PERGAMON

Chemosphere 52 (2003) 673–682

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

# Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge

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Received 4 September 2002; received in revised form 3 January 2003; accepted 25 January 2003

**Abstract**

Phthalic acid esters (PAE) are commonly found in the sludge generated in the wastewater treatment plants. Anaerobic digestion followed by land application is a common treatment and disposal practice of sludge. To date, many studies exist on the anaerobic biodegradation rates of PAE, especially of the easily biodegradable ones, whereas the higher molecular weight PAE have reported to be non-biodegradable under methanogenic conditions. Furthermore, there is no information on the effect of the PAE on the performance of the anaerobic digesters treating sludge. In this study, the anaerobic biodegradation of di-*n*-butyl phthalate (DBP), di-ethyl phthalate (DEP) and di-ethylhexyl phthalate (DEHP) was investigated and their relative rates of anaerobic degradation were calculated. Also, the biological removal of PAE during the anaerobic digestion of sludge in bench-scale digesters was investigated using DBP and DEHP as model compounds of one biodegradable and one recalcitrant PAE respectively. The degradation of all the PAE tested in this study (DEP, DBP and DEHP) is adequately described by first-order kinetics. Batch and continuous experiments showed that DEP and DBP present in sludge are rapidly degraded under mesophilic anaerobic conditions (a first-order kinetic constant of $8.04\times10^{-2}$ and $13.69\times10^{-2}$–4.35 $day^{-1}$ respectively) while DEHP is degraded at a rate between one to two orders of magnitude lower ($0.35\times10^{-2}$–$3.59\times10^{-2}$ $day^{-1}$). It is of high significance that experiments with anaerobic sludge of different origin (US and Europe) showed that degradation of DEHP occurs under methanogenic conditions. Accumulation of high levels of DEHP (more than 60 mg/l) in the anaerobic digester has a negative effect on DBP and DEHP removal rates as well as on the biogas production.


*Keywords:* Anaerobic degradation; Kinetics; DEP; DBP; DEHP; Phthalate esters

## 1. Introduction

Great interest has been devoted in recent years for the disposal of wastes generated by modern society. For instance, biologically treated sewage sludge, farm manure, organic industrial wastes and organic household wastes can, with great advantage, be recycled and used in farmland as fertilizers and as soil improving components. Therefore, the responsible management of wastewaters, wastes and sludge is of substantial economic and environmental interest. This type of wastes may, however, contain hazardous components in amounts preventing

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0045-6535/03/$ - see front matter 
doi:10.1016/S0045-6535(03)00126-7

their recycling and making their final disposal problematic. Specifically, many anthropogenic, toxic organic compounds (xenobiotics) are found in the sludge generated in municipal wastewater treatment plants.

Phthalic acid esters (PAE) are widely used industrial chemicals serving as additives in polyvinylchloride, polyvinylacetate, cellulosic and polyurethane resins (Staples et al., 1997). They are characterized by low solubility in water and high octanol/water partition coefficients. In fact, the longer the alkyl-chain, the lower the solubility and the higher the octanol/water partition coefficient (Staples et al., 1997). The PAE are commonly found in the primary and secondary sludge of municipal wastewater treatment plant. PAE have been found in sewage sludge at levels of 12–1250 mg/kg-TS. It is the high hydrophobicity and the low solubility of PAE that allows them to be adsorbed to suspended organic matter and subsequently to be transferred to primary and secondary sludge streams. During sewage treatment, the PAE can be enriched in the sewage sludge solids at concentrations several orders of magnitude higher than in the influent sewage. Anaerobic digestion followed by land application is a common treatment and disposal practice for sludge. PAE not degraded during the digestion process and introduced into the soil through the agricultural utilization of sewage sludge can be subject to further degradation. However, the most persistent PAE have been detected in soil (Michael et al., 1984). It has further been reported that more than 41% of the DEHP in sludge-amended soil escapes mineralization after one year (Madsen et al., 1999). Widespread occurrence of PAE in the environment raised concern about their possible toxicity to humans and other organisms since some of them are considered as potential carcinogens, teratogens and mutagens (Giam et al., 1982). Specifically, it has been reported that di-ethylhexyl phthalate (DEHP), one of the more recalcitrant phthalate ester, has xeno-estrogenic, carcinogenic and mutagenic effects (Beliles et al., 1989; Schulz, 1989; Nielsen and Larsen, 1996).

Many studies exist on the degradation of phthalates under aerobic (Eaton and Ribbons, 1982), nitrate reducing (Benckiser and Ottow, 1982) and methanogenic conditions (Ziogou et al., 1989) with mixed cultures or well-defined pure cultures of microorganisms. The most commonly studied PAE are—as it is anticipated—the ones most commonly found in the environment, i.e. di-ethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), butyl benzyl phthalate (BBP), DEHP and di-octyl phthalate (DOP) with DEHP and DBP being the most abundant ones.

Since anaerobic digestion of primary sludge is a common treatment method prior to sludge disposal, the understanding of the potential for anaerobic degradation of PAE in sludge and the measurement of their degradation rates is of great importance. The initial step in the anaerobic degradation of phthalates is believed to be the hydrolysis of the ester side chains of the di-alkyl phthalate through mono-alkyl phthalate (Shelton et al., 1984) leaving phthalic acid (PA) and alkyl alcohols available for further conversion to methane and carbon dioxide. In some cases, intermediates have been identified in traces, i.e. PA and mono-ethyl phthalate during the anaerobic degradation of DEP (Ejlertsson et al., 1996a), PA and mono-butyl phthalate during the anaerobic degradation of BBP (Shelton et al., 1984). Hydrolysis of PAE could be a complex process to be kinetically described, especially when suspended organic matter is present where PAE are adsorbed to; however, first-order kinetics are widely used in the literature and adequately simulate the PAE degradation (Ziogou et al., 1989; Madsen et al., 1999; Wang et al., 2000).

To date it is well known that PAE with shorter alkyl-chains, i.e. DEP and DBP, are very easily biodegraded while PAE with longer alkyl-chains, i.e. DOP and DEHP, are poorly degraded under anaerobic conditions. O'Grady et al. (1985), confirmed that a correlation exists between increasing length of the ester side-chain and decreasing biodegradability. However, controversial results exist concerning the anaerobic degradation of DEHP. Many researchers have reported that DEHP is non-degradable under methanogenic conditions (Shelton et al., 1984; Battersby and Wilson, 1989; Painter and Jones, 1990; Ejlertsson et al., 1996b, 1997). O'Connor et al. (1989) observed that little mineralization of DEHP under anaerobic conditions occurred and reported indications that DEHP is biodegraded to methane. On the other hand, Parker et al. (1994) reported a surprisingly high degradation of DEHP (83.3%) under methanogenic conditions in a two-stage system, which treated a mixture of primary and secondary sludge and was operated in a total retention time of 53 days at 35 °C.

Information regarding biodegradation and toxicity of phthalates were obtained through batch experiments by O'Connor et al. (1989). The results of this study indicated that PA, di-methyl phthalate, DEP and di-butyl phthalate were susceptible to anaerobic degradation. DBP did not suppress methanogenesis up to a concentration of 300 mg/l while DEP and DEHP exhibited a relatively high toxicity to methanogenesis over 20 and 100 mg/l respectively.

Very few studies are available on the relative degradation rates of phthalate esters and removal of phthalates during the actual wastewater treatment processes. Furthermore, there is no information on the effect of the recalcitrant phthalates on the performance of anaerobic digesters treating sludge and on the removal of biodegradable phthalates. In this study the anaerobic biodegradation of DEHP, DBP and DEP was investigated and their relative rates of anaerobic degradation were measured. Also, the biological removal of phthalates during the anaerobic digestion of sludge in bench-scale

digesters was investigated using DBP and DEHP as model compounds of one biodegradable and one recalcitrant PAE respectively.

## 2. Materials and methods

### 2.1. Analytical and computational methods

Determinations of the total (TSS) and volatile (VSS) suspended solids and total volatile solids (VS) were carried out according to standard methods (APHA, 1989). For the quantification of volatile fatty acids, the samples were analysed after acidification with 17% $H_3PO_4$ on a gas chromatograph (Hewlett Packard 5890 series II) with a flame ionisation detector and a capillary column (Hewlett Packard FFAP 30 m, inner diameter 0.53 mm, film 1 μm). Biogas production in continuous experiments was measured using compact automated displacement gas metering systems (Angelidaki et al., 1992) and its composition in methane was quantified with a gas chromatograph (Shimadzu GC-8A) with a flame ionisation detector and a packed column (Porapak Q, 80/100-mesh). Extraction of PAE was carried out in 10 ml glass centrifuge tubes. One milliliter of sample was extracted three times with 1 ml of di-chloromethane. The pH of the sample was adjusted to 12–14 with addition of 1 N NaOH solution and BBP was added and served as internal standard. Subsequently, the combined di-chloromethane extract was evaporated under $N_2$ gas supply and the residue was taken with 1 ml of di-chloromethane and filtered through a 0.45 μm PTFE membrane filter prior to GC–MS analysis. A Hewlett Packard model HP 6890 series gas chromatograph equipped with a HP 5973 mass selective detector (MS) and a 30.0 m × 250 μm × 0.25 μm HP-5MS column was used for the identification and quantification of PAE. The oven was programmed from 50 to 310 °C at a rate of 20 °C/min, held isothermal at 50 °C for 2 min and 310 °C for 10 min. The injector temperature was set at 280 °C and helium was used as carrier gas at 1.2 ml/min. The medium (BA medium) used in batch experiments was prepared from the following stock solutions (chemicals in g/l of distilled water): (A) $NH_4Cl$, 100; NaCl, 10; $MgCl_2 \cdot 6H_2O$, 10; $CaCl_2 \cdot 2H_2O$, 5; (B) $K_2HPO_4 \cdot 3H_2O$, 200; (C) resazurin, 0.5; (D) trace metals and selenite solution: $FeCl_2 \cdot 4H_2O$, 2; $H_3BO_3$, 0.05; $ZnCl_2$, 0.05; $CuCl_2 \cdot 2H_2O$, 0.038; $MnCl_2 \cdot 4H_2O$, 0.05; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2 \cdot 6H_2O$, 0.05; $NiCl_2 \cdot 6H_2O$, 0.092; ethylene-di-amine-tetra-acetate, 0.5; $Na_2SeO_3 \cdot 5H_2O$, 0.1; HCl 37%, 1 ml; (E) vitamin solution according to Wolin et al. (1963). The following volumes of stock solutions were added to 916 ml of distilled water: (A) 10 ml; (B) 2 ml; (C) 1 ml; (D) 1 ml; (E) 10 ml. About 50 ml of a 52 g/l $NaHCO_3$ solution were added as well. The medium was gassed with 80% $N_2$–20% $CO_2$, dispensed and autoclaved. Before inoculation the medium was reduced with a 25 g/l $Na_2S \cdot 9H_2O$ solution to a ratio of 0.1 ml/10 ml of medium. Simulation of non-steady state continuous and batch experimental data and calculation of the corresponding $K_h$ value was made using a modified non-linear least squares fitting method based on converting the differential mass balances to algebraic, upon discretization of time. This enabled the application of standard non-linear programming optimization algorithms. The least-squares objective was to select the best kinetic parameter values so as to minimize the squared residuals between the experimentally measured and the respective predicted concentration.

### 2.2. Continuous experiments in lab-scale digesters

#### 2.2.1. Experiments with DEHP at low concentration

This study was carried out at the Biocentrum-DTU and the influent was primary sludge collected from the Lundtofte municipal wastewater treatment plant located at Lyngby in Denmark. Two 3-l (useful volume) continuous stirred tank reactor (CSTR) type digesters ($A_D$ and $B_D$) were started up using as inoculum anaerobic sludge from the Lundofte plant and operated at the mesophilic temperature range (35 °C). During the first phase of the continuous experiment both digesters were fed with primary sludge at a 20 days retention time for a period of approximately six months. During the second phase of the experiment digester $A_D$ served as control while digester $B_D$ was being fed with primary sludge with DEHP added at a final concentration of 10 mg/l.

#### 2.2.2. Experiments with DBP and DEHP at high concentration

The concentrations of PAE used in the previous continuous experiments are similar to the ones reported for municipal wastewater. Much higher concentrations of PAE can be found in industrial effluents. In order to evaluate the effect of such high PAE concentrations on the performance of anaerobic digesters treating industrial wastewater, continuous experiments were run with sludge from the Hyperion wastewater treatment plant (HWTP) located in Playa del Rey, CA, USA. This study was carried out at the UCLA and the influent was a mixture of primary and secondary sludge with a high content in DBP (around 250 mg/l). Two 2.5-l (useful volume) CSTR type digesters ($A_U$ and $B_U$) were started up using as inoculum anaerobic sludge from the HWTP and operated at the mesophilic temperature range. After three months of operation at 20 days retention time (stabilization period), digester $B_U$ started being fed with sludge to which a mixture of DEHP and DBP was added at a high concentration (100 additional mg/l of each phthalate ester) while digester $A_U$ served as control.

### 2.3. Batch kinetic experiments

Batch kinetic experiments were carried out in 58 ml serum vials in order to calculate the relative rates of the degradation of DEP, DBP and DEHP during the anaerobic digestion of primary sludge. Anaerobic mixed liquor either from digester $B_D$ or digester $A_D$ fed with Lundtofte sludge was used as inoculum. The phthalates were added to the primary sludge in the form of concentrated solution in ethanol. The mixture was stirred for 15 min to allow the phthalates to be distributed in the sludge. Subsequently, 5 ml of this mixture and 10 ml of the anaerobic inoculum were transferred into the serum bottles, which contained 30 ml of BA medium. The experiments were carried out in duplicates at 37 °C. Duplicates that have been autoclaved served as controls in order to make sure that no abiotic degradation of phthalates takes place under the described experimental conditions.

## 3. Results and discussion

### 3.1. Continuous experiments in lab-scale digesters

#### 3.1.1. Experiments with DEHP at low concentration

The characteristics of the two collections of primary sludge used are shown in Table 1. During the first phase of the experiment the degradation of background levels of DEHP present in primary sludge from the Lundtofte wastewater treatment plant in Denmark was studied. Both digesters were operated similarly during a six months stabilization period. The characteristics of the two digesters $A_D$ and $B_D$ during the first experimental phase are shown in Table 2. One can observe that DEHP concentration in the digesters is lower than in the influent primary sludge (Table 1). However, due to the very low DEHP level and to the relatively high standard deviation of the DEHP measurement it was uncertain whether removal of DEHP occurred or not. In order to verify that DEHP removal was actually occurring and that the relevant activity was present in the digesters, one transition experiment took place (second phase) during which the concentration of the influent DEHP increased to 10 mg/l in the test digester (digester $B_D$). DEHP concentration in the digesters versus time was followed. DEHP level in the effluent of the test digester increased over time and after approximately 10 weeks it reached a stable level. In Fig. 1 the expected increase of DEHP concentration in case of no removal is compared with the experimental DEHP concentration. Assuming that: (a) the first step of the PAE biodegradation is the hydrolysis (Shelton et al., 1984) and (b) the hydrolysis of PAE follows first-order kinetics (Madsen et al., 1999; Wang et al., 2000), the DEHP mass balance in a CSTR is as following:

$$\frac{dS}{dt} = D \cdot S_{in} - D \cdot S - K_h \cdot S \quad (1)$$

where $S_{in}$ and $S$ is the influent and effluent DEHP concentration respectively, $D$ is the dilution rate and $K_h$ is the hydrolysis constant.

Optimal value for the hydrolysis constant, $K_h$, obtained through a least squares fitting method was $K_h = 0.993 \times 10^{-2}\ \text{day}^{-1}$. The simulated DEHP profile is shown in Fig. 1 as well and adequately fits the experimental DEHP concentration.

Table 1
Characteristics of the two collections of primary sludge from the Lundtofte wastewater treatment plant

| | First collection | Second collection |
|---|---|---|
| TS (g/l) | 11.51 ± 0.40 | 14.47 ± 0.31 |
| VS (g/l) | 8.42 ± 0.40 | 10.72 ± 0.27 |
| TSS (g/l) | 10.52 ± 0.35 | 13.22 ± 0.18 |
| VSS (g/l) | 8.11 ± 0.22 | 10.17 ± 0.17 |
| DEHP (mg/l) | 1.05 ± 0.17 | 1.22 ± 0.44 |

Table 2
Characteristics of the digesters $A_D$ and $B_D$ during the first and second experimental phase; mean values with their relative standard deviation are presented

| | First phase | | Second phase | |
|---|---|---|---|---|
| | Digester $A_D$ | Digester $B_D$ | Digester $A_D$ | Digester $B_D$ |
| pH | 6.9 | 6.9 | 6.9 | 6.9 |
| Biogas production (ml/day) | 655 ± 83 | 720 ± 90 | 587 ± 89 | 696 ± 95 |
| Biogas composition in methane (%) | 61 ± 2 | 61 ± 3 | 62.7 ± 5.4 | 61.4 ± 2.5 |
| TS (g/l) | 12.70 ± 0.76 | 12.66 ± 0.76 | 11.37 ± 0.34 | 12.52 ± 0.31 |
| VS (g/l) | 6.20 ± 0.26 | 6.45 ± 0.66 | 5.60 ± 0.00 | 7.14 ± 0.16 |
| TSS (g/l) | 11.00 ± 0.27 | 11.25 ± 0.62 | 10.26 ± 0.79 | 11.09 ± 0.60 |
| VSS (g/l) | 6.13 ± 0.32 | 6.66 ± 0.22 | 5.20 ± 0.33 | 6.56 ± 0.34 |
| DEHP (mg/l) | 0.70 ± 0.37 | 0.81 ± 0.48 | 0.74 ± 0.52 | Transition experiment |
| VFA | Not detected | Not detected | Not detected | Not detected |

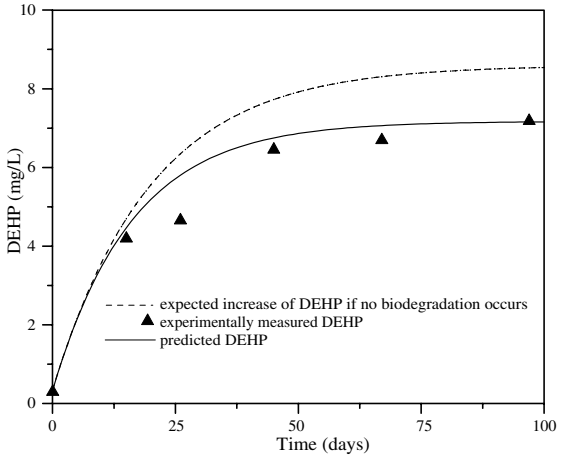


Fig. 1. Experimental and simulated ($K_h = 0.993 \times 10^{-2}$ day$^{-1}$) DEHP concentration versus time during the transition experiment in comparison with the expected accumulation of DEHP in case that degradation did not occur.

### 3.1.2. Experiments with DBP and DEHP at high concentration

The characteristics of the sludge used for these experiments are shown in Table 3. One can see that it is characterized by a high content in DBP while its DEHP content was low. Also average operational parameters for both digesters $A_U$ and $B_U$ during the three months stabilization period before PAE addition are reported in Table 3, showing that the performances of both digesters were similar. The amounts of biogas and $CH_4$ produced and removal of VS from both digesters were as expected for a mesophilic sewage sludge digester.

Applying a steady state solution to Eq. (1), the hydrolysis constant for PAE, $K_h$, can be calculated from the following equation:

$$K_h = D \cdot (S_{in} - S) \cdot \frac{1}{S} \quad (2)$$

It was calculated that $K_h$ for DBP hydrolysis in $A_U$ and $B_U$ lied between 1.98 and 4.35 day$^{-1}$. On the other hand, average concentrations of DEHP in the influent and effluent of the digesters were very small (Table 3). Similarly to the experiments with $A_D$ and $B_D$, due to the very low DEHP level and to the relatively high standard deviation of the DEHP measurement it was uncertain whether removal of DEHP occurred or not.

After the three months stabilization period, digester $B_U$ was fed with sludge to which a mixture of DEHP and DBP was added at a high concentration (100 additional mg/l of each phthalate ester) while digester $A_U$ served as control. The addition of PAE stopped after 80 days. DEHP and DBP concentration in the digester $B_U$ was monitored during this period and the results are presented in Fig. 2a and b, respectively. The mean concentration of DBP and DEHP in the influent and effluent of the control digester, $A_U$, remained stable as reported in Table 3.

During the first 40 days DBP concentration in the effluent of the $B_U$ gradually increased and finally it was stabilized at an average concentration of 68.59 (±8.64) mg/l corresponding to a DBP removal of 78.6%. After the 80th day, the addition of PAE was stopped and DBP concentration returned to the initial low level (removal of 99%). In order to get a clearer picture of the kinetics of the DBP removal during this experiment, a first-order kinetic model was fitted to the experimental values (Eq. (1)). The experimental period of 17 weeks was split up to four shorter periods (see Fig. 2a) since the hydrolysis constant, $K_h$, seemed to vary throughout the experiment. Optimal value for the hydrolysis constant, $K_h$, obtained through a least squares fitting method and the simulated DBP profile is shown in Fig. 2a. The mean DBP concentration in the influent and the calculated $K_h$ value for each time period is shown in Table 4. Also, the theoretical predictions using Eq. (2) for the effluent DBP concentration and percent of removal at steady state are presented in Table 4.

Similarly to DBP, DEHP concentration in the effluent of the $B_U$ started to increase after the PAE increase in the influent and reached a maximum around day 50. After the 80th day addition of phthalate esters was stopped and the concentration of DEHP decreased again to its initial level. Similarly to the DBP case, a first-order kinetic model was fitted to the experimental

Table 3
Characteristics of the Hyperion sludge (influent sludge) and the digesters $A_U$ and $B_U$ after the three months stabilization period; mean values of the last four weeks of operation with their relative standard deviation are presented

| | Influent sludge | Digester $A_U$ | Digester $B_U$ |
|---|---|---|---|
| VS (g/l) | 16.42 ± 2.28 | 7.46 ± 0.56 | 7.21 ± 0.57 |
| DBP (mg/l) | 255.4 ± 83.1 | 2.9 ± 4.5 | 6.3 ± 3.2 |
| DEHP (mg/l) | 3.1 ± 0.5 | 2.5 ± 0.9 | 1.8 ± 1.1 |
| VS loading rate (mg/day) | – | 821 ± 114 | 821 ± 114 |
| Biogas production (ml/mg VS loaded) | – | 1.27 | 1.34 |
| Biogas composition in methane (%) | – | 54.9 ± 4.7 | 55.1 ± 4.5 |
| VS removal (%) | – | 55.5 | 54.1 |

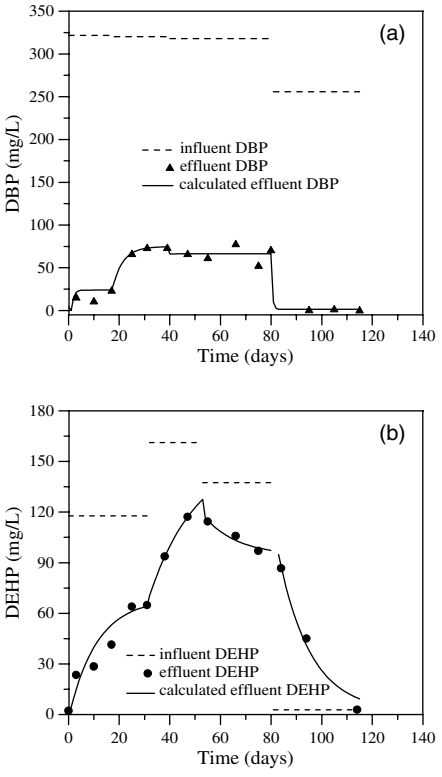


Fig. 2. Experimentally measured influent and effluent DBP and DEHP concentration and model predicted DBP and DEHP effluent profile during the experiment with $B_U$.

values (Eq. (1)). The experimental period of 17 weeks was split up to four periods (Fig. 2b) and optimal values for the hydrolysis constant, $K_h$, obtained through a least squares fitting method. The model predicted DEHP profile is compared with the experimental data in Fig. 2b. The mean DEHP concentration in the influent and the estimated $K_h$ value for each time period is shown in Table 5. Theoretical predictions for the effluent DEHP concentration and % removal at steady state are presented in Table 5 as well.

A decreased DBP efficiency and corresponding $K_h$ had been calculated during the second and third period of the experiment with $B_U$ (Fig. 2a, day 20th–80th) although the influent DBP concentration was almost stable. The decreased efficiency of DBP degradation is correlated with the accumulation of high levels of DEHP in the digester (Fig. 2b) suggesting that increased concentration of DEHP had a negative effect on DBP removal. Also, the efficiency and corresponding $K_h$ of DEHP removal was very much negatively affected when the DEHP influent concentration increased over 150 mg/l. The inhibition of both DBP and DEHP removal from high concentration of DEHP was reversible, since the level of PAE concentration decreased to their original value after PAE addition stopped, namely 6.2 and 1.9 mg/l of DBP and DEHP (Tables 4 and 5) compared with the 6.3 and 1.8 mg/l of DBP and DEHP respectively after the three months stabilization period (Table 3). Consequently, the kinetic constants and removal efficiencies increased to their original level as well when the addition of the 100 mg/l PAE stopped.

Hydrolysis constants estimated for DBP removal were one to three orders of magnitude higher than these calculated for DEHP. Also hydrolysis of DEHP was faster during experiments with $B_U$ compared with the

Table 4
Mean DBP concentration in the influent and calculated $K_h$ value for each time period during the experiment with $B_U$

| Period | Days | Influent DBP (mg/l) | $K_h$ (day$^{-1}$) | Model predictions for steady state | |
|---|---|---|---|---|---|
| | | | | Effluent DBP (mg/l) | Percent of removal |
| I | 0–17 | 321.79 ± 66.99 | $62.02\times10^{-2}$ | 24.01 | 92.5 |
| II | 17–39 | 320.25 ± 14.11 | $16.40\times10^{-2}$ | 74.83 | 76.6 |
| III | 39–80 | 318.06 ± 33.90 | $18.92\times10^{-2}$ | 66.47 | 79.1 |
| IV | 80–115 | 256.02 ± 23.58 | 2.00 | 6.24 | 97.6 |

Also theoretical (model) predictions for the effluent DBP concentration and percent of removal at steady state are presented.

Table 5
Mean DEHP concentration in the influent and calculated $K_h$ value for each time period during the experiment with $B_U$

| Period | Days | Influent DEHP (mg/l) | $K_h$ (day$^{-1}$) | Model predictions for steady state | |
|---|---|---|---|---|---|
| | | | | Effluent DEHP (mg/l) | Percent of removal |
| I | 0–31 | 117.76 ± 17.59 | $3.59\times10^{-2}$ | 68.55 | 41.8 |
| II | 31–53 | 161.26 ± 19.11 | $0.20\times10^{-2}$ | 155.10 | 3.8 |
| III | 53–80 | 137.54 ± 10.24 | $2.30\times10^{-2}$ | 94.23 | 31.5 |
| IV | 80–115 | 2.88 ± 0.78 | $2.58\times10^{-2}$ | 1.90 | 34.0 |

Also theoretical (model) predictions for the effluent DEHP concentration and percent of removal at steady state are presented.

experiment in $B_D$. This could be attributed either to the presence of hydrolytic enzymes (esterases) due to the high influent concentration of DBP or to the addition of secondary sludge in the influent of $A_U$ and $B_U$ or to both of them. However, the experimental results from both $B_D$ and $B_U$ digesters indicate that DEHP is degraded at a very slow rate (see also the results of batch kinetic experiments of this study) and it accumulates to a great extent in the digesters. It was also clear that recalcitrance to anaerobic degradation is an inherent DEHP characteristic since anaerobic digested sludge from different sources showed the same difficulty to degrade it. On the other hand, the fact that $B_U$ exhibited a higher ability for DEHP degradation than $B_D$ might be an indication of indirect acclimation of biomass to DEHP as well due to the presence of high DBP concentration.

The performance of the control, $A_U$, and test, $B_U$, digester regarding biogas production, VS removal and VFA concentration is presented in Table 6. Addition of phthalates did not affect the test digester performance during the first 50 days of PAE addition. The amount of gas produced, and percent of removal of VS were similar for both $B_U$ ($1368 \pm 235$ ml/day and $56.7 \pm 4.3$ respectively) and $A_U$ ($1393 \pm 245$ ml/day and $54.8 \pm 4.4$ respectively) digesters and also similar to the levels observed before phthalate addition. Likewise, during this period no effect was observed on the $CH_4$ concentration, which remained stable at approximately 55% in the biogas from both digesters. At the end of the 50-days period a decrease in the gas production of both the control ($1261 \pm 109$ ml/day) and test ($628 \pm 100$ ml/day) digester, was observed. However, the decrease was dramatically more pronounced in the test digester, and gas production in $B_U$ never returned to the level of gas production in the control digester for the rest 30 days of PAE addition (Table 6). The decrease in biogas production that was observed in the test digester may be evidence of a process imbalance. It is interesting, however, that the levels of VFA during the whole 80-days period of PAE addition were similar for both the test and control digester whereas a process imbalance is usually accompanied by a rise in levels of VFA (Ahring et al., 1995). Also, the removal of VS was very similar for both digesters (Table 6).

This important decrease of methane production correlated with the high concentration of DEHP in the $B_U$ digester suggests that the DEHP has inhibitory effects on methanogenesis. O'Connor et al. (1989) investigated the effect of the concentration of DEHP on the methanogenesis in batch experiments. They reported

Table 6
Performance of the digesters $A_U$ and $B_U$ during and after the 12 weeks period of PAE addition

| Weeks | Days | Biogas production (ml/day) | | VS removal (%) | | VFA (mM) | |
|---|---|---|---|---|---|---|---|
| | | $A_U$ | $B_U$ | $A_U$ | $B_U$ | $A_U$ | $B_U$ |
| 1st | 1 | 1080 | 1070 | 53.54 | 52.53 | 0.74 | 1.59 |
| | 2 | 1062 | 1031 | | | | |
| | 4 | 1308 | 1081 | 50.62 | 53.81 | 1.73 | 2.91 |
| 2nd | 9 | 1008 | 1155 | 57.26 | 59.35 | 0.54 | 0.95 |
| 3rd | 16 | 1380 | 1470 | 55.77 | 59.53 | 0.42 | 0.55 |
| 4th | 23 | 1523 | 1433 | | | | |
| | 29 | 1594 | 1604 | 51.27 | 53.80 | 0.30 | 0.77 |
| 5th | 30 | 1607 | 1630 | | | | |
| | 36 | 1379 | 1356 | 63.23 | 64.29 | 0.91 | 0.30 |
| 6th | 37 | 1377 | 1330 | | | | |
| | 43 | 1658 | 1602 | 52.28 | 54.04 | 0.51 | 1.03 |
| 7th | 44 | 1747 | 1654 | | | | |
| 8th | 51 | 1446 | 710 | | | | |
| | 54 | 1266 | 716 | 55.91 | 56.84 | 0.48 | 0.51 |
| 9th | 58 | 1356 | 650 | 55.57 | 57.53 | 1.01 | 2.04 |
| 10th | 65 | 1264 | 666 | | | | |
| | 67 | 1298 | 728 | 48.08 | 48.45 | 0.13 | 1.98 |
| 11th | 72 | 1106 | 490 | 52.87 | 55.78 | 0.30 | 0.73 |
| 12th | 79 | 1190 | 476 | | | | |
| | 80 | 1164 | 586 | 49.01 | 45.03 | 0.52 | 0.20 |
| 13th | 86 | 1192 | 828 | | | | |
| 14th | 93 | 1074 | 434 | | | | |
| 15th | 100 | 1128 | 294 | | | | |
| 16th | 107 | 872 | 502 | | | | |
| 20th | 114 | 862 | 322 | | | | |
| 18th | 121 | 1010 | 342 | | | | |

that DEHP exhibited a relatively high toxicity to methanogenesis over 100 mg/l. Battersby and Wilson (1988) also studied the effect of DEHP concentration on biogas production by digested sludge. It was found that gradual increase in the concentration of DEHP from 25 to 200 mg of carbon/l caused a respective decrease in biogas production (from 48% to 6% of the theoretical value) over a period of 60 days. Although the level of DBP also increased in $B_U$ up to an average concentration of 68.39 (±8.02) mg/l, this concentration was well below the background concentration in the influent sludge, so it should not affect gas production. Furthermore, O'Connor et al. (1989) reported no toxic effect of DBP on methanogenesis at similar concentrations.

The toxic effects observed in the present study may be due to the accumulation of DEHP by itself or to the accumulation of a primary metabolite, i.e. 2-ethyl hexanol that is released during the hydrolysis of DEHP. It is potentially significant that Ejlertsson et al. (1997) reported a negative effect of 2-ethyl hexanol on the methanogenic process. A similar toxic effect on methanogenesis caused by long chain fatty acids (LCFA) coming from lipids hydrolysis (i.e. oleic acid coming from the hydrolysis of glycerol trioleate) has been reported (Angelidaki and Ahring, 1992). Irreversible decrease in methane production not followed by an accumulation of VFA and a low removal rate for the toxic compound were observed as well. Further experiments demonstrated that LCFA like oleic and stearic acids inhibited all steps on the anaerobic thermophilic biogas process and that the inhibition of methane production was irreversible. An effect of LCFA on the membrane of microbial cells was hypothesized as the reason for the inhibition and it is reasonable to hypothesize that LCFA and long chain alcohols, like 2-ethyl hexanol, might have similar inhibitory effects. However, more experimental work would be necessary to test this hypothesis.

### 3.2. Batch kinetic experiments

Batch studies in serum bottles were performed in order to define the relative degradation rates of easily biodegradable and recalcitrant PAE. The biodegradation of the easily biodegradable DEP and DBP and the recalcitrant DEHP was studied using anaerobic sludge from the control digester $A_D$ which was being fed with primary sludge from the Lundtofte treatment plant. Biodegradation rates for each phthalate ester were calculated using a first-order kinetic model and applying a least squares fitting method. The concentration of PAE in the control vials was stable throughout the experiment suggesting that abiotic degradation or loss of PAE did not occur under the specific experimental conditions. The experimental and model predicted (first-order hydrolysis, Eq. (1)) degradation curves of DEP, DBP and DEHP are shown in Fig. 3. Estimated values for hydrolysis constant, $K_h$, and half-lives of PAE, $t_{1/2}$, (calculated according to Eq. (3)) are shown in Table 7. Biodegradation rates for DEP and DBP were approximately in the same order of magnitude and between one to two orders of magnitude higher than the biodegradation rate for DEHP.

$$t_{1/2} = \frac{\ln 2}{K_h} \tag{3}$$

The influence of DEHP-acclimation on the degradation rates of the three aforementioned PAE was also studied using anaerobic sludge from the test digester $B_D$ that was being fed with primary sludge containing 10 mg/l DEHP. The estimated values for hydrolysis constant are also shown in Table 7 and are comparable with the values obtained from the kinetic experiments with non-acclimated anaerobic sludge from digester $A_D$. Therefore, DEHP-acclimation at the low concentration of 10 mg/l did not have a positive effect on the degradation rates of phthalate esters.

It is noticeable that the continuous transition experiment in the digester $B_D$ and the batch experiments resulted in similar values for the DEHP hydrolysis constant $K_h$, $0.99\times10^{-2}$ and $0.4\times10^{-2}$ day$^{-1}$ respectively. Also, experiments with the digester $B_U$ resulted in hydrolysis constants lying between $0.20\times10^{-2}$ and $3.59\times10^{-2}$ day$^{-1}$. This is strong evidence that degradation of DEHP occurs under methanogenic conditions, although

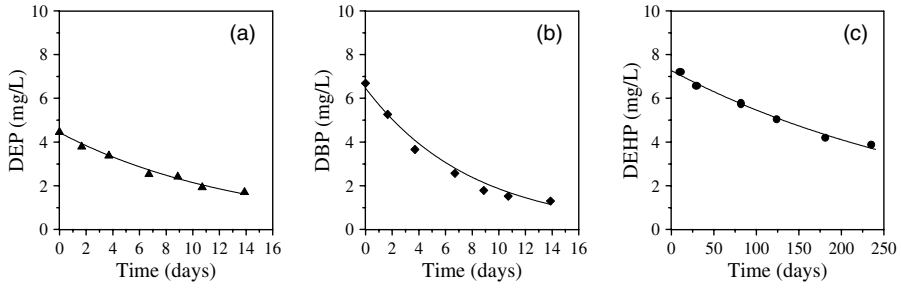


Fig. 3. Model predicted and experimental concentration profiles of DEP (a), DBP (b) and DEHP (c) during batch experiments with anaerobic sludge from digester $A_D$ used as inoculum.

Table 7
Optimal values of kinetic constant, $K_h$, with their relative standard deviation and half-lives of DEP, DBP and DEHP under methanogenic conditions

| PAE studied | Kinetic constant $K_h$ ($day^{-1}$) | Coefficient of correlation $R^2$ | Half-lives $t_{1/2}$ (day) |
|---|---|---|---|
| *Experiments with anaerobic sludge from control digester $A_D$* | | | |
| DEP | $8.04\times10^{-2}\pm1.27\times10^{-2}$ | 0.86 | 8.6 |
| DBP | $13.69\times10^{-2}\pm1.78\times10^{-2}$ | 0.97 | 5.1 |
| DEHP | $0.35\times10^{-2}\pm0.09\times10^{-2}$ | 0.97 | 198 |
| *Experiments with anaerobic sludge from digester $B_D$ (acclimated to 10 mg/l DEHP)* | | | |
| DEP | $6.34\times10^{-2}$ | 0.94 | 10.9 |
| DBP | $11.18\times10^{-2}\pm1.24\times10^{-2}$ | 0.99 | 6.2 |
| DEHP | $0.40\times10^{-2}\pm0.02\times10^{-2}$ | 0.95 | 173 |

very slow. The slightly enhanced degradation rate in the continuous reactor $B_D$ compared to the batch experiment is probably due to the mixing conditions. To our knowledge, this is the first time that degradation kinetics of DEHP under methanogenic conditions were measured and reported. It is very interesting though, that the degradation rates of DEHP calculated in the present study are in agreement with these calculated in the study of Madsen et al. (1999) concerning DEHP mineralization in sludge amended soil. In the latter study, first-order kinetics with a $K_h$ lying between of $0.23\times10^{-2}$ and $1.27\times10^{-2}$ $day^{-1}$ were reported for DEHP in sludge-amended soil incubated under aerobic conditions whereas a $K_h$ of $0.23\times10^{-2}$ $day^{-1}$ was calculated for DEHP in sludge-amended soil incubated under anaerobic conditions.

In the study of Ziogou et al. (1989) first-order degradation kinetic constants for DEP and DBP have been reported to be $14.40\times10^{-2}$ and $25.44\times10^{-2}$ $day^{-1}$ respectively under methanogenic conditions. Also in the study of Wang et al. (2000) a first-order constant for DBP has been calculated to be $51.84\times10^{-2}$ $day^{-1}$. They are in the same range with these calculated in the present study albeit higher. This could be attributed to the different kind of sludge used and to the stirring conditions.

Concluding, degradation of all the phthalate esters tested in this study (DEP, DBP and DEHP) is adequately described by first-order kinetics. Batch and continuous experiments showed that DEP and DBP present in sludge are rapidly degraded under mesophilic anaerobic conditions while DEHP is degraded at a rate between one to two orders of magnitude slower than DEP and DBP. It is of high significance that experiments with anaerobic sludge of different origin (US and Europe) showed that degradation of DEHP occurs under methanogenic conditions, although very slow. Furthermore, acclimation of the anaerobic sludge to 10 mg/l DEHP does not have a beneficial effect on the degradation rates of all three PAE tested. Accumulation of high levels of DEHP in the anaerobic digester had a negative effect on DBP and DEHP removal rates as well as on biogas production. Consequently, the level of PAE is a critical parameter to measure in sewage sludge going to anaerobic digestion in cases involving industrial influents with high concentrations of poorly biodegradable or recalcitrant PAE (e.g. DEHP or DOP) or municipal wastewater plants receiving wastewater from industrial sources where a shock load of recalcitrant PAE can be experienced.

## Acknowledgements

This work was funded by grants from (a) the Danish Environmental Research Program (Center for Sustainable Land Use of Waste), (b) the Commission of the European Communities (under Marie Curie grant no. HPMF-CT-1999-00371) and (c) the University of California Los Angeles. The authors would like to thank Dr. Ioannis V. Skiadas for critical review of the manuscript.

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