Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge

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Abstract Anaerobically stabilized sewage sludge has potential to partially substitute synthetic fertilizers. The main risk with the recycling of urban sludge on agricultural soils is the accumulation of unwanted products, such as trace metals and organic micropollutants. In this context, the polycyclic aromatic hydrocarbons (PAHs) are particularly monitored because of their toxic properties at low concentrations and their high resistance to biological degradation. The aim of the present study was to optimize PAHs removal during anaerobic digestion of contaminated sewage sludge. Thirteen PAHs were monitored in laboratory-scale anaerobic bioreactors under mesophilic (35°C) and thermophilic (55°C) methanogenic conditions. Abiotic losses were statistically significant for the lightest PAHs, such as fluorene, phenanthrene and anthracene. It was shown that PAH removal was due to a specific biological activity. Biological PAHs removal was significantly enhanced by an increase of the temperature from 35°C to 55°C, especially for the heaviest PAHs. Bioaugmentation experiment was also performed by addition of a PAH-adapted bacterial consortium to a non-acclimated reactor. Significant enhancement of PAHs removal was observed. It was finally shown that PAH removal efficiencies and methanogenic performances were closely linked. The rate of biogas production may be used as an indicator of bacterial activity on PAH removal.

Keywords Bioaugmentation; biogas yield; mesophilic conditions; methanogenesis; sewage sludge; thermophilic conditions

Introduction

Recycling of sewage sludge by spreading on agricultural lands is common throughout the European Community. In France, 60% of urban sludge is recycled on land versus 25% for landfilling and 15% for incineration (Wiart *et al.*, 1999). Considering that production of sewage sludge reaches more than 8×10^6 tons of dry matter per year in Europe, their treatment and their disposal constitute a great problem for environmental protection and local communities management.

Sewage sludges are usually treated before disposal or recycling in order to reduce their water content, their fermentation propensity and the presence of pathogens. The sludge stabilization processes include anaerobic digestion, aerobic digestion, lime stabilization and composting (Lue-Hing *et al.*, 1992). Although sludge composting is widely used, anaerobic digestion of sewage sludge is a good alternative choice for high sludge-producing units. Anaerobic digestion presents several advantages, such as low rate of biomass production, high disinfecting potential, low needs of energy and production of biogas. This biogas, which is typically composed of 65% methane and 35% carbon dioxide, can be combusted directly in modified gas boilers to produce energy. However, the anaerobic digestion processes require high investments and considerable maintenance. This process is strictly reserved to the highest sludge-producing wastewater treatment plants. Anaerobic digestion can be performed under mesophilic (30–35°C) or thermophilic (about 55°C) conditions. Thermophilic systems offer more advantages, including higher methane production, higher dilution rate, better pathogen reduction and the production of a consistent liquid residue. However, thermophilic systems are more expensive and require greater levels of control. In both cases, production of methane results from synergistic interactions between various

groups of bacteria, including hydrolytic bacteria, fermentative acidogenic–acetogenic bacteria and methanogens (Bitton, 1994). One of the characteristics of methanogens is that microbial activity correlates well with biogas production rates depending on the substrate and the bacterial consortium composition (Bitton, 1994). Biogas production for sludge digestion traditionally ranges from 0.2 to 0.4 litre per gram of degraded COD according to sludge origin and bacterial activity (Arundel, 2000).

One of the main risks with recycling of urban sludge is the accumulation in agricultural soils of unwanted products, such as trace metals and organic micropollutants. The PAHs are particularly monitored in this context because of their high toxic and high carcinogenic properties even at low concentrations. PAHs are widely distributed in the environment due to numerous sources of production. In the case of wastewater treatment plants, they are easily concentrated into sewage sludge because of their low water solubility and their high affinity for organic compounds. The PAHs are known to be highly recalcitrant to biodegradation due to a low bioavailability. Nevertheless, their biodegradation has been largely studied under aerobic conditions. In comparison, less is known under anaerobic conditions. In a first study, Mihelcic and Luthy (1988) reported PAH biodegradation under anaerobic-denitrifying conditions for low molecular weight PAHs, such as naphthalene and acenapthene. Several authors have since reported biodegradation of naphthalene, phenanthrene and fluoranthene under more reduced sulfate-reducing conditions, in marine sediments (Coates *et al*., 1996; Rockne and Strand, 1998). So far only non-significant PAH biodegradation under methanogenic conditions (Coates *et al*., 1997; Karthikeyan and Bhandari, 2001; Kirk and Lester, 1990) has been reported. However, the degradation potential of methanogenic ecosystem is well extended and, after hard natural selection, methanogenic microorganisms participate in the degradation of uncommon compounds, such as aliphatic hydrocarbons and low aromatic compounds, benzene, toluene, benzoate and naphthalene (Schink, 1988). Recently, in our laboratory significant disappearance of 13 PAHs was demonstrated under methanogenic conditions (Trably *et al*., 2002a). A specific biological PAH degradation by a long-term adapted anaerobic consortium was suspected.

The aim of the present study was to optimize biological PAH removal during anaerobic digestion of sewage sludge. This paper describes the influence of the temperature on PAH removal during methanogenic digestion of PAH-contaminated sewage sludge by a long-term PAH-adapted ecosystem. PAHs behavior was characterized under mesophilic (35°C) and thermophilic (55°C) methanogenic conditions. Bioaugmentation experiments in a non-adapted complex ecosystem were also performed by addition of a PAH-adapted bacterial consortium. Finally, methanogenic performances were studied as an indicator of bacterial activity on PAH removal.

Methods

Anaerobic reactor

Studies were performed in laboratory-scale continuous stirred tank reactors. The operating parameters were fixed to simulate the main conditions encountered in industrial sewage sludge digesters. The reactors were performed under perfectly mixed conditions and in a continuous mode with a hydraulic retention time of 40 days and a daily organic load of 1 $kg_{COD}.m^{-3}.d^{-1}$. The reactor volumes were of 5 litres and their homogeneity was provided by magnetic stirring. pH was not regulated but did not evolve significantly between the assays (7.6 ± 0.1 pH units). Biogas outlet was cooled to avoid water losses which would be particularly significant in the case of thermophilic processes. The biogas was collected in storage column of 5 litres in order to estimate the daily biogas production. The analysis of gases was performed by gas chromatography – catharometer detection. The temperature

was maintained at 35°, 45°C or 55°C with a water bath. All of the reactors were continuously fed with a long-term PAH-contaminated sewage sludge. In the substrate storage tank, microbial degradation was limited by cooling the substrate until its entrance to the reactor. After reaching steady state, PAHs removal efficiencies were calculated for each reactor by an average of 5 to 7 outlet samples. Sterile reactors were used to estimate PAH abiotic losses of the mesophilic and thermophilic anaerobic reactors. These control reactors were chemically sterilized by addition of 100 mM sodium azide (NaN_3 – Riedel de Haën).

The reactors were initially charged with 5 litres of an anaerobic methanogenic ecosystem. Two starting inoculum were used: the PAH-adapted ecosystem corresponded to the outlet of a long-term PAH-contaminated methanogenic digester. It was hypothesized that a natural selection of the microbial consortium occurred in the contaminated digester. In order to evaluate the influence of the inoculum origin, a non-adapted ecosystem was also used. This non-adapted inoculum corresponded to the outlet of a methanogenic digester which had never been in contact with significant PAH contamination. The bioaugmentation experiment was performed by starting inoculation with a sludge mixture composed of 10% of adapted microbial consortium and 90% of non-adapted ecosystem. In the inlet of the reactors, the substrate corresponded to a mixture of primary and secondary sewage sludge *in situ* contaminated with PAHs. The use of a long-term *in situ* contaminated sludge is strongly recommended for the consideration of the highly complex interactions between PAHs and organic solids. Spiked sludge would not provide the real bioavailability of PAHs to micro-organisms. PAH concentrations in contaminated sewage sludge were as follows: fluorene, 15 ± 0.3 μg.l^{-1}; phenanthrene, 100 ± 2 μg.l^{-1}; anthracene, 20 ± 0.4 μg.l^{-1}; fluoranthene, 295 ± 6 μg.l^{-1}; pyrene, 200 ± 4 μg.l^{-1}; benzo(a)anthracene, 120 ± 2.4 μg.l^{-1}; chrysene, 140 ± 3 μg.l^{-1}; benzo(b)fluoranthene, 105 ± 2 μg.l^{-1}; benzo(k)fluoranthene, 75 ± 2 μg.l^{-1}; benzo(a)pyrene, 120 ± 3 μg.l^{-1}; dibenzo(ah)anthracene, 20 ± 0.5 μg.l^{-1}; benzo(ghi)perylene, 40 ± 1 μg.l^{-1} and indeno(123cd)pyrene, 100 ± 2 μg.l^{-1}.

PAH analysis

Analytical methods were previously tested and validated in the laboratory by the use of a certified matrix (Trably *et al*., 2002b). They exhibit repeatability and reproducibility errors lower than 2%. The reactor sludge samples corresponded to a three-day collection of the outlet of each reactor. The minimum volume of sample for PAH analysis was about 350 ml. The substrate feeding tank was changed once a week and an aliquot of 350 ml was taken for analysis. Total solids were determined by drying 20 ml of the sludge sample in an oven at 110°C for 24 hours. The remaining other 300 ml of the sludge sample were first centrifuged (20,000 g, 25 min.). Aqueous phases were stored at –20°C for further solid-phase extraction. Solid pellets were ground with glass beads (diameter 4 mm) and were dried in a ventilated oven (60 hours at 40°C). Dry samples were sieved on grid (diameter 2 mm) and were stored at –20°C for further accelerated solvent extraction.

In a first step, PAHs were extracted from the liquid phase by solid-phase extraction on PAH-affinity column (Supelco ENVI-18™) according to the Supelco recommendations. Regardless of the operating conditions, the aqueous concentrations of the PAHs were not significant in comparison with PAH concentrations in solids. The liquid/solid PAH ratio was always lower than 1% for all of the PAHs (data not shown). Therefore, the aqueous PAH values were neglected in all the assays. In a second step, PAHs were extracted from dried and sieved samples with a ASE-200 system (DIONEX™). The extracting solvent was a mixture of hexane/acetone (50/50, v/v). Extracting parameters were as follows, temperature, 120°C; pressure, 100 bars; cycles of extraction, 2; static time, 5 minutes; cell flush, 60% and purge time, 120 sec. The extracting cells were filled with 0.5 g of the dry sample, 1g of polar-molecules purifying alumina (provided by SIGMA® A-1522) and 1.5 g

of hydromatrix-celite dispersing compound (provided by VARIAN®). The extract was evaporated under nitrogen flow to dryness. Residues were dissolved in 3.9 g of acetonitrile. The extract was vigorously agitated (2 min.), let diffused (30 min.) and analyzed by reverse phase-high performance liquid chromatography. The analytical chain of RP-HPLC was composed of a multi-sample injector (Waters-717-Plus), a solvent degasser (Waters inline Degasser), a peristaltic pump system (Waters-600 controller) and a fluorimetric detector (JASCO FP-1520). PAH separation was performed with a PAH high-selective column (Bakerbond™ PAH 16-plus). The elution temperature was maintained constant at 25°C. The flow rate was fixed at 0.3 $ml.min^{-1}$. The linear gradient elution (35 min.) started after 5 min. of elution with solvent mixture from 40% acetonitrile – 60% water to 100% acetonitrile. After 70 min., the column was rinsed with 40% acetonitrile – 60% water. Total analytical time was about 95 minutes. The fluorimetric detector was calibrated by injection (20 μl) of standard solutions (Ultra Scientific PM-612-1 PAH mixture). The fluorimetric PAH detecting program was optimized for each PAH and was as follows: fluorene (266/312), phenanthrene (250/370), anthracene (250/400), fluoranthene (280/430), pyrene (320/404), benzo(a)anthracene (280/430), chrysene (268/384), benzo(b)fluoranthene (234/420), benzo(k)fluoranthene – benzo(a)pyrene (270/400), dibenzo(ah)anthracene – benzo(ghi)perylene (300/407) and indeno(123cd)pyrene (300/500).

Results and discussion

Effect of temperature on PAH removal

Anaerobic digestion of PAH contaminated sewage sludge was performed under mesophilic (35°C), intermediate (45°C) and thermophilic conditions (55°C). All of the reactors were conducted under methanogenic conditions. Methanogenic activity was identified by production of a characteristic biogas with methane content about 70–75%. No volatile fatty acids were detected. Solids reduction rates were about 50% for the adapted bioreactors (ADA35, ADA45 and ADA55). All these results were highly consistent with a complete methanogenic degradation pathway.

PAH abiotic losses were estimated with sterile control reactors working at 35°C, 45°C and 55°C, respectively named CR35, CR45 and CR55. PAH removal efficiencies were calculated by comparison of inlet and outlet PAH concentrations of each control reactor after reaching steady state. The results are presented in Figure 1. PAH abiotic losses were strictly limited to the lightest PAHs, such as fluorene, phenanthrene and anthracene. They became highly significant with the increase of the temperature: more than 50% of fluorene were lost at 55°C versus 25% at 35°C. However, PAH abiotic losses depend greatly on the operating conditions, such as temperature, stirring and source of PAH contamination (natural or spiked sludge). Kirk and Lester (1990) previously reported significant abiotic losses for all of the PAHs during methanogenic digestion of spiked sewage sludge. As a long-term PAH-contaminated sludge was used as substrate in our case, PAHs were hardly linked to the organic matrix. They were less dependent on abiotic losses than PAHs of spiked sludge. It was also observed that larger amounts of heavy PAHs were recovered in the outlet of the thermophilic control reactor, from benzo(b)fluoranthene to indeno(123cd)pyrene. As no water losses were measured during the process, it was suggested that PAH diffusion was greatly enhanced under thermophilic conditions. PAH mass transfer occurred from non-extractable to extractable fraction of solids implying higher concentrations in the outlet compared to the inlet. As a part of the heaviest PAHs was hardly linked to organic compounds in long-term contaminated sewage sludge, they were not totally recovered by the analytical method and were more available to PAH extraction after diffusion in solids.

PAH removal efficiencies were also calculated in the case of biological reactors working under methanogenic conditions at 35°C (ADA35), 45°C (ADA45) and 55°C (ADA55)

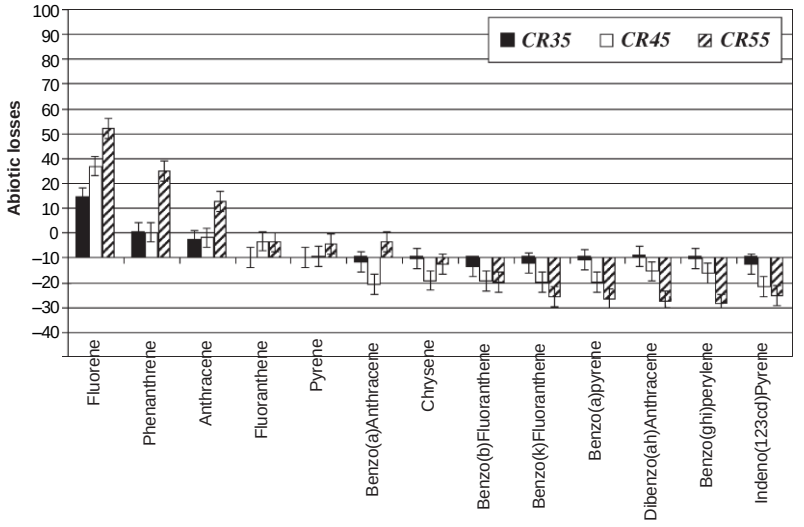

Figure 1 PAH abiotic losses in control reactors at 35°C (CR35), 45°C (CR45) and 55°C (CR55)

(Figure 2). Under mesophilic conditions (35°C), two groups of PAH performances were distinguished: in the first group, removals of the lightest PAHs, such as fluorene, phenanthrene and anthracene, were increased by abiotic losses. In the second group, other removals were statistically similar for all of the PAHs at 35°C with an average of 46 ± 4%. Removals were influenced by neither the PAH molecular weight nor the PAH concentration in sewage sludge. They rather seemed to be associated or limited by the solid reduction rate of approximately 50%. Similar results were observed at 45°C, with an average removal of 40 ± 6%. PAH removal efficiencies were slightly lower compared to the two other cases, indicating that this temperature was a transient condition between mesophilic and thermophilic conditions. For all the PAHs, removals were greatly enhanced under thermophilic conditions (55°C). A large part of the lightest PAH removals resulted from a significant increase of the abiotic losses. For the heaviest PAHs, removals were enhanced because of the putative increase of PAH diffusion rate from solids to more bioavailable compartment. Furthermore, the solids reduction rate was also about 50% under thermophilic conditions. In contrast to mesophilic and intermediate conditions, PAH removal efficiencies were 53 ±

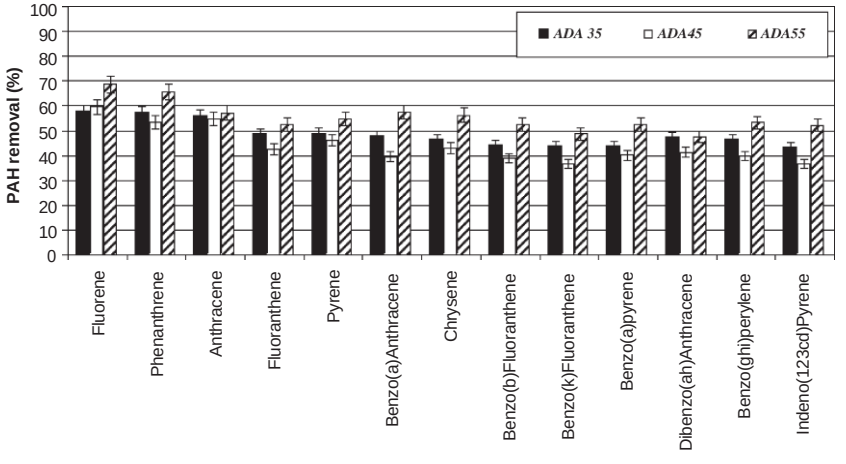

Figure 2 PAH removal in methanogenic reactors at 35°C (ADA35), 45°C (ADA45) and 55°C (ADA55)

6% and were significantly higher than the solid reduction rate. Therefore, PAH concentration in solids, which are targeted in legislative procedures, decreased significantly during the thermophilic process.

Regardless of the operating conditions, disappearance of 13 PAHs was observed with a significant change of temperature. However, it could not yet be concluded to be effective biodegradation. The PAH disappearance could be the result of either non-specific transformation or PAH incorporation into a non-extractable fraction. In order to confirm the involvement of the biological activity in PAH removal, experiments with non-acclimated and bioaugmented ecosystems were performed.

Bioaugmentation experiments and methanogenic performances

Anaerobic biological reactors were initially charged with 5 litres of methanogenic ecosystem. Bioaugmentation experiment was performed by addition of a long-term PAH-adapted ecosystem to a non-adapted inoculum (10% of the total volume). PAH removals were calculated for PAH-adapted, non-adapted and bioaugmented bioreactors, respectively ADA35, NADA and NADA10%. Results are presented in Figure 3. As expected, PAH removals of the non-adapted NADA reactor were significantly lower than PAH removal efficiencies of the PAH-adapted ADA35 reactor, especially the heaviest PAHs. Removals were significantly enhanced by the addition of the adapted ecosystem into the non-adapted reactor (NADA10% reactor). However, removals were significantly influenced by the number of rings of PAH in both the non-adapted reactors. Under aerobic conditions, it is well known that the heaviest PAHs have the lowest biodegradability (Sutherland *et al*., 1995). The low heavy-PAH removals in non-adapted reactors may be the result of biological limitation. A long-term acclimation of the inoculum was necessary to remove PAHs, especially for the heaviest ones. Moreover, solids reduction rates were about 25% for NADA reactor and 35% for NADA10% reactor. As previously hypothesized for adapted reactors, PAH removals may be the consequence of PAH incorporation into non-extractable fraction of solids. In this case, all of PAH removals would be about 25% for the NADA reactor and 35% for the NADA10% reactor. In contrast, PAH removals of non-adapted reactors were lower than the solids reduction rates. Therefore, disappearance of PAH in the adapted reactors was mainly due to a specific biological activity and did not only result from PAH incorporation into the non-extractable fraction of solids.

In the NADA10% bioaugmented reactor, the addition of adapted ecosystem was not

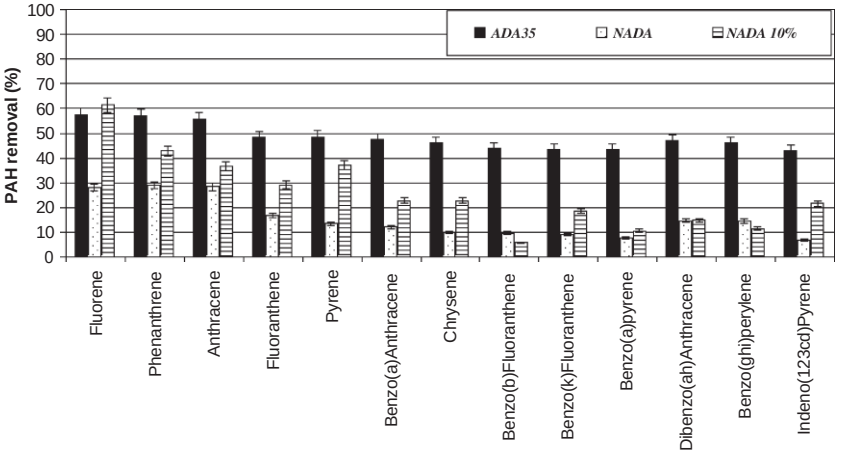

Figure 3 PAH removal in methanogenic reactors ADA35, NADA and NADA10%, inoculated respectively with PAH adapted, non-adapted and bioaugmented (10%) ecosystems

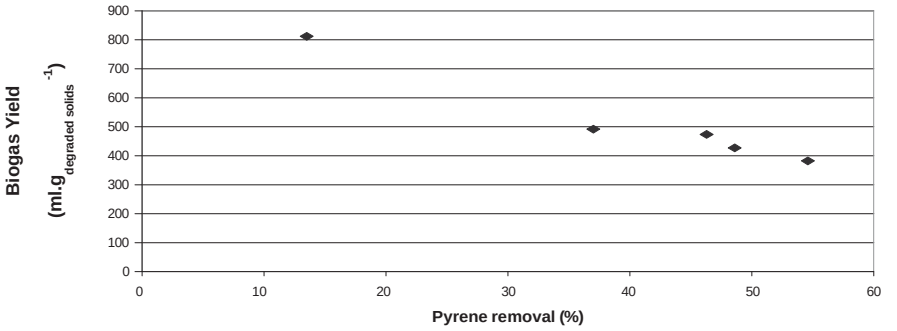

Figure 4 Correlation between PAH removal (%) and the biogas yield ($ml_{biogas} \cdot g_{degraded\ solids}^{-1}$) for pyrene

sufficient to reach performances similar to those of the totally adapted reactor. The gain of PAH removal performances between non-adapted and bioaugmented reactors did not depend on the PAH molecular weight. It appeared that this gain was 33.4% for fluorene and 23.4% for pyrene. For the other PAHs, the increase of their removal was between 10 to 15%. Benzo(b)fluoranthene, dibenzo(ah)anthracene and benzo(ghi)perylene removals were not enhanced by addition of adapted bacterial consortium. As PAH concentrations in sewage sludge were very low, from 20 to 300 μg per litre of sludge, implementation and selection of PAH-degrading bacteria require a long time in the hardly selective and complex environment of methanogenic digesters, to obtain sufficient adapted consortium activity.

Moreover, it was observed that PAH removal efficiencies seemed to be closely associated to the biogas production rate (Figure 4). Biogas yield decreased proportionally with the increase of PAH removal. Methanogenic activity was not inhibited because of the constant methane content between the different assays. The variation of yield of biogas production between reactors was due to similar biogas production in spite of different solids reduction rates (50% for the adapted reactors and 25% for NADA reactor and 35% for NADA10% reactor). The lowest biogas production rates corresponded to the PAH-adapted reactors. This result suggested a difference of bacterial population composition between the assays and a change of the substrate specificity. It cannot be directly concluded that PAHs were the sole origin of the change of biogas yields because of the very low PAH concentration in sludge. However, with regard to the result, the yield of biogas may be used as an indicator of the bacterial activity on PAH removal.

Conclusions

Removal of 13 priority PAHs was evaluated under methanogenic anaerobic digestion of contaminated sewage sludge. An increase of the temperature significantly enhanced PAH removal, especially for the heaviest PAHs. Mass transfer diffusion rate and PAH bioavailability were probably increased under thermophilic conditions. PAH abiotic losses were only limited to the lightest PAHs during the anaerobic process. Biological PAHs removal was suggested under methanogenic conditions. PAH removal required a long-term acclimation of the methanogenic ecosystem and seemed to be limited by the solids reduction rates in adapted reactors. In contrast, the non-adapted ecosystem presented low PAH removal efficiencies and its activity was significantly limited by the number of rings of PAHs traducing a biological limitation. Bioaugmentation of a non-acclimated ecosystem was successful with a significant increase of the PAH removal. A hard and long-time selection of PAH-degrading micro-organisms in such complex environment is necessary to obtain effective removal of PAH. Moreover, it was shown that removal efficiency correlated well with the methanogenic activity. The biogas yield was directly associated to

PAH removal. It may be used to evaluate the bacterial activity on PAH removal in contaminated ecosystems. As an indicator of the microbial population composition, it may reveal the involvement of methanogenic bacteria on PAH removal.

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