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CHLOROPHYLL, NITROGEN, AND PHOTOSYNTHETIC PATTERNS DURING GROWTH AND SENESCENCE OF TWO BLUE-GREEN ALGAE[1,2]

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SUMMARY

A standardized, multiflask, batch culture system was developed to study the processes of algal senescence in Anacystis nidulans *and* Phormidium molle *Gom. var.* tenuior *W. et G. West. Growth data over a 3-year period gave reproducible and comparable time-course curves.*

Although A. nidulans *is unicellular and* P. molle *filamentous, the patterns of change with age were similar. Mean logarithmic doubling times and carbon yields were, respectively, 6.9 hr and 390 mg C/liter for* A. nidulans *and 7.2 hr and 710 mg C/liter for* P. molle. *Chlorophyll concentration and photosynthetic capacity per unit carbon rose rapidly during the logarithmic phase to maximum levels in either late log phase* (P. molle) *or early linear phase* (A. nidulans), *then fell throughout the declining growth phase to low levels in the stationary phase. Nitrate was rapidly exhausted from the medium during the period of logarithmic growth and stoichiometrically converted to particulate organic form; very little subsequent fixation of molecular nitrogen occurred. The phycocyanins were rapidly destroyed during the logarithmic phase while the carotenoids remained relatively constant throughout the whole growth period and then slowly declined. Preliminary electron micrographs showed a progressive deterioration in cellular ultrastructure, especially a reduction in the number of photosynthetic thylakoids, commencing in the linear growth phase.*

Analysis of the results suggests that occurrence of linear growth kinetics and termination of culture growth were caused by exhaustion of nitrate. The observed decreases in chlorophylls, phycocyanins, and photosynthetic capacity during active culture growth show that senescence effects may not be, as assumed, restricted to the stationary phase of growth.

INTRODUCTION

Many species of algae in limited-volume culture undergo extensive physiological and morphological changes following the cessation of growth. The term senescence has frequently been applied to these events. The few descriptive studies undertaken to date have demonstrated that declines in chlorophyll, carbohydrate, and nucleic acid concentrations can occur with age (***10,19,31***), while lipids and carotenoids may accumulate (***10,21***). Rates of both photosynthesis and respiration may also decline (***26, 34***), and in some species, ultrastructural changes, such as secondary wall formation and breakdown of lamellar structure, have been observed (***25***). Many of these studies have focused on only one or a few aspects of the process in widely differing species of algae. In consequence, the causes of senescence are not well understood. Since the termination of culture growth and the onset of senescence are often coincident, it has frequently been assumed that the same factors that limit growth, such as nutrient exhaustion (***9***) or accumulation of autoinhibitors (***27***), trigger the aging responses. However, experimental verification of a direct causal link between the 2 processes seems to be lacking, even though no evidence has been found in the algae for strictly endogenous senescence changes such as occur in certain mammalian tissues *in vitro* (***16***).

Algal senescence is of interest as a model system for studying not only higher organism senescence, but also certain aspects of phytoplankton dynamics and primary production processes in lakes (***10***). In addition, the algal senescence system may prove valuable in lacustrine paleolimnology as a simple but experimentally accessible analogy to the processes of chlorophyll degradation in the water column (***4***). In these ecological connections, it is significant that only fragmentary and largely qualitative data are available (***10,25***) on aging effects in the important blue-green algae.

Difficulties with culture methodology appear to have complicated these studies. For correlation purposes, accurate time-course information on the many chemical, physiological, and biochemical parameters that may change with time are needed. Repeated, large-volume sampling of single cultures for a variety of analyses is difficult to do without bacterial contamination and alteration of the future growth responses of the culture. On the other hand, multiple-flask culture series often do not provide the necessary reproducibility between different samples (*ie*, flasks) to permit comparisons over time, nor sufficient total volume per culture to permit precise,

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TABLE 1. *The modified No. 11 medium of Hughes* et al. (*17*).

Salt, mg per liter		Salt, mg per liter	
$NaNO_3$	496	Na_2CO_3	20
$K_2HPO_4 \cdot 3H_2O$	39	Gaffrons minor	
$MgSO_4 \cdot 7H_2O$	75	element solution	0.08 ml
$CaCl_2 \cdot 2H_2O$	36	Fe-EDTA[a]	4 ml

[a] Sterilized separately and added to other components when the solutions had cooled to room temperature. Composition: Na_2-EDTA, 7.76 g per liter and $FeSO_4 \cdot 7H_2O$, 5.80 g per liter.

simultaneous measurements of a large number of variables.

In this note we describe a simple, standardized, multiflask culture system which in the course of experimental pigment studies (*4*) has permitted frequent measurements of many variables under axenic and reproducible conditions of cultivation. Data on changes in several important physiological parameters during growth and senescence of 2 morphologically different blue-green algae are presented and evaluated in relation to the factors controlling senescence.

MATERIALS AND METHODS

Culture procedures. *Anacystis nidulans* (strain unknown) and *Phormidium molle* Gom. var. *tenuior* W. et G. West (isolated as a contaminant from a culture of *Scenedesmus bohyanum*) were both cultivated in a modification (Table 1) of Gorham's No. 11 medium (*17*). Sodium silicate caused heavy precipitation of several components in this medium during autoclaving. It was therefore deleted after tests when both organisms failed to show any growth impairment in its absence. Cultures were rendered axenic by lysozyme treatment (*2*) and repeated subculturing of individual cells or filaments isolated from agar plates (*12*). Bacterial contamination was evaluated at the time of harvesting by incubating 1-ml culture aliquots at 25 C on 1.5% bacto-agar plates prepared with the No. 11 medium. Reagent grade chemicals and glass-redistilled water were used throughout.

Maintenance cultures were grown at 32 ± 1 C under continuous fluorescent illumination [2×10^4 ergs /(cm²)(sec)] in low-form Fernbach flasks containing 1 liter of medium. Flasks were flushed once daily with ca. 150 ml of CO_2. A standard subculturing regimen was followed.

The experimental growth system (Fig. 1) consisted of 2 adjustable culture benches, each accomodating 10 culture vessels (16-oz, clear glass prescription bottles). Cultures were grown at 32 C and illuminated by fluorescent lamps (Gro-Lux, Sylvania Corp.) mounted to provide ca. unilateral light. Each flask contained 300 ± 10 ml of half-strength medium. CO_2-enriched air entered the cultures through bent glass tubes which were mounted in rubber stoppers sealed into the mouth of the flasks with silicon cement. The growth conditions for *A. nidulans* were: light intensity, 6×10^4 ergs/(cm²)(sec); CO_2 concentration, 0.5%; aeration rate, 140 ml/min per flask; and for *P. molle*: light intensity, 4.5×10^4 ergs/(cm²)(sec); CO_2 concentration, 2%; aeration rate, 75 ml/min per flask.

Experimental cultures were prepared by a "bulk" inoculation procedure. The required cell concentrate was added aseptically to a 20-liter carboy containing the full volume of medium for the run. Following mixing, the inoculated medium was siphoned into individual flasks. To ensure uniformity in the physiological condition of inocula between successive runs, maintenance cultures were serially subcultured twice for 40 hr on the culture benches and the third subculture was then used as the experimental run. This procedure caused clumping of *P. molle* but not of *A. nidulans*. The clumps were dispersed by gently agitating the inoculum for 5–10 min in an ultrasonic bath (Branson Corp.).

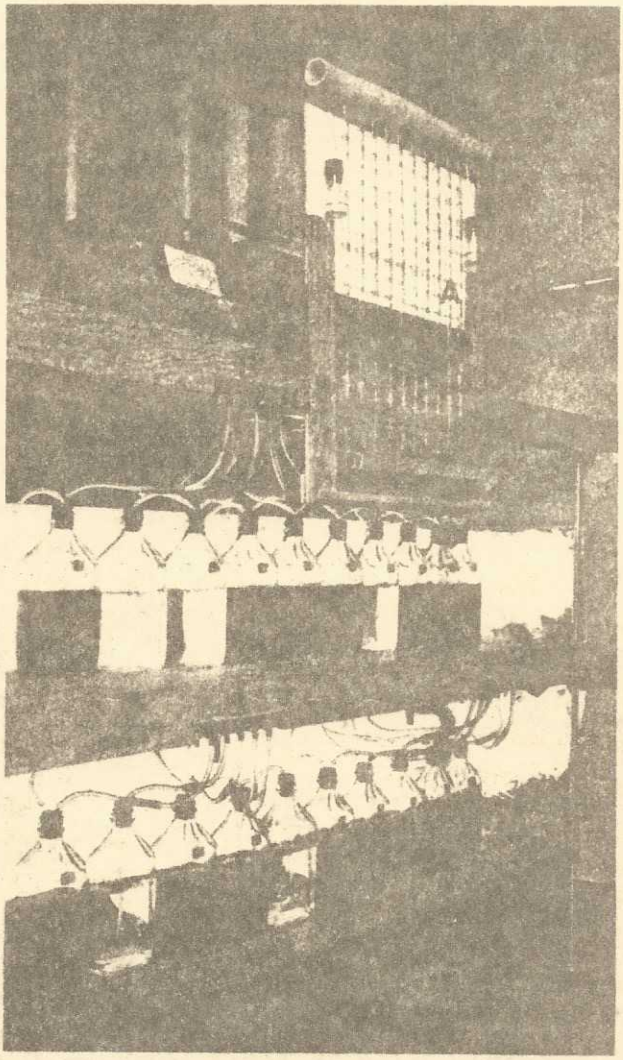

FIG. 1. A general view of the algal culture benches and aeration system. Flow rates to all bottles were equalized by use of the outflow meter (*A*); the outflow gas from each bottle was bubbled through a soap solution into inverted pipettes. By adjusting the inflow needle valves (*B*), equal bubbling rates in the pipettes and hence equal aeration rates in the culture flasks were obtained.

The composition of the gas mixture was regulated by calibrated flowmeters located in the air and CO_2 lines ahead of a large mixing bottle. Gas flow to the culture vessels was controlled by individual needle valves. Aeration rates were equalized by a soap-bubble "outflow meter" (Fig. 1), and the absolute flow rates were then set by adjusting the total gas flow to each bench.

Growth was monitored in 1 flask for the first 20 hr of growth using a Klett photoelectric colorimeter (No. 42 blue filter). The point in time at which the optical density of this culture reached 35 Klett units was arbitrarily designated as 0 hr for all runs, thereby compensating for differences in initial cell concentrations and lag times from one series to another. One flask was harvested at 5–10 hr intervals throughout the run, tested for axeneity and diluted to 300 ml with distilled

water as a correction for water loss. One or more of the following measurements were then made: soluble and particulate organic carbon (C), nitrate-nitrogen (NO_3-N), total organic nitrogen (organic N), chlorophyll, and apparent photosynthetic oxygen evolution under saturating light intensities (photosynthetic capacity).

Analytical procedures. Cell biomass was determined either by optical density (at cell concentrations below 80 mg C/liter) or by particulate organic C estimation. Under the standardized culture conditions employed here, a linear relationship existed between particulate C and absorbance from 25 to 200 Klett units (for both *P. molle* and *A. nidulans*). Organic C was determined by a modification of the Walkley-Black wet oxidation technique (*3*): comparisons with a dry combustion method confirmed that complete oxidation of the algal carbon was achieved. Maximum error (*30*) did not exceed 5%. Both the total C content of unfiltered, lyophilized samples and the soluble C remaining in the medium after filtration through 0.45-μ membrane filters (Millipore Corp.) were measured. Particulate C was then calculated as the difference between the two.

Organic N was determined with a direct-combustion carbon, hydrogen, nitrogen analyzer (Hewlitt Packard, Model 185) calibrated with cyclohexanone-2,4 dinitrophenyl-hydrazone (B.D.H. Ltd., analytical standards grade). Two lyophilized replicates of the cultures were analyzed by ratio-recording for each sample. The measurements thus include both particulate and dissolved organic N.

Nitrate was converted to nitrite on cadmium-copper reduction columns using the ammonium-ammonium chloride buffer of Grasshof (*15*). Nitrite was then measured by diazotization with sulfanilamide, coupling with N-1 naphthylethylenediamine and colorimetric measurement at 540 nm (*13*). Interfering phosphate ion was removed by precipitation with 0.1 M $MgSO_4$ and 0.2 N NH_4OH for 0.5 hr.

Oxygen evolution measurements were made for *P. molle* by the direct one-vessel manometric method of Warburg & Krippahl (*33*), and subsequently for both *P. molle* and *A. nidulans* by the O_2 electrode technique of Grant *et al.* (*14*). For manometry, 4 replicates were determined per sample. Two ml of cell concentrate which had been resuspended in the original growth medium were placed in the main space of each Warburg vessel. Then 2 ml of the appropriate carbonate-bicarbonate mixture together with 1.3 mg of carbonic anhydrase (Sigma Chem. Corp.) were introduced into the large side arm of the vessel. Manometric changes were recorded for 1 hr at 32 ± 0.5 C under light and CO_2 conditions determined in preliminary experiments (*3*) to be saturating but not inhibitory for photosynthesis. The contents of each flask were then quantitatively transferred to tared extraction vessels, lyophilized, and analyzed for organic C content (*3*). For the oxygen electrode procedure, 5 replicate samples, 2 ml each, were removed directly from the culture without dilution or resuspension and measured under saturating light intensities at 32 ± 0.5 C.

Measurements of incident and transmitted light intensity for the cultures and of saturating light intensities for photosynthesis using the O_2 electrode procedure were made with a radiometer (Y.S.I., model 65). Saturating light intensities for the Warburg measurements were determined with a calibrated selenium cell meter (*3*).

Chlorophyll was determined according to the procedure of Daley *et al.* (*6*). Aliquots of culture suspensions were filtered through glassfiber filters and the filters extracted ultrasonically in a mixture of methanol, acetone and water. Extracts were then fractionated by reversed-phase, thin-layer chromatography and estimated directly on the chromatograms by scanning filter fluorometry. Reproducibility with 4 replicates was ca. 11%. Since carotenoids are also resolved on these chromatograms, an approximate estimation of their changes with age was obtained by visual comparison of extracts from equal quantities of cells of different ages. Similar preliminary estimates of phycobilin changes were made by visual comparison of the color of the cell residue remaining on the (solvent-resistant) membrane filters after the extract containing the chlorophylls and carotenoids had been removed by filtration.

RESULTS

In preliminary replicated experiments, high variations in growth kinetics were observed both between different culture runs and between different flasks within runs. The principal sources of variation were gas mixture composition, aeration rate, medium concentration, initial cell densities, and the physiological condition of the cell inoculum. Growth rates showed the typical saturation response to increasing percentages of CO_2 in the gas stream, so that above the saturation point, compositional variations were without effect on growth. However, increases in aeration rate for a given CO_2-air mixture caused a decline in growth rate in *P. molle*, presumably as a result of sensitivity to increased turbulence (*11*). Similar turbulence effects were not observed with *A. nidulans*. Variations in initial cell densities primarily affected the duration of the lag phase without altering the kinetic pattern once growth had commenced. On the other hand, differences in the physiological status of the inoculum affected both the duration of the lag phase and the subsequent rate of logarithmic growth. Variations in medium concentration altered only the magnitude of the final yield.

When these variables had been standardized as described above, the reproducibility of the culture system was again assessed. In 4 *P. molle* runs over a 3-year period, time-course curves of particulate organic C appeared by visual inspection to be uniform and superimposable (Fig. 2*A*). Comparisons of oxygen exchange data from series 1 and 2 in Fig. 2*A* demonstrated similar reproducibility. In addition, because individual cultures were not opened until harvested for analysis, axeneity was maintained throughout the runs. On the basis of these results we have concluded that data obtained from different culture runs can be directly compared on the same time scale.

Time-course curves of particulate carbon, NO_3-N, organic N, chlorophyll, and photosynthetic capacity for *P. molle* and *A. nidulans* are shown in Fig. 2 and 3, respectively.

P. molle. Five growth phases were recognized from the particulate C measurements (Fig. 2*A*): a lag phase (not shown), a logarithmic phase, a linear phase, a period of declining relative growth rate, and a long stationary phase. No death phase, as judged by a decrease in the particulate C concentration following cell lysis, was observed. Soluble organic C remained at undetectable levels throughout the experiments. The logarithmic growth rate was high, with a mean doubling time of 7.2 hr (upper and

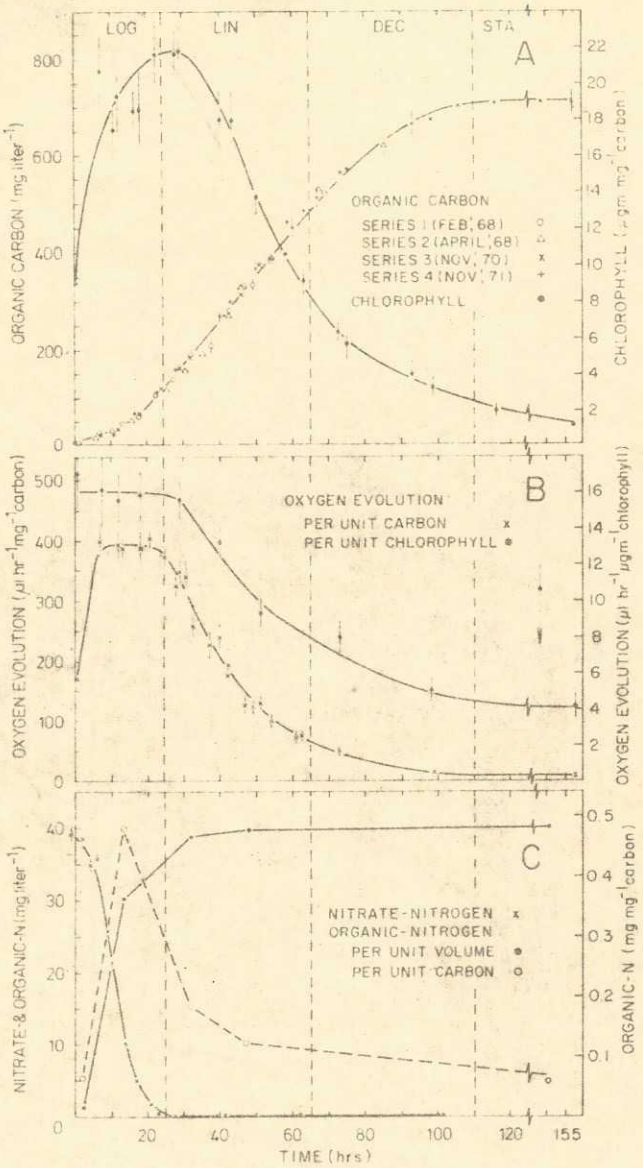

FIG. 2. Changes with age in the patterns of growth and chlorophyll concentration (*A*), photosynthetic capacity per unit carbon and per unit chlorophyll (*B*), NO_3-N and organic N per unit volume, and organic N per unit carbon (*C*) in batch cultures of *P. molle*. Standard deviations indicated, where possible, by vertical bars. *LOG*, logarithmic growth phase; *LIN*, linear growth phase; *DEC*, phase of declining relative growth; *STA*, stationary or senescence phase.

lower 95% confidence limits, 6.8 and 7.6 hr, respectively). The final yield was 710 mg C/liter.

Chlorophyll (Fig. 2*A*) and photosynthetic capacity (Fig. 2*B*) per unit carbon rapidly rose to maxima early in the log phase of growth, remained constant until the beginning of the linear growth phase, and then declined continuously throughout the linear, declining, and stationary phases. On a per unit chlorophyll basis, photosynthetic capacity remained ca. constant until 30 hr and declined slowly thereafter (Fig. 2*B*). The minimum light intensity needed to saturate photosynthesis fell from 6×10^4 ergs/(cm²)(sec) at 10 hr to 2.5×10^4 ergs/(cm²)(sec) at 90 hr; the photosynthesis vs. light curves were of the typical hyperbolic form. Between 0 and 25 hr, the intensity of the light transmitted through the culture flasks declined from 3.8×10^4 ergs/(cm²)(sec) to 0.

In the first 25 hr of growth, NO_3-N was rapidly depleted from the medium, while organic N accumulated to a maximum. Beyond 25 hr the organic N concentration per unit volume remained unchanged (Fig. 2*C*). Hence organic N levels per unit carbon peaked in the log phase and then declined to a minimum in the stationary phase (Fig. 2*C*). Preliminary measurements of phosphorus exchange (W. O. Buddle & S. R. Brown, unpublished data) indicate that the patterns and rates of phosphate and nitrate uptake in this alga are similar.

The phycobilins declined rapidly to low levels during the first 15 hr of linear growth, the color of the cell debris changing from bright blue to grey-white. Carotenoid levels remained relatively constant throughout both the growth and senescence phases. Consequently, the color of the cultures changed rapidly from the characteristic blue-green of logarithmically growing cells to green in the linear phase and orange-yellow early in the stationary phase.

Marked variations in cell morphology and buoyancy were also seen. The trichomes increased in length from 10 to 30 cells in the inoculum (as a result of presonication) to many hundreds of cells in intertwined filaments by the end of the linear growth phase. Between 70 and 80 hr the gelatinous clumps of filaments abruptly dispersed and filament length rapidly decreased. Despite aeration, a mat of short filaments had formed on the bottom of the flasks by 175 hr. Preliminary electron micrographs (S. R. Brown, unpublished data) showed a progressive deterioration in intracellular structure, together with a reduction in thylakoid area beginning in the linear growth phase.

A. nidulans. The kinetic patterns for growth, chlorophyll synthesis, photosynthetic capacity, and nitrogen metabolism (Fig. 3*A*, *B*, *C*) in this unicellular species were similar to those for *P. molle*. The rate of logarithmic growth was the same (G = 6.9 hr, with upper and lower 95% confidence limits of 7.2 and 6.5 hr, respectively), while the durations of both the linear and declining growth phases were shorter than for *P. molle* (Fig. 3*A*). Final cell yield was 390 mg C/liter and soluble organic C accumulation was negligible.

By the middle of the linear growth phase all NO_3-N had been removed from the medium and stoichiometrically converted to organic form (Fig. 3*C*). The organic N concentration remained unchanged per unit volume beyond the first 35 hr of growth, but organic C synthesis continued until

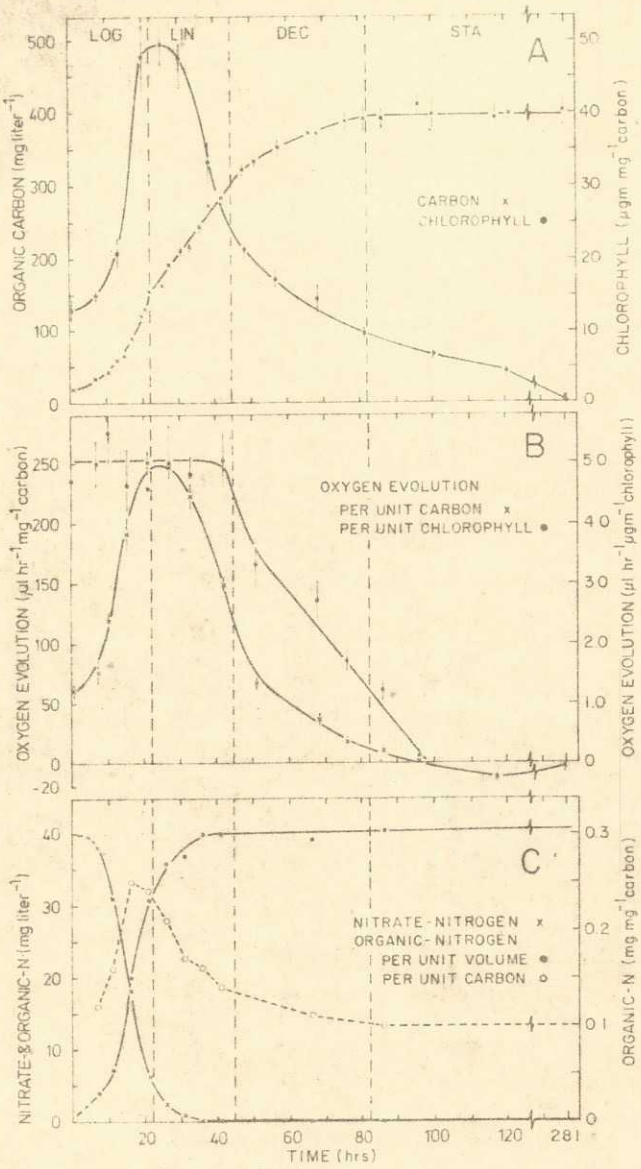

FIG. 3. Changes with age in the patterns of growth and chlorophyll concentration (*A*), photosynthetic capacity per unit carbon and per unit chlorophyll (*B*), NO_3-N and organic N per unit volume, and organic N per unit carbon (*C*) in batch cultures of *A. nidulans*. Standard deviations shown by vertical bars. Abbreviations as in Fig. 2.

80 hr. Hence, on a per unit carbon basis, the organic N content declined from a maximum of 0.25 mg/mg C at 15 hr to 0.10 mg/mg C at growth termination (Fig. 3*C*).

Chlorophyll concentration (Fig. 3*A*) and apparent photosynthetic capacity (Fig. 3*B*) expressed per unit carbon followed similar patterns with age. Both rose rapidly during logarithmic growth, peaking broadly in the first half of the linear growth phase, and then fell gradually to low levels in the stationary phase. Photosynthetic capacity was proportional to chlorophyll during the logarithmic and linear phases but fell more rapidly than did chlorophyll subsequently. Thus the photosynthetic capacity remained constant per unit chlorophyll for the first 40 hr and then declined as growth slowed and ceased (Fig. 3*B*). In the stationary phase, *A. nidulans* exhibited a small, net uptake of oxygen in the light, which declined to undetectable levels by 280 hr.

The phycobilins were rapidly destroyed during the linear growth phase, while the carotenoids appeared to remain unchanged during active growth of the culture and then decline slowly to very low levels by 280 hr. The resultant color change of *A. nidulans* was similar to that of *P. molle* throughout the growth period, but unlike *P. molle*, continued to change in the stationary phase from orange-yellow through yellow to grey-white.

Little alteration with age in the size or morphology of *A. nidulans* cells was observed. However, incidental ultrastructural observations made during a study of flagellate ingestion of this alga (*5*) indicated that a similar deterioration in intracellular organization to that seen in *P. molle* occurred with time.

DISCUSSION

Few detailed studies of growth kinetics and senescence changes have been undertaken for the blue-green algae. Hence only general comparisons can be made between the present results and those from other groups of algae.

The growth pattern of *P. molle* under the present culture conditions is similar to that of *A. nidulans* and other unicellular cyanophytes (*10,23*). The growth rates of both species are high, with generation times as short or shorter than most other cultured blue-green algae (see *18,20,22*). The soluble organic C measurements indicate that neither species excreted significant amounts of fixed carbon nor underwent cell lysis. This latter observation is somewhat surprising given the unfavorable environmental conditions that exist late in the stationary phase of growth and the previously reported sensitivity of some other species of blue-green algae to lysis (*20,22*).

The dissimilarities seen here between the patterns of nitrate uptake and carbon fixation have also been documented in a variety of other marine and freshwater algae (*7,32*). Such "luxury consumption" processes would seem to be widely distributed in the algae. From the kinetic data (Fig. 3*C*, 4*C*) it is clear that the surplus assimilated nitrogen was stored in an organic rather than inorganic form in both algae. Cyanophycin granules, consisting of reserve polypeptides (*29*), are known to occur in cyanophytes. Since the total organic nitrogen levels remained constant following the depletion of nitrate from the medium, it would appear that neither species was capable of fixing significant amounts of molecular nitrogen under the present culture conditions. The carbon/nitrogen ratios at the end of the growth period (Fig. 2*C*, 3*C*) are similar in magnitude to those considered

by Fitzgerald (*8*) as characteristic of nitrogen-deficient cells.

Age-rate changes in photosynthetic capacity have been reported for several species of *Chlorella* (*26,28, 34*), but inadequate consideration of changes in saturating light intensities with age in these studies make specific comparisons with the present results difficult. Nevertheless, a general decline in photosynthetic capacity per cell with time would appear to be a common pattern in all studies to date. During the logarithmic and linear phases of growth in *A. nidulans* and the log phase in *P. molle*, oxygen evolution rates were a function of chlorophyll concentration. In subsequent growth phases, capacity was limited by the rate of the dark reactions of photosynthesis. Similar declines in photosynthetic capacity have been seen when logarithmically growing cultures were transferred to medium devoid of nitrogen (*10*).

The decreases in chlorophyll with time in both the *P. molle* and *A. nidulans* cultures are similar to those seen in many diatoms and green algae, both during senescence (*19,31*) and following transfer to nutrient-deficient medium (*9,35*). They differ, however, from the results of Allen & Smith (*1*) for *A. nidulans* and 9 other blue-green algae. These authors observed a rapid loss of phycobilins under conditions of nitrogen starvation, but no degradation of chlorophyll. Such differences in response may be due in part to nonenzymic photooxidation of chlorophyll late in the senescence phase of the present experiments (*4*). The kinetic patterns for the phycobilins and the carotenoids observed here and by Allen & Smith (*1*) are similar. The relative stability or decline in carotenoids in blue-green algae contrasts markedly with the pattern in certain green algae, where carotenoids accumulate to high levels (*10,21*).

Changes in cell morphology and ultrastructure similar to the preliminary observations reported here have been described for other blue-green algae (*22, 24,25*).

In view of the presumed intimate relationship between algal growth and senescence, it is of interest to consider to what extent the present data permit identification of the factor(s) limiting growth of *P. molle* and *A. nidulans*. Of the 4 commonly recognized causes of growth termination in batch cultures —namely, pH, CO_2, light, and nutrients—neither CO_2 nor pH would appear to have been responsible, since the former was provided in excess and the latter remained approximately constant throughout the growth period. A distinction between light and nutrients is possible, at least in theory (*10,23*), from an analysis of culture growth kinetics. Thus the occurrence of a linear growth phase followed by an extended phase of declining growth rate is indicative of light limitation, while nutrient depletion results in an abrupt transition from the logarithmic to the stationary growth phase without an intervening linear phase. That the observed period of linear growth, at least in *P. molle* (Fig. 1*A*), results from light limitation is reinforced by our observation that the light intensity transmitted through the culture (*23*) declines to zero at the beginning of the linear growth phase.

However, several other observations are at variance with this interpretation and suggest instead that nutrient limitation was the cause of growth stoppage. First, during light-limited linear growth, photosynthetic capacity (as opposed to the prevailing photosynthetic rate in the cultures) should remain constant per unit carbon. It is clear from Fig. 2*B* and 3*B* that the opposite result was obtained, with the photosynthetic capacity declining throughout. Since low light intensity, per se, is not known to effect permanent cell damage, the observed linear kinetics would not appear to have been caused by light limitation alone. Second, final yields were found to be proportional to medium strength in tests with *P. molle* and to nitrate concentration with *A. nidulans*, but did not change in response to increases in light intensity (in half-strength medium, unpublished data). Last, 90-hr cultures of *P. molle* rapidly "regreened" when nitrate was added to the medium, but did not do so when the effective culture illuminance was increased by dilution of the culture with nitrogen-free medium (*3*).

These apparent contradictions in the data may be a result of the "luxury-consumption" pattern of nutrient uptake. In the case of nitrogen, for example, the quantity of organic N in the cultures following the depletion of nitrate was constant per unit volume and, hence, if limiting, may have generated linear growth kinetics. Because of such differences in the rates of nutrient utilization and growth, the normal kinetic pattern for nutrient limitation, *ie*, the absence of a linear growth phase, would not occur. "Nutrient-limited" linear growth, unlike light limitation, would be expected to cause permanent physiological changes, such as the observed decreases in photosynthetic capacity (per unit biomass) following logarithmic growth. The coincidence in the onset of linear growth and the depletion of NO_3-N implicate nitrogen as the limiting nutrient in both *P. molle* and *A. nidulans*. However, the possibility that concurrent or near-concurrent limitation by other nutrients such as phosphorus (which in *P. molle* shows similar "luxury" uptake kinetics) cannot be eliminated without further study.

As a consequence of these nutrient uptake patterns, changes in pigments and photosynthetic capacity that are commonly termed senescence effects commence during the period of active carbon assimilation. It is thus essential that accurate growth data be determined as a prelude to all studies of algal senescence.

The culture procedures described here are sufficiently reproducible to permit direct correlations between physiological parameters, even when these are obtained from different series of cultures. Thus additional time-course information, as it accumulates, can be compared with the data reported here, in the hope of providing a better understanding of growth and senescence in these 2 blue-green algae.

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