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# GROWTH PATTERNS OF BACTERIA

# IN PRESSURIZED SEWAGE

ΒY

# DENNIS MAZZEI

# A THESIS

# PRESENTED IN PARTIAL FULFILLMENT OF

### THE REQUIREMENTS FOR THE DEGREE

# OF

#### MASTER OF SCIENCE

# AΤ

# NEW JERSEY INSTITUTE OF TECHNOLOGY

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Newark, New Jersey 1977

#### ABSTRACT

Primary sewage was pressurized at 100 psi for one hour. The effects of this pressure on the growth patterns of general and nitrifying bacteria were examined daily for eighteen consecutive days using Standard Plate Counts and Most Probable Number (MPN) estimates. In conjunction with these tests, the degree of biodegradability was also determined by measuring both daily biochemical oxygen demands (BOD's) and dissolved oxygen levels.

Graphical analysis of experimental data compare the BOD's exterted by microorganisms to their respective numbers of colonies as determined by plate counts of both pressurized and non-pressurized sanitary sewage diluted to 3.00%. The analysis of the combined data revealed the following: (1) pressurization increased the initial population of general bacteria by 55% and 100%, and (2) the second-stage BOD's of pressurized sewage which began three to four days earlier than that of the non-pressurized sewage was reduced by as much as 17.7%.

In addition, the effects of pressure on the population growth of nitrifying bacteria were examined. Serial 10-fold and 5-fold dilution-to-extinction techniques were use to obtain the MPN estimates of the  $NH4^+$ -oxidizers group. This is a common indirect method used to enumerate the population growth of nitrosomas bacteria.

ii

The graphs of the MPN's indicate that the population and the rate of growth of nitrosomas bacteria were greater in the pressurized sewage. Similar tests to determine the growth patterns of nitrobacter bacteria failed.

It was concluded that pressurization initiates the propagation of accelerated bacterial growth, thereby increasing the assimilative capacity of the bacteria and reducing biodegradability of sanitary sewage. APPROVAL OF THESIS

GROWTH PATTERNS OF BACTERIA IN

PRESSURIZED SEWAGE

BY

DENNIS MAZZEI

FOR

CIVIL AND ENVIRONMENTAL ENGINEERING

ΒY

FACULTY COMMITTEE

APPROVED:

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NEWARK, NEW JERSEY MAY, 1977

#### PREFACE

Since 1972, graduate environmental engineering students at the New Jersey Institute of Technology have been measuring the overall biodegradability of sanitary sewage exposed to varying pressures in terms of biochemical oxygen demand, chemical oxygen demand, and dissolved oxygen.

The results of the reasearch, in part, indicate that the rate of organic degradation of sanitary sewage by microbial activities is increased in the presence of moderately low pressures. Specifically, the beginning of the secondstage biochemical oxygen demands (BOD's) of pressurized sewage began three to four days earlier and was reduced by as much as 25% less than that of the corresponding BOD's of the non-pressurized sewage.

Early in 1976, Dr. Su Ling Cheng, the thesis advisor, suggested continued research in this area should begin to examine the growth patterns of microorganisms in pressurized sanitary sewage.

This thesis, a partial fullfillment of the requirements of the Master Degree of Science, is a study of the growth patterns of bacteria and its relationship to the biochemical oxygen demands of sanitary sewage subjected to 100 psi of pressure with an one hour detention time.

v

The conclusion that pressurization can be used to reduce the biodegradability of sanitary sewage by increasing the initial bacterial-population growth was based on experimental evidence.

It is hoped that the reproducibility of the experimental day generated in this thesis will be tested by other outside independent researcher and perhaps spark enthusiasm for further investigation.

At this point I would like to acknowledge my sincere graditude and respect to Dr. Cheng. His expert guidance and seemingly unlimited resources provided the strength needed to bring this research to a successful conclusion.

On occassions, Dr. Eugene Golub, now department chairman, and Dr. Robert Dresnack discussed the course of research during its development and provided technical assistance.

To my wife Arunee, this paper is dedicated.

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### GENERAL OUTLINE OF EXPERIMENTS

# Part I

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Dummere

e	rur pose:	bacteria in non-pressurized and pres- surized sewage samples diluted to 3.00, 0.10, 0.01 and 0.001%'s
II.	Source:	Effluent end of primary settling tank at Tanglewood Lane Sewage Treatment Plant in Chatham, New Jersey
III.	Tests:	a. BOD b. DO c. Standard Plate Count
IV.	Graphs:	<ul> <li>a. BOD curves</li> <li>b. D0 levels</li> <li>c. Growth patterns of general bacteria diluted to: <ul> <li>1. 3.00%</li> <li>2. 0.10%</li> </ul> </li> </ul>

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# Part II

I.	Purpose:	To first confirm the results of Part I
		and then to examine the growth patterns
		of nitrifying bacteria in pressurized and
		non-pressurized sewage diluted to 3.00%

3. 4.

- II. Source: Effluent end of primary settling tanks at the Water and Wastewater Treatment Plant in Verona, New Jersey
- III. Tests: a. BOD
  - b. DO
    - c. Standard Plate Count
    - d. Multiple-tubes; Serial 5-fold and 10-fold dilution-to-extinction

0.01%

0.001%

- IV. Graphs: a. BOD curves
  - b. DO levels
  - c. Growth patterns: 1. general bacteria 2. nitrifying bacteria
  - d. All the above excepting b. for pressurized samples
  - e. All the above for non-pressurized samples

#### INTRODUCTION

#### Background Information

Experimental evidence during the last few years by independent researchers has tended to confirm the fact that significant reductions of organic matter in sanitary sewage can be obtained by pressurization.

In 1960, researchers at Purdue University compared the effects of limited and unlimited supplies of oxygen upon pressurized and non-pressurized samples of diluted sewage.<sup>1</sup> During each experiment one group of test samples received an ample supply of oxygen with a flow rate of two cubic feet per hour while another group, subjected to the same pressure, received a limited supply of oxygen with a flow rate of 0.5 cubic feet per hour. The samples and the controls were analyzed for chemical oxygen demands (COD's) and dissolved oxygen (DO's) levels after a threehour detention time and at further predetermined intervals.

The results indicated that the pressurized samples which received the ample supply of oxygen did not show any improvement compared to the control samples; however, among the oxygen-limited samples, reduction of organic

<sup>&</sup>lt;sup>1</sup> Richard Lawrence, Robert Reynolds, and Robert Schilling, "The Effects of Pressure on the Degradation of Organic Wastes by Microorganisms," paper presented at the Middle West Regional Conference of Chemical Engineers, 1971, p.11 ff.

matter was doubled under pressure, and the overall percentage of organic matter was significantly less than that of the original control samples.<sup>2</sup>

From the results of the experiments, Lawrence et al. concluded that increased reduction of organic waste in an oxygen-limited environment is attributed in part to the stimulating effects of pressure upon the activity of microorganisms.<sup>3</sup>

John Chack, who was the first of a series of researchers from the New Jersey Institute of Technology to study the effects of pressure upon diluted sewage samples.<sup>4</sup> Samples were placed in compressible plastic bottles at pressures of 100, 400,700, 1000 and 1500 psi in a high pressure simulator for periods of one, two and three days. Chack concluded that for a given detention time the oxygen demand doubled or tripled under the influence of pressure.<sup>5</sup> But, although the oxygen demands of the pressurized samples were greater, the total demand approached that of the control samples. The maximum rate of oxygen consumption

3<sub>Ibid</sub>.

<sup>4</sup>John Chack, "Biodegradability and Coliform Die-away in a High-pressure Ocean Environment" (master's thesis, New Jersey Institute of Technology, 1972), p.19.

<sup>5</sup>Ibid., p.20.

<sup>&</sup>lt;sup>2</sup>Lawrence, p.14.

occurred at 100 psi and decreased slightly with higher pressures until it reached that of the control samples.

In 1975, Nusser conducted another series of tests to determine the effects of moderately increased pressures at different detention times upon diluted sanitary sewage saturated with dissolved oxygen.<sup>6</sup> Samples were subjected to pressures ranging from 29.4 to 100 psi of pressure for periods of ten, thirty, and sixty minutes in a modified Triaxial Soils Testing Cylinder connected to a Self-Compensating Mercury Control Apparatus. The dissolved oxygen (DO) levels and the biochemical oxygen demands (BOD's) were recorded daily for 17 to 21 days. The results indicated that the second-stage BOD's samples pressurized at 100 psi began three to four days earlier than the control samples did, and that the maximum first-stage or carbonaceous BOD was as much as 22.7% less than that of the non-pressurized control samples.<sup>7</sup>

Nusser concluded that pressurization decreases the biodegradability of sanitary sewage. The rate of bacterial decomposition increased with pressure, indicating greater assimilation of waste by bacteria. It is obvious that some difference between pressurized and non-pressurized

<sup>&</sup>lt;sup>6</sup>D. Nusser, "The Effects of Pressure on the Biodegradability of Sanitary Sewage" (master's thesis, New Jersey Institute of Technology, 1975), p.22.

<sup>&</sup>lt;sup>7</sup>Ibid., p.27.

populations of bacteria must be responsible for the differences in first - and second-stage BOD curves.<sup>8</sup>

Only Chack, however, experimented with actual population growths of bacteria, but his study was limited to the coliform group.<sup>9</sup> Therefore, the experiments that follow are for the purpose of finding the cause of the growth abnormality of: (1) the bacterial density of sanitary sewage as determined by Standard Plate Counts which represents a general population of bacteria, and (2) autotrophic nitrifying bacteria in sanitary sewage, specifically, the nitrosomas and nitrobacter bacteria.

<sup>8</sup>Ibid., p.32. <sup>9</sup>Chack, p.35.

## PART I

#### Experimental Objective

The main objective of this part of the study was to examine the influence of pressure upon the population growth of general (gross) bacteria. Studies by two independent researchers have concluded that sewage samples subjected to 100 psi of pressure had reduced the BOD of sanitary sewage by as much as 25%. <sup>10</sup>

In addition, Nusser observed sewage samples which were pressurized to 100 psi for one hour, maintaining a 0.45 ratio of air to sewage by volume and subsequently diluting the samples to 3.00%, producing the BOD curves in Figure 1, below.<sup>11</sup> From the BOD curves, Nusser concluded that the



<sup>10</sup>Nusser, p.51. 11 Ibid., p.32.

second rise in the graph or the second-stage BOD began on the thirteenth day in the pressurized sample. three days before it did in the non-pressurized control sample, It was also 15.5% less than that of the control sample. Each point on both BOD curves was calculated from the average of two DO tests per sample per day. Thus, a total of four DO tests were taken per day for twenty consecutive days.

Nusser's experiment was repeated in the beginning of Part I, indicating the reproducibility of the curves and providing bacteria of pressurized and non-pressurized sewage diluted to 3.00%, (These test samples were further diluted to 0.01%, 0.01% and 0.001%.) Coinciding with the DO test was the daily preparation of three replicates of each dilution for eighteen consecutive days. This wide range of dilutions was to provide plate counts within 30 to 300 colonies as recommended by Standard Methods for the Examination of Water and Wastewater. 13 Each Plate was counted 24 hours after incubation on a darkfield Quebec colony counter with the aid of a hand counter. Three points each representing one of three separate plate counts of nonpressurized sewage sample, and three more points representing three separate plate counts of pressurized sewage sample of the same dilution were plotted on the day which they were plated. These plates were then discarded and new plates were inoculated with samples which had been incubated

<sup>&</sup>lt;sup>13</sup>American Public Health Association, et al., 13th ed. (Washington, D.C., 1974), p. 661.

in airtight vials at the beginning of the experiment. A line on the graph connecting the mean number of colonies in pressurized sewage between each two consecutive days represents the growth pattern of that sample for that 24-hour period. The growth patterns of the entire population of pressurized and non-pressurized samples was represented by a separate graph for each dilution.

Relationships existing between the growth patterns of the BOD curves are examined graphically in the topic of Discussion in Part I.

# Source of Sample

Wastewater was obtained from the Tanglewood Lane Sewage Treatment Plant in Chatham, New Jersey, a small plant that provided tertiary treatment to domestic sewage at a rate of 3.5 million gallons a day.

Five gallons of primary sewage which passed over the weirs of the primary settling tank was collected in a large plastic container. The cold month of February provided excellent refrigeration during transportation.

#### Apparatus

Sewage samples were pressurized in a modified Wykeham Farrance Model WF1020 Triaxial Solis Testing Chamber (Figure 2). A Wykeham Farrance Self-compensating Mercury Control Apparatus Model WF12760 (Figure 3). connected to the testing chamber, was used to maintain a constant  $100 \pm 0.05$  psi of pressure.

A modified pressure cooker was substituted for an autoclave. The cover portion was equipped with a safety valve, manual pressure-release valve, and gauge to measure both temperature  $(F^{O})$  and pressure (psi). The pressure was controlled by regulating the temperature of two large electric heaters in conjunction with the pressure-release valve.

An Isotemp<sup>®</sup> incubator by Fisher Scientific was used to incubate test samples. This incubator with a 2.5 cubic foot capacity, Model Number 302, provided ample space and good sensitivity - less than  $\pm$  0.5° change when maintained at 37°C.

A darkfield Quebec colony counter and a small hand counter were used to determine Standard Plate Counts. More than seven hundred disposal plastic petri dishes, size 100 X 15mm, were used for the experiments in Part I.

# Chemicals

1. Dehydrated tryptone glucose-extract agar was used to prepare standard plates.

2. Hach chemicals were used to determine the dissolved oxygen level.





Operational Procedure for Wykeham Farrance Self Compensating Mercury Control Apparatus Model WF1276

# Preliminary Steps:

- 1. Close all valves
- 2. Turn on city water; open valve 1
- 3. Using rachet winch, lower pot A
- 4. Fill cylinder by turning piston
- 5. Close valve 1; open valve 5
- 6. Force water into pot D by turning screw piston, this raises mercury into pot D to pot B and forces water into pot C. Mercury in pot C will rise halfway into pot A.
- 7. Valve is closed at proper mercury level

# Pressurizing Steps:

- 1. Open valves 1 and 9; read pressure on ten inch dia. Bourdon gauge (lbs./sq.inch) 2. Close valve 1; open valve 5
- 3. Open valve 8
- 4. Open valves 10, 11 and SI valve; fill chamber and close all valves
- 5. Close valve 1; open valves 5, 6, 8 and PI. Close valve 5 at desired pressure

Figure 3

# Procedures

Preliminary Preparation. Two hundred glass containers similar to small BOD bottles were needed for the first part of this experiment. Each container was to contain one of eight different dilutions (4 pressurized and 4 non-pressurized) of wastewater samples, incubated from one to twenty days.

Small 12-ml vials with plastic closures, originally part of Hach Chemicals' Coliform Presumptive Test Kits, were used. They were washed with chromic sulfuric acid, a glass cleaning solution, and rinsed throughly with distilled water. The vials, with closures screwed loosely on, were autoclaved at 20 psi for ten minutes. Disposable sterile plastic gloves were worn while handling sterlized glassware.

Eighty BOD bottles were washed with glass cleaning solution and rinsed with distilled water. Two BOD bottles for each pressurized and non-pressurized samples diluted to 3.00% were required for a period of twenty days.

Two large 13-gallon plastic containers, each with a spigot at the bottom, were cleaned and rinsed with distilled water. In each container was placed 13.33 liters of dilution water. The dilution water was prepared in accordance with <u>Standard Methods for the Examination of</u> <u>Water and Wastewater</u> by adding one milliliter of the following chemicals to each gallon of distilled water: magnesium sulfate, phosphate buffer solution, calcium chloride, and ferric chloride.<sup>14</sup> Air was pumped into the dilution water in each container just prior to the addition of the wastewater sample. A total of thirty gallons of distilled water was required to rinse the necessary glassware and to prepare all dilutions needed for this experiment.

Pressurization. A portion of the collected sample was poured into a large perspex holding container with a spigot at the base, elevated above the modified triaxial compression chamber. Primary sewage flowed from this container by gravity via polyvinyl chloride tubing into the base of the chamber. The perspex chamber, graduated from the top in increments of 25 ml., has a total volume of 1170 ml before expansion of the latex membrane. The chamber was filled with 805 ml of wastewater, leaving 365 ml of air above to be compressed. A 0.45 ratio of air to effluent by volume was maintained during pressurization. Experimental evidence indicates that this ratio of air to wastewater provides microorganisms with a sufficient amount of oxygen at 100 psi of pressure for one hour.<sup>15</sup> A large glass jar with a metal cap was also filled with a 0.45 ratio of air to effluent at normal atmospheric pressure.

Operational procedures for pressurizing samples are

<sup>14</sup>American Public Health Association, et al., p. 491. <sup>15</sup>Nusser, p. 32.

listed step by step in Figure 3. Pressure is supplied to the effluent in the chamber by expansion of the latex membrane. Approximately 40 psi of pressure was provided by the city water line and another 60psi from distilled water that flowed from the elevated dash pots, located 10 feet above the chamber, into the latex membrane. Valves 5, 6 and 8 (see Figure 3) were closed when 100 psi was maintained on the Bourdon gauge. The chamber was inverted every five minutes, preventing suspended solids from settling to the base of the chamber during one hour of pressurization.

<u>Plating (General Bacteria)</u>. Four hundred milliliters of plate count agar had been prepared in accordance to instruction printed on the package of dehydrated tryptone glucose-extract agar medium before pressurization was completed. This agar solution and at least eight onemilliliter pipets, one for each dilution, were autoclaved at 21 psi of pressure for fifteen minutes.

Thirty-two disposable sterile plastic petri dishes, size 100 X 15mm, enough to prepare three replicate plates for 4 pressurized and 4 non-pressurized dilution samples, for plating. More than seven hundred petri dishes were used during 21 days of testing.

After one hour of pressurization, the pressure in the chamber was released by opening the upper air valve (number

11 in Figure 3), and the metal cap was removed from the jar containing the control sample.

Three dilutions of pressurized and three matching dilutions of non-pressurized samples were prepared by adding exact amounts of raw sewage to pre-measured volumes of dilution water. The amount of sewage required to prepare each dilution is listed in a chart located in the appendix on page 48.

Each of the thirty-two plates was prepared by pipetting one milliliter of well-mixed dilution sample into a petri dish. In addition, control plates with plain agar and agar with one milliliter of distilled water were also prepared. The covers on the petri dishes were lifted with care, permitting only the tips of the pipets to enter without touching. The cover of each dish was then closed promptly after receiving the sample. Only one sterilized pipet was used per dilution of each type of sample.

The pour plate technique was used. Approximately ten milliliters of warm agar was throughly mixed with each sample by tilting and rotating the bottom of each dish.

An erlenmeyer flask of warm agar, stoppered with a piece of sterile cotton, was place in a water bath and maintained at a constant temperature of  $45^{\circ}$ C during plating. Each plate was then placed in an incubator

preheated to 37°C.

Forty of the eighty BOD bottles were filled with 3.00% dilution of pressurized sewage which was contained in one of the two large thirteen-gallon plastic jugs. The remaining forty bottles were filled with 3.00% dilution of non-pressurized sewage. All the bottles were water sealed with plastic caps.

A total of one hundred and eighty-fortyvials was separated into eight sets of twenty-three vials, each set corresponding to one of the eight different dilutions. the vials were opened and each filled with diluted sewage sample to a maximum capacity. The caps, inverted and also filled were carefully screwed onto the vials, preventing air bubbles. The vials were place in a prone position and incubated at 37°C.

The azide modification of the Winkler Method with phenyl arsenyl oxide (PAO) as the titrant and starch as the indicator was used to determine the DO of pressurized and non-pressurized sewage immediately after pressurization, just a few minutes prior to plating. Four DO tests were taken, two for each sample. Both plate counts and DO's were continued at the same 24-hour intervals for the duration of the experiment.

The petri dishes were removed from the incubator every 24 hours after plating. Standard Plate Counts of general bacteria were made on a darkfield Quebec colony counter with the aid of a small hand counter. The colony counter divides the petri dish into 56 equal square areas and further subdivides a select few into 9 smaller sections. All cultures of 200 colonies or more were calculated from the mean number of colonies in three representative subsections times its appropriate multiplication factor. Each dilution of both pressurized and non-pressurized samples was represented by three replicate plates. Control plates with plain agar and agar inoculated with plain distilled water were also examined for contamination. The mean valve of these plates was recorded as the general population growth for that particular dilution for that day.

# Discussion

The BOD curve of pressurized primary sewage diluted to 3.00% and the BOD curve of its corresponding nonpressurized control are represented in Figure 4.

As indicated by the curves, the second-stage BOD of the pressurized sewage began on the seventh day, three days earlier than that of its non-pressurized control. This three-day difference between the beginning of the second-stage BOD of pressurized and non-pressurized sewage was also observed by Nusser. In addition, the 16.8% reduction of first-s age or carbonaceous BOD obtained is within  $\pm$  5.0% of those observed by Nusser.<sup>16</sup>

<sup>16</sup>Nusser, p.51.



However, the second-stage BOD as indicated by Nusser's curves began on the tenth instead of the seventh day for the pressurized sewage and the thirteenth instead of the tenth day for the non-pressurized sewage. This may be due to differences in graphical interpretations. The first and - second-stage BOD's of the non-pressurized sewage were consistently higher than those of the pressurized sewage throughout the entire eighteen consecutive days of testing.

The growth patterns of general and nitrifying bacteria in pressurized and non-pressurized sewage diluted to 3.00% are represented by the graph in Figure 5. Primary growth-the highest peak--in the non-pressurized sewage reached 32,000 colonies per ml of sample on the ninth day. The next prominent peak to occur after the highest peak is referred to as the secondary growth in this study. Interestingly, both the second-stage BOD (Figure 4) and secondary growth of the non-pressurized sewage began on the tenth day (Figure 5).

The largest number of colonies of pressurized sewage diluted to 3.00% in Figure 5 is represented as a plateau during the first two days after pressurization. The population declined rapidly from the second to third days and continued as a stable population of relatively few colonies for the following three days. Secondary

# General Population of Non-pressurized & Pressurized Samples Diluted to 3.00%



FIGURE 5

growth of the pressurized sewage began on the sixth day (Figure 5), just one day before the beginning of its corresponding second-stage BOD (Figure 4).

Secondary growth of the pressurized sewage began almost four days earlier than that of the non-pressurized sewage (Figure 5), and also the second-stage BOD of the pressurized sewage (Figure 4) began three days earlier than that of the non-pressurized sewage. Both the secondary growth and the second-stage BOD of the pressurized sewage began four and three days, respectively, before their corresponding secondary growth and second-stage BOD of the non-pressurized sewage did.

Initially, the population of the pressurized sewage was greater than that of the non-pressurized sewage. However, the population of the non-pressurized sewage was consistently greater from the first day to the eighteenth day after incubation.

It is interesting to note that while the DO level of the pressurized sample immediately after pressurization was 0.6ppm higher than that of the non-pressurized control (Figure 6) the growth pattern of the pressurized population at that time was declining (Figure 5). This would seem to indicate that bacteria which received oxygen under pressure had assimilated enough to reach maximum



growth during pressurization. The DO content in the pressurized sewage remained consistently higher than that of the non-pressurized sewage. Conversely, the bacteria in the non-pressurized sewage could not assimilate enough oxygen during pressurization, and subsequently primary growth did not begin until much later — the ninth day (Figure 5). The rate of DO consumption by the bacteria in the non-pressurized sewage exceeded that of the pressurized sewage within 24 hours after incubation.

The graphs in Figure 7 represent the number of colonies of general bacteria in pressurized sewage diluted to 0.10% and non-pressurized sewage diluted to 0.10%. The graph of the pressurized sewage indicates the population was in rapid growth from the first to the second days (Figure 7) and a declining growth from the second to fourth days. The population was approaching extinction during the interval between the fourth and seventh days by a horizonal line representing very little growth. The secondary growth pattern of pressurized sewage diluted to 0.10% and the second-stage BOD of the pressurized sewage diluted to 3.00% (Figure 4) both began on the seventh day.

The growth patterns of the non-pressurized sewage diluted to 0.10% in Figure 7 also indicates that the population was in rapid growth between the first and second days and a declining growth between the second and fourth days.





This pattern continues to show a rapid decline during the fourth and fifth days and follows with a gradual decline until it reaches its lowest number of colonies on the tenth day. The secondary growth pattern of the nonpressurized sewage diluted to 0.10% and the second-stage BOD of the non-pressurized sewage diluted to 3.00% both began on the tenth day.

Again, the number of colonies of the non-pressurized sewage was higher after the first day of incubation until the eighteenth day.

The graphs in Figure 8 represent the general population of pressurized sewage diluted to 0.01% and its corresponding non-pressurized control. The growth patterns of the pressurized sewage shows rapid growth from the first to second days, continued with a moderate decline and rise to the fourth day. The plate counts are more dispersed and less reliable. A rapid decline in growth continued from the fourth to sixth days. This growth pattern as observed in sewage diluted to 0.01% Figure 7 increased from a three-day interval to six-days at 0.01% dilution (Figure 8). A low-colony period did not occur. The end of declining growth occured on the ninth instead of the tenth (as for the 3.00% and 0.10% dilutions) before beginning its secondary growth. The population of the pressurized sewage, here again, was initially greater


than that of the non-pressurized sewage.

The pattern of growth of the non-pressurized sewage went from a rapid growth during the first three days and began to stabilize during the following three days before declining to its lowest population level on the ninth day. The secondary growth pattern of both the non-pressurized and pressurized sewage began on the ninth day. Pressure had little or no effects upon the general population diluted to 0.01%.

The graphs in Figure 9 represent the growth patterns of general bacteria in pressurized sewage diluted to 0.001% and its corresponding non-pressurized control. The growth patterns of both pressurized and non-pressurized sewage are very similar. However, two recurring patterns persist: first, the pressurized population was initially greater than that of the non-pressurized control; second, growth patterns of general bacteria in both pressurized and nonpressurized sewage indicates secondary growth began on the tenth day.



#### PART II

## Experimental Objective

The growth patterns of general and nitrifying bacteria in pressurized and non-pressurized sewage collected from a source different from that of Part I were examined in the second part. The procedure for determining the BOD's and D0 levels as well as preparing the standard plate in Part I for 3.00% dilution samples were duplicated. The growth patterns of the pressurized and non-pressurized sewage were determined by plotting the daily most probable number of serial 5-fold and 10-fold dilutions. The relationship of first - and second-stage BOD curves to the growth patterns of general and nitrifying bacteria in pressurized and nonpressurized sewage can be easily observed by comparing graphs.

Nitrification, which is primarily responsible for the second-stage BOD, is caused by the independent activities of nitrosomas and nitrobacter bacteria. The nitrosomas bacteria reduce organic waste by oxidizing ammonia to nitrites, and then the nitrobacter continue by oxidizing nitrites to nitrates. Fortunately, the nitrifying bacteria grow only on silica gels or in certain mineral solutions containing ammonium salts and will not interfere

with Standard Plate Counts.<sup>17</sup> Mineral media used in this experiment are based upon those described by Finstein.<sup>18</sup> The medium of nitrosomas bacteria is the same as that of the nitrobacter with the exception of pH, the former being 7.8 and the latter 8.0.

A medium inoculated with nitrifying bacteria will take at least four to five days before a strong nitrite reaction develope.<sup>19</sup> However, this growth is inhibited in the presence of other microorganisms and will take place only in its absence. The culture tubes containing the media were incubated at  $24^{\circ}$ C instead of the optimum temperature of  $28^{\circ} \pm 1^{\circ}$ C. Significant growth of nitrifying bacteria was not expected until after the fifth day.

#### Source of Sample

Wastewater was collected from the Water and Wastewater Treatment Plant in Verona, New Jersey, a small trickling filter plant which provides secondary treatment to domestic sewage at a rate of 2.5 million gallons a day.

<sup>&</sup>lt;sup>17</sup>Edwin Jordan, <u>A Text-Book of General Bacteriology</u>, 9th ed. (Philadephia, 1929), p. 666.

<sup>&</sup>lt;sup>18</sup>M.S. Finstein et al., "Length of Incubation for Encumerating Nitrifying Bacteria Present in Various Environments," <u>Applied Microbiology</u> (February, 1975), pp. 265-268.

<sup>&</sup>lt;sup>19</sup>Jordan, p. 667.

A five-gallon composite sample of primary sewage which passed over the weirs of the primary settling tanks was collected in a large plastic container.

## Apparatus

The same equipment used in the first part was also required for Part II. An accurate pH meter and spot depression plates and two hundred glass culture tubes, size 16 X 100mm, with polypropylene closures were the only additional equipment required.

## Chemicals:

The chart below lists the weights of different chemical ingredients required to prepare two liters of medium for nitrifying bacteria. Twenty-five grams of calcium

CHEMICAL NAMES	CHEMICAL SYMBOLS	GRAMS
Sodium Chloride	NaCl	4.00
Potassium Phosphate	K <sub>2</sub> HPO4	1.00
Magnesium Sulfate	MgS04•7H20	0.10
Calcium Chloride ,	CaCl2·H20	0.04
Potassium Bicarbonate	KHC03	0.04
Sodium Molybdate	NaMo04•2H <sub>2</sub> 0	4.8 ug
Ammonium Sulfate	(NH4)2S04	1.00

carbonate is needed as a substrate for the bacteria in the culture media.

<u>Test Reagents</u>. Sulphanilamide solution- Dissolve 5 grams of sulphanilamide in a mixture of 50 ml of concentrated hydrochloric acid and 300 ml of distilled waster. Dilute to 500 ml with distilled water. Solution is stable for many months. N-(1-Naphthyl) - Ethylenediamine Dihyrochloride solution, commonly referred to as Marshall's solution. Dissolve 50 grams of dihydrochloride in 500 ml of distilled water. The solution must be remewed once every month.

Phosphate Stock Solution. To prepare phosphate buffer solution dissolve 34.0 grams of potassium dihydrogen phophate, KH<sub>2</sub>PO<sub>4</sub>, in 500 ml of distilled water. Adjust this solution to pH 7.2 with 1N NaOH and dilute to one liter with distilled water. Dilute 1.25 ml of the stock phosphate buffer solution to 1 liter with distilled water.

<u>pH Adjustment with NaOH</u>. Forty grams of NaOH pellets were dissolved into one liter of distilled water, making a 1N solution. This solution was autoclaved at 20 psi for fifteen minutes.

J.D.H. Strickland and T.R.Parsons, "A Manual of Sea Analysis," <u>Canada Fisheries Research Board</u>, Bulletin No.125 (Ottawa, 1960), p. 63.

### Procedures

The chemical components of the nitrifying medium are the same for both nitrosomas ( $NH_4^+$  oxidizer) and nitrobacter ( $NO^{-2}$  oxidizer). Each component listed in the chart on the previous page was autoclaved separately at 20 psi of pressure for fifteen minutes and then dissolved in two liters of distilled water. Half of this solution, 1 liter, was adjusted to pH 7.8 ( $NH_4$  medium) and the other ( $NO^{-2}$  medium) to 8.0 by titrating with NaOH.

One hundred and seventy glass culture tubes (16 X 150 mm) with polypropylene closures were autoclaved at 20 psi for fifteen minutes. Six - milliliter portions of  $NH_4^+$  medium were transferred to eighty-five tubes, and six - milliliter portions of  $NO^{-2}$  medium were transferred to the remaining eighty-five tubes. Approximately 0.1 gram of calcium carbonate was added to each tube. Five tubes with only  $NH_4$ + medium and five more tubes with  $NO^{-2}$  medium were capped and labelled as control samples for their respective media.

Eight consecutive 10-fold dilutions of raw nonpressurized sewage were prepared by first diluting 100 ml. of raw sewage to 1000 ml with distilled water. Then 100 ml. of this dilution  $(10^{-1})$  was diluted to another 1000 ml. with distilled water. This dilution series continued until eight consecutive dilutions were prepared.

One-milliliter portions of non-pressurized sample were transferred to 5 replicate tubes per dilution for each type of medium. The procedure for pressurizing sewage as described in Part 1 was repeated with the wastewater sample collected in Part II. Eight consecutive 10-fold dilutions of pressurized sample were prepared as described on the previous page for the non-pressurized sewage samples. One-milliliter portions of the pressurized sewage sample were transferred to 5 replicate tubes of each dilution for each type of medium.

The procedures were repeated for measuring the daily DO levels and population growths of both pressurized and non-pressurized sewage diluted to 3.00%. A portion of the remaining sample that was pressurized for the nitrifying tests was used to prepare dilutions for standard plates. The BOD bottles and the culture tubes, slanted to promote aeration, were incubated together at 24°C. The plates were incubated separately at 35°C. Refer to the procedures in Part I for details.

The medium in each tube was examined daily by aseptically removing a few drops to a spot plate containing three drops of sulphanilamide solution and three drops of Marshall's reagent. These test reagents turned bright red in the presence of nitrites. Samples that had weak reactions (such as pink) or no reactions were not scored,

while those that exhibited strong reactions were scored positive (+).

## Discussion

Primary sanitary sewage tested in Part I and in Part II are from two different sources. Yet, the BOD curve of the pressurized sewage diluted to 3.00% and the curve of its corresponding non-pressurized control (Figure 10) are similar to the BOD curves of sewage with the same dilution in Part I (Figure 4).

In fact, the second-stage BOD of the pressurized sewage began, again, on the seventh day, three days earlier than that of the non-pressurized control.

The maximum first-stage BOD (80.00 ppm) of nonpressurized sewage was reduced to 65.83 ppm in the pressurized sewage. This 17.7% reduction obtained here, and the 16.8% reduction in Part I are within  $\pm$  5.0% of those observed by Nusser.<sup>20</sup>

Again, the daily BOD's of the pressurized sewage were consistently less than that of the non-pressurized control during eighteen consecutive days of testing.

The growth patterns of general bacteria in both pressurized and non-pressurized sewage diluted to 3.00% are

<sup>&</sup>lt;sup>20</sup>Nusser, p. 51.

represented by the graphs in Figure 11. The graph of the pressurized sewage indicates rapid growth from the first to the third days followed by a rapid decline during the third, fourth and fifth days. This population remained stable for the next 24 hours before secondary growth began on the sixth day. This secondary growth began three days earlier than that of its corresponding non-pressurized control. The second-stage BOD of the pressurized sewage (Figure 10) appears to have been triggered by a secondary decline (Figure 11) which began on the seventh day.

The D0 level of the pressurized sewage (7.9 ppm) immediately after pressurization was 0.6 ppm higher than that of the non-pressurized control (7.3ppm) in Figure 12. The growth pattern indicated by the graphs in Figure 11 show both pressurized and non-pressurized bacteria to have been in rapid growth during the first day. Although the initial population growth of the non-pressurized bacteria is much more rapid than that of the pressurized bacteria (Figure 11), the number of colonies of the non-pressurized (10,000) is almost half the number of colonies of the pressurized sample (17,500) in Figure 11, suggesting that the pressurized population exerted its initial predominant growth earlier than the predominant growth of bacteria in the non-pressurized sewage.





с.

This early accelerated growth can only be attributed to the fact that more DO was available during pressurization, or that the pressure stimulated the bacteria to assimilate more oxygen.

This situation, however, changes during the first day of incubation. A parallel relationship of DO depletion to growth patterns can be easily recognized by examining the slopes of the DO levels and the rates at which bacteria grew during the first 48 hours (Figure 14 and 15). The non-pressurized bacteria utilized more oxygen at a faster rate than the pressurized bacteria did during the first 24 hours in both experiments -- Figures 5 and 6 (Part I) and ll and l2 (Part II). This fact is clearly reflected by the very rapid population growth in the non-pressurized sewage during this same 24-hour period in both Parts I and II.

The numbers of nitrosomas bacteria per day and the general shapes of the growth patterns of both pressurized and non-pressurized sewage were basically the same (Figure 13), the difference being a three-day lag by the non-pressurized sewage started to decline during its secondary growth on the seventh day (Figure 15), corresponding with the second-stage BOD (Figure 19). The nitrosomas population of the non-pressurized sewage surged on the tenth day, corresponding with the beginning of the second-stage BOD of the non-pressurized sewage (Figure 10). This nitrosomas





FIGURE 13





FIGURE 15

population also began to multiply rapidly during the time the general population of the non-pressurized sewage started to decline during its secondary growth. The BOD curve of the non-pressurized sewage arched upward, is due to its predominant growth of general bacteria (Figure 14). The nitrosomas population of the pressurized sewage grew most rapidly from the tenth to eleventh days. This growth, which must have exerted BOD, was not represented by the curve (Figure 15), as expected.

Enumeration of nitrifying bacteria was based on values of the MPN for 10 tubes inoculated from each of three successive 10-fold dilutions. The data listed in the appendix proved to be inconsistent and further investigation revealed a lack of phosphate buffer solution in the dilution water. The pH of the liquid medium was not buffered and the general population interfered with the population growth of the nitrifying bacteria. A second test for nitrifying bacteria was continued using only 5 replicate tubes instead of 10 and of course buffer solution. These results indicate that the test for nitrosomas bacteria was successful, while the test for nitrobacter bacteria completely failed. A value estimated from MPN of less than one continued ten days after the nitrosomas population stabilized.

#### CONCLUSION

1. The results of independent tests indicate that the beginning of the second-stage BOD's of pressurized primary sewage samples from different sources started three days earlier than the beginning of the second-stage BOD's of their corresponding non-pressurized sewage samples (columns 1 and 5 of the Chart Summary on the following page). Additionally, the 17.7% and 16.8% reductions of second-stage BOD's by pressurized samples (column 6) obtained in this study are within  $\pm$  5.0% of the 22.7% and 15.0% reductions of second-stage BOD's observed by Nusser. From this data, it appears that pressure could be used as a viable means to decrease the biodegradability of domestic sewage.

2. The DO levels of pressurized samples were initially higher and continued to be consistently higher throughout the entire duration of both experiments. The BOD levels of the pressurized samples were consistently lower than those of the control samples.

3. The initial increases of the pressurized populations were always higher than those of the non-pressurized controls. This indicates that pressurization fostered an initial bacterial growth even though less growth occurred later.

	(1)	(2)	(3)	(4)	(5)	(6)
	Source	Activated sewage treatment plants	Beginnir 2nd-stag 14.7 psi	ng of ge BOD's 100 psi	(3)-(4)	Reduction of 1st stage BOD
PART I	Tanglewood Lane Water & Waste- water Treatment Plant Chatham, NJ	Sanitary sewage collected from primary settl- ing tank	10 days*	7 days*	3 days	17.7%
PART II	Verona Water & Wastewater Treatment Plant Verona, NJ	composite sani- tary samples collected from primary settl- ing tanks	10 days*	7 days*	3 days	16.8%
"Biodegrad- ability of Sanitary	Tanglewood Lane Water & Waste- water Treatment	Sanitary sewage collected from primary settl-	13 days*	10 days*	3 days	22.7%
Sewage" by Nusser	Chatham, NJ	LIG VALUE				15.5%

\* days after incubation

CHART SUMMARY

4. With increasing dilution there is an increase of similarity between the growth patterns of pressurized and non-pressurized sewage samples (Figures 5,7,8 and 9).

5. The graphical similarity of the growth patterns of nitrosomas bacteria (Figure 13) indicate that the rate of growth of both pressurized and non-pressurized populations were basically the same except for the three-day lag by the non-pressurized bacteria. The second-stage BOD increases of Part II were exerted at the same time the nitrosomas population began to multiply rapidly. The small number of nitrobacter bacteria may be attributed to the less-than-optimum incubation temperature used.

6. The general populations of both pressurized and non-pressurized bacteria began to approach death during the fourteenth day in Part I and during the sixteenth day in Part II.

7. In Part I the nitrification of both pressurized and non-pressurized sewage coincided with the initiation of the secondary growth phase. In Part II, nitrification of both types coincided with the decline of the secondary phase.

#### RECOMMENDATIONS

Although this project proved successful under controlled laboratory conditions, further experimentation is needed to proved its feasibility on a larger scale.

Determination of its feasibility depends largely on the cost effectiveness of this type of system as compared to other conventional methods of treatment.

A study examining the present worth value of this prototype system considering the capital cost, the amount of energy required, the operation and maintenance cost, environmental factors and so forth is needed to place the applicability of this type of treatment in proper perspective.

## <u>PART I</u>

## Dilution Schedule

400 ml of Raw Sewage	dilute to 13,300ml with distilled water	400 ml / 13,330 ml = 0.0300	0.300 X 100 = 3.00 %
100 ml of 3.00% above	dilute to 3000ml with distilled water	(100 ml)(.30)/.001 = 3000 ml concentration = 0.001	0.0010 X 100 = 0.10 %
100 ml of 0.10% above	dilute to 1000ml with distilled water	(100 ml)(.001)/.0001 = 1000 ml concentration = 0.0001	0.0001 X 100 = 0.010 %
100 ml of 0.01% above	dilute to 1000ml with distilled water	(100 ml)(.0001)/.000001 = 1000 ml concentration = 0.00001	0.00001 X 100 = 0.001 %

# APPENDIX

PART	Ι

# DISSOLVED OXYGEN - 3.00 % DILUTION

Pressure = 100	psia	Pre	ssure	= 14.7	psia
D.O. D.O. DAYS MAX. MIN.	BOD AVE.	DAYS	D.O. MAX.	D.O. MIN.	BOD AVE.
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	36.67 61.67 83.33 93.33 96.67 110.83 107.50 102.50 106.66 110.83 123.33 126.66 130.00 133.33 138.33 146.67 154.16	012345678901234567 1111111111	765544444433333222	7654444339986500500 7654444339986520500 7652687	58.33 68.33 101.67 108.33 119.17 123.17 123.17 129.17 129.17 139.99 139.99 139.99 1450.00 170.00 175.00

## PART I - DATA

Standard Frate	Courts of General Dactors
PRESSURE = 100 psi	PRESSURE = 14.7 psi
2/7	776
14,100 - 3.000 %	5,189 - 3.000 %
13,430 - 3.000 %	8,400 - 3.000 %
14,280 - 3.000 %	6,048 - 3.000 %
12,432 - 0.100 %	201 - 0.100 %
11,256 - 0.100 %	384 - 0.100 %
13,943 - 0.100 %	180 - 0.100 %
10,080 - 0.010 %	194 - 0.010 %
6,720 - 0.010 %	30 - 0.010 %
6,720 - 0.010 %	155 - 0.010 %
2,950 - 0.001 %	74 - 0.001 %
3,156 - 0.001 %	19 - 0.001 %
2,277 - 0.001 %	21 - 0.001 %
2/8	3/76
7,635 - 3.000 %	36,181 - 3.000 %
15,456 - 3.000 %	24,360 - 3.000 %
17,472 - 3.000 %	22,008 - 3.000 %
27,720 - 0.100 %	27,720 - 0.100 %
37,464 - 0.100 %	27,522 - 0.100 %
30,912 - 0.100 %	19,656 - 0.100 %
40,320 - 0.010 %	15,120 - 0.010 %
34,912 - 0.010 %	16,296 - 0.010 %
41,832 - 0.010 %	16,968 - 0.010 %
40,320 - 0.001 %	2,221 - 0.001 %
40,320 - 0.001 %	116 - 0.001 %
40,320 - 0.001 %	51 - 0.001 %

Standard Plate Counts of General Bacteria

PRESSURE	= 100 psi		PRESSURE	= 14.7 p	si
		2/9/76			
466 - 229 -	3.000 % 3.000 %		21,000 - 23.016 -	3.000 %	
53 -	3.000 %		14,952 -	3.000 %	
16,800 -	0.100 %		29,736 -	0.100 %	
15,624 - 10,920 -	0.100 % 0.100 %		25,872 - 22,600 -	0.100 %	
22.848 -	0.010 %		50.000 -	0.010 %	
25,032 = 20.832 =	0.010 %		43,000 -	0.010 %	
10,00° -			),000 -		
40,000 -	0.001 %		33,000 -	0.001 %	
31,752 -	0.001 %	2/10/76	35,000 -	0.001 %	
613 -	3.000 %	2/10/70	980 -	3.000 %	
54 - 48 -	3.000 % 3.000 %		10,584 -	3.000 %	
2 557 -	0 100 %		38 136	0 100 ø	
28 -	0.100 %		37,464 -	0.100 %	
- 220	0.100 %		14,704 -	0.100 %	
33,096 - 36,960 -	0.010 % 0.010 %		50,000 - 35,000 -	0.010 %	
34,272 -	0.010 %		40,000 -	0.010 %	
50,000 - 40,000 -	0.001 %		37,128 -	0.001 %	
50,000 -	0.001 %		40,152 -	0.001 %	
~	0 000 1	2/11/76		1	
	3.000 % 3.000 %		53 <b>-</b> 99 <b>-</b>	3.000 % 3.000 %	
44 -	3.000 %		2,352 -	3.000 %	
1,344 -	0.100 % 0.100 %		10,584 -	0.100 %	
1,250 -	0.100 %		12,768 -	0.100 %	
17,976 -	0.010 %		33,600 -	0.010 %	
20,832 -	0.010 %		34,944 -	0.010 %	
32,424 -	0.001 %		30,072 -	0.001 %	
35,616 - 34,776 -	0.001 % 0.001 %		37,464 - 36,624 -	0.001 %	

PRESSURE	= 100	psi		PRESSUR	E =	= 14.7	7 psi
			2/12/76				
485 - 15 - 28 -	3.000 3.000 3.000	50 % 70 %		4,760 10,416 8,904		3.000 3.000 3.000	% % %
- 51 - 1,250 1,325 -	0.100 0.100 0.100	50 50 50		13,776 18,984 14,952	- (	0.100 0.100 0.100	% % %
6,384 - 6,384 - 7,392 -	0.010 0.010 0.010	% % %		34,272 41,747 39,000	( (	0.010 0.010 0.010	% % %
23,688 - 28,056 - 25,500 -	0.001 0.001 0.001	% % %		31,920 40,000 40,000	( (	0.001 0.001 0.001	% % %
			2/13/76				
2,314 - 3,005 - 3,117 -	3.000 3.000 3.000	%%% %		2,427 1,699 1,848	-	3.000 3.000 3.000	% % %
1,007 - 541 - 2,445 -	0.100 0.100 0.100	50 50 50		8,232 11,256 10,081		0.100 0.100 0.100	% % %
14,616 - 14,784 - 11,760 -	0.010 0.010 0.010	5% 5% 5% 5%		27,048 33,432 33,264		0.010 0.010 0.010	50 50 50
26,880 - 29,400 - 32,088 -	0.001	50 50 50		18,480 45,000 45,000	-	0.001 0.001 0.001	50 50 50
			2/14/76				
3,845 - 8,568 - 3,864 -	3.000 3.000 3.000	50 % %		12,936 12,432 12,096		3.000 3.000 3.000	50 50 50
2,384 - 3,080 - 4,629 -	0.100 0.100 0.100	50 50 F0		15,960 16,800 17,304		0.100 0.100 0.100	50 50 50
6,552 - 9,744 - 8,232 -	0.010 0.010 0.010	50 50 50		18,312 22,680 19,985		0.010 0.010 0.010	50 50 50
24,694 - 23,016 - 33,600 -	0.001 0.001 0.001	% % %		23,184 24,528 25,536		0.001 0.001 0.001	% % %

PRESSURE =	= 100 psi	PRESSURE = $14.7$ psi
		2/15/76
6,386 - 3	3.000 %	27,384 - 3.000 %
10,080 - 3	3.000 %	34,104 - 3.000 %
6,216 - 3	3.000 %	33,264 - 3.000 %
5,376 - 0	).100 %	10,584 - 0.100 %
5,712 - 0	).100 %	11,256 - 0.100 %
7,404 - 0	).100 %	11,928 - 0.100 %
2,817 - 0	).010 %	10,920 - 0.010 %
1,325 - 0	).010 %	11,424 - 0.010 %
1,288 - 0	).010 %	7,244 - 0.010 %
24,696 - 0	0.001 %	19,488 - 0.001 %
29,906 - 0	0.001 %	15,219 - 0.001 %
27,888 - 0	0.001 %	40,000 - 0.001 %
		2/16/76
2,618 - 3	3.000 %	2,632 - 3.000 %
1,549 - 3	3.000 %	3,211 - 3.000 %
3,211 - 3	3.000 %	2,799 - 3.000 %
1,027 - 0	0.100 %	14,107 - 0.100 %
1,699 - 0	0.100 %	16,552 - 0.100 %
1,195 - 0	0.100 %	17,056 - 0.100 %
31,890 - 0	0.010 %	23,856 - 0.010 %
38,640 - 0	0.010 %	23,016 - 0.010 %
36,792 - 0	0.010 %	28,506 - 0.010 %
23,520 - (	0.001 %	11,088 - 0.001 %
26,880 - (	0.001 %	21,336 - 0.001 %
29,824 - (	0.001 %	28,741 - 0.001 %
		2/17/76
3,192 -	3.000 %	9,744 - 3.000 %
3,360 -	3.000 %	10,752 - 3.000 %
5,880 -	3.000 %	20,157 - 3.000 %
2,800 - (	0.100 %	16,296 - 0.100 %
6,720 - (	0.100 %	18,144 - 0.100 %
7,728 - (	0.100 %	17,808 - 0.100 %
8,568 - (	0.010 %	29,568 - 0.010 %
9,576 - )	0.010 %	34,104 - 0.010 %
9,408 - )	0.010 %	31,416 - 0.010 %
30,072 -	0.001 %	50,000 - 0.001 %
28,555 -	0.001 %	11,928 - 0.001 %
24,026 -	0.001 %	34,776 - 0.001 %

PRESSURE = 100 psi	PRESSURE = $14.7$ psi
	2/18/76
10,920 - 3.000 %	19,488 - 3.000 %
11,592 - 3.000 %	19,313 - 3.000 %
11,088 - 3.000 %	17,976 - 3.000 %
1,288 - 0.100 %	13,937 - 0.100 %
2,931 - 0.100 %	17,976 - 0.100 %
9,072 - 0.100 %	15,792 - 0.100 %
16,296 - 0.010 %	31,080 - 0.010 %
32,592 - 0.010 %	24,864 - 0.010 %
33,432 - 0.010 %	26,040 - 0.010 %
19,488 - 0.001 %	21,336 - 0.001 %
21,000 - 0.001 %	26,544 - 0.001 %
20,664 - 0.001 %	22,512 - 0.001 %
	2/19/76
8,904 - 3.000 %	4,368 - 3.000 %
7,560 - 3.000 %	6,720 - 3.000 %
8,064 - 3.000 %	10,248 - 3.000 %
13,600 - 0.100 %	10,584 - 0.100 %
5,040 - 0.100 %	12,600 - 0.100 %
5,712 - 0.100 %	17,304 - 0.100 %
9,576 - 0.010 %	39,480 - 0.010 %
9,576 - 0.010 %	31,584 - 0.010 %
13,608 - 0.010 %	35,616 - 0.010 %
20,328 - 0.001 %	43,680 - 0.001 %
20,192 - 0.001 %	38,304 - 0.001 %
24,024 - 0.001 %	32,928 - 0.001 %
	2/20/76
1,680 - 3.000 %	10,080 - 3.000 %
1,064 - 3.000 %	9,576 - 3.000 %
3,416 - 3.000 %	9,912 - 3.000 %
2,875 - 0.100 %	11,256 - 0.100 %
2,371 - 0.100 %	14,952 - 0.100 %
3,864 - 0.100 %	7,392 - 0.100 %
11,928 - 0.010 %	25,872 - 0.010 %
14,616 - 0.010 %	26,376 - 0.010 %
14,112 - 0.010 %	35,616 - 0.010 %
33,096 - 0.001 %	38,000 - 0.001 %
33,600 - 0.001 %	39,312 - 0.001 %
26,376 - 0.001 %	33,096 - 0.001 %

PRESSURE = 100 psi		PRESSURE =	14.7 psi
	2/21/76		
1,157 - 3.000 %		5,880 - 3	.000 %
1,605 - 3.000 %		5,376 - 3	.000 %
1,363 - 3.000 %		8,904 - 3	.000 %
- 0.100 %		7,392 - 0	).100 %
- 0.100 %		10,752 - 0	).100 %
- 0.100 %		11,088 - 0	).100 %
5,882 - 0.010 %		15,288 - 0	).010 %
2,277 - 0.010 %		18,648 - 0	).010 %
2,595 - 0.010 %		19,320 - 0	).010 %
10,584 - 0.001 %		30,744 - 0	).001 %
13,104 - 0.001 %		29,232 - 0	).001 %
16,464 - 0.001 %		26,880 - 0	).001 %
	2/22/76		
164 - 3.000 %		1,344 - 3	3.000 %
429 - 3.000 %		635 - 3	3.000 %
728 - 3.000 %		1,027 - 3	3.000 %
355 - 0.100 %		11,592 - (	).100 %
392 - 0.100 %		14,280 - (	).100 %
747 - 0.100 %		10,920 - (	).100 %
9,408 - 0.010 %		22,848 - (	).010 %
12,936 - 0.010 %		21,168 - (	).010 %
11,928 - 0.010 %		24,696 - (	).010 %
13,104 - 0.001 %		16,464 - (	).001 %
13,944 - 0.001 %		41,160 - (	).001 %
14,448 - 0.001 %		40,000 - (	).001 %
	2/23/76		
163 - 3.000 %		560 -	3.000 %
449 - 3.000 %		579 -	3.000 %
448 - 3.000 %		896 -	3.000 %
299 - 0.100 %		6,552 -	0.100 %
1,232 - 0.100 %		11,592 -	0.100 %
690 - 0.100 %		14,212 -	0.100 %
2,688 - 0.010 %		13,440 -	0.010 %
1,524 - 0.010 %		25,697 -	0.010 %
3,360 - 0.010 %		25,704 -	0.010 %
33,096 - 0.001 %		9,072 -	0.001 %
27,720 - 0.001 %		10,248 -	0.001 %
33,096 - 0.001 %		13,440 -	0.001 %

PRESSURE = 100 psi		PRESSURE	Ξ	14.7	psi
	2/24/76				
356 - 3.000 % 1,064 - 3.000 % 933 - 3.000 %		1,624 - 1,923 - 3,024 -	333	•000 •000 •000	% % %
1,083 - 0.100 % 1,120 - 0.100 % 1,307 - 0.100 %		21,000 - 3,696 - 5,208 -		.100 .100 .100	% % %
10,416 - 0.010 % 9,576 - 0.010 % 5,208 - 0.010 %		20,664 - 22,008 - 26,376 -		.010 .010 .010	% % %
13,104 - 0.001 % 12,264 - 0.001 % 15,960 - 0.001 %		11,592 - 19,152 - 13,272 -	- C - C	0.001 0.001 0.001	% % %
	2/25/76				
6,048 - 3.000 % 4,704 - 3.000 % 5,544 - 3.000 %		1,213 - 1,213 - 2,277 -		3.000 3.000 3.000	%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
31,248 - 0.100 % 30,576 - 0.100 % 27,397 - 0.100 %		11,088 - 10,920 - 12,432 -	- C - C	.100 .100 .100	50 50 50
1,885 - 0.010 % 1,904 - 0.010 % 1,587 - 0.010 %		19,992 - 22,008 - 23,352 -	- C	).010 ).010 ).010	50 50 50
12,432 - 0.001 % 12,768 - 0.001 % 11,592 - 0.001 %		14,112 - 13,104 - 18,984 -	- () - ()	).001 ).001 ).001	50 50 50
	2/26/76				
9,072 - 3.000 % 6,552 - 3.000 % 7,896 - 3.000 %		5,880 - 5,880 - 5,712 -		3.000 3.000 3.000	5~ % %
8,400 - 0.100 % 7,392 - 0.100 % 6,216 - 0.100 %		9,072 · 10,248 · 9,072 ·	- ( - (	).100 ).100 ).100	% % %
10,920 - 0.010 % 12,096 - 0.010 % 13,796 - 0.010 %		19,320 19,320 24,472	- ( - (	0.010 0.010 0.010	50 50 50
23,016 - 0.001 % 23,189 - 0.001 % 23,352 - 0.001 %		39,312 33,432 36,685	- (	0.001 0.001 0.001	5% 5% 5%

PRESSURE = 100 psi	PRESSURE = 14.7 psi
2/27/76	
16,128 - 3.000 %	2,520 - 3.000 %
12,432 - 3.000 %	5,040 - 3.000 %
13,776 - 3.000 %	4,536 - 3.000 %
7,392 - 0.100 %	17,640 - 0.100 %
11,592 - 0.100 %	15,624 - 0.100 %
15,456 - 0.100 %	15,960 - 0.100 %
40,000 - 0.010 %	22,176 - 0.010 %
38,000 - 0.010 %	21,840 - 0.010 %
40,000 - 0.010 %	18,144 - 0.010 %
21,168 - 0.001 %	21,336 - 0.001 %
24,528 - 0.001 %	21,336 - 0.001 %
21,840 - 0.001 %	23,352 - 0.001 %

# PART II

## DISSOLVED OXYGEN - 3.00 % DILUTION

Pres	ssure =	= 100	psia	Pre	essure	= 14.	<u>7 psia</u>
	D.O.	D.O.	BOD		D.O.	D.O.	BOD
DAYS	MAX.	MIN.	AVE.	DAYS	MAX.	MIN.	AVE.
01234567890123456 111111	77766666555555555555555555555555555555	77766655555555555555555555555555555555	20.83 25.83 40.00 41.67 55.00 70.00 65.83 74.17 75.83 76.67 78.33 79.16 83.33 85.83 85.83 88.33 90.00	0123456789011234567 111211111	766555005500000000005555500055000550005	7.6.55 5.6.8.98650 5.5.54 4.4.4 4.4 9.1.000 0.000	35.80 43.33 47.50 50.83 60.00 71.60 80.00 80.00 80.00 80.00 80.00 80.00 100.00 102.50 105.00 105.00 105.00 111.67

## PART II - DATA

Standard Plate	Counts of Gene	ral Bacteria
PRESSURE = 100 psi	PRESS	URE = 14.7 psi
14,400 - 3.000 % 15,000 - 3.000 % 15,600 - 3.000 %	10,40 8,90 9,60	0 - 3.000 % 0 - 3.000 % 0 - 3.000 %
	5/2/76	
23,500 - 3.000 % 20,000 - 3.000 % 21,500 - 3.000 %	36,62 34,60 37,60	0 - 3.000 % 0 - 3.000 % 0 - 3.000 %
	5/3/76	
25,500 - 3.000 % 27,400 - 3.000 % 25,000 - 3.000 %	25,40 31,60 20,80	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %
	5/4/76	
28,200 - 3.000 % 18,300 - 3.000 % 22,700 - 3.000 %	25,90 26,00 26,50	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %
	5/5/76	
19,500 - 3.000 % 18,200 - 3.000 % 13,300 - 3.000 %	17,60 22,20 20,30	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %
	5/6/76	
21,000 - 3.000 % 17,800 - 3.000 % 17,000 - 3.000 %	36,50 31,00 34,60	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %
	5/7/76	
25,000 - 3.000 % 27,200 - 3.000 % 28,700 - 3.000 %	17,30 16,80 22,20	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %
	5/8/76	
17,800 - 3.000 % 24,500 - 3.000 % 21,300 - 3.000 %	50,00 50,00 50,00	00 - 3.000 % 00 - 3.000 % 00 - 3.000 %

PRESSURE = 100 psi	PRESSURE = 14.7 psi
5	5/9/76
23,200 - 3.000 %	17,300 - 3.000 % 20.300 - 3.000 %
21,600 - 3.000 %	20,300 - 3.000 %
5	5/10/76
16.600 - 3.000 %	19,800 - 3.000 %
17,400 - 3.000 %	22,800 - 3.000 %
15,300 - 3.000 %	21,700 - 5.000 %
E C	5/11/76
10,100 - 3,000 %	7,600 - 3.000 %
14,600 - 3.000 %	7,200 - 3.000 %
12,000 - 3.000 %	7,400 - 3.000 %
	5/12/76
7,000 - 3.000 %	5,000 - 3.000 %
9,400 - 3.000 %	7,700 - 3.000 % 12.000 - 3.000 %
0,100 )1000 /	
	5/13/76
2,000 - 3.000 %	11,800 - 3.000 %
4,200 - 3.000 %	11,600 - 3.000 %
1	5/14/76
-	, , , , , , , , , , , , , , , , , , , ,
3,700 - 3.000 %	2,700 - 3.000 %
6,000 - 3.000 %	2,000 - 3.000 %
	5/17/76
13 800 - 3 000 %	5.500 - 3.000 %
14,300 - 3.000 %	6,300 - 3.000 %
13,100 - 3.000 %	5,200 - 3.000 %
	5/18/76
11.300 - 3.000 %	17.000 - 3.000 %
11,600 - 3.000 %	12,000 - 3.000 %
12,000 - 3.000 %	12,000 - 3.000 %
	5/19/76
9.400 - 3.000 %	3.500 - 3.000 %
9,600 - 3.000 %	4,400 - 3.000 %
7,700 - 3.000 %	4,200 - 3.000 %

## PART II - DATA

MPN Estimates of Nitrosomas Pouplation (Serial 5-Fold Dilutions)

Pressure = 100 psi

Davra	$S_{a}$	Dilutions	Probable Number Times
Days	SCOLET (4)	$0 \qquad 1 \qquad 2$	DITUTION PACTOR
1	0,0,0	10, 10, 10	
2	0,0,0	$10^{\circ}, 10^{1}, 10^{2}$	
3	0,0,0	$10^{\circ}, 10^{1}, 10^{2}$	0
4	1,1,0	$10^{\circ}, 10^{1}, 10^{2}$	$0.40 \times 10 = 0.40$
5	3,1,0	$10^{\circ}, 10^{\circ}, 10^{\circ}$	$1.10 \times 10 = 1.10$
6	5,5,1	10, 10, 10,	35.0 X 10 = 35.0
7	5,1,0	10, 10, 10	3.30 X 10 = 330
8	5,2,0	10, 10, 10	$4.90 \times 10 = 4,900$
9	3,0,0	10, 10, 10	$0.78 \times 10^{-2} = 7,800$
10	3,1,0	10, 10, 10	1.10 X $10 = 11,000$
11	4,1,1		2.10 X 10 = 21,000 $\mu$
12	4,2,0	10 <sup>4</sup> , 10 <sup>5</sup> , 10 <sup>6</sup>	$2.20 \times 10 = 22,000$
13	4,2,0		$2.20 \times 10 = 22,000$
14	4,2,1		$2.60 \times 10 = 26,000$
15	4,2,1	10, 10, 10	$2.60 \times 10 = 26,000$

16 No Change
continued

Pressure = 14.7 psi					
Days	Scored (+)	Dilutions	Probable Number Times Dilution Factor		
1	0,0,0	$10^0$ , $10^1$ , $10^2$			
2	0,0,0	$10^0$ , $10^1$ , $10^2$			
3	0,0,0	$10^0$ , $10^1$ , $10^2$			
4	0,0,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>			
5	0,0,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>			
6	1,1,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>	$0.40 \times 10^{\circ} = 0.40$		
7	2,1,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>	$0.68 \times 10^{0} = 0.68$		
8	3,1,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>	$1.10 \times 10^{0} = 1.10$		
9	5,5,1	$10^{0}$ , $10^{1}$ , $10^{2}$	$35.0 \times 10^0 = 35$		
10	5,0,0	$10^2$ , $10^3$ , $10^4$	$2.30 \times 10^2 = 230$		
11	5,1,0	$10^3$ , $10^4$ , $10^5$	$3.30 \times 10^3 = 3,300$		
12	2,1,0	$10^4$ , $10^5$ , $10^6$	$0.68 \times 10^4 = 6,800$		
13	3,1,0	$10^4$ , $10^5$ , $10^6$	1.10 X $10^{4}$ = 11,000		
14	3,2,0	$10^4$ , $10^5$ , $10^6$	1.40 X $10^4 = 14,000$		
15	4,2,0	$10^4$ , $10^5$ , $10^6$	2.20 X $10^{44} = 22,000$		
16	4,2,0	$10^4$ , $10^5$ , $10^6$	$2.20 \times 10^4 = 22,000$		
17	4,3,0	10 <sup>4</sup> , 10 <sup>5</sup> , 10 <sup>6</sup>	2.70 X $10^4 = 27,000$		
18	4,3,0	10 <sup>4</sup> , 10 <sup>5</sup> , 10 <sup>6</sup>	$2.70 \times 10^4 = 27,000$		

19 No Change

MPN Estimates of Nitrosomas Population (Serial 10-Fold Dilutions) Pressure = 100 psiProbable Number Times Days Scored (+) Dilutions Dilution Factor  $10^{\circ}$ ,  $10^{1}$ ,  $10^{2}$   $10^{\circ}$  X 0.2 = 0.2 1 2,0,0  $10^{\circ}$ ,  $10^{1}$ ,  $10^{2}$   $10^{\circ}$  X 0.32 = 0.32 3,0,0 2  $10^{0}$ ,  $10^{1}$ ,  $10^{2}$   $10^{0}$  X 1.01 = 1.01 7,0,0 3  $10^{\circ}$ ,  $10^{1}$ ,  $10^{2}$   $10^{\circ}$  X 1.28 = 1.28 4 8,0,0  $10^1$ ,  $10^2$ ,  $10^3$   $10^1$  X 1.39 = 13.9 5 6.4.0  $10^1$ ,  $10^2$ ,  $10^3$   $10^1$  X ? 7,9,2 6 Pressure = 14.7 psi

Days	Scored (+)	Dilutions	Probable Number Times Dilution Factor
1	3,0,0	$10^{0}$ , $10^{1}$ , $10^{2}$	$10^0 \times 0.09 = 0.09$
2	3,0,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>	$10^0 \times 0.09 = 0.09$
3	5,0,0	$10^0$ , $10^1$ , $10^2$	$10^0 \times 0.85 = 0.85$
4	6,1,0	10 <sup>0</sup> , 10 <sup>1</sup> , 10 <sup>2</sup>	$10^0 \times 0.92 = 0.92$
5	5, <u>7</u> ,2	$10^1$ , $10^2$ , $10^3$	10 <sup>1</sup> X ?
6	8,3, <u>4</u>	10 <sup>1</sup> , 10 <sup>2</sup> , 10 <sup>3</sup>	10 <sup>1</sup> X ?

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