

Kinetics of microbial mineralization of organic carbon from detrital *Skeletonema costatum* cells

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ABSTRACT: Long-term (6 mo) studies of the mineralization of detrital ^{14}C -labelled *Skeletonema costatum* cells by coastal microbial communities demonstrated the existence of 4 main pools (G_i) of 'utilizable' organic material. The most labile fraction, G_{01} material, comprised 13 % of the algal biomass and was consumed via first-order kinetics in 14 h. The G_{02} , G_{03} and G_{04} fractions (54, 29 and 4 % of the algae respectively) had turnover times on the order of days to weeks, months and years, respectively. Definition of pool size and lability was based on changes in first-order consumption rates of particulate and dissolved organic carbon (POC and DOC) released from intact cells during the experimental period. Sodium azide inhibition of bacterial metabolism resulted in DOC accumulation at rates approximating POC mineralization rates and community metabolism, but problems with incomplete inhibition of the uptake of G_{01} materials negated conclusions concerning natural DOC release rates from algae. Conflicting opinions on the response of benthic communities and deep-water bacterioplankton to sedimenting algal blooms may be resolved through consideration of the amount of G_{01} and G_{02} -organic matter which remain in detrital algae.

INTRODUCTION

In recent reviews on the fate of organic matter in the sea, Azam and co-workers (Azam & Ammerman 1984, Azam 1986, Azam & Cho 1987) suggest there is a tight coupling between the production and the consumption of 'utilizable' dissolved organic matter (DOM) by the bacterioplankton. Most of the original studies summarized in these reviews have concentrated on the fate of labile fractions of algal exudates, or labile substrates such as glucose and glutamate in short-term experiments (times on the order of a day or less). Utilization of the more refractory pools of exudate DOM and of DOM hydrolyzed from detrital particulate organic matter (POM) has not been as rigorously examined. As noted by Biddanda (1988), there is little intrinsic difference in how bacteria respond to POM or DOM; mineralization rates of refractory materials will be a function of the frequency of contact of bacteria and substrate, a necessary requirement for surface-bound exoenzyme hydrolysis (Hollibaugh & Azam 1983, Rego et al. 1985), and of the speed at which the bacterial assemblage can produce the requisite enzymes.

Laboratory studies of the decay of algae and other POM in water and sediment indicate that POM is composed of at least 2 labile fractions and a non-

reactive fraction (Garber 1984, Westrich & Berner 1984, Henrichs & Doyle 1986, Grant & Hargrave 1987). The disappearance of each of the reactive fractions (G_i) over time can be predicted by first-order reaction kinetics in Berner's (1980) multi-G model:

$$\frac{dG_i}{dt} = -k_i G_i \quad (1)$$

where k_i = a first-order decay constant. The total quantity of POM (G_T) at any time is the sum of the exponentially declining fractions (G_i) plus the non-reactive fraction (G_{NR}):

$$G_T(t) = \sum G_i \exp(-k_i t) + G_{NR} \quad (2)$$

For microbial-mediated decay of POM to occur, bacteria must first solubilize each of the fractions into DOM, or await passive leakage of intracellular DOM across cell membranes (Bjørnsen 1988). The few studies of POM decay which have considered DOM cycling note that production and consumption were closely matched resulting in little loss of DOM to the external environment (Kepkay & Andersen 1985, Schwinghamer & Kepkay 1987). However, these studies were concerned with the fate of *Spartina* litter buried in sediments, an environment with significantly slower rates of diffusion and material transport than the

water column. Newell et al. (1981) and Biddanda (1988) considered the short-term fates of both POM and DOM in seawater samples, but unfortunately used phytoplankton debris, consisting of cells exploded by osmotic shock or freeze-heat cycles, rather than intact cells.

If bacterial metabolism is limited by DOM quality and the rates at which different fractions of DOM leak out of cells or are hydrolyzed from POM, as suggested by Westrich & Berner (1984), Fuhrman (1987) and Biddanda (1988), experiments need to be performed on intact POM to more fully understand the dynamics of bacteria-POM-DOM interactions in natural systems. In this paper, I present results of a laboratory simulation of water column degradation of intact ^{14}C -labelled phytoplankton designed to examine reaction kinetics during the long periods required to mineralize the cells.

MATERIALS AND METHODS

Approximately 2 l of a ^{14}C -labelled *Skeletonema costatum* culture was prepared by incubating an algal inoculum in Guillard & Ryther's (1962) f/2 medium supplemented with 37 MBq of $\text{NaH}^{14}\text{CO}_3$ for 2 wk at 15°C under continuous light. This period should be adequate for uniform labelling of *S. costatum* cells according to Welschmeyer & Lorenzen (1984). Then, 50 ml aliquots of the culture were centrifuged at 10 000 rpm for ½ h to separate particulate (PO^{14}C) from dissolved organic carbon (DO^{14}C) and residual inorganic label ($^{14}\text{CO}_2$). The particulate algal fraction was washed into a flask with dialyzed seawater (<1000 molecular weight fraction of the same seawater used to prepare f/2 medium; Amicon DC10 unit used), and bubbled with carbon dioxide for 3 h followed by nitrogen for another 9 h to purge residual $^{14}\text{CO}_2$ and kill the algae. This stock suspension was stored at 2°C in darkness under a nitrogen atmosphere until the experiment began 12 h later. Prior to use, less than 2% of the ^{14}C label in the stock suspension was found in the $^{14}\text{CO}_2$ fraction; the balance was in PO^{14}C (76%), and DO^{14}C that leaked out of the cells (22%). The fate of algal culture DO^{14}C is examined in another paper (Pett unpubl.).

On Day 0 of the experiment (10 May 1987), 5 ml portions of the ^{14}C -labelled algal suspension, consisting of ca 40 μmol of POC and 5.4×10^6 dpm, were added to 98 plastic bottles containing 250 ml of seawater taken the previous day from a depth of 35 m at a central station in Bedford Basin, Nova Scotia, Canada (70 m maximum depth). The quantity added was chosen on the basis of total POC concentrations normally observed at the time of sample collection, near the end of the spring bloom (Parrish 1987). Sodium azide at a final concentration of 15 mmol l^{-1} (Rosson et al. 1984)

was added to half of the samples to inhibit respiration and gain further insight into the kinetics of DOC release and consumption. The samples were placed on a slowly-rotating plankton wheel (2 rpm) or reciprocating table in a dark, 10°C cold-room for incubations of 0, 3, 6, 9, 12, 18, and 24 h, and 2, 4, 8, 16, 30, 60, 90, 120, 150, and 180 d. At each interval, triplicate samples were taken for analysis of PO^{14}C , DO^{14}C , $^{14}\text{CO}_2$, total POC, total DOC, and bacteria counts.

The fractionation of ^{14}C into PO^{14}C , DO^{14}C and $^{14}\text{CO}_2$ employed methods similar to those outlined by Novitsky (1986). A 5 ml portion from each bottle was added to 60 ml serum bottles and sealed with a sleeve-style stopper fitted with a polypropylene cup containing a folded filter wick saturated with 0.15 ml of β -phenethylamine. Then 0.1 ml of 2 N HCl was injected into the sample to liberate $^{14}\text{CO}_2$. After at least 1 h of gentle mixing, the filter wick was removed and assayed for radioactivity in a liquid scintillation counter. Preliminary experiments on the efficacy of $^{14}\text{CO}_2$ extraction indicated asymptotic levels were attained after 45 min. The remaining liquid was further acidified with 4.9 ml of ice-cold 2N HCl and after ca 1 h of mixing, filtered through a Millipore GS (0.22 μm) membrane filter to separate DO^{14}C and PO^{14}C fractions. The membrane filter was washed twice with 5 ml of 1N HCl, and dissolved in 1.5 ml of methyl cellosolve prior to liquid scintillation counting.

Total pools of DOC and POC were determined from samples passing through, or retained on organic-free (450°C ashed), Whatman GF/F glass fibre filters rather than the organic Millipore filters used in ^{14}C -fractionation. DOC was measured by the UV-oxidation technique of Gershey et al. (1979), while POC was measured after high temperature combustion of the filters in a Leco furnace. Both techniques employed a non-dispersive infra-red analyzer for detection of CO_2 .

Samples for bacteria counts were preserved with 2% filtered (0.2 μm) formaldehyde buffered with sodium borate, and stored in a refrigerator until counted. The samples were enumerated by epifluorescence microscopy following 2 min staining with prepared acridine orange (0.01%, Becton-Dickinson No. 4940), filtration onto Sudan-B Black stained 0.2 μm Nuclepore filters, and immediate mounting in Cargille Type A immersion oil on a glass slide (Schwinghamer 1988). At least 400 cells were enumerated on each filter.

RESULTS AND DISCUSSION

Over the short term, community metabolism appeared to be fuelled mainly by consumption of labile algal leachates that were present in the original inoculum (Fig. 1a, b). Approximately 58% of this DOM

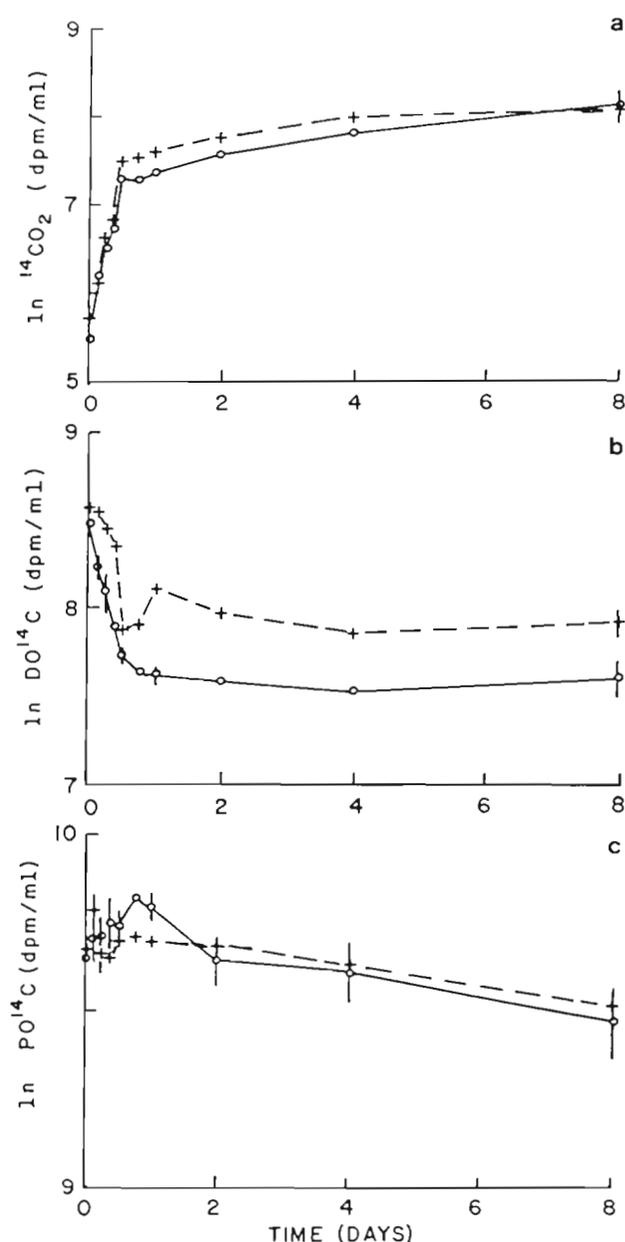


Fig. 1. Time-series measures of the natural log of (a) ^{14}C -labelled carbon dioxide, (b) dissolved organic carbon, and (c) particulate organic carbon concentrations during early stages of mineralization of *Skeletonema costatum*. (o) Mean \pm 1 standard deviation of non-poisoned samples; (+) mean \pm 1 standard deviation of sodium azide inhibited samples

leachate or 13% of added ^{14}C , hereafter called G_{01} -DOC, was consumed via first-order kinetics within 14 h. Linearity of various portions in these plots verifies first-order consumption or production of organic pools, and the slopes give us the reaction rate constants (when natural logarithms are used). Uptake rates of G_{01} -DOC that had leached out of the algae in the 24 h between centrifugation and sample amendment were

calculated to be on the order of 0.06 h^{-1} (1.51 d^{-1}), comparable with the highly labile exudates released by living phytoplankton as observed by Lancelot (1979, 1984) and Pett (unpubl.). Approximately 50% of this material appeared to be directly metabolized to $^{14}\text{CO}_2$; the balance going into bacterial biomass (Fig. 1c). The remaining leachates were mineralized at rates 2 to 3 orders of magnitude slower (Table 1).

Table 1 A summary of first-order rate constants determined from time series measurements of ^{14}C -labelled particulate and dissolved organic matter and carbon dioxide (PO^{14}C , DO^{14}C , $^{14}\text{CO}_2$, respectively) during mineralization of intact *Skeletonema costatum* cells in non-poisoned and sodium azide inhibited seawater samples. Positive values indicate production; negative values consumption. NS: rate constant not significantly different from zero

Treatment	Pool	Rate constant (d^{-1})		
		PO^{14}C	$^{14}\text{CO}_2$	DO^{14}C
Non-poisoned	G_{01}	0.20	3.31	-1.51
	G_{02}	-0.08	0.10	-0.01
	G_{03}	-0.04	0.03	< -0.01
	G_{04}	-0.02	< 0.01	-
Sodium azide inhibited	G_{01}	NS	3.55	-1.27
	G_{02}	-0.05	NS	0.07
	G_{03}	-0.01	NS	0.03
	G_{04}	NS	NS	NS

Within 2 to 3 d of the removal of G_{01} -DOC, bacterial numbers also rapidly declined from a high of $3 \times 10^6\text{ ml}^{-1}$ at time zero to pre-addition levels of 0.5 to $1.0 \times 10^6\text{ ml}^{-1}$. As observed by Newell et al. (1981) and Biddanda & Pomeroy (1988), this was also the time when numbers of protozoans became abundant. No further changes in bacterial density were observed over the following 6 mo.

The commonly-used sodium azide concentration of 15 mmol l^{-1} (0.1%, e.g. Rosson et al. 1984) which was used here as a broad spectrum inhibitor of microbial metabolism, had little effect on bacterial respiration and only reduced uptake of G_{01} -DOC by 11% in the first 8 d (Fig. 1a, b). As a result, the concentration was increased to 30 mmol l^{-1} on Day 10 and increased further by 10 mmol l^{-1} every 15 d or so until Day 90, and then by 5 mmol l^{-1} until the end of the experiment. This stopped $^{14}\text{CO}_2$ production and caused DO^{14}C to accumulate (Fig. 2). Higher starting concentrations of sodium azide (30 to 45 mmol l^{-1}) in other experiments examining the uptake of algal culture DO^{14}C (unpubl.) still did not completely eliminate uptake of G_{01} -DOC. It should be noted however, that this fraction had already leaked out of the algae before sample amendment, and little metabolism of POM or DOM leachates occurred between the time of G_{01} -DOC consumption and addition of more sodium azide. Unfortunately, this poor

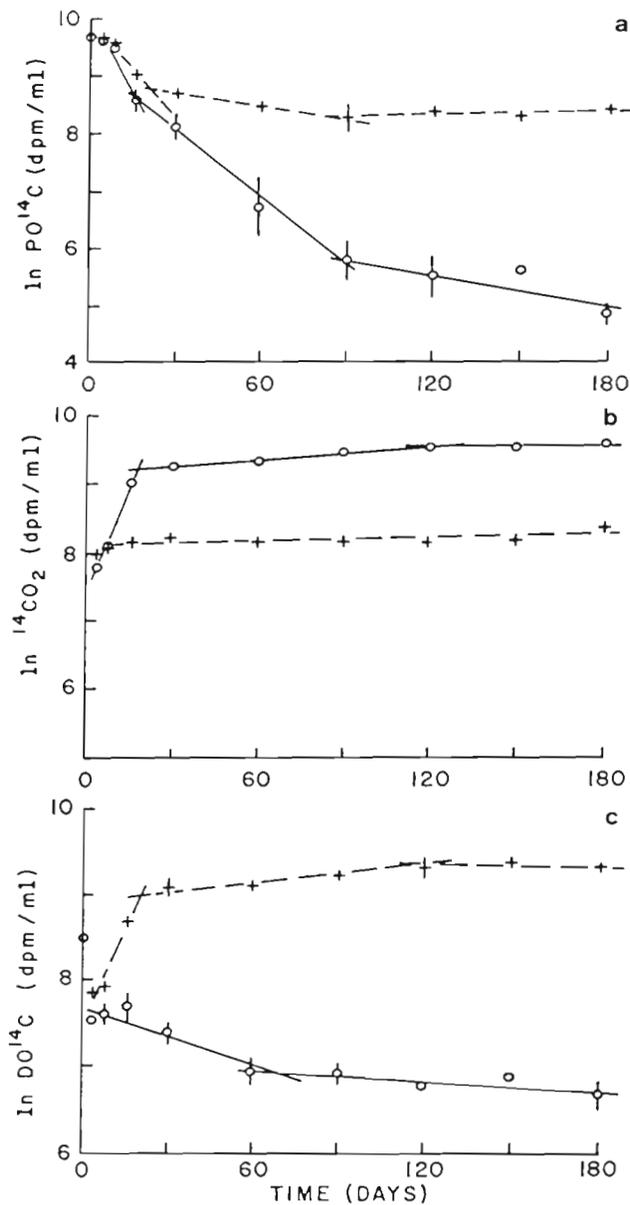


Fig. 2. Time-series measures of the natural log of (a) ^{14}C -labelled particulate organic carbon, (b) carbon dioxide, and (c) dissolved organic carbon concentrations during later stages of mineralization of *Skeletonema costatum*. Lines determined by linear least-squares regression analysis. (o) Mean \pm 1 standard deviation of non-poisoned samples; (+) mean \pm 1 standard deviation of sodium azide inhibited samples

inhibition of DOC uptake by sodium azide amendment coupled with the possibilities that (1) the azide-inhibited bacteria may have altered the permeability of algal cell walls in the early part of the experiment and (2) enzymes released from azide-killed bacteria could be responsible for part of the DOC release from algae later on in the experiments, precludes an outright acceptance of the long-term DO^{14}C accumulation rates as natural production rates of DOC from detrital algae.

Utilization of PO^{14}C material was not evident until after ca Day 4 (Figs. 1c and 2a). Thereafter the long-term rates of $^{14}\text{CO}_2$ production (Fig. 2b) were very comparable to decay rates of PO^{14}C (Table 1) and total POC. Total POC and PO^{14}C behaved similarly throughout the experiment confirming uniform labelling of algal POM (correlation coefficient = 0.96, $p < 0.01$). The largest fraction of the algae, G_{02} -type organic matter, comprising 54 % of added ^{14}C , was consumed in ca 17 d at a rate of 0.08 d^{-1} . After this time, there was a distinct change in the slope of PO^{14}C consumption and $^{14}\text{CO}_2$ production indicating another change in POM lability (G_{03} -organic matter, Fig. 2a, b). Since DO^{14}C was released at comparable rates in poisoned samples (0.05 d^{-1} calculated from data in Fig. 2c) and never accumulated in the non-poisoned samples, there appeared to be a tight coupling of POC breakdown and uptake of resultant DOC. Although subject to the limitations of sodium azide poisoning mentioned earlier, the G_{02} -DOC was apparently consumed as fast as it leaked from the algae.

The remaining 23 % of algal PO^{14}C , which was degraded from Day 17 to ca Day 90 at a rate of 0.04 d^{-1} , also appeared to be mineralized as fast as DO^{14}C accumulated in sodium azide inhibited samples, i.e. at 0.03 d^{-1} (Table 1). As a consequence of this 'apparent' tight coupling of G_{02} and G_{03} -DOC production to community metabolism and POC breakdown, there was no significant change in the total DOC pool throughout the 6 mo experiment. G_{03} -particulate decay rates were nearly identical to mineralization rates for the more long-lived leachates in the original inoculum (and in experiments on the fate of culture exudates, Pett unpubl.), and are well within Ogura's (1972, 1975) estimates for his F_1 fraction of bulk seawater DOM and Westrich & Berner's (1984) estimates for G_{01} plankton material.

After ca 90 d, community metabolism and degradation rates of POM and DOM were again considerably reduced. All but 2 % of the original algal PO^{14}C and 16 % of the DO^{14}C leachate (4 % of total added ^{14}C) were consumed by the end of the experiment at rates of 1.8 and 0.7 yr^{-1} , respectively. Studies by Ogura (1972, 1975) and Westrich & Berner (1984) on what I call G_{04} fractions of POC and DOC gave the same order of magnitude results: 0.4 to 1.1 yr^{-1} .

Although this experiment only provides kinetic data for the mineralization of 1 algal species, on 1 occasion, it does help explain or clarify several recent controversies concerning bacteria-POM interactions and the fate of POM in the marine environment. In many coastal environments, where there is (1) more frequent mixing of the water column, (2) enhanced bacterial productivity arising from sediment resuspension (Wainright 1987), and (3) more abundant zooplankton populations

than were present in my 250 ml bottle samples, most of the G_{01} and G_{02} -organic matter in sedimenting phytoplankton (67% of *Skeletonema costatum* biomass), would leak out and probably be consumed in a matter of days or less, long before detrital algae finally settle onto the seabed. Rapid loss of large amounts of low molecular weight DOM after cell death is expected due to the large concentration gradient across phytoplankton cell walls (Bjørnsen 1988). Indeed this has been shown by several investigators in short-term experiments with the consequent development of bacteria-POM aggregates (e.g. Newell et al. 1981, Garber 1984, Biddanda 1988).

Since most benthic and deep-water microbial communities are dependent upon POM settling out of surface waters, their response to 'fresh' inputs of POM will be related to nutritional status of both the POM and DOM after it has been subject to degradation in the water column. Depending upon the sinking rate, most of the G_{01} and G_{02} -organic matter remaining in sinking particles (including bacteria themselves) would be rapidly recycled by particle fragmenters or be released into bulk seawater to subsequently enter the suspended matter microbial loop. Considering an average particle residence time of 30 d in the upper mixed layer of the oceans (Lande & Wood 1987), sub-pycnocline microbial communities typically receive G_{03} and more resistant POM that has undergone considerable modification by zooplankton and bacteria (Altabet 1988, Wakeham & Canuel 1988). Consequently, mineralization rates of sinking particulate matter and bacterioplankton growth rates decrease with increasing depth (Cho & Azam 1988, Karl et al. 1988) and we often see little stimulation of benthic activity following sedimentation of algal blooms (Hargrave 1980, Czytrich et al. 1986, Kannevorf & Christensen 1986, Rudnick & Oviatt 1986). Clearly though, some relatively undegraded algae (or even living cells) do sink to the seabed very rapidly initiating an immediate, but short-lived pulse of benthic activity (Graf et al. 1983, Meyer-Reil 1983, Graf 1987a, b, Lochte & Turley 1988).

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