Oxidative Transformations of Contaminants in Natural and in Technical Systems

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Abstract. In this paper, we present case studies of oxidative transformations of contaminants by oxidants which are relevant in natural and in technical systems. These oxidants are reactive oxygen species (ROS), manganese(III,IV), iron(III), and molecular dioxygen (O_2). Regarding ROS, we discuss *i*) their concentrations and their efficiencies as oxidants in natural waters and in water treatment, *ii*) reactions occurring in bromide-containing waters in the presence of ROS, *iii*) role of iron in the formation of ROS, and *iv*) quantitative structure-activity relationships (QSAR) of reactions of ROS with contaminants. Concerning Mn^{III} and Mn^{IV} as oxidants, we present experimental studies on the oxidation of anilines by δ-MnO_2. With respect to oxidative transformation of the hydrophilic organic contaminants ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA), we show that these organic complexing agents are efficiently oxidized by Fe^{III} and O_2, respectively, if catalyzed by light (for the oxidation of EDTA by Fe^{III}) and by enzymes (for the oxidation of NTA by O_2).

Introduction

In oxic environmental and in technical systems, several oxidants play pivotal roles in the oxidative transformations of contaminants: molecular dioxygen in its electronic ground state (O_2) and reactive oxygen species (ROS), particularly the hydroxyl radical (HO·), carbonate radicals (HCO_3·/CO_3·⁻), singlet oxygen ($^1O_2$), the hydroperoxyl/superoxide radicals (HO_2·/O_2·⁻), hydrogen peroxide (H_2O_2), and ozone (O_3). In addition, also iron(III) and manganese(III,IV) are key players in en-

vironmental oxidative transformation processes.

Although O_2 is the ultimate electron acceptor for many oxidative transformations in environmental systems, O_2 in its electronic ground state, which is a triplet state, is a poor oxidant on its own, both from a thermodynamic (*Table 1*) and kinetic point of view. Therefore, oxidative transformations of contaminants by O_2 are only effective in the presence of biotic or abiotic catalysts, *i.e.*, microorganisms or photocatalysts, respectively. Unlike O_2, ROS may be efficient oxidants also in the

absence of catalysts, where HO· is the strongest oxidant among the ROS (*Table 1*). In aquatic systems, ROS are formed in photochemical and subsequent thermal reactions involving natural dissolved organic matter and other water constituents, *e.g.*, nitrate, as well as transition metals, in particular iron. In technical systems, reactive oxygen species (H_2O_2, O_3) are added during water treatment and may be transformed into the more reactive HO· during the process.

Regarding Fe^{III}, Mn^{III}, and Mn^{IV} as oxidants, the following two issues are of particular importance: *i*) Under oxic conditions, the thermodynamically stable forms of iron and manganese are amorphous and crystalline Fe^{III}-, Mn^{III}-, and Mn^{IV}-(hydr)oxides (where the notation (hydr)oxides includes oxides and hydroxides). Therefore, when considering Fe^{III}, Mn^{III}, and Mn^{IV} as oxidants, surface reactions play pivotal roles. *ii*) The speciation of these metals determines their standard reduction potential and thus their efficiency as oxidants. In environmental systems, Fe^{III} acts usually as an oxidant only if it is supplied with additional free energy, in particular in the form of near UV light. Thereby, ligand-to-metal charge-transfer transitions play crucial roles.

In this paper, we present our studies on oxidative transformations of contaminants with ROS, Mn^{IV}, Fe^{III}, and O_2 as oxidants involving thermal, photochemical, and microbial processes. These studies were carried out with well-defined laboratory models that are relevant for environmental and technical systems, and were aimed to identify the most important factors which determine the kinetics of oxidative transformations of contaminants in complex environmental systems (see also *Wehrli* and *Schwarzenbach* [3], this issue). In particular, we want to illustrate *i*) the role of oxidative transformations in water treatment, *ii*) the role of light and of microorganisms in oxidative transformations of contaminants, and *iii*) the role of surfaces in these processes.

Table 1. *One-Electron Standard Reduction Potentials of Some Environmentally and Technically Important Oxidants*

Oxidant	Reaction			E^0 [V] *vs.* NHE
HO·	HO· + e⁻	⇌	OH⁻	+1.90[a])
HCO_3·/CO_3·⁻	CO_3·⁻ + e⁻	⇌	CO_3^{2-}	+1.59[b])
ClO_2	ClO_2 + e⁻	⇌	ClO_2^-	+0.93[a])
$^1O_2$	$^1O_2$ + e⁻	⇌	O_2·⁻	+0.83[a])
HO_2·/O_2·⁻	HO_2· + e⁻	⇌	HO_2^-	+0.75[a])
O_2	O_2 + e⁻	⇌	O_2·⁻	–0.16[a])

[a]) *Stanbury* [1], and references therein.
[b]) *Huie et al.* [2].

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Reactive Oxygen Species as Oxidants

As pointed out in the introduction, reactive oxygen species (ROS) play crucial roles as oxidants of contaminants both in environmental and in technical systems. In order to determine half-lives of pollutants due to oxidative transformation by ROS, steady-state concentrations of ROS have to be assessed. This can be done by three different methods: *i*) by direct measurement, *e.g.*, [4], *ii*) by indirect methods using probe molecules with known second-order rate constants of reaction with a given ROS, *e.g.*, [5][6], and *iii*) by estimating production and consumption rates of a given ROS in a considered system.

Concentrations of Reactive Oxygen Species in Natural Waters and in Water Treatment

Fig. 1 shows the relevant ROS and their approximate concentrations in different aqueous phase compartments. The technical systems shown in *Fig. 1* refer to drinking-water treatment through ozonation and by advanced oxidation processes. For sunlit surface waters (top layer, < 1 cm) and for cloud waters, the concentrations are given for summer noon conditions. The concentrations of the various ROS in the various compartments shown in *Fig. 1* span over a range of *ca.* 13 orders of magnitude. The highest concentrations usually occur in technical systems, whereas in surface and cloud waters, ROS concentrations are much lower, but frequently in a similar range for both compartments. The relatively wide range of the ROS steady-state concentrations in a given environmental compartment stems mostly from the varying water matrix.

From measured second-order rate constants of ROS reactions [7][8], pseudo-first-order rate constants that include the ROS concentrations, k' [s^{-1}], can be calculated. The numerical values are shown in the three boxes to the right of the concentration ranges in *Fig. 1* for three selected model micropollutants, atrazine (top), phenol (middle), and trichloroethene (TRI, bottom), and for the upper and lower concentration limits of the corresponding ROS. As illustrated in *Fig. 1*, these rate constants can cover a range of more than 10 orders of magnitude in different compartments. A comparison of pseudo-first-order rate constants for different oxidants within the same compartment (drinking water, surface water, cloud water) allows to estimate the main oxidation pathway for a particular micropollutant. For example, atrazine is oxidized mainly by HO$^\cdot$ or carbonate radicals during drinking-water
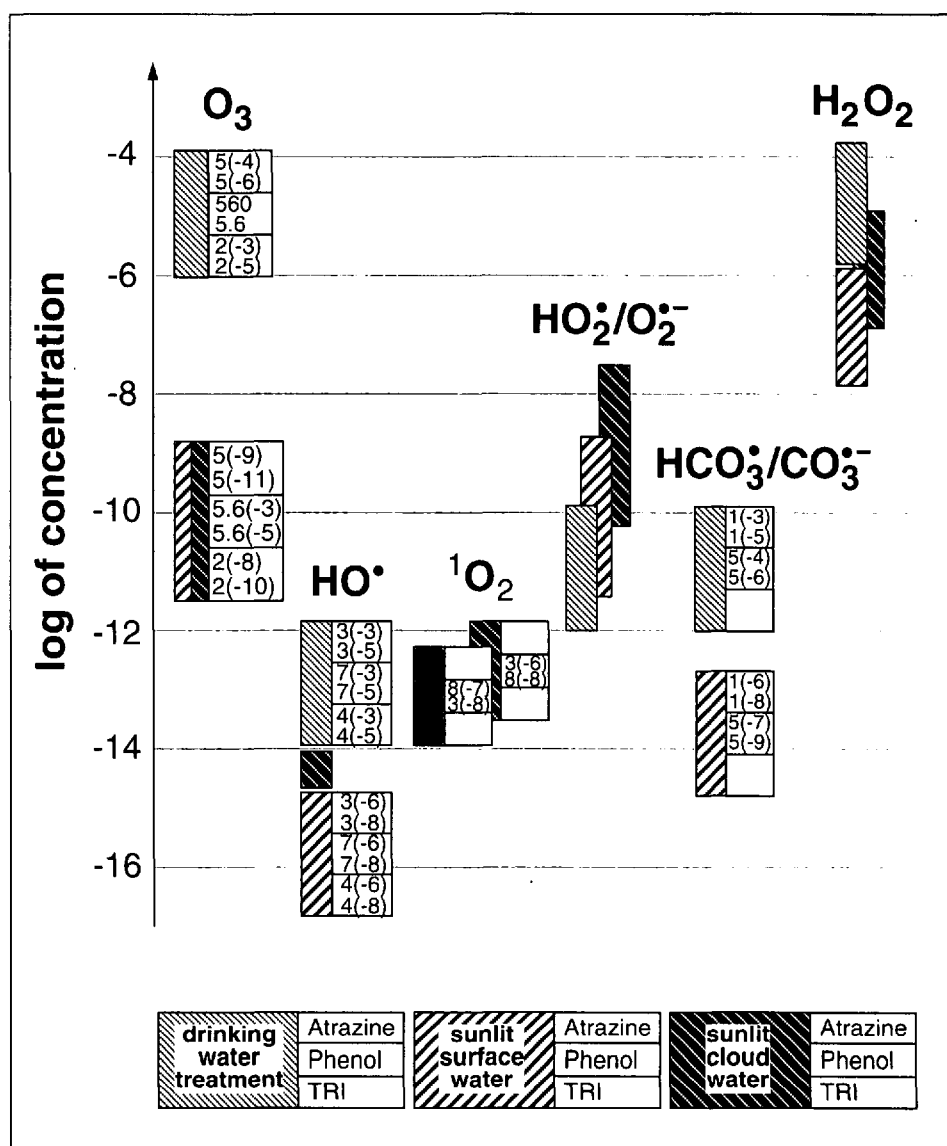

Fig. 1. *Ranges of steady-state concentrations of reactive oxygen species (ROS) in drinking water, and in sunlit surface and cloud waters.* The values in the boxes to the right of the concentration ranges are pseudo-first-order rate constants (in s^{-1}) of oxidation of three micropollutants (atrazine, phenol, and trichloroethene (TRI)) for the upper and lower concentration limits of the corresponding ROS. (Numbers in parantheses are power of 10.)

ozonation, whereas ozone is the main oxidant for phenols. In sunlit cloud and surface waters, the same qualitative pattern is observed, although the rate constants are 3–5 orders of magnitude smaller with a corresponding increase of the half-lives, $t_{1/2}$ (where $t_{1/2} = \ln 2/k'$).

Reactive Oxygen Species Related to Water Treatment

The demand for enhanced treatment of drinking waters and wastewaters leads to an increasing application of Advanced Oxidation Processes (AOP). The common goal of all AOPs currently applied in water treatment practice is the production of high concentrations of HO$^\cdot$ to guarantee the oxidation of micropollutants which are only slowly oxidized by O$_2$ and ROS such as O$_3$ and H$_2$O$_2$. In current water treatment practice, the most frequently

applied AOPs are combined processes such as H$_2$O$_2$/UV, O$_3$/UV, and O$_3$/H$_2$O$_2$. The common feature of these three processes is the presence of H$_2$O$_2$ in combination with HO$^\cdot$ and other oxidants.

For bromide-containing drinking waters, these processes have to be carefully evaluated because oxidation of bromide may eventually lead to bromate which is highly undesired due to its potential carcinogenicity. In the 1990s, strict drinking water standards have therefore been set. The reaction mechanism for bromate formation during H$_2$O$_2$-based oxidative processes, as suggested from our work, is shown in *Scheme 1*: HO$^\cdot$ and/or O$_3$ can oxidize bromide to hypobromous acid (HOBr) or hypobromite (OBr$^-$) [9–11]. HOBr/OBr$^-$ is the critical reaction intermediate from where several reaction pathways are possible: *i*) reaction with organic

matter and formation of brominated organic compounds, *ii*) further oxidation of HOBr/OBr⁻ to bromate, and *iii*) reduction of HOBr/OBr⁻ with H_2O_2 to bromide [12]. Therefore, a competition between addition, oxidation, and reduction of HOBr/OBr⁻ can occur during H_2O_2-based AOPs. The rate of reduction becomes decisive for the amount of bromate and brominated organic compounds that can be formed. The processes depicted in *Scheme 1* are encountered in H_2O_2-based AOPs in engineered systems (*e.g.*, drinking-water treat-

ment, marine aquaria, bleaching processes, and cooling water) as well as in natural systems (*e.g.*, cloud waters and surface waters).

## Roles of Iron in the Formation of Reactive Oxygen Species

In iron-rich environmental and technical systems, iron plays significant roles in catalyzing ROS production. For example, the iron-catalyzed production of ROS has been shown to be of key importance for the oxidation of contaminants in atmospheric

waters (see, *e.g.*, [13][14]). We have studied the various processes that result in formation (and consumption) of ROS and that are coupled to the light-induced redox cycling of iron under oxidation of an electron donor in heterogeneous, acidic experimental systems, which are representative for acidic surface waters (*e.g.*, acidic rivers and lakes) (*Scheme 2*) [15]. As electron donor, we used Suwannee River Fulvic Acid (SRFA) as a model compound for natural dissolved organic matter. The process which initiates iron redox cycling in these heterogeneous systems is photoreduction of Fe[III] on the surface of an Fe[III]-(hydr)oxide under oxidation of the adsorbed electron donor, and transfer of part of reduced surface iron into solution. The following issues are of particular interest with regard to formation and consumption of ROS in these experimental systems: *i*) At both pH 3 and 5, a considerable fraction of reduced surface iron is reoxidized as a surface species resulting in ROS formation on the surface of the solid phase. *ii*) In these acidic experimental systems, molecular dioxygen does not play a significant role as an oxidant of dissolved Fe[II] but is a key player in the formation of HO·/O_2·⁻. *iii*) Fulvic acid enhances the rate of Fe[II] oxidation by H_2O_2 (the Fenton reaction) and is an effective scavenger of HO· produced thereby, resulting in recycling of HO_2·/O_2·⁻ [16].

Scheme 1. *Bromine Reactions During Oxidation Processes (O_3/H_2O_2) in Drinking-Water Treatment.* The major sinks are bromate and bromo-organic compounds.
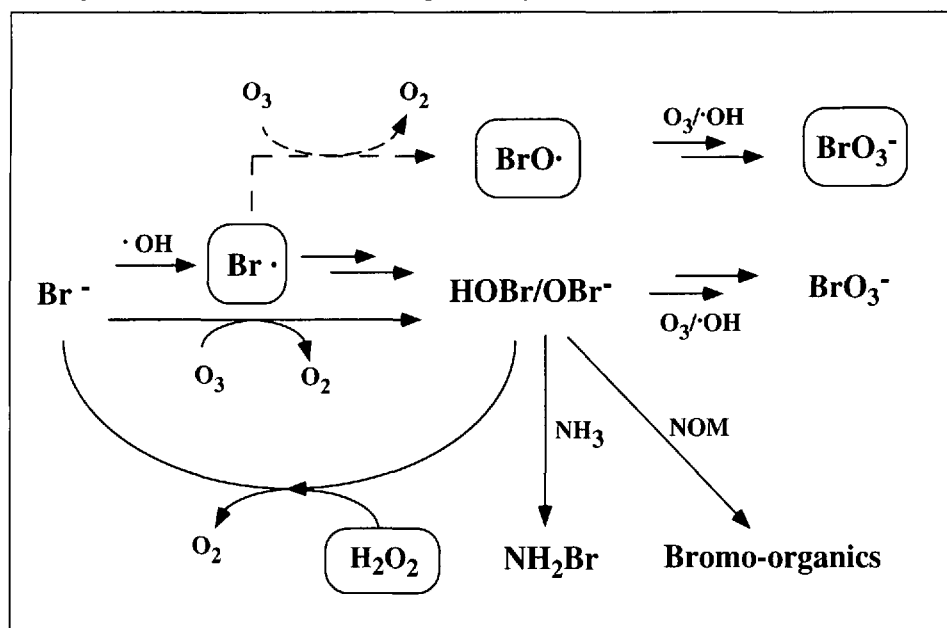
Scheme 2. *Iron-Catalyzed Production and Consumption of Reactive Oxygen Species* (modified from *Voelker* and *Sulzberger* [15]). The numbers in italic indicate the amount of iron (in μM) oxidized or reduced by each of the depicted processes after 300 minutes illumination of aerated aqueous suspensions containing 40 μM of iron in the form of initially lepidocrocite (γ-FeOOH) and 10 mgl⁻¹ Suwannee River Fulvic Acid (SRFA) at pH 3 (bold numbers) and at pH 5 (numbers below). These relative rates were assessed by measuring net formation of dissolved Fe[II], total dissolved iron, and H_2O_2, and by simulating the data with kinetic modeling [15][16].
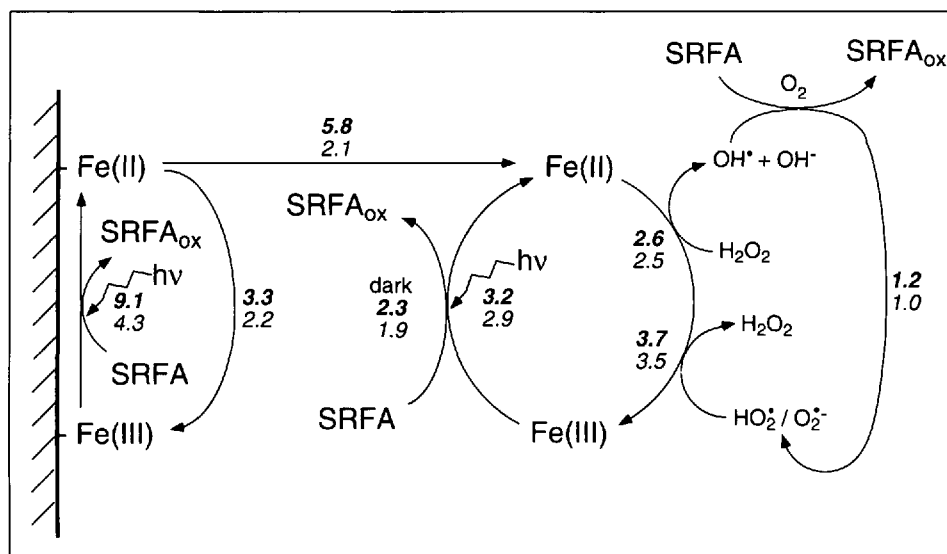
Quantitative Structure-Activity Relationships (QSAR) of Reactions of Reactive Oxygen Species with Contaminants

As pointed out in the first paragraph of this section, we need to know both second-order rate constants of ROS reactions with contaminants and ROS steady-state concentrations in order to determine half-lives of contaminants that are subject to oxidative transformations by ROS. The reactivity of different ROS varies a lot. For example, hydroxyl radicals react unselectively with the majority of organic contaminants as well as with natural dissolved organic matter with rate constants that are limited by diffusion within two orders of magnitude. Unlike HO·, O_3 and $^1O_2$ are very selective oxidants that can, *e.g.*, add to C=C double bonds to form cyclic compounds that are then further degraded. Also the reactivity of contaminants with a given ROS can vary significantly. Because of the large number of organic chemicals in daily use (*ca.* 100 000, see also *Wehrli* and *Schwarzenbach* [3], this issue), it is impossible to determine second-order rate constants for reactions
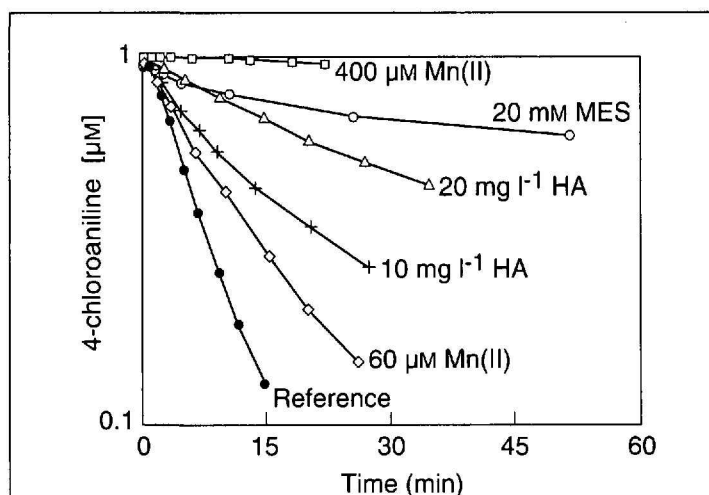
Fig. 2. *Effect of added co-solutes on the oxidation rate of 4-chloroaniline in stirred δ-MnO_2 suspensions (0.55 g l^{-1}) at pH 4.8 (25°)* (modified from *Klausen et al.* [27]). HA = Aldrich humic acid; MES = 2-(*N*-morpholino)ethane sulfonic acid. 'Reference' refers to 4-chloroaniline degradation without added co-solutes.
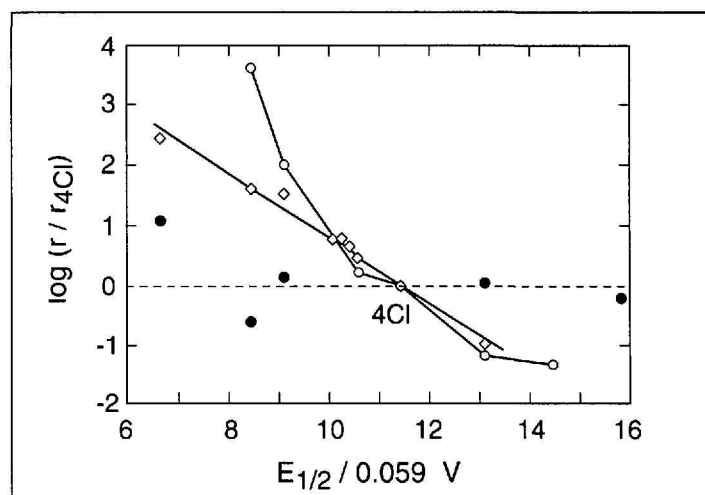
Fig. 3. *Rates of oxidation of a series of monosubstituted anilines as a function of their half-wave potential, relative to the rates of 4-chloroaniline oxidation, r_{4Cl}, in the presence of δ-MnO_2 (25°)* (modified from *Klausen et al.* [27]). ($\Diamond$) Initial rates obtained from batch experiments with 60 nmol aniline per m^2 δ-MnO_2, without added Mn^{II} (pH 6.4); (O) quasi-steady-state rates obtained from mixed-flow reactor experiments with 12 nmol aniline per m^2 δ-MnO_2, and with added Mn^{II} (0.5 mM) at pH 4.9, and ($\bullet$) quasi-steady-state rates obtained from mixed-flow reactor experiments with 12 nmol aniline per m^2 δ-MnO_2, and with added Mn^{II} (5 mM) at pH 6.4.

of ROS with these chemicals individually with experimental methods. Instead, it is desirable to develop predictive tools that allow to estimate rate constants with the help of quantitative structure-activity relationships (QSAR), which can save a lot of time and resources. One of the features common to many ROS and other oxidants is that they can undergo one-electron reduction, thereby initiating the oxidation of contaminants. Therefore, the one-electron standard reduction potential of contaminants is a major factor in determining their rate constants of oxidation by a given ROS. An example of such a QSAR is the oxidation of phenoxide ions by $^1O_2$, where the one-electron standard reduction potential was used as the predictor for the rate constants [17]. A linear free-energy relationship (LFER) was used in this case. A more general type of QSAR for one-electron transfer reactions is the *Marcus* relationship (see, *e.g.*, [18]). It was used successfully to model rate constants of various substituted phenoxide ions reacting with ClO_2 [19]. Our QSAR studies suggest that the *Marcus* relationship may be generally useful for quantifying photosensitized oxidative transformation processes in natural waters [20]. An important difference between LFER and *Marcus* relationship is that only the latter can be used to model rate constants which approach diffusion limit. Therefore, *Marcus* relationships are better suited for studying the reactivity of oxidants with relatively high reduction potentials, *e.g.*, the carbonate radical ($CO_3^{\cdot-}$) (see *Table 1*).

Manganese(III,IV) as Oxidant

Colloidal Mn^{III}- and Mn^{IV}-(hydr)oxide phases may be important oxidants of organic pollutants, particularly if reactive surface sites are continuously regenerated, *e.g.*, *via* microbially catalyzed reoxidation of Mn^{II} and subsequent precipitation. In this section, we discuss the factors that are likely to control the kinetics of abiotic oxidation of an important type of organic pollutants, namely aromatic amines, by δ-MnO_2 under environmental conditions. Aromatic amines (anilines) are often formed by reductive transformations of the corresponding nitroaromatic compounds (*e.g.*, explosives or pesticides) under reducing conditions, where Fe^{II} is the reductant [21] (see also *Schwarzenbach et al.* [22], this issue). Under these environmental conditions, *e.g.*, under reducing subsurface conditions, many aromatic amines are persistent, since anaerobic biodegradation of these compounds is not effective. However, they can be transported in the aqueous phase (*e.g.*, *via* groundwater streams) to more oxic environments where they can undergo oxidative transformation with Mn^{III}- and Mn^{IV}-(hydr)oxides acting as oxidant. Thereby a variety of products is formed, some of which are, unfortunately, of even greater (eco)toxicological concern than the reactants. (Note, that in these environments, sunlight is not a factor, and hence, ROS are not involved in oxidative transformations of these compounds.)

Previous work [23][24] has shown that the kinetics of oxidation of organic compounds such as phenols and anilines by Mn^{III}- and Mn^{IV}-(hydr)oxides depends, like most surface reactions [25], on pH [26], on the concentration of reactive surface sites as well as on the *Gibbs* free energy of the one-electron transfer from the adsorbed electron donor to the surface metal. In the case of oxidative transformation of anilines by δ-MnO_2, the polarographic half-wave potential, $E_{1/2}$, of anilines ($ArNH_2$) has proven to be a useful predictor for relative rate constants of these oxidative transformations [27]:

$$ArNH_2 \xrightarrow{E_{1/2}} ArNH^{\cdot} + e^- + H^+ \rightarrow Products \qquad (1)$$
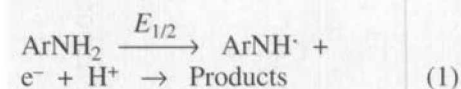
The availability of reactive surface sites for adsorption of anilines can be significantly decreased by co-solutes, in particular by Mn^{II} which is formed during reductive dissolution of δ-MnO_2 and which can get re-adsorbed on the surface of δ-MnO_2, where the extent of adsorption increases with increasing pH. *Fig. 2* shows the effects of added Mn^{II}, 2-(*N*-morpholino)ethanesulfonic acid (MES), and *Aldrich* humic acid (HA) on the rate of 4-chloroaniline oxidation by δ-MnO_2 [27]. HA was used as a model compound for dissolved natural organic matter. With increasing concentrations of the co-solutes the rate of 4-chloroaniline oxidation decreased. The decrease in the rate of 4-chloroaniline oxidation by MES and HA, as compared to that in the absence of co-

solutes, can in part be explained by reductive dissolution of δ-MnO$_2$ also by MES and HA, and re-adsorption of Mn^{II} thus blocking reactive surface sites for the adsorption of 4-chloroaniline.

Rates of surface reactions often decrease with time due to loss of reactive surface sites. A loss of reactive surface sites can have several causes: i) product accumulation on the surface (*e.g.*, re-adsorption of Mn^{II} formed upon reductive dissolution of Mn^{III}- and Mn^{IV}-(hydr)-oxides), ii) 'aging', and iii) dissolution of the solid phase. Due to aging of solids (*i.e.*, transformation into thermodynamically more stable phases), initial rates are often higher than quasi-steady-state rates. We have demonstrated the effects of aging and of blocking surface sites by Mn^{II} on

the oxidation of substituted anilines in the presence of suspended δ-MnO$_2$, using batch reactors as well as mixed flow-through reactors which were operated under quasi-steady-state (qss) conditions [27][28]. The effect of added Mn^{II} on both initial and qss oxidation rates of a particular aniline was essentially the same, *i.e.*, rates decreased with increasing Mn^{II} concentrations, although qss rates were generally substantially smaller than initial rates [27]. Furthermore, also with respect to relative oxidation rates of different anilines, the concentration of Mn^{II} had a significant effect, such that at high surface coverage with Mn^{II} (pH 6.4), the polarographic half-wave potential of the anilines, $E_{1/2}$, could no longer be used as a descriptor for the relative reactivity of the
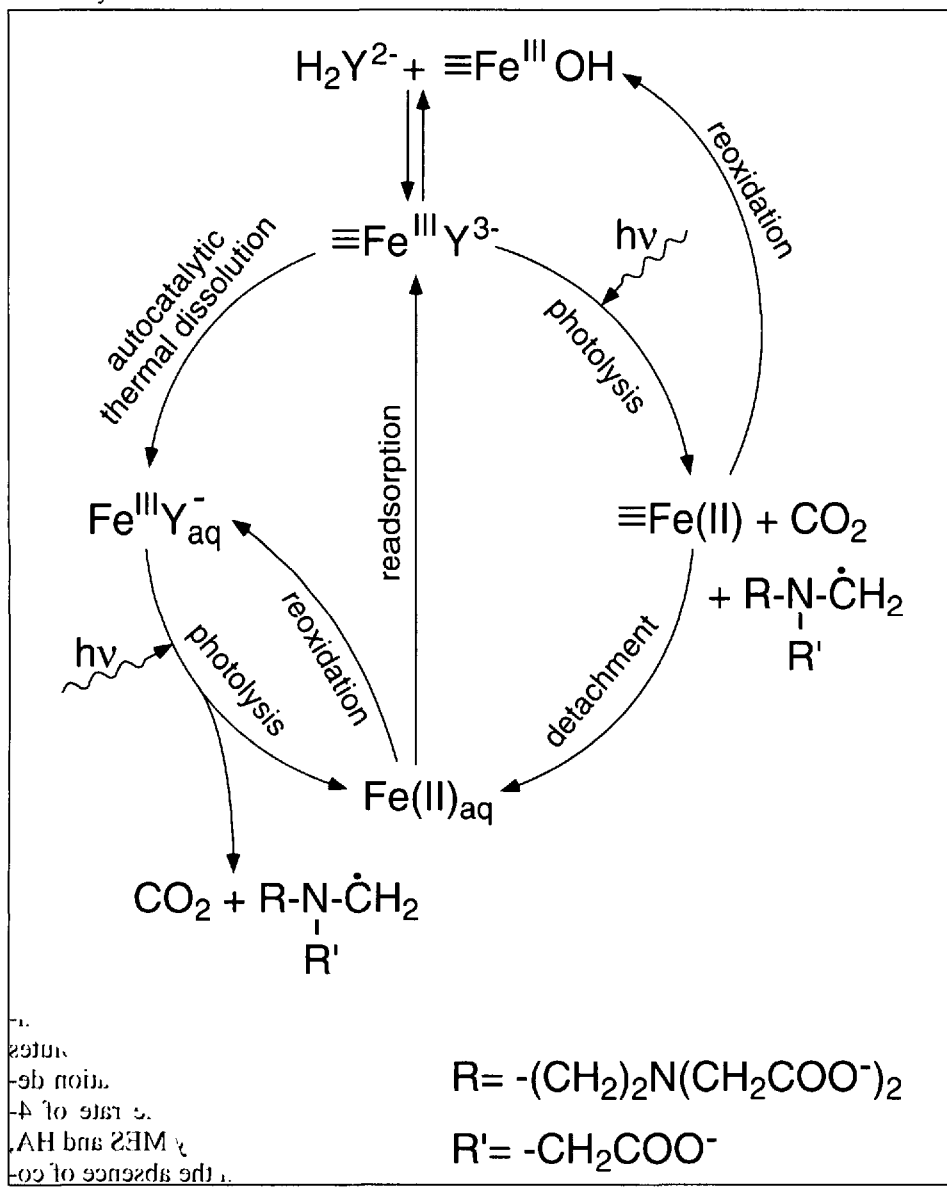
different compounds (*Fig. 3*). It appears that in this case, the availability of reactive surface sites was controlling the rates and not $E_{1/2}$.

Iron(III) as Oxidant

Many hydrophilic organic contaminants form complexes with Fe^{III}, and these complexes are often subject to photolysis. A typical case of such a pollutant is ethylenediaminetetraacetate (EDTA). (It should be mentioned that EDTA on its own does not absorb solar light in the spectral region that reaches the Earth' surface, whereas the Fe^{III}EDTA complex does.) In the following, we present experimental studies on the photooxidation of EDTA with Fe^{III} as oxidant both in homogeneous and in heterogeneous experimental systems.

EDTA is a powerful chelating agent being widely used for the complexation of metal ions interfering with industrial processes, *e.g.*, photographic developing, paper production, and textile dyeing. In food and cosmetics production it is used as iron-complexing agent to prevent goods from being spoiled by microorganisms. Although EDTA-degrading bacteria have been isolated recently [29], biodegradation does not occur or is slow in sewage treatment plants. Therefore, considerable EDTA concentrations have been found in sewage effluents and in river waters [30]. Studies on the fate of EDTA in the River Glatt have shown that a significant fraction of EDTA leaves sewage treatment plants located along this river as the Fe^{III}EDTA complex [30]. In order to predict photolysis rate coefficients and thus half-lives of EDTA due to photolysis of Fe^{III}EDTA in the River Glatt, quantum yields of Fe^{III}EDTA photolysis as a function of wavelength and pH were determined [31]. Although the speciation of Fe^{III}EDTA depends on pH, the quantum yield of Fe^{III}EDTA photolysis was found to be largely pH-independent in the pH range between 4–10, but decreased with increasing irradiation wavelength (between 313–405 nm). Using quantum yields and absorption coefficients determined in the laboratory, we estimated half-lives of EDTA in the River Glatt of *ca.* 30 min on a clear summer day, and of *ca.* 4 h on a clear winter day (using 24 h-averaged light intensities and not taking into account shading by water plants) [31]. These estimates were made with help of tabulated photon fluxes [32] for the geographical conditions of the River Glatt and with measured attenuation coefficients of this river. We were also able to simulate EDTA

Scheme 3. *Conceptual Model of Light-Induced Oxidation of Initially Uncomplexed EDTA in the Presence of a Colloidal Fe^{III}-(hydr)oxide Phase* (modified from *Karametaxas et al.,* [36]). The symbol Y^{4-} designates the EDTA anion, the short-hand notations $\equiv$Fe^{III}OH and $\equiv$Fe^{III}Y^{3-} stand for surface hydroxide groups of the Fe^{III}-(hydr)oxide and for the Fe^{III}EDTA surface complex, respectively, and RR'NCH$_2$ stands for the reducing radical formed upon photooxidation and subsequent decarboxylation of EDTA.

$$H_2Y^{2-} + \equiv Fe^{III}OH$$

$$\equiv Fe^{III}Y^{3-}$$

reoxidation

autocatalytic thermal dissolution

hv

photolysis

$$Fe^{III}Y^-_{aq}$$

readsorption

$$\equiv Fe(II) + CO_2$$

$$+ R-N-\overset{\bullet}{C}H_2$$

$$R'$$

hv

photolysis

reoxidation

detachment

$$Fe(II)_{aq}$$

$$CO_2 + R-N-\overset{\bullet}{C}H_2$$

$$R'$$

$$R = -(CH_2)_2N(CH_2COO^-)_2$$

$$R' = -CH_2COO^-$$

concentrations along the River Glatt [33]. The predictions of the chosen model showed good general agreement with data from field investigations, suggesting that the major transformation process of EDTA in the River Glatt is photolysis of dissolved $Fe^{III}EDTA$ (see also *Pacini et al.* [34], this issue).

EDTA forms complexes with Fe^{III} also on the surface of colloidal Fe^{III}-(hydr)-oxides [35]. Experimental studies [36] suggest that also the $Fe^{III}EDTA$ surface complex is subject to photolysis. *Scheme 3* shows a conceptual model of EDTA photooxidation in a heterogeneous experimental system containing a colloidal Fe^{III}-(hydr)oxide phase such as lepidocrocite (γ-FeOOH). Photooxidation of adsorbed EDTA is accompanied by formation of surface Fe^{II} which is either detached from the lattice of the solid phase and transferred into solution or reoxidized as a surface species, the relative rates depending on pH and on the thermodynamic stability of the Fe^{III}-(hydr)oxide phase [37]. Re-adsorption of photochemically produced Fe^{II}_{aq} on surface sites which are coordinated with EDTA results in catalysis of the thermal dissolution of the solid phase [38], yielding dissolved $Fe^{III}EDTA$ which is readily photolyzed [31]. The primary photooxidation product undergoes decarboxylation, and the remaining radical, $RR'N\dot{C}H_2$, reacts with O_2 and H_2O to form formaldehyde (CH_2O), ethylenediaminetriacetate, and $HO_2 \cdot /O_2 \cdot^-$ [36].

The most important feature of *Scheme 3* is that the oxidant (Fe^{III}) is recycled by reoxidation of Fe^{II} either in solution or on the surface of the Fe^{III}-(hydr)oxide. How efficiently Fe^{II} re-oxidation occurs depends on the Fe^{II} speciation and thus on the standard reduction potential of the corresponding Fe^{III}/Fe^{II} redox couples (*Fig. 4*). It is noteworthy that the potential of adsorbed iron is similar to that of the iron-monohydroxo complex, and that structural iron in some minerals exhibits a reduction potential that is even more negative than that of iron in ferredoxin (see also *Schwarzenbach et al.* [22], this issue).

Molecular Dioxygen as Oxidant

As pointed out in the introduction, oxidation of anthropogenic chemicals by O_2 is only efficient in the presence of photocatalysts or of microorganisms acting as catalysts. Enzyme-catalyzed reactions with O_2 as ultimate electron acceptor play crucial roles in oxidative transformations of contaminants that are bioavail-
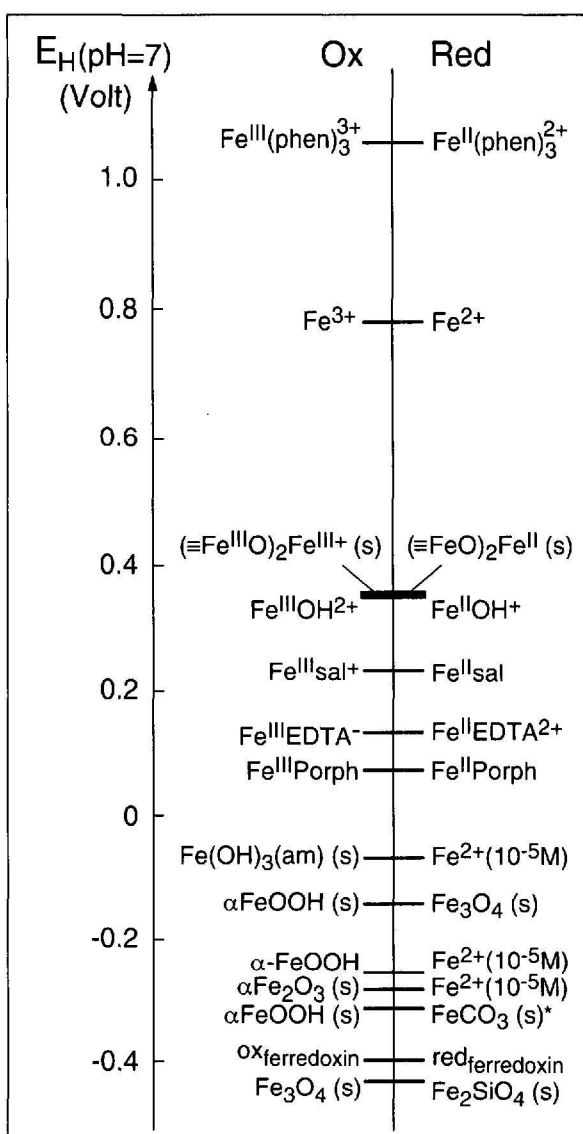
Fig. 4. *Representative redox couples at pH 7* (modified from *Stumm* and *Sulzberger* [39]). phen, phenanthroline; sal, salicylate; porph, porphyrin.

Table 2. *Some Properties of Key Enzymes Involved in the Degradation of NTA in Obligately Aerobic, and Facultatively Denitrifying Gram-Negative Bacteria* (adapted from *Egli* [41])

Enzyme properties	NTA-MO cA/cB	NTA-DH	IDA-DH
Localization	cytoplasmic	cytoplasmic	membrane-bound
Molecular mass			
SDS (kD)	47/36	78	–
native (kD)	99/88	162	–
Dubstrate(s)	NTA	NTA	IDA
Products	IDA + Glyoxylate	IDA + Glyoxylate	Glycine + Glyoxylate
Redox component	NADH, FMN	FAD[a]	(cyt b)[b]
e^--Acceptor	O_2	O_2	Respiration chain (Q10?)
e^--Mediators	–	PMS[c]	–
Metal requirement	Mg^{2+}	none	none (Ca^{2+})[d]
K_m(NTA)	0.5 mM[e]	0.19 mM[f]	–
K_m(IDA)	–	–	8 mM[g]

NTA-MO, NTA monooxygenase; cA, monooxygenase component; cB, FMN-reducing component; NTA-DH, NTA dehydrogenase; IDA-DH, IDA dehydrogenase; FAD, flavine adenine dinucleotide; Q10, ubiquinone-10; PMS, phenanzine methosulfate; K_m, *Michaelis-Menten* constant.
[a]) Covalently bound FAD; [b]) indicated by spectral properties; [c]) *in vivo* e^--mediator unknown; [d]) stimulation observed, but Ca^{2+} not strictly necessary for activity; [e]) measured for Mg^{2+}-complexed NTA; [f]) measured for uncomplexed NTA; [g]) measured for Ca^{2+}-complexed IDA.
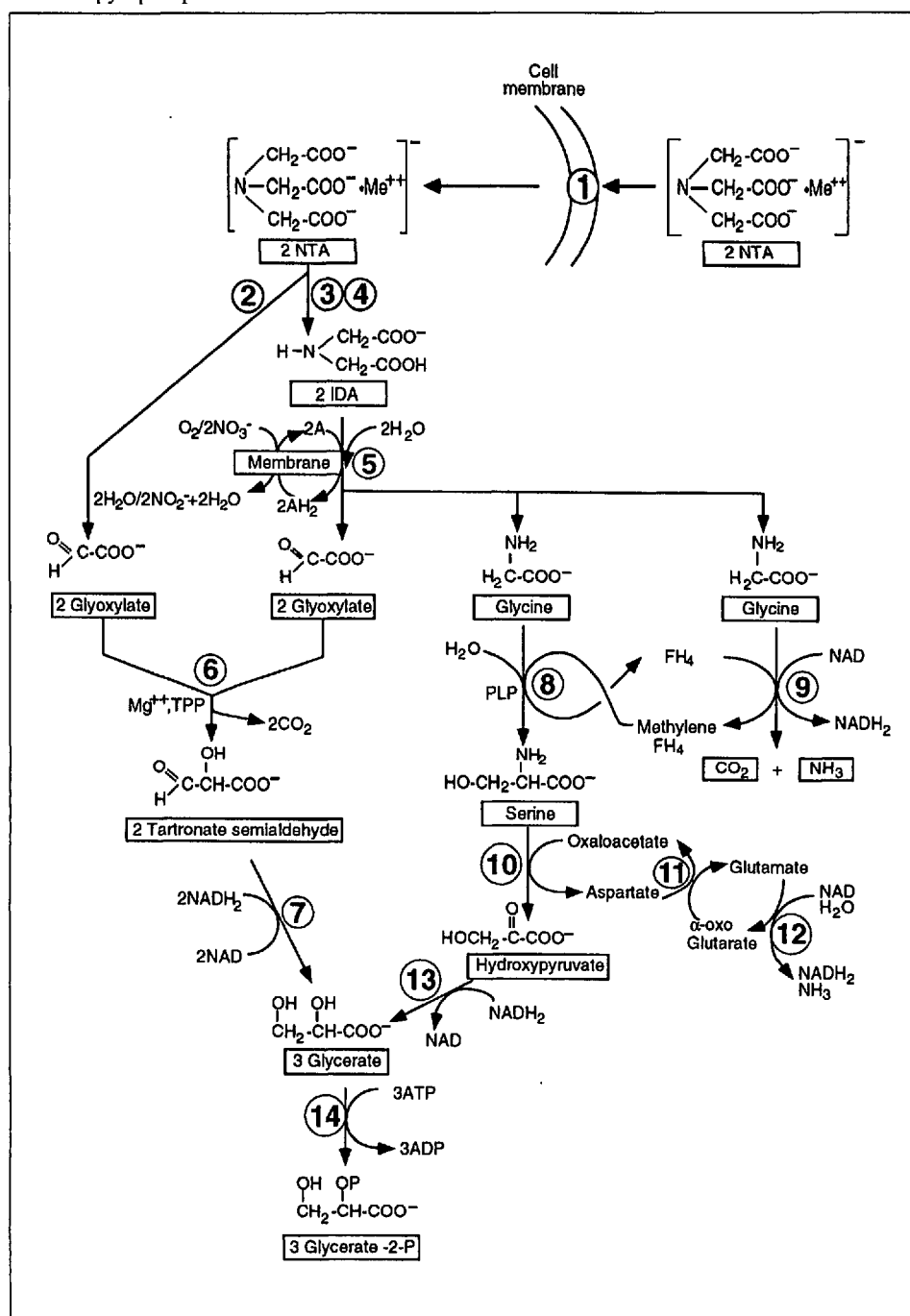
able, both in environmental and in technical systems. In this section, we present our work on the microbial degradation of nitrilotriacetic acid (NTA), an organic complexing agent that is used, besides many other industrial applications, as substitute for phosphate to reduce the phosphate load introduced into surface waters. Over the last decade, various aspects of the biodegradation of NTA in environmental and in technical systems have been stud-

ied in our laboratory [40–42]: *i*) the type and ecology of microorganisms able to degrade this complexing agent, *ii*) the biochemistry and genetics of the enzymes involved in NTA metabolism, and *iii*) the physiological growth behavior of these microbes.

Results from an extensive isolation and characterization program of NTA-degrading bacteria indicate that the ability to degrade NTA is not restricted to a

specific microbial specialist strain but that it is a property that can be found in quite diverse microorganisms. Microscopical detection and enumeration of NTA-degrading bacteria in wastewater treatment plants and natural waters were possible using an indirect immunofluorescence test with cell-surface-specific antibodies raised against whole cells of a *Chelatobacter* and a *Chelatococcus* strain [41][43]. The results obtained give strong evidence that these two new genera are ubiquitously distributed in the environment. In surface waters, the fraction of immunopositive cells was in the range of 0.01–0.1% of the total cell count, whereas in sewage treatment plants, this fraction was between 0.1 and 1%, suggesting a tenfold enrichment of NTA-degrading bacteria in treatment plants. Surprisingly, the number of NTA-degrading bacteria was not significantly different in treatment plants that differed considerably with respect to their NTA elimination efficiency, indicating that NTA degradation might be regulated at the level of enzyme synthesis.

Biochemistry of NTA Degradation

Both, the oxygen-dependent and oxygen-independent metabolic breakdown of NTA has been investigated in our laboratory, and the resulting metabolic pathway of NTA degradation is shown in *Scheme 4*. Presently, three different enzymes are known that catalyze the oxidative cleavage of NTA (*Table 2*), all of them forming iminodiacetate (IDA) and glyoxylate. From an obligately aerobic *Chelatobacter* strain a soluble $NADH/Mg^{2+}/O_2$-dependent monooxygenase was isolated and characterized [41][42]. This enzyme showed a very narrow substrate specificity: out of some twenty structurally related compounds it accepted only NTA as a substrate. Recent studies have demonstrated that in NTA monooxygenase the flavin-containing component cB can be replaced by probably any redox enzyme which is able to supply the reduced redox mediator flavin mononucleotide (FMN) for the monooxygenase component such as, *e.g.*, the oxidoreductase from the luciferase enzyme system in *Photobacterium fischeri*. An oxygen-independent enzyme, a soluble NTA dehydrogenase, was isolated from the denitrifying strain [41]. Neither of the NTA-utilizing enzyme systems described in *Table 2* are able to accept iminodiacetate as their substrate. In *Chelatobacter* and *Chelatococcus* strains as well as in the denitrifying NTA-utilizing isolate, iminodiacetate is further metabolized by a membrane-bound dehydrogenase, the products formed being glycine and glyox-

Scheme 4. *Metabolic Pathway Proposed for NTA Oxidation in Obligately Aerobic, and Facultatively Denitrifying Gram-Negative Bacteria* (from *Egli*, [41]). 1, transport enzyme; 2, NTA monooxygenase; 3, NTA dehydrogenase; 4, NTA dehydrogenase/nitrate reductase enzyme complex; 5, IDA dehydrogenase; 6, glyoxylate carboligase; 7, tartronate semialdehyde reductase; 8, serine hydroxymethyl transferase; 9, glycine synthase (decarboxylase); 10, serine oxaloacetate aminotransferase; 11, transaminase; 12, glutamate dehydrogenase; 13, hydroxypyruvate reductase; 14, glycerate kinase; (methylene)FH_4, (N5,N10-methylene) tetrahydrofolic acid; PLP, pyridoxal phosphate; TPP, thiamine pyrophosphate.

ylate [41]. Hence, once NTA is transported into the cell, two enzymatic steps are sufficient to transform NTA into harmless products that are found in every biological cell.

Since microbial NTA degradation occurs readily in many different environments including sewage treatment plants, whereby harmless products are formed, NTA, unlike EDTA, is not considered to be of ecotoxicological concern.

Conclusions

The presented examples on oxidative transformations of contaminants illustrate that these processes are extremely complex, even in well-defined model systems. The main purposes of such studies are to identify the most important reaction pathways occurring in these complex systems, and to determine rate constants of individual reactions. Furthermore, with these studies we also aim to develop tools which allow prediction of kinetic constants on the basis of thermodynamic data, using, e.g., the *Marcus* relationship.

In order to predict half-lifes of contaminants due to oxidative transformations in environmental and in technical systems, it is not sufficient to determine or predict kinetic constants. Also 'environmental factors' have to be assessed. In oxidative transformation processes, important environmental factors are the steady-state concentrations of oxidants, e.g., of reactive oxygen species (ROS). For photochemical reactions, including photoproduction of ROS, the spectral photon flux as a function of latitude, altitude, and season as well as attenuation coefficients of a considered water body are the most important environmental factors. If we are faced with oxidative transformations where biotic or abiotic catalysts are involved, also the concentration, type, and reactivity of these catalysts have to be assessed in a considered compartment (see also *Hug et al.* [44], this issue).

Most environmental and some technical aquatic systems are heterogeneous where reactions at solid/water interfaces play pivotal roles (where solids are minerals, organic matter, and microorganisms). In dealing with surface reactions, it is not sufficient to determine initial rates, since rates of surface reactions often decrease with time due to aging or dissolution of the solid phase, or to product accumulation on the surface. Furthermore, in heterogeneous aqueous systems, oxidative transformations occur mostly as an interplay of surface and solution reactions. Thereby it is important to realize that the reactivity of surface species is often significantly different from that of dissolved species.

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