

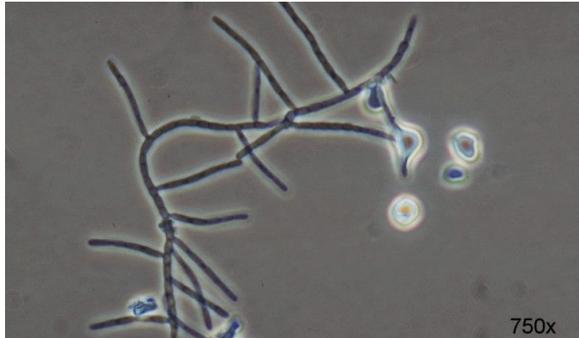
## Nocardioforms

*Resembles:* -

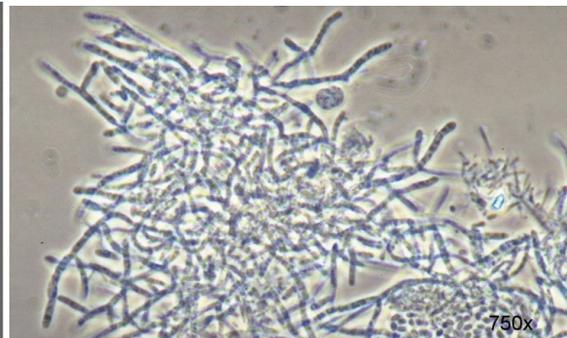
*Probes:* phylum specific probe: HGC69a [6] and species specific probe: GOR0596 [1], see remarks

*Frequency occurrence* (200 samples; 175 plants):

- observed with a FI  $\geq$  1 in 24 samples
- observed with a FI  $\geq$  3 in 5 samples



*Skermania piniformis*



*Gordonia sp.*

### Characteristics

- short, truly branched filaments, frequently occurring in small 'colonies' of tangled filaments;
- inside the flocs as well as free in the liquid between the flocs;
- filament length variable but usually  $< 200 \mu\text{m}$ ;
- cell diameter  $0.6 - 2.0 \mu\text{m}$ , depending on the morphotype present;
- septa visible in more robust species;
- more or less square cells;
- no sulphur storage, but other stored granules might be present;
- usually Gram positive, but Gram variable morphotypes have been observed as well;
- only Neisser positive if poly-P-granules are present;

### Remarks

Nocardioforms, or mycolata or mycolaic acid containing *Actinomycetes*, belong, just like "*Candidatus Microthrix parvicella*" and "*Candidatus Microthrix calida*" to the phylum *Actinobacteria* and class *Actinomycetes*. The *Actinobacteria* were formerly known as high mol % G+C Gram positive bacteria. Unfortunately, the phylum specific probe HGC69a does not target all members of the *Actinobacteria*. Positive signals with this probe and the species specific probe GOR0596 were obtained in 53 % and 20 % respectively of the samples where nocardioforms were observed with a FI  $\geq$  1 by conventional microscopy. Other species specific nocardioforms probes were not applied during the Dynafilm project.

The group of the nocardioforms includes many different species [4]. Five 'morphotypes' were observed during the Macobs and Dynafilm projects. Except for *Skermania piniformis*, a reliable morphology based identification and classification is not possible, however. Probes are now available for a limited number of species. GOR0596, a probe by which *Gordona amarae* among other *Gordonia sp.* can be identified, was used during the Dynafilm project.

### Physiology

The reader is referred to the literature [e.g. 5 or 7] for detailed information about this subject. Generally speaking, it can be stated that nocardioforms can use a broad spectrum of substrates, including complex compounds, for their growth. Hydrophobic substrates ( lipids, olive oil, etc.) favour their growth, particularly. Most strains isolated from activated sludge require molecular oxygen for their growth.

### ***Occurrence in activated sludge***

Nocardioforms are notorious for their role in foaming followed by scum formation in activated sludge plants, especially in WTPs where the water temperature is higher than about 15 °C. Due to their selective flotation they enrich themselves at the water surface. Consequently, they are not removed with the surplus sludge, which implies that the population size is not controlled by the sludge age applied. Transport of scum into the sludge digestion tank will also cause a scum layer to occur in this tank. The negative effect on the settling velocity of the flocs is usually small. The following process conditions are favourable for the growth of nocardioforms:

- fats or other hydrophobic components in the influent;
- surface active materials in the influent;
- internal recycling of floating material;
- water temperature higher than approximately 15°C;
- ???

The question marks indicate that the available knowledge is still incomplete. Gram positive bacteria often have a hydrophobic cell surface through which fats bind well to their surfaces and thereby can be absorbed from the water. Fats and surface active compounds are always present in domestic waste water and also in many industrial effluents. In spite of this, nocardioforms are not always present, even at higher water temperatures. This can be partially explained by the sludge load applied:

Nocardioforms are usually found at somewhat higher sludge loading levels ( 0.1 - 0.7 kg BOD/kg MLSS.day). At lower sludge loadings, other Gram positive filamentous bacteria such as "*Candidatus M. parvicella*" are present, which also use the hydrophobic compounds entering the WTP. Floc forming bacteria that can use this substrate also exist. It is not yet known which factors are decisive in the competition for this substrate.

During Macobs and Dynafilm, large populations were observed in WTPs treating wastewater from chemical, fish, rendering and food industries. Thus, it is not possible to correlate the occurrence of nocardioforms with a specific wastewater.

### ***Control options***

1. Good 'house-keeping'.
2. Systematic skimming , followed by removal/destruction of scum. This is by far the most effective option. Scum should never be internally recycled (for example to the primary sedimentation tank). In case of very persistent and massive foam and scum, a separate flotation unit can also be considered for 'separating' the nocardioforms from the sludge.
3. A drastic reduction of the sludge age is sometimes effective, but is not possible if extensive nitrification is required.
4. Dosing compounds which can change the cell surface from hydrophobic to hydrophilic (Al-salts, certain clay types) might be effective.
5. Reduction of the concentration of fats and so on, in the influent of the WTP.
6. Controlling symptoms, viz. applying physical or chemical methods aimed at a destruction of the filaments or at improving the settling velocity of the flocs by increasing their weight.

References for further reading about process control: 2, 3, 5 and 8.

### ***References***

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6. Roller, C., M. Wagner, R. Amann, W. Ludwig and K. H. Schleifer (1994) *In situ* probing of Gram positive bacteria with high DNA G+C content using 23S rRNA-targeted oligonucleotides. *Microbiology* **140**, 2849-2858.
7. Seviour, R. J. and L. L. Blackall (1999) *The microbiology of activated sludge*. Kluwer Publishers, Dordrecht, The Netherlands.
8. Tandoi, V., D. Jenkins and J. Wanner (2005) *Activated sludge separation problems – Theory, Control Measures, Practical Experiences*. IWA Publishing, London, UK.

### ***Slide show images***

Including one morphotype observed during a previous project, micrographs of six morphotypes are included in the slide show:

- 1-9: Nocardioforms-1: diameter ca. 0.7  $\mu\text{m}$  and hardly visible septa; probably *Gordona amarae*
  - 1-6: morphology
  - 7-8: Gram stained
  - 9: FISH image; probe GOR-0596
- 10-18: *Skermania piniformis*. Characteristic V-shaped branching; diameter ca. 0.9  $\mu\text{m}$ 
  - 10-11: low magnification
  - 12-17: morphology at a high magnification
  - 18: Gram stained
- 19-23: Nocardioforms-3: short branches, almost perpendicular to the main branch; diameter ca. 0.7  $\mu\text{m}$  and septa hardly visible
  - 19-21: morphology at a high magnification
  - 22: Gram stained
  - 23: Neisser stained  $\rightarrow$  many poly-P granules
- 24-32: Nocardioforms -4: less frequently branched, Gram variable and a diameter of about 1.2  $\mu\text{m}$ 
  - 24-29: morphology at a high magnification
  - 30: Gram stained  $\rightarrow$  Gram variable
  - 31: Neisser stained  $\rightarrow$  poly-P granules
  - 32: FISH image; HGC probe
- 33-37: Nocardioforms (?) -5: Robust, less frequently branched filaments with a diameter of about 2.0  $\mu\text{m}$ . Gram variable and septa clearly visible. The features of this morphotype are rather uncommon for nocardioforms. A tiny fungal species is the alternative option.
  - 33-34: morphology at a low magnification
  - 35-36: morphology at a high magnification
  - 37: Gram stained
- 38-39: Nocardioforms -6: Cells largely filled with granules; diameter ca. 1.5  $\mu\text{m}$ 
  - 38: branched filaments inside a sludge floc
  - 39: FISH image; probe HGC
- 40-41: Miscellaneous
  - 40: filaments almost covered by attached growth
  - oil droplets adhering to a filament