9
Biodegradation of Special Organic Compounds

9.1
Introduction

Until today, industrial effluents loaded with particular organic compounds, which are difficult to biodegrade, have frequently been mixed with other effluents and "treated" together in WWTPs. Over time, the disadvantages of this technology have become obvious:

- The chemical oxygen demand (COD) of the effluent is considerably higher than legal limits, resulting in high discharge fees or the requirement to supplement the process with activated carbon treatment.
- Stripping of a part of the organic volatile compounds leads to air pollution problems and the necessity to cover the aerated basins and to treat the waste air.
- Certain effluents are only diluted, such as those from chemical plants with a high concentration of non-biodegradable compounds.
- Effluents containing toxic compounds reduce the removal rate in activated sludge plants.
- Endocrine-disrupting compounds discharged into wastewater can pass through WWTPs and enter aquatic environments.

Many more reasons can be cited for the need to turn away from end-of-the-pipe technology. New approaches are necessary, especially when new production units are to be constructed or old units have to be modernized. Furthermore, local circumstances have to be taken into consideration. In industrialized countries, a large percentage of industrial effluent is discharged into municipal WWTPs. But a significant number of large cities around the world do not have any municipal WWTP, so that industrial wastewater treatment is absolutely necessary to protect ground water reservoirs and to guarantee a minimum quality of raw water for the production of clean drinking water. Since clean water is also an essential raw material for industry and agriculture, many national economies will be affected by the need for clean water in the near future.

This makes it necessary for industry to conserve water by reducing its consumption. In principle, there are essentially three ways to conserve water:

Fundamentals of Biological Wastewater Treatment. Udo Wiesmann, In Su Choi, Eva-Maria Dombrowski

ISBN: 978-3-527-31219-1

1. Design new production processes with lower water consumption.
2. Recycle cooling and process water.
3. Develop economical solutions for the treatment of effluents with different types and strengths of problematic and non-problematic compounds (see Chapter 13).

The treatment of wastewater and process water should be carried out in most cases in the immediate proximity of the production unit. Both production and waste management have to be designed and optimized by a single team; and both units have to be operated by a single team. Wastewater treatment must be integrated into the production process!

Physical, chemical and biological processes must all be considered to find the best solution. Biological and chemical oxidation and reduction processes are of special interest if almost complete transformation to compounds such as CO_2, H_2O, N_2 and CH_4 is possible. The pollution in the effluents from such optimized processes is no longer characterized by thousands of different chemicals, rather there are often only three or four single compounds which dominate; and a knowledge of stoichiometry as well as kinetic coefficients may be helpful for a better understanding and optimization of the biodegradation processes.

Before we pick up this topic in Chapter 13, this chapter will discuss groups of problematic dissolved materials, which are not easily mineralized by microorganisms.

9.2 Chlorinated Compounds

9.2.1 Chlorinated *n*-Alkanes, Particularly Dichloromethane and 1,2-Dichloroethane

9.2.1.1 Properties, Use, Environmental Problems and Kinetics

Chlorine, a by-product from the electrolysis of chloroalkalis, was used in the beginning of the 20th century for the production of chloroorganic compounds and is known for its high reactivity with organics. Of the 114 organic priority pollutants listed by the United States Environmental Protection Agency (EPA) in 1975, 22 are chlorinated alkanes (Patterson 1985). Some of these are cited in Table 9.1. Because of their toxicity and tendency to bioaccumulate in animals, they must be removed as completely as possible from all liquid and gaseous effluents.

Inspection of the compound properties listed in Table 9.1 (Wiesmann and Libra 1999) shows that, with increasing chlorine content, the Henry coefficient rises, whereas solubility and biodegradability show a reversed trend. Therefore, tri- and tetrachloroethene are normally air pollutants and are removed from water and air by activated carbon adsorption. The discussion here will concentrate on dichloromethane (DCM) and 1,2-dichloroethane (DCA).

The maximum concentration permitted in drinking water for DCM is 20 μg L^{-1} and for DCA is 30–50 μg L^{-1} (WHO 1987). Toxicity experiments with rats led to a

Table 9.1 Some chlorinated n-alkanes, their properties and uses (Patterson 1985).

Compound	c^* (mg L^{-1})[a]	H[b]	LD_{50}[c]	Uses	EPA No.[d]
Dichloromethane (CH_2Cl_2)	16 700	0.13	167–2400	Solvent for intermediate products Cleaning agent for metal surfaces Propellent for polyurethane production	
1,2-Dichloroethane ($C_2H_4Cl_2$)	8 700	0.046	730	Intermediate product in the production of polyurethane solvent Fuel additive	10
Trichloromethane ($CHCl_3$)	7 800	0.14	450–800	Solvent in pharmaceutical industry	
1,1,1-Trichloroethane ($C_2H_3Cl_3$)	4 800	0.21	10 000–14 300	Solvent for dyes and ink Cleaning agent for cars and textiles	11
Trichloroethene (C_2HCl_3)	1 000	0.49	4 200–7 200	Solvent for dyes and ink Cleaning agent for cars and textiles	87
Tetrachloroethene (C_2Cl_4)	150	1.2	4 000–5 000	Solvent for dyes and ink Cleaning agent for cars and textiles	85

[a] Solubility, 20 °C.
[b] Henry coefficient, 20 °C.
[c] Rat, oral (mg kg^{-1} body weight).
[d] EPA list of organic pollutants (EPA 1975).

LD_{50} of 0.167–2.4 g kg^{-1} live weight. The LD_{50} value gives the mass of the toxic compound per mass of the animal for which there is a 50% probability that the animal would die within a given time. Mutagenic effects in rats could not be proven (Jongen et al. 1978). Loew et al. (1984) and Rannug (1980) indicate that DCA may be carcinogenic. Data on the worldwide production of DCA during 1960–1981 show an increasing production from $93 \cdot 10^3$ t a^{-1} to $825 \cdot 10^3$ t a^{-1}, however, production decreased in Germany from $137 \cdot 10^3$ t a^{-1} to $67 \cdot 10^3$ t a^{-1} in the 3-year period 1990–1993 (Herbst 1995). Study of the mechanism and the kinetics of DCM degradation began in the late 1970s. Studies of the catabolic reactions were first published by Leisinger (1988).

The influence of DCM concentration on the specific growth rate can be described by Haldane kinetics, and the influence of oxygen by Monod kinetics. These two influences are combined by multiplication:

$$\mu = \mu_{max} \frac{S}{K_S + S + S^2/K_i} \cdot \frac{c'}{K' + c'} \tag{9.1}$$

The kinetic and stoichiometric coefficients are listed in Table 9.2. The variability of the values can be explained by the use of different methods for kinetic measurements and different microbial cultures. Some authors used pure cultures, some mixed.

Table 9.2 Kinetic and stoichiometric coefficients for aerobic and anoxic degradation of DCM and DCE (Herbst 1995, expanded).

Compound; reference	Bacteria	$Y_{O_2/S}$ (mol mol^{-1})	$Y_{B/S}$ gMLSS (g DCM)$^{-1}$	μ_{max} (d^{-1})	K_S (mg L^{-1} DCM)	K_i (mg L^{-1} DCM)	K' (mg L^{-1} O_2)
DCM							
Brunner (1982)	*Pseudomonas*		0.158	2.6	17.0–42.5	995	
Dirks and Ottengraf (1991)	*Hyphomicrobium*		0.17	2.6		300	0.055
Hauschild et al. (1992)	*Pseudomonas*			5.3	4	1470	
Niemann (1993)	Mixed culture	0.62			3–10	200–2500	0.5–0.9
Herbst (1995)	Mixed culture	0.56	0.105	0.77	11.6		
Freedman et al. (1997)	*Acinetobacter* sp.		0.118[a)]	1.1		405	
DCA							
Freedman et al. (1997)	Mixed culture	0.58[b)]	0.087	0.77			
Janssen et al. (1985)	*Xanthobacter*				109		
Sallis et al. (1990)	*Rhodococcus*			2.5	26		
Rudek (1992)	*Xanthobacter*	1.12	0.12	3.4			0.055
Freitas dos Santos and Livingston (1995)	*X. autotrophicus*	1.65	0.166	4.6	7.6		0.1
Herbst (1995)	Mixed culture	1.7	0.17	4.6	57	125	0.5

[a)] g MLVSS (g DCM)$^{-1}$. [b)] $Y_{NO_3/S}$ mol mol^{-1}.

We can conclude from a mean K_S value of 10 mg L^{-1} DCM that very low DCM concentrations below 1 mg L^{-1} can only be obtained by a physical or chemical second step, e.g. adsorption on activated carbon or ozonation.

A hypothetic pathway for the catabolism of 1,2-dichloroethane oxidation was published by Janssen et al. (1985).

9.2.1.2 Treatment of Wastewater Containing DCM or DCA

DCM balances in activated sludge systems show that DCM is almost totally stripped by air (Gerber et al. 1979). Therefore, trickling filters are also unsuitable.

Only 11% of the DCM added were biodegraded, 75% were desorbed into the air and 14% remained in the effluent (Winkelbauer and Kohler 1989).

In further laboratory- and pilot-scale studies, fixed and fluidized bed reactors with solid particles as support material for bacteria were used. In fixed-bed reactors, a high fluid recycle rate is necessary for aeration in external absorption tanks (Kästner 1989) or for the addition of H_2O_2 by mixing (Stucki et al. 1992). Similarly, in two-phase fluidized-bed reactors, a high recycle rate must be used for oxygen addition and fluidization (Gälli 1986; Burgdorf et al. 1991; Stucki et al. 1992; Niemann 1993; Herbst 1995). In order to avoid total substrate desorption, oxygen can be added in the absorption tank using non-porous membranes (Herbst 1995). Figure 9.1 presents a laboratory-scale fluidized bed reactor for the mineralization of DCA and DCM. The production of HCl makes it necessary to control the pH to 7–8 by adding NaOH.

During growth, bacteria formed pellets with a diameter of 3–5 mm, which were kept fluidized in the reactor with the upflowing water containing the DCM or DCA. The pellets settled inside the enlarged head of the reactor; and the solid-free water passed a non-porous tubular membrane where oxygen was taken up by diffusion. This water was mixed with inflowing wastewater containing DCA or DCM before being recycled into the reactor.

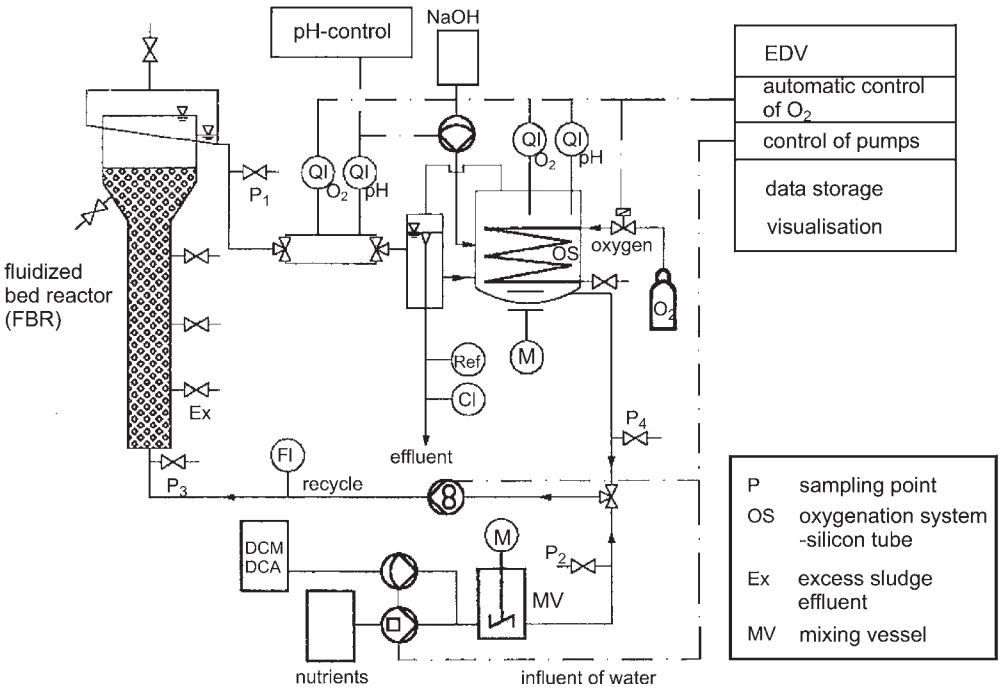

Fig. 9.1 Laboratory-scale plant with fluidized-bed reactor and oxygenation membrane for bubble-free aerobic mineralization of dichloromethane (DCM) and dichloroethane (DCA; Herbst and Wiesmann 1996).

The avoidance of direct aeration by using a membrane prevented desorption of the volatile substrates. A special membrane bioreactor to mineralize DCA has been tested successfully (Freitas dos Santos and Livingston 1995). DCA was kept separated from the biofilm by a membrane and only the part of the reactor with the biofilm and without DCA was aerated. This kept the DCA concentration inside the region of the membrane and biofilm so low that almost none was stripped by air bubbles.

High bacterial concentrations growing on the surface of small sand particles (Gälli 1986; Stucki et al. 1992; Niemann 1993), in porous glass particles (Burgdorf et al. 1991) or in dense flocs weighted by $CaCO_3$ (Herbst 1995; Herbst and Wiesmann 1996) yielded high mean reaction rates of up to 1400 mg L^{-1} h^{-1} DCM and 400 mg L^{-1} h^{-1} DCA. HCl was formed during the reaction and a relatively large amount of NaOH or $Ca(OH)_2$ had to be dosed. Lonjaret (1996) successfully tested a biotrickling filter for the treatment of DCM-loaded exhaust air.

9.2.2 Chlorobenzene

9.2.2.1 Properties, Use and Environmental Problems

Of the 12 known chlorobenzenes, six are of commercial importance (MCB, 1,2-DCB, 1,4-DCB, 1,2,4-TCB, 1,2,4,5-TeCB and HCB). Compounds with less chlorine are characterized by higher water solubility and higher vapor pressure or Henry coefficient (Table 9.3).

The toxicity of the chlorobenzenes also increases with increasing number of chlorine atoms. German production decreased steadily through the 1980s and 1990s as a result of stricter regulations. Emissions in the state of North-Rhine-Westphalia (Bayer Leverkusen, Germany) were estimated at 2886 t a^{-1} MCB and 1055 t a^{-1} DCB for 1992 (Döpper and Stock 1995). Three years earlier (1989), these emissions for the state of Hessen (Hoechst AG, Germany) were higher by a factor of nearly ten (Döpper and Stock 1995). These reductions result from the decrease in production and increase in efficiency of wastewater treatment.

9.2.2.2 Principles of Biological Degradation

A suitable auxiliary substrate is necessary to facilitate anaerobic dechlorination (acetate, acetone and others). The anaerobic degradation of MCB and benzene is obviously possible only in combination with the reductive dechloronation of higher-chloronated benzenes. The hydrogen replacing the chlorine atom probably comes from water (Nowak 1994).

Some papers report the production of the stable intermediate 1,2,3-TCB (Bosma et al. 1988; Ramanand et al. 1993). An example of the formation of intermediates during the anaerobic dechlorination of a mixture of 1,2,3-TCB, 1,3,4-TCB and 1,3,5-TCB in batch experiments (20 mg L^{-1} of each compound, 1-L flask tests) is presented in Fig 9.2.

Table 9.3 Some chlorobenzenes, their properties and uses at T = 25 °C. For symbols used, see Table 9.1.

Compound	c^* (mg L^{-1})	H	LD_{50} for rat (mg kg^{-1})	Uses	EPA No.
Monochlorobenzene (MCB)	460[a] T = 20–30 °C	0.16[a]	2900[a]	– Intermediate in the production of dyes, agrochemical, synthetics, aids for textile industry[a]	7
1,2-Dichlorobenzene (1,2-DCB)	130[a] T = 20–30 °C	0.10[a]	1500	– Intermediate in the production of 3,4-dichloroaniline decolorizer, degreaser[a]	
1,4-Dichlorobenzene (1,4-DCB)	73[a] T = 20–30 °C	0.079[a]	500	– Deodorizer, moth repellent – Intermediate in production of dyes[a]	
1,2,4-Trichlorobenzene (1,2,4-TCB)	30[b]	0.059		– Intermediate in the production of trichloronitrobenzene, etc. – Solvent	8
1,2,4,5-Tetrachlorobenzene (1,2,4,5-TeCB)	0.5–2.4[c] T = 25 °C	K_p = 0.12 kPa m^3 mol^{-1}[c]		– Intermediate in the production of tetrachloronitrobenzene and trichlorophenol[c]	
Hexachlorobenzene (HCB)	0.006[b]	0.071		– Intermediate in the production of pentachlorothiophenol and PCP production (in Germany until 1985)[c]	9

[a] Rippen (1991). [b] Patterson (1985). [c] Nowak (1994).

The mixed culture performing this degradation was obtained from methanogenic sediments of the Saale River near Jena, Germany. Acetone was used as an auxiliary substrate. It is remarkable that all three DCBs were observed in different amounts, presumably as a result of different degradation rates. Special cultures of aerobic bacteria seem to be capable of mineralizing all chlorinated benzenes from 1,2,4,5-TeCB (Sander et al. 1991) and 1,2,3,4-TeCB (Feidieker 1993) to MCB. Prieger-Kraft (1995) proved that both compounds were mineralized as the only source of carbon and energy. Currently, there is no confirmation of the aerobic elimination of PCB and HCB. In contrast to anaerobic dechlorination, during aerobic reactions the ring of the MCB is opened and chloro-*cis* together with *cis*-muconic acid are formed. Only then can the chlorine atom be separated.

Aerobic pure cultures use chlorinated benzenes as the only source of carbon and energy, with μ_{max} = 13.2 d^{-1} with MCB (Reineke and Knackmuss 1984), μ_{max} = 4.3 d^{-1} with 1,2-DCB (Haigler et al. 1992) and μ_{max} = 1.7 h^{-1} with 1,3-DCB (De Bont et al. 1986).

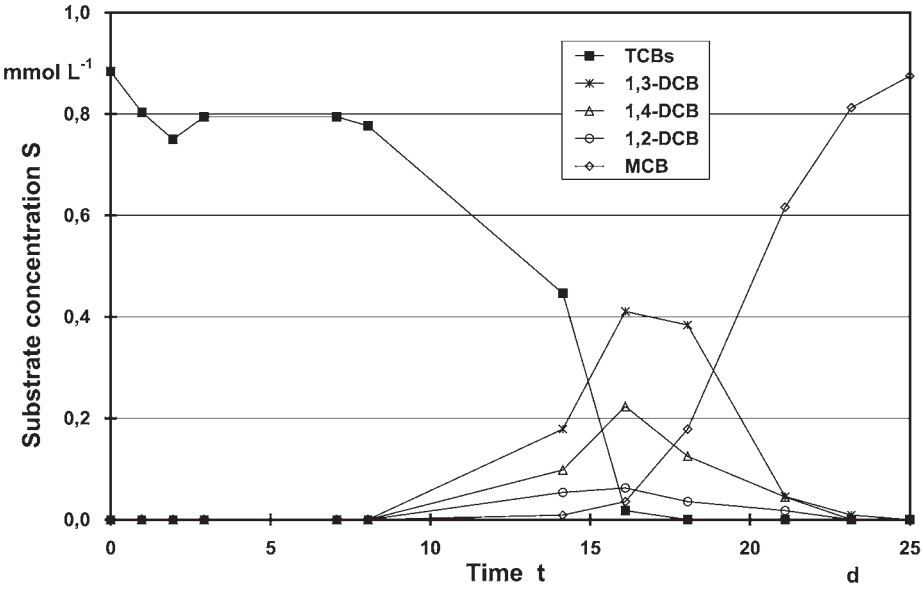

Fig. 9.2 Anaerobic reduction of three tetrachlorobenzenes (three isomeric TCBs, 20 mg L^{-1} of each), T = 28 °C, pH 7.28, auxiliary substrate: acetone (Adrian et al. 1995).

9.2.2.3 **Treatment of Wastewater Containing Chlorobenzene**

In all known continuous biological processes: (a) only laboratory-scale experiments have been carried out and (b) the pure or mixed bacterial cultures used were immobilized on solid particles.

As shown above, the dechlorination of HCB and PCB is only possible by using anaerobic conditions and auxiliary substrates. Fathepure and Vogel (1991) used a biofilm reactor and were able to transform HCB to 1,2,3,4-TeCB with methanol and to 1,2,3-TCB and 1,2-DCB with acetate as an auxiliary substrate. Nowak (1994) was successful in reducing 1,2,3,4-TeCB, all three TCBs and all three DCBs as well as MCB in a fluidized bed reactor with a mixture of methanol, acetate, acetone, ethanol, propanol and a mixed culture from a sediment of the Saale River.

Aerobic mineralization of MCB, the three DCBs and 1,2,4-TCB were achieved in fixed bed reactors by Bouwer and McCarthy (1982) with small glass beads for biofilm growth, by Van der Meer et al. (1987) with sand particles from the Rhine River and by Schäfer (1994) with polyurethane foam particles with and without activated carbon as carriers for mixed cultures, or *Pseudomonas* sp. (Van der Meer et al. 1987). In the experiments by Schäfer (1994), no auxiliary substrates were needed as the source of energy or carbon.

9.2.3 Chlorophenols

Of the ten known chlorophenols, five are produced as intermediates or end-products in the chemical industry (Table 9.4). Compared with chlorobenzenes, chlorophenols have a higher solubility in water and a lower Henry coefficient of nearly two orders of magnitude. Because of its high toxicity and its low biodegradability, pentachlorophenol (PCB) was used during the 1970s as an herbicide, fungicide and disinfectant worldwide in amounts of several 10 000 t a^{-1}. As a result of stronger regulations, production decreased during the 1980s (BRD in 1983 produced 1800 t a^{-1}, EU in 1989 produced 1000 t a^{-1}; Rippen 1991).

Khandker (1992) studied the aerobic degradation of 4-MCP as the only source of carbon and energy by a culture with three strains of bacteria ($Y^{\circ}_{X/S}$ = 0.63 MLSS $(gMCP)^{-1}$, μ_{max} = 3.6 d^{-1}, K_S = 21.1 mg L^{-1} MCP). The relatively high K_S value points to a large region of substrate limitation.

The oxidation of 2-CP, 3-CP, 4-CP and 2,4-DCP by H_2O_2 was catalyzed by an immobilized peroxidase (Siddique et al. 1993). High oxidation rates were obtained, but high-molecular-weight polymerization products arose which precipitated as small solid particles.

Table 9.4 Some chlorophenols, their properties and use. For symbols used, see Table 9.1.

Compound	c^* (mg L^{-1}; at 20 °C)	H (at 20 °C)	LD_{50} for rat (mg kg^{-1})	Uses	EPA No.
2-Monochlorphenol (2-CP)	29.0	0.49	670	Intermediate in the production of phenols, phenol resins, dyes, bactericide, fungicide	24
4-Monochlorophenol (4-CP)	27.0	$0.025 \cdot 10^{-3}$	250–670	Intermediate in the production of 2,4-DCP, TCP and TeCP solvent for mineral oil industry	
2,4-Dichlorophenol (2,4-DCP)	4.4	$0.131 \cdot 10^{-3}$	580	Intermediate in the production of 2,4-dichlorophenoxy acetate herbicide, toxin for moths	31
2,4,5-Trichlorophenol (2,4,5-TCP)	1.2	$0.28 \cdot 10^{-3}$	820	Intermediate in the production of 2,4,5-trichlorophenoxyacetate herbicide, fungicide, preservation aid	8
Pentachlorophenol (PCP)	19	$0.029 \cdot 10^{-3}$	50	Herbicide, fungicide Wood preserver Disinfectant	

9.3 Nitroaromatics

9.3.1 Properties, Use, Environmental Problems and Kinetics

The most prominent compound of this group is 2,4,6-trinitrotoluene (TNT). It has been produced in large amounts since 1900 and is used particularly as an agent of warfare. The soil of nearly all production sites is polluted by TNT and the products of its slow, natural biodegradation. Cleaning of these sites will be a task for many years to come. Because of its relatively low solubility in water (130 mg L^{-1}), TNT causes mainly soil pollution problems.

In contrast, several other nitroaromatics are characterized by a higher solubility in water (Table 9.5). 2,4-Dinitrotoluene (2,4-DNT) and 4-nitrophenol (4-NP), for example, are intermediates in the production of TNT. Therefore, they are more likely to be found in wastewater; and our knowledge about their biodegradability will be discussed briefly below.

Rippen (1991) reported an annual worldwide production of 60 000 t a^{-1} and a German production of 10 000 t a^{-1} TNT. Usually, 4-NP is produced in batch reactors on the basis of purchase orders. After each production run, the reactor, pipes and stirring vessels are washed with water and the remaining raw materials, prod-

Table 9.5 Some nitroaromatic compounds, their properties and uses. For symbols used, see Table 9.1.

Compound	c^* (mg L^{-1})	H	Uses	EPA No.
Nitrobenzene,	2000,	0.0029	Intermediate in the production of aniline	
$C_6H_5NO_2$	20 °C		Intermediate in the production of dyes Rubber, pharmaceutical products, photochemicals	
4-Nitrophenol, $C_6H_4OHNO_2$	13 700, 20 °C	$0.021 \cdot 10^{-6}$	Intermediate in the production of pesticides, azo and sulfur dyes, as well as chemicals for photo industry	58
2,4-Dinitrophenol, $C_6H_3OHN_2O_4$	200, 12.5 °C	$0.65 \cdot 10^{-6}$	Pesticide, fungicide Intermediate in the production of dyes, explosives and chemicals for photo industry	
2,4-Dinitrotoluene, $C_7H_6N_2O_4$	250, 20–25 °C	$2.4–200 \cdot 10^{-6}$	Intermediate in the production of polyurethane and of 2,4,6-dinitrotoluene	
2,4,6-Trinitrotoluene $C_7H_5N_3O_6$	130, 20 °C	$0.30 \cdot 10^{-8}$	Explosive Intermediate in the production of dyes and pharmaceuticals	

ucts and by-products enter the wash water, which is eventually discharged to the next wastewater treatment plant. Because of the multiple dilution processes, the concentration of 4-NP in water is normally relatively low and specialized cultures of bacteria cannot establish themselves in activated sludge plants. Therefore, 4-NP must be removed from the concentrated industrial effluents near the source by biological or physico-chemical processes. Its human toxicity is based on damage caused to the function of liver, kidney and central nervous system (Thiem and Booth 1991). The respiration rate of activated sludge is inhibited by 50% at a 4-NP concentration of 110 mg L^{-1} (Pagga et al. 1982).

With 660 000 t a^{-1} worldwide and 97 000 t a^{-1} in Germany, the annual production of 2,4-DNT is much higher than that of 4-NP (Rippen 1991). 2,4-DNT is mainly used as an intermediate in the production of TNT and polyurethane. Rippen (1991) reported concentrations of 9.7 mg L^{-1} in TNT production effluents and about 14 mg L^{-1} in the wastewater of special production processes for organic chemicals. Biodegradation experiments with anaerobic bacteria have been unsuccessful (Hu and Shieh 1987; Battersby and Wilson 1989).

Table 9.6 shows published stoichiometric coefficients for the description of aerobic catabolism and anabolism. It follows from these coefficients that 67.5% of the carbon is used for catabolism (formation of CO_2) and 24% of the nitrogen for anabolism, resulting in 76% being transformed to NO_2 ($Y^{\circ}_{NO_2/S\text{-}N}$ = 0.76 mol NO_2–N (mol S-N)$^{-1}$).

The low value of μ_{max} = 0.072 d^{-1} (Heinze 1997) for 4-NP degradating bacteria results in a long generation time of t_G = 9.6 d, but this value obtained with a mixed culture is considerably longer than that obtained by Schmidt et al. (1987) with *Pseudomonas* sp.

Table 9.6 Stoichiometric and kinetic coefficients for aerobic degradation of 4-NP (Heinze et al. 1995; Heinze 1997).

Compound, reference	Bacteria	$Y^{\circ}_{O_2/S}$ [mol O_2 (mol C)$^{-1}$]	$Y^{\circ}_{X/S}$ [g MLSS (g DOC)$^{-1}$]	$Y^{\circ}_{NO_2/S-N}$ [mol N (mol S-N)$^{-1}$]	$Y^{\circ}_{CO_2/S}$ [mol CO_2 (mol C)$^{-1}$]	μ_{max} (d^{-1})	K_S (mg L^{-1} DOC)
4-NP							
Jensen and Lautrup-Larsen (1967)	*Pseudomonas* sp.			0.52			
Jakobezyk et al. (1984)	Mixed culture			0.76			
Schmidt et al. (1987)	*Pseudomonas* sp.					7.4	1.1±0.2
Ou and Sharma (1989)	*Pseudomonas* sp.				0.675	0.048	
Heinze (1997)	Mixed culture	0.82	0.41	0.76		0.072	<1.0
2,4-DNT							
Bausum et al. (1992)	Mixed culture				0.64		
Heinze (1997)	Mixed culture	0.71	0.62	0.80		2.4	2.6–4.9

Bausum et al. (1992) carried out measurements with 2,4-DNT labelled by ^{14}C. Offgas measurements of $^{14}CO_2$ made it possible to determine $Y^{o}_{CO_2/S}$ = 0.64 mol CO_2 (mol C)$^{-1}$ (Table 9.6). The same authors used first-order kinetics to describe the influence of 2,4-DNT concentration, but their results for the reaction rate showed a relatively high degree of scattering. Further kinetic results were published by Heinze et al. (1995) and Heinze (1997; Table 9.6). It is remarkable that the maximum growth rate μ_{max} = 2.4 d^{-1} is higher by a factor of 33 than for the mineralization of 4-NP. Additionally, the higher $Y^{o}_{X/S}$ and the lower $Y^{o}_{O_2/S}$ show that a higher amount of carbon was used in anabolism, although two NO_2^- ions have to be separated from one 2,4-DNT molecule!

9.3.2
Treatment of Wastewater Containing 4-NP or 2,4-DNT

Laboratory-scale experiments were successfully carried out with an activated sludge reactor at an influent concentration of 400 mg L^{-1} 4-NP by Jakobezyk et al. (1984). If very low effluent concentrations (μg L^{-1} range) are required, bioreactors must be used (e.g. fixed-bed reactors) which have activated carbon as a support material for bacteria (Speitel et al. 1989). For the treatment of highly loaded synthetic wastewater (630–2500 mg L^{-1} 4-NP), Heitkamp et al. (1990) used a fixed-bed reactor with an immobilized culture of *Pseudomonas* sp. At a mean retention time of only 2.3 h, they obtained a mineralization efficiency of 93%. These experiments show that high reaction rates are possible after some time if slow-growing bacteria are immobilized. An effluent from a 2,4-DNT production plant of BASF Schwarzheide GmbH has been treated since 1995 by a two-step anoxic/aerobic activated sludge plant. In the first step, 2,4-DNT is most likely reduced to amino-nitrotoluene and 2,4-diaminotoluene, which are mineralized in the second step (Socher 1997).

9.4
Polycyclic Aromatic Hydrocarbons and Mineral Oils

9.4.1
Properties, Use and Environmental Problems

Polycyclic aromatic hydrocarbons (PAHs) are contained mainly in the tar of hard coals and in all kinds of mineral oils. During the various preparation processes for intermediate products in the chemical industry and for numerous fuels, PAHs enter the wastewater, groundwater and solid wastes. Because of their toxicity, 13 PAHs are included in the EPA list. Four of these and two further PAHs are presented in Table 9.7. Two characteristics are of notable importance: their low solubility in water, but high solubility in mineral oil, and their toxicity (LD_{50}). With increasing number of rings and molecular mass, their solubility in water decreases and their toxicity increases dramatically.

Table 9.7 Some polycyclic aromatic compounds, their properties, and use (Rippen 1991). For symbols used, see Table 9.1.

Compound	c^* (mg L^{-1})	LD_{50} (mg kg^{-1})	Use	EPA No.
Naphthalene	25 (20 °C)	Rat: 1780	Raw material for the production of dyes	
Anthracene	0.048 (20 °C)	*Daphnia magna*: 3 mg L^{-1}	Intermediate in the production of dyes and anthrachinone	78
Phenanthrene	0.95 (20 °C)	Rat: 700	Raw material for the production of explosives, pharmaceutical products, drugs, herbicides, tanneries	81
Benzo(e)pyrene	0.0038 (25 °C)			
Benzo(k)fluorethene	0.0006 (25 °C)			75
Acenaphthene		Rat: 10 000	Raw material for the production of textile pigment dyes and synthetics, insecticides and fungicides	77

Therefore, toxic PAHs are mostly dissolved in other organics such as mineral oils. Mineral oils and their biodegradability will be discussed first before continuing with the biodegradability of PAHs.

9.4.2 Mineral Oils

Mineral oils are composed of the following compounds (Berwick 1984):

- saturated hydrocarbons, *n*-alkanes, branched alkanes, cyclic alkanes,
- unsaturated hydrocarbons,
- heterocyclic alkanes,
- asphalts.

Saturated hydrocarbons can be mineralized by bacteria and yeasts and have been used as an energy and carbon source for the production of single-cell protein (SCP). *n*-Alkanes are very important components; and their content in distillation products increases as their boiling points decrease. The solubility in water is only about 0.002 mg L^{-1} for compounds with 12 or more C-atoms (Mackay and Shiu 1981). For solutions with concentrations above solubility, the oil phase can be dispersed as small droplets with adequate energy input, e.g. with a rotor-stator system. A stable oil–water emulsion can be produced with high energy input and the addition of an emulsifier.

An emulsion produced from *n*-dodecane (750 mg L^{-1}) with a surfactant (Eumulgin ET5, Henkel; 150 mg L^{-1}) and at a rotational speed of 10 000 min^{-1} for 1 min was designated as a standard emulsion (Cuno 1996). The distribution of oil droplets of this standard emulsion is presented in Fig. 9.3.

It could be demonstrated by biodegradation experiments of emulsions with mean droplet diameters between 1 and 15 µm that the bacterial growth rate was completely independent of the droplet size (Fig. 9.4).

These results can only be understood if the following mechanism of the oil biodegradation is assumed: the bacteria were covered by oil layers during collisions and then oil diffused into the cells. It is probable that the microkinetics of oil biodegradation is rate-limiting.

The following results of a kinetic biodegradation study were obtained with the standard emulsion mentioned above (Cuno 1996): T = 20 °C, $Y^{\circ}_{X/S}$ = 1.34 g MLSS (g DOC)$^{-1}$, μ_{max} = 4.08 d^{-1}, K_S = 22.9 mg DOC L^{-1}, k_d = 0.96 d^{-1}.

The result makes it probable that, in these experiments, mass transfer was not rate-limiting.

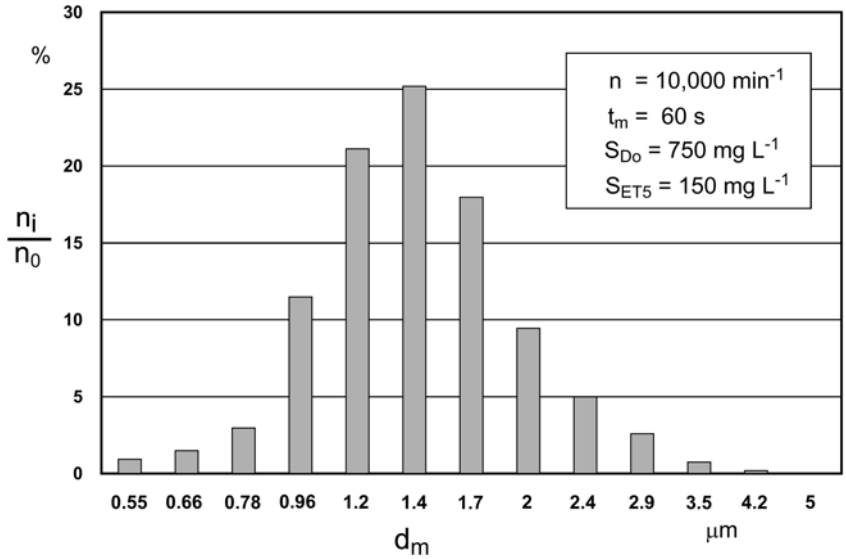

Fig. 9.3 Oil droplet distribution of the standard emulsion (Cuno 1996). n = stirrer speed, S_{Do} = concentration of *n*-dodecane, n_i = number of droplets of each size range, n_0 = total number of droplets, t_m = mixing time, S_{ET5} = concentration of the emulsifier, d_m = mean diameter of droplets.

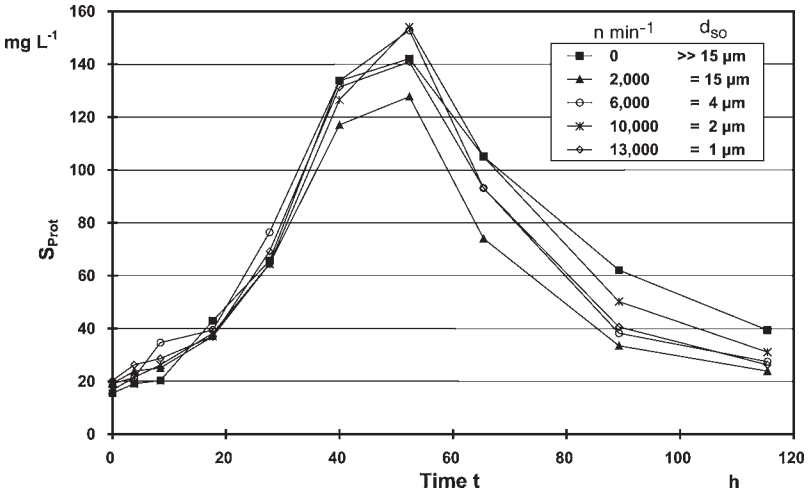

Fig. 9.4 Concentration of protein S_{prot} from growing bacteria for *n*-dodecane emulsions with different mean diameter of the droplets (Cuno 1996). n = stirrer speed, d_{50} = diameter of 50% of the droplet distribution, t = time, S_{prot} = concentration of protein.

9.4.3
Biodegradation of PAHs

9.4.3.1 PAHs Dissolved in Water

Because of their low solubility in water and the frequently rate-limiting dissolution of crystals, PAH biodegradation can be more easily studied by using solving agents such as dimethylsulfonoxide (DMSO; Cuno 1996). With the help of DMSO, the solubility of acenaphthene can be increased remarkably. Figure 9.5 shows the results of a batch experiment with a mixed culture of PAH-degrading bacteria. The concentration of the culture using acenaphthene as an energy and carbon source could not be measured. Therefore, in the solution of the model:

$$\frac{dX}{dt} = \mu_{max} \frac{S}{K_S + S} X - k_d X \tag{9.2}$$

$$\frac{dS}{dt} = -\frac{\mu_{max}}{Y^{\circ}_{X/S}} \frac{S}{K_S + S} X \tag{9.3}$$

assumptions must be made for $Y^{\circ}_{X/S}$ and k_d. The line in Fig. 9.5 shows the solution which agrees with the experimental data very well. Table 9.8 shows some further kinetic coefficients for the aerobic biodegradation of acenaphthene and other PAHs. The maximum growth rate decreases from naphthalene over phenanthrene, acenaphthene to anthracene. Further results for PAHs with four rings were published by Cuno (1996).

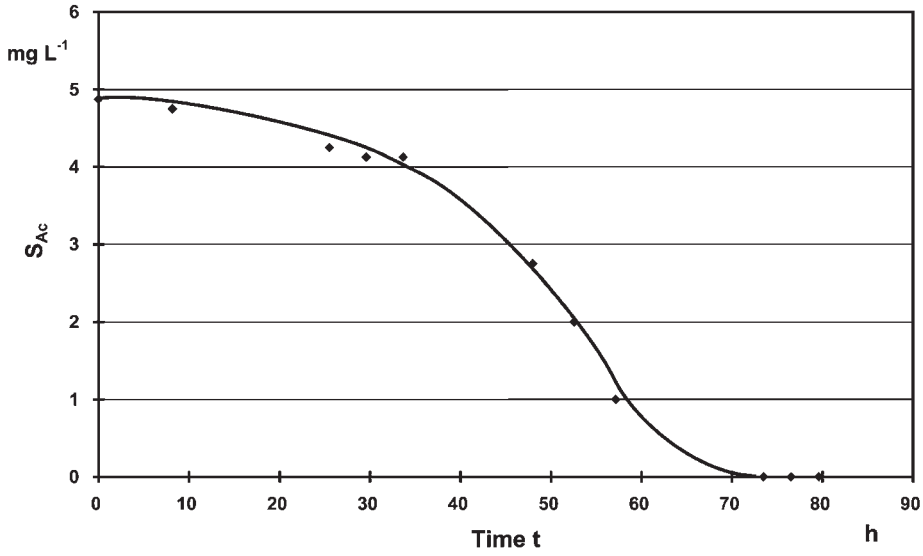

Fig. 9.5 Biodegradation of acenaphthene dissolved in water using a solving agent (5 vol% dimethylsulfonoxide; Cuno 1996), modelled using Eqs. (9.2) to (9.3).

Table 9.8 Stoichiometric and kinetic coefficients for aerobic degradation of some PAHs.

Reference	Bacteria	Compound	$Y^{\circ}_{X/S}$	μ_{max} (d^{-1})	K_S (mg L^{-1} S)	k_d (d^{-1})
Wodzinski and Johnson (1968)	*Pseudomonas* sp.	Naphthalene	0.5[a)]	7.2		
Guha and Jaffé (1996)	Mixed culture	Phenanthrene	0.3[a)]	0.026	0.09	
Springfellow and Aitken (1995)	*P. stutzeri*	Phenanthrene			0.24	
Komatsu et al. (1993)	*Pseudomonas* sp.	Acenaphthene		2.8		
Cuno (1996)	Mixed culture	Acenaphthene	0.7[b)]	1.48	0.065	0.0048
Breure et al. (1990)	*Pseudomonas* sp.	Anthracene		0.072		0.048
Cuno (1996)	Mixed culture	Anthracene		0.55	0.18	

[a)] g MLVSS (g S)$^{-1}$, [b)] g Pr (g S)$^{-1}$

The cometabolism of two PAH by a pure culture was studied by Springfellow and Aitken (1995). *Pseudomonas stutzeii* is able to use both naphthalene and phenanthrene as the only source for carbon and energy. The influence of both substrates on the oxygen consumption rate could be described by using a model for competitive inhibition:

$$\frac{r_{O_2}}{r_{O_2,max}} = \frac{S}{K_S\left[1 + \frac{S_i}{K_i}\right] + S} \tag{9.4}$$

with S as the concentration of phenanthrene and S_i as the concentration of naphthalene.

9.4.3.2 PAHs Dissolved in n-Dodecane Standard Emulsion

An interesting question regarding the biodegradation of *n*-dodecane droplets (standard emulsion, Section 9.4.2) and the biodegradable PAHs dissolved inside the droplets is: *will both be oxidized at the same reaction rate?*

Indeed, the results published by Cuno (1996) seem to confirm this assumption. Although *n*-dodecane, acenaphthene and anthracene are biodegraded by bacteria at different growth rates (Tables 9.7 and 9.8), the results in Fig. 9.5 can be described approximately by Eqs. (9.2) and (9.3), using the same kinetic coefficients for all three substrates.

A possible model to gain insight into these results is the transfer of very small oil droplets with dissolved PAHs into the bacterial cell. PAHs such as pyrene, dissolved in non-biodegradable, and in water-insoluble substances such as heptamethylnonane, can be used by bacteria such as *Rhodococcus* sp. at a considerably higher rate than those dissolved in the aqueous medium (Bouchez et al. 1997). PAHs need a solving agent which may be biodegradable (*n*-alkanes) or not (heptamethylnonane). Naphthalene and phenanthrene as well as the solving agent hexadecane can also be mineralized by sulfate-reducing bacteria, which could be proven with ^{14}C-labeled substances (Coates et al. 1997; Zhang and Young 1997).

9.5 Azo Reactive Dyes

9.5.1 Properties, Use and Environmental Problems

Nearly half of the dyes being used in textile finishing processes are reactive dyes which are soluble in water (Schönberger 1996). The molecular structure is characterized by three functional parts:

- a hydrophilic group,
- a group which produces the colour,
- a group which reacts with the textile fiber.

Figure 9.6 demonstrates the fixation of a reactive dye at a textile fiber. HSO_4^- is first separated from the reactive anchor group (step 1) before the vinyl sulfate group is hydrolyzed (step 2) and reacts directly with the fiber (step 3).

But a part of the vinyl sulfate groups formed is hydrolyzed and is not able to react with the fiber (step 4). It is lost for this dyeing process and remains as an impurity in the wastewater arising from the washing processes. The most important groups of reactive dyes are azo dyes. They are characterized by an azo group: –N=N–. Reactive black 5 (RB 5) is the most commonly used azo dye. It is a diazo dye with two azo groups. Figure 9.7 presents RB 5 in its vinyl sulfonic form, before fixation to the fiber and before hydrolysis. In this form with two vinyl sulfonic anchor groups, the RB 5 molecule can be fixed at one or two points on the fiber.

One vinyl sulfonic group can be hydrolyzed, resulting in a weaker fixation. No reaction with the fiber occurs if both groups are hydrolyzed.

Azo dyes occur in wastewater from textile works and also occur together with naphthalene sulfonic acids (NSA) in wastewater from the production of azo dyes. Let us discuss briefly some experiences showing the biodegradation of NSA.

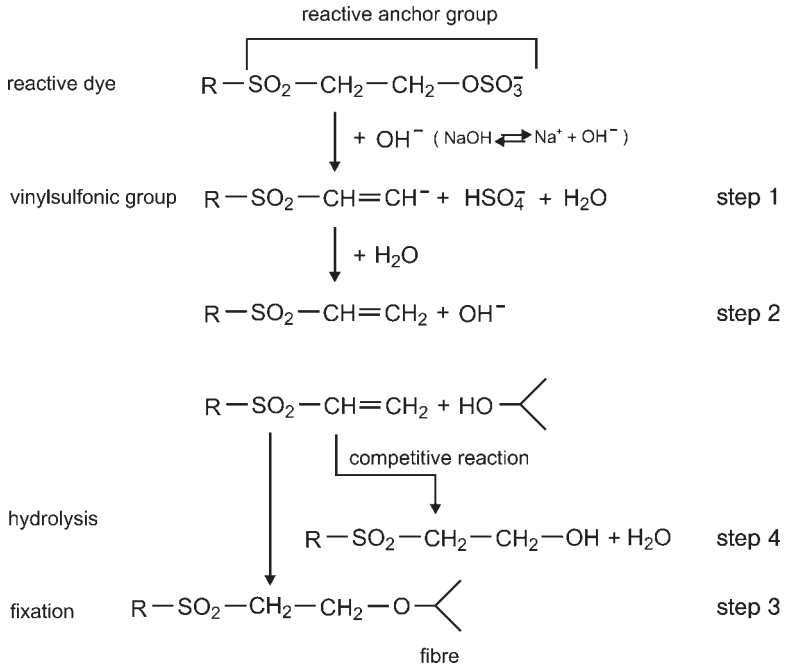

Fig. 9.6 Formation of the vinyl sulfonic group in reactive dyes, fixation at textile fibers and hydrolysis.

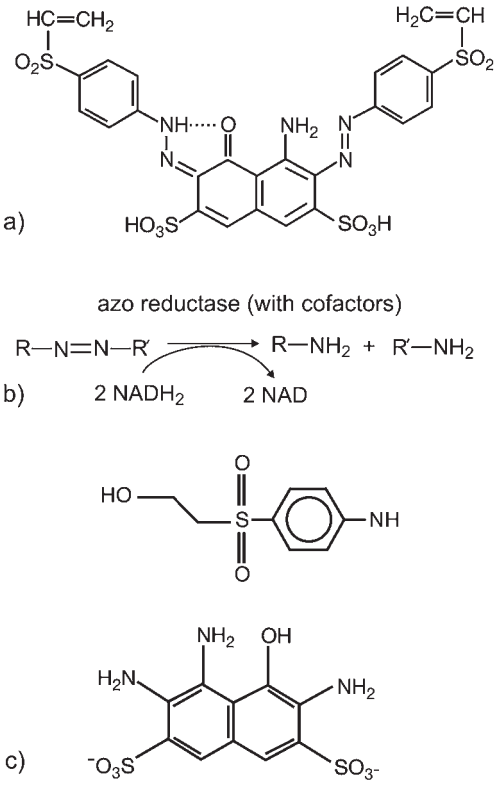

Fig. 9.7 Reactive black 5 (RB 5) and metabolites of anaerobic treatment. (a) RB 5 with two vinyl sulfonic groups, ready for fixation on textile fibers. (b) Reduction of an azo dye. (c) Metabolites formed by anaerobic treatment: aminobenzene-2-hydroxyethylsulphonic acid (above; p-Base) and naphthalene metabolite (below; TAHNDS). (After Sosath 1999; Borchert 2002; Wiesmann 2002).

9.5.2
Production of Azo Dyes in the Chemical Industry – Biodegradability of Naphthalene Sulfonic Acids

NSAs and their substituted analogs are commonly found in the wastewater from the production of azo dyes together with 1-hydroxonaphtalene, wetting agents, dispersants and other surfactants (Nörtemann and Hempel 1991) at concentrations up to 4300 mg L^{-1} COD (Krull and Hempel 1993). Naphthalene sulfonic acids which are of high significance for the production of azo dyes are, for example, naphthalene-2-sulfonic acid (2 NSA) or 6-amino naphthalene 2-sulfonic acid (6-A 2-NSA; Fig. 9.8a). In aerobic WWTPs they are non-biodegradable because bacteria which are able to use these compounds as a source of carbon, nitrogen or energy are washed out because of their low growth rates. Nörtemann and Hempel (1991) were successful in obtaining aerobic enrichment cultures of adapted bacteria with 6-A 2-NSA as the only substrate. Results for different dilution rates (D) are presented in Fig. 9.8b (Diekmann et al. 1988).

In Fig. 9.8b, the experimental results are compared with a model on the basis of Monod kinetics (solid line). With a dilution rate $D = D_C = 0.2\ d^{-1}$, a maximal growth rate of $\mu_{max} = 4.8\ d^{-1}$ follows (see Table 9.9; Diekmann et al. 1988; Diekmann and

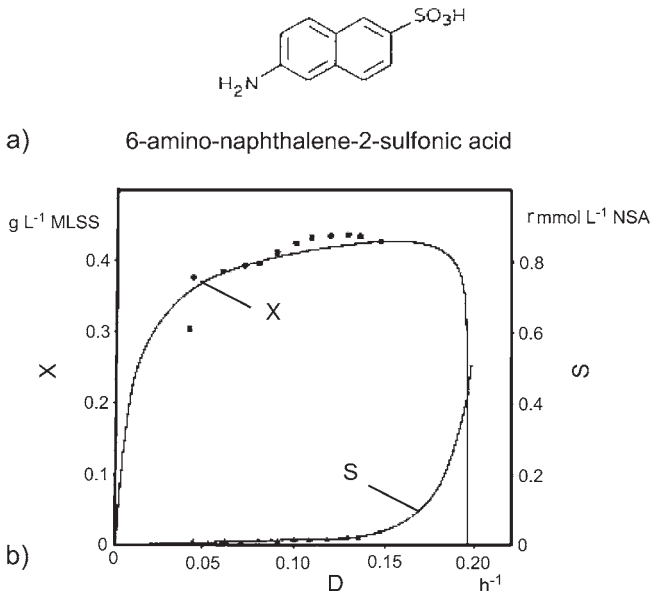

Fig. 9.8 Concentration of 6-aminonaphthalene-2-sulfonic acid (6-A-2-NSA) and bacterial concentration depending on dilution rate D.

Hempel 1989). Experiments with an airlift-loop reactor and sand as support material for bacteria were very successful (Diekmann et al. 1988).

Table 9.9 includes other kinetic coefficients regarding the biodegradation of naphthalene sulfonic acids obtained by the same group.

Some NSAs seem to be non-biodegradable. Until now, it has not been possible to transform some NDSAs, e.g. 1,5-naphthalene disulfonic acid. Breithaupt et al. (2001b) were successful in mineralizing this compound only by a two-step process of ozonation and biodegradation (Wiesmann 2002).

Table 9.9 Stoichiometric and kinetic coefficients for aerobic degradation of naphthalene sulfonic acids.

Reference	Bacteria	Compound	$Y^{\circ}_{X/S}$ [a]	μ_{max} [b]	K_S [c]	m_S [d]
Krull et al. (1998)	*P. testosteroni* A3	1NS/2NS	0.84	12	3	0.034
Krull and Hempel (1993)	Defined mixed culture	1NS/1,6 2,6NDS	0.37	0.89	41	0.004
Diekmann et al. (1988)	Defined mixed culture	6-A-2-NSA	1.47	4.8	2	0.009
Diekmann and Hempel (1989)	Defined mixed culture	6-A-2-NSA	1.28	4.8	2	0.007

[a] g MLSS (g COD)$^{-1}$; [b] d^{-1}; [c] mg L^{-1} COD; [d] Maintenance coefficient, g COD (kg MLSS · d)$^{-1}$.

Only about 70–90% of the azo dye is fixed to the fiber during the colorization process. The rest is hydrolyzed by a competitive reaction and remains in the wastewater generated by washing and rinsing with clean water (Fig. 9.6). We distinguish three main effluents with different concentrations of dyes and other compounds, such as fixers and surfactants (Sosath 1999):

- wastewater produced by the first rinsing processes, with concentrations of 4000–12 000 mg L^{-1} COD (60–70%);
- wastewater produced by the following processes, with concentrations of 200–2000 mg L^{-1} COD;
- used dye bath liquors.

The COD of the dye liquor is very high, but it cannot be used for further dying because of its excessive water content and various impurities. It is separated, transported and often incinerated with solid wastes. Dye houses with a direct discharge into surface water have to treat the wastewater after mixing with other waste streams. An activated sludge process is often used without a noticeable degree of mineralization of the used dyes. Dye houses with an indirect discharge into the sewerage system are generally not required to pretreat the wastewater.

We are convinced that this situation must be changed in future. Suitable pretreatment processes have to be developed (Sosath and Libra 1997).

9.5.3 Biodegradation of Azo Dyes

9.5.3.1 Direct Aerobic Degradation

The aerobic degradation of azo dyes by adapted bacteria has been reported (Meyer et al. 1979; Kulla et al. 1984; Blümel et al. 1997), but the relevance of such specialized bacteria to textile wastewater treatment is probably very limited. The aerobic decolorization of a number of azo dyes by different species of white-rot fungi has been demonstrated successfully (Spadaro et al. 1992; Ollikka et al. 1993; Heinfling et al. 1997; Borchert and Libra 2001; Borchert 2002). Their lignin-degrading systems (LDS) are relatively non-specific and so are able to oxidize and decolorize a variety of high-molecular-weight compounds. However, the complete mineralization of azo dyes by white-rot fungi seems impossible.

The most common approach to the biological treatment of azo dyes is the combination of anaerobic reduction with aerobic degradation of the aromatic amines. This will be discussed in more detail.

9.5.3.2 Anaerobic Reduction of Azo Dyes

It has long been known that azo dyes can be reduced by anaerobic bacteria in the human intestine to aromatic amines (Dieckhues 1960). The reduction equivalents gained by the oxidation of an auxiliary substrate through $NADH_2$ reduce the azo bond to form aromatic amines and thus decolorize the solution (Fig. 9.7b). We use

the simplified notation of the energy carrier here as $NADH_2$, as opposed to the proper notation as $NAD(P)H + H^+$ (see Chapter 3).

Putting it into the perspective of the classic three-step anaerobic degradation of complex carbon substrates, the anaerobic reduction of the dye is thought to take place in the first of the three steps by acidogenic bacteria.

The hypothesis for the mechanism of the biological reduction of azo dyes through the redox equivalents varies, depending on the enzyme (azo reductase) which participates in the decolorization. The enzymatic reduction theory was proposed by Zimmermann et al. (1982), Rafii et al. (1990), Haug et al. (1991), and Chung and Stevens (1993). Although most of the microorganisms reported to produce azo reductase are facultative anaerobic bacteria (Chung and Stevens 1993), some obligate anaerobic bacteria have been isolated from human intestinal microflora which produce azo reductase (Rafii et al. 1990). They found that the enzyme was extracellular and did not require induction by an azo dye for production.

Yoo et al. (2000) presented evidence that sulfur-reducing bacteria (SRB) can play an important role in the reduction of azo dyes. The azo bond is most likely chemically reduced through the sulfide produced by *Desulphovibri* from the sulfate often found in dye wastewaters. The biomediated chemical reduction rate was much faster than the rate found for fermenting bacteria.

The inhibition of *Vibrio fischeri* by the products of anaerobically treated RB 5 was studied by Libra et al. (2004). The EC_{50} value was decreased from 29.6 mg L^{-1} (completely hydrolyzed RB 5) to 1.5 mg L^{-1} (partly hydrolyzed RB 5; Libra et al. 2004).

9.5.3.3 Aerobic Degradation of Metabolites

Various authors have investigated the pathways of aromatic amine degradation in pure cultures (Meyer et al. 1979; Nörtemann et al. 1986; Haug et al. 1991). However, determination of the degree of degradation in mixed cultures is difficult. An auxiliary substrate is usually added to facilitate anaerobic decolorization, often in concentrations (measured as DOC) very much higher than that of the metabolites. Reduction in the effluent DOC is usually not a reliable indication of metabolite degradation. It is possible to follow the metabolites analytically, e.g. with HPLC; however, some of the metabolites are instable in the presence of oxygen and are not measurable for a longer time as a result of auto-oxidation, although no change in the DOC concentration occurs (Sosath and Libra 1997).

Using the commercial preparation of the RB 5 metabolite p-Base (Fig. 9.7c), Soewondo (1997) showed nearly complete mineralization in aerobic batch experiments. The results could be described using Monod kinetics.

9.5.4 Treatment of Wastewater Containing the Azo Dye Reactive Black 5

Sosath (1999) used a laboratory-scale two-step process with an anaerobic step and a subsequent aerobic step. In both reactors, bacteria were immobilized on the surface of rotating discs. He used a mixture of yeast extract and acetate as an auxiliary sub-

strate. In step 1, 66% decolorization was observed without remarkable consumption of the auxiliary substrate DOC, which was removed nearly completely in step 2, down to the remaining DOC of the dye. Obviously, the metabolites of decolorization are not biodegradable in this system. The mineralization of RB 5 seems only to be possible by chemical oxidation or a combination of chemical oxidation and biodegradation. Krull et al. (1998) treated a highly concentrated dyehouse effluent by using combination of biological and chemical methods. Breithaupt et al. (2001a) were successful in using a recycle reaction system consisting of an aerobic biological rotation disc reactor and an ozone batch reactor with automatic process control. Nearly 88% of the DOC could be removed. Approximately 66% of the ozone which would be needed for a 90% chemical oxidation could be saved (Wiesmann 2002).

An oxidation step with H_2O_2/UV and subsequent aerobic biodegradation operating in recycle mode was suitable to reduce the RB 5 DOC of 450 mg L^{-1} to about 33% by chemical oxidation and to reduce it by a further 33% by biological oxidation (Mohey El-Dein 2002). Of more practical interest is the treatment of more realistic dyehouse effluent, a mixture of RB 5 (18% DOC), a fixer (e.g. Akrofil PGM, 75%) and a surfactant (7%). Because of the biodegradability of these latter compounds, the most effective reactor system would consist of a first aerobic biological stage, followed by an ultrafiltration membrane to hold back microorganisms, an ozone reactor and an aerobic rotating disc reactor to mineralize products of chemical oxidation. The DOC could be reduced down to about 18% (α=82%), including the remaining molecules of all three model components (Rapp and Wiesmann 2003; Rapp 2005).

9.6 Final Remarks

In this chapter, we discussed some of the problems which arise from products of chemical industry, e.g.:

- chlorinated organics,
- nitro aromatics,
- polycyclic aromatic hydrocarbons,
- azo dyes.

It is very seldom that any of these are produced in nature. Nevertheless, some bacteria and yeasts “learn” to use their carbon, nitrogen and/or energy for growth after going through mutations, which can occur within a relatively short time due to the brevity of each generation. However, the processes of mineralization are often very difficult; and several different strains must cooperate. In some cases, toxic compounds are produced which are more dangerous to animals and human beings than the original compounds.

Most of these compounds cannot be removed in activated sludge plants or trickling filters; and thus it is necessary to develop tailored processes for their treatment. For high removal efficiencies it is necessary to combine different processes.

One alternative is to utilize precipitation, adsorption and membrane separation to obtain concentrates which can be disposed by other methods, such as incineration. Another alternative is the use of biological and chemical oxidation processes which result in the production of biomass and CO_2. We have shown some examples in which the combination of both can be successful. However, in nearly all cases the treatment costs are relatively high.

The best way to avoid these problems, therefore, is to use more and more natural dyes (Seweko 1988) and thus stop the production of water-polluting products by the chemicals industry which cannot be biodegraded, or only with great difficulty. The production of chlorinated organics was remarkably reduced in Europe over the past two decades. The production of azo dyes, however, is still increasing. We shall need much time to reach this goal, but we must start.

References

Adrian, L.; Manz, W.; Szewzyk, U.; Görisch, H. **1995**, Anaerober Abbau von Trichlorbenzonen in hochangereicherter Mischkultur, *Schriftenreihe Biologische Abwasserreinigung* 6, 81–103.

Battersby, N.S.; Wilson, V. **1989**, Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge, *Appl. Environ. Microbiol.* 55, 433–439.

Bausum, H.T.; Mitshell, W.R.; Major, M.A. **1992**, Biogradiation of 2,4- and 2,6-dinitrotoluene by fresh water microorganisms, *J. Environ. Sci. Health* A 27, 663–695.

Berwick, P.G. **1984**, Physical and chemical conditions for microbial oil degradation, *Biotechnol. Bioeng.* 26, 1294–1305.

Blümel, S.; Kudlich, M.; Stolz, A. **1997**, Mögliche Strategien für den mikrobiellen Abbau sulfonierter Azofarbstoffe, in: *Treatment of Wastewaters from Textile Processing* (*7 Kolloquium des Sonderforschungsbereiches 193*), Technical University Berlin.

Borchert, M. **2002**, Transformation von Reaktivfarbstoffen durch den Weißfäulepilz *Trametes versicolor* und bakterielle Mischkulturen (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 240.

Borchert, M.; Libra, J.A. **2001**, Decolorization of reactive dyes by the white rot fungus *Trametes versicolor* in sequencing batch reactors, *Biotechnol. Bioeng.* 75, 313–321.

Bosma, T.N.P.; van der Meer, J.R.; Schraa, G.; Tros, M.E. **1988**, Reductive dechlorination of all trichloro- and dichlorobenzene isomers, *FEMS Microbiol. Ecol.* 53, 223–229.

Bouchez, M.; Blanchet, D.; Vandecasteele, J.R. **1997**, An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain, *Microbiology* 143, 1087–1092.

Bouwer, E.J.; McCarty, P.L. **1982**, Removal of trace chlorinated organic compounds by activated carbon and fixed film bacteria, *Environ. Sci. Technol.* 16, 836–843.

Breithaupt, T.; Choi, I.S.; Wiesmann, U. **2001a**, Optimierung eines Systems aus Bioreaktor und Ozonreaktor zur Abwasserreinigung und Prozesswasseraufbereitung, *DFG Abschlussbericht*, 2001.

Breithaupt, T.; Reemtsma, T.; Jekel, M.; Storm, T.; Wiesmann, U. **2001b**, Combined biological treatment/ozonation of wastewaters for the mineralization of non-biodegradable naphtalene-1,5-disulphonic acid, *Acta Biotechnol.* 23, 321–333.

Breure, A.M.; Volkering, F.; van Andel, J.G. **1990**, Biodegradation of polycyclic aromatic hydrocarbons, in: *DECHEMA Biotechnology Conferences 4*, VCH, Weinheim.

Brunner, W. **1982**, *Bakterieller Abbau von Dichlormethan und 1,2 Dichlorethan*, PhD thesis, ETH Zurich.

Burgdorf, R.; Donner, C.; Weber, A.; Wiesmann, U. **1991**, Bakterien knacken CKW – Biologischer Abbau im Wirbelschichtreaktor, *Chem. Ind.* 11, 13–18.

Chung, K.-T.; Stevens, S.E., Jr. **1993**, Degradation of azo dyes by environmental microorganisms and helminths, *Environ. Toxicol. Chem.* 12, 2121–2132.

Coates, J.D.; Woodward, J.; Allen, J.; Philipp, P.; Lovley, D.R. **1997**, Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments, *Appl. Environ. Microbiol.* 63, 3589–3593.

Cuno, M. **1996**, Kinetische Untersuchungen zum biologischen Abbau von Mineralölen und polyzyklischen aromatischen Kohlenwasserstoffen (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 148.

De Bont, J.A.M.; Vorage, M.J.A.W.; Hartmann, S.; von den Tweel, W.J.J. **1986**, Microbial degradation of 1,3-dichlorobenzene, *Appl. Environ. Microbiol.* 52, 677–680.

Dieckhues, B. **1960**, Untersuchungen zur reduktiven Spaltung der Azofarbstoffe durch Bakterien, *Zbl. Bakteriol. Parasitenkd. Infektionskr. Hyg.* 1, 244–249.

Diekmann, R.; Hempel, D.C. **1989**, Production rates of CO_2 by suspended and immobilized bacteria degrading 6-aminonaphthalene-2-sulphonic acid, *Appl. Microbiol. Biotechnol.* 32, 113–117.

Diekmann, R.; Nörtemann, B.; Hempel, D.C.; Knackmuss, H.-J. **1988**, Degradation of 6-aminonaphthalene-2-sulphonic acid by mixed cultures: kinetic analysis, *Appl. Microbiol. Biotechnol.* 29, 85–88.

Dirks, R.; Ottengraf, P. **1991**, Verification of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter, *Bioprocess Eng.* 6, 93–99.

Döpper, M.; Stock, W. **1995**, Herkunft, Eintrag und Verbleib von Chloraromaten in der Umwelt, in: Mikrobielle Eliminierung chlororganischer Verbindungen, Vol. 6, *Schriftenreihe Biologische Abwasserreinigung Sonderforschungsbereich* 193, Berlin.

EPA **1975**, Development document for effluent limitation guidelines, in: *New Source Performance Standards for the Pressed and Blow Glass Segment of the Glass Manufacturing Point Source Category* (US EPA 440/1-75/034-a), U.S. Environmental Protection Agency, Washington, D.C.

Fathepure, B.Z.; Vogel, T.A. **1991**, Complete degradation of polychlorinated hydrocarbons by a 2-stage biofilm-reactor, *Appl. Environ. Microbiol.* 57, 3418–3422.

Feidieker, D. **1993**, *Untersuchungen zur mikrobiologischen Sanierbarkeit eines mit Chlorkohlenwasserstoffen kontaminierten Industriestandortes*, PhD thesis, Technical University Berlin.

Freedman, D.L.; Smith, C.R.; Noguera, D.R. **1997**, Dichloromethane biodegradation under nitrate-reducing conditions, *Water Environ. Res.* 69, 115–122.

Freitas dos Santos, L.M.; Livingston, A.G. **1995**, Novel membrane bioreactor for detoxification of VOC wastewaters: biodegradation of 1,2-dichloroethane, *Water Res.* 29, 179–194.

Gälli, R. **1986**, Optimierung des mikrobiellen Abbaus von Dichlormethan, in: *BUA-Stoffbericht 6*, VCH, Weinheim.

Gerber, V.; Gorobets, L.; Loakimis, E. **1979**, Effects of urea and dichloromethane on biochemical treatment of refinery wastewater, in: *Chemistry and Technology of Fuels and Oils, vol. 1,5 no. 3/4*, Consultens Bureau Enterprise, New York, p. 269–272.

Guha, S.; Jaffé, P. **1996**, Determination of Monod kinetic coefficients for volatile hydrophobic organic compounds, *Biotechnol. Bioeng.* 50, 693–699.

Haigler, B.E.; Pettigrew, C.Y.; Spain, J.C. **1992**, Biodegradation of mixtures of substituted benzenes by *Pseudomonas* sp. strain JS150, *Appl. Environ. Microbiol.* 58, 2237–2244.

Haug, W.; Schmidt, A.; Nörtemann, B.; Hempel, D.C.; Stolz, A.; Knackmuss, H.-J. **1991**, Mineralization of the sulfonated azo dye Mordant Yellow 3 by a 6-aminonaphthalene-2-sulfonate-degrading bacterial consortium, *Appl. Environ. Microbiol.* 57, 3144–3149.

Hauschild, I.; Horn, T.; Rudek, M.; Safak, T.; Starnick, J. **1992**, The effect of substrate and product inhibition on the microbial degradation of chlorinated hydrocarbons, in: *DECHEMA Biotechnology Conferences 5*, VCH, Weinheim, p. 1061–1967.

Heinfling, A.; Bergbauer, M.; Szewzyk, U. **1997**, Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adusta*, *Appl. Microbiol. Biotechnol.* 48, 261–266.

Heinze, L. **1997**, Mikrobiologischer Abbau von 4-Nitrophenol, 2,4-Dinitrophenol und 2,4-Dinitrotoluol in synthetischen Abwässern, (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 167.

Heinze, L.; Brosius, M.; Wiesmann, U. **1995**, Biologischer Abbau von 2,4-Dinitrotoluol in einer kontinuierlich betriebenen Versuchsanlage und kinetische Untersuchungen, *Acta Hydrochim. Hydrobiol.* 23, 254–263.

Heitkamp, M.A.; Camel, V.; Reuter, T.J.; Adams, W.J. **1990**, Biodegradation of p-nitrophenol in an aqueous waste stream by immobilized bacteria, *Appl. Environ. Microbiol.* 56, 2967–2973.

Herbst, B. **1995**, Biologischer Abbau von Dichlormethan und Dichlorethan durch Mischkulturen im Wirbelschichtreaktor (PhD thesis), *VDI-Forschungsberichte, Reihe 17*, 126.

Herbst, B.; Wiesmann, U. **1996**, Kinetics and reaction engineering aspects of the biodegradation of dichloromethane and dichloroethane, *Water Res.* 30, 1069–1076.

Hu, L.Z.; Shieh, W.K. **1987**, Anoxic biofilm degradation of monocyclic aromatic compounds, *Biotechnol. Bioeng.* 30, 1077–1083.

Jakobezyk, J.; Kwiatkowski, M.; Ostrowska, J. **1984**, Untersuchung der biologischen Zersetzung aromatischer Nitroverbindungen in Abwässern, *Chem. Tech.* 36, 112–116.

Janssen, D.B.; Scheper, A.; Dijkhizen, L.; Witholt, B. **1985**, Degradation of halo-organic compounds by *Xanthobacter autotrophicus* GJ10, *Appl. Environ. Microbiol.* 49, 673–677.

Jensen, H.L.; Lautrup-Larsen, G. **1967**, Microorganisms that decompose nitro-aromatic compounds, with special reference to dinitro-ortho-cresol, *Acta Agric. Scand.* 17, 115–126.

Jongen, W.; Alink, G.; Koeman, J. **1978**, Mutagenic effect of dichloromethane on *Salmonella typhimurium*, *Mutat. Res.* 56, 245–248.

Kästner, M. **1989**, Biodegradation of volatile chlorinated hydrocarbons, in: *DECHEMA Biotechnology Conferences 3B*, DECHEMA, Frankfurt, p. 909–912.

Khandker, S.A.K. **1992**, *Mikrobieller Abbau von aromatischen und chloraromatischen Verbindungen unter aeroben und denitrifizierenden Bedingungen*, PhD thesis, Technical University Berlin.

Komatsu, T.; Omori, T.; Kodama, T. **1993**, Microbial degradation of the polycyclic aromatic hydrocarbons acenaphthene and acenaphthylene by a pure bacterial culture, *Biosci. Biotechnol. Biochem.* 54, 864–865.

Krull, R.; Hempel, D.C. **1993**, Biodegradation of naphthalene-sulphonic acid-containing sewages in a two-stage treatment plant, *Bioprocess. Eng.* 10, 229–234.

Krull, R.M.; Hemmi, P.O., Hempel, D.C. **1998**, Combined biological and chemical treatment of a highly concentrated residual dyehouse liquors, *Water Sci. Technol.* 36, 339–346.

Kulla, H.G.; Krieg, R.; Zimmerman, T.; Leisinger, T. **1984**, Experimental evolution of azo dye-degrading bacteria, in: *Current Perspectives in Microbial Ecology*, ed. Klug, M.J.; Reddy, C.A., American Society of Microbiology, Washington, D.C., p. 663–667.

Leisinger, T. **1988**, Mikrobieller Abbau problematischer Komponenten in Abluft, in: *Biosensors and Environmental Biotechnology* (Biotech. Ser. Vol. 2), ed. Hollenberg, C.; Sahm, H., Gustav Fischer, Stuttgart, p. 125–133.

Libra, J.A.; Borchert, M.; Vigelahn, L.; Storm, T. **2004**, Two stage biological treatment of a diazo reactive textile dye and the fate of the dye metabolites, *Chemosphere* 56, 167–180.

Loew, G.; Regbagatti, M.; Poulsen, M. **1984**, Metabolism and relative carcinogenic potency of chloroethanes: a quantum chemical structure–activity study, in: *Cancer Biochemistry and Biophysics*, Vol. 7, Gorden and Breach, New York, p. 109–132.

Lonjaret, J.-P. **1996**, *Entwicklung und mathematische Modellierung eines Biotropfkörperfilters für den biologischen Abbau von Dichlormethan in Abluft*, Wissenschaft und Technik Verlag, Berlin.

Mackay, D.; Shiu, W.J. **1981**, A critical review of Henry's law constants for chemical environmental interest, *J. Phys. Chem. Ref. Data* 10, 1175–1199.

Meyer, U.; Overney, G.; Wattenwyl, A. **1979**, Über die biologische Abbaubarkeit von Azofarbstoffen, *Textilveredelung* 14, 15–20.

Mohey El-Dein, A.A. **2002**, Oxidation kinetics of the azo dye Reactive Black 5 using H_2O_2/UV and aerobic biological treatment (PhD thesis), *VDI-Forschungsberichte, Reihe 3*, 762.

Niemann, D. **1993**, *Biologische Abluftreinigung mit Biofilm-Wirbelschicht-Reaktoren*, PhD thesis, ETH Zurich.

Nörtemann, B.; Hempel, D.C. **1991**, Application of adapted bacterial cultures for the degradation of xenobiotic compounds in industrial wastewaters, in: *Biological Degradation of Wastes*, ed. Martin, A.M., Elsevier Applied Science, London, p. 261–279.

Nörtemann, B.; Baumgarten, J.; Rast, H.G.; Knackmuss, H.-J. **1986**, Bacterial communities degrading amino- and hydroxynaphthalenesulphonates, *Appl. Environ. Microbiol.* 52, 1195–1202.

Nowak, J. **1994**, *Umsatz von Chlorbenzolen durch methanogene Mischkulturen aus Saale-Sediment in Batch- und Reaktorversuchen*, PhD thesis (Berichte zur Siedlungswasserwirtschaft Nr 4), Technical University Berlin.

Ollikka, P.; Alhonmäki, K.; Leppänen, V.; Glumoff, T.; Raijola, T.; Suominen, I. **1993**, Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol.* 59, 4010–4016.

Ou, L.T.; Sharma, A. **1989**, Degradation of methyl parathion by a mixed bacterial culture and a *Bacillus* sp. isolated from different soils, *J. Agric. Food Chem.* 37, 1514–1518.

Pagga, U.; Haltrich, W.G.; Günthner, W. **1982**, Untersuchungen über die Wirkung von 4-Nitrophenol auf Belebtschlamm, *Vom Wasser* 59, 51–65.

Patterson, J.W. **1985**, *Industrial Wastewater Treatment Technology*, Butterworth, Boston, Mass., p. 305–306.

Prieger-Kraft, A. **1995**, *Mikrobieller Abbau von 1,2,3,4- und 1,2,3,5-Tetrachlorbenzol*, PhD thesis, Technical University Berlin.

Rafii, F.; Franklin, W.; Cerniglia, C.E. **1990**, Azoreductase activity of anaerobic bacteria isolated from human intestinal microflora, *Appl. Environ. Microbiol.* 56, 2146–2151.

Ramanand, K.; Balba, M.T.; Duffy, J. **1993**, Reductive dehalogenation of chlorinated benzenes and toluenes under methanogenic conditions, *Appl. Environ. Microbiol.* 59, 3266–3272.

Rannug, U. **1980**, Genotoxic effects of 1,2-dibromomethane and 1,2-dichloroethane, *Mutat. Res.* 76, 269–295.

Rapp, T. **2005**, Untersuchungen zur biologischen Behandlung und Ozonisierung eines synthetischen Textilabwassers (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 53.

Rapp, T.; Wiesmann, U. **2003**, Biologisch-chemische Verfahrenskombination zur Reinigung von Mischabwässern der Textilveredlung, *Chem. Ing. Tech.* 75, 453–457.

Reineke, W.; Knackmuss, H.-J. **1984**, Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene-degrading bacterium, *Appl. Environ. Microbiol.* 47, 395–402.

Rippen, G. **1991**, *Handbuch Umweltchemikalien: Stoffdaten, Prüfverfahren, Vorschriften, Landsberg/Lech*, Ecomed, Berlin.

Rudek, M. **1992**, *Mikrobieller Abbau von 1,2-Dichlorethan*, PhD thesis, Technical University Berlin.

Sallis, P.; Armfield, S.; Bull, A.; Hardman, D. **1990**, Isolation and characterization of a haloalkane halidohydrolase from *Rhodococcus erythropolis* Y2, *J. Gen. Microbiol.* 136, 115–120.

Sander, P.; Wittich, R.M.; Fortnagel, P.; Wilkey, H.; Franke, W. **1991**, Degradation of 1,2,4-trichloro- and 1,2,4,5-tetrachlorobenzene by *Pseudomonas* sp. strains, *Appl. Environ. Microbiol.* 57, 1420–1440.

Schäfer, M. **1994**, *Untersuchungen zum Abbau chlorierter Benzole durch Mischkulturen in kontinuierlich betriebenen Reaktoren*, PhD thesis, Technical University Berlin.

Schmidt, S.K.; Scow, K.M.; Alexander, M. **1987**, Kinetics of p-nitrophenol mineralization by a *Pseudomonas* sp.: effects of second substrates, *Appl. Environ. Michrobiol.* 53, 2617–2623.

Schönberger, H.: Zur Abwässerfrage der Textilveredlungsindustrie (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 196.

Seweko, U. **1988**, Naturfarbstoffe – eine Alternative zu synthetischen Farbstoffen, *Melliand Textilberichte* 4, 271–276.

Siddique, M.H.; St. Pierre, C.C.; Biswas, N.; Bewtra, J.K.; Taylor, K.E. **1993**, Immobiliz-

ed enzyme-catalyzed removal of 4-chlorophenol from aqueous solution, *Water Res.* 27, 883–890.

Socher, R. **1997**, personal communication, BASF Schwarzheide.

Soewondo, P. **1997**, *Zweistufige anaerobe und aerobe biologische Behandlung von synthetischem Abwasser mit dem Azofarbstoff C.I. Reactive Orange 96*, PhD thesis, Technical University Berlin.

Sosath, F. **1999**, Biologisch-chemische Behandlung von Abwässern der Textilveredelung mit Reaktivfarbstoffen (PhD thesis), *VDI-Forschungsberichte, Reihe 15*, 215.

Sosath, F.; Libra, J.A. **1997**, Biologische Behandlung von synthetischen Abwässern mit Azofarbstoffen, *Acta Hydrochim. Hydrobiol.* 25, 259–264.

Spadaro, J.T.; Gold, M.H.; Renganathan, V. **1992**, Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol.* 58, 2397–2401.

Speitel, G.E. Jr; Luc, C.-J.; Turakhia, M.; Zhu, X.-J. **1989**, Biodegradation of trace concentrations of substituted phenols in granulated activated carbon columns, *Environ. Sci. Technol.* 23, 68–74.

Springfellow, W.T.; Aitken, M.D. **1995**, Competitive metabolism of naphthalene, methylnaphthalene, and fluoriene by phenanthrene-degrading *Pseudomonas*, *Appl. Environ. Microbiol.* 61, 357–362.

Stucki, G.; Thüer, M.; Bentz, R. **1992**, Biological degradation of 1,2-dichloroethane under groundwater conditions, *Water Res.* 26, 273–278.

Thiem, K.-W.; Booth, G. **1991**, Nitro compounds, aromatic, in: *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A17, 5th edn, VCH, Weinheim.

Van der Meer, J.R.; Roelofsen, W.; Schraa, G.; Zehnder, A.J.B. **1987**, Degradation of low concentrations of dichlorobenzenes and 1,2,4-trichlorobenzene by *Pseudomonas* sp. strain P51 in non-sterile soil columns, *FEMS Microbiol.* 27, 1538–1542.

WHO **1987**, *Methylene Chloride Health and Safety Guide, Environmental Health Criteria, No. 32: Methylene Chloride IPCS (International Program on Chemical Safety)*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, p. 42.

Wiesmann, U. **2002**, Versuche zur Entfärbung und Mineralisierung des Azo-Farbstoffs C.I.Reactive Black 5, *GWF Wasser Abwasser* 134, 329–335.

Wiesmann, U.; Libra, J.A. **1999**, Special aerobic wastewater and sludge treatment processes, *Biotechnology* 11a, 373–415.

Winkelbauer, W.; Kohler, H. **1989**, Biologischer Abbau von leichtflüchtigen Chlorkohlenwasserstoffen im offenen System am Beispiel von Dichlormethan in einem Tauchtropfkörper, *GWF Wasser Abwasser* 130, 13–20.

Wodzinski, R.; Johnson, M. **1968**, Yields of bacterial cells from hydrocarbons, *Appl. Microbiol.* 16, 1886–1891.

Yoo, E.S.; Libra, J.A.; Wiesmann, U. **2000**, Reduction of azo dyes by *Desulfovibrio desulfuricans*, *Water Sci. Technol.* 41, 15–22.

Zhang, X.; Young, L.Y. **1997**, Carboxylation as an initial reaction in the anaerobic metabolism of naphtaline and phenanthrene by sulfidogenic consortia, *Appl. Environ. Microbiol.* 63, 4759–4764.

Zimmermann, T.; Kulla, H.G.; Leisinger, T. **1982**, Properties of purified orange II azoreductase, the enzyme initiation of azo dye degradation by *Pseudomonas* KF46, *Eur. J. Biochem.* 129, 197–203.