JOUR. FAC. SCI., SHNSHU UNIV., vol. 8, pp. 135—145, December, 1973

Studies on the blooming of Microcystis aeruginosa. II. Rapid accumulation of phosphate by Microcystis aeruginosa.

TOKIO OKINO

Suwa Hydrobiological Station, Faculty of Science,
Shinshu University.

Synopsis

The rapid accumulation of phosphate by *Microcystis aeruginosa* was studied. Gelatinous matter in which the cells were embeded could be dissolved with dilute HCl solution. Thus obtained extract contained a large quantity of phosphate. In the enrichment experiment of pond water with phosphate, *M. aeruginosa* accumulated phosphate linearly with time in the first 30 to 60 minutes and then reached the maximum. The rate of accumulation was also studied by means of P^{32} tracer technique. The accumulation rate was dependent on temperature, the optimum at about 30C.

Colonies of *M. aeruginosa* could be divided into the supernatant and the precipitating parts by centrifugation. Both of the rate and the maximum amount of phosphate accumulation were higher in the former than the latter, *i. e.*, three times in the rate and six times in the amounts. The vigorous growth of *Microcystis* seems to be caused by the rapid accumulation of phosphate into the algal colonies. It was suggested by a series of experiments by P^{32} that the gelatinous matter surrounding the cells should play an important role in the accumulation of phosphate.

Introduction

There have been many studies on the effects of nutrients on the growth of algae under the natural and experimental conditions. GERLOFF and SKOOG (1952, 1954, 1957a, 1957b) undertook a series of culture experiments on the minimum nutrient requirements by using a blue-green alga, *Microcystis aeruginosa*. They found that the nutrients content in the culture medium which induces the maximum growth does not always coincide with that which brings about the maximum content of them in algal cells. These things may suggest that this alga tends to

* Contributions from the Suwa Hydrobiological Station, Shinshu University, No. 12

accumulation some nutrients in excess in cells, when sufficient nutrients, especially phosphate and nitrate, are available.

Concerning the accumulation of phosphate, ROREM (1955) reported that the polysaccharide of bacterial cell wall absorbed rubidium and phosphate well. WHITTON (1967) observed rapid absorption of phosphate by *Nostoc*. Recently, SHAPIRO and BARETT (1968) reported that phosphate was absorbed rapidly by *M. aeruginosa*. These phenomena may be important concerning the relation of growth of algae to the utilization of nutrients. If the production of organic matter by algal population is limited by the amount of nutrients, the growth of each species population in mixed algal community may be strongly influenced by the species specific modes of utilization of nutrients. In natural waters, it is common that the amounts of nutrients are too small to give rise to the maximum growth of the algae. From this point of view, it may be important to study the mode of life of each alga in natural community. Regarding to this problem, the author has studied the rapid accumulation of phosphate by cells of *Microcystis aeruginosa*.

Material and methods

The blue-green alga, *M. aeruginosa*, which grew in a pond ($9 \times 9 m^2$, 0.45m in deep) in the garden of the Research Institute for Natural Resources in Tokyo, was used for the experiments. It has been known that the alga often grows densely in eutrophic water in summer. As the cell of *M. aeruginosa* are aggregated by the gelatinous matter to form colonies, it is difficult to count the number of cells directly. The cells in the colony, however, can be dispersed by the treatment with 0.01-N HCl or by sonification. The treatment with HCl was used for the dissolution of the gelatinous matter.

The sonification in the band of 200 KC/sec for one minute was used for cell number counting. The time of treatment over one minute was not suitable because of the rising of the temperature in the samples. This method is inconvenient to apply to the mixed algal population, because of its difficulty to distinguish the dispersed cells of *M. aeruginosa* from other unicellular species with the similar form or size.

M. aeruginosa cells were collected by centrifugation of the pond water at 3,000 rpm for 15 minutes. Then the precipitated algal colonies were suspended in 0.01-N HCl solution of the same volume as initial. The treatment was carried out twice to obtain the algal cells free from gelatinous matter.

The phosphate amounts of pond water free from algal colonies and HCl extract of algal colonies were measured with the colorimetrical method. The amount of carbohydrate was determined by the anthrone method. The algal colonies which did not precipitate by centrifugation were separated by filtration

with glass fibre filter (Millipore Co. Ltd.).

The following experiments were carried out to obtain some information about the accumulation of phosphate in the gelatinous matter of colonies. The pond waters containing *M. aeruginosa* population and the algal free pond water were enriched with phosphate and incubated at about 20 C. Two liters of each sample were poured into a three liter beaker and stirred gently by a magnetic stirrer.

Pond water containing *M. aeruginosa* was preserved in the dark over night at room temperature (about 20 C), and then the centrifuged algal colonies were suspended in distilled water and centrifuged again to rinse the colonies. Thus obtained colonies were resuspended in the modified Chu's No. 10 medium containing P^{32} labelled phosphate. In this experiment, 1 ml of the concentrated algal suspension was added to 10 ml of the medium. The experiment of P^{32} uptake was carried out under the conditions of 400 lux, pH 7.5, and 25 ± 1 C except for the experiments on the dependence of accumulation rate of phosphate on temperature. The accumulation of phosphate into the colonies of *M. aeruginosa* was measured by means of determination of radioactivities of the residue obtained by filtration of the incubated culture solution. The radioactivity was counted by a GM-counter. The amount of chlorophyll was determined by the method of Parsons and STRICKLAND (1966) in which the pigment was extracted with 90 % acetone. The photosynthetic activity was measured with the determination of 0_2 evolution in the sealed bottles of about 100 ml volume at about 15,000 lux under which the maximum rate of the photosynthesis occurred.

Result

As shown in Table 1, the concentration of phosphate phosphorus was 0.018 ppm in the algal free pond water prepapred by filtration, and 0.027 ppm in centrifuged sample. The concentration of phosphate accumulated in the gelatinous matter corresponded to 0.070 PO_4-P ppm. When the pond water containing the algal colonies was acidified to 0.01-N with HCl solution, the concentration of phosphate released in the medium was 0.092 ppm. The cell density of *M. aeruginosa* population used in this experiment was 7.56×10^6/ml. as shown in Fig. 1, the amount of phosphate accumulated in the gelatinous matter of algal colonies increased simultaneously in contrast to the rapid elimination of phosphate from the medium. After phosphate was added to pond water containing *M. aeruginosa* colonies, the detailed observations were performed on the accumulation of phosphate labelled with P^{32}. The rapid accumulation of phosphate was clearly recognized as shown in Fig. 2. The radioactivities of the algal colony increased linearly during first ten minutes and reached the maximum after about 15 minutes. The maximum value of 10,820 cpm was measureed after about 30 minutes. This

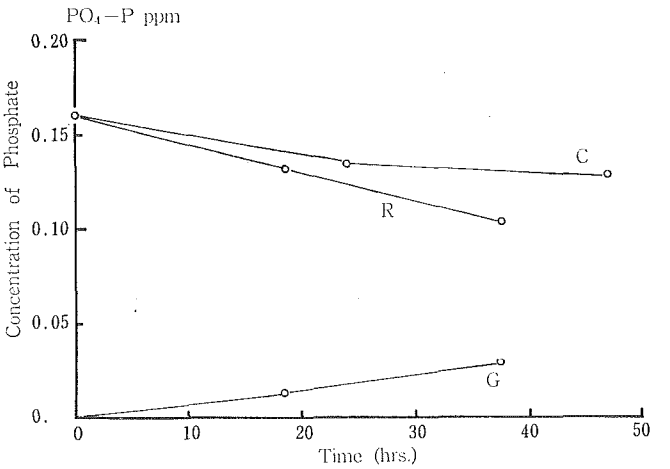

Figure 1. The accumulation of phosphate into the gelatinous matter of the colonies of *Microcystis aeruginosa*. The alphabets C, G, and R in figure indicate the concentration in the medium without algal colonies, the phosphorus content in the gelatinous matter and the concentration of phosphate remaining in the medium, respectively.

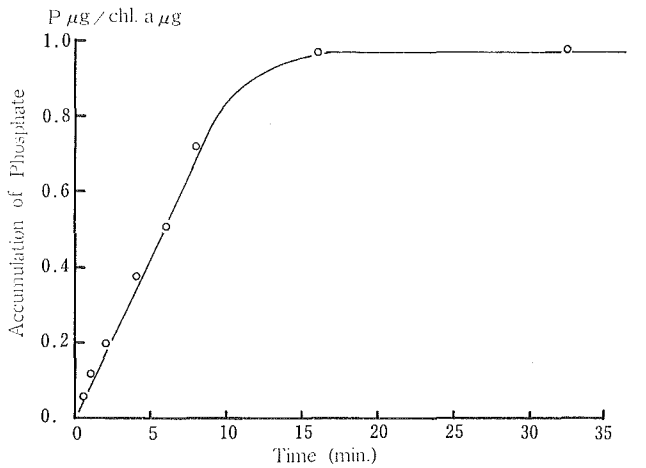

Figure 2. The rate of accumulation of phosphate into the colonies of *Microcystis aeruginosa*. The experiment was carried out under the condition of 142μg chl. a/L, 400 lux, pH 7.5 and 25 C.

value was corresponding to the amount of phosphorus accumulated in the colonies, being about 1.0 μg P/μg chl. a.

It is difficult to distinguish the phosphorus amount contained in the algal cells from that accumulated in the gelatinous matter of algal colony. The algal cells used in this experiment showed high photosynthetic activity, and the net produc-

Table 1. Phosphate phosphorus accumulated in the gelatinous matter of *Miorocystis* colony. The volume of diluted HCl solution to dissolve the gelatinous matter is equal to that used for pond water containing the algal colonies.

	Concentration of carbohydrate as glucose mg/L	PO_4–P mg/L
Filtrate of pond water. Algal colonies were removed by filtration with glass fibre filter.	6.5	0.018
Supernatant of pond water. Algal colonies were removed by centrifugation.	7.0	0.027
HCl extract, free from algal cells and contained dissolved gelatinous matter.	24.0	0.092

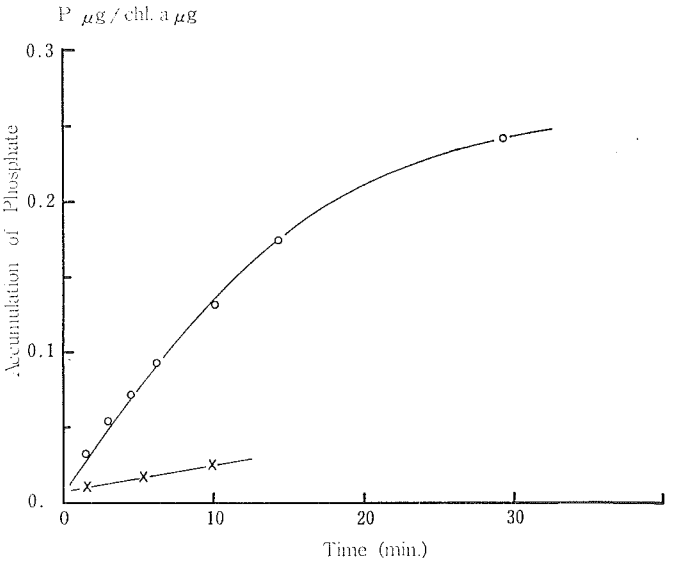

Figure 3. The comparison between the accumulation rate of phosphate of the starved colony and the colony with sufficient supply of it.
o———o ; starved colony, x———x ; sufficient colony.

tion was 31.0 mg 0_2/mg chl. a/hr. at 19C. After the maximum accumulation of phosphate was attained, the algal colonies were incubated again in the medium containing P^{32}. The rate of the phosphate accumulation into the algal colonies has, however, decreased remarkably as compared with that into starved ones (Fig. 3). The relation between the accumulation rate and temperature was studied with two series of experiments. The results obtained were shown as the relative

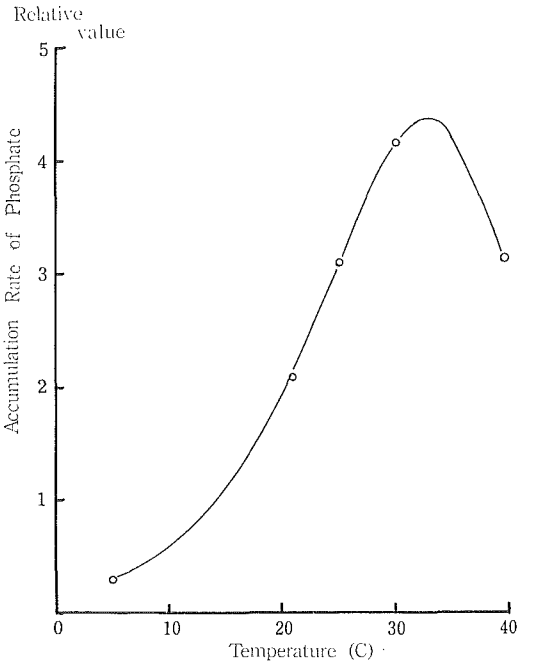

Figure 4. The relation of relative rate of the accumulation of phosphate into the algal colonies to the temperature.

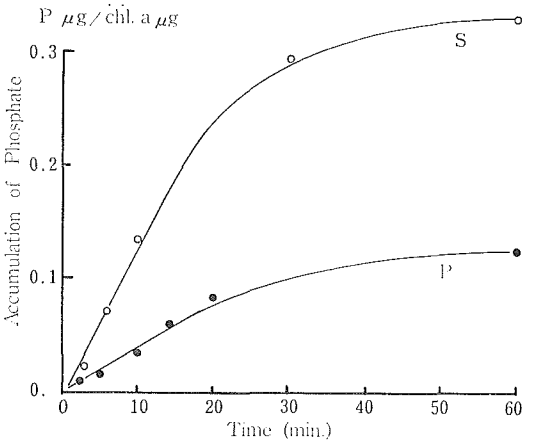

Figure 5. Relation of accumulation rates of phosphate of the supernatant to the precipitating parts of the colonies of *Microcystis aeruginosa*. S ; supernatant part, P ; precipitating part.

values, as the phosphate concentrations in the medium and chlorophyll contents of used algal population were different in respective series of experiments. The rate increased with the raising of temperature, and reached the maximum at

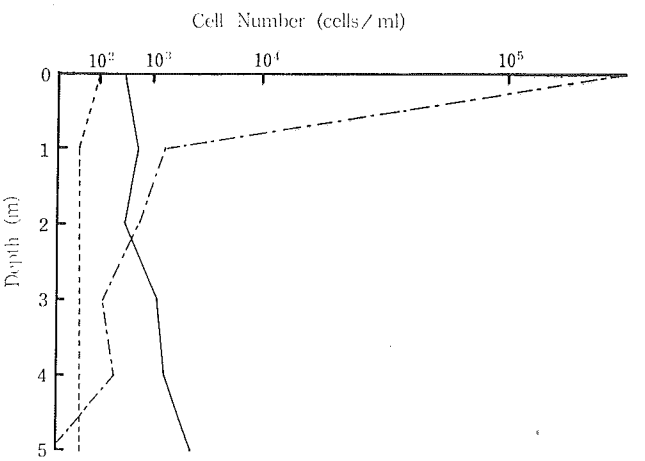

Figure 6. The vertical distribution of the number of algae at 11:00 a.m. in August 1, 1967 in Lake Suwa.
(—•—•— ; *Microcystis aeruginosa*, ———— ; Bacillariophyceae, ... ; Chlorophyceae).

about 30C and then decreased rapidly (Fig. 4).

It has been known that colonies of *M. aeruginosa* are often divided into two parts by centrifugation, namely one in supernatant and the other in precipitating part. The characters of both parts seemed to be different in the appearance and the physiological states. The content of nitrogen of the algal cells in supernatant part was higher than that in the precipitates, namely the former was 8.67% and the latter 7.53% on dry weight basis. The photosynthetic activities of both parts differed remarkably, and the former showed far higher activity, *i. e.*, 5.05 mg O_2/mg chl. a/hr. as compared with 0.60 mg O_2/mg chl. a/hr of the latter. The accumulation rates of phosphate by both parts were compared. The rate in the former was six times as high as that in the latter (Fig. 5).

Discussion

In most of eutrophic lakes and ponds, *M. aeruginosa* increases very vigorously and brings about a large standing crop in summer season, which becomes frequently the cause of sudden summer death of fishes or so called "Susumizu" (Iwai 1960, Yamagishi and Okino 1967). This alga has a character to gather together on the surface layer and often to form a thick film at the surface. According to our measurement carried out at 11:00 a.m. in August 1st, 1967 in Lake Suwa, the cell number in the surface water was 2×10^5 cells/ml being 200 times as much as that at one meter depth (Fig. 6).

Although such a high population of algal cells at the water surface seems to

be unfavorable for sufficient supply of nutrients to each cell, the extensive bloom of this alga has often been observed in eutrophic waters as presented above. The concentration of available nutrients in lake water is, however, usually very low during heavy blooming of this alga. In Lake Suwa, the concentration of phosphate in surface water was 0.006—0.036 PO_4-P ppm during the blooming period. Contrary to this, the phosphorus content of this algal colonies was 0.36%. This value corresponds nearly to the maximum value reported by GERLOFF et al. (1954). According to their results, the phosphorus content of this algal colonies reached the maximum of 0.4%, when the initial concentration of phosphate phosphorus in the medium was higher than 0.8 ppm.

Moreover, in their culture experiments, the initial concentration of phosphate phosphorus which induced the maximum yield of the algal colonies was 0.4 ppm. This value did not agree with the value which brought about the maximum content of phosphorus of algal colonies. Taking these results into consideration, it may be suggested that this alga accumulates phosphate in excess by some processes. In the studies on *Nostoc*, WHITTON (1967) found that phosphate was absorbed from the medium by some nonactive means and the accumulation rate of phosphate was reduced by pre-treating the colonies with chelating agents.

On the other hand, there are some reports concerning the effects of physiological conditions of the organisms upon the accumulation rate of phosphate (CUSHING and WATSON 1968, GUTKNECHT 1961, 1965, SHAPIRO 1967). As seen in the present study, *M. aeruginosa* accumulated phosphate rapidly and the accumulation rate was about 0.08 μg P/μg chl. a/min. at 25C. The rate may be affected by the physiological conditions at the experiment. If there are some differences in the physiological conditions between the alga in supernatant and precipitating parts, in which the nitrogen content differs from each other, the physiological differences between both parts might also bring about different accumulation rates of phosphate. As a matter of fact, the supernatant part showed higher rate than another (Fig. 4). The rate was about 3.5 times higher and the concentration of phosphorus accumulated into the algal colonies was 3 times higher in the supernatant part than those of precipitating part.

According to our observation in some lakes and ponds, *M. aeruginosa* showed the tendency to rise to the surface early in the morning, and to scatter down toward the evening because of increase of the specific gravity of algal colony. Such daily rhythm in the vertical distribution of this alga is considered to be favorable for the uptake of nutrients as well as the production of organic matter by photosynthesis. Because the algal colonies follen down to deeper layer may have the chance to obtain the phosphate released by excretion by animals and bacterial decomposition. In blooming season, the algal density is usually lower,

and the amount of available phosphate may be relatively large in the deeper layer as compared with those in the surface layer. When the algal colonies rise to the surface due to the decrease in the specific gravity caused by consumption of the stored photosynthetic product, the high activity of phosphate accumulation recognized in the supernatant part may be favorable for the uptake of phosphate. As the photosynthetic activity of the alga is high in the morning and decreases in the afternoon in clear day, the vertical movement of this alga brings about no serious effect reducing the daily photosynthetic production.

The accumulation rate of phosphate measured by use of P^{32} seems to depend on water temperature, and the optimum was attained at about 30 C. According to the previous studies the appearance of bloom of *M. aeruginosa* is limited in warm season. In Lake Suwa, this alga begins to grow remarkably at the surface water temperature of over 20C, and to decrease in autumn when the thermocline is broken down. The highest standing crop of this alga was obtained early in August when the surface water temperature was 30 C. This fact seems to have some relations to the high accumulation rate of phosphate as mentioned before.

In the colonies of *M. aeruginosa*, the algal cells are embedded in gelatinous matter of carbohydrate nature. When the accumulation rate of phosphate of this alga is constant, the increase with time of phosphate absorbed into the colony mostly depends on accumulation in the gelatinous matter. As shown in Table 1, the concentration of phosphate in the solution increased simply by centrifugation. It seems to suggest that some part of phosphate in gelatinous matter is able to be released easily by centrifugation from the algal colony. According to SHAPIRO (1967), activated sludge flocs composed of bacteria, protozoa, and others were able to release or uptake actively a large amount of phosphorus according to environmental conditions, and the phosphorus was detectable mostly in the acid-extract fraction. The rapid accumulation of phosphate by the algal colonies may be explained in terms of rapid absorption of gelatinous matter, neverthless this was not solved clearly in the present study.

The rapid absorption of phosphate from the medium may be advantageous nature of this alga for its rapid growth. It is assumed that the phosphate accumulated temporarily in the gelatinous matter will be transported into the algal cells, and used for the growth. As a rule, the concentration of nutrients, particularly that of phosphate, is always extremely low in natural water. This suggests that the phosphate released by the decomposition of organic materials is absorbed immediately by the other organisms. Under such conditions as phosphate being insufficient, the growth of each species may be influenced by its accumulation rate of phosphate.

In the present study, the accumulation of phosphate was investigated only

with *Microcystis*, but in future those of other many species should be investigated from the same point of view.

Acknowledgement

The author would like to express his appreciation to Prof. K. HOGETSU, Dr. Y. KITAZAWA and Dr. Y. TEZUKA of Tokyo Metropolitan University for their useful suggestions and a critical reading of the manuscript, and to Prof. H. KURASAWA of Shinshu University for offering him valuable field data. He also wishes to thank to Dr. A. OTSUKI of Tokyo Dental College and to the members of ecological laboratory of Tokyo Metropolitan University for their useful discussions.

Reference

1. CUSHING C. E. and D. G. WATSON (1968) Accumulation of ^{32}P and ^{65}Zn by living and killed plankton. *Oikos*, **19**, 143–145.
2. GERLOFF G. C., G. P. FITZGERALD and F. SKOOG (1952) The mineral nutrition of *Microcystis aeruginosa*. *Amer. J. Bot.*, **39**, 26–32.
3. GERLOFF G. C. and F. SKOOG (1954) Cell contents of nitrogen and phosphorus as a measure of their availavility for growth of *Microcystis aeruginosa*. *Ecology*, **35**, 551–556.
4. GERLOFF G. C. and F. SKOOG (1957) Nitrogen as a limiting factor for the growth of *Microcystis aeruginosa* in southern Wisconsin Lakes. *Ecology*, **38**, 556–561.
5. GUTKNECHT J. (1961) Mechanism of radioactive zinc uptake by *Ulva lactica*. Limnol. *Oceanogr.*, **6**, 426–431.
6. GUTKNECHT J. (1965) Uptake and retension of cesium 137 and zinc 65 by sea weeds. *Limnol. Oceanogr.*, **10**, 58–66.
7. HAUG A. and O. SMIDSRD (1967) Strontium, calcium and magnecium in broun algae. *Nature*, **215**, 1167–1168.
8. LEVIN G. V. and J. SHAPIRO (1965) Metabolic uptake of phosphorus by waste water organisms. *J. Wat. Pollut. Cont. Fed.*, **37**, 800–821.
9. OKINO T. (1967) The diurnal changes of cell numbers and activity of *Microcystis* in the surface water of fish pond. *Miscel. Rep. Res. Inst. Nat. Res.*, **70**, 11–15, (in Japanese).
10. OKINO T. (1973) Studies on the blooming of *Microcystis aeruginosa*. I. Characteristics of the water bloom of *Microcystis*. *Jap. J. Bot.*, **20**, 381–402.
11. OKINO T. (1968) The diurnal variation of photosynthetic activity. *Miscel. Rep. Res. Inst. Nat. Res.*, **72**, 1–5.
12. ROREM E. S. (1955) Uptake of rubidium and phosphate ions by polysaccharide-producing bacteria. *J. Bact.*, **70**, 691–701.
13. SHAPIRO J. (1967) Induced rapid release and uptake of phosphate by microorganisms. *Science*, **155**, 1269–1271.
14. WHITTON B. A. (1965) Extracellular products of blue-green algae. *J. Gen. Microbiol.*, **40**, 1–11.

15. WHITTON B. A. (1967) Phosphate accumulation by colonies of *Nostoc*. *Plant Cell Physiol.*, 8, 293-296.