Kamm

Bacterial Purification Of Sewage.

BACTERIAL PURIFICATION OF SEWAGE

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Wilbur Fred Kamm

ENTITLED Bacterial Purification of Sewage

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in General Science

Edward Barbour.
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BACTERIAL PURIFICATION OF SEWAGE.

INTRODUCTION.

Although air has always played an important role in sewage disposal, treatment by aeration in the presence of sludge is the latest development. The earliest use of air was in the exposure of sewage on the ground or in shallow pools. The disposal of sewage by irrigation is an aeration process. No more sewage can be disposed of on land than can be thoroughly oxidized. The disposal of sewage by dilution in streams depends also on the amount of air present, the amount of sewage that can be purified by a stream being limited by the amount of dissolved oxygen present. Intermittent sand filtration, with sewage added intermittently to sand beds, is an aeration process for, between the periods of flooding with sewage, air is allowed to enter the pores of the sand. The intermittent contact bed system is also an aeration process because after every "dose" of sewage the beds are aerated. Sprinkling filters depend upon aeration obtained by spreading the sewage in a finely divided state into the air.

Sewage disposal by means of dilution, intermittent sand filtration, irrigation, contact beds, and by sprinkling filters are all aeration processes. In all these systems the essential

requirements are,- the sewage must be brought into intimate contact with a sufficient quantity of fresh air; b, the air must be introduced in such a manner as to insure its absorption; c, the sewage must be retained subject to bacterial action, with an adequate air supply, long enough to permit the completion of the process of purification.

DISPOSAL OF SEWAGE BY DILUTION.

The cheapest method of sewage disposal is by dilution in a body of water if sanitary conditions are not interfered with. Applied to raw sewage, this method of disposal allows solid particles to sink to the bottom of the stream where they putrefy or float to the shore, where they undergo decomposition, and cause a nuisance. Care must be taken to make sure that no use is made of the water of a stream for a considerable distance below the sewer outlet.

DISPOSAL OF SEWAGE BY IRRIGATION

Sewage irrigation has for its object the use of the sewage for fertilizer as well as the sewer water for irrigation. The sewage is applied intermittently to the land at a rate so slow that it does not interfere with the raising of crops. The western cities with their dry climate and sandy soils have found this system to be of most advantage. Irrigation farms.

still exist today at Pasadena, California; Fresno, California; Alhambra, California; Salt Lake City, Utah; and Greeley, Colorado[1] . In 1899 Paris had 12,000 acres of sewage irrigation farms and was continually increasing the area. Berlin put its first irrigation farm into service in 1876 and since that time they have gradually increased in area until in March, 1914, the total area was 43,000 acres. The average volume of sewage treated by the Berlin farm amounted in 1914 to about 77 million United States gallons daily.

DISPOSAL OF SEWAGE BY INTERMITTENT SAND FILTRATION

This process consists of delivering the sewage to a specially prepared field of sand for a certain period, and then applying sewage to other similar fields, returning to the first field again after it has recovered from the first application. The object of this system is to dispose of the sewage and not to raise crops. It may be applied to crude sewage, or to an effluent from a preliminary process. If treated by some preliminary process the sewage can be filtered more rapidly. The usual method of preliminary treatment may consist in using settling tanks, grit chambers, digestion tanks, screens, or chemical precipitation.

DISPOSAL OF SEWAGE BY CONTACT BEDS.

Intermittent contact bed systems of sewage disposal

operate by alternately filling and emptying receptacles containing broken stone, gravel, cinders or other material having a large proportion of voids. The liquid is allowed to remain in the bed for a given period in contact with the bacterial growth which attaches to the surface of the granular material. After contact the liquid is discharged and air is admitted to the bed to be absorbed by the bacteria before additional sewage is admitted. Contact beds are usually operated in conjunction with preliminary treatment, so that the liquid entering them is freed from the large solid particles.

DISPOSAL OF SEWAGE BY SPRINKLING FILTERS.

The sprinkling filter was the most practical up to the time of the suggestion of the activated sludge. Its success depends upon the aeration obtained by spreading the sewage in a finely divided state into the air and then allowing it to trickle over crushed stone. The main province of the sprinkling filter is to reduce the non-settling colloidal and dissolved organic matters to a point where the effluent will no longer putrefy. Fron this viewpoint sprinkling filters have been successful in that stable non-putrescible effluents can be obtained at high rates. The method of application varies. At Madison, Wisconsin the sewage is applied to a coarse-grained filter through lines of perforated tile pipe laid about two feet apart. At Mount Vernon, New York the sewage

is sprayed into the air by means of fixed nozzles.

DISPOSAL OF SEWAGE BY AERATION

More recently aeration of sewage has been undertaken with surprisingly good results. It had long been known that sewage exposed to air in thin sheets or permitted to trickle over rocks was partially nitrified and clarified, however, it was not until 1912 that promising results were obtained by experiments by the direct aeration of sewage.

In November 1912, Dr. Fowler, who had been impressed by the results obtained at Lawrence, Massachusetts by aeration of sewage in tanks containing slate, suggested to E. Ardern, M. S. C. and W. T. Lockett[2] that they experiment with sewage disposal by means of aeration. Their first work was performed in eighty ounce bottles partially filled with sewage through which air was blown. After aerating the sewage for a period of five weeks complete nitrification was produced. After the sludge had completely settled the effluent was drawn off and more raw sewage added. The process was repeated until complete nitrification was obtained in six hours. The accumulated sludge had a high nitrogen content and settled very readily, leaving a clear stable effluent. Since then the work has been repeated and extended by Ardern and Lockett and at Illinois[3].

Aeration of sewage has been tried at Milwaukee.[4] A two million gallon sewage aeration plant was constructed in

1915 and according to reports excellent results are being obtained.

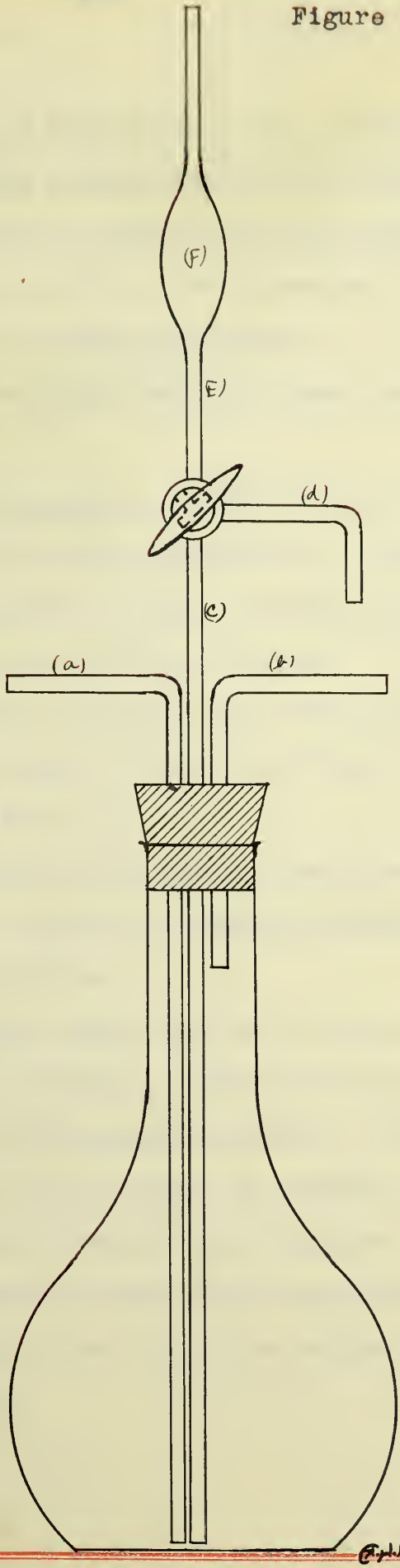
In order to determine whether the oxidation of the sewage during aeration was produced by the oxygen in the air alone in the presence of bacteria, or perhaps of some other organisms Robbins Russel[5] made a study of the organisms found in activated sludge and isolated bacteria which changed ammonia to nitrite and to nitrate. We have repeated and extended Russel's work.

ISOLATION OF NITRIFYING BACTERIA.

Pure cultures of the nitrifying bacteria were secured by means of special apparatus and special media.

Apparatus. A modified "Beesley aeration apparatus", [6] was used (Fig.I). A wide mouthed liter flask was fitted with a three-hole rubber stopper, one of the holes contains a piece of glass tubing bent at right angles, (a) extending to the bottom of the flask, a second hole contains a similar tube (b) but extending just through the stopper. One limb of a three-way stop cock passes through the third hole, to the bottom of the flask; a second limb (d) is bent at right angles, while on the third (e) a bulb (f) of about ten cc capacity, is blown, the top of this limb is plugged with cotton. By opening the stopcock a solution may be aseptically drawn from the flask through tube (c) into the bulb (f) and upon closing the stop-

Figure I



cock it may be run off through tube (d). When it is desired to aerate the solution with purified air, compressed air is blown through a series of wash bottles and attached to tube (a) containing in order,- cotton, sterile water, sulphuric acid, and a weak solution of sodium hydroxide.

Media. Silica jelly,[7] purified agar, and mineral agar have been used.

Silica Jelly.Reagents. Sodium silicate containing from 40 to 50 grams of silica anhydride in 1000 cc.

Hydrochloric acid, one cc. of which will exactly neutralize one cc. of the silica solution.

Sodium carbonate solution for isolation of nitrite bacteria containing 3 gm. of Sodium carbonate and 5 gm. of ammonia sulphate in 500 cc.

Sodium carbonate solution for isolation of nitrate bacteria containing 3 grams of sodium carbonate and 5 grams of sodium nitrite in 500 cc.

Procedure. Add slowly 106 cc of the acid to 100 cc of the sodium silicate solution. After thoroughly mixing the two, add 0.2 grams of potassium phosphate, 0.04 grams of magnesium sulphate, and 0.1 grams of calcium chloride. Tube the mixture in ten cc.portions and sterilize it at 120° for 15 minutes. In order to cause the solidification of this medium add to each tube one cc.of the sodium carbonate containing the salt desired.

Purified Agar. Soak ordinary agar in distilled water for ten days, the water being changed twice daily. After the complete removal of the soluble matter dry the agar.

Purified Ammonia Agar.
(for nitrite bacteria).

2 grams NH_4MgPO_4
0.5 gm. K_2SO_4
3 drops of a 10 per cent solution of $FeCl_3$
3 drops of a 10 per cent solution of $CaCl_2$
15.0 gm. purified agar.
1000 cc distilled H_2O.

Purified Nitrite Agar.
(for nitrate bacteria).

2.0 gm. $NaNO_2$
0.5 gm. K_2HPO_4
15.0 gm. purified agar.
1000 cc distilled H_2O.

Sterilize at 120° for 15 minutes.

Mineral Agar. Make up agar according to standard methods of water analysis[8] and add to each liter the following salts,-

Ammonia Agar.

(for nitrite bacteria)

1 gm. $(NH_4)_2SO_4$

1 gm. K_2HPO_4

0.5 gm. $MgSO_4$

0.4 gm. $Fe_2(SO_4)_3$

2.0 gm. NaCl

0.5 gm. $CaCO_3$

Nitrite Agar.

(for nitrate bacteria).

0.5 gm. $NaNO_2$

0.5 gm. K_2HPO_4

0.5 gm. NaCl

0.5 gm. $MgSO_4$

0.5 gm. $CaCO_3$

3 drops of a 10 per cent solution of $FeCl_3$

Ammonia Broth.

(for nitrite bacteria)

1 gm. $(NH_4)_2SO_4$

1 gm. K_2HPO_4*

0.5 gm. $MgSO_4$

3 drops of a 10 per cent solution of $FeCl_3$

2.0 gm. NaCl

10.0 gm. $CaCO_3$**

Nitrite Broth.

(for nitrate bacteria).

0.5 gm. $NaNO_2$

0.5 gm. K_2HPO_4*

0.5 gm. NaCl

2 drops of a 10 per cent solution of $FeCl_3$

0.5 gm. $MgSO_4$

10.0 gm. $CaCO_3$**

*K_2HPO_4 should be dissolved separately.

**The $CaCO_2$ should be sterilized separately.

ISOLATION OF BACTERIA

One aeration apparatus containing sterile ammonia broth and another containing nitrite broth were inoculated with 2 cc of activated sludge. After nitrification had continued for several days 10 cc. from each flask was inoculated into similar fresh flasks. When this procedure had been repeated four times according to the "Accummulation method of Beijerinck" fairly pure cultures should be obtained. After the last inoculation nitrification was permitted to go to completion. The nitrite broth required 18 days and the ammonia broth 20 days for complete oxidation (Fig. II and III). After the 20th day the nitrite broth showed an increase in ammonia and nitrite, which was afterwards proven to be due to denitrifying bacteria. The ammonia broth showed but very slight denitrification. During the nitrification process, lasting 24 days, the bacteria decreased (Fig. IV and V) in both broths for about 15 days after which there was a gradual increase. After the lowest point in the bacterial reduction had been reached ammonia and nitrite silica jelly plates were inoculated, with ammonia and nitrate broths respectively. After 14 days, growth appeared on the ammonia silica jelly and five days later, growth appeared on the nitrite silica jelly. The nitrite organism was transferred to purified ammonia agar and the nitrate bacteria to purified nitrite agar.

From the two flasks after denitrification had set in, 8 different organisms were isolated. Of these, 6 proved to be

Figure II

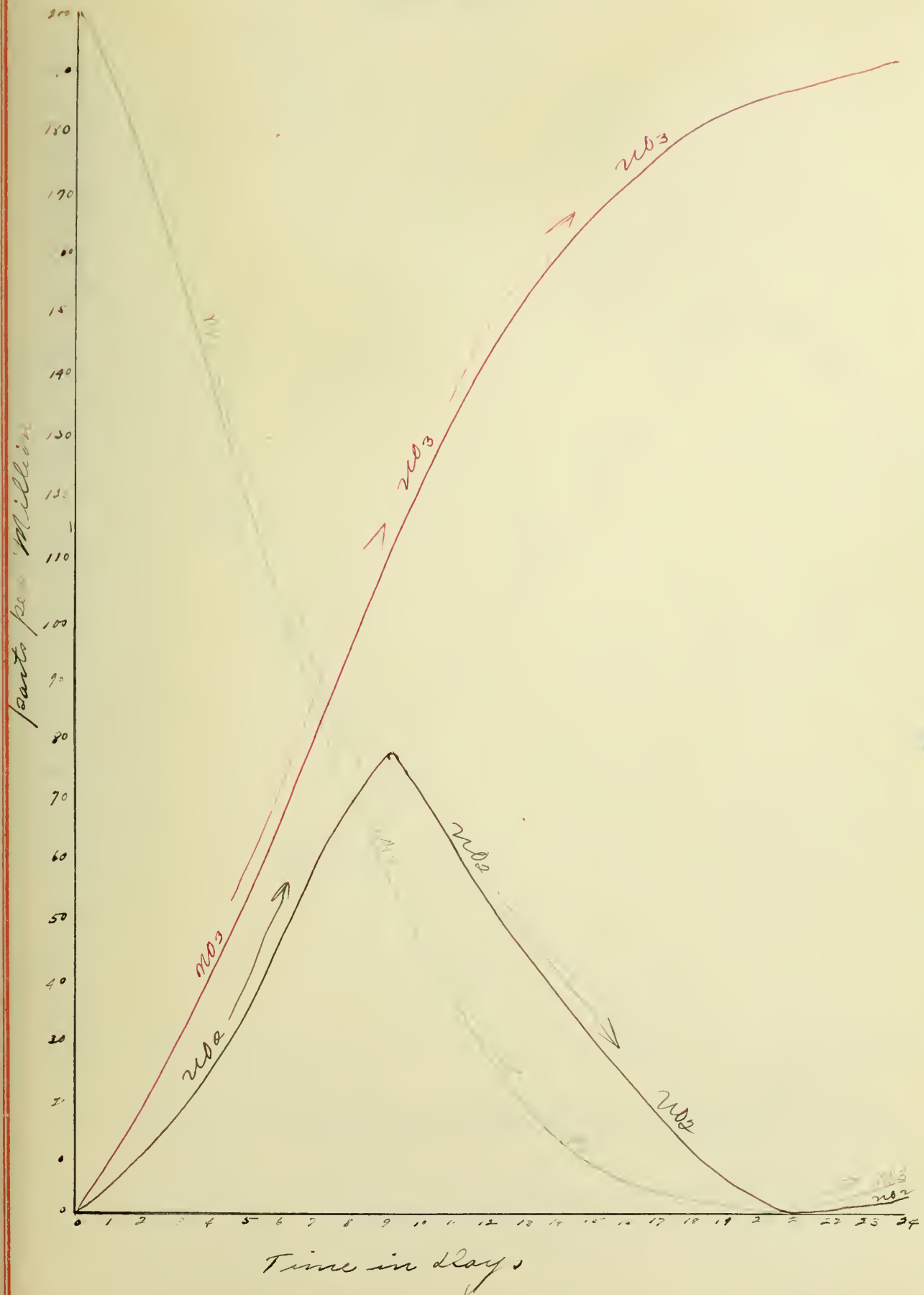


Figure III

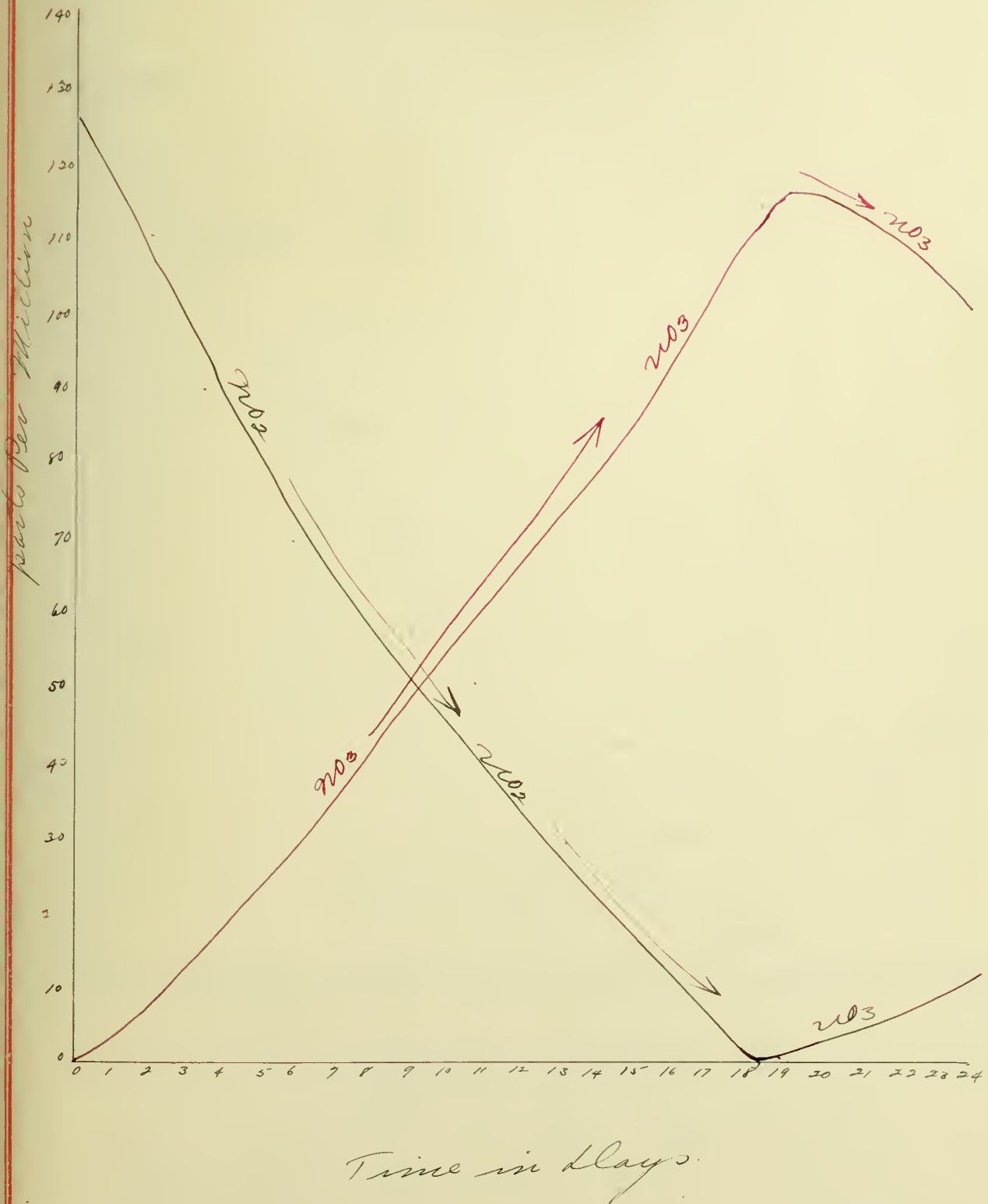

Parts per Million
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
NO2
NO3
NO3
NO2
NO3
NO3
Time in days.

Figure IV

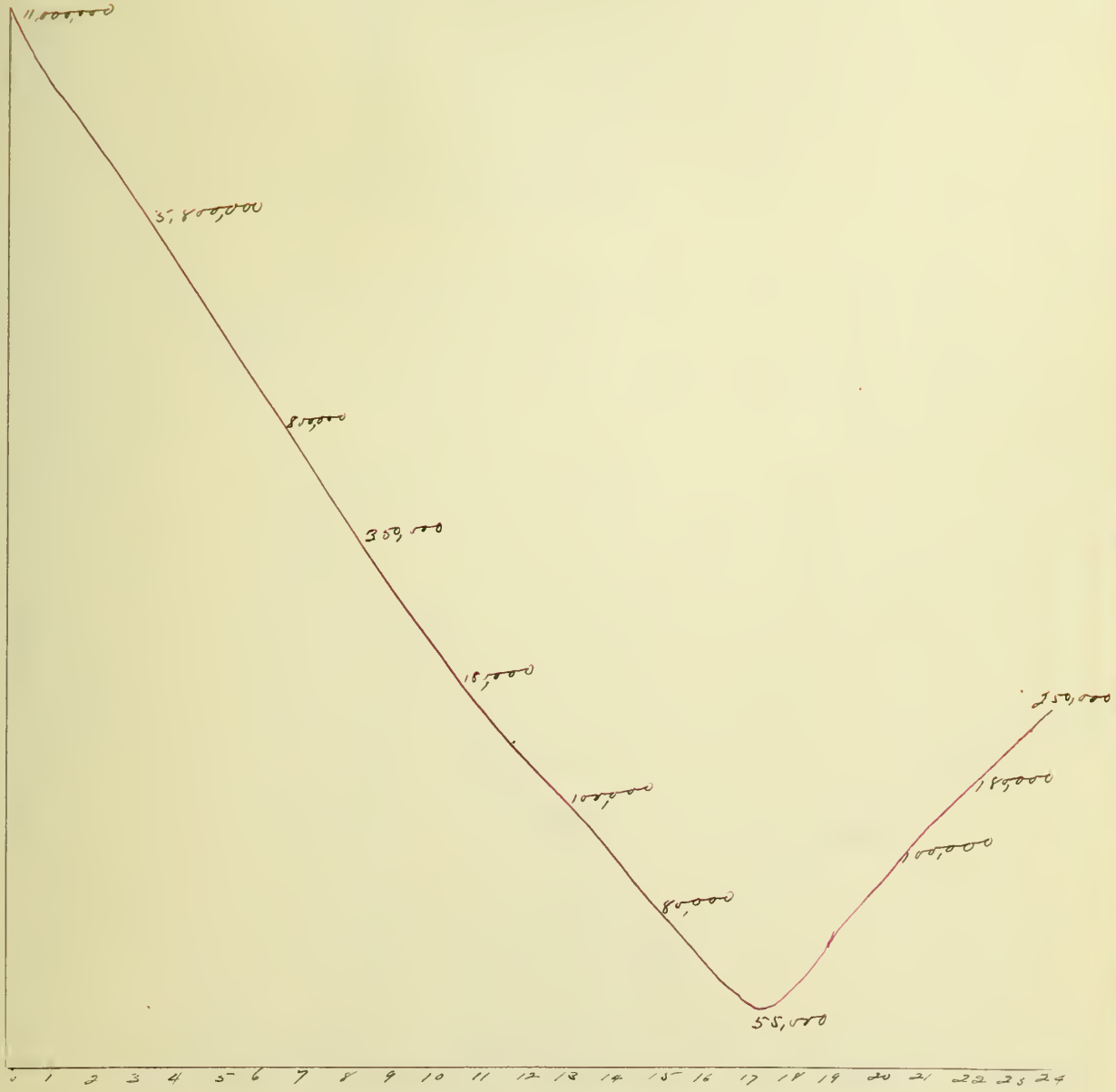
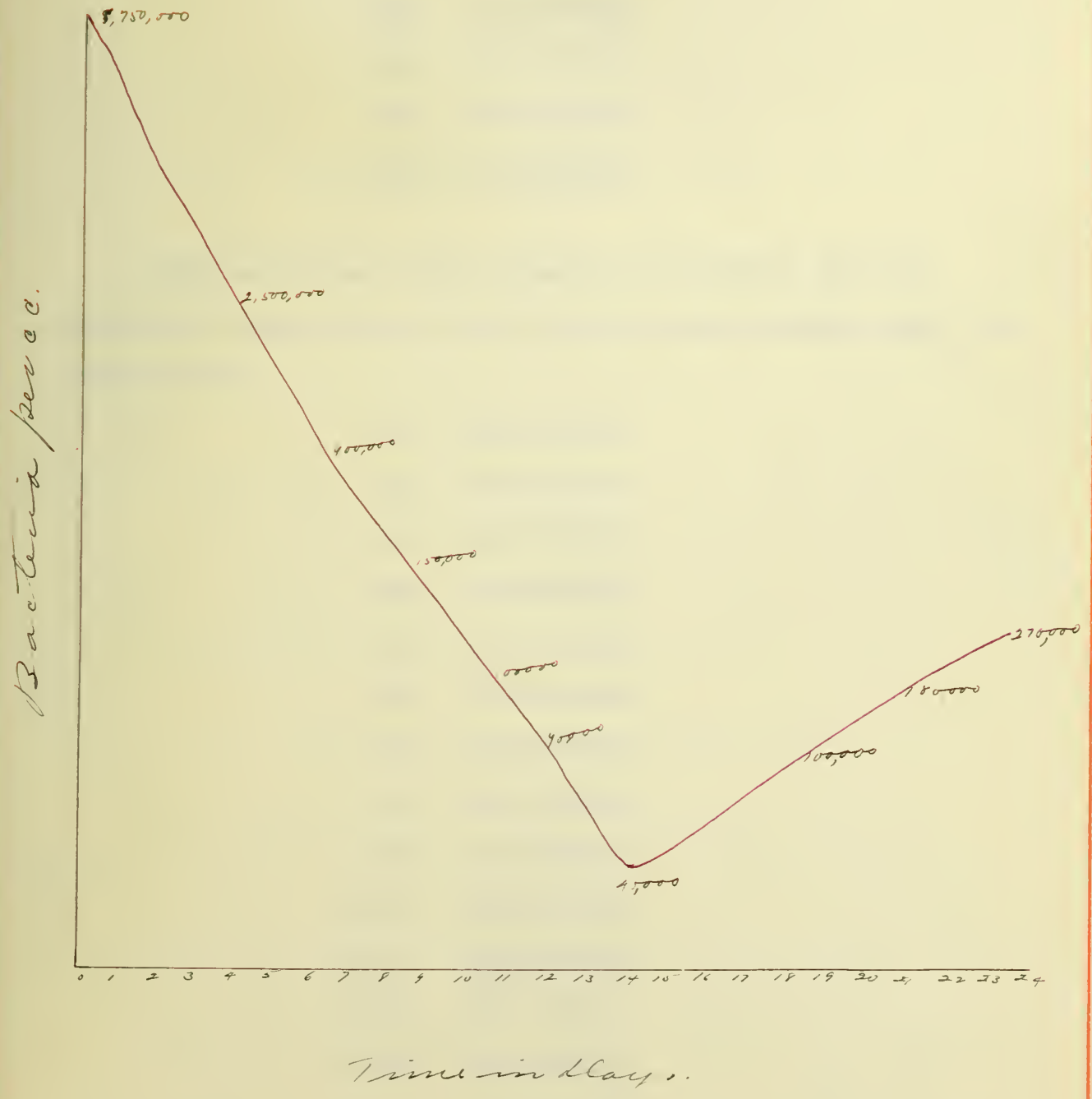

11,000,000
3,800,000
800,000
300,000
15,000
100,000
80,000
55,000
150,000
1,500,000
3,000,000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time in days.

Figure V


Bacteria per c.c.
5,750,000
2,500,000
400,000
150,000
60000
40000
4,000
100,000
180000
270,000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Time in days.

denitrifying bacteria with the following group numbers,-

(1). 111.3313013

(2). 111.1333513

(3). 111.3333023

(4). 211.2333013

(5). 211.3333023

(6). 111.3331813

From activated sludge nineteen organisms, typical activated sludge flora, were isolated upon standard agar and identified,-

(1). 111.2222012

(2). 111.2222022

(3). 111.2222033

(4). 111.2224012

(5). 111.2322022

(6). 111.3332023

(7). 111.3333513

(8). 112.2223022

(9). 112.2322013

(10). 112.2331013

(11). 112.3332013

(12). 121.1233011

(13). 121.2332512

(14). 121.2332913

(15). 122.2332523

(16). 122.3332823

(17). 212.1332513

(18). 211.3333813

(19). 222.1113022.

EXPERIMENTS ON NITRIFICATION

The bacteria isolated from the mineral broths were nitrifiers. Four days after sterile ammonia broth had been inoculated with the nitrite bacteria and sterile nitrite broth with nitrate bacteria, the ammonia broth showed a very high nitrite content with traces of nitrate and the nitrite broth showed a great reduction in nitrite and a corresponding increase in nitrate. Experiments to determine the action of these nitrifying bacteria on sewage were undertaken.

ACTION OF BACTERIA ON STERILE SEWAGE.

Fresh sewage obtained from the Champaign sewer was sterilized in 400 cc portions in "Beesley aeration flasks" at 120° for 15 minutes on two consecutative days. Various combinations of bacteria were added to the sterile sewage.

Flask 1 sterile sewage + nitrite bacteria (See Fig. 6).

" 2 " " + nitrate " (Fig. 7)

" 3 " " + nitrite + nitrate bacteria (Fig.8)

Figure VI

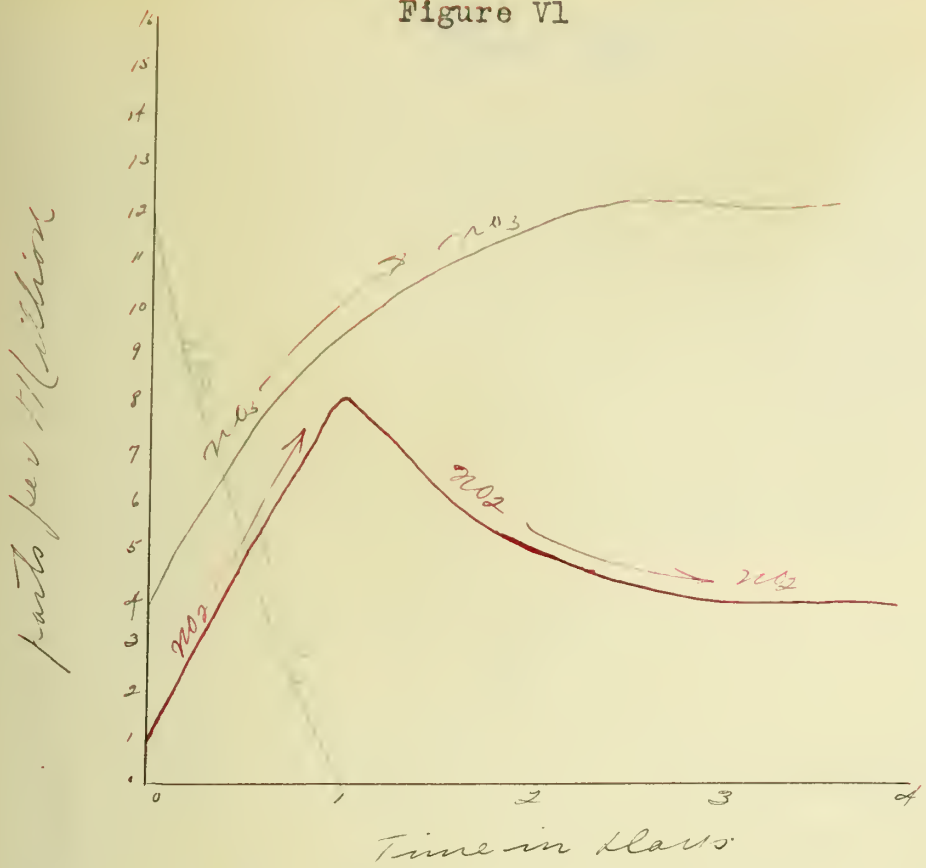


Figure VII

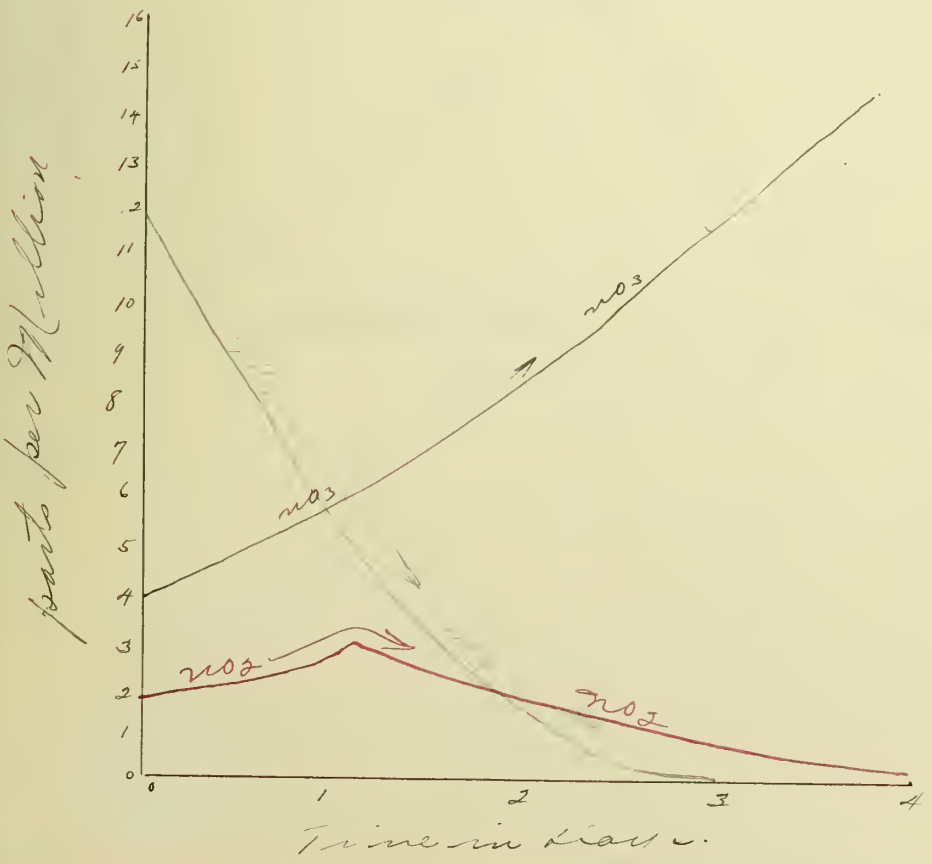
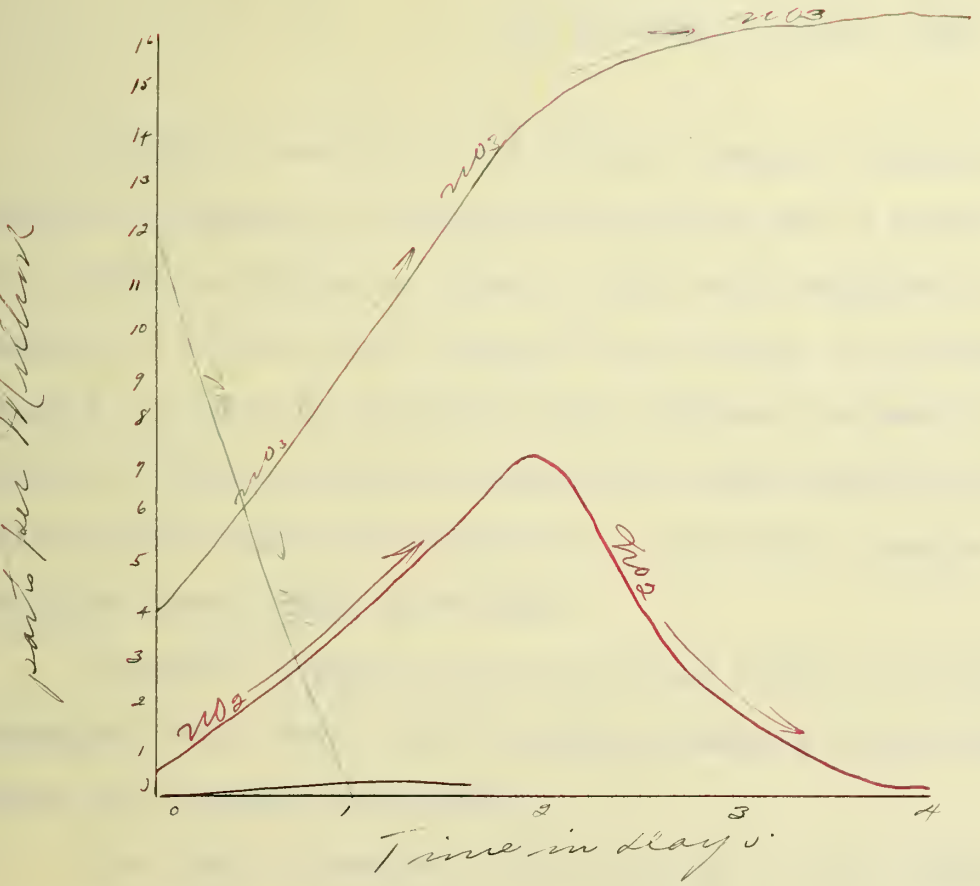


Figure VIII



Flask 4 sterile sewage as a blank or check (Fig. 9)

" 5 " " + 19 cultures + nitrite bacteria (Fig 10)

" 6 " " + 19 " + nitrate bacteria bacteria (Fig. 11).

" 7 " " + 19 cultures + nitrite + nitrate bacteria (Fig. 12).

" 8 " " + 19 cultures (See Fig. 13).

" 9 " " as a blank or check (Fig. 14).

Before inoculation the sterile sewage contained 12 parts per million of ammonia, 0.75 parts of nitrite and 4 parts of nitrate. The nitrite cultures in flask 1 which were supposed to change ammonia to nitrite also oxidized the nitrite to nitrate. In flask 2 the nitrate bacteria also oxidized the ammonia to nitrate. Because of the unexpected results the experiment was repeated twice later with similar results. A thorough examination of the cultures proved them to be pure.

Kaserer[9] claims to have isolated single nitrifying organisms which were able to oxidize ammonia to nitrate. He named the organism B nitrator.

In flask 3 complete nitrification of both ammonia and nitrite was obtained.

Flask 4 containing sterile sewage as a blank, showed practically no oxidation.

Flask 5, containing cultures of nitrite, and the 19 other organisms showed complete oxidation after a period of 3 days.

Figure IX

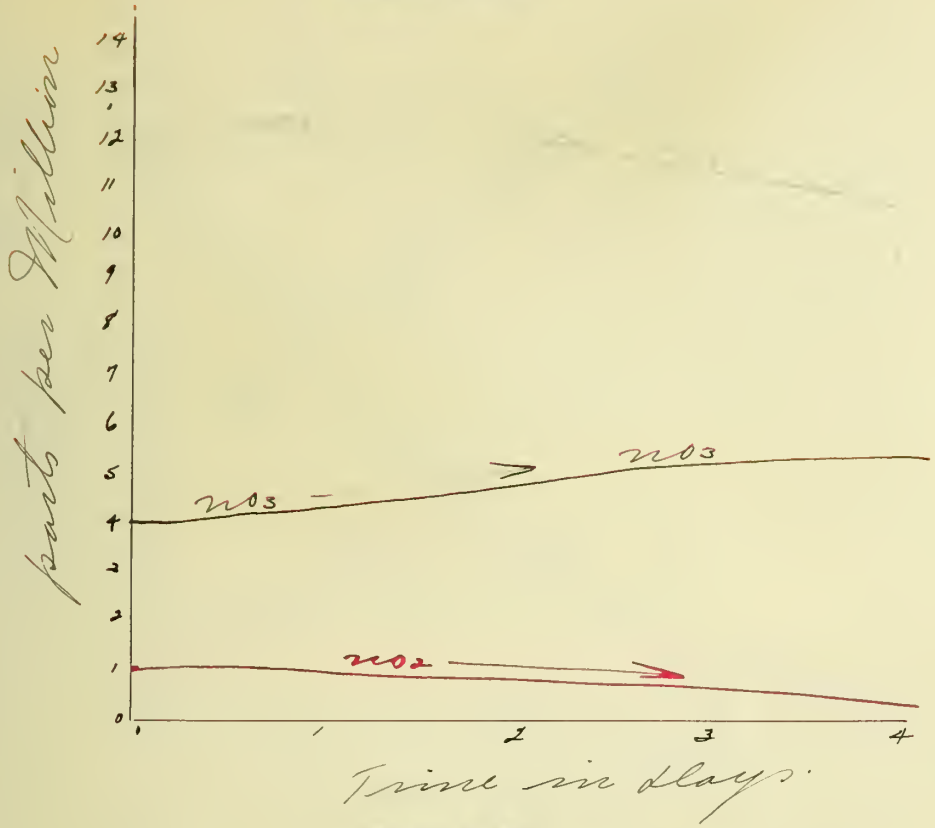


Figure X

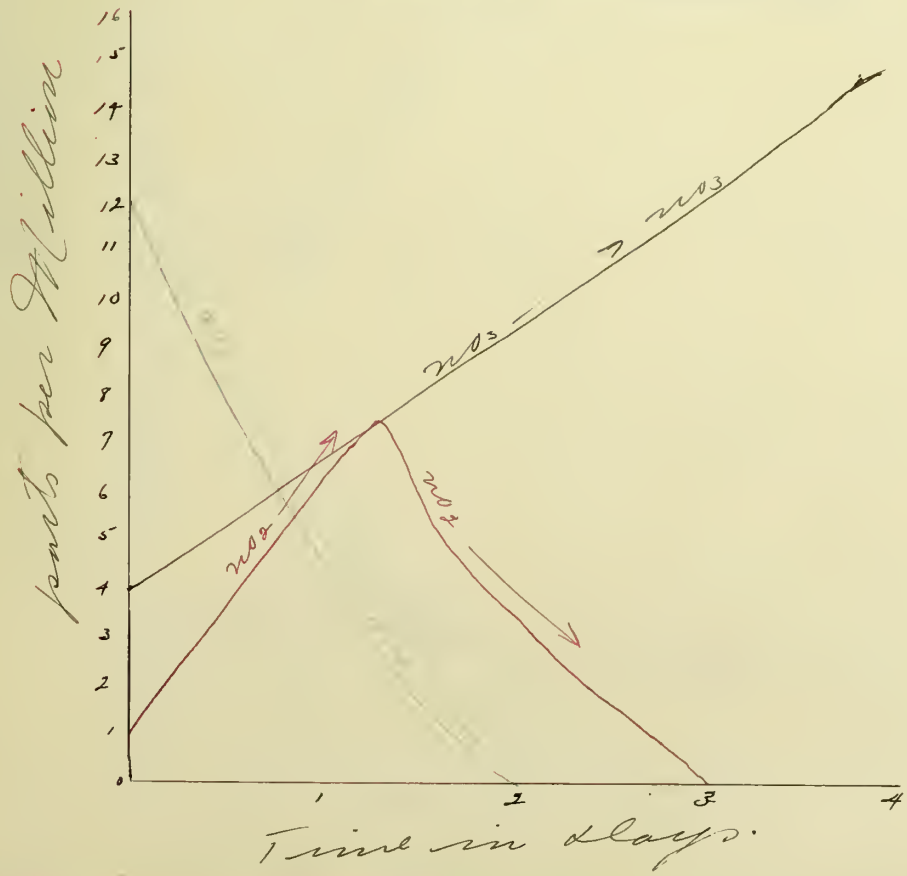


Figure XI

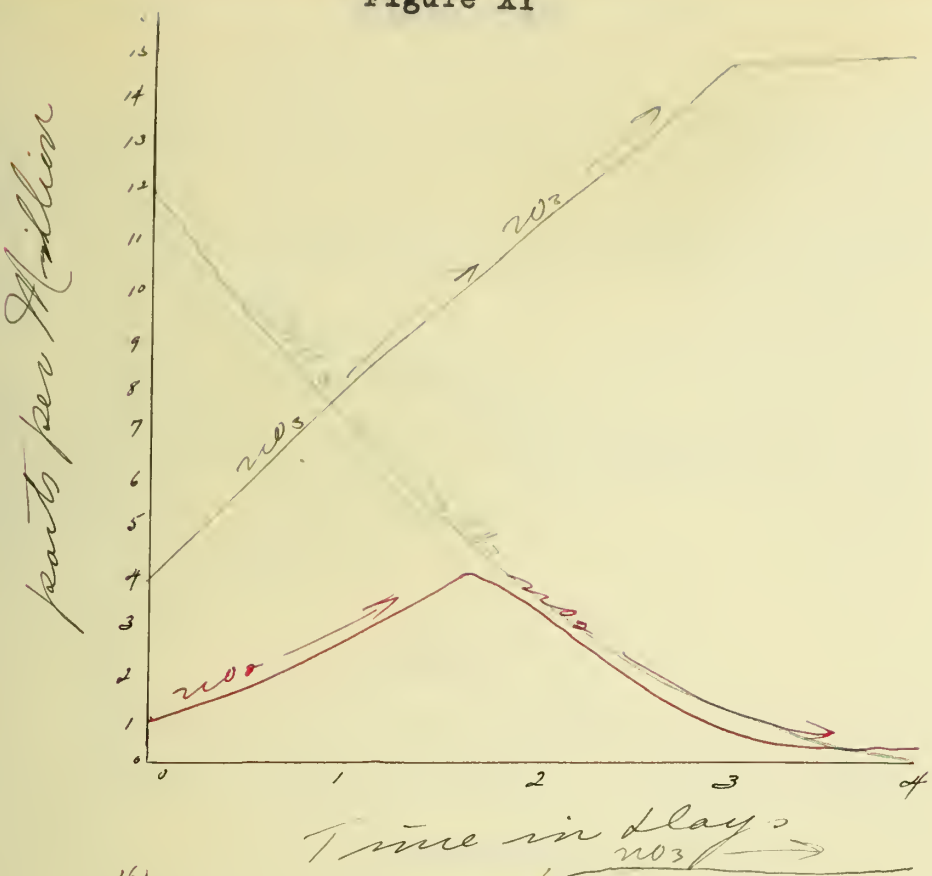


Figure XII

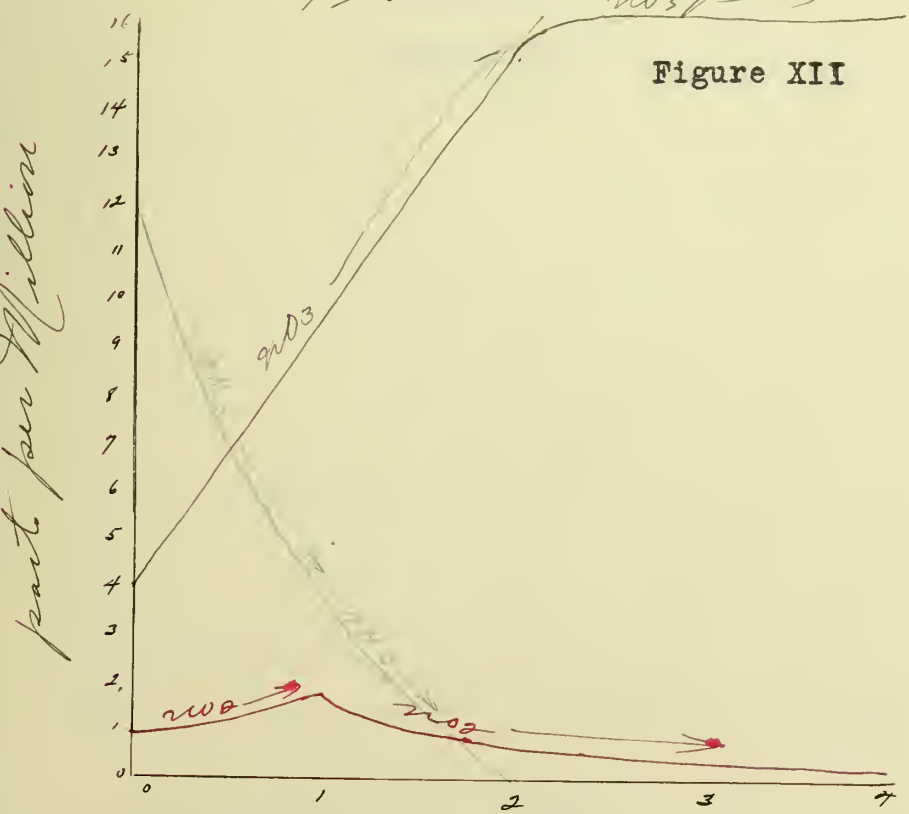


Figure XIII

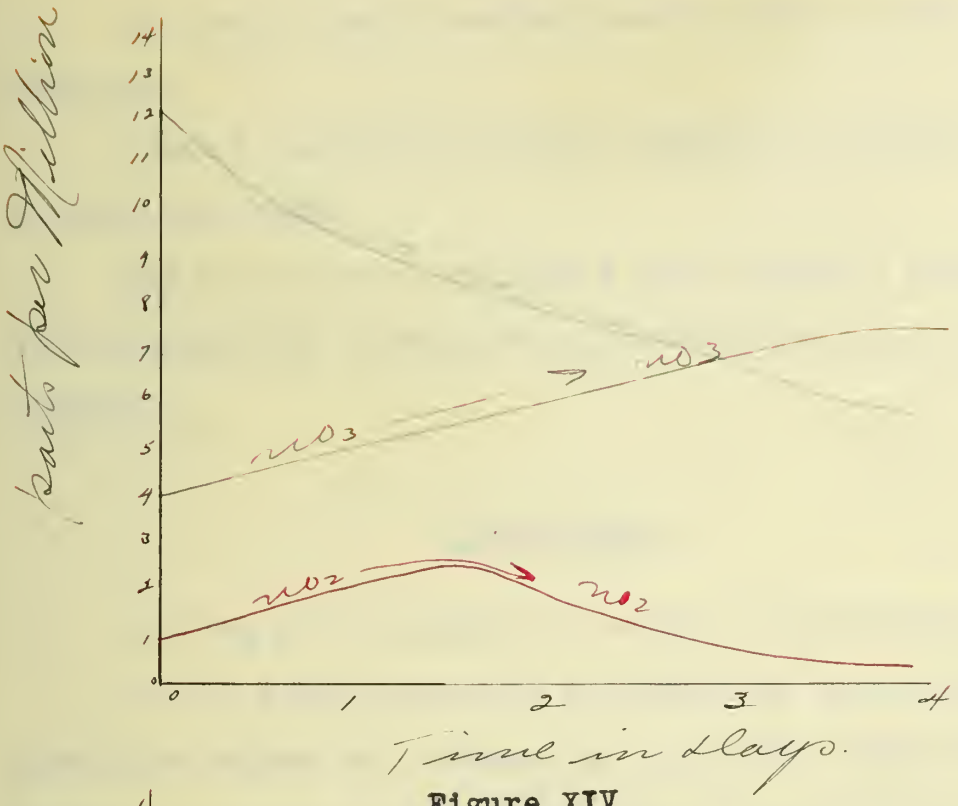
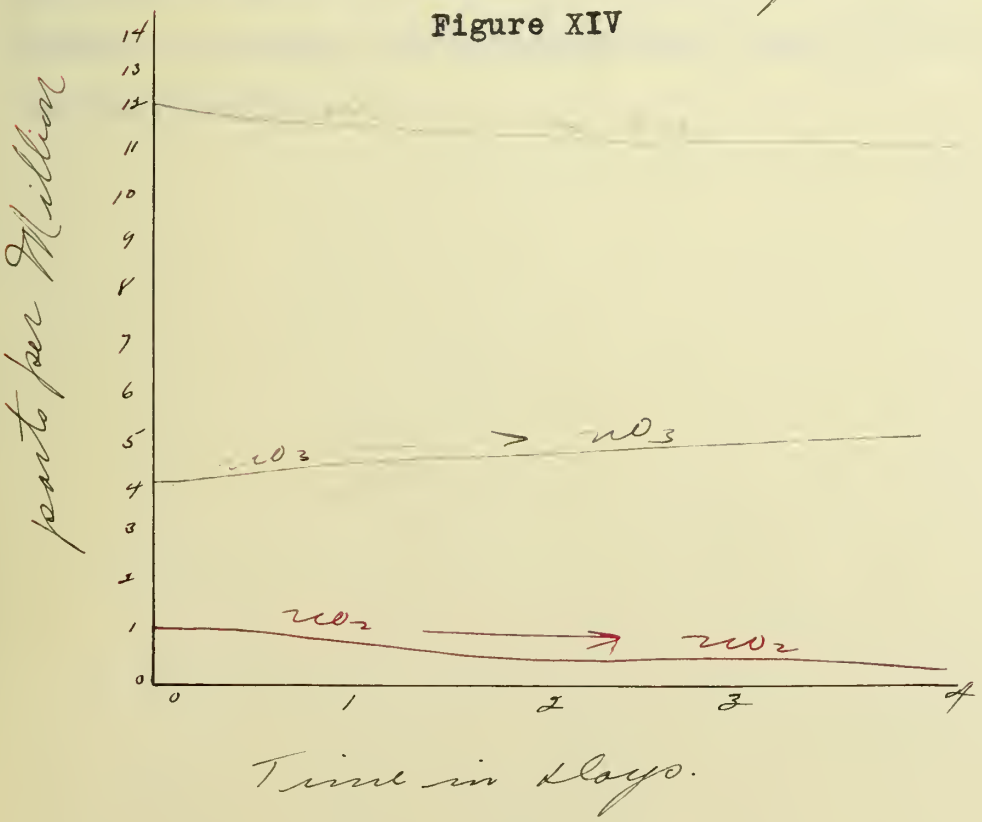


Figure XIV



In flask 6 and 7 similar results as in flask 5 were obtained.

Flask 9 containing sterile sewage as a blank showed no nitrification.

The 19 cultures in flask 8 also showed a slight amount of nitrification, showing that probably weak nitrifiers were present.

CONCLUSIONS.

a. The nitrification of sewage is produced by bacteria.

b. If sewage could be nitrified by pure cultures of nitrifiers alone on a commercial scale the sludge would be higher in nitrogen value because none would be lost through denitrification.

EXPERIMENTS ON DENITRIFICATION

As previously stated the ammonia and nitrite broths were completely nitrified and then showed a decrease in nitrate with a corresponding increase of nitrite and ammonia (Figs. 4 and 5). From agar plates inoculated with broth eight different bacteria were isolated. Because of the denitrifying action which had been going on, the cultures were inoculated into sterile standard potassium nitrate broth in fermentation tubes. After four days the potassium nitrate broth was tested for nitrite and ammonia and six of the cultures proved to be denitrifiers.

In order to determine whether the six denitrifying organism would denitrify under aerobic conditions, two flasks of sterile nitrate broth, (composed exactly like the nitrite and ammonia broth from which the cultures were isolated, except that instead of the sodium nitrite and ammonium sulphate equal amounts of sodium nitrate was substituted in both cases), were inoculated with a mixture of the 6 cultures;at the end of 7 days one of the flasks showed 5 parts per million of nitrate reduction and the other 6 parts. A blank or check showed no denitrification. Because of this slight amount of reduction it was readily seen that the organisms could not perform their function rapidly in a strictly mineral broth. Organic nitrate broths of the following composition were prepared and inoculated.

Organic Nitrate Broth

A.

0.5 gm. $NaNO_3$

0.5 gm. $MgSO_4$

0.5 gm. NaCl

0.5 gm. K_2HPO_4

3 drops $FeCl_3$ 10 per cent solution.

10 gm. $CaCO_3$

1. gm. proteim

1000 cc H_2O

Organic Nitrate Broth

B.

0.5 gm. $NaNO_3$

0.5 gm. $MgSO_4$

0.5 gm. NaCl

0.5 gm. K_2HPO_4

3 drops $FeCl_3$ 10 per cent solution.

10 gm. $CaCO_3$

1 gm. protein.

1 gm. meat extract.

1000 cc H_2O.

The presence of the organic matter in both cases enabled the bacteria to reduce the nitrate very rapidly. (Fig. 15a for Broth a and Fig. 15b for Broth b).

Similar experiments were carried out using the individual bacteria. The experiments were performed with aeration and with surface exposure. In the surface exposure experiments wide bottomed flasks stoppered with cotton plugs were used. In all cases reduction of nitrate to nitrite and ammonia resulted, however, the reduction in the aeration flask was twice as great as in the surface oxidation flasks.

DENITRIFICATION OF NITRIFIED SEWAGE.

Nitrified sewage was taken from the University aeration tanks and sterilized at 120° for 15 minutes on two consecutative days. The nitrified sewage,in order that it should not be entirely free from sludge, was taken from the tanks 10 minutes after aeration had ceased. The sample thus obtained was well nitrified and contained a small amount of finely divided organic matter in suspension.

The sterile sewage contained 14 parts per million of nitrates, 1 part of nitrite, and 2 parts of ammonia. Two flasks of the sterile nitrified sewage were then inoculated with a mixture of the 6 denitrifying cultures and aerated. The cultures in both flask of nitrified sewage produced almost identical results. At the end of 5 days the nitrate had

Figure XV a

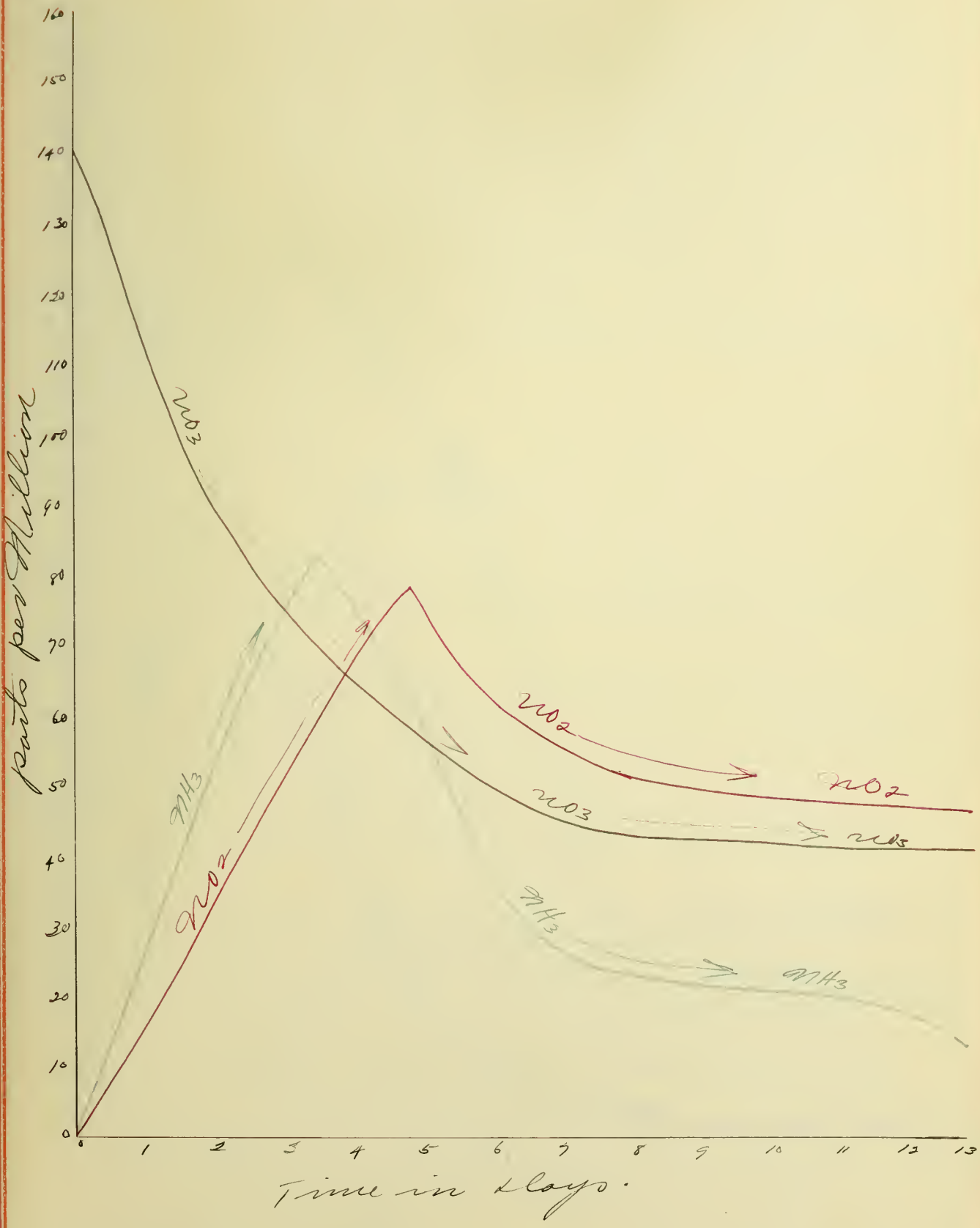
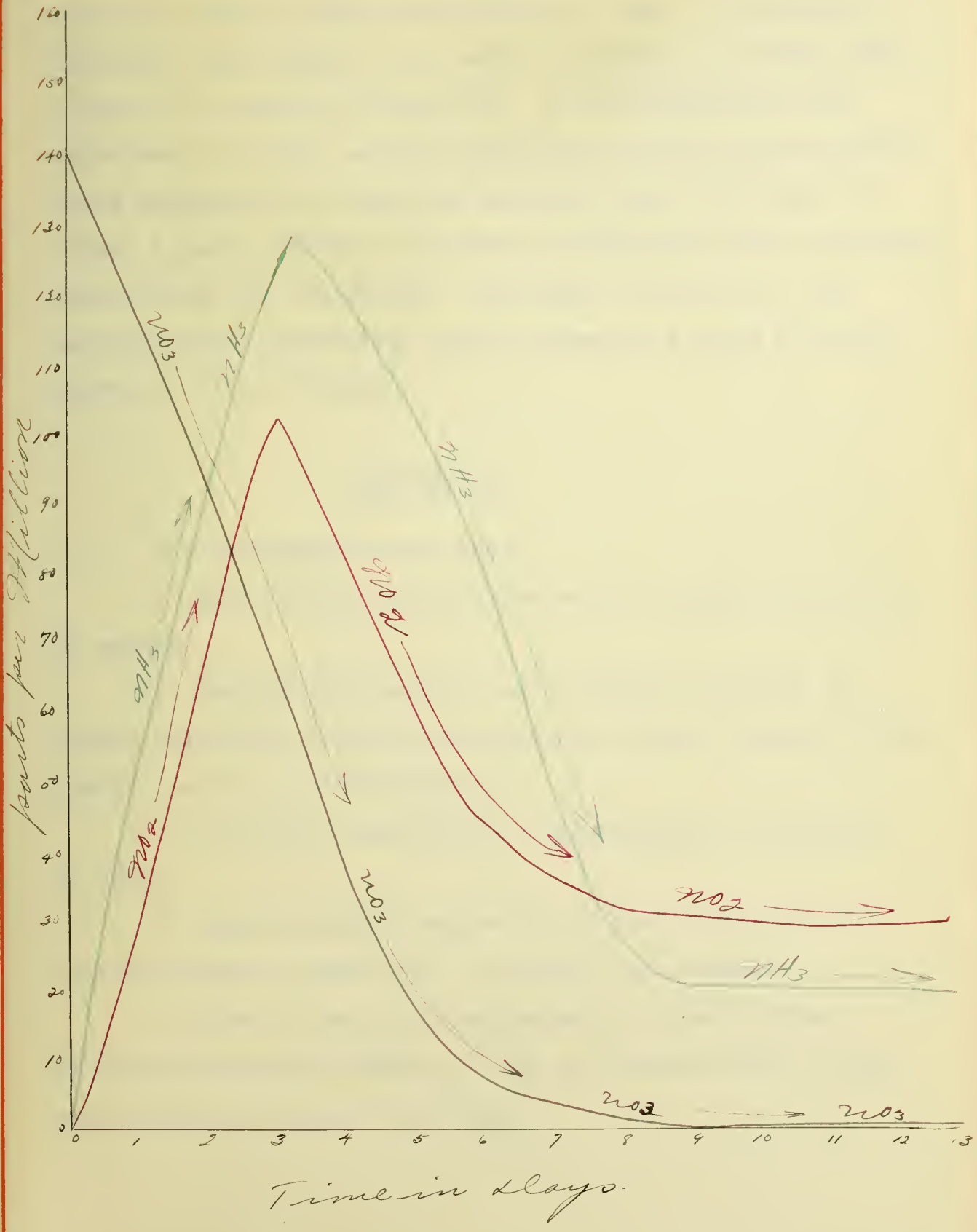

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Parts per Million
NO_3
NH_3
NO_2
NO_2
NO_3
NO_3
NH_3
NH_3
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time in days.

Figure XV b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0 1 2 3 4 5 6 7 8 9 10 11 12 13
Parts per Million
Time in days.
NO3
NH3
NO2
NO3
NH3
NO2
NH3
NO3
NO2
NO3

decreased from 14 parts per million to 1 part. The ammonia increased from 2 parts to 4, while the nitrite increased from 1 part to 2 (See Fig. 16 and 17). At the beginning of the experiment the total amount of nitrogen present in the various forms amounted to 17 parts per million, while at the end of 5 days 7 parts remained. The form in which the loss of nitrogen resulted was not determined. (See Figs. 16 and 17). The aeration flask containing sterile sewage as a blank or check showed no denitrification.

CONCLUSION.

The experiment proves that,-

a. Denitrifying bacteria reduce the nitrate and nitrite in sewage.

b. Denitrifying bacteria are constantly present in sewage, but their action is usually not noticed because of the greater amount of nitrification.

c. In all the experiments a large amount of nitrogen is lost.

d. Denitrification may be one of the reasons why the aeration tanks at times fail to nitrify the sewage.

e. This is the first mention made of denitrifying bacteria having been proved to play an important role in the purification by means of aeration.

Figure XVI

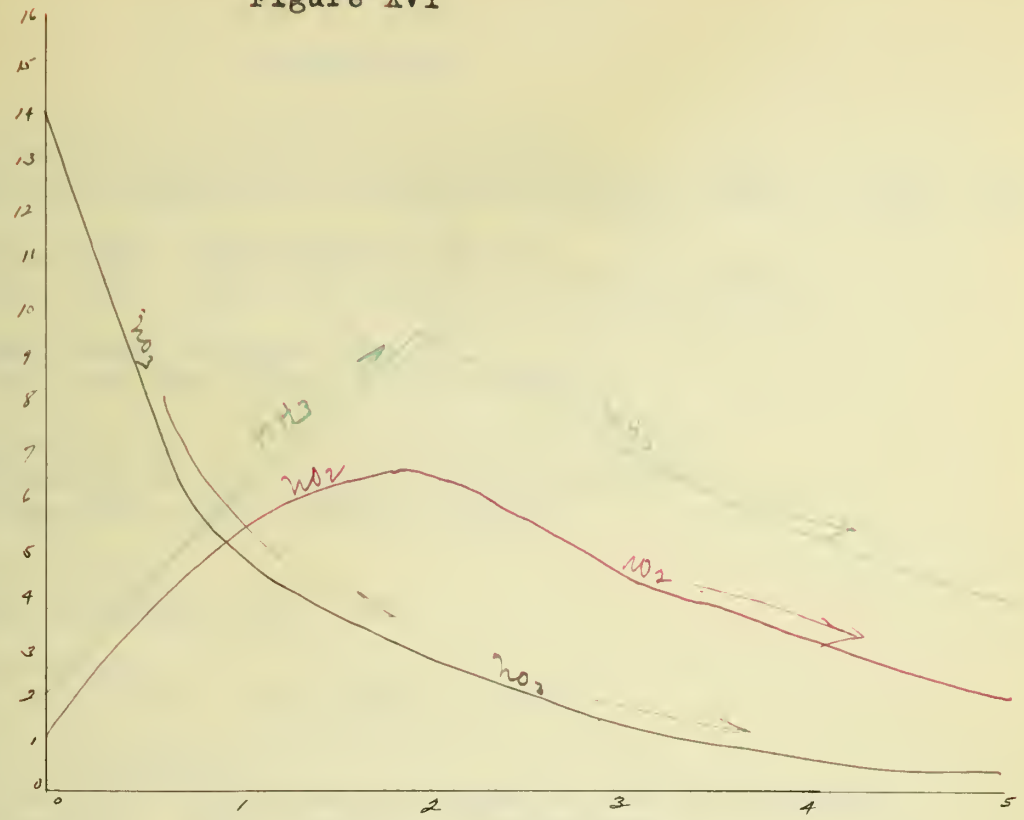
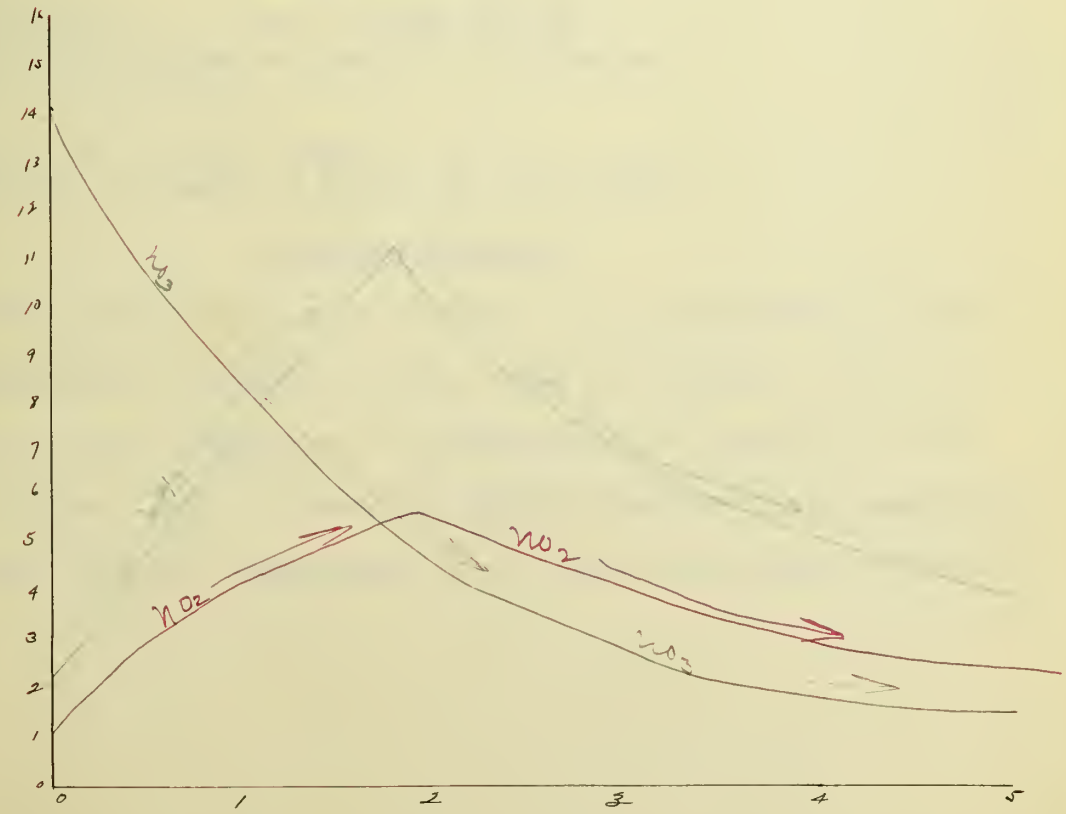


Figure XVII



REFERENCES.

8. American Public Health Association.
Report of Committee on Standard Methods, 1905, p. 105, 108.

2. Ardern, Edw., and Lockett, Wm. T.,
J. Soc. Chem. Ind., 33, 523-39, 1122-24 (1914).

3. Bartow, E.and Mohlman, F. W.
J. Ind. and Eng. Chem. 7, 318 (1915).

6. Beesley, R. M.
Experiments on the Rate of Nitrification.
J. Chem. Soc. 105, 1014-57 (1914).

1. Fuller, G. W.
Sewage Disposal,1912.

4. Fuller G. W.
Eng. News, Vol., 74, 1146-47 (1915).

9. Kaserer, Hermann.
Ueber einige neue Sticktoffbakerien mit autotropher Lebensweise. Zeit. land iv. Versuchsw.
Oestrreich 10, 37-42, 1907
Chem. Centr. 87, 1, 652 (1907).

5. Russel, R.
A New Method of Water Purification.
Thesis for degree of Master of Science.
Uni. of Ill., 1915.

7. Stevens, F. L. and Temple, J. C.
Centralb. f. Bakt. 2 Abt., 21, 84 (1908).

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