Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.22280

134 (2018) 1–6
December

The aerobic biodegradation of EDDHA and EDDHSA in water under the static test conditions

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Received 19 December 2017; Accepted 27 March 2018

ABSTRACT

The aim of the study was to determine the degree of aerobic biodegradation of ethylenediamine-*N,N′*-di([*ortho*-hydroxyphenyl]acetic acid) (EDDHA) and ethylenediamine-*N,N′*-di([2-hydroxy-5-sulfophenyl)acetic acid) (EDDHSA) chelators under static test conditions. For comparative purposes, tests were also carried out for iminodisuccinic acid (IDHA), *N,N′*-di(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (HBED), ethylenediaminetetraacetic acid (EDTA) ligands, which are also used for production of micronutrient fertilizers. The tests were carried out in accordance with PN-88-C-0561 "Study on the aerobic biodegradation of organic compounds in water under the static conditions." This method is used to predict the susceptibility to biochemical degradation of organic compounds entering surface water or into biological sewage treatment plants. For 20 d, the chelator concentration was measured daily in an organic medium inoculated with standard activated sludge under aerobic conditions without light and at room temperature. The obtained results allowed to determine the degree of distribution of ligands used in the fertilizer industry. EDDHA and EDDHSA chelators belong to the compounds difficult to biodegrade under the conditions used. Only the IDHA ligand has been completely degraded and can be classified as easily degradable. Chelates EDTA and HBED also have a slight decomposition and should be classified as difficult to decompose.

Keywords: Biodegradation; EDDHA; EDDHSA; Micronutrient; Chelators

1. Introduction

The increase in population has led to an increase in the area of cultivated fields. It was necessary to fertilize the macro- and microelements. Micronutrients are often found in undissolved forms, not used by plants, especially on calcareous and alkaline soils [1].

Microelements have biochemical functions and are an important factor influencing the growth and yielding of plants. Fertilizers are produced on the basis of copper, zinc, iron, manganese, boron, and molybdenum ions. These six elements are directly involved in metabolic processes. Among the microelements, fertilizers can be distinguished as follows: technical salts, macronutrients enriched with micronutrients, enamel glaze, chelates, and industrial waste and minerals. To be effective, they must have a variety of chemical compositions and physicochemical properties [1,2].

At present, chelating compounds are often used to produce micronutrient fertilizers. The Regulation of the European Parliament and of the Council EC No 2003/2003 of 13 October 2003 includes 11 synthetic chelating agents belonging to the group of aminopolycarboxylic compounds: ethylenediaminetetraacetic acid (EDTA), 2-hydroxyethylethylenediaminetriacetic acid, ethylenetriaminepentaacetic acid (DTPA), ethylenediamine-*N,N′*-di([*ortho*-hydroxyphenyl] acetic acid) ([*o,o*] EDDHA), ethylenediamine-*N*-([*ortho*-hydroxyphenyl]acetic acid)-*N′*-([*para*-hydroxyphenyl]acetic acid)

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Presented at the 13th Conference on Microcontaminants in Human Environment, 4–6 December 2017, Czestochowa, Poland.

([*o,p*] EDDHA), ethylenediamine-*N,N′*-di([*ortho*-hydroxy -methylphenyl]acetic acid) ([*o,o*] EDDHMA), ethylenediamine-*N*-([*ortho*-hydroxy-methylphenyl]acetic acid)-*N′*-([*para* -hydroxy-methylphenyl]acetic acid) ([*o,p*] EDDHMA), ethylenediamine-*N,N′*-di([*5*-carboxy-2-hydroxyphenyl]acetic acid) (EDDCHA), ethylenediamine-*N,N′*-di([2-hydroxy-*5*-sulfophenyl]acetic acid) (EDDHSA), iminodisuccinic acid (IDHA),*N,N′*-di(2-hydroxybenzyl)ethylenediamine-*N,N′*-dia cetic acid (HBED). Complexes with these chelators are thermally and chemically durable. The structure of the chelates facilitates the movement of the microelement in plant cells in which gradual dissociation results in the release of the microelement. Metal ions in the form of chelate, which are not subject to sorption and binding in the soil, are less leached from the soil and do not react with phosphates, carbonates, and other substances contained in fertilizers or in soil solution. Chelates provide an effective way to deliver micronutrients, even on basic soils, as they stabilize microelement ions in a wide pH range. Often they are a component of multicomponent fertilizers or a component of one-component fertilizers whose properties are adapted to the needs of particular agricultural crops. Microchelates can be applied to foliar or soil. Both the pH and other chemicals in soil should have minimalized influence on chelated micronutrients. The degree of complexation of the micronutrient, as required by the European Parliament, should be at least 80% of the declared total water-soluble metal ions content [1,2].

Environmental impact is important. The degradation time of the complexing agent should be similar to the time of uptake of microelement by plant. High concentration of chelator in soil or sewage can cause displacement of microelements by other cations in the soil. The toxic effect on the environment results in the ability of ligands to increase bioavailability and phytotoxicity of heavy metals and radioactive elements. This involves remobilization of sediments, bottom sediments, and river basins. Consequently, this leads to increased absorption of toxic elements, not only by plants, but also by all living organisms. The high concentration of chelators also contributes to water eutrophication due to the presence of bioavailable nitrogen in their structure. EDTA, still the most commonly used chelator in the fertilizer and other industries, is the compound of the highest concentration of anthropogenic origin in European inland waters. Some consumer products have replaced this compound with other biodegradable derivatives [3–7].

Despite the widespread use of chelate fertilizers, the mechanism of decomposition of most of them is still unknown. The results of the study allow to evaluate the susceptibility of EDDHA and EDDHSA and three other ligands used in the fertilizer industry [8].

To supply iron ions and to combat the effects of chlorosis due to its lack, EDDHA and its derivatives such as EDDHSA, EDDHMA, or EDDCHA are most commonly used. Microfertilizers based on this chelator contain 6% chelated iron ions. Commercial products based on EDDHA are used for chlorosis-sensitive plants grown on high-quality soils due to their longer and durable effect compared with other ligands with lower stability constants. Fe-EDDHA is used for hydroponic cultivation and for soil solution. It is used mainly on high-pH land, in the Mediterranean, and in the Middle East, where 20%–50% of fruit trees show symptoms of iron deficiency. EDDHA can occur as three regioisomers: (*o,o*) EDDHA, (*o,p*) EDDHA, and (*p,p*) EDDHA. Isomer (*ortho, ortho*) is characterized by low reactivity in calcareous soils and high efficiency of supplementing iron deficiency. This compound offers up to six bonds to the microelement ion. The isomer (*ortho, para*) can coordinate the ion with five bonds and is characterized by a lower stability than the (*ortho, ortho*) isomer. The formation of (*p,p*) EDDHA complex with microelement ions is sterically prevented [8–14].

EDDHSA was first synthesized in 1958. It stabilizes cations in a wide pH range of 4–12. The presence of the sulfonic group affects the acidity of the compound, which contributes to its greater efficiency on calcareous soils. No isomers are produced in the EDDHSA synthesis due to the blocked *para* position. The solubility of this chelator is almost four times higher than that of EDDHA. Fe-EDDHSA chelates are commonly used to control chlorosis and to supplement iron deficiency. Fertilizers based on EDDHSA are used in Spain, France, and Italy. It is recommended for many different plant species, especially fruit trees such as citrus, apricot, avocado, plum, and peach. It is also used for growing grapes, small shrubs, and strawberries [14,15].

In spite of the fact that EDDHA and other structurally related phenolic ferric chelating agents are the most efficient iron chelates employed to relieve chlorosis in plants, their degradation pathways are still unknown. Fe-EDDHA and its analogues have very low reduction potential, which make them unreactive in photochemically and chemically induced electron transfer processes. Schenkeveld et al. [16,17] observed that in period of time, after soil application of Fe-EDDHA, the concentration of chelate decreases. He hypothesized that this could be related to the biodegradation process. Further studies on calcareous soil show that the activity of microorganisms does not affect the concentration of chelate. Similar results were obtained for sterilized soil. In conclusion, biodegradation does not affect the effectiveness of fertilizer application to soil [16,17].

To the best of our knowledge, there are no available studies where EDDHA and EDDHSA were biodegraded in an organic medium inoculated with standard activated sludge. Activated sludge organisms are involved in the oxygen distribution of chemical compounds present in wastewater. The presented experiments were conducted to show direct effect of decomposition of EDDHA and EDDHSA and other chelators entering both the surface waters and biological sewage treatment plants. The aim of this study was to determine the degree of aerobic degradation of two chelators used in the production of microelement fertilizers. These were EDDHA and EDDHSA. For comparative purposes, tests were carried out for EDTA, IDHA, and HBED. The decomposition process under static test conditions was determined by the reduction of chelator concentration.

2. Materials and methods

Following chelating agents were used in the tests: EDDHA, EDDHSA, HBED (Production-Consulting Firm ADOB, Poland), EDTA (35% aqueous solution of sodium salt, Boruta-Zachem SA, Poland), and IDHA (33% aqueous solution of sodium salt, Production-Consulting Firm ADOB, Poland). EDDHA and EDDHSA were synthesized according

to the Pertee method, which is used in the industrial production of these chelators [18–20]. Phenol (POCH, Gliwice) or 65% aqueous solution of *para*-hydroxybenzosulfonic acid (POCH, Gliwice), dichloroacetic acid (Merc Millipore, USA), sodium hydroxide (POCH, Gliwice), ethylenediamine (POCH, Gliwice), and ethyl acetate (POCH, Gliwice) were used for the synthesis. All chemical agents used for synthesis were analytically pure. Sample EDDHA contained *o,o*-EDDHA, *o,p*-EDDHA, *p,p*-EDDHA, and other by-products typically obtained in the industrial synthesis of *o,o*-EDDHA. In such a form, these ligands are used for the production of micronutrient fertilizers. All chelators have fertilizer purity.

Assessment of the biodegradation of fertilizer micronutrients complexing agents was carried out according to Standard PN-88/C-05561–Examination of the aerobic biodegradation of organic compounds in the aqueous environment under the conditions of the static test and Organization of Economic Co-operation and Development tests [21,22]. This method is used to predict the degree of decomposition of organic compounds entering the surface waters or biological sewage treatment plants. For 20 d, the chelator concentration was measured daily in an organic medium inoculated with standard activated sludge under aerobic conditions without light and at room temperature. The activated sludge was bred in laboratory conditions. The wastewater treatment plant, for the city of about 24,000 residents, was used for the inoculation, which amounted to 2,900 m^3 of sewage per day. They are mainly domestic wastewater; about 13% per volume are industrial wastewater. The activated sludge contained the minimum amount of iron that affected the biodegradation process. In the sludge used for testing the mixed liquor volatile suspended solids, the concentration of the activated sludge biomass was equal 5 g L^{-1} and dissolved oxygen concentration was equal 2.0 mg L^{-1} [21–24].

Mineral medium and standard activated sludge were placed in three flasks. Solution of the tested organic compound was inserted into first two flasks. The concentration of 7 mmol L^{-1} was used for all tested compounds. Moreover in the second flask, the glucose has been applied as a factor supporting the biochemical decomposition. Glucose is a factor supporting the process of biodegradation. It is an easily decomposed source of energy and carbon for microorganisms. It is added to compare the results obtained under standard conditions, and when an additional source of energy needs to be provided to microorganisms. The third flask served as a control test. Flasks prepared in that way were secured with corks and supplied with wires delivering compressed air. Then they were put on electromagnetic mixers, in the overshadowed place, at room temperature. Determined amount of the sample prepared in such way was collected from each of three flasks immediately after preparation and then after 1, 3, 6, and 24 h and then every day for a duration of 20 d. After draining them off, they were used for indicating the concentration of tested compound and the chemical oxygen demand (COD) value [25]. The concentration of tested compounds was determined using complexometric titration. The titrant was 0.001 mol L^{-1} magnesium chloride solution. The analysis was made in alkaline environment at pH = 10. The Eriochrome Black T (indicator) was added.

Degree of compound biodegradation in the time (t) has been calculated according to the formula:

$$A_t = \frac{c_0 - c_t}{c_0} \cdot 100 \quad (1)$$

where A_t is degree of chelator reduction (%), C_0 is concentration of compound in the time t = 0 (mol L^{-1}), and C_t is concentration of compound after the t time (mol L^{-1}).

The value of COD was determined by the dichromate method in accordance with the PN-ISO 6060: 2006 Standard [25]. The method consists in determining the potassium dichromate (VI) used for the oxidation of organic compounds and some of the inorganic components present in the test sample. Oxidation occurs in an acidic environment at the boiling temperature in presence of a catalyst (silver and mercury ions), which also eliminates the influence of chloride ions. Potassium dichromate is reduced to chromium (III). The excess of potassium dichromate is titrated with a standard solution of ammonium and iron (II) sulfate against ferroin. The COD value was calculated according to the following formula:

$$Z = \frac{(V_4 - V_3) \times n \times 8 \times 1000}{V} \quad (2)$$

where Z is chemical oxygen demand (mg O_2 L^{-1}), V_4 is volume of ammonium and iron (VI) sulfate (VI) used for titration of the control (mL), V_3 is volume of ammonium and iron (VI) sulfate (VI) used for titration of the tested sample (mL), n is normality of ammonium and iron (II) sulfate (mL), and V is volume of the sample used for the analysis (mL).

Degree of COD reduction was calculated according to following formula:

$$B_t = \frac{\mathrm{COD}_0 - \mathrm{COD}_t - \mathrm{COD}_c}{\mathrm{COD}_0} \times 100 \quad (3)$$

where B_t is degree of COD reduction (%), COD_0 is COD value at t = 0 time (mg O_2 L^{-1}), COD_t is COD value after the t time (mg O_2 L^{-1}), and COD_c is COD value in the control test at t = 0 time (mg O_2 L^{-1}).

3. Results and discussion

The aim of the study was to investigate the degree of aerobic biodegradation of EDDHA and EDDHSA, used in the production of micronutrient fertilizers, under static test conditions. Three other chelators, used in the fertilizer industry, were also tested for comparative purposes: EDTA, HBED, and IDHA.

Figs. 1 and 2 show the results of the degree of reduction of EDDHA and EDDHSA with and without glucose by 20 d.

Figs. 3 and 4 show the degree of COD reduction change in 20 d for EDDHA and EDDHSA.

EDDHA ligand concentration decreased by about 20% without additional energy source. Glucose supplementation resulted in 23% of decomposition. Degree of COD reduction

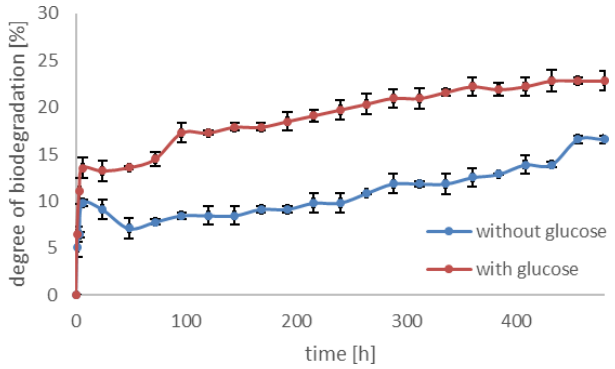

Fig. 1. Reduction of concentration of EDDHA under the static test conditions.

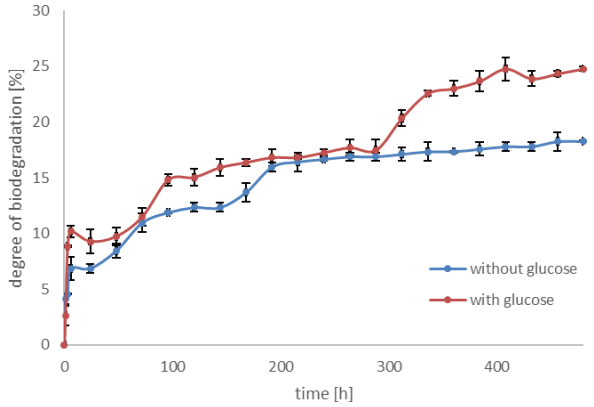

Fig. 2. Reduction of concentration of EDDHSA under the static test conditions.

was equal 12.6%, and the addition of sugar caused its drop by 15.0%.

EDDHSA concentration decreased by 20% after 20 d of experiment under static test conditions. When glucose was added, the ligand degradation was about 25%. The COD reduction was 15.1%, and the carbohydrate additive caused the reduction to be 15.6%. Glucose is a factor supporting the process of biodegradation. It is an easily decomposed source of energy and carbon for microorganisms. Its addition has increased the degree of biodegradation of EDDHA after 300 h of running the experiment. Most likely, the chelator was decomposed into other organic substances that did not have complexing properties (intermediate products), which resulted in a difference in concentration and COD reduction. The time of adaptation of microorganisms is the time when the greatest changes were observed in concentration and COD in the analyzed system. The adaptation time of the microorganisms was the same for both compounds and it was 4 d.

Table 1 shows the degree of degradation of all tested compounds, the degree of COD reduction, and time of adaptation of microorganisms for all tested chelating agents after the biodegradation process of the medium without additional energy and with glucose.

The results in Table 1 show that, under static test conditions, the IDHA ligand was the most rapidly degraded. The reduction rate of this compound was 100% already on

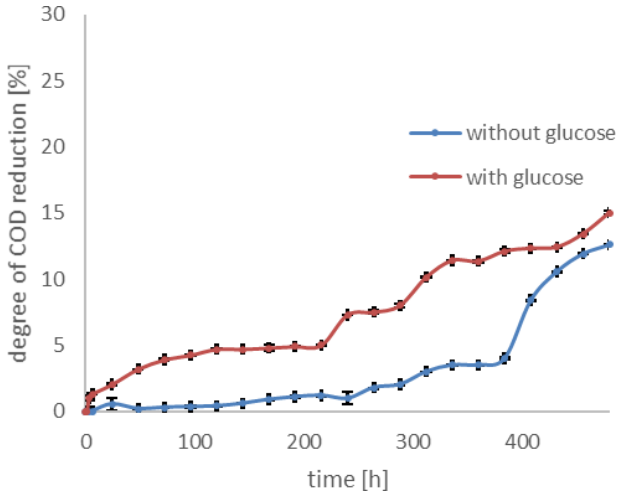

Fig. 3. Reduction of COD of EDDHA under the static test conditions.

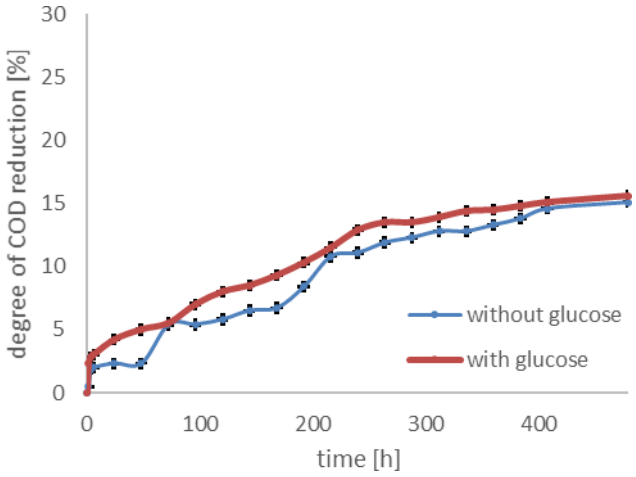

Fig. 4. Reduction of COD of EDDHSA under the static test conditions.

the 4th day of the experiment in a glucose flask and on the 5th day of the experiment in a flask without glucose. The adaptation time of the microorganisms was also short and took 2 d. The most commonly used chelator EDTA has been degraded about 30%. The adaptation time of the microorganisms was the longest for this compound and took 1 week. The breakdown rate for HBED was about 20%, and the addition of glucose led to the breakdown of nearly 30%. The adaptation time was 2 d.

The EDDHA, EDDHSA, EDTA, and HBED biodegradation occurs slower than IDHA. According to the standard, IDHA can be considered as easily degradable because its concentration has been biodegradable above 70% within 5 d of the experiment. EDDHA, EDDHSA, EDTA, and HBED should be considered to be difficult to decompose as their biodegradation rates do not exceed 50% over the duration of the experiment.

The same chelating agents were studied for biodegradability in activated sludge under kinetic test conditions for 42 d. This method consists of determination of

Table 1
Results of aerobic biodegradation of fertilizer chelating agents under the static test conditions

Chelating agent	With glucose		Without glucose		Time of adaptation of microorganisms (h)
	Degree of concentration reduction (%)	Degree of COD reduction (%)	Degree of concentration reduction (%)	Degree of COD reduction (%)	
EDDHA	23.5	15.0	19.7	12.6	96
EDDHSA	25.2	15.6	19.1	15.1	96
EDTA	32.2	30.5	26.9	26.0	168
IDHA	100	98.7	100	95.4	48
HBED	27.8	26.4	18.2	17.8	48

partial biodegradation of organic substances of specified concentrations in synthetic wastewater by activated sludge microorganisms. Also, only IDHA was completely degraded after 5 d of experiment. The degree of biodegradation of EDDHA was 55% and of EDDHSA was 34%. Chelators EDTA and HBED have been decomposed in a small extent. In conclusion, EDDHA, EDDHSA, EDTA, and HBED belong to compounds that are difficult to biodegraded under kinetic test conditions [26].

Photochemical and chemical degradation can be a good alternative method in the removal of chelating agents and chelates from water. The effect of exposition to sunlight and in the presence of oxygen leads to decomposition of, commonly used, Fe-EDTA and Fe-DTPA to products that are found to be readily biodegradable. Gomez-Gallego et al. [8] have conducted research on EDDHA and Fe-EDDHA photoreactivity in UV/Vis and visible light, in presence of O_2. At the environment pH (4–8), the chelate and chelator were resistant to photodegradation. Hernandez-Apaolaza and Lucena [30] continued the research begun by the Gomez-Gallego et al. [8]. The aim of their studies was to evaluate how the period of sunlight exposure and the concentration of irradiate Fe-EDDHA solution influence the photostability on the chelate at a constant pH. They had demonstrated that this chelate photodecomposition was highly correlated with the concentration of the solution exposed. Extending the exposure time, up to 30 d, and reducing the concentration to the values used in agriculture have caused Fe-EDDHA to undergo photodegradation. The photodecomposition products were salicylaldehyde, salicylic acid, and salicylaldehyde ethylenediamine diimine. None of the decomposition products affected the growth and development of soybeans [8,27–30].

4. Conclusion

In conclusion, EDDHA and EDDHSA belong to compounds that are difficult to biodegrade under static test conditions. The degree of decomposition was similar to the HBED and EDTA biodegradation rates. Fe-EDDHA, Fe-EDDHSA, and Fe-HBED contain high content of chelated iron ions and may be used in smaller amounts than other ligands. It results in less impact on environment and pollution of inland waters.

The investigated studies allow to predict the susceptibility of biochemical degradation of ligands in aerobic conditions. The results show that the use of Fe-EDDHA and Fe-EDDHSA chelates in too high doses may result in both compounds being found in surface water and wastewater. Compounds that are not biodegradable get along with wastewater to biological wastewater treatment plants. Surface water is a source of drinking water. High concentration of nonbiodegradable compounds in surface water and treated wastewater will affect their presence in drinking water. The chelators concentration in the effluents and surface waters should be monitored, as their presence may have negative effects on the environment and living organisms.

Both reduction of the chelate dose and using longer acting products should be considered for compounds that are not easily biodegradable. This reduces the number of applications and the amount of substance introduced into the soil. The breakdown of the compounds depends primarily on the chemical and soil properties, so further evaluation is needed to assess the effect of chelators and their degradation products on environment. An alternative to the decomposition of these compounds may be photodegradation or chemical degradation.

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