

# Significance of Algal Excretory Products for Growth of Epilimnetic Bacteria

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Light-stimulated uptake of  $^{14}\text{CO}_2$  and differential filtration through Nucleopore filters were used to estimate the significance of phytoplankton excretion as a source of bacterial carbon in water samples collected at different seasons of the year in Lake Mendota, Wis. On an annual basis, about 14% of the estimated bacterial production was accounted for by algal excretion, although at certain times of year the fraction of bacterial carbon derived from algal excretion was considerably higher. About 20% of the annual primary production was estimated to pass through the bacterial component.

Dissolved organic carbon (DOC) may serve as a primary growth substrate for free-living bacteria in aquatic systems. The DOC pool in lakes has several sources: runoff from the land, defecation by aquatic animals, lysis of plankton cells, and excretion by phytoplankton. Much of the DOC pool may consist of highly polymerized macromolecules, such as humic acids derived from terrestrial sources, and may thus be partially unavailable for use by the epilimnetic bacteria. The portion of the DOC pool consisting of low-molecular-weight compounds, such as simple sugars, carboxylic acids, amino acids, and short peptides, originates primarily from the plankton in the water column and is probably turned over rapidly. It is commonly accepted that bacteria play a major role in consuming the low-molecular-weight organic materials.

The DOC released by phytoplankton may be the result of cell lysis upon the crash of an algal bloom or may be excreted during photosynthesis by a healthy population. Release by a healthy population is generally defined as "excretion."

It is generally accepted that heterotrophic bacteria directly utilize products excreted by algae (5, 12, 19, 20) and that bacterial production and primary production are closely correlated (16). However, carbon excreted from phytoplankton may be of greater or lesser significance for bacterial growth, depending on the ecosystem under study. Excretion of dissolved carbon by phytoplankton and subsequent uptake by heterotrophic bacteria has been studied with  $^{14}\text{C}$  tracer and differential filtration methodology in natural samples (5-7, 12, 21). However, wide variation in algal excretion rates, bacterial production rates, and DOC fluxes have been reported in the literature. Thus, the question arises as to the general significance for heterotrophic bacteria of algal excretion.

The purpose of the work presented here was to use differential filtration to assess the importance to epilimnetic bacteria of carbon excreted by algae in Lake Mendota, Wis. This was done by estimating with the  $^{14}\text{C}$  technique the uptake by the bacteria of algae-excreted photosynthetically fixed carbon and relating the uptake to total bacterial production rates (15). The carbon cycle has been extensively studied in Lake Mendota, and the seasonal cycle of phytoplankton biomass, particulate organic carbon concentrations (8), and planktonic bacterial production (15) have been

measured. Thus, the fraction of the total bacterial production that can be supported by the excreted carbon can be estimated. In the present investigation, the flux of added  $^{14}\text{C}$  bicarbonate was measured in natural lake samples through the phytoplankton to the soluble DOC pool and on into the heterotrophic bacteria, using differential filtration.

## MATERIALS AND METHODS

**Natural samples.** Investigations were carried out with natural populations of phytoplankton obtained from Lake Mendota, which is a eutrophic hardwater lake under ice cover from late December to early April. A thermocline develops in late June, and the hypolimnion becomes anaerobic until fall turnover in October. In the summer there is usually a heavy bloom of cyanobacteria in the epilimnion and smaller blooms of diatoms and green algae in the spring and fall (8). Bacterial production generally follows algal production over time (15).

Samples were collected in 1-liter polyethylene bottles from a depth of 0.5 m. The samples were kept cool and dark, and isotope experiments were immediately carried out in a nearby laboratory. The time between sampling and initiation of incubation was generally 15 to 20 min.

**$^{14}\text{C}$  bicarbonate uptake, fixation, and excretion.** Each water sample was distributed into a series of sterile 25-ml screw-capped test tubes with no headspace. The samples were preincubated in a water bath at in situ temperature for 45 min. The light intensity was set at 130 microeinsteins/m<sup>2</sup> per s, provided by two fluorescent cool white lamps. This light intensity is generally just saturating for phytoplankton photosynthesis but does not cause inhibition (V. J. Watson and T. D. Brock, submitted for publication).  $\text{NaH}^{14}\text{CO}_3$  (10  $\mu\text{Ci}$ ; New England Nuclear Corp.) was added to each tube with a sterile 1-ml syringe, and the tubes were quickly capped with Teflon-lined screw caps, inverted several times, and placed in a water bath.

At each time point, tubes were removed from the water bath and placed on ice in the dark until filtration. Filtration was begun immediately and completed within 1 h. Each sample was filtered through a series of polycarbonate filters (Nucleopore) held in polycarbonate filter holders (Nucleopore). The 3- and 1- $\mu\text{m}$  fractions were filtered by vacuum, and the filtrate was then poured into a 20-ml syringe and pushed slowly through the 0.4- and 0.2- $\mu\text{m}$  filters, the final filtrate being collected in a 50-ml test tube. The filters were rinsed with 10 ml of 0.2- $\mu\text{m}$ -filtered lake water, dried, and

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placed in HCl fumes overnight. The acid fumes remove any inorganic carbon compound (i.e.,  $\text{CaCO}_3$ ) that may have formed during the incubation period. The filters were then placed in 10 ml of scintillation fluid and counted in a liquid scintillation counter. Corrections for the efficiency of the scintillation counter and quenching were made by using commercially prepared quench standards and the channels-ratio method.

To measure excretion, 5 ml of the final filtrate was placed in a scintillation vial, 5 drops of concentrated HCl was added, and the solution was allowed to equilibrate for 24 h before the addition of Aquasol (New England Nuclear) liquid scintillation fluid. Tests showed that all radioactive carbon dioxide was removed by this procedure. In each experiment, parallel nonradioactive samples were filtered through the various pore-size filters for determination of bacterial number (see below) and analysis of chlorophyll (as a measure of the sizes of phytoplankton colonies).

**Acridine orange direct counts.** Bacterial counts were performed by the acridine orange method of Hobbie et al. (10), using 0.2- $\mu\text{m}$  polycarbonate filters (Nuclepore) that had been stained with acid black stain. The samples were observed with a Zeiss fluorescence microscope equipped with a II FL vertical illuminator, a high-pressure mercury bulb (HBO; 200 W), exciter filters BG 12 and 38, barrier filter 50, and an oil immersion 100 $\times$  objective with 12.5 $\times$  eyepieces. At least 600 bacteria were counted in 10 or more fields for each sample. Controls were run for each counting session using filtered lake water or acridine orange alone.

**Chlorophyll assay.** Chlorophyll was measured by the method of Strickland and Parsons (17).

**Calculations.** The total light-stimulated carbon fixation was defined as the sum of disintegrations per minute found in all fractions, particulate and soluble, less uptake by samples incubated in the dark. Radioactivity from samples incubated in the dark was similar to that from samples incubated in the light in the presence of 1% (vol/vol) formaldehyde. The percent total excretion was calculated by dividing the sum of the particulate fractions less than 3  $\mu\text{m}$  (assumed to be excreted radioactivity incorporated into bacteria) and the soluble fraction by the total carbon fixed. The percent net excretion (that carbon not taken up by the bacteria) was calculated by dividing the soluble fraction by the total carbon fixed. Assays of dissolved inorganic carbon in the lake water (8) were used to convert disintegrations per minute to amount of carbon fixed.

The uptake rate of carbon excreted by bacteria was calculated from the rate of uptake in the 0.2- to 3- $\mu\text{m}$  particulate fraction. An assumption of this calculation is that the specific radioactivity of the excreted material is not diluted by nonradioactive material already present in the water. It is likely that this assumption is false, so that the bacterial uptake rates should be higher than the calculated rates by some unknown factor.

An average value for total bacterial production,  $4 \times 10^3$  mg of C per g of bacterial C per day, was used in some calculations. This value was derived from the dark sulfate uptake data of Pedros-Alio and Brock (15). It was also assumed (15) that the average bacterial cell in Lake Mendota has a carbon content of  $15 \times 10^{-15}$  g.

## RESULTS

**Phytoplankton and bacteria in the lake.** Phytoplankton biomass, as measured by chlorophyll, varied over the year from a low of 1.7  $\mu\text{g/liter}$  in late spring to a high of 44.8  $\mu\text{g/liter}$

in the late summer. During the year of the present study (1981 to 1982) there were three major peaks of chlorophyll, late May and early September 1981 and middle April 1982. Other data on phytoplankton development in Lake Mendota can be found in papers by Konopka and Brock (11), Fallon and Brock (8), Pedros-Alio and Brock (15), and Watson and Brock (submitted).

The concentration of bacteria in the water samples varied over the year from about  $5 \times 10^4$  cells per ml in late spring to  $4 \times 10^9$  cells per ml in the late summer (this latter number represents an unusually high value during the crash of an algal bloom). However, for most of the year the concentration was  $5 \times 10^6$  to  $8 \times 10^6$  cells per ml. These data are similar to those reported by Pedros-Alio and Brock (15).

**Algal photosynthesis and excretion.** For the majority of the uptake experiments, most of the radioactivity was taken up by the >3- $\mu\text{m}$  fraction. A measurable amount of radioactivity was left in the soluble fraction, and a measurable amount of the radioactivity was associated with the 0.4- to 3- $\mu\text{m}$  particulate fraction. No chlorophyll was ever found in the smallest fraction (0.2 to 0.4  $\mu\text{m}$ ), and only a small amount (less than 5%) of chlorophyll passed through the 3- $\mu\text{m}$  filter. (As described by Fallon and Brock [8], nanoplankton that would pass through a 3- $\mu\text{m}$  filter are rare or nonexistent in Lake Mendota.) Of the free-living bacteria (not attached to particulate material), about half passed through the 0.4- $\mu\text{m}$  filter and were retained on the 0.2- $\mu\text{m}$  filter, but few were retained by the 3- $\mu\text{m}$  filter. These latter observations are similar to earlier data of Pedros-Alio (C. Pedros-Alio, Ph.D. thesis, University of Wisconsin, Madison, 1981). The >3- $\mu\text{m}$  fraction can be considered the algal fraction, and the 0.2- to 3- $\mu\text{m}$  fraction can be considered the bacterial fraction. The photosynthetically fixed carbon that is excreted by the phytoplankton should be in two fractions, the soluble fraction (<0.2- $\mu\text{m}$  fraction) and the bacterial fraction (0.2- to 3- $\mu\text{m}$  fraction), since all of the radioactivity fixed by the heterotrophic bacteria must pass through the algae. Total excretion was thus calculated as the sum of all these fractions. The percent bacterial uptake was calculated as the proportion of radioactivity found in the 0.2- to 3- $\mu\text{m}$  fraction relative to the total amount of radioactivity fixed.

A summary of the algal excretion data is presented in Table 1. For all experiments, incubation times of 1, 2, and 4 h were used. To a first approximation, the rates of algal photosynthesis and excretion and bacterial uptake were

TABLE 1. Algal photosynthesis and excretion

Sampling date (1981)	Total carbon incorporated (mmol of C/liter/h)	Algal excretion, soluble only (% of incorporated)	Algal excretion, soluble + bacterial (% of incorporated)
3/27	$6.6 \times 10^{-4}$	28	56.3
5/21	$3.69 \times 10^{-4}$	29.4	55.4
6/2	$7.88 \times 10^{-5}$	25	26.5
6/26	$8.81 \times 10^{-4}$	9.9	23.7
8/4	$6.08 \times 10^{-4}$	28.6	43.9
9/3	$1.01 \times 10^{-3}$	74.5	74.5
9/10	$1.37 \times 10^{-3}$	22.9	36.3
9/29	$6.98 \times 10^{-4}$	18.6	38.2
10/6	$5.89 \times 10^{-4}$	2.4	26.4
10/13	$5.24 \times 10^{-4}$	0	32.8
11/3	$2.12 \times 10^{-4}$	26.9	48.1
4/14 <sup>a</sup>	$9.28 \times 10^{-4}$	0.8	34.2
Average		22.3	41.4

<sup>a</sup> 1982.

TABLE 2. Bacterial carbon production

Date (1981)	Total bacterial count $\times 10^6$	Bacterial carbon production			Bacterial carbon uptake (% of total algal excretion)
		Calculated from total count (mmol of C/liter/h)	From assimilation of algal products (mmol of C/liter/h)	From algal products (%)	
3/27	4.96	$1.03 \times 10^{-3}$	$1.85 \times 10^{-4}$	17.9 (8.9) <sup>a</sup>	28
5/21	0.049	$1.02 \times 10^{-5}$	$7.54 \times 10^{-5}$	>100	20
6/2	7.01	$1.46 \times 10^{-3}$	$1.53 \times 10^{-5}$	1.0 (0.5)	19
6/26	8.46	$1.76 \times 10^{-3}$	$1.49 \times 10^{-5}$	8.5 (4.2)	17
8/4	7.73	$1.61 \times 10^{-3}$	$6.6 \times 10^{-5}$	4.1 (2.0)	11
9/3	6.89	$1.43 \times 10^{-3}$	$1.04 \times 10^{-4}$	7.3 (3.6)	10
9/10	3,550	$7.39 \times 10^{-1}$	$1.65 \times 10^{-4}$	0.17 (0.008)	12
9/29	2,000	$4.17 \times 10^{-1}$	$1.36 \times 10^{-4}$	0.033 (0.016)	19
10/6	4.08	$8.54 \times 10^{-4}$	$1.41 \times 10^{-4}$	16.6 (8.3)	24
10/13	2,800	$5.83 \times 10^{-1}$	$1.62 \times 10^{-4}$	0.028 (0.014)	31
11/3	5.78	$1.20 \times 10^{-3}$	$2.55 \times 10^{-5}$	2.1 (1.0)	12
4/14	2.50	$5.21 \times 10^{-4}$	$3.58 \times 10^{-4}$	68.6 (34.3)	39
Average				(13.6)	

<sup>a</sup> Numbers in parentheses are the values obtained if it is assumed that 50% of the bacterially assimilated carbon is respired rather than incorporated into cell material.

<sup>b</sup> 1982.

linear over this time period, so that it seemed appropriate to present the data as average hourly values calculated from the total incubation period.

As found in other studies (8, 11; Pedros-Alio, Ph.D. thesis), there was considerable variation in algal photosynthesis throughout the year. There was also considerable variation in algal excretion rate, with an average excretion rate for the year of 41.4% of the total light-stimulated <sup>14</sup>C incorporation. The excretion values are higher than those presented previously (11) because in earlier work excretion was estimated only from the fraction of radioactivity that passed the 0.4- $\mu$ m filter, so that the excreted material present in the bacterial fraction would have been missed.

**Bacterial production and incorporation of algal excretory products.** A summary of the bacterial data is given in Table 2. In this table, total bacterial counts are given as well as the rate of bacterial uptake of excreted material. Bacterial carbon assimilation rates in this table were calculated in two ways: (i) from the radioactivity present in the >0.2- to <3- $\mu$ m fraction and (ii) from the total bacterial count in the sample, assuming a carbon content per cell of  $15 \times 10^{-15}$  g and an average production rate of  $4 \times 10^3$  mg of C per g of bacterial carbon per day (15). Also presented in Table 2 are calculations intended to express the percentage of bacterial growth (calculated from total numbers and average production rates) that could be accounted for by algal excretion. Throughout most of the year a significant proportion of bacterial production can be accounted for by algal excretion. Only during the fall of the year, when extensive algal decomposition was occurring, did excretion account for only a small fraction of the bacterial production. It should be noted that during this fall period, a marked increase in bacterial numbers took place. It is likely that the bacteria seen at that time in the water had developed primarily on decomposing algal material, whereas at other times of the year they were consuming algal excretory products and nonalgal-derived organic materials, such as humic acids. An additional assumption that must be made in deriving bacterial production from carbon excretion to bacterial production from total counts is the fraction of the bacterially incorporated radioactive carbon that is not assimilated into cell material but is respired. In the present work, it was assumed that

50% of the algal excretory material taken up by the bacteria is respired rather than assimilated.

## DISCUSSION

These results, taken together with the earlier work of Pedros-Alio and Brock (15), provide strong support for the idea that epilimnetic bacterial production in Lake Mendota is closely tied to algal production and that throughout much of the year a significant portion of bacterial carbon is derived from algal excretion. These results also agree with the work of others on both freshwater and marine systems (5, 12).

A variety of interactions between algae and bacteria occur in aquatic systems (5), but one of the major processes is the production by algae of organic carbon materials suitable for bacterial assimilation. Straskrbova and Komarkova (16), from studies of seasonal changes in bacterioplankton and phytoplankton, showed that bacterial peaks usually lagged slightly behind algal maxima, and similar results have been obtained by Pedros-Alio and Brock (15) in Lake Mendota. Although it is obvious that other sources of bacterial organic carbon exist in aquatic systems, phytoplankton constitute the major source in most cases. In aquatic habitats such as deeply stained lakes, where poor light penetration limits phytoplankton production and high humic acid content provides a significant source of bacterial nutrients, phytoplankton are likely not to be the major source of bacterial carbon and energy. Sediment resuspension also may constitute a significant source of bacterial carbon at certain times of the year (13). However, in large, clear water lakes, the open oceans, and other habitats in which loading with allochthonous materials occurs at low rates, bacteria must be virtually dependent on phytoplankton.

On the other hand, the dependence of bacteria on phytoplankton need not be a direct one via immediate utilization of excreted organic carbon by living phytoplankton. Extensive decomposition of phytoplankton does occur (5, 8). In Lake Mendota, it seems that phytoplankton decomposition constitutes the major source of bacterial carbon during the fall of the year, when phytoplankton breakdown occurs at an extensive rate. Although phytoplankton blooms do crash during the summer (8), the extent of decline is not as great as

in the fall, and the contribution of decomposition to bacterial production is consequently probably considerably less.

Excretion of organic materials by living phytoplankton has been extensively studied in many systems (5, 9, 14, 18). Diel cycles of dissolved carbohydrate in the oceans can also be interpreted in terms of phytoplankton release and bacterial incorporation (2). In recent years it has become widely accepted that excretion measurements that do not take account of the rapid assimilation of organic carbon by bacteria may considerably underestimate true algal excretion rates. In the present work, it has been shown by differential filtration that about half of the material excreted by algae is quickly assimilated by bacteria, so that the actual excretion rate is about twice as high as the measured excretion rate. In earlier work, the measured excretion rates (assessed by counting radioactivity on material passing through a 0.4- $\mu\text{m}$  filter) had been considered to be low enough so that excretion could be ignored in productivity calculations. Since the average annual excretion rate in the Lake Mendota epilimnetic phytoplankton is now calculated to be 41%, this means that earlier primary production determinations (8) were underestimates.

Bacterial incorporation of excreted products using differential filtration has been measured in other aquatic systems by Cole et al. (5), Larsson and Hagstrom (12), Wolter (21), and Bell et al. (1). The results of these studies have been similar to ours, namely, that a major proportion of the algal exudates are used by bacteria and that a major proportion of the bacterial organic carbon is derived from algal excretion. In Mirror Lake, Cole et al. (5) found up to 40% of the gross algal exudate to be consumed by the bacteria and about 33% of the total bacterial production to be supported by algal excretion. Larsson and Hagstrom (12) found that exudate constituted 16% of primary production and that phytoplankton supplied about 50% of bacterial production in a eutrophic gradient in the northern Baltic Sea. Wolter (21) reported that an average of 21% of algal exudates were utilized by bacteria in Kiel Fjord. The study by Bell et al. (1) dealt with Lake Norrviken, a eutrophic lake with many similarities to Lake Mendota. Bacterial production rates in Lake Norrviken ranged from  $1.75 \times 10^{-5}$  to  $5.9 \times 10^{-4}$  mmol/liter per h, similar to our values for Lake Mendota (Table 2). Bell et al. found that 10 to 80% of the total bacterial production (estimated from [ $^3\text{H}$ ]thymidine incorporation) was accounted for by algal excretion (similar to earlier values found for the same lake [6]). In Lake Mendota about 14% of bacterial production is supported by algal excretion, and about 20% of algal exudate is consumed by bacteria.

Although in several other studies, picoplankton (defined as chlorophyll-containing particles passing filters smaller than 2  $\mu\text{m}$ ) have complicated analyses, we did not have this problem in Lake Mendota. During spring blooms, large diatoms constitute the major fraction of the phytoplankton population, and during the summer blooms fairly large-sized cyanobacteria are dominant (8). Thus, the use of differential filtration was possible without special precautions to exclude small-sized phytoplankton cells from the bacterial fraction. We did, however, check for picoplankton by routine chlorophyll measurements on small-pore filters.

The values reported in our work are averages of samples showing wide seasonal variance. It is clear that at certain times of year, or with certain phytoplankton populations, bacterial production is almost totally supported by algal excretion, whereas at other times of the year, when algal decomposition is high, only a very small fraction of the bacterial production is derived from materials excreted by living phytoplankton.

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