Nostocoida limicola III

Resembles:

- *N.limicola* I: diameter filaments < 1.0 µm and does not hybridise with probe NLIMII-175
- Several *Alphaproteobacteria*: probe ALF-968 positive and usually Neisser negative

Probes: phylum specific: HGC-69a [13]; species specific: NLIMII-175 [10, 15]; see remarks below
Frequency occurrence (200 samples; 175 WTPs):

- observed with a FI ≥ 1 in 9 samples
- observed with a FI ≥ 3 in 1 sample

Characteristics

- bent/coiled, frequently tangled filaments;
- frequently mainly present inside the sludge flocs;
- not motile;
- not branched;
- filament length > 200 µm;
- cell diameter 1.2 – 1.5 µm;
- sheath absent;
- attached growth absent;
- septa usually hardly visible;
- discoid or more disc shaped cells;
- no sulphur storage;
- Gram positive;
- Neisser positive: filaments stain entirely grey-violet.

Remarks

The *Nostocoida limicola* story is a little bit complicated and confusing. Three, Gram and Neisser positive, *N. limicola* morphotypes can be distinguished through applying phase-contrast microscopy [4]:

- *N. limicola* I: cell septa hardly visible; diameter 0.6 – 0.8 µm
- *N. limicola* II: cell septa visible; spherical to disc shaped cells; diameter: 1.0 – 1.2 µm
- *N. limicola* III: cell septa visible; discoid to disc shaped cells; diameter: 1.2 – 1.5 µm. Note: The larger diameter range mentioned in reference 4 was based on outdated evidence and is therefore not valid any longer.

As *N. limicola* II is not very often observed and, moreover, closely resembles *N. limicola* III, it has been proposed to use the latter name for both morphotypes [5]. However, the name *N. limicola* II is still mentioned in published papers.

Filamentous morphotypes resembling *N. limicola* filaments are frequently observed in WTPs treating industrial wastewater. It is now known that many of these morphotypes belong to the *Alphaproteobacteria.* Group specific (ALF-968) and several species specific probes are available and are needed to distinguish them from the *N. limicola* morphotypes.

Several papers have been published in recent years in which it is stated by the authors that pure cultures of *N. limicola* resembling filaments were obtained [2, 8, 9, 14]. Subsequently, probes were developed based upon these pure cultures [10, 14, 15].

1. It has been assumed that probe NLIMI-91 should hybridise with *N. limicola* I [10, 15]. A fluorescent signal with this probe was obtained in 12 out of 126 Dynafilm samples. The size of the population giving a signal was usually small (1 on a scale ranging from 0 to 4). The probe positive filaments were, one sample excluded, very short (length < 50 µm) and composed of almost spherical cells with a diameter of ca. 1.3 µm. Thus, the appearance of the probe NLIMI-91 positive, short filaments present in the Dynafilm samples was very similar to that in the original paper [10]. Considering that morphotype *N. limicola* I looks completely different (long curled and tangled filaments with a diameter of circa 0.8 µm), it is concluded that probe NLIMI-91 does not hybridise with *N. limicola* I. Filaments with the appropriate *N. limicola* I morphology, were observed in just one Dynafilm sample. These filaments did not hybridise with probe NLIMI-91. Hence, it is concluded that identification of morphotype *N. limicola* I through FISH is still not possible. A German research group reached a similar conclusion [11]. Probe NLIMI-91 can only be used for the identification of *Trichococcus* species, filamentous micro-organisms which have only a minor role in bulking of activated sludge.
2. Probe NLIMII-175 was developed starting with sequences of pure cultures, described in reference 2 as "*Candidatus* N. limicola", which have been classified as members of the *Actinobacteria*. The pure cultures stained Gram and Neisser positive. This probe should allow the *in situ* identification of *N. limicola* II [10, 15]. Filaments hybridising with this probe were present in 9 Dynafilm samples and they were identified by phase-contrast microscopy as *N. limicola* III filaments. Thus, probe NLIMII-175 cannot be used for the *in situ* identification of morphotype *N. limicola* II, but targets *N. limicola* III.
3. Probe NLIMIII-301 should allow the identification of *N. limicola* III through FISH [9, 10, 15]. A fluorescent signal with this probe was obtained in 13 samples. The filaments hybridising with probe NLIMIII-301 were rather short and composed of spherical cells with a diameter ranging from 1.5 µm to 1.8 µm. These filaments did not at all resemble the characteristic long, tangled *N. limicola* III filaments, composed of disc shaped cells, but instead those of a bacterium known as an *Isosphaera* sp. This conclusion is in agreement with the evidence presented in reference 11.
4. Ten *Nostocoida limicola*-like pure cultures, with an almost identical 16S rRNA sequence were isolated from domestic WTPs in Germany [14]. They were classified as members of the *Chloroflexi* (green non-sulphur bacteria). The pure cultures stained Gram positive and Neisser negative. Based upon their sequence, probe AHW-183 was developed and applied for the *in situ* identification of filaments in activated sludge. Fluorescent signals were only obtained with domestic sludges. The filaments hybridising with AHW-183 indeed closely resemble those of morphotype *N. limicola*. They were mainly observed inside sludge flocs. Considering the Neisser negative staining results, it seems likely that this probe does not hybridise with *N. limicola* I or III. Unfortunately, probe AHW-183 was not applied during the Dynafilm research program.

In conclusion, when the *Alphaproteobacteria* are excluded, four *N. limicola* morphotypes can be observed in activated sludge:

- *N. limicola* I: long, coiled and tangled filaments with a diameter of ca. 0.8 µm. The cell septa are hardly visible. Gram and Neisser positive. The phylogenetic position is not known and a probe is not yet available.
- *N. limicola* II: compared with *N. limicola* III, more spherical shaped cells and a somewhat smaller diameter. A probe is not yet available.
- *N. limicola* III: long, coiled and tangled filaments composed of discoid cells with a diameter of ca. 1.3 µm. Gram and Neisser positive. *N. limicola* III is a member of the *Actinobacteria* and can be identified through applying probe NLIMII-175.
- *N. limicola* IV: long, coiled and tangled filaments composed of discoid cells with a diameter of ca. 1.0 µm. Gram positive and Neisser negative. *N. limicola* IV is a member of the *Chloroflexi* and can be identified through applying probe AHW-183.

The morphotypes *N. limicola* II and IV are not presented in separate lemmas on this CD-ROM.

Physiology

The pure cultures described in reference 2 ("*Candidatus* N. limicola", targeted by probe NLIMII-175) could use a broad range of carbon sources for their growth: several short chain fatty acids and sugars, Tween 80, peptone and glycerol. Citrate, succinate, oleic acid, oleate and propanol were not taken up. Growth at anoxic or anaerobic conditions was not observed.
Although probes were not applied by Nowak and Brown [12], their description of the filamentous morphotype investigated in pure culture completely fits the characteristics of *N. limicola* III. Good growth was obtained in complex media with various sugars as a carbon source. Short chain fatty acids hardly stimulated their growth. Some growth was observed if anaerobic conditions were applied. More or less similar results were obtained in MAR studies with activated sludge [1]. However, the identity of the *N. limicola* filaments present was unfortunately not confirmed by applying FISH.
The pure cultures described in reference 14 (probe AHW-183 positive) were isolated on sludge hydrolysate agar [3], a poorly defined complex medium. None of the substrates tested supported their growth in pure culture.

Occurrence in activated sludge

Morphotype *N. limicola* III was observed in nine WTPs during the Macobs and Dynafilm research program. Larger populations were mainly present in plants receiving a significant percentage of domestic wastewater as well. Thus, it seems that morphotype *N. limicola* III, just like morphotype *N. limicola* I, needs components present in domestic wastewater for its growth. It cannot be excluded, however, that the combination of domestic and industrial wastewater favours its growth.

Large populations of *N. limicola* III are mainly observed in domestic plants where a load of about 0.1 kg BOD/kg MLSS.day is applied. In general, the population shows a seasonal rhythm, with its maximum during winter.
Due to the tangled growth of *N. limicola* III, this filamentous species does not affect the settling velocity of the flocs very much.

Most papers dealing with *N. limicola* in industrial plants were published when probes were not available to confirm the identity of the filaments present. Considering (1) that *N. limicola* III was hardly ever observed in WTPs treating 100 % industrial wastewater during the Macobs and Dynafilm program and (2) that *N. limicola* III resembling *Alphaproteobacteria* were observed in many industrial WTPs, it seems very likely that the name *N. limicola* should be replaced by *Alphaproteobacteria* in many published papers.

Control options

The common possibilities aimed at solving a bulking problem are listed below (1-7). As a consequence of the confusion discussed above, the value of most reports dealing with control of *N. limicola* is very limited. Aerobic selectors have been reported to be effective. Nutrient removal conditions, viz. an anoxic selector successfully controlled *N. limicola* III [12].
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: 5, 6, 7 and 16.

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 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 a destroying the filaments or at improving the settling velocity of the flocs by increasing their weight.

References

1. Andreasen, K. and P. H. Nielsen (1997) Application of microautoradiography to the study of substrate uptake by filamentous microorganisms in activated sludge. *Appl. Environ. Microbiol*. **63**, 3662-3668.
2. Blackall, L. L., E. M. Seviour, D. Bradford, S. Rosetti, V. Tandoi and R. J. Seviour (2000) *Candidatus* Nostocoida limicola, a filamentous bacterium from activated sludge. *Int. J. Syst. Evol. Microbiol*. **50**, 703-709.
3. Eikelboom, D. H. (1975) Filamentous organisms observed in activated sludge. *Water Research* **9**, 365-388.
4. Eikelboom, D. H. and H. J. J. van Buijsen (1981) Microscopic sludge investigation manual. IMG-TNO, Report A94a, Delft, The Netherlands.
5. Eikelboom, D. H. (2000) *Process control of activated sludge plants by microscopic investigation*. IWA Publishing, London, UK.
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. Lemmer, H und G. Lind (2000) *Blähschlamm, Schaum und Schwimmschlamm – Mikrobiologie und Gegenmassnahmen.* F. Hirthammer Verlag, München, Germany.
8. Liu, J. R., P. Burrell, E. M. Seviour, J. A. Soddell, L. L. Blackall and R. J. Seviour (2000) The filamentous bacterial morphotype "*Nostocoida limicola* I " contains at least two previously described genera in the low G + C Gram positive bacteria. *Syst. Appl. Microbiol*. **23**, 528-534.
9. Liu, J. R., C. A. McKenzie, E. M. Seviour, R. I. Webb, L. L. Blackall, C. P. Saint and R. J. Seviour (2001) Phylogeny of the filamentous bacterium "*Nostocoida limicola* III " from activated sludge. *Int. J. Syst. Evol. Microbiol.* **51**, 195-202.
10. Liu, J. R. and R. J. Seviour (2001) Design and application of oligonucleotide probes for fluorescent in situ identification of the filamentous bacterial morphotype *Nostocoida limicola* in activated sludge. *Environ. Microbiol*. **3** (9), 551-560.
11. Müller, E., M. Schade and H. Lemmer (2005) Filaments in scum: detection and identification quality of classical microscopic sludge analysis vs. fluorescence in situ hybridisation. In: Proc. 4th Activated Sludge Population Dynamics Conference, Brisbane, Australia and submitted for publication.
12. Nowak, G. and G. D. Brown (1990) Characteristics of Nostocoida limicola and its activity in activated sludge suspension. *Res. J. Wat. Poll. Contr. Fed*. **62**, 137-142.
13. 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
14. Schade, M., C. Beimfohr and H. Lemmer (2002) Phylogenetic and physiological characterization of a "*Nostocoida limicola*"- like organism isolated from activated sludge. *Wat. Sci. Technol.* **46** (1-2), 91-97.
15. Seviour, R. J., R. J. Lui, E. M. Seviour, C. A. McKenzie, L. L. Blackall and C. P. Saint (2002) The *"Nostocoida limicola"* story: resolving the phylogeny of this morphotype responsible for bulking in activated sludge. *Water Sci. Technol*. **46** (1-2), 105-110.
16. 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: coiled/tangled filaments
- 2-7: morphology at a high magnification; usually discoid or disc shaped cells
- 8: rarely 'parallel' growth
- 9: Gram stained (plus "*Candidatus* M. parvicella")
- 10: Neisser stained

- 11-12: characteristic FISH images with probe NLIMII-175.