8. Dynamics of Dissolved Organic Matter and Microbes in Seawater through Sub-Micron Particle Size Analyses

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Introduction

The dissolved organic matter (DOM) pool in seawater is one of the largest exchangeable reservoirs of reduced carbon on our planet (Carlson, 2002). Its magnitude (~685 Gt carbon) is roughly of the same order as that of the atmospheric carbon dioxide pool (Hansell and Carlson, 1998; Hedges and Keil, 1995; Fasham et al., 2001). However, DOM constitutes only a miniscule fraction of seawater, as it is contained within an electrolyte-laden solution in which the mass of inorganic salts outweigh organics by several thousand-fold (Ratanathanawongs and Keil, 1997). The dilute nature of DOM in seawater makes exploring its formation, dynamics and fate an immense challenge. An intriguing aspect of DOM is that a large fraction of this dissolved organic pool is not readily available for biological consumption (Koike et al., 1990; Wells and Goldberg, 1991; Pakulski and Benner, 1994; Benner, 1997). Radioisotope studies have shown that the average residence time of DOM in the deep ocean can be of the order of ~ 6000 years, a time period long enough for these compounds to be cycled several times in the oceans before being degraded (Williams and Druffel, 1987; Druffel et al., 1992). Because of these extremely slow turnover times, it is believed that the semi-refractory fraction of DOM could be playing an unexpectedly significant role in mitigating the build-up of carbon dioxide in the earth’s atmosphere and reducing global warming (Hedges, 1992; Benner, 1997). In view of the above, understanding the processes regulating the dynamics and distribution of DOM in seawater, assumes tremendous importance for global biogeochemistry and climate change studies.

Phytoplankton are the primary source of oceanic DOM and supply, on an average, about 0.5 Gigatons of Carbon per year (Mague et al. 1980; Fogg, 1983; Kirchman et al., 1991). This constitutes between 5 to 25% of the total carbon photosynthesized (Fogg, 1983). However, this amount can vary depending on the environmental conditions during phytoplankton photosynthesis and growth (Fogg, 1983; Cota et al., 1996). For instance, during a bloom event, phytoplankton excrete on average between 20 to 40% of newly fixed carbon (Kirchman et al., 1991), which can increase to about 60% when nutrients become limiting towards the end of a bloom (Norrmann et al., 1995). Other factors that contribute to the DOM pool are death and decay of phytoplankton following cessation of a bloom (Mague et al., 1980), sloppy grazing by protozoans and copepods and, in some cases, lysis of phytoplankton cells by viruses (Balch et al., 2002). Since the DOM pool represents a large fraction of the atmospheric carbon dioxide that is sequestered into the oceans, mechanisms responsible for its formation and transformation as well as its fate have been the subject of considerable study (Bjorrensen, 1988; Zlotnik and Dubinsky, 1989; Amon and Benner, 1994; Carlson and Ducklow, 1995; Hansell and Carlson, 1998).

Most investigations of the biological processes responsible for the dynamics of DOM rely on monitoring the consumption and breakdown of specific compounds of phytoplankton origin, generally, through the use of labeled precursors and substrates (Kirchman et al., 1991; Fry et al., 1996; Rich et al., 1997). Considerable efforts have also gone into studying physical and chemical processes (abiotic processes), which impact the dynamics and turnover rates of DOM (Kepkay, 1994, Moran and Zepp, 1997; Chin et al., 1998). However, integrating biotic and abiotic influences on DOM has proven challenging. For example, we now know that nanometer-sized DOM particles can assemble spontaneously to form tiny (~5µm) polymer gels (Chin et al., 1998). Once formed, these microgels can either be colonized by bacteria and re-enter the food chain (Chin et al., 1998; Wells, 2002) or become non reactive (Carlson, 2002). We strongly believe that information on such intermediate compounds, either by way of their size or their susceptibility to biotic and abiotic transformations, could provide important clues...
as to how DOM becomes refractory, or how the formation of different reactive groups of organic compounds impacts the capacity of the oceans to mitigate the build-up of CO₂ in earth’s atmosphere (Najjar et al., 1992).

At present, the exact mechanisms involved in the transformation and degradation of organic polymers remain elusive primarily because of the lack of suitable analytical techniques to examine DOM intermediates (Benner et al., 1992). The miniscule proportion of DOM with respect to inorganic salts in seawater (1:35,000) has only served to compound the problem (Benner et al., 1992). The operational pre-concentration step in most techniques imposes severe limitations on the true nature of compounds in the DOM pool (Vaillancourt and Balch, 2000). There have been attempts to determine the molecular weight or size spectrum of DOM using size exclusion chromatography, but these have generally proven unsatisfactory (Beckett et al., 1987). One reason is that DOM is exposed to high shear stresses during separation by exclusion chromatography. Furthermore, a sizeable amount of DOM is lost because of adsorption onto the support matrix. Ultrafiltration, another widely utilized although problematic technique has provided valuable data on the relative amounts of organic carbon. However these separations have been confined to broadly defined size categories (Maurer, 1976; Benner, 1991). Resistive-pulse particle counting and laser-light scattering have also enabled the analysis of DOM particles in their native environment with minimal sample manipulation, but these techniques are severely limited by detector sensitivity to particles less than 0.4μM (Koike et al., 1990; Chin et al., 1998). Transmission electron microscopy and atomic force microscopy have allowed direct observation of DOM particles down to sizes close to 1 nm (Harris, 1977; Wells and Goldberg, 1991, Santschi et al., 1998). However, the major difficulty with these microscopic methods is that the sample can be exposed to rapid changes in ionic strength and pH, which alter the diameter of organic particles (Vaillancourt and Balch, 2000).

During the past two years, we have been investigating the utility of the Flow Field Flow Fractionation (FFFF) technique for examining submicron-sized particles in seawater. The FFFF is a family of chromatography-like techniques for separating and sizing of particles in suspension (Giddings et al., 1976; Giddings et al., 1978; Beckett et al., 1987) and has found great success in industry and medicine. However, the technique has yet to be exploited in oceanic sciences. Unlike previous methods, the FFFF does not depend on sample pre-concentration prior to its injection into the FFFF channel. Since the integrity of the sample is maintained, it allows for analysis of the sample in its native state.

Here we present results from a wide range of studies undertaken in our laboratory on the formation, fate and transformation of submicron colloidal particles that demonstrate the immense potential of the FFFF technique for ocean biogeochemistry, marine optics and remote sensing studies.

The FFFF technique and approach

The underlying principles of the separation of compounds by the FFFF system are similar to those involved in separation by chromatography, except that the compounds are separated in a thin ribbon-like channel by interaction with an external flow field rather than an internal stationary phase (Fig. 1). The sides

![Diagram of FFFF](image-url)

Fig. 1. Schematic representation of the FFFF depicting the channel and cross flow, the Rheodyne® injector, pumps and detector. Detailed inset shows the semi-permeable bounding walls of the channel and illustrates the normal mode of separation of particles inside the flow channel. Figure adapted from Beckett et al. (1987)
of the thin ribbon-like channel are composed of porous frits bounded on one side by a semi-permeable membrane. Filtered seawater as a carrier solution is pumped by two precision pumps through the channel at right angles to each other. The flow along the long axis of the channel (called the channel flow) is laminar and has a parabolic profile with faster flow velocities in the center than at the edges. For separation and sizing of particles, the sample is injected into the channel flow stream. The secondary stream, known as the cross-flow, is pumped into the channel at right angles to the channel flow through a porous frit. The cross-flow stream exits through the semi-permeable membrane and in doing so helps drive the particles in the channel flow-stream towards the semi-permeable membrane which is also known as the accumulation wall. When the particle sizes in the sample are <1µm, the forcing of the particles towards the accumulation wall is opposed by the particles' Brownian motion, which is related to the diffusion coefficient or the molecular size of the particles. When the particles reach equilibrium state within the channel, the larger particles are compressed against the accumulation wall, which causes them to be carried slowly by the channel flow because of the slower velocities of the channel flow near the accumulation wall. In contrast, the smaller particles tend to be dispersed further away from the accumulation wall. As a result, they travel more rapidly and are eluted faster because of the parabolic nature of the channel flow. In this mode of operation, known as the normal mode, the size measurement capabilities of the FFFF are based on a theoretical relationship between the retention volume \( V_r \) and the ordinary diffusion coefficient \( D \). Particle hydrodynamic diameters \( (d_h) \) can then be obtained from \( D \) using the Stokes-Einstein equation \( d_h = kT/3\pi\eta D \), where \( k \) is Boltzmann’s constant, \( T \) is the absolute temperature, and \( \eta \) is the viscosity of the carrier solution (Giddings et al., 1976, 1978). Upon exit from the channel, a variety of optical or other characterization techniques can be used in-line to detect the fractionated components. In the case of larger particles (usually > 1µm) however, the mechanism of particle migration through the channel undergoes a transition from the normal to the steric mode. In the steric mode, larger particles (with negligible diffusion), when driven forcefully by cross-flow towards the accumulation wall, are lifted back towards the long axis of the channel by hydrodynamic forces and carried out of the channel faster than the smaller particles. The exact relationship between the lift force and the channel flow velocity is not well-characterized and calibrations with standard monodispersed particles (>1µm) are necessary to calibrate an FFFF fractogram into a size distribution plot.

The FFFF instrument utilized in this study was a model F-1000® universal Fractionator (Postnova Analytics, Salt Lake City, UT, USA) equipped with two high precision pumps (one computer controlled) for maintaining the rates of the channel and cross-flow during particle separation. A regenerated cellulose membrane of nominal molecular weight cutoff of 10,000 Daltons served as the semi-permeable membrane within the channel. A Rheodyne® injector with a 100 µl sample loop was used to introduce the samples into the channel. Channel flow exited the channel at two points towards the end of the channel, one at the detector outlet and the other just upstream called the frit outlet.

A needle valve placed at the frit outlet helped restrict the channel flow and redirect the carrier solution enriched with particles to detector outlet. An ultraviolet absorbance detector (Alltech® Model 450 UV at 254 nm) set at its highest sensitivity of 0.005 absorbance units was used to detect particles. 0.02 mm filtered seawater from the Gulf of Maine containing a non-ionic surfactant (Pluronic® F68, BASF, final concentration 0.1% v/v) served as the carrier solution in the FFFF. Surfactant was added to the carrier solution prior to filtration in order to minimize interactions between individual particles and reduce particle adhesion to the semi-permeable membrane during sample runs through the FFFF. Prior to an experiment, mono-disperse suspensions of polystyrene latex beads (Coulter®, Polysciences® and Duke®) ranging in diameter from 19 nm to 900 nm were run through the FFFF to establish a standard curve, which provided an elution time dependent measure of particle diameter under a given set of conditions (Fig.2)

![Fig. 2. Standard curve of elution time versus particle size of monodispersed polystyrene beads.](image-url)
Since the primary purpose of this communication is to demonstrate the potential and the promise of the FFFF technique for ocean sciences, the results presented below highlight the diversity of the experiments rather than the kinetics of the processes. Detailed protocols have been omitted, but all pertinent references have been listed and results have been discussed exhaustively. For clarity, we have deliberately limited this section to only a detailed description of the FFFF while details on individual experiments both laboratory and field, along with first interpretations of the results have been included as sub-sections in the following section.

Observations from Laboratory Experiments

Species related size spectra of phytoplankton exudates

The purpose of this experiment was to compare the size spectra of exudates released by phytoplankton from different phylogenetic groups growing under identical conditions. Actively growing, axenic cultures of several species were obtained from Bigelow Laboratory’s Culture Collection for Marine Phytoplankton (CCMP), transferred to f/2 media and grown as batch cultures under a 14L:10D light cycle and at 18°C until the cells reached exponential phase. Growth in this medium and under these conditions for over four generations ensured that the cultures were acclimated. Following the fourth transfer and during the exponential stage of growth (established by fluorescence measurements of whole cells in a Turner Designs Fluorometer), cells were gravity filtered through a 1 µm pore size filter. The filtrate was carefully collected and 100 µl aliquots were immediately injected into the FFFF to obtain the size spectra of the exudates.

The FFFF fractograms for six species in Fig. 3 show distinct interspecies differences in the nature of dissolved organic material from phytoplankton. The particle size distribution of the exudates ranged from 10-700 nm and bore no relationship to the size or the phylogeny of the phytoplankton species examined. Two diatoms, one dinoflagellate, two Prymnesiophytes and one Chlorophyte, although grown under identical conditions, produced a wide range of particles with peaks in size range extending from 10 to 500 nm. The largest range of particles was observed in Dunaliella sp. while the dinoflagellate Amphidinium carterae produced the smallest amount of particles that were also the least diverse.

Size spectra of phytoplankton exudates during growth cycle

In the next set of experiments, the size spectra of phytoplankton exudates were examined in relation to the growth phase and age of the cultures. The axenic cultures were grown in conditions described above. Cultures were sub-sampled at various stages of growth monitored fluorometrically, gravity filtered through a 1 µm pore size filter and the filtrates were then injected into the FFFF. The resulting fractograms (Fig. 4) revealed large differences in the size spectra of the exudate pool during the growth cycle of the cultures.
Because of space constraints, only two representative fractograms (Dunaliella sp. and Emiliana huxleyi) have been displayed. During the early and mid-exponential growth phases, particles sizes within the exudates were much smaller compared to those in the late exponential and stationary growth phases.

During the early and mid exponential phase, Dunaliella sp. produced only small sized particles in the 100-150 nm range. In the late exponential and stationary phase particles in the 200-300 nm range also appeared in the exudate. In contrast, the coccolithophore E. huxleyi produced larger particles (400-1200 nm) throughout the entire growth cycle, with particle sizes of about 400-800 nm in the early and mid exponential phase and larger ones (1000-1200 nm) in the later phases. In both cultures, size of the particles was clearly tied to growth phase of the cells, with larger particles associated with aging cultures. These differences in particle size of exudates from early exponential to stationary phase cultures can be attributed either to accumulation and aggregation of the particles in the culture medium as the culture grew and aged or simply due to differences in the composition of the exudates as the cultures aged.

Bacterial uptake mediated changes in the size spectra of phytoplankton exudates

In the third set of experiments, 20 ml of suspension from three stationary phase cultures (Fig. 5) was filtered through 1 µm filters, transferred to sterile tubes and incubated in the dark at 16°C with and without the marine bacterium, Vibrio sp. isolated from the Gulf of Maine. Four ml aliquots of exudates inoculated with this bacterium and controls were sub-sampled soon after inoculation, and following 12 and 36 h of incubation. The aliquots were gravity filtered prior to injection into the FFFF. Fractograms of the exudates with and without bacteria (Fig. 5) at 0 h and after 12 and 36 hrs of incubation showed a distinct uptake of phytoplankton exudates by bacteria. Bacterial uptake of exudates reduced the DOM pool in all three cultures to almost half its size at the end of 36 hours as evidenced by the size of particle peaks. Larger aggregates (>400 nm) appeared to be more susceptible to consumption by the bacterium than smaller (200 to 400nm) particles. Even though uptake rates have not been calculated (although possible based on the detector response), the difference in the amount of exudates between controls and bacterial samples shows that uptake of the exudate from the dinoflagellate Heterosigma akashiwo was greatest while that from the diatom Chaetoceros sp. was the least. This is in spite of the fact that the size range of particles in the 3 exudates was similar (200-800 nm). A reasonable assumption is that particles produced by the three cultures, although of the same size range, were structurally different. Interestingly, at the end of 36 h, a sizeable fraction of particles remained unconsumed. We suspect that this unconsumed fraction could represent the semirefractory component of algal exudates (Koike et al., 1990; Wells and Goldberg, 1991; Pakulski and Benner, 1994; Benner, 1997). Our ability to size and separate particles with the FFFF opens immense possibilities for more specific and sophisticated chemical analyses that could provide us with a better understanding of the characteristics of this fraction.

Dynamics of viral, bacterial and phytoplankton interactions

In previous studies, (Balch et al., 2000; 2002) we have shown that viral infection of biological particles rapidly transforms bacteria and phytoplankton into optically active material, viz. DOM and cell debris. The FFFF was used to investigate the kinetics of particle size dynamics during viral infection of host bacterial and phytoplankton cells. Marine bacteriophages and host bacterial cells were isolated from seawater samples collected in West Boothbay Harbor, Maine as
described in Balch et al. (2000). Bacteria were isolated by filtering seawater onto 0.4 mm membrane filters which were then placed on pads containing 2216 Marine Broth and incubated overnight at 25°C.

Six strains were isolated by picking the colonies from the pads and growing them overnight in the 2216 broth to produce pure cultures. Bacteriophage isolation involved an enrichment technique in which 100 host bacterial colonies of each species were concentrated by centrifugation, resuspended in 5ml broth and inoculated into 500ml flasks containing 100ml triple strength broth and 300ml of the original sea water sample. Following an incubation period of 1-3 days, aliquots from each enriched flask were filtered (0.22 µm filter) and spot tested on beds of original host bacterial cells and observed for development of typical virus plaques over a 24-48 h period. Depending on the speed with which the phage types could lyse their host cells, subsets of the viral phages were selected for further study. A total of 6 viral types were isolated.

Of the several bacteria-virus experiments performed, only the infection of Pseudomonas perfectomarina by the viral strain LS05 is shown (Fig. 6). The kinetics of the infection process and the effect of viral lysis on the optical properties of the bacterial suspension are described in Balch et al. (2002). The infection and lysis of P. perfectomarina and the formation of submicron size particles was observed within 3 hours following the transfer of the viral phage (Fig. 6). The viral peak, which ranged in size from 150-200 nm increased sharply with time especially from 180 to 350 min. After that, the 650-minute increase was smaller. Note that since the species of P. perfectomarina used in these experiments exceeded 1mm in diameter, its early elution from the FFFF seen in the fractogram is to be expected.

A similar viral lysis experiment was carried out in a mesocosm in West Boothbay Harbor. P. perfectomarina was grown in 2 outdoor 5m², 100 L bags, one of which served as control, while the other was infected with the LS05 virus (for more details on the mesocosm experiment refer to Balch et al., 2002). Viral infection was observed approximately 22 hrs after infection of the host cells (Fig. 7). Particles produced following the infection were in two size ranges: 150-200 nm (viral particles) and 400-600 nm (bacterial debris). It must be noted that the bacterial debris peak was not distinct in laboratory culture experiments using the same bacteria and virus (Fig. 6). It is probable that flocculation and aggregation of particles under natural conditions produced this fraction. Although the size range of the particles remained consistent from 0 to 49 h, gradual increases in both viral particle and bacterial debris peaks were observed with time. Also noteworthy is the fact that the FFFF helped detect viral infection of the control sample during the course of the experiment from contamination of the peristaltic pump used for subsampling of the mesocosms.

In order to study phytoplankton-viral interactions, laboratory experiments were conducted in which the cyanobacterium Synechococcus sp. grown in f/2 medium was infected with the lysate of S-1 viral strain. Viral infection of the cells was noticed as early as 6 days into the experiment (Fig. 8). Viral size measured by the FFFF was confirmed by TEM (see Fig.1 in Balch et al., 2002). The viral peak at 300-400 nm was highest on the 17th day of infection but almost disappeared on the 24th day. Instead, the 24th day showed an increase in the large size fraction (500-600 nm) thought to be sub-micron particles formed after viral lysis. No other sample in this experiment showed a peak in this size range. A large amount of
exudate from the *Synechococcus* sp was collected in the control samples on the 17th day although this peak disappeared by the 24th day possibly because of particle aggregation to form particles greater than 1 mm.

**Observations from Open Ocean studies**

In order to test the ability of the FFFF to separate and size submicron particles in natural seawater samples, the instrument was operated on two cruises to the North Atlantic Ocean. These two cruises Chalk-Ex I and II were undertaken in Nov. 2001 and June 2002 to perform large-scale manipulative experiments in which several tons of calcium carbonate (CaCO$_3$) particles were dispersed into the sea to simulate a coccolithophore bloom. In addition to obtaining data that tracked and monitored the fate of the chalk, the FFFF ably demonstrated spatial as well as temporal differences in the size distribution of submicron particles in the Gulf of Maine during pre-Chalk sampling.

Two stations were selected to depict the vertical size spectra (0-60m) of samples analyzed by the FFFF during the Chalk-Ex cruise of November 2001. The South Station was characterized by shallow and stratified waters, while the North Station was well mixed. At the South Station (Fig. 9A), the FFFF fractograms showed conspicuous vertical differences in the size distribution of particles, with a greater fraction of smaller sized particles (100-700 nm) residing in the surface waters and larger particles (400-900 nm) at deeper depths. Smaller particles in the upper 2m comprised the bulk of particles sampled in the 0-60 m water column. The presence of smaller sized particles (which as seen in the culture experiments are within the typical size range of particles released by phytoplankton) in surface seawater samples could be DOM produced from the typical fall phytoplankton blooms observed in the Gulf of Maine (Townsend, 1991). The decrease in smaller sized particles and increase in larger particles with depth could be ascribed either to preferential consumption of the smaller particles by heterotrophs and/or the aggregation of small particles to form larger particles at depth. The consistency of these results for samples at stations with similar physical characteristics suggest that larger particles within the 800-900 nm size fraction represent the semi-refractory component which plays an important role in the transfer of carbon to deeper waters.

The scenario at the North Station was very different. Following a storm, the waters were well mixed and no significant differences were observed with depth throughout the water column (Fig. 9B). The bulk of the particles was located within the 600-900 nm size range. The fact that the physical conditions and their impact on the water column were clearly observed in the fractograms substantiates its effectiveness as a tool to study particle dynamics in coastal and open ocean environments.

As part of the large scale experiments performed to simulate the coccolithophore bloom, cretaceous coccolith calcite (mean size = 2µm diameter, all
particles smaller than 10µm) was added to seawater from several depths in the South Station to attain final concentrations of ~0.02mM which are typically representative of coccolithophore blooms. About 4 h after addition of calcite, the broad peak at 100-900 nm observed in pre-Chalk samples (Fig. 9A) disappeared almost completely as seen in the fractograms of Fig. 10. No peaks were seen at any of the depths sampled. These observations were consistent with seawater samples collected immediately following the dispersal of chalk. The concentration of dissolved organic carbon (DOC) in samples from the laboratory experiment (measured using a Shimadzu TOC-5000 according to Sugimura and Suzuki, 1988) also showed a decline over a time scale of hours. The reduction in DOC after the chalk was added to the water, clearly demonstrates the removal of DOM from seawater due to its adsorption onto cretaceous chalk particles.

In order to create conditions that would be more representative of the natural marine environment, additional experiments were performed using calcite ooze sediments from two cores KNP73 and LL394, obtained from the Woods Hole core collection. The ooze was oven dried at 40°C, and added to exudates of the diatom, *Pleurosigma* sp. and the macrophyte, *Fucus vesiculosiss* which had been pre-filtered through a 1.0µm filter. After addition of the calcite ooze, the exudates were left for 4 hours before the fractograms were run. Calcite ooze sediments have some DOC adsorbed onto them, so measurements of the amount of DOC that can be absorbed by calcite are more realistic than those with commercially available cretaceous chalk, which is usually treated. FFFF was used to detail the sub-micron particle size distribution of the exudates before and after addition of calcite ooze sediments (Fig. 11A-B). The experiment also used untreated exudates as controls as well as exudates containing commercial cretaceous chalk for comparison. Comparisons of the FFFF derived size spectra of the DOM pool before and after addition of the calcite sediment confirmed that the sediment samples were still reactive for DOM (Fig. 11A-B). Sediments from both cores removed a large proportion of the DOM in both exudates over a time scale of hours. As suspected, cretaceous chalk adsorbed a larger amount of DOM than the calcite ooze core samples.

**Utility and Potential of FFFF**

All FFFF fractograms obtained from the carefully planned and executed experiments confirm the remarkable dissimilarity of the particle size spectra of exudate pools of different phytoplankton groups. Culture studies have shown that phytoplankton release a wide variety of photosynthetic compounds including nitrogenous compounds, such as amino acids and proteins and organic acids such as glycollate and lipids (Hellebust, 1965; Fogg, 1983). The proportion of these compounds within the culture medium could have a significant influence on submicron particle sizes within the DOM pool.

The FFFF technique clearly showed that the size of particles in the extracellular pool of phytoplankton is related to the growth phase of the cells, with larger particles associated with aging cultures. In addition to
age, factors such as nutrients (Norrman et al., 1995) and light (Mague et al., 1980) also influence the composition of the exudates. Actively growing, nutrient enriched cultures tend to release a greater proportion of amino acids than carbohydrates (Biddanda and Benner, 1997). On the other hand, the nutrient impoverishment that usually accompanies the transition of phytoplankton growth from active exponential cells to stationary phase cells in batch cultures, results in phytoplankton releasing a larger amount of carbohydrates rather than amino acids because nitrogenous compounds become limiting (Biddanda and Benner, 1997; Bronk, 1999). In general, polysaccharides tend to aggregate much more rapidly than amino acids, potentially explaining the larger sizes of sub-micron particles in cells nearing senescence and decay (Biddanda and Benner, 1997).

It is possible that these differences in the released exudates and their differential ability to spontaneously aggregate or immediately form colloidal complexes with trace metals within the media would have an important bearing on the eventual size spectra of sub-micron particles within the exudates. The aggregation of sub-micron particles is thought to represent an important step in the transformation of DOM into particulate organic matter (POM) in seawater (Chin et al., 1997) which accumulates and aggregates slowly in the media over time as the cultures age. Chin et al. (1997) showed that the aggregation of DOM into larger particles could readily be understood in terms of the polymer gel theory, which posits that the eventual equilibrium size of the assembled particles is dependent on the total amount of available free polymers. In older cultures, the presence of a greater amount of DOM coupled with the tendency of polysaccharides to aggregate faster than other compounds would have contributed to the formation of larger particles than seen in younger and healthier cultures.

The high detection capacity of this technique provided fresh evidence that uptake of exudates by bacteria could be related to the size and nature of the particles within the extracellular pool. Presently, we are uncertain whether this preferential uptake is related to the surface area presented by the DOM particles to bacterial cells or whether it is related to the chemical composition of the DOM particles themselves. For instance, data on the fluorescence excitation-emission spectra (Coble, 1996) of the fractions separated by FFFF could provide valuable information that would allow us to be more conclusive. Also of consequence is the impact of ultraviolet radiation on phytoplankton exudates and its implications for bacterial uptake of DOM. Exposure to ultraviolet radiation breaks down more refractory and larger molecules of DOC through photodegradation making DOM more accessible to heterotrophs (Tranvik and Bertilsson, 2001).

An increase in viral particles and the lack of bacterial debris flowing viral infection of bacteria corroborates optical measurements undertaken in similar experiments by Balch et al. (2002) wherein they observed a sharp drop in backscattering coefficients several hours after infection accompanied by an increase in viral counts. However, their optical measurements in mesocosm experiments did not document the large amount of bacterial debris (400-600 nm) observed by the FFFF. Instead they found a large increase in beam attenuation in the dissolved fraction that passed through 0.2 μM pore size filters which they attributed to chromophoric dissolved organic matter (cDOM) released following lysis of host cells. The large peak in the 400-600 nm obtained in these experiments and ascribed to bacterial debris could be optically inactive. However Balch et al. (2002) hypothesize that it could be particles flexible enough to pass through 0.2 μM pore size filters and contribute to the blue attenuation that was observed by them.

Results from the FFFF using chalk and calcite oozes suggest a wide range of processes by which biogenic particles could aid in transporting DOM to the deep oceans. There are conflicting reports as to whether DOC adsorbed onto mineral particles is less or more prone to bacterial attack (Keil and Kirchman, 1994; Mayer, 1994) but it has been demonstrated that DOM adsorption prevents the dissolution of sinking mineral particles in seawater (Troy et al., 1997). It has also been shown that the mineral surface itself acts to prevent or reduce bacterial decomposition (Hedges and Keil, 1995). It follows therefore that organic compounds that are able to adhere to mineral particles will be more successfully preserved. Away from riverine and continental shelf regions, biogenic particles account for a large fraction of the inorganic particles present in seawater, the most abundant being the silica cell walls of diatoms and the CaCO₃ coccoliths of coccolithophores (2.7 kg L⁻¹) which are significantly denser than seawater (Buitenhuis et al., 1996). DOC adsorbed onto calcite surfaces could be more quickly scavenged from surface waters and transported into the deep sea. If organic matter associated with PIC particles sinks faster there would be less time for DOC to be remineralized by bacteria and consequently more organic carbon would escape from surface waters. Although DOM sorption on to sinking mineral particles has been proposed as an abiotic removal mechanism (Nagata and Kirchmann, 1996; Druffel et al., 1998), the magnitude of its importance in the oceanic carbon cycle is unknown.
because of the lack of data (Druffel et al., 1998). The ability of the FFFFF to detect this process opens the possibility of its estimation.

Future prospects

The above experiments demonstrate the ability of the FFFFF to greatly further our understanding of the DOM pool, the removal processes, the characteristics of labile and refractory components of the pools and the factors that lead to DOM accumulation. The results raise intriguing questions about the dynamics and fate of organic compounds in seawater. Based on the accuracy and consistency of data, we believe the FFFFF could be used in future studies that relate to the formation, transformation and fate of DOM in seawater and in doing so provide answers to central questions concerning the marine and global cycles of major bioactive elements.

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