Environmental fate and microbial degradation of aminopolycarboxylic acids

Margarete Bucheli-Witschel ¹, Thomas Egli *

Swiss Federal Institute for Environmental Science and Technology, Department of Microbiology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

Received 18 January 2000; received in revised form 30 August 2000; accepted 30 August 2000

Abstract

Aminopolycarboxylic acids (APCAs) have the ability to form stable, water-soluble complexes with di- and trivalent metal ions. For that reason, synthetic APCAs are used in a broad range of domestic products and industrial applications to control solubility and precipitation of metal ions. Because most of these applications are water-based, APCAs are disposed of in wastewater and reach thus sewage treatment plants and the environment, where they undergo abiotic and/or biotic degradation processes. Recently, also natural APCAs have been described which are produced by plants or micro-organisms and are involved in the metal uptake by these organisms. For the two most widely used APCAs, nitrilotriacetate (NTA) and ethylenediaminetetraacetate (EDTA), transformation and mineralisation processes have been studied rather well, while for other xenobiotic APCAs and for the naturally occurring APCAs little is known on their fate in the environment. Whereas NTA is mainly degraded by bacteria under both aerobic and anaerobic conditions, biodegradation is apparently of minor importance for the environmental fate of EDTA. Photodegradation of iron(III)-complexed EDTA is supposed to be mostly responsible for its elimination. Isolation of a number of NTA- and EDTA-utilising bacterial strains has been reported and the spectrum of APCAs utilised by the different isolates indicates that some of them are able to utilise a range of different APCAs whereas others seem to be restricted to one compound. The two best characterised obligately aerobic NTA-utilising genera (Chelatobacter and Chelatococcus) are members of the α-subgroup of Proteobacteria. There is good evidence that they are present in fairly high numbers in surface waters, soils and sewage treatment plants. The key enzymes involved in NTA degradation in Chelatobacter and Chelatococcus have been isolated and characterised. The two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied. Under denitrifying conditions, the two first catabolic steps are catalysed by a monooxygenase (NTA MO) and a membrane-bound iminodiacetate dehydrogenase. NTA MO has been cloned and sequenced and its regulation as a function of growth conditions has been studied.
1. Introduction

1.1. Aminopolycarboxylic acids (APCAs) – an important group of chelating agents

APCAs, also known as complexones, are compounds that contain several carboxylate groups bound to one or more nitrogen atoms (Fig. 1). Hence, they are essentially derived from the amino acid glycine [1,2].

The major chemical property of APCAs is their ability to form stable and water-soluble complexes with many metal ions. APCAs form a special kind of metal complexes because they co-ordinate the metal ion by forming one or more heteroatomic rings (Fig. 2). This ring formation leads to a higher stability of the complexes as compared to metal–ligand complexes in which no such rings are present, a phenomenon which is called ‘chelate effect’.

Additionally, the presence of basic secondary or tertiary amino groups and the large negative charge of APCAs contributes to the high stability of metal–APCA complexes. To describe the stability of such a metal–ligand complex, the equilibrium constant $K$ is used. It is defined as

$$K = \frac{[\text{MeL}]}{[\text{Me}] [\text{L}]}$$  (1)

where $[\text{Me}]$ is the concentration of the metal ion, $[\text{L}]$ the concentration of the ligand, and $[\text{MeL}]$ that of the ligand–metal complex at equilibrium [2].

In many industrial processes and products, the presence of free metal ions causes problems such as the formation of insoluble metal salt precipitates or the catalysis of the decomposition of organic compounds. Chelation masks the metal ions and restricts them from playing their nor-
mal chemical role and from entering potentially harmful and unwanted reactions. Because of their industrial importance, chelating agents are produced and used in large quantities. Among the chelating agents presently employed, polyphosphates are the most widely used compounds, followed by APCAs [3]. Therefore, much attention has been paid to the environmental fate of APCAs, with interest focused on nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA), the two most important APCAs. In the case of NTA, research concentrated on biodegradation because this was found to be the major environmental elimination pathway for this compound. Several reviews have already been dedicated to this topic [4–6]. For EDTA, however, biodegradation does not seem to be a relevant elimination mechanism and so far an abiotic mechanism has been identified as major process for the degradation of EDTA in natural systems [7]. However, an increasing number of reports on the biodegradation of EDTA have been published recently and also other APCAs are receiving more and more attention concerning their occurrence and behaviour in the environment.

In this review, we will summarise the information concerning the microbial breakdown of APCAs. At the same time, we would like to present the most important aspects of the abiotic degradation of this group of compounds to provide a current summary of the knowledge of the fate of APCAs in the environment.

1.2. Important APCAs

The predominant representatives of APCAs are the synthetically produced compounds EDTA, NTA, diethylenetriaminepentaaetate (DTPA) and hydroxyethylideneacetic acid (HEDTA). Figures on the total amounts produced of the most important synthetic APCAs are difficult to obtain and the estimated amounts of these chelating agents produced or used in the USA and Western Europe are listed in Table 1. Their chemical structure is shown in Fig. 1A. In recent years, an increasing number of APCAs of biological origin have been described, most of which are involved in metal acquisition by organisms (Fig. 1B).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>USA</th>
<th>Western Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>42</td>
<td>13.6</td>
</tr>
<tr>
<td>NTA</td>
<td>32</td>
<td>8.3</td>
</tr>
<tr>
<td>DTPA</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>HEDTA</td>
<td>18</td>
<td>2.0</td>
</tr>
<tr>
<td>Other APCAs</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodiumpolyphosphates</td>
<td>586</td>
<td>1111</td>
</tr>
<tr>
<td>Organophosphonates</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Hydroxyacarboxylic acids</td>
<td>110</td>
<td>15</td>
</tr>
</tbody>
</table>

For comparison, data are also shown for other industrially important complexing agents. Figures for the USA are based on production, those for Europe are estimated from information on consumption (adapted from [3]).

1.2.1. Synthetically produced APCAs

In 1862, the first chemical synthesis of an APCA, namely that of NTA, was described [8]. It was based on the reaction of monochloroacetic acid in an ammonium solution. Much later, in 1935, the synthesis of EDTA was reported by I.G. Farbenindustrie. Again, the synthesis was based on monochloroacetic acid reacting with ethylenediamine in the presence of sodium hydroxide. Alternatively, EDTA is produced from ethylenediamine reacting with sodium cyanide and formaldehyde in the presence of sodium hydroxide. Depending on the amine employed, also other APCAs, for instance 1,3-propylenediaminetetraacetic acid (PDTA), can be produced by this type of reaction [2].

The most important function of APCAs is to form metal chelates. This chelation has a strong influence on their environmental fate, including biodegradation. We will therefore summarise the properties of the most intensively investigated APCA chelates, i.e. the metal complexes of NTA and EDTA.

NTA contains four donor atoms and is a so-called quadridentate chelating ligand. It forms 1:1 complexes with metal ions by establishing three chelate rings with four co-ordination sites of the metal occupied by NTA. Since most cations have a co-ordination number of six, the remaining two sites are normally occupied by water molecules, resulting in octahedral co-ordination of the ion (Fig. 2A) [1].

EDTA contains six donor atoms and acts as a hexadentate ligand. It can form a maximum of five chelate rings. Ideally, EDTA should form an octahedral complex in which both the metal ion and EDTA have a co-ordination number of six (Fig. 2A). However, this octahedral co-ordination seems to be only possible with cations of relatively small size. With larger cations, constraints within the structure of the EDTA ligand prevent this ideal structure, and the complexed metal ion may still remain accessible to other ligands such as water molecules. Indeed, X-ray analysis has shown that the structure of most metal EDTA complexes differs from the ideal octahedral structure and that the cations exhibit often higher co-ordination numbers than six (Fig. 2B). On the other hand, in some complexes, such as those with Cu$^{2+}$ or Ni$^{2+}$, EDTA does not fully utilise its donor capacity by leaving one carboxylate group not co-ordinated. Octahedral co-ordination is instead completed by a water molecule (Fig. 2B) [1,2].

Stability constants for 1:1 complexes of EDTA and NTA are listed in Table 2. For each metal, the stability of its NTA complex is several orders of magnitude lower than that of its EDTA complex, as can be expected from
the lower chelating capability of NTA. Although Fe(III)EDTA has a stability constant of log $K = 25.0$, this is still not high enough to keep it from decomposing at pH values above 8–9 due to precipitation of iron(III)-hydroxide. In the search for more effective chelating agents at higher pH values, DTPA and HEDTA were developed. They are preferentially employed instead of EDTA for sequestering iron(III) ions in the pH range of 8–10. EDDHA with a log $K$ of 33 (Fig. 1A) is able to complex iron(III) ions more selectively than EDTA, DTPA or HEDTA and it does not decompose even in the most strongly alkaline solutions [2].

Lately, the synthesis of several new APCAs was reported, namely $\beta$-alaninediacetate ($\beta$-ADA), serinediace-tate (SDA), asparaginic acid diacetate (ASDA) and methyglycinediacetate (MGDA) (Fig. 1). They are derived from amino acids by substituting the amino group with two acetyl groups. The stability of their metal complexes seems to be closer to NTA complexes than to EDTA–metal complexes [9]. Also, the synthesis of $N$-acyl deriva-
tives of ethylenediaminetriacetate (ED3A), chemicals that act both as a surfactant and powerful chelating agent, given the short name chelactant, has been described [10].

1.2.2. Naturally occurring APCAs

Recently, it was shown that APCAs are also naturally occurring compounds. Some even contain an ethylenediamine central moiety, a feature that was considered to
be restricted to xenobiotic chelating agents such as EDTA.

1.2.2.1. Ethylenediaminedisuccinate (EDDS). The first natural APCA described, EDDS, was isolated from culture filtrate of the actinomycete *Amycolatopsis orientalis*. It was detected in an antibiotic screening program due to its ability to inhibit activity of the Zn$^{2+}$-dependent phospholipase C [11]. In the actinomycete, EDDS is most probably involved in Zn$^{2+}$ uptake, because its production was found to be totally repressed at Zn$^{2+}$ concentrations higher than 2.5 $\mu$M in the growth medium [12], whereas below 2.5 $\mu$M it increased with decreasing Zn$^{2+}$ concentrations. In contrast, other trace metals (Fe$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Mn$^{2+}$, Mo$^{2+}$) exerted no repressive effect on EDDS production [13]. However, there is no preferential complexation of Zn$^{2+}$ by EDDS, for the stability constants for Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ are higher than that for Zn$^{2+}$ (Table 2).

**Table 2**

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Log $K_{MeNTA}$</th>
<th>Log $K_{MeEDTA}$</th>
<th>Log $K_{MeEDDS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg$^{2+}$</td>
<td>5.47</td>
<td>8.83</td>
<td>5.82</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>6.39</td>
<td>10.61</td>
<td>4.23</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>7.46</td>
<td>13.81</td>
<td>8.95 (+)</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>10.66</td>
<td>16.44</td>
<td>13.49 (+)</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>10.38</td>
<td>16.26</td>
<td>14.06</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>12.94</td>
<td>18.7</td>
<td>18.36</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>11.34</td>
<td>17.88</td>
<td>12.7 (+)</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>9.78</td>
<td>16.36</td>
<td>10.8 (+)</td>
</tr>
<tr>
<td>Al$^{3+}$</td>
<td>11.4</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>8.33 (+)</td>
<td>14.27</td>
<td></td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>15.9</td>
<td>25.0</td>
<td>22.0 (+)</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>11.5</td>
<td>18.52</td>
<td>16.79</td>
</tr>
</tbody>
</table>

Fig. 2. Ideal octahedral structure of metal-NTA and metal-EDTA complexes (A) as well as steric structure of NiEDTA and Fe(III)EDTA as determined by X-ray analysis of solid complexes [255,256] (B).
Rhizopus microzoferrin was first isolated from cultures of a representative of APCAs, rhizoferrin, was detected. Rhizopus [Rhizobium] really transported by EDDS into cells of S,S existence of three different stereoisomers, [S,S]-, [R,S]-, and [R,R]-EDDS. A. orientalis exclusively produces the S,S-isomer. For the biosynthesis of [S,S]-EDDS, two pathways were proposed by Cebulla [12], starting from either l-aspartate and serine, or from oxaloacetate and 2,3-diaminopropionic acid. In both cases, N-(2-aminoethyl) aspartic acid (AEAA) would be an intermediate reacting then with oxaloacetate to form [S,S]-EDDS.

EDTA is rather recalcitrant towards biological and chemical degradation in the environment and there is increasing pressure to replace it by other powerful complexing agents. For this, EDDS seems to be a promising candidate. Consequently, its chemical and biotechnological production has been investigated. Two routes exist to synthesise EDDS chemically: firstly, the reaction of maleic anhydride with ethylenediamine yielding a mixture of the three stereoisomers of EDDS [15,16], and secondly, the reaction of aspartic acid with 1,2-dibromoethane leading to the formation of either pure [S,S]-EDDS or [R,R]-EDDS, depending on the stereoisomer of the aspartic acid employed [17]. As an alternative, both the fermentation conditions for the production of [S,S]-EDDS by A. orientalis and the subsequent purification procedure were optimised [18]. Highest [S,S]-EDDS productivity was obtained by fed-batch cultivation with a medium containing very low Zn\(^{2+}\) concentrations, glycerol as carbon source, a mixture of glutamate and urea as nitrogen source, and high initial phosphate concentrations. Under optimised conditions, final EDDS concentrations of some 20 g l\(^{-1}\) were obtained. EDDS can be purified using a three-step procedure consisting of an acid precipitation, an ethanol washing step and a final crystallisation leading to 92% purity with an overall yield close to 50% [18].

1.2.2.2. Rhizobactin. In 1984, Smith and Neiland [19] described the formation of the APCA rhizobactin by Rhizobium mellioti strain DM4 when cultured on a low-iron medium. Consequently, rhizobactin is considered a siderophore. As EDDS, rhizobactin contains an ethylenediamine group with one amine function linked to pyruvic acid, and the second to the carbon skeleton of lysine carrying malic acid on the N\(^6\) amino group [20]. The absolute configuration of rhizobactin was \(\psi^{\alpha}, \psi^{\beta}, \gamma^{\text{malate}}, \delta^{\text{amino acid}}\), suggesting that rhizobactin is biochemically related to the pyruvic acid-derived opines which have a configuration of \(\psi^{\alpha}, \psi^{\beta}, \gamma^{\text{amino acid}}\).

1.2.2.3. Rhizoferrin. Also among fungal siderophores, a representative of APCAs, rhizoferrin, was detected. Rhizoferrin was first isolated from cultures of Rhizopus microsporus var. rhizopodiformis [21]. Apparently, rhizoferrin is a widespread siderophore within the class of Zygomycetes [22]. It is composed of two citric acid groups linked via amid bonds to a 1,4-diaminobutane (putrescine) molecule [23]. The asymmetric carbon atoms of the citric acid residues are always in \(R,R\)-configuration. A value of 10\(^{23.5}\) was reported for the stability constant of the iron(III) rhizoferrin complex [24]. Thus, rhizoferrin is not a particular powerful iron chelator at pH 7.4 compared to other fungal and bacterial siderophores of the hydroxamate or catecholate type but it becomes much more competitive at pH values near 4.5, the growth optimum for the producing fungi [24].

Transport experiments with \(^{55}\)Fe-labelled rhizoferrin confirmed that the compound acted as siderophore [21]. In the producing fungal strain, Absidia spinosa, reductively inert gallium rhizoferrin, and the kinetically inert chromium and rhodium complexes were also taken up, indicating that the intact metal–siderophore complex was transported into the cell [25].

Rhizoferrin is able to deliver iron not only to the producing organisms but to other micro-organisms as well. It showed strong siderophore activity in nearly all bacterial strains tested from the Proteus–Providencia–Morganella group, although these strains possess their own efficient iron uptake system based on keto- or hydroxycarboxylate iron complexes [26]. The rates of iron uptake in Morganella morugani mediated by rhizoferrin were equivalent to those measured in the presence of some of its own siderophores. Recently, a rhizoferrin receptor from M. morganii has been cloned [27]. This transport system (called Rum, for rhizoferrin uptake into Morganella spp.) was specific for ferric rhizoferrin and staphyloferin A (described below), while citrate and the citrate derivative aerobactin were not transported. The system consisted of two proteins, RumA located in the outer membrane and the periplasmic RumB. Homology analyses indicated a close relationship of RumA to the outer membrane receptor FecA of the Escherichia coli ferric citrate transport system. RumB, however, showed little homology to other binding proteins with the exception of certain signatures of conserved amino acid residues characteristic of binding proteins of the siderophore transport family.

The biotechnological production of rhizoferrin and some of its analogues was also studied [28]. From several producing strains tested, Cunninghamamella elegans and Mucor rouxii exhibited highest productivity and were selected for process optimisation. Moreover, C. elegans was used to produce analogues of rhizoferrin by feeding analogues of the two building blocks (1,4-diaminobutane and citric acid). Feeding citric acid analogues resulted in only low amounts of derivatives being formed. However, feeding of 1,5-diaminopentane or 1,3-diaminopropanol caused a considerable formation of analogues containing bridges with five or three methylene residues, respectively. Also, bis-(2-aminoethyl)-ether and branched diamine precursors were used as building blocks but only low product yields were obtained. Finally, two interesting effects were observed when feeding the ketone 1,4-diamino-2-butanol as a pre-
Staphylococcus agents much more powerful than citrate alone [29]. Together of two citric acid residues provides an iron-binding ornithine [30]. As in the case of rhizoferrin, the tying to- where addition of D-ornithine resulted in a signiﬁcant ornithine led to a preferential excretion of rhizoferrin whereas addition of ω-ornithine resulted in a signiﬁcant reduction of rhizoferrin produced. All these biologically produced rhizoferrin analogues had siderophore activity in growth promotion assays with M. morganii with activities comparable to or only slightly lower than that of rhizoferrin. In contrast, the citric acid analogues exhibited signiﬁcantly lower activity. These results show clearly that also the structure of biologically produced APCAs can be manipulated, a point which might become interesting with respect to a possible future exploitation of naturally occurring APCAs.

1.2.2.4. Staphyloferrins. Structurally related to rhizo- ferrin is staphyloferrin A, a siderophore originally isolated from a culture of Staphylococcus hyicus [29,30]. It consists of two citric acid residues linked by two amid bonds to ω-ornithine [30]. As in the case of rhizoferrin, the tying together of two citric acid residues provides an iron-binding agent much more powerful than citrate alone [29]. Staphylococci produce also another siderophore of the complex- one type, staphyloferrin B, the chemical structure of which is quite different from that of staphyloferrin A. Composed of 2,3-diaminopropionic acid, citrate, ethylenediamine and 2-ketoglutaric acid as structural components, it lacks the symmetry of staphyloferrin A and rhizoferrin, and it is therefore a less potent chelator of iron [26]. Both staphyloferrin siderophores are produced by a wide range of Staphylococcus strains, some of which were even found to produce both types [31]. Transport measurements in S. hyicus with ferric staphyloferrin A and B revealed uptake of $^{55}$Fe within the range of 1–15 min with staphyloferrin B being less effective than staphyloferrin A [26].

1.2.2.5. Plant siderophores. Siderophores from plants were described which also fit into the group of APCAs. An example is nicotianamine, an essential constituent of higher plants which is important for cellular iron transport and/or metabolism. Nicotianamine contains six donor groups (three nitrogen atoms and three carboxylate groups) with an arrangement ideal for the formation of chelate rings [32]. Presently, there is no evidence that nicotianamine plays a role in iron uptake from the rooting media, whereas this is known to be the case for the chemi- cally similar mugineic acid and its acidic amino acid ana-

logues, as well as avenic acid (Fig. 1B). These phytosidero-

phores are apparently widespread among grass species and cultivars [33]. Mugineic acids might also be produced and excreted by plants stressed by zinc deﬁciency [34]. Mass production of mugineic acid and its derivatives is proposed because of their potential application in agriculture, medical science and pharmacy. Possible production methods take advantage of cell cultures of barley or wheat, or are based on water cultures of intact plants, in both cases exposing the cells or plants to iron deﬁcient conditions [35].

1.3. Fields of application for APCAs

Due to their metal sequestering capacity, synthetic APCAs are used in many industrial processes or products in order to (i) prevent the formation of metal precipitates, (ii) to hinder metal ion catalysis of unwanted chemical reactions, (iii) to remove metal ions from systems, or (iv) to make metal ions more available by keeping them in solution.

1.3.1. Use of APCAs to prevent formation of precipitates

In addition to active washing ingredients, detergents contain a large proportion of metal-complexing agents to inhibit the formation of insoluble Ca$^{2+}$ and Mg$^{2+}$ salts, and thus prevent the deposition of scale on both textile fibres and washing machine parts. The ﬁrst complexing agents employed in modern detergents were di- and tri- phosphates [36]. However, it was soon found that these contribute to the eutrophication of lakes and rivers. During the search for substitutes, NTA was proposed as an alternative detergent builder and NTA-containing detergents were ﬁrst marketed in Sweden in 1967. Since then, NTA-based detergents have been used in Canada, Finland and Sweden, the US, and Switzerland, although they have met considerable opposition [4] (note that EDTA is not used as a substitute for polyphosphates in household detergents, see below). In industrial cleaning agents, NTA, EDTA, and recently also MGDA, are used to prevent precipitation of calcium, magnesium and heavy metal salts [37–39]. For the same purpose, EDTA is especially used in the textile and photographic industry, as well as in electro-plating processes instead of cyanide [37]. In the photographic industry, FeNH$_2$EDTA is also employed as oxidising agent for silver [37], but recently it is being partly replaced by β-ADA and PDTA [39].

1.3.2. Use of APCAs to prevent catalysis mediated by metal ions

Small amounts of EDTA have been included in many detergent formulations to stabilise the bleaching agent per- borate by preventing metal-catalysed decomposition of the compound [38]. In the bleaching process of the pulp and paper industry, hydrogen peroxide is increasingly employed instead of chlorine compounds and the addition
of either EDTA or DTPA avoids the decomposition of hydrogen peroxide catalysed by manganese or iron ions [40]. APCAs are also used as additives for pharmaceuticals, cosmetics and food to prevent the transformation of the ingredients or rancidity due to metal-catalysed reactions [37,41].

1.3.3. Use of APCAs to remove metal ions

Multidentate chelating agents are widely applied in the nuclear industry for decontamination of reactors and equipment because they form water-soluble complexes with many radionuclides. Although the exact composition of most of the decontamination reagents is proprietary information, it is assumed that mainly NTA, EDTA, HEDTA or DTPA are present in these formulations [42]. In a single decontamination operation, hundreds of kg of chelating agents are employed [42,43]. The wastes obtained, containing radionuclide–chelator complexes, are solidified and disposed of [42–44]. Concern was raised that due to the presence of powerful chelators in such wastes the migration of radioactive transition metals, rare earths and transuranics may be enhanced [45]. APCAs have also been proposed for application in the remediation of metal-contaminated soils or sediments, either to serve as washing agents [46,47] or to support electrokinetic extraction processes [48,49]. Apparently, APCAs have also the potential to be employed in phytoremediation strategies due to their ability to increase metal desorption from soil and to facilitate metal uptake by plants [50]. Moreover, in medical treatment, CaEDTA is used as antidote for the treatment of lead or other heavy metal intoxications. EDTA complexes the toxic metal and accelerates its excretion [51].

1.3.4. Use of APCAs to increase metal availability

Since the early 1950s, synthetic chelating agents have been used to improve plant nutrition [52]. Especially APCA chelators are employed in fertilisers to supply plants with trace metals such as iron, copper, zinc and manganese. Most commonly, the Fe(III) chelates of EDTA, HEDTA, DTPA, EDDHA and ethylenediamine-di(o-hydroxy-p-methylphenyl) acetic acid and the Cu(II), Zn(II) and Mn(II) chelates of EDTA are present in fertilisers [53].

1.4. Concentrations of APCAs in the environment

Since the 1970s, NTA has received much attention because of its use in laundry detergents and this has led to monitoring programs during which its concentration was measured in various environmental compartments. More recently, interest was also focused on EDTA and the determination of EDTA concentrations was often included in the monitoring of NTA. In contrast, little is known about the occurrence and concentrations of other APCAs in the environment.

1.4.1. Concentrations of NTA and EDTA in sewage treatment plants

In Canada, where NTA accounts for up to 15 volume % of laundry detergents, the typical NTA loading of raw wastewater averaged 2500 μg l⁻¹ [54]. Several case studies in Swiss municipal wastewater treatment plants revealed similar average concentrations in the influent of 100–1000 μg NTA l⁻¹, whereas EDTA concentrations in raw wastewater were determined to be somewhat lower ranging from 10 to 500 μg EDTA l⁻¹ [55–57]. However, the EDTA concentrations in secondary effluents were approximately five times higher than those of NTA, and did not differ significantly from the EDTA concentrations in the influent [57]. Obviously, a large portion of the incoming NTA, but not of EDTA, was eliminated from the wastewater during the treatment process.

1.4.2. Concentrations of NTA and EDTA in surface waters

Despite the dramatic increase of NTA usage in Canada since 1970, no accumulation of NTA in Canadian surface waters was observed [58]. From 1971 until 1975, a median concentration of 50 μg NTA l⁻¹ was found in Canadian streams [59]. In Canadian coastal waters of the Atlantic and the Pacific Ocean and in samples from the port area of Halifax, no NTA was detectable more than 2 years after its first usage in detergents [60].

The concentrations of NTA and EDTA found in European rivers are in the range of 0–20 and 0–60 μg l⁻¹, respectively [37,38,61]. For EDTA, however, occasionally concentrations higher than 100 μg l⁻¹ were found in German and Swiss rivers [62,63].

In Swiss lakes, NTA concentrations measured were below 10 μg l⁻¹. At the bottom of the lakes, NTA was present at approximately the detection limit of 0.1–0.2 μg l⁻¹. EDTA concentrations of 1–4 μg l⁻¹ were found throughout the whole waterbody and in contrast to NTA, the concentrations of EDTA were only slightly subject to temporal or spatial variations [61].

In several sediment cores from Lake Greifensee, Switzerland, EDTA concentrations in the range of 60 μg kg⁻¹ to 1170 μg kg⁻¹ were found [64]. The water content of the sediments was 80% with an EDTA concentration in the pore water equal to that found in the overlying water column, namely around 6 μg l⁻¹, indicating an enrichment of EDTA in the sediments of this lake. A similar enrichment of EDTA in the sediment, ranging between 80 and 310 μg EDTA kg⁻¹, was found in the Southern part of the Finish Lake Saimaa [65]. This lake receives a high load of treated wastewaters from pulp and paper industries, which use EDTA and DTPA in their production process [65, 66].

1.4.3. Concentrations of NTA and EDTA in ground- and drinking water

NTA concentrations determined in ground- and drinking water ranged normally between 1 and 5 μg l⁻¹
In Swiss groundwaters, EDTA concentrations of 0.1 to nearly 15 \( \mu \text{g l}^{-1} \) were found [61]. Drinking water obtained from surface water by bank infiltration from the river Ruhr showed rather high EDTA concentrations (median of 25 \( \mu \text{g l}^{-1} \)), similar to those found in the supplying river (median of 26 \( \mu \text{g l}^{-1} \)). This indicates that no elimination took place in the water along the infiltration passage. For NTA, however, the concentrations decreased by a factor of 10 during infiltration, resulting in a median concentration of 0.7 \( \mu \text{g l}^{-1} \) in the drinking water [67]. Due to the observed persistence of EDTA during infiltration, the compound was proposed to be used as tracer to analyse how far infiltrated river water penetrates into groundwater aquifers [68].

### 1.4.4. Environmental concentrations of DTPA

Only little data are available on concentrations of DTPA in freshwater. In the few samples collected from German rivers, DTPA concentrations between 2 and 15 \( \mu \text{g l}^{-1} \) were determined [38]. Rather high concentrations for both DTPA and EDTA (10–20 \( \mu \text{g l}^{-1} \)) were observed in the Southern part of the Finnish Lake Saimaa. In contrast to EDTA, which was found in all samples, DTPA was only detected in the vicinity of the point sources and it was not found in the sediments despite its presence in the overlaying waterbody [65,66]. Even though in these investigations the analytical detection limit for DTPA was higher than that for EDTA and the extractability from the sediments might have been lower, this suggests that DTPA was more readily degraded in this particular lake water than EDTA.

### 1.4.5. Environmental concentrations of other APCAs

The occasional samples originating from various German rivers revealed the presence of \( \beta \)-ADA and PDTA at concentrations around 1–5 \( \mu \text{g l}^{-1} \). In the same samples, MGDCA was not detectable. Furthermore, HEDTA was measured at two sampling points in the river Elbe (Germany) at rather high concentrations of about 40 \( \mu \text{g l}^{-1} \) [39,69]. These preliminary data indicate that it is not sufficient to analyse only the ‘classical’ representatives NTA, EDTA and DTPA but that also other complexing compounds have to be considered when monitoring the pollution of surface waters with APCAs.

### 1.4.6. Concentrations of APCAs in soils

We are not aware of reports on the concentration of APCAs in agriculturally used soils despite their usage in fertilisers. Eventually, the highest environmental APCA concentrations were reported for the Hanford site, USA, where wastes from nuclear decontamination processes containing both complexing agents and radioactive metals were dumped into the ground [70]. In the mixed waste, approximately 9 g EDTA \( l^{-1} \), 10 g HEDTA \( l^{-1} \) and 1–2 g NTA \( l^{-1} \) were detected plus a wide variety of chelator fragments, also present in the mg–g \( l^{-1} \) range.

### 1.5. Speciation of APCAs in the environment

In addition to environmental concentrations, information on the speciation of APCAs is essential to understand their environmental fate. So far, only data on the speciation of NTA and EDTA have been reported.

Considering typical concentrations of metal ions and APCAs in natural systems, the chelating agents can be expected to occur mostly as metal complexes. Unfortunately, reliable analytical methods for monitoring the various metal species of APCAs in natural waters are still lacking. Yet, in the case of EDTA, a method based on high performance liquid chromatography has been developed and first promising results have been obtained. However, this method is still merely applicable to high concentrations of the various EDTA species [71]. Attempts to exploit capillary electrophoresis have been made to assess the speciation of APCAs, in particular of EDTA and DTPA. But this method is restricted to rather stable complexes and its sensitivity is still too low for detecting complexes in environmental samples [72,73]. Nonetheless, making use of the photolability of Fe(III)EDTA and the slow exchange kinetics of NiEDTA with Fe(III) ions, analytical methods were developed to distinguish Fe(III)-EDTA and NiEDTA from other EDTA complexes even when present at low, environmentally relevant concentrations [74,64].

Generally, equilibrium calculations based on the stability constants (see Eq. 1) have to be used to predict the speciation of APCAs in ecosystems. However, two major difficulties affect the prediction of APCA speciation under environmental conditions. Firstly, other natural ligands including inorganic anions such as hydroxide, bicarbonate and phosphate anions and organic compounds such as humic acids compete with APCAs for the metals. Whereas concentrations of inorganic ligands can be measured and thus their effect on APCA speciation can be predicted, it is more difficult to estimate the influence of organic ligands on metal complexation. For marine systems, methods to analyse the complexation of a variety of metal ions with natural ligands were described [74–77]. For freshwater systems, such methods exist for Cu\(^{2+}\) and Zn\(^{2+}\) [78,79] and Co\(^{2+}\) and Cd\(^{2+}\) [80,81]. The second difficulty is that speciation calculations assume that the chemical equilibrium has been reached. However, and this applies in particular to EDTA, due to the slow kinetics of some of the metal exchange reactions, true speciation in a natural system may differ considerably from the calculated equilibrium. Under conditions as they are found in natural waters, where a large excess of Ca\(^{2+}\) and Mg\(^{2+}\) over trace metals exists, exchange reactions of EDTA complexes have been shown to occur at slow rates with time scales of hours to days [82,83]. Especially Fe(III)EDTA was observed to exchange rather slowly with other metals and for the exchange reactions with divalent cations, half-life times of about 20 days were determined in river water [84]. Hence,
the complexation of EDTA in river water depends not only on the dissolved concentrations of the various cations and other ligands which determine the equilibrium speciation but also on the initial speciation of EDTA in which it is released. This explains why Fe(III)EDTA makes up a considerable portion of total EDTA in surface waters, because it is mainly this species which is released into the environment from both photographic industry and wastewater treatment plants using iron salts for phosphate precipitation.

Several authors presented speciation calculations for NTA or EDTA, taking into account the above mentioned problems to a larger or smaller extent. Early predictions for NTA speciation in freshwater including both inorganic and other heavy metals (Fig. 3) [85]. The contradicting modelling data underline the need for more information on the effect of natural ligands on metal speciation.

To determine the speciation pattern of EDTA in a river system, a combination of measurements and equilibrium calculations was applied (Fig. 3). According to measurements, the Fe(III)EDTA and NiEDTA fractions were set at 31% and 10%, respectively. Including into the model these values and the concentrations of natural ligands for Cu and Zn, the following distribution was predicted: 31% Fe(III)EDTA, 30% ZnEDTA, 15% Mn(II)EDTA, 12% CaEDTA, 10% NiEDTA, 2% PbEDTA and 0.5% CuEDTA [86]. Hence in contrast to NTA, co-ordination with heavy metals seems to be more important for EDTA, while the rather unstable CaEDTA complex makes up only a quite small portion of the total EDTA.

1.6. Environmental risks

The main concerns which have been raised in the discussion on the risks of the occurrence of APCAs in the environment were: (1) adverse effects on the operation of wastewater treatment plants, (2) toxic effects of APCAs on aquatic and mammalian organisms, (3) the contribution of nitrogen from APCAs to eutrophication, and (4) the potential to mobilise metals.

1. No negative effects on the normal operation of wastewater treatment plants or sludge disposal systems have been reported so far for both EDTA and NTA at environmentally relevant concentrations [4,37].

2. Acute and chronic toxicity of NTA towards more than 50 species of freshwater and marine organisms has been studied [4]. Chronic ‘no observed effect concentrations’ of NTA for aquatic life were at least one order of magnitude higher than measured environmental concentrations [4]. Furthermore, acute or chronic effects were only reported when the NTA concentrations used in the tests were equal to or in excess of the concentration of divalent metal ions. In analogy, also EDTA and DTPA were only weakly to moderately toxic for aquatic organisms when they were complexed with metal ions [37,87,88]. Given the fact that in surface waters always a large stoichiometric excess of Ca and other divalent metal ions is present and that the actual NTA, EDTA and DTPA concentrations are many orders of magnitude below the observed toxic concentrations, no adverse effects on aquatic life can be anticipated for these APCAs.

In toxicity studies, NTA was only moderately toxic to mammals during acute oral exposure. It was not teratogenic itself or in presence of heavy metals and it was non-genotoxic. NTA was not metabolised but was rapidly excreted by the kidney. Findings that urinary tract tumours can develop as a consequence of chronic exposure to high doses of NTA were explained by changes in Zn and Ca distribution between urinary tract tissues and urine. But again, thresholds determined for such effects were much higher than human exposure resulting from the low environmental NTA concentrations [4]. Also EDTA is only weakly toxic to man (in fact, it is used as antidote and is permitted as food additive in some countries). WHO fixed the acceptable daily intake of EDTA to 2.5 mg kg⁻¹.
body weight. Considering average concentrations of EDTA in drinking water, the daily uptake of EDTA is far below this value [37].

3. It was suspected that widespread usage of NTA or EDTA (both contain nitrogen) might enhance eutrophication. However, the contribution of both compounds to the environmental nitrogen loading was found to be insignificant for any direct eutrophication effect [4]. It has also been discussed whether or not NTA or EDTA would stimulate algal growth indirectly by extracting essential metals from sludges, sediments or humic acid and making them better available or – alternatively – by protecting organisms against the toxic effects of certain heavy metals. While in laboratory systems indirect effects were demonstrated at relatively high NTA and EDTA concentrations, they are thought to be negligible in surface waters [4,37].

4. The potential of NTA and EDTA to alter heavy metal distribution has been thoroughly studied in model systems (for reviews, see [4,37,89]). It was concluded that metal mobilisation by NTA and EDTA will not be significant at environmental concentrations and that variations in pH will have larger effects on aqueous concentrations of heavy metal ions. However, shock loadings in wastewater treatment plants with concentrations of free NTA or EDTA in the mg l⁻¹ range can cause mobilisation of Zn²⁺ leading to elevated Zn²⁺ concentrations in the effluent [55,90]. Moreover, at pH 7–8 the mobilisation of toxic heavy metals (e.g. Pb or Cd) sorbed to particles such as iron(hydr)oxides resulted from an exchange of Fe(III) complexed by EDTA against the toxic metal, a reaction which is catalysed by the particle surface [91]. Thus, Fe(III)EDTA, which is an important EDTA species in many rivers, can remobilise adsorbed heavy metals during infiltration or in groundwater aquifers in calcareous regions characterised by pH values > 7.0. In fact, such unwanted exchange reactions have already been observed in a field investigation of an infiltration passage [91]. On the other hand, in aquifers with lower pH values (< 7.0), Me(II)EDTA chelates will react with iron(hydr)oxides resulting in the dissolution of the mineral and the formation of Fe(III)EDTA. The metal liberated from the original EDTA complex will then adsorb to the surface and thus become immobilised. Hence, under such conditions, no remobilisation but rather an immobilisation of toxic metals is to be anticipated [92].

2. Abiotic elimination of APCAs in the environment

The elimination of various APCAs from the environment is based on different biotic and abiotic processes. Despite the chemical and structural similarity of the APCAs, the mechanism primarily responsible for their elimination has to be considered separately for each compound. Numerous laboratory and field investigations have shown that biodegradation is the key mechanism for the removal of NTA from the environment [4], whereas EDTA is predominantly eliminated via photodegradation [62]. Before focusing on the biodegradation of APCAs, we will therefore summarise the most important abiotic processes leading to either transformation of APCAs or to their elimination from environmental compartments.

2.1. NTA

For both Fe(III)NTA and CuNTA, photochemical degradation by sunlight was reported, and this may – although only to a minor extent – contribute to the decomposition of NTA in the photic zones of lakes and marine systems [4,93,94]. Photodegradation of other metal complexes is not likely, since no significant decrease in NTA concentrations was measured when solutions containing NTA and an excess of Cd²⁺, Pb²⁺, Mg²⁺ or Cr³⁺ were exposed to light of 350 nm, a wavelength at which both CuNTA and Fe(III)NTA were degraded. The half-life for Fe(III)NTA during irradiation with sunlight was determined to be approximately 1.5 h, whereas that for CuNTA was more than 100 times higher. The decomposition probably originates from a ligand to metal charge transfer resulting in a reduced metal ion and the formation of a ligand radical which then undergoes sequential decarboxylation giving rise to CO₂ and formaldehyde, and iminodiacetate (IDA). Fe(III)IDA and Cu(II)-IDA were further photodegraded with glycine being formed. The latter, however, degraded more slowly than Fe(III)IDA, because of a shift of the absorption spectrum of Cu(II)IDA towards shorter wavelengths and thus a smaller overlap with the solar emission spectrum [95].

2.2. EDTA

The recalcitrance of EDTA towards biodegradation in wastewater treatment plants or the environment has directed much attention to other mechanisms of elimination. Thermic hydrolysis and indirect photolysis of EDTA are obviously negligible for its fate in natural systems [7]. Direct photolysis, oxidation by metal(hydr)oxides and, to a smaller degree, also sorption of EDTA to particles and subsequent sedimentation of these EDTA-loaded particles seem to be important processes for the partial elimination of EDTA from aquatic systems.

2.2.1. Photolysis

The process considered to be the most important for the elimination of EDTA from surface waters is direct photolysis, which results from the fraction of sunlight below 400 nm [62]. Apparently, only Fe(III)EDTA is susceptible to sunlight irradiation, whereas other environmentally rel-
relevant EDTA species (complexes with Mg$^{2+}$, Ca$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$ and Hg$^{2+}$) will not photolyse. Under laboratory conditions, Mn(II)EDTA and Co(III)EDTA also photodecomposed, although at rates approximately one order of magnitude lower than that of Fe(III)EDTA [96,97]. Interestingly, initially uncomplexed EDTA was photodegraded in the presence of lepidocrocite, an iron oxide [98], indicating that EDTA adsorbed to the surface of the iron hydroxide can be photooxidosed. Although free EDTA is not present in natural systems, the authors suggested that metal complexes might undergo photodegradation in an analogous manner by adsorbing to iron oxides and forming ternary surface complexes.

Several researchers [40,99,100] have calculated the photolysis half-lives of Fe(III)EDTA for surface waters at various geographical locations. They ranged between only 11.3 min to more than 100 h depending on the light conditions employed for modelling. A comparison of field data with model calculations demonstrated that photolysis by sunlight is really the most important process for the degradation of EDTA in the Swiss river Glatt [7]. While on cloudy days no significant decrease in the EDTA concentrations could be detected along the river, all available Fe(III)EDTA was eliminated by photodegradation under sunny conditions within approximately 1 day. The remaining EDTA found on sunny days consisted of photostable EDTA complexes.

As products of the photolysis of Fe(III)EDTA, ED3A, both possible isomers of ethylenediaminediacetate (EDDA) ($N,N$-EDDA and $N,N'$-EDDA), IDA and ethylenediaminomonoacetate (EDMA) were found, suggesting that the reaction mechanisms are similar to those already described for the photolysis of NTA. Photolysis clearly does not result in the total mineralisation of EDTA but more easily biodegradable metabolites are formed during this process [101]. After a rather short irradiation (6.5 h) of a Fe(III)EDTA solution with sunlight, almost all of the initial EDTA was transformed into ED3A, EDDA and EDMA. When this mixture of photolysis metabolites was incubated with activated sludge, a bioelimination of the photodegradation products was 92%. These results indicate a higher resistance of ED3A towards biological mineralisation when compared with EDDA and EDMA. Clearly, only investigations with pure compounds can give a final answer. If ED3A actually turns out to be rather persistent, then further field investigations should be considered which do not only include the monitoring of the disappearance of Fe(III)EDTA but also the fate of ED3A.

### 2.2.3. Redox processes

Lately, attention was drawn to the possible oxidation of APCAs by +III metal oxidants in natural systems [106,107]. Such oxidants comprise manganese(III/IV)-(hydr)oxides, Co(III)-containing mineral phases (e.g. Co(III) stemming from the oxidation of Co(II) adsorbed onto manganese(III/IV)- and iron(III)-(hydr)oxides) as well as iron(III)-(hydr)oxides. While the potential of the Fe(II)/Fe(III) half reaction ($E^\circ = +0.67$ V) is too low to allow oxidation of EDTA, those for the Mn(III)/Mn(II) and Co(III)/Co(II) half reactions ($E^\circ = +1.50$ V and $E^\circ = +1.48$ V, respectively) are sufficiently high. Indeed, interactions of EDTA with Mn(III)OOH and Co(III)OOH have been shown to result in the oxidation of EDTA, yielding the deacetylated breakdown products ED3A and EDDA. Mn(III) is a more reactive oxidant than Co(III) and, therefore, the reactions of EDTA with Mn(III)OOH were significantly faster than those with Co(III)OOH. Apparently, EDTA does not persist in the presence of manganese(III/IV)-(hydr)oxides and reactions with such minerals were proposed to represent an important sink for APCAs in the environment [107]. However, most experiments were done with free EDTA, and the influence of metal complexation of the APCAs in the oxidative process still has to be investigated in detail.

### 2.3. Other APCAs

Photodegradation was also reported for Fe(III)DTPA but the products of the reaction were not determined [40]. A theoretical half-life of Fe(III)DTPA lower than that of Fe(III)EDTA was calculated. Hence, the rates of photodegradation of the iron(III) chelates apparently in- crease in the order NTA$^6 >$ EDTA$^6 >$ DTPA.

DTPA as well as HEDTA also adsorb to surfaces with pH-dependent charge, i.e. aluminium- or iron oxides [108]. Yet, we are not aware of data on the adsorption of DTPA and HEDTA in aquatic systems and their role for the elimination of these APCAs from the waterbody.
3. Microbial degradation of APCAs during wastewater treatment and in the environment

It has been demonstrated in numerous studies that NTA is easily biodegradable during wastewater treatment, in natural waters and in soils. In addition, many investigations indicate that biodegradation is complete and that IDA, a metabolite, is formed intracellularly during the microbial breakdown of NTA, does not accumulate in nature [4]. In contrast to NTA, contradictory results have been published concerning the biodegradation of EDTA or DTPA in the environment and only few reports can be found in the literature dealing with the biodegradability of other APCAs. Hence, the question of the importance of biodegradation for the environmental fate of these chelating agents is still unanswered.

3.1. NTA

3.1.1. During wastewater treatment

During the treatment of wastewater, NTA is mainly eliminated in biological treatment steps such as oxidation ponds and lagoons, activated sludge systems, or trickling filters. Systems that previously had not been exposed to NTA frequently require an adaptation period of 1-4 weeks before maximum degradation activity is achieved [4]. Elimination efficiencies for NTA reported for different wastewater treatment plants range between 70 and >90%. Less efficient NTA removal (in some cases merely around 50%) was only found at low temperatures during the winter months or in treatment plants characterised by elevated sludge loading rates [54,109–115]. NTA elimination during wastewater treatment was successfully simulated with a simple activated sludge model [116] which included only temperature-dependent growth of NTA-degrading microorganisms with NTA \( \mu_{\text{NTA}}(T) = 2.2 \, \text{day}^{-1} \exp(0.07 (T–20^\circ\text{C})) \), but the model neglected decay of NTA degraders and their growth with carbon sources other than NTA (the two effects probably compensating for each other).

Due to lack of sorption, NTA is accumulated neither in primary nor in secondary sludge and, therefore, the removal of NTA during anaerobic sewage sludge digestion is not of utmost importance. Nevertheless, Klein [117] and Kirk and co-workers [118] demonstrated NTA reductions in digesters varying from 8 to 45%, elimination of NTA close to 100% was observed when activated sludge already acclimated to NTA was added to the digestion process [119].

3.1.2. In the aquatic environment

According to numerous laboratory studies, NTA is biologically degraded in freshwater [4,120]. Contradictory results have been reported on its degradation in marine and estuarine water samples. Whereas Kirk and co-workers [121] found no NTA degradation by a marine bacterial population in coastal marine waters under aerobic or anoxic conditions, several other researchers found NTA degradation in estuarine and offshore environments [122–124]. In particular, in samples from an estuarine system which had been pre-exposed to NTA for several years, NTA was rapidly degraded with no lag period at the low NTA concentration of 10 \( \mu \text{g NTA l}^{-1} \) [123].

Apparently, NTA degradation follows first order kinetics, especially at the low NTA concentrations (below 2 \( \mu \text{g NTA l}^{-1} \)) found in surface and groundwaters [125]. Similar rate constants (about 0.3 day\(^{-1}\)) were determined for all freshwater systems situated in areas where NTA was not extensively used in detergents at the time of the study. They were approximately 3-4 times lower than those found in a river from a region where NTA-containing detergents were marketed. This again underlines the crucial role of acclimation of the microbial population present [123,126]. Similar to activated sludge from sewage treatment plants a few days to 4 weeks were typically required for an unadapted microbial population to acquire NTA-degrading ability. Acclimation to NTA and subsequent biodegradation occurred even at low environmental concentrations between 5 and 50 \( \mu \text{g NTA l}^{-1} \) [126], but the time period needed for developing degradation ability seemed to decrease with increasing NTA concentrations, as tested for a concentration range from 0.02 mg NTA l\(^{-1}\) to 20 mg NTA l\(^{-1}\) [127].

Analysis of measured NTA concentrations from a field study along a Swiss river receiving a high load of treated wastewater indicated biological NTA elimination with a half-life of about 8 h [62,128]. This agrees with the results of the laboratory studies described above [126]. Also the effect of temperature on NTA degradation was investigated [62,126,129]. While in summer about 90% of NTA was eliminated from the river Glatt over a flow distance of 22 km, only 65% of the NTA disappeared in winter [62]. Similarly, model calculations suggested that the behaviour of NTA in a Swiss lake can be attributed to biological degradation as only removal process with a constant degradation rate of 0.035 day\(^{-1}\), corresponding to a half-life of 20 days [105].

3.1.3. In aquifers and soils

In laboratory column systems with aquifer material from a natural river water/groundwater infiltration site, NTA was rapidly mineralised under both aerobic and denitrifying conditions [130]. According to these data, it was expected that very low NTA concentrations (<2 \( \mu \text{g l}^{-1} \)) will be found in the groundwater even in cases where the infiltration distance was only a few meters. Hence, even a significant increase in NTA concentration in river waters should not lead to higher concentrations in the groundwater. Low NTA concentrations measured in various groundwater samples, also in those obtained from infiltration areas in close distance to the feeding river, confirmed these predictions [38,61,67,131].
Also in soils, NTA readily decomposed under aerobic conditions with half-lives ranging from 3 to 7 days [132–134]. In contrast to Tiedje and Mason [132], both Tabetabai and Brenner [135] and Ward [133] observed NTA degradation also under anaerobic conditions where nitrate substituted molecular oxygen as a terminal electron acceptor. While nitrate concentrations in the system had no influence on the initial rates of NTA degradation, they affected the extent of the mineralisation under anaerobic conditions [133].

3.2. EDTA

3.2.1. During wastewater treatment and in laboratory test systems

Balances of the EDTA load in the influent and effluents of municipal wastewater treatment plants with sampling periods of several days gave no indication for a significant elimination of EDTA neither by biological nor by physicochemical processes [55, 56, 136, 137]. At the same time, elimination of EDTA neither by biological nor by physical processes was observed [138–141]. However, efficient EDTA elimination of about 80% in an industrial wastewater treatment plant has been reported lately [142]. In activated sludge samples taken from the very plant, almost 100% of added EDTA (measured as DOC) was degraded within 10 days.

Several reports have already described biologically mediated EDTA degradation under laboratory conditions. The first report [143] demonstrated decomposition of EDTA by microbial populations from an aerated lagoon receiving EDTA-containing industrial effluents. The authors followed [14C]CO2 formation from the iron(III) complex of radioactively labelled EDTA when incubated in the dark to prevent photodegradation. After an incubation period of 5 days, about 90% of the initially present EDTA had disappeared. 27% of the initial radioactivity of the acetate-labelled and 31% of the ethylene-labelled EDTA was recovered as 14CO2, indicating that both the ethylene backbone and the acetyl groups were attacked. During EDTA degradation, the concentrations of ED3A and IDA increased transiently. Most likely, these two compounds were intermediates of the microbial EDTA degradation. In contrast, other possible intermediates such as NTA, N,N′-EDDA and N,N′-EDDA, EDMMA and glycine were found only at very low concentrations. Supplementing the culture with NTA or ethylenediamine, EDTA in a marine ecosystem. In microcosms containing sea water and sediment illuminated with UV light, about 50% of the initially added Fe(III)EDTA complex was converted after 17 weeks [150]. Unfortunately, the poor experimental set-up used does not allow clear distinction between biodegradation and photodecomposition of the Fe(III)EDTA chelate.

3.2.2. In soils and sediments

Contradictory results have been published concerning
EDTA degradation in soils and sediments. Some groups found no biological EDTA breakdown [151,152], whereas others observed a slow microbial EDTA decomposition under aerobic conditions [153–156]. No EDTA mineralisation was found under anaerobic conditions [154,156].

3.3. DTPA

3.3.1. Wastewater

From measurements of EDTA and DTPA concentrations in wastewater effluents from pulp and paper mills, in the influent and effluent of wastewater treatment plants of these mills [157] as well as in the receiving lake [66], it was inferred that DTPA is biologically and/or chemically more readily degradable than EDTA. Indeed, in an activated sludge process, a reduction of DTPA amounting to 50–70% was observed while only 30% of EDTA was eliminated from the wastewater. Also in model wastewater treatment plants, the initial DTPA concentration was reported to be reduced by about 50% [158]. However, elimination could not unequivocally be attributed to microbial activity. In contrast, Hinck and co-workers [140] did not observe DTPA breakdown in activated sludge although the inoculum for the biodegradability tests was taken from a treatment plant that had been in contact with DTPA for over 5 years.

3.3.2. Soils and sediments

In soils and sediments, several groups observed the microbial breakdown of DTPA [151,155,156,159] and only Allard and co-workers [152] were unable to detect disappearance of DTPA in sediments. Both NTA and EDTA were formed as products of DTPA breakdown apparently resulting from the cleavage of a C–N bond within one of the ethylenediamine parts of the DTPA molecule [151]. Further metabolites were identified as incompletely substituted APCAs such as diethylenetriaminetetraacetate, diethylenediaminetetraacetate and ED3A which will spontaneously cyclise under neutral and especially acidic conditions to form oxopiperazinepolycarboxylic acids [159]. Unfortunately, it was not possible to distinguish whether these cyclised substances were formed during DTPA transformation itself or only subsequently during the analytical procedure. Nevertheless, the authors proposed that they might be formed during DTPA degradation and then accumulate in the environment due to their high stability. Some of these metabolites were also detected in samples taken from different German rivers during a subsequent screening program. The dominant metabolite was ED3A and its corresponding cyclised form [159].

3.4. Other synthetic APCAs

Only one report is available which describes the slow degradation of HEDTA by soil micro-organisms [155] according to which after 173 days of incubation the measured HEDTA concentration was below 5% of that initially supplied. In sterile controls, the fraction of non-biologically degraded HEDTA in this experiment was determined as 27%. Nevertheless, recently HEDTA was considered to be biologically not degradable [160].

Typical biodegradation tests using activated sludge as an inoculum and aerobic conditions showed that the amino acid derivatives β-ADA, SDA and MGDA are readily mineralised by micro-organisms. Biodegradation of ASDA was found to be stereospecific and only L-ASDA was easily biodegradable [9].

3.5. Naturally occurring APCAs

3.5.1. Biodegradation of EDDS

In some applications, EDDS is suggested to replace the poorly degradable EDTA. Several biodegradation studies with activated sludge from different sources have consistently demonstrated the stereospecificity of EDDS breakdown [141,161]: whereas [S,S]-EDDS and [R,S]-EDDS disappeared rapidly, [R,R]-EDDS was recalcitrant. However, only the S,S-isomer was completely mineralised [161] and the transformation of [R,S]-EDDS led to the production of a recalcitrant intermediate (AEAA), indicating the removal of one succinyl residue only. This suggests that only [S,S]-EDDS should be employed for the application in both domestic and industrial products.

Takahashi and co-workers [141] investigated the degradation of propanediaminedisuccinate (PDDS), a compound very similar to EDDS and also occurring as three different stereoisomers. In contrast to EDDS, all three isomers of PDDS were easily biodegradable. However, it was not determined whether the compound was completely mineralised.

3.5.2. Other naturally occurring metal-chelating compounds

There is little information on the possible biodegradation of naturally occurring APCAs. Nevertheless, several studies indicate that plant-produced chelating organic compounds, such as the APA mugineic acid, are degraded by rhizosphere micro-organisms under environmental conditions [162–165]. In this way, the microbial flora may reduce iron uptake of plants by both the degradation of the phytosiderophores and by competition for iron.

4. Degradation of APCAs by pure microbial cultures

Up to now, successful isolation of pure, APA-degrading microbial cultures has been restricted to microbial strains that are able to grow with NTA or EDTA. Some of these isolates have lately been shown to also be able to utilise [S,S]-EDDS (see Section 5.3). Reports on strains utilising DTPA or HEDTA, two other industrially important APCAs, have not been published so far, and we are
not aware of any attempts to isolate micro-organisms that grow with representatives of the new generation of APCAs such as β-ADA or MGDA.

4.1. Pure cultures utilising NTA

The isolation of NTA-degrading micro-organisms from a wide range of ecosystems has been described including river waters, activated sludge, sediments and soils [166–178]. Most of the pure cultures consisted of Gram-negative, obligately aerobic rods capable of utilising NTA as sole source of carbon/energy and nitrogen. Although for the majority of Gram-negative strains detailed characterisation was missing, reports before 1985 mostly allocated them to the genus *Pseudomonas* [166–170,172–175]. One Gram-negative strain was identified as *Listeria* sp. [139]. Two Gram-positive isolates were described and one of them was identified as a *Bacillus* sp. [175], whereas for strain TE3 [177] further work is needed to define its exact taxonomical position (morphological and physiological studies indicate its affiliation to the *Corynebacterium sensu stricto, Mycobacterium, Nocardia, Rhodococcus* group [179]). In addition to the strictly aerobic bacteria, denitrifying strains have also been isolated [171,178], whereas – so far – the enrichment of sulfate-reducing NTA degraders has not been successful [171,180].

A more detailed taxonomical study of Gram-negative NTA-utilising isolates [177–179,181], including most of the isolates still available at the time, revealed that none of them can be assigned to the genus *Pseudomonas* as defined by de Vos and de Ley [182]. These isolates formed three distinct groups (Table 3): the first group contains obligately aerobic, motile rods which are mostly pleomorphic and can utilise sugars and also a wide range of other substrates including methylated amines, hence indicating their ability to assimilate C1 units. These strains are phylogenetically localised in the *Agrobacterium–Rhizobium* branch of the α-subclass of *Proteobacteria* and they have been combined to form the new genospecies *Chelatobacter heintzii* [181]. Most recent data indicate that *Chelatobacter* strains are closely related to bacteria from the genus *Aminobacter* which are able to grow with methylated amines [183,184]. It is therefore possible that in the future both groups will be combined to a single genus [185].

The second group of isolates (Table 3) consisted of obligately aerobic, non-motile short rods or diplococci unable to grow with sugars. They again could not be assigned to an existing genus and, therefore, were established as the new genospecies *Chelatococcus asaccharovorans* [181]. This genus belongs to the α-subclass of *Proteobacteria* and 16S rRNA sequence comparison indicated *Rhodopseudomonas* as nearest neighbour.

The range of APCAs, which can be used as growth substrates by *Chelatococcus* and *Chelatobacter* strains, comprised NTA and IDA, while EDTA did not support growth [177]. Moreover, *C. asaccharovorans* grew with [S,S]-EDDS, whereas *Chelatobacter* strains did not [186]. No further APCAs have so far been tested as growth substrates for the NTA-degrading strains.

The third group of NTA degraders presently consists of only one strain (TE11) that is facultatively denitrifying.

### Table 3

<table>
<thead>
<tr>
<th>Isolation substrate</th>
<th>Strain [reference]</th>
<th>Taxonomical position</th>
<th>Main properties</th>
<th>Respiration</th>
<th>Growth with APCAs other than isolation substrate</th>
<th>μmax (h−1) on isolation substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTA</td>
<td><em>C. asaccharovorans</em> strain TE1 and TE2 [181]</td>
<td>α-2 branch of <em>Proteobacteria</em></td>
<td>Gram-negative, diplococci, non-motile, with S-layer, not utilising sugars</td>
<td>obligately aerobic</td>
<td>[S,S]-EDDS</td>
<td>0.08–0.1</td>
</tr>
<tr>
<td></td>
<td><em>C. heintzii</em>, strains TE4-TE10 and ATCC 29600 and ATCC 27109 [181]</td>
<td>α-2 branch of <em>Proteobacteria</em>, within the <em>Agrobacterium–Rhizobium</em> branch</td>
<td>Gram-negative, rods, pleomorphic, motile (1–3 subpolar flagella)</td>
<td>obligately aerobic</td>
<td>IDA</td>
<td>0.1–0.15</td>
</tr>
<tr>
<td></td>
<td>strain TE11 [178]</td>
<td>γ-branch of <em>Proteobacteria</em>, closely related to <em>Xanthomonas</em></td>
<td>Gram-negative, rods, motile</td>
<td>aerobic and denitrifying</td>
<td></td>
<td>0.08 (aerobic); 0.03 (denitrifying)</td>
</tr>
<tr>
<td>EDTA</td>
<td><em>A. radiobacter</em> ATCC 55002 [190]</td>
<td></td>
<td></td>
<td></td>
<td>PDTA (only when complexed with Fe(III))</td>
<td></td>
</tr>
<tr>
<td></td>
<td>strain BNC1/DSM 6780 [191]</td>
<td>α-branch of <em>Proteobacteria</em></td>
<td>Gram-negative, rods degrading EDTA only when complexed with Fe(III)</td>
<td>aerobic</td>
<td>NTA, IDA, ED3A</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>strain DSM 9103 [192]</td>
<td>α-branch of <em>Proteobacteria</em>, within the <em>Agrobacterium–Rhizobium</em> branch</td>
<td>Gram-negative, diplo-rods, non-motile when grown with EDTA</td>
<td>aerobic</td>
<td>NTA, IDA, N,N'-EDDA, [S,S]-EDDS</td>
<td>0.06–0.07</td>
</tr>
</tbody>
</table>
(Table 3). Apart from its ability to denitrify and its inability to grow with IDA, this Gram-negative motile isolate differs only slightly from C. heintzii with respect to its physiological and nutritional characteristics. Polyamine and quinone patterns, however, discriminate TE11 from the latter and suggest an allocation to the γ-subclass of Proteobacteria. The presently available data suggest that TE11 is closely related to but still clearly distinct from members of the genus Xanthomonas [178].

Surface antibodies were raised against C. heintzii and C. asaccharovorans and were used to investigate their distribution in the environment [187,188]. In surface waters, cells reacting with antibodies made up for 0.01–0.1% of the total (acridine orange) cell number. In activated sludges of wastewater treatment plants, the proportion was about 10-fold higher. Surprisingly, no significant difference in the fraction of cells reacting with antibodies was observed between samples collected from treatment plants, which differed considerably with respect to their efficiency in NTA elimination [187]. Despite the immunological detection of bacteria of the genera Chelatobacter and Chelatococcus in activated sludge and in the environment, the contribution of these strains to the degradation of NTA is still unknown. To tackle this question, an experimental approach would be required which consists of the testing of individual cells present in environmental samples for both their serological reaction with the antibodies above described and for NTA degradation.

It should be added here that in several Pseudomonas strains (P. aeruginosa, P. fluorescens and P. cepacia), NTA stimulated growth by promotion of the iron uptake, although it did not serve as growth substrate [189]. Apparently, NTA assisted the intracellular incorporation of iron through an active transport system, as do naturally occurring siderophores. In contrast, other chelators such as EDTA, EDDS and EDDHA had an inhibitory rather than a stimulating effect on growth of the tested bacterial strains. Hence, in the environment, NTA might act as a siderophore-like compound for some micro-organisms. However, the interesting question of the intracellular fate of NTA used by micro-organisms as a siderophore has not been further studied.

4.2. Pure cultures utilising EDTA

Most likely because of the poor biodegradability of EDTA, the successful isolation of EDTA-degrading bacteria has only recently been reported [190–192] (Table 3). While the isolates described by Nörtemann [191] and Witschel and co-workers [192] exhibit many similarities, the pure culture isolated by Laufl and co-workers [190] has rather different properties with respect the EDTA degradation characteristics.

The latter strain, identified as Agrobacterium radiobacter, was isolated from a secondary waste treatment facility that had received wastes containing Fe(III)EDTA for several years. Consequently, the isolate grew with Fe(III)EDTA as sole source of carbon, whereas no significant growth was observed with uncomplexed EDTA [190]. The strain was able to degrade Fe(III)EDTA at initial EDTA concentrations higher than 100 mM but it failed at low concentrations, and the residual EDTA concentrations were always in the range of approximately 3–5 mM. In batch culture, the extent of degradation was more complete for lower initial pHs (probably due to a slower increase of culture pH from excreted NH₃ during EDTA breakdown). Addition of peptone or yeast extract to the medium did not affect EDTA degradation but that of glycerol reduced the amount of EDTA degraded drastically. It was suggested that in the presence of glycerol, the Agrobacterium strain utilised EDTA merely as nitrogen source and no longer as carbon and energy source. Other APCAs were tested for growth of the Agrobacterium strain, but only Fe(III)PDTA was degraded and no growth was observed with NTA, IDA or EDDDA, although the latter compounds are supposed to be intermediates in the breakdown of EDTA [143,193]. Furthermore, the strain was able to utilise a wide range of sugars, carboxylic acids and amino acids as growth substrates.

From an industrial sewage treatment plant receiving EDTA-containing wastewater, Nörtemann [191] was able to isolate a mixed culture of strain BNC1 and BNC2 that grew at considerably lower initial EDTA concentrations (≤1 mM) than the Agrobacterium strain. In the mixed culture, only strain BNC1 degraded EDTA and its metabolic activity was stimulated in the presence of other micro-organisms probably providing vitamins. Indeed, addition of vitamins was demonstrated to significantly enhance growth of a pure culture of strain BNC1 [194]. As long as EDTA was complexed with alkaline earth metal ions, strain BNC1 tolerated EDTA concentrations up to approximately 16 mM. However, the presence of uncomplexed EDTA considerably reduced the viability of cells and no growth with free EDTA was observed [194]. A similar observation had already been reported for an NTA-degrading strain [176]. Such adverse effects are probably due to interactions of free EDTA or NTA with metal ions that stabilise the bacterial cell surface because destabilising effects of free EDTA on various Gram-negative bacteria are well known [195,196]. A taxonomic analysis indicated an allocation of strain BNC1 to the α-subgroup of Proteobacteria [193]. However, a more detailed taxonomical study including 16S rRNA sequencing is needed to more exactly allocate this strain, in particular to determine its relation to the other EDTA-degrading strains known so far.

Recently, another EDTA-degrading bacterial isolate DSM 9103 has been described [192]. Starting with the mixed culture from Gschwind [144] a Gram-negative, obligately aerobic bacterium was obtained which also belongs to the α-subgroup of Proteobacteria. Both polar lipid pattern and 16S rRNA sequence of the strain are
indicative of members of the *Agrobacterium-Rhizobium* branch. Indeed, the 16S rRNA sequence of the isolate showed highest similarity (96.1%) to that of *Rhizobium loti* [186], now renamed *Mesorhizobium loti* [197]. When added to raw wastewater or activated sludge from a municipal wastewater treatment plant cells strain DSM 9103 were able to degrade EDTA to completion (initial concentration approximately 3.5 mM) [192]. However, supplementation with mineral medium (containing high concentrations of alkaline earth metal ions) was necessary to obtain efficient EDTA removal in activated sludge, indicating again the importance of EDTA complexation for its biodegradability. Interestingly, strain DSM 9103 is not only able to grow with EDTA but also with other APCAs such as NTA, IDA and [S,S]-EDDS [198].

Although ferrichrome and ferrioxamine type siderophores do not belong to the class of APCAs, it should be mentioned here that pure cultures of ferriochrome A-degrading micro-organisms had been isolated already in the 1960s and that the breakdown of these siderophores has been studied [199–207]. Interestingly, based on 16S rRNA homology, a deferrioxamine B-degrading bacterium was found to be a strain of *R. loti* [205] and, therefore, it seems a close relative of some of the EDTA-degrading strains presently known (see above). Speciation was found to be affecting degradability with only the iron-free compound (deferrioxamine B, but not ferrioxamine B) being degraded [202,203,206].

### 4.3. Potential of bacterial isolates for the treatment of APCA-containing wastewaters

NTA is removed efficiently during biological wastewater treatment (see Section 3) and, therefore, the application of NTA-degrading isolates for a special treatment of NTA-containing wastewaters has not been investigated. In contrast, elimination of EDTA in most wastewater treatment plants is negligible [55,56,137]. Thus, the use of EDTA-degrading strains for the treatment of wastewaters containing high concentrations of EDTA was proposed and also patented [208–212].

A process based on a mixed culture of strain BNC1 and BNC2 for treating industrial EDTA-containing wastewaters has been developed. The cells were fixed on carrier material and employed in a biofilm airlift-loop reactor [211]. The reactor was operated at dilution rates between 0.06 and 0.3 h⁻¹ and allowed to achieve an EDTA elimination efficiency between 95 and 98% for an EDTA concentration of 0.45 g l⁻¹ in the feed medium. To mimic the situation in real wastewater treatment plants where EDTA is rarely the sole source of carbon and nitrogen, a mixture of EDTA and glycerol (each 300 mg l⁻¹) was fed to the reactor (D = 0.2 h⁻¹). Initially, when feeding the mixture, no significant reduction of EDTA elimination efficiency was observed but after more than 10 days, the extent of EDTA degradation started to decrease gradually, probably as a result of changes in the composition of the microbial population. In fact, the biofilm on the carrier particles was found to become more filamentous and voluminous when feeding the substrate mixture. Hence, in wastewaters containing a high fraction of readily degradable carbon, a two-step process might be of advantage where first the easily degradable carbon sources are eliminated before EDTA is removed with the help of strain BNC1 in a second step [212].

Industrial wastewaters often contain the Fe(III) complex or other heavy metal complexes of EDTA, but strain BNC1 is not able to attack these chelates (see Section 7). To treat wastewaters containing such EDTA complexes, a precipitation step was established before the EDTA solution was supplied to the bioreactor. By the addition of a large excess of calcium hydroxide to a Fe(III)-EDTA-containing synthetic wastewater, Fe³⁺ was exchanged against Ca²⁺ and then precipitated as iron hydroxide. A solution containing mainly CaEDTA was obtained, and after adjusting the pH, the Fe(III)EDTA elimination efficiency by strain BNC1 was about 98% [213].

Eventually, the system was tested for its performance in eliminating EDTA in real wastewaters originating from the dairy industry, vat cleaning in a brewery, the pulp and paper industry, and from the photographic industry. The data indicated that all these wastewaters were treatable with immobilised EDTA-degrading bacteria but occasionally specific pre-treatment steps were required. EDTA in wastewater from pulp and paper manufacturing was efficiently removed without any pre-treatment. Wastewaters from the dairy industry could be treated in a two-step process because of the presence of high amounts of non-EDTA DOC, which must be removed first. Wastewaters from vat cleaning or the photographic industry again contain high amounts of heavy metals and a precipitation step (or a similar process to remove these metals) has to be carried out before biological treatment is possible [212]. The results are quite promising with respect to a potential utilisation of immobilised cells of strain BNC1 for the biological treatment of EDTA-containing wastewaters. Nonetheless, a note of caution should be added because all tests for EDTA degradation in industrial wastewaters have so far been performed under constant conditions, a situation that is rarely encountered in real life. Therefore, important data are still missing with respect to overall elimination efficiencies in case of variations in wastewater composition or other fluctuations of parameters (e.g. in pH or feeding rate).

### 5. Biochemistry and genetics of APCA degradation

Information on the biochemistry and genetics of APCA degradation is of course restricted to those compounds for which isolation and cultivation of pure bacterial cultures has been reported, i.e. NTA, EDTA and [S,S]-EDDS.
Again, of all APCAs, NTA has clearly received most attention.

5.1. NTA degradation

Research mainly focused on the intracellular metabolism of NTA and the question of its transport into cells was rarely addressed. One can merely speculate that NTA is actively transported across the cell membrane because [14C]NTA uptake was inhibited by KCN [169,214].

5.1.1. NTA catabolism

Based on identification of products accumulated during NTA degradation and of enzymatic activities enhanced in cell-free extracts of NTA-grown cells, already some 25 years ago a pathway for the aerobic NTA breakdown was proposed (Fig. 4) [166,170,174,215]: NTA was thought to be metabolised in two steps via IDA to glycine and glyoxylate, with the first step being catalysed by a monooxygenase (MO) [166,170]. The products were then channeled into the central metabolism. A comparison of enzyme activities in cell-free extracts of glucose- and NTA-grown cells gave strong evidence for the involvement of glycine decarboxylase and serine hydroxymethyl transferase in the subsequent metabolism of glycine, and that glyoxylate carboligase and tartronic semialdehyde dehydroge-

Fig. 4. Metabolic pathway of NTA in obligately aerobic and facultatively denitrifying Gram-negative bacteria. (1) Transport enzyme; (2) NTA MO; (3) NTA DH; (4) NTA DH/NtrR complex; (5) IDA DH; (6) glyoxylate carboligase; (7) tartronate semialdehyde reductase; (8) serine hydroxymethyl transferase; (9) glycine synthetase (decarboxylase); (10) serine-oxaloacetate aminotransferase; (11) transaminase; (12) glutamate DH; (13) hydroxypyruvate reductase; (14) glycine kinase; (methylene)FH4, (N<sub>5</sub>,N<sub>10</sub>-methylene) tetrahydrofolic acid; PLP, pyridoxal phosphate; TPP, thiamine pyrophosphate (from [6] with permission).
nase (DH) took care of the transformation of glyoxylate into glycerate [170].

The proposed pathway was confirmed by purification and characterisation of two enzymes from C. heintzii ATCC 29600 that catalyse the degradation of NTA and IDA, respectively [216,217]. A MO (NTA MO) was found to catalyse the attack on NTA leading to the formation of IDA and glyoxylate. In a second step, IDA is oxidatively split into glycine and glyoxylate, not by NTA MO as originally supposed [166], but by a membrane-bound IDA DH. Both enzymatic activities were not only detected in C. heintzii but also in Chelatococcus strains [218].

5.1.1.1. Characteristics of NTA MO. NTA MO from C. heintzii ATCC 29600 was first purified and characterised by Uetz and co-workers [216] and later more information was obtained by sequencing the corresponding genes [219,220] (Table 4). NTA MO was originally thought to consist of two weakly associated components, cA and cB, both of which must be present to catalyse the transformation of NTA [216]. More recently, it was demonstrated [220] that cA and cB are two distinct enzymes, with cA being the true MO catalysing the oxidative cleavage of NTA with the consumption of FMNH₂ and molecular oxygen, and cB being an oxidoreductase providing FMNH₂ for the MO by transferring reducing equivalents from NADH to FMN (Fig. 5). cB can be replaced by other NADH₂:FMN oxidoreductases, for instance the oxidoreductase from Photobacterium fischeri which normally supplies FMNH₂ to the luciferase enzyme. Therefore, FMN and FMNH₂ most presumably act here as real coenzymes not being tightly bound to cB. Recently, similar two-enzyme systems have been described, also consisting of a NADH₂:FMN oxidoreductase and a MO, namely those involved in the oxidation of dibenzothiophene [221] and in the synthesis of antibiotics such as pristinamycin IIₐ [222], actinorhodin [223] or valanimycin [224]. It therefore is not surprising that cA and cB exhibited the highest homology with proteins of this two-enzyme system group when the amino acid sequences were compared [220, 224,225]. Eventually, sequence comparison revealed a significant similarity between FMNH₂-dependent MOs from E. coli, Bacillus subtilis and Pseudomonas putida catalysing the desulfonation of alkanesulfonates and several of the above mentioned MOs (including NTA MO) [226]. In this study, also a conserved domain present in all MOs was detected which might be part of the active site of these enzymes. This domain, however, contained none of the protein motifs known so far.

Also from Chelatococcus strain TE2, a FMNH₂-dependent MO was purified which exhibited many similarities with cA from C. heintzii ATCC 29600. The N-termini of NTA MO from C. heintzii ATCC 29600 was first purified and characterised by Uetz and co-workers [216] and later more information was obtained by sequencing the corresponding genes [219,220] (Table 4). NTA MO was originally thought to consist of two weakly associated components, cA and cB, both of which must be present to catalyse the transformation of NTA [216]. More recently, it was demonstrated [220] that cA and cB are two distinct enzymes, with cA being the true MO catalysing the oxidative cleavage of NTA with the consumption of FMNH₂ and molecular oxygen, and cB being an oxidoreductase providing FMNH₂ for the MO by transferring reducing equivalents from NADH to FMN (Fig. 5). cB can be replaced by other NADH₂:FMN oxidoreductases, for instance the oxidoreductase from Photobacterium fischeri which normally supplies FMNH₂ to the luciferase enzyme. Therefore, FMN and FMNH₂ most presumably act here as real coenzymes not being tightly bound to cB. Recently, similar two-enzyme systems have been described, also consisting of a NADH₂:FMN oxidoreductase and a MO, namely those involved in the oxidation of dibenzothiophene [221] and in the synthesis of antibiotics such as pristinamycin IIₐ [222], actinorhodin [223] or valanimycin [224]. It therefore is not surprising that cA and cB exhibited the highest homology with proteins of this two-enzyme system group when the amino acid sequences were compared [220, 224,225]. Eventually, sequence comparison revealed a significant similarity between FMNH₂-dependent MOs from E. coli, Bacillus subtilis and Pseudomonas putida catalysing the desulfonation of alkanesulfonates and several of the above mentioned MOs (including NTA MO) [226]. In this study, also a conserved domain present in all MOs was detected which might be part of the active site of these enzymes. This domain, however, contained none of the protein motifs known so far.

Also from Chelatococcus strain TE2, a FMNH₂-dependent MO was purified which exhibited many similarities with cA from C. heintzii ATCC 29600. The N-termini of NTA MO from C. heintzii ATCC 29600 was first purified and characterised by Uetz and co-workers [216] and later more information was obtained by sequencing the corresponding genes [219,220] (Table 4). NTA MO was originally thought to consist of two weakly associated components, cA and cB, both of which must be present to catalyse the transformation of NTA [216]. More recently, it was demonstrated [220] that cA and cB are two distinct enzymes, with cA being the true MO catalysing the oxidative cleavage of NTA with the consumption of FMNH₂ and molecular oxygen, and cB being an oxidoreductase providing FMNH₂ for the MO by transferring reducing equivalents from NADH to FMN (Fig. 5). cB can be replaced by other NADH₂:FMN oxidoreductases, for instance the oxidoreductase from Photobacterium fischeri which normally supplies FMNH₂ to the luciferase enzyme. Therefore, FMN and FMNH₂ most presumably act here as real coenzymes not being tightly bound to cB. Recently, similar two-enzyme systems have been described, also consisting of a NADH₂:FMN oxidoreductase and a MO, namely those involved in the oxidation of dibenzothiophene [221] and in the synthesis of antibiotics such as pristinamycin IIₐ [222], actinorhodin [223] or valanimycin [224]. It therefore is not surprising that cA and cB exhibited the highest homology with proteins of this two-enzyme system group when the amino acid sequences were compared [220, 224,225]. Eventually, sequence comparison revealed a significant similarity between FMNH₂-dependent MOs from E. coli, Bacillus subtilis and Pseudomonas putida catalysing the desulfonation of alkanesulfonates and several of the above mentioned MOs (including NTA MO) [226]. In this study, also a conserved domain present in all MOs was detected which might be part of the active site of these enzymes. This domain, however, contained none of the protein motifs known so far.

Also from Chelatococcus strain TE2, a FMNH₂-dependent MO was purified which exhibited many similarities with cA from C. heintzii ATCC 29600. The N-termini of NTA MO from C. heintzii ATCC 29600 was first purified and characterised by Uetz and co-workers [216] and later more information was obtained by sequencing the corresponding genes [219,220] (Table 4). NTA MO was originally thought to consist of two weakly associated components, cA and cB, both of which must be present to catalyse the transformation of NTA [216]. More recently, it was demonstrated [220] that cA and cB are two distinct enzymes, with cA being the true MO catalysing the oxidative cleavage of NTA with the consumption of FMNH₂ and molecular oxygen, and cB being an oxidoreductase providing FMNH₂ for the MO by transferring reducing equivalents from NADH to FMN (Fig. 5). cB can be replaced by other NADH₂:FMN oxidoreductases, for instance the oxidoreductase from Photobacterium fischeri which normally supplies FMNH₂ to the luciferase enzyme. Therefore, FMN and FMNH₂ most presumably act here as real coenzymes not being tightly bound to cB. Recently, similar two-enzyme systems have been described, also consisting of a NADH₂:FMN oxidoreductase and a MO, namely those involved in the oxidation of dibenzothiophene [221] and in the synthesis of antibiotics such as pristinamycin IIₐ [222], actinorhodin [223] or valanimycin [224]. It therefore is not surprising that cA and cB exhibited the highest homology with proteins of this two-enzyme system group when the amino acid sequences were compared [220, 224,225]. Eventually, sequence comparison revealed a significant similarity between FMNH₂-dependent MOs from E. coli, Bacillus subtilis and Pseudomonas putida catalysing the desulfonation of alkanesulfonates and several of the above mentioned MOs (including NTA MO) [226]. In this study, also a conserved domain present in all MOs was detected which might be part of the active site of these enzymes. This domain, however, contained none of the protein motifs known so far.

Also from Chelatococcus strain TE2, a FMNH₂-dependent MO was purified which exhibited many similarities with cA from C. heintzii ATCC 29600. The N-termini of
both proteins were virtually identical with only one amino acid being different. In addition, the overall amino acid composition of the two MOs was very similar (however, the whole amino acid sequence of cA from *C. asacharovorans* has not been unraveled yet). In contrast, no protein equivalent to cB has yet been isolated from *C. asacharovorans*, but the existence of such a protein is compulsory as indicated by the fact that cB from *C. heintzii* could form a functional, NTA-oxidising enzyme complex with cA from *C. asacharovorans* [218].

Consistently, studies with antisera raised against cA and cB from *C. heintzii* ATCC 29600 indicated the presence of cA in both *Chelatobacter* and *Chelatococcus* strains, while cB was found to be restricted to *Chelatobacter* strains [6,218]. cA and cB could immunologically only be detected in cells which were grown with NTA. Interestingly, in trimethylamine-cultivated *C. heintzii* bacteria, a protein of 70 kDa, thus significantly larger than NTA MO, cross-reacting with cA antiserum was found. In cell extracts, however, no NTA MO activity but only trimethylamine-reacting cA in both *Chelatobacter* species was detectable [218].

Also DNA–DNA hybridisation data suggested that the gene encoding cA is more conserved than the cB gene [225] (see Section 5.1.4). This confirms the crucial role of cA (NTA MO) for NTA degradation in contrast to that of cB, which probably can be replaced by any other enzyme, providing reduced FMN. Here it is noteworthy that – apart from the luciferase system and the two-enzyme systems listed above – other NADH₂:FMN oxidoreductases providing free reduced flavins have been described which seem to be involved in the reduction of iron complexed to siderophores [227]. However, no similarity at the amino acid sequence level among the various classes of these oxidoreductases has so far been found.

An unusual property of the oxidoreductase cB from *C. heintzii* was that the reduction of FMN was highly stimulated by the addition of NTA. Other structurally related compounds, among them also IDA, had no such stimulating effect when tested in reaction mixtures containing both cB and cA [216]. According to these NADH₂:FMN oxidoreductases providing free reduced flavins have been described which seem to be involved in the reduction of iron complexed to siderophores [227]. However, no similarity at the amino acid sequence level among the various classes of these oxidoreductases has so far been found.

An unusual property of the oxidoreductase cB from *C. heintzii* was that the reduction of FMN was highly stimulated by the addition of NTA. Other structurally related compounds, among them also IDA, had no such stimulating effect when tested in reaction mixtures containing both cB and cA [216]. According to these NADH₂:FMN oxidoreductases, the substrate spectrum of the NTA MO (cA) was stated to be rather narrow. Yet, catalysis by the NTA MO of the oxidative splitting of IDA to glycine and glyoxylate 

5.1.2. Enzymatic degradation of IDA

Since the NTA MO does not catalyse the cleavage of IDA, an additional enzyme must exist. Indeed, IDA-oxidising activity was detected in the particulate membrane fraction from cells of *C. heintzii* and *C. asacharovorans*, which was subsequently attributed to a membrane-bound IDA DH [217] (Table 4). IDA DH from *C. heintzii* was distinctly different from other membrane-bound DHs such as succinate DH and it merely catalysed the oxidative splitting of IDA to glycine and glyoxylate. IDA DH is probably not only associated with the membrane but exists as an integral membrane protein which feeds electrons from the oxidation of IDA into the electron transport chain via the ubiquinone pool [217]. Partial enrichment of IDA DH was achieved by extracting the enzyme from membranes with the help of cholate and incorporating it into soybean phospholipid vesicles. In this artificial system, IDA DH activity was fully reconstituted upon addition of the major quinones in the *Chelatobacter* genus as intermediate electron carriers, i.e. ubiquinone Q₁ or ubiquinone Q₁₀, and of iodonitrotetrazolium chloride as terminal electron acceptor [217].

5.1.3. NTA degradation in denitrifying bacteria

Obviously, the initial step in the metabolism of NTA in denitrifying NTA degraders has to proceed via an oxygen-independent step. In membrane-free extracts of strain TE11 grown under anoxic conditions, a protein complex consisting of two enzymes, a NTA DH and a nitrate reductase (NtrR), catalysed the first step of NTA oxidation resulting in the formation of IDA and glyoxylate [228] (Table 4). Only with both enzymes present, NTA was transformed, and activity was coupled to a phenazine-methosulfate (PMS)-dependent transfer of electrons from NTA to nitrate which was reduced to nitrite. Apart from the artificial dye PMS, no naturally occurring compound has been found so far which mediates the electron transfer from NTA DH to NtrR. As for NTA MO, the substrate spectrum of the DH seems to be restricted to NTA.

N-Ethylmaleimide, a thiol-binding reagent, inhibited NTA DH but an excess of dithiothreitol partly restored enzymatic activity, indicating the involvement of thiol groups in reaction catalysis by NTA DH [229]. As a redox component NTA DH contains a covalently bound FAD moiety. Additionally, an iron content of about four iron atoms per mol of enzyme was found. Since results from difference spectra of oxidised versus reduced enzyme argued against the presence of heme chromatophores, NTA DH is supposed to contain two 2Fe–2S clusters, one in each monomer of the homodimeric enzyme [229]. Thus, electrons derived from NTA are presumably first transferred to FAD, then to the iron–sulfur cluster, and finally to PMS or the so far unknown in vivo electron carrier.

The in vitro found NTA DH/NtrR complex is rather unusual and in fact the first enzyme complex reported where a catabolic DH is so tightly associated with a NtrR. Therefore, the in vivo localisation of both NTA DH and NtrR was investigated by immunochromical labeling of the enzymes to address the question of whether the complex is an artefact produced during cell disruption or whether it really exists in the cells. NTA DH was detected in the cytoplasm, whereas NtrR was associated with or
integrated in the cytoplasm membrane [225]. The reductase from the NTA DH/NtrR complex appears to belong to the dissimilatory type of NtrR [225] because NtrR expression was not observed under conditions where only the assimilatory type of NtrRs should be produced, i.e. during aerobic growth with nitrate as sole nitrogen source. In contrast, ammonium did not repress the reductase expression under anoxic conditions. Moreover, NtrR production was independent of the presence of NTA in the growth medium while NTA DH was only formed during cultivation with NTA [225].

NTA DH was also purified from aerobically grown strain TE11, and seemed to be identical to the enzyme expressed under anoxic conditions [229]. However, during aerobic growth, electrons were transferred to the respiration chain, but again, only in the presence of PMS. Although the NTA DH/NtrR complex from denitrifying cells and NTA DH from aerobically grown cells catalysed the same overall reaction, association of NTA DH with NtrR led to a marked change in the kinetic properties with an increase in affinity for NTA [6]. However, it remains to be tested whether this is real or an artefact originating from the purification procedure.

The interesting aspect of the process of in vivo electron transfer from NTA DH to either NtrR or to the respiration chain is a still unsolved and intriguing question, especially when bearing in mind that NTA DH apparently is not associated with the cytoplasmic membrane. In analogy to the situation found in case of the functionally related trimethylamine DH [230], an electron-transferring flavoprotein was suggested to be involved [229], but this hypothesis has not yet been confirmed.

Also in the denitrifying strain TE11 – similar to the obligately aerobic Chelatobacter and Chelatococcus strains – IDA is further oxidised by a membrane-bound IDA DH [229].

5.1.4. Genetic information concerning NTA metabolism

Quite recently, the NTA-metabolising enzymes described above were also investigated at the genetic level [219,220,225]. The genes of the two enzymes, cA and cB (ntaA/nmoA and ntaB/nmoB, respectively), were cloned from C. heintzii ATCC 29600. They were oriented divergently with an intergenic region of 307 bp, a rather unusual organisation for genes whose products act so closely together. Downstream of the gene for cA, additional open reading frames (ORFs) (ORF1/nmoR and nmoT) were found. In its N-terminus, the 24.4 kDa gene product of ORF1/nmoR exhibited a DNA-binding motif (helix-turn-helix) which is characteristic for a family of bacterial regulatory proteins called GntR family. Hence, the ORF1/nmoR gene product might be involved in the regulation of the expression of the genes for cA and cB. Unfortunately, the attempts to further characterise the putative NTA regulator have failed so far. Sequence similarities of nmoT with several transposases indicate that it may be part of an insertion element.

To test the presence of genes homologous to ntaA and ntaB in other NTA-degrading strains, DNA–DNA hybridisation experiments were performed using different restriction fragments from cloned parts of the cA and cB genes from C. heintzii ATCC 29600 as probes [225]. Surprisingly, in C. heintzii TE6, only regions homologous to the cA gene and ORF1 but not to the gene for cB were revealed although previous immunological studies had indicated the presence of proteins similar to cA and cB [218]. Conversely, no crossreacting proteins similar to cB were detected in C. asaccharovorans TE2, whereas at the DNA level, regions highly homologous to those of ORF1 and the genes encoding cA and cB in C. heintzii ATCC 29600 were found. However, shortly after the start of the ntaB equivalent gene from strain TE2, the homology with ntaB from C. heintzii ATCC 29600 ended. Apparently, the gene for cB in C. asaccharovorans TE2 is disrupted in its reading frame, and most probably no intact cB is synthesised.

An attempt was made to clone the gene for NTA DH from denitrifying strain TE11 [225] starting with oligonucleotide probes derived from the N-terminal amino acid sequence of the enzyme [229]. However, because the specificity of the available sequence information was too low, the probes synthesised hybridised with many different DNA fragments. This is consistent with the observation that the N-terminal amino acid sequence of NTA DH exhibited high similarity to other flavin-containing enzymes all possessing a common FAD-binding segment (βββ-fold) in their N-terminus [229].

5.2. Catabolism of EDTA and the similarity of EDTA MO with NTA MO

Due to the successful isolation of EDTA-degrading bacterial strains within the 1990s, first information on the metabolism of EDTA could be obtained. However, so far only strain BNC1 and DSM 9103 have been studied biochemically while the pathway for EDTA breakdown in the Agrobacterium strain growing with Fe(III)EDTA is still unknown.

Uptake of EDTA into cells of strain DSM 9103 was mediated by an energy-dependent carrier, which is most probably driven by the proton motive force. This transport system had a high apparent affinity for EDTA. It was rather specific for EDTA because, of several APCAs and other structurally related compounds, only DTPA competitively inhibited the transport of EDTA [231].

Based on products excreted by a mixed microbial culture during EDTA degradation, Belly and co-workers [143] proposed two possible pathways for the microbial breakdown of EDTA, i.e. either the successive removal of acetyl groups from EDTA or the cleavage of a C–N bond within the ethylenediamine part of the molecule. The former mechanism, the removal of acetyl groups, was re-
cently confirmed in cell-free extracts obtained from strain BNC1 [193,232] and from strain DSM 9103 [198]. The EDTA-splitting activity required molecular oxygen, NADH2 and FMN and yielded glyoxylate and transiently ED3A, the latter being further degraded under the same assay conditions resulting in the formation of another glyoxylate and N,N'-EDDA. In both strains, no N,N'-EDDA production was observed, indicating regiospecific removal of the second acetyl group from EDTA.

From strain DSM 9103, a two-enzyme system was purified which catalysed the removal of two acetyl groups from EDTA (Fig. 5, Table 4) [198]. Most likely, one enzyme (cA) is a MO catalysing the oxidative cleavage of EDTA and ED3A while consuming oxygen and reduced FMN (it has not been demonstrated yet that molecular oxygen is incorporated into water and glyoxylate). The second enzyme (cB) is a NADH2:FMN oxidoreductase that provides FMNH2 for the MO. As in case of NTA MO, the oxidoreductase from P. fischeri could take over the function of cB. Furthermore, cB from the NTA-oxidising enzyme system was able to replace cB in the EDTA-degrading enzyme system, and vice versa.

In contrast to the NTA-degrading enzyme system, EDTA MO exhibited a broader substrate spectrum. It cleaved not only EDTA and ED3A but also other APCAs such as NTA, DTPA, HEDTA, PDTA and 1,3-diaminopropanol-tetraacetic acid. N,N'-EDDA was not further transformed by EDTA MO but a cofactor-independent N,N'-EDDA-degrading activity was detected in cell-free extracts of strain DSM 9103 [198]. Additionally, N,N'-EDDA transformation was also observed in membrane fractions of DSM 9103 cells. By both, the soluble and the particulate fraction, N,N'-EDDA was oxidatively split resulting in the formation of one glyoxylate molecule. Apparently, an additional acetyl residue was removed from the ethylenediamine backbone probably leaving EDMA (Fig. 5). Preliminary data indicate the involvement of a DH [233]. However, the enzyme(s) responsible for this activity still remain(s) to be isolated.

One can speculate that EDMA is further metabolised by elimination of the last acetyl moiety leaving ethylenediamine which can be transformed to glycine. Alternatively, EDMA might be cleaved within the ethylenediamine part of the molecule yielding finally two molecules of glycine. As a third possibility, EDMA might be converted into IDA by the removal of the primary amine group. IDA can then be oxidised to form glycine and glyoxylate. The metabolic pathway for the products of EDTA degradation, glycine and glyoxylate, is assumed to be similar to that described previously for the NTA degrader C. heintzii [233].

All in all, important similarities between the NTA me-
tabolism in \textit{C. heintzii} and the EDTA breakdown in strain DSM 9103 are discernible. In both cases, a MO catalyses the first attack on the chelating agent and the MO is dependent on an accessory enzyme providing reduced FMN. Less acetylated intermediates of the metabolism (carrying only two acetyl groups) are then further converted by a DH. The close taxonomical neighbourhood of the organisms as well as these biochemical similarities foster the hypothesis that the enzymes have common ancestors. Only genetic studies, in particular on strain DSM 9103, can give an answer to this question.

Just recently, an EDTA MO from bacterium BNC1 was purified and characterised [234]. In contrast to EDTA MO from strain DSM 9103, this enzyme catalysed the removal of only one acetyl group from EDTA resulting in the formation of ED3A and glyoxylate, whereas formation of EDDA could not be detected. However, whether or not ED3A is accepted as a substrate by the EDTA MO from strain BNC1 was not tested. On the other hand, the EDTA MOs from both strains share the property of acting together with a distinct oxidoreductase providing reduced FMN.

5.3. \textit{EDDS} catabolism

\textit{EDDS} is a structural isomer of EDTA and it can also be used as a growth substrate by the EDTA-degrading strain DSM 9103. Therefore, it was speculated that \textit{EDDS} might be transported into the cells by the EDTA carrier and subsequently be cleaved by EDTA MO. This, however, proved not to be the case [198,231,235]. Whereas uptake of \textit{EDDS} by strain DSM 9103 has not yet been studied, the initial step in the intracellular \textit{EDDS} breakdown has been elucidated [235].

The catabolism of \textit{EDDS} was initiated by a carbon--nitrogen lyase catalysing the non-hydrolytic cleavage of the C–N bond between the ethylenediamine part of the molecule and one of the succinyl residues without any cofactors being required (Fig. 6, Table 4). The reaction led to the formation of fumarate and AEAA. Also in \textit{C. asaccharovorans}, a similar [\(S,S\)]-EDDS-splitting activity was detected requiring no cofactors and resulting in the formation of the same products as those found in DSM 9103, thus indicating the action of the same type of enzyme [235].

The further degradation of AEAA remains still to be unravelled. To date, one can merely speculate that, catalysed by a DH or a MO, the C–N bond between the succinyl residue and the ethylenediamine part of the molecule is split, or that an aspartyl residue is removed by the cleavage of a C–N bond within the ethylenediamine part of AEAA [233].

Out of the three stereoisomers of \textit{EDDS} ([\(S,S\)], [\(R,R\)], and [\(R,S\)]-EDDS), the lyase accepted only [\(S,S\)]- and [\(R,S\)]-EDDS. Probably only the S-configuration of the chiral C-atom can be attacked by the enzyme. Apparently, the enzymatic degradation of AEAA is also stereospecific [161]. While a microbial consortium mineralised [\(S,S\)]-EDDS totally, utilisation of [\(R,S\)]-EDDS resulted in the formation of a dead-end product, identified as AEAA which was supposedly present in the \(\alpha\)-configuration. Consequently, only l-AEAA, which should be formed during [\(S,S\)]-EDDS breakdown, can be expected to be further metabolised.

6. Regulation of the APCA degradation and enzyme expression

6.1. Regulation of \textit{NTA} degradation

The ability to metabolise NTA was inducible in \textit{Chelatobacter}, \textit{Chelatococcus} and denitrifying strains. NTA- and IDA-degrading activity was only detected in cell-free extracts and membrane fractions, respectively, of bacteria grown with either NTA or IDA [100,209]. This, however, provides only limited information concerning the regulation processes taking place under conditions found in wastewater treatment plants or in the environment which are characterised by rather low substrate concentrations. Therefore, experiments with \textit{C. heintzii} ATCC 29600 were conducted in carbon-limited continuous culture fed with either NTA or glucose to get a better insight in possible regulation mechanisms of NTA breakdown during growth under conditions similar to those found in the environment [236,237].

In a glucose-limited continuous culture, i.e. in the absence of NTA, both the NTA- and IDA-oxidising activ-
ties were very low and close to the detection limit, independent of the growth rate applied. When cultivated with NTA as sole source of carbon and nitrogen, the specific activity of NTA MO increased with decreasing dilution rates. The cells exhibited a maximum specific activity (approximately 180 μmol NTA (g protein)^{-1} min^{-1}) at dilution rates between 0.02 and 0.03 h^{-1}, whereas at a dilution rate close to μmax of _C. heintzii_, the specific NTA MO activity was 4–5 times lower. Thus, at high growth rates with NTA, repression of the NTA MO activity was observed, a pattern often found for enzymes catalysing the first step in catabolic pathways [238,239]. Under the same conditions, IDA DH activity was approximately constant at growth rates above 0.03 h^{-1} and only decreased considerably at lower growth rates.

In both nature and wastewater treatment plants, NTA-degrading micro-organisms will not only encounter NTA as sole substrate but they will grow in the presence of a complex mixture of potential carbon and/or nitrogen sources [240]. Indeed, under laboratory batch conditions, both _Chelatobacter_ and _Chelatococcus_ spp. are capable of consuming NTA in combination with a suitable carbon substrate generally resulting in an enhanced specific growth rate [177]. To investigate the degradation of NTA during mixed substrate growth in more detail, _C. heintzii_ was cultivated with different mixtures of glucose and NTA in a carbon-limited chemostat and special attention was given to the expression of enzymes involved in NTA metabolism [236]. Synthesis and activity of NTA-degrading enzymes was controlled by the ratio of substrates rather than their actual concentrations in the feed. During growth with mixtures containing less than 1% of the total carbon as NTA, the amount of cA and cB produced was close to the detection limit. Although the specific activities of NTA MO and IDA DH in cell extracts were very low, the bacteria were able to degrade the small amounts of NTA provided. Either the constitutive enzymatic activity was sufficient to consume the NTA supplied or an alternative, currently unknown enzyme system was involved. Feeding a NTA/glucose mixture containing 3.6% of NTA–carbon triggered induction of NTA enzymes above the constitutive background level and a further increase of the NTA fraction resulted in clearly enhanced activities of both NTA MO and IDA DH. Assuming that under environmental conditions NTA-degrading enzymes are similarly regulated by the proportion of NTA to the total carbon utilised, no significant induction of these enzymes can be expected in rivers receiving a high load of treated wastewater where NTA may contribute only to some 0.1–1%. In wastewater treatment plants, however, NTA might contribute to some 1–10% of the total utilisable carbon, and here, induction of NTA-degrading enzymes can be expected to play a significant role.

Obviously in the engineered or natural environment, NTA-degrading bacteria will rarely experience steady-state conditions, but rather changes in substrate concentration and spectrum caused by shock loading, diurnal and weekly cycles, heavy rainfalls, etc. Consequently, cells have to adapt to the changing conditions, and the lag phase resulting from these adaptation processes can severely affect the efficiency of biodegradation over long time periods [241].

To investigate the dynamic behaviour of NTA degradation in a continuous culture of _C. heintzii_ under transient conditions, the feed was switched from a medium containing glucose only to one containing either NTA only or mixtures of glucose and NTA [237]. When glucose-pregrown cells were suddenly confronted with NTA as sole source of carbon, nitrogen and energy, a long lag phase of about 25 h was measured until NTA MO expression started. This lag phase was considerably shortened when the cells were supplied with mixtures of NTA and glucose instead of NTA only. Possibly in the latter case, the portion of glucose available after the switch was able to support the rearrangement of the cellular metabolism, particularly to provide energy and building blocks for the synthesis of NTA-degrading enzymes. Hence one can expect that alternative carbon substrates have a considerable positive effect on the expression of other enzymes under changing environmental conditions.

Although such steady-state and dynamic experiments with pure cultures of _C. heintzii_ provide some basic information on the behaviour of this bacterium, one still cannot infer how NTA degradation is really regulated in wastewater treatment plants or in surface waters. In particular, the question of whether regulation proceeds at the level of enrichment of NTA-degrading micro-organisms or rather at the level of induction/repression of the pollutant-specific enzyme systems remains unanswered. Nevertheless, a number of observations suggest that for short-term fluctuations in the supply with NTA regulation takes place at the enzyme level [188]. Firstly, when NTA was added to activated sludge in laboratory batch cultures, only expression of NTA MO but no enrichment of NTA-degrading bacteria from the genera _Chelatobacter_ and _Chelatococcus_ (assessed with surface antibodies) was detected within about 100 h. Also, only a slight rise of _Chelatobacter_ bacteria was observed after activated sludge in porous pot plants that had been fed with synthetic wastewater containing high concentrations of NTA for more than 1 month. Secondly, no enrichment of _C. heintzii_ was found in the Swiss wastewater treatment plant on the mountain Saentis which was periodically exposed to unusually high inflowing NTA concentrations. NTA MO, however, became detectable within a few days after the ratio of NTA–carbon to DOC had increased over 10% in the influent, resulting in a markedly higher NTA removal efficiency [188].

In contrast to these data [188], in situ hybridisation with 16S rRNA probes specific for bacteria from the _Rhizobiaceae_ group revealed a steady raise of _Rhizobiaceae_ in a fed-batch culture of activated sludge daily amended with 1 g l^{-1} NTA [242]. Most of the APCA-degrading strains
known to date belong to this family of Rhizobiaceae. However, cells detected by the Rhizobiaceae probe did not react with probes specific for C. heintzii, which might indicate that important NTA-degrading bacteria from the Rhizobiaceae group are still unknown. Unfortunately, in these studies, measurements of NTA concentrations or enzyme activities in the culture were lacking to prove whether or not the observed enrichment was really linked to the degradation of NTA.

6.2. Regulation of degradation of EDTA and EDDS

As for the NTA-degrading enzymes, EDTA MO is also inducible in the two EDTA-degrading strains BNC1 and DSM 9103. When strain DSM 9103 had been grown with EDTA or ED3A, the rate of EDTA consumption in cell-free extract was stimulated 5-10-fold over the constitutive level of bacteria cultivated with glycerol. Growth with NTA or IDA, however, did not result in such a stimulation [198]. This partly contrasts with the observations reported for EDTA-degrading strain DSM 9103: compared to EDTA-grown cells, both EDTA transport and EDTA-oxidising activity were 10 times lower when cells had been grown with IDA or fumarate, whereas it was similarly high (about 80% of the activity of EDTA-grown cells) when the strain had been cultivated with NTA or [S,S]-EDDS. This also suggests that transport and catabolic enzymes are regulated in a co-ordinate manner [198,235].

Likewise, [S,S]-EDDS-degrading activity in the two strains DSM 9103 and C. asaccharovorans was inducible. Lyase-catalysed EDDS transformation was only detected in cell extracts of [S,S]-EDDS-grown bacteria but not in fumarate-, EDTA- or NTA-grown cells [235].

7. Influence of metal speciation on microbial APCA degradation

In the previous chapters on microbial and enzymatic APCA degradation, one important point was not touched, namely the fact that in both growth media and the environment APCAs are generally complexed with metal ions. Consequently, metal ions must be expected to have a substantial influence on the biodegradability of APCAs. In this context, several aspects have to be considered: initially, the APCA molecule has to be transported across the cytoplasm membrane metal-free or in association with a metal ion. If the latter were the case, a cell would encounter an enormous influx of metal ions. To deal with this situation, the cell can either excrete the metal ions or inactivate the cations by precipitating them intracellularly with a suitable anion (e.g. phosphate). On the other hand, if only the free APCA molecule enters the cell, then the metal has to dissociate from the complexing agent prior to uptake. This could be achieved by destabilising the metal–APCA complex during transport, resulting in the release of the metal ion at the outer side of the cytoplasmic membrane. Alternatively, the cells could transport only free APCA existing in equilibrium with the metal-complexed APCA in the surrounding medium. This question of APCA speciation arises also at the level of enzyme-catalysed breakdown in the cytoplasm.

As to the properties of the metal–APCA species itself, one is left with the question of whether the thermodynamic stability of a complex, its dissociation kinetics, or its structure governs the uptake into a cell or the enzymatic transformation.

Several difficulties are encountered when investigating the effect of APCA speciation, in particular in assays with whole cells. Firstly, metal toxicity has to be considered. Secondly, the presence of metal ions as well as metal-complexing ligands on the bacterial surface should be taken into account, because both might significantly affect the complexation of the APCA added to the system. However, it is difficult to assess the influence of these surface-bound ligands or metals. Thirdly, although the speciation of APCAs at the beginning of an assay can be predicted rather easily, changes in the course of such an experiment are difficult to assess.

7.1. In whole cells

7.1.1. NTA

Investigations of the biodegradability of different NTA species were first performed in the 1970s. The systems studied (activated sludge, river water, soil) were rather complex – with many other ligands and metal ions being present beside the NTA complex – and, therefore, no conclusive information was obtained. Nevertheless, the data suggested that NTA complexed with Cu²⁺, Fe³⁺, Mn²⁺ or Pb²⁺ is probably easily biodegradable under environmental conditions whilst NTA bound to Cd²⁺, Cu²⁺, Hg²⁺ or Ni²⁺ might be more recalcitrant [132,243–246].

Somewhat later, degradation of metal–NTA complexes by a ‘Pseudomonas’ species (now C. heintzii ATCC 29600) was studied under more defined conditions [247]. However, also here buffer systems were used which themselves have complexing properties and, therefore, the speciation of NTA was not precisely defined. When metal concentrations were kept low enough to avoid toxicity, the bacterial strain was able to attack Ca²⁺, Mn²⁺, Mg²⁺, Cu²⁺, Zn²⁺, Cd²⁺ and Fe³⁺ chelates of NTA, as well as free NTA as determined by oxygen consumption and ¹⁴CO₂ evolution from ¹⁴C-labelled NTA. Only NiNTA was resistant towards degradation.

Recently, more elaborate data on the effects of NTA speciation on microbial degradation were presented in which calculations of the chemical speciation were included [248]. Degradation assays with C. heintzii ATCC 29600 revealed first order kinetics for the decomposition of several degradable NTA species at concentrations ranging from 0.05 to 5.23 µM NTA with degradation rates in the
following order: H2NTA− > CoNTA− > FeOHNTA− ~ ZnNTA− > AIOHNTA− > CuNTA− > NiNTA−. This order indicates no relationship with the chemical stability of the different complexes. However, a relationship between the rates of degradation and the lability of the various metal–NTA complexes was established. This implies that the rates of formation of H2NTA− from MeNTA, i.e. the dissociation rates of the complexes, determine the degradation rates. Two models can explain these results: the first model is based on the assumption that only H2NTA− is taken up into the cells, with the extracellular dissociation of the metal–NTA complex being the rate-limiting step in the degradation. The second model assumes that all species, i.e. H2NTA− and metal–NTA complexes, enter the cytoplasm, where the species accepted by the NTA MO has then to be generated. Previously, Uetz and co-workers [216] had demonstrated that the preferred substrate of NTA MO is MgNTA, whereas CaNTA or H2NTA− are not accepted by the enzyme. Therefore, an exchange of Mg2+ for the metal complexed to NTA must take place intracellularly before NTA degradation can proceed. This exchange reaction will be the rate-limiting step which is again dependent on the dissociation kinetics of the metal complexes. Unfortunately, the data collected are not sufficient to establish which of the processes actually occurs. For this, short-term studies of NTA uptake into cells, preferably carried out with membrane vesicles, are required. Such data are still lacking and experience from our laboratory indicates that it will be very difficult to prepare membrane vesicles from C. heintzii [214].

Somewhat contrasting results were reported by Madsen and Alexander [139]. Based on speciation models, defined media were prepared whose composition favoured the presence of one predominant species of NTA. Under such conditions, an undefined microbial consortium mineralised CaNTA but not AINOTA, MgNTA, Fe(III)NTA or free NTA. The same pattern was found for a Listeria sp. isolated in the course of the study although the isolation medium contained mainly free NTA. Confirmatory tests with this strain in the presence of constant NTA and varying Ca concentrations (≤ NTA concentration) showed that the extent of NTA mineralisation increased with increasing Ca concentrations.

### 7.1.2. EDTA

Several reports on the degradability of various EDTA complexes suffer from the same drawbacks as those described above for NTA, i.e. that undefined, rather complex systems were used. In such systems, EDTA was found to be degraded by mixed microbial consortia when added in the free form or as chelate of Cu2+, Cd2+, Zn2+, Ni2+, Mn2+, Ca2+ or Fe3+ [149,153].

Contrasting requirements of pure EDTA-degrading cultures with respect to EDTA complexes supporting growth were reported. On the one hand, growth of an Agrobacterium sp. was only observed when EDTA was supplied as Fe(III)EDTA. Free EDTA as well as complexes with Ni, Cu or Co(III) did not support growth [190,249]. On the other hand, the EDTA-degrading strain BNC1 was not capable of utilising Fe(III)EDTA [194]. Growth of this strain on EDTA mineral salt medium always stopped before the substrate was completely degraded and the remaining concentration of EDTA corresponded to the Fe(III) concentration in the medium. Nevertheless, growth of BNC1 was dependent on the presence of metal-complexed EDTA because free EDTA appeared to interact negatively with the cell walls and completely inhibited bacterial growth.

Experiments with resting cells of strain BNC1 revealed that EDTA complexes with low stability constants (log K < 14) were readily degraded. CaEDTA, BaEDTA and MgEDTA were consumed at the highest rates, followed by MnEDTA. Free EDTA was also degraded, although growth with this species was observed to slow down. Generally, chelates with stability constants exceeding 1014 were not degraded. Only ZnEDTA (log K = 16.5) was slowly oxidised [232]. Similar results were reported recently for strain DSM 9103 [250]. Complexes with stability constants (log K < 16, i.e. the Mg2+, Ca2+, Mn2+ complexes and free EDTA) were degraded by EDTA-grown resting cells to completion at a constant rate. For more stable EDTA chelates (with Co2+, Cu2+, Zn2+ and Pb2+), the data suggested that these complexes were not used directly but had to dissociate prior to degradation. Only CdEDTA and Fe(III)EDTA were not degraded within 48 h. In the case of CdEDTA, the toxicity of free Cd2+ ions most likely prevented a significant degradation of EDTA, whereas in the case of Fe(III)EDTA, a combination of metal toxicity and the very slow dissociation of the complex was probably responsible for the absence of degradation [250].

For transport in strain DSM 9103, uptake experiments with [14C]EDTA in presence of various metal ions revealed a nearly identical pattern to that described for resting cells of strain BNC1 [231]. Free EDTA as well as chelates of Ca2+, Ba2+ and Mg2+ were readily incorporated at similar rates. Uptake of MnEDTA proceeded at an intermediate rate and all other, more stable complexes (those with Zn2+, Co2+, Cu2+, Ni2+ or Fe3+) were not transported in these short-term assays. In addition, Ca2+ uptake of strain DSM 9103 was considerably increased in the presence of EDTA, indicating that Ca2+ is probably transported together with EDTA into the cells. Cells of strain DSM 9103 seem to utilise both proposed mechanisms, i.e. intracellular precipitation and excretion of the metal, to cope with the increased incoming flux of metal ions due to EDTA degradation.

All these studies indicate that for the two strains BNC1 and DSM 9103, the EDTA chelates of Zn2+, Cu2+, Ni2+, Co2+ and Fe3+ are not directly accessible. They can only be degraded after dissociation, a process that depends on the dissociation rate constant of the complex. However, this rate constant is rather low for some of the heavy metals, making it unlikely that EDTA-grown cells can utilise these complexes. The only known exception to this rule is ZnEDTA, which is not degraded by EDTA-grown resting cells due to its relatively low dissociation rate constant.
metal–EDTA complexes, such as Fe(III)EDTA, NiEDTA or CuEDTA [91]. Consequently, the environmental recalcitrance of EDTA towards biological degradation might – at least partly – be attributed to the presence of species which do not favour microbial attack, in particular when considering that in a typical river system about 70% of the total EDTA is predicted to be present as stable heavy metal complexes (Fig. 3) [85].

7.2. At the enzyme level

At the enzymatic level, NTA MO from C. heintzii ATCC 29600 [216,251], NTA DH from strain TE11 [229,252], EDTA MO from strain DSM 9103 [198], cell-free extracts and EDTA MO from strain BNC1 [232,234], and finally EDDS lyase from strain DSM 9103 [235] were used to test the effects of APCA speciation (see Table 4).

With respect to the metal complexes that were accepted as substrates, both NTA MO from C. heintzii and EDTA MO from strain DSM 9103 exhibited a similar behaviour. Neither of the MOs transformed the free complexing agent or the Ca2+ chelate, whereas complexes of NTA or EDTA with Mg2+, Mn2+, Co2+ and Zn2+ were degraded. NTA MO attacked also Fe(III)NTA and NiNTA. The highest specific transformation activity was found for the Mg2+ complexes. Since Mg2+ is the most abundant intracellular divalent cation [253], NTA and EDTA can be expected to be mainly present as Mg2+ chelate in the cytoplasm. Therefore, it is not surprising that Mg complexes of both APCAs were the best substrates. All in all, no clear relationship is discernible between the stability of a NTA or EDTA complex or its dissociation rate constant and its MO-catalysed oxidation. Therefore, it was suggested that the structure of a complex might be a determining factor [198].

It should be added that for the EDTA-degrading strain BNC1, conflicting results were published. In cell-free extracts, Klüner and co-workers [232] found a similar pattern to that described above for the two MOs, with free EDTA and CaEDTA not being oxidised. In contrast, these two species, as well as various other EDTA complexes with di- and trivalent cations, were degraded in enzyme assays with purified EDTA MO performed by Payne and co-workers [234]. Currently, this discrepancy cannot be explained.

In contrast to NTA MO and EDTA MO from DSM 9103, NTA DH accepted free NTA. Addition of divalent metal ions almost completely inhibited enzymatic NTA breakdown [252]. This inhibition was reversed upon addition of EDTA, strongly suggesting that only uncomplexed NTA is a substrate of the DH.

Similarly, EDDS lyase accepted free [S,S]-EDDS as a substrate but also EDDS complexes with Mg2+, Ca2+, Ba2+ and Mn2+ [235]. All these complexes have stability constants lower than 109. MnEDDS with the highest stability constant of all degradable species was transformed at a very low rate. All other complexes which were not attacked by the lyase exhibited stability constants higher than 1010. Hence, in the case of EDDS, the stability of a complex apparently rules the enzymatic degradability. One can speculate that during substrate-binding, the metal ion has to be exchanged or removed from the complex with the EDDS molecule. This assumption is supported by the observation that metal ions had a strong influence on the equilibrium between EDDS and the cleavage products which is reached in the lyase-catalysed elimination reaction (Fig. 6). In presence of metal ions (especially those forming non-degradable complexes), this equilibrium was shifted towards the educt EDDS probably because free EDDS was removed from the equilibrium by the formation of metal complexes, indicating that only free EDDS is the actual substrate of the lyase.

8. Outlook

To date, the industrially most important APCAs are the synthetically produced compounds NTA, EDTA, DTPA and HEDTA. Recently, a number of new APCAs, such as PDTA, β-ADA, SDA or MGDA, have been synthesised which may have the potential to substitute the classical representatives in various applications and they are currently being tested. In addition, several naturally occurring APCAs produced by micro-organisms or plants, also with considerable potential for application, have been described in the literature. The producing organisms exploit the metal-complexing capacity of APCAs for enhancing their metal uptake, in most cases that of iron. On top of their good complexing ability, a considerable advantage of these natural APCAs is that they are probably better biodegradable than most synthetic APCAs. This is because, firstly, the metal they are transporting into the cell has to be freed intracellularly and, secondly, because organisms have been exposed to them for a longer time than to the xenobiotic ones. However, to date, there is little information available on these aspects. First studies with respect to a biotechnological production of some of these APCAs have already been reported and they seem promising. Nevertheless, considerably more research is still needed before such biotechnologically produced APCAs may compete with the established and cheap synthetic APCAs NTA and EDTA.

Once released into the environment, the fate of APCAs is determined either by abiotic or biotic processes (Fig. 7). For each of the different compounds, it has to be assessed individually because no general pattern applicable to all of them has emerged. The environmental fate of NTA is determined by biodegradation with abiotic processes playing a minor role for its elimination from both wastewaters and ecosystems. Hence, NTA most likely does not present a major risk for the environment. In contrast, EDTA, DTPA and HEDTA seem to be – if at all – only poorly biodegradable and therefore abiotic elimination mecha-
nisms are of greater significance. Indeed, for EDTA it has been demonstrated that the most important elimination process in rivers is its photolysis, which is, however, restricted to sunny days and to only one EDTA species, i.e., Fe(III)-EDTA. To what extent abiotic oxidative processes are important for EDTA (and perhaps also other APCAs) breakdown in soils, especially in comparison to biodegradation, remains to be investigated. Furthermore, one should not forget that abiotic processes usually do not lead to a mineralisation of the complexing agents, but merely to a transformation into compounds which still have metal-complexing properties. Therefore, future research should also focus on the metabolites of these abiotic degradation processes such as ED3A. Especially in monitoring programs for EDTA and DTPA potential breakdown products should be included, too, for a better assessment of the pollution of a system with these xenobiotic APCAs. As mentioned above, new APCAs of both synthetic and biological origin are being tested for their potential to replace the classical ones. Some of them appear to be easily biodegradable, at least under aerobic conditions. Though, for all of them, the information available is still too limited to conclude that they are harmless from an environmental point of view.

So far, the isolation of aerobic strains growing with NTA, EDTA or [S,S]-EDDS has been successful. Virtually all strains have been found to be members of the Agrobacterium–Rhizobium group in the α-branch of Proteobacteria. A possible future isolation and taxonomic characterisation of bacterial strains growing with DTPA, HEDTA, β-ADA, SDA, ASDA or MGDA might unravel an interesting picture with respect to the evolution of microbial degradation of xenobiotic APCAs. In the absence of molecular oxygen, NTA is the only APC for which degradation has been reported under denitrifying conditions. Interestingly, this strain is not a member of the α-branch of Proteobacteria.

Some of the NTA- and EDTA-degrading strains pres-
ently isolated seem to exhibit an extended spectrum of APCAs they can utilise as growth substrates. One can therefore expect that testing the newly marketed chelating agents such as PDTA, β-ADA, EDDS or MGDA for degradation by NTA- or EDTA-utilising bacteria might yield interesting results. Furthermore, the fact that both the EDTA-degrading strain investigated in our laboratory and the NTA-degrading Chelatococcus strains are able to degrade not only one but two or more APCAs indicates that such pure cultures could perhaps be used for special treatment of wastewaters which contain high amounts of rather poorly biodegradable APCAs such as DTPA, HEDTA or PDTA. In case of EDTA, the application of pure cultures has already shown rather promising results in laboratory experiments [212,213], whereas large scale treatment still remains to be investigated.

Due to the isolation of pure bacterial cultures, the metabolic pathways for NTA, EDTA and [(5,5)-EDDS] degradation have been – at least partly – elucidated. The first transformation of all three compounds consists of the cleavage of the bond between a nitrogen atom and a carboxylic residue (an acetyl or succinyl group). So far, de-carboxylation or cleavage within the ethylenediamine part of the EDTA or EDDS molecule has not been observed as first metabolic step. In this context, it is worth mentioning that the biochemistry of EDTA degradation in the Agrobacterium strain which grows only with iron(III)-complexed EDTA [190], a complex that is not touched by the strains investigated so far, has not been studied yet. Perhaps, the co-ordinated iron ion plays a role in the enzymatic breakdown of EDTA in this bacterium.

Of course, for the characterisation of degradative pathways for other APCAs such as DTPA or the amino acid derivatives β-ADA, SDA, ASDA or MGDA, pure cultures able to degrade these chemicals should be available. However, one can speculate that the aerobic breakdown will also include the attack of an MO on the compounds as seen for NTA and EDTA degradation in strains C. heintzii, BNC1 and DSM 9103. This would lead to the removal of an acetyl group. In fact, EDTA MO has been shown not only to catalyse the deacetylation of EDTA but also that of DTPA and HEDTA. Therefore, one can speculate that β-ADA, SDA, ASDA or MGDA should all be accepted as substrates by both NTA MO and NTA DH due to the structural resemblance of these compounds with NTA. In analogy to the NTA metabolism for the amino acid derivatives, a second acetyl group can be supposed to be cleaved off in a reaction catalysed by a DH and generating the corresponding amino acid.

From a number of studies, it has become clear that the speciation of APCA molecules greatly influences their environmental fate. But especially in case of biological elimination, not enough information is currently available to suggest how exactly this process is affected by speciation. So far, it has not been shown for any APCA-degrading bacterium which species of the complexing compound is/are really taken up by the cells. This information, however, seems essential for understanding why the biodegradability of different APCAs varies so tremendously. If only free APCA molecules or rather unstable species are transported into the cells, then this might explain why APCAs such as EDTA or DTPA forming very stable complexes (especially with heavy metals) are not as well biodegradable as NTA. This could also explain the positive effect of a pH rise on the microbial elimination of EDTA as it was observed in a sewage treatment plant because it is known that heavy metal–EDTA complexes are destabilised at higher pH values.

All in all, the enormous influence speciation has on the fate of APCAs in nature shows clearly the necessity to include all important species of new APCAs in degradation and toxicity tests in order to be really able to predict and judge the compound’s environmental behaviour.

Acknowledgements

The authors would like to thank Kay Fox, Laura Sigg and Bernd Nowack for careful reading of and valuable comments on this manuscript. TE would like to express his thanks to Hansueli Weilenmann for the fruitful collaboration over the years (not only in the field of complexing agent biodegradation) as well as to all the research students that have added mosaic stone by mosaic stone without which it would have been impossible to get to the present level of understanding. Furthermore, the generous financial support from the Swiss National Science Foundation, The Research Commission of the Swiss Federal Institute of Technology Zürich, Lever Switzerland and Unilever Research Laboratories Port Sunlight (UK), the Swiss Agency for the Protection of the Environment (BUWAL), and last, but not least, of the Swiss Federal Institute for Environmental Science and Technology is gratefully acknowledged.

References


of EDTA by a bacterial isolate. Poster presented at the 54 Annual Meeting of the Swiss Society for Microbiology, Lugano.


Xi, L., Squires, C.H., Monticello, D.J. and Childs, J.D. (1997) A


Silver, S. and Lusk, J.E. (1987) Bacterial magnesium manganese and...

