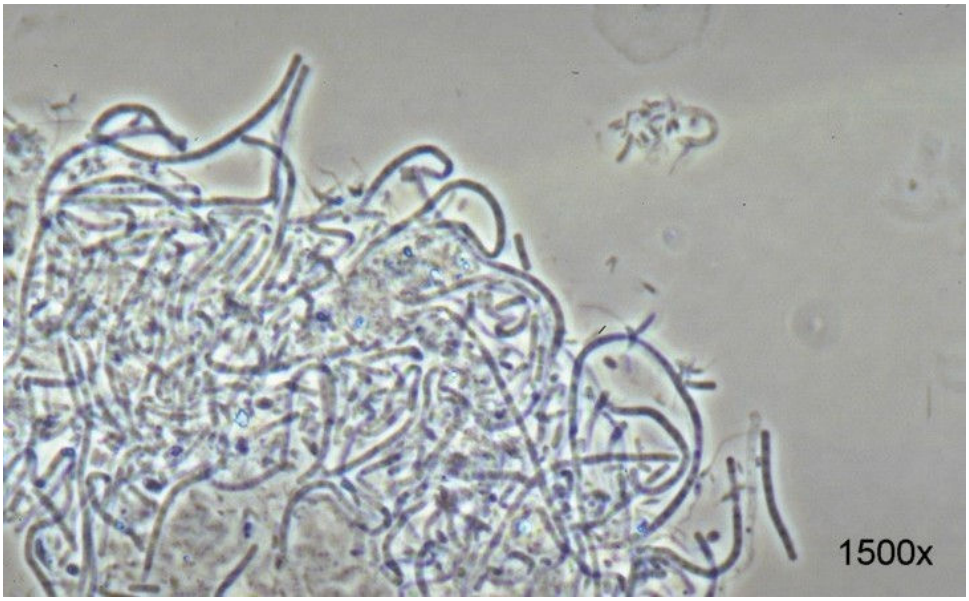
"Candidatus Microthrix parvicella"

Resembles: "*Cand.* M. calida" (see remarks below) and *N. limicola* I (filaments stain completely grey-violet with Neisser)
Probes: not targeted by the phylum specific probe HGC69a; species specific probes: MPA-645 [6] and MPAall-1410 [9]
Frequency occurrence: (200 samples; 175 WTPs):

- observed with a FI ≥ 1 in 14 samples
- observed with a FI ≥ 3 in 3 samples



Characteristics

- bent/curled filaments, often tangled;
- free in the liquid phase as well as inside or around the flocs;
- filament length variable;
- filaments not branched;
- not motile;
- cell diameter 0.5 – 0.6 µm;
- no sheath;
- rarely significant attached growth;
- septa not clearly visible;
- no sulphur storage, but often poly-P-granules inside the cells;
- Gram positive;
- often Neisser positive (poly-P-granules), but as these granules are not always present, filaments might stain Neisser negative.

Remarks

Two *Microthrix parvicella* resembling morphotypes can be distinguished by conventional microscopy in sludges from industrial wastewater treatment plants [4, 9]. 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 [9].
"*Candidatus* Microthrix parvicella" and "Candidatus *Microthrix calida*" are members of the phylum *Actinobacteria*, class *Actinomycetes* [2, 9].

Initially, only one *M. parvicella* probe (MPA-645) was available [6]. It turned out that *M. parvicella* resembling filaments present in industrial sludges frequently did not hybridise with MPA-645. Populations composed of 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

Due to its rather peculiar nutritional requirements, "*Candidatus* M. parvicella" is difficult to cultivate in pure culture [3, 4, 12, 15]. It can only use long chain fatty acids (LCFAs) such as palmitic acid or oleic acid as a carbon source and for its energy supply [1, 11, 13]. In addition to this, pure culture studies have showed that this bacterium requires reduced nitrogen and sulphur compounds for its growth [13].

Its hydrophobic cell surface is a great advantage in the competition with other 'sludge bacteria' for the poor water soluble LCFAs entering the treatment plant. The LCFAs preferentially enrich themselves along hydrophobic surfaces. "*Cand.* M. parvicella" has a high storage capacity for LCFAs, excrete several exo-enzymes for LCFA degradation and is capable of taking up and storing this substrate in aerobic as well as in anoxic and anaerobic zones in a treatment plant [11]. All stored compounds are further metabolised in the aerobic and anoxic zones, where reduced sulphur and nitrogen compounds should be present to allow cell synthesis.

"*Cand.* M. parvicella" appears to be microaerophilic, which means that low oxygen concentrations (< 1.0 mg/l) favour its growth.

Pure culture studies [13] revealed an optimal growth rate at 22 ºC, very little growth in the temperature range of 25-30 ºC, but still a considerable growth at 7 ºC.

Finally, "*Cand.* M. parvicella" needs only small amounts of substrate for maintenance, viz. to keep the cells alive during periods without influent supply.

This combination of the physiological features favours "*Cand.* M. parvicella" in its competition with the other 'sludge bacteria' for the available substrate.

However, "*Cand.* M. parvicella" grows relatively slowly [13] which imply that this bacterium cannot maintain itself in WTPs where a short sludge age is applied.

Occurrence in activated sludge

The classical "*Cand.* M. parvicella" commonly occurs in low loaded domestic treatment plants, in particular when nutrient removal conditions are applied. It will mainly occur in industrial plants if a mixed influent (domestic + industrial) is treated or when an industrial effluent rich in LCFAs (e.g. wastewater from slaughterhouses or fish industries) is discharged to the WTP. "*Cand.* M. parvicella" is the most important cause of bulking sludge in domestic plants in many countries and is also frequently responsible for foaming and scum formation. Transport of surplus sludge with a large "*Cand.* M. parvicella" population to the sludge digestion tank can also cause scum to arise in this tank. The

population size of "*Cand.* M. parvicella" shows a distinct seasonal pattern which has not yet been explained: the population size is at its maximum at the end of the winter and at its minimum in late summer/autumn.

The following process conditions favour "*Cand.* M. parvicella" [5]:

- sludge age > about 10 days;
- alternating aerobic, anoxic and anaerobic conditions, viz. nutrient removal conditions;
- waste water containing a substantial amount of LCFAs, such as oleic acid. The size of the LCFAs fraction in common domestic wastewater frequently amounts to 20-30 % of the COD;
- conditions in which the fats/lipids present in the influent are hydrolysed before they reach the aeration tank. This releases the LCFAs, which improves their availability to "*Cand.* M. parvicella". Consequently, a long hydraulic retention time in the sewer, the primary sedimentation tank or in the anaerobic zone (Bio-P process) is favourable to"*Cand.* M. parvicella";
- an oxygen concentration < 1.0 mg O_2/l in the aerobic zone of the treatment plant;
- a large (> 40% of the total volume) anoxic zone in the aeration tank;
- water temperature below ca. 15°C. "*Cand.* M. parvicella" grows principally in the late autumn and winter;
- incomplete nitrification in the aerobic zone of the WTP;
- supply of reduced sulphur and nitrogen compounds with recycled water from the sludge dewatering unit.

This combination means that the process conditions in nutrient removal plants extremely favour "*Cand.* M. parvicella".

Microthrix resembling filaments were observed in about 25 Dynafilm samples:

- Small "*Cand.* M. calida" populations (FI ≤ 2) were present in four WTPs treating effluent from 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

1. Dosing aluminium salts (2 - 3.5 g Al/kg MLSS. day) in the recycled activated sludge flow for at least three weeks [5]. Positively charged polyaluminium (Al^{3+}) is recommended for *Microthrix* control. Up until now, this has been the only method that has an almost "guaranteed" effect and which hardly ever negatively influences the treatment results. Any scum/foam will usually disappear within a couple of days. It will take about two weeks before the sludge settling properties start to improve. However, a reduction in the average floc size must be taken into account.
2. Strong reduction of the retention time in anoxic zones in the plant. This action is often not feasible in nutrient removal plants.
3. This is also valid for a major reduction of the sludge age (loss of nitrification).
4. Aiming at an almost complete nitrification in the aerobic zone of the treatment plant.
5. A mixing phase (just for a few minutes) of raw waste water and recycled sludge - before they reach the aeration tank - followed by alternating anoxic and aerobic process conditions (Bio-Denitro). This method is effective for largely controlling "*Cand.* M. parvicella", but stimulates the development of

Type 0041, which does not grow quite so massively and does not seriously contribute to the occurrence of scum , however.
6. Experiences with various types of selectors for controlling "*Cand.* M. parvicella" have not been consistent up to the present. A non-aerated selector has been introduced between the anaerobic zone (Bio-P) and the anoxic tank in several treatment plants in The Netherlands. Experiences with this configuration (BCFS process) have been positive [10].
7. Removal of hydrophobic compounds from the influent [14].

References for further reading about process control: 5, 7, 8 and 16.

References

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Slide show images

- 1-4: morphology at a low magnification
- 5-11: morphology at a high magnification.
    - diameter somewhat variable
    - some attached growth might be present
    - image 7: plus Type IF-70
    - images 10 and 11: filaments which only hybridise with probe MPAall-1410 (= nr 3)
- 12-13: Gram stained
- 14-15: Neisser stained
- 16: FISH image with probe MPA-VIT (=MPAall-1410 + MPA-645)