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OPTIMIZATION OF CONDITIONS FOR THE GROWTH OF WATER HYACINTH IN BIOLOGICAL TREATMENT

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ABSTRACT

The experiments recently carried out with the water hyacinth for energy production and tertiary depuration of slurry of pig manure, proved that the hyacinths did not grow and even died when the culture solution reached or exceeded a pH value of 8. The symtomatology started with the appearance of chlorosis in the apical leaves, later spreading to the rest of the plant. At the same time, the roots turned black and the plants died. In order to eliminate these problems and to correct these effects, an experiment in the growth solution with three different pH values (5.5 to 6.0, 6.7 to 7.3 and 8.0 to 8.5), and three different iron concentrations (0, 5 and 10 mg/L) were carried out. Furthermore, two chemical forms of iron (Mohr salt and Chelate), were used in the experiments to add necessary iron to the solution. The results showed the best growth of the plants in the solution containing 5 mg/L Fe. It was also shown that the plants produced more cellular matter when iron was added in the form of Chelate than iron added in the form of Mohr salt.

RESUMEN

En ensayos realizados con el jacinto de agua para la producción de energía y la depuración terciaria de purines de cerdo, se comprobó que cuando la solución de cultivo superaba un pH de 8, la planta necrosaba y terminaba muriendo. La sintomatología se iniciaba con la aparición de clorosis en las hojas apicales extendiéndose posteriormente al resto de la planta al mismo tiempo que las raíces se iban oscureciendo. Para conocer estas causas y corregir este comportamiento se diseñaron ensayos de experimentación y se estudiaron tres intervalos de pH (5.5 a 6.0, 6.7 a 7.3 y 8.0 a 8.5), tres concentraciones de hierro (0, 5 y 10 mg/L) y dos compuestos de hierro (sal de Mohr y Quelato) en la solución de cultivo. Los resultados de productividad mostraron una clara respuesta del jacinto cuando se aplicó una concentración de 5 mg/L de Fe, siendo más eficaz y duradero utilizarlo en forma de Quelato.

INTRODUCTION

The water hyacinth, *Eichhornia crassipes* (Mart) Solms, has been the subject of more intensive study than any other aquatic plant in recent years. A native of South America, this floating aquatic species adapts extremely well to almost every area to which it has been introduced. In the southern United States, it is the most common and important pest species among the aquatic plants. Due to its vegetative reproduction and high growth rate, water hyacinth spreads rapidly, clogging drainage ditches, shutting out other aquatic vegetation and interfering with shipping and recreation (Holm and Weldon 1969, Raynes 1972). Much effort and many funds have been devoted to the control of this prolific weed (Bates and Hentges 1976).

The information on the role of Fe in plant matabolism started historically in 1970 by the observation made by Baeyens indicating that Fe is necessary for the maintenance of chlorophyll in the plants (Baeyens 1970, Gómez and Mellado 1981). The iron is clearly as essential as the component of the many heme and non heme Fe enzymes and carriers. However, it is now generally accepted that Fe does not play a role in the enzymatic synthesis of porphyrins, neither in plants nor in porphyrin-secreting bacteria (Hewitt 1963, Bollar and Butler 1966).

The pH is a factor influencing the availability of the micronutrient cations (Domínguez 1983). With an increase of the pH, the ionic forms of the micronutrient cations change to hydroxides or oxides. An example is the iron ion as typical element of the micronutrient cations group (Brady 1974, Burriel *et al.* 1974). The higher valence states of iron form hydroxides more insoluble than its lower valence counterpart.

If ferric sulfate was added to the growth solution, a reaction would occur assuming that the hydroxyl ions would come from the soil solution (Alexeiev 1975): most of the ferric ion would be changed into the insoluble form $Fe(OH)_3$ (Vogel 1963). By using an iron Chelate instead of the iron sulfate, the ferric ion will be protected from its precipitation.

Research has been realized on the subject of the freshwater macrophyte or water hyacinth cultivation in pig manure slurry as a potential biomass source which can be converted into methane or into nutritive feed due to its high nutrient content. Moreover, its absorption index of heavy metals has recently been used for the tertiary depuration of pig manure slurry.

MATERIALS AND METHODS

The experiments with the water hyacinth were carried out in a greenhouse at optimum temperature (28-30°C) for the growth. The plants used were all healthy and at a similar stage of growth, they had roots of the same size and leaves of the same color. The experiments were carried out as follows: eighteen 200-L trays isolated from each other were placed in the greenhouse. They were filled with 40 liters of slurry containing pig manure as nutrient source. An equivalent weight of plants was placed on each tray.

The plant growth at three different pH ranges (5.5 to 6.0, 6.7 to 7.3 and 8.0 to 8.5) was evaluated. Three different iron concentrations were used: 0, 5 and 10 mg/L, in two different chemical forms: Mohr salt $Fe(NH_4)_2(SO_4)_2 6H_2O$ and Chelate (Ethylenediaminodi-o-hydroxiphenylacetic-HFe) in addition to the solution **(Table I).**

TABLE I. pH AND $[Fe^{2+}]$ CONCENTRATIONS IN THE TEST SOLUTIONS

Chemical forms of iron	$[Fe^{2+}]$ (mg/L)	pH
Mohr salt $Fe(NH_4)_2(SO_4)_2 6H_2O$	0	5.5-6.0 6.7-7.3 8.0-8.5
	5	5.5-6.0 6.7-7.3 8.0-8.5
	10	5.5-6.0 6.7-7.3 8.0-8.5
Chelate (EDDHA-HFE)	0	5.5-6.0 6.7-7.3 8.0-8.5
	5	5.5-6.0 6.7-7.3 8.0-8.5
	10	5.5-6.0 6.7-7.3 8.0-8.5

Analytical procedures

The pH was determined by a Digit 501 pH-meter. The iron and potassium (these were measured to evaluate their decrease in the slurry) concentrations were determined using an Instrumentation Laboratory model IL 357, Atomic Absorption Spectrophotometer and Atomic Emission Spectrophotometer, respectively.

The chlorophyll A and B were determined by extraction of the plant material with acetone and the optical density measured at 645 and 663 nm, respectively, using Beckman model B Spectrophotometer (Catsh and Jarvis 1971).

The rotting of the roots was determined visually according to the percentage of surface that had turned black. The total nitrogen in the growth substrate was determined by the Kjeldahl method (Hesse 1971). The ammonium nitrogen and nitrate nitrogen were determined by the method described by Bremmer (1965). The phosphorus concentration was determined after extraction with sodium bicarbonate by colorimetry using a Beckman model B Spectrophotometer at 882 nm (Olsen and Cole 1954). The chemical oxygen demand (COD) was determined by the potassium dichromate method according to APHA-AWWA-WPFC (1971).

The wet weight of the water hyacinth was determined at the end of every week. The plants were weighed after retrieval from the growth substrate followed by draining for five minutes.

RESULTS AND DISCUSSION

Among the three ranges of pH studied, the first to show a smaller variation during the culture was the 6.7 to 7.3 range followed by the 5.5 to 6.0 range. The greatest changes were found in the 8.0 to 8.5 range. This indicated that the hyacinth had a tendency to neutralize the environment. Therefore, it was necessary to add a base or an acid in order to maintain required pH range. We can conclude that this neutrophile plant has a buffering effect, providing alkaline elements when the environment is acid and vice versa.

This phenomenon could be explained by the presence of the following components in the plant: potassium oxalate and oxalic acid, which are both pH regulating compounds. Due to an exosmosis phenomenon, the hyacinth excretes those salts through the roots (Francois 1969).

The potassium ion of potassium oxate would be interchanged when the environment is acidic [1]. When the environment becomes alkaline, the hydrogen ion of oxalic acid will become the interchanged ion [2] (Francois 1969), as follows:

Acid

$$H^+ + C_2O_4HK \rightarrow C_2O_4H_2 + K^+ \qquad [1]$$

Alkaline

$$OH^- + C_2O_4H_2 \rightarrow C_2O_4H^- + H_2O \qquad [2]$$

Productivity studies showed that the optimum growth rate is in a substrate of pH 6.7 to 7.3 with concentrations of Fe^2 5.0 mg/L **(Table II).** At this pH plants produced more cellular matter because they did not need to regulate and maintain the neutral pH solution (Parija 1934). **Figures 1** and **2** show the total chlorophyll concentration (µg/cm²) related to time. **Figure 1** shows a decrease in chlorophyll concentration with time when the iron was added as Mohr salt. This may be due to the changes of chemical species of iron under different redox conditions. A decrease of metal in the plant is accompanied by a decrease of chlorophyll concentration because the iron is an element that acts in its biosynthetic process in the transformation from III-Coproporphirinogen to IX-Protoporphirinogen (Barceló and Nicolás 1980) **(Fig. 3).**

TABLE II. PRODUCTIVITY OF WATER HYACINTH

$[Fe^{2+}]$ (mg/L)	pH range	Productivity (tons/ha/year)	
		Mohr Salt	Chelate
0.0	6.7-7.3	290.7	277.6
0.0	8.0-8.5	242.7	140.3
0.0	5.5-6.0	249.7	225.1
5.0	6.7-7.3	453.3	405.2
5.0	8.0-8.5	236.2	384.1
5.0	5.5-6.0	296.5	375.1
10.0	6.7-7.3	381.2	374.4
10.0	8.0-8.5	313.4	166.5
10.0	5.5-6.0	381.2	315.1

When iron was added in Chelate form, the reduction in chlorophyll content was smaller than in the experiment with added iron in Mohr salt **(Fig. 2).** It appears that in its form of Chelate the activity of iron changes less when it is in form of Mohr salt, penetrating and migrating inside the plant. No precipitation of iron was observed when sulfuric acid was added to the solution.

The absorption of iron from the Chelate is unknown but it is assumed that both the iron and the Ethylenedia-

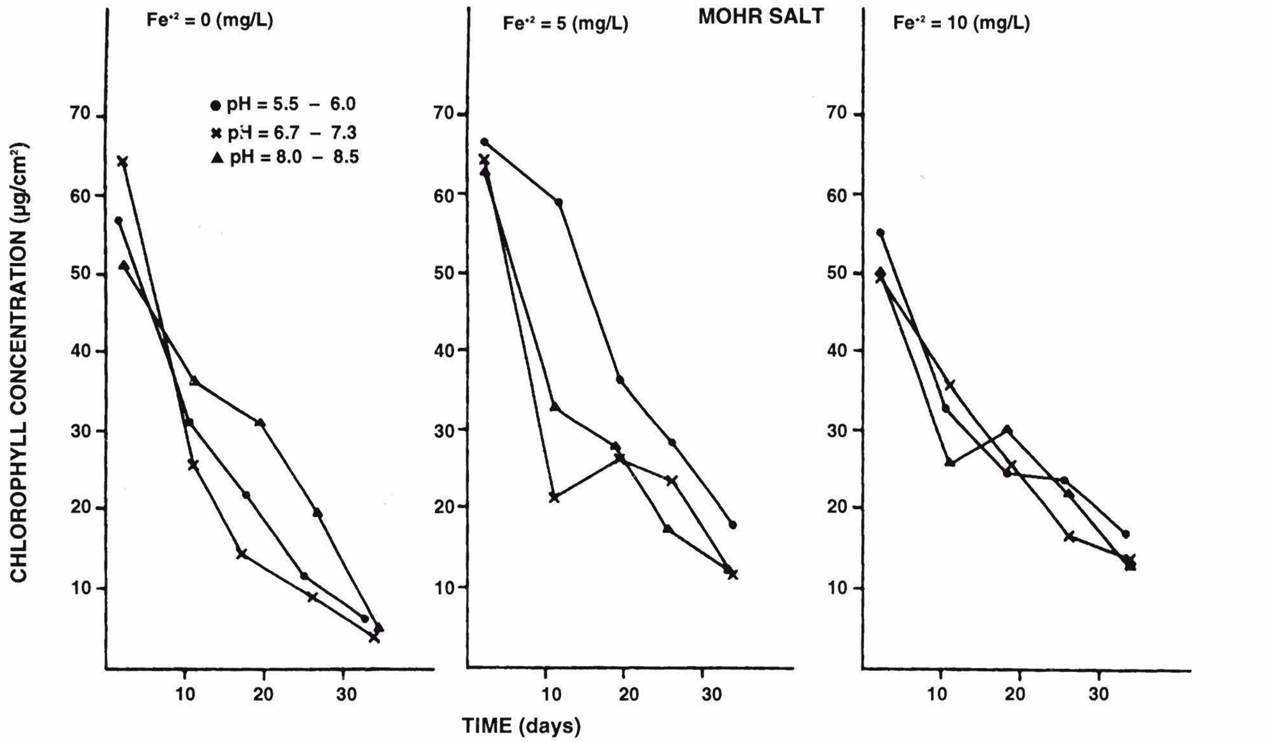

Fig. 1. Chlorophyll concentration (µg/cm²) versus time (days) for Mohr salt

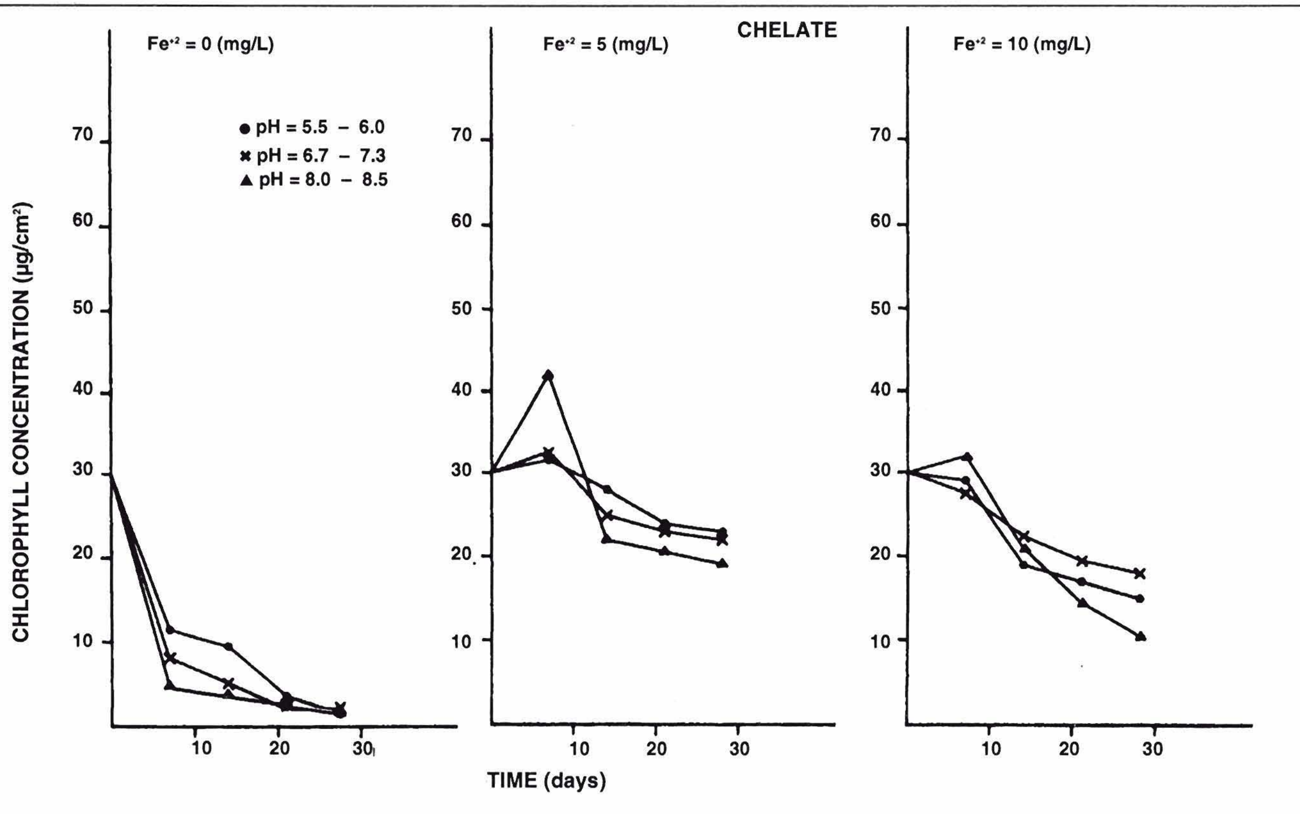

Fig. 2. Chlorophyll concentration (µg/cm²) versus time (days) for chelate

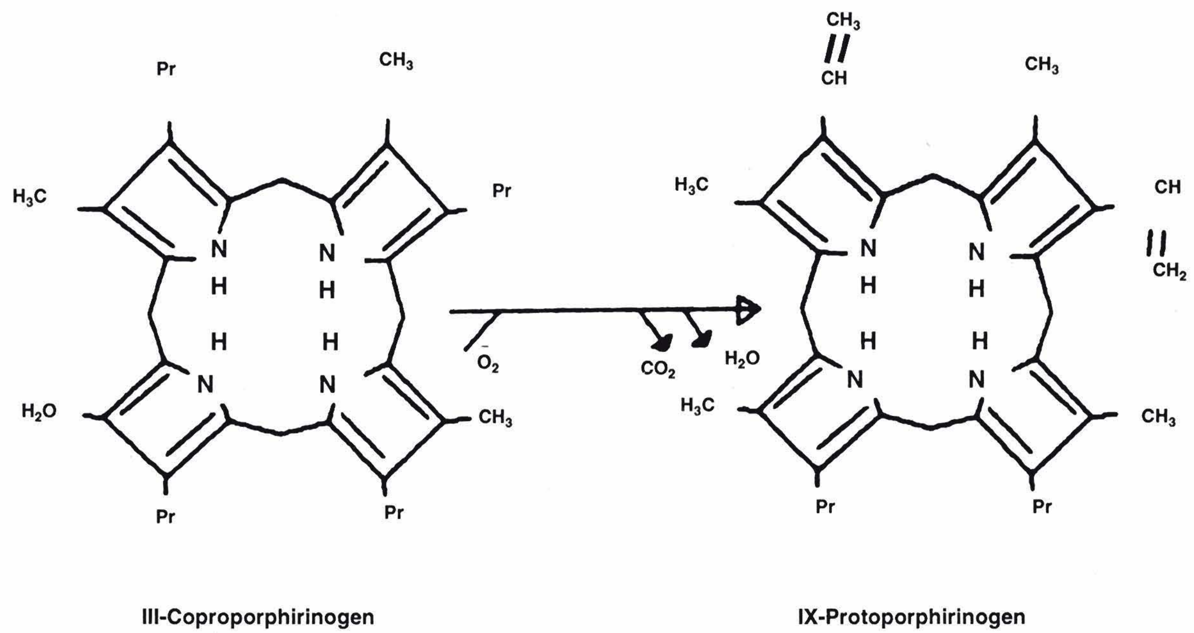

Fig. 3. Transformation of III-Coproporphirinogen to IX-Protoporphirinogen

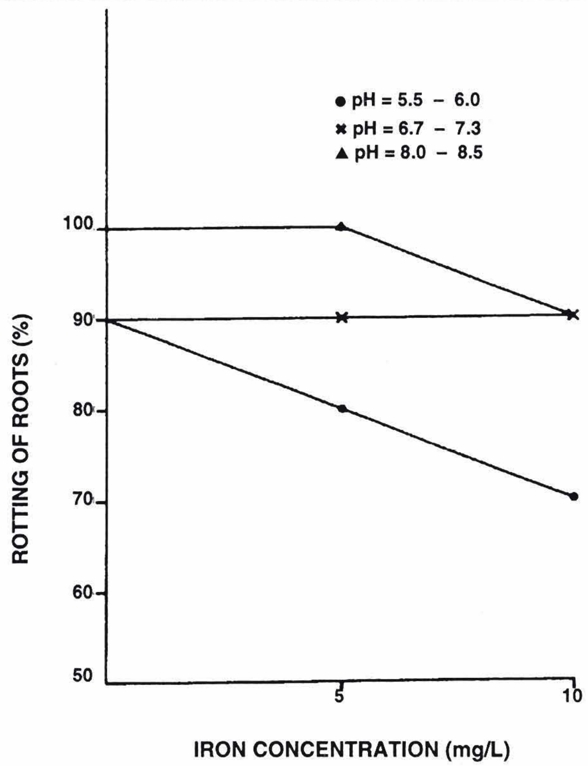

Fig. 4. Rotting of roots (%) versus iron concentration (mg/L)

minodi-o-hydroxiphenylacetic are assimilated separately, despite of a great stability constant of reaction (Primo and Carrasco 1973).

When the pH level increased the rotting of the roots became greater **(Fig.4).** The slurry with the pig manure contains the nutritive elements necessary for the growth of the water hyacinth. The amount of the nutrients absorbed by this plant from the slurry were: N-NH_4 90%, N-NO_3 72%, P 83%, K 70%, Kjeldahl-N 32% and COD 90% (**Table III**). The water hyacinth seems to prefer ammonium ions rather than nitrate ions. However, in the absence of ammonium N, a high growth can also be achieved with nitrate as the only source of N (Reddy and Hueston 1982).

TABLE III. CONCENTRATIONS OF NUTRIENTS IN THE SLURRY USED IN THE EXPERIMENT

Nutrients (mg/L)	Initial	Final	Reduction (%)
Kjeldahl-N	29.20	19.90	32
N-NH_4	10.50	1.00	90
N-NO_3	18.00	5.00	72
P	18.20	3.00	83
K	100.00	30.00	70
COD	1070.00	110.00	90

CONCLUSIONS

- Productivity studies showed that the optimum growth rate is obtained in a substrate of pH 6.7 to 7.3 with concentrations of Fe^2 5.0 mg/L.
- Chlorophyll concentration decreased with time when the iron was added as Mohr salt.
- When iron was added in Chelate form, the reduction in chlorophyll content was smaller than in the experiment with added iron in the form of Mohr salt.
- The pig manure slurry contains the nutritive elements necessary for the growth of the water hyacinth. The amount of the nutrients absorbed by this plant from the slurry were: N-NH_4 90%, N-NO_3 72%, P 83%, K 70%, Kjeldahl-N 32% and COD 90%.

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