Microbial Behavior And Sediment Stabilization

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LONG-TERM GOALS

Our goal is to understand how microbial behavior influences the mechanical stability of illuminated littoral marine sediments. We define "behavior" as the interaction of bacteria and diatoms with environmental signals that are either auto- produced, or arise from another source. A further goal is to investigate whether these processes, which define sediment optical signatures, can be influenced by anthropogenic intervention.

OBJECTIVES

Our objectives are to isolate representative bacteria and diatoms from stabilized sediments and use these organisms in axenic and defined mixed cultures to examine the microbially-driven sediment aggregation process *in vitro***.** The surficial chemistry necessary for adhesion is an important variable in sediment particle colonization by microorganisms. We will measure the influence of this parameter on the adhesion process as well as the possibility for cell/cell interaction in the attached cell layer.

APPROACH

It is well accepted that marine sediment stabilization against mechanical disturbance is dependent on the activities of the indigenous microorganisms. There is controversy however as to which groups of microorganisms are most involved. The majority of workers favor the diatom fraction of the population as being the most active, but others support the idea that bacteria are the drivers of the system. Our approach is to examine the relative contributions of both types of organisms and to measure the degree to which there is metabolic interaction or synergism. Stabilization requires that the interstitial spaces of the sediment particles be bridged by microbially-derived polymeric adhesives. This leads to an increase in the threshold force needed to move them and to an decrease in the hydraulic permeability of a bed of such particles. It is this second parameter that we intend to measure. Columns of glass beads will provide the substrata and growth medium flow with time will be measured. We will also quantify the production of the polymers formed by each organism alone, and in concert with the other type of cell, i.e., bacteria or diatoms alone and together in defined mixtures. This will be accomplished by raising specific antibodies to each polymer and using them in an ELISA assay. We will use surfaces of defined chemistry to investigate the influence of surface chemistry on the particle colonization by using dynamic image analysis to measure the numbers of attached cells, their speed and direction over the surface. All experiments will involve newly isolated organisms. B.Wigglesworth will perform the experiments

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needing image analysis and supervise the three work study undergraduates performing growth measurements. Graduate student D. Walker will continue to optimize the production of EPS from the bacterial cultures. Similarly, K.Cooksey will carry out work to produce diatom EPS and continue the sediment permeability experiments with glass bead-containing mini-columns. Both types of EPS will be purified and used eventually for the production of antibodies.

WORK COMPLETED

A series of organisms [10 bacterial clones and 8 diatoms species] have been isolated into axenic culture and several have been identified. The diatoms have been seen for two years in the Spring and Fall at the study site on San Juan Is, WA, and thus are representative of the area. The design for the silt - containing columns to be used in the hydraulic permeability studies is at a point where they can be used to collect data, however they will continue to be improved. The influence of surface wettability on diatom attachment and motility has been completed for one organism. A protocol for the production of bacterial extracellular polymers [EPS] has been scaled up so that cells are now grown in a 2L. airlift fermentor. Appproximately 125 mg EPS can be prepared from each experiment The procedure for the accumulation of diatom EPS has been developed. This project is progressing on several fronts simultaneously.

RESULTS

Using SEM of acid cleaned frustules we identified the diatoms isolated as species of *Navicula, Amphora* and *Auricula*. We believe that this is the first time this latter species has been cultivated and studied physiologically. All diatoms isolated were present in sediments over a two year period and are therefore appropriate organisms for study. Two clones of the bacteria have been identified as *Pseudoalteromonas haloplanktis* and *Bacillus simplex* by fatty acid methyl ester and / or 16S rRNA analyses. The EPS from the *Pseudoalteromonas* has been isolated. Its FTIR spectrum is distinct from that of *Alteromonas atlantica* - the organism most frequently selected from culture collections for studies of marine bacterial adhesion. We have almost completed the production of a genetic library of the *Pseudoalteromonas* organism so that eventually, genetic regulation of its EPS synthesis can be studied. [other bact work here].

We have made growth rate versus light level measurements for several of the diatoms. The results vary from organism to organism. In some cases, cells grow equally well at low and high light, but in other cases, there is a large reduction in growth rate at a lower light level. Even this simple experiment shows that the activities of diatoms in sediments are species specific and that all diatoms cannot be regarded similarly in a modeling effort. It is clear that sediment chlorophyll levels will not give information concerning diatom activity, only their presence. Two species of diatom have been grown individually on silt- sized glass beads[50-150μm] contained in 5mL columns. They caused the permeability as measured by the flow through the column of beads, to fall by 40%. This technique is now ready to be used to collect data. All of our diatoms in culture form aggregates in high Ca^{2+} medium and some of them disperse from the aggregate using what appears to be negative taxis to a self-generated signal. Our hypothesis is that this signal may be high oxygen concentration or some product of related to photorespiration, such as glycolate. This is being tested. Self-generated dispersal would promote colonization throughout the sediment. This phenomenon proved not to be universal in the diatoms we have in culture, so once again, it is not possible to generalize when it comes to the activities of diatoms

in sediments.

Amphora coffeaeformis is found commonly on wetted surfaces in the sea, especially sediment particles and ships. Although studies of the adhesion of diatoms to substrata as a function of differential surface chemistries has been published, none of these have used substrata where the differential surface properties were generated by the packing of a single chemical functional group. We prepared wettability gradients generated by the relative proximity of covalently bound methyl groups on glass surfaces. *Amphora* was not motile on surfaces where the contact angle was greater than 40°, although the cells did attach to these surfaces, albeit poorly. During these experiments we found that *Amphora* can change the wettability of a surface in the direction of increased hydrophilicity with time, i.e., *Amphora* can autocondition a surface. Measurement of the speed of the *Amphora* cells on the surfaces gave an indirect measure of the physicochemical interaction of the diatom motility polymer with the surface. This has great importance in the design of fouling release coatings as well as in our understanding of the properties of natural surfaces conducive to diatom colonization.

IMPACT/APPLICATION

Mines are often buried in shallow marine sediments. If placement of these weapons disturbs the natural optical signature of the sediments, it is possible that they can be detected by this means. The work will provide information concerning the biogeochemical activities of microorganisms in nearshore marine sediments.

TRANSITIONS

Dr. Brenda Little of the Naval Research Laboratory, Stennis Space Center has expressed interest in using the gradient wettability approach to study the adhesion of diatoms to optical surfaces on naval vessels.

RELATED PROJECTS

None

PUBLICATIONS

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