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# **SMALL SCALE WASTE MANAGEMENT PROJECT**

## **Microbial Clogging of Wastewater Infiltration Systems**

by

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## Microbial Clogging of Wastewater Infiltration Systems

Amy B. Ronner and Amy C. L. Wong\*

## ABSTRACT

We have studied the bacteria and bacterial extracellular polymers (ECP) associated with clogged soil pores of onsite wastewater infiltration systems and reduced infiltration. Approximately 160 bacteria have been isolated from the clogging layer of a ponded Wisconsin mound. We developed an *in vitro* screening system using infiltration columns in plastic syringe barrels to determine the clogging potential of these isolates. The columns were inoculated with single or multiple cultures, and the infiltration rate of a synthetic sewage broth was monitored. Biofilm formation on sand grains, ECP production, and structure development in the columns were analyzed by plate counts and photometric methods, 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. These isolates represented a variety of bacterial genera.

**Keywords.** Bacteria, Columns, Infiltration, Onsite wastewater treatment, Polysaccharides, Septic tank effluent, Wastewater

## OVERVIEW OF BACTERIAL CLOGGING

The phenomenon of clogging, or reduced hydraulic conductivity of onsite wastewater systems, has usually been studied in terms of the physical parameters of soil or 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. Some researchers believe that networks of extracellular polysaccharides produced by bacteria adhering to sand grains cause clogging. Mitchell and Nevo (1964) found a positive correlation between clogging in sand columns and the accumulation of polysaccharides produced by indigenous dune sand bacteria. Harris et al. (1964) found that the aggregate stability of soils was a function of microbial synthesis of soil-binding substances rather than the total number of microorganisms. Lindenbach and Cullimore (1989) were able to create clogging in sand columns using *Pseudomonas*, *Acinetobacter*, and *Bacillus* isolates from clogged golf green soils, and they attributed this clogging to a clear slime or biofilm produced by the bacteria.

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However, Siegrist (1987) found that decreasing concentrations of polysaccharides corresponded with increased clogging severity in pilot-scale infiltration cells dosed with domestic septic tank effluent, and concluded that polysaccharide materials were not responsible for soil clogging. In several experiments using pure cultures of soil isolates (an *Arthrobacter* strain, and variants of an unidentified bacterium that were positive and negative for slime and capsule production), Vandevivere and Baveye (1992a; 1992c) observed that aggregates of nonmucoid bacterial cells could clog soil pores.

The objectives of this study were to identify bacteria associated with the clog zone of onsite systems, and assess the ability of these organisms to initiate clogging in sand columns. Ultimately we hope to elucidate the various mechanisms of clogging, and develop methods for controlling clogging *in situ*.

## MATERIALS AND METHODS

### Clog sampling site

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. Samples were obtained by digging into the side of the mound to the gravel layer. The clog zone was identified by a black slimy deposit at the gravel and sand interface. Samples of this black slime were collected in plastic bags, packed on ice and returned to the laboratory within 2 hours.

### Isolation of clog zone organisms

Clog zone samples were diluted 1:10 (w/v) in 0.5% peptone water and plated on a variety of selective and non-selective solid media including brain heart infusion agar, MacConkey agar, triple sugar iron agar, eosin methylene blue agar, pseudomonas isolation agar, potato dextrose agar, and plate count agar. Duplicate platings were incubated aerobically and microaerophilically at 18°C and 30°C for up to 8 days. Colony types that were predominant or appeared slimy were isolated on trypticase soy agar (TSA), characterized by Gram stain, morphology, motility, and catalase and pellicle production, and were stored in 88% glycerol solution at -70°C. Presumptive identifications were made with diagnostic kits (API-20E, NFT; BioMerieux-Vitek) and standard biochemical tests. Our studies to date have focussed on aerobic isolates only.

### Columns

Columns were constructed from 60 cc plastic syringe barrels. Four aeration holes, two on opposite sides, were drilled into the barrels using a size 15 (4.6 mm diameter) drill bit. Approximately 25 mm of PVC autoclavable tubing (3.2 mm ID, 4.8 mm OD) was inserted into each aeration hole so that the inner end of the tubing was flush with the syringe wall. A

piece of tubing approximately 15 cm long was affixed to the syringe outlet. The outer joints of all the tubings were sealed with high-temperature silicon sealant, and the sealant was allowed to cure for several days. The aeration hole tubing was plugged with glass wool, and a thin layer of glass wool was placed inside the barrel to cover the outlet hole, in order to prevent outflow of sand. The ends of all the tubings were wrapped loosely with aluminum foil, and the columns were placed upright in plexiglass racks. Each column was filled with approximately 50cc (85 g) of Ottawa Sand (mesh 20-30). The columns were closed with black rubber stoppers and covered tightly with aluminum foil. The columns were autoclaved for one hour, at 121°C and 15 psi, on two successive days. The repeated autoclaving prevented the growth of spore-forming organisms which might have been heat shocked by the first autoclaving. The columns were allowed to cool for at least 24 hours before inoculation.

#### Column inoculation and feeding

Groups of up to 16 isolates were randomly selected to be screened for clogging ability in sand columns. Frozen isolates were thawed and passaged twice on TSA before inoculation. Colonies were picked with sterile wooden applicators and suspended in 5.5 ml of 0.01 M phosphate buffered saline (PBS, pH 7.4) to an optical density of 0.5 McFarland Standard. One half ml of each suspension was removed for quantification of the inoculum, and the remaining 5 ml was poured onto the top surface of the sterile column. Control columns were inoculated with PBS only. The columns were stoppered and incubated aerobically at 18°C. Two hours after inoculation, the columns were fed with 5 ml of synthetic sewage (SS) broth, a minimal medium for maintaining bacterial growth (James, 1964). Thereafter, the columns were fed every 48 hours with 10 ml of SS broth, and the time for the SS broth to infiltrate into the sand surface was recorded. Any outflow from the lower ports was collected in sterile tubes, the volume of outflow was recorded, and aliquots were plated on TSA or SS agar for quantification of non-adherent organisms. Development of clogging was indicated by an increase in infiltration time, delay in outflow from the drainage tube, or reduced volume of outflow. Occasionally we noted the development of structure in the upper sections of clogged columns, possibly due to the presence of bacterial extracellular polymers.

Columns were maintained for 2 weeks. After the columns were dismantled, sand from the upper, middle, and lower portions of each column was plated to determine microbial load, and samples from each section were examined by scanning electron microscopy (SEM), according to a method adapted from Vandevivere and Baveye (1992b).

### RESULTS

Approximately 160 clog zone organisms have been isolated. Sixty-three percent of the isolates are gram negative. Eighty-one isolates have been screened so far, and 24 (30%) of these have caused stable clogging in sand columns. These isolates represent many different bacterial genera. Most are organisms commonly found in soil such as *Pseudomonas*, *Aeromonas*, *Xanthomonas*, *Agrobacter*, and *Acinetobacter*. Others such as coliforms, *Enterobacter* and *Serratia* may come from soil or septage. All of the 3 *Serratia* isolates

tested have caused clogging. Infiltration time for uninoculated control columns generally ranged from 3-5 seconds, and drainage from the outflow tube began immediately. As clogging developed in inoculated columns, the infiltration time often increased to 20-30 seconds, or as long as several minutes, and drainage was usually delayed. In some instances clogging was evidenced by a delay of up to 10 minutes in the start of outflow, while infiltration time remained short. Clogging usually became evident within 5-7 days, although some isolates started after 12 days. Some isolates caused only short-term or sporadic clogging, and were not considered stable cloggers.

Using SEM, we observed large numbers of bacteria adhering to sand grains of both clogged and unclogged columns. Bacterial ECP was often visible on both clogged and unclogged samples, appearing as networks of materials surrounding and interlinking bacterial cells and the sand surfaces. We found no correlation between clogging and bacterial numbers in column outflow or on sand grains. Production of extracellular ECP, determined by toluidine blue staining (Tsai et al., 1988) was not predictive of clogging ability. There may be qualitative rather than quantitative differences in the ECP produced by various bacteria which would cause stable clogging.

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