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Optimization of Chelates Production Process for Agricultural Administration of Inorganic Micronutrients

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The iron chlorosis is one of the most diffused plant disease, which affects their growth and reduces the yield of harvests. This disease is caused by the iron deficiency and it is highlighted by the progressive yellowing of plants due to the reduction of chlorophyll production. The most efficient and diffused therapy against the iron chlorosis is the use of chelating agents and, among them, the o,o-EDDHA/Fe^{3+}, the most stable isomer of EDDHA, is the most used due to its capacity to guarantee a prolonged treatment.

The aim of this work is to develop a production process environment friendly, based on the recovering and recycling of organic solvents to minimize the waste produced. The feed organic solvents ratio has been varied evaluating the synthesis yield and the percentage of o,o-EDDHA/Fe^{3+} produced to identify the best feeding conditions. Several products have been then tested on lettuce plants to determine their usability.

1. Introduction

To guarantee and preserve the plants growth, the presence in the soil of all the necessary nutrients has to be ensured and, among them, the iron is particularly important due to its participation in the chlorophyll formation and as catalyst in some metabolic reactions. The iron deficiency causes the iron chlorosis, a plant disease which affects the plants development and reduces the yields of crops. It appears with a progressive yellowing of plant, starting from the younger leaves and proceeding till the older parts, due to the decrease of the chlorophyll content. The iron chlorosis is related to the iron bioavailability for the plants, which is mainly controlled by its oxidation state. In fact the plant adsorbs, by the roots, the Fe^{3+} present in the soil after a reduction to Fe^{2+} by enzymatic reactions (Marsh Jr et al., 1963), for this reason one of the most important causes of iron chlorosis is the nature of soil. In the calcareous soils the iron is readily converted in an insoluble form for the plant absorption (Chen and Barak, 1982) and, moreover, the presence of $CaCO_3$ increases the alkalinity of soil and further reduces the possibility to solubilize the necessary iron, causing the iron deficiency in the plants (Yunta et al., 2003b). Another cause of the iron deficiency could be the presence of antagonist microelements (such as Mn, Cu, Zn, Co, Ni and Cd) which can generate a competitive adsorption (Mengel, 1994). Several therapies against the iron chlorosis have been proposed, such as the use of iron salts or the application of foliar spray. Unfortunately both these methods have been ineffective because, if iron salts are used in the soil, the iron is swiftly converted in useless form for the plant, if foliar spray are used, only surfaces treatment can be performed and the spray can be subjected to leaching phenomena.

In the agricultural practice the most common method to correct the iron chlorosis is the fertilization with chelating agents, organic macromolecules able to bind with more than one chemical bond a metal ion, stabilizing and increasing its availability (García-Marco et al., 2006). The use of chelated iron ensures the necessary iron requirement because the chelating agents act as carrier of iron ion (Fe^{3+}) which it is released at the interface with the plant roots, where the iron is adsorbed. The chelating agent, free of ions, returns in the soil available to bond ions present in the soil and restart the cycle.

Six chelating agents, all polyamine-carboxylic acids, are authorized by the European directive (98/3/EC) for the agricultural uses and, among them, the most efficient is the EDDHA, a phenolic compound with a polyamine-carboxylic acid backbone, able to form ferric complexes with high stability (Yunta et al., 2003a).

The EDDHA is effective because offers up to six bonds to link the iron ion ensuring the necessary stability of metallic complex to treat the iron deficiency (Rodrigues et al., 2011).

Due to the large use of chelated iron, many production processes have been proposed but all of them drove to the production of a mixture of the three position isomers of EDDHA/Fe^{3+}: o,o-EDDHA/Fe^{3+}, the more stable and consequently the more incisive in the iron chlorosis treatment, which have six bonds with the iron ion, o,p-EDDHA/Fe^{3+}, and the p,p-EDDHA/Fe^{3+}, which establish respectively five and four bonds with the metallic ion (Gómez-Gallego et al., 2002).

Moreover, the current industrial production processes of chelated iron involve a very large use of organic solvents, chlorinated substances and water, which determine an expensive post processing and a large quantity of wastes. The starting point of research work has been the one step glyoxylat reaction scheme introduced by Petree (Petree et al., 1978), which proposed a reaction between phenol, ethylenediamine, glyoxylic acid and an aqueous solution of a base to obtain the EDDHA, with a stoichiometric molar ratio respectively 1:0.5:1:1 and a suggested molar ratio respectively 13:0.5:1:1. The large excess of phenol, respect to the stoichiometric, is necessary to maximise the yield of EDDHA. The dosing of reagents has to occur at 45 °C while the reaction has to occur at 75 °C for 2 h. At the end of reaction, the mixture has to be cooled down at 25 °C and the reaction mixture is washed with water and carbon tetrachloride to separate the oil phase, containing the organic solvent, from the water phase, containing the EDDHA produced and the unreacted reagents. The water phase obtained is extracted twice more with carbon tetrachloride and transferred in the chelating vessel. During the chelation, a concentrated solution of iron(III) chloride was slowly added to the water phase and the pH is stabilized to pH 7.5. To obtain the prevalence of ortho-ortho isomer of EDDHA (the desired product), the pH 7.5 has to be reached as soon as possible. The obtained product was dried at 80 °C overnight to get the typical red-brown powder.

1.1 Aim of the work

The aim of this work is to develop an EDDHA/Fe^{3+} production process environmentally compatible with a simple and expensive post processing. For this reason, this work has been focused on the elimination of chlorinated substances and organic solvents, dangerous to manage and responsible of corrosion issues to the industrial plants. To evaluate the effect of the feeding solvent ratio on the final product, the yield and the percentage of o,o-EDDHA/Fe^{3+} are evaluated, and the fertilizers product tested on lettuce plants.

2. Materials and methods

2.1 Materials

The reagents necessary to the production of EDDHA were: phenol (CAS number 108-95-2), ethylenediamine (CAS number 107-15-3), sodium hydroxide solution 50 % wt/wt aqueous solution (CAS number 1310-73-2), glyoxylic acid solution 50 % wt/wt aqueous solution (CAS number 298-12-4), all purchased from Sigma Aldrich, Milan, Italy. The solvent used for the extraction phases were toluene (CAS number 108-88-3, purchased by Delchimica, Naoles, Italy) and deionized water. During the chelation process, as iron salt has been used the iron nitrate nonahydrate (CAS number 10421-48-4, purchased by Delchimica, Naples, Italy), as basic solution has been used sodium hydroxide solution 50 % wt/wt aqueous solution (CAS number 1310-73-2) and as acid solution has been used hydrochloric acid 37 % wt/wt aqueous solution (CAS number 7647-01-0) purchased from Sigma Aldrich, Milan, Italy.

2.2 Production process

The production of o,o-EDDHA/Fe^{3+} has been performed in a plant designed and realized ad hoc composed by two batch vessels, the first one for the synthesis of EDDHA and the second one for its chelation with the iron salt. The first reactor is equipped with a glass jacket for the regulation of the reacting mixture temperature by the action of a computer managed thermal bath, the chelation was carried out working at room temperature. The control of process parameters (pH and temperature) has been performed equipping each vessel with pH probe and temperature sensor (thermocouple) connected with an acquisition data board managed by a computer. The desired temperature (75 °C) in the jacket during the synthesis of EDDHA is ensured by the hot water circulation. For the pH regulation during the chelation, an ad hoc software has been developed using the Labview environment (National Instruments). Based on the difference between the measured value and the set point (pH 7.5 is the more favourable condition during the chelation step), the software can activate the two peristaltic pumps for the dosing of acid (HCl 37 % wt/wt aqueous solution) or basic solutions (NaOH 50 % aqueous solution) to correct the pH of the solution.

In this work, the process illustrated (Petree et al., 1978) has been varied to reduce the toxic and chlorinate solvents:

1. the carbon tetrachloride has been replaced with toluene, used as organic solvent to promote the phases separation during the extraction phases;
2. the number of extraction phases, after a preliminary observation of experimental data, has been reduced from 3 to 2;
3. the volumes of water and organic solvents have been reduced, with a global saving in terms of costs, time and post processing operation
4. based on this process, with the aim to further reduce the use of phenol, its partial substitution with toluene, a less expensive and toxic solvent, has been performed, maintaining constant the molar ratio between organic mixing and the limiting reagent (ethylenediamine);
5. with the global goal to save hazardous substances, the toluene used during the separation phases has been recycled and used during all the following operations of extraction limiting the polluting waste. Five feeding organic composition (phenol/toluene) have been tested (Table 1):

Table 1. Reaction pattern proposed

Name of process	Feeding composition phenol/toluene
Feed 1	1.000/0.000
Feed 2	0.700/0.300
Feed 3	0.540/0.460
Feed 4	0.390/0.610
Feed 5	0.078/0.922

According to the Feed 1 reaction scheme, the reaction between 3.9 mol of phenol, 0.15 mol of ethylenediamine, 0.3 mol of glyoxylic acid and 0.3 mol of sodium hydroxide has been performed at 75 °C for 2 h. At the end of reaction time, the vessel has been cooled down and 750 mL of toluene and 500 mL of water have been added for the extraction phases. After a vigorous stirring, the mixing has been subjected to a rest phase and, in few minutes, two layers have been clearly visible. The water phase (on the bottom of the vessel) has been extracted again with 300 mL of toluene and 100 mL of water, obtaining the final water phase ready for the chelation. Then, the obtained water phase has been transferred in the chelation vessel and chelated by the adding of iron salt solution of 88 mg of iron (III) nitrate nonahydrate in 50 mL of deionized water. The obtained highly viscous solution has been dried overnight and quantitatively recovered.

The starting point has been the organic solvents composed only by phenol (Feed 1), whereas the Feed 5 ratio provides only the phenol stoichiometric necessary for the reaction, the remaining part of solvent has been replaced with toluene. In the Table 1, from Feed 2 to Feed 4, the excess of phenol in the feed mixture has been decreased with the increase of toluene used, maintaining constant the total molar amount of organic solvent fed to the reactor.

2.3 Analytical methods

The obtained products have been characterized in terms of EDDHA yield and chelated iron content by the application of the official method of determination (Standardization, 2007), based on the use of high-performance liquid chromatography (HPLC). The HPLC analyses have been performed using a C-18 column (an Agilent Zorbax Eclipse Plus C18) with internal diameter of 3.9 mm and lengths 150 mm, with a flow rate of the eluent phase, composed by acetonitrile, 0.5 % TBA % v/v and water up to 1 L volume, of 1 mL/min. The detection has been performed at a wavelength of 280 nm (Standardization, 2007).

The powders obtained from the syntheses have been dissolved in a weak alkali solution (.2 g/L of NaOH in deionized water) with a concentration of 60 mg/50 mL and filtered with 45 µm filter before the HPLC analysis.

Analysing the chromatograms obtained, it is possible to identify the peaks of two stereoisomers of o,o-EDDHA/Fe^{3+}: racemic-o,o-EDDHA/Fe^{3+} and meso-o,o-EDDHA/Fe^{3+} and, after a proper calibration technique (Garamella, 2012), calculate the yield of reaction and the percentage of chelated iron as ortho ortho isomer.

2.4 Key parameters

To evaluate the results of the syntheses, two key parameters have been calculated: the yield of reaction and the percentage of iron chelated as ortho ortho isomer.

The reaction yield has been calculates as:

$$Yield = \frac{n_{Fe,react}}{n_{rl}} \cdot 100 [=] \% \qquad (1)$$

where:
$n_{Fe,react}$ are the mol of obtained product;
n_{rl} are the mol of limiting reactant (ethylenediamine).
The percentage of iron chelated as ortho ortho isomer has been evaluated as the ratio between the mol of o,o-EDDHA/Fe^{3+} and the total amount of solid product (C_p) obtained in the batch after the drying step:

$$\%_{o,o-EDDHA/Fe} = \frac{n_{Fe,react} \cdot MW_{Fe}}{C_p} \cdot 100 \qquad (2)$$

2.5 Product testing

To evaluate the efficacy of treatment with the chelated iron and to compare the effects of different products, a test on a seedling of lettuce plants have been performed. For 43 days three lettuce samples, starting from chlorotic condition, have been treated:

- untreated: the plants have been watered every two days for 43 days;
- commercial: the plants have been watered every two days and treated with commercial chelated iron every 5 days (2 g/L chelated iron concentration solution);
- T5A: the plants have been watered every two days and treated with the chelated iron produced according to the Feed 1 process every 5 days (2 g/L iron chelated concentration solution).

The seedling have been photographed every day to monitor the plants conditions and evaluate the progress in the iron chlorosis therapy.

3. Results and discussion

Based on the yield of reaction and the percentage of iron chelated as ortho ortho isomer a comparison between the results obtained changing the feed solvent ratio can be performed. In Figure 1 on the abscissa the molar fraction of phenol in the organic solvent feeding has been reported. Thus, starting from the left side of the graph to the right side, the feeding ratio from Feed 5 to Feed 1 have been reported (see Table 1).
In Figure 1 the yield of the process (on the left) and the percentage of o,o – EDDHA/Fe^{3+} (on the right) have been represented. The Feed 1 process, which represents the reference point for the design of the other experiments, results in a yield of 26 % with a percentage of chelated iron as ortho ortho of 2.7 %. The Feed 2 process results in a yield of 23 % with a 2 % of ortho-ortho chelated iron and these percentages tend to decrease reaching the Feed 5, which shows a yield of 2.5 % and 0.2 % of o,o-EDDHA/Fe^{3+}. As could be seen from the graph, the process where only phenol is fed to the reaction mixture results in a better yield and product formation. This characteristic is balanced by the fact that in this process a large quantity of the expensive and toxic solvent has to be used and there is no possibility to recycle the phenol in the separation phase. Thus, beside the fact that the toluene adding tends to get worst the process yield, the toluene in the feeding mixture can be used as organic solvent in the separation process, with a consistent advantage in solvent recover. For these reasons, in the choice of the suitable feeding solvent ratio two principal aspects have to be taken into account: the diminution of the costs (both in economical and in environmental field) due to the solvent recover, which leads to increase the toluene molar fraction, and the total yield of the process, which leads to decrease the toluene molar fraction. A balance between these aspects has to be found.

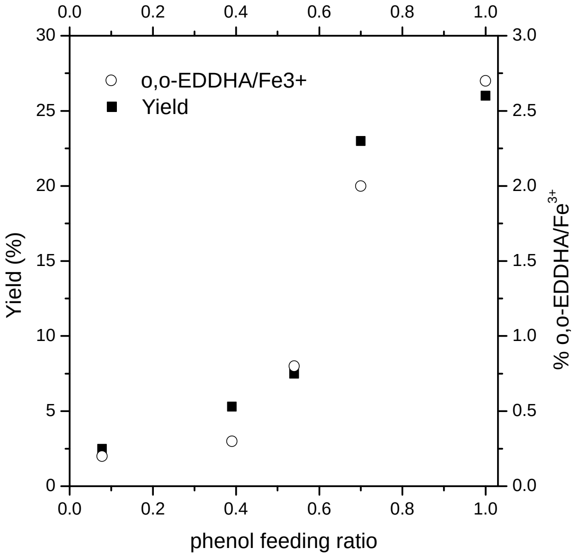

Figure 1. Yield (■) and o,o-EDDHA/Fe^{3+} (○) evolution with phenol feeding ratio.

The product obtained from the Feed 1 process has been used for the tests on the seedling of lettuce plants, in order to have a direct comparison of the effect of the different products on plants. From the Figure 2 the evolution of chlorotic conditions of the lettuce plants can be observed. It is clear as the product obtained from the Feed 1 process is more efficient than the commercial one. In particular, till the 15th day the plants seem to be in the same quite good condition while after the first month of test, the better effect on chlorosis treatment of T5A product is clear. In fact, whereas both the untreated samples and those treated with the commercial product starts to dry out, probably due to a depletion of the nutrition elements in the soil, the T5A samples continue to grow and to show an healthy green color. After 43 days, even if the T5A samples show only a reduced drying out of the plants, the other samples are substantially died.

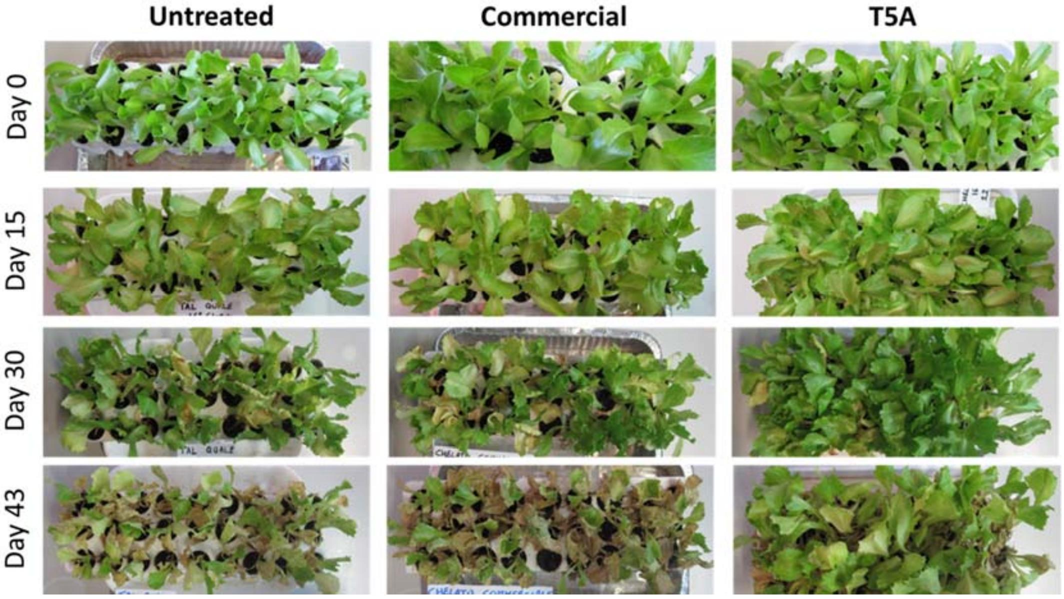

Figure 2. Effects of different products on the iron chlorosis treatment.

4. Conclusions

The aim of this work is to develop a more sustainable production process of o,o-EDDHA/Fe^{3+}, which is the most worldwide used fertilized to treat the iron chlorosis. Different production process have been tested varying the composition of the organic solvents fed in the alimentation mixing. All these processes were based on the reduction of the total amount of the used organic solvents and chlorinate substances, which are the principal cause of environment pollution. Starting from the literature, the process was modified according to the so called 'Feed 1' process (Table 1), obtaining a good process yield. Moreover, the Feed 2 process shows an interesting industrial result in terms of reaction yield and o,o-EDDHA/Fe^{3+} percentage, while the extreme reduction of phenol and its substitution with toluene (Feed 5) does not allow to obtain interesting processes due to low reaction yield and the small fraction of iron chelated as ortho ortho.

Finally, the product obtained from the Feed 1 process was used for the tests on the seedling of lettuce plants, in order to have a direct comparison of the effect of the different products on plants, showing encouraging results in the treatment of iron chlorosis.

The next step of research process could be the complete recycling of organic used in the process in order to realize a closed loop. Moreover, in order to optimize the process and reduce the costs, the reduction of water and of iron salt used could be tested.

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References

Chen Y. & Barak P. 1982. Iron nutrition of plants in calcareous soils. *Adv. Agron,* 35**,** 217-240.

Garamella A. 2012. Progettazione e realizzazione di un impianto su scala di laboratorio per la produzione di chelati per l'agricoltura. *Chemical Engineering Degree Thesis*.

García-Marco S., Torreblanca A. & Lucena J. J. 2006. Chromatographic determination of Fe chelated by ethylenediamine-N-(o-hydroxyphenylacetic)-N'-(p-hydroxyphenylacetic) acid in commercial EDDHA/Fe3+ fertilizers. *Journal of agricultural and food chemistry,* 54**,** 1380-1386.

Gómez-Gallego M., Sierra M. A., Alcázar R., Ramírez P., Piñar C., Mancheño M. J., García-Marco S., Yunta F. & Lucena J. J. 2002. Synthesis of o, p-EDDHA and its detection as the main impurity in o, o-EDDHA commercial iron chelates. *Journal of agricultural and food chemistry,* 50**,** 6395-6399.

Marsh Jr H., Evans H. & Matrone G. 1963. Investigations of the role of iron in chlorophyll metabolism I. Effect of iron deficiency on chlorophyll and heme content and on the activities of certain enzymes in leaves. *Plant physiology,* 38**,** 632.

Mengel K. 1994. Iron availability in plant tissues-iron chlorosis on calcareous soils. *Plant and Soil,* 165**,** 275-283.

Petree H. E., Jelenevsky A. M. & Myatt H. L. 1978. Preparation of phenolic ethylenediaminepolycarboxylic acids. US Patent 4,130,582.

Rodrigues V. L., Camacho J. L. P., Toledo M. C. M. & Taqueda M. E. S. 2011. Organophosphated Fertilizers Production in Humifert Process. *Chemical Engineering Transactions,* 25**,** 1067-1072.

Standardization E. C. F. 2007. Fertilizers - Determination of chelating agents in fertilizers by chromatography - Part 2: Determination of Fe chelated by o,o-EDDHA and o,o-EDDHMA by ion pair chromatography. *European Standard EN 13368-2:2007*.

Yunta F., García-Marco S. & Lucena J. J. 2003a. Theoretical speciation of ethylenediamine-N-(o-hydroxyphenylacetic)-N'-(p-hydroxyphenylacetic) acid (o, p-EDDHA) in agronomic conditions. *Journal of agricultural and food chemistry,* 51**,** 5391-5399.

Yunta F., García-Marco S., Lucena J. J., Gómez-Gallego M., Alcázar R. & Sierra M. A. 2003b. Chelating agents related to ethylenediamine bis (2-hydroxyphenyl) acetic acid (EDDHA): synthesis, characterization, and equilibrium studies of the free ligands and their Mg2+, Ca2+, Cu2+, and Fe3+ chelates. *Inorganic chemistry,* 42**,** 5412-5421.