PERGAMON

Water Research 37 (2003) 1260–1269

WATER RESEARCH

www.elsevier.com/locate/watres

Toxicity of di-(2-ethylhexyl) phthalate on the anaerobic digestion of wastewater sludge

F. Alatriste-Mondragon[a], R. Iranpour[b], B.K. Ahring[a,*]

[a] *Department of Civil and Environmental Engineering, University of California Los Angeles, 5732E Boelter Hall, Los Angeles, CA 90095-1593, USA*

[b] *Applied Research, City of LA Bureau of Sanitation, Hyperion Treatment Plant, 12000 Vista Del Mar, Playa del Rey, CA 90293, USA*

Abstract

Previous studies on the microbial degradation of individual phthalic acid esters (PAEs) have demonstrated that the compounds with short ester hydrocarbon chains are easily biodegraded and mineralized, but PAEs with long ester chains are less susceptible to degradation and some of them are considered recalcitrant. Moreover, they inhibit methanogenesis. However, studies have not been made on the effect of feeding a combination of recalcitrant and biodegradable PAEs into anaerobic digesters treating wastewater sludge. The present study was conducted with wastewater sludge from the Los Angeles Bureau of Sanitation's Hyperion Treatment Plant. Di (2-ethylhexyl) phthalate (DEHP), the most common persistent PAE found in wastewater, and di-*n*-butyl phthalate (DBP), a common PAE with short ester chains, were sorbed into the sludge fed to a bench-scale digester for a period of 12 weeks. DEHP degradation was always poor, and accumulation of DEHP was correlated with inhibition of the microbial degradation of DBP and with process instability of the test digester. Inhibition of the DBP removal was completely reversed after DEHP addition was discontinued, but biogas production never recovered to the level observed in a control digester. Other process parameters of digester performance were not affected by DEHP accumulation. These results are similar to the toxic effects of long chain fatty acids on sludge digestion, suggesting that DEHP or its degradation products affect all the microbial populations in the anaerobic bioreactor. Our results imply that high levels of DEHP or other recalcitrant PAEs in wastewater sludge are likely to compromise methanogenesis and removal of biodegradable PAEs in sludge digesters.

Keywords: Phthalic acid esters; Anaerobic biodegradation; Wastewater sludge; Anaerobic digestion; DEHP; DBP

1. Introduction

Phthalic acid esters (PAEs) or phthalate esters are chemical compounds widely used as plasticizers giving plastics flexibility and durability. Plasticizers hold 65% of the 7.5 million ton world market for plastic additives. The majority of this, about 90% is used for polyvinyl chloride (PVC)-based plastics [1]. Industrial applications of PVC-based plastics include coatings, plumbing, and construction materials and in the manufacture of common plastic products such as vinyl upholstery, tablecloths, and shower curtains. PAEs are also present in plastic products for human use, e.g. teething rings, pacifiers, soft squeeze toys, plastic bottles, and enclosures for food containers and in medical products, e.g. flexible devices for administering parenteral solutions, and vinyl gloves [2]. Formerly, they were used in an even wider range of applications.

PAEs are ubiquitous compounds in the environment. Earlier studies reported the presence of phthalate esters in the air and precipitation at remote marine locations [3] and surface waters and sediments [4]. Phthalates are further found in waste such as source-sorted household

*Corresponding author. Present address: BioCentrum, The Technical University of Denmark, Block 227, 2800 Lyngby, Denmark. Tel.: +45-45883276; fax: +45-45256183.

E-mail address: bka@biocentrum.dtu.dk (B.K. Ahring).

0043-1354/03/$ - see front matter
PII: S0043-1354(02)00387-1

solid waste [5]. A recent survey reported detectable levels of phthalate esters in samples of foodstuffs, human mother's milk, dust, and textiles with di (2-ethylhexyl) phthalate (DEHP) and di-*n*-butyl phthalate (DBP) being the most abundant [6].

Widespread occurrence of PAEs in the environment raise concerns about their toxicological effects on living organisms. The existing studies have indicated a variety of biological effects on humans and other organisms. Estrogenic effects of PAEs on humans are further being discussed [7]. DEHP is included in class B2 (probable human carcinogen) while butyl-benzyl phthalate (BBP) is in class C (possible human carcinogen) [8]. Other commercial phthalates (DBP, di-ethyl phthalate (DEP) and di-methyl phthalate (DMP)) were included in class D (have not been classified as human carcinogens) [8].

Once PAEs enter in the environment they partition between air, water, soil and sediments. However, given the low solubility and highly hydrophobic nature of these compounds, they will preferentially be sorbed to the organic fraction of soil or sediments, as well as to the organic matter suspended in water. PAEs are present in sewage through several non-point sources such as domestic and commercial discharges, street runoff, and aerial deposition and point sources such as industrial discharges. During the sewage treatment, primary and ultimate degradation of PAEs occur during the aerobic phase of sewage treatment [9]. PAEs sorbed to the suspended organic matter will escape aerobic degradation and will be transferred to primary and secondary sludge streams. These streams are normally treated by anaerobic digestion of the sewage sludge. Batch experiments using digested sludge as inoculum demonstrated the microbial degradation of phthalate esters under methanogenic conditions [10–13]. Members of the PAEs with lower molecular weight (i.e. DMP, DEP, DBP and BBP) were easier to degrade than members with higher molecular weight (i.e. DOP and DEHP). Results from a bench-scale digester study [14] and a pilot plant digester study [15] were in good agreement with the earlier batch studies.

Sorbed PAEs that are not degraded during the anaerobic digestion of sewage sludge will accumulate in the sewage sludge solids (biosolids) at concentrations several orders of magnitude higher than in the influent wastewater. The USA National Sewage Sludge Survey reported detectable levels of DEHP in the range from 55.1 to 163.3 mg/kg dry weight [16]. Similar levels were reported in a Canadian survey [17] and in a more recent survey in Germany [18].

Anaerobic digestion followed by land application is a common treatment and disposal practice for biosolids. Continuous practice of land application of biosolids may eventually lead to accumulation of the most persistent toxic organics in the soil, with a consequent threat to the ecosystem and to human beings in particular. Therefore, it is of utmost importance to operate the anaerobic digesters treating wastewater sludge under conditions that guarantee an efficient removal of the most common toxic organic compounds found in sewage sludge.

Only scarce information exists on the effect that the most common persistent phthalate ester found in wastewater sludge, such as DEHP, have on both the performance of anaerobic digesters treating wastewater sludge and on the removal of biodegradable phthalate esters. Therefore, our goal in this study was to evaluate the biological removal of PAEs during the anaerobic digestion of wastewater sludge in bench-scale digesters using DBP and DEHP as model compounds representing a biodegradable and a recalcitrant PAE, respectively.

2. Materials and methods

The study was carried out in two phases. A preliminary toxicity study was done in small serum bottles to determine an initial concentration of DBP and DEHP that would be low enough not to harm the performance of a bench-scale digester. Once this was determined, the main phase began, with a test digester being fed with sludge to which DEHP and DBP had been added, and a control digester receiving a feed containing no PAEs but otherwise identical to the test digester feed.

For both phases the seed culture was digested sludge obtained from full-scale anaerobic digesters at the Hyperion Wastewater Treatment Plant, located at Playa del Rey, California. The feed for both phases was a mixture of primary sludge and secondary mixed liquor from the same plant.

2.1. Toxicity study

The goal of our toxicity batch experiment was to simulate as much as possible the conditions existing in the bench-scale digester while testing the toxic effects of three concentrations of DBP and DEHP: 5, 50 and 500 mg/l. Methane production was followed in 60-ml serum bottles in which the seed sludge was diluted with a mineral medium to a TVS concentration similar to the one used in the bench-scale digesters, and the feed sludge carried the PAEs.

Each serum bottle contained 10 ml of mineral medium. The composition of the mineral medium was as follows in mg/l: NH_4Cl 1000, NaCl 10, $MgCl_2 \cdot 6H_2O$ 100, $CaCl_2 \cdot 2H_2O$ 50, $K_2HPO_4 \cdot 3H_2O$ 400, $NaHCO_3$ 2600, $Na_2S \cdot 9H_2O$ 250, $FeCl_2 \cdot 6H_2O$ 2.38, H_3BO_3 0.050, $ZnCl_2$ 0.050, $CuCl_2 \cdot 2H_2O$ 0.038, $MnCl_2 \cdot 4H_2O$ 0.050, $(NH_4)_6$ $Mo_7O_{24} \cdot 4H_2O$ 0.050, $AlCl_3$ 0.050, $COCl_2 \cdot 6H_2O$ 0.050, $NiCl_2 \cdot 6H_2O$ 0.092, EDTA 0.5, $Na_2SeO_3 \cdot 5H_2O$ 0.066. Mineral medium was prepared

following anaerobic standard procedures to avoid presence of oxygen. Headspace in the bottles contained an O_2-free mixture of N_2/CO_2 (80/20 v/v). DBP and DEHP were dissolved in methanol and added from concentrated stock solutions directly to the feed sludge. Sewage sludge and phthalates were stirred for 1 h to allow the phthalates to be sorbed onto the sludge. Then, 10 ml of the sludge containing phthalates and 10 ml of seed sludge were transferred to the serum bottles. The final concentration of total volatile solids (TVS) in the serum bottles was 15 g/l. Controls without PAEs addition and controls without feed sludge were included. The serum bottles were incubated at 35°C. The amount of biogas produced and the concentration of CH_4 in the biogas were measured at successively increasing time intervals.

The specific methanogenic activity was calculated as the ratio of the cumulative amount of methane produced to the initial content of TVS present in the sewage sludge. The specific methanogenic activities were corrected for the seed contribution.

The toxic effect of the PAEs tested was measured as "relative activity" as previously described [19]. In our study, we defined the relative methanogenic activity (RMA) at any given time as

$$\mathrm{RMA} = \frac{\mathrm{SMA\ (PAE)}}{\mathrm{SMA\ (control)}},$$

where SMA (PAE) is the specific methanogenic activity at the tested concentration, and SMA (control) the specific methanogenic activity in the control without PAEs addition.

2.2. Operation of digesters

Two 5-l digesters were used for the experiment. Magnetic stirring plates and magnetic bars were used to continuously mix the contents of the digesters (2.5 l). A hot water jacket and a water recirculation bath were used to maintain the temperature of the digesters at 35°C. Mesophilic digested sludge obtained from the Hyperion plant was used as initial seed. The digested sludge from Hyperion was diluted 1:1 with warm tap water to a final concentration of 7.5 g TVS/l. Digesters were fed once a day with sewage sludge (a mixture of primary sludge and secondary mixed liquor) containing in average 15 g TVS /l. Digesters were operated with a 20-days HRT. Steady state was indicated by stable biogas production, biogas composition (CH_4/CO_2), TVS removal and levels of volatile fatty acids (VFAs).

When PAE addition to the test digester began, DBP and DEHP were dissolved in methanol, and then added to the sludge feedstock and stirred for 1 h to allow sorption of the phthalates. The resulting concentration of each phthalate ester added to the feed sludge was 100 mg/l, giving a concentration of at least 5 mg/l in the digester before any accumulation had occurred. The control digester was fed with sludge to which the same amount of methanol had been added, except that no phthalates were dissolved in the methanol.

PAE addition began after an acclimation period of nearly 3 months for both digesters. Average background concentrations of DBP and DEHP in the feed sewage sludge were measured over the last month of the acclimation period. The addition of PAEs to the test digester started on day 86 and continued for nearly 12 weeks. Starting at day 166 no more PAEs were added to the test digester, but both test and control digesters were fed for another 6 weeks with sludge amended with the same amount of methanol.

2.3. Digester performance

The amount of biogas produced was continuously measured with a gas meter constructed by our group [20]. Biogas composition was measured with a gas chromatograph equipped with a TCD. A stainless-steel column (8 ft × 2.1 mm i.d.) packed with 60/80 Carboxen 1000 was used. The oven temperature was 85°C and the He flow was 30 ml/min. TVS, ammonia and alkalinity were measured as described by the Standard Methods for Analysis of Water and Wastewater [21]. pH was measured with a pH electrode. VFA levels were quantified as free acids with a gas chromatograph equipped with an FID. A glass column (6 ft × 2 mm ID) packed with 3% Carbowax 20 M, 1% H_3PO_4 on 60/80 Carbopack C was used. The oven temperature program was 100°C (1 min); 15°C/min, 200°C (3 min). The temperature in the detector and injector was 220°C. The He flow was 20 ml/min.

2.4. Levels of phthalate esters

The levels of DBP and DEHP were measured by extracting them with dichloromethane (DCM) and measuring the concentrations with a gas chromatography assay. One-milliliter samples of feeding sludge and digested sludge were extracted three times with DCM (1:1 volume). DCM extracts were passed through a small glass column containing anhydrous Na_2SO_4 to eliminate contaminating water. The three extracts were pooled and concentrated in a water bath at 35°C to a final volume equal to 100 µl. Concentrations of DBP and DEHP in the DCM extracts were measured with a gas chromatograph equipped with an FID. A high-performance capillary column (30 m × 0.32 mm i.d.) with a film of HP-5 (crosslinked 5% PH ME siloxane; 0.25 µm film thickness) was used. The oven temperature program was 150°C (5 min); 5°C/min, 220°C; 3°C/min, 275°C (13 min). The temperatures in the detector and injector were 320°C and 275°C, respectively. The He flow was

1.2 ml/min. A 2-μl sample was injected by an auto-sampler in splitless mode.

3. Results

3.1. Toxicity study

Table 1 shows the combined effect of both DBP and DEHP on the RMA. At 5 mg/l more than 93% of the methanogenic activity relative to the control was observed at any given time. At 50 mg/l only 70% of the activity was initially observed and after 360 h the activity increased to approximately 90%. The most dramatic effect was observed at 500 mg/l. Less than 20% of the methanogenic activity relative to the control was initially observed followed by an increase of up to 75% after 360 h.

Table 1
Combined toxicity of DBP and DEHP on methanogenesis from sewage sludge

Time[a] (h)	Relative methanogenic activity[b] Individual concentration of DBP or DEHP (mg/l)[c]		
	5	50	500
24	93.7	72.1	15.9
60	99.7	64.1	24.5
360	97.1	88.6	74.2
696	98.1	92.8	69.1

[a] Indicated times are the elapsed times after addition of phthalate esters after which the methanogenic activity was measured.

[b] Relative methanogenic activity (RMA) is defined as the percentage of the methanogenic activity at the concentration tested relative to the methanogenic activity of the control without phthalate esters addition. See text for the full definition of RMA.

[c] DBP and DEHP were added together sorbed into fed sludge. Indicated concentrations correspond to each individual phthalate.

3.2. Digester performance

Average operational parameters for both digesters during the acclimation period are reported in Table 2, showing that the performances of both test and control digesters were similar. The amounts of biogas, CH_4 produced, and removal of TVS from both digesters were as expected for a mesophilic sewage sludge digester with added methanol.

Figs. 1 and 2 show that additon of phthalates did not affect the test digester performance during the first 7 weeks of PAEs addition. The amount of gas produced (Fig. 1), and removal of TVS (Fig. 2) were similar for both the test and the control digester and also similar to the levels observed before phthalate addition. Likewise, during this period there was no change in the previous CH_4 concentration of approximately 55% in the gas from both digesters.

At the end of the 7-week period, a new batch of feeding sludge coincided with a decrease in the gas production of both the control and test digester, and hence a portion of the decrease in gas production of the test digester is attributed to the new feed. However, the decrease was dramatically more pronounced in the test digester, and gas production in the test digester never returned to the level of gas production in the control digester (Fig. 1) for the next 5 weeks of PAEs addition.

The decrease in biogas production observed in the test digester is evidence of a process imbalance. However, a process imbalance is also commonly indicated by a rise in levels of VFAs [22], so it is interesting that the levels of VFAs during the 12-week period of PAEs addition were similar for both the test and control digester (Fig. 1). Also, the removal of TVS was very similar for both digesters during the 12 weeks of phthalate addition (Fig. 2).

Table 2
Basic operational parameters of the test and control digester[a]

Parameter	Influent[b]	Effluent[b]	
		Test digester	Control digester
TVS (mg/l)	16416 (±2281)	7208 (±575.249)	7456.818 (±561)
VS load (mg/l·day)	821 (±114)		
VS removal (%)		55.5 (±5.4)	54.1 (±4.4)
Biogas (l/g vs. loaded)		0.94 (±0.12)	0.9 (±0.11)
CH_4 (% volume)		55.1 (±4.5)	54.9 (±4.7)

[a] Digesters were operated at 35°C and 20 days HRT.

[b] Average values over a 4-week period before phthalate addition to the test digester. Numbers in parenthesis represent the standard deviation.

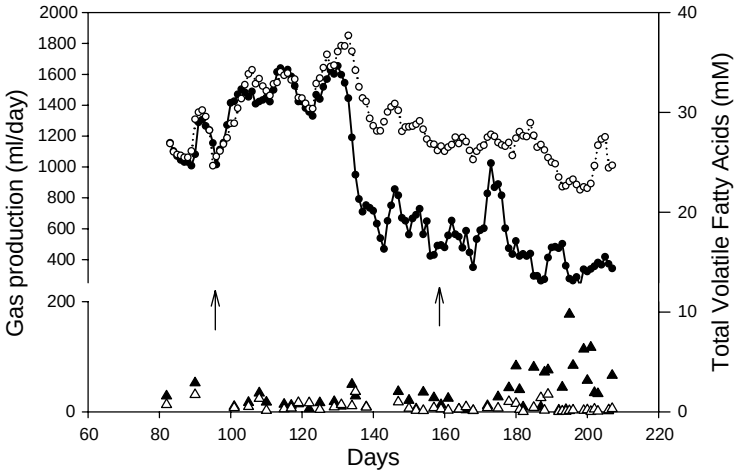

Fig. 1. Effect of DBP and DEHP addition on gas production on volatile fatty acids level. The arrows indicate the begining (day 86) and end (day 166) of the PAEs addition period. DBP and DEHP were sorbed into the sewage sludge and fed at a load of 12.5 mg/day of each phthalate. (—●—) gas (test digester); (-----○-----) gas (control digester); (▲) VFAs (test digester); and (△) VFAs (control digester).

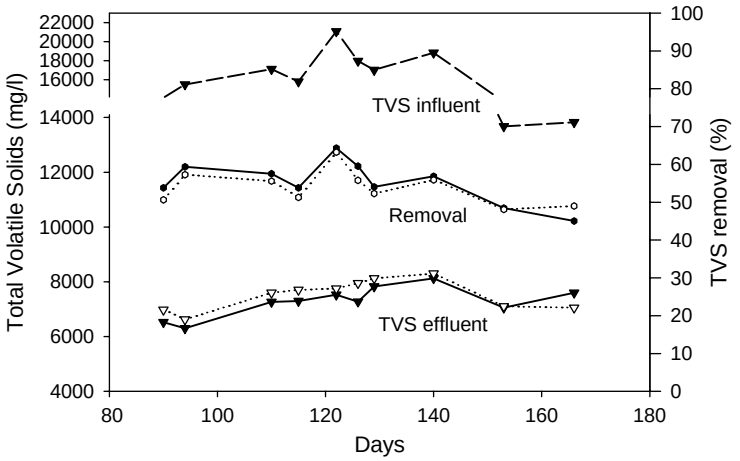

Fig. 2. Total volatile solids in influents and effluents and TVS removal during the 12-weeks PAEs addition period. Solid symbols: test digester; and open symbols: control digester.

3.3. Removal of phthalates in digester

During the last month of the acclimation period, background concentrations in the feed sludge were 255.4 (±83.1) mg/l and 3.1(±1.8) mg/l for DBP and DEHP, respectively.

Levels of DBP in the influent and effluent of the test and control digesters are shown in Fig. 3. Average DBP concentration in the influent and effluent of the control digester during the 12-weeks period of PAEs feeding were 212.7 (±49.6) and 2.9(±4.5) mg/l. Average removal of DBP in the control digester was 96.9(±3.4)% for the complete period of PAEs addition (Table 3). Average DBP concentration in the influent of the test digesters for the 12-week period was 323.8 (±38.3) mg/l. Levels of DBP in the effluent of the test and control digesters were similar for the first 2 weeks of the addition period (Fig. 3). After the second week DBP concentration in the effluent of the test digester increased (Fig. 3) and reached a stable level around day 110 with an average concentration equal to 64.4 (±14.1) mg/l. Removal of DBP in the test digester was near 100% during the first 2 weeks of phthalate addition followed by a decrease to an average removal equal to 78.3 (±3.8)% for the rest of the addition period (Table 3). After addition of phthalate esters was stopped, removal of DBP increased again to around 100% (Table 3).

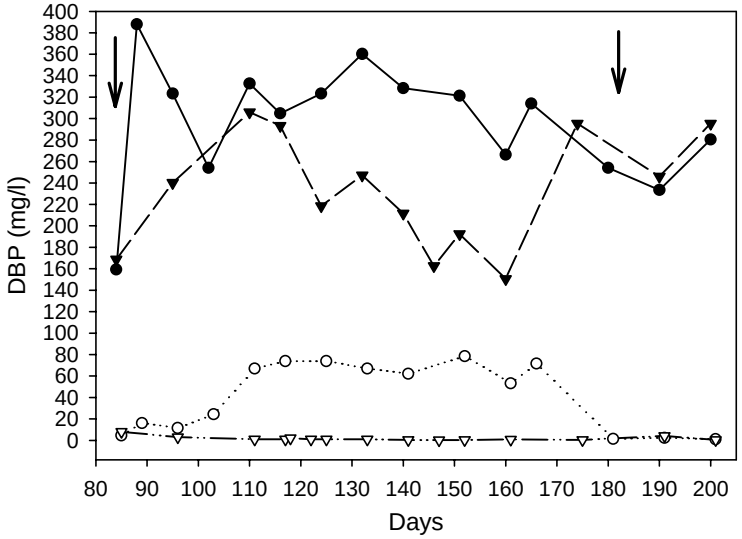

Fig. 3. DBP concentrations in digesters. Arrows indicate the 12-weeks period with PAEs addition.(—●—) influent test digester; (----○----) effluent test digester; (—▼—) influent control digester; and (—▽—) effluent control digester.

Concentrations of DEHP in the influent and effluent of the control digester were 2.3(±0.5) and 2.5(±0.9) mg/l, respectively, showing no detectable DEHP removal of these small concentrations. Average measured concentration of DEHP in the influent of the test digester over the 12-week addition period was 134.9 (±23.1) mg/l. The concentration of DEHP in the effluent of the test digester increased after a few days of PAEs addition (Fig. 4). After 7 weeks, DEHP concentration in the effluent reached a stable level (Fig. 4) that lasted until the end of the PAEs addition 5 weeks later. During this 5-week period the influent and effluent average concentrations of DEHP were equal to 147.3(±17.6) and 109.5(±7.9) mg/l, respectively. A two-tailed z-test with a 95% confidence level indicated that these two average concentrations are statistically different ($P = 0.00001$ for the null hypothesis of equal means). Therefore, a 26.3(±8.1)% average removal of DEHP in the test digester was observed during the period when DEHP effluent concentration was stable.

4. Discussion

4.1. Toxicity study

Previous studies by O'Connor et al. [23] and Battersbery and Wilson [24] reported non- or low toxicity at low concentrations of phthalate esters and a clear toxic effect at the higher concentrations tested. Our toxicity batch experiment was different from the ones reported by O'Connor et al. [23] and Battersbery and Wilson [24] in several ways. In those experiments, individual PAEs were added directly to serum bottles before sludge addition, instead of using a combination of them sorbed in the feeding sludge. However, the two approaches yielded similar results. Our results indicated that 95% of the methanogenic activity as compared to the control was present at a concentration of 5 mg/l at any given time during the testing period of the batch experiment (29 days). The other two concentrations tested, 50 and 500 mg/l, were initially inhibitory. However, by the end of the 29-day testing period 93% and 70%, respectively, of the methanogenic activity as compared to the controls was observed at these higher concentrations. Based on these results, step increments of the PAEs concentration in the digester equivalent to 5 mg/l of each phthalate was considered to be safe enough to avoid initial problems with the digester performance.

4.2. Methanogenesis inhibition

Since gas production decreased in test and control digesters after 7 weeks of PAEs feeding in the test digester, the use of a new lot of feed sludge evidently caused some of the reduction in the gas from the test digester. However, the greater decrease in gas production observed in the test digester is strong evidence of inhibition of methanogenesis by an effect associated with PAE build-up, particularly in view of our direct observation of build-up obtained from measurements of the influent and effluent concentrations of DBP and DEHP. These measurements indicate that DEHP is only degraded to a limited degree, so that it accumulates in the test digester over time.

Table 3
Removal of DBP and DEHP in test and control digesters

Time (days)	% Removal		
	Test digester[a]		Control digester[b]
	DBP	DEHP	DBP
82	98.1[a]	n.d.[a]	n.m.[c]
83	97.1[a]	n.d.[a]	89.0
85	90.2	84.6	n.m.[c]
88	97.0	79.0	97.2
95	92.5	77.4	97.1
102	87.0	55.9	n.m.[c]
110	79.8	39.8	99.2
116	75.7	53.2	98.6
122	77.1	36.6	97.4
132	82.7	34.5	99.1
140	76.1	16.1	n.m.[c]
150	83.4	31.0	99.6
160	73.1	23.8	99.1
180	99.4[d]	n.d.[d]	98.9
190	99.5[d]	n.d.[d]	91.6

[a] Addition of phthalate esters to the test digester started on day 84. Reported values for DBP removal on days 82 and 83 correspond to the removal of DBP already present in the fed sludge. DEHP removal was not detected (n.d.) on days 82 and 83 because existing levels of DEHP in the fed sludge were very low.

[b] Values for DBP removal in the control digester correspond to removal of the background levels of DBP present in the fed sludge. Levels of DEHP in the control digester were very low and no removal was detected.

[c] n.m.: not measured (sample was not available).

[d] Phthalate esters addition finished on day 166. Reported values for DBP removal on days 180 and 190 correspond to removal of background levels of DBP present in the fed sludge. Existing levels of DEHP in the fed sludge after day 166 were very low and DEHP removal was not detected (n.d.)

A clear decrease of methane production was observed when the measured concentration of DEHP in the test digester reached an average concentration of 109.5($\pm$7.9) mg/l after 7 weeks of PAEs feeding. This value is somewhat higher than the concentration that was found to be initially toxic in our batch experiment (50 mg/l) and it is in the same range of concentrations reported to be toxic by O'Connor et al. [23] and Battersby and Wilson [24]. O'Connor et al. [23] reported that when 200 mg/l of DEHP was present, methane production was only 37% relative to the active control. Battersby and Wilson [24] found that increasing concentrations of DEHP (25, 50, 100 and 200 mg of carbon/l) produced decreasing amounts of biogas (from 48% to 6% of the theoretical gas production) over a period of 60 days.

Although the level of DBP also increased up to an average concentration equal to 64.4 ($\pm$14.1) mg/l, this concentration was well below the background concentration in the feed sludge, so it should not affect gas production. Also, our initial toxicity screening showed that the addition of up to 50 mg/l of DBP and DEHP had a mild and reversible inhibitory effect on methanogenesis. Prior batch studies [23] also reported no toxic effect of DBP on methanogenesis at similar concentrations.

4.3. PAEs degradation

The high concentrations of DBP and low concentrations of DEHP found in the Hyperion sewage sludge indicate that the Hyperion digested sludge used as seed to start up our bench-scale digesters, was a DBP-acclimated sludge. Therefore, it was expected to be very active degrading DBP and to have a lower degradation activity for DEHP. This assumption was confirmed by our results that showed that the removal of DBP was a very efficient process and that DEHP removal was negligible in our control digester. Degradation of DBP and persistency of DEHP were consistent with results from prior batch studies that investigated the susceptibility to anaerobic degradation of PAEs in digested sludge [23,12].

During the first 2 weeks of addition of phthalate esters, the measured level of DEHP in the test digester were low. This initially low and stable level can be explained by sorption of DEHP to the glassware surface of the digester and to the initial dilution of DEHP in the digester. After this 2-week period, a continuous increase in the level of the DEHP was observed in the test digester until it stabilized around day 131 and for the next 5 weeks of PAEs feeding. Our data suggested that an average 26.3%($\pm$8.1) of the DEHP added was degraded during this 5-week period. This result indicates that after 7 weeks of DEHP feeding, a bacterial population capable of slowly degraded DEHP was established in the anaerobic digester.

The DEHP removal observed in our study is consistent with the degradation of DEHP observed by Govind et al. [14] and Parker et al. [15]. These authors reported the degradation of mixtures of toxic organics that included several PAEs. The concentrations tested in those studies were similar to the ones found in municipal sewage. Govind et al. [14] found 26% degradation of DEHP and 96% degradation of DBP in bench-scale digesters dosed with 0.5 mg/l. In a pilot plant study, Parker et al. [15] reported a 61% removal of DEHP and 93% removal of DBP in an anaerobic digester that was dosed with 10 mg/l of DEHP. In our study, a much higher concentration of DBP and DEHP was tested (100 mg/l).

The high levels of DEHP in our test digester was correlated with both methanogenesis inhibition and decreased efficiency of DBP degradation. This

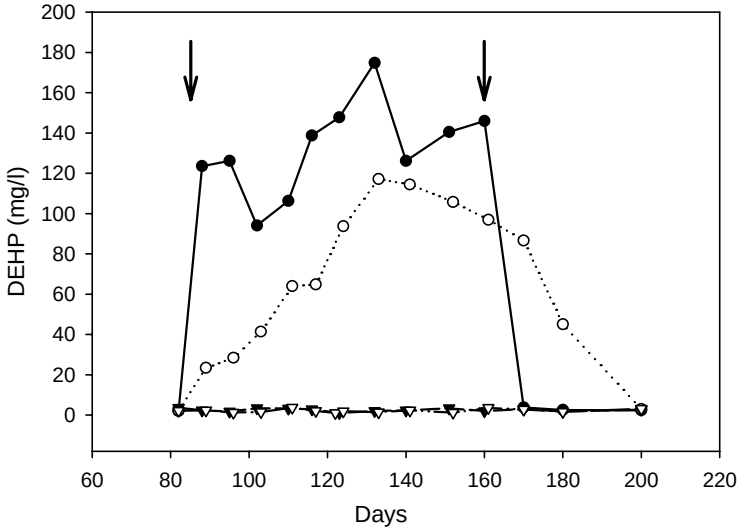

Fig. 4. DEHP concentrations in digesters. Arrows indicate the 12-weeks period with PAEs addition. (—●—) influent test digester; (----○----) effluent test digester; (—▼—) influent control digester; and (—▽—) effluent control digester.

Table 4
Selected[a] DEHP industrial wastewater discharge data obtained during a survey in the wastewater collection system, City of Los Angeles Bureau of Sanitation

Industrial class[b]	Number of industrial units in class	Average concentration (mg/l)[c]
Aircraft manufacturing, service and maintenance	12	114.9
Chemical manufacturing and packing	5	312.0
Etchers and engravers	1	456.0
Metal treating	14	135.4

[a] The four classes reported in the table are those with the highest DEHP concentration among other 77 classes in the survey.
[b] Industrial classes are based on industrial classification, City of Los Angeles Bureau of Sanitation.
[c] Average concentrations for 77 classes range from 0.51 to 456.0 mg/l.

observation suggests that the accumulation of DEHP in the digester was the cause of both problems. Furthermore, the degradation of DBP increased to its original level of almost 100% after DEHP addition stopped. The discrepancy between our results and those reported by Govind et al. [14] and Parker et al. [15] can be attributed to the higher concentration used in our study.

As part of a source identification study, concentrations of DEHP in industrial wastewater discharges were evaluated in a survey in the collection system, City of Los Angeles Bureau of Sanitation [25]. Samples were obtained from industrial units representing the industrial classifications that are potential DEHP contributors. In addition, the entire historical data inventory covering the period of 1990–2000 was used to determine the average DEHP concentration for each industrial classification. Table 4 shows the average DEHP concentrations in wastewater discharges of the industrial classifications with the highest concentration of DEHP. Concentrations range from 114.9 to 456 mg/l. Furthermore, dewatering of sewage sludge before anaerobic digestion would result in much higher concentrations of DEHP than found in raw wastewater. This data is an indication that the DEHP concentrations tested in our study can be found in industrial wastewater and that the toxic effects observed may be relevant for anaerobic digesters receiving this type of industrial effluent.

High concentrations of DEHP found in household solid waste [5] can be another source of high loads of this compound to sewage sludge co-digested with this type of waste.

4.4. Toxicity mechanism of DEHP

The toxic effect observed in our study may be due to the accumulation of DEHP by itself or to the accumulation of a primary metabolite. Indirect evidence of primary transformation of DEHP was reported by

O'Connor et al. [23]. They observed a decrease in the initial concentration of DEHP in their experiments, as measured by UV scans. Furthermore, the lack of methane production above the active controls used in that study suggested a primary transformation of DEHP to an unknown intermediate(s) that was not further mineralized to methane. The authors interpreted these observations as a partial metabolism of DEHP. However, no direct evidence of the presence of such intermediate(s) was provided. Indeed, the authors recognized that the apparent loss of DEHP may be explained by partition into particulate matter in the medium.

One obvious possible early step in the degradation of DEHP is hydrolysis to release 2-ethyl hexanol, or perhaps other long chain alcohols (LCA). Hence, it is potentially significant that a negative effect of 2-ethyl hexanol and decanol on the methanogenic process has been reported by Ejlertsson et al. [26]. These authors performed batch experiments with serum bottles inoculated with a BBP-degrading culture isolated from an anaerobic pilot-plant biogas reactor treating municipal solid waste. Degradation of several LCA and PAEs was studied. All alcohols tested were degraded, but methane production from 2-ethyl hexanol showed an initial lag phase of approximately 3 days and smaller methane production for the next 7 days than the control without alcohol addition. Decanol showed a similar adaptation period with a longer initial lag phase (13 days) and a smaller methane production as compared to the control for the next 20 days.

Angelidaki and Ahring [27] reported a similar toxic effect on methanogenesis by the long chain fatty acids (LCFA) oleate and stearate. Their results were even more similar to ours, i.e. an irreversible decrease in methane production not followed by an accumulation of VFA, and a low removal rate for the toxic compound. They concluded that oleate and stearate inhibited all steps on the anaerobic thermophilic biogas process. An effect of LCFA on the membrane of microbial cells was hypothesized as the reason for the inhibition. As PAEs with long side-chain alcohols or their LCA metabolites would also interact in a similar fashion with the cell membrane, it is reasonable to hypothesize that LCFA and LCA might have similar inhibitory effects. However, more experimental work would be necessary to test these hypotheses.

5. Conclusions

(A) PAEs with long side-chains such as DEHP can only be biodegraded at low rates during anaerobic digestion of wastewater sludge with a consequent accumulation in the reactor to a toxic level when sewage sludge with high concentrations of PAEs is digested.

(B) DEHP accumulation coincided with a decrease in both gas production and in the efficiency of removal of DBP.

(C) The results from our bench-scale study confirm expectations based on previous batch and bench-scale studies that did not as closely simulate the operation of full-scale digestion systems for municipal wastewater plants.

(D) The level of PAEs is, therefore, a relevant parameter to measure in sewage sludge going to anaerobic digestion in cases involving industrial influent with high concentrations of poorly biodegradable or recalcitrant PAEs (e.g. DEHP or DOP) or municipal wastewater plants receiving wastes from industrial sources where a shock load of recalcitrant PAEs can be experienced.

(E) These results are reminiscent of observations of toxicity of long-chain fatty acids and long-chain alcohols, but additional experiments would be needed to determine whether this apparent similarity results from the same or related mechanisms.

Acknowledgements

This work was funded by a grant from The Danish Environmental Research Program (Center for Sustainable Land Use of Waste) and The University of California Los Angeles. Miguel Zermeno at the Hyperion Wastewater Treatment provided technical assistance to obtain sludge samples used in this study. Anthony Taglieri and Amilcar Diaz provided laboratory help at the UCLA C&EE Department.

References

[1] Cavanaugh T. Plastic additives '95. In: Chemical Marketing Reporter, p SR, 1995.

[2] IARC, 1982. Some industrial chemicals and dyestuffs. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon, France: International Agency for Research on Cancer, p. 416.

[3] Atlas E, Giam CS. Global transport of organic pollutants: ambient concentrations in the remote marine atmosphere. Science 1981;211(4478):163–5.

[4] Michael PR, Adams WJ, Werner AE, Hicks O. Surveillance of phthalate esters in surface waters and sediments in the US. Environ Toxicol Chem 1984;3(3):377–89.

[5] Hartmann H, Ahring BK. Phthalic acid esters found in municipal organic waste: enhanced anaerobic degradation under hyper-thermophilic conditions. Proceedings of the Third International Symposium on Anaerobic Digestion of Solid Wastes, Munich, 18–20 September 2002.

[6] Bruns-Weller E, Pfordt J. Determination of phthalic acid esters in foodstuffs, mother's milk, dust, and textiles (research article). Umweltwissenschaften Schadstoff-Forschung 2000;12(3):125–30.
[7] Editorial, Male reproductive health and environmental oestrogens. Lancet 1995;345(8955):933–5.
[8] USEPA, List of substances on IRIS. In: United State Environmental Protection Agency, Integrated Risk System Information, 2000.
[9] Subba-Rao RV, Rubin HE, Alexander M. Kinetics and extent of mineralization of organic chemicals at trace levels in freshwater and sewage. Appl Environ Microbiol 1982; 43(5):1139–50.
[10] Battersby NS, Wilson V. Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. Appl Environ Microbiol 1989;55(2):433–9.
[11] O'Connor OA, Rivera MD, Young LY. Methanogenic degradation of 5 phthalate esters and 11 chloro nitro and methyl substituted phenols. In: Abstracts of the Annual Meeting of the American Society for Microbiology, 1986. p. 287.
[12] Shelton DR, Boyd SA, Tiedje JM. Anaerobic biodegradation of phthalic acid esters in sludge. Environ Sci Technol 1984;18(2):93–7.
[13] Ziogou K, Kirk PWW, Lester JN. Behavior of phthalic acid esters during batch anaerobic digestion of sludge. Water Res 1989;23(6):743–8.
[14] Govind R, Flaherty PA, Dobbs RA. Fate and effects of semivolatile organic pollutants during anaerobic digestion of sludge. Water Res 1991;25(5):547–56.
[15] Parker WJ, Monteith HD, Melcer H. Estimation of anaerobic biodegradation rates for toxic organic compounds in municipal sludge digestion. Water Res 1994; 28(8):1779–89.
[16] USEPA, National Sewage Sludge Survey. Fed Reg 1990;55(218):47210–83.
[17] Webber MD, Lesage S. Organic contaminants in Canadian municipal sludges. Waste Manage Res 1989;7(1):63–82.
[18] Schnaak W, Kuechler T, Kujawa M, Henschel KP, Suessenbach D, Donau R. Organic contaminants in sewage sludge and their ecotoxicological significance in the agricultural utilization of sewage sludge. Chemosphere 1997;35(1-2):5–11.
[19] Young JC, Tabak HH. Multilevel protocol for assessing the fate and effect of toxic organic chemicals in anaerobic treatment processes. Water Environ Res 1993; 65(1):34–45.
[20] Angelidaki I, Ellegaard L, Ahring BK. Compact automated displacement gas metering system for measurement of low gas rates from laboratory fermentors. Biotechnol Bioeng 1992;39(3):351–3.
[21] APHA (American Public Health Association). Standard methods for the examination of water and wastewater, 17th ed. Washington: APHA, 1989.
[22] Ahring BK, Sandberg M, Angelidaki I. Volatile fatty acids as indicators of process imbalance in anaerobic digestors. Appl Microbiol Biotechnol 1995;43(3):559–65.
[23] O'Connor OA, Rivera MD, Young LY. Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environ Toxicol Chem 1989;8(7):569–76.
[24] Battersby NS, Wilson N. Evaluation of a serum bottle technique for assessing the anaerobic degradability of organic chemicals under methanogenic conditions. Chemosphere 1988;17(12):2441–60.
[25] Personal communication, 2002. Donald C. Tilman Wastewater Reclamation Plant. Source Identification Study. City of Los Angeles Bureau of Sanitation. From the desk of Reza Iranpour (mailto: riz@san. lacity. org).
[26] Ejlertsson J, Alnervik M, Jonsson S, Svensson BH. Influence of water solubility, side-chain degradability and side-chain structure on the degradation of phthalic acid esters under methanogenic conditions. Environ Sci Technol 1997;31:2761–7.
[27] Angelidaki I, Ahring BK. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Appl Microbiol Biotechnol 1992;37:808–12.