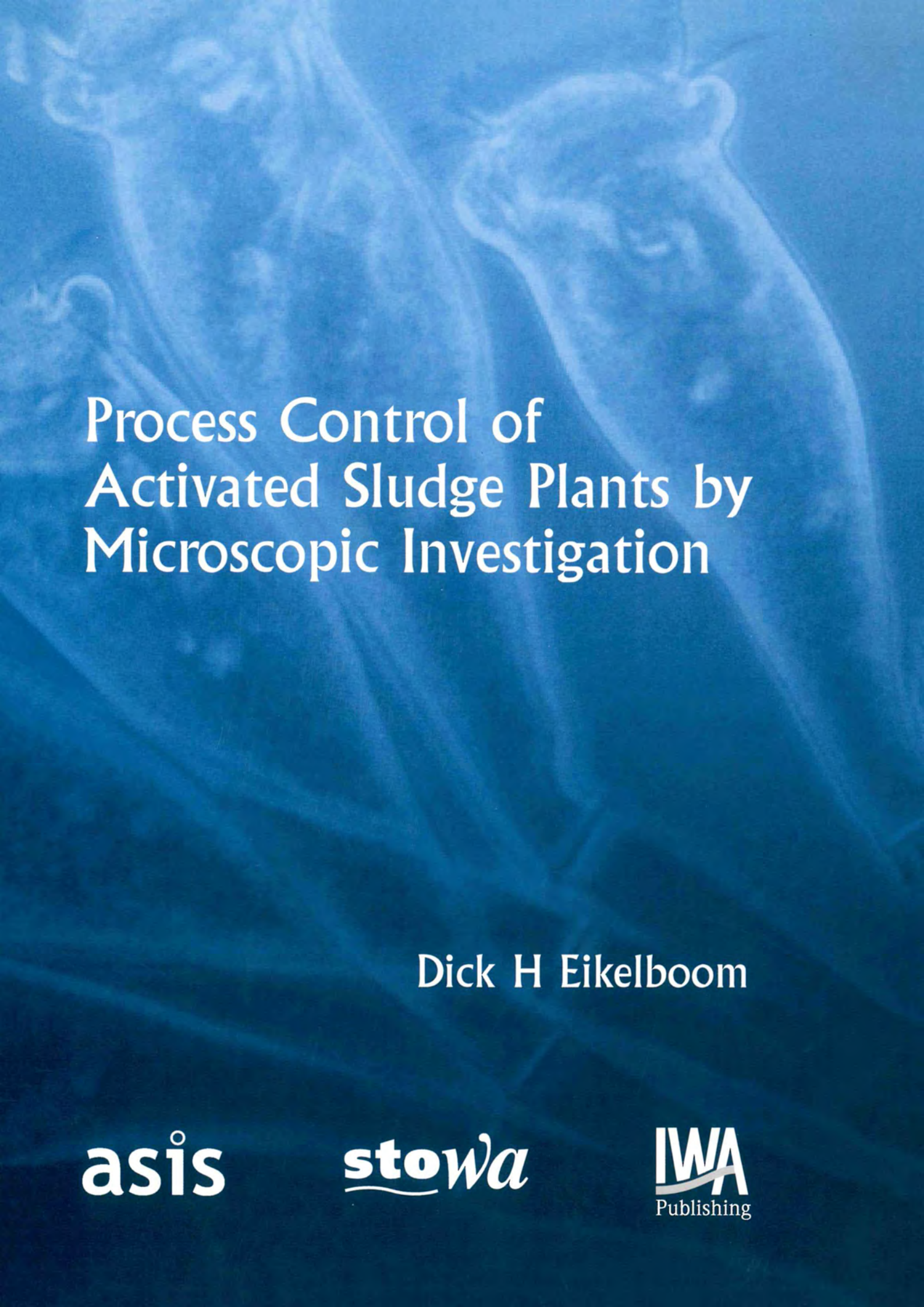

# Process Control of Activated Sludge Plants by Microscopic Investigation

Dick H Eikelboom

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by

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# Contents

# Foreword

The activated sludge process is widely used for treating waste water. Process stability and final effluent quality largely depends upon the composition of the biomass in an activated sludge plant. Operational problems such as bulking and scum formation occur when the 'wrong' micro-organisms are dominating the sludge population. Microscopic sludge investigation is therefore a necessity for process control and stable plant operation.

The knowledge concerning diagnosis and solution of operational problems in activated sludge plants has greatly expanded during the past 20 years. Many papers have been published in scientific journals and books. However, for those working in the field this information is not easily accessible. Therefore, a multi-media package dealing with the biology of activated sludge has been compiled. It includes a CD-ROM and a manual. The manual deals with the theory, which is further explained and visualised on the CD-ROM through 75 minute audio recording, almost 100 short videos, more than 650 photographs and several animations.

This package is the sequel to the '*Microscopic sludge investigation manual*' (Eikelboom and van Buijsen, 1981), a classic in the waste water literature which has been out of print for several years. The methods described in this manual have become more or less standard all over the world. Compared with the 1981 manual, not only are updated microscopic techniques described in this package, but solutions for most operational problems are now presented as well. It is an expert system aimed at improving process stability in activated sludge plants. Although the manual and CD-ROM can be used separately, they actually belong together. It is the combination that makes this package a unique tool for process operators and other people involved in waste water treatment.

These multi-media products have been produced through cooperation between ASIS (Activated Sludge Information Systems), TNO MEP (TNO Institute of Environment, Energy and Process Innovation) and STOWA (Foundation for Applied Water Research). ASIS (www.asissludge.com) owns the full copyrights of the entire package. However, the English edition is only available through IWA Publishing.

Dick H. Eikelboom
Zutphen, May 2000

# 1 Introduction

The activated sludge process is widely used for treating waste water. Even though various modifications of this process are in use, the basic principles for all these designs are comparable. Biology forms the key to the whole process. Operational problems are often a direct result of an 'incorrect' biomass composition. Information concerning the composition of the sludge is therefore indispensable for the correct interpretation and subsequent correction of the treatment process. For example, a turbid effluent cannot only be caused by poor settlement of the flocs in the final clarifier Owing to a massive growth of filamentous micro-organisms, but also by flocs that are too small, by the absence of protozoa or by dispersed bacterial growth. Even though the ultimate effect is more or less similar, different causes are responsible. It is obvious that the exact problem must be taken into consideration, to solve a specific problem efficiently. Microscopic sludge investigation is thereby essential, as this is the only method for acquiring information about the factual quality of the sludge flocs.

A method was developed around 1975, by which important quality characteristics of the biomass in activated sludge plants could be determined in a quick and easy manner through microscopic investigation. This method of analysis was recorded in the '*Microscopic sludge investigation manual*' (D.H.Eikelboom and H.J.J. van Buijsen, TNO Report A94A, 1981) and is now used all over the world.

However, the knowledge concerning this matter has greatly expanded since 1981. Nowadays, much more information is available, particularly concerning (1) the influence of the process conditions on the quality of the sludge and (2) how microscopic observations can be used for plant control. In addition, the effluent requirements have been sharpened, which means that process stability has become more important than ever. For these reasons, it has been decided to compile a completely updated version of the 20-year-old manual.

Together with the manual, a CD-ROM has been made on this subject. The two components of this multi-media package complement each other. The manual deals with the theory. The visual aspects are presented in detail on the CD by means of numerous animations, photographs and videos. The 'living character' of the sludge population is also extensively demonstrated. The subjects are quickly accessible on account of the interactive structure (see Appendix A).This combination forms a unique tool for process control, education and training.

This manual contains two main sections: Microscopic sludge investigation (chapters 2 to 7) and Process control (from chapter 8). In the first section, it is explained how the various characteristics of activated sludge can be assessed by microscopic investigation. This section is especially intended for the microscopist. In the second section, the nature of the activated sludge and the factors that determine the composition of the sludge floc are further expanded. Process control and trouble-shooting are the key words that best describe this section.

The manual does not give a complete overview of everything that is known about the biology of the activated sludge process. However, it includes all the information necessary to interpret the microscopic image and to convert it into practice. It has been taken into account that users of this manual have mostly followed a chemical and/or technological education and, therefore, know little of the biology of the activated sludge process. Chapter 13 gives references for further study of the subjects covered in this book.

# 2 Microscopy

The human eye is unable to clearly distinguish subjects with diameters smaller than 0.1 mm. The diameter of bacterial cells is typically about 1 μm (= 0.001 mm) and they can therefore only be observed with a microscope.

## 2.1 Basic principles

The maximum magnification that can be achieved with a microscope depends upon the 'resolution'. This means its capability for distinguishing two close-together points from one another. In other words: the smallest diameter that can still be sharply observed.

The resolution of a microscope is determined by the wavelength (λ) of the radiation (e.g. light) used and the so-called numerical aperture (NA) of the objective. The following formula is used:

$$Resolution = \frac{\lambda}{2 \times NA}$$

The numerical aperture comprises the refractive index of the lens, the thickness of the layer between the study object and the objective lens, etc. The wavelength of visible light lies between 0.4 μm and 0.7 μm. A wavelength of 0.5 μm and an NA of 1.25 (a rather large NA) results in a resolution of 0.2 μm. This means that with a normal light microscope, only particles, or components of them, larger than ca. 0.2 μm can be observed.

An electron microscope is not operated with visible light but with electrons. As the wavelength of electrons is much shorter than that of visible light, the resolution of an electron microscope is also much greater, reaching ca. 0.001 μm. This type of microscope will not be further referred to in this manual as a relatively simple light microscope is sufficient for investigating activated sludge.

Such a light microscope comprises the following components (Fig. 1):

- a support stand;
- a stage, on which the slide is placed. The slide can be moved by means of the adjustment screws;
- a revolver with three or four objectives;
- two eyepieces;
- a diaphragm, which is used to focus the microscope properly;
- a light source, which is usually positioned in the stand;
- a condenser, which concentrates the light exactly onto the slide. The height of the condenser can be altered with a knob and the light can be centered by means of the adjustment screws;
- a coarse and a fine adjustment, so as to achieve the correct distance between the objective and the slide.

The magnification of the object to be studied is achieved by a twofold lens system, i.e. an objective and an ocular (eyepiece). The total magnification is similar to the product of each individual lens. A 10× ocular is usually used, although others with magnifications of 6×, 15× or 20× also exist. Laboratory microscopes are equipped with 3 to 4 objectives. Even though other objectives are commercially available, objectives that magnify the image 10, 40 and 100 times are usually employed. This results in a total magnification (using a 10× ocular) of 100×, 400× or 1000×. The choice of objective is dependent upon the size of the object to be studied. This will be returned to in paragraph 3.4.

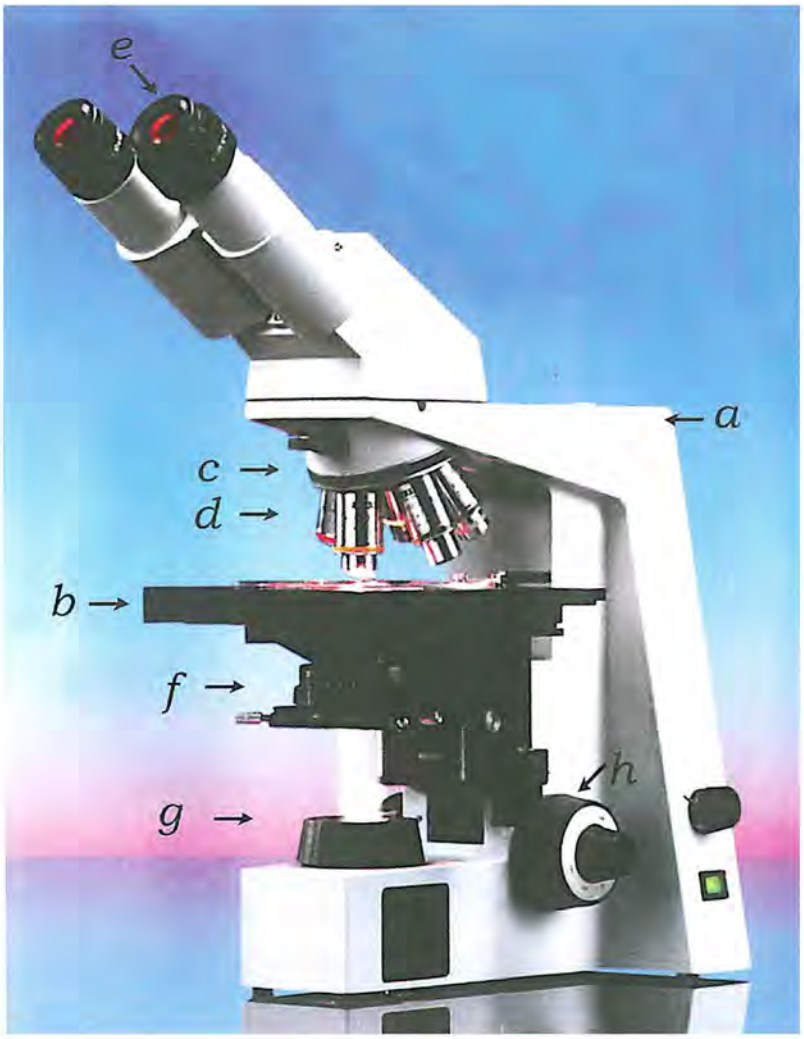



*Figure 1* *Schematic depiction of a microscope.*

| | |
|---|---|
| *a: stand* | *e: eyepieces (oculars)* |
| *b: stage* | *f: condenser* |
| *c: revolver* | *g: light source + diaphragm* |
| *d: objectives* | *h: coarse/fine adjustment* |

At high magnifications (100× objective), the space between the objective and the cover slip of the slide must be filled up with immersion oil. This oil has the same refractive index (1.52) as the glass of the lenses and the slide. In this way, the light beams are not bent away at the glass/air interface but can pass through it unhindered.

## 2.2 Bright field and phase contrast

When light passes through a medium that is not uniform in composition, e.g. a drop of water containing small sludge flocs, a part of the light beam will be scattered or bent more than the rest. This is a direct consequence of variations in the refractive indices (of the water phase and with particles present). Small differences can hardly ever be observed with a so-called bright field microscope. This means that bright field is less suitable for viewing low contrast slides, such as those of activated sludge. This optic (meaning here the objective plus matching condenser) is only used for studying richly contrasting micro-organisms: e.g. organisms possessing a very definite structure; a characteristic colour; stained slides, etc.

Phase contrast equipment is necessary for examining organisms in activated sludge. The phase contrast optics 'translate' relatively small differences in refractive indices into clearly observable variations in brightness. As a result, this equipment is ideal for viewing relatively contrast poor organisms such as bacteria. The structure of the cells of micro-organisms can also be much more clearly observed with phase contrast.

## 2.3 Preparing a slide

A wet mount is used for observing living activated sludge. When preparing stained slides (see paragraph 2.6), a fixed smear is used to start with.

***Wet mount***

A drop of the activated sludge is placed on a glass slide. This slide must be clean and properly de-greased. Subsequently, a clean cover slip is placed on the drop. Shutting in air bubbles must be avoided as much as possible. The slide is now ready and can be observed. If an oil immersion objective is used, a drop of immersion oil must first be put on the cover slip.

It is important that the water droplet is neither too big nor too small. In the latter case, insufficient liquid is present to totally fill the space under the cover slip, resulting in many air bubbles being trapped. Drying out of the slide, as a result of evaporation along the edges of the cover slip, then occurs very rapidly. This causes the liquid in the slide to flow, making it difficult to study. By contrast, if the droplet is too large, the water layer between the slide and the cover slip is too thick. This causes problems with the depth of focus in the slide. Use of an oil immersion lens can cause further problems because the cover slip moves while the slide is being positioned (the cover slip actually floats on the water droplet). This is not only very tiring for the eyes, but also disturbs the slide. The placing of a water droplet of the correct size (about 5 µl) can be best achieved by using a pipette with a small opening (e.g. a Pasteur pipette). Excess liquid can be removed by placing a filter paper against the edges of the cover slip.

A properly made wet mount slide can be used for 30 minutes before it begins to dry out. Drying out can be prevented by smearing liquefied (by heating) vaseline, using a small painting brush, along the edges of the cover slip. The slide is now sealed and can be used for hours.

The layer of water between the cover slip and the slide must be evenly thick all over. The presence of some larger particles (e.g. sand grains) can sometimes hinder this. It is then not possible to fill up completely the space between the slide and cover slip with water. In this case it is better to prepare a new slide

***Fixed smear***

A small drop of sludge suspension is placed on a slide and spread over a surface area of ca. 1 $cm^2$. The slide is subsequently dried in the air. Fixing, by drawing the slide through the pilot flame of a Bunsen burner, is not necessary for activated sludge. Further treatment, e.g. staining, can now be done.

## 2.4 Adjusting and using a microscope

The slide is placed on the microscope stage. Depending upon the size of the object to be viewed, an objective and a corresponding condenser position are selected. The distance between the two oculars is checked against the eyes. Viewing with both eyes also needs some practice. Subsequently, the distance between the slide and the objective is reduced by the coarse adjustment until the object comes vaguely into view. Fine adjustment sharpens the image. The image can now be turned by means of the adjustment screws on the stage.

The quality of the microscopic image is determined to an important degree by whether the microscope is properly adjusted or not. This is particularly relevant for phase contrast. Without going fully into this matter - the manner in which the microscope is set up is heavily dependent upon the manufacturer and, therefore, the supplier can explain this better - several frequently made mistakes are given below.

The condenser should concentrate the light on the slide. An incorrect condenser height results in less light on the slide and a less sharp image. Therefore, it must be regularly checked that the condenser is correctly adjusted. Doing this is, in fact, very easy. After almost closing the diaphragm, the condenser height is sought at which the diameter of the illuminated circle, such as is observed in the microscope, is at a minimum. Finally, the illuminated circle is centered with the adjustment screws.

Regular checking is also required for the phase contrast adjustment. Using the adjustment eyepiece provided, it must be checked now and then whether the phase rings are properly concentric. Care must also be taken that the position of the condenser and the objective used correspond correctly with one another.

Objectives must be regularly cleaned, and on every occasion after the use of immersion oil. The use of lens paper is preferable to ordinary pocket tissues, and such like, as these products often leave fluff. In addition, it is necessary to give the microscope an annual service.

No air bubbles must be present in the droplet of immersion oil (100× objectives) on the cover-slip. An air bubble has a different refractive index than the oil and therefore scatters the light beams. If an air bubble is present, no sharp image can be obtained.

Finding the microscopic image is sometimes a problem when using the 100× objective. The space between the objective and the surface of the slide (the so-called working distance) is then extremely small (ca. 0.15 mm). There is an actual risk that the objective can be lowered too far when seeking the image. The objective can touch the slide through which it can become damaged. This can be avoided by not looking through the microscope when bringing the slide and the objective towards one another but, instead, bringing them as close as possible together by eye. Then, one should look into the ocular and, by moving the slide, a recognisable image (e.g. an activated sludge floc) can be brought into focus with the fine adjustment.

## 2.5 Measuring and counting

To measure the sizes of the particles on a microscopic slide, a micrometer must be present in one of the oculars. This measuring ocular must first be calibrated with a simple slide on which 1 mm is divided into 100 units. It can be established in this manner how large the distance between the 'stripes' is at the different magnifications.

To establish the number of particles (e.g. free-living cells, characteristic colonies or certain filaments) in a given activated sludge, a rough estimate is sufficient for routine investigation. The microscopic image is compared to several reference photographs (e.g. see Figs 29-38). Occasionally, the number of particles can be more exactly determined by using a counting chamber. In fact, this is a wet slide of which the volume of liquid is known.

More advanced methods for quantification are mostly based on the photographic or digital registration of characteristics, followed by image analysis. These are very useful methods for research purposes, but such operating procedures are still too work intensive for "daily" use. It is still uncertain to what extent microscopic investigation can be automated in the future. The composition of activated sludge is extremely heterogeneous and a great many different characteristics are simultaneously assessed.

## 2.6 Staining techniques

It is possible to improve the visibility of various cell components by employing specific staining methods. This handbook only deals with Gram and Neisser staining. The principle of most staining methods is that a certain cell component binds a stain more strongly than the other parts of that cell.

### *2.6.1 Gram staining*

Gram staining is an indispensable aid when identifying bacteria. This staining first colours the bacteria blue using carbol gentian violet. The cells are then washed with an alcohol solution. The cells of some bacterial strains re-release the absorbed blue dye during this process. These bacteria are known as Gram negative. In the case of Gram positive bacteria, the absorbed carbol gentian violet cannot be removed by washing with alcohol. The colourless Gram negative bacteria are subsequently restained with safranine, which gives them a red colour. This is the result of differences between Gram positive and Gram negative bacteria in the composition of the cell wall.

***Necessary solutions***

| | | |
|---|---|---|
| A | *Carbol gentian violet solution* | Dilute 10 ml of the stock solution with 90 ml of a 5% phenol solution. |
| | Stock solution | Carbol gentian violet 10 g, alcohol (96%) 90 ml. |
| B. | *Lugol's iodine solution* | Dissolve 3 g KI in a few mls of distilled water, mix in 1 g $I_2$ and dilute to 300 ml with distilled water. |
| C. | *Alcohol solution* | Dilute 7 ml of the stock solution with 1000 ml (96%) alcohol. |
| | Stock solution | $I_2$ 100 g<br>KI 40 g<br>alcohol (96%) 1250 ml<br>distilled water 100 ml. |
| D. | *Safranine solution* | Dissolve 0.25 g safranine in 10 ml (96%) alcohol and dilute with 100 ml distilled water. |

***Staining procedure***

- Prepare a fixed smear (see paragraph 2.3).
- Apply solution A for a contact period of 60 seconds; subsequently allow the excess dye to run off the slide.
- Apply solution B for a contact period of 60 seconds; subsequently allow the excess dye to run off the slide.
- Dip the slide in solution C for 30 seconds. Move the slide gently to and fro in this solution.
- Rinse the slide clean with tap water by allowing the water to flow gently over the back of the slide.
- Apply solution D for a contact period of 120 seconds; subsequently, rinse the slide again with tap water.
- Allow the slide to dry and view with a 100x bright field objective. A blue filter strengthens the contrast. Drying can be speeded up by first removing most of the water with filter paper.

***Results***

Gram negative and Gram positive bacteria stain red and blue, respectively (Figs 2 and 3). The blue colour can vary from light blue to almost black. Sludges from high loaded plants mostly comprise Gram negative bacteria, while many Gram positive strains are also present in sludges from lower loaded plants. The presence of Gram positive species contributes to a more robust floc (see also paragraph 4.1).Fungi and protozoa/metazoa do not stain evenly, or not at all, with this staining method.

For some filamentous bacteria, particularly Type 0041, not all parts of a filament stain in the same manner. This is usually caused by the attachment of other bacteria to the filaments. These bacteria screen off the filaments to some extent so that the blue stain cannot be sufficiently absorbed. When assessing the result of the staining in such a case, attention is principally paid to the 'clean' tip of the filament.

The result of the staining depends upon the age of the cell for some species. Young cells stain red whereas older ones stain blue. This can also result in two colours occurring in one filament.

***Remarks***

- The solutions can be bought ready made.
- Numerous different recipes for Gram staining are mentioned in the literature. The recipe described provides filamentous organisms with a good contrast.
- Most solutions can be retained for an almost unlimited time. Solution C (not the stock solution) must be renewed once a month.
- The slides must be properly de-greased.
- The slides must be viewed with bright field. The difference between red and blue is less clear with phase contrast.
- The slide must not contain too many sludge particles, as excess dye can then no longer be removed by rinsing. Large 'blobs' of dye can be seen when viewing. If this is the case, the staining must be repeated with fewer sludge particles on the slide.

### *2.6.2 Neisser staining*

Staining according to Neisser is a test for the presence of polyphosphates stored in the cells (= storage materials). This method is an indispensable aid to the identification of certain strains of filamentous bacteria. Furthermore, this staining method can make the Bio-P bacteria, responsible for biological phosphate removal, visible.

*Necessary solutions*

| | | | |
|---|---|---|---|
| A. | Methylene blue | 0.1 | g |
| | Glacial acetic acid | 5 | ml |
| | Ethanol 96% | 5 | ml |
| | Distilled water | 100 | ml |
| B. | Crystal violet, 10% in 96% ethanol | 3.3 | ml |
| | Ethanol 96% | 6.7 | ml |
| | Distilled water | 100 | ml |
| C. | Chrysoidin Y, 1% aqueous solution | 33.3 | ml |
| | Distilled water | 100 | ml |

***Staining procedure***

- Prepare a fixed smear.
- Place a freshly made mixture of 2 parts solution A and 1 part solution B onto the slide for a contact period of 10-15 seconds. Afterwards, allow the excess dye to run off the slide.
- Add solution C for a contact period of 45 seconds.
- Rinse the slide with tap water (with the flow against the back of the slide).
- Allow the slide to dry and then view with a 100x bright field objective. Drying can be speeded up by removing most of the water carefully with filter paper.

***Results***

Neisser negative cells stain hardly or not at all (slightly brown or yellow; Fig. 4). Three main groups of Neisser positive bacteria can be distinguished.

1. Filamentous bacteria which stain completely grey-violet (Fig. 5). This almost always applies to *Nostocoida limicola* or Type 0092.

2. Filamentous bacteria which contain blue-black coloured polyphosphate globules (Fig. 6). Without staining, these globules cannot be clearly observed with a light microscope. They are indeed clearly visible if a much higher magnification (electron microscopy) is used (Fig. 7). These globules, which are present in pairs, are an important identification characteristic for *Microthrix parvicella*.

3. Colonies of blue-black coloured cells (Fig. 8). These comprise Bio-P bacteria. There are some variations in the manner in which these types of colonies stain with Neisser. The shade is sometimes much lighter (Fig. 9), or only a part of the cell stains darkly.

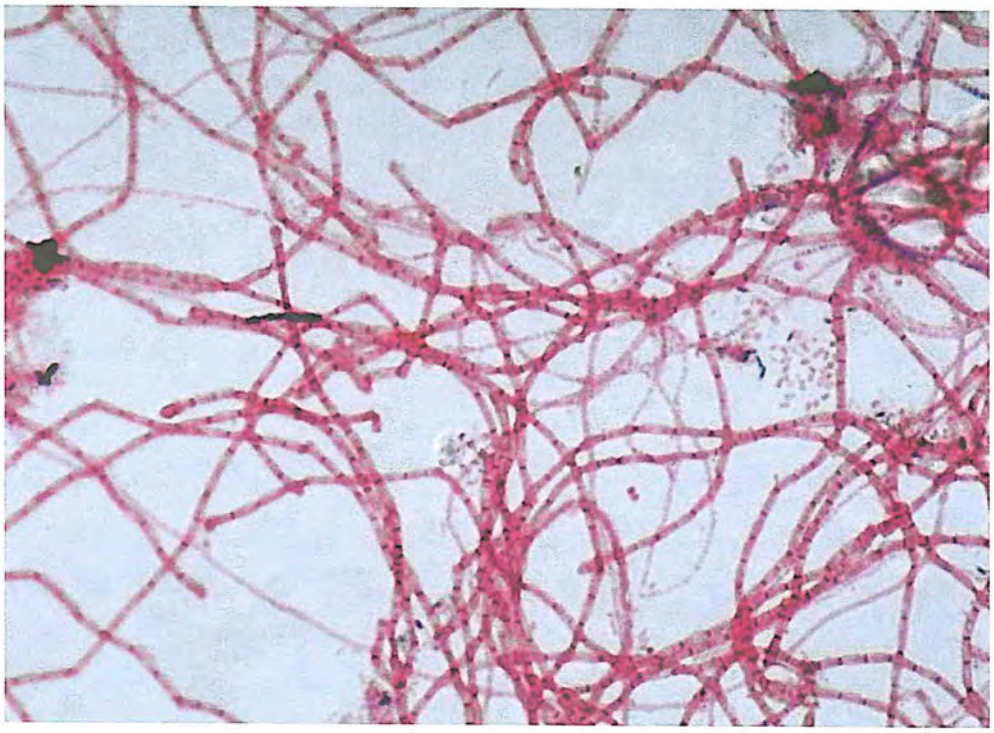


*Figure 2* *Gram negative bacteria (1250×).*

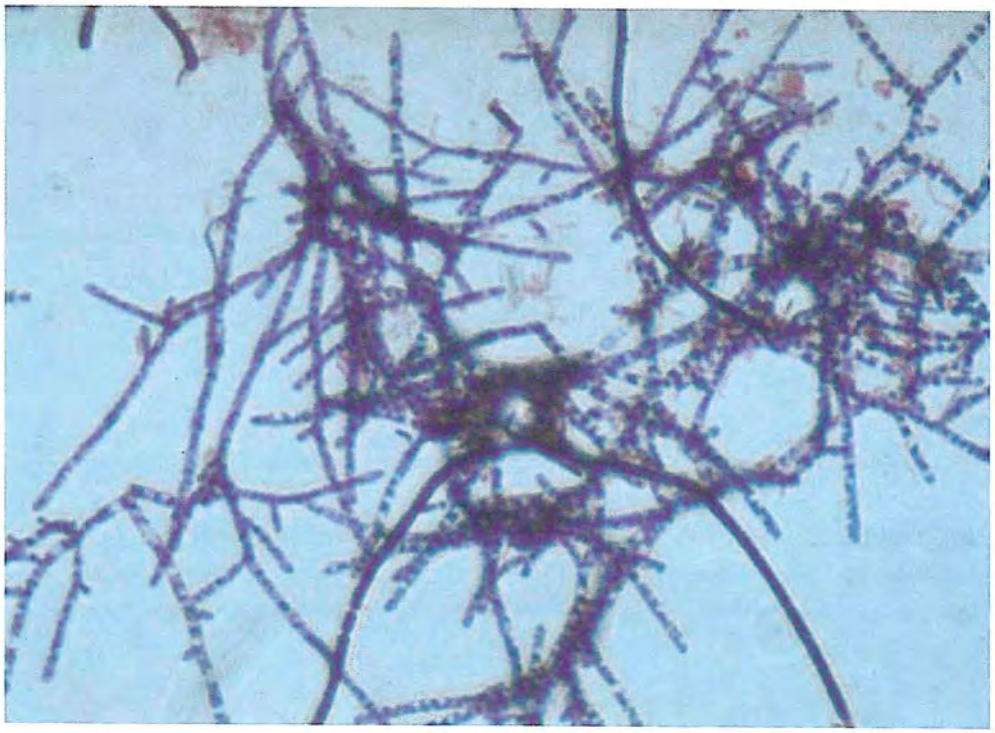


*Figure 3* *Gram positive species (1250×).*

*Remarks*

- Solutions A to C can be retained for a practically unlimited time. They can be bought ready made.
- Neisser negative filaments have little colour, so they are often difficult to find on a stained slide.
- For *M. parvicella*, the polyphosphate granules are often largest in the season in which this bacterium grows best (winter).
- Occasionally, strongly refractive sulphur globules are also darkly stained with Neisser.
- Retaining the sludge for a few days in a refrigerator has little effect on the results of Neisser staining. Therefore it is not necessary to refresh the sludge by e.g. aeration before the staining is carried out.

## 2.7 Sulphur storage test

Some filamentous bacteria can store sulphur globules in their cells if they grow in the presence of reduced sulphur compounds, such as sulphides.These globules comprise elementary sulphur that is stored as an intermediary product (storage material). *Beggiatoa, Thiothrix,* Type 021 N (sometimes) and Type 0914 may store S-granules inside their cells. However, the sulphur globules are not always present, which complicates a correct identification. By adding $Na_2S$ to activated sludge, *Thiothrix* strains especially, store many S granules in a short period of time.

*Method*

Mix a volume of activated sludge with a similar volume of an $Na_2S$ solution (200 mg $Na_2S \bullet 7\ H_2O$ per 100 ml). Aerate the mixture for 15–30 minutes. Then check microscopically if the cells have stored any sulphur globules. These globules are clearly visible at a minimum magnification of 400×.

*Result*

*Thiothrix* sp. store large, strongly refractive granules (Fig. 10). Small globules are black, however. Usually only small, or no S globules at all are formed in the cells of other filamentous bacteria. Type 021 N, which sometimes also stores sulphur, is an exception.

## 2.8 Other microscopic techniques

*Stereo microscopy*

A stereo microscope provides a three dimensional image of the activated sludge flocs. Unfortunately, the magnification is limited (maximum ca. 50×), as a result of which many details are invisible or poorly visible. This instrument is sometimes used for counting large organisms in sludge, for example, certain worm species.

*'Genetic probes'*

It is currently possible to mark a fragment of genetic material from a micro-organism with a fluorescent label. When such a 'probe' is added to sludge, it binds itself very specifically to the organism which possesses the same genetic code. This organism can be very easily traced within the mixed sludge population by using fluorescence microscopy (Fig. 11). This is a very good technique for research purposes which, in addition, is rapidly developing. It can be expected that a type of sensor will become available within the next 5(?) years, which will enable important micro-organisms groups in activated sludge (nitrifying bacteria, Bio-P bacteria, most filamentous species, etc.) to be quickly detected and quantified.

However, this position has not yet been reached. Normal phase contrast microscopy is still superior for the routine monitoring of activated sludge, because:

1. Various sludge quality aspects can be simultaneously examined using phase contrast microscopy. Information is gathered concerning not only filamentous bacteria, but also concerning the sludge floc characteristics, the protozoa population, etc. The sludge quality can only be assessed on the basis of this total picture. Probes only provide information on filamentous bacteria.

2. No probe is yet available for several commonly occurring filamentous species.

3. At the moment, application of probes is still too labour intensive. Using phase contrast microscopy, an experienced microscopist can carry out a complete assessment of activated sludge in about 30 min. Use of probes takes markedly more time.

4. At present, the equipment necessary is still quite expensive.

So, for the time being, normal phase contrast microscopy remains an indispensable aid to quickly establishing a diagnosis whenever a treatment plant does not function properly.

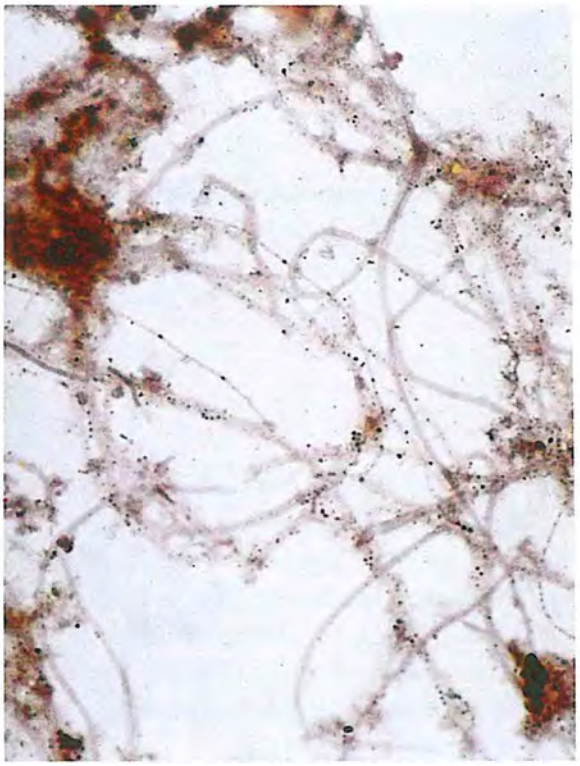


*Figure 4 Neisser negative filaments (900x).*

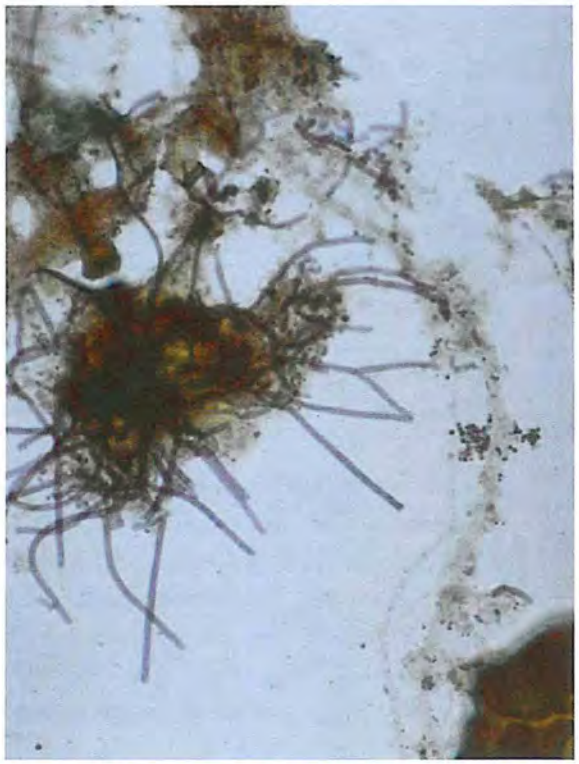


*Figure 5 Type 0092 filaments stained grey-violet by the Neisser method (900x).*

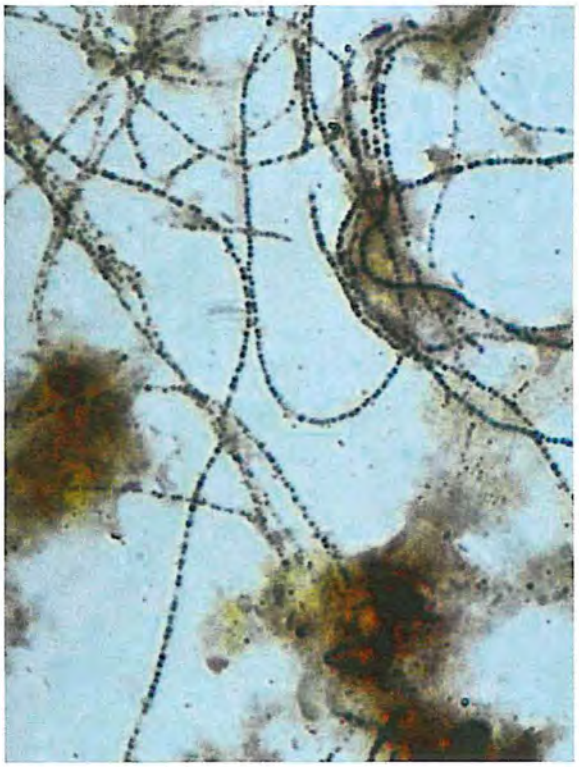


*Figure 6 Neisser stained blue-black globules in* M. parvicella *filaments (900x).*

*Figure 7 Electron microscope image of poly-P granules in* M. parvicella *(26,300x).*

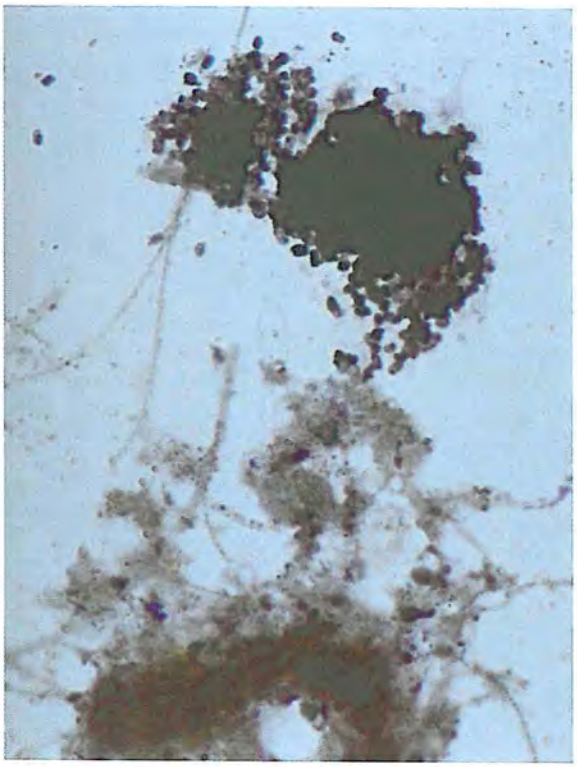


*Figure 8 Neisser stained colony of poly-P bacteria in activated sludge (900x).*

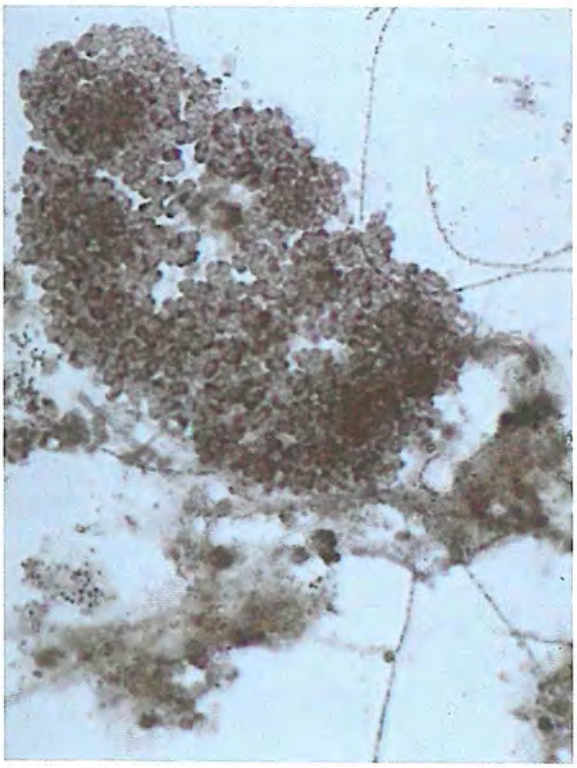


*Figure 9 Neisser stained colony of poly-P bacteria in activated sludge (900x).*

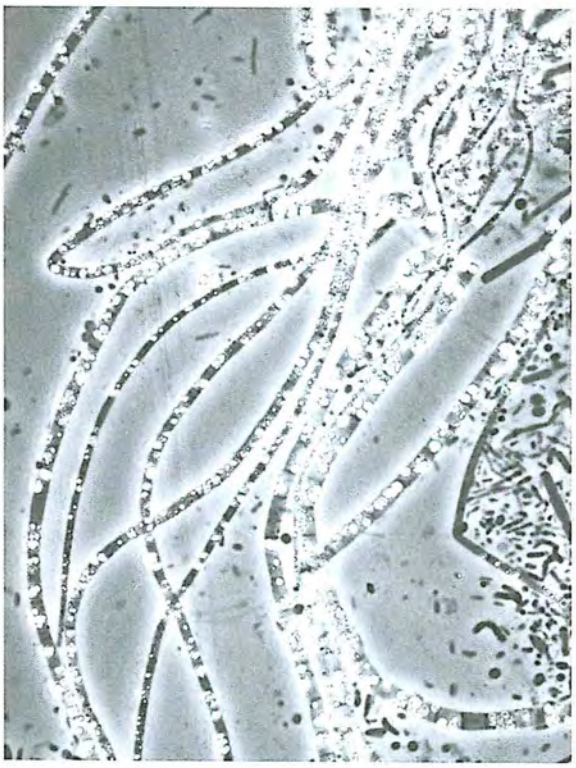


*Figure 10 Sulphur granules in* Thiothrix *filaments (900x).*

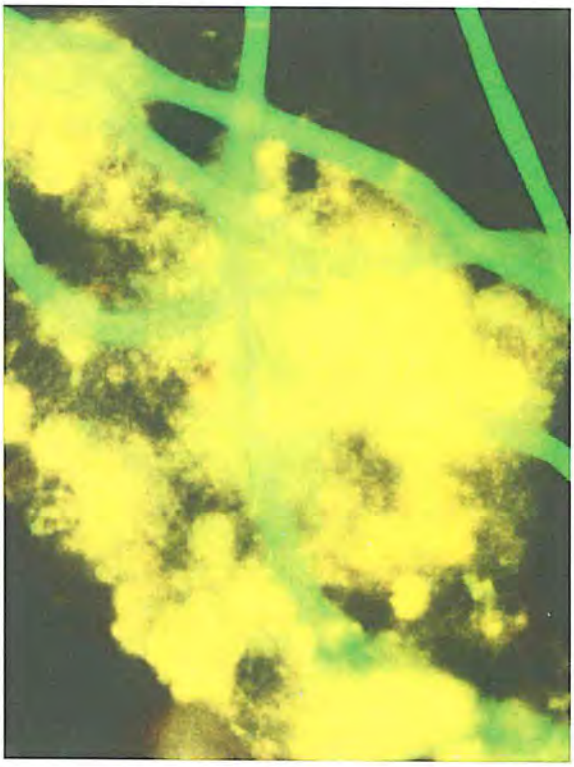


*Figure 11 Type 021 N filaments in activated sludge. Filaments labeled with a fluorescent 'probe'(900x). Photograph provided by Bioclear.*

# 3 Microscopic investigation of activated sludge

The microscopic image provides information concerning several visually observable properties of activated sludge. This information is relevant to the qualitative assessment of the sludge. In addition, it can help with making a diagnosis if the treatment plant is not functioning to expectation. The analysis does not provide any direct information on the activity of the biomass, however. This must be determined in a different way.

The frequency with which sludge investigation is carried out is linked to the sludge age. A frequency of once per week or fortnight is necessary in order to identify changes in the composition of the sludge if the sludge age is short (days). In plants with much longer sludge ages (weeks), a frequency of once every 1 to 2 months is adequate.

## 3.1 Sampling and handling samples

The investigation is done with sludge taken from the aeration tank. It is advisable to always sample from the same location e.g. the overflow to the final settlement tank. The bottle is one third filled with sludge, so as to maintain aerobic conditions in the sludge as long as possible. The sludge must not be thickened. Preservation, apart from cooling, is also not allowed as all living micro-organisms would then die.

The microscopic investigation should be carried out with sludge that is as fresh as possible. Samples that cannot be analysed directly must be kept cool (4–7 °C), preferably in open bottles. The samples must not be frozen as this can affect the structure of the floc. The properties of the sludge change gradually during storage. This occurs faster if the sludge is more highly loaded. Zoogloea colonies, spirochaetes, and some species of protozoa/metazoa disappear first. Owing to disintegration of the floc, the number of free cells subsequently increases. Micro-organisms which are characteristic of anaerobic conditions, such as spirils, might appear. Sludges from high-loaded treatment plants (no nitrification) must therefore be analysed within 2–3 days. Sludge from low-loaded plants (nutrient removal conditions) can be retained for 1 to 2 days longer before the characteristics really begin to change.

## 3.2 Method for the analysis

The contents of the sample bottle must of course be properly mixed by shaking it gently by hand. Slides are now prepared for the microscopic investigation and for the two stainings. Through good mixing, one drop of sludge should be representative of the contents of the sample bottle and therefore the complete contents of the aeration tank!

The conclusions concerning the quality of a given activated sludge must always be based on a large number of observations. So, examining just a few sludge flocs is not sufficient. Many flocs must always be viewed on a given slide before anything can be concluded about the average quality of the floc in the sludge involved. This is also valid for most of the other

characteristics. This means that the slide must be observed systematically (Fig. 12). If viewing is not done in this manner – and if the microscope stage is more or less haphazardly turned – then an insufficient proportion of the slide will often be observed.

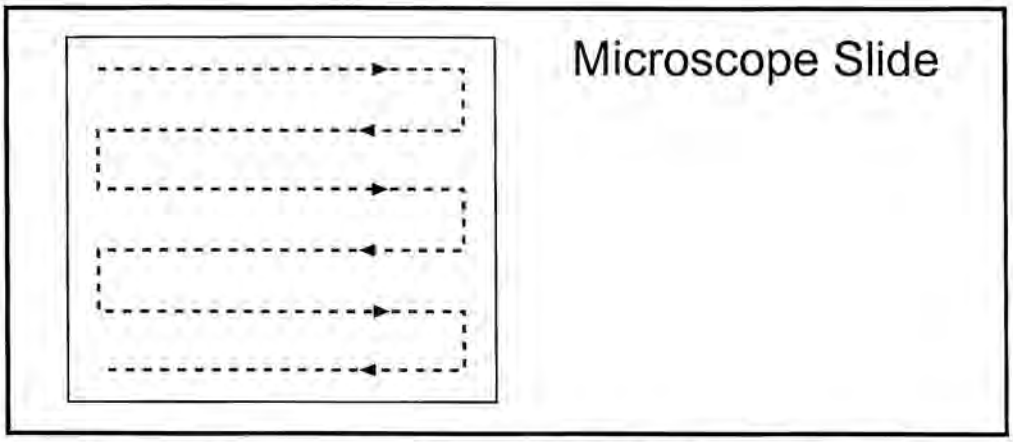



*Figure 12* *Schematic outline of the manner in which a slide should be viewed.*

## 3.3 The microscopic image

The untrained eye sees a mixture of heterogeneously composed and irregularly shaped conglomerates (= flocs) through a microscope. Particles that move around can be seen here and there, while filamentous structures are sometimes present between the flocs. The colour of the floc components can vary from grey-yellow to brown-black.

A more experienced observer will notice that not all flocs look alike, but that there are clearly observable differences. These visually observable variations are related to:

- *form, structure and dimensions of the flocs;*
- *composition of the flocs.* If there is a clearly visible variation in the micro-organisms, then characteristic bacterial groups can be absent or present, or many (in)organic dead particles, etc., can be present in the flocs.
- *filamentous micro-organisms.* If these organisms are present in the sludge, which species can be distinguished?
- *Bacteria which are not bound to the flocs.* If practically all the cell material is present in the form of sludge flocs or if there are many free (moving) bacterial cells present between the flocs;
- *other organisms.* Protozoa, etc., which are present in the sludge and their numbers.

Information on all of these sludge characteristics, which tell something about the process conditions in the treatment plant, can be gathered by phase contrast microscopy.

The correct magnification is an important aspect. This must correspond with the size of the subject to be studied. It does not make much sense to observe a particle with a diameter of ca. 1 µm at a total magnification of 100× as it is still only 0.1 mm across and therefore hardly anything can be seen.

## 3.4 Choosing the correct objective

A magnification of 100× to 200× is used to begin with when carrying out the analysis. This magnification is the most suitable for compiling the following information:

- the form, structure and dimensions of the sludge flocs;
- counting and identifying protozoa and metazoa;
- estimating the size of the filamentous population (the Filament Index);
- establishing some characteristics of the filaments, such as their shape, the length of the filaments, the attachment of other micro-organisms to their surfaces and if the filaments are bound to the flocs or whether they are just free in the liquid. This is an aid in recognizing filamentous species again at higher magnifications or on stained slides;
- observing so-called monocolonies. These are flocs or components of flocs which seem to comprise of one type of cell. Monocolonies sometimes have a characteristic shape. Bio-P bacteria usually grow in monocolonies. However, monocolonies are not always present;
- counting any fibers or inorganic particles, such as sand grains, present.

The magnification is increased subsequently to 400×, by which particles with diameters of 0.5 µm to 1.0 µm can be observed. Attention can now be paid to the following aspects:

- the structure and the dimensions of filamentous bacteria and of smaller protozoa, such as flagellates;
- bacteria that are not bound to the flocs. These sometimes have a characteristic shape (spirochaetes and spirils);
- the strength (firmness) of the flocs;
- the variation between the cell shapes in the floc (diversity) and the presence of small monocolonies.

It will sometimes turn out to be necessary to adjust slightly the observations recorded at a lower magnification.

Finally, the 1000× magnification is used. The last characteristics of the filamentous bacteria can now be registered. This involves (1) determining whether the cell septa are observable and, if they are visible, what shapes the cells are, (2) the diameter of the filaments and (3) the presence of storage materials.

## 3.5 Recording the observations

The observations are recorded on an analysis form (see the following page). The analysis now needs to be ended with a quality assessment: i.e. good, moderate or bad (see chapter 7). For process control, it is also necessary that the results are compared with those obtained

from previous analyses. In this manner it can be established whether any changes have occurred with time (trend).

When registering, or quantifying the observations, several problems arise. The result of a chemical analysis can be expressed as a figure: the concentration of a given compound. In this way, a given situation is adequately described and made understandable for others. However, observations from a microscopic sludge analysis cannot easily be expressed in exact figures. Counting and measuring all particles/cells is exceptionally labour intensive. It is also senseless as only the order of magnitude of most of the characteristics is relevant for assessment of the quality of the sludge. Quantification of the visual observations must therefore be done in a different way. Care must be taken that subjective criteria do not hinder the comparison of results. A given floc structure must not be characterised as 'reasonably good' by one researcher and as 'poor' by another.

It has been shown that this problem can be largely overcome by using reference photographs. By comparing the microscopic image with a reference for that characteristic, the category can be assigned. In addition, numbers of micro-organisms must sometimes be approximately estimated. Some examples of this are given on the analysis form. So, practically all observations can be expressed as a number. This matter is discussed in more detail in the following chapters.

## Result of microscopic sludge investigation

Water treatment plant: .....

| Sampling date | Analysed by |
|---|---|
| Sample number | Date of analysis |

| **Sludge quality** | |
|---|---|
| Good | |
| Moderate | |
| Bad | |
| **Trend** [1] | |
| Better | |
| Similar | |
| Worse | |
| irrelevant | |

**(specific photograph)**

1) in comparison with the last sampling

| **Filamentous organisms**[a] **FI =** | | | | **Proto-/Metazoa**[b] | | **Various characteristics** | |
|---|---|---|---|---|---|---|---|
| *M. parvicella* | | Type 021N | | Ciliates | | Floc type [d] | |
| Type 0041/0675 | | *Thiothrix* | | Flagellates | | Free-living cells [c] | |
| Type 0092 | | *S. natans* | | Amoeba | | Zoogoea [b] | |
| Type 1851 | | *H. hydrossis* | | Test. amoeba | | Poly-P colonies [b] | |
| Type 0803 | | *N. limicola* | | Heliozoa | | Other monocolonies [b] | |
| Type 0914 | | Type 1701 | | Rotifers | | Spirils [b] | |
| Actinomycetes | | | | Nematodes | | Spirochaetes [b] | |
| | | Various species | | Worms | | Fibres [b] | |

a) Scale 0 - 5 = none - numerous filamentous organisms
b) Scale 0 - 3 = none - numerous cells/colonies per slide
c) Scale 0 - 3 = none - hundreds of cells per field of view

d) 1 characteristic of low sludge load + surface aerator
2 characteristic of low sludge load + diffused air aeration
3 characteristic of high sludge load
4 markedly many small flocs (< ca 25µm)
5 otherwise; see remarks

Remarks:
.....
.....
.....
.....
.....

# 4 Characteristics of activated sludge flocs

Activated sludge flocs are conglomerates of living and dead bacterial cells, often also including filamentous strains, precipitated salts, trapped inorganic particles (sand) and organic fibers. They are held together by a slime matrix, comprised of polymeric compounds surrounding the cells, and by chemical bonding forces, in which divalent cations, such as $Ca^{2+}$, play an important role. Free-living bacteria, protozoa and occasionally higher organisms are present around the flocs and in the water between the flocs.

The percentage of living cells increases as the sludge load in the treatment plant is higher. The difference between living and dead cells is often not distinctly visible anyway. It can only be established by carrying out further investigations.

The process operator prefers robust, compact flocs which settle rapidly. In practice, this 'ideal floc' does not always occur. Monitoring of the floc quality is therefore an important aspect of microscopic sludge investigation.

Several characteristics of activated sludge flocs are outlined in this chapter, including some groups of organisms that strictly speaking do not belong to the floc as they are present free in the water phase.

For information about filamentous organisms, bulking sludge and protozoa/ metazoa, the reader is referred to the relevant chapters.

## 4.1 Morphological characteristics

The word 'morphology' is derived from the Greek word 'morphe', which means 'shape'. The meaning is used more broadly in this handbook, covering also the structure, strength and size of the activated sludge flocs.

When examining the morphological characteristics of the activated sludge in a given treatment plant, there will always be a considerable variation in the different characteristics. Therefore, a global characterisation of the biomass can/must be conducted. It only needs to be established whether the morphological characteristics are reasonably in accordance with those of the floc type in the plant involved (see paragraph 4.4). If this is not the case, the sludge must be characterised on the basis of the characteristics listed below.

*Shape*

The shapes of activated sludge flocs can vary from more or less round (Fig. 13) to distinctly irregular (Fig. 14). The settling velocity of the flocs is reduced if they are irregularly shaped. As illustrated in Fig. 13, rounded flocs are not really round, however, but rather angular. Precisely rounded flocs hardly ever occur. The flocs in many treatment plants are more or less rounded. So, this is the most commonly occurring floc shape. Through a combination of

diffused air aeration and a relatively high sludge load (> ca. 0.3 kg BOD/kg MLSSday), the flocs are sometimes markedly irregularly shaped. The growth of floc forming bacteria along the filaments that protrude from the flocs can also result in flocs of this shape.

*Structure*

When describing floc structure, the following extremes can be distinguished:

- compact flocs, in which the bacteria are stacked close to one another. The flocs are mostly brown (Fig. 15);
- open flocs, in which the water can actually flow through the floc particles (Fig. 16).

Flocs settle faster if they are more compact.

The combination of diffused air aeration and a sludge load < ca. 0.3 kg BOD/kg MLSSday is associated with compact flocs even if many filamentous bacteria are present, With a higher sludge load and this aeration system, the flocs are often irregularly shaped and are, consequently, more open.

If surface aerators are applied, less compact flocs are usually formed. It is often obvious that the floc is actually comprised of various smaller floc particles. This is the consequence of the turbulence in the zone near the aerator. Flocs are battered to smaller pieces. If filamentous bacteria are absent, the floc particles will subsequently reaggregate to larger units, but it remains obvious that these are in fact comprised of 'sub-flocs'. If filamentous bacteria are present, particularly if *Microthrix parvicella* is involved, agglomerates can arise. These are open flocs in which floc particles are bound together by filaments (Fig. 16).

*Strength*

When carrying out microscopic sludge investigation, a distinction must be made between 'firm' (Fig. 17) and 'weak' (Fig. 18) flocs.
Where a firm floc is concerned, the floc itself and the surrounding liquid are distinctly separated. This is not the case with a weak floc, where the interface between the floc and the liquid is not sharply defined because many cells are present at the edges of the flocs and it is not certain if they are indeed bound to the flocs. Weak flocs can also be easily damaged.

Bacteria form flocs so that they can maintain themselves in nutrient poor environments. Flocs offer protection against consumption by protozoa and they help the bacteria to avoid being washed out. The necessity for floc forming decreases if more food is available and if fast growing species in the population come to predominate. Therefore, the strength of the flocs is mainly determined by the sludge load applied: the higher the load, the weaker the flocs are. This shift in the population is also demonstrated by the Gram staining results. Flocs originating from high loaded plants are chiefly comprised of Gram negative bacteria, while many Gram positive cells are also present if a low sludge load is applied. Gram positive bacteria possess a hydrophobic (≈ water repellent) cell surface. As a result of this, they remain attached to one another in preference to being suspended in the water phase.

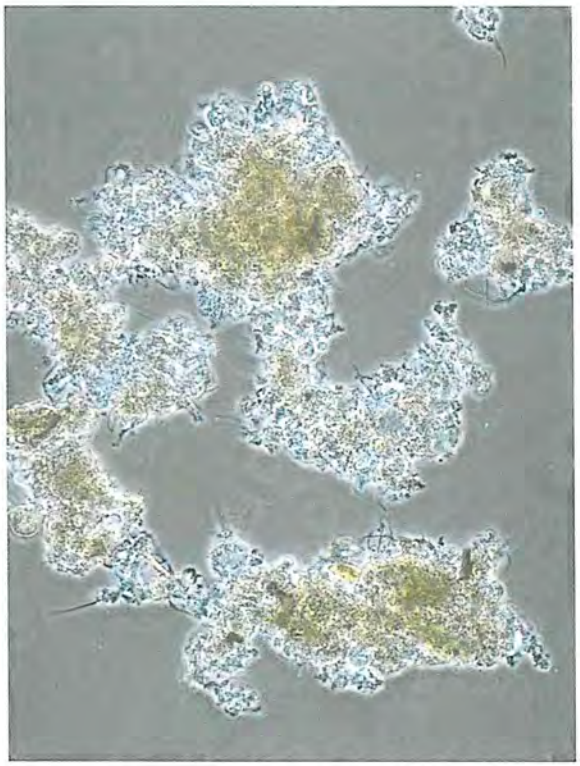


*Figure 13 Rounded sludge flocs (150×).*

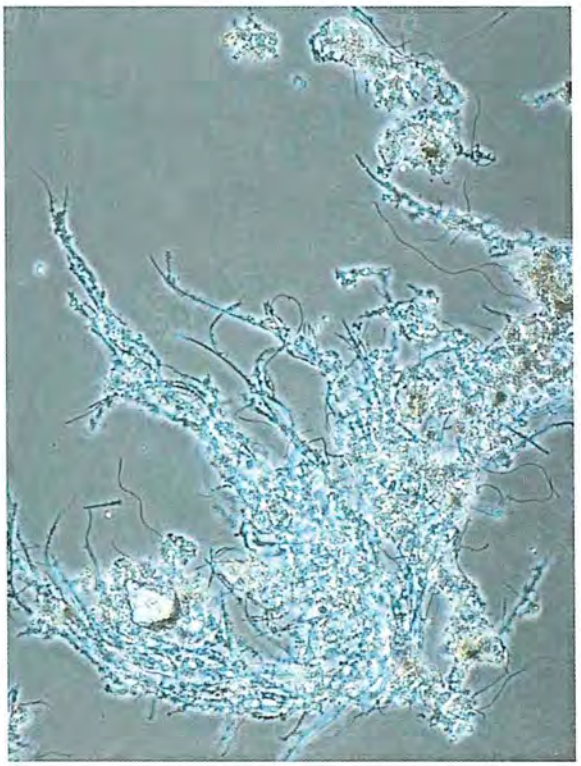


*Figure 14 Irregularly shaped flocs (150×).*

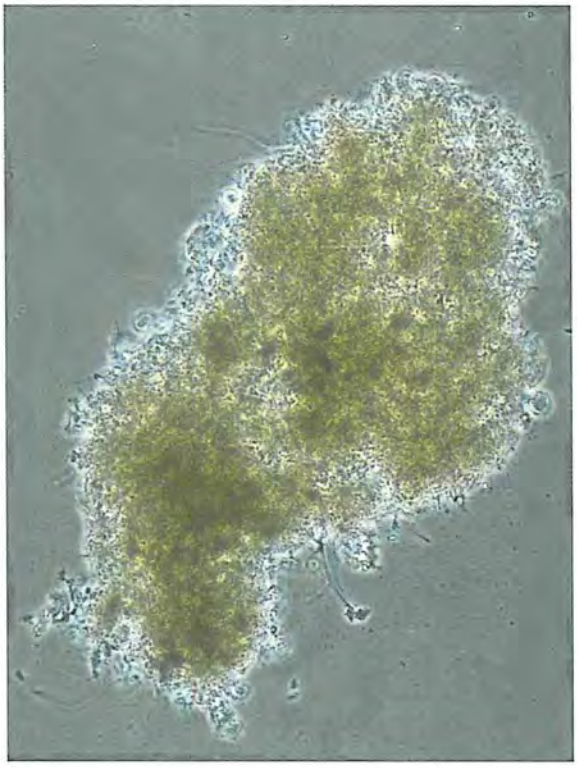


*Figure 15 A compact sludge floc (150×).*

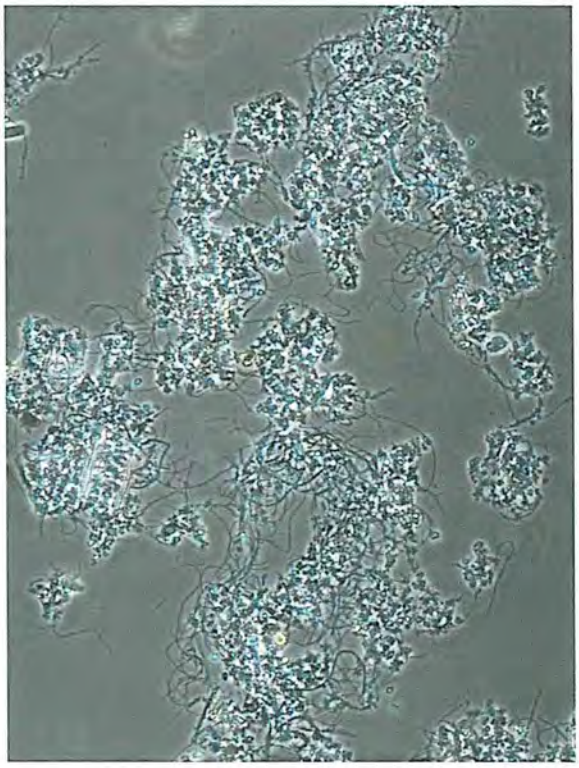


*Figure 16 An open floc (150×).*

*Size*

Where the dimensions of a sludge floc are concerned, the size of macroscopic sludge flocs which are formed when the sludge mass settles, is not intended. These macroscopic flocs, with diameters of ca. 10 mm, hardly show any cohesion and disintegrate easily. However, these loose conglomerates comprise of numerous much smaller particles, i.e. the real activated sludge flocs. The sizes of these flocs can vary from 10 to 20 µm to sometimes as much as a few mm. The diameters of the flocs are determined by means of a standard micrometer. Filaments which protrude from the floc are not included when establishing the diameter.

Three size classes are distinguished:

- small flocs : diameter < 25 µm
- medium-sized flocs : diameter 25–250 µm
- large flocs : diameter > 250 µm

Compact flocs settle more rapidly if they are larger. The floc size in a given activated sludge is considerably variable. Small flocs are nearly always present. If their percentage is not too high, they are removed from the water by being taken up in the sludge blanket (final clarifier). A high percentage of small flocs (> 25%) can actually result in sludge being discharged with the effluent, however.

The aeration system used has a significant influence on the floc size in a treatment plant. When surface aerators are used, the size of the flocs can vary from 25 µm to 250 µm. With diffused air aeration, the flocs are noticeably larger (range: 25–1000 µm; often >500 µm).

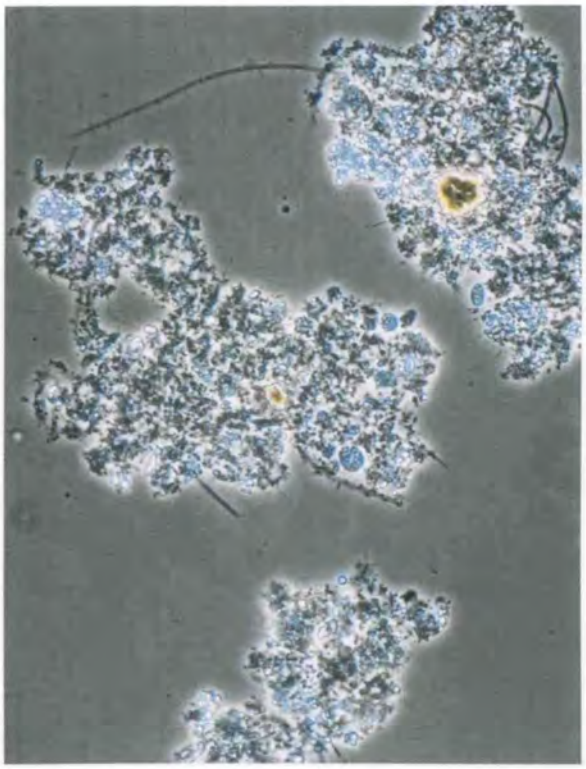


*Figure 17 A robust floc (300×).*

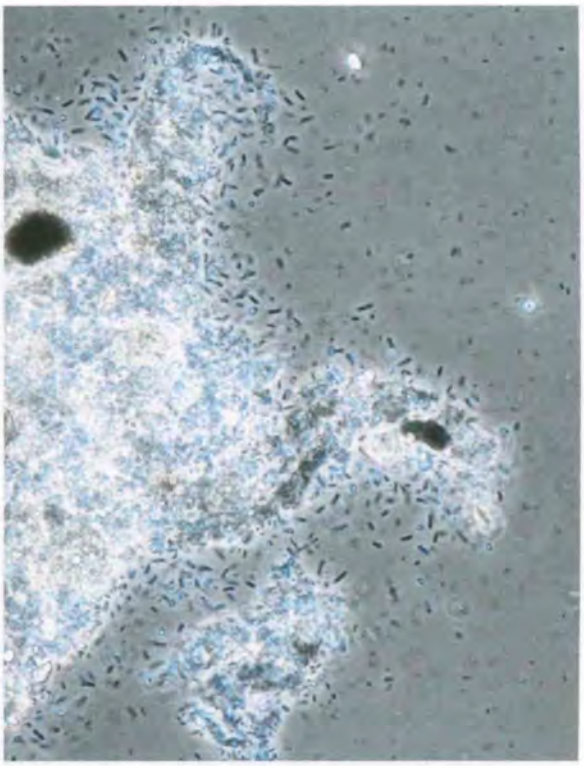


*Figure 18 A weak floc (300×).*

*Figure 19 Floc with a low diversity (900×).*

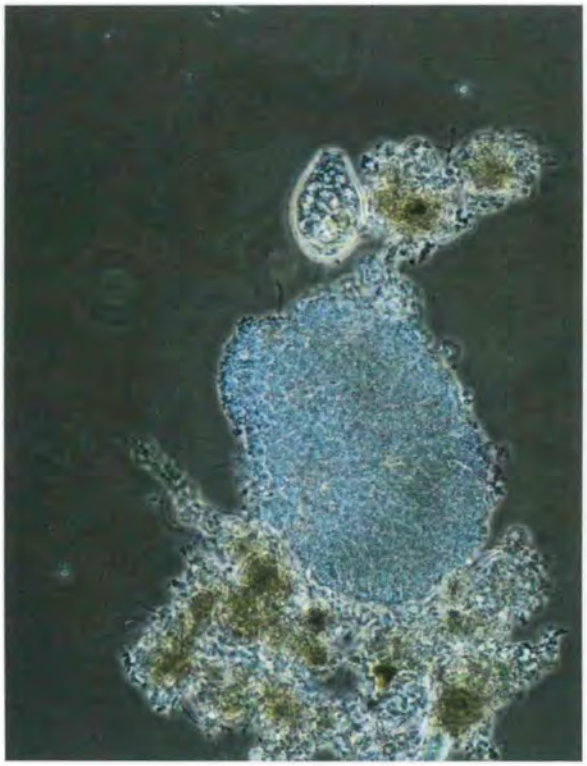


*Figure 20 A monocolony (150×).*

A high percentage of small flocs (> 20–30%) in any treatment plant can have different causes:

- complexing agents (e.g. EDTA) in the influent. Such compounds extract the divalent cations from the floc, causing them to fall apart into smaller particles;
- an extremely low sludge load (< 0.025 kg BOD/kg MLSS. day). The flocs disintegrate owing to the degradation/consumption of the polymeric capsules around the cells in the floc;
- extremely turbulent conditions in the aeration tank;
- the addition of Al salts. Aluminium ions probably change the surface properties of the (hydrophobic) cells in the floc;
- poisoning of the sludge.

## 4.2 Composition of the floc

*Diversity*

A sludge floc is usually comprised of a range of micro-organisms. Owing to this large species diversity, an activated sludge system is very flexible and many different compounds can be degraded simultaneously. This multiplicity of species can be observed microscopically (100× objective). Attention should be paid to the variation of the shapes and measurements of the cells in the flocs. Furthermore, the cells are usually imbedded in amorphous organic material (humic remains and such like)

With a small range of diversity (Fig. 19), the plant becomes more vulnerable because the treatment performance is completely dependent upon the functioning of a limited number of of bacterial strains. If there is a larger range of species, the role of bacteria which cease to function can often be taken over by other micro-organisms.

A low diversity only occurs in high-loaded industrial treatment plants fed with an unbalanced influent (lack of specific nutrients). It is seldom observed, and for this reason it is also not mentioned on the analysis form.

*Monocolonies*

Conglomerates are sometimes present in and between the sludge flocs, which appear to be composed of one type of cell (Fig. 20). These are held together by a slime matrix around the cells and are known as monocolonies. They are principally observed in nutrient removal plants. The size and shape of monocolonies are very variable and they can reach the size of activated sludge flocs. Four groups can be distinguished.

1. Zoogloea colonies often have a characteristic finger-like shape (Fig. 21). Their presence is indicative of a load > ca. 0.1 kg BOD/kg MLSS. day and/or shortages of certain nutrients. In addition, Zoogloea colonies can only be clearly observed in fresh samples. They disappear within 2 to 3 days if the sludge is stored in a refrigerator.

2. Bacteria that are involved in biological phosphate removal form compact, somewhat rounded colonies. They can be present in the floc (Fig. 22) as well as in the water phase (Fig. 20). In the latter case, they are distinctly larger than the monocolonies in the flocs, of which the diameter is often no more than 10 µm to 20 µm. Bio-P monocolonies are stained dark with Neisser (Figs 8 and 9).

3. Monocolonies can also be present which resemble those of Bio-P bacteria, but which stain yellow-brown with Neisser (Fig. 23). There are indications that these are monocolonies of denitrifying bacteria.

4. The fourth group contains monocolonies in which the cells are not stacked closely together (Fig. 24). Here, the cells are surrounded by a thick layer of slime. Their presence indicates, as does that of Zoogloea's, a high sludge load and/or shortages of certain nutrients.

To record the number of monocolonies, a scale varying from 0 (= absent) to 3 (= numerous colonies/slide) is used.

*(In)organic particles*

The greatest part of an activated sludge floc is comprised of living and dead bacterial cells. In addition, macro-molecular (in)organic particles, which are obviously not of bacterial origin, are also often present. These are particles which have been transported with the influent and which have been subsequently encapsulated by the sludge flocs. Apart from their size, the organic particles can principally be recognised by their fibrous structure (Fig. 25). The inorganic particles, mainly sand grains, etc., possess a larger refractive index than the other floc material. They are conspicuous on account of their relatively great brightness (Fig. 26). The extent to which these types of particle are present is mainly determined by the absence or presence of a grit chamber and/or a pre-settlement tank

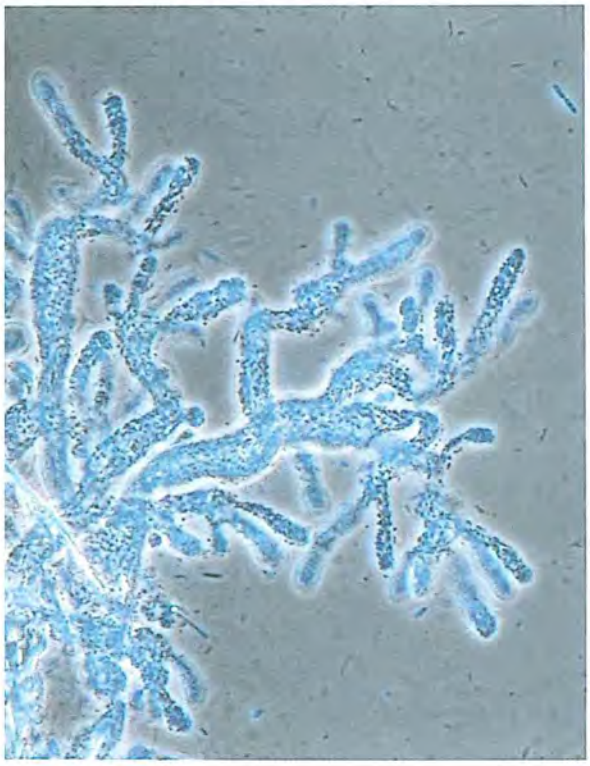


*Figure 21 Zoogloea colony (300×).*

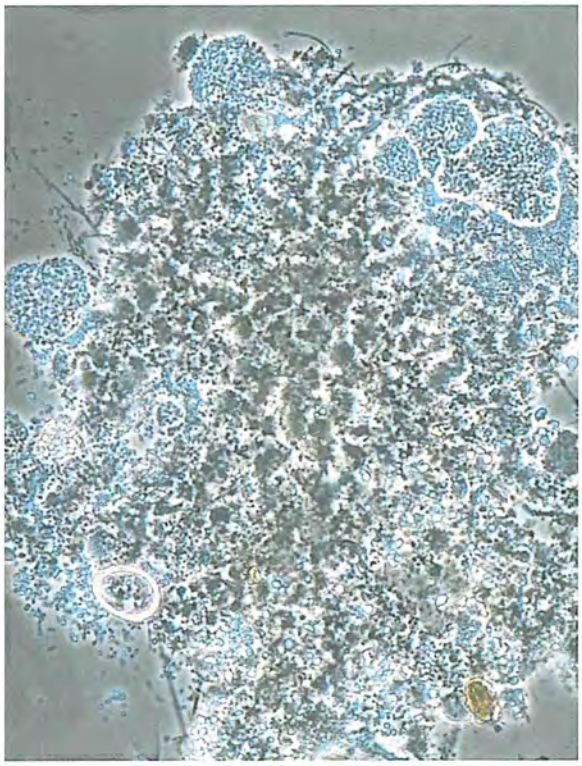


*Figure 22 Bio-P colonies in the floc; not stained (300×).*

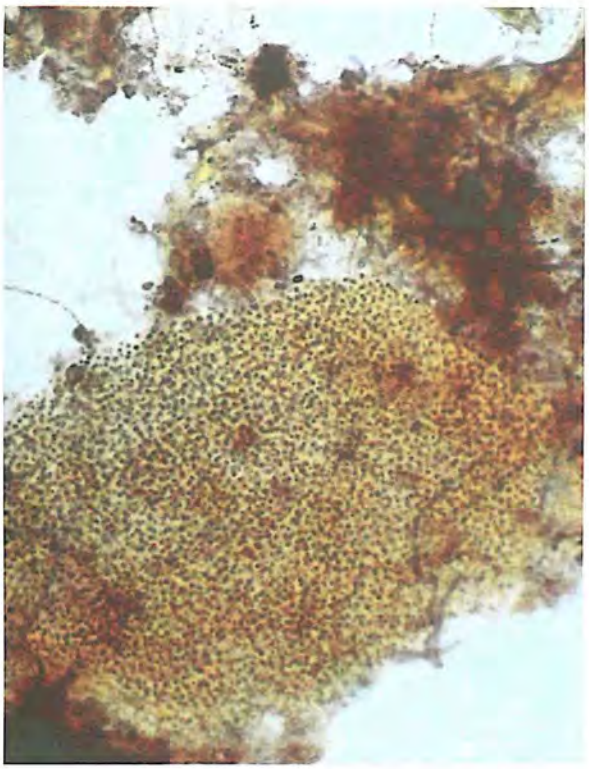


*Figure 23 Monocolony of denitrifying (?) bacteria after Neisser staining (900×).*

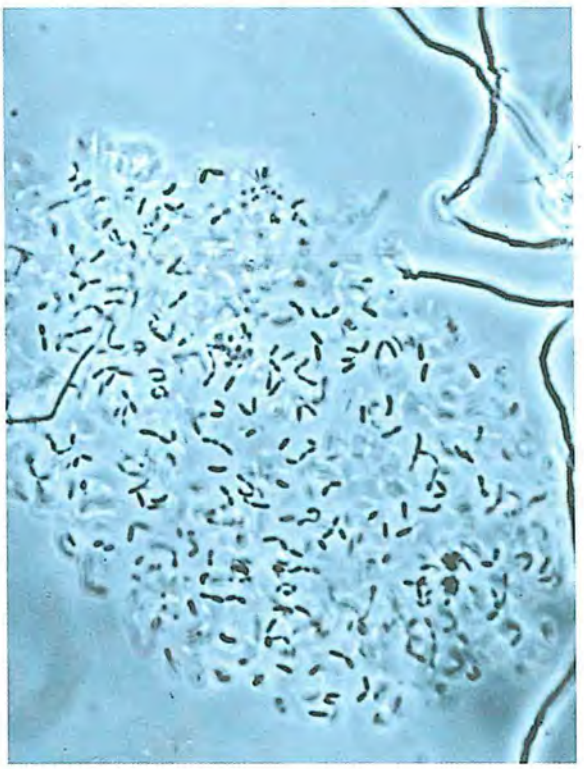


*Figure 24 Monocolony of cells with a slime matrix (900×).*

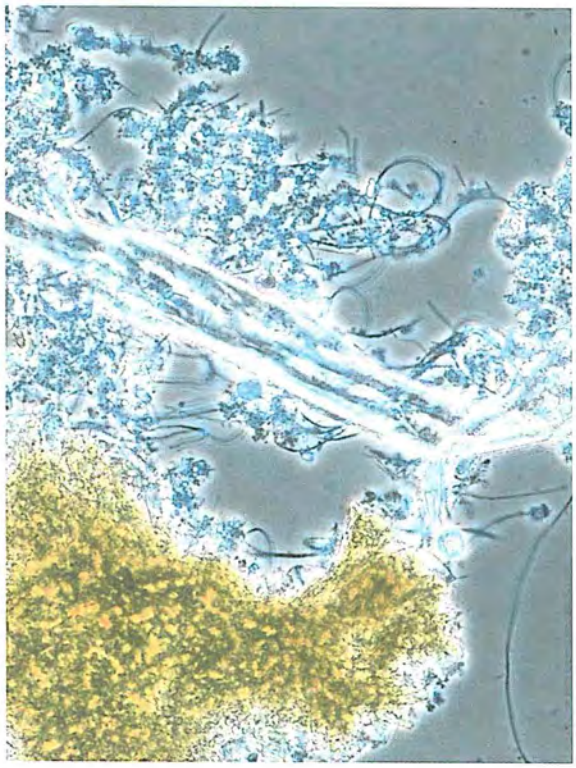


*Figure 25 An organic fibre in activated sludge (500×).*

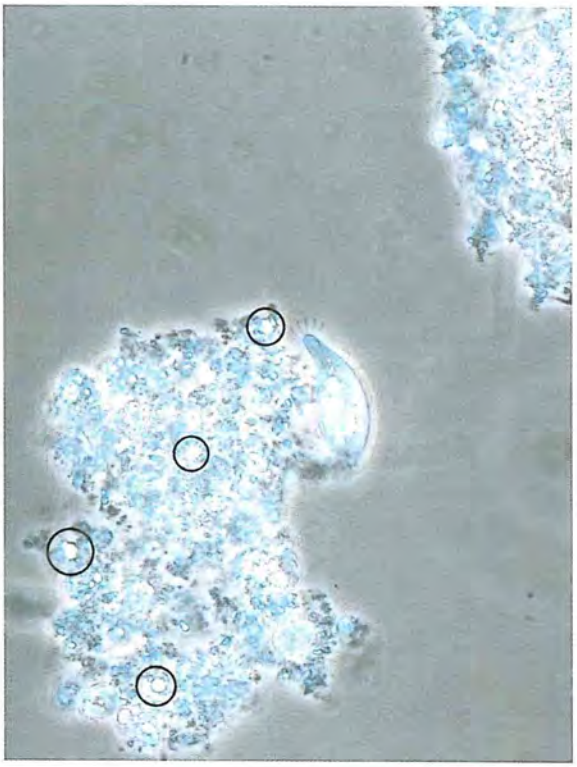


*Figure 26 Sand grains in activated sludge (150×).*

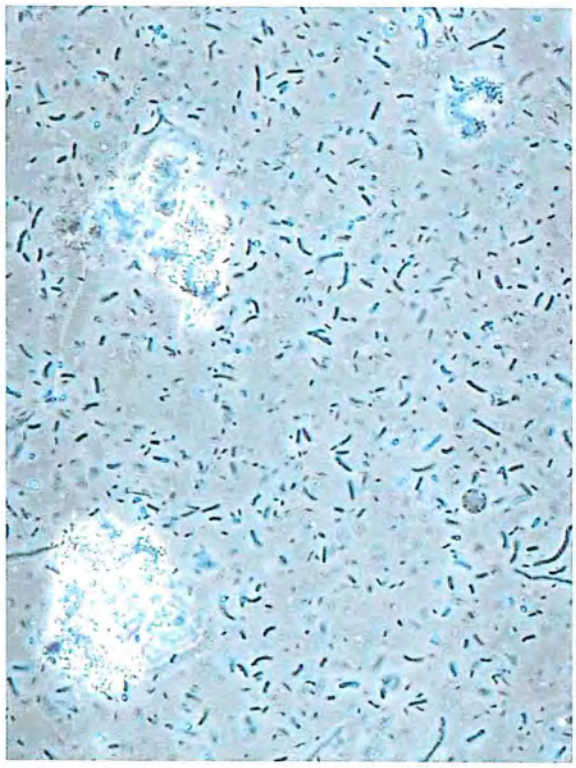


*Figure 27 Many free-living cells (300×).*

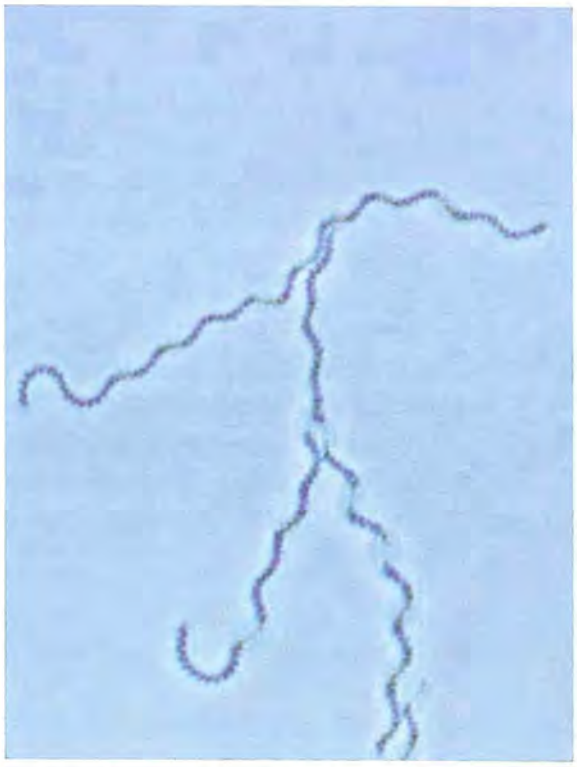


*Figure 28 Spirochaetes (900×).*

## 4.3 Free-living cells, spirochaetes and spirils

*Free- living cells*

The term 'free-living cells' refers to cells that are not bound to the floc but which are loose in the water phase (Fig. 27). They do not settle in the final clarifier. Therefore, free-living cells adversely affect the quality of the final effluent.

To register the number of free-living cells, a scale varying from 0 (= absent) to 3 (= hundreds of cells/field of view) is used.

Supply together with the influent as well as 'erosion' of the flocs are both sources of free-living cells. The free cells are removed extensively by the ciliates in particular. These protozoa are therefore indispensable for maintaining a clear effluent. If the sludge load rises, the number of 'available' free-living cells increases (more supply with the influent and a less robust floc), as a result of which the size of the protozoa population also increases. However, with short sludge ages protozoa can no longer be maintained, as they do not grow fast enough. The limit is a sludge load of 0.3 to 0.4 kg BOD/kg MLSS. day. If this load is exceeded, many free cells but hardly any predators are present in the sludge. The presence of many free cells at a low sludge load is indicative of a lack of oxygen or of the presence of toxic components in the influent.

*Spirochaetes*

Spirochaetes are extremely flexible bacteria which 'wriggle' through the water in a very characteristic manner. The cell forms a double spiral with one long and one short coil (Fig. 28). The cells usually have a diameter of ca. 0.5 µm and are 20 µm to 30 µm in length. To register the number of spirochaetes, a scale varying from 0 (= absent) to 3 (= numerous cells/slide) is used.

Spirochaetes commonly occur in low loaded activated sludge plants. Numerous cells per field of view are sometimes present. Their number can increase during microscopic investigation because spirochaetes are often 'hidden' in the flocs. Spirochaetes disappear if the sludge sample is stored for a few days in a refrigerator.
Spirochaetes are principally observed in plants with anoxic conditions (denitrification) in the centre of the floc or in a separate section of the aeration tank.

*Spirils*

Spirils have bowed cells which often move rapidly through the water. They move in a straight line with a very characteristic, 'corkscrew like' movement which can stop abruptly. The diameter can vary from 0.2 µm to 1.7 µm and their length reaches 20–30 µm.
To register the number of spirils in activated sludge, a scale varying from 0 (= absent) to 3 (= numerous cells/slide) is used.

The presence of spirils in an activated sludge is indicative of a lack of oxygen in the treatment plant.

## 4.4 Floc types

The major influence that the process conditions have on the characteristics of the flocs ensures that sludges from similar treatment plants closely resemble each other. This is also valid for the composition of the protozoa and metazoa populations (paragraph 6.3). It must be verified during microscopic investigation, whether the floc type observed is characteristic of the treatment plant involved. Any deviations must of course be noted. In (Dutch) domestic treatment plants, more than 90% of all sludges belong to the following three floc types.

*Floc type 1*

Floc type 1 is characteristic of treatment plants with a sludge load < ca. 0.2 kg BOD/MLSS.day using surface aerators for the oxygen supply. This group includes oxidation ditches and carrousels.

The flocs have the following characteristics if filamentous bacteria are largely absent:

- medium size (25–250 µm);
- robust (i.e. few free-living cells);
- rounded to angular;
- not really compact. It is often clearly visible that the floc is in fact comprised of separate, compact 'sub-flocs'.

The presence of filamentous bacteria, especially *M. parvicella*, results in the formation of agglomerates (Fig. 16), causing the average floc size to increase. The development of *M. parvicella* displays a definite seasonal pattern, with the minimum occurring in summer and the maximum at the end of the winter. This means that a summer and winter image are often discerned with floc type 1. In addition, closing up of the agglomerates indicates that the floc-forming bacteria again have the upper hand.

Biological nutrient removal may result in an increase of the number of monocolonies, especially in summer. The number of free-living bacteria cells often somewhat increases under nutrient removal conditions, probably as a result of the long anoxic periods.

Adding iron salts for chemical phosphate removal is associated with flocs having a brown colour. Dosing with aluminium salts for this purpose causes a reduction of the average floc size.

*Floc type 2*

Floc type 2 is distinctive of treatment plants with a sludge load < ca. 0.2 BOD/kg MLSS day and diffused air aeration for the oxygen supply. The flocs have the following characteristics:

- (medium)large; range 25–1000 µm, often > 500 µm;
- robust (i.e. few free-living cells);
- rounded;
- compact;
- sometimes a frayed edge.

On account of the lesser turbulence in the water, the flocs are larger, on average, and more compact than those of floc type 1. This difference also results in floc type 2 having a darker colour.

Filamentous bacteria in general hardly affect the morphology of the flocs. They mainly grow at the edges of the flocs or in the water phase between the flocs. More irregularly shaped flocs only arise if filaments with much attached growth are present (e.g. Type 0041).

Refer to floc type 1 for information concerning the effects of (biological) nutrient removal on the morphological features of the floc.

*Floc type 3*

Floc type 3 is characteristic of treatment plants with a sludge load > 0.2 to 0.3 kg BOD/kg MLSS.day. The flocs are not particularly robust, causing many free-living cells to be present. With short sludge ages, the most important bacteria consuming protozoa (the ciliates) cannot be maintained in the plant because they do not grow fast enough. This also contributes to the increase in the number of free-living cells.

Monocolonies can be present, especially the very characteristic Zoogloea colonies.
The size and the shape of the flocs are determined by the the aeration system applied. The flocs are often large, but irregularly shaped, in treatment plants with diffused air aeration. They are smaller and more rounded with surface aerators. The flocs are often open structured even if filaments are absent.

Microscopic investigation establishes which floc type is present. The floc type number is registered on the analysis form. The number is combined with a 4 (e.g. 1 - 4) if the percentage of small flocs is greater than 20 to 30%. Other deviations are mentioned under 'remarks'.

*Industrial treatment plants*

Sludges from industrial treatment plants often display large variations in the morphology of the floc and cannot be classified into a few groups. Therefore, the following comments must suffice:

1. The floc in various industrial sectors (particularly in those treating 'chemical' waste water) is not really robust and the use of surface aerators is commonly accompanied by small flocs and floc fragments. The sludge often appears 'messy'.

2. A growth *en masse* of filamentous bacteria, often results in large but markedly irregularly shaped flocs.

3. In fact, the 'normal' floc quality must be established individually for every industrial treatment plant. This must occur on the basis of the criteria below:

- shape
- structure
- strength
- size
- any other characteristics

The floc quality established thus can subsequently be used as a reference for the routine monitoring of the activated sludge in this plant.

# 5 Filamentous micro-organisms

Filamentous micro-organisms are bacteria, fungi and algae whose cells do not become detached from one another following cell division. Filaments comprising several cells arise in this manner. Sometimes the cells cannot detach as they are surrounded by a sheath (a sort of cover). Transverse walls or septa are always present between the cells in a filament. These septa are not always clearly visible with light microscopy, however.

Growth in the form of a filament is characteristic for certain bacteria species. These bacteria form filaments under practically all conditions. The supposition sometimes put forward, that most filamentous bacteria form free-living cells under other circumstances and that growth in the form of a filament is an abnormality, is therefore incorrect.

Some 30 different filamentous species have been observed in activated sludge. These are principally bacteria. Of these 30, 10 are frequently encountered. It may be expected that unknown species will be present in industrial treatment plants and the inventory is therefore incomplete. Most filaments do not have a name, but a number, because their characteristics are not yet (completely) known.

An identification key has been developed for the most commonly occurring species, with which their identity can be relatively easily determined.

Filamentous species are normal micro-organisms which are virtually always present in activated sludge and contribute to the treatment process. They can be present free in the liquid as well as bound to the flocs.

A growth *en masse* of filamentous micro-organisms results in the formation of bulking sludge and a deterioration of the settling and dewatering properties of the sludge. In addition, some species are also responsible for scum formation.

## 5.1 The filament index

The size of the filamentous micro-organism population can vary considerably. For controlling the process, it is necessary that the population size can be quantified in some way. In principle, it is possible to fairly exactly determine the number of filaments, and their length, in a given sludge by counting and measuring. However, this is not only an extremely time consuming task, but it can only be conducted if relatively straight filaments are concerned. Coiled filaments, which often form into tangles and run right through the floc, cannot be measured in this manner. Compared with the determination of the Filament Index (FI), various other quantification methods referred to in the literature are also too labour intensive for the routine investigation of activated sludge.

The filament index is a measure of the number of filamentous micro-organisms in activated sludge. A scale of 0 to 5 is used (from none to very many filaments). There is a difference of approximately a factor of 10 between the consecutive FI classes. The FI is established by comparing the microscopic image of the sludge, at a low magnification, with a series of

reference photographs of the various FI classes (Figs 29–38). The sludge receives the FI value of the photograph which best corresponds with the number of filaments in the microscopic image.

The number of filaments is, therefore, visually quantified when determining the FI. This seems to be not too exact but, in practice, it has been shown that this method is not only quick to carry out but it also provides very relevant information. With an FI of 1 or 2, the effect of the filaments on the settling velocity of the sludge is still slight. If the FI is 3, the settling properties often deteriorate noticeably, especially if robust filaments are present. Bulking sludge usually occurs at higher FI values.

The settling velocity of the flocs is not just determined by the number of filaments but also by the type of filaments, the ash content of the sludge, etc. (see chapter 10). Therefore, the correlation between FI and SVI is not always consistent, especially if the results from different treatment plants are combined.

Finally, the following points are important when determining the FI:

- it is a visual estimation of the number of filaments. This means that minute details are not examined as the screening of the slide must be done quickly;
- the difference between e.g. $3^+$ and $4^-$ is small. The choice of a given FI is therefore sometimes arbitrary. It is of course also possible to fill in 3.5 on the analysis form;
- in case of doubt, the FI is rounded up if few flocs are present on the slide and rounded down if many flocs are present;
- if there is an uneven distribution of the filaments on the slide, their number is visually averaged;
- the FI division is mainly based on observations made at a magnification of 100–200×. However, sometimes it appears to be necessary to revise this classification if the sludge is viewed at a higher magnification. This is the consequence of the fact that thin filaments are sometimes missed at lower magnifications;
- filaments that are hidden in the floc cannot be properly observed and, therefore, are not included when establishing the FI. The effect of these filaments on the settling velocity of the flocs is limited (only indirectly through the influence of the floc structure). These filaments are clearly visible after Gram staining, as a result of which many more filaments sometimes seem to be present than was first estimated.

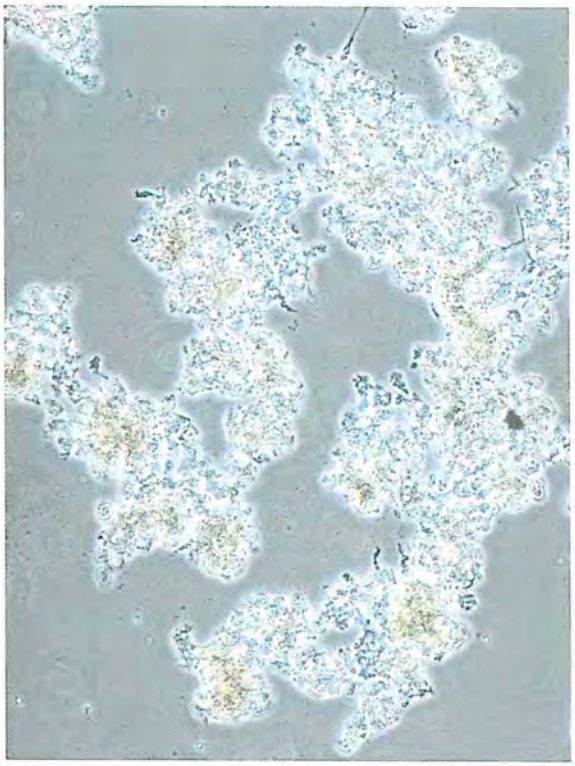


*Figure 29 FI = 0 (150×).*

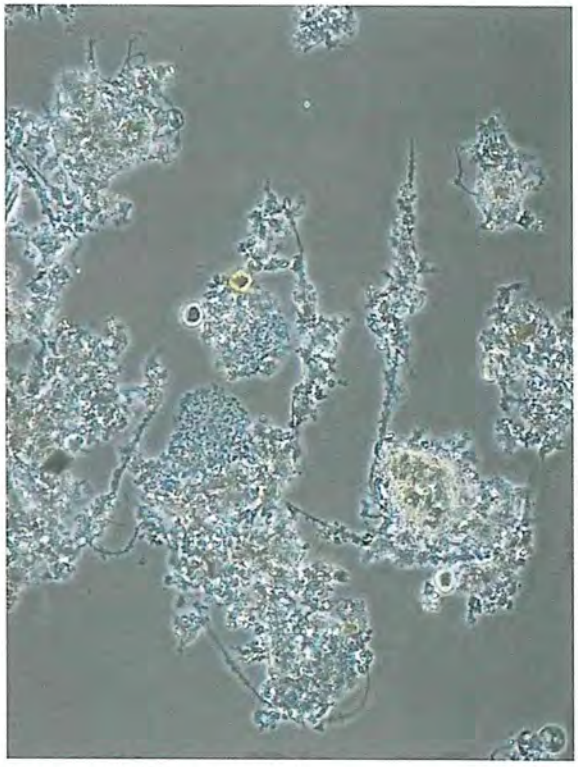


*Figure 30 FI = 1 (150×).*

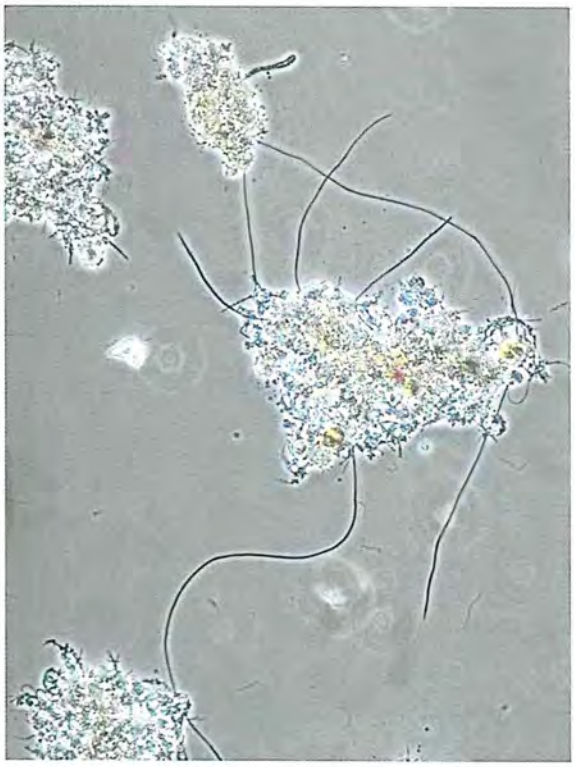


*Figure 31 FI = 2; robust filaments (150×).*

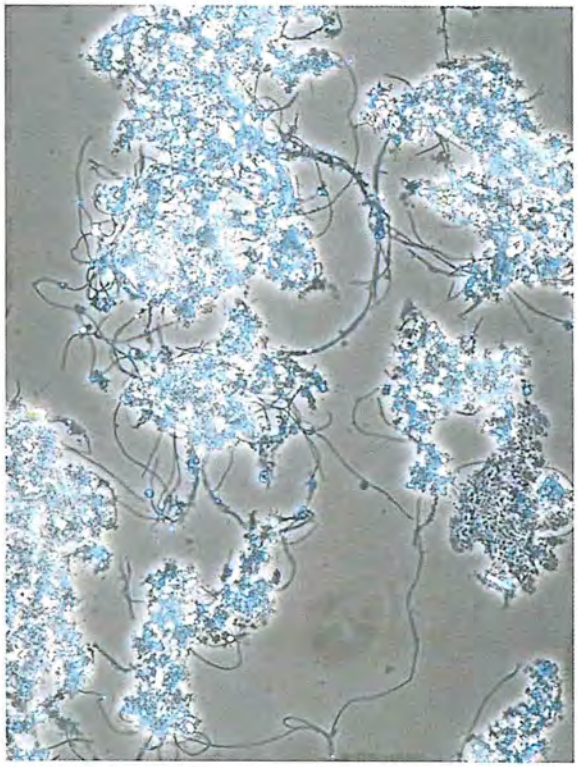


*Figure 32 FI = 2; thin filaments (300×).*

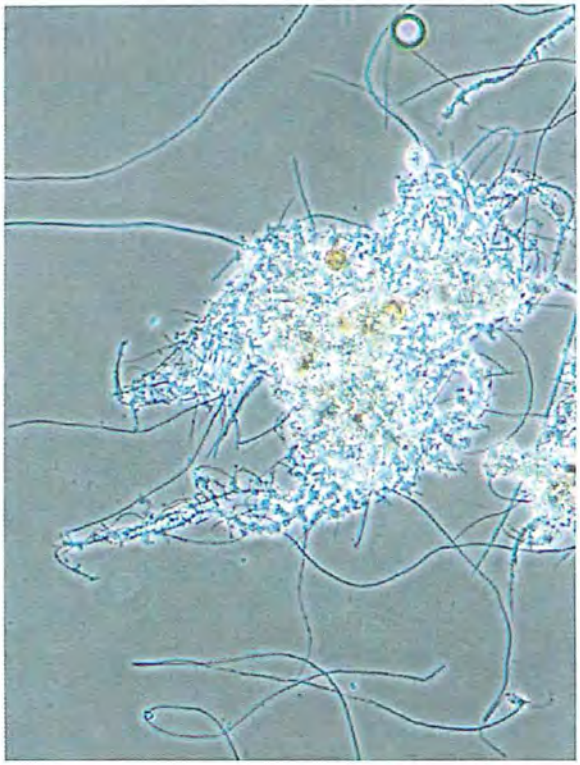


*Figure 33 FI =3; robust filaments (150×).*

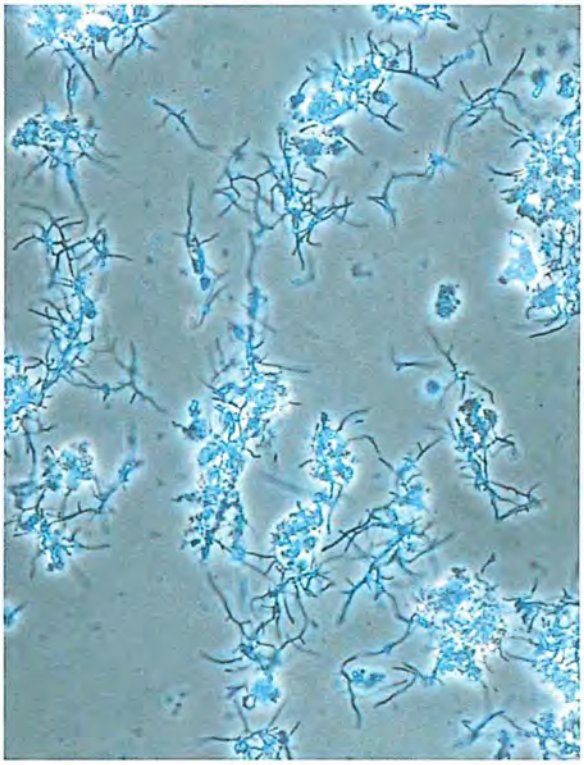


*Figure 34 FI = 3; thin filaments (300×).*

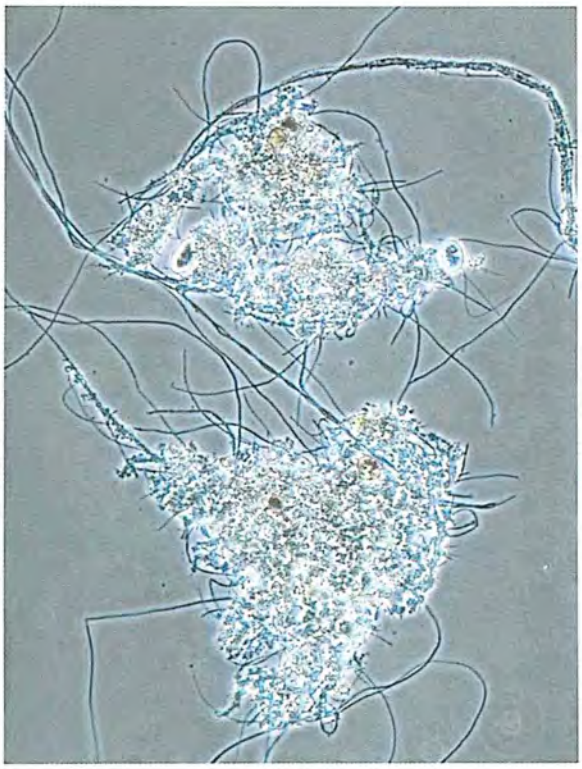


*Figure 35 FI =4; robust filaments (150×).*

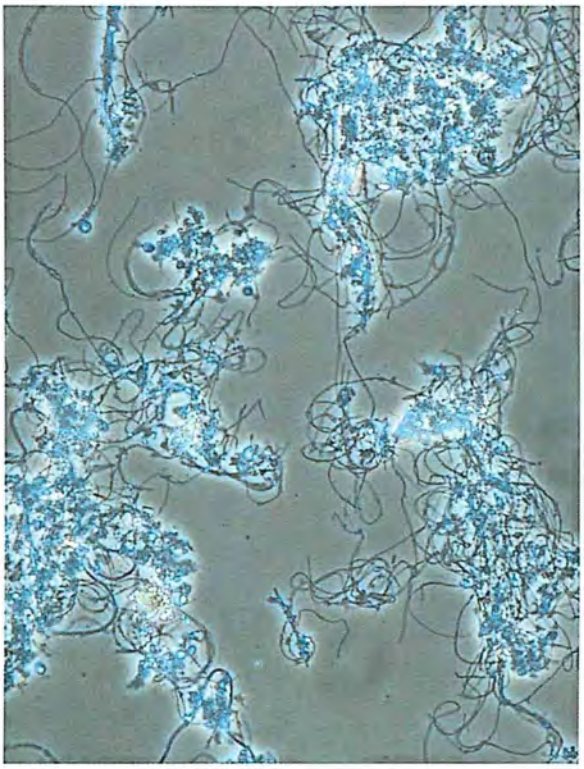


*Figure 36 FI = 4; thin filaments (300×).*

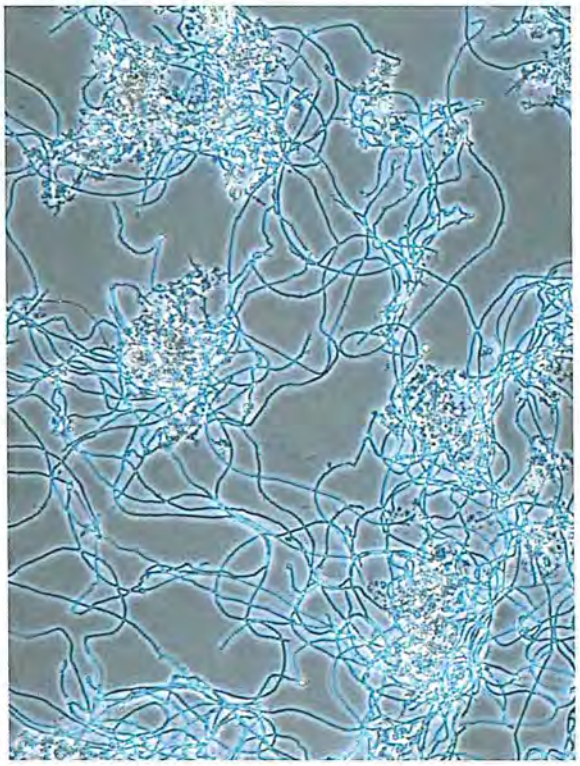


*Figure 37 FI =5; robust filaments (150×).*

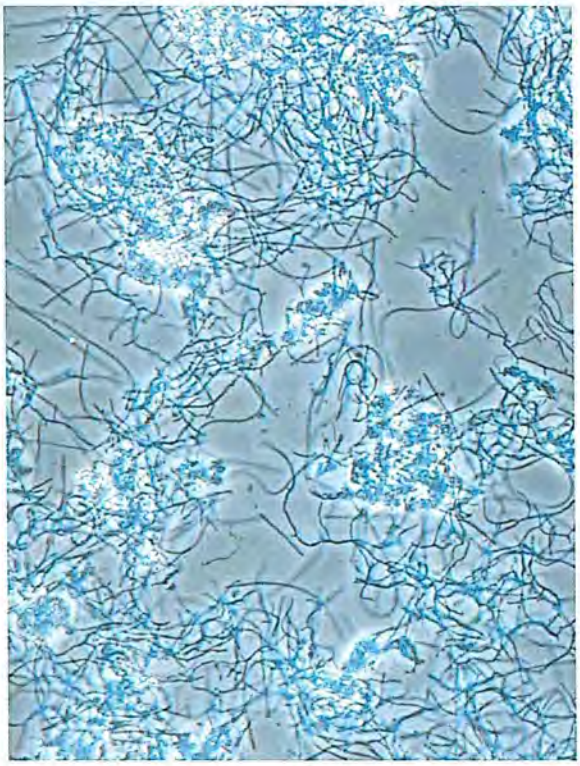


*Figure 38 FI = 5; thin filaments (300×).*

## 5.2 Identifying filamentous micro-organisms

As will be discussed in chapter 8, the activated sludge population has a dynamic character. This means that the size and the composition of the population of filamentous micro-organisms varies in relation to changes in the process conditions. In process control, it is important for three reasons to know which filamentous micro-organisms are present.

- the nature and the extent of problems with the process stability are dependent upon the filamentous species present.
  The development of 'notorious' filamentous organisms must therefore be detected as soon as possible;

- no general bulking sludge control methods exists. So it must first be established which species is present;

- certain species can be used as indicator organisms for the process conditions.

### *5.2.1 Identification characteristics*

A total of 11 characteristics are determined in order to identify filamentous micro-organisms. Some of these characteristics are illustrated in Figs 39 and 40.

*Mobility*

Only a few filamentous bacteria (*Beggiatoa* and *Flexibacter)* are able to move freely. This occurs by means of 'gliding movement' resembling the manner in which a worm propels itself.

If it is thought that a certain filament appears to move, then it should be verified that there is no liquid flow on the slide owing to evaporation of the water along the edges.

*Branching*

The absence or presence of branching is an important characteristic for identifying filamentous bacteria in activated sludge. **Real** and **false** branches are distinguished.

Actinomycetes and fungi show **real** branching. The side shoot actually sprouts from the main branch. With real branching, the cell in fact grows in two directions.

Sheath forming bacteria, principally *Sphaerotilus natans* and Type 1701, form **false** branches. These arise because 'swarming cells' attach themselves to the sheath around the filament and subsequently, through cell division, develop into side shoots (including a sheath).

Swarming cells are cells which have split away from the free end of the filament and temporarily, i.e. as long as they have not yet become attached, are present in the sludge as free-living cells. A side branch can also be formed at a spot where damage to the sheath has occurred.

In the case of false branching, a very small space is often visible between the cells in the main branch and those in the side shoot. This space is lacking in the case of real branching. False branches are always 'V' shaped, while real branches often stand 'dead straight' on the main branch (but not in the case of the actinomycete *Skermania piniformis*). Actinomycetes always form numerous real branches. Several branched filaments gathered together clearly resemble a bunch of sticks. Fungi are easily distinguishable from the sheath forming bacteria and the actinomycetes on account of their much more robust filaments.

Attachment of single-celled bacteria to a filament (= attached growth) is sometimes mistaken for branching.

Occasionally in the sludge, filaments are observed which are attached to each other at their bases. This is known as a rosette of filaments. These bacteria secrete substances with which they attach themselves. *Thiothrix* and *Leucothrix* strains mainly form rosettes.

*Filament shape*

Three groups of filament shapes are distinguished:
- 'straight'
- bowed/bent
- twisted/coiled

The word 'straight' has been placed between inverted commas, as only *Haliscomenobacter hydrossis* forms very straight filaments. Straight examples are indeed somewhat bent, especially where relatively long filaments are concerned. During microscopic investigation, a distinction should be made between (1) straight to slightly bent and (2) bent to coiled filaments.

*Attached growth*

The surface (= the outside wall) of filamentous micro-organisms is usually 'clean'. Other cells or particles of floc are sometimes attached to the surface, however, and partially cover it. This is known as 'attached growth'. The filaments of bacteria which have a sheath around the cells are often surrounded by attached growth.

When identifying filaments, a distinction must be made between (1) little or no attached growth and (2) much attached growth.

*Filament diameter*

Filamentous micro-organisms are divided into three groups on the basis of their diameters:
- diameter < 1 μm
- diameter 1–2.5 μm
- diameter > 2.5 μm

For some filaments (Type 021N and *Thiothrix* strains), the cell diameter sometimes gradually decreases towards the tip of the filament.

*Septa or transverse walls*

Transverse walls or septa are the walls between consecutive cells of a filament. Septa are not always clearly visible with a light microscope. When identifying filamentous organisms, a distinction should be made between (1) clearly visible and (2) poorly visible/invisible. Where filaments possess much attached growth, attention should be paid to their clean extremities for assessing this feature.

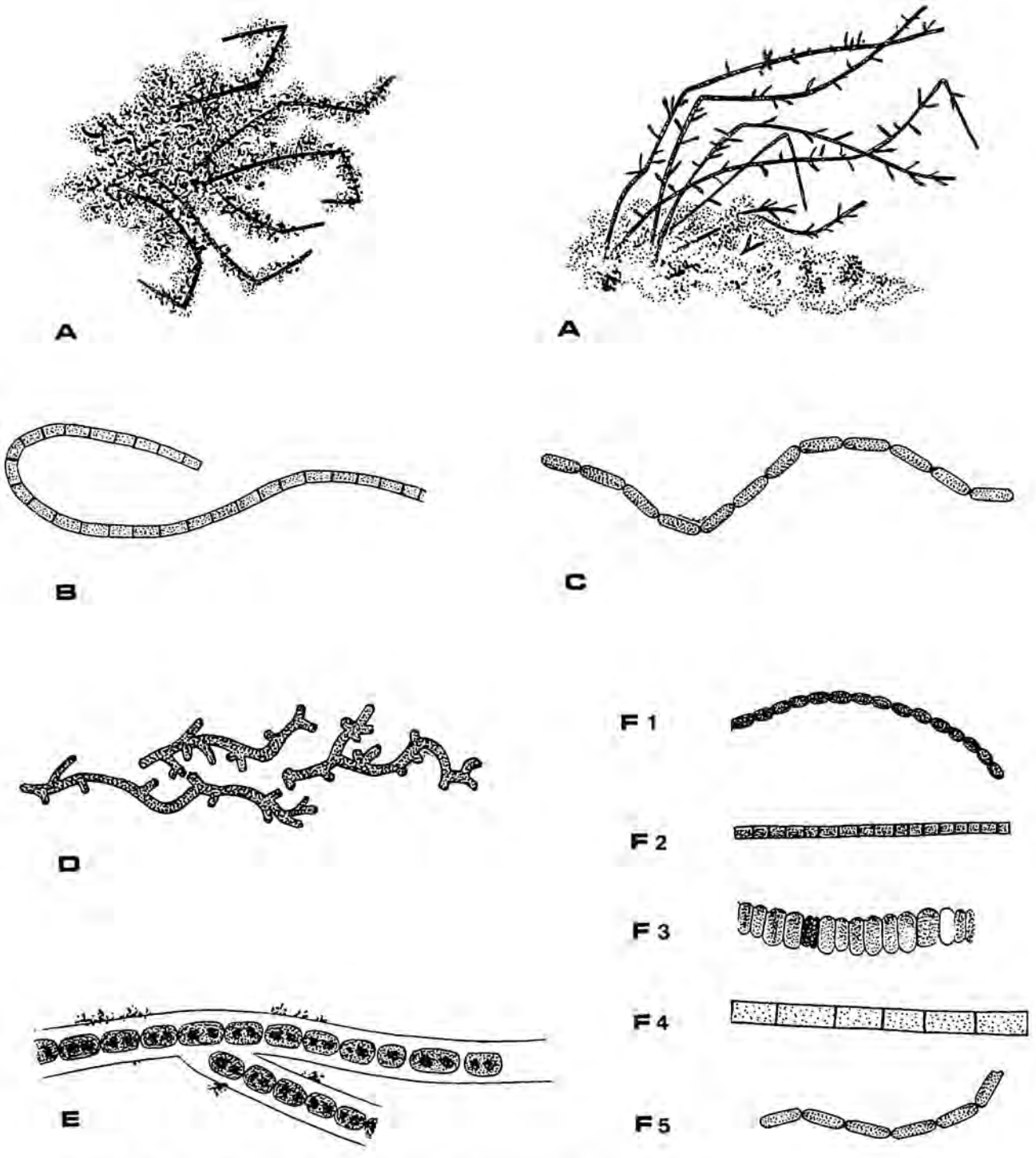



*Figure 39* *Various morphological characteristics*
*A: Attached growth to the filaments*
*B: No constrictions of the outside wall*
*C: Definite constrictions present*
*D: Real branching*
*E: False branching; the cells are surrounded by a sheath*
*F: Cell shapes; (1) almost round, (2) square, (3) disc shaped, (4) rectangular, (5) rod shaped.*

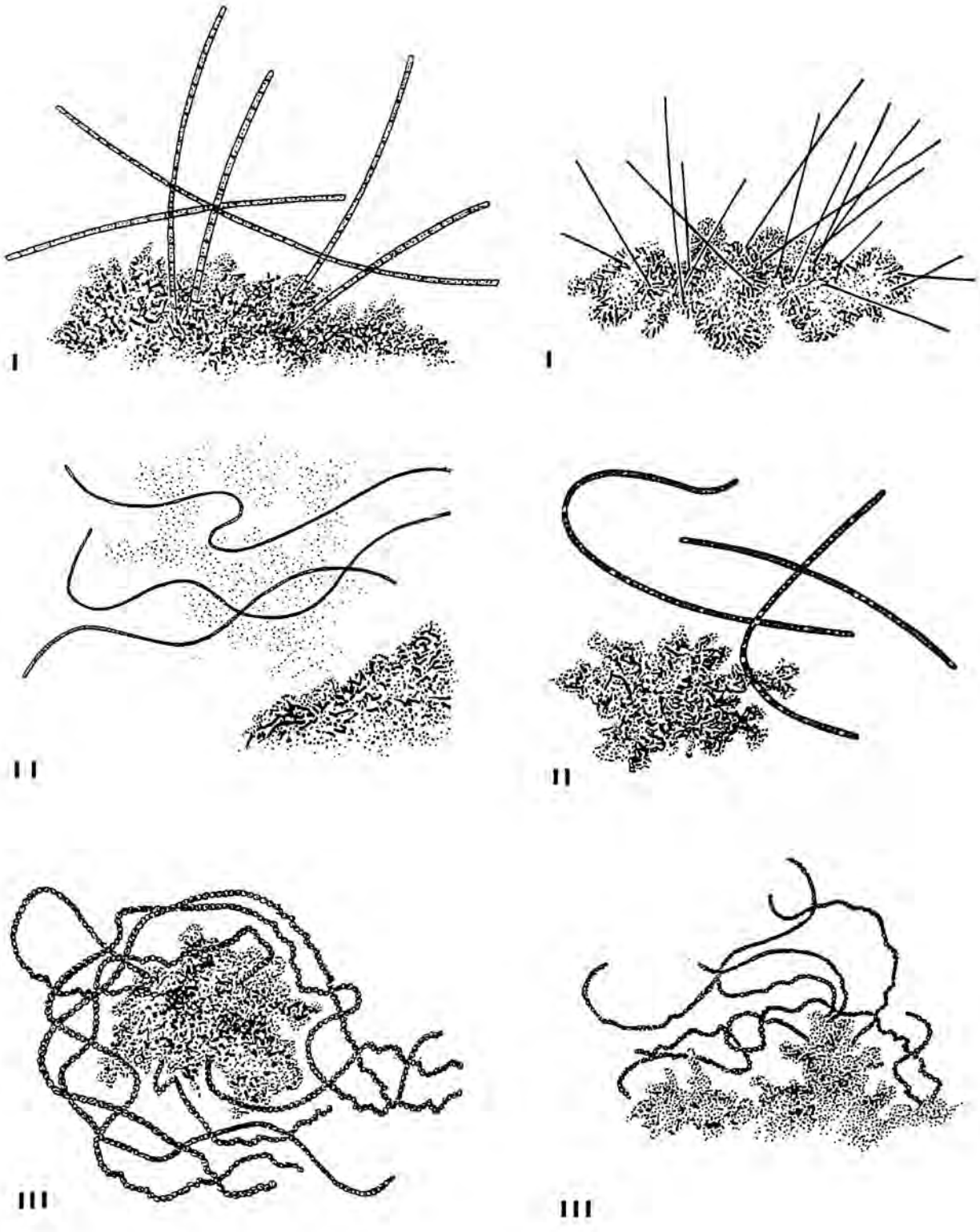



*Figure 40* *Shape of the filaments*
**I**: *straight filaments*
**II**: *bent filaments*
**III**: *twisted filaments.*

*Cell shape*

The following cell shapes can be distinguished in free-living bacterial cells:

- spherical or coccus : the cells are approximately round;
- rod shaped : the length of the fairly straight cells is clearly greater than the cell diameter;
- spiral shaped : the length of the spiral turned cells is always much greater than the cell diameter. Spirochaetes (flexible cell) and Spirils (non-flexible cell) are hereby distinguished;
- 'vibrio' shaped : the cells are somewhat bowed.

Filamentous micro-organisms comprise coccus or rod shaped cells or other cell shapes derived from these shapes. This means that the cells in a filament are square or rectangular, if there are no constrictions of the communal outside wall at the position of the transverse wall.

The cell type can actually only be determined if the septa are clearly visible. The length of the cells in a given filament is often highly variable. The cell length of some filamentous species (Type 021N, *S. natans* and *Thiothrix*) can increase considerably towards the tip of the filament.

*Sheath*

The cells of certain filamentous species are surrounded by a transparent cover known as a sheath, which is not often visible with a light microscope. The sheaths of some species are indeed visible following Gram staining. The sheath is sometimes more easily visible if the sludge sample is stored for a few days in a refrigerator. A lot of attached growth often indicates that a sheath is present.

In addition, it should be noted that empty cells in a filament are sometimes mistaken for sheaths. The transparent empty cells are generally very short. This means that long, transparent covers must be visible in or on the extremities of the filaments before it can be definitely concluded that a sheath is present. As this characteristic is often not properly established, it is of subsidiary importance for the identification of filamentous bacteria in activated sludge.

*Granules*

Granules are globules of storage material in the cells. Three types of granule are distinguished with microscopic investigation (see also paragraphs 2.6.2 and 2.7):

- polyphosphate granules. Without Neisser staining these granules are poorly or not visible at all;

- sulphur granules. These globules are of a dark shade when still small. On the other hand, larger examples are strongly refractive and are consequently clearly visible;

- other storage materials. This usually involves poly-β-hydroxybutyrate (PHB). PHB granules are hardly ever observed in filamentous microorganisms in domestic treatment plants. Like the sulphur granules, the PHB granules are also strongly refractive. Sulphur granules have a blue-yellow sheen while PHB granules are yellowish. The colour difference is not so easy to distinguish. By allowing alcohol to flow through the slide (a drop of ethanol on one side and a filter paper on the other side), it can be established which storage material is present. The sulphur globules disappear (dissolve) and the PHB granules remain intact.

*The results of Gram and Neisser stainings*

For information on carrying out these staining methods, refer to paragraph 2.6.

### *5.2.2 The concepts of 'predominant' and 'secondary'*

In a given activated sludge, various filamentous micro-organisms are usually present, as a result of which their individual identification is more difficult. Nevertheless, this problem is usually less complicated than it at first appears because the various species are mostly not present in similar quantities. Predominating and secondary strains are in fact always present. It is a general rule of thumb that a certain species is 'secondarily present', if its numbers are one tenth to one twentieth those of the predominating species. One or two predominating and a variable number of secondary species are usually present in low loaded domestic plants. The number of predominating strains can increase at higher sludge loads ( > ca. 0.1 kg BOD/kg MLSS. day).

The distinction between predominating and secondary strains is also separate from the FI classification. Therefore, a distinction must be made between predominating and secondary species with every FI. Only at low FI values (1 or 2) can it occasionally occur that a distinct predominating filamentous species is absent.

### *5.2.3 Identification procedure*

Identification involves several steps, including observing the stained slides.

Depending on the condition of the filaments, characteristics such as the shape of the cells or the diameter of the filaments can somewhat vary. It is therefore important that several filaments of a given species are always viewed before the characteristics are noted.

It is not so easy for less experienced microscopists to simultaneously identify all the species present. This easily results in mistakes. To learn the method properly, it is advisable at the beginning not to carry out identifications simultaneously but one after the other.

Using a low magnification, it is first estimated how many different filamentous species appear to be present. Predominating and secondary strains are distinguished. The morphological characteristics of the filaments are noted. The sheet shown in Appendix B of this handbook can be used for this purpose. It is also established whether the filaments are free in the water phase or are present at the edges of the flocs. The shape as well as the positioning of the filaments are both aids to their detection at a high magnification.

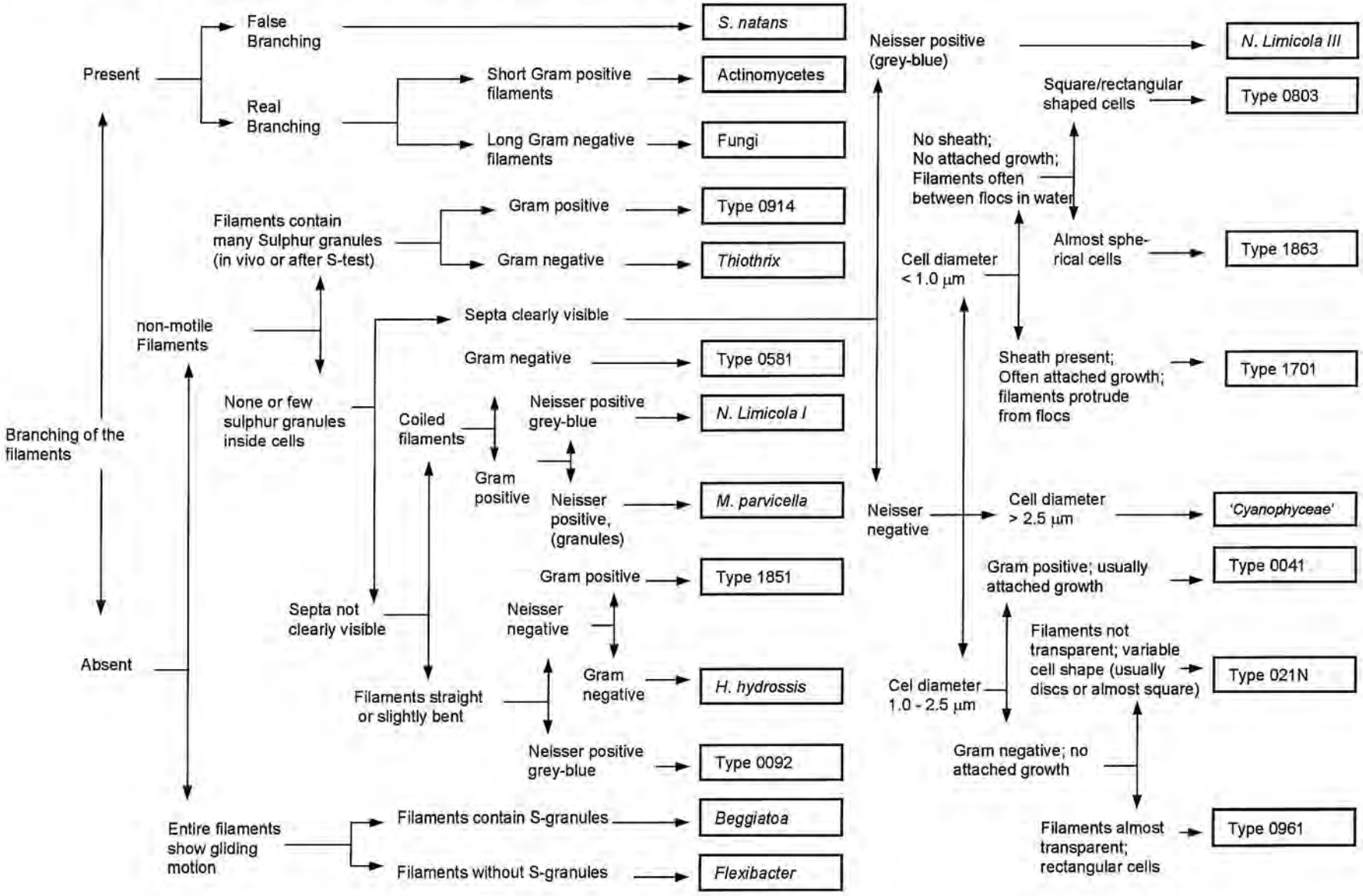
A magnification of 400× is subsequently used and finally one of 1000×. The species that have been observed at the low magnification are sought again on the slide. The various characteristics necessary for identification are now gathered and registered. Finally, the stained slides are viewed at 1000× bright field. Neisser negative filaments are often particularly difficult to recover on the slide.

Identification can now proceed with a complete set of data. Two identification keys have been developed for this. These are presented in the following pages. The same 21 species are included in both keys. The staining results (Gram and Neisser) are the main entries in the second key. The choice of key is a matter of personal preference. Furthermore, several rare strains are not included in the keys.

Whenever a name or a number is established, it should be checked that the conclusion is correct. Filaments in the microscopic image are now compared with photographs of the species involved. In addition, it should be verified that the registered characteristics are largely correct (see paragraph 5.3 and table 1). The observations must again be critically assessed if there are clear differences. The clear presence or absence of the septa is a potential source of confusion. The correct adjustment of the microscope is essential for assessing this characteristic properly. Following the other key is sometimes helpful. In describing the filamentous micro-organisms (next paragraph), the species with which they can easily be confused are mentioned.

Occasionally, where industrial plants are concerned, filamentous micro-organisms can be present in activated sludge which are actually unknown or which are not included in the identification keys.

## Identification key 1 of filamentous organisms in activated sludge



# Identification key 2 of filamentous organisms in activated sludge

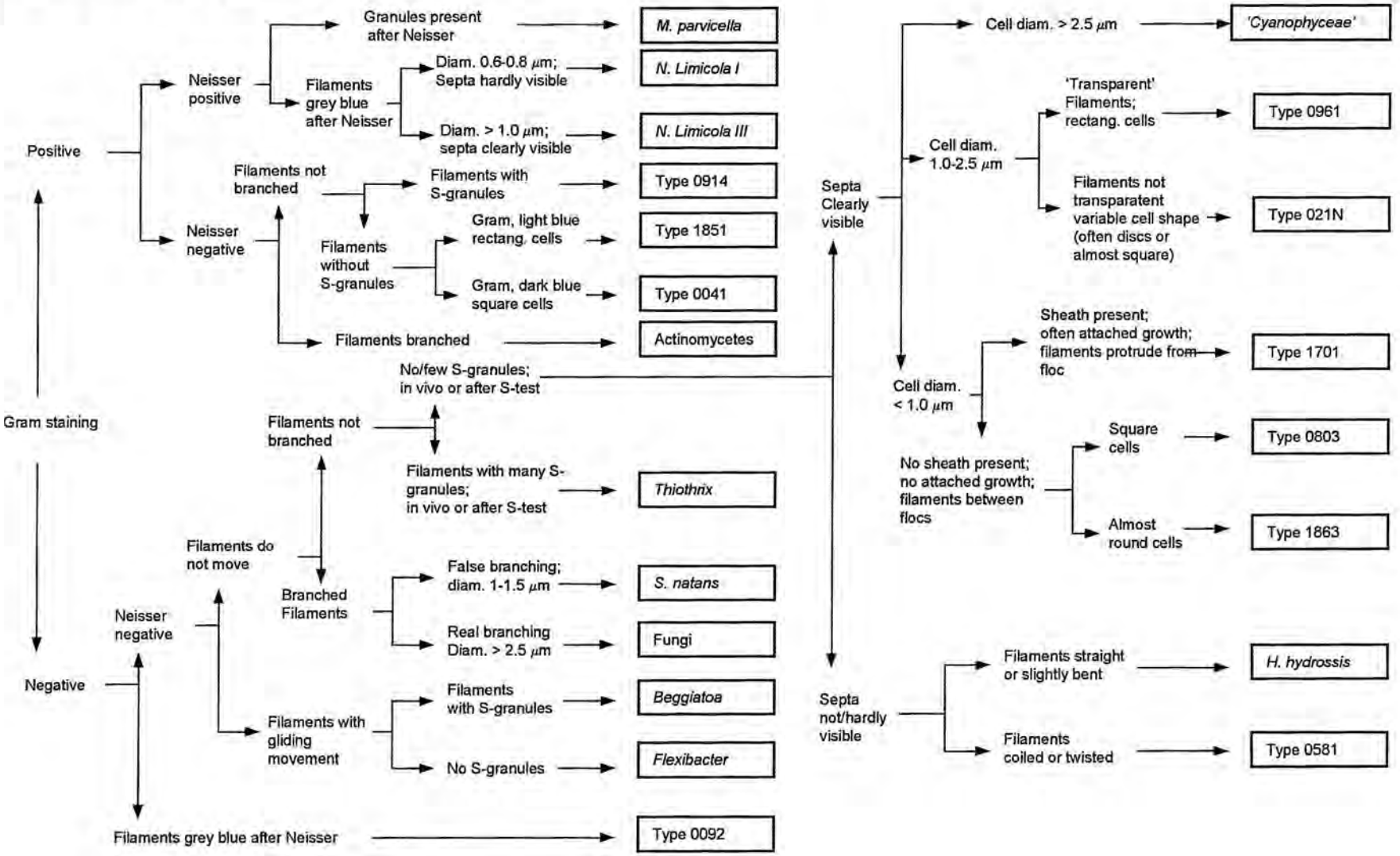


*Table 1* *Characteristics of the filamentous micro-organisms included in the identification key*

| | Phase contrast microscopy | | | | | | | | | | | | | | | | Bright-field Microscopy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Filament shape | | | | | | diameter cells | | | | | S-granules in cells | | Gram stain | | Neisser stain | |
| | Branching | Motion | straight or somewhat bent | Coiled or twisted | Filament length < 200 µm | Attached growth | Sheath present | Septa clearly visible | < 1.0 µm | 1.0-2.5 µm | > 2.5 µm | Cell shape square or rectangular | Cell shape round, disc or rod | In vivo | After Sulphur test | Positive | Negative | Positive | Negative |
| Actinomycetes | + | | | + | + | | | | + | | | ∞ | ∞ | | | + | | ± | + |
| Beggiatoa | | + | + | | ± | | | | + | + | + | ∞ | ∞ | + | | | + | | + |
| Cyanophyceae | | | + | | | | ± | + | | | + | + | + | | | + | + | | + |
| *H.hydrossis* | | | + | | + | | + | | + | | | ∞ | ∞ | | | | + | | + |
| Flexibacter | | + | + | | + | | | ± | + | | | ∞ | ∞ | | | | + | | + |
| *M.parvicella* | | | | + | + | | | | + | | | ∞ | ∞ | | | + | | +[6] | |
| *N.limicola I* | | | | + | ± | | | ± | + | | | | + | | | + | | +[7] | |
| *N.limicola III* | | | | + | ± | | | + | + | + | | | + | | | + | [9] | +[7] | [9] |
| Fungi | + | | + | | | | | + | | + | + | + | | | | [4] | [4] | | + |
| *S. natans* | +[1] | | + | | | ± | + | + | | + | | | + | | | | + | | + |
| *Thiotrix* | [2] | | + | | ± | | ± | +[3] | + | + | | +[3] | +[3] | ± | + | | + | | + |
| Type 0041 | | | + | | ± | + | + | + | + | + | | + | | | | + | | | + |
| Type 0092 | | | + | | + | | | | + | | | ∞ | ∞ | | | | + | +[7] | |
| Type 021N | | | + | | ± | | | + | ± | + | | + | + | | ±[8] | | + | | + |
| Type 0581 | | | | + | + | | | | + | | | ∞ | ∞ | | | | + | | + |
| Type 0803 | | | + | | + | | | + | + | | | + | | | | ± | + | | + |
| Type 0914 | | | + | | + | | | +[3] | + | | | +[3] | | + | | + | | | + |
| Type 0961 | | | + | | | | | + | | + | | + | | | | | + | | + |
| Type 1701 | | | + | | + | + | + | + | + | | | | + | | | | + | | + |
| Type 1851 | | | + | | ± | + | + | | + | | | ∞ | ∞ | | | | | +[5] | + |
| Type 1863 | | | | + | + | | | + | + | | | | + | | | | + | | + |

± = sometimes; ∞ = cell shape can not be discerned with phase contrast ;1) False branching ; 2) Sometimes rosettes; 3) Cells can be discerned after S-globules are dissolved ; 4) Fungi can not be stained with this method ; 5) light blue ; 6) granules ; 7) filaments stain grey blue ; 8) small granules ; 9) Gram and Neisser negative species exist too.

## 5.3 Description of the various filamentous species

A total of 27 filamentous organisms are described in this paragraph. The process conditions under which the various strains are mainly present are also given. Information on this subject is not yet complete. The reader is referred to Chapter 10 for information on control strategies. The descriptions are in alphabetical order and for the organisms that do not yet have a name, in numerical order.

### 5.3.1 Actinomycetes

*Description*
Actinomycetes (Fig. 41) possess the following characteristics:

- real branching;
- non motile;
- 'twisted' filaments (bunch of sticks), in and around the flocs;
- filament length < 200 μm;
- cell diameter < 1.0 μm (often 0.5–0.7 μm);
- no attached growth;
- no sheath;
- septa invisible;
- no sulphur storage;
- Gram positive;
- sometimes Neisser positive (poly-P granules).

The Actinomycetes include several different strains that can only be distinguished from one another by further research. On account of the extremely characteristic branching, confusion with other filamentous species is practically impossible. *M. parvicella* is related to the Actinomycetes.

*Occurrence in activated sludge*
Actinomycetes are notorious for forming scum, particularly in countries where the average water temperature is higher than that in The Netherlands. The transportation of floating material to the sludge digestion tank can also cause scum in this tank. The negative effect on the SVI is limited. Actinomycetes float preferentially, meaning that the population in the scum is often much larger than the one in the sludge that is in suspension. The following process conditions are favourable to the growth of Actinomycetes:

- fats or other hydrophobic constituents in the influent;
- surface-active materials in the influent;
- internal recycling of any floating material;
- water temperature higher than ca. 15°C.

The available knowledge is still incomplete. Gram positive bacteria usually have a hydrophobic surface on which fats, etc. bond well and, in this manner, can be selectively taken up from the water phase. Fats and surface active materials are always present in domestic waste water. In spite of this, Actinomycetes are not always present, also not at higher water temperatures. This can be partially explained by the sludge load applied: Actinomycetes are mainly encountered at higher sludge loading levels (0.1 - 0.7 kg BOD/kg.MLSS.day). Other Gram positive bacteria such as *M. parvicella*, which also grow on the fat fraction from the influent, are frequently present at lower loaded plants. Floc formers which can use this substrate also exist. It is not yet known which factors are decisive in the competition for this substrate.

### 5.3.2 *Beggiatoa*

*Description*
*Beggiatoa* (Fig. 42) possesses the following characteristics:

- not branched;
- mobile (gliding movement);
- straight/bowed filaments, free in the water between the flocs;
- variable filament length;
- cell diameter 1.5–2.5 μm;
- no attached growth;
- septa occasionally visible;
- rectangular cells;
- *in vivo* sulphur storage;
- Gram negative;
- Neisser negative.

The combination of sulphur granules in the filaments and a gliding motion is so characteristic that confusion with other strains is unlikely.

*Occurrence in activated sludge*

*Beggiatoa* occurs in treatment plants with many reduced sulphur compounds in the influent ($H_2S$!) and/or a shortage of oxygen. The filaments do not substantially affect the settling velocity of the flocs. *Beggiatoa* functions as an indicator organism.

### 5.3.3 'Cyanophyceae'

*Description*
The 'Cyanophyceae'(Fig. 43) comprise a group of filamentous organisms possessing the following characteristics:

- not branched;
- immobile;
- straight/bowed filaments free living in the water between the flocs;
- filament length > 200 µm;
- cell diameter > 2.5 µm;
- no attached growth;
- occasionally a sheath;
- septa clearly visible, unless the filaments contain much storage material. These storage materials often disappear if the sludge is stored in a refrigerator for a few days.
- variable cell form;
- no sulphur storage;
- usually Gram negative;
- usually Neisser negative

Filaments are mainly classified in this group on the basis of their cell diameters, which are strikingly big. The cell form and the staining can vary strongly, indicating that this group comprises different species. The somewhat thinner species can be mistaken for Type 021N. The inverted commas around the name indicate that these filaments morphologically resemble Cyanophyceae (= blue-green algae) but, owing to metabolic processes, differ from them considerably. Instead of solar energy (photosynthesis), they use organic compounds to grow.

*Occurrence in activated sludge*
'Cyanophyceae' are not often observed in activated sludge. The following process conditions are favorable to these filaments:

- sludge load > ca. 0.1 kg BOD/kg MLSS.day;
- waste water with a high organic acid content, such as acetic and propionic acid;
- complete mixing in the aeration tank;
- probably also a shortage of nutrients.

This means that these filaments are hardly ever present in domestic treatment plants with nutrient removal. In industrial plants, 'Cyanophyceae' can develop massively in the case of large amounts of organic acids in the influent, leading to high SVI values.

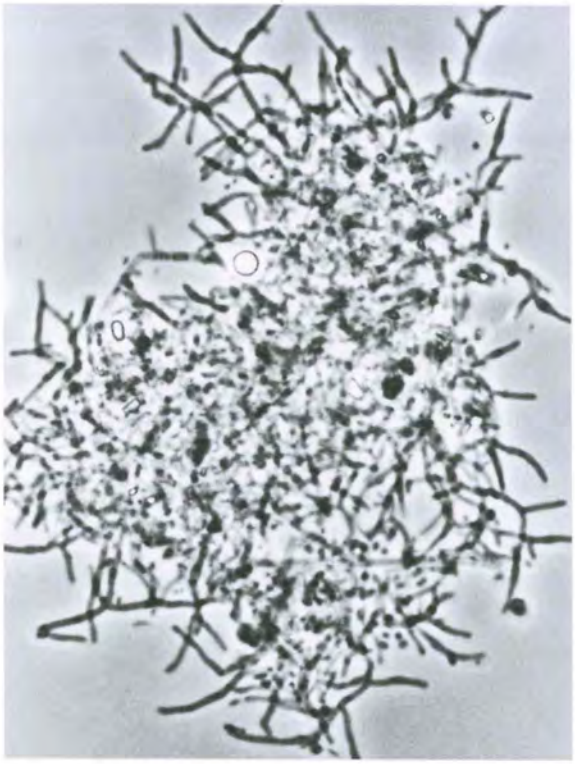


*Figure 41 Sludge floc with Actinomycetes (900x).*

*Figure 42* Beggiatoa *(900x).*

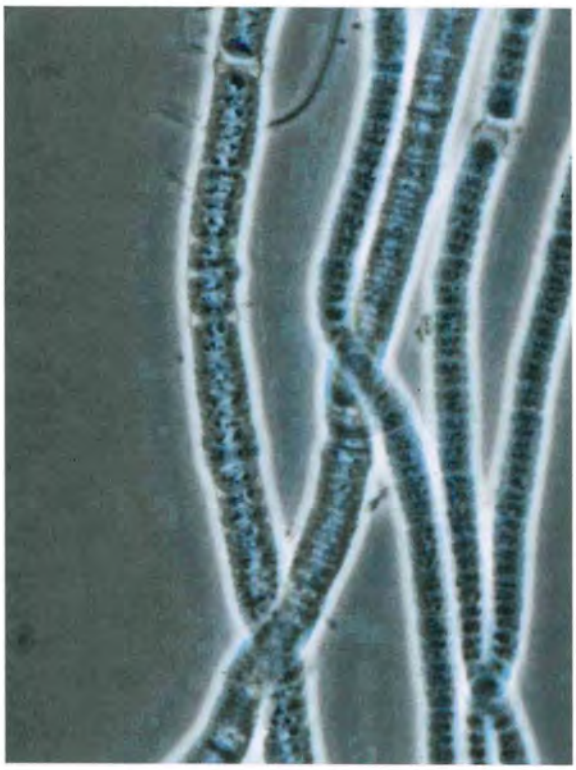


*Figure 43 Example of 'Cyanophyceae' (1150x).*

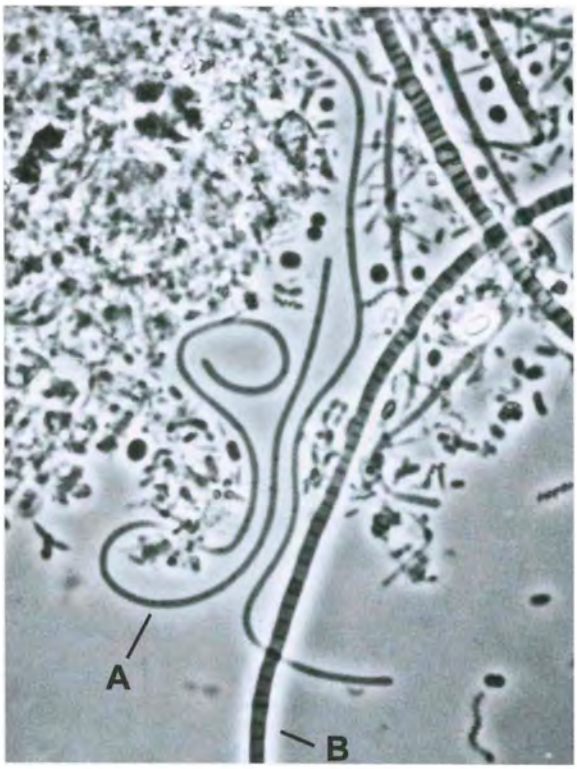



*Figure 44* Flexibacter *(A) and Type 021N (B) (900x).*

### 5.3.4 *Flexibacter*

*Description*
*Flexibacter* (Fig. 44) possesses the following characteristics:
- not branched;
- gliding motion;
- bowed filaments, free moving in the liquid;
- filament length < 200μm;
- cell diameter ca. 0.7 μm;
- no attached growth;
- no sheath;
- septa not clearly visible;
- no sulphur storage;
- Gram negative;
- Neisser negative.

Because of their gliding motion and the absence of sulphur granules, there is little chance of confusion with other filamentous species.

*Occurrence in activated sludge*
*Flexibacter* filaments are occasionally observed at high sludge loading levels. It is not known which other factors determine the growth of this filamentous species. *Flexibacter* filaments have no effect on the settling velocity of the flocs.

### 5.3.5 *Haliscomenobacter hydrossis*

*Description*
*Haliscomenobacter hydrossis* (Fig. 45) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight filaments, protruding from the flocs;
- filament length < 200 μm;
- cell diameter 0.3–0.4 μm;
- rarely attached growth;
- sheath present;
- septa not visible;
- no sulphur storage;
- Gram negative;
- Neisser negative.

The thin straight filaments of *H. hydrossis* are so characteristic that they are not easily confused with other filamentous species. However, the thin stems of *Hyphomicrobium* cells (not a filament) can indeed be mistaken for *H. hydrossis* filaments.

*Occurrence in activated sludge*
*H. hydrossis* commonly occurs in activated sludge plants. The following process conditions are favourable to the growth of this filament:

- sludge load (domestic waste water) > ca. 0.2 kg BOD/kg MLSS.day;
  *H. hydrossis* can also develop *en masse* at lower loadings in industrial plants,;
- many low molecular compounds in the influent (agro industry);
- an influent with a high concentration of nitrogen compounds;
- complete mixing in the aeration tank;
- a low oxygen concentration in the aeration tank;
- probably also a deficiency of phosphate.

The *H. hydrossis* population is always small in domestic treatment plants with nutrient removal. This species can indeed develop *en masse* in industrial plants where many easily biodegradable compounds are present in the influent.

### 5.3.6 *Leucothrix*

*Description:*
*Leucothrix* (Fig. 46) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- bowed/twisted filaments, free in the water between the flocs;
- filament length >> 200 µm;
- highly variable cell diameter (1.5 to 3.5 µm);
- no attached growth;
- no sheath;
- septa/constrictions clearly visible, so long as many storage materials are absent;
- variable cell form (disc shaped, round, rod shaped);
- no sulphur storage;
- Gram negative;
- Neisser negative.

*Leucothrix* can only be distinguished from morphologically similar strains ('Cyanophyceae', Type 021N) by carrying out further investigation. Therefore, *Leucothrix* is not included in the taxonomic key.

*Occurrence in activated sludge*
*Leucothrix* occasionally occurs in industrial treatment plants. Growth of this filament can result in extremely high SVI values. The following process conditions are favourable to *Leucothrix*:

- sludge load > ca. 0.1 kg BOD/kg MLSS.day;
- waste water containing many easily biodegradable compounds, such as fatty acids etc.;
- complete mixing in the aeration tank;
- a relatively high salt concentration (10 - 40 g/l).

### 5.3.7 *Microthrix parvicella*

*Description*
*Microthrix parvicella* (Fig. 47) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- bowed/twisted filaments, free in the water or in/around the flocs;
- filament length often < 200 μm;
- cell diameter ca. 0.5 μm;
- occasionally a little attached growth;
- no sheath;
- septa not clearly visible. Poly-P granules are actually sometimes mistaken for septa;
- no sulphur storage;
- Gram positive;
- Neisser positive (poly-P granules). These granules become smaller as the growth stagnates (in summer).

*M. parvicella* resembles *N. limicola* I. However, this latter organism forms more robust filaments and stains grey-blue with Neisser staining. If a mistake is made when carrying out Gram staining, causing the filaments to be stained red and not blue, the identification key will indicate Type 0581.

*Occurrence in activated sludge*
*M. parvicella* commonly occurs in low loaded domestic treatment plants. This filamentous species is the most significant cause of bulking sludge in many countries (including The Netherlands), and is also frequently responsible for scum formation. The transportation of surplus sludge which contains many *M. parvicella* filaments to the sludge digestion tank can also cause a scum layer to arise in this tank. The population size shows a distinctive seasonal pattern: the population is at its maximum at the end of the winter and at its minimum in summer. The following process conditions are favourable to the growth of *M. parvicella*:

- sludge load < ca. 0.2 kg BOD/kg MLSS.day;
- waste water containing a substantial concentration of higher fatty acids, such as oleic acid. This is always the case with normal domestic waste water;
- circumstances in which the fats [triglycerides, etc.] present in the influent are hydrolysed before they reach the aeration tank. This releases the higher fatty acids. A long retention time in the sewer system, the primary settling tank or in the anaerobic zone with Bio-P processes is favourable to *M. parvicella*;
- a low oxygen concentration in the aeration tank and/or a large anoxic zone (> 40% of the content of the aeration tank);
- water temperature of < ca. 15°C. *M. parvicella* grows principally in the late autumn and winter;
- supply of reduced sulphur and nitrogen compounds is also a possible cause. For this reason, recycling of water from the sludge dewatering unit is 'suspect'.

This combination means that the process conditions in domestic nutrient removal plants are extremely favorable to the growth of *M. parvicella*. This species hardly ever occurs in industrial treatment plants.

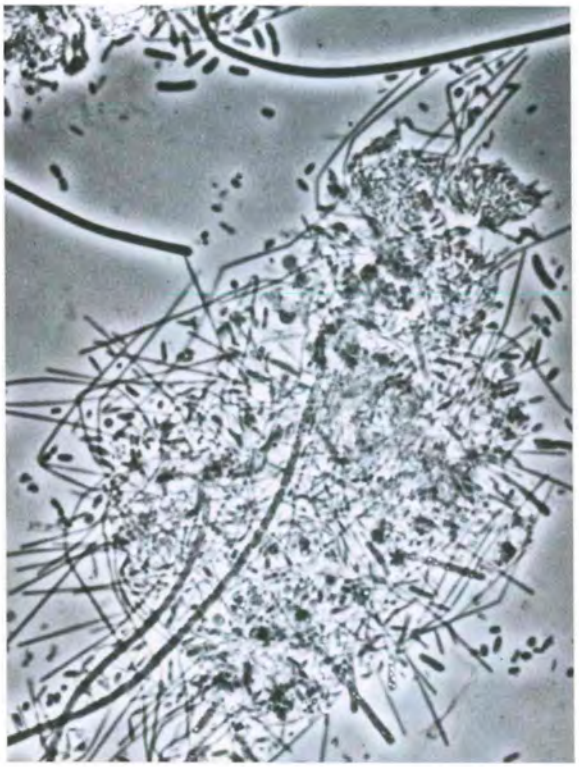


*Figure 45* H. hydrossis *(1000×)*.

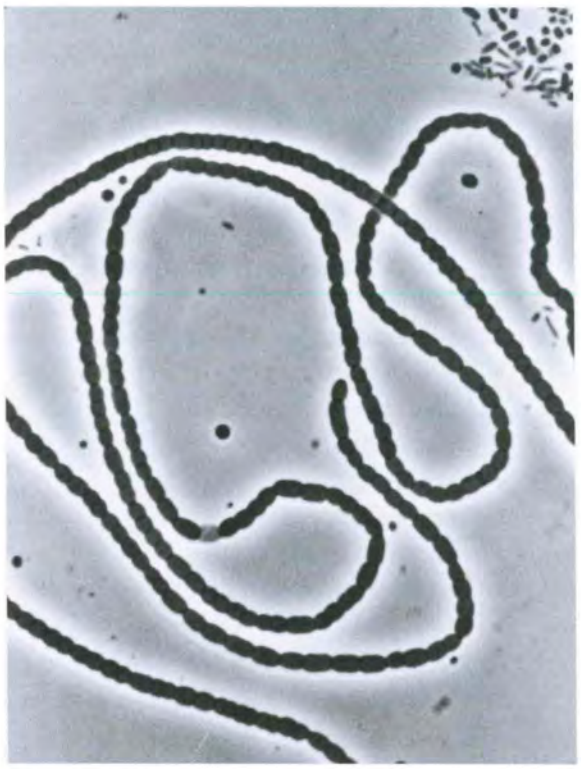


*Figure 46* Leucothrix *(900×)*.

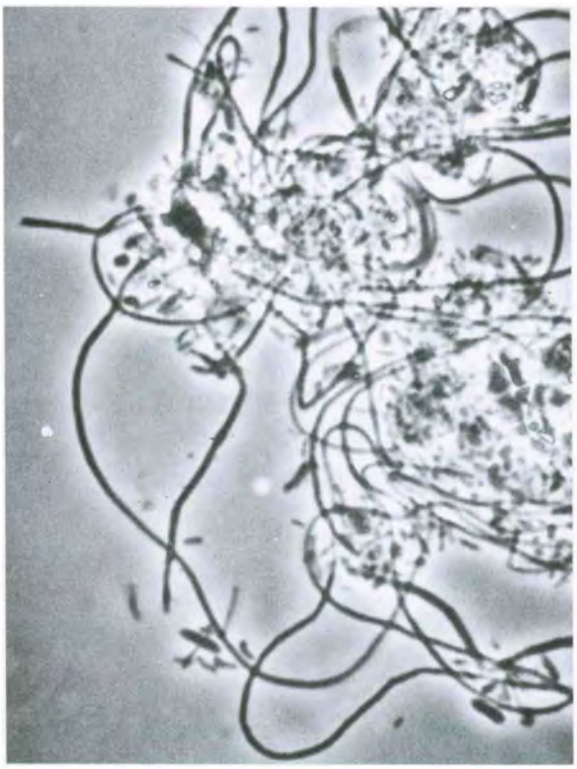


*Figure 47* M. parvicella *(1200×)*.

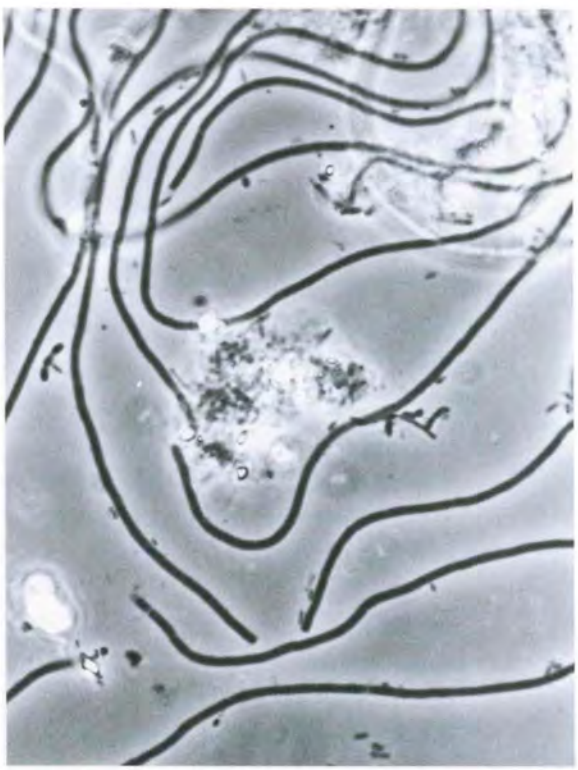


*Figure 48* N. limicola *I (900×)*.

### 5.3.8 *Nostocoida limicola* I

*Description*
*Nostocoida limicola* I (Fig. 48) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- variable filament shape. The filaments can be present in the flocs as well as free in the liquid phase;
- filament length < 200μm;
- cell diameter 0.6–0.7 μm;
- no attached growth;
- no sheath;
- septa not clearly visible;
- no sulphur storage;
- Gram positive;
- Neisser positive. The cells sometimes actually stain grey instead of grey-blue.

*N. limicola* I resembles *M. parvicella* and can easily be mistaken for it. The filaments of *N. limicola* are in fact more robust and the result of Neisser staining is different for these filaments. N.B.: See also *N. limicola* III.

*Occurrence in activated sludge*
*N. limicola* I occasionally occurs in low loaded plants (oxidation ditch conditions) treating a domestic influent. It is not known which factors determine the growth of this species. The presence of *N. limicola* I may result in flotation of the sludge, which means that a scum layer arises. The negative effect on the SVI is limited.

### 5.3.9 *Nostocoida limicola* III

*Description*
*Nostocoida limicola* III (Fig. 49) is a collective name for a group of filamentous species, including the previously distinguished *N. limicola* II, which possess the following characteristics:

- not branched;
- immobile;
- bowed to very twisted filaments which sometimes form tangles inside the flocs;
- variable filament length, but usually > 200μm;
- cell diameter 0.8–2.0 μm;
- no attached growth;
- no sheath;
- septa visible;
- disc shaped to round cells;
- no sulphur storage;
- usually Gram positive;
- usually Neisser positive.

The problem with identifying *N. limicola* III arises from the inconsistent results of the staining methods. All of the combinations (four) can be observed, mainly in sludges from industrial treatment plants. As the diameter is also extremely variable, *N. limicola* III probably contains a group of mutually related strains. On account of their morphological characteristics, these strains are easily distinguishable from other filamentous species.

*Occurrence in activated sludge*
*N. limicola* III is very frequently observed in activated sludge. The population in Dutch low loaded domestic treatment plants (nutrient removal) is actually always low. Large populations can be present if higher loadings are applied and in industrial plants. As the (tangles of) filaments are often taken up in the flocs, the effect on the SVI is limited, but their presence can result in the formation of scum. Although it is not yet fully established, the following process conditions are probably favorable to *N. limicola* III:

- a sludge load of 0.1–0.3 kg BOD/kg MLSS.day;
- industrial waste water containing many easily biodegradable compounds (agro industry);
- pre-settlement of the influent;
- low water temperatures;
- possibly also nutrient shortages;
- complete mixing in the aeration tank.

The available information is still incomplete, however.

### 5.3.10 Fungi

*Description*
Fungi (Fig. 50) are filamentous micro-organisms possessing the following properties:

- real branching;
- immobile;
- straight/bowed filaments;
- filament length > 200 μm;
- cell diameter is usually > 2.5 μm;
- no attached growth;
- no sheath;
- septa clearly visible;
- rectangular cells;
- no sulphur storage;
- Gram 'negative': fungal filaments often do not stain evenly;
- Neisser negative.

Fungi form robust, branched filaments (hyphae), which, at low magnification, can be easily distinguished from other filamentous organisms.

*Occurrence in activated sludge*
Fungi are only seldom observed in domestic treatment plants, and they never dominate. Fungi also almost never cause bulking sludge in industrial plants. If fungi do come to dominate the sludge population the reason is usually a very low pH level.

### 5.3.11 *Sphaerotilus natans*

*Description*
*Sphaerotilus natans* (Fig. 51) is a filamentous bacterium possessing the following charactristics:

- often false branching;
- immobile;
- straight to slightly bowed filaments;
- filament length >200 μm;
- filament diameter: 1.0–1.5 μm;
- occasional attached growth;
- sheath present (often only visible after Gram staining);
- septa clearly visible;
- rectangular to rod shaped cells;
- no sulphur storage. Other storage materials can be present in industrial treatment plants;
- Gram negative;
- Neisser negative.

If branching is absent, this species can be confused with Type 0041 (Gram positive, shorter cells), Type 021N (shorter cells, no attached growth, no sheath) or with Type 0961 (cells clearly longer and distinctly transparent).

*Occurrence in activated sludge*
The following process conditions are favourable to *S. natans*:

- shortages of $O_2$, N or P;
- a large fraction of low molecular compounds in the influent (agro industry);
- sludge load > ca. 0.2 kg BOD/kg sludge.day;
- complete mixing in the aeration tank.

*S. natans* rarely occurs in modern treatment plants with biological nutrient removal. It regularly causes bulking sludge in industrial treatment plants containing a large fraction of easily biodegradable compounds in the influent. It often also grows excellently in pilot plants (synthetic influent). Growth *en masse* of *S. natans* results in an extremely high SVI.

### 5.3.12 *'Streptococcus'*

*Description*
'Streptococcus' (Fig. 52) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- bowed/twisted filaments, free in the water between the flocs;
- filament length < 200 μm;
- cell diameter ca. 0.7 μm;
- no attached growth;
- no sheath;
- septa/constrictions clearly visible;
- round cells;
- no sulphur storage;
- Gram positive;
- Neisser negative.

This filamentous species morphologically resembles streptococci and is also Gram positive. It has not yet been definitely established if this name is correct. 'Streptococcus' resembles Type 1863, which is Gram negative. 'Streptococcus' is not included in the taxonomic key.

*Occurrence in activated sludge*
'Streptococci' are seldom observed in activated sludge and have no effect on its settling velocity. The factors determining their growth in treatment plants are not known.

### 5.3.13 *Thiothrix*

*Description*

The name *Thiothrix* encompasses a group of filamentous bacteria possessing the following characteristics:

- not branched;
- immobile;
- straight/bowed filaments, which mostly protrude from the flocs;
- filament length: highly variable;
- cell diameter also variable (0.5–1.5 μm), sometimes narrowing within the filament towards the tip (= tapering);
- no attached growth;
- a sheath is present for some strains;
- septa clearly visible so long as the cells are not filled with sulphur globules;
- square to rectangular cells;
- occasional rosettes;
- sulphur granules present (*in vivo* or after the S test);
- usually Gram negative;
- Neisser negative.

*Thiothrix* filaments are usually attached to the flocs. The sulphur globules are very characteristic (Fig. 53). *Thiothrix* can easily be confused with Type 021N if only small S granules are present after the sulphur deposit test. N.B.: Sulphur storing filamentous bacteria with a different morphology are sometimes present in industrial plants.

Besides low molecular compounds such as fatty acids, *Thiothrix* also uses reduced sulphur compounds for growth. The sulphides are oxidised and elemental sulphur is temporarily stored in the cell as an intermediary product. These are the bright globules that can be microscopically observed. They disappear if the sludge is stored for a few days in a refrigerator.

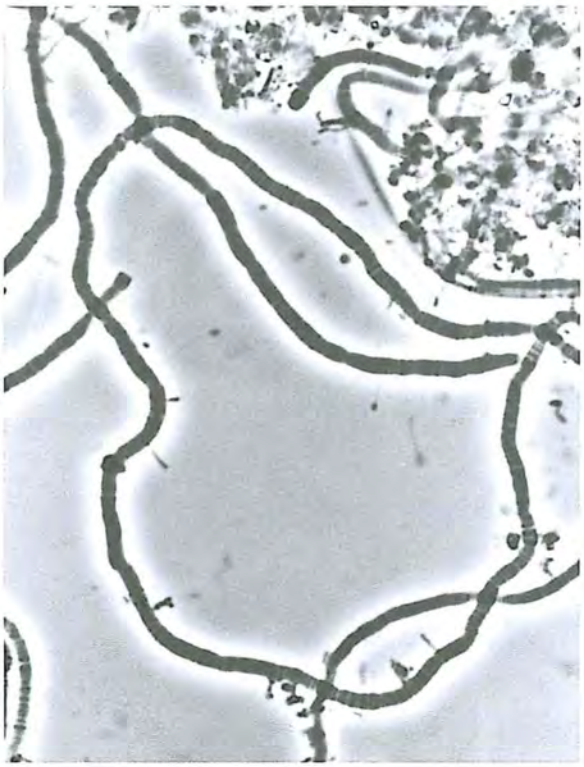


*Figure 49* N. limicola III *(900×).*

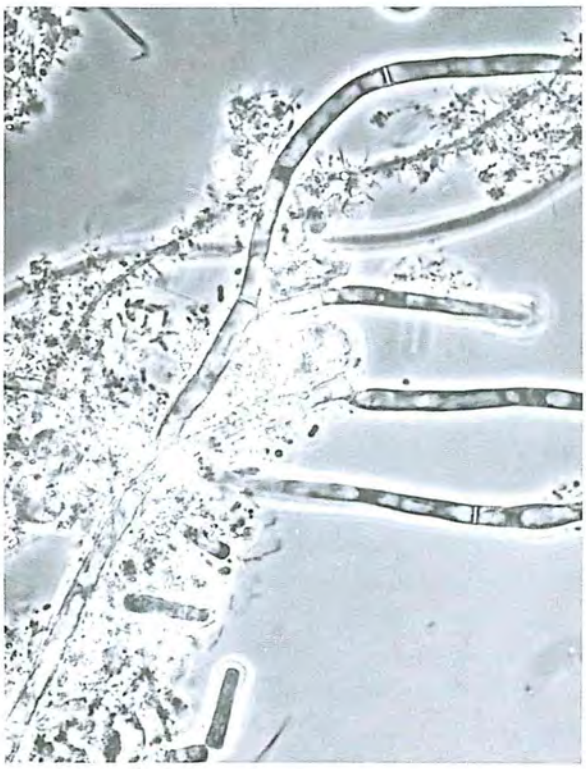


*Figure 50 Example of a fungus (400×).*

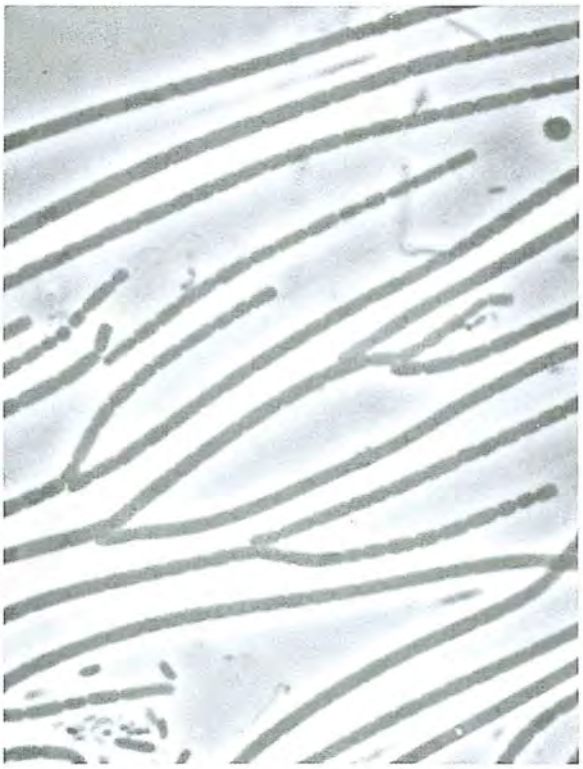


*Figure 51* S. natans *(900×).*

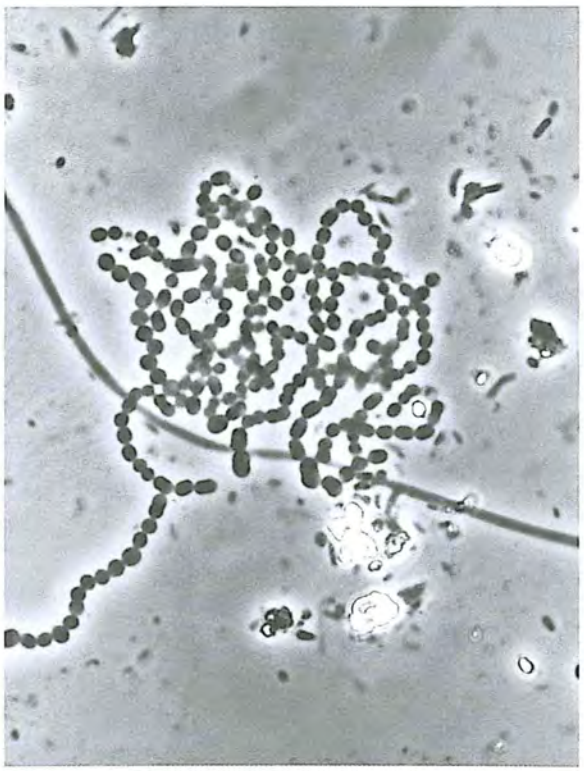


*Figure 52* 'Streptococcus' *(900×).*

*Occurrence in activated sludge*
The following process conditions are favorable to *Thiothrix*:
- reduced sulphur compounds in the influent;
- a high concentration of low molecular carbon compounds in the influent;
- sludge load > ca. 0.1 kg BOD/kg MLSS.day;
- lack of $O_2$, N or P;
- complete mixing in the aeration tank.

*Thiothrix* hardly ever occurs in modern low loaded treatment plants (biological nutrient removal), unless unusually high sulphide levels are present (stale sewage). Large *Thiothrix* populations, which cause bulking sludge, can be present at high sludge loading levels and in industrial treatment plants.

### 5.3.14 Type 0041/0675

*Description*
Type 0041 (Fig. 54) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/slightly bowed filaments, occurring free in the water as well as attached to the flocs;
- filament length is variable;
- cell diameter is variable (0.6-1.5 µm);
- much attached growth always present in domestic plants; 'clean' filaments sometimes present in industrial plants;
- sheath present;
- septa visible (if a lot of attached growth is present only visible at the tip of a filament);
- square cells;
- no sulphur storage;
- Gram positive;
- Neisser negative.

Type 0041 includes Gram positive, sheath forming bacteria, including the previously described Type 0675, but excluding Type 1851. The latter stains light blue with Gram and also consist of much longer, rectangular cells. Type 0041 comprises several species which differ from one another in regard to their cell diameters.

The somewhat thicker filaments of Type 0041 can be mistaken for those of *S. natans* (Gram negative and rectangular cells), Type 021N (Gram negative) or for *Thiothrix* filaments (sulphur granules and Gram negative).

*Occurrence in activated sludge*
Type 0041 occurs very commonly in activated sludge. The following process conditions are favourable to this filamentous species:

- sludge load < ca. 0.2 kg BOD/kg MLSS.day;
- pre-mixing of raw urban influent and returned sludge before these flows enter the aeration tank. This means that Type 0041 in domestic treatment plants mainly grows on the particulate fraction present in the influent. It is not known if this is also valid for industrial plants;

Little is yet known concerning the factors that determine the growth of this species.
The Type 0041 population is always small in domestic plants with nutrient removal, if no pre-mixing is carried out. Pre-mixing stimulates the growth of Type 0041, however. This practically never causes high SVI values (stabilisation usually at 120 to 130 mg/l).
The development of Type 0041 in industrial treatment plants can indeed result in high SVI values.

### 5.3.15 Type 0092

*Description*
Type 0092 (Fig. 55) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/slightly bowed filaments;
- filament length < 200 µm;
- cell diameter 0.5–0.7 µm;
- no attached growth;
- no sheath;
- septa not clearly visible;
- no sulphur storage;
- Gram negative;
- Neisser positive (grey-blue filaments).

Type 0092 is for the most part 'hidden' in the flocs in domestic treatment plants, as a result of which only the short extremities of the filaments are visible on unstained slides. Type 0092 can be confused with *H. hydrossis* (Neisser negative and the filaments are usually longer). As the filaments of Type 0092 are largely concealed in the flocs, the population size of this species can easily be underestimated. It is only possible to see how large the population really is after Neisser staining.

*Occurrence in activated sludge*
Type 0092 commonly occurs in activated sludge. The following process conditions are favourable to this species:
- sludge load < ca. 0.1 kg BOD/kg MLSS.day;
- raw influent;
- water temperature > ca. 15 °C.

Therefore, not much is yet known about the factors governing the growth of this species.
The population size displays a seasonal rhythm in domestic treatment plants, with the maximum occurring in summer/autumn. As the filaments are largely present inside the flocs, the effect on the SVI is limited. In many treatment plants, the disappearance of *M. parvicella* in April/May is coupled with an increase of Type 0092. This suggests that both of these filamentous organisms can use the same substrate. This theory has not yet been verified.
Practically nothing is known of the physiology of this bacterium.
Type 0092 is occasionally observed *en masse*, free in the water between the flocs, in industrial plants (e.g. dairy industry).

### 5.3.16 Type 0211

*Description*
Type 0021 (Fig. 56) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- bowed/twisted filaments (in and around the flocs);
- filament length < 200 μm;
- cell diameter 0.2- 0.3 μm;
- no attached growth;
- no sheath;
- septa/constrictions clearly visible;
- rod shaped cells;
- no sulphur storage;
- Gram negative;
- Neisser negative.

Type 0211 could be mistaken for Type 1863, although this latter is noticeably thicker. Type 0211 is not included in the taxonomic key.

*Occurrence in activated sludge*
Type 0211 is occasionally observed in highly loaded activated sludge. The factors determining the growth of this filamentous species in treatment plants are not known.

### 5.3.17 Type 021N

*Description*
Type 021N (Fig. 57) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/bowed filaments, usually free in the liquid between the flocs;
- filament length > 200 μm;
- cell diameter variable, usually 1.5–2.0 μm, but sometimes reduced towards the tip of the filament;
- no attached growth;
- no sheath;
- septa clearly visible;
- variable cell form (disc shaped, almost square and rod shaped). A few almost black cells are sometimes present in the filaments;
- little or no sulphur storage;
- Gram negative;
- Neisser negative.

Type 021N can easily be confused for *Thiothrix*. Both strains are related to each other. *Thiothrix* forms many sulphur granules (*in vivo* or during the S test) while Type 021N hardly stores sulphur. If attached growth is absent on Type 0041, its filaments also resemble those of Type 021N. Type 0041 is Gram positive, however.

*Occurrence in activated sludge*

Type 021N is frequently observed in activated sludge. The following process conditions are favourable to this species:

- a broad spectrum of sludge loading levels (0.05–0.4 kg BOD/kg MLSS.day) with a greater chance of massive growth at a sludge load > 0.1 kg BOD/kg MLSS.day;
- influent containing many easily biodegradable compounds (fatty acids, etc.). These compounds can arise in the sewer (stale sewage) as well as originating from industry;
- shortages of nutrients, including oxygen;
- complete mixing in the aeration tank.

The substrate required by Type 021N in order to grow is removed from the water by floc forming bacteria under anoxic/anaerobic conditions in domestic treatment plants with biological nutrient removal. Type 021N, which is strictly aerobic, is only encountered as a secondary species under these process conditions. Type 021N frequently causes bulking sludge in industrial treatment plants and in domestic plants without nutrient removal.

### 5.3.18 Type 0411

*Description*

Type 0411 (Fig. 58) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- bowed/'twisted' filaments, often occurring along the edges of the flocs;
- filament length < 200 μm;
- cell diameters 0.5–0.7 μm;
- no attached growth;
- no sheath;
- septa/constrictions clearly visible;
- rod shaped cells;
- no sulphur storage;
- Gram negative;
- Neisser negative.

Type 0411 is not included in the taxonomic key.

*Occurrence in activated sludge*

Type 0411 is occasionally observed in highly loaded treatment plants (> ca. 0.3 kg BOD/kg MLSS.day). The factors which determine the growth of Type 0411 in activated sludge are not known. This species has only a minor effect on the settling velocity of the sludge flocs.

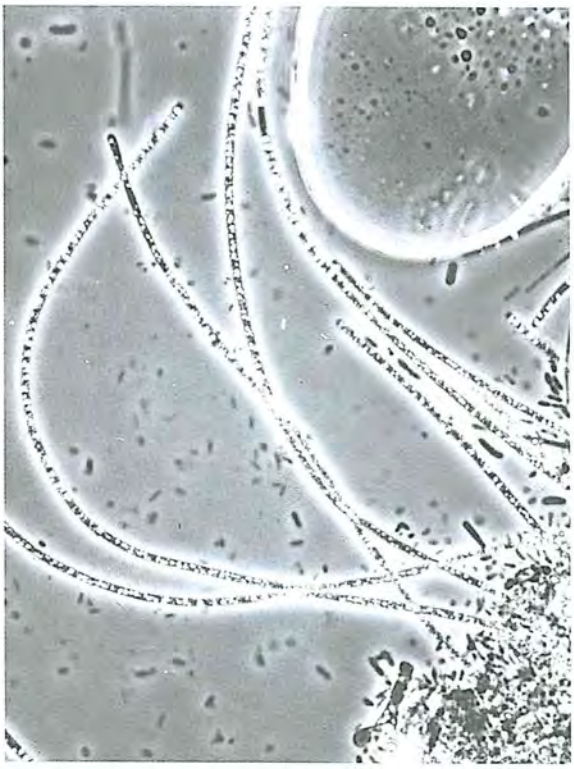


*Figure 53 Sulphur globules in* Thiothrix *filaments (900×).*

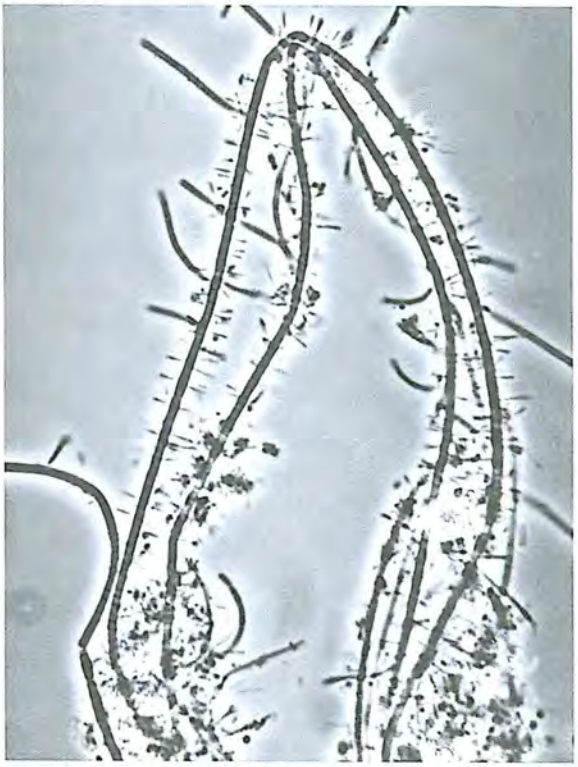


*Figure 54 Type 0041 (900×).*

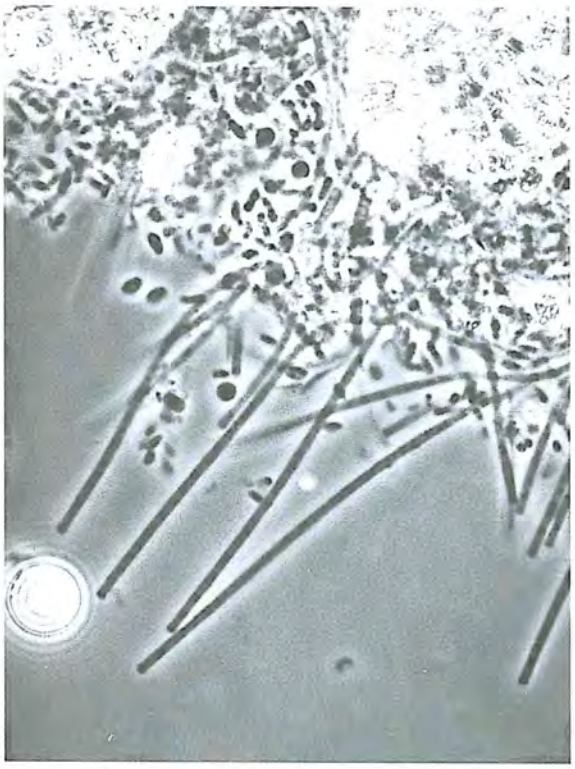


*Figure 55 Type 0092 (900×).*

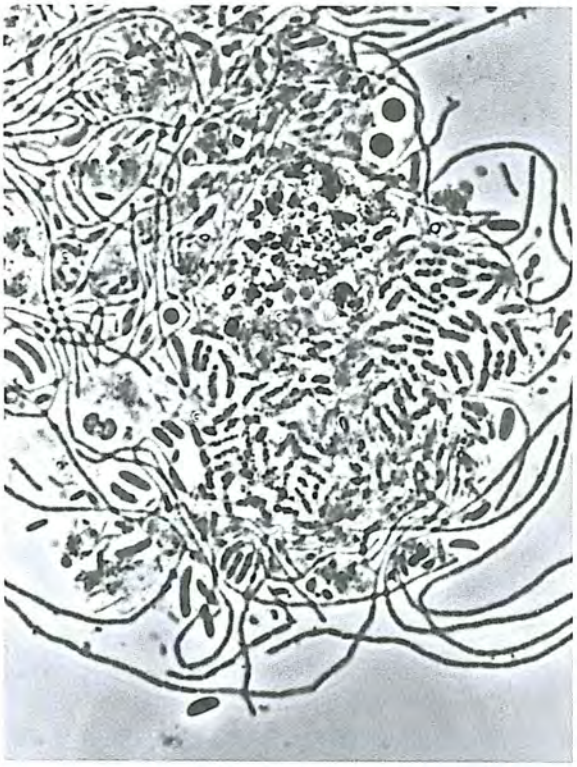


*Figure 56 Type 0211 (900×).*

### 5.3.19 Type 0581

*Description*
Type 0581 (Fig. 59) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- bowed/twisted filaments, free in the water or along the flocs;
- filament length < 200 µm;
- cell diameter 0.3–0.4 µm;
- no attached growth;
- no sheath;
- septa not visible;
- no sulphur storage;
- Gram negative;
- Neisser negative.

Type 0581 morphologically resembles *M. parvicella*. Clear distinctions between both organisms can only be obtained by Gram and Neisser staining. Type 0581 is also a little thinner. Despite these differences, it is possible that 2 growth forms of the same species are concerned.

*Occurrence in activated sludge*
Type 0581 is occasionally observed in low loaded domestic treatment plants and a discontinuous influent supply.

### 5.3.20 Type 0803

*Description*

Type 0803 (Fig. 60) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/slightly bowed filaments. These are often attached to a piece of inorganic material (sand grains and such like);
- filament length < 200 µm;
- cell diameter 0.7–0.8 µm;
- no attached growth;
- no sheath;
- septa clearly visible, no constrictions;
- square cells
- no sulphur storage, but small, dark globules are sometimes present in the cells. The reaction to the S test is, in fact, negative;
- usually Gram negative, but sometimes Gram positive;
- Neisser negative.

If the septa are not properly observed during analysis, use of the identification key can indicate Type 1851 (light blue after Gram staining and much attached growth often present), *H. hydrossis* (thin, usually dead straight filaments) or Type 0092 (Neisser positive). N.B. See also Type 0914. Both strains are probably closely related.

*Occurrence in activated sludge*
Type 0803 is regularly observed in activated sludge. The following process conditions are favourable to this species:

- sludge load < ca. 0.2 kg BOD/kg MLSS.day;
- industrial waste water (textiles, pharmaceuticals);
- possibly anaerobic pre-treated waste water also.

Type 0803 is frequently present, but practically never *en masse*, in domestic treatment plants. Pre-denitrification results in Type 0803 disappearing (and Type 0914 also). The population shifts towards Type 0914 with Bio-P processes. Type 0830 can indeed be present *en masse* and cause bulking sludge in treatment plants with (principally) industrial influent.

### 5.3.21 Type 0914

*Description*
Type 0914 (Fig. 61) is a filamentous bacterium possessing the following characteristics:

- not branched;
- immobile;
- straight/slightly bowed filaments, usually along the edges of the flocs
- filament length < 200 µm;
- cell diameter 0.6- 0.8 µm;
- no attached growth;
- no sheath;
- septa poorly visible or invisible;
- sulphur granules present *in vivo*;
- Gram positive;
- Neisser negative.

The short, sulphur globule filled, filaments of Type 0914 are very characteristic. The chance of confusion with species that also can store sulphur is not very big. *Thiothrix* usually forms longer filaments and is Gram negative while *Beggiatoa* has a characteristic gliding movement.

If the sulphur granules are removed from Type 0914, the filaments resemble those of Type 0803. The populations of both strains are often complementary to one another. If one of them disappears the other appears and *vice versa*. Therefore, it is quite probable that the Types 0914 and 0803 represent growth forms of the same bacterium.

*Occurrence in activated sludge*
Type 0914 occurs in treatment plants where the influent contains reduced sulphur compounds and in which a sludge load less than ca. 0.2 kg BOD/kg MLSS.day is applied. The sulphur compounds can be transported with the influent, but can also be formed in the plant. Pre-settlement of the influent and use of Bio-P processes (anaerobic period) stimulate the development of Type 0914. However, the population is always small in plants with a mainly domestic influent. Many Type 0914 filaments are occasionally present in industrial plants.

### 5.3.22 Type 0961

*Description*
Type 0961 (Fig. 62) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/slightly bowed filaments which are often strikingly transparent;
- filament length > 200 μm;
- cell diameter 1.0–2.0 μm;
- no attached growth;
- it is not certain whether a sheath is present or not;
- septa clearly visible, no constrictions;
- square/rectangular cells
- no sulphur storage;
- Gram negative;
- Neisser negative.

Type 0961 can be mistaken for robust *Thiothrix* filaments (S storage and not so transparent) and Type 1852 (cell diameter ca. 0.7 μm).

*Occurrence in activated sludge*
Type 0961 is not very frequently observed in activated sludge. The following process conditions are favorable to this species:
- sludge load >ca. 0.2 kg BOD/kg MLSS.day;
- waste water containing a large fraction of easily biodegradable components (agro-industry).

Information concerning this species is also still very limited. Type 0961 can sometimes develop *en masse* in industrial treatment plants, in nutrient removal plants this bacterium is hardly ever observed.

### 5.3.23 Type 1701

*Description*
Type 1701 (Fig. 63) is a filamentous bacterium possessing the following characteristics:
- occasional false branching;
- immobile;
- straight/slightly bowed filaments, often attached to the flocs
- filament length < 200 μm;
- cell diameter 0.7- 0.9 μm;
- attached growth often present;
- sheath present;
- septa visible (if attached growth is present, then only at the clean tip of the filament);
- rod shaped cells
- no sulphur storage;
- Gram negative;
- Neisser negative.

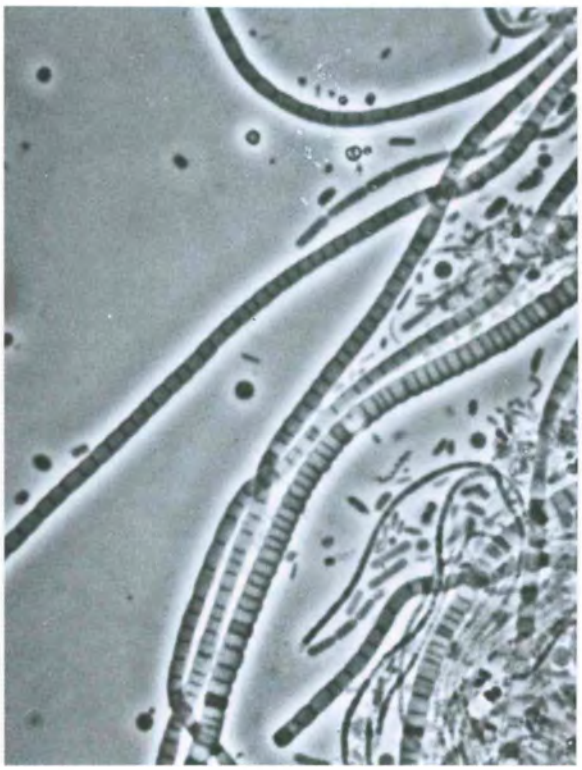


*Figure 57 Type 021N (900×).*

*Figure 58 Type 0411 (750×).*

*Figure 59 Type 0581 (1150×).*

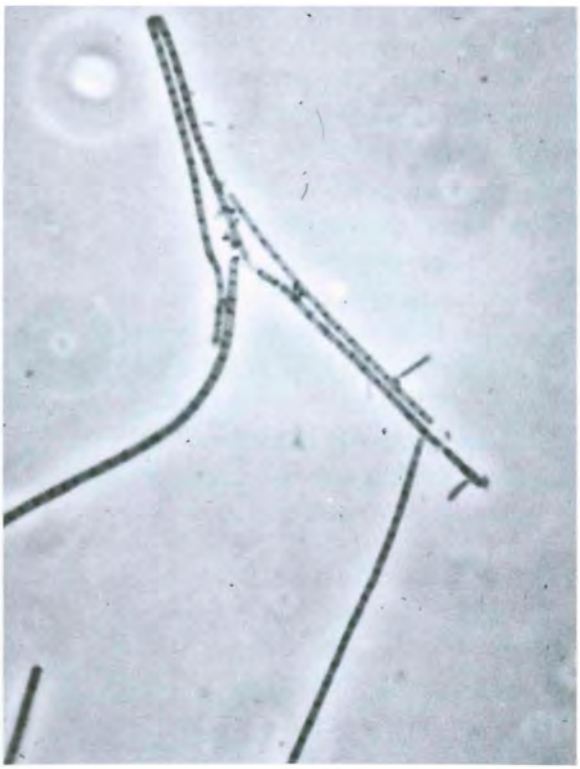


*Figure 60 Type 0803 (1200×).*

If branching is observed, the identification key can indicate *S. natans* (markedly thicker filaments). If the septa are not observed, Type1701 can be confused with Type 1851 (Gram positive), *H. hydrossis* (thinner filaments without attached growth) or Type 0092 (Neisser positive).

*Occurrence in activated sludge*
Type 1701 is not very often observed in activated sludge. The following process conditions are favourable to this filamentous bacterium:
- sludge load > ca. 0.2 kg BOD/kg MLSS.day;
- waste water containing a high level of carbohydrates, especially starch;
- low oxygen levels in the aeration tank;
- complete mixing in the aeration tank;
- relatively high water temperatures (> ca. 15°C).

This means that Type 1701 practically never occurs in domestic treatment plants where nutrient removal is implemented. Type 1701 grows mainly in plants in which waste water originating from certain agro-industries is treated and in which it can cause high SVI values.

### 5.3.24 Type 1702

*Description*
Type 1702 (Fig. 64) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight/slightly bowed filaments which protrude from the flocs;
- filament length < 200 μm;
- cell diameter ca. 0.6 μm;
- no attached growth;
- sheath present;
- septa invisible;
- no sulphur storage;
- Gram negative;
- Neisser negative.

Type 1702 can be mistaken for *H. hydrossis* (thinner filaments) and Type 0092 (Neisser positive). Type 1702 is not included in the taxonomic key.

*Occurrence in activated sludge*
Type 1702 is only occasionally observed in activated sludge. It is not known which process conditions stimulate its growth.

### 5.3.25 Type 1851

*Description*
Type 1851 (Fig. 65) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight filaments which protrude from the flocs and sometimes form into bundles;
- filament length variable, but usually > 200 µm;
- cell diameter 0.5–0.7 µm;
- usually much attached growth, but sometimes clean filaments in industrial treatment plants;
- sheath present;
- septa often not clearly visible;
- rectangular cells;
- no sulphur storage;
- Gram positive. The light blue colour is very characteristic;
- Neisser negative.

Type 1851 can be confused with thin Type 0041 filaments (dark blue after Gram staining and shorter cells). If septa are observed in Type 1851, the identification key may indicate Type 1701 (rod shaped cells and Gram negative).

*Occurrence in activated sludge*
Type 1851 is regularly observed in low loaded plants, but it almost never predominates in domestic treatment systems. Large populations of Type 1851 can be present in industrial treatment plants (agro-industry). The following process conditions are favorable to it:
- sludge load < ca. 0.15 kg BOD/kg MLSS.day;
- low molecular compounds (agro-industry).

The knowledge concerning this species is still very limited.

### 5.3.26 Type 1852

*Description*
Type 1852 (Fig. 66) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- straight filaments which often appear to be transparent;
- filament length > 200 µm;
- cell diameter 0.6- 0.8 µm;
- no attached growth;
- sheath present;
- septa clearly visible;
- rectangular cells;
- no sulphur storage;
- Gram negative;
- Neisser negative.

*Figure 61 Type 0914 (900×).*

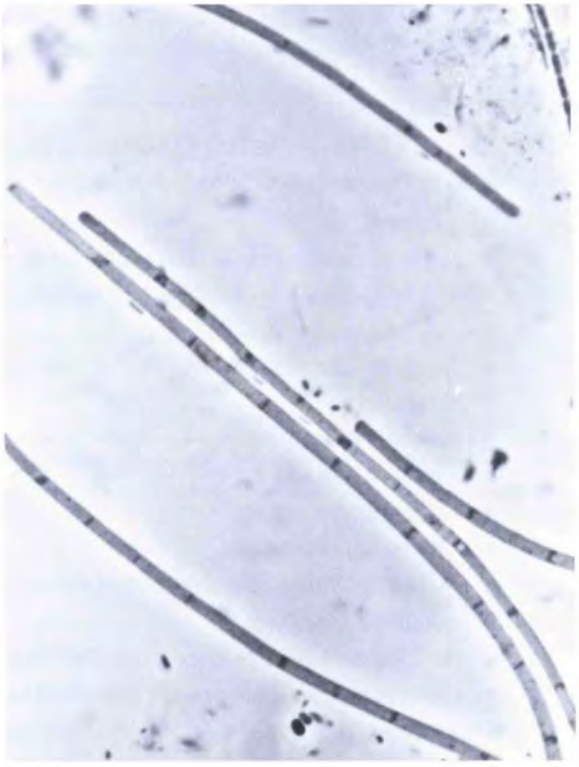


*Figure 62 Type 0961 (900×).*

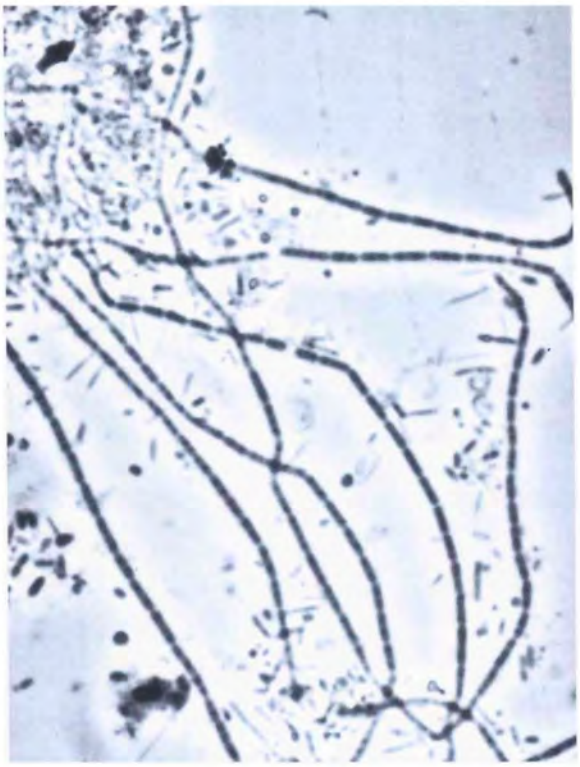


*Figure 63 Type 1701 (900×).*

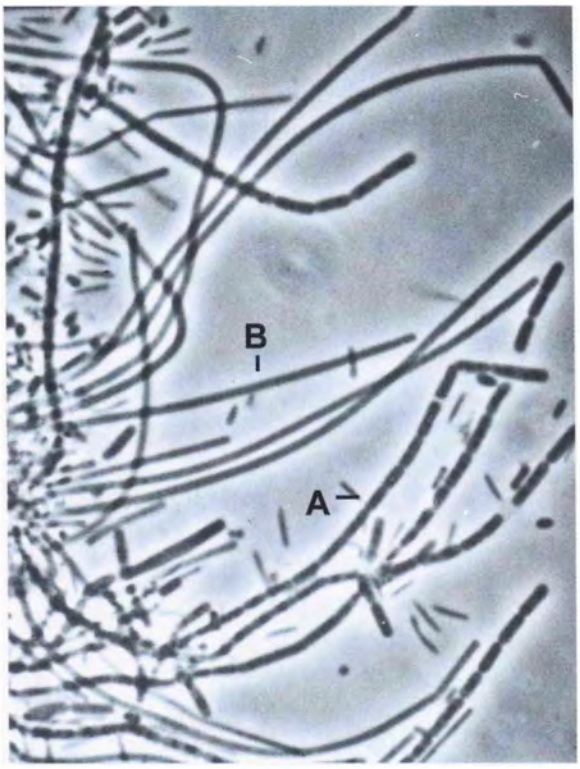



*Figure 64 Type 1701 (a) and 1702 (b) (1250×).*

Type 1852 can be confused with thin *Thiothrix* strains (which store sulphur granules) and with Type 0961 (cell diameter > 1.0 µm).

*Occurrence in activated sludge*
Type 1852 is only rarely observed and is not included in the identification key. The process conditions that stimulate the growth of Type 1851 are not known.

### 5.3.27 Type 1863

*Description*
Type 1863 (Fig. 67) is a filamentous bacterium possessing the following characteristics:
- not branched;
- immobile;
- bowed/twisted filaments, free in the liquid;
- filament length < 200 µm;
- cell diameter 0.5–0.8 µm;
- no attached growth;
- no sheath;
- septa clearly visible;
- round cells;
- no sulphur storage;
- Gram negative;
- Neisser negative.

The small chains of Type 1863 can be mistaken for 'Streptococci' (Gram positive) and for Type 0211 (thinner filaments).

It has recently been established that Type 1863 comprises several morphologically identical but physiologically varying species.

*Occurrence in activated sludge*
Type 1863 is principally observed at high sludge loading levels (0.3 - 0.6 kg BOD/kg MLSS.day). It is not known which other process conditions also promote the growth of this filament. Type 1863 has no effect on the settling velocity of the flocs but can, even if the filaments are Gram negative, contribute to the formation of scum.

*Figure 65 Type 1851 (650×).*

*Figure 66 Type 1852 (625×).*

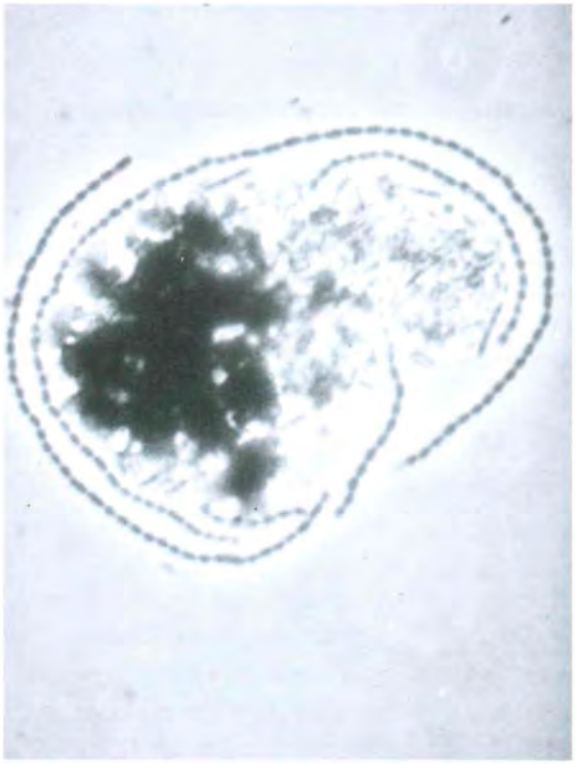


*Figure 67 Type 1863 (900×).*

# 6 Protozoa and metazoa

Protozoa, and frequently also metazoa, are almost always present in activated sludge. Some species are attached to the flocs whereas others are free in the water between the flocs. These organisms are (much) larger than bacteria, their 'length' varying from 10 to 10,000 µm (table 2). In addition, they also possess a characteristic shape. On account of the combination of characteristics, they are very conspicuous on a microscopic slide.

*Table 2* *Order of magnitude of (micro-)organisms in activated sludge*

| Group | Cell length (µm) | Remarks |
|---|---|---|
| ***bacteria*** | 1–5 | occasional colonies or filaments |
| ***protozoa*** | | |
| -flagellates | 10–30 | occasional colonies |
| -amoeba | 30–400 | |
| -testate amoeba | 30–200 | |
| -heliozoa | 40–200 | |
| -ciliates | 25–400 [1)] | occasional colonies |
| ***metazoa*** | | |
| -rotifers | 100–500 | |
| -nematodes | 500–3,000 | |
| -tardigrades | 200–1,200 | |
| -worms | 3,000–10,000 | |

[1)] A few species are much longer (up to 1000 µm)

Many protozoa/metazoa mainly feed on bacterial cells which are present free in the liquid or at the edges of the flocs. In this manner they remove many bacterial cells that are not firmly bound to the flocs. Free bacterial cells cannot be separated from the treated water through settling in the final clarifier. Protozoa are also indispensable for a far-reaching COD reduction, viz. a clear effluent.

Furthermore, protozoa/metazoa that consume sludge flocs (→ reduction of the sludge production) or consume other protozoa also exist.

The presence of certain species is relevant for the process conditions in the treatment plant. Assessing the composition of the population is, therefore, an important aspect of microscopic sludge investigation. This indicator function is further referred to in paragraph 6.3.

More than 200 different strains can be observed in activated sludge. Identification of these organisms is not really very easy and requires specialist knowledge. It is also unnecessary to identify all of these species for the purpose of process monitoring, distinguishing the most important main groups is sufficient. The following paragraphs contain a short description of these main groups, referring to several species that are regularly observed in activated sludge.

## 6.1 Protozoa

Like bacteria, protozoa are single celled organisms. As long as the sludge loading level is not extremely high, they are present in practically every activated sludge. Their population is always much smaller than that of the bacteria. The biomass of protozoa present in the treatment plant comprises, at most, a few percent of the total biomass.

Protozoa are divided into four groups:
- ciliates
- flagellates
- amoeba;
- testate amoeba;
- heliozoa

### 6.1.1 Ciliates

Ciliates are characterised by the presence of cilia (= vibrating hairs) on their cell surfaces. For some ciliates, the surface is completely covered with cilia whereas other species are only partly covered. Ciliates also exist which cilia are only present during a certain phase of their lives.

For many species, the cilia are arranged in a certain manner around the mouth openings. As a result, these cilia make the water flow in their vicinity. They fan, as it were, the food towards their mouth openings. These nutrient particles are subsequently taken up from the water by filtration. In addition, cilia contribute to the locomotion of several species.

Ciliates take in food particles by means of their mouth openings. According to the type of organism, this food can comprise organic fragments as well as bacterial cells or other protozoa. Therefore, predation plays an important part within such a mixed population. Some examples are given below:

- ciliates that mainly eat bacteria: *Aspidisca*, *Blepharisma*, *Carchesium*, *Colpidium*, *Chilodonella*, *Epistylus*, *Opercularia*, *Vorticella*;
- omnivorous species that consume bacteria as well as flagellates and small ciliates: *Euplotes*, *Stentor*;
- carnivorous species that mainly eat other ciliates: *Litonotus*, *Trachelophyllum*, *Hemiophrys*, *Suctorea*.

Bacterial cells transported with the influent form an important source of nourishment for ciliates. Sewage contains some tens of mg/l of bacterial cell material, a level that can also vary markedly according to the type of sewer, the weather conditions, the time of year, etc. The number of cells that are consumed is closely linked to the format of the organism. In this manner, small ciliates consume ca. 10 cells/min., larger ones (e.g. *Opercularia)* more than 10 times as much whereas some nematode strains consume 5000 cells/min. Ciliates mainly exploit their food for the production of new cells. Movement costs little energy. As a rule of thumb, it is reckoned that 50% of the available food is used for cell synthesis.

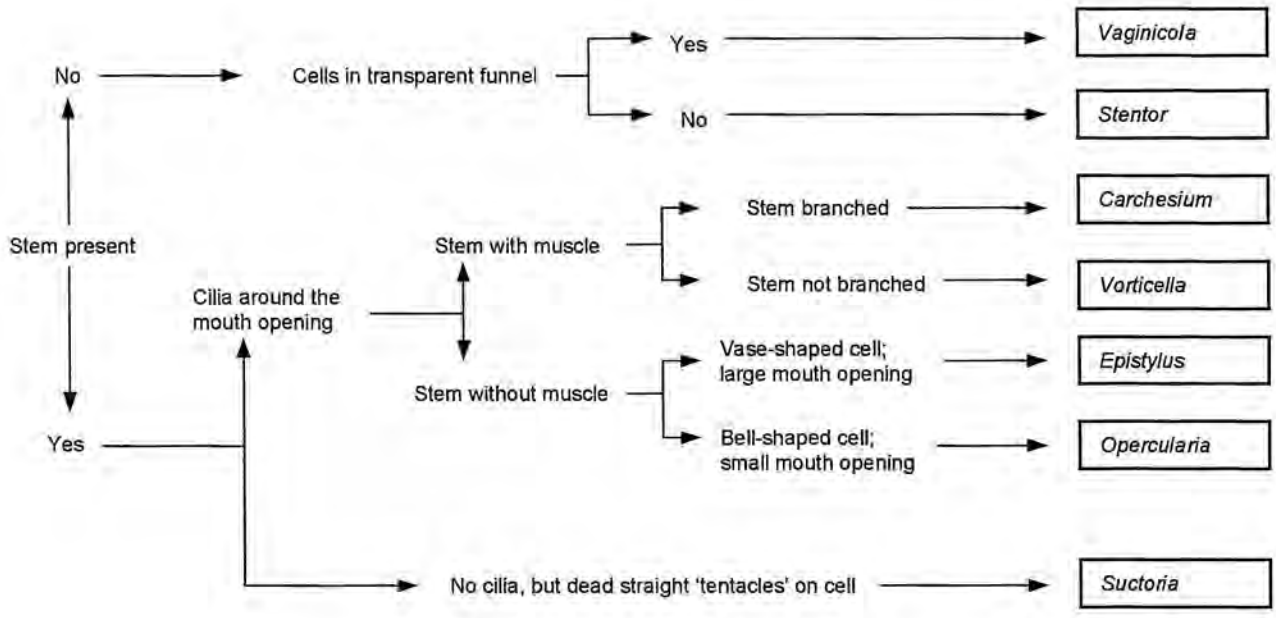
If the oxygen concentration is adequate, the size of the population is principally determined by the sludge load applied and the related sludge age. The population is at its maximum at a sludge load of ca. 0.3 kg BOD/kg MLSS.day (order of magnitude: $10^3$–$10^4$ cells per ml). Fewer bacterial cells are available at lower sludge loading levels (floc more robust and relatively less transport with the influent), leading to a smaller ciliate population. At higher sludge loading levels, the sludge age is so short that the relatively slow-growing ciliates cannot be maintained in the sludge any more.

Quantities of thousands of specimens per millilitre are very suggestive of, and lead easily to, an over-estimation of the contribution of the ciliates to the total treatment process. The influence of a given group of organisms is not actually determined by quantity, but in terms of its contribution to the total amount of biomass in the treatment plant. The mass of several strains that frequently occur in activated sludge can vary from 0.1 ng to 10 ng, with peaks to 80 ng for *Stentor roesili*, a 'giant' among the ciliates. With 1 ng and 5000 specimens/ml and a total sludge concentration of 4 g/l, only 0.5% of the total biomass will comprise ciliates. The actual contribution of ciliates (and that of the other protozoa) to the total treatment performance is consequently very subordinate to that of bacteria.

Ciliates are subdivided into sessile (= attached), crawling and free-living species.

### *6.1.1.1 Sessile ciliates*

The cells of sessile ciliates are usually positioned on a stem which is often attached to a sludge floc. The stems are branched in some strains, through which colonies of sometimes numerous cells arise. Fig. 68 represents an identification key for distinguishing sessile ciliates commonly occurring in activated sludge.

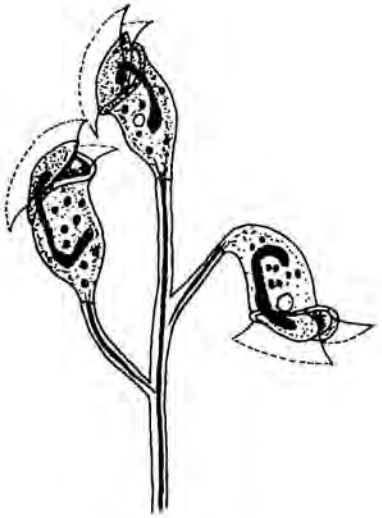


### *Carchesium*

*Carchesium* is a sessile ciliate with bell-shaped cells. These cells have a diameter of 50–125 µm. There is a wreath/ring of cilia around the mouth opening and the rest of the cell surface is bare.

The stem contains a contracting 'muscle'. The stems are mostly branched, thereby creating colonies. These colonies can reach a diameter of a few mm. The 'muscle' in the stem is interrupted at the points where branching occurs.

*Carchesium* sp. commonly occur in activated sludge at a sludge load less than ca. 0.2 kg BOD/kg MLSS.day.



### *Epistylis*

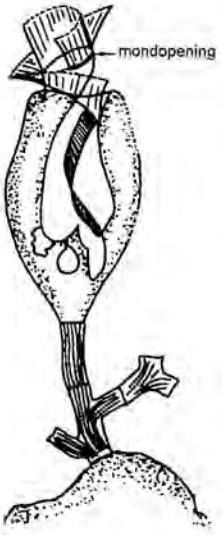
*Epistylis* has somewhat 'vase'-shaped cells. They have a diameter of 70–100 µm. The stem is usually branched, which causes colonies to arise, but does not contain a 'muscle'. Therefore, the stems cannot contract (the cell actually does). The colonies can reach macroscopic dimensions (mm).

*Epistylis* sp. occur commonly in activated sludge, especially at sludge loading levels of 0.1 to 0.2 kg BOD/kg MLSS.day.

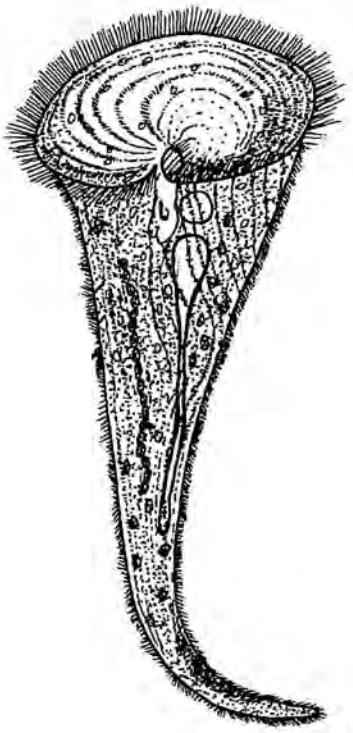
### *Opercularia*

*Opercularia* has somewhat bell-shaped cells. The cell diameter is approximately 140 µm. The stems are branched, causing colonies to form. A non-contracting 'muscle' is present in the stem. In comparison with the other sessile ciliates, *Opercularia* has a small mouth opening.

*Opercularia* sp. mainly occur at higher sludge loading levels (0.2 to 0.3 kg BOD/kg MLSS.day).



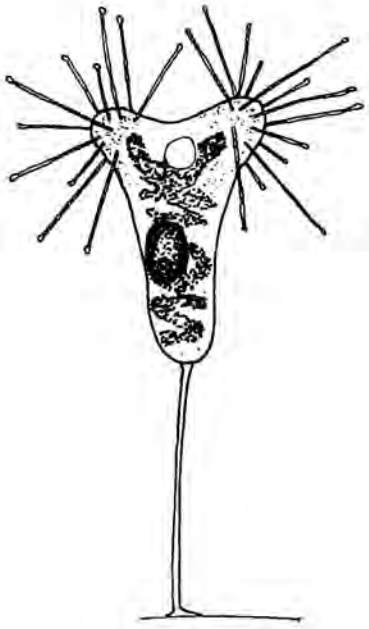
### *Stentor*



*Stentor* is a ciliate with a very characteristic trumpet-shaped cell. It is usually, but not always, attached to a sludge floc. A wreath of cilia for transporting water and nutrient particles to the mouth opening is present at the broad extremity. The cell length can vary from 150 to 500 µm. It is, therefore, a large ciliate. *Stentor* does not possess a stem.

*Stentor* is chiefly observed in low-loaded treatment plants.

### Suctoreans



Suctoreans are ciliates, although cilia are absent on adult cells. The cilia disappear when the cell forms a stem with which the organism can attach itself to a surface, e.g. an activated sludge floc. Adults are therefore sessile. Some very characteristic, dead straight tentacles are present on the cell surface. These tentacles are thicker than the straight pseudopoda of the heliozoa. For most suctoreans, a small knob is present at the extremities of all the tentacles. This is a type of mouth for catching protozoa, which are sucked empty. Protozoa form the most important source of food for suctoreans. Depending upon the strain, both round and vase shaped cells can be observed in activated sludge. The cells in activated sludge usually measure 50–100 µm in length.

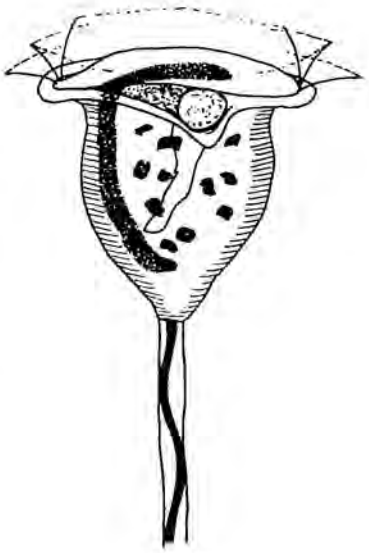
Suctoreans are regularly observed in activated sludge. The population is generally always small.

### *Vaginicola*

*Vaginicola* is a sessile ciliate with a trumpet-shaped cell. The cell is contained in a distinctly transparent funnel. Two cells are often present in this funnel. The cell can contract, meaning that it can disappear completely into the funnel. A wreath of cilia, which transports water and nutrient particles to the mouth opening, is present on the 'head' of the cell. The cell measures approximately 100 µm in length.

*Vaginicola* is chiefly observed in low loaded plants (oxidation ditch conditions).

### *Vorticella*



*Vorticella* is a sessile ciliate whose cells measure 50–150 µm. These are somewhat bell shaped. A wreath of cilia is present around the mouth opening by which the organism directs water (containing nutrients) towards the mouth opening. The stem is never branched, meaning that *Vorticella* is a solitary organism. A contracting muscle is present in the stem.

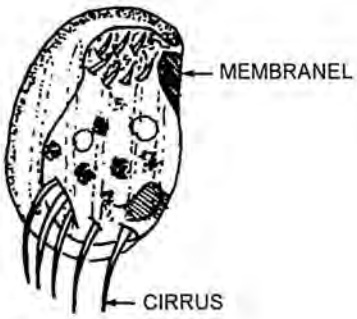
*Vorticella* sp. commonly occur at sludge loading levels < ca. 0.4 kg BOD/kg MLSS.day.

### *6.1.1.2 Crawling ciliates*

The representatives of this group 'crawl' over the sludge flocs and graze their surfaces. Bacterial cells that are not firmly attached to the flocs are removed in this manner.

#### *Aspidisca*

*Aspidisca* is a ciliate that crawls, often at a high speed, over the flocs. Seen from above, the cell is round whereas the side view is more oval shaped. A distinct mouth opening is absent. Five ridges are present on the 'back'. The cilia are not individually implanted on the body but are present as cirri. Cirri are small bundles of cilia that come to a point and look like small feet. There are seven cirri on the front ventral side and five or more on the back of the cell. The cilia also form a membranel ('strips' of cilia that are stuck together).



Onderkant

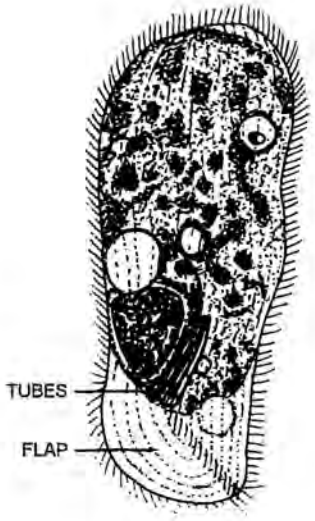

Zij-aanzicht

The sizes of the different *Aspidsca* strains can vary from 30 to 50 µm. The very common species *Aspidisca costata* has a diameter of 30µm. *Aspidisca* resembles *Euplotes,* which is larger (30–100 µm) and does not crawl over the flocs.

*Aspidisca* occurs very frequently. As long as the sludge load is not too high (< ca. 0.4 kg BOD/kg MLSS.day), this ciliate can be observed in almost every activated sludge.

#### *Chilodonella*

*Chilodonella* is a crawling/grazing ciliate whose most important characteristic is an almost transparent flap on the front side. This flap often curls up when the organism crawls over the floc. Seen from the side, this flattened flap is also clearly recognisable. The mouth opening is surrounded by a bulge in the shape of a short tube. The cell length can vary from 40 to 125 µm.




*Chilodonella* commonly occurs in activated sludge if the sludge load is < ca. 0.2 kg BOD/kg MLSS.day.

***Trachelophyllum***

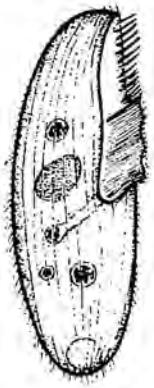
*Trachelophyllum* species are characterised by their flat, elongated cells, which are completely covered with cilia. The cilia are longer around the mouth opening and are ordered in a characteristic manner (bowed 'moustache hairs'). *Trachelophyllum* usually move rapidly through the water between the flocs, but can also be observed crawling over them. The most common representative of this strain is *Trachelophyllum pusillum*, which has a cell length of 30 to 50 µm.

*Trachelophyllum* is a very commonly occurring ciliate at sludge loading levels less than ca. 0.4 kg BOD/kg MLSS.day.

### *6.1.1.3 Free-living ciliates*

As the name suggests, these ciliates move freely in the water between the flocs. The speed at which they move is highly variable.

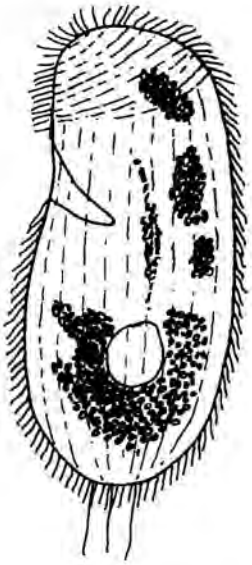
***Blepharisma***



The (light) pink cells of *Blepharisma* are very characteristic. These free moving ciliates have somewhat oval cells of 200 µm in length. A zone with strips of relatively long cilia is present near the mouth opening. The cilia transport water, containing nutrients, to the mouth opening.

*Blepharisma* is mainly observed at some higher sludge loading levels (0.1–0.4 kg BOD/kg MLSS.day).

### *Colpidium*



*Colpidium* is a free-moving ciliate possessing a kidney-shaped cell. The cell surface is covered with cilia. A few long vibrating hairs are present on the back of the cell. The zone around the mouth opening is dented inwards. The most usual species (*Colpidium colpoda*) has a cell length of ca. 100 µm.

*Colpidium* is less frequently observed than the other free-moving ciliates. This ciliate probably occurs most commonly at sludge loading levels of 0.1–0.4 kg BOD/kg MLSS.day.

### *Euplotes*

*Euplotes* is a free-swimming ciliate with oval cells. The cilia are stuck together as cirri. There are nine cirri present on the front side and five on the back. These cirri function as pseudopoda. There is also a characteristic strip of joined cilia present on the cell, which transports nutrients to the mouth opening. Six ridges are present on the upper surface of the cell. The sizes of the different strains can vary from 30 to 100µm.

*Euplotes* resembles *Aspidisca*, but is usually bigger and is free swimming.

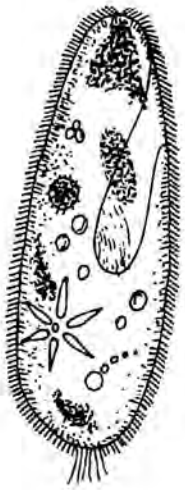
*Euplotes* commonly occurs in activated sludge, principally at sludge loading levels of 0.1–0.2 kg BOD/kg MLSS.day.

### *Litonotus* (*Lionotus*)

The cells of *Litonotus* are shaped like a bottle (amphora). The neck, which is almost as long as the rest of the cell, is slightly bowed. The total length of the cell is ca. 100 µm. The cilia on the outside of the neck are longer than those on the remainder of the cell surface. *Litonotus* usually moves through the water in an 'elegant' manner.

*Litonotus* commonly occurs in sludge loading levels lower than ca. 0.4 kg BOD/kg MLSS.day.

***Paramecium***



*Paramecium* is a free-moving ciliate whose cells slightly resemble slippers. It is a large ciliate. Cell length can vary from 180 to 300 µm. The cell surface is covered with cilia. The nucleus of the cell is large and usually clearly visible. The contractible star-shaped vacuole is very characteristic.

*Paramecium* is primarily observed at sludge loading levels of 0.1–0.3 kg BOD/kg MLSS.day.

***Spirostomum***

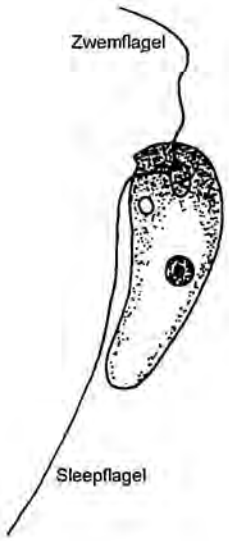

*Spirostomum* has a markedly elongated, flexible cell covered with cilia, with a characteristic vacuole present at the extremity. *Spirostomum* usually moves quickly through the liquid between the flocs. It is the largest ciliate present in activated sludge, the cell length varying from 500 to 900 µm.

*Spirostomum* commonly occurs in activated sludge, particularly in treatment plants with sludge loading levels less than ca. 0.2 kg BOD/kg MLSS.day.

### 6.1.2 Flagellates

Flagellates derive their name from the fact that they possess one or more (usually no more than eight) flagella. The flagella are longer than the cilia of ciliates. On account of their rapid movement, the flagella are quite often not clearly visible. The flagella assist the movement of the cell. Some species have a mouth opening with which they can consume nutrient particles such as bacteria cells. Other strains absorb dissolved nutrients through their cell walls. Because of their diameters of 10–30 µm, flagellates are noticeably smaller than ciliates. Therefore, a 40× objective should be used for microscopic viewing.

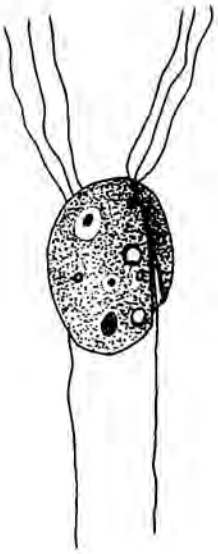
### *Bodo*




*Bodo* is a free-swimming flagellate, which propels itself with a characteristic jerky movement. There are two flagella present, one swimming flagellum and one trailing flagellum at the rear. The flagella are longer than the oval cell. The cell wall is dented at the point where the flagella are implanted.The flagella are not always clearly visible. The cells are approximately 15 µm in length.

The presence of *Bodo* sp. indicates a high sludge load (> ca. 0.4 kg BOD/kg MLSS.day) and/or a lack of oxygen.

### *Hexamitus*



*Hexamitus* is a free-swimming flagellate which moves rapidly in a straight line through the water (but does not rotate like *Trepomonas*). There are two groups of three flagella on the front side and two trailing flagella behind. The cell is somewhat oval-shaped and has a diameter of approximately 20 µm.

The presence of *Hexamitus* indicates a high sludge load (>ca. 0.4 kg BOD/kg MLSS.day) an/or a lack of oxygen.

### *Peranema*

*Peranema* is a free-swimming flagellate with a very characteristic, long, thick flagellum. A second much thinner (trailing) flagellum is also present, but this is hardly ever visible. Only the tip of the flagellum moves when the cell is in motion. The cell is usually 20–30 µm long.

This flagellate is regularly observed in activated sludge. In contrast to most other flagellates, the presence of *Peranema* is not indicative of specific process conditions such as a high sludge load or a lack of oxygen.

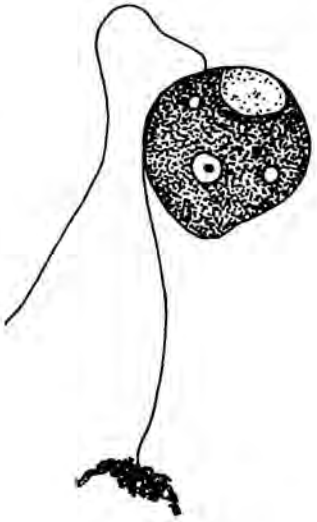
### *Monosiga*

The cell is spherical to oval. A collar is present on its 'top' through which the flagellum emerges. *Monosiga* is usually fixed to a sludge floc. The cells are 1–15 µm in length.

The presence of *Monosiga* indicates a high sludge load (> ca. 0.4 kg BOD/kg MLSS.day) and/or a shortage of oxygen

### *Pleuromonas*

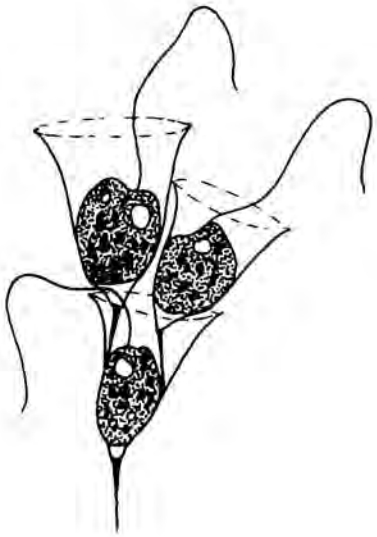
*Pleuromonas* is a flagellate that moves in a characteristically rapid and jumpy manner. The cells are usually not free-living in the water but are attached to the flocs. *Pleuromonas*, like *Bodo*, has two flagella. The cell is attached to the floc by the longest flagellum. Cell diameter is 5–10 µm.



The presence of *Pleuromonas* indicates a high sludge load (> ca. 0.4 kg BOD/kg MLSS.day) and/or a shortage of oxygen.

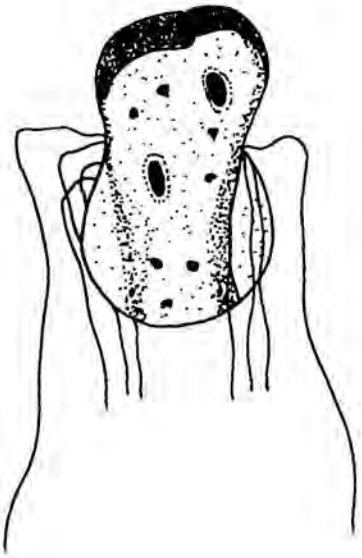
### *Poteriodendron*

*Poteriodendron* is a flagellate whose most important characteristic is that it forms colonies of cells. The cells are contained in transparent funnels. These funnels are joined together into colonies by stems on their undersides. The diameters of the individual cells are ca. 20 µm. The cells have a flagellum which allows them to move inside the funnel but they cannot leave it.



*Poteriodendron* colonies are regularly observed in activated sludge. It is not known if this flagellate can be used as an indicator organism.

***Trepomonas***



*Trepomonas* is a free-swimming flagellate which moves through the water in a characteristic rotating manner. Seen from above, the cell is oval and from the underside it is spherical. The cells measure approximately 20 µm in length. *Trepomonas* possesses two groups of four flagella which are implanted on the side of the cell. Besides two long (20 µm) flagella, six short (8 µm) ones are also present

The presence of *Trepomonas* indicates a high sludge load (> ca. 0.4 kg BOD/kg MLSS.day) and/or a lack of oxygen.

### 6.1.3 Amoeba, testate amoeba and heliozoa

These organisms have in common that they can form pseudopoda. These pseudopoda are temporary extensions of the cell content.

**Amoeba**

Amoeba are single-celled organisms possessing a flexible cell membrane which allows the shape of the cell to constantly change. They absorb nutrient particles (bacteria cells, other protozoa, etc.) by engulfing them. This resembles the uptake of food by a mouth opening. The sizes of the various strains range from 50 to 400 µm. Amoeba can be as big as a sludge floc and their particular structure sometimes resemble sludge flocs. Apart from the odd exception, amoeba move extremely slowly (by means of the pseudopoda).

Amoeba are characteristic of somewhat higher sludge loading levels (0.1–0.4 kg BOD/kg MLSS.day) and/or shortages of oxygen. They are seldom observed in low loaded nutrient removal plants.

## Testate amoeba

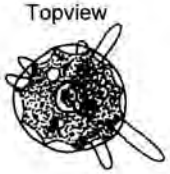
In this group of amoeba, the cell is surrounded by a type of shell. There is an opening in the shell through which the pseudopoda can protrude. These pseudopoda are seldom visible on a microscopic slide. The cell form (round, beaker shaped, etc.) depends on the species in question.

*Arcella* is a testate amoeba with a shell which, when seen from above, is round. The side view resembles the top of a toadstool. The shell has a clearly observable structure. In general, the shells are practically transparent, but in activated sludge they are usually coloured yellow-brown on account of the precipitation of iron compounds on their surfaces. The round opening on the underside of the shell is very characteristic.

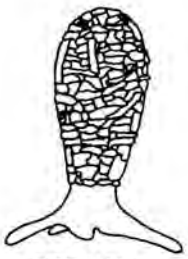
The structure of *Euglphya* shells largely resembles that of a honeycomb. The sizes of the various testate amoeba can vary from 30 to 200 µm.

Testate amoeba are almost always present, and often in large numbers, in low-loaded activated sludge plants. *Arcella* is the most commonly occurring species. It mainly occurs under nitrifying conditions.

ARCELLA
Topview



ARCELLA
Sideview



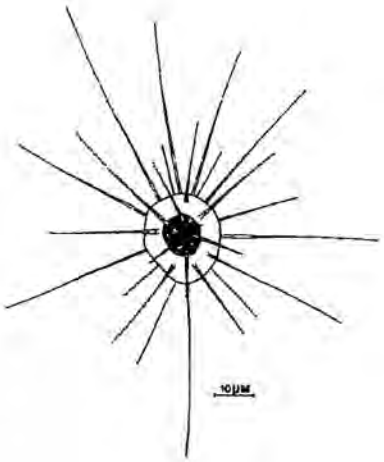
DIFFLUGIA

EUGLYPHA

## Heliozoa

Heliozoa belong to the amoeba. They have spherical cells which are surrounded by needle thin, dead straight, retractable pseudopoda. The pseudopoda are not used for propelling the cell but for catching bacteria and protozoa. Organisms that come into contact with the pseudopoda are 'paralysed' after which they are consumed by the heliozoa. The cell diameter can vary from 40 to 100 µm.

Heliozoa are principally observed in low-loaded activated plants. The population is usually small.



## 6.2 Metazoa

In contrast to bacteria and protozoa, metazoa are multi-cellular micro-organisms, meaning that they are 'higher' organisms. The sizes of the different species can vary from 100 µm to sometimes as much as 1–2 cm. Most metazoa feed on free-living bacteria cells or on very small floc particles. Species also exist that can consume whole sludge flocs.

The following metazoan groups can be present in activated sludge:
- Rotifers
- Nematodes
- Worms
- Tardigrades

Apart from exceptional cases, metazoa play a subsidiary role in activated sludge treatment plants. They are mainly observed at sludge loading levels lower than ca. 0.15 kg BOD/kg MLSS.day. To register the number of metazoa, a scale varying from 0 (= absent) to 3 (= numerous organisms/slide) is used.

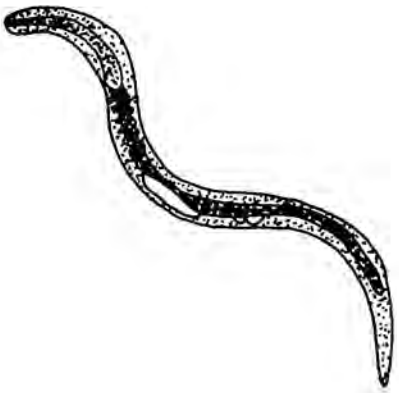
### 6.2.1 Rotifers

Rotifers are relatively large, distinctly mobile and elongated multi-celled organisms. The body length can vary from 100 to 500 µm and is surrounded by a type of armour into which the head and tail can be withdrawn. A few bunches of cilia are present on the head of the organism. These cilia can create a current in the direction of the mouth opening. They have a set of 'jaws' around their mouth openings by which they can crack particles filtered from the water. Particles larger than about 10 µm cannot actually fit in this mouth opening. Rotifers, therefore, principally consume free-living bacteria cells and small floc particles.

The tail is branched in a characteristic manner and is important to the movement of the organism. With this, a rotifer first attaches itself, after which the body is stretched. The tail is subsequently released, the head stays in place and the abdomen is drawn up. This manner of propulsion resembles the manner in which a slug moves.

Rotifers commonly occur in activated sludge with low loading levels. The size of the population is nearly always very limited in domestic treatment plants. Large numbers of rotifers are sometimes present in plants treating waste water from the agro-industry.

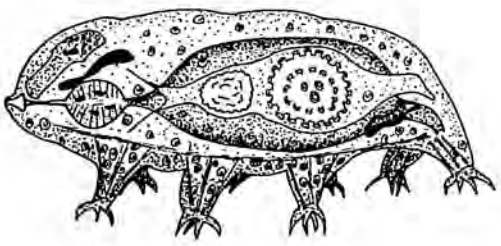
### 6.2.2 Nematodes



Nematodes have elongated, cylindrical and very flexible bodies. The extremities of this multi-celled micro-organism are thinner than its central section. The cell length can vary from 0.5 mm to 3 mm. They are larger than most protozoa. They principally consume free-living bacteria cells and very small floc particles. They swallow/engulf food particles by contraction of the oesophagus. Owing to the fact that they do not possess 'jaws' and that their mouth openings are small, they cannot consume intact flocs. These exceptionally mobile animals are sometimes difficult to keep in focus during microscopic investigation. In addition, nematodes often 'crawl away' into the flocs and it can be a while before they reappear.

Nematodes are regularly observed in activated sludge with a low loading level, but they are almost never present in large numbers. It is not known if nematodes can be used as indicators for given process conditions.

### 6.2.3 Tardigrades

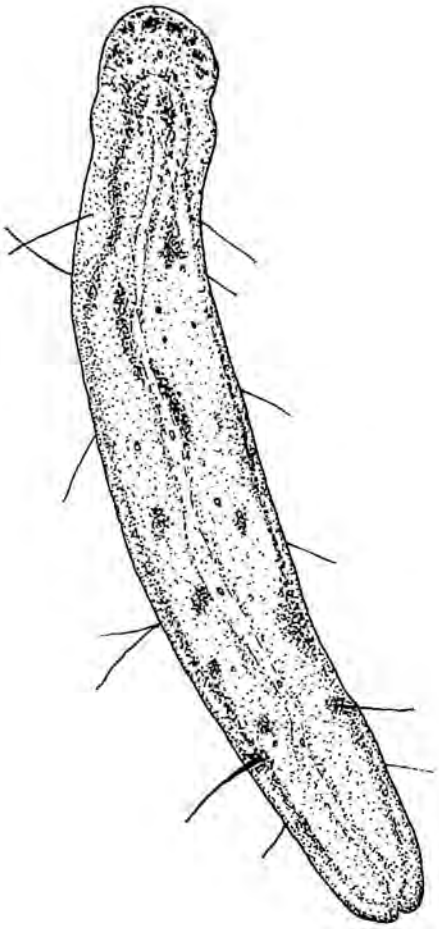


This is a multi-celled organism with a very striking form. A tardigade, sometimes known familiarly (to the experts) as a water bear, has pseudopoda with small claws, with which it crawls over the flocs and grazes them. This organism moves around somewhat awkwardly when free in the water. The size can vary from 200 to 1200 µm.

Tardigrades are occasionally observed at sludge loading levels of < 0.1 kg BOD/kg MLSS.day.

### 6.2.4 Worms

Worms are the largest organisms observed during the microscopic investigation of activated sludge. With diameters of approximately 0.1 mm and lengths to a maximum of 10 mm, their presence is very apparent on a wet slide. Oligochaeta are almost always involved, often *Nais elingius* and *Aelosoma* spp. Worms are able to consume sludge flocs or particles of flocs. A worm bloom (ca. $10^5$ worms/l) is also connected with a reduction in sludge production.



Little is known about the growth of worms in activated sludge plants. They do not grow particularly slowly. Under the best of conditions they have a generation time of a few days. More than enough nutrients, in the form of sludge flocs, seem to be present. Consequently, one would expect worms to be present in virtually all treatment plants. However, they are not often observed at low sludge loading levels (oxidation ditch conditions). Sludges containing many worms usually originate from treatment plants with a sludge load of ca. 0.1 kg BOD/kg MLSS.day and in which the influent is pre-settled. A definite connection between their presence or absence and the process conditions is usually missing.

## 6.3 Indicator function

A new activated sludge plant can be started by opening the sewage supply and switching on the aeration system. Sludge flocs subsequently arise spontaneously in the aeration tank. These are removed in the final clarifier and recycled into the aeration tank. By constantly adding fresh sewage, without withdrawing the sludge from the system, the desired biomass concentration can be cultivated within a few weeks. During the start phase, the sludge loading level is constantly reduced and the treatment performance is increased.

A shift within the protozoa population takes place simultaneously, which is actually a characteristic succession of species. Many free-living bacteria cells ($\geq 10^8$ cells/ml) are present during the first days and the compounds, which have been transported with the sewage, are not yet fully processed by the bacterial biomass. An excess of food for

flagellates and amoeba is created, and they also develop *en masse*. After a few days also free swimming ciliates appear. The numbers of flagellates and amoeba are reduced considerably thereafter. Because of the increase in the sludge concentration, the sludge load decreases constantly during such an initial phase. A subsequent scarcity of nutrients automatically results in a decrease in the growth rate of the free-living ciliates.

Many species disappear as soon as their population doubling rate exceeds the hydraulic retention time in the aeration tank. At this stage, the ciliates that are attached to the sludge flocs (e.g. *Vorticella*), or those that crawl over them (e.g. *Aspidisca),* appear and, therefore, are not easily washed out. This explains the domination of attached and crawling species at low sludge loading levels. Thecamoeba, and sometimes metazoa, also eventually appear with long sludge ages. By this time, a high level of treatment performance, including extensive nitrification, takes place; the number of bacterial cells that are not bound to the flocs is less than $10^6$/ml.

Therefore, other strains grow as the treatment performance rises. This fact can also be used in the opposite sense: the presence of certain strains says something about the treatment results and the process conditions.

The connection between the sludge load and the composition of the population is shown systematically in Fig. 69. During the microscopic investigation of the sludge, it should be checked that the species present are characteristic for the load applied in the treatment plant. If the sludge load is not too high, mainly ciliates, testate amoeba and occasionally some metazoa should be present. A shortage of oxygen in a low loaded plant results in a decline of the COD removal rate and causes, therefore, a shift towards flagellates and amoeba within the population

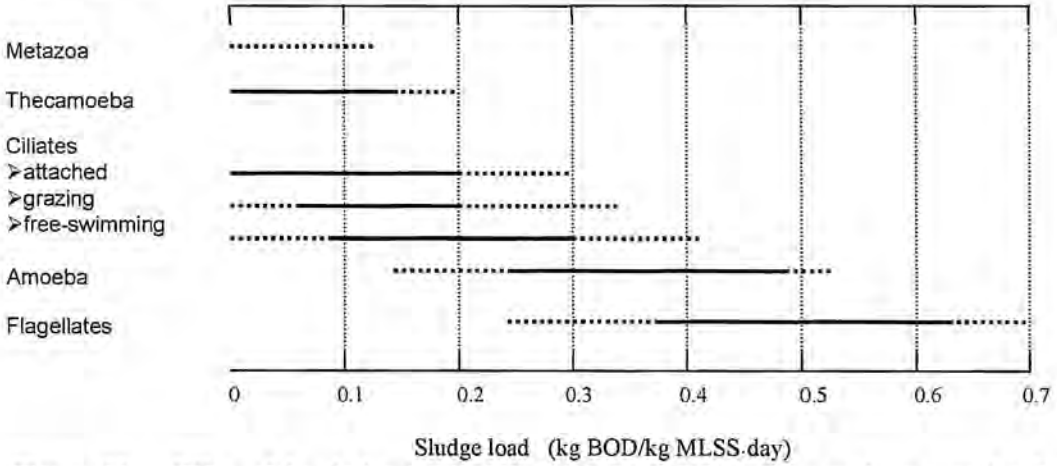



*Figure 69 Effect of the sludge load on the occurrence of protozoa and metazoa (no lack of oxygen).*

If protozoa and metazoa suddenly disappear, then toxic components are present in the influent. This can be followed within a few days by an explosive increase in the number of protozoa, because the number of free-living bacterial cells has considerably increased during their absence.

A strong increase in the number of worms in activated sludge can cause a decrease in the production of surplus sludge (see paragraph 9.7).

# 7 Conclusions of sludge investigation

Microscopic sludge investigation should result in a conclusion concerning the quality of the activated sludge expressed as 'good', 'moderate' or 'poor'.

When establishing the quality of the sludge, the observations concerning floc morphology, filamentous population, etc. are integrated into a final conclusion. This is not so easy as, in fact, very different things are being compared. The various aspects are also not of equal importance. For instance, a high filament index is more problematic than the presence of many free-living cells. The importance attached to the various criteria during assessment is shown in table 3. If only one aspect is assessed as 'moderate' and the rest as 'good', then the overall sludge quality is considered as 'good'. Two to three 'moderate' assessments also result in a final conclusion of 'moderate'. The sludge quality is considered 'poor' if there are higher scores in the 'moderate' column, or if aspects are directly assessed as 'poor'.

*Table 3 Criteria for establishing the sludge quality*

| | Good | Moderate | Poor |
|---|---|---|---|
| Filament Index | < 3 | 3–4 | 4–5 |
| Free-living cells | 0–1 | 2–3 | ≥ 3 |
| Spirils | 0 | 1 | ≥ 2 |
| Ciliates/Testate amoeba | ≥ 1 | < 1 | 0 |
| Flagellates/Amoeba | 0 | 1–2 | ≥ 3 |
| %flocs > 25 µm | > 80 to 90 | > 50 to 70 | < 50 |
| Floc structure | compact | open | — |
| Floc 'strength' | robust | weak | — |
| Floc shape | rounded | irregular | — |

N.B. In some treatment plants, a moderate/poor quality is inherent to the design.

# 8 The activated sludge process

## 8.1 Use and acceleration of natural cyclic processes

The processes that take place in biological water treatment plants are derived from natural material cycles. The sun drives these cycles. Energy is always essential for growth/synthesis. Algae, plants and trees use solar energy to grow (photosynthesis). They take up elements such as nitrogen, phosphorus and sulphur from the ground and from water. Together with carbon from the $CO_2$ in the atmosphere, these elements are processed into the organic building blocks which create algae, plants and trees. The cell material which is formed can subsequently serve as food (energy) for people and animals which, in their turn, are sometimes consumed by other organisms.

All life is finite. Sooner or later only dead organic material remains. Everyone knows that leaves, even entire tree trunks and dead animals, disappear in the passage of time. Numerous micro-organisms, principally bacteria and fungi, are responsible for this degradation process. The elements (C, N, P, S, etc.) confined in the organic material are released by this degradation process (= mineralisation) and can subsequently be reused by photosynthetic organisms. This closes the cycle. The recycling of elements in natural material cycles is as old as the earth itself. An example of the nitrogen cycle is given in Fig. 70.

Numerous micro-organisms are, by nature, involved in the degradation of organic material. This is because dead organic material forms their only source of food. Just like all other living creatures, micro-organisms need constant energy for their life processes. To provide for this, the available organic compounds are partially broken down. This produces energy which is exploited so as to convert the remaining portion of the nutrients into cell components. This involves:

- replacing defective cell components;
- forming storage materials, so as to survive periods of food shortage. The quantity of storage material which can be stored is also dependent upon the type of organism;
- forming new cells, as long as sufficient nourishment is available. This growth is re-encountered during the treatment of waste water as surplus or excess sludge.

Micro-organisms that derive energy from the conversion of inorganic compounds also exist. The nitrifying bacteria (see Fig. 70) are known examples of this.

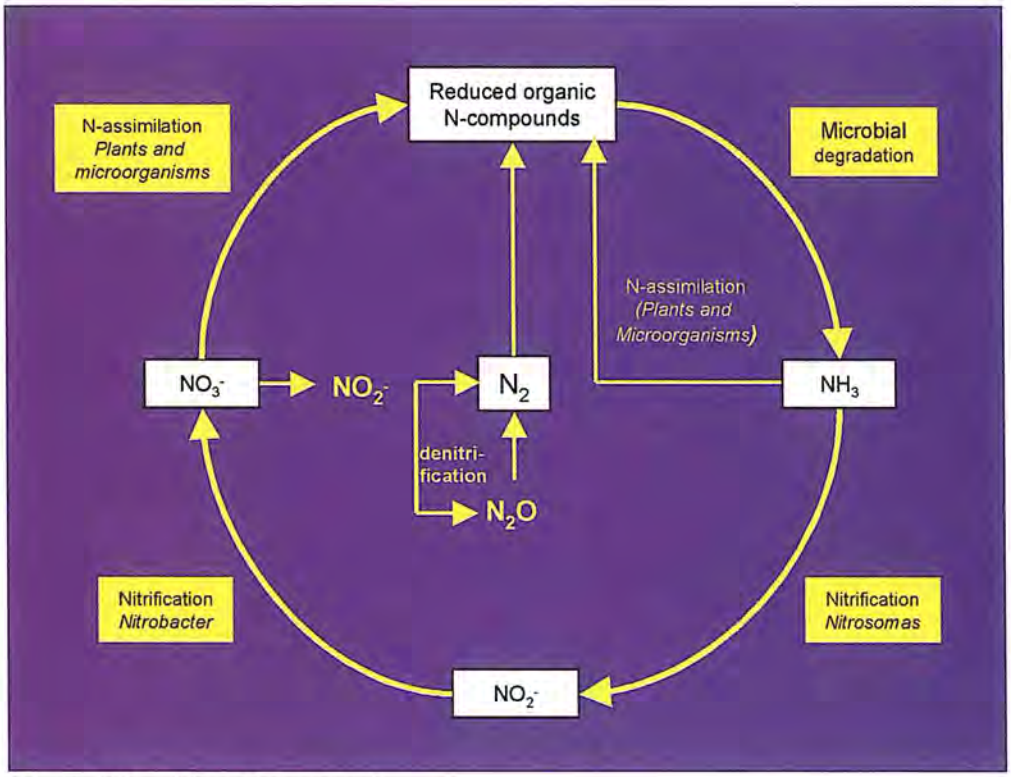



*Figure 70 The nitrogen cycle.*

Sewage contains too few micro-organisms to allow the degradation/conversion to pass off quickly and efficiently. The activated sludge process overcomes this difficulty. By maintaining a large quantity of 'pre-cultured' biomass in the treatment plant, degradation can be accelerated and can be carried out under controlled conditions.

## 8.2 Formation of activated sludge flocs

Aeration of waste water spontaneously leads to the formation of biomass flocs with the passage of time. These settle out as soon as aeration ceases. After the supernatant, of which the pollution level is substantially reduced, is removed, the settled sludge can be used for the treatment of a new batch of waste water. This causes the amount of material which can be settled to increase and the treatment process to progress more rapidly. The activated sludge process is based on this discovery (made at the end of the nineteenth century).

The following processes take place during the aeration of the waste water, in which many bacteria are naturally present. The nutrient concentration (= F) is high at the beginning. This means that aerobic bacteria can grow quickly and multiply themselves through cell division. Therefore, the quantity of biomass (= M) increases. The increase in M as well as the reduction in F run logarithmically, the F : M relation consequently reduces quickly during aeration. This continues until the nutrients run out. If the F/M ratio is low, the cells of many bacterial strains 'stick together'. Conglomerates of cells arise: i.e. the activated sludge flocs.

In activated sludge treatment plants, the flocs and the effluent are separated by means of settlement. The settled biomass is largely returned to the aeration tank, as a result of which a low F/M ratio is maintained. This is not only necessary for floc formation, but also to allow the treatment process to proceed quickly. The increase of the biomass is removed as surplus sludge.

Bacteria form flocs to survive nutrient-poor environments. This helps them to avoid being washed away. Floc formation also provides protection against many bacteria consuming protozoa as these are unable to 'bite off' parts of the floc.

Flocs are more robust if the sludge loading level (F/M ratio) is lower. This is connected with the relative increase in the number of Gram positive bacteria strains in the floc, as referred to in paragraph 4.1.

Floc formation is a complicated and as yet not completely understood phenomenon. Several factors are known to contribute to floc formation:
- many bacteria form slime capsules around their cells comprised of polymer compounds. This glues, as it were, the cells together;
- bacteria are negatively charged. Positively charged ions, especially divalent ions such as $Ca^{2+}$, contribute to the bonding of the cells or of floc components;
- some bacteria form a network of extremely thin filaments (fibrils) around their cells. This network also contributes to the bonding of cells and to entrapment of other bacteria and particles.

## 8.3 Factors that determine the composition of activated sludge flocs

Activated sludge flocs are conglomerates of:
- living micro-organisms, principally bacterial cells. Many different species are almost always present;
- dead cells. The percentage of dead cells increases if the sludge loading level is lower;
- 'undigested' organic fragments which have been entrapped by the floc;
- sand grains and precipitated inorganic compounds, such as salts. The size of this fraction (the ash content) can vary from 10–60% (in weight).

The composition of the flocs in a given treatment plant is heavily dependent upon the process conditions. The most important process conditions are explained in paragraphs 8.3.1. to 8.3.5. Previous to this, three biological aspects are discussed.

*Mixed population*

In a biological treatment plant, a large number of different (in)organic compounds are brought into contact with a large diversity of micro-organisms. The compounds present in the influent are exploited by the micro-organisms for their energy supply and for the formation of cell components.

Any given compound cannot actually be utilized by every micro-organism, and large differences arise. Some micro-organisms are almost 'omnivores', whereas on the other hand, other bacteria (e.g. nitrifyers) are highly specialised. This diversity in the bacterial population makes it possible to remove so many different components simultaneously from the waste water.

*Competition*

Many compounds in waste water are used as food by various micro-organisms. There is always a lack of nutrients as a result of the low F/M ratio. This combination results in a strong competition between the various micro-organisms on account of the scarcity of nutrients. The winners dominate the sludge population. To maintain a stable process, it is necessary that conditions are created in which the desired bacterial strains can consume the largest part of the available nutrients.

*Not static, but dynamic*

The activated sludge population is apparently in equilibrium. In practice, this population actually changes constantly owing to the influence of the seasons, changes in the composition of the influent, etc. This is not usually noticeable in the overall treatment performance, because changes in the sludge population are 'driven' by the best possible exploitation of the nutrients on offer. The mostly excellent treatment results are a direct consequence of this. The fact that the population is not static, but really dynamic, means that the composition can be deliberately influenced by the process conditions. The most important process conditions are : the sludge load applied, the composition of the waste water, the oxygen concentration, the configuration of the plant, the pH and the temperature. In practice, there is practically always a combined effect of these conditions on the population composition.

### 8.3.1 Sludge load and sludge age

Micro-organisms duplicate themselves by means of cell division, as a result of which two new cells arise from one. The minimum time that is necessary for such cell division can vary, according to the different micro-organisms, from 30 min to a few days. Considerable differences in growth rate therefore occur.

In addition, the growth rate of a given species is not constant but is heavily dependent upon the prevailing process conditions. The availability of nutrients, the temperature, the pH and the oxygen supply all affect the growth rate. The maximum growth rate is only achieved under optimum conditions. It is the case for most bacteria in an activated sludge plant that the actual growth rate is often significantly lower than the maximum possible division time.

The sludge loading level (in kg BOD or COD per kg MLSS per day) indicates how much food is available to the micro-organisms. The population will grow faster at a higher loading level. As a constant sludge concentration is maintained in the treatment plant, the quantity of discharged sludge increases and the sludge age decreases as the loading level rises. The sludge load and its associated sludge age therefore have a large influence on the composition of the sludge population. Only micro-organisms with generation times less than the sludge age can be maintained in the sludge. All other strains are completely removed with the discharged sludge.

A lack of nutrients hardly ever occurs at high loading levels (sludge age: a few days), meaning a maximum growth rate for many species. The fastest growers consume the most nutrients and therefore come to dominate the population. Under these conditions, the floc is not very robust and many free-living cells are often present. As the floc is comprised of fast growing strains, the composition of the sludge population can completely change within a few days.

Relatively little food is available at low sludge loading levels (sludge age: weeks), causing the micro-organisms to grow slowly. The bacteria which exploit the scarce nutrients efficiently are now in a favourable competitive position. An important percentage of the cells in the floc are dead or in a sort of state of rest.

Owing to the high sludge age, micro-organisms that degrade 'special' compounds, and which often grow slowly, can now also be maintained. The presence of a nitrifying population is a clear example of this. A low sludge loading level therefore goes hand in hand with a greater diversity of the population. The flocs are usually robust and firm. The composition does not change quickly.

### 8.3.2 Composition of the waste water

The origin and the pretreatment of the waste water determine the composition of the influent.

*Nutrient supply*

Micro-organisms require a range of nutrients, such as C, N, P, O and S, for optimum growth. These elements must also be present at a given ratio in order to be fully exploited for the growth of the cell. With the exception of carbon, this ratio must correspond with that in the cell (table 4). Of the carbon compounds in the influent, approximately half are completely degraded for energy provision. This carbon escapes as $CO_2$. For this reason the level of carbon in the nutrients must be twice as high as that necessary for cell synthesis.

*Table 4 Composition of a bacterial cell*

| Element | % of dry weight |
|---|---|
| Carbon | 50 |
| Oxygen | 20 |
| Nitrogen | 14 |
| Hydrogen | 8 |
| Phosphorus | 3 |
| Sulphur | 1 |
| Potassium | 1 |
| Sodium | 1 |
| Calcium | 0.5 |
| Magnesium | 0.5 |
| Chloride | 0.5 |
| Iron | 0.2 |
| Others (Mn, Mo, Zn, etc.) | 0.3 |

The element ratio in waste water is practically always different from that shown in table 4. Simply put, the growth stagnates as soon as one element from the necessary series is (practically) used up. The element which is depleted first is often known as the growth restricting or limiting factor. Growth limiting factors play an important role in the formation of bulking sludge (see chapter 10).

If growth-limiting conditions occur in practice, then shortages of carbon, nitrogen, phosphorus or, if aeration is insufficient, oxygen are usually involved. The remaining elements are almost always present in sufficient quantities.

A shortage of carbon compounds occurs during the treatment of urban waste water. On account of this, so much N and P remain that additional treatment steps must be introduced into the process so that these elements can still be removed.

In industrial waste water often an excess of carbon compounds is present. Nitrogen and/or phosphorus must be dosed in the ratio BOD: N : P = 100 : 5 :1. The desired dosage can sometimes also be achieved by analysing the effluent. For this, 'traces' (0.1–0.2 mg/l) of the elements involved must be present in a form that can be taken up biologically.

*Carbon compounds*

In most treatment plants, the carbon compounds in the influent form the most important source of food/energy for the micro-organisms. The composition of the population is consequently also dependent upon the quality/nature of the carbon compounds present in the waste water. As a result, different bacterial strains to those in domestic treatment plants grow in industrial plants. Individual differences also occur within industry (chemical, dairy, paper, etc). This illustrates the dynamic nature of the activated sludge population: the supply of different nutrients leads to the selection of other species.

Finally, compounds can be present in industrial waste water which can only be degraded by very specialised micro-organisms. Culturing such a population sometimes takes (a lot of) time.

*Particle size*

Apart from the chemical composition, the size of the nutrient particles present in the influent affects also the population composition. In the case of particle size, the difference between 'dissolved' and 'undissolved' is mainly concerned. Micro-organisms can only take up dissolved compounds directly from the water phase. Components present in the particulate/collodial fraction must first be hydrolysed by enzymes outside the cell. Dissolved compounds, such as sugars, amino acids and fatty acids are formed from the macro-molecules. These are subsequently taken up by the cell. Micro-organisms which can manufacture the necessary enzymes are therefore in an advantageous position if there is a large supply of undissolved substrate.

Hydrolysis takes time, as a result of which the undissolved components in the influent are less rapidly absorbable than the dissolved compounds in the waste water. In substrate that has yet to be hydrolysed, a distinction can again be made between slowly and fast hydrolysable particles. An important part of the slowly hydrolysable fraction is removed in the primary settling tank.

Fresh urban waste water contains a large fraction of undissolved substrate. This fraction is reduced by hydrolysis processes when the waste water spends longer under way before it reaches the aeration tank. A long retention time in the transport sewer and/or the pre-settlement tank therefore results in a significant increase in the dissolved fraction in the waste water. The changes occur faster at higher temperatures. Very roughly, raw domestic waste water in The Netherlands poses the following COD composition as it reaches the treatment plant:

- settleable : 30 %
- hydrolysable : 35 %
- dissolved/directly biologically absorbable : 30 %
- inert : 5 %

The 'settleable' and 'hydrolysable' fractions also contain bacterial cell material present in the influent.

The low molecular fraction is important for biological nutrient removal, but it also contributes to the 'bulking sludge' phenomenon (chapter 10).

The size of the dissolved fraction present in industrial influents (agro industry!) is often markedly larger compared to urban waste water.

### 8.3.3 Oxygen

In principle, the activated sludge method is an aerobic process. Aerobic means that molecular oxygen ($O_2$) is available for the biological processes. Many micro-organisms are strictly aerobic, just like humans. Their metabolic processes stagnate without $O_2$. Many species can actually survive longer oxygenless periods (hours), e.g. while the activated sludge remains in a final clarifier. Micro-organisms also exist which can switch to anaerobic metabolic processes if molecular oxygen is lacking.

At an oxygen concentration of 2 mg/l in the aeration tank, all flocs with a diameter less than ca. 400 µm are aerobic as far as their centres. The centres of larger flocs are devoid of oxygen. As a consequence, denitrification, for example, can take place in the centres of such flocs. A reduction of the oxygen concentration in the water means that the anaerobic part of the flocs becomes greater.

Lack of oxygen leads to a reduction in the processing rate of the substrate, as a result of which it remains longer in solution. This contributes to bulking sludge arising, among other things.

### 8.3.4 Configuration

*Nutrient concentration*

The configuration determines the nutrient concentration during the mixing of waste water and activated sludge and has therefore a large influence on the composition of the sludge population. The nutrient concentration in several configurations is demonstrated schematically in Fig. 71.

The influent is diluted with the entire content of the aeration tank in a water treatment plant (WTP) with complete mixing (I). The resulting nutrient concentration in the aeration tank is low and equals the BOD or COD in the final effluent. The concentration becomes higher when the load of the plant is increased.

In a WTP with plug flow (II), the influent is mixed up with a smaller amount of sludge, viz. the recycled sludge flow. As a result, the dilution effect is much less. The nutrient concentration is high in the front section of the tank, but is reduced as the substrate is consumed by the micro-organisms. This causes a substrate gradient to arise longitudinally in the tank. The course of the gradient depends upon the nutrient level during mixing at the front of the tank (known as floc load) and the rate at which the micro-organisms consume the nutrients. Substantial differences are possible in this regard between plants. Consequently, different gradients are shown in Fig. 71.

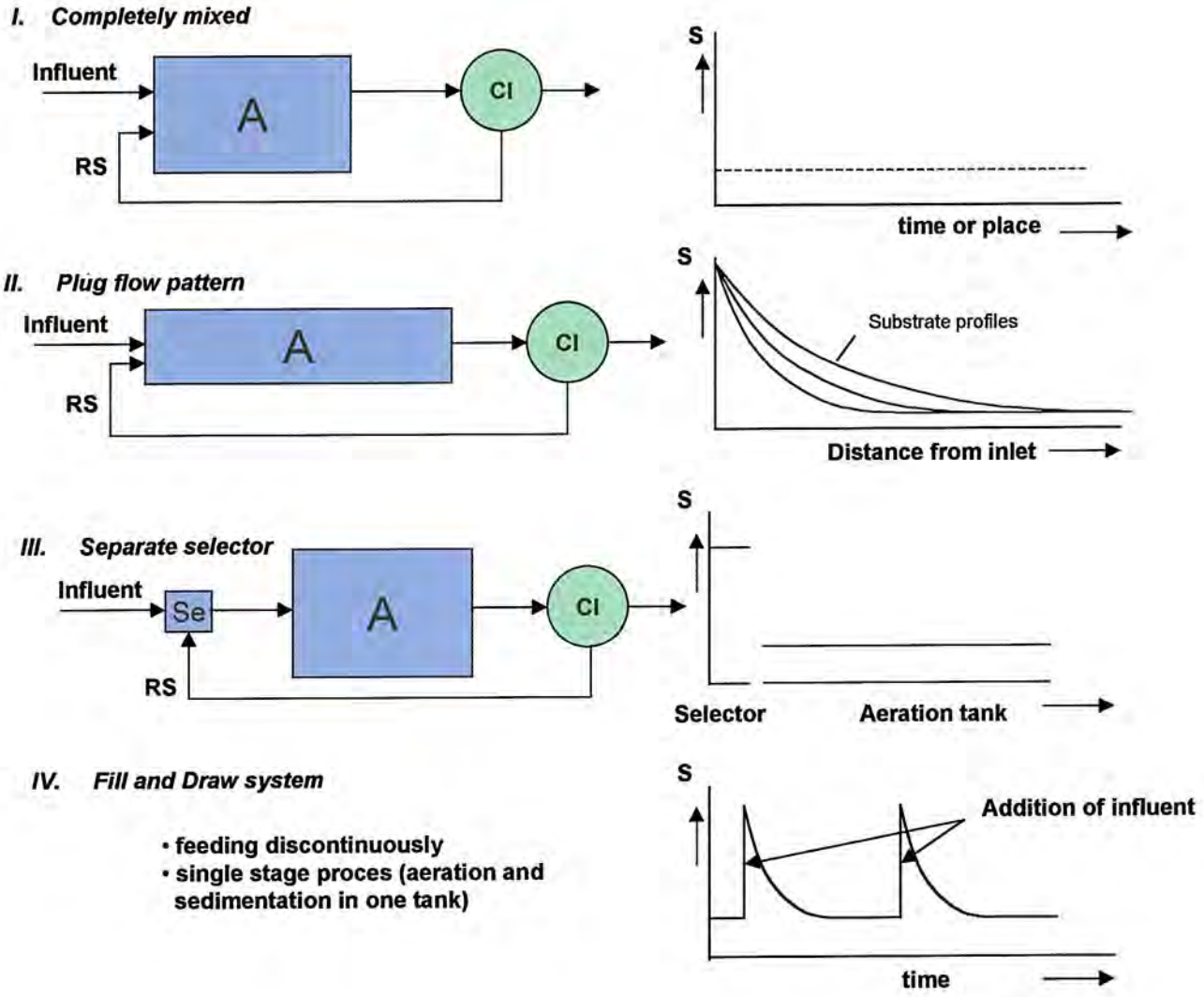



*Figure 71* *The effect of the influent dosing regime (left) on the substrate concentration in the aeration tank (right).*
*Infl.: influent; RS: returned sludge; A: aeration tank; C: final clarifier; Se: contact tank or selector; S: nutrient concentration.*

In configuration III, the influent is not directly introduced into the aeration tank but is first mixed with the returned sludge in a separate contact tank or selector. After being left in this tank for some time, the mixture flows into the aeration tank, in which a considerable dilution takes place. This process implies a high substrate concentration in the selector and a low level in the aeration tank.

An intermittent process is employed in plants with a fill-and-draw configuration (IV). The entire treatment process is carried out in one tank. A relatively large quantity of waste water is added periodically. The nutrient concentration is high directly after this addition, but reduces during aeration. After some time, aeration is stopped. The sludge settles, after which the supernatant (effluent) can be removed. More waste water is subsequently added, etc. The original oxidation ditch was just such a sequential batch reactor (SBR).

Configuration I is advantageous to micro-organisms which 'grow efficiently' at permanently low nutrient concentrations. Alternating conditions prevail in systems II to IV: a short period in which many nutrients are available is followed by a relatively long period in which no new nutrients are supplied. The following selection criteria now determine the composition of the sludge population to an important extent:

- the (maximum) growth rate during nutrient excess;
- the capability to take up nutrients rapidly and to store them inside the cells (hoarding);
- the energy consumption during the long period in which no substrate is added. Species whose energy use is high 'consume themselves' and therefore die.

It is obvious, that this feeding pattern results in a different population composition to those in treatment plants using complete mixing in the aeration tanks.

*Nutrient removal*

Removal of nitrogen and phosphorus using biological techniques means that an anoxic and respectively an anaerobic zone must be introduced into the process sequence. An example of a nutrient removal plant is provided in Fig. 72. Anoxic means that molecular oxygen ($O_2$) is absent but that nitrate ($NO_3^-$) is available. The nitrate/nitrite, which is formed by nitrification in the aerobic zone, is denitrified to nitrogen gas under anoxic conditions. This nitrogen gas is dispersed into the atmosphere. Anaerobic conditions arise if the nitrate/nitrite is also used up. The alternation from aerobic to anaerobic or from anoxic to anaerobic conditions leads to the growth of bacteria which can store extra phosphate in their cells and, in this way, are responsible for the biological removal of P.

For comprehensive information concerning biological N and P removal, the reader is referred to other handbooks (see chapter 13). Here, it is sufficient to remark that both processes are combined with the removal of especially easily biodegradable carbon compounds from the waste water (the dissolved fraction). As it takes place under anoxic/anaerobic conditions, these nutrients are no longer available to strictly aerobic micro-organisms.

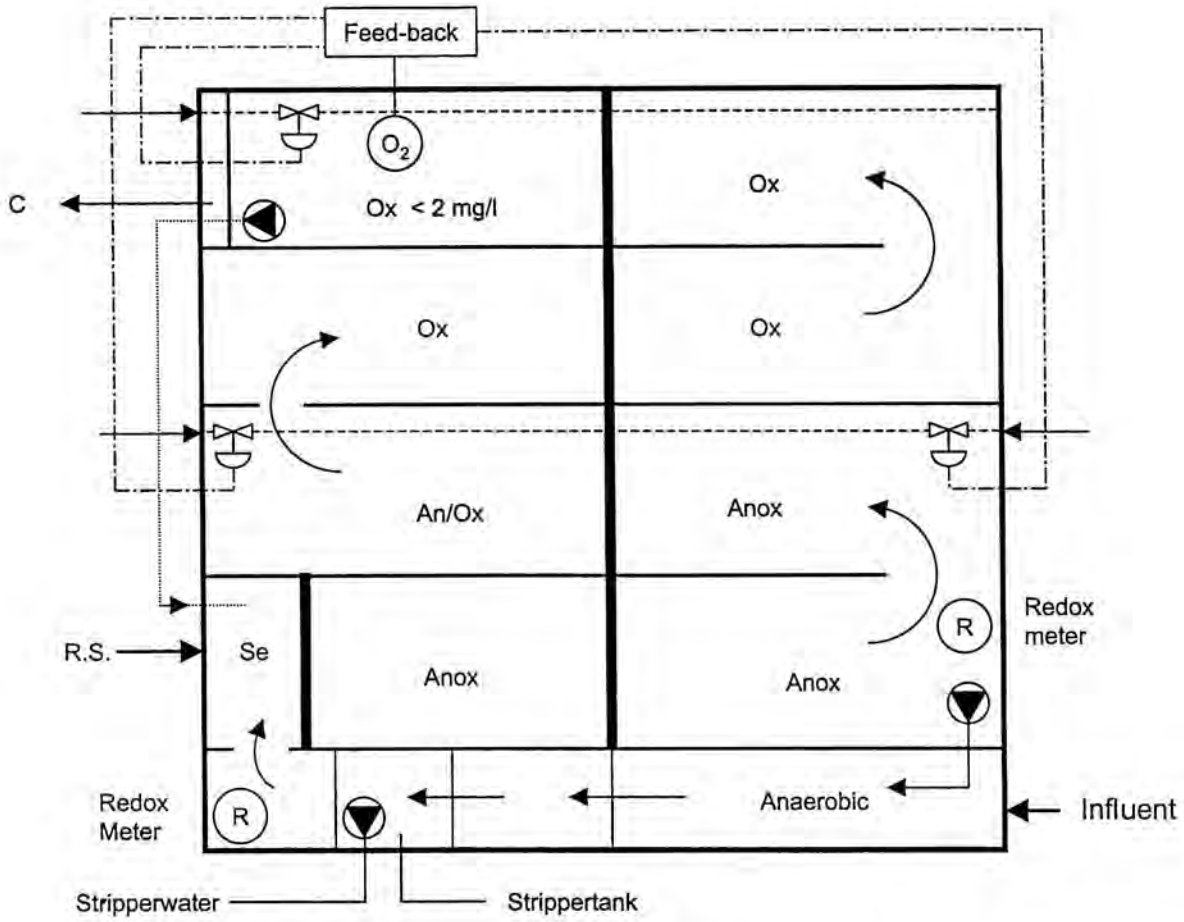



*Figure 72 Example of a treatment plant with biological N and P removal Infl = pre-settled influent; RS =returned sludge; C =final clarifier; Se = contact tank or selector; Anox = anoxic; Ox =oxic or aerobic.*

### 8.3.5 Temperature and pH

Every micro-organism has a specific temperature range in which it can grow (Fig. 73). No growth is possible above the maximum temperature or below the minimum temperature. Within this range, the activity and growth rate increase as the temperature rises until the maximum is reached, after which the activity is reduced abruptly at further temperature increases. As is shown in Fig. 73, the temperature ranges for different micro-organisms vary greatly. The optimum growth temperature can vary from 5–10 °C (Arctic regions) to almost 100 °C (bacteria that live in geysers in areas with volcanic activity)

Changes in the water temperature in the treatment plant therefore result in shifts within the sludge population. One very definite example of such a shift is the occurrence of scum in domestic plants. This usually occurs in winter in The Netherlands and is often the consequence of the growth of *Microthrix parvicella,* a filamentous bacterium which grows well at low water temperatures.

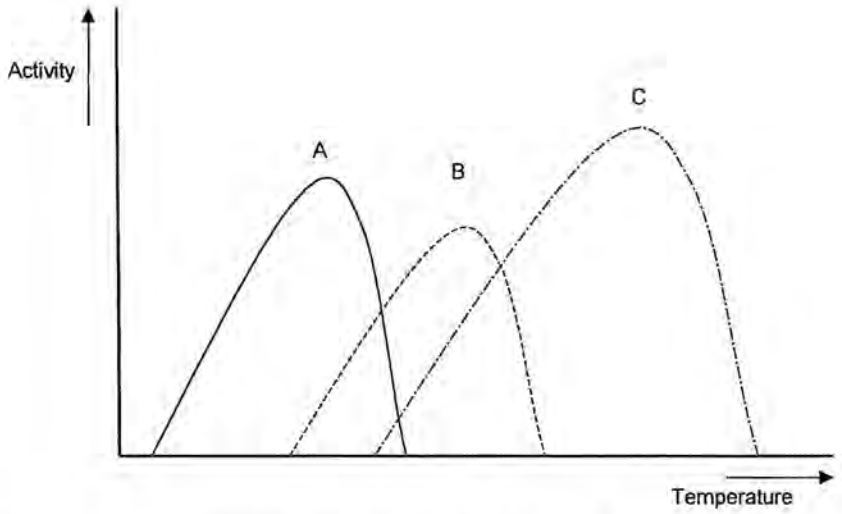



*Figure 73* *Schematic representation of the effect of temperature on the activity/growth rate of three different micro-organisms.*

The same situation as for temperature is valid for pH, and every micro-organism has a specific pH range with a minimum and a maximum.

Although exceptions to this exist – the bacteria which cause corrosion in sewers grow in dilute sulfuric acid with a pH of 1 to 2 – bacteria principally grow at a pH > 5 to 6 whereas, in contrast, fungi prefer lower pH values.

## 8.4 Uptake of nutrients by the floc

During mixing of waste water and activated sludge, the various components in the waste water are taken up by the flocs. The size/nature of the particles in the waste water determines the manner in which they are bound by the flocs:

- low molecular, dissolved compounds can pass the cell wall directly and are therefore immediately taken up from the water phase by the micro-organisms. Consumption costs energy, which means that no more will be taken up than can be processed in the cell. This processing rate, which also includes the synthesis of storage materials, determines the rate at which the dissolved fraction is removed from the water phase;
- high molecular weight, dissolved compounds and colloidal components are bound to the floc by sorption processes and are subsequently hydrolysed (enzymes), after which uptake by bacteria is possible. The hydrolysation processes take place quickly. The uptake of the low molecular weight compounds usually forms the rate determining step;
- larger particles are captured by the flocs and also subsequently hydrolysed. The surfaces of particles are often already colonised by bacteria during transport in the sewer (Fig. 74).

All of these processes together are known as 'biosorption'. The size/rate of biosorption is dependent upon (1) the composition of the waste water, (2) the properties of the activated sludge and (3) the mixing ratio of the activated sludge and the waste water. Points (1) and (2) are further considered in chapter 10.

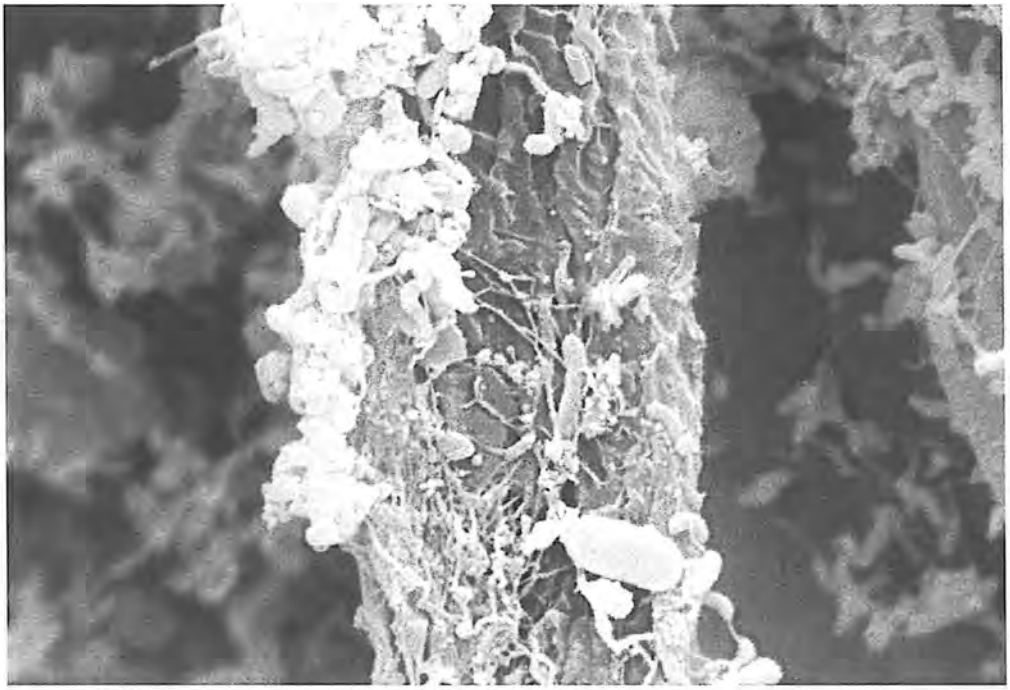


*Figure 74* *The surface of this fibre is colonised by bacterial cells (5000×).*

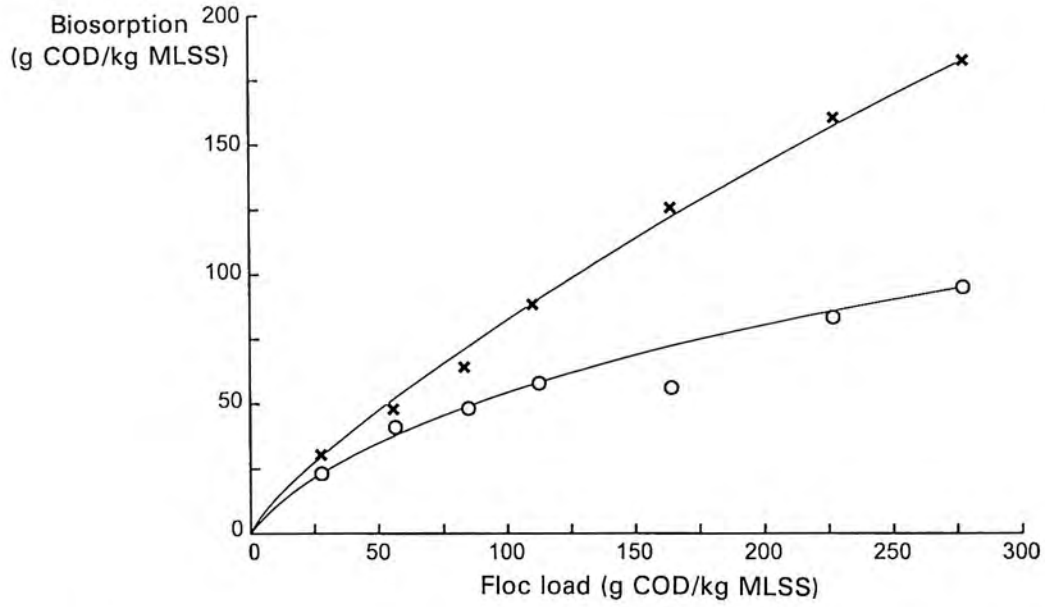



*Figure 75* *Effect of the floc load and the contact time on the biosorption by an activated sludge*
o—o*: after 1 min. contact time;*×—×*: the same after 10 min.*

The mixing ratio of waste water and sludge determines the so-called floc load (in g BOD or COD per kg MLSS).The floc load is a measure of the nutrient concentration which the micro-organisms 'experience' during the mixing of waste water and sludge. The floc load is calculated as follows:

$$floc\ loading = \frac{(COD_i - COD_e)Q_i}{X_r \cdot Q_r}(g\ COD/kg\ MLSS)$$

| | | |
|---|---|---|
| $COD_i$ | : influent COD | ($g/m^3$) |
| $COD_e$ | : effluent COD | ($g/m^3$) |
| $Q_i$ | : influent flow | ($m^3$/hour) |
| $Q_r$ | : returned sludge flow | ($m^3$/hour) |
| $X_r$ | : solids concentration in the returned sludge | ($kg/m^3$) |

The level of the floc load is the driving force for the biosorption (Fig. 75). Because the substrate penetrates deeper into the floc, more nutrients are removed from the water phase in proportion to the increased floc load. The time effect shown in Fig. 75 is the direct result of the biological activity, i.e. the conversion of substrate previously taken up.

# 9 Operational problems

The treatment of waste water is always aimed at producing a clear effluent with an as low as possible level of residual pollution. An extensive separation of the sludge from the effluent in the final clarifier is therefore of crucial importance. This separation step, achieved by settlement, is the weakest part of the activated sludge process. A considerable list exists of the possible causes of an increased suspended solids concentration in the effluent.

1. Many free-living cells are present which cannot be removed by settling of the sludge. Possible causes are:
   - a high sludge load;
   - overloading—i.e. a chronic lack of oxygen;
   - poisoning—i.e. the disappearance of the protozoa.

2. Deflocculation of the sludge
   Possible causes are:
   - too turbulent conditions in the aeration tank;
   - poisoning of the sludge;
   - an extremely low sludge loading level (<< 0.05 kg BOD/kg MLSS.day);
   - the presence of chelating compounds in the influent.

3. The sludge flocs do not settle fast enough. Therefore, the sludge volume index is too high. This is known as bulking sludge. Possible causes are:
   - filamentous bacteria in the sludge;
   - a strong water binding in the floc.

4. A fraction of the sludge flocs settles too fast, disturbing the filtration of the sludge blanket in the final clarifier. This is known as heavy sludge. Possible causes are:
   - precipitation of salts in the flocs;
   - transportation of fast settling flocs with the influent (e.g. from the primary settling tank during pre-precipitation).

5. The sludge flotates in the final settlement tank and/or in the aeration tank. Possible causes are:
   - the presence of certain filamentous bacteria;
   - denitrification in the final clarifier;
   - anaerobic conditions on the bottom of the final clarifier;
   - the presence of fats, and such like, in the sludge;
   - constructional reasons, causing the entrapment of flotated material in the aeration tank.

6. The design or the functioning of the final clarifier is not optimal. The following reasons can be considered:
   - hydraulic overloading;
   - too much sludge in the final settlement tank (the sludge volume index is too high and/or the returned sludge flow is too low);
   - returned sludge flow is too high;

- short circuits;
- the sludge scraper does not function properly;
- uneven weir loading (caused by the tank not being level or by wind effects);
- turbulence in the final settlement tank, caused e.g. by a difference in temperature between the tank and its surroundings.
- churning up of the sludge caused by an unevenly or incorrectly directed input flow in the final sedimentation tank.

Most of these problems can be solved, in principle. To tackle a given problem efficiently, it is necessary that a proper diagnosis is first established. Consequently, microscopic sludge investigation is indispensable. When the results of this analysis are combined with those from a simple settling experiment carried out in a settling glass, the direction in which the solution must be sought can usually be quickly arrived at (Fig. 76). For the sake of completeness, a reduction of the sludge production is also included in this scheme.

## 9.1 High sludge load and/or lack of oxygen

Microscopic sludge investigation establishes that many free-living bacteria cells are present between the flocs. Ciliates are absent, but many flagellates, and occasionally also amoeba, are present. This is a characteristic picture of sludge from a treatment plant with a high sludge load (> 0.3 to 0.4 kg BOD/kg MLSS.day). Overloading of a low loaded system also results (to a limited extent) in an increase in the number of free-living cells and the number of flagellates. Lack of oxygen - long anoxic/anaerobic period during nutrient removal - produces the same effect. The substrate that is transported with the influent is not actually taken up by the micro-organisms in the flocs rapidly enough and, as a consequence, growth in the suspension is possible. The absence of the most important consumers of free bacterial cells (ciliates) enhances this effect.

The following actions should be done to establish the cause exactly:
- check the actual sludge load;
- check the oxygen concentration;
- determine the respiration rate of the activated sludge. A level that is too high also indicates a high load and/or a lack of oxygen.

In some treatment plants, the (too) high sludge load is inherent in the design. In such a case, the effluent quality can only be improved by enlarging the plant (or by lowering the COD load entering the WTP).

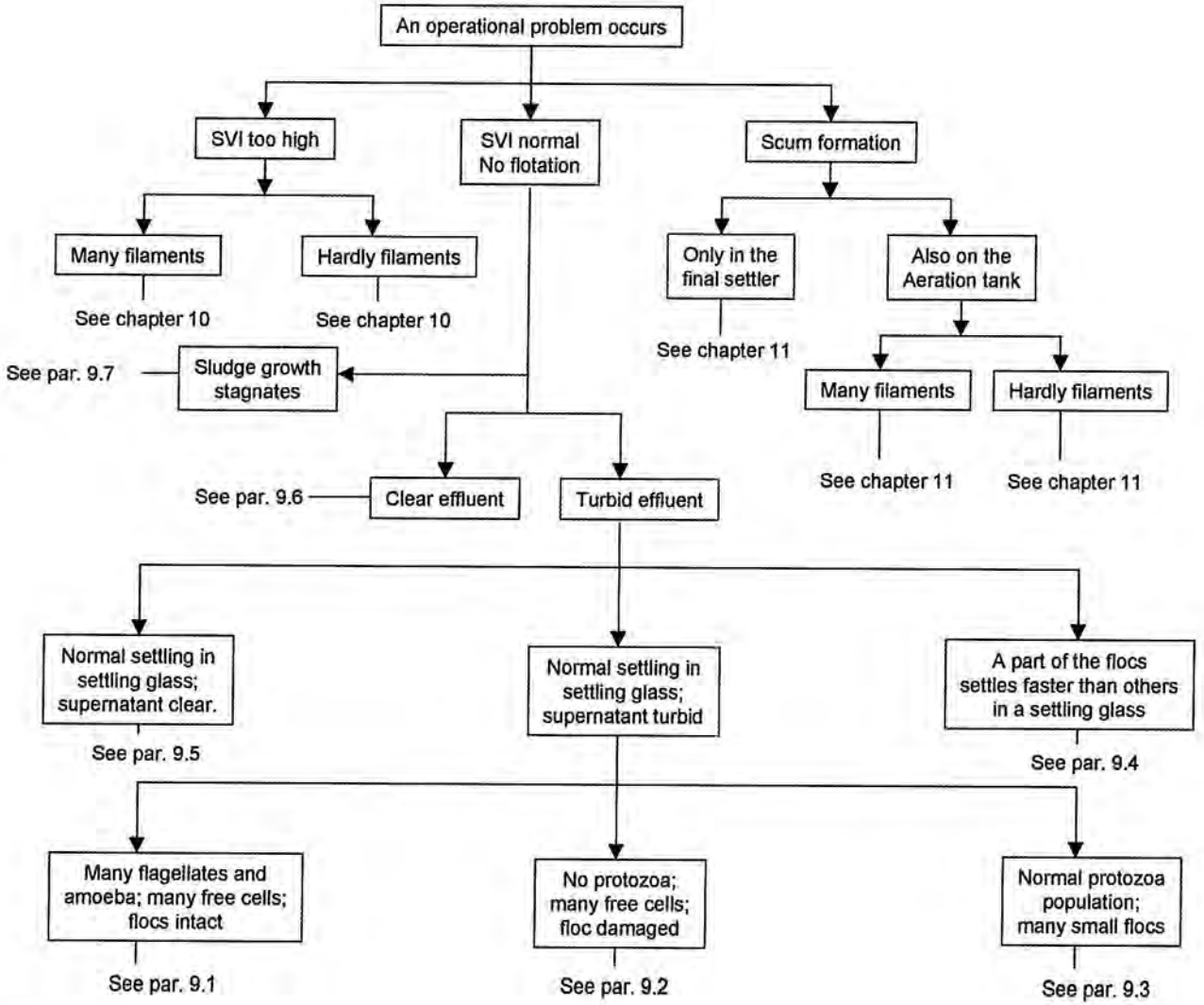



*Figure 76 Trouble-shooting scheme.*

## 9.2 Poisoning of the sludge

Depending upon the nature and the concentration of the components involved, the transportation of toxic compounds with the influent affects the quality of the activated sludge as follows:

- protozoa disappear abruptly. These micro-organisms are present free in the water phase and are therefore fully exposed;
- the exterior of the floc is damaged, as a result of which the number of free-living cells increases. The absence of ciliates, etc. enhances this effect;
- in extreme cases, large groups of bacteria in the floc die, which might result in loss of nitrification and deflocculation.

The last mentioned problems does not occur very readily, because the bacteria in the flocs are reasonably protected against exposure to toxic compounds. In addition, many poisonous components are quickly rendered harmless through sorption to the organic sludge matrix.

Contamination can be followed within a few days by a fast increase in the number of ciliates, as a large food source (free-living bacteria cells) is temporarily available. The return of the ciliates also means that the toxic effect has largely disappeared. The effluent quality usually returns to normal within a few days.

The chances of poisoning occurring can be reduced by the following means

- tracing and clearing up potential sources;
- buffering the influent in order to avoid peak loading with toxic components
- monitoring the influent by using a toxicity meter.

## 9.3 Many small flocs; deflocculation

The presence of many small flocs, where there is no evidence of toxic compounds entering the plant, can have various causes:

- mechanical damage from the use of surface aerators. The heavy turbulence that is caused by surface aerators is the most important cause of the large fraction of small flocs in the sludge, especially at high sludge loading levels (the floc is not robust) and in many industrial treatment plants (idem);
- deflocculation owing to the presence of chelating compounds, such as EDTA, in the influent. This type of compound withdraws divalent ions ($Ca^{2+}$ and others) from the floc as a result of which they disintegrate;
- the slime matrix surrounding the cells, which contributes to their bonding, is partially broken down at very low sludge loading levels (<< 0.05 kg BOD/ kg MLSS.day);
- dosing with aluminium salts (for phosphate removal and/or controlling bulking sludge) results in a significant reduction to the average floc size. The reason for this is still not known.

Effective measures aimed at an improvement of the strength or the size of the floc in treatment plants are still largely unknown. Loss of solids owing to deflocculation can only be prevented by dosing with flocculants. Post-treatment of the effluent (sand or membrane filtration) can offer a possible alternative.

## 9.4 Heavy sludge

The name 'heavy sludge' is somewhat misleading, because only a fraction of the flocs settle fast. This is also the cause of the problem. The heavy flocs settle so rapidly (within a few minutes) that the sludge blanket in the final clarifier is disturbed. The filtration effect of the sludge blanket is missing, resulting in a turbid effluent.

Heavy sludge does not occur very often. It is sometimes caused by transport of fast settling flocs originating from elsewhere. The following causes can be considered:

- pre-treatment of the waste water in a trickling filter;
- supply with the waste water;
- overflow from the pre-settlement tank during pre-precipitation;
- precipitation *en masse* of salts in large sludge flocs.

## 9.5 The final clarifier does not function well

The combination of (1) a turbid effluent and (2) good/complete settling with a clear supernatant in a settling glass implies usually that the final clarifier does not function well. The increased solids concentration in the final effluent, therefore, does not have a biological cause. As stated in the introduction to this chapter, the following aspects must now be checked:

- the actual and according to the design specifications, the maximum permissible hydraulic loading of the final settlement tank;
- the solids loading level of the secondary sedimentation tank and the height of the sludge blanket;
- the actual returned sludge flow;
- a possible 'chimney effect' in tanks with double overflow weirs;
- possible short circuited flows in the final settlement tank (tracer investigation);
- the performance of the sludge scraper;
- whether the overflow weir is level. Wind effects can also cause uneven weir loadings;
- differences in the temperature between the tank and its surroundings can possibly result in turbulence in the final settlement tank;
- the positioning of the inflow to the final settlement tank.

## 9.6 Treatment performance inadequate; but effluent is clear

It sometimes occurs that the effluent is perfectly clear while the treatment results (removal of COD, N or P) are still insufficient. Microscopic investigation has shown that there are no possible toxic effects. In the case of this combination, something is certainly wrong with the setting of important process parameters (sludge age, the length of the anoxic period, etc.).

## 9.7 Decline in sludge production

If the sludge production decreases, while the sludge load has not been changed, the following two causes must be considered:

- settling of sludge 'somewhere' in the plant, e.g. caused by heavy sludge (see paragraph 9.4);
- the growth of worms in the plant.

Worms are the largest (micro-) organisms observed in activated sludge. They possess a diameter of ca. 0.1 mm and a length to a maximum of 1–1.5 cm. Oligochaeta are almost always involved: often *Nais elingius* and *Aelosoma* species. Worms are able to consume whole sludge flocs and floc fragments. A significant fraction of the consumed organic matter is converted into energy. A worm bloom (order of magnitude: $10^5$ worms per litre) is therefore associated with a reduction in sludge production (Fig. 77).

In addition, growth *en masse* of this type of organism can result in a change, which can be positive (improved settling characteristics) as well as negative (nitrification loss) to the properties of the sludge.

The growth of worms in activated sludge is surrounded by many questions. They do not grow very slowly and under optimum conditions their generation time is only a few days. More than sufficient nutrients, in the form of sludge flocs, appear to be present in a WTP. Under dry weather conditions, their numbers in the returned sludge flow are twice as high as they are in the sludge in the aeration tank. Therefore, there is no question of an extensive washing out of worms under these conditions. It is not known what is happening with storm water conditions, but many worms can be expected to occur in almost all treatment plants. In spite of this, worms do not often occur *en masse* in low loaded domestic treatment plants in The Netherlands. Sludges with a large population usually originate from plants with a sludge load of ca. 0.1 kg BOD/kg MLSS. day and a pre-settled influent. In plants treating industrial waste water (some agro industries), on the other hand, worms are frequently observed *en masse* at lower sludge loading levels. This indicates that the properties of the sludge (e.g. energy content or the nature of the inorganic fraction) possibly play an important role.

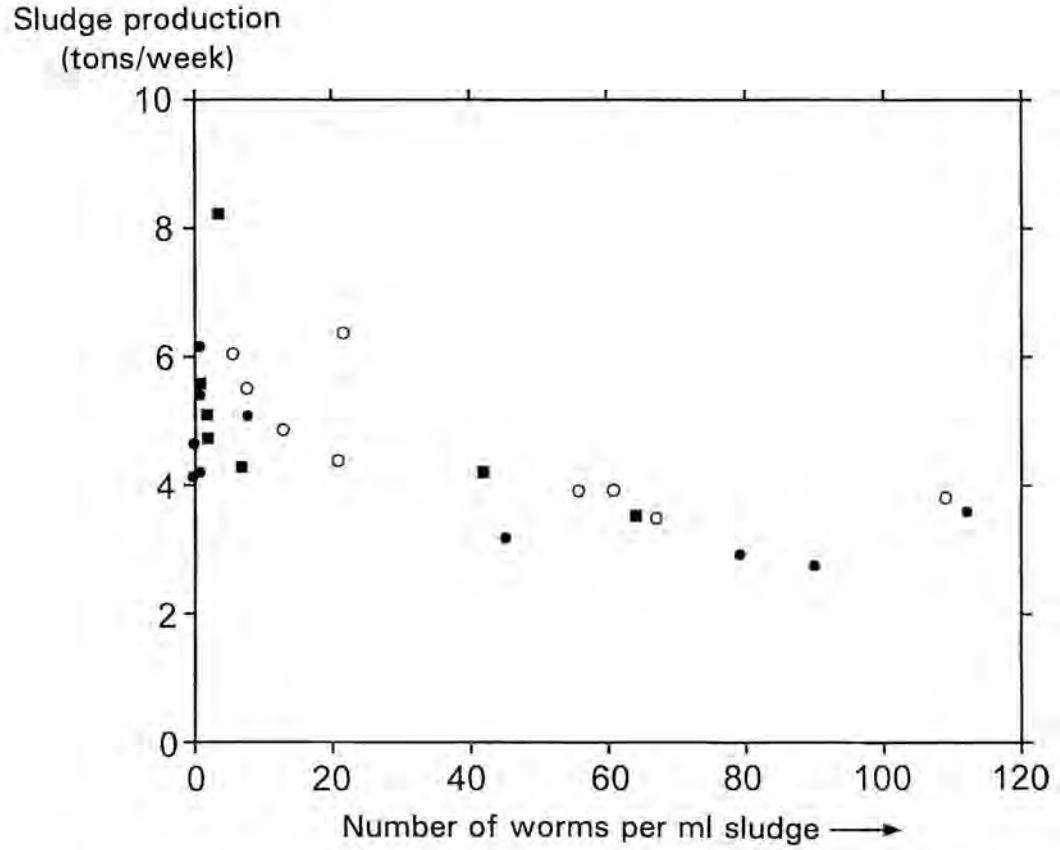



*Figure 77* *Sludge production and the number of worms in a given treatment plant (C. Ratzak. Grazer induced sludge reduction in waste water treatment plants. Thesis, VU Amsterdam, 1994).*

Worms will not survive long periods (days) without oxygen, which is a condition that can therefore be used as a control option.

Finally, it can be stated that the presence of a 'sheltering place', e.g. the use of a specific biomass support material in the aeration tank, can increase the chance of maintaining a large worm population in the treatment plant.

# 10 Bulking sludge

Activated sludge flocs comprise a mixed micro-organism population. The composition of the population is determined by the quality of the waste water and the process conditions in the WTP, which are, in principle, controllable. From the point of view of the micro-organisms, there is a chronic shortage of nutrients in most WTPs which results in strong competition between them. The species which consume the largest portion of the scarce nutrients will come to dominate the population and if these are filamentous micro-organisms, bulking sludge will occur. Additionally, the presence of certain components in the influent, especially reduced sulphur compounds (including $H_2S$) can further stimulate the growth of specific filamentous species.

Filamentous micro-organisms are part of the normal sludge population. More than 30 different species are known, principally bacteria. Most species do not have a name but a number, as their characteristics are not yet fully known. Of these 30, about 10 species frequently cause operational problems while the remaining strains are less often observed in activated sludge. For more detailed information regarding filamentous micro-organisms, the reader is referred to chapter 5.

As long as the number of filaments is limited, their presence has no measurable effect on plant performance. However, an increase in the population usually results in a decrease of the settling velocity of the flocs which causes the sludge volume index (SVI) to deteriorate. This phenomenon is known as 'bulking sludge'. As soon as the SVI exceeds the level of 150 ml/g, bulking sludge occurs.

A form of bulking sludge, known as Zoogloea bulking sludge (see paragraph 10.5), also occurs in which filamentous organisms play no part.

Bulking sludge can cause severe operational problems and increases the treatment costs. The problems mainly involve the high risk of sludge losses with the final effluent and the deteriorated dewatering and thickening properties of the sludge.

Bulking sludge with SVI values of 400–600 ml/g, and occasionally even higher levels, occurred very frequently in the past (in 50–60% of all WTPs). However, information on controlling and preventing bulking sludge has significantly increased over the last thirty years. The process stability has considerably improved, especially in domestic treatment plants. Bulking sludge still occasionally occurs, especially in winter, but the SVI usually remains under the level of 250–300 ml/g in Dutch domestic WTPs. In contrast, bulking sludge still causes major operational problems in many industrial WTPs., particularly in plants with a high concentration of easily biodegradable compounds in the waste water (agro industry).

## 10.1 Filament index and sludge volume index

If microscopic sludge investigation is not included in the monitoring programme of a WTP, the increase in the number of filaments can only be noticed when the sludge settling velocity deteriorates significantly. The settled volume increases gradually at first, and then increases exponentially. This is the reason why process operators are often caught unawares by bulking sludge. Through routine microscopic investigation, the growth of filaments (= the increase of the filament index) can be observed sooner and measures can be taken in time.

Filamentous micro-organisms can affect the settling characteristics of the sludge, including compression of the flocs at the bottom of the final clarifier, in three ways:

- filamentous organisms which protrude from the flocs, or which are mainly present in the water phase between the flocs, prevent the flocs from coming close together. The effect is more prominent if the filaments are straighter, robuster or longer. However, the structure of the floc is not affected. Some examples are: *S. natans*, *Thiothrix*, Type 021N and Type 0803/0914;

- owing to the presence of other filamentous species open structured flocs (agglomerates) can sometimes arise. Large agglomerates settle relatively slowly and compact poorly. Agglomerates consist of small flocs which are bound together by filaments. Some filamentous bacteria also form tangles of filaments, around which an open structured floc subsequently arises. Examples of filamentous species which often cause agglomerates to occur are: Actinomycetes, *M. parvicella*, *N. limicola III*, Type 0092 and Type 1701;

- filamentous micro-organisms that cause sludge flotation as a result of which a scum layer arises. The reader is referred to chapter 11 for more information on this matter.

Together with the population size, the shapes and length of the filaments are decisive in their effect on the settling characteristics of the activated sludge (Table 5).

*Table 5 Effect of filamentous organisms on the SVI in domestic WTPs*

| Large | Medium | Small |
|---|---|---|
| *M. parvicella* | Actinomycetes | *N. Limicola* |
| *S. natans* | *H. hydrossis* | Type 1863 |
| *Thiothrix* | Type 0041 [1)] | |
| Type 021 N | Type 0092 [1)] | |
| Type 1851 | Type 0803/0914 | |
| | Type 1701 | |

[1)] the negative effect is often greater in industrial WTPs

Some organisms virtually never cause bulking sludge because they form flexible, tiny filaments (Type 1863) or actually grow as flocs (*N. limicola*).

The size of the population and the morphogy of the species involved are important, but not the only, factors determining the settling velocity of the flocs. The physical–chemical properties also play a role. These involve:

- the shape of the floc. A 'rounded' floc settles better than an irregularly shaped floc;
- the size of the floc;
- the structure of the floc. Compact flocs settle faster than open agglomerates;
- the size of the inorganic fraction (the ash content). Sludges with an inorganic fraction > 40–50% always settle rapidly, even if many filaments are present;
- the extent to which water is bonded by the floc (Zoogloea bulking sludge);
- the surface charge of the floc.

This means that the FI and the SVI are not always very clearly correlated, especially when the results of many different WTP's are combined. An example is given in Fig. 78.

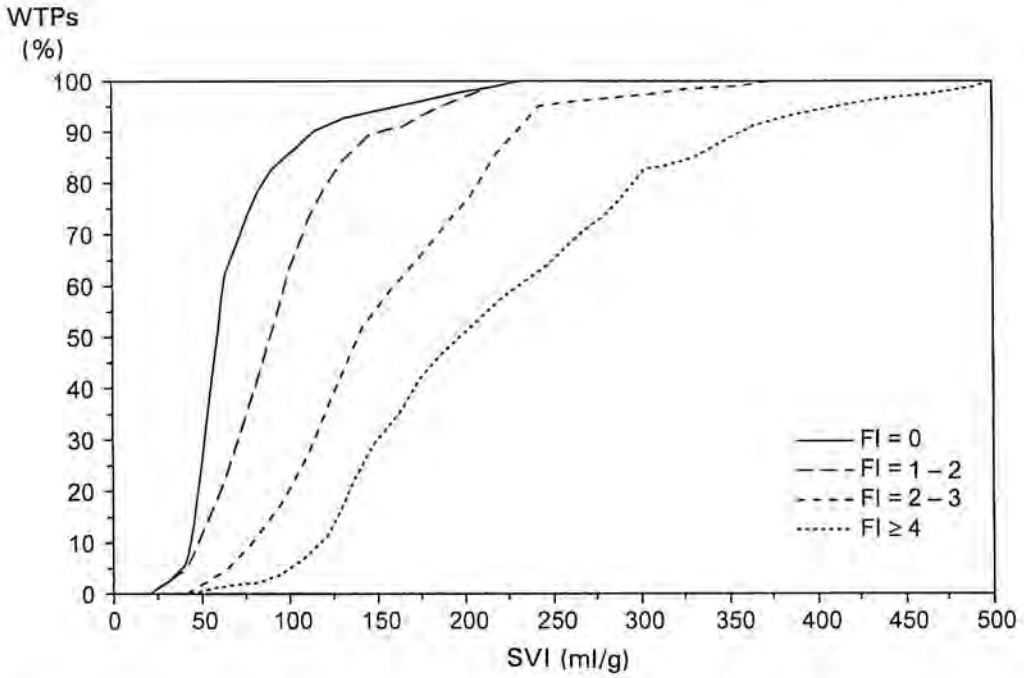



*Figure 78* *The relation between the FI and the SVI from a study of the settling characteristics of activated sludge in ca. 100 oxidation ditches.*

## 10.2 Growth of filamentous micro-organisms

As covered in chapter 8, the differential growth rates of the micro-organisms in activated sludge are particularly inconstant, but are dependent upon the process conditions. A micro-organism grows at its maximum rate if all the necessary nutrients are present in excess and also with optimum pH and temperature. As soon as growth limiting conditions arise, the growth rate reduces. It can be stated generally that the growth rate of floc forming bacteria decreases more than that of the filamentous species. Therefore, filamentous bacteria still grow relatively fast at growth limiting conditions. They can make maximum profit from their specific morphological and physiological characteristics, which eventually results in the occurrence of bulking sludge.

Lack of carbon compounds in treatment plants with 'complete mixing' (see paragraph 8.3.4.) is an important cause of bulking sludge. This is also valid for shortages of oxygen. Industrial waste water often contains little nitrogen or phosphorus. Shortages of other elements can play an incidental part. The effects are cumulative, therefore, filamentous micro-organisms benefit when the number of growth limiting elements is greater.

In addition, some compounds in the influent can further stimulate the growth of certain filaments because these components cannot be used by many other micro-organisms (→little competition). Sulphides (including $H_2S$) are a known example. Reduced sulphur compounds stimulate the development of *Thiothrix*, Type 021 N, *Beggiatoa* and Type 0914. Apart from Type 021 N, these filaments can also be used as indicator organisms for sulphide entering the plant.

Practically all of the filamentous micro-organisms investigated up to the present can only take up nutrients and grow if $O_2$ is available. They are strictly aerobic but can still grow relatively fast at low oxygen concentrations, Their growth stops totally, however, under anoxic/anaerobic conditions. Many floc forming bacteria can actually grow under conditions where oxygen is absent. This fact forms the basis of some bulking sludge control strategies. It will be further elaborated in the following paragraphs.

Up to the present, *Microthrix parvicella* is the only known exception to this rule. *Microthrix parvicella* cannot actually grow under anoxic/anaerobic conditions, but is capable of adsorbing substrate from the water phase under these circumstances.

If the conditions are favourable to filamentous micro-organisms, the remaining process conditions determine which species will develop. In principle, filamentous bacteria can grow under almost every commonly practiced combination of process conditions (sludge age, quality of the influent, temperature, etc.). The filaments only really lose the competition at very high sludge loading levels. Their maximum growth rate is insufficient to maintain themselves at very short sludge ages. Therefore, practically no filaments are observed at sludge loading levels above 1 to 1.5 kg BOD/kg MLSS.day.

## 10.3 Process conditions and population composition

This paragraph deals with the effect of the process conditions on the compostion of the population, using several examples. The information that is presented chiefly involves domestic treatment plants.

Table 6 shows the effect of the sludge load applied on the filamentous population present. Occasional observations (predominating in less than 5% of the WTPs) are not included in the table. The information presented is based on an inventory in The Netherlands and concerns the situation before the introduction of nutrient removal conditions in many WTPs.. However, the group of WTPs with sludge loading levels < 0.1 kg BOD/kg MLSS.day consist mainly of oxidation ditches and carrousels, plants in which simultaneous denitrification was also standardly used, but not yet optimised, in the past.

Table 6 shows that two groups of filamentous species can be distinguished.

*Table 6 Effect of the sludge load applied on the filamentous bacteria population in Dutch domestic WTPs without (optimised) nutrient removal*

*+++ : dominant in more than 40% of the WTPs*

*++ : dominant in 15% - 40% of the WTPs*

*+ : dominant in 5% - 15% of the WTPs*

| Filamentous organisms | Sludge loading level (kg BOD/kg MLSS.day) | | |
|---|---|---|---|
| | < 0.1 [1] | 0.1 - 0 2 [2] | > 0.2 [2] |
| *M. parvicella* | +++ | +++ | + |
| Type 0041 | ++ | + | |
| Type 0092 | ++ | + | |
| Type 0803 | + | + | |
| Type 0914 | | + | |
| Type 0581 | + | | |
| Type 1851 | + | + | |
| Actinomycetes | + | | |
| *N. limicola* | + | ++ | + |
| Type 021 N | ++ | ++ | +++ |
| *H. hydrossis* | + | ++ | +++ |
| Type 1701 | | | +++ |
| *S. natans* | | | +++ |
| *Thiothrix* | | + | ++ |
| Type 1863 | | | ++ |
| Type 0411 | | | ++ |

1) chiefly treatment plants with (1) raw influent, (2) extensive nitrification and (3) simultaneous denitrification (not optimised)

2) pre-settled influent and occasional incomplete nitrification

### 10.3.1 Sludge load > 0.2 kg BOD/kg MLSS.day and domestic influent

The group that is encountered in domestic treatment plants, mainly at sludge loading levels ≥ca. 0.2 kg BOD/kg MLSS.day, chiefly comprises Gram negative bacteria. These filamentous species are often present *en masse* in industrial WTPs, also even at low sludge loading levels.
Most species in this group have been isolated from activated sludge. Research conducted with pure cultures has shown that they grow on carbon compounds which reach the WTP in a dissolved form (sugars, lower fatty acids such as acetic acid, etc.). These low molecular weight compounds arise from anaerobic conversions in the supply sewer and/or in the primary sedimentation tank. For these reasons, a long transport route ('old' waste water) increases the chance of bulking sludge occurring. Sulphides, which further stimulate the growth of certain filaments, as is stated in the previous paragraph, also arise during anaerobic conversions.

A large proportion of the COD of industrial waste water is often comprised of easily biodegradable, low molecular weight compounds. This is the reason why these Gram negative species grow frequently and *en masse* in industrial WTPs.

### 10.3.2 Characteristic of low sludge loading levels

The second group is characteristic of sludge loading levels ≤ ca. 0.1 kg BOD/kg MLSS.day. *M. parvicella* is the most important filament in this group. In the literature, these species are often designated by the term 'low F/M filamentous micro-organisms'. Most species in this group stain Gram positive. Various species of this group also play a decisive role in the formation of scum (chapter 11).

With the exception of *M. parvicella* and some actinomycetes, not much is known with any certainty about the physiological characteristics of the low FM-micro-organisms (e.g. which carbon compounds they use for their growth, etc).

*M. parvicella* is dependent on higher fatty acids (oleic acid, palmitic acid, etc.) to grow. In The Netherlands, 20–30% of the COD of urban waste water is comprised of higher fatty acids, which are also important sources of carbon in the influent. Higher fatty acids mainly reach the treatment plant in the form of fats and such like, which means that they are chiefly present in the undissolved particulate fraction in the influent. The fats must first be broken down before *M. parvicella* can consume the fatty acids. Just as with other Gram positive species, *M. parvicella* has a hydrophobic cell surface through which the fats and fatty acids can easily be sorbed.

Actinomycetes can use a broad spectrum of carbon compounds, including the fat/lipid fraction from the waste water. There are indications that this fraction can be used as well by the Types 0041, 1851 and 0092. Type 0803/0914 almost certainly grows on compounds from the dissolved fraction in the influent.

*M. parvicella* is very characteristic of domestic waste water and industrial discharges hardly ever result in an increase to its population (Fig. 79). This diagram also shows that several filaments in domestic WTP's mainly grow on compounds originating from industrial discharges. These species can also be present *en masse* in industrial WTPs.

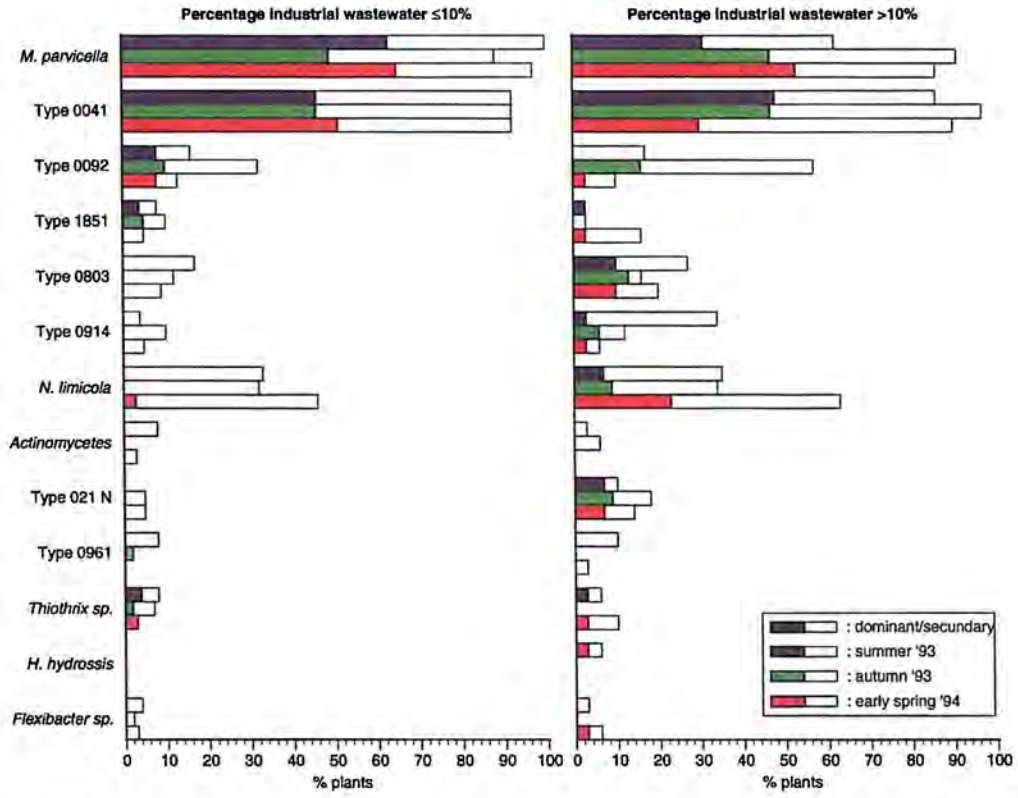



*Figure 79* *The effect of industrial waste water on the filamentous population in Danish domestic treatment plants.*

*N.B.* *1. Only plants with an FI > 2.*

*2. Principally low-loaded plants with nutrient removal.*

*M. parvicella* is a filamentous bacterium whose population size displays a distinctly seasonal rhythm. The number of filaments is at its highest at the end of the winter and at its lowest in summer (Fig. 80). This also results in a very characteristic SVI pattern in many domestic treatment plants (Fig. 81).

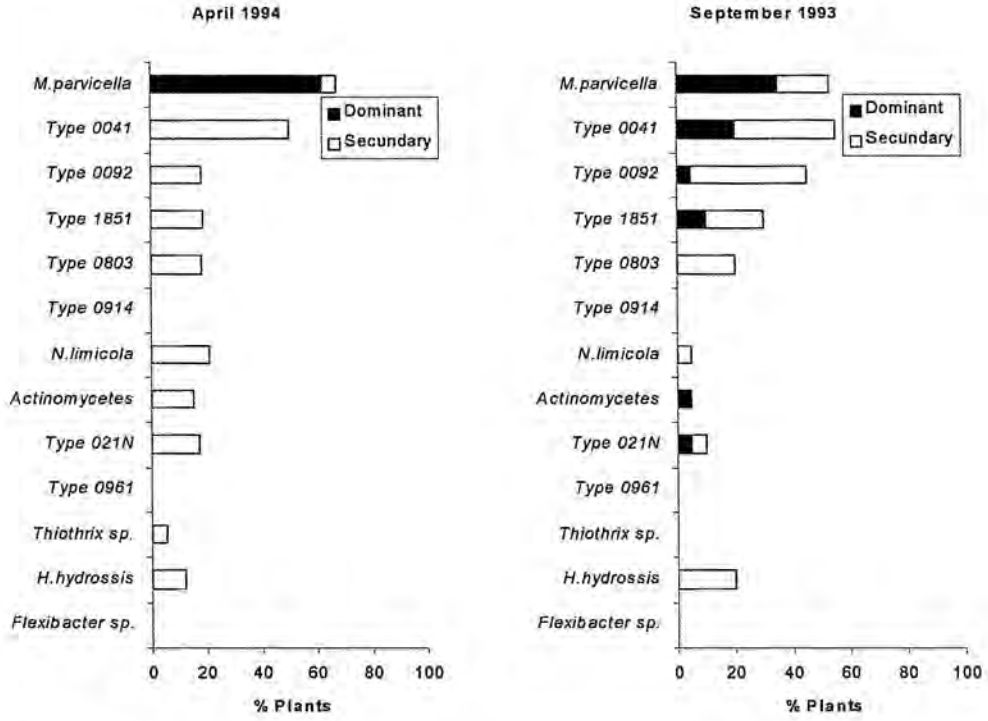



*Figure 80 Seasonal effect on the population in Dutch nutrient removal plants.*

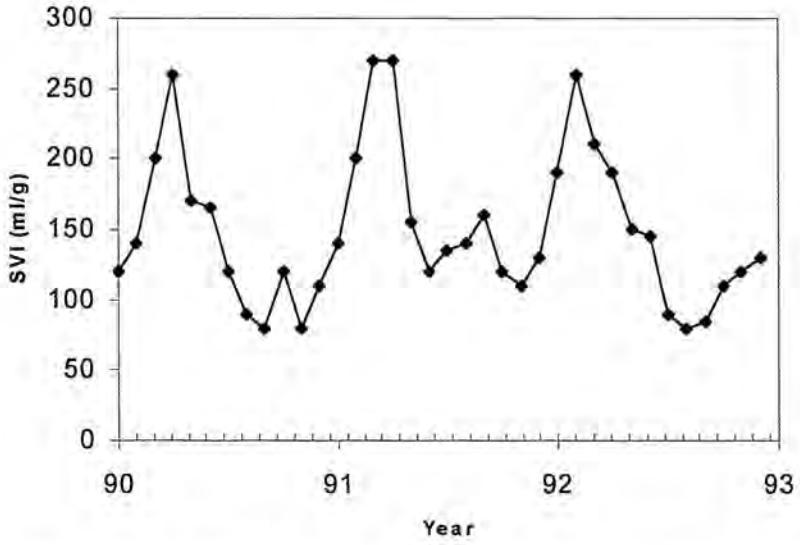



*Figure 81 Characteristic SVI pattern in treatment plants with seasonal growth of M. parvicella.*

### 10.3.3 The effect of biological nutrient removal

The introduction of biological nutrient removal conditions means that anoxic and/or anaerobic zones are created in the treatment plant. Consequently, the dissolved fraction is largely removed from the waste water through denitrification and/or Bio-P bacteria. Filamentous bacteria, apart from *M. parvicella*, cannot take up substrate if molecular oxygen is lacking. This means that filamentous species that grow on the dissolved fraction in the influent are eliminated under such conditions. These bacteria are therefore largely absent in Fig. 80.

In contrast, nutrient removal stimulates the development of *M. parvicella*. As is illustrated in table 7, *M. parvicella* is by far the most important filament in nutrient removal plants. The numbers of Type 0041 in Danish WTPs are also high, but the growth of this filamentous species hardly ever results in high SVI values in domestic treatment plants. For modern domestic plants, the bulking sludge question has in fact been reduced to the following question: how can the growth of *M. parvicella* be controlled? In areas with temperatures higher than those in Western Europe, actinomycetes also play an (important) role.

*Table 7* *Dominant filamentous micro-organisms in WTPs with nutrient removal during the early spring of 1994.*
*Numbers: % WTPs in which the species concerned was present*

| Species | The Netherlands | | | | Denmark | | | Germany | Greece |
|---|---|---|---|---|---|---|---|---|---|
| | O.S. | Car. | Schr. | V.C.[1] | Bio-P | Bio-Denitro | P.D. | V.C.[2] | V.C.[3] |
| *M. parvicella* | 58 | 66 | 44 | 67 | 74 | 28 | 43 | 75 | 55 |
| Type 0041 | 6 | | 11 | | 32 | 38 | 20 | | |
| Type 0092 | 3 | | | | 5 | 9 | | | 10 |
| Type 1851 | | | 11 | | | 3 | | | |
| Type 0803 | | | | | 5 | 3 | | 12 | |
| Type 0914 | | | | | 5 | | | | |
| *N. limicola* | | | | | 16 | 3 | 10 | | |
| Actinomycetes | | | | | | | | | 20 |
| Type 021N | | 3 | 11 | | | 6 | | | |
| *Thiothrix sp.* | | | | | | 6 | | | |
| *S. natans* | 3 | | 11 | | | | | | |
| N WTP's. | 31 | 32 | 9 | 21 | 19 | 32 | 30 | 8 | 11 |

O.D. = oxidation ditch
Car. = carrousel
Schr. = Schreiber
V.C. = various configurations
P.D. = pre-denitrification

1) incl. 4 bio-P WTPs
2) incl. 3 Bio-P WTPs
3) incl. 2 Bio-P WTPs

In addition, the pre-mixing of influent and sludge (contact period: a few minutes), which is practically a standard practice in Denmark, is the reason for the strikingly high figures of Type 0041 in Danish WTPs. The populations of *M. parvicella* and Type 0041 are complementary to one another: if the population of one increases, that of the other decreases and *vice versa*.

There is still no definite evidence available for the cause of the excessive growth of *M. parvicella* in nutrient removal plants. However, there are strong indications that the degradation of fats, lipids, etc. under anoxic/anaerobic conditions (→ free oleic acid, etc.) favours *M. parvicella*. This bacterium can adsorb the free higher fatty acids from the water phase at anoxic/anaerobic conditions, whereas many floc forming bacteria can only take up and process these components under aerobic conditions.

### 10.3.4 The competition between filamentous organisms

In the competition for nutrients, the filamentous bacteria not only compete against the floc formers, but often against each other as well. As a consequence, changes can take place within the population but the number of filaments is not reduced. This phenomenon can occasionally be observed in WTPs with some parallel and more or less identical lanes. Here, the same filamentous micro-organisms are present but in varying numerical ratios. Apparently unstable equilibria occur within the population. Some examples of filamentous species which alternate with one another are:

- *S. natans* / *Thiothrix* / Type 021 N
- *M. parvicella* / Actinomycetes / Type 0041 / Type 0092

## 10.4 Controlling bulking sludge

Filamentous micro-organisms can be controlled in various ways.

*Control of symptoms only*

Various methods have been developed over the years by which only symptoms are suppressed and the cause is not removed. These involve:

- chemical destruction of the filaments by, e.g. the addition of chlorine to the returned sludge;
- mechanical destruction of the filaments by, e.g. ultrasonic treatment of the sludge;
- enlarging the flocs by dosing with flocculants;
- increasing the weight of the flocs by adding calcium compounds or talc;
- addition of Fe or Al salts. The effect of these products is not only based on increasing the weight of the floc by precipitation of hydroxides and/or phosphates. Dosing often also results in a reduction to the number of filaments. *M. parvicella*, for example, can be often effectively controlled by dosing of Al salts.

Several drawbacks are attached to these control methods, such as side effects on the floc population, a larger sludge production, the significant costs and the only temporary effect. Symptom control should therefore only be carried out for (1) occasional bulking sludge problems, (2) situations in which a sudden considerable loss of solids is threatened or (3) where no structural solutions are available.

*Structural solutions*

Structural solutions require a change of the process conditions, aimed at the removal of the strong competitive position of the filamentous micro-organisms. This involves measures that bring about a situation in which the largest part of the available carbon compounds is actually taken up by floc forming bacteria.

For a systematic approach, the following sequential approach is recommended:

Preventing a possible limitation of growth Owing to a lack of certain elements, particularly nitrogen and/or phosphorus (see paragraph 8.3.2.). This step can be passed over in the case of domestic waste water.

Monitoring whether the oxygen supply is (still) adequate. An oxygen level < 2 mg $O_2$/l is a potential risk, particularly in fully loaded treatment plants. The substrate will consequently not be processed rapidly enough and remains available to the filamentous bacteria for longer. They are further benefited because, at low oxygen levels, their growth rate reduces less than that of the floc formers. Additionally, changing the conditions in a section of the aeration tank from oxic to anoxic reduces the time available for aerobic conversion of the substrate.

Monitoring whether sulphides are a possible cause. If microscopic investigation establishes that many sulphur oxidising filamentous bacteria are present, it should be worked out how the reduced sulphur compounds can be removed from the waste water before it reaches the aeration tank. Options are: pre-aeration, chemical oxidation, preventing sulphides arising, etc.

Monitoring whether the influent contains a large fraction of easily biodegradable carbon compounds (e.g. lower fatty acids). These stimulate the growth of many filamentous species (see paragraph 10.3.1) particularly if there are also shortages of N, P or $O_2$. For industrial waste water, removal of this fraction in a separate pre-treatment stage is occasionally a realistic option. For this purpose, e.g. an anaerobic pre-treatment or a two-stage configuration can be considered.

Check whether a selector and/or a selective zone can be incorporated in the process in order to benefit the floc forming bacteria selectively. This option is further dealt with in the following paragraphs.

### 10.4.1 Selection of floc forming bacteria by the application of a high floc load

The configuration of the treatment plant determines the nutrient concentration that is available to the micro-organisms during the mixing of influent and sludge (paragraph 8.3.4). The floc load is very low if complete mixing is applied and growth limiting conditions arise owing to a lack of carbon compounds. The floc load is high in WTPs with a plug flow pattern, in a separate contact tank/selector or if the influent is supplied discontinuously (SBR process). A substrate gradient (in time or location) occurs in these configurations, which markedly reduce the chance of bulking sludge occurring.

The affect of a high floc loading level on the uptake of substrate by the floc is schematically displayed in Fig. 82.

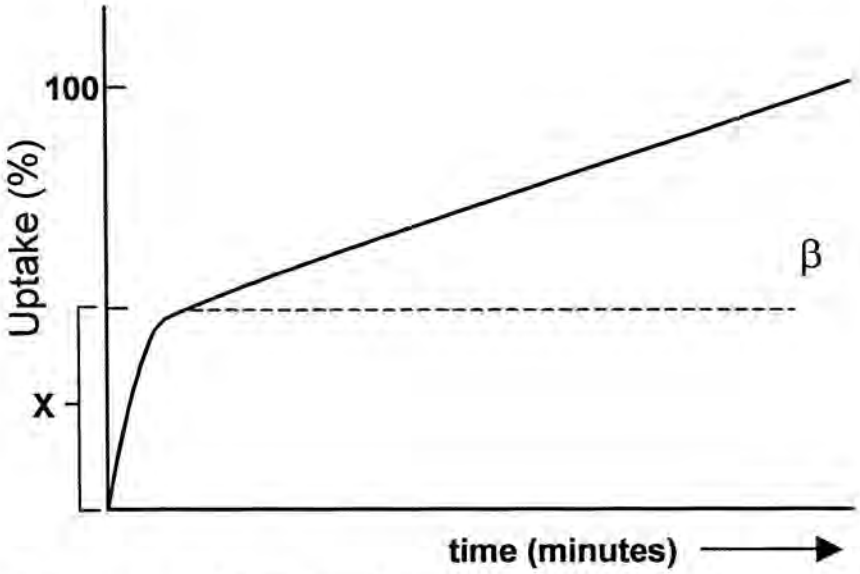



*Figure 82* *Substrate uptake pattern at a high floc load.*

Within one minute, viz. almost instantaneously, a part of the COD is bound by the floc. This is a physical-chemical removal mechanism (sorbtion and entrapment) in which $O_2$ has no part. The size of the fraction that is removed in this way (= X) is dependent upon:

- the floc load. More substrate is bound at a higher floc loading level Owing to the fact that it penetrates deeper into the floc. The removed percentage is reduced as the floc load increases, however;
- the composition of the waste water. Mainly undissolved components in the waste water are bound by the floc during instantaneous biosorption. Therefore, with pre-settled waste water, X is substantially less than is the case with raw influent.

A short contact time between (raw) influent and sludge is often sufficient to prevent an excessive growth of filamentous species which grow on the particulate fraction in the waste water. This removal mechanism is e.g. responsible for the remarkably low numbers of *M. parvicella* in Bio-Denitro treatment plants (Table 7) in which such a pre-mixing step is standardly used. With the mixing ratio of influent and returned sludge that is in common practice (r = 0.5), the floc load in the mixing zone measures 100–150 g COD/kg MLSS. At

an hydraulic retention time of 5 minutes this corresponds with a sludge load of 1.2–1.8 kg COD/kg MLSS.h. This load is expressed per hour of influent supply because averaging the loading level per day will result in an underestimation of the actual nutrient level during mixing of influent and sludge in domestic plants.

Fig 82 shows that the instantaneous biosorption is followed by a more gradual uptake process. This concerns consumption of easily biodegradable components from the waste water (dissolved and rapidly hydrolysable compounds) by micro-organisms. The rate at which this occurs depends upon the biological activity. This activity can be manipulated. The application of a high floc loading level results in the growth or selection of floc forming bacteria which can quickly hoard substrate. Approximately 75% of the substrate taken up is stored in the cell. With the exception of *M. parvicella*, which grows on another fraction in the influent, filamentous micro-organisms cannot store much substrate quickly in their cells.

Therefore, by introducing a high floc loading level, a sludge can be cultured in which floc forming bacteria largely remove the easily degradable substrate from the water phase in 10 - 20 minutes. An example of this is shown in Table 8. The likelihood of bulking sludge arising is consequently considerably reduced. The selection of a population capable to store substrate rapidly usually takes a few weeks.

*Table 8* *Biosorption by two types of activated sludge. Sludge A originates from a WTP with a selector*

| | Floc load (mg COD/g MLSS) | Time (min.) | Biosorption (mg COD/g MLSS) | Removed (%) |
|---|---|---|---|---|
| sludge A + influent A | 123 | 1 | 80 | 65 |
| | | 10 | 104 | 85 |
| | | 30 | 107 | 96 |
| sludge A + influent B | 50 | 1 | 20 | 39 |
| | | 10 | 46 | 92 |
| | | 30 | 47 | 93 |
| sludge B + influent B | 37 | 1 | 5 | 13 |
| | | 10 | 13 | 36 |
| | | 30 | 13 | 36 |
| sludge B + influent A | 91 | 1 | 14 | 15 |
| | | 10 | 22 | 24 |
| | | 30 | 33 | 27 |

A second pre-condition must be fulfilled to allow this selection mechanism to function properly: after passing the zone with the high floc loading level, the micro-organisms must be given enough time to process the hoarded substrate. The biosorption capacity must be totally regenerated when the floc forming bacteria, capable to store substrate, again reach the selector. This regeneration process takes time. The retention time of the biomass in the aeration tank is too short in highly loaded plants (load > ca. 0.4 kg BOD/kg MLSS.day) for a complete regeneration of the biosorption capacity. This problem can sometimes be overcome by pre-aeration of the returned sludge. The critical value of about 0.4 kg BOD/kg MLSS.day is lower if an oxygen concentration <2 mg $O_2$/l is maintained in the aeration tank. More time for regeneration is also necessary at low water temperatures (→ lower activity of the micro-organisms).

The aerobic selector is based on the above mentioned pre-conditions. In recent years, it has been applied successfully for controlling bulking sludge in industrial WTPs. The same result can be achieved with a properly designed plug flow configuration or with an SBR plant. An aerobic selector is less suitable for controlling bulking sludge in domestic treatment plants, because the substrate necessary for denitrification and/or Bio-P removal would be removed under aerobic conditions.

### 10.4.2 Designing an aerobic selector

The basic principle is that the retention time in the selector must be long enough to enable almost complete removal of the easily degradable fraction from the waste water.
Equation (1) represents how the volume of the selector can be determined (Prendl, 1997):

$$V_{sel} = \frac{Q \cdot S_{sol}}{OS_{sel} \cdot r_{sub}} \qquad (1)$$

$V_{sel}$ : volume of the selector ($m^3$)

$Q$ : influent flow ($m^3/h$)

$S_{sol}$ : concentration of easily degradable compounds in the influent(kg COD/$m^3$)

$QS_{sel}$ : amount of easily degradable compounds in the influent (kg COD/h)

$MLVSS_{sel}$ : organic matter concentration in the selector (kg/$m^3$)

$r_{sub}$ : specific substrate removal rate at an adequate oxygen supply by sludge that is adapted to a high floc load (kg COD/kg MLVSS.h)

In principle, the complete returned sludge flow should pass the selector. However, if this results in a floc load << 100 g COD/kg MLSS, then a part of the returned sludge flow should be sufficient. The influent should always completely pass through the selector.

The size of the inorganic fraction often amounts to 20–30%. In industrial WTPs, however, this percentage can be much higher. The size of the easily biodegradable fraction in the influent can be estimated by determining the BOD/COD ratio of the dissolved fraction. The ratio is almost 1 for easily degradable compounds.

The specific substrate removal rate should be established experimentally. If this is not possible, it can be worked out for industrial waste water, if $S_{sol}$ is greater than 200 mg/l, by $r_{sub}$ = 0.5 kg COD/kg MLVSS.h. This rate must be lowered proportionally if less than 200 mg/l of easily biodegradable compounds are present in the influent. However, it is not advisable to use for $r_{sub}$ values < 0.2 kg COD/kg MLVSS.h (→ over sized selectors).

These design criteria result in selectors with hydraulic retention times (sludge + influent) of 10–30 minutes.

To establish the necessary aeration capacity, it must be known which part of the substrate taken up is already completely degraded in the selector ( the remainder is converted into storage materials). If this percentage is unknown, a value of 25% can be used, which consequently results in an aeration capacity of $0.25.Q.S_{sol}$ (kg $O_2$/h) in the selector.

The selector must be compartmentalised, preferably consisting of at least four compartments. A plug flow configuration can also be used. The very high sludge load in the first compartment/section benefits the substrate uptake by the flocs. If strong fluctuations in the COD of the influent occur, the selector must be designed on peak loading levels. The selector is then over-sized during periods of low supply, but Owing to the compartmentalisation, the load is still high enough in the first compartment.

Sludge can store a maximum of ca. 0.4 g of substrate per gram of organic material. In order to allow the selection mechanism to operate properly, a maximum of 50% of this storage capacity is exploited. This means that the floc load (with 'dissolved' substrate) can amount to no more than 200 mg COD/g MLVSS. Therefore, an aerobic selector cannot be used without caution for very concentrated waste water.

### 10.4.3 Combination with nutrient removal

Treatment plants for biological nutrient removal are primarily designed on an extensive removal of nitrogen and/or phosphorus. A low sludge load is applied in order to achieve complete nitrification. The dissolved COD fraction in the waste water is consumed under anoxic/anaerobic conditions by floc forming denitrifying and/or Bio-P bacteria in the sludge. Consequently, mainly low F/M filamentous species, especially *M. parvicella*, are encountered in nutrient removal plants (Fig. 83).

However, the incorporation of a zone with a high floc load has some effect on the growth of low F/M species as well. From the results of an inventory in four European countries, it was concluded that nutrient removal plants can be classified according to a decreasing size of the filamentous population thus:

- complete mixing + simultaneous denitrification;
- complete mixing + intermittent (de)nitrification;
- alternating anoxic/oxic process conditions, extended with an anaerobic tank for Bio-P (Bio-Denipho);
- alternating anoxic/oxic conditions (Bio-Denitro);
- pre-denitrification.

A large *M. parvicella* population is also, however, often encountered with pre-nitrification. A high floc load is obviously insufficient for controlling the growth of *M. parvicella* adequately in nutrient removal plants.

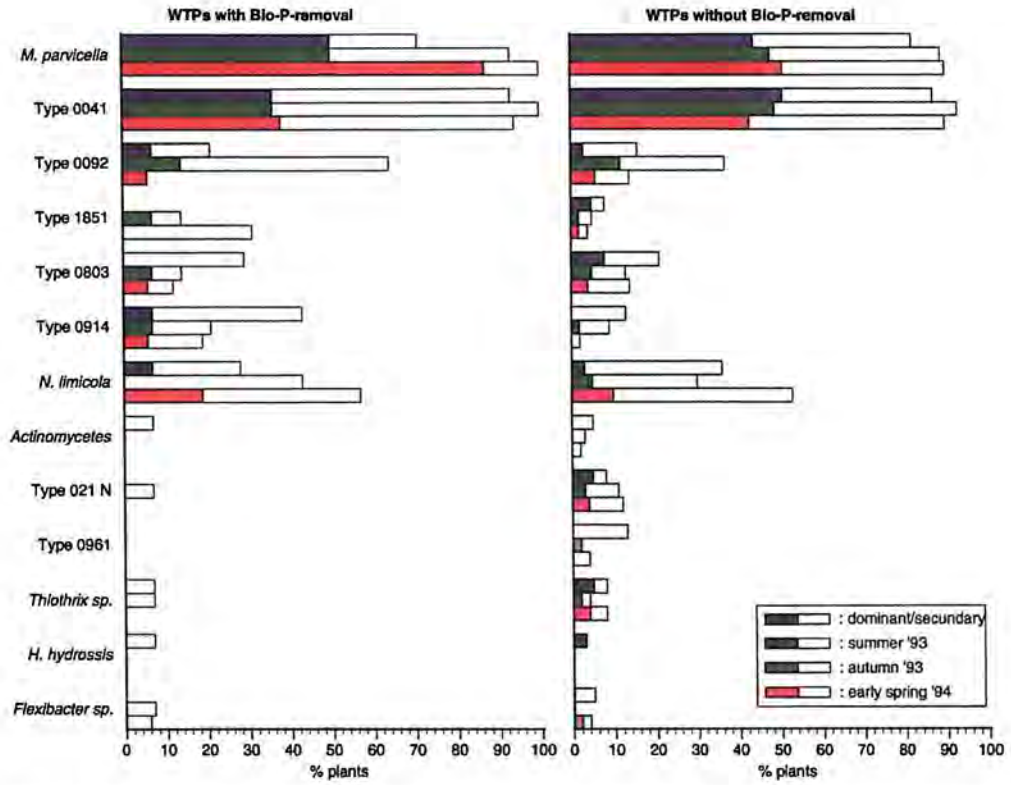



*Figure 83 Filamentous population in Danish treatment plants with nutrient removal and an FI of ≥ 2.5.*

It is well established that the introduction or optimising of nutrient removal conditions stimulates the growth of *M. parvicella*. Consequently, an investigation in 25 Dutch WTPs confirmed that the SVI in 60% of the plants had risen after changes in the process conditions aimed at a better removal of N and/or P. In 16 of the plants, the excessive growth of *M. parvicella* caused bulking sludge during the winter. This did also occur in plants in which *M.parvicella* was effectively controlled in the past by the incorporation of a selector in the process. The number of plants with bulking sludge has also increased in other countries as a result of the introduction of nutrient removal conditions.

A consistent explanation for this phenomenon is lacking for the time being. Therefore, a summing up of some points related to the growth of *M. parvicella* must suffice:

- the combination of raw influent + a selector for the removal of particulate substrate + oxic conditions in the aeration tank is effective in controlling the growth of *M. parvicella*. The results are less consistent with pre-settled waste water;
- changing the conditions in a section of the aeration tank from oxic to anoxic often results in the development of *M. parvicella*, also WTPs with a selector. Bulking sludge practically always occurs with the anoxic volume of > 40% - 50% in the aeration tank;

- in contrast, the combination of (1) a selector and (2) alternating anoxic/oxic conditions (Bio-Denitro configuration) is effective in controlling an excessive growth of *M. parvicella*. This process does indeed stimulate Type 0041, but this species only seldom causes bulking sludge in domestic plants;
- a preceding anaerobic tank (HRT: 1-3 hours, with or without compartmentalisation) for Bio-P removal is associated with a substantial increase of the *M. parvicella* population. On account of the heavy Bio-P clusters in the flocs, the SVI does not increase accordingly;
- conditions in which fats are broken down under anoxic or anaerobic conditions (→ free higher fatty acids) almost certainly enhance the competitive position of *M. parvicella*. In order to control the growth of this species, the higher fatty acids must be irreversibly bound by the floc before they can be taken up by *M. parvicella;*
- recently, a non-aerated selector was admitted between the anaerobic and anoxic zones in some WTPs in the Netherlands (Fig. 72; Van Loosdrecht *et al*, 1998). First experiences with this configuration are positive, but the number of WTPs using it is too small to draw any definite conclusions.

As structural solutions are not yet available, symptom control is often used against *M. parvicella*. Dosing with aluminium salts (3 g $Al^{3+}$/kg MLSS.day) is nearly always effective. In this way, chemical phosphate removal is combined with bulking sludge control. Within 2–3 weeks, dosing results in a considerable reduction in the number of filaments and any possible scum that may be present has often already disappeared.

## 10.5 Zoogloea bulking sludge

Zoogloea bulking sludge is not caused by the growth of filamentous bacteria, but by the strong bonding of water by the floc. This form of bulking sludge only occurs occasionally. The microscopically easily recognisable zoogloea colonies are not always present. Strong water bonding is caused by a high level of bio-polymers in the floc.

Zoogloea bulking occasionally occurs in highly loaded plants. If a high sludge load is the cause, then only symptom control can be used.

A non-balanced nutrient supply, particularly an excess of carbon compounds, can also result in the formation of many bio-polymers. In this case, the remedy consists of dosing the missing nutrients.

# 11 Scum formation

Flotation of activated sludge results in the formation of scum in the aeration tank and/or the final clarifier. This often starts with large bubbles of darkly coloured foam on the surface of the aeration tank. It is definitely not detergent foam, which is much whiter. Where this foam coagulates, a scum layer arises. In extreme cases, the surface of the tank can become completely covered by a thick layer of scum which can reach tens of centimetres in thickness, and in which the solids concentration can increase up to 30–50 g/l (Fig. 84). Formation of scum is, in addition to bulking sludge, the second largest stability problem in activated sludge plants. The introduction of biological nutrient removal conditions has led to a large increase in the number of WTPs with scum problems. In The Netherlands, ca. 50% of all low loaded plants are affected by scum during winter.

Scum results in loss of solids in the final effluent, odour problems, aesthetic drawbacks, less safe working conditions and extra cleaning work. Transportation of the flotation material to the sludge digestion tank often causes a scum layer on this tank also. When the scum freezes, the walls of the tanks and the sludge scraper in the final clarifier can be damaged.

In domestic treatment plants, filamentous micro-organisms are almost always the main cause of scum occurring. Therefore, bulking sludge and scum formation are frequently associated with one another. Before this subject is continued, the following paragraph will first briefly describe some other possible causes.

## 11.1 Scum formation in which filamentous species play no decisive role

*Denitrification in the final clarifier*

If the sludge only floats in the final sedimentation tank tank, the surface of the aeration tank is therefore virtually clean, the formation of scum is usually caused by denitrification. This process results in small nitrogen gas bubbles. These are bound in the aggregated flocs at the bottom of the final clarifier. The sludge often floats in lumps which slowly rise to the suface. The small nitrogen gas bubbles are not strongly bound to the flocs. Owing to slight turbulence, e.g. by stirring or by spraying effluent on the flotated sludge, the bubbles are released. The flocs consequently settle again. Filamentous bacteria can increase the flotation process, but are not the main cause.

*Figure 84 The aeration tank is completely covered by scum.*

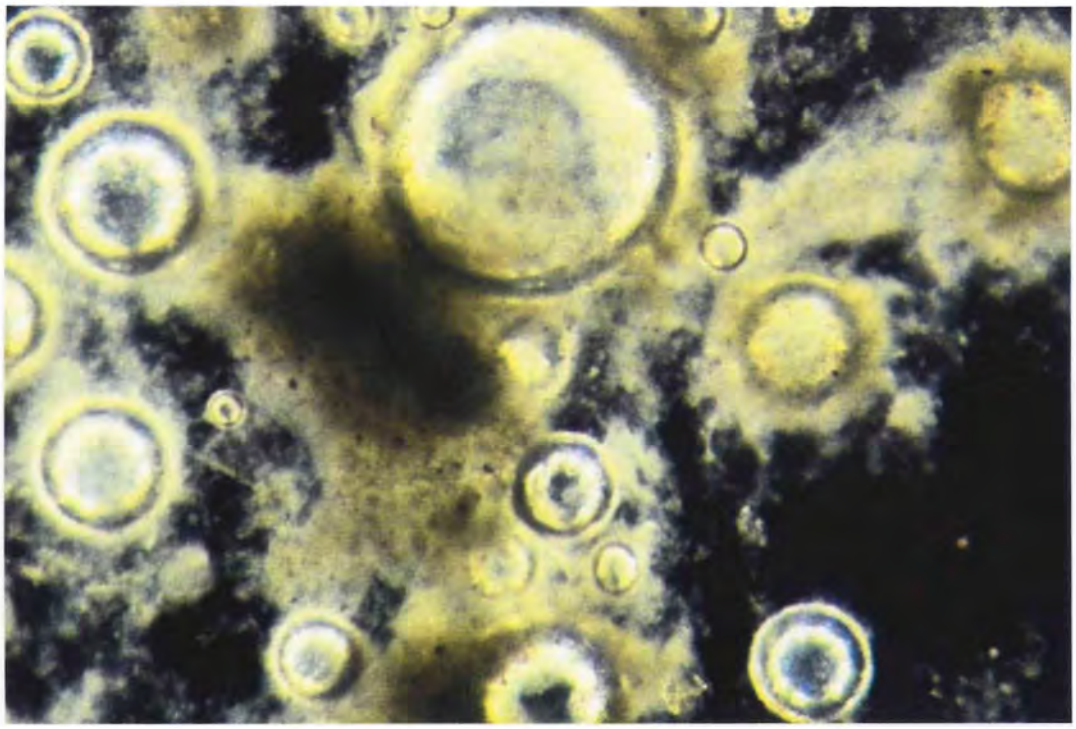


*Figure 85 Stabilised gas bubbles in scum (50×).*

This phenomenon is usually the result of, as yet, unprocessed substrate that is present in the floc. Consequently, the mineralization process is not complete when the sludge enters the final clarifier. Owing to the lack of oxygen, nitrate is switched to for the metabolic processes (→ denitrification). The process is boosted by a high concentration of nitrate in the sludge and by the sludge remaining for a(n) (overly) long time in the final sedimentation tank.

*Constructional causes*

The following combination occasionally occurs:
- no scum in the final clarifier;
- scum present in the aeration tank;
- hardly any filamentous micro-organisms in the sludge;
- no industrial discharges.

If this is the case, the cause of the scum layer is usually constructional.

Some material is always to be found floating on the surface of the aeration tank. This material is normally re-suspended by the aeration in the tank or transferred to the final settlement tank and there removed. In carrousel systems, however, the aerators are sometimes covered. If the cover/wall extends to under the water surface, everything that floats accumulates in front of it. Scum occurs almost spontaneously. In addition, if the overflow to the final sedimentation tank is positioned behind the surface aerators in carrousel systems, a scum layer can easily arise. The flow pattern around the aerator results in the retention of floating material in the zone right in front of the aerator. This material is therefore trapped in the aeration tank. A baffle placed in the overflow to the final sedimentation tank has the same effect. As filamentous micro-organisms are absent, these types of scum layer are not stabilised. They can be easily destroyed by spraying with effluent.

*Industrial discharges*

Process operators of domestic treatment plants often hold local industry responsible for causing scum. Discharges of fats or detergents can indeed bring about scum. However, scum formation in domestic WTPs is almost always primarily caused by filamentous micro-organisms that grow on components present in domestic waste water.

## 11.2 Stabilized gas bubbles

Persistent scum arises owing to the attachment of small, but exceedingly stabilized, gas bubbles to the flocs (Fig. 85). Only a small quantity of gas is necessary to reduce the density of the activated sludge to such an extent that it remains floating: 0.44 $cm^3$air/g dry matter.

The origin and the composition of the gas bubbles can vary. They are often just ordinary air bubbles produced through the aeration system or otherwise. Every form of turbulence can cause air bubbles, e.g. pumping the returned sludge by Archimedes screws or turbulent

conditions at the overflow to the final clarifier. Gas bubbles are also formed during denitrification ($N_2$-gas).

The life time for gas bubbles in activated sludge is usually short. In so far as they do not disappear into the atmosphere, small bubbles (25–100 µm) dissolve in the water within 10–20 minutes. This process can be slightly delayed if surface-active compounds are present in the water, such as detergents, as is always the case with domestic waste water. These types of compound stabilise the gas–water interface surrounding the bubbles thereby increasing their life time. Scum occurs thus.

However, the interface around the bubbles is unusually robust if small hydophobic particles are also present together with the surface-active compounds. Hydrophobic means water repellent. A greasy surface, for instance, is hydrophobic. If such hydrophobic particles are present, the interface is extremely stabilised. The life time of the bubbles is then almost endless and they can no longer be destroyed by adding anti-foam products.

Gram positive filamentous bacteria usually possess a hydrophobic cell surface. This group of bacteria also plays a decisive part in the formation of persistent scum layers in domestic treatment plants (Table 9).

*Table 9* *Results of the determination of the Scum Index (SI) with two activated sludges.*
*Sludge T: no filaments; SVI = 60 mlg/g*
*Sludge B: many filaments; SVI = 200 ml/g*

| Sludge | Influent (25 vol. %) | SI (%) | SVI [1] (ml/g) |
|---|---|---|---|
| T | T | 0 | 60 |
| T | B | 0 | 60 |
| B | T | 75 | 75 |
| B | B | 48 | 105 |

[1] SVI of the fraction that settled after the flotation test.

The Scum Index (SI) can be used as a measure of the flotation properties of activated sludge. In this test, it is established under standardized conditions which percentage of the sludge will float in 10 minutes.

Only sludge B (many *M. parvicella* filaments present) flotates extensively. In addition, the fraction that settles after the test has a much lower SVI than the original material, indicating that any filaments present are largely flotated.

## 11.3 Effect of the size and composition of the filamentous population on scum formation

In April and May of 1990, an investigation was done in The Netherlands into the nature and extent of scum in the aeration tanks in carrousel treatment plants. Approximately 70 WTPs were involved. The investigation was repeated in September and October of the same year.

Five groups of WTPs were distinguished:
- : no scum layer present;
± : 5% of the surface covered with scum;
+ : 25% of the surface covered with scum;
++ : 50% of the surface covered with scum;
+++ : 70–100% of the surface covered with scum.

During spring and autumn, scum was present on 54% and 31%, respectively, of the carrousels (Table 10). The percentage of WTPs with extensive scum layers (++/+++) reduced during the summer from 34% to 12%. Therefore, there is a definite seasonal effect on the occurrence of scum.

*Table 10 The number of carrousels with scum in their aeration tanks*

| | Spring | | Autumn | |
|---|---|---|---|---|
| Extent of scum layer | no. WTPs | % | no. WTPs | % |
| - | 33 | 46 | 46 | 69 |
| ± | 4 | 6 | 10 | 15 |
| + | 10 | 14 | 3 | 5 |
| ++ | 16 | 23 | 3 | 5 |
| +++ | 8 | 11 | 5 | 7 |

Table 11 shows that the possibility of scum occurring is greater if more filamentous bacteria are present in the sludge. This connection was very obvious during spring, particularly. Occurrence is practically 100% with an FI > 4. The layer of scum was usually also larger when more filaments were present in the sludge.

*Table 11 The connection between the Filament Index (FI) of the activated sludge and the presence of scum*

| FI | Spring | | Autumn | |
|---|---|---|---|---|
| | no. WTPs | % with scum [1] | no. WTPs | % |
| 0 | 0 | — | 1 | 0 |
| 1 | 1 | 0 | 10 | 13 |
| 2 | 20 | 25 | 27 | 15 |
| 3 | 22 | 36 | 18 | 22 |
| 4 | 23 | 87 | 11 | 44 |
| 5 | 4 | 100 | 0 | — |

1) percentage of the number of WTPs in the concerned group

Table 12 compares the filament-indices of the floating sludges with that of the suspended activated sludges in the same WTPs. The number of filaments was often (noticeably) greater in the scum than in the suspended sludge in the aeration tank. Occasionally, the scum appeared to consist practically of a pure culture of filaments. Therefore, selective flotation of filamentous bacteria and/or growth of these bacteria in the scum occur. In general, many filaments were also present in small layers of scum.

*Table 12* *FIs of suspended and floating sludges in the same WTPs. Expressed in the number of WTPs*

| FI | Spring | | Autumn | |
|---|---|---|---|---|
| | suspended | scum | suspended | scum |
| 1 | — | — | 2 | 0 |
| 2 | 5 | 0 | 6 | 2 |
| 3 | 6 | 3 | 5 | 3 |
| 4 | 16 | 6 | 6 | 7 |
| 5 | 4 | 19 | 0 | 5 |
| extreme[1)] | 0 | 3 | 0 | 2 |

1) almost a pure culture

Microscopic investigation revealed that *M. parvicella* was particularly responsible for scum occurring in these WTPs.

The comparison between the April/May composition of the population in the suspended sludges with that of the floating biomass shows some striking differences (Fig. 86):

- *M. parvicella* was even more frequently dominant in the scum (from 84% to 94%);
- the percentages in the scum were also slightly higher for actinomycetes and *N. limicola*;
- all other strains were less frequently observed in the scum than in sludges in suspension;
- the scum was almost a pure culture in about 50% of the plants.

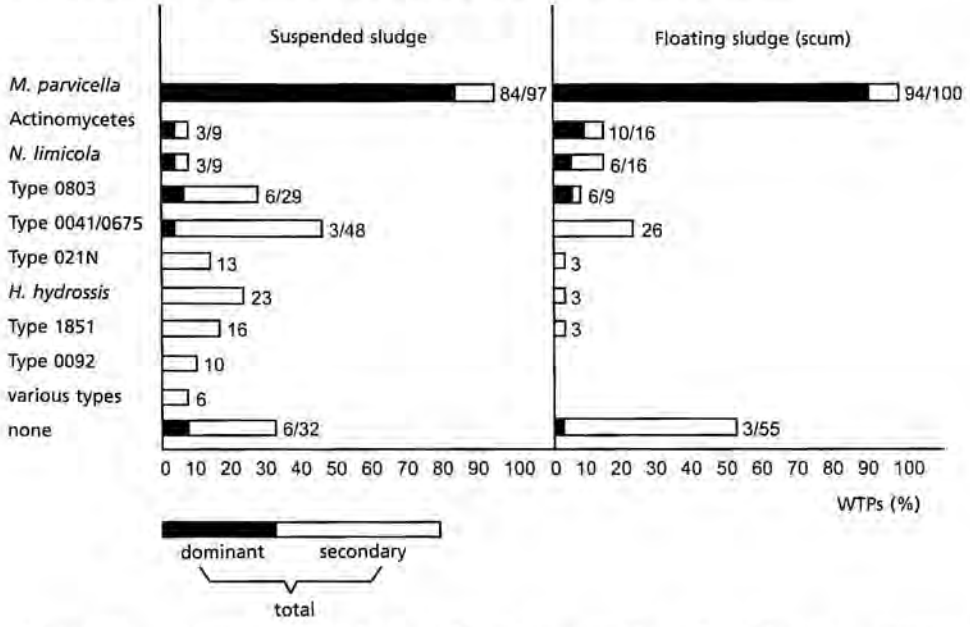



*Figure 86* *Comparison of the population composition of the sludge in suspension with that of the scum in the same WTPs.*
*Expressed in % of the number of WTPs*
*Period: April/May; no. WTPs = 31*

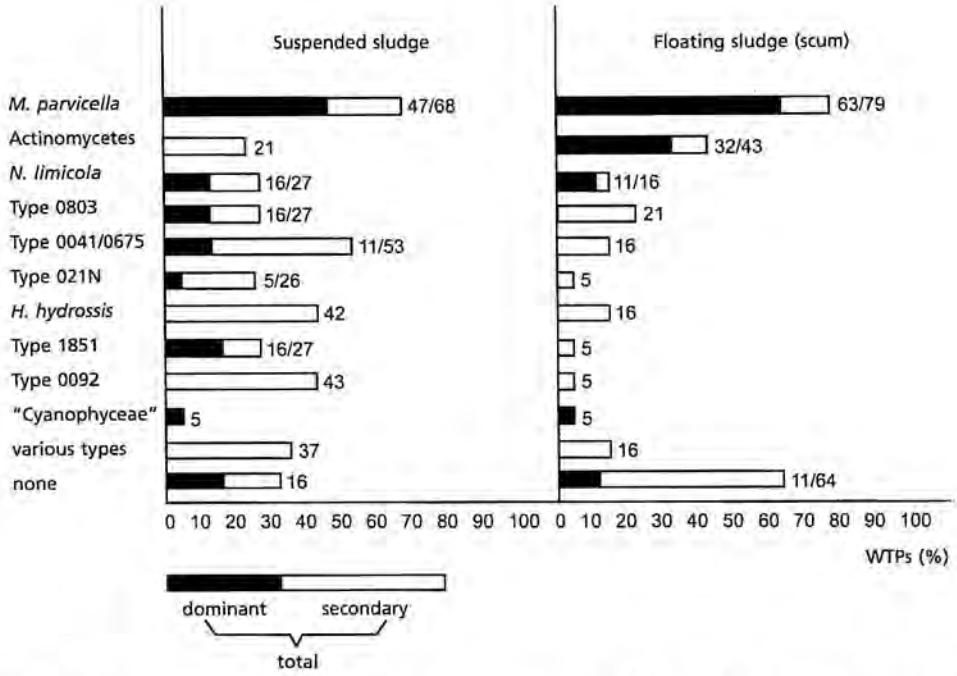



*Figure 87* *Comparison of the population composition of the sludge in suspension with that of the scum in the same WTPs.*
*Expressed in % of the number of WTPs*
*Period: September/October; no. WTPs = 19*

The differences between these two sludges were even greater during the autumn (Fig. 87). The high percentage (32%) of WTPs with scum in which actinomycetes were dominant is quite remarkable. These species often seem to appear from 'nowhere'. Actinomycetes were only secondarily present in 21% of the activated sludges in suspension. In contrast, they were predominating in 32% of the scum samples, indicating that that the growth of actinomycetes is practically always followed immediately by flotation (→ extreme flotation properties). There are some countries in which the contribution of actinomycetes to the occurrence of scum is strikingly larger than it is in The Netherlands (and the role played by *M. parvicella* is correspondingly less). Both species compete for the same nutrients (the fat fraction in the influent). A higher water temperature and/or a higher sludge load benefit the competitive position of the actinomycetes.

It appears from these results that only *M. parvicella*, actinomycetes and, to a limited extent, *N. limicola* flotate selectively. It is certainly not coincidental that these organisms play such an important part in the stabilisation of gas bubbles and the occurrence of scum. All three species are Gram positive and are therefore hydrophobic. They do not really feel at home in a watery environment, meaning that they prefer the likewise hydrophobic surfaces of gas bubbles. *M. parvicella* and *N. limicola* form flexible filaments which easily 'bend' themselves into a bowed interface. This is also the case for the small branched networks of the actinomycetes.

Of the commonly occurring filamentous bacteria in low loaded activated sludge treatment plants, Types 0041/0675 and 1851 are also Gram positive. However, the cells of these filaments are surrounded by a sheath through which contact between the cell surface and its surroundings is prevented. In addition, the filaments are straight and usually covered with attached single celled bacteria (attached growth).

The fat fraction supplied with the influent, as well as the micro-organisms that consume these compounds, prefer the gas–water interface above the water phase. This comes suspiciously close to 'trusting the cat to keep the cream'. In spite of the fact that this has not yet been proven, this combination appears to be an important selection advantage for some filamentous species in the competition for the available nutrients.

In countries with moderate temperatures, the occurrence of scum is very strongly associated with the growth of *M. parvicella*. Because of its growth being connected to the seasons, scum disappears during summer in many plants.

The introduction and optimizing of nutrient removal conditions has strengthened the competitive position of *M. parvicella,* as a result of which the number of plants with scum has increased in recent years.

## 11.4 Controlling scum

As long as the scum index is not extremely high (SI < 10-15%), several methods are available for controlling scum:

1. Systematic skimming, removal and destruction of sludge that floats in the aeration tank. The skimmed material must not be circulated (e.g. to the primary sedimentation tank), as this can lead to a build-up of a large population of scum-producing bacteria in the plant. Transport to the sludge digestion tank is also strongly advised against.
2. Removal of the baffle in the overflow from the aeration tank to the final clarifier. Floating material can then flow through to the final sedimentation tank and is removed with existing or expanded skimming facilities. It was established during the study referred to in the previous paragraph, that the presence of a baffle in autumn determined to a large extent the occurrence of scum in the aeration tank (no baffle: 15% with scum; baffle present: 43% with scum). This difference was insignificant in early spring (*M. parvicella* in excellent condition and still growing relatively fast).
3. Spraying effluent on the scum, by which floating material is returned into suspension.

However, these measures are inadequate where sludges with an SI > 20–30% are concerned. Controlling the growth of the responsible filamentous species is then necessary for solving the problem. As is mentioned in chapter 10, *M. parvicella* can be effectively controlled by dosing with Al salts and probably by a BCFS configuration as well. It is not yet known whether actinomycetes can be controlled in the same manner.

Separation of sludge and final effluent by dissolved air flotation might be an alternative on special occasions. This option is not often used in practice, however.

# 12 Closing remarks

An aeration tank is not a tank full of sludge, but a bio-reactor filled with micro-organisms.

Micro-organisms can be very effective, as long as they are treated properly.

Micro-organisms work for 24 hours a day and 7 days a week, but they do this at their own pace. Changes to the population can sometimes take. Therefore, patience is a virtue!

# 13 Literature

For more information on the biology and the process stability of activated sludge treatment plants, the reader is referred to the titles below.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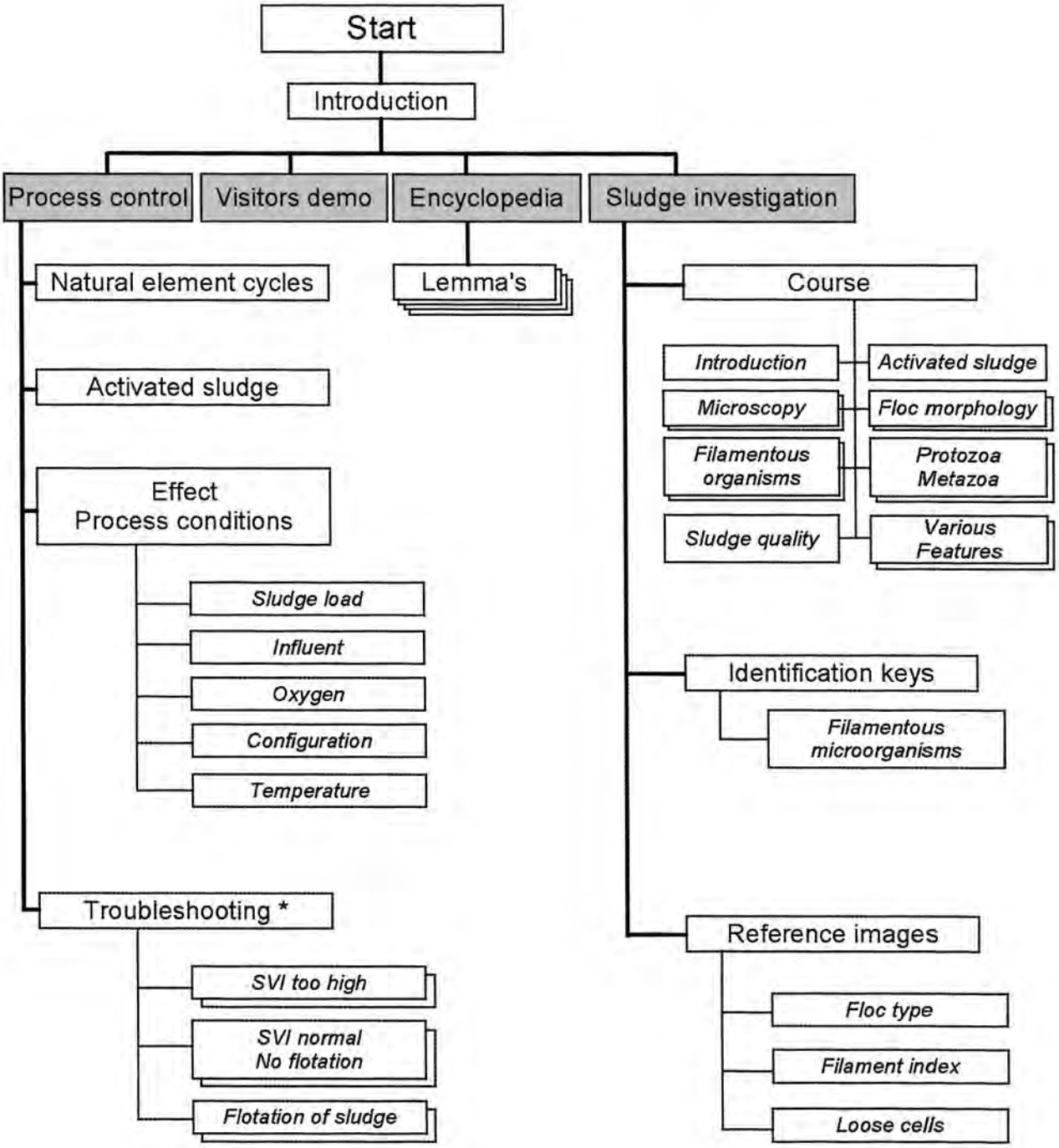
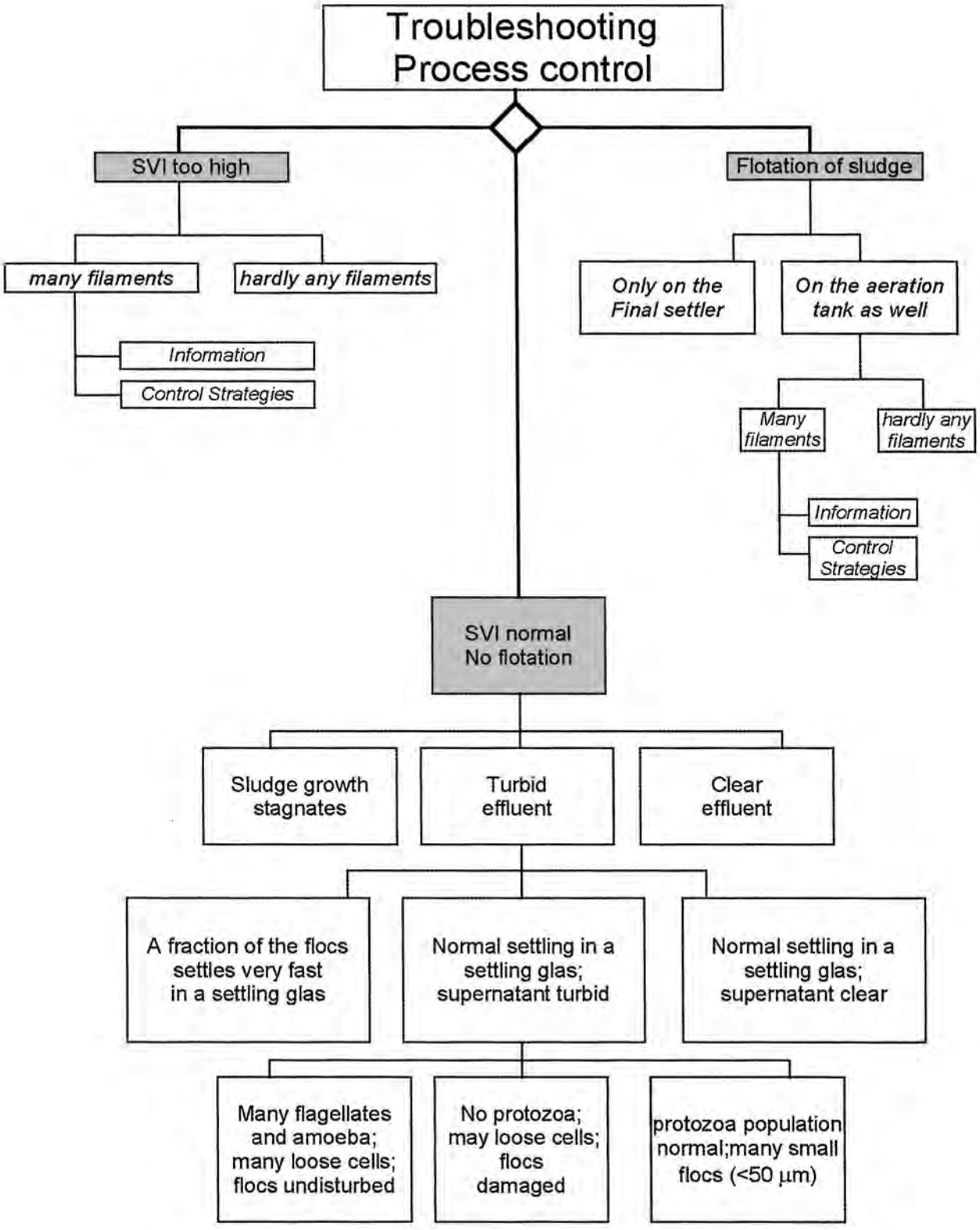
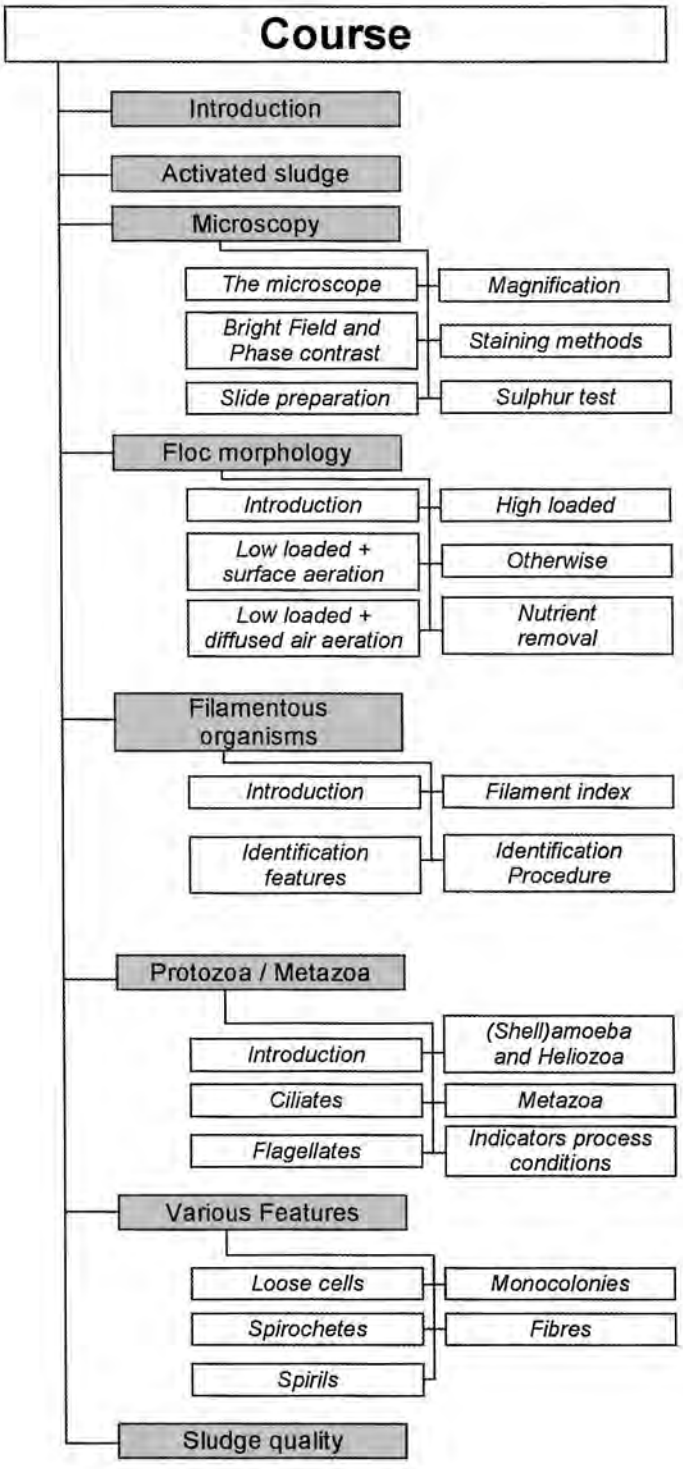
# CD-I Architecture
# Process control of activated sludge plants by microscopic sludge investigation




Troubleshooting
Process control
SVI too high
Flotation of sludge
many filaments
hardly any filaments
Only on the Final settler
On the aeration tank as well
Information
Control Strategies
Many filaments
hardly any filaments
Information
Control Strategies
SVI normal
No flotation
Sludge growth stagnates
Turbid effluent
Clear effluent
A fraction of the flocs settles very fast in a settling glas
Normal settling in a settling glas; supernatant turbid
Normal settling in a settling glas; supernatant clear
Many flagellates and amoeba; many loose cells; flocs undisturbed
No protozoa; may loose cells; flocs damaged
protozoa population normal;many small flocs (<50 μm)


Course
Introduction
Activated sludge
Microscopy
The microscope
Magnification
Bright Field and Phase contrast
Staining methods
Slide preparation
Sulphur test
Floc morphology
Introduction
High loaded
Low loaded + surface aeration
Otherwise
Low loaded + diffused air aeration
Nutrient removal
Filamentous organisms
Introduction
Filament index
Identification features
Identification Procedure
Protozoa / Metazoa
Introduction
(Shell)amoeba and Heliozoa
Ciliates
Metazoa
Flagellates
Indicators process conditions
Various Features
Loose cells
Monocolonies
Spirochetes
Fibres
Spirils
Sludge quality

Appendix 2 Form for recording the characteristics of filamentous micro-organisms

| **Name Plant** | **Date** | **Filamentous organisms** | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|
| ..... | ..... | 1 | 2 | 3 | 4 | 5 | 6 | |
| Dominating | | | | | | | | |
| Secondary | | | | | | | | |
| Branching | absent | | | | | | | |
| | false branching | | | | | | | |
| | real branching | | | | | | | |
| Motility | | | | | | | | |
| Many S-granules | present in vivo | | | | | | | |
| | present after S-test | | | | | | | |
| Septa | clearly visible | | | | | | | |
| | not/hardly visible | | | | | | | |
| Filament shape | straight | | | | | | | |
| | bent/bowed | | | | | | | |
| | coiled/twisted | | | | | | | |
| Neisser staining * = positive | granules* | | | | | | | |
| | cells grey-blue* | | | | | | | |
| | negative | | | | | | | |
| Gram staining | positive (blue) | | | | | | | |
| | negative (red) | | | | | | | |
| Filament diameter | < 1.0 µm | | | | | | | |
| | 1.0-2.5 µm | | | | | | | |
| | > 2.5 µm | | | | | | | |
| Attached growth | substantial | | | | | | | |
| | absent/scanty | | | | | | | |
| Constrictions | clearly observable | | | | | | | |
| Cell shape | disc shaped | | | | | | | |
| | spherical/coccus | | | | | | | |
| | rod-shaped | | | | | | | |
| | square | | | | | | | |
| | rectangular | | | | | | | |
| Sheath | present | | | | | | | |

Conclusions:

1 = _____

2 = _____

3 = _____

4= _____

5 = _____

6 = _____

Appendix 3

## Result of microscopic sludge investigation

Water treatment plant: .....

| Sampling date | Analysed by |
|---|---|
| Sample number | Date of analysis |

| **Sludge quality** | |
|---|---|
| Good | ❒ |
| Moderate | ❒ |
| Bad | ❒ |
| **Trend** [1)] | |
| Better | ❒ |
| Similar | ❒ |
| worse | ❒ |
| irrelevant | ❒ |

**(specific photograph)**

1) in comparison with the last sampling

| **Filamentous organisms**[a)] **FI =** | | | | **Proto-/Metazoa**[b)] | | **Various characteristics** | |
|---|---|---|---|---|---|---|---|
| *M. parvicella* | | Type 021N | | Ciliates | | Floc type [d)] | |
| Type 0041/0675 | | *Thiothrix* | | Flagellates | | Free living cells [c)] | |
| Type 0092 | | *S. natans* | | Amoeba | | Zoogoea [b)] | |
| Type 1851 | | *H. hydrossis* | | Test. amoeba | | Poly-P colonies [b)] | |
| Type 0803 | | *N. limicola* | | Heliozoa | | Other monocolonies [b)] | |
| Type 0914 | | Type 1701 | | Rotifers | | Spirils [b)] | |
| Actinomycetes | | | | Nematodes | | Spirochaetes [b)] | |
| | | Various species | | Worms | | Fibres [b)] | |

a) Scale 0 - 5 = none - numerous filamentous organisms
b) Scale 0 - 3 = none - numerous cells/colonies per slide
c) Scale 0 - 3 = none - hundreds of cells per field of view

d) 1 characteristic of low sludge load + surface aerator
2 characteristic of low sludge load + diffused air aeration
3 characteristic of high sludge load
4 markedly many small flocs (< ca 25µm)
5 otherwise; see remarks

Remarks:

.....

.....

.....

.....

.....

The activated sludge process is widely used for treating waste water. Process stability and final effluent quality largely depends upon the composition of the biomass in an activated sludge plant. Operational problems such as bulking and scum formation occur when the 'wrong' micro-organisms dominate the sludge population. Microscopic sludge investigation is therefore a must for process control and stable plant operation.

Knowledge concerning diagnosing and solving operational problems in activated sludge plants has greatly expanded during the past 20 years. Many papers have been published in scientific journals and books. However, for those working in the field this information is not easily accessible. Therefore, this multi-media package has been compiled; it includes a CD-ROM and a manual. The manual deals with the theory, which is further explained and visualised on the CD-ROM through 75 minutes of audio presentation, almost 100 short videos, about 650 photographs and several animations.

This package is the sequel to the 'Microscopic sludge investigation manual' (Eikelboom and van Buijsen, 1983), a classic in the waste water literature. The methods described in this book have become more or less standard all over the world. Compared with the 1983 manual, not only updated microscopic techniques are described in this package, but solutions for most operational problems are now presented as well. It is an expert system aimed at improving process stability in activated sludge plants. Although the manual and CD-ROM can be used separately, they actually belong together. It is the combination that makes this package a unique tool for process operators and other people involved in waste water treatment.

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