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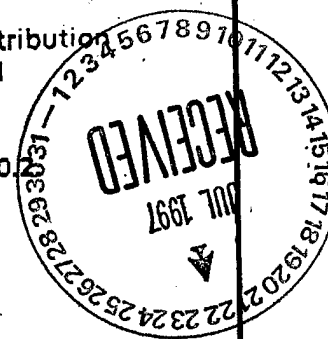
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Franklin College of Arts and Sciences  
School of Marine Programs  
*Department of Marine Sciences*

November 29, 1996

Stacey Curtis  
NCCOSC RDTE Div  
53475 Strothe Road  
San Diego, CA 92152-6310

Dear Stacey,

Enclosed please find the final report for our project on bacterial degradation of paper slurries. The report details the results of chemical analyses (elemental and sugar composition of the slurries) and describes the microbiological studies conducted at the University of Georgia over the past 1.5 years.

Our final set of experiments for the temperature/nutrient matrix study is still ongoing, but will be complete by early January. We will send you a revised version of our report at that time.

If you would like an electronic version of the text or a word processing file on a disk, please let me know, and include information on the word processing program you use.

We enjoyed collaborating with you, Chuck, and Bart on this project, and hope our data will be a useful part of your final analysis of paper slurry release at sea. Please let us know if we can provide any further information.

Sincerely,

Mary Ann Moran  
Assistant Professor

19970728 185

BACTERIAL DEGRADATION OF CELLULOSIC WASTES AT SEA

FINAL REPORT FOR GRANT# N00014-95-0570  
- 1 -  
^

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NOVEMBER 1, 1996

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## INTRODUCTION AND BACKGROUND

Concern over potential environmental degradation of marine habitats by anthropogenic wastes prompted an evaluation by the U.S. Navy of their waste disposal practice of releasing cellulosic wastes (shredded office paper and other paper products) from ships into open ocean waters. One of the important factors determining the ecological consequences of releasing cellulosic wastes into open ocean water is the rate of bacterial decomposition of the material within the water column and on the sea floor. Relatively little is currently known about the biological fate of cellulosic material in the open ocean or the cellulolytic abilities of natural oceanic bacterial assemblages, primarily because cellulose is not naturally abundant in such environments. Such information is critical, however, to understanding the fate of cellulosic particles released into the ocean, to determining where in the environment the bulk of decomposition is likely to occur, to understanding the potential local impacts of releases on marine food webs, and to predicting the time frame necessary for complete decomposition. This report describes studies designed to measure rates and kinetics of bacterial decomposition in seawater and determine the importance of various environmental factors (temperature and nutrient concentrations) in controlling the activity of cellulolytic bacteria in the marine pelagic environment.

Cellulose is a natural and abundant component of coastal marine environments, and previous research in these systems provide a framework for designing studies and interpreting research results. It has been found that rates of mineralization of plant polysaccharides (cellulose and hemicellulose from wood and grasses) range from 0.2 to 2.0% d<sup>-1</sup> (for particles < 500  $\mu\text{m}$  in diameter under aerobic conditions) in tropical and subtropical coastal marshes and mangrove swamps (Benner et al. 1984, 1985, Benner and

Hodson 1984). Temperature is an extremely important environmental determinant of polysaccharide decomposition rates in these coastal marine systems, with  $Q_{10}$  values of 3 - 4 for a temperature increase from 10 to 20°C (Benner et al. 1986). Furthermore, decomposition kinetics of plant polysaccharides can be complex, and mass loss does not always follow a simple negative exponential (first order) model. Instead, the specific rate of decomposition may continually decrease, so that no single decomposition parameter ( $k$ ) is appropriate for describing the entire degradation process (Moran et al. 1989a).

The degradation of cellulosic waste materials at sea will be influenced by factors not operating in the shallow coastal systems that have served as the focus for previous studies. First, temperatures in the bulk of the ocean are lower than those in temperate and subtropical coastal systems, and particles spend significant amounts of time at temperatures less than 10°C. Second, particle sinking rate influences the temperature, pressure, and redox conditions experienced by the attached bacterial community. For example, larger particles (> 500  $\mu\text{m}$  diameter) sinking at a rate of 50 to 100  $\text{m d}^{-1}$  are likely to move out of the upper mixed layer in less than one day and reach the sea floor (with 1-2 °C temperatures and ~ 500 atm pressure) in 40 to 80 days. By contrast, smaller particles (< 50  $\mu\text{m}$  in diameter) sinking at rates of approximately 1  $\text{m d}^{-1}$  may stay in the warmer surface waters for several weeks. Third, because the background abundance of cellulolytic organisms is likely to be low in oceanic waters, bacterial community composition may constrain the rate of degradation, at least initially and perhaps overall.

The general mechanism by which organic particles are degraded in seawater is still poorly understood and much debated (Smith et al. 1992). Observations of naturally-occurring particles in the ocean surface waters have shown that most particles in the sea are

heavily colonized by bacteria (Alldredge and Gotschalk 1990). However, measurements of bacterial growth rates using radiotracer techniques indicate that the attached bacteria are growing very slowly (Ducklow et al. 1982, Simon et al. 1990). If bacterial growth and respiration were the only sinks of particulate carbon, then such slow rates would suggest that it takes months to years for complete degradation of the particles, and therefore that much of the particulate material in the upper ocean sinks to the sea floor undegraded. On the other hand, it has recently been hypothesized that attached bacterial communities have intense hydrolytic activities, even though they are not growing rapidly (Smith et al. 1992). According to this hypothesis, the bacteria are solubilizing particles at much faster rates than predicted from growth rates alone, and thus much of the particulate material is being released as partially-degraded soluble material before the particle sinks to the sea floor. If so, the hydrolytic activity of attached bacteria would result in more of the particle being degraded before it reaches the sea floor. This controversy over the decomposition of natural particles is extremely relevant to understanding the fate of cellulosic wastes at sea, particularly with regard to the rate at which the cellulosic particles decompose and the amount of particulate material that survives passage through the water column to be deposited on the sea floor.

In the studies described herein, degradation of cellulosic particles by marine bacterioplankton was studied over a range of environmental conditions typical of various locations and depths in the ocean. Direct measure of particle mass, measures of bacterial production, and measures of bacterial respiration were used to estimate rates and kinetics of bacterial degradation of cellulosic wastes under a number of nutrient and temperature regimes. Studies were also designed to examine the effect of particle size and bacterial



community composition (inoculum source) on rates of decomposition. Ancillary studies were also conducted to characterize the chemical composition of the cellulose slurries.

## EXPERIMENTAL DESIGN AND RESULTS

### Preliminary Studies

**Chemical Composition of the Cellulosic Particles** - Although the paper slurries contain cellulose as the primary solid constituent, inorganic fillers added to pulped wood during the paper making process will also be a component of the particulate matrix. Clays, calcium carbonate, and titanium dioxide are among the most commonly used fillers and are usually added to improve the whiteness and the printing properties of the paper. In most paper-making processes, fillers account for 15% or less of the total weight of the finished paper.

White paper and mixed paper slurries were analyzed for inorganic content by filtering slurry subsamples through a Whatman GF/F glass fiber filter, rinsing thoroughly with distilled water, drying at 110°C, and weighing. The filters were then combusted at 550°C for 4 hrs to remove all organic materials, and the weight of the remaining inorganic fraction was determined. The elemental composition of the paper slurries (carbon, hydrogen, and nitrogen content) was determined on a Perkin-Elmer 240C CHN analyzer.

Chemical analysis revealed that the white paper and mixed paper particles were 92% organic matter by weight (Table 1). Thus the biologically non-reactive inorganic fillers used in the paper-making process can only make up a small percentage (<8%) of the particle weight. CHN analysis indicated that nitrogen content of the particles is extremely low (below detectable levels) for the white paper slurry and only 0.02% of the mixed paper by weight (Table 1). The weight ratio of carbon to nitrogen (C:N ratio) is therefore >1000,

a value far from the optimal C:N ratio for a bacterial substrate (C:N of 15 or less). These data suggest that inorganic nitrogen availability in seawater may be a critical factor in determining rates of particle degradation in the ocean.

**Sugar Analysis of Mixed Paper Slurry** - Two replicate samples of mixed paper slurry were analyzed at the University of Georgia Complex Carbohydrate Research Center. The purpose of the analysis was to obtain detailed information about the sugar composition of the paper slurry material. HPLC analysis of acid-treated mixed paper slurry indicated that glucose was the major component of the paper material, making up 80% of the total sugar components. Minor constituents included xylose (14%) and mannose (6%). About half of the mixed paper was insoluble in strong sulfuric acid, however, and thus could not be analyzed for carbohydrate content by standard methods.

In consultation with Dr. Russ Carlson of the Complex Carbohydrate Research Center, we conducted an additional sugar analysis on the mixed paper slurry which involved a stronger hydrolysis step than in the HPLC method described above. In this approach, the paper sample was pretreated with a phenol/concentrated sulfuric acid solution at 100°C for 40 minutes, and then analyzed for total sugar spectrophotometrically based on absorbance at 490 nm (relative to a glucose standard). Although this method destroyed many of the side groups that can be used to identify individual sugar components, it successfully hydrolyzed all of the slurry material so that an accurate measure of total sugar could be obtained. Results of analysis of six replicate samples of mixed paper indicated that sugars accounted for  $98 \pm 4$  % of the paper slurry weight. We previously measured ash content of the slurry to be 6-8% of the total dry weight; thus all important components of the slurry have been identified (Table 1).

Table 1. Elemental analysis of white paper and mixed paper slurries.

Sample	Percent C	Percent N	C:N (weight ratio)	Percent Inorganic Matter	Percent Organic Matter	Percent Sugar
White paper	38.5	below detectable levels	NC	3.8	96.2	ND
Mixed paper	38.2	0.02	1908	8	92	98 ± 4

NC---not calculated  
 ND---not determined

**Nutrient-free Seawater** - In order to measure decomposition rates as a function of temperature and nutrients, it was necessary to identify a nutrient-free artificial seawater matrix in which to suspend the paper slurry; this would allow careful control of the concentration of nutrients available to bacterial degraders. A commercial artificial seawater source (Sigma Sea Salts; St. Louis, MO) was found to contain ammonium at concentrations > 4  $\mu$ M and phosphate at concentrations > 2.5  $\mu$ M. Another artificial seawater made in our laboratory according to the method of Lyman and Flemings (1940) contained even higher inorganic nutrient concentrations, and further testing of stock solutions made from individual salts indicated measurable ammonium present in even high-purity preparations of many sea salt chemicals. The relatively high nutrient levels in coastal seawater also precluded our use of this natural seawater source for low-nutrient experiments.

A low-nutrient artificial seawater source was therefore prepared in our laboratory using chemicals that were baked overnight at temperatures just below their melting point and then dissolved in low-organic deionized water (Harrison et al. 1980). The artificial seawater

concentrate (2.5 X) was boiled for 2 hr to drive off any residual ammonium immediately before dilution to standard seawater salinity. With this method, we produced artificial seawater containing only trace levels of inorganic N and P (typically 0.06  $\mu\text{M}$  ammonium and 0.015  $\mu\text{M}$  phosphate).

### **Decomposition Method Comparisons**

Three methods were tested to determine the best approach to measuring bacterial degradation of cellulosic particles: i) gravimetric, ii) bacterial protein production, iii) bacterial respiration. These methods were tested with two types of cellulose slurry (white paper only and mixed paper/cardboard) to compare relative rates of decomposition.

**Gravimetric measurements** involved determining the weight of the cellulosic material at the beginning and end of a given decomposition period. Both white and mixed paper slurries were added to Pyrex flasks and suspended in natural seawater freshly collected from the Georgia nearshore at concentrations of 1 mg (dry weight)  $\text{ml}^{-1}$ . The seawater was amended with 5  $\mu\text{M}$  N (as nitrate and ammonium) and 1  $\mu\text{M}$  P (as  $\text{PO}_4$ ). Flasks were incubated at 28°C while shaking at 200 rpm. All analyses were conducted in triplicate. After a one month degradation period, flask contents were filtered through an 8  $\mu\text{m}$  pore-size Nuclepore filter. Filters were washed to remove salt and dried at 60°C overnight before determination of the weight of the remaining cellulose.

**Bacterial protein production** measurements track increases in bacterial cell biomass by giving instantaneous measurements of the rate of incorporation of radiolabeled leucine into protein. For these experiments,  $^3\text{H}$ -leucine was added to the medium in low concentrations; bacteria assimilated this amino acid and incorporated it into the bacterial

proteins being synthesized within the cell. Rates of  $^3\text{H}$ -leucine incorporation were thus proportional to rates of growth, and could be converted to carbon equivalents (e.g., amount of bacterial carbon production) by a standard conversion factor that accounts for internal isotope dilution of the leucine and the percent leucine typically found in bacterial protein (Simon and Azam 1989). To conduct the assay, subsamples (1.7 ml) were removed from incubation flasks at daily-to-weekly intervals and pipetted into 2-ml microcentrifuge tubes.  $^3\text{H}$ -leucine was added at a final concentration of 20 nM, and cells were allowed to incorporate the leucine for a 1 hour period. After the incubation period, cells were killed and proteins precipitated by the addition of trichloroacetic acid (TCA). Following a series of washes to remove unincorporated  $^3\text{H}$ -leucine, bacterial cell components were pelleted by centrifugation and radioactivity in bacterial protein was determined by scintillation counting. Typically, three replicate samples and 1 control (killed sample) were analyzed from each flask at each time point.

**Bacterial respiration** rates were determined by cumulative measures of oxygen consumption. The microbial consumption of oxygen was tracked by measuring oxygen concentration at various intervals during the incubations with automated precision Winkler titrations.

For this method, incubations were set up in replicate 60 ml glass BOD bottles and placed in temperature controlled incubators or water baths. At the beginning of the experiment and at intervals of 2-days to several weeks, five replicate BOD bottles were sacrificed for determination of dissolved oxygen concentration. Oxygen concentrations were measured using an automated precision Winkler method. Oxygen was first chemically fixed in each bottle by the addition of both manganese chloride and sodium

hydroxide-sodium iodide solutions. A brown precipitate indicated that oxygen was present and had reacted with the manganous hydroxide, forming manganic basic oxide. Upon the addition of sulfuric acid, this precipitate was dissolved and at the same time, iodine was liberated. The quantity of iodine was determined by titrating a portion of the solution with a standard solution of sodium thiosulfate using a Mettler DL21 automatic titrator.

**Results of the methods comparison** Based on gravimetric measurements, rates of decomposition of mixed paper particles averaged 15.9% ( $\pm 4.8\%$ ) over a 32 day period; based on  $^3\text{H}$ -leucine incorporation, rates of decomposition averaged 11.2%; based on bacterial respiration, rates of decomposition averaged 11.26%. Assuming that bacterial decomposition of cellulosic particles occurs via first order kinetics, the measured rates translate into a decay constant ( $k$ ) of approximately  $-0.005 \text{ d}^{-1}$ . Thus the organic matter in mixed paper has a minimum half life of approximately 140 d (4.3 months) in the ocean under temperature and nutrient conditions similar to those used in this experiment. The half life would be longer if environmental conditions are suboptimal for some or all of the decomposition period. During the 32 day time period, bacterial numbers varied from  $7.8 \times 10^6 \text{ ml}^{-1}$  to  $4.0 \times 10^7 \text{ ml}^{-1}$ . These numbers are significantly higher than numbers commonly found in coastal seawater (approximately  $2 \times 10^6 \text{ ml}^{-1}$ ) and reflect the additional substrate added to the seawater in the form of cellulosic particles. Microscopic examination of the flask contents reveals that most of the bacteria are attached to the particle surfaces, rather than free-living.

Although the gravimetric approach is the most straightforward method for measuring decomposition rates, we found fairly large variations among replicates and low sensitivity. It was difficult to add identical weights to replicate incubations and to completely recover

thousands of small particles for weight loss determination. The measurement of bacterial growth rates via uptake of tritiated leucine was found to be a more sensitive approach, although this measure provides an instantaneous growth rate that has to be interpolated between time points, and it only provides information on cellulose converted to bacterial biomass; mineralization losses must be calculated based on an estimate of the efficiency of bacterial biomass production.

The respiration method was found to be the most appropriate approach for measuring cellulose decomposition in this study. Measurements are cumulative and precision among replicates are good. This method was chosen for the remainder of the decomposition studies using two types of respiration assays: 1) short-term assays (8 days or 30 days in length) to systematically examine factors likely to control the decomposition of cellulose in seawater (particle size, temperature, nutrients, water source); 2) long-term assay (three months) to examine the kinetics of decomposition and determine whether first order degradation is the appropriate model for paper slurry degradation.

### **Particle Size Studies**

The cellulosic particles present in the waste slurries span a fairly large size range (from 30  $\mu\text{m}$  to over 3 mm in diameter) and, upon release into the ocean, will sort in the water column based on size and sinking rate. Because bacterial decomposition of particles in the ocean is primarily a surface phenomenon, it is possible that large differences in the surface-to-volume ratio among the particles results in differences in specific decomposition rates. Thus smaller particles with larger surface-to-volume ratios might degrade more rapidly, and have a larger decay constant, than larger particles with smaller surface-to-volume ratios.



Mixed paper slurry was size-fractionated into five size categories by resuspension in distilled water and sequential filtration through a series of sieves and polyethylene mesh screens with 2 mm, 1 mm, 500  $\mu\text{m}$  and 150  $\mu\text{m}$  openings. Particles caught on the 2 mm sieve were repeatedly resuspended and re-filtered to ensure that smaller particles were not attached to larger ones and that the sieve openings were not being occluded. Final size classes were as follows:  $>2000 \mu\text{m}$ ,  $<2000$  but  $>1000 \mu\text{m}$ ,  $<1000$  but  $>500 \mu\text{m}$ , and  $<500$  but  $>149 \mu\text{m}$ .

Size fractionation of the mixed paper slurry into five categories showed that most of the particles did not pass the largest size sieve. We found that 97.8% of the particles (by weight) were retained on the 2 mm mesh. The 1-2 mm category contained 1.4% of the particle weight; the 500  $\mu\text{m}$  - 1 mm category contained 2.3% of the weight; the 150-500  $\mu\text{m}$  category contained 1.1% of the weight; and the  $<150 \mu\text{m}$  category contained 0.4% of the weight.

To measure the effect of the size of cellulose particles on rates of decomposition of mixed paper wastes, bacterial respiration rates were measured individually for each of the particle size categories. Treatments were set up such that all size categories contained the same initial weight of mixed paper ( $0.1 \text{ mg cellulose ml}^{-1}$ ) and five replicates of each size class were established. To measure decomposition, microbial consumption of oxygen was followed over an 8-day period.

Results of the first experiment showed no statistical differences (ANOVA;  $P=0.29$ ; Fisher LSD) among size classes. Assuming first order kinetics, rates were consistent with

degradation of 12.4% of the cellulose in 30 days. In a second experiment, we included an additional treatment which contained the full range of particle sizes (i.e., unaltered mixed paper slurry). Again, there were no differences among the size classes or between the size classes and the unsized treatment (Figure 1) (ANOVA;  $P=0.35$ ; Fisher LSD). Rates of respiration indicated a decomposition rate of 9.4% in 30 days, assuming first order kinetics. Although accurate sizing of the particles was difficult (many particles are linear, so the angle at which they hit the screen has a significant effect on whether or not they pass through; screens clogged easily for the larger size classes), these experiments indicate that particle size is not a critical factor in determining decomposition rate. This is possibly due to the highly linear shape of most of the particles, which increases the effective surface area:volume ratio for all size classes, or to the ability of bacteria to access and colonize individual cellulose fibers within the particle matrix, regardless of its overall size.

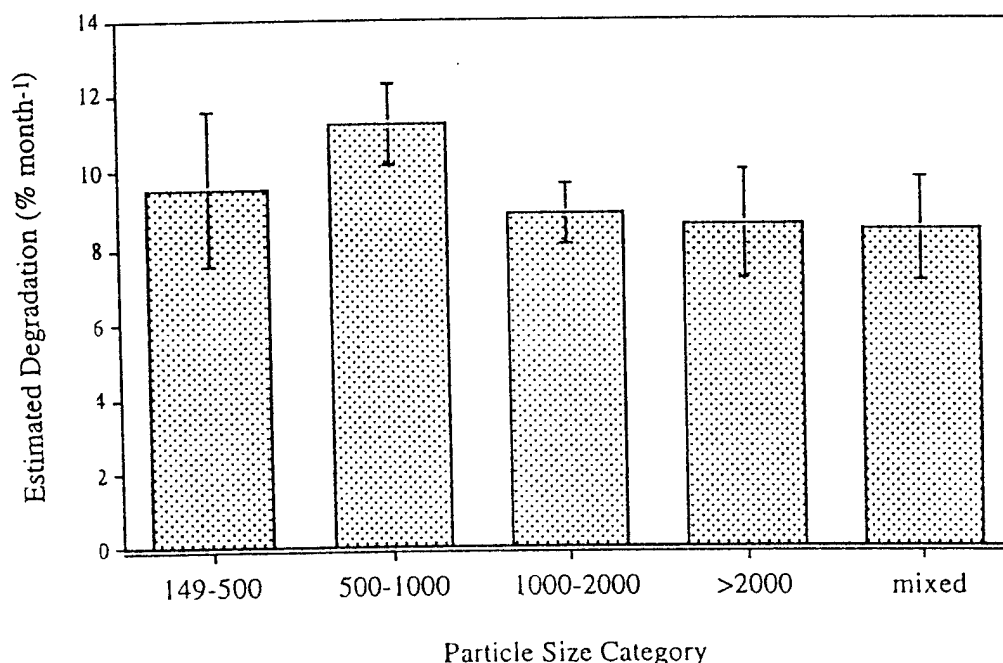


Figure 1. Results of particle size study #2. Bacterial respiration rates were measured for four different size classes of mixed paper particles and for unfractionated mixed paper slurry over an 8 d period.

### Long-Term Decomposition Kinetics

A study was conducted to examine the kinetics of mixed paper slurry decomposition over a 3-month time frame to determine whether the first order kinetics apparent in shorter studies (8 d to 1 month) could be extrapolated to long-term degradation. Decomposition of the paper slurries was carried out in coastal Georgia seawater at 28°C with unlimited inorganic nutrient supply (starting concentrations of 100  $\mu\text{M}$  N and 20  $\mu\text{M}$  P). At intervals of 1-2 weeks, decomposition of the cellulose was determined from cumulative changes in oxygen concentrations in gas-tight incubation bottles (BOD bottles). After 11 weeks of decomposition, 30.7% of the paper slurry had been decomposed. The experiment was terminated at this point because oxygen concentrations inside the BOD bottles had dropped from 206 to 64  $\mu\text{M}$  and there was concern that low oxygen availability might begin to affect decomposition rates.

Kinetics of decomposition generally followed a simple first order decay model where  $-k$  is the first order decomposition constant and equals  $-0.0048 \text{ d}^{-1}$ . The  $r^2$  value for a natural log plot of the data fit with a linear model is 0.982. The equation predicts 50% decomposition of paper slurry in 145 days (21 weeks) and 90% decomposition in 480 days (69 weeks) when decomposition occurs in warm coastal seawater without nutrient limitation (Figure 2).

### Effects of Bacterial Community Source on Decomposition Rates

Laboratory studies were conducted to explore inherent differences in bacterial communities from different marine environments to degrade the cellulose slurries. Degradation rates were determined by bacterial respiration (oxygen consumption) in 8 d bioassays. Seawater was collected in polyethylene carboys from a number of coastal and

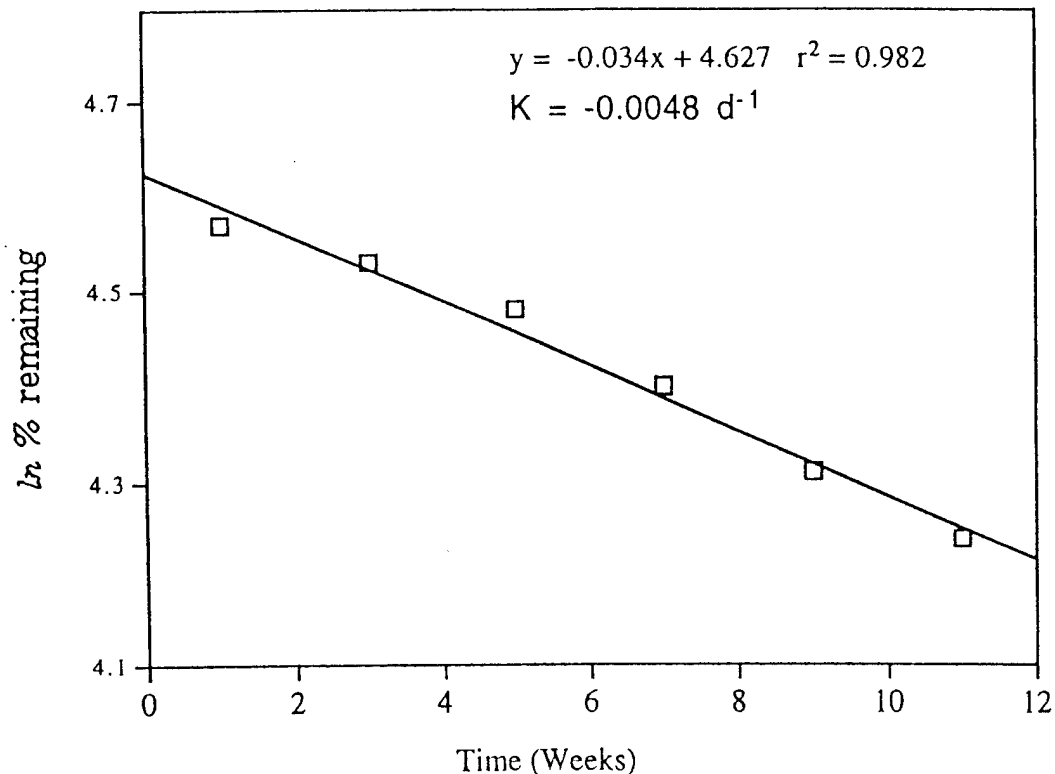


Figure 2. Results of long-term decomposition experiment. Cellulose decomposition was measured as changes in oxygen content in BOD bottles containing  $0.01 \text{ mg ml}^{-1}$  mixed paper slurry.

oceanic sites at a variety of depths (from surface to 3522 m). The water was shipped to our laboratory under refrigeration and used in decomposition studies within 2 days. Rates of cellulose decomposition were measured both with and without supplements of inorganic nutrients (ammonium and phosphate) to control for possible nutrient limitations on bacterial activity. For all samples, degradation rates were measured at  $28^\circ\text{C}$  to control for temperature effects on bacterial activity; for some samples, degradation rates were also measured at the ambient temperature at the time of collection.

Samples were collected from three oceanic sites in tropical, temperate, and arctic regions (Eastern Northern Pacific, Sargasso Sea, Bering Strait, and Chuckchi Sea); from a continental shelf site in a temperate region (Southeastern U.S. shelf); and from a nearshore coastal site in a temperate region (Georgia coastal) (Table 2).

Results showed that cellulose was degraded slowly in all oceanic water samples (average = 0.85% in 30 d at 28°C) (Figure 3 and Table 3), with rates approximately ten-fold lower than those measured for the southeastern US shelf (8.21% in 30 d at 28°C) or coastal Georgia seawater (10.7% in 30 days at 28°C). Differences between oceanic samples and either the shelf or coastal samples were statistically significant (ANOVA;  $P < 0.001$ ; Fisher's LSD). These differences occurred despite the fact that temperature, cellulose slurry concentration, and nutrient availability were identical in all experiments. The low rates of degradation measured for oceanic water may be due to an absence of cellulolytic bacteria in the open ocean. Alternatively, it may be due to injury to bacteria when brought to 1 atmosphere pressure (for deep water samples), or to injury to or inactivity of bacteria when brought from low temperatures to 28°C.

We examined the possibility that the standard temperature chosen for these experiments (28°C) adversely affected rates of decomposition measured in oceanic samples, in which bacterial populations may be adapted to significantly lower ambient temperatures. In these decomposition experiments, water samples from the Chuckchi Sea (Arctic 28 m) and Bering Strait (Arctic 5 m) were incubated at temperatures similar to the temperature of the seawater at the time of collection (4°C) during 8 d bioassays. Rates of decomposition were determined to be 0.88% and 0.50% in 30 days, respectively, values

which are not significantly different from those measured for these same samples at the higher temperature (0.46% and 0.46% at 28°C, respectively) (T-tests; P=0.68 and P=0.58).

Table 2. Water samples collected for bacterial community source studies.

Site	Collection Date	Collection Temp (°C)	Collection Depth (meters)
Arctic (Chuckchi Sea)	July, 1996	-1.5	28
Arctic (Chuckchi Sea)	July, 1996	-0.3	3522
Arctic (Bering Strait)	July, 1996	0.9	5
Sargasso Sea #1	February, 1996	ND	surface
Sargasso Sea #1	February, 1996	ND	750
Sargasso Sea #2	May, 1996	7.5	1000
Northern Pacific Ocean	August, 1995	ND	1500
U.S. Shelf	April, 1996	17	surface
Coastal Georgia	May, 1995	18	surface

\* ND--Not determined.

We also considered the possibility that injury to bacteria due to changes in pressure (when deep water samples were brought to the surface) adversely affected measured rates of cellulose decomposition for oceanic samples. Surface water samples from the Chuckchi Sea (Arctic 28 m) and Bering Strait (Arctic 5 m) contained bacterial populations that were adapted to both cold temperatures (typical of deep ocean water) and surface pressures. Thus the bacteria in these water samples experienced no stress with regard to either temperature or pressure during cellulose decomposition experiments conducted at lower temperatures. Rates of cellulose slurry decomposition for these samples were 1.24% and 0.50% (in 30 days at 4°C), which are similar to rates measured for deep water samples.

For two samples collected at the same site (Chuckchi sea), we found no statistical difference between rates in surface and deep water (T-test,  $P=0.75$ ). Thus low rates of degradation of cellulose by oceanic bacterial communities appear to be related to inherent differences in the community structure with regard to the ability to utilize cellulosic material, at least during the initial stages of decomposition, and not to effects of manipulating the temperature or pressure of the samples.

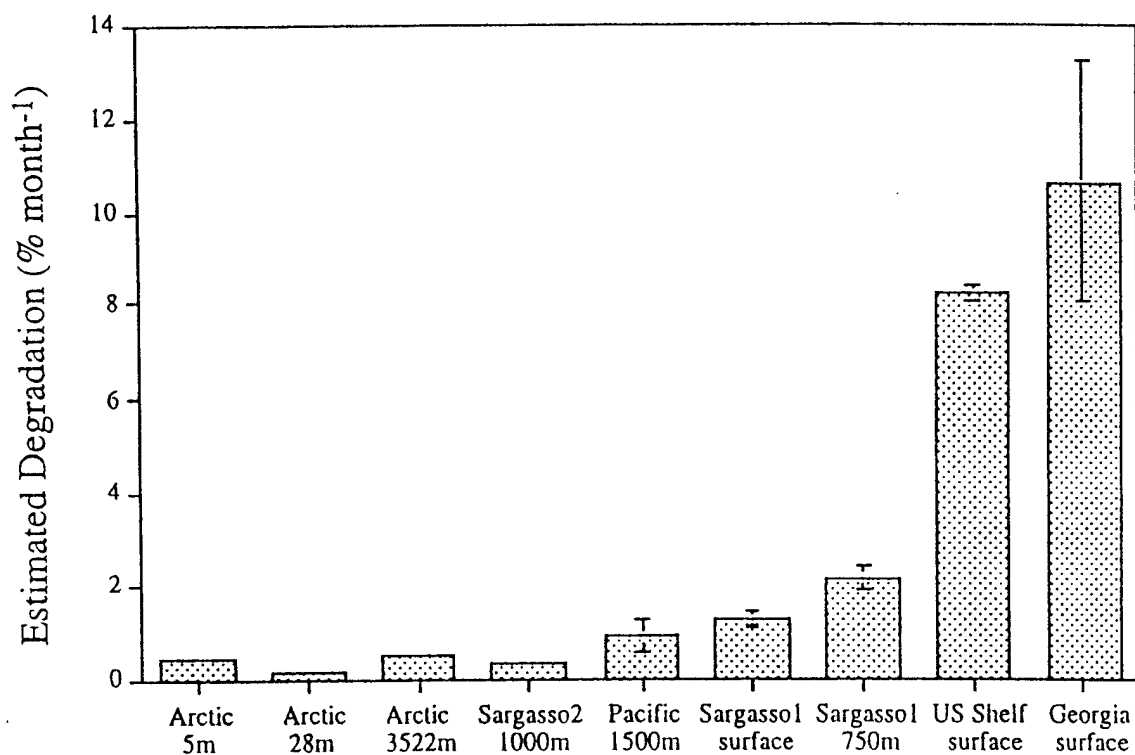


Figure 3. Rates of degradation of mixed paper cellulose slurry by bacterial populations from oceanic and coastal sites. Degradation was determined by measuring oxygen consumption in 8 d bioassays at 28°C; 5  $\mu\text{M}$  N and 1  $\mu\text{M}$  P were added to the seawater prior to incubation.

We found no effect on decomposition rates in these studies when inorganic N and P were added to seawater (5  $\mu\text{M}$   $\text{NH}_4^+$  and 1  $\mu\text{M}$   $\text{PO}_4$ ) for either surface or deep seawater. The lack of a nutrient effect likely results from sufficient N and P levels in the original seawater; for example, nitrate levels in the Eastern Tropical Pacific were 42  $\mu\text{M}$ .

Table 3. Rates of degradation of mixed paper slurry by bacterial populations from oceanic and coastal sites. A "-" in the nutrient addition column indicates that inorganic nutrients were not added to the seawater; a "+" indicates the addition of 5  $\mu$ M N and 1  $\mu$ M P prior to incubation.

Site	Temperature (°C)	Nutrient Addition	% Decomposition (30 d)	k
Pacific 1500 m	28	-	1.38 $\pm$ 0.67	-0.00046
	28	+	0.88 $\pm$ 0.36	-0.00030
Sargasso #1, surface	28	-	1.72 $\pm$ 0.24	-0.00058
	28	+	1.25 $\pm$ 0.19	-0.00042
Sargasso #1, 750 m	28	-	1.58 $\pm$ 0.34	-0.00053
	28	+	2.12 $\pm$ 0.27	-0.00072
Sargasso #2, 1000 m	7.5	-	0.25 $\pm$ 0.09	-0.00008
	7.5	+	0.62 $\pm$ 0.25	-0.00021
	28	-	0.44 $\pm$ 0.18	-0.00015
	28	+	0.29 $\pm$ 0.11	-0.00010
Arctic, 5 m	4	-	0.49 $\pm$ 0.21	-0.00016
	4	+	0.50 $\pm$ 0.15	-0.00017
	28	-	0.50 $\pm$ 0.06	-0.00017
	28	+	0.46 $\pm$ 0.21	-0.00015
Arctic 28 m	4	-	1.24 $\pm$ 0.42	-0.00042
	4	+	0.88 $\pm$ 0.13	-0.00029
	28	-	0.24 $\pm$ 0.06	-0.00008
	28	+	0.11 $\pm$ 0.04	-0.00004
Arctic, 3522 m	4	-	0.49 $\pm$ 0.02	-0.00016
	4	+	0.56 $\pm$ 0.18	-0.00019
	28	-	0.54 $\pm$ 0.26	-0.00018
	28	+	0.50 $\pm$ 0.08	-0.00017
SE Continental Shelf	17	-	0.28 $\pm$ 0.16	-0.00009
	17	+	2.58 $\pm$ 0.33	-0.00087
	28	-	0.70 $\pm$ 0.38	-0.00023
	28	+	8.21 $\pm$ 0.2	-0.00286
Georgia Coastal	28	+	10.63 $\pm$ 2.6	-0.00375



**Temperature and Nutrient Matrix Studies** - Decay rates of cellulose particles were determined under the temperature and nutrient conditions representative of "special area" sites which were chosen by the RDTE Division as the focus of their modeling efforts. Conditions for these studies were based on seasonal variations and vertical profiles from the Baltic Sea, the Mediterranean Sea, and the Caribbean Sea. Chosen temperatures for these studies spanned a range of 4°C to 28°C, inorganic nitrogen concentrations ranged from 0 (trace levels) to 5  $\mu\text{M}$ , and inorganic phosphorus concentrations from 0 (trace levels) to 1  $\mu\text{M}$ .

The bacterial inoculum for all the experiments in this matrix was identical, to eliminate any potential effects of differences in composition of the bacterial community among experiments. The inoculum was prepared by incubating 1.0  $\mu\text{m}$ -filtered coastal seawater overnight in the laboratory to allow growth of the natural bacterial community in the absence of any protozoan grazers or metazoans. After incubation, the inoculum was divided up into a number of aliquots for long-term storage. Each seawater aliquot (800  $\mu\text{l}$ ) was mixed with glycerol (200  $\mu\text{l}$ ) in 1.5 ml plastic cryogenic vials and stored at -70°C. To obtain a fresh bacterial inoculum for each experiment, an aliquot was thawed, inoculated into fresh seawater, and allowed to grow for 48 hr. Bacterial cells were then harvested by centrifugation at 14,000 rpm for 20 minutes, resuspended in low-nutrient artificial seawater and used to inoculate each experiment.

Low-nutrient artificial seawater was made according to the procedures described above and inoculated with 0.8 ml of the standard bacterial inoculum. Cellulosic particles from mixed paper waste was added to the seawater to a final concentration of 0.001  $\text{mg ml}^{-1}$ .

Nutrient-free seawater/cellulose slurries were amended with inorganic nutrients to establish the following treatments: 5  $\mu\text{M}$  N and 1  $\mu\text{M}$  P; 3.75  $\mu\text{M}$  N and 0.75  $\mu\text{M}$  P; 2.5  $\mu\text{M}$  N and 0.5  $\mu\text{M}$  P; 1.25  $\mu\text{M}$  N and 0.25  $\mu\text{M}$  P; 0.064  $\mu\text{M}$  N and 0.015  $\mu\text{M}$  P (trace levels in the seawater preparation). Seawater of each nutrient concentration was dispensed into 60 ml glass BOD bottles; thirty replicate BOD bottles were set up for each nutrient treatment. Dissolved oxygen concentrations were measured by precision Winkler titrations in 3 replicate bottles at days 0, 3, 7, and then weekly for 3 additional weeks. In all cases, a "minus cellulose" control treatment was established in which paper slurry was not added; oxygen consumption in this treatment represented bacterial respiration at the expense of dissolved organic matter in the coastal seawater, and not at the expense of cellulose. Oxygen consumption in the treatments with cellulose were corrected for the consumption in the control treatments for final calculations of decomposition rate. Experiments were run at 28°C, 18°C, 8°C, and 4°C.

Rates of cellulose degradation for the temperature/nutrient matrix studies are shown in Tables 4 and 5. Temperature had a significant effect on rates of paper slurry degradation and is likely to be an important control on the rate at which paper is degraded in seawater. Rates of degradation at 28°C were approximately 10-fold those measured at 4°C. Inorganic nutrient concentrations were much less important in determining rates at which cellulose slurries degraded in seawater at most temperatures, and we observed little or no effect of 100-fold variations in N and P concentrations on decomposition rates at 4°C, 8°C, and 18°C. Initial results, however, indicate that nutrient concentrations will have a larger effect on decomposition rates at the highest temperature (28°C). (These experiments will be completed by January 1997).

Table 4. Results of temperature/nutrient matrix studies presented as percent decomposition of paper slurry in 30 d. High = 5  $\mu\text{M}$  N and 1  $\mu\text{M}$  P; Medium High = 3.75  $\mu\text{M}$  N and 0.75  $\mu\text{M}$  P; Medium = 2.5  $\mu\text{M}$  N and 0.5  $\mu\text{M}$  P; Medium Low = 1.25  $\mu\text{M}$  N and 0.25  $\mu\text{M}$  P; and Low = 0.064  $\mu\text{M}$  N and 0.015  $\mu\text{M}$  P.

Temperature	High	Medium High	Medium	Medium Low	Low
28 C	10.63				
18 C	5.55	5.87	6.04	5.56	5.20
8 C	3.52	3.25	3.09	3.83	3.03
4 C	1.32	0.53	1.00	1.59	1.13

Table 5. Results of temperature/nutrient matrix studies presented as the decomposition constant (k) assuming first order degradation kinetics. Nutrient treatments are as described in Table 4.

Temperature	High	Medium High	Medium	Medium Low	Low
28 C	-0.00375				-
18 C	-0.00190	-0.00202	-0.00208	-0.00191	-0.00178
8 C	-0.00120	-0.00110	-0.00105	-0.00130	-0.00103
4 C	-0.00045	-0.00018	-0.00033	-0.00053	-0.00038

For some experiments, seawater/cellulose slurries were incubated in 15-L aluminized mylar bags (with polyethylene inner coating) instead of BOD bottles to determine the effect of vessel type on measured decomposition rates. These gas-tight bags allowed measurement of decomposition via bacterial oxygen consumption, and sequential sampling from the same bag further increased sensitivity by providing cumulative measures of

oxygen consumption. For these experiments, three BOD bottles were filled via a Tygon tube from the polyethylene bag just prior to dissolved oxygen measurements.

Rates of cellulose decomposition were similar in polyethylene bags compared to BOD bottles. At 28°C with high nutrient levels (5  $\mu\text{M}$  N and 1  $\mu\text{M}$  P), 9.58% of the paper slurry in the polyethylene bags was degraded after 30 days. By contrast, 10.39% and 11.93% of the paper in the BOD bottles was degraded in two separate experiments. Similarly, at 28°C with low nutrient levels (0.064  $\mu\text{M}$  N and 0.01  $\mu\text{M}$  P), rates of degradation were 3.89% in the bags and 4.46% in the bottles. Although there was a statistically significant difference between bags and bottles at high nutrient levels ( $P=0.05$ ) [there was no difference at low nutrient levels ( $P=0.051$ )], the magnitude of the difference was low and we conclude that rates of degradation in the two types of incubation vessels are similar (Figure 4).

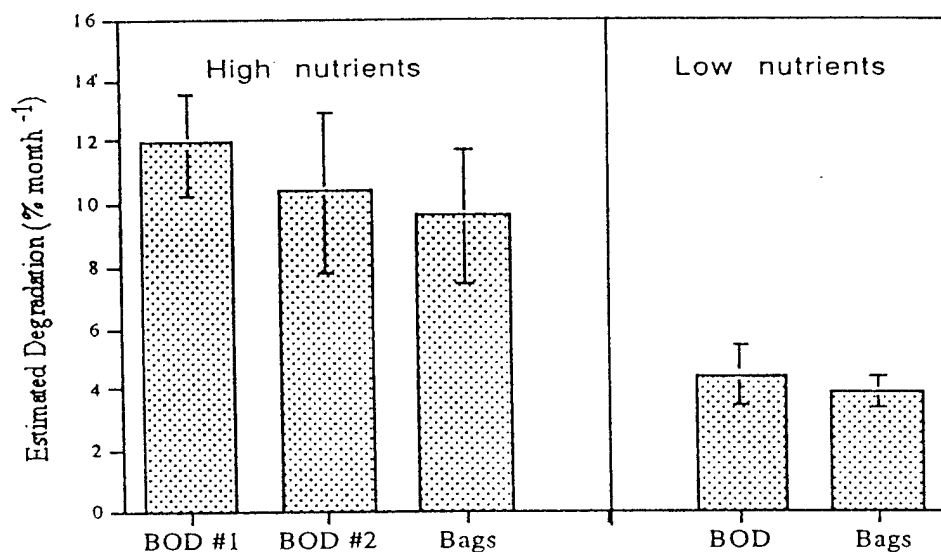


Figure 4. Comparison of degradation rates in two types of incubation systems: glass BOD bottles and 15-liter polyethylene/mylar bags.

## CONCLUSIONS

- 1) Decomposition of mixed paper slurries in seawater follows first order degradation kinetics. Decay constants ( $k$ ) range from  $-0.004 \text{ d}^{-1}$  at  $28^\circ\text{C}$  to  $-0.0004 \text{ d}^{-1}$  at  $4^\circ\text{C}$ . These correspond to half lives of 173 and 1733 days, respectively.
- 2)  $Q_{10}$  values for cellulose decomposition average 1.7 for temperatures  $10^\circ\text{C}$  and above.
- 3) Inorganic nutrient concentrations appear to significantly affect decomposition rates of paper slurries only at high temperature ( $28^\circ\text{C}$ ). At  $18^\circ\text{C}$  and below, bacterial degradation is generally limited by temperature rather than nutrient availability over a range of nutrient concentrations representative of coastal and open ocean water. (These experiments are not complete).
- 4) Decomposition rates are not affected by the size of the paper particles; particles ranging from  $> 2 \text{ mm}$  to  $< 500 \mu\text{m}$  are degraded at similar rates.
- 5) Bacterial communities from coastal seawater degrade cellulose more rapidly than communities from open ocean seawater, at least in short-term (8 d) studies. Rates of decomposition under identical conditions differ by about 10-fold.

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