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Microbiology of the Aquatic Environment

Microbiology of the Aquatic Environment

Edited by Phillip E. Greeson

Briefing Papers on Water Quality

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FOREWORD

In August 1974, the Water Resources Division of the U.S. Geological Survey introduced the first of a series of briefing papers that were designed to increase the understanding of its employees of the significance of various aspects of water quality. Numerous briefing papers have been prepared under the direction of the Quality of Water Branch. Other papers will be prepared as the need arises. Each paper addresses a separate topic and is written in a nontechnical, easy-to-understand manner for distribution within the organization.

Because of the favorable reception that the papers have received and their apparent effectiveness in accomplishing the objective stated above, it would appear that their wider distribution would serve a useful purpose. It is hoped that a wide range of persons, including those interested in the quality of our Nation's water resources but who have little or no technical training, will find value in reading the papers. Furthermore, it is hoped that the papers will be suitable for supplemental reading in secondary education programs and in beginning college-level courses.

The U.S. Geological Survey plans to publish several U.S. Geological Survey Circulars that contain compilations of briefing papers on particular aspects of water quality. This fifth Circular contains three papers describing the microbiology of the aquatic environment.

Philip Cohen
Chief Hydrologist

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BRIEFING PAPERS ON WATER QUALITY

Microbiology of the Aquatic Environment

Edited by Phillip E. Greeson

ABSTRACT

This is the fifth of several compilations of briefing papers on water quality prepared by the U.S. Geological Survey. Each briefing paper is prepared in a simple, nontechnical, easy-to-understand manner. This U.S. Geological Survey Circular contains three papers on the microbiology of aquatic environment. Briefing papers are included on "The ecological role(s) of aquatic micro-organisms in lakes and reservoirs," "Effects of bacteria on the chemical and physical state of iron," and "Micro-organisms in stormwater: A summary of recent investigations."

The Ecological Role(s) of Aquatic Micro-organisms in Lakes and Reservoirs

By Bruce L. Kimmel[1]

ABSTRACT

Micro-organisms form vital links in the structure of aquatic ecosystems by virtue of their roles in organic matter production and decomposition, nutrient uptake and regeneration, foodweb transfers, and biogeochemical transformations. By the nature of their biological activities, autotrophic and heterotrophic micro-organisms are sensitive indicators of the ecological and water-quality status of aquatic environments. However, only by thoroughly understanding how micro-organisms function in healthy aquatic ecosystems can we hope to recognize or accurately predict their response to water-quality changes.

INTRODUCTION

Much attention in the field of water quality is focused on micro-organisms as indicators of pollution (for example, fecal coliform and fecal streptococci bacteria), threats to public health (for example, pathogenic bacteria and viruses), or symptoms of water-quality deterioration (for example, nuisance algal blooms). Because characteristics common to water-quality problems are readily noticeable, the public often receives the mistaken impression that the presence of micro-organisms signals the demise of a body of water. As an example, the term "algae" may connote surface scums and foul odors to the layman, while "bacteria" implies disease-causing contamination. In fact, such extreme conditions are rare, and usually occur only in systems which have been altered significantly by excessive loading of nutrients, organic matter, and/or toxic contaminants. Under typical circumstances, micro-organisms such as algae and bacteria form vital links in the structure of aquatic ecosystems, and indeed are the operative factors in organic matter production, decomposition, and nutrient regeneration. As professionals in the field of water quality and communicators of water-quality information to other agencies and to the general public, it is important to maintain a perspective in regard to the ecological significance of aquatic micro-organisms. The purpose of this discussion, therefore, is to review the ecological roles of aquatic micro-organisms in lakes and reservoirs.

AN OVERVIEW OF ECOSYSTEM STRUCTURE

Before specifically addressing aquatic micro-organisms and their ecological roles, let us briefly consider the basic structure of lentic (that is, lake-like or standing-water) ecosystems. Photosynthetic production usually forms the organic matter base of aquatic foodwebs. Organic matter formed photosynthetically by primary producers (for example, algae) is consumed by herbivores (for example, zooplankton), which are fed upon by higher consumers (carnivores; for example, fish). Predatory transfers, the ingestion and assimilation of living primary producers by herbivores and herbivores by carnivores, collectively are referred to as the "grazer pathway." All of these foodweb levels produce excreted or defecated material and dead organisms which are utilized by "decomposers" (for example, bacteria and fungi). Decomposers promote the breakdown of organic materials to inorganic compounds, and therefore, generally are assigned the role of nutrient regeneration. Nonpredatory transfers, which involve the use of nonliving organic matter by decomposers, collectively are termed the "detritus pathway" (fig. 1).

[1] Biological Station and Department of Zoology, University of Oklahoma, Norman, Oklahoma.

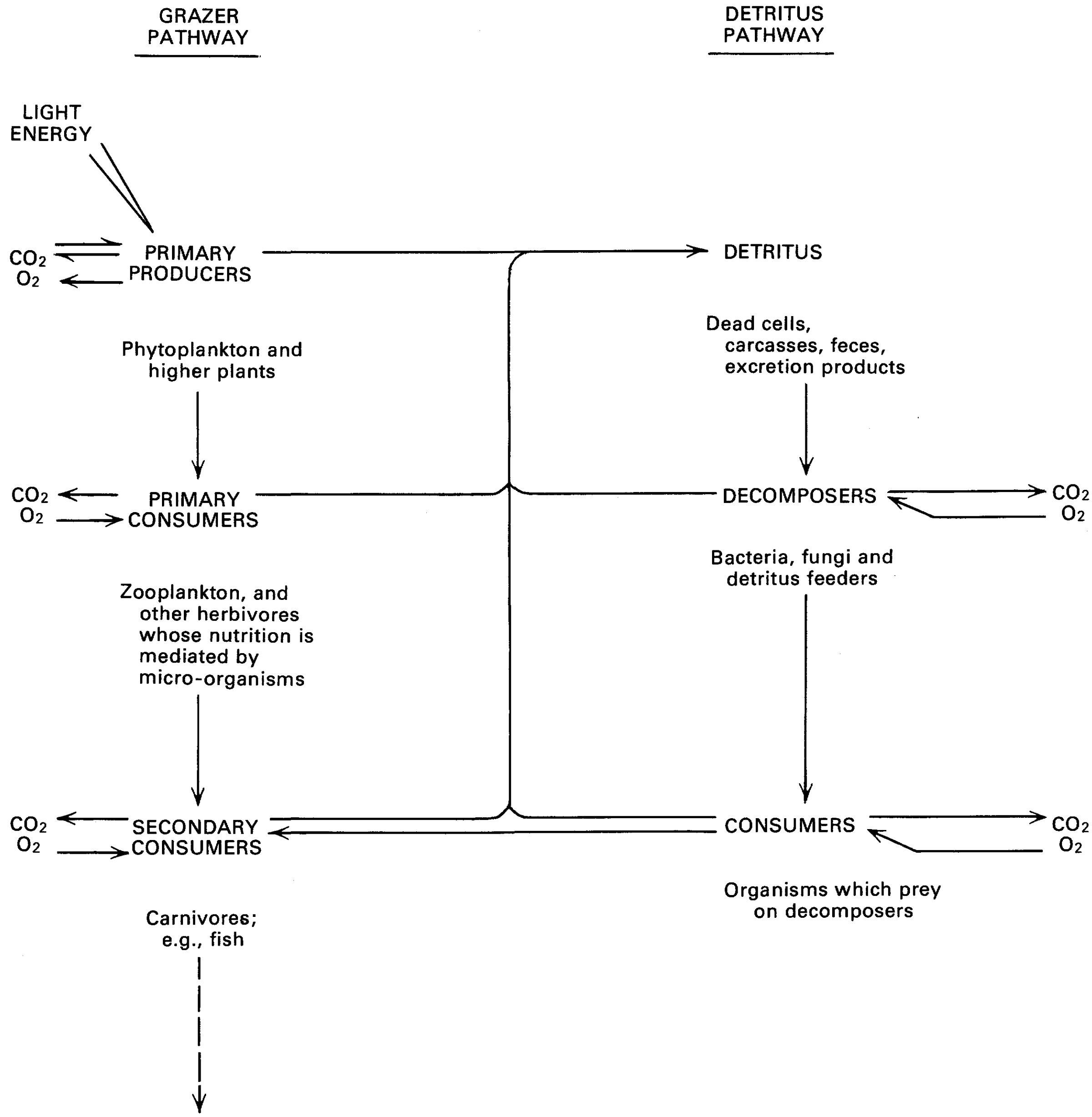


FIGURE 1.—The grazer and detritus pathways (modified from Campbell, 1977).

Although the flow of energy and materials through both grazer and detritus pathways is recognized, the relative importance of the two pathways in various types of ecosystems appears not to be. It is often generalized that the grazer pathway dominates ecosystem energy flow in grasslands and pelagic (open-water) systems, while the detritus pathway is more important in forests, marshes, and streams. However, this generalization should not be accepted unquestioningly. Although the magnitude of detritus accumulation is obvious in some terrestrial and semiterrestrial systems in which litter (for example, fallen leaves and dead marsh grass) is readily visible, it is much less apparent in the open waters of lakes and reservoirs where the "litter" (for example, dead algal cells and zooplankton fecal pellets), as well as many of the producers and consumers, is of microscopic size.

This discussion considers primarily those aquatic micro-organisms which fall into the "Producer" and "Decomposer" categories described above;

however, as will be seen, some of these micro-organisms also perform roles assignable to "Herbivores" and "Consumers." Our discussion will focus on two fundamental aspects of the ecological roles of micro-organisms in lakes and reservoirs:

(1) Algae as primary producers, and bacteria as decomposers and nutrient regenerators;

(2) Bacteria and fungi as particle producers and potential food sources for higher consumers.

ORGANIC MATTER PRODUCTION AND DECOMPOSITION

Algae and certain bacteria produce organic matter by the process of photosynthesis, which is the formation of organic compounds from carbon dioxide (CO_2) and other inorganic nutrients by way of light energy and the chlorophyll pigment in plants. Some bacteria produce organic compounds by chemosynthesis, in which energy-yielding chemical reactions, rather than sunlight, provide the energy necessary for CO_2 fixation. Both photosynthesis and chemosynthesis result in the formation of organic material from inorganic components, and, therefore, are considered to be autotrophic (primary production) processes.[2] Although some bacteria are autotrophic, most are heterotrophic and like other heterotrophs (for example, herbivores and higher consumers) they grow by assimilating organic materials previously synthesized by autotrophs. The metabolic processes of all organisms are energy consumptive, and require adenosine triphosphate (ATP, formed during cellular respiration) as their "energy currency." All living organisms, in order to continue living, must conduct cellular respiration, which is in some ways the reverse of photosynthetic carbon fixation (fig. 2). Organic matter decomposition, a collective term describing the net conversion of organic material back to inorganic compounds (for example, CO_2, H_2O, and nutrients), occurs by virtue of the respiratory activities of those heterotrophic micro-organisms using the organic material for growth.

Carbon dioxide and oxygen are reactants in the photosynthesis-respiration equilibrium (fig. 2), and

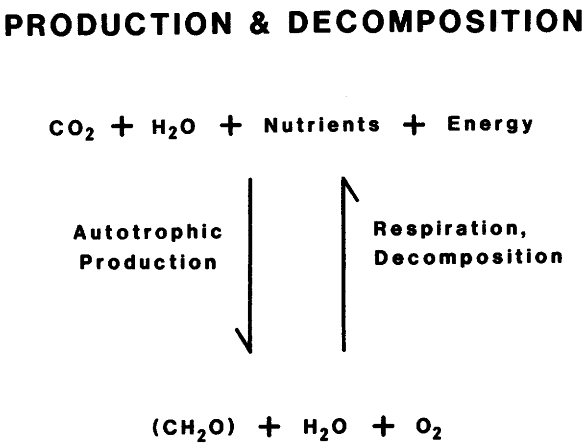


FIGURE 2.–The "equilibruim" relationship between autotrophic (photosynthesis, chemosynthesis) and heterotrophic (respiration, decomposition) processes. The photosynthetic conversion of light energy into chemical potential energy results in nonequilibrium concentrations of carbon, nitrogen, sulfur, and phosphorus in reduced organic compounds of high potential energy. Heterotrophic processes tend to restore the equilibrium by decomposing organic materials by way of energy-yielding oxidation-reduction reactions.

the balance or imbalance of these processes within a water body can have profound chemical and biological consequences for the entire system. The photosynthetic production of oxygen is restricted to the upper, lighted regions of the water column (the euphotic zone). Respiration is not directly light-dependent, but is dependent on temperature. Respiration and organic matter decomposition occur through the water column, and depending on the amount of organic matter present and the extent of wind-mixing, can deplete the dissolved oxygen supply in aphotic (unlighted) layers. The chemical and biological conditions associated with such oxygen depletion (for example, increased nutrient release from sediments, exclusion of oxygen-dependent fauna, and less complete decomposition of sedimented organic matter) are marked, and often characteristics of lake trophic status. In oligotrophic (nutrient-poor, unproductive) lakes, insufficient organic matter is produced for decomposition processes to affect available oxygen levels to any significant extent. In contrast, organic matter decomposition in eutrophic (nutrient-rich, productive) lakes can rapidly deplete dissolved oxygen in aphotic parts of the water column.

[2] However, most chemosynthesis results from energy released in the oxidation of reduced compounds (S^{-2}, NH_4^+, CH_4) derived from the decomposition of previously formed organic matter, and thus is actually a special type of secondary production. A notable exception, where chemosynthesis appears to be a true autotrophic process, is represented by the recently discovered Galapagos Rift hydrothermal vent ecosystems. Here, chemosynthetic bacteria fix CO_2 with energy derived from the oxidation of geothermically reduced sulfur compounds emitted from the ocean-floor vents (see Karl and others, 1980).

MICROBIAL NUTRIENT TRANSFORMATIONS AND REGENERATION

Thus far, organic matter production and decomposition have been discussed primarily in terms of carbon dioxide, water, and oxygen exchanges. However, living organisms are not composed entirely of C, H, and O, but also contain small amounts of many other elements. Table 1 compares the elemental composition of living freshwater plants to the environmental availability of elements for plant growth (that is, demand-vs-supply). Phosphorus, nitrogen, and carbon are the least available elements relative to plant growth requirements, and thus are most likely to limit biological productivity in aquatic systems.

Although essential nutrients are incorporated into organic compounds during organic matter synthesis, they are regenerated as a result of excretion processes and organic matter decomposition. Organic matter decomposition (fig. 3) occurs by:
(1) Hydrolysis of large (high molecular weight) organic polymers into smaller (low molecular weight) compounds; and
(2) Mineralization of these smaller organic molecules to inorganic compounds (for example, H_2S, NH_4^+, and PO_4^{-3}). Bacterial decomposition of organic materials accounts for a large fraction of the total nutrient regeneration which occurs; however, organisms such as phytoplankton, zooplankton, and fish excrete NH_4^+, PO_4^{-3}, and low molecular weight organic compounds, and therefore are also important nutrient regenerators.

In an oxygenated environment, organic matter exists in a chemically reduced state (low Eh, high potential energy) relative to most of its inorganic components (fig. 2). The oxidation of certain mineralization products (for example, $S^{-2} \rightarrow S^{\circ} \rightarrow SO_4^{-2}$, $NH_4^{=} \rightarrow NO_2^{-} \rightarrow NO_3$, and $CH_4 \rightarrow CO_2$) provides the energy for bacterial chemosyntheses. The basic types of microbially mediated transformations important in carbon, nitrogen, and sulfur cycling are summarized in tables 2, 3, and 4; and integrated into the overall nutrient cycling patterns for these elements in lake systems in figures 4, 5, and 6.

Phosphorus does not undergo the oxidation-reduction transformations characteristic of C, N, and S cycles (fig. 7). Inorganic phosphorus occurs in aquatic environments primarily as orthophosphate (PO_4^{-3}), and is incorporated directly by algae and bacteria into phospholipids, nucleic acids, and ATP. Although phosphorus turnover time (the time required for phosphorus assimilation and regeneration) is often only a matter of minutes in surface waters during the summer (Lean, 1973), sedimentation of organic particles and precipitation of inorganic phosphorus with iron and manganese complexes can cause phosphorus depletion in the mixed layers of lakes

TABLE 1.—*Concentrations of essential elements for plant growth in the living tissues of freshwater plants (demand), in mean world river water (supply), and the approximate ratio of elemental concentrations required to those available (that is, demand:supply ratio) (after Vallentyne, 1974)*

Element	Symbol	Demand by plants (%)	Supply in water (%)	Demand: Supply ratio
Oxygen	O	80.5	89	1
Hydrogen	H	9.7	11	1
Carbon	C	6.5	.0012	5,000
Silicon	Si	1.3	.00065	2,000
Nitrogen	N	.7	.000023	30,000
Calcium	Ca	.4	.0015	<1,000
Potassium	K	.3	.00023	1,300
Phosphorus	P	.08	.000001	80,000
Magnesium	Mg	.07	.0004	<1,000
Sulfur	S	.06	.0004	<1,000
Chlorine	Cl	.06	.0008	<1,000
Sodium	Na	.04	.0006	<1,000
Iron	Fe	.02	.00007	<1,000
Boron	B	.001	.00001	<1,000
Manganese	Mn	.0007	.0000015	<1,000
Zinc	Zn	.0003	.000001	<1,000
Copper	Cu	.0001	.000001	<1,000
Molybdenum	Mo	.00005	.0000003	<1,000
Cobalt	Co	.000002	.000000005	<1,000

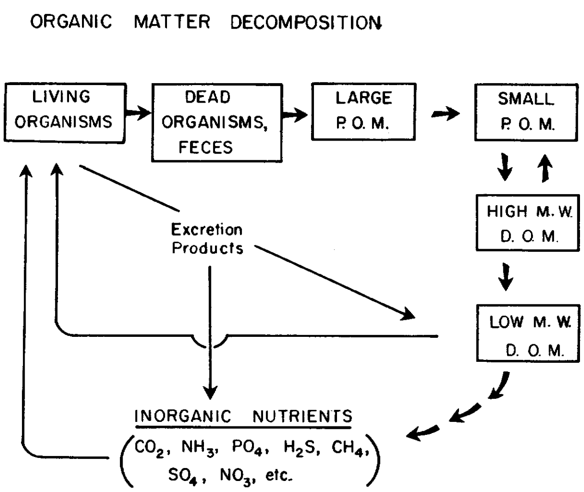


FIGURE 3.—Processes involved in the decomposition and mineralization of organic matter in aquatic environments. POM = particulate organic matter, DOM = dissolved organic matter, MW = molecular weight (modified from Sorokin and Kadota, 1972).

TABLE 2.—*General categories of microbially mediated carbon transformations*
[E = energy]

MICROBIAL TRANSFORMATIONS: CARBON

1. Inorganic C Assimilation (photosyn., chemosyn.)

$$CO_2 + H_2A + E \longrightarrow (CH_2O) + H_2O + 2A$$

2. Respiration, Aerobic Decomposition

$$(CH_2O) + O_2 \longrightarrow CO_2 + H_2O + E$$

3. Anaerobic Decomposition

$$(CH_2O) + X \cdot O \longrightarrow CO_2 + H_2O + E$$
$$CO_2 \longrightarrow CH_4 + E$$

TABLE 3.—*General categories of microbially mediated nitrogen transformations*
[E = energy]

MICROBIAL TRANSFORMATIONS: NITROGEN

1. Inorganic N Assimilation

$$N_{inorg.} + E \longrightarrow (\text{-}NH_2\text{-})$$

2. N_2 Fixation

$$N_2 + H_2 + E \longrightarrow NH_3 \longrightarrow (\text{-}NH_2\text{-})$$

3. Ammonification

$$\text{Org.-N} + O_2 \longrightarrow CO_2 + H_2O + NH_3 + E$$

4. Nitrification

$$NH_3 \xrightarrow{O_2} NO_2 \xrightarrow{O_2} NO_3$$
$$+E: \; CO_2 \longrightarrow (CH_2O) \qquad +E: \; CO_2 \longrightarrow (CH_2O)$$

5. Denitrification

$$NO_3 \longrightarrow N_2, N_2O + E$$
$$(CH_2O) \longrightarrow CO_2 + H_2O$$

TABLE 4.—*General categories of microbially mediated sulfur transformations*
[E = energy]

MICROBIAL TRANSFORMATIONS: SULFUR

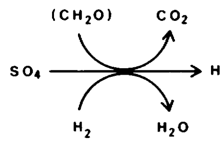
1. Inorganic S Assimilation

$$S_{inorg.} + E \longrightarrow (\text{-}SH\text{-})$$

2. Organic Matter Decomposition

$$(\text{Org.-SH}) + O_2 + H_2O \longrightarrow CO_2 + H_2O + E + H_2S$$

3. Inorganic S Reduction (anaerobic)



4. Inorganic Sulfur Oxidation

a. Chemosynthesis

$$H_2S + O_2 \longrightarrow S^{\circ} + H_2O + E$$
$$CO_2 \longrightarrow (CH_2O)$$

$$S^{\circ} + O_2 + H_2O \longrightarrow H_2SO_4 + E$$
$$CO_2 \longrightarrow (CH_2O)$$

$$S_2O_3, S^{\circ} + HCO_3 \longrightarrow SO_4 + CO_2 + H_2O + E$$
$$NO_3 \longrightarrow N_2$$

b. Photosynthesis

$$CO_2 + H_2S + E \longrightarrow (CH_2O) + H_2O + S$$

and reservoirs. Naturally low levels of available phosphorus in aquatic environments, the essentiality of phosphorus for plant growth, and the high phosphorus concentration in certain byproducts of our modern society (for example, fertilizers, detergents, and municipal sewage) make phosphorus an especially important element in regard to water quality. In short supply, phosphorus availability can severely limit biological productivity, while an overabundance of phosphorus can result in nuisance conditions concomitant with accelerated

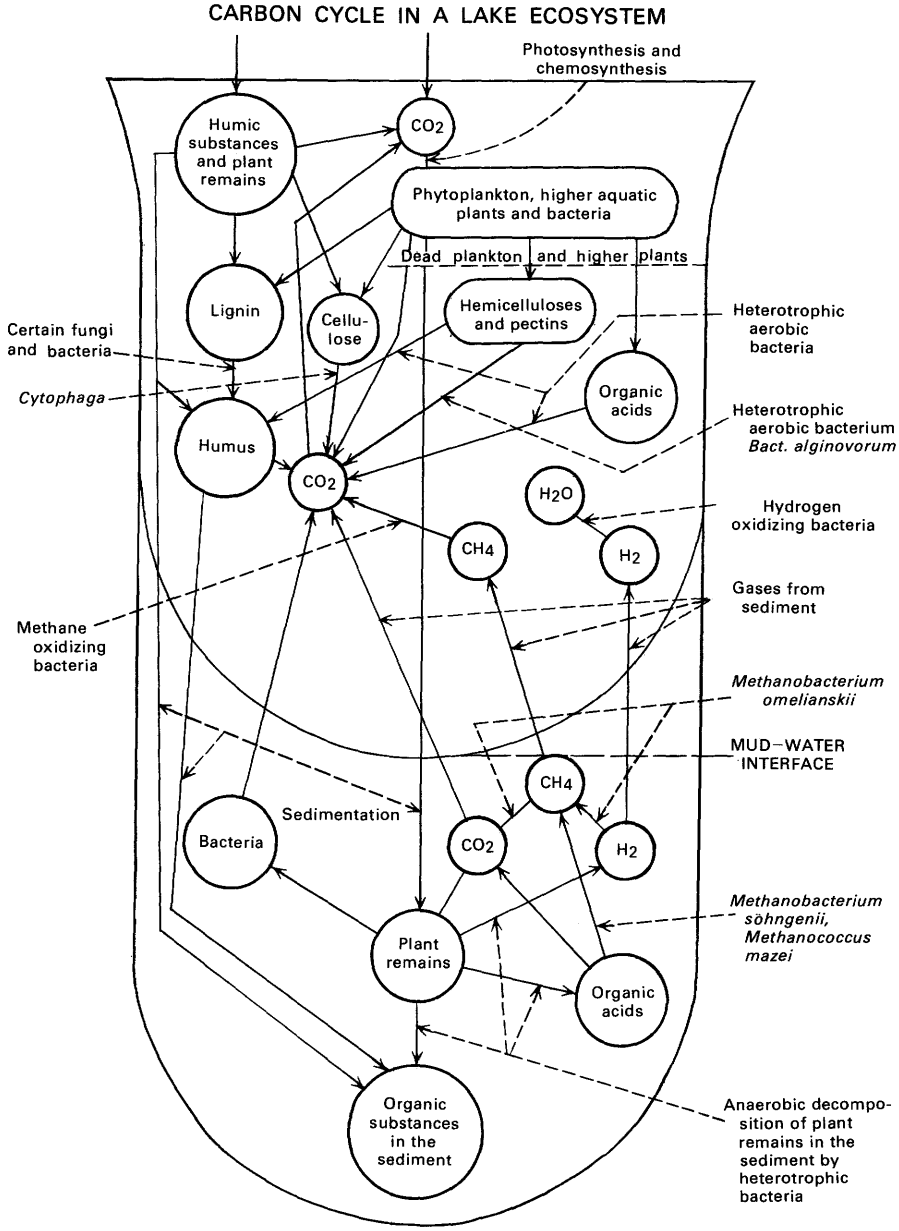


FIGURE 4.—Patterns of carbon cycling in lakes and reservoirs (from Brock, 1966, after Kuznetsov, 1959).

eutrophication.[3] Since phosphorus is often the least available inorganic nutrient in aquatic environments (that is, relative to demand, see table 1), extensive efforts have been undertaken in the past decade to minimize phosphorus loading of natural waters.

Although biogeochemical cycles often are discussed individually by element for the sake of simplicity, it should be realized that in nature they are inseparable. Carbon, nitrogen, sulfur, phosphorus, and other essential elements are assimilated simultaneously during organic matter synthesis, and similarly, are released simul-

[3] Eutrophication is a natural process involving the slow accumulation of nutrients and organic matter, and gradually increased productivity in an aquatic system. Accelerated eutrophication results from excessive nutrient loading from the inflow of municipal wastes and/or drainage from fertilized agricultural land, and often is associated with nuisance algal blooms, taste and odor problems, and severe oxygen depletion accompanied by fishkills.

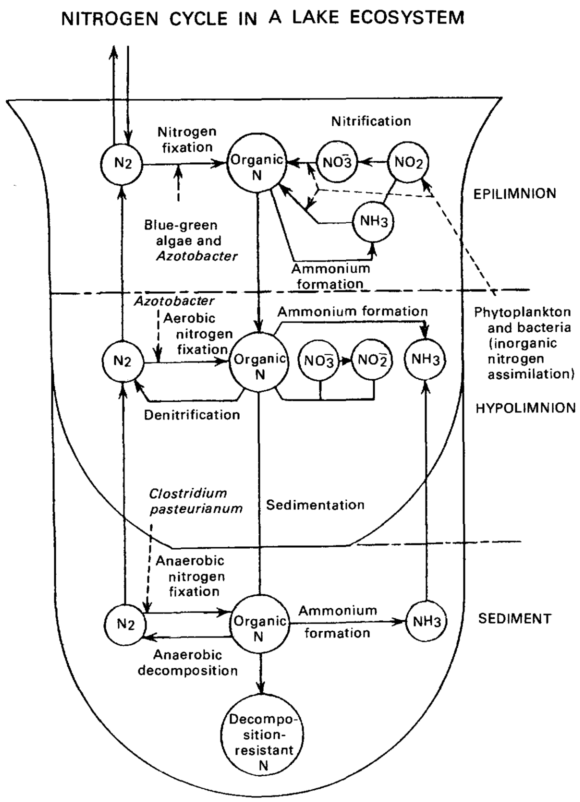


FIGURE 5.—Patterns of nitrogen cycling in lakes and reservoirs (from Brock, 1966, after Kuznetsov, 1959).

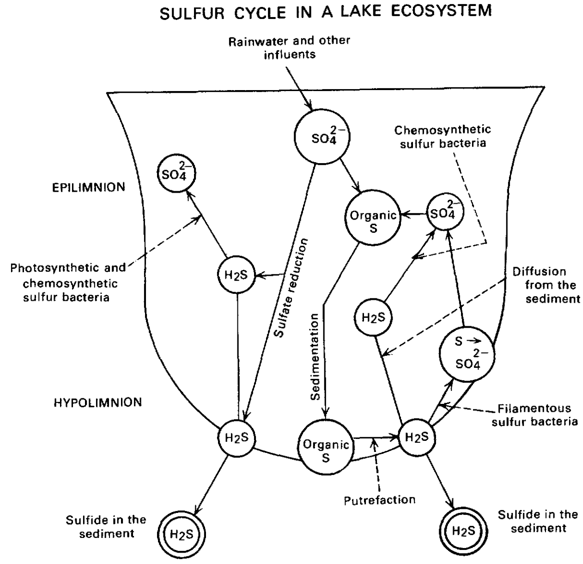


FIGURE 6.—Patterns of sulfur cycling in lakes and reservoirs (from Brock, 1966, after Kuznetsov, 1959).

taneously during organic matter decomposition. Because the availability of certain elements (for example, P, Fe, and Mo) influences the uptake or transformations of others (C, N), the elemental cycles are highly interdependent.

BACTERIA AS PARTICLE PRODUCERS AND POTENTIAL FOOD SOURCES FOR HIGHER TROPHIC LEVELS

The photosynthetic conversion of inorganic compounds of organic matter is only the initial process in a sequence of trophic interactions collectively referred to as the "foodweb." The most familiar foodweb sequence (primary producer→grazer→higher consumer; the grazer pathway) may present an oversimplified view of trophic interactions in lakes and reservoirs by ignoring nonpredatory transfers (primary producer→detritus→bacteria→grazer→higher consumer; the detritus pathway). More specifically, the conversion of dissolved organic compounds (derived from algal extracellular excretion or cell lysis, zooplankton excretion, and/or allochthonous sources[4]) to organic particles by way of bacterial uptake and growth represents a potentially important mechanism for reintroducing otherwise unavailable (that is, unharvestable) dissolved organic matter to the foodweb (fig. 8). For pelagic marine systems, Pomeroy (1974) indicated that the producer-detritus-bacteria-grazer sequence could provide at least 30 percent more energy to higher trophic levels than estimated from consideration of only a producer-grazer pathway.

However, whether bacterial secondary production provides a significant source of particulate organic matter for planktonic grazers is a controversial question at present. Free-living (unattached) bacteria are probably too small (<1 μm) to be efficiently harvested by most zooplankton; however, bacteria colonizing suspended detritus or otherwise associated with aggregates of detritus, algal cells, and/or silt particles may be grazed readily by virtue of their greater effective particle size. Planktonic ciliates (a type of Protozoa) may help bridge the "particle-size gap" between free-living bacteria and grazers by ingesting bacteria and then becoming prey for filter-feeding zooplankton (Porter and others, 1979). The occurrence of attached-vs-unattached bacteria in pelagic

[4] The term "allochthonous" refers to organic matter originating from outside the lake (e.g., material from the watershed). In contrast, "autochthonous" material is that produced within the lake (for example, phytoplankton production).

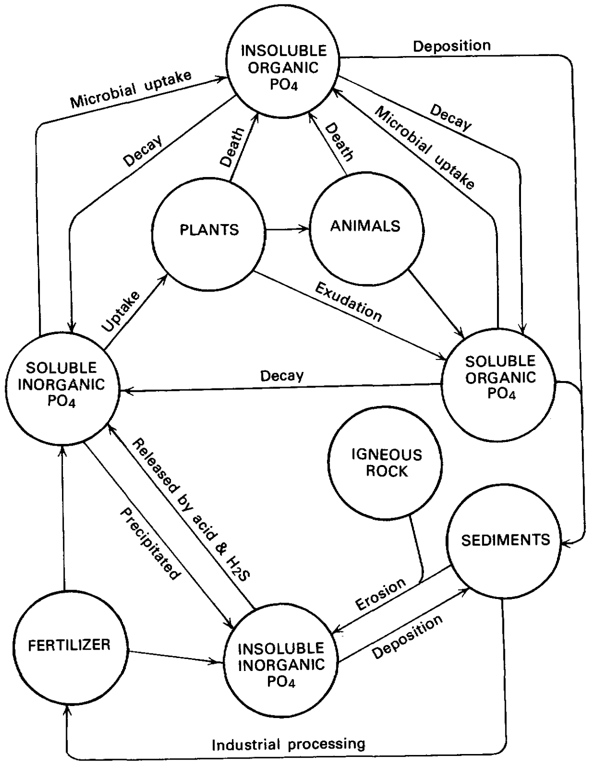


FIGURE 7.–Environmental phosphorus cycling (after Campbell, 1977).

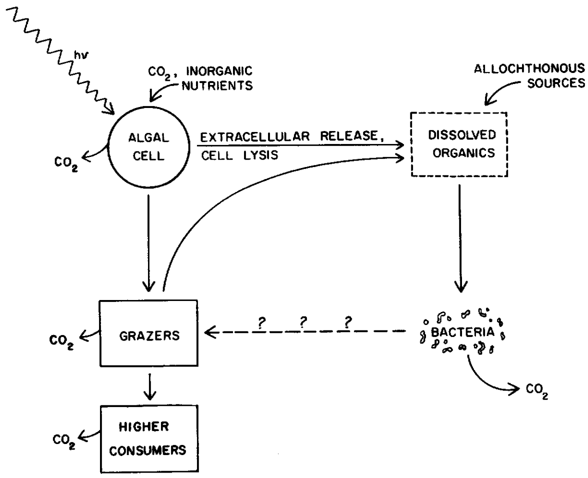


FIGURE 8.–Organic carbon conversions of potential importance in pelagic foodwebs. Some photosynthetic-produced algal biomass is harvested directly by filter-feeding grazers. Dissolved oxygen compounds (present by virtue of allochthonous input, zooplankton excretion, algal excretion, or cell lysis) are unavailable to grazers, but may be returned to a particulate state by bacterial uptake. However, the relative availability of free-living bacteria and bacterial-detrital aggregates to pelagic grazers is uncertain.

environments appears to be highly variable, both temporally and among systems, as numerous investigators have reported contradictory results and conclusions.

Although algal photosynthesis is the primary mechanism of organic matter production in most lakes and reservoirs, the potential significance of heterotrophic micro-organisms as both secondary producers (by conversion of dissolved organic matter to a particulate form) and consumers (by respiration) of organic matter should be recognized. Indeed, bacterial production may be of particular ecological importance in many reservoirs for the following reasons:

(1) Reservoirs usually receive large quantities of allochthonous organic materials by tributary inflow from their watersheds. Some labile (biologically available) fraction of this allochthonous input may provide major supplements of organic substrates for bacterial production;

(2) Erosion products from the watershed (for example, suspended silt and clay particles) are potential sites for adsorption of dissolved organic compounds and bacterial attachment/aggregation, and thereby may constitute a harvestable source of organic matter for planktonic grazers. Alternatively, such microbial-detrital aggregates may sediment rapidly from the water column and result in the transport of organic matter and nutrients to benthic communities;

(3) In turbid, well-mixed reservoirs, phytoplankton production is often limited more by available light than by nutrients. In contrast to photosynthesis, bacterial production is not directly light-dependent and, therefore, may form a predominant fraction of the particulate organic matter supply available to grazers in light-limited systems. Especially for reservoir foodwebs, in which bacteria may be of particular importance, an improved understanding of algal and bacterial production and respiration processes, and the relative availability of these primary and secondary particle producers (that is, phytoplankton and bacteria, respectively) to grazers is required.

SUMMARY

The classical trophic-dynamics approach (Lindeman, 1942) persists as an ecological paradigm even though the nondiscrete nature of trophic levels was recognized by Lindeman and is well known to most ecologists. Although useful as a conceptual framework, the trophic level approach may lead to a simplistic view of energy and materials flow in nature (Wetzel and others, 1972; Rigler, 1975; Goldman and Kimmel 1978). Most aquatic organisms can be considered as article producers (by either autotrophic or heterotrophic production of protoplasm), consumers (assimilation of dissolved or particulate organic compounds), and decomposers (by conversion of organic materials to inorganic materials by respiration and excretion). Nutrient regeneration, the classical role of bacteria and fungi, takes place at virtually every level of the foodweb. Figures 9 and 10 summarize contrasting views of energy and nutrient flow in lakes and reservoirs. Particularly for reservoirs, our present level of understandings of ecosystems dynamics is inadequate to determine the relative accuracy of either view.

Micro-organisms, by virtue of their pivotal roles in organic matter production and decomposition, foodweb transfers by dissolved to particulate organic matter conversions, nutrient uptake and regeneration, and biogeochemical transformations, are essential to the ecological functioning of aquatic systems. By the nature of their biological activities, autotrophic and heterotrophic micro-organisms are sensitive indicators of the ecological and water-quality status of aquatic environments. However, only by thoroughly understanding how micro-organisms function in healthy aquatic ecosystems, can we hope to recognize or accurately predict their responses to water-quality changes or other environmental disturbances.

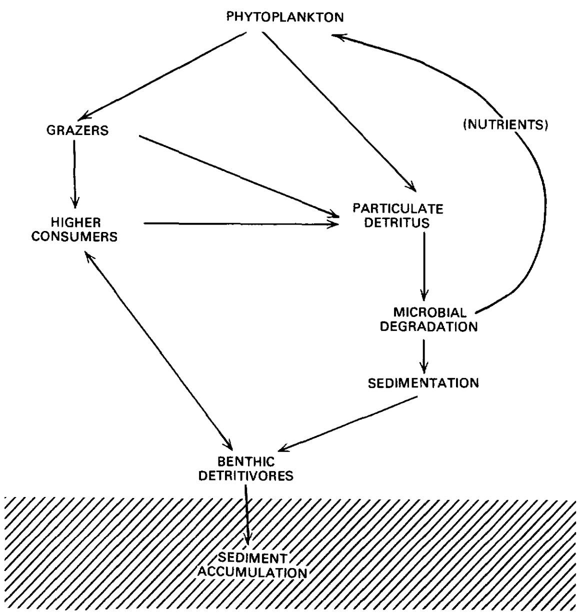


FIGURE 9. – A classical view of energy and nutrient flow in a lake or reservoir ecosystem. As symbolized by the relative size of the arrows, most of the energy and nutrients flow through the grazer pathway, and the detritus pathway is assigned the role of nutrient regeneration (after Goldman and Kimmel, 1978).

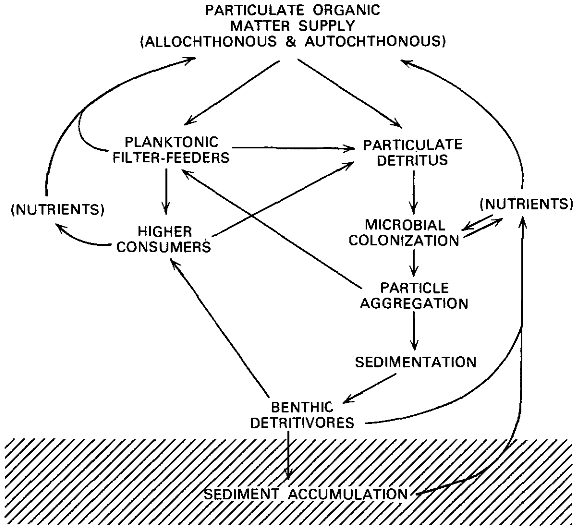


FIGURE 10. – A more complex view of energy and nutrient flow in a lake or reservoir ecosystem. Allochthonous inputs of dissolved and particulate organic materials directly enter the detritus pathway, and support bacterial growth and detritivores (detritus-feeders). Much of the primary production may also enter the detritus pathway directly via phytoplankton mortality unrelated to grazing. Adsorption of dissolved organic compounds, microbial colonization, and particle aggregation produces microheterotroph-detrital aggregates which are, by virtue of their particle size, available to planktonic grazers. Sedimentation of detrital aggregates provides a major energy and nutrient source for benthic detritivores which become prey items for higher consumers. Nutrient regeneration occurs at virtually every foodweb level, and only a small fraction of the total organic matter supply accumulates as permanent sediment (after Goldman and Kimmel, 1978).

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Effects of Bacteria on the Chemical and Physical State of Iron

By Gail E. Mallard

ABSTRACT

Bacterial activity can bring about transformations in the chemical and physical state of iron such as oxidation, reduction, solubilization, precipitation, and formation and decomposition of organic-iron complexes. A brief overview of the chemical and biological reactions of iron in soils and water is presented for the nonspecialist who has some familiarity with chemistry and microbiology. The physiology and ecology of certain bacteria that commonly affect iron are outlined and references for procedures used in the cultivation of these organisms are provided.

INTRODUCTION

This report presents, for the nonspecialist who has some familiarity with chemistry and microbiology, a brief overview of the chemical and biological reactions of iron in soils and water. It mentions some of the resulting problems and describes several of the principal organisms involved. Emphasis is placed on the bacteria that oxidize or reduce iron as an integral part of their metabolism, but organisms that cause reactions indirectly also are considered.

In soils and water, iron and compounds of iron exist as dissolved ions, solids, and colloidal suspensions. Bacterial activity can bring about the following transformations in the chemical or physical state of iron: oxidation, reduction, solubilization, precipitation, and formation and decomposition of organic-iron complexes. These alterations may be enzyme-catalyzed, in which case they probably contribute to the cell's metabolism, or they may be due to nonspecific chemical reactions occurring in response to changes in the environment, which are in turn induced by microbial activity. In either case, the presence and activity of certain microorganisms will cause a change in the state of iron. Although the bacteria associated with iron in soils and water commonly are referred to as "iron bacteria," this is actually a general term applied to a diverse group of organisms having greatly differing metabolic capabilities and living in a wide variety of habitats.

The two most serious problems associated with iron bacteria are the adverse effects on flow and water quality in water-supply systems, and acidification of water draining from mines ("acid-mine drainage"). Water-supply systems typically are affected by the deposits of ferric hydroxide and the gelatinous growth of iron bacteria, both of which lead to reduced potability (unpleasant taste and odor), discoloration of water, clogged well screens, and a reduction in flow through pipes. In coal-mining operations, the bacterium, *Thiobacillus ferrooxidans,* produces large quantities of sulfuric acid as a waste product of its metabolism, and when this acid waste flows over land and enters a body of water, the land and receiving water become unfit for many terrestrial and aquatic organisms.

CHEMICAL REACTIONS OF IRON

Microbiological changes in suspended or dissolved iron may accompany naturally occurring chemical changes and in some case will compete with them. Additionally, alterations in the environment brought about by microbial activity may shift chemical equilibria and thereby change the dominant form of iron. For these reasons, the chemical reactions of iron, exclusive of biological activity, are described briefly below. The chemistry of iron in natural waters has been extensively reviewed and explained in numerous papers by Hem (1960, 1961, 1970, 1972; and Hem and Cropper, 1959).

In natural waters, iron occurs as ferrous (reduced, Fe^{+2}) and ferric (oxidized, Fe^{+3}) ions as well as various oxides and hydroxides. Some of the pertinent reactions of iron are listed below:

$$Fe^{+2} \rightleftharpoons Fe^{+3} + e^- \quad (1)$$
$$Fe^{+2} + H_2O \rightleftharpoons FeOH^{+2} + H^+ + e^- \quad (2)$$
$$Fe^{+2} + 2H_2O \rightleftharpoons Fe(OH)_2^+ + 2H^+ + e^- \quad (3)$$
$$Fe^{+2} + 3H_2O \rightleftharpoons Fe(OH)_3 + 3H^+ + e^- \quad (4)$$
$$FeOH^+ + 2H_2O \rightleftharpoons Fe(OH)_3 + 2H^+ + e^- \quad (5)$$
$$Fe(OH)_2 + H_2O \rightleftharpoons Fe(OH)_3 + H^+ + e^- \quad (6)$$
$$Fe^{+2} + HCO_3^- \rightleftharpoons FeCO_3 + H^+ \quad (7)$$
$$Fe^{+3} + H_2O \rightleftharpoons FeOH^{+2} + H^+ \quad (8)$$
$$FeOH^{+2} + H_2O \rightleftharpoons Fe(OH)_2^+ + H^+ \quad (9)$$
$$Fe(OH)_2^+ + H_2O \rightleftharpoons Fe(OH)_3 + H^+ \quad (10)$$

Hydrogen ions, electrons, or both appear in all of the above equations; therefore, the final equilibrium concentrations of the iron compounds are, in part, dependent upon Eh and pH. The effect of Eh and pH on iron, and the ranges in which particular species of ions or solids will be stable at chemical equilibrium, may be depicted in stability-field diagrams as shown in figure 11. Such diagrams depict one or more solid components of known chemical composition in contact with water. The concentration of the dissolved ions will affect the location of the boundaries. It should be noted that in some cases, stability-field diagrams do not reflect the true situation because the equilibrium conditions assumed in their preparation may never be attained.

Some of the characteristics of iron that are important in determining its solubility and availability were listed by Hem (1972, p. 443) and are quoted below:

(1) Iron may occur in the ferric (oxidized) or ferrous (reduced) state, and oxidation or reduction is readily accomplished;
(2) Oxidized forms tend to have low solubility, especially in alkaline solutions;
(3) Reduced metals can form compounds with carbonate or sulfide that are low in solubility;
(4) Organic and inorganic solute complexes are formed with many ligands and this tends to increase the solubilities of iron species;
(5) Colloidal dispersions of ferric hydroxide can occur.

In general, when Eh and pH are low, iron solubility is high. "Water in contact with air will have an Eh of approximately 0.35 to 0.50 volt, and usually its pH is greater than 5." Under these conditions, "ferric hydroxide or ferric oxide is the stable form and iron solubility is low" (Hem, 1961, p. 227).

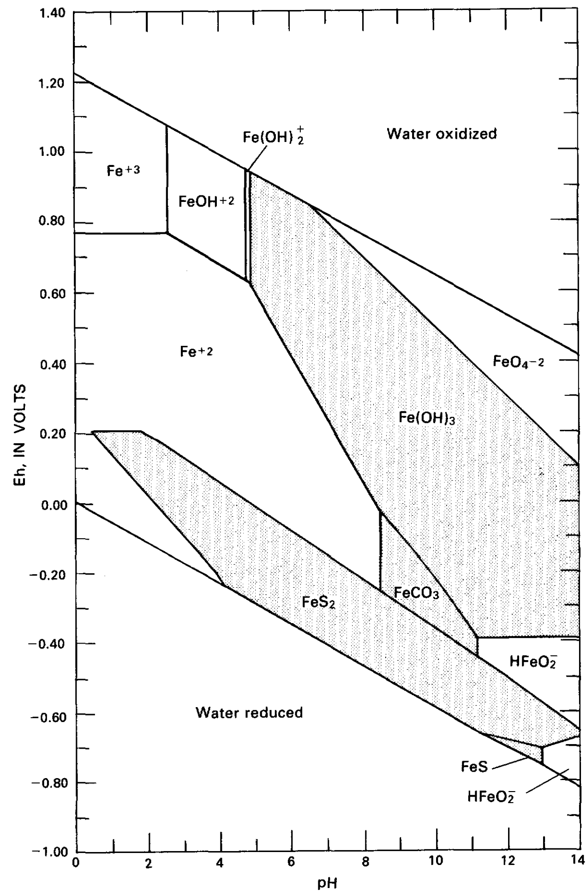

FIGURE 11.–Fields of stability for solid and dissolved forms of iron as a function of Eh and pH at 25°C and 1 atmosphere of pressure. Activity of sulfur species 96 mg/L as SO_4^{-2}, carbon dioxide species 1,000 mg/L as HCO_3^-, and dissolved iron 0.0056 mg/L (from Hem, 1970, p. 118).

EFFECTS OF MICROBIAL ACTIVITY ON IRON

The chemical and physical state of iron can be altered both directly and indirectly by microbial activity. Bacteria can oxidize and reduce iron by direct enzymatic mechanisms; they also can consume oxygen, produce and degrade organic compounds, and change the Eh and pH of the environment. Microbiologically mediated changes in iron, and environmental changes that affect iron, are illustrated in figure 12. Iron-oxidizing and iron-reducing bacteria cause a direct, enzymatic change in iron that is important in energy generation for the cells. The other changes in iron illustrated in figure 12 are caused indirectly, by products of bacterial metabolism; the cells responsible may be unaffected by the presence or absence of iron. Some of the changes in iron and the bacteria that cause these changes are discussed in the following section.

DECOMPOSITION AND FORMATION OF ORGANIC-IRON COMPOUNDS

Sugars, simple organic acids, and highly polymerized humus constituents can combine with ferrous and ferric iron to form iron-organic complexes (Alexander, 1977). Because iron is more soluble and stable when complexed with organic compounds than when alone (Hem, 1960), microbial activity leading to the production or decomposition of such compounds may affect the chemical and physical state of iron. Bacterial activity may stabilize iron if the organic acids produced as waste products of microbial metabolism accumulate and combine with the iron. Conversely, if the organic part of an iron-organic complex is degraded by microbial metabolism, its stabilizing effect will be removed and iron will be subject to oxidation and (or) precipitation. For example, bacterial metabolism causes iron to precipitate from solutions of the citrate, lactate, acetate, malate, malonate, oxalate, and gallate salts of iron (Alexander, 1977). Some of the heterotrophic organisms that bring about the precipitation of iron from solution by using the organic part of an iron-organic complex are *Enterobacter, Serratia, Bacillus, Klebsiella, Alcaligenes, Moraxella, Corynebacterium, Caulobacter, Mycobacterium, and Escherichia* (Cullimore and McCann, 1977).

IRON REDUCTION

The reduction of iron requires an organic energy source and a low redox potential. The reduction may be either an indirect chemical process due to changes in Eh or a direct enzymatic reduction. Ottow (1968) demonstrated that bacteria capable of reducing iron were distributed through a column of soil and were present at levels of 10^4 to 10^5 per gram of dry soil. Some of the genera containing species able to reduce iron are *Bacillus, Clostridium, Klebsiella, Pseudomonas,* and *Serratia* (Alexander, 1977).

Many heterotrophic organisms potentially are able to bring about a sequence of events that result in indirect iron reduction. As organic compounds are metabolized, oxygen is consumed and reducing compounds are produced; this lowers the Eh and shifts the equilibrium in the direction of ferrous iron. Another, more direct, mechanism is the transfer of electrons from a reduced carbon compound to ferric iron, possibly resulting in the generation of energy for the cells responsible. These bacteria are not highly specialized organisms; they seem to be using iron as one of several possible terminal electron acceptors. Evidence indicates that at least two different enzymes or enzyme systems are involved. Ottow (1968) investigated the physiology of iron reduction with pure cultures of *Enterobacter aerogenes, Escherichia coli,* and six species of *Bacillus.* Because nitrate suppressed iron reduction in cultures that had the enzyme nitrate reductase, Ottow suggested that ferric iron was acting as an alternative terminal electron acceptor for nitrate reductase. Later studies (Ottow and Munch, 1978) with a *Clostridium* species that did not possess the enzyme nitrate reductase, but was able to reduce iron, led to the suggestion that at least two different enzymes are involved in iron reduction – nitrate reductase and another enzyme able to reduce iron (and manganese), but unable to reduce nitrate.

Iron reduction may be responsible for the production of gleys – sticky bluish-gray soils – in waterlogged areas (Alexander, 1977). The color of the gleyed zone is due to the ferrous sulfide produced when the end product of iron reduction (Fe^{+2}) reacts with hydrogen sulfide, the end product of bacterial sulfate reduction.

IRON OXIDATION AND PRECIPITATION OF IRON COMPOUNDS

The oxidation of ferrous to ferric iron and the subsequent precipitation of insoluble ferric hydroxides commonly is associated with a heterogeneous group of bacteria. *Thiobacillus ferrooxidans* lives in environments of low pH, oxidizes iron enzymatically, and thereby derives its energy. *Gallionella* also obtains energy from iron oxidation, although it requires certain environmental conditions (neutral pH and the presence of oxygen) that force it to compete with the spontaneous chemical oxidation of ferrous iron. In addition, a variety of organisms living in aerobic environments at neutral pH deposit ferric hydroxide

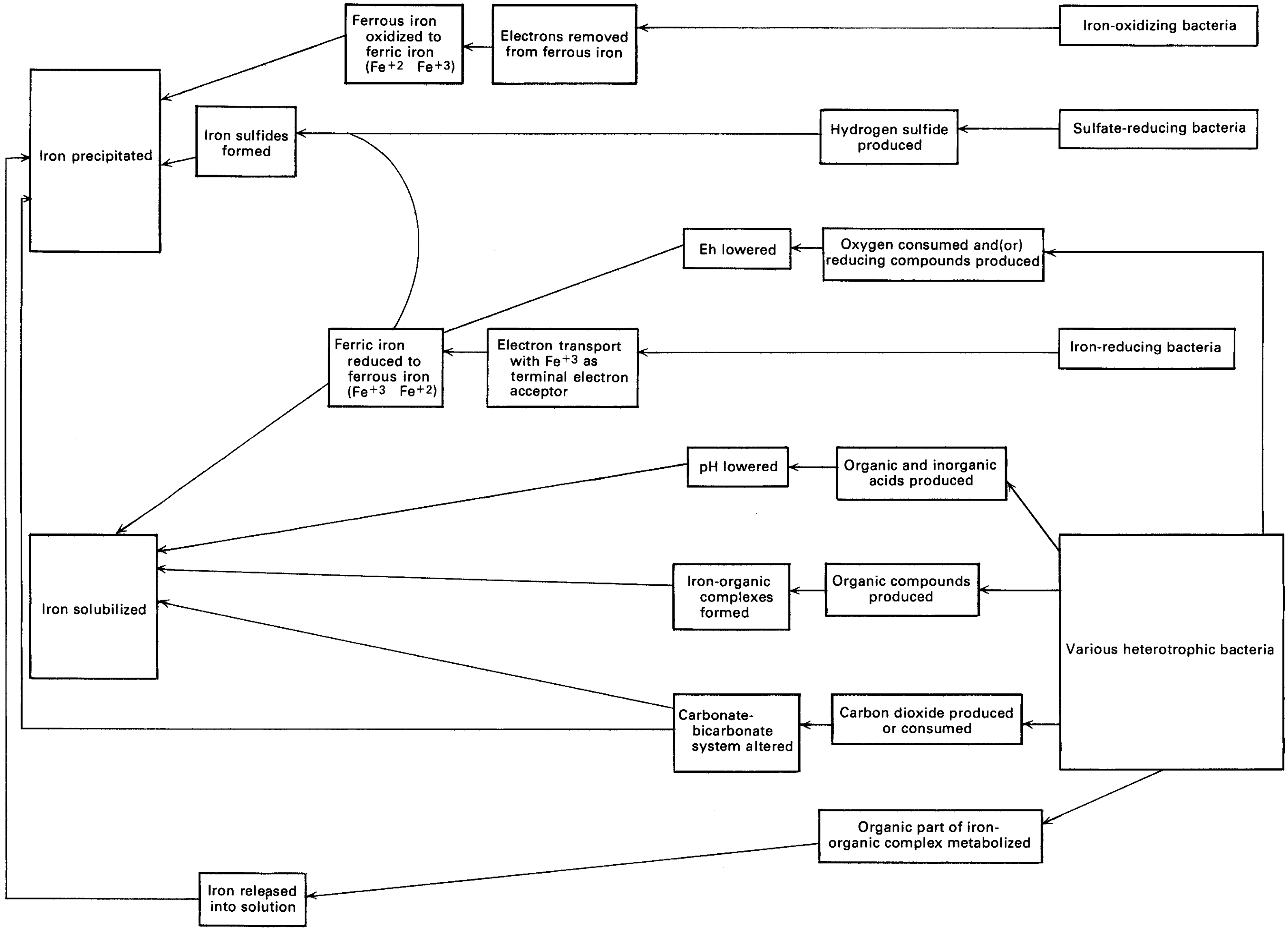

FIGURE 12.—Direct and indirect changes in iron resulting from microbial activity.

on the external surface of the cell (cell wall, capsule, or sheath). For these bacteria, it is likely that the oxidation of ferrous iron is nonenzymatic and that the bacteria derive no energy from the process, but merely serve as convenient sites for precipitation of the ferric compounds generated by spontaneous chemical reactions. The following sections summarize information about the ecology and physiology of bacteria that oxidize and (or) precipitate iron.

Thiobacillus ferrooxidans

Thiobacillus ferrooxidans, an acidophilic bacterium requiring a pH range of 2.5–5.8, is able to grow without organic compounds and derives energy from the oxidation of ferrous iron and cell carbon from carbon dioxide. The ability to grow at low pH is significant for the cell's energy-generating metabolism because it is only below pH 5 that ferrous iron is stable in the presence of the oxygen that is required by this organism. In nature, *Thiobacillus ferrooxidans* uses ferrous sulfate ($FeSO_4$), pyrite (FeS_2), and other reduced iron compounds, and forms ferric oxides, hydroxides, sulfates, and phosphates as end products (Lundgren and others, 1972). It also is able to oxidize elemental sulfur, thiosulfate, and possibly other reduced sulfur compounds.

Electrons removed from iron are transferred to oxygen, giving the following overall reaction:

$$4\ Fe^{+2} + O_2 + 4\ H^+ \rightarrow 4\ Fe^{+3} + 2\ H_2O$$

The energy yield of this reaction is low [about 10–11 kilocalories of energy per mole of iron (55.8 g) oxidized], and energy requirements for cell synthesis are high [about 115 kilocalories of energy to fix 1 mole of carbon (Lundgren and others, 1972)]; therefore, *Thiobacillus ferrooxidans* must convert a large amount of substrate for a relatively low cell yield. Estimates of the ratio of iron oxidized to cell material formed a range as high as 500:1 (Wolfe, 1964).

In mining operations, the action of iron-oxidizing bacteria in degrading pyrite and other metal sulfide minerals, and the large amounts of waste products produced (sulfuric acid, ferrous iron, and other metal ions), leads to the major water-quality problem referred to as acid-mine drainage. This acid effluent has obvious detrimental effects on both the land over which it flows and upon the receiving waters. It has been estimated that "10,000 miles of streams and 29,000 surface acres of impoundments and reservoirs are seriously affected by surface mining operations and that deep mining (and other types of mining) add to these figures significantly." (Lundgren and others, 1972, p. 69). Alexander (1977, p. 370) cites an estimate that "the bituminous mines of western Pennsylvania alone add about a million tons of sulfuric acid each year to the Ohio River drainage area."

The reactions leading to the production of acid-mine waters are presented below with pyrite used as an example (Lundgren and others, 1972):

$$\underset{\text{pyrite}}{2\ FeS_2} + 2\ H_2O + 7\ O_2 \rightarrow \underset{\text{ferrous sulfate}}{2\ FeSO_4} + \underset{\text{sulfuric acid}}{2\ H_2SO_4} \quad (1)$$

$$\underset{\text{ferrous sulfate}}{4\ FeSO_4} + O_2 + 2\ H_2SO_4 \rightarrow \underset{\text{ferric sulfate}}{2\ Fe_2(SO_4)_3} + 2\ H_2O \quad (2)$$

$$\underset{\text{ferric sulfate}}{Fe_2(SO_4)_3} + 6\ H_2O \rightarrow \underset{\text{ferric hydroxide}}{2\ Fe(OH)_3} + 3\ H_2SO_4 \quad (3)$$

The initial oxidation of pyrite or other metal sulfide may be biological or nonbiological. The slow nonbiological oxidation of pyrite is accelerated greatly by the addition of a small amount of acid-mine water, which may contain as many as 10^6 iron-oxidizing organisms per milliliter (Lundgren and others, 1972). The catalytic activity is attributed to the bacteria because it is eliminated by sterilization and is negligible at 0°C (Alexander, 1977). In the absence of a catalyst, the oxidation of ferrous to ferric iron (reaction 2, above) proceeds very slowly below pH 4 and is the rate-limiting step in the oxidation of iron pyrite and the formation of acidity. Microbial activity has been shown to accelerate this reaction by a factor of larger than 10^6 (Lundgren and others, 1972). Reaction 3 (above) is nonbiological.

Pyrite oxidation also may occur by an alternative mechanism in which the ferric iron generated biologically in reaction 2 spontaneously oxidizes the iron sulfide as in the following reaction:

$$FeS_2 + 14\ Fe^{+3} + 8\ H_2O \rightarrow 15\ Fe^{+2} + 2\ SO_4^{-2} + 16\ H^+ \quad (4)$$

The ferrous iron produced from this reaction may then be oxidized by the iron bacteria.

Gallionella

Gallionella is a unique stalked bacterium that lives at neutral pH and has been reported to obtain energy by oxidizing iron. In nature, these organisms are found in soil and water containing ferrous iron; they also may be found in water-supply systems, where the iron hydroxide deposits associated with this organism may lead to reduced flow in the lines, discolored water, and other problems.

Gallionella is probably autotrophic. The cells, which are found in waters low in organic carbon, oxidize ferrous to ferric iron and fix measurable quantities of $^{14}CO_2$. Furthermore, *Gallionella* can grow on a simple medium (Wolfe, 1964) containing mineral salts, carbon dioxide, and ferrous sulfide as an energy source. The organisms are microaerophilic and grow well under low oxygen concentrations (0.1 to 0.2 mg/L of O_2); higher levels (>2.75 mg/L of O_2) inhibit growth (Cullimore and McCann, 1977). The optimum pH for growth is 6 to 7. In laboratory culture, *Gallionella* exhibits zonal growth in an area of the test tube where the organisms can compete for ferrous iron as well as oxygen and in the same region where Fe^{+3} is deposited in uninoculated tubes (Wolfe, 1964).

The type species for the genus is *Gallionella ferruginea.* Other species may exist, but are not well described. The cells of *Gallionella* sp. are kidney-shaped or rounded and have a long stalk that is secreted from the side of the cell perpendicular to its long axis. The stalk, which is composed of bundles of helically twisted fibrils and covered with ferric hydroxide, usually is attached to a substrate. It elongates as the cell grows and may bifurcate at the time of cell division.

Sphaerotilus-Leptothrix GROUP

The two genera, *Sphaerotilus* and *Leptothrix,* are frequently discussed together because they are similar in morphology and because there is some confusion in the literature as to their differentiation and classification. Although both are commonly referred to as "iron bacteria" and deposit ferric hydroxide, it is unlikely that either is able to obtain energy from iron oxidation.

The genus, *Sphaerotilus,* contains only one species; the genus, *Leptothrix,* contains five. All of these organisms are rod-shaped and generally occur in chains encased within a sheath, but some occur as single cells that are motile. In the latest issue of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974), the recognized source of taxonomic information on bacteria, the two genera are differentiated on the basis of sheath coating: *Leptothrix* sheaths typically are encrusted with iron or manganese oxides, whereas *Sphaerotilus* sheaths rarely are encrusted with iron and never with manganese oxides. Although a complete discussion of the taxonomy of these organisms is beyond the scope of this paper, the uncertainty of differentiation between the two groups is indicated in the following statement by Mulder and van Veen in their description of *Sphaerotilus natans* (in Buchanan and Gibbons, 1974, p. 129): "S. *natans* may thus behave like an iron bacterium and according to some authors it is identical with *Leptothrix ochracea* * * *. However, the present authors have clearly shown that the two organisms are distinct * * *." The taxonomy and classification of *Sphaerotilus* and *Leptothrix* have been reviewed by Wolfe (1964), Mulder (1964), and Dondero (1975), in addition to the discussion by Mulder and van Veen (in Buchanan and Gibbons, 1974).

Although the sheath of *Leptothrix,* and rarely *Sphaerotilus,* may be encrusted with iron hydroxides, it is unlikely that the bacteria oxidize iron or derive energy from it. Because both organisms grow at the same pH, near neutrality, at which ferrous iron (Fe^{+2}) is chemically oxidized to ferric iron (Fe^{+3}), it is probable that the sheaths serve only as a site for the deposition of the insoluble ferric hydroxides. Dondero (1975, p. 419) described studies by Cataldi, who was:

> * * * unable to grow 255 isolates of *Leptothrix* and *Sphaerotilus* on purely mineral media, although they grew on media containing 0.005% peptone and 0.01% manganese acetate. The absence of additional iron or manganese in the medium was not detrimental to growth, nor was the presence necessarily beneficial. In pure culture, iron was never oxidized * * *. Oxidation of the iron or manganese could be produced with dead cells, as well as with live cultures.

Similarly, Wolfe (Heukelekian and Dondero, 1964, p. 108), in a discussion of Mulder's paper, reported that ferric hydroxide was deposited on the sheaths of *Sphaerotilus natans* cells that had been killed. It is, therefore, unlikely that the *Sphaerotilus-Leptothrix* group causes the oxidation of iron or obtains any energy from the process.

Both *Sphaerotilus* and *Leptothrix* are found attached to submerged rocks, plants, and so on in slowly running waters. In this type of habitat, the flowing water provides the organisms with a continuous source of nutrients and also carries away their waste products. *Leptothrix* is found in unpolluted water containing iron, whereas

Sphaerotilus is associated with water having a higher concentration of organic material.

These organisms have some economic importance because "they grow in pipes along with deposits of iron or manganese and interfere with the passage of water in wells and filtration plants" (Dondero 1975, p. 408). Massive growth of *Sphaerotilus* in streams and sewage treatment plants is a common problem, although this phenomenon is not associated with iron deposition.

OTHER IRON-DEPOSITING BACTERIA

In addition to the bacteria described above, *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) lists 14 other genera that become coated or encrusted with iron deposits (chiefly ferric hydroxide). Most of these organisms have not been cultivated in the laboratory or grown in pure culture; thus, details of their physiology and metabolism are unclear. However, because all of these bacteria require oxygen and live at near neutral pH, it is likely that the cells or associated sheaths or capsules serve only as a site for precipitation of chemically oxidized iron. It is unlikely that these organisms obtain energy from iron oxidation.

A list grouping the iron-depositing bacteria into four major categories and giving some of their pertinent characteristics as in *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) is presented below:

I. Gliding Bacteria
- *Toxothrix:* no pure cultures
  - not cultivated in the laboratory
  - iron not needed for growth
  - optimum pH 6.5–7.2
  - develops best at reduced oxygen tensions
  - found in cold springs, bogs, ponds, and lakes containing ferrous iron.

II. Sheathed Bacteria
- *Lieskeella:* no pure cultures
  - deposits ferric hydroxide on capsule
  - found in upper layers of mud in bodies of water.
- *Crenothrix:* not grown on artificial media in pure culture
  - sheath may be encrusted with iron oxides
  - found in stagnant and running waters containing organic matter and iron salts.
- *Clonothrix:* not grown in pure cultures on artificial media
  - sheath may be encrusted with iron compounds giving yellowish brown color
  - found attached to iron fittings in well water.

III. Budding and (or) Appendaged Bacteria
- *Pedomicrobium:* pure cultures available
  - ferric iron compounds deposited
  - grow at neutral pH
  - microaerophilic to aerobic
  - widely distributed in soil and water.
- *Seliberia:* pure culture available
  - deposits iron hydroxide in iron-containing media and in soils
  - facultatively anaerobic
  - common inhabitant of soils.
- *Planctomyces:* not grown on artificial media
  - cells encrusted with ferric hydroxide
  - aerobic
  - found in surface layers of lakes.
- *Metallogenium:* have been grown in pure culture
  - iron oxides deposited
  - optimum pH 6.8–7.0
  - found in soil and water.
- *Caulococcus:* have not been cultured in the laboratory
  - deposits ferric iron
  - microaerophilic
  - found in bottom mud of lakes.
- *Kusnezovia:* not cultured in the laboratory
  - oxidizes and deposits iron
  - microaerophilic
  - found in mud samples from lakes.

IV. Gram-negative, Chemolithotrophic Bacteria

Four genera are placed in the Family Siderocapsaceae. The main characteristic of this group is its ability to deposit iron and (or) manganese oxides on or in capsules when present, or on extracellular material. G. A. Zavarzin, writing about this family in *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974, p. 464), makes the following observations:

> Few of the organisms in this family have been cultivated and the validity of a taxon based on deposition of iron and manganese oxides is doubtful, as it is now known that many other bacteria have this ability. The four genera recognized have been observed and described by numerous investigators and they are of geological importance. Differentiation is mainly on morphological features.

The following editorial note also is included in the introductory material for the group (Buchanan and Gibbons, 1974, p. 464):

> The author has questioned the validity of the family and to the editors, its placement has presented a problem.
>
> The four genera included have only one feature in common—the ability to deposit or to cause the deposition of iron and/or manganese oxides. Most are organotrophs. *Siderococcus* reproduces by budding and could be placed in Part 4. Dr. Dubinina has recently obtained *Siderocapsa* in pure culture and found it indistinguishable from *Arthrobacter globiformis*. *Ochrobium* may be an alga.
>
> As it is impossible to place the family, as here constituted, in a more logical place it is included with the chemolithotrophs, even though it is known that in many instances the deposition of iron or manganese is the result of the metabolism of the organic ion of the compound.

Siderocapsa: pure culture available
- capsule encrusted with iron compounds
- generally aerobic, but may grow at reduced oxygen tensions
- found in neutral to slightly alkaline conditions
- common in freshwater.

Naumanniella: capsule coated with deposits of iron
- complex organic compounds of iron are decomposed
- widely distributed in iron-bearing waters.

Ochrobium: cells surrounded by capsule containing iron
- widely distributed in iron-bearing freshwater.

Siderococcus: ferric hydroxide deposited in medium, cells not encrusted with iron compounds
- growth observed only in natural habitat and isolated mud samples
- found in mud habitats with low oxygen concentrations and neutral pH
- widely distributed in freshwaters and bottom deposits.

CULTIVATION AND IDENTIFICATION OF IRON BACTERIA

Bacteria which are able to bring about a change in the chemical or physical state of iron are a diverse group living in many different habitats and having greatly differing requirements for growth. Because it would be impossible to cultivate all of these organisms in the same medium and by the same procedures, a list of all media and procedures used is necessary, but beyond the scope of this report. Instead, references for techniques used to screen water samples for iron bacteria and additional references on methods to cultivate specific types of bacteria are presented. The best source of information for the identification of iron bacteria is *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974).

Iron-reducing bacteria require an organic source of carbon and energy and may be grown on a general bacteriological medium with the addition of ferric iron to test for iron reduction. Ottow (1968) and Ottow and Munch (1978) describe some of the media and procedures used to detect iron-reducing bacteria.

General screening methods for iron bacteria are given by Rodina (1972) and Cullimore and McCann (1977). These methods include differential staining followed by microscopic observation, as well as several enrichment techniques in which visible color changes and (or) distinctive patterns of growth indicate the presence of iron bacteria.

A medium suitable for the growth of heterotrophic, iron-precipitating bacteria is given in Standard Methods (American Public Health Association and others, 1976). Rodina (1972) lists several media for the cultivation of iron-oxidizing and iron-precipitating bacteria; some of these media are evaluated by Cullimore and McCann (1977). Other references for media and procedures to culture specific types of iron bacteria are:

(1.) *Thiobacillus ferrooxidans*
- a. Silverman and Lundgren (1959)
- b. Lundgren and others (1972)
- c. American Public Health Association and others (1976).

(2.) *Metallogenium* sp.
- a. American Public Health Association and others (1976).

(3) *Gallionella* sp.
- a. Wolfe (1958)
- b. American Public Health Association and others (1976).

(4.) Budding Bacteria
- a. Ghiorse and Hirsch (1978).

(5.) *Sphaerotilus-Leptothrix*
- a. American Public Health Association and others (1976)
- b. Dondero (1975).

SUMMARY

Bacteria are able to bring about changes in both the chemical and physical state of iron in water and soils. Many of the changes result indirectly from microbial alterations of the environment; for example, iron increases and decreases in solubility as a result of Eh and pH changes associated with the growth of many heterotrophic bacteria. Iron can be affected more directly when iron-organic complexes are destroyed as the organic part is used by micro-organisms. Finally, some bacteria are able to bring about the direct enzymatic oxidation or reduction of iron as a part of their energy generating process. The physiology and ecology of certain bacteria that commonly affect iron are outlined in this paper. Emphasis is placed on those organisms that change iron directly as a significant part of their metabolism; although, indirect reactions also are described. References for procedures used in the cultivation of these organisms are provided.

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Micro-organisms in Stormwater

A Summary of Recent Investigations

By Gail E. Mallard

ABSTRACT

The major concepts and considerations in microbiological analysis of storm runoff are summarized and documented to provide a basis for use by community planners, water managers, and others interested in studies of stormwater. Twenty-five published reports dating since 1964 are cited.

All storm runoff contains a variety of bacteria, including total coliforms, fecal coliforms, and fecal streptococci, that are derived from the land over which the water flows. Most of the total coliforms are native soil organisms, whereas the fecal coliforms and fecal streptococci originate from the feces of wild and domestic animals. Urban runoff has been reported to contain pathogenic organisms, but this probably presents little direct threat to human health because the runoff is not ingested. Runoff water, however, can have other negative effects such as contamination of surface waters, which may result in beach closures, or contamination of shellfish. This type of contamination is generally of short duration because indicator bacteria and pathogens die rapidly in the aquatic environment. Similarly, bacteria and viruses deposited on soil by stormwater are inactivated by drying, competition from soil microflora, and a variety of other processes.

Every storm producing runoff is unique in terms of the number and type of micro-organisms, for they vary from site to site, from storm to storm, and over time during any one storm event. Stormwater to be examined for micro-organisms must be collected in sterile containers and processed immediately.

INTRODUCTION

Any attempt to find micro-organisms in stormwater generally will meet with success. Bacteria and viruses are normal inhabitants of soil, water, human and animal skin and gut, plant surfaces, and indeed almost every place on earth. Only certain extremely hostile environments and the internal tissues of plants and animals are free of micro-organisms. Stormwater running over the land surface inevitably will become contaminated, but this should not be a cause for concern, unless it threatens human health or well-being. Does stormwater present a serious threat? Unfortunately, no unequivocal answer can be given. Stormwater carrying micro-organisms may enter a drinking-water source and cause an outbreak of disease, or it may lead only to the temporary shutdown of recreation facilities, such as beaches. Depending on local conditions, the study of microbial contamination of runoff may be of real importance to a community or it may be of only academic interest.

PURPOSE AND SCOPE

This summary presents general information gained from recent investigations and describes problems that may arise in future investigations. It is hoped that this information will be of value in planning studies that involve micro-organisms in stormwater.

BASIC CONCEPTS IN MICROBIOLOGY

All scientific and technical fields have concepts, procedures, assumptions, and terminology that are unfamiliar to the nonspecialist. Although many of the terms and concepts of microbiology are familiar to the general public, the exact meanings and limitations may be poorly understood. For this reason, the main discussion of micro-organisms in storm runoff is preceded by a brief introduction to some of the principles of microbiology. A glossary of biological and microbiological terms is provided by Greeson and others (1977).

INDICATOR ORGANISMS

Water that has been polluted by human sewage is hazardous because several diseases are transmitted by way of the fecal-oral route, such as typoid fever, cholera, dysentery, and hepatitis. When presented with a water sample that is suspect, the microbiologist typically does not try to isolate and identify the pathogens, but instead determines numbers of "indicator" organisms–total coliforms, fecal coliforms, and fecal streptococci. These bacteria are known as indicator organisms because they indicate the presence of sewage and ideally are correlated with the number of pathogens in a water sample.

The growth characteristics and metabolic reactions of the indicator organisms have been investigated for many years, and relatively easy and straightforward methods for their detection and enumeration are available (American Public Health Association, 1975; Greeson and others, 1977). In contrast, methods for detection of pathogens are complicated, tedious, and time consuming.

In addition to being easier to isolate and work with in the laboratory than pathogens, indicator bacteria are used because water contaminated by human waste typically will contain many more of these organisms than pathogens. Indicator organisms are present at relatively high levels in all members of the population and are being shed at all times. In contrast, only a few clinical cases and carriers will be shedding pathogens at any given time.

Most of the characteristics of an ideal indicator organism are listed in *Drinking Water and Health* (National Research Council, 1977, p. 71) and are summarized as follows:

(1) Applicable to all types of water;
(2) Present in sewage and polluted waters, when pathogens are present;
(3) Number is correlated with the amount of pollution;
(4) Present in greater numbers than pathogens;
(5) No aftergrowth in water;
(6) Greater survival time than pathogens;
(7) Absent from unpolluted waters;
(8) Easily detected by simple laboratory tests in the shortest time consistent with accurate results;
(9) Has constant characteristics;
(10) Harmless to man and animal.

Although no organism or group of organisms is a perfect indicator, coliforms are ideal in most ways and have been used by water microbiologists and others concerned with the public health for many years. The U.S. Environmental Protection Agency's safe drinking-water standards are written in terms of coliforms, rather than pathogens (American Public Health Association, 1975), as are standards for recreational waters. As recently as 1977, the use of coliforms as indicators of fecal contamination was endorsed by the Safe Drinking Water Committee of the National Research Council (1977). Yet, the popularity of coliforms as an indicator does not preclude critical reviews of their use (Dutka, 1973) or suggestions that other organisms or groups would provide more accurate determinations (Carberry and Stapleford, 1970). Additionally, continuing efforts are being made by many investigators to refine the methods used to detect indicator organisms.

The effectiveness of total coliforms, fecal coliforms, and fecal streptococci as indicators of viral contamination is particularly suspect. Several reports indicate that bacterial indicators are inadequate predictors of viruses in ground water (Marzouk and others, 1979; Vaughn and others, 1978), as well as in fresh and marine surface water (Vaughn and others, 1979). This lack of a reliable indicator for enteroviruses presents a serious problem because detection of viruses in water samples requires expensive and time-consuming procedures. Studies of viruses in the environment, however, are becoming more commonplace, and methods for their detection and enumeration are being improved.

The use of indicator organisms in stormwater-runoff studies may pose a special problem. Most of the fecal coliforms and fecal streptococci in stormwater are derived from animal feces, so that the level of these indicator organisms may be high at all times, regardless of the number of pathogens in the water sample. Olivieri and others (1977) found little or no correlation between indicator and pathogenic bacteria in storm samples. Negative correlations, although not significant, were observed between indicator bacteria and enteric viruses.

RATIOS OF FECAL COLIFORMS TO FECAL STREPTOCOCCI

Aside from the question of the reliability of indicator organisms, a significant question remains unanswered–how is human fecal contamination differentiated from other fecal contamination?

Since fecal material from many warmblooded animals contains coliforms and streptococci, the presence of these groups does not mean that human waste was the source of contamination. Geldreich and Kenner (1969) approached this problem by examining feces from various warmblooded animals (including humans), domestic wastewater, stormwater, food processing wastes, agricultural waters, and recreational waters. They concluded that the ratio of fecal coliforms to fecal streptococci (FC:FS) in human feces and in water polluted with human waste is always greater than 4.0, whereas the ratio of fecal coliforms to fecal streptococci in feces from farm animals, cats, dogs, and rodents, and in separate stormwater systems and farmland drainage, is less than 0.7.

Although the ratio of fecal coliforms to fecal streptococci in a water sample gives some indication of the source of the contamination, these ratios must be interpreted with caution. First, the water samples must be taken near the source of contamination, because once the organisms have entered the receiving body of water, variables such as temperature, pH, metal concentration, nutrient availability, and other environmental factors will alter the interrelationship between the two indicator systems. McFeters and others (1974) investigated the question of survival of indicator bacteria in natural waters and found that the initially high FC:FS ratio of human sewage decreased with time, whereas the initially low ratio for waste from domestic livestock increased. This reinforces the advice given by Geldreich and Kenner (1969, p. R349) that "the use of a ratio relationship for stream samples would be valid only during the initial 24-hour travel downstream from point of pollution discharge into the receiving stream." Even if used correctly, the FC:FS ratio should not be regarded as a "magic number," especially for samples that contain water from a mixture of nonpoint sources, because the ratio of fecal coliforms to fecal streptococci does not reflect absolute numbers. For example, if most of the contamination in a sample were from nonhuman sources, a small amount of human sewage might not be sufficient to shift the overall ratio upward enough to cause concern. As a result, the presence of human pathogens in human sewage would be masked by the indicator ratio characteristic of animal waste, and a real danger would go undetected.

MICRO-ORGANISMS IN STORMWATER

BACTERIAL LOAD OF STORMWATER

Rain contains very few bacteria before it reaches the ground. Geldreich and others (1968) collected rain from 49 storms in sterile collectors and tested for total coliforms, fecal coliforms, and fecal streptococci. In 42 of the samples, counts of these bacteria were less than one organism per 100 mL; in the remaining seven samples, total coliform densities ranged between 1 and 92 per 100 mL. When this "contaminated" rain was filtered and the filter examined microscopically, the authors observed soil particles and insect and vegetation fragments, which they believed were the source of the bacteria. Whenever rain comes in contact with land, it becomes contaminated with bacteria. Counts of total coliforms, fecal coliforms, and fecal streptococci in stormwater, compiled from several references (table 1), range as follows: 10^3–10^5 total coliforms per 100 mL, 10^2–10^4 fecal coliforms per 100 mL, and 10^2–10^5 fecal streptococci per 100 mL.

SOURCES OF BACTERIA IN STORMWATER

Geldreich and others (1968) examined 843 stormwater samples and observed that fecal coliforms constituted an average of 8.6 percent of the median total coliforms present. After examining over 7,000 separate strains of coliforms and determining their physiological type, they concluded that the remaining 91.4 percent of the total coliforms came from the soil. The distribution of strains was found to be the same in soil, surface waters, and stormwater. This exhaustive analysis leaves little doubt that most total coliforms in stormwater are native soil organisms that are washed off soil particles by water running over the land surface.

If most of the total coliforms come from the soil, what is the source of the other two pollution indicators, fecal coliforms, and fecal streptococci? By definition these are contributed by warmblooded animals. Because the ratio of fecal coliforms to fecal streptococci in urban stormwater is usually much less than 1 (see table 5), it is assumed that most of the bacteria in stormwater are of nonhuman origin. In an urban environment, these bacteria probably originate from fecal material of dogs, cats, rodents, and other small animals; whereas in rural areas, larger domestic animals would make a significant contribution. In either case, the amount of waste generated by animals should not be underestimated. For example, a large city such as New York could easily have half a million dogs, and these dogs will deposit about 150,000 pounds of feces and 90,000 gallons of urine each day on streets, sidewalks, and park areas. Even a small number of farm animals can have a significant impact because one cow will generate as much manure as 16.4 humans, a pig will produce as much as 1.9 humans, and 12 chickens as much as one human (Geldreich, 1976). Pollution from wild animals also must be considered. A beach in Madison, Wisconsin, was closed to swimming because of high bacterial counts in 1978. An investigation revealed that fecal coliforms deposited by wild ducks had multiplied in the beach sands and were carried into the water by storm runoff (Standridge and others, 1979).

VARIATION IN NUMBER OF MICRO-ORGANISMS IN STORMWATER

The microbiological character of runoff from any given storm and any given area is likely to be unique. This presents obvious problems, if one is to compare runoff between areas or within the same area over time. First, the land use and topography of the drainage area will have a significant effect on quality of the runoff. For example, urban stormwater and runoff from a feedlot will be very different from each other. Even within the general category of urban runoff, water from residential areas is likely to differ considerably from water from commercial areas in terms of micro-organisms.

If runoff from only one area is considered, time is the most significant variable because bacterial counts vary from storm to storm (Davis and others, 1977) and with season (see table 5). However, the amount of time elapsed between storms does not seem to affect microbiological quality, as indicated by Olivieri and others (1977, p. 88), who, after studying storms in Baltimore, Maryland, for a 12-month period, concluded that "the levels of fecal coliforms observed in the storm runoff appear to be independent of the time between storms." Upon first consideration, this conclusion may seem unlikely because waste material will accumulate between storms. However, fecal organisms begin to die as soon as they leave their normal habitat, the gastrointestinal tract of warm-blooded animals, so that only freshly deposited fecal bacteria are likely to affect the counts in runoff.

Changes in bacterial counts during a storm have been documented by Davis and others (1977), who studied an area near Houston, Texas, that was being developed into a planned community. Figure 13, which shows a hydrograph and bacteriological data from one storm at a small stream site, indicates that the densities of both fecal coliforms and fecal streptococci rise sharply at the beginning of the storm and gradually decay over the next 60 hours. The time from 20–50 hours represents the bulk of the hydrograph. During this period, the fecal coliform density decreases by slightly less than 1 log unit (base 10), while the fecal streptococcal density decreases by just over 1 log unit. Qureshi and Dutka (1979) conducted a similiar study of stormwater draining a residential area in Burlington, Ontario, and emptying into a small creek. In that study, samples of runoff were collected at the outfall before entering the stream.

Changes in bacterial counts at this site (arithmetic mean of 12 storms) are shown in figure 14. Data for individual storms show no predictable pattern for maximum bacterial populations (Dutka, 1977; Qureshi and Dutka, 1979). In a report on the microbiological quality of stormwater from residential and commercial areas (3 sites), Qureshi and Dutka (1979) concluded:

> "There appeared to be little relationship between the duration, intensity, and amount of rainfall and the occurrence of peak microbial populations. As a result, no typical pattern of time-related distribution of indicator and pathogenic bacteria could be established in this investigation."

The amount of time micro-organisms spend in the stormwater before collection and analysis has a significant effect on the final counts obtained. In general, storm runoff is a hostile environment for fecal indicator organisms and pathogens because these organisms require high nutrient levels and warm temperatures for growth. Furthermore, salts, organics, and other chemicals that may be in stormwater will have an adverse effect on micro-organisms, so that the number of organisms in stormwater will decline with time. In addition, the relative concentrations will change with time because the strains die at differing rates. A more complete discussion of the importance of prompt analysis is presented in the section "Methods and Procedures."

TABLE 5.—*Densities of indicator bacteria in stormwater*

Source of stormwater	Total coliforms per 100 mL	Fecal coliforms per 100 mL	Fecal strep per 100 mL	FC:FS Ratio	Reference
Detroit - urban street catch basins, 1949	25,000 - 930,000	not measured	not measured	not measured	Weibel and others, 1964
Detroit - urban street catch basins, 1950	2,300 - 430,000	Do.	Do.	Do.	Do.
Seattle - street gutters 1959-1960	up to 16,100	Do.	Do.	Do.	Do.
Stockholm, Sweden - streets and parks, 1945-1948	median: 4,000 high: 200,000	Do.	Do.	Do.	Do.
Pretoria, South Africa - residential, park, school, sports ground areas	240,000	Do.	Do.	Do.	Do.
Pretoria, South Africa - business and flat area	230,000	Do.	Do.	Do.	Do.
Cincinnati, Ohio - residential; 1962-1963	median 58,000	median 10,900	median 20,500	0.53	Do.
Cincinnati, Ohio - 1962-1964					Geldreich and others, 1968
Wooded hillside					
Spring	2,400	190	940	0.20	
Summer	79,000	1,900	27,000	0.70	
Autumn	180,000	430	13,000	0.03	
Winter	260	20	950	0.20	
Street gutters					
Spring	1,400	230	3,100	0.07	
Summer	90,000	6,400	150,000	0.04	Do.
Autumn	290,000	47,000	140,000	0.34	
Winter	1,600	50	2,200	0.02	
Business district					Do.
Spring	22,000	2,500	13,000	0.19	
Summer	172,000	13,000	51,000	0.26	
Autumn	190,000	40,000	56,000	0.71	
Winter	46,000	4,300	28,000	0.15	
Rural					Do.
Spring	4,400	55	3,600	0.02	
Summer	29,000	2,700	58,000	0.05	
Autumn	18,000	210	2,100	0.10	
Winter	58,000	9,000	790,000	0.01	
Baltimore - Inner city, residential commercial - Pop. density 92.5/acre, 1974-1975	mean of 24 storms 380,000	mean of 24 storms 83,000	mean of 24 storms 560,000	90% less than 4.0; 80% less than 1.0	Olivieri and others, 1977.
Baltimore - Residential/shopping center - Pop. density 26.6/acre, 1974-1975	mean of 21 storms 38,000	mean of 21 storms 6,900	mean of 21 storms 50,000	90% less than 4.0; 80% less than 1.0	Do.
Business district	not measured	13,000	51,000	0.26	Geldreich and Kenner, 1969
Residential	Do.	6,500	150,000	0.04	Do.
Rural	Do.	2,700	58,000	0.05	Do.
Burlington, Ontario - single-family residential	10,000	4,000	4,000	1.0	Qureshi and Dutka, 1979

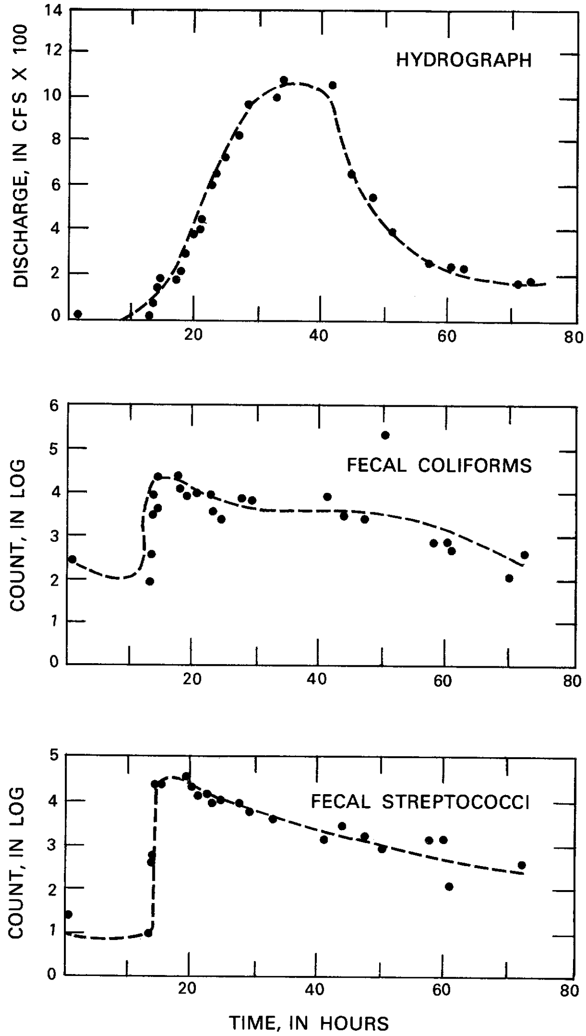

FIGURE 13.–Hydrograph and graphs of densities of fecal coliforms and fecal streptococci bacteria in a small stream near Houston, Texas, during a storm (modified from Davis and others, 1977; reproduced with permission of Water Resources Bulletin).

PATHOGENS IN STORMWATER

Olivieri and others (1977) investigated the occurrence of pathogens in urban stormwater in Baltimore. An analysis of stormwater samples from a site not known to have any sewage overflows gave the results presented in table 6.

It is obvious that urban stormwater can contain pathogens, although at relatively low levels. Could this represent a threat to human health? One way to begin to answer this question is to consider the number of bacteria and viruses necessary to establish infection. Unfortunately, some strains are more virulent than others. The susceptibility of a human population is also variable and depends on such considerations as age, general health, and degree of immunity. Nevertheless, rough estimates can be made from available data. A dose of 10^5 *Salmonella* may be necessary to establish infection, but in the case of some *Shigella* strains, 10^2 or 10^3 viable organisms is enough to infect (National Research Council, 1977). The situation with viruses is not as well documented; but, in general, a conservative approach is taken and one virus particle is considered sufficient to establish infection. It should be pointed out, however, that infection does not always lead to disease.

By combining the known densities of pathogens in stormwater with the infective doses just cited, one might be able to make an estimate of the direct health hazard of urban runoff. From this line of reasoning, the amount of stormwater an individual would have to consume to acquire a *Salmonella* infection would be on the order of hundreds of gallons. The danger from virus infection may be greater because dilution will not destroy infectivity. It is unlikely that any single individual will consume enough stormwater to insure virus infection; but, if many individuals drink slightly contaminiated water, it is possible that one or a few may become infected.

Stormwater does not need to be ingested to pose a health hazard. It is, perhaps, ironic that contamination of saltwater, which is not consumed, may be more dangerous than contamination of drinking water. For example, oysters and clams are known to concentrate viruses, and several well-documented outbreaks of hepatitis have been caused by virus-contaminated shellfish (Vaughn and Landry, 1977). Also, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are associated with eye, ear, and skin diseases and might become a problem in areas where stormwater or surface waters, heavily contaminated with stormwater, are used for recreational activities.

All of the pathogens mentioned above are of human origin and, as expected, are present in urban stormwater in relatively low numbers. The report on the Baltimore study discusses the issue at some length and concludes that urban stormwater probably represents little threat to public health (Olivieri and others, 1977). However, Van Donsel and others (1967, p. 1362) cite a U.S. Public Health Service analysis of waterborne-disease out-

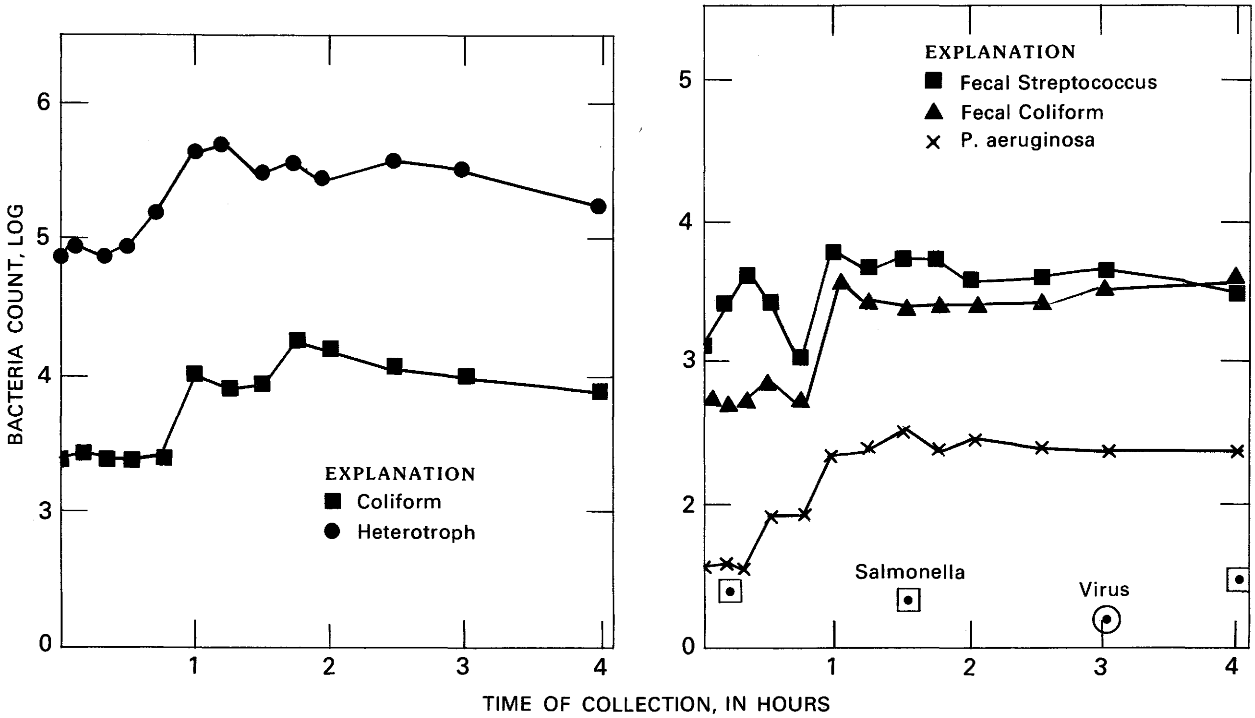

FIGURE 14.–Bacterial populations in runoff samples taken at stormwater outfall, Burlington, Ontario. Arithmetic mean of bacteriological data, based on 12 storms (from Qureshi and Dutka, 1979, reproduced from Water and Sewage Works, vol. 23, no. 3, March 1979, with permission).

breaks occurring in the United States and Puerto Rico from 1946 to 1960:

"At least 29 outbreaks involving 9,233 cases were associated with stormwater runoff, caused either by rainfall washing human and animal feces or sewege into wells, springs, streams, reserviors, and open-water mains, or by wide-spread flooding of individual and public water systems."

FATE OF MICRO-ORGANISMS IN STORMWATER

As long as stormwater containing bacteria and viruses is confined to gutters, ditches, and storm sewers, it poses little threat to humans; but when it enters the receiving body, the viruses and bacteria may become a problem. Fortunately, bacteria that are adapted to live in or on a human host (pathogens and fecal indicators) are not well adapted for competition and survival in the aquatic ecosystem. McFeters and others (1974) placed enteric bacteria from well water (temperature of 9.5°C to 12.5°C) inside membrane dialysis chambers. The average time required for a 50 percent reduction in the initial population (half-time) was 17 hours for fecal coliforms and 22 hours for enterococci. Among a group of pathogens studied, the longest half-time was 26.8 hours (*Shigella* sp.) and the shortest, 2.4 hours (*Salmonella* sp.). The authors observed no growth of the bacteria studied.

Although viruses will not grow outside of their hosts, they apparently do not disappear as rapidly as bacteria. Studies cited by Vaughn and others (1979) indicate that human viruses may persist in aquatic environments for long periods of time, as much as 154 days in one example.

TABLE 6.–*Densities of pathogens in stormwater from Baltimore, Maryland*[1]

	Percent occurrence in collected samples	Geometric mean densities
Salmonella species	52	5.7 per 10 L
Pseudomonas aeruginosa	100	592 per 100 mL
Staphylococcus aureus	82	12 per 100 mL
Animal virus	83	--
Polio virus	42	--
Coxsackie Virus B	50	--
Echovirus	33	--
Other unidentified virus	8	--
Enterovirus	--	170 Plaque forming units per 10 L

[1] Data from Olivieri and others (1977).

Van Donsel and others (1967) studied the survival of indicator bacteria in soil using tracer strains that could be identified. They found that in summer, the fecal coliforms survived slightly longer than the fecal streptococci (3.3 days as compared to 2.7 days for 90 percent reduction); whereas in spring and winter, the fecal streptococci survived much longer than the fecal coliforms. In autumn, survival time was the same for both types, and the shortest survival time for both groups was at an exposed site in summer. This study produced evidence of aftergrowth of nonfecal coliforms in the soil as a result of temperature and rainfall variations, and Van Donsel and others (1967) pointed out that such aftergrowth may contribute to variations in the bacterial count of storm runoff and that these variations have no relation to the sanitary history of the drainage area. The problem of aftergrowth of fecal coliforms deposited by ducks on a beach in Wisconsin (Standridge and others, 1979) already has been described.

Generally, soil is effective in removing bacteria and viruses. Gerba and others (1975) presented an extensive review of the topic. Factors that affect the survival of bacteria in soil are moisture content and moisture-holding capacity, temperature, pH, sunlight, availability of organic matter, and competition and antagonism from soil microflora. Movement of bacteria into the ground water is limited by the straining of bacteria at the soil surface and by adsorption to clays in the soil. Deterioration of ground-water quality from the downward movement of micro-organisms is likely to be a problem only where the water table is near land surface.

A recent study (Yeager and O'Brien, 1979) concluded that drying is the most important factor in the inactivation of viruses in soils. Virus removal by soils depends mainly on adsorption by clays (Gerba and others, 1975). However, viruses that are adsorbed to clays can retain their infectivity (Schaub and Sagik, 1975). Binding of virus particles to clay depends on the net charge of the virus, which in turn is dependent on pH. Unfortunately, the viruses are not bound irreversibly to the soil particles and may be desorbed by rain (Landry and others, 1979). Wellings and others (1975) have isolated viruses from ground water under a recharge basin after a period of heavy rain.

The movement of pathogens to ground water may be a problem in any area where storm runoff is stored in basins or used for artificial recharge. The largest source of information on this hazard is the literature on artificial recharge of ground water and land treatment of sewage. Both practices present a greater potential contamination problem than storm runoff, since pathogens likely will be applied to the same land area in greater numbers over a long period of time. Several studies indicate that ground-water contamination from artificial recharge is possible. For example, Vaughn and others (1978) found viruses and bacteria in ground water beneath recharge basins at two Long Island, New York, sewage treatment plants where the depth to water was 18 and 30 feet, but found no viruses under basins that were 80 feet above the water table. However, even where viruses and bacteria were found in the ground water, the numbers had been reduced considerably during passage through the soil. Although the numbers of bacteria and viruses are likely to be much lower in urban stormwater than in treated wastewater, Vaughn and Landry (1977) recovered viruses from ground water beneath a stormwater-recharge basin on Long Island. The source of these viruses has not been definitely established; however, their presence suggests the possibility of ground-water contamination by stormwater.

METHODS AND PROCEDURES

A detailed discussion of the methods used to grow and enumerate micro-organisms in stormwater is beyond the scope of this paper. Other sources (American Public Health Association, 1975; Greeson and others, 1977) give much information on the procedures used to cultivate indicator bacteria (fecal coliforms and fecal streptococci). Pathogenic organisms are more difficult to cultivate, and great care must be exercised to avoid laboratory-acquired infections. Viruses must be grown inside a living host cell, and virus concentration and propagation are complicated processes that should be left to specialists in virology.

Setting aside any discussion of the methods used in processing a water sample, an important issue remains. If the sampling program is improperly designed or if the water sample is collected improperly, the data will be at best meaningless or at worst misleading. Some of the points that must be considered in sampling are addressed below.

SAMPLE COLLECTION

STORMWATER

Water samples for microbiological analysis should be collected by hand in sterile containers by a trained individual (American Public Health Association, 1975). Two early reports (Burn and Vaughn, 1966; Weibel and others, 1964) mentioned allegedly sterile automatic-sampling devices for stormwater; however, the descriptions of the devices are somewhat incomplete and leave doubt as to the integrity of the sample. Almost all recent papers indicate that stormwater samples were collected by hand in sterile containers. This is obviously expensive, time consuming, and some may feel unnecessary. It might be argued that since stormwater is already heavily contaminated, slight additional contamination from the sampling device will be negligible. However, this is circular reasoning, and the true level of contamination in the stormwater can never be established by this approach. Another argument might be that fecal coliforms and fecal streptococci are unlikely to be in the air or in washed bottles and automatic samplers. This argument may seem reasonable; nevertheless, microbiological data reported for samples collected under nonsterile conditions always will be suspect.

RECEIVING WATERS

Collecting stormwater at the outfall of a storm drain or sewer will give the best estimate of the microbiological quality of the runoff. However, to judge the impact of stormwater on some environments, it may be necessary to sample a stream or other receiving water. In lakes, reservoirs, deep rivers, and estuaries, bacterial abundance may vary transversely, with depth, and with time of day. The time variable was pointed out by Davis and others (1977) in their study of a stream receiving storm runoff. In their study, bacterial densities were not constant in stream waters over a period of 24 hours, even during constant low-flow conditions. Their data showed highly variable batch flows, and they concluded that grab samples reflected the condition and content solely at the time at which the sample was taken. A possible solution to this problem might be to collect samples upstream from the discharge point to serve as a control for each stormwater sample taken.

STORAGE OF STORMWATER SAMPLES

The rule when working with micro-organisms is that the more rapidly the samples are processed, the more accurate the results will be. Bacterial samples must be refrigerated if held more than 1 hour before processing; the maximum transport time is 6 hours, and the samples must be processed within 2 hours after arrival at the lab (American Public Health Association, 1975; Greeson and others, 1977).

A measure of the potential for survival of various bacterial groups in stormwater is presented in figure 15, taken from Geldreich (1976). In that study, urban stormwater was collected, filtered to remove the "native" bacterial populations, and pure cultures were added to the water. The culture densities then were determined over a period of 2 weeks. Problems with this approach are that the laboratory cultures might have declined at a different rate than those occurring naturally in stormwater and also that the added bacteria might have survived longer because they did not have to compete with the "native" population.

As figure 15 indicated, the apparent number of bacteria decreased significantly with storage time and different types of bacteria died at different rates. In general, the bacteria survived longer when stored at 10°C (fig. 15*A*) than at 20°C (fig 15*B*), but even when stored at 10°C (fig 15*A*), 50 percent of the *Salmonella typhimurium* died within 2 days. Fecal coliforms survived slightly longer, with 50 percent surviving for 14 days, whereas *Streptococcus bovis* (a component of animal feces but not human feces) disappeared within the first day at 10°C and within 3 days at 20°C. These data clearly support the general rule that rapid processing of microbiological samples is of utmost importance.

DESIGN OF SAMPLING PROGRAM

When designing a program to determine the microbiological quality of stormwater, one must consider the following points:

(1) The number and type of bacteria in runoff will be influenced by the intensity of the storm, duration of the storm, season of the year, and land use of the area being drained. Therefore, great care must be taken in any extrapolation from one area to another or from one storm to another.

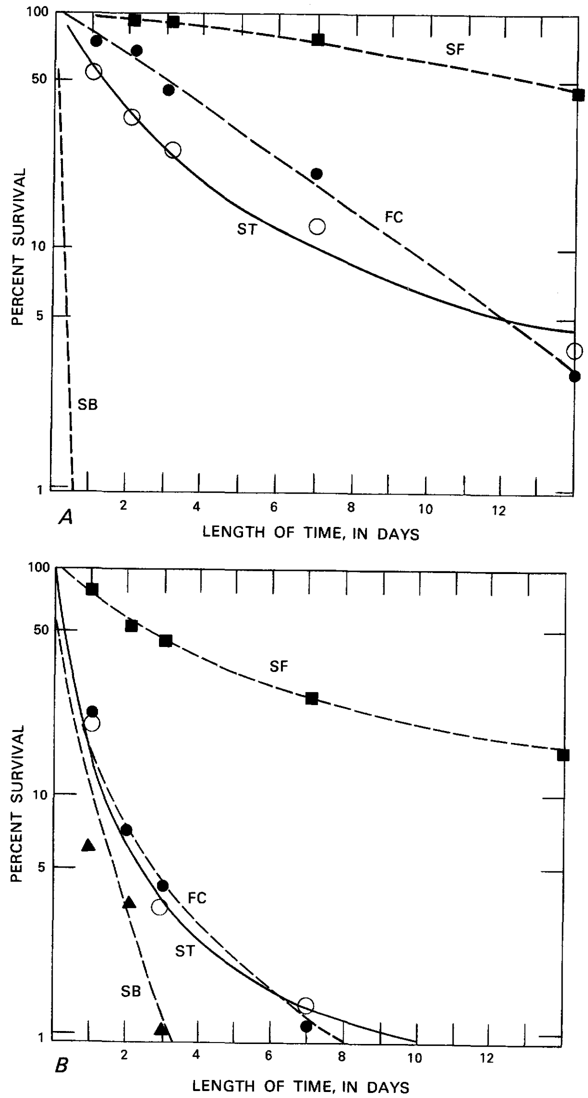

FIGURE 15.—Persistence of selected bacterial strains in stormwater: *A*, stored at 10°C; *B*, stored at 20°C. SF=*Streptococcus faecalis*, FC=fecal coliform bacteria, ST=*Salmonella typhimurium*, SR=*Streptococcus bovis* (modified from Geldreich, 1976; reprinted with permission from Critical Reviews in Environmental Control, copyright by the Chemical Rubber Company, CRC Press, Inc.).

(2) Provision must be made for a nearby laboratory to handle all samples. Samples must be processed within hours of collection.

(3) No good predictor or indicator for the presence of pathogens in stormwater is known. Pathogenic bacteria and viruses seem to occur in a random fashion throughout the storm.

SUMMARY AND CONCLUSIONS

Information obtained from recent investigations of micro-organisms in stormwater has been reviewed and summarized with the intent of providing assistance to those planning studies of stormwater. The following conclusions are indicated by this review:

(1) Most stormwater contains relatively large numbers of total coliforms, fecal coliforms, and fecal streptococci.

(2) Most of the total coliforms in runoff are native soil organisms.

(3) The source of most fecal coliforms and fecal streptococci in runoff is probably wild or domestic animals.

(4) A report from Baltimore, Maryland, has documented the occurrence of hazardous organisms in urban runoff. The source of the contamination is unknown.

(5) Stormwater probably presents little direct threat to human health because it is not ingested, but it can produce other negative effects such as contamination of beaches or shellfish.

(6) Contamination of surface waters by bacteria in stormwater is of short duration because indicators and pathogens die rapidly in the aquatic environment. Viruses apparently do not disappear as rapidly as bacteria and may be concentrated by shellfish.

(7) Bacteria and viruses deposited on soil by stormwater are inactivated by drying, competition from soil microflora, and a variety of other processes. The movement of pathogens to ground water is likely to be a problem only where the water table is near land surface.

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