Appl Microbiol Biotechnol (1999) 51: 751–759

MINI-REVIEW

B. Nörtemann

Biodegradation of EDTA

Received: 14 September 1998 / Received revision: 9 December 1998 / Accepted: 11 December 1998

Abstract The chelating agent ethylenediaminetetraacetate (EDTA) is not degraded by conventional biological and physicochemical methods for the treatment of wastewater and the purification of drinking water. Of the measurable organic compounds it is the one present at the highest concentration in many surface and drinking waters. In recent years, however, studies have demonstrated that EDTA can be degraded by specially enriched bacterial cultures and in wastewater treatment plants receiving EDTA-containing effluents. The amounts of EDTA released into the aquatic environment could thus be reduced by establishing appropriate biological wastewater treatment plants. This article describes the degradation of EDTA and its metal chelates by different bacterial cultures, catabolic steps in EDTA degradation, and biological methods for the removal of this chelating agent from wastewaters.

Introduction

Many aminopolycarboxylic acids such as those shown in Fig. 1 form strong and water-soluble complexes with most alkaline earth and heavy metal ions. These complexes are chemically and thermally very stable and can be used for a wide variety of industrial applications, e.g., for controlling the oxidation potential of metal ions, for adjusting metal ion concentrations, for masking free metal ions to avoid metal-catalyzed spoilage or the precipitation of poorly soluble salts, and for cleansing processes where precipitates of salts in tubes, bottles, and on membranes must be removed. Of the aminopolycarboxylic acids, ethylenediaminetetraacetate (EDTA) is technically the most important and is used in large quantities [sales quantities in Europe 1997: EDTA, 32 550 tons; nitrilotriacetate (NTA), 18 600 tons; diethylenetriaminepentaacetate (DTPA), 14 000 tons].

For a long time, EDTA was thought to be resistant to biodegradation (Bunch and Ettinger 1967; Madsen and Alexander 1985; Alder et al. 1990; Bolton et al. 1993). In contrast, NTA was found to be readily biodegradable. A summary of NTA-degrading bacteria isolated from various habitats was given by Kemmler and Egli (1990). Even in municipal wastewater treatment plants, high degrees of NTA degradation were observed, e.g., 97% in the Glatt sewage treatment plant of the city of Zurich (Siegrist et al. 1988). As to the biodegradability of DTPA, very little information is available, and to date no axenic or mixed bacterial culture capable of growing with DTPA as sole source of carbon and energy has been described.

In recent years, the biodegradability of EDTA has been increasingly recognized and investigated from different points of view. One of these is that EDTA has become the measurable organic compound at the highest concentration in many surface waters and in drinking waters prepared from them (Dietz 1985; Brauch and Schullerer 1987; Dietz 1987; Brauch and Kühn 1988; Frimmel et al. 1989; Schullerer and Brauch 1989; Alder et al. 1990; Haberer 1991; Nusch et al. 1991; Klopp and Pätsch 1994). EDTA is not toxic to mammals at the concentrations found in the aquatic environment, but there has been some concern about its potential to remobilize heavy metals out of river sediments and sewage sludges even though this could not be proved under natural conditions (Dietz 1987; Siegrist et al. 1988; Alder et al. 1990; Xue et al. 1995; Kari and Giger 1996; Nowack et al. 1997). Since, in principle, drinking waters should not contain xenobiotic compounds, the amount of EDTA released into aquatic systems should be reduced as far as technically possible. One approach to achieving this goal would be the development of special biological systems for the treatment of EDTA-contain-

B. Nörtemann
Institut für Bioverfahrenstechnik, Technische Universität Braunschweig, Gauss-Str. 17, D-38106 Braunschweig, Germany
e-mail: B.Noertemann@tu-bs.de
http: // www.tu-bs.de/institute/ibvt
Tel.: +49 531 391 7654
Fax: +49 531 391 7652

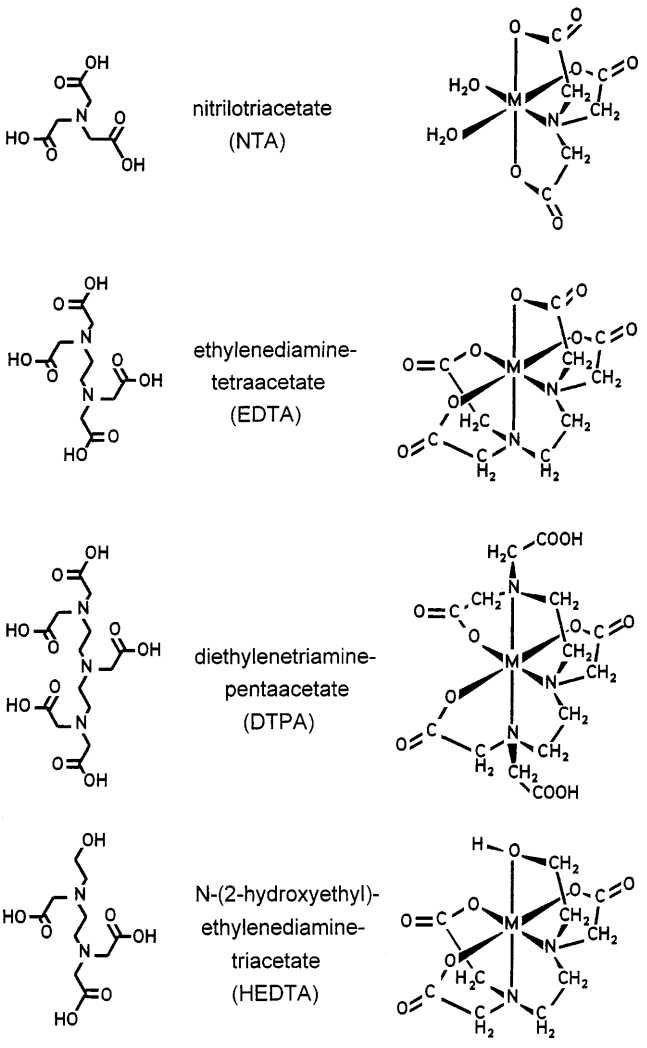

Fig. 1 Aminopolycarboxylates of technical importance for use as chelating agents (*left side* free acids, *right side* metal chelates without the charge)

ing wastewaters. This would require enrichment of suitable bacteria, knowledge of the optimum physiological conditions for efficient EDTA degradation and of parameters affecting it, investigations of catabolic pathways, and the development of special reaction systems ensuring high degradation rates even under suboptimal growth conditions.

Another reason to investigate the biodegradation of EDTA is the fact that this compound can be used for the remediation of sites contaminated with heavy metals or radionuclides, and for the decontamination of nuclear power plant equipment (Ayers 1970; Mcfadden 1980; Macaskie 1991). In addition, EDTA in groundwater could undesirably enhance the migration of heavy metals or radionuclides from contaminated soils or disposal sites (Means et al. 1978; Means and Alexander 1981). Therefore, it is of interest to understand the biodegradation of EDTA and to develop new approaches to stimulate it (Palumbo et al. 1994; Bolton et al. 1996; Thomas et al. 1998).

EDTA-degrading microorganisms and the influence of chemical speciation on the biodegradability of EDTA

Although Tiedje (1975) and Belly et al. (1975) found a slow oxidation or co-metabolism of EDTA by microorganisms from several habitats, it is only recently that mixed bacterial cultures (Nörtemann et al. 1991; Gschwind 1992; Thomas et al. 1998) and axenic cultures (Lauff et al. 1990; Nörtemann 1992; Palumbo et al. 1994; Witschel et al. 1995) with the capability to grow with this chelating agent as sole carbon source were described. As reported for NTA, citrate, and oxalate, the nature of the metal ion bound by a complexing agent can determine the biodegradability of the ligand (Firestone and Tiedje 1975; Madsen and Alexander 1985; Francis et al. 1992; Bolton et al. 1996). Since EDTA in industrial wastewaters or natural waters is usually associated with metal ions, the range of metal-EDTA chelates that is degradable by axenic or mixed bacterial cultures is important.

Of the metal-EDTA chelates tested, only Fe(III) EDTA supported growth of an *Agrobacterium* species isolated by Lauff et al. (1990). This chelate was oxidized at high initial concentrations of approximately 30 mmol/l, but consumption of the substrate ceased when the concentration decreased to approximately 3 mmol/l. Although this residual concentration of EDTA represented 10% of the initial content, the remaining chemical oxygen demand (COD) was about 30% of the initial value, possibly indicating the accumulation of partial degradation products. Contamination by other microorganisms or the addition of readily degradable compounds (sucrose and yeast extract) caused an inhibition of EDTA oxidation.

Degradation of Fe(III)EDTA at lower concentrations was reported by Miyazaki et al. (1997). The bacterial strain described, possibly belonging to the genus *Pseudomonas*, degraded the chelate at an initial concentration of less than 100 mg/l (about 0.3 mmol/l), whereas growth was inhibited at higher concentrations. The Fe(III)EDTA chelate was also slowly oxidized by cells of another *Agrobacterium* species (Palumbo et al. 1994) and by cells of a mixed bacterial culture (Thomas et al. 1998). The aim in both of these studies was the degradation of EDTA chelates of heavy metals and radionuclides. However, the *Agrobacterium* species only degraded Fe(III)EDTA, and in most experiments, residual EDTA concentrations of more than 40% of the initial content remained in the culture fluid. The mixed culture utilized Fe(III)-, Cu-, Co-, Ni-, and CdEDTA as substrates for growth (initial concentrations, 5 mmol/l), but the oxidation rates for all chelates tested were rather low[1] and

[1] Thomas et al. (1998) state a very high oxidation rate, e.g., for Fe(III)EDTA, they report a rate of 15 μmol/μg protein and hour (i.e., 250 mmol/g protein and minute) and attribute an even higher rate to the *Agrobacterium* species described by Lauff et al. (1990). These rates appear to be in error and are not consistent with values estimated from the graphic presentation of the results.

decreased significantly at relatively high concentrations. With the exception of Fe(III)EDTA, consumption of the chelates ceased completely at residual concentrations of 3–4 mmol/l. Although inorganic phosphate was added to the culture fluids to promote precipitation of heavy metal ions released during degradation of the ligand EDTA, it is possible that the cells were inactivated by free heavy metal ions. From the time-course presented it is obvious that the concentration of dissolved heavy metal ions not chelated by EDTA increased significantly during degradation of EDTA so that toxic effects could have arisen.

The range of metal-EDTA chelates that are degraded by cells of a mixed bacterial culture containing the gram-negative strain BNC1 as a primary utilizer differed from that of the cultures described above (Nörtemann et al. 1991; Nörtemann 1992; Klüner et al. 1994; Klüner 1996; Klüner et al. 1998). The degradability of the complexes by whole cells of strain BNC1 depended strictly on their thermodynamic stabilities (expressed as conditional constants at pH 8, 20 °C, and an ionic strength of I = 0.1 mol/l). With the exception of ZnEDTA (conditional stability constant, $K = 10^{14.2}$), only metal-EDTA chelates with stability constants below 10^{12} were degraded. The maximum turnover rate for Ba- ($K = 10^{5.5}$), Mg- ($K = 10^{6.4}$), and CaEDTA ($K = 10^{8.4}$) was approximately 20 μmol/g protein and minute. The EDTA chelate of Mn^{2+} ($K = 10^{11.7}$) was oxidized at a rate of approximately 15 μmol/g protein and minute. Metal-EDTA chelates with stability constants above 10^{12} such as Fe(III)-, Co-, Cd-, Pb-, Ni-, and CuEDTA ($K = 10^{13.7}$, $10^{13.9}$, $10^{14.2}$, $10^{15.2}$, $10^{16.2}$, and $10^{16.2}$, respectively) were not metabolized. However, the oxidation of uncomplexed EDTA or degradable metal-EDTA complexes was not inhibited by these strong chelates. In the presence of Fe-, Co-, Cd-, Pb-, Ni-, or CuEDTA, a given surplus of uncomplexed EDTA was always consumed at the same rate as observed in the absence of additional metal ions.

The range of degradable EDTA chelates was not described for cells of a mixed culture enriched by Gschwind (1992) or the gram-negative strain DSM 9103 isolated from it (Witschel et al. 1995). However, experiments carried out in order to determine the taxonomic position of several EDTA-degrading organisms revealed that Mg-, Ca-, and MnEDTA are degradable by cells of strain DSM 9103. Very recently, other EDTA-degrading bacteria have been isolated from different habitats, each of them continuously receiving EDTA-containing wastewaters (B. Nörtemann et al., unpublished results). These new isolates are phylogenetically closely related to the EDTA-degrading strains BNC1 and DSM 9103. All of them belong to a new genus within the α-subclass of proteobacteria and seem to degrade the same or a similar range of metal-EDTA chelates.

The fact that EDTA is not necessarily resistant to biodegradation was also confirmed by studies of van Ginkel et al. (1997) and Kaluza et al. (1998) who observed biodegradation of this chelator in conventional wastewater treatment plants. Notably, van Ginkel et al. (1997) observed no degradation of EDTA at pH 7, whereas a high degree of EDTA degradation was obtained at pH values greater than 8. It is not known whether this phenomenon can be explained by physiological requirements of the EDTA-degrading organisms or by the composition of the different metal-EDTA species, which is dependent not only on the metal ions present but also on the pH.

Initial reactions in EDTA and NTA degradation

Because of the poor biodegradability of EDTA, only a few studies on the biochemistry of EDTA degradation have been reported. The identified or proposed catabolic steps in EDTA degradation are illustrated in Fig. 2. Belly et al. (1975) identified ethylenediaminetriacetate (ED3 A) and iminodiacetate (IDA) as main metabolites of EDTA oxidation by highly concentrated biomass from an aerated lagoon receiving EDTA-containing wastewaters. In addition, *N*,*N*- and *N*,*N*′-ethylenediaminediacetate (*N*,*N*-EDDA; *N*,*N*′-EDDA), ethylenediaminemonoacetate (EDMA), NTA, and glycine were detected in rather low concentrations (less than 0.065 mmol/l). However, some of these compounds could have been artificially formed during derivatization before gas chromatography/mass spectrometry analysis. Klüner (1996) identified ED3A, *N*,*N*′-EDDA, and glyoxylate as metabolites of EDTA oxidation by cell-free extracts of strain BNC1, whereas *N*,*N*-EDDA was not an intermediate. In addition to the identified metabolites, it was suggested that IDA is a metabolite formed from ED3A in a further reaction (Klüner 1996; Klüner et al. 1998).

Both reactions in the sequential oxidation of EDTA to *N*,*N*′-EDDA via ED3A by cell-free extracts of strain BNC1 require NADH, FMN, and O_2 which, on the basis of knowledge of the products formed, suggests that they are catalyzed by one or more monooxygenases. Sequential oxidative removal of two acetyl groups from EDTA catalyzed by only one enzyme has been shown for strain DSM 9103 by Witschel et al. (1997). The two-enzyme system converts EDTA to *N*,*N*′-EDDA via ED3A. Component A′ of this system is most likely a monooxygenase which catalyzes the cleavage of EDTA into ED3A and glyoxylate, and of ED3A into *N*,*N*′-EDDA and glyoxylate, while consuming O_2 and $FMNH_2$. The latter is provided by component B′, a $NADH_2$:FMN oxidoreductase that can be replaced by other $NADH_2$:FMN oxidoreductases (e.g., from *Photobacterium fischeri*). A similar system has been found for the oxidation of NTA by a two-component monooxygenase from *Chelatobacter* ATCC 29600 (Uetz et al. 1992). This enzyme consists of two weakly associated components A and B and occurs as a tetramer ($\alpha_2\beta_2$). The FMN-containing component B exhibited a NTA/Mg^+-stimulated NADH-oxidizing activity but was unable to hydroxylate NTA. Hydroxylation of NTA with the formation of IDA and glyoxylate required the

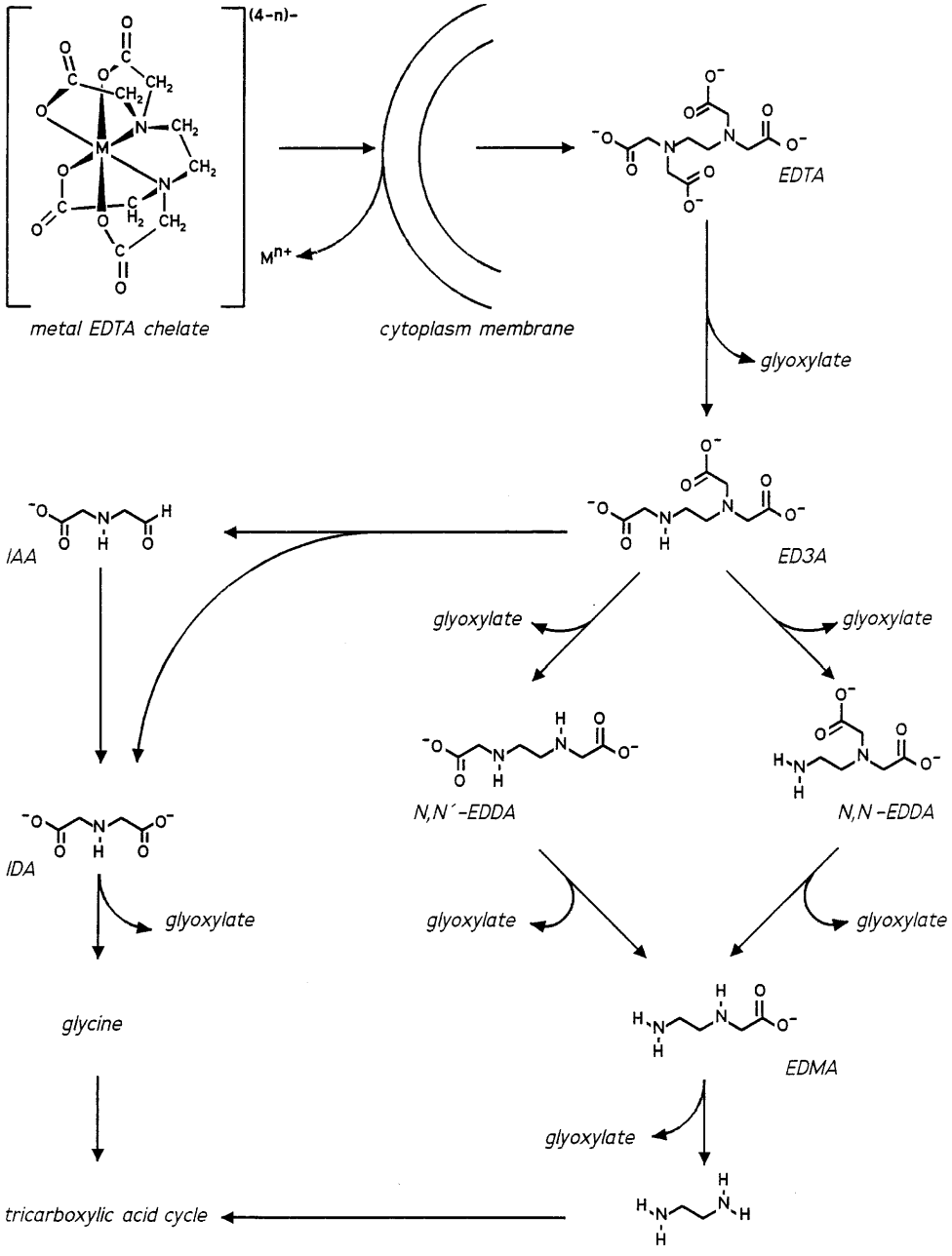

Fig. 2 Postulated pathways for the catabolism of EDTA. For the purpose of a simplified presentation, only the most relevant catabolic steps are presented, and intracellular EDTA oxidation is described using the uncomplexed structures. *ED3A*, ethylenediaminetriacetate; *N,N′-* and *N,N-EDDA*, *N,N′-* and *N,N*-ethylenediaminediacetate; *EDMA*, ethylenediaminemonoacetate; *EDA*, ethylenediamine; *IAA*, iminoacetaldehydeacetate; *IDA*, iminodiacetate; *n* is the positive charge of metal ion

presence of component A. The molecular weights of the monomers of components A and B (obtained by SDS-PAGE analysis) were 47 kDa and 36 kDa, and their native molecular masses were determined to be 99 kDa and 88 kDa, respectively. Of 26 compounds tested, NTA was the only substrate for the NTA monooxygenase. Cloning of the corresponding genes (Knobel et al. 1996) revealed that the deduced amino acid sequence of *ntaA* (gene coding for component A) showed significant homology only to SoxA (40.1% in a 451-amino-acid region overlap), a 50-kDa protein involved in the conversion of dibenzothiophene-5,5′-dioxide to an unidentified metabolite, and to SnaA (49.2% in a 250-amino-acid region) which is involved in pristamycin synthesis. Therefore, the evolutionary origin of the NTA monooxygenase still remains to be elucidated.

Very recently, Payne et al. (1998) described the initial enzyme for EDTA degradation by strain BNC1. This enzyme is also a two-component enzyme system consisting of an EDTA monooxygenase and a NAD(P) H_2:FMN oxidoreductase which can be replaced by other NAD(P)H_2:FMN oxidoreductases. The two-component enzyme converts EDTA to ED3A and glyoxlate but does not oxidize ED3A. The molecular weights (obtained by SDS-PAGE analysis) of the monomers of both EDTA monooxygenases described by Witschel et al. (1997) and Payne et al. (1998) are quite similar at 44 kDa (strain DSM 9103) and 45 kDa (strain BNC1),

whereas their N-terminal sequences are completely different. Moreover, gel filtration experiments indicate that the EDTA monooxygenase component of strain BNC1 is a monomer in its native state, whereas the corresponding component A′ of strain DSM 9103 is a tetramer.

So far, nothing is known about the genetic loci coding for the EDTA-degrading enzymes and their evolutionary origin. In contrast to NTA monooxygenase, the EDTA monooxygenases accept a broad range of aminopolycarboxylates such as NTA, DTPA, *N*-(2-hydroxyethyl)ethylenediaminetriacetate (HEDTA), and 1,3-diaminopropane-2-ol-tetraacetate (1,3-DPTA). This broad range of substrates leads to the question whether a natural substrate for the EDTA monooxygenases exists or whether these enzymes have evolved by adaptation processes to the xenobiotic compound EDTA. A natural structural isomer of EDTA, S,S-ethylenediaminedisuccinate (S,S-EDDS), is degraded by strain DSM 9103, but this compound is not a substrate for the EDTA monooxygenase of this strain and is converted by a completely different type of enzyme (Witschel et al. 1997; Witschel and Egli 1998). Although EDTA and NTA monooxygenases revealed no similarities with regard to their NH_2-terminal sequences or in immunoblots, both enzymes have many characteristics in common so they could have evolved from the same ancestor. However, this remains to be elucidated by further studies.

The carrier for the transport of EDTA into the cytoplasm of strain BNC1 is inducible and appears to be a high-affinity system, because the Monod constant for EDTA degradation by whole cells is only 8 μmol/l EDTA (Henneken et al. 1994; Henneken 1995; Henneken et al. 1996), whereas under optimum conditions the Michaelis constant of EDTA oxidation by cell-free extracts is 120 μmol/l. The K_m value of the EDTA-oxidizing enzyme from strain DSM 9103 is approximately 800 μmol/l (Witschel et al. 1997), whereas it is only 8.5 μmol/l for the purified EDTA monooxygenase of strain BNC1 (Payne et al. 1998). For all of these EDTA-converting systems, the MgEDTA chelate is the favored substrate.

Influence of chemical speciation on the oxidation of EDTA by cell-free extracts and EDTA monooxygenases

A dependency of the degradability of EDTA chelates on their thermodynamic stability constants, as found with whole cells of strain BNC1, was not observed with ultrafiltered (Klüner 1996; Klüner et al. 1998) or dialyzed (Payne et al. 1998) cell-free extracts of this organism. Remarkably, the range of metal-EDTA chelates which can be oxidized by these extracts is not identical. Payne et al. (1998) reported that glyoxylate was released from all of the 18 metal-EDTA chelates tested using dialyzed cell-free extracts, whereas Klüner (1996) found that only Ba-, Co-, Mg-, Mn-, and ZnEDTA were oxidized using ultrafiltered extracts. Moreover, the purified EDTA monooxygenase of strain BNC1 also converts uncomplexed EDTA (Payne et al. 1998), which is not metabolized by ultrafiltered extracts (Klüner 1996). The reason for these discrepancies is not yet known.

The range of metal-EDTA chelates converted by the EDTA monooxygenase of strain DSM 9103 (Witschel et al. 1997) is very similar to that described for ultrafiltered extracts of strain BNC1 (Klüner 1996; Klüner et al. 1998). In both cases, CaEDTA ($K = 10^{8.4}$) is not a substrate for the first catabolic enzyme whereas CoEDTA ($K = 10^{13.9}$) is oxidized. Since CaEDTA but not CoEDTA can be degraded by whole cells of strain BNC1, it is probable that at least for some metal-EDTA chelates the uptake of the ligand into the cytoplasm is accompanied by a change in the metal speciation. This appears to be possible only for chelates with low thermodynamic stability constants and/or high dissociation rates. The conditional thermodynamic stability constants of some metal-EDTA chelates increase in the order BaEDTA < MgEDTA < CaEDTA < MnEDTA < Fe(III)EDTA < CoEDTA < ZnEDTA < CdEDTA < PbEDTA < CuEDTA < NiEDTA (Ringbom 1963), and a rough estimate for the dissociation rate for the IDA segment of metal-EDTA complexes is stated by Nowack and Sigg (1997) in a series Fe(III) < Ni < Cu < Co < Zn < La < Pb < Ca. This could explain why CaEDTA but not CoEDTA is degraded by whole cells of strain BNC1. In contrast, the range of metal-EDTA complexes which can be oxidized by the first catabolic enzyme of strain BNC1 or the EDTA monooxygenase of strain DSM 9103 is not strictly affected by their thermodynamic stabilities or the dissociation rates and must thus be influenced by another determining factor. Since not all metal-EDTA chelates have the postulated ideal octahedral structure, the coordination of the metal ion could affect the degradability of the ligand. Actually, the central ion of the CaEDTA complex which can not be oxidized by the first catabolic enzyme of strain BNC1 or strain DSM 9103 has a coordination number of 8, whereas that of the degradable MgEDTA chelate has a coordination number of 7 (Anderegg 1987). However, the central ion of Fe(III)EDTA also has a coordination number of 7, but the chelate is not a substrate for the first catabolic enzyme. It must be assumed that at least two or more factors in combination determine the enzymatic degradability of metal-EDTA chelates.

A comparable transport mechanism with a coupled de- or *trans*-complexation was proposed for NTA catabolism (Egli 1994), and the relative turnover rates for metal-NTA chelates determined with whole cells of *Chelatobacter heintzii* ATCC 29600 also differed from that obtained in studies with the first catabolic enzyme, NTA monooxygenase (Bolton et al. 1996; Xun et al. 1996). The degradability of various metal-NTA complexes by whole cells of *C. heintzii* was found to be related to the corresponding dissociation rates, but the lability of the complexes did not strictly influence their

degradability by NTA monooxygenase. These common characteristics in the degradation of EDTA and NTA support the assumption that both catabolic systems–transport systems and initial catabolic enzymes–could have been evolved from the same ancestor.

Methods to reduce the amount of EDTA discharged into the aquatic environment

The amounts of EDTA released into the aquatic environment can be decreased by biological and by several nonbiological methods. One of the nonbiological methods is the optimization of technical processes, as was, for example, realized in the production of EDTA. In this case, the load of EDTA in the wastewater was reduced by approximately 60% from 1991 to 1994, and by more than 94% from 1986 to 1994 (BUA-Stoffbericht 1996).

Another nonbiological method is the substitution of EDTA by more readily degradable complexing agents such as tartrate, citrate, alaninediacetate, methylglycinediacetate, and NTA. However, the thermodynamic stabilities of the corresponding metal complexes are relatively low compared to metal-EDTA chelates. They are thus not suited for many industrial processes or products where strong chelating properties are required. In addition, technically applicable complexing agents must often be very resistant to chemical and thermal effects as well as to biodegradation (e.g., cleansing processes, process baths). For these technical processes, EDTA is still the best compound known because it is cheap to produce and to apply and is not toxic to mammals. In contrast, other compounds with chelating properties comparable to those of EDTA are usually more expensive, and have been less intensively investigated with regard to their toxic or ecotoxic behaviour.

The development of methods for the elimination of EDTA from process waters or wastewaters would allow continued application of this cheap and technically well-suited chelating agent while reducing environmental problems. Physicochemical methods are as a rule very expensive and are often not efficient enough. However, recent reports indicate that EDTA could be mineralized efficiently and inexpensively by biological systems. There are two main approaches for the elimination of EDTA from wastewaters that contain the chelating agent at relatively high concentrations. One of them is the removal of EDTA in conventional activated sludge plants, as demonstrated by van Ginkel et al. (1997) and Kaluza et al. (1998). Although the treatment plant described by van Ginkel et al. (1997) received predominantly domestic wastewater, the EDTA concentration in the influent was relatively high at 25–36 mg/l (0.086–0.12 mmol/l). The mean hydraulic retention time was 1 day and the sludge retention time 20 days. Under these conditions high degrees of EDTA degradation, between 78% and 95%, were achieved. However, experiments carried out in semicontinuous activated sludge units revealed that efficient EDTA removal was only possible at high sludge retention times of 12 or more days and at pH values greater than 8.

Kaluza et al. (1998) reported EDTA removal at about 80% in a Finnish wastewater treatment plant with a sludge retention time of 9 days and a mean hydraulic retention time of 1 day. The plant was operated at a pH value of 7.6–7.9 and at a temperature of 35–40 °C . The mean concentrations of EDTA in the influent and effluent were 28 mg/l (about 0.1 mmol/l) and 5.8 mg/l (0.02 mmol/l) respectively. Hence, the removal of EDTA by conventional activated sludge plants appears to be an efficient and cheap method, especially when the treatment plant is operated at high sludge retention times and receives alkaline wastewater.

In contrast to the wastewater treatment plants described above, municipal or even conventional industrial wastewater treatment plants usually receive wastewaters with low concentrations of EDTA between a few micrograms and a few milligrams per liter. Such concentrations might be too low to stimulate adaptation processes for the microbial degradation of EDTA. However, most of the EDTA is used for special industrial applications where the process waters or partial wastewater streams with high concentrations of this compound could be treated separately before they are mixed with other wastewaters or released into the aquatic environment. To establish such a decentralized and efficient wastewater treatment system, the mixed bacterial culture containing the EDTA-degrading strain BNC1 was investigated extensively (Henneken et al. 1994; Henneken 1995; Henneken et al. 1995, 1996; Brüggenthies 1996; Klüner et al. 1996).

Despite the low and varying growth rate of the culture (μ_{max} = 0.03–0.07 per hour), high degradation rates were achieved by immobilizing the cells on solid carriers in airlift-loop reactors (Fig. 3). Because a high biomass concentration was obtained by improved cell support, the dilution rate could be increased to more than 1.2 per hour (hydraulic retention time, less than 50 min) without a decrease in the degree of EDTA

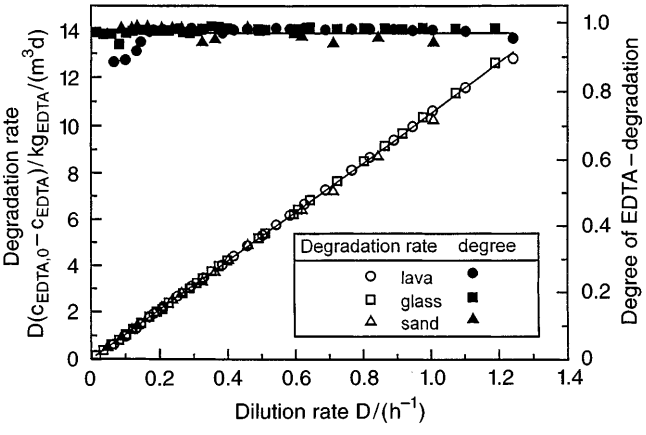

Fig. 3 Rate and degree of EDTA degradation ($c_{EDTA,0}$ = 450 mg/l) under continuous flow conditions in an airlift-loop reactor with immobilized cells of an EDTA-degrading bacterial mixed culture

degradation (97–98%) and without reaching the capacity limit. Under these conditions, a maximum rate of EDTA degradation of 12.8 kg/(m^3 × day) was achieved. The concentration of dissolved organic carbon (DOC) always corresponded to the DOC calculated theoretically from the residual EDTA concentration, indicating that no organic metabolites were accumulated in the culture fluid. In addition, the constant and high degree of degradation reflects the favorable ecological conditions for mixed cultures with mutual interactions between different species. It was also shown that the immobilized cells are very resistant to suboptimal or inhibitory conditions such as shock-loading with uncomplexed EDTA or low temperatures. After the transient alteration of process parameters or the sequential reduction of the temperature, the degree of EDTA degradation always returned rapidly to its original value.

Quantitatively, the most important species of EDTA in industrial wastewaters are Ca- and MgEDTA (from industrial cleansing processes and pulp and paper production), Fe(III)EDTA (from photographic bleaching processes) and, to a lesser extent, MnEDTA (from pulp and paper production). It was shown on the laboratory scale that EDTA can be eliminated very efficiently from most of the wastewaters from cleansing processes and pulp and paper manufacture (EDTA concentrations ranging from 0.1 mmol/l to approximately 3 mmol/l). The degree of EDTA degradation was always high, reaching maximum values of more than 99% (Brüggenthies 1996).

Wastewaters from photographic laboratories contain the stable Fe(III)EDTA chelate ($K = 10^{13.7}$) which is not degraded by cells of strain BNC1 or the mixed culture. Nevertheless, the elimination of Fe(III)EDTA was achieved in a two-stage process combining a physicochemical pretreatment step and the biological system (Henneken 1995; Henneken et al. 1995; Brüggenthies 1996; Klüner et al. 1996). In the first stage, lime wash [a suspension of $Ca(OH)_2$] was added to the wastewater, resulting in a stoichiometric excess of Ca^{2+} compared to Fe(III)EDTA and an increased pH value of higher than 10. This resulted in a *trans*-complexation reaction according to the equation: $[Fe(III)EDTA]^- + Ca^{2+} + 3\ OH^- \rightarrow [CaEDTA]^{2-} + Fe(OH)_3\downarrow$. At high concentrations of Ca^{2+} and high pH values Fe^{3+} is almost completely displaced from the ligand and precipitated as the hydroxide which can be removed by sedimentation. The CaEDTA chelate was then readily degraded in a second stage, an airlift-loop reactor with immobilized biomass. In addition to EDTA, other recalcitrant organic compounds could be eliminated, indicating that the mixed culture is suited for the biological treatment of industrial wastewaters.

Conclusions and perspectives

Although it was once thought that EDTA was nonbiodegradable, many recent reports indicate that it can be oxidized by microorganisms. Our present understanding of the biochemical basis of EDTA degradation seems to indicate that the initial catabolic enzymes for the degradation of NTA and EDTA share many characteristics in common. It can thus be assumed that they may have evolved from the same ancestor even though it still remains to be identified.

The continued use of large quantities of EDTA as chelating agent will depend particularly on the costs and material properties of potential substitutes. Cheap and readily degradable substitutes, provided that they exist or can be developed, could be utilized for technical processes and products if the chelating properties were strong enough and no resistance against undesired biodegradation during application (or in the technical products) were required. At present, however, EDTA is the cheapest and most suitable complexing compound for many technical purposes. In these cases, the elimination of EDTA from the corresponding process waters or partial wastewater streams by special treatment systems is an appropriate approach to reduce the amounts of EDTA released into the aquatic environment.

In contrast to most of the physicochemical methods, biological treatment systems for wastewaters containing EDTA can be operated economically and very efficiently. Under certain conditions, EDTA can be removed even in conventional wastewater treatment plants. For this, a relatively high hydraulic and sludge retention time, an alkaline pH value of the wastewater or of the reaction system, and a relatively high concentration of EDTA in the wastewater (more than 20–30 mg/l or approximately 0.07–0.1 mmol/l, respectively) are required or favorable.

However, municipal or conventional industrial treatment plants usually receive wastewaters containing EDTA at low concentrations, at which adaptation processes for the degradation of this recalcitrant compound are not observed. Because most EDTA sold is used for special industrial purposes which produce wastewaters with high concentrations of EDTA, it would be advantageous to treat those wastewaters before they are mixed with others that are essentially EDTA-free. Small and compact bioreactors with immobilized cells of an EDTA-degrading culture could be integrated into production or application processes as a final stage and operated inexpensively at high rates and degrees of EDTA degradation.

Further studies on the biodegradation of aminopolycarboxylates such as EDTA and DTPA should also consider a possible accumulation of ketopiperazinecarboxylates. Ketopiperazinecarboxylates can be formed by spontaneous cyclization of primary metabolites which are yielded by the removal of an acetyl group (released as glyoxylate) from the respective chelating agent. As an example, such a cyclization is shown for the first metabolite of EDTA degradation, ED3A, in Fig. 4. Ternes et al. (1996) identified ketopiperazinecarboxylates in many surface waters and drinking waters, indicating that EDTA and DTPA are partly and incompletely

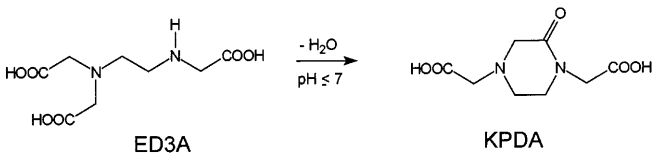

Fig. 4 Spontaneous formation of ketopiperazinedicarboxylate (KPDA) from ED3A

metabolized in the aquatic environment. Notably, the ketopiperazinecarboxylates appear to be resistant to biodegradation, and nothing is known about their toxic or ecotoxic behavior. Therefore, it must be ensured that these compounds are not formed during biological or nonbiological treatment of EDTA- or DTPA-containing wastewaters. In the experiments with cells of the EDTA-degrading mixed culture containing strain BNC1, the DOC concentration always corresponded to the remaining EDTA content, implying that no remarkable accumulation of metabolic intermediates occurred. However, it is not known if sub-optimal conditions or disturbances in the operation of wastewater treatment systems could lead to the release of these metabolites by the mixed bacterial culture or other EDTA-degrading bacteria. These factors need to be examined, but experience so far with the biological mineralization of EDTA by the mixed culture containing strain BNC1 suggests that a successful outcome is possible.

Acknowledgements Parts of the research described in this paper were supported by grants from the Deutsche Forschungsgemeinschaft (DFG, no. He1515/5-1 + 2), the Bundesministerium für Bildung und Forschung (BMBF, no. 0310458A), and the Bundesministerium für Wirtschaft via the Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke" e.V. (AiF, no. 11031 N/1).

References

Alder AC, Siegrist H, Gujer W, Giger W (1990) Behaviour of NTA and EDTA in biological wastewater treatment. Water Res 24: 733–742

Anderegg G (1987) Complexones. In: Wilkinson G (ed) Comprehensive coordination chemistry. Pergamon Press, Oxford

Ayers JA (1970) Decontamination of nuclear reactors and equipment. Ronald Press, New York

Belly RT, Lauff JJ, Goodhue CT (1975) Degradation of ethylenediaminetetraacetic acid by microbial populations from an aerated lagoon. Appl Microbiol 29: 787–794

Bolton H, Li SW, Workman DJ, Girvin DC (1993) Biodegradation of synthetic chelates in subsurface sediments from the southeast coastal plain. J Environ Qual 22: 125–132

Bolton H, Girvin DC, Playmale AE, Harvey SD, Workman DJ (1996) Degradation of metal-nitrilotriacetate complexes by *Chelatobacter heintzii*. Environ Sci Technol 30: 931–938

Brauch HJ, Kühn W (1988) Organische Spurenstoffe im Rhein und bei der Trinkwasseraufbereitung. gwf Wasser Abwasser 129: 189–196

Brauch HJ, Schullerer S (1987) Verhalten von Ethylendiamintetraacetat (EDTA) und Nitrilotriacetat (NTA) bei der Trinkwasseraufbereitung. Vom Wasser 69: 155–164

Brüggenthies A (1996) Biologische Reinigung EDTA-haltiger Abwässer. In: Hempel DC (ed) ibvt-Schriftenreihe des Instituts für Bioverfahrenstechnik, Technische Universität Braunschweig, vol 3. FIT-Verlag, Paderborn

BUA-Stoffbericht 168(1996) Ethylendiamintetraessigsäure/Tetranatriumethylendiamintetraacetat. Hirzel, Stuttgart

Bunch RL, Ettinger MB (1967) Biodegradability of potential organic substitutes for phosphate. Proceedings industrial waste conference, 22nd Purdue Eng Extn Ser no 129: 393–396

Dietz F (1985) Gewässerbelastung durch EDTA – eine neue Herausforderung an den Gewässerschutz. Korrespondenz Abwasser 11: 988–989

Dietz F (1987) Neue Messergebnisse über die Belastung von Trinkwasser mit EDTA. gwf Wasser Abwasser 128: 286–288

Egli T (1994) Biochemistry and physiology of the degradation of nitrilotriacetic acid and other metal complexing agents. In: Ratledge C (ed) Biochemistry of microbial degradation. Kluwer Academic, Dordrecht, pp 179–195

Firestone MK, Tiedje JM (1975) Biodegradation of metal-nitrilotriacetate complexes by a *Pseudomonas* species. Appl Environ Microbiol 29: 758–764

Francis AJ, Dodge CJ, Gilow JB (1992) Biodegradation of metal citrate complexes and implications for toxic-metal mobility. Nature 356: 140–142

Frimmel FH, Grenz R, Kordick E, Dietz F (1989) Nitrilotriacetat (NTA) und Ethylendiamintetraacetat (EDTA) in Fliessgewässern der Bundesrepublik Deutschland. Vom Wasser 72: 175–184

Gschwind N (1992) Biologischer Abbau von EDTA in einem Modellabwasser. gwf Wasser Abwasser 133: 546–549

Haberer K (1991) Rheinwasser als Rohstoff für die Grundwasseranreicherung in Wiesbaden. gwf Wasser Abwasser 132: 60–64

Henneken L (1995) Biologischer Abbau des Komplexbildners EDTA – Reaktionskinetik und Verfahrensentwicklung. Fortschrittsberichte series 15: Umwelttechnik. VDI-Verlag, Düsseldorf

Henneken L, Klüner T, Nörtemann B, Hempel DC (1994) Abbau von EDTA mit freien und immobilisierten Bakterien. gwf Wasser Abwasser 135: 354–358

Henneken L, Nörtemann B, Hempel DC (1995) Influence of physiological conditions on EDTA degradation. Appl Microbiol Biotech 44: 190–197

Henneken L, Brüggenthies A, Nörtemann B, Hempel DC (1996) Teilstrombehandlung EDTA-haltiger Abwässer mittels Bio-Wirbelbettreaktoren. Chem Ing Tech 68: 310–314

Kaluza U, Klingelhöfer P, Taeger K (1998) Microbial degradation of EDTA in an industrial wastewater treatment plant. Water Res 32: 2843–2845

Kari FG, Giger W (1996) Speciation and fate of ethylenediaminetetraacetate (EDTA) in municipal wastewater treatment. Water Res 30: 122–134

Kemmler J, Egli T (1990) Nitrilotriacetat-abbauende Bakterien. gwf Wasser Abwasser 131: 23–27

Klopp R, Pätsch B (1994) Organische Komplexbildner in Abwasser, Oberflächenwasser und Trinkwasser, dargestellt am Beispiel der Ruhr. Wasser Boden 8: 32–37

Klüner T (1996) Chemie und Biochemie des mikrobiellen EDTA-Abbaus. Cuvillier, Göttingen

Klüner T, Henneken L, Gehle M, Brüggenthies A, Nörtemann B, Hempel DC (1994) Katabolismus von Ethylendiamintetraacetat (EDTA). Bioforum 17: 284–288

Klüner T, Nörtemann B, Hempel DC, Brüggenthies A, Henneken L (1996) EDTA biologisch eliminieren? mo 50: 392–395

Klüner T, Hempel DC, Nörtemann B (1998) Metabolism of EDTA and its metal chelates by whole cells and cell-free extracts of strain BNC1. Appl Microbiol Biotechnol 49: 194–201

Knobel HR, Egli T, van der Meer JR (1996) Cloning and characterization of the genes encoding nitrilotriacetate monooxygenase of *Chelatobacter heintzii* ATCC 29600. J Bacteriol 178: 6123–6132

Lauff JJ, Steele DB, Coogan LA, Breitfeller JM (1990) Degradation of the ferric chelate of EDTA by a pure culture of an *Agrobacterium* sp. Appl Environ Microbiol 56: 3346–3353

Macaskie LE (1991) The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. Crit Rev Biotechnol 11: 41–112

Madsen EL, Alexander M (1985) Effects of chemical speciation on the mineralization of organic compounds by microorganisms. Appl Environ Microbiol 50: 342–349

Mcfadden KM (1980) Organic components of nuclear wastes and their potential for altering radionuclide distribution when released to soil. PNL 2563. National Technical Information Service, Springfield, Va, USA

Means JL, Alexander CA (1981) The environmental biogeochemistry of chelating agents and recommendations for the disposal of chelated radioactive wastes. Nucl Chem Waste Manage 2: 183–196

Means JF, Crerar DA, Duguid JO (1978) Migration of radioactive wastes: radionuclide mobilization by complexing agents. Science 200: 1477–1481

Miyazi H, Suzuki S, Imada K (1997) Isolation and characterization of a bacterium that decomposes (ethylenediaminetetraacetato) ferrate (III) complex. Environ Sci 10: 257–262

Nörtemann B (1992) Total degradation of EDTA by mixed cultures and a bacterial isolate. Appl Environ Microbiol 58: 671–676

Nörtemann B, Imberg B, Hempel DC (1991) Biodegradation of ethylenediaminetetraacetate (EDTA). In: Verachert H, Verstraete W (eds) International Symposium on Environmental Biotechnology, 22–25 April 1991, Ostend, Belgium. Koninklijke Vlaamse Ingenieursverenigung ZVW, Antwerpen, pp 259–262

Nowack B, Sigg L (1997) Dissolution of Fe(III) (hydr)oxides by metal-EDTA complexes. Geochim Cosmochim Acta 61: 951–963

Nowack B, Xue H, Sigg L (1997) Influence of natural and anthropogenic ligands on metal transport during infiltration of river water to groundwater. Environ Sci Technol 31: 866–872

Nusch EA, Eschke HD, Kornatzki KH (1991) Die Entwicklung der NTA- und EDTA-Konzentrationen im Ruhrwasser und daraus gewonnenem Trinkwasser. Korrespondenz Abwasser 38: 944–949

Palumbo AV, Lee SY, Boerman P (1994) The effect of media composition on EDTA degradation by *Agrobacterium* sp. Appl Biochem Biotechnol 45: 811–822

Payne JW, Bolton H, Campbell JA, Xun L (1998) Purification and characterization of EDTA monooxygenase from the EDTA-degrading bacterium BNC1. J Bacteriol 180: 3823–3827

Ringbom A (1963) Complexation in analytical chemistry. Wiley, New York

Schullerer S, Brauch HJ (1989) Oxidative und adsorptive Behandlung EDTA- und NTA-haltiger Wässer. Vom Wasser 72: 21–29

Siegrist H, Alder A, Gujer W, Giger W (1988) Behaviour of the organic complexing agents NTA and EDTA in activated sludge treatment. gwf Wasser Abwasser 68: 101–109

Ternes T, Stumpf M, Steinbrecher T, Brenner-Weiß G, Haberer K (1996) Identifizierung und Nachweis neuer Metabolite des DTPA in Fließgewässern und Trinkwässern. Vom Wasser 87: 275–290

Thomas RAP, Lawlor K, Bailey M, Macaski LE (1998) Biodegradation of metal-EDTA complexes by an enriched microbial population. Appl Environ Microbiol 64: 1319–1322

Tiedje JM (1975) Microbial degradation of ethylenediaminetetraacetate in soils and sediments. Appl Microbiol 30: 327–329

Uetz T, Schneider R, Snozzi M, Egli T (1992) Purification and characterization of a two-component monooxygenase that hydroxylates nitrilotriacetate from "Chelatobacter" strain ATCC 29600. J Bacteriol 174: 1179–1188

Van Ginkel CG, Vandenbroucke KL, Stroo CA (1997) Biological removal of EDTA in conventional activated-sludge plants operated under alkaline conditions. Bioresource Technol 59: 151–155

Witschel M, Egli T (1998) Purification and characterization of a lyase from the EDTA-degrading bacterial strain DSM 9103 that catalyzes the splitting of [S,S]-ethylenediaminedisuccinate, a structural isomer of EDTA. Biodegradation 8: 419–428

Witschel M, Weilenmann H-U, Egli T (1995) Degradation of EDTA by a bacterial isolate. Poster presented at the 54th Annual Meeting of the Swiss Society of Microbiology, Lugano, March 23–24, 1995. (Abstract page 146)

Witschel M, Nagel S, Egli T (1997) Identification and characterization of the two-enzyme system catalyzing oxidation of EDTA in the EDTA-degrading bacterial strain DSM 9103. J Bacteriol 179: 6937–6943

Xue H, Sigg L, Kari FG (1995) Speciation of EDTA in natural waters: exchange kinetics of Fe-EDTA in river water. Environ Sci Technol 29: 59–68

Xun L, Reeder RB, Playmale AE, Girvin DC, Bolton H (1996) Degradation of metal-nitrilotriacetate complexes by nitrilotriacetate monooxygenase. Environ Sci Technol 30: 1752–1755