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CALLE HONORIO DELGADO No. 932 – TELF. 016772
URBANIZACION INGENIERIA – SAN MARTIN DE PORRAS
AP. 5045
LIMA – PERU

Second Interim Progress Report

Project N° 304-1486

"NATURALLY OCCURRING PLASMIDS AND HEAVY METAL RESISTANCE OF THIOBACILLUS FERROOXIDANS"

This report cover the period between January to June 1985.

The advances are the following:

The laboratory is already implemented, and the laboratory equipment is operating.

In relation to the Technical Consultation, Miss Jasmin Hurtado, M. Sc. carried out during October 6 to March 8 at University of Georgia, Anne O. Summers's Laboratory the following experiments:

1.- Transposition of Tn5 (E. coli pAJ 5000: Tn5) to Thiobacillus versatus

2.- Improvement recovery of T. versatus Tn5 mutants.

4.- Characterization of T. versatus Tn5 mutants

5.- Plasmid screen in parentals and T5 mutants strains

6.- Plasmid screen in metal resistant strains. Use of restriction enzymes.

7.- Transformation. Cloning Vectors

8.- Hybridization

Experiments performed during March 8 to May 8 at Ohio State University at Olli H. Tuovinen's Laboratory.

1.- Carboys culture of T. ferrooxidans

2.- Protein determination of T. ferrooxidans

3.- Stimulation/inhibition of sulfur, and sulfur compounds on ferrous oxidation by T. ferrooxidans: clark electrode (oxygen consumption) and ferrous oxidation.

In relation to the techniques to be used in the project, the oxido-reduction and complexemetric techniques in order to determinate Fe^{++}, Fe^{+++} and total Fe are standarizated.

Rec'd in SCI: AUG 8 1985

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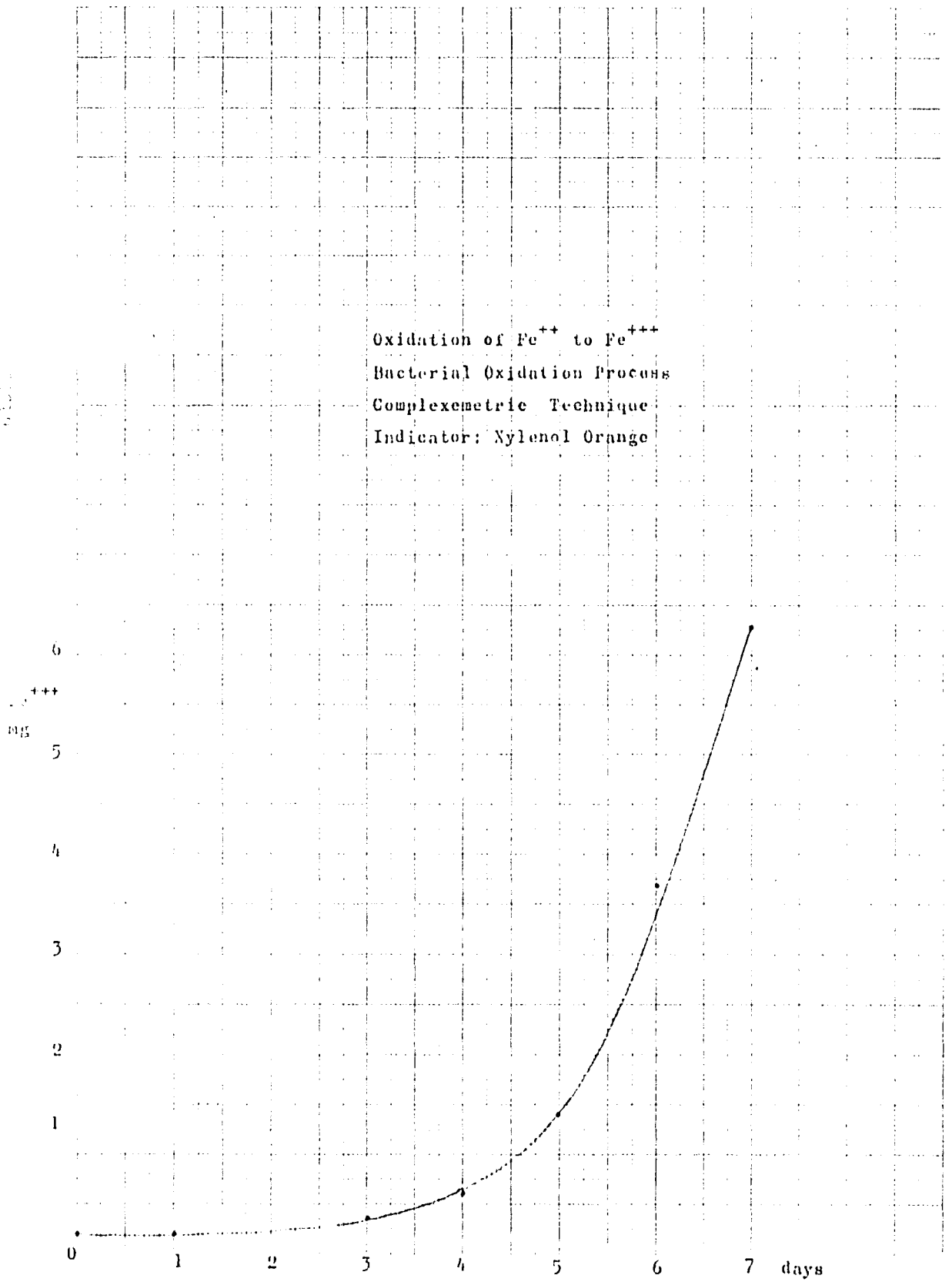
These techniques are used in order to carry out the quantification of iron in the kinetic studies of the chemical and bacterial oxidation of Fe^{++} to Fe^{+++}.

When we make the ferrous determination using oxido-reduction technique with $KMnO_4$ like valoraizer, we find that this technique doesn't have the sensibility that we need when the iron concentration is low (< 10 mg/ml). On the other hand when we use the complexometric technique with EDTA-Na like valoraizer and salicilic acid or xylenol orange like indicator, we find a very good sensibility, working at low concentration of iron.


Oxidation of Fe++ to Fe+++
Bacterial Oxidation Process
Complexemetric Technique
Indicator: Xylenol Orange
+++
mg
6
5
4
3
2
1
0
1
2
3
4
5
6
7
days

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Fe^{+++} Determination

Complexometric Technique

Valoraizer: EDTA-Na Indicator: Xylenol Orange

A blue complexo of Fe^{+++} - Xylenol Orange is formated and by titulation with EDTA-Na Solution cause a change of the blue color and a yellow color appear.

In order to determinate total Fe, is necessary to oxidize the sampl with 3% H_2O_2 and make the total Fe valuation like Fe^{+++}.

Oxidation of Fe^{++} to Fe^{+++}

Bacterial Oxidation Process

Complexometric Technique

Kelly medium

Time days	EDTA (ml.) Exhausted volume	Fe^{+++} mg.
0	0.3	0.1680696
1	0.35	0.1694701
3	0.6	0.3641508
4	1.0	0.6022494
5	2.5	1.4005800
6	6.6	3.7253248
7	11.2	6.2745984

In relation to the sample colection, we have samples from Arequipa, Lima and Cerro de Pasco, and we are working with the microorganismos isolated from these mine waters.
The growth on solid media is solving with the use of purified agar, and now we can obtain isolated colonies of our strains.

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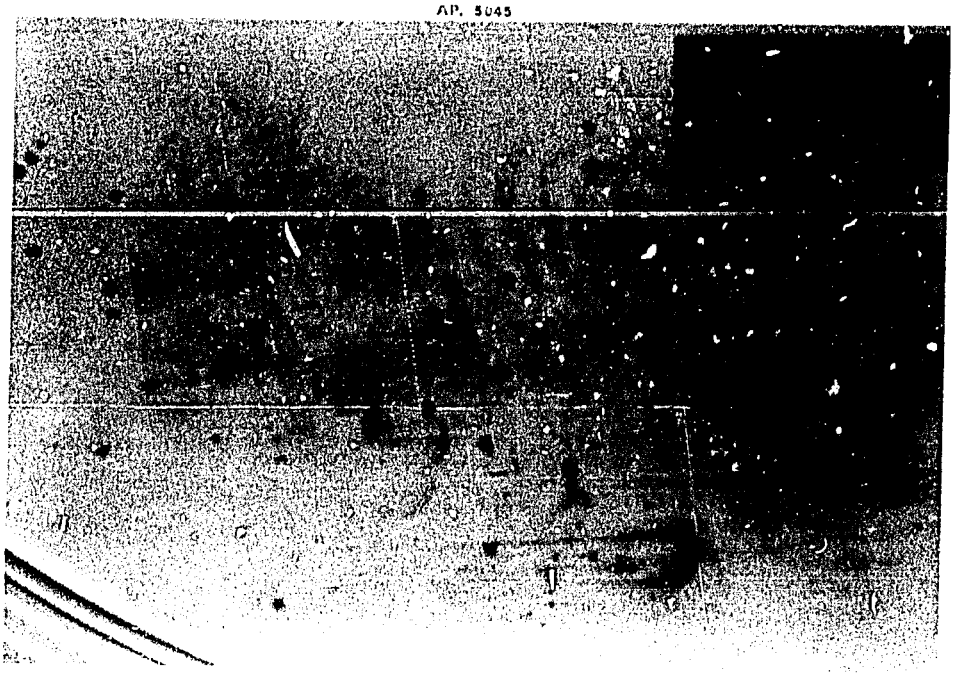

The apparition of precipitate in our liquid media was solving using the Ferrous Iron Medium (Tuovinen-Kelly, 1973) which has the following formulation.

Mineral Salts Solution:

K_2HPO_4	0.5 g.
$(NH_4)_2SO_4$	0.5 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
H_2SO_4 0.11 N	1000 ml.

(sterilize by autoclaving)

Ferrous Iron Solution (Per liter)

$FeSO_4 \cdot 7H_2O$	167 g.
H_2SO_4 1 N	50 ml.

(sterilize by filtration).

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Ferrous Iron Medium:

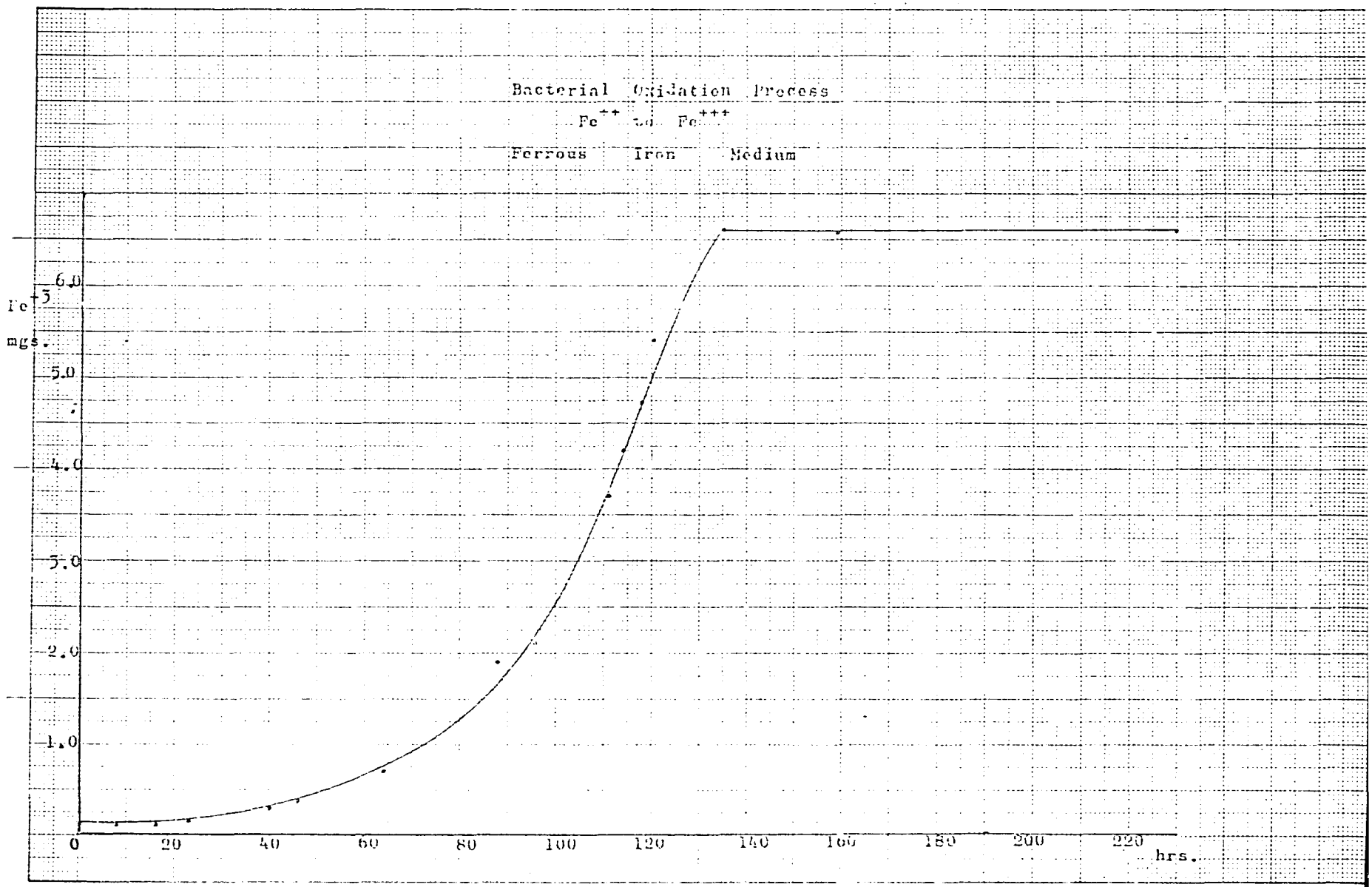
Mix 1 part ferrous iron solution to 4 parts mineral salts solution. The pH of the medium is 1.6.

Bacterial Oxidation of Fe^{++}
Complexometric Technique
Ferrous Iron Medium

Time Hours	EDTA (ml) Exhausted Volume		Fe^{+++} mg
0	0.1,	0.2	0.1052722
8	0.2,	0.2	0.1052722
16	0.2,	0.2	0.1052722
23	0.3,	0.3	0.1579084
40	0.5,	0.5	0.263157
46	0.6,	0.7	0.3421349
64	1.25,	1.25	0.6579517
88	3.5,	3.7	1.894901
96	4.0,	4.0	2.1054456
111	7.0,	7.1	3.7108478
114	8.0,	8.0	4.2108912
117.5	9.1,	9.1	4.7898887
120.5	10.2,	10.2	5.3688862
135.0	12.65		6.6584717
159.0	12.65		6.6584717
231.0	12.65		6.6584717

Total Ferrous Determination
Complexometric Technic

Medium	EDTA (ml) Exhausted Volume	Fe^{+++} mg
Ferrous Iron Medium	12.65	6.67161630


Bacterial Oxidation Process
Fe^{++} to Fe^{+++}
Ferrous Iron Medium
Fe^{+3}
mgs.
6.0
5.0
4.0
3.0
2.0
1.0
0
20
40
60
80
100
120
140
160
180
200
220
hrs.

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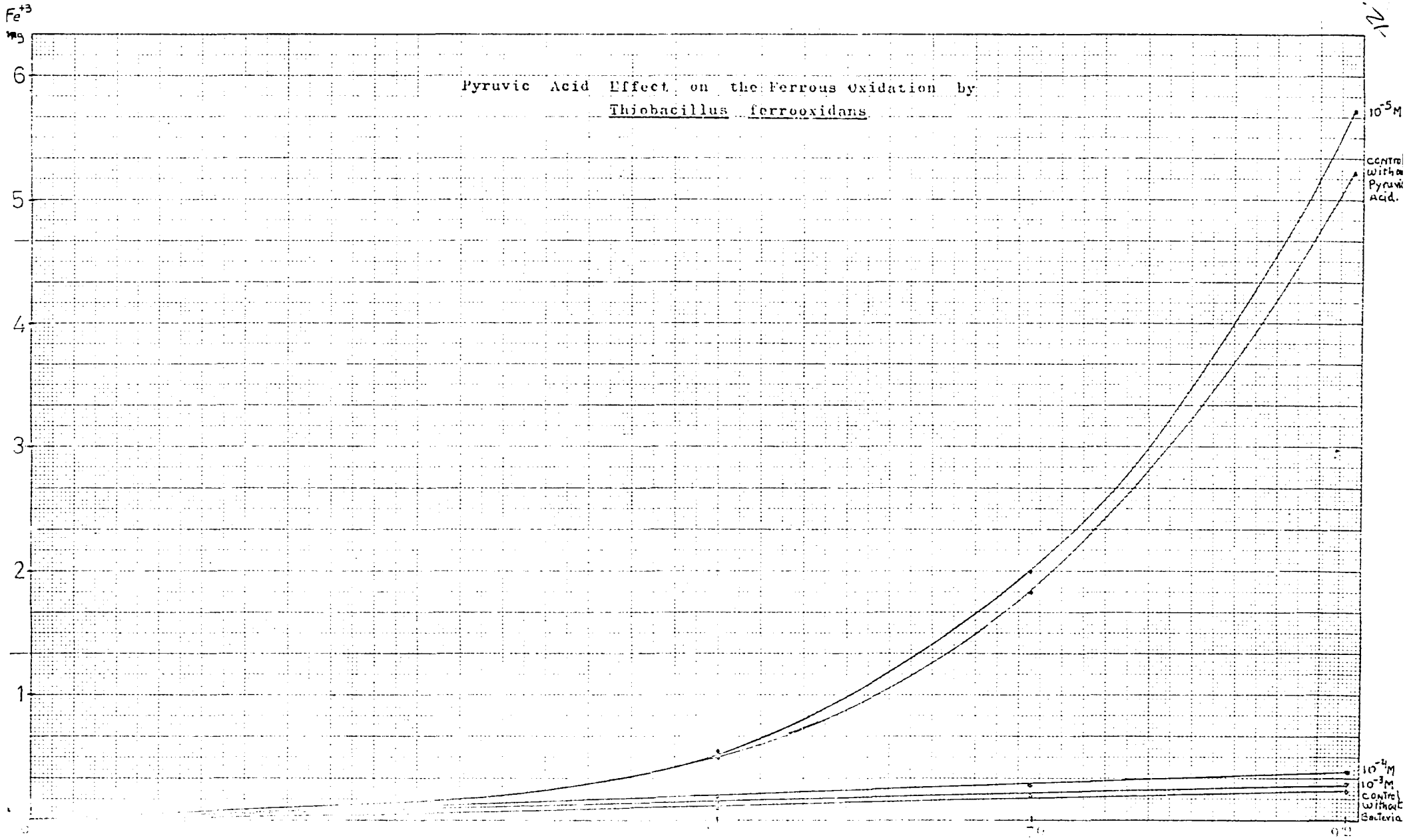
In order to investigate the inhibitory effect of the bacterial growth, from organic compounds, we are investigating the effect of the pyruvic acid on the Ferrous Oxidation by Thiobacillus ferrooxidans.
The sodium pyruvate concentration used were the following 10^{-3}M, 10^{-4}M, 10^{-5}M. We find that sodium pyruvate 10^{-3}M and 10^{-4}M, produce inhibition of the bacterial ferrous oxidation, but when we use sodium pyruvate 10^{-5}M the effect to seen stimulating.

Pyruvic Acid Effect on the Ferrous Oxidation by Thiobacillus ferrooxidans.-

Culture with Sodium Pyruvate	Fe^{+3} mg/ml 22 hrs.	Fe^{+3} mg/ml 48 hrs.	Fe^{+3} mg/ml 70 hrs.	Fe^{+3} mg/ml 92 hrs.
10^{-3}	0.092	0.131	0.189	0.294
10^{-4}	0.105	0.184	0.255	0.381
10^{-5}	0.118	0.523	1.946	5.76
Control without Pyruvate	0.118	0.504	1.87	5.22
Control without bacteria	0.071	0.105	0.226	0.273

Bacterial Growth Control.-

Culture	Bacterial Number Initial	Bacterial Number Final (92hrs.)
Without Sodium Pyruvate	1×10^5 bact./ml.	9×10^7 bact./ml.
With Sodium Pyruvate 10^{-5}M	1×10^5 bact./ml.	1.08×10^8 bact./ml.


Pyruvic Acid Effect on the Ferrous Oxidation by
Thiobacillus ferrooxidans
Fe+3
mg
6
5
4
3
2
1
10-5M
control without Pyruvic Acid.
10-4M
10-3M
control without Bacteria