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EFFECT OF SELECTED DYES ON THE GROWTH OF THE FILAMENTOUS BLUE-GREEN ALGA, *LYNGBYA MAJESCULA*

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ABSTRACT

The effect of four dyes [rose bengal, methylene blue, zinc phthalocyaninetetrasulfonate (ZPS), and erythrosin] on the short-term growth of the filmentous alga, *Lyngbya majescula* Harv., was measured using a Warburg apparatus. Rose bengal and methylene blue reduced the rate of oxygen production by 43 and 35%, respectively; ZPS and erythrosin by about 25%. Reduction in the rate of oxygen evolution was ascribed to photodynamic action caused by production of singlet oxygen with the dye serving as a sensitizer. The hypothesis was verified by a diagnostic test: the inhibiting effect of sodium azide (10^{-4} *M*) on the sensitizer (rose bengal, μM). Presumably the other dyes function in a similar fashion, and their activity is consistent with their ability to generate singlet oxygen. The growth of the alga was measured in outside studies using 5-g samples of *L. majescula* in plastic trays under ambient conditions. Again, rose bengal and methylene blue were the most effective in inhibiting the growth of the alga (measured as change in weight as a function of time); ZPS and erythrosin were much less effective.

Key words: *Lyngbya majescula*, blue-green, filamentous, photodynamic action, rose bengal, dyes

INTRODUCTION

Filamentous algal species comprised the sixth most abundant aquatic plants encountered in Florida in 1984 (14). Some increases occurred because of another problem plant, *Hydrilla verticillata* Royle (14). A calming of wave action occurred as hydrilla reached the surface, allowing filamentous mats to establish.

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Of the various filamentous algae that exist in Florida and the Southeastern United States, *Lyngbya* sp. may be the most troublesome because of its tenacity, resistance to herbicide treatment, and tendency to emit odorous compounds such as geosmin (*trans*-1, 10-dimethyl-*trans*-9-decanol). This compound and others, produced by algae and actinomycetes (7,8,11), can be taken up by fish through several routes and impart off-flavors to the fish flesh (12). Several species of *Lyngbya* are known to produce geosmin, (13) and a *Lyngbya* sp. was abundant in several ponds where fish had extreme off-flavor (4).

Algal growth may be managed in a variety of ways (3,4,5,6,9). An alternate approach, photodynamic action, is considered in this study. Photodynamic action refers to the light-dependent lethal effect that certain colored organic molecules have on organisms. The phenomenon has been researched and reviewed (1,2). It depends upon conversion of ordinary oxygen into an electronically excited singlet state ($^1\Delta_g$) termed singlet oxygen. An application of this phenomenon to management of aquatic plants includes research on the marine dinoflagellate *Ptychodiscus brevis* (1), and on *Hydrilla verticillata* (2).

MATERIALS AND METHODS

Laboratory studies. Four dyes were selected on the basis of previous experience (1) to represent a range of structures as well as a range of effectiveness as sensitizers for the production of singlet oxygen. Methylene blue is a cation; rose bengal, erythrosin, and ZPS (zinc phthalocyaninetetrasulfonate) are anions; and rose bengal and erythrosin are structurally related (both being xanthenylbenzoates that differ in the number and kind of halogens that are present).

Lyngbya sp. was collected from a 30-acre infestation in Horseshoe Lake, Lakeland, Florida. The sample was identified as *Lyngbya majescula* by Ms. Maria Socorro Chan and Professor Clinton J. Dawes, Department of Biology, University of South Florida.

The alga was cultured in 10% Hoagland's solution initially, subjected to gentle shaking to remove contaminants, then cultured in sterile modified Gorham's medium with shaking, followed by transfer using appropriate technique to minimize contamination.

Modified Gorham's medium consisted of: $NaNO_3$, 496 mg; K_2HPO_4, 39 mg; $MgSO_4$ (anhydrous), 36.6 mg; $CaCl_2 \cdot 2H_2O$, 36 mg; $Na_2SiO_3 \cdot 9H_2O$, 58 mg; $Na_2CO_3 \cdot H_2O$, 23.4 mg; Citric acid, 6 mg; NaFeEDTA, 7 mg; dissolved and diluted to 1 liter with glass-distilled water.

Gorham's solution (4 ml) was pipetted into the outer annulus of Warburg flasks. A known amount of alga (10-20 mg) was added to the solution. Central wells of the Warburg flasks were filled with filter paper strips saturated with 10% aqueous KOH to absorb CO_2; the side arms contained 1 ml of 5×10^{-6} *M* dye dissolved in Gorham's medium. The atmosphere was N_2 for photosynthesis experiments. Illumination was provided by fluorescent lamps arranged vertically around the water bath. Observed intensity was 100 $\mu E/m^2/sec$ as measured by a model Li 185A LiCor radiometer/photometer. Water temperature was maintained at 25°C by means of a Sargent-Welsh thermoregulator.

Initial manometer readings were recorded every 15 minutes for 1 hour. Contents of the side arm flasks were then added to the suspension of filamentous alga, and measurements of the manometer changes with time were then repeated after a 30-min. incubation period. During the initial-rate measurement, the side-arm containing the dye was shielded with foil to prevent photodegradation.

Manometer readings, recorded as a function of time (in minutes), were fitted to the best straight line by a least-squares procedure. Slopes of the lines were taken as a measure, in arbitrary units, of the rates of photosynthesis.

Outside Studies. Lyngbya majescula (5 g fresh weight) was cultured in 5 L of Gorham's medium in plastic tubs (33 × 30 cm; 13 cm deep) and maintained on the roof of the Science Center. The light intensity was 1500-1800 $\mu E/m^2/sec$. The temperature range was 20–40°C. There was no difference in the temperature of test or control solutions. The medium was maintained at constant level by daily addition of distilled water. *Lyngbya* samples were removed periodically to check the fresh weight, which was measured by placing the plant sample in Nylon mesh and inserting it in a Teflon centrifuge tube above Kimwipe® laboratory tissue to absorb water during centrifugation at 6000 rpm for 5 min using a Sorvall-DuPont SS-3 centrifuge.

The change in fresh weight (% fresh wt) was taken as the difference in fresh weight at a given time and the initial fresh weight. The percent change in fresh weight was calculated using this equation:

$$\% \text{ change in fresh weight} = \frac{\text{change in fresh weight}}{\text{initial fresh weight}} \times 100$$

RESULTS AND DISCUSSION

The results in Table 1 indicate that rose bengal and methylene blue were the most effective dyes of those studied, for reducing the rate of oxygen evolution. Erythrosin and ZPS were significantly less effective.

The mechanism of inhibition was presumed to be photodynamic action via the production of singlet oxygen. A conventional diagnostic test for singlet oxygen depends upon the fact that 0.1 mM azide decomposes singlet oxygen (1), so if the effectiveness depends upon singlet oxygen, then the effect should diminish in the presence of sodium azide. The effect of azide on the effectiveness of rose bengal in reducing the rate of oxygen production by *Lyngbya majescula* is summarized in Table 2. In the presence of μM dye, a 42% reduction in rate was obtained, a result

TABLE 1. RATE OF OXYGEN EVOLUTION BY *LYNGBYA MAJESCULA* IN PRESENCE OF SELECTED DYES (μM).

Dye	Per cent Decrease in rate after adding dye
Methylene blue	35 ± 1[a]
Rose bengal	43 ± 4
ZPS	23 ± 3
Erythrosin	25 ± 5

[a]mean ± standard error for four different experiments

TABLE 2. EFFECT OF ADDED AZIDE ON THE EFFECTIVENESS OF METHYLENE BLUE IN REDUCING THE RATE OF OXYGEN GENERATION OF *LYNGBYA MAJESCULA* AT 25°C IN MODIFIED GORHAM'S MEDIUM.

System	Normalized Rate[a] Control	Test	% Reduction
Dye, 10^{-6} *M*	1.00 ± 0.05	0.58 ± 0.05	42
Azide, 10^{-4} *M*	1.00 ± 0.002	0.99 ± 0.02	1
Dye (10^{-6} *M*) and Azide (10^{-4} *M*)	1.00 ± 0.03	0.82 ± 0.02	18

[a]Normalized rate of oxygen production, rate of production relative to rate for control for each system. Mean ± S.D.

that was consistent with previous observations (Table 1). In the presence of azide (10^{-4} *M*), only a 1% reduction was observed, a result that was within experimental error. In the presence of μ*M* dye and azide (10^{-4} *M*), the reduction was only 18%, a result which was consistent with the decomposition of singlet oxygen by azide, and thus is consistent with the mechanism that is generally recognized, but which should be verified for individual cases.

The study was extended to examining *Lyngbya majescula* grown outside in small tubs (Fig. 1, 2) during June and July. Consistent results were obtained for experiments, done at two different times. Specifically, the percent change in fresh weight as a function of time was a linear function with a slope of 0.13 ± 0.02 for the first control series (early June) and the slope was 0.110 ± 0.006 for the second control series (late June-early July). There was no statistically significant difference between the two slopes.

The greater light intensity outside resulted in such rapid photodegradation of the dyes that, at the concentration used in the laboratory studies, the color of the dye was not perceptible at the end of the day. For example, for rose bengal (10^{-5} *M*, 30°C, 300 μE/m²/sec) in Gorham's medium exhibited pseudo first-order rate constants for degradation of 0.160 ± 0.012 and 0.243 ± 0.010 hr^{-1} for nitrogen and air atmospheres, respectively (10). This would be equivalent to a half life of 3 hours in air. Thus,

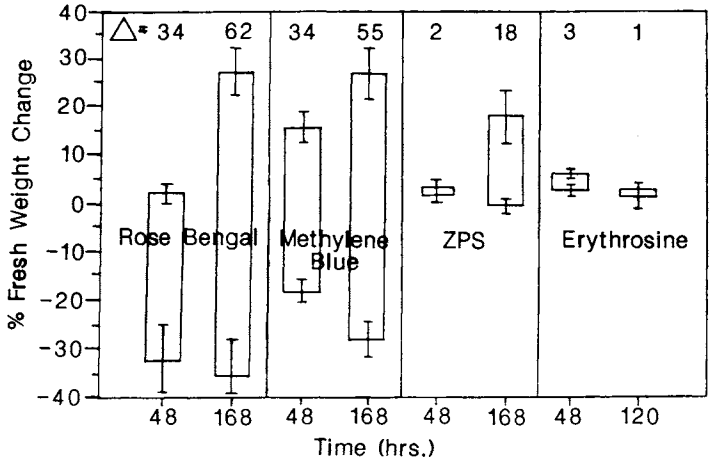

Figure 1. Effect of selected dyes (10^{-4} *M*) on the growth of *Lyngbya majescula* under outside ambient conditions. Change in fresh weight is indicated for control (upper bars) and test (lower bars) with vertical lines showing ± S.D. Δ = algebraic difference in the average % fresh weight change between test and control groups.

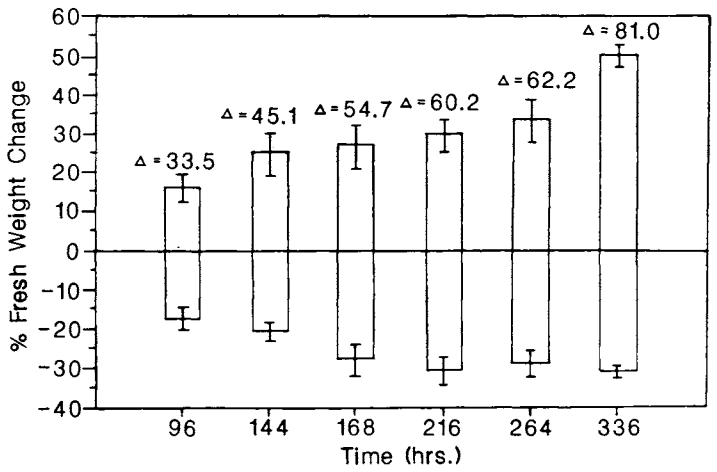

Figure 2. Effect of methylene blue (10^{-4} *M*) on the fresh weight of *Lyngbya majescula* under outside ambient conditions. Change in fresh weight is indicated for control (upper bars) and test (lower bars) as a function of time in hours. The vertical lines indicate ± 1 S.D. Δ = algebraic difference in the average % fresh weight change between test and control groups.

higher concentrations were used in the initial outside studies.

The four dyes were screened for effect on *Lyngbya majescula* (Fig. 1) by comparing the change in fresh weight after 48 and 168 hours. While the absolute values can be compared, it may be more effective to compare the algebraic differences between the fresh weight changes for test and control; the changes in fresh weight as absolute values on a given day were not the same. Based on this comparison, (Fig. 1), rose bengal and methylene blue were again the most effective of the dyes studied on the basis of differences (test versus control) at 48 or 168 hours. Erythrosin was the least effective which is consistent with previous observations (1).

On the basis of the results indicated in Figure 1, the effect of methylene blue was examined in a further study (Fig. 2). Results showed (Fig. 2) the control algae thrived during the course of the study (366 hours), forming mats which floated to the surface, whereas the dye-treated algae were bleached and remained in the bottom of the tubs.

In conclusion, the results of these experiments demonstrated the potential value of photodynamic action using certain dyes (sensitizers) for the management of filamentous algae. Future studies should be directed at more effective application of the dye(s), and at obtaining a cheaper dye that would be more practical for large-scale control projects.

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