Aminopolyphosphonates - chemical features and practical uses, environmental durability and biodegradation

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Abstract

Growing concerns of the quality of the environment lead to introduction of complex system of safety assessment of synthetically manufactured and common applied chemicals. Sometimes however, our knowledge of consequences that result from the usage of these substances, appears far later, than in the beginning of their application. Such situation is observed in relation to aminopolyphosphonates as the subgroup of organophosphonate compounds. These substances, which are anyhow not easy determined in environmental samples and suspected to influence the ecological equilibrium in aquatic ecosystems, seem to induce interesting biotechnological prospects.

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Introduction

The organophosphonates are a group of both synthetic and biogenic organophosphorus compounds, characterized by the presence of a covalent single bond of carbon to phosphorus (C-P). Because of its high energy of dissociation this bond is very stable comparing with the more labile O–P, N–P, and H–P linkages [1]. The presence of C-P bond significantly affects durability of phosphonates considering resistance to chemical hydrolysis and thermal decomposition [2], as well as their susceptibility to biodegradation by microorganisms [3]. Interestingly, phosphonates were identified as naturally occurring compounds in all living organisms, especially prokaryotic ones [4-6]. This finding strongly suggests that enzymatic systems for the cleavage of carbon to phosphorus bond may exist, that can allow to degrade also phosphonates of an anthropogenic origin.

Among the synthetically manufactured organophosphonates, *N*-phosphonomethylglycine, the active ingredient of the popular Monstanto's herbicide *Roundup*®, seems to be the most important [7]. However, this molecule contains only one phosphonic acid group attached to a carbon atom. Another group of aminophosphonates of economic interest consists of the so-called polyaminopolymethylenephosphonates in which typically all available N-H functions are saturated with methylenephosphonate groups. They are categorized as complexing agents and have received high and still increasing attention over the last 20 years due to their enormous importance for industrial and domestic processes. For example, they have become components of washing powders and other detergents, antiscaling and anticorrosion agents, fire retardants, and dispersants in ceramic and cement industries. As a consequence of their wide and increasing industrial applications, several

thousand tons of organophosphonates are introduced every year into the environment worldwide. In 1998 the consumption of phosphonates on a global scale was estimated to 58,000 tons [8]. More recent data for Germany indicate the usage of more than 4.000 tons of aminomethylenephosphonates and more than 5.000 tons of hydroxyethanediphosphonate as components of detergents and cleaning agents only in 2001. The most widely used representatives of (poly)aminopolyphosphonates are shown in Table 1.
The number of registered commercial cleaning products containing such phosphonates exceeds 2700, a fact that underlines the importance of this substance category [8].

In this paper the overview of the most popular used aminopolyphosphonates, the existing data on their fate in environment, as well as the available information on their biodegradation will be summarised.

Aminopolyphosphonates – substances of special and growing importance

(Poly)amino(poly)methylenephosphonates have become available on a larger scale based on the pioneering synthetic work of Moedritzer and Irani [9]. They developed a simple and efficient route in water as solvent to convert primary and secondary alkyl amines into the corresponding aminomethylenephosphonates. In fact, this method is applicable to a great variety of substrates but over the last decades only a couple of different structures have attracted the interest of academic and industrial researchers because of their outstanding properties and efficiency in several technical applications. These most relevant structures are shown in Figure 1. Furthermore, the synthesis gives rise to a sustainable approach by using phosphorous acid that originates from other chemical processes like manufacture of fatty acid chlorides for special surfactant types.
These aminomethylenephosphonates are very strong complexing agents towards alkaline earth, transition, and heavy metal ions. The complex formation constants of the water soluble (1:1)-complexes are similar to those of the well-known aminopolycarboxylates (e.g. EDTA) [10, 11]. However, the phosphonates discussed here may also form multinuclear complexes of varying stoichiometry which make them more efficient. A second and even more important property of the aminomethylenephosphonates is their ability to prevent the precipitation of hardly soluble salts of alkaline earth metals (e.g. $CaCO_3$, $BaSO_4$) under substoichiometric conditions by interfering already in the early stages of the crystallization processes of these salts. It should be emphasized that under certain conditions only one molecule of a phosphonate is required to keep more than 10 000 Calcium ions in solution. As final result of this interaction, phosphonates also modify the shape of the alkaline earth salt crystals and

change their morphology to amorphous solids that can be easily removed from such systems. Due to their high charge density they are recognized as polyelectrolyte compounds offering additionally superior dispersing functionalities for inorganic materials.

Based on the functional properties described above, these aminomethylenephosphonates are state-of-the-art chemicals for the resource-efficient and thus sustainable formulation of chemicals for a great variety of applications including detergents, water treatment chemicals, auxiliaries for pulp&paper and textile treatment, metal treatment, flotation of ores, oilfield chemicals and others. Water treatment applications in general vover the majority of uses (Figure 2). The following two examples shall illustrate more deeply the technical advantages provided by using these phosphonates:

a) Aminomethylenphosphonates are used in modern detergents and cleaning agents to inhibit the precipitation of water hardness, to disperse and prevent the redeposition of insoluble inorganic matters and to avoid the metal-catalysed catalytic decomposition of hydrogen peroxide in bleaching active detergents. Typical concentrations of aminomethylenphosphonates are between 0.1 to 5 % in final formulations which reduces dramatically the phosphorous release to the environment compared to earlier used formulations containing typically a tenfold or higher concentration of polyphosphates.

b) Modern water treatment technologies use such phosphonates in concentrations typically lower than 20 ppm to keep cooling water circuits or reverse osmosis devices free of scale. Earlier applied technologies used again polyphosphates in concentrations 50 (!) times higher.

Generally, it can be stated that the introduction of aminomethylenephosphonates led to a significant reduction of phosphorous release, therefore reducing the eutrophication potential . Until now there are still growing demands for aminopolymethylenephosphonates in industrial applications and new technologies and application areas are being developed (flame retardancy, electricity storage) [12-14].

Of course, in this context the environmental behaviour of these substances becomes of great importance. Earlier studies have shown that aminomethylenephosphonates can be effectively removed in waste water treatment plants mainly by adsorption and precipitation processes rather than by biological degradation [15]. Elimination degrees are above 90% whereas adsorption onto mineral oxides and humic acids both contribute to this efficient removal [16]. This fact, however, does not change the situation that these compounds, even adsorbed or precipitated, still exist in the form of their original chemical structure.

Environmental fate and assessment of the presence of aminopolyphosphonates

Recalcitrant sequestering substances are of environmental concern because of their persistence and strong metal chelating ability, being potentially capable of remobilizing heavy metals from sediments and treated sludges [17]. However, as a general rule, complexing agents show low acute toxicity toward both vertebrates and invertebrates [18]. This is true also for most aminopolyphosphonates, for which LD_{50} values ranging from 200 to 500 mg L^{-1} have been reported [19]. Toxicity was found to vary as a function of pH, water hardness and the complexed metal ion, being free acids generally more toxic than sodium or calcium salts [18]. Hence, comparison of data from different studies is made difficult by the lack of strictly standardized assay conditions. As expected for highly water-soluble compounds, low bioconcentration factors in fish suggested a negligible bioaccumulation of polyphosphonates [20]. Accordingly, toxicity data from long-term and short-term studies did not differ significantly. Despite the low toxicity to mammals, fishes and water invertebrates, these compounds were found to inhibit algal growth at relatively low doses (LD_{50} values < 50 mg L^{-1}) [20]. Such effect is believed to rely upon the ability to complex materials (*e.g.* essential mineral nutrients), and not upon toxicity *per se*. This notwithstanding, their release in the environment seems therefore susceptible to induce adverse consequences on water ecosystems, either reducing algal biomass or altering population dynamics and equilibria among competing species. On the whole, and despite the increasing use in industry and housecleaning products, possible environmental effects of polyphosphonates have been poorly investigated to date.

The lack of information on the fate of phosphonates in the environment is at least in part due to analytical problems, namely to the lack of methods for their determination at trace-level concentrations in the environment. Although determination of some phosphonates in wastewater treatment plants has been carried out by Nowack in the late 90-ties, with application ion pair HPLC after Fe(III) complexation [15], a comprehensive survey of (poly)aminopolyphosphonates concentrations in natural waters has not been done so far. The analytical procedures, which are proposed for the determination of these substances are usually time-consuming, complex, costly and non-selective. Moreover, these methods possess high detection limits of about 1 µM or more, that hampers their usage for the evaluation of the presumably lower concentrations of polyaminophosphonates in environmental samples [8]. Additionally, described xenobiotics do not absorb the light in UV and Vis range, thus their analysis is rather difficult and require derivatization, for instance post column reaction with Fe(III) followed by detection with UV-Vis spectrometry [21]. Other non selective methods

using colorimetric detection require post-column oxidation of phosphonates to phosphates [22, 23]. Another attempt, however also moderately successful in solving this analytical problem, was the usage of ribonucleotide electrolytes for measurement of polyaminophosphonates by capillary electrophoresis (CE) and indirect photometric detection (IPD) [24]. Even ion-pair HPLC methods, that have been effectively used for the separation of oxidative breakdown products of derivatives of aminopolyphosphonates and some phosphonates in reactions with 2,4-dinitrophenylhydrazine and 9-fluorenyl methylchloroformate [24], which allowed the identification of these chemicals with a detection limit of 0.05 mM [25], were not reliable enough to be introduced as appropriate standards.

Consequently, the lack of sensitive and commonly accepted analytical proceduresmakes that the applicability of such compounds, even if acted as xenobiotics, is not regulated in most EU countries.

Biodegradation of aminopolyphosphonates by microorganisms

The discovery of naturally-occurring organophosphonates [4] prompted the interest in searching the pathways for the biosynthesis, the biotransformation and the biodegradation of such compounds, especially in case of prokaryotic organisms [26]. However, the above-mentioned difficulties in the selective isolation and determination of phosphonates in biological matrices limited these efforts to the most popular herbicide worldwide, glyphosate [27].

Regarding (poly)aminopolyphosphonates, very low degradability rates have been reported in standard tests, evaluated as both ready biodegradability (usually ranging from 0 to 29%) and inherent biodegradability (from "no significant biodegradation“ to ~ 40%) [20, 28]. They can therefore be considered as recalcitrant compounds. Numerous studies have indeed shown that little, if any, primary or ultimate biodegradation occurs for any phosphonate in standard biodegradation tests, such as the OECD screening test, BOD_{20} test, sapromat test and closed bottle test [29]. This most likely depends on the mechanism for bacterial metabolization of a wide array of C–P compounds, which relies upon the activity of a membrane-bound C–P lyase able to act upon unsubstituted alkyl and aryl phosphonates. Its activity was found to depend upon the phosphate status of the cell, with both substrate uptake and C–P bond cleavage being under the direct control of the pho regulon, which is expressed only during phosphate starvation [30]. If inorganic phosphate is concomitantly present, it is used preferentially, and organophosphonates are not metabolized at all.

In some instances, polyphosphonates have been found to serve as a carbon source, but only if present at high concentrations. DOC removals of 23-33% of 1-hydroxyethane (diphosphonic acid) and nitrilo-tris(methylenephosphonic acid) were observed in a Zahn-Wellens test [20]. On the other hand, almost no information is available to date concerning the anaerobic biodegradability of polyphosphonates. For the two aforementioned compounds, however, less than 4% of the labeled carbon was found to be converted to $^{14}CO_2$ / $^{14}CH_4$ in a model digestor [20].

The application of Nuclear Magnetic Resonance (NMR) spectroscopy, especially the ^{31}P NMR (phosphorus NMR) technique, recently allowed a significant progress in the research on biodegradation of aminophosphonates. With respect to the usage of ^{14}C-labeled substances, or the usage of ^{13}C NMR spectroscopy that needs the samples to be enriched in ^{13}C isotope, the ^{31}P NMR technique shows several advantages. Firstly the nucleus ^{31}P possesses an isotopic abundance of 100% and a relatively high magnetogyric ratio. The value of nuclear spin is ½, making spectra relatively easy to interpret, what although limited to phosphorus compounds, broadens applicability of ^{31}P NMR technique for metabolomic studies of samples of natural origin as well as to monitor the energy status of cells [31, 32]. Moreover, the direct phosphorus NMR measurements have been successfully used for identification of natural organophosphonate compounds present in the cells of living organisms – e.g. eukaryotic protozoan [33]. Worth noting is that ^{31}P NMR spectroscopy enables both semiquantitative determination of phosphorus compounds and structural changes of organophosphonates molecules, what is essential in examination of metabolites, which are formed during process of their biodegradation [34-36].

In our earlier papers we described the usefulness and simplicity of this method to track the biotransformation of organophosphonates in separated culture media (*ex vivo* ^{31}P NMR) and directly in cultures containing whole living cells growing in NMR tubes (*in vivo* ^{31}P NMR). *In vivo* ^{31}P NMR spectroscopy has been effectively used for analysis of metabolic activity of whole living cells of taxonomically various organisms growing in NMR tubes in media containing an aminophosphonate - glyphosate [35]. The results confirmed the ability of halophylic cyanobacterium *Spirulina platensis* and bacterial strain *Streptomyces lusitanus* to biodegrade the herbicide, and the same skill was proved for the first time for a fungus, *Fusarium dimerum*. Based on *in vivo* ^{31}P NMR measurements we found that this microorganism is able to use glyphosate as a sole source of phosphorus , as shown by disappearing of the signal (peak) of this compound with time (Figure 3).

Because of their well-developed enzymatic systems and the ability to adaptat to new, often harsh conditions of growth, fungi are good candidates for bioremediation purposes. The abilities of these microorganisms (especially filamentous fungi) to transform and decompose onerous xenobiotics, including phosphonates, are widely reported in literature [34, 35, 37-39]. However, there is only limited information about biodegradation of aminopolyphosphonates by several fungal species, among others: *A. alternata, F. dimerum, A. terreus, P. variotti* [36]. These microorganisms were found to be insensitive toward two aminopolyphosphonates: hexamethylenediaminetetra (methylenphosphonic) acid and aminotris(methylenphosphonic) acid, added to the medium in the range of concentrations from 1 to 10 mM. What is more important, some of these molds were able to degrade tested aminopolyphosphonates and to utilize the phosphorus, which was build-in in their structures, as a nutrient [36]. Biodegradation of these xenobiotics occurs via formation of indirect phosphonic metabolites, whose structures have not been determined, yet.

Both cyanobacteria and fungi are known to adapt easily to contaminated environments [40], additionally a few species have been found to exhibit tolerance to glyphosate and be able to growth in media containing phosphonates as the only sources of phosphorus [41, 42]. Furthermore, cyanobacteria being phylogenetically the oldest autotrophs and inhabiting all natural water reservoirs, seem to posses a special position in relation to organophosphonates. This hypothesis may be correlated with the natural production of a phosphonic compound, by *Aphanizomenon flos-aquae* [43] and *Trichodesmium erythraeum* [44], what suggests that this kind of metabolic features – the pathway of phosphonate transformation - might be an evolutional achievement, which allows these microorganisms for diversification of the sources of nutritive phosphorus.

An interesting support of this hypothesis is the finding that the cyanobacterium *Spirulina platensis* is able to mineralize the polyaminopolyphosphonate hexamethylenediamine-N,N,N,N-tetrakis(methylphosphonic) acid as a nutrient source [45]. Although the utilization of the tested aminopolyphosphonate was incomplete, the use of this species for bioremediation purposes is promising, due to the lack of toxins, rapid growth and content of valuable nutrients inside the cells. Nowadays the members of genus *Spirulina* and *Arthrospira* evoke increasing interest, considering various biotechnological purposes.

Biotechnological approaches for polyphosphonate removal from wastewaters

Because of the uncertainty of their environmental fate, an attractive possibility would be represented by the build up of specific plants for polyphosphonate removal from sewage waters. A biotechnological approach for the development of cheap and efficient remediation systems requires the isolation in pure culture of microbial strains able to degrade the recalcitrant compounds. Although several bacterial strains have been reported to catalyze the breakdown of the C-P moiety in various simple phosphonates [46], the microbial ability to degrade polyphosphonates has been poorly investigated till now. Some early studies indeed provided evidence that a complete breakdown with respect to the C–P bond cleavage could be achieved by some bacteria that seem ubiquitously present in both contaminated and uncontaminated environments (*e.g.* [47]). However, also due to the unavailability of efficient methods for their quantization, microbial metabolism was assessed only indirectly, as the ability to utilize a given compound as the sole phosphorus source for growth. Thus the question remains on whether degradation does occur also in wastewaters, where inorganic phosphate or other, more physiological P-sources are usually present. Recently, two cyanobacterial strains were found to metabolize the [hexamethylenediamine-tetra(methylenphosphonic acid) potassium salt], a $CaSO_4$ inhibitor used for boiler treatment and reverse osmosis desalination at alkaline pH values [45]. The compound served as sole source of phosphorus for cyanobacterial growth, but -interestingly- its metabolization was not affected by the presence of Pi. A lab-scale pilot plant, consisting of a series of sequentially connected vessels containing an actively proliferating algal culture, was built and tested for wastewater treatment (Figure 4). Results showed 50% removal of the polyphosphonate added to an initial concentration of 1.2 mg L^{-1} [45]. However, at low substrate levels metabolism rates were lower, suggesting that, similarly to other phosphonates [42], a concentration-driven uptake may represent a limiting step for its biodegradation.

The use of cyanobacteria for bioremediation purposes is particularly promising, since the produced biomass can be used thereafter to feed livestock (at least in the case of non toxin-producing strains, like *Spirulina*) or as a source of compounds of high-added value. Because polyphosphonate utilization was incomplete, and limited to actively proliferating cultures, the process requires further optimization. Work is currently under way in our laboratories within this perspective. Preliminary data suggest the possibility to increase significantly the rate of polyphosphonate metabolization by treating cultures with sublethal doses of detergents with a high hydrophile-lipophile balance (Forlani, G. *et al*., unpublished

results). Moreover, a screening of other cyanobacterial species, as well the assessment of their biodegradative potential against a wider range of polyphosphonates, are in progress.

Conclusion

Although phosphorous is an essential nutrient for life, the thermodynamical stability of phosphonates, particularly the presence of direct carbon to phosphorus (C-P) bond, makes these substances durable in physiological conditions. Based on the current knowledge about their degradation and adsorption behavior in sewage treatment plants the possibility that polyphosphonates from the effluent may enter surface waters in significant amounts cannot be excluded. The utilization of organophosphonates by microorganisms therefore represents a highly interesting and sustainable approach to close the gap between industrial synthesis of (poly)aminopolyphosphonates, their applications and environmental concerns. The possibility that aminopolyphosphonates may act as eutrophication agents is indeed another, yet underestimated risk. Should some cyanobacterial strains or eukaryotic microalgae be able to use these compounds as a phosphorus source, this would lead to algal blooming, with subsequent massive fish death and severe reduction of water quality. Fortunately, however, the ability to utilize organophosphonates as nutritive compounds possessed by some cyanobacteria and filamentous fungi, may act as significant factor promoting the usage of these microorganisms in waste water treatment and for other biotechnological purposes.

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Captions to figures

Figure 1. Structures of relevant polyaminophosphonates and the general way of their synthesis

Figure 2. Estimated percentage of phosphonate application worldwide

Figure 3. ^{31}P NMR spectra of dense culture cell of *Fusarium dimerum* in Czapek medium with glyphosate as the sole source of phosphorus; just before the addition of the phosphonate (A), 1 h (B), 1 (C), 3 (D), 5 (E), 7 (F), 14 (G) and 21 days (H) thereafter. On presented spectra, the chemical shift of the peak corresponding to the aminophosphonate is about 8-10 ppm, whereas inorganic phosphorus peak lays in the range 3-5 ppm [35]

Figure 4. A lab-scale plant used to evaluate the suitability of cyanobacterial strains for polyphosphonate removal from wastewaters. A series of vessels containing an actively proliferating culture of *Spirulina platensis* were loaded with a 2.5 mM solution of the potassium salt of [hexamethylenediamine-tetra(methylenphosphonic acid); overflow devices and a constant inflow of 100 ml day^{-1} determined a residence time of 7 days. Before being saturated after two weeks of functioning, the plant allowed the removal of about 50% of the compound [45]

Captions to table:

Table1 The most widely used aminopolyphosphonates

Figure 1

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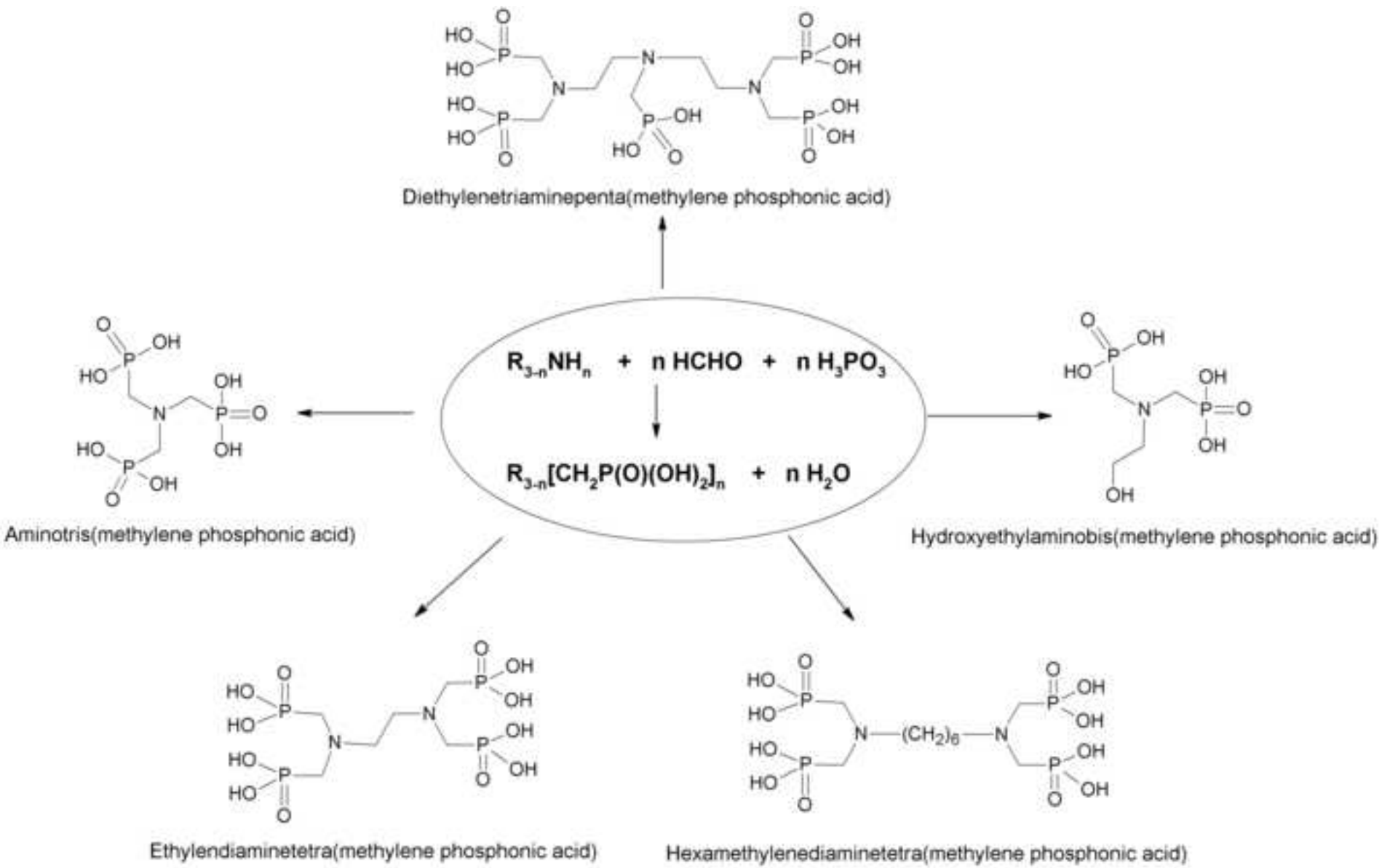

Figure 2

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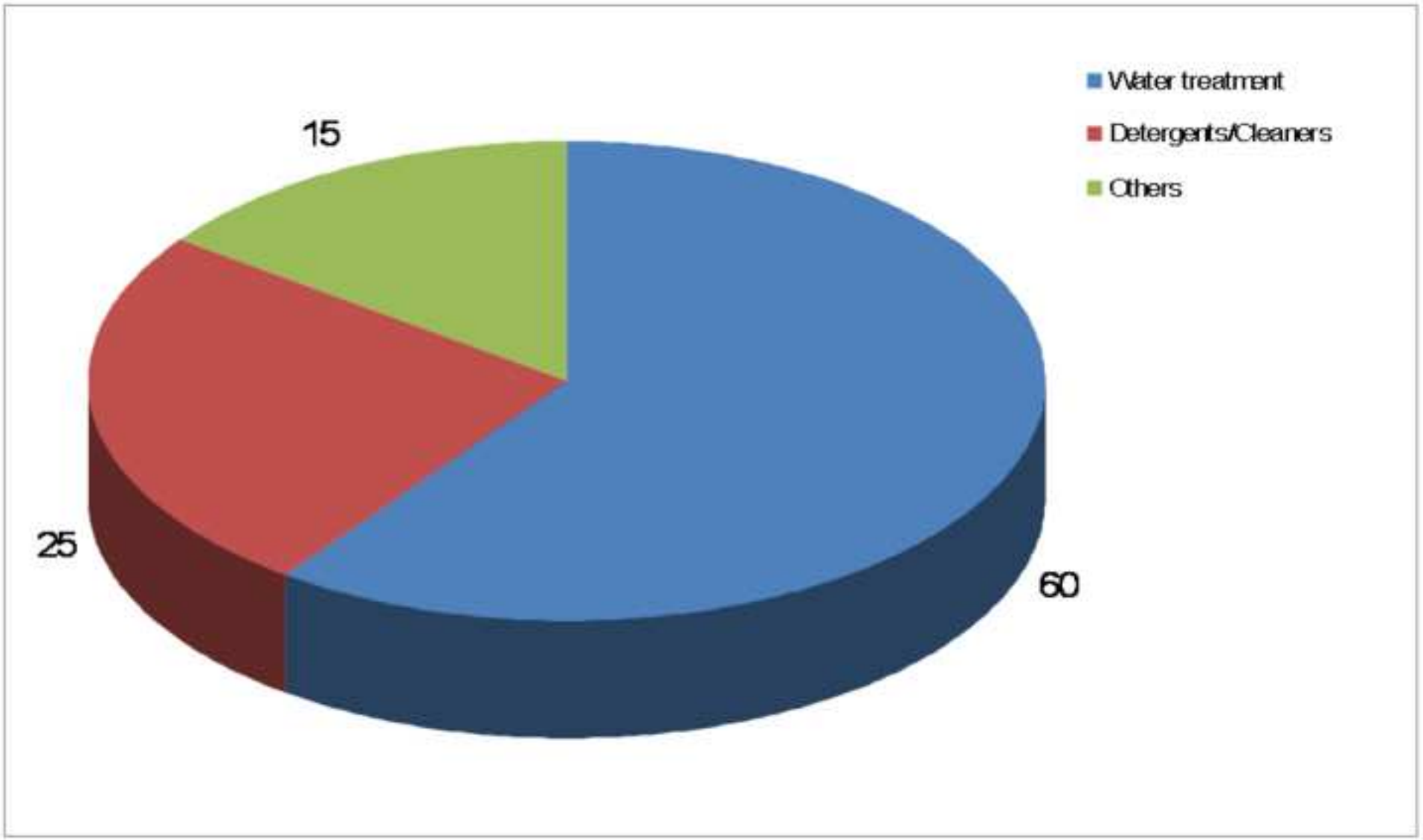

Figure 3

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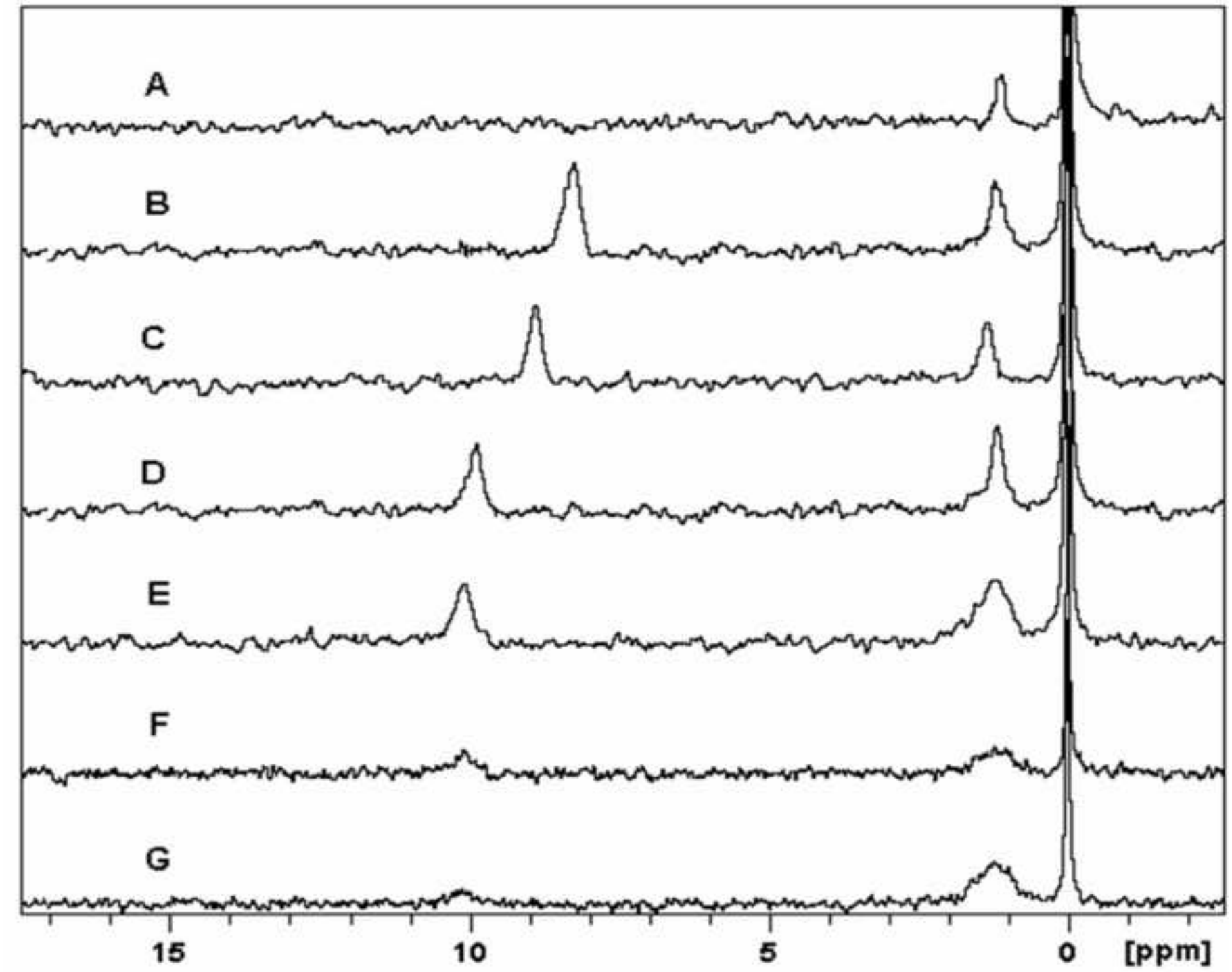

Figure 4

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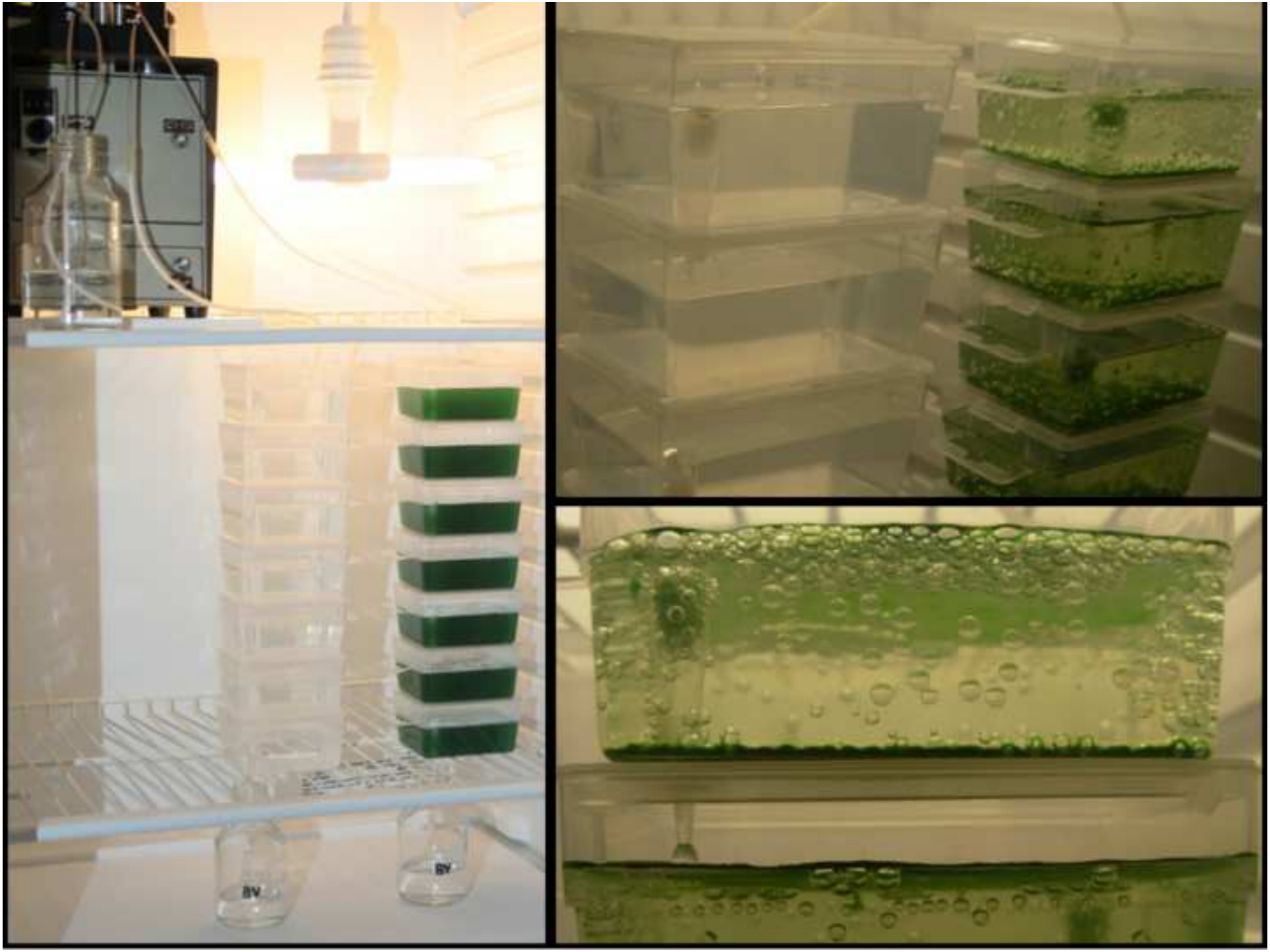

Table 1

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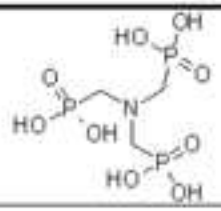
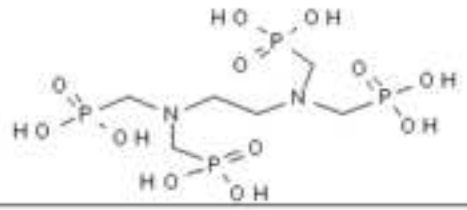
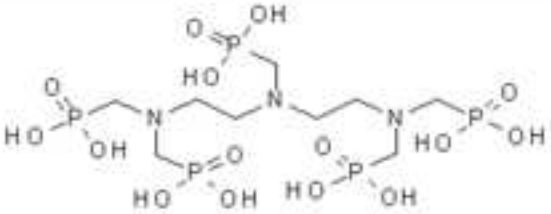
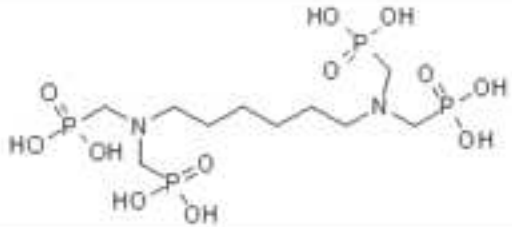
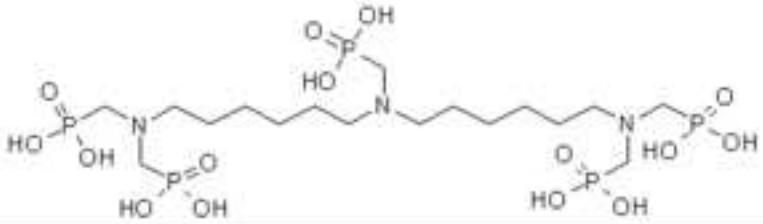
Structure	Name	Application*
	Amino tris(methylene - phosphonic acid) ATMP	Scale inhibitor, deflocculant, chelating agent, additives for detergent
	Ethylenediamine tetra(methylenephosphonic acid) EDTMP	Scale inhibitor, deflocculant
	Diethylenetriamine penta(methylene phosphonic acid) DTMP	Scale inhibitor, deflocculant, additives for detergents, stabilizer of hydrogen peroxide
	Hexamethylenediamine tetra(methylene phosphonic acid) HDTMP	Scale inhibitor (particularly for gypsum)
	Bis(hexamethylene triamine penta(methylenephosphonic acid) BHMTMP	Scale inhibitor (particularly for high temperature and high pressure)

* data from Beilstein Data Base and other sources