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The Identification, Cultivation and Control of Iron Bacteria in Ground Water

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1. Introduction

(a) *General introduction*

IRON BACTERIA may be defined as "that group of aerobic bacteria which appear to utilize the oxidation of ferrous and/or manganous ions as an essential component in their metabolic functioning." The resultant production of ferric arid/or manganic salts (usually the hydroxide) within the cell or cell coatings gives the bacteria their typical brown coloration. Studies of pyrite deposits laid down 300 million years ago have shown them to contain a range of fossil bacteria including representatives of two major groups of iron bacteria, *Gallionella* and *Sphaerotilus* (Schopf *et al*. 1965).

Fossil iron bacteria were first reported by Ehrenberg (1836); while analysing ochre microscopically he mistook them for diatoms but named them *Gallionella ferrunginea*. Other genera of iron bacteria were first described from living specimens such as *Leptothrix ochracea* which is mainly responsible for tile formation of bog ore (Kutzing 1843) and *Crenothrix polyspora* discovered and described by Cohn (1870) while studying the brown flocculent precipitates which had rendered well waters undrinkable. In addition to a very detailed description, Colin stained the bacteria using the Prussian blue reaction in which potassium ferricyanide with hydrochloric acid stained with an intensity varying with the concentration of ferric compounds present. Colin's Studies also highlighted the nuisance value of iron bacteria.

Throughout history, there are reports of water from wells, rivers and creeks being stained blood red or brown and becoming undrinkable presumably due to the growths of iron bacteria. In Berlin in 1877, the whole water main system had to be replaced because it was clogged with iron bacteria growing within the system. Typical symptoms of iron bacterial growths in water supplies are: (a) discoloration of the waters (yellow to rust-red or brown); (b) reduction in flow rates through the system caused by coatings of iron bacteria inside the pipes, (c) development of thick red or brown coatings on the sides of reservoirs, tanks and cysterns; sometimes sloughing off to form either fluffy specks in the water or gelatinous clumps of red to brown filamentous growths: (d) rapid clogging of filter screens: (e) heavy surface and sedimented growths of a red or brown color sometimes irridescent (ochre), in water.

Frequently, the heavy growths of' iron bacteria form a substrate for other bacteria which may degrade these materials anaerobically to form acidic products and hydrogen sulphide These in turn call cause taste, odour and corrosion problems. Controlling the growth of iron bacteria has always posed a problem due to the heavy deposits of ferric and/or manganic salts around the cell and the cell coatings themselves, forming a natural barrier to any bacteriocidal agent. Stott (1973) summarized the difficulty of controlling iron bacteria by the statement "Iron bacteria are tenacious and continue to grow even after the severest kind of treatment . . . and if relief is to be had it is likely to be temporary."

It was the nuisance nature of iron bacteria in water supplies that focussed attention upon their activities and led Winogradsky (1888) to postulate a biological grouping of the organisms characterized by their relation to iron. He concluded from his cultural studies that the iron bacteria were chemoautotrophic and derived energy from the transformation of iron from the ferrous to the ferric state. Winogradsky's postulate of obligate chemoautotrophy was shown to be incorrect at least for sonic iron bacteria since they grew well heterotrophically (Molisch 1892). Two schools of thought generated around these two opposing postulates of chemoautotrophic and heterotrophic nutritional pathways for the iron bacteria (Pringsheirn 1952). Mulder (1974 reviewed the literature with particular emphasis on the chemolithotrophy of the sheath-forming iron bacteria. Confusion in determining their true nutritional status has been made more intense because these organisms grow only at pH values above 6, a pH range in which ferrous ions will oxidize rapidly by purely chemical reaction. Mulder contends that it is therefore very difficult to that the energy released from such a reaction can be utilized by the

bacteria. He postulates that "it is highly probable that in many cases the so-called biological iron oxidation by these bacteria is confined to absorption of chemically oxidised iron by the sheaths or slime layer surrounding the sheaths." Mulder (1964) does, however, demonstrate that the organisms of the *Leptothrix* group are able to convert manganous ions readily to manganic oxide over the pH range of 6.0-7.5. There is evidence that this conversion is due to the presence of proteinaceous substances promoting manganese oxidation on the outside of the sheaths.

Many attempts have been made to differentiate the iron bacteria into groups based upon differences in nutritional requirements. Stott (1973) divided them into three groups. viz., group 1, those that precipitate ferric hydroxide from solutions of ferric bicarbonate, using the carbon dioxide set flee and the available energy of the reaction for their life processes; group 2, those that do not require ferrous bicarbonate for their vital processes but which cause the deposition of ferric hydroxide when either inorganic or organic salts are present; group 3, those that attack iron salts of organic acids, utilizing heterotrophically the organic acid radical while eventually converting the basic salt to ferric hydroxide. In practice, the iron bacteria are almost always differentiated by their morphological characteristics.

(b) *The classification of iron bacteria*

(i) *Based* on Bergey's Manual of Determinative Bacteriology

Considerable changes have occurred in the classification of the iron bacteria over the last decade. In the 7th edition of *Bergey's Manual of Determinative Bacteriology,* (Breed *et al.* 1957), the iron bacteria were listed in the Caulobacteriaceae, Siderocapsaceae, Chlamydobacteriaceae, and Crenotrichaceae, while in the 8th edition (Buchanan & Gibbons 1974), they were incorporated in 15 genera listed in the following parts: part 2, Gliding bacteria, *Toxothrix;* part 3, Sheathed bacteria, *Sphaerotilus, Leptothrix, Lieskeella, Crenothrix* and Clonothrix part 4, Budding and/or appendaged bacteria, *Pedomicrobium, Gallionella, Metallogenium* and *Kusnezovia* and part 12, Grain negative chemolithotrophic bacteria, *Thiobacillus* (one species only, *Thiobacillus ferrooxidans, Siderocapsa, Naumanniella, Ochrobium* and *Sideococcus.*

Diagrammatic presentations of these genera are shown in Fig. 1, their dominant habitats in Table 1, and a proposed dichotomous key for their identification in Table 2.

(ii) Other genera reported to include iron bacteria

Although the 8th edition of *Bergey's Manual of Determinative Bacteriology* considerably reduces the confusion in the classification of iron bacteria, species of iron bacteria have been reported in other bacterial genera.

Mann & Quastel (1946) showed that the manganese oxide present in soil was mainly the result of biological oxidations. Van Veen's (1973) review showed in studies *in vitro* that several different types of fungi and sonic bacterial genera, *(Criyptococcus, Pseudomonas* and *Hyphomicrobium)* were all capable of oxidizing manganese independently of hydrocarboxylic compounds. *Bacillus* spores and the chlamydospores of several fungi could become impregnated with manganic oxides after prolonged incubation in agar media enriched with $MnSO_4$ or $MnCO_3$. *Arthrobacter* (strain 216) and *Coniothyrium fuckelii* were both found to oxidize manganese salts the most rapidly. Schweisfurth (1973) studied a range of manganese-oxidizing strains of *Pseudomonas* and proposed a species group, Ps. *mangonoxudans.* Clark *et al. (1967)* briefly reviewed the definition of the term iron bacteria and considered that it included all organisms capable of precipitating iron biologically. Using synthetic media containing ferric ammonium citrate, they found that isolates of *Aerobacter aerogenes, Serratia indica* and *Bacillus pumulis* could all precipitate iron mainly through the utilization of the citrate. Ivarson &

Heringa (1972) in a study of the micro-organism oxidizing manganese in manganese deposits in soil, isolated sonic organisms resembling *Hyphomicrobium* and also a fungus belonging to the genus *Cephalosporium,* both of which could actively oxidize manganese, but failed to detect any of the noted genera of soil iron bacteria. In similar studies of Illinois waters, A. *aerogenes* was most commonly isolated. All bacteria possessing the iron precipitating characteristic had two features in common, viz., the ability to utilize citrate and the possession of capsular material. Clearly, in the presence of citrate and iron or manganese organic molecules, the degradation in total or in part of the organic fraction could lead to a biological precipitation of the iron or manganese. This is a pattern of precipitation different from that associated with the traditional grouping of iron bacteria.

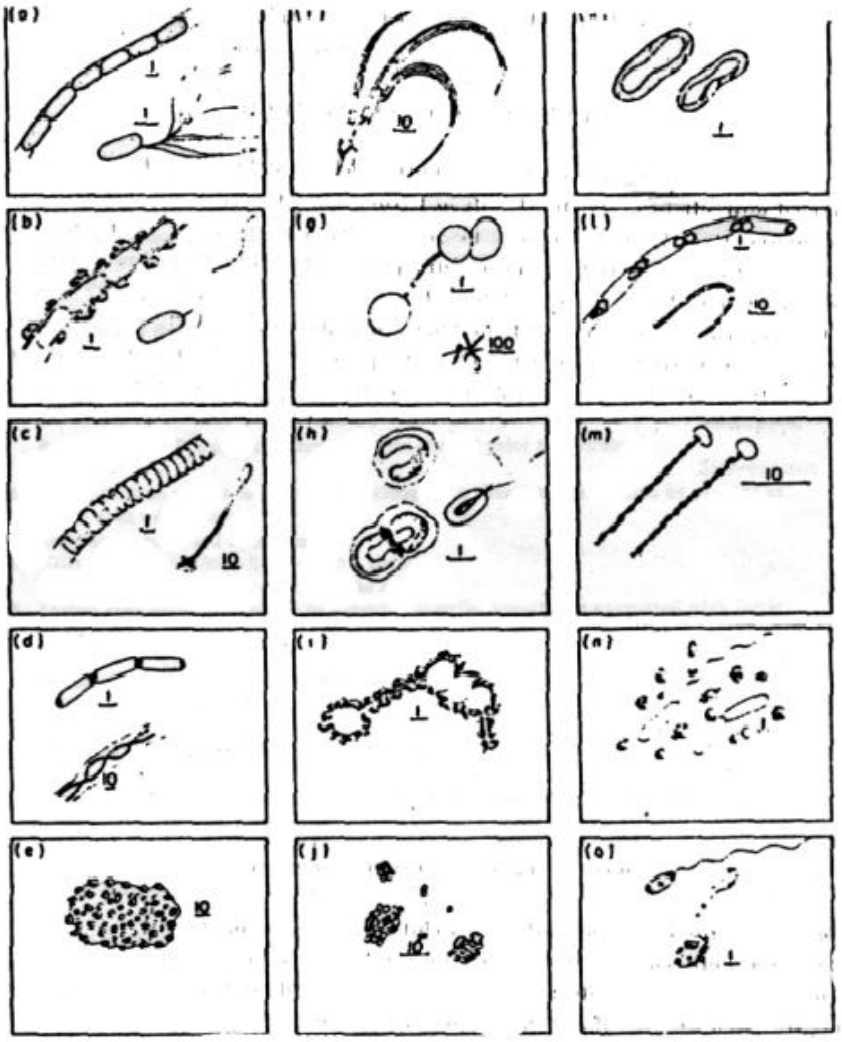

Fig. 1. The principal morphological features of iron bacteria. (a) *Sphaerotilus, upper:* shows cells in sheath lower: single cell. (b) *Leptothrix, upper:* shows cells in encrusted sheath; lower: single cell. (c) *Crenothrix, upper:* shows cells in sheath; lower: complete filament. (d) *Lieskeella, upper:* cells stained with methylene blue: lower: spirally wound filaments in capsule. (e) *Siderocapsa*, section of cells, embedded in common capsule encrusted with iron and manganic compounds. (f) *Clonothrix*, apical tips of filaments. (g) *Metallogenium,* upper: free cells; lower: microcolony, (h) *Ochrobium,* upper: single cell; lower left: paired cell; lower right motile cell. (i) *Kusnezovia,* cells and interconnecting filaments heavily encrusted with manganic deposits and oxalic acid. j) *Siderococcus*, cell arrangements showing encrustations of micro-colonies with ferric hydroxides and oxides. (k) *Naumanniella*, single cells. (l) *Toxothrix,* upper: cells in filaments; lower: U-shaped trichome. (m) *Gallionella*, single cells. (n) *Thiobacillus ferrooxidans,* cells among precipitated iron. (o) *Pedomicrobium,* upper: swarmer cell; lower: stained preparation, mother cell encrusted with deposits giving off a hypha with bud at tip. The scale number is in Fm. *Thiobacillus* refers only to the species, *T. ferrooxidans.*

Table 1

Range of habitats in which iron bacteria occur and their ability to oxidize reduced iron and manganese salts

Genus	Habitat					Oxidizes			
	Soil	Fresh water	Bogs	Mud sediments in lakes and rivers	Well water and piped systems	Acidic mine drainings	Fe only	Mn only	Fe and/ or Mn
Toxothrix	-	+	+	±	-	-	+	-	-
Sphaerotilus	-	+	-	+	+	-	-	-	+
Leptothrix	-	+	-	+	+	+	-	-	+
Lieskeella	-	+	-	+	-	-	+	-	-
Crenothrix	-	+	-	-	+	-	-	-	+
Clonothrix	-	+	-	-	+	-	-	-	+
Pedomicrobium	+	+	-	-	-	-	-	-	+
Gallionella	+	+	+	+	+	-	+	-	-
Metallogenium	+	+	-	+	-	-	-	-	+
Kusnezovia	-	-	-	+	-	-	-	+	±
Thiobacillus ferrooxidans	+	+	+	+	+	+	+	-	-
Siderocapsa	-	+	-	-	+	-	-	-	+
Naumanniella	+	+	-	-	+	-	-	-	+
Ochrobium	-	+	-	-	+	-	+	-	-
Siderococcus	-	-	-	+	-	-	+	-	-

(c) Studies on the major genera of iron bacteria

(i) Gallionella

Although described first by Ehrenberg (1836), a very detailed morphological study of *Gallionella* was reported by Cholodny (1924). Isolation techniques and some of the difficulties of obtaining pure cultures were outlined by Nunley & Krieg (l968), who found that pure cultures of *Gallionella ferruginea* could be obtained by incubating for 1 -2 days in Wolfe's medium containing 0.5% of formalin to prevent contaminant heterotrophic growth. Enrichment of *Gallionella* cultures was reviewed by Basashova (1967) and excellent growth was obtained in an atmosphere of 6% 0_2. 59% N_2 and 35% CO_2. The ultrastructure (Balahova & Cherni 1970; Hanert 1970), and the form of iron in the filaments (Maradanyan & Balashova 1971), have been subjected to intensive investigation. These studies revealed that *Gallionella* was a mycoplasmodial organism with a stalk consisting of helically coiled uniquely mineralized oxidized fibres in a structure quite different from that of' the common brown compounds of oxidized iron. The nature of the cell led Balashova (1969) to suggest that a relationship could be drawn between *Gallionella, Metallogenium,* and *Mycoplasma* since all three bacterial genera lacked a cell wall. Extensive physiological studies of *G. ferruginca* were undertaken by Hanert (1968), who found that it grew well under low oxygen concentrations (0.1-0.2 $mg.1^{-1}$ of O_2), while higher levels (>2.75 $mg.1^{-1}$ of O_2) were inhibitory. It was also able to fix $^{14}CO_2$ in measurable quantities, and to undertake autoxidation of ferrous iron stimulated by carbon dioxide in a medium containing ferrous sulphide.

Table 2

Dichotomous key to the genera of iron bacteria

1. Cells reproduce by budding, never possess tapering filaments	2
Cells do not reproduce by budding unless filaments are tapered	4
2. Cells possess cellular extensions resembling hyphae	*Pedomicrobium*
Cells do not possess cellular extensions resembling hyphae	3
3. Do not possess rigid cell walls, buds are atypical 'elementary bodies'	*Metallogenium*
Possess rigid cell walls, buds resemble vegetative cells	*Kusnezovia*
Possess rigid cell walls, cells become pear-shaped prior to budding	*Siderococcus*
4. Cells occur in chains or filaments enclosed in a sheath and may also be present as single cells or swarmers	5
Cells never enclosed in a sheath	8
5. Cells occur in chains when within a sheath	6
Cells occur in filaments when within a sheath	7
6. Motile cells possess a bundle of subpolar flagella, sheath not encrusted with ferric or manganic oxides	*Sphaerotilus*
Motile cells possess a single polar flagellum sheaths tend to be encrusted with ferric or manganic oxides	*Leptothrix*
7. Tapering filament enclosed in distinct sheath	*Clonothrix*
Filament not tapering and may exhibit false branching, sheath thin and indistinct	*Crenothrix*
8. Cells possess a torus or marginal thickenings, resembling a diatom	9
Cells do not possess torus or marginal thickenings	10
9. Torus resembles horseshoe, cells often in pairs	*Ochrobium*
Torus does not resemble horseshoe, never in pairs	*Naumanniella*
10. Cells occur in long filaments, frequently U shaped	*Toxothrix*
Cells never in long filaments	11
11. Cells possess a long spirally twisted stalk arising from centre of cell	*Gallionella*
Cells do not possess stalks	12
12. Cells embedded in common capsule	*Siderocapsa*
Cells not embedded in common capsule	13
13. Cells derive energy from oxidation of reduced sulphur compounds, cells never in spiral arrangement	*Thiobacillus ferrooxidans*
Cells do not derive energy front oxidation of reduced sulphur compounds, cells occur in double spiral arrangement	*Lieskeella*

(ii) Sphaerotilus/Leptothrix *group*

The *Sphaerotilus/Leptothrix* group of sheathed bacteria has been extensively reviewed by Dondero (1975) and earlier by Phaup (1968). A further role of *Sphaerotilus* as a component of 'sewage fungus' was discussed by Curtis (1969), and its ultrastructure described by Bissett & Brown (1969). These bacteria (in particular, *Sphaerotilus natans* are a major component of 'sewage fungus', defined by Curtis (1969) as "massive growth of slimy cotton-wool-like plumes (white, grey or brown) which can rapidly colonise surfaces." Typically it is found in rivers below sources of organic pollution. Dondero (1975) indicated that members of this group could also grow in pipes and along with deposits of iron and manganese, reduce the rate of flow of water through the system. However, most reported occurrences are of surface water infestations. the slimy growths interfering with the development of benthic animals and consequently of fish (Avery 1970).

(iii) Toxothrix

Krul *et al.* (1970) in a review and study *of Toxothrix* found it to be present in a large number of the iron springs in and around Lansing East Michigan. They used a partially submerged microscopic technique and also grew the organisms in the laboratory. It was noted that the trichomes would disintegrate rapidly during laboratory observation, a feature of the genus which may have restricted its observation in routine screening studies. Hasselbarth & Ludemann (1967) observed *Toxothrix* in well waters in districts of Germany.

(iv) Thiobacillus ferrooxidans

Thiobacillus ferrooxidans is a unique species obtaining energy simultaneously from the oxidation of inorganic sulphur compounds and iron (Temple & Colmer 1951), a feature which led to its implication as a causative factor in corrosion problems involving acidic mine waters. The difficulties in culturing and enumerating *T. ferrooxidans* were overcome by Tuovinen & Kelly (1973) using a medium with a pH of 1.3 and $FeSO_4$ - $7H_2O$, $(NH_4)_2SO_4$. $MgSO_4$ - $7H_2O$ and H_2SO_4 as the sources of sulphur and iron. Enumeration was performed using selected non-inhibitory membrane filters (Tuovinen *et al.* 1971*a*) and agar base. The mechanisms of sulphur and iron oxidation have been studied (Temple & Colmer 1951 ; Vestal & Lundgren 1971). High tolerances to sonic metals (Zn, Ni, Cu, Co, Mn and Al) have been reported (Tuovinen *et al.* 1971*b*); the organism was resistant to these at concentrations of more than 10 $g.1^{-1}$ Other metals, for example Ag, Te, As and Se were more toxic, and could not be tolerated at concentrations above 50-100 $mg.1^{-1}$. Increased tolerance was noted during oxidation of sulphur and iron. This may be a significant ecological feature favouring the growth of *T. ferrooxidans* in heavy metal solutions toxic to other potential microbial competitors.

2. Problems Caused by Iron Bacteria

(a) General survey

The occurrence of iron in geological strata is already well established (Lepp 1975), but little attention has been paid to the role of iron bacteria in the strata or aquafers, mainly because of the difficulty of monitoring the organisms. The biological cycling of manganese and iron in water has been reviewed (Mulder 1972) and the chemical factors affecting the equilibria and availability of iron and manganese have been discussed (Hem 1972). For soils, minerological aspects of the biological iron and manganese cycles have been given by lwasa (1970), all of which clearly illustrates a diverse potential habitat for the iron bacteria. It is not therefore surprising that problems have been reported in well and

mining operations, piped water and sewage systems and in open bodies of water.

Iron bacteria have caused problems in water supplies since the dawn of civilization and there are many references in history to 'red' water, undrinkable water covered in slime, and plugged wells.

In wells, the major problems are (a) growths plugging the screens; (b) coating of the piped systems, impellars and motors, thereby reducing flow rates; (c) reduced potability of the water and finally; (d) total plugging of the well. Hasselbarth & Ludemann (1972) reported encrustations of wells which caused rapid decreases in yields particularly at times of maximum demand. The encrustations were considered by Hasselbarth & Ludemann (1972) to be caused by: (a) iron and manganese bacteria, various species of which exist in the soil and could presumably enter a well during the initial boring operations or by seepage into the aquafer feeding the well; (b) sulphate-reducing bacteria which reduce sulphates to sulphides to meet their respiratory needs (the sulphides when excreted react with the iron to form iron sulphide deposits): (c) corrosion of metallic tubing and extension pipes.

Mogg (1972) elaborated on the remedial aspects of encrustations in wells, and recommended that the reduction or elimination of this problem could be achieved by providing an increase in screen open area in newly designed wells, reduced draw down in wells through lower pumping from the screened area of the well by the use of packers and vacuum seals and periodic chemical treatment (sulphamic or hydrochloric acids) to reduce microbial numbers. Mogg (1972) considered the dominant iron bacterial genera in well encrustations to be *Gallionella, Crenothrix* and *Leprothrix* and postulated that cells of iron bacteria may be transported easily from one well to another on well repair tools and equipment. He cites an example of a North Carolina well which became rapidly infected after undergoing minor repairs, Grainge & Lund (1969) indicated that "iron bacteria cause serious uncontrolled fouling in a high proportion of the wells throughout the world" and further indicated that "there is a proliferation of recommended controls, many of which we have found to be ineffective. . ." There is considerable evidence to support this (see Section 4).

Rao (1970) reported that in the Howrah district of India, iron bacteria occurred widely in the wells and water supply system, causing serious clogging or corrosion of the pipes. The iron content of the wells was between 0.2 and 0.8 $mg.1^{-1}$ and the dominant genus was *Clonothrix*. Other genera also frequently encountered were *Crenothrix, Leptothrix* and *Siderocapsa.* The occurrence of iron bacteria in water supplies was described (Anon. 1939) in which the major problems were stated to be the limitation of hydraulic efficiency owing to the
presence of growths up to 1.5 cm thick on the inside of pipes, impaired water quality (taste, odour and colour), and potential plugging of filters and pipes. Some of these aspects were further described by Omerod (1974). Considerable problems have been generated in mines and mine drainage which is rich in dissolved reduced sulphur compounds and iron. In the presence of air, *T. ferrooxidans,* in conjunction with other sulphide-oxidizing bacteria, generates a very acidic product due to the release of' sulphuric acid as a terminal metabolic product. The factors involved have been extensively studied (Lorenz & Stephan 1967; Baker & Wilshire 1970), and ill particular the rate at which it occurs (Singer & Stumm 1970). Methods for reducing the acidity in drainage water
were proposed by Walsh & Mitchell (1972) and included (a) the partial neutralization of the water to a pH value above 4.3 to inhibit stalked bacteria and *T. ferrooxidans,* (b) the use of surface active agents to detach stalks (holdfasts) at the site of pyrite degradation and (c) the introduction of heterotrophic bacteria capable directly or indirectly of parasiting upon the stalked bacteria. Most treatments currently employed utilize (a).

(b) Global distribution of iron bacteria

Apart from frequent statements indicating that the problems involving iron bacteria in ground

water are worldwide, there has been no attempt to determine the true extent of the problem. Cullimore & McCann (1974) carried out a survey of 150 countries by correspondence with the respective Departments of Environment or equivalent bodies. The global distribution of iron bacterial problems in ground water was drafted on the basis of the governmental replies Fig. 2). Some countries elaborated extensively on the nature of the problems (summarized in Table 3).

From this survey based on responsive countries only, it is clear that iron bacteria occur on all continents (except Antartica for which no data is available) and other countries including China, Germany, Yugoslavia and Holland presumably also experience problems since papers on the subject have originated from them. Indeed, it seems likely that iron bacteria are present in the ground water and surface waters throughout the temperate and tropical zones of the world.

(c) Local problems with iron bacteria in Saskatchewan

The southern half of Saskatchewan is prairie, with an annual rainfall of 20-40 cm, and is fed by one major river system and several large aquafers running from the northern half of the province which is a part of the Canadian Shield. In the south, the dominant industry is agriculture (grain crops). The low rainfall restricts the source of water to spring run-off and well supplies. Problems with iron bacterial growth in wells occur across the southern part of the province (Fig. 3) to a varying degree *(Anon.* 1972). . . Meneley (pers. comm.) has calculated that the approximate annual cost of remedial measures to the provincial economy is four to six million dollars. The principal effects of iron bacterial growths were listed *(Anon* 1972) as being the corrosion of water pumps pressure tanks, galvanized pipes and fittings; the clogging of metal and plastic pipes; the reduction of water flow and water pressure and the coating of the resin beds of water softeners with slime, reducing efficiency and imparting unpleasant tastes and odours to the water. Dominant iron bacteria listed were *Gallionella* and *Sphaerotilus.* The authors, in a survey of well water samples from across the southern half of Saskatchewan, found that over 90% of the wells contained iron bacteria (see Section 3c) and that the dominant genera were, in decreasing order of percentage occurrence: *Crenothrix, Leptothrix, Gallionella, Sphaerotilus* and occasionally *T. ferrooxidans* and *Siderocapsa.* Control methods used include treatment with hypochlorite ('shock' chlorination); sulphamic or sulphuric acids and a number of proprietary products, but there is a high incidence in the reoccurrence of problems after all types of treatment. Some attempts are made to enforce higher hygienic standards during construction and maintenance operations in an effort to prevent cross-contamination of wells.

3. The Growth and Enumeration of Iron Bacteria

(a) Factors influencing the growth of iron bacteria

The impact of iron bacterial growths on the quantity and quality of water supplies has focussed some attention on the factors controlling their growth. Each chemical and physical factor will be discussed separately.

(i) Iron

As suggested by the name iron bacteria, iron can perform a key role in controlling the growth of the organisms. Wolfe (1960) isolated a strain of *Clonothrix* growing in waters devoid of manganese and containing only 0.02 $mg.1^{-1}$ of iron, but in general, growths occur only at substantially higher concentrations of iron. Hasselbarth & Ludemann (1972) in their review of the large iron and manganese bacteria *(Gallionella, Leptothrix)* found that in static water conditions growth occurred at between

1.6 and 12 mg.1^{-1} iron, and was prevented at l4 mg.1^{-1} iron. In flowing water, such as a pumping well, encrustrations of iron bacteria could be expected if the iron concentration exceeded 0.2-0.5 mg.l^{-1} because of the continuous flow of nutrients. Similar concentrations of iron (mg.l^{-1}) are reported elsewhere in the literature, for example, 0.2-0.8 (Rao 1970); 0.3 (Luthy 1964) and 1 (Mogg 1972). Starkey (1945) and Stephenson (1950) calculated the reaction dynamics assuming that the oxidation of ferrous ions generated the sole source of energy for the synthesis of cell material. The ratios calculated were 500:1 and 448:1 (for iron to cell material) respectively, clearly indicating that very large deposits and encrustration of iron bacteria occur relative to cell mass.

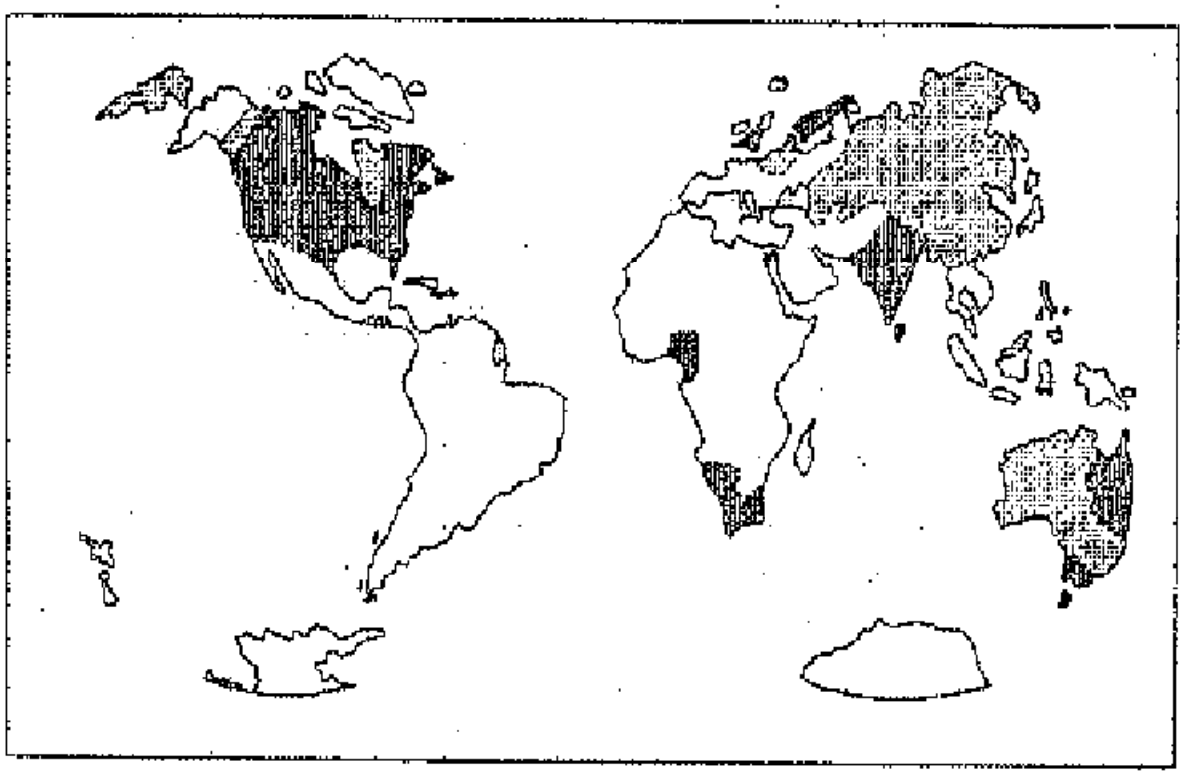

Fig. 2. Global distribution of iron bacteria problems acknowledged by government agencies. The shaded areas represent districts where governments have indicated problems from iron bacteria. Documented information and locally severe occurrences are shown in the dark shade. The light shade indicates that these problems are suspected to be caused by iron bacteria. Unshaded areas represent the countries which failed to respond to the survey and about which there is no specific mention in the literature concerning the distribution of the bacteria or which reported ignorance of the occurrence of iron bacteria. The map does not therefore give a complete distribution.

(ii) *Manganese*

This may fulfil the same role as iron in the metabolism of iron bacteria, but has been ignored to some extent in earlier well monitoring programmes. Luthy (1964) considered 0.05 mg.1^{-1} of manganese to be undesirable since it would cause staining but stated that the problem became more severe at manganous ion concentrations >0.15 mg.1^{-1} . Studies carried out by the authors appeared to indicate that for most strains of iron bacteria, the uptake of iron and manganese did not interact with each other in any fixed ratio. In bioassays of freshly isolated iron bacteria (mainly *Crenothrix sp.),* most displayed a preferential growth in media containing higher concentrations of ferrous ions than in media containing the equivalent molar concentrations of manganous ions. However, for one strain of *Crenothrix* subjected to intensive uptake studies, it was noted that: (a) the rate of reduction of dissolved manganese was affected by the concentration of iron present; (b) when iron was in excess of' manganese (>5:1)

then the rite of utilization of manganese was accelerated (c) when the total concentration of iron and manganese was low (<10 mg.1^{-1} combined iron and manganese), then the excess of iron necessary to stimulate manganese uptake was more extreme (>100:1). In these bioassays, few strains grew more efficiently on manganese than on iron. Clearly, the alternate use of manganous ions by iron bacteria requires more study, and the name iron bacteria may be a poor one since it focusses attention on that element rather than on manganese and perhaps some other metallic elements.

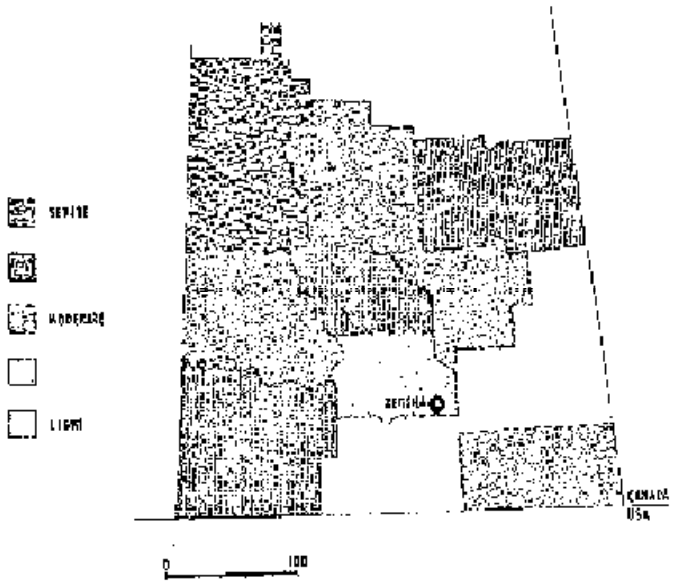

Fig. 3. Intensity of iron bacterial contamination of wells in the agricultural region of Saskatchewan. Severity of the contamination problem is riven in a sliding shade scale displayed to the left of the map. The scale is in miles. The clear zone to the north is outside the agricultural region of Saskatchewan.

(iii) *pH*

Iron bacteria. except *T. ferrooxidans,* generally grow well over the pH range of 5.4 to 7.2 (Hasselbarth & Ludemann 1972). The pH of individual culture media are within this range: Prevot's medium, pH 16.0, iron bacteria medium, pH 6.5: *Leptothrix* medium pH 5.9-6.8; Lieske's medium, pH 6.6; van Niel's medium pH 6-7 (Rodina 1965). Under more alkaline conditions (that is, pH of 7.5-9.0). ferrous and manganous ions lend to oxidize rapidly by normal physicochemical processes and become less available as a potential energy source.

(iv) *Oxygen*

All iron bacteria are either aerobic or microaerophilic and massive growths of iron bacteria have been reported in wells containing less than 5 mg.1^{-1} O: (Hasselbarth & Ludemann 1972). Growth may be suppressed tinder saturated oxygen conditions. In addition, Weart & Margrave (1957) noted that the growth of some species of iron bacteria was restricted to a defined oxidation-reduction range.

(v) *Temperature*

There has been no direct study of the growth range of iron bacteria directly isolated from wells but some ranges are listed in *Bergey's Manual of' Determinative Bacteriology* (Buchanan & Gibbons 1974) and are presented in Table 4. Studies on the growth of iron bacteria in the wells of southern Saskatchewan would suggest that all the strains are obligate or facultative psychrophiles, since the water temperature varies between 3 and 14EC. Although only limited thermal gradient growth studies

have been undertaken, evidence from field studies suggests that temperature elevation in well water may trigger off iron bacterial growths. For example, in one refinery well (#6 Consumers Co-operative Refinery, Regina), significant increases in iron bacterial numbers followed a rise in the water temperature above 5.5-5.8EC. When the temperature returned to a value below this level the bacterial numbers declined

Table 3

Information received from various governmental agencies concerning the occurrence of *iron bacterial infestations*

Country	Area	Problem-specified	Causative agent
Australia	Victoria and Queensland	Irrigation jets plugged, plugged pipes	*Gallionella* dominant in ground water (Queensland)
Canada	Most provinces	Extensive growth in wells	*Gallionella, Crenothrix. Leptothrix, Clonothrix, Sphaerotilus* and *Siderocapsa* (rare)
El Salvador	Apopa and Soyapango	Clogging well screens	Iron bacteria, not specified
Guyana	-	Water supplies	Not specified
India	Widespread	Reduction in flow rates and potability	Clonothrix predominant in Calcutta area
Malaya	-	Contamination of wells and irrigation water for rice culture	Not specified
Nigeria	Bomore	Plugging of screens	Not specified
Norway	Widespread	Growths in systems of hydroelectric power plants	*Gallionella*
Singapore	City	Deterioration in water supply	Not specified
South Africa	Widespread	Pipe scaling	Not specified
Sri Lanka	Widespread	Plugging through water supply systems	*Crenothrix*
Sweden	Widespread	Discoloured water, plugged pipes	*Gallionella, Crenothrix. and Leptothrix*
United Kingdom	-	Discolouration of water supplies	*Gallionella* (less organic mat ter present) or *Leptothrix/ Crenothrix* (organic matter relatively high)
United States of America	Alabama	5% wells discoloured, 62% wells contain iron bacteria	*Gallionella* and *Sphaerotilus*

Table 3, continued

	Indiana, New England, New Jersey, Ohio	Problems with iron bacteria particularly common	Not specified
	California, Colorado, Indiana, Kansas, Kentucky, Louisiana, Missouri, New York, Ohio, South Carolina, Vermont, West Virginia	969 communities surveyed and 10% of water examined exceeded 0.3 $mg.1^{-1}$ iron and could support iron bacterial infestations	Not specified
Union of the Socialist States of Russia	Widespread	Not specified	Not specified

(vi) *Carbon*

Most wells contain sufficient dissolved carbon dioxide/bicarbonate (Hasselbarth & Ludemann 1972) to meet the growth requirements for the chemoautotrophic iron bacteria *(Gallionella) so* that carbon is not likely to be restrictive for this genus. The other genera requiring organic carbon may be restricted by the availability (qualitative and quantitative of these compounds. In Rodina's (1965) listed media, organic carbon is provided in a variety of forms - citrate, acetate, glucose, peptone, asparagine and infusates of various leaves (for example, lettuce and willow). Comparative studies indicate that the most universal source of carbon is citrate (as ferric ammonium citrate). It is difficult to predict a threshold concentration below which iron bacterial growth would be restricted or totally inhibited since the total available organic carbon would be a function of flow rate as well as concentration.

The range of cultural procedures which have been used for the iron bacteria are shown in Table 5.

Table 4

Growth temperature ranges for iron bacteria

Genus	Growth temperature range (EC) minimal	optimal	Maximal
Sphaerotilus	15	25 - 30	37
Leptothrix	10	20 - 25	35
**Crenothrix*	6	26 - 28	34
Thiobacillus ferrooxidans	?	15 - 20	25

Cullimore & McCann (unpublished).

(b) *Cultural differentiation of iron bacteria*

Although there has been a considerable level of activity in determining the types arid physiological grouping of the traditional iron bacteria, they remain a poorly defined group (Macrae & Edwards 1972). This stems from the fact that many bacteria can become encrusted with iron precipitated from a sol, as demonstrated by Macrae & Edwards (1972) for *K. pneumoniae, Micrococcus, E. coli, Corynebacterium pseudodiphtheriticum, Ps. fluorescens, Mycobacterium phlei* and *Caulobacter.* Macrae *et al. (1973)* cast further doubt on the delineation of iron bacteria by reporting that isolates of *Pseudomonas, Alcaligenes* and *Moraxella* were able to utilize organic iron complexes (as ferric ammonium citrate and in some cases, as ferric malonate arid/or ferric galate). In all cases iron was precipitated, often as a heavy red precipitate. Clearly, the definition of iron bacteria needs resolving if an accurate determination is to be conducted into the relative importance of iron bacteria in well waters.

Of primary concern must be the name iron bacteria which in itself does not accurately define their function since many bacteria in this group can alternatively use manganese or even use manganese exclusively. Furthermore, there is no evidence which would exclude the use of other metallic elements from performing the same function, albeit in a minor role. Therefore, the group could be more precisely defined by the term metallo-oxidizing bacteria (MOB). This would eliminate from the group the heterotrophic bacteria capable of utilizing the organic component of iron or manganese organic complexes leading to the precipitation of iron or manganese (i.e. the metallo-precipitating non-oxidizing bacteria, MPNB). While the MOB group would oxidize specifically either manganous or ferrous compounds, with encrustation and/or precipitation of the metal as the oxides or hydroxides, the MPNB would cause only precipitation of the metal. with encrustation only when iron is present in a colloidal form (Table 6). In most instances, however, the MOB group would be encrusted with the metallic oxides or hydroxides while the MPNB group would cause precipitation. Confirmation of the types of bacteria present in any primary isolation/enumeration would therefore have to include at least microscopic examination for the types of bacteria present. Many of the media recommended for isolating MOB will allow the growth of the MPNB. Rodina (1965) has listed a range of media suitable for culturing iron bacteria (that is, MOB). Cullimore & McCann (1975), using a range of these media at pH values of 6.0 and 7.4 to culture MOB from 15 water samples taken from Saskatchewan wells, found by means of a microscopic examination of pellicular growths of the liquid media that MOB had grown to differing extents in all media (Table 7). Genera recovered included *Crenothrix, Leptothrix, Sphaerotilus. Gallionella* and *Siderocapsa.* with *Gallionella* and *Crenothrix* being the most commonly isolated. The Winogradsky and the iron peptone media gave the best recovery rates. *Gallionella* could not, however, be reliably subcultured on these media but *Crenothrix. Leptothrix* and *Sphaerotilus* grew well.

Table 5
Cultural procedures for iron bacteria

Method	Specific notes	References
Thiobacillus *Ferrooxidans*		
9K medium	9000 mg.1-1 Fe, pH 3.3 developed by	Silverman & Lundgren (1959)
9K medium	used by	Margalith *et al.* (1966), Remsen & Lundgren (1966), Korezynski *et al.* (1967) Wang, *et al.*(1970). Howard & Lundgren (1970), Vestal & Lundgren (1971), Lapteva *et al.*(1971),Niemela & Tuovinen (1972)
9K medium	Added 0.5M solution $FeSO_4.7H_2O$	Vestal & Lundgren (197 1)
9K medium	Omitted chloride salts	Howard & Lundgren (1970)
Medium for use in acid bituminous coal mine effluents	$FeSO_4.7H_2O$, 2000 mg.l^{-1}:$MgS0_4.7H_20$,0.1%; $(NH_4)_2SO_4$, 0.05%, pH adjusted using H_2SO_4 to 2-2.5	Temple & Colmer (1951)
Medium for coal mine effluents	$(NH_4)_2SO_4$, 0.015%; KCl, 0.0005%, $MgSO_4.7H_2O$, 0.05%; KH_2PO_4, 0.005%; $Ca(NO_3)_2$, 0.001%- $FeSO_4.7H_2O$ added to final strength of 0.1%	Leathen *et al.* (1956)
Solid agar medium	2% agar added to basal medium	Temple & Colmer (1951)
Solid agar medium	Enumeration using agar media fails due to inhibition of organisms	Bryner & Jameson (1958), Unz & Lundgren(1961)

Continued

Table 5 - Continued

Method	Specific notes	References
Silica gel as gelling agent in media	Variable success	Leathen *et al.*(1951), Leathen *et al.*(1956) Bryner & Jameson (1958), Beck (1960), Lapteva *et al.* (1971)
Enumeration of viable organisms	K_2HPO_4, 0.08% $MgSO_4.7H_2O$, 0.08%, $(NH_4)_2SO_4$, 0.08%, $FeSO_4$, $7H_2O$,. 6.66% pH 1.55. Prewashed membrane filters. Agarose at 0.3-0.5%, (w/v)	Tuovinen & Kelly (1973)
Incorporation of organic compounds in medium	Reported *T. ferrooxidans* inhibited by specific compounds	Kelly (1971). Tuovinen *et al.* (1971b),Usami Sugitani (1971)
Incorporation of organic compounds in medium	Only some strains inhibited	Shafia & Wilkinson (1969), Tabita & Lundgren (1971)
Medium containing glucose, basal salts, p-amino benzoic acid	Transition from chemolithotrophy to organotrophy	Shafia *et. al.* (1972)
Sphaerotilus/Leptothrix Cultured	Autotrophically on thiamin. biotin, cyanocobalamin-supplemented medium	Ali & Stokes (1971)
Methionine synthesis	Cyanocobalamin shown to be essential	Johnson & Stokes (1965)
Growth on manganous ions	*Sphaerotilus discophorus* could exclusively utilize manganous rather than ferrous ions	Ali & Stokes (1971)
Heterotrophic media	Recommended for group	Mulder & Van Veen (1963). Petitprez *et. al.* (1969), Stokes& Powers (1965 and 1967). Bisset & Brown (1969)
Carbon substrate utilization by strains of *Sphaeroitilus*	Wide range of sugars could be metabolized	Lackey & Wattie (1940), Stokes (1954). Scheuring & Hohnl (1956)
Carbon substrate utilization by strains of *Sphaerotilus*	Fructose utilized	Hohnl(1955). Mulder & Van Veen (1963)
	Fructose utilized by some strains	Lackey & Wattie (1940), Scheuring & Hohnl (1956). Razumov (1961)
	Lactose not utilized	Stokes (1954)
	Xylose and ribose utilized	Curtis (1969)
	Glucose suppressed the oxidation of other organic compounds	Stokes & Powers (1967)
	Organic alcohols could be utilized but not methanol	Stokes (1954)
	Glycerol utilization confirmed	Hohnl(1955), Mulder & Van Veen (1963)
	Succinic, fumaric, butyric, lactic, pyruvic and acetic acids utilized	Stokes(1954)
	Additional acids including malic, propionic, Citric, glucolic, amlonic and tartaric all utilized	Scheuring & Hohnl (1956)
Simple medium	Gelatin supplemented with mineral salts	Pringshiem (1942), Zikes (1915)

Continued

Table 5 - Continued

Method	Specific notes	References
Amino acid utilization	0.1 $mg.l^{-1}$ methionine stimulates uptake	Wilson (1960)
	Asparagine, aspartic acid, glutamine and glutamic acid could serve as carbon and nitrogen sources only	Scheuring & Hohnl (1956)
	Methionine, threonine, tyrosine glycine and cystine could serve as nitrogen sources only	Scheuring & Hohnl (1955)
	Disparity in results postulated to be due to different isomers or concentrations	Harrison & Heukelekian (1958), Hohnl (1955)
Inorganic nitrogen	Nitrate utilized	Linde (1913)
	Grew with ammonium or nitrate ions	Cataldi (1939), Lackey & Wattie (1940)
	Ammonium ion utilized only when carbon source was sucrose, glycerol or succinate	Stokes (1954)
	Nitrate proved superior source of nitrogen	Hohnl (1955), Razumov (1961), Phaup(1968)
	Cyanocobalamin (B_{12}) stimulated uptake of NH_4, NO_2 and NO_3	Dias & Heukelekian (1967), Mulder & VanVeen (1962), Okrend &Dondero (1964)
	B_{12} stimulation of uptake could be duplicated using higher concentrations of' methionine	Wuhrmann & Koestler(1950)
	Thiamine and biotin also essential for uptake in some strains	Stokes & Johnson (1965) Johnson & Stokes (1965)
Mineral salt requirements	Basic medium suggested	Lackey & Wattie (1940)
	FeC_{l3} could cause inhibition	Johnson & Stokes (1966)
	Filament formation calcium dependent	Dias & Dondero (1967)
	Strontium could not be used as a substitute for calcium dependency	Dias *et al.* (1968)
	Phosphate inhibitory at	
	0.15 M	Hohnl (1955)
	0.05 M	Gaudy & Wolfe (1961)
	Sphaerotilus dominant component of sewage fungus' at 150 Fg.1^{-1} phosphorus	Wuhrmann *et al.* (1966) Ornerod *et al.* (1906)
Basic medium	Enrichment medium using ferrous sulphide at pH 6.0	Kucera & Wolfe (1957)
	Modified above medium to monitor pH changes with indicators	Wolfe (1960)
	Eliminated contaminants by 0.5% formalin pretreatment for 2 days	Nunley & Krieg (1969)
Preservation of viable cultures	15% glycerol at -80EC	Nunley & Kreig (1969)
Optimization of growth	Maximal at 0.1-0.2 $mg.l^{-1}$	Hanert (1968)
	1% $C0_2$ enrichment	Van Iterson (1958)
Recommended medium	Lieske's medium containing 3% (w/v) iron filings or flat plates	Rodina (1965)

(c) *Qualitative examination* of *'iron' bacteria*

Many techniques have been developed for the rapid screening of water samples for the detection of iron bacteria. Direct microscopic examination. the original preferred technique (Biswas 1937; *Anon.* 1939), is still widely used (Barbic & Bracilovic 1974). The most successful stain in the author's exper-

Table 6

Genera comprising *the metallo-oxidizing bacteria and the metallo-precipitating* non-oxidizing *bacteria*

Group	Genus	Autotrophic	Heterotrophic	Non-colloidal metal encrusted on cell	Metal precipitated only
MOB	*Gallionella*	+	-	+	-
MOB	*Toxothrix*	-	+	K	±
MOB	*Sphaerotilus*	-	+	-	+
MOB	*Leptothrix*	-	+	+	-
MOB	*Lieskeella*	-	+	+	-
MOB	*Crenothrix*	-	+	+	-
MOB	*Clonothrix*	-	+	+	-
MOB	*Pedomicrobium*	-	+	+	-
MOB	*Metallogenium*	-	+	+	-
MOB	*Kusnezovia*	-	+	+	-
MOB	*Thiobacillus*	-	+	±	K
MOB	*Siderocapsa*	-	+	+	-
MOB	*Naumanneilla*	-	+	+	-
MOB	*Ochrobium*	-	+	+	-
MOB	*Sideococcus*	-	+	±	K
*MPNB/MOB	*Pseudomonas*	-	+	K	+
*MPNB/MOB	*Hyphomicrobium*	-	+	K	+
*MPNB/MOB	*Arthrobacter*	-	+	K	+
MPNB	*Aerobacter*	-	+	-	+
MPNB	*Serratia*	-	+	-	+
MPNB	*Bacillus*	-	+	(K)	+
MPNB	*Klebsiella*	-	+	-	+
MPNB	*Alcaligenes*	-	+	-	+
MPNB	*Moraxella*	-	+	-	+
MPNB	*Corynebacterium*	-	+	-	+
MPNB	*Caulobacter*	-	+	-	+
MPNB	*Mycobacterium*	-	+	-	+
MPNB	*Escherichia*	-	+	-	+

* Individual strains capable of oxidizing ferrous and/or manganous inorganic salts have been reported.

rience is that of Meyers (1958) in which the cells stain red and the iron deposits blue. The technique consists of: (a) separating the cells by centrifugation (if water is to be examined): (b) smearing the centrifuged pellet onto a slide (if a culture is to be examined then direct smear of the pellicle may be made); (c) air drying slide: (d) placing it in methanol for 15 *min; (C)* heating to boiling point a 1:1 mixture of potassium ferricyanide and 5% acetic acid (both solutions in distilled water); (f) immersing slide in boiling mixture for 2 min; (g) washing it gently with distilled water after cooling: (h) staining for 5 to 10 min with 2% aqueous safranin; (i) rinse, dry and examine. Alternatively, wet mounts of water samples rich in iron bacteria can be efficiently examined using phase contrast microscopy (V. G. Collins, pers. comm.). For samples low in bacterial numbers, Leuschow & Mackenthun (1962) used

filtration through a 0.45 Fm pore size membrane filter, followed by drying at 100EC and saturating the filter with immersion oil having the same refractive index as the filter material. The filter is examined under an oil immersion and the types of iron bacteria observed and enumerated. Leuschow & Mackenthun (1962) applied the technique to wells in Wisconsin and found that using 100 ml samples, 55% were positive for iron bacteria.. Dominant bacteria were Gallionella and *Leptothrix* and the highest counts $>10^7$ cells. mL^{-1} in reddish and turbid waters

Table 7
Percentage occurrence of *different genera* of *metallo-oxidising bacteria on twelve media using, well water samples*

Medium	pH	*Crenothrix*	*Gallionella*	*Leptothrix*	*Sphaerotilus*	*Siderocapsa*
Winogradsky	6	60	27	0	7	0
Winogradsky	7.4	80	33	7	0	0
Prévot	6	40	20	13	13	0
Prévot	7.4	27	13	7	0	0
Iron peptone	6	87	20	13	0	0
Iron peptone	7.4	87	13	7	7	0
Leptothrix	6	33	13	7	0	7
Leptothrix	7.4	27	20	0	7	7
Ferric ammonium citrate	6	20	13	0	7	0
Ferric ammonium citrate	7.4	73	27	0	0	0
Lieske's	6	20	13	13	7	0
Lieske.s	7.4	20	13	0	0	0

Formulae as listed in Rodina (1965).

Several very simple cultural techniques have been developed to indicate presence or absence of iron bacteria in water samples. These include the following:

1. Water is placed in a wide-necked sample bottle and left overnight. The appearance of flakes resembling cotton wool indicates the of iron bacteria and is confirmed by microscopic examination of the flakes (Rodina 1965)

2. Water is placed in an aquarium jar together with sediment. A cork with several cover glasses inserted vertically into its lower side is floated on the water. The appearance of rust spots and/or cotton-like accumulates above the precipitated sediment indicates the presence of iron bacteria and examination of the cover glasses (air dried and stained) after 24 h incubation will reveal the types of any iron bacteria which have become attached to the glass (Cholodny 1953).

3. The simple is placed in a conical flask to which a chemically cleaned soft steel washer is added. An extruded plastic rod is now placed vertically in the water. After two days a translucent filamentous growth occurs at and below the water line on the rod and develops a brown tint indicative of the presence of iron bacteria (Grainge & Lund 1969). The authors recommended tile use of this technique to ascertain the potential effectiveness of control programmes.

4. Cullimore & McCann (1975) have developed a three-day Field test for detection of both the MOB and MPNB in water, based on a modification of Winogradsky's medium with nutrient levels established at the minimal concentration of each element to achieve optimal growth assuming an adequate supply of all other nutrients. using a strain of *Crenothrix* (Table 8). The test is performed in a 25 mL capacity screw-capped tube containing 0.75 mL of concentrated medium (X20) evaporated to dryness

at 65EC under aseptic conditions. The water sample is placed directly into the tube whereupon the medium is rehydrated and returns to its normal concentration; and incubated in the dark at room temperature (22 ±3EC) for three days. In Saskatchewan, this period has been found to be sufficiently long for growth to occur. In general. the iron bacteria develop a thick pellicle or flaky deposit on the surface of the medium which itself becomes yellow or brown. If no bacteria are present then the medium will slowly auto-oxidize to a green colour. Several alternative reactions can occur in this test (Table 9). Reaction patterns A to D all indicate the presence of iron bacteria but no clear categorization of the genera can thereby be ascertained. Reaction patterns D and E are both the result of the presence of hydrogen sulphide-producing micro-organisms since the black deposits are iron sulphides. In pattern E, no iron bacteria have been recovered and the test indicates the presence of sulph ide -producing-micro-organisms only.

Table 8

Critical minimal concentrations for maintenance of optimal growth of a strain of Crenothrix

Element	Concentration ($mg.1^{-1}$)	
	Original medium*	Modified medium
Fe	1000	600
N	750	450
C	2500	1500
Na	140	0
K	220	220
P	90	90
Mg	50	50
S	67	67
Ca	90	0
Cl	16	0

pH adjusted to 7.4.

*Winogradsky's medium.

Table 9

Alternative reactions occurring in the field test

Reaction type	Visible changes in medium						
	Colour of medium			Brown pellicle or plug.	Brown flakes on surface	White effervescense	Black deposit
	colourless	yellow	brown				
A	-	K	±	+	K	-	-
B	-	K	±	-	+	-	-
C	-	+	-	-	-	-	-
D	K	K	±	+	K	-	+
E	+	-	-	-	-	+	+

Cullimore & McCann (1975).

The test therefore indicates not only the presence of iron bacteria but also of the corrosive hydrogen sulphide producers. Tests on ground waters in Saskatchewan have been 95% positive for the presence of iron bacteria and microscopic examination of the surface growths has revealed the dominant types to be *Crenothrix, Lcptothrix, Sphaerotilus* and *Gallionella.*

(d) *Quantitative examination* of '*iron ' bacteria*

Modifications of several of the qualitative procedures can be used to achieve quantitative results by the application of a serial dilution technique prior to the test procedure, or by the use of the membrane filtration method of Leuschow & Mackenthun (1962). Another MF technique has been developed by Cullimore & McCann (1975) in which the water sample (100 mL, 10 mL equiv.; 0.1 mL equiv.) is membrane filtered using a 0.22 Fm pore size filter, and subsequently cultured on the modified Winogradsky's medium containing 2% agar and incubated at 28EC for 3 days. The iron bacteria grow as brown colonies, sometimes irridescent. with irregular edges, such colonies being counted as iron bacteria. A typical pattern of iron bacterial populations in well waters northeast of Pilot Butte (east of Regina) is given in Fig. 4 and Table 10. This clearly shows that the numbers present are subject to considerable variation depending upon factors such as the water temperature, level of pumping activity and quality of the water.

Table 10

Distribution of iron bacteria in wells north east of Pilot Butte, Saskatchewan

Well site (see Fig. 4)	Iron bacterial numbers (organisms.mL^{-1})	
	Before run-off	After run-off
1	88	13
2	25	>3000
3	85	1
4	50	336
5	100	>3000
6	300	27
7	>3000	1
8	30	376
9	10	>3000
10	>3000	>3000
11	300	400
12	200	-
13	300	>3000
14	0	446
15	50	70

Run-off is the period of snow melt which occurs each spring.

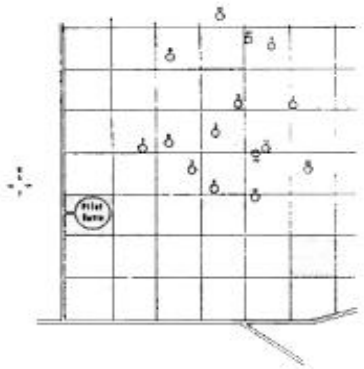

Fig.4. Distribution of wells tested for iron bacteria north cast of Pilot Butte, Saskatchewan. The grid pattern divides the land into square miles sections.

TABLE 11
Recommended chemical treatments for the control of iron bacteria in ground water,

Treatment*	Site	Reference
110 lb oxalic acid, 50 lb sulphamic acid, 50 lb wetting agent, 0.25 lb inhibitor	Wells	Grainge & Lund (1969)
Elimination of dissolved CO_2 by elevation of pH to above 8.3	Wells	Ellis (1932)
Residual Chlorine. 0.2 $mg.1^{-1}$	Lab tests	Grainge & Lund (1969)
Hydrogen peroxide, 100 $mg.1^{-1}$ and phosphate inhibitor	Lab tests	Grainge & Lund (1969)
Hypochlorite, 0.438%	Wells	Machmeier (1971)
Residual chlorine, 50- 100 $mg.1^{-1}$ for 2 h	Wells	Machmeier (1971)
Shock chlorination with 5.25% hypochlorite	Wells	Machmeier (1971)
Hydrochloric acid (muriatic acid), 14-21%	Screened wells	Schafer (1974)
Sulphamic acid, 7.5 -107, (several hours contact time)	Screened wells	Schafer (1974)
Hydroxyacetic acid, 4.7-7% (contact time related to pH of water)	Screened wells	Schafer (1974)
Chlorine gas to give 500 $mg.1^{-1}$	Wells	Schafer (1974)
LBA(Liquid Antibacterial Acid, USA Patent 3085929), 5% (treat for 36 h)	Wells	Luthy (1964)
Recycling of hypochlorite solutions	Water supplies	Rao (1970)
Hydrochloric acid treatment followed by 300 $mg.1^{-1}$ chlorine, 18 h contact	Wells	Mogg (1972)
Calcium hypochlorite, 715 $mg.1^{-1}$	Wells	Schafer (1974)
Lithium hypochlorite, 0.14%	Wells	Schafer (1974)
Sodium hypochlorite, 0. 14%	Wells	Schafer (1974)
Chlorine dioxide gas (limited use)	Wells	Schafer (1974)
Potassium permanganate 0.1-0.2%	Wells	Schafer (1974)
Conyinuous chlorination	Wells	Woods(1973)
Acrolcin, 0.1 - 30 $mg.1^{-1}$ (restricted use)	Water systems	Woods (1973)

*All concentrations mentioned refer to final concentrations in ground water

4. Control of Iron Bacteria in Ground Water Supplies

(a) *Chemical control of iron bacteria*

Many chemical treatments have been suggested for the control of iron bacteria in ground water supplies including bacteriocidal compounds, halogens and halogenated compounds, organic and inorganic acids, copper and copper salts. Many of the individual treatments recommended are summarized in Table 11; most frequently used are calcium or sodium hypochlorite, hydrochloric acid, sulphamic acid and some proprietary preparations. Cullimore & McCann (1975), using an automatically recording densitometer examined the ability of some of these compounds to inhibit the growth of strains of *Crenothrix* and *Gallionella* in Winogradsky's medium (pH 7.4). The effectiveness of each compound differed very significantly with the number of cells present in the 15 mL culture (Table 12). Javex (a commercial preparation of sodium hypochlorite prevented growth at 250 $mg.1^{-1}$ for up to 100 cells.15 mL^{-1} while 1% was necessary to prevent the growth of 7 x 10^5 to 1 x 10^6 cells.15 mL^{-1} . Clearly, the

selection of concentration for the chemical control must reflect the number of cells present within the treated system The most effective of the tested compounds was an iodine plymer synthesized by Levine, Chemistry Department, University of Regina. the structural formula of which (Fig. 5) *is* similar to that of crystal violet. Field trials have yet to be conducted on the polymer since little is known of its potential environmental effect. Potassium permanganate was also highly effective but is perhaps potentially dangerous since it contains manganese which could become a substrate for iron bacterial regrowths.

Table 12
Extrapolated effective control concentration (mg.1^{-1}) for five disinfecant against a range of cell concentrations of iron bacteria

Disinfectant	Cell concentration of iron bacteria (cells. 15 ml-')				
	+30 to 100	300 to 1000	+3000 to 10,000	70,000 to 100,000	+700,000 to 1,000,000
Javex*	250	750	850	10,000	10,000
HTH*	2000	5000	7500	100,000	100,000
IP (iodine polymer)	10	20	30	NC	NC
$CuSO_4$	500	1000	5000	10,000	10,000
LBA	50,000	50,000	50,000	ND	N D
$KMnO_4$	10-20	100	250	5000	500

All data for pH of 7.4.
+ Extrapolated data.
NC, no control since the solubility of IP in water is very low; ND, not determinable from data.
* Both based on hypochlorite concentration.

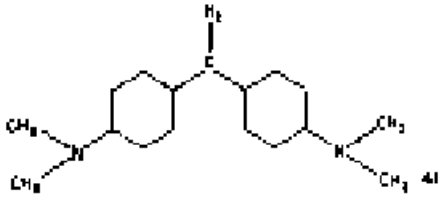

Fig. 5. Molecular structure of iodine polymer.

In Saskatchewan, recommended chemical treatments are widely applied but there is a history of recolonization, of the treated wells over even short periods of time; this has led to some doubt as to the effectiveness of control by chemical means. A summary of some of the data for limited field studies on the efficiency of recommended treatments applied to wells in Saskatchewan is shown in Fig. 6. It will be seen that inadequate control practices frequently lead to a post-treatment surge in the iron bacterial populations after a few days. The mechanisms which could influence this may be postulated to be as follows.

1. The chemical control agent may have a differential effect on the iron bacteria growing in clumps or as a slime coating. Inhibition may first occur in the outermost cells; penetration of the inner

(protected) cells might be more a function of the ability of the agent to become transported through the cell and the copious slime coatings than upon its toxic potential. Thus, as a result of poor penetration, very high concentrations of an agent (for example, sodium hypochlorite) could be employed without achieving adequate control.

2. Iron bacteria may be growing extensively outside of the treatment zone, and be pulled back into the treatment zone upon the resumption of pumping.

3. The control agent may become neutralized by dead organic material and non-target bacteria, thus reducing its effectiveness.

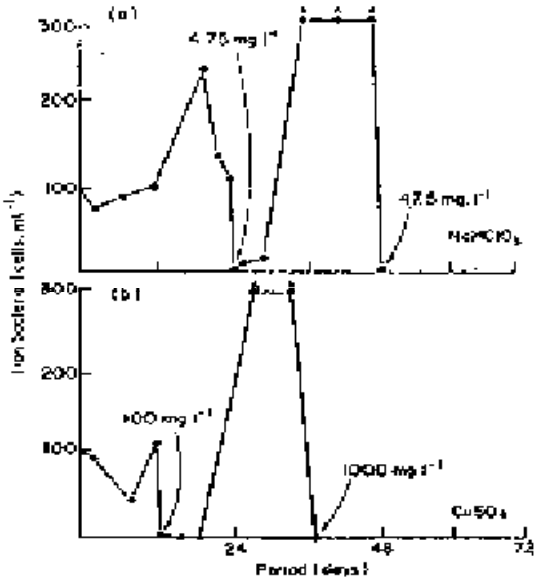

Fig. 6. Effect of two treatments of sodium hypochlorite (a) and copper sulphate (b) on the iron bacterial population in a shallow bore well in Saskatchewan. Arrows represent termination of treatment.

4. Water temperature may be a critical factor affecting the metabolic activity of the iron bacteria. Well water temperatures in Saskatchewan vary between 4 and 12EC and there is some evidence (unpublished) that the iron bacteria are by and large facultative psychrophiles with growth initiation between 5 and 9EC. In one industrial well which was closely monitored, the population of iron bacteria increased rapidly when the well water temperature rose between 5.4 and 6.0EC. Laboratory studies on a strain of *Crenothrix* indicated that its minimum growth temperature was 7.5EC. The optimum growth temperature was found to be 26 to 29EC, and inhibition occurred at 34EC. Further studies are underway to determine the temperature growth range of a number of iron bacteria. The temperature. by influencing the rate of metabolic activity, would affect the rate at which chemical control agents would be taken tip and transported into the cell (when an active transport system is necessary).

These factors Undoubtedly contribute to the sometimes ineffective chemical treatment of wells and have led to the search for alternate systems for achieving control.

(b) Physical *control* of *iron bacteria*

Comparatively little attention has been paid to the physical control of iron bacteria using ultrasonics, heat, cathodic protection and u.v. irradiation. Mogg (1972) discussed some of the physical factors involved in the construction of a well which could influence subsequent growths of iron bacteria, and suggested that metal or plastic parts should be resistant to hydrochloric or sulphamic acids and recommended (in decreasing order of resistance and suitability) most plastics and fibreglass, stainless steel types 304 and 316, silicon manganese bronze, silicon red brass, armco iron, low carbon steel and concrete. He disputed the beliefs that non-conductors such as plastics, fibreglass, concrete and transite would not be subject to encrustations by iron bacteria. Mogg also recommended that problems could be reduced by provision of a greater screen open area in the well; by reduction of draw-down through more efficient design and lower pumping rates; by provision of regular chemical treatment at recommended rates regardless of the appearance of symptoms; and by keeping oxygen away from the screened areas with vacuum seals and packets. Mogg (1972) also commented that "there seem to be more cases or iron bacteria today, and we believe that iron bacterial spores (cells) can be transported from one well to another." He illustrated this by citing a trouble-free well which had been in service for a number of years and became plagued with iron bacterial problems after the pump had been removed for minor repair. Indeed, it is now recommended practice in many parts of the world to disinfect all tools, equipment and drilling materials prior to drilling a new well, in order to reduce the risk of contamination by iron bacteria from another site. In some instances, a new well is treated immediately with hydrochloric or sulphamic acid.

The direct physical treatment of well water has received little attention. but an initial laboratory-based study was undertaken by Cullimore & McCann (1975). Since sonication (at between 500 and 900 kHz) is a well-documented method of disintegrating microbial cells (Carpenter 1972) it was thought that sonication of wells contaminated with iron bacteria might control the growth of the organisms. Several experiments were conducted on cultures *of' Crenothrix* and *Gallionella* but no significant reductions in cell numbers were observed even after 60 min of exposure to a tissue sonicator operating at maximum power (800 kHz). This abnormal survival capability is at least in part due to the extensive mucoid and gelatinous coatings of the cell which serve to dampen the effects of the sonication. Sonication is therefore an unsatisfactory method for the control of iron bacterial growth. Similarly, no real control of iron bacteria was achieved by providing cathodic protection.

On the other hand, studies on the heat sensitivity of 22 cultures of iron bacteria (Fig. 7) revealed that all were fairly sensitive to temperatures close to the pasteurization range used for milk (Table 13). Laboratory trials in a simulated well (a 3.1 m length of 10 cm plastic casing) gave complete elimination of all iron bacteria (3000 cells.mL1^{-1}) in a 2 m head of water within 20 min of reaching holding temperatures of 52, 55, 60 or 70EC. This indicates that pasteurization should eliminate completey, all iron bacteria from the system by the end of the holding temperature phase. The initial field trials were conducted on Well #24, Imperial Oil Refinery, Regina, using high pressure steam injected down the well as the heat source for pasteurization. The temperature was raised to 65EC and held for 40 min before being lowered by pumping out the treated water. This water was turbid and dark, brown in colour. Two pasteurization treatments (Fig. 8) had the composite effect of elevating the hourly flow rate from 3000 to 7000 imperial gallons.h^{-1}. Previous to this treatment, the well had been subjected to a number of chemical treatments (HTH, A9 bactericide and sulphamic acid), some of which gave increased flow rates which were however of short duration as the iron bacterial level in the well built up again. This also occurred after the two steam pasteurizations but did not reach a level which would affect refinery operations. No further Studies were conducted at this well since the refinery was closed down and operations moved to Edmonton Alberta.

Table 13
Time-temperature combinations necessarily to kill 100% of the cells in 9 nil amounts of 22 cultures of iron bacteria

Holding time (min)	Temperature (EC)
100	56
50	57.5
30	58
20	59
10	59.5
5	62. 5
3	64
2	65
1	66
0.1	7.5

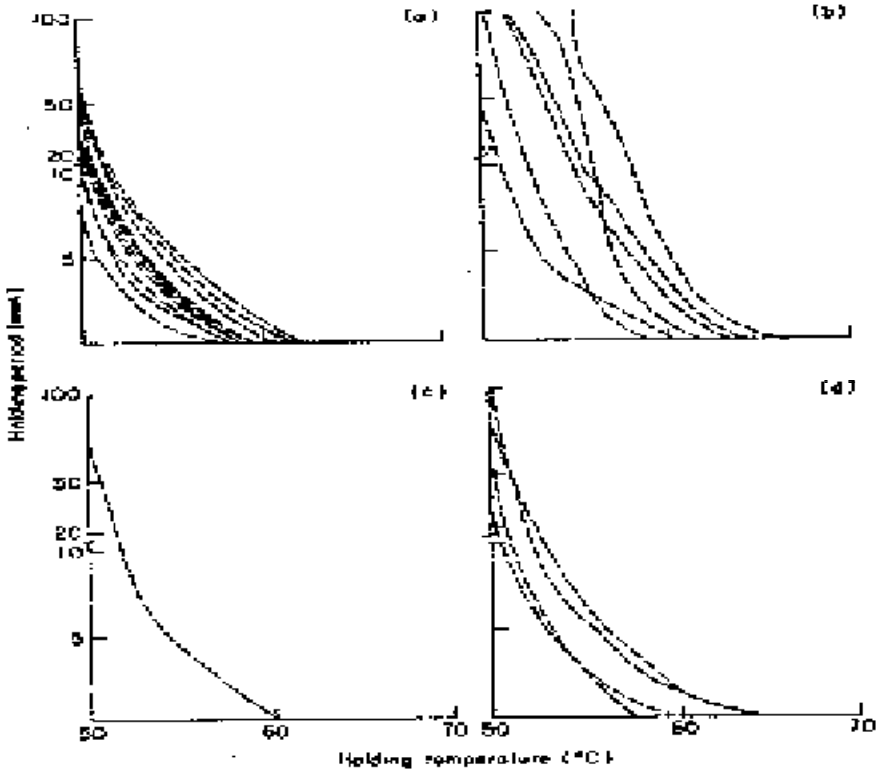

Fig. 7. Influence of holding time (min) and temperature on the survival of cells of 22 strains of iron bacteria. (a) *Crenothrix*, (b) *Sphaerotilus*, (c) *Gallionella*, (d) C + G. The individual graphs indicate time-temperature combinations just sufficient to kill all cells.

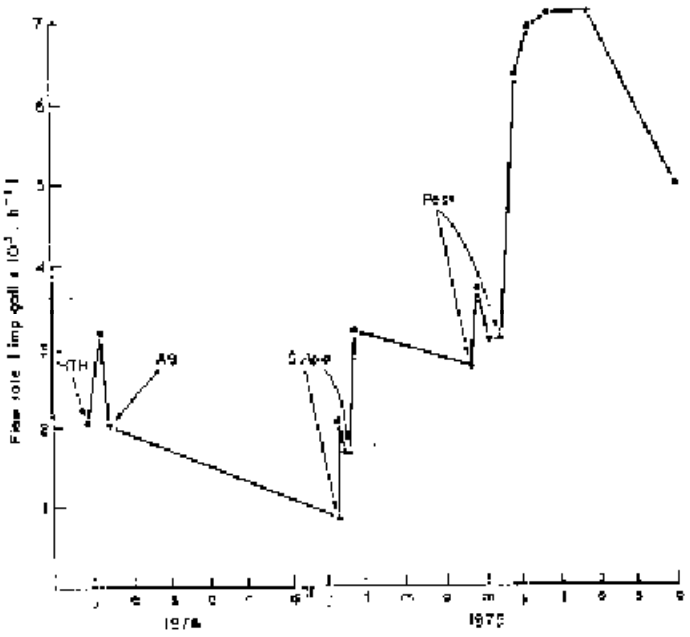

Fig. 8. Influence of treatments to eliminate iron bacteria on the flow rate of well*#24,* Imperial Oil Refineries, Regina. HTH, commercial preparation of sodium hypochlorite; A9, disinfectant; Sulp. a., sulphamic acid; Past., pasteurized by steam to 65EC for 40 min. Arrows represent termination of treatment. Iron bacterial growths will reduce flow rates. Flow rate is in imp. gall x $10^{3\ -1}$.

During the spring of 1975, a pilot pasteurization system was installed in Well #6. Consumers Co-operative Refineries Ltd (Fig. 9). Despite several technical difficulties in providing the steam supply, three pasteurization runs were performed and the influence of these on the iron bacterial population was recorded (Fig. 9). In each case. steam was applied gradually until the well water and pump-out water registered between 60 and 70EC. The system was left for 40 min and then the contents were pumped out. This water was dark brown and contained very high numbers of iron bacteria. As pump-out continued the water became clear. In Well #6, the pump-out water after pasteurization continued to contain iron bacteria at populations of between 30 and 300 cells mL^{-1} . If the well had been pasteurized effectively (and all indications are that it was), then the recurrence of a low level population would indicate that iron bacteria were growing or surviving in the sand and aquafer outside of the screen and were being pulled into the well with the pumping activity. From this it can be deduced that a secondary build-up in iron bacterial numbers will occur in the well on the screen, casings and pumping equipment, so that maintenance of high flow rates would be dependent upon regular pasteurization depending on the rapidity of the secondary build-up. Further trials are now being planned to develop all automatic well pasteurization unit and also to investigate its potential for the treatment of farm wells.

(c) General discussion

From all this, it is clear that the iron bacteria are in general very resistant to chemical methods of control, perhaps due to the protective slime layers and other coatings which surround the cells, together with the tendency for the cells to clump and/or form thick layers. Furthermore, these coatings are often heavily impregnated with ferric and manganic oxides and hydroxide deposits which could restrict the diffusion of the chemical agents and perhaps enter into some direct chemical reaction with them. Clearly the lack of success of chemical treatments is in part due to insufficient concentration and/

or contact time to allow total penetration of the iron bacteria in the wells, or is “too little, too late”. To overcome this, more attention needs to be paid to the accurate categorization of wells to determine the necessary level of treatment if chemical methods are to be used. One possible scheme is given below.

Category A. No iron bacteria present: iron bacteria test negative.

Category B. Iron bacteria present in low numbers but no surface colonial growth present; iron bacteria test positive. Iron bacteria numbers: 1 to 300 cells.mL^{-1} (In isolated cases, there could be as many as 5000 cells.mL^{-1}

Category C. Iron bacteria present in moderate numbers with some surface colonial growth present; iron bacteria test positive. Iron bacteria numbers: 300-5000 cells.mL $^{-1}$.

Category D. Iron bacteria present in high numbers with extensive surface colonial growth but no ‘plugging’, iron bacteria test positive (may also show sulphide reduction, i.e. a black precipitate). Iron bacteria numbers: 5000 to 50.000 cells.mL^{-1}.

Category E. Iron bacteria present in excessive numbers with such extensive surface colonial growth that ‘plugging’ occurs; iron bacteria test positive (frequently accompanied by heavy sulphide reduction, (i.e. a black precipitate). Iron bacteria numbers: 50,000-10,000,000 cell, mL^{-1}.

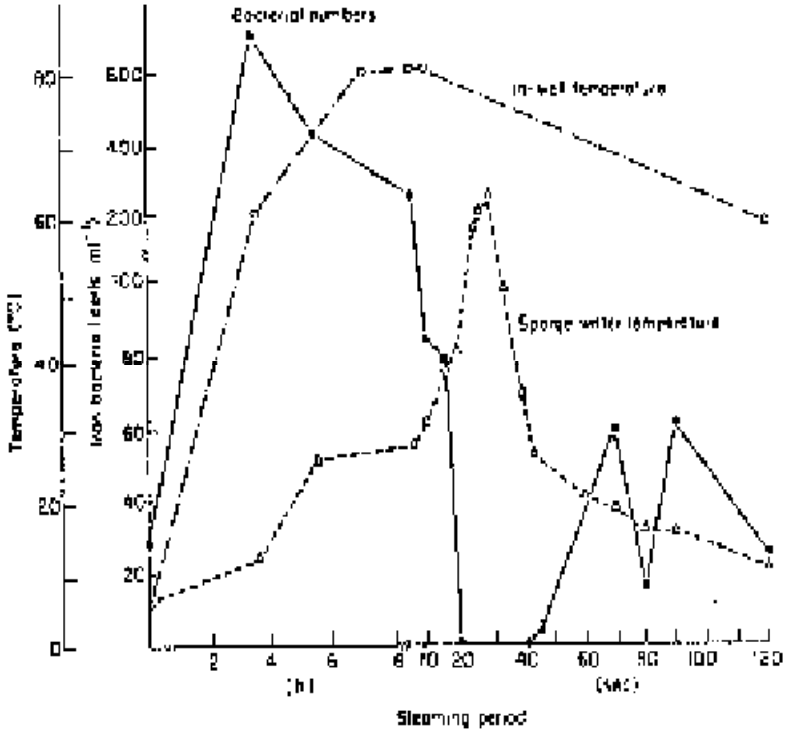

Fig. 9. Impact of steam pasteurization of Well #6, Consumers Cooperative Refineries Lid, Regina. Bacterial numbers peaked during the steaming period of 8 h due to the slime breaking up on screen and internal walls and fixtures prior to temperature becoming lethal. Recurrence of iron bacteria after treatment due to bacteria being drawn into well through screen after pasteurization.

Note that screens could become plugged in any of categories B to E but that the rate of plugging would increase dramatically with the severity of the problem.

Three category groups may be established using the simple field test system developed by Cullimore & McCann (1975). These are: category A wells, no reaction: category B and C, reaction type A, B or C (see Table 9); category D and E, reaction type D, E or occasionally A, B or C.

In Saskatchewan, the vast majority of wells (80% of those tested) fit into the middle group (category B, 60%; category C, 20%). Less than 5% of the wells are in category A and the remaining 15% of those tested are in category D or E, many of which have been abandoned. A possible relationship between chemical treatment and the effective control of iron bacteria may be achieved, at least in theory, for category B, C and D wells (Table 14), but not for category E wells, since the iron bacterial coatings would probably be too thick to allow complete penetration of the chemical even over a prolonged period. The frequency of treatment would depend upon the rate at which the quality of the well water degenerated. In some instances, very rapid growth of iron bacteria have been observed to appear on screens in a matter of hours (Oliver 1975). Steam pasteurization offers a more rapid (total operation time 1 1/2 to 6 h) and complete destruction of the iron bacteria. It is therefore ideally suited to the treatment of high capacity wells such as those employed in industry and by water authorities, where it can be installed as a permanent facility. For small wells, treatment by steam pasteurization can be achieved using portable steam generators. In conclusion. some reduction in the level of iron bacterial infections could be achieved by steam pasteurization of: (a) all newly installed wells: (b) all wells undergoing major repairs: (c) all category C, D, and E wells, on a routine basis until the wells return to a stable category B or A stage whereupon chemical treatment should suffice.

(a) and (b) would reduce the risk of a well unaffected by iron bacteria from becoming infected with contaminants from the soil, drilling equipment, or repair and replacement materials. (c) would reduce the population of iron bacteria dramatically within the well and keep the population down to manageable levels by subsequent regular treatments.

Table 14
Potential treatment time-chemical concentration for the control of iron bacteria in wells in categories B to D

Well category		Chemical treatment		
		Sodium hypochlorite	Sulphamic acid	Hydrochloric acid
B	Cone (%)	0.5	7.5	14
	Time (h)	6	12	6
C	Cone (%)	1	10	18
	Time (h)	24	24	12
D	Cone (%)	10	10	21
	Time (h)	48	48	24

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