

Problem Organisms in Water: Identification and Treatment

MANUAL OF WATER SUPPLY PRACTICES

M7



American Water Works
Association



Documents

Document Sharing Hub

Problem Organisms in Water: Identification and Treatment

AWWA MANUAL M7

Third Edition



**American Water Works
Association**

Science and Technology

AWWA unites the drinking water community by developing and distributing authoritative scientific and technological knowledge. Through its members, AWWA develops industry standards for products and processes that advance public health and safety. AWWA also provides quality improvement programs for water and wastewater utilities.

MANUAL OF WATER SUPPLY PRACTICES—M7, Third Edition

Problem Organisms in Water: Identification and Treatment

Copyright © 1966, 1995, 2004 American Water Works Association

All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information or retrieval system, except in the form of brief excerpts or quotations for review purposes, without the written permission of the publisher.

Project Manager/Copy Editor: Melissa Christensen
Production Editor: Carol Stearns

Library of Congress Cataloging-in-Publication Data

Problem organisms in water : identification and treatment.--3rd ed.

p. cm. -- (AWWA manual ; M7)

"Replaces AWWA manual M7, Animals associated with potable water supplies: operators' identification guide (1966) by William M. Ingram and Alfred F. Bartsch"--Foreword.
Includes bibliographical references and index.

ISBN 1-58321-292-2

1. Aquatic pests--Identification. 2. Aquatic pests--Control. I. Ingram, William Marcus, 1913- Animals associated with potable water supplies. II. American Water Works Association. III. Series.

TD491 .A49 no. M7 2003

[TD427.A68]

628.1 s--dc22

[628.1'61]

2003060084

Printed in the United States of America

American Water Works Association
6666 West Quincy Avenue
Denver, CO 80235-3098

ISBN 1-58321-292-2



Printed on recycled paper



Documents

Document Sharing Hub

Contents

List of Figures, v

List of Tables, ix

Foreword, xi

Acknowledgments, xiii

Introduction, xv

Chapter 1 Actinomycetes 1

Biology and Ecology, 1
Significance for Water Supplies, 4
Control Strategies, 5
References, 5

Chapter 2 Iron Bacteria 7

Biology and Ecology, 7
Significance for Water Supplies, 11
Control Strategies, 12
References, 15

Chapter 3 Sulfur Bacteria 17

Biology and Ecology, 17
Significance for Water Supplies, 20
Control Strategies, 21
References, 22

Chapter 4 Nitrifying Bacteria 23

Biology and Ecology, 23
Significance for Water Supplies, 25
Control Strategies, 26
References, 27

Chapter 5 Nematodes 29

Biology and Ecology, 29
Significance for Water Supplies, 32
Control Strategies, 32
References, 33

Chapter 6 Bloodworms or Midges (Chironomid Larvae) 35

Biology and Ecology, 35
Significance for Water Supplies, 37
Control Strategies, 38
References, 39

Chapter 7 Crustacea	41
Biology and Ecology, 41	
Significance for Water Supplies, 45	
Control Strategies, 45	
References, 46	
Chapter 8 Rotifers	47
Biology and Ecology, 47	
Significance for Water Supplies, 49	
Control Strategies, 49	
References, 50	
Chapter 9 Zebra Mussels	51
Biology and Ecology, 51	
Significance for Water Supplies, 53	
Control Strategies, 54	
References, 56	
Chapter 10 Algae	57
Biology and Ecology, 57	
Significance for Water Supplies, 57	
Algal Divisions, 59	
Control Strategies, 66	
References, 66	
Chapter 11 Protozoa	69
Biology and Ecology, 70	
Significance for Water Supplies, 76	
Control Strategies, 77	
References, 77	
Appendix A AWWA Survey on Nuisance Organisms, 79	
Appendix B Troubleshooting Guide for Problem Organisms, 87	
Appendix C Suggestions for Optimizing Conventional Water Treatment, 91	
Appendix D Actinomycete Culture Agars, 95	
Appendix E Iron Bacteria Presence/Absence and Quantity Methods, 99	
Appendix F Color Section, 101	
Abbreviations, 137	
Index, 139	
List of AWWA Manuals, 145	

Figures

- 1-1 Actinomycetes, phase contrast 1,000×, 2
- 1-2 Actinomycetes, dark-field illumination, 400×, 3
- 1-3 Bacterial colonies—typical colony versus actinomycete colony, 50×, 3
- 2-1 Single-celled iron bacterium *Siderocapsa treubii*, 8
- 2-2 Filaments of *Sphaerotilus natans*, 9
- 2-3 Laboratory culture of *Gallionella ferruginea*, 9
- 2-4 Stalk fragments of *Gallionella ferruginea* and iron–manganese precipitate, 10
- 2-5 Filamentous iron bacteria recovered from well water. DIC microscopy, 1,000×, 10
- 2-6 Partly filled impeller channel, 11
- 2-7 Largely clogged bowl volutes, 12
- 2-8 Column pipe with iron biofilm, 12
- 2-9 Clean versus clogged impellers, 13
- 3-1 Side view of a tubercle, 18
- 3-2 Cross section of a slime, 18
- 3-3 Cross section of a tubercle, 18
- 3-4 An SRB *Desulfovibrio desulfuricans*, 20
- 3-5 *Beggiatoa*, a colorless sulfur bacterium, 20
- 3-6 An operating water well being pumped, influence (suppressive) on SRB activity (theoretical), 21
- 3-7 Operational water well during period of quiescence (not pumping), causing the SRB activity to increase and the hydrogen sulfide to spread (theoretical), 22
- 4-1 Electron micrograph showing cross section of a nitrifying bacterium, 24
- 4-2 Water quality parameters during a 1985 nitrification episode at Garvey Reservoir, Los Angeles County, Calif., 26
- 5-1 Roundworms (phylum Nematoda), 30
- 5-2 Nematode in bright-field, 200×, 31
- 5-3 Nematode at 250×, 32
- 6-1 Two-winged flies (order Diptera), 36
- 6-2 Pupa of a chironomid (Insecta, Chironomidae), 20×, dorsal view, 37
- 6-3 Parthenogenetically formed eggs in abdomen of a chironomid pupa (Insecta, Chironomidae), ventral view, 70×, 39
- 7-1 Crustaceans, 42
- 7-2 Crustaceans, 43

- 7-3 Crustacean *Bosmina* with eggs, iodine stain, bright-field microscopy, 60×, 44
- 7-4 *Asellus aquaticus* (Crustacea, Isopoda), dorsal view, 5×, 45
- 7-5 The amphipod *Hyallela azteca* (1.0 cm) isolated from a point-of-use filter, 46
- 8-1 Rotifers (phylum Rotifera), 48
- 8-2 Rotifer, 200×, 49
- 9-1 Life cycle of the zebra mussel, 52
- 9-2 Adult zebra mussel, 53
- 9-3 Zebra mussels encrusted on structure, 53
- 9-4 Veliger larvae of the zebra mussel, showing the velum extended at lower right, 54
- 9-5 Impact of zebra mussels on screens, 54
- 10-1 Algae, Chrysophyte, *Dinobryon* (3 cells), and others, unstained bright-field, 240×, 62
- 10-2 Algae, Diatom, *Asterionella*, unstained bright-field microscopy, 120×, 63
- 11-1 *Arcella* (0.1 mm), 70
- 11-2 *Paramecium* (0.2 mm), 70
- 11-3 *Giardia* cysts and *Cryptosporidium* oocysts stained by immunofluorescent antibody, 4',6-diamidino-2-phenylindole (DAPI), and Nomarski differential interference contrast (DIC) microscopy, 72
- F-1 Iron bacteria. *Leptothrix* (note sheath), 1,000×, 102
- F-2 Iron bacteria. *Caulobacter* (large “colony”), 1,000×, 102
- F-3 Iron bacteria. *Thiobacillus ferro oxidans* (note precipitated iron), 1,000×, 103
- F-4 Iron bacteria. *Pseudomonas*, 1,000×, 103
- F-5 Sulfur bacteria. *Thiobacillus* (note precipitated sulfur), 1,000×, 104
- F-6 Sulfur bacteria. *Beggiatoa* (note sulfur granules), 1,000×, 104
- F-7 Sulfur bacteria. *Thiothrix* (note sulfur granules), 1,000×, 105
- F-8 Sulfur bacteria. *Thiopedia* (note sulfur granules), 1,000×, 105
- F-9 Nitrifying bacteria. *Nitrosomonas* (cocci), 1,000×, 106
- F-10 Nitrifying bacteria. *Nitrobacter* (rods), 1,000×, 106
- F-11 Nematode larva, 60×, 107
- F-12 Nematode, 250×, 107
- F-13 Chironomid. Head of a pupa, dorsal view, 1:7, 108
- F-14 Chironomid. Pupa (Insecta, Chironomidae), ventral view, 1:20, 108
- F-15 Chironomid. Pupa (Insecta, Chironomidae), ventral view, 1:70, 109
- F-16 *Daphnia* (2 mm), 110
- F-17 *Daphnia* sp. Water Flea (Crustacea), 110
- F-18 *Cyclops* (2 mm), 111

- F-19 *Cyclops* sp. Water Flea (Crustacea), 111
- F-20 *Asellus aquaticus* (5 mm), 112
- F-21 *Asellus aquaticus* (Crustacea, Isopoda), dorsal view, 1:5, 112
- F-22 *Asellus aquaticus* (Crustacea, Isopoda), lateral view, 1:20, 113
- F-23 *Asellus aquaticus* (Crustacea, Isopoda), head, dorsal view, 1:25, 113
- F-24 *Asellus aquaticus* (Crustacea, Isopoda), head, ventral view, 1:100, 114
- F-25 Faecal pellets of *Asellus aquaticus*, 1:4, 114
- F-26 Faecal pellets of *Asellus aquaticus* containing bitumen, 1:40, 115
- F-27 Faecal pellets of *Asellus aquaticus* containing iron-rust, 1:40, 115
- F-28 Rotifer. *Monostyla*, 60×, 116
- F-29 Rotifer. Gastrotrich (*Chaetonotus*), 200×, 116
- F-30 Protozoan. Ciliate, *Vorticella*, unstained, bright-field microscopy, 120×, 117
- F-31 Rotifers. Rotifer, 200×, 117
- F-32 Gastrotrich, 200×, 118
- F-33 Zebra mussels. Variations, 118
- F-34 Zebra mussel larvae showing some disintegration, 119
- F-35 Algae. Chlorophyte, *Scenedesmus*, unstained, bright-field microscopy, 120×, 119
- F-36 Algae. Chrysophyte, *Dinobryon* (2 cells) and others, unstained, bright-field microscopy, 240×, 120
- F-37 Algae. Diatom, *Asterionella*, unstained, bright-field microscopy, 120×, 120
- F-38 Algae. Diatom, *Fragilaria*, unstained, bright-field microscopy, 240×, 121
- F-39 Algae. Diatom, *Hannaea*, unstained, bright-field microscopy, 240×, 121
- F-40 Algae. Euglenophyte, *Phacus*, unstained, bright-field microscopy, 120×, 122
- F-41 Algae. *Anabaena Scherenietieri*, bright-field microscopy, 400×, 122
- F-42 Protozoa. Amoeba, 200×, 123
- F-43 Protozoa. Flagellates, 1,000×, 123
- F-44 Protozoa. Free ciliate, 200×, 124
- F-45 Protozoa. Stalked ciliate (stalks), 200×, 124
- F-46 Protozoa. *Giardia lamblia*, 240×, 125
- F-47 Protozoa. *Cryptosporidium*, 125
- F-48 *Cryptosporidium* oocyst exhibiting sporozoites, DIC microscopy, 1,000×, 126
- F-49 *Cryptosporidium* oocyst exhibiting internal structures known as sporozoites, phase contrast, 1,000×, 126
- F-50 *Giardia* cyst and *Cryptosporidium* oocyst, stained with fluorescent antibody stain, 1,000×, 127

- F-51 *Giardia* cyst exhibiting internal structures—nuclei, axonemes, median bodies, DIC microscopy, 1,000×, 127
- F-52 Taste-and-odor algae, 128
- F-53 Filter-clogging algae, 129
- F-54 Polluted-water algae, 130
- F-55 Clean-water algae, 131
- F-56 Plankton and surface algae, 132
- F-57 Reservoir algae, 133
- F-58 Wastewater-treatment-pond algae, 134
- F-59 Estuarine pollution algae, 135

Tables

- 11-1 Taxonomic listing of protozoa potentially associated with potable water, 73
- B-1 Troubleshooting guide for problem organisms, 88
- C-1 Suggestions for optimizing conventional water treatment systems, 92

This page intentionally blank.

Foreword

The drinking water community shares its water systems with a world of microscopic living creatures. These creatures may contribute to numerous operational challenges, from problems in source water through finished water, including encrustation or corrosion within the water system and water quality problems at the customer's tap. When water treatment problems arise due to these organisms, it is often difficult for an operator to locate help. This manual provides information regarding nuisance organisms, the problems they cause, treatment options, and references to literature resources. It is intended to be a "first stop" toward finding answers.

This third edition replaces AWWA Manual M7, *Problem Organisms in Water: Identification and Treatment*, 2nd edition. This complete revision of AWWA Manual M7 stems from the results of the 1989 AWWA Survey of Nuisance Organisms (appendix A) that demonstrated a need for information to help identify and control these organisms.

This page intentionally blank.

Acknowledgments

The AWWA Organisms in Water Committee thanks the following authors for sharing their knowledge and time:

Chapter 1: *John Lisle*, University of South Florida, Tampa, Fla., and *Bruce MacLeod* and *Mark Simpson*, Manatee County Public Works Department, Bradenton, Fla.

Chapter 2: *Jennifer L. Clancy*, Clancy Environmental Consultants, St. Albans, N.Y., and *Stuart A. Smith*, Ground Water Science, Upper Sandusky, Ohio

Chapter 3: *D. Roy Cullimore*, Regina Water Research Institute, Regina, Sask.

Chapter 4: *Nancy I. Lieu* and *Roy L. Wolfe*, Metropolitan Water District of Southern California, La Verne, Calif.

Chapter 5: *Richard C. Lorenz*, City of Westerville, Westerville, Ohio

Chapter 6: *Jeffrey Hoshaw*, Peerless-Midwest Inc., Mishawaka, Ind.

Chapter 7: *Eugene W. Rice*, Risk Reduction Engineering Laboratory, USEPA, Cincinnati, Ohio

Chapter 8: *Glenn Matherene*, Terre Bonne Parish Waterworks, Houma, La.

Chapter 9: *James F. Lubner*, University of Wisconsin Sea Grant Institute, Milwaukee, Wis.

Chapter 10: *George Izaguirre*, Metropolitan Water District of Southern California, La Verne, Calif.

Chapter 11: *Frank W. Schaefer III*, Risk Reduction Engineering Laboratory, USEPA, Cincinnati, Ohio, and *Susan N. Boutros*, Environmental Associates Ltd., Ithaca, N.Y.

Thanks also to Angela Podesta for organizing the Nuisance Organisms survey and the chapters in the manual. The committee hopes that this manual will be a useful resource for the water industry.

Organisms in Water Committee

M.M. Marshall, Chair, University of Arizona, Tucson, Ariz.

R.M. Hoffman, Vice-Chair, Wisconsin State Laboratory of Hygiene, Madison, Wis.

Zia Bukhari, American Water, Belleville, Ill.

Kevin Connell, DynCorp I&ET, Alexandria, Va.

Ricardo DeLeon, Metropolitan Water District of Southern California, La Verne, Calif.

G.D. Di Giovanni, Texas A&M University System, El Paso, Texas

C.R. Fricker, CRF Consulting, United Kingdom

N.H. Hall, University Hygienic Laboratory, Iowa City, Iowa

P.T. Klonicki, CH Diagnostic & Consulting Services Inc., Loveland, Colo.

A.B. Margolin, University of New Hampshire, Durham, N.H.

W.J. Robertson, Health Canada, Ottawa, Ont.

P.A. Rochelle, Metropolitan Water District of Southern California, La Verne, Calif.

F.W. Schaefer, III, USEPA, Cincinnati, Ohio

D.M. Sharp, Tampa Water Department, Tampa, Fla.

J.L. Sharpley, HDR Engineering, Bellevue, Wash.

Michelle Smith, Baldwin City, Kan.

Jon Standridge, Wisconsin State Laboratory of Hygiene, Madison, Wis.

Greg Sturbaum, CH Diagnostic & Consulting Service, Inc., Loveland, Colo.

Giovanni Widmer, Tufts University, North Grafton, Mass.

J.T. Wierenga, City of Grand Rapids, West Olive, Mich.

This page intentionally blank.

Introduction

In 1989 the Organisms in Water Committee of the American Water Works Association (AWWA) conducted a survey to determine problems that water utilities encounter due to organisms in water. Of the 80 systems surveyed, only 7 indicated that they experienced no problems due to organisms in water. The other 73 systems had their hands full. These problems went beyond finding low levels of coliform bacteria in routine monitoring samples. The difficulties were related to “nuisance organisms,” microorganisms that cause problems with plant operations or with customers because of undesirable tastes, odors, color, or their mere presence in what is supposedly a clean glass of water.

NUISANCE ORGANISMS SURVEY

Appendix A presents the results of the Nuisance Organisms survey. Algal cells were identified as the greatest problem by 60 percent of the respondents. Blue–green algae causing taste-and-odor problems generated the majority of these complaints.

Tied for the second-most prevalent problem was iron bacteria within the distribution system or at the wellhead. These bacteria caused consumer taste-and-odor complaints. More than half the respondents sought help from a wide variety of sources, including other utilities, state or US Environmental Protection Agency (USEPA) laboratories, private consultants, laboratories, and universities. Typical control measures, such as increased chlorination, were only marginally effective.

Another common problem for utilities were actinomycetes, which cause particularly difficult taste-and-odor problems. Activated carbon and copper sulfate were found to be more effective controls than increased chlorination.

Midge larvae and other types of larvae were problematic, because it is the customer who usually discovers the problem. Increased chlorination and filter backwash were marginally effective, but utilities found no “sure-fire” approaches to this challenge.

Sulfur bacteria presented problems for 9 percent of the respondents. Because humans are particularly sensitive to hydrogen sulfide, small amounts of sulfur bacteria can cause offensive taste-and-odor problems.

The following chapters provide basic information and resources to assist the water treatment operator in troubleshooting and resolving problems caused by nuisance organisms. When problems arise, there is often no place for an operator to turn for help. This manual provides a resource to find more information regarding nuisance organisms in water. Each section provides a brief description of the organism, the problems that it causes, information on how to treat the problem, and references to recent literature to aid the operator in solving water treatment problems caused by organisms. Guides for troubleshooting the organisms discussed in this manual and for optimizing conventional water treatment systems may be found in appendixes B and C.

This page intentionally blank.



Chapter 1

Actinomycetes

Actinomycetes are a collection of nine different groups of bacteria. The most familiar of the group are the Streptomyces. These organisms are widely distributed in nature and account for a large part of the normal microbial population of soils and water sediments.

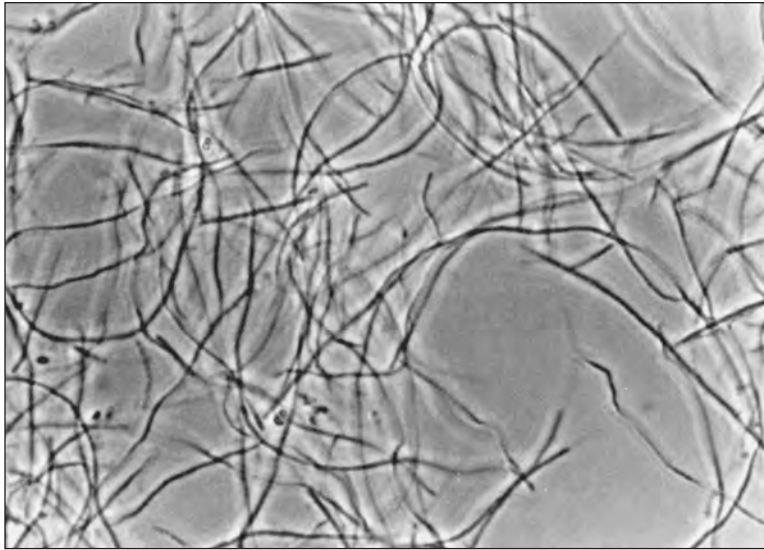
Actinomycetes' significance to the potable water industry relates to their ability to produce, as part of their normal metabolic activities, earthy–musty–moldy taste-and-odor compounds. Five taste-and-odor compounds have been attributed to the actinomycete group. These compounds are

- geosmin or *trans*-dimethyl-*trans*-9-decalol, which imparts an earthy–musty taste and odor
- 2-methylisoborneol (MIB), which imparts an earthy–musty, camphor-like taste and odor
- 2-isopropyl-3-methoxy/pyrazine, which is similar to MIB
- 2-isobutyl-3-methoxy/pyrazine, which has an earthy–musty taste and odor sometimes described as similar to bell peppers
- 2,3,6-trichloroanisole, which is described as musty

BIOLOGY AND ECOLOGY

Actinomycete strains include three types—anaerobic, aerobic, and microaerophilic (requiring a small amount of oxygen to live). This diversity is an advantage in regard to their survival and reproduction in soils and water sediments. They have two different cell forms, filamentous and spore. The most frequently found form is determined by the growth conditions in their immediate environment. The filamentous or vegetative stage is the attached life stage, shown in Figure 1-1, during which they reproduce, producing taste-and-odor compounds.

The alternative stage is the spore form. Although they do not produce taste-and-odor compounds during this stage of the life cycle, it is in this resistant stage that they are easily dispersed in air, land, and water.



Source: Scott Tighe, Analytical Services Inc.

Figure 1-1 Actinomycetes, phase contrast 1,000×

When nutrition and environmental conditions are favorable, spores can germinate into the vegetative stage and produce taste-and-odor compounds. Studies indicate that the vegetative stage grows best in the temperature range of 15 to 38°C. This observation undoubtedly explains why taste-and-odor problems generally occur in the spring, summer, and fall months in temperate areas.

Actinomycetes have been isolated from various aquatic habitats. In general, these organisms prefer the following conditions:

- eutrophic ponds and lakes rich in nutrients, with low concentrations of dissolved oxygen
- shallow ponds
- both clear and turbid lakes
- sediments, although actinomycetes can be found throughout the water

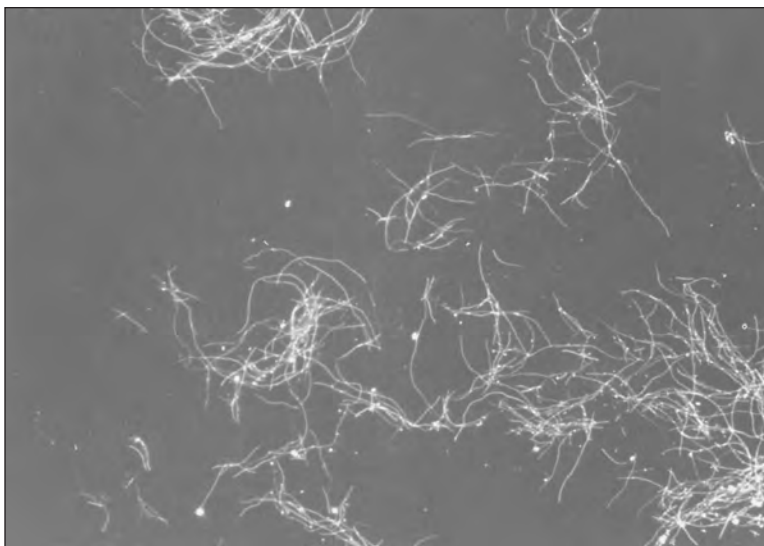
Identification

The transparent body of the vegetative actinomycetes that appears in Figure 1-2 makes these organisms difficult to observe with a compound microscope.

Actinomycetes' general size and shape can be easily confused with other organisms that are not involved in taste-and-odor production. The spore stage, which is even more difficult to observe because of its very small size, is visible at higher magnifications with the light microscope.

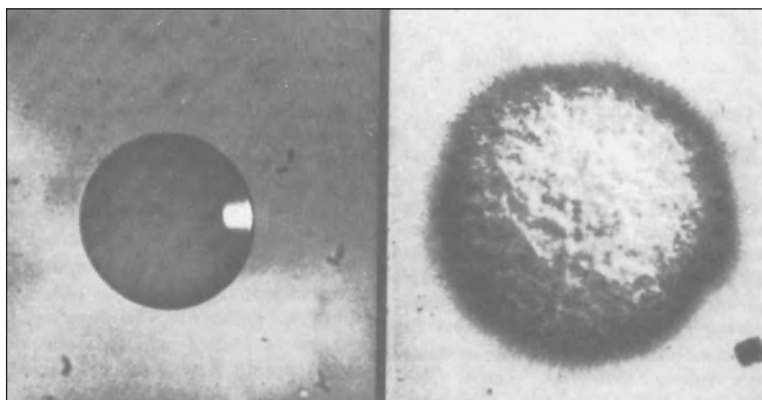
Shown on the left in Figure 1-3 is a typical bacterial colony characterized by a smooth mucous-like appearance and a smooth border. On the right is an actinomycete colony characterized by branching filaments that give it a fuzzy appearance at its border and a dull powdery appearance.

Direct examination of soils, water, and sludges. Actinomycetes are most easily identified by culturing and microscopic examination. Because this process requires from four to seven days to complete, it is not practical for immediate operational control. The customer will be aware of the taste and odor long before the cultures



Source: Scott Tighe, Analytical Services Inc.

Figure 1-2 Actinomycetes, dark-field illumination, 400×



Source: Standard Methods for the Examination of Water and Wastewater.

Figure 1-3 Bacterial colonies—typical colony versus actinomycete colony, 50×

are ready for examination. However, regular sampling and examination for actinomycetes will enable plant staff to develop baseline data for a source water. With these data, staff may predict when problems will occur and make operational changes before the organism becomes a problem.

Cellular morphology. The vegetative stage is characterized by branching and filamentous cells. The filaments can be extremely short, making them hard to distinguish from nonfilamentous, nonactinomycete organisms. Individual filaments vary in diameter from 0.5 to 2.0 μm . Spores are most easily observed when they are attached to filamentous branches. If the spore chains do not break, they may appear as a straight or looped chain. A simple stain, such as crystal violet or safranin, will increase contrast to view specimens more easily under the microscope.

Culture characteristics. The most productive method of observing actinomycetes is to grow them in isolated colonies on a specific agar. This method is more efficient than isolating a single vegetative cell from a reservoir. Samples take from

four to seven days to incubate before microscopic examination. Five media that can be used to isolate actinomycetes are listed in appendix D.

Indirect methods of examination. Odor and flavor testing are useful to determine the concentration of earthy or musty odors that accompany the presence of actinomycetes. There are currently four methods used to determine the concentrations of odors produced by actinomycetes, including threshold odor, flavor profile analysis, gas chromatography, and gas chromatography–mass spectroscopy. The first two tests, threshold odor and flavor profile analysis, are sensory. They are qualitative tests but have the advantage of being quick, inexpensive, and require little training to perform. The threshold odor test is often sufficient for MIB detection, because that compound has a strong smell.

The flavor profile analysis test can be used only on samples safe for consumption. These methods are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Sections 2160 and 2170. The flavor profile method is more quantitative than the threshold odor test. It requires an intermediate level of training and expertise and a panel of at least four trained members to describe the intensities and characteristics of tastes and odors. Reference samples of earthy–musty compounds are required, as well as periodic calibration of the panelists. The panel determines the concentration based on the flavor intensities of the samples.

Two other tests—gas chromatography or gas chromatography–mass spectroscopy—can also be used to measure actinomycete-caused taste-and-odor compounds. The ability of humans to detect these tastes and odors at low levels, however, requires a concentration step before analysis. These methods require expensive equipment and highly trained analysts to yield quantitative information down to 5.0 parts per trillion. These methods are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9250.

SIGNIFICANCE FOR WATER SUPPLIES

Actinomycetes have been isolated from soils, source water, and drinking water treatment plants. The presence of these organisms in soil may become a problem for water utilities when organisms are carried in runoff into source water supplies, establish themselves, and begin producing odors.

It is difficult to positively correlate actinomycete numbers with a taste-and-odor problem since some actinomycetes' spore colonies do not produce tastes and odors.

Actinomycete spores or vegetative cells in the source water may be drawn into a water plant's intake. They can associate with floc and settle in the sludge blanket or in algal mats along the walls of treatment basins. If growth conditions are favorable, actinomycetes may release taste-and-odor compounds. These compounds may also be introduced into the potable water supply when the following conditions occur:

- organisms or spores are retained on treatment plant filters
- soil enters the distribution system during installation of water pipes and repair of main breaks
- a cross connection occurs with an actinomycetes-contaminated source, such as an irrigation pond system connected to standard plumbing without check valves

These taste-and-odor compounds can be detected by humans at extremely low concentrations, for example, 2.0 parts per trillion for MIB. Although consumption of these bacteria or their compounds is not considered a health hazard, the water may still be aesthetically unacceptable to the consumer.

CONTROL STRATEGIES

Water treatment techniques for controlling taste-and-odor compounds produced by actinomycetes depend on the source of the difficulty. Distribution system problems are generally easier to correct than problems in the source water or treatment plant.

Flushing is an effective course of action when the problem is in an isolated area of the distribution system. Actinomycete filaments and spores are susceptible to chlorination. If the problem is persistent, the operator should consider isolating the line and superchlorinating. Both pigging and superchlorination may be necessary if actinomycetes are associated with biofilms.

Taste-and-odor problems due to actinomycete growth at the treatment plant can be controlled by minimizing sludge depth in the sedimentation basin and by maintaining an active basin-cleaning program. Taste-and-odor problems in the source water are the most difficult to control. An alternate source that is taste-and-odor free should be considered first. Sometimes varying the intake depth can be useful.

The oxidants chlorine, chloramines, chlorine dioxide, and potassium permanganate are generally not effective in reducing taste-and-odor compounds caused by actinomycetes. Although some utilities have reported success using these oxidants, others have found their use increases the intensities of tastes and odors.

Ozone, and recently, hydrogen peroxide and ozone, called PEROXONE, have been effective in reducing the concentration of these odorants. Capital costs for this treatment technique are expensive and must be justified on a case-by-case basis.

Activated carbon adsorption has proven effective in many cases. Powdered activated carbon can be added during taste-and-odor episodes to the raw water at an application point that maximizes contact time. Granular activated carbon has been used successfully either as a filter medium or as a postfilter contactor. When considering carbon, it is important to minimize other organics that may interfere with the adsorption of the target odorants and maximize activated carbon and odorant contact time.

REFERENCES

- American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1992. *Standard Methods for the Examination of Water and Wastewater*. Latest edition. Washington, D.C.: American Public Health Association.
- Arai, Tadashi. 1976. *Actinomycetes—The Boundary Microorganisms*. Tokyo, Japan: Toppan Company.
- Bartel, J.H.M., B.M. Brady, and I.H. Suffet. 1989. *Taste and Odor in Drinking Water—Phase I and II*. Denver, Colo.: American Water Works Association Research Foundation and American Water Works Association.
- Burlingame, G.A., and M.J. McGuire. 1994. Simple Test Flags Changes in Taste and Odor. *Opflow*, 20(10):1,6.
- Difco Laboratories Inc. 1984. *Difco Manual*. 10th ed. Detroit, Mich.
- Faust, S.D., and O.M. Aly. 1983. *Chemistry of Water Treatment*. Boston, Mass.: Ann Arbor Science Books.
- Goodfellow, M., M. Mordarski, and S.T. Williams. 1983. *The Biology of Actinomycetes*. London, England: Academic Press.
- Goodfellow, M., S.T. Williams, and M. Mordarski. 1988. *Actinomycetes in Biotechnology*. San Diego, Calif.: Academic Press.
- Mallevialle, J., and I.H. Suffet (eds.). 1987. *Identification and Treatment of Tastes and Odors in Drinking Water*. Denver, Colo.: American Water Works Association Research Foundation and American Water Works Association.
- Silvey, J.K.G. 1988. Tastes and Odors. In *Manual of Water Utility Operations*. 8th ed. C.K. Foster, ed. Austin, Texas: Texas Water Utilities Association.
- Waksman, S.A. 1967. *The Actinomycetes: A Summary of Current Knowledge*. New York, N.Y.: Ronald Press.

This page intentionally blank.



Chapter 2

Iron Bacteria

Iron-related or iron-precipitating bacteria (iron bacteria) are a diverse group of microorganisms widely distributed in nature. They are found in fresh and salt waters, in soils, and on desert rock surfaces. Iron bacteria do not normally cause diseases to humans or animals, but they are nuisance microorganisms. These bacteria are capable of transforming iron and sometimes manganese to an insoluble form that can cause severe fouling problems in wells, treatment plants, and distribution systems. Similar problems are caused by bacterial formation of sulfur slimes, which are formed by a separate group of bacteria (see chapter 3). Iron bacteria can also cause fouling in industrial cooling and boiler waters.

BIOLOGY AND ECOLOGY

Iron bacteria convert soluble iron (Fe^{2+}), either naturally present in water or corroded from pipe surfaces, to the insoluble form (ferric iron [Fe^{3+}]), which is deposited on or outside the bacterial cells. In combination with bacterial polymers, these insoluble forms are responsible for fouling. Iron bacteria are able to accumulate large amounts of this material, well in excess of their biomass. As can be seen in Figure 2-1, cells are surrounded by a deposit of ferric hydrate. On average, individual cells are 0.4 to 1.5 μm wide and 0.8 to 2.5 μm long.

Bacteria that can be associated with iron biofouling include the following:

1. The sheathed bacteria, such as *Leptothrix* and *Clonothrix*. These produce sheaths of oxidized iron that surround their cells. Oxidized iron or manganese is deposited outside the sheaths. *Sphaerotilus*, a form that may be related to *Leptothrix*-type bacteria, is familiar as “sewage fungus” and can form large flocs that may interfere with activated sludge processes.
2. Bacteria with appendages, such as *Hyphomicrobium*, *Caulobacter*, and *Gallionella*. *Gallionella* form long stalks on which iron is deposited outside the cells. The bacteria *Hyphomicrobium* and *Caulobacter* are associated with manganese precipitation.
3. *Thiobacillus* bacteria can thrive in aerobic or anaerobic environments and can oxidize both reduced sulfur and reduced or ferrous iron. These are the only known iron-oxidizing bacteria and are considered to be both iron and sulfur bacteria.



Source: Standard Methods for the Examination of Water and Wastewater.

Figure 2-1 Single-celled iron bacterium *Siderocapsa treubii*

4. Heterotrophic iron-precipitators in the environment precipitate iron mostly by using the organic part of iron-organic complexes and discarding the iron. This group includes a variety of coliforms and members of *Pseudomonas* and related bacteria.

Iron bacteria are common in water. The *Sphaerotilus–Leptothrix* group is thought to proliferate under low organic nutrient conditions in flowing water. They attach to surfaces in biofilms and thrive because nutrients are constantly provided and waste products are washed away. Figure 2-2 shows *Sphaerotilus natans* with cells within filaments and some free “swarmer” cells. Filaments show false branching and some areas without cells. Individual cells within the sheath may vary in size, averaging 0.6 to 2.4 μm wide by 1.0 to 12.0 μm long. Most strains are 1.1 to 1.6 μm wide and 2.0 to 4.0 μm long.

Lakes rich in suspended solids may contain high concentrations of *Gallionella*, *Hyphomicrobium*, and *Clonothrix*. Such bacteria are present in almost all aquifers studied worldwide. Examples of iron bacteria, including *Leptothrix*, *Caulobacter*, *Thiobacillus*, and *Pseudomonas*, appear in the color section of this manual.

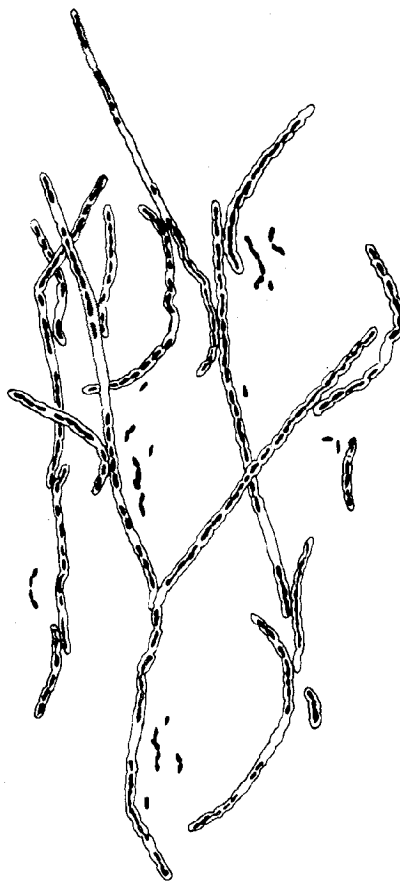
Figure 2-3 illustrates a laboratory culture of *Gallionella ferruginea*, showing cells, stalks excreted by cells, and branching stalks where cells have divided. Inorganic iron on and around the stalks often blurs the outlines. Cells at the tip of the stalk average 0.4 to 0.6 μm wide by 0.7 to 1.1 μm long.

Environmental factors including temperature, light, pH, redox potential (Eh), organic matter, and iron or manganese content determine whether or not these bacteria will thrive and proliferate to an extent that results in fouling and, thus, requires treatment. Figure 2-4 shows a mixture of fragments of stalks of *Gallionella ferruginea* and inorganic iron–manganese precipitate found in natural samples from wells. Stalks will appear golden yellow to orange when examined under the microscope.

Soluble inorganic and organic material in groundwater can be oxidized microbially to a soluble state when exposed to oxygen. Sheaths and slimes may be produced that can impart color, tastes, and odors to water. The sheaths and slime can become encrusted with iron; plug sand filters, pump impellers, and column pipe; and interfere with filtration and distribution of drinking water.

Identification

Culture methods for a number of types of iron-oxidizing bacteria can be found in *Standard Methods for the Examination of Water and Wastewater*, Section 9240. Culture methods are useful for two reasons. They provide an “early warning” of iron-precipitating



Source: Standard Methods for the Examination of Water and Wastewater.

Figure 2-2 Filaments of *Sphaerotilus natans*



Source: Standard Methods for the Examination of Water and Wastewater.

Figure 2-3 Laboratory culture of *Gallionella ferruginea*



Source: Standard Methods for the Examination of Water and Wastewater.

Figure 2-4 Stalk fragments of *Gallionella ferruginea* and iron-manganese precipitate



Source: Scott Tighe, Analytical Services Inc.

Figure 2-5 Filamentous iron bacteria recovered from well water. DIC microscopy, 1,000×

heterotrophic bacteria, which can easily be missed by microscopic methods. They also can be used to confirm the presence of living bacteria in iron deposits.

Direct examination. When iron bacteria have become so numerous that they affect water quality, culture methods are rarely needed. Microscopic examination of the slime, floc, or other masses of microbial material is usually all that is required to determine whether or not iron bacteria (at least filamentous or stalked types) are the problem (see Figure 2-5). Routine cultural examinations provide confirmation that the iron precipitation is “alive.” Early identification enables plant staff to change operational practices before the iron bacteria form intractable deposits.



Source: Stuart Smith.

Figure 2-6 Partly filled impeller channel

Microscopic methods to determine presence or absence and quantity of iron bacteria in a sample are taken from *Standard Methods for the Examination of Water and Wastewater*, Section 9240, and may be found in appendix E.

SIGNIFICANCE FOR WATER SUPPLIES

Bacterial iron precipitation, or iron biofouling, can cause taste-and-odor problems, frothing, color, and increases in turbidity. Iron bacteria can mask the presence of pathogenic bacteria, reduce the efficiency of disinfection, and increase disinfectant demand. Biofilms surrounding iron bacteria harbor corrosion-producing bacteria and accelerate corrosion. Once established, biofilms are often unaffected by the chlorine levels usually found in drinking water distribution systems.

Iron bacteria can multiply rapidly in distribution piping under favorable conditions. Accumulations of insoluble iron minerals and biofouling on the pipes themselves reduce flow.

The sequence shown in Figures 2-6 through 2-9 demonstrate iron biofouling in a well pump (lineshaft turbine) in western Ohio. The figures show iron biofouling typically occurring over 2 to 10 years in this area. Yield had declined from over 380 gpm to less than 200 gpm over 10 years. Corrosion of castings under the iron biofilm weakened this pump beyond repair. The encrusted material is mostly ferrihydrite, but there are some filamentous forms.

These chemical and physical changes on the pipe surfaces can lead to the growth of other types of microorganisms that can cause further taste, odor, and corrosion problems.

Iron bacterial fouling deteriorates piping in distribution lines, reduces distribution capacities, and increases operating and maintenance costs. Iron biofouling especially affects well pumps and column pipe, causing plugging, wear, and corrosion. Inorganic iron deposits and organic slimes produced by these bacteria clog pump intakes, well screens, pipelines, and filters.

Severe fouling in private wells has ruined washers, dishwashers, toilets, tubs, and clothing. In addition, iron biofouling allows protected anaerobic environments to form, promoting corrosion, forming hydrogen sulfide and black ferrous sulfide deposits.



Source: Stuart Smith.

Figure 2-7 Largely clogged bowl volutes



Source: Stuart Smith.

Figure 2-8 Column pipe with iron biofilm

CONTROL STRATEGIES

Controlling iron bacteria in a water system may be achieved either by thorough removal or inactivation of the bacteria in the system or by removal of the bacteria from the water.

Preventing Iron Bacteria Fouling in Wells

The major factors creating iron bacteria fouling include the following:

- the presence of the bacteria
- the presence of dissolved or complexed iron or manganese
- an environment that encourages bacterial survival and growth



Source: Stuart Smith.

Figure 2-9 Clean versus clogged impellers

Aggravating factors are controllable by sound engineering, construction, and operating practices. Controlling the following factors will limit the impact of iron bacteria on a well and its connected system:

- well design, construction, and use
- water system design and construction
- choices in water treatment

Design or construction flaws allow the entry of bacteria, increase chemical oxidation, restrict inlet channels, and provide havens for growth. Cascading water from breaks or fractures above the pumping water level encourages heavy biofouling growth and pump plugging.

Periods of nonuse or occasional use allow fouling growths to increase. However, in some cases, intermittent use may dissipate heavy biofouling. Overuse may aggravate fouling buildup by encouraging sand or mineral clogging and extra oxidation.

The use of corrodible metals in the well and pumping system, bimetallic connections (bronze/cast impeller assemblies), and the mixing of waters of differing redox potential encourage corrosion supported by bacterial activity.

The key to catching growth before it causes problems is ongoing, preventive monitoring and testing. Official “standard” methods to detect either bacteria involved in biofouling or the biofouling itself do not yet exist. However, the cost-effective methods described earlier enable identification of bacteria before symptoms occur. Hydraulic testing (drawdown and yield) should be conducted periodically to check for reduced function. Turbidity testing can also detect bacterial particle breakthrough and indicate problems in the system.

Tests should be run soon after a well is drilled or serviced in any way, and also at regular intervals—monthly, quarterly, or annually. On highly valuable wells, borehole camera surveys are useful in monitoring the progress of biofilm buildup in the well.

Valuable wells should be tested hydraulically using step-test procedures one to four times a year. Such wells should be equipped for convenient flow, line pressure, and drawdown measurement. Valved diversions allow pumping tests to be conducted without concern for varying system total dynamic head (TDH). Measurement apparatus

should be kept in good working order by cleaning such equipment as flowmeters and air lines. Step tests allow an analysis of changes in specific capacity and total yield and can be used to distinguish pump from aquifer problems. If caught early, regular chlorination and increased vigilance can keep bacterial problems under control.

It is important to avoid introducing iron bacteria into a well during drilling or repair work, although the most likely bacteria source is the aquifer itself. Chlorinate all drilling fluid mix, makeup, and mist water, even if it is from a chlorinated municipal supply. Because of chlorine's instability, add it periodically to maintain an appropriate free-chlorine residual. Drilling-mud manufacturers have recommendations on maximum chlorine levels. The removal of phosphate and organic polymer additives should be confirmed by tests during well development.

To avoid contamination from one well to another, disinfect drilling rods, bits, and tools thoroughly with chlorine between jobs. Chlorinate pumps, pipe, and filter-pack material before installation. Carefully washing pack material with chlorinated water ensures it is free of organic matter. The well should be chlorinated (refer to ANSI/AWWA C654, Standard for Disinfection of Wells, latest edition) and sealed immediately to prevent the introduction of airborne contamination, unless it is tested and put into service immediately after construction or repair.

Controlling Iron Bacteria Fouling in Wells

Once iron bacteria become established in a well, chemical and physical treatment may be required to control bacterial problems. Shock chlorination of the well may eliminate the problem for one to three years, after which the procedure will need to be repeated. Once established, such treatments tend to be necessary at increasingly shorter intervals, decreasing to monthly or weekly.

In some systems, continuous chlorination may be required to keep the iron bacteria from proliferating. Chlorination followed by greensand or conventional filtration has been effective in a number of systems. Chlorine, hypochlorites, and potassium permanganate have been used for chemical control of iron bacteria. For maximum effectiveness, shock chlorination should be accompanied with physical agitation of the well. This may include jetting, air surging, air-lift pumping, or valved surge blocks. After shock chlorination, the well should be treated with a dissolution–dispersion step using acidification and surfactants, then surged. A maintenance contract between a competent well-cleaning contractor and the well owner is advisable.

Physical methods of removing iron bacteria from wells have included heat pasteurization, explosives, and ultrasound. The latter two methods have not been reported to be successful and should be avoided in most cases. High-frequency sonic methods, however, may be used to remove hard outer deposits when treating iron biofouling.

One method of chemical control involves injecting chlorine into ground wells drilled around supply wells. Another procedure involves the injection of oxygenated degassed air into the subsurface as a means of in situ biooxidation. This method encourages bacterial growth near the wells, theoretically reducing growth near the source water well. Increasing the redox potential of groundwater leading to precipitation of iron and manganese has been used in some sand and gravel aquifers to isolate iron in the groundwater before water reaches the well, but this treatment has had limited application.

Further information on control of well iron biofouling can be found in *Evaluation and Restoration of Water Supply Wells* (1993), published by AWWA and the AWWA Research Foundation. Employ an experienced well-remediation contractor and include testing in contract specifications to apply remediation methods exactly.

Control of Iron Bacteria in Filters

Controlling iron bacteria growth in filters may be difficult, because the conditions permitting their proliferation (for example, nutrients in the source water, light, pH, and Eh of the water) may be beyond the operator's control. Once established, bacteria can be removed by backwashing and superchlorination. If the problem is short-lived or seasonal, increasing the chlorine level at the prechlorination step may be necessary to keep the bacteria in control. Additional treatment, such as greensand filtration, may be required on a long-term basis.

Control of Iron Bacteria in the Distribution System

In both groundwater and surface water systems, problems associated with iron bacteria may not appear until the water is in the distribution system. The quality of water leaving the treatment plant or wellhead may deteriorate at some point in the distribution system, and fouling occurs. Conditions in piping materials, both chemical and physical, can enhance the growth of a relatively low level of iron bacteria to the point that they become a nuisance.

The result may lead to corrosion and tuberculation of the pipes. When levels of iron and iron bacteria in the finished water are already low, immediate attention should be focused on the pipes. Severely corroded pipes may require replacement. Shock chlorination of the lines may reduce high levels of iron bacteria on pipes. For dead-end areas, looping water lines may be necessary to maintain adequate chlorine residual for bacterial growth control. Physical removal of corroded materials, followed by the use of a backwashable, corrosion-control chemical, and filtration to prevent or limit reseeding of iron bacteria, may be necessary to prevent future water quality declines because of iron bacteria.

Consistent monitoring is essential to prevent a full-blown resurgence of iron bacteria contamination. Bacteria tend to grow back. If regrowth persists, periodic chlorination should be instituted.

REFERENCES

- American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1992. *Standard Methods for the Examination of Water and Wastewater*. Latest edition. Washington, D.C.: American Public Health Association.
- American Water Works Association. 1987. ANSI/AWWA Standard C654, *Disinfection of Wells*. Latest edition. Denver, Colo.: American Water Works Association.
- Cliver, D., R. Newman, and J. Cotruvo, eds. 1987. Drinking Water Microbiology. *J. Environmental Pathology, Toxicology, and Oncology*, 7:5,6.
- Cullimore, D.R. 1990. An Evaluation of the Risk of Microbial Clogging and Corrosion in Boreholes. In *Water Wells Monitoring, Maintenance, Rehabilitation*. P. Howsam, ed. pp. 25–34. London, England: E. & F.N. Spon.
- Dondero, N. 1975. The Sphaerotilus–Leptothrix Group. *Ann. Rev. Microbiol.*, 29:407–428.
- Driscoll, F.D., ed. 1986. Well Failure Caused by Iron Bacteria. In *Groundwater and Wells*. pp. 646–655. St. Paul, Minn.: Johnson Filtration Systems Inc.
- Mansuy, N., and C. Nuzman. 1990. Well Problems Identification and Its Importance in Well Rehabilitation. P. Howsam, ed. In *Water Wells Monitoring, Maintenance, Rehabilitation*. pp. 87–99. London: E. & F.N. Spon.
- Ridgway, H.F., and B.H. Olson. 1981. Scanning Electron Microscope Evidence for Bacterial Colonization of a Drinking-Water Distribution System. *Applied and Environmental Microbiology*, 41:274–287.
- Smith, S. 1982. Culture Methods for the Enumeration of Iron Bacteria From Water Well Samples—A Critical Literature Review. *Ground Water*, 20:482–485.

- Smith, S.A. 1992. *Methods for Monitoring Iron and Manganese Biofouling in Water Wells*. Denver, Colo.: American Water Works Association Research Foundation and American Water Works Association.
- Smith, S.A., and M.S. Borch. 1993. *Evaluation and Restoration of Water Supply Wells*. Denver, Colo.: American Water Works Association Research Foundation and American Water Works Association.
- Starkey, R.L. 1945. Transformations of Iron by Bacteria in Water. *Jour. AWWA*, 37:963–984.
- Tuovinen, O.H., and E.L. Nurmiaho. 1979. Microscopic Examination of Bacteria in Fe(III)-oxide Deposited From Ground Water. *Microbial Ecology*, 5:57–66.



Chapter 3

Sulfur Bacteria

Sulfur bacteria are serious nuisance organisms in water because they can cause severe taste-and-odor problems as well as corrosion. As sulfur bacteria grow, they use large quantities of sulfates, other forms of oxidized sulfur, and/or elemental sulfur to generate hydrogen sulfide gas. These bacteria, referred to as sulfate-reducing bacteria (SRB), create smells like “rotten eggs,” initiate corrosive processes in metal fittings, and react with dissolved metals, such as iron, to generate black deposits. Note that rotten-egg smells also are created by other bacteria, including many of the coliforms.

Usually the events described above mean that somewhere upstream major bacterial fouling has removed oxygen from the water and allowed these bacteria to dominate. Bacteria commonly vent hydrogen sulfide into waters when oxygen is absent and sufficient amounts of dissolved organic materials exist. Forcing oxygen into the water eliminates the SRB, since oxygen is toxic to their activities.

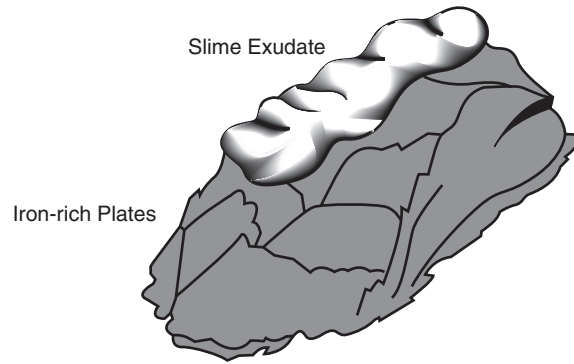
BIOLOGY AND ECOLOGY

SRB usually protect themselves by cohabiting slimes and tubercles with other slime-forming bacteria. A tubercle may appear as a slightly raised area on the metal surface, as shown in Figure 3-1.

Figure 3-2 illustrates a cross section of a slime, with the sulfate-reducing bacteria located at the metal surface and other bacteria overlaying the slime. This creates a bubble-like structure composed of hardened iron-rich plates overlaying an active slime formation. Figure 3-3 shows the cross section of a tubercle.

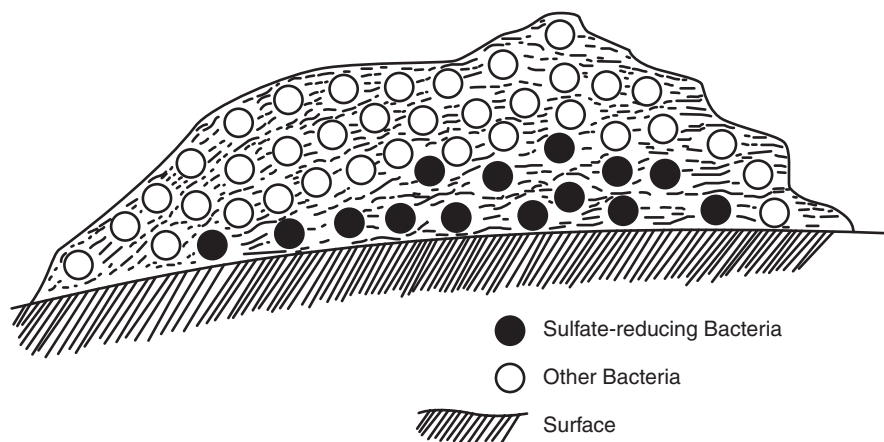
Rotten-egg smells will occur more commonly when a water system or well is not used for an extended period of time. In these cases, the oxygen in the water is used up by various bacteria. Once the oxygen is gone, the growth and activities of the SRB group become rampant, with intense production of the rotten-egg smell.

Once an SRB group establishes itself on a surface within a biological slime or tubercle, it begins to generate hydrogen sulfide gas. This gas can trigger a complex electrolytic corrosion process on some metallic surfaces. Corrosion begins with pitting and cavitation and terminates with perforation of the supporting structures, such as metal pipe walls, and system failure.



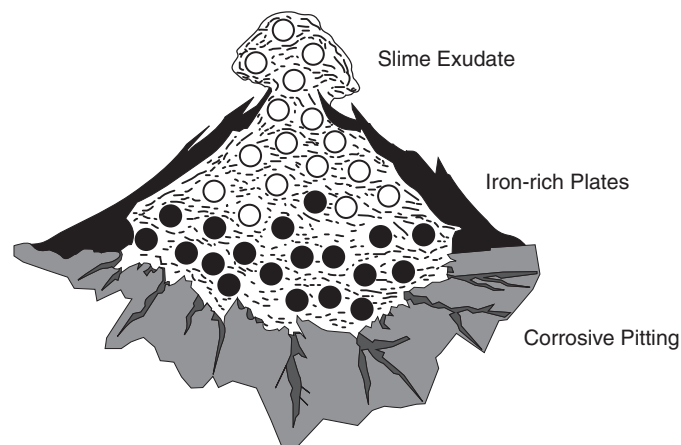
Source: D.R. Cullimore.

Figure 3-1 Side view of a tubercle



Source: D.R. Cullimore.

Figure 3-2 Cross section of a slime



Source: D.R. Cullimore.

Figure 3-3 Cross section of a tubercle

At the same time, various slime-forming bacteria that inhabit the same sites may magnify the problem by generating various organic acids, which are also corrosive. These include sulfur-oxidizing bacteria, colorless sulfur bacteria, and purple and green sulfur bacteria. Examples of SRB *Thiobacillus*, *Beggiatoa*, *Thiothrix*, and *Thiopedia* are shown in the color section of this manual. Of these groups, the sulfur-oxidizing bacteria are the most documented because of their association with acidic mine tailings and leaching of ores. These bacteria require oxygen to grow and they convert various sulfides to acidic products, such as sulfuric acid.

The colorless sulfur bacteria do not usually produce acidic products but do convert hydrogen sulfide and other sulfides to sulfates. These bacteria are sometimes found growing in water wells and distribution systems where sulfides are present and where there is also a significant level of dissolved oxygen.

Oxygen is toxic to the purple or green sulfur bacteria which, like plants, are able to photosynthesize. Unlike plants, however, these bacteria oxidize sulfides to elemental sulfur, which becomes deposited in and around the cells. Common habitats for these bacteria include septic ponds, where they occasionally dominate and turn the water red, and deeper lakes that have become stratified, or layered, and where these bacteria form distinctive plates of floating growth.

Identification

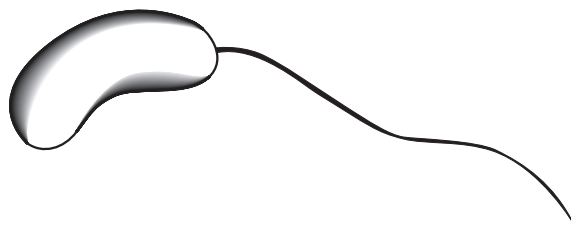
Because the SRB cause corrosion while growing on a surface, tests on the water itself may be negative. Tests will be positive when bacteria are present in the water while moving from one corrosion site to colonize another.

The following is a taxonomic listing of the major genera of sulfur bacteria associated with potable water.

- Sulfate-Reducing Bacteria (SRB)
Desulfovibrio
Desulfotomaculum
- Sulfur-Reducing Bacteria (SRB, but use sulfur)
Desulfuromonas
- Sulfur-Oxidizing Bacteria (produce acidic products)
Thiobacillus
- Colorless Sulfur Bacteria (need hydrogen sulfide)
Beggiatoa
Thiothrix
- Sulfur-Oxidizing Photosynthetic Purple and Green Sulfur Bacteria
Chlorobium
Chromatium

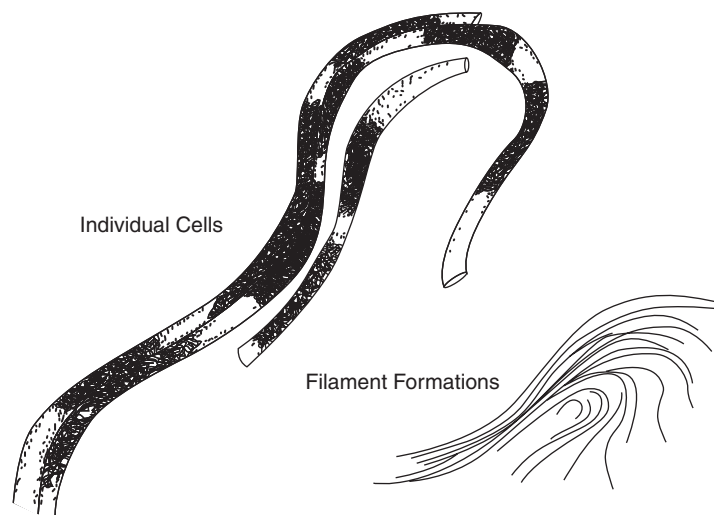
For more detailed information on SRB, see *Standard Methods for the Examination of Water and Wastewater*, Section 9240. Figures 3-4 and 3-5 illustrate two sulfur-reducing bacteria.

Primary indicators of an SRB problem include the presence of odor, tubercles, corrosion inside metallic equipment, and black slimes. Bacteriological tests can provide confirmation of SRB involvement. The SRB grow in protected places, often surrounded by other types of bacteria that may mask their presence. These growths make microscopic examination difficult. Most culture methods, like those in Section 9240C of *Standard Methods for the Examination of Water and Wastewater*, confirm the presence



Source: D.R. Cullimore.

Figure 3-4 An SRB *Desulfovibrio desulfuricans*



Source: D.R. Cullimore.

Figure 3-5 *Beggiatoa*, a colorless sulfur bacterium

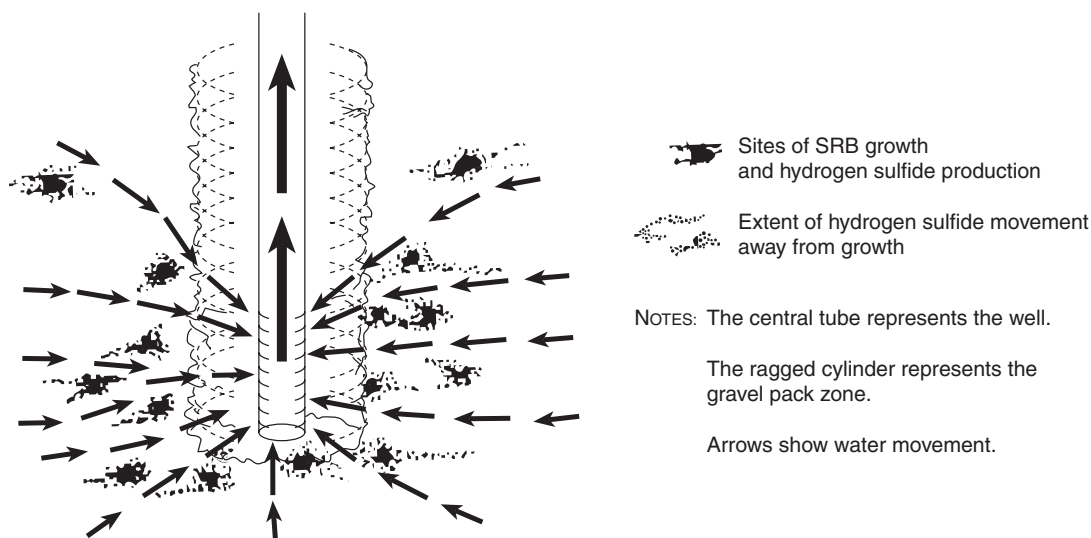
of SRB when the bacteria produce visible black sulfides. Section 9240C of *Standard Methods for the Examination of Water and Wastewater* provides information on media to grow the more common types of SRB. These black deposits appear in the liquid culture medium or as distinctive black growths in or around SRB colonies. The lag time before these black deposits become visible indicates the aggressivity of the SRB group. For example, a rapid two-day delay would indicate a more aggressive population than an eight-day delay period.

SIGNIFICANCE FOR WATER SUPPLIES

An active SRB infestation may present multiple difficulties, including corrosion; severe taste, odor, and colored water problems; loss of efficiency or equipment failures in treatment processes; and increasing consumer complaints.

Corrosion is difficult to control once SRB become established. Slimes or tubercles inhibit chemical treatments using chlorine, acids, and cleaning agents. As a result, higher dosages and longer exposure times become necessary to control corrosion.

Where significant amounts of iron, manganese, or other metallic materials occur in the water, hydrogen sulfide can react with these compounds to form metallic sulfides. Many of these chemical compounds are black in color and can cause the slime to become black in appearance. When these blackened slimes break up, the water may



Source: D.R. Cullimore.

Figure 3-6 An operating water well being pumped, influence (suppressive) on SRB activity (theoretical)

contain threadlike strings of black slime-like material. These black growths frequently are not accompanied by rotten-egg smells, because the hydrogen sulfide gas has been converted into black sulfides. The slimes flow down walls and across surfaces and may change in color to browns, reds, yellows, and greys as the oxygen in the air stimulates aerobic bacterial activity in the slime.

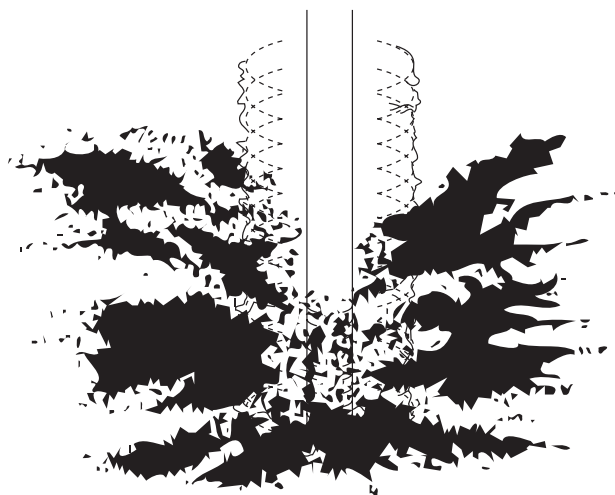
CONTROL STRATEGIES

Unfortunately, the ability of SRB to grow in places where they are protected by either copious “overburdens” of slime or within tubercles makes control difficult. Treatments that have been recommended include a variety of disinfection, acidification, and cleaning practices. It is essential when applying these various practices to follow the suggestions below.

- Step 1 Ensure that the system has been flushed and cleaned thoroughly before starting the treatment program.
- Step 2 Apply the highest recommended dosage and use the longest contact time for the selected treatment program in order to maximize effectiveness.
- Step 3 Increase the dissolved oxygen in the water to suppress SRB activity.
- Step 4 Consider ongoing or routine application of disinfectants and penetrants to reduce the rate of recovery of the SRB from the “shock” effect of step 2.

Pump operation influences the effect of SRB, as the increased flow to an operating pump will tend to discourage SRB growth. A quiescent or inactive pump causes the spread of SRB, as oxygen is used up. When pumping is resumed, the hydrogen sulfide is flushed out (see Figures 3-6 and 3-7).

Routine testing for residual dissolved oxygen (DO) is a simple and useful step to monitor for SRB problems. A dramatic decline in DO may be taken as an early



NOTE: When oxygen is used up, hydrogen sulfide spreads through to the well. This is flushed out when the pumping starts again.

Source: D.R. Cullimore.

Figure 3-7 Operational water well during period of quiescence (not pumping), causing the SRB activity to increase and the hydrogen sulfide to spread (theoretical)

warning signal that conditions are becoming more supportive for SRB growth. Simple analytical methods can be used to monitor the presence and aggressivity of SRB in water.

REFERENCES

- American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1992. *Standard Methods for the Examination of Water and Wastewater*. Latest edition. Washington, D.C.: American Public Health Association.
- Cullimore, D.R. 1990. An Evaluation of the Risk of Microbial Clogging and Corrosion. In *Water Wells Monitoring, Maintenance, Rehabilitation*. P. Howsam, ed. pp 25–34. London: E. & F.N. Spon.
- Pfennig, N., F. Widdel, and H.G. Truper. 1991. The Dissimilatory Sulfate Reducing Bacteria. In *The Prokaryotes*, Vol. 1. pp. 926–940. New York: Springer-Verlag.
- Postgate, J.R. 1984. *The Sulphate Reducing Bacteria*. 2nd ed. Cambridge, England: Cambridge University Press.
- Tiller, A.K. 1990. Biocorrosion in Civil Engineering. In *Microbiology in Civil Engineering*. P. Howsam, ed. pp. 24–38. FEMS Symposium No. 59. London: E. & F.N. Spon.
- Widdel, F. and N. Pfennig. 1984. Dissimilatory Sulfate or Sulfur-Reducing Bacteria. In *Bergey's Manual of Systematic Bacteriology*. Vol. 1. pp. 663–679. N.R. Krieg and J.G. Holt, eds. Baltimore, Md.: Williams & Wilkins.



Chapter 4

Nitrifying Bacteria

Nitrifying bacteria are a group of microorganisms capable of converting ammonia and other reduced nitrogen compounds to more oxidized nitrogen compounds, e.g., nitrate. This process is referred to as nitrification. There are two major groups of nitrifiers (heterotrophs and chemolithotrophs), which are differentiated based on how they obtain energy for growth. This chapter discusses only the chemolithotrophs because of the water quality problems they cause in distribution systems.

Nitrifying bacteria can be helpful in biologically active filtration by decreasing unwanted ammonia entering a treatment plant. However, these bacteria can become a nuisance in some distribution systems where chloramines are used for disinfection. Partial or incomplete nitrification may produce excess nitrite, accelerating the breakdown of chloramines. Chlorine and ammonia may decrease to the extent that disinfection becomes ineffective and regrowth occurs.

BIOLOGY AND ECOLOGY

Nitrifying bacteria are found in a variety of shapes, including rods, curved rods, spheres, spirals, and lobular forms. See Figure 4-1 for an electron micrograph of a nitrifying bacterium. These bacteria range in size from approximately 0.3 to 1.7 μm .

Chemolithotropic nitrifying bacteria are gram-negative and strictly aerobic. Chemolithotropic means that these organisms can obtain energy from oxidizing simple inorganic compounds, such as ammonia or nitrite, and use dissolved carbon dioxide to make sugars for cell material. Many other bacteria commonly found in water obtain their energy from organic compounds. Those organisms are known as heterotrophic bacteria.

Within the cells of nitrifying bacteria are membranes that are believed to be where the nitrification process occurs (Watson, Valos, and Waterbury 1981). In order to successfully complete the nitrification process, two subgroups of nitrifiers (ammonia oxidizers and nitrite oxidizers) are necessary. Ammonia-oxidizing bacteria (AOB) convert ammonia to nitrite, and nitrite-oxidizing bacteria (NOB) convert nitrite to nitrate. Because the nitrification process by which they obtain their energy is relatively inefficient, the growth rate of nitrifiers is much slower than that of heterotrophic bacteria (Wood 1986).



Source: Lieu, Wolfe, and Means (1993).

Figure 4-1 Electron micrograph showing cross section of a nitrifying bacterium

The most common AOB genus recovered from water is *Nitrosomonas* (Painter 1970; Rittmann and Snoeyink 1984; Wolfe et al. 1988 and 1990). Other genera found in water and other habitats include *Nitrosovibrio*, *Nitrosococcus*, and *Nitrospira*. The most common NOB is *Nitrobacter*. Other NOB genera are *Nitrococcus* and *Nitrospina*, which occur in marine habitats.

Nitrifying bacteria can occur in a variety of habitats, including freshwater, marine, soil, brackish water, wastewater, and treated drinking water. Although nitrifiers can survive in a wide range of temperatures (5 to >30°C), their growth rate is controlled by the concentration of ammonia or nitrite, pH, dissolved oxygen, light, and temperature. The optimum pH for the growth rate of nitrifiers is from 7.5 to 8.2 and the optimum temperature is from 25 to 30°C (Bock et al. 1986; Watson, Valos, and Waterbury 1981). Because nitrifiers are sensitive to ultraviolet irradiation, they typically grow in dark environments.

Nitrification is part of a larger process, called the nitrogen cycle, in which elemental nitrogen may be converted to more complex forms of nitrogenous compounds. To complete the loop, these compounds may be converted back to the elemental form of nitrogen gas.

Although there are other groups of organisms and physical means by which nitrification can occur, nitrifying bacteria make up the major group of organisms that can perform nitrification. In addition to the nitrification process, AOB and NOB may play a role in the carbon cycle by excreting organic by-products, which, in turn, support the growth of heterotrophic bacteria (Jones and Hood 1980). Consequently, nitrifiers play a major role in the ecosystem for the existence of life.

Identification

It can be tedious and difficult to identify nitrifying bacteria. This is due, in part, to the slow growth rate of nitrifiers and their poor isolation and colony development on agar culture plates. Photographs of nitrifiers *Nitrosomonas* and *Nitrobacter* are included in the color section of this manual.

Nitrifying bacteria may be identified by observation of growth on selective inorganic media, detection of nitrite or nitrate production, and observation of the physical structure of internal membranes with an electron microscope. A list of media for culturing ammonia-oxidizing and nitrite-oxidizing bacteria is described by Watson, Valos, and Waterbury (1981).

SIGNIFICANCE FOR WATER SUPPLIES

Depending on the treatment application and type of disinfectant, nitrification can be either a benefit or a nuisance to the water industry. Nitrification is beneficial whenever the elimination of natural sources of ammonia from source water is desirable or planned, as ammonia has a considerable chlorine demand.

In the planned approach, nitrifying bacteria are allowed to increase to high levels, typically in the filter beds or the clarifiers, where they convert ammonia to nitrate (Rittman and Snoeyink 1984; Kurtz-Crooks et al. 1986).

Because nitrate does not have an appreciable chlorine demand, complete nitrification results in a decrease in the cost of chlorination. In addition, elimination of free ammonia may lead to increased biological stability of water in the distribution system.

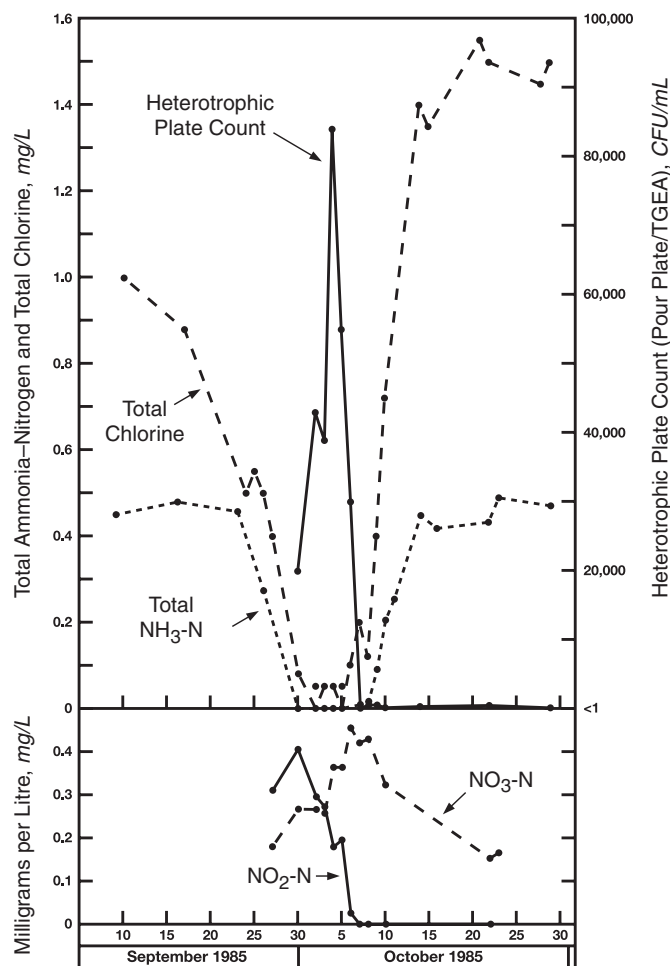
However, partial or incomplete nitrification in distribution systems using chloramines can significantly impact the water quality. As Figure 4-2 indicates, these effects include decay of the chloramine and ammonia residuals and increases in nitrite and heterotrophic bacteria (Wolfe et al. 1988).

Water quality deteriorates because of a buildup of nitrite from the first step of nitrification. The second step, conversion of nitrite to nitrate, either does not occur or occurs at a very slow rate in drinking water. AOBs, which are highly resistant to chloramines, have tolerated monochloramine levels up to 5.0 mg/L (Kirmeyer, Foust, and LeChevallier 1991; Cunliffe 1991; Wolfe et al. 1988 and 1990; Lieu et al. 1993), converting the excess ammonia used to form chloramines into nitrite. NOBs convert nitrite to nitrate at a slower rate and may be more sensitive to antimicrobial agents, including drinking water disinfectants, than AOB (Bock, Koops, and Harms 1986). This leads to an accumulation of nitrite in the water.

Nitrite has a significant chlorine demand, consuming five parts of chlorine for each part of nitrite. In addition, nitrite appears to accelerate the breakdown of the chloramine residual. Decreased disinfectant levels may cause a substantial increase in heterotrophic bacteria, possibly even coliforms. Also, heterotrophic bacteria may increase because they can use the organics secreted by AOB. Thus, nitrification can impair a utility's ability to meet the provisions of the US Environmental Protection Agency's (USEPA's) Surface Water Treatment Rule, which requires utilities to maintain a disinfectant residual or have a heterotrophic plate count below 500 colonies/mL in 95 percent of the distribution system samples analyzed per month.

USEPA has also established a maximum contaminant level (MCL) for nitrite at 1.0 mg/L (as nitrogen). Depending on the level of ammonia in the drinking water, the nitrite standard can be exceeded by severe cases of nitrification.

Incomplete nitrification in chloraminated waters occurs predominantly in water with low turnover rates at temperatures greater than 15°C. Typical sites for nitrification problems are in storage tanks and dead ends within the distribution system.



Source: Wolf, Means, Davis, and Barrett (1988).

Figure 4-2 Water quality parameters during a 1985 nitrification episode at Garvey Reservoir, Los Angeles County, Calif.

CONTROL STRATEGIES

Several methods have been used successfully to control nitrification problems in distribution systems containing chloraminated waters. These include flushing the distribution pipeline, superchlorinating reservoirs and storage tanks, increasing the chlorine-to-nitrogen ($\text{Cl}_2\text{:N}$) weight ratio, decreasing the detention time in reservoirs and distribution system pipelines (Lieu, Wolfe, and Means 1993), and switching to chlorine disinfection in the distribution system.

Flushing the distribution system is the easiest and quickest method of controlling nitrification. However, it is generally the least effective, particularly when nitrification is severe. Superchlorination will eliminate nitrification because nitrifiers are highly sensitive to free chlorine. This method may be costly and result in the temporary production of elevated disinfection by-products, such as trihalomethanes. Increasing the $\text{Cl}_2\text{:N}$ weight ratio may prevent nitrification by reducing the amount of excess ammonia available to the AOB (Wolfe et al. 1988; Lieu, Wolfe, and Means 1993). Decreasing the detention time in reservoirs or storage tanks will help prevent increases in the numbers

of slow-growing AOB. Finally, periodic switches to free chlorine in the distribution system should prevent biofilm formation that harbors nitrifiers.

The best preventive program involves a comprehensive monitoring system and knowledge of the flow pattern of the water within the distribution system. Parameters that need to be monitored regularly include total and free chlorine, total and free ammonia, nitrite, temperature, dissolved oxygen, and pH (Wolfe et al. 1990). When the system is monitored regularly to detect nitrification early, a greater number of control options are available. In addition, monitoring information helps to optimize chloramine treatment in order to minimize nitrification problems (Lieu, Wolfe, and Means 1993).

REFERENCES

- Bock, E., H.P. Koops, and H. Harms. 1986. Cell Biology of Nitrifying Bacteria. *Nitrification*. J.I. Prosser, ed. Washington, D.C.: IRL Press.
- Cunliffe, D.A. 1991. Bacterial Nitrification in Chloraminated Water Supplies. *Applied and Environmental Microbiology*, 57:3399.
- Jones, R.D., and M.A. Hood. 1980. Interaction Between an Ammonium Oxidizer, *Nitrosomonas* sp. and Two Heterotrophic Bacteria, *Nocardia atlantica* and *Pseudomonas* sp., A Note. *Microbial Ecology*, 6:271.
- Kirmeyer, G., G. Foust, and M. LeChavallier. 1991. Optimization of Chloramination for Distribution System Water Quality Control. In *Proceedings of the 1991 Annual Conference in Philadelphia, Pa.* Denver, Colo.: American Water Works Association.
- Kurtz-Crooks, J., V.L. Snoeyink, M.D. Curry, and M.L. Reynolds. 1986. Technical Note: Biological Removal of Ammonia at Roxana, Illinois. *Jour. AWWA*, 78:5:94.
- Lieu, N.I., R.L. Wolfe, and E.G. Means III. 1993. Optimizing Chloramine Disinfection for the Control of Nitrification. *Jour. AWWA*, 85:2:84.
- Painter, H.A. 1970. A Review of Literature on Inorganic Nitrogen Metabolism in Microorganisms. *Water Res.*, 4:393.
- Rittmann, B.E., and V.L. Snoeyink. 1984. Achieving Biologically Stable Drinking Water. *Jour. AWWA*, 76:10:106.
- Watson, S.W., F.W. Valos, and J.B. Waterbury. 1981. The Family Nitrobacteraceae. *The Prokaryotes*. M.P. Starr et al., eds. Berlin: Springer-Verlag.
- Wolfe, R.L., E.G. Means, M.K. Davis, and S.E. Barrett. 1988. Biological Nitrification in Covered Reservoirs Containing Chloraminated Water. *Jour. AWWA*, 80:9:109.
- Wolfe, R.L., N.I. Lieu, G.I. Izaguirre, and E.G. Means. 1990. Ammonia-Oxidizing Bacteria in a Chloraminated Distribution System: Seasonal Occurrence, Distribution, and Disinfection Resistance. *Applied and Environmental Microbiology*, 56:451.
- Wood, P.M. 1986. Nitrification as a Bacterial Energy Source. *Nitrification*. J.I. Prosser, ed. Washington, D.C.: IRL Press.

This page intentionally blank.



Chapter 5

Nematodes

Nematodes are unsegmented roundworms belonging to the phylum Nematoda, which includes both parasitic and free-living forms. These roundworms, a name that aptly describes their anatomy, are prevalent throughout the environment. It has been said that if the earth and everything on it except nematodes were to disappear, the earth's surface would be outlined by the worms. The top layer of soil can contain over 1 million nematodes per square meter. The greatest number of nematodes are the free-living forms that are commonly associated with soils and aquatic environments.

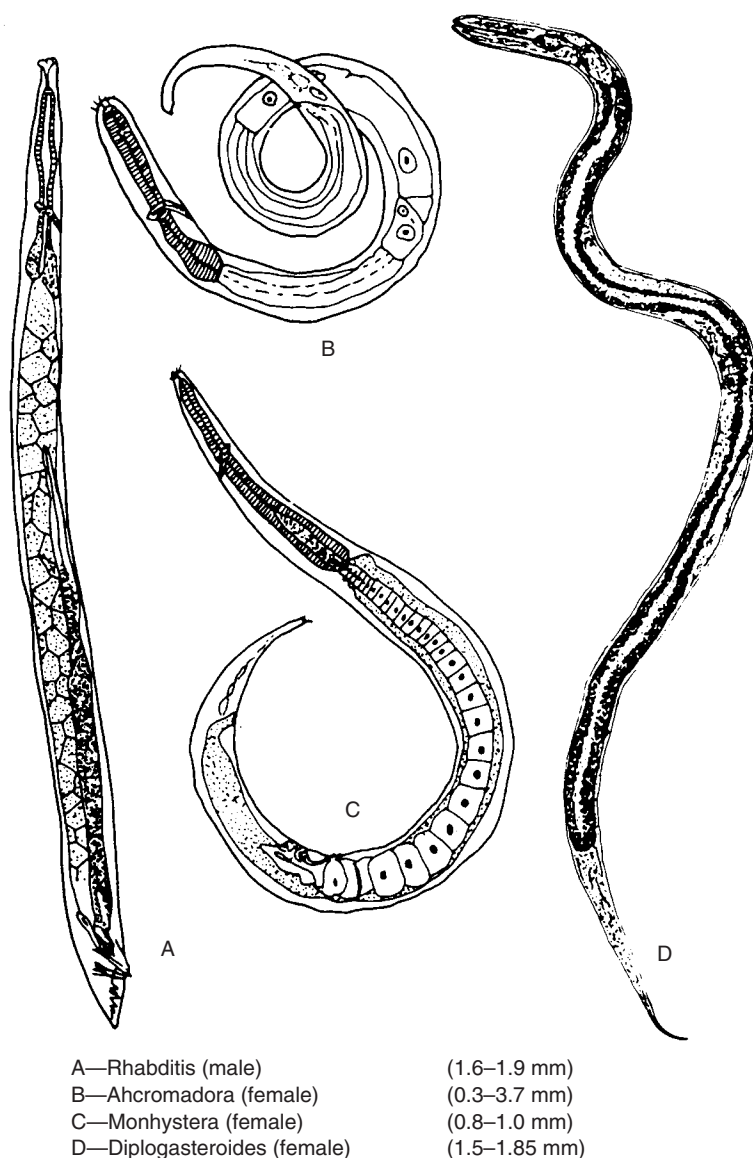
BIOLOGY AND ECOLOGY

The free-living nematodes found in fresh water are generally microscopic in size, ranging from 5 to 50 μm in width and 100 to 1,000 μm in length. The distinctive characteristics of these animals are their smooth (no cilia) cylindrical shape and whiplike movements that form C- and S-shape outlines. The thrashing motion of these organisms is the result of muscles that run lengthwise and only allow for a side-to-side motion, as shown in Figure 5-1. This method of locomotion is rather ineffective in water but effective in denser materials, such as soils.

The body of a nematode is plump and tapers to a rather broad head and to a tail that may be elongated. The head consists of a lipped mouth, which is followed by an esophagus and intestine that runs the length of the body. The tail is posterior to the anus. Because most nematodes are light beige to clear in color and translucent, they are interesting specimens to view under the microscope. See Figure 5-2.

Nematodes feed on a wide variety of food, depending on the species, including both live and dead plants, animals, and bacteria. Mouth parts of many of the nematodes are specialized for feeding on their choice food. Many of the predatory worms have a rigid, hollow stylet that can extend from the mouth to penetrate the prey and suction cell fluids.

The life cycle of the nematode consists of an egg, several larval stages, and the adult. Larval stages appear similar to the adults except they lack reproductive organs and are smaller. Both male and female are known for most species of nematodes. However, in some cases, reproduction is by the development of an unfertilized egg (parthenogenetic).



Source: Standard Methods for Examination of Water and Wastewater.

Figure 5-1 Roundworms (phylum Nematoda)

Adult females are slightly larger than males. All stages of nematode life, but especially the egg, are resistant to adverse environmental conditions.

Nematodes occur in almost every ecological habitat. In aquatic environments, the greatest numbers are usually found in the aerobic benthic sediments of lakes and rivers. Large numbers are found in wastewater treatment plants and secondary sewage effluent.

The majority of nematodes found in source waters for drinking water plants result from soil runoff, high river flows that suspend the benthic organisms, or from sewage effluent. Nematode levels in source waters generally increase with increasing turbidities and river flow. The distance from a sewage treatment plant also greatly affects the number of nematodes found in the water column, as nematodes flourish in aerobic biological wastewater treatment plants. The number of nematodes in rivers varies



Source: Scott Tighe, Analytical Services Inc.

Figure 5-2 Nematode in bright-field, 200×

greatly depending on the watershed and local sources. A 1979 study by the US Environmental Protection Agency (USEPA) indicated that a direct relationship exists between nematode density in finished water and rainfall, as the scouring action of increased streamflow washes organisms into streams and lakes. River water often contains 0–3 nematodes/L, but on occasion, can reach 800 or more/L.

Identification

Large volumes of water should be examined when sampling for nematodes. A common method of enumeration is to filter 1–20 L of tap water through a 5- μm or smaller membrane filter. A standard coliform bacteria filter of 0.45 μm can be used but will limit the quantity of water examined due to quicker clogging. Place the filter on a moist, absorbent pad, and observe for nematodes and other organisms using a stereoscopic microscope at its highest power (40–50 $\times$).

An alternative technique is to rinse the membrane filter with several millilitres of distilled water and examine in a counting dish or Sedgwick–Rafter cell. Live nematodes can be distinguished by observing them for up to a minute to detect movement. A compound microscope is required to identify and count the smallest nematodes (see Figure 5-3). A technique for counting and identifying nematodes may be found in *Standard Methods for the Examination of Water and Wastewater*, Section 10750.

In natural waters, nematodes are often irregularly dispersed and the quantity of organisms can vary greatly over time. For these reasons, it is important to sample large volumes of water over several time periods to adequately assess representative populations in source waters. For turbid raw waters, smaller volumes must be filtered through several membrane filters. Larger pore-size soil sieves (number 500 mesh) or plankton nets (10–35 μm) can be used to concentrate raw waters. Although this will only be a qualitative method, because many of the smaller nematodes will be missed, this method still provides meaningful information. To determine relative numbers, a 35- μm plankton net can be used to collect nematodes from fire hydrants or natural waters.



Source: R.C. Lorenz.

Figure 5-3 Nematode at 250×

SIGNIFICANCE FOR WATER SUPPLIES

Nematodes can be found in many drinking waters that have surface water sources. Their density in finished water leaving several water plants in the eastern United States that have monitored for nematodes averaged between 0.13 and 0.4 nematodes/L. Many of the nematodes observed in tap water are alive, as determined by movement. Live nematodes have also been collected from residential faucets and fire hydrants in distribution systems.

The highest density of nematodes in the distribution system is commonly observed when flushing hydrants in low-water-usage, dead-end areas. The greatest numbers of nematodes are frequently noted after generating a scouring velocity by flushing the hydrant. Nematode concentration in these areas are often much greater than in the water leaving the plant.

In many cases, live nematodes are collected in water having a free-chlorine residual. Other aquatic animals are also frequently observed in these samples. In some areas, the nematodes and associated community of organisms appear to be surviving and reproducing in the distribution system.

Nematodes found in source and potable waters have not been identified as pathogenic forms, and they themselves do not present a health threat. However, the presence of live worms does not portray a quality product to the public and may also compromise the microbiological integrity of the drinking water.

Nematodes present in finished water are capable of ingesting human pathogens, such as *Salmonella*, *Shigella*, and viruses. Pathogens ingested by the worm can survive within the worm for several days, which may allow passage into the distribution system. In some areas, the waterborne nematodes may include plant parasitic forms that potentially could have a negative effect when finished water is used for irrigation.

CONTROL STRATEGIES

Conventional water treatment processes are not highly effective in removing nematodes from the supply. Nematodes are very resistant to inactivation by free chlorine and, unless inactivated, nematodes can pass through rapid sand filters. Optimizing