II. Microbial degradation of chemical waste, an alternative to physical methods of waste disposal

Chemical wastes and their biodegradation – an overview

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The Global 2000 Report points out that: 'Unless there are very significant structural changes in the world's economies, increased economic activity can be expected to produce larger quantities of waste materials and more residual wastes. Whether these residual wastes actually enter the environment as pollutants depends on policies for, and expenditures on, environmental protection. The projected economic growth will have one of two effects (or a combination of both): increased release of wastes and pollutants into the environment or increased costs of keeping the waste and pollution out of the environment ... unless there are innovations in production processes, by which 'wastes' are recycled and used (as in the pulp and paper industry, which now uses 'waste' as an energy source)'[1].

More than 5 million chemical compounds were described in Chemical Abstracts by the year 1980. Some 45,000 substances (as such, or in a vast variety of mixtures) are traded worldwide, and 70,000 are in US commerce. Some 1000 new chemicals are brought on the market annually. 150 chemicals are produced in excess of 50,000 tons per annum. The total world production of synthetic organic chemicals is estimated at 300 million tons per year. Ecotoxicological data are available for less than 1000 compounds[1,10,19,20].

The contributions of different countries to the world production of chemicals (data from 1974) are: USSR 11.3%; USA 23.8%; Japan 9.8%; U.K. 5.9%; France 5.3%; Germany (FRG) 9.2%; and other countries 34.7%[20].

The dangers arising from the environmental exposure to chemicals and their decomposition products and the effects and hazards they cause are not known for the majority of the commercially traded substances[20]. Environmental chemicals have been defined as 'substances which enter the environment through human activities and which can appear in such concentrations that they become hazardous to living things, in particular man himself. Among these substances are chemical elements and/or compounds of organic and inorganic nature, of natural and/or synthetic origin. The human activities may be direct or indirect, deliberate or accidental. The term living things encompasses human beings and their environment, including animals, plants and microorganisms'[20].

Waste disposal in the chemical industry

The production of specialities such as dyestuffs, pigments, drugs, agrochemicals etc. is generally associated with the formation of large amounts of undesired by-products and wastes. These wastes (transformation losses, solvents, auxiliary chemicals, by-products, catalysts etc.) amount to about 50–60% of the raw materials used and cause a wide range of environmental problems[4]. The chemical composition of such wastes is often rather complex and involves a large number of compounds. For the disposal of chemical wastes the following strategies are generally applied[4]:

- Improvement and modification of production processes and plants: reduction of wastes by recycling of acids, salts and gases; reduction of transformation losses; improved reaction specificities; simplified product isolation.
- Chemical hydrolysis (esters etc.).
- Thermal oxidation e.g. high-temperature incineration of organic or aqueous process effluents (high specific energy consumption).
- Wet-air oxidation in a reaction chamber at 280 °C/120 bar where a stream of atmospheric oxygen with or without catalyst serves to oxidize pollutants. In this process the heat of reaction is recovered and used for pre-heating the input to the reaction temperature. The oxidation yield is generally very high (over 98%).
- Microbial oxidation or biodegradation:
- Degradation in sewage treatment plants.
- Biodegradation with spezialized (salt tolerant) microorganisms in pure or mixed cultures.
- Development or isolation of fast growing microorganisms with wide spectra of biodegradative activities.

For more detailed information on advantages and disadvantages of the methods of disposal listed above, refer to Bretscher[4].

In this review I shall concentrate on biological processes for waste disposal and try to summarize which chemical pollutants are relevant and what is known about their biodegradability. I shall exclude information on the wastes from pulp and paper industries (refer to Gasche[7]).

Priority pollutants according to the US Environmental Protection Agency (EPA)

The behavior of toxic pollutants in the environment depends on a variety of chemical processes (such as hydrolysis, photolysis, oxidation, reduction, hydration etc.), physical- or transport processes (sorption, volatilization etc.) and biological processes (bioaccumulation, biotransformation, biodegradation). Literature on these processes was collected and evaluated by the US Environmental Protection Agency for a selection of 129 specific compounds and elements. EPA published a two-volume report 'Water-Related Environmental Fate of 129 Priority Pollutants' (1979)[5].

The 9 pollutant groups evaluated in the EPA study were: metals and inorganics, pesticides, PCB's and related compounds, halogenated aliphatic hydrocarbons, halogenated ethers, monocyclic aromatics, phthalate esters, polycyclic aromatic hydrocarbons, nitrosamines and miscellaneous compounds. The data provided by the EPA study consist of hard experimental facts, comparisons with related compounds but also model-based assumptions. Therefore, the reliability of the data is not always high. According to EPA statistics from 1980[16,18] based on over 3100 analyzed individual effluent samples from a total of 35 industrial categories and subcategories, those pollutants observed with highest frequency are: phthalates (ubiquitous), chloroform (37% of samples), methylene chloride (36%), benzene (26%), toluene (28%), phenol (24%), ethylbenzene (15%), naphthalene (10%), phenanthrene/anthracene (10%), tetrachloroethylene (10%), trichloroethylene (10%) and trichloroethane (11%).

Patterson and Kodukala published a complementary biodegradation study on the 114 organic priority pollutants from the EPA list (without cyanide, asbestos and the 13 metals)[16].

The organic priority pollutants are generally considered to be toxic. The extent of toxicity is a function of both the relative toxicity and the absolute concentration of the individual pollutant[16]. The biological processes observed during the classical biological treatment of an industrial waste mixed with sewage, or in soil and surface waters, depend on mixed and complex communities of microorganisms. In aerobic or anaerobic biological waste treatment processes priority pollutants can yield different types of responses or combinations thereof:

- Inhibition and deterioration of the overall treatment efficiency.
- Non-biodegradability: compound passes unchanged through the system.
- Biochemical conversion → formation of different compounds, not necessarily simpler ones.
- Biodegradation (mineralization) → CO_2, H_2O, nitrate, sulfate, biomass.
- Acclimatization/degradation → population shift or adaption.
- Sorption onto soil particles, primary sludge etc.
- Volatilization.

In treatment systems it is difficult to distinguish the relative importance of biotransformation, biodegradation, acclimatization, sorption and volatilization (stripping), as all these processes result in the disappearance of the investigated compound from the soluble phase in the treatment system! Therefore, the disappearance of a pollutant does not necessarily mean that the compound is really biodegraded. This restriction may explain the contradictory statements quite often found in the literature, and therefore also in table 1 of this review. For some of the pollutants biosorption factors for sludge have been reported[16]. Furthermore, a treatment system may readily acclimatize to and biodegrade a particular compound present at low concentrations, whereas a higher concentration might be toxic. In natural aquatic systems the small numbers of microorganisms present may not be able to metabolize even small concentrations of pollutants fast enough to prevent long-term toxicity to higher organisms.

Table 1 is a compilation of the most important facts extracted from EPA documents, but also from other sources[2,3,5,6,11-18,21-23,25].

Further important pollutants not included in the EPA list

One of the most important sources for the EPA report[5] was a paper by Thom and Agg[25] from the British Department of the Environment. In this report pollutants were divided into the following 3 groups: easily biodegraded substances; resistent substances; and substances which may be removed if suitable acclimatization can be achieved. The study by Thom and Agg[25] comprises information on the removal during biological treatment processes of more than 200 compounds considered to be the most important synthetic organic chemicals of concern in the UK from the standpoint of water pollution.

Another important source for the EPA report[5] was a study by Tabak et al.[24] on the microbial metabolism of aromatic compounds. Bacteria (*Pseudomonas, Flavobacterium, Achromobacter* and *Xanthomonas* sp.) from soil and related environments were selected or adapted (206 isolates) to metabolize and utilize phenol, hydroxyphenols, nitrophenol, chlorophenols, methylphenols, alkylphenols and arylphenols as the sole source of carbon. A phenol-adapted culture was then tested for utilization of 104 compounds (respirometric experiments) in concentrations of 100-500 ppm.

Data from these and other sources are compiled in table 2.

Table 1. Environmental fate and biodegradation of priority pollutants (EPA selection)

Abbreviations:
IE, pollutant detected in industrial effluents[5,16,18]; R, pollutant detected in the Rhine river[12]; V, volatilization; S, sorption; CS, chemical speciation; T, transport downstream; P, photolysis; H, hydrolysis; O, oxidation and photooxidation; BA, bioaccumulation; BM, biomagnification; BCF, bioconcentration factor; LOC, compound is among the 100 leading organic compounds (US-production in 1970: $2.4 \cdot 10^3$ to $3.4 \cdot 10^6$ tons/year)[3]; R–XY, rank among the top 50 in 1981 (US-market: $5.3 \cdot 10^5$ to $13 \cdot 10^6$ tons/year; the list also includes inorganic compounds)[14]

Name of the priority pollutant (EPA)[5]	Occurrence/importance[3,5,12,14,16,18]	Physical and chemical processes in the environment[5]	Bioaccumulation/biomagnification[5]	Biotransformation/biodegradation in natural aquatic environments[5] or by cultures of organisms isolated from such environments[11]	Static flask acclimation/degradation studies with settled domestic sewage and 5 mg/l of pollutant[16]		Removal in biological treatment systems[16]		Predicted biodegradability for biological treatments[25]
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
Metals and inorganics									
Antimony		S, T, CS	Slight BA, BCF 40 (fish), 16,000 (invertebrates)	Methylation may occur, oxidation by *Stibiobacter senarmonti*					
Arsenic	R	V, S, T, CS	Slight BA, BCF 333 (fish, invertebrates, plants), toxic	Methylation and reduction by *Methanobacterium* or fungi, yeasts					
Asbestos		T	No evidence	No evidence					
Beryllium		S, T, CS	Slight BA, no BM, BCF 100 (fish, invertebrates, plants)	No evidence					
Cadmium	R	S, T, CS	Strong BA, BCF 1000–250,000, highly toxic	*Klebsiella aerogenes* can be adapted to Cd→CdS[2]					
Chromium		S, T, CS	BA, BCF 400–4000, essential nutrient	Probably not important					
Copper		S, T, CS	BA, BCF 700–1700, essential nutrient, no BM	No evidence					
Cyanide	IE	V, P, CS	No BA, toxic	Biodegraded at low concentration by almost all microorganisms					
Lead		S, T, CS	BCF 60–200	Methylation					
Mercury	R	V, S, T, CS, P	BCF 1000–100,000	Methylation and reduction					
Nickel		S, T, CS	BCF 100–400, algae 40,000	No evidence Ni-enzymes known					
Selenium		S, T, CS	BCF 400–800 Se-Met, Se-Cys	Utilized by *Bacillus* sp. SS[15]					
Silver		S, T, CS	BCF 200–10,000, toxic	Not important					
Thallium		S, T, CS	Strong BA, BCF 100,000–150,000, inhibits photosynthesis	No evidence					
Zinc		S, T, CS	Strong BA, BCF 2000–100,000	More than 25 Zn-containing enzymes identified so far					
Pesticides									
Acrolein		No data ev. P	Probably not significant	Oxidation, hydration, biodegradable by unknown pathway	≥ 90		No data		
Aldrin	R	S, V, (P)	BCF 1000–10,000	Aldrin→dieldrin epoxidation by most organisms	0	0	–	100 (1)	Not degraded
Chlordane	R	S, V, (P)	BCF 1000–10,000	Normally persistent, utilized as a substrate by *Aspergillus niger*	0	0	No data, tends to concentrate in sludge		

Table 1: continuation 1

Name of the priority pollutant (EPA)^{5}	Occurrence/ importance 3,5,12,14, 16,18	Physical and chemical processes in the environment^{5}	Bioaccumulation/ biomagnification^{5}	Biotransformation/ biodegradation in natural aquatic environments^{5} or by cultures of organisms isolated from such environments^{11}	Static flask acclimation/ degradation studies with settled domestic sewage and 5 mg/l of pollutant^{16}		Removal in biological treatment systems^{16}		Predicted biodegradability for biological treatments^{25}
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
DDD (Dichlorodiphenyldichloroethane)	R	V, S	BCF 1000–100,000	Cometabolism, reductive dechlorination aerobic/anaerobic^{11,13}	0	0	0.2–0.3 tends to concentrate in sludge (3)	17–100	
DDE (Dichlorodiphenyldichloroethene)	R	V, S, P	BCF 10,000–100,000	Cometabolism, reductive dechlorination^{11,13}	0	0	– Tends to concentrate in sludge (1)	37	
DDT (Dichlorodiphenyltrichloroethane)	R, LOC	V, S, (P), (H)	BCF up to 10^6	DDT→DDD, DDE by many organisms, complete mineralization with *Fusarium oxysporum*^{11}	0	0	– Tends to concentrate in sludge (1)	100	Not degraded
Dieldrin	R	V, S, P	BCF 100–10,000	Degradation to CO_2 reported for bacteria and fungi^{13}	0	0	– Tends to concentrate in sludge (1)	72	Not degraded
Endosulfan and endosulfan sulfate	R	V, S, P, H, O	BCF 50–30,000, probably not significant	Degraded to endosulfate and endosulfandiol or lactone by soil bacteria and fungi^{13}	0	0	No data		
Endrin and endrin aldehyde	R	P	BCF 1000–10,000	Isomerization, dechlorination and hydroxylation by soil bacteria	0 Aldehyde not tested	0	No data		
Heptachlor	R	V, S, P, H	BCF up to 10,000	Oxidized to its epoxide in soil and metabolized to chlordene etc.^{13}	0	0	6.3	76 (1)	
Heptachlor epoxide	R	(S)	BCF 100–10,000	No evidence	0	0	No data		
Hexachlorcyclohexanes (α, β, δ-BHC isomers)	R	S	BCF 10–500, not important	Transformed by *Clostridium sphenoides* (α-BHC)^{13}	0	0	Most likely as lindane		Not degraded
γ-Hexachlorcyclohexane (Lindane)	R	S	BCF 10–300, not important	γ-BHC→α, β, δ-BHC, by different bacteria, utilized and degraded by soil microorganisms as the sole carbon source (anaerobic)	0	0	– Tends to concentrate in sludge (1)	0	Not degraded
Isophorone		P	Not likely to be important	Possibly oxidation	≥ 90		3–10	0–33 (2)	
TCDD (Dioxin)		S, P	BCF up to 10,000	Very slow^{17}	Not tested		No data		
Toxaphene		S, V	BCF 1000–10,000	Reduction, dechlorination (anaerobic), rel. persistent	Not tested		No data		
PCB's and related compounds									
Polychlorinated biphenyls	R	S, V, P	BCF up to 10^6	Only important for those with fewer than 4 chlorines per molecule *Alcaligenes, Actinobacter, Achromobacter*	1242:37 1254:11 1248: 0 1210: 0 1016:44	66 0 0 0 48	Tend to concentrate in sludge		Not degraded
2-Chloronaphthaline		P, (S)	Probably a short term process	Can be utilized as the sole carbon source by soil bacteria, chlorosalicylate as metabolite	≥ 90		2–2000	50–100 (4)	

Table 1: continuation 2

Name of the priority pollutant (EPA)[5]	Occurrence/importance [3,5,12,14,16,18]	Physical and chemical processes in the environment[5]	Bioaccumulation/biomagnification[5]	Biotransformation/biodegradation in natural aquatic environments[5] or by cultures of organisms isolated from such environments[11]	Static flask acclimation/degradation studies with settled domestic sewage and 5 mg/l of pollutant[16]		Removal in biological treatment systems[16]		Predicted biodegradability for biological treatments[25]
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
Halogenated aliphatic hydrocarbons									
Chloromethane (Methyl chloride)	LOC	V, (P), O	Probably not significant	Probably not degraded by microorganisms[22]	Not tested		56 Possibly removed by stripping (1)	> 91	
Dichloromethane (Methylene chloride)	IE, R, LOC	V, (P), O	Probably not significant	Utilized by strains of *Pseudomonas* and *Hyphomicrobium* as the sole carbon source[22,23]	≥ 90		9–1100 Inhibits anaerobic digestion of sludge[25]	35–65(6)	Should be degraded
Trichloromethane (Chloroform)	IE, R, LOC	V, (P), O	No evidence, possibly weak to moderate BA, no BM	Probably only slow degradation by microorganisms[22,23]	49	100	4–2600 Inhibits anearobic digestion of sludge[25]	5–98 (7)	Should be degraded
Tetrachloromethane (Carbon tetrachloride)	R, LOC	V, P	No BM	Probably only very slow degradation by microorganisms[22,23]	87	100	95–250 Inhibits anaerobic digestion of sludge[25]	94–98(2)	Should be degraded
Chloroethane (Ethyl chloride)	LOC	V, (P), O, H	Probably not significant	No evidence, perhaps at very slow rates, anaerobic?	Not tested		Yes[11]		
1,1-Dichloroethane (Ethylidine chloride)	R	V, (P), O	Probably not significant	No evidence, perhaps at very slow rates[22,23]	50	91	1.7–7.1	6–92 (3)	
1,2-Dichloroethane (Ethylene dichloride)	R, LOC, R-17	V, (P), O	Probably not significant	Utilized by mixed cultures as the sole carbon source[22]	26	63	1.5–8.4 Inhibits anaerobic digestion of sludge [25]	65–85(2)	Should be degraded
1,1,1-Trichloroethane (Methyl chloroform)	R, LOC	V, (P), O	Probably not significant	No evidence, perhaps at very slow rates[22], anaerobic?	29	83	9–1800	70–99 (5)	Should be degraded
1,1,2-Trichloroethane	IE, R	V, (P), O	Probably not significant	No evidence, perhaps at very slow rates, anaerobic?	6	44	11	> 9 (1)	
1,1,2,2-Tetrachloroethane	R	V, (P), O	Possible, but importance not assessed	No evidence[22]	0	29	12.8	> 22 (1)	Should be degraded
Hexachloroethane	R	(V), (P)	Possible, but importance not assessed	No evidence	≥ 90		No data		
Chloroethene (Vinyl chloride)	IE, R, LOC, R-21	V, O	Probably not significant	Evidence for persistence	Not tested		No data		
1,1-Dichloroethene (Vinylidene chloride)	R	V, O	Probably not significant	Probably at very slow rate	78	100	43.2	97 (1)	
1,2-trans Dichloroethene	R	V, O	Probably not significant	Probably at very slow rate	67	95	Yes[11]		
Trichloroethene (Trichloroethylene)	IE, R, LOC	V, O	Possible, but probably not important; no BM	Metabolized by higher organisms →dichloroacetate which is degraded by microorganisms	64	87	60–120	73–90 (3)	Should be degraded
Tetrachloroethene (Perchloro-ethylene)	IE, R, LOC	V, O	BCF 100, no BM	Metabolized by higher organisms →trichloroacetate which is degraded by microorganisms	45	87	6–560 Concentrated in sludge, BCF 6	60–99 (5)	Should be degraded
1,2-Dichloropropane (Propylene chloride)	IE, R	V, (S), O	Possible, but no evidence	Utilized as a carbon source by some soil microorganisms (slowly)	42	89	16	> 67 (1)	Should be degraded

Table 1: continuation 3

Name of the priority pollutant (EPA)[5]	Occurrence/importance [3,5,12,14,16,18]	Physical and chemical processes in the environment[5]	Bioaccumulation/biomagnification[5]	Biotransformation/biodegradation in natural aquatic environments[5] or by cultures of organisms isolated from such environments[11]	Static flask acclimation/degradation studies with settled domestic sewage and 5 mg/l of pollutant[16]		Removal in biological treatment systems[16]		Predicted biodegradability for biological treatments[25]
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
1,3-Dichloropropene	R	V, (S), O, H	Probably not important	Utilized as a carbon source by some soil microorganisms (slowly)	55	85	Yes[11]		
Hexachlorobutadiene (HCBD)	IE, R	S, V	BA may be significant; no BM BCF up to 1000	Very persistent	≥ 90		No data		
Hexachlorocyclopentadiene (HCCPD)	IE	S, V, P, H, (O)	BCF 300–2000	Slightly metabolized but no products identified	≥ 90		No data		
Bromomethane (Methyl bromide)		V, H, O	Not significant	Probably not significant	Not tested		No data		
Bromodichloromethane		(V), (S)	Possible, but no evidence	Some invertebrates seem to metabolize halogenated methanes and halogens	35	59	54	> 99	
Dibromochloromethane	IE	(V), (S)	Possible, but no evidence	As bromodichloromethane	25	39	81	> 99	
Tribromomethane (Bromoform)		(V), O	Possible, but no evidence	Tribromomethane occurs naturally in seaweed (*Asparagopsis*), therefore possibly metabolized by some microorganisms	11	48	910	89 (1)	
Dichlorodifluoromethane (Freon-12)	LOC	V, P	No evidence	No evidence, persistent, volatility probably precludes biodegradation	Not tested		No data		
Trichlorofluoromethane (Freon-11)	LOC	V, P	Possibly not significant	As Freon-12 probably not degradable[22]	59	73	48–920	19–99 (3)	
Halogenated ethers									
Bis(chloromethyl) ether (BCME)		(V), O, H	Too toxic and hydrolytically unstable →HCHO + HCl	Not likely to be a competing process	Not tested		59	83 (1)	
Bis(2-chloroethyl) ether		(V), O, H	Probably not important	Possibly after acclimation	≥ 90		0.12–19	47–92(2)	
Bis(2-chloroisopropyl) ether	IE	(V), O, H	Probably not important	No evidence	85	100	0.16–2	0–62 (2)	
2-Chloroethyl vinyl ether	IE	V, (S), O, H	Probably not important	Persistence is expected	76	100	No data		
4-Chlorophenyl phenyl ether		(V), S, P	BCF 800 in fish	Potential persistence in natural waters	0	1	Yes, rapidly degraded by acclimatized sewage sludge[5]		
4-Bromophenyl phenyl ether		(V), S, P	Probably similar as chloroanalog	Probably similar as the chloroanalog	0	0	360	95 (1)	
Bis(2-chloroethoxy) methane	IE	P, H	Probably not important	No evidence	0	0	25	> 60 (1)	
Monocyclic aromatics									
Benzene	IE, R R-16	V, (S), O	Low potential	Utilized as sole carbon source by some soil microorganisms *Pseudomonas putida*, *Achromobacter* sp.	49	100	8–10,200	66–100 (7) 90–100[6]	Should be degraded

Table 1: continuation 4

Name of the priority pollutant (EPA)[5]	Occurrence/ importance [3,5,12,14,16,18]	Physical and chemical processes in the environment[5]	Bioaccumulation/ biomagnification[5]	Biotransformation/ biodegradation in natural aquatic environments[5] or by cultures of organisms isolated from such environments[11]	Static flask acclimation/ degradation studies with settled domestic sewage and 5 mg/l of pollutant[16]		Removal in biological treatment systems[16]		Predicted biodegradability for biological treatments[25]
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	%removal (number of tests)	
Chlorobenzene	IE, R, LOC	V, S, O	BCF 1000–10,000	Very persistent, utilized by *Pseudomonas putida* when pregrown on toluene, via 3-chlorocatechol	89	100	15–1900	67–99 (3)	Should be degraded
1,2-Dichlorobenzene	IE, R, LOC	V, S, O	High potential, BA probably as chlorobenzene	Very persistent, utilized by *Pseudomonas putida* when pregrown on toluene	45	29	1.8–250 Concentrated in sludge, BCF 7-8	28–97 (5)	Not degraded
1,3-Dichlorobenzene	R	V, S, O	As 1,2-isomer	As 1,2-isomer	59	35	3.1 Concentrated in sludge, BCF 5-28	86 (1)	Not degraded
1,4-Dichlorobenzene	IE, R, LOC	V, S, O	As 1,2-isomer	As 1,2-isomer	55	16	30–53	> 82 (2)	Not degraded
1,2,4-Trichlorobenzene	IE, R	V, S, O	BCF 100–1000	As dichlorobenzenes	54	24	0.6–285	67–83 (2)	
Hexachlorobenzene	R	S	BCF 100–10,000, possibly BM	Very persistent, metabolized to pentachlorophenol	56	5	0.75–10	0–47 (2)	
Ethylbenzene	IE, R, LOC, R-20	V, (S), O	Low potential, possibly not important	Utilized as a carbon source by some soil bacteria	≥ 90		29–882	78–99 (4) 90–100[6]	Should be degraded
Nitrobenzene	LOC	(S), P	Low potential, possibly not important	Slow degradation by soil bacteria	≥ 90		10 Yes[11]	0 (1)	Should be degraded
Toluene	IE, R, LOC, R-15	V, S, O	Probably not important	Some soil bacteria (*Pseudomonas putida* etc.) can utilize toluene as the sole carbon source	≥ 90		70–680 Concentrated in sludge, BCF 48	52–99 (5) 70–90[21]	Should be degraded
2,4-Dinitrotoluene	IE, LOC	(V), S, P, O	Probably not important	Biodegraded by *Azotobacter* (slow), reduction of the nitro group	77	27	2000	75 (1)	
2,6-Dinitrotoluene	IE, LOC	(V), S, O, P	Probably not important	As 2,4-isomer	82	29	12–1900	~ 80 (2)	
Phenol	IE, LOC R-35	(V), P, O	Probably not important	Utilized as the sole carbon source via catechol by *Pseudomonas putida*, yeasts etc.	≥ 90		25–440	41–99 (5)	Easily degraded
2-Chlorophenol	IE, R	P	No evidence	Slowly degraded by pure and mixed cultures: *Pseudomonas*, *Arthrobacter*	86	100	10	46 (1)	Should be degraded
2,4-Dichlorophenol	IE, R	P	Probably not important	Readily biodegraded by soil bacteria: *Pseudomonas*, *Achromobacter*, *Arthrobacter* etc.	≥ 90		13.3	> 25 (1)	Should be degraded
2,4,6-Trichlorophenol	R	(V), P	Probably not important	Degraded by soil and sludge bacteria	≥ 90		703–1000	36–99 (2)	Should be degraded
Pentachlorophenol	IE, R	S, P	BCF 100–1000	*Pseudomonas* sp. and *Arthrobacter* sp.[21] utilize PCP as the sole carbon source	19	100	34–5333 Inhibits anaerobic digestion of sludge[25]	71 (2)	Not degraded

Table 1: continuation 5

Name of the priority pollutant (EPA)^5	Occurrence/ importance ^{3,5,12,14,16,18}	Physical and chemical processes in the environment^5	Bioaccumulation/ biomagnification^5	Biotransformation/ biodegradation in natural aquatic environments^5 or by cultures of organisms isolated from such environments^{11}	Static flask acclimation/ degradation studies with settled domestic sewage and 5 mg/l of pollutant^{16}		Removal in biological treatment systems^{16}		Predicted biodegradability for biological treatments^{25}
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
2-Nitrophenol	IE	S, P, O	Probably not important	Very persistent, inhibits microbial growth in natural aquatic systems: some pure cultures utilize 2-NP as a sole carbon source	≥ 90		40	> 90 (1)	Should be degraded
4-Nitrophenol	IE	S, P, O	Probably not important	As the 2-isomer	≥ 90		13–90	23–99 (3)	Should be degraded
2, 4-Dinitrophenol		S, P, O	Probably not important	As mononitrophenols	60	100	Not tested		
2, 4-Dimethylphenol	IE	P, (O)	Probably not important	Persistent in natural aquatic systems, but readily degraded by activated sludge culture as a sole carbon source	≥ 90		40	> 32 (1)	
p-Chloro-m-cresol	IE	P	Low potential, toxic	Natural systems unclear, effectively degraded in sewage treatment plants	78	100	Not tested		Should be degraded
4, 6-Dinitro-o-cresol (DNOC)	IE	S, P, O	Probably not important, toxic	Degraded by pure cultures as the sole carbon source, natural systems unclear	52	51	11	99 (1)	Not degraded
Phthalate esters									
Dimethyl phthalate	IE	S, (V), (H)	BCF 100/10,000 no BM	Utilization as carbon source by *Serratia, Penicillium lilacinums, Enterobacter aerogenes* in soil, water and activated sludge, hydrolysis by esterases, decarboxylation	≥ 90		8–60	25–60	Should be degraded, concentrated in sludge, BCF 6–172
Diethyl phthalate	IE				≥ 90		6–140	0–98	
Di-n-butyl phthalate	IE				≥ 90		1–23	0–68	
Di-n-octyl phthalate	IE				0	92	5000	0	
Bis(2-ethylhexyl) phthalate	IE, LOC				0	95	1–102	37–83	
Butyl benzyl phthalate	IE				≥ 90		6–18	0–91	
Polycyclic aromatic hydrocarbons									
Acenaphthene		S, P, (V)	BCF 100–(10,000) short-term process	Naphthalene is utilized as the sole carbon source by a number of bacteria (*Pseudomonas* etc.), generally PAH's with less than 4 rings are degraded by microbes	≥ 90		1–15	0–95	
Acenaphthylene					≥ 90		0.2–5	0–80	
Fluorene	IE				82	77	1.7–2	94–99	
Naphthalene	IE, R				≥ 90		1.3–56 All PAH's concentrate in sludge BCF 9–100	0–64 70–90^6	Should be degraded
Anthracene	IE	S, P, (V)	BCF 100–10,000	All these aromatics utilized by *Pseudomonas* sp. as the sole carbon source	43	92	8.5	> 60	
Fluoranthene					0	100	0.6–2	0–92	
Phenanthrene	IE				≥ 90		3 BCF 14–45 for all these PAH's	0–91	Should be degraded
Benzo[a]anthracene	IE	S, (P), (V)	BCF ~ 10,000 short-term process	Probably slow, generally PAH's with 4 or less rings are degraded by microbes, metabolized via hydroxylation and ring-fission	16	0	No data		
Benzo[b]fluoranthene	IE				Not tested		No data		
Benzo[k]fluoranthene	IE				Not tested		No data		
Chrysene	IE				0	38	No data		
Pyrene	IE				71	100	2.3–3	16–67 (2)	

Table 1: continuation 6

Name of the priority pollutant (EPA)[5]	Occurrence/ importance 3,5,12,14, 16,18	Physical and chemical processes in the environment[5]	Bioaccumulation/ biomagnification[5]	Biotransformation/ biodegradation in natural aquatic environments[5] or by cultures of organisms isolated from such environments[11]	Static flask acclimation/ degradation studies with settled domestic sewage and 5 mg/l of pollutant[16]		Removal in biological treatment systems[16]		Predicted biodegradability for biological treatments[25]
					1st culture % degrad.	4th culture % degrad.	Concentration (µg/ml)	% removal (number of tests)	
Benzo[ghi]perylene	IE	S,P,(V)	BCF 100–100,000 short-term process	Probably very slow, benzo a pyrene with *Pseudomonas* sp., generally PAH's with 4 or more rings are degraded slowly by microorganisms but easily metabolized by multicellular organisms	Not tested		No data		
Benzo[a]pyrene	IE				Not tested		3	33	BCF 44
Dibenzo[a,h] anthracene					Not tested		No data		
Indeno[1,2,3-cd] pyrene					Not tested		No data		
Nitrosamines and miscellaneous compounds									
Dimethylnitrosamine		P	Probably not significant	Persistent, very slow degradation in sewage and soil	Not tested		2000	> 95	
Diphenylnitrosamine		(S),P	Possible, but importance not assessed	Seems to be more easily degraded than dialkylnitrosamines; synthesized and degraded by intestinal bacteria	87	100	3–5.3	67–84(2)	
Di-n-propylnitrosamine		P	Probably not significant	Persistent, slow degradation by sewage and soil bacteria	27	50	0.5–11	0–99(2) BCF 100	
Benzidine	IE, R	S,P,O	No BA	Not easily degraded by microorganisms, inhibits sewage treatment	Not tested		4–12	0–41(2)	
3,3'-Dichlorobenzidine		S,P,(O)	BA in fish, toxic	Persistent, also in activated sludge	Not tested		No data		
1,2-Diphenylhydrazine (Hydrazobenzene)	IE	S,P,O	Highly probable no data	No evidence, metabolized in rats	80	77	14–2000	0–100	
Acrylonitrile	LOC, R-39	O	Probably not significant	Degraded in sewage sludge by *Pseudomonas* sp.	≥ 90			70–90[6]	

Outlook

Most of the biodegradation data found in the literature are restricted to aerobic processes as most of the existing sewage or waste treatment facilities involve aerobic processes. However, some anaerobic waste treatment processes including methane formation from organic substrates such as waste sugars operate on a large-scale (sugar industries). With the present knowledge about biodegradation processes one would certainly prefer an aerobic treatment for large-scale chemical waste. Many of the priority pollutants listed in table 1 are leading organic chemicals and occur either in industrial effluents or in rivers such as the Rhine. The biodegradation data found in different papers are sometimes contradictory and sometimes rather incomplete. The data in both tables 1 and 2 indicate that there is still much work to be done in order to decrease environmental pollution and make waste treatment more efficient. Waste treatment facilities should probably become more specific and better adapted to defined effluents. This goal could be reached either by separate treatment for such defined wastes (process effluents etc.) combined with recycling of materials and energy or by inoculating classical sewage treatment facilities with specialized strains for difficult degradation problems.

Table 2. Fate of synthetic organic chemicals in biological sewage treatment or in pure cultures (compounds not comprised in the EPA-List, table 1)

Abbreviations: R, pollutant detected in the Rhine river[12]
LOC, compound is among the 100 leading organic compounds (US-production in 1970: $2.4 \cdot 10^3$ to $3.4 \cdot 10^6$ tons/year)[3]
R-XY, rank among the top 50 in 1981 (US-market: $5.3 \cdot 10^5$ to $13 \cdot 10^6$ tons/year; the list also includes inorganic compounds)[14]

1. Compounds that are easily degraded in biological sewage treatment[25]

Acetaldehyde (LOC)
Acetic acid (LOC, R-34)
Acetone (LOC, R-38)
Acrylic acid (LOC)
Aniline (LOC)
Benzoic acid
Benzyl alcohol
1-Butanol (LOC)
Butyraldehyde
Butyric acid
Catechol
Citric acid
o-, m-, p-Cresol
Decanoic acid
Ethanol (LOC, R-50)
2-Ethoxyethanol
Ethyl acetate (LOC)
Ethylene glycol (LOC, R-29)
Formic acid
Glucoheptonic acid
Gluconic acid
Glycerol (LOC)
Isobutyraldehyde
Lactic acid
Lauric acid
Maleic acid/Maleic anhydride
Oxalic acid
Palmitic acid
Propionic acid
Salicylic acid
Tartaric acid
Urea (LOC, R-13)

Nearly all these compounds occur naturally (as intermediates in metabolic cycles or as catabolites) as well as being produced industrially and all are readily used by microorganisms as sources of carbon and energy.

2. Compounds that may be degraded if suitable acclimatization can be achieved[25]

Acetonitrile
Acrylamide
Adipic acid (LOC, R-49)
Alkanesulfonates
Alkylbenzenesulfonates (LOC)
Alkyl sulfates*
Allyl alcohol
Benzaldehyde
Benzenesulfonic acid
Biphenyl
1,3-Butadiene (R-32)
2-Butanol
2-Butoxyethanol
Butyl acrylate (LOC)
ε-Caprolactam
Chloroacetic acid
o-, m-, p-Chloroaniline (R)
o-, m-, p-Chlorobenzoic acid
3-Chloropropene (R)
Chlorthiamid
Cyanuric acid (R)
Cyclohexane (LOC, R-43)
Cyclohexanol
Cyclohexanone (LOC)
Cyclohexylamine
2,4-D (R)
Dalapon
2,4-DB
Dichlobenil
Dichloroprop
Dichlorvos
Diethanolamine (LOC)
Diethylamine
Diethylene glycol (LOC)
Dimethylamine (LOC)
Dimethyldithiocarbamic acid
Dimethylformamide
Diuron
1,2-Epoxypropane
Ethanolamine (LOC)
Ethoxylated aliphatic acids
Ethoxylated aliphatic alcohols
Ethoxylated alkyl amides
Ethoxylated alkylamines
Ethoxylated alkyl sulfates
Ethyl acrylate (LOC)
Ethylamine
Ethylenediamine (LOC)
2-Ethyl-1-hexanol (LOC)
Fluoroacetamide
Fluoroacetic acid
Formaldehyde (LOC, R-25)
Formamide
Fumaric acid (LOC)
Glycollic acid
Hexane
4-Hydroxybenzenesulfonic acid
Malathion
Maleic hydrazide
MCPA
Mecoprop
Methanol (LOC, R-18)
2-Methoxyethanol
Methyl acrylate
Methylamine
Methylene bisthiocyanate
Methyl metacrylate (LOC)
1-Methylnaphthalene
2-Methyl-2,4-pentanediol
2-Methyl-2-pentanone
2-Methyl-2-propanol
Morpholine
Nitrilotriacetic acid
o-, m-, p-Nitrotoluol
Octadecylamine
1-Octanol
Oleic acid
Paraformaldehyde
Parathion (R)
Pentaerythritol (LOC)
Phenylacetic acid
o-Phenylenediamine
Phloroglucinol
Phthalic acid
Phthalic anhydride (LOC)
Picloram
Polyethylene glycols
Polypropylene glycols (LOC)
Polyvinyl acetate
Polyvinyl alcohol
1,2-Propandiol
2-Propanol
Pyrethrum
Pyridine
Pyrogallol
Quinol
Resorcinol
Rotenone
Stearic acid
Tetrahydrofuran
Thiourea
Thiram
Toluene-2,4-diisocyanate (LOC)
p-Toluenesulfonic acid
o-, m-, p-Toluidine
1,1,2-trichloro-1,2,2-trifluoroethane
Triethanolamine (LOC)
Triethylamine
Triethylene glycol (LOC)
Trimethylamine
Vinyl acetate (LOC, R-40)
o-, m-, p-Xylene (LOC, R-22, 30)
Xylenols (6 isomers)

* see also part II of this series of papers[8]

The removal of these compounds may in some treatment facilities be incomplete, whereas in others it could be quite satisfactory. For acclimatization the initial concentration of these compounds might be very low and then increased rather slowly to the final level wanted for the treatment, which then should be held constant (fluctuations may result in the loss of acclimatization). The pollutant level held in acclimatized systems might in some cases be toxic to unacclimatized organisms. Not many of the compounds in this group occur free in nature, yet they are all potentially biodegradable.

3. Compounds resistant to biodegradation[25]

Alkylbenzenesulfonates (branched-chain)
Alkylnaphthalenesulfonates
Atrazine
1,2-Benzisothiazol-3-one
1H-Benzotriazole
Bis(tributyltin)oxide (R)
Bromoxynil
tert-Butylphenol
Carboxymethylcellulose (LOC)
Chlorendic anhydride
Chlorfenvinphos
Dichlorophen
2,4-Dichloro-3,5-xylenol
Dimethylsulfoxide**
Dinoseb
Diphenyl ether
Diquat
Ethoxylated alkylphenols
Ethylenediaminetetraacetic acid
Hexachlorophene
Hexamethylenetetramine (LOC)
2-Mercaptobenzothiazole
Paraquat
Pentachlorophenyl laurate
Phenylmercuric acetate
o-Phenylphenol**
Picric acid
Quaternary ammonium compounds***
Simazine
2,4,5-T**

** pure cultures degrading these compounds have been described, *** see also part III of this series of papers[9]
Most of these chemicals are resistent to degradation and can be harmful to wildlife.

4. Aromatic compounds that are degraded by pure cultures (degradation of parent substrate)[24]

Rapidly degraded	Less rapidly degraded	Slowly degraded
Phenol	o-, m-, p-Nitrophenol	2,4-Dinitrophenol
Catechol	2,4,6-Trinitrophenol	2,6-Dimethylphenol
Resorcinol	2-Chloro-4-nitrophenol	2,4-Dichlorophenol
Quinol	2,6-Dichloro-4-nitrophenol	2,4,6-Trichlorophenol
Phloroglucinol	m-, p-Chlorophenol	
o-Cresol	o-Phenylphenol	
m-Cresol	Thymol	
p-Cresol		

Phenol-adapted bacteria degraded a variety of hydroxyphenols, aminophenols, alkylphenols, arylphenols, chloronitrophenols, cresols, dimethylphenols, benzoic acids etc. at significant rates. For details see Tabak et al.[24].

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Biodegradation and utilization of monomethyl sulfate by specialized methylotrophs

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Methylations in organic syntheses are often carried out with dimethyl sulfate (DMS) as the methyl donor. Usually in such reactions, only one methyl group of DMS is transferred to the methyl accepting group (hydroxyl, mercapto, amino or imino group) and monomethyl sulfate (MS) is formed as a by-product in stoichiometric quantities. Therefore, the production of large-scale chemicals such as agrochemicals or dyestuffs involving methylations with dimethyl sulfate yields large volumes of monomethyl sulfate containing mother liquors. Only in rare cases can MS be used for further methylations. The recycling and remethylation of MS to DMS is very expensive and the disposal of MS by hydrolysis can create security problems (formation of dimethyl ether by alkaline hydrolysis or formation of chloromethane by acid hydrolysis with HCl). One of the methods best suited for its disposal is the incineration of the MS-containing mother liquors. As the biodegradation of monomethyl sulfate would be an alternative to physical or chemical methods of disposal, we searched for MS degrading microorganisms in order to establish a biological waste treatment process for MS-containing mother liquors.