# Thiothrix-1

Resembles: -

*Probes*: class specific Gam42a [10] and group specific G123T [7] and TNI [16] *Frequency occurrence* (200 samples; 175 WTPs):

- observed with a FI  $\geq$  1 in 15 samples
- observed with a  $FI \ge 3$  in 4 samples



#### **Characteristics**

- straight, bent or somewhat twisted filaments;
- filaments frequently protruding from or attached to the flocs; occasionally attached to other filaments;
- often tapering of the filaments;
- filament length variable;
- filaments not branched;
- not motile;
- cell diameter may vary from 0.6 μm (tip filament) to 1.2 μm (basal cells);
- sheath absent;
- without attached growth;
- septa only visible in thicker filaments and when S granules are missing;
- rectangular cells;
- often already *in situ* sulphur granules; fast storage of granules with the S-test;
- Gram negative;
- Neisser negative.

#### Remarks

All *Thiothrix* morphotypes belong to the *Gammaproteobacteria*; class specific probe: Gam42a [10].. G123T and TNI are group specific probes. A number of classified *Thiothrix* species might occur in WTPs [1, 4, 7, 8] and probes are available by which various subgroups can be distinguished [7]. However, additional FISH tests, aimed at further identification of the morphotypes observed by applying such subgroup specific *Thiothrix* probes, were not carried out during the Dynafilm project.

Thiothrix-1 includes Thiothrix-3 and 5, two previously distinguished separate morphotypes [3].

### Physiology

Most classified *Thiothrix* species use low molecular compounds, such as short chain fatty acids and often also sugars for their growth and they also derive energy from the oxidation of reduced sulphur compounds such as  $H_2S$  or thiosulphate. It has even been demonstrated that acetate is taken up under anoxic or anaerobic conditions by some *Thiothrix* strains if thiosulphate is present [13]. Some *Thiothrix* strains absolutely require reduced sulphur compounds for their growth.

References for further reading about the physiology of *Thiothrix*: 1, 5, 11, 12, 13, 14, 17 and 18.

### Occurrence in activated sludge

*Thiothrix*-1 was often observed in WTPs treating wastewater from pulp & paper industries and on occasion also in plants receiving wastewater from chemical, dairy and food industries. Thus, it is not possible to correlate this morphotype with a specific industrial branch. However, it is very likely that growth of *Thiothrix*-1 will mainly occur in plants treating wastewater rich in reduced sulphur compounds.

## **Control strategies**

The common possibilities aimed at solving a bulking problem are listed below (1-7). Selectors or two step configurations are often effective methods to control filamentous species using low molecular compounds for their growth. If sulphur is stored very easily and in massive amounts by the *Thiothrix* morphotype present, reduced sulphur compounds, supplied with the influent, most likely play a decisive role in the competition of that Thiothrix sp. with other sludge bacteria. Reduced sulphur compounds can be removed in an aerated, highly loaded first stage (option 3). Other low molecular compounds, also stimulating growth of *Thiothrix* sp., will be largely removed simultaneously. It is always recommended to start with a pilot scale experiment before a selected control method is applied on full scale.

References for further reading about process control: 2, 6, 9 and 15.

1. Good "House-keeping"

2. Remove deficiencies:  $O_2 > 2 \text{ mg/l}$  and BOD:N:P =100:5:1.

3. Two step configuration (aerobic/aerobic or anaerobic/aerobic), in order to remove most of the easily degradable influent fraction before this enters the aeration tank.

4. Aerobic selector.

5. Anoxic zone if sufficient nitrite/nitrate is available for removal of the dissolved fraction from the influent through denitrification.

6. Anaerobic zone if a combination with a Bio-P process is an option.

7. Controlling symptoms, viz. applying physical or chemical methods aimed at destroying the filaments or at improving the settling velocity of the flocs by increasing their weight.

## References

1. Aruga, S., Y. Kamagata, T. Kohno, S. Hanada, K. Nakamura and T. Kanagawa (2002) Characterization of filamentous Eikelboom type 021N bacteria and description of *Thiothrix disciformis* sp. nov. and *Thiothrix flexilis* sp. nov. *Int. J. of Syst. and Evol. Microbiol.* **52**, 1309-1316.

2. Eikelboom, D. H. (2000) *Process control of activated sludge plants by microscopic investigation*. IWA Publishing, London, UK.

3. Eikelboom, D. H. and B. Geurkink (2002) Filamentous microorganisms observed in industrial activated sludge plants. *Water Sci. Technol.* **46** (1-2), 535-542.

4. Howarth, R., R. F. Unz, E. M. Seviour, L. L. Blackall, R. W. Pickup, J. G. Jones, J. Yaguchi and I. M. Head (1999) Phylogenetic relationships of filamentous sulfur bacteria (*Thiothrix spp.* and Eikelboom type 021N bacteria) isolated from wastewater treatment plants and description of *Thiothrix eikelboomii* sp. nov., *Thiothrix unzii* sp. nov., *Thiothrix fructosivorans* sp. nov. and *Thiothrix defluvii* sp. nov. *Int. J. Syst. Bacteriol.* **49**, 1817-1827.

5. Hudson, R., C. Williams, R. J. Seviour and J. A. Soddell (1994) Variation in phenotypic characters of type 021N from activated sludge systems. *Water Sci. Technol.* **29**, 143-147.

6. Jenkins, D., M. G. Richard and G. T. Daigger (2004) *Manual on the causes and control of activated sludge bulking, foaming and other solids separation problems.* IWA Publishing, London, UK.

7. Kanagawa, T., Y. Kamagata, S. Aruga, T. Kohno, M. Horn and M. Wagner (2000) Phylogenetic analysis of and oligonucleotide probe development for Eikelboom type 021N filamentous bacteria isolated from bulking activated sludge. *Appl. Environ. Microbiol.* **66** (11), 5043-5052.

8. Kim, S. B., M. Goodfellow, J. Kelly, G. Saddler and A. C. Ward (2002) Application of oligonucleotide probes for the detection of *Thiothrix* spp. of activated sludge treatment plants of paper and board mill wastes. *Water Sci. Technol.* **46** (1-2), 559-564.

9. Lemmer, H und G. Lind (2000) Blähschlamm, Schaum und Schwimmschlamm – Mikrobiologie und Gegenmassnahmen. F. Hirthammer Verlag, München, Germany.

10. Manz, W., R. Amann, W. Ludwig, M. Wagner and K. H. Schleifer (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: problems and solutions. *Sys. Appl. Microbiol.* **15**, 593-600.

11. Nielsen, P. H., K. Andreasen, M. Wagner, L. L. Blackall, H. Lemmer and R. J. Seviour (1998) Variability of type 021N in activated sludge as determined by *in situ* substrate uptake pattern and *in situ* hybridization with fluorescent rRNA targeted probes. *Water Sci. Technol.* **37**, 423-440.

Nielsen, P. H., K. Andreasen, N. Lee and M. Wagner (1999) Use of microautoradiography and fluorescent *in situ* hybridization for characterization of microbial activity in activated sludge. *Wat. Sci. Technol.* **39** (1), 1-9.
Nielsen, P. H., M. A. de Muro and J. L. Nielsen (2000) Studies on the in situ physiology of *Thiothrix* spp. present in activated sludge. *Environ. Microbiol.* **2** (4), 389-398.

14. Rosssetti, S., L. L. Blackall, C. Levantesi, D. Uccelletti and V. Tandoi (2003) Phylogenetic and physiological characterization of a heterotrophic, chemolithotrophic Thiothrix strain isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* **53**, 1271-1276.

15. Tandoi, V., D. Jenkins and J. Wanner (2005) Activated sludge separation problems – Theory, Control Measures, Practical Experiences. IWA Publishing, London, UK.

16. Wagner, M., P. Amann, P. Kämpfer, B. Assmus, A. Hartmann, P. Hultzler, N. Springer and K. Schleifer (1994) Identification and *in situ* detection of Gram-negative filamentous bacteria in activated sludge. *System. Appl. Microbiol.* **17**, 405-417.

17. Williams, T. M. and R. F. Unz (1985) Filamentous sulphur bacteria of activated sludge: characterization of *Thiothrix, Beggiatoa* and Eikelboom Type 021N strains. *Appl. Environ. Microbiol.* **49**, 887-898.

18. Williams, T. M. and R. F. Unz (1989) The nutrition of *Thiothrix*, Type 021N, *Beggiatoa* and *Leucothrix* strains. *Water Res.* 23, 15-22.

#### Slide show images

- 1-3: low magnification; filaments often attached to the floc or to other filaments
- 4-6: diameter variable (often tapering)
- 7: occasionally more twisted filaments
- 8-20: examples S-storage, *in situ* or after applying the sulphur deposit test.
  - o 10: S. natans does not store S
  - small S granules are almost black
- 21-22: FISH images with probe TNI