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SMALL SCALE WASTE MANAGEMENT PROJECT

Characterization of Microbial Clogging in Wastewater Infiltration Systems

by

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Characterization of Microbial Clogging in Wastewater Infiltration Systems

Amy B. Ronner and Amy C. Lee Wong*

ABSTRACT

We studied the bacteria and bacterial extracellular polysaccharides (EPS) associated with clogged soil pores and reduced infiltration of onsite wastewater infiltration systems Approximately 160 bacteria have been isolated from the clogging layer of a ponded south-central Wisconsin mound system. We developed an *in vitro* screening system using sand columns in plastic syringe barrels to determine the clogging potential of these isolates. The columns were inoculated with monocultures of these isolates, incubated at 18°, 12°, and 4°C, and the infiltration rates of various liquid media were monitored. Biofilm formation on sand grains and EPS production in the columns were analyzed by plate counts and and carbohydrate assays, and visualized by scanning electron microscopy. Thirty percent of the isolates tested so far induced varying degrees of clogging within 2 weeks after column inoculation. Some isolates induced ponding within 1-3 days of inoculation. The isolates represent a variety of bacterial genera. Neither bacterial numbers in outflow or adhering to sand grains, nor gross EPS production, were predictive of clogging ability. Much of the EPS produced by attached cells appears to be water soluble, but more EPS may adhere to sand grains at colder temperatures.
Keywords: Bacteria, Onsite wastewater treatment, Polysaccharides.

INTRODUCTION

The phenomenon of clogging, or reduced hydraulic conductivity of onsite wastewater systems, has usually been studied in terms of wastewater effluent quality. It is believed that bacterial populations also play a major role in the development of clogging, but there has been little effort to identify the organisms involved in clogging, or to elucidate the mechanisms by which they influence the infiltrative system.

The ability of bacteria to colonize mineral surfaces, and to protect themselves from environmental stresses by production of EPS (Costerton, et al., 1981), creates situations where some degree of clogging may be inevitable. In order to be able to control clogging in these systems, we need to know which bacteria are causing clogging, and to what extent clogging is caused by the production of bacterial EPS.

Many researchers have attributed clogging to the presence of EPS, or slime, produced by bacteria adhering to sand grains (Avnimelech and Nevo, 1964; Frankenberger et al., 1979; Harris, et al., 1964; Lindenbach and Cullinane, 1989; Mitchell and Nevo, 1964; Simons and Magdoff, 1979; Vandevivere and Baveye, 1992). The slime is believed to fill pore spaces between sand grains, along with bacterial aggregates, and prevent infiltration. Bacterially induced clogging has been shown to occur even in severely oligotrophic, or low nutrient, conditions (Gupta and Swartzendruber,1962).

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Soil temperature has also been thought to influence clogging. DeVries (1972) and Simons and Magdoff (1979) noted that cold and wet conditions apparently induced clogging of mound system sand filters, but that dry and hot conditions "rejuvenated" the infiltrative surfaces by allowing aerobic decomposition of slime and effluent solids in sand pore spaces. Kristiansen (1981) reported more intense clogging in sand filter trenches maintained at 12-15°C than in trenches at ambient temperatures ranging from 4-16°C, but did not determine amounts of EPS present in those systems.

The objectives of this study were to assess the ability of individual organisms associated with the clog zone of onsite systems to initiate clogging in sand columns, and to determine the influence of bacterially produced EPS on the clogging process. Our long term goals are to elucidate the various mechanisms of clogging, and develop methods for controlling clogging *in situ*.

MATERIALS AND METHODS

Isolation of Clog Zone Organisms

Samples of the clog zone of a ponded mound-type septic system at the Arlington, Wisconsin Agricultural Experiment Station were collected twice, in October 1992 and in July 1993. The mound had been ponded for about 2 years prior to the first sampling. Samples were obtained by digging into the side of the mound to the gravel layer. The location of the clog zone was identified by a black slimy deposit at the gravel and sand interface. Clog zone samples were plated on a variety of selective and non-selective solid media, and incubated aerobically and microaerophilically at 18°C and 30°C for up to 8 days. Colony types that were common or that appeared slimy were isolated on trypticase soy (TS; Difco Laboratories, Detroit, MI) agar, characterized by Gram stain, morphology, motility, and catalase and pellicle production, and were stored frozen at -70°C or lyophilized. Presumptive identifications were made with diagnostic kits (API-20E, NFT; BioMeriux-Vitek, Hazelwood, MO) and standard biochemical tests. Our studies to date have focussed on aerobic isolates only.

Columns

Details of column construction have been described previously (Ronner and Wong, 1994). Briefly, columns were constructed from 60 cc plastic syringe barrels. Each column was filled with 85.0 ± 0.1 grams (approximately 50 cc) of Ottawa Sand (mesh 20-30; Fisher Scientific Co., Itasca, IL). The columns were closed with rubber stoppers, sterilized, and placed upright in plexiglass racks.

Screening for Clogging Ability

Bacterial isolates were randomly selected to be screened for clogging ability in sand columns. Pure colonies were picked from TS agar subcultures, suspended in 0.01 M phosphate buffered saline (PBS, pH 7.4) to a concentration of approximately 10^7 cfu/gram sand. Fiveml of suspension was poured evenly onto the top surface of the sterile column. Control columns were inoculated with PBS only. The columns were stoppered and incubated aerobically at 18°, 12°, or 4°C . These temperatures represent a range of seasonal temperatures commonly recorded for mound system soils (J. Converse, personal communication; Simons and Magdoff, 1979). Two hours after inoculation and cell attachment, growth in the columns was supported by adding 5 ml of test media: either TS broth, which contains 0.25% glucose; or synthetic sewage (SS) broth (James, 1964), a minimal medium for maintaining bacterial growth which contains meat extract, peptone, and mineral salts but no added sugars. Thereafter, the columns were fed every 48 hours with 10 ml of test media, and the time for the media to infiltrate into the sand surface was recorded with a stopwatch. Outflow was collected and the volume was recorded. If outflow was delayed, the approximate time from infiltration of the test media until the beginning of outflow

was recorded. Samples of the outflow were plated on TS or SS agar for quantification of non-adherent organisms, and were assayed for total carbohydrates and glucose. Columns were maintained for 2 weeks. The columns were then dismantled, and portions of sand from each column were plated to determine counts of adherent bacteria. Sand was also sampled for scanning electron microscopy (SEM), and for quantification of carbohydrates.

Carbohydrate Assays

Extracellular polysaccharides from column samples were assayed for total carbohydrates by the phenol reaction, as described in Gerhardt, et al. (1994). EPS was removed from duplicate 30 g samples of column sand by vigorous agitation of 3 successive washes with 10 ml of PBS. The wash suspension was centrifuged at 5,000g for 20 minutes to remove bacterial cells. The supernatant was assayed for total carbohydrate in micrograms ml^{-1} calculated against a standard curve of D-glucose. Residual glucose was determined with a Sigma Diagnostics Glucose Kit (Sigma Diagnostics, St. Louis, MO).

RESULTS

Approximately 160 organisms have been isolated from the Arlington mound system clog zone. Sixty-three percent of the isolates are Gram negative. Eighty-one isolates were screened for clogging ability, and 24 (30%) of these have caused stable clogging in sand columns. These clogging isolates represent many different bacterial genera. Most are organisms commonly found in soil such as *Pseudomonas*, *Aeromonas*, *Bacillus*, *Xanthomonas*, *Agrobacter*, and *Acinetobacter*. Others such as coliforms, *Enterobacter*, *Klebsiella*, *Staphylococcus*, and *Serratia* may come from soil or septage. From the screened isolates, a group of 19 clogging and non-clogging organisms was selected for further study. Organisms in this group that were reliable cloggers were presumptively identified as members of the genera *Bacillus*, *Pseudomonas*, *Serratia*, *Klebsiella*, and *Staphylococcus*. It is apparent, however, that many different types of organisms, irrespective of genus or morphology, have the potential to cause clogging in sand columns.

Definition of Clogging

Criteria were developed to define clogging. Columns that we considered to be clogged exhibited at least 3 of the following characteristics:

1. Infiltration time approaching 15-20 seconds. Uninoculated control columns, unclogged columns, and almost all newly inoculated columns had infiltration times of approximately 4-6 seconds. Columns undergoing clogging showed a progressive or a sudden increase in infiltration time. Completely ponded columns showed no discernible infiltration in more than 15 minutes.

2. Delayed outflow. Start of outflow from control or unclogged columns was usually immediate or within 5-10 seconds after addition of liquid. A delay of more than 30 seconds indicated some degree of clogging.

3. Reduced outflow volume. An outflow return of 8 ml or less of the 10 ml of added liquid indicated retention of liquid within the sand pore spaces. Control and unclogged columns usually returned at least 9 ml of liquid. Ponded columns often gave no outflow at all, or liquid would exude from the aeration ports instead of the outflow tube.

4. Slow, dropwise outflow. Outflow from control and unclogged columns was rapid. Outflow that continued for several minutes, or that came out slower than one drop per second, indicated restricted movement of liquid within the column.

Clogging ability could not be predicted by bacterial counts. Counts of outflow bacteria for almost all the isolates screen so far have been 10^8 to 10^9 cfu/ml, except for most of the *Bacillus* isolates which were 10^6 cfu/ml. Counts of bacteria adhering to sand grains were generally 10^7 to 10^8 cfu/gram of sand, except for most *Bacillus* isolates which were 10^6 cfu/gram.

SEM examination of sand grains shows that all of the isolates screened so far were able to adhere well to sand, and to produce visible amounts of extracellular materials, presumably EPS. Therefore gross EPS production was not an indicator of clogging ability.

Clogging was observed with some reliability with several organisms (Table 1) from the isolates we are studying. We observed two patterns of clogging. The more common pattern was a steadily progressive increase in infiltration time and a decrease in outflow speed and volume. The other clogging pattern, that appeared occasionally, was dramatic ponding of medium at the top of the column, with little or no infiltration observed over 48 hrs, and no return from the outflow tube. In most cases liquid dripped out of the aeration ports, although sometimes these ports also became clogged and liquid remained perched above the sand column between feedings. Typically the ponding began within 1-3 days of column inoculation, and continued throughout the 2 weeks of the trial. This severe clogging was seen occasionally with several isolates when they were grown in TS broth at 18°C, although usually these isolates demonstrated the more gradual clogging pattern. However there were 2 isolates that reliably demonstrated the dramatic ponding pattern at all temperatures and even when grown with SS medium. These 2 isolates have been presumptively identified as *Pseudomonas fluorescens* and *Xanthomonas maltophilia*. The rapid onset of this drastic ponding, and the fact that it occurs reliably at 4°C as well as at 18°C, warrants further study of the prevalence of these organisms in the clog zone, and of their impact on the system.

EPS

EPS produced by adherent cells in the columns was apparently water soluble, since much greater amounts of EPS were found in outflows than attached to sand grains. More EPS was produced by cells fed with TS broth than SS broth. Only 3- 30% of the total carbohydrate from both TS and SS outflows was glucose, so the majority of the measured EPS was presumed to be bacterially produced complex polysaccharides rather than residual simple sugars from the media.

Some of the isolates we have tested so far did not grow well in SS broth at 4°C within the 2 week duration of the trials. However, many isolates were capable of growth at 4°C. Many of the isolates that grew well at 4°C produced more EPS at 4°C than at 18°C, even though cell counts may sometimes have been lower. Also, 10-20 times as much EPS remained attached to sand grains at 4°C than at 18°C.

A possible explanation for the greater adherence of EPS to sand at colder temperatures is our observation that colonies of some bacterial cultures stored on agar plates at 4°C for several weeks have a different texture than freshly grown colonies. These colonies have a rubbery, more cohesive texture than freshly grown, slimy colonies. The rubbery texture may persist for several weeks when the plates are transferred to room temperature conditions. We are trying to determine whether this textural change is caused by the production of different types of slime at different temperatures, or simply a dehydration or gellation effect of the cold storage. In either case, this change in slime texture may be a reason for *in situ* clogging of infiltration systems when the weather becomes cold.

Table 1. Clogging efficiency of some organisms that cause clogging in sand columns.

Isolate	Temperature °C	Clogging Efficiency†
Bacillus 2 isolates	18	4/15 (27%)
	12	2/11 (18%)
	4	2/12 (17%)
Pseudomonas 2 isolates	18	7/13 (54%)
	12	6/9 (67%)
	4	3/8 (38%)
Serratia 2 isolates	18	7/17 (41%)
	12	8/13 (62%)
	4	1/12 (8%)
Klebsiella 1 isolate	18	2/8 (25%)
	12	0/5 (0%)
	4	2/6 (33%)
Staphylococcus 3 isolates	18	8/14 (57%)
	12	2/7 (28%)
	4	0/8 (0%)

†Number of columns that clogged/number of column trials.

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