Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion

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Abstract Anaerobic digestion for the treatment of sludge in wastewater treatment plants has been reported to produce a low organic loaded effluent with an acceptable economic cost. But in the last years, new regulations and the increasing sludge production invite us to find an alternative and/or to improve the process efficiency. Moreover, the use of the effluent as fertilizer in agriculture imposes more restrictions on digestion process product and its micropollutant contents to protect the environment.

In this study, a performance of the anaerobic digestion under mesophilic and thermophilic conditions at different hydraulic retention times (HRT) is assessed and the removal efficiencies of two important compounds or family compounds (Polycyclic Aromatic Hydrocarbons, PAH, and Di-2-(Ethyl-Hexyl)-Phthalate, DEHP) are evaluated. A positive effect of thermophilic temperature was observed on both micropollutants’ biodegradation. However, HRT effect also had an important role for DEHP and low molecular weighted PAH removal.

Keywords Anaerobic; biodegradation; DEHP; HRT; mesophilic; PAH; sludge; thermophilic

Introduction
The increasing amount of sludge from wastewater treatment plants (WWTPs) and the restrictive legislations on their management and final destination invite us to enhance the actual treatment processes and/or to find a reliable alternative. In Europe, since new legislations have been approved, it was estimated that the sludge produced has increased more than 50% from 1992 to 2005 (CEC, 2000). Nowadays, the main destinations of this product are landfilling, incineration and soil organic fertilization. This last destination is the preferred option of the EU legislation due to a positive effect on nutrients recycling and organic material reconstitution in the soil.

However, sludge reuse in agricultural soil would satisfy healthiness conditions in order to avoid unwanted agents and transference of harmful elements to the receptor medium. Within these elements, organic micropollutants are distinguished. In the third draft presented to EU commission (CEC, 2000) some compounds and family compounds were selected for limiting their content in the dry sludge. Polycyclic Aromatic Hydrocarbons (PAH) family and Di(2-ethyl-hexyl)-phthalate (DEHP) were within the selected organic compounds.

The PAH compounds are the product of incomplete combustion of organic substances such as fossil fuel, wood and mineral oil. DEHP represents 90% of the annual total phthalate amount production (4.2 million Mt) and, generally, has an industrial use, mainly as plasticizer. Due to the hydrophobic character of both PAH and DEHP, these compounds tend to accumulate in the solid matter such as the sludge. Typical PAH and DEHP contents in the sludge lie in the range of 1−10 mg/kg dw and 10−100 mg/kg dw, respectively (CEC, 2000).
PAH
Anaerobic stabilization is a common treatment reserved to sludge in WWTPs. However, to reduce some organic pollutant content, some studies showed doubts about their efficiency (Klecka et al., 1990; Volkering et al., 1993; Ghoshal et al., 1996). In contrast, other researchers (Coates et al., 1996; Rockne and Strand, 1998) observed an acceptable PAH degradation rate, especially for low molecular weight (LMW) PAH, such as naphthalene, acenaphthene and phenanthrene. Chang et al. (2003) reported 3.5 d as the half-life of some PAH compounds under methanogenic conditions, but without clear preference for LMW PAH. In a recent study carried out by Trably et al. (2003), a biodegradation of 13 PAH compounds including high molecular weight (HMW) PAHs was observed.

Temperature effect on PAH degradation was studied by some authors (Feitkenhauer and Märk, 2003; Feitkenhauer et al., 2003) who stated an increase of naphthalene solubility (approximately 10%) when the temperature was increased from 20 to 75°C under aerobic conditions. Besides, Trably et al. (2003) observed an increase from 46% to 53% in the elimination rate of 13 PAH when temperature was increased from 35°C to 55°C. Christensen et al. (2004) reported an enhancement from 10–50% to 60–85% in naphthalene biodegradation rate when the temperature range was changed from mesophilic to thermophilic conditions. This improvement was related to compound transference to aqueous medium, which is intensified with increasing temperatures. However, this assumption must be verified since temperature effect under anaerobic conditions on diffusion and sorption phenomena is still not well defined (Holliger and Zehnder, 1996).

On the other hand, from sterilized reactors, an important abiotic removal rate was observed, especially for fluorescence PAH (phenanthrene, fluorene and anthracene). It was estimated to be more than 50%, 40% and 25% at 55, 45 and 35°C, respectively (Trably et al., 2003). Similar results were reported by Christensen et al. (2004) for naphthalene.

DEHP
DEHP removal from sludge before its use as a soil conditioner is an important preventive action, since the aged xenobiotic in soil is more recalcitrant (Bollag, 1992; Alexander, 1995; Hatzinger and Alexander, 1995; Madsen et al., 1999). DEHP recalcitrance is due to its high hydrophobicity index and its tri-dimensional structure which obstructs the enzymatic hydrolysis (Ejlertsson et al., 1997).

DEHP aerobic removal was verified by different studies. Marttinen et al. (2004) reported 4%, 33–41% and 50–62% as elimination percentage at a retention time of 1, 7 and 28 d, respectively. Likewise, Banat et al. (1999) observed a 30–40% of DEHP elimination in an activated sludge system at 20°C. However, from various studies carried out on DEHP monitoring in anaerobic systems, anaerobic DEHP removal was not confirmed (Horowitz et al., 1982; Shelton et al., 1984; O’Conner et al., 1989; Ziogou et al., 1989; Ejlertsson et al., 1996; Ejlertsson and Svensson, 1996). Ejlertsson et al. (1997) observed a removal efficiency within a range of 87–91% for some phthalates with high solubility water index (11.2–50 mg/L) at incubation time ranging from 35 to 100 d, but no removal index was detected for DEHP, whose solubility coefficient was estimated as 3 μg/L. On the other hand, Reinhart and Pohland (1991) experienced the total disappearance of DEHP from municipal solid waste incubated in a methanogenic lysimeter after 4 years. Madsen et al. (1999) estimated a mineralization portion of 32% of the initial DEHP content (1.6 mg/kg dw) in sludge-amended soil after 1 year of incubation period under anaerobic conditions at 20°C.

The anaerobic degradation of DEHP was reported to depend on the inoculum used. The use of landfill leachate was verified as an efficient seed for phthalates biodegradation
including DEHP (Angelidaki et al., 2000). Gavala et al. (2003), estimated DEHP anaerobic degradation adsorbed in primary sludge between 0.0035 and 0.0099 d⁻¹ with half-life time in the range of 198–70 d after anaerobic mesophilic digestion.

Recently, the temperature effect on DEHP biodegradation was investigated by Banat et al. (1999), who observed an increase of DEHP biodegradation from 22% to 31% and 44% with temperature increase from 20°C to 52°C and 62°C, respectively, in activated sludge aerobic treatment. On the other hand, Marttinen et al. (2004) recorded DEHP elimination rate in the range of 30–60% in composting process for primary, activated and anaerobic sludge treatment with initial content between 57–77 mg/kg dw.

The use of hyperthermophilic processes (68°C and 5 d as HRT) for treating thermophilic anaerobic digester effluent enhanced DEHP elimination by an increase from 9.6% to 34–53% (Hartmann and Ahring, 2003).

The aim of this study is to compare the efficiency of mesophilic and thermophilic conditions on PAH and DEHP micro-pollutants’ biodegradation at different hydraulic retention times (HRT), during anaerobic sewage sludge digestion.

Materials and methods

Experimental set-up

Two completely mixed and jacketed anaerobic digesters (5 L) were used in this study. Each one was seeded with 3.5 L of waste activated sludge (WAS) from a municipal WWTP of the Barcelona Metropolitan Area. The operating temperature was controlled by means of two heating systems (Haake DC 40) at 55°C and 35°C for the thermophilic and mesophilic reactors, respectively.

The seed acclimation to anaerobic conditions and the adaptation to mixture of primary and secondary sludge lasted 60 and 80 days for mesophilic and thermophilic digester, respectively. After the process start-up, the steady HRT reached was 35 days (mesophilic) and 30 days (thermophilic). Subsequently, the operating HRT of both digesters was reduced following the procedure proposed by Rimkus et al. (1982), where the successive increase amount was ~ 14% of initial VS reactor content. In the mesophilic digester, the HRT was reduced during 13 weeks until an HRT of 18 days was reached. In the thermophilic digester, the minimum HRT obtained was 8 days (this process lasted 30 weeks), since below this HRT value, the biological system became unstable (it was inhibited by high volatile fatty acids (VFA) accumulation inside the anaerobic reactor).

The influent and effluent samples corresponding to selected HRTs (26, 22, 18, 12 and 8 days) were collected in crystal vessels and kept at −10°C for micro-pollutant analysis (see Table 1).

Analytical methods

PAH and DEHP were analysed using solvents and chemicals of analytical grade (Merck, Barcelona), simultaneously with other micropollutants. 16 PAH compounds mixture (including EU-PAH: sum of low molecular weight (LMW) PAH (acenaphthene, phenanthrene, fluorene) and high molecular weight (HMW) PAH (fluoranthene, pyrene,

| Table 1 | Total solid content (g TS/L) in feed (F), thermophilic (T) and mesophilic (M) sludge at selected HRTs |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| HRT (d)         | 26              | 22              | 18              | 12              | 8               |                 |
| F (TS)          | 41.21           | 39.83           | 38.91           | 43.21           | 42.21           |                 |
| T (TS)          | 32.28           | 33.39           | 34.14           | 34.32           | 34.41           |                 |
| M (TS)          | 31.42           | 31.56           | 31.53           | –               | –               |                 |
benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3-c,d)pyrene) was supplied by Supelco (Barcelona). DEHP (95% purity) and anthracene d_{10} were supplied by Fluka (Barcelona) and Sigma-Aldrich (Barcelona), respectively. Sample analysis was carried out as follows: 10 g of lyophilized samples, initially spiked with anthracene d_{10}, were reflushed in a Soxhlet apparatus filled with a mixture of Dichloromethane: n-Hexane “DCM: Hex” (1:1) during 24 hours. The extract was purified and dried on alumina-sodium sulphate column by means of several elution solvents with different polarities, namely, (i) Hexane, (ii) Hex: DCM (1:1), (iii) DCM: AcEt “ethyl acetate” (8:2) and (iv) DCM:MeOH “methanol” (9:1). Then, eluates were concentrated under a gentle N_{2} stream and were reconstituted in 1 mL Hex, before their injection into GC/MS. The GC was equipped with an HP-5MS column. Both PAH and DEHP compounds were recovered in the second fraction (ii) of the elution solvents used (DCM: Hex).

For PAH analysis, the samples were injected splitless into a gas chromatograph (Shimadzu QP2010) by an automatic sampler (Shimadzu AOC-20i+S). The injector temperature was 280°C and the flow 1 mL/min. The oven was set to an initial temperature of 60°C for 1 min followed by an increasing temperature (10°C/min) up to 320°C which was kept for 5 min. Helium was used as gas carrier. The MS was run in SIM mode, which differentiated two groups, the first from 128 to 188 m/z and the second from 202 to 278 m/z.

For DEHP analysis, the flow was set at 1.01 mL/min and the injector temperature was 280°C. The oven temperature programme had an initial temperature of 60°C for 3 min, followed by an increase (15°C/min) to 200°C; subsequently, the increasing rate was changed to 3°C/min until 300°C that was kept for 5 min. The last increasing rate was 5°C/min, until 320°C was reached. At this temperature, the oven was kept in isothermal conditions during 3 min. The SCAN mode was employed for identifying the DEHP corresponding peaks (primary ion 149 m/z and the secondary ions 167 and 279 m/z).

To estimate method recovering index, for DEHP, PCB and NP/E, another sample from feed sludge was spiked with the corresponding component with an amount corresponding to 50 mg/kg dw of DEHP.

Although not shown here, analyses of total chemical oxygen demand (COD_{t}), total solids (TS), volatile solids (VS), pH, bicarbonate alkalinity, gas production, composition of gas (methane and carbon dioxide fraction) and individual VFAs were performed according to the Standard Methods (1992) to assure the good digester behaviour.

**Results and discussion**

**PAH biodegradation**

PAH recovery indexes obtained in this study are presented in Table 2. From Table 3, it is observed that the anaerobic digestion treatment of sewage sludge results in a biodegradation of PAH, since PAH content in the fresh sludge was higher than in the treated sludge. Apparently, the total PAH content in the treated sludge was more influenced by its initial amount in the feed than by the HRT tested. In Table 3, it is stated that all values of total PAH in the thermophilic treated sludge for every HRT tested were below the cut-off

**Table 2 PAH mixture (anthracene d_{10}) and DEHP recovery indexes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HRT (d)</th>
<th>26</th>
<th>22</th>
<th>18</th>
<th>12</th>
<th>8</th>
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<tbody>
<tr>
<td>Anthracene d_{10}</td>
<td>Feed (%)</td>
<td>83.22</td>
<td>96.44</td>
<td>88.05</td>
<td>85.64</td>
<td>91.23</td>
</tr>
<tr>
<td></td>
<td>Meso (%)</td>
<td>82.00</td>
<td>90.51</td>
<td>93.05</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Thermo (%)</td>
<td>77.65</td>
<td>89.87</td>
<td>87.76</td>
<td>84.60</td>
<td>91.70</td>
</tr>
<tr>
<td>DEHP</td>
<td>Feed (%)</td>
<td>85</td>
<td>92</td>
<td>96</td>
<td>93</td>
<td>89</td>
</tr>
</tbody>
</table>
limit content (6 mg/kg dw) cited in the third draft (CEC, 2000). However, the mesophilic sludge PAH content was higher than 6 mg/kg dw at an HRT of 26 d. These results demonstrate that thermophilic anaerobic conditions imply a higher PAH removal efficiency than the mesophilic temperature range.

Figure 1(a) illustrates the total PAH elimination percentage in the mesophilic and the thermophilic anaerobic digesters. Under thermophilic conditions, this percentage oscillated between 51.01%–65.46% without a clear tendency with the HRT. In the mesophilic digester, the total PAH elimination rate was lower than in the thermophilic digester,

<table>
<thead>
<tr>
<th>HRT (d)</th>
<th>26</th>
<th>22</th>
<th>18</th>
<th>12</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>11.44</td>
<td>8.5</td>
<td>5.26</td>
<td>11.57</td>
<td>5.64</td>
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<tr>
<td>M</td>
<td>6.50</td>
<td>5.39</td>
<td>3.07</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>T</td>
<td>3.95</td>
<td>3.82</td>
<td>1.88</td>
<td>4.73</td>
<td>2.76</td>
</tr>
</tbody>
</table>

Table 3 Sludge total PAH content (mg/kg dw) in the fresh and treated sludge

Figure 1 Anaerobic elimination efficiencies of (a) total PAH, (b) LMW PAH and (c) PAH with abiotic characters (phenanthrene and fluorene)
where 36.56–43.20% was registered as the interval values of PAH removal efficiency. These results were coherent with the fact that high temperatures can enhance organic micropollutants’ biodegradation, concordantly with the results reported by Christensen et al. (2004) and Trably et al. (2003). Similarly to the thermophilic digester, the mesophilic removal efficiencies did not have a clear tendency with HRT reduction.

On the other hand, high removal percentages of LMW PAH in both digesters were observed (see Figure 1(b)). LMW PAH elimination efficiency was in the range of 59.82–81.72% and 52.60–57.07% in the thermophilic and the mesophilic digester, respectively, with an HRT dependency effect observation. At higher HRT, the elimination efficiency of LMW PAH was more important than at lower HRT.

Furthermore, LMW PAH removal percentage was more pronounced than the removal efficiency of total PAH. As an example, at HRT 22 d, the LMW PAH removal efficiency was achieved with 20% more than the total PAH removal efficiency value in both digesters (see Figure 1). These observations can correlate with the fact that the PAH with HMW have a higher coefficient sorption (Kow) than LMW PAH, which is correlated with the diffusion and bioavailability phenomena (Coates et al., 1996; Rockne and Strand, 1998). However, these results do not agree with the Chang et al. (2003) observations, where another removal order was reported without molecular weight influence, probably because of the seed used and the implemented adaptation mode (adapted to phenanthrene).

In Table 4 the percentage of LMW PAH with respect to the total PAH of the sludge for every studied case is presented. The effect of LMW PAH high elimination efficiency on the total PAH elimination efficiency was limited because of their low percentage with respect to the total PAH, except at HRT 12 days.

For some compounds (such as phenanthrene and fluorene), the removal percentage values were higher than the other PAH including the remaining LMW PAH (see Figure 1(c)). A higher performance of the elimination rate of these compounds was probably due to the abiotic elimination phenomena cited by Trably et al. (2003), who reported values greater than 50% as the abiotic degradation contribution, especially when high temperature, agitation or pH modification was adjusted to the optimum conditions.

DEHP biodegradation

DEHP recovery indexes obtained in this study are presented in Table 2. As illustrated in Figure 2(a), both thermophilic and mesophilic anaerobic digestion led to a reduction in the DEHP content of the treated sludge. Moreover, the decrease in DEHP content was more pronounced under thermophilic conditions. In Figure 2(b) the DEHP removal efficiency under thermophilic and mesophilic conditions at the tested HRTs is presented. From this Figure, it is clear that the percentage of removal at thermophilic conditions (31.7–46.7%) was higher than at mesophilic conditions (21.7–37.8%). These results are convergent with the conclusions extracted from the studies of Banat et al. (1999) and Fauser et al. (2003).

In both digesters, the HRT reduction effect can be appreciated but with different impacts. In the mesophilic digester, the DEHP removal efficiency decreased along with

<table>
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<tr>
<th>HRT (d)</th>
<th>26</th>
<th>22</th>
<th>18</th>
<th>12</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>12.76</td>
<td>6.12</td>
<td>18.54</td>
<td>72.92</td>
<td>22.08</td>
</tr>
<tr>
<td>T</td>
<td>6.75</td>
<td>3.91</td>
<td>14.49</td>
<td>69.58</td>
<td>18.11</td>
</tr>
<tr>
<td>M</td>
<td>9.64</td>
<td>4.17</td>
<td>15.06</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
HRT decrease, where elimination percentage deceleration was lower at 22–18 d HRT reduction (it was decreased by 6% only) than at 26–22 d HRT reduction (10.1%).

In the thermophilic digester, two DEHP removal efficiency values intervals can be distinguished. The first at higher HRTs (26–18 d), where the removal efficiency values had 46% as the average value, and the second interval, at lower HRTs (12–8 d), where removal values had an average of 32%. The dependency of the DEHP elimination on HRT can be observed as previously reported by Marttinen et al. (2004). The assessed DEHP removal values in this study were in the range stated by Marttinen et al. (2004) and Angelidaki et al. (2000), which is 23–61% for an anaerobic system.

Apparently, the positive effects of high temperature and residence time on DEHP removal observed under aerobic conditions (Knudsen et al., 2000; Fauser et al., 2003) were also stated at anaerobic conditions in this study.

**Conclusions**

An enhancement in Polycyclic Aromatic Hydrocarbons (PAH) elimination at the thermophilic temperature range (55°C) was observed with respect to the mesophilic temperature range (35°C).

The effect of the HRT on the PAH degradation was appreciated only for LMW PAH, but the high content of HMW PAH and its very recalcitrant characteristics avoided the detection of an HRT effect on the total PAH removal.
Under thermophilic conditions, the DEHP elimination efficiency was increased by 25% to 50% with respect to mesophilic anaerobic conditions. Furthermore, high HRT had a clear positive effect on DEHP biodegradation.

Additionally, from biogas production and composition results (not shown in this paper), it can be concluded that thermophilic anaerobic digestion at HRT between 12 and 18 d offers the optimum conditions for PAH and DEHP removal from sewage sludge.

Acknowledgements

The authors would like to thank the Spanish Environmental Ministry (Project 4.3-188/2005/3-B) for the financial support of this study.

References


