Effect of anaerobic digestion at 35, 55 and 60 °C on pharmaceuticals and organic contaminants
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ABSTRACT

The application of treated sewage sludge on farmland is a suggested method for recycling nutrients and reducing demand for commercial fertilizer. However, sludge needs to be safe from possible contaminants which can cause acute and long-term health and environmental problems. Residual pharmaceuticals and organic contaminants are mentioned as emerging threats since wastewater treatment plants are not designed to degrade these substances. The aim of this study was to screen and evaluate the presence, and reduction, of pharmaceuticals and polycyclic aromatic hydrocarbons (PAHs) during anaerobic digestion of mixed primary and waste-activated sludge at 35, 55 and 60 °C and during pasteurization at 70 °C. The study showed the difficulty of analysing pharmaceutical compounds in low concentrations in the sludge matrix. No general reduction of these compounds was seen during treatment, but for individual substances some reduction occurred. The PAHs were generally not reduced during digestion or pasteurization, but for three substances (indenol[1,2,3-cd]pyrene and dibenzo[a,h]anthracene (analysed together) and benzo[g,h,i]perylene) reduction (up to 60%) during digestion was seen. Digestion at 35 and 55 °C resulted in about the same order of reduction of the three individual PAHs, which was higher than for digestion at 60 °C.

Key words | anaerobic digestion, organic contaminants, pharmaceuticals, sludge

INTRODUCTION

To be able to apply treated sewage sludge on farmland, to recycle nutrients and reduce demand for commercial fertilizer, the sludge needs to be rendered safe from possible contaminants which can cause health and environmental problems. Pathogens, residual pharmaceuticals and organic contaminants in sludge are mentioned as emerging threats since wastewater treatment plants (WWTPs) are not designed to degrade or mineralize these substances, thus yielding an accumulation of sludge (Naturvårdsverket 2008).

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated bi-phenyls (PCBs) are micropollutants with known toxic and carcinogenic properties both for humans and aquatic organisms (Økland et al. 2005). The groups include some barely degradable compounds with long degradation times (Naturvårdsverket 2003; Økland et al. 2005). The proposal for a new EU Water Framework Directive (WFD) (EC 2012) is mostly focused on PAHs since the use of PCBs is banned and PCB concentrations in sludge have been observed to decrease during recent decades in both the EU and other parts of the world (Erhardt & Prieß 2007; Clarke et al. 2008, 2010). The studies by Clarke et al. (2008, 2010) also showed that the degradation increased with higher temperature and that some PAHs were more efficiently removed during thermophilic digestion. In Trably et al. (2003) it was shown that removal efficiency correlated with methanogenic activity.

Environmental effects from pharmaceutical residues in wastewater are of great interest since humans excrete pharmaceuticals and their metabolites, which can possibly end up in the environment with the treated wastewater (to recipients) or as sludge being used as fertilizer on agricultural land. Effects on aquatic organisms in recipients have been seen from endocrine disruptors, and the eventual increase of resistance to antibiotics in bacteria caused by residues in wastewater effluents or re-used sludge is now a matter of discussion. No statutory limit on concentrations for...
pharmaceutical residues in effluent wastewater or stabilized sludge from WWTPs exist, but the proposal for a new EU WFD suggests keeping track of Diclofenac, Ethinylestradiol and estradiol (EC 2012).

There are two possibilities for making the sludge less harmful for the environment and possible to re-use as fertilizer: (1) the contaminants can be avoided in the original wastewater by up-stream work (mitigation near the source of the pollution, e.g. by reducing industrial discharges, local treatment of wastewater at hospitals and by educating inhabitants); and (2) the contaminants could possibly be reduced/degraded in the sludge treatment processes at the WWTP, such as by anaerobic digestion or, if occurring, a separate hygienization step or other pre/post-treatment methods. This study has focused on finding out the potential of the second possibility by evaluating the presence and reduction of 99 pharmaceuticals and 15 PAHs in sludge during anaerobic digestion at 35, 55 and 60 °C. These analyses were made in connection with measuring pathogen reduction after semi-continuous digestion at different temperatures with different minimum exposure times (time between feeding). Different exposure times were evaluated since they, in combination with temperature, are the most important factors for reduction of pathogens and are likely important for the reduction of pharmaceutical residues and micropollutants as well. Specific combinations of temperature and minimum exposure times were suggested by the Swedish EPA (Environmental Protection Agency) as approved hygienization methods in the forthcoming legislation, e.g. 55 °C-min 6 h and 60 °C-min 2.5 h. Also included in the study was a separate hygienization step in the form of pasteurization at 70 °C for 1 h, which has been suggested as a means of reducing pathogens in sludge; more details are found in Kjerstadius et al. (2013). The substrate used was mixed primary and waste-activated sludge from a medium-sized 300,000-person-equivalent municipal WWTP in southern Sweden.

**METHODS**

**Sludges**

Sludge consisting of a mix of primary sludge (75%) and waste-activated sludge (25%) from the Sjölunda wastewater treatment plant in Malmö, Sweden, was pasteurized at 70 °C for 1 h or digested in anaerobic reactors. The sludge was collected from the full-scale facility on a weekly basis.

**Digestion test procedure**

The anaerobic digestion test setup was a series of 20 L semi-continuously fed stirred-tank reactors, each set to a combination of temperatures (35, 55 and 60 °C) and hydraulic retention times (7 and 15 days). The reactors were started up with sludge originating from the same wastewater treatment plant (collected on a weekly basis) and operated for 5 months before a steady state was reached. Steady state was defined as the state when all reactors maintained a stable temperature, methane content and gas production and the levels of the inhibitory substances, total ammonia nitrogen and volatile fatty acids (acetate and propionate), were low and stable as well. Operational conditions in detail are found in Kjerstadius et al. (2013). At steady-state conditions and after minimum exposure times of 2, 2.5, 6 and 24 hours samples were removed and analysed for concentrations of the pharmaceutical substances and PAHs. Minimum exposure time is here defined as the time between feeding of new sludge and withdrawal of digested residue used when operating the semi-continuously fed digesters. Pasteurization of sludge was done by heating up raw sludge for 30 min in a water bath and then keeping it at 70 °C for 1 hour. Total solids (TS) and volatile solids (VS) were determined according to DS/EN 872:1997.

**Analysis of pharmaceutical residues**

All sludges were freeze-dried and 0.1 g (dry weight) sample aliquots were used for extraction, to which internal and surrogate standards were added before extraction. Sequential extraction was performed using ethyl acetate and methanol (1:1) followed by methanol and water (7:3) with 5% triethylamine. Samples were homogenized for 4 min, at 42,000 oscillations per minute, using a Mini Beadbeater (BioSpec. Bartlesville, USA) with zirconium beads and then centrifuged at 14,000 rpm for 10 min. This protocol was done for both eluent mixtures and the supernatants were combined, evaporated to 20 μL and reconstituted in 1 ml water and acetonitrile (95:5 mixture) with 0.1% formic acid.

All pharmaceuticals were analysed with the same methodology as reported in Grabic et al. (2012). In short, a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) were used as the analytical system. Twenty μL of the sample was loaded
onto a Hypersil GOLD aQ™ column (50 × 2.1 mm ID, 5 µm particles, Thermo Fisher Scientific, San Jose, CA, USA) preceded by a guard column (2 × 2.1 mm ID, 5 µm particles) of the same packing material and from the same manufacturer. Both heated electrospray and atmospheric pressure photoionization in positive and negative ion modes were used for ionization of target compounds. The same method was used to investigate the fate of APIs in wastewater treatment by Hörsing et al. (2011) and Hey et al. (2012) and a full method evaluation and detailed description is given in Grabic et al. (2012).

Analysis of PAHs

The sludge samples (digested, pasteurized and untreated sludge from the four test rounds with minimum exposure times 24, 6, 2 and 2.5 h) were freeze-dried and then stored at 4°C until analysis. Microwave assisted extraction of 0.5 g freeze-dried sludge was performed in a Multiwave 5000SOLV (Anton-Paar, Graz, Austria) using hexane and acetone (3:2). Thereafter the extracts were filtered through glass wool, and 2 ml isooctane (2,2,4-trimethylpentane) containing 100 µg/l naphthalene-d8, pyrene-d10 and phenanthrene-d10 as internal standards was added before evaporation to 2 ml (with N2 or compressed air). Conditioned solid phase extraction (SPE) columns (LC-Florisil) were used for clean-up and the analytes were eluted by hexane and toluene (4:1). SPE-extracts were evaporated to 2 ml and subjected to analysis. Gas chromatography mass spectrometry (GC-MS) was used for separation and detection of all PAHs simultaneously in each sample. Out of the US EPA list of 16 PAHs, 15 were determined (acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene and pyrene).

RESULTS AND DISCUSSION

Pharmaceutical residues

The concentrations of 99 pharmaceutical substances in the sludges were analysed using duplicate or triplicate samples. The samples were taken out before and after treatment. The treatments were pasteurization (1 h at 70°C) or digestion at 55, 55 or 60°C using two different minimum exposure times (2 h or 24 h) and a hydraulic retention time (HRT) of 15 days (also 7 days for digestion at 55°C).

Some (72) of the substances were found in at least one sludge sample, but only 19 of the substances were found in untreated, pasteurized and digested sludge. Measured concentrations of pharmaceuticals in the raw and treated sludge ordered by their medical effect are shown in Figure 1. The 24-h-exposure time was chosen since the largest effect was expected for the longest exposure time. The results show that the precision of the measurements is low and that the variation is great, which leads to the conclusion that in general no clear effect of any treatment can be seen. Complete analysis results are found in Appendix 1 (available online at http://www.iwaponline.com/wst/069/016.pdf).

Average measured concentrations of chosen substances are presented in Table 1, together with standard deviations (SD) to show the difficulty of measuring such low concentrations in the sludge matrix. The chosen substances are two of the ones mentioned in the water framework directive.

![Figure 1](https://iwaponline.com/wst/article-pdf/69/6/1282/472388/1282.pdf) | Concentrations of analysed pharmaceuticals in the sludge samples sorted according to therapeutic effect (exposure time 24 hours).
proposal (Diclofenac and Ethinylestradiol), substances that deviate widely from other reported values in the literature (Wahlberg et al. 2010; Fick et al. 2011) (Bupropion, Ciprofloxacin and Miconazole), substances occurring in the highest concentrations (Ciprofloxacin, Dipyridamol, Sertraline, Irbesartan and Ketoconazole) and substances that seem to be reduced during anaerobic digestion (Irbesartan and Trimethoprim).

The concentrations of pharmaceuticals in the mesophilic sludge are on the same level as results in recent studies on Swedish sludges (Wahlberg et al. 2010; Fick et al. 2011). The results clearly showed the difficulty of analysing pharmaceutical compounds in low concentrations in the sludge matrix, which is a far more complicated medium than wastewater. The SD of the analysed samples (with \( N = 2 \) or 3) were substantially higher than the SD of the reference material used during method development (data not shown here), where the number of replicates were higher (\( N = 6 \)). However, the preliminary results indicate that there is no general reduction of pharmaceuticals at any temperature or minimum exposure time. Two compounds (Irbesartan and Trimethoprim) could, according to the results, possibly be reduced by pasteurization and/or anaerobic digestion (Table 1) with higher reduction for thermophilic digestion. Irbesartan (a blood-pressure lowering substance) was seen to be reduced during both treatments while Trimethoprim (an antibiotic substance) was only reduced by anaerobic digestion, not by pasteurization. Some structural similarities are present between Trimethoprim and Irbesartan, e.g. both have heterocyclic groups that contain nitrogen (pyrimidine- and tetrazole-groups respectively), see Figure 2. However, they have different physico-chemical properties, e.g. Trimethoprim is a weak base and Irbesartan is a weak acid and they also differ in logP (0.44 and 5.5 respectively), which makes it very difficult to draw any conclusions.

The concentrations of some substances were higher after digestion, which is only partly related to the degradation of TS during digestion. This indicates either a high uncertainty in the method resulting in high standard deviations or that the substances actually occur in higher concentrations after digestion. The uncertainty in the

<table>
<thead>
<tr>
<th>Substance (LOQ, μg/kg TS)</th>
<th>Untreated (4.9% TS)</th>
<th>35 C, 15 days HRT (2.0% TS)</th>
<th>55 C, 15 days HRT (2.0% TS)</th>
<th>60 C, 15 days HRT (2.3% TS)</th>
<th>70 C, 60 min (4.9% TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac (10)</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Ethinylestradiol (10)</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Bupropion (0.1)</td>
<td>5</td>
<td>1</td>
<td>13</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Miconazole (5)</td>
<td>36</td>
<td>19</td>
<td>13</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Ciprofloxacin (10)</td>
<td>2,100</td>
<td>800</td>
<td>4,100</td>
<td>600</td>
<td>1,500</td>
</tr>
<tr>
<td>Dipyridamol (50)</td>
<td>190</td>
<td>130</td>
<td>490</td>
<td>30</td>
<td>470</td>
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<tr>
<td>Sertraline (10)</td>
<td>280</td>
<td>10</td>
<td>670</td>
<td>40</td>
<td>810</td>
</tr>
<tr>
<td>Ketoconazole (50)</td>
<td>200</td>
<td>60</td>
<td>160</td>
<td>50</td>
<td>220</td>
</tr>
<tr>
<td>Irbesartan (0.5)</td>
<td>1,900</td>
<td>2,600</td>
<td>540</td>
<td>380</td>
<td>20</td>
</tr>
<tr>
<td>Trimethoprim (0.1)</td>
<td>19</td>
<td>21</td>
<td>&lt; 0.1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: unit: μg/kg TS. Digested sludges with 24 hours exposure time. \( N = 2 \) for all samples except 70 C, 60 min, where \( N = 3 \). The total solids (TS) content indicated for each sludge was reduced by \( \sim 60\% \) by anaerobic digestion.

Figure 2 | Reduction of three PAHs (indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene (analysed as a group) and benzo[g,h,i]perylene) during digestion and pasteurization.
method is a probable reason, because in 70% of the cases with higher concentrations after digestion, the standard deviations are really high for both concentrations in untreated and digested sludge. If the substances actually occur in higher concentrations after digestion there are two possibilities. The digestion may lead to extraction of pharmaceuticals from the sludge by reduction of the particles on which the pharmaceuticals can adsorb. The other reason could be that degradation products, which have been released in conjugated form after metabolization in the human body and then end up in wastewater entering the WWTP, can be converted back to the mother substance. This phenomenon has been discussed for wastewater in other studies (Naturvårdsverket 2008; Wahlberg et al. 2010).

High standard deviations (15%–118%) for the results were seen for samples analysed in both duplicate and triplicate and are explained by the difficulties of making analyses of the sludge matrix. Recovery tests performed by the same laboratory as in the study on both wastewater and sludges showed in general lower degrees of recovery and higher standard deviations for sludge. More reliable analysis methods for sludge are consequently needed.

**Polycyclic aromatic hydrocarbons**

Samples were analysed in duplicate and certified reference materials (LGC6182, LGC Standards, Middlesex, UK) in triplicate, yielding relative standard deviations (RSD) of 2%–17%, and recoveries of 98%–112% (benzo[g,h,i]perylene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and naphthalene, i.e., the lightest and the heaviest PAHs had the same excellent RSD 5%–18% and, as expected, lower recoveries of 25%–32%). The limit of quantification varied between the compounds, from 0.1 to 0.4 mg/kg TS.

Out of the 15 PAHs analysed, 11 were detected and quantified. Fluoranthene (0.15–0.66 mg/kg TS), phenanthrene (0.10–0.49) and pyrene (0.13–0.56) were the three PAHs detected in all samples. Anthracene, benzo[a]anthracene and fluorene were present in only a few samples above the quantification limit (1, 3 and 8 samples out of 28 analysed). Benzo[b]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene were also detected above the detection limits. The summary concentrations of the eight PAHs (anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, fluoranthene, indeno[1,2,3-cd]pyrene and naphthalene) included in the proposal for the new WFD (EC 2012) are found in Table 2.

The results show that no statistical significant reduction of the total PAH was achieved during digestion or pasteurization, a result that is also valid for several of the individual PAHs in the study. For single PAHs, reduction up to 60% during digestion was seen, namely, for indeno[1,2,3-cd] pyrene and dibenzo[a,h]anthracene (analysed together) and for benzo[g,h,i]perylene, see Figure 2. Digestion at 55 and 60 C resulted in similar reduction of these three PAHs while digestion at 60 °C resulted in lower reduction. No statistical correlation between reduction and exposure time was seen. No reduction of the three PAHs was seen during pasteurization. The total concentration of sum PAH (1.4–2.8 mg/kg TS) was high compared to the results found in Olofsson et al. (2015), where sludges from seven Swedish WWTPs were analysed (ranging from 0.47 to 1.7 mg/kg TS).

Reduction of the same three heavy PAHs was also seen in a study by Trably et al. (2003) but that study also saw reduction of other PAHs which could be explained by the long HRT (41 days) used in their digestion tests, since a long retention time may lead to increased degradation. However, no significant difference in reduction between the different HRTs (7 days and 15 days) applied in this study was seen. In Christensen et al. (2004) naphthalene was reduced during digestion with much longer exposure time than in this study. Higher reduction at longer exposure times could be explained by a favouring of PAH reduction when easily degradable matter has already been consumed. This reasoning is supported by the results in Trably et al. (2003) which showed that PAH reduction increased when biogas production decreased. Alternatively the decrease in gas production could be a result of inhibition of methanogens.

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**Table 2** | Summary concentrations of PAH-7 mentioned in the WFD proposal (2012) in the sludges after different treatments/digestion (results in mg/kg TS)

<table>
<thead>
<tr>
<th>Minimum exposure time (h)</th>
<th>Untreated</th>
<th>70 °C, 60 min</th>
<th>35 °C, 15 days HRT</th>
<th>55 °C, 15 days HRT</th>
<th>60 °C, 15 days HRT</th>
<th>55 °C, 7 days HRT</th>
<th>60 °C, 7 days HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1.7</td>
<td>1.8</td>
<td>2.3</td>
<td>2.3</td>
<td>2.5</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>1.6</td>
<td>1.6</td>
<td>2.4</td>
<td>2.3</td>
<td>2.5</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>2.5</td>
<td>1.6</td>
<td>1.7</td>
<td>2.5</td>
<td>2.2</td>
<td>2.4</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>1.7</td>
<td>2.4</td>
<td>2.1</td>
<td>2.2</td>
<td>2.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Benzo[b]fluoranthene was not measured, but dibenzo[a,h]anthracene was included.*
from the increase of hydrogen produced during degradation of PAH shown by Christensen et al. (2004). The latter is more unlikely since the hydrogen production from reduction of PAH should be insignificant compared to the other hydrogen produced in the biogas process.

The influence of digestion temperature on reduction is contradictory to other studies. The results for the three PAHs that were reduced showed that the reduction was lower at 60 °C than at 35 °C or 55 °C. Christensen et al. (2004) and Trably et al. (2005) state that the reduction is increased with increased temperature and with adjusted microbial population. A possible explanation for the lower reduction seen at 60 °C could be a sub-optimal microbial population with anaerobic digestion metabolism deriving from the higher temperature of digestion. Such a hypothesis is supported by the fact that methane production was 10% less in reactors operated at 60 °C than at 55 °C (Kjerstadius et al. 2003). This could be an effect of the shift in methanogenic genera from Methanosarcina to Methanaota which occurs around 60–62 °C (Zehnder 1988; Zinder 1990; Van Lier et al. 1993). However, since the complete degradation pathway of PAH in anaerobic digesters with sludge as feed was unknown, a sub-optimal microbial population remains a hypothesis.

General discussion

This screening for micropollutants in sludge before and after anaerobic digestion under different operating conditions and after merely pasteurizing indicates that there is not much reduction from such treatments. The longest HRT used in the digestion experiments resulted in a low organic loading rate (1.9 kg VS/m³, day) which is on the same level or lower than what is seen in most full-scale digesters. It can therefore be assumed that not much reduction will be seen in full-scale digesters. If the concentration of micropol-lutants in sludge is considered a risk when recycling sludge to farmland (it was not the aim of this work to evaluate the risk) there are other possibilities for reduction: (1) more work with source control; and (2) more advanced sludge treatment methods. If none of these are applicable and the concentrations of the pollutants are considered to be a risk, alternative handling of the sludge than recycling to farmland should be considered, e.g. incineration.

CONCLUSIONS

The screening and evaluation of the presence and reduction of 99 pharmaceuticals and 15 PAHs performed clearly shows the difficulty of obtaining reliable results in the sewage sludge matrix. The potential for improving sludge quality by reduction of harmful contaminants during anaerobic digestion or pasteurization at 70 °C seems to be low, but more studies are recommended on specific target compounds. However, the following conclusions can be drawn from this data:

- Pharmaceutical residues were generally not reduced during digestion at any investigated temperature (35, 55 and 60 °C) or by pasteurization (70 °C for 1 h). In individual cases, some reduction occurred but this cannot be determined due to the low accuracy when analyzing sludge samples.
- PAHs were generally not reduced during digestion or pasteurization, but were reduced to some extent (up to 60%) during digestion in three individual cases (indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene (analysed together) and benzo[g,h,i]perylene). Digestion at 35 and 55 °C resulted in about the same order of reduction of the three individual PAHs, which was higher than for digestion at 60 °C.

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