New Jersey Institute of Technology

Digital Commons @ NJIT

Dissertations   Electronic Theses and Dissertations

Spring 5-31-1984

The effects of pressure on the kinetics and mechanisms of the bod reaction

Peter Sereico
New Jersey Institute of Technology

Follow this and additional works at: https://digitalcommons.njit.edu/dissertations

Part of the Environmental Engineering Commons

Recommended Citation

INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

8416341

Sereico, Peter

THE EFFECTS OF PRESSURE ON THE KINETICS AND MECHANISMS OF THE BOD REACTION

New Jersey Institute of Technology   D.Eng.Sc.   1984

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

THE EFFECTS OF PRESSURE ON THE KINETICS

AND MECHANISMS OF THE BOD REACTION

BY

PETER SEREICO

A DISSERTATION

PRESENTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE

OF

DOCTOR OF ENGINEERING SCIENCE

AT

NEW JERSEY INSTITUTE OF TECHNOLOGY

Newark, New Jersey

May, 1984

APPROVAL OF DISSERTATION

THE EFFECTS OF PRESSURE ON THE KINETICS
AND MECHANISMS OF THE BOD REACTION

BY

PETER SEREICO

FOR

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

FACULTY COMMITTEE

APPROVED:_____CHAIRMAN

_____

_____

_____

_____

_____

Newark, New Jersey
May 1984

VITA

Name: Peter Sereico

Degree and date to be conferred: D. Eng. Sc., 1984

Secondary education: Seton Hall Prep., South Orange, N.J. 1956

Collegiate institutions attended Dates   Degree   Date of Degree

Newark College of Eng.	1956-61	BS	1961
N.J.I.T.	1967-71	MS	1971
N.J.I.T.	1978-84	D.Eng.Sc.	1984

Major: Environmental Engineering

Publications: "A Convenient Method for Detergent Phosphate
                Analysis", Chemistry Magazine, March 1972.

A chapter dealing with pressurization as a wastewater
treatment in a textbook resulting from the presentation
at the 3rd International Conference on Physical-Chemical
Methods for Water and Wastewater Treatment, Lubin, Poland.

A chapter on the mechanisms of BOD reduction due to
chlorination in a textbook resulting from the presentation
at the International Conference Concerned with Chemistry
for the Protection of the Environment, Toulouse, France.

A chapter dealing with receiving water analysis to be
published in the forthcoming Biotechnology Handbook
edited by P. Cherimissinoff, Ann Arbor Press.

In progress: a book dealing with reaeration in streams
and wastewater treatment plants.

Positions held:

1983 - present   Somerset Consulting Services, Fort Lee,
                       N.J., Director of Environmental Services.

1982 - 1983   Member, Township of Verona Advisory
                       Committee for the Infiltration/Inflow
                       and Facilities Planning Study.

1981 - 1983   Lakland Laboratories, Wharton, N.J.,
                       Director of water quality laboratory
                       specializing in NPDES requirements.

1981 - present   Lecturer, PE Review Course. Responsible
                       for environmental section including waste-
                       water, water treatment, and stream analysis
                       in New Jersey and New York City.

1978 - present   PRP Associates, West Caldwell, N.J.
                       Principal partner, environmental consulting
                       firm.

1973 - present   New Jersey Institute of Technology, Newark,
                       N.J. Adjunct Professor, Graduate Division.
                       Teach graduate courses in the Civil and
                       Environmental Engineering Division.

1967 - 1971   New York University, Washington Square,
                       New York, N.Y. Adjunct Lecturer, Chemistry
                       in the School of Continuing Education.

ABSTRACT

The research was concerned with the kinetics and
mechanisms of BOD reduction due to pressurization. The
initial investigations involved attempts to reproduce
and interpret the results of previous investigators.
Thereafter, actual wastewater samples were pressurized
and changes in the wastewater characteristics were analyzed
and recorded. Based on the findings, possible mechanisms
for creating the changes noted were proposed.

To test the mechanisms considered, samples of pure
compounds were prepared and pressurized. The results
of this analysis eliminated all of the mechanisms but
the actual one. The mechanism responsible for changes
in BOD profile under pressure conditions is the hydrolysis
of large molecular weight organic molecules into smaller
fractions. Specific members of these families were
analyzed and BOD changes and kinetic data were recorded.

The final phase of the research involved the preparation
of composite synthetic wastewater samples subjected to
pressure, and analyzing the similarities in their responses
to pressure compared to that of actual wastewater samples.

DEDICATION

The author wishes to dedicate this research effort
to his son Russell, who provided the inspiration with
his sense of humor, understanding, and sincere help
and interest in the project. This work, may, in some
little way, extend the frontiers of science so that
when Russ becomes older it will be a cleaner and nicer
world.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to each
of the committee members for their guidance and encouragement
during the course of this research effort. A special
thanks is to be accorded to Professor Robert Dresnack,
Ph.D., for his initial encouragement and guidance throughout
the long period of time required to accomplish this project.
The author would also like to acknowledge the assistance
provided by Professor David Kristol, Ph.D., who spent
many an afternoon with the author discussing the progress
of the effort.

The author wishes to acknowledge the assistance
provided by Mrs. Elizabeth Price who's basic knowledge
and understanding of chemistry and microbiology proved
extremely important in overcoming several "roadblocks".

Finally, Mrs. Kathie Nolte, for the skillful typing
of the manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

I. INTRODUCTION

The need for more efficient wastewater treatment
and reduction is becoming increasingly important as
input loadings are constantly increasing and legislation
is placing more constraints and limitations on treated
wastewaters being discharged into the environment. The
need for improved technologies to meet these requirements
is apparent.

Process alteration on existing wastewater facilities
can provide the means to upgrade treatment efficiencies
on existing influents, or it can allow higher loadings
to achieve the same efficiencies. One area of process
alteration that has been the subject of little attention
is the application of pressure to wastewaters to achieve
Bod reduction. Several investigations (21-27) have
been made regarding its use, but the reported results
have been confusing and contradictory. To date, several
laboratory bench models and one operational pilot plant
(28) have been constructed. Data from these facilities
indicates that the increase in the first stage of decompo-
sition of pressurized organic matter can be as high
as 36 percent. This represents a potential for increased

1

wastewater treatment handling and removal efficiencies.

The initial objective to this research effort was
to confirm that there is change in the BOD profile as
a result of pressurization based on the studies and
findings of prior investigators. The scope of study
then proceeded along two paths; the first was to reproduce
some of the earlier investigations to obtain quantitative
data to support qualitative statements in prior investiga-
tions, and to substantiate and expand upon quantitative
data presented by others; the second area of investigation
was to investigate and establish the reasons why these
changes took place. The main focus was to examine the
chemistry of the reactions involved. Once the mechanisms
responsible for the observed changes were understood,
the means to alter variables such as amount and extent
of pressure to achieve optimum operating conditions
in a given designed system could be attained. Furthermore,
the phenomenon can then also be applied to existing
systems to improve wastewater removal efficiencies therein.

This research is confined to the investigation
of the kinetics and mechanisms involved when wastewaters

subjected to biodegradation are pressurized. Further studies will be needed to develop the engineering design data to apply the findings herein optimally to wastewater treatment plants.

II. BACKGROUND OF THE STUDY

Of all the parameters used to measure the strength of wastewater, the most common indicator is the Biochemical Oxygen Demand (or BOD) test. By definition, the BOD is the amount of oxygen required by microorganisms while stabilizing decomposable organic matter under aerobic conditions (1). This condition can be represented by the following reactions:

(a) $CHON + O_2 \xrightarrow{[M.O.]} CO_2 + H_2O + NH_3$ (ammonia)

(b) $2NH_3 + 3O_2 \xrightarrow{[M.O._1]} 2H^+ + 2H_2O + 2NO_2^-$ (nitrite)

(c) $2NO_2^- + O_2 \xrightarrow{[M.O._2]} 2NO_3^-$ (nitrate)

Where: 1.   CHON represents organic compounds containing carbon, hydrogen, oxygen, and nitrogen.

2. $[M.O.]$, $[M.O._1]$, $[M.O._2]$ are concentrations of the species of microorganisms necessary for the reactions to proceed.

4

These reactions are slow, and they usually require
10 to 30 days to reach completion, depending on temperature,
pH, microorganism concentration, etc. In the absence
of ammonia, the only oxygen required is the amount shown
in reaction (a), and the total oxygen demand is referred
to as the first stage, or carbonaceous, BOD. If nitrogen
is present, under the proper conditions, reactions (b)
and (c) will proceed exerting an additional BOD referred
to as the second stage or nitrogenous oxygen demand
(NOD). A graphical representation of these reactions
is shown in Figure No. 1. The flat plateau portion
of the carbonaceous curve represents the total 1st stage
oxygen required and is given the designation BOD_L.

During the course of the development of the BOD
test, a convenient time reference of five days was chosen
to report oxygen depletion values. The designation
BOD_5 was used to report these values, and, as a rule,
for raw sewage the value of BOD_5 usually ranges from
60 to 70 percent of the BOD_L value. Before the advent
of dissolved oxygen probes, it was very difficult in
the wet chemical analysis to daily record BOD values
because the daily samples were reacted and rendered

Figure No. 1

Generalized BOD Curves For First
And Second Stage Oxygen Demands

useless. Also, an obvious disadvantage of taking a reading at the end of only one day, say day 5, would be that any unusual events before or after five days time would not be noticed.

One of the major assumptions underlying the nature of the BOD reaction is that the oxidations shown in reactions (a) to (c) are all first order reactions and that these reactions can be described by first order kinetics. If this is true, then the organic decomposition is directly proportional to the concentration of organic material present. The derivation of the first order expression is as follows:

(1) $\quad -\dfrac{dc}{dt} = k_1 C$

where the derviative is negative to show a decreasing amount.

C = concentration
k_1 = first order specific rate constant.

After rearranging terms and integrating expression (1) becomes:

(2) $\quad \ln C/C_o = k_1 t$ or

(2A) $\quad C/C_o = 10^{-k_1 t}$

Expression (2) and (2A) is used to determine any concentration, C, at any time, t, based on a starting concentration of C_o, decomposing at a rate given by k_1. This expression can also be used to determine any amount C at any time t, based on a fixed final concentration L_o. Replacing C with L_r to represent the concentration of organics remaining, expression (2A) can be rewritten as:

(3) $L_r/L_o = 10^{-k_1 t}$ Rearranging (3) gives
                                         (4) in terms of L_r.

(4) $y = L_o (1-10^{-k_1 t})$

This is the BOD formula, which relates the amount of oxygen consumed or demanded during an organic decomposition at any time t until the reaction is over when y = L.

That the first stage BOD reaction is not the single kinetic event as shown above is well discussed by Gaudy (2). The rate at which the reaction actually proceeds is a function of the type of substrate, amount of substrate, and the concentrations of microorganisms which, in turn,

is dependent on substrate, pH, temperature, and their position on the growth curve. To account for these variables, researchers such as Young (3) have suggested that the oxygen demand may in fact be a second order reaction. The first order approximation has become so imbedded in environmental engineering principles that it is used as a kinetic principle rather than the approximation it actually is. There may be instances where the data will not corroborate the principles of a first order reaction, and in these cases one must realize the limitations of the first order approximation.

In a strict chemical sense in a chemical reaction, the principle that the rate of reaction is proportional to the concentration of material present is valid. For a first order reaction, this is expressed as:

(5)   rate $= k_1$ [concentration]^1

where the exponent that the concentration is raised to is the action order. However, in catalyzed reactions, the concentration of the catalyst must be taken into account and a term incorporated into the rate expression.

$$(6) \quad \text{rate} = k_1 \, [\text{concentration}]^1 \, [\text{catalyst}]^x$$

where the order of the reaction now is the sum of 1
+ x. x can have fractional values and this gives rise
to fractional order reactions such as the decomposition
of peroxide in the presence of iodide ion which is first
order in peroxide and 3/4 order in iodide. Methods
to evaluate the order of the catalyst are described
in Fraga, et. al. (4) and Nechamkin (5).

The BOD reaction can be shown as:

$$(d) \quad \text{organic matter} + \text{oxygen} \xrightarrow{[\text{M.O.}]} CO_2 + H_2O$$

where the microorganisms serve as the catalyst in the
reaction. The oxygen in the reaction serves the needs
of the aerobic microorganisms. In kinetic form, this
can be expressed as:

$$(7) \quad \text{rate} = k_1 \, [\text{organic matter}]^1 \, [O_2]^0 \, [\text{MO}]^x$$

where [MO] is the microorganism concentration in numbers/
vol.

It is known that above a certain minimum dissolved oxygen (DO) concentration (of about 1-2 ppm) the reaction proceeds independently of this concentration (6). For that reason, the order of the oxygen is zero. The reaction order term for the microorganism would not be a constant because during the reaction the concentration of microorganisms would always be changing, the amount of change depending on their position on the growth curve which, in turn, is a function of the predator-prey relationship for the microorganism animal concentration. Figure 2A shows a microorganism growth curve in its different phases. The fastest rate of growth occurs during log growth due to the interrelationship between food (organisms), microorganisms, and microorganism predators. As the food supply diminishes and microorganisms population increases the predator concentration increases, and declining growth occurs. As the situation continues growth is arrested during the stationary phase leading to declining microorganism populations culminating in the final or death phase.

The growth of microorganisms can be further complicated due to the aftergrowth phenomenon, which is defined

Lag Phase | Log Growth | Stationary | Increasing Death | Log Death | Death

Number Of Organisms, N

Figure No. 2A

Growth Pattern For Numbers of Organisms

Log Number Of Organisms, N

Log Growth Curve for Organisms Experiencing Aftergrowth

Log Growth Curve for Non-Aftergrowth Organisms

Figure No. 2B

Growth Patterns For Organisms Experiencing Normal Growth and Aftergrowth

Organisms Destroyed by Environmental Stress

as the accelerated, unexpected growth of microorganisms

as a result of an environmental stress. Reproducing

at a faster rate would require more energy, leading

to a more rapid oxidation of the organic material present.

Environmental stresses, such as chlorination or dilution

have been shown to distort the food-microorganism-predator

relationship which cause the microorganisms to proliferate

at a faster rate than their predators until such time

that the predators reassert themselves and a population

equilibrium is again established. But until that time

the microorganisms have increased at a faster rate than

if no stress had been applied. It is left to determine

if pressurization is such a stress. Population profiles

for aftergrowth, non-aftergrowth situations are shown

in Figure 2B. For a more complete description concerning

aftergrowth consult references (7-10).

In many articles in the literature (11-17), reference

is made to the term BOD Reduction where the reduction

is measured relative to some control. Wastewater samples

are subject to physical or chemical stresses and there

appears to be an apparent BOD_5 or BOD_L reduction as

a result. Also, the rate of decomposition in some cases

for the stressed sample appears to be greater implying
that there are kinetic changes also taking place. Two
different curves are presented dependent on the stressing
process and these are shown in Figure 3A and 3B. Both
curves display lower BOD_L values and the kinetic rates
required to reach these BOD values are different.

If, in fact, BOD curves for stressed and control
samples are drawn and compared and assuming no loss
of carbon material the BOD_L values for both should be
the same. The shapes of the curves may differ indicating
different pathways to the final oxidation products along
with different kinetic rates. Figure 3C shows the effect
on the BOD curve of different samples of the same concen-
tration oxidizing at different rates.

The intent of these preliminary investigations
was to establish patterns and relationships between
the variables that could influence the mechanisms and
rate by which wastewater samples are decomposed. The
important variables are concentration and nature of
the wastewater and the accompanying population of micro-
organisms. Recognizing the same, the experiments that

Figure No. 3A

Variations In First Stage BOD
As A Result Of An Environmental
Stress

Figure No. 3B

One Type Of First Stage BOD
Reduction As A Result Of An
Environmental Stress

BOD$_L$ (u)

BOD$_L$ (s)

Δ BOD

BOD, mg/L

Time - Days

——— Unstressed BOD Curve
– – – Stressed BOD Curve

Figure No. 3c

Effect On The BOD Curve For Different Materials Oxidizing
At Different Rates (L Values Are The Same)

$K_3 > K_2 > K > K_1$

Time - Days

BOD, mg/L

follow will take these variables into account.

III. LITERATURE REVIEW

There is very little reported background literature
dealing with pressurization as a wastewater removal
systems operation. For what little that is reported,
there is no consistency regarding the types of samples
pressurized, pressure detention time, amount of pressure,
and conditions under which pressurization was applied.
The results are for the most part qualitative and the
results, when compared, present a puzzling and confused
description of the pressurization process. The following
presentation discusses the previous investigations and
the major contribution, if any, of each.

PREVIOUS INVESTIGATIONS EXAMINING THE EFFECTS
OF PRESSURE TO WASTEWATER RESEARCH

LeNOBLE

With the advent of new and improved pressure producing
equipment, experiments were conducted prior to 1963
investigating the role of pressure on the mechanism
and equilibrium yields or an organic reaction (19).
The equilibrium under study was of the type;

19

$$A \underset{}{\overset{k_1}{\rightleftharpoons}} B \underset{}{\overset{k_2}{\rightleftharpoons}} C$$

Where A = one isomer of an organic compound

C = another isomer of the organic compound

B = intermediate transition compound

k = forward and reverse specific rate constants

Overall, the reaction can be considered as being of the type:

$$A \rightleftharpoons C$$

The equilibrium constant can be expressed as:

$$\frac{[C]}{[A]} = K_e$$

The transitory compound, B, was cyclical in the study performed, whereas compounds A and C were unsaturated aliphatic allyl azides. Specifically, the reaction was:

$$CH_2=CH-\underset{\underset{CH_3}{|}}{CH}-N_3 \rightleftharpoons CH_3-CH=CH-CH_2-N_3$$

α-methylallyl azide $\qquad\qquad$ γ-methylallyl azide

The reaction involved closing and opening of a ring
structure intermediate to achieve final equilibrium.
The rate of isomerization as well as the equilibrium
constant increased as the applied pressure was increased.
Although the results are specific for one type of organic
reaction; equilibrium, and ring closing and opening
of an intermediate, and for use of a very high pressure
(up to 7,000 atms) they do indicate that pressure does
influence both the rate and equilibrium concentrations
of organic reactions.

JANNACH

The first significant discovery dealing with the
effects of pressure on the degradation of organic material
was the report of Jannach (19). In 1968 the Woods Hole
Oceanographic Institute's research submarine ALVIN sank
to a depth of 5,000 feet (2165 psig) off the coast of
Woods Hole, Massachusetts. After ten months of submersion,
the vessel was recovered and food materials found inside
were in a relatively well-preserved state with little
or no degradation. When placed into a refrigerator
at 30°F in the lab, it spoiled in only a few weeks.

Subsequent experiments substantiated these findings

with the conclusion that the rates of microbial degradation

were 10 to 100 times less at the elevated pressures.

It was postulated that the slow-down is caused by pressure

exerting an effect on the cells, which, in turn, raises

their minimal growth temperature. In an environment

of low temperature and elevated pressure as found in

the deep sea, microbial activity will decrease and eventually

cease; increased pressure causes the minimal growth

temperature to rise and finally surpass the environmental

temperature.

LAWRENCE

Lawrence's work (20) consisted of pressurizing

waste sludge, diluted with distilled water at one, two,

and three atmospheres; and then measuring the resultant

chemical oxygen demand (COD). Samples were withdrawn

after 30 minute intervals for a run of 3 hours. Figure

4 illustrates Lawrence's results. What is not clear

is whether a control was run for comparison or if the

one atmosphere sample was in fact, the control. In

any event there is a general reduction in COD as a result

of a pressure stress.

Figure No. 4
COD Variation In Waste Water Samples
Pressurized To Three Atmospheres

KAPLAN AND KLEI

An investigation of reaction kinetics of a complete-mix activated sludge process operating at pressures of 0 to 100 psig was conducted by Kaplan and Klei (21). The flow-through pressure cylinder was constructed from stainless steel and was equipped with instruments for monitoring dissolved oxygen, temperature, pH, and flowrate. Activated sludge from a wastewater treatment plant was adjusted to a pH of 6.8 to 7.2, and temperatures of 68°F to 72°F, and placed in the pressure cylinder. Samples of influent and effluent wastes were analyzed for COD.

Detention times of one to two hours resulted in the best operating conditions. Kaplan and Klei reported that little quantitative data was obtained due to the unsteady state conditions in the reactor. However, it was concluded that the growth yield coefficient decreased with increased pressure and that the kinetics of the biological reaction with pressure seemed to follow second order kinetics.

No mention was made or shown of a non-pressurized

control as a basis of comparison. It does appear that
pressure can decrease the COD depending on the amount
of pressure applied.

CHACK

Chack (22) investigated the effects of pressure
on the Biochemical Oxygen Demand (BOD) of diluted sewage;
also, on the coliform die-away. High pressures (100
psig to 400 psig) were employed in an attempt to simulate
the degradation and die-away as would be encountered
in an ocean environment. His results indicated that
the BODs and kinetic rate constant increased with the
application of pressure, as shown in Figure No. 5. In
time, the total carbonaceous and nitrogenous BOD for
the non-pressurized sample approached the oxygen demands
for the pressurized samples. It would appear that the
increase in pressure from 100 psig to 400 psig produced
no significant increase in the BOD_L value. There is
a faster rate of BOD exertion for the pressurized samples
and there is a lack of the characteristic two-stage
BOD curve.

Figure No. 5

Variation Of The BOD Of Pressurized Wastewater Samples - Chack

Another aspect of Chack's work involved enumerating the coliform density after pressure was applied using the Most-Probable-Number (MPN) method. His results are semi-quantitative at best, but the indication is that there is a decrease in population with increasing pressure.

The significance of Chack's work is that his results showed an increase in the rate of BOD exertion as well as the absence of the second stage, or nitrogenous BOD. It would appear that pressurization increased the rate of conversion of organic nitrogen to ammonia from whence the nitrification proceeded simultaneously with carbonaceous oxidation, thus eliminating the classical first-stage - second-stage BOD curve.

NUSSER

Nusser (23) investigated the pressures below the values used by Chack ($<$ 100 psig) and found the BOD to be reduced rather than increased. His optimum BOD reduction occurred for 27.4 psig (2 atm) and one hour detention time where an apparent 25 percent BOD_L reduction was realized. This is shown in Figure No. 6.

Figure No. 6

Pressure Effects On The BOD Profile - Nusser

Pressurized — — —
Unpressurized ———

First Stage BOD

Time - Days

BOD (mg/L)

Another aspect of Nusser's work was the occurrence
of the second stage BOD up to three days earlier in
the pressurized samples, as compared to the unpressurized
samples. This would suggest that pressurization accelerated
the organic nitrogen to ammonia to nitrate conversion
reactions, thereby exerting an earlier oxygen demand.

MEZEI AND MAZZEI

Mezei (24) and Mazzei (25) showed BOD reductions
for pressurized wastewater samples of up to 16 percent
at 100 psig and one hour detention time. Mazzei attempted
to extend Chack's work with coliform die-away but his
results were very erratic. However, it did indicate
the appearance and concentration of nitrifying bacteria
earlier and increasing for pressurized samples in conjunction
with the earlier appearance of the second stage BOD
for pressurized samples. This would reinforce the hypothesis
that pressurization increased the organic nitrogen to
ammonia to nitrite to nitrate conversion because ammonia
and nitrite are substrates for the nitrifying organisms
necessary to carry out these reactions.

NOLTE

Nolte (26) constructed a laboratory pilot plant activated sludge reactor as well as the pressurization vessel to carry out his investigations. Synthetic samples consisted of sugar as the main organic constituent and the effects of pressure showed that the rate constant increased, and at the same time there was an apparent 16 percent reduction in BOD_L. Nolte also showed a positive correlation between BOD reduction and the mixed liquor suspended solids (MLSS) in the activated sludge chamber.

EDWARDS

Edwards (27) constructed a pilot plant pressurization unit which went on stream in 1979 at the Caldwell-West Caldwell Sewage Treatment Plant (28) in West Caldwell, New Jersey. This system is primarily a physical-chemical operation but it is important because it is the only large scale pressurization process reported.

In this process, sulfuric acid was added to raw wastes to lower the pH to approximately 1, and the acid-

ified wastes were then pressurized with on-site generated ozone to approximately 40 psig for 30 minutes. The effluent was neutralized with caustic to a pH of 7 with the effects being a dissolved oxygen (DO) of 30 mg/l which quickly dissipated and no apparent sludge. Edwards claims that the acid/ozone/pressure combination oxidizes the sludge to the soluble state and provides BOD reductions of 50 percent. Unused ozone was recycled and repressurized for use in the next batch.

DISCUSSION OF THE RESULTS OF PREVIOUS INVESTIGATIONS

The results of the prior investigations are summarized in Table No. 1. As can be seen, the pressures, detention times, samples, and conditions vary considerably. Obviously, there is a need to discuss these results in order to resolve many of the apparent contradictions and confusions.

The work involving pressurization conducted outside of New Jersey Institute of Technology was exploratory and would appear to be overlapping, yet the results and conclusions are contradictory. Lawrence (20) and Kaplan and Klei (21) both used COD as their measuring parameter with Lawrence reporting reductions but not

TABLE 1.
SUMMARY OF PRESSURE INVESTIGATIONS

NAME	PRESSURE	ANALYSIS	DETENTION TIME	SAMPLE	REDUCTION	REMARKS
LAWRENCE	14.7 psig 29.4 psig 44.1 psig	COD COD COD	30 min. 30 min. 30 min.	diluted sludge	– 56% 37%	increased pressure decreased COD reduction. 3 atms kept water saturated.
KAPLAN KLEI	0 to 100 psig	COD	1 to 2 hours	activated sludge	N/A	biological reaction was 2nd order. Pressure no effect on rate constant.
CHACK	100 to 400 psig	BOD	1 to 5 days	sewage diluted in nutrient water	BOD_5 doubled BOD_L same	increase in pressure increased BOD. Rate also increased.
NUSSER	0 to 400 psig	coliform	2 to 8 days	diluted sewage		.
	14.7 to 100 psig	BOD	10 to 360 minutes	sewage		
	100 psig	BOD	30 min.	sewage	12%	2nd stage BOD occurred 3 days earlier.
	100 psig	BOD	60 min.	sewage	16%	Higher kinetic rates.
	100 psig	BOD	360 min.	sewage	19%	Amt. of air above liquid surface no factor.
	14.7 psig	BOD	60 min.	sewage	21.3%	

33

TABLE NO. 1 (Continued)
SUMMARY OF PRESSURE INVESTIGATIONS

NAME	PRESSURE	ANALYSIS	DETENTION TIME	SAMPLE	REDUCTION	REMARKS
NUSSER	29.4 psig	BOD	60 min.	sewage	25.1%	
	73.5 psig	BOD	60 min.	sewage	12.5%	
MEZEL	40 psig	BOD	60 min.	primary effluent	2%	
MAZZEI	100 psig	BOD	60 min.	primary effluent	16.5%	Nitrifying organisms were higher. 2nd stage occurred sooner.
NOLTE	0 to 40 psig	BOD		synthetic waste	16%	Rate constants increased with increasing pressure and higher MLSS.
EDWARDS	40 psig	sludge	60 min.	sewage		

compared to any non-pressurized control. Using sludge
as he did, the COD may not be the best parameter to
measure because both inorganic and organic materials
are oxidized during the COD procedure. Also, in Lawrence's
work he shows the COD increasing after a certain value
of pressure (2 atm) is applied.

In the work conducted at NJIT, there is more continuity
as each new researcher attempts to extend from where
his predecessor left off. Chack (22) begins by pressurizing
up to 400 psig and notes that BOD_5 is doubled as a result.
This is an obvious contradiction to the results obtained
by Jannach (19). In an effort to resolve this problem,
Nusser (23) finds that at pressures up to 100 psig there
is BOD reduction. Nusser's work is a partial agreement
to Lawrence's (20). Nusser was the first to observe
the increase in the second stage BOD as a result of
pressure. Chack left unexplored the possibility of
aftergrowth as an explanation as to why the BOD and
rate increased even though he measured coliform die-
away. Nolte (26) prepared a synthetic waste and then
subjected it to pressure in a simulated activated sludge
reactor. He found that the BOD was reduced while at

the same time the rate of decomposition increased. The
BOD can be summarized in Figure No. 7, which compares
the generalized BOD curves for the principle researchers.

The core of the problem is that all varieties of
samples are used for pressurization as well as pressure
devices and pressure detention time. The fact that
microorganisms are an integral part of the BOD reaction
underlies the need to take this into consideration.
Only, Chack, indirectly, did any investigation into
the microorganism concentration as a result of pressure.
The overall conclusion from all these effects is that
there is a response of BOD pressure. To best show this
conclusion in Figure No. 7, which shows generalized
BOD curves for pressurized and non-pressurized samples
with three areas open to investigation:

    1.   There is a reduction in the BOD due to pressure;

    2.   Second stage nitrification occurs faster in
        the pressurized sample.

    3.   The kinetics of the first stage BOD are altered
        because of pressure.

Figure No. 7

Graphical Summary Of The BOD Results Obtained By The Principal Researchers In The Pressure Field

Control

Nolte, Nusser, and Mazzei

Chack

② ③ ① ③

Time - Days

BOD - mg/L

Areas Of Investigation

① Apparent BOD$_L$ Reduction
② Earlier Appearence of Nitrification
③ Kinetic Rates Altered

IV. SPECIFIC OBJECTIVES OF THE RESEARCH

It is the intent of this research to reproduce many of the previously mentioned findings in order to obtain better quantitative data regarding the pressurization phenomenon. Assuming these results are achieved, the main thrust of the research will be an attempt to answer the following questions:

1. Why was there a reduction in the first stage BOD for pressurized samples as compared to non-pressurized samples?

2. Why did the second stage BOD in fact occur sooner in pressurized samples as compared to non-pressurized samples?

3. Why were the kinetics of the BOD reaction altered due to the application of pressure?

To account for these observations, the focus of the research will be to ascertain what the phenomenon at elevated pressures is due to:

1. Was there a change in the dissolved oxygen con-

centration caused by pressure to supply sufficient

oxygen so that if any reductions took place

they were simply caused by chemical oxidation?

2. Did the phenomenon simply result because of

of the nature and concentrations of the micro-

organisms present in the seeded dilution water?

3. Did the wastewater sample simply oxidize naturally

within the detention time period in the pressur-

ization vessel to account for the BOD reduction?

4. Did the pressurization reduce the microorganism

population to such a level that they experienced

an aftergrowth potential?

5. Did the pressurization increase the rate of

change of the components present in sewage to

different organic forms, making its oxidation

easier and faster?

6. Did the pressurization increase the conversion

of organic nitrogen to ammonia to nitrite to

nitrate faster so that the second stage BOD

appeared sooner than would be the case for

non-pressurized samples?

After these questions have been answered based on the review of actual wastewater samples subjected to pressure, the results will be compared with findings on a prepared and pressurized synthetic sewage sample. If the results for the synthetic sample are consistent with the results for the actual samples, then it can be theorized that the phenomenon noted is real and reproducible.

This theory can then ultimately be utilized to design a systems operation related to pressurization in wastewater treatment facilities.

V. EQUIPMENT DESIGN AND DESCRIPTION OF ITS OPERATION

The research was conducted with a pressure vessel
previously constructed by a prior researcher (26) at
NJIT. This vessel was used in all subsequent pressurization
studies. This device was constructed from one-quarter
inch thick lucite plastic formed into a tubular shape
one foot in diameter and three feet high. The vessel
was reinforced with three metal straps around the circum-
ference. Two 2-inch clear plastic plates were circularly
grooved three-eighths of an inch deep on one side and
rubber O-ring gaskets inserted as caps for either end
of the tube. The cylinder was supported upright on
four aluminum legs which passed through the lower and
upper plate caps. The upper ends of the legs are threaded
so that nuts could be applied to tighten the end plates
against the cylinder. Midway through the research the
aluminum rods were replaced by steel rods as the aluminum
threads tended to strip when tightened too much. Figure
8 shows a diagram of the apparatus.

Air was supplied by a Champion "Porta Champ" air
compressor. Air was diffused through porous stones

Air relief valve

Air

Pressure Regulator

Influent

Liquid level switch and float

Air flowmeter

pH probe

D.O. Probe

Effluent line

Paddle shaft

Effluent flow meter

Settling Chamber

Baffle

Baffle

Effluent line

Paddle

Waste sludge line

Motor for paddle

Figure No. 8

Pressure Vessel Used For All Pressurization Studies

placed on the bottom of the cylinder. The device was
rated to handle 50 psig as a maximum. Operating pressures
in this study never exceeded 40 psig. The air pressure
maintained in the vessel was set by a pressure regulator
and a pressure gage was installed. A relief valve was
placed on the upper plate so that a continuous flow
of air was always maintained.

The lower plate contained the drain which was controlled
by a valve. Also, a motor-activated rotating paddle
was placed in the center of the bottom plate to insure
the contents were kept suspended during pressurization.

VI. EXPERIMENTAL PROCEDURE

During the course of the investigation several laboratory techniques were employed to measure and collect experimental data. The major parameter measured was oxygen consumption and this was accomplished using both the Biochemical Oxygen Demand, BOD, and the Chemical Oxygen Demand, COD, methods. To measure changes in other chemical compounds spectrophotometric and potentiometric procedures were employed due to the very small concentrations of the compounds involved. Following will be presented a detailed description of the BOD and COD procedures (29) with the individual spectrophotometric methods included in Appendix D.

BOD

The BOD test was performed using a Weston and Stack model 330 Dissolved Oxygen meter. The meter is calibrated at ambient temperature with the probe in water vapor saturated air. The meter is standardized with dilution water using the modified Winkler Method.

For the daily BOD analyses 5 samples and 2 blanks were prepared. Two blanks were used in the event one proved faulty. When both blank values were obtained

43

an average value was used.

Raw sewage from the Caldwell treatment plant usually
runs about 200-250 mg/l BOD and is very consistent.
Dilutions were made so that the smallest dilutions would
not deoxygenate even up to twenty days. The larger
samples would deoxygenate but they were used because
they provided meaningful oxygen depletions during the
early phases of the incubation. With a careful range
of dilutions chosen there would always be oxygen remaining
after twenty days for a dissolved oxygen reading.

Depletion curves for the blanks were also drawn
as a precaution against erroneous blank readings. Occasion-
ally this would occur if the DO probe required a new
diaphram, change in the potassium iodide solution, or
some other adjustment. During the course of the twenty
day incubation period the blank may experience a one
mg/l depletion due to the decomposition of organics
present in the seed material used to prepare the dilution
water. However, BOD values are determined by the differences
between samples and blanks, so any diviation with the
blank in automatically corrected for.

The average of the five daily readings was taken
as the BOD for that day up to day seven in order that
accurate curves could be drawn. Daily values were needed
so that the specific rate constant could be determined
accurately as possible. This sampling process was repeated
each detention time of pressurization that the wastewater
sample was subjected. Usual detention times were for
2, 4, 8, 24, and 48 hours. Besides the pressurized
samples controls and aerated samples were run after
the same detention times. This means that within 48
hours 18 sets of 7 bottles (5 samples and 2 blanks)
were prepared and 126 daily readings were required.

The advantage of the DO probe was that the same
sample bottle could be used every day for a dissolved
oxygen reading. In the wet chemical method the contents
of the bottle are reacted every day so that would mean
using 126 times 15, or approximately 1800 bottles for
the entire analysis. When it was seen that the DO readings
were constant after a certain time the bottle was analyzed
using the wet chemical method as a verification. The
results were reproducible.

The results using the probe were very good compared

to using only the wet chemical method. When plotting

BOD curves the points tended to give a good BOD profile

without the need for curve fitting. When using the

wet chemical method only there is too much variance

in daily readings and when the points are plotted the

BOD profiles were not well defined and curve fitting

techniques had to be applied. The advantages using

the DO prove for an extended time cannot be over emphasized.

For an excellant discussion concerning the theory of

the BOD test, readers are referred to Ciacchio (30).

COD

Because the composition of the raw sewage used

during the research was essentially domestic, it was

decided to use the modified Jeris method for COD determin-

ations (31). This eliminates the need for the lengthy

reflux period as required in Standard Methods COD proce-

dure (32). Into Erlenmeyer flasks are placed blanks

and sample solutions, and to these are added a combined

potassium dichromate, sulfuric acid, and silver sulfate.

These are heated on the hot plate to the boiling point,

cooled, and then titrated with ferrous ammonium sulfate.

From the titration values, sample size, and the molarity

of ferrous ammonium sulfate, the COD value can be calculated.
The derivation of the COD formula is presented in Appendix
C.

For purely domestic sewage wastes, the Jeris Method
can be used in place of the Standard Methods procedure
without appreciable error. This has been shown by Wells
(33) and also by the author in determining COD's for
known compounds and comparing these values to the theoret-
ical COD of the compound. The results are in excellant
agreement.

Midway through the research a Hach COD reactor
was obtained. This method is essentially the Jeris
procedure but the blanks and samples are added instead
to screw cap vials containing the reagent. These vials
are then heated in a thermstatically controlled heating
mantle for one hour at 150°C. As in the BOD test 3
samples and 2 blanks are run. Averages of the blanks
are used when they were obtained. Care must be used
so that the volume of the sample is not greater than
the volume of the reagent solutions in the vials which
may cause dilutions leading to erroneous results. After
digestion, the samples can be titrated or analyzed

spectrophotometrically (34). Calibration curves for this procedure are shown in Appendix D. This Hach method is now EPA approved (35).

SPECTROPHOTOMETRIC

During the course of the research the analysis for various organic compounds was performed using spectrophotometric means. The components tested for spectrophotometrically were glucose, ammonia, and proteins. For the analysis of these compounds calibration curves were prepared from the pure components being tested for. Each analysis involved adding specific reagents to samples producing a characteristic color whose intensity was measured. Using the calibration curve and the measured intensity concentrations can then be determined. For a discussion of the chemistry involved and a listing of the reagents and procedures used see Appendix M. Calibration curves are found in Appendix D.

VII. POSSIBLE MECHANISMS TO EXPLAIN OBSERVED PHENOMENA

After examining the results of the previous investigations
(19-26), and keeping in mind the kinetic principles previously
described, possible mechanisms were proposed in order
to attempt to describe the following:

1. Why was there an apparent BOD reduction
   with the use of pressure?

2. Why did the second-stage BOD appear earlier
   in pressurized samples?

3. Why and how were the kinetic rate constants
   altered due to pressure?

The next phase of the research was to determine
the exact mechanisms which contributed to the BOD and
COD reductions that occurred due to pressure. This will
be done by a step-by-step investigation of all the possible
mechanisms thought to be responsible.

DETENTION TIME IN THE PRESSURE VESSEL

In the works reported by Nusser (23), Mazzei (25),
and Nolte (26), they all showed generalized BOD curves

49

for pressurized samples to be less than for non-pressurized
samples for the same time period monitored. These results
were shown in Figure 7, page 36. The initial question
posed in this investigation was would this reduction
in BOD phenomena noted by others occur within the pressure
vessel herein both for pressurized and non-pressurized
conditions. To address this question, two samples were
analyzed; one was pressurized up to 48 hours, and the
other, non-pressurized, was aerated to prevent it from
possibly going septic. This will not affect the end
result for this investigation because the detention times
selected are such that the sample should not go septic.
Another phase of the investigation will focus on the
role of the dissolved oxygen concentration as a cause
of the BOD phenomenon. Aliquots from both were drawn
off at 2, 4, 8, 12, 24 and 48 hours after initiation
of the test, and their BOD and COD values determined.
The graphical and tabulated results are shown in Appendix
E.

For all times of pressurization, the difference
in the BODs for the pressurized sample ranged from 10
to 20 mg/l. When the COD results were obtained, these
showed the same differences for the same pressurization
times. Also, kinetic rate constants were evaluated,

and these show a decrease in value for a corresponding decrease in BOD material. The k values were greater for the pressurized samples compared to the aerated samples. These data are summarized in Table 2.

The experimental results of Δ BOD, Δ COD, and k rates indicate that pressurization does in fact have a positive effect on the amount and rate of organic decomposition. This is consistent with prior findings by other authors such as Nusser (23), Mazzei (25), and Nolte (26). What these results show is that for all other variables kept constant, i.e., Dissolved Oxygen Saturation Values, sufficient microorganisms in the seeded dilution water, and sample composition, there is the positive effect on apparent demand reduction due to pressurization. The effects of each of these variables individually are examined below.

ROLE OF MICROORGANISMS

Because of the nature of the BOD reaction, microorganisms play an important role in the amount and rate of organic decomposition. For this reason, investigations were

TABLE 2
VARIATION OF BOD_L AND COD VALUES FOR
AERATED AND PRESSURIZED WASTEWATER SAMPLES

PRESSURE TIME: HOURS	AERATED (1)			PRESSURIZED (2)			CHANGES IN (3)	
	BOD mg/l	COD mg/l	k_1 1/day	BOD mg/l	COD mg/l	k_1 1/day	Δ BOD mg/l	Δ COD mg/l
0	130	320	0.19	130	320	0.19	--	--
2	125	280	--	115	270	--	10	10
4	125	235	0.17	111	220	0.21	14	15
8	114	195	0.14	104	180	0.19	10	15
12	--	185	--	--	170	--	--	15
24	75	152	0.10	64	130	0.16	11	22
48	55	150	0.09	35	128	0.13	20	22

(1)   Indicates the changes in BOD, COD, and k_1 after increased periods of aeration for the aerated, non-pressurized sample.

(2)   Indicates the changes in the BOD, COD, and k_1 after increased periods of pressurization for pressurized samples.

TABLE 2 (Continued)
VARIATION OF BOD_I AND COD VALUES FOR
AERATED AND PRESSURIZED WASTEWATER SAMPLES

(3)   Indicates the change in BOD (Δ BOD) for the aerated
v.s. the pressurized sample after the specified time
period, and the change in COD (Δ COD) for the aerated
v.s. pressurized sample, again for the same specified
time period.

conducted to assess that role. The approach used was
to pressurize a sample, determine the microorganism
population, and then perform a BOD. BODs would be performed
in both seeded and unseeded BOD dilution water.

Sewage samples were pressurized and from aliquots
drawn off after increasing times of pressurization,
total plate counts and total coliform enumerations were
performed. After 48 hours of pressurization, total
coliform organisms were reduced to extinction. Total
bacteria were also reduced but sufficient numbers remained,
even after 48 hours of pressurization, to effect the
BOD reduction as noted below. Table No. 3 summarizes
the microbial concentrations as a result of pressurization.

The BOD curves obtained from seeded and unseeded
dilution water are shown in Appendix F.

What this trial shows is that the pressurization
does not affect the microorganisms responsible for bio-
chemical oxidation as evidenced by the BODs for pressure
times of two to 48 hours; and also that the seeding
of the dilution water does not in any way influence
the BOD results. If the pressurization destroyed the

TABLE NO. 3
SUMMARY OF MICROORGANISM POPULATION
AND BOD AFTER PRESSURIZATION

| PRESSURE TIME: HOURS | MICROORGANISMS | | BOD_L RESULTS, mg/l | |
	TOTAL PLATE COUNT: #/100 ml	TOTAL COLIFORM #/100 ml	SEEDED DILUTION WATER	UNSEEDED DILUTION WATER
0	TNTC	10^6-10^8	$\overline{}100$	$\overline{}100$
2	TNTC	10^8	80	80
4	TNTC	10^4	80	85
8	TNTC	300	68	68
24	10^{12}	30	45	50
48	10^{10}	0	22	22

TNTC - Too Numerous To Count.

organisms necessary for the organic decomposition by
biochemical means, then there should be no BOD exertion
in the unseeded dilution water as neither the sample
nor the dilution water would contain the kinds and amounts
of the necessary organisms to accomplish a BOD exertion.
Table No. 3 summarizes the BOD results for seeded and
unseeded dilution water.

CHEMICAL OXIDATION

Using compressed air as the source of pressure,
the result would be to increase the concentration of
dissolved oxygen in the pressure chamber. Compressed
air is introduced through the stone diffusor which,
in effect, acts like an aerator. One could then speculate
that any changes in the BOD profile could be caused
by the increased concentration of oxygen. At 40 psig
pressure, dissolved oxygen readings ranged from 8.6
to 13.0 mg/l, depending on the length of time subject
to pressurization.

Referring to the kinetic expression for the BOD
reaction, the question to resolve is what is the effect

on the BOD profile of the increased oxygen concentration. The simplified expression states that, for the reaction shown in (e):

(e)   organics   +   D.O. $\xrightarrow{[MO]}$ CO_2 + H_2O

it can be theorized that an increase in dissolved oxygen would increase the rate by which the organic material degrades. By making more oxygen available to the micro-organisms, they may metabolize the organics faster, resulting in a faster rate of decomposition. A generalized BOD curve for such a situation is shown in Figure 9.

To study the effect of oxygen concentration, three trials were prepared and run: (1) pressurized and aerated, the aeration coming from the compressor through the diffusor; (2) non-pressurized and aerated with an air pump; and (3) samples non-pressurized and non-aerated, henceforth called quiescent samples. At selected times, aliquots were drawn from all three samples and BOD analyses performed. The plots are shown in Appendix G, with the results tabulated in Table No. 4.

58

Figure No. 9

Composite BOD Curves Including Pressurized Detention Time

TABLE No. 4
BOD AND RATE CONSTANT VALUES FOR SAMPLES
WHICH WERE QUIESCENT, AERATED, AND PRESSURIZED

QUIESCENT SAMPLES		
TIME, HOURS	BOD mg/l	RATE CONSTANT DAY-1
0	120	0.11
4	88	0.11
8	75	0.12
24	53	0.11

AERATED SAMPLES		
TIME, HOURS	BOD mg/l	RATE CONSTANT DAY-1
0	115	0.11
4	90	0.11
8	70	0.12
24	53	0.11

PRESSURIZED SAMPLES		
TIME, HOURS	BOD mg/l	RATE CONSTANT DAY-1
0	115	0.17
4	–	–
8	70	0.16
24	55	0.13

What the results show is that the aerated and quiescent
samples display almost identical BOD curves for all
the times samples were taken. Leaving raw wastewater
samples standing for 24 hours results in D.O. levels
dropping almost to zero and the samples going septic.
For that reason, 24 hours was the time chosen to terminate
the experiment. The D.O. level was at saturated conditions,
consistently in the aerated sample, indicating the air
pump was supplying oxygen faster than it was being consumed
in the organic decomposition.

The interesting aspect of the BOD profiles for
the three systems analyzed is that the general shape
approximates the speculated shape but apparently for
a different reason. The kinetic rate constants are
higher for the pressurized sample as compared to the
aerated and quiescent samples which are identical. Also,
the BOD_L value for the pressurized sample appears to
be a little less than the BOD_Ls for the aerated and
quiescent samples, giving rise to an apparent BOD reduction.

The results of this experiment indicate that the
oxidation reaction is not oxygen limiting. That is,

above a certain dissolved oxygen concentration, the
reaction carries on independently of oxygen concentration.
This minimum dissolved oxygen concentration is what
is necessary to maintain the aerobic nature of the micro-
organism population. Nusser (23) conducted experiments
in which he altered the oxygen volume in the pressure
cylinder above the liquid surface prior to pressurization
and concluded from his investigation that there was
no difference in BOD response. Also, Gaudy (6) indicates
that a certain "critical" D.O. concentration is needed
to support microbial respiration and above this value
increases in D.O. concentration do not alter microbial
or organic decomposition rates. He quotes a minimum
value of 0.5 mg/l but suggests that a value of 2 mg/l
be used.

To prove that BOD decomposition is independent
of oxygen above a certain limit the following experiment
was performed. A raw sewage sample was collected,
screened to remove solid particles, and separated as
follows: a sample to be pressurized at 40 psi and 12-
13 mg/l of DO; a sample aerated at atmospheric pressure
resulting in a DO of 8-9 mg/l, called the aerated sample;
and a sample allowed to stand at atmospheric pressure

with no aeration, called the quiescent sample. The
differences between the aerated and quiescent samples
are that during the course of the trial the aerated
sample would decompose lowering the BOD available but
would maintain a saturated DO value. The quiescent
sample would also decompose lowering the BOD but also
it would experience a reduction in DO. BOD's would
then be taken at intervals up to 24 hours with samples
taken at 4, 8, and 24 hours after the trials commenced.
DO's were monitored for the aerated and quiescent samples
and these are shown in the following chart.

| TIME HR.S | DISSOLVED OXYGEN, mg/l | | DO |
	AERATED	QUIESCENT	Δ
4	10	5.1	4.9
8	10	3.8	6.2
24	10	2.4	7.6

If there were changes in the BOD profile then oxygen
concentration was a parameter to consider; if not, then
the BOD reaction was independent of the DO concentration.
The BOD profiles for samples analyzed at 4, 8, and 24
hours are shown in Figures 10 & 11. The curves are

Figure No. 10

Variation Of BOD For Quiescent And Aerated
Wastewater Samples For 4 Hours

Figure No. 11

Variation Of BOD For Quiescent And Aerated
Wastewater Samples For 8 and 24 Hours

the same for each time samples. Also, the kinetic rate

constants were determined for each BOD curve and plotted

(Figure No. 12). The variations of k curves for the

aerated and quiescent samples are the same and they

show the characteristic decrease indicating that the

rate decreases as the organic concentration decreased.

To summarize:

(8)   rate = k $[CHO]^1 [DO]^0$

(9)   rate =   k $[CHO]^1$

Equation (8) indicates that the rate of decomposition

is independent of DO as   DO for each trial is different

but the rates and BOD_L values are the same. Also, expression

(9) indicated what is assumed in the first order decompo-

sition law; the rate decrease is proportional to the

decrease in organic material. Gaudy (6) has indicated

the same in his research.

These generalizations can be summed up as follows:

1)   The rates of decomposition and the BOD_L values

Figure No.12

Variation Of The Specific Rate Constant For The BOD Reaction After
Various Times Of Pressurization For Aerated And Non-Aerated Samples

for the aerated and quiescent samples are the same.

2)   The pressurized sample has a lower BOD_L value but
a faster rate of decomposition as compared to the aerated/
quiescent sample.

3)   Any changes that do take place are not the result
of increased DO concentration due to pressurization.

The final conclusion is that for any change that
does occur in the BOD profile due to pressure, the contri-
buting cause must be changes taking place in the nature
and composition of the organic materials being pressurized.
It is the intent of this research to show that this
is the case by investigating all the ways the nature
and composition of organic materials can be changed,
chemically and microbiologically, and comparing these
BOD profiles to control profiles to observe any changes
taking place.

AFTERGROWTH

As mentioned earlier, the BOD reaction is catalyzed

by aerobic microorganisms which derive their energy
from the breakdown of organic components. The extent
by which they can function depends on their nature,
temperature, pH, salt concentration, predator-prey relation-
ship, and their relative position on the growth curve.
Mention was made that environmental stresses, such as
dilution and disinfection, can lower the total microbial
population to such a degree that single cell plant bacteria
can then experience a population explosion far greater
than what could normally be expected. The causes respon-
sible for this phenomenon are (1) reduced predator population
whose reproduction rate is much less than the bacteria,
and (2) a higher ratio of food to microorganisms which
result in unlimited exponential growth for the microorganisms.
In this flurry of activity, the microorganisms would
degrade the organics at a faster rate, resulting in
a more rapid oxygen demand. As conditions normalize,
this increased rate would then revert to the equilibrium
conditions that existed before the environmental stress
was applied.

To determine if aftergrowth is a viable factor,
two series of experiments were performed. In the first

trial, a sewage sample was pressurized for periods of 1/2, 1, and 3 hours. After each time interval, aliquots were drawn off and allowed to stand for up to three days. BODs were taken each day and compared to a control. The important relationship to determine is if there was any increase in BOD utilization, and, if so, were these increases due to microbial activity. Since first-stage BOD activity was the focus of the investigation, any nitrification which occurred beyond the first stage was ignored.

The curves shown in Appendix H show no abnormal BODs being exerted. In fact, the curves resemble those obtained when investigating the role of detention time of the wastes in the pressure vessel. Also, the rate constants were determined and these exhibited the decrease that was shown before for decreasing BOD concentrations.

For the second trial, it was decided to repeat the experiment but this time to increase the time of pressurization to 24 hours. Then the samples would be allowed to stand for up to four days and BODs would be determined. The results obtained were astonishing.

As the sample stood after pressurization, the rate of
BOD exertion and the rate constant both increased up
to a maximum value. See Appendix I. As the BOD is
exerted faster for days one to three, the BOD curves
cross over at days 5 and 7, and each succeeding day
there is less BOD_L. This difference is accounted for
in the more rapid degradation of the organisms during
the initial phase of the oxidation.

From Figure 61 in Appendix I, it can be seen that
the rate constant increases rapidly to a maximum and
then declines. After 24 hours of pressurization, this
could be enough of an environmental stress to reduce
the original microorganism population to an aftergrowth
potential. As the organisms multiply, now that the
stress is removed, their rate of oxygen utilization
will be greatly increased, resulting in a higher rate
constant value. These findings show that there is an
aftergrowth potential when using pressure.

HYDROLYSIS

The final mechanism to consider was that the material
being pressurized was subject to molecular changes due

to hydrolysis. Hydrolysis simply involves reactions of larger molecular weight organic compounds with water to produce smaller organic molecular weight fractions. An example would be the acid catalyzed hydrolysis of ethyl acetate, $C_2H_5COOCH_3$, to ethanol and acetic acid:

(F) $C_2H_5COOCH_3 + H_2O \xrightarrow{[H^+]} C_2H_5OH + CH_3COOH$

In an attempt to demonstrate that pressure could increase the rate of hydrolysis, it was decided to study a known hydrolysis reaction which was easy to monitor and measure. Pressurized and non-pressurized samples would be run and any changes in the hydrolysis rates would be due to the pressure effect.

The compound chosen was para-nitro-phenyl-acetate, $C_8H_7NO_4$, which is a slightly soluble, yellow powder, which in water hydrolyzes to paranitro-phenol and acetic acid:

(g) NO_2⟨◯⟩$-O-C-CH_3 + H_2O \rightarrow NO_2$⟨◯⟩$-OH + CH_3COOH$

 O

   $(C_8H_7NO_4)$ $(C_6H_5NO_3)$

The para-nitro-phenol produced is an intense yellow
color whose absorbence can be measured spectrophoto-
metrically and thus the concentration can be monitored
as it is being produced. The reaction is acid or base
catalyzed, thus it would have to be performed in a buffer
solution as the product, acetic acid, would tend to
lower the pH as it is being formed. In a basic buffer
solution, the following reaction takes place:

$$(h) \quad C_8H_7NO_4 + H_2O \xrightarrow{[OH]} NO_2^- \bigcirc \cdot O^- + CH_3COOH + H^+$$

The para-nitro-phenoxy formed has a maximum absorbence
at 400 nm, and this will be the species monitored. For
the actual experiment, 30 liters of 7.5 pH buffer solution
(36) was prepared from sodium hydroxide and potassium
dihydrogen phosphate solutions added to de-ionized water.
Para-nitro-phenyl acetate was added to make a 100 mg/l
solution. (Editor's note: Data pertaining to chemicals
used in all experiments can be found in Appendix J).
Pressurized and non-pressurized samples were run and
aliquots taken off intermittently up to 160 hours. The
experiment was repeated this time preparing the solution
to be pH of 7.7 (37) by altering the amounts of sodium
hydroxide and potassium dihydrogen phosphate solutions.

All pHs were verified, using the Coleman model 39 pH
meter. All spectrophotometric readings were performed
on the Bausch and Lomb Spectronic 21 spectrophotometer,
using distilled water as the blank. The results of
the two trials are shown in Figures 13 and 14. The
pressurized samples did show an increased rate of hydrolysis
at both pH's. For the non-pressurized samples, plateaus
were reached which signalled the end of the reaction,
but for the pressurized samples the curves descended.
Para-nitro-phenoxy compounds are known to dimerize and
trimerize (38) which are not colored and do not absorb.
This does not detract from the fact that the pressurization
does in fact cause an increase in the rate of hydrolysis.
To show that reaction (g) is irreversible, solutions
of nitro-phenol were prepared and pressurized, and these
exhibited no change in absorbence for 200+ hours. Samples
were also drawn off and analyzed for phenol,   -OH,
using the aminoantipyrine method as described in Standard
Methods (39). The test results indicated no phenol
present.

Now that pressure has been shown to definitely
influence the rate of hydrolysis, the main components

74

Figure No.13

The Effects Of Pressure On Para-Nitro Phenyl Acetate
As Compared To An Aerated Sample Conducted @ pH 7.5

Figure No. 14

The Effects Of Pressure On Para-Nitro Phenyl Acetate As Compared To An Aerated Sample Conducted @ pH 7.7

75

found in sewage were investigated in terms of their hydrolysis potential. The major organic compounds found in sewage as well as their known hydrolysis reactions to their final decomposition forms are shown in Figure 15 (40). The complex organic compounds, Line 1, are capable of hydrolyzing to smaller molecular weight fractions as shown on Line 2. The compounds in turn, are then oxidized to carbon dioxide and water with the aid of microorganisms. Proteins can also hydrolyze to form ammonium ion which can then be oxidized to the nitrite/nitrate forms. Compounds listed in Line 1 and Line 2, Figure 15, were obtained and the next phase of the research involved pressurizing each individual compound and measuring any change in the rate of hydrolysis as compared to a non-pressurized sample.

NITRIFICATION

Another phase of the investigation called for samples to be allowed to oxidize to 1st and 2nd stage oxygen demands so that the nitrification effects can be realized. In all of the previous trials only the times at which nitrification began were noted but the samples were

Figure No. 15

Diagram Showing Decomposition Mechanisms
For Organics Found In Sewage

never allowed to fully nitrify and reach the second
stage plateau. This trial was performed to show changes
in the NOD profile due to pressure. The strategy was
as follows:

1. Obtain a sample containing carbon and nitrogen compounds.

2. Add nitrifying organisms to one BOD dilution water
bottle and none to the other BOD dilution bottle.

3. Pressurize wastewater sample and prepare a BOD analysis
for it and also for the aerated non-pressurized control.

4. Allow the incubation to proceed for 25 days.

Two dilution water bottles were prepared: one,
filled with nitrifyers, and the second without nitrifyers.
Two sets of trials were run for the 24 hour pressure
detention time and the 24 hour aerated detention time:
one with nitrifyers and the second without. In this
way it will be seen if the first stage BOD was really
the first stage with no other complications producing
the profile. The pressure detention time was 24 hours
and the results compared to a control.

It was decided to use a prepared synthetic sample
because this sample contained no readily nitrifyable
ammonia and the BOD and NOD values were known. The
sample contained the following ingredients:

Acetic Acid	CH_3COOH
Starch	$C_6H_{10}O_5$
Glutamic Acid	$C_5H_9O_4N$
Urea	CON_2H_4
Glucose	$C_6H_{12}O_6$
Histidine	$C_6H_9N_3O_2$

The theoretical BOD was 8,000 mg/l and the NOD
was 6,500 mg/l. A 1/50 dilution was prepared and amyl-
glucosidase enzyme was added to increase the hydrolysis
rate of starch. The results are shown in Figure No.
16 and Table No. 5. From this Figure the following
can be seen:

1. Lowering of the 1st stage BOD due to increase of
pressure time. The 24 hour detention time sample has
less BOD because any difference from the control was
the amount decomposed in the pressure vessel for that
period of time. The pressurized trial has a higher

Figure No. 16

Variation of BOD and NOD for Pressurized and
Aerated Samples Compared to the Control

Legend

●———	Control, with Nitrifiers	BOD = 160 NOD = 100
○———	Control, without Nitrifiers	BOD = 160
▲———	24 Hours pressurized, with Nitrifiers	BOD = 124 NOD = 100
△———	24 Hours pressurized, without Nitrifiers	BOD = 124
▣———	24 Hours aerated, with Nitrifiers	BOD = 135 NOD = 100
□———	24 Hours aerated, without Nitrifiers	BOD = 135

Time - Days

BOD, mg/L

TABLE NO. 5

SAMPLE	TIME, DAYS									
	1.1	2.4	3.3	4.2	6.6	8.3	11.2	13	15.8	21.3
Control, w/nitrifyers	87	125	133	150	160	166	174	240	262	260
Control, wo/nitrifiers	81	126	136	143	157	168	154	158	164	–
24 hours, pres., w/nitrifiers	75	100	113	122	121	139	167	211	232	230
24 hours pres., wo/nitrifiers	72	104	107	126	123	125	125	126	123	–
24 hours aerated, w/nitrifiers	64	94	121	130	138	141	152	184	244	240
24 hours aerated wo/nitrifiers	59	97	118	127	121	137	135	136	139	–
	1.4	2.3	3.3	5.8	7.3	10.2	12	14.5	19.5	22

BOD values for samples allowed to nitrify with and without nitrifying organisms supplied.

rate but lower BOD because induced hydrolysis caused
more conversion to smaller molecular weight fractions
which oxidized easier and faster, and the aliquots drawn
off would oxidize faster during the BOD incubation because
they contained a higher amount of these fractions.

2. The pressurized samples nitrifyed faster; i.e.,
the pressurized sample began to nitrify at day seven
whereas the control began after day 10. This is in
agreement with previous trials.

3. For the aliquots drawn off in the non-nitrified
dilution water first stage effects were the only effects
noticed.

4. The 1st stage BOD for the control was approximately
180 mg/l which agrees with the BOD of the prepared sample.

5. The NOD was approximately 120 mg/l and this was
the same for both the aerated, pressurized, and the
control, meaning there was no loss in the nitrification
potential of the sample and the control as a result
of pressurization.

The third investigation involved adding a nitrification supressor, allyl thiourea (ATU), to an actual sewage sample, pressurizing and aerating for a certain detention time and then comparing to a control. The purpose was to obtain BOD profiles for nitrification suppressed samples and then comparing these to the profiles where nitrification was not suppressed. The curve for the situations, Figure 17, was the same indicating that in actual wastewater samples nitrification did not take place in days 0 to 10.

The important findings made during this trial was that pressure induced nitrification started earlier and that NOD values were the same indicating no nitrification taking place during the 1st stage.

Figure No. 17

BOD Curves For Wastewater Samples Plus
Allyl Thiourea as a Nitrification Supressor

VIII   THE EFFECT OF PRESSURE ON THE HYDROLYSIS RATES OF CHEMICAL FAMILIES

The experimental tests just completed have eliminated the following as possible causes for the reduction in the apparent BOD, and changes in the kinetic rate constants:

1)   Detention time in the pressure vessel.

2)   The concentration of microorganisms as effected by pressure.

3)   Chemical oxidation.

Experimental results with para-nitro-phenyl acetate indicate that the rate of hydrolysis could be increased by the application of pressure. With that as the basis, the experimentation will now focus on two areas; the breakdown of sewage components to easier oxidized forms, and the conversion of ammonia to nitrite and nitrate.

Now that pressure has been shown to definitely influence the rate of hydrolysis, the hydrolysis potential of the main components found in sewage will be performed.

85

POLYSACCHARIDES

Polysaccharides are complex organic compounds of which starch was chosen as the typical example for these studies. Starch has the general formula, $C_6H_{10}O_5$, and when hydrolyzed, the monosaccharide formed is glucose (41).

(i) $(C_6H_{10}O_5)_x + xH_2O \longrightarrow x\ C_6H_{12}O_6$

The glucose can then be analyzed spectrophotometrically using the ortho-toluidine method (42). The reaction of glucose with o-toluidine, heated in acid, produces a blue-green color proportional to the glucose concentration which can be measured in the 620-650 nm range. Calibration curves can be prepared from standard glucose solutions and these appear in Appendix D.

This particular experiment posed several problems before results were finally obtained. The first time it was performed, there was no BOD exertion after several days, whereas the CODs were identical and closely approximated the theoretical. Because the CODs were the same as

the theoretical COD for the known starch concentration,
it indicated there was no hydrolysis or oxidation taking
place. It was then realized that enzymes were needed
to break down the starch into the disaccharide and mono-
saccharide forms.

Amylase enzyme was suggested, and this was obtained
from Chemalog Chemical. Solutions were prepared and
aliquots analyzed but no results were obtained. It
was then realized that amylase enzyme hydrolyzed starch
only to the disaccharide, maltose, $C_{12}H_{22}O_{11}$, but the
o-toluidine test was only specific for the monosaccharide,
$C_6H_{12}O_6$, form.

Amylglucosidase enzyme was then selected and obtained
and this enzyme is able to hydrolyze starch only to
glucose. Two starch solutions of pH 7.0 and 4.5 were
prepared and the amylglucosidase was added and the experiment
proceeded. The results indicated that the hydrolysis
was successful and proceeded faster at pH of 7, which
is the approximate pH range of sewage. Now that the
proper conditions and rates of hydrolysis were determined,
the pressurization began.

A 500 mg/l starch solution plus 2.4 ml of amyl-glucosidase was prepared and divided into two parts. One was pressurized at 40 psig and the other aerated at atmospheric pressure. Glucose readings were obtained from the calibration curve and these results are shown in Figure 18. Apparently, an initial lag time exists in which the enzymes become acclimated to the material but once this is overcome, the reaction proceeds, with the pressurized sample showing less lag time and a faster rate of hydrolysis.

The next phase involved in this investigation was to determine if, in fact, the glucose produced by hydrolysis was in turn hydrolyzed or oxidized faster as a result of pressurization. To do this, glucose samples were prepared, pressurized, and aerated, and aliquots were drawn off and analyzed. The results obtained indicated no hydrolysis taking place for either sample.

To summarize the results of the hydrolysis reactions, polysaccharides are hydrolyzed at a faster rate due to pressure, but the hydrolysis stops at the monosaccharide stage. The reactions are shown in equation (j):

Figure No. 18

The Rate Of Hydrolysis Of Starch Catalyzed By Amylglucosidase
Enzyme For Pressurized And Non-Pressurized Samples

$$(j) \quad (C_6H_{10}O_5)_x + xH_2O \xrightarrow{[-ASE]} x \ C_6H_{12}O_6$$

$$k_p,>k_a$$

$$C_6H_{12}O_6 + O_2 \xrightarrow{[M.O.]} 6CO_2 + 6H_2O$$

FATS

The next family of compounds to be investigated were the fat and fatty acid series and their hydrolysis potential. Fats are esters of trihydroxy alcohols and they are capable of hydrolysis to fatty acids. The fat to be investigated was tristearin, $C_{57}H_{110}O_6$, which is the ester of glycerol and stearic acid. Formula (k) shows the hydrolysis reaction for tristearin:

(k)

$$
\begin{array}{l}
\qquad\quad\overset{\displaystyle O}{\overset{\displaystyle \|}{C_{17}H_{35}-C-O-CH_2}} \\
\qquad\quad | \\
C_{17}H_{35}-\underset{\underset{\displaystyle O}{\displaystyle \|}}{C}-O-CH + 3 \ H_2O \longrightarrow 3 \ C_{17}H_{35}COOH + C_3H_8O_3 \\
\qquad\quad | \\
\qquad\quad | \qquad \overset{\displaystyle O}{\overset{\displaystyle \|}{}} \\
C_{17}H_{35}-C-O-CH_2
\end{array}
$$

This reaction is catalyzed by base solution and this

process is referred to as saponification (41). Tristearin
is very insoluble in most solvents with tetrahydrofuran
being one of the few exceptions. A tristearin/tetrahydro-
furan solution was added to a sodium hydroxide solution
such that the tristearin was 0.0013M in 0.0077M sodium
hydroxide. As the tristearin hydrolyzed to stearic
acid, this would neutralize some of the excess base
solution. To monitor this reaction, samples were drawn
off and titrated with standard .005M hydrochloric acid
solution. All titrations were performed potentiometrically,
using the Corning Model 7 pH meter. Curves were drawn
and inflection points determined. After six days the
base concentration was reduced to 0.0061M due to hydrolysis.
The neutralization reaction is shown in equation (1).

$$(1) \quad C_{17}H_{35}COOH + NaOH \longrightarrow C_{17}H_{35}COONa + H_2O$$

The difference in sodium hydroxide molarity, 0.0077 -
0.0061 = 0.0016, represents the stearic acid formed.
Dividing by 3 to convert to an equivalent amount of
tristearin and determining the percentage conversion
gives approximately 60 percent hydrolysis conversion
of tristearin to stearic acid under normal quiescent

conditions.

For the pressure/hydrolysis reaction, it was decided
to dispense with the tetrahydrofuran solvent and only
add the tristearin to a standardized sodium hydroxide
solution. Two samples were prepared: one for pressur-
ization at 40 psig and the other sample aerated at atmos-
pheric pressure. The base solution was prepared to
be 0.0025M and this standardized with 0.005M hydrochloric
acid. After the tristearin was added, the solution
was standardized and the starting base concentration
was 0.00247M (Figure 18) which closely agreed with the
prepared molarity.

Again, pressurized and non-pressurized samples were
compared after 100 hours. The results are shown in
Table No. 6, and in Figures 19, 19A, 19B, 19C, 19D.

TABLE NO. 6
THE RATES OF HYDROLYSIS OF TRISTEARIN TO STEARIC ACID
FOR PRESSURIZED AND NON-PRESSURIZED SAMPLES

MOLARITIES AFTER TIMES OF PRESSURIZATION			
SAMPLE	0 HOUR	50 HOUR	100 HOUR
Pressurized	0.00247M	0.0014M	0.0001M
Aerated	0.00247M	0.002M	0.00048M

Figure No. 19

Titration Of Tristearin Na oH With 0.005m HCl
At The Start Of The Experiment

Figure No. 19A

Titration Of Sample Pressurized For 50 Hours

Base Molarity = 0.0014 M

mL HCl

pH

Figure No. 19 B

Titration Of Quiescent Tristearin Sample After 50 Hours

Figure No. 19 C

Titration Of Pressurized Tristearin After 100 Hours Of Pressure

Base Molarity = 0.0001M

ml. HCL

pH

Figure No. 19 D

Titration of Quiescent Tristearin Sample After 100 Hours

Base Molarity = 0.00048

ml. HCL

PH

Again, the effect of pressure is that the rate of hydrolysis is increased. The results are shown in the following figures:

Figure No. 18: Tristearin is dissolved 0.0025M sodium hydroxide solution and a 50 ml aliquot is titrated with 0.005M hydrochloric acid potentiometrically at pH equal to 7 the required volume of HCl was 24.7 and, which converts to 0.00247M as the starting base/tristearin solution. So little time has elapsed that there has been very little hydrolysis.

Figure 19A: After 50 hours the equivalence point (pH=7) occurs at 20 ml indicating that the sodium hydroxide/ tristearin molarity is 0.002M.

$$(50ml\ NaoH/TS)\ (M) = (0.005M\ HCl)\ (20ml)$$
$$M\ NaOH = 0.002M$$

In 50 hours 20% of the tristearin has reacted due to hydrolysis.

$$\frac{.0025 - .002}{.0025}\ (100) = 20\%$$

Figure 19B: After 50 hours of pressurization the equivalence point occurs at 14 ml HCl indicating that after 50 hours the pressurized sample has undergone additional hydrolysis resulting in the NaOH/Tristearin molarity being 0.0014M.

$$(50 \text{ ml NaOH/TS}) \ (M \text{ NaOH}) = (0.005M \text{ HCl}) \ (14 \text{ ml})$$
$$M = 0.0014M$$

In 50 hours of pressurization the percentage conversion of tristearin to stearic acid is:

$$\frac{(0.0025M - .0014M)}{(.0025M)} \ (100) = 44\%$$

or twice the hydrolysis with no pressure.

Figure 19C: After 100 hours of pressurization the equivalence point occurs at 4.8 ml HCl. The resulting NaOH/Tristearin molarity is 0.0001M determined in the same manner as in curve 14A & 14B. This represents a conversion of tristearin of:

$$\frac{(.0025M - .0001M)}{.0025M} \ (100) = 96\%$$

Figure 19D: After 100 hours at atmospheric pressure
the molarity of NaOH/Tristearin is 0.00048M representing
a conversion of:

$$\frac{(.0025M - .0048)}{(.0025)} \, (100) = 81\%$$

When this series was completed, stearic acid was
next to be investigated.   Stearic acid is a white insoluble
solid with a molecular weight of 284.   The compound
is a saturated linear seventeen carbon chain carboxylic
acid whose formula is $C_{17}H_{35}COOH$.   The length of the
chain would suggest that the compound is resistant to
chemical oxidation.   This was borne out by literature
data and the fact that COD results were inconclusive
and inconsistent.   BOD analyses were performed for the
pressurized and non-pressurized samples and the kinetic
rate constants determined with the results shown in
Table No. 7.

TABLE NO. 7
BOD$_L$ AND SPECIFIC RATE CONSTANTS FOR PRESSURIZED
AND NON-PRESSURIZED STEARIC ACID SOLUTIONS

	PRESSURIZED				AERATED			
SAMPLE	BOD	Δ BOD	% RED.	k	BOD	ΔBOD	% RED.	k
0 hr.	187	---	--	.17	190	---	----	.18
12 hr.	165	22	11	.16	165	25	8.3	.16
24 hr.	132	55	29	.16	120	60	33.0	.17
36 hr.	82	105	59	.16	75	105	58.0	.16

The BOD$_L$ results show that the samples responded exactly the same, indicating no pressure effect. Also, the kinetic rate constants were almost identical suggesting no changes in the oxidation conditions for both the pressurized and non-pressurized samples.

To summarize these results, pressure did have an effect on the initial step, viz., the hydrolysis of tristearin to stearic acid, with little or no effect on the breakdown of stearic acid.

The scheme can be shown in the following reaction:

(m)   tristearin + H_2O $\xrightarrow[k_p\ k_a]{pressure}$ stearic acid,

where $k_p > k_a$

(n)   stearic acid + O_2 $\xrightarrow[k_p, k_a]{}$ $CO_2 + H_2O$

where $k_p = k_a$

AMINO ACIDS AND PROTEINS

The last series of compounds to be analyzed is the protein/amino acid series. Proteins are complex combinations of the twenty or so amino acids and are a vital constituent for life processes of plants and animals.

Amino acids are carboxylic acid molecules with the amino group, NH_2, attached to the first carbon atom in the chain, known as the alpha carbon; and hence are referred to as alpha-amino acids. The compound formed by the union of two amino acids is known as a dipeptide

and is formed by a loss of water between an amino and
carboxylic group, as shown:

$$\text{(o)} \quad R\text{-}\underset{\underset{NH_2}{|}}{\overset{\overset{H}{|}}{C}}\text{-COOH} + H\text{-}\underset{\underset{H}{|}}{\overset{}{N}}\text{-}\underset{\underset{H}{|}}{\overset{\overset{R}{|}}{C}}\text{-COOH} \longrightarrow R\text{-}\underset{\underset{NH_2}{|}}{\overset{\overset{H}{|}}{C}}\text{-}\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{N}}\text{-}\overset{\overset{R}{|}}{C}\text{-COOH} + H_2O$$

The hydrolysis of proteins involves degradation to several
intermediates before the final α - amino acids are
produced. The sequence is as follows:

(p) protein $\longrightarrow$ proteoses $\longrightarrow$ peptones $\longrightarrow$ polypeptides

amino acids $\longleftarrow$ dipeptides

NH_3 fatty acids

One of the end products is ammonia and this will be
monitored also, because of the tendency of the pressurized
samples to exhibit an earlier nitrification appearance
during the BOD analysis.

The method of protein analysis most commonly used
is the Kjeldahl method which involves digestion of organic
samples followed by distillation and measurement of

the ammonia released. The protein content may then
be calculated on the assumption that proteins contain
16.5 percent nitrogen. This method required considerable
time and may become quite laborious when many samples
are to be determined. A more simplified test can be
used, taking advantage of the peptide bond characteristic
of all proteins.

When biuret, $NH_2-CO-NH-CONH_2$, or any compound containing
two or more peptide bonds are treated with alkaline
copper sulfate, a characteristic purple color is formed,
hence the name biuret test. The color intensity follows
Beer-Lambert's Law and thus this method can be used
to measure protein concentration. The color formation
is probably due to the formation of a chelate involving
one atom of copper and four atoms of nitrogen from the
peptide groups as shown in the following Figure No.
20 (42).

The test procedure involves preparing the biuret
reagent (copper sulfate in sodium hydroxide solution),
standard solutions of protein and preparing a calibration
curve from which unknown sample concentrations can be
estimated. The actual procedure is given in (43).

FIGURE NO. 20
DIPEPTIDE BONDS WITH COPPER

The calibration curve was prepared from solutions of Albumin Bovine, Fraction V, treated with the biuret reagent, and allowed to stand for 30 minutes. This curve is shown in Appendix D. The result of the experiment is shown in Figure No. 21. The pressurized sample does exhibit an accelerated rate of hydrolysis due to pressure.

With the protein analysis completed, the amino acid selected for use was glutamic acid, $C_5H_9NO_4$, with a molecular weight of 147 and the following structure:

$$HOOC-CH_2-CH_2-CH(NH_2)-COOH$$

Figure No.21

The Effect Of Pressure On The Hydrolysis Rate Of Protein

A solution containing 6.12 grams of glutamic acid
and 2.12 grams of ammonium choride was prepared in 40
liters of deionized water. BODs and CODs were taken
at various intervals up to one day. The CODs were performed,
using the Hach reactor with the accompanying vials containing
all the necessary reagents. Samples were added to the
vials, digested, and then titrated to obtain the COD
values. Figures in Appendix L show the degree of BOD
reduction encountered in the samples and Table No. 8
summarizes the results of the BOD and COD determinations.

Ammonia determinations were performed using the
Nessler Reagent method and measuring color intensities
developed spectrophotometrically (21). Initially, a
calibration curve was prepared from standard ammonia
solutions and the Nessler reagent and this shown in
Appendix D. The results of this trial is shown in Figure
22 where the pressurized sample shows a greater concentration
of ammonia as compared to the aerated sample.

Even though the concentration of the amino acid
was not measured directly it can be assumed from inference
that changes were taking place. The BOD and COD results
show increased reduction for the pressurized samples

TABLE NO. 8
BOD AND COD RESULTS FOR GLUTAMIC ACID HYDROLYSIS

SAMPLE	PRESSURIZED			AERATED			QUIESCENT		
	BOD_L	ΔBOD	% BOD REDUC.	BOD_L	ΔBOD	% BOD REDUC.	BOD_L	ΔBOD	% BOD REDUC.
C	107	--	----	118	--	----	113	--	----
4	97	10	9.3	110	8	6.8	109	4	3.5
8	92	15	14.0	105	13	11.0	103	10	8.9
24	80	27	25.2	98	20	17.0	98	15	13.3

SAMPLE	PRESSURIZED			AERATED			QUIESCENT		
	COD	ΔCOD	% COD REDUC.	COD	ΔCOD	% COD REDUC.	COD	ΔCOD	% COD REDUC.
C	110	--	--	108	--	--	99	--	--
4	87	23	21	81	27	35	90	9	8
8	77	33	30	79	29	26	74	25	23
24	55	55	50	62	46	42	78	21	21

compared to the quiescent and aerated samples, and the concentration of ammonia was greater in the pressurized sample suggesting an increased rate of hydrolysis.

NITRIFICATION EFFECTS

Up to this point, the research was concentrated on finding reasons for the apparent change in BOD_L

Figure No. 22

Variation Of Ammonia Produced In A Pressurized Sample Versus
A Non-Pressurized Sample Measured Using The Nessler Reagent

and the change in kinetic rate constants because of pressure. The experimental results would indicate that hydrolysis plays a major role in causing these phenomena. The focus now will be on investigating why second-stage, or nitrogenous BOD occurs earlier, in pressurized wastewater samples. Of the series of chemical compounds studied, only protein/amino acid compounds contain organic nitrogen which would be the major potential source of nitrification in raw sewage.

The next set of trials will study pressure effects regarding nitrification, using proteins and then amino acids. Solutions will be prepared, pressurized with a control, and analyzed for ammonia to determine if pressure does, in fact, cause nitrification to occur earlier.

AMINO ACID HYDROLYSIS

A glutonic acid solution was prepared with the intent to measure the amount of ammonia present for the pressurized and non-pressurized samples. Ammonia determinations were performed using the Nessler Reagent method and measuring color intensities developed spectro-photometrically (23). Initially, a calibration curve

was prepared from standard ammonia solutions and the
Nessler reagent and this is shown in Appendix D. The
results of this trial is shown in Figure No. 44 where
the pressurized sample shows a greater concentration
of ammonia as compared to the aerated sample.

Even though the concentration of the amino acid
was not measured directly, it can be assumed from inference
that changes were taking place. The BOD and COD results
show increased reduction for the pressurized samples
compared to the quiescent and aerated samples, and the
concentration of ammonia was greater in the pressurized
sample suggesting an increased rate of hydrolysis.

PROTEIN NITRIFICATION

Nusser (23) first reported the exertion of the
second stage BOD appearing earlier in pressurized samples
as compared to controls. The same observation was made
by Mazzei (25) and he proceeded to culture and enumerate
nitrifying organisms. He determined that the nitrifyer
population was higher in the pressurized samples and
correlated the increase in nitrifyer organism population
to the appearance of the second stage BOD. Mazzei
determined ammonia concentrations before and after

Figure No. 23

Variation Of Ammonia Produced In A Pressurized Sample Versus
A Non-Pressurized Sample Using The Nessler Method

pressurization of primary sewage in his model activated sludge reactor and observed higher ammonia values after pressurization. Putting these facts together it would appear that pressurization increases the rate by which proteins and other organic nitrogen compounds degrade to ammonia. As the coliform population diminishes due to pressurization, the increase of ammonia and the apparent lack of effect on the nitrifyers provide all the necessary requirements for the increase in second stage BOD.

Protein samples (Serum Albumin, Bovine Fraction V) were again prepared and pressurized and trials were run with the goal to measure the ammonia concentration. The starting sample concentration was 1,000 mg/l and the samples were run for 166 hours. Ammonia readings were obtained, using the spectrophotometric Nessler process with concentrations being read from the calibration curve. The results are shown in Figure No. 22 & 23 where, after 160 hours, the rate of ammonia production increases but is higher in the pressurized sample. The conclusion is that pressure is a positive factor, causing nitrification to occur earlier.

IX. DISCUSSION OF RESULTS

Using the results of the previous investigators
as a starting point together with results obtained from
this study - provide the answers to the question presented
in the thesis objective. Restated, these questions
are:

1) Why was there an apparent BOD reduction for pressurized
samples when compared to a control?

2) Why did nitrification occur sooner in pressurized
samples?

3) In what manner were the kinetics altered?

4) What may be the application of this process to existing
wastewater technology?

Of all the mechanisms considered and tested, experi-
mental evidence indicates that hydrolysis alone is the
cause of any changes that do take place in the BOD profiles
as a result of pressurization. The pressurization process

114

is responsible for converting large molecular weight
compounds found in wastewater to smaller molecular weight
factors which in turn are oxidized easier and faster.
That pressure increased the rate of hydrolysis in general
was shown by the conversion of a buffered solution of
para-nitro-phenyl-acetate (PNPA) to Para-nitro-phenol
(PNP). One solution of PNPA was pressurized and analyzed
for PNP. With increasing detention time the concentration
of PNP for the pressurized sample was greater than for
the non-pressurized control. This series of experiments
established the rate of pressurization in increasing
the hydrolysis rate. The next phase of the project
was to identify the major components of sewage, subject
each component to pressurization and determine if, in
fact, the hydrolysis rate was altered by pressurization.
The experimental results indicate the following conversions
occur more rapidly due to pressurization.

1) Polysaccharides ⟶ Monosaccharides

    (starches)   (sugars)

2) Fats ⟶ Fatty Acids

3) Proteins ⟶ Amino Acids

4) Amino Acids $\longrightarrow$ End Products + NH_3

Literature reference (43) cites the fact that these compounds (fats, proteins, and amino acids) are subject to hydrolysis and it was to determine if the hydrolysis rate could be altered by pressurization.

To intelligently discuss the results as they appeared in the BOD curves obtained in this study as well as by the other researchers it is necessary to separate the wastewater into the time spent in the pressure vessel being pressurized and the time of incubation after pressurization during the BOD testing phase. It must be established that the term "BOD reduction" is a misnomer since to reduce BOD, it is necessary to reduce the amount of carbon contained in organic compounds. The pressure process converts carbon compounds to different forms of carbon compounds but the amount of carbon remains the same. It is only in the oxidation of carbon compounds to carbon dioxide and water that the amount of organic carbon changes. The idea that pressurization leads to "BOD reduction" can be explained as follows.

During pressurization in the pressure vessel, an
increased rate of hydrolysis takes place as shown by
experimental results. This means that during the pressur-
ization detention time, more of the larger molecular
weight fractions are being hydrolyzed than would hydrolyze
under non-pressurized, quiescent conditions. While
under pressurization, oxidation is taking place. Because
the pressurization process produces more chemical fractions
easier to oxidize, oxidation takes place at a faster
rate. This means that after the pressure detention
time, the material in the pressure chamber will have,
compared to the non-pressurized control.

1) Less total organic carbon due to more carbon being
oxidized after hydrolysis.

2) A greater concentration of more easily oxidized
organic material.

There is no way to measure or analyze the compounds
during pressurization in the pressure vessel so any
conclusions regarding these changes must be made from
inference by analyzing the material after pressurization.

The changes that do take place can be shown in Figure
No. 24, "Part A" where the dashed curve indicates the
pressurized sample and the solid curve the non-pressur-
ized. Two facts are evident from the illustration;
there is an increase in oxidation of the organic material,
and the oxidation takes place at a faster rate.

After pressurization for a specified duration is
completed, samples are drawn off and BOD's performed.
BOD's also were performed for non-pressurized samples
which acted as the control. The resulting BOD profiles
are shown in Figure 24, "Part B" which shows two important
facts; the 1st stage BOD, BOD_L, is lower for the pressurized
sample and the pressurized sample oxidized at a faster
rate. This was determined by evaluating the specific
rate constant, k_1, for both control and pressurized
samples. For the specific rate constant to increase
while the concentration of starting material decreased
would appear to be a violation of the first law of kinetics,
on which the entire BOD relationship is predicated.
Restated, the first law is:

$$- \frac{dc}{dt} = k_1 c$$

Figure No. 24

BOD Curves Showing The Entire Time Of Pressurization and Incubation

Part "A"

BOD Change In
Pressure Vessel

Part "B"

BOD Change In
Pressurized Effluent

If pressurization causes an increase in hydrolysis
with the subsequent higher oxidation rate of the hydrolysis
products, then it follows that the pressurized effluent
would be lower in BOD material concentration than the
control. With a smaller concentration, for the fixed
specific rate constant, the rate of reaction, $-dc/dt$,
would be less. But this is not the case. The only
explanation for a decrease in concentration resulting
in an increase in specific rate constant is that the
nature of the organic material had been changed.

Thus, the rate constant, k_1, is also a function
of the type of material being reacted as well as concen-
tration changes. With pressurization increasing the
hydrolysis rate in the pressure chamber, the material
discharged will have a higher concentration of smaller
molecular weight fractions, much easier to oxidize than
would be in the control. Less total organic concentration
but higher in smaller molecular weight fraction, for
the pressurized effluent leads to the BOD curves shown
in Figure 25. What appears as a BOD reduction in Figure
25 is a real reduction, with the amount of reduction
directly proportional to the detention time in the pressure
vessel.

Figure No. 25

Variations In BOD Curves For Pressurized, Aerated, And Control Samples

——— Control, Zero Detention Time
— — — Non-Pressurized After $t = T_1$
— - — Pressurized After $t = T_1$

$K_1 > K_3 > K_2$

BOD_{L1}

ΔBOD_{1-2}

BOD_{L2}

BOD_{L3}

ΔBOD_{2-3}

ΔBOD_{1-3}

K_1

K_2

K_3

1. ΔBOD_{1-2} Natural Decomposition In $t = T_1$
2. ΔBOD_{1-3} Decomposition In $t = T_1$ + Pressure Effects
3. ΔBOD_{2-3} Apparent BOD Reduction

BOD, mg/L

Time, Days

The longer the pressurization the greater the hydrolysis taking place producing more easily oxidized compounds. More oxidation takes place during pressurization producing an effluent of lower potential BOD, and a product lending itself to easier and faster oxidation.

BOD reductions of 10% to 15% can be attained in the pressurized times of 12 to 48 hours. The $\triangle$ BOD becomes constant when all the hydrolyzable material has been hydrolyzed and hydrolysis is complete. The only reaction now is oxidation and the rates of reaction for the non-pressurized control and pressurized sample would be the same.

Throughout the discussion the emphasis was placed on the fact that hydrolysis alone was the cause of any increased oxidation and reduction in BOD. Other mechanisms were also considered and these include oxidation, premature nitrification, and microorganism effects.

While in the pressure vessel, the D.O. of the waste-water was maintained at 13 mg/l, a value considerably

higher than the saturation value of approximately 8-
9 mg/l for the particular temperature of the vessel.
Also, as the BOD reaction progresses, the oxygen concen-
tration does not decrease as it would for a non-pressurized
oxidation. At first glance, it would appear that only
any increase in the oxidation of the material would
be oxygen induced, however, that is not the case.

Above a certain D.O. value the BOD reaction is
independent of D.O. concentration, as reported by Gaudy
(44). The figures vary (from 1-2 mg/l) for different
researchers and it was felt that because of the special
nature of pressurization, the role of oxygen had to
be determined.

To study the effects of dissolved oxygen on wastewater,
samples were prepared in the following manner. A wastewater
sample was obtained, filtered, and divided with one
part allowed to decompose under atmospheric pressure
until it became anaerobic. For the sample strength
this would take 2-3 days. This sample was designated
"quiescent". Into the other sample part was inserted
an aerator similar to the aerators used in fish tanks
and this was able to maintain a D.O. concentration of

9-10 mg/l for the entire time of decomposition because
the aeration rate was for greater than any loss due
to oxidation. For all trials after specified times
(8-48 hours) the BOD curves for the aerated and quiescent
samples are identical, indicating that above a certain
critical D.O. value the oxidation of organic material
is independent of D.O. concentration.

When the BOD curves are drawn for the pressurized
sample being oxidized in the pressure vessel it is dis-
placed higher than the control BOD curve. Another consid-
eration could be that hydrolysis is causing an increase
in the nitrification rate with a subsequent increase
in BOD. For raw wastewater under ordinary conditions
nitrification does not occur until 7-10 days have elapsed
in the BOD analysis. There are several reasons why
this lag time is so pronounced; one, raw wastewaters
contain very small concentrations of nitrifying organisms
responsible for nitrification and these autotropic organ-
isms cannot compete in the same regime with aerobic
organisms present during biological decomposition. After
7-10 days the first stage oxidation is complete and
the aerobes responsible are declining in number. Also,
organic nitrogen compounds would have hydrolyzed producing

an increase in ammonia which is the starting substrate
for nitrification. Under pressurization it was not
known if any of these conditions were altered to such
an extent so as to have significant nitrification occuring
before the customary 7-10 day time period.

With that in mind a series of experiments were
performed with the emphasis on having nitrification
occur for the control and pressurized sample. The strategy
was as follows: because raw wastewaters do not contain
significant nitrifying populations a source of nitrifying
organisms was obtained and these were added to the samples.
To insure that nitrification would not take place in
the comparison samples a nitrification supressor, allyl
thiourea, ATU, was added. This chemical has the ability
to supress the growth of nitrifyers with the consequent
elimination of 2nd stage NOD. A sample would be pressurized
for 24 hours and compared to a non-pressurized sample.
Also, there would be aerated samples with and without
nitrifyers. A synthetic sample had to be used for two
reasons. The first was consistency; the pressurized
samples, with and without nitrifyers had to be run at
different times, and if different raw wastewaters were
used they would be different and there would be no basis

for comparison. A synthetic sample was prepared with a known BOD and it also contained organic nitrogen compounds. The experimental results indicate the following:

1) BOD curves for the control sample with and without nitrifyers were identical for the first stage BOD with nitrification taking place beginning at day 7 in the nitrified samples.

2) Pressurized samples with and without nitrifyers were identical for the first stage BOD.

3) Aerated samples with and without nitrifyers were identical for first stage BOD.

4) The amount of NOD for the pressurized as well as the aerated samples were the same indicating no NOD lost in the first stage. If the first stage BODs were the same, and the NODs the same, this means there has been no nitrification taking place during the opening phases of decomposition.

The final phase investigated the role of pressure on the microorganisms concentration as it could effect

the BOD oxidation process. Two different dilution waters were prepared, one seeded with microorganisms, and the other, non-seeded. BOD analyses were performed and the BOD curves drawn were identical for the control and pressurized samples. Organism counts were also conducted up to 48 hours pressure time. There was a significant drop in microorganism population ($10^{14} \longrightarrow 10^8$/100 ml) but so many remained that the BOD reaction was able to proceed without any interruption.

The other significant development produced during pressurization was the appearance of the 2nd stage NOD earlier in the pressurized sample. Several reasons account for this. The first is that the hydrolysis rates of organic nitrogen compounds, proteins and amino acids, are enhanced by pressurization resulting in higher concentrations of ammonia available for nitrification. Also, pressurization up to 48 hours extinguish the coliform population which inhibit the action of the nitrifyers. Thus, a climate favorable for earlier nitrification is produced which is shown taking place 2 days earlier than for the non-pressurized samples.

X. VERIFICATION OF HYPOTHESIS

MOLECULAR SIZE

It has been shown that pressure does in fact increase the rate of hydrolysis of the large molecular weight fractions found in sewage which, in turn, reduces the apparent BOD_L. But the question still remains as to the exact mechanism involved to account for these lower BOD_L values. A possible consideration is that the longer it takes large molecular weight fractions to break down, the longer will be the time for the aerobic microorganisms to adjust to the end products. During this time, the microorganisms will exert an oxygen demand due to their aerobic nature. This can be expressed in chemical equation form as follows:

(q) $\qquad C_aH_bO_c + H_2O \xrightarrow[k_p \ k_u]{[M.O.]} C_xH_yO_z + C_mH_nO_0$

(r) $\qquad C_xH_yO_z + O_2 \xrightarrow{[M.O.]} CO_2 + H_2O$

(s) $\qquad C_mH_nO_0 + O_2 \xrightarrow{[M.O.]} CO_2 + H_2O$

128

where subscripts a, b, c > subscripts x, y, z, m, n, o.

k_u = rate of unpressurized sample

k_p = rate of pressurized sample

$k_p > k_u$

Specific examples of these reactions applicable to this research are:

(t)   starch + water $\xrightarrow[\text{[ase]}]{k_p \ k_u}$ glucose $\xrightarrow{\text{[M.O.]}}$ CO_2 + H_2O

where [ase] = Enzyme concentration

(u)   proteins + H_2O $\xrightarrow{k_p \ k_u}$ amino acids $\xrightarrow{\text{M.O.}}$ CO_2 + H_2O

where it has been shown that $k_p > k_u$.

If the smaller compounds are formed faster and these are more biodegradable than the larger fractions, the rate of degradation should be greater, requiring more oxygen

to be demanded during the early phases of the BOD reaction.
Graphically, this can be shown in Figure No. 24, with
the rate of decomposition of the pressurized sample
is greater in the incubation time 0 to 5 days but with
a lower BOD_L. What has occured is a lower BOD_L after
pressurization due to the changes in chemical composition
taking place during the pressurization process.

As an example, assume a 500 mg/l starch solution
is prepared, pressurized, and compared to the non-
pressurized control. If after one time unit of pressuriza-
tion, assume 100 mg/l of starch is converted to glucose
in the pressurized sample compared to 50 mg/l conversion
in the non-pressurized sample, as in the following reaction:

$$\text{Starch} + H_2O \xrightarrow{[-ASE]} \text{Glucose, where}$$

Molecular
Weights: 162   18   180

[-ASE] = enzyme concentration added

$$k_p > k_u$$

This would produce 111.1 mg/l glucose for the pressurized
sample and 55.5 mg/l glucose for the control, as shown
in the following stoichiometric relationships:

$$\frac{100 \text{ mg}}{162} = \frac{X}{182}, \quad X = 111.1 \text{ mg/l}$$

$$\frac{50 \text{ mg/l}}{162} = \frac{Y}{180}, \quad Y = 55.5 \text{ mg/l}$$

The glucose is then converted to carbon dioxide and water as shown:

$$C_6H_{12}O_6 + 6 \ O_2 \xrightarrow{[MO]} 6 \ CO_2 + 6 \ H_2O, \text{ where}$$

Molecular
Weight: 180   32

[MO] are microorganisms present

$k_p = k_u$ because pressure has no effect on glucose oxidation.

111 mg/l of glucose would have a theoretical BOD of 118 mg/l for the pressurized sample, whereas the control, which would only have a 50 mg/l conversion, would have a BOD of 59 mg/l, as shown by the following stoichiometry:

$$\frac{111}{180} = \frac{X \text{ mg/l } O_2}{(6) \ (32)}, \quad X = 118 \text{ mg/l}$$

$$\frac{55.5}{180} = \frac{Y \text{ mg/l } O_2}{(6) \ (32)}, \quad Y = 59 \text{ mg/l}$$

This would cause the pressurized BOD curve to show a higher demand for that particular time period. After the time period of pressurization, there would be 400 mg/l of starch left to react in the pressurized sample and 450 mg/l in the control. With sufficient time, the starch in the pressurized sample would revert to 444 mg/l glucose with a theoretical BOD of 474 mg/l. In the non-pressurized control, with 450 mg/l starch, it would be converted to 500 mg/l of glucose with a theoretical BOD of 533 mg/l. This would appear in the BOD curves as the apparent difference in the first stage BOD but, in truth, both samples would have exerted the same oxygen demand.

Pressurized: 118 mg/l + 474 = 592 mg/l

Non-Pressurized: 59 mg/l + 533 = 592 mg/l

As far as the term "BOD reduction" is concerned, this is a misnomer because there cannot be a real BOD reduction since the number of carbon atoms present to be oxidized remains the same. What does happen is that certain carbon compounds are more easily oxidized than others and this exerts more oxygen demands in the earlier

part of the BOD curve, leaving the BOD_L after pressuri-
zation to be lower by the additional oxidation in the
pressure vessel.

To show if this hypothesis is viable, the following
experiment was conducted. Various combinations of starch
and glucose were prepared and their BOD_L determined
to ascertain any change in BOD_L and the rate of decompo-
sition. As an example, a 75 percent starch-25 percent
glucose solution was compared to a 50 percent starch-
50 percent glucose solution. This simulates a condition
where pressurization has caused a 25 percent increase
in glucose. If the hypothesis is correct, the 50 percent
starch-glucose should have a lower BOD_L but a higher
rate of decomposition.

The range of samples that were prepared were as
follows:

1) 100 percent starch -   0 percent glucose

2)   75 percent starch - 25 percent glucose

3)   50 percent starch - 50 percent glucose

4)   25 percent starch - 75 percent glucose

5)   0 percent starch - 100 percent glucose

The chemical reactions are as follows:

(v) Starch

$$(C_6H_{10}O_5)_x + H_2O + 6x\ O_2 \longrightarrow 6x\ CO_2 + 5x\ H_2O$$

(w) Glucose

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ H_2O + 6\ CO_2$$

Stoichiometric amounts of sugar and starch were weighed out to obtain the proper proportions. Also, amylglucosidase enzyme was added. The trial was run and the results are shown in Figure 26. As the percentage of glucose increased, the rates of reaction increased, indicating that for organic molecules which are smaller and less complex, they are more easily metabolyzed. If the specific rate constants are determined and plotted, the results are shown in Figure 27. The rates are approximate because from the curves there are greater lag times for the higher starch concentrations, indicating that the enzymes and microorganisms required a greater time to acclimate themselves to the substrate.

The results show that as molecular size and complexity decrease, it is easier for the microorganisms to metabo-

Figure No. 26

BOD Curves For Various Sugar-Starch Solutions

Figure No.27

Variation of the Specific Rate Constant as a Function of
the Amount of Glucose in a Glucose/Starch Mixture

lize them, thus exerting more oxygen demand earlier
than if the compounds were more complex. In the time
required for the large molecules to degrade to the smaller
forms, microorganisms require oxygen to sustain their
life processes. If this time could be accelerated,
the microorganisms would use the oxygen earlier in the
decomposition.

SNYTHETIC SAMPLES

With all of the experimental studies performed
and evidence obtained and presented herein relating
to the causes of BOD reduction, the next procedure was
to prepare a synthetic sample closely approximating
compounds found in domestic sewage, subjecting it to
pressurization, and then observing if the same effects
were produced as in actual sewage. Care must be exercised
in preparing a synthetic sample in order for it to be
as representative as possible in nature and BOD requirements.

A simulated sample was prepared from the sewage
analysis given in references (49) and (50). Based on
the analysis of the soluble fraction, the following

recipe was prepared in Table No. 9. 5.02 grams were
dissolved in 40 liters of water to approximate the BOD
of an actual sewage sample. BOD tests were performed
and BOD data plotted showed the identical BOD profiles
as were obtained for the actual sewage samples used
in the study. These curves are shown in Figures 28
and 29.

The results can be summarized as follows:

1) The BOD profile for the synthetic sample was very
similar to the curves obtained for the actual sewage
sample.

2) For longer detention times the apparent BOD_L decreased
as well as the specific rate constant.

3) The BOD profile for the pressurized and non-pressur-
ized samples after pressurization detention time elapsed,
showed the higher rate constant for the pressurized
sample.

4) Ammonia values were taken and these were found to

Figure No.28

BOD Variations of Synthetic Wastewater Samples
Pressurized And Non-Pressurized

Figure No.29

BOD Variations of Synthetic Wastewater Samples
Pressurized And Non-Pressurized

Control ●———●
24 Hours Pressurized ◇----◇
24 Hours Non-Pressurized ○———○

BOD - mg/Lt.

Time - Days

ΔBOD

be higher in the pressurized sample compared to the non-pressurized.

5) The BOD samples were allowed to stand for an excess of 20 days, but no nitrification occurred. Obiviously, there was a lack of nitrifying organisms in the seeded dilution water.

These results are summarized in Table No. 10.

TABLE NO. 9
COMPOSITION OF SYNTHETIC SEWAGE

MATERIAL	PERCENTAGE	GRAMS
Glucose	9.	0.45
Sucrose	9.	0.45
Albumin	13.	0.65
Glutamic Acid	13.	0.65
Acetic Acid	18.	0.90
Starch*	22.	1.10
Tristearin	8.3	0.41
Stearin	8.3	0.41
	100.0%	5.02 g.

* Amylglucosidase also added to breakdown the starch.

TABLE NO. 10

SUMMARY OF BOD AND SPECIFIC RATE
CONSTANT VALUES FOR SYNTHETIC SAMPLES

CONTROL			AERATED, 8 HR			AERATED, 24 HR	
time hr	BOD mg/L	k l/day	time hr	BOD mg/L	k l/day	BOD mg/L	k l/day
1.1	52	.22	1.0	34	.15	40	.22
2.08	79	.19	1.9	46	.13	50	.19
3.18	104	.18	2.9	76	.15	63	.19
4.0	--	.18	4.0	--	.15	--	.18
5.0	123	.19	4.9	101	.16	91	
6.2	134		6.1	111		86	
7.1	140		7.0	115		94	
			9.1	120		105	
			11.0	120		111	

PRESSURE, 8 HR			PRESSURE, 24 HR	
time hr	BOD mg/L	k l/day	BOD mg/L	k l/day
1.1	13	.09	18	.18
2.1	25	.11	32	.16
3.0		.12		.15
4.1	46	.12	37	.16
5.0		.12		.16
5.4	61	.12	48	
6.4	65		57	
10.0	78		65	
13.0	75		68	

XI. APPLICATION

Now that the role of pressure in reducing the BOD profile is finally understood the question to address is the possible application of the process to existing treatment technology. The current state of the art in wastewater treatment employs primary treatment to remove solids and some BOD, secondary treatment to remove BOD to prescribed limits, tertiary or advanced treatment, if necessary, and disinfection prior to discharge into the receiving body of water. The disadvantages of conventional wastewater treatment are:

1) Effluent often of too poor water quality.

2) At high flows (I and I) influent bypasses the sewage treatment plant and enters directly into the receiving body of water.

3) Chlorinated effluents have a detrimental effect on the ecological balance.

4) Too much land required if tertiary or advanced treatment is required.

5)   Large holding tanks required for flow equalization.

6)   Land around the sewage treatment plant required
as a buffer for odors, vermin, etc.

The pressurization process can be incorporated
at several locations in current wastewater treatment
operation.   Pressurized effluent possesses several ad-
vantages.   These are:

1)   It is saturated with dissolved oxygen with concen-
tration dependent on the temperature and salt content.

2)   It has a higher percentage of smaller molecular
weight fractions due to the hydrolysis of the larger
molecular weight fractions, which are easier to oxidize
to more stable forms.

3)   By keeping the process aerobic longer it stimulates
a greater ratio of aerobic/anaerobic organisms which
is beneficial if further treatment is required.

4)   Coliform organisms are rendered harmless and reduced
to extinction.

5) Suspended solids reduction takes place during pressurization as the process solublizes solid particles which are then hydrolyzed and oxidized to stable forms.

6) Ammonia concentrations are made higher due to hydrolysis of organic nitrogen compounds which can then be nitrified or removed.

Specific applications of the pressure process to existing wastewater treatment can occur in any one of several places or in combination during the treatment operation. These will be shown in the following discussion.

PRESSURIZATION OF PRIMARY EFFLUENT

The pressurization process can be accomplished as a batch or continuous operation depending on the flow rates and hydraulic loadings. A hyperbaric pressure vessel system for treating wastewaters is shown in Figure 30. In the pressure vessel shown in the diagram several concurrent chemical and physical driving forces can be used to minimize the detention time and increase treatability of the waste for subsequent operations.

Figure No. 30

A Sewage Treatment Plant Using Pressurization
As A Pre-Treatment Operation

Wastewater is received through conventional bar screens,
grit chambers, and comminutors, picked up at a wet well,
and then pumped into the pressure vessel. Within the
tanks the waste stream can impinge onto impact-type
comminutors or baffles to help breakup solid particles.
Pressure can be maintained at 40 psi which will provide
a DO of 12-13 mg/l. Baffles can be arranged to prevent
mixing of the influent with partially treated water,
with the water moving lengthwise in the tank within
the prescribed detention time. Because of the turbulent
action there should be no settling of sludge within
the pressure vessel.

When the effluent leaves the pressure vessel it
will be oversaturated in DO, have a higher percentage
of smaller molecular weight organic fractions, and a
reduced microorganisms concentration. It could now
be directed to a primary treatment tank for solid settling
and BOD reduction. Two forces can now act to achieve
a greater BOD reduction; due to the increased rate of
hydrolysis induced by pressure the compounds are now
more easily oxidized and the surviving microorganisms
are free to multiply now that the physical killing force

has been removed. They will experience a log growth
increase because the food to microorganism ratio, F/M,
has been increased and with the over-saturation of DO
because of pressure, hence the rate of oxidation of
the remaining organic compounds should increase rapidly
until microorganism growth begins to decline. The material
could then be subjected to secondary treatment and the
conventional treatment process continued.

PRESSURIZATION OF SLUDGE

The problems inherent with sludge handling and
disposal are enormous and has been recounted ad infinitum.
Anerobic digestion is the most common method of sludge
praticed today because of the high loading rates encountered
although aerobic digestion is preferred due to current
effluent standards. The advantages of aerobic digestion
are:

1) Lower BOD in supernatent.

2) An end product with less odors, biologically stable,
and easier to handle.

3)   An end product with excellent dewatering properties
as compared to the anaerobic end product.

4)   Higher fertilization value in the end product.

Of course, there is no methane production with aerobic
digestion which may be a disadvantage to the process.
However, with the advantages stated above aerobic digestion
may become the digestion method of the future once the
dynamics and kinetics and mechanisms are understood.
The pressure process can be used to advantage in pressur-
izing conventional primary sludge and this process is
shown in Figure 31.

Another digestion alternative would be to add sul-
furic acid to the sludge to reduce the pH to 0-1 prior
to pressurization. This would dissolve the solids easier
to more soluble forms, the pressurization plus H^+ ions
would hydrolyze the organics more rapidly than without
acid, neutralize with caustic, and then discharge or
recycle back to primary or secondary treatment. This
effluent would be neutral, saturated with DO, and contain
more compounds much easier to oxidize in later treatment
steps. Some inert materials will remain, and these

Figure No.31

A Sewage Treatment Plant Using Pressurization To Treat Sludge

can be treated easier because there will be less bulk,
and the material would possess better dewatering proper-
ties.

LAGOONS

An interesting approach using pressure could be
as a means to eliminate conventional secondary treatment
(trickling filters, activated sludge, etc.) by pumping
pressurized effluent to oxidation ponds or lagoons.
The pressurized effluent's characteristics are that
it is oversaturated in DO, the microorganism population
reduced to a pont where it can experience an accelerated
growth is given the opportunity, and high in hydrolyzed
fractions plus increased concentrations of ammonia hydrolyzed
from proteins, amino acids, and other organic nitrogen
compounds. When released into a lagoon the microorganism
dynamics take over causing a rapid oxidation of organics,
and a rise in microorganism concentration. In laboratory
studies the specific rate constant increased to a maximum
after four days detention time and then decreased sig-
nalling a drop in the F/M ratio causing a decline in
microorganism growth reflected by a lower specific rate

constant.   (See curves presented in Appendix H and I).

With an increase in the ammonia concentration resulting
from hydrolysis nitrifying organisms could be placed
into the lagoon to effect nitrification. Studies on
Rivers (49) have shown that immediate nitrification
can occur if the river water contains a sufficient nitrifer
population and if the effluent is high in ammonia. The
classic two stage BOD profile which occurs in extended
laboratory bottle tests is not applicable to an in-
vito situation and both nitrification and oxidation
can occur simultaneously. The lagoon could accept pressurized
pre-treated primary effluent or pressurized waste directly
without secondary treatment. A possible treatment scheme
is shown in Figure 32.

CHLORINATION ALTERNATIVE

With detention times of 24 hours and a pressure
of 40 psi microorganism population is diminished whereas
the coliforms are reduced to extinction. The advantages
of using pressure prior to discharge are that it provides
the last opportunity to hydrolyze organics and nitrogen
compounds, saturate the effluent with DO, and destroy

Figure No. 32

A Sewage Treatment Plant Eliminating Secondary Treatment
By Discharging Pressurized Waste Directly Into A Lagoon

all coliforms. The only question that arises is whether
pressurization has any effect on pathogens and viruses.
The assumption made with chlorination is that if there
are no coliforms there are pathogens since the pathogens
are less resistent to chlorination than the coliforms.
Studies have shown (52) that at a pH of 6 and chlorine
dose of 30 mg/1 and 10 seconds duration time there is
a 99.93% viral inactivation. At the time of this writing
there is nothing available concerning the viral inacti-
vation due to pressurization, so before pressurization
could be definitely proposed as a chlorination alternative
data would be necessary regarding pressurization-kill
of virus organisms. A flow sheet is shown in Figure
33.

These brief descriptions are only a partial listing
of the potential of pressurization in wastewater treatment.
Engineering studies are needed to obtain optimum operating
conditions, equipment size, and selection.

155

Figure No. 33

A Sewage Treatment Plant Using Pressure To Treat
Clarifier Effluent Prior To Discharge

XII.   CONCLUSION

As a result of this investigation the reason why the BOD changes that do occur due to pressure have now been firmly established. The answer lies in the manner the BOD curves are prepared with their proper time relationships to also include the time the sample was being pressurized in the pressure vessel. The situation can be summarized by the following generalized curve as shown in Figure 34.

The BOD reaction is taking place in the vessel during pressurization but at a faster rate due to the increased rate of hydrolysis producing more easily oxidized components than in the non-pressurized control. The aliquot which is now removed for BOD study has a smaller organic concentration than the control, hence a smaller BOD, but it would oxidize at a faster rate because of the increased concentration of smaller molecular fractions. The BOD_L values for the pressurized aliquot would be less because of the increased BOD experienced during pressurization. Nitrification would occur sooner for the pressurized sample because the pressure induced hydrolysis would convert more organic nitrogen found in proteins and amino acids

156

Figure No. 34

Generalized BOD Curves For Pressurized and Control Samples

to ammonia. The BOD_L for both samples would be the same except that the longer the detention time in the pressure vessel the greater the BOD increase, consequently, the lower the BOD in the aliquot drawn off.

What was interpreted as BOD reduction in the past was the BOD curve for the pressure aliquot compared to the control prepared at the beginning of pressurization. Obviously, the detention is the factor which must be accounted for. This investigation made that adustment and also elucidated the exact mechanism to explain why these changes took place. To summarize the findings of this investigation the following BOD and kinetic events occur due to pressurization:

1) Pressurization causes an increase in the rate of hydrolysis of large organic components to smaller molecular weight fractions.

2) Increased BOD takes place in the pressure vessel due to hydrolysis.

3) The longer the waste is pressurized the greater the BOD increase in the pressure vessel.

4)   After pressurization the effluent will have a lower
concentration of organics, but a higher concentration
of smaller molecular weight fractions.

5)   The pressurization effluent will oxidize at a faster
rate after discharge into a receiving body of water.

6)   Pressurization reduces coliform organisms to extinc-
tion but leaves sufficient organisms remaining to carry
on the BOD reaction.

7)   Pressurization effluents will be oversaturated in
dissolved oxygen.

     Pressurization holds interesting possibilities for
advanced wastewater treatment. Now that the nature of
the phenomenon involved is finally understood, this under-
standing can contribute to useful applications of this
process to existing wastewater facilities.

APPENDIX A

DEMAND CURVES OF WASTEWATER SAMPLES RESULTING

FROM ENVIRONMENTAL STRESSES

160

Figure No.35

Variation Of The BOD Of Pure Sewage And Diluted
Samples After Standing For One Day

Figure No.36

Variation Of The BOD Of Pure Sewage And Diluted
Samples After Standing For Three Days

Figure No.37

Variation Of The BOD Of Pure Sewage And Diluted
Samples After Standing For Two Days

Figure No. 38

Variation of The BOD of Pure Sewage And Diluted
Samples After Standing For Four Days

Figure No.39

Decrease Of BOD_L Values For Samples Allowed
To Stand Under Quiescent Conditions

Figure No. 40

Variation Of The Specific Rate Constant In The BOD Equation
As A Function Of The Time The Sample Is Allowed To Stand

Figure No. 41

Variation Of The Specific Rate Constant For Sewage
And Diluted Samples After Being Allowed To Stand

APPENDIX B

TABLES OF RECORDED DATA RELATING TO DEMAND

CURVES OF WASTEWATER SAMPLES SUBJECT TO

STRESSES

168

Day	12/9	12/10	12/11	12/12	12/15	12/16	12/17
0	0	2	3	4	7	8	9

100%

	12/9	12/10	12/11	12/12	12/15	12/16	12/17
B_1	7.6	6.6	5.9	5.2	4.4	4.0	4.1
B_2	7.5	6.6	5.7	5.0	4.3	4.1	4.1
Ave	53	72	100	122	178	169	172 / 165
4	6.8 / 56	5.7 / 67	4.6 / 90	3.4 / 127	2.1 / 169	1.8 / 172	1.8 / 172 / 2.0 / 165
5	6.6 / 57	5.4 / 72	3.8 / 120	3.3 / 108	1.2 / 189	x	
6	6.6 / 47	5.8 / 78	4.0 / 90	2.5 / 130	0.8 / 177	x	
8	5.6 / 73	4.2 / x	x	x	x	x	
10	5.8 / 52	4.0 / 78	4.6 / x	2.1 / 90	x		

67%

	12/9	12/10	12/11	12/12	12/15	12/16	12/17
B_1	7.6	7.0	6.9	6.6	6.0	5.9	5.9
B_2	7.5 / 1	7.1 / 2	6.9 / 3	6.0 / 4	5.9 / 8	5.9 / 8	8
12	6.5 / 31	5.4 / 41	4.5 / 62	3.7 / 73	1.3 / 117	1.2 / 118	1.2 / 118
10	5.4 / x	x	x	x	x	x	x
8	68 / 6.8	6.3 / 28	5.6 / 49	4.9 / 64	2.8 / 120	2.4 / 131	2.8 / 131
6	6.9 / 32	6.6 / 22	6.2 / 56	5.1 / 75	3.2 / x	x	x
5	7.1 / 27	x	x	x	x	x	x
Ave	29	34	54	71	118	125	

BOD DATA FOR DILUTED SAMPLES WITH NO STANDING TIME

	Day 0	12/9	12/10 2	12/11 3	12/12 4	12/15 7	12/16 8	12/17 9	10
33%	B₁	7.3	6.8	6.3	5.9	5.2	5.2	5.2	5.2
	B₂	7.2 / 1	7.0 / 2	6.7 / 3	6.4 / 4	5.3 / 7	5.2 / 8	5.2 / 9	5.3 / 10
	20	5.8 / 22	4.8 / 31	3.4 / 46	2.0 / 62	0.4 / 73	.2 / 75	X / X	X / X
	18	6.0 / 21	4.9 / 33	3.4 / 51	1.9 / 71	0.5 / 79	0.4 / 80	X / X	X / X
	15	6.2 / 21	5.2 / 34	3.7 / 56	3.2 / 60	X / X	X / X	X / X	X / X
	12	7.1 / X	5.7 / 30	4.5 / 50	4.3 / 46	2.3 / 75	2.3 / 72	2.3 / 72	2.3 / 72
	10	7.2 / X	6.0 / 27	5.2 / 39	4.5 / 49	2.8 / 73	2.9 / 69	2.7 / 75	2.8 / 74
	Ave	21	29	49	57	75	75	75	73

BOD DATA FOR DILUTED SAMPLES WITH NO STANDING TIME
(CONTINUED)

Day 1		12/10 1	12/11 2	12/12 3	12/15 6	12/16 7
100%	B1	5.8	5.2	4.5	3.8	3.6
	12 ml	4.1 / 4.2	2.5 / 67	1.3 / 80	0.3 / 88	X / X
	10 ml	4.5 / 39	3.0 / 66	1.7 / 84	0.4 / 102	X / X
	8	4.9 / 34	3.8 / 52	2.5 / 75	0.5 / 124	X / X
	6	5.0 / 40	X / X	X / X	X / X	X / X
	5	5.2 / 36	4.4 / 48	X / X	X / X	X / X
	Ave	38	61	80	104	X / X
67%	B1	6.0	5.6	5.1	4.2	
	B2	5.8	5.3	4.6	3.6	
	15	5.0 / 18	3.8 / 33	2.5 / 47	0.5 / 68	
	14	5.0 / 19	4.1 / 29	X / X	X / X	
	12	5.2 / 18	X / X	X / X	X / X	
	10	5.4 / 15	4.5 / 28	3.3 / 46	1.7 / 66	
	8	5.6 / 11	4.7 / 28	3.9 / 35	1.8 / 79	
	Ave	16	29	43	71	

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 1 DAY

33%								
Day 1	12/10 / 1	12/11 / 2	12/12 / 3	12/15 / 6	12/16 / 7			
B₁	6.0 / 1	5.5 / 2	5.1 / 3	4.5 / 6	4.7 / 7	4.7 / 8	5.0 / 9	4.8 / 10
B₂	6.2 / 1	5.8 / 2	5.5 / 3	5.0 / 6	5.1 / 7	5.3 / 8	5.5 / 9	5.3 / 10
25	5.7 / 5	4.6 / 12	4.2 / 13	3.0 / 21	2.8 / 25	2.8 / 26	2.6 / 32	2.4 / 32
20	6.0 / 12	4.8 / 15	4.3 / 22	3.3 / 24	3.3 / 24	3.3 / 26	3.2 / 31	2.9 / 32
18	X / X	5.2 / 8	4.6 / 12	3.7 / 17	3.6 / 22	3.5 / 25	X / 31	X / 32
15	X / X	5.3 / 7	4.8 / 10	X / X	X / X	X / X	X / X	X / X
12	X / X	5.3 / 11	4.9 / 11	X / X	X / X	X / X	4.1 / 29	30 / X
Ave	9	12	15	20	24	25	31	32

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 1 DAY (CONTINUED)

Day 2	12/11 (1)	12/12 (2)	12/15 (5)	12/16 (6)	12/17 (7)	12/18 (8)	12/19 (9)
100%							
B1	5.9	5.8	5.5	5.5	5.5	5.8	5.5
B2	6.1	5.8	5.5	5.5	5.5	5.5	5.5
15	3.9	3.0	2.0	1.7	1.0	X	X
14	4.0 / 43	3.0 / 60	2.0 / 71	1.8 / 79	1.0 / 96	1.3 / 105	1.2 / 107
12	4.2 / 45	3.6 / 55	2.4 / 77	1.9 / 90	1.5 / 100	2.2 / 99	2.2 / 99
10	4.4 / 48	3.9 / 57	2.7 / 84	2.5 / 90	2.1 / 102	X / X	X / X
8	X / X	X / X	X / X	X / X	X / X	X / X	X / X
Ave	46	57	79	84	97	99	103
67%							
B1	5.9	5.6	5.2	5.3	5.3	5.5	5.2
B2	5.9	5.7	5.3	5.4	5.4	5.5	5.5
25	4.3 / 19	3.6 / 24	2.3 / 35	1.8 / 43	1.5 / 46	1.4 / 49	1.4 / 47
20	4.6 / 19	4.1 / 23	2.8 / 37	2.2 / 47	2.4 / 44	2.3 / 48	2.2 / 47
15	4.9 / 20	4.6 / 21	3.6 / 33	3.5 / 37	3.4 / 39	3.5 / 40	X / X
12	5.0 / 22	4.7 / 24	3.7 / 39	3.5 / 46	3.5 / 46	3.4 / 52	X / X
10	X / X	X / X	X / X	X / X	X / X	X / X	X / X
Ave	20	23	36	45	44	47	47

BOD DATA FOR DILUTED SAMPLES
AFTER STANDING TIME OF 2 DAYS

33% Day 2	12/11 (1)	12/12 (2)	12/15 (5)	12/16 (6)	12/17 (7)	12/18 (8)	12/19 (9)
B1	5.8	5.6	5.2	5.2	5.2	5.2	5.0
B2							
30	5.4 / 1	5.2 / 2	6.5 / 5	7.5 / 6	7 / 7	10 / 8	
25	5.8 / 0	5.4 / 2.4	4.4 / 4.2	4.4 / 7	4.2 / 7	4.2 / 10	
20	5.7 / 2	5.4 / 2	4.7 / 5	4.6 / 8	4.5 / 10	4.6 / 10	
18	5.7 / 2	5.4 / 2	4.7 / 6	4.6 / 9	4.5 / 11	4.6 / 11	
15	X / X	5.5 / 2	4.7 / 5	4.8 / 8	4.7 / 10	4.8 / 8	
Ave	3	2	5	8	10	10	9

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 2 DAYS (CONTINUED)

Day 3		12/12 1	12/15 4	12/16 5	12/17 6	12/18 7	12/19 8
100%	B₁	6.0	5.5	5.6	5.6	5.8	5.7
	B₂	6.1	5.7	5.8	5.8	6.0	6.0
	25 ml	4.5 / 18	2.3 / 39	1.8 / 47	1.2 / 54	0.8 / 61	X / X
	20	4.7 / 20	2.9 / 40	2.6 / 47	2.0 / 55	1.6 / 64	1.5 / 65
	15	X / X	X / X	X / X	X / X	X / X	X / X
	12	5.0 / 26	3.8 / 45	X / X	X / X	X / X	X / X
	10	5.3 / 22	4.2 / 42	3.7 / 60	3.6 / 63	3.5 / 72	3.4 / 73
	Ave	21	41	51	57	66	69
67%	B₁	6.2	5.8	5.8	5.8	6.0	5.8
	B₂	5.7	5.2	5.2	5.0		
	25	5.7 / 1	4.8 / 4	4.7 / 5	4.1 / 6	4.0 / 7	4.0 / 8
	20	5.7 / 6	4.8 / 12	4.7 / 13	4.2 / 20	4.3 / 24	4.1 / 22
	18	5.9 / 8	4.8 / 15	4.5 / 19	4.2 / 24	4.4 / 26	4.2 / 26
	15	5.9 / 6	5.0 / 16	4.5 / 26	4.3 / 25	4.4 / 26	4.6 / 27
	12	5.9 / 7	5.1 / 17	4.7 / 26	4.4 / X	X / X	
	Ave	7	15	23	26	25	26

BOD DATA FOR DILUTED SAMPLES
AFTER STANDING TIME OF 3 DAYS

Day 3	12/12 1	12/15 4	12/16 5	12/17 6	12/18 7	12/19 8
B$_1$	5.9	5.8	5.7	5.6	6.0	5.9
B$_2$	6.0	5.6	5.6	5.5	5.8	5.8
30	6.1 / 1	5.4 / 4	5.0 / 7	4.9 / 6	5.0 / 9	5.0 / 9
28	6.1 / X	5.4 / 3	5.1 / 7	4.8 / 8	4.9 / 11	4.9 / 10
25	5.7 / 3.6	4.9 / 9.6	4.7 / 11	4.7 / 10	5.1 / 11	4.7 / 14
22	6.0 / X	5.3 / 5	5.1 / 8	4.6 / 13	4.9 / 13	4.9 / 13
20	6.3 / X	5.5 / 3	X / X	X / X	X / X	X / X
Ave	36	4	9	9	11	12

(Table heading: 33%)

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 3 DAYS (CONTINUED)

Day 4	12/15 (3)	12/16 (4)	12/17 (5)	12/18 (6)	12/19 (7)
100%					
B_1	5.7	5.7	5.5	5.8	5.7
B_2	5.7	5.6	5.5	5.8	5.6
25 ml	4.0 / 20	3.7 / 24	2.6 / 35	2.2 / 43	1.9 / 45
20	X / X	X / X	X / X	X / X	X / X
18	4.2 / 25	3.9 / 29	3.1 / 40	2.9 / 48	2.8 / 47
15	4.4 / 26	3.8 / X	3.4 / 42	3.5 / 46	3.2 / 49
12	4.7 / 25	4.3 / 34	3.9 / 40	4.0 / 45	3.8 / 46
Ave	24	31	39	45	47
67%					
B_1	5.0	4.9	4.7		
B_2	5.2	5.0	4.7		
40	4.5 / X	X / X	X / X	X / X	X / X
35	3.7 / 12	2.6 / 20	2.2 / 21	1.7 / 26	1.4 / 27
30	3.6 / 15	2.9 / 21	2.4 / 23	2.0 / 28	1.6 / 30
25	32 / 23	26 / 28	2.1 / 31	X / X	X / X
20	X / X	X / X	X / X	X / X	X / X
Ave	15	23	25	27	28

BOD DATA FOR DILUTED SAMPLES
AFTER STANDING TIME OF 4 DAYS

Day 4	33%				
	12/15	12/16	12/17	12/18	12/19
B1 / B2	7.4 / 3	7.2 / 4	7.3 / 5	7.6 / 6	7.5 / 7
40	5.4 / X	6.0 / X	5.9 / 13	6.1 / 13	5.8 / 14
35	6.4 / 9	6.1 / 10	6.1 / 12	6.3 / 13	6.0 / 14
30	6.6 / 8	6.1 / 11	6.1 / 12	6.6 / 12	6.4 / 13
25	6.7 / 8	6.5 / 9	6.3 / 12	X / X	X / X
20	7.0 / 6	6.7 / 8	X / X	X / X	X / X
Ave	8	10	12	13	14

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 4 DAYS (CONTINUED)

100%, L=175			66%, L=125			33%, L=75		
y	TIME	k	y	TIME	k	y	TIME	k
45	1	.13	30	1	.12	15	1	.096
	2	.13	50	2	.11	30	2	.11
105	3	.13	68	3	.11	47	3	X
120	4	.13	85	4	.12	60	4	X
135	5	.13	95	5	.12	70	5	X
	Ave	.13		Ave	.115		Ave	.10

100%, L=110			66%, L=72			33%, L=32		
y	TIME	k	y	TIME	k	y	TIME	k
38	1	.18	20	1	.14	6	1	.09
61	2	.17	35	2	.14	11	2	.09
77	3	.17	45	3	.14	15	3	.09
90	4	.18	55	4	.14	19	4	.1
95	5	.17	62	5	X	22	5	.1
	Ave	.174		Ave	.14		Ave	.094

100%, L=85			67%, L=47			32%, L=10		
y	TIME	k	y	TIME	k	y	TIME	k
	1	.306	15	1	.17	3	1	.15
57	2	.24	25	2	.16	5	2	.15
72	3	.27	30	3	.15	6	3	.13
78	4	.27	37	4	.17	7	4	.13
82	5	.29	41	5	.17	8	5	.14
	Ave	.27		Ave	.17		Ave	.14

100%, L=55			66%, L=25			33%, L=.14		
y	TIME	k	y	TIME	k	y	TIME	k
21	1	.21	7	1	.14	3	1	.11
32	2	.19	12	2	.14	5	2	.10
41	3	.20	16	3	.15	7	3	.11
47	4	.21	18	4	.14	9	4	.13
50	5	.21	22	5	.14	10	5	.13
	Ave	.21		Ave	.14		Ave	.11

100%, L=48			67%, L=28			33%, L=13		
y	TIME	k	y	TIME	k	y	TIME	k
1	1	.01	4	1	.07	2	1	.07
17	2	.09	9	2	.08	4	2	.08
25	3	.10	14	3	.10	6	3	.09
31	4	.11	21	4	X	8	4	.1
38	5	X	24	5	X	10	5	.11
	Ave	.1		Ave	.083		Ave	.09

APPENDIX C

DERIVATION OF THE COD FORMULA

DERIVATION OF COD FORMULA

A. COD procedure attempts to duplicate natural oxidation using sugar as the example the reaction is:

1. $C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$

    a. There is a 6 to 1 mole ratio of sugar to oxygen.

    b. The time for completion is 10-20 days.

B. Laboratory Reaction: To speed the reaction a stronger oxidizing agent and heat will be used to oxidize the organic compounds.

1. $C_6H_{12}O_6 + 4\ K_2Cr_2O_7 + 4\ H_2SO_4 \longrightarrow 6\ CO_2 + 10H_2O +$
$$4\ Cr_2O_3 + 4\ K_2SO_4$$

    a. XS Dichromate used.

    b. Reaction heated to boiling point.

    c. 4 to 1 ratio between Dichromate and sugar.

2. Back titrate XS Dichromate with Ferrous Sulfate.

$$K_2Cr_2O_7 + 6\ FeSO_4 + 4\ H_2SO_4 \longrightarrow Cr_2O_3 +$$

$$3\ Fe_2\ (SO_4)_3 + K_2SO_4 + 4\ H_2O$$

a. There is a 6 to 1 mole ratio between PDC and FS.

C. COD formula:

1. Based on mole relationships

   a. moles of Dichromate added

      1. This would ordinarily be VM

      2. Cannot be used because some is oxidized during heating.

      3. A blank is used, V_B

      4. The equivalent amount of Potassium Dichromate would be

$$\frac{V_B M}{6} = \text{mole PDC}$$

2. Moles of XS Dichromate remaining

   1. $V_S M$ = mole Ferrous Sulfate

   2. $V_S M/6$ = mole XS PDC remaining

3. Moles of Dichromate which reacted

$$\frac{V_B M}{6} - \frac{V_S M}{6} = PDC \ reacted$$

4. Moles of organic present

$$[(V_B-V_S)M/6]/4 \quad (B-1-c)$$

5. Moles of oxygen required

$$\frac{[(V_B-V_S)M/6]}{4}x \ 6 \quad (A-1-a)$$

6. Milligrams of oxygen required

$$\frac{[(V_B-V_S)M/6] \ (6) \ (32,000)}{4} = COD$$

7. Final form after simplification

$$\frac{(V_B-V_S) \ (M) \ 8,000}{Sample \ Size} = mg/L \ COD$$

APPENDIX D

CALIBRATION CURVES USED IN THE STUDY

1. HACH COD METHOD
2. O-TOLUIDINE METHOD FOR GLUCOSE
3. BIURET METHOD FOR PROTEIN
4. AMMONIA BY THE NESSLER METHOD

Figure No. 42

Calibration Curve For Hach COD
Method @ 600 NM.

3 mL solution

COD Concentration, mg/l

Absorbance

Figure No.43

Calibration Curve For Glucose Determination
Using O-Toluidine Method @ 635 NM.

Figure No. 44

Calibration Curve For Ammonia
Determination By Nessler Procedure

Absorbance

Ammonia Concentration, mg/L

Figure No. 45

Calibration Curve For Protein Using The Biuret
Reaction @ 540 NM.

APPENDIX E

RESULTS OF INVESTIGATIONS REGARDING DETENTION

TIME IN THE PRESSURE VESSEL

Figure No. 46

Variation Of BOD For Pressurized And Non-Pressurized
Wastewater Samples For Four Hours Detention Time

192

Figure No. 47

Variation of BOD For Pressurized And Non-Pressurized
Wastewater Samples For The Same Detention Time

Figure No. 48

Variation Of BOD For Pressurized And Non-Pressurized
Wastewater Samples For The Same Detention Time

APPENDIX F

DEMAND CURVES OBTAINED FROM SEEDED AND

UNSEEDED DILUTION WATER

Figure No. 49

Variation Of BOD For Wastewater Samples Prepared In Seeded And
Unseeded Dilution Water Pressurized For Different Detention Times

196

Figure No. 50

Variation of BOD For Wastewater Samples Prepared In Seeded And
Unseeded Dilution Water Pressurized For Different Detention Times

Figure No. 51

Variation Of BOD For Wastewater Samples Prepared In Seeded And
Unseeded Dilution Water Pressurized For Different Detention Times

Figure No. 52

Variation of COD of Pressurized And
Non-Pressurized Wastewater Samples

Figure No. 53

Variation Of Specific Rate Constant For The Decomposition Of
Wastewater For Pressurized And Non-Pressurized Samples

APPENDIX G

DEMAND CURVES FOR QUIESCENT, AERATED, AND
PRESSURIZED WASTEWATER SAMPLES TO STUDY THE
EFFECTS OF DISSOLVED OXYGEN

200

Figure No. 54

Variation Of BOD For Samples Aerated And
Pressurized For 24 Hours Versus The Control

Figure No. 55

Variation Of BOD For Samples Aerated And
Pressurized For 8 Hours Versus The Control

APPENDIX H

DEMAND CURVES FOR SAMPLES PRESSURIZED FOR

UP TO THREE HOURS AND ALLOWED TO STAND

Figure No. 56

Variation Of BOD With Time For Samples Not Pressurized And
Allowed To Settle For A Period Of Three Days

Figure No. 57

Variation of BOD With Time For Samples Pressurized For
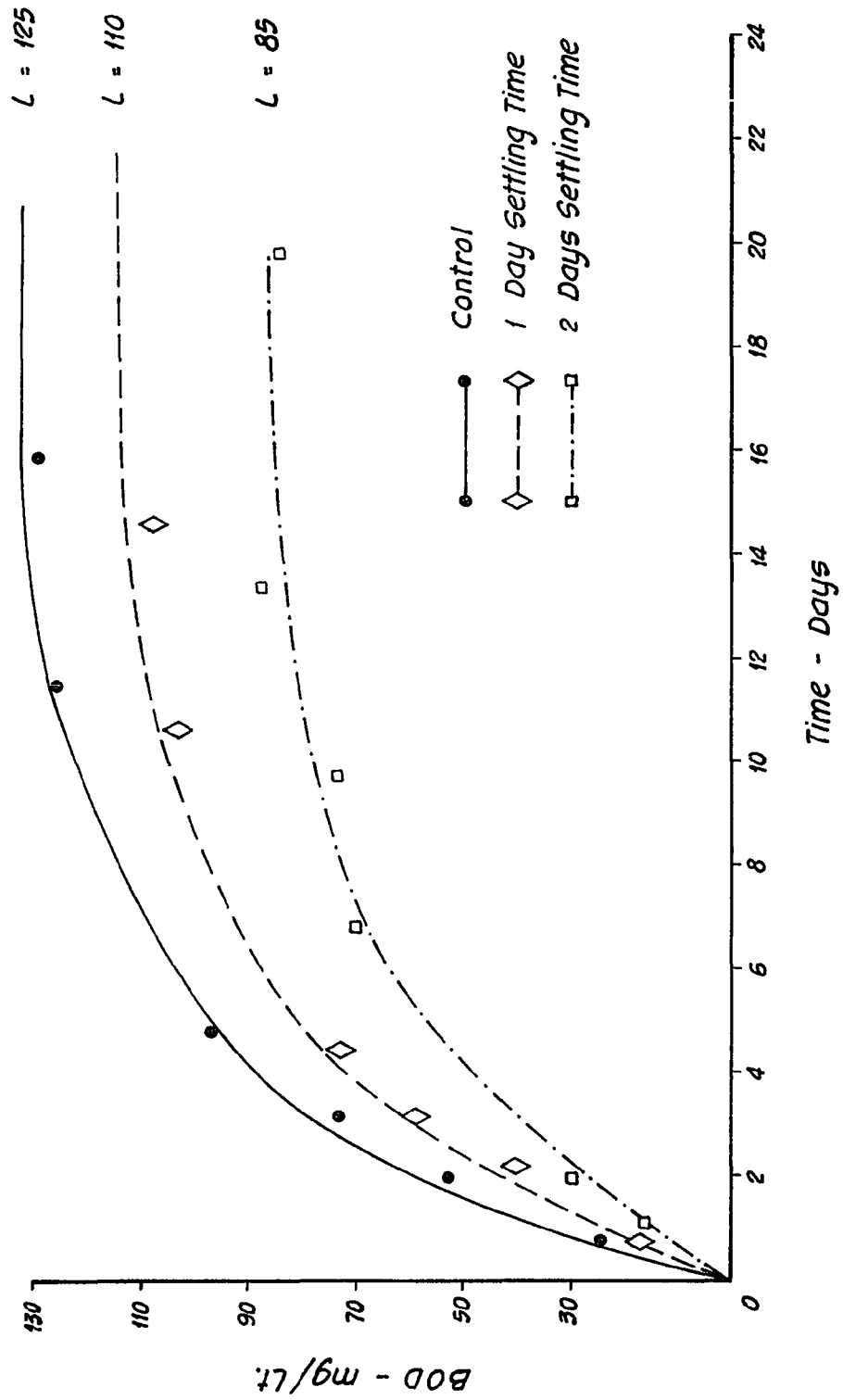Half Hour And Allowed To Settle For A Period Of Three Days

Figure No. 58

Variation Of BOD With Time For Samples Pressurized For
1.5 Hours And Allowed To Settle For A Period Of Three Days

Figure No.59

Variation Of BOD With Time For Samples Pressurized For
Three Hours And Allowed To Settle For Up To Three Days

Figure No. 60

Variation Of BOD With Time For Samples Pressurized For
One Hour And Allowed To Settle For A Period Of Three Days

APPENDIX I

VARIATIONS IN THE BOD AND SPECIFIC RATE CONSTANT FOR SAMPLES PRESSURIZED FOR 24 HOURS AND ALLOWED TO STAND

Figure No. 61

Variation Of The BOD With Time For A Sample Pressurized For 24 Hours @ 40 psi. And Allowed To Stand For Four Days

Figure No. 62

Variation Of The Specific Rate Constant For A Pressurized
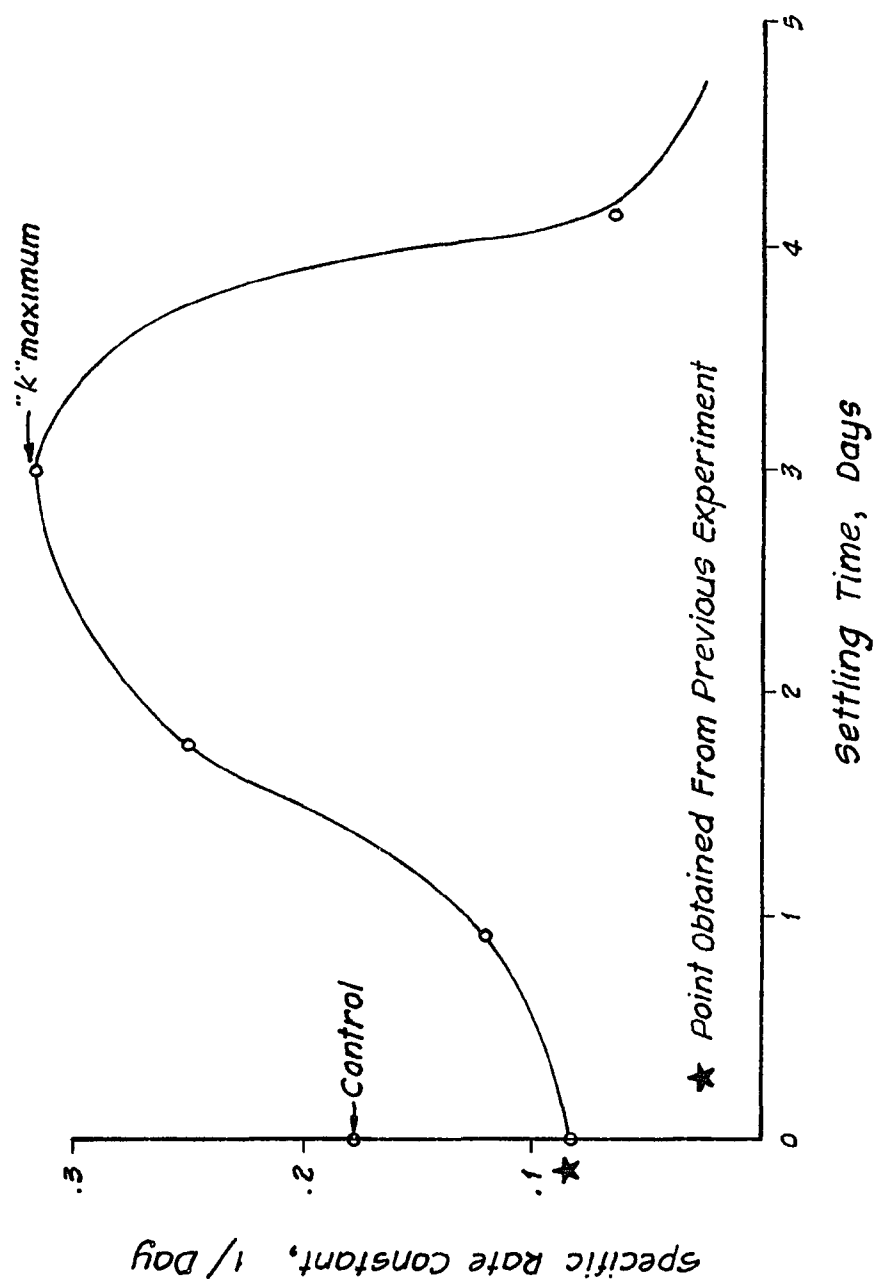Sample Allowed To Stand For A Period Of Three Days

Figure No. 63

Variation of The Specific Rate Constant For A Pressurized
Sample Allowed To Stand For A Period Of Four Days

APPENDIX J

SUPPLIERS OF CHEMICALS AND SUPPLIES

USED IN THE STUDY

SUPPLIERS OF CHEMICALS AND SUPPLIES USED IN THE STUDY

1. Aldrich Chemical Co., Milwaukee, Wisconsin

    a. Para-Nitro-Phenyl Acetate, Ni, 990-7

    b. 4-Dimethyaminoantipyrine, Di3, 910-6

2. Baker Chemical

    a. O-Toluidine, 7W-122

    b. Tristearin, 3-X177

    c. Serum Albumin Fraction Five, 2-A464

3. Chemalog Chemical

    a. Amylase, 06-5960-00

4. Fisher Scientific, Springfield, New Jersey

    a. Sodium Hydroxide

    b. Potassium Di-Hydrogen Phosphate

    c. O-Nitro-Phenol, N-105

    d. Starch, Potato, S-516

    e. Cupric Sulfate

5. Merck Chemicals, Rahway, New Jersey

    a. Stearic Acid, 04281

6. National Biochemicals

    a. Glutamic Acid, 7160

7. Sigma Chemical, St. Louis, Missouri

    a. Amylglucosidase, A-3514

8. Wards Chemical

    a. Glucose, 58W 3200

APPENDIX K

DEMAND CURVES FOR PRESSURIZED AND
NONPRESSURIZED STEARIC ACID SOLUTIONS

Figure No. 64

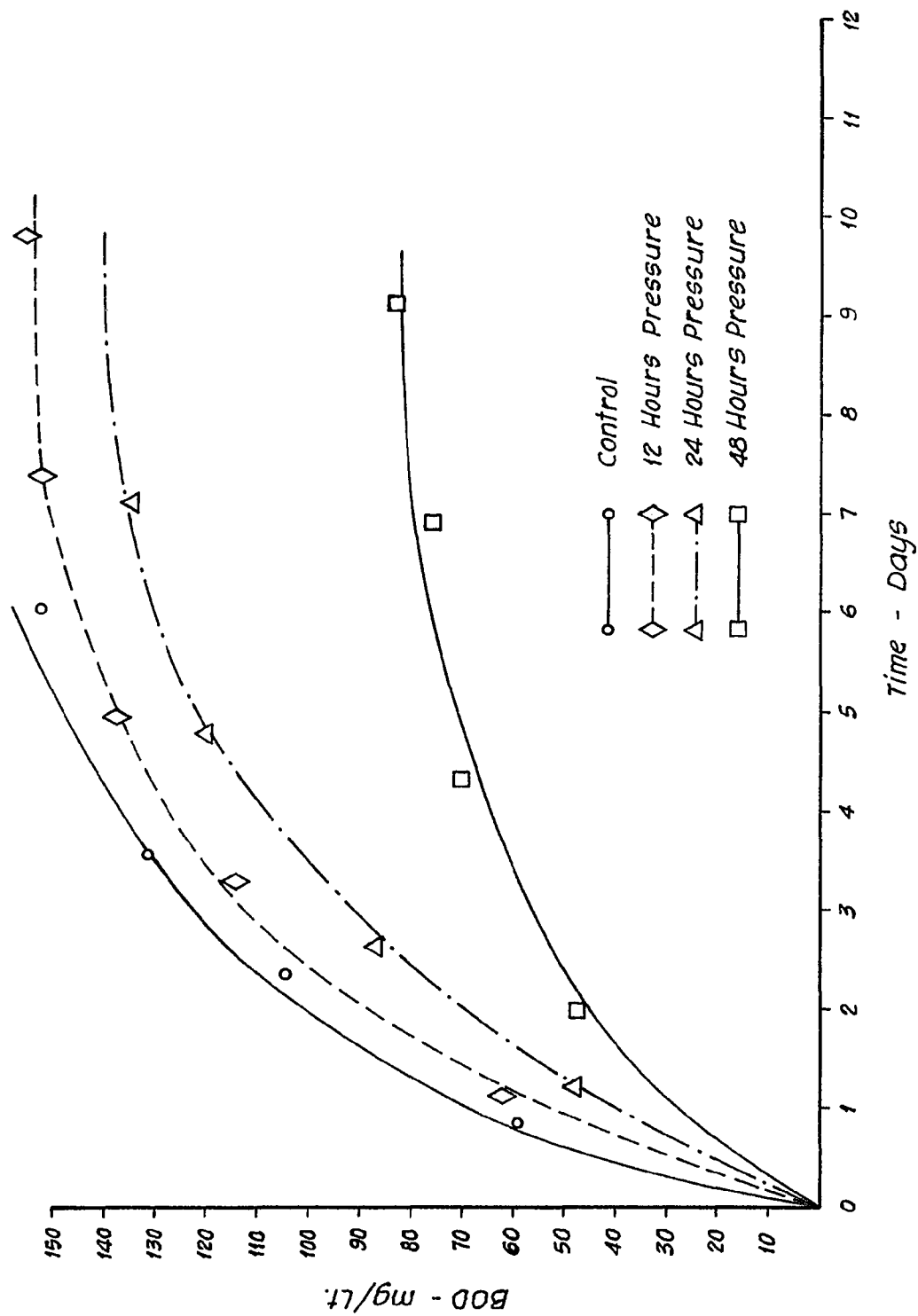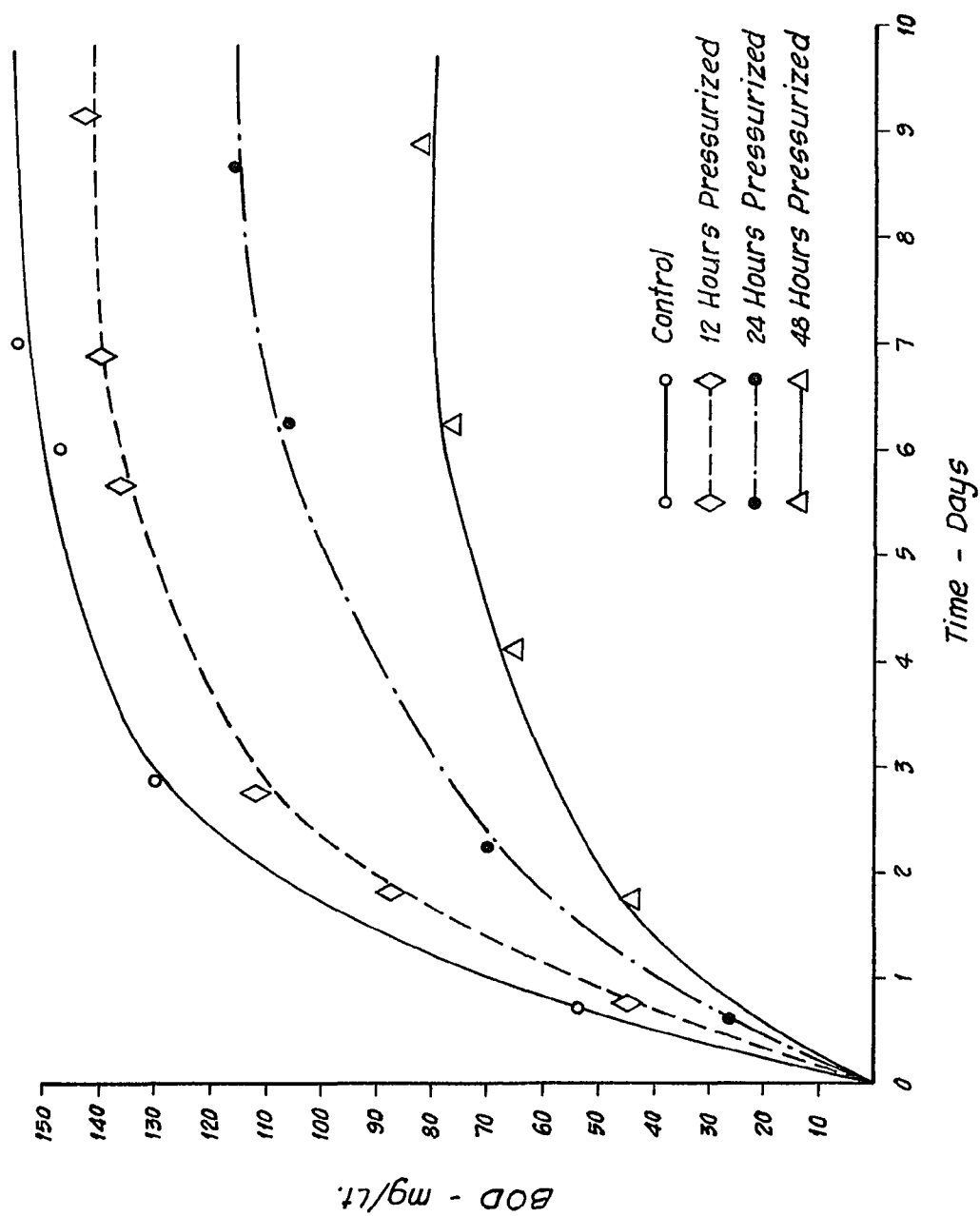Variation Of BOD Values Of Stearic Acid For Pressurized Samples

Figure No. 65

Variation of BOD Values of Stearic Acid For Aerated Samples

BOD - mg/Lt.

Time - Days

○——○ Control
◇——◇ 12 Hours Pressurized
●——● 24 Hours Pressurized
△——△ 48 Hours Pressurized

APPENDIX L

THE EFFECTS OF PRESSURE ON THE HYDROLYSIS

RATE OF PROTEINS TO PRODUCE AMMONIA

220

Figure No. 66

Variation Of The Specific Rate Constant As A Function
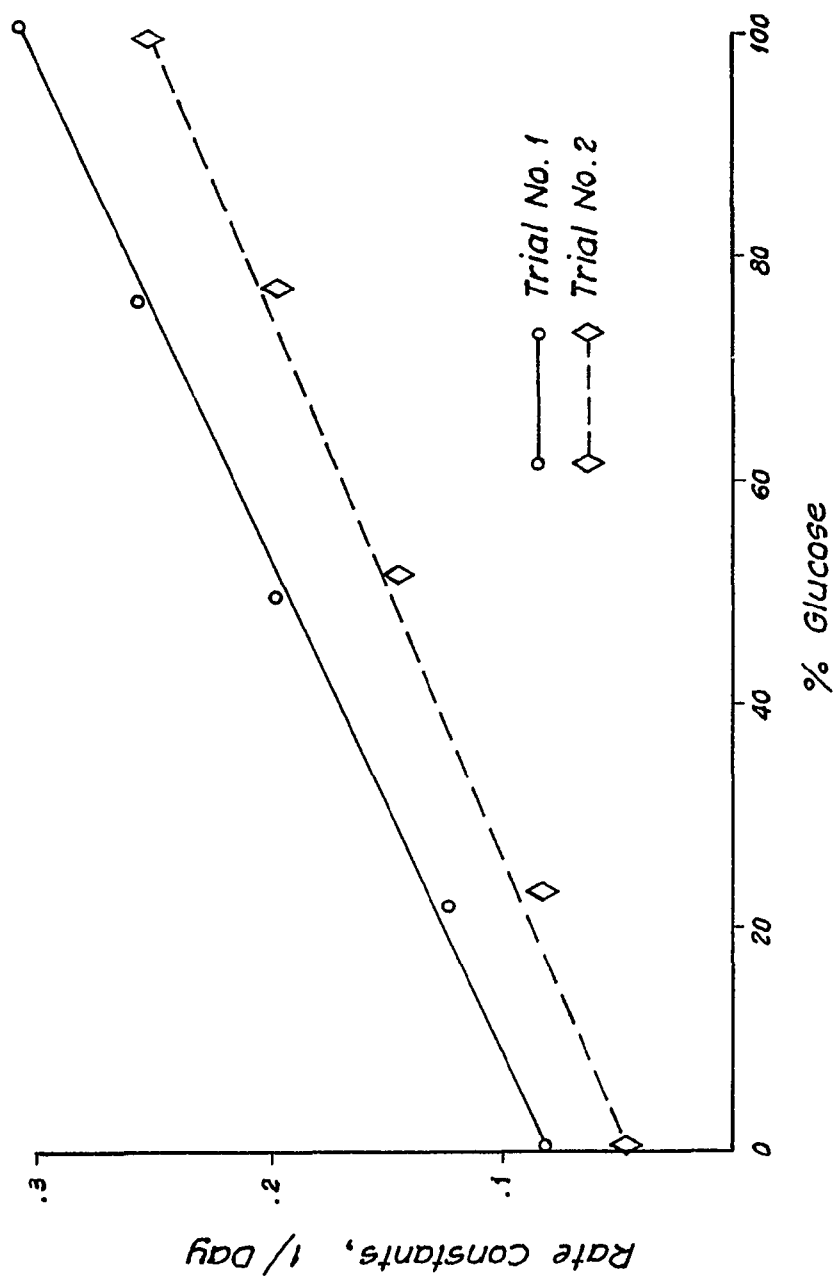Of The Amount Of Glucose In A Glucose/Starch Mixture

Figure No. 67

The Effect Of Pressure On The Hydrolysis
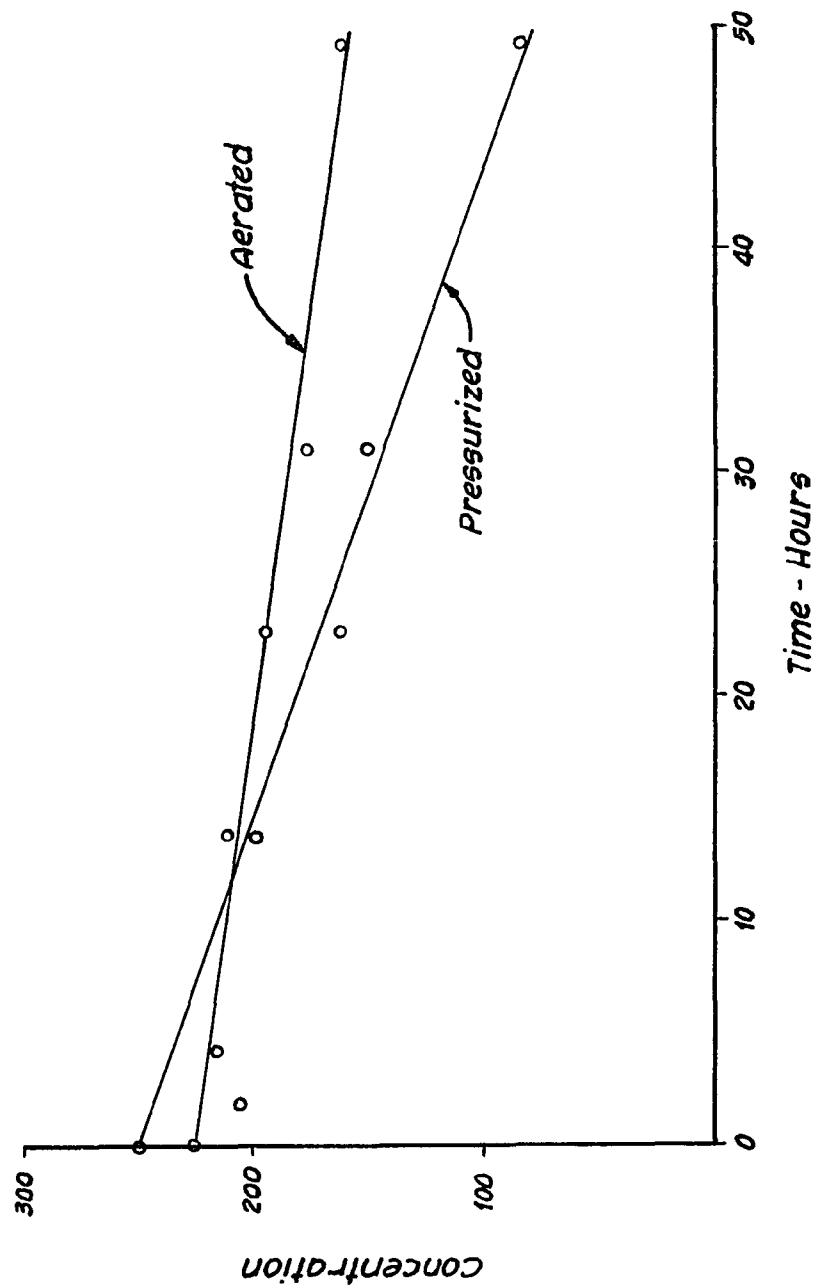Rate Of Protein Versus Non - Pressurization

Figure No. 6B

Variation In The Ammonia Concentration For Pressurized
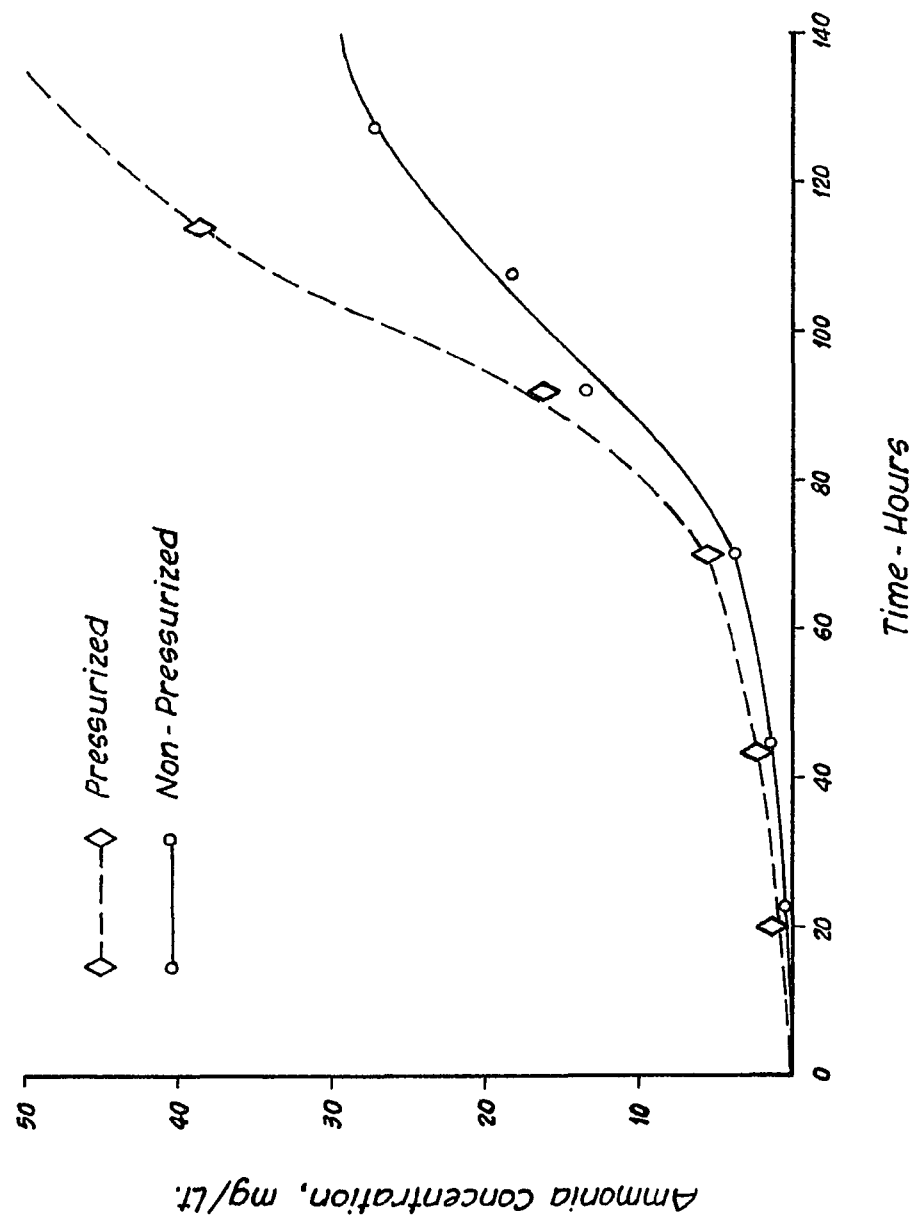And Non-Pressurized Protein Solutions

223

Figure No. 69

Variation In The Ammonia Concentration For Pressurized
And Non-Pressurized Protein Solutions, Concentration
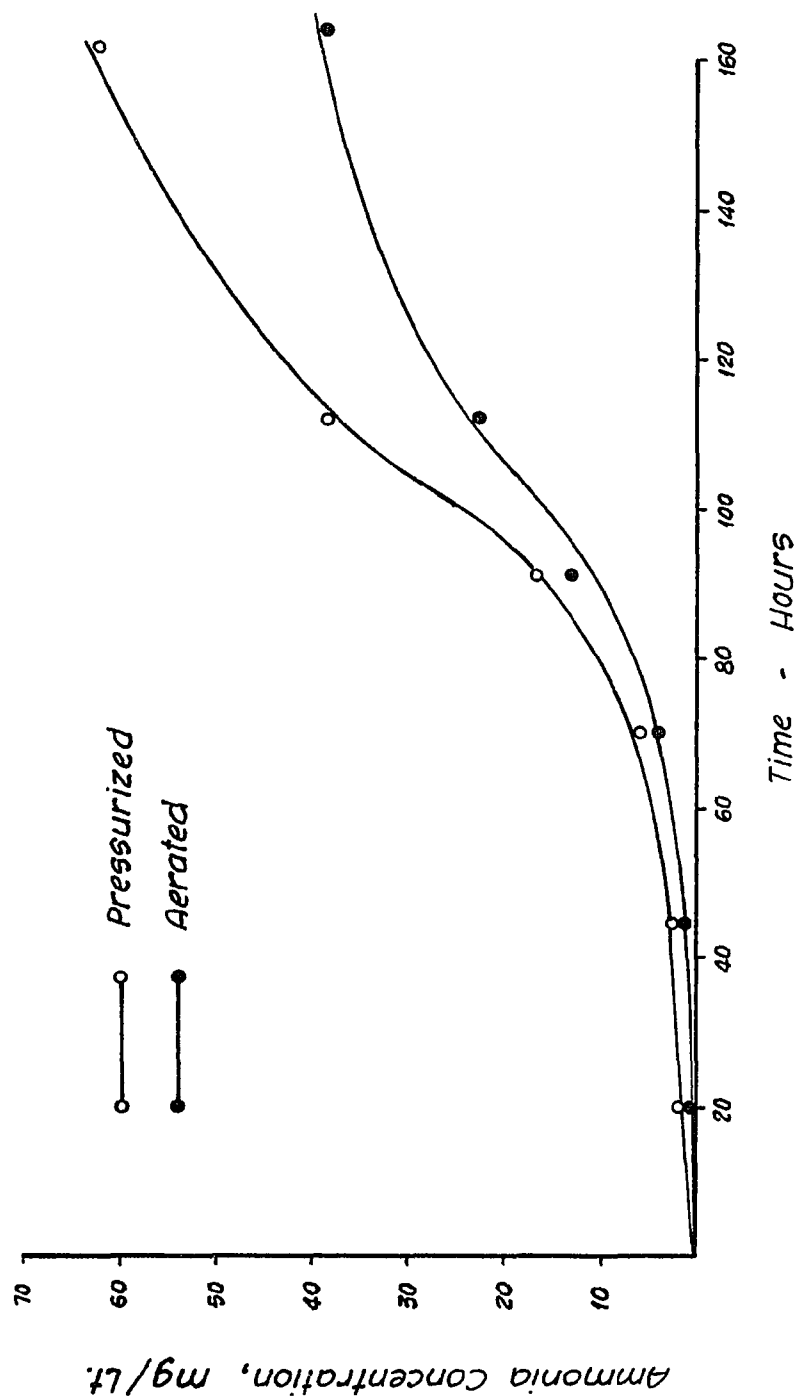Measured Using The Nessler Procedure

Figure No. 70

Variations of BOD Values For Glutamic Acid
For An Aerated Sample

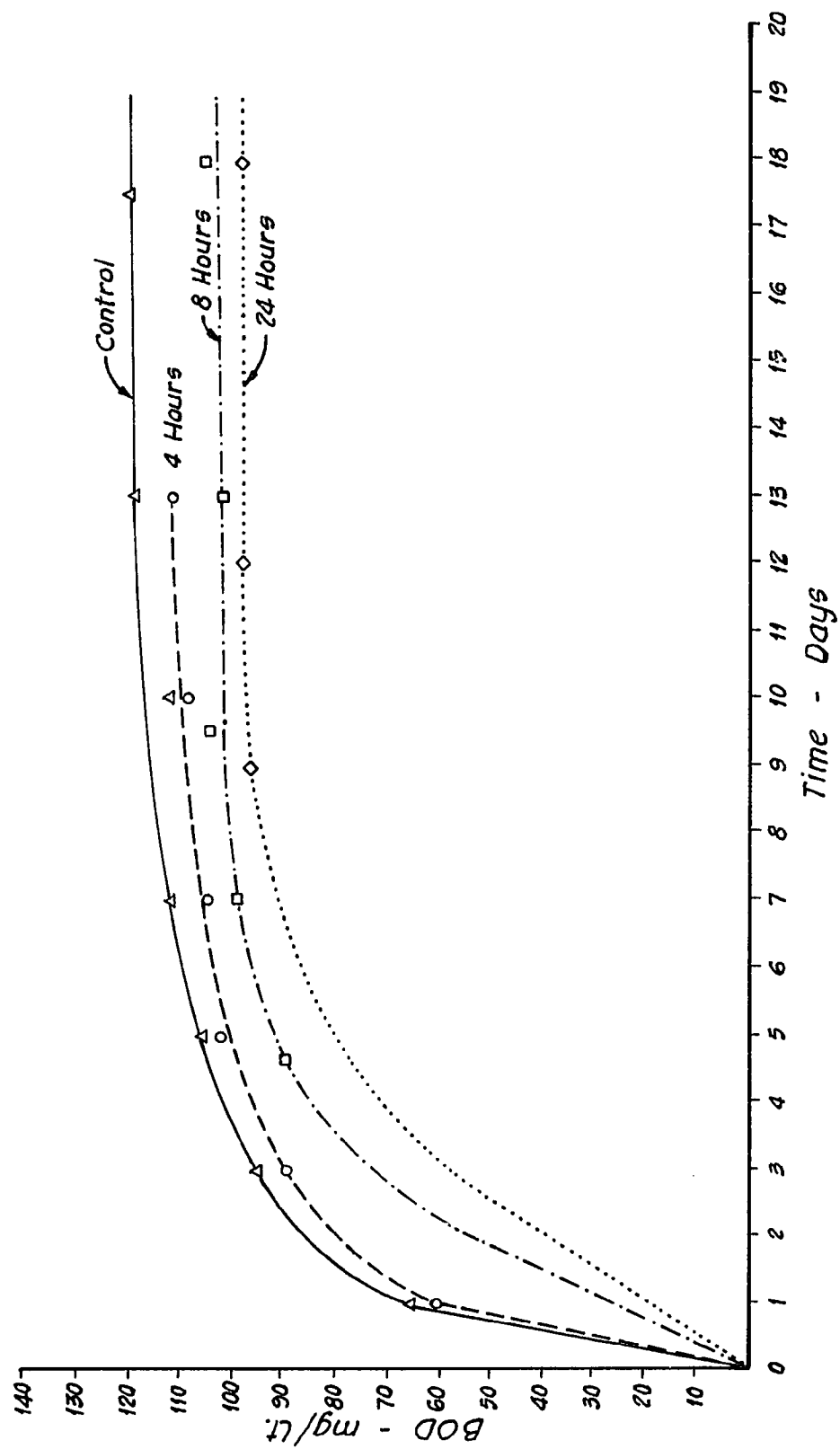Figure No. 71

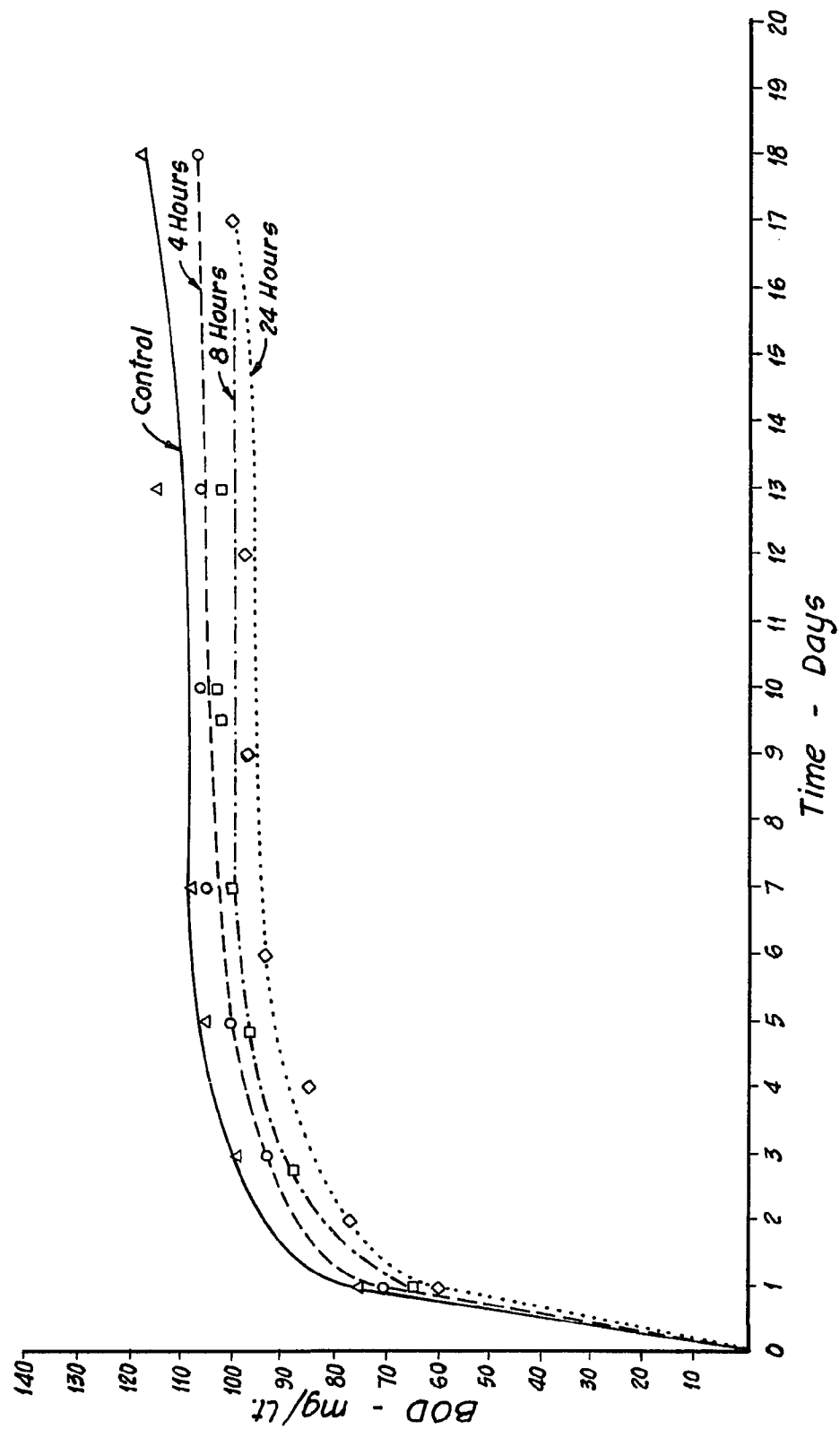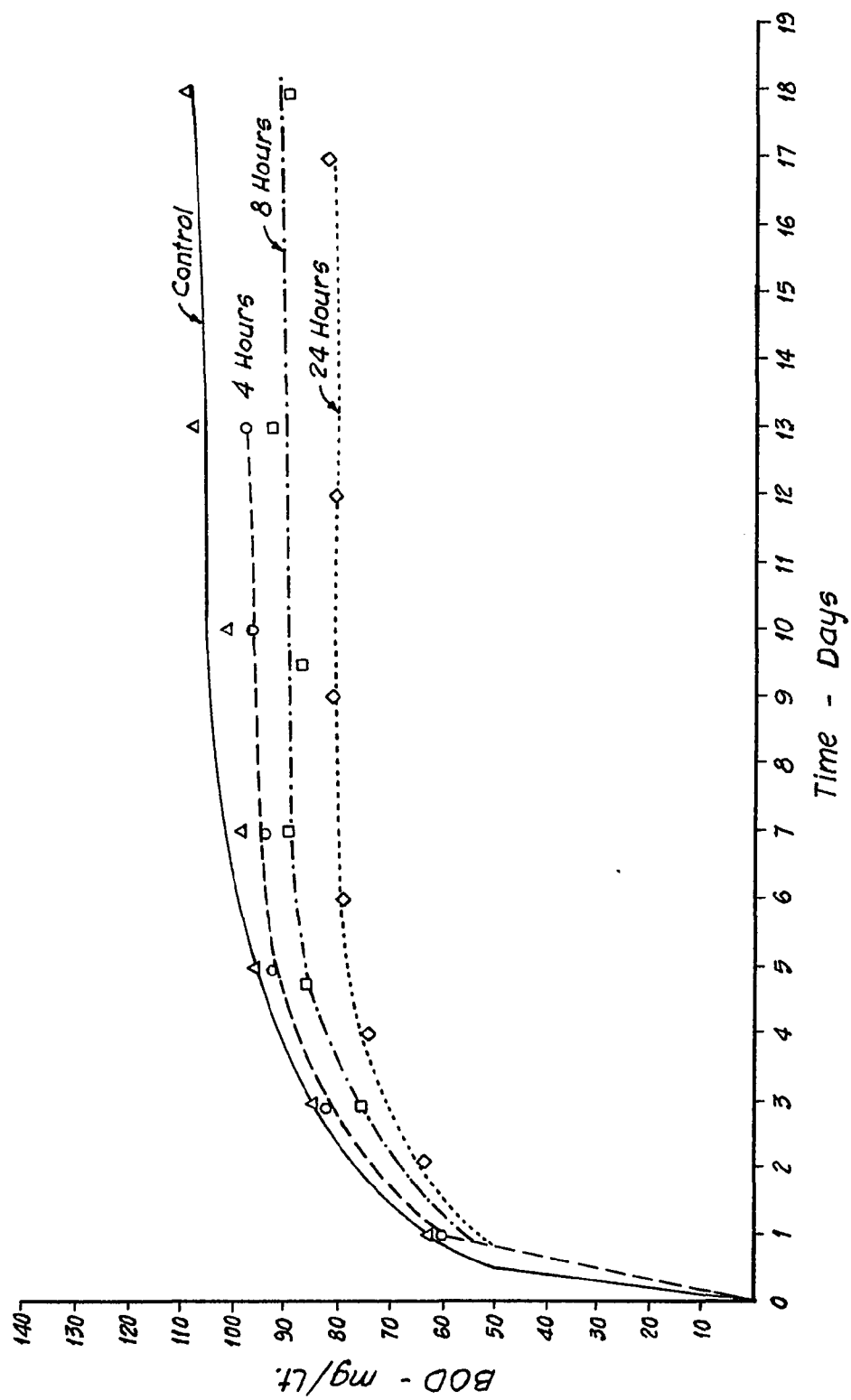Variation of BOD Values For Glutamic Acid
For A Quiescent Sample

226

Figure No. 72

Variation of BOD Values For Glutamic Acid
For Pressurized Samples

APPENDIX M

EXPERIMENTAL PROCEDURE

1. GLUCOSE ANALYSIS
2. PROTEIN ANALYSIS

APPENDIX M

GLUCOSE ANALYSIS

REAGENTS	EQUIPMENT
1. 6% O-Toluidine in concentrated Acetic Acid	1. Spectrophotometer, set at 635 nm.
2. Stock glucose solution, 10,000 mg/l	2. Hot water bath, set at 100°C.
3. Working glucose solution, 100 mg/l	3. Timer

REACTION

$$CH_3C_6H_4NH_2/HAc + C_6H_{12}O_6 \quad \text{green-blue complex}$$

(O-toluidine)   (glucose)

The reaction involved preparing a 1,000 mg/l starch solution $(C_6H_{10}O_5)_x$ and adding glucosidase enzyme to aid in the hydrolysis of starch to glucose. The hydrolysis is shown as follows:

$$(C_6H_{10}O_5) + x H_2O \xrightarrow{[ase]} x C_6H_{12}O_6$$

The glucose produced was then measured as shown above.

PROCEDURE

1. Prepare glucose solutions of 500 to 2,500 mg/l from the stock solution according to the table below:

ml Glucose Solution	Water	Concentration mg/l
0.5	9.5	500
1.0	9.0	1000
1.5	8.5	1500
2.0	8.0	2000
2.5	7.5	2500

2. Add 0.10 ml of distilled water to a cuvette and 0.10 ml of each diluted glucose solution to cuvettes.

3. Add 5 ml of O-toluidine/acetic acid solution.

4. Place in boiling water for 10 +/-0.1 minutes.

5. Remove, place in cold water bath.

6. Read the absorbance, and prepare calibration curve.

7. Repeat steps 3 to 6 for the sample. Multiple samples were run (0.05, 0.10, 0.20 ml) so that the different volume samples would converge on a common value.

PROTEIN ANALYSIS

REAGENTS	EQUIPMENT

1. Biuret reagent

    (1.5 g $CuSO_4 \cdot 5\ H_2O$)
    (6 g $NaK_4C_4O_6 \cdot 4\ H_2O$)
    (300 ml of 10% NaOH
    diluted to 1 L)

2. NaCl solution, 0.9%

3. Sodium Deoxycholate

4. Albumin; 10,000 mg/l

1. Spectrophotometer, set
   at 540 nm.

REACTION

Albumin solution is allowed to hydrolyze in the sequence
as shown:

    Protein $\longrightarrow$ Polypeptide $\longrightarrow$ Dipeptide $\longrightarrow$ Amino Acid

                      Fatty Acid + NH_3 $\longleftarrow$

The dipeptide produced is reacted with biuret reagent
to produce a purple complex indicated below:

PROCEDURE

1. To B & L cuvettes add the reagents as indicated in the following table:

CONCENTRATION	00	100	200	500	1000	1400
Albumin	0.0	0.1	0.2	0.5	1.0	1.4
Nadeoxycholate	0.1	0.1	0.1	0.1	0.1	0.1
NaCl, 0.9%	1.4	1.3	1.2	0.9	0.4	000
Biuret reagent	1.5	1.5	1.5	1.5	1.5	1.5

2. After 30 minutes read absorbance, plot calibration curve.

3. Perform analysis for the sample.

BIBLIOGRAPHY

1. Sawyer, C.N. and P.L. McCarty, Chemistry for Enviromental Engineering, New York, McGraw-Hill, Third Edition, p. 416.

2. Gaudy, Anthony and Elizabeth Gaudy, Microbiology for Environmental Scientists and Engineers, McGraw-Hill, New York, 1980, pp. 487-504.

3. Young and Clark, "Second Order Equation for BOD Formula", Journal Sanitary Engineering Division, Proceedings of ASCE, SA 1, February, 1965, pp. 43-51.

4. Fraga, E.R., J. Schifino, and I. Gomez, "Determination of Catalytic Coefficient for a First Order Reaction", Journal of Chemical Education, Vol. 52, No. 11 November, 1975, p. 749.

5. Nechamkin, Howard, Elhannan Keller and Jerome Goodkin, "Reaction Rates for a Homogeneously Catalyzed Reaction", Journal of Chemical Education, Vol. 54, No. 12, December, 1977, p. 755.

6. Gaudy, Anthony and Elizabeth Gaudy, Microbiology for Environmental Scientists and Engineers, McGraw-Hill, New York, 1980, p. 191

7. Heukelekian, Henry, "Effect of Dilution of Sewage on Bacterial Numbers", Sewage Works Journal, Vol. 5, No. 5, September, 1933, pp. 774-787.

8. Eliassen, Rolf, "Coliform Aftergrowth in Chlorinated Storm Overflows", Journal Sanitary Engineering Division, SA 2, April, 1968, pp. 371-380.

9. Finstein, Melvin S., Pollution Microbiology, Marcel Dekker, New York, 1972, pp. 137-41.

10. Heukelekian, Henry, "Disinfection of Sewage With Chlorine; Part IV. Aftergrowth of Coliform Organisms in Streams Receiving Chlorinated Sewage", Sewage and Industrial Wastes, March, 1951, pp. 173-77.

11. Jennelle, E.M., and A.F. Gaudy, "Studies on the Kinetics and Mechanisms of BOD Exertion in Dilute Solutions", Biotechnology and Bioengineering, Vol, Xii, 1970, pp. 519-39.

12. Barty, H.G. and F.M. Bell, "Reduction of Sewage by Chlorination", Sewage Works Journal, Vol. 1, 1929, p. 279.

13. Barty, H.G., "Some Effects of Sewage Chlorination Upon the Receiving Stream", Sewage Works Journal, Vol. 5, 1933, p. 429.

14. Griffin, E.E. and N.S. Chamberlain, "Exploring the Effects of Heavy Dosages of Chlorine in Sewage", Sewage Works Journal, Vol. 17, 1945, p. 730.

15. Snow, W.B., "BOD of Chlorinated Sewage", Journal Sewage and Industrial Wastes, Vol. 24, 1952, p. 689.

16. Zoloum, R., "Reduction of Oxygen Demand of Treated Wastewater by Chlorination", JWPCF, Vol. 46, No. 12, December 1974, p. 2770

17. Susag, R.H., "BOD Reduction by Chlorination", JWPCF, Vol. 40, No. 11, Part II, 1968, p. 434.

18. LeNoble, W.J. "The Effect of Pressure on the Equilibrium of Alpha and Gamma - Methylallyl Azide", Journal of Physical Chemistry, Vol. 67, November 1963, pp. 2451-2453.

19. Jannach, Holger W., Kjell Einjellen, Carl O. Winsen, and A. Farmanfarmanian, "Microbial Degradation of Organic Matter in the Deep Sea", Science, February 1971, pp. 672-75.

20. Lawrence, Richard, Richard Reynolds and Robert Schilling, "The Effects of Pressure on the Biological Degradation of Organic Wastes by Microorganisms", paper presented at the Middle West Regional Conference of Chemical Engineers, 1971, pp. 11-15.

21. Kaplan, Leonard J. and Herbert Klei, "A Study of the Reaction Kinetics of a Completely Mixed Activated Sludge Reactor", University of Connecticut, 1972.

22. Chack, John J., "Biodegradability and Coliform Die-
    Away in a High Pressure Ocean Environment",
    Master's Thesis, New Jersey Institute of Tech-
    nology, 1972, p. 22.

23. Nusser, Donald O., "The Effects of Pressure on the
    Biodegradability of Sanitary Sewage", Master's
    Thesis, New Jersey Institute of Technology, 1975.

24. Mezei, Nicholas, "The Effects of Pressure on the Bio-
    deradability of Sanitary Sewage in a Model Acti-
    vated Sludge Reactor", Master's Thesis, New
    Jersey Institite of Technology, 1977.

25. Mazzei, Dennia, "Growth Patterns of Bacteria in
    Pressurized Sewage", Master's Thesis, New Jersey
    Institute of Technology, 1977.

26. Nolte, Wayne F., "Kinetic Responses to Elevated
    Pressure of a Complete Mix Activated Sludge
    Process", Doctoral Dissertation, New Jersey
    Institute of Technology, 1979, pp. 3-21.

27. Edwards, R.N., "The Synergetic Oxyozonation Synthesis
    Processes for the Treatment of Industrial and
    Domestic Wastewater and Sludge", Private Con-
    fidential Report, 1979.

28. "Caldwell Sees New Treatment at Waste Plant", Sunday
    Star Ledger, Section One, p. 9, October 18, 1979.

29. Standard Methods for the Examination of Water and
    Wastewater, 14th Edition, American Public Health
    Association, Washington, D.C., 1975, p. 543-50.

30. Ciaccio, Leonard L., Editor, Water and Water Pollution,
    Vol. 3, Marcel Dekker, New York, 1972, pp. 801-28.

31 Jeris, John S., "A Rapid COD Test", Water and Wastes
    Engineering, May, 1967, pp. 89-91.

32. Standard Methods, op. cit, p. 550.

33. Wells, W.N., "Evaluation of the Jeris Rapid COD Test",
    Water and Sewage Works, April, 1970, pp. 123-129

34. Jerka, A.M. and M.J. Carter, "Micro Semi-Automated Analyses of Surface and Wastewaters for COD", Analytical Chemistry, Vol. 27, No. 8, 1975, pp. 1397-1401.

35. Federal Register, Vol. 45, No. 105, May 29, 1980, p. 36136.

36. Lange's Handbook, 6th Edition, Handbook Publishers, Sandusky, Ohio, 1946, p. 1103.

37. Lange's Handbook, ibid, p. 1103.

38. Morrison, R.T. and P.N. Boyd, Organic Chemistry, Allyn and Bacon, New York, 1959, pp. 577-80.

39. Standard Methods, op. cit., p. 505.

40. Vowles, P.D. and D.W. Connell, Experiments in Environmental Chemistry, Pergamon Press, New York, 1980, p. 23.

41. Sawyer, C.N. and P.L. McCarty, Chemistry for Sanitary Engineers, 2nd Edition, McGraw-Hill, New York, 1978, p. 141.

42. The Colorimetric Determination of Glucose", Sigma Chemical Co., St. Louis, Mo., Technical Bulletin No. 635.

43. Sawyer, C.N. and P.L. McCarty, op. cit.

44. Gaudy, A.F., "Colorimetric Determination of Protein and Carbonhydrate", Industrial Water and Wastes, January-February, 1962, pp. 17-27.

45. Rendina, George, Experimental Methods in Modern Biochemistry, Saunders, Philadelphia, 1971, pp. 75-78.

46. Standard Methods, op. cit., p. 412.

47. Nusser, D.O., op. cit., p. 50.

48. Mazzei, op. cit., p. 50.

49. Ciaccio, L., op. cit., pp. 340-46.

50. Hunter, Joseph V. and Henry Heukelekian, "The Compo-
        sition of Domestic Sewage Fractions", Journal
        Water Pollution Control Federation, Vol. 37,
        No. 8, August, 1965, pp. 1142-63.

51. Finstein, M.L., "The Question of Nitrification in
        the Passiac River, New Jersey, Analysis of
        Historical Data and Experimental Investigation",
        Water Research, Vol. 13, 1979, pp. 525-537.
        Permangm Press, London, UK.

52. Culp, G.L. and R.L. Culp, New Concepts in Water
        Purification, pp. 197-204, Van Nostrand-Reinhold,
        1974, New York.