Review of Iron Bacteria in Water Distribution and their Identification in a Simulated Cast Iron Water Distribution System

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Abstract

Iron bacteria are considered a major source of microbial corrosion in many iron environments having iron surfaces. Groundwater wells are good examples of iron environment. There are different types of iron bacteria present in nature. The growth of iron bacteria depends on the surrounding water environment with specific iron and manganese concentrations, pH and other chemistry. Though iron bacteria do not have health effect, there are various other effects that can be contributed by these microbes. These are stain, corrosion and unexpected use of disinfection. There are several ways to control iron bacteria. However, these processes are often ineffective in treating biofilms generated in distribution systems. This paper included a review on the presence of iron bacteria in water distribution systems, the effect of iron bacteria and ways to control iron bacteria in such systems. This paper also includes innovative experiments to investigate the presence of iron bacteria in centralized drinking water distribution systems. The experiments also investigated the effect of types of disinfection and exposure time on the presence of iron bacteria in distribution systems. Experimental results showed the presence of Leptothrix and Clonothrix types of iron bacteria present in the water distribution system. The presence of iron bacteria was not affected by the type of disinfection and also by the length of time.

Keywords: Iron bacteria; Drinking water distribution; Microbial corrosion; Disinfection; Annular Reactor

Introduction

Corrosion is a severe problem in water distribution system. To date cast iron are used worldwide for water distribution system and they are easily susceptible to corrosion. The major types of corrosion being considered in cast iron water distribution systems are microbial in nature (Microbial induced corrosion). This type of corrosion is therefore, affected by the presence of microscopic, one-celled living organisms, including algae, fungi, and bacteria (Smart 1997). Microbiological corrosion is not fundamentally different from any other type of electrochemical corrosion (Stott 1988). Iron bacteria have been considered as the key factor for microbial corrosion in metals. Iron bacteria are specific species of bacteria that are grown in iron environment.

Drinking water is mostly carried through cast iron distribution system. Drinking water distribution in traditional practice is the pipe that is used to supply water from groundwater wells. However, in recent years most of the developed world has adapted their infrastructure in context of centralized distribution system with a centralized water treatment facility. Cast iron distribution systems are mostly used as a pipe material for centralized water distribution system. However, there has not been any study for the presence of iron bacteria in drinking water distribution system.

The objective of this paper is to review different aspects of iron bacteria in context to drinking water distribution system. The paper focused on the presence of iron bacteria in drinking water wells, effects of iron bacteria on health and other related issues and protecting distribution system from the presence of iron bacteria. The paper also examined the presence of iron bacteria in a laboratory scale simulated water distribution system from a centralized water treatment plant.

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Types of Iron Bacteria

The name iron bacteria identifies a number of organisms that are categorized as autotrophic, meaning they derive their carbon from the carbon dioxide (CO_2) in the air, and their energy from consuming (oxidizing) dissolved iron or manganese. Iron bacteria are approximately 1-2 μm wide and 3-15 μm long. There are various types of iron bacteria that normally exist. Some of the most common genus of iron bacteria and there characteristics are described below.

Siderocapsa Sp

These organisms remain as true bacteria. These are capsulated coccoid or short rods, occurring in groups of 1 to 30 but generally les than 10, surrounded by mucoid capsule (Fig. 1). The deposit surrounding the capsule is rust-brown due to the presence of hydrous ferric oxide.

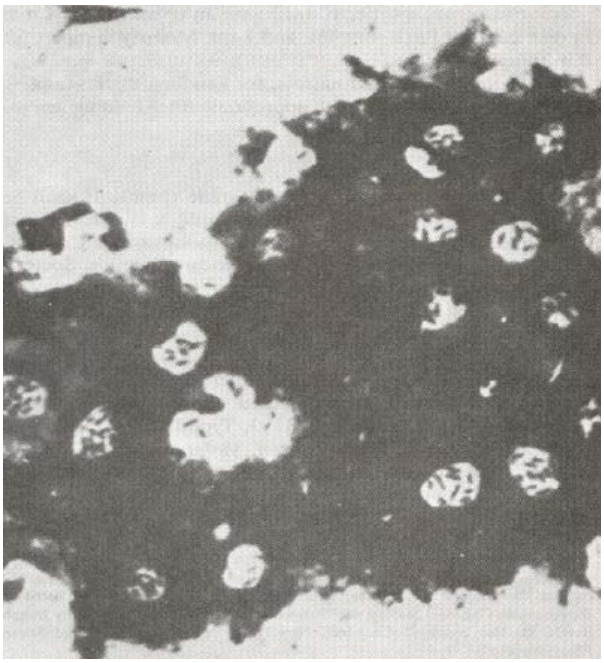

Fig. 1 *Siderocapsa sp*. in multiple colonies surrounded by ferric hydrate in 500x magnification (ASTM 2001)

Gallionella Sp.

These organisms are normally stalked bacteria. These are twisted or straight bands resembling a ribbon or a row of beads (Fig. 2). Bacteria are rod-shaped and borne at the top of the stalk. The stalks are slender (1-3 μm), dichotomously branched, composed of colloidal hydrous ferric oxide. The bacteria are frequently overlooked and the stalk considered as the bacterium. There has been evidence of this type of bacteria in water distribution system (Ridgway et al. 1981).

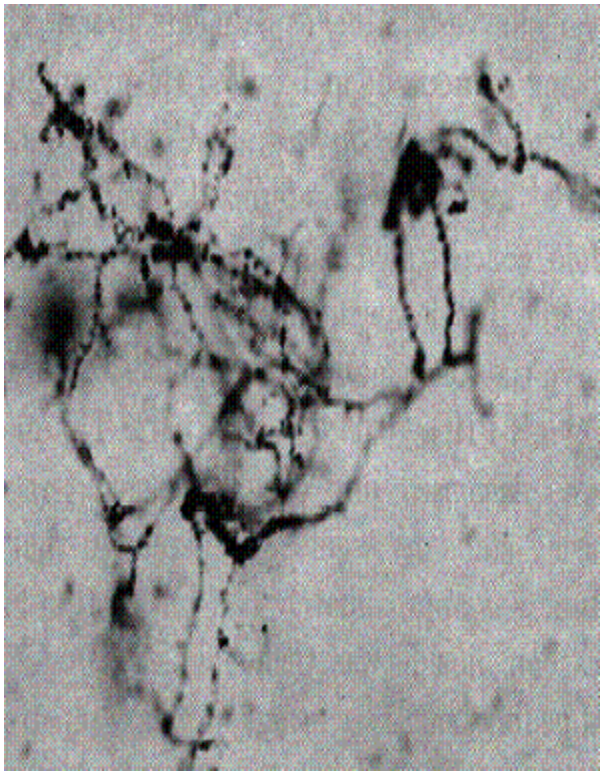

Fig. 2 *Gallionella sp*. in 900x magnification (Kucera and Wolfe 1957)

Fig. 3 *Sphaerotilus* sp. in 1625x magnification (Van Veen et al. 1978)

Sphaerotilus Sp.

This is a filamentous type of bacteria, which is not encrusted with iron. The filaments are attached, colorless, may show false branching (Fig. 3). The cells are rod-shaped or oval, 1.5–4 μm in diameter, surrounded by a firm sheath which is entirely organic and not impregnated with iron. There was evidence of *Sphaerotilus* bacteria in groundwater well (Van Veen et al. 1978).

Crenothrix Sp.

This type of bacteria is also filamentous (Fig. 4). But, it is encrusted with iron. The filaments are usually not branched and attached to a firm substrate, and are differentiated into a base and a tip. The sheath is plainly visible and is thin and colorless at the tip, becoming thick and encrusted with iron oxide at the base. The cells vary from cylindrical to spherical, the diameter being between 2 – 9 μm. Spherical, nonmotile reproductive bodies are formed. False branching may occur due to germination of spores within the sheath.

Fig. 4 *Chrenothrix sp*. in 345x and 380x magnification (ASTM 2001)

Leptothrix Sp.

These types of bacteria are also filamentous. However, these bacteria may be branched and contain colorless, cylindrical cells which first have a thin colorless sheath that later becomes encrusted with iron oxide. In general, these bacteria cells ranges from 0.5 – 1 μm in diameter. These bacteria are not encrusted with iron (Fig. 5). These types of bacteria are observed in wells (Van Veen et al. 1978).

Clonothrix Sp.

These bacteria are also filamentous, not encrusted with iron (Fig. 6). However the size of these bacteria varies within 2-7 μm. These filaments are attached and show false branching. The sheaths are organic and encrusted with iron hydroxide or manganese, are broader at the base, and taper to the tip. The cells are colorless, cylindrical. The filaments are colorless when young, becoming dark, yellowish-brown with age.

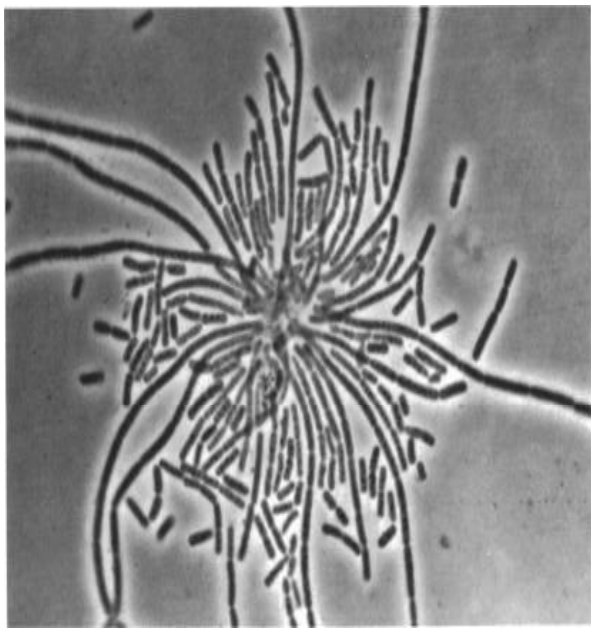

Fig. 5 *Leptothrix sp*. cells coming out of their sheath at 1625x magnification (Van Veen et al. 1978)

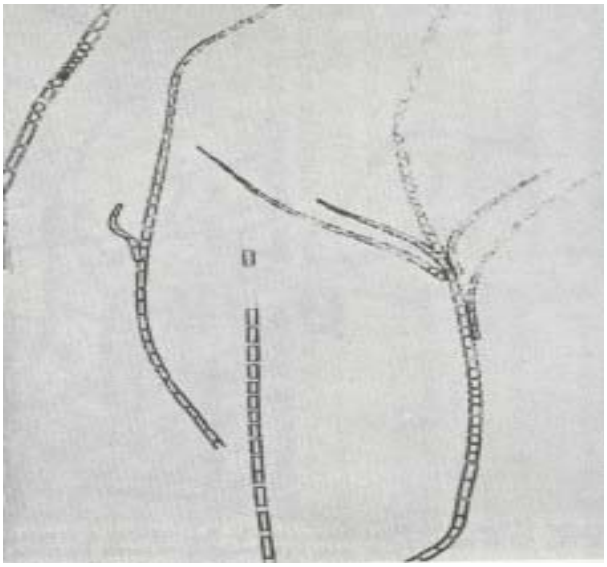

Fig. 6 Clonothrix sp. in 430x magnification (ASTM 2001)

Growth Conditions for Iron Bacteria

Temperature, light, pH, and oxygen supply are critical to the growth of iron bacteria. They are naturally occurring organisms in the environment. The presence of elevated levels of iron or manganese in wells often gives rise to the growth of iron bacteria. These organisms produce a filamentous deposit as they grow that is analogous to a snake shedding its skin. They thrive in water, which contains 0.5 to 4 mg/L of dissolved oxygen, and as little as 0.01 mg/l-dissolved iron (Capital Health 2002). They prefer a temperature range of 5 to 15°C. In general the bacteria prefer lower pH but are known to grow at pH which range from 5.5 to 8.2 with an optimum pH around 6.5. These organisms are not affected by light and have been found to grow in exposed areas, in shade as well as complete darkness (Albatros Fine Chemicals Ltd. 2003).

Water wells and distribution systems almost always produce these conditions. Iron bacteria need other essential nutrients that include carbon, nitrogen, phosphorous etc. They also exploit the oxidation reaction of inorganic species (iron) for energy supply. In addition, they act as an inorganic (H^+/e^-) donor and utilize CO_2 as carbon source. That is why they are some times known as iron oxidizing bacteria and for the adoption of this type of metabolism they are called Chemolithotrophic autotroph bacteria.

Iron Bacteria in Groundwater Wells

The presence of iron bacteria in groundwater wells was well documented in various literatures since early 1930s (Brown 1934, Lueschow and Mackenthun 1962, Smith 1985). There were discrete researches conducted on the presence of iron bacteria in different times. Apparently, the presence of iron bacteria was often ignored as there was little evidence for its health effects (Smith 1984). However, due to its effect on corrosion and high regulatory requirement on drinking water quality, there were growing importances on the presence of iron bacteria

Effects of Iron Bacteria in Water Distribution

HealthConsideration

Iron is not considered hazardous to health (New Hampshire Department of Environmental Services 2002). In the same way iron bacteria is not a threat to human health. In fact, iron is essential for good health because it transports oxygen in human blood. In the United States, most tap water probably supplies less than 5 percent of the dietary requirement for iron. Under Department of Natural Resources (DNR) rules, iron is considered a secondary or "aesthetic" contaminant. The present recommended limit for iron in water of 0.3 mg/l, is based on taste and appearance rather than on any detrimental health effect (Wisconsin Department of Natural Resources 2003). Private water supplies are not subject to the rules, but the guidelines can be used to evaluate water quality.

Taste and Food

Dissolved ferrous iron gives water an unpleasant taste. When the iron combines with tea, coffee and other beverages, it produces an inky, black appearance and a harsh, unacceptable taste. Vegetables cooked in water containing excessive iron turn dark and look unappealing. This unpleasant odors and taste are due to the death of the bacteria

Stains

Concentration of iron as low as 0.3 mg/l will leave reddish brown stains on fixtures, tableware and laundry that is very hard to remove. These can be both stains from iron deposit or from iron bacteria.

Corroding

Iron bacteria can cause electrons from ferrous iron (Fe_2) to be converted to ferric iron (Fe_3). As a result, it increases oxidation (corrosion) of water pumps, pipes, pressure tanks, filters, screens, valves and thereby reduces the life of these equipments.

Clogging

For watering systems, iron above 0.5 mg/L has caused blockages in small diameter pipes (States of Queensland 1994). To survive, the bacteria utilize the iron, leaving behind a reddish brown or yellow slime that can clog plumbing and

cause an offensive odor. This slime or sludge is noticeable in the toilet tank when the lid is removed.

Reducing Pressure

Reduction of water flow capacities and pressure in pipes and fittings is the result of hardening. This is in fact increase in energy costs from pumping water through constricted pipes.

Metal Deterioration

The activity of iron bacteria deteriorates metal fittings rapidly, which causes failure of distribution systems. This deterioration is mainly from the microbial induced corrosion within the fixtures.

Softener Problems

Zeolite water softeners lose their softening capacity due to slime coating. Most of the softeners would react with these biofilms. This would reduce the efficiency of the softener.

Sulfur Bacteria Infestation

The slime produced by iron bacteria increases chances of sulfur bacteria infestation. Sulfate reducing bacteria is also observed along with iron bacteria in microbial corrosion sites of environment.

Chlorinating Problem

By providing an environment for other more harmful bacteria to live, the slime reduces the ability of chlorine to kill bacteria. Free chlorine available in the distribution system is taken up to kill other bacteria.

Toxicity to Vegetation

Growth in plants can be retarded when iron is greater than 2.0mg/L. If it is more than 5.0 mg/L it can be toxic to plants (States of Queensland 1994). So it's a problem if this water is used for vegetation or irrigation.

Mitigation of Iron Bacteria

Iron bacteria are naturally present in surface waters and are also often found in the soil. Because it is difficult to get rid of iron bacteria once they exist in distribution systems, prevention is the best safeguard against accompanying problems. For distribution system prevention means disinfecting everything that goes into the distribution system with a strong (250 ppm) chlorine solution. Iron bacteria are nourished by carbon and other organics, and it is essential that these are not introduced into any part of the distribution system during water supply (Lenntech Water Treatment 2003). Treatment techniques, which may be successful in removing or reducing iron bacteria, include physical removal pasteurization, and chemical treatment. Treatment of heavily infected wells may be difficult, expensive, and only partially successful.

Physical removal is typically done as a first step in heavily infected piping system. The pumping equipment in the system must be removed and cleaned. The pipes are then scrubbed by use of brushes or other tools. Physical removal is usually followed by chemical treatment.

Pasteurization has been successfully used to control iron bacteria. Pasteurization involves a process of injecting steam or hot water into the distribution system and maintaining a water temperature in the well of 60^{o}C (140^{o}F) for 30 minutes. Pasteurization can be effective; however, the process may be expensive.

Chemical treatment is the most commonly used iron bacteria treatment technique. The three groups of chemicals typically used include: surfactants; acids (and bases); and disinfectants**,** biocides, and oxidizing agents (Minnesota Department of Health 2003). Surfactants are detergent-like chemicals such as phosphates. Surfactants are generally used in conjunction with other chemical treatment. It is important to use chlorine or another disinfectant if phosphates are used, since bacteria may use phosphates as a food source. Depending on the type of polyphosphate used, water with 1 to 3 ppm of iron can be adequately treated (Seelig et al. 1992). Acids have been used to treat iron bacteria because of their ability to dissolve iron deposits, destroy bacteria, and loosen bacterial slime. The acid solution may be able to penetrate thick incrustations of bacteria that the chlorine solution was unable to kill. Acids are typically part of a series of treatments involving chlorine, and at times, bases. Acid and chlorine should never be mixed together. Disinfectants are the most commonly used chemicals for treatment of iron bacteria, and the most common disinfectant is household laundry bleach, which contains chlorine (Minnesota Department of Health 2003). Dissolved iron can be removed by oxidizing the iron into the insoluble form and then filtering out the precipitated solids. Iron in water can be oxidized by aeration, by filtration using an oxidizing media, or by addition of a chemical-oxidizing agent such as chlorine bleach, hydrogen peroxide, or ozone.

Chlorine also acts as a disinfectant, which kills iron bacteria on contact. The effectiveness of the treatment will depend on dosage rates, pH of the water, contact time, water temperature and turbidity (States of Queensland 1994). Chlorine successfully kills other mircroorganism but can not kill microorganism under the shelter of biofilm. However, the hypohalite (OCl-) is actually very effective at oxidizing the extracellular polysaccharide and the proteinaceous attachment structures. Therefore, the use of chlorine in alkaline waters can still be extremely effective. This can achieve both oxidation of the extracellular material and sufficient kill of the microorganis. Nonoxidizing microbicides are also effective in controlling biofilm. Combining the use of nonoxidizing and oxidizing microbicides is a very effective means of controlling biofilm. Sufficient time should be allowed for the nonoxidizing microbiocide to work before resuming oxidant feed unless an oxidant compatible microbiocide is being used (i.e., polyquat) (Aquadyn Technology 2001). Study also showed that use of other disinfectant, like chlorite and chlorine dioxide resulted in decreased or similar corrosion rates and monochloramine and free chorine increased corrosion rates (Eisnor 2002).

Enzyme technologies that will break down the extracellular polysaccharides and degrade bacterial attachment structures (fimbriae) are currently being developed and patented. These technologies, although expensive, may provide biofilm control where microbiocide use is environmentally restricted (Aquadyn Technology 2001).

Experimental Techniques

The presence of iron bacteria has been investigated before in various iron environments, including groundwater wells. However, there has not been any investigation done on drinking water distribution systems in centralized systems. The presence of iron bacteria has also not been investigated in cast iron water distribution system. Therefore, identification of iron bacteria in drinking water distribution

system would provide a new approach for iron bacteria related problems in drinking water industry. Experimental approach was used to identify the presence of iron bacteria. The presence of disinfectant was also investigated.

The drinking water distribution system is not an easy system to model using experiments. Several types of experiments are normally used to model drinking water distribution system. One of the recent types of experiment to model biological growth in a distribution system is using annular reactor (AR).

The bench-scale equipment used for this project will be the Model 1120 LS Laboratory Model Regrowth Monitor and Annular Reactor from BioSurface Technologies Corporation. The monitor consists of two (2) concentric cylinders. The inner cylinder can rotate at speeds between 25 and 430 rpm and is controlled by a variable speed DC motor. This rotation is used to simulate the shear stress encountered on the pipe wall in a distribution system. Flush-mounted on the inner cylinder are twenty (20) removable coupons on which the biofilm accumulates. These coupons are easily removed for biofilm sampling. The outer cylinder is stationary.

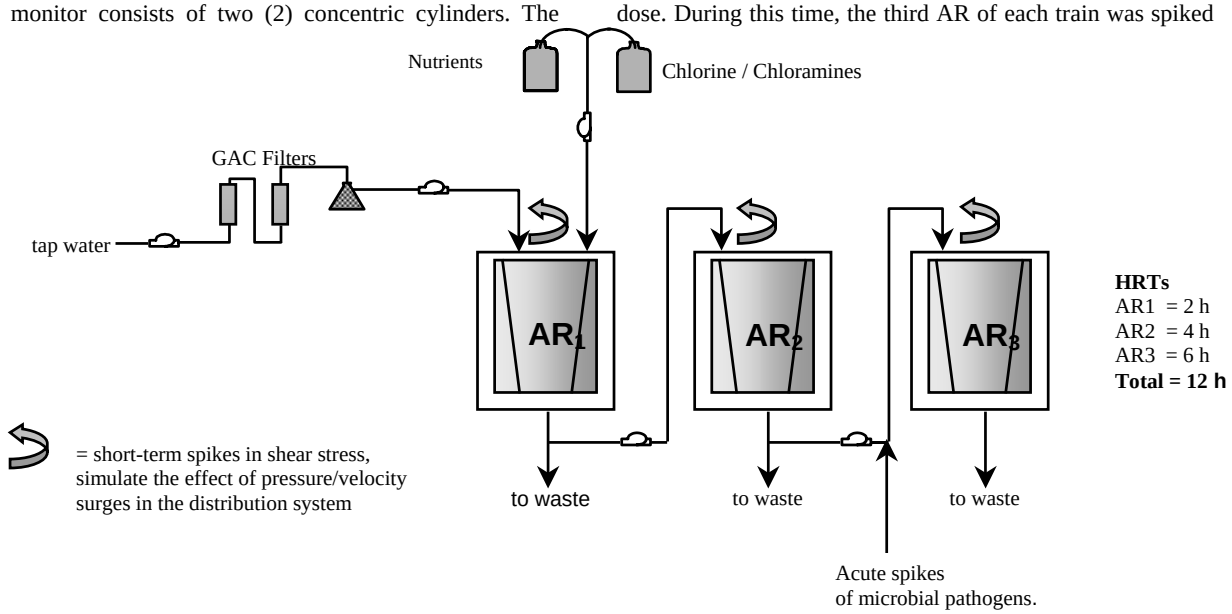

Fig. 7 Bench scale AR setup

Bench Scale Setup

Two trains of three ARs in parallel were used to simulate a distribution system. These two AR trains contained cast-iron coupons. Tap water supplied by the Halifax Regional Water Commission (HRWC) was used as the primary process water for the ARs. On average, the treated surface water had an alkalinity of 35 mg/L as $CaCO_3$, a pH of 8.5, and a total organic carbon (TOC) concentration of approximately 1.5 mg/L. Prior to entering the ARs the tap water was passed through a filter containing fresh granular activated carbon (GAC) media to neutralize any free chlorine present in the tap water. A second GAC filter operating biologically removed any background biological organic matter (BOM) in the water.

The filtered tap water, nutrient material and a disinfectant were pumped into the first AR of each train (Fig. 7). The nutrients were dosed at a concentration of 100 µg/L representative of an average level of BOM for most North American full-scale plants (LeChevallier 1996). To ensure a carbon limiting system, the carbon, nitrogen and phosphorus cocktails were pumped into the ARs to obtain a molar C:N:P ratio of 100:20:5. The disinfectants to be tested are chlorine and monchloramine. Each AR was maintained at a fixed rotational speed of 50 rpm, which results in a shear stress of 0.25 N/m^2 at the outer wall. All exposed surfaces were covered by opaque plastic to reduce the potential of phototrophic growth in the bench-scale system. Experiments were conducted at a liquid phase temperature range of 20 ± 1ºC.

Annular Reactor Operation

After a three-week acclimation period of no disinfectant, the disinfectants were ramped up to 0.5 mg/L for free chlorine and 1.0 mg/L for monochloramine in the effluent of AR1. This level of disinfection was to simulate a low disinfectant dose. During this time, the third AR of each train was spiked with wastewater for a period of 24 hours to simulate a breech in infrastructure integrity. The wastewater was attained from Mill Cove Wastewater Treatment Plant in HRM and was fully characterized prior to spiking. The wastewater was spiked at a flow rate equivalent to between 10% and 20% of total flow for that AR. This flow rate corresponded to the average distribution system water loss as reported.

Sampling

Biofilm samples were collected after 158 and 168 days of the beginning of the operation. Samples from distribution system were also taken from annular reactor. The sampling procedures were adapted from ASTM guidelines and Standard Methods to identify the presence of iron bacteria (ASTM 2001, Lueschow and Mackenthun 1962, Wolfe 1958, Clesceri et al. 1998). Biofilm samples were collected from scratching and diluting the samples. The samples were collected and stored in the freeze. The storing may not keep the number of bacteria intact. However, it was expected to show qualitative bacteria samples in a standard microscope. The samples were too brown, and appear to contain iron precipitate. Therefore, enough time were given to settle those iron samples. It provided rather diluted samples, and thus would provide better visualization of iron bacteria samples.

Image Analyzer

These samples were taken to Kirlzess Image analyzer. ASTM suggests 400-1000x magnification for identification of iron bacteria samples. 1000x magnification were used for observation of the samples. The images observed from the

image analyzer were tested for further investigation. These probable bacteria samples were then compared with standard iron bacteria samples.

Results and Discussion

Visual Observation

Samples from various biofilm showed red color water initially. Rusty slime deposits were also observed in some cases. This indicated high probabilities of the presence of iron bacteria in these samples. Though the tests conducted were qualitative in nature, there were no significant visual differences observed between the samples with different disinfectant. However, the water samples from the distribution system in most of the cases did not show significant difference than clean water. Therefore, from visual observation, it was not possible to predict the presence of iron bacteria.

Fig. 8 Microscopic view of biofilm samples in cast iron coupons in AR2 for 168 days with mono chloramine disinfectant.

Observation with Magnification

The samples were observed in 1000x magnification. The samples observed were matched with various genus of iron bacteria. Most of the samples observed were in close similarity to *Leptothrix orchracea* (Fig. 8). These Figures showed the presence of iron bacteria in the biofilm samples. It also indicates of the extent of iron bacteria in the biofilm coupons to some extent. *Chrenothrix* types of iron bacteria were also observed in a few biofilm samples (Fig. 9).

Various Species

There were various species of iron bacteria that normally exist in the environment. The sampling collection procedures used precipitation of iron hydroxides. Therefore, some of the species, which were more attached to iron precipitates, might have been removed automatically from the samples. Therefore, the samples might not contain some of the species of iron bacteria. The summary of the presence of different types of iron bacteria are shown in Table 1. *Leptothrix* type of bacteria was the most commonly observed genus of bacteria in the samples. In one samples, *Clonothrix* type of bacteria was observed. Other genuses of bacteria were more attached to the precipitates. It might also be possible that these bacteria could not survive for long time periods.

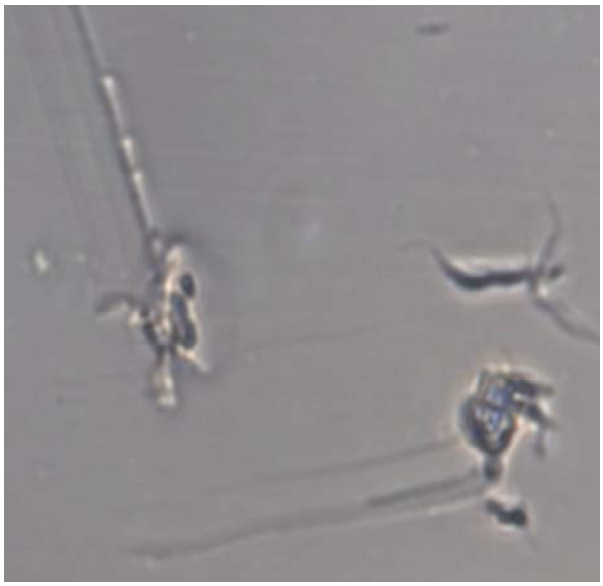

Fig. 9 Microscopic view of biofilm samples in cast iron coupons in AR2 for 158 days with mono chloramine disinfectant

Effect of Disinfectants

The methodologies included only two types of disinfectants; chlorine and chloramines. This work was aimed at preliminary evaluation and thus did not include any quantitative analysis. However, the observation did not show any differences between chlorine and chloramines in terms of its ability to kill iron bacteria in the biofilm. The effluents did not show iron bacteria with chlorine. The results also indicated that low concentrations of chlorine may not be suitable for killing iron bacteria completely.

Sizes of Iron Bacteria

The sizes of bacteria observed were similar to the standard sizes of bacteria for each of the species (ASTM 2001). However, the ranges of size observed were less than the standard sizes for these species of bacteria (Table 2). This can be due to smaller number of samples. The samples were also stored for some time. This can also affect the sizes of the bacteria in the sample. However, irrespective of the differences in size, these spots in the samples were expected to be iron bacteria.

Time

The existence of iron bacteria on the sample/coupon was not affected with the increase of time during the growth of biofilm. Therefore, it can be inferred that changes in time frame did not affect the presence of iron bacteria. However, quantitative analyses can bring light to this research.

Conclusions

The experiments were conducted on a more qualitative nature. It was hard to draw conclusions. However, there were several conclusions that can be drawn from this investigation. Iron bacteria were present in the cast iron distribution system. These iron bacteria were found to exist in various forms and persisted for long periods without their suitable environment. Furthermore, iron bacteria were responsible for the iron deposits leading to microbial corrosion occurring in the cast iron distribution system. Chlorine and monochloramine did not disinfect iron bacteria within the biofilm.

Table 1. Presence of different genus of iron bacteria in the samples

Days	*Disinfectant*	*Siderocapsa*	*Gallionella*	*Sphaerotilus*	*Crenothrix*	*Leptothrix*	*Clonothrix*
158 AR1	Chlorine	No	No	No	No	Yes	No
158 AR2	Chlorine	No	No	No	No	Yes	No
158 AR2	Chloramine	No	No	No	No	Yes	No
168 AR1	Chlorine	No	No	No	No	Yes	Yes
168 AR2	Chlorine	No	No	No	No	Yes	Yes
168 AR1	Chloramine	No	No	No	No	Yes	No
158 water AR2	Chlorine	No	No	No	No	No	No
158 water AR2	Chloramine	No	No	No	No	Yes	No

Table 2. Sizes of iron bacteria*

Types of bacteria	Standard size	Size observed
Leptothrix ochracea	0.5-1 μm	0.4-0.8 μm
Crenothrix polyspora	2-9 μm	1-3 μm

* standard sizes are taken from ASTM 2001

Low doses of chlorine might not be able to destroy iron bacteria completely from the water. Low disinfectant doses of mono chloramines (1 mg/l) and chlorine (0.5 mg/l) were not sufficient to destroy iron bacteria from the biofilm.

Proper understanding of kinetics of iron bacteria was important for controlling microbially induced corrosion. Quantitative analysis of these experiments is needed to better understand this research.

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