

# THE EFFECTS OF BACTERIA ON THE FLOW BEHAVIOR OF CLAY-SEAWATER SUSPENSIONS

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**ABSTRACT:** The presence of glue-like exopolymer produced by the marine benthic bacterium *Aeromonas atlantica* present in concentrations comparable with typical marine muds can enhance the yield stress of dilute clay-seawater suspensions typical of the sediment-water interface by 60%. This effect is inferred to be due mainly to bacterial attachment to and exopolymeric bridging between clay domains under nutrient-poor conditions. The relative change in the yield stress of a clay-seawater suspension, and by inference its erosion resistance, is dependent on the availability of nutrients and the history of microbial attachment. This conclusion applies regardless of the constitutive model used to describe the behavior of the suspension under shear. Our results are relevant to rheological studies of fine-particle suspensions in general because inevitable contamination of "abiotic" slurries often occurs under the very conditions (nutrient poor) that can result in maximal binding effects.

## INTRODUCTION

Microbes and their exudates are ubiquitous in marine sediments (Meadows and Anderson 1968; Deflaun and Mayer 1983; Weaver 1989; Decho 1990). These organisms can alter the mechanical properties and the erosion resistance of sediments, and so influence the navigability, water quality, and geologic record of marine environments. Although microbial stabilization of sands has been well demonstrated (e.g., de Boer 1981; Grant et al. 1986; Grant and Gust 1987; Patterson 1989; Dade et al. 1991), there has been surprisingly little consideration of microbial effects on the transport behavior of clays (cf. Parchure and Mehta 1985; Dade and Nowell 1991). We report here the first observations of the effects of a common benthic marine bacterium, *Aeromonas atlantica*, on the rheology of clay-seawater suspensions.

Our study was specifically designed to test the null hypotheses that *A. atlantica* has no effect on the flow behavior of the clay-rich suspensions as a function of either time or nutrient availability. Clay-rich muds make up a significant fraction of seafloor sediments overall, and kaolinite, the clay considered here, is an important component of muds in many estuaries and on continental shelves (Weaver 1989). The solids concentration of the suspensions used in our study approximates that in the uppermost millimeters of natural, cohesive beds in subaqueous environments (e.g., Hayter 1986). We chose to examine the effects of the pseudomonad *A. atlantica* because it is a common member of natural microbial communities in shallow marine sediments and a prolific producer of exopolymer that contains uronic acids, the glue-like component of the exopolymer (Fazio et al. 1982; Dade et al. 1991).

We observed that bacterial activities can measurably enhance the yield stress and the viscosity of clay-seawater suspensions. The yield stress defines the stress required to mobilize a cohesive suspension under shear, and can be related directly to the erosion resistance of clay-rich, seafloor muds (Dade et al. 1992). Once a suspension is mobilized, its viscosity reflects a resistance to further deformation under shear. Both properties are of interest to sedimentologists and engineers alike.

## METHODS

We inoculated mixtures of twice-autoclaved kaolinite clay and 0.2  $\mu\text{m}$ -filtered artificial seawater with *Aeromonas atlantica* (ATCC 19262). Each

suspension comprised clay solids at 14% of its initial volume (corresponding approximately to 370 kg of clay per  $\text{m}^3$ ), and was maintained by vigorous daily mixing until sampled. Low nutrient suspensions were generated by the introduction of bacteria alone (resulting in an overall organic content characterized by 0.2 mg N and 1.2 mg C per g of dry sediment). High nutrient suspensions were generated by the addition of bacteria plus growth media (yielding 0.8 mg N and 12 mg C per g of dry sediment). Control inocula were prepared identically to those for the respective treatments but without the bacterial component. Once inoculated, treatment and control suspensions were adjusted to pH 8.0–8.3 with 0.2  $\mu\text{m}$ -filtered NaOH and kept at 25°C.

The clay-seawater suspensions consisted of kaolinite ("Goldart", Cedar Heights Clay Co., Oak Hill, Ohio) and artificial seawater (Sigma Chemical Company, St. Louis, Missouri). The kaolinite clay has a median diameter of 1.5  $\mu\text{m}$ , a specific surface area of 19.8  $\text{m}^2$  per g of dry sediment, and loss upon ignition amounting to 9.3% of its dry weight (Dade 1992).

Bacterial inoculations were prepared as follows. Cells of *Aeromonas atlantica* from stock culture were grown overnight in 6 ml of marine broth (DIFCO, Detroit, Michigan) at 25–30°C. One ml aliquots of the inoculated broth were then added to 200 ml of growth media (described in Dade et al. 1991) and incubated for an additional 4–6 d at 25°C while agitated at 130 rpm on a shaker table. Bacteria were harvested for introduction into clay-seawater suspensions by centrifugation at 17,000 g for 10 min. High-nutrient inocula consisted of concentrates of *Aeromonas* cells resuspended in 15 ml of the harvest supernatant, which contained dissolved exopolymer and unspent growth media. Low-nutrient inocula were prepared from harvest concentrates by twice washing them in 0.2  $\mu\text{m}$ -filtered artificial seawater followed by reharvest and addition to 15 ml of filtered seawater.

Within 12 hours and then again on days 9, 30, and 51 following inoculation, selected suspensions and their controls were sampled in true replicates and subjected to measurements of bacterial abundance (3 replicates from each of the treatment and control suspensions), uronic acids content (2–3 replicates) and rheology (3–4 replicates). Uronic acids were assayed with a standard method (Montreuil et al. 1986) modified as follows to accommodate the clay-bearing samples. A pellet of clay plus adsorbed polymer generated by centrifugation was rinsed three times in deionized  $\text{H}_2\text{O}$ , dried overnight, ground finely, and subdivided into triplicate portions of 0.5 g each. The material resulting from this procedure was rehydrated with deionized  $\text{H}_2\text{O}$  and then hydrolyzed in sodium tetraborate-sulfuric acid. Clay was removed by centrifugation before addition of a color reagent for polymer-component uronic acids, and the acids were assayed by means of spectrophotometric absorbances at 520 nm. All absorbances were corrected for residual turbidity. We report concentrations of exopolymer adsorbed onto mineral particles only. The concentrations of supernatant exopolymer observed in all treatments and their controls were negligible.

The flow behavior of each suspension (adjusted to pH 8.0–8.3) was evaluated with a Brookfield viscometer adapted for small sample volumes and low shear rates of between 0.6  $\text{s}^{-1}$  and 121.9  $\text{s}^{-1}$  (see Barnes et al. 1988 for an exposition of the principles of viscometry). The device was calibrated before the experiment with standard fluids with known rheological properties (Dade 1992). No significant flow hysteresis was detected in our measurements of suspension properties. Various measures of the yield stress and the viscosity of each suspension were derived by analyzing the relationship between shear stress and shear rate in terms of constitutive models commonly used to relate the total stress in and rate of strain of

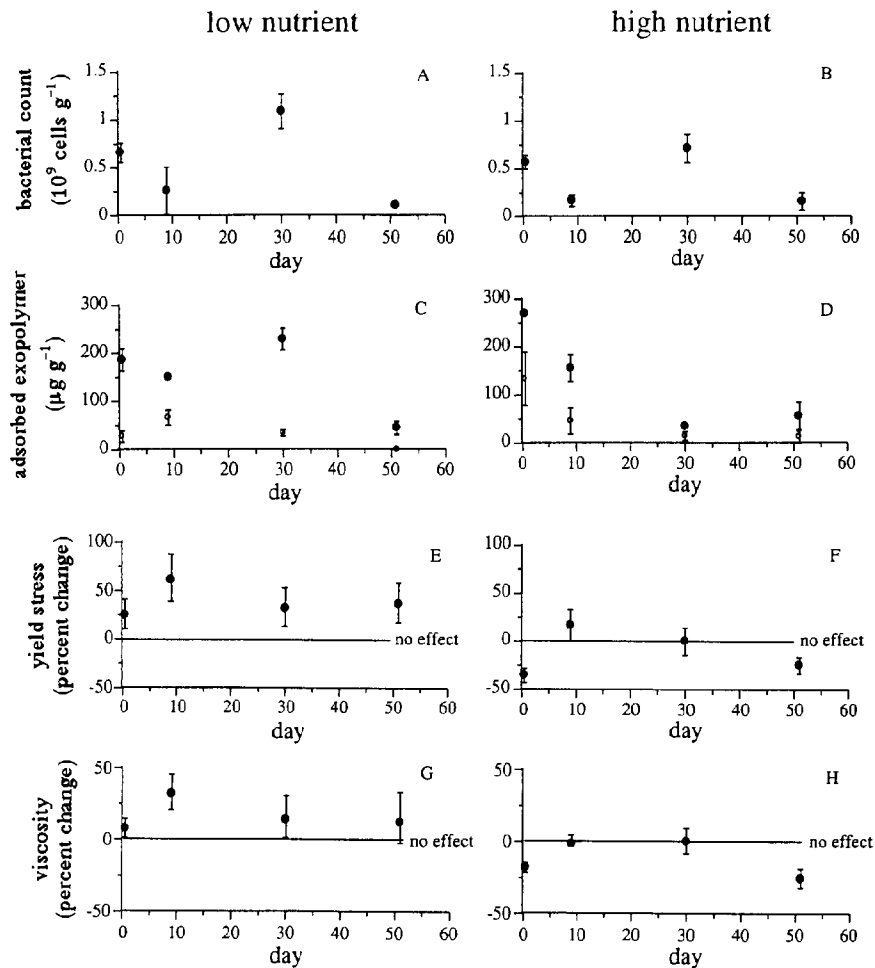


FIG. 1.—Presence and rheological effects of the benthic marine bacterium, *Alteromonas atlantica*, in kaolinite-seawater suspensions as functions of nutrient loading and time. Solid symbols indicate observations of treatments. Open symbols indicate control values in C and D. Vertical bars indicate  $\pm 1$  standard error of 2–4 true replicates recovered from the corresponding ANOVAs. The Bingham yield stresses of the initial control suspensions for the low-nutrient and high-nutrient treatments were 0.46 Pa and 0.25 Pa, respectively. The viscosity of the control suspensions for both treatments was 5 centipoise. A, B) Bacterial abundance per g of dry clay. C, D) Concentration of adsorbed exopolymer per g of dry clay. E, F) Relative change [= (treatment – control)/control  $\times$  100%] in Bingham yield stress of suspensions for treatment effect and control values measured at each time. G, H) Relative change in Bingham viscosity of suspensions delineated at each time.

cohesive materials. These included Bingham, Herschel-Bulkley, and Casson flow models (e.g., Barnes et al. 1988; Dade and Nowell 1991).

## RESULTS

Initial bacterial populations in both treatments were approximately  $10^9$  cells per g of dry sediment. This level persisted for over 30 days, and then began to diminish at times approaching 2 months (Fig. 1). Bacteria were undetected in the sterile controls at all times. Sources of exopolymer include material initially present on the clays, material introduced with the inocula, and material produced by microbial activities in the respective treatments. Approximate balances between microbial population growth and exudate production and the inevitable processes of decay of organic materials, presumably due to enzymatic activities, can result in standing stocks of bacterial cells and adsorbed exopolymer which are relatively constant. For the first 30 d in low-nutrient treatments, adsorbed exopolymer was apparently maintained by just such a balance at approximately 100  $\mu\text{g}$  or more per g of dry sediment in excess of the controls. In nutrient-rich suspensions, however, the exopolymer introduced with the inocula or initially present on clay particles was not maintained by ongoing microbial production but instead systematically decayed over a period of several weeks. Other interpretations of the data summarized in Figure 1A–D are possible, of course, but the key point is that these observations are consistent with established notions of bacterial growth and decay in batch culture, and with the effects of nutrient stress on exopolymer production by *A.*

*atlantica* in particular (Fazio et al. 1982; Uhlinger and White 1983; Dade et al. 1991).

Treatment effects on the flow behavior of the clay-seawater suspensions are shown in Figure 1E–H. We report the relative magnitude of these effects, because absolute measures of flow properties depend on the choice of the constitutive model used to describe suspension behavior. The results shown in Figure 1 are for Bingham flow properties, but virtually identical patterns emerge, in relative terms, for any of the measures of yield stress and viscosity from the various flow models considered. Relative values, moreover, provide the relevant measure of bacterial effects given that there was a drift in the rheological properties of the control suspensions over the course of the experiment, owing in part to the natural decay of any glue-like exopolymer initially present in the suspension. Overall, changes in the flow parameters of suspensions attributable to both treatment and time were statistically significant ( $p \ll 0.001$ , Weighted Means Model ANOVA on yield stress and viscosity data rendered homoscedastic by, respectively, logarithmic and power transformations).

In particular, both the yield stress and the viscosity of the low nutrient treatments were significantly different, overall, from the properties of the corresponding controls ( $p \ll 0.001$ , adjusted for multiple comparisons). The yield stress of the inoculated suspensions increased by up to 60% ( $\pm 25\%$ ) over that of the controls, while the viscosity of the inoculated suspensions increased by up to 30% ( $\pm 10\%$ ). These differences manifested themselves in time between day 0 and day 9 ( $p = 0.002$  in the case of

yield stress, and  $p = 0.05$  in the case of viscosity; both adjusted for multiple comparisons). Temporal changes in flow behaviors of the treatments relative to their controls were statistically undetectable thereafter.

In contrast, the yield stress of the nutrient-rich suspensions hosting *A. atlantica* was not significantly different, overall, from the corresponding controls. At least qualitatively, however, yield stress of the high-nutrient suspensions decreased immediately following inoculation relative to the high nutrient controls. This initial difference was mirrored in the viscosity of the inoculated suspensions to a statistically significant degree ( $p = 0.05$ , adjusted for multiple comparisons).

In short, the null hypotheses—that the bacterium *Aalteromonas atlantica* has no effect on the flow behavior of the suspensions, and that microbial effects are independent of time and nutrient availability—must be rejected. A discernible increase in the flow resistance of nutrient-poor, clay-seawater suspensions occurs with time when the suspensions host the microbe *A. atlantica* in batch culture. There is, in contrast, a suggestion of a short-lived decrease in the flow resistance of high-nutrient suspensions immediately following inoculation with *A. atlantica*, but these effects are only marginally significant if at all.

#### DISCUSSION AND CONCLUSION

When motile bacteria first gain access to attachment sites, settlement is reversible (Marshall et al. 1971). When stressed, cells commonly lose their motility in time and attach to surfaces by way of exopolymer secretions, although this behavior is by no means universal among pseudomonads (cf. Williams and Wimpenny 1977; Uhlinger and White 1983; Marqués et al. 1986). The 0.5–9 d required in our experiments for the development of significant increases in the flow resistance of the inoculated, low-nutrient suspensions apparently corresponds to the time for *A. atlantica* to achieve a phase of growth accompanied by attachment and exudate production. The attachment of bacteria and the secretion of exopolymer are accompanied, in turn, by the formation of extensive macromolecular bridges between clay domains (Bennett et al. 1991). Exopolymer bridges simultaneously reinforce and increase the volume of the existing network of cohesive particles. As a result, the resistance of a suspension to transport under shear is measurably enhanced. The sustained microbial effects seen qualitatively in the flow behavior of the nutrient-poor suspensions at day 51, even after the microbial population and exopolymer amounts began to diminish, may reflect the influence of residual exopolymer.

The contrasting reduction in the flow resistance of clay-seawater suspensions immediately following the introduction of a relatively high concentration of nutrients and microbial material is due to interactions between particles and abundant organic material that suppress physicochemical cohesion between clay grains (Dade and Nowell 1991). Clay domains with relatively thick coatings of organic material derived from high nutrient loads (and not microbial exopolymer), for example, may not achieve close contact to produce a strongly cohesive network. When nutrient levels are high, moreover, bacterial cells are unstressed and remain unbound to particle surfaces, and so produce little or no new binding exopolymer. The eventual breakdown of introduced or native organic material ultimately results in flow behavior that is comparable with the uninoculated, organic-rich controls. This interpretation is supported by our observations (not shown) of the final volumetric fraction of solids of suspensions allowed to settle undisturbed. The presence of *A. atlantica* and its exudates produced under low-nutrient conditions results in a space-filling network of clay and exopolymer that is significantly more extensive, and also less dense, than the cohesive networks generated in the other suspensions.

Our results should give pause to rheologists who pursue accurate descriptions of particle-water mixtures used in industrial, engineering, and sedimentological settings. Unintentional but unavoidable introduction of bacteria to such suspensions can result in significant, time-dependent changes in suspension properties due to the low availability of nutrients.

This problem is especially acute when polyvalent cations, which enhance the gel-like, cohesive properties of microbial exudates (Sutherland 1983), are present, as in seawater. Conversely, our findings are consistent with long-established practices that result in microbial contamination and the enhancement with time of desirable properties of clay slurries used in ceramic applications (Spurrer 1921).

The mineralogy and the concentrations of solids, organic material, bacteria, and microbial exopolymer in our low-nutrient suspensions are comparable to conditions seen at the sediment-water interface of many marine muds. So, although the effects we describe here are more subtle than those attributed to the thick microbial mats that carpet sands in some shallow marine settings (Neumann et al. 1970; Grant and Gust 1987), they are almost certainly more characteristic of typical fine-grained sediments in the sea.

Interestingly, the relative magnitude of cohesion enhancement due to *A. atlantica* in our kaolinite-seawater suspensions is also less than that previously reported for otherwise noncohesive sand subjected to similar treatments (Dade et al. 1991). This difference is presumably due to a similar amount of exopolymer distributed over a specific surface area of clay one to two orders of magnitude greater than that of sand. In both sand and clay subjected to these treatments the maximal cohesive force imparted by microbial exopolymer was about  $10^7$  times the exopolymer weight. But whereas bacterial exopolymer only augments existing interactions between cohesive particles in clay suspensions, the presence of microbial exopolymer in sand results in a new, adhesive force that can contribute significantly to the ability of individual, larger and otherwise noncohesive particles to resist entrainment by an overlying flow.

Bacterial enhancement of the yield stress of the nutrient-poor clay suspensions studied here is comparable to previously reported microbial effects on the erosion resistance of kaolinite beds in laboratory flumes (Parchure and Mehta 1985). These effects are striking if one considers that they can result, as in our experiments, from (approximately) only 100  $\mu\text{g}$  of bacterial exopolymer per g of dry sediment. This amount is equivalent to a uniform distribution of 1 ng of exopolymer per  $\text{cm}^2$  of sediment surface, or an amount of exopolymer per typical particle of clay that is of the order of 0.01% of the particle weight. The corresponding approximately 60% increase in the shear stress required to erode a seabed composed of such microbially bound clays can be significant for sedimentologists and engineers who wish to know the total time that a threshold for sediment transport is exceeded during diurnal or monthly tidal cycles.

While natural sediments are more complex than the simple systems we have studied so far, our experiments demonstrate that bacteria measurably affect the flow behavior of marine muds in a way that depends on nutrient availability and on the history of microbe-sediment interactions leading to exudate secretion. These microbe-sediment relationships, in turn, can influence geophysical and biochemical interactions on much larger scales.

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