The Microscopic Investigation of Microorganisms in Sewage Purification Plants

Introduction

Biological sewage purification relies on the biodegradation of organic material in waste water by bacteria, fungi and protozoa in combination with oxygen. The biodiversity of these microorganisms is of decisive importance for the cleaning performance because it is only this biodiversity that guarantees a great variety of sewage components to be metabolized.

Qualitative and quantitative variations in the family of microorganisms are indicators for disturbances and suggest causes of particular variations. Monitoring these microorganisms is therefore of extraordinary importance for sewage purification plants.

Microscopic Images with Different Contrasting Techniques

1. Stained Specimens: Brightfield Images

With a brightfield microscope, the user gets high-contrast images of stained microorganisms and their nuclei. Staining, however, always involves one additional process step and requires consumables.

A. Neisser Staining

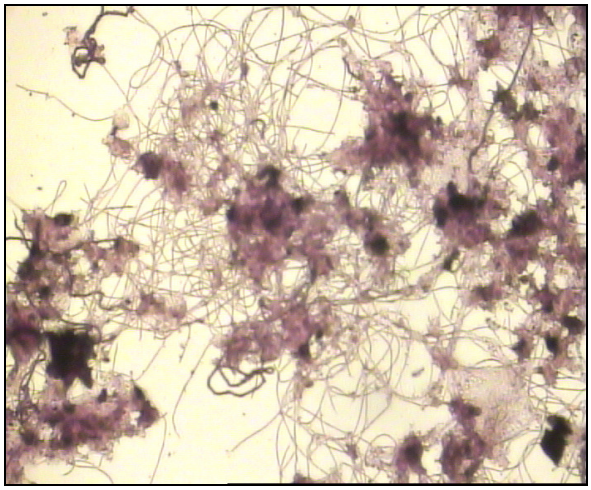

Fig. 1: Magnification: 400x.

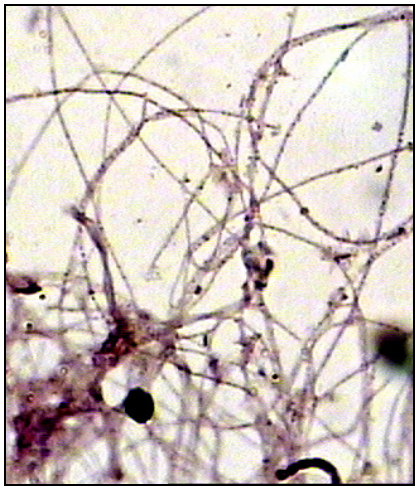

Fig. 2: Magnification: 1000x.

The Neisser-positive bacteria clearly show dark granules (Fig. 2).

B. Gram Staining

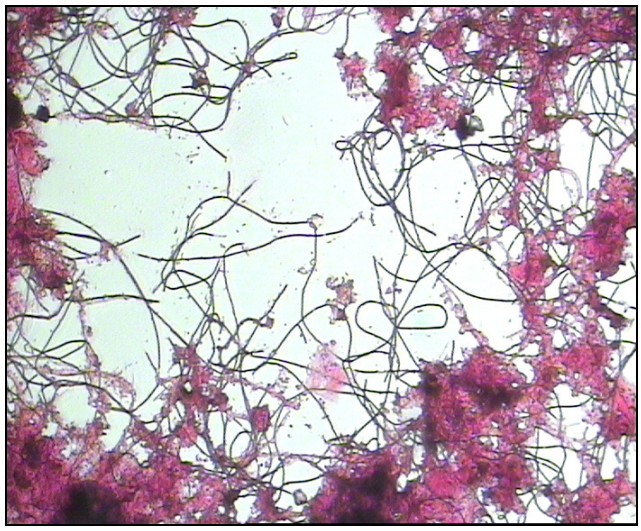

Fig. 3: Gram staining, magnification: 400x.

Depending on the composition of their respective cell walls, the bacteria are stained red or dark blue.

C. Violet Staining

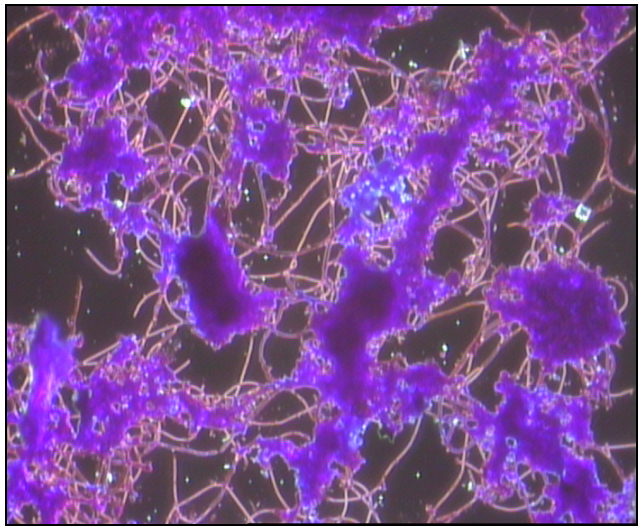

Fig. 4: Violet staining, darkfield image. Magnification: 400x.

2. Unstained Specimens

In a brightfield microscope, the image contrast can be increased by closing the aperture diaphragm. However, resolving power gets lost so that the user has to find an optimal adjustment (Figs. 5 and 6).

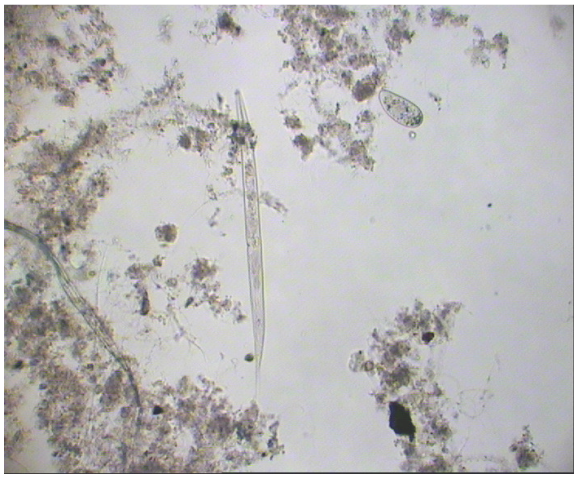

Fig. 5: Nematode (links) and paramecium (right), magnification: 100x.

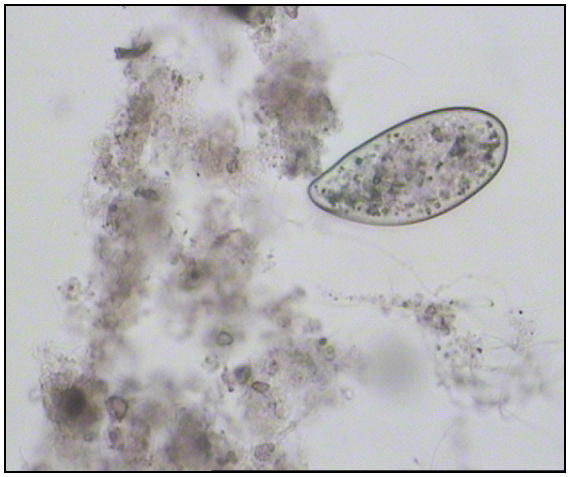

Fig. 6: Detail of Fig. 5, paramecium, magnification: 400x.

The influence of the aperture diaphragm is illustrated in the overview images of Figs. 7 and 8. Vorticella and paramecium are hardly visible with the aperture diaphragm fully opened.

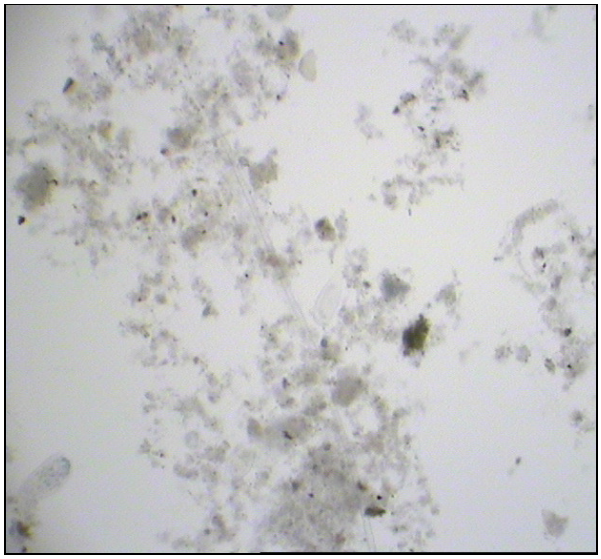

Fig. 7: Aperture diaphragm fully opened. Magnification: 100x.

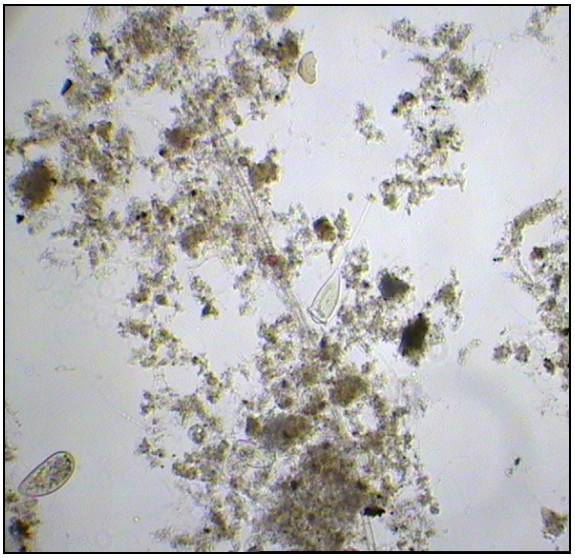

Fig. 8: Same as Fig. 7, aperture diaphragm closed.

Images of even higher contrast can be reached with phase-contrast or darkfield microscopy (Figs. 9 and 10).

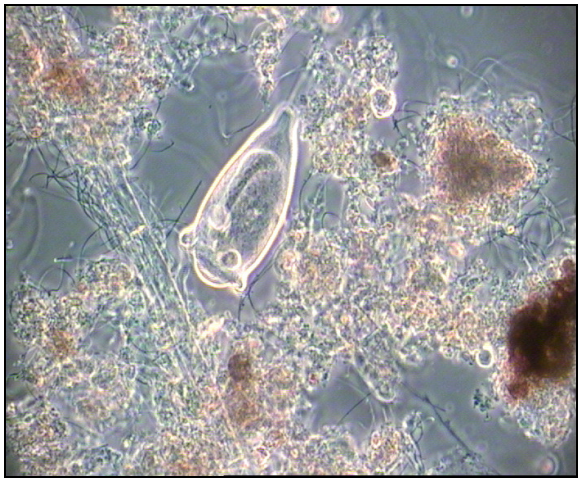

Fig. 9: Vorticella, phase-contrast image. Magnification: 400x.

Fig. 10: Same as Fig. 9, darkfield image.

Figures 11 – 14 show the different techniques in the microscopic imaging of filamentous bacteria.

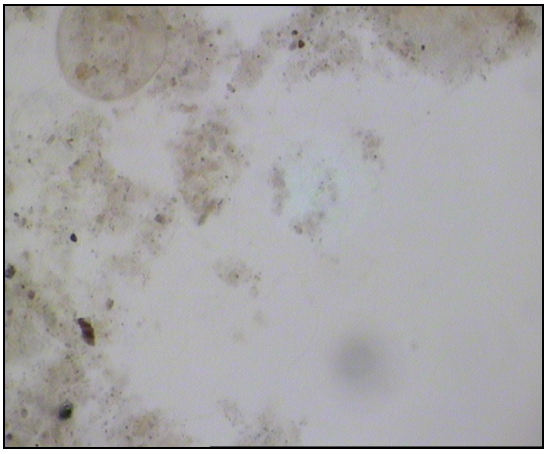

Fig. 11: Brightfield, aperture diaphragm fully opened, magnification: 400x.

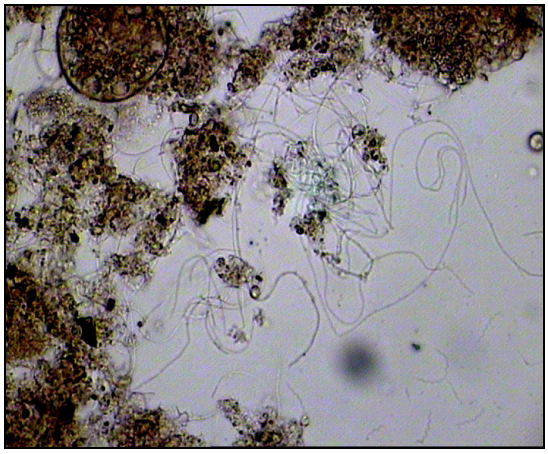

Fig. 12: Same as Fig. 11, aperture diaphragm closed.

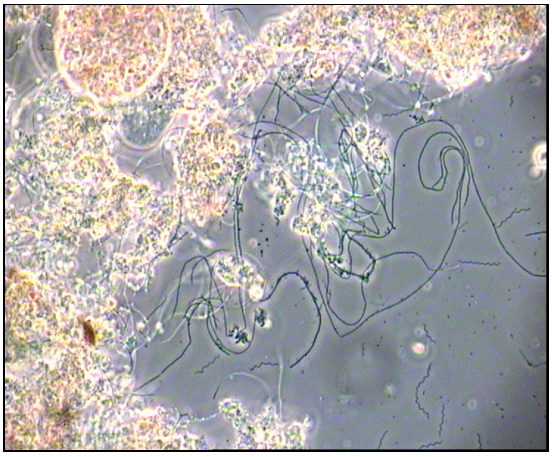

Fig. 13: Same as Fig. 11, phase contrast..

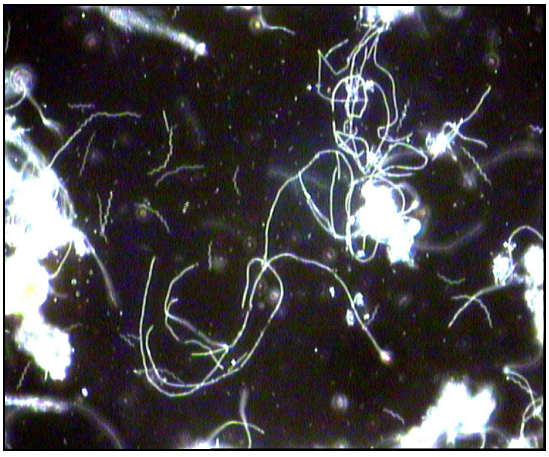

Fig. 14: Same as Fig. 11, darkfield.

The filamentous bacteria are hardly visible with the aperture diaphragm fully opened. Upon closing the aperture diaphragm, the contrast increases, but the resolving power decreases – as can be observed as slight image blur.

Phase contrast and darkfield microscope, once again, yield high-contrast images of the microorganisms.

Additional Contrasting Techniques

Recently, genetic probes are more and more often employed, particularly for the identification of bacteria. The probes specifically stain only certain DNA sections. This makes it possible to determine bacteria in the original specimen directly and rapidly, i. e., without the necessity of culturing. There are very specific genetic probes for important waste-water microorganisms, e. g. for filamentous bacteria that produce bulking and floating sludge, for nitrating and denitrating bacteria or for those bacteria that contribute to the biological removal of phosphate.

These genetic probes are marked with fluorescence stains. When the probes are then added to a specimen and if they find their target bacteria, these will characteristically light up in the fluorescence image. This, however, requires a fluorescence microscope into which the H 600 BS can be transformed in two ways:

- Installation of an HBO incident-light fluorescence illuminator with mercury high-pressure lamp;
- Installation of an LED incident-light fluorescence illuminator.

While the first alternative can be equipped with a variety of fluorescence filter sets, it is quite expensive. The second alternative is less flexible, but significantly more economic.

Waste-Water investigation with the Hund H 600 BS

The Hund H 600 BS (Fig. 15) is a microscope equipped according to the demands of waste-water investigations. It is based on the known H 600 microscope stand which guarantees high stability during operation and observation. In addition, Hund offers a wide variety of accessories which allows to add further contrasting techniques to the microscope.

Also, the large stand base is designed as a hand rest so that the low-lying knobs for coarse and fine focusing drives ensure comfortable microscopic work with good ergonomics.

With a spring lever, the specimen is safely held in the specimen holder on the microscope stage. The stage can be moved into two dimensions through a coaxial drive. It is ball-bearing guided which guarantees smooth movements, even after years of use.

The stage has a size of 160 mm x 130 mm, and the traveling range is 76 mm x 52 mm, thus well-suited for standard microscope slides.

The halogen illumination (12 V/30 W) is integrated into the stand base. Its intensity can be adjusted continuously, and it is bright enough to allow the attachment of digital or microscope cameras.

The binocular observation tube with its viewing angle of 30° allows the ergonomically convenient observation of the microscopic image with both eyes. Eyepieces with magnifications of 10x or 12.5x can be inserted. There are also special eyepieces for spectacle wearers. Different acuities can be corrected for with the diopter adjustments on both eyepiece tubes. Rubber eyecups on the eyepieces block disturbing stray light.

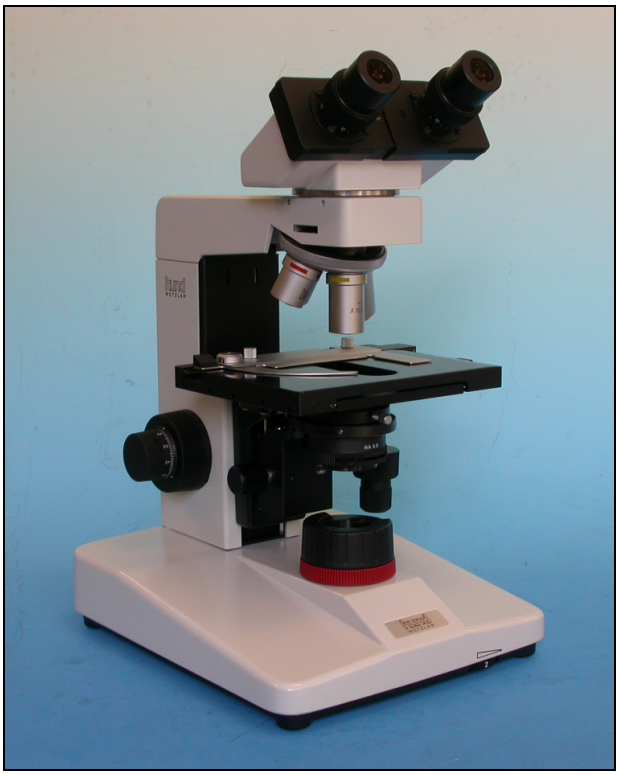

Fig. 15: The H 600 BS for waste-water investigations.

The nosepiece can receive four objectives and is inclined backwards so that the view onto the specimen is unobstructed. The ball bearing guarantees a long lifetime and constant quality of the rotary motion. For the microscopic investigation of waste water, we recommend objectives with magnifications of 10x, 20x and 40x. Only for the observation of filamentous organisms, an objective 100x with oil immersion is necessary.

The objectives can be chosen both as brightfield and as phase-contrast objectives. Phase-contrast, but also darkfield observations yield high-contrast images of almost transparent microorganisms and, thus, allow their simple identification.

The user can switch between the contrasting techniques simply by rotating the disc of the combination condenser (Fig. 16). Additional adjustments of the phase-contrast components are not necessary.

Fig. 16: Combination condenser with rotating disc (arrow).

Image Documentation

For documentation purposes, e. g. as a record for a certain situation in the sewage purification plant, the H 600 BS can be equipped with a trinocular tube. The microscopic image can then be oserved either with a camera or visually (switchable 100/100 tube), or in both ports simultaneously (30/70 tube with fixed light distribution). This offers the possibility to attach different camera types:

- CMOS or CCD cameras with C-Mount;
- digital single-lens reflex camera bodies.

For both alternatives, dedicated adapters are available. C-Mount cameras are usually operated via the USB interface of a PC and come with a software for storing and evaluating the resulting images.

With these camera options, the live images of the microscope can be displayed, e. g. for visitor groups. Digital single-lens reflex cameras usually store the image files on memory cards, but more and more often, they come with software for remote release and image archiving. Observing the live image via the camera's LCD monitor is not very convenient because with the camera attached to the microscope, the monitor points upwards. However, camera manufacturers also offer cables to connect TV monitors (depending on the functional range of the camera).

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