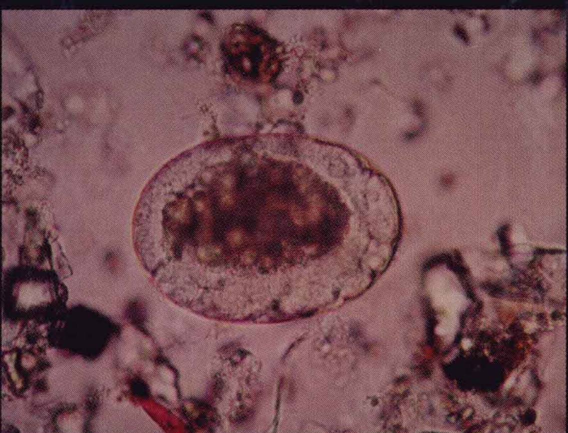
WASTEWATER ORGANISMS

A COLOR ATLAS






SHARON G. BERK
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Preface

The organisms of the treatment system are key players in an effective wastewater treatment process. Upon close inspection of the wastewater, a variety of organisms can be observed having different sizes, shapes, life cycles, and roles in the processes. This book is intended to serve as a guide to the organisms commonly found in wastewater treatment facilities. It should be of interest to plant operators and other personnel, as well as to those interested in an overview of the organisms. It should aid the novice in identifying organisms, usually to the genus level. For those interested in species-level identification, more detailed techniques are given in the literature cited.

Most of the samples examined in the preparation of this book were taken from treatment plants in Tennessee, except for the parasites, which were collected from all across the U.S. The parasite photographs were kindly contributed by Dr. M. D. Little of Tulane University. Although most of the samples were from Tennessee, organisms found there have been reported by others from a variety of sites in the U.S. and Europe. The authors have not, however, found all species reported by other investigators. Fungi, for example, were not observed often enough in the samples examined to photograph; yet they are occasionally found in certain types of treatment processes.

Living specimens rather than fixed or stained specimens were photographed using differential interference contrast or phase contrast microscopy, often combined with a flash system. Although differential interference contrast is not usually acquired for non-research labs, it can show more details of living cells than other optical methods. It is our intention to provide the reader with the best view of such details of living organisms. This information may be useful in interpreting the structures of organisms viewed under the more commonly used brightfield or phase contrast techniques. The three-dimensional appearance of objects viewed with differential interference contrast optics also helps the viewer obtain a better understanding of the form and dimensions of the organisms. Although we chose to photograph living organisms, the chapter on enumeration provides literature and techniques for fixing and staining, which are often required for identification to the species level.

Sharon G. Berk
John H. Gunderson

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Mr. Jim South, Cookeville Wastewater Treatment Plant, Cookeville, TN
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We thank Dr. V. Dean Adams, former Director of the Center for the Management, Utilization and Protection of Water Resources at Tennessee Technological University, for encouraging us to work on this book. We also thank Dr. Dennis George, Associate Director of the Center for the Management, Utilization, and Protection of Water Resources, for helping us with questions concerning treatment processes. We are especially grateful to Dr. Hollings Andrews of the Biology Department of Tennessee Technological University for identifying the algae for us. Finally, we are very indebted to Dr. M. Dale Little of the School of Public Health and Tropical Medicine of Tulane University for contributing nearly all the photographs of parasites from sludge.

Dedication

We dedicate this book to the memory of Mr. Mark Kirk, who was a plant operator at the Cookeville Wastewater Treatment Plant. Mark helped us get started on the project, and many of the photographs in the book are from samples that he helped us collect. He was a fine gentleman and colleague who took his work seriously, yet had a cheerful attitude. He enjoyed conversing with us about the organisms, and he was also an amateur photographer. We are grateful for his help, and we will miss him.

About the Authors

John Gunderson received a B.S. in zoology and Russian from the University of Nebraska and his Ph.D. in zoology from the University of California at Berkeley. He held a postdoctoral position at the National Jewish Center for Immunology and Respiratory Medicine in Denver, Colorado in the laboratory of Mitchell Sogin where he used ribosomal RNA sequences to study protistan phylogeny. He also worked for a year in the Center for Molecular Evolution at the Marine Biological Laboratory in Woods Hole, Massachusetts before becoming an adjunct assistant professor in the Center for Management, Utilization and Protection of Water Resources at Tennessee Technological University. His research interests are in the field of protistan evolution. He serves on the Board of Reviewers for the *Journal of Eukaryotic Microbiology* and is a member of the Awards Committee of the Society of Protozoologists.

Sharon Berk received a B.S. in biology from the University of Illinois, a M.S. in zoology from the University of Maryland, and a Ph.D. in microbiology from the University of Maryland. She spent five years in the Department of Environmental Sciences of the University of Virginia as a research assistant professor. In 1987 she joined the faculty of the Center for the Management, Utilization and Protection of Water Resources at Tennessee Technological University, where she is currently a professor of biology. Her research interests lie in various aspects of environmental microbiology and include studies of protozoan-bacterial trophic interactions, and the development of toxicity tests using protozoa. Her recent projects include interactions of protozoa and *Legionella*, the bacterium that causes Legionnaires' Disease, and the use of artificial wetlands for wastewater treatment. She serves on *ad hoc* review panels of the National Institutes of Health and on the Standard Methods Committee for development of *Standard Methods for the Examination of Water and Wastewater*. She has been a long-time member of the Society of Protozoologists and the American Society for Microbiology. Courses she has taught include pollution microbiology, marine microbiology, microbial ecology and microscopy.

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CHAPTER 1

Treatment Processes and Organisms

TREATMENT PROCESSES

Aerobic biological processes utilize microorganisms, primarily bacteria, to degrade colloidal or fine particulate matter and dissolved organics, resulting in carbon dioxide, water, and new cell biomass. Such processes produce flocculent settlable solids that can be removed in sedimentation tanks. Two of the most commonly used biological treatments include the activated sludge process and the trickling filter process.[1] The activated sludge process is most often used for large treatment systems. Most samples for the present study were collected from wastewater treatment plants utilizing activated sludge processes, and a few were from those using trickling filter systems.

In general, as sewage enters a wastewater treatment plant, it will initially be screened to remove coarse solids, then transferred to a grit chamber to remove sand and abrasive coarse material. From there, it is transferred to a primary settling tank to further remove other coarse solids. The portion of the contents of the primary settling tank containing fine particulates and dissolved organics is transferred to aeration tanks, which may be aerated by a variety of methods depending on the treatment plant. In the aeration tanks, much of the biological degradation takes place, and diverse communities of microorganisms thrive. The effluent from aeration tanks flows into another settling tank (or clarifier) to allow settling of flocculent settlable solids (activated sludge) produced during aeration. Some of the settled biological residuals from the clarifier are reintroduced into the aeration tank to ensure that an active microbial population is present. The process has become known as the activated sludge process because of the production of masses of active microorganisms produced and reintroduced during the treatment process.

Trickling filters consist of circular beds containing rocks or sheets of corrugated plastic over which the wastewater flows. Microorganisms attach to the surfaces of the rocks or plastic and degrade organic matter in the wastewater.

Residual solids (sludge) removed from primary sedimentation tanks can be digested anaerobically. It is placed in sealed tanks that may or may not be mechanically mixed. In a standard-rate digester, the contents are not usually heated or mixed, but in a high-rate process, the contents are heated and mixed. Heating will help destroy pathogens. Biological residuals from clarifiers are often digested aerobically, although it may be more costly than anaerobic digestion. For further details on these and other treatment processes, see Metcalf and Eddy, Inc.[1]

BACTERIA

The majority of bacteria present in activated sludge are heterotrophic, Gram-negative, motile rods.[2] Many are brought in with feces and may include human pathogenic bacteria. Their role in activated sludge is to oxidize organic waste to carbon dioxide and water and to produce flocculent settlable solids. Some may be chemolithotrophic and oxidize ammonia *(Nitrosomonas),* nitrite *(Nitrobacter),* and hydrogen sulfide *(Thiothrix).* Filamentous bacteria such as *Sphaerotilus* may be found to cause bulked sludges, i.e., biological solids with poor settling characteristics and compactability.[1] Such filamentous bacteria have been referred to as "sewage fungi".

Anaerobic bacteria are active in anaerobic sludge digesters. They may originate from fecal material, land drainage, stormwater, and biological industrial effluents.[3] The role of these bacteria is to degrade sludges, which usually contain proteins, fats, and polysaccharides, into end products such as methane (which can be captured and used as an energy source in the treatment plant), carbon dioxide, water, and new bacterial biomass. Anaerobes include obligate anaerobes (those unable to live in the presence of free oxygen), facultative anaerobes (those able to switch from aerobic to anaerobic metabolism), and micro-aerophilic forms (those growing best in low oxygen concentrations). Most of the sulfide produced from sewage is from bacteria that reduce sulfates, such as *Desulfovibrio desulfuricans.* Another example of an anaerobic genus is *Methanobacterium,* which is responsible for methane production.

Identification of bacteria, even to the genus level, is tedious, requiring many biochemical (metabolic) tests or genetic analysis. The field of bacterial taxonomy is in flux, particularly with the appearance and use of ribosomal RNA analyses. It is virtually impossible to identify bacteria simply by observation under the microscope, and an educated guess is all that can be made based on appearance of cells. Very few have unusual characteristic morphologies that set them apart from the others. *Zoogloea,* for example, forms clusters of finger-like projections that few others do. Only a small proportion of all species are filamentous or contain sulfur granules. Most are small rods or curved rods. The photographs in this book serve primarily to demonstrate the variety of morphological types and sizes of bacteria encountered in wastewater treatment systems. Included in this section are the cyanobacteria, often still called blue-green algae. On the basis of cell structure, presence of murein, 70S ribosomes, and other features, these organisms are prokaryotes.[4] Cyanobacteria such as *Oscillatoria* photosynthesize as green plants do, evolving oxygen. The cyanobacteria of wastewater treatment systems can often be found on clarifier walls and trickling filters where they are exposed to sunlight.

ALGAE

Algae are chlorophyll-bearing plants that are distinguished from other chlorophyllous plants by their modes of sexual reproduction.[5] Some algae are unicellular, while others may be multicellular. Some are suspended in the water column and are termed planktonic, while others may be attached to substrates such as rocks or as in the case of treatment facilities, clarifier walls or trickling filters.

Many species of algae are photoautotrophic, i.e., they get energy from light and all other nutrients from inorganic compounds such as carbon dioxide and ammonia. Some also require other growth factors such as vitamins and are termed photoauxotrophic. A few species are facultatively heterotrophic, which means they can switch to a mode of nutrition that utilizes organic compounds. Still a few others, in addition to photosynthesizing, may ingest solid particles.

In wastewater treatment systems, algae grow on the nutrients in the water and get energy from sunlight. Generally they are found in highest numbers on surfaces such as trickling filters or walls of clarifiers, where the biologically treated waters trickle over the top of the clarifiers. In such an environment are found many filamentous or branched forms attached to the substrate. Algae play a minor or insignificant role in wastewater treatment. They may even cause problems of clogging or eutrophic blooms after discharge of treated wastewater to receiving waters. However, they are some of the loveliest specimens to observe with a microscope.

PROTOZOA

Several groups of protozoa are represented in wastewater treatment plants. These include flagellates, ciliates, amoebae, and sporozoa. The sporozoa are parasites of humans and animals and therefore are included in a separate section on parasites. The taxonomic division of predominately unicellular eukaryotes into algae and protozoa, and the protozoa into flagellates, ciliates, amoebae, and sporozoa does not reflect their true evolutionary relationships. However, this traditional scheme is followed here, solely for convenience. It is the scheme that will be found in the references cited. In several texts on wastewater treatment, suctorians have been regarded as a separate class from ciliates. However, they are ciliates that lack cilia in the adult stage, but possess cilia in the larval stage.

Protozoa are abundant and diverse in activated sludge, where they feed primarily on bacteria and organic nutrients. Protozoa demonstrate a variety of trophic (feeding) modes. Some feed on bacteria, some on algae, some on other protozoa, and some feed on a mixture of food types such as algae and bacteria. They may originate from various components of sewage or from material of the conduits collecting sewage and runoff. Many species form cysts that can be airborne or picked up by birds and aquatic insects. Several anaerobic species have also been observed in Imhoff tanks and digesters and are thought to feed on sulfur bacteria.

The primary role of protozoa is to clarify the effluent through predation on bacteria. In addition, protozoa may cause flocculation, which is thought to result from secretion of a mucous-like substance from certain species.[6] However, protozoan-induced flocculation may not be significant in clarification of the effluent. Protozoa are capable of utilizing dissolved organic substrates; however, the extent to which this affects the effluent is unknown.[6] In addition to removal of bacteria, predation or grazing of bacteria by protozoa plays another role. Grazing on bacteria, especially on surfaces, has been shown to enhance bacterial community metabolism. By cropping bacteria, the protozoa remove senescent bacteria and maintain the population in a youthful physiological state increasing the rate of bacterial assimilation of organic matter.[7]

The structure of the protozoan community is believed to reflect the quality of the effluent. Ciliates, in particular, have been considered indicators of a good treatment process.[6] However, it should be remembered that incoming wastewater may derive from many different sources and therefore contain different nutrients or toxic compounds. Protozoa (and other organisms) may adapt to a particular chemical composition of wastewater, resulting in different community structures, depending on the composition of wastewater. In samples from various treatment plants examined for this book, the dominant species differed from plant to plant; yet no plant reported a poor quality effluent. More research on community structure and quality of treatment processes is necessary.

PARASITES

Parasites of humans and animals are different from many other sewage organisms, in the sense that they are not part of an active community of organisms interacting with the sewage and other organisms, but rather, stages that just exist in the sewage as resistant forms, usually as eggs or cysts. For these reasons, the parasitic protozoa and other parasitic invertebrates have been placed in a section of this book separate from the free-living protozoa and invertebrates.

Parasites enter the treatment system with feces of humans and animals. Although most are killed during sludge treatment, some such as ascarids can survive the harsh environment of the treatment process. Anaerobic digestion does, however, destroy more parasites than aerobic digestion of sludges. Some parasitic forms can even resist the concentrations of chlorine used as a disinfectant prior to release of a treated effluent.[8] In general, exposure of raw sewage to treatment processes shortens the survival time of the parasites. The parasites do not play a role in the treatment processes. More details concerning characteristics and life cycles of sewage parasites are provided in the photograph section of this book.

INVERTEBRATES

Two of the most common groups of invertebrates encountered by the authors were the rotifers and nematodes. Rotifers are multicellular invertebrates usually between 100 and 500 µm. Most are found in freshwater. The term "rotifer" (= "wheel-bearer") derives from the fact that most of the species have a ciliated anterior end (corona) that resembles a pair of rotating wheels due to the synchronous beating of the cilia. Natural populations are almost all females, and reproduction is parthenogenetic.[9] Males are unknown for many species, and in those species in which they occur, males are rare and short-lived. Nematodes are roundworms, usually less than 1 cm long. They are difficult to indentify and, as a group, are poorly known.[9] They move with a whip-like motion. Rotifers are most common in activated sludge, and nematodes are usually found in trickling filter material or in treatment plants with percolating filters. The roles of rotifers and nematodes are not clear. Both serve to consume and digest particulate matter and other smaller organisms. Annelids, with similar roles, are found among filamentous material.

Tardigrades (water bears) were encountered in only one treatment plant, and gastrotrichs were found less frequently than rotifers and nematodes. Fox et al.[8] found copepods and cladocerans in sewage of the Chicago area; however, these groups are not considered to be normal inhabitants of a wastewater system. These groups were not observed in any samples of the present study. More details on structure and habits are provided in the photograph section.

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CHAPTER 2

Observation and Enumeration of Organisms