

Anaerobic degradation of xenobiotics by organisms from municipal solid waste under landfilling conditions

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Abstract

The potential for biological transformation of 23 xenobiotic compounds by microorganisms in municipal solid waste (MSW) samples from a laboratory scale landfill reactor was studied. In addition the influence of these xenobiotic compounds on methanogenesis was investigated. All R11, 1,1 dichloroethylene, 2,4,6 trichlorophenol, dimethyl phthalate, phenol, benzoate and phthalic acid added were completely transformed during the period of incubation (> 100 days). Parts of the initially added perchloroethylene, trichloroethylene, R12, R114, diethyl phthalate, dibutyl phthalate and benzylbutyl phthalate were transformed. Methanogenesis from acetate was completely inhibited in the presence of 2,5 dichlorophenol, whereas 2,4,6 trichlorophenol and R11 showed an initial inhibition, whenafter methane formation recovered. No transformation or effect on the anaerobic microflora occurred for R13, R22, R114, 3 chlorobenzoate, 2,4,6 trichlorobenzoate, bis(2 ethyl)hexyl phthalate, diisodecyl phthalate and dinonyl phthalate. The results indicate a limited potential for degradation, of the compounds tested, by microorganisms developing in a methanogenic landfill environment as compared with other anaerobic habitats such as sewage digester sludge and sediments.

Abbreviations: BBP – benzylbutylphthalate, DEHP – bis(2 ethylhexyl) phthalate, 3 CB – 3 chlorobenzoate, R22 – chlorodifluoromethane, CFC – chlorofluorocarbon, R13 – chlorotrifluoromethane, *cis*1,2 DCE – *cis* 1,2 dichloroethylene, DBP – dibutyl phthalate, R12 – dichlorodifluoromethane, 1,1 DCE – 1,1 dichloroethylene, R114 – dichlorotetrafluoroethane, 2,5 DCP – 2,5 dichlorophenol, DEP – diethyl phthalate, DiDP – diisodecyl phthalate, DMP – Dimethyl phthalate, DNP – dinonyl phthalate, MSW – municipal solid waste, PCE – perchloroethylene, PA – phthalic acid, PAE – phthalic acid esters, R11 – trichlorofluoromethane, 2,4,6 TCB – 2,4,6 trichlorobenzoate, 2,4,6 TCP – 2,4,6 trichlorophenol, VC – vinylchloride

Introduction

Landfilling has long been a widespread method for disposing of municipal solid waste (MSW). The succession of biological processes during the transformation of MSW results in a landfill life-cycle. This cycle can be divided in five development phases characterized by different levels of oxygen availability as well as fermentative and methanogenic activities (cf. Barlaz et al. 1989; Christensen & Kjeldsen 1989).

MSW may contain or give rise to a variety of organic pollutants which are released in the leachate and the gaseous phase of landfills (cf. Willumsen et

al. 1988; Laugwitz 1990; Pohland 1991; Öman & Hynning 1993; Christensen et al. 1993a). The fate of such compounds in landfills is therefore of considerable concern, since several of them are regarded as priority pollutants (KEMI 1990) and may contaminate the surrounding environment.

The formation, appearance and disappearance of a specific pollutant depends on several factors including the chemical and physical properties of the compound itself, e.g. its chemical reactivity, water solubility, sorption capacity, volatility and biodegradability. Furthermore, these compound-specific properties interact with the physical and chemical conditions pre-

vailing in a landfill such as temperature, moisture, pH, types of waste and the capability of the microflora (Pohland 1991; Reinhart & Pohland 1991). As a result of the dynamic nature of the landfill environment and complex interactions occurring, the potential for transformation and degradation of the pollutants varies over time (Pohland 1991).

Halogenated as well as nonhalogenated aromatic and aliphatic compounds, have been shown to be completely mineralized to methane and carbon dioxide. Others were reportedly transformed to various organic compounds in anaerobic incubations of sediments and sewage digester sludge samples as well as in enrichment and pure culture studies (cf. recent reviews by Mohn & Tiedje 1992 and Elder & Kelly 1994). In a study where a few of the pollutants likely to occur in landfills were added to pilot-plant landfill reactors, most of them were not recovered after four years of incubation (Pohland 1991). Furthermore, the occurrence of organic metabolites and plausible end-products provided strong evidence that the added pollutants had been transformed (Reinhart & Pohland 1991).

The purpose of the present study was to investigate the capacity of the anaerobic microflora, developing under landfilling conditions, to degrade or transform some of the pollutants known to occur in landfill leachate or in the gaseous phase. An array of pollutants were studied to determine whether they could be transformed and whether they could be utilized as carbon and/or energy sources. In addition, their influence on the degradation of the indigenous substrate in the inoculum was assessed.

Materials and methods

Chemicals

Test substances used in the study and their abbreviations are presented in Table 1. The CFC's R11, R12, R13, R21, R22 and R114 were purchased from ALFAX (Stockholm). *Cis* 1,2 DCE, 2,4,6 TCP, 2,5 DCP, 2,4,6 TCB and 3 CB were Aldrich products. All other chemicals used in this investigation were MERCK products.

MSW used as inocula

The inoculum originated from a reactor simulating the different development phases of a landfill as origi-

nally described by Stegmann (1981) and modified by Lagerkvist & Chen (1993). The model was filled with 40–45 kg unsorted and milled MSW with a particle size of approximately 1 cm. Water was added to give a dry solid (DS) content of about 30%. By the time of sampling (500 g), the reactor had been producing methane for nine months and simulated the so-called stable methanogenic phase (cf. Barlaz et al. 1989; Christensen & Kjeldsen 1989). The inoculum was prepared by diluting sub-samples of 200 g in a mixture of 1.7 litres phosphate buffer and 100 ml C1 (see below) supplemented with 2 ml 1M sodium sulphide, while flushing with N₂. The mixture was homogenised in a pebble mill for 30 min and then finally sieved (mesh 8 mm) while flushing with N₂.

Preparation of experimental bottles and sampling

A mineral medium, (pH=7.0) was prepared as described by Zehnder et al. (1980) except for the following additions: 33 $\mu\text{g l}^{-1}$ Na₂WO₄·H₂O, 0.5 g cystein HCl and 0.2 g yeast extract (Oxoid). The media were made up from three stock solutions: the phosphate buffer with resazurin, the salt, trace element and vitamin solution (C1) and, finally, the solution containing bicarbonate, yeast extract, cystein and sulphide (C2). All handling with medium and inoculum were done with regular techniques for cultivation of strict anaerobic microorganisms. The inoculum was added in 5 ml portions to the experimental bottles (118 ml) using a 10 ml cut-off pipette.

In the incubations with CFC's and chlorinated ethylenes, phosphate buffer was prepared and distributed among the experimental bottles as described above. C1 was then added, followed by the inoculum. The bottles were closed with Viton stoppers (Maagtechnic, Dübendorf, Switzerland) and sealed with aluminium crimps. The gas phase was replaced with N₂/CO₂ (80:20; < 2 ppm O₂) and C2 was added. Each CFC and chlorinated ethylene was added to a 118-ml bottle with a N₂/CO₂ atmosphere where they were allowed to evaporate, to give a concentration of 1 $\mu\text{mol.ml}^{-1}$. One ml each of the aliphatic compounds (= 20 μM) and 20 ml of H₂/CO₂ (80:20; < 2 ppm O₂), of which H₂ served as an electron donor, were added to the experimental bottles.

The aromatic compounds were added to a final concentration of 50 mgC.l⁻¹. Phthalic acid esters (PAE) were added to the bottles as pure compounds with a 10 μl Hamilton syringe, followed by phosphate buffer. The other aromatic compounds were separately dis-

Table 1. Extent of degradation, products formed, and influence on methanogenesis by the MSW-samples.

Compound	Fate ⁽¹⁾	End products	Methane yield % ⁽²⁾	Curve number ³
Perchloroethylene (PCE)	PT	TCE <i>cis</i> 1,2 DCE	X	1
Trichloroethylene (TCE)	PT	<i>cis</i> 1,2 DCE	X	1
1,1 Dichloroethylene (1,1 DCE)	PT	Not identified	X	1
Vinylchloride (VC)	NT		X	1
Trichlorofluoromethane (R11)	CT	R31	X	4
Dichlorodifluoromethane (R12)	PT	R22	X	1
Chlorotrifluoromethane (R13)	NT		X	1
Chlorodifluoromethane (R22)	NT		X	1
Dichlorotetrafluoroethane (R114)	PT	C ₂ HClF ₄	X	1
2,4,6 Trichlorophenol (2,4,6 TCP)	CT	2,4 DCP	0	4
2,5 Dichlorophenol (2,5 DCP)	NT		0	5
2,4,6 Trichlorobenzoate (2,4,6 TCB)	NT		0	1
3 Chlorobenzoate (3 CB)	NT		0	1
Dimethyl phthalate (DMP)	CD	CH ₄ & CO ₂	90	2
Diethyl phthalate (DEP)	PD	monoethyl phthalate CH ₄ & CO ₂	10	3
Dibutyl phthalate (DBP)	PD	monobutyl phthalate CH ₄ & CO ₂	19	3
Benzylbutylphthalate (BBP)	PD	monobutyl phthalate monobenzyl phthalate CH ₄ & CO ₂	11	3
Bis(2 ethylhexyl) phthalate (DEHP)	NT		0	1
Diisodecyl phthalate (DiDP)	NT		0	1
Dinonyl phthalate (DNP)	NT		0	1
Phthalic acid (PA)	CD	CH ₄ & CO ₂	76	3
Phenol	CD	CH ₄ & CO ₂	75	2
Benzoate	CD	CH ₄ & CO ₂	85	2

¹ CD = complete degradation, PD = part of the amount added was degraded, CT = complete transformation, PT = part of the amount added was transformed, NT = not transformed.

² Methane yield is given as per cent of the theoretical methane yield resulting from a complete degradation of the compounds. X = no methane or no methane to be expected from the target compound or the amount of methane would be too low to be distinguished from the background formation.

³ Curve number refers to Fig 1.

solved in the phosphate buffer by vigorous shaking before distributing the mixture among the experimental bottles. C1 was added, followed by the inoculum. The bottles were closed with EPDM rubber stoppers and sealed with aluminium crimps. The gas phase was replaced with N₂/CO₂ (80:20) and C2 was added.

All incubations, as well as the controls used to check for methane production from waste material in the inoculum, were made in triplicate and incubated at 37 °C for at least 100 days. Since hydrogen was added, to serve as an electron donor in some cases, inoculated controls with hydrogen were included. Autoclaving for 2 h at 121 °C did not sufficiently sterilize the cultures inoculated with MSW. Therefore, the extent of

abiotic influence on the test compounds could not be determined. However, inoculated bottles were incubated at 70 °C, which should inhibit mesophilic microbial activity and probably enhance pure chemical transformations. These bottles were therefore regarded as controls for detecting any non-biological transformations. The structure of the inoculum in these controls should have been considerably less affected than by the sterilization procedure, and thus, more closely mimicking conditions in the experimental bottles.

Analyses of halogenated aliphatics and methane were made on day 0, 3, and 9 and every ten days thereafter (see below). Liquid samples were collected from cultures containing with water-soluble aromat-

ics. Before sampling, the bottles were gently shaken for 30 s before 0.5 ml culture liquid was withdrawn with a 1 ml syringe and transferred to an Eppendorf tube. The samples were frozen and stored at -20°C until HPLC-analysis (see below). Bottles with dibutyl phthalate (DBP), benzylbutyl phthalate (BBP) and bis(2-ethylhexyl) phthalate (DEHP) were frozen on day 278 for GC and HPLC analyses later on (see below).

Enrichment cultures were started from bottles transforming 1,1 dichloroethylene (1,1 DCE). Transformation studies were made with these cultures in the following way: Sterile anaerobic bottles (118 ml) containing 45 ml phosphate buffer, C1, C2, 20 ml H_2/CO_2 and 2 μmoles of 1,1 DCE were placed at 37°C . Parallel bottles, whose contents were identical in all respects except that hydrogen had been excluded, were incubated as well. After 14 days of incubation the bottles were inoculated with 5 ml culture liquid from cultures able to transform 1,1 DCE and placed at 37°C and 70°C respectively. Two bottles were kept sterile (uninoculated) to serve as controls.

Analytical procedure

Headspace samples from the incubations with chlorinated aliphatics were analysed by gas chromatography (Chrompack 9001) and separated on a $25\text{ m} \times 0.53\text{ mm}$ Poraplot Q column ($df = 20\mu$). The gas sample was split after separation for simultaneous detection by flame ionization and electron capture. Helium was used as carrier gas at $5\text{ ml}\cdot\text{min}^{-1}$, and nitrogen was used as make-up gas at $25\text{ ml}\cdot\text{min}^{-1}$. Injector and detector temperatures were set at 250°C . A temperature program was used to separate the aliphatic compounds studied and the methane produced from indigenous substrates: For the CFCs, the initial temperature was 100°C (3 min), thereafter it was increased by $10^{\circ}\text{C}\cdot\text{min}^{-1}$ to 150°C and held at this temperature for 4 min. For the chlorinated ethylenes, the initial temperature was 150°C (3 min); thereafter it was increased by $10^{\circ}\text{C}\cdot\text{min}^{-1}$ to 200°C and then held at this temperature for 7 min.

Gas samples (0.3 ml) for methane analysis were withdrawn from the incubations with aromatic compounds and quantified by GC (Örlygsson et al. 1993). Overpressure in the bottles was accounted for. Analyses of volatile fatty acids (VFA) were performed according to Örlygsson et al. (1993). GC-MS analyses were kindly performed by Dr. P. Borgå, Dept. of Chemistry, Swedish Univ. of Agr. Sci, Uppsala.

DBP, BBP and DEHP were extracted from the culture liquid by adding 15 ml of hexane to the experimental bottles which were shaken for at least 12 h at 350 rpm. The bottles were then centrifuged at 2000 rpm for 10 min to separate the water and organic phases. After separation 0.8 μl of the hexane phase was injected on a GC (Chrompack 9000 with auto injector 901) with a split flow of $25\text{ ml}\cdot\text{min}^{-1}$. DBP, BBP and DEHP were separated isothermally at 230°C using a $25\text{ m} \times 0.53\text{ mm}$ CP-SIL 5 CB ($df = 5\mu$). Helium was used as carrier gas at $5\text{ ml}\cdot\text{min}^{-1}$, and nitrogen was used as make-up gas at $25\text{ ml}\cdot\text{min}^{-1}$. Injector and detector temperatures were set at 250°C .

All other aromatic compounds as well as the monoesters from the PAE of interest, were analysed using HPLC-technique. Frozen samples were thawed, acidified, by adding 50 μl 5 M H_3PO_4 to 0.5 ml sample, and centrifuged (Heraeus Biofuge 15) in Eppendorf tubes at 12,000 rpm. Control experiments showed that none of the compounds studied adhered to the plastic of the Eppendorf tubes. Supernatants ($\text{pH} < 2$) were injected (25 μl) with an autoinjector (WISP 710) on WATERS HPLC systems with a 6000 A pump. Separation was done at room temperature on a Millipore C-18 column using a degasified mobile phase of 0.03 M H_3PO_4 in 60% methanol and 40% distilled water at a flow of $1.2\text{ ml}\cdot\text{min}^{-1}$. The UV-detector 490 (WATERS) was set at 280 nm (phenols and PAE) or 254 nm (benzoates).

Results and discussion

Methane formation due to the degradation of the target compound, and/or parts of the indigenous substrate present in the inoculum available for conversion to methane followed the patterns shown in Fig. 1.

Freon R11 was completely reductively transformed to CH_2FCl (R31) within nine days of incubation (Table 1). The formation of R31 from R11 was confirmed by GC-MS. Traces of CHFCl_2 (R21) were observed as a transient intermediate during the R11 transformation. The transformation of R11 seemed to be coupled to biological activity as indicated by the finding that neither R31 nor methane was formed in incubations at 70°C (data not shown). After 100 days of incubation, a 5% portion of the added R12 was transformed to R22, and less than 1% of the added R114 was converted to C_2HClF_4 . The formation of C_2HClF_4 was confirmed with GC-MS. The R13 and R22 freons were not transformed (Table 1). Methane accumulation fol-

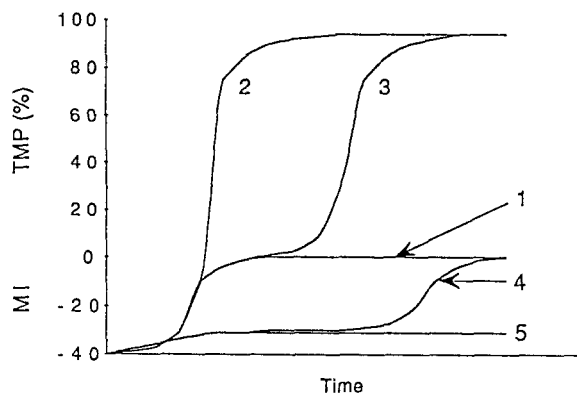


Fig. 1. Patterns of methane formation observed in the incubations amended with xenobiotic compounds. Curve no. 1, degradation of the indigenous substrate; curve no. 2, complete degradation of the test compound when no lag phase was observed; curve no. 3, partial or complete degradation of the test compound with a significant lag phase; curve no. 4, significant initial inhibition of methane formation by the test substance, which recovered later on; curve no. 5, significant inhibition of methane formation throughout the period of incubation. TMP = theoretical methane potential from the target compound; MI = methane formed as a result from degradation of indigenous substrate in the inoculum.

lowed curve no. 4 (Fig. 1) during incubations with R11 and remained retarded for as long as R11 was detectable ($< 0.001 \mu\text{moles}$), whenafter the methane formation rate increased to levels observed in the controls. Methane accumulation in the other incubations with freons followed the pattern observed in the control (Curve no. 1; Fig. 1). Reductive dehalogenation of R11 has been shown to be catalyzed by corrinoids active in the Wood-pathway (Egli et al. 1990; Krone et al. 1991; Krone & Thauer 1992). The corrinoids studied by Krone & Thauer (1992) transformed R11, with CO as the major end-product. R21, formate, R31, R41, $\text{C}_2\text{F}_2\text{Cl}_4$ and $\text{C}_2\text{F}_2\text{Cl}_2$ were identified as side-products at low concentrations. Deipser & Stegmann (1994) observed a transformation of R11, with R21 as the sole end-product during degradation of MSW in a 110-l landfill reactor. In their system, R11 was transformed to R21 during both the acidogenic and methanogenic phase. Observations by Krone et al. (1991) and Deipser & Stegmann (1994) indicate that R12 is more resistant to transformation than R11. However, transformations of both R11 and R12 have been shown to occur during anaerobic incubations of samples from freshwater sediments and soils (Lovley & Woodward 1992). Since no intermediates or end-products were detected, these

authors suggested that the freons studied were transformed via CO as reported by Krone et al. (1991).

After 100 days of incubation of the bottles with perchloroethylene (PCE) and trichloroethylene (TCE), about half of the amounts added were reductively transformed to TCE and traces of *cis* 1,2 dichloroethylene (*cis* 1,2 DCE) and *cis* 1,2 DCE, respectively. We concluded that the transformations of PCE and TCE were mediated by biological activity since no transformation occurred in incubations at 70°C . The reductive formation of TCE and *cis* 1,2 DCE at the expense of PCE and TCE, respectively, is in agreement with earlier studies performed with sewage digester sludge (cf. Vogel & McCarty 1985; Freedman & Gossett 1989). A slow transformation of PCE to TCE was also reported for landfill leachate samples by Christensen et al. (1993b).

In contrast to PCE and TCE, 1,1-DCE was transformed to products that have yet to be identified. The expected intermediates and/or end-products vinylchloride (VC) and ethylene were not detected during the transformation of 1,1 DCE. Enrichment cultures were therefore started to further investigate this transformation. An experiment was performed with the enrichment cultures growing in the presence of 1,1-DCE under different conditions (Fig. 2). At 70°C and 37°C without hydrogen addition, the transformation of 1,1 DCE did not take place or proceeded at a very slow rate. This strongly indicated that the transformation of 1,1 DCE was due to biological activity and that reducing equivalents were needed. A conversion of 1,1-DCE via acetaldehyde to CO_2 may have occurred, as suggested by Vogel & McCarty (1985) for the VC oxidation to CO_2 . VC was not transformed by the waste samples during the experimental period, and the formation of methane from indigenous substrate and hydrogen was not affected by the presence of chlorinated ethylenes.

2,4,6 trichlorophenol (2,4,6, TCP) was observed to be dechlorinated to 2,4 dichlorophenol after a lag phase of 150 days. 2,4,6 TCP partially inhibited the methanogenic flora, as indicated by the observation that methane formation began directly after starting the incubation, but ceased after approximately 15 days. The formation of methane started again after 85 days of incubation; thus, methane accumulation followed curve no. 4 (Fig. 1). Between day 15 and 85 the indigenous material was fermented to acetate which accumulated in the cultures (Fig. 3). Bacteria producing methane at the expense of acetate were probably responsible for the methane formation occurring

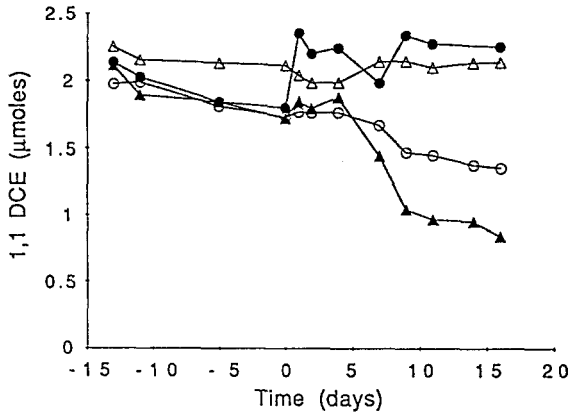


Fig. 2. Transformation of 1,1 DCE by enrichment cultures from MSW-samples. ▲ = incubation at 37 °C with H₂; ○ = incubation at 37 °C without H₂; ● = incubation at 70 °C with H₂; △ = uninoculated and sterile control incubated at 37 °C with H₂. The bottles were pre-incubated for 14 days before inoculation at day 0.

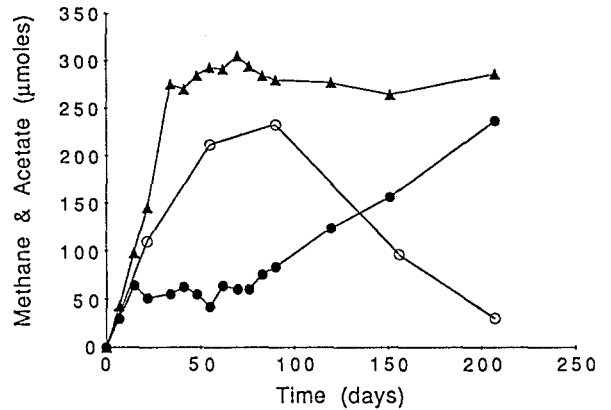


Fig. 3. Accumulation of acetate and methane during transient inhibition of methane formation from acetate in incubations with 2,4,6 TCP. ● = methane formed in incubations with 2,4,6 TCP, ▲ = methane formed in controls and ○ = acetate accumulation as compared to controls.

after day 85 (Fig. 3). As discussed by Madsen & Aamand (1992), methanogenic bacteria seem to be sensitive to chlorinated phenols. According to these authors, the positions of the chlorines on the aromatic ring can strongly influence the intensity of such an inhibition. 2,5 dichlorophenol (2,5 DCP) inhibited the methanogenic population more strongly than 2,4,6 TCP did, since methane accumulated according to curve no. 5 (Fig. 1). The acetate formed through the transformation of indigenous substrate in incubations with 2,5 DCP was not further degraded during the experimental period. 2,5 DCP, 3-chlorobenzoate and 2,4,6 trichlorobenzoate were resistant to microbial degradation during the experimental period.

Dimethyl phthalate (DMP), one of the short-chained PAE, was completely degraded to methane and carbon dioxide, and formation of the former followed the curve no. 3 pattern (Fig. 1). Phthalic acid (PA) was formed in equimolar amounts as a transient intermediate and disappeared after an intermediary lag phase of 25 days (Fig 4). Monomethyl phthalate was detected in trace amounts. Diethyl phthalate (DEP), DBP and BBP were transformed to the corresponding monoesters by the solid waste sample. None of the three esters were transformed to PA. Approximately 40% of the DEP added was degraded to monoethyl phthalate by the end of the experiment, whereas DBP was completely hydrolysed to monobutyl phthalate. At the termination of the experiment we were able to recover more than 90% of the added DEHP but only about 30% of the BBP added. BBP was trans-

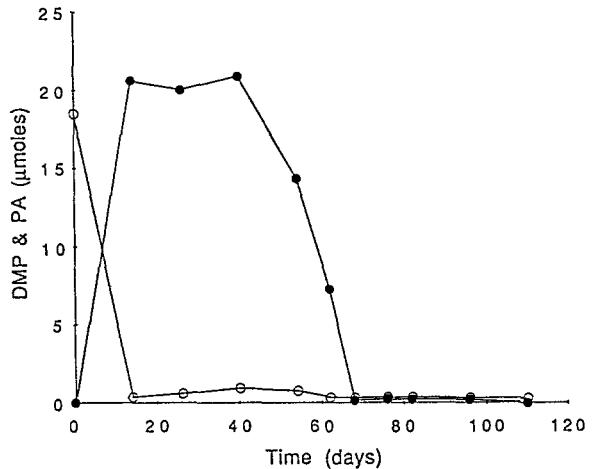


Fig. 4. Transformation of DMP (○) to the transient intermediate PA (●).

formed to monobutyl phthalate, monobenzyl phthalate and benzoate. In the bottles with DEHP, no monoesters could be detected. Ethanol, butanol and benzyl alcohol were likely set free by hydrolyses of DEP, DBP and BBP and then degraded to methane and carbon dioxide. This was indicated by the fact that the levels of methane accumulation exceeded those resulting from the degradation of indigenous substrate (cf. Table 1). The diisodecyl phthalate (DiDP) and dinonyl phthalate (DNP) incubations, in which methane formation was the only process studied, followed curve no. 1 (Fig. 1), suggesting that no degradation had occurred.

However, we cannot exclude the possibility that minor hydrolyses to the corresponding monoesters and alcohols occurred. PAE have been shown to be degraded to methane and carbon dioxide during the incubation of samples from a variety of anaerobic habitats (Horowitz et al. 1982; Shelton et al. 1984; Shelton & Tiedje 1984; O'Connor et al. 1989). Generally, the length of the side-chain is considered to determine the degradability of PAE under methanogenic conditions (Shelton et al. 1984). Thus, short-chain PAE, such as DMP, DEP and DBP, should be more susceptible to biodegradation than DEHP, DiDP and DNP. However, in agreement with our results, Battersby & Wilson (1989) observed that DBP was only partially degraded to methane and carbon dioxide in samples from primary sewage sludge. Additional evidence indicating that PAE is persistent in the landfill environment is the presence of DEP, DBP and BBP in leachate water samples as observed by Öman & Hynning (1993).

PA, benzoate and phenol are not considered to be priority pollutants. However, since these compounds are known to be formed as intermediates during the transformation of PAE and chlorinated phenols and benzoates, they were included in the investigation. PA was completely degraded to methane and carbon dioxide after a lag phase of approximately 65 days, thus following methane-formation curve no. 3 (Fig. 1; Table 1). Phenol and benzoate were both degraded to methane and carbon dioxide without a lag phase (curve no 2; Fig. 1). Benzoate was formed as a transient intermediate during phenol transformation before subsequent degradation to methane and carbon dioxide. The reductive formation of benzoate as an obligatory intermediate during phenol degradation under methanogenic conditions have recently been reported (cf. Knoll & Winter 1989; Bécharde et al. 1990; Zhang & Wiegel 1990; Elder & Kelly 1994).

Conclusions

Our results show that anaerobic microorganisms developing in the landfill environment are able to transform several xenobiotic compounds found in the aqueous and gaseous phases of landfills. One great advantage of studying the transformations of target compounds in waste subsamples, as reported here, is that it is easier to follow the formation of intermediates and end-products. Furthermore, the interactions between a specific compound and the microbial population are possible to observe as exemplified by R11, 2,4,6 TCP

and 2,5 DCP in our study. It is impossible to distinguish the toxic effects of a specific compound when present in a mixture of xenobiotics added to a waste sample. Although these experiments showed that certain compounds were transformed, they will not necessarily be transformed *in situ*. However, with the technique reported here, it is possible to compare different landfill environments concerning their potential for transforming xenobiotics. We are now continuing this work with MSW-samples from different landfills and MSW-samples from laboratory reactors simulating the different developing phases of landfills. Knowledge of the biotransformation pathways dominating during different landfill phases should help us to develop environmentally acceptable landfill techniques.

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