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Microbial response to organic particle generation by surface coagulation in seawater

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ABSTRACT: Coagulation of organic material onto bubbles and the production of organic particles as the bubbles either dissolve or burst at an air-water interface had a large and well-defined effect on the concentration of dissolved organic carbon (DOC) in a coastal seawater. After only 15 min of bubbling, DOC was reduced by 43 %, and the rapid development of a transient microbial community was associated with the production of labile organic particles. Half of the DOC removed by this surface coagulation was respired to CO_2 within 3 d, and mineralization rates (209 to 212 μgC l^{-1} d^{-1}) were comparable to primary production rates in even the most productive coastal waters. While surface coagulation is obviously not primary production, it does appear to physically regenerate carbon which remains less accessible to the biota when in the dissolved and colloidal state. This physical regeneration of DOC may be an important part of any pelagic ecosystem based on the consumption of organic particles, but when combined with rapid mineralization of the regenerated material, it could also be a mechanism for recycling a large reservoir of apparently refractory carbon back to CO_2 at the ocean surface.

INTRODUCTION

Coagulation of colloids and particles to form aggregates has long been of interest for understanding the mass flux of carbon and other elements in aquatic systems. Particle interactions due to Brownian motion, laminar and turbulent shear, differential settling, inertial impaction, convective diffusion and biological activity have all been suggested as mechanisms to explain particle distributions in natural waters (Alldredge 1972, 1979, McCave 1975, 1984, Lerman 1979, Hunt 1982). The aggregation of molecules, colloids and particles onto the surfaces of bubbles by surface coagulation is an important, additional consideration in any study of the air-sea interface and seawater near the ocean surface.

Surface coagulation has commonly been associated with the conversion of dissolved organic material (DOM) to particulates by bubbles which either dissolve or burst at the air-water interface after rising through the water column. Sutcliffe et al. (1963) were the first to describe the conversion of DOM to particulate organic matter (POM) by bubbling. Menzel (1966), however, questioned these data after finding no particles when he bubbled triple-filtered seawater, and Barber (1966) added the suggestion that bacteria were required for coagulation. Batoosingh et al. (1969) discovered that larger colloidal materials in the size range of 0.2 to 1.2 μm were required for the conversion of significant amounts of dissolved organic carbon (DOC) to particulate organic carbon (POC). They also found that continued bubbling did not produce more particulates until existing particles had been removed by filtration. Wallace & Duce (1978) concluded that bubbling unfiltered seawater produced no particles and that perturbation of the system by filtration was responsible for particle formation. After bubbling mixtures of seawater and an algal extract, Corner et al. (1974) found that organic aggregates were formed, but concluded that bacteria were not responsible for the aggregation. In contrast, Biddanda (1985) reported that bubbling a mixture of seawater and a seaweed-organic extract resulted in large amounts of bacterial-particle aggregates. On this basis, he came to the opposite conclusion, that aggregates were formed only when active bacteria were present.

To reconcile this large body of conflicting data, Johnson et al. (1986) combined both theory and experiment

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to delineate the natural conditions under which surface coagulation will operate. They confirmed that filtration of DOM was critical in determining the amount of particles formed, but also found that bubble size was an important factor. Filtering seawater through anything finer than a 2 µm pore size tended to remove colloids – the most important source of DOM for coagulation onto bubble surfaces. In addition, rising bubbles of about 500 µm in diameter were far more efficient at scavenging DOM and subsequently producing particles than bubbles < 200 µm in diameter. With proper control of filtration and bubble size, bacteria were not required for particle formation, but did become an important component of aggregates within hours of particle generation. In this paper, we show that surface coagulation can remove large amounts of DOC from a coastal seawater and that an active microbial population responds to the production of organic particles as bubbles dissolve or burst at the air-sea interface.

MATERIALS AND METHODS

Seawater preparation. On 3 Nov 1987, seawater from a water depth to 10 m in the North West Arm of Halifax Harbour (Canada) was collected from the unfiltered intake line of the Aquatron seawater system at Dalhousie University. As recommended by Johnson et al. (1986), the natural seawater was filtered through a 2 µm Nuclepore membrane to remove larger particles without removing the colloidal fraction of DOM. Eight liters of the seawater were bubbled for 15 min at room temperature (20 °C) in an enclosed glass column described by Johnson et al. (1986). Compressed air was passed through a 10 to 25 µm glass frit at a flow rate of 100 ± 20 ml min^{-1} to produce bubbles 340 to 1960 µm in diameter (average diameter: 487 µm). The air supplied to the frit was prefiltered through activated carbon and a sterile, disposable 0.22 µm filter (Millipore) to ensure that contaminants were not introduced on bubble surfaces. After 15 min of bubbling, the seawater was divided into two 4 l batches. Bubbling of one batch was continued for the duration of a 15 d experiment to determine the effects of continuous bubbling. The other batch was stirred gently at room temperature to provide a system generated by minimal bubbling. In addition, a 4 l batch of filtered seawater was stirred as an unbubbled control. At intervals, samples were taken from all seawaters for determinations of bacterial and protozoan numbers, dissolved organic carbon and the rate of oxygen consumption.

Oxygen consumption rate. Microbial consumption of oxygen was measured using the microgradient technique of Kepkay et al. (1986). Samples (10 ml) were pipetted into acrylic chambers and filtered through 0.2 µm Nuclepore filters. The same volume of filter-sterilized (0.2 µm-filtered) seawater was added to each chamber and oxygen gradients were measured to a distance of 5 mm above the material collected on the surface of each filter. The gradients allowed calculation of the downward flux of oxygen over a given length of time and, thus, the rate of consumption per cm^2 of surface or per ml of original sample.

Bacterial and protozoan numbers. Counts of particle-associated microbiota were carried out using Schwinghamer & Kepkay's (1987) protocol for fluorescence microscopy. A 5 ml sample from each batch of seawater was fixed with 200 µl of 50 % (v/v) gluteraldehyde which had been filtered through 0.2 µm membranes (Nuclepore). Sub-samples were diluted in seawater filtered through 0.2 µm membranes (Nuclepore), stained with acridine orange and counted on 0.2 µm Nuclepore filters which had previously been stained with Sudan black. A Leitz epifluorescence microscope equipped with a 100× oil immersion objective was used to count bacteria and a 40× objective was used to count larger protozoa (with cell diameters of 15 to 25 µm).

Dissolved organic carbon. An automated analyzer was used to determine DOC in each of the 3 batches of seawater. Design of the analyzer is similar to the system developed by Cauwet (1984) and is based on the UV oxidation of seawater samples which had previously been filtered through 0.2 µm filters and acidified. The CO_2 produced by DOC oxidation was then passed through a reduction column to be converted to methane, and analysed on a Perkin Elmer gas chromatograph (Model 3200) equipped with flame ionization detector.

RESULTS

The results from our experiment were similar to less complete data sets from pilot studies carried out on surface water from the North West Arm. Within 2 h of particle production by bubbling, the rate of oxygen consumption was enhanced by a factor of 3.1 to 3.5 (Fig. 1A). Consumption continued to increase until, by 4 h, it had reached a maximum in the bubbled seawater. During the first 6 h, attached bacteria underwent 3.3 doublings in the bubbled and stirred seawater, and 2.2 doublings in the seawater which was bubbled continuously (Fig. 1B). This was far faster than the 1.5 doublings apparent over 32 h in the unbubbled control. The maximum cell numbers apparent in the bubbled seawaters at 6 h lagged behind oxygen uptake by a maximum of 2 h; the reason for this lag is not known.

The number of larger protozoans (primarily

flagellates with cell diameters of 15 to 25 μm) remained at about 150 cells ml^{-1} from 0 to 12 h, but reached a short-lived peak of between 600 and 800 cells ml^{-1} at 27 h in the bubbled seawaters. This peak declined to 180 cells ml^{-1} by 32 h. A smaller, secondary peak of oxygen consumption at 27 h (Fig. 1A) may have been associated with the predominance of these larger protozoans, but a direct link cannot be established between cell number and consumption. Smaller flagellates and ciliates in the size range of 1 to 3 μm (Wright & Coffin 1984) and 2 to 20 μm (Fenchel 1982, Sherr & Sherr 1984, Sherr et al. 1986) may have also been an important component of the microbial population. Numerous cells in the appropriate size ranges were observed in samples taken after 6 h, but could not be unequivocally identified as protozoans. Our preservation and staining techniques were only reliable for counts of bacteria and the larger protozoans. Methods such as the double-staining technique of Sherr & Sherr (1983) would be required to obtain accurate counts of the smaller protozoans.

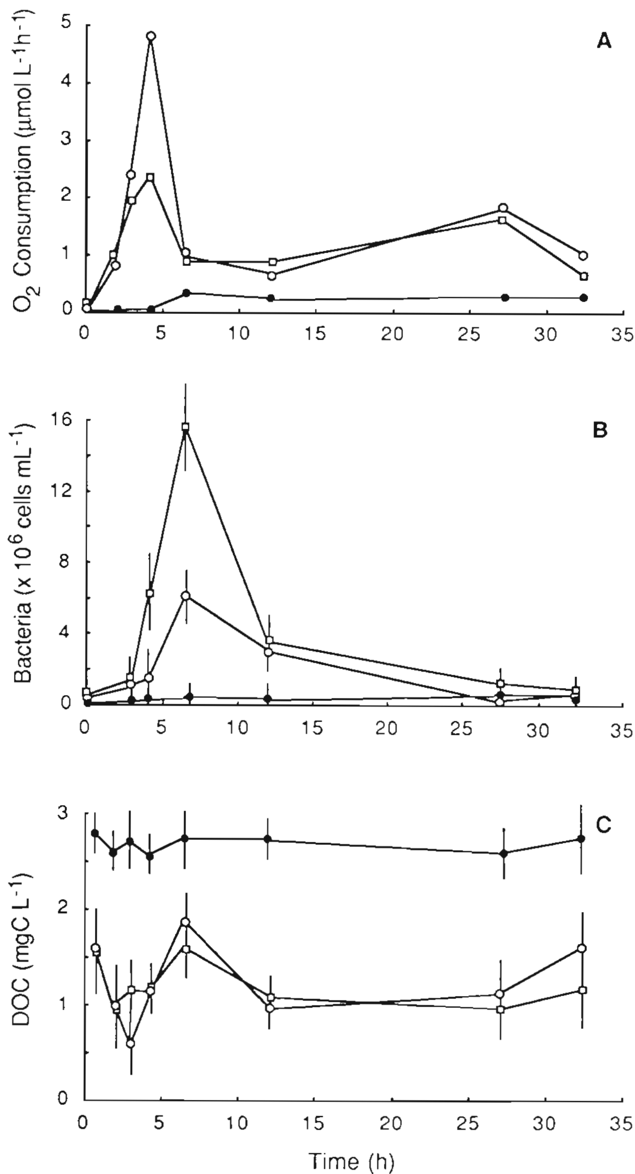

Fig. 1 Rate of oxygen uptake (A), bacterial numbers (B) and DOC (C) in unbubbled, stirred seawater (closed circles), seawater bubbled for 15 min prior to time zero and then stirred (open squares), and in seawater bubbled continuously (open circles). Results are from the first 32 h of a 15 d experiment. Only number of bacteria attached to particles are shown; error bars delineate the range around means calculated from duplicate samples. Error bars for oxygen uptake in selected duplicate samples were all smaller than the dimensions of the symbols. Error bars from DOC measurements delineate 2× standard deviation around means calculated from triplicate analyses

Oxygen consumption, bacterial number and protozoan number all remained low in the bubbled seawaters from 32 h to 15 d. In the unbubbled control, protozoan numbers were low throughout the experiment, whereas bacterial numbers and oxygen uptake increased slowly and steadily during the first 75 h. After 75 h and for the duration of the experiment, bacterial numbers and oxygen consumption remained at similar, low values in all of the seawaters.

In the unbubbled control (Fig. 1C) DOC remained largely unchanged from 0 to 32 h. In contrast, DOC was reduced by 43 % when seawater was bubbled for only 15 min (Table 1) and fluctuated during the first 32 h of relatively intense biological activity (Fig. 1C). From 32 h to 15 d, there was a significant reduction of DOC in both the bubbled seawaters and the unbubbled control. This was associated with the long-term, physical coagulation of organic particles (B. D. Johnson & P. E. Kepkay unpubl.) rather than any extensive biological activity.

Cumulative oxygen consumption over 15 d was similar in the 2 bubbled seawaters (Fig. 2) and leveled off by Day 3. The oxygen consumed over this period (54.7 to 55.8 μmol l^{-1}) was equivalent to the respiration of 656 to 670 μgC l^{-1} to CO_2. This was 55 to 56 % of the DOC incorporated into particles by 15 min of bubbling and 24 % of the DOC originally available in the unbubbled seawater (Table 1). When Lee & Fuhrman's (1987) conversion factor of 2.0×10^{-8} μgC $cell^{-1}$ was applied to the mean number of attached bacteria (1.6×10^6 cells ml^{-1}) in the bubbled seawaters from 32 h to 15 d, only 32 μgC l^{-1} (or 1.2 % of the original DOC pool) remained as bacterial cell carbon after the initial period of microbial activity and growth. Cumulative oxygen consumption in the unbubbled control did not level off by Day 3 (Fig. 2). Instead, it gradually increased and reached a value similar to the bubbled seawaters (47.5 μmol l^{-1} by Day 15). However, the rate of oxygen uptake over this period (3.4 μmol l^{-1} d^{-1}) was about 5 times less than the oxygen consumed over 3 d in the bubbled seawaters (17.4 to 17.7 μmol l^{-1} d^{-1}).

Table 1. Partitioning of DOC into organic particles, CO_2 and attached bacterial cell carbon

DOC fraction	$\mu gC\ l^{-1}$	% of original DOC pool	% of DOC converted to particles by surf. coagulation
DOC in original SW	2790	–	–
DOC removed by 15 min of bubbling	1190	42.7	–
Cum. CO_2 prodn. in bubbled SW (over 3.1 d)	656–670	23.5–24.0	55.1–56.3
Cum. CO_2 prodn. in unbubbled SW (over 14.1 d)	570	20.4	47.9
CO_2 prodn. d^{-1} in bubbled SW	209–212	7.5–7.6	17.6–17.8
CO_2 prodn. d^{-1} in unbubbled SW	40.8	1.5	3.5
Bacterial cell C remaining from 32 h to 15 d	32.0	1.2	2.7

DISCUSSION

The production of organic particles after surface coagulation initiated a rapid bacterial response. The rate of oxygen uptake reached a maximum by 4 h in the bubbled seawaters (Fig. 1A), and was associated with 2.2 to 3.3 doublings of attached bacteria by 6 h (Fig. 1B). This means that bacteria associated with particles had a generation time of 2 to 3 h, far faster than our estimate of 21 h for growth in the unbubbled control and outside the range of 10 to 100 h for heterotrophic growth in surface waters (Van Es & Meyer-Reil 1982). After 6 h, a sharp reduction of bacterial numbers may have been, in part, associated with the activities of relatively large flagellates (about 20 µm in diameter), but smaller flagellates and ciliates in the size range of 1 to 3 µm (Wright & Coffin 1984) and 2 to 20 µm (Fenchel 1982, Sherr & Sherr 1984, Sherr et al. 1986) were probably an important part of the protozoan population which was not accounted for. These smaller protozoans may have been responsible for the rapid reduction in the number of attached bacteria between 6 and 12 h (Fig. 1B). However, by 32 h, the net result of a rapid microbial response to particle generation was certainly not a large number of cells. Instead, oxygen consumption remained low, and microbial numbers were indistinguishable from those found in the unbubbled control over times up to and including 15 d. If our sampling had not been sufficiently rapid at the beginning of the experiment, we would have missed the dynamics associated with transient communities and inferred that bubbling had no effect.

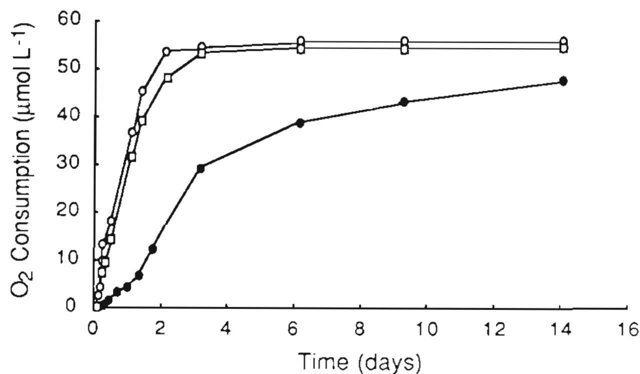

Fig. 2. Cumulative oxygen uptake over 15 d in seawater bubbled continuously (open circles), seawater bubbled for 15 min and then stirred (open squares), and in seawater left unbubbled, but stirred (closed circles)

The reasons why organic particles caused such a rapid microbial response remain unclear, but the answer may lie in the results of Baylor et al. (1962) and Graham et al. (1979), which show that bubbling seawater for 18 to 45 min can concentrate dissolved phosphorus into the particulate or adsorbed phase by a factor of 4 to 200. Given that much of the DOC converted to particles was released back into solution during the first 32 h of intense microbial activity (Fig. 1C) and that only half was eventually mineralized over 3 d (Table 1), the microbial community was probably not carbon limited. As a result, the degree to which limiting nutrients such as phosphorus and nitrogen are involved in surface coagulation may ultimately regulate the magnitude of a microbial response to particle generation.

Large amounts of DOC were removed from solution by bubbling (Fig. 1C). The result of only 15 min of bubbling was a 43 % reduction of the DOC originally available in unbubbled seawater (Table 1). To our knowledge, this is the first demonstration that surface coagulation can have a large and easily-discernible effect on the concentration of DOC in a natural seawater. Our experiment succeeded because we followed the precautions taken by Batoosingh et al. (1969), Johnson & Wangersky (1985) and Johnson et al. (1986). Filtering seawater through anything finer than a 2 µm pore size removes larger colloids in the 0.2 to 1.2 µm size range – a primary source of material for surface coagulation. This means that interpretation of data from experiments designed to show the effect of bubbling will be seriously compromised if colloidal material has been removed by filtration.

It still remains to be seen if bubble spectra from our experiment are typical of the spectra under a breaking

wave, but it is well known (see Blanchard 1983) that bacteria are concentrated by many hundreds of times into the particles generated after surface coagulation. The rapid metabolic response of the bacteria (Fig. 1A) leaves little doubt that these particles are labile. Our results show that the microbial community mineralized almost half the DOC regenerated to particles in as little as 3 d (Fig. 2, Table 1). This was equivalent to 24 % of the DOC available in our seawater sample before bubbling (Table 1). Only 1.2 % of the original DOC pool could be accounted for as bacterial cell carbon after the initial burst of microbial activity and growth during the first 32 h. In addition, when cumulative oxygen consumption (Fig. 2) was expressed as rate of CO_2 production (Table 1), between 209 and 212 $\mu gC\ l^{-1}\ d^{-1}$ were mineralized in the bubbled seawaters. This was comparable to primary production in the most productive of coastal waters. Even on Georges Bank, primary production does not exceed 500 $\mu gC\ l^{-1}\ d^{-1}$ (O'Reilly et al. 1987). While it is obvious that surface coagulation is not primary production, bubbling appears to physically regenerate organic carbon which remains a good deal less accessible to the biota when in the colloidal state.

Our data are in conflict with a generally accepted view that >90 % of the DOC in seawater is refractory, and is mineralized to CO_2 over a time scale of years in surface waters (Ogura 1972, 1975) or thousands of years in the deep ocean (Williams et al. 1969, Williams & Druffel 1987). The apparently refractory nature of DOC in seawater is also at odds with Hedges' (1987) observation that the amount of carbon which can accumulate in the oceanic DOC pool per year (Williams & Druffel 1987) has to be much less than 1 % of the organic matter synthesized annually by marine phytoplankton. A quantitative indication of the role surface coagulation plays in converting the dissolved organic fraction of primary production back to CO_2 is required even for a partial resolution of these conflicts.

Different amounts and different fractions of DOC may be made available when other seawaters are bubbled, but the *rapidity* of the microbial response to surface coagulation in our experiment is a factor which cannot be ignored. The physical regeneration of DOC by bubbling may well be an important process in any pelagic ecosystem fueled by organic particles, but when combined with rapid mineralization of the regenerated material, it could also be a key element of the mechanism required by Hedges (1987) to recycle a globally important reservoir of carbon back to CO_2 at the ocean surface.

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