Microbial Behavior And Sediment Stabilization

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Award # NOOO149610879
http://www.onr.navy.mil/sci_tech/ocean/onrpgahj.htm

LONG-TERM GOALS

Our goal is to understand how microbial activity and behavior influence the mechanical stability of illuminated littoral marine sediments. We define Abehavior@ 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 sediment surficial chemistry implicated in adhesion processes is an important variable in sediment particle colonization by microorganisms. We have measured 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 (EPS). This leads to an increase in the threshold force needed to move these particles and to a concomitant decrease in the hydraulic permeability of the sediment bed. It is this second parameter that we have measured. Columns of glass beads provide the substrata for cellular attachment and growth medium flow with time through the bed of beads has been 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. All

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 1999		**00-00-1999 to 00-00-1999**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Microbial Behavior And Sediment Stabilization	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Montana State University,Department of Microbiology,Bozeman,MT,59717**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**4**	

experiments will involve newly isolated organisms. Other aspects of this work involve the auto-production of a putative cellular dispersal factor that leads to a stimulation of spatial colonization of surfaces and a study of the ability of diatoms to metabolize organic substances likely to be found in sediment porewater. B. Wigglesworth will perform the experiments needing image analysis and supervise the two undergraduates performing growth measurements. K. Cooksey will analyze EPS produced by diatoms and continue the sediment permeability experiments with glass bead-containing mini-columns.

WORK COMPLETED

A series of organisms [10 bacterial clones and 8 diatoms species] isolated into axenic culture have been provided to Dr. Michael Franklin for use in his ONR- funded study of cell -cell interaction at the genetic level. The design for the silt - containing columns to be used in the hydraulic conductivity studies has been considerably improved. A study of the growth physiology of two diatom species has been completed.

RESULTS

We have developed methods to assess growth of diatoms and bacteria in percolation columns containing glass beads ranging in size from 50-125?m (sediment fraction = Afine silt@)in addition to methods to measure cellular utilization of PO4''' and production of carbohydrate-like polymers. These polymers are the soluble extracellular polymers related to diatom cell motility, and the less soluble matrix polymer(s). We have also improved the column to column reproducibility and minimized the reduction in flow of uninoculated control columns due to the packing of the beads with time. Results of other workers who have used this methodology have been clouded because of problems of this type. In a typical experiment the flow rates of columns after diatom growth are reduced by 25% ? 4%(n=15), whereas in uninoculated controls the flow is reduced 5%?1%(n=3). Our experiments have shown the following : (a). Diatoms of two species (Amphora coffeaeformis and a species of Navicula) grow to the same extent (4-5 generations) in the columns before entering stationary phase; (b). The reduction in flow rate of the columns measured after diatoms have grown is dependent on the number of cells used to inoculate the columns. Where cells have grown from small inocula, the flow rates are reduced more, compared to uninoculated controls than in situations where a larger inoculum is used, even though the final biomass levels in each case are similar. This implies that it a product of growth that reduces the flow rate, not the presence of the cells themselves; (c). Time-course experiments where flow reduction is measured against diatom biomass production and PO4''' utilization show that flow rates do not fall until all the PO4''' is used and biomass increase is ended. We interpret these results to mean that soluble diatom motility polymers produced during logarithmic growth are not responsible for sediment stabilization, but it is the less soluble matrix polymers formed when the cells enter stationary phase that are active in this regard. This does not support current wisdom wherein motility polymers are considered to play a primary role in sediment stabilization. Our conclusions are supported by the fact that diatoms grown in flasks containing medium with reduced PO4''' do not produce increased levels of soluble carbohydrate polymers. In order to relate our results to the situation in the field, samples of porewater taken from areas of stabilized (obvious diatom bloom) and non-stabilized sediments (no obvious diatoms) in False Bay, San Juan Island were analyzed for soluble PO4'''. Bloom areas contained 6-10?M PO4''' whereas control areas had PO4'''concentrations of more than 20?M..

These concentrations are of the same order as in our in vitro experiments. In all cases where the flow rate through experimental columns had been reduced by diatom activity, the bead bed was stabilized against mechanical disturbance. This was tested by tilting the columns through 60? and measuring the angle adopted by the putatively stabilized and non-stabilized (uninoculated) controls. The surface of the control bead bed became horizontal in one hour, i.e., it Aavalanched@, whereas the stabilized bead beds adopted the angle of tilt, i.e., no movement was seen. These experiments justify the use of hydraulic permeability measurements as indicators of sediment stabilization. Cryostage /SEM examination (i.e., unfixed preparations) of the stabilized beads showed presence of diatoms in the interstices and that these cells were enveloped in a polymer matrix the volume of which exceeded that of the cells.
Two types of experiments have been performed with bacteria and diatoms inoculated into the same percolation column. In one series medium containing 0.05% of both D-glucose and yeast extract was used to provide heterotrophic bacteria with growth substrates, and in another there was no added organic substrates. In this second series, bacterial growth was dependent on secreted products of diatom metabolism. In neither case was there a significant decrease in flow attributable to the presence of bacteria in the system over that caused by diatoms alone, however at this time only Pseudoalteromonas haloplanktis has been used in these experiments. It remains to be seen whether our lack of finding a bacterial effect is a general one. One effect of the addition of heterotrophic growth substrates to the medium in the columns was that diatoms were present not just as a biofilm but became distributed throughout the bead bed. The addition of yeast extract to the medium in effect increased the available PO4''' sevenfold. We were able to show that it was the presence of assimilable organic substrates that was responsible for the increase in diatom biomass and not the increased level of PO4'''. Two species of Navicula have been investigated for their ability to grow heterotrophically and mixotrophically (light = 6?moles m-2 sec-1, or 6% of the autotrophic level). Navicula sp.1 grew heterotrophically on yeast extract and mixotrophically on aspartate, alanine, glutamate, DBglucose and yeast extract whereas Navicula sp. 3 grew heterotrophically and mixotrophically on both glutamate and aspartate, but not on glucose. As some diatoms we have tested previously have been shown to be chemotactic to such organics, these results support the possibility that diatoms in marine sediments are able to utilize organic compounds in their environment and that this capability may enhance their role in stabilizing such sediments. To conclude the growth physiological measurements we have shown that in every case, the diatoms isolated from False Bay sediments require Ca 2+ for both attachment to surfaces and for their subsequent motility.

The structural chemistry of microbial polymers is somewhat similar from one organism to another which makes simple analysis of one polymer in the presence of another difficult. This project does not need detailed structural information, but would be aided if the polymers with which we are dealing contained what might be termed structural finger prints. In order to ascertain if this is a possibility, we have embarked on a preliminary Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) analysis of the surface attached footprint material left when diatom cells are removed from a surface using a mechanical shear. Results so far indicate that reliable spectra can be collected from the footprint of a single cell. This technique appears promising and will be continued.

IMPACT/APPLICATIONS

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 provides new information concerning the biogeochemical activities of microorganisms in nearshore marine sediments. Our results imply that current models of sediment stabilization by marine microorganisms may need revision.

TRANSITIONS

Dr. Brenda Little of the Naval Research Laboratory, NRL/Stennis Space Center has asked that we provide her with samples of diatom and bacterial exopolymers for use in an investigation of heavy metal binding by such polymers. The study concerns heavy metal pollution remediation in harbor sediments. We have supplied Dr. Michael Franklin with organisms for use in a project funded by Biomolecular and Biosystems, ONR. Dr. Recep Avci has submitted a proposal to ONR in which he will investigate the microbial exopolymer/substratal adhesive forces using chemical force microscopy. KEC has been invited to work on the program committee of an international meeting on microbial extracellular polymers to be held in Mulheim, Germany in 2000.

RELATED PROJECTS

The National Science Foundation has funded a small project wherein the technology developed here will be used to investigate the role of thermophilic cyanobacteria in stream bed stabilization in Nymph Creek, Yellowstone National Park.

PUBLICATIONS

1. Wiggleworth-Cooksey, B. Van der Mei, H., Busscher, H.J.and Cooksey K.E. [1999]. The influence of surface chemistry on the control of cellular behavior : Studies with a marine diatom and a wettability gradient.Colloids and Surfaces B: Biosurfaces 15 : 71-79.

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