Microfauna of Activated Sludge
Helmut Berger

INTRODUCTION

Activated sludge reactors are the most common type of sewage treatment plants. The purification of the waste water follows the same biological processes as self-purification in natural waters. The activated sludge treatment is a truly aquatic process in which the sewage and the organisms are aerated together in tanks for several hours. The organisms form flocculent growths, that is, activated sludge, which may then be easily separated from the fluid phase in settling tanks. Bacteria account for about 96% of the total biomass (predominantly in the form of sludge flocs) in the aeration tank where the organic wastes are mainly degraded. The flocs are irregularly formed masses, usually 50–300 μm in size, and composed of inorganic and organic material. They consist of (1) carbonates and phosphates, iron and aluminium hydroxide and organic substance including fibrous material and starch grains and (2) the biochemically active agglutinated bacteria embedded in a gelatinous matrix. In the interstices of the flocs are suspended bacteria (mainly intestinal bacteria such as Escherichia coli) that form the major food source for the eukaryotic activated sludge organisms, including flagellates, naked and testate amoebae, ciliates, rotifers and nematodes (Bernerth 1978; Curds 1992; Ettl 2000; Foissner 1991).

Many of the protozoa found in polluted rivers also occur in sewage treatment plants. However, the diversity is usually much lower in a given sample of sludge, there being hardly more than 15 protozoan and three metazoan species. The activated sludge microfauna can be used as an indicator of plant performance and sludge quality.

The species composition and the dominance depend on the type of plant (Table 1). High-rate plants usually degrade only carbon compounds and are characterized by a low sludge age of less than four days (the average floc age of the sludge in the system) together with a high sludge loading, that is, a high concentration of easily degradable material. This process is often used for the partial treatment of used water with the objective of reducing the loading of pollutants. By contrast, conventional plants have a low sludge loading and a high sludge age (10–30 days). Thus, they harbour not only organisms with a short generation time (bacteria, flagellates), but also taxa with a longer generation time, such as ciliates, testate amoebae, rotifers, nematodes and sometimes even small oligochaete worms. Conventional plants not only degrade carbon compounds, but also reduce the concentration of ammonia (nitrification), limit the oxidized nitrogen (denitrification), and – in some cases – even remove phosphorus (for reviews see Hawkes 1983; Stier et al. 2003). The degradation and mineralisation of the pollutants is mainly done by the floc bacteria. The protozoans and, to a smaller extent the micrometazoans, have three major functions in the activated sludge process (Curds 1992; Foissner 1991): (1) Various protozoan species, for example the ciliates Aspidisca and Chilodonella, graze on the surface of
the flocs which then become compact and easier to separate from the purified water. This is important because a high abundance of filamentous bacteria causes the phenomenon known as “bulking”, in which the sludge becomes difficult to settle and as a result may be discharged in the effluent. (2) Hundreds of species of intestinal bacteria, including pathogens which are not incorporated in flocs would make even purified waste water turbid. Usually, however, these dispersed bacteria are ingested by filter feeding protozoa, for example, Vorticella and Epistyli. They make the effluent clear! (3) The grazing impact of the microfauna increases the turnover of the system by stimulation of bacterial growth.

Table 1. Comparison of some biological features of high-rate waste water treatment plants and conventional activated sludge plants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>high-rate plants</th>
<th>conventional plants</th>
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<tbody>
<tr>
<td>Dominant bacteria</td>
<td>non-flocculating</td>
<td>flocculating</td>
</tr>
<tr>
<td>Dominant protozoa</td>
<td>flagellates</td>
<td>ciliates</td>
</tr>
<tr>
<td>Rotifers</td>
<td>usually lacking</td>
<td>few to many</td>
</tr>
<tr>
<td>Nematodes</td>
<td>usually lacking</td>
<td>few</td>
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PRACTICAL TECHNIQUES

Since activated sludge always contains pathogens, safety has to be a primary concern!

Activated sludge plants are now present in most larger villages. Contact the operator before sampling. Use fresh activated sludge, which is taken from the aeration tank with a plastic beaker on a long handle. Put about 300 ml sludge into a 500 ml wide-necked, screw-capped plastic bottle and take it to the laboratory under cool conditions.

For investigation, shake the bottle, take a drop with an ordinary pipette, put it on a microscope slide, and cover the preparation with a coverslip. If three replicates are investigated, reliable data on the species present are obtained. Put parts of the sample into Petri dishes or aerate the sample to avoid fouling when the sludge is kept for a longer period.

In practice, a semiquantitative investigation with a rating scale will be sufficient. However, quantitative investigation is also possible and easily performed with the method by Aescht & Foissner (1992). Sludge quality can be assessed with the sludge biotic index (SBI) of Madoni (1994) or the method by Grossmann et al. (1999).

COMMON ORGANISMS IN ACTIVATED SLUDGE

Almost 300 protozoan species have been recorded from activated sludge plants (Curds 1992; Ganner et al. 2002). All species are freshwater inhabitants usually occurring in moderately (betamesosaprobic) to strongly (polysaprobic) polluted rivers. Thus, most protozoan taxa can be identified with the guides given in the bibliography of this chapter. Activated sludge bacteria can be identified with the guide of Eikelboom & Buijsen (1999). The metazoan fauna of the activated sludge usually consists
of rotifers and nematodes and only rarely tardigrads (*Thulinia*) and annelids such as *Aelosoma*.

**Heterotrophic Flagellates**

Heterotrophic flagellates ("zooflagellates", "Zoomastigophora") do not form a phylogenetically coherent group. Thus they are defined operationally as free-living protists moving and/or feeding by the use of flagella and feeding exclusively by heterotrophic means or, if with plastids, then also capable of ingesting particles (mixotrophy). Heterotrophic flagellates occur in all activated sludge samples, but a high abundance indicates poor plant performance.

**Hexamita** spp. O. Diplomonadida, Fam. Hexamitidae (Fig. 1, 2, 22, 23)

*Hexamita* species are 10–13 × 8–10 μm (*H. pusilla*) to 24–35 × 14–18 μm (*H. crassa*) in size. The body is soft, ellipsoidal, and of twofold rotational symmetry, that is, all organelles (nuclei, flagellar system, cytostome) are doubled. The nuclei are in the anterior part of the cell between the two flagellar (mastigont) systems. One or two, rarely more contractile vacuoles circulate in the cytoplasm which often contains small, highly refractive inclusions. Eight long flagella are arranged in two subapical bundles. The cytostomes are about in mid-body. The movement of *Hexamita* species is creeping, rotating, or shooting like a flash. Species of *Hexamita* and of other genera of the Diplomonadida (*Trepomonas*, Fig. 3, 4, 24, 25; *Trigonomonas*, Fig. 5) lack mitochondria and dictyosomes, and thus have an anaerobic metabolism, that is, they normally occur at sites with very little or no oxygen. They therefore indicate insufficient aeration and/or high loading of the activated sludge.

**Bodo saltans** O. Kinetoplastea, Fam. Bodonidae (Fig. 26, 27)

*Bodo saltans* is a bean-shaped, slightly flexible organism with a usual body length of 5–9 μm. The nucleus is slightly behind the mid-body, the contractile vacuole subapical. Behind the flagellar pocket is the so-called kinetoplast, that is a widened, DNA-rich part of the single, tubular mitochondrion. The two flagella emerge from the anterior of the body; the anterior (swimming) flagellum is of about body length, while the posterior (trailing) flagellum is two to three times as long. The subapical oral apparatus is a tiny gap recognizable in the microscope at a magnification of × 1000. *Bodo saltans* is easily recognizable by its dancing-springing movement while it is attached to the substrate by the trailing flagellum. *Bodo saltans* feeds on bacteria and is therefore common in activated sludge, where it is usually attached to the flocs (Fig. 27). However, it is often rare in fresh sewage and highly loaded plants.

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**Fig. 1 – 11.** Some common protozoa in activated sludge. 1: *Hexamita fusiformis*, a diplomonadid flagellate. 2: *Hexamita pusilla*. 3, 4: *Trepomonas agilis communis*. Broad side (3) and narrow side view (4) showing the twofold rotational symmetry. 5: *Trigonomonas compressa*. *Trigonomonas* has only 3 flagella per bundle. 6: *Hartmannella* sp., a naked amoeba. 7: *Amoeba proteus*. The cytoplasm is usually dark due to many refractive inclusions. 8: *Euglypha rotunda*, a testate amoeba. 9: *Euglypha* sp., a testate amoeba with spiny test. 10, 11: Morphology of the peritrich ciliate *Orculata asynmettica*. 10: Small colony with two extended and one contracted specimens. 11: Ciliate and nuclear apparatus of a swarmer (50 μm) after protargol impregnation.
Hexamita fusiformis
22 – 27 μm

Hexamita pusilla
10 – 13 μm

Trepomonas agilis
communis
13 – 25 μm (3, 4)

Trigonomonas compressa
24 – 33 μm

Hartmannella sp.
30 μm

Amoeba proteus
300 – 600 μm

Euglypha rotunda
40 μm

Euglypha sp.

Small colony
length of extended specimens 28 – 75 μm

Ciliature and nuclear apparatus of a swarmer

Opercularia asymmetrica (10, 11)
Naked Amoebae

Naked amoebae ("Gymnamoebae") lack a test. They live in all aquatic and terrestrial habitats including activated sludge. Gymnamoebae are bacteriovores or predators of other protists and small metazoans such as rotifers. An increased abundance of small naked amoebas indicates low performance of the plant due to very high loading, not easily degradable wastes, or technical problems. Common genera in activated sludge are Amoeba, Hartmannella, Naegleria and Vahlkampfia. Their identification is rather difficult and representations of only two genera are figured (Fig. 6, 7).

Testate Amoebae

Like heterotrophic flagellates, the testate amoebae ("Testacea") do not form a monophyletic group. In activated sludge, moderate or high abundances of testate amoebae indicate a high sludge age of 10 days or more, stable plant conditions, and nitrification (oxidation of ammonia via nitrite to nitrate). Usually, only one or two testaceous species occur in an activated sludge sample, but they may be very abundant. Common genera are Arcella, Diffugia, Euglypha, Frenzelina, Centropyxis, and Pyxidicula. As an example, Euglypha is briefly described. Further genera are contained in the chapter Testate Amoebae in Mosses and Forest Soils, p. 97 – 110.

Euglypha spp. SCl. Testaceafilosia, O. Euglyphida, Fam. Euglyphidae (Fig. 8, 9, 28)

Euglypha species have a 20–50 μm (E. rotunda) to 135–170 μm (E. aspera) long, ovoid test built of overlapping, endogenous siliceous plates that, in some species, bear spines. The aperture of the test (pseudostome) is surrounded by denticulate "mouth plates". The pseudopodia are filiform. The nucleus and the contractile vacuoles are in the rear portion of the cell. The cytoplasm is usually colourless, and movement is an amoeboid gliding, often with erect test. Euglyphids feed mainly on bacteria, yeast, and algae.

Ciliates

Ciliates (Ciliophora) are usually the most striking protozoa in activated sludge. Important groups are the peritrichs, the hypotrichs, and the cytophorpholds.

Opercularia asymmetrica SCl. Peritrichia, O. Sessilida, Fam. Operculariidae (Fig. 10, 11, 32)

Opercularia asymmetrica is 28–75 × 13–40 μm in size (without stalk) and spindle-shaped with the ventral side slightly shorter than the dorsal side (therefore the name

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**Fig. 12 – 21.** Some common protozoa and micro-metazoan in activated sludge. 12: Vorticella convallaria, a peritrich ciliate. 13: Ventral view of Aspidisca cicada, a hypotrich ciliate. 14 – 16: Cross sections (above) and dorsal views (below) of Aspidisca turrita (14; with spines), Aspidisca cicada (15; with ridges), and Aspidisca lyncus (16; smooth). 17: Euplotes affinis. 18, 19: Morphology of the cytophoral ciliate Chilodonella uncincta. 18: Ventral view in vivo. 19: Ciliature of ventral side after protargol impregnation. 20: The rotifer Philodina. 21: A nematode.
**Vorticella convallaria**  
60 – 80 μm

**Aspidisca cicada**  
30 μm

**Euplotes affinis**  
40 – 70 μm

**Chilodonella uncinata**  
Ventral view in vivo  
40 μm (18, 19)

**The rotifer Philodina**  
400 μm

**A nematode**  
100 – 1000 μm

Comparative morphology of *Aspidisca* species
asymmetrica). Contracted cells are globular. The cells (zooids) are frequently without a stalk but, if one is present, it is thin (about 2 μm in diameter), short and usually unbranched. Thus the zooids are often solitary and only rarely do colonies composed of between 2 and 14 specimens occur. The peristomial disc is narrow and usually projects slightly beyond the thin peristomial collar. The vestibulum is comparatively large and extends vertically to mid-body. The oral ciliature and the silverline pattern are clearly recognizable only after silver impregnation. The macronucleus is bean-shaped and accompanied by a 3–4 μm micronucleus. The contractile vacuole is in the anterior third of the cell on the dorsal vestibular wall. The cell surface (pellicle) is very narrowly transversely striated. Opercularia asymmetrica is, like all peritrichs, a filter feeder devouring mainly suspended bacteria (2000–3000 per hour). Thus, high numbers of peritrichs distinctly reduce the number of free bacteria and the turbidity of the purified waste water.

Vorticella convallaria group SCI. Peritrichia, O. Sessilida, Fam. Vorticellidae (Fig. 12, 33)

Extended cells are usually 70 × 40 μm in size (without stalk) and bell-shaped when extended. They are globular when contracted. The peristomial collar projects slightly beyond the body proper. The peristomial disc is slightly convex and raised, while the vestibulum is moderately large and extends obliquely to the centre of the body in feeding specimens. The oral ciliature and the silverline pattern are clearly defined only after silver impregnation. The cytoplasm is colourless to distinctly yellowish (Vorticella citrina). The macronucleus is J-shaped and the contractile vacuole lies on the ventral wall of the vestibulum. The cell surface (pellicle) is very narrowly transversely striated. The stalk is unbranched, 4–7 μm across, 50–800 μm (usually about 150 μm) long, and contracts helically due to a distinct muscle (myoneme). Vorticella convallaria is attached to sludge flocs; when abundant, it may form pseudocolonies. The species is common in healthy activated sludge where up to 18,000 specimens/ml occur; in stabilization ponds up to 19,200 individuals/ml have been counted. It is very likely that V. convallaria indicates increased nitrification. It ingests up to 14,000 bacteria per day, and thus contributes significantly to the reduction of floating bacteria.

Aspidisca spp. Cl. Spirotrichea, O. Euplotida, Fam. Aspidiscidae (Fig. 13 – 16, 29 – 31)

The size of both Aspidisca cicada and Aspidisa turrita is 25–40 × 20–40 μm, while Aspidisca lyneus is slightly larger (35–50 × 30–45 μm). The body is rigid and shows

Fig. 22 – 32. Light (22–27, 32) and scanning electron (28–31) micrographs of some activated sludge protozoa. 22, 23: Hexamita species, a diplomonad flagellate (length about 20 μm). 24: Specimens of Trepomonas sp. among bacterial flocs (length about 15 μm). 25: A Trepomonas species (length about 15 μm) with conspicuous, concave buccal areas. 26, 27: Bodo saltans (length about 7 μm), a common heterotrophic flagellate in activated sludge, has a swimming and a trailing flagellum. Many specimens are often seen attached to a sludge floc. 28: Test of Euglypha rotunda (length 40 μm). Note the serrated mouth platelets. 29, 30, 31: The dorsal side of the hypotrich ciliate Aspidisca. A. cicada has ridges (29), while that of A. turrita has a conspicuous horn (30); by contrast, the dorsal side of A. lyneus (31) is smooth. 32: Three solitary specimens of the peritrich ciliate Opercularia asymmetrica attached to a bacteria floc.
22 Hexamita sp.
23 Hexamita sp.
24 Trepomonas sp.
25 Trepomonas sp.
26 Trepomonas sp.
27 Bodo saltans, many specimens attached to a sludge floc
28 Euglypha rotunda
29 Aspidisca cicada
30 Aspidisca turrita
31 Aspidisca lyneceus
32 Opercularia asymmetrica attached to a sludge floc
a roundish-triangular outline, that is, the right cell margin is convex, the left almost straight, the anterior is rather broadly rounded, and the rear transversely truncated. The ventral side is plane, the dorsal slightly to distinctly vaulted in A. lynceus. By contrast, A. cicada has 6–8 distinct dorsal ridges, and A. turrita has a dorsal horn. The macronucleus is horseshoe-shaped, the contractile vacuole is in the right posterior part of the body. The ciliature of Aspidisca is very conspicuous being composed of thick cirri arranged as shown in figure 13. By contrast, the dorsal side bears 5–6 longitudinal rows of very short bristles. The oral apparatus is bipartite: anteriorly are three small frontal membranelles; in the left posterior part of the body is a bowl-shaped buccal cavity with about 11 adoral membranelles.

Aspidisca species belong to the most common and often most abundant ciliates (up to 40,000 specimens/ml) in activated sludge. They indicate low to medium sludge loading and sufficient oxygen supply. Like the cyrtophorids (see below), Aspidisca species graze on the sludge flocs making them compact.

Euplotes spp. Cl. Spirotrichea, O. Euplotida, Fam. Euplotidae (Fig. 17, 34, 35)

The size of the common Euplotes species ranges from 40–70 × 25–45 μm (E. affinis) to 140–230 × 95–35 μm (E. eurystomus). The body is rigid and has an elliptical outline. The ventral side is roughly plane, while the dorsal side is vaulted and more or less distinctly longitudinally ribbed. The macronucleus is C- to 3-shaped and the contractile vacuole is in the right posterior part of the body. The ciliature of Euplotes spp. is conspicuous and consists of nine (Euplotes affinis, E. aediculatus, E. eurystomus) or 10 (E. moebiusi) thick frontoventral cirri and five distinct transverse cirri and 3–4 caudal cirri near the posterior end of the cell. On the dorsal side are 6–11 longitudinal rows of very short bristles. The oral apparatus is huge: it commences at the anterior end and extends along much of the left body margin. Euplotes species are common inhabitants of sewage treatment plants, showing abundances of up to 10,000 individuals/ml. E. affinis (Fig. 17, 34) is characteristic of activated sludge plants with sludge stabilization, and indicates a good effluent quality. Like Aspidisca and cyrtophorids, Euplotes species are grazers, ingesting bacteria from the surface of the sludge flocs.

Fig. 33 – 38. Common ciliates in activated sludge. 33: Vorticella convallaria, a peritrichous ciliate attached to a bacterial floc, length 70 μm. 34, 35: Ventral view of the hypotrichous ciliate Euplotes affinis (34) and silverline pattern of Euplotes moebiusi (35). 36 – 38: The cyrtophorid ciliate Chilodonella uncinata (length about 40 μm) is a common inhabitant of activated sludge which grazes bacteria from the surface of the sludge flocs. 36: Ventral view showing, inter alia, the excretory pores of the contractile vacuoles and the non-ciliated field behind the mouth (oral basket). 37: Lateral view after protargol impregnation, showing the macronucleus and the oral basket whose rear end is conspicuously rolled up. 38: Ciliature and silverline pattern (fine meshes) of the ventral side after dry silver nitrate impregnation. (Fig. 1 - 5 from Pascher & Lemmermann 1914; Fig. 6 from Bovee 1985; Fig. 7 from Liebmann 1962; Fig. 20 from Bunke & Schmidt 1976, Philodina citrina (Rotatoria) – Film E 2332 IWF Göttingen; Fig. 21 from Schwab 1995; Fig. 32 from Aescht & Foissner 1992)
Vorticella convallaria

Euplotes affinis
Ventral view

Euplotes moebiusi
Silverline pattern

Ventral view in vivo

Lateral view, protargol impregnation

Ventral side, ciliature and silverline pattern

Chilodonella uncinata (36 – 38)
**Chilodonella uncinata** Cl. Phyllopharyngea, O. Cyrtophorida, Fam. Chilodonellidae (Fig. 18, 19, 36 – 38)

*Chilodonella uncinata* has a usual size of 45 × 30 μm and ellipsoidal outline with a small beak left anteriorly. The cell is flattened ventrally and more or less distinctly vaulted dorsally. The macronucleus is globular and usually near the rear end of the cell. *Chilodonella* has two contractile vacuoles, the anterior immediately behind the oral apparatus, the posterior subequatorially near the left margin of the ventral side. The ventral ciliature is composed of 10–12 longitudinal rows arranged as shown in figures 18, 19, 36 and 38, that is, in two groups that leave blank the postoral area. The oral opening is in the median of the anterior quarter; the oral basket, consisting of 9–11 strong rods extending dorsally, has its rear end rolled up. The preoral ciliary row is continuous (not fragmented as in *Pseudochilodonopsis* which also occurs in activated sludge) and extends from the oral opening to the anterior beak. The dorsal side is unciliated, except for a short, subapical row, the so-called dorsal brush. *Chilodonella uncinata* is common in freshwater habitats and in activated sludge where it grazes, like the euplotids, on bacteria adhering to the flocs which therefore become compact so that the settling of the sludge is improved. Up to 1200 specimens/ml have been observed but an oxygen content below 1 mg/l is hardly tolerated.

**Micro-Metazoa**

The abundance of metazoa is usually very low in activated sludge. Often only some rotifers (Fig. 20) and nematodes (Fig. 21) are present. They indicate – like testate amoebae – high sludge age. Occasionally one can find other metazoa, for example, *Aeolosoma hemprichi* which is easy to recognize by the red “oil-droplets” in the epidermis. This minute (~ 1 mm) oligochaete can reach up 180 individuals/ml and then drastically reduces activated sludge biomass. Metazoans can be eliminated from the system by increasing sludge loading and so reducing sludge age.

**BIBLIOGRAPHY**


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