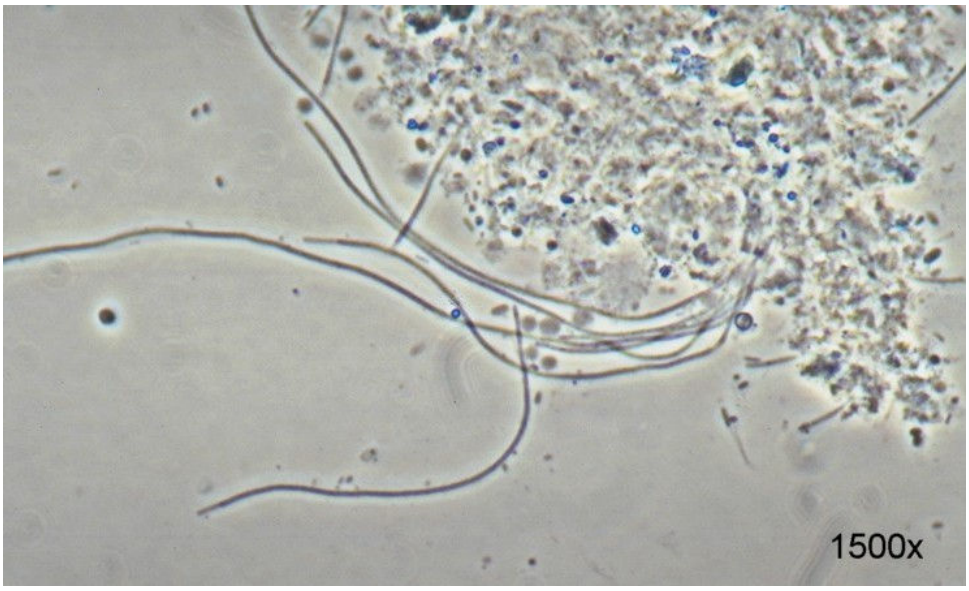
"*Candidatus* Microthrix calida"

Resembles: "*Candidatus* Microthrix parvicella" (see remarks) and Type IF-59 (Gram negative)
Probes: MPAall-1410 and MPA-T1-1260 [6]; see remarks
Frequency occurrence (200 samples; 175 WTPs):

- observed with a FI ≥ 1 in 4 samples
- observed with a FI ≥ 3 in 0 samples



Characteristics

- bent/curled filaments, often tangled;
- free in the liquid phase as well as inside or around the flocs. Frequently, however, mainly present inside the flocs;
- filament length variable;
- filaments not branched;
- not motile;
- cell diameter ca. 0.3 µm;
- no sheath;
- rarely significant attached growth;
- septa not visible;
- no sulphur storage, but occasionally small poly-P-granules inside the cells;
- Gram positive;
- Neisser negative or somewhat positive (very small poly-P-granules).

Remarks

Two *Microthrix parvicella* resembling morphotypes can be distinguished by conventional microscopy in sludges from industrial wastewater treatment plants [1, 6]. Both are characterised by curled and tangled, obvious Gram positive filaments, but they differ from each other in cell diameter : 0.5 – 0.6 µm (= "*Candidatus* Microthrix parvicella") and ca. 0.3 µm (= "*Candidatus* Microthrix calida") respectively. Both *Microthrix* morphotypes have been obtained in pure culture. 16S rRNA gene sequence analysis revealed maximal 96.7 % sequence similarity between both species [6]. "*Candidatus* Microthrix parvicella" and "Candidatus *Microthrix calida*" are members of the phylum *Actinobacteria*, class *Actinomycetes.*

Initially, only one *M. parvicella* probe (MPA-645) was available [3]. It turned out that *M. parvicella* resembling filaments present in industrial sludges frequently did not hybridise with MPA-645. Tiny,

M. parvicella resembling filaments were nearly completely missed by applying FISH and even more robust *M. parvicella* filaments occasionally did not hybridise with MPA-645 either. To solve this problem, two new *Microthrix* probes have been developed during Dynafilm: MPAall-1410, a more general *Microthrix* probe and MPA-T1-1260, specific for "*Candidatus* Microthrix calida".

Nearly all *M. parvicella* resembling filaments in the industrial sludges tested gave a fluorescent signal with probe MPAall-1410. Therefore, it is more or less a universal *Microthrix* probe. By applying various combinations of the three probes now available, different *Microthrix* strains can be distinguished in the industrial sludges:

1. Positive FISH result with MPA-645 as well as with MPAall-1410, but not with MPA-T1-1260: the classical "*Candidatus* Microthrix parvicella".
2. Positive FISH results with MPA-T1-1260 and MPAall-1410, but not with MPA-645: the tiny Microthrix = "*Candidatus* Microthrix calida".
3. Positive FISH results with MPAall-1410, but not with MPA-645 and MPA-T1-1260: filaments morphologically very similar to "*Cand.* M. parvicella", but completely Neisser negative and with slightly thicker filaments.
4. Positive FISH results only with MPA-645. Morphologically this filament resembles the tiny "*Candidatus* Microthrix calida".

Physiology

Pure culture studies have provided very little useful information so far. "*Candidatus* Microthrix calida" requires a relatively high pH (> 7.8) for its growth and grows at temperatures up to 36.5 ºC. From MAR experiments it has been concluded that "*Candidatus* M. calida", just like "*Candidatus* M. parvicella", can use long chain fatty acids as a carbon source. However, this MAR study was carried out with an axenic culture which means that data of *in situ* measurements are not yet available.

Occurrence in activated sludge

Microthrix resembling filaments were observed in about 25 Dynafilm samples:

- Small "*Candididatus* M. calida" populations (FI ≤ 2) were present in four WTPs treating effluent from a fish industry, calf manure (2x) and chemical wastewater, respectively. High temperatures (30-38 ºC) are very common in at least three of these plants. This might explain the occurrence of "*Cand.* M. calida" in these plants. The occurrence of tangled "*Cand.* M. calida" filaments inside the flocs suggests that lysis products of other bacteria might support growth of this bacterium.
- Except for two WTPs treating calf manure, the classical *"*Cand. *M. parvicella"* was only observed in considerable amounts in WTPs treating a mixture of domestic and industrial wastewater.
- The "*Cand.* M. parvicella" resembling filaments which only hybridised with probe Mpaall-1410 were mainly observed in WTPs treating chemical wastewater and, occasionally, if effluents from food or textile industries were received. This unknown species was observed in 6 samples, two times with a FI ≥ 3.

Control options

The common possibilities aimed at solving a bulking problem are listed below (1-7). Full scale experience with controlling this filamentous morphotype is not available. However, considering the hydrophobic cell surface properties of "*Candidatus* M. calida", it seems likely that this bacterium, just like "*Candidatus* M. parvicella", can be effectively controlled by dosing Al^{3+}.
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, 4, 5 and 7.

1. Good "House-keeping".
2. Remove deficiencies: O_2 > 2 mg/l and BOD:N:P =100:5:1.
3. Two step configuration (aerobic/aerobic or anaerobic/aerobic), in order to remove largely 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

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2. Eikelboom, D. H. (2000) *Process control of activated sludge plants by microscopic investigation*. IWA Publishing, London, UK.
3. Erhart, R., D. Bradford, R. J. Seviour, R. Amann and L. L. Blackall (1997) Development and use of fluorescent *in situ* hybridisation probes for the detection and identification of *Microthrix parvicella* in activated sludge. *System. Appl. Microbiol*. **20**, 310-318.
4. 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.
5. Lemmer, H und G. Lind (2000) *Blähschlamm, Schaum und Schwimmschlamm – Mikrobiologie und Gegenmassnahmen*. F. Hirthammer Verlag, München, Germany.
6. Levantesi, C., S. Rossetti, K. Thelen, C. Kragelund, J. Krooneman, D. Eikelboom, P. H. Nielsen and V. Tandoi (2006) Phylogeny, physiology and distribution of "*Candidatus* Microthrix calida", a new *Microthrix* species isolated from industrial activated WWTPs. Accepted for publication in Environmental Microbiology
7. Tandoi, V., D. Jenkins and J. Wanner (2005) *Activated sludge separation problems – Theory, Control Measures, Practical Experiences*. IWA Publishing, London, UK.

Slide show images

- 1-3: thin, curled filaments. Image 1: plus Type IF-49
- 4: frequently tangled inside the flocs
- 5-6: Gram stained
- 7: FISH image with probe MPA-T1-1260