APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1983, p. 252–259 Vol. 46, No. 1
0099-2240/83/070252-08$02.00/0

Partitioning of CO_2 Fixation in the Colonial Cyanobacterium *Microcystis aeruginosa*: Mechanism Promoting Formation of Surface Scums

HANS W. PAERL

Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina 28557

Received 2 February 1983/Accepted 19 April 1983

Constraints on inorganic carbon (C_i) availability stimulated buoyancy in natural, photosynthetically active populations of the colonial blue-green alga (cyanobacterium) *Microcystis aeruginosa*. In nonmixed eutrophic river water and cultures, O_2 evolution determinations indicated C_i limitation of photosynthesis, which was overcome either by CO_2 additions to the aqueous phase or by exposure of buoyant colonies to atmospheric CO_2. Microautoradiographs of *M. aeruginosa* colonies revealed partitioning of $^{14}CO_2$ fixation and photosynthate accumulation between peripheral and internal cells, particularly in large colonies. When illuminated colonies were suspended in the aqueous phase, peripheral cells accounted for at least 90% of the $^{14}CO_2$ assimilation, whereas internal cells remained unlabeled. However, when $^{14}CO_2$ was allowed to diffuse into colonies 15 min before illumination, a more uniform distribution of labeling was observed. Resultant differences in labeling patterns were most likely due to peripheral cells more exclusively utilizing CO_2 when ambient C_i concentrations were low. Among colonies located at the air-water interface, internal cells showed an increased share of photosynthate production when atmospheric $^{14}CO_2$ was supplied. This indicated that C_i transport was restricted in large colonies below the water surface, forcing internal cells to maintain a high degree of buoyancy, thus promoting the formation of surface scums. At the surface, C_i restrictions were alleviated. Accordingly, scum formation appears to have an ecological function, allowing cyanobacteria access to atmospheric CO_2 when the C_i concentration is growth limiting in the water column.

Recent independent studies have critically examined the role that inorganic carbon (C_i) availability plays in promoting aquatic blue-green algal (cyanobacterial) blooms (5, 7, 12, 13). Blooms can lead to the formation of surface scums, which are of environmental concern, because a rapid deterioration in water quality often results. Walsby (15) and Reynolds and Walsby (12) attributed scumming to excess buoyancy brought on by intracellular gas vacuole formation. The major freshwater scum-forming genera *Anabaena, Aphanizomenon, Microcystis*, and marine *Oscillatoria* (*Trichodesmium*) all exhibit gas vacuolation. Buoyancy regulation has been linked to cellular turgor pressure; when turgor pressure is high, gas vacuolation, and thus buoyancy, is low, whereas low turgor pressure promotes gas vacuolation (12). Cellular turgor pressure is controlled by the production of osmotically active substances, the foremost of which are recently produced products of photosynthesis (photosynthate). Hence, when photosynthate pools are depleted, gas vacuolation commonly increases in these genera (4, 16). Control of photosynthesis is, therefore, a potential mechanism for explaining buoyancy regulation and resultant scum formation. The three most commonly observed environmental constraints on photosynthesis are: (i) photosynthetically active radiation (PAR) availability, (ii) nutrient (most often nitrogen and phosphorus) availability, and (iii) access to a source of CO_2. In focusing on the relationship (in either time or space) of these factors to buoyancy, some relevant information exists. Previous studies have shown that PAR requirements of natural cyanobacterial populations are similar to those of eucaryotic phytoplankton (12). Therefore, it seems unlikely that unusual PAR requirements might initiate the dramatic surface accumulations reported in aquatic environments. Field studies revealed that diel surface scum appearances of *Anabaena* and *Microcystis* populations were poorly related to PAR intensity in a variety of nutrient-rich Canadian and New Zealand lakes (6). Booker and Walsby (1) subsequently

showed that, although minimal PAR intensities were essential for eliciting photosynthetically driven buoyancy alteration, the diffusion of CO_2 into *Anabaena* cultures ultimately limited buoyancy regulation. Later, we (10) observed that buoyancy alteration in natural *Anabaena* and *Microcystis* populations was independent of the intensity or direction of PAR and the magnitudes of nitrogen and phosphorus enrichment but heavily dependent on ambient pH and C_i concentrations. In a separate study, Klemer et al. (8) reported that limitation of the C_i concentration enhanced buoyancy (independent of PAR), whereas limitation of nitrogen decreased buoyancy in *Oscillatoria* populations. Collectively, these recent studies point to C_i concentration constraints, and particularly to CO_2 availability, as a potential environmental factor promoting cyanobacterial surface scums. This possibility was examined in natural and laboratory populations of *Microcystis aeruginosa*.

MATERIALS AND METHODS

The colonial genus *Microcystis* is extremely buoyant and prolific during blooms (12). Because of this it was chosen as a relevant genus. Natural *M. aeruginosa* populations were taken from surface waters of the Neuse River, North Carolina, a slow-flowing eutrophic river currently receiving excessive nitrogen and phosphorus loading, while having relatively low C_i concentrations ranging from 1 to 5 mg of C · liter^{-1} (1,000 to 5,000 ppb). During sampling, *M. aeruginosa* accounted for 92% of the phytoplankton biomass, typical of monospecific blooms in the Neuse River. Excessive buoyancy was observed in *M. aeruginosa* throughout summer bloom periods. In situ primary productivity measurements made during sampling periods indicated that surface populations had high photosynthetic rates, revealing maximal specific (per unit of chlorophyll *a*) rates of CO_2 fixation when compared with subsurface populations (H. W. Paerl, J. Tucker, and P. T. Bland, Limnol. Oceanogr., in press). This indicated that scums were formed by actively growing populations. Surface populations were successfully grown in xenic 500-ml batch laboratory cultures, using ASM-J (11) medium at 28°C and 700 microeinsteins of PAR (cool white fluorescent) · m^{-2} · s^{-1}. To detect constraints on C_i concentration availability, O_2 evolution was monitored in response to CO_2 enrichment in triplicate filled and sealed 125-ml glass jars, each fitted with a magnetic stirrer, a pH electrode (Fisher Scientific Co. combination type), and a Clark-type O_2 electrode (YSI Co. model 5750). A 0.005 M Tris buffer addition was made through a septum in the polyethylene jar lid to minimize pH shifts after CO_2 additions. By stabilizing pH conditions, the effects of CO_2 additions on photosynthesis could be examined independent of pH influences. This Tris concentration assured effective buffering without significantly altering O_2 evolution rates (10). A 700-microeinstein · m^{-2} · s^{-1} PAR source was provided for illumination. The chlorophyll *a* content of *M. aeruginosa* was determined for colonies sonically disrupted in 90% acetone. Spectrophotometric absorbances entered into a trichromatic formula were used to quantify chlorophyll *a* concentrations (2).

The assimilation of $^{14}CO_2$ in similar-size jars was followed by microautoradiography. $NaH^{14}CO_3$ (5 μCi; Amersham Corp.), having a specific activity of 58 mCi · mmol^{-1}, was injected into the jar, followed by incubation identical to that of O_2 evolution experiments. Additions of $NaH^{14}CO_3$ were made both before and during illuminated periods to examine the diffusion of $^{14}CO_2$ into colonies under light and dark conditions. Formalin (2% [vol/vol])-killed suspensions were treated identically to live samples as a check on abiotic ^{14}C retention by colonies, as well as on chemography and background labeling in microautoradiographs. In additional experiments, jars were partially filled with *M. aeruginosa* suspensions, leaving 60 ml of headspace. A 2.5-μCi $^{14}CO_2$ (generated from $NaH^{14}CO_3$) sample was added to the headspace and was incubated. Microautoradiographs were prepared by gravity filtering 1.5-ml subsamples of suspension on 25-mm HA membrane filters (Millipore Corp.). Suspensions were allowed to filter for 1 to 2 min until approximately 2 mm of liquid remained on top of the filter. Filters were then quickly placed on 25-mm-diameter metal stubs and immersed in liquid N_2, followed by thin sectioning and freeze-dry fixation. Sectioning was performed immediately on the frozen concentrate left on each filter. Frozen stubs were mounted in a cooled (−20°C) International Cryostat CTI sectioning apparatus, in which 10-μm-thick sections were cut, followed by freeze-drying. Dried sections were then placed on clean HA Millipore filters, which were optically cleared by exposure for 1 s to acetone fumes (9). This technique has been shown to be ideal for retention of fixed ^{14}C as well as preservation of phytoplankton cell integrity, including gas vacuoles (H. W. Paerl, manuscript in preparation). Microautoradiographs were prepared from filters by a thin-emulsion-layer grain density technique described by Paerl and Stull (9). Isotopic labeling was observed both on a colony-wide basis (×200) and at the cellular level (×1,000) with a phase-contrast Zeiss model K research-grade microscope having oil immersion objectives. Photomicrographs were recorded on Illford Pan-F film developed with Agfa Rodinal, an ultrafine grain developer. Because autoradiographic emulsions and *Microcystis* sections were situated in different planes, high-magnification photography generally proved to be difficult. Accordingly, low-magnification photographs revealing acceptable colony, as well as silver grain, development detail were used for illustrative purposes. In interpreting microautoradiographs, background exposure and potential chemography were taken into consideration. The number of silver grains exposed per cell was used as a measure of relative photosynthetic activities in various regions of colonies. Silver grains appearing within a 3-μm distance of cells minus background grain development were considered in evaluating cellular radioactivity (9).

Earlier experiments with natural and laboratory *Anabaena* populations revealed increased buoyancy in response to CO_2 depletion (10). Similar experiments were conducted with both natural and cultured *M. aeruginosa* populations. Buoyancy responses were tested by distributing a homogeneous sample among a set of 200-ml Pyrex graduated cylinders, each cylinder

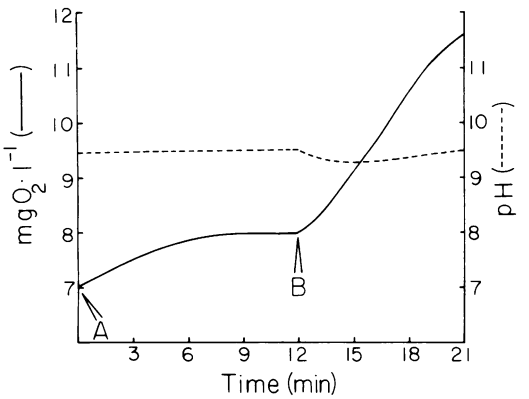

FIG. 1. O_2 evolution and pH changes in freshly sampled Neuse River surface water dominated by *M. aeruginosa* colonies incubated in filled jars (no headspace). *M. aeruginosa* accounted for 92% of phytoplankton biomass at this time. The chlorophyll *a* content was 3.80 mg · liter^{-1}. At point A (0 min), jars were sealed and measurements were initiated. The effects of C_i availability on photosynthesis became apparent within 12 min. At this point (B), CO_2 at 5 mg of C · liter^{-1} was introduced through a septum, and an immediate stimulation of O_2 evolution followed. The slope of CO_2-stimulated O_2 evolution proved to be 2.7 times the slope of O_2 evolution, given natural C_i conditions. The pH remained virtually constant during O_2 evolution measurements due to Tris buffering; however, a slight gradual decline in pH was observed (due to CO_2 hydration) after the time of CO_2 enrichment.

containing 150 ml of river or culture water. Cylinders were illuminated from all sides by a 700-microeinstein · m^{-2} · s^{-1} PAR source (cool white fluorescent) to avoid vertical illumination gradients. Contents of all cylinders were buffered to pH 9 with 0.005 M Tris. A range of $NaHCO_3$ concentrations was then added, in triplicate, for each concentration. Relative buoyancy changes were measured by sampling the upper 10 ml of each cylinder for chlorophyll *a* accumulation after 1 h of exposure to each $NaHCO_3$ concentration.

RESULTS AND DISCUSSION

Oxygen evolution determinations were used to test for C_i concentration constraints on photosynthesis. In filled jars, freshly sampled *M. aeruginosa* populations immediately exhibited constraints in C_i concentration availability significant enough to impair photosynthetic O evolution. Oxygen evolution rates at natural C_i concentrations determined within 1 min after sealing the jars were consistently lower than rates after CO_2 additions (CO_2 was added to bring the C_i concentration from 2.1 to 7.1 mg of C · liter^{-1}) (Fig. 1). Triplicate assays gave excellent agreement, with O_2 evolution rates under both natural C_i concentrations and CO_2-amended conditions having standard errors not exceeding ±1.5% (Fig. 1). A paired Student *t* test of O_2 evolution rates revealed a highly significant ($P < 0.005$) difference between natural C_i concentrations and CO_2-amended conditions. Because the time required for CO_2 hydration ($CO_2 + H_2O \rightarrow H_2CO_3$) is relatively long (the time required for half a CO_2 addition of 5 mg of C · liter^{-1} to hydrate in Neuse River water was 4.9 min at 28°C), O_2 evolution rates could be determined within the time required for CO_2 hydration; hence, measurements allowed for direct observations of CO_2 utilization by *M. aeruginosa*.

Additions of HCO_3^-, as $NaHCO_3$, likewise stimulated O_2 evolution over natural C_i concentration conditions, although to a lesser extent per milligram of carbon liter^{-1} than CO_2 (Table 1). This is likely because a strong preference for CO_2 over HCO_3^- as a C_i source occurs in *M. aeruginosa* as well as other natural bloom-form-

TABLE 1. Oxygen evolution rates in freshly sampled *M. aeruginosa* populations exposed to a natural C_i concentration, which was 2.1 mg of C · liter^{-1}, and equimolar additions (5 mg of C · liter^{-1}) of various C_i sources[a]

Treatment	O_2 evolution rate (mg O_2 · mg of chlorophyll $a^{-1} \cdot h^{-1}$)[b]
Natural C_i concn	4.45 ± 0.15
CO_2 addition of 5 mg of C · liter^{-1}	11.12 ± 0.12
HCO_3^- addition of 5 mg of C · liter^{-1}	6.91 ± 0.23
CO_3^{2-} addition of 5 mg of C · liter^{-1}	4.65 ± 0.18

[a] A 0.005 M Tris addition was used to buffer suspensions at pH 8.50. All rate measurements were made during the first 3 min of exposure to various C_i regimes.

[b] Standard errors of triplicate determinations are given.

TABLE 2. Effects of various pH regimes (established by 0.005 M Tris buffering) on O_2 evolution rates after CO_2 enrichment of 5 mg of C · liter^{-1} to Neuse River water[a]

Treatment (pH)	O_2 evolution rate (mg of O_2 · mg of chlorophyll $a^{-1} \cdot h^{-1}$)[b]
7.20	10.55 ± 0.23
8.15	10.34 ± 0.19
8.62	10.45 ± 0.31
9.23	10.11 ± 0.43
10.05	10.16 ± 0.27
8.15 (no CO_2 addition)	5.02 ± 0.18

[a] All rate measurements were made during the first 3 min of exposure to CO_2 enrichment.

[b] Standard errors of triplicate determinations are given.

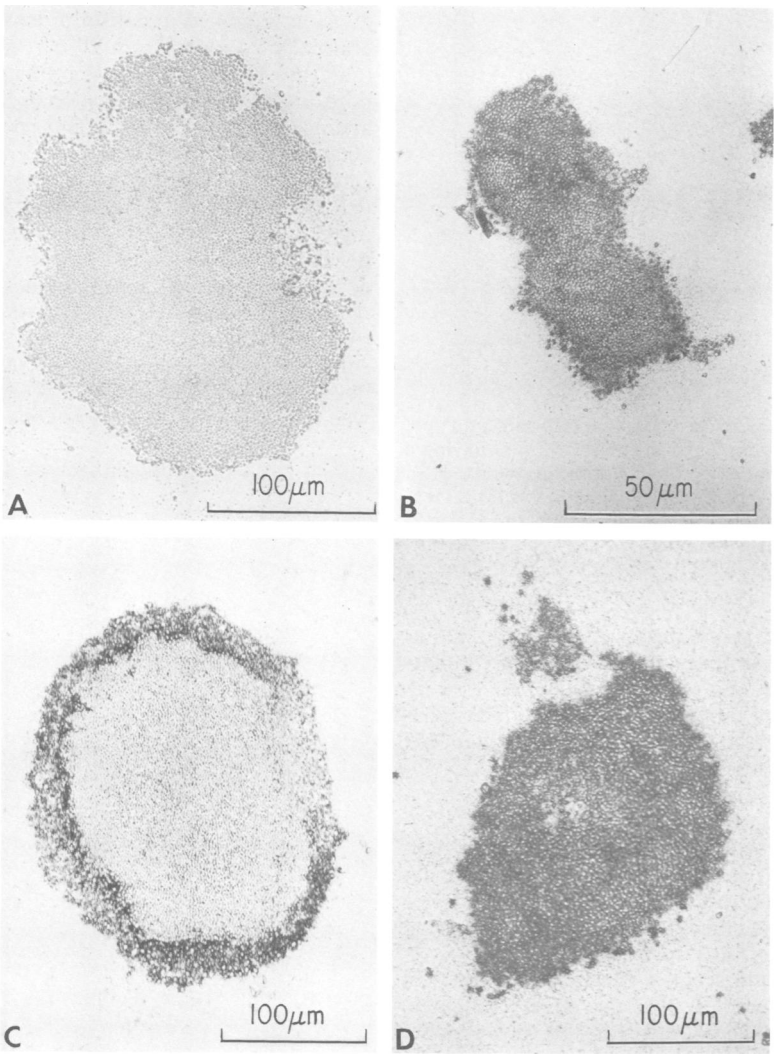

FIG. 2. Autoradiographs of $^{14}CO_2$ assimilation patterns in freeze-sectioned *M. aeruginosa* colonies. (A) Intact Formalin-killed (control) colony sectioned and processed for autoradiography. Note that silver grain development (shown as a darkening of the photographic emulsion overlaying colony sections) is absent in this sample, indicating no chemography or excessive background exposure problems. (B) Small, irregular (linear dimensions, 25 by 75 μm) colony showing near-uniform $^{14}CO_2$ assimilation among internal and peripheral cells after 4 h of incubation. Autoradiographic exposures can be seen as a general darkening of photographic emulsion overlaying the colony. (C) Partitioning of $^{14}CO_2$ assimilation in large (200-μm-diameter) colonies incubated for 4 h in aqueous medium. Approximately 90% of the silver grain deposition was seen overlaying peripheral cells, whereas internal cells remained virtually free of labeling. (D) Large (185-μm-diameter) buoyant colony incubated in a jar having a 60-ml headspace supplied with atmospheric $^{14}CO_2$. Under these conditions, colonies became more uniformly labeled.

ing cyanobacteria (10). In this study, both forms of C_i enrichment significantly and consistently stimulated photosynthesis in natural *M. aeruginosa* bloom populations. Cultured *M. aeruginosa* revealed similar C_i-limited characteristics.

Since pH changes after CO_2 (and HCO_3^-) additions were within 0.3 U, CO_2 stimulation of O_2 evolution due to pH alterations could probably be ruled out. In an earlier study of cyanobacterial populations in the Chowan River, North Carolina, pH was likewise eliminated as having a significant impact on O_2 evolution (10). The C_i concentration, and specifically the CO_2, levels present dictated on O_2 evolution rates in these studies. To further examine potential effects of pH changes on O_2 evolution rates, 0.005 M Tris

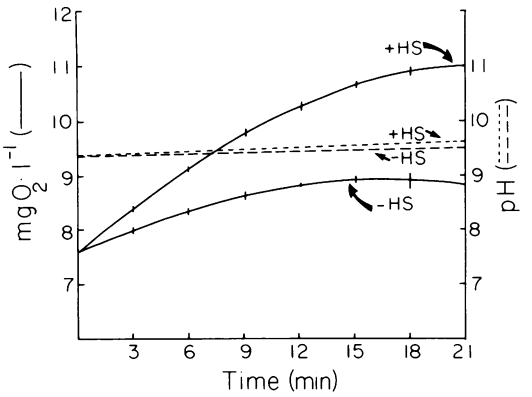

FIG. 3. Comparison of O_2 evolution and pH changes in *M. aeruginosa* incubated in jars having a 60-ml headspace (+HS) versus no headspace (−HS). The chlorophyll *a* content (3.20 mg · liter^{-1}) was identical for each treatment. Error bars show the variability among triplicate samples.

was employed to create a range of pH regimes typical of Neuse River conditions. After buffering, CO_2 additions of 5 mg of C · liter^{-1} were made, followed by O_2 evolution rate determinations. Results (Table 2) showed that ambient pH regimes had little consequence on rates of O_2 evolution per unit biomass, whereas the presence of CO_2 enrichment was a dominant factor in enhancing these rates. It is therefore concluded that the C_i concentrations, and specifically the CO_2 concentrations, present in Neuse River water were insufficient for maintaining optimal photosynthetic rates during blooms.

Microautoradiography revealed the fate of $^{14}CO_2$ assimilated by *M. aeruginosa* colonies. Colony diameters varied from 20 to well over 200 μm, with a weighted mean of 110 μm. During a 2-h incubation of freshly sampled Neuse River water a contrasting pattern of ^{14}C labeling was observed between small (<40-μm) or irregular and large (>40-μm) freeze-sectioned colonies. In suspensions incubated for 15 min with $NaH^{14}CO_3$ in the medium, slightly higher labeling (10 to 20%) was observed in peripheral than in internal cells in small or irregular colonies. After 15 min, nearly uniform labeling was observed among all cells in these colonies, and this pattern persisted through 4 h of incubation (Fig. 2B). In terms of silver grain counts per cell corrected for background exposure, peripheral cells averaged from 18 ± 6 grains per cell (outermost layer of cells) to 16 ± 5 grains per cell (inner portions of peripheral regions). Within the same colonies the internal cells averaged 14 ± 3 grains per cell. Large colonies, however, showed a sharp division in labeling between peripheral and internal cells (Fig. 2C). This division remained intact for at least 4 h. Peripheral cells averaged from 20 ± 7 grains per cell (outermost layer) to 12 ± 4 grains per cell (inner portions of peripheral regions). Internal cells averaged from 4 ± 3 grains per cell to no detectable grains per cell in the colony centers. Chemography and abiotic ^{14}C retention were not detected in any microautoradiographs. Accordingly, no corrections for false exposures were necessary. Microautoradiographs indicated that access to ambient C_i in aqueous medium was restricted in large *M. aeruginosa* colonies. The restrictions were alleviated when $^{14}CO_2$ was supplied in the headspace of jars containing buoyant colonies (Fig. 2D). Thus, when $^{14}CO_2$ was only available in the aqueous phase, major

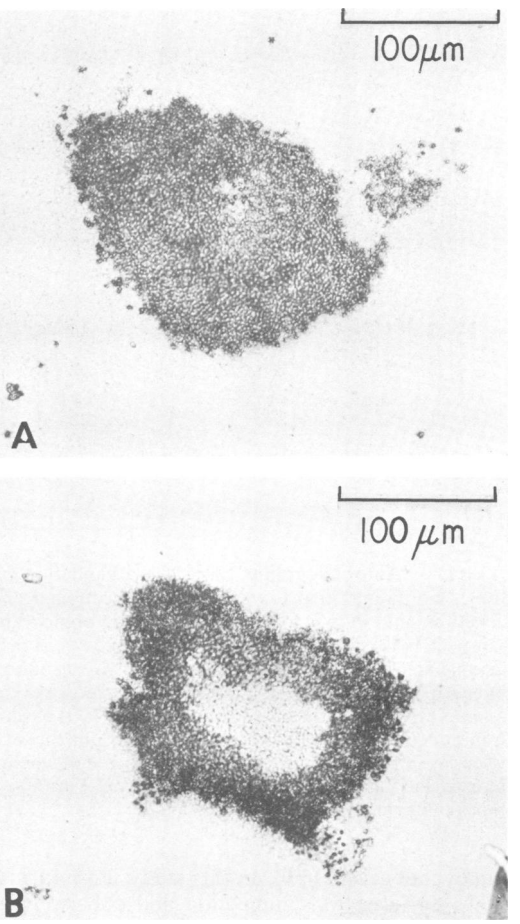

FIG. 4. Autoradiographs revealing differences in the $^{14}CO_2$ assimilation patterns between sectioned *M. aeruginosa* colonies initially exposed to a 15-min dark period after the $NaH^{14}CO_3$ addition before illumination (A) and colonies illuminated immediately after the $NaH^{14}CO_3$ addition (B).

portions of large *M. aeruginosa* colonies experienced limited access to this carbon source. Even with gentle stirring, the internal cells received only 10 to 15% of the ^{14}C labeling in peripheral cells. In contrast, $^{14}CO_2$ supplied in the atmospheric phase was more effectively utilized by internal cells. Initial slopes of O_2 evolution were significantly ($P < 0.01$) higher in jars provided with a 60-ml headspace than in headspace-free jars, substantiating the above CO_2 assimilation results (Fig. 3).

Among large colonies, C_i concentration availability constraints could have been due to photosynthetically active peripheral cells assimilating a bulk of the available ambient CO_2 supplies, thereby impairing diffusive transport of CO_2 to internal cells. By adding $NaH^{14}CO_3$ in the dark, such blockage should have been alleviated, allowing free $^{14}CO_2$ to diffuse into the internal regions of colonies. When $NaH^{14}CO_3$ was added at the start of a 15-min dark period followed by a 15-min illumination (700-microeinstein $\cdot m^{-2} \cdot s^{-1}$) period, microautoradiographs of colonies revealed nearly uniform ^{14}C photosynthate labeling throughout the colonies (Fig. 4). In contrast, when $NaH^{14}CO_3$ was supplied during continuous illuminated periods, peripheral cells became exclusively labeled, as observed in previously discussed field and laboratory samples (Fig. 2 and 4). When Neuse River water was initially enriched with unlabeled $NaHCO_3$, increasing the C_i concentration from 2.1 to 10 mg of C $\cdot$ liter^{-1}, and $NaH^{14}CO_3$ was subsequently added during illuminated periods, ^{14}C labeling was more uniform throughout large colonies.

The above experiments indicate that CO_2 transport to internal portions of *M. aeruginosa* colonies is constrained when relatively small amounts of the C_i concentration are present in waters supporting actively growing colonies. During periods of vigorous photosynthesis in the Neuse River, CO_2 demands by peripheral cells are high enough to set up a CO_2 diffusion barrier to internal cells. When colonies are situated near atmospheric CO_2 supplies at the air-water interface or when CO_2 is directly added to the aqueous phase, these constraints are minimized.

The degrees of buoyancy were closely related to the C_i concentrations in Neuse River water. At a buffered pH of 9.00, increased C_i concentration enrichment led to a steady decrease in buoyancy (Fig. 5). Hence, gas vacuole formation, the mechanism assuring buoyancy in *Microcystis* populations (14), appeared to be inversely related to C_i availability. High-magnification views of *Microcystis* colonies sampled from buoyancy experiments substantiated this relationship. Detailed observation of large *Microcystis* colonies also showed differences in degrees of gas vacuolation between peripheral and internal cells (Fig. 6). Since higher photosynthetic activities were confined to peripheral cells (Fig. 2), the finding that gas vacuolation is inversely related to photosynthetic (photosynthate) production (4, 15) can be confirmed in *M. aeruginosa*. Buoyancy among large colonies found in the Neuse River was repeatedly attributable to a high degree of gas vacuolation in internal cells; the same proved to be true in cultures maintained under relatively low C_i concentration conditions. Very large colonies (150 to 300 μm) constituted a bulk of the surface-dwelling *Microcystis* populations in this river as well as in other freshwater habitats thus far examined (10).

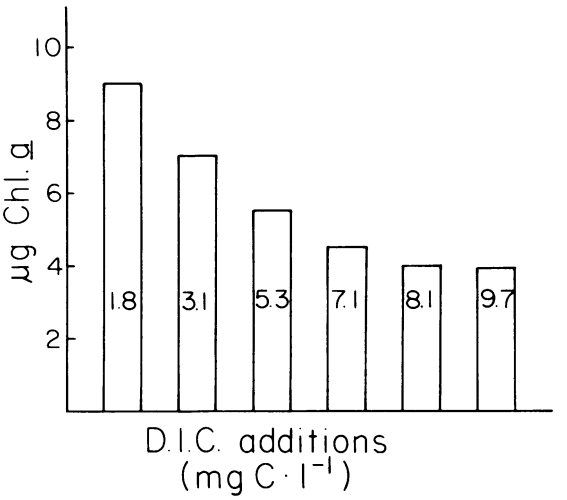

FIG. 5. Buoyancy of freshly sampled *M. aeruginosa* colonies in pH 9.00 Tris-buffered Neuse River water in response to increasing dissolved inorganic carbon (DIC) (as HCO_3^-) additions. The natural river dissolved inorganic carbon concentration was 1.8 mg of C $\cdot$ liter^{-1}. Decreased buoyancy, reported here as the decreased chlorophyll *a* (Chl. *a*) accumulation in the upper 10 cm of graduated cylinders, occurred with increasing dissolved inorganic carbon concentrations.

Ecological implications. Autoradiographic and O_2 evolution results point to similar conclusions, namely, that access to naturally occurring C_i and CO_2 can be restricted in *M. aeruginosa* colonies. Such restrictions are likely to be most profound in low C_i concentration and high-nutrient (both nitrogen and phosphorus) systems as typified by the Neuse and other large river systems in eastern North Carolina. To obtain optimal O_2 evolution rates, CO_2 supplementation is necessary (Fig. 1). This implies that photosynthetic performance in natural populations is limited to some extent by CO_2 availability to the internal cells of colonies. The most likely explanation for such limitation of photosynthesis is that CO_2 transport constraints commonly occur in inter-

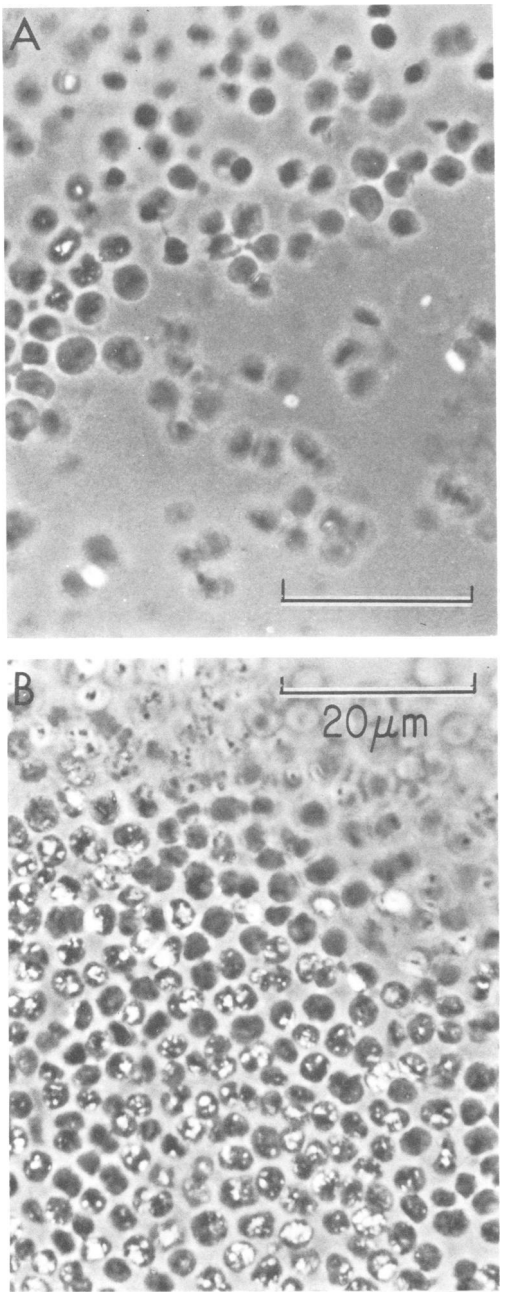

FIG. 6. Phase-contrast observations of freshly sampled large, freeze-fixed, whole (unsectioned) *M. aeruginosa* colonies. (A) Vacuole-free peripheral cells near the edge of the colony. (B) Highly vacuolated internal cells. Vacuoles appear as bright, white cellular inclusions. Since whole colonies were three-dimensional, cells in the edge of each field are out of the focal plane.

nal portions of colonies. Small or irregular colonies experience minimal problems with regard to CO_2 transport from the ambient environment to internal cells.

Consideration of free CO_2 supplies in aqueous and atmospheric phases is helpful in developing a rationale for scum formation. During calm nonmixed periods, when surface scums occur and a pH of 8.0 to 9.5 characterizes Neuse River water, virtually no free CO_2 would be expected in solution. An influx of "new" CO_2 would have to come from the atmosphere, invading surface waters by molecular diffusion, an extremely slow process (14). The dominant C_i sources under these conditions are HCO_3^- and CO_3^{2-}. This study, as well as earlier studies, has shown a strong preference for CO_2 over HCO_3^- and CO_3^{2-} as a photosynthetic carbon source among a variety of cyanobacteria, including *Microcystis* populations (10). Under such circumstances the atmosphere represents an attractive source of CO_2. At a mean pH of 9.00 and a C_i concentration of 3 mg of C · liter^{-1} during *M. aeruginosa* blooms in nonmixed Neuse River water, free (CO_2) in the aqueous phase is approximately 0.009 mg of C · liter^{-1}. In contrast, the atmospheric CO_2 concentration is approximately 330 μg/ml. As a result, during nonmixed periods, the atmospheric CO_2 concentration is at least $10^5 >$ the concentration of river water. This calculation incorporated the assumption that molecular diffusion and microbial recycling were the main routes of CO_2 replenishment in the water column during such bloom periods. The direct utilization of atmospheric CO_2 by *Microcystis* colonies at the air-water interface would therefore represent an alternative to diffusion-limited transport of CO_2 and the resultant limitation of photosynthesis in nonturbulent aqueous media.

The ^{14}C labeling patterns and buoyancy characteristics observed here are similar to the observations of Carpenter and Price (3) of marine *Oscillatoria* (*Trichodesmium*) colonies. Like *Microcystis* colonies in stagnant river waters, surface *Trichodesmium* colonies (trichomes) predominated in calm ocean waters. By autoradiography, they also found that peripheral cells were more heavily labeled with fixed $^{14}CO_2$ than were internal cells, indicating that photosynthate production was largely confined to peripheral cells. They noted that internal cells appeared to be less pigmented than were peripheral cells. Poor pigmentation of internal cells could have been due, in part, to a relatively high proportion of cell volumes being occupied by gas vacuoles. Their findings may therefore be related to the explanation for a high degree of buoyancy in *Microcystis* colonies presented here.

I have presented a rationale, based on microscopic examinations of localized photosynthetic activity, for excessive buoyancy in *Microcystis*

colonies. Excess buoyancy is most likely a response to CO_2-limited growth conditions, bringing colonies to the air-water interface where atmospheric CO_2 can be readily intercepted and can be utilized by peripheral and internal cells. The persistence of surface blooms, therefore, appears to be an ecological strategy poised at optimizing photosynthetic production. One question this work raises is that if CO_2 transport is restricted to internal cells, then why are all *Microcystis* colonies not small or irregular to circumvent this limitation. It would seem likely that a large colony size is advantageous for other reasons. Perhaps the inability of zooplankton grazers to consume large colonies and the intimate association of large colonies with attached bacteria and protozoans serve to benefit the survival and proliferation of *Microcystis* blooms.

ACKNOWLEDGMENTS

I thank H. Page, V. Page, and B. Bright for assistance with manuscript preparation, R. T. Barber, J. J. Kohlmeyer, and J. C. Goldman for their reviews and critiques, and A. E. Walsby for helpful suggestions concerning experimental techniques.

This work was supported by National Science Foundation grant DEB 8104689, Office of Water Research and Technology, Department of Interior, grant B 127-NCB, and North Carolina Sea Grant.

LITERATURE CITED

1. **Booker, M. J., and A. E. Walsby.** 1981. Bloom formation and stratification by a planktonic blue-green alga in an experimental water column. Br. Phycol. J. **16**:1–11.
2. **Burnison, B. K.** 1980. Modified DMSO extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquat. Sci. **37**:727–733.
3. **Carpenter, E. J., and C. C. Price.** 1976. Marine *Oscillatoria* (*Trichodesmium*): explanation for aerobic nitrogen fixation without heterocysts. Science **176**:1278–1280.
4. **Dinsdale, M. T., and A. E. Walsby.** 1972. The interrelations of cell turgor pressure, gas vacuolation and buoyancy in a blue-green alga. J. Exp. Bot. **23**:561–570.
5. **Goldman, J. C.** 1973. Carbon dioxide and pH: effect on species succession of algae. Science **182**:306–307.
6. **Kellar, P. E., and H. W. Paerl.** 1980. Physiological adaptations in response to environmental stress during an N_2-fixing *Anabaena* bloom. Appl. Environ. Microbiol. **40**:587–595.
7. **King, D. L.** 1970. The role of carbon in eutrophication. J. Water Pollut. Control Fed. **42**:2035–2051.
8. **Klemer, A. R., J. Feuillade, and M. Feuillade.** 1982. Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on buoyancy of *Oscillatoria*. Science **215**:1629–1631.
9. **Paerl, H. W., and E. A. Stull.** 1979. In defense of grain density autoradiography. Limnol. Oceanogr. **24**:1166–1169.
10. **Paerl, H. W., and J. F. Ustach.** 1982. Blue-green algal scums: an explanation for their occurrence during freshwater blooms. Limnol. Oceanogr. **27**:212–217.
11. **Parker, D.** 1982. Improved procedures for the cloning and purification of *Microcystis* cultures. J. Phycol. **18**:471–477.
12. **Reynolds, C. S., and A. E. Walsby.** 1975. Water blooms. Biol. Rev. Camb. Philos. Soc. **50**:437–481.
13. **Shapiro, J.** 1973. Blue-green algae: why they become dominant. Science **179**:382–384.
14. **Stumm, W., and J. J. Morgan.** 1970. Aquatic chemistry. John Wiley & Sons, Inc., Interscience, New York.
15. **Walsby, A. E.** 1972. Structure and function of gas vacuoles. Bacteriol. Rev. **36**:1–32.
16. **Walsby, A. E., and M. J. Booker.** 1980. Changes in buoyancy of a planktonic blue-green alga in response to light intensity. Br. Phycol. J. **15**:311–319.