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18 January 2022

Biodet Ref: 22/44990
Client Ref: Opihi

Timaru District Council
PO Box 552
TIMARU 7940

Attn: Pauline Robertson

Dear Pauline

Re: **EXAMINATION OF WATER SAMPLES FOR IRON-RELATED MICROORGANISMS**

Four water samples were received 11 January 2022 for iron-related bacteria analysis. The samples were identified as follows:

Laboratory Number	Sample Identification	Sample Type and Description
44990/1	Opihi River 1 10/1/2022	Clear, colourless with abundant tiny pale particulates.
44990/2	Opihi River 2 10/1/2022	Clear, colourless with a moderate level of tiny pale particulates.
44990/3	Opihi Gallery 1 10/1/2022	Clear, colourless with occasional tiny pale particulates.
44990/4	Opihi Gallery 2 10/1/2022	Clear, colourless with occasional tiny pale particulates.

METHODOLOGY

Microbiological tests using selective procedures were carried out on the samples. (Methods are available on request).

Heterotrophic Iron-Precipitating Bacteria: Vol 31, No 6-Ground Water-Nov-Dec 1993, Microbiological Analysis of Iron-Related Biofouling in Water Wells.

Filamentous Iron-Oxidising/ Iron-Precipitating Bacteria: 7-day water slide culture, ASTM Standard Method D932-85 (2009)

Analysis commenced: 11 January 2022.

MICROSCOPIC EXAMINATION

This was carried out at 1000X magnification under phase contrast using a 7-day water slide culture.

Microscopic examination:	44990/1 Opihi River 1	44990/2 Opihi River 2	44990/3* Opihi Gallery 1	44990/4* Opihi Gallery 2
Amorphous/ Sludge	+	+	+	+
Siliceous material (sand, silt etc)	+	+	0	0
Diatoms	+++	+++	0	0
Algae (unicellular and filamentous)	++ Unicellular + Filamentous	++ Unicellular + Filamentous	0	0
Unicellular bacteria	+++	++	++	++
Filamentous bacteria (Iron-oxidising and iron-precipitating bacteria)	++ Unidentified sheathed filamentous bacteria +++ Unidentified unsheathed filamentous bacteria	+++ Unidentified sheathed filamentous bacteria +++ Unidentified unsheathed filamentous bacteria	+ Unidentified sheathed filamentous bacteria ++ Unidentified unsheathed filamentous bacteria ++ Dark brown clusters of material including empty filamentous bacterial sheathes**	+ Unidentified sheathed filamentous bacteria ++ Unidentified unsheathed filamentous bacteria ++ Dark brown clusters of material including empty filamentous bacterial sheathes**
Yeast/Filamentous fungi	0	0	0	0
Nematodes	0	0	0	0
Protozoa (non-*Giardia*)	++	+++	+	+
Other	++ *Amoeba*	+ *Amoeba*	+ *Amoeba*	+ *Amoeba*
Iron-related particles (Iron spot test)	++	++	+++	+++

Key:	Abundant	+++++	Light levels	++
	High levels	++++	Sporadic	+
	Moderate levels	+++	Absent	0

*A noticeable, brown-tinged gelatinous slime was present on the gallery slides, but not the river slides.

**The dark brown clusters of material included empty filamentous bacteria sheathes that became blue when the iron-spot test reagents were added. Much of the cluster did not turn blue, suggesting the possibility of Mn-oxidising filamentous bacteria also being present. We were unable to definitively confirm this. These brown clusters were not observed in the river samples.

Culture Results

The following results were obtained and are shown as colony-forming units (cfu) per millilitre of sample. Limit of detection is 1 cfu per ml.

Culture	44990/1 Opihi River 1	44990/2 Opihi River 2	44990/3 Opihi Gallery 1	44990/4 Opihi Gallery 2
Heterotrophic Iron-precipitating Bacteria (non-filamentous)	290	160	560	3,700

Note: The filamentous iron-precipitating bacteria cannot be cultured on conventional agar and are identified from the microscopic slide-culture examination only.

Discussion

Biofilms and MIC:

Corrosion is an electrochemical process caused by a flow of electricity from one metal to another metal or to another sort of electron sink. This flow requires a solution (electrolyte) to conduct the electron flow. River water and many industrial waters are good electrolytes permitting the flow of electrons from a negative area to a positive one.

Microbially influenced corrosion (MIC) is metal loss caused by or accelerated by microbial action at either the anode or the cathode. Biofilm formation leads to important modifications of the metal/solution interface inducing changes in type and concentration of ions, pH, oxygen levels, flow velocity and buffering capacity. All these are important considerations in understanding MIC, biofilms and their interactions.

The role of microorganisms in appears to start with heterotrophic microbial growth in the water producing Extracellular Polysaccharide Substances (EPS). This EPS settles out on surfaces in which the microorganisms become embedded producing a biofilm. The attachment of this biofilm to an immersed metal surface, and the activity of the aerobic organisms embedded in it, depletes the oxygen at the metal surface thereby creating an oxygen differential and establishing corrosive, oxygen-differential areas. The iron-precipitating bacteria in these biofilms have enzymes that are capable of dissolving and utilising metallic iron. This type of corrosion does not depend on active growth, as even with the death of the organisms, there is a mechanical barrier to the ingress of oxygen.

Sulphate-reducing Bacteria (SRB) can be trapped in the biofilm so if anaerobic conditions arise, these organisms can flourish. Other anaerobic bacteria such as *Clostridium,* produce corrosive organic acids. However, MIC processes can take place in the absence of anaerobes.

The heterotrophic bacteria (*Pseudomonas* and the iron-precipitating bacteria) derive their energy from carbon and are thus readily cultured. *Pseudomonas* is an important slime-forming organism.

Filamentous iron-oxidising bacteria (the true iron bacteria) have the capacity to oxidise ferrous iron to ferric iron, which is deposited in the extracellular bacterial sheath as ferric hydroxide ($Fe(OH)_3$). They derive their energy for growth by this oxidation process and so are termed autotrophic. (These autotrophic bacteria are difficult to culture. The heterotrophic bacteria derive their energy from carbon and are readily cultured).

In carrying out this oxidation process, the true iron bacteria result in rusty slime formations that produce large amounts of iron precipitates in a very short time. The biotic oxidation rates are up to 10 -100 times the abiotic rates. The $Fe(OH)_3$ deposits are in very much larger amounts than the bacterial biomass causing poor water quality, clogging, biofouling and serious corrosion problems. Typically the inner surface of the pipe is plugged with a brownish rust sediment layer containing tubercles of various sizes. Deposited layer thicknesses can vary from 1- 6cm. Severe pitting-attack with a pit length of up to 10mm can often be detected beneath these deposits. This type of pitting is also called crevice corrosion and is commonly found in carbon steel. [1]

The iron-oxidising bacteria result in corrosion due to the formation of condensed oxygen zones and partition of the metal surface into small anodic sites, under the colony, with large surrounding cathodic areas. The dense rust deposits formed by these iron-oxidising bacteria may also create oxygen concentration zones initiating further crevice corrosion, thus accelerating the process.

The true iron bacteria are aerobic and the genus *Sphaerotilus* is the most generally recognised species associated with this type of corrosion. However, the anaerobic SRB (Sulphate-Reducing Bacteria) can also be involved.

FINDINGS

Opihi River Water Samples

- Both river water samples exhibited low to moderate levels of filamentous iron-oxidising bacteria and low levels of non-filamentous heterotrophic iron-precipitating bacteria. Iron-related particles were present at low levels.

- Diatoms, unicellular and filamentous algae were also observed in both river water samples at sporadic to moderate levels.

Opihi Gallery Water Samples

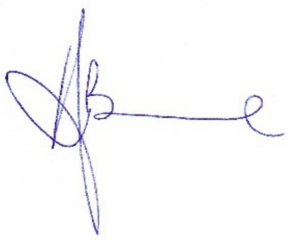
- Diatoms and other algae were not observed in the Gallery samples.

- Both Gallery water samples exhibited slightly lower levels of filamentous iron-oxidising bacteria when compared to the river water samples, but non-filamentous heterotrophic iron-precipitating bacteria were in higher levels in the Gallery samples compared to the river samples.

- A distinct gelatinous slime was associated with the Gallery samples, but not the river samples. It is possible that the iron bacteria may be producing this.

I hope this information is of help to you. If you have any queries, please do not hesitate to contact me.

Yours faithfully



Adrienne Burnie
B.Sc., NZCMT

The samples were tested as received.
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[1] 'Pitting Corrosion of Carbon Steel caused by the Iron Bacteria', Starosvetsky, Armon, Yahalon and Starosvetsky; International Biodeterioration and Biodegradation 47 (2001) 79-87