

# Elimination of Estrogenic Endocrine Disrupters from Laboratory-Derived Sewage Sludge by Means of Simultaneous Aerobic Sludge Stabilisation

**Please note corrections given at the end of the paper.**

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## 1. ABSTRACT

Behaviour and elimination of major estrogenic endocrine disrupting compounds (EDCs) during wastewater and sewage sludge treatment have been studied in laboratory experiments simulating simultaneous aerobic sludge treatment. Elimination and, in part, degradation rates, and mass balances of the anoxic and aerobic elimination of steroids, bisphenol A, and alkylphenols from synthetic wastewater as well as from laboratory-derived sewage sludge are presented. The elimination of 17 $\beta$ -estradiol (E2) amounted to 98.6 to 99.6 % (n = 3). 69.6 to 99.0 % of the E2 was eliminated in the first, anoxic treatment vessel simulating denitrification. Distinct degradation to Estrone (E1) was observed. On molar basis, the overall elimination rate of E2+E1 amounted to 89.4 to 97.9 %. A similar behaviour was observed for bisphenol A (BPA). 40.2 to 67.8 % of the BPA was eliminated during denitrification, and 79.9 to 98.0 % overall (n = 4). In contrast, 4-*tert*-octylphenol, 4-nonylphenol, 17 $\alpha$ -ethynyl-estradiol, and mestranol have been eliminated by about 60 to 90 % indicating a lower biodegradability. Simultaneous aerobic sewage sludge treatment, thus, can be estimated being suitable for eliminating the EDC studied from synthetic wastewater and the thereby derived sewage sludge if the process was optimised.

**KEYWORDS:** estrogenic endocrine disrupters, wastewater, sewage sludge treatment, alkylphenols, steroids, bisphenol A

## 2. INTRODUCTION

Endocrine disruption has become one of the most important environmental issues causing scientific, political, legislative, and administrative action (European Commission, 2001; US Congress, 1995). One important class of endocrine active compounds are natural occurring and xenobiotic substances that mimic endogenous estrogens through direct activation of estrogen receptors (Metzler, 2001a, 2001b). Some of the major estrogenic so-called endocrine disrupting compounds (EDCs) are the natural steroid hormone 17 $\beta$ -estradiol (E2), the E2 degradation products estrone (E1) and estriol (E3), the synthetic steroids and oral contraceptives 17 $\alpha$ -ethynylestradiol (EE2) and mestranol (ME), alkylphenols (AP) and their short-chained ethoxylates representing degradation products of non-ionic surfactants, and bisphenol A, a plastics monomer and plasticiser additive (Metzler, 2001a, 2001b). These compounds are frequently determined in raw and treated wastewater as well as sewage sludge (Metzler, 2001a; Tennhardt *et al.*, in press). A broad variety of aquatic species was shown to be affected by estrogenic pollutants released to the environment with wastewater treatment

plant effluents as well as surface run-off from agricultural soils after sewage sludge application (Metzler, 2001a; Tyler *et al.*, 1998). The effective or even exhaustive elimination of endocrine active compounds from wastewater and sewage sludge, therefore, is a very important task for environmental engineers. Amongst other topics, occurrence, elimination, and degradation of these compounds during municipal wastewater and sewage sludge treatment are studied at the Department of Waste Management, Dresden University of Technology, Germany.

### 3. MATERIALS AND METHODS

The laboratory wastewater treatment plant (WWTP) type used (behrotest<sup>®</sup> KLD4, Behr, Duesseldorf, Germany) and the realisation of the experiments basically met the regulations of OECD guideline 303A (OECD, 2001) and the German guideline DIN EN ISO 11733 (DIN, 1998). Each WWTP consists of a diet reservoir, an anoxic and an aerobic treatment vessel ( $V = 3.0\text{ l}$ , respectively), a separator vessel ( $V = 2.0\text{ l}$ ), oxygen and temperature probes, stirrers, and an aeration system (see fig. 1).

Potable water (PW) and the target compound/diet solution (AD, stored at 4°C) were separately supplied to the denitrification vessel (DV) resulting in a total target input of 0.5 l/h. The sludge suspension subsequently flowed through the aeration vessel (AV) and the separator (SP). The denitrification as well as the aeration vessel have been continuously stirred. Both activated sludge and separated sludge (excess sludge) circulated in closed loops.

The aeration system automatically regulated the oxygen concentration in the aeration vessel between 2 and 4 mg O<sub>2</sub>/l. Weekly composite samples of 1 litre volume of the mixed influent solutions as dropping into the denitrification vessels, of the denitrification and nitrification sludge suspensions, and of the effluents were taken, respectively. The sludge age amounted to 20 – 21 d as frequently set in full-scale WWTPs applying simultaneous aerobic sludge treatment. The units have not been cross-linked except from the facts that they were inoculated by parts of the same municipal sewage sludge and that the diet influent solutions were supplied from the same stock. The diet was composed according to the guidelines mentioned above. In order to control the general performance of the biocoenosis, chemical oxygen demand (COD), dissolved organic carbon (DOC), nitrate-N, nitrite-N, and ammonia-N concentration, pH-value, temperature, and conductivity of the influents, the effluents, and the supernatants of the denitrifying as well as the activated sludge suspensions were determined, respectively.

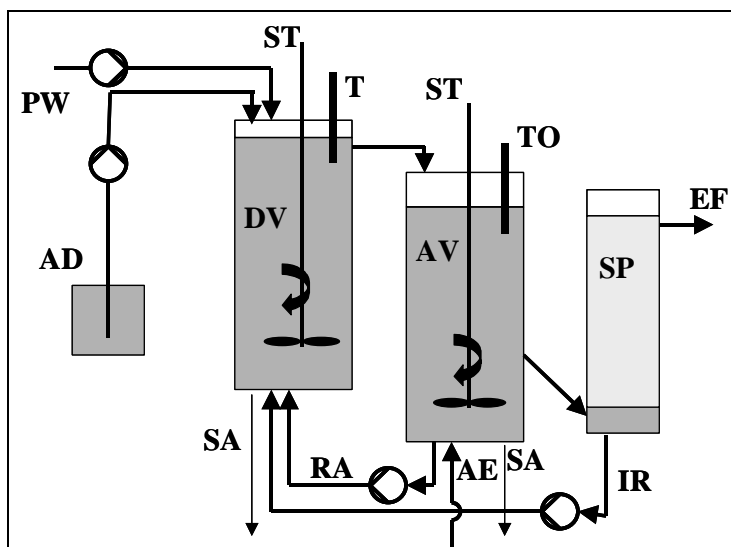


Figure 1. Experimental set-up and realisation schematic diagram; for explanation of acronyms see text

4-tert-octylphenol (OP), 4-nonylphenol (NP), 17 $\beta$ -estradiol (E2), ethynylestradiol (EE2), mestranol (ME), and bisphenol A (BPA) were chosen as target compounds. Two experiments were carried out simultaneously, respectively, in order to study the elimination of the target compounds when supplied either as a mixture component (experiments m1, m2; “m” for “mixture”) or individually (experiments e1, e2, b1, b2; “e” for “estradiol”, “b” for “bisphenol”). The target influent concentrations amounted to 1  $\mu$ g/l, respectively, except for BPA in experiments b1 and b2 and NP in experiments m1 and m2 which have been supplied at 10  $\mu$ g/l, respectively. Since only in the case of E2 metabolites have been determined, in the strict sense, only in these cases elimination can be formally attributed to microbiological degradation.

The sample preparation and EDC determination procedures are described in detail elsewhere (Weltin *et al.*, in press; see also Tennhardt *et al.*, 2003). Liquid samples and suspensions are centrifuged and the supernatants are conserved and drawn through a solid phase extraction cartridge. Solids are freeze-dried and subsequently extracted by means of Soxhlet extraction and separated through size exclusion chromatography. Additional clean-up by means of silica gel and derivatisation (silylation) follow, respectively. The silylmethyl derivatives finally are exchanged into 1 ml of toluene containing mirex as internal standard. EDC determination is carried out by means of gas chromatography/mass spectrometry. Bisphenol A-d<sub>16</sub>, 17 $\beta$ -estradiol-17-acetate, and 4-*n*-nonylphenol are used as surrogate standards.

In order to calculate the mass balances, the result “not detectable (n.d.) in the GC/MS vial” was replaced by 1.5 x method detection limit, “n.d. in the sample” by limit of detection minus blank, and “not quantifiable (n.q.) in the sample” by limit of quantification (LOQ) minus blank. Thus, the values of the mass flux data represent the upper margin of the possible interval, respectively. Overall elimination rates were calculated as differences between influent and effluent mass fluxes minus sampling mass fluxes, vessel elimination rates were calculated in relation to system influent mass fluxes. The respective LOD was calculated as mean blank plus three times standard deviation of blanks and LOQ as mean blank plus six times standard deviation of blanks. In tables and figures, the following data are presented: week 5 of experiments m1 and m2 (final sampling), week 8 of experiments b1 and b2 (final sampling), week 8 of 12 of experiment e2 (steady state).

#### 4. RESULTS AND DISCUSSION

In most cases, the corollary parameters kept the target values/intervals regulated by the OECD and DIN EN ISO guidelines. Thus, taking some minor restrictions into account, a valid interpretation of the data is possible. In three cases, corollary parameters continuously exceeded the respective target values: the nitrite-N effluent concentrations of experiments m1 and e1, and the ammonia-N effluent concentration of experiment m2. However, the general performance of experiments m1 and m2 was more satisfying than that of experiments e1 and e2 which have been carried out first (for details see Gehring *et al.*, 2002a; Tennhardt *et al.*, in press). Therefore, predominantly the results of experiments m1 and m2 will be discussed within this paper.

#### 4.1 Natural Hormones

The steroid mass balances of experiment e2 have been calculated by means of the target volume fluxes regulated before the start of the sampling period for steroid determination, respectively (PW 446 ml/h, AD 54 ml/h, RA 1000 ml/h, IR 550 ml/h). The influent E2 concentrations varied and differed from the target concentration of 1 µg/l. E1 was determined in all influent samples but not a constituent of the AD solution. Thus, very rapid initial E2 degradation in the diet reservoir and/or in the supplying conduction must be stated. The performance of the experiments was distinctly improved with time. In week 5 of experiments m1 and m2, the influent E2 concentrations amounted to 0.654 µg/l and 0.707 µg/l, respectively (Tennhardt *et al.*, in press).

After the resultant concentrations of COD, DOC, and the target compounds reached the steady state, respectively, the E2 effluent concentrations of experiments m1 and m2 amounted to <10 ng/l. E1 was determined in the effluents at 10 – 20 ng/l and 10 – 70 ng/l, respectively. In the two treatment vessels, the E2 and E1 concentrations amounted to <30 ng/l and <100 ng/l in the supernatants and <50 µg/kg DM and <120 µg/kg DM in the solid phases, respectively. In almost all cases, the E1 concentrations exceeded those of E2 except in the effluents.

In table 1 the degradation (positive values) and formation rates (negative values) of E2, E1, and E3 calculated for the three treatment vessels and for the complete system are summarised. The elimination of E2 amounted to 97.9 + 1.1 % (n = 9; SD = 1.7; experiments m1 and m2).

Table 1. Degradation [%] of 17β-estradiol during simultaneous aerobic sewage sludge stabilisation, synthetic wastewater, laboratory experiments; for explanations of acronyms see text

| substance | no. of exp.     | denitrification | aeration | separator | system <sup>a</sup> |
|-----------|-----------------|-----------------|----------|-----------|---------------------|
| E2        | m1              | 99.0            | 0.2      | 0.4       | 99.6                |
|           | m2              | 69.6            | 29.1     | 0.2       | 99.0                |
|           | e2 <sup>1</sup> | 83.0            | 15.7     | - 0.2     | 98.6                |
| E1        | m1              | - 163           | 230      | 18        | 84                  |
|           | m2              | - 650           | 680      | - 22      | 9                   |
|           | e2 <sup>1</sup> | - 5,500         | 5,420    | 136       | 60                  |
| E2+E1     | m1              | 51.5            | 42.0     | 0.4       | 96.8                |
|           | m2              | - 6.9           | 98.4     | -2.2      | 89.4                |
|           | e2 <sup>1</sup> | - 10.3          | 106.1    | 2.1       | 97.9                |

<sup>1</sup> calculated using target volume flux values, see text

Up to 99.0 % of the E2 has been eliminated within the first treatment stage simulating denitrification. Simultaneously, very high E1 formation rates and very limited adsorption of E2 to sludge have been observed (see also fig. 2, 3). These results clearly show that E2 was very rapidly degraded to E1 under anoxic conditions. Subsequently, E2 is nearly exhaustively degraded within the second, aerated treatment stage resulting in water phase concentrations below or hardly above the limit of detection. Despite of its very high biodegradability E1 is not completely eliminated. The limited hydraulic retention time as well as the comparatively high water solubility of E1 probably contributed to this effect. The overall elimination of E2+E1 during the steady state phases amounted to 96.4 ± 1.2 % (n = 23; SD = 2.9; weekly

determinations). Since n.d. and n.q. have been replaced by LOD and LOQ, respectively, this value represents the lower margin of the possible interval. E3 is the secondary degradation product of E2. Since E3 was n.d. in 93 % of all cases, we conclude that E3 was either not formed at all as proposed by Ternes *et al.* (1999) or degraded immediately below our LOD. The very high variance in the E1 data is caused by decreased influent E1 concentrations due to the improved performance of the experiments m1 and m2. Note that elimination rates have been calculated in relation to the respective AD influent.

Figures 2 to 4 display the mass balances calculated for the degradation of E2, E1, and E2+E1. Obviously, the rapid and extensive degradation of E2 to E1 in the denitrification stage (fig. 2) caused adsorption of E1 to sludge (fig. 3) despite of the comparable high biodegradability and water solubility of E1. During nitrification the water phase E2 concentrations as well as the solid phase E1 concentrations are reduced to levels hardly above or below the respective LOD (see fig. 2, 3). A small portion of the E2 adsorbed to sludge was detracted from degradation (fig. 2, 4). However, 96.4 % of the sum E2+E1 have been eliminated from the synthetic wastewater within approximately 16 h hydraulic retention time. No systematic difference in the rate or the extent of the degradation of E2 and E1 was observed when E2 was supplied either as sole target compound or as a constituent of a

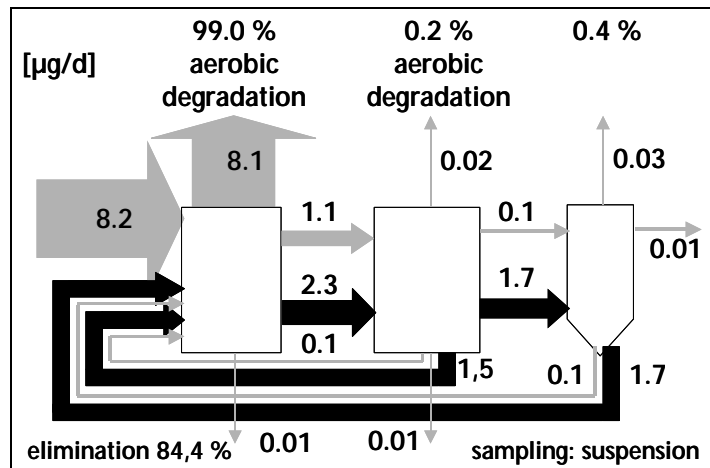


Figure 2.  $17\beta$ -estradiol mass balance of experiment m1; all values [ $\mu\text{g/d}$ ] except otherwise stated

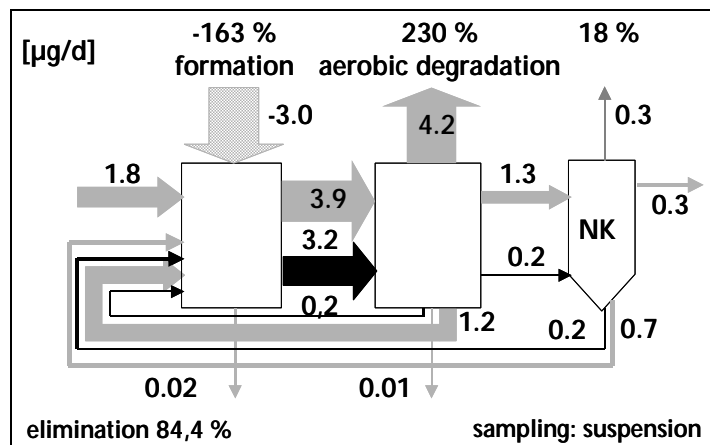


Figure 3: estrone mass balance of experiment m1, all values [ $\mu\text{g/d}$ ] except otherwise stated

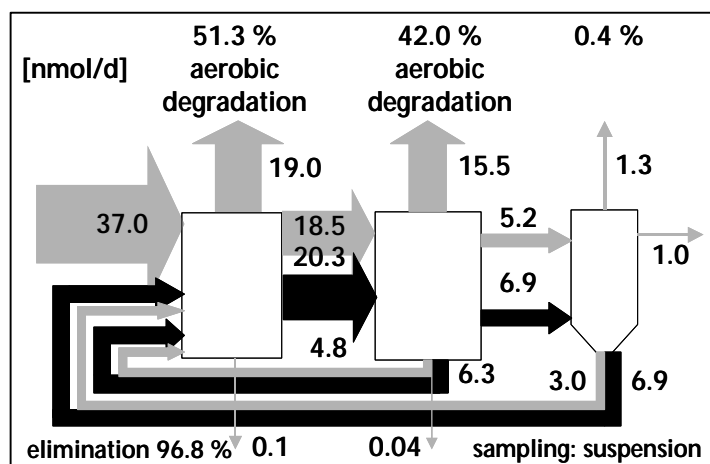


Figure 4. Cumulated ( $17\beta$ -estradiol + estrone) mass balance of experiment m1, all values [ $\text{nmol/d}$ ] except otherwise stated

target compound mixture. The E2 and E1 effluent concentrations of 4 – 38 nmol/l and 0.024 – 0.265 nmol/l determined in weeks 1 to 5 of experiments m1 and m2, respectively, presumably would be capable affecting sensitive aquatic organisms if released to water environments at a high volume proportion of the receiving water body (Routledge *et al.*, 1998).

#### 4.2 Bisphenol A

In table 2 the degradation rates of bisphenol A are listed (for denotation of experiments see chapter 3). 79.9 to 98.0 % of the BPA have been eliminated from the synthetic wastewater. The BPA degradation rates in experiment m2 and the BPA water and sludge concentrations in experiment m1 showed a slightly different behaviour in comparison to the other three experiments, respectively. The results of experiments b1 and b2 have already been discussed (Gehring *et al.*, 2002b). The data have been reviewed and, therefore, the bisphenol A data show minor differences to earlier publications (e.g. Gehring *et al.*, 2002b).

Table 2. Elimination of bisphenol A during simultaneous aerobic sewage sludge stabilisation, synthetic wastewater, laboratory experiments [%]

| no. of exp. | denitrification | aeration | separator | system |
|-------------|-----------------|----------|-----------|--------|
| m1          | 59.0            | 28.9     | 3.7       | 91.6   |
| m2          | 40.2            | 40.1     | 0.4       | 79.9   |
| b1          | 53.8            | 44.3     | - 0.1     | 98.0   |
| b2          | 67.8            | 29.7     | 0.0       | 97.5   |

In the course of the experiments, the BPA influent concentrations ranged from 0.674 to 0.888 µg/l, from 0.705 to 0.902 µg/l, from 4.06 to 12.66 µg/l, and from 4.21 to 15.33 µg/l (experiments m1, m2, b1, and b2, respectively; Gehring *et al.*, 2002a; Tennhardt *et al.*, in press). The varying influent BPA concentrations did not noticeable influence the resultant BPA effluent concentrations which were comparably low in three of four experiments. The water phase and the solid phase concentrations of BPA of the respective final week of the four experiments are summarised in table 3.

Table 3. Bisphenol A concentrations after simultaneous aerobic sewage sludge stabilisation, synthetic wastewater, laboratory experiments; for explanations of acronyms see text

| no. of exp. | influent | DV water phase | DV solid phase | AV water phase | AV solid phase | effluent |
|-------------|----------|----------------|----------------|----------------|----------------|----------|
|             | [µg/l]   | [µg/l]         | [µg/kg DM]     | [µg/l]         | [µg/kg DM]     | [µg/l]   |
| M1          | 0.869    | 0.126          | 43             | 0.103          | 86             | 0.071    |
| M2          | 0.860    | 0.223          | 55             | 0.167          | 26             | 0.302    |
| B1          | 12.66    | 0.380          | 415            | n.d.           | 169            | 0.220    |
| B2          | 12.28    | 0.240          | 392            | n.d.           | 214            | 0.280    |

In each experiment, the solid phase BPA concentrations reached a steady state but this possibly has to be questioned in case of the water phase BPA concentrations in the aeration stages during experiments m1 and m2. Whilst the steady state solid phase BPA concentrations of experiments b1 and b2 were significantly higher than those of experiments m1 and m2, probably caused by the much higher BPA influent mass fluxes, the BPA concentrations in the aeration vessel water phases were reduced below the LOD in experiments b1 and b2 but not

in experiments m1 and m2 (table 3). This leads to the assumption that degradation was limited if BPA was supplied as a constituent of the multi-compound EDC mixture supplied in experiments m1 and m2. Desorption of BPA from suspended biomass to water must have occurred during separation in experiments b1 and b2 causing effluent BPA concentrations of 0.220 µg/l and 0.280 µg/l, respectively.

BPA is well-known to be rapidly biodegradable under aerobic conditions (Dorn *et al.*, 1987; Lobos *et al.*, 1992). Elimination rates of 40 – 68 % in the denitrification vessels (fig. 5) make clear that BPA is also rapidly degraded by adapted sewage sludge biota under anoxic conditions. Anoxic degradation was not exhaustive and distinct adsorption to sludge occurred. The BPA remaining after denitrification was extensively degraded in the aeration stage resulting in water phase BPA concentrations of n.d. to 0.2 µg/l.

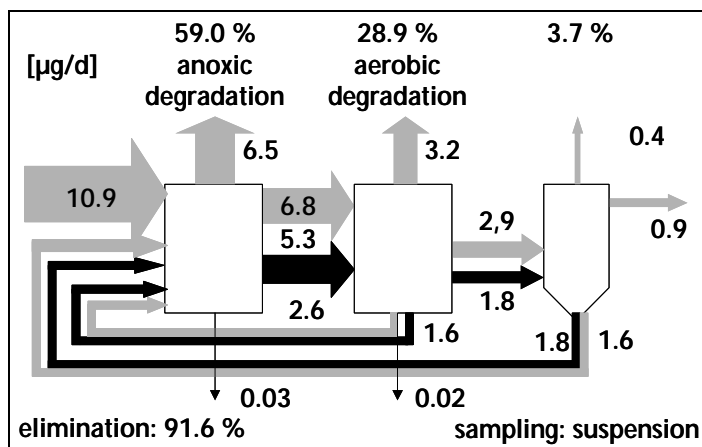


Figure 5: Bisphenol A mass balance experiment m1, all values [nmol/d] except otherwise stated

#### 4.3 Alkylphenols and Synthetic Steroids

In this chapter, preliminary results of experiments m1 and m2 are presented regarding the degradation of the pharmaceuticals and alkylphenols studied. Unfortunately, the river Elbe flood event of summer 2002 caused loss of samples, laboratory equipment, chemicals, and time. Therefore, the results presented, i.e. regarding the alkylphenolic compounds, are and probably will remain incomplete. The two short-chained nonylphenol ethoxylates determined (NP1EO, NP2EO) have not been supplied as target compounds. Thus, contamination with the PW influent must be stated. However, since AP ethoxylates are degraded to AP only under anaerobic conditions (Maguire, 1999) this contamination does not restrict the interpretation of the AP data set. In both experiments, the influent concentrations i.e. of the APs were distinctly lower than the respective target concentrations (see Tennhardt *et al.*, in press).

The results of experiments m1 and m2 are very similar. The overall elimination rates of the APs in week 5 amounted to 73 – 89 % (table 4). Obviously, OP was degraded to a lesser extent than NP. The overall elimination of the two pharmaceuticals studied amounted to 63 – 80 %. Though ME was less degraded in 4 of 5 weeks of experiment m1 the differences were not significant. In experiment m2 varying degradation rates of ME and EE2 were observed (Tennhardt *et al.*, in press).

Table 4. Elimination of EDC [%] during simultaneous aerobic sewage sludge stabilisation; synthetic wastewater, laboratory experiments, for explanations of acronyms see text

| no. of exp. | OP | NP              | EE2 | ME |
|-------------|----|-----------------|-----|----|
| m1          | 73 | 83 <sup>a</sup> | 80  | 63 |
| m2          | 74 | 89              | 71  | 76 |

<sup>a</sup> not considering mass fluxes with sampling

Table 5 lists the effluent concentrations of the target compounds in week 5 of the two experiments. The NP concentrations exceeded the OP concentrations by more than 3 fold. Since both alkylphenols have comparably high log  $k_{ow}$  values of approximately 4 this probably was caused by the much higher NP influent mass flux or/and a lower biodegradability of NP. The stronger hydrophobicity of ME compared to EE2 caused higher ME concentrations in sludge paired with lower concentrations in the supernatants (data not shown, see Tennhardt *et al.*, in press) and, thus, the effluents. These results indicate a lower biodegradability of ME compared to EE2 due to a lower bioavailability.

Table 5. Effluent concentrations of endocrine disrupters [ $\mu\text{g/l}$ ] after simultaneous aerobic sewage sludge stabilisation; synthetic wastewater, laboratory experiments <sup>a</sup>

| no. of exp. | OP    | NP    | NP1EO | NP2EO | ME    | EE2   |
|-------------|-------|-------|-------|-------|-------|-------|
| m1          | 0.034 | 0.155 | 0.052 | 0.036 | 0.101 | 0.159 |
| m2          | 0.023 | 0.102 | 0.065 | 0.047 | 0.074 | 0.229 |

<sup>a</sup> for explanations of acronyms see text

## 5. CONCLUSIONS

Simultaneous aerobic sewage sludge treatment predominantly applied at small and medium-size municipal wastewater treatment plants is estimated being very effective in eliminating bisphenol A and 17 $\beta$ -estradiol and its metabolites from synthetic wastewater and the thereby derived sludge. BPA as well as E2 have been extensively degraded under denitrifying as well as under aerobic conditions. Under anoxic conditions, E2 was metabolised to estrone. Subsequently, E1 as well as the remaining E2 have been almost exhaustively degraded under aerobic conditions. 4-tert-octylphenol, 4-nonylphenol, 17 $\alpha$ -ethynylestradiol, and mestranol have been overall eliminated to a lesser extent indicating a lower biodegradability. Thus, within one class of chemicals the extent of degradation by adapted sewage sludge microbiota seems to depend mainly on the water solubility of the respective compound. An aerobic downstream treatment stage is recommended for WWTP effluents. Sewage sludge should be stabilised under anaerobic conditions (degradation of AP precursors) in combination with downstream aerobic treatment, e.g. composting (degradation of APs).

Whereas the BPA and AP influent concentrations represented high but realistic levels of municipal wastewater pollution in Germany (Tennhardt *et al.*, in press; Wenzel *et al.*, 1998) the steroid influent concentrations were set unrealistic high. Despite of elimination rates of 63 – 99.6 % the effluent concentrations of the natural and synthetic steroids obtained in these experiments most likely would affect sensitive aquatic organisms if released to water environments at a significant volume proportion even if additivity of the estrogenic effects did not occur. The AP and BPA effluent concentrations are unlikely to affect exposed organisms.

Future research should comprise determinations of additional metabolites and precursors such as long-chained APnEO and AP carboxylates. Steroids should be supplied at lower concentrations in order to obtain more realistic conditions. Additional important EDC such as the androgenic steroid testosterone should be included. Above all, it has to be investigated how the effluent steroid concentrations can further be reduced. The influence of an increased hydraulic retention time and/or sludge age should be studied as well as the effects of additional downstream treatment e.g. in maturation ponds. The laboratory results has to be confirmed in field studies.

## 6. ACKNOWLEDGEMENTS

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## Corrections

Page 554, Figure 1: abbreviations: AE = aeration, EF = effluent, IR = intern reflux (excess sludge) RA = reflux of activated sludge, SA = sampling, ST = stirrer, T = temperature probe, TO = temperature and oxygen probe

Page 556, Table 1: <sup>a</sup> calculated from difference between system influent and effluent mass fluxes

Page 557, Figure 2: 99.0 % *anoxic* (not aerobic) degradation in denitrification vessel; 99.9 % total elimination (not 84.4)

Page 557, Figure 4: 51.3 % *anoxic* (not aerobic) degradation in denitrification vessel

Page 559, Figure 5: data given in [µg/d] except elimination rates in [%], not [nmol/d]

Page 559, paragraph 2: “Elimination rates of 40 – 68 % in the denitrification vessels (*table 3 not fig. 5*)...”

Please refer to as:

Gehring, M., Tennhardt, L., Vogel, D., Weltin, D., Bilitewski, B. (2003): Elimination of Estrogenic Endocrine Disruptors from Laboratory-Derived Sewage Sludge by Means of Simultaneous Aerobic Sludge Stabilisation. In: Pullammanappallil, Pr., McComb, A., Diaz, L. F., Bidlingmaier, W. (eds.): Proc. 4 th Int. Conference ORBIT Ass. on Biol. Process. Organics: Adv. Sustain. Soc., April 30 - May 02, 2003, Perth, Australia, vol. 2, 553 - 562.

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