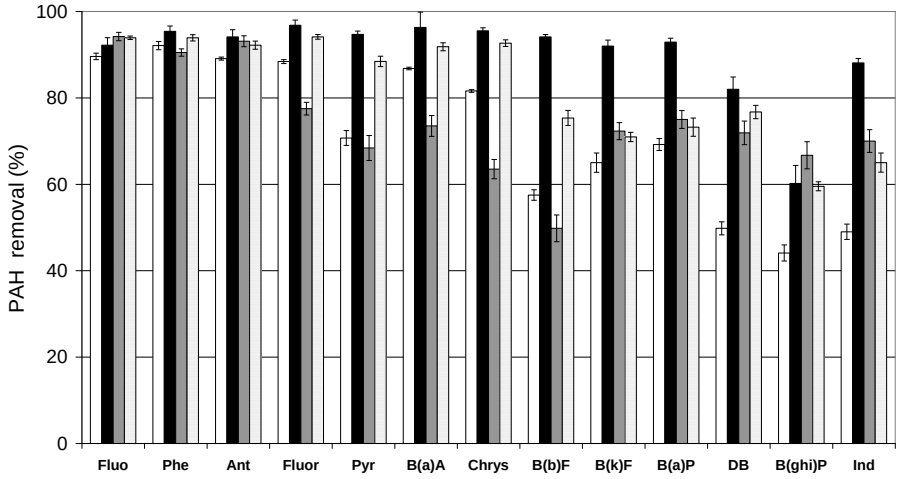

Fig. 2: PAH removal in biological reactors at 35°C (white), 45°C (black), 55°C (gray), and at 35°C with addition of methanol in reactor inlet (dots). Error bars correspond to 95% confidence intervals

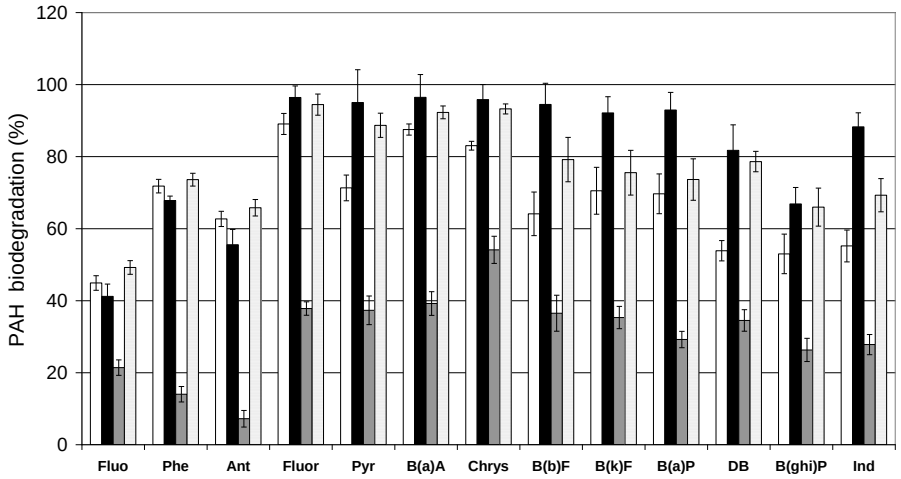

Fig. 3: PAH biological degradation in bioreactors at 35°C (white), 45°C (black), 55°C (gray), and at 35°C with addition of methanol in reactor inlet (dots). Error bars correspond to 95% confidence intervals

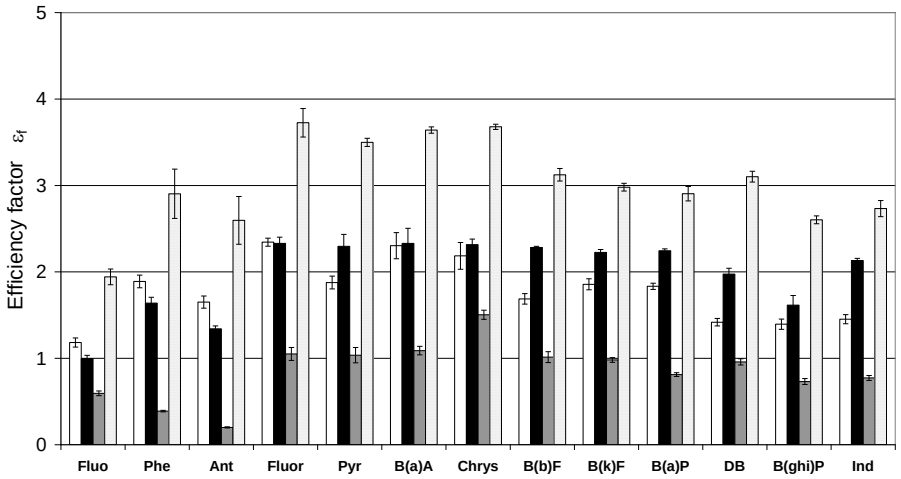

Fig. 4: Biological efficiency factor (ε_f) in bioreactors at 35°C (white), 45°C (black), 55°C (gray), and at 35°C with addition of methanol in reactor inlet (dots). Error bars correspond to 95% confidence intervals

Table 1: Average PAH concentrations (in $mg \cdot kg_{DW}^{-1}$) in sludge before and after aerobic treatment at 35°C, 45°C, or in presence of methanol (250 mM). Relative standard deviation errors are lower than 10% for all values (data not shown). The 'French max values" correspond to the PAH concentrations allowed in sludge to be spread on agricultural land, according to the current French regulation

PAH	Fluo	Phe	Ant	Fluor	Pyr	B(a)A	Chrys	B(b)F	B(k)F	B(a)P	DB	B(ghi)P	Ind
Untreated	0.50	3.36	0.71	8.79	6.54	3.70	4.43	3.45	2.34	4.06	0.59	2.76	2.94
Treated [35°C]	0.09	0.45	0.13	1.64	3.27	0.80	1.30	2.41	1.34	2.24	0.48	2.54	2.52
Treated [45°C]	0.06	0.25	0.07	0.59	0.93	0.24	0.33	0.43	0.32	0.46	0.16	1.72	0.61
Treated [55°C]	0.04	0.55	0.12	3.5	4.2	2.07	2.81	3.34	1.22	1.85	0.38	1.43	2.09
Treated [methanol]	0.04	0.25	0.07	0.86	1.6	0.42	0.42	1.43	0.91	1.36	0.17	1.38	1.42
French max values	-	-	-	5	-	-	-	2.5	-	2	-	-	-