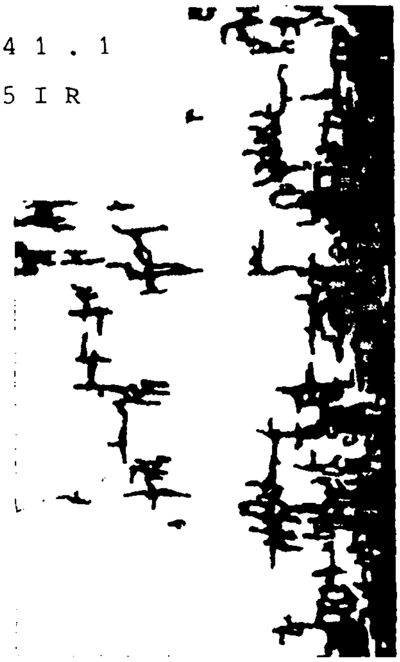
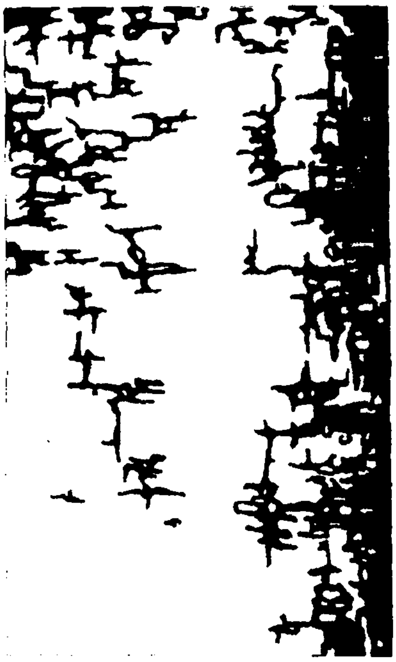
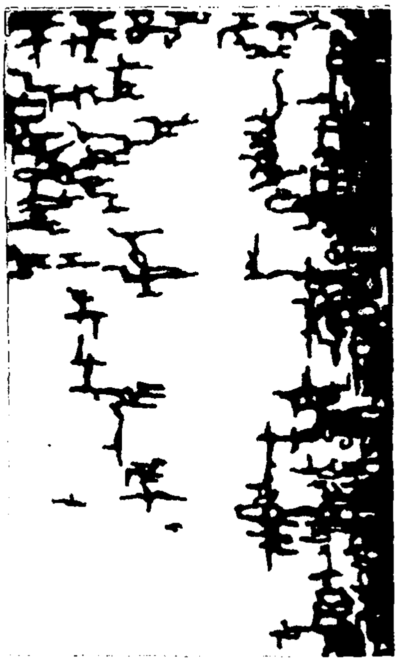
Iron Bacteria Occurrence, Problems & Control Methods In Water Wells







by

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U.S. Army Corps
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Waterways Experiment
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I. INTRODUCTION

Objectives and Scope

This report represents a state-of-the-art document on iron-precipitating bacteria in ground water and wells. The basic objective of this document is to review what is currently known about the nature and occurrence of iron-precipitating bacteria in ground water so that this information might be utilized to guide the development of effective strategies for controlling the growth of these bacteria in wells. "Iron-precipitating" bacteria include a wide variety of different organisms. Information on the physiology and ecology of this diverse group of bacteria is presented along with a review of the problems attributed to the growth of iron-precipitating bacteria in wells. Control measures used to rehabilitate wells clogged with the troublesome accumulation of these bacteria and their associated iron deposits are also discussed.

This document was prepared to assist government officials, engineers and ground-water industry representatives who have an interest in the problems associated with iron-depositing bacteria in wells and who are either responsible for or involved in well maintenance and rehabilitation practices. Impetus for the development of this report was provided by a U.S. Army Corps of Engineers research program addressing the repair, evaluation, maintenance and rehabilitation needs of older hydraulic structures constructed as part of the Corps civil works program. One aspect of this research program has been concerned with the maintenance needs associated with relief wells and underground drainage systems which are installed as appurtenant structures to dams and levees. The decline in efficiency of these wells and drains as a result of clogging by iron-precipitating bacteria has been regarded as a common and routine maintenance problem. Efforts are therefore underway to identify effective treatment methodologies for keeping these relief wells and drainage systems free from iron-precipitating bacteria and to maintain these wells and underground drainage systems in optimum operating condition.

Background

Since the mid-nineteenth century, certain filamentous bacteria have been recognized for their ability to remove iron from solution by precipitating insoluble ferric hydroxide outside their cells (Harder, 1919). These "classical" iron-precipitating bacteria include the stalked *Gallionella* species and members of the *Sphaerotilus-Leptothrix* group of filamentous iron-precipitating bacteria. Subsequent studies of bacterial iron transformations in water, however, have shown that the deposition of insoluble ferric hydroxide is associated with an even more heterogeneous group of bacteria, including many nonfilamentous forms, such as the encapsulated *Siderocapsa* species (Starkey, 1945). Although the bacteria associated with iron precipitation in water are commonly referred to as "iron bacteria," this is a general term used to describe a diverse group of organisms which occur in a wide range of habitats including lakes, ponds, swamps, bogs, drainage ditches and chalybeate springs (Ghiorse, 1984).

In addition to their ability to deposit ferric hydroxide around their cells, various iron bacteria are also capable of excreting extracellular polysaccharides which have been variably described in the literature as gelatinous, mucilaginous or slime-like substances (Gaudy and Wolfe, 1962). This extracellular material coats the external surface of the organism's cell wall or sheath and serves as a site for the accumulation of precipitated ferric hydroxide. Over time, this extracellular material may become impregnated and encrusted with iron oxides resulting in a brown to reddish colored slimy mass which typifies the growth of iron-precipitating bacteria in natural environments.

Iron bacteria which occur in water-distribution systems, wells and underground drains are considered significant fouling agents as a result of their dual ability to excrete extracellular slimes and to precipitate large amounts of ferric hydroxide. Historically, prolific growths of filamentous iron bacteria in water supply systems have been called "water calamities" (Starkey, 1945). In these instances, the gelatinous growth of iron bacteria and their associated deposits of ferric hydroxide have resulted in discolored water, unpalatable taste and odors and reductions in flow through pipes. Additionally, iron bacteria have been implicated as indirect agents of corrosion in water distribution pipes as a result of their ability to create a suitable growth environment for other organisms directly involved in metal corrosion processes (Starkey, 1945).

Iron bacteria infestations in water wells are regarded as the most prevalent cause of bacterial degradation of well performance (Smith, 1984). These infestations are often characterized by a severe and rapid clogging of the well screen openings and the pores of the materials surrounding the well bore (Mogg, 1972). This physical blockage of the well intake area results in decreased hydraulic efficiency, manifested by significant reductions in specific capacity. In severe cases, steel well components may be corroded anodically and tuberculated (Smith, 1980).

A quantitative survey of the economic impact of iron bacterial well degradation has never been conducted, although a survey of water authorities by Cullimore and McCann (1977) demonstrated worldwide familiarity with the problem. Reports describing the occurrence and effect of iron bacteria growth in wells have been published periodically and involve locations as geographically diverse as Germany (Hasselbarth and Ludemann, 1972), Yugoslavia (Barbic et al., 1974), India (Roa, 1970), Finland (Tuovinen and Nurmiaho, 1979) and North America (Cullimore and McCann, 1977). In addition to these widespread reports, representatives of the water well industry involved in well rehabilitation work have speculated that iron bacterial populations are spreading (Mogg, 1972). This speculation, however, is based on empirical observations rather than on a quantifiable assessment of iron bacteria in aquifers.

Despite this overall awareness of the iron bacterial problem in wells, there is a paucity of knowledge regarding effective methods for treating wells contaminated with these nuisance organisms. Throughout the published literature, the description of remedial treatment methods used to control iron bacteria in wells are frequently anecdotal and the techniques used are often lacking scientific investigation. Grainge and Lund (1969) noted that there was a proliferation of recommended controls for iron bacteria in wells, however, they found most of these methods to be ineffective. They further concluded that this lack of advancement in control strategies for iron bacteria was due to the difficulties in culturing iron bacteria for experiments.

The problems associated with culturing various iron-precipitating bacteria on laboratory media have been extensively reviewed in the literature (Pringsheim, 1949). Because there are no known simple procedures for cultivating many of these organisms in the laboratory, most studies have been limited to microscopic observations (Starkey, 1945). As a result, detailed information on these organisms is usually in regards to their physical structure (morphology) as opposed to their metabolic or growth requirements (physiology).

In general, the role which iron plays in the metabolic function of iron bacteria is poorly understood and is the subject of conflicting ideas in the research literature. In a review of the historical literature on iron bacteria, Pringsheim (1949) indicated that the metabolic controversy originated from two opposing postulates which were based on early cultural studies of iron bacteria in the late 1800s. Winogradsky postulated in 1888 that iron bacteria were chemoautotrophs; therefore, they utilized the energy released by the oxidation of ferrous iron to ferric iron in order to assimilate inorganic carbon for cell growth and development. Later research by Molisch in 1892 and 1910, however, showed that some iron bacteria grew well heterotrophically; therefore, they used organic carbon for an energy source and cell development. As a result of the research of Molisch, it is the contention that many, if not most, of the iron bacteria live on organic materials and effect the precipitation of iron incidentally during their growth. Although it is recognized today that not all iron precipitating bacteria are alike physiologically, this general controversy over the chemoautotrophic versus heterotrophic growth requirements of many specific iron-precipitating bacteria continues.

Though knowledge of the specific aspects of iron deposition by these bacteria may not be necessary for dealing with their troublesome accumulations in wells, more definitive information on the physiology and ecology of iron bacteria could help to guide the development of effective control strategies. By reviewing what is known about the specific growth requirements of various iron bacteria genera and by identifying key environmental conditions for their occurrence in ground water, control strategies may be better directed toward the destruction of the organisms themselves or the alteration of ground water and well environments so as to be unfavorable for their growth. Any means which can be used to prevent their development may be expected to aid in preventing fouling.

Discussion of Major Works

Current knowledge of the nature and occurrence of iron bacterial populations in ground water and wells is based on a relatively small body of literature. Researchers in this field typically cite older studies when compared to more active areas of microbial ecology research. This seems to be due to the lack of systematic study of iron-precipitating bacteria over the years. References on the subject are oftentimes obscure. Nevertheless, a number of available studies on iron bacteria are consistently cited in the literature and serve as primary references for what is presently known about the physiology and ecology of these organisms.

A monograph by Harder (1919) on the potential role of iron bacteria in the formation of iron-bearing ores represents the earliest known comprehensive study of the environmental occurrence of iron-precipitating bacteria. Although much of the taxonomic, morphologic and physiologic termnology used in this report is outdated and inaccurate by modern day standards, Harder presented some solid observations and postulates regarding the nature of these organisms. He observed and concluded that: 1) iron-depositing bacteria are ubiquitous in the iron-bearing waters of mines, wells and springs; 2) common bacteria in water and soil, other than the classical filamentous iron bacteria, are capable of precipitating iron from solutions containing iron salts of organic acids; and 3) these organisms are capable of being introduced into fissures or other large underground openings by surface waters infiltrating through larger soil and rock openings. Harder contended that these subsurface populations of iron bacteria are responsible for the deposition of iron-bearing sedimentary and bog ores. This postulate still represents the basis for some present-day views that iron bacteria are indigenous to certain underground formations which also serve as aquifers.

Starkey (1945) presented the earliest known comprehensive review of iron-precipitating bacteria in water distribution systems. This article, still used as a primary reference for water system workers (Smith, 1982), contains a clear discussion of the physiological aspects of bacterial iron transformations, including iron deposition by nonspecific heterotrophic bacteria as well as by the classical filamentous iron bacteria. Starkey also introduced the concept that bacterial iron hydrates on iron and steel provided a suitable substrate and anaerobic microenvironment for the growth of sulfate-reducing bacteria which are responsible for metal corrosion. He further acknowledged the difficulty of culturing iron bacteria in laboratory media and pointed out that the study of these bacteria may be limited only to microscopic observations.

Pringsheim (1949) provided the first critical review of the historical literature on iron bacteria. Noting the existence of "a vast literature of unequal value," Pringsheim attempted to separate established facts from what he perceived to be an overwhelming mass of rash and controversial statements about iron bacteria. He discusses in detail the problems involved in the cultural investigation of these organisms as well as the unresolved controversy over the chemoautotrophic versus heterotropic theory of iron bacteria growth. Pringsheim also attempted to simplify the taxonomy of iron bacteria by inserting all the members of the *Sphaerotilus*-*Leptothrix* group of sheathed bacteria into one genus, *Sphaerotilus*, recognizing only two species, *S. natans* and *S. discophorus*. In doing so, however, he created some new problems in nomenclature. Later researchers rejected Pringsheim's taxonomic scheme in favor of one in which the genera *Sphaerotilus* and *Leptothrix* were maintained (van Veen et al., 1978).

Procedures for culturing the classical stalked iron bacteria *Gallionella*, which is common in well clogging problems, have been the subject of numerous studies. Kucera and Wolfe (1957) were successful in formulating such a medium which was subsequently improved by Wolfe (1958) to enable continuous subculturing of *Gallionella* in the laboratory. Wolfe reported, however, that he was unable to achieve pure cultures of *Gallionella* with this medium due to a small but persistent growth of *Pseudomonas*. Two reports of successful pure cultures of *Gallionella* were published in 1968 (Hanert, 1968; Nunley and Krieg, 1968). Hanert used Wolfe's medium without agar and claimed pure cultures by serially diluting out contaminants. Nunley and Krieg employed Wolfe's medium containing 0.5 percent formalin which produced cultures without contamination in seven out of ten trials. Subsequent attempts by other researchers to corroborate both these isolation techniques, however, have failed (Christian, 1975).

Hasselbarth and Ludemann (1972) published the earliest known aquifer-wide environmental research involving an iron bacteria problem in wells. They examined municipal wells located within six water catchment areas in Germany which were experiencing bacterial encrustation problems resulting from the growth of *Gallionella* and *Siderococcus* species. By comparing the ground-water quality between these wells and wells located within two other water-catchment areas which were not experiencing iron bacterial problems, they concluded that the mass development of iron bacteria in wells was a function of the chemical nature of the well water. They found that massive growth was associated with well water having a specific pH, Eh (oxidation-reduction potential) and dissolved ferrous iron range. Hasselbarth and Ludemann concluded that four conditions were necessary for iron bacterial clogging of wells: 1) the presence of iron bacteria; 2) specific dissolved ferrous iron concentrations; 3) specific Eh and rH index (an adjusted oxidation-reduction potential taking into consideration temperature and pH values) ranges; and 4) elevated ground-water flow velocities, which are common at well intakes.

Valkenburg et al. (1975) reported on a state-wide survey of 109 wells in Alabama which were sampled to determine the occurrence and distribution of iron bacteria. In the majority of samples collected, *Gallionella* and/or *Sphaerotilus* species were identified both microscopically and by cultural methods. At least one genera of iron bacteria was found in 27 of the 35 counties where samples were collected, demonstrating widespread occurrence of these organisms throughout Alabama aquifers. Valkenburg et al. (1975) compared the occurrence of iron bacterial populations to well depth and water chemistry parameters, including temperature, dissolved ferrous iron, pH, conductivity and dissolved oxygen. Selected bacteriocides and heat treatments for controlling *Gallionella* were also tested under laboratory conditions. Valkenburg et al. argued that iron bacteria are probably introduced into wells from the surface and not via the water-bearing zone, thus implicating drilling and well service contractors as the primary vector for the spread of iron bacteria. They recommended well drillers use clean drilling water, circulation tanks and standard development techniques when constructing wells and that all completed and repaired wells should be properly disinfected in order to prevent the future spread of iron bacteria.

Cullimore and McCann (1977) discussed extensive iron bacteria research which had taken place at the University of Regina in Saskatchewan since 1971. The research was initiated in response to an intractable iron bacterial problem that affected over 90 percent of the wells across the southern half of Saskatchewan, resulting in remedial measures costing four to six million dollars annually. The predominant genera of iron bacteria identified in the well clogging problems were Crenothrix, Leptothrix, Gallionella and Sphaerotilus. Cullimore and McCann reviewed a variety of chemical and physical iron bacteria control measures which were the subject of a series of later reports (Cullimore, 1979 a and b). Based on field trials, they reported that the heat treatment of wells represented a promising method for rehabilitating wells clogged with iron bacteria. By raising ground water temperatures to near pasturization levels, they indicated that iron bacterial growths, which had previously resisted chemical treatments, were successfully removed from wells.

II. CLASSIFICATION AND OCCURRENCE

Taxonomy

According to Bergey's Manual of Determinative Bacteriology (1974), eighteen genera of bacteria have been characterized as having the ability to deposit ferric hydrate outside their cells. These genera represent a diverse group of bacteria with differing morphology and metabolic capabilities as well as widely varying habitats (Table 1). The ability to precipitate iron from solution has apparently evolved among many unrelated organisms. In the past, it has been generally assumed that the iron has played some role of importance to the cells preceding its precipitation.

Despite this general listing of bacteria associated with iron deposition, the taxonomy or classification of iron bacteria can best be described as intricate and disputed. As an example, one group in Bergey's Manual (1974), the Siderocapsaceae family comprising the four genera _Siderocapsa_, _Naumanniella_, _Ochrobium_ and _Siderococcus_, has been questioned as to its validity as a taxonomic unit. The editors of Bergey's Manual (1974) note that recent research showed some _Siderocapsa_ species were indistinguishable from _Arthrobacter_ species and that _Ochrobium_ may in fact be an alga. They further note that despite including this family with the chemolithotrophs, members of the family are heterotrophic, depositing iron only as the result of the metabolism of the organic ion portion of iron salts of organic acids. As a result, it is easy to see that the taxonomy of this group is in disarray and requires further study.

TABLE 1. Genera of Bacteria That Become Coated or Encrusted With Iron Deposits (after Bergey, 1974)

	Genus	Notes
Gliding bacteria	Toxothrix	No pure cultures; found in cold springs, bogs, ponds and lakes containing ferrous iron.
Sheathed bacteria	Sphaerotilus	Pure cultures of some species claimed; found in slowly running water attached to submerged plants and rocks; occurs in activated sludge.
	Leptothix	Pure cultures of some species claimed; found in slowly running waters attached to submerged rocks and plants; growth causes problems in water works.
	Lieskella	No pure cultures; found in upper layers of mud in bodies of water.
	Crenothrix	No pure cultures; found in stagnant and running waters containing organic matter and iron salts.
	Clonothrix	No pure cultures; found attached to iron fittings in well waters.

TABLE 1. Genera of Bacteria That Become Coated or Encrusted With Iron Deposits (after Bergey, 1974) (continued)

	Genus	Notes
Budding and/or appendaged bacteria	Gallionella	Pure cultures claimed; found in ferrous iron-bearing waters and soils; growth causes problems in water works.
	Pedomicrobium	Pure cultures; found widely distributed in soil and water.
	Seliberia	Pure cultures; common inhabitant of soils.
	Planctomyces	No pure cultures; found in surface layers of lakes.
	Metallogenium	Pure cultures; found in soil and water.
	Caulococcus	No pure cultures; found in bottom mud of lakes.
	Kusnezovia	No pure cultures; found in mud samples from lakes.
Gram-negative, chemolithotrophic bacteria	Thiobacillus	Pure cultures; found in acid waters of high iron content and in soils containing pyrite and marcasite.
	Siderocapsa	No pure cultures; common in fresh water.

TABLE 1. Genera of Bacteria That Become Coated or Encrusted With Iron Deposits (after Bergey, 1974) (continued)

	Genus	Notes
Gram-negative, chemolithotropic bacteria (Continued)		
	Naumanniella	Pure cultures; widely distributed in iron-bearing waters.
	Ochrobium	No pure cultures; widely distributed in iron-bearing waters.
	Siderococcus	No pure cultures; widely distributed in fresh waters and bottom deposits.

In addition to disputed taxonomy, contradicting nomenclature used to describe certain genera of sheathed bacteria has caused problems when reviewing the literature on iron bacteria. The cause of the nomenclature problem was the demonstration by Pringsheim (1949) that forms of the sheathed iron bacteria could change under different culturing conditions in the laboratory. As a result of his observations, Pringsheim argued that most forms previously designated as various species of *Leptothrix* and *Clonothrix* should be included in the genus *Sphaerotilus* along with *S. natans*. He proposed abolishment of the genus names *Leptothrix* and *Clonothrix* and recognized instead only two species; *S. natans* and *S. discophorus*. *S. discophorus* was distinguished by its ability to deposit ferromanganese oxide in the sheath, while *S. natans* deposited primarily iron hydroxide. This nomenclature, however, has been disputed by Mulder and vanVeen in their description of the sheathed bacteria in Bergey's Manual (1974). They contend that the diversity of the various species in this group is not in accordance with Pringsheim's simplified classification system and that they have shown that members of the *Sphaerotilus* and *Leptothrix* genera are distinctly different organisms. Since publication of the eighth edition of Bergey's Manual (1974), Mulder and vanVeen's classification of the *Sphaerotilus-Leptothrix* group has gained acceptance. However, such acceptance has not been universal (Ghiorse, 1984).

It should also be realized that many of the problems facing the taxonomy of iron bacteria are the result of the inability of researchers to isolate pure cultures of many of these organisms (Table 1). Without isolated cultures, these organisms can only be studied from environmental samples or enrichments of such samples by using microscopy. Therefore, detailed knowledge of various iron bacteria is often limited to structural features and this information has not always proved adequate for taxonomic purposes.

For the purposes of this report, the remaining focus will be on the taxonomic genera of iron bacteria which have been commonly observed in ground water and well environments. This mainly includes the stalked *Gallionella*, members of the sheathed *Sphaerotilus*-*Leptothrix* group and nonfilamentous, heterotrophic forms such as *Siderocapsa* and *Siderococcus*. This does not imply that the other organisms are not important in iron deposition. As an example, *Thiobacillus ferrooxidans* is a known chemolithotrophic iron bacterium which is acidophilic, and frequently associated with acid mine drainage (Bigham et al., 1985). Acidophilic bacteria, however, are rarely described as occurring in water well environments, outside of those aquifers which might be affected by acid mine drainage. Investigators should be aware of the possibility of this or any other unusual ground-water condition when looking at iron bacteria problems in wells. However, it is unlikely that these or other unique iron-depositing species will be a prevalent problem.

Morphological Characteristics

The morphological (structural) characteristics of iron bacteria represent the major basis of identification and classification. Because of their size and unique extracellular structures, iron bacteria can often be identified in water or substrate samples by light microscopy. Identification is made by comparing the microscopic images with available drawings or photographs of iron bacteria (Standard Methods, 1976). Traditionally, the iron bacteria have been categorized into three morphological groups: stalked forms which are oriented from top to base; filamentous forms composed of chains of cells enclosed in a sheath; and typical shaped coccoid cells or short rods occurring in irregular aggregates (Starkey, 1945).

Gallionella is the unique example of a stalked iron bacterium. These organisms are characterized by a small bean-shaped cell from which originates a long slender twisted stalk (Figure 1). The small oval cell is typically 0.5x1.2 microns in size and the ribbon-like stalk may attain a length of 200 microns or more. The composition of the stalk has been the subject of much study and controversy (Ghiorse, 1984). Figure 2 is an electron micrograph showing the Gallionella apical cell, the area of fine fibrillar material near the surface of the cell and the dense stalk fibrils originating from the cell. Although it has been generally assumed that the Gallionella stalks were entirely inorganic extrusions of ferric hydrate, current research indicates that the stalk fibrils may contain protein to which the ferric hydrate is bound (Ghiorse, 1984). Regardless the composition of the Gallionella stalk, the organisms grow attached to surfaces by their stalk and this sessile characteristic must be kept in mind when sampling for these bacteria (Hanert, 1981a).

The filamentous forms of iron bacteria are represented by the members of the Sphaerotilus-Leptothrix group. These organisms are structurally characterized by filaments which are composed of a series of cells enclosed

FIGURE 1. Gallionella with their characteristic bean-shaped cells from which are excreted twisted stalks encrusted with ferric hydrate. Mag. approximately 1180x (Starkey, 1945).

FIGURE 2. Electron micrograph of Gallionella showing bean-shaped cell and ferric hydrate coated stalk fibrils originating from it. Bar = 0.5 microns (Ghiorse, 1984).

in a sheath (Figure 3). Studies of *Sphaerotilus natans* have shown that the sheath of this organism is composed of a protein-polysaccharide-lipid complex which is chemically distinct from the cell wall (Romano and Peloquin, 1963). Little is known, however, about the chemical composition of *Leptothrix* sheaths. In addition to sheath structures, members of the *Sphaerotilus*-*Leptothrix* group excrete extracellular polysaccharides which result in a cohering slime layer that covers the sheath (Gaudy and Wolfe, 1962). Both the sheath and the slime layer of these organisms typically become encrusted with ferric hydrate resulting in large masses of filamentous growth and iron deposits (Figure 4). Additionally, the sheaths of *Leptothrix*, especially if heavily impregnated with iron, are frequently empty (vanVeen et al., 1978). The filamentous iron bacteria are also characterized by their sessile nature, with some species of the *Sphaerotilus*-*Leptothrix* group developing special holdfasts which are used to attach the organism to solid surfaces (vanVeen et al., 1978).

The third morphological group of iron bacteria are comprised of nonfilamentous, unicellular bacteria which grow in irregular clusters (Figure 5). These organisms include members of the Siderocapsaceae family, particularly the genera *Siderocapsa*, *Naumanniella* and *Siderococcus.* A common structural feature of this group is the development of an extracellular capsule composed of an excreted gelatinous material of unknown chemical composition (Hanert, 1981b). These slime-encapsulated bacteria are characterized by their ability to form large masses of ferric hydrate which impregnates the capsular material. Unlike the stalked and filamentous forms of iron bacteria, many of these unicellular organisms are

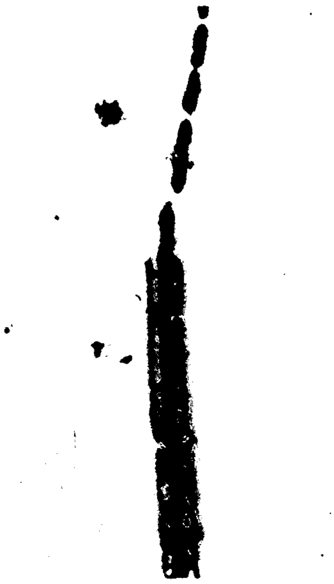

FIGURE 3. Leptothrix spp. filament comprised of a series of cells coming out of their sheath. Mag. approximately 2200x (Starkey, 1945).

FIGURE 4. Sheaths of Leptothrix from an accumulation of precipitated ferric hydrate in iron-bearing water. Mag. approximately 390x (Starkey, 1945).

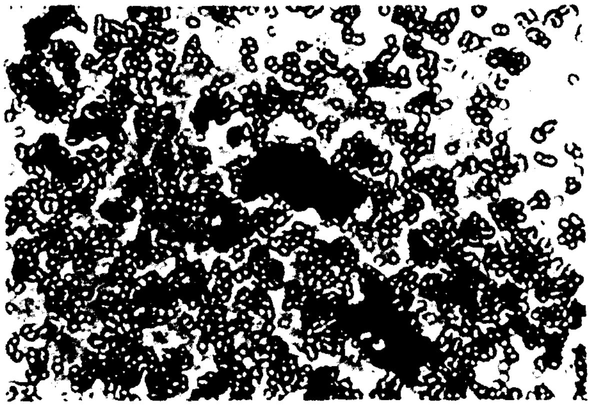

FIGURE 5. Siderococcus organisms showing coccoid forms in irregular clusters along with precipitated ferric hydrate (Hasselbarth and Ludemann, 1972).

planktonic, not sessile. In addition to the Siderocapsaceae family, Macrae and Edwards (1972) demonstrated that seven heterogeneous genera of unicellular, rod-shaped bacteria, including *Pseudomonas* and *Eschericha*, were capable of precipitating colloidal iron from solution by passively adsorbing the iron onto their cell walls. Where these heterotrophic bacteria occur in mixed populations with stalked and filamentous iron bacteria, they may also contribute to iron deposition and potential clogging problems (Smith, 1984).

Physiological Characteristics

Despite numerous nutritional and physiological studies of various iron bacteria genera over the past one hundred years, many aspects of the metabolic and growth requirements of these organisms remain unknown. Researchers often attribute this lack of progress to the failure to isolate axenic (pure) cultures of these organisms (Wolfe, 1964). Without pure cultures for study, the incontrovertible evidence needed to resolve many physiological questions about iron bacteria cannot be easily obtained.

Oxidation of ferrous iron is often thought to be one of the most typical characteristics of iron bacteria genera. This characteristic is based on the original postulate by Winogradsky in 1888 that iron bacteria are chemoautotrophic (Pringsheim, 1949). Winogradsky claimed that iron in the ferrous state is oxidized to ferric iron and that the energy obtained from this oxidation is used by the iron bacteria to assimilate or fix carbon dioxide into organic compounds necessary for metabolism. Although the precise mechanism for the biochemical oxidation of iron has never been confirmed, Baas-Becking and Parks (1927) proposed one reaction by which iron bacteria may live as chemoautotrophs:

$$4\ FeCO_3 + O_2 + 6H_2O \longrightarrow Fe(OH)_3 + 4\ CO_2 + 81{,}000\ cal$$

Utilizing this equation, Starkey (1945) estimated that the growth of bacteria on ferrous iron in solution will result in an approximate 500 to 1 ratio of precipitated ferric hydrate to cell material. Wolfe (1964) suggested that the small amount of energy released by this exothermic reaction was responsible for the large quantities of ferric hydrate precipitated by iron bacteria.

Although the Winogradsky theory that iron bacteria are chemoautotrophic has received additional support from the results of other studies, the concept has not been universally accepted. In a review of the historical literature on iron bacteria, Pringsheim (1949) relates the details of other theories which have been advanced to explain the physiological deposition of iron by these bacteria. Molisch, in a series of studies from 1892 through 1910, was able to grow cultures of iron bacteria on organic medium, without the intentional addition of iron. He established for the first time that some iron bacteria were heterotrophic. Based on Molisch's results and later studies which have followed, researchers have shown that many of the iron bacteria are able to grow on organic material, and there is reason to believe that in the case of certain organisms classified as iron bacteria, precipitation of iron is the result of the decomposition of organic compounds of iron and not the oxidation of inorganic iron compounds (Harder, 1919). Recognizing the potential for both chemoautotrophic and heterotrophic growth among differing iron bacteria genera, Starkey (1945) concluded that where iron bacteria are the sole cause of a fouling problem, there will be an abundance of ferric hydrate with relatively few bacterial cells. However, if the clogging material is composed of large amounts of filamentous growth and slime with little accompanying ferric hydrate, then the bacteria probably developed on the organic constituents of the water or some material other than ferrous iron.

With regard to the iron bacteria genera which characteristically inhabit ground water and well environments, *Gallionella* species are generally considered to be chemoautotrophic, although some researchers believe that this has not been demonstrated conclusively (Ghiorse, 1984). The best evidence of chemoautotrophic growth of *Gallionella* was presented by Hanert (1968) in which he used carbon 14 labelling to show that carbon dioxide was fixed (i.e. assimilated into organic compounds for metabolism) in measurable amounts by these organisms. Furthermore, *Gallionella* can be grown on an inorganic medium containing mineral salts, carbon dioxide and ferrous sulfide, which is used as an energy source (Kucera and Wolfe, 1957). Notwithstanding this evidence, Ghiorse (1984) argues that rigorous proof of chemoautrophy in *Gallionella* is still lacking. One reason is that pure cultures of these organisms have not been easily obtained for study. Despite reports by Hanert (1968) and Nunley and Krieg (1968) claiming growth of pure cultures of *Gallionella*, their methods have not been corroborated by other researchers (Christian, 1975). Secondly, research of chemoautotrophy in *Gallionella* has been impeded by abiotic ferrous iron oxidation which naturally occurs in the organism's growth environment. Since *Gallionella* is a microaerophilic bacterium and grows near neutral pH conditions, Kucera and Wolfe (1957) have suggested that the organism must compete with atmospheric oxygen for the oxidation of ferrous iron. This creates a question as to whether the ferric hydrate precipitated during *Gallionella* growth is the result of either strict chemical or bacterial processes.

vanVeen et al. (1978) reviewed the question of chemoautotrophy among members of the filamentous *Sphaerotilus*-*Leptothrix* group of iron bacteria. Similar to the problems encountered with *Gallionella*, they stated that the main experimental difficulty in testing members of this group for chemoautotrophy is that they are aerobic and grow between a pH of 6 and 8. At these pH values, ferrous iron is readily oxidized to ferric iron nonbiologically, so that it is difficult to determine whether or not the bacteria contribute to the oxidation of ferrous iron. There are, however, many reported studies which have established that some of these organisms can grow on organic compounds and do require organic matter for growth (Starkey, 1945, vanVeen et al., 1978). Conversely, no modern studies have been conducted which show the ability of members of the *Sphaerotilus*-*Leptothrix* group to grow chemoautotrophically. vanVeen et al. (1978) concluded that although the sheath of *Leptothrix*, and rarely *Sphaerotilus*, may be encrusted with ferric hydrate, it is unlikely that the bacteria oxidize iron or derive energy from such a process. Because both organisms grow at near neutral pH where ferrous iron is chemically oxidized to ferric iron, it is probable that the sheaths serve only as a site for the deposition of insoluble ferric hydrate.

Studies of the Siderocapsaceae family indicate an obvious dependence on organic material for growth (Hanert, 1981b). In addition, no studies have ever reported the growth of these aerobic heterotrophs in inorganic media. A lack of pure cultures of the genera _Siderocapsa_, _Ochrobium_ and _Siderococcus_ has resulted in very little being known about the nutritional and physiological requirements of these organisms. They are believed, however, to be involved in the precipitation of ferric hydrate as a result of metabolizing the organic carbon portion of iron humates in soil and water.

Environmental Conditions

Although the various genera of iron bacteria have been shown to vary physiologically with respect to their role in the precipitation of iron, there is no reason to doubt that these organisms characteristically become encoated with ferric hydrate and that their normal habitat is iron-bearing waters. In addition, the bacteriological precipitation of dissolved or suspended iron in water may also be accompanied by naturally occurring chemical changes and in some cases will compete with them (Mallard, 1981). Therefore, in order to better define the relationships between iron bacteria and their potential development in ground water and well environments, the chemical variables influencing iron transformations in natural waters will be discussed in conjunction with the environmental conditions where iron bacteria are known to occur.

The principal chemical variables which influence iron solubility in natural waters include the pH and redox potential (Eh) (Hem and Cropper, 1959). The terms "redox potential (Eh)," "oxidation potential" and "oxidation-reduction potential" are interchangeably used in the literature of water chemistry to represent the relative intensity of oxidizing and reducing conditions in solutions. Eh, simply defined, is the measure of the voltage resulting from the flow of electrons in solution and is influenced by the water temperature and the concentrations of electrically charged ions in solution. Positive values for Eh signify oxidizing conditions in a solution and negative Eh values represent a reducing environment. The effect of Eh and pH on iron, and the ranges in which particular ion or solids will be stable at chemical equilibrium, may be depicted in a stability field diagram or "Eh-pH diagram" (Figure 6). The shaded regions of the diagram depict conditions where insoluble compounds of iron will occur and the unshaded regions represent conditions where dissolved iron forms are predominant. The upper and lower diagonal boundaries across the diagram represent the area in which water is chemically stable. Above the upper boundary line water is oxidized to form oxygen gas and below the lower boundary line water is reduced to form hydrogen gas. It should be noted, however, that Eh-pH diagrams are theoretical and are calculated on the basis of selected conditions for temperature, pressure and ionic concentrations. Therefore, a stability field diagram may not reflect an actual situation because the assumptions made in the preparation of the diagram may not be representative of the site. Nevertheless, the diagram is a useful and convenient device for summarizing the aqueous chemistry of iron in solution and solid phases (Hem, 1970).

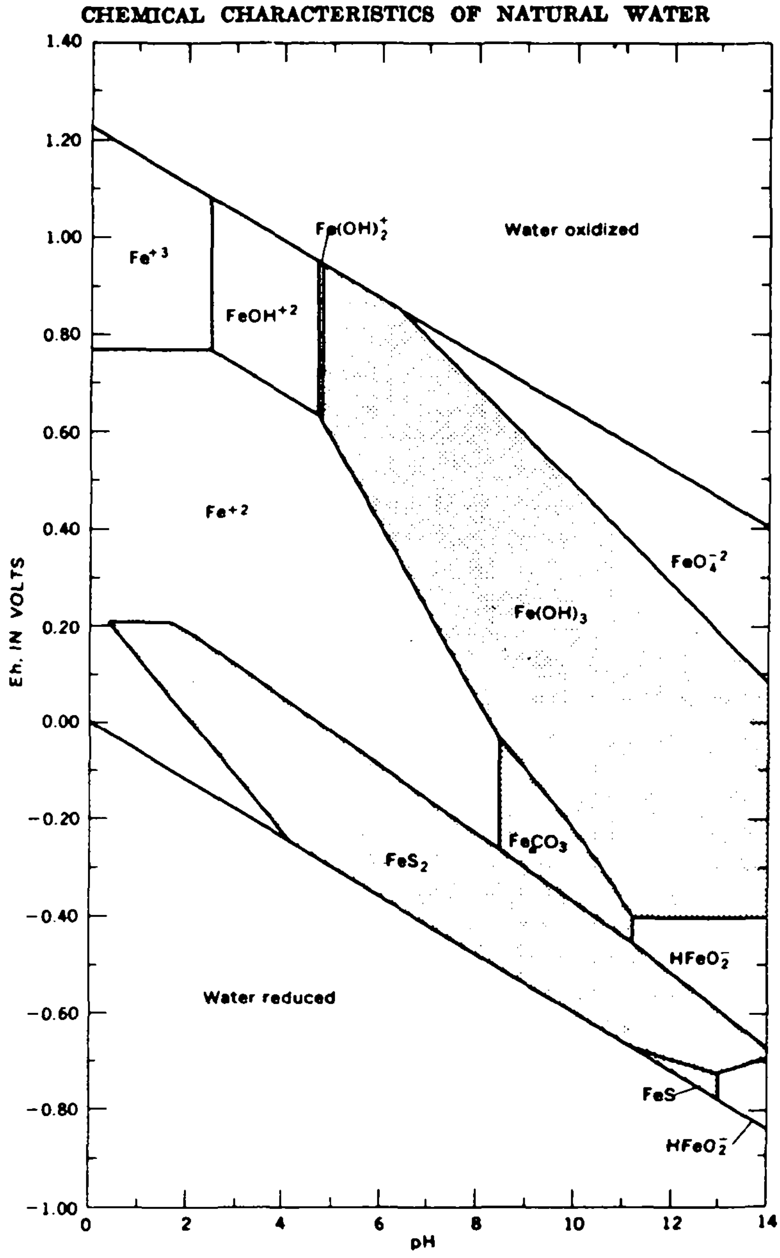

FIGURE 6. Fields of stability for solid and dissolved forms of iron as a function of Eh and pH at 25°C and 1 atmosphere of pressure. Activity of sulfur species 96 mg/l as SO_4^{-2}, carbon dioxide species 1,000 mg/l as HCO_3^- and dissolved iron 0.0056 mg/l. (Hem, 1970).

Based on the Eh-pH diagram for iron shown in Figure 6, it can generally be concluded that when the Eh and pH of a solution are low, the solubility of iron is high. Conversely, the higher the pH or Eh value, the lower the solubility of iron. As a general reference, water in contact with air will have an Eh of approximately 0.35 to 0.50 volt, and usually a pH greater than 5 (Hem, 1970). Under these conditions, the stable form of iron is ferric hydroxide or ferric oxide and iron solubility is low.

The chemical principles, as represented in the Eh-pH diagram, exert a well-defined control over the solubility of iron in ground water. Ground water with a pH between 6 and 8 can be significantly reducing where ground-water flow precludes the entrainment of oxygen into the water and where dissolved oxygen levels are depleted by contact with reduced iron minerals. Under such conditions, ground water can carry significant concentrations of ferrous iron in solution (Figure 6). However, where the ground water encounters oxygenating conditions, which commonly occurs during pumping of a well, the ferrous iron will oxidize rapidly to the ferric iron form. This is evidenced where ground water is clear when first drawn from a well, but soon becomes cloudy and then brown from precipitating ferric hydroxide. Under such oxygenating conditions, the Eh value of the water is raised above 0.40 volts and the solubility of iron is low (Figure 6).

Investigations of the development of iron bacteria in ground water and wells have shown that these organisms characteristically occur within a defined Eh range. Hanert (1981a) reported that Gallionella occur most abundantly in nonorganic, iron-bearing waters characterized by a low redox potential in an Eh range of +200 to +320 millivolts (mV). He also cited that the pH environmental limits for Gallionella range from 6.0 to 7.6 and that dissolved oxygen measurements show a range of 0.1 to 1.0 mg/l. These Eh and pH limits characterize Gallionella as a gradient organism that develops neither under strongly oxidizing conditions nor in a highly reducing zone. Based on stability field diagrams for iron, the Eh and pH environmental ranges for Gallionella are within the zone where ferrous iron is stable (Figure 6). Hanert (1981a) argues that the stability of ferrous iron is the essential factor in the environmental conditions for Gallionella, and is much more important than temperature and dissolved oxygen values. Other observations of the water quality of Gallionella habitats include 5 to 25 mg/l of ferrous iron, greater than 20 mg/l of carbon dioxide, a chemical oxygen demand (COD) of less than 12 mg/l and a temperature range of 8 to 16°C (Hanert, 1981a).

Hasselbarth and Ludemann (1972) also reviewed the chemical nature of ground water where Gallionella and Siderococcus were implicated in well clogging problems. By comparing the chemical water quality between these wells and other control wells which had not experienced iron bacteria problems, they concluded that an Eh value greater than -10mV ± 20mV, was necessary for the mass development of these organisms. They also stated that the measured range of pH values for the infected wells was 6.5 to 7.6

and they used these values to calculate an index of "reductivity intensity" (rH), where:

$$rH = Eh \div 0.0992T + 2pH$$

Eh = oxidation-reduction potential referred to normal hydrogen electrode;

T = absolute temperature in degrees Kelvin.

According to this index, Hasselbarth and Ludemann (1972) concluded that ground water with a rH value greater than 14.5 ± 1 was necessary for iron bacteria growth. This value is in general agreement with the observations of Hanert (1981a) that rH values for Gallionella habitats vary from 19 to 21. Other water quality factors or conditions cited by Hasselbarth and Ludemann (1972) for mass iron bacteria growth included dissolved oxygen concentrations below 5 mg/l, a minimum ferrous iron range of 0.2 to 0.5 mg/l and an adequate velocity of flow which must markedly exceed normal ground-water movement.

In 1975, a state-wide survey was conducted in Alabama to determine the distribution and occurrence of iron bacteria in water wells. Valkenburg et al. (1975) reported that as a result of sampling 109 water wells, they found the widespread development of Gallionella and "Sphaerotilus-like" genera in Alabama wells. Based on their sampling program, they concluded that the environmental conditions most conducive to iron bacteria growth included shallower well depths (less than 400 feet), water temperatures of about 10°C, dissolved ferrous iron concentrations greater than 0.25 mg/l, a pH range between 6 and 8, dissolved oxygen levels around 1.0 to 3.0 mg/l and a moderate to high conductance range of 300 to 700 micromhos/cm.

The environmental conditions for the mass growth and development of members of the Siderocapsaceae family have also been studied (Hanert, 1981b). Observations of the growth of Siderocapsa, Naumanniella and Siderococcus in lake environments have shown these organisms to be microaerophilic, developing in zonal water quality areas characterized by dissolved oxygen levels less than 1.0 mg/l, an rH index less than 19 and a dissolved ferrous iron concentration between 1.0 and 2.0 mg/l. Hanert (1981b) also noted that optimal growth of Siderocapsaceae seems to take place where environmental conditions change from a highly reduced situation to an oxidized condition, under near neutral pH values.

A summary listing of the various environmental conditions reported for the growth of iron bacteria is shown in Table 2. Based on these values, iron bacteria genera can generally be described as occurring in iron-bearing water and soil with neutral pH conditions and relatively low redox potentials. These organisms are predominantly microaerophilic and require limited organic material for growth, with the possible exception of chemoautotrophy in Gallionella. This typical environmental profile of iron bacteria growth conditions should be kept in mind when reviewing iron bacteria problems in ground water and wells. Where water quality analyses indicate alkaline pH, high redox potentials and oxygen rich waters, clogging problems from ferric hydrate precipitate may be simply attributed to chemical processes rather than to an iron bacteria infestation.

TABLE 2. Environmental conditions characterizing the growth of iron bacteria

Parameter	Range	Organism	Reference
Redox potential (Eh)	+200 to +320mV	Gallionella	Hanert (1981a)
	>-10mV ± 20mV	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
pH	6.5 to 7.6	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
	6.0 to 8.0	Gallionella and Sphaerotilus	Valkenburg et al. (1975)
	6.0 to 7.6	Gallionella	Hanert (1981a)
	6.0 to 8.7	Siderocapsaceae	Hanert (1981b)
	6.7 to 7.7	Clonothrix, Crenothrix, Leptothrix and Siderocapsa	Rao (1970)
Reductivity intensity (rH)	>14.5 ± 1	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
	19 to 21	Gallionella	Hanert (1981a)
	<19	Siderocapsaceae	Hanert (1981b)
Dissolved ferrous iron	5 to 25 mg/l	Gallionella	Hanert (1981a)
	>0.2 to 0.5 mg/l	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
	>0.25 mg/l	Gallionella and Sphaerotilus	Valkenburg et al. (1975)

TABLE 2. Environmental conditions characterizing the growth of iron bacteria (continued)

Parameter	Range	Organism	Reference
Dissolved ferrous iron	1.0 to 2.0 mg/l	Siderocapsaceae	Hanert (1981b)
	0.2 to 5.0 mg/l	Clonothrix, Crenothrix, Leptothrix and Siderocapsa	Rao (1970)
Dissolved oxygen	0.1 to 1.0 mg/l	Gallionella	Hanert (1981a)
	<5 mg/l	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
	1.0 to 3.0 mg/l	Gallionella and Sphaerotilus	Valkenburg et al. (1975)
	<1.0 mg/l	Siderocapsaceae	Hanert (1981b)
Chemical oxygen demand (COD)	<12 mg/l	Gallionella	Hanert (1981a)
	>5 to 25 mg/l	Gallionella and Siderococcus	Hasselbarth and Ludemann (1972)
Temperature	8 to 16°C	Gallionella	Hanert (1981a)
	10°C	Gallionella and Sphaerotilus	Valkenburg et al. (1975)
Carbon dioxide	>20 mg/l	Gallionella	Hanert (1981a)

III. IRON BACTERIA RELATED PROBLEMS

Physical Clogging

Iron bacteria are predominantly regarded as fouling agents which result in physical clogging problems in underground drainage systems (Ford and Tucker, 1975; Ford, 1979), water supply systems (Starkey, 1945) and wells (Rao, 1970; Mogg, 1972; Hasselbarth and Ludemann, 1972). These organisms are responsible for significant clogging problems as a result of their ability to precipitate ferric iron as well as produce extracellular polysaccarides or similar "slime-like" organic polymers which encapsulate the sheaths and cell walls of these bacteria.

With regard to water wells, the accumulation of iron hydrates and extracellular bacterial slimes can clog well screens, gravel packs, pump intakes, pipelines, filters and other water well system components. This plugging phenomena is manifested by inefficient well hydraulics, including reduced specific capacities and lower pumping levels. Iron bacteria clogging in wells is usually distinguished from chemical iron deposition problems by the rapid reduction of well flows, in which yields may decline as much as 75 percent in a year's time (Luthy, 1964). Mogg (1972) presented records kept over a ten-year period for a well infested with Gallionella (Figure 7). These records show specific capacity reductions of greater than 50 percent in addition to the frequent need for well redevelopment.

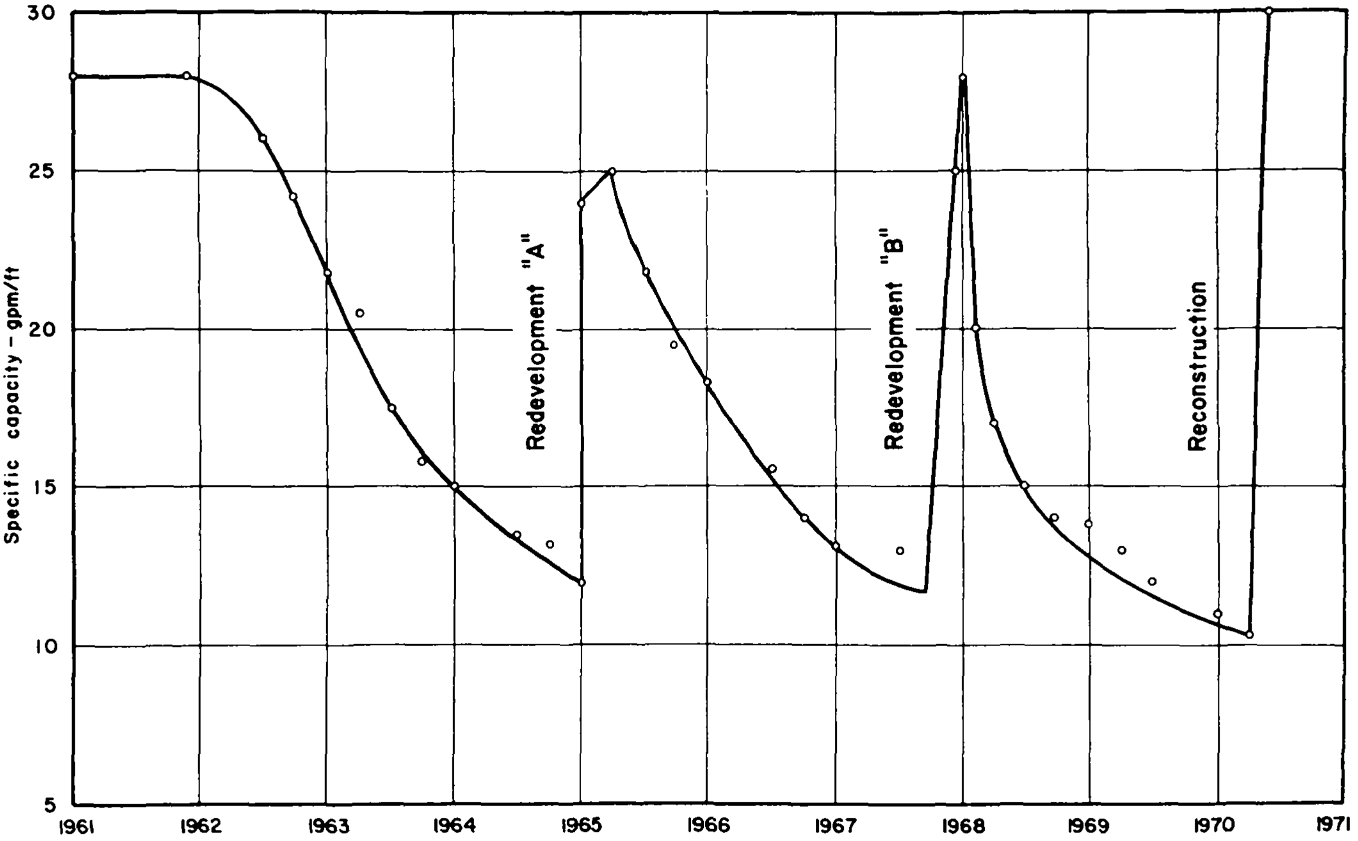

FIGURE 7. The effect of Gallionella clogging on the specific capacity of a well (Mogg, 1972).

Iron bacteria clogging problems in ground water and wells ultimately result in reduced life of well equipment, reduced well capacity and redevelopment expense. Cullimore and McCann (1977) reported that iron bacteria problems in the southern half of Saskatchewan were responsible for well maintenance and rehabilitation costs of four to six million dollars, annually. This reference serves to highlight the economic impact of iron bacteria problems in wells.

Bacteriologically Assisted Corrosion

An early misconception regarding iron bacteria was that these organisms directly attacked ferrous metal surfaces in water in order to utilize the iron during their growth. Later research, however, showed that iron bacteria are capable of growing on nonferrous materials submerged in water, and that their nutritional iron requirements can be met by dissolved ferrous iron already in solution. Although iron bacteria are no longer viewed as direct agents of corrosion, Starkey (1945) introduced the theory that iron bacteria may aid certain electrolytic processes and may enhance the growth of other bacteria which are directly involved in metal corrosion.

Figure 8 depicts a typical electrolytic corrosion process involving anodic and cathodic areas on a metal surface. The anodes and cathodes represent regions with differing electrical charge and between which are established weak electrical potentials. In galvanic cells involving metals, corrosion always takes place at the anode where the metal is dissolved (oxidized) so as to form ions along with the release of electrons. The electrons pass through the metal to the cathode where they combine with hydrogen ions from the water to form atomic hydrogen (H). This deposition of cathodic hydrogen inhibits further corrosion reactions by reducing the electrical potential between the anodic and cathodic areas (Clark, 1980). In addition, where iron corrosion occurs, the dissolved ferrous iron from the anodic area will combine with hydroxyl ions in the surrounding water and deposit a low soluble compound, ferrous hydroxide (Figure 8). If oxygen is present in the water, this compound will be oxidized to ferric hydroxide which will collect on the metal surface. The ferric hydroxide, like cathodic hydrogen protects the metal surface from additional corrosion by reducing the electrical potential between the anode and cathode. Smith (1980) contended that iron bacteria can accelerate the corrosion process by utilizing the ferrous iron from corroding iron and precipitating ferric hydrates around their cells, as opposed to directly on the metal surface. By displacing the ferric hydroxide, the iron bacteria preserve the electrical potential between the anode and cathode and thus enhance corrosion.

Starkey (1945) proposed that the iron bacteria were associated with corrosion processes as a result of their extracellular slimes and ferric hydrate deposits which would provide a suitable growth environment for sulfate-reducing bacteria. The sulfate-reducing bacteria are anaerobic and are capable of developing within anoxic zones between metal surfaces and the accumulated organic and iron hydrate products of iron bacteria (Starkey, 1945). By reducing sulfates to sulfides, these bacteria obtain

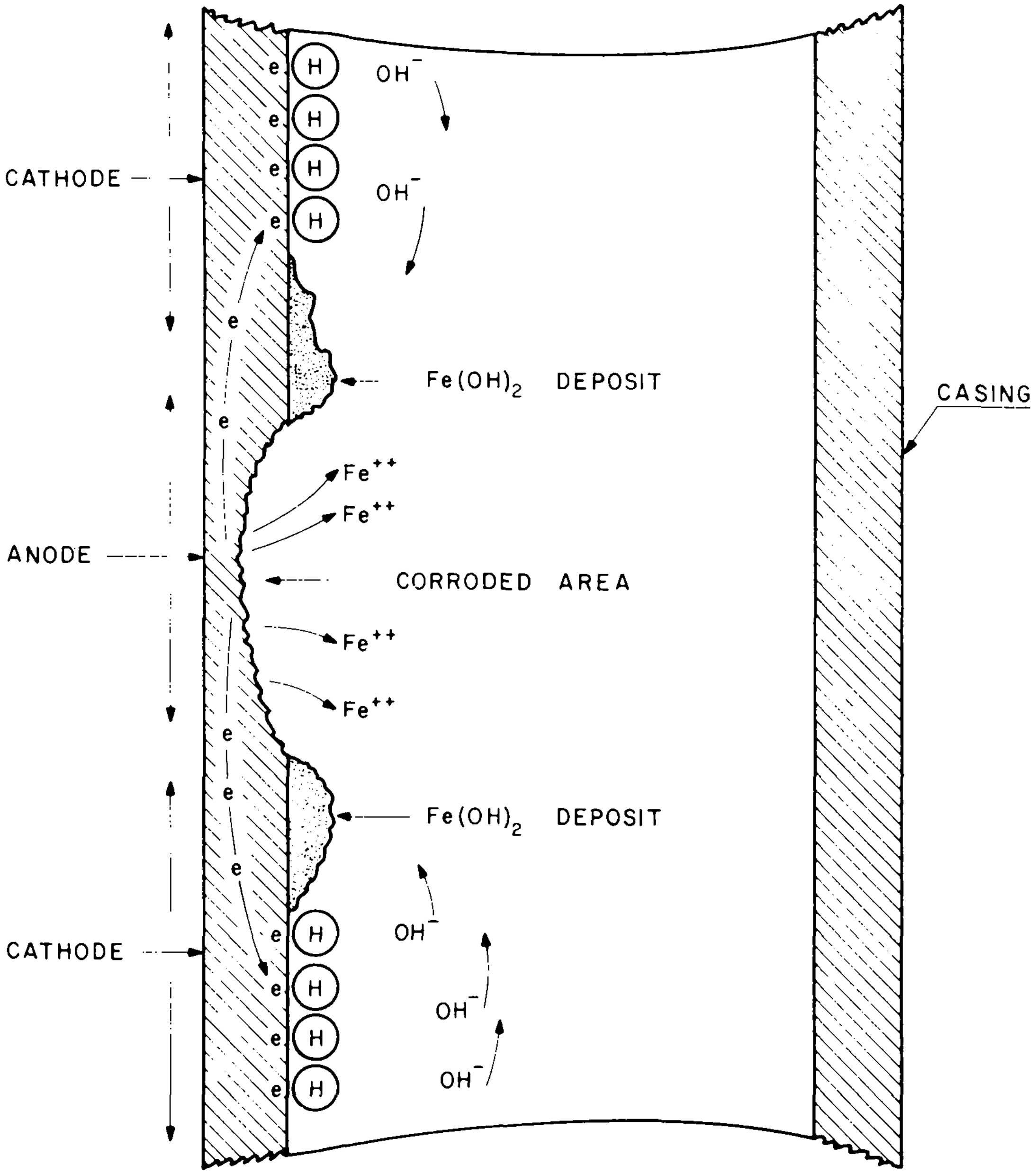

FIGURE 8. Typical electrolytic corrosion process (Clarke, 1980).

the necessary energy to oxidize organic material for cell growth. The resulting sulfides, however, combine with cathodic hydrogen to form hydrogen sulfide. Corrosion processes are therefore enhanced as the cathode is left unprotected and the electrical potential between the anodic and cathodic areas is maintained. In addition, hydrogen sulfide reacts with ferrous iron to form ferrous sulfide which is anodic to iron, and therefore a corrosive agent in and of itself (Figure 9). Smith (1980) further showed that these bacterial corrosion sites become tuberculated, being characterized by soft, raised features on badly corroded pipe. These tubercles perpetuate the galvanic corrosion process by providing a suitable environment for sulfate-reducing bacteria and by creating new electrical potentials between the anoxic interior of the tubercle and the surrounding water which contains dissolved oxygen (Figure 10).

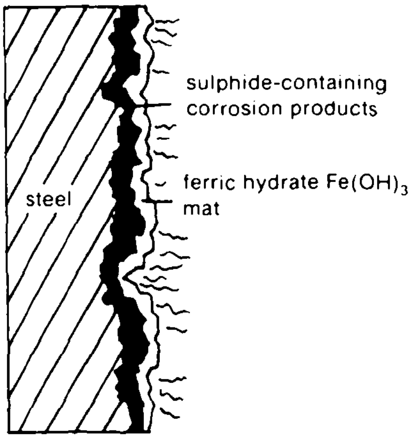

FIGURE 9. Diagram showing anoxic, corrosive sulfide zone beneath ferric hydrate and gelatinous deposits from iron bacteria (Smith, 1980).

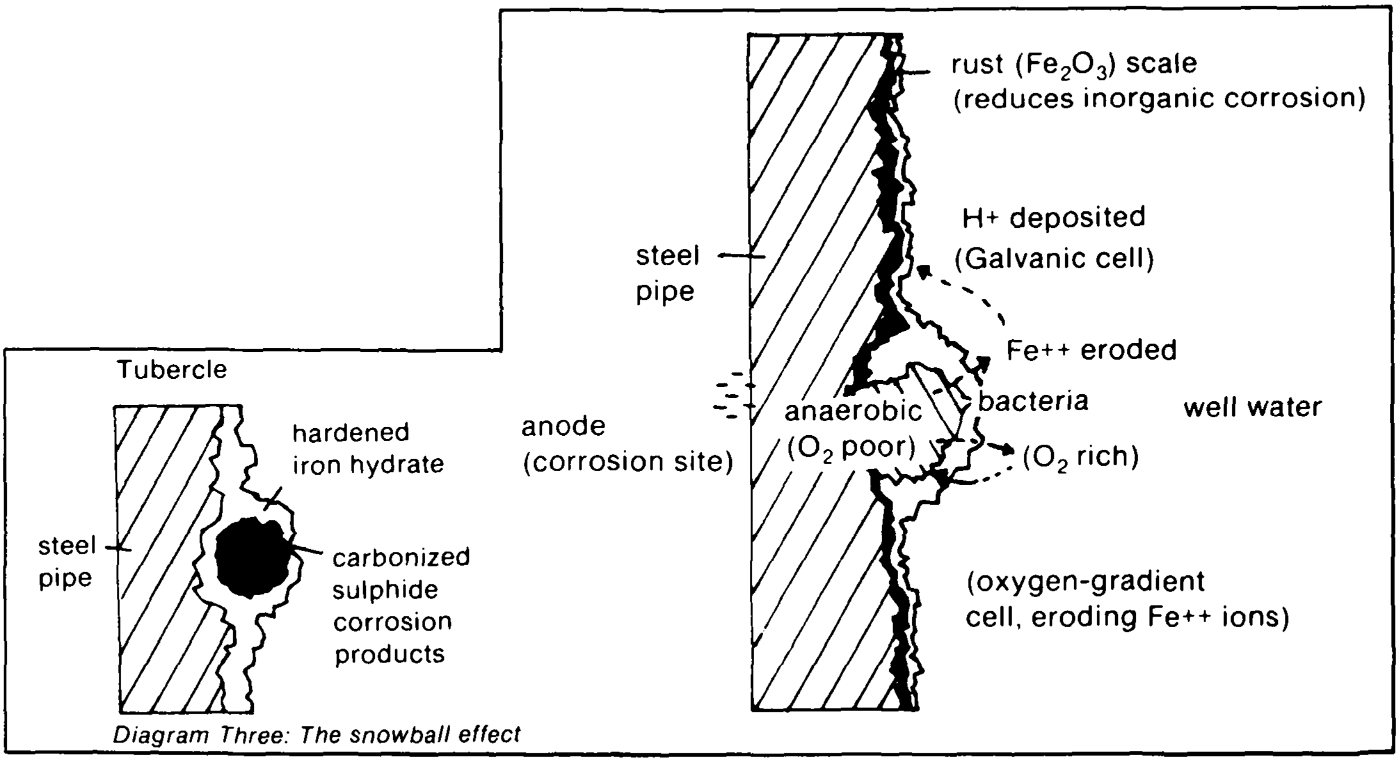

FIGURE 10. Diagram showing a tubercle and the mechanism in which these areas contribute to the metal corrosion process (Smith, 1980).

IV. CONTROL OF IRON BACTERIA

Chemical Methods

Many chemical treatments have been suggested for the control of iron bacteria in ground water and wells including various disinfectants, copper salts, organic and inorganic acids, surfactants and an array of proprietary products. Among these chemical agents, calcium and sodium hypochlorite, hydrochloric (muriatic) acid and sulfamic acid are widely recommended and used for rehabilitating wells affected by iron bacteria (Stott, 1973; Schafer, 1974). The success of these and other chemical treatment methods, however, has been variable as exemplified by numerous reported incidences of recolonization or regrowth in the treated wells, sometimes within short periods of time (Mogg, 1972; Cullimore and McCann, 1977). In these instances, the chemical treatment must be repeated at regular intervals in order to control the growth of these organisms.

A number of reasons have been postulated as to why iron bacteria present such a challenge to chemical control methods. One reason is that the chemical agent may be ineffective or limited in its ability to penetrate the ferric hydrate deposits and extracellular organic slimes or sheaths which accumulate around the iron bacteria cells. In addition, ground-water temperatures are cold, resulting in reduced chemical activity. Concomitantly, iron bacteria are psychrophilic (growing best at colder temperatures) and have a slower metabolic rate, resulting in less absorption of the chemical agent. Many chemical agents are also neutralized or inactivated by the presence of other organic or inorganic substances in the ground water, thereby depleting the concentration of the chemical below levels necessary for an effective bactericidal kill. Similarly, concentrations of the chemical agent may be diluted to ineffectual levels by ground-water hydraulics at the treated well site. Finally, the iron bacteria may be capable of growing outside the chemical treatment zone and may be later carried by ground-water flow back to the treated well.

Grainge and Lund (1969) noted that despite a continued proliferation of recommended chemical treatments for the control of iron bacteria in ground water and wells, many of these methods were found to be ineffective. They observed that although these chemical treatment strategies are directed at the destruction of the iron bacteria themselves, the bactericidal effectiveness of many of these compounds had not been scientifically evaluated under controlled conditions. This lack of bactericidal data was attributed to the difficulties involved in culturing many of these organisms for study (Grainge and Lund, 1969).

The lack of success of some chemical treatments, however, is due in part to inappropriate techniques employed by the user as opposed to deficiencies with the treatment process (Cullimore and McCann, 1977). Descriptions of remedial treatment procedures are often anecdotal and the techniques used are sometimes based on empirical guesswork. To overcome this, more attention needs to be given to assessing and characterizing the degree of iron bacteria problems in wells to determine the necessary level of chemical treatment. Details regarding the chemical treatment process must include chemical concentrations, contact times and chemical application methods which are sufficient to allow total penetration of the iron bacteria, by the chemical agent, in the well.

Chemical treatments for the control of iron bacteria in ground water and wells have typically employed the use of three categories of chemicals: 1) disinfectants; 2) acids; and 3) surfactants (Gass et al., 1983). These chemicals may serve as biocides and agents for dissolving or dispersing the ferric hydrate and organic slime deposits associated with iron bacteria growth. The chemical treatments may be used individually or used in an alternating series, such as acid treatment followed by disinfection.

Disinfectants

Chemical agents which are employed to directly destroy or inactivate the cells of iron bacteria are classified as disinfectants. Within the water well industry, chlorine is the most widely used disinfectant for treating iron bacteria problems in wells (Schafer, 1974). Chlorine compounds have several advantages over other chemical disinfectants in that they are inexpensive, readily available and proven effective against a wide variety of bacteria.

The effectiveness of chlorine as a disinfectant is the result of the strong oxidizing nature of chlorine and its aqueous compounds. When chlorine gas is added to water, a mixture of hypochlorous (HOCl) and hydrochloric (HCl) acids is formed:

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

The formation of hypochlorous acid is important because this compound retains the oxidizing property of chlorine and it is in this form that the principal disinfecting action of chlorine occurs. It is generally thought that the death of bacterial cells results from hypochlorous acid oxidizing essential bacterial enzymes thereby disrupting the metabolism of the organism. In addition, hypochlorous acid has a small molecular size and is electrically neutral, thereby allowing rapid penetration through the bacterial cell wall (American Water Works Association, 1973).

Hypochlorous acid in solution, however, ionizes or dissociates into hydrogen and hypochlorite (OCL^-) ions; the degree of dissociation depends on water pH and temperature:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

Where the pH of the water is below 6, the chlorine will exist predominantly in the hypochlorous acid form. Between a pH range of 6.0 to 8.5, however, hypochlorous acid rapidly dissociates into hypochlorite ion, and above a pH of 8.5, the hypochlorite ion is the predominent form of chlorine. This is important to the disinfection process because the hypochlorite ion is not as strong an oxidizing agent as hypochlorous acid and the negative charge of the ion impedes its ability to penetrate the organism's cell wall.

Hence, the hypochlorite ion is not as effective a disinfection agent as hypochlorous acid. The pH of ground water is normally within the range where chlorine may exist as both hypochlorous acid and hypochlorite ion. Chlorine existing in water as hypochlorous acid and hypochlorite ions is defined as "free residual chlorine."

Chlorine-based compounds, such as calcium hypochlorite ($Ca(OCl_2)$, with 70 percent available chlorine) and sodium hypochlorite ($NaOCl$, typically with 5 to 12 percent available chlorine) are commonly used for treating wells with iron bacteria problems. These compounds ionize in water, similar to chlorine gas, and yield hypochlorite ions:

$$Ca(OCl)_2 + H_2O \rightleftharpoons Ca^{+2} + 2\ OCl^- + H_2O$$

$$NaOCl + H_2O \rightleftharpoons Na^+ + OCl^- + H_2O$$

These hypochlorite ions also combine with hydrogen to form hypochlorous acid, as shown in the previous equilibrium equation. Therefore, the same free residual chlorine in water is formed regardless of whether chlorine gas or hypochlorite chlorine compounds are used.

Along with an understanding of the term "free residual chlorine," the effective use of chlorination for the control of iron bacteria in ground water and wells requires an understanding of the terms "chlorine dosage," "chlorine demand" and "contact time." The total amount of chlorine added to water, usually measured in either parts per million or milligrams per liter is referred to as the chlorine dosage. However, as quickly as chlorine is added to water, a portion of the chlorine dosage will be lost as the chlorine oxidizes both organic matter and reduced inorganic minerals. These organic and inorganic compounds which react with and neutralize chlorine in water are said to exert a chlorine demand. If the chlorine dosage to a water supply is equal to the chlorine demand, no free residual chlorine will be formed, resulting in no disinfection action. Therefore, when chlorinating a well for the treatment of iron bacteria problems, the chlorine dosage must exceed the chlorine demand exerted by extraneous organic and inorganic materials in the ground water in order to produce a free residual chlorine which will have a bactericidal effect on the iron bacteria. In addition, the concentration of free residual chlorine formed during the treatment process will determine the amount of time the chlorine must be in contact with the iron bacteria in order to achieve an adequate bactericidal kill. This length of exposure of the targeted bacteria to the free chlorine residual is termed contact time.

Although the technology for chlorinating wells clogged by iron bacteria has existed since the early 1900's (White, 1972), Grange and Lund (1969) were the first researchers to demonstrate, under controlled laboratory conditions, the bactericidal effectiveness of chlorine against iron bacteria. They reported that free chlorine residuals between 300 and 500 mg/l over an 18 hour contact time effectively killed iron bacteria. In addition, they noted that at a lower free residual chlorine concentration of 25 mg/l, a minimum contact time of 24 hours would be required to dislodge filamentous iron bacteria deposits from the walls of wells.

The results of the study by Grainge and Lund (1969), coupled with empirical observations made over the years, has resulted in a set of generally recognized procedures for chlorinating wells with iron bacteria problems (Johnson Division, 1972; White, 1972). This procedure involves the use of either calcium hypochlorite or sodium hypochlorite in order to provide a chlorine dosage which will result in a 200 to 500 mg/l free residual of chlorine in the well water. After introducing the chlorine into the well, the chlorinated water is forced out into the water-bearing formation by one of several physical surging techniques, including air lifting or the use of a mechanical surge block (Gass et al., 1983). The surging action not only forces the chlorinated water out into the formation, but also physically agitates the ferric hydrate and organic slime deposits so as to break up these deposits. This action results in a more thorough contact between the iron bacteria and the chlorine residual. A contact time of 24 hours is usually prescribed, during which time the well is periodically surged and the free residual chlorine concentration checked. If the free residual chlorine concentration falls below the necessary level for an effective bactericidal kill, the chlorine dosage is increased. After 24 hours, the well is pumped free of chlorinated water and normal well operations resumed. In some instances, three or four successive chlorine treatments are performed to increase the liklihood that the chlorinated water is flushed out into the formation around the well (Johnson Division, 1972).

Under controlled laboratory conditions, Valkenburg et al. (1975) investigated the bactericidal effectiveness of selected chlorine and quaternary ammonium compounds on iron bacteria. Using Gallionella cultures, they demonstrated that two quaternary ammonium compounds, Bardac-22™ and benzethonium chloride, were effective in destroying Gallionella at low concentrations. Figure 11 shows that 0.5 mg/l of Bardac-22™ and 3.0 mg/l of benzethonium chloride were reported to have killed Gallionella after a 3 hour exposure at 20°C. The active bactericidal ingredient in quaternary ammonium compounds is n-alkyl dimethyl benzyl ammonium chloride which, in addition to being a strong disinfectant, possesses cleaning (detergent) properties due to its sufactant activity. Valkenburg et al. (1975) postulated that the successful destruction of Gallionella by the quaternary ammonium compounds was due to the detergent action of these disinfectants and the resulting ability of the compound to penetrate ferric hydrate deposits around the organisms. In the same study, Valkenburg et al. (1975) also demonstrated the bactericidal effectiveness of chlorine against Gallionella by using Clorox™, which is a sodium hypochlorite solution with 5.25 percent available chlorine, and trichloroisocyanuric acid, which is an organic chlorine compound with 90 percent available chlorine (see Figure 11). Despite these laboratory findings, Valkenburg et al. (1975) concluded that future field experimentation was necessary to fully assess the long-term effectiveness of all these disinfectants against iron bacteria populations in wells.

Cullimore and McCann (1977) also examined the ability of several different disinfectants to inhibit the growth of Gallionella and Crenothrix cultures. They demonstrated that the effectiveness of most disinfectants

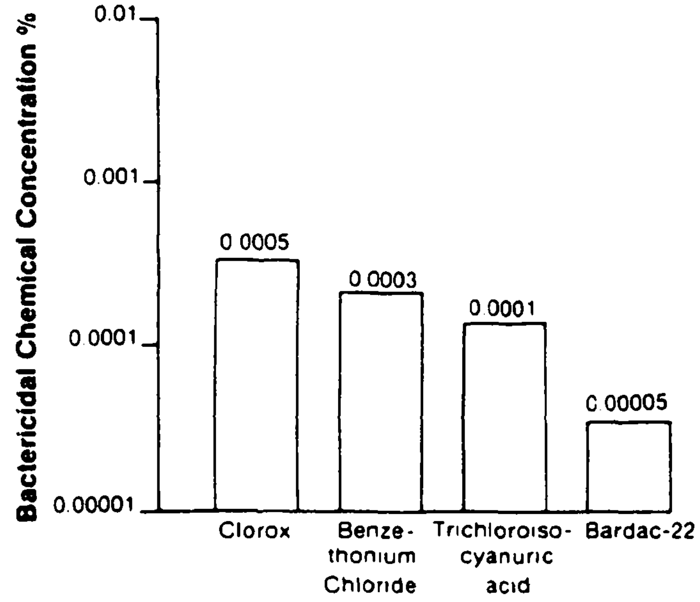

FIGURE 11. Bactericidal chemical concentrations necessary to kill <u>Gallionella</u> organisms after 3 hours of exposure at 20°C (after Valkenburg et al., 1975).

differed significantly with the number of cells present in each 15 ml culture (Table 3). For example, Javex™, which is a sodium hypochlorite solution containing 5.5 percent available chlorine, prevented the growth of iron bacteria at a free chlorine residual concentration of 250 mg/l, where the density of bacteria cells was less than 100 per 15 ml. However, where the density of bacteria cells was 700,000 to 1,000,000 cells per 15 ml, the concentration of chlorine residual required to prevent bacterial growth was 10,000 mg/l. Cullimore and McCann (1977) concluded that the selection of a disinfectant concentration for the control of iron bacteria in wells must reflect the density of cells within the proposed treatment area.

Cullimore (1979a) also conducted a series of laboratory trials to determine the relative effectiveness of different chemical disinfectants against strains of Gallionella, Crenothrix and Sphaerotilus iron bacteria. The results of these trials are shown in Table 4. Cullimore (1979a) showed that two chlorine compounds, chlorine dioxide and hypochlorites, were more effective than the other compounds based on an "assimilated rating." This rating involved averaging the effectiveness of a particular chemical to kill the iron bacteria over a concentration range which varied from 100 to 10,000 mg/l over a maximum contact time of 6 days. In addition to chlorine, quaternary ammonium compounds were also shown to be generally effective against the iron bacteria. Cullimore (1979a), however, cautioned that the laboratory findings may not be indicative of actual field results because the laboratory trials were conducted with the iron bacteria in suspension, so that the disinfectant was readily able to penetrate small groups of cells. These trials, therefore, did not evaluate the relative

TABLE 3. Extrapolated effective control concentration (mg/l) for five disinfectants against a range of cell concentrations of iron bacteria (Cullimore and McCann, 1977)

	Cell concentration of iron bacteria (cells per 15 ml)				
Disinfectant*	+30 to 100	300 to 1,000	+3,000 to 10,000	70,000 to 100,000	+700,000 to 1,000,000
Javex** (sodium hypochlorite, 5.5% available chlorine)	250	750	850	1,000	10,000
HTH** (calcium hypochlorite, 70% available chlorine)	2,000	5,000	7,500	10,000	100,000
IP (iodine polymer)	10	20	30	40	NC
$CuSO_4$	500	1,000	5,000	50,000	10,000
$KMnO_4$	10-20	100	250	500	5,000

* All data for pH of 7.4

\+ Extrapolated data

** Based on hypochlorite concentration

NC, no control since the solubility of IP in water is very low

ND, not determinable from data

TABLE 4. Relative effectiveness of selected disinfectants against Gallionella, Crenothrix and Sphaerotilus (Cullimore, 1979a)

Compound	Assimilated Rating*	Code**
Chlorine dioxide	82	Cl
Hypochlorite	74	Cl
San Q-5	69	QAC
Hyamine	68	QAC
QA	64	Form
Triton X100	39	S
Iodine	33	I
Rexol	30	S

* Rating of the chemical to kill iron bacteria over a concentration range from 100 to 10,000 mg/l over a maximum 6 day contact time.

** Active component; Cl-chlorine; QAC-quaternary ammonium compound; Form - a special formulation of quaternary ammonium compound, surfactant, iodine, citric acid and inorganic salt nutrients; S - surfactant; I - iodine.

effectiveness of these disinfectants against an iron bacterial slime growth on a fixed surface. Cullimore (1979a) concluded that field evaluations of chlorine and quaternary ammonium compounds were needed to determine the bactericidal effectiveness of these compounds when applied to actual iron bacterial infestations in ground water and wells.

Acids

Acids are another category of chemicals which may be used for rehabilitating wells clogged by iron bacteria. The main value of acids is the ability to chemically dissolve the ferric hydrate deposits which accumulate around these organisms. In addition, some acids in high concentrations may serve as disinfectants and may also help to disperse and loosen the extracellular slime material deposited by the iron bacteria (Mogg, 1972). Valkenburg et al. (1975) showed that a concentration of 40 mg/l of acetic acid and 73 mg/l of hydrochloric acid were capable of inhibiting Gallionella growth under laboratory conditions (Figure 12). Luthy (1964) and Cullimore and McCann (1977) also reported that a proprietary organic acid, LBA™ (liquid antibacteria acid), was effective in controlling the growth of iron bacteria when used at a manufacturer's recommended concentration of 5 percent (50,000 mg/l) over a 36 hour contact time.

The acids most commonly used as chemical treatments for the control of iron bacteria in wells include hydrochloric acid (HCL) and sulfamic acid (NH_2SO_3H) (Schafer, 1974; Cullimore and McCann, 1977). Hydrochloric (muriatic) acid is commercially prepared as a solution and is available in

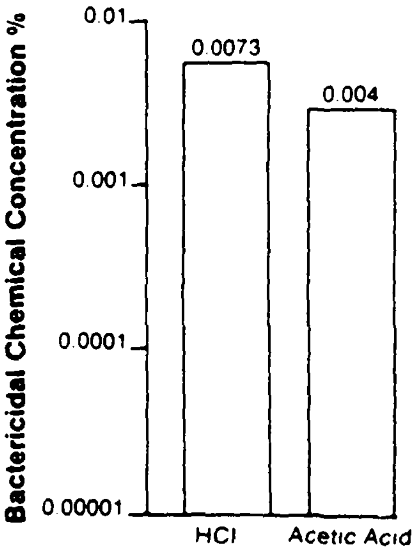

FIGURE 12. Lowest concentration of acids that will kill <u>Gallionella</u> organisms after 3 hours of exposure at 20°C (after Valkenburg et al., 1975)

several strengths, the most common being 28 percent. This acid may also be sold with an inhibiting agent, such as a gelatin-based compound, which serves to lessen the tendency of the acid to attack and corrode metal surfaces during the well treatment process. Sulfamic acid is available as a dry, granular material and forms a strong acid when dissolved in water. The solubility of sulfamic acid in water increases with temperature, ranging from 15 to 20 percent by weight at most ground-water temperatures (Johnson Division, 1972). Sulfamic acid reportedly is a slower-acting acid than hydrochloric acid and therefore has a less corrosive effect on metal well components.

Similar to disinfectants, the concentration and contact times for hydrochloric and sulfamic acids, when used to treat iron bacteria problems in wells, should vary depending on the density of the organisms in the treatment zone. Table 5 contains one proposed scheme recommended by Cullimore and McCann (1977). As can be seen from Table 5, the necessary acid concentrations may vary from 7.5 to 21 percent and recommended contact times may range from 6 to 24 hours.

In addition, the method used to apply the acid to a well during treatment for iron bacteria is important. Because these acids are heavier than water, the acid should be introduced to the proposed treatment zone by pouring the solution down a conductor pipe. This allows the concentrated acid to be initially emplaced in the areas of the well which require direct treatment. Once in the well, however, the acid solution should be agitated so that a uniform mixture is achieved throughout the treatment zone. As

TABLE 5. Potential acid treatments listing time-chemical concentrations for the control of iron bacteria (Cullimore and McCann, 1977)

Density of iron bacteria		Chemical treatment	
		Hydrochloric acid	Sulfamic acid
1 to 300 cells/ml	Conc. (%)	14	7.5
	Time (hrs.)	6	12
300 to 5,000 cells/ml	Conc. (%)	18	10
	Time (hrs.)	12	24
5,000 to 50,000 cells/ml	Conc. (%)	21	10
	Time (hrs.)	24	48

with disinfectants, agitation can be accomplished with a jetting tool, surge block or compressed air in order to force the acid out into the formation and to break up the ferric hydrate and bacterial slime deposits.

Most chemical treatment strategies for controlling iron bacteria in ground water and wells use a series of separate treatments involving acid followed by a disinfectant. This strategy recognizes the primary capability of acid to dissolve encrusted deposits of ferric hydrates and to loosen iron bacteria slimes. Once the spent acid is removed from the treated well, a disinfectant is used to achieve the desired bactericidal kill. This treatment series may be repeated several times in order to effectively remove all the iron bacteria deposits and suppress the growth of these organisms.

Surfactants

Surfactants are another category of chemicals which may be used, especially in conjunction with disinfectants, for treating wells with iron bacteria problems. These chemicals have a detergent capability in which they attach onto ionic particles, such as clays, colloids and metal ions, forming large complex particles with the same electrical charge. Because similar electrically charged particles tend to repel each other, these particles are broken up and dispersed. Chemicals which can create these uniform charged particle complexes are referred to as "surface-active agents" or surfactants.

Within the water well industry, glassy sodium phosphates (polyphosphates) are useful in the treatment of wells with iron bacteria problems because they can effectively disperse the ferric hydrate deposits surrounding these organisms. Polyphosphates are typically applied at concentrations of approximately 3 percent (Gass et al., 1972). In addition a minimum 50 mg/l free chlorine residual is always applied along with the polyphosphate in order to suppress the growth of bacteria which may utilize the polyphosphates as a nutrient source. A chemical treatment strategy for the control of iron bacteria in wells might therefore utilize polyphosphates as a pretreatment to disinfection or may use polyphosphates concurrently with a compatible disinfectant in order to promote a more effective penetration of the iron bacteria by the bacteriocide. Cullimore (1979a) also showed that surfactants did kill some iron bacteria in laboratory studies, presumably due to the dispersion of the protective slime coating which forms around the cells (Table 4).

Regarding the method of application, polyphosphates are dissolved slowly in a mixing tank and introduced into the well as a solution. As with all other chemical treatments, once the polyphosphates are placed in the well, vigorous mechanical agitation, such as surging or jetting, is employed to enhance loosening and dispersion of the ferric hydrate deposits. Two or more successive treatments of polyphosphates are usually performed in order to increase the likelihood that the chemical solution will be forced out into the surrounding formation.

Physical Methods

In contrast to chemical methods, physical treatment methods for controlling iron bacteria in ground water and wells has only received attention within the last 10 years. Much of this attention has been brought about by researchers attempting to find alternatives to traditional chemical treatment strategies which may not have produced desired rehabilitation results. Unlike the traditional chemical treatment strategies for iron bacteria in wells, physical control methods have centered on the destruction of the organisms themselves and on the alteration of ground water and well environments so as to manipulate the growth of these bacteria. The more prominent physical control methods investigated to date include: 1) ultraviolet light; 2) ultrasonic vibration; 3) heat treatment (pasteurization); and 4) changing the redox potential of ground water.

Ultraviolet Light

Ultraviolet light has been known to be an effective physical bactericidal disinfectant since the 1940s (National Academy of Sciences, 1980). The mechanism of disinfection action is believed to be the denaturation of essential nucleoproteins, which results from the direct absorption of ultraviolet energy by the organism. For disinfection purposes, ultraviolet lamps are designed to provide a maximum amount of light at the proper wavelength (2,537 Angstrom units) for the greatest bactericidal action. In order for an ultraviolet disinfection unit to be effective, however, the water must be circulated to expose a maximum surface area of the bacteria to the ultraviolet light. Turbid water will significantly decrease the bactericidal effectiveness of ultraviolet light as a result of bacteria being "shadowed" by particulate matter, thereby preventing the killing rays of ultraviolet light from reaching the targeted organisms (Lehr et al., 1980).

Cullimore (1981a) conducted preliminary investigations into the use of "in-well" ultraviolet lamps to directly irradiate selective areas of a well which were subject to iron bacteria clogging. He concluded, however, that these bacteria were less sensitive to this treatment method because the organisms were shielded from the ultraviolet light by encrusted ferric hydrate deposits and extracellular polysaccharide slimes. As a result of these deposits and the turbid conditions which may occur within the well, Cullimore (1981a) contended that ultraviolet light cannot be considered an effective treatment method for controlling iron bacteria infestations in wells.

Ultrasonic Vibration

Cullimore and McCann (1977) discussed their investigation of ultrasonic vibration (sonication) as a possible physical treatment method for controlling the growth of iron bacteria in wells. Sonication involves utilizing the energy produced by sound waves so as to disintegrate individual bacterial cells. The ultrasound is generated by a probe which could conceivably be installed permanently or be periodically lowered into a well for treatment. Cullimore and McCann (1977) theorized that the ultrasonic probe in the well could be used to remove the ferric hydrate and organic slimes deposited by the iron bacteria and to disintegrate the individual cells. They reported, however, that a series of experiments on Gallionella and Crenothrix showed that no significant reduction in the number of cells occurred, even after 60 minutes exposure time in a ultrasonic tissue dismembranator operating at 800,000 cycles per second. Cullimore (1981a) later noted that there was even some evidence of reproduction occurring in the cultures. Cullimore and McCann (1977) credited this atypical survival capability of the iron bacteria to the extracellular gelatinous cell coatings which serve to lessen the effects of sonication. As a result, they concluded that sonication would not represent an effective physical treatment method for controlling iron bacteria in wells.

Pasteurization

Pasteurization involves the application of heat to destroy microorganisms. Although the bactericidal effects of heat have been known since the 1860s when Louis Pasteur demonstrated that a temperature of 60°C for 30 minutes could kill spoilage bacteria, pasteurization is a relatively new approach to disinfecting water supplies. With regard to controlling iron bacteria in ground water and wells, preliminary studies have demonstrated that these organisms are heat sensitive and that growth in wells can be controlled by repeated heat treatments.

Valkenburg et al. (1975) first reported the sensitivity of Gallionella to heat. They demonstrated under laboratory conditions that 48°C was bactericidal to Gallionella following a 10 minute exposure. Valkenburg et al. (1975) concluded that pasteurization may be of potential use in treating water wells contaminated with iron bacteria.

In addition, extensive laboratory and field studies conducted throughout the 1970s by researchers at the Regina Water Research Institute, University of Regina in Saskatchewan have shown that iron bacteria, as a group, are very sensitive to the elevation of water temperatures (Cullimore, 1979b). Figure 13 depicts the influence of temperature on the growth of iron bacteria. As previously noted in this report, iron bacteria are psychrophilic and have an optimum growth temperature at approximately 10°C. When water temperatures are elevated to temperatures ranging from 32°C to 45°C, however, the organisms cease to grow and their bacterial slimes are dispersed. As the water temperature raises above 54°C, the iron bacterial cells are rapidly killed. In practice, therefore, the use of steam or hot water to elevate ground-water temperatures above 54°C for a prescribed period of time, should represent an effective method for controlling iron bacteria growth in wells.

Cullimore and McCann (1977) reviewed the findings of initial pasteurization field tests which were conducted on two wells near Regina, Saskatchewan using high-pressure steam injection. In both instances, the water temperature was raised to 65°C for 40 minutes and this treatment resulted in the dispersion of encrusted slime deposits and the killing of bacterial

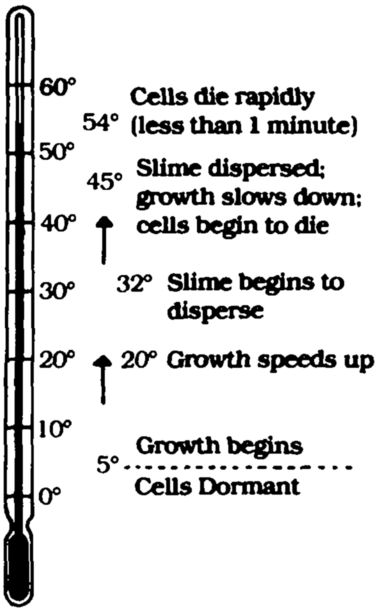

FIGURE 13. Effect of temperature on iron bacteria (Cullimore,1980).

cells. Although these heat treatments were initially effective in restoring well yields, the recurrence of iron bacteria populations after treatment was noted for both wells. Cullimore and McCann (1977) postulated that this recurrence of iron bacteria populations was the result of organisms, surviving in the aquifer outside the treated zone, recolonizing the well after the pasteurization treatment. They concluded, therefore, that the control of iron bacteria problems would require regular pasteurization treatments of the wells.

Cullimore (1979b) also reported on a recycling hot water pasteurization system which was installed in a well at Bulyea, Saskatchewan. This field test demonstrated that the pasteurization temperatures had to reach a minimum of 38°C to cause dispersion of the iron bacterial slimes and 54°C to kill 99 percent of the cells in the treated volume of water. Although Cullimore (1979b) concluded that the pasteurization process was successful in restoring the yield of this iron bacteria clogged well, he acknowledged that the system had energy-efficiency problems. Most of the energy (between 60 and 81 percent) was lost by radiation of heat through the insulated pipes and also from the free discharge of hot water back down the well. In addition, recurring iron bacteria growth in the well resulted in a recommendation that the pasteurization treatment be repeated a minimum of once every three months. Cullimore (1981b) indicated that alternative energy-efficient and reliable portable or permanent heater systems were needed.

Changing the Redox Potential

Another physical treatment strategy which can be used to control iron bacteria problems in wells is to alter the redox potential of the ground water. As was earlier discussed in this report, redox is a measure of the oxidation or reduction potential occurring in water. Where dissolved oxygen is readily available in water, the redox potential (Eh) will be high and metals, such as iron and manganese, will be oxidized and precipitated from solution. In oxygen-deficient ground water, the Eh values will be low and the solubility of these metals will be high. With regard to iron bacteria occurrence in ground water, these organisms have been shown to favor relatively low redox potentials (between -10mV and +350mV) where they can compete with limited amounts of oxygen for the oxidation and deposition of iron. As a result, processes which influence the redox potential of ground water may be used to manipulate the growth of these organisms in aquifers and in wells.

One technique which attempts to selectively alter the redox potential of ground water not only for the removal of iron and manganese but also for the control of iron bacteria is known as the Vyredox™ method. This system was first developed in Finland in 1955 and is a patented process (Zienkiewicz, 1985). The Vyredox™ method actually makes use of existing iron precipitating bacteria in the ground water to control iron-related problems in a production well. This is done by using aeration wells to enhance bacterial growth in an area removed from the production wells. In this process, a percentage of water pumped from a producing water well is degassed, then aerated and returned to the aquifer through several small injection wells surrounding the producing well (Figure 14). This oxygenated water creates a zone, away from the producing well, favorable to both iron bacteria growth and the precipitation of iron and manganese oxides. As a result, dissolved iron and manganese in the ground water moving through the zone of elevated Eh is precipitated out of solution both chemically and biologically. Ground water recharging the producing well will therefore be low in iron and manganese concentrations and will be free of iron bacteria.

The percentage of water from the production well which is aerated and returned to the aquifer may vary from a miniumm of 4 percent to as high as 33 percent, depending on the hydrology of the site and the desired degree of water treatment (Zienkiewicz, 1985; Cullimore, 1981a). The size and dimensions of the recharge zone are determined from pilot studies and geological investigations of each proposed treatment site. The recharge zone must be large enough to allow adequate contact time for the precipitation of iron and manganese and must have an adequate porosity for the accumulation of these deposits (Zienkiewicz, 1985). According to Armstrong (1978), the Vyredox™ method is primarily applicable to shallow sand and gravel aquifers (less than 300 feet).

Concerns over long-term blockage of the production well as a result of accumulated iron and manganese oxides and iron bacteria growth in the surrounding aquifer has led some researchers to question the Vyredox™ process. Hallberg and Martinell (1976), however, argued that such deposits would

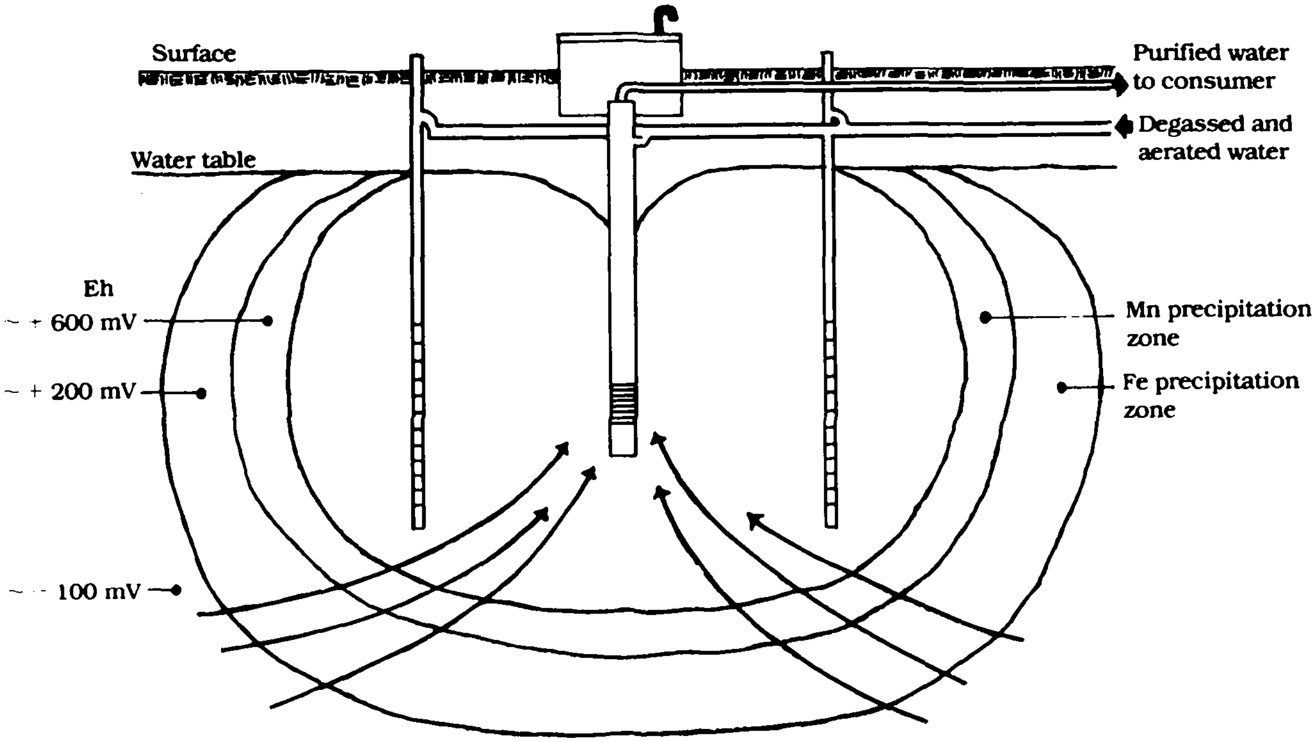

FIGURE 14. Vyredox™ method showing an iron and manganese precipitation zone which is created in the aquifer (after Zienkiewicz, 1985).

take hundreds of years to significantly reduce the yield of the production well. Since this process has only been in use in the United States since 1979, adequate time has not lapsed to document the validity of either view point. Consideration of capital costs, pumping costs and system maintenance, however, have limited the application of this process to commercial and municipal well use. Additionally, Cullimore (1981a) stated that an extensive cost/benefit study should be performed when considering the use of this procedure for controlling iron bacteria in wells.

V. CONCLUSIONS

"Iron bacteria" is a general term which refers to a wide variety of organisms which are quite diverse in morphology, physiology and environmental occurrence. As a result of their ability to deposit ferric hydrate around their cells and to excrete extracellular polysaccharide slimes, these organisms are capable of causing significant clogging problems in ground water and wells, water-distribution systems and underground drainage systems. Additionally, iron bacteria are involved in bacteriologically assisted corrosion processes due to the incidential harborage of sulfate-reducing bacteria in their ferric hydrate and organic slime deposits. Iron bacteria problems in ground water and wells are recognized worldwide and may be locally responsible for multimillion dollar annual well maintenance and rehabilitation costs.

Despite the widespread familarity with iron bacteria problems in wells, these organisms have not been the subject of systematic study. One reason for the lack of active microbial research on iron bacteria is that these organisms are difficult to culture for experimental study, and pure cultures of many of these organisms have never been obtained. As a result, much of the knowledge regarding the growth requirements of iron bacteria is rudimentary and an understanding of the environmental conditions necessary for their development is limited.

The lack of definitive information on the physiology and ecology of iron bacteria has hampered the development of effective treatment strategies for controlling the growth of these organisms in ground water wells. Remedial methods used to control iron bacteria in wells are frequently based on colloquial observations and the techniques used are often lacking scientific documentation. As a result, the success of many recommended iron bacteria treatment strategies has been variable, and recolonization or regrowth in the treated well is common.

Studies have demonstrated that selected chemicals are capable of effectively killing iron bacteria under laboratory conditions. Among these chemicals, chlorine and quaternary ammonium compounds have been shown to be the most effective disinfectants. The use of these and other chemicals for controlling iron bacteria populations in wells, however, requires systematic field evaluation in order to define the chemical concentrations, contact times and chemical application methods which will effectively remove actual iron bacteria infestations in wells.

Physical methods for controlling iron bacteria in ground water and wells, including heat treatment and the alteration of the ground water redox potential, have also been shown to be effective in pilot studies. Neither treatment process, however, has been commercially developed for small-scale use. Energy efficient and reliable equipment associated with the heat treatment of wells for iron bacteria control is not yet commercially available. In addition, the Vyredox™ method of altering ground-water redox potential, while proven effective in pilot and full scale installations, is a proprietary process and involves capital and operational maintenance costs which typically limit this method to municipal or commercial uses.

VI. RECOMMENDATIONS

Iron bacteria infestations in water wells are regarded as the most prevalent cause of bacterial degradation of well performance due to their ability to clog well intakes and surrounding aquifer materials with their characteristic deposits of ferric hydrate and gelatinous slime. Despite this well recognized problem, effective treatment strategies have been slow to develop as a result of what little is known about the physiology and ecology of this diverse group of organisms. In order to develop an effective treatment strategy for the control of iron bacteria problems in wells, further study is needed in the following areas:

1) Techniques for culturing and isolating iron bacteria need to be refined in order that these organisms be available for physiological and environmental control studies. These studies could generate additional knowledge about the specific growth requirements of various iron bacteria genera and identify key environmental conditions for their occurrence in ground water.

2) Additional field studies are needed to characterize the nature of iron bacteria populations in wells and to determine whether these organisms are indigenous to shallow water table aquifers. These studies should attempt to relate the occurrence and mass development of these bacteria to "indicator" ground water quality parameters such as redox potential, pH, dissolved oxygen, ferrous iron, total organic carbon and temperature.

3) Continued research on techniques or methods for sampling and enumerating iron bacteria in aquifer materials is required. This research is needed to quantifiably assess the ability of these organisms to migrate with ground-water flow out into the aquifer away from an infected well.

4) Systematic field evaluations of some of the more promising disinfectants, including chlorine and quaternary ammonium compounds, are needed in order to determine the effectiveness of these compounds when applied to actual iron bacterial infestations in ground water and wells. Closer attention needs to be paid to the details of the chemical treatment process, including chemical concentrations, contact time and chemical application methods. The degree of success of these treatments should be quantified by the enumeration of bacterial cells before and after treatment.

5) Field evaluations are needed to assess the effectiveness of a combination of chemical treatment processes used in successive series, such as the use of acids or polyphosphates prior to disinfection. Such processes may assist the disinfectant in reaching and contacting the iron bacteria cells located beneath the heavier layers of ferric hydrate deposits and organic slimes.

6) A field demonstration project utilizing an in-well immersion heater to pasteurize iron bacteria clogged wells is needed to determine

whether such a process would be energy-efficient and suitable for development as a reliable portable, or permanently installed, heat treatment unit.

7) Additional methods or concepts for altering ground-water redox potentials, such as anoxic blocks in a well, require research as a basis for controlling the growth of iron bacteria in wells. By creating redox conditions unfavorable for the growth of these organisms in wells, clogging may be prevented.

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