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STUDY ON THE AEROBIC BIODEGRADATION OF CHELATING AGENTS UNDER THE STATIC CONDITIONS

BADANIE TLENOWEJ BIODEGRADACJI ZWIĄZKÓW CHELATUJĄCYCH MIKROELEMENTY NAWOZOWE W WARUNKACH TESTU STATYCZNEGO

Abstract: In this paper results of biodegradation of DTPA, HEEDTA and a new, perspective chelating agents – MGDA and GLDA are shown. The assessment of susceptibility of these compounds to biodegradation was carried out in accordance with the Polish Standard PN-88/C-05561 – Study on the aerobic biodegradation of organic compounds in water under the static conditions. Analysis showed, that GLDA and MGDA are characterized by considerably quicker and more effective biodegradation than DTPA or HEEDTA and in this connection they can replace it with success in many branches of industry.

Keywords: Biodegradation, chelating agents, static test

Nowadays exists a possibility of providing for the agriculture the new generation of fertilizers of greater effectiveness and controlled activity, having the maximally limited negative influence on the quality of the water sources, soil and atmosphere. Liquid micronutrient fertilizers are the fertilizers meeting such requirements. Micronutrients contained in them are found in a form of chelate – the complex with the organic compound. These connections guarantee fast and safe micronutrients uptake by plants creating fertilization as the effective method of supplementing nutrients. However the stability and the period of the availability of micronutrients are dependent on the properties of the ligand. Thus studying the specificity – first of all the biodegradability and the potential time of affecting in the soil – of chelating agents applied in micronutrient fertilizers is essential [1–6].

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For complexation of micronutrients different chelating agents are being used. The role of the ligands perform both natural occurring organic substances and synthetic compounds. Natural organic complexones are for example lignosulphonic acids, sulphonic tannins or humic acids. However for the production of liquid micronutrient fertilizers it is beneficial to apply first of all synthetic organic compounds, which by the formation a few ionic or ligand bonds, are able to enclose element permanently. Among them most commonly used compounds are the following: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxy-2-ethylenediaminetriacetic acid (HEEDTA), nitrilotriacetic acid (NTA) and their salts [1, 2, 5].

However research indicate, that so far applied chelates are characterized by insufficient biodegradability and do not meet the primary rules of environmental protection. Their negative influence on the environment and related danger for people lead to start searching for new compounds, of the same or even higher effectiveness, but first of all being characterized by a fast and effective decomposition through microorganisms. The example of such biodegradable substitute can be methylglycinediacetic acid (MGDA) and glutamic acid *N,N*-diacetic acid (GLDA) [1, 2, 4, 5].

Diethylenetriaminepentaacetic acid – DTPA

Diethylenetriaminepentaacetic acid (DTPA) is universally used chelating agent which complex metal cations. It creates complexes via five carboxylic and three amino groups with free pairs of electrons. DTPA has broad applications in different branches of industry. It is used in production of household detergents, herbicides, in the paper and fertilizer industry. Moreover its ability to bind as well as to remove radioactive substances from the organism such as plutonium, americium and the curium finds application in medicine [6–11].

Several information are known about the influence of this compound on the natural environment. However some sources inform that high concentrations of DTPA inhibit growth of microorganisms. It can suggest the toxicity of this substance. Furthermore above-mentioned chelate has small degree of biodegradability or it is even non-biodegradable [11, 12].

Hydroxy-2-ethylenediaminetriacetic acid – HEEDTA

HEEDTA belongs to hydroxycarboxylic acids and differs from aminopolycarboxylic acids with it, that in its structure one carboxylic group is replaced with the hydroxyl group. Such structure of HEEDTA contributes to increasing its solubility in the water [8, 13].

This chelate, similarly to DTPA, is a compound with strong complexing properties. It has also similar applications, however its world scale consumption is much lower. It usually appears in the trisodium salt form – Na_3HEEDTA [8, 13].

The biochemical decomposition process of this compound is difficult and is carried out slowly. It is assumed that the degree of its biodegradation does not exceed the 20 % [8].

Methylglycinediacetic acid – MGDA

Methylglycinediacetic acid is a new, synthetic chelating agent which forms complexes of the high stability in a wide range of the pH and the temperature values. Nowadays it has been applied already in detergents, household detergents, cosmetics, in processes of galvanization and in the textile industry [14, 15].

Many standard OECD tests has proved that MGDA is a readily biodegradable chelating agent. Moreover, unlike other complexing substances, MGDA does not require adapted bacteria for decomposition, but instead is degraded under the standard conditions defined by the OECD. Furthermore after carrying out many toxicological and environmental tests it has been proved that MGDA is completely safe for the human health and it is not triggering off negative effects in the natural environment [5, 13, 15].

Glutamic acid *N,N*-diacetic acid – GLDA

GLDA is, similarly to MGDA, new, strongly complexing synthetic compound. In a wide range a pH range is characterized by a good solubility as well as the unprecedented stability in high temperatures. Moreover it has been not observed that applying this compound triggered off any ecological or toxicological effects [16, 17].

GLDA molecule is built from two carboxymethyl groups linked to the atom of nitrogen of glutamate. This synthetic chelate has been released on the market relatively recently and it is produced under the commercial name Dissolvine® GL. However this product includes only L-GLDA, isomer D-GLDA is not undergoing the biodegradation [16–18].

L-GLDA is considered to be biodegradable. Degree of its biodegradation in the sequence of 28 days in the test of the closed bottle exceed the 60 %. Moreover it was possible to isolate microorganisms that were able to carry out the effective decomposition of this isomer. Microorganisms that carried out the L-GLDA biodegradation were Gram negative bacteria from the *BG-1* strain. They were characterized by the 100 % homology with *Rhizobium radiobacter* and therefore they were categorized to *α-Proteobacteria* [16–18].

The optimal balance between the degree of the biodegradation and the ability of strong and permanent complexes formation by GLDA causes that this compound is an excellent alternative for the conventional synthetic chelating agents and can be applied without reservations in different branches of industry [16].

Materials and methods

Assessment of the biodegradation of fertilizer micronutrients complexing agents was carried out according to Polish Standard PN-88/C-05561 – Examination of the aerobic

biodegradation of organic compounds in the aqueous environment under the conditions of the static test [19].

The progress of the process of the biochemical disintegration of these compounds was determined on the basis of the decay of the substance in the sample and with the usage of changes in the COD degree reduction.

The degree of the organic compound decay and the chemical oxygen demand – COD reduction, during the biodegradation test under the static test conditions, inform us about the susceptibility to the biochemical decomposition. It is assumed that compounds are readily biodegradable when the degree of elimination reach level of 70 % during 5 days or the 45 % after twenty-four hours. The substance is being regarded biodegradable when its elimination reaches 50–70 % in sequence 20 days or 70 % in the sequence of 6–20 days. If the decay reaches level below the 50 % in the sequence of 20 days such compound can be classified as a hardly biodegradable. The compound is considered to be resistant to the biodegradation, when during the time of the test (20 days) is not undergoing the biodegradation [19].

Study on the aerobic biodegradation of organic compounds in the aqueous environment under the static test conditions – PN-88/C-05561

The method is based on determining the degree of degradation of organic compound placed in mineral medium inoculated with standard activated sludge. The process of the biodegradation lasted maximal for 20 days and it was carried out under aerobic conditions, in the room temperature, without the access of the light.

Mineral medium and standard activated sludge were placed in three flasks. Solution of the tested organic compound was inserted into first two flasks. The third flask served as a control test. Moreover in the process it has been applied, for comparison, the glucose as the factor supporting the biochemical decomposition.

Flasks prepared in this 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.

Immediately after preparing samples and connecting apparatus, after 1, 3, 6, 24 hours, and every next day through maximal 20 days from each of three flasks a determined amount of the sample has been collected. After draining them off they were used for indicating the concentration of tested compound and the COD value.

COD was examined with the usage of two methods: potassium dichromate and potassium permanganate. The concentration of tested compounds was determined using complexometric titration [20, 21].

Diethylenetriaminepentaacetic acid (DTPA), hydroxy-2-ethylenediaminetriacetic acid (HEEDTA), 40 % trisodium salt of methylglycinediacetic acid (Trilon®M, BASF, Germany) and 38 % tetrasodium salt of glutamic acid-*N,N*-diacetic acid (Dissolvine®GL, Akzo Nobel, The Netherlands) were subjects to the research on the biodegradation.

Results and discussion

In this paper the determination of biodegradation degree of chelating agents, applied in micronutrient fertilizers manufacturing: the DTPA and HEEDTA as well as MGDA and GLDA – compounds that may displace from fertilizer market the ones so far applied, were the aim of presented researches. The progress of the process of the biochemical degradation of tested substances was determined on the basis of the concentrations reduction and with the usage of the nonspecific indicator of mass depletion, which is the COD reduction.

Reduction of the compound concentration (degree of compound reduction, degree of compound biodegradation) in the time (t) has been calculated according to the formula:

$$X = \frac{C_0 - C_t}{C_0} \cdot 100 \ [\%]$$

in which: C_0 – concentration of compound in the time t = 0, [g/dm^3],
C_t – concentration of compound after the t time, [g/dm^3].

Results of the degree of reduction of all tested compounds are shown in Fig. 1. It represents the reduction of the DTPA, HEEDTA, MGDA and GLDA concentration in samples with and without the addition of glucose.

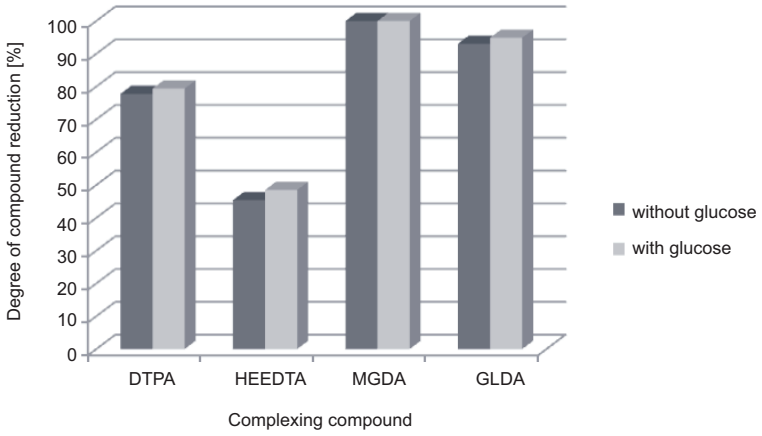

Fig. 1. Degree of reduction of complexing compounds: DTPA, HEEDTA, MGDA and GLDA

The highest degree of biodegradation for all tested compounds, both in the presence as well as at the lack of glucose, was characterized by the MGDA. It is totally biodegradable and the addition of glucose accelerated its decomposition. In the case of the second alternative chelating agent – GLDA, the reduction of the concentration reached the level of 94 % in the sequence of 20 days in both kinds of samples. Thus it is possible to classify this substance among biodegradable substances. Moreover the addition of glucose did not influenced on the rate of its decay.

The most difficult biodegradable chelating agent turned out to be the compound, commonly used so far, HEEDTA. The degree of its degradation reached level of 48.5 % in the case of the addition of glucose and 45.4 % when the biodecay occurred without its presence. Glucose did not also influenced on the rate of the DTPA elimination in the greater degree and the degree of its biodegradation reached level about 78 %.

COD was examined with the two methods: potassium permanganate and potassium dichromate appropriately according to standards: PN-ISO 6060:2006 and PN-EN ISO 8467:2001. The degree of COD reduction for both methods was calculated according to following formula:

$$Y = \frac{a - b - c}{a} \cdot 100 \ [\%]$$

in which: a – COD value at t = 0 time, mg O_2/dm^3,
b – COD value after the t time, mg O_2/dm^3,
c – COD value in the control test at t = 0 time, mg O_2/dm^3.

The degree of COD reduction of organic compounds in examined samples with and without the addition of the glucose is presented in Fig. 2.

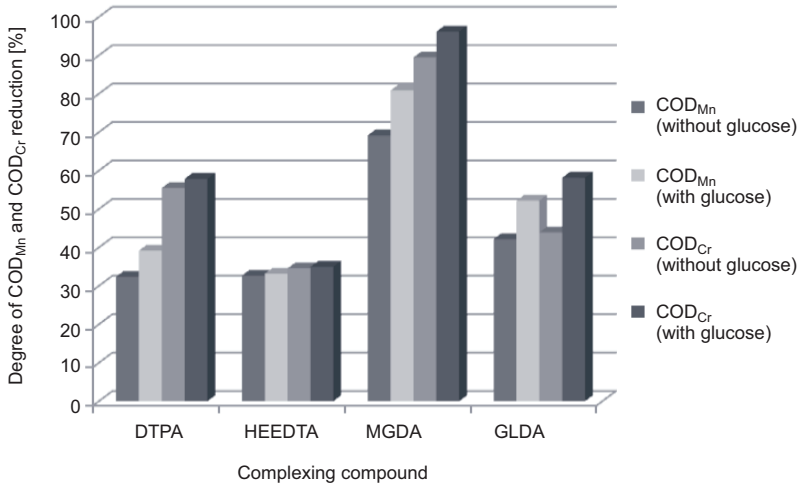

Fig. 2. Degree of COD_{Mn} i COD_{Cr} reduction for DTPA, HEEDTA, MGDA and GLDA

The highest degree of COD reduction, for all tested compounds, both with potassium permanganate as well as potassium dichromate was characterized by MGDA. This prospective chelating agent, compared with different chelates, was characterized by about 70–80 % degree of COD_{Mn} reduction and about 93 % degree of COD_{Cr} reduction. For GLDA the degree of the COD_{Mn} reduction reached level about 43 % and 54 % with the potassium dichromate method. Glucose had the influence on the degree of COD reduction of this compound, because in flasks containing its addition in the case of the

potassium permanganate method the degree of the COD reduction increased about 10 %, and in the case of the potassium dichromate method about 14 %.

The lowest COD reduction was found for HEEDTA. Value of the degree of the COD reduction in this case in both methods and irrespective of the glucose presence oscillated from 32 to 35 %. Also degree of DTPA COD_{Mn} reduction reached level over 30 %, however the degree of COD_{Cr} reduction was much higher and reached level about 56 %.

The list of results of the biodegradation for all tested chelating agents are shown in Table 1 and Table 2.

Table 1

Biodegradation of complexing substances under static conditions without glucose

Complexing compound	Degree of compound reduction [%]	Degree of COD reduction [%]	
		COD_{Mn}	COD_{Cr}
DTPA	77.8	32.4	55.5
HEEDTA	45.4	32.7	34.6
MGDA	100 (216 h)	69.2	89.5
GLDA	93.1	42.2	43.9

Table 2

Biodegradation of complexing substances under static conditions with glucose

Complexing compound	Degree of compound reduction [%]	Degree of COD reduction [%]	
		COD_{Mn}	COD_{Cr}
DTPA	79.4	39.2	57.9
HEEDTA	48.5	33.2	35.0
MGDA	100 (192 h)	80.9	96.2
GLDA	94.9	52.2	58.2

The biodegradation tests carried out under the static test conditions has shown, that the most difficult degradable by microorganisms was HEEDTA. The degree of the concentration reduction of this compound reached level 48.5 % in the presence of glucose and 45.4 % with lack of it. The presence of the glucose influenced on increase in the degree of the HEEDTA biodegradation only insignificantly. COD for HEEDTA, examined both with potassium permanganate as well as potassium dichromate method, reached level of degree of COD reduction on average 33 %. The 10 % difference between the degree of the concentration and COD reduction can suggest that during the process of the decomposition of this compound intermediates products of decomposition could be formed.

The degree of the DTPA biodegradation reached level of the 78 %, furthermore glucose only insignificantly influenced the reduction degree of this substance. According to COD results obtained with potassium dichromate it came out that DTPA is undergoing the biochemical disintegration in about 55 %, and in the case of the

potassium permanganate method only in about 37 %. The disproportionate reduction in estimated COD to obtained value of the DTPA concentration reduction indicates indirect products of the degradation of this compound appearing in the sample.

The degree of MGDA biodegradation reached level of the 100 %. Total degradation of this chelating agent occurred at 216 hour of the process. The results of the degree of COD_{Mn} and COD_{Cr} reduction reached appropriately level about 70–80 %, without presence of additional glucose, and 89–96 %, when glucose was being added.

The reduction of GLDA concentration reached the level of 94 % and glucose only slightly influenced its rate. However COD reduction examined with both methods reached level 55 % in the presence of the factor supporting the biodegradation and about 43 % at its lack. In this case it is also possible to suspect that during the process of the biodegradation indirect products of the GLDA disintegration could be formed.

Conclusions

On the basis of the carried out researches on the biodegradation under the static test conditions it can be found, that:

1. Complexing compounds such as DTPA and HEEDTA, that are the ones so far applied in the fertilizer industry, were underwent biodegradation in the range of 45 to 80 % in the 20 days of process.

2. The most hardly biodegradable chelating agent was HEEDTA and its biochemical decomposition reached level about 47 %.

3. In the case of conventional chelating agents glucose did not have considerable influence on the rate of their biochemical decay.

4. Researches on the biodegradation of relatively new chelating agent, which is MGDA, showed that it was characterized by a fast and effective degradation by microorganisms. The biodegradation reached level of 100 % in time less than 11 days.

5. The presence of glucose as a readily available source of carbon and energy had influence on MGDA biodegradation rate.

6. The degree of the GLDA biodegradation, as a new prospective chelating agent, reached level about 95 %, however much lower degree of the COD reduction of this compound showed that during its decomposition the intermediates could be formed.

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BADANIE TLENOWEJ BIODEGRADACJI ZWIĄZKÓW CHELATUJĄCYCH MIKROELEMENTY NAWOZOWE W WARUNKACH TESTU STATYCZNEGO

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Abstrakt: Przedstawiono wyniki biodegradacji DTPA i HEEDTA oraz nowych, alternatywnych związków kompleksujących – MGDA i GLDA. Ocena podatności tych związków na biorozkład przeprowadzona została zgodnie z Polską Normą PN-88/C-05561 – Badanie tlenowej biodegradacji związków organicznych w środowisku wodnym w warunkach testu statycznego. Badania wykazały, iż GLDA i MGDA odznaczają się znacznie szybszą i skuteczniejszą biodegradacją niż DTPA czy HEEDTA i w związku z tym mogą zastąpić je z powodzeniem w wielu gałęziach przemysłu.

Słowa kluczowe: biodegradacja, związki chelatujące, test statyczny