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Decomposition of Blue-Green Algal (Cyanobacterial) Blooms in Lake Mendota, Wisconsin

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Decomposition of natural populations of Lake Mendota phytoplankton dominated by blue-green algae (cyanobacteria) was monitored by using oxygen uptake and disappearance of chlorophyll, algal volume (fluorescence microscopy), particulate protein, particulate organic carbon, and photosynthetic ability ($^{14}CO_2$ uptake). In some experiments, decomposition of ^{14}C-labeled axenic cultures of *Anabaena* sp. was also measured. In addition to decomposition, mineralization of inorganic nitrogen and phosphorus were followed in some experiments. Decomposition could be described as a first-order process, and the rate of decomposition was similar to that found by others using pure cultures of eucaryotic algae. Nitrogen and phosphorus never limited the decomposition process, even when the lake water was severely limited in soluble forms of these nutrients. This suggests that the bacteria responsible for decomposition can obtain all of their key nutrients for growth from the blue-green algal cells. Filtration of lake water through plankton netting that removed up to 90% of the algal biomass usually did not cause a similar decrease in oxygen demand, suggesting that most of the particulate organic matter used for respiration of the decomposing bacteria was in a small-particle fraction. Short-term oxygen demand correlated well with the particulate chlorophyll concentration of the sample, and a relationship was derived that could be used to predict community respiration of the lake from chlorophyll concentration. Kinetic analysis showed that not all analyzed components disappeared at the same rate during the decomposition process. The relative rates of decrease of the measured parameters were as follows: photosynthetic ability > algal volume > particulate chlorophyll > particulate protein. Decomposition of ^{14}C-labeled *Anabaena* occurred at similar rates with aerobic epilimnetic water and with anaerobic sediment, but was considerably slower with anaerobic hypolimnetic water. Of the various genera present in the lake, *Aphanizomenon* and *Anabaena* were more sensitive to decomposition than was *Microcystis*. In addition to providing a general picture of the decomposition process, the present work relates to other work on sedimentation to provide a detailed picture of the fate of blue-green algal biomass in a eutrophic lake ecosystem.

Eutrophic lakes often exhibit massive developments of blue-green algae (cyanobacteria), and the decomposition of these blue-green algal populations has been considered to have a number of detrimental effects on lake ecosystems. A number of studies have been carried out in which the rates and processes involved in the decomposition of various eucaryotic algal blooms have been examined, but little work has been done on the decomposition of blue-green algae, despite the fact that blue-green algal blooms are often the most serious manifestation of eutrophication.

Gunnison and Alexander (11) compared various algae for susceptibility to decomposition and showed that blue-green algae lost cell structure rapidly during the decomposition process. Jewell and McCarty (13) and Foree and McCarty (5) have carried out extensive studies of the decomposition of freshwater algal material (from cultures) in laboratory systems. During long-term decomposition studies, they showed that aerobic and anaerobic incubations reached similar endpoints after 100 to 200 days, although the anaerobic systems showed slower initial rates than the aerobic systems. They also showed that there was a fraction of the material which was resistant to decomposition for times as great as 300 days and that this resistant fraction averaged about 40% of the initial amount of material in both aerobic and anaerobic situations. In some lake studies estimates have been made that decomposition could proceed almost to completion. Thus, Kuznetzov (17)

and Moss (21) have estimated from calculations of respiration versus production that 100% of produced material was decomposed in 1 year. It is likely that these estimates were high, due to the difficulty of separating respiration of newly produced material from respiration of old material and of material originating outside the lake system.

Mineralization of material during algal decomposition has received a great deal of attention in recent years, especially because of the interest in phosphorus regeneration in aquatic systems (31). Kinetic data suggest that phosphorus is regenerated quite rapidly during the initial stages of the decomposition process (3, 8). Phosphorus often appears to be released at a more rapid rate than carbon is mineralized, as shown by changes in the C:P ratio of particulate material during the decomposition process (14, 22). Some of the phosphorus released during decomposition appears as free orthophosphate in the water and probably arises as a result of the release of stored phosphorus products during the autolysis process (7) or bacterial lysis (33). Loss of nitrogen from decomposing algal material has also been shown to be more rapid than the loss of carbon in many cases (12, 27, 28). Mineralization of nitrogen also appears to be slower under anaerobic conditions than under aerobic conditions (28).

In the present work, detailed decomposition studies have been carried out on natural phytoplankton populations dominated by blue-green algal species. Decomposition was measured by oxygen uptake (biological oxygen demand [BOD]), by changes in particulate carbon, protein, and chlorophyll, and by microscopic measurement of blue-green algal volume. During the decomposition process, release of inorganic nitrogen and phosphorus were also measured in some experiments. In the present work, careful attention was paid to the influence of various environmental factors on the decomposition process, especially temperature, inorganic nutrients, and aerobiosis. In some experiments, decomposition was carried out by using ^{14}C-labeled blue-green algal material, permitting an analysis of the various forms of carbon which were produced during the decomposition process. Decomposition studies were carried out on samples collected weekly throughout the period when blue-green algal blooms dominated the system, for the 2 years 1976 and 1977. The study was done in Lake Mendota, a eutrophic hard water lake at Madison, Wisc. Lake Mendota has been the subject of a large number of limnological investigations, and during the time that the present studies were done, other work in this same laboratory dealt with the kinetics and physiology of the blue-green algal blooms which were dominating the lake.

MATERIALS AND METHODS

Routine sampling methods. Water samples were collected with 2-liter polyvinyl chloride Van Dorn bottles. Upon recovery, samples were immediately transferred to polyethylene bottles or Teflon bags which were stored in an insulated cooler in the dark until return to the laboratory. Routine limnological data for oxygen, temperature, and light were also obtained at the time samples were collected. For most decomposition studies surface water samples were used, although for a few experiments samples from deeper in the lake were also obtained.

Incubation and oxygen uptake during decomposition. Samples for decomposition studies were placed in 300-ml standard BOD bottles. Samples were incubated in the dark at 20°C. Oxygen concentrations were followed daily or at longer intervals with a Yellow Springs Instrument Co. model 5420 BOD probe with an automatic stirrer and a Yellow Springs Instrument Co. model 54 oxygen meter. Oxygen uptake was followed for approximately 21 days, with readings taken after the first 12 to 24 h of incubation and thereafter every 48 to 72 h. To extend the incubations to 3 weeks, it was often necessary to bubble air through the bottles intermittently to resaturate the water with oxygen. Without such saturation, oxygen initially present in the water samples would have been consumed, in most cases in 24 to 216 h, depending upon the respiratory activity in the sample. Oxygen values were never allowed to fall below 1 mg/liter over the entire period of incubation.

In some experiments anaerobic conditions were used, the samples being gassed with argon and maintained under an argon atmosphere during the incubations.

Chemical analyses. Protein was assayed by the method of Lowry et al. (18). Chlorophyll *a* was measured by extraction in 90% acetone (in 1976) or in a mixture of 90% acetone and dimethyl sulfoxide (1:1, vol/vol) (in 1977). The procedure for chlorophyll measurements followed that of Strickland and Parsons (34), which permitted a measurement of both chlorophyll *a* and phaeophytin.

Two methods were used during this study to measure organic carbon in particulate material. During 1976, the acid-dichromate wet oxidation procedure of Strickland and Parsons (34) was used for all samples. In 1977 an alternate procedure was developed, which involved wet oxidation of the sample in a sealed ampoule, followed by headspace analysis of CO_2 on a Packard model 419 gas chromatograph (Fallon and Brock, submitted for publication). Standard curves were constructed by using glucose standards at concentrations of 50 to 5,000 μg/liter, along with reagent blanks. The gas chromatograph method had the advantage that it permitted analysis of samples over a wider range of organic matter content and also eliminated possible interference from reducing material other than organic carbon. Whenever organic matter

is mentioned in the text, the value was obtained by multiplying the organic carbon value by 2.5 (9). It should be recognized that the conversion of carbon to organic carbon using this factor is only an approximation.

Algal biovolume. To estimate blue-green algal volume in various samples, the fluorescent microscope counting procedure of Brock (1) was used. Different genera were recognized by their characteristic sizes and morphologies. For each sample 35 fields were counted. In some cases where a particular organism was unusually predominant, this organism would be counted to about 500 individuals and thereafter only the subdominant types were counted until 35 fields were reached. Filamentous organisms were recorded as intersections with horizontal and vertical lines on an eyepiece grid (1). By knowing the diameter of the various filamentous organisms, volumes can be calculated using the formula of Olson (26). For the nonfilamentous species, individual cells were counted, and volumes were calculated from formulas for geometric shapes appropriate for the cell.

Inorganic and organic chemical analyses. Particulate phosphorus was analyzed by the persulfate oxidation method of Menzel and Corwin (19). After digestion, soluble phosphate was analyzed by the method of Murphy and Riley (24), as outlined by Strickland and Parsons (34). Assays for nitrite and ammonia as well as soluble reactive phosphorus were carried out on membrane-filtered samples by methods described by Strickland and Parsons (34). The ammonia was analyzed by the hypochlorite method (34). Nitrate was assayed by the procedure of Mullen and Riley (23), in which nitrate is reduced by hydrazine to nitrite.

Photosynthesis. In some decomposition experiments the physiological state of algae was measured by estimating photosynthetic ability, using $^{14}CO_2$ as a tracer. Samples were removed from the treatments during the course of the experiment, and light-stimulated $^{14}CO_2$ uptake ability of the population was measured under standardized conditions (25°C; light intensity, 50 μE/m^2 per s with cool white fluorescent light; 24-h incubation time). In these experiments 1 μCi of [^{14}C]bicarbonate was added to each 18-ml vial to follow the uptake of carbon dioxide. After approximately 24 h of incubation, the contents of the vials were fixed with Formalin and filtered onto Whatman GF/C glass fiber filters. The filters were dried overnight and then exposed to HCl fumes for 3 to 4 h to remove any radioactivity that might have been present as an inorganic carbonate precipitate. Radioactivity of the filters was then determined by liquid scintillation counting.

Labeled algae experiments. One series of decomposition experiments was carried out with ^{14}C-labeled *Anabaena*. The *Anabaena* was prepared by first growing a thick axenic suspension at 30°C in mineral salts medium (6) under 200 μE/m^2 per s. After 5 days of growth, 1 additional volume of fresh mineral salts medium was added to the culture along with 100 μCi of [^{14}C]bicarbonate in a total volume of approximately 250 ml. The culture was then allowed to continue growth for 4 days in the presence of the radioisotope. After the labeling period, the cells were harvested under sterile conditions by centrifugation and washed three times in sterile mineral salts medium. The cells were then dried at 50°C for later use in decomposition studies. The cells were killed by drying, as indicated by lack of metabolic activity in sterile controls. Killing was done in an attempt to ensure that the organic matter contained in them would not be significantly altered, but that the cells would be dead so that the decomposition of material would be due to decomposer activity in the water sample only and not to autolytic processes in the cells. For the decomposition experiments in which the labeled culture was used, water samples were collected from different locations in the lake in 300-ml glass-stoppered BOD bottles. For the anaerobic samples, argon-gassed sampling bottles were used, and the water was allowed to flush through the BOD bottles five times before they were stoppered. Upon return to the laboratory, 5-ml subsamples were removed to 10-ml serum vials closed with neoprene rubber stoppers. For the anaerobic regimes, the vials were flushed with argon. For the aerobic samples, 30-ml vials were used to insure sufficient oxygen in the larger headspace. After 3 h of preincubation at appropriate temperatures, 0.5 ml of the ^{14}C-labeled algal suspension was added to the experimental vials. At each time point the radioactivity in the vials was partitioned into three fractions, gaseous, soluble, and particulate. Soluble and particulate radioactivities were operationally defined based on filtration through a GF/C glass fiber filter (nominal pore size, 1.2 μm at a flow rate of 10 ml/s). Gaseous radioactivity was monitored in two ways. At each time point a 0.4-ml sample of headspace was injected into the gas chromatograph-proportional counter system described by Nelson and Zeikus (25). This system allowed the quantification of $^{14}CH_4$ and $^{14}CO_2$ in the sample. After this, the liquid in the vials was acidified to pH 1 to 2 by the addition of 0.3 ml of 6 N H_2SO_4. The vials were then attached to a nitrogen gas train and bubbled for 0.5 to 1 h at about 300 ml/min through a trapping solution of phenethylamine, methanol, and liquid scintillation fluid (1:1:3). This system quantitatively trapped the carbon dioxide which had been liberated by acidification. The scintillation vials containing trapped carbon dioxide were then counted with liquid scintillation counting. After collection of the carbon dioxide from the vials, the liquid was filtered onto a glass fiber filter. The filters were dried overnight and then counted by liquid scintillation spectrometry. A 0.5-ml sample of the filtrate from each of the sample vials was counted in Aquasol (New England Nuclear Corp.); this provided an estimate of the soluble radioactivity. Correction for quenching was made by the channel ratio method.

RESULTS

Effect of nitrogen and phosphorus on the decomposition process. During the summer period, as a result of the development of massive blue-green algal blooms, soluble nitrogen and phosphorus were virtually eliminated from the epilimnion of Lake Mendota. Because the bacteria carrying out the decomposition process should require nitrogen and phosphorus for

growth, it was considered possible that nitrogen and phosphorus availability might limit decomposition of the blue-green algal material. Ward and Brock (37) had already shown that hydrocarbon-oxidizing bacteria were nitrogen and phosphorus limited during the summer in Lake Mendota. Thus, during 1976 some of the decomposition experiments were set up so that phosphate and/or nitrogen was added to some of the bottles in the form of potassium phosphate or sodium nitrate solutions. These were added such that an additional 150 μg of nitrate nitrogen per liter and/or 150 μg of phosphate phosphorus per liter were added to the amended bottles. Such concentrations were shown by Ward and Brock (37) to be sufficient to overcome N and P limitations in hydrocarbon-oxidizing bacteria in Lake Mendota. Data comparing nutrient-amended samples with unamended controls are presented in Table 1. In no case did addition of N and/or P significantly increase oxygen uptake. The lack of any significant effect of added N or P on oxygen uptake is in contrast to the results of Ward and Brock (37) on hydrocarbon decomposition. It is likely that in the present case, the necessary N and P for the decomposing bacteria were obtained from the blue-green algal material itself, so that the decomposing bacteria were never limited for these elements. Because of the lack of effect of added N and P, all future experiments were done with natural phytoplankton samples without any amendments of inorganic nutrients.

Effect of filtration on oxygen demand. To determine what fraction of the particulate material in the lake was undergoing decomposition, experiments were done in which filtration was carried out to remove different size fractions. In some cases the water sample was filtered through 16 layers of a nylon mesh of 153-μm pore size, which removed most particles larger than about 60 μm (based on microscopic observation). At other times filtration was done through prewashed Whatman no. 1 filter paper, which has a nominal pore size of 11 μm. The filtered samples were then compared with unfiltered samples for oxygen uptake. Data for a number of experiments in 1977 are shown in Fig. 1; similar data were obtained in 1976. The data showed clearly that filtration frequently had little effect on the oxygen demand of the material, although in some cases filtration did significantly reduce the rate or extent of oxygen uptake. Despite the small effect which filtration had on oxygen uptake, filtration removed a large amount of algal biomass, as shown by chlorophyll *a* and protein measurements. Table 2 presents a comparison of the effect of the removal of particulates on the long-term oxygen uptake. The values represent the initial reduction of chlorophyll and protein by filtration and the reduction in BOD which resulted from this filtration. It can be seen that under most conditions over 50% of the chlorophyll and protein was removed by filtration, yet this led to only a very small reduction in oxygen demand. These results appear to indicate that often the bulk of the oxygen demand in the water samples was in smaller particles.

TABLE 1. *Effects of nitrate and phosphate additions on oxygen uptake in surface water samples from Lake Mendota in 1976*

Starting date	Oxygen uptake (mg/liter)[a]			
	Control	$+PO_4^{3-}$	$+NO_3^-$	$+PO_4^{3-} + NO_3^-$
6 July	12.7	13.0	12.4	13.3
12 July	15.7	14.3	15.9	16.1
19 July	11.0	9.4	9.1	11.5
26 July	13.2	15.2	13.1	16.7
2 August	10.0	11.6	9.9	9.1
10 August	10.9	7.4	7.3	6.6
16 August	47.5	43.4	43.4	47.0
23 August	5.2	7.2	4.9	4.8
2 September	6.2	5.5	7.1	5.8
27 October	16.3	17.9	17.3	15.9

[a] Data represent the mean values of duplicate samples in each treatment. Average standard deviation for all treatments was equal to 30% of the mean. Oxygen uptake is given for the duration of each experiment, which was generally 20 days. Additions of nitrate were 150 μg of N per liter, and additions of phosphate were 150 μg of P per liter.

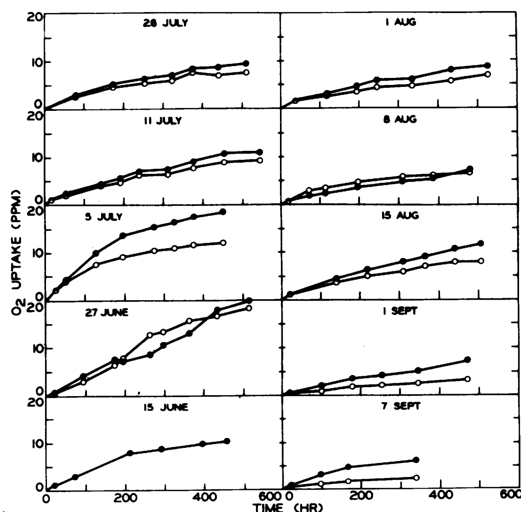

FIG. 1. *Cumulative oxygen uptake versus time for incubations in 1977. The 1976 data were similar. Symbols: ●, unfiltered samples; ○, filtered samples. See Table 2 for filter sizes.*

TABLE 2. *Effect of removal of particulates on long-term oxygen uptake (18 to 22 days) in 1977*[a]

Starting date	Chlorophyll *a* concn (μg/liter)	Protein concn (μg/liter)	Reduction of BOD (mg/liter)
15 June	39.5/40 (99)[b]	1,460/1,970 (74)	1.7/11.2 (15)
27 June	22/60 (37)	1,070/2,570 (42)	1.5/20.4 (7)
5 July	62/110 (56)	1,800/3,980 (45)	6.8/12.0 (57)
11 July	13/41 (33)	1,250/2,870 (44)	2.0/11.3 (18)
26 July	26/61 (43)	1,670/3,970 (42)	1.7/9.2 (18)
11 August	20/47 (43)	3,040/4,250 (72)	1.6/8.6 (19)
8 August	7/22 (32)	1,420/3,460 (41)	0.6/7.0 (9)
15 August[c]	16/27 (58)	1,330/2,030 (66)	3.3/7.9 (31)
1 September[c]	35/38 (92)	3,470/3,940 (88)	4.2/7.6 (55)
7 September[c]	57/60 (95)	1,390/2,130 (65)	4.1/6.4 (64)

[a] Samples were filtered through 16 layers of nylon plankton net to give an effective pore size of 50 to 60 μm. Data for 1976 were similar.

[b] Value for filtered sample/value for unfiltered sample. Numbers in parentheses are percentages and were calculated as follows: (concentration in unfiltered sample − concentration in filtered sample)/(concentration in unfiltered sample) × 100.

[c] These samples were filtered through washed Whatman no. 1 filters (approximate pore size, 11 μm).

TABLE 3. *Oxygen uptake versus suspended solids in sample*[a]

Starting date (1976)	Oxygen uptake/mg of suspended solids[b]	Starting date (1977)	Oxygen uptake/mg of suspended solids
6 July	0.29[c]	15 June	0.12[c]
12 July	0.18	27 June	0.11
19 July	0.23	5 July	0.11
26 July	0.19	11 July	0.11
2 August	0.24	26 July	0.08
16 August	0.05	1 August	0.09
23 August	0.21	8 August	0.10
6 September	0.11	15 August	0.07
27 October	0.05	22 August	0.08
		1 September	0.09
		7 September	0.08

[a] Values are oxygen uptake (milligrams of O_2 per liter) per milligram of suspended solids per day and are based on total oxygen uptake in unfiltered control samples over the first 90 to 160 h of incubation. These data should be comparable to values for 5-day BOD incubation data from other workers.

[b] The values for suspended solids were based on initial chlorophyll concentration and concentration of chlorophyll *a* per milligram (dry weight) of suspended solids from water column data (R. D. Fallon, Ph.D. thesis, University of Wisconsin, Madison, 1978).

[c] The means of 1976 and 1977 data are 0.17 and 0.09 mg of O_2 per liter per mg of suspended solids per day, respectively.

Kinetic analyses. The data in Fig. 1 also indicate that oxygen uptake for the most part could be approximated by first-order kinetics (correlation coefficient, 0.8 for a semilogarithmic plot). From the oxygen uptake values and from the analytical data on the samples incubated, it was possible to calculate oxygen uptake rate per milligram of suspended solids per day (Table 3). The calculations were done for incubation periods of 90 to 160 h, which should provide values comparable to 5-day BOD incubations of other workers. The oxygen uptake data per milligram of suspended solids which we obtained are much higher than values reported by Jewel and McCarty (13) for algal cultures, although our values were similar to values reported by Fitzgerald (4) for Lake Mendota. Thus, it appears that lake water samples may tend to show oxygen uptake values per milligram of suspended solids per day which are higher than those seen in samples from cultured algae, perhaps due to greater heterotrophic activity in the lake water.

From the oxygen uptake data, first-order decay constants were calculated (Table 4). The k value yields an estimate of the rate of loss per day of oxygen demand, and this is an indication of the rate of organic matter decomposition. Although there was considerable week-to-week variability in both years, the average first-order decay constants for both years were virtually the same (Table 4). From the average first-order decay constant calculated (0.042 day^{-1}), it can be estimated that about 85% of the oxygen demand present would on the average be expended in a 20-day period, indicating only a moderate rate of decomposition. This first-order decay constant is much lower than numbers reported for sewage and polluted water systems, which are usually between 0.1 and 0.3 day^{-1} (32). This may indicate a lack of large amounts of readily decomposable soluble organic material in the

TABLE 4. *First-order decay coefficients for BOD incubations*

Starting date (1976)	k (day^{-1})[a]	Starting date (1977)	k (day^{-1})
6 July	0.053	15 June	0.026
12 July	0.050	27 June	0.021
19 July	0.061	5 July	0.054
26 July	0.047	11 July	0.041
2 August	0.063	26 July	0.042
10 August	0.11	1 August	0.037
16 August	0.004	8 August	0.037
23 August	0.036	15 August	0.051
6 September	0.005	22 August	0.06
11 October	0.01	1 September	0.026
27 October	0.01	7 September	0.067
9 November	0.04		

[a] The coefficients (k) were calculated from the following equation: $L_T = L(1 - 10^{-kt})$, where L_T is the BOD at any time t, L is the total BOD, and t is the time in days, according to the graphical method of Thomas (35). The mean and standard deviation for the 1976 data were 0.041 and 0.031 day^{-1}, respectively; those for 1977 were 0.042 and 0.015 day^{-1}.

Lake Mendota samples, such as are often present in heavily polluted systems and are often the cause for rapid rates of oxygen uptake.

Community respiration. Based on changes in oxygen between time zero and the first time point measured, usually 20 to 92 h, one can estimate the short-term oxygen demand per unit of chlorophyll *a* per hour in the water column. From this value one can also estimate the oxygen demand per unit of particulate matter based on the ratio of chlorophyll *a* to particulate organic matter, which had been determined in other measurements. This value yields an estimate of community respiration rate for epilimnetic waters in Lake Mendota. Ratios of oxygen uptake to chlorophyll *a* and oxygen uptake to particulate organic matter for 1976 are presented in Table 5; similar data were obtained in 1977. Calculations were also made of the correlation between initial chlorophyll *a* concentration and long-term BOD (475 h) (Fig. 2). There was a reasonable correlation between chlorophyll *a* and long-term BOD. It is interesting that this correlation exists because, as noted in Table 2, it is sometimes possible to filter much of the chlorophyll out of the sample without having an equivalent effect on the BOD. How is it possible for the BOD of a sample to correlate with the chlorophyll *a* concentration of a sample even if most of the chlorophyll *a* can be removed without any effect on BOD? This might occur if the oxygen-demanding material present predominantly in the small-particle fraction was derived from the algal biomass which was present primarily as a large-particle fraction. It can be hypothesized that the large algal colonies are continually breaking down a subfraction, which is liberating small particles which are then the dominant decomposable material of the water.

Loss of chlorophyll and protein during decomposition. Changes in chlorophyll *a* and particulate protein were also measured during the decomposition experiments, and in 1977 algal volume counts (fluorescence microscopy) were also made (Table 6). The first-order rate

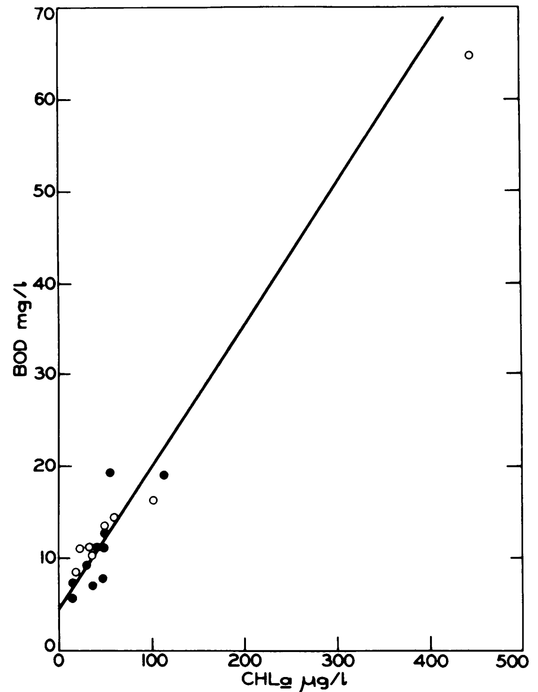

FIG. 2. *Linear correlation of initial chlorophyll a (CHLa) concentration and total oxygen uptake after 450 to 500 h of incubation. Symbols: ○, 1976 data; ●, 1977 data.*

TABLE 5. *Short-term oxygen uptake versus initial concentration of chlorophyll a and estimated initial concentration of particulate organic matter in 1977*[a]

Starting date	Incubation period (h)	Chlorophyll *a* concn (µg/liter)	POM concn (mg/liter)[b]	O_2 uptake (mg/liter)	O_2 uptake (µg/h)/µg of chlorophyll *a*[c]	O_2 uptake (mg/h)/mg of POM[d]
15 June	22	40	3.6	0.8	0.9	0.01
27 June	92	60	5.5	4.2	0.76	0.008
5 July	25	110	6.1	2.6	0.95	0.017
11 July	25	41	2.3	1.4	1.4	0.024
26 July	76	61	5.5	2.8	0.6	0.007
1 August	42	47	4.3	1.6	0.8	0.009
8 August	24	22	3.7	0.6	1.1	0.007
15 August	23	47	7.8	1.0	0.9	0.006
22 August	22	27	2.7	1.3	2.2	0.022
1 September	27	38	2.9	0.6	0.58	0.008
7 September	22	60	4.6	0.8	0.61	0.008

[a] Data for 1976 were similar.

[b] POM, Particulate organic matter. Based on initial chlorophyll *a* concentration and the measured value of chlorophyll *a*/POM for surface waters on the stated date.

[c] The mean and standard deviation for these values were 0.98 and 0.47 µg/h per µg of chlorophyll *a*.

[d] The mean and standard deviation for these values were 0.011 and 0.006 mg/h per mg of POM.

constants appeared to be fairly similar throughout both 1976 and 1977, indicating that the decomposition rate during most of the time was relatively constant. Results of observations on changes in algal volume during 1977 incubations are presented in Table 7. Most blue-green algal genera showed rapid loss, with 95% or more of the initial volume having disappeared in 8 to 22 days. The three filamentous blue-green algal genera *Aphanizomenon*, *Anabeana*, and *Phormidium* all showed volume loss of 99% or more by day 8 of incubation. On the other hand, both types of *Microcystis* species often did not show such rapid decay rate, and the cells appeared better able to maintain themselves in the dark. In some cases no significant change at all was seen in the volume of the two *Microcystis* species over the first 8 days of incubation. This apparent ability of *Microcystis* to maintain cell morphology is consistent with observations (unpublished data) that *Microcystis* sediments readily out of the water column and is able to maintain itself at the mud-water interface through the winter, when there is virtually no light in the bottom of the lake.

Mineralization of algal material. The re-

TABLE 6. *Decay coefficients for chlorophyll a and particulate protein for BOD incubations (unfiltered samples), based on initial and final concentrations*[a]

Starting date (1976)	Chlorophyll *a* k (day^{-1})	Particulate protein k (day^{-1})	Starting date (1977)	Chlorophyll *a* k (day^{-1})	Particulate protein k (day^{-1})
12 July	0.087[b]	0.04[b]	19 May		0.009
19 July	0.065	0.037	15 June	0.096	0.031
2 August	0.052	0.019	27 June	0.065	0.028
10 August	0.048	0.026	5 July	0.074	0.027
16 August	0.048	0.031	11 July	0.048	0.023
11 October	0.04	0.023	26 July	0.083	0.052
18 October	0.038	0.028	1 August	0.078	0.033
25 October	0.065	0.035	8 August	0.052	0.030
			15 August	0.056	0.013
			22 August		0.011
			1 September	0.061	0.031

[a] The decay coefficients (k) are for the following equation: $C_t = C_0\ (10^{-kt})$, where C_t is the final concentration as a percentage of the initial concentration, C_0 is 100%, and t is the time in days.

[b] For 1976 the mean and standard deviation of k for chlorophyll *a* were 0.057 and 0.017 day^{-1}, respectively; these for particulate protein were 0.030 and 0.009 day^{-1}, respectively.

[c] For 1977 the mean and standard deviation for chlorophyll *a* were 0.07 and 0.017 day^{-1}, respectively; those for particulate protein were 0.026 and 0.016 day^{-1}, respectively.

TABLE 7. *Loss of recognizable algal volume (fluorescence microscopic quantification) in BOD incubations in 1977*[a]

Starting date	No. of days after starting date	% of the original volume lost with the following algal genera:				
		Aphanizomenon	*Anabaena*	*Phormidium*	Large-celled *Microcystis*	Small-celled *Microcystis*
15 June	19	>99	>99	>99	ND[b]	ND
5 July	19	>99	>99	ND	ND	—[c]
11 July	19	>99	>99	>99	ND	ND
26 July	13	99	99	96	85	—
	24	>99	ND	ND	99	—
1 August	11	ND	99	95	70	46
	22	ND	ND	ND	86	98
8 August	9	99	ND	95	4	0[d]
	22	>99	ND	ND	70	70
15 August	8	95	97	ND	5	76
	21	>99	ND	ND	88	93
22 August	8	98	ND	ND	0	18
	23	>99	ND	ND	95	>99

[a] Values indicate the percentage of the original volume lost, as measured on the day of incubation.

[b] ND, Not detected (present in starting date sample, but not detected in the sample on the incubation day indicated).

[c] —, None of the indicated genus in the sample on the starting date.

[d] A value of zero means that there was no loss in volume of the indicated genus over the period indicated.

sults of a typical experiment showing the decrease in particulate protein and chlorophyll *a* and the increase in inorganic nitrogen and soluble phosphorus are shown in Fig. 3; increase in soluble phosphate occurred to about the same extent under both aerobic and anaerobic conditions. On the other hand, loss of chlorophyll was almost virtually absent, and loss of particulate protein and liberation of inorganic nitrogen were much slower under anaerobic conditions. The initial form of liberated inorganic nitrogen during decomposition was in the form of ammonia, but in the later stages of some aerobic incubations some of the ammonia was converted to nitrate, probably by a bacterial nitrification process. Although in the experiment presented in Fig. 3 only particulate protein loss was measured, in some other experiments loss of total particulate organic carbon was also measured, and similar results were obtained for the rates of loss of both these parameters.

In some experiments the loss in photosynthetic ability of the blue-green algae during dark incubation was followed by the ^{14}C technique, as described above. In most of the experiments the loss in photosynthetic ability during dark incubation was very rapid and occurred at a rate which was much more rapid than the rate of loss of particulate chlorophyll or protein. In a few experiments this loss of photosynthetic ability did not occur; the difference from one experiment to another may have been due to the nature of the blue-green algal populations present in the water at the time the experiment was done. Unfortunately, insufficient experiments were done to obtain a clear picture of the relationship between algal species and sensitivity to dark incubation.

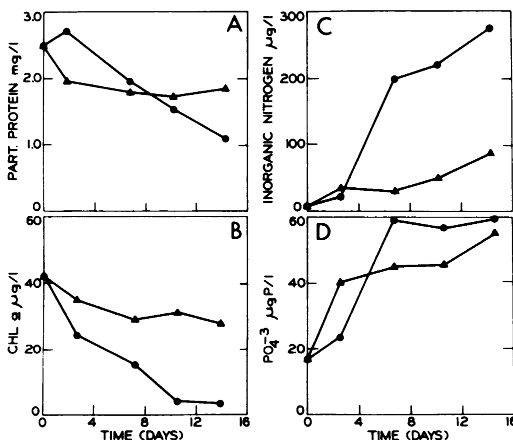

FIG. 3. *Decomposition of natural algal samples. Starting date, 11 July 1977. (A) Particulate protein. (B) Chlorophyll a. (C) Soluble inorganic nitrogen* (*$[NO_3^-]$ + $[NO_2^-]$ + $[NH_4^+]$*). *(D) Soluble reactive phosphorous. Symbols: ●, aerobic incubation; ▲, anaerobic incubation.*

Experiments with ^{14}C-labeled algae. As an alternate method to examine carbon turnover during blue-green algal decomposition, a number of experiments were performed in which ^{14}C-labeled *Anabaena* which had been heat killed by drying at 50°C was used. This material was incubated with water samples obtained from one of the three locations in the lake, the aerobic epilimnion, the anaerobic hypolimnion, and the anaerobic sediment. The results of a representative experiment are shown in Fig. 4. There was an initial rapid rate of decrease of particulate radioactivity, followed by a slowly decreasing rate of decomposition. This initial rapid rate probably represented the rapid solubilization of low-molecular-weight compounds from the cells,

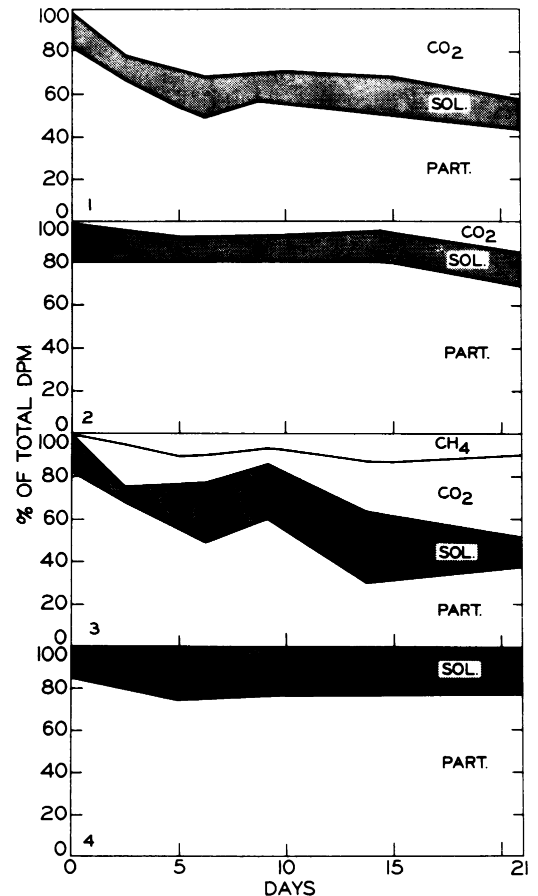

FIG. 4. *Decomposition of ^{14}C-labeled Anabaena. The graphs indicate the relative proportion of radioactivity in each fraction at the various incubation times. (1) Surface water, aerobic, 25°C. (2) Hypolimnetic water, anaerobic, 17°C. (3) Sediment slurry, anaerobic, 17°C. (4) Formalin-killed control, 25°C.*

which were then rapidly mineralized by bacteria in the system. However, even in the control experiment (Formalin-killed material) about 20% of the labeled material was solubilized, presumably due to the presence of freely diffusible soluble materials in the cells. In the material which was not Formalin treated and therefore contained living bacteria, the soluble portion of the material was a smaller fraction, usually around 10%. This soluble fraction probably represented a balance between the formation of soluble material by breakdown of the algal cells and the rapid mineralization of the soluble material to carbon dioxide by bacteria in the system. During aerobic incubation, about 55% of the particulate radioactivity had been lost after 21 days of incubation, and about 84% of the decomposed material appeared as carbon dioxide. In the sample incubated with hypolimnetic water, very little decomposition took place, probably because of the anaerobic conditions and the low density of anaerobic bacteria in the hypolimnetic water. On the other hand, incubation under anaerobic conditions with material from the sediment-water interface led to rapid decomposition of particulate organic matter. Approximately 64% of the particulate ^{14}C had been lost after 21 days of incubation, with approximately 58% of the decomposed material appearing as radioactive carbon dioxide and 14% as methane. However, radioactive methane was not formed in all experiments. These results show that the anaerobic mud-water interface is capable of decomposing blue-green algal carbon at a rate comparable to the aerobic system, even at the lower incubation temperatures. Thus, there seems to be no inhibition of decomposition under anaerobic conditions, provided that an appropriate population of bacteria from the sediment was available.

DISCUSSION

From the data presented in this paper it can be concluded that decomposition of blue-green algal material is a rapid process and that it can occur under both aerobic and anaerobic conditions. Although there is a good correlation between chlorophyll content of the sample and 3-week BOD, observations also showed that removal of most of the chlorophyll in a sample by filtration through a plankton net often did not similarly reduce the BOD. This is consistent with earlier observations on Lake Mendota made by ZoBell (38). It has been generally considered that small particles in aquatic systems often account for the majority of the respiratory activity (30), and our results are consistent with this conclusion. However, in some samples removal of large particles by filtration did result in a substantial reduction of BOD. These samples probably contained material with a lower ratio of nanoplankton to net plankton or contained net plankton with elevated rates of respiration, such that the contribution by the net plankton to overall respiration was greater.

Our results also show that nitrogen and phosphorus never become limiting for decomposition in the blue-green algal blooms, even under conditions where there is no soluble nitrogen or phosphorus in the water. Our results are in contrast with the work of Ward and Brock (37) on hydrocarbon degradation in Lake Mendota and with the considerable work on the decomposition of macrophytes or seaweeds, materials generally richer in carbohydrate material than the blue-green algae (J. Ustach, M. S. Thesis, North Carolina State University, Raleigh, 1969). In the present system, in which blue-green algae dominate the phytoplankton, it appears that nitrogen and phosphorus never become limiting. It is likely that this is due to the fact that the elemental composition of the substrate (blue-green algal cells) is similar to the elemental composition of the decomposer population.

Mineralization studies in the present work showed that the blue-green algal systems under study had rates of remineralization that were similar to those reported by previous workers. Mineralization resulted in the liberation of soluble phosphate and soluble nitrogen. The nitrogen species first liberated was ammonia, but nitrification could occur, resulting in the conversion of some or most of the ammonia to nitrate. Decomposition and mineralization were similar under aerobic and anaerobic conditions, provided that a population of bacteria adapted to the particular conditions was available. However, in hypolimnetic water, where conditions are anaerobic but high population densities of anaerobic bacteria are not present, the rate of anaerobic decomposition was quite slow. If, however, a sediment-water mixture was used which contained a mature anaerobic population, then anaerobic decomposition rates were quite similar to aerobic rates, even though the temperature of the sediment system was lower than that of the aerobic system.

A picture which emerges from the decomposition studies is that loss of chlorophyll is much slower under anaerobic than under aerobic conditions. It is generally thought that the initial breakdown of the porphyrin ring requires the presence of molecular oxygen. However, despite the fact that chlorophyll breakdown was considerably delayed or completely inhibited, decomposition of other components of the algal material could proceed quite well under anaerobic conditions.

Another consistent observation was that the recognizable algal cellular structure disappeared very rapidly during dark incubation, and the rate of disappearance of algal structure was much more rapid than the rate of disappearance of particulate organic carbon. It is assumed that under dark conditions algal autolysis takes place, leading to the loss of morphological integrity, but that the autolyzed material remains in a particulate form, where it then undergoes decomposition. Bacterial lysis may also contribute to losses, but densities of lytic bacteria are maximally 10^3 cells per ml (Fallon and Brock, manuscript in preparation), whereas Daft et al. (2) could only demonstrate rapid lysis of blue-green algal populations at densities of 10^6 cells per ml. The sensitivity of the algae to dark incubation was also indicated by the rapid loss of photosynthetic ability when the algae were incubated under dark conditions. Even 1 or 2 days of incubation in the dark frequently led to a rapid loss of photosynthetic ability by the algae. These results suggest that in the lake the blue-green algal populations have a rapid turnover rate. However, not all of the blue-green algae underwent loss of morphological integrity at identical rates. *Microcystis* seemed to be much more resistant to autolysis than the other blue-green algae when incubated under anaerobic conditions. The rapid loss of cell morphology in blue-green algae has also been noted by Gunnison and Alexander (11), who showed that the cell walls of a number of species of blue-green algae were among the least resistant to decomposition of a number of phototrophic organisms tested. The lack of resistance by the blue-green algae was thought to be due to the chemical nature of cell wall structures of the species tested.

When the Lake Mendota data are compared with those of other workers (3, 5, 20, 27, 28), it appears that loss of particulate organic material in natural samples from Lake Mendota was toward the high end of the ranges observed in other experiments. However, in most of the other studies laboratory cultures were used, which in general tend to be much more stable, probably because they have been grown under conditions of high nutrient input. Overall, decomposition in the Lake Mendota phytoplankton populations showed processes similar to those observed with eucaryotic algae. Carbon tended to be lost more slowly than nitrogen or phosphorus from the particulate material. Also, a tendency for slower initial rates of decomposition under midwater anaerobic conditions was observed. As noted above, this tendency may be due to a lack of a proper bacterial population for decomposition, and it can be concluded that blue-green algae are decomposing more slowly in the upper reaches of the hypolimnion in Lake Mendota than in the surface waters or at the sediment-water interface. Also, comparisons of chemical analyses of particulate material from the water column with analyses of sedimented material (unpublished data) also tended to support evidence from the laboratory studies that organic material, as it settles through the lake, tends to become enriched in carbon relative to nitrogen and phosphorus.

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