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Microbial degradation of chelating agents used in detergents with special reference to nitrilotriacetic acid (NTA)

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Abstract

The extensive use of phosphate-based detergents and agricultural fertilizers is one of the main causes of the world-wide eutrophication of rivers and lakes. To ameliorate such problems partial or total substitution of phosphates in laundry detergents by synthetic, non-phosphorus containing complexing agents is practiced in several countries. The physiological, biochemical and ecological aspects of the microbial degradation of the complexing agents most frequently used, such as polyphosphates, aminopolycarboxylates (especially of nitrilotriacetic acid), and phosphonates are reviewed.

Abbreviations: AODC – Acridine orange direct counts, ATMP – Aminotrimethylphosphonate, DTPA – Diethylenetriaminepentaacetate, DTPMP – Diethylenetriaminepentamethylphosphonate, EDTA – Ethylenediaminetetraacetate, EDTMP – Ethylenediaminetetramethylphosphonate, ED3A – Ethylenediaminetriacetate, HEDP – Hydroxyethylidenediphosphonate, HEDTA – Hydroxyethylethylenediaminetriacetate, IDA – Iminodiacetate, IFT – Immunofluorescence test, MW – Molecular weight, NTA – Nitrilotriacetate, PA – Polyacrylate, PHC – Polyhydroxycarboxylate, PMS – Phenazine methosulfate, SDS-PAGE – Sodium dodecylsulfate polyacrylamide gel electrophoresis, SPP – Tetrasodiumpyrophosphate, STP – Pentasodiumtriphosphate

Introduction

The washing of textiles is a complex process in which dirt (e.g., fats, proteins or inorganic and organic salt incrustations) is either emulsified or dissolved in the aqueous phase without damaging the textile fibers. To achieve this, detergents consisting of surface-active substances (tensides), bleaching agents and various additives (enzymes, perfumes etc.) are used. Furthermore, to prevent deposition of scale on both textiles and washing machine parts and to support performance of tensides, metal sequestering and/or chelating agents (so-called 'builders') have to be added because, in alkaline washing-liquid, bivalent metal ions such as calcium and magnesium form water insoluble salts with carbonate and washing active tensides (Jakobi et al. 1983). Historically, deposition of scale on textile fibers was circumvented by removing bivalent ions from the washing suds by precipitation with excess soap, sodium carbonate or sodium silicate (Berth et al. 1983; Jakobi et al. 1983). The invention of mechanical washing machines required adoption of a different strategy from precipitation, whereby Ca^{2+} and Mg^{2+} ions were kept in solution in the form of water soluble complexes during the washing process.

The compounds which have been employed for

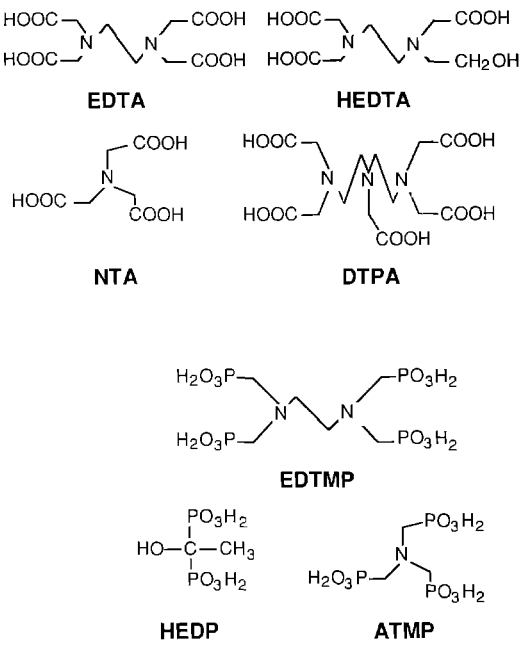

Fig. 1. Chemical structure of some synthetic chelating agents.

this purpose and which have been used in increasing amounts over the past 50 years were polyphosphates, mainly STP, because of its high complexing capacity, low toxicity and low price (Table 1). Over the last thirty years it has been shown in numerous studies that phosphates in domestic wastewater and agricultural fertilizers are the principal sources of phosphorus contributing to the widespread process of eutrophication of inland surface waters (Vollenweider 1968; Hauptausschuss Phosphate BRD 1978). Hence, in order to reduce the phosphorus load introduced into surface waters via wastewater various countries have implemented laws such as restriction of the phosphorus content in laundry detergents from typically 30–40% STP to a level of 4–5% STP (e.g. Canada, FRG, Italy) or the complete ban of phosphates from laundry detergents (e.g. the State of Indiana (USA) or Switzerland). This is because in wastewater usually a considerable part (40–70%) of the phosphorus stems from phosphate-based detergents, the remainder originating mainly from feces (Bunch & Ettinger 1967; Epstein 1972; Hauptausschuss Phosphate 1978).

Although worldwide the most frequently used chelating agent is still STP, alternative compounds have been suggested as potential builders (Kemper et al. 1975; Matzner et al. 1973) some of which are presently employed to partially or completely replace STP in detergents (Bernhardt 1990). The most important of them are the organic complexing agents NTA and citrate, but lately also silicates with cationic exchanging capacity such as Zeolites (Table 1). In addition to builders a range of other metal complexing agents such as phosphonates and polymeric carboxylic acids are frequently included in detergents in smaller quantities (1–2% in domestic laundry detergents) because of their ability to either slow down or inhibit crystal growth (Fig. 1).

Due to a number of facts (Mottola 1974; Tiedje 1980), the biodegradation of NTA has been the focus of considerable interest and, as a consequence, it is the synthetic chelating agent for which most information is available concerning both chemo-dynamic behaviour in the environment and biodegradation. This is probably due to the fact that it was essentially the first compound proposed as an efficient and cost-effective alternative to STP

Table 1. Estimated use of detergents and of chelating agents in the USA and Western Europe in 1981 (in 1000 metric tons). Data from Schneider (1984) and Egli (1988).

	USA	Western Europe
Detergents (total)[a]	7848[b]	7735
Synthetic laundry detergents	5209[b]	5643
Per capita consumption 1980	30.2[b,c]	18.9
Chelating agents		
STP	567	1100
SPP	19	11
EDTA	42	13.6
NTA	32	8.3
DTPA	4	0.5
HEDTA	18	2.0
Others	2.5	
Citric acid[d]	102	7.5
Gluconic acid	8	7.5
Organophosphonates	10	10

[a] Including soaps, soap based laundry detergents, synthetic laundry detergents and other cleaning agents.
[b] Figure given for North America.
[c] in kg
[d] Only approximately 30% used in detergents, rest is used in food and beverage industry.

in detergents. However, even today its environmental impact is assessed differently in different countries and whereas the use of NTA in laundry detergents is prohibited in Italy, it has been permitted in countries such as Canada, Sweden, Norway, Finland, Holland and Switzerland. Therefore, particular attention will be given in this review to various aspects of the biochemistry, physiology and ecology of the biodegradation of this aminopolycarboxylic acid. Although the carboxylic acids citrate and gluconate are frequently used as complexing agents in detergents for certain special purposes, they are common growth substrates and intermediates in microbial metabolism and they will not be considered here (for information see Egli 1988).

Nitrilotriacetic acid

NTA-degrading microorganisms

A wide range of microorganisms including *Pseudomonas, Bacillus* (Pickaver 1976), *Listeria* (Madson & Alexander 1985), and yeast species (Pickaver 1976) have been reported in the literature to be able to degrade NTA. Nevertheless, in the majority of detailed studies, Gram-negative motile, both obligately aerobic and facultatively denitrifying rods have been repeatedly isolated in pure culture primarily from soil and wastewater. Originally these isolates were assigned to the genus *Pseudomonas* (Cripps & Noble 1973; Tiedje et al. 1973; Focht & Joseph 1971; Kakii et al. 1986) and this led to the general view that NTA degradation in nature is primarily mediated by specialized *Pseudomonas* strains. However, in all cases this allocation was based on limited taxonomic information. A more detailed study in our laboratory in which a number of recently isolated obligately aerobic (Egli et al. 1988; Wehrli & Egli 1988) and one denitrifying strain (Wanner et al. 1990) were investigated, revealed that none of the isolates could be assigned to the genus *Pseudomonas* (as defined by de Vos & de Ley (1983)). Presently three distinctly different groups of Gram-negative, NTA-utilizing bacteria can be recognized and their key characteristics are given in Table 2. In addition to the information given in this table concerning the taxonomic characterization of these isolates, SDS-PAGE of soluble proteins, serological cross reactions, DNA: DNA-hybridization studies and rapid sequencing of 16S rRNA (El-Banna 1989; Wanner et al. 1990; El-Banna et al. unpubl.) all suggest that the three groups of Gram-negative, NTA-degrading bacteria are representatives of at least two, if not three, new genera. To date the phylogenetic position of the new genus consisting of strains 4–10 together with

Table 2. Key characteristics of the three different groups of NTA-degrading Gram-negative bacteria (data from Egli et al. 1988; Wanner et al. 1990, and El-Banna 1989).

Strains	Morphology	Sugars utilized	Respiration	G + C ratio	Main quinone	Main polyamine
TE 4–10, ATCC 27109, ATCC 29600	Motile rods, mostly pleomorphic	+	obligately aerobic	62.1–63.4	Q-10	*s*HSPD
TE 1, 2	Short rods or diplococci, non-motile, S-layer	–	obligately aerobic	63.3–63.5	Q-10	*s*HSPD
TE 11	Motile rods	+	facultatively denitrifying	65.3	Q-8	SPD

G + C ratio is given in mol% guanine plus cytosine; Q-10(9,8), Ubiquinone with 10(9,8) isoprene units in the side chain; *s*HSPD, *sym*-homospermidine; SPD, spermidine. For comparison: *Pseudomonas fluorescens* has a G + C ratio of 60.2 and contains mainly putrescine and Q-9 (Auling G & Busse J, pers. comm.).

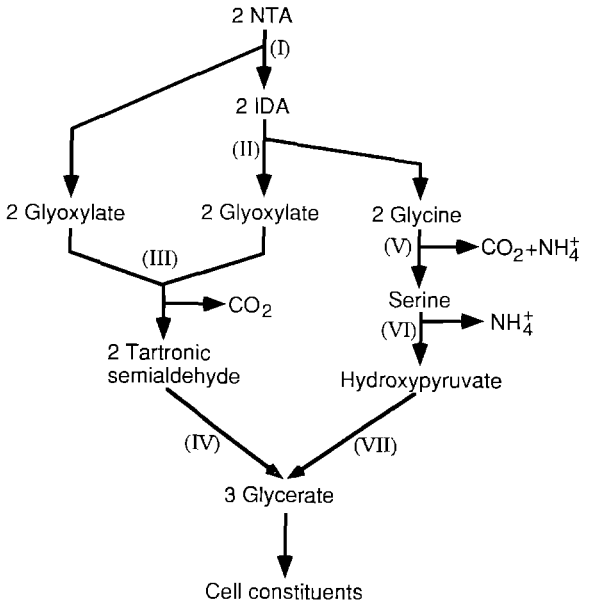

Fig. 2. Metabolic pathway for nitrilotriacetic acid proposed by Cripps & Noble (1973) for the Gram-negative obligate aerobic strain T23. I, Nitrilotriacetic acid mono-oxygenase; II, Unknown enzyme; III, Glyoxylate carboligase; IV, Tartronic semialdehyde reductase; V, Glycine decarboxylase and serine hydroxymethyltransferase; VI, Serine oxaloacetate aminotransferase; VII, Hydroxypyruvate reductase.

the two strains from the ATCC is best defined by a location in the α-2 branch of *Proteobacteria* where it is separated from its closest phytogenetic neighbours in the Rhizobia/Agrobacteria branch by a similarity coefficient (S_{AB}) of 0.59 (G. Auling, E. Stackebrandt, pers. comm.). There is also strong evidence that the second group consisting of strains TE 1 and 2 are representatives of a new genus in the α-branch of *Proteobacteria*. However, its exact taxonomic position in the α-2 branch still remains to be elucidated. Although, except for its ability to denitrify, no striking differences were observed between isolate TE 11 and the group of motile Gram-negative rods with respect to nutritional and physiological properties (Egli et al. 1988; Wanner et al. 1990), the presence of ubiquinone Q-8 and the polyamine spermidine clearly suggests a distant relationship to the group of obligately aerobic NTA-degraders and indicates that it should be allocated to the γ-subgroup of *Proteobacteria*. The combined presence of Q-8 and spermidine suggest allocation to the genus *Xanthomonas* (Ikemoto et al. 1980; Webb & G. Auling, pers. comm.). However, a number of physiological properties that are different to those typically exhibited by *Xanthomonas* spp. makes such an allocation of strain TE 11 rather unlikely and the possibility remains that a new genus will have to be established (Wanner et al. 1990).

Although none of the other strains isolated and described in the literature has been characterized to such an extent, the information available suggests that they may be members of the three groups of NTA-degraders described above. Furthermore, information presently available indicates that the ability to utilize NTA is not restricted to either a special genus or even a species but that it is a feature exhibited at least by members of both the α- and the γ-group of *Proteobacteria*. Further, a NTA-utilizing Gram-positive member of the *Rhodococcus* group has been isolated (Egli et al. 1988), thereby indicating that the ability to utilize NTA is not restricted to the Gram-negative bacteria.

Biochemistry

The biochemical pathway for NTA degradation was first investigated in two virtually physiologically and probably taxonomically identical bacteria, namely the obligately aerobic Gram-negative strains T23 (Cripps & Noble 1973) and ATCC 29600 (Firestone & Tiedje 1978). For both bacteria a mono-oxygenase was reported to be responsible for the conversion of NTA to IDA and glyoxylate. It was impossible to demonstrate further degradation of IDA in cell-free extracts. Based on enzyme induction patterns in NTA- and glucose-grown cells, Cripps & Noble (1973) proposed the biochemical pathway shown in Fig. 2. In an attempt to purify NTA mono-oxygenase, a protein, exhibiting NTA-stimulated NADH oxidation but completely devoid of NTA-splitting activity, was isolated from ATCC 29600 (Firestone et al. 1978; Snozzi & Egli 1987). These results indicate that NTA mono-oxygenase is a multi-component enzyme. This has been confirmed recently by the isolation of a functional enzyme consisting of two proteins, a flavin-containing component B with a MW of 32000 on

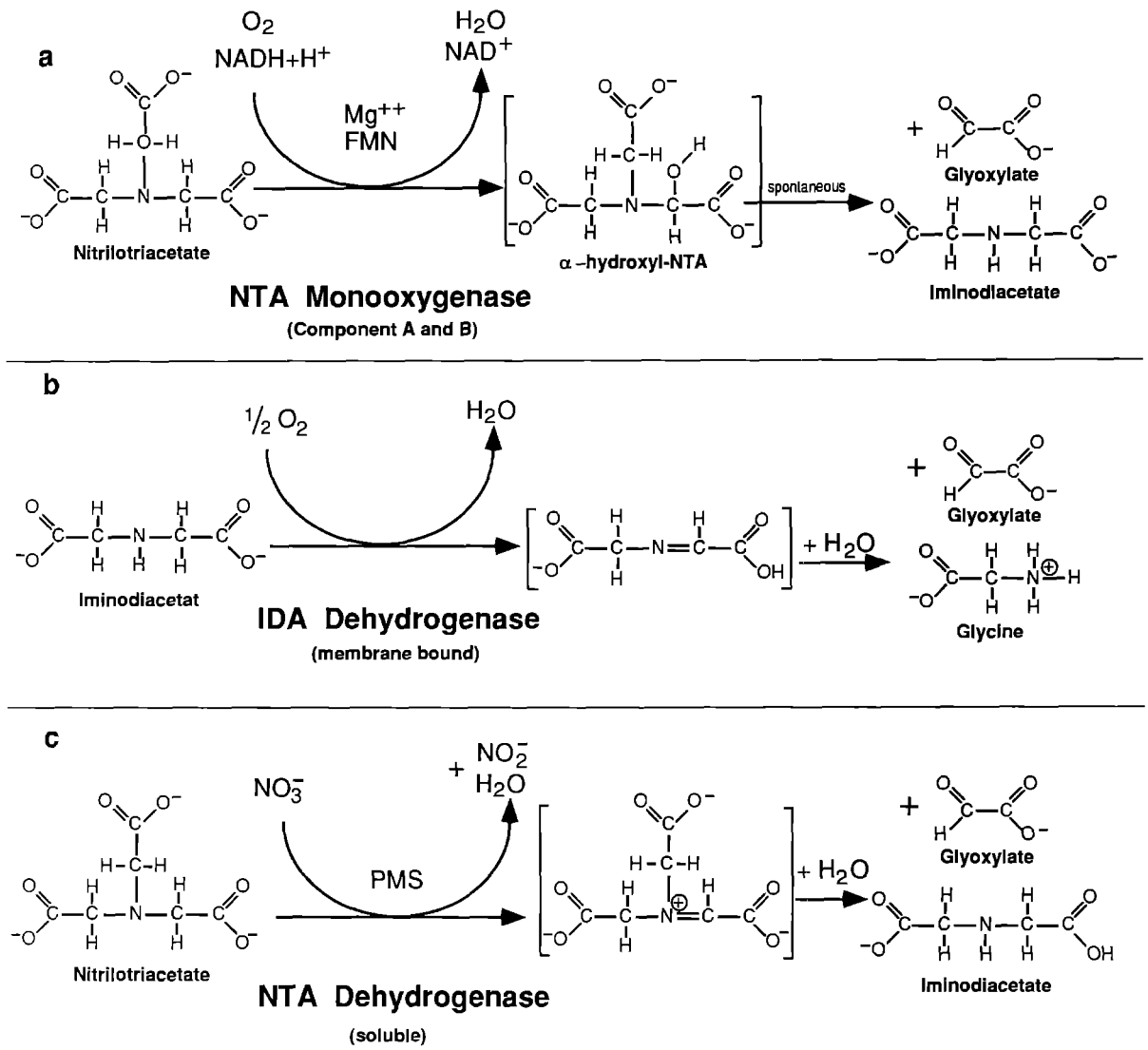

Fig. 3. Reactions catalyzed by nitrilotriacetate mono-oxygenase (*a*) and the membrane-bound iminodiacetate dehydrogenase (*b*) in the Gram-negative obligate aerobic strain ATCC 29600 and of nitrilotriacetate dehydrogenase (*c*) in the denitrifying strain TE 11.

SDS-PAGE exhibiting NTA/Mg^{2+}-stimulated NADH oxidation and a second component A (MW 50000 on SDS-PAGE) for which presently no catalytic activity is known. Catalytic activity, i.e., the formation of glyoxylate and IDA from NTA (Fig. 3a) was obtained only in the presence of components A and B, FMN and magnesium ions. Furthermore, the purified NTA mono-oxygenase did not accept IDA as a substrate (T. Uetz, unpubl.). This indicated that the subsequent metabolism of IDA was catalyzed by an additional enzyme and not by NTA mono-oxygenase as previously suggested by Firestone et al. (1978). Recently, IDA oxidation in the membrane fraction of NTA-grown cells of strain ATCC 29600 has been demonstrated (Fig. 3b). Glyoxylate was identified as a product of the reaction which was dependent on molecular oxygen and was inhibited by potassium cyanide (T. Uetz, unpubl. results). Whether NTA is degraded via NTA mono-oxygenase and IDA dehydroge-

nase in all obligately aerobic, Gram-negative isolates is not yet known.

It is obvious that in the facultatively denitrifying isolate TE 11 the metabolism of NTA must proceed via a different pathway during growth in the absence of molecular oxygen. In this bacterium, activity of a soluble NTA dehydrogenase which was dependent on the presence of PMS and nitrate was detected in membrane-free cell extract. In the reaction catalyzed (Fig. 3c) NTA was broken down stochiometrically to IDA and glyoxylate with the concommitant reduction of nitrate to nitrite (Wanner et al. 1989). Whether this dehydrogenase is also involved in the breakdown of NTA in aerobically grown cells and the fate of IDA in strain TE 11 still await elucidation.

To date, little biochemical information is available on the transport of NTA across the cell membrane. Wong et al. (1973) observed that transport of ^{14}C-labelled NTA was inhibited by both cyanide and azide which indicates that it is energy dependent. Experiments reported by Firestone & Tiedje (1975), in which the oxygen consumption of whole cells of strain ATCC 29600 incubated in the presence of various NTA-metal chelates was measured showed that this was highest when NTA was supplied in the presence of magnesium, manganese, calcium, iron, or sodium, whereas with nickel the rate of oxygen consumption was negligible. This suggests that transport of NTA into the cell is influenced by the nature of the metal-NTA complex. However, it is not yet known whether NTA is transported across the membrane in the form of a metal-NTA complex or whether before or during the process of transport the initially complexed metal ion has to be exchanged by other cations. In any case, the complexed cation being transported across the cytoplasmic membrane together with NTA has to be excreted to avoid intracellular accumulation.

Regulation of NTA catabolic enzymes

As has been shown in the previous section not all enzymes involved in the degradation of NTA have been identified. Nevertheless, the data presented by Cripps & Noble (1973) clearly demonstrated that growth on NTA led to enhanced activities of a series of enzymes in cells of strain TE23, including NTA mono-oxygenase and several enzymes involved in the transformation of glyoxylate and glycine to glycerate (compare Fig. 2). In our laboratory, the specific activity of NTA mono-oxygenase was recently measured as a function of both specific growth rate and of the nature of the growth-limiting substrate in chemostat cultures of strain ATCC 29600 (Wilberg 1989). It was found that the specific activity of NTA mono-oxygenase increased with decreasing specific growth rates during growth with NTA as the sole source of carbon, nitrogen and energy (Fig. 4). In addition, derepression of the synthesis of this enzyme was also observed at low growth rates when the cells were cultivated with glucose and ammonium. These results, shown in Fig. 4, suggest that although NTA is not absolutely necessary for expression, it stimulates additional induction of the synthesis of this enzyme. However, several questions with respect to the regulation of the synthesis of enzymes involved in NTA metabolism remain to be elucidated. Of particular interest are studies on the expression of the transport system for NTA, of NTA mono-oxygenase and of IDA dehydrogenase under different growth conditions, such as pulses of NTA to cultures exponentially growing with substrates other than NTA or the effect of different carbon/nitrogen substrates and ratios.

Ecological aspects of NTA degradation

The two crucial questions which remain to be answered with respect to NTA degradation under environmental conditions are firstly, how many and what kind of microorganisms capable of potentially degrading NTA are present in different ecosystems and secondly, how is the capacity to degrade NTA regulated in these microorganisms during growth in such systems.

Two different methods have been reported in the literature for the enumeration of NTA-degrading microorganisms in different ecosystems. Larson and co-workers used a ^{14}C-most-probable-number

technique for enumeration of NTA-degrading microorganisms in estuarine water samples, a method which potentially allows enumeration of all organisms able to grow with NTA. In samples of different salinity the fraction of NTA-degrading microorganisms ranged from 0.0002 to 0.26% of the total bacterial cell count as measured by AODC (Larson & Ventullo 1986; Pfaender et al. 1985). Their results (Table 3) indicated that the distribution of NTA-degrading microorganisms was influenced neither by salinity nor by the dissolved organic carbon concentration in the water and they concluded that NTA degraders were indigenous members of the estuarine microbial community. In our own laboratory we used antibodies raised against whole cells of two different NTA-degrading isolates, namely strains ATCC 29600 and TE 2, in an indirect immunofluorescence assay to microscopically enumerate positively reacting cells in both wastewater treatment plants and natural waters (Wilberg 1989; Kemmler & Egli 1990). In this study it was found that the fraction of IFT-positive cells in surface waters was in the range of 0.01–0.1% of the total cell number assessed with AODC, whereas in activated sludge this number ranged from 0.1–1.0%, suggesting a ten-fold enrichment of NTA-degrading bacteria in treatment plants (Table 3). No significant difference in the fraction of IFT-positive cells was observed between samples collected from treatment plants which differed considerably with respect to their efficiency in NTA elimination. It should be stressed that these results might be influenced by both cross reactivity of the antisera used with non-NTA-degrading bacteria and that the sera might not recognize all NTA degraders present in the samples. This question could be answered by using a method in which sera of high specificity and uptake of ^{14}C-labelled NTA were combined.

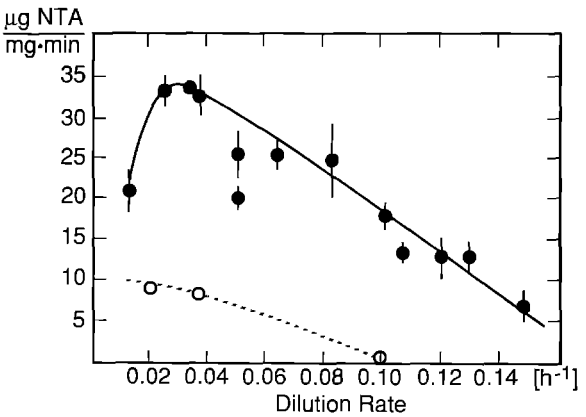

Fig. 4. Specific activity of nitrilotriacetate mono-oxygenase in cell-free extracts of strain ATCC 29600 grown in carbon-limited continuous culture with either nitrilotriacetic acid (●) with glucose/ammonium (○) as the sources of carbon, nitrogen and energy, as a function of the dilution (growth) rate. Data from Wilberg (1990).

There is considerable information available in

Table 3. Detection and enumeration of NTA-degrading microorganisms in samples collected from different ecosystems and wastewater treatment plants.

Origin of sample	AODC[a]	IFT-positive[b] or MPN[c] (in % of AODC)	Reference
Activate sludge from treatment plant exhibiting good NTA elimination (> 98%)	$1.1 \cdot 10^9$	0.026%	Wilberg (1989)
Activated sludge from treatment plant exhibiting reduced NTA elimination (41%)	$2.4 \cdot 10^9$	0.192%	Wilberg (1989)
Kriesbach, small river eutrophic	$1.3 \cdot 10^9$	0.014%	Wilberg (1989)
River Rhine, surface water	$1.4 \cdot 10^9$	0.062%	Wilberg (1989)
Garden soil	$1.1 \cdot 10^8$	0.003%	Wilberg (1989)
Estuary, 0.8–1.9‰ salinity	$5 \cdot 10^8$–$9.2 \cdot 10^9$	0.0002–0.26%	Larson & Ventullo (1986)
Estuary, 1.4‰ salinity	$0.3 \cdot 10^9$	0.22%	Pfaender et al. (1985)

[a] Acridine orange direct counts.
[b] Immunofluorescence test positive cells with antibodies raised against strain ATCC 29600.
[c] Most probable number.

the literature suggesting the necessity for an adaptation period in order to obtain NTA degradation in environmental samples. However, such studies do not answer the question as to whether this adaptation period was due either to enrichment of competent microorganisms or to induction of NTA-degrading enzymes in microorganisms originally present in these samples. Only detailed studies in which microscopic techniques are combined with serological or radiolabelling methods, or the use of gene probes can yield information on the presence and expression of enzymes involved in the degradation of NTA under such complex conditions. Another approach to study the regulation of the capacity to degrade NTA would be the exposure of pure cultures of NTA-degrading bacteria in membrane chambers to the environment. Using this method, McFeters et al. (1990) were able to demonstrate that strain ATCC 29600, pre-grown in the laboratory under non-inducing conditions, was able to induce the catabolic enzymes for NTA within 6–8 hours upon transfer to a wastewater treatment plant.

Other aminopolycarboxylic acids

Presently, no report on the successful isolation and cultivation of either a microbial enrichment culture or a pure culture able to degrade EDTA, DTPA, or HEDTA is available in the open literature. Nevertheless, either biologically mediated transformation or degradation of all three compounds has been reported to occur. Whereas a number of studies dealing with the degradation of EDTA have been published the authors are aware of only one report concerning the degradation of DTPA and HEDTA in soil slurries (Means et al. 1980).

The biodegradation of EDTA is very important with respect to environmental considerations because it is a compound which is currently used in quantities equal to or higher than NTA (Table 1). Biologically-mediated, aerobic degradation of EDTA has been reported in soils, river and lake sediments (Tiedje 1975 and 1977; Means et al. 1980) and in sludge from an aerated wastewater treatment lagoon (Belly et al. 1975). Using ^{14}C-labelled EDTA, Belly and co-workers demonstrated the microbial breakdown to ^{14}C-CO_2 of both the ethylene and the acetate part of the molecule. Because the addition of easily degradable carbon sources stimulated oxidation of EDTA, it was suggested that EDTA was co-metabolized by certain members of the microbial population rather than utilized as a sole source of carbon or nitrogen by a specific EDTA-degrading bacterium (Tiedje 1975 and 1977). In contrast to soil where no significant accumulation of breakdown intermediates could be detected (Tiedje 1975) a range of compounds, probably derived from microbial degradation of EDTA were identified by Belly et al. (1975) in wastewater. Primarily IDA and ED3A and also glycine and NTA were found as products. This and the fact that similar intermediates were found during aerobic photodegradation of Fe(III)-EDTA (Lockhart & Blakeley 1975) points to the possible involvement of light and NTA-degrading microorganisms in the breakdown of EDTA. Despite this, EDTA seems to be rather persistent in wastewater treatment plants (Madsen & Alexander 1985; Bunch & Ettinger 1962; Gerike & Fischer 1979). This is supported by the recent results obtained during the Swiss NTA/EDTA monitoring programme where no significant degradation of EDTA (in contrast to NTA) was detected during aerobic wastewater treatment and in aerobic groundwater infiltration zones. Despite the greatly increased use of NTA in laundry detergents since the Swiss ban of STP in July 1986, a higher residual concentration of EDTA (10–20 $\mu g\, l^{-1}$) than of NTA (2–5 $\mu g\, l^{-1}$) was found in larger Swiss rivers (Giger et al. 1987). Similar observations have been made for the River Ruhr in Germany (Dietz 1987).

Polyphosphates

Polyphosphates are relatively stable compounds that chemically hydrolyse only very slowly. Half-lives in sterile water of the two polyphosphates used as detergent builders, STP and SPP, at pH 7.0 and 30° C were reported to be in the range of 1–3

years (Griffith et al. 1973; Zinder et al. 1981). The biologically catalyzed rate of STP hydrolysis in both natural waters and wastewater treatment plants is two to three orders of magnitude faster (Heinke et al. 1969).

A wide range of eukaryotic and prokaryotic cells are known to accumulate and store high molecular weight polyphosphates within their cells. Under certain conditions bacteria and yeasts have been reported to accumulate as much as 20% of their dry weight as polyphosphate (Kulaev & Vagabov 1983). Hence, it is not surprising that many microorganisms are equipped with the ability to hydrolyse polyphosphates and use them as a source of phosphorus.

With respect to the transport of polyphosphates into cells relatively few data have been published and the basic question of whether polyphosphates can be transported into the cell at all still awaits clarification. According to Kulaev & Vagabov (1983) tripolyphosphate (TPP) seems to be the form in which polyphosphate is taken up most efficiently by cells. Nevertheless, because of numerous reports about extracellular and periplasmic polyphosphate-degrading phosphatases (Kulaev & Vagabov 1983), it seems justified to assume that extracellular polyphosphates are first hydrolysed to inorganic phosphate before being transported into the cell. Considerable evidence for such a mechanism was recently found for *Escherichia coli* (Rao et al. 1987). In this bacterium growth on polyphosphate (chain length approximately 100 phosphate residues) as a sole source of phosphorus was dependent on the presence of alkaline phosphatase. Mutants which were not able to either induce the phosphate regulon or were deficient in an active alkaline phosphatase lost the capability to grow on polyphosphate (Rao et al. 1987; Torriani-Gorini 1987).

Until now several different enzyme types (eq. I–III) hydrolyzing polyphosphates (including TPP) have been described in the literature, most of them exhibiting low specificity with respect to the degree of polymerization of the polyphosphate accepted as the substrate. As discussed by Kulaev (1979) it is not yet known whether this lack of specificity is a general property of these enzymes or whether it originates from the problem of separating individual phosphatases with differing substrate specificities during purification.

I Alkaline and acid phosphatases
$R\text{-}P + H_2O \rightarrow ROH + P_i$

IIa Polyphosphate-phosphohydrolases
$PP_n + H_2O \rightarrow PP_{n-1} + P_i$

IIb $TPP + H_2O \rightarrow PP + P_i$

III Polyphosphate-depolymerases
$PP_n + H_2O \rightarrow PP_{n-x} + PP_x$

In the second group there are specific tripolyphosphatases as they have been described for many fungi, yeasts and bacteria (Kulaev & Vagabov 1983). In eucaryotic cells these tripolyphosphatases were found mainly in the mitochondrial fraction and therefore might be of minor importance in the hydrolysis of extracellular TPP, whereas the localization of tripolyphosphatase in bacteria is still obscure. In general, in both eukaryotes and prokaryotes polyphosphatases are known to be bound to the outside of the cytoplasmic membrane (Kulaev & Vagabov 1983). Their synthesis was reported to be repressible by extracellular phosphate and derepression of these enzymes is usually observed during phosphate starvation (Harold 1966; Torriani-Gorini 1987).

Phosphonates

In earlier reports metal-complexing phosphonic acids were generally considered to be rather stable compounds towards microbial attack. Nevertheless, a range of microorganisms is known to be able to split the C-P bond and remove the phosphorus group from structurally simple alkyl-phosphonates by means of a specific phosphonatase (La Nauze et al. 1978).

Only recently, isolation of microorganisms able to degrade HEDP, EDTMP and ATMP has been described in the literature. Pipke et al. (1987) reported the isolation of an *Arthrobacter* sp. (strain GLP-1) that was able to grow with the phosphonate

herbicide glyphosate as the only source of phosphorus. Subsequent investigations showed that this strain was able to utilize a wide range of phosphonic acids when they were supplied as the only source of phosphorus, including the complexing agents HEDP, EDTMP and DTPA during growth with which doubling times in the range of 12–15 hours were measured (Pipke & Amrhein 1988). This was confirmed by Schowanek & Verstraete (1990a) who tested several bacteria with respect to their potential to utilize a range of phosphonates. Whereas most strains were able to obtain phosphorus for growth from structurally simple mono-phosphonates only *Arthrobacter* sp. GLP-1 was able to use the four complexing phosphonates HEDP, EDTMP, ATMP and DTPMP as the sole source of phosphorus. The same authors demonstrated enrichment and isolation of three pure cultures of EDTMP-, DTPMP- and HEDP-degrading bacteria from seven different ecosystems that had been previously exposed to these phosphonates suggesting that microbes capable of degrading these chelating agents are widely distributed in polluted natural waters. One of the isolates was a Gram-negative, non-motile rod (tentatively identified as *Pseudomonas paucimobilis*) which grew in batch culture with HEDP as the sole source of phosphorus with a doubling time of 9.4 hours (Schowanek & Verstraete 1990b). Experiments with *Arthrobacter* sp. GLP-1 indicated that this strain was not able to assimilate nitrogen from these complexing phosphonates under nitrogen/phosphorus-limited growth conditions. This suggests that only the phosphonate group(s) in these compounds was removed and utilized as a growth substrate (Schowanek & Verstraete 1990a). Such action would be consistent with the observations made by Daughton et al. (1979a) who reported that during growth of *Pseudomonas testosteroni* with several alkylphosphonates the alkyl groups were released into the growth medium in the reduced form. One might speculate therefore that the microbial attack of ATMP and HEDP via a similar mechanism would yield methylamine and ethanol, respectively.

With respect to the regulation of phosphonate metabolism, it has been demonstrated for a variety of alkylphosphonate-degrading Gram-negative and Gram-positive bacteria that both transport of phosphonates and phosphonatase are inhibited/repressed in the presence of ortho-phosphate (Rosenberg & La Nauze 1967; Daughton et al. 1979b; Lerbs et al. 1990). Evidence that this pattern of regulation might not hold for all organisms was recently presented by Schowanek & Verstraete (1990b) who found that methylphosphonate was used simultaneously with ortho-phosphate during batch growth of their isolate MMM101a.

Polymeric polycarboxylates

Although these compounds are no chelating agents in the strict sense they are frequently included in today's detergents because of their ability to prevent growth and deposition of crystals on textile fibres (Opgenorth 1987). To date, isolation of a microbial culture able to degrade either PA or PHC has not been reported. In closed-bottle tests degradation of such compounds has proved to be slow and highly dependent on the degree of polymerisation (Haschke & Marlock 1974; Metzner & Nägerl 1982). Studies performed in treatment plants indicated that these polymers are not mineralized during wastewater treatment but are eliminated by either sorption to activated sludge or by precipitation as calcium salts (Opgenorth 1987).

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