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The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater

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Abstract

Experiments were performed to examine the role of surface-active polysaccharides in the formation of transparent exopolymer particles (TEP) by bubble adsorption in seawater. Filtered (1.0 μm, 0.45 μm) and ultrafiltered (0.1 μm, 100 kDa, and 10 kDa) seawater samples were bubbled in a glass foam tower. The neutral sugar composition, concentration of TEP, and concentration of surface-active carbohydrates in generated foam samples were determined. Three different surface seawater samples (Monterey Bay, California; Shannon Point, Anacortes, Washington; and East Sound, Orcas Island, Washington) were used in the experiments. Significant concentrations of pre-existing and new TEP were extracted into foam by bubble adsorption. Newly generated TEP accounted for 28–52% of the TEP collected in the foam samples by bubbling 1.0-μm–filtered samples. Neutral sugar composition analyses of foam samples derived as a function of bubbling time indicated that two types of surface-active carbohydrates were extracted by bubble adsorption: highly surface-active carbohydrates, which were extracted initially, and less surface-active carbohydrates. As in our previous mesocosm study (Mopper et al. 1995), highly surface-active polysaccharides were enriched in deoxysugars (fucose and rhamnose), whereas the less surface-active polysaccharides and residual (bubble-stripped) water were glucose rich. In addition, the highly surface-active fraction was strongly enriched in covalently bound sulfate. The concentrations of TEP and surface-active carbohydrates that were extracted into the foam both decreased sharply with decreasing filter size used to filter samples prior to bubbling, in agreement with theoretical considerations. The results of this study suggest that bubble adsorption of sulfate-rich surface-active carbohydrates is an important pathway for the formation of TEP in surface waters, especially during algal blooms.

Nonliving particulate organic matter (POM) has important roles in microbial processes, carbon and nutrient cycling, and food web dynamics in the pelagic zone of the ocean. Nonliving particles can be formed in seawater from dissolved organic matter (DOM) and from colloids by various physical processes, such as shear (Passow and Azetsu-Scott unpubl. data) and bubble adsorption–collapse (Riley 1963; Johnson 1975; Johnson and Cooke 1980; Kepkay 1994). POM may also be formed abiotically at the expense of DOM by adsorption to clays or detritus (Jensen and Søndergaard 1982) because of salting-out effects (Sholkovitz 1976) or spontaneously as a function of dissolved organic carbon (DOC) concentration (Jensen and Søndergaard 1982; Chin et al. 1998).

A new class of nonliving POM, called transparent exopolymer particles (TEP), has been discovered to be abundant (as many as 10^4 ml^{-1}) in oceanic surface waters and in algal cultures (Alldredge et al. 1993; Passow et al. 1994). TEP are discrete particles (operationally defined as being >0.4 μm in diameter) that are formed from acidic polysaccharide precursors released from diatoms and other algae as dissolved and colloidal matter (Passow and Azetsu-Scott unpubl. data). Different species of diatoms release various amounts of these precursors (Passow and Alldredge 1994), and the rate of TEP accumulation in cultures has been found to be proportional to the growth rate (Waite et al. 1995). In general, the highest concentrations of TEP in situ are found at or shortly after the peak of diatom blooms (Passow et al. 1994; Passow and Alldredge 1995*b*).

TEP appear to be important for particle aggregation in the ocean (Logan et al. 1995), which in turn impacts particulate flux and carbon export from the surface of the ocean (Alldredge et al. 1993). The percentage of diatoms enclosed by TEP increases during the course of a bloom until >70–90% of all cells are included in TEP-diatom flocs (Passow et al. 1994; Passow and Alldredge 1995*b*), which eventually sink (Alldredge and Gotschalk 1989).

In a previous study (Mopper et al. 1995), we showed that TEP can be produced in seawater from surface-active polysaccharides by bubble scavenging (i.e., surface coagulation). We hypothesized that this process might be an important pathway for the formation of TEP in surface waters. The main goal of the current study was to obtain evidence to test this hypothesis and to ascertain the size fractions and neutral sugar and bound-sulfate compositions of DOM that are primarily responsible for the formation of TEP by bubble adsorption.

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Study areas

Surface seawater samples from three different sites (Monterey Bay, California; Shannon Point, Anacortes, Washington; and East Sound, Orcas Island, Washington) were collected, filtered, ultrafiltered, and bubbled to extract TEP into foam samples within 48 h of collection.

Monterey Bay, California—Monterey Bay is a site of an upwelling of high nutrient water (Graham 1993), and high densities of phytoplankton and aggregates (flocs) have been observed there (Treat et al. 1978). Surface seawater samples were collected (using 30-liter Niskin bottles) at a depth of approximately 10 m on 27 July 1993. On that day, the phytoplankton was a typical mixed summer community (recycling-type system) with silicoflagellates, large dinoflagellates, and coccolithophorids, as well as some small *Chaetoceros* spp. (Alldredge 1995). The chlorophyll *a* (Chl *a*) concentration in the surface layer (upper 10 m) was ~5–6 μg liter^{-1}, and marine snow (up to several millimeters), consisting primarily of larvarean houses, was abundant. The water was decanted into 20-liter fluorinated polypropylene Jerry cans (Cole Parmer) and stored onboard in the dark for ~2 h. The water samples were then transported to the laboratory at the University of California-Santa Cruz to be filtered and bubbled.

East Sound (Orcas Island, Washington)—The East Sound sampling site was located ~18 km northeast of the Friday Harbor Marine Laboratory. The average depth of the site is ~30 m. During the spring, strong tidal currents transport nutrient-rich deep Pacific water into the Sound across a narrow sill (8 to 22 m deep) at its southern end (Kiørboe et al. 1996). These conditions induce intense, bimonthly algal blooms in the Sound. Sampling was conducted daily from 14 April (day 1) 1994 to 24 April (day 11) 1994 at a 28-m-deep station in the middle of the northern part of the Sound (48°40′02″N, 122°54′01″W) aboard the *RV Barnes*. About midway through this period (18 April 1994, day 5), a diatom bloom began and remained in the log-growth phase (~0.2 d^{-1}) until the end of the study. By the end of the sampling period, the bloom was dominated, in terms of cell numbers, by *Chaetoceros* spp., followed by *Thalassiosira, Pseudonitzschia, Nitzschia,* and *Rhizosolenia* spp. (Kiørboe et al. 1995, 1996). In terms of biomass, the diatom *Thalassiosira mendiolana* was the dominant species (~75% of total algal C) (Kiørboe et al. 1996). Although chlorophyll concentrations in the surface water were high, reaching about 20 μg liter^{-1}, no aggregation was visible (Kiørboe et al. 1996). Samples were collected each day from 0800 to 1000 h in the upper 10 m using Niskin bottles attached to a conductivity-temperature-depth (CTD) rosette. The water was decanted into 20-liter fluorinated polypropylene Jerry cans and stored onboard in the dark for ~4 h. The samples were processed immediately upon the ship's return to the Friday Harbor Marine Laboratory.

Shannon Point (Anacortes, Washington)—On 14 January 1994, surface seawater samples (from a 5-m depth) were collected at the Shannon Point Marine Laboratory (Western Washington University) using their seawater pumping system. The water inlet was ~150 m from the shore. At the time of the sampling, the algal activity in the water column was very low (<0.1 μg Chl *a* liter^{-1}). Seawater samples were collected in 20-liter fluorinated polypropylene Jerry cans and stored at about 4°C until filtered and bubbled 2 d later in the home laboratory. This allowed the study of the formation of TEP in surface seawater under nonblooming conditions.

Materials and methods

Sample filtration and bubbling experiments—Seawater samples were gravity-filtered through 1.0-μm (and 0.45 μm for the East Sound samples) Polycap capsules (Whatman) and ultrafiltered through 0.1-μm, 100-kDa, and 10-kDa membranes (Amicon). The 100- and 10-kDa membranes were spiral-wound polysulfone, whereas the 0.1-μm membrane was hollow-fiber polysulfone. The samples were filtered (or ultrafiltered) and bubbled batch-wise (i.e., 1 aliquot of seawater for each filter or ultrafilter size), as opposed to sequentially, to minimize contamination and to avoid carryover artifacts (Buesseler et al. 1996; Mopper et al. 1996). Ultrafiltration was performed using an Amicon DC-10 instrument. Prior to use, each cartridge was cleaned with acid and base, according to the manufacturer's recommendations, rinsed three times with 30 liters of high-purity deionized water, and finally preconditioned with 20–30 liters of seawater sample.

Two main types of bubbling experiments were performed: size fractionation and time series (kinetics). To extract TEP and surface-active polysaccharides, sample water was bubbled using a 15.3-liter glass–Teflon foam tower (Fig. 1), as described in Mopper et al. (1995). Purified zero air was forced through a sintered glass frit (pore size 15–25 μm) at 250 ml min^{-1}. The resultant bubble size spectrum resembled that produced by a breaking wave (Cloke et al. 1991; Haines and Johnson 1995). For size-fractionation experiments (type 1), each filtrate or permeate was bubbled for 1 h, and the generated foam was continuously collected to give integrated samples (Mopper et al. 1995). These size-fractionation experiments were conducted twice, once with water from Shannon Point (14 January 1994) and once with water collected on 17 April 1994 at East Sound (day 4). For the second type of experiment (kinetics), foam samples were collected as a function of bubbling time. These time-course experiments were conducted with filtered (<1.0 μm) seawater samples from Monterey Bay (27 July 1993) and East Sound (19 April 1994, day 6, bloom). In addition to the experiments described above, prefiltered water (<1 μm), collected from days 1–11 from the Chl *a* maximum in East Sound, was bubbled for 1 h to examine TEP and surface-active polysaccharide variations over the course of the bloom (Zhou 1996).

In all experiments, filtered seawater (before bubbling), foam, and residual (bubble-stripped) samples were collected for measurements of TEP and total carbohydrates (*see below*). The neutral sugar composition of acid hydrolysates was determined for time-series and size-fractionation exper-

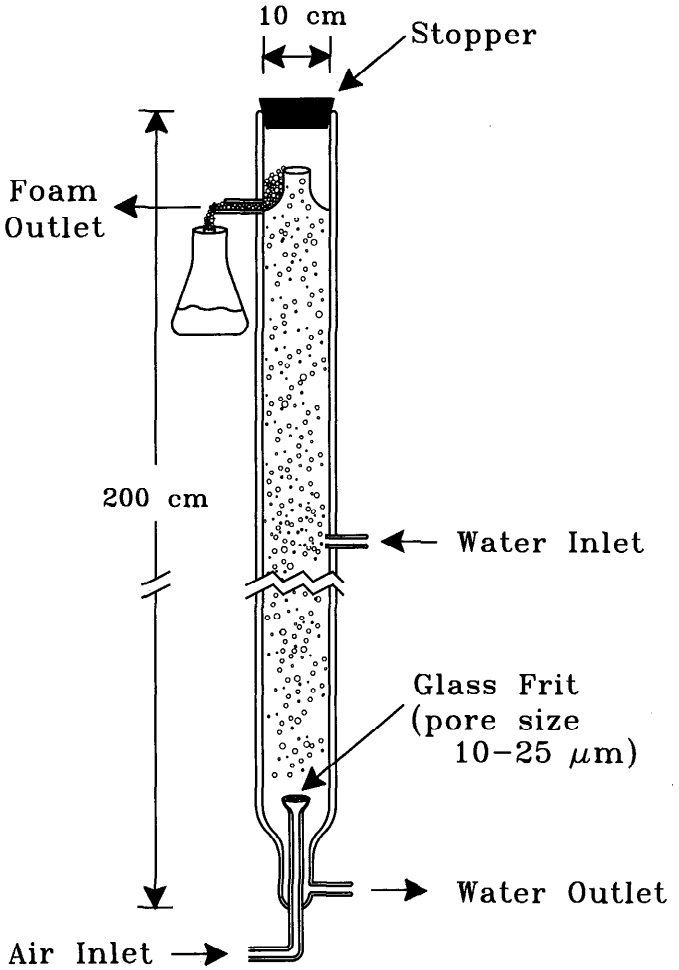

Fig. 1. Bubble absorption column for extracting surface-active DOM as particles. The design is a modification of that used by Baylor and Sutcliffe (1963). The average bubble size was ~500 μm.

iments. Bacterial concentrations were determined in filtered seawater, foam, and residual water samples for the size-fractionation experiments (*see below*). The collected foam samples actually consisted of foam plus varying amounts of background seawater. Thus, all foam measurements were corrected for this background—that is, concentrations in the background water were subtracted. To facilitate data comparison, the background-corrected TEP and carbohydrate concentrations of the foam were volume normalized, that is, the concentrations were multiplied by the volume of the foam sample and divided by the original volume of filtered seawater placed in the bubble extraction column (15.3 liters). The overall reproducibility for foam tower extractions, based on TEP and carbohydrate determinations, is estimated at ±10–20% (n = 3).

Determination of TEP—Concentrations of TEP were measured using the technique of Passow and Alldredge (1995*a*). Briefly, 30–100-ml foam or 500-ml seawater samples were filtered at a low and constant vacuum (~150 mm Hg) onto polycarbonate filters (0.4 μm). The filters were stained for ~2 s with a 0.02% aqueous solution of Alcian blue (8GX; Sigma) in 0.06% acetic acid (pH 2.5; 0.2 μm filtered). The stained filters were gently rinsed with distilled water to remove excess dye and then transferred into 25-ml beakers. Six milliliters of 80% H_2SO_4 were added and the filters soaked for 2 h. The beakers were agitated 3–5 times during this period. The absorbance of the sulfuric acid solution was measured at 787 nm. Filter controls consisted of stained blank filters and were subtracted from the absorbances of samples. The calibration standard was prepared by grinding ~15 mg of gum xanthan into 200 ml of distilled water. Dry weights of the standard solution were determined by filtering 0.5–3-ml aliquots onto preweighed filters. The concentration of Alcian blue–stainable particles in the standard was measured by filtering 0.5–3 ml of the calibrated standard and following the procedure described above. TEP concentrations are given in units of gum xanthan equivalents (micrograms per liter). The precision was ±0.2 μg (n = 3), corresponding to a relative standard deviation of ±5–10% for most analyses but only about ±20–30% near the detection limit (~0.1 μg liter^{-1} at signal-to-noise ratio = 2).

Determination of total carbohydrate concentration and neutral sugar composition—Foam and water samples were kept frozen until analyzed. Sample aliquots (four replicates of 25 ml) were dried under vacuum using a Speedvac Plus model SC210A concentrator (Savant Instruments). The dry residue was treated with cold (0°C) 12 M sulfuric acid (~72% w/w), sonicated for 15 min, stored for 2 h at room temperature, and then diluted to 1.2 M (on ice) and hydrolyzed for 4 h at 100°C (Mopper 1977). Total carbohydrates in the hydrolysate were measured spectrophotometrically by the 3-methyl-2-benzothiazolinone hydrazone hydrochloride method (Pakulski and Benner 1992). Glucose was used as a standard. The precision was usually ±5–10% (relative standard deviation, n = 4) at the 1 μM level.

Chromatographic analysis of neutral sugars in the acid hydrolysates involved neutralization and deionization steps before injection into the high-performance liquid chromatography (HPLC) system. Details of these steps are given in Mopper et al. (1992). Briefly, 6-deoxy-glucose was added into the hydrolysates as an internal standard (final concentration, 1 μM) before the hydrolysates were neutralized with an equivalent amount of barium hydroxide powder. The internal standard served to correct for sugar losses during the subsequent workup steps. The neutralized samples were centrifuged to remove the barium sulfate precipitate. The supernatants (~2 ml) were desalted by passing them through a glass column containing a 5-ml mixed bed of equal amounts of anion (AG2-X8, 20–50 mesh, carbonate form) and cation (AG50-X8, 50–100 mesh, hydrogen form) exchange resins (Bio-Rad). The flow rate was about 2 ml min^{-1}.

The neutral sugar composition in the hydrolysates was determined by anion exchange HPLC at a pH of >12, followed by electrochemical detection in the pulsed amperometric mode (Rocklin and Pohl 1983). The separations were obtained on a 4 × 250–mm Carbo-Pac PA1 analytical column (Dionex) at a flow rate of 1.0 ml min^{-1} and at ambient

Table 1. The amount (gum xanthan equivalent, μg) and percentage of TEP extracted and generated by bubbling <1.0 μm-filtered seawater (15.3-liter) samples.

Sample	Water (0 h)	Residual	Loss (extracted) from water	% Lost (extracted) from water	Foam	% Excess (new) TEP in foam	Mass balance
Monterey Bay 27 Jul 93	459	306	153	33.3	452	66	165.1
Shannon Point 14 Jan 94	367	275	92	25.1	112	18	105.4
East Sound 17 Apr 94	398	169	235	59.0	200	−18	91.1
East Sound 19 Apr 94	223	141	82	36.8	172	52	140.4

TEP in "Water (0 h)" means the total amount of TEP (μg) in the <1.0-μm–filtered seawater before bubbling; TEP in "Residual" means the total amount of TEP (μg) in the same filtered seawater after bubbling (usually 1 h); TEP "Lost (extracted) from water" is the TEP in the initial water (0 h) minus the TEP (μg) in the "residual" water; TEP "% Lost (extracted) from water" is defined as [Lost/(Water 0 h)] × 100%; TEP in "Foam" means total amount of TEP (μg) collected in the foam samples; "% Excess (new) TEP in foam" corresponds to the amount of TEP in the foam that is newly generated (not extracted) from the seawater sample and is defined as [1− (Lost/Foam)] × 100%; "Mass balance" is equal to [(Residual + Foam)/Water (0 h)] × 100%.

temperature. In the current study, isocratic elution was used for most samples. The isocratic mobile phase was 0.25 mM acetic acid in 7.5 mM NaOH solution. For the East Sound samples, gradient elution was used because it was found to give a somewhat better resolution, especially for rhamnose and xylose. The gradient used was 7% B, 0–15 min; 7–100% B, 15–25 min; 100% B, 25–35 min; where A mobile phase was water and B mobile phase was 1.5 mM acetic acid in 50 mM NaOH. Postcolumn addition of base (0.8 M NaOH added at 0.8 ml min^{-1}) was used to stabilize the baseline (Rocklin and Pohl 1983).

Determination of bacterial concentrations—Bacterial concentrations were determined from replicate slides that were prepared directly after sampling by staining 3–10 ml of sample with Acridin orange according to the method of Hobbie et al. (1977). Bacteria were enumerated on a Zeiss fluorescence microscope at a magnification of ×1,250. A total of 1,000 cells were counted on 10–20 frames per replicate for all foam samples and for all water samples filtered through 1.0- or 0.45-μm filters. A total of fewer than 50 cells on 30 frames each were considered to represent background values.

Determination of covalently bonded sulfate—Free sulfate and other salts were removed from foam and seawater samples using an Amicon stirred cell ultrafiltration system (model 8400). The membrane filter size was 1,000 Daltons. Approximately 150 ml of sample was placed in the cell, and high-purity nitrogen gas (~60 psi) was used to displace the salts through the membrane filter with deionized water. Usually, about 6,000 ml of deionized water was needed to remove all salts and free sulfate, based on a negative silver chloride test. The retentate was concentrated to about 40 ml and collected for analysis of bound sulfate, total carbohydrate, and total organic carbon (by high temperature catalytic combustion). A 25-ml aliquot of the desalted retentate was dried under vacuum using a Speedvac Plus model SC210A concentrator (Savant Instruments). The dried samples were digested in 0.2 ml concentrated nitric acid–sodium carbonate solution (10 mg $NaCO_3$ ml^{-1} HNO_3) at 200°C for 15 min (Falshaw and Furneaux 1994). The residual HNO_3 was evaporated at 200°C for 1 h. Digested samples were diluted in deionized water to 25 ml for sulfate measurement. The undigested samples were also analyzed to determine background sulfate concentrations, which were usually negligible. Sulfate measurements were made on a Dionex series 4000i ion chromatography system equipped by an HPIC-AS4A anion exchange column, a micromembrane suppressor, and a conductivity detector. Analyses were performed isocratically at a flow rate of 2 ml min^{-1}. The mobile phase consisted of 0.75 mM sodium bicarbonate with 0.2 mM sodium carbonate. The anions were identified and quantified by comparing retention time and peak areas with those of external standards. The bound-sulfate content was determined by subtracting the background concentration from the concentration of the digested sample.

Results and discussion

Extraction and formation of TEP in <1.0-μm-filtered samples—Significant amounts of pre-existing TEP were removed (25–59%; "% lost"; Table 1) from <1.0-μm filtered seawater samples after bubbling for about 1 h. These extracted TEP were assumed to be completely transferred from the bulk water into the foam fraction as a result of adhesion to the bubbles—that is, we assumed that there was no dissolution or disaggregation of pre-existing TEP or significant adsorption to the walls during the experiment. We further assume that the total amount of TEP in the foam was equal to the amount of TEP removed from the water (i.e., extracted, pre-existing TEP) plus any newly generated TEP formed by bubbling. The latter contributed 66, 18, and 52% of TEP in the foam from Monterey Bay, Shannon Point, and East Sound 19 April 1994 samples, respectively ("% excess"; Table 1). The amount of TEP in the foam fraction of the East Sound sample collected on 17 April was actually smaller (−18%) than the amount of TEP removed from the bulk sample during bubbling, indicating that TEP was lost from the system. This suggests that the assumption noted above

Table 2. Concentrations of surface-active carbohydrate and TEP in foam samples collected after bubbling filtered seawater samples for 1 h. Concentrations were background-corrected (*see* Materials and Methods).

Seawater	Filter size	Surface-active carbohydrate in foam, normalized (glucose equivalent, μg liter^{-1})	Surface-active TEP in foam, normalized (gum xanthan equivalent, μg liter^{-1})	Bacteria in foam,† normalized* 10^3 ml^{-1}	Mass balance on bacteria,† (%)
Shannon Point	1.0 μm	8.09	6.61	7.5	54
14 Jan 94	0.1 μm	2.34	1.02	1.3	—
	100 kDa	<3.4	1.86	2.2	—
	10 kDa	<3.8	1.29	6.6‡	—
East Sound	1.0 μm	16.3	12.2	71	69
17 Apr 94 (day 4)	0.45 μm	7.56	0.63	4.2	81
	0.1 μm	6.52	0.28	3.3	—
	100 kDa	10.2	1.15	1.9	—

* Volume normalized, i.e., the concentrations were multiplied by the volume of the foam sample and divided by the original volume of filtered seawater placed in the bubble extraction column (15.3 liters).

† Concentrations of bacteria in prefiltered water (before bubbling) were 0.25, 0.9, and 0.07 × 10^6 ml^{-1} for 1.0-μm Shannon Point and 1.0-μm and 0.45-μm East Sound samples, respectively. Bacterial concentrations in water prefiltered through 0.1-μm, 100 kDa, or 10 kDa were too low for reliable counts.

‡ Contamination suspected.

that there was no loss of TEP to the walls or dissolution is not correct; that is, we may be underestimating the amount of newly formed TEP in the other samples. Even though the 18% loss (and 18% gain for the Shannon Point sample) is close to our precision, some loss of TEP to the walls is supported by bacterial results (*see below*).

The formation of new TEP is reflected in the mass balances (Table 1). Abundant TEP were newly generated in samples from Monterey Bay and East Sound (19 April 1994) in which phytoplankton activity was high. In contrast, the Shannon Point sample and the East Sound sample collected during the prebloom period (17 April 1994), both of which showed low algal activity, yielded negligible new TEP on bubbling. Bubbling generates TEP (defined as retained on a 0.4-μm filter) from colloidal precursors (<0.4 μm). Thus, from the above results these precursors appear to consist mainly of relatively fresh material released by phytoplankton as opposed to pre-existing (background) organic matter, which is in agreement with previous observations (Passow and Alldredge 1994, 1995*b*; Passow et al. 1994). The results of Kepkay et al. (1990) may also be relevant in this context. Those investigators found that respiration in seawater samples was greatly enhanced by bubbling as a result of bubble adsorption–coagulation. However, this enhancement was found to be important only for high productivity surface water samples.

Size fractionation experiments—Surface-active carbohydrates, TEP, and bacteria were enriched in all foam samples of the size-fractionation experiments (enriched relative to nonbubbled waters). Enrichment of bacteria in foam samples should result from the extraction of bacteria from the initial (unbubbled) water by bubbles (Blanchard and Syzdek 1974; Blanchard 1983; Weber et al. 1983; Biddanda 1985), whereas enrichment of TEP in foam should represent both extracted pre-existing and newly formed TEP. Enrichment of total carbohydrates in foam represents the extraction of surface-active carbohydrates, a fraction of which will include carbohydrates from TEP and bacteria.

Concentrations of surface-active carbohydrates, TEP, and bacteria in foam extracts decreased sharply when decreasingly smaller-sized filters were used to size-fractionate the samples before bubbling, as shown in Table 2. From this table, it can be seen that the concentration of TEP dropped off more rapidly than that of surface-active carbohydrates (about 7–20-fold versus about 2–4-fold) with filter-pore sizes decreasing from 1.0 μm to 0.1 μm (1.0 μm to 0.45 μm for the East Sound sample). These results indicate that bubbling extracts surface-active carbohydrates from both >0.1-μm and <0.1-μm colloidal substances. In contrast, bubbling extracts TEP from surface-active carbohydrate precursors that are mainly >0.1 μm (or >0.45 μm for the East Sound sample), indicating that concentrations of TEP precursors were much higher in the larger-size fractions compared with the smaller ones. However, it should be pointed out that because of the way TEP are defined (>0.4 μm), any TEP formed from the smaller-size fractions (<0.1 μm) must be newly generated. Compositional evidence (*see below*) suggests that the latter may have been derived from background DOM and background colloidal substances rather than from freshly released algal material. Thus, background substances appear to be less effective than fresh colloidal material at producing particles (e.g., TEP) by bubble adsorption, which is consistent with the results of Kepkay et al. (1990). TEP results from these size fractionation experiments are also in agreement with theoretical and empirical studies that have demonstrated that bubble scavenging primarily affects colloids in the size range of about 0.2–1.2 μm (Batoosingh et al. 1969; Johnson et al. 1986; Kepkay and Johnson 1989).

Alternatively, the difference in the drop-off seen in extraction rates for TEP versus surface-active carbohydrates (Table 2) may have been because of the formation of TEP that were too small to qualify as TEP. TEP were operationally defined by retention on 0.4-μm filters, whereas surface-

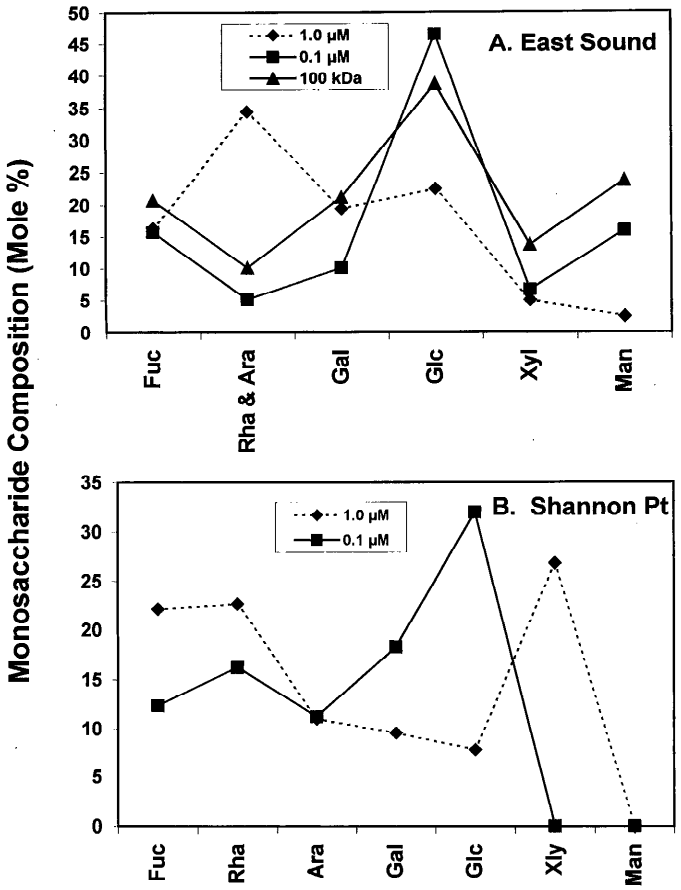

Fig. 2. (A) Mole percent neutral sugar composition of foam samples (0–1.0-h bubbling interval) extracted from filtered East Sound surface seawater samples of different sizes, collected 17 April 1994 (day 4). Fuc = fucose; Rha = rhamnose; Ara = arabinose; Gal = galactose; Glc = glucose; Xyl = xylose; Man = mannose. (B) Mole percent neutral sugar composition of foam samples (0–1.0-h bubbling interval) extracted from filtered low-productivity Shannon Point surface seawater samples of different sizes, collected 14 January 1994. The data for the <100-kDa fractions are not shown because they were not considered reliable because of possible interference and low sugar concentrations. The lines were drawn to indicate trends.

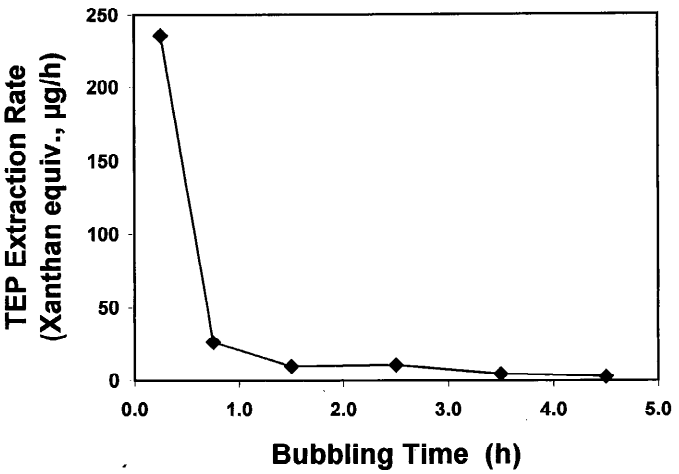

Fig. 3. TEP extraction rate as a function of bubbling time. Results were from bubbling <1.0-μm–filtered East Sound surface seawater collected 19 April 1994 (day 6).

active carbohydrates were measured in whole foam samples. Thus, colloidal TEP precursors that passed a 0.4-μm filter may have represented an important fraction of the material produced or extracted by bubbling but would not have been quantified using the current procedure. Filter sizes smaller that 0.4 μm (e.g., 0.2 or 0.1 μm) will be used in future studies to quantify the amount of "smaller TEP" that may be formed by bubbling.

Mass balances for bacteria in the size-fractionated samples indicate that only 54–80% of bacteria initially present (before bubbling) could be accounted for after the 1-h bubbling period (*see Table 2*). This loss of bacteria was presumably due to adsorption to container walls. Thus, these results suggest that our estimates for the formation of TEP, which are based on the assumption that no TEP were lost, may be low because TEP are very adherent and because loss rates of TEP due to adsorption are probably comparable to those of bacteria.

Inspection of Table 2 reveals that the concentrations of surface-active carbohydrates and TEP in foam samples extracted from 100- and 10-kDa ultrafiltered seawaters were larger than those extracted from 0.1-μm ultrafiltered seawater. The reason for this surprising result is not clear. One possibility may be the differences in the construction of the ultrafilters. The 0.1-μm cartridge has a hollow-fiber design, whereas the other ultrafilters (100 and 10 kDa) are spiral wound. It has been previously shown that the size cutoff given by the manufacturer for a membrane can be grossly inaccurate when applied to seawater and other dilute media. For example, during a recent colloid extraction intercomparison experiment, it was found that a membrane with a cutoff rated by the manufacturer as 1 kDa was actually closer to 50 kDa, as determined by standards added to seawater (Buesseler et al. 1996). Another possible explanation may be related to differences in the filtration times. Filtering through 100- and 10-kDa membranes usually took two to four times longer than through the 0.1-μm membrane. The greater resistance offered by the smaller pore–sized membranes might have caused stress-related changes in surface-active properties of the colloids. In support of the latter explanation, in a previous study (Mopper et al. 1996) we noted marked changes in fluorescence and absorbance spectra of colloids after ultrafiltration of seawater samples.

Neutral sugar composition of size-fractionated samples—Compositional analyses revealed that surface-active polysaccharides in the foam extracts of the <1.0-μm–filtered East Sound and Shannon Point samples contained a higher percentage of deoxysugars (fucose plus rhamnose) relative to glucose (Fig. 2A,B), in agreement with our group's previous results (Mopper et al. 1995). In contrast, the surface-active polysaccharides in the <0.1-μm and <100-kDa ultrafiltered seawater fractions were enriched in glucose and thus may have represented background substances and food reserve

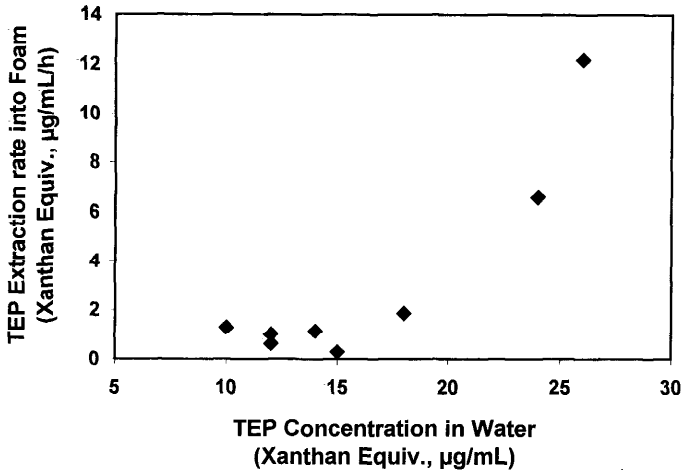

Fig. 4. Extraction rate of TEP in the foam as a function of initial TEP concentration before bubbling. Results were from bubbling seawater samples from the size-fractionation experiments from East Sound 17 April 1994 (1.0 μm, 0.45 μm, 0.1 μm, 100 kDa) and from Shannon Point 14 January 1994 (1 μm, 0.1 μm, 100 kDa, 10 kDa) (same samples as in Table 2).

glucans (Mopper et al. 1995). The neutral sugar composition of the samples is discussed further below.

Kinetics experiments—For the two time-series experiments (Monterey Bay and East Sound on 19 April 1994), the extraction rate of TEP from <1.0-μm filtered seawater was high initially and dropped rapidly after the first 30 min of bubbling, as shown in Figure 3 for the East Sound sample. The data in Figure 3 can be fit to either first-order or second-order kinetics. A first-order fit (i.e., log [TEP] versus bubbling time) implies that the extraction rate of TEP was directly related to the concentration of TEP precursors initially present in the water. A second-order fit (i.e., 1/[TEP] versus bubbling time) implies that the rate was linearly related to the square of the TEP–precursor concentration—that is, two TEP molecules were involved in the transition state (O'Melia and Tiller 1993). Second-order kinetics fit these data better (r^2 = 0.93 for second-order versus r^2 = 0.51 for first-order), which is in agreement with our previous results (see fig. 11 in Mopper et al. 1995).

However, particle formation caused by surface coagulation on rising bubbles should theoretically follow first-order kinetics because the rate of particle formation is linearly dependent on the particle collection efficiency and the concentration of colloids or particles present (Johnson et al. 1986; Weber et al. 1993). Figure 4 shows that the rate of TEP enrichment into the foam was found to have a nonlinear relationship to the initial concentration of TEP in unbubbled water. A possible explanation for the nonlinearity is that TEP in foam samples were derived from two sources: pre-existing TEP (i.e., TEP already present before bubbling) and newly formed TEP (Table 1). Consequently, it is likely that neither first- nor second-order kinetic analysis is appropriate for describing the TEP enrichment rate into the foam. In contrast to these TEP results, and in agreement with theory, the extraction rate of bacteria was found to have a linear relationship to the concentration of the bacteria initially present in the filtered water (before bubbling; r^2 = 0.99, n = 4).

The second-order kinetics, which the TEP data appear to follow (r^2 = 0.93), may also be explained by the extraction of two types of materials at different rates: one type that is formed rapidly and another that is extracted at a slower rate. In support of this explanation, the results of our previous mesocosm study showed that there were two major types of compositionally different surface-active polysaccharides present in seawater during an algal bloom (Mopper et al. 1995). These types were "highly surface-active" polysaccharides that are extracted initially (after less than 30 min of bubbling), and "less surface-active" polysaccharides, which may have been background substances in the seawater. Thus, the former consists of colloidal material freshly released by algae during the bloom, whereas the latter may have consisted of more aged colloidal material present in the water before the bloom. Neutral sugar results of the present study further support this explanation (*see below*).

As described above for TEP, the rate of extraction of surface-active carbohydrates into the foam samples was also high initially and dropped off quickly after the first 30 min of bubbling (plot resembles Fig. 3). A fair correlation (r^2 = 0.57, n = 26) was obtained between TEP and surface-active carbohydrates in foam samples for size-fractionated samples collected on different days during the East Sound experiment. A correlation was expected because it has been shown previously that particles and surface-active carbohydrates both follow similar extraction kinetics during bubbling (Mopper et al. 1995). This correlation suggests that TEP represent a high percentage of surface-active polysaccharides extracted by bubble adsorption. Based on weight, we estimate that TEP represents ~20–80% of surface-active polysaccharides in foam extracted from 1-μm filtered seawater (e.g., Table 2). This estimate is crude because the exact molecular weight of the reactive subunit within the xanthan standard used to calibrate TEP is not known.

In contrast to TEP, the concentration of bubble-extracted bacteria was poorly correlated with concentrations of surface-active carbohydrates in various foam samples (r^2 = 0.03, n = 8). The poor correlation is probably related to the different extraction kinetics, that is, first-order versus apparent second-order, as discussed above. The poor correlation indicates that coextracted bacteria represent a low percentage of surface-active polysaccharides (and TEP) extracted by bubble adsorption.

Neutral sugar composition of time-series samples—The neutral sugar composition of the time-series foam samples from the Monterey Bay experiment further supports the idea that there are two major types of surface-active materials that can be extracted at different rates by bubbling. Figure 5A shows the mole percent neutral sugar composition for foam samples collected at different bubbling times as well as the source seawater (<1.0 μm) before bubbling and the residual seawater after bubbling. The neutral sugar composition of the 0–1.5-h foam fraction ("highly" surface-active carbohydrates) is quite different, that is, enriched in fucose and depleted in glucose, relative to the subsequent time fractions and the source (and residual) water. In fact, the compositions

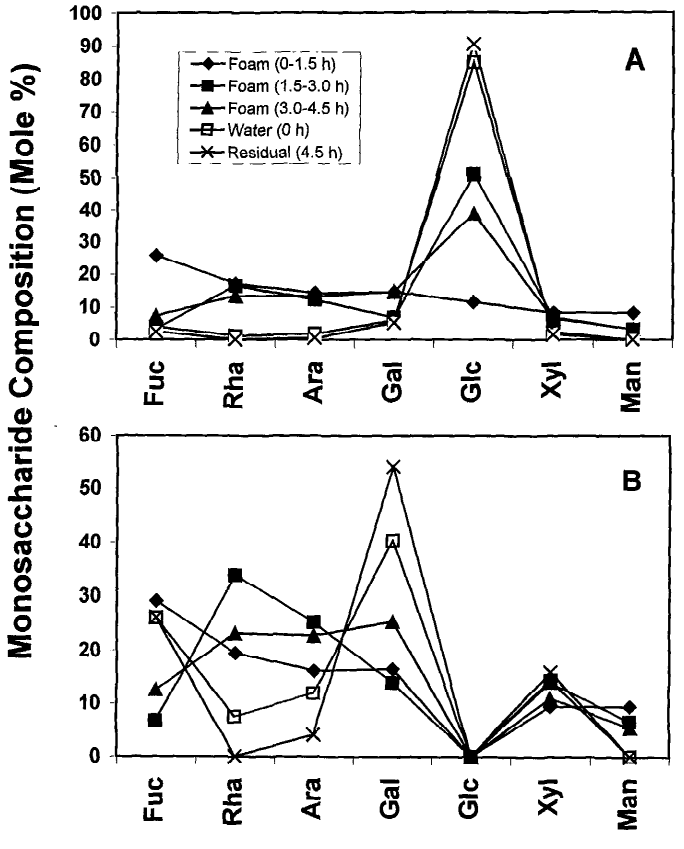

Fig. 5. (A) Mole percent neutral sugar composition of foam samples, filtered seawater (<1.0 μm), and residual seawater (after bubbling 4.5 h). The results are from bubbling <1.0-μm filtered Monterey Bay surface seawater collected 27 July 1993. Fuc = fucose; Rha = rhamnose; Ara = arabinose; Gal = galactose; Glc = glucose; Xyl = xylose; and Man = mannose. (B) Same as in panel (A), but on a glucose-free basis (mole percent glucose was set to zero).

of the subsequent foam fractions (>1.5 h) appear to approach that of the source, that is, enriched in glucose. However, the high concentration of glucose in the subsequent foam fractions and the source water make it difficult to determine whether deoxysugars are in fact preferentially scavenged by bubbling or whether they are just passively concentrated as glucose remains behind in the residual water. Therefore, the data were replotted on a glucose-free basis (Fig. 5B). From this plot, it can be seen that the deoxysugars (fucose plus rhamnose) are indeed significantly higher in the earliest foam fraction than in source and residual waters, that is, 48% versus 33% and 26%, respectively. Even more striking is the preferential extraction of rhamnose plus arabinose into the later foam fractions, especially the 1.5–3.0-h fraction, that is, 59% versus 19% and 4% for source and residual waters, respectively. Figure 5B also shows that in addition to glucose galactose is left behind in the residual water, although it starts to become extracted into the last foam fraction. In summary, the highly surface-active foam fraction (0–1.5 h) is enriched in deoxysugars, whereas the less surface-active foam fractions (>1.5 h) are enriched in glucose, rhamnose, and arabinose, and the residual water (non–surface-active carbohydrates) is enriched in glucose and, to a lesser extent, in galactose.

From these marked changes in composition, it is evident that highly surface-active polysaccharides, which include TEP and their precursors, were extracted into the foam quickly, <1.5 h and, probably, <0.5 h (Mopper et al. 1995). Mopper et al. (1995) found that highly surface-active polysaccharides and TEP also contained relatively high concentrations of deoxysugars (fucose and rhamnose), whereas less surface-active polysaccharides and the source water were glucose rich. Similar results were obtained for the <1.0-μm filtered East Sound and Shannon Point samples (Fig. 2A,B). Deoxysugars constituted about 43, 42, and 44% of the total neutral sugars in the initial foam extracts (0–1 h) from <1.0-μm filtered Monterey Bay, East Sound, and Shannon Point seawater samples, respectively.

Frew et al. (1990) examined the monosaccharide composition of surface-active carbohydrates released by a marine diatom species (*Phaeodactylum tricornutm*). Complex changes in the monosaccharide composition as a function of growth stage were also found, but glucose (from small glucans) dominated surface-active material in the latter stages. The apparent lesser importance of deoxysugars (compared with our results; Mopper et al. 1995) may be for several reasons. Frew et al. (1990) extracted surface-active substances at a pH of 5 with C_{18} Sep-Pak solid-phase cartridges (Waters Associates). Thus, it is possible that a different fraction of the surface-active material was extracted by solid-phase cartridges than by bubble adsorption. For example, C_{18} cartridges may preferentially extract a fraction that is enriched in glucose (such as the less surface-active fraction discussed above). Alternatively, the exudate of the diatom species examined by Frew et al. (1990) may simply have contained much fewer deoxysugars than the species present in our study. Frew et al. (1990) also suggested (based on staining results) that some of the surface activity of the carbohydrate-containing solid-phase extracts was due to conjugation with proteins and lipids. Mopper et al. (1995) also found that proteins were coextracted with surface-active carbohydrates by bubble adsorption (lipids were not determined). However, it seems likely that these two different extraction methods (i.e., solid-phase adsorption and bubble adsorption) would have different affinities for protein-conjugated carbohydrate-containing surface-active exudates. This difference in affinities may have also contributed to the monosaccharide composition differences found between the two studies.

Bound sulfate in foam samples—It has been shown that deoxy- and galactose-rich algal (especially diatom) exudates contain high concentrations of sulfate half esters (Percival 1968; Allan et al. 1972). The presence of sulfate half ester groups renders these polysaccharide-rich exudates stainable by Alcian blue (Rasmus 1977) and somewhat sticky because of their tendency to form hydrogen bonds and metal ion bridges, especially with calcium and other alkaline earth ions. Sulfate analyses support the presence of high concentrations of covalently bonded sulfate within the colloidal/DOM pool extracted by bubbling different seawater samples, as shown in Table 3. Although the data set is small, several

Table 3. Bound sulfate (or sulfate half ester) content of foam extracted from seawater samples by bubbling.

Sample	Foam fraction (h)	% Sulfate (w/w-OM)*	Sulfate concentration (volume-normalized)† (μg liter $^{-1}$)
East Sound (Apr 23)	0–1	52.5	420
East Sound (Apr 24)	0–1	44.5	330
Monterey Bay (day 1)	0–1	0	0
Santa Barbara mesocosm (1993*)			
Day 7	0–1	0	0
Day 7	1–5	0	0
Day 8	0–1	23.0	16
Day 8	1–5	0	60
Day 9	0–1	0	0
Day 9	1–5	0	0
Day 10	0–1	11.5	17
Day 10	1–5	0	0
Day 11	0–1	43.0	94
Day 11	1–5	0	0
Day 12	0–1	7.1	14
Day 12	1–5	9.3	42
Day 13	0–1	15.4	80
Day 13	1–5	0	0

* Weight percent sulfate was calculated as weight of sulfate in foam divided by the weight of organic matter (OM) in foam (estimated as two times the extracted TOC) times 100%. A description of the mesocosm experiment and samples is given in Mopper et al. (1995).

† For the volume normalization calculation, the concentrations of bound sulfate in the foam were multiplied by the volume of the foam sample and divided by the original volume of filtered seawater placed in the bubble extraction column (15.3 liters).

trends can be noted. Covalently bound sulfate was found mainly in the highly surface-active foam fraction (0–1 h), as opposed to the less surface-active fraction (1–5 h). Also, covalently bound sulfate was found mainly during high algal activity, for example, in the East Sound near the end of the experiment and in samples from the latter half of the mesocosm experiment, corresponding to late log and stationary growth phases of the diatom bloom (Mopper et al. 1995). There appears to be a relationship between covalently bound sulfate in the foam (as a percentage of bubble-extracted organic matter) and the concentration of highly surface-active carbohydrates for the mesocosm samples. Although the correlation is only fair ($r^2 = 0.57$, $n = 7$), the similarity in the trends is evident from Figure 6. The peak in the percentage of sulfate seen on day 8 occurred 1 d before the first detectable flocs (>0.5 mm) (Alldredge et al. 1995). The drop-off in percentage of sulfate after day 11 may have been caused by a dilution effect, that is, the release of non–sulfate-containing surface-active material, in particular glucans, by senescent algae as the bloom crashed (Mopper et al. 1995).

Another interesting trend shown in Table 3 is that bound sulfate (as a percentage of bubble-extracted organic matter) and the absolute concentration of bound sulfate both are significantly higher in the East Sound samples than in the mesocosm samples, even though the latter system flocculated and the former did not. Thus, sticky sulfated polysaccharides, which were strongly enriched in the highly surface-active foam fraction (e.g., 0–1 h of bubbling), may have had an important role in floc formation in the mesocosm system but not in the East Sound system. Clearly, factors other than just the production of sticky polymers are needed for algal flocculation to occur (Jackson 1990; Kiørboe et al. 1994; Dam and Drapeau 1995).

Conclusions

The results of this study indicate that high-molecular-weight colloidal substances are the main source of the TEP (Alcian blue–stainable particles >0.4 μm) and surface-active polysaccharides that were extracted by bubble adsorption. The size range of the bubble-extracted colloidal precursors (>0.1 μm and <1.0 μm) lies within the optimal range for particles available for surface coagulation in seawater (Johnson et al. 1986; Johnson and Kepkay 1992). Apparently, this optimal range represents a minimum between two different particle transport mechanisms: convective diffusion and turbulent shear (Kepkay 1994). Thus, particles in this range, including TEP, are less accessible to bacteria and can accumulate in seawater in the absence of surface adsorption or other coagulation mechanisms (Kepkay and Johnson 1989). Bubbling of filtered seawater has been shown to considerably enhance bacterial activity as a result of organic matter degradation on the newly formed aggregates (Kepkay and Johnson 1988). In turn, this enhanced degradation should significantly speed up the cycling of carbohydrates and other algae-released colloids in surface waters.

Waters with high algal activity produced the highest concentrations of surface-active carbohydrates and new TEP when bubbled, indicating that substances released by algae are preferentially scavenged by bubbles (Corner et al. 1974; Kepkay et al. 1990). For example, a 1-h bubbling time increased TEP concentrations by ~150% for biologically productive waters in Monterey Bay and East Sound, whereas no significant increases were observed for low-productivity Shannon Point and prebloom East Sound samples (Table 1). These results suggest that bubble adsorption of surface-active material has a role in the formation of TEP in surface

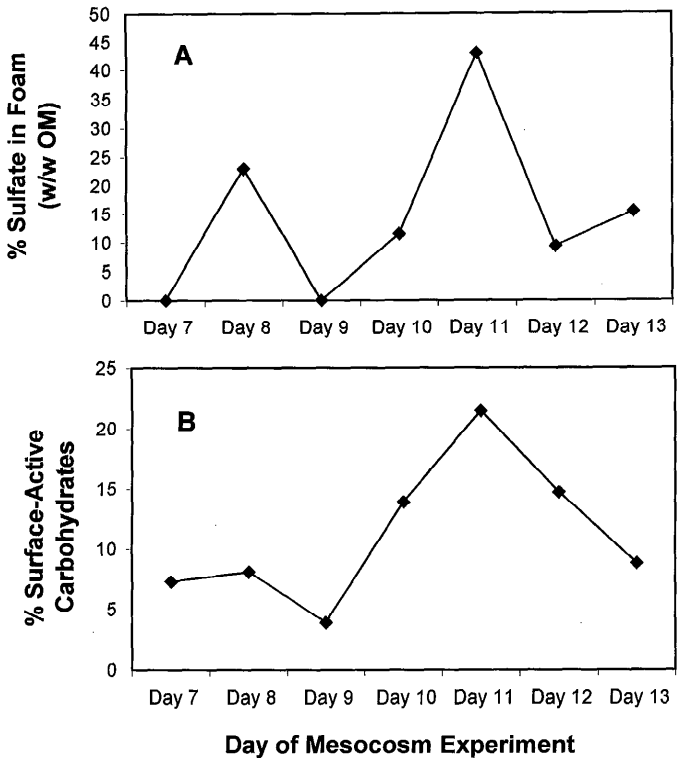

Fig. 6. (A) Covalently bound sulfate concentration (as w/w percentage of organic matter, OM) within the highly surface-active (0–0.5 h) foam fraction extracted from filtered mesocosm water (<1.0 μm) on different sampling days (Mopper et al. 1995). (B) Percent of total carbohydrates (MBTH reactive) in filtered mesocosm water (<1.0 μm) that could be extracted as highly surface-active carbohydrates (in 0–0.5-h foam) on different sampling days (after Mopper et al. 1995).

seawater, especially during algal blooms. However, the overall importance of bubble adsorption in TEP formation in the sea cannot be readily quantified from this study because the results from the bubbling of a fixed volume of seawater inside a bubbling column cannot be easily extrapolated to bubble adsorption that occurs in surface waters. Although the bubble population generated in the column resembles that formed during the breaking of a wave (Cloke et al. 1991; Haines and Johnson 1995), the relative coagulation efficacy in the column may be low by as much as a factor of 2–20 (Kepkay and Johnson 1989). Because bubbles formed during the breaking of a wave can be injected as deep as 20 m or more before they dissolve (Kanwisher 1963), bubble adsorption may impact TEP formation well below the sea surface. Large bubbles (from 200 to >600 μm) that are typically formed during the breaking of waves are more effective than smaller background bubbles (<200 μm) at producing particles by surface adsorption (Johnson et al. 1986). Thus, the production of TEP by bubble adsorption during low-wind conditions is expected to be less important than that during high-wind events.

In addition to bubble adsorption (surface coagulation), TEP are known to be formed from laminar and turbulent shear (Passow and Azetsu-Scott unpubl. data) by the coalescence of colloidal particles that follow streamlines and eddies. Future studies need to address the question of whether TEP formed from bubble adsorption are compositionally and structurally different from TEP formed by shear. The answer to this question could give new insights into the mechanisms of abiotic particle formation in the sea.

A fair correlation was found between the concentration of TEP and the concentration of surface-active polysaccharides in the foam samples, which suggests that TEP in foam were derived from and coextracted with these polysaccharides. However, it is not clear whether the reverse holds true, that is, whether surface-active polysaccharides are composed mainly of TEP. As a rough estimate, bubble-extracted surface-active polysaccharides may be composed of 20–80% TEP by weight.

In agreement with results from a previous study by our group (Mopper et al. 1995), highly surface-active polysaccharides (i.e., polysaccharides extracted into the foam within a very short time, usually <1 h) are enriched in deoxysugars (fucose and rhamnose) and galactose and are sulfated. The finding of relatively high concentrations of sulfated deoxysugars in different coastal waters is consistent with the exudate composition of the algal species found in the sampled waters (Percival 1968; Allan et al. 1972; Myklestad et al. 1972). These results suggest that algal exudation is the main source for surface-active sulfated polysaccharides extracted by bubbling.

The results of this study have implications beyond that of TEP formation and the resultant particle flux. Several recent studies have shown that polysaccharides are the dominant component of high-molecular-weight DOM and colloidal material (>~1,000-Dalton ultrafiltrates) isolated from surface and deep oceanic sites (McCarthy et al. 1993; Aluwihare et al. 1997; Skoog and Benner 1997). These studies have shown that these polysaccharides have a high content of deoxysugars and galactose. Based on these findings and the results of the present study, we hypothesize that the highly surface-active sulfate-containing polysaccharides released by phytoplankton become preferentially incorporated into high-molecular-weight DOM and colloidal substances by abiotic reactions. These reactions include hydrogen bonding and cationic (e.g., calcium ion) complex formation involving covalently bound sulfate. Further analyses of high-molecular-weight marine DOM and colloidal substances for measurement of covalently bonded sulfate are needed to test this hypothesis of abiotic DOM and colloid formation.

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