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Influence of Blue-Green Algae on the pH and Buffer Capacity of Culture Media

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Three strains of the blue-green algae *Anabaena cylindrica*, *Anacystis nidulans* and *Nostoc muscorum* are able to adjust the pH of culture medium from stress levels (pH 4-6) to levels favorable

for growth (pH 7-10). Over a period of one week, these strains increased the buffer capacity of their media with time. The pH of maximum buffer capacity after one week was very close to the pK_a of phosphoric acid. Algae grown in a medium initially buffered with Na_2CO_3 shifted the pH of maximum buffer capacity from the pK_a of carbonic acid to that of phosphoric acid.

INDEX DESCRIPTORS: Buffer Capacity, Blue-Green Algae, *Anabaena cylindrica*, *Anacystis nidulans*, *Nostoc muscorum*.

The ability of freshwater algae to produce and release organic substances into their aquatic environment is well documented (Fogg, 1962, 1966, 1971; Fogg and Westlake, 1953; Fogg *et al.*, 1965; Lefèvre, 1964; Lewin, 1956; Nalewajko, 1966; Nalewajko and Lean, 1972; Watt, 1966; Whitton, 1965). Physiological effects on other organisms ranging from stimulatory to toxic have been reported for these extracellular products, and investigators have attributed to them the competitive success of certain species and the mutually beneficial associations of others (Fogg *et al.*, 1973; Whitton, 1973).

Less is known about the relationships between freshwater algae and the pH of their environment or their potential for altering the pH by secreting metabolic products. Brock (1973) concluded from the literature and his own observations that blue-green algae are completely absent from natural and man-made habitats of pH less than 4, where eukaryotic algae may grow abundantly in numbers and in taxonomic diversity. When Shapiro (1973) subjected a mixed population of algae to high levels of carbon dioxide and nutrients under lake conditions, he found a dramatic change in the taxa of dominant species. Blue-green algae, which dominated the control community, were replaced by green algae. When the pH was adjusted to 5-6 with nutrients added, a similar shift occurred.

Sorokin (1964, 1965) and Sorokin and Mitrofanov (1966) described the ability of a high-temperature strain of the green alga *Chlorella* to change the pH of suspending fluid in the dark and to produce a buffer system whether or not the original medium was buffered. High-temperature strains of *Chlorella* and *Stichococcus* grown in the light were able to alter the pH and buffering characteristics of Tris or phosphate-buffered media (Sorokin, 1971).

During a study of extracellular products released by blue-green algae, we noticed that the initial pH of medium in which *Anabaena cylindrica*, *Anacystis nidulans* or *Nostoc muscorum* was grown was altered as much as two pH units, up or down, over a period of one week. Cultures initiated at low pH (4-6) adjusted the pH of the medium upward; those initiated at high pH (10-12) adjusted downward. Additionally, the buffer capacity of the medium was increased with time by the algae.

MATERIALS AND METHODS

Stock cultures of *Anabaena cylindrica* (Culture Collection of Algae at Indiana University, CCAIU B629) and *Anacystis nidulans* (CCAIU 625) were grown in Kratz-Myers C medium (Kratz and Myers, 1955) at 22° C under 1600 lux cool-white fluorescent and incandescent light on a 12-hr per day light cycle. *Nostoc muscorum* (Kaiser Research Foundation M-12.4.1) was grown under the same conditions in modified Chu 10 medium (Gerloff *et al.*, 1950) containing 40 ml of sterile soil extract per liter.

A series of 10 cultures of each alga was prepared by inoculating the respective sterile medium (100 ml per 250-ml cotton-plugged Erlenmeyer flask) with stock culture (15 ml) and immediately adjusting the pH with 0.1 N HCl or 0.1 N NaOH to give a range from pH 3 to 12. *Anabaena* was grown in both Kratz-Myers and Chu media. Illumination and temperature during incubation were the same as for stock cultures. Packed cell volumes (PCV) were measured every two days, and pH was measured daily with a Beckman Zeromatic II pH meter for one week.

Cultures of each alga in its respective medium without adjustment of initial pH were grown for one week under the same conditions. The cells were harvested by centrifugation and membrane filtration, and titration curves were determined for the supernatants. Titration curves also were obtained for uninoculated Chu and Kratz-Myers media and for 0.005 M Na_2CO_3 and 0.0057 M K_2HPO_4 in water.

Values for the buffer index, β , as a measure of buffer capacity were calculated by the method of Sorokin (1965) using Van Slyke's equation:

$$\beta = dB \text{ (or } dA) / dpH$$

where dB and dA are increments of base and acid added. Buffer capacity was measured at 0.1 to 0.2 pH intervals. β was expressed as the absolute value of the result. The midpoint of the two pH readings used to calculate each value of β was chosen as the corresponding pH value for purposes of plotting β vs pH. β values were expressed in milliequivalents of acid or base necessary to cause a pH shift of one unit in one liter of medium. Titration of Na_2CO_3 was done on 50 ml of 0.005 M Na_2CO_3 , and the β values were expressed as if the titration had been done on 0.0002 M Na_2CO_3 (the concentration in Chu medium). The titration of K_2HPO_4 was done on 50 ml of 0.0057 M K_2HPO_4 , which also is the concentration in Kratz-Myers medium.

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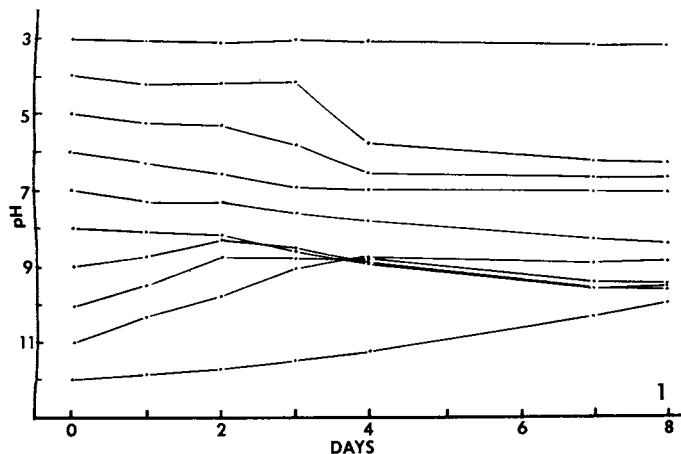


Figure 1. Changes in pH caused by *Anabaena cylindrica* in Kratz-Myers medium with different initial pH levels.

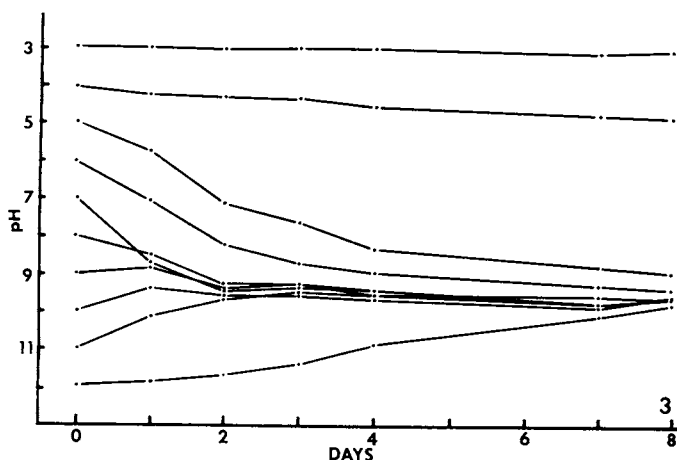


Figure 3. Changes in pH caused by *Nostoc muscorum* in modified Chu medium with different initial pH levels.

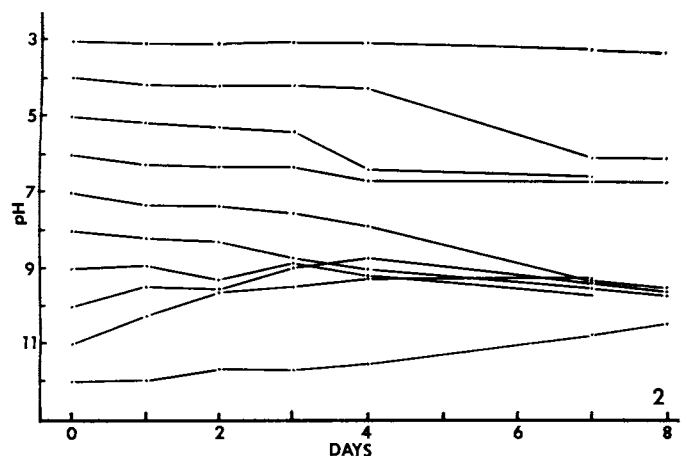


Figure 2. Changes in pH caused by *Anacystis nidulans* in Kratz-Myers medium with different initial pH levels.

RESULTS

The culture series of *Anabaena* in Kratz-Myers medium which had been adjusted to initial pH values of 3 to 12 showed a pattern similar to that for *Anacystis* in the same medium (Figures 1 and 2). At pH 3, no significant change in pH was effected over one week, cells appeared white and packed cell volumes indicated no growth. The media of both algae initiated at pH 4-6 changed with time over one week to pH 6.1-7.1. Cultures of *Anabaena* initiated at pH 7-11 changed with time over one week to pH 8.5-9.7. Cultures of *Anacystis* initiated at pH 7-11 changed over one week to pH 9.5-9.7. Cultures of *Anabaena*, *Anacystis* and *Nostoc* initiated at pH 12 adjusted more slowly to a lower pH (Figures 1-3). The cultures of *Nostoc* in Chu medium initiated at pH 3 and 4 failed to grow or to significantly change the pH. *Nostoc* cultures initiated at pH 5-11 altered the pH of the medium significantly within one day and reached pH 8.9-9.8 after one week (Figure 3). Growth (as measured by PCV) of *Anabaena*, *Anacystis* and *Nostoc* was greatest in cultures initiated at pH 8-11.

When buffer indices (β) were calculated according to

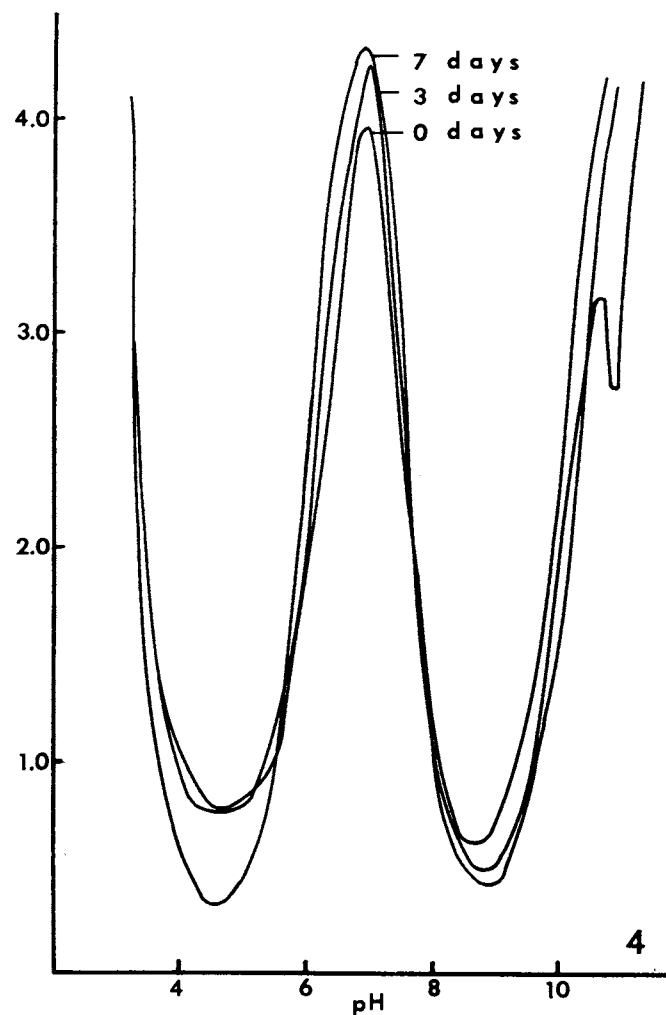


Figure 4. Buffer index (β) plotted against pH for supernatants from 0-, 3- and 7-day cultures of *Anacystis nidulans* in Kratz-Myers medium.

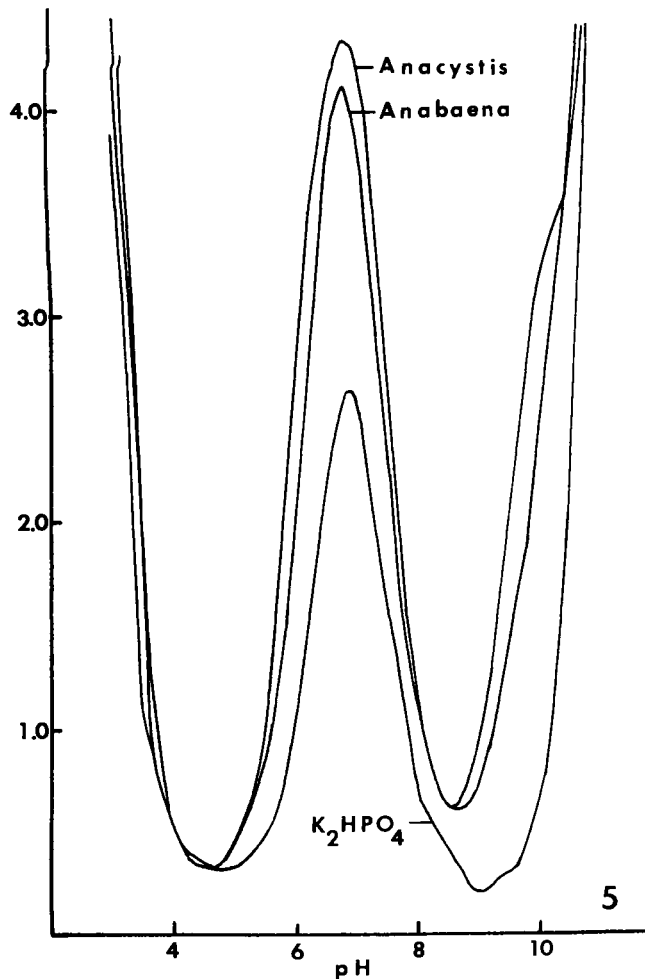


Figure 5. Buffer index (β) plotted against pH for 0.0057 M K_2HPO_4 and supernatants from 7-day cultures of *Anabaena cylindrica* and *Anacystis nidulans* grown in Kratz-Myers medium.

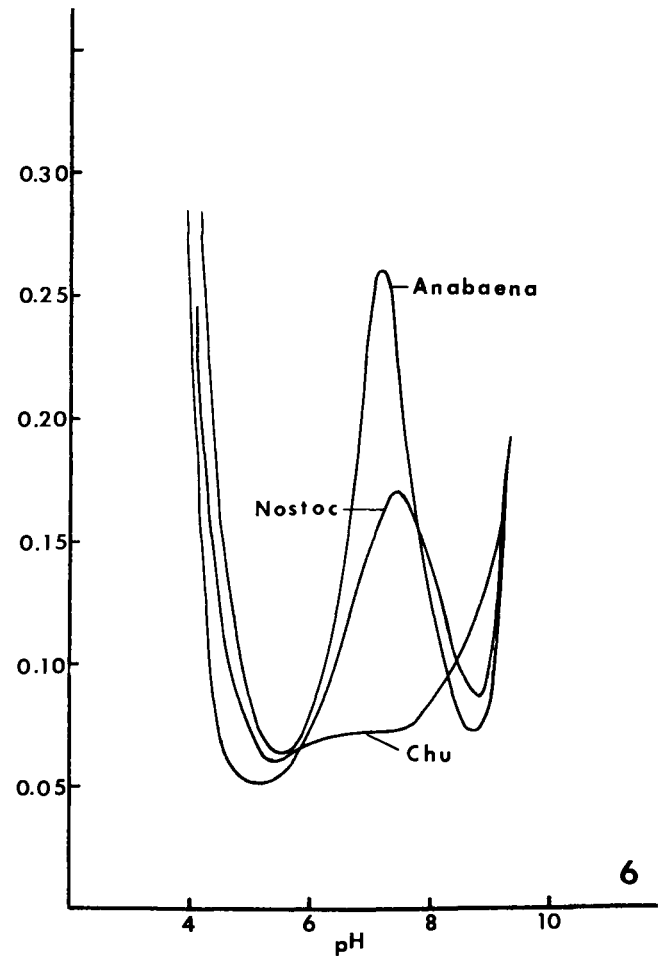


Figure 6. Buffer index (β) plotted against pH for uninoculated Chu medium and supernatants from 7-day cultures of *Anabaena cylindrica* and *Nostoc muscorum* grown in Chu medium.

Sorokin (1965) and plotted against pH for filtered supernatants from 0-, 3- and 7-day cultures of *Anacystis* in Kratz-Myers medium, a time-related increase in buffer capacity in the pH range of 6-8 was observed (Figure 4). The buffer capacity at day 0 may be equated to the initial buffer capacity of the medium since the algae were harvested, and the supernatant titrated, immediately after inoculation. When β is plotted against pH for the supernatants from 7-day cultures of *Anacystis* and *Anabaena* in Kratz-Myers medium and for 0.0057 M K_2HPO_4 (the concentration of K_2HPO_4 in the original medium), buffer capacity is maximum at approximately the same pH for all three (Figure 5). Figure 6 shows β plotted against pH for uninoculated Chu medium and for supernatants from 7-day cultures of *Anabaena* and *Nostoc* grown in Chu medium. In Figure 7, β is plotted against pH for the same supernatants and for 0.0002 M Na_2CO_3 (the concentration of Na_2CO_3 in the original medium).

Kratz-Myers medium initially contains no added carbonate but includes 0.0057 M K_2HPO_4 and 0.00057 M sodium citrate. The modified Chu medium contains 0.0002 M Na_2CO_3 , 0.000057 M K_2HPO_4 , 0.000015 M citric acid and 0.000012 M sodium citrate. The buffer capacity of Kratz-Myers me-

diu is considerably greater than that of Chu medium (Figures 4 vs. 6). Maximum buffer capacity for Kratz-Myers medium occurs at pH 7.1, which is very close to the pK_2 for phosphoric acid (7.2). The pH of maximum buffer capacity in Chu medium is shifted by *Anabaena* to pH 7.1, and by *Nostoc* to pH 7.3. Neither maximum coincides with the pK_1 for carbonic acid (6.4), although the molar concentration of carbonate added to the initial medium was 4-10 times greater than that of the other two buffer components, phosphate and citrate, in this dilute medium.

DISCUSSION

Failure of *Anabaena*, *Anacystis* and *Nostoc* to grow in media below pH 4 is consistent with Brock's (1973) report that blue-green algae cannot tolerate pH levels below 4. He cited the erroneous classification of eukaryotic *Cyanidium caldarium* as a blue-green species that had been found in acid waters in Japan. However, Lind and Campbell (1970) reported low species diversity for an acid strip-mine lake (pH 3.2-4.1) but identified the blue-green alga *Aphanizomenon*

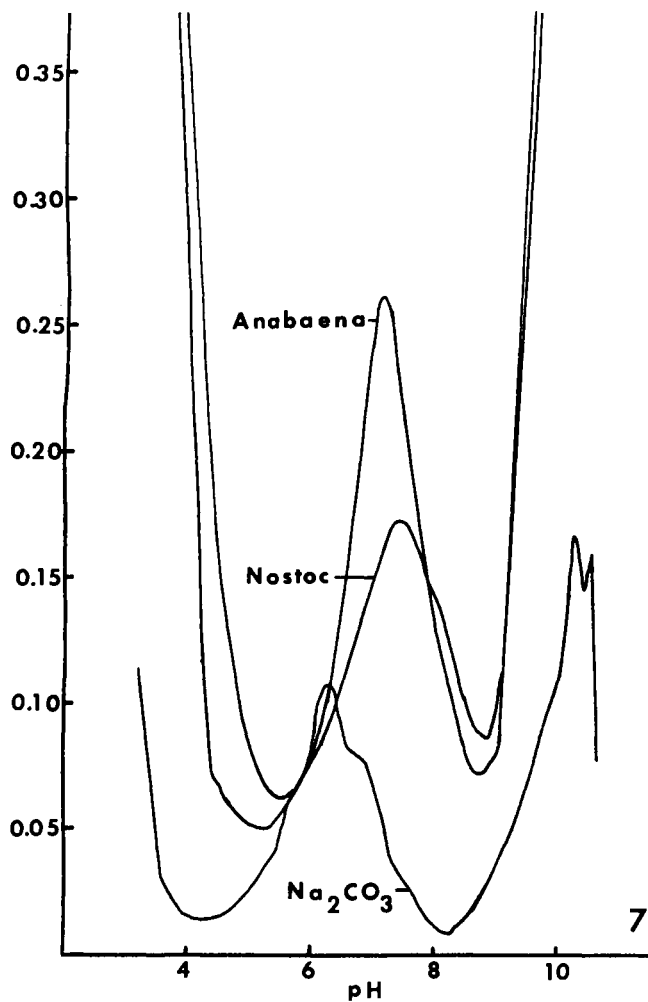


Figure 7. Buffer index (β) plotted against pH for 0.0002 M Na_2CO_3 and supernatants from 7-day cultures of *Anabaena cylindrica* and *Nostoc muscorum* grown in Chu medium.

flos-aquae as one of the three most common species among the 17 taxa of phytoplankton.

The ability of our three strains to alter the pH of culture media provides evidence that blue-green algae as well as the green algae studied by Sorokin (1964, 1965, 1971) and Sorokin and Mitrofanov (1966) possess this property. When Sorokin (1971) chose as experimental organisms the high-temperature strains of the green algae *Chlorella* (7-11-05) and *Stichococcus* (6-17-35), he grew them at 40° C to take advantage of the correspondingly high metabolic rates. He used a nutrient medium with higher levels of phosphate (0.01 M) and other inorganic nutrients than in our study, and 5% CO_2 -in-air was bubbled through the medium. Our strains of blue-greens were grown at 22° C in Kratz-Myers medium initially buffered by 0.0057 M K_2HPO_4 and 0.00057 M sodium citrate, and in Chu medium initially buffered by 0.0002 M Na_2CO_3 , 0.000015 M citric acid, 0.000012 M sodium citrate and 0.000057 M K_2HPO_4 , without benefit of agitation or air bubbled through the medium. Despite these disadvantages, all three strains were able to adjust the pH of their media from stress levels to favorable levels, a property that

could provide an important advantage in natural aquatic environments. Growth as monitored by packed cell volume was maximum in cultures initiated at favorable pH levels (pH 7-10), however.

Sorokin (1965) identified bicarbonate as the ingredient responsible for buffer capacity produced by *Chlorella* cells which he maintained 44 hr in the dark with CO_2 -free air bubbled through the suspending fluid. He attributed bicarbonate formation to CO_2 provided by cellular respiration. When Sorokin and Mitrofanov (1966) repeated this experiment using medium initially buffered with Tris or citric acid-sodium citrate, the algal cells altered the buffering characteristics of the suspending fluid. Buffer capacity increased with time and was maximum at pH 6.4. In citrate buffer, the pH at which the suspending fluid had maximum buffer capacity shifted from the pK values of citric acid to the pK₁ of carbonic acid, 6.4. When buffer index (β) was plotted against pH for bicarbonate and for the supernatants of his 44-hr cultures of *Chlorella*, the peaks coincided in pH level. Sorokin (1971) found that high-temperature strains of *Chlorella* and *Stichococcus* grown under illumination in nutrient medium buffered with Tris or phosphate increased the buffer capacity of the medium and shifted the pH of maximum buffer capacity from that in the initial medium toward the pK of the buffer system produced by the algae.

The information in Figures 4-7 indicates that the increased buffer capacity generated by the blue-green strains in our study probably is not due to bicarbonate since buffer capacity is not maximum at pH 6.4 (pK₁ for carbonic acid). Carbonate is not an ingredient in Kratz-Myers medium, and the concentration of Na_2CO_3 in Chu medium is only 0.0002 M. All CO_2 for photosynthesis must be obtained from the atmosphere, an exchange that is slow at best (Kuentzel, 1969).

A mutualistic symbiosis between bacteria and blue-green algae has been credited with promoting enhanced population growth of the latter (Boyd, 1972; King, 1970; Kuentzel, 1969, 1970; Lange, 1967, 1970; Lingg, 1973). According to this concept, organic matter produced by algae supports growth of bacteria which, in turn, evolve CO_2 during respiration. The CO_2 is essential to blue-green algal photosynthesis, and increased levels would be expected to stimulate "bloom" level populations in eutrophic waters where atmospheric CO_2 has become the limiting factor. However, this symbiotic relationship can be discounted as a source of CO_2 in Sorokin's experiments since he reported using axenic cultures. In our study, the *Anacystis* and *Nostoc* strains were axenic.

Kratz-Myers medium has considerably greater buffer capacity than does Chu medium because of its higher phosphate concentration (Figures 4 and 5). In Kratz-Myers medium, *Anacystis* increased the buffer capacity in the pH range of 6-8 with time (Figure 4) but did not shift the pH of maximum buffer action, which corresponds to the pH of maximum buffer capacity for phosphate (Figure 5). The *Anabaena* cultures also increased the buffer capacity of Kratz-Myers medium without shifting the pH of highest buffer index (β) (Figure 5). This suggests that phosphate or some other acid with a similar pK is released during growth of these species. In Chu medium, where the slight initial buffer action is due primarily to carbonate, cultures of *Anabaena* and *Nostoc* increased the buffer capacity of the suspending fluid in the pH range corresponding to the pK₂ of phosphoric acid, 7.2. Blue-green algal cells are capable of storing excess

phosphorus in polyphosphate bodies and of using this accumulation as a source of phosphorus when exogenous phosphorus is limiting (Stewart and Alexander, 1971). Kuenzler (1970) has reported excretion of extracellular organic phosphorus compounds by planktonic algae and reassimilation of this source of phosphorus by the species that produced it and by other species. Batterton and van Baalen (1968) found that *Anacystis nidulans* cells could continue to divide slowly when the phosphorus content of the cells was reduced to 10-15% of the normal level. Fitzgerald and Nelson (1966) have shown that production of the enzyme alkaline phosphatase is induced in planktonic algae when they are deprived of orthophosphate.

The cells of the blue-green strains in our study appeared to be excreting phosphate, including stored phosphate, during the one-week duration of the experiments. This could account for the increase in buffer capacity with a maximum β very close to the pK_2 of phosphoric acid. Further studies will be required to determine whether phosphate is released from blue-green algal cells to account for the buffer system and, if so, what minimum level of internal concentration can be tolerated.

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