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NOTE

ALGAL ORGANIC CARBON EXCRETION IN LAKE MICHIGAN[1]

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ABSTRACT. Algal external metabolites have been claimed to be an important carbon source for aquatic heterotrophic bacteria. We measured the release by algae of recently-fixed carbon by following the accumulation of ^{14}C-organics in filtrates of Lake Michigan samples incubated under natural light for 8 to 26 hours. Pretreatment of samples with an antibiotic and a suite of nonradioactive amino acids, to prevent microbial uptake of excreted products, did not affect the apparent release rates which ranged between 2% and 21% ($X \pm SD = 11.34 \pm 9.32$ mg-C m^{-3} day^{-1}) of short-term autotrophic production. Comparison of our release rates of 0.42 to 1.54 mg-C m^{-3} d^{-1} ($X \pm SD = 0.84 \pm .40$ mg-C m^{-3} day^{-1}) to estimates of bacterial demand made during a simultaneous study suggests that organic carbon released from recently-fixed internal pools may not alone support bacterial production.
ADDITIONAL INDEX WORDS: Bacteria, phytoplankton, carbon cycle, carbon-14.

INTRODUCTION

Although organic compounds are released by phytoplankton, the rates and mechanisms of their transfer into the dissolved organic pool are unclear. Estimates of algal external metabolite production in various environments range between 1 and 70 percent of primary production (Chrost and Faust 1983, Conveney 1982, Iturriaga and Zsolnay 1983, Mague *et al.* 1980, Cole *et al.* 1982). Some evidence suggests, however, that phytoplankton do not suffer large carbon losses through excretion except during stationary and declining phases of growth (Gardner and Lee 1975, Sharp 1977, Sharp 1984, Riemann and Sondergaard 1984). Rather, most organic carbon contributed to the dissolved organic carbon (DOC) pool by phytoplankton comes from degradation of dead cells or spillage during zooplankton grazing (Sharp 1977, Sharp 1984).

Recent evidence indicates that, in some environments, extracellular organic carbon released by phytoplankton may constitute from 10–50% of the carbon assimilated by bacteria (Riemann *et al.* 1982, Larsson and Hagstrom 1982, Brock and Clyne 1984, Bell and Sakshaug 1980). The potential importance of this organic carbon source is suggested by observed coupling of extracellular release and bacteria production. For example, Riemann *et al.* (1982) found that during the period of increase in diatom abundance, release of extracellular organic compounds and bacterial uptake remained low. However, after the peak of the bloom, DOC release increased four-fold (from 3% to 12% of the total ^{14}C fixation), and 30–50% of these extracellular products were utilized by bacteria during the 5-hour incubation. Also, increases in both bacterial abundance and algal extracellular products are at times greater in the daytime than at night, supporting the hypothesis that bacterial growth can be significantly and directly coupled to algal DOC release (Nalewajko *et al.* 1980).

To investigate the importance of phytoplankton excretion to bacteria in Lake Michigan, we measured algal external metabolite production in epilimnetic samples between May and August 1984. We show that metabolite excretion rates ($\bar{X} \pm SD = 0.84 \pm 0.40$ mg-C m^{-3} day^{-1}) are low (20%–60%) relative to recent estimates of carbon needed by bacteria in Lake Michigan.

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MATERIALS AND METHODS

Lake water was collected from the epilimnion of Lake Michigan in opaque Niskin bottles and transferred to dark 4-L LPE carboys at a 100-m station, 26 km west of Grand Haven, Michigan. At shore, within 2-4 h after collection, 0.225 mCi of ^{14}C-bicarbonate (New England Nuclear) were added to 550 mL of lake water yielding a final concentration of 0.409 μCi mL^{-1}. The sample was then split into 76-mL tissue culture flasks. Initial and final bacteria samples were perserved in glutaraldehyde (2% final concentration) and samples were taken for total ^{14}C determination. The 76-mL samples were incubated at ambient temperature under screened natural light for up to 24 h and flask contents were filtered periodically by filtering through 47-mm diameter, 0.2-μm pore size Nuclepore filters. To prevent errors due to release of intracellular ^{14}C into the filtrate, only the first 15-20 mL were collected in glass scintillation vials. The filtrates were then acidified and bubbled with air for 15 minutes to purge the remaining inorganic carbon. Initial tests showed that radioactivity of added inorganic ^{14}C stock was purged to background levels from samples in less than 5 minutes. Five mL of filtrate were removed and placed in a clean scintillation vial followed by 12 mL of scintillation cocktail, forming a gel. The remaining sample was filtered and the filter was placed in a second scintillation vial. To remove inorganic ^{14}C, we used the decontamination procedure of Lean and Burnison (1979). Filters and filtrates were acidified by adding 0.4 mL of 0.5 M HCL. After 3 hours, the filters were dissolved with 0.2 mL of a 10:1 solution of methylene chloride and ethanolamine (Lean and White 1983). Twelve mL of scintillation cocktail (Research Products International, 3a70b) were then added to the filters. Radioactivity was assayed on a Packard Tricarb liquid scintillation spectrometer with sample quenching determined by the external standard method.

There is potential for ^{14}C-organic contamination in the ^{14}C-bicarbonate stock solution (Williams and Yentsch 1976). In this study, however, we used linear regression of time course determinations (N = 4-6); acidified filtrates at time zero served as blanks and also indicated the organic contamination in that particular stock. While these blanks ranged between 17.4 and 257.2 dpm mL^{-1} over the season, blanks within each stock solution varied only slightly.

Bacterial abundance was determined by the acridine orange direct count (AODC) method (Hobbie *et al.* 1977).

Two treatments were done to minimize bacterial uptake of ^{14}C-labeled algal excreta. In the first treatment, a solution of 17 amino acids (SIGMA) was added to 550 mL of raw lake water yielding a final concentration of 0.435 μM. Amino acids were added to provide an alternative organic substrate for the bacteria, presumedly diluting uptake of ^{14}C-labeled organics released by the phytoplankton. This amino acid concentration did not affect the autotrophic activity (Fig. 1). For the second treatment, the antibiotic gentamicin sulfate (SIGMA) was added to 550 mL of lake water (yielding a concentration of 0.02 mg mL^{-1}) to reduce bacterial uptake of ^{14}C. Preincubation at this concentration for 90 minutes is bactericidal (Chrost 1978); thus, to ensure bacterial inhibition, we preincubated for 90 minutes in the dark. Gentamicin also did not affect ^{14}C uptake. This is consistent with similar studies using gentamicin (Iturriaga and Zsolnay 1981). These samples were then treated as described above for the raw lake water.

RESULTS

Autotrophic ^{14}C uptake rates for samples treated with gentamicin or amino acids were not substantially different from the raw lake water samples, indicating that the treatments did not affect photosynthesis (Table 1). Bacteria abundances, determined at the beginning and end of each experiment, also showed no significant changes (pairwise t-test α = .05). Bacteria concentrations ranged

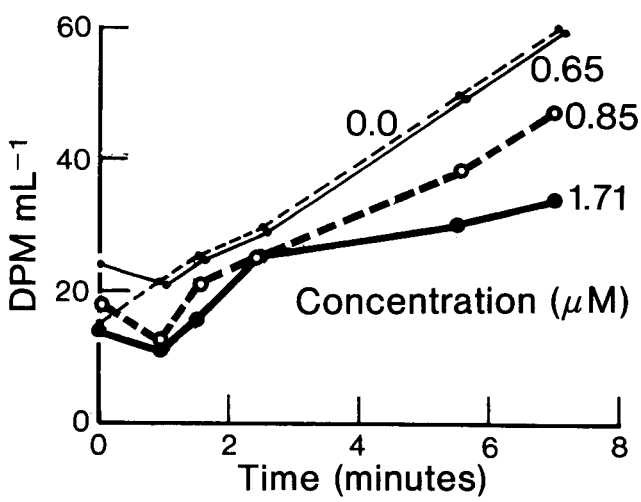

FIG. 1. Time-course uptake of ^{14}C-bicarbonate in the presence of different added amino acid concentrations.

TABLE 1. Uptake of ^{14}C-bicarbonate during 6 to 8-hour incubations under different treatments (mg C m^{-3} day^{-1}).

Date	Treatment		
	Raw	Amino Acids	Antibiotic
23 May	2.66	1.59	2.83
11 June	43.27	43.52	36.09
20 June	39.52	30.59	25.66
26 June	4.09	4.87	5.19

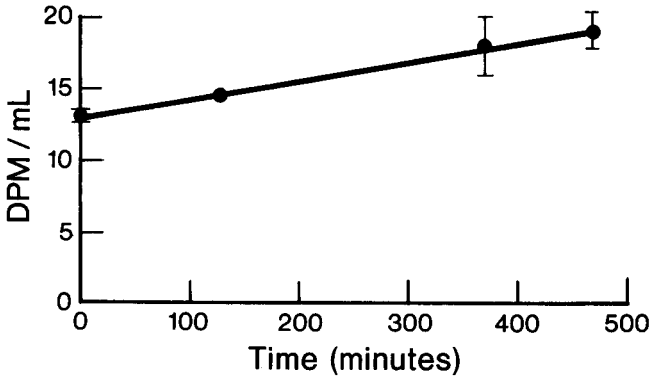

FIG. 2. Accumulation of radioactivity in filtrates of the 23 May 1984 experiment. Error bars represent +/− one standard deviation of the mean of treatments. Regression is significant at the 95% confidence level.

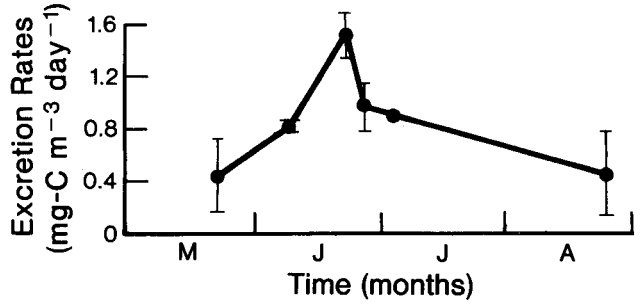

FIG. 3. Rates of release of recently fixed carbon for epilimnetic samples from Lake Michigan. Error bars represent +/- one standard error of the mean of the different treatments.

from 4.78×10^5 - 11.68×10^5 cells mL^{-1} over the season.

Organic carbon release rates were determined from accumulation of radioactivity in filtrates over time ranging from 8 to 26 hours. Plots of activity versus time remained linear (Fig. 2), demonstrating that DOC release rates were relatively constant during both light and dark periods. This is consistent with culture studies (Mague *et al.* 1980). Excretion rates were determined for each treatment separately; however, because we found no difference among treatments, the data were combined and a single excretion rate was determined for each date. Resulting rates from May through August 1984 ranged between 0.42 and 1.54 mg-C m^{-3} day^{-1}, with highest rates found in late June and lowest rate in late May (Fig. 3).

DISCUSSION

In our experiments we measured excretion of recently assimilated carbon while attempting to reduce heterotrophic uptake of excreted labeled organics without affecting primary production. We found that neither treatment had an apparent affect on the excretion rates. As both of these treatment should have reduced uptake of labeled excreta by bacteria, these results suggest that bacteria were not using this exudate of recently assimilated carbon as a significant carbon source. However, these results may also be explained by rapid cycling of the excreted carbon assimilated by the bacteria in the untreated sample. Sufficient grazing on the bacteria by microheterotrophs could return labeled carbon to the water both by grazer excretion and respiration and by release during filtration (Goldman and Dennett 1985). This may be of particular concern if the grazers are similar to the delicate flagellates reported in other environments (Fahnenstiel *et al.* 1986, Caron *et al.* 1985, Sieburth 1984), because grazing appears to be an important control on bacteria populations in Lake Michigan (Scavia *et al.* 1986; Gardner *et al.*, Great Lakes Environmental Research Lab, Ann Arbor, Michigan, unpublished manuscript).

There is also evidence that phytoplankton release freshly labeled low molecular weight products (< 10,000 daltons) initially, followed by high molecular weight compounds (Sondergaard and Schierup 1982, Iturriaga and Zsolnay 1983, Iturriaga 1981, Chrost and Faust 1983). Bacteria utilize low molecular weight compounds at a rapid rate (Iturriaga 1981, Nalewajko and Lean 1972, Nalewajko *et al.* 1976) and then may also release high molecular weight products (Iturriaga and

Zsolnay 1983, Nalewajko and Lean 1972, Sondergaard and Schierup 1982, Dunstall and Nalewajko 1975). This could also explain why ^{14}C-DOC in the raw lake water filtrate was as high as in the samples treated to inhibit bacterial uptake; bacteria may rapidly take up the recently assimilated, low molecular weight carbon released by algae and subsequently excrete the labeled carbon as high molecular weight ^{14}C-DOC. Both this and the grazing scenarios suggest hypotheses for unaltered samples to be as high as those treated to reduce uptake of the released isotope by bacteria. Another suggestion could be that the bacteria are not substrate limited and that the small amount of organics being released by phytoplankton is of little significance to bacteria as a carbon source. A study done on Lake Michigan in 1984 showed that the uptake of added amino acids was slow and constant during incubations, indicating that the organic carbon concentration in the lake is not controlling the bacterial growth (Gardner *et al.* unpublished data). We may also have been unable to detect differences among treatments because excretion rates were very low. Excretion rates may have been underestimated because the algal excretory pools were not labeled immediately (Kaplan and Bolt 1982, Mague *et al.* 1980). In our experiments, accumulation of isotope in the filtrate was linear over time suggesting that the active pools were sufficiently labeled. This linearity may also be expected, however, if excretory pools were labeled very slowly. While incubations in the present study were for up to 26 hours only, analysis of a recent experiment (Scavia and Laird unpublished data) revealed linear accumulation of filtrate isotope for 53 hours. With typical algal generation times during midsummer of 2-5 days (Fahnenstiel and Scavia, Great Lakes Environmental Research Laboratory, Ann Arbor, MI; unpublished data), one would expect significant internal pools to have been labeled during this experiment. Most other potential artifacts of excretion experiments lead to overestimates; for example, cell-breakage, filtration artifacts (Goldman and Dennett 1985, Fuhrman and Bell 1985) and insufficient purging of inorganic ^{14}C. Thus, we feel true release rates of recently fixed autotrophic carbon are likely no higher than our estimates.

Expressed as a percentage of our short-term autotrophic production rates ($X \pm SD$ = 11.34% ± 9.32%), these excretion values are similar to those reported for other oligo-mesotrophic lakes (7.35%–48.93%, Chrost and Faust 1983; 6%–13%, Lovell and Konopka 1985; 10%–13%, Chrost 1978) and eutrophic Lake Mendota (23%–56%, Brock and Clyne 1984). Our production values were sometimes lower than those determined from *in situ* incubations or from depth- and time-integration of production-versus-irradiance curves established in larger bottles and shorter incubations (Fahnenstiel and Scavia, Great Lakes Environmental Laboratory, Ann Arbor, MI, unpublished data). For the experiments in which our uptake rates were similar to the *in situ* rates, excretion percentages ranged between 2% and 5%. These values are comparable to those reported for Lake Erie (1.2%) and Lake Ontario (4.4%) (Lee and Nalewajko 1978).

One motive for this study was to evaluate the flux of carbon from primary producers as a source to planktonic heterotrophic bacteria. In a related study (Scavia *et al.* 1986), bacterial net production was estimated for the same region in Lake Michigan as this study and during the same time periods. While it is difficult to assess actual carbon production requirements from their thymidinie-based experiments, Scavia *et al.* (1986) suggest that even under the most conservative set of assumptions concerning thymidine-to-growth conversion factors, bacteria carbon content, and growth efficiency, our excretion rates of 0.42–1.54 mg-C m^{-3} day^{-1} can support only between 20% and 60% of the bacteria production. Because less stringent assumptions are likely, other carbon sources must be sought to balance the bacterial production. Labile dissolved organic matter may be derived from sources other than extracellular release of recently fixed carbon by actively photosynthesizing phytoplankton (Storch and Saunders 1978, Sharp 1984). Other sources may include algal autolysis, "sloppy-feeding" (cellular pool spillage during zooplankton feeding on phytoplankton), and zooplankton excretion (Azam and Ammerman 1984, Sharp 1984, Rai 1984, Copping and Lorenzen 1980). These sources originate ultimately from autotrophic pelagic production and are therefore subject to the same constraint. That is, only a small portion of algal production can be transferred to organic carbon available to bacteria via these routes. Otherwise the existence of substantial algal and zooplankton populations would not be possible. While production of low-molecular weight DOC from high-molecular weight DOC of allochthonous origin is one source that is distinct from phytoplankton production and is important in smaller lakes (Cole *et al.* 1984), mass balance

calculations indicate that it must be unimportant in Lake Michigan. Temporal and spatial disequilibrium of autotrophic DOC production and subsequent bacterial use could possibly explain the apparent imbalance in Lake Michigan (Scavia *et al.* 1986).

While we show that phytoplankton in Lake Michigan do release organic carbon, data reported here indicate that the contribution of excreta derived from recently assimilated carbon is not sufficient to satisfy the immediate demands of pelagic bacteria. Our rates, expressed as a percent of autotrophic production, are in the lower range of those reported for smaller meso- and eutrophic lakes, but similar to those reported for other Great Lakes. It is not clear at this time why these larger lakes might be different; however, the key to future examination of alternative carbon sources and pathways may lie in the different physical regimes experienced by the plankton of large versus small lakes.

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