

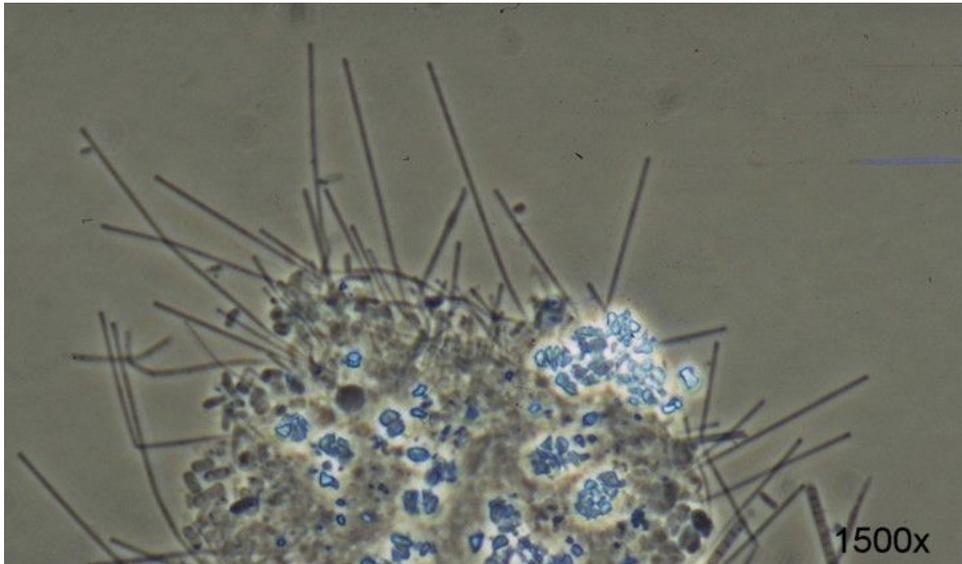
## ***Haliscomenobacter hydrossis***

*Resembles:* see remarks

*Probes:* group specific: CF319a [6]; species specific: HHY [8], Hhyall-654 [4], Hhy-T8-463 [4] and Hhy-T5-654 [4], see remarks

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

- observed with a FI  $\geq$  1 in 58 samples
- observed with a FI  $\geq$  3 in 14 samples



### ***Characteristics***

- short straight (needle-like appearance) or longer slightly bent filaments, usually protruding from the flocs;
- occasionally bundles of long filaments;
- filaments not branched;
- not motile;
- filament length variable;
- cell diameter ca. 0.4  $\mu$ m;
- the cells are surrounded by a sheath;
- attached growth occasionally present;
- septa not visible;
- no sulphur storage;
- Gram negative;
- Neisser negative.

### ***Remarks***

The following morphotypes have in common that they form thin, straight or bent filaments in which the cell septa are not clearly visible:

1. *H. hydrossis*: Gram and Neisser negative; usually protruding from the flocs.
2. Type IF-33: very short filaments, mainly inside the flocs.
3. Type IF-45: Neisser positive (small granules).
4. Type IF-46: Gram and Neisser positive (small granules).
5. Type IF-47: bent filaments free in the water phase.
6. Type IF-53: compared with *H. hydrossis* more robust filaments, occasionally tangled.
7. Type 0092: compared with *H. hydrossis* more robust filaments, which stain entirely grey-violet with Neisser.

The numbers 2-6 are not targeted by the *H. hydrossis* probes, or by the group specific probe.

*H. hydrossis* belongs to the *Cytophaga-Flexibacter*-group of the Bacteroidetes, that is targeted by the group-specific probe CF-319a [6]. Unfortunately, not all filamentous bacteria affiliated within this group hybridise with probe CF-319a. Only one species-specific probe was available at the start of Dynafilm: HHY [8], a probe based upon the nucleotide sequence of a *H. hydrossis* strain in the DSMZ collection. It turned out during Dynafilm that the FISH results obtained with this probe did not correlate very well with the conventional microscopic analysis observations; large populations of *H. hydrossis* resembling filaments were frequently completely missed by FISH analysis. Therefore, several new probes were developed during Dynafilm:

- probe Hhyall-654: A degeneracy was introduced into the published probe sequence of HHY. This new probe now targets another *H. hydrossis* isolate apart from the DSMZ type strain. The new isolate showed a 97.5 % sequence similarity with the DSMZ strain.
- probe Hhy-T8-463: This probe was developed for another new isolate, also morphologically similar to *H. hydrossis*. The 16S rDNA analysis of this bacterium showed that it is a new species, not previously described and sharing only 83.1 % 16S rRNA gene sequence similarity to the DSMZ *H. hydrossis* strain.
- probe Hhy-T5-654: This probe was based upon a RT-PCR product (filaments obtained through micromanipulation) and shares 87.8 % 16S rRNA gene sequence similarity to *H. hydrossis* (DSMZ strain).

All Dynafilm samples were screened by applying the new probes. Fluorescent signals with probe Hhy-T8-463 were only rarely obtained, which means that this *H. hydrossis* resembling strain hardly ever occurs in industrial sludges. The application of the two other probes resulted more frequently in fluorescent signals. However, in about 40 % of the samples, where a large population of *H. hydrossis* resembling filaments was observed by conventional phase contrast microscopy, none or hardly any hybridisation signals were obtained with FISH. It is therefore evident that morphotype *H. hydrossis* includes at least four species and that more probes are required to allow a reliable identification of this morphotype through FISH.

In general, the characteristic *H. hydrossis* morphology (needle-like filaments) was more clear in FISH images with probe Hhy-T5-654 than when fluorescent signals were obtained with the probes HHY and Hhyall-654. The filaments were often less straight or even curled in FISH images with the latter two probes.

### **Physiology**

The Dynafilm results clearly show that morphotype *Haliscomenobacter hydrossis* includes various species. Consequently, the information in papers published in the past, dealing with the physiology of *H. hydrossis*, but without any information about the taxonomic position of the strains investigated, is unfortunately not very useful any longer. The Dynafilm results at least explain the sometimes conflicting evidence mentioned in the literature. Pure culture studies that have been published, mainly state that *H. hydrossis* filaments appear to be highly specialized [3].

### **Occurrence in activated sludge**

Large population of probe Hhy-T5-654 positive filaments were often observed in WTPs treating wastewater from chemical industries as well as, on occasion, in WTPs receiving effluents from fish, starch and food industries.

Probe Hhyall-654 positive filaments were mainly observed in WTPs treating wastewater from tannery, rendering, chemical, dairy and agro-industries.

Filaments resembling *H. hydrossis* but not hybridising with these two probes were observed in several WTPs receiving wastewater from potato industries and, on occasion, also if wastewater from fish, chemical and starch industries was received for treatment.

The large variety of industries shows that it is not possible to correlate the various “*H. hydrossis*” strains with a specific wastewater.

### **Control options**

The common possibilities aimed at solving a bulking problem are listed below (1-7). Full scale experience of controlling this filamentous morphotype is hardly available and it is not known which *H. hydroxsis* resembling species were present in papers dealing with control of this bacterium. It is only known that aerobic selectors have occasionally been applied with success.

Large populations of probe Hhy-T5-654 positive filaments were present in two Dynafilm WTPs where an anaerobic pre-treatment of the wastewater was in operation. Thus, it seems to be that filaments hybridising with this probe cannot be controlled by the anaerobic/aerobic two step configuration. It is always recommended to start with a pilot scale experiment before a selected control method is applied on a full scale.

References for further reading about process control: 1, 2, 5 and 7.

1. Good 'House-keeping'.
2. Remove deficiencies:  $O_2 > 2 \text{ mg/l}$  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 in 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. Kämpfer, P. (1995) Physiological and chemotaxonomic characterization of filamentous bacteria belonging to the genus *Haliscomenobacter*. *Syst. Appl. Microbiol.* **18** (3), 363-367.
4. Kragelund, C., C. Levantesi, A. Borger, K. Thelen, D. Eikelboom, V. Tandoi, Y. Kong, J. Krooneman, T. R. Thomson and P. H. Nielsen (2007) Identity, abundance and ecophysiology of different filamentous *Bacteroides* species in industrial activated sludge treatment plants. In preparation.
5. Lemmer, H und G. Lind (2000) *Blähschlamm, Schaum und Schwimmschlamm – Mikrobiologie und Gegenmassnahmen*. F. Hirthammer Verlag, München, Germany.
6. Manz, W., R. Amann, W. Ludwig, M. Vancanneyt and K. H. Schleifer (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiol.*, **142**, 1097-1106.
7. Tandoi, V., D. Jenkins and J. Wanner (2005) *Activated sludge separation problems – Theory, Control Measures, Practical Experiences*. IWA Publishing, London, UK.
8. Wagner, M., P. Amann, P. Kämpfer, B. Assmus, A. Hartmann, P. Hultzler, N. Springer and K. Schleifer (1994) Identification and *in situ* detection of Gram-negative filamentous bacteria in activated sludge. *System. Appl. Microbiol.* **17**, 405-417.

### **Slide show images**

- 1-6: short filaments: dead straight and protruding from the flocs
  - 4: plus Type IF-23
- 7-10: longer, bent filaments
- 11-13: bundles of long filaments
- 14-15: occasionally with attached growth
- 16: Gram stained
- 17: FISH Image with probe HHY-23a
- 18: FISH image with probe Hhy-T5-654