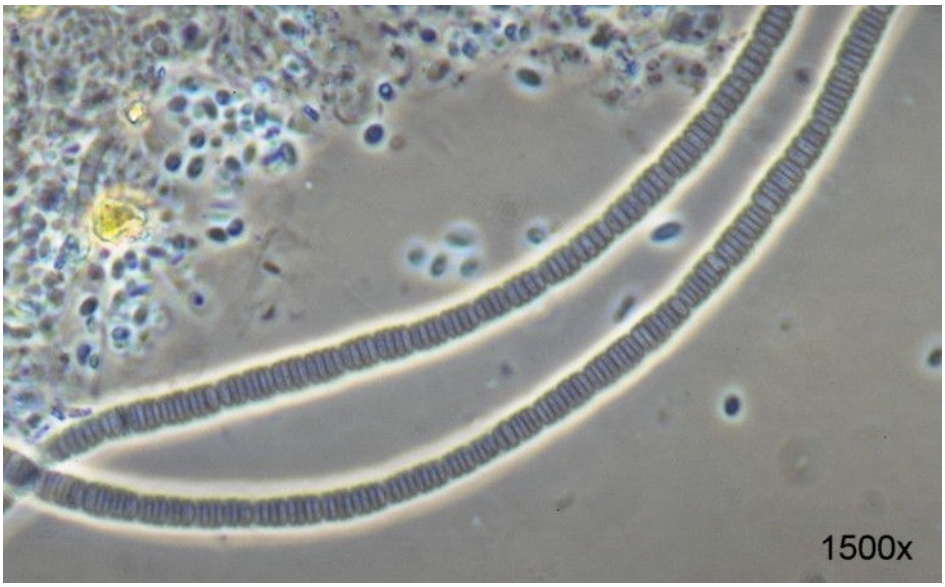
"*Candidatus* Alysiomicrobium bavaricum"

Resembles: several other *Alphaproteobacteria* and *N. limicola* I and III, see remarks
Probes: class specific: ALF-968 [7]; species specific: PPx3-1428 [8]
Frequency occurrence (200 samples; 175 WTPs):

- observed with a FI ≥ 1 in 24 samples
- observed with a FI ≥ 3 in 4 samples



Morphotype C

Characteristics
Filaments which give a clear fluorescent signal with probe PPx3-1428 do have in common that they are not branched and not motile, they do not have a sheath and only rarely attached growth, they do not store sulphur and the cell septa and constrictions are clearly visible. However, if the size and the shape of the cells are considered, four different morphotypes can be distinguished:

A. Bent or curled filaments comprised of almost spherical or somewhat discoid cells with a diameter varying from 0.9 µm to 1.3 µm. Gram positive or Gram variable. Usually Neisser positive, due to staining of the cell septa.
B. Bent or curled filaments comprised of square or rectangular cells with a diameter 1.3 µm. Gram positive. Neisser positive, due to staining of the cell septa.
C. Very robust, bent or curled filaments comprised of discus shaped or spherical cells with a diameter varying from 1.8 µm to 3.0 µm. Gram negative or Gram variable. Usually Neisser positive, due to staining of the cell septa.
D. Robust, bent or curled filaments. Discoid cells and less clear constrictions of the outer cell wall. Cells frequently filled with stored compounds. Gram negative or Gram variable. Usually Neisser positive, due to staining of the cell septa.

Thus, the morphology of "*Candidatus* A. bavaricum" is extremely variable. Without conclusive FISH results with probe PPx3-1428, it is hardly believable that all these morphotypes belong to only one bacterial species. Other filamentous bacteria showing a similar morphological variation are not known. Filaments surrounded by a layer of slime with adhering bacterial cells were observed in several samples with the morphotypes C or D predominating.

Remarks
The group of filamentous *Alphaproteobacteria*, observed in activated sludge so far, includes seven classified species: "*Candidatus* Alysiomicrobium bavaricum", "*Candidatus* Alysiosphaera europaea", "*Candidatus* Catenimonas italica", "*Candidatus* Combothrix italica", "*Candidatus* Monilibacter

batavus", "*Candidatus* Sphaeronema italicum" and *Meganema perideroedes* [3, 4, 6, 8, 10, 11] and two morphotypes whose phylogenetic position is unknown: Type IF-10 and Type IF-43. Due to problems arising with the developed probe, "*Candidatus* Catenimonas italica" is not presented further on this CD.

Most filamentous *Alphaproteobacteria* resemble each other and frequently occur together. Moreover, the morphological features of these species are not constant but depend upon the actual growth conditions. Consequently, a reliable identification of these species requires the application of FISH and probes are now available for all but one classified species [6, 8, 10]. Due to their completely different morphology, it is easy to distinguish Types IF-10 and 43 (species specific probes not available) from each other.

Filamentous *Alphaproteobacteria* also resemble the morphotypes *Nostocoida limicola* I and III. However, the latter two morphotypes do not hybridise with probe ALF-968.

Physiology

The filamentous *Alphaproteobacteria* include two physiological groups, as is shown in table 1 [4]. Group A species mainly use short chain fatty acids for their growth, whereas, in addition, also sugars, amino acids and alcohol are taken up by the members of Group B. They all prefer aerobic conditions, but in MAR experiments some activity was observed with nitrate or even nitrite as electron-acceptor. Moreover, a remarkable ability to store PHA (polyhydroxyalkanoates; common storage products in many bacteria) from different substrates and under aerobic as well as anoxic conditions has been ascertained.

Table 1

Name	***Carbon sources used***		***PHA storage***
	fatty acids	sugars, amino acids, ethanol	
"*Cand.* A. bavaricum"	+	+	++
"*Cand.* A. europaea"	+	+	+
"*Cand.* M. perideroedes"	+	+	++
"*Cand.* M. batavus"	+	-	+
"*Cand.* S. italicum"	+	-	++

N.B.: Nothing is known about the physiology of other *Alphaproteobacteria*: *"Candidatus* Combothrix italica" and the Types IF-10 and 43

Occurrence in activated sludge

Large populations of filamentous *Alphaproteobacteria* were observed in WTPs treating a variety of industrial effluents. Thus, it is not possible to correlate these filamentous bacteria with a specific wastewater. However, *Alphaproteobacteria* were almost completely absent in plants treating waste water from dairy industries (14 WTPs).

Several, more frequently observed *Alphaproteobacteria* showed a remarkable and so far unexplained geographical distribution in the Dynafilm samples (table 2). Thus, the occurrence in Italy is almost opposite to that in Denmark

Table 2

Name or number	*The Netherlands*	*Denmark*	*Italy*
"Candidatus C. italica"	±	-	+
"Candidatus A. europaea"	+++	-	+
"Candidatus A. bavaricum"	++	++	-
"Candidatus S. italicum"	+	±	+
"Candidatus M. batavus"	+	-	±
Type IF-43	+	+	+

-: absent; ± : rare ; + : occasional ; ++ : often ; +++ : frequent

Control strategies

The common possibilities aimed at solving a bulking problem are listed below (1-7). Option 3, a highly loaded first stage, where the easily degradable influent fraction is largely removed, offers better prospects than options 4 and 5 for controlling filamentous species with a large substrate storage capacity.

It is always recommended to start with a pilot plant before control methods are applied on a full scale. References for further reading on process control: 1, 2, 5 and 9.

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

1. Eikelboom, D. H. (2000) *Process control of activated sludge plants by microscopic investigation*. IWA Publishing, London, UK.
2. 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.
3. Kragelund, C., J. L. Nielsen, T. R. Thomson and P. H. Nielsen (2005) Ecophysiology of the filamentous *Alphaproteobacterium Meganema perideroedes* in activated sludge. *FEMS Microbial Ecology* **54** (1), 111-122
4. Kragelund, C., Y. Kong, J. van der Waarde, K. Thelen, D. Eikelboom and P. H. Nielsen (2006) Ecophysiology of different filamentous *Alphaproteobacteria* species from industrial wastewater treatment plants. Submitted for publication.
5. Lemmer, H und G. Lind (2000) *Blähschlamm, Schaum und Schwimmschlamm – Mikrobiologie und Gegenmassnahmen.* F. Hirthammer Verlag, München, Germany.
6. Levantesi, C., C. Beimfohr, B. Geurkink, S. Rossetti, K. Thelen, J. Krooneman, J. Snair, J. van der Waarde and V. Tandoi (2004) Filamentous *Alphaproteobacteria* associated with bulking in industrial wastewater treatment plants. *Sys. Appl. Microbiol*. **27** (6), 728-736.
7. Neef, A. (1997) *Anwendung der in situ Einzelzell Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen*. Ph. D Thesis. Technische Iniversität München, Germany.
8. Snaidr, J., C. Beimfohr, C. Levantesi, S. Rossetti, J. van der Waarde, B. Geurkink, D. Eikelboom, M. Lemaitre and V. Tandoi (2002) Phylogenetic analysis and *in situ* identification of "*Nostocoida limicola*"-like filamentous bacteria in activated sludge from industrial wastewater treatment plants. *Water Sci. Technol*. **46** (1-2), 99-104.
9. Tandoi, V., D. Jenkins and J. Wanner (2005) *Activated sludge separation problems – Theory, Control Measures, Practical Experiences*. IWA Publishing, London, UK.
10. Thomsen, T. R., L. L. Blackall, M. A. de Muro, J. L. Nielsen and P. H. Nielsen (2006) *Meganema perideroedes* gen. nov., sp. nov., a filamentous *Alphaproteobacteria* from activated sludge. Accepted for publication in *Int. J. Syst. Evol. Microbiol*.

11. Waarde, J. van der, J. Krooneman, B. Geurkink, A. van der Werf, D. Eikelboom, C. Beimfohr, J. Snaidr, C. Levantesi and V. Tandoi (2002) Molecular monitoring of bulking sludge in industrial wastewater treatment plants. *Water Sci. Technol*. **46** (1-2), 551-558.

Slide show images

- 1-6: morphotype A
    - 1-4: morphology at a high magnification
    - 5: Neisser stained
    - 6: FISH image with probe PPx3-1428
- 7-13: morphotype B
    - 7-10: morphology at a high magnification
    - 11: Gram stained
    - 12: Neisser stained
    - 13: FISH image with probe PPx3-1428
- 14-23: morphotype C
    - 14: morphology at a low magnification
    - 15-17: morphology at a high magnification
    - 18-19: filaments surrounded by a layer of slime
    - 20: more spherical cells
    - 21: Gram stained
    - 22: Neisser stained
    - FISH image with probe PPx3-1428
- 24-34: morphotype D
    - 24-27: morphology at a high magnification
    - 28-29: surrounded by a layer of slime with adhering cells
    - 30-31: cells filled with stored compounds
    - 32: Gram stained → variable
    - 33: Neisser stained
    - 34: FISH image with probe PPx3-1428