

THE EFFECT OF THE H ION CONCENTRATION ON THE AVAILABILITY OF IRON FOR CHLORELLA SP.*

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The H ion concentration of the culture medium is now recognized as being an important factor in the growth of all classes of plants. In certain special cases this may even become the limiting factor for growth. However, in experiments with higher plants growing in soils of different pH values a correct interpretation of results is difficult, because it is frequently impossible to isolate the direct effects of the hydrogen ion concentration from other contributing factors. The following experiments with a unicellular green alga, *Chlorella sp.*, growing in mineral nutrient solutions under pure culture conditions, were performed in an effort to obtain a more exact analysis of the effect of the H ion concentration on the rate of growth of a green plant.

Methods.

Uniform amounts of a pure culture of *Chlorella* were introduced into liquid culture solutions in pyrex Erlenmeyer flasks of 150 cc. capacity. For each culture 50 cc. of a mineral nutrient solution was supplied. In each experiment the salt content of the several solutions was the same in all cultures, except for the varying amounts of the phosphate buffers used to obtain the range of pH desired. The dry weight of the crop produced was used as the criterion of growth.

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Growth of Chlorella in Complete Nutrient Solution at Varying H Ion Concentrations.

Each culture solution in this series of experiments consisted of equal portions of a mineral nutrient solution, designated Solution A, and of a phosphate buffer mixture, Solution B. Solution A contained 2.95 gm. $\text{Ca}(\text{NO}_3)_2$, 0.4 gm. MgSO_4 , a trace of $\text{Fe}_2(\text{SO}_4)_3$, and

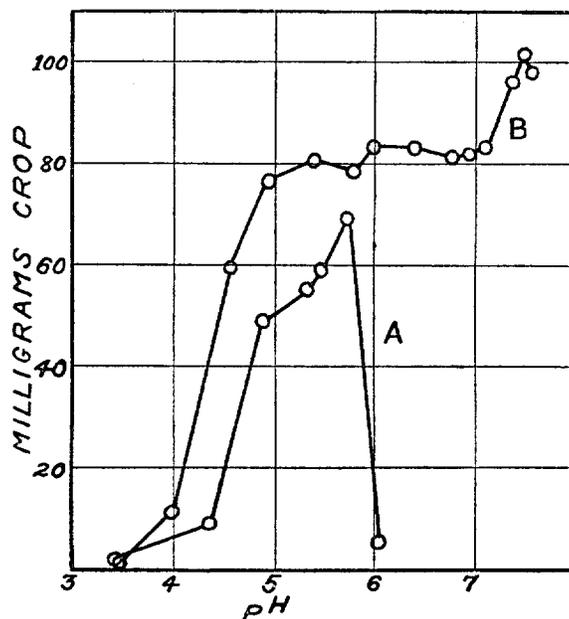


FIG. 1, A. Growth-pH curve with normal culture solution from which the iron was precipitated at the higher pH values.

B. Growth-pH curve with culture solution in which the calcium of the normal culture solution was replaced by ammonium and in which the iron was held in solution in the more alkaline cultures by the addition of sodium citrate.

20 gm. glucose per liter. The buffer mixtures (B) were prepared from $M/7.5$ solutions of KH_2PO_4 , K_2HPO_4 , and H_3PO_4 in such proportions as to give a range of H ion concentrations from pH 2.8 to pH 7.13. Four replications of each pH were prepared; one was used for the initial pH determinations and the remaining three in each case were inoculated. The H ion determinations were made electrometrically using a bubbling type of electrode, and were checked

by Gillespie's colorimetric method. The cultures were inoculated with $\frac{1}{2}$ cc. of a suspension of the algal cells in a balanced solution consisting of 95 cc. of 0.6 per cent NaCl and 5 cc. of 2.2 per cent CaCl_2 . The usual aseptic precautions were observed throughout the preparation of solutions and the inoculations.

2 weeks after inoculation determinations were made of the dry weight of the crop of each culture and of the final H ion concentration of each solution. The contents of individual culture flasks were centrifuged in large pyrex test-tubes, a portion of the clear supernatant liquid poured off for pH determination, and the algal cells then thrown on the asbestos mat of a Gooch crucible. The weight of the crop was ascertained after drying *in vacuo* at 80°C . for 18 hours. The results obtained in one of the experiments are presented graphically in Fig. 1, A.

The data for the final pH determinations showed that the H ion concentrations of the solutions remained very constant even where there was considerable growth of the alga. The average crop determinations, plotted in Fig. 1, A, show so far as this experiment is concerned that maximum growth appeared at pH 5.7. The acid limit may be placed at about pH 3.4 and the alkaline at about pH 6.1. Substantially the same results were obtained in two duplications of this experiment.

Precipitation of Salts in the Nutrient Solutions.

In the above experiments all the solutions on the acid side of pH 5.7 were perfectly clear, whereas considerable precipitate occurred on the alkaline side of this point. The precipitate consisted chiefly of magnesium and calcium phosphates, and it seemed possible that the iron, supplied as ferric sulphate, was also largely thrown down. Lack of growth in solutions more alkaline than pH 5.7 could not be interpreted, therefore, as due to a direct effect of the H ion concentration, but might be rather the result of the unavailability of some essential mineral element. To test this assumption a considerable quantity of the complete nutrient solution was prepared, using a buffer mixture which gave an initial H ion concentration of pH 6.8. The copious precipitate which formed was filtered off and the clear filtrate adjusted to pH 5.5 with concentrated HCl of low iron con-

tent. This adjusted solution, though apparently near the optimum pH, permitted only a slight growth when inoculated with *Chlorella*. The addition of ferric sulphate increased the growth three fold, and of magnesium sulphate two and a half fold, whereas growth was depressed by the addition of calcium chloride. In the unadjusted solution at pH 6.8 no growth of the organism was apparent. These results indicate that in solutions of pH 6.0 and above the precipitation of iron is a factor in the growth of *Chlorella*.

The Solubility of Ferric Iron in Buffered Culture Solutions.

In order to determine the extent of the precipitation of iron quantitative tests were performed on filtered buffer solutions, and at the same time an attempt was made to keep the iron in solution by means of salts of certain organic acids. For this purpose a phosphate buffer mixture having a pH value of 7.0 was prepared and iron was added to this as ferric sulphate. To various portions of this solution sodium citrate, sodium tartrate, and sodium potassium tartrate were added. At the time of mixing all these solutions were turbid except the ones to which sodium citrate had been added. After standing overnight the solutions were filtered and the clear filtrates tested for iron by the procedure of Marriott and Wolf.¹ This method, slightly modified by the writers, was found to be highly satisfactory for the determination of such small amounts as 0.0005 mg. ferric iron. The tests showed that about $\frac{1}{100}$ of the iron was left in the phosphate buffer solution to which no organic salt was added, whereas in the solutions containing sodium citrate all the iron remained in solution. The two tartrate salts were not effective in holding the iron in solution. Additional tests showed that as little as 0.005 gm. of sodium citrate in 50 cc. of buffer solution was sufficient to hold all the iron in solution at pH 7.0, and further that the presence of magnesium sulphate did not cause its precipitation. When, however, an attempt was made to keep the iron in an available form in the complete nutrient solution by the addition of sodium citrate it was found that in all solutions of pH 6.0 and above, this was not effective. The loss of the iron in

¹ Marriott, W. McK., and Wolf, C. G. L., The determination of small quantities of iron, *J. Biol. Chem.*, 1905-06, i, 451.

these more alkaline solutions may be accounted for by the formation of an amorphous precipitate of calcium phosphate on which the iron was apparently adsorbed. It was evident therefore that with a considerable amount of calcium present it would be impossible to hold the iron in a form available for growth in solutions of pH 6.0 and above.

Effect of the H Ion Concentration on the Growth of Chlorella in Solutions Lacking Calcium.

As it has been shown by Schramm² that very little if any calcium is necessary for the growth of *Chlorella*, a series of solutions of differing pH was prepared from which calcium was entirely omitted. The same procedure was followed as in the first experiments recorded in this paper. Solution A in this case, however, consisted of 1.0 gm. NH_4NO_3 , 0.4 gm. MgSO_4 , 0.4 gm. sodium citrate, 0.8 mg. FeCl_3 , and 20 gm. glucose per liter. The ferric chloride was added from a standard solution of iron wire in hydrochloric acid. The usual buffer mixtures were designed to give a range of pH from 3.5 to 7.6. The completed solutions were all clear, and with sodium citrate present the iron was neither precipitated chemically nor removed by adsorption. Initial iron tests showed as much soluble ferric iron in the most alkaline solution (pH 7.5) as in the most acid one (pH 3.5). Six replications of each solution were prepared, five of which were inoculated, and the sixth used for the initial pH and iron determinations.

Crop determinations made at the end of 2 weeks showed the usual rapid increase in the amount of growth from pH 3.5 to pH 5.4. Between pH 5.4 and 7.15 the rate of growth was approximately the same in all cultures. This was followed by a second increase in the dry weight of the crops at pH 7.4 and 7.5. The results are shown graphically in Fig. 1, B.

The final H ion concentration determinations showed in nearly all cases that there was a slight increase in the acidity of the solutions. Estimations of the residual ammoniacal and nitrate nitrogen

² Schramm, J. R., A rapid and delicate method involving pure cultures of algæ for mineral nutrition studies. Paper presented before the Botanical Society of America, Boston Meeting, Dec. 29, 1922.

in the solutions demonstrated that this change in reaction was due to a differential absorption of the ions of ammonium nitrate.

CONCLUSIONS.

The data obtained in these experiments indicate clearly that unless the necessary precautions are taken to keep the iron of the culture medium in solution the results obtained by varying the H ion concentration will not represent the true effect of this factor on growth. The availability of iron in nutrient solutions has been the subject of numerous recent investigations and it is now known that iron is precipitated at the lower hydrogen ion concentrations, that the iron of certain iron salts is less likely to be precipitated than that of others, and that certain salts of organic acids tend to keep the iron in solution. In general, ferric citrate seems to be the most favorable source of iron. In addition to chemical precipitation, however, it is also possible for the iron to be removed by adsorption on an amorphous precipitate such as calcium phosphate. As this precipitate is frequently formed when nutrient solutions are made alkaline, this may account for the discordant results reported in the literature as to the availability of certain forms of iron. By omitting calcium from the culture solution iron can be maintained in a form available for growth in alkaline solutions by the addition of sodium citrate. In such solutions the maximum growth of *Chlorella* occurred at pH 7.5. The alkaline limit for growth has not been established as yet.

In investigating the availability of iron at varying concentrations of the hydrogen ion, changes in the pH value of the solution during the course of an experiment should also be taken into account. This is especially important in unbuffered solutions. The differential absorption of the ions of ammonium salts may cause a marked increase in the hydrogen ion concentration, which in turn will cause an increase in the solubility of iron. In strongly buffered solutions as used in these experiments this effect is slight.