Biofouling of Groundwater Distribution System by *Thiothrix* spp. (U)

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1 BIOFOULING OF GROUNDWATER DISTRIBUTION SYSTEMS BY
2 THIOTHRIX SPP.

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16

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1 **ABSTRACT**

2 Thiothrix spp., sulfide oxidizing filamentous bacteria, were
3 found to be the main bacterial component of aquatic biofilms
4 causing biofouling in selected municipal water storage tanks,
5 private wells, and drip irrigation systems in Florida. The water
6 originated from the upper Floridan aquifer and associated aquifers
7 in Central and North Florida. Samples were examined where visible
8 biofilms had a white, slimy, filamentous appearance indicative of
9 Thiothrix spp. The detection of Thiothrix spp was confirmed by
10 enzyme-linked immunosorbent assay (ELISA). These observations
11 confirm that these bacteria and associated extracellular material
12 play an important role in formation of biofilms, which in turn may
13 induce physical changes leading to significant biofouling. These
14 studies suggest that Thiothrix spp.-associated biofouling occurs
15 at an interface where reduced sulfide-containing water contacts
16 aerated water and a surface or substrate is available for
17 attachment.

1 INTRODUCTION

2 Biofouling has been defined as the undesirable accumulation
3 of microbial and extracellular materials of microorganisms
4 (Chracklis, 1990). Thiothrix spp. have been described as a
5 component of biofilms resulting in biofouling of water wells
6 (Lackey et al., 1965), irrigation systems (Ford and Tucker, 1975),
7 and drainage systems (Spencer et al., 1963). Thiothrix spp. have
8 also been indicated as a cause of filamentous bulking in
9 wastewater treatment plants (Williams and Unz, 1985). However,
10 little is known of the microbial ecology of biofouling by
11 Thiothrix spp.

12 Thiothrix spp. are chemomixotrophic bacteria that obtain
13 energy from the oxidation of sulfide to elemental S and sulfate
14 (SO_4^{2-}), (Strohl and Schmidt, 1984, Odintsova et al., 1993).
15 Thiothrix spp. can attach to a variety of substrata with slimelike
16 holdfast material in flowing water with sulfide concentrations of
17 at least 0.1 mg/L and a pH near neutral (Larkin and Nelson, 1987,
18 Larkin and Strohl, 1983). In the springs issuing from and
19 underwater limestone caves within the Floridan aquifer, visible
20 colonies of Thiothrix spp. have been observed as thin white mats
21 or tufts on rocks and sediments on cave floors, walls, and
22 ceilings (Brigmon et al., 1994b).

23 In this report we use a Thiothrix spp.-specific enzyme-linked
24 immunosorbent assays (ELISA), and scanning electron microscopy
25 (SEM) methods for identification and characterization of Thiothrix
26 spp. in biofilms. We describe the biofouling caused by Thiothrix
27 spp. in different aquatic systems.

MATERIALS AND METHODS

Sampling Sites. Sites sampled were from three biofouled aquatic systems at Northcentral Florida sites including a spring water bottling plant at Orange Springs, municipal water storage tanks and aerators at Palatka, and an agricultural drip irrigation system in Polk County. At each of these selected sites visible colonies of white filamentous sulfur bacteria were evident.

Orange Springs in Marion County, Florida emanated from a cave formed in the Hawthorn Formation, a heterogeneous mixture of clays, sands, and limestone over-lying Ocala limestone (Brigmon et al., 1994b). Orange Spring water was sulfurous with a distinct smell of sulfide and with highly visible colonies of Thiothrix in the spring basin and spring run. The source of the bottling plant's water source was a 4 inch diameter polyvinyl chloride (pvc) pipe inserted inside a cavern below the spring basin at a depth of 25 m. Pumping rate was 2000 gal/h. The Orange Springs water was causing sand and cartridge filters for a drinking water bottling system to completely clog up within 24 hr of pumping initiation. Fresh spring water tested weekly over a six month period at the source contained sulfide concentrations of 5-10 ppm. On close examination, the cause of the biofouling was found to be a biofilm that was composed predominately of Thiothrix spp.

Samples were collected from the City of Palatka municipal water system in Putnam County, Florida. The Palatka water well field consisted of several wells screened in the upper Floridan aquifer at depths ranging from 75 to 125 m below ground level.

1 All well casings were 26 cm in diameter and pumped at an average
2 rate of 400 gal/min. The aeration system was on one of the city
3 of Palatka's 500,000 gallon water storage tanks supplied by the
4 well field. Profuse growths of Thiothrix were observed with
5 filamentous mats up to 8 cm thick and 3 m long in the aerator
6 (Figure 1). The biofilm was causing interference with flow
7 through the aerator. The metal pans in the aerator were corroded
8 on the bottom. On the inside of the water storage tank, long
9 strands of Thiothrix spp. were evident. The City of Palatka
10 biofouling came to our attention when reports of weakness in the
11 structural roof of one of three concrete storage tanks was
12 correlated with a large formation of "sulfur slime." The inside
13 of the roof of the storage tank was corroded through the concrete
14 to the metal reinforcement rods. The air inside of the tank
15 contained hydrogen sulfide concentrations of 30 mg/L when first
16 opened while the water in the tank contained 3-7 mg/L hydrogen
17 sulfide.

18 Sections of biofouled irrigation pipe were submitted for
19 examination to the University of Florida's Department of
20 Microbiology and Cell Science by the Institute of Food and
21 Agricultural Science. A drip irrigation system had ceased to
22 function as a result of blockage of pipe and emitters due to
23 "sulfur slime." Farmers had lost part of their crops to drought
24 due to this biofouling problem. No water chemistry data were
25 available on this site. The Florida Cooperative Extension Service
26 reported this to be a seasonal problem occurring most often in the
27 fall (Pitts et al., 1990).

1 Thiothrix spp. Identification. Bacteria were cultured in
2 lactate-thiosulfate HEPES (LTH) growth-medium (Williams and Unz,
3 1989) made using 0.2 µm filtered water from the site of collection
4 (Brigmon et al., 1994a; McGlannan and Makemson, 1990). To isolate
5 Thiothrix spp., environmental bacteria samples were vortexed and 1
6 ml was pipetted into 9 ml of LTH and incubated for 48 h at room
7 temperature. Serial dilutions of the enrichments were made with
8 filtered autoclaved site water. Pour plates were made with 0.1-ml
9 samples from dilutions mixed with 0.3% Na₂S₂O₃*5H₂O in 1% agar
10 made with the natural water. Thiothrix spp. colonies were
11 tentatively identified for further examination by their white,
12 rough filamentous appearance.

13 Monoclonal antibodies (MAB) specific for Thiothrix spp. were
14 employed to test the environmental samples in an ELISA (Brigmon et
15 al., 1995). Bacteria samples from LTH medium after 24 to 48 hr
16 incubation at room temperature were centrifuged and tested on
17 ELISA plates with Thiothrix spp. specific MAB (Brigmon et al.,
18 1995).

19 **Scanning Electron Microscopy.** Colonies of filamentous
20 bacteria were concentrated near drip irrigation emitter openings.
21 Small pieces (ca. 5 mm²) of black plastic irrigation pipe were
22 excised and fixed for 1 hr in 2.5% glutaraldehyde buffered with
23 sodium cacodylate to pH 7.2. Samples were then dehydrated in a
24 standard ethanol series and finally soaked in hexamethyl
25 disilazane before air drying. The dry samples were sputter-coated
26 with gold and examined in a Hitachi S-4000 field emission scanning
27 electron microscope (SEM).

1 RESULTS AND DISCUSSION

2
3 Thiothrix spp. were identified in samples of biofouled
4 material taken from all three sites. Thiothrix spp. were
5 identified by their characteristic filamentous appearance,
6 motility, ability to form sulfur granules, and presence of
7 rosettes. All Thiothrix spp. observed in this study were enclosed
8 in a sheath. All biofouling samples discussed in this report
9 tested positive for Thiothrix spp. using microbiological
10 procedures and ELISA.

11 The SEM of the biofouled pipe samples revealed the complex
12 filamentous nature of the bacterial sulfur slime (Fig. 1). Fig.
13 1A shows a cross section of the pipe indicating the blockage of
14 the pipe caused by the formation of the biofilm. Fig. 1B shows
15 that the walls of the pipe are also covered with a filamentous
16 biofilm. Fig. 1C is a photomicrograph of the biofouled pipe
17 showing particles enmeshed in the filamentous mat.

18 A photograph of the Thiothrix spp. biofouled aerator from the
19 City of Palatka, Florida is shown in Fig 2. The thick white mat
20 was composed almost entirely of Thiothrix spp. Underneath the
21 surface of the Thiothrix spp. were strands of filamentous algae.

22 The presence of Thiothrix spp. in six of eight underwater
23 caves sampled in a previous study (Brigmon et al., 1995b) suggests
24 that Thiothrix spp. may be an important part of the S cycle in the
25 Floridan aquifer. Periodic changes in conduit and diffuse aquifer
26 water flow could result in substantial localized changes in oxygen
27 and sulfide concentrations that could affect the growth of

1 Thiothrix spp. in water supplying these systems (Brigmon et al.,
2 1994b). This may partly explain the reported regional and
3 seasonal biofouling of drip irrigation systems in the area. The
4 ability of Thiothrix spp. to attach and thrive in optimal
5 environmental conditions make it hard to control (Bland and
6 Staley, 1978).

7 A previous study demonstrated that Thiothrix spp. has the
8 potential to contribute to acidolytic porosity generation in
9 carbonate aquifers with conduit flow (Brigmon et al., 1995). The
10 presence of Thiothrix spp. in the aquatic systems sampled in this
11 study implies the possibility of corrosion due to sulfuric acid
12 generation by Thiothrix spp. Significant oxidation rates of
13 sulfide to sulfate and elemental sulfur to sulfate by Thiothrix
14 spp. have been reported (Schmidt et al., 1987, Strohl and Schmidt,
15 1984). Extreme corrosion of metal alloy as well as concrete on
16 the inside of the storage tank was noted at the Palatka site. No
17 Thiobacillus or Desulfovibrio spp. were observed in microscopic
18 examinations of corroded concrete as has been previously described
19 (Kulpa and Baker, 1990). However, mixtures of sulfur and iron-
20 sulfur compounds resulting from microbial metabolism and chemical
21 reactions can be highly corrosive (Nielson, et al., 1993).
22 Periodic mechanical removal of the biofilm as well as chlorination
23 was necessary to control the biofilm after the tanks were
24 repaired.

25 At a spring water bottling facility in Orange Springs,
26 Florida both cartridge and sand filters became plugged within 24 h
27 of the initiation of flow. Frequent backwashing of sand filters

1 and increasing the pore size of the cartridge filters did not
2 control the fouling problem. Installation of aeration tanks and a
3 chlorination system were successful at eliminating the biofouling
4 of drinking water filters.

5 Thiothrix spp. have frequently been associated with
6 wastewater as the predominate species in treatment plants
7 (Farquhar and Boyle, 1972). Methods for control of Thiothrix spp.
8 biofouling in wastewater treatment plants include chlorination and
9 adjusting the dissolved oxygen concentration (Jenkins et al,
10 1984). These same methods may be used in other environments where
11 Thiothrix spp. are a problem.

12 Blockage of micro-irrigation systems can be caused by
13 physical, chemical, or biological conditions. Causes of physical
14 plugging problems include sand and suspended debris in supply
15 water. Chemical plugging can result from scale formed by
16 precipitation of calcium, magnesium, iron, or manganese compounds.
17 Biological causes of blockage include bacteria and algae. It is
18 evident from close examination of the clogged pipe section that
19 the matrix of the biofilm contains particulate matter and
20 precipitated minerals including pyrite and possibly other iron
21 sulfides (Fig 1C). The presence of iron sulfide within the
22 biofilm is an indication of localized back-reduction of
23 biologically oxidized sulfur. This may be related to the fact
24 that sulfide oxidizes at a lower redox potential than does ferrous
25 iron. The sulfur bacteria problem can be minimized in irrigation
26 systems if there is no air-water contact until water is discharged
27 (Pitts et al., 1990). Defective valves or fittings on the suction

1 side of the irrigation pump can contribute to sulfur bacteria
2 problems (Ford and Tucker, 1975). The application of chlorination
3 to solve biofouling can be detrimental due to potential crop
4 damage and environmental concerns. The limited penetration of
5 chlorine into an established biofilm has been proven to be a
6 limiting factor in the effectiveness of the biocide as a means of
7 control (de Beer et al., 1994). Digging new wells or replacement
8 of equipment can be expensive (Pitts et al., 1990). In this case,
9 equipment was replaced and monitored for leaks.

10 Typically, there are other sulfur bacteria as well as various
11 iron bacteria that make up the matrix causing the fouling
12 associated with Thiothrix spp. (Spencer et al., 1963). In this
13 study, other bacteria were observed but not characterized.
14 Thiothrix spp. appeared to be the predominant species in these
15 particular biofouled systems. We observed smaller bacteria, mostly
16 rods, beneath the Thiothrix spp. in the pipe sections in the
17 exopolysaccharide biofilm matrix (Fig 1D).

18 We have observed that biofilms composed principally of
19 Thiothrix spp. can cause biofouling on a variety of surfaces
20 including metal pans and concrete at the Palatka municipal
21 drinking facility, organic polymers like the pvc irrigation pipe
22 at the farm in Polk County, as well as sand and cartridge filters
23 at the spring water bottling plant in Marion County.

24 Much of our interest in the microbial biogeochemistry of
25 subsurface aquatic systems is due to the economically important
26 fresh water aquifers situated there. The biofouling problems
27 described here are of economic concern. We have previously

1 reported on *Thiothrix* spp. with respect to the water chemistry
2 (Brigmon et al., 1994a) and the hydrogeology (Brigmon et al.,
3 1994b) of the Orange Springs and Palatka sites.

4 Identification of specific biofouling microorganisms can be
5 useful in the development of methods for their control. In
6 addition, this knowledge can be applied to the microbial ecology
7 of biofouling in specific environments, thus it is useful to
8 analyze for specific bacteria. The detection of Thiothrix spp.
9 can serve as an indicator of biofouling in groundwater collection,
10 storage, and delivery systems.

11 12 **ACKNOWLEDGMENTS**

13
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FIGURE CAPTIONS:

Figure 1. Scanning electron micrographs (SEM) of Thiothrix in irrigation pipe from Polk County, Florida. A) Cross section of Thiothrix spp. biofilm at emitter connection which clogged pipe. B) Attached Thiothrix spp. from side of pipe. C) Thiothrix spp. and particulate matter in the biofilm matrix. D) Biofilm of smaller bacteria and exopolysaccharide beneath Thiothrix spp.

Figure 2. City of Palatka, Florida water storage tanks aerator 3 M long strands of Thiothrix spp. forming mat covering surface of aerator.

FIGURES

Figure 1. Scanning electron micrographs (SEM) of Thiothrix spp. in irrigation pipe from Polk County, Florida. A) Cross section of Thiothrix spp. biofilm at emitter connection which clogged pipe.

Figure 1. B) Attached Thiothrix spp. from side of pipe

Figure 1. C) Thiothrix spp. and particulate matter in the biofilm matrix.

Figure 1. D) Biofilm of smaller bacteria and exopolysaccharide
beneath Thiothrix spp.

Figure 2. City of Palatka, Florida water storage tanks aerator 3 M
long strands of Thiothrix spp. forming mat covering surface of
aerator.

LEGEND

- State Route
- County Boundary
- Street, Road
- Hwy Ramps
- Major Street/Road
- State Route
- Railroad
- River

Open Water

Scale 1:50,000 (at center)

5000 Feet

1000 Meters

Mag 13.00
Wed Aug 02 11:45:55 1995

FIGURE 2

CROSS-SECTION
SCALE: 1"=20'

NOTES:

1. EMBANKMENT SLOPE PROTECTION MAT OF ASPHALTIC CONCRETE TO BE REINFORCED WITH 6" x 6" #10 - #11 GAGE WELDED WIRE FABRIC.
2. SEAMS OF WELDED WIRE REINFORCING ARE TO BE TIED AT 6 INCHES ON CENTER ALONG HORIZONTAL SEAMS. TIES ARE REQUIRED ALONG SLOPING SEAMS.
3. SLOPE PROTECTION MAT TO BE ANCHORED TO SLOPE WITH 2'-0" LONG STAKES OF NO. 6 REINFORCING-STEEL BARS SPACED 10'-0" ON CENTER ALONG TOP AND TOE OF MAT AND ON SLOPE.
4. SUITABLE COMPACTION OF ASPHALTIC CONCRETE MAT IS TO BE OBTAINED BY USE OF HAND-OPERATED ROLLERS SUFFICIENT TO PRODUCE A HOMOGENEOUS MASS.

MAT DETAIL
NOT TO SCALE

FIGURE 3