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Anaerobic degradation of phthalic acid esters during digestion of municipal solid waste under landfilling conditions

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Abstract

Anaerobic microorganisms in municipal solid waste samples from laboratory-scale landfill reactors and a pilot-plant biogas digestor were investigated with the aim of assessing their ability to transform four commercially used phthalic acid esters (PAEs) and phthalic acid (PA). The PAEs studied were diethyl phthalate (DEP), butylbenzyl phthalate (BBP), dibutyl phthalate (DBP) and bis(2-ethylhexyl) phthalate (DEHP). No biological transformation of DEHP could be detected in any of the experiments. Together with waste samples from the simulated landfilling conditions, the PAEs (except DEHP) were hydrolytically transformed to their corresponding monoesters. These accumulated as end products, and in most cases they were not further degraded. During incubation with waste from the biogas digestor, the PAEs (except DEHP) were completely degraded to methane and carbon dioxide. The influence of the landfill development phase on the transformations was investigated utilizing PA and DEP as model substances. We found that during both the intense and stable methanogenic (but not the acidogenic) phases, the microoganisms in the samples had the potential to transform PA. A shorter lag phase was observed for the PA transformation in the samples from the stable methanogenic phase as compared with earlier phases. This indicates an increased capacity to degrade PA during the aging phases of the municipal solid waste in landfills. No enhancement of the DEP transformation could be observed as conditions in the methanogenic landfill model changed over a year's time. The results indicate that microorganisms developing in a methanogenic landfill environment have a substantially lower potential to degrade PAEs compared with those developing in a biogas reactor.

Abbreviations: butylbenzyl phthalate – BBP; bis(2-ethylhexyl) phthalate – DEHP; coenzyme A – CoA; dibutyl phthalate – DBP; diethyl phthalate – DEP; dry solids – DS; monobenzyl phthalate – MBeP; monobutyl phthalate – MBuP; monoethyl phthalate – MEP; municipal solid waste – MSW; phthalic acid – PA; phthalic acid ester(s) – PAE(s); volatile fatty acids – VFA

Introduction

Phthalic acid esters (PAEs) represent a group of pollutants found in leachate from landfills. For example, diethyl phthalate (DEP), di-n-butyl phthalate (DBP) and butylbenzyl phthalate (BBP) were present at concentrations of up to 100 μg/l in leachate from the Gryta landfill in Sweden (Öman & Hynning 1993). The main bulk of PAEs produced is used as plasticizers in polyvinyl chloride (PVC) plastics. PAEs are also used in cosmetics, insect repellents, inks, munitions, etc. (Giam et al. 1984). During 1989, 24 000 tons of PAEs were used in Sweden, amounting to approximately 2.7 kg per Swedish inhabitant (KEMI 1990). The characteristics of the PAEs are determined by the length of the alcohols esterifying the phthalic acid (PA) molecule. Due to the extensive use of PAEs, traces can be found at most trophic levels in any ecosystem (Giam et al. 1984).

In earlier studies, several commercially used PAEs were found to be transformed to methane and carbon dioxide in samples from anaerobic environments, whereas others were resistant to degradation (Shelton et al. 1984; Ejlertsson et al. 1996). During degradation,

PAEs are hydrolysed to PA and the corresponding alcohols via monoalkyl phthalate. PA and the alcohols are then converted to methane and carbon dioxide. Presumably, the anaerobic degradation of PA follows the pathway used by denitrifying bacteria, i.e. a decarboxylation of PA and a condensation with Coenzyme A (CoA), which will be transferred to benzoyl-CoA (Nozawa & Maruyama 1988). Benzoyl-CoA is then further degraded via the benzoate pathway to H_2 and acetate (cf. Elder & Kelly 1994). The degradation of benzoate to H_2 and acetate is a thermodynamically unfavourable reaction under standard conditions, i.e. $\Delta G^{01} > 0$. However, in methanogenic systems the hydrogenotrophic methanogens keep the partial pressure of H_2 low enough allowing the benzoate degrader to gain energy for growth from this chemical reaction. Therefore, a coordinated interaction by methanogenic bacteria and fermentative bacteria is needed for the complete degradation of PA. It should be noted that this is also the case for the alcohols released from the hydrolysis of the PAEs studied, which are degraded firstly to the corresponding carboxylic acid and H_2. The acids are then further degraded either via β-oxidation or the benzoate pathway to H_2 and acetate.

During the landfilling of MSW, five characteristic phases in the microbial degradation of the waste can be identified (Christensen & Kjeldsen 1989). Initially, composting will occur, during which period the oxygen trapped within the waste is consumed. The resulting oxygen depletion causes a metabolic shift towards fermentative processes, giving rise to products such as volatile fatty acids (VFA), H_2, ethanol, etc. This leads to the second phase characterized by a high VFA concentration and low pH (ca 5). A third phase of intense methanogenesis follows when methanogenic bacteria consume H_2, formate and acetate. During this phase, the decrease in the partial pressure of H_2 will lead to conditions favouring the degradation of organic acids, resulting in turn in a rapid increase in methane formation. The transformation of acids into methane and carbon dioxide leads to an increase in pH. Phase four (stable methanogenic) starts once the production of methane and gas has stabilized. This occurs when hydrolysis and fermentation processes are balanced by methanogenesis. After several years, the rate of gas production will decline, owing to a reduction in substrate quality, and air may enter the landfill. This will lead to the fifth aerobic post-composting phase of the landfill. The length and intensity of each phase varies with factors such as MSW-quality, degree of compaction and climate.

Authorities in the Scandinavian countries have recently become concerned about the fate of PVC-plastics in soil and landfills. Thus they are currently asking questions such as: "Are parts of the PVC plastic degraded? What intermediates and end products are formed? Are they toxic?" In conjunction with the debate about the possible transformations of PVC-plastics in landfills, the fate of the plasticizers in PVC (i.e. the PAEs) in such environments has received special attention. To address these questions we started the present investigation, the aim of which was to study the potential for degradation of PA and four commercially used PAEs in MSW under landfill conditions. We also wanted to elucidate whether degradation rates of PAEs differ between the development phases of a landfill. For comparative purposes, we also made corresponding analyses of MSW treated in a pilot-plant biogas digestor. The PAEs investigated were: DEP, DBP, BBP and bis(2-ethylhexyl) phthalate (DEHP).

Materials and methods

The investigation was divided into three experiments: 1) First, the ability of landfill microorganisms to degrade PAEs during the different anaerobic aging phases of a landfill was studied as follows: Solid waste from two reactors (referred to as "models" below) simulating the reaction sequence of a landfill was sampled on three occasions, each representing one of the typical anaerobic aging phases, i.e. the acidogenic, intense and stable methanogenic phases. DEP and PA were used as model substances. 2) In the second experiment, the degradation dynamics of DEP, DBP, BBP, DEHP and PA were studied using waste samples taken from a model at the stage of stable methane production. 3) Finally, the same PAE compounds and PA were used in a similar study with solid waste samples from a biogas reactor treating MSW.

Chemicals

All chemicals used in this investigation were MERCK products, except for monoethyl phthalate (MEP) and mono(2-ethylhexyl) phthalate (MEHP), which were gifts from Neste Oxo AB, Stenungsund, Sweden.

Origin and sampling of MSW

The first four of the five development phases of a landfill were simulated by reactor models as origi-

nally described by Stegmann (1981) and modified by Lagerkvist & Chen (1993). The models (volume of 100 litres) were built of stainless steel and equipped with a water recirculation system and sampling ports for water, gas and solids. Each of two models were filled with 40-45 kg unsorted and milled MSW (particle size approximately 1 cm). Water was added to give a dry solid (DS) content of about 30%. Due to rapid fermentation, acidogenic conditions (pH ca 5.5) were established within a few days after the filling and closing. One model was kept this way throughout the experiment. In the other model air was blown through the reactor, thus, fermentation products and some of the easily degradable material were degraded aerobically in this case. After about eight weeks, the air flow was stopped and the model turned anaerobic. This treatment facilitated the rapid development of a methanogenic flora. Samples of 500 g were taken from the two models on three occasions: at "time zero" (point of transformation between the acidogenic phase and the phase of intense methanogenesis) and six and 11 months later. The last two sampling times represented the intensive and stable methanogenic phases, respectively.

The pilot-plant digester was operated as a two-stage biogas process under mesophilic conditions (37°C). The leachate water from a reactor (15 m^3) loaded with MSW was recirculated through a methanogenic filter (3 m^3). The latter was used to treat the initial pulse of easily degradable substances extracted from the MSW. Water was added to the MSW in order to arrive at a DS content of 30%. Initially the MSW digester worked as a hydrolysis reactor, but it gradually became a one-stage biogas reactor. Once methane production in the system had leveled off, the contents of the reactor were replaced by a new batch of waste. The new MSW was mixed with 10% (w/w) of the material from the previously treated MSW to facilitate the restart of the biogas process. Samples were taken from the hydrolysis reactor after three such replacements. By that time, the biogas plant had been operating for 18 months. Samples of 500 g were taken from the top of the MSW reactor.

Preparation of inoculum and experimental bottles

A defined mineral medium, (pH=7.0) was used as described by Ejlertsson et al. (1996). To obtain a medium with pH=5.5 the mineral medium was modified as follows: Na_2HPO_4 was omitted, and the final concentrations of KH_2PO_4 and $NaHCO_3$ were changed to 20 mM and 5 mM, respectively. Both media were made up from three stock solutions: the phosphate buffer with resazurin, a solution containing trace elements, vitamins and mineral salt (C1), and finally the bicarbonate, cystein, and sulphide solution (C2). During all sampling, medium preparation and transfers between vials, gasing techniques (N_2 <10 ppm O_2 or N_2/CO_2, 4:1; <2 ppm O_2) for cultivating strictly anaerobic bacteria were used.

Inocula were prepared from subsamples (200 g) diluted in 1.7 l phosphate buffers (pH 7 and 5.5) and 100 ml solution C1 supplemented with 2 ml 1M sodium sulphide. The mixtures were homogenised further in a pebble mill for 30 min and then finally sieved (mesh 8 mm).

The phosphate buffers were prepared and distributed among the experimental bottles which were closed with EPDM rubber stoppers, sealed with aluminium crimps and then autoclaved at 121°C for 30 min. Solution C1 was added, and 5 ml (118 ml bottles) or 15 ml (250 ml bottles) inoculum was added with a cut-off pipette. The bottles were closed, and the gas phase was replaced by N_2/CO_2. After addition of solution C2, the bottles were incubated in the dark at 37°C. Final liquid volumes were 50 ml (118 ml bottles) and 150 ml (250 ml bottles).

In experiment 1, DEP and PA were separately dissolved in the medium by vigorous shaking to give a final concentration of 250 mg/l. Duplicate bottles (118 ml) were incubated and sampled for DEP, PA and methane on ten occasions during 100 days. After sampling for methane (see below), the bottles were gently shaken for 30 s, whereupon culture liquid (0.5 ml) was withdrawn with a 1 ml syringe, transferred to an Eppendorf tube, immediately frozen and stored at -20°C until HPLC-analysis according to Ejlertsson et al. (1996).

For experiments 2 and 3, DEP, DBP, BBP, DEHP were added to the experimental bottles by using a Hamilton 10-μl-pipette. PA was dissolved in the medium by vigorous shaking. In experiment 2 the initial concentrations were 50 mgC/l (70-80 mgPAE/l). Four bottles (118 ml) of each PAE were prepared and inoculated. Two of them were immediately frozen and stored at -20°C to be used as time-zero controls. The other two bottles were incubated and sampled for methane. On day 237, these bottles were also frozen until HPLC analysis of PAEs and intermediates according to Ejlertsson et al. (1996). In experiment 3, the bottles (250 ml) were incubated for 100 days with an initial PAE concentration of 150 mg/l. However, high

amounts of methane produced from indigenous substrates in the MSW, made the methane measurements nonconclusive. Therefore diluted cultures were started to confirm the complete transformation of each PAE to methane and carbon dioxide. The previously incubated experiment bottles with PA, DEP, DBP, BBP and DEHP were seperately used as inoculum for duplicate dilution cultures in the following way: 5 ml of culture fluid from an experiment bottle was transferred to a 118 ml bottle containing 45 ml fresh medium (see above) with PA or PAE concentration of 150 mg/l.

Analytical procedure

Gas samples (0.3 ml) for methane analysis were withdrawn from the experimental bottles and quantified by gas chromatography as described by Örlygsson et al. (1993). PAEs and their degradation products were analysed according to Ejlertsson et al. (1996).

Monoesters used for peak identification were synthesised from phthalic anhydride and butanol or benzyl alcohol. Equimolar amounts of phthalic anhydride and alcohol were dissolved in toluene and refluxed for 12 h. After cooling, the mixtures were transferred to a separation funnel, and 25 ml of water was added. After a vigorous shaking and equilibration, the water phase was removed. The cleaning procedure with water was done three times. The organic phase was thereafter concentrated in a rotary evaporator. Recrystallisation was done in a boiling ethanol/water solution. Crystals were allowed to dry on filter paper (Whatman No. 1). GC-MS and NMR analyses were used for confirmation and for assessing the purity of the monoesters.

Results and discussion

Experiment 1

Samples from both the acidogenic and methanogenic (at intense and stable methanogenic phases) landfill models transformed 20-25% of the added DEP during 80 days of incubation at about equal rates (Fig. 1). Monoethyl phthalate (MEP) and PA were detected as intermediates with samples from the model operating under methanogenic conditions (Fig. 1), whereas only traces of these intermediates were observed with samples from the model under acidogenic conditions. No VFA or alcohols that could have been intermediates were observed in the analyses made with the latter samples. Ethanol, from the hydrolysis of DEP, was

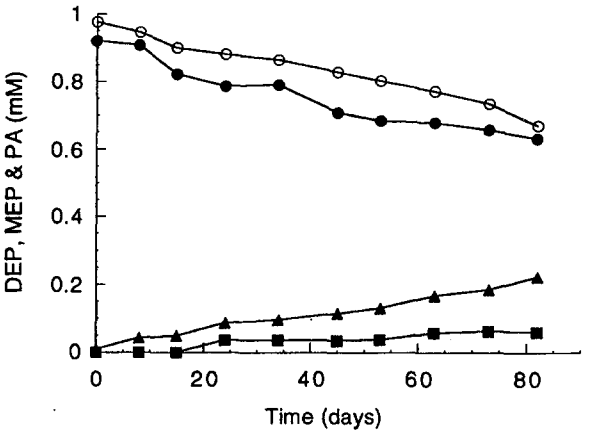

Figure 1. Transformation of DEP in waste samples from the acid (○) and the methanogenic (●) landfill models. Formation of MEP (▲) and PA (■) occurred only under methanogenic conditions. Waste samples were withdrawn from the models when the methanogenic models were at the stage of intense methane production.

most likely degraded to methane and carbon dioxide, as indicated by the excess of methane that had formed in relation to the controls by the end of the experiments (data not shown). The excess methane (approximately 25 μmoles) was equivalent to the amount expected to be formed from the complete degradation of ethanol. The rate of hydrolysis observed in this study was much faster than the reported half-life associated with abiotic (non enzymatic) hydrolysis. For dimethyl phthalate, which has properties similar to DEP, the half-life associated with abiotic hydrolysis is ca 3 years (cf. Giam et al. 1984). Therefore, it is highly unlikely that the rate of hydrolysis observed could have been due exclusively to abiotic degradation. This means that an enzymatic hydrolysis of the DEP molecules took place, rather than abiotic hydrolysis. Since the reaction kinetics does not indicate a coupling to growth it is possible that unspecific hydrolysis took place. This is supported by the findings by Kurane et al. (1984) who showed that lipases hydrolysing olive oil had hydrolytic activity on PAEs.

DEP in sewage sludge can be partly transformed to methane and carbon dioxide (Horowitz et al. 1982; O'Connor et al. 1989), whereas it can be resistant to decomposition in sediments (Horowitz et al. 1982). The methane recovery attributed to the degradation of DEP within these sludge samples was 16-32% of the amount theoretically obtained from a complete degradation of DEP to methane and carbon dioxide. Since this quantity of methane corresponds roughly to the

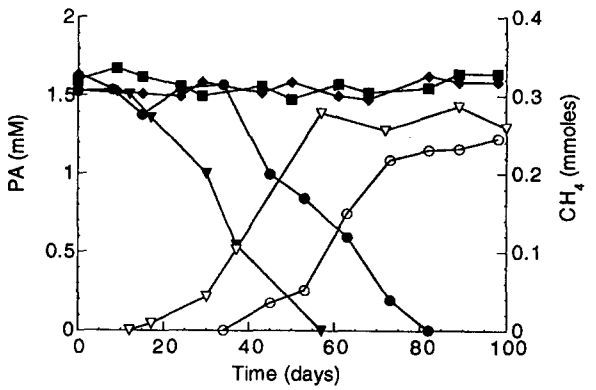

Figure 2. Degradation of PA under the acidogenic phase (■), the phase of initial methanogenic activity (time zero sample in the methanogenic model) (◆), the phase of intense methane formation (●) and the phase of stable methane formation (▼). Methane formation under the phase of intense methane formation (○) and the phase of stable methane formation (▽).

amount that can be derived from one ethanol moiety of the DEP added, these data indicate that DEP was hydrolysed to ethanol and MEP and that the MEP was unaffected. This would then resemble the behaviour of the waste samples in our acidogenic landfill model and in the model working in the two methanogenic phases (Fig. 1).

Microorganisms in samples taken during time zero in the methanogenic model were unable to degrade PA, whereas those in samples taken six months later, during the intensive methanogenic phase, were able to transform all of the added PA (Fig. 2). The shorter lag phase observed for the PA-transformation in samples taken later on, when the stable methanogenic phase was reached, indicates that the capacity to degrade PA continued to increase. There are at least two possible, non-mutually exclusive explanations for this phenomenon: i) Organisms able to degrade simple aromatic compounds did develop during the six months of methanogenic activity in the model; ii) The methanogenic population was not able to keep the partial pressure of H_2 low enough to allow fermentative bacteria to transform PA to H_2 and acetate via benzoate in the samples from the initial part of the unstable methanogenic phase. The first explanation is as likely as the second.

Experiment 2

DEP, DBP and BBP were transformed to the corresponding monoester(s) after 278 days of incubation with the samples from the methanogenic model working in the phase of stable methanogenesis (Table 1). PA was degraded completely to methane and carbon dioxide. Approximately 40% of the DEP added was converted to MEP and, thus, followed the pattern observed in experiment one.

DBP was transformed to monobutyl phthalate (MBuP) which was the only end product detected (Table 1). The butanol formed by hydrolysis of DBP was most likely decomposed to methane and carbon dioxide. Probably, the low recovery of MBuP was due to the low solubility of MBuP in the water phase, which was the phase analysed by the HPLC method used. Partial degradation of DBP to methane and carbon dioxide has also been observed to occur in sewage sludge samples (Horowitz et al. 1982; Battersby & Wilson 1989), while Johnson & Lulves (1975), Horowitz et al. (1982), Shelton et al. (1984) and O'Connor et al. (1989) reported a complete transformation of DBP added to sewage digester sludge or sediment samples. The findings of Engelhardt et al. (1975), in their study of the aerobic degradation of DBP, may explain the observations made in the studies above. These authors showed that several aerobic isolates formed MBuP as the end product during growth with DBP. The butanol released by DBP hydrolysis was used for growth by these organisms. The same observation was made for a denitrifying *Pseudomonas* sp. (Benckiser & Ottow 1982). Engelhardt et al. (1975) concluded that a large number of aerobic bacteria can form monoesters and degrade PA, whereas transformation of the monoester seems to be more difficult, with only a small group of aerobic microorganisms being capable of doing so. Our findings suggest that anaerobic microorganisms developing under landfilling conditions have similar features.

In the BBP incubations, 30% of the amount added remained at the end of the experiment. By then, BBP had been transformed to MBuP, monobenzyl phthalate (MBeP), PA and benzoate (Table 1). The occurrence of MBuP and PA is in agreement with the results obtained by Shelton et al. (1984) who observed these compounds as intermediates during the transformation of BBP by anaerobic microorganisms from a sewage sludge digester. However, our investigation shows that MBeP and MBuP also may be end products during anaerobic BBP transformation. The benzoate formed in BBP incubations probably originated from the oxidation of benzyl alcohol released from the hydrolysis of BBP. Therefore, the amount of benzoate given in Table 1 should not be included in the mass balance

Table 1. Degradation of DEP, DBP, BBP, DEHP and PA under methanogenic conditions at a concentration of 50 mgC/l and formation of intermediates and methane above control values. The amount of methane formed from PAE is compared with the theoretical maximum amount that can be formed. The experiments were terminated after 278 days, except for PA and DEP which were terminated after 110 days. Inoculum originated from the methanogenic model, sampled at the phase of stable methane production. MEP=monoethyl phthalate, MBuP=monobutyl phthalate, MBeP=monobenzyl phthalate and Benz=benzoate, bd=below detection limit.

	Initial PAE conc. (mg/l)	(μM)	Conc. at termination (mg/l)	(μM)	Products formed	(μM)	Methane formed (μmol)	Methane (%)
PA	86	520	bd		bd		74	76
DEP	77	350	44	200	MEP	140	11	10
					PA	20		
DBP	70	230	bd		MBuP	130	23	19
BBP	87	280	20	60	MBuP	80	17	11
					MBeP	120		
					PA	40		
					Benz	60		
DEHP	70	180	60	150	bd		bd	bd

for the conversion of BBP, which then fits within +/- 10%. The recovery of benzoate was too low to match the hydrolysis of the BBP, as implied by the measured concentration of PA and MBuP. Presumably, the missing amount was present in the form of benzyl alcohol. Unfortunately, it was not possible to detect this alcohol with the analysis method used. The butanol formed according to the observed hydrolysis of BBP to PA and MBeP (Table 1) would theoretically give rise to the amount of methane recovered in these bottles. As in the case of the ethanol released from DEP hydrolysis, presumably, the butanol was converted to methane and carbon dioxide. Thus, there are strong evidences to believe that the benzyl alcohol was still present at the termination of the experiment.

Nearly 85% of the added DEHP remained in the bottles at the end of the experiment. None of the likely end products were observed and no excess of methane was found in these incubations as compared with the controls. Thus, the decline in the DEHP concentration observed may be due to binding to the waste matrixes of the inoculum. Similar difficulties connected with the anaerobic conversion of DEHP have been reported by several authors (Johnson & Lulves 1975; Horowitz et al. 1982; Shelton et al. 1984; Battersby & Wilson 1989; O'Connor et al. 1989; Ziogou et al. 1989). In contrast to these findings, Reinhart & Pohland (1991) reported that DEHP had completely disappeared after four years of codigestion with MSW. However, since no attempt was made to analyse intermediates or products in their study, it is not clear whether DEHP was completely degraded, transformed to MEHP or irreversibly adsorbed to the waste matrix.

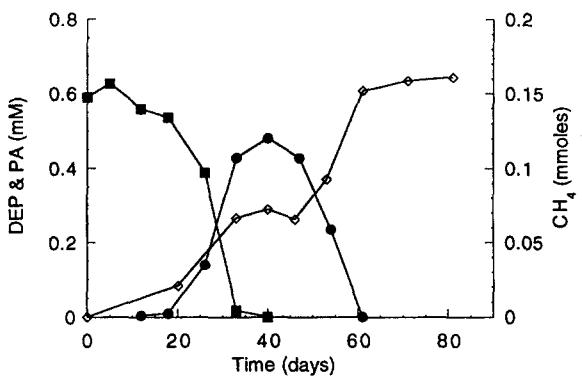

Figure 3. Degradation of DEP (■) via PA (●) to methane (◇) at a DEP concentration of 0.7 mM (-150 mg/l). Waste samples originated from the pilot plant digester treating MSW.

Experiment 3

PA, DEP, DBP and BBP were completely transformed by waste samples from the pilot plant digestor and an excess of methane as compared to controls was observed after 60-80 days of incubation. The diluted cultures started to confirm a degradation of added PAE to methane and carbon dioxide, had completely transformed PA, DEP, DBP and BBP after 40-60 days of incubation. DEP was degraded via MEP (traces) and PA, to methane and carbon dioxide (Fig. 3). The excess

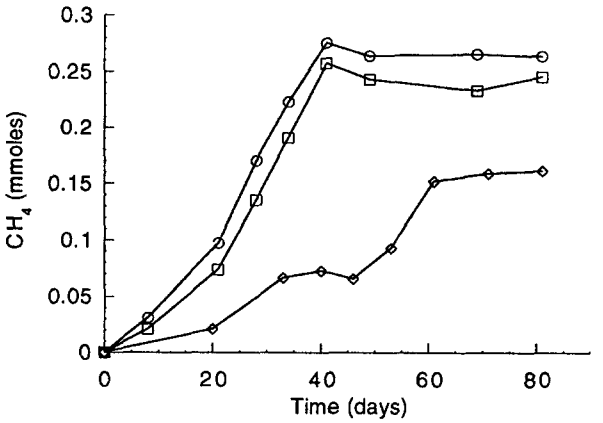

Figure 4. The formation of methane from diluted cultures amended with DEP (◇), DBP (□) and BBP (○) at 150 mg/l as source of energy and carbon. The diluted cultures originated from incubations with waste from the pilot plant digestor.

of methane during the first 30 days was most likely due to the degradation of the two ethanol moieties released from the hydrolysis of DEP. Methane formation that started again on day 45 resulted from the degradation of PA. It can be concluded that both the formation and degradation of PA was coupled to growth, since rates of transformation and formation of methane increased (Fig. 3). The diluted cultures growing on DBP or BBP were able to transform the PAE added to methane and carbon dioxide (Fig. 4). Incubations with DEHP did not produce more methane than the controls.

In contrast to microorganisms from the landfill models, those from the biogas reactor were able to completely degrade the PAEs added (except for DEHP) to methane and carbon dioxide. This difference may be due to the longer acclimatisation period that had been experienced by the populations developing in the four successive loadings of the biogas reactor. The need for an acclimatisation period for microorganisms in MSW was also indicated by the observations made for PA in experiment one.

Conclusions

The complete degradation of PAEs (except for DEHP) obtained when using inoculum from the pilot-plant biogas reactor clearly show that anaerobic microorganisms digesting MSW can attack compounds of this type. The kinetics observed during the complete degradation of the PAEs studied indicated that the microorganisms were able to acquire energy for growth from these reactions. Our results also show that an adaptation period is needed before anaerobic microorganisms in landfills can degrade PAEs. Furthermore, an active methanogenic bacterial population also seems to be required in order to achieve a rapid hydrolysis of PAEs. This finding is supported by results from Örlygsson et al. (1993) demonstrating that the presence of hydrogenotrophic methanogens promoted protein hydrolysis. The present study also shows that the monoesters formed from PAEs can form stable end products under landfilling conditions. These compounds are more water soluble than the corresponding "mother substances" and are probably more inert as well (Giam et al. 1984). The resistance to hydrolysis of the monoesters was shown by Wolfe et al (1980), who measured the base catalysed hydrolysis rates for DMP, DBP and DEHP. They observed that the diesters were hydrolysed twelwe times faster than the monoesters. Since DEP was hydrolysed to MEP under acidogenic conditions, it is also possible that other PAEs could be hydrolysed under such conditions. Thus, PAEs and, to an even greater extent, their more water-soluble transformation products may be released from the waste and transported out of the landfill with leachate water. Such a scenario might be mitigated by landfilling techniques promoting the rapid development of an active methanogenic microflora.

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