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THE POTENTIAL USE OF FERROMANGANESE-
DEPOSITING BACTERIA AS A TREATMENT
METHOD FOR HEAVY-METAL-
CONTAMINATED WATER;
A LITERATURE STUDY

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<p>Sammendrag:</p> <p>Norway is particularly susceptible to potential pollution of its water resources by heavy metals due to: (i) the importance of its fishing and water-related interests, (ii) the large number of industries, including mining, which produce heavy metal-containing wastes, (iii) the significant number of landfill sites inadequately protected against leaching of heavy metals into underlying aquifers or surface drainage waters.</p> <p>Microbes, including bacteria, can immobilise high levels of heavy metals, and there is evidence that microbially related processes can be competitive with existing methods for treatment of heavy metal-contaminated water.</p> <p>Ferromanganese-depositing bacteria have been used in biological systems for the removal of iron and manganese from drinking water, and in processes for the treatment of acidic, iron-rich mine-drainage waters. Evidence from the literature and from recent studies suggests that these ubiquitous organisms have potential for use in the treatment of other heavy metal-contaminated water e.g. landfill leachate. More detailed studies are necessary before a full assessment of their potential can be made.</p>				
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The potential use of ferromanganese-depositing bacteria as a treatment method for heavy metal-contaminated water; a literature study.

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1. Introduction

Heavy metals present in the hydrosphere may be derived both naturally and anthropogenically (Drever, 1988). The weathering of rocks, particularly those containing sulphidic deposits, can result in relatively high levels of heavy metals occurring in water and soils. A typical example is the common occurrence of waters rich in iron, derived from weathering of iron-containing rocks and mobilisation in an acidic or reducing environment. The presence of high iron levels is undesirable in drinking water, but is often an entirely natural phenomenon. Similarly, high levels of other metals, such as nickel and copper, may be naturally-derived and should not be assumed to be due to pollution by man's activities (Bølviken, 1990). Nevertheless, for several decades there has been growing concern over the potential damage, both to public health and to the environment, that can be caused by increasing levels of anthropogenically-derived heavy metals.

An obvious source of anthropogenic heavy metal contamination is industrial effluent, including leachate from mine wastes, which releases metals directly into the hydrosphere. Mining also indirectly releases metals into the environment by exposing fresh rock surfaces to oxidation. Mobilized heavy metals may then enter the hydrosphere via mine drainage waters. Activities such as smelting introduce metals into the atmosphere, from whence they are subsequently washed out by rain into surface waters. Waste tips are another potential source, due to leaching of heavy metals (Appendix 1). Microbial breakdown of organic material present in the waste creates reducing conditions suitable for the solubilisation and mobilisation of many metal ions. Landfill sites containing domestic waste as well as those containing hazardous waste can pose problems where landfills were designed and sited without sufficient consideration of their pollution potential. Examples of all these sources of heavy metal pollution can be found in

Norway and will be described in more detail in section 1.3. First, however, the types of problems which may arise due to high levels of heavy metals in the hydrosphere will be discussed.

1.1. Problems caused by heavy metals.

The problems caused by high levels of most heavy metals, whether in terrestrial or aquatic environments, are due to both their potential toxicity and to their non-biodegradability.

Although certain heavy metals, e.g. Fe, Zn, Cu, Mo, Co, are essential trace elements, required by plants, animals and microbes in small amounts, all of these, with perhaps the exception of Fe, become toxic above threshold levels which may still be low relative to amounts discharged to the environment by man's activities. Other heavy metals, e.g. Hg, Cd, Pb, have no physiological value and are potentially toxic at even lower levels (Duxbury, 1985). A typical effect of heavy metal toxicity is inhibition of microbial activity. This can result in inhibition of the degradation of organic material, with a particularly adverse effect on ecosystems which depend on nutrient recycling, such as forests (Domsch, 1987). Among higher organisms, toxic effects may first become apparent when populations of certain species drastically decline. For example, high levels of copper, zinc, iron and lead in Norwegian fresh waters have affected salmonid fisheries (Grande and Arnesen, 1988). Toxic effects manifested in humans include the development of unusual diseases, a well-known example being Itai-Itai disease, suffered by villagers living along the Jinzu River in Japan; the causative agent was thought to be cadmium present in mine waters which were discharged into the river and subsequently accumulated in the soils of adjacent paddy fields (Salomons and Forstner, 1984).

The toxicity of heavy metals is exacerbated by their non-biodegradability, which results in their concentration within the food chain, with potentially devastating effects on higher consumers. For example, heavy metals which are assimilated by sessile bacteria in the bottom sediments of aquatic environments can be passed on to benthic invertebrates and vertebrates. Metals may thus accumulate in fish and ultimately in consumers feeding on the fish, such as humans (e.g. Patrick and Loutit, 1978; Homewood, 1991). A dramatic example of this was the mercury-poisoning of the people living around Minimata Bay (Japan) as a result of eating fish contam-

inated by mercury-laden effluent from a plastics factory. To date 2252 people have been designated as "Minimata disease" victims, of whom 1228 have died. Although the discharges were eventually stopped in 1968, the legal battles for compensation are still continuing (Howe,1976; New Scientist,1992). Consumption of agricultural produce grown on soil contaminated by high levels of heavy metals may also have a deleterious effect on the consumers. This is likely to have been a factor in the case of Itai-Itai disease mentioned previously. It has also necessitated the regulation of the use of activated sewage sludge, derived from waste water treatment plants, on agricultural land (Sterritt and Lester,1986; Morper,1986; Andersen,1991).

Although the release of anthropogenically-derived heavy metals can have disastrous consequences, this is not necessarily always the case. The extent and nature of observable pollution effects depend on a wide range of physicochemical and biological factors. For example, the susceptibility of the biota to heavy metal toxicity may be affected by the species present (different species may be more resistant than others) and the ages of individual populations (cf. Grande and Arnesen,1988) as well as by the speciation of the metals present. Metal ions will be subject to hydraulic processes as well as to water/rock/soil interactions, such as acid-base reactions, oxidation/reduction, precipitation, complexation and adsorption (Moore and Ramamoorthy,1984; Klamberg et al,1987). These processes determine the concentrations of particular metal ions which are available for interactions with the biota. The immobilisation of heavy metals by precipitation (usually in association with oxidation/reduction) and by adsorption is an important means by which toxic metals are rendered less harmful. For example, several authors have found that heavy metals leached from landfills were attenuated by precipitation and adsorption in the underlying sediments (Mather et al, 1983; Ross,1985; Misund and Sæther,1991). Precipitation reactions appeared to be dependent on changes in pH or Eh, which were determined, for example, by the degree of biodegradation occurring in the landfill waste and by the lithology of the underlying rock (Mather et al, 1983; Ross,1985). It is important to realise, however, that metal ions may also be remobilised. This may be caused by a subsequent reduction in pH due, for example, to the exhaustion of the buffering capacity of the sediment (Ross,1985). Alternatively, it may be brought about by microbial action. Francis and Dodge (1990) have demonstrated that Cd, Cr, Ni, Pb and Zn which were coprecipitated with goethite (iron oxide) were remobilised by the action of an anaerobic *Clostridium* sp. which brought about the reduction of ferric iron.

The study of the toxic effects of heavy metals on ecosystems and the assessment of the pollution potential of heavy metals discharged to the environment is a complex area of research which is discussed in more detail by, for example, Moore and Ramamoorthy (1984).

1.2. Problems related to iron

Iron is not considered to be a potentially toxic heavy metal. Indeed, iron salts are used for a variety of purposes in water treatment (e.g. sludge settling, phosphate and sulphide precipitation, disinfection), primarily due to their lack of toxicity and to their natural abundance (Morper, 1986; Solo and Waite, 1989). Nevertheless, iron can, and does, cause major problems in water supplies due to the formation of iron precipitates. These are formed both chemically and biologically and can cause severe plugging problems in the supply system, as well as turbidity, discolouration and unpleasant odours.

In the Nordic countries, it is estimated that 25% of groundwater may have an excessive iron concentration (Malkki, 1988a). The concentration of iron generally regarded as acceptable in drinking water is 0.1 mg/l, although highest permissible levels can be as great as 1.0 mg/l (WHO, 1971; SIFF, 1987), but this is frequently exceeded for both natural and anthropogenic reasons. For example, it is not unusual in Norway for unpolluted groundwater to contain several mg/l iron (SIFF, 1987).

Iron-rich waters usually occur either due to leaching of iron from bedrock and superficial deposits containing mafic-silicate minerals and iron oxides or sulphides (Vuorinen et al., 1987) or due to leaching from waste tips, particularly wastes from the mining industry, along with other heavy metals. Leaching occurs under acidic, reducing conditions which commonly arise due to microbial breakdown of organic material. Oxidation of organic material consumes oxygen and produces carbon dioxide, thus lowering both the pH and the redox potential (Eh) of the pore water (Seppanen, 1988). At an Eh below about +240mV (Seppanen, 1988), iron is reduced to its soluble ferrous form and is leached from the rock into the surrounding pore water. This is the case in developing peat bogs, where the breakdown of plant material results in leaching of iron from the interface between peat formation and the adjacent mineral material, e.g. granite (Halbach and Ujma, 1978; Carlson et al, 1978). In waste tips, breakdown of organic material may result in leaching of iron from the waste material or from the mineral

material underlying the tip. In either case, iron is mobilised in its soluble ferrous form, with or without other heavy metals. It will usually remain in solution until a sufficient increase in Eh (as may occur on contact with air) and pH causes its oxidation and precipitation as ferric iron. However, if the water also contains a high concentration of humic substances, with which the ferrous ions form complexes, chemical oxidation and precipitation will be hindered. Much Norwegian drinking water is derived from bog-rich catchments. The acidic, reducing conditions prevalent in these, together with the availability of humic substances for complexation, account for many of the high iron levels recorded in such waters.

In the case of iron-rich groundwater, precipitation will usually occur as the water approaches a pumping well; increased oxygenation, which causes an increase in Eh, favours the conversion of ferrous to ferric iron and initiates chemical precipitation of ferric oxides and hydroxides. In addition, the increase in Eh often initiates the development of biofilms of ferromanganese-depositing bacteria at the aerobic/anaerobic interface; these catalyse the oxidation of ferrous iron and become encrusted with iron hydroxides (Tyrrel and Howsam, 1990). Among the most common ferromanganese-depositing bacteria which have been identified in groundwater wells are *Gallionella* sp. which are thought to obtain energy by the oxidation of iron. The development of biofilms of these bacteria depend on sufficient ferrous iron being available and will usually occur under conditions where chemical oxidation of iron is restricted. Aerobic/anaerobic interfaces present ideal sites, as the low oxygen concentration restricts chemical oxidation, but is sufficient to support bacterial growth. Several genera of ferromanganese-depositing bacteria which are not obligate iron-oxidisers also appear to favour low oxygen concentrations, though the reason for this is less clear.

Certain acidophilic bacteria may also cause the precipitation of ferric hydroxides in mine waste waters, where the pH is too low for chemical oxidation to occur. The ferromanganese-depositing bacteria will be discussed in more detail in section 2.3.

The formation of iron precipitates and the proliferation of ferromanganese-depositing bacteria cause turbidity, discolouration and unpleasant odours which are unfavourable in drinking water supplies. In addition, particles of precipitate can block UV-light rays used to disinfect water at some water treatment plants and thus cause an indirect health hazard. The development of iron-encrusted biofilms can rapidly result in blocking of aquifers, filter screens, pumps and pipes,

etc and is believed to be the cause of yield problems at several Norwegian boreholes (Banks,1992). Furthermore, it has been found that sulphate-reducing bacteria are also often associated with the biofilms. These anaerobic bacteria are able to grow in localised anaerobic environments created below the surface of the biofilm and produce sulphides. The reduction reaction consumes hydrogen and thus enhances corrosion of metal parts (Howsam,1988). The overall result is usually deterioration and even complete failure of the supply system. Similar problems have been encountered with heat pumps in regions of iron-rich water (Pedersen and Hallbeck,1985).

Awareness of the role played by bacteria in iron-related problems of water supply has increased significantly over the years, as reflected by the literature dedicated to "biofouling" and the successful maintenance of supply systems (Cullimore and McCann,1977; Cullimore,1987; Howsam,1988; Howsam,1990; Banks,1992). One way to avoid such problems is to effectively remove excessive quantities of iron and manganese from the water, before it reaches susceptible parts of the supply system. Current methods for doing this will be discussed in section 2.2.

1.3. Heavy metal pollution; the Norwegian perspective.

In recent years there has been an increased awareness of problems related to heavy metal pollution of soils, groundwater and surface water in Norway. The potential for such pollution is significant due to the large number of industries which can release heavy metals into the environment (Table 1).

The discharge of polluting effluents is, on paper, controlled by the Pollution Control Act (Forurensningsloven) of 1981, which requires companies with polluting discharges to have a discharge permit. Permits are issued on a case by case basis, which is considered an appropriate approach for the number of companies involved. General guidelines only exist for the electroplating industry. However, although the regulation of discharges may have been improved since 1981, the issue of permits does not guarantee that standards are maintained; in 1987, 50% of companies inspected were found to be out of compliance with the limits set down (Jordfald,1988).

Table 1: Industries in Norway with potential to cause heavy metal pollution.

Industry	Potentially polluting heavy metals used/discharged	Use
Chlor-alkali	Hg	liquid electrodes
Galvo-technic	Cr,Zn,Cu	plating agents
Wood treating	Cr,Cu,As	impregnating agents
Wood pulp	Hg	previously used as fungicide
Mining and smelting	Fe,Cu,Ni	metallic products/biproducts
Shipbuilding	various	hull protection agents, dust from sanding

Where effluents are discharged directly into fjords (large volumes of water with restricted outlets to the open sea) heavy metals often accumulate close to the point of discharge, in the sediments and local biota (Moore and Ramamoorthy,1984). In the past, excessive levels of mercury (90-110 mg/kg) arising from a chlor-alkali factory have been reported in sediments from Gunnekleivfjorden (Skei, 1978). Even greater levels of lead, zinc, copper and cadmium were present in sediments from Sørfjord close to a metal mine (Skei et al.,1972). High levels of the same metals were found in brown algae near to another mine at the head of Hardanger fjord. Metal residues were also found in zooplankton, mussels and cod - reflecting the accumulation of metals within the food chain. (Stenner and Nickless,1974; Skei et al,1976).

Several of Norway's river systems, including the Orkla and Folldal rivers, have been significantly polluted by drainage waters from both operational and disused ore mines (Table 2). Two types of pollution may occur. The first is due to acid mine drainage from waste deposits and tailings, which is characterised by a low pH and high levels of iron, copper and zinc. As the drainage water moves downstream, the pH rises and characteristic ochre deposits are formed. The second type of pollution arises due to return water from the flotation process. This water is characterised by high levels of calcium and sulphate and a pH above 7 (Aanes,1988). The altered chemistry of the river water, together with associated sedimentation and precipitation, can have a significant effect on the river ecosystem.

A recent survey by NGU revealed that heavy metal-containing compounds were among the most common pollutants identified in both municipal and industrial waste landfills in Norway (Misund et al, 1991a,b). At municipal landfills there was, until recently, little control over the types of waste which were deposited. As a result many of these sites contain industrial (and often hazardous) waste as well as domestic waste. However, a study of six Norwegian landfill sites containing mixed domestic and industrial waste has indicated that heavy metal concentrations in the landfill leachates were not significantly higher than those in domestic waste water. The one exception was the concentration of iron (Johansen and Carlson,1976). It is likely that the majority of any heavy metals which were leached from the waste were attenuated in the local sediments. Thus the potential for heavy metal pollution from such sources may be relatively small. A greater problem is likely to be presented by designated hazardous waste sites, in which the amounts of heavy metals deposited will be much larger. The potential hazard due to such sites depends, in part, on their design and construction. Ideally landfills should be designed so as to minimise the amount of leachate which can enter the underlying subsoils and groundwater. Preferably the landfill should have either an impermeable basal lining or a lining of a material, often clay-rich, which will adsorb leached inorganic pollutants. It should also have an low-permeability top covering to prevent water entering the landfill, e.g. via rain and snow, thus minimising the amount of leachate produced. One example of a landfill constructed along these lines is the metal residue disposal site at Sluppen, Trondheim. In this case leakage of heavy metal-contaminated leachate into the surrounding sediments and local aquifer has been successfully prevented by good construction. Unfortunately, however, no provision was originally made for the treatment of heavy metals present in the drainage water. This water is subsequently discharged via the sewage network into Trondheimsfjord (Morland,1990). Furthermore, this leachate has been found to contain relatively high levels of nickel and zinc (4.13 mg/l and 128.3 mg/l respectively) (Misund, 1989). Many of the other landfills surveyed by NGU appear to have been constructed without any consideration of their potential for polluting water supplies, even though many are sited on sand and gravel deposits which could represent future drinking water resources.

Elevated levels of heavy metals may also occur naturally (Table 2) or as a secondary result of soil acidification. Soil acidification may arise due to acid rainfall or due to reforestation of land previously used for agriculture. In either case, acidification of the soil can result in increased mobilisation of heavy metals. In areas where sewage sludge is applied to land, the potential for

acidification and subsequent remobilisation of any heavy metals present should always be considered (Andersen,1991).

Given the importance of Norway's fishing and other water-related interests, as well as the damage which may be caused to the environment as a whole, potential pollution of Norway's water resources by heavy metals is a major concern. In order to minimise pollution, discharges to the environment must be controlled. The issue of discharge permits and the disposal of hazardous waste in specially designated, suitably constructed sites goes some way towards this. To ensure that discharge permits are complied with necessarily requires routine self-monitoring by the industries themselves as well as regular inspections and penalisation when limits are exceeded. At the same time, the development and introduction of efficient and economical technologies for recycling or disposing of waste metals should be encouraged.

Table 2: Specific examples of heavy metal pollution in Norway.

1. Natural "pollution",
e.g. Ni derived from Kautokeino greenstone belt, N.Norway (Bølviken,1986)
2. Airborne pollution from smelting works,
e.g. Ni pollution on Norwegian-Russian border, derived from Soviet smelting works (Bølviken,1986; 1990)
3. Industrial effluent/leakage/leachate from industrial activity, e.g.
 - (i) Hg contamination derived from Hydro Porsgrunn (chloralkali) plant on Herøya (Skei,1978; Loken et al.,1988),
 - (ii) suspected heavy metal-contamination of groundwater due to leakage from the closed-down Røros Chrome electroplating works (Morland,1990)
4. Mine drainage waters/leachate from mine waste tips, e.g.
 - (i) pollution of the Folla river by high levels of Fe as well as Cu and Zn, particularly due to acid mine drainage from disused mines (Aanes,1988;Johannessen,1988),
 - (ii) pollution of the Orkla river by copper and zinc (Grande and Arnesen,1988),
 - (iii) pollution of the Gaula river by copper and zinc due to acid mine drainage (Johannessen,1988)
5. Leachate from landfill sites, e.g.
 - (i) high levels of Fe in leachate from domestic landfills (Johansen and Carlson,1976),
 - (ii) high levels of nickel and zinc in drainage water from Sluppen hazardous waste site, Trondheim (Misund,1989)

2. Heavy metal removal by microbially-mediated methods.

Microorganisms, such as fungi, yeast, algae and bacteria, are capable of accumulating large amounts of heavy metals and radionuclides from their environments. They do so by a variety of mechanisms which include metabolism-independent (i.e. passive) adsorption as well as processes which are dependent on metabolism (i.e. active) (Gadd,1990; Brierley,1990). A great deal of research effort has been applied, in recent years, to investigating the potential use of microbes, or products derived from them, for the treatment of water contaminated by unacceptably high levels of heavy metals (Lundgren and Malouf,1983; Hutchins et al,1986; Eccles and Hunt,1986; Gadd,1988; Ehrlich and Brierley,1990; Gadd,1990). Most research has been directed towards the treatment of industrial waste waters to remove and recover valuable metals (e.g. Au, Ga) or radionuclides. Much less attention has been paid to removal of less valuable heavy metals (e.g. Pb,Cu,Zn) which may be discharged in huge quantities (Gadd,1990) or to the treatment of water contaminated, for example, by leachate from landfill sites, which may contain complex mixtures of different heavy metals.

There is, however, increasing evidence that microbial technology can be both economic and competitive in comparison with traditional treatment methods, such as ion exchange, used in the mining and metallurgy industries (Brierley et al,1986; Whitlock,1989; Gadd,1990) and a number of microbial systems have already been employed or assessed commercially. These include large-scale systems such as biofilm-covered rotating biological contactors to treat lead-mining effluent (Whitlock,1989), the removal of iron from acid drainage waters, using rotating biological contactors or sequencing batch reactors (Olem and Unz,1977; Theis and Engelmann, 1982) and the treatment of lead/zinc smelting effluent by bacteria and algae in artificial switchback channels (Wixon,1985; Gale,1985; Hutchins et al.,1986). Two smaller scale immobilised microbial systems which have been successfully developed are the AMT-Bioclain system (Brierley et al,1986) and the AlgaSORB system (Barkley,1991) -both of which are based on "biosorption", i.e. the passive adsorption of metals by biological material -in this case, by microbial cell walls.

This report will focus on heavy metal immobilisation and removal from aqueous systems by bacteria. For further discussion of other aspects of microbially-mediated metal removal

processes, including algal and fungal systems, the reader is referred to the literature previously cited.

2.1. Heavy metal immobilisation by bacteria.

Modern bacteria play a central role in the cycling of major elements including the metals, Fe, Mn, Cu, Zn, and Co (Zajic, 1969; Olson, 1983, McLean and Beveridge, 1990) and are thought to be important in controlling fluxes of metal ions in aqueous environments (Geesey and Jang, 1990). The occurrence of fossil bacteria in ancient sediments -usually associated with metal deposits- has led to speculation about the bacterial origin of such deposits (Beveridge, 1978; Muir, 1978; McLean and Beveridge, 1990) and the possible role of ferromanganese-depositing bacteria in the formation of deep-sea nodules has received particular attention (e.g. see Krumbein, 1978).

Although all bacteria require low levels of metals such as Fe, Co, Cu, Mo, Mn, Ni and Zn, as essential inorganic nutrients (McLean and Beveridge, 1990; Gadd, 1990), most of these can rapidly become toxic over a relatively narrow concentration range. Nevertheless, bacterial populations do exist in waters where levels of these metals and of metals with no physiological function (e.g. Cd, Hg), undergo significant variation and may greatly exceed toxicity thresholds. One mechanism which enables bacteria to survive under such conditions is their ability to immobilise metals, by a variety of methods, on the outside of the cell. Entry of excessive amounts of unwanted metals into the cell is thereby prevented, enabling the cell to function normally, even in potentially toxic environments (McLean and Beveridge, 1990; Ghiorse, 1986). External immobilisation may be achieved by a number of methods including: (i) direct binding of metal ions to the cell wall, (ii) precipitation and (iii) extracellular complexation.

2.1.1 Metal binding

The structure of bacterial cell walls, in relation to metal binding, has been discussed in detail by McLean and Beveridge (1990), but some of the main principles will be summarised here: Firstly, most bacteria possess cell walls which are anionic and thus able to attract cationic metals. Gram-positive bacteria (mainly *Bacillus* sp) possess a cell wall consisting of a "thick network" of peptidoglycan with attached molecules of teichoic acid and teichuronic acid. The phosphodiester groups of teichoic acid and the carboxylate groups of teichuronic acid make an

important contribution to the ion-exchange capacity of the cell wall (Brierley, 1990; McLean and Beveridge, 1990). In contrast, Gram-negative bacteria have cell walls comprised of a thin layer of peptidoglycan sandwiched between two membranes. A major component of the outermost membrane is lipopolysaccharide, which is unique to Gram-negative bacteria and which, along with phospholipids in the membrane, is able to bind metal ions. The contribution of peptidoglycan to metal binding in these bacteria is probably quite small, given that it is less accessible than in the case of the Gram-positive cell wall.

Binding of metal ions to bacterial cell walls requires the metal ions to be in solution and is affected by the same physico-chemical factors as the binding of metal ions to inorganic surfaces. Although any metal ions in solution in an aqueous environment will tend to concentrate on surfaces (the "interfacial effect"), adsorption is both pH dependent and affected by the presence of chelating agents, e.g. EDTA, humic substances, which may be present (Davis and Leckie, 1978; McLean and Beveridge, 1980). However, bound metal ions can also act as nucleation sites for the precipitation of additional metal species, resulting in greater amounts of metals being immobilised than might otherwise have been expected (Beveridge et al, 1983; Ferris et al, 1989).

2.1.2 Precipitation

Precipitation of metal ions extracellularly may be brought about by bacteria in several ways. According to Brierley (1990), precipitation is where a substance produced by the bacterium reacts chemically with a metal in solution to form an insoluble metal compound. A typical example of a bacterium which does this is the H₂S-producing bacterium *Desulphovibrio*. The H₂S produced when the bacterium reduces sulphate, reacts with metal ions to form insoluble metal sulphides. The activity of these and other species of sulphate-reducing bacteria is observed in natural anaerobic environments such as swamps and anoxic soils, and has been utilised in man-made impoundments created for the removal of soluble metals from acid mine drainage waters (Brierley and Brierley, 1980). It is also an important component in artificial wetlands, which are now being considered as a potential method for reducing the acidity and the levels of metal ions present in run-off from abandoned mines and associated solid mine wastes (Brierley, 1990).

Precipitation of metal ions may also be brought about either by the utilisation of the organic parts of organic-metal ion complexes or by oxidation. Both of these mechanisms are important in the precipitation of iron and manganese by certain ferromanganese-depositing bacteria, which will be discussed in the next section.

2.1.3 Extracellular complexation

Extracellular complexation is where substances capable of metal binding are either excreted or produced extracellularly by the bacterial cell. The substances may be (i) low molecular weight chelating agents, such as siderophores, or (ii) high molecular weight polymers, including polysaccharides and proteins (Brierley,1990).

(i) Siderophores

Siderophores are produced by certain Gram-negative bacteria, such as *Pseudomonas* and *Arthobacter*, when these bacteria encounter an iron-poor environment, and form complexes with ferrous ions present in solution. The complexes subsequently bind to specialized proteins in the outer membrane and the iron is transported into the cell. Although this is obviously a mechanism for obtaining essential iron (rather than a means of protecting the cell against excessive iron levels) characterisation of siderophores, which are usually catechol or hydroxamate derivatives, has led to patenting of the synthesis of a series of similar molecules. These have been used to treat aqueous nuclear waste and other industrial waste waters containing toxic heavy metals (Holbein et al,1984; DeVoe and Holbein,1985).

(ii) Extracellular polymers

Observations of a wide variety of natural microbial habitats have shown that bacteria frequently grow in flocs or in biofilms, consisting of a matrix of extracellular polymers surrounding both live and dead cells (Costerton et al 1978;Scott et al, 1986; Geesey and Jang,1990). The extracellular polymers may form definite capsules (also known as sheaths) in which the polymers are closely associated with the cell surface, or may form a "slime", in which the polymers are more loosely associated with the cells and can readily slough off the cell into the external medium. The term "slime" is often also used, particularly in engineering circles, to refer to biofilms (i.e. extracellular polymers plus cells) which develop on submerged surfaces.

Geesey and Jang (1990) have discussed metal binding by bacterial extracellular polymers in detail. It has been suggested by several authors that the presence of extracellular polymers effectively detoxifies the cell's environment by maintaining a "buffer" zone between the cell and the surrounding medium. This buffer zone provides physical protection for the cell and defends it against excessive intracellular uptake or direct cell surface adsorption of toxic substances (Scott et al,1986). It must, however, also allow the uptake of essential ions.

Bacterial capsules or slime generally consist of polysaccharides. Large amounts of protein, as well as some nucleic acid material are also often recovered in crude extracellular polymer preparations. It is not clear whether the nucleic acids make any contribution to metal binding (Geesey and Jang,1990), although their presence in bacterial slime is presumably inevitable, as they will be released due to the lysis of dead cells. There is, however, no doubt that strains of bacteria which produce capsules or sheaths, are able to accumulate greater concentrations of metal ions on their exterior surfaces than those which do not. This has been shown for *Zoogloea ramigera*, the ubiquitous sewage treatment organism (Friedman and Dugan,1967) and for species of *Arthobacter* (Scott et al.,1986).

Adsorption of heavy metals by extracellular polymers is an important aspect of activated sewage treatment. Although the activity of the bacterial population can be inhibited by the presence of too high concentrations of heavy metals, the sewage sludge bacteria can cause a significant decrease in the levels of heavy metals discharged in the treated effluent. The extracellular polymers produced adsorb soluble metal ions and enable flocculation of insoluble one. This has been particularly studied by, for example, Brown and Lester (1979), Rudd et al. (1984) and Sterritt and Lester (1986). Adsorption of metal ions to the extracellular polymers present is affected by a number of factors including the type of metal ion (Sterritt and Lester found that nickel is not as readily adsorbed as copper and zinc), the relative concentrations of different metal species present, the concentration of organic chelating substances (e.g. humic and fulvic acids) and sludge age. As mentioned previously, however, high levels of heavy metals adsorbed by the sludge may not only have an adverse effect on the viability of the sewage bacteria but can also render the sludge too toxic for "disposal" by use on agricultural land (Morper,1986; Andersen,1991).

Several workers have studied the adsorption of metal ions by mono-cultures of *Zoogloea ramigera* in more detail, with a view to the feasibility of also recovering the metals (Norberg and Persson, 1984; Norberg and Rudin, 1984; Kuhn and Pfister, 1989). It is apparent that metal immobilisation by mono-cultures is much more efficient than by the mixed cultures present in sewage sludge, partly because the latter may include species with little metal immobilising ability (Macaskie and Dean, 1989). In the case of *Zoogloea ramigera*, it has also been shown that the production of metal-adsorbing polymer can be maximised, for a mono-culture, by adjusting the growth conditions (Norberg and Enfors, 1982).

The mechanisms described above include both active and passive processes. Immobilisation of metals by precipitation, whether via a chemical precipitant, oxidation, or in association with degradation of organo-metallic complexes, requires the presence of actively metabolising cells. Extracellular complexation also requires the presence of live cells to produce the complexing agents, although, in the case of extracellular polymers, the subsequent metal adsorption takes place passively and could therefore occur in the absence of metabolising cells. Adsorption of metals by bacterial cell walls is a passive process and therefore does not require the bacterial biomass to be living. The passive processes of adsorption, whether by cell walls or by extracellular polymers are examples of "biosorption". Interest in the potential of biosorption-based systems, using bacterial or other microbial material, for the treatment of water polluted by heavy metals has existed for some time and is on the increase. It is a phenomenon which is likely to have a key role in many systems developed for heavy metal removal in the future.

2.2 Microbial methods for the removal of iron from groundwater.

Removal of dissolved iron and/or manganese from groundwater requires oxidation, with the subsequent precipitation of insoluble ferric and manganic oxides/hydroxides. The simplest method must be the overland run-off method, which was still in use in 1988, and in which oxidation occurs due to natural aeration (Malkki, 1988a). The main problems associated with this method are the large surface area required and the fact that the water being treated is open to the air and therefore exposed to all atmospheric pollutants, both chemical and biological.

Oxidation may also be brought about chemically, in water treatment plants, by the addition of chemicals such as potassium permanganate (KMnO_4), chlorine dioxide (ClO_2) and ozone (O_3). Following flocculation of the precipitated hydroxides, the flocs are separated from the treated water by, for example, the use of filters. However, the most interesting methods, from the point of view of this report, are those which exploit the ferromanganese-depositing bacteria, i.e. the organisms which cause many of the iron-related problems in the first instance.

A number of different "biological" processes for the removal of iron from groundwater have already been in operation in different countries for many years. These include: (i) slow/rapid sand filtration (Czekalla et al., 1985; Malkki, 1988a and b; Hatva, 1988; Seppanen, 1988; Lahermo and Vuorinen, 1988), (ii) re-infiltration (Agerstrand, 1979; Malkki, 1988a) and (iii) the Vyredox, VYR or recharge method (Hallberg and Martinell, 1976; Rott, 1981; Braester and Martinell, 1988; Rott, 1990).

In sand filtration, aerated water is passed through one or more filters. Populations of ferromanganese-depositing bacteria develop within the filters and cause the precipitation of ferric hydroxides. Manganese oxides may also be precipitated. The precipitates are retained within the filters and ultimately result in clogging, at which point the filters are cleaned, often by back-flushing. The rate at which slow sand filters become blocked can be reduced, and the overall efficiency of iron removal improved, by the introduction of pre-treatment units. These are also biological filters incorporating ferromanganese-depositing bacteria, and may take a variety of forms, some of which are discussed in the references given above. Simple filters, involving aeration followed by oxidation within a gravel filter, have also been used on a small scale to remove iron from water supplies in rural communities (Chibi, 1991).

Re-infiltration is a method which was originally used in the 1950's and was further developed in Sweden by Agerstrand (1979), with Nordic conditions in mind. It is essentially based on pre-oxidation followed by slow sand filtration. The groundwater is aerated, pre-oxidised in a surface unit, passed into two basins where slow sand filtration takes place and finally re-infiltrated further into the aquifer, where it is stored before being abstracted using conventional wells.

The Vyredox method was developed in Finland at the end of the 1960s. Oxygen-enriched water is injected into the aquifer, thus creating an oxidised zone around the well. Ferrous iron is

oxidised both chemically and biologically and the precipitated hydroxides are immobilised within the oxidised zone. When pumping to abstract water is underway, groundwater is filtered through this zone. Any soluble ferrous ions present are adsorbed onto both the oxidised precipitates and bacterial surfaces and are subsequently also oxidised.

One major concern with the Vyredox method, is whether or not such treatment is likely to result in the aquifer effectively becoming clogged after a certain length of time. Braester and Martinell (1988) maintain that as long as the oxygenated zone is developed properly and the treatment plant is run correctly, the pore volume of the aquifer is sufficiently large to only be affected very slowly by the accumulation of precipitates (see also Hallberg and Martinell, 1976). They describe a plant which had been in successful operation for five years, with no reported decrease in yields from the production well. However, a more critical factor for the abstraction of groundwater is the permeability of the aquifer. This is largely governed by the size of the "bottlenecks" between pore spaces. The aperture of these bottlenecks is significantly smaller than that of the total pore volume and may thus become clogged more rapidly than the pore volume as a whole. It is therefore possible that the aquifer may be adversely affected by hydroxide precipitation rather sooner than expected (D. Banks, personal communication).

Another problem can arise if the aquifer contains a high organic carbon content. In this case, a significant amount of oxygen may be consumed in the oxidation of organic material and less oxygen is available for the oxidation of iron. The use of the Vyredox method at Sunndalsøra has been of limited success for this reason (Aura Avis, 1991).

The involvement of ferromanganese-depositing bacteria in the processes above has been demonstrated and discussed in the references cited. It is now appropriate to describe these bacteria in more detail.

3. The ferromanganese-depositing bacteria.

The ferromanganese-depositing bacteria include bacteria which oxidise ferrous and/or manganous ions, resulting in the deposition of ferric /manganic salts (usually hydroxides) within the cell or cell coating, as well as a large number of species which cause the deposition of iron and manganese salts by a variety of methods other than oxidation. In either case, the iron/-

manganese precipitates formed typically impart an orange-brownish colour to the bacterial growths, which frequently occur as biofilms (Cullimore and McCann, 1977).

The main genera of metal-oxidising bacteria include *Gallionella*, *Leptothrix* and *Sphaerotilus*. In addition, one species of *Thiobacillus* (*Thiobacillus ferrooxidans*) and *Metallogenium* spp. oxidise iron/manganese, though under more acidic conditions than members of the previous genera.

3.1 *Gallionella*

Gallionella spp. are widely reported as inhabitants of water wells and pipes throughout the World, often occurring as part of a mixed bacterial population. They have also been identified in Norwegian hydroelectric power plant systems and in undersea tunnels (Cullimore and McCann, 1977; Smith and Tuovinen, 1985; Banks, 1991). These Gram-negative bacteria are characterised by rod- or "bean-" shaped cells, each of which excretes a twisted stalk comprised of fibrils of ferric hydroxide and extracellular polymeric material. Although they are usually isolated from biofilms, it appears that, in their exponential phase, the bacteria exist as flagellated, motile forms. They only become sessile and produce the characteristic stalks during their stationary phase or when their growth is retarded due to one or more environmental factors becoming unfavourable (Hallbeck and Pedersen, 1987). By adopting a sessile form, the bacteria are constantly supplied with fresh nutrients as the water flows past them; the stalks may act as "Archimedian-screws", causing nutrient-bearing water to spiral down to the sessile cell (Cullimore, 1990).

Environmental factors which are said to favour growth of *Gallionella* biofilms are: a supply of ferrous ions (5 to 25 mg/l), a low oxygen level (0.1 to 1 mg/l), a pH between 5.5 and 7.6, a redox potential between +200 and +320 mV, a temperature between 8 and 16°C (although they tolerate lower temperatures) and the presence of dissolved CO₂ (>20 mg/l) (Hanert, 1981a; Smith and Tuovinen, 1985; Pedersen and Hallbeck, 1985). The redox potential is the main determining environmental factor, as it is this that determines the level of ferrous iron present and ferrous iron is essential for *Gallionella*'s growth (Hanert, 1981a): If the level of oxygen present rises significantly, resulting in a rise in the Eh, increased chemical oxidation of ferrous iron will occur. This will reduce the concentration of ferrous iron available to the bacteria and their growth may be restricted.

It is generally believed that *Gallionella* are chemoautotrophic, obtaining energy by the oxidation of ferrous iron and assimilating carbon dioxide. This has not been proved conclusively but the bacteria certainly thrive in environments containing very little dissolved organic carbon. Two groups of workers (Hallbeck and Pedersen, 1987; Ghiorse, 1987) have observed the presence, within *Gallionella* cells, of inclusions resembling carboxysomes, i.e. polyhedral bodies of ribulose-1,5-diphosphate, which are essential for CO₂ fixation. Hanert and co-workers have found two other key enzymes, ribulose biphosphate carboxylase and phosphoribulokinase, present in a *Gallionella* strain (Ghiorse, 1987). All of these observations support the idea of autotrophic nutrition for *Gallionella*.

The ability of *Gallionella* to thrive in environments containing little organic carbon means that, in plants/processes designed for the microbial removal of iron from groundwater, these bacteria are usually the first to proliferate (e.g. Hatva, 1988).

3.2 *Leptothrix* & *Sphaerotilus*

Sphaerotilus and *Leptothrix* are usually considered together as the two genera are very similar. Both are Gram-negative, filamentous sheathed bacteria capable of oxidising ferrous iron and, in the case of *Leptothrix*, manganese. Unlike *Gallionella*, these bacteria are heterotrophs and require an organic source of carbon. However, whereas *Sphaerotilus* requires a high level of dissolved organic carbon and, in fact, is a common inhabitant of sewage sludge, *Leptothrix* prefers a relatively low organic carbon concentration (<2ppm, according to Cullimore, 1990). *Leptothrix* is often one of the early colonisers in iron removal plants, along with *Gallionella*.

The sheaths of these bacteria consist of carbohydrate, protein and lipid and accumulate iron and manganese oxides. Work carried out by Ghiorse (1987) on *Leptothrix* has shown that manganous, and possibly ferrous, ions are oxidised extracellularly by a protein, or proteins, with no energy gain to the cell. Furthermore, manganous ions (> 500mM) inhibit the growth of *Leptothrix* cells, suggesting that the role of manganese oxidation is detoxification.

Other environmental conditions which have been quoted for the growth of these bacteria include, a requirement for calcium (for sheath formation), a ferrous iron concentration between 0.25 and 14 mg/l (greater concentrations are inhibitory), pH 5.4 to 8.0 and a low concentration of dissolved oxygen (Smith and Tuovinen, 1985).

3.3 *Thiobacillus ferrooxidans*

Thiobacillus ferrooxidans is a unique bacterium which obtains energy by oxidising ferrous iron whilst simultaneously oxidising inorganic sulphur compounds. Furthermore, its chemoautotrophic nature has been conclusively shown (Tuovinen and Kelly, 1972). Unlike *Gallionella*, which requires a relatively neutral pH, *Thiobacillus ferrooxidans* is an acidophile, thriving in a pH range of 1.5 to 3.0. This, together with its requirement for inorganic sulphur (e.g. sulphides) and ferrous iron means that it is a frequent inhabitant of acid mine drainage waters but does not usually occur in aquifers or in water wells. The activity of *Thiobacillus ferrooxidans* has been utilised in systems designed to remove excess ferrous iron from such waters (Olem and Unz, 1977; Theis et al., 1982). The oxidation of ferrous iron is an aerobic process and occurs on the cell surface, with electron transfer ultimately to a cytochrome oxidase in the cell membrane (Norris, 1990).

Thiobacillus ferrooxidans also plays an important role in the leaching of metals from sulphidic minerals such as pyrite, chalcopyrite and uraninite. Further information on bacterial leaching can be found in articles by Lodi et al (1989), Tuovinen (1990), and Merson (1992).

3.4 *Metallogenium*

Metallogenium spp. catalyse ferrous iron and manganese oxidation in the pH range 3.5 to 5.0, thus filling an ecological niche between the acidophilic *Thiobacillus ferrooxidans* and bacteria, such as *Gallionella*, *Leptothrix* and *Sphaerotilus*, which prefer a more neutral pH. Although not usually discussed in relation to ground water, they could occur in ground water near to coal seams (Smith and Tuovinen, 1985). According to Hatva (1988) and Seppanen (1988), *Metallogenium* have been found below the surface of filter sand in slow sand filters used for iron removal from groundwater. If these are the same organisms as those referred to by Smith and Tuovinen, it may be that localised regions of reduced pH occur in the filters due to the decay of other bacterial material.

3.5 Other bacterial species

Other bacteria associated with iron deposition are members of the genera *Siderocapsa*, *Pseudomonas*, *Arthobacter*, *Pedomicrobium*, *Hyphomicrobium* and *Crenothrix*, to name a few (Cullimore and McCann, 1977; Smith and Tuovinen, 1985; Ghiorse, 1986).

Siderocapsa spp. frequently occur in ground water (Hanert, 1981b). These non-filamentous, slime-encapsulated bacteria apparently cause the precipitation of iron by utilising the organic part of iron/manganese humates. They may have potential for use in iron-removal plants where significant amounts of these complexes are present (Seppanen, 1988).

Pseudomonas and *Arthobacter* spp. have also been shown to cause iron precipitation by utilising the organic parts of organic-iron complexes (Macrae et al., 1973), rather than specific oxidation of ferrous iron. *Pseudomonas* is found widely in ground water and is increasingly implicated in plugging of water wells by iron-containing slimes formed when the precipitated metal oxides are adsorbed by the bacterial surfaces. The siderophores mentioned in section 2.1 are also produced by certain species of these genera.

Pedomicrobium and *Hyphomicrobium* spp. are hyphal budding bacteria, most of which deposit iron oxides, though not all deposit manganese oxides. Ghiorse (1986) found that the oxides were deposited on extracellular acidic polymers. Furthermore, he found that manganese oxide deposition appeared to involve an extracellular enzyme, but that the deposition of iron oxides did not.

Crenothrix polyspora is a sheathed bacterium and is included here as it is often dominant among bacterial populations in water wells suffering from plugging by ferromanganese-depositing bacteria. However, according to Ghiorse (1987), it does not itself deposit either iron or manganese extensively and is likely to be a methane-oxidising bacterium. Nevertheless, it will contribute to plugging problems due to the large filamentous aggregates which it forms.

Table 3 summarises some of the main features of the ferromanganese depositing bacteria.

3.6 Summary

In summary, the main mechanisms by which the ferromanganese-depositing bacteria discussed above are known to, or are likely to, cause iron/manganese precipitation, are: (i) intracellular oxidation, (ii) oxidation by an extracellular enzyme/protein, (iii) precipitation, without bacterially-mediated oxidation, following utilisation of organic-metal ion complexes. These are all active mechanisms as they require active metabolism by living cells and they each bring about what may be termed "primary precipitation". However, equally important is secondary

Genus	Chemoautotrophic (C) or heteroautotrophic (H)	Preferred pH range	Other comments
Gallionella	C	5.5 - 7.6	Oxidises Fe ^{II} ; microaerophilic; common in biofilms in water supply systems, iron removal plants, hydroelectric power plants.
Leptothrix	H	5.4 - 8.0	Requires low concentrations of soluble organic C; oxidises Mn ^{II} and Fe ^{II} extracellularly; microaerophilic; often in biofilms together with Gallionella.
Sphaerotilus	H	5.4 - 8.0	Similar to Leptothrix, but requires high concentration of soluble organic C; common in sewage sludge.
Thiobacillus ferroxidans	C	1.5 - 3.0	Oxidises Fe ^{II} and inorganic S compounds simultaneously; aerophilic; frequent in acid mine drainage waters.
Metallogenium	H	3.5 - 5.0	Oxidises Fe ^{II} and Mn ^{II} .
Siderocapsa	H	-	Utilises organic part of Fe/Mn humates; common in ground water.
Pseudomonas Arthobacter	H H	-	Utilise organic parts of Fe/organic complexes; some species produce siderophores. Pseudomonas common in groundwater.
Pedomicrobium Hyphomicrobium	H H	-	Most deposit Fe oxides, some deposit Mn oxides.

Table 3. Main features of different genera of ferromanganese depositing bacteria.

precipitation/oxidation, in which further iron/manganese ions become adsorbed and are subsequently oxidised chemically (e.g. Czekalla et al. 1985). The additional ions may be both adsorbed by the primary precipitate and "biosorbed" by the polymeric sheath/slime material enveloping the bacterial cells. Adsorption of transition metals to oxides and hydroxides, especially those of Al, Fe and Mn, is a well known phenomenon (Drever, 1988; Francis and Dodge, 1990) and a number of workers have investigated the potential for removal of heavy metals from waste waters via adsorption on chemically precipitated iron oxides (Benjamin et al., 1982; Schultz et al., 1987). Biosorption of metals was described in section 2.1. In either case, the bacterial material/hydroxide deposits effectively act as catalytic surfaces, so that the rate of secondary oxidation is enhanced, resulting in the rapid accumulation of further precipitates.

4. The potential for the use of ferromanganese-depositing bacteria in the treatment of groundwater contaminated by heavy metals.

As has been discussed in the previous sections, ferromanganese-depositing bacteria bring about the precipitation of iron and manganese from water by a variety of mechanisms. Their ability to do this can give rise to severe problems in water supplies but has also been exploited in water treatment plants designed to reduce iron and manganese concentrations to acceptable levels. Primary precipitation of iron/manganese oxides and hydroxides is accompanied by rapid secondary precipitation as further ions are immobilised by adsorption to both the precipitates and to the bacterial material (biosorption). However, it is not only iron and manganese ions which become adsorbed in the secondary phase. Ghiorse (1986) states that the manganese oxide precipitate, deposited by biofilms of bacteria such as *Leptothrix*, *Pedomicrobium* and *Hyphomicrobium*, will coprecipitate other metals present in solution. This would result in the selective accumulation of manganese and the non-selective accumulation of other metals. In the presence of iron, the iron hydroxides deposited would coprecipitate other metal ions non-selectively. Furthermore, one would also expect biosorption of other metal ions to the extracellular polymeric material present in the biofilm.

Analysis of the metals present in biofilm samples collected from the Hvaler tunnel in southern Norway (Banks et al, 1992a,b) provide evidence that biofilms of ferromanganese-depositing bacteria do indeed accumulate metals other than iron and manganese (see Appendix 3). The biofilms, in which the main bacterium was *Gallionella ferruginea*, were found growing on the tunnel wall at sites where leakage of seawater into the tunnel was occurring. An initial study showed that the concentrations of iron, lead, molybdenum, vanadium and cadmium, were significantly greater than the concentrations of these metal ions in the leakage water. The accumulation factors for Fe, Pb, Mo, V and Cd respectively were: 50000, > 120, > 100, > 160 and > 105 (Banks,1991). In a later study, the maximum accumulation factors for Fe, Mn, Cu, Zn, Ba, Ti and Cr were: >3440000, 105, 840, 1650, 176, 1130 and >49. In addition, Zr, Be and La had high accumulation factors with maximum values of >2000, > 1100 and >470, respectively (D.Banks, 1991, unpublished data). In all cases, the accumulation factors given were calculated relative to those for ions assumed to be "non-adsorbed", such as Na.

The biofilms analysed in the above studies were clearly accumulating a mixture of metal ions from solution (though in the case of Pb some may also have come from petrol fumes). This suggests that the use of such bacteria in the treatment of iron-rich waters (see 2.2), might be extended to the treatment of non-acidic waters contaminated by other heavy metals. For waters with a low organic carbon content, *Gallionella* or *Leptothrix* spp. could be utilised, whilst for waters with a higher organic carbon content, species such as *Sphaerotilus*, *Pseudomonas* and *Siderocapsa* spp. might be used. For acidic waters containing little organic carbon, *Thiobacillus ferrooxidans* has potential; it has already been used in the treatment of iron-rich mine waste-waters (Olem & Unz, 1977; Theis et al, 1982) and has been found to be tolerant to relatively high levels of metals such as zinc, nickel, copper, cobalt and aluminium (Cullimore and McCann,1977). However, acidic effluent would need to be effectively neutralised, for example by limestone treatment (Theis et al, 1982) following heavy metal removal.

Given that many of the ferromanganese-depositing bacteria are ubiquitous in aquifers and water supplies, including those of the Nordic lands, the simplest approach to treatment of contaminated water may be to design a system in which the indigenous bacterial population is able to proliferate. This is effectively what happens in biological filters designed for iron removal. Similarly, in the pilot study undertaken by Olem & Unz (1977), removal of iron from acid mine drainage waters was performed by an indigenous population of *Thiobacillus ferrooxidans* which

colonised the surface of rotating biological contactors immersed in the water. The same approach has more recently been used in a system designed for the treatment of mine waste waters contaminated by cyanide and heavy metals (Whitlock, 1989). In this case, the indigenous bacteria, which formed biofilms on a series of rotating biological contactors, were mainly *Pseudomonas* spp.. The system was self-regulating, i.e. the bacterial population changed as periodical changes in the waste water components occurred, so that performance was more or less maintained over five years of operation. Changes in the bacterial population are also observed in iron removal plants, the first bacteria to populate the filters being chemoautotrophs (e.g. *Gallionella*), with heterotrophs only appearing at a later stage, when the level of organic carbon has increased due to the decay of other bacterial cells (Hatva, 1988). In theory, a stable population would eventually develop, however the clogging of filters prevents continual operation of individual filters over a long period. In the design of any heavy metal removal plant, the potential for rapid clogging would need to be considered and, preferably, minimised.

Other factors which need to be considered when assessing the potential use of ferromanganese bacteria in a system for heavy metal removal include:

- the maximum heavy metal concentrations in water that can be tolerated by the biofilms, before toxicity effects adversely affect metal removal,
- the effect, on metal removal, of the presence of organic chelating agents, such as EDTA, humic acids, and of other cations which commonly occur in water, such as Ca^{2+} and Mg^{2+} ,
- the overall efficiency of metal removal, the mode of disposal of the immobilised metals and whether metal recovery is required.

If ferromanganese bacteria are to be used for the treatment of heavy metal-contaminated water, it is important that the biofilms are not adversely affected by the concentrations of heavy metals likely to be present in the water. Although accumulated metal ions may cause toxic effects in localised regions within the biofilm, these are unimportant as long as the overall growth and integrity of the biofilm does not deteriorate. Within an established bacterial population toxic effects could be expected to result in increased cell death. This would result in degradation of both cellular and extracellular organic material and would be accompanied by the release of

bound metal ions back into the water. Such would be the case if an established bacterial system was subjected to a heavy metal load which exceeded its tolerance levels for any length of time. Of course, if the heavy metal load initially exceeded tolerance levels, a healthy biofilm would not develop in the first place. It is therefore important that the tolerance levels of the ferromanganese-depositing bacteria, for heavy metals in solution, are determined. Tolerance levels will vary for different bacterial species and for different metals.

As has been mentioned earlier (2.1), heavy metal immobilisation by bacteria can be adversely affected by complexation of the metal ions with organic chelating agents. This will be particularly relevant in the treatment of waters containing significant levels of, for example, humic acids. The extent to which such substances decrease metal accumulation by ferromanganese bacteria should therefore be investigated. *Siderocapsa*, *Pseudomonas* and *Arthobacter* spp. may be of particular interest in this situation, as they are known to precipitate iron by their utilisation of the organic part of iron-organic complexes (2.3). It may be that they are also able to utilise other metal-organic complexes.

Commonly occurring cations, such as Ca^{2+} and Mg^{2+} , may also compete with heavy metal ions for binding sites on the bacterial biofilms. Although the results from the Hvaler tunnel studies suggested that these ions were not adsorbed to any extent, it may still be necessary to assess their effect if they are likely to be present in significant concentrations in any given system.

Any assessment of whether or not a system based on ferromanganese-depositing bacteria has realistic potential for heavy metal removal will depend on the efficiency with which it is able to achieve this. Ideally one would hope for >90% metal ion removal but certainly that metal ion concentrations would be reduced to below the levels considered as permissible by the legislative bodies. The capacity of the bacteria to accumulate metal ions from solution will be affected, not only by the factors mentioned above but also by design factors, particularly the area of biofilm surface exposed to the solution and the contact time between the solution and the biofilm. From this point of view, the pros and cons of different systems, such as rotating biological contactors and biological fluidised beds, need to be considered, along with rates of flow and the system's dimensions. These are essentially the engineering aspects of the problem and some discussion of them may be found in Brierley et al. (1986), Macaskie and Dean (1989) and Brierley (1990). One should also consider what should happen to the heavy metals once they have been removed

from the water and are bound to the bacterial biomass. The alternatives are, either to contain the used biomass-plus-metals in a specially-designed site, where the potential for re-leaching of the metals is inhibited, or to recover the metals from the biomass so that they can be re-used. Recovery of metals is the usual choice for valuable metals and much of the research into biosorption has been undertaken with the ultimate aim of recovery. It can be achieved by incineration (though this may have the undesirable effect of volatilising certain metals and thus redistributing them aerially) or by either biological or chemical reduction (Morper, 1986; Ghiorse, 1986).

Whether or not a technically sound metal recovery process can be put into operation usually depends on its economic viability. Hence the attention that has been previously paid to the use of biosorption to recover valuable metals, as their value offsets the total running costs. Where the greatest benefit is to be gained is "merely" an environmental one, running costs may need to be rather lower than those of a process yielding a valuable product. The use of indigenous biomass, such as ferromanganese-depositing bacteria, should minimise at least one of the components of the running costs, i.e. the cost of the adsorptive medium.

5. Conclusion

Norway is particularly susceptible to potential pollution of its water resources by heavy metals due to the importance of its fishing and other water-related interests, the large number of industries which produce heavy metal-containing wastes and the significant number of landfill sites which contain heavy metals yet are inadequately protected against leaching of the metals into underlying aquifers or into surface drainage waters.

Microbes, including bacteria, are able to accumulate high levels of heavy metals and there is evidence that treatment processes utilising microbes can be competitive with existing treatment methods. Ferromanganese-depositing bacteria display a number of characteristics which suggest that they have potential for use in such treatment systems. Further investigation of the capacity of these bacteria for metal uptake, including determination of their tolerance levels, should be the first step towards an assessment of that potential.

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Appendix 1

Typical composition of Norwegian landfill leachate

Compiled by David Banks

Landfill		Gronno	Brekstadalen	Yggeseth	Isi I	Isi II	Tararod	Dal Stog	Randsund	Holskogen	Holskogen	Sluppen
Kommune		Oslo	Stadsno	Asker	Barnum	Barnum	Sem	Ullensaker	Kristiansund	Kristiansund	Kristiansund	Tromsheim
Commenced		1969	1970/71	1970/72	1967	1974	1973	1979/81	1982	1960	1972	
Completed		In use	1990	In use	1974	In use		In use	In use	1982	In use	In use
Waste Type		P,I,S,F,D	D,I,S	D	D,I,S	P,D,S	D	D	I	I	D	MS
Sampling date		Gronno ²	Brekstadalen ²	Yggeseth ²	Isi I ²	Isi II ²	Tararod ²	Dal Stog ⁴	Randsund ⁵	Holskogen ⁵	Holskogen ⁵	Sluppen ⁶
		Gronno ³	pre-1976	pre-1976	pre-1976	pre-1976	pre-1976	8/89	ca.6/90	ca.6/90	ca.6/90	9/89
pH		1977	6,8	5,9	7	6,4	6,2	5,3	7,63	7,39	7,22	7,21
TOC	mgC/L	1978	100	1700	180	30	800	9760	37,8	37,7	436	9,1
COD	mgO/L	310	470	9425	825	110	3455	60900	110	110	1700	
BOD	mgO/L		320	5250	590	50	2300					
Total N	mgN/L	137	182	250	155	16,6	156	710	9,96	49,6	254	
NH4-N	mgN/L	28	120	227	141	10,2	84	501				
NO3-N	mgN/L		0,04	0,04	0,02	0,79	0,68	6,8				
NO2-N	mgN/L											
Organic N	mgN/L		62	23	14	6	71					
Total P	mg/l	0,45	0,6	7,7	3,3	0,1	1,6	1,6	0,17	0,23	13	
Alkalinity	meq/L		30	39	31	6,2	21,6					
E.C.	uS/cm	3310	3210	3380	3050	655	4530	14120	1130	1970	3860	
Cl	mg/L		1060	370	590	68	340	1270				
SO4	mg/L	9	10	100	37	41	100	660				

Waste type
 Hazwaste mapping reference no.

- References:
- 1 = Mather, 1992.
 - 2 = Johansen & Carlson, 1976.
 - 3 = Traaen et al, 1979.
 - 4 = Karstensen, 1989.
 - 5 = Karstensen, 1990.
 - 6 = Miaurd, 1989.
- Waste type
- P = production waste
 - I = industrial waste
 - S = sewage sludge
 - F = ash from waste incineration plant
 - D = domestic
 - MS = metal sludge from galvotechnic industry
- Hazwaste mapping reference no.
- Gronno 0301 0038
 - Brekstadalen 0231 014
 - Yggeseth 0220 003
 - Isi I 0219 010
 - Isi II 0219 011
 - Dalstog 0233 008
 - Randsund 1001 012
 - Holskogen I 1001 010
 - Holskogen D 1001 011
 - Sluppen 1601 005

Landfill	Mather 1992	Mather 1992	Gronmo	Gronmo	Brånadalalen	Yggeøeth	Isi I	Isi II	Tararud	Dal Stog	Randsaund	Holskogen	Holskogen	Sluppen
Kommune			Oslo	Oslo	Slesdalen	Asker	Bærum	Bærum	Sern	Ulensaker	Kristiansand	Kristiansand	Kristiansand	Tromsheim
Commenced			1969	1969	1970/71	1970/72	1967	1974	1973	1979/81	1982	1960	1972	
Completed			In use	In use	1990	In use	1974	In use		In use	In use	1982	In use	In use
Waste Type			P,I,S,F,D	P,I,S,F,D	D,I,S	D	D,I,S	P,D,S	D	D	I	I	D	MS
Sampling date														
Al					<0,05			<0,05		0,18	<0,03	<0,03	0,66	<1
B					5,7			1,6		20,8	0,6	1,2	3,3	
Ba					0,4			0,3		2,6	0,11	1,1	0,24	<0,25
Ca					1,54	400	173	130	218	1460	66	99	137	1100
Cd					0,0001	0,0009	0,002	0,0005	0,0008	0,05	<0,01	<0,01	<0,01	<0,06
Co					0,009	0,07	0,018	0,004	0,033	0,34	<0,01	<0,01	0,019	<0,2
Cr					0,035	0,06	0,027	0,002	0,17	0,11	<0,01	0,02	0,066	<0,1
Cu					0,011	0,022	0,009	0,008	0,021	0,09	<0,005	<0,005	0,034	0,128
Fe					78	234	37,7	11,5	68,9	440	2,8	0,25	5,1	<0,1
K					320	187	219	21,3	214	>1000	78	63	360	8,15
Mg					68	54	58	13	40	193	15,7	49	69	29,25
Mn					1,3			4,3		64	0,66	0,44	1,6	7,9
Na					540	206	312	34,8	197	650	81	134	380	50,8
Ni					0,03	0,03	0,015	0,005	0,12	1,1	0,04	0,03	0,18	4,13
Pb					0,17	0,01	0,001	0,001	0,015	0,44	<0,05	<0,05	<0,05	<0,9
Zn					0,11	0,65	0,085	0,12	2,63	6,6	0,02	0,013	0,16	128,3
Hg					<0,05			<0,05		<0,05				<0,02

Appendix 2

National & international standards for heavy metals in drinking water & sewage sludge

Table A: WHO, EEC and SIFF standards for heavy metals in drinking water.				
	WHO	EEC	SIFF	SIFF
	(MPL)	(MAC)	(G)	(NA)
As	0,05	0,05	0,01	0,01
Ba	1,0	0,1	1,0	1,0
Cd	0,01	0,005	0,001	0,005
Cr	0,05	0,05	0,01	0,05
Cu	0,05	0,1	0,1	0,3
	(3,0)	(3,0)	(1,0)	-
Fe	0,1	0,2	0,1	0,2
Hg	0,001	0,001	5,0e-05	5,0e-04
Mn	0,05	0,05	0,05	0,1
Ni	ns	0,05	ns	ns
Pb	0,1	0,05	0,005	0,02
Se	0,01	ns	ns	ns
Zn	5,0	0,1	0,3	0,3
	-	(5,0)	(1,0)	-
NOTE: All values are in mg/l.				
For Cu and Zn, first values given are for water at the water treatment works.				
Standard limits for water that has been standing in pipes for 10 to 16 hours are given in brackets.				
MPL= Maximum Permissible Level				
MAC= Maximum Acceptable Level				
G= Good: Concentrations less than these values indicate good water quality.				
NA= Not Acceptable: Concentrations greater than these values are not acceptable without further treatment.				
ns= no standard given				
Sources:				
WHO, (1970): "European standards for drinking water", 2nd edition, Geneva				
EEC, (1980): "Directive on the quality of water for human consumption".				
SIFF, (1977): "Drikkevann G2: Kvalitetsnormer for drikkevann".				

Table B. Columns 1 & 2 = SFT limits for heavy metals in sewage sludge for use on land (after Andersen, 1991).			
Column 3 = sludge from Korsvikfjorden treatment plant, Kristiansand, which receives leachate from Randsund Industrial Landfill (after Karstensen, 1990)			
	SFT limit - agriculture/forestry/gardens	SFT limit - "green" (natural) sites	Korsvikfjorden
Cd	4	10	<1
Co	-	20	2,7
Cr	125	200	6,6
Cu	400	1500	27
Fe	-	-	1190
Hg	3	7	1,9
Mn	-	500	214
Ni	80	100	7,8
Pb	100	300	39
Zn	700	3000	95
Ag			3
Al			3090
Ba			540
Sb			14
Sn			12
Ti			190
V			2,9
NOTE: All values are in mg/kg dry weight.			
NOTE: Randsund landfill is locality 1001 012 in Misund & Tvedt, 1990			

Appendix 3

Accumulation of heavy metals in Gallionella biofilms from the Hvaler tunnel

The Hvaler biofilms were found growing on several salt-water leakages from fractures in the Iddefjord granite of the Hvaler road tunnel (Banks et al. 1992a,b). Samples of biofilms were collected, dissolved in acid (in all cases, c.100 % dissolution was experienced) and analysed for metallic elements. Samples of leakage water were also taken and analysed (although it must be recognised as possible that passage through biofilm material may have reduced the water's in-situ content of bioaccumulable elements in such leakage water).

As the biofilms were growing on salt water leakages, they appear to contain much Na, Mg and other seawater elements, due to the water content of the biofilm slime. In order to compare the composition of the biofilms with the leakage water, it has been assumed that the most soluble cations (Na, Mg, K) have not been preferentially adsorbed on the biofilm, but merely represent the biofilm's intra- and extracellular salt-water content. The biofilm concentrations have thus been divided by a normalising ratio to render Na, Mg and K concentrations similar to those in the leakage water. This appears to be a successful technique.

Sample 1: Collected at chainage 4120 on 23rd March 1990 in tunnel. Biofilm filtered to remove excess water and dissolved in acid. Concentrations of elements in acid-dissolved biofilm measured but not related back to biofilm's dry weight. Cation content of both leakage water, and filtrate (derived from filtering of biofilm) determined. Leakage water contained 11600 ppm Cl⁻. Microscopic analysis revealed biofilm to consist almost entirely of bacteria encrusted in iron deposits; 90-95% of biological component appeared to be *Gallionella*, 5-10% another fibrous bacterial type.

Samples 2 & 3: Collected at (respectively) chainage 3615 and 4120 in tunnel on 29th May 1991. Leakage water contained (respectively) 17000 and 17300 ppm Cl⁻. A portion of both biofilms was "rinsed" four times with distilled water to remove seawater and loosely adsorbed elements. Rinsed and unrinsed samples were dried (2 days' drying at 50°C and 1 day at 110°C), dissolved in conc.HNO₃, and analysed for cations (calculated as mg/Kg dry weight).

Weight loss on heating the dried sample (see above) at 550°C for 1 hour was also calculated, and gave the following results (NGU results).

Sample 2: 18.65 % weight loss with respect to dried biofilm.
Sample 2 (rinsed): 20.43 % weight loss

Sample 3: 19.71 % weight loss
Sample 3 (rinsed): 20.68 % weight loss

A similar weight-loss analysis of sample 3 at Norges Tekniske Høgskole, Trondheim gave a weight loss of c. 10.9 % with respect to dried biofilm. Such weight loss tests do not provide a very satisfactory measure of the biofilm's organic content, as the weight-loss is presumably due both to the oxidation of organic material and the dehydration of iron hydroxide.

In all cases the extremely high Fe content of the biofilm may have rendered the determination of some minor elements (especially beryllium) by emission spectroscopy somewhat inaccurate (*pers.comm.* Magne Ødegård - NGU laboratory).

Data from sample 1 partially published in Banks, 1991.
Data from sample 2 - unpublished data, D.Banks, 1991.

Hvaler biofilm - sample 1						
	Filtrate	Filtrate	Water leakage	Biofilm	Normalised biofilm	Accumulation factor
	ppm	ppm	ppm	ppm	ppm	
	F	FS				
Si	4,08	4	4,52	27500	1447,37	320,21
Al	<10	<10	<10	244	12,84	>1,28
Fe	0,066	0,077	0,298	282500	14868,42	49894,03
Ti	<0,4	<0,4	<0,4	13,1	0,69	>1,72
Mg	1200	1200	1100	23000	1210,53	1,10
Ca	1100	1100	1100	34400	1810,53	1,65
Na	7500	7600	7400	150200	7905,26	1,07
K	158,6	139	143,7	2600	136,84	0,95
Mn	3,1	3,1	2,8	645,7	33,98	12,14
Cu	<0,1	<0,1	<0,1	10,7	0,56	>5,63
Zn	<0,6	<0,6	<0,6	144,2	7,59	>12,65
Pb	<9	<9	<9 (<0,09)	202,3	10,65	>1,18 (>118)
Ni	<4	<4	<4	<40	<2,11	
Co	<2	<2	<2	39,5	2,08	>1,04
V	<0,7	<0,7	<0,7 (<0,027)	83,6	4,40	>6,29 (>163)
Mo	<1	<1	<1 (<0,03)	58,9	3,10	>3,10 (>103)
Cd	<0,6	<0,6	<0,6 (<0,006)	11,9	0,63	>1,04 (>105)
Ba	<2,5	<2,5	<2,5	31,3	1,65	>0,66
Be	<0,1	<0,1	<0,1	85,3	4,49	>44,89
Sr	12,07	12,35	12	675,7	35,56	2,96
Li	<0,5	<0,5	<0,5	<5	<0,26	

Figures in parentheses are based on assumed limits of metal content in leakage water, from values of metals in other seawater and groundwater samples from the Hvaler area (Banks 1991).

F = filtered

FS = filtered and subsequently acidified with a small amount of ultrapure HNO₃.

Hvaler biofilm - 2nd sample							
	Water leakage	Biofilm	Normalised biofilm ppm	Accumulation factor		Rinsed biofilm	Ratio rinsed/unrinsed
	ppm	mg/Kg dry weight (ppm)				mg/Kg dry weight (ppm)	
Si	3,31	129	17,20	5,20		113	0,876
Al	1,2	1300	173,33	144,44		1500	1,154
Fe	<0,01	258200	34426,67	>3442667		383200	1,484
Ti	0,0138	116,6	15,55	1127		123,9	1,063
Mg	1100	10100	1346,67	1,22		3500	0,347
Ca	929,1	17400	2320,00	2,50		15300	0,879
Na	9500	58300	7773,33	0,82		753,9	0,013
K	201	1800	240,00	1,19		180,7	0,100
Mn	2,03	1600	213,33	105,09		2100	1,313
P	<0,1	1300	173,33	>1733,33		2000	1,538
Cu	0,003	18,8	2,51	835,56		24	1,277
Zn	0,0134	165,9	22,12	1650,75		219,2	1,321
Pb	<0,05	<5	<0,67			<5	
Ni	<0,04	<2	<0,27			<2	
Co	<0,01	4,2	0,56	>56,00		5	1,190
V	<0,005	<1	<0,13			<1	
Mo	<0,01	<2	<0,27			<2	
Cd	<0,01	<2	<0,27			<2	
Cr	<0,01	3,7	0,49	>49,33		4,6	1,243
Ba	0,0232	30,7	4,09	176,44		42	1,368
Sr	11,71	467	62,27	5,32		531	1,137
Zr	<0,005	75,5	10,07	>2013,33		98,4	1,303
Ag	<0,01	<1	<0,13			<1	
B	3,38	255,5	34,07	10,08		225,4	0,882
Be	<0,002	17,3	2,31	>1153,33		24,6	1,422
Li	0,156	2,1	0,28	1,79		1,2	0,571
Sc	<0,002	<0,5	<0,067			<0,5	
Ce	<0,05	20,2	2,69	>53,87		12,1	0,599
La	<0,01	35,5	4,73	>473,33		48,7	1,372
Y	<0,002						

Hvaler biofilm - sample 3							
	Water leakage	Biofilm	Normalised biofilm	Accumulation factor		Rinsed	Ratio rinsed/unrinsed
	ppm	mg/Kg dry weight (ppm)	ppm			mg/Kg dry weight (ppm)	
Si	4,06	55,6	6,54	1,61		74,1	1,33
Al	1,24	124,5	14,65	11,81		235,1	1,89
Fe	<0,01	289200	34023,53	>3402353		468700	1,62
Ti	0,0233	9,6	1,13	48,47		21,7	2,26
Mg	1100	11100	1305,88	1,19		1600	0,14
Ca	1400	23900	2811,76	2,01		16800	0,70
Na	9000	63100	7423,53	0,82		822,4	0,013
K	161,1	1800	211,76	1,31		55,4	0,031
Mn	2,77	532,5	62,65	22,62		739,9	1,39
P	<0,1	601,8	70,80	>708,00		949,9	1,58
Cu	0,0043	0,2	0,024	5,47		<0,2	<1,00
Zn	0,013	62,6	7,36	566,52		91,7	1,46
Pb	<0,05	<5	<0,59			<5	
Ni	<0,04	<2	<0,24			<2	
Co	<0,01	3	0,35	>35,29		4,2	1,40
V	<0,005	<1	<0,12			<1	
Mo	<0,01	<2	<0,24			<2	
Cd	<0,01	<2	<0,24			<2	
Cr	<0,01	<1	<0,12			<1	
Ba	0,0287	30	3,53	122,98		44,6	1,49
Sr	12,87	498,5	58,65	4,56		527,6	1,06
Zr	0,0057	18,5	2,18	381,84		26,9	1,45
Ag	<0,01	<1	<0,12			<1	
B	2,59	242,1	28,48	11,00		201,5	0,83
Be	<0,002	18,7	2,20	>1100,00		26,5	1,42
Li	0,181	1,8	0,21	1,17		<0,5	<0,28
Sc	<0,002	<0,5	<0,059			<0,5	
Ce	<0,05	<3	<0,35			<3	
La	<0,01	27,6	3,25	>324,71		31,9	1,16
Y	<0,002						