Part I

Overview

1

Introduction

The microscope provides the wastewater treatment plant operator with a special tool for process control and troubleshooting of the activated sludge process. The microscope may be used on a routine or as-needed basis to determine the impact of various operational conditions on the biomass and the treatment efficiency of the activated sludge process. Sampling and frequency of microscopic examinations will be determined by manpower availability, severity of problems, and quantity and quality of industrial discharges. Frequencies from daily, weekly, and monthly to once every mean cell residence time (MCRT) have been used. However, during undesired operational conditions the microscope may be used more frequently to provide useful data with respect to the causative factors for the operational condition and progress of corrective measures.

Although the use of the microscope may be confusing at first and the amount of time spent on microscopic examinations considerable, the amount of time spent on microscopic examinations decreases greatly as microscopic techniques and the identification of organisms improve. Additionally, the operator need not be a microscopist to be able to use the microscope. Therefore, the use of the microscope may be incorporated as a standard analytical tool for process control and troubleshooting of the activated sludge process.

The microscope enables an operator to see the "bugs" or organisms in the treatment process. Each treatment process has its own profile of organisms when operating at a steady-state condition. By seeing the organisms the operator is able to correlate the organisms with existing operational conditions. These conditions may be acceptable or not acceptable. Therefore, the operator can "read" the organisms and determine whether operational conditions are acceptable or not acceptable, that is, use the organisms as indicators or "bioindicators."

Microscopic Examination of the Activated Sludge Process, by Michael H. Gerardi

The activated sludge process contains a large number and a large diversity of organisms. The major organisms by numbers and roles performed in the activated sludge process are the bacteria and protozoa (Figure 1.1). The minor organisms are the metazoa or multicellular, microscopic animals and macroscopic invertebrates (Figure 1.2). The metazoa commonly found in the activated sludge process include rotifers, free-living nematodes, water bears, and bristleworms. Additional organisms that are found in the activated sludge process include algae, fungi, immature insects, water fleas, and tubaflex.

Bacteria enter the activated sludge process through fecal waste and inflow and infiltration (I/I) as soil and water organisms and exist in the activated sludge process as dispersed and often motile cells, flocculated cells, and filamentous organisms. Dispersed cells include many young bacteria and the nitrifying bacteria *Nitrosomonas* and *Nitrobacter*. As bacteria age they lose the flagella that provide them with locomotion, and they produce a sticky polysaccharide coat that permits flocculation and floc particle development. *Escherichia* and *Zoogloea* are floc-forming bacteria, and quickly flocculate and initiate floc formation and development of floc particles. The dispersed cells that do not flocculate are (1) adsorbed to floc particles by compatible charge, (2) adsorbed to floc particles by change to compatible charge by the coating action of secretions from ciliated protozoa and metazoa, or (3) consumed or cropped by protozoa and metazoa.

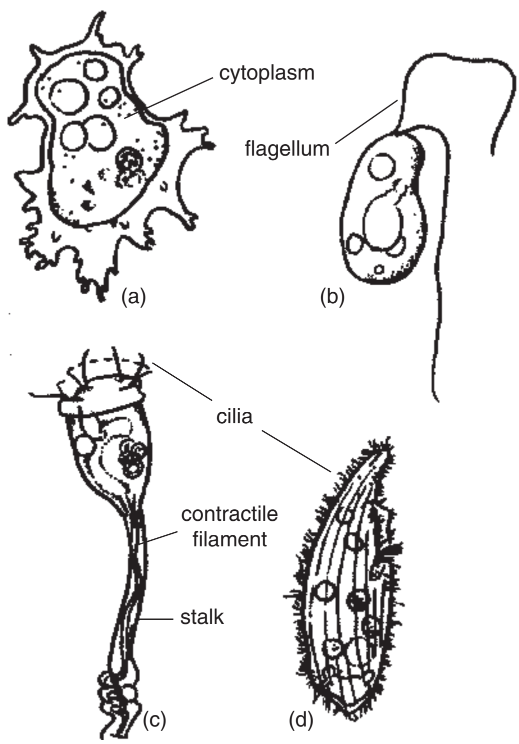

Figure 1.1 *Protozoa in the activated sludge process. There are three, commonly observed types of protozoa in the activated sludge process. These types include amoebae such as* Acanthamoeba (a), *flagellates such as* Bodo (b), *and ciliates such as* Vorticella (c) *and* Blepharisma (d).

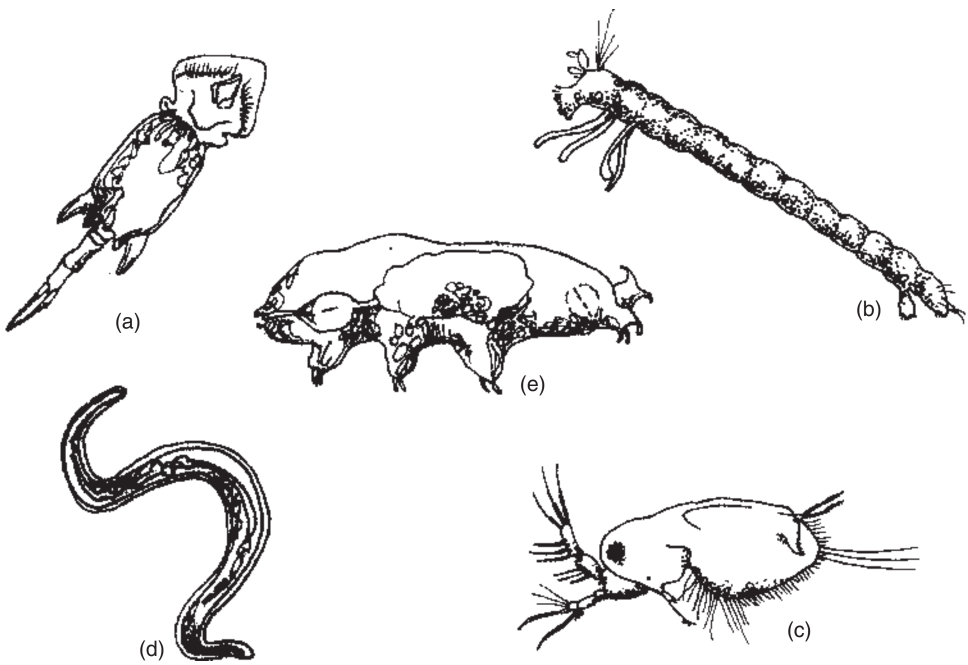

Figure 1.2 *Multicellular organisms in the activated sludge process. Several multicellular organisms commonly observed in the activated sludge process include the rotifer* (a), *the bloodworm* (b), *the water flea* (c), *the free-living nematode* (d), *and the water bear* (e).

Although they are single-celled organisms, some bacteria grow in chainlike fashion as trichomes or filaments. Large numbers of filamentous organisms enter activated sludge processes through three sources. They enter through I/I as soil and water organisms. They grow in the biofilm in sewer systems and enter through the sloughing action of the biofilm as wastewater flows over the biofilm, and they enter through the effluent of biologically, pretreated industrial wastewater. Examples of commonly occurring filamentous organisms in activated sludge processes include the Nocardioforms (Figure 1.3), *Microthrix parvicella* (Figure 1.4), and *Sphaerotilus natans* (Figure 1.5).

Bacteria are found in the activated sludge process in millions per milliliter of wastewater and billions per gram of solids. Bacteria are responsible for the degradation of organic wastes, nutrient (nitrogen and phosphorus) removal, floc formation, and removal of colloids, dispersed growth, particulate material, and heavy metals.

Protozoa are single-celled organisms and often are referred to as animallike or plantlike in composition. Most protozoa are free-living and enter the activated sludge process through inflow I/I as soil and water organisms. Depending on operational conditions protozoa are found in highly variable numbers from <100 per milliliter to tens of thousands per milliliter. Although wastewater protozoa have been placed in four, five, and six groups, wastewater protozoa most often are placed in five groups in the activated sludge process. These groups include the amoebae, flagellates, free-swimming ciliates, creeping or crawling ciliates, and stalked ciliates (Figure 1.6).

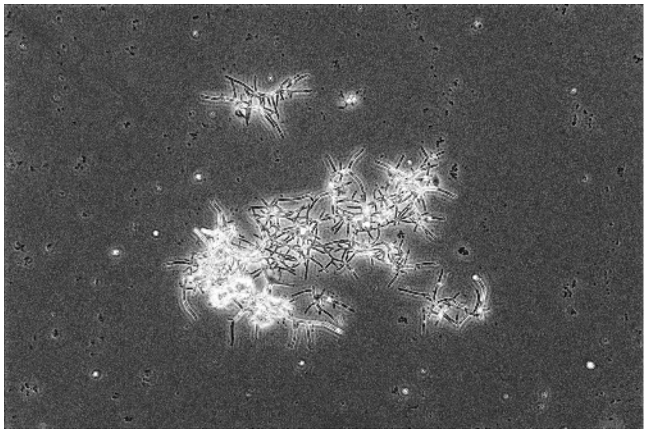

Figure 1.3 *Nocardioforms. Nocardioforms or Nocardia are short (<20μm), branched, Gram-positive (blue) foam-producing filamentous organisms. Branching is true in Nocardioforms. There is a continuity of growth of cellular material between the branches, that is, there is no "gap" between the branches. Also, the branches are not surrounded by a transparent sheath.*

Figure 1.4 Microthrix parvicella. Microthrix parvicella *is 100–400μm in length and is a non-branched, Gram-positive (blue) foam-producing filamentous organism. The Gram-positive color of the filamentous organism often appears as a "chain of blue beads."*

The gut content or cytoplasm of the amoebae is jellylike, and its cell membrane is very thin and flexible. The cytoplasm "flows" against the cell membrane and provides locomotion for the organism. The stretching and contracting motion of the amoebae is called a "false foot," or pseudopodia, mode of locomotion. This means of locomotion also enables the amoebae to trap particulate material and bacteria in order to obtain nourishment.

There are two types of amoebae, naked and testate. The naked amoebae such as *Amoeba proteus* (Figure 1.7) do not have a protective covering or testate. The testate amoebae such as *Difflugia* (Figure 1.8) do have a protective covering or testate. The testate consists of calcified material. It provides protection and allows the organism to drift in water currents. Amoebae move slowly through the wastewater and often

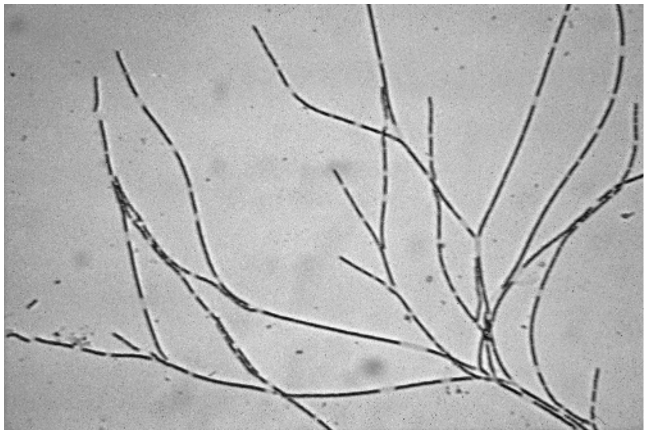

Figure 1.5 Sphaerotilus natans. Sphaerotilus natans *is a relatively long (>500 μm), branched, Gram-negative (red) filamentous organism. Branching is false in Sphaerotilus natans. The branches are surrounded by a transparent sheath and have a "gap" or no cellular material between the branches.*

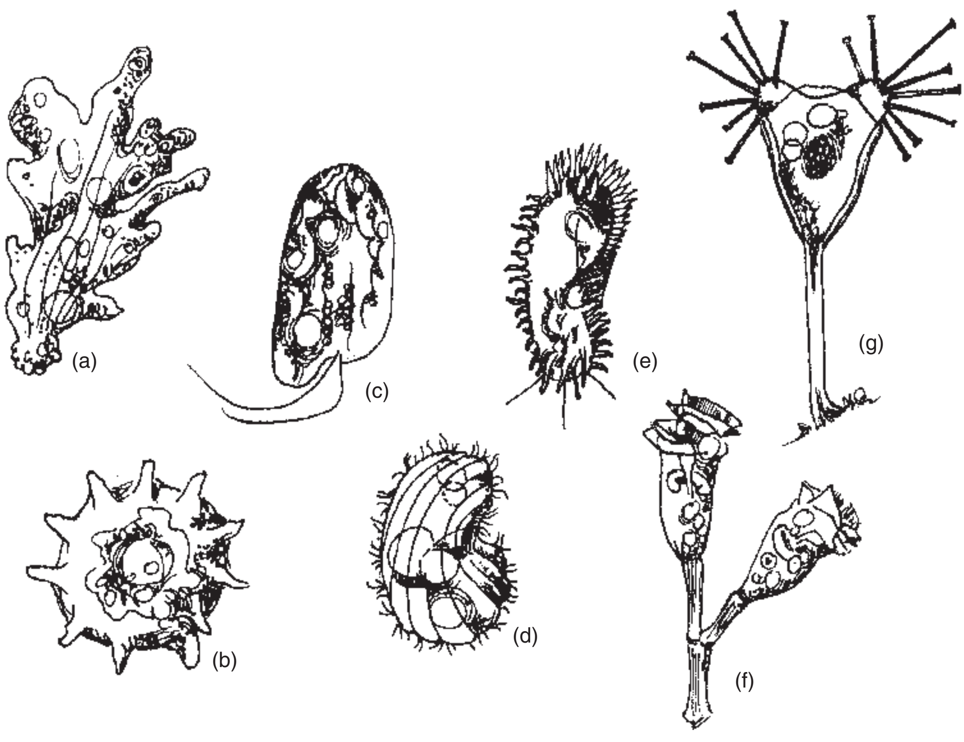

Figure 1.6 *Groups of protozoa. Representatives of the five basic groups of protozoa in the activated sludge process include the amoebae* Amoeba (a) *and* Arcella (b); *the flagellate* Cryptodifflugia (c); *the free-swimming ciliate* Colpoda (d); *the crawling ciliate* Styloncychia (e), *and the stalked ciliate* Epistylis (f). *Occasionally, a tentacled stalk ciliate such as* Acineta (g) *may be observed.*

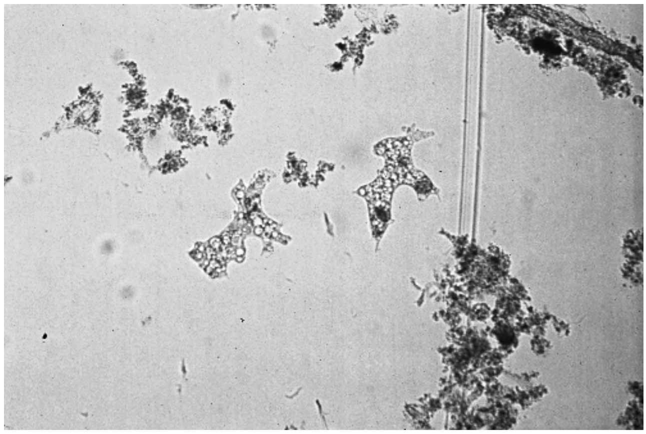

Figure 1.7 *Amoeba proteus. Naked amoebae such as* Amoeba proteus *often are difficult to observe because they move slowly and often have little contrast with their surrounding environment.*

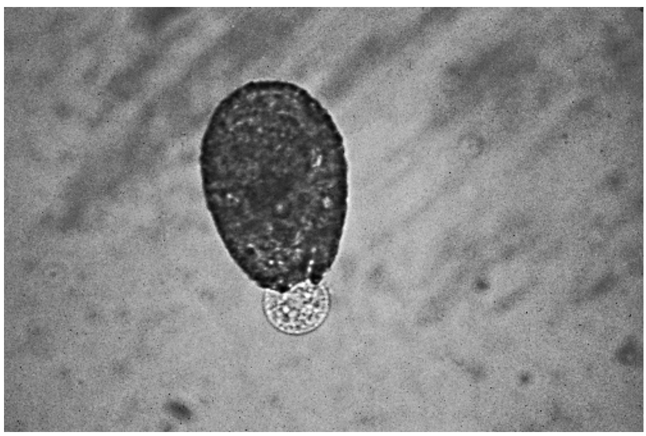

Figure 1.8 Difflugia. *Testate amoebae such as* Difflugia *move by the streaming of cytoplasm under the testate or drift in water currents.*

are overlooked during microscopic examinations of mixed liquor. Often, testate amoebae are misidentified as cysts, pollen grains, or other bodies.

Flagellates are oval-shaped and possess one or more whiplike structures, flagellum (singular) or flagella (plural). The flagella are located at the posterior portion of the organism, and their beating action provides locomotion. The anterior portion of the organism moves in the opposite direction of the beating action of the flagella. Therefore, flagellates have a quick, corkscrew pattern of locomotion.

There are two types of flagellates, plantlike and animallike. Plantlike flagellates such as *Euglena* (Figure 1.9) contain chloroplasts and are capable of photosynthesis. These flagellates also are known as motile algae. In the presence of excess phosphorus, pigmented flagellates may proliferate rapidly, resulting in population sizes >100,000/mL. Because pigmented flagellates are phototrophic, the swimming action toward the sunlight may cause bulking in secondary clarifiers when present in such

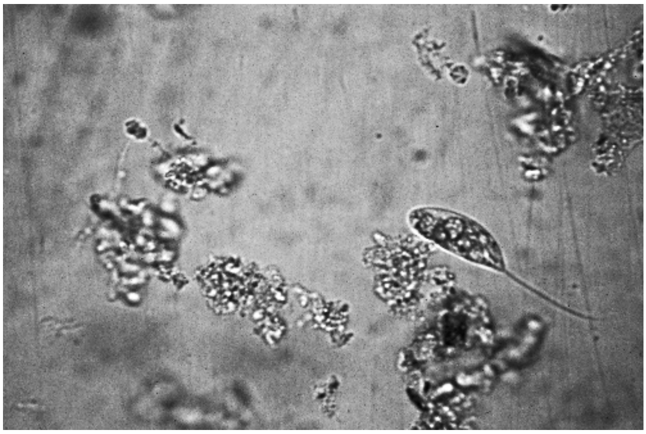

Figure 1.9 Euglena. Plantlike motile algae or pigmented flagellates such as Euglena are green because of the presence of photosynthetic pigments or chloroplasts. These organisms are phototrophic, that is, they swim toward sunlight. In addition to phototrophic ability, plantlike flagellates move by the whipping action of the flagella.

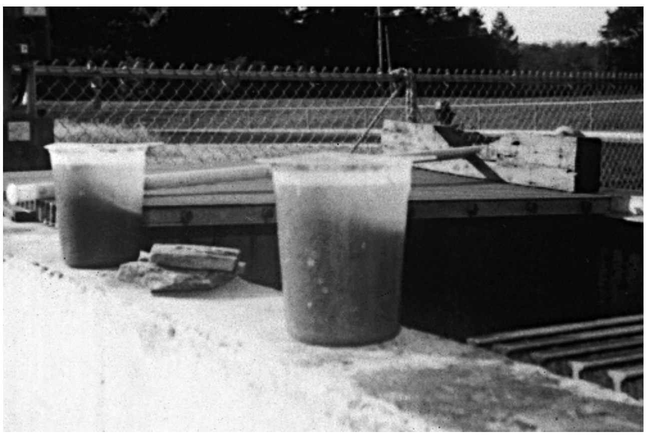

Figure 1.10 Swarming of pigmented flagellates. Relatively large numbers (>100,000 per mL) of pigmented flagellates or motile algae are capable of "pushing" secondary solids toward the sunlight in outdoor settleability tests and clarifiers.

large numbers (Figure 1.10). Animallike flagellates such as *Bodo* (Figure 1.11) do not contain chloroplasts.

Free-swimming ciliates swim freely in the bulk solution, that is, they do not attach to floc particles. Free-swimming ciliates such as *Paramecium* (Figure 1.12) and *Stentor* (Figure 1.13) possess numerous, short hairlike structures or cilia that are found in rows on the entire surface of the organism. The cilia beat in unison for locomotion and produce water currents for feeding purposes. The water current draws suspended or dispersed bacteria to the mouth opening on the bottom or ventral surface of the organism.

Crawling ciliates also possess cilia that are found in rows. However, the rows of cilia are located only on the ventral surface of the organism. Because of the reduced

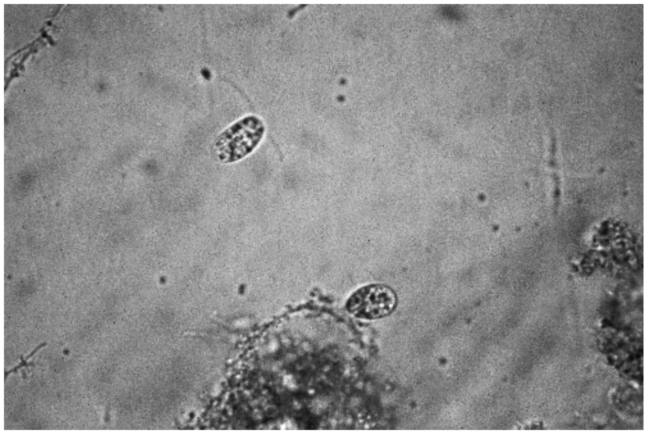

Figure 1.11 Bodo. *Animallike flagellates such as* Bodo *do not contain chloroplasts and are not phototrophic. Nonpigmented flagellates move exclusively by the whipping action of the flagella.*

Figure 1.12 Paramecium*. Free-swimming ciliates such as* Paramecium *have rows of short, hairlike structures or cilia over the entire surface of the body. The beating action of the cilia provides locomotion and draws bacteria into the mouth opening.*

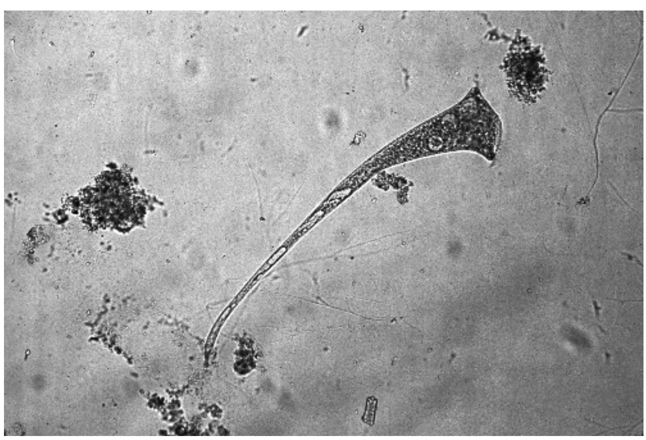

Figure 1.13 Stentor. Stentor *is a trumpet-shaped, free-swimming ciliate.*

number of cilia, crawling ciliates such as *Aspidisca* (Figure 1.14) and *Euplotes* (Figure 1.15) are poor swimmers and prefer to remain on the surface of floc particles. Some of the cilia at the anterior portion and/or posterior portion of the rows are modified to form "spikes," or cirri, that anchor the organism to the floc particles. Once anchored, the beating action of the cilia produces water currents that draw dispersed bacteria to the mouth opening on the ventral surface of the organism.

Stalked ciliates possess a circular row of cilia around the mouth opening. The cilia serve two purposes. First, they produce water currents to draw dispersed bacteria to the mouth opening. Second, they serve as a "propeller" that permits the organism to swim from a low dissolved oxygen concentration (≤0.5 mg/L) to a high dissolved oxygen concentration (Figure 1.16).

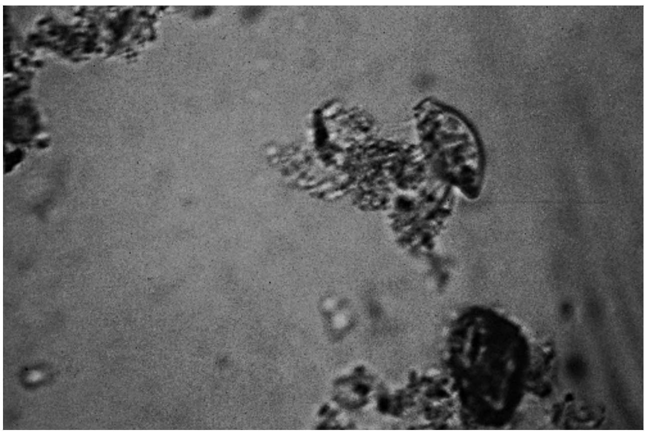

Figure 1.14 Aspidisca*. Crawling or creeping ciliates such as* Aspidisca *have rows of cilia only on the ventral surface of the body. The beating action of the cilia appears as numerous small "legs" as the protozoa "crawls" over the surface of a floc particle.*

Figure 1.15 Euplotes*. Under high-power magnification, the cilia of* Euplotes *can be seen in contact with the surface of a floc particle. Some of the cilia have been modified to form "spikes," or cirri, that help anchor the protozoa to the surface of the floc particle.*

Figure 1.16 *Free-swimming stalked ciliate. Under low dissolved oxygen concentrations (<0.5 mg/L), stalked ciliates such as* Vorticella *detach from floc particles and swim towards higher dissolved oxygen concentrations. The stalked ciliates use their stalk or "tail" as a rudder and their cilia around the mouth opening as a "propeller."*

Stalked ciliates typically are sessile or attached to the floc particles but do swim freely under a low dissolved oxygen concentration. Stalked ciliates may be solitary (individual) such as *Vorticella* (Figure 1.17) or colonial such as *Carchesium* (Figure 1.18). Some stalked ciliates such as *Vorticella* possess a contractile filament that permits "springing" action (Figure 1.19). The springing action provides for a water vortex that draws more bacteria to the mouth opening. Some stalked ciliates such as *Opercularia* (Figure 1.20) do not have a contractile filament and cannot spring.

The protozoa, especially the ciliated protozoa, perform several significant and beneficial roles in the activated sludge process. These roles include:

- The removal of dispersed bacteria through cropping action and coating action. Cropping action is the consumption of bacteria, while coating action is the covering of the bacterial cell with secretions that make the surface charge of the bacterial cell compatible for adsorption to floc particles.
- Improved settling of floc particles (solids) in secondary clarifiers by adding weight to the floc particles when the protozoa are crawling on or attached to the floc particles
- Recycling mineral nutrients, especially nitrogen and phosphorus, through their excretions or waste products

Metazoa are multicellular organisms that enter the activated sludge process through I/I as soil and water animals. They are strict aerobes and do not tolerate adverse operational conditions such as low dissolved oxygen concentration, high pollution, and toxicity. The most commonly observed metazoa in the activated sludge process are the rotifer (Figure 1.21) and the free-living nematode (Figure 1.22). Although present in relatively small numbers (several hundred per milliliter), they too perform several significant and beneficial roles. These roles include:

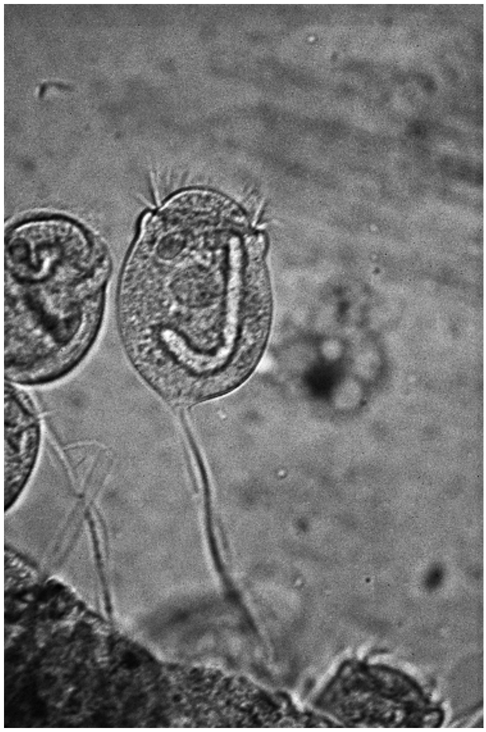

Figure 1.17 Vorticella. Vorticella *is a solitary protozoa.*

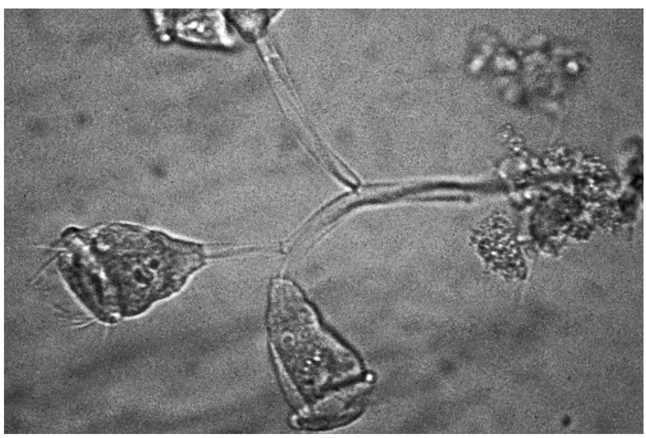

Figure 1.18 Carchesium. Carchesium *is a colonial protozoa.*

- The removal of dispersed bacteria through cropping action and coating action
- Improved settling of floc particles in secondary clarifiers by adding weight to the floc particles when they are crawling on floc particles or burrowing into floc particles

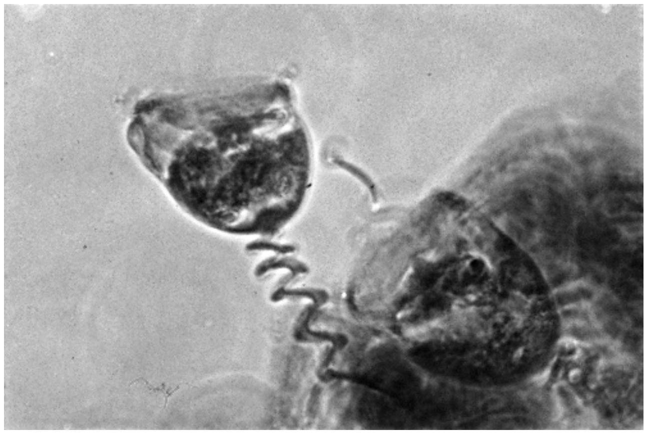

Figure 1.19 *Contractile filament. Some stalked ciliates such as* Vorticella *have a contractile filament or myoneme in the stalk that permits "springing" action. The springing action produces a water vortex that draws bacteria into the mouth opening.*

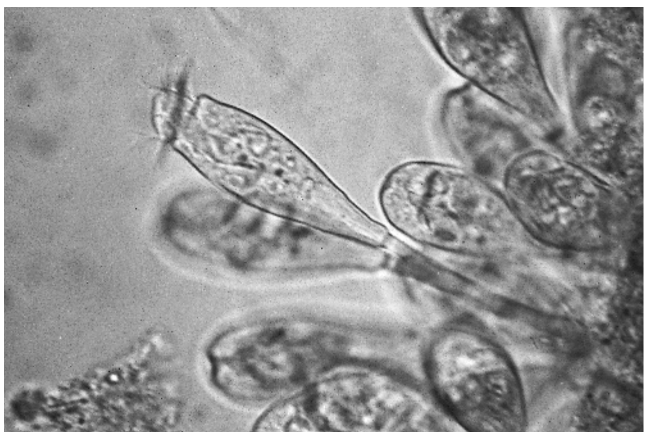

Figure 1.20 Opercularia*. Some stalked ciliates such as* Opercularia *do not have a contractile filament and are not capable of springing.*

- Recycling mineral nutrients, especially nitrogen and phosphorus, through their excretions or waste products
- The initiation of floc formation by secreting bundles of wastes that serve as attachment sites for bacteria
- Stimulating bacteria activity within floc particles by snipping away at floc particles or burrowing into floc particles. The snipping action and burrowing action permit free molecular oxygen (O_2), nitrate (NO_3^-), substrate or biochemical oxygen demand [(BOD)], and nutrients to penetrate to the core of the floc particles.

When the activated sludge process matures and operates successfully at a steady-state condition, the process has its own mixed liquor biota or "fingerprint" biota

Figure 1.21 *Rotifer. Rotifers such as* Philodina *are the most commonly observed metazoa in the activated sludge process.*

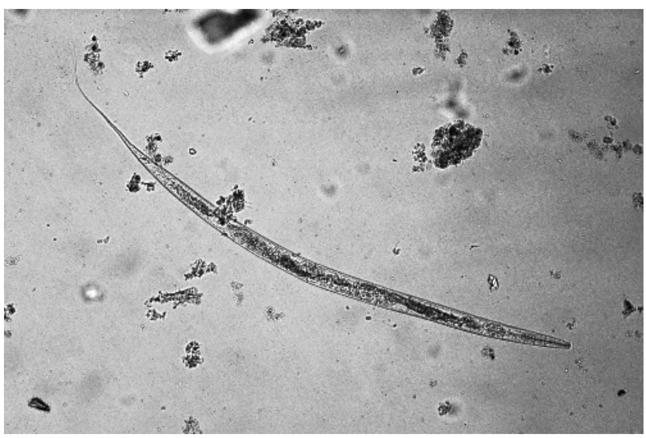

Figure 1.22 *Nematode. The free-living nematode is a commonly observed metazoa in the activated sludge and has mouthparts that are capable of biting into the floc particles.*

that reflects the steady-state environmental or operational condition of the process. This condition is noted in numerous parameters including (1) an acceptable range of values for the sludge volume index (SVI), (2) an acceptable range of values for the food-to-microorganism ratio (F/M), and (3) an acceptable range of values for the mean cell residence time (MCRT). At the steady-state condition the mixed liquor would consist of healthy life-forms that would be indicative of an acceptable mixed liquor effluent. For many activated sludge processes a mature and healthy mixed liquor biota under a steady-state condition (Figure 1.23) may contain the following:

- Little or insignificant dispersed growth in the bulk solution
- Little or insignificant particulate material in the bulk solution
- Mostly medium (150–500 µm) and large (>500 µm) floc particles
- Mostly irregular and golden-brown floc particles
- Mostly firm and dense floc particles as revealed through methylene blue staining
- Insignificant interfloc bridging and insignificant open floc formation
- Large and diverse population of ciliated protozoa

An undesired change in the mode of operation of the activated sludge process or an undesired change in industrial wastewater discharge can produce numerous changes in the mixed liquor biota that can be observed with microscopic examinations of the mixed liquor. Changes in the biota usually can be observed within 24–36 hours after the change in mode of operation or industrial discharge. Changes in the biota that can be observed include the following:

- Number of organisms
- Dominant and recessive groups of protozoa
- Activity and structure of protozoa and metazoa
- Quantity of dispersed growth and particulate material

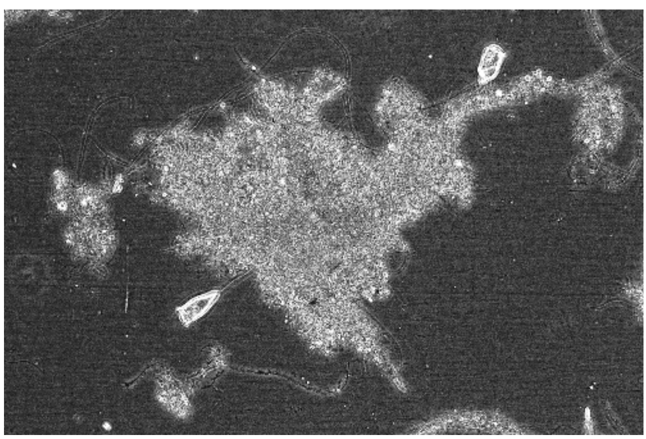

Figure 1.23 *Mature floc particle. In a healthy, steady-state condition a mature floc particle would be golden-brown in color, irregular in shape, and medium (150–500 µm) or large (>500 µm) in size. The floc particle would possess limited filamentous organisms growth. The bulk solution would contain little dispersed growth and little particulate material. Crawling ciliates and stalked ciliates may be found on the floc particle.*

TABLE 1.1 Reasons for Periodic Microscopic Examinations of Mixed Liquor

Correlate healthy and unhealthy biota to operational conditions
Correlate healthy and unhealthy biota to industrial discharges
Evaluate the impact of changes in the mode of operation
Evaluate the impact of industrial discharges
Identify factors responsible for loss of settleability
Identify factors responsible for loss of solids
Identify factors responsible for foam production
Identify appropriate process control measures
Monitor and regulate process control measures
Provide supportive data for industries and regulatory agencies

- Quantity of stored food in floc particles
- Strength and density of floc particles
- Dominant shape and size of floc particles
- Range in sizes of the floc particles

Periodic microscopic examinations of the mixed liquor can be used for many process control and troubleshooting purposes (Table 1.1). These purposes include in-plant and discharge monitoring and regulation. In-plant monitoring may simply be the use of target organisms or bioindicators to quickly determine deteriorating or improving conditions and appropriate process control measures. Discharge monitoring may be limited solely to the identification of problematic wastewaters.