BIOLOGICAL, CHEMICAL AND PHYSICAL FACTORS CONTROLLING THE CONCENTRATION OF MANGANESE IN THE HYPOLIMNION OF IMPOUNDMENTS

By

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ABSTRACT

It has been known that biological activity is needed to remove the dissolved oxygen from the hypolimnion, but the literature continues to have claims for the direct intervention of bacteria in lowering the valence of and thus dissolving the manganese. If there is direct contact between the higher valence state of manganese and bacteria then the more active the bacteria or the more favorable the conditions for their growth the more manganese should be dissolved. An inoculated culture medium should dissolve more manganese than a sterile medium. Many results were obtained that lent some support to the active participation of bacteria, but a refinement in the procedures gave completely negative results. The sterile medium dissolved more manganese; the more favorable the medium for bacterial activity, the less manganese dissolved. Because iron is frequently found in lake and well waters with manganese, the question arose as to whether the iron in the medium could aid the solution of manganese. No aid was found from iron in increasing the amount of manganese in solution. All our data supports the conclusion that after bacteria remove the dissolved oxygen, the manganese is dissolved according to purely chemical factors in the environment of the manganese dioxide. The physical condition of the manganese dioxide is also very significant.

DESCRIPTORS-- / *manganese/ *hypolimnion/ *impoundment limnology/ microbiological metabolism/ anaerobic conditions/ biological reduction/ bacterial activity

IDENTIFIERS-- / Piedmont Plateau/ formaldoxine procedure/ phenanthroline prodedure

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I. INTRODUCTION

In previous work[1], shown in the enclosed reprint, we studied the effects of temperature, pH, a single bacterial inoculum, and a mixed bacterial inoculum on the dissolving of manganese dioxide in nutrient broth enriched with 0.5g glucose per liter.

Our present work concerns the effects of added ferrous ions or those produced from ferric oxide in dissolving manganese from its dioxide at two different temperatures. The temperatures selected were 23°C (room temperature) and 36°C.

In an earlier phase of this project we tried oxalic acid, thioglycolic acid, tannic acid, thiomalic acid, L-cystine, glutathione, sodium sulfate, sodium thiosulfate and sodium sulfite as reducing agents (0.5 gm of each in 1 liter of glucose-enriched nutrient broth). At the time we were interested in establishing that some bacteria aid in dissolving manganese from its dioxide (MnO_2) while some do not. In the earlier studies the bacteria used were Escherichia coli and Pseudomonas fluorescens, while the persulfate method was used to determine manganese. It was decided that the reducing agent in the inoculated or uninoculated media did not contribute to any appreciable extent in dissolving the manganese from its dioxide. It has subsequently been found that the persulfate method for determining manganese gives poor results in the presence of the unfortified nutrient broth, with added reducing agents no appreciable manganese was recovered. Previously it had been concluded that the controls did not dissolve MnO_2. However, with new procedures both analytical and environmental during incubation the controls do indeed dissolve manganese from its dioxide. Later findings indicate that the persulfate data was in error. Further, tests should be run using the formaldoxime method or atomic absorbtion with controls and mixed bacteria.

In our present work two types of manganese dioxide were used. One of these was freshly precipitated from a stoichimetric mixture of solutions of manganous sulfate and potassium permanganate. The precipitated manganese dioxide was washed with distilled water, sterilized and again washed with sterile distilled water. It was then introduced into the sample tubes. The second type of manganese dioxide consisted of the commercially available, dense, crystalline form.

In lake and river bottoms one is likely to find hydrous manganese dioxide which was formed by precipitation of the manganese during the fall turnover. The experiments using the freshly prepared manganese dioxide are believed to approach the conditions actually found in lakes and rivers. Utilization of dense manganese dioxide crystals, on the other hand, may aid in an understanding of soluble manganese from mineral formations which are present in the soil of certain deep lakes.

The effect of ferrous ions on the solution of manganese was studied because observations of natural samples indicate that iron and manganese are generally found together in natural water samples. The question was raised as to whether the soluble ferrous ions aided the solution of the manganese or whether the iron is only incidental to the manganese.

The exact mechanism of the reactions of dissolving manganese from its dioxide which occur in the experimental media (and in nature) is unknown. The material from the reprint report of earlier work on this problem (project) indicates that the solution of manganese is believed to be basically chemical in nature. When an increased rate of manganese solution in the inoculated media does occur, however, it is believed that this increase is caused by the gas and acid by-products of the microorganisms in the mixed media used rather than biological reduction per se (contact of the bacterium with the higher valence manganese ion or molecule).

II. PROCEDURES

The studies of the reactions of manganese dioxide with its environment were carried out in 20-25 ml samples of bacterial media in screw capped test tubes that could be sterilized, modified, sealed and set aside for observation at daily intervals. All tubes contained nutrient broth enriched with 0.5 gram glucose enrichment per liter. The organic matter and minerals were sterilized separately and the minerals added aseptically after the medium had cooled. After inoculating one set of tubes, all tubes were sealed with a vaspar plug to exclude oxygen as far as possible and then each one was capped with a screw cap to reduce oxygen circulation.

In place of commercial manganese dioxide, part of the studies used a freshly prepared material which was obtained by mixing manganous sulfate and potassium permanganate to produce manganese dioxide stoichimetrically. The precipitate was removed on a filter, washed, sterilized in an autoclave and rewashed with sterile water and then added to the tubes with cold, sterile nutrient broth.

The inoculated tubes were given 1 ml of a mixture of five different pure cultures. The mixed culture in previously reported work had been found to dissolve the most manganese. These organisms included 16 hour broth cultures of Pseudomonas fluorescens, Escherichia coli, Rhodaspirillum rubrum, Sarcina aurantiaca, Pseudomonas fragi.

After contact periods, five replicate tubes of each set, were filtered through a membrane filter (0.45 mμ pore size). The filtrate of each tube was analyzed separately for manganese and ferrous iron. The formaldoxine procedure (3) was used for manganese and the phenanthroline procedure (5) for iron. The results of each replicate were averaged and the results plotted.

III. RESULTS

A summary of the results obtained with the various conditions is shown in Table I. The data shows considerable variation in the amount of manganese dissolved. When dense or commercially available manganese dioxide was added to the bacterial medium, very little manganese was dissolved. When freshly prepared, hydrous manganese dioxide was used, more than a 100 fold increase in soluble manganese occurred. The highest value does not represent a solution of all the manganese originally present in suspension; there was always an excess of the oxide on the filter after incubation. Approximately, four g/ℓ both dense and/or hydrous manganese dioxide was added to each tube originally.

Bacteria caused more manganese to dissolve than the media alone produced in seventy percent of the runs. Frequently, the manganese concentration in solution increased and then decreased before the end of the observation period. Typical runs are presented in Figures 1 and 2. All other basic data is presented in the Appendix.

Because soluble iron is frequently found in natural waters along with manganese the possible effect of iron on the solubility of manganese has been studied. There is no clear cut increase in dissolved manganese when iron was also present in the medium during incubation. The data of Table I also show that very little iron dissolves in the medium whether manganese is present or not. When a small amount of ferrous ion was added initially, the concentration in the filtrate at the end of the incubation period was generally a small fraction of the concentration added as shown in Table II. The conditions in the tubes did not favor the attainment or maintenance of iron in solution.

TABLE I.

EFFECT OF VARIOUS CONDITIONS ON THE MAXIMUM AMOUNT OF MANGANESE AND IRON WHICH DISSOLVES

Data from Table # in Appendix	Character of manganese oxide		Temperature of incubation		Iron		Control time to maximum		Inoculated time to maximum	
	Dense	Hydrous	23°	36°	Soluble	Oxide	Hours	mg/ℓ	Hours	mg/ℓ
I	X		X				120**	1.0	90	2.4
II	X		X			X	110	1.2	110	2.1
III	X		X		X		120**	1.8	120**	1.6
IV		X	X		X		70	670	70	800
V		X		X	X		40	370	70	790
VI		X	X				40	780	70	690
VII		X		X			40	700	70	640
VIII		X	X			X	60	310	90**	720
IX		X		X		X	70	570	50	730
				The Dissolving of Iron						
X			X			X	20*	1.0	110**	1.34
XI	X		X			X	40	.5	90	0.90
XII		X	X			X	70	.35	70	.50
XIII		X		X		X	70	.36	70	.61

* Initial observation of that series.
** Final observation of that series.

TABLE II.

EFFECT OF VARIOUS CONDITIONS ON THE MINIMUM SOLUBLE IRON VALUE FROM FERROUS AMMONIUM SULFATE

Date from Table # in Appendix	Character of manganese oxide		Temperature of incubation		Iron		Control time to minimum		Inoculated time to minimum	
	Dense	Hydrous	23°	36°	Soluble	Oxide	Hours	mg/ℓ	Hours	mg/ℓ
XIV	X		X		X		110	0.09	20	0.2
XV		X	X		X		90	0.10	60	0.7
XVI		X		X	X		90	0.05	90	0.05

FIGURE 1. - MANGANESE CONCENTRATION VS TIME IN CONTROLS

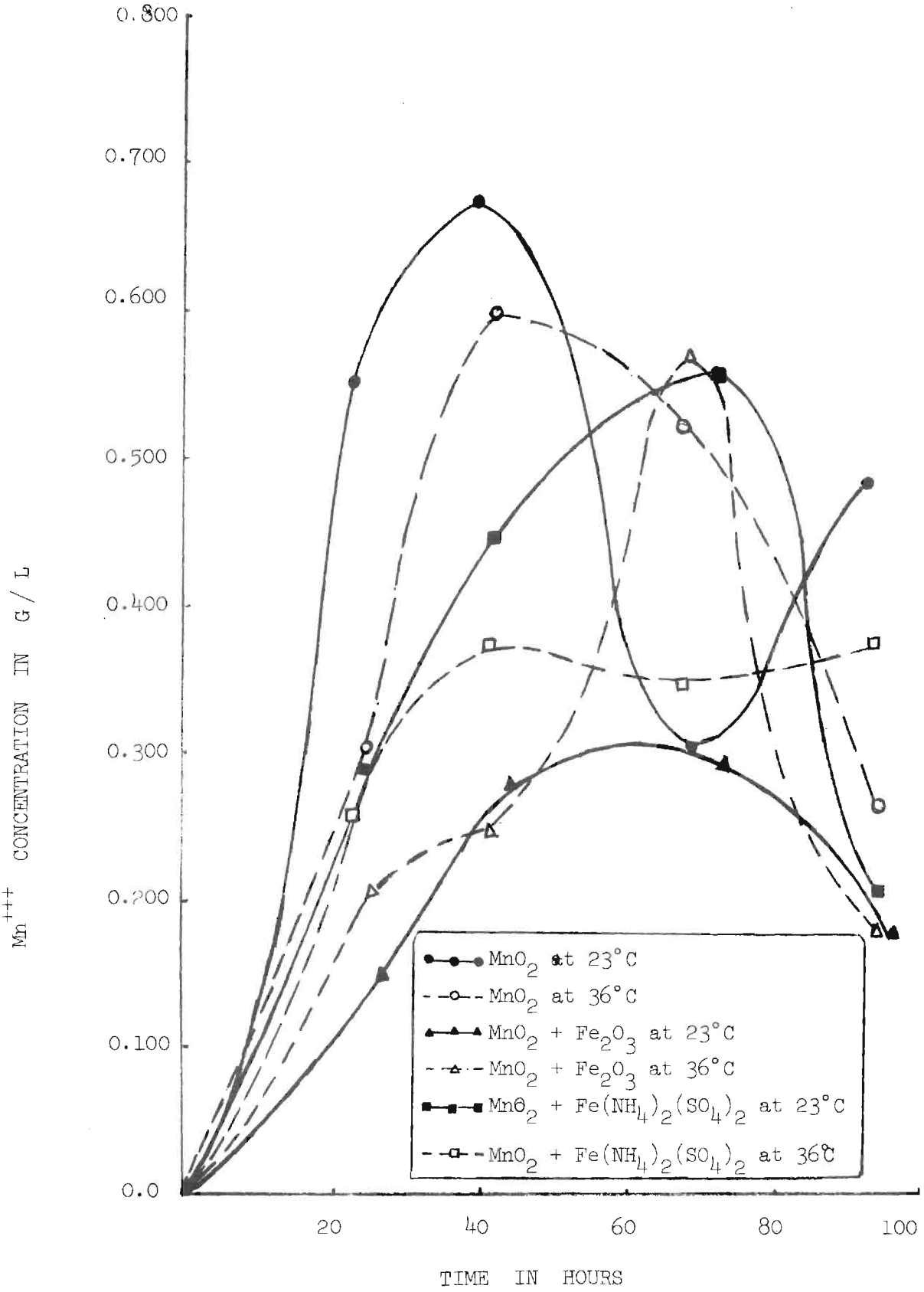
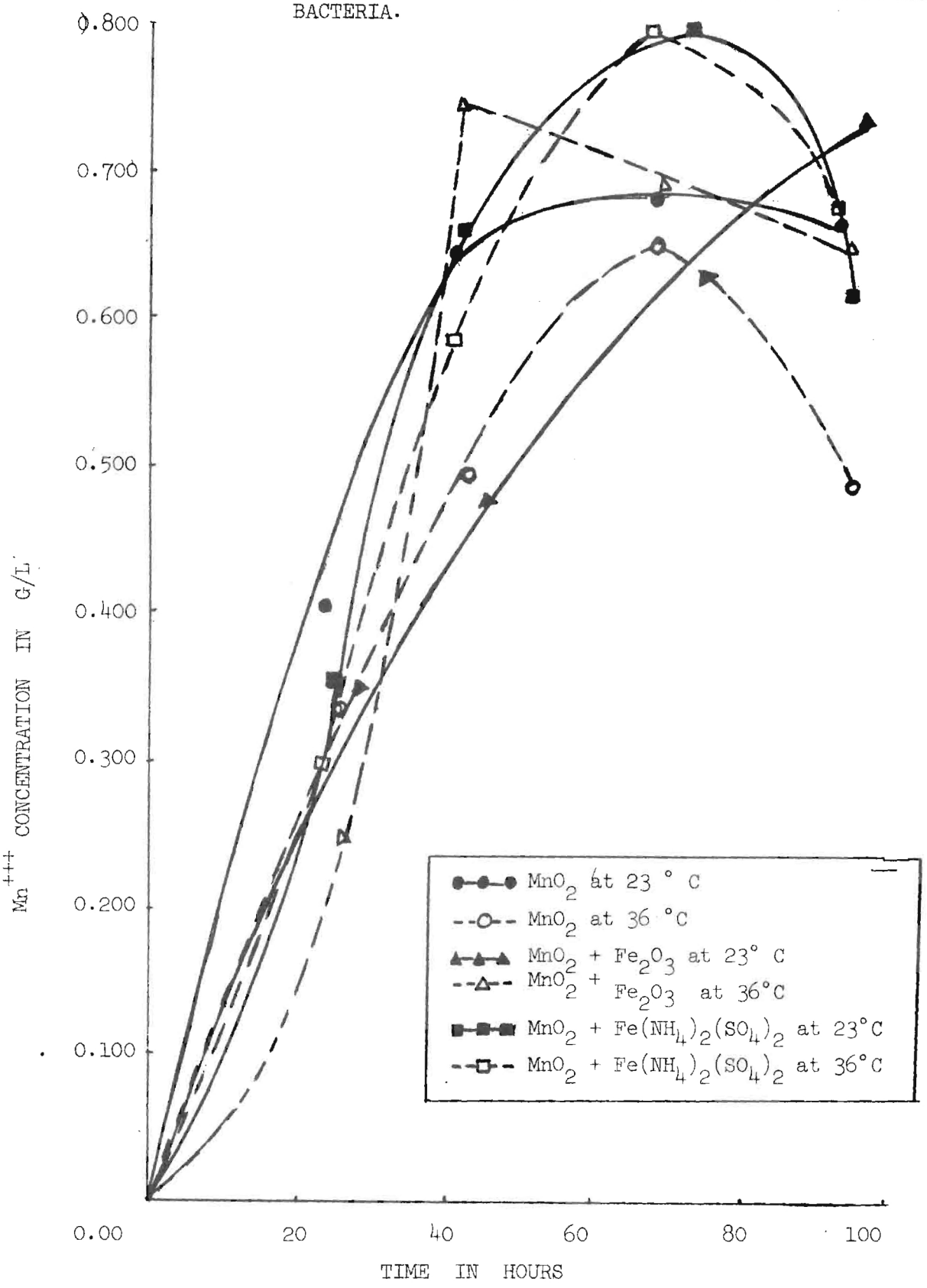


FIGURE 2.- MANGANESE CONCENTRATION VS TIME IN MEDIA WITH GROWING BACTERIA.



IV. DISCUSSION

While it is comparatively easy to carry out the technique of an analysis, it is much more difficult to obtain information that can be used for theoretical considerations. Thus, in determining manganese with persulfate which should change all manganese to the permanganate ion for color comparison some known interferences such as chlorides can be eliminated if they are present in a limited concentration, but less is known about other interferences. When organic compounds are used to reduce and dissolve manganese dioxide, these may also interfere with the permanganate test. Thus, many earlier conclusions must now be reviewed with newer analytical techniques and with more adequately controlled environmental conditions.

In this project, the high quantities of manganese dissolved without a bacterial inoculum indicates that an adequate analytical procedure is available with the formaldoxime reagent and that the equipment and procedures for attaining and maintaining anaerobiosis is adequate. The frequent attainment of a peak concentration at 60 to 90 hours and a subsequent drop may indicate the introduction of dissolved oxygen but it is believed that a better explanation may be that the manganese coaleses or increases to such a size that it is removed by the filter. The attainment of a maximum concentration occurs regardless of either manganese concentration or the presence of metabolizing bacteria. Since the bacterial medium can consume oxygen readily after the bacteria have grown anaerobically, it is believed that the bacteria would maintain the solution of manganese free of dissolved oxygen after more than 60 hours incubation or after the maximum manganese value had been attained. Therefore, the decrease in manganese which occurs after 60 hours should not be from oxygen contamination in the inoculated

samples and hopefully is not in the uninoculated tubes.

From observations of the diffusion rate of manganese in natural lake water it is known that manganese diffuses more slowly than truly ionic material (such as calcium). The ferrous ion diffuses at the same rate as calcium and it is believed therefore to be ionic.

The fact that very little ferrous ion develops or that when added very little remains in solution during the incubation period is not understood. The iron concentration from a diffuse or hydrous ferric oxide might also have increased to a much higher concentration, but this would not have answered any questions concerning manganese.

It is believed that enough ferrous ion was present to have effected the manganous ion concentration if the ferrous ion had an important role in dissolving the manganese. No consistent effect of the presence or absence of the ferrous ion was noted in the amount of manganese dissolved as shown in Table III. Three comparable controls showed some greater quantity of manganese; two did not. In inoculated tests two showed a greater concentration of manganese three did not.

The bacteria in the inoculated tubes had no favorable effect on the rate of manganese solution but did permit the development of more soluble manganese in seven out of ten runs. In general different temperatures had very little effect on the quantity or rate of manganese dissolved.

TABLE III.

SUMMARY TABLE OF FACTORS STUDIED

Control	Inoculated
Effect of iron on manganese solution	
2 out 5 positive	3 out 5 positive
Effect of bacteria on the rate of manganese solution	
5 negative	2 positive of 10
Effect of bacteria on maximum concentration of manganese	
3 negative	7 positive
Effect of temperature on manganese solution	
2 negative 1 positive	1 neg. 2 no dif.
Effect of temperature on iron solution from oxide	
None	None

V. CONCLUSIONS

The rate and amount of manganese dissolved from its dioxide has been studied under a variety of conditions.

The condition of the manganese dioxide at the time of the study had the greatest effect on the amount of manganese dissolved. A freshly prepared manganese dioxide releases more than 100 times as much manganese into solution as dissolves from dense, commercial manganese dioxide. When either soluble or insoluble iron was added to the medium with manganese dioxide, there was no consistent increase in the rate or amount of manganese dissolved. When the mixture of manganese dioxide and organic medium both inoculated and uninoculated was allowed to react at different temperatures no consistent pattern was observed. When bacteria of 5 species were inoculated into the medium, there was generally more manganese dissolved than in the same medium with no bacterial inoculum. It is believed, however, that the beneficial effect is a by-product of the organisms rather than the activity of the organisms per se.

In most runs the manganese concentrations reached a peak value and then decreased by asubstantial amount. It is believed that the "soluble" material coalesed into filterable particles with time.

VI. RECOMMENDATIONS

It is recommended that future work include:

1. A reevaluation of the effect of many highly reducing organic compounds on the rate and quantity of manganese which can dissolve from its dioxide. This study should use atomic absorption for both manganese and iron concentrations.
2. Studies of the migration diffusion rate of manganese in the laboratory to determine the relative size of the particles.

VII. APPENDIX

TABLE I.

QUANTITY OF MANGANESE DISSOLVED FROM DENSE CRYSTALS OF MnO_2 at 23°C

Time in hours	Concentration of Mn+++ in mg/ℓ	
	Control	Bacteria
20	0.4	0.5
30	0.58	0.83
40	0.66	1.26
50	0.70	1.55
60	0.68	1.78
70	0.63	1.94
80	0.60	2.00
90	0.66	2.4
100	0.80	1.96
110	0.94	1.80
120	1.0	1.40

TABLE II.

QUANTITY OF MANGANESE DISSOLVED FROM DENSE CRYSTALS OF MnO_2 at 23°C IN PRESENCE OF Fe_2O_3

Time in hours	Concentration of Mn+++ in mg/ℓ	
	Control	Bacteria
20	0.16	0.28
30	0.36	0.84
40	0.55	1.70
50	0.62	1.90
60	0.66	1.58
70	0.63	1.48
80	0.70	1.66
90	0.80	1.98
100	0.90	2.07
110	1.18	2.12
120	1.18	2.04

TABLE III.

QUANTITY OF MANGANESE DISSOLVED FROM DENSE CRYSTALS OF MnO_2 at 23°C IN PRESENCE OF $Fe(NH_4)_2(SO_4)_2$

Time in hours	Concentration of Mn+++ in mg/ℓ	
	Control	Bacteria
20	0.24	0.48
30	0.50	0.78
40	0.80	1.08
50	0.90	1.28
60	0.66	1.30
70	0.56	1.30
80	0.90	1.32
90	1.48	1.34
100	1.68	1.36
110	1.78	1.45
120	1.80	1.58

TABLE IV.

QUANTITY OF MANGANESE DISSOLVED FROM HYDROUS MnO_2 IN PRESENCE OF $Fe(NH_4)_2(SO_4)_2$ AT 23°C

Time in hours	Concentration of Mn^{++} in g/ℓ	
	Control	Bacteria
20	0.240	0.245
30	0.350	0.500
40	0.450	0.640
50	0.505	0.720
60	0.540	0.775
70	0.665	0.800
80	0.500	0.795
90	0.240	0.725

TABLE V.

QUANTITY OF MANGANESE DISSOLVED FROM HYDROUS MnO_2 IN PRESENCE OF $Fe(NH_4)_2(SO_4)_2$ at 36°C

Time in hours	Concentration of Mn^{++} in g/ℓ	
	Control	Bacteria
20	0.225	0.260
30	0.340	0.440
40	0.370	0.580
50	0.365	0.700
60	0.355	0.760
70	0.355	0.785
80	0.360	0.765
90	0.370	0.700

TABLE VI.

QUANTITY OF MANGANESE DISSOLVED FROM HYDROUS MnO_2 AT 23°C

Time in hours	Concentration of Mn+++ in g/ℓ Control	Bacteria
20	0.540	0.395
30	0.745	0.570
40	0.775	0.650
50	0.685	0.670
60	0.350	0.680
70	0.310	0.685
80	0.370	0.680
90	0.475	0.660

TABLE VII.

QUANTITY OF MANGANESE DISSOLVED FROM HYDROUS MnO_2 AT 36°C

Time in hours	Concentration of Mn+++ in g/ℓ Control	Bacteria
20	0.265	0.265
30	0.485	0.385
40	0.700	0.490
50	0.685	0.575
60	0.550	0.630
70	0.510	0.640
80	0.430	0.600
90	0.310	0.520

TABLE VIII.

QUANTITY OF MANGANESE DISSOLVED FROM FRESH HYDROUS MnO_2 IN PRESENCE OF Fe_2O_3 AT 23°C

Time in hours	Concentration of Mn^{++} in g/ℓ	
	Control	Bacteria
20	0.105	0.265
30	0.180	0.350
40	0.260	0.435
50	0.295	0.510
60	0.310	0.575
70	0.295	0.625
80	0.270	0.680
90	0.215	0.715

TABLE IX.

QUANTITY OF MANGANESE DISSOLVED FROM HYDROUS MnO_2 IN PRESENCE OF Fe_2O_3 AT 36°C

Time in hours	Concentration of Mn+++ in g/ℓ	
	Control	Bacteria
20	0.160	0.165
30	0.230	0.350
40	0.250	0.700
50	0.320	0.730
60	0.425	0.715
70	0.570	0.695
80	0.280	0.680
90	0.200	0.650

TABLE X.

QUANTITY OF IRON DISSOLVED FROM Fe_2O_3 AT 23°C IN PURELY ORGANIC MEDIUM

Time in hours	Concentration of Fe++ in mg/ℓ Control	Bacteria
20	0.97	0.18
30	0.78	0.35
40	0.53	0.53
50	0.32	0.60
60	0.21	0.64
70	0.16	0.70
80	0.18	0.83
90	0.22	1.12
100	0.23	1.28
110	0.19	1.34

TABLE XI.

QUANTITY OF IRON DISSOLVED FROM Fe_2O_3 IN PRESENCE OF DENSE MnO_2 CRYSTALS AT 23° C

Time in hours	Concentration of Fe++ in mg/ℓ	
	Control	Bacteria
20	0.28	0.14
30	0.40	0.22
40	0.47	0.27
50	0.45	0.28
60	0.24	0.24
70	0.20	0.21
80	0.25	0.28
90	0.38	0.91
100	0.33	0.65
110	0.23	0.30

TABLE XII.

QUANTITY OF IRON DISSOLVED FROM Fe_2O_3 IN PRESENCE OF HYDROUS MnO_2 AT 23°C

Time in hours	Concentration of Fe++ in mg/ℓ Control	Bacteria
20	0.20	0.17
30	0.245	0.19
40	0.185	0.21
50	0.20	0.35
60	0.26	0.48
70	0.35	0.50
80	0.32	0.47
90	0.10	0.25

TABLE XIII.

QUANTITY OF IRON DISSOLVED FROM Fe_2O_3 IN PRESENCE OF HYDROUS MnO_2 AT 36° C

Time in hours	Concentration of Fe++ in mg/ℓ Controls	Bacteria
20	0.15	0.0
30	0.195	0.01
40	0.180	0.05
50	0.20	0.16
60	0.335	0.40
70	0.355	0.61
80	0.21	0.40
90	0.05	0.10

TABLE XIV.

QUANTITY OF IRON REMAINING IN SOLUTION FROM 10 mg/ℓ $Fe(NH_4)_2(SO_4)_2$ IN PRESENCE OF DENSE CRYSTALS OF MnO_2 AT 23°C

Time in hours	Concentration of Fe++ in mg/ℓ Control	Bacteria
20	0.15	0.20
30	0.25	0.44
40	0.53	0.69
50	0.55	0.73
60	0.21	0.68
70	0.11	0.74
80	0.12	0.96
90	0.12	1.12
100	0.11	1.19
110	0.09	1.22

TABLE XV.

QUANTITY OF IRON REMAINING IN SOLUTION FROM 10 mg/ℓ $Fe(NH_4)_2$ $(SO_4)_2$ IN PRESENCE OF MnO_2 AT 23°C

Time in hours	Concentration of Fe++ in mg/ℓ Control	Bacteria
30	0.46	1.50
40	0.385	0.82
50	0.380	0.72
60	0.42	0.68
70	0.425	0.69
80	0.35	0.76
90	0.105	0.90

TABLE XVI.

QUANTITY OF IRON REMAINING IN SOLUTION FROM 10 mg/ℓ $Fe(NH_4)_2\ (SO_4)_2$ IN PRESENCE OF MnO_2 AT 36°C

Time in hours	Concentration of Fe++ in mg/ℓ Control	Bacteria
30	0.225	0.62
40	0.560	0.61
50	0.600	0.66
60	0.615	0.850
70	0.605	0.96
80	0.450	0.32
90	0.05	0.05

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