

The Search for Microbial Manganese Reduction in Two Antarctic Lakes, Lake Hoare and Lake Fryxell

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ABSTRACT

Measurements, including temperature and oxygen, were made to establish the water column profiles for two Antarctic lakes. Several incubations with concentrated lake water and particulate manganese oxides were set up from each lake. Samples were taken at various times during the incubation and measured for soluble manganese concentration to determine if manganese reduction was occurring. Manganese reduction was not observed in the incubations from Lake Hoare. Water concentrated from 9 to 9.5 meters in Lake Fryxell did show some reduction when amended with nutrients. Also, at various depths throughout the water columns, water samples were used to inoculate minimal media plates overlaid with manganese oxides and R2A plates. Colonies were only observed on a few of the manganese overlay plates, none of which reduced manganese. More growth was seen on the R2A plates where colonies were observed for approximately half of the depths sampled at Lake Hoare and most of the depths at Lake Fryxell. The colonies that grew on R2A plates were transferred to manganese overlay plates to test their ability to reduce manganese. Five of the organisms from Lake Hoare and twenty-nine from Lake Fryxell were identified as possible manganese reducers.

INTRODUCTION

Studies have shown that certain bacteria have the ability to solubilize particulate manganese oxides (2,4,6). It is possible that this ability may play an important role in aquatic geochemical cycles, particularly of trace metals, because manganese oxides have an enormous surface area, thus providing many adsorption points for metal and other cations (1,5,7). Trace metals are transported downward through the water column with the manganese oxide particles and released upon solubilization of the manganese oxides.

The purpose of this study was to search for the presence of manganese reducing bacteria in Lake Hoare and Lake Fryxell, both located in Antarctica. These lakes were chosen because of the permanent ice cover that has been present for over 1000 years (5). An ice barrier like this prevents outside factors, such as wind, from changing the characteristics of the water column. Also, these lakes provide a simple system for study because of the lack of complex life forms and interactions such as fish and agriculture.

A relatively simple screen was used to test for bacterial manganese reduction. Manganese reducing bacteria were easily distinguished from non-reducing bacteria because colonies of reducers were surrounded by a clear zone due to the solubilization of the particulate manganese oxides. Oxidic manganese reduction assays were used in addition to the simple screening technique to determine which regions of the water column had the potential for microbial manganese reduction.

METHODS

Sampling. Dissolved oxygen, in parts per million (ppm), and temperature ($^{\circ}\text{C}$) profiles were done for one location at each lake using a YSI oxygen meter that also measured temperature. Water samples were also collected using a MasterflexTM pump with an attached hose that was lowered to specific depths.

Isolation and characterization of manganese reducers. The water samples were used to inoculate plates of R2A (Difco), a general purpose media, and were incubated at ambient temperatures (-4 to 4°C). Once colonies formed, isolated colonies were transferred onto R2A plates repeatedly until colonial growth was from one organism. Once isolated, colonial morphology, cellular morphology, and Gram stains were recorded. Colonies from the isolated plates were then transferred to AMR plates, which is another general purpose media, overlaid with manganese oxides (2). If the organism was a reducer, a clear halo formed around the colony creating a very obvious screen for which organisms were reducers.

Oxic manganese reduction assay. Samples were taken from different portions of the lakes and concentrated twenty-fold. Each concentrated water sample was used to set up the following set of enrichments in duplicate: 1- only lake water, 2- water and manganese oxides, 3- water, manganese oxides, and nutrients (0.02% yeast extract and peptone), and 4-water, manganese oxides, nutrients, and azide (respiration inhibitor). The enrichments were incubated in the dark, stationary, at 0 - 4°C . At given timepoints samples were taken from the enrichments (2) and the concentration of soluble manganese was determined by the formaldoxime assay (3).

RESULTS

Initially, lake profiles for dissolved oxygen and temperature were determined for Lake Hoare and Lake Fryxell. The oxygen profile of Lake Hoare was steady from the ice/water interface down to approximately 21 m at which point it dropped from 20 ppm to about 1 ppm at 29 m (Fig. 1). Temperature remained constant at 0°C throughout the water column. The oxygen concentration of Lake Fryxell was 20 ppm from the ice/water interface down to 10 m at which point it dropped dramatically over a space of one meter to approximately 1 ppm (Fig. 2). Temperature in the Lake Fryxell water column was fairly constant, ranging from 0° to 3°C .

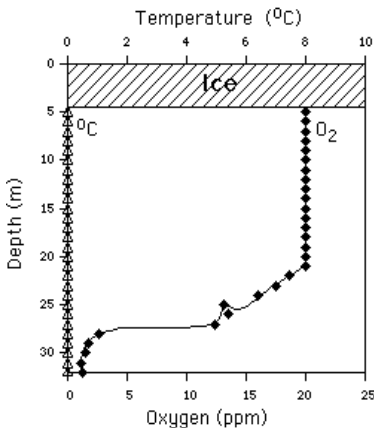


Figure 1. Measurements of temperature ($^{\circ}\text{C}$) and dissolved oxygen (ppm) in Lake Hoare, Antarctica.

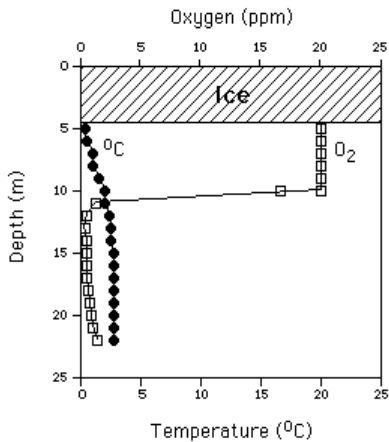


Figure 2. Measurements of temperature(°C) and dissolved oxygen (ppm) in Lake Fryxell, Antarctica.

Oxic manganese reduction assays were conducted on the concentrated water samples from 27-28 m and 28-28.75 m of Lake Hoare. An increase in Mn (II) was observed in the water samples amended with manganese oxides and nutrients, however, due to the fluctuating nature of the curves it is not clear that the increase is due to biological reduction (Fig. 3A & B). The addition of sodium azide, a respiratory inhibitor, had no effect on the results observed.

The results of the oxic manganese reduction assays conducted on the concentrated water samples from 9-9.5 m in Lake Fryxell contain the most promising evidence for the presence of biological manganese reduction (Fig. 3C). When the 9-9.5 m concentrated water sample was amended with both manganese oxides and a nutrient solution containing peptone and yeast extract, there was a steady increase in the concentration of Mn (II) during the first 180 hr of incubation. None of the enrichments containing concentrated water from 9.5-10 m showed evidence of manganese reduction (Fig. 3D). Again, the presence of sodium azide had no effect on manganese reduction.

A search for microorganisms able to reduce manganese was conducted by inoculating minimal media plates overlaid with manganese oxides and R2A plates with water samples from various depths of Lake Hoare and Lake Fryxell. Colonies were only observed on a few of the manganese overlay plates, none of which were surrounded by a clear zone which would have indicated manganese reduction. More growth was observed on the R2A plates. Colonies from the R2A plates were then transferred to manganese overlay plates in order to test for the ability to reduce manganese. Five of the organisms from Lake Hoare and twenty-nine from Lake Fryxell were identified as possible manganese reducers. Thus far, 3 of the 5 reducers from Lake Hoare and 7 of the 29 reducers from Lake Fryxell have been partially characterized (Table 1). None of the reducers isolated from Lake Fryxell came from 9 to 9.5 m where the oxic manganese reduction assay indicated reducers may be found.

CONCLUSIONS

Oxic manganese reduction assays were conducted with concentrated water samples from both Lake Fryxell and Lake Hoare. The 9-9.5 m Lake Fryxell assays had the most promising results supporting the presence of biological manganese reduction. An overall increase in Mn

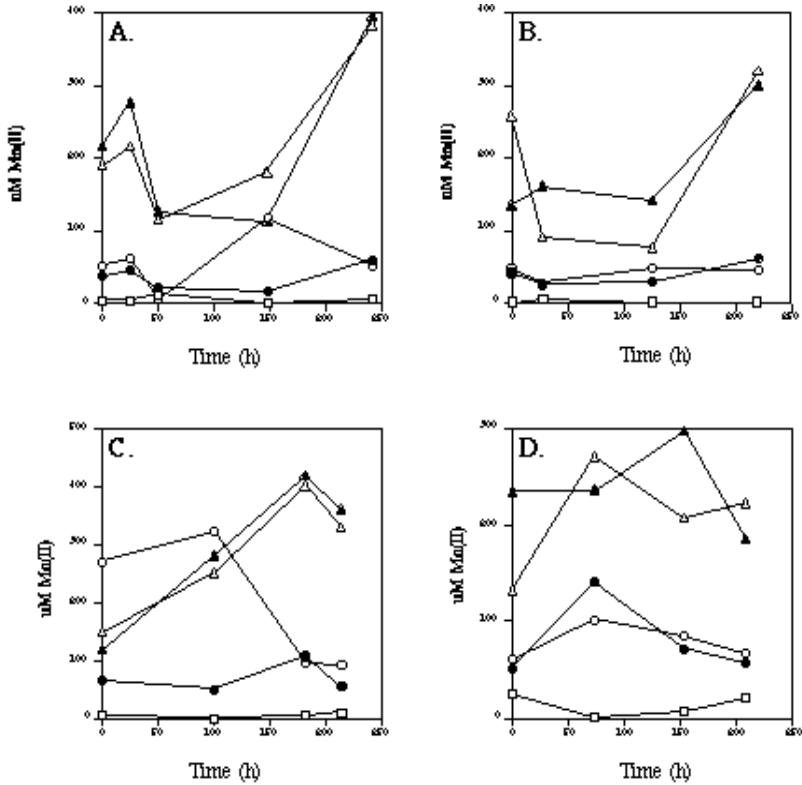


Figure 3. Water samples were concentrated from 27-28 m (A) and 28-28.75 m (B) from Lake Hoare and 9-9.5 m (C) and 9.5-10 m from Lake Fryxell. The concentrated water samples were incubated with and without amendments (—□— none, —●— manganese oxides, —○— manganese oxides and azide, —▲— manganese oxides and nutrients, and —△— manganese oxides, nutrients and azide) and then observed over time for the reduction of manganese.

(II) concentration was seen over time when the assays were amended with manganese oxides and nutrients. A similar setup without nutrients showed no overall increase in Mn (II) concentration. This apparent stimulation of manganese reduction by nutrients is indicative of microbial activity. Although sodium azide did not have any effect, this does not rule out the possibility of microbial activity since sodium azide does not inhibit fermentative bacteria. Manganese reduction was carried out by fermentative bacteria in another antarctic lake study (2).

Ten manganese reducers from Lake Hoare and Lake Fryxell (three and seven, respectively) have been partially characterized. The majority of the manganese reducers were gram positive cocci with the exception of two manganese reducers isolated from Lake Fryxell which were gram negative rods. None of the manganese reducers identified were isolated from the 9-9.5 m depth in Lake Fryxell where the oxic manganese reduction assays indicated

Table 1. Manganese reducers isolated from Lake Hoare and Lake Fryxell

Depth (m)	Strain designation	Gram stain	Cell morphology	Colonial characteristics ¹
Lake Hoare:				
9	LH9	+	cocci, clusters	shiny, lemon yellow, circular
12	LH12	+	cocci, clusters	circular, white, transparent, shiny
29	LH29	+	cocci/ovoid	yellow, circular, shiny
Lake Fryxell:				
7	LF7	+	cocci, singles/double	circular, white
8	LF8Y1	+	cocci, pairs	circular, yellow, shiny, opaque
	LF8Y2	+	cocci, pairs	circular, light yellow, shiny, transparent
	LF8Y3	+	cocci, singles	circular, light yellow, shiny, transparent
	LF8C1	+	irregular cocci, chains	circular, cream, opaque, shiny
	LF8C2	-	rods, pairs	circular, cream, opaque, shiny
	LF8P	-	rods, pairs	irregular, purple/pink, opaque

¹Grown on R2A plates at room temperature for 5-7 days.

that reducing microorganisms may be located. The apparent contradiction between the results from the two techniques may be due to differences in the microorganisms found at the various depths. A span of 1-1.5 m, such as that between 8 m and 9-9.5 m, can result in substantially different microbial environments and, therefore, different microbial communities. Although manganese reducers were isolated from 8 m, the microbial growth requirements of the microbes of interest at 9-9.5 m may be sufficiently different that they were unable to grow on the media provided. Competition between microorganisms, where the manganese reducers were outgrown or inhibited by other microbes, is another plausible reason for these results.

Overall, we did find some evidence for oxic manganese reduction - more so in Lake Fryxell than Lake Hoare. Future plans include continuing to characterize the other isolates and confirming their ability to reduce manganese. A second molecular-based approach will also be used to try to detect manganese reducers or depths containing potential reducers which the culture-based techniques may have missed.

ACKNOWLEDGMENTS

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REFERENCES

1. Balistrieri, L.S., and J.W. Murray. 1986. The surface chemistry of sediments from the Panama Basin: the influence of Mn oxides on metal adsorption. *Geochim. Cosmochim. Acta* **50**:235-243.
2. Bratina, B.J., B.S. Stevenson, W.J. Green, and T.M. Schmidt. 1998. Manganese reduction by microbes from oxic regions of the Lake Vanda (Antarctica) water column. *Appl. Environ. Microbiol.* **64**:3791-3797.
3. Brewer, P.G. 1971. Colormetric determination of manganese in anoxic waters. *Limnol. Oceanogr.* **16**:107-110.
4. Davidson, W. 1993. Iron and manganese in lakes. *Earth-Sci. Rev.* **34**:119-163.
5. Green, W.J., D.E. Canfield, Y. Shengsong, K.E. Chave, T.G. Ferdelman, and G. Delanois. 1993. Metal transport and release processes in Lake Vanda: the role of oxide phases, p. 145-163. *In* W.J. Green and E.I. Friedmann (ed.), *Physical and biochemical processes in Antarctic lakes*. American Geophysical Union, Washington, D.C.
6. Lovley, D.R. 1991. Dissimilatory metal reduction. *Annu. Rev. Microbiol.* **47**:263-290.
7. Stumm, W., and J.J. Morgan. 1970. *Aquatic chemistry: an introduction to emphasizing chemical equilibria in natural waters*, p. 514-563. John Wiley & Sons, New York, NY.