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

New Jersey Institute of Technology

D.ENG.SC.

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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

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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

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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.

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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 decomposition of pressurized organic matter can be as high as 36 percent. This represents a potential for increased

wastewater treatment handling and removal efficiencies.

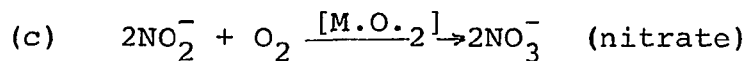
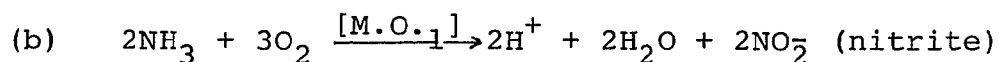
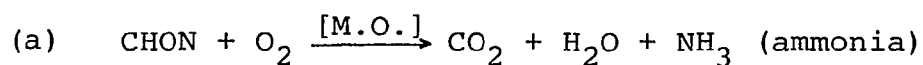
The initial objective to this research effort was to confirm that there is change in the BOD profile as a result of pressurization based on the studies and findings of prior investigators. The scope of study then proceeded along two paths; the first was to reproduce some of the earlier investigations to obtain quantitative data to support qualitative statements in prior investigations, 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:

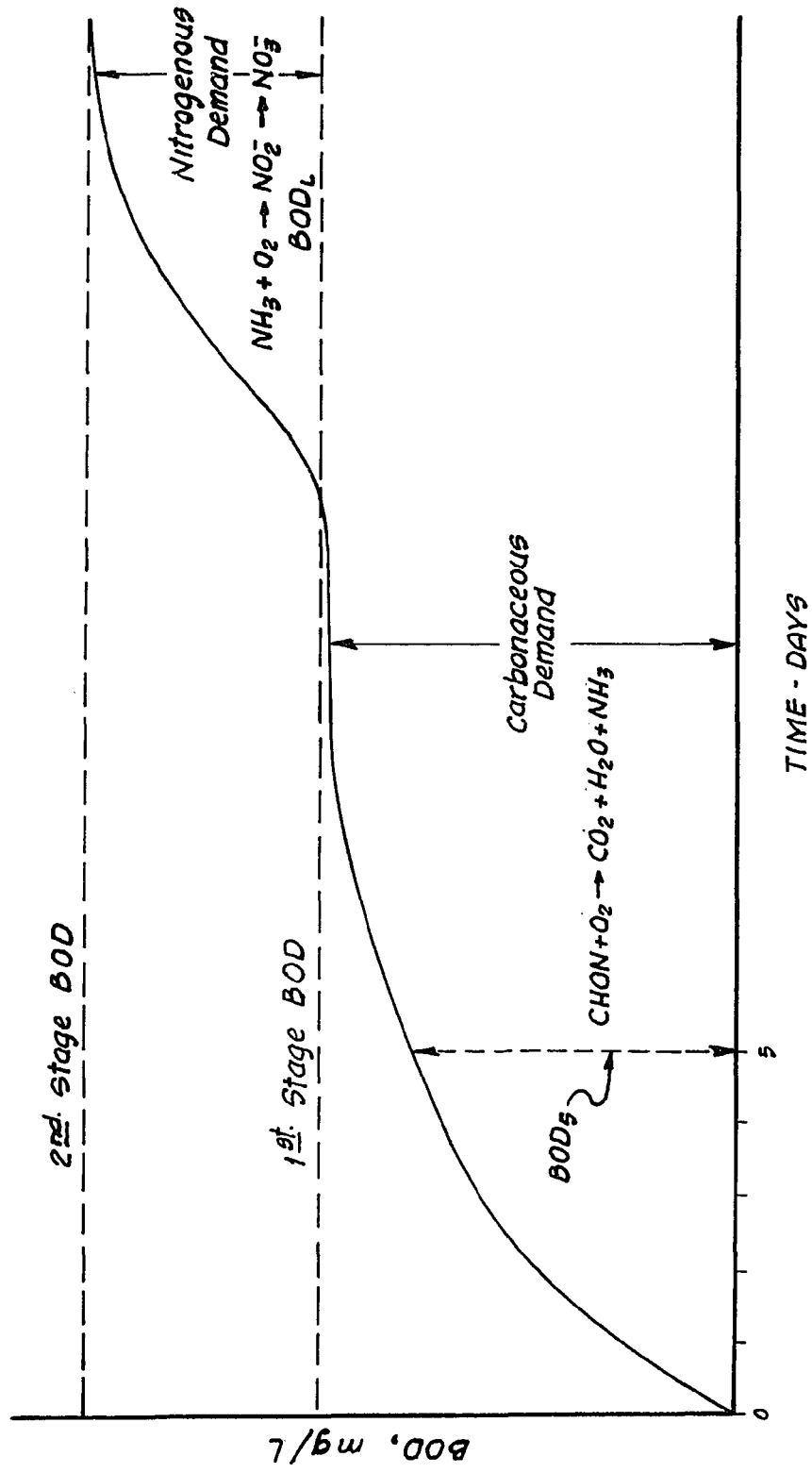


- Where:
1. CHON represents organic compounds containing carbon, hydrogen, oxygen, and nitrogen.
 2. [M.O.], [M.O.₁], [M.O.₂] are concentrations of the species of microorganisms necessary for the reactions to proceed.

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:

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

where the derivative is negative to show a decreasing amount.

C = concentration
 k_1 = first order specific rate constant.

After rearranging terms and integrating expression (1) becomes:

$$(2) \quad \ln C/C_0 = k_1 t \text{ or}$$

$$(2A) \quad C/C_0 = 10^{-k_1 t}$$

Expression (2) and (2A) is used to determine any concentration, C , at any time, t , based on a starting concentration of C_0 , 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_0 . Replacing C with L_r to represent the concentration of organics remaining, expression (2A) can be rewritten as:

$$(3) \quad L_r/L_0 = 10^{-k_1 t} \quad \text{Rearranging (3) gives} \\ (4) \quad \text{in terms of } L_r.$$

$$(4) \quad y = L_0 (1 - 10^{-k_1 t})$$

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:

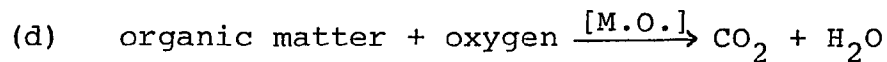
$$(5) \quad \text{rate} = k_1 [\text{concentration}]^1$$

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:



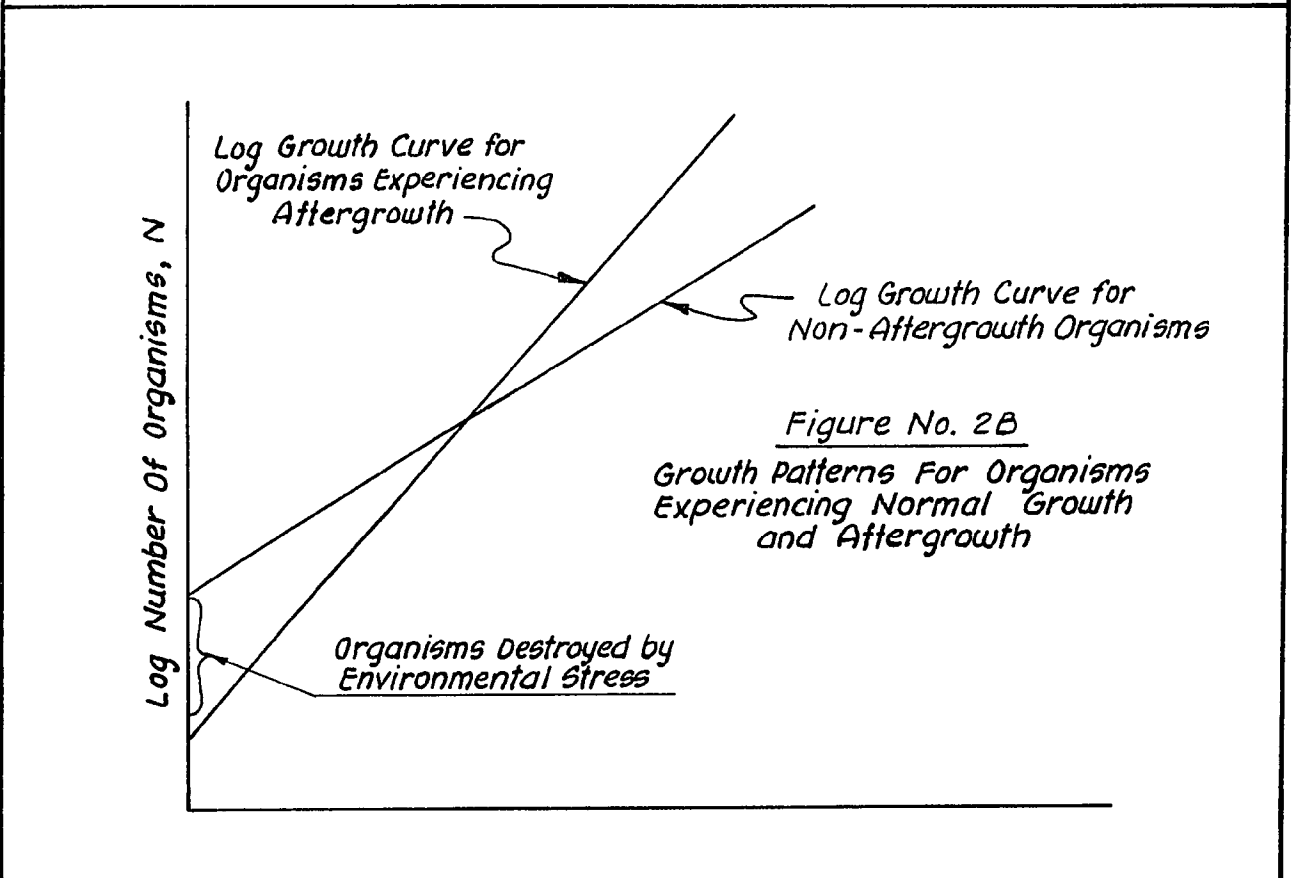
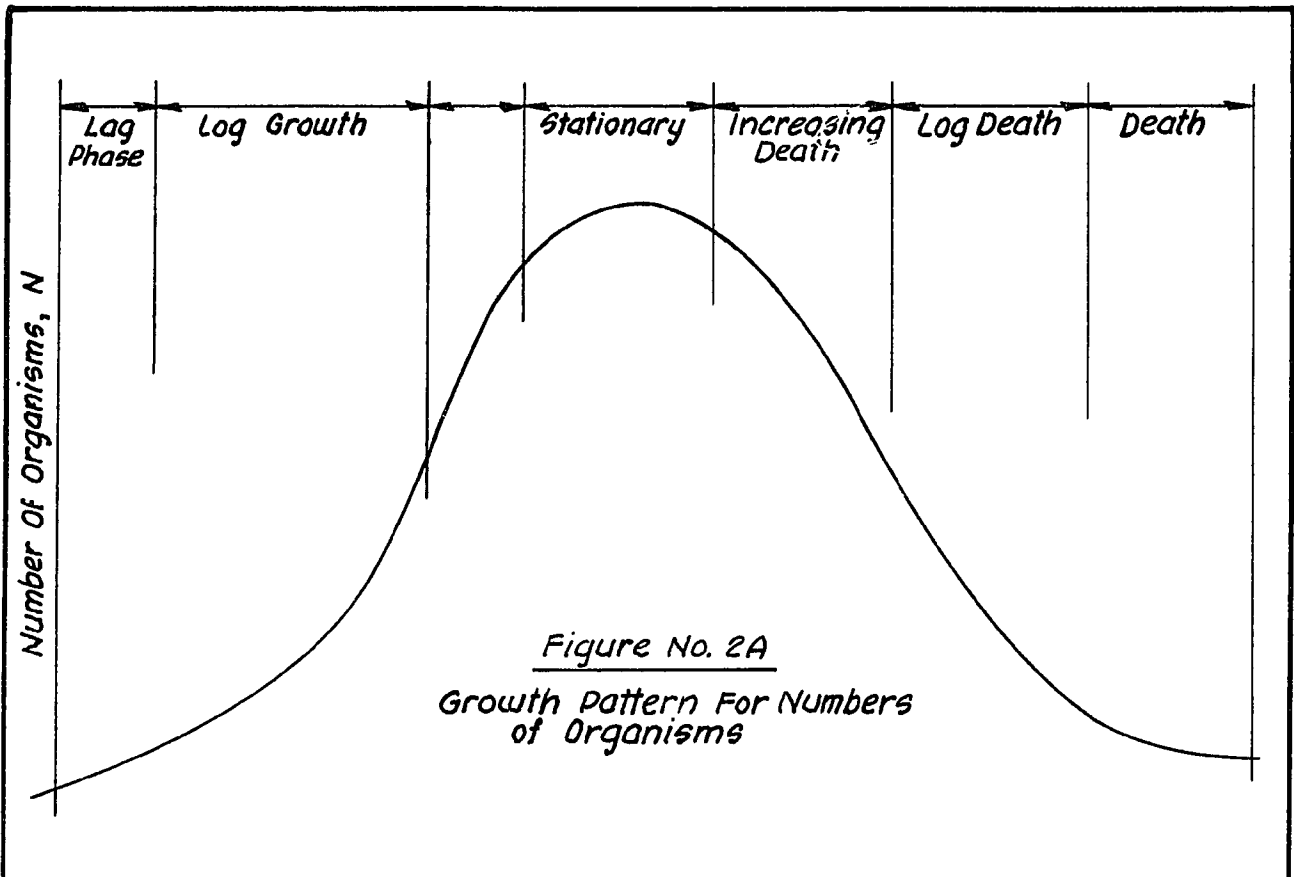
where the microorganisms serve as the catalyst in the reaction. The oxygen in the reaction serves the needs of the aerobic microorganisms. In kinetic form, this can be expressed as:

$$(7) \quad \text{rate} = k_1 [\text{organic matter}]^1 [\text{O}_2]^0 [\text{MO}]^x$$

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



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 concentration 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 microorganisms. Recognizing the same, the experiments that

Figure No. 3A
 Variations In First Stage BOD
 As A Result Of An Environmental
 Stress

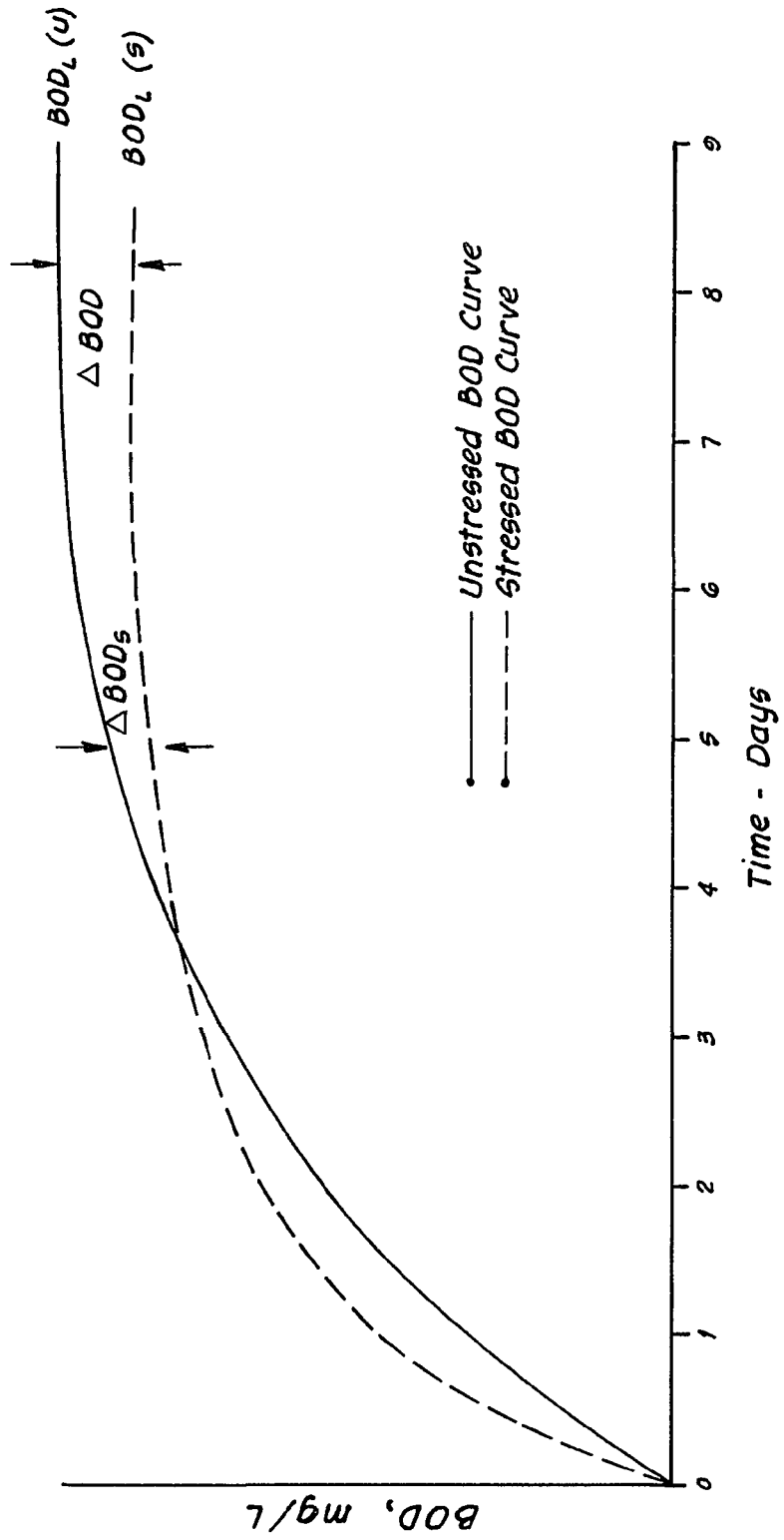


Figure No. 3B
One Type Of First Stage BOD
Reduction As A Result Of An
Environmental Stress

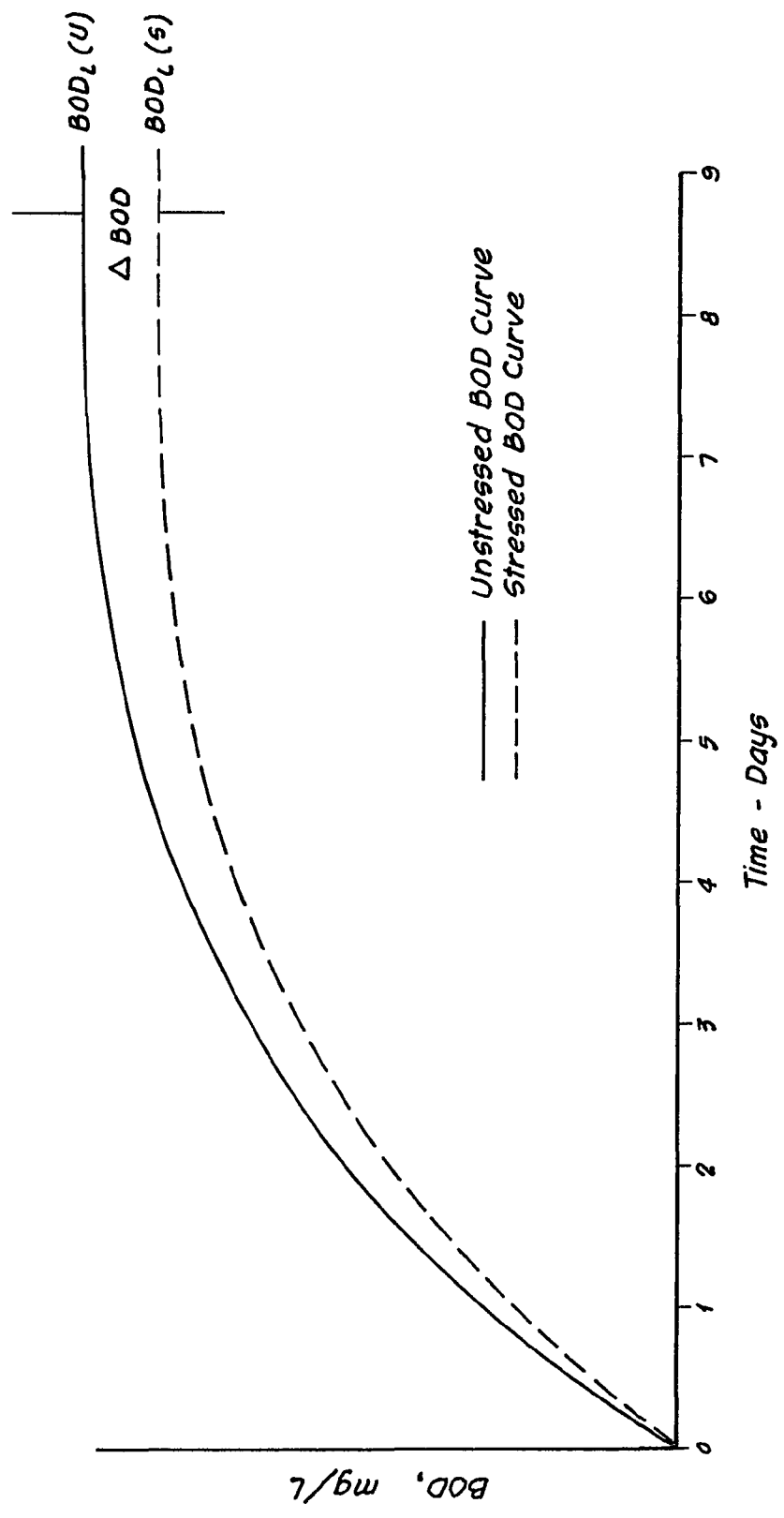
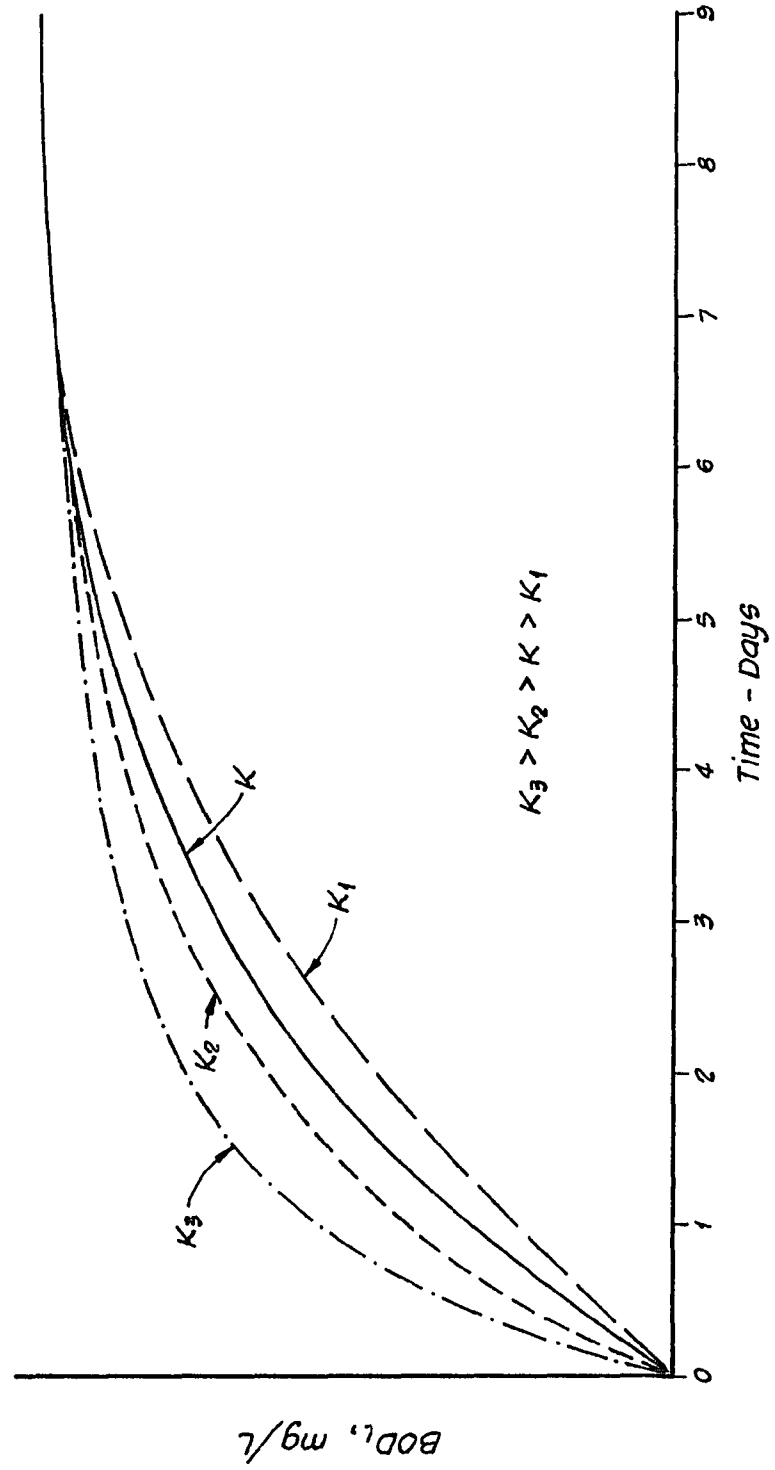


Figure No. 3C
Effect On The BOD Curve For Different Materials Oxidizing
At Different Rates (L Values Are The Same)



follow will take these variables into account.

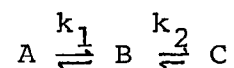
III. LITERATURE REVIEW

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 of an organic reaction (19). The equilibrium under study was of the type;



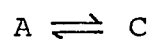
Where A = one isomer of an organic compound

C = another isomer of the organic compound

B = intermediate transition compound

k = forward and reverse specific rate constants

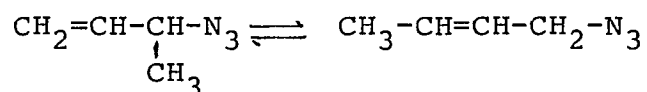
Overall, the reaction can be considered as being of the type:



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:



β -methylallyl azide

α -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

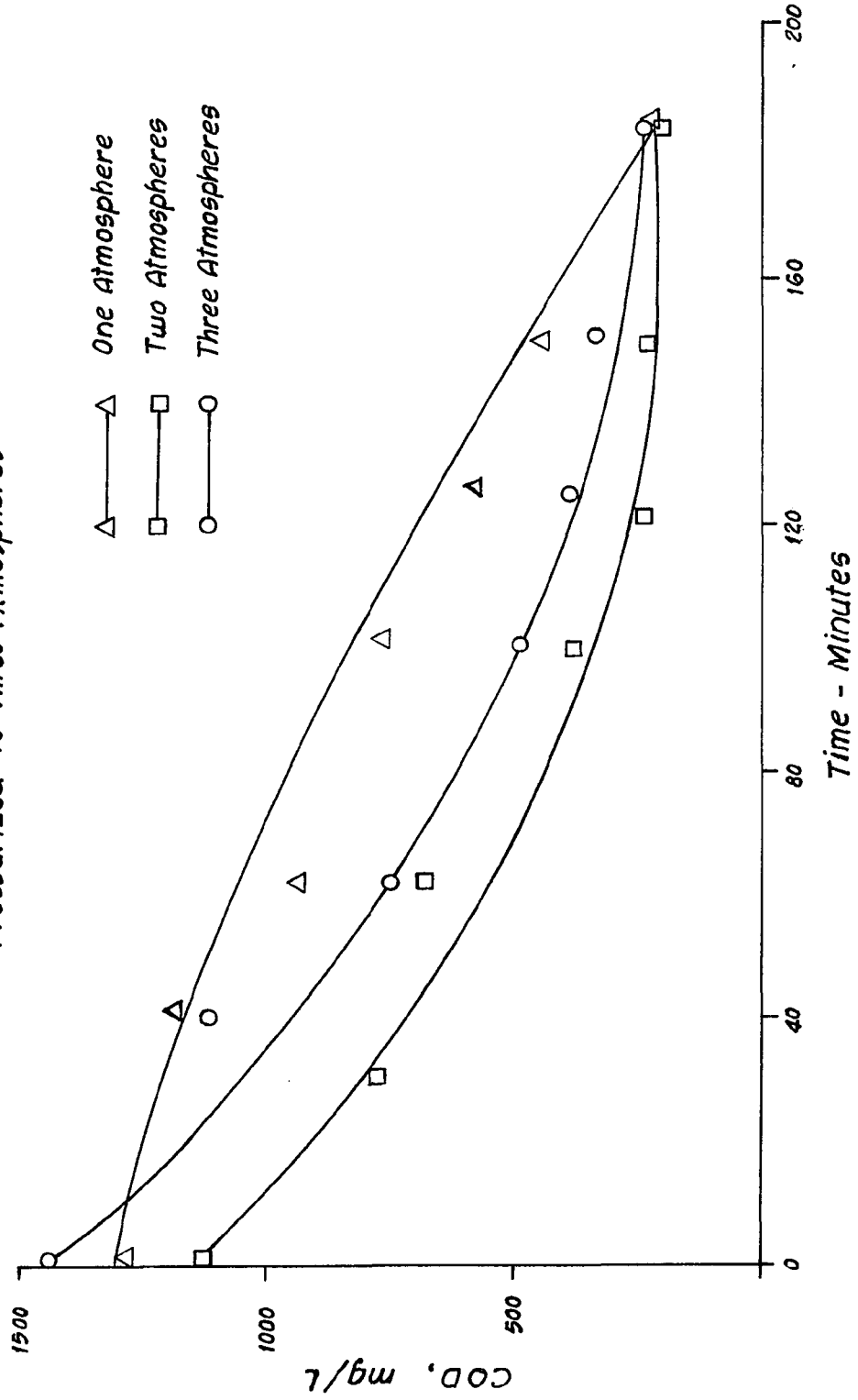
The first significant discovery dealing with the effects of pressure on the degradation of organic material was the report of Jannach (19). In 1968 the Woods Hole Oceanographic Institute's research submarine ALVIN sank to a depth of 5,000 feet (2165 psig) off the coast of Woods Hole, Massachusetts. After 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.

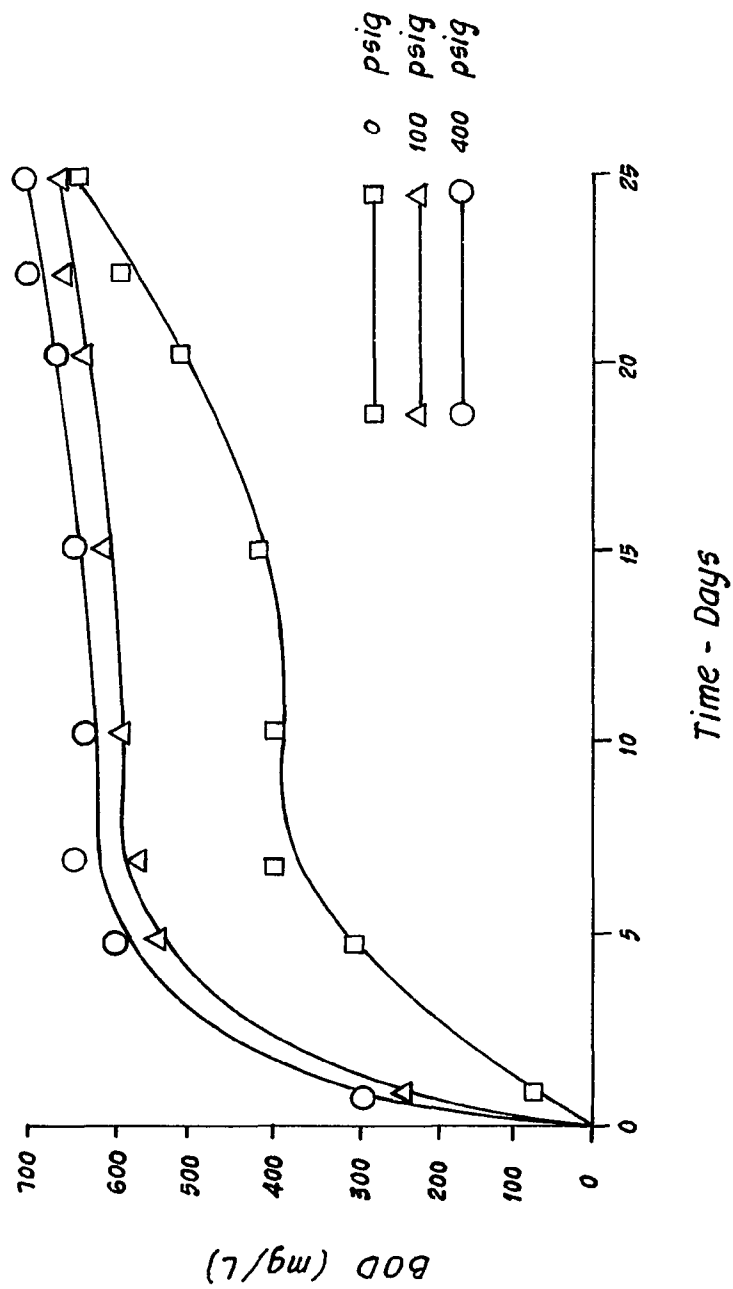
No mention was made or shown of a non-pressurized

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



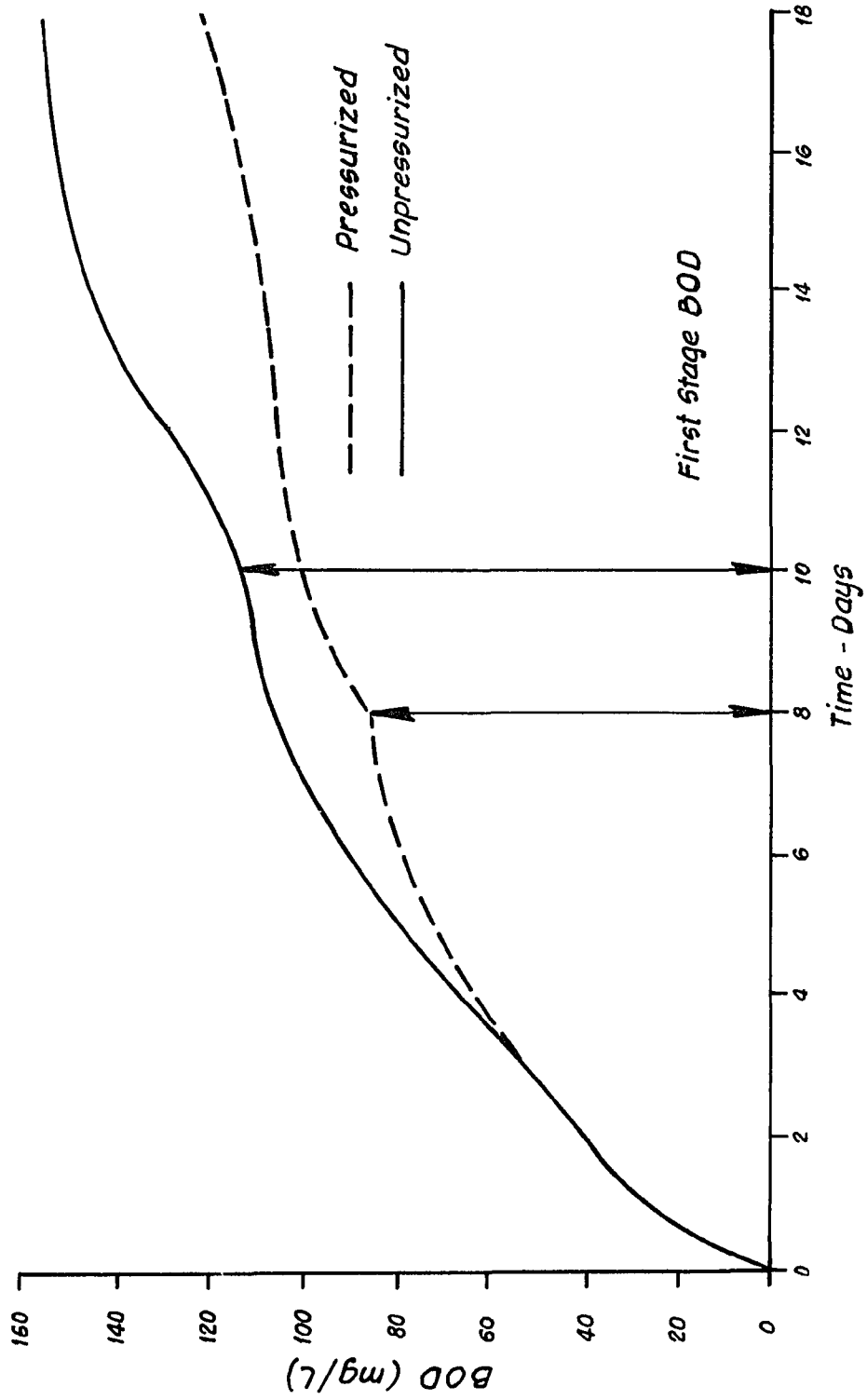
Another aspect of Chack's work involved enumerating the coliform density after pressure was applied using the Most-Probable-Number (MPN) method. His results are semi-quantitative at best, but the indication is that there is a decrease in population with increasing pressure.

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



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

<u>NAME</u>	<u>PRESSURE</u>	<u>ANALYSIS</u>	<u>DETENTION TIME</u>	<u>SAMPLE</u>	<u>REDUCTION</u>	<u>REMARKS</u>	
LAWRENCE	14.7 psig	COD	30 min.	diluted	-	increased pressure	
	29.4 psig	COD	30 min.	sludge	56%	decreased COD reduc-	
	44.1 psig	COD	30 min.		37%	tion. 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 di- luted in nutrient water	BOD ₅ dou- bled 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		12%	2nd stage BOD occurred 3 days earlier.
	100 psig	BOD	30 min.	sewage		16%	Higher kinetic rates. ³
100 psig	BOD	60 min.	sewage		19%	Amt. of air above liquid surface no factor. ²	
14.7 psig	BOD	60 min.	sewage		21.3%		

TABLE NO. 1 (Continued)
SUMMARY OF PRESSURE INVESTIGATIONS

<u>NAME</u>	<u>PRESSURE</u>	<u>ANALYSIS</u>	<u>DETENTION TIME</u>	<u>SAMPLE</u>	<u>REDUCTION</u>	<u>REMARKS</u>
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

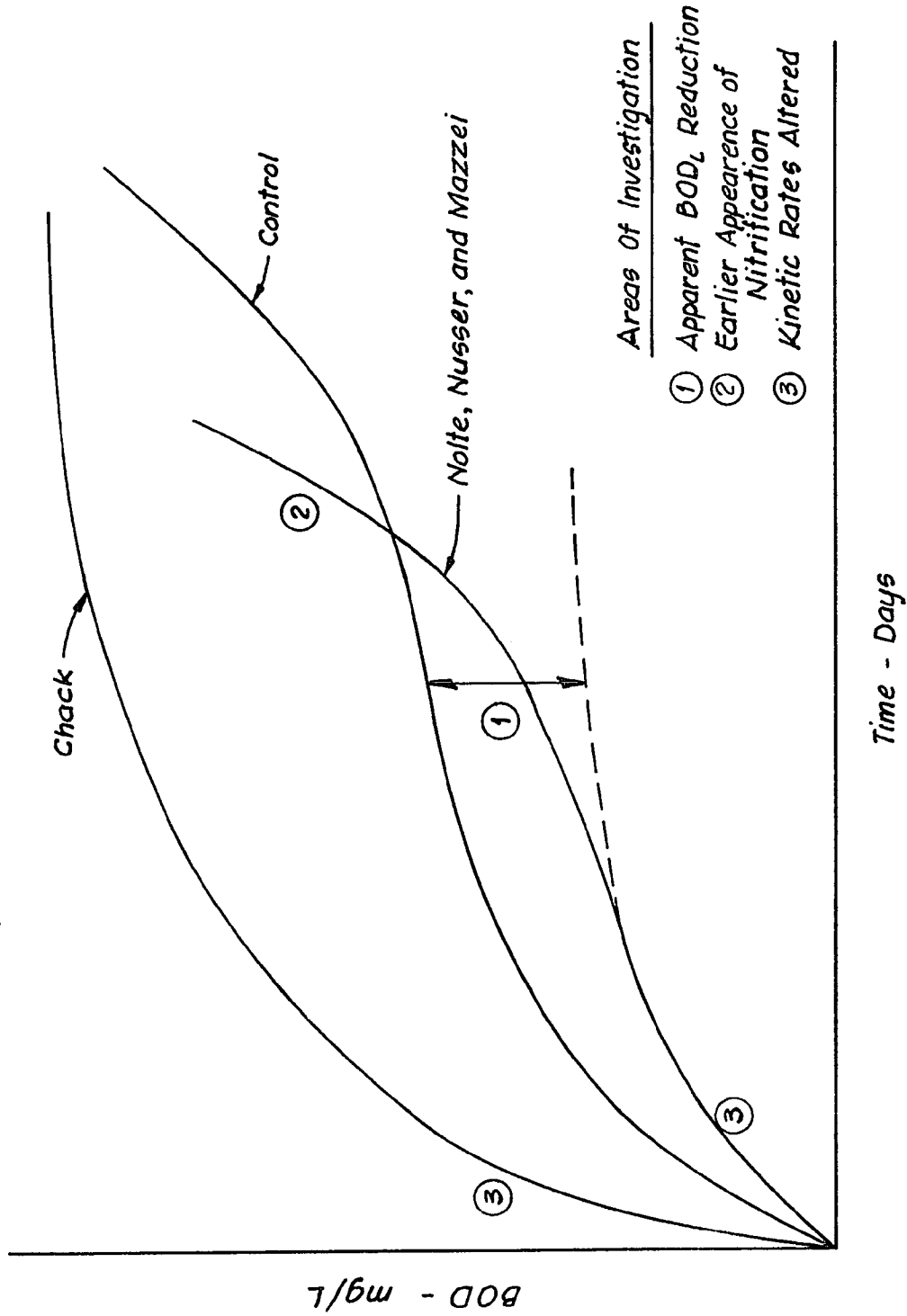
the same time the rate of decomposition increased. The BOD can be summarized in Figure No. 7, which compares the generalized BOD curves for the principle researchers.

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



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 microorganisms present in the seeded dilution water?
3. Did the wastewater sample simply oxidize naturally within the detention time period in the pressurization 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 circumference. 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

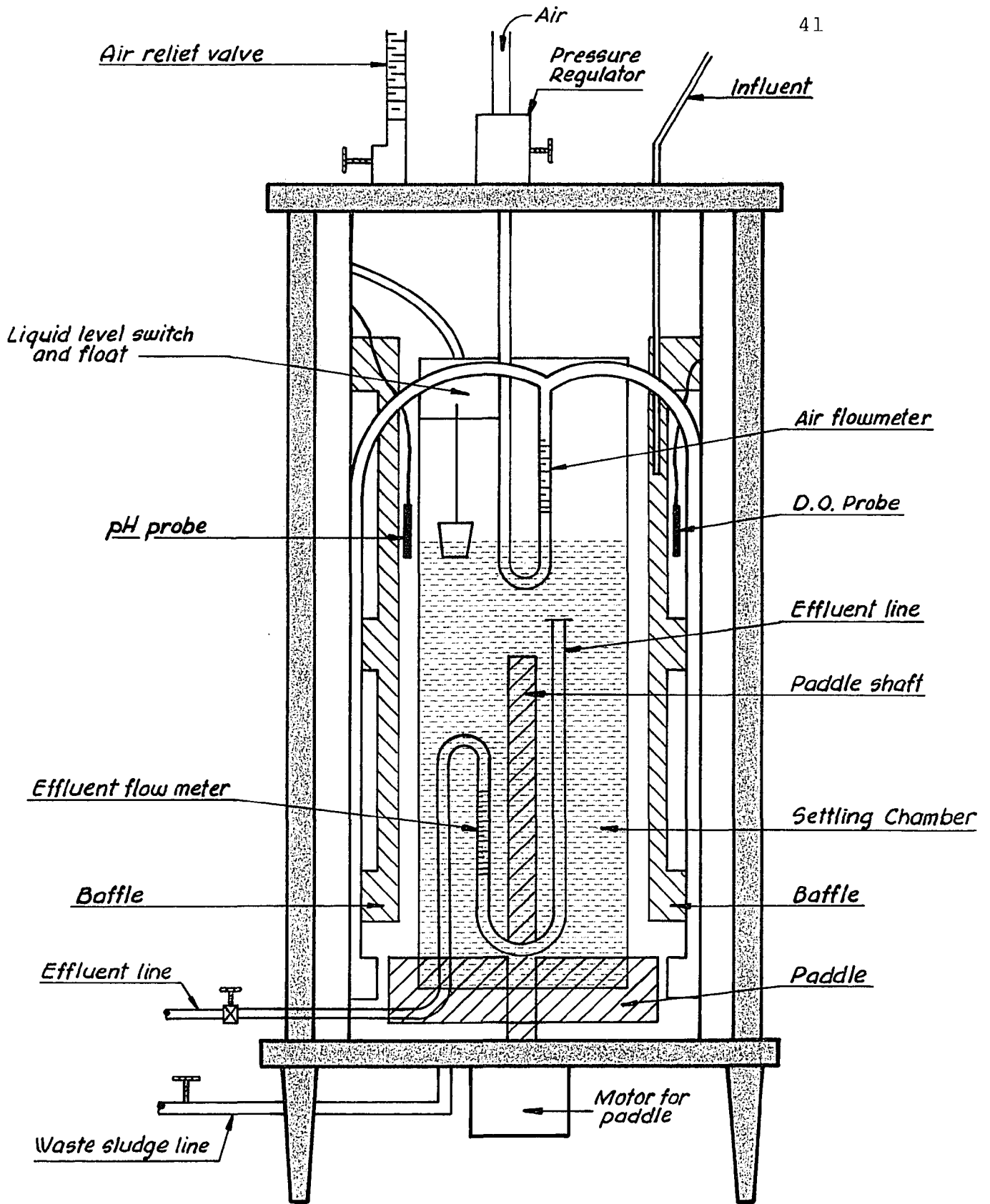


Figure No. 8

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

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. Occasionally this would occur if the DO probe required a new diaphragm, 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 deviation with the blank is 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 probe for an extended time cannot be over emphasized. For an excellent 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 determinations (31). This eliminates the need for the lengthy reflux period as required in Standard Methods COD procedure (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 theoretical COD of the compound. The results are in excellent 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 thermostatically 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

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 BOD₁ AND COD VALUES FOR
 AERATED AND PRESSURIZED WASTEWATER SAMPLES

PRESSURE TIME: HOURS	(1)			(2)			(3)	
	AERATED			PRESSURIZED			CHANGES IN	
	BOD	COD	k ₁	BOD	COD	k ₁	Δ BOD	Δ COD
	mg/l	mg/l	l/day	mg/l	mg/l	l/day	mg/l	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 k₁ after increased periods of aeration for the aerated, non-pressurized sample.

(2) Indicates the changes in the BOD, COD, and k₁ after increased periods of pressurization for pressurized samples.

TABLE 2 (Continued)
VARIATION OF BOD_t 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 biochemical 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	UNSEDED DILUTION WATER
0	TNTC	10^6-10^8	100	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 diffuser 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):



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 microorganisms, 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 diffuser; (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.

Figure No. 9
Composite BOD Curves Including Pressurized Detention Time

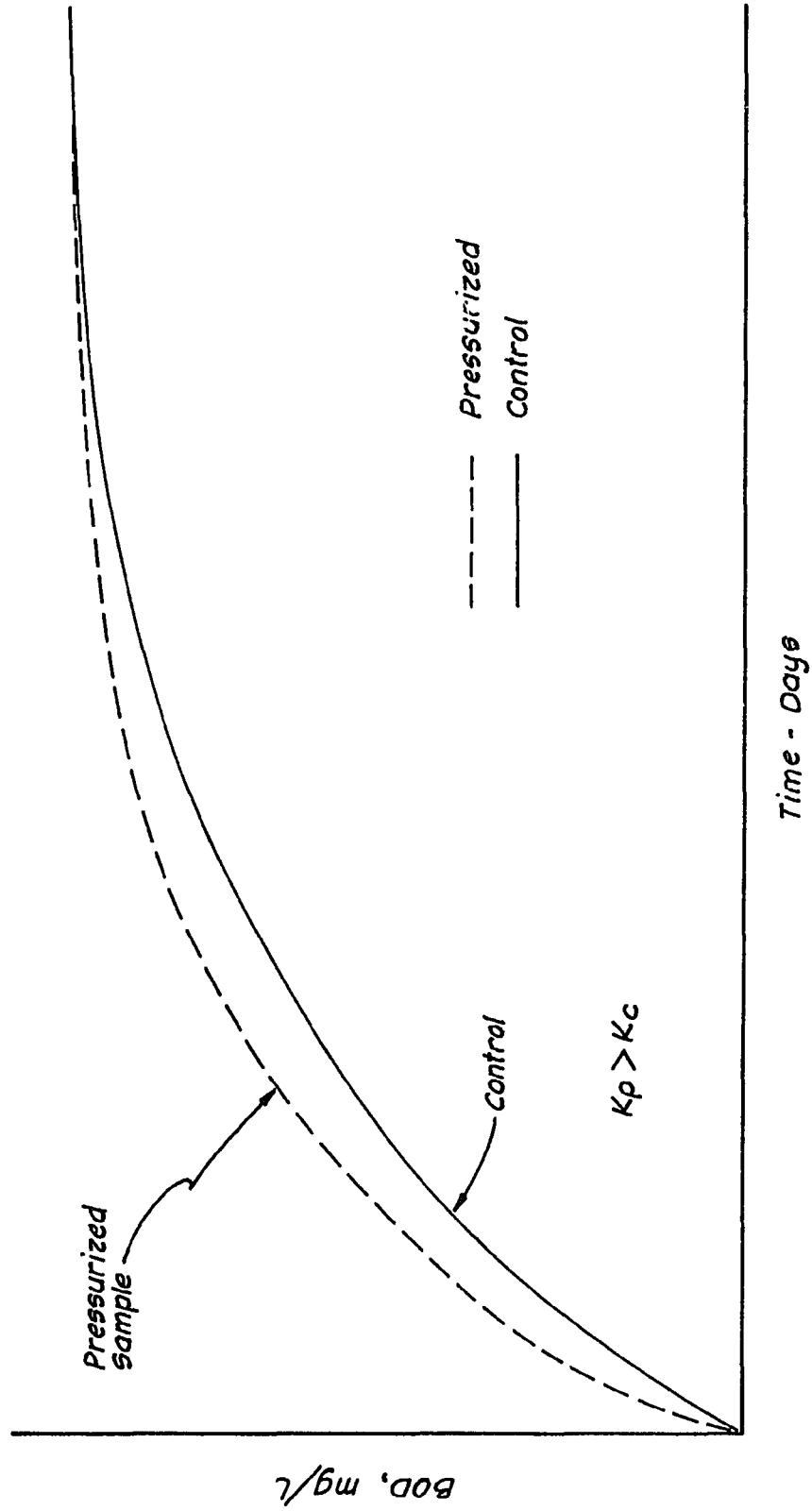


TABLE No. 4
BOD AND RATE CONSTANT VALUES FOR SAMPLES
WHICH WERE QUIESCENT, AERATED, AND PRESSURIZED

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_L s 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	△
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

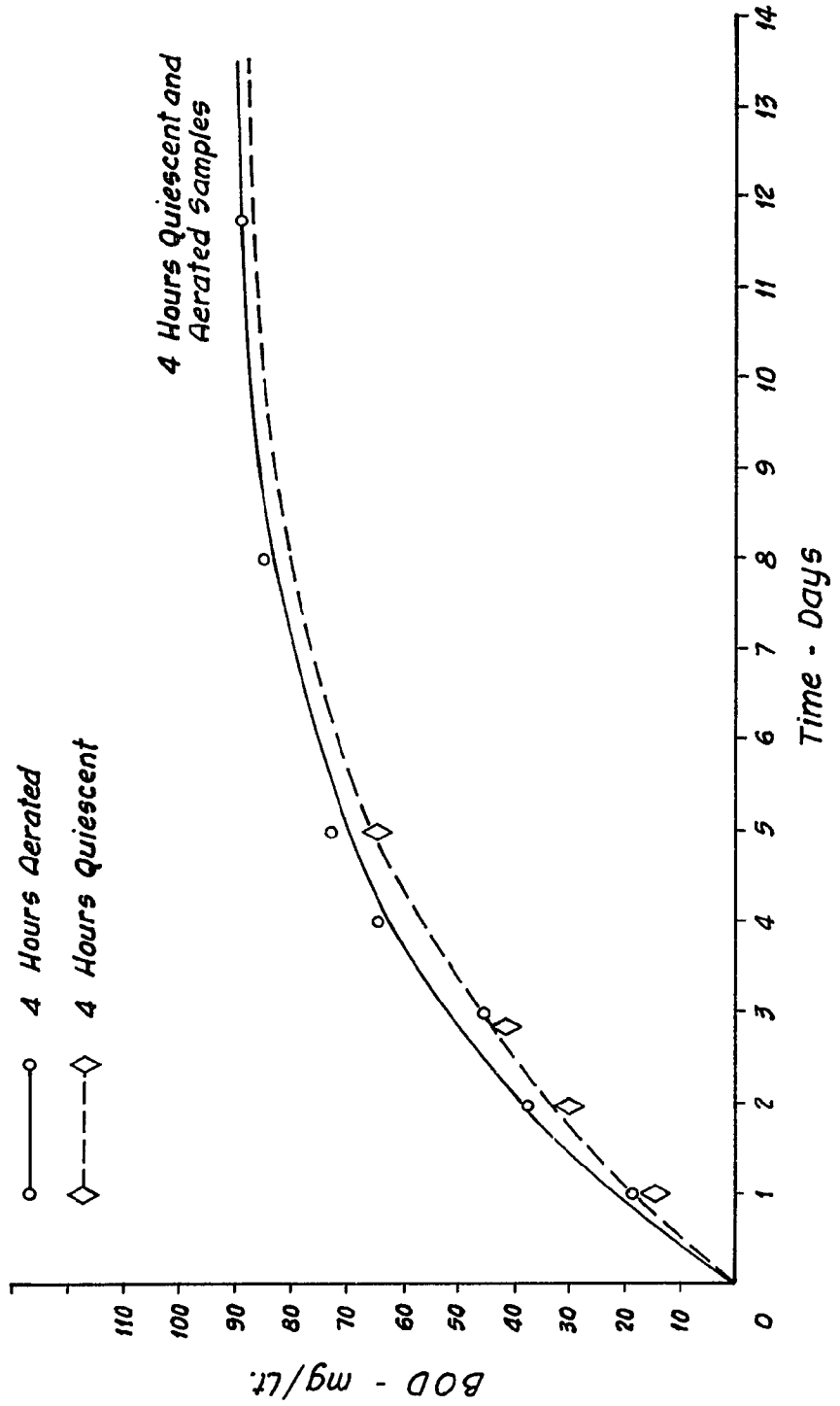
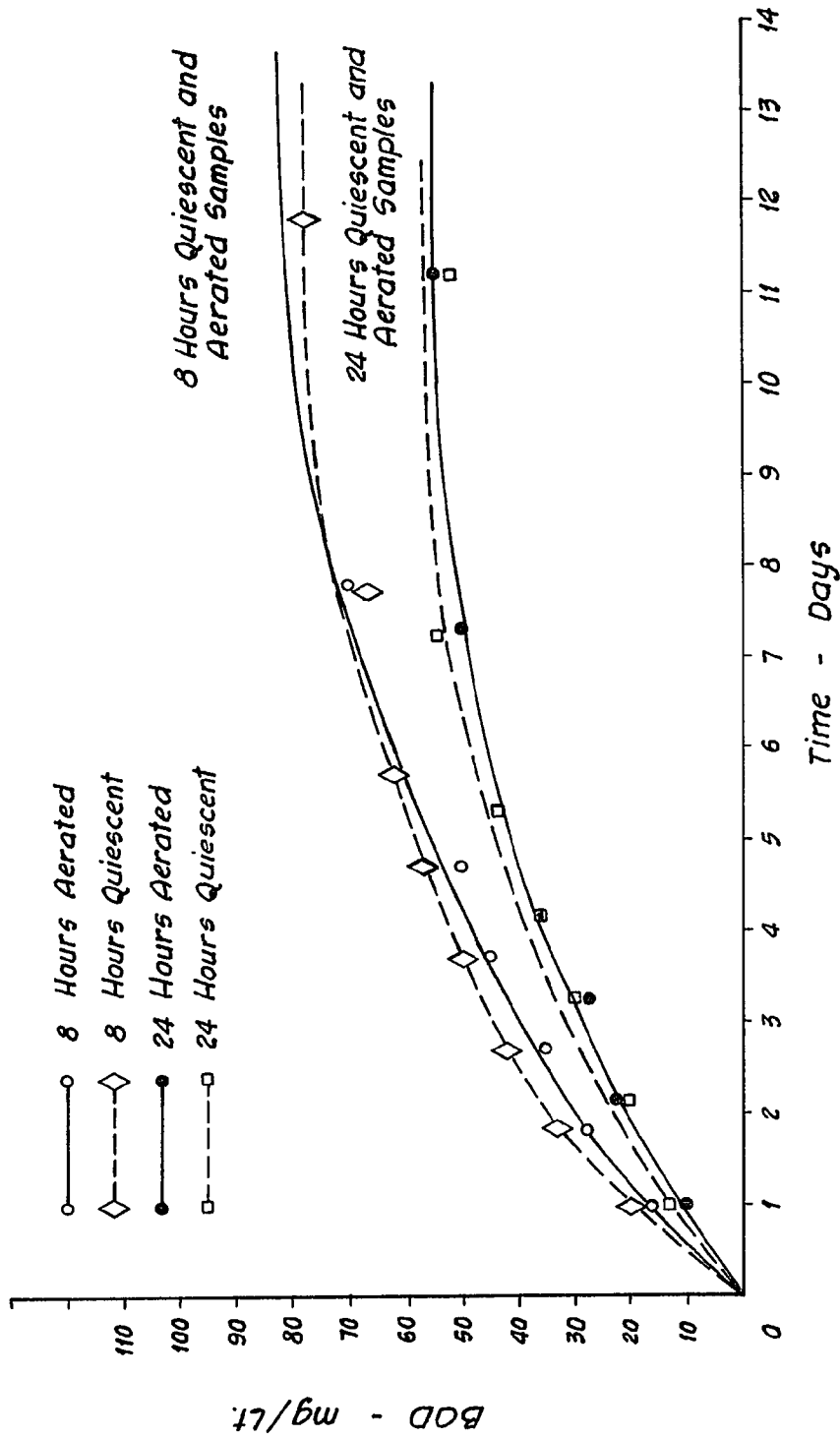


Figure No. 11
 Variation Of BOD For Quiescent And Aerated Wastewater Samples For 8 and 24 Hours



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:

$$(8) \quad \text{rate} = k [\text{CHO}]^1 [\text{DO}]^0$$

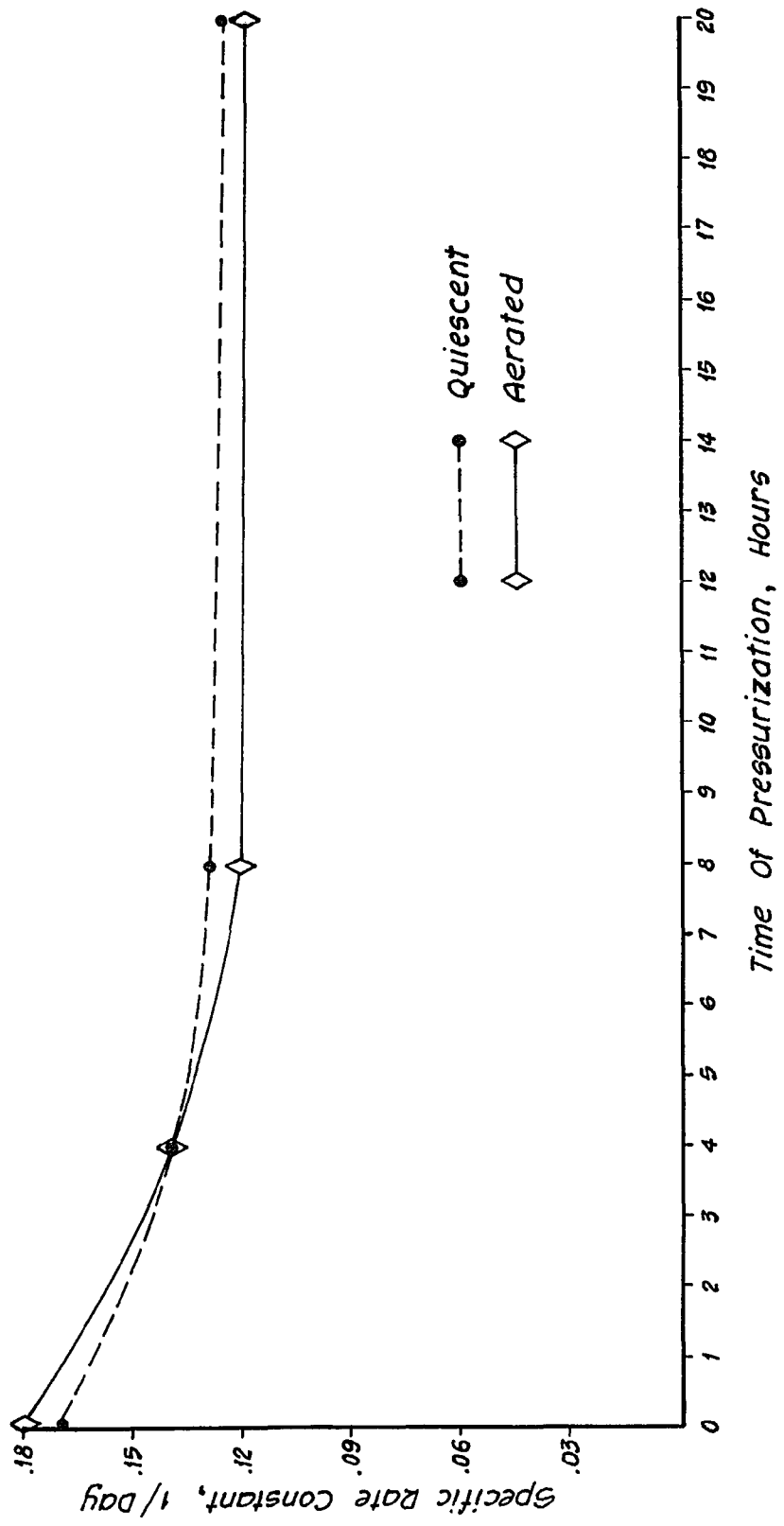
$$(9) \quad \text{rate} = k [\text{CHO}]^1$$

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 decomposition 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 contributing 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 relationship, 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 responsible 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.

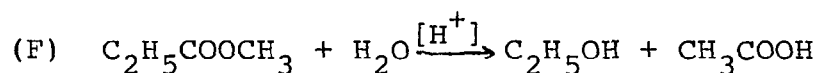
As the sample stood after pressurization, the rate of BOD exertion and the rate constant both increased up to a maximum value. See Appendix I. As the BOD is exerted faster for days one to three, the BOD curves cross over at days 5 and 7, and each succeeding day there is less BOD_L . This difference is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

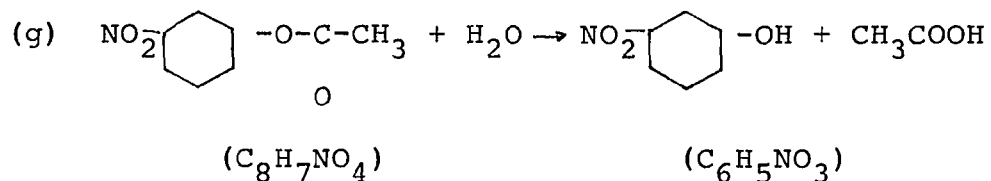
The final mechanism to consider was that the material being pressurized was subject to molecular changes due

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:

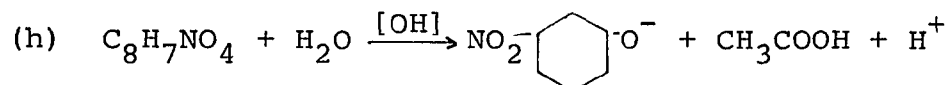


In an attempt to demonstrate that pressure could increase the rate of hydrolysis, it was decided to study a known hydrolysis reaction which was easy to monitor and measure. Pressurized and non-pressurized samples would be run and any changes in the hydrolysis rates would be due to the pressure effect.

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:



The para-nitro-phenol produced is an intense yellow color whose absorbance can be measured spectrophotometrically 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:



The para-nitro-phenoxy formed has a maximum absorbance 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

Figure No. 13

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

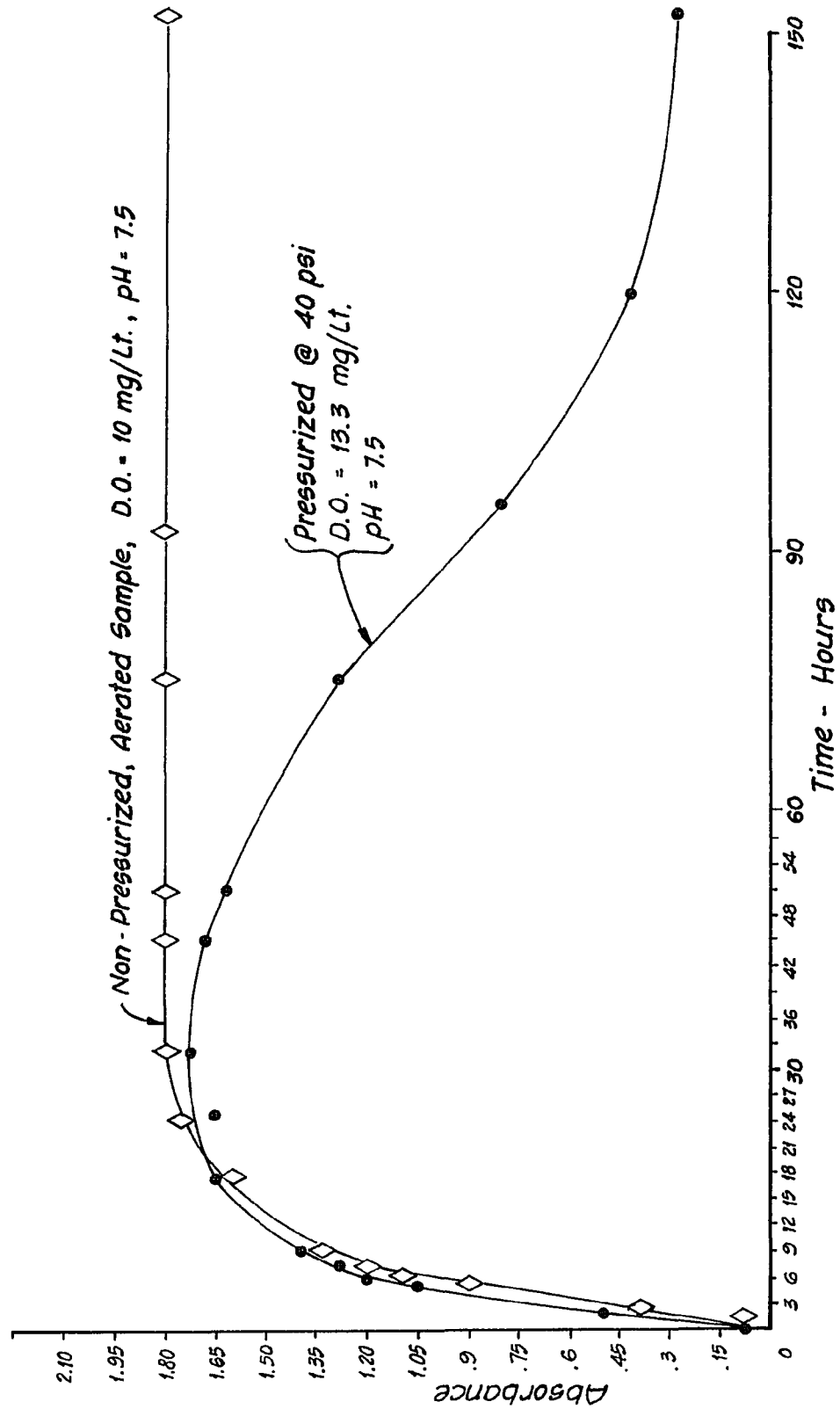
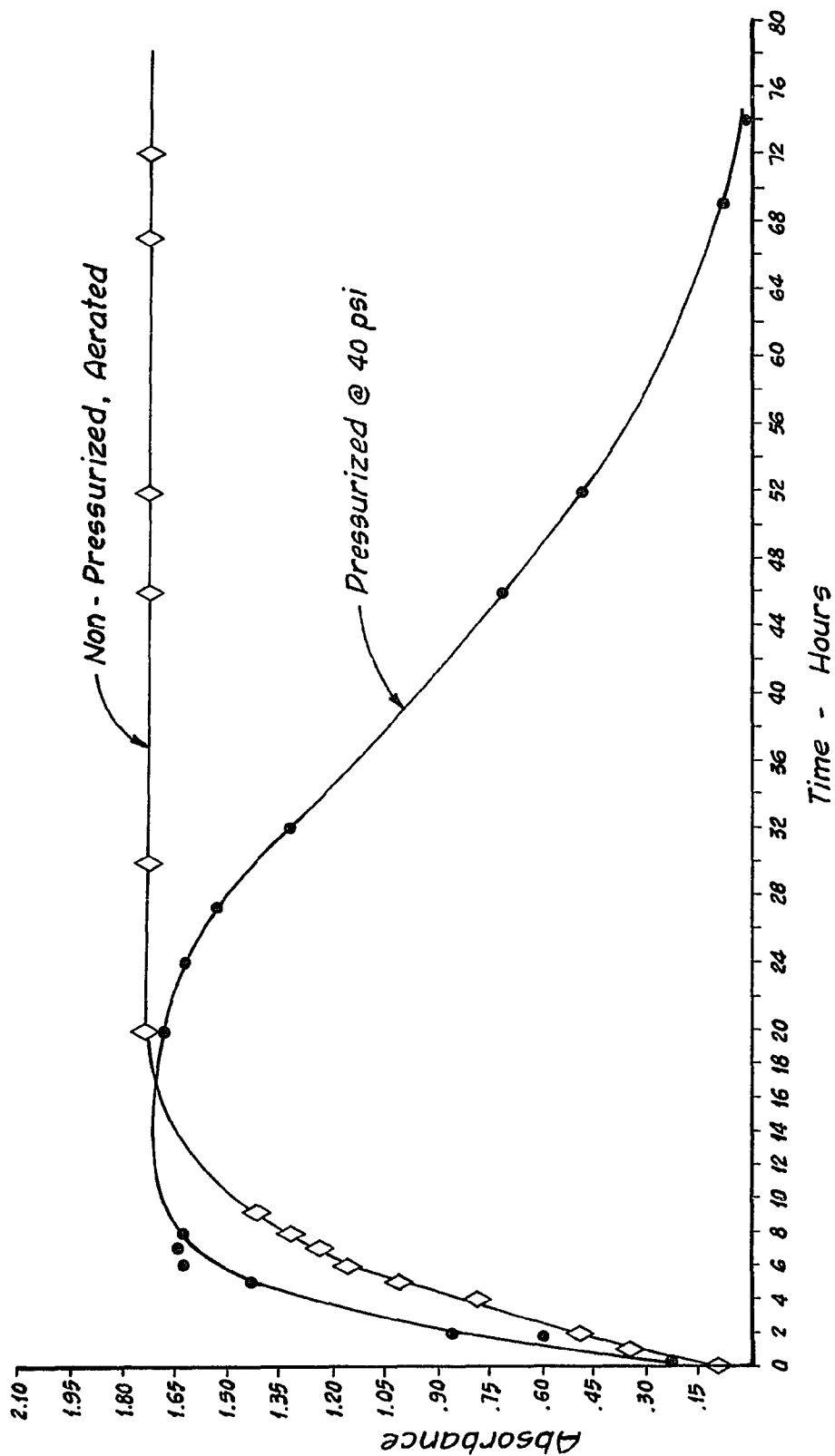


Figure No. 14
*The Effects Of Pressure On Para - Nitro Phenyl Acetate
As Compared To An Aerated Sample Conducted @ pH 7.7*



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

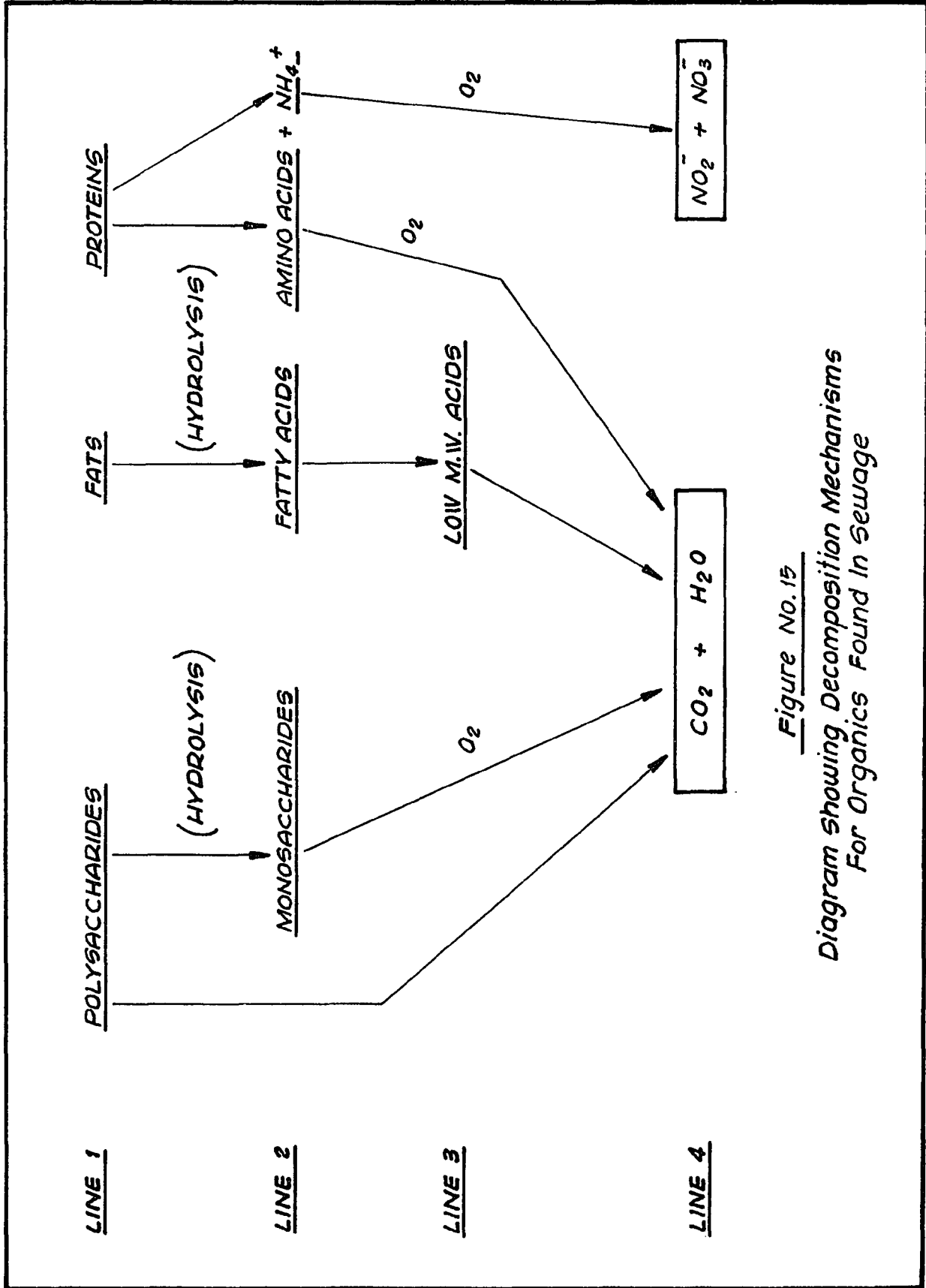


Figure No. 15
*Diagram showing Decomposition Mechanisms
For Organics Found In Sewage*

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 nitrifiers, and the second without nitrifiers. Two sets of trials were run for the 24 hour pressure detention time and the 24 hour aerated detention time: one with nitrifiers 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 nitrifiable ammonia and the BOD and NOD values were known. The sample contained the following ingredients:

Acetic Acid	CH_3COOH
Starch	$\text{C}_6\text{H}_{10}\text{O}_5$
Glutamic Acid	$\text{C}_5\text{H}_9\text{O}_4\text{N}$
Urea	CON_2H_4
Glucose	$\text{C}_6\text{H}_{12}\text{O}_6$
Histidine	$\text{C}_6\text{H}_9\text{N}_3\text{O}_2$

The theoretical BOD was 8,000 mg/l and the NOD was 6,500 mg/l. A 1/50 dilution was prepared and amyloglucosidase 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

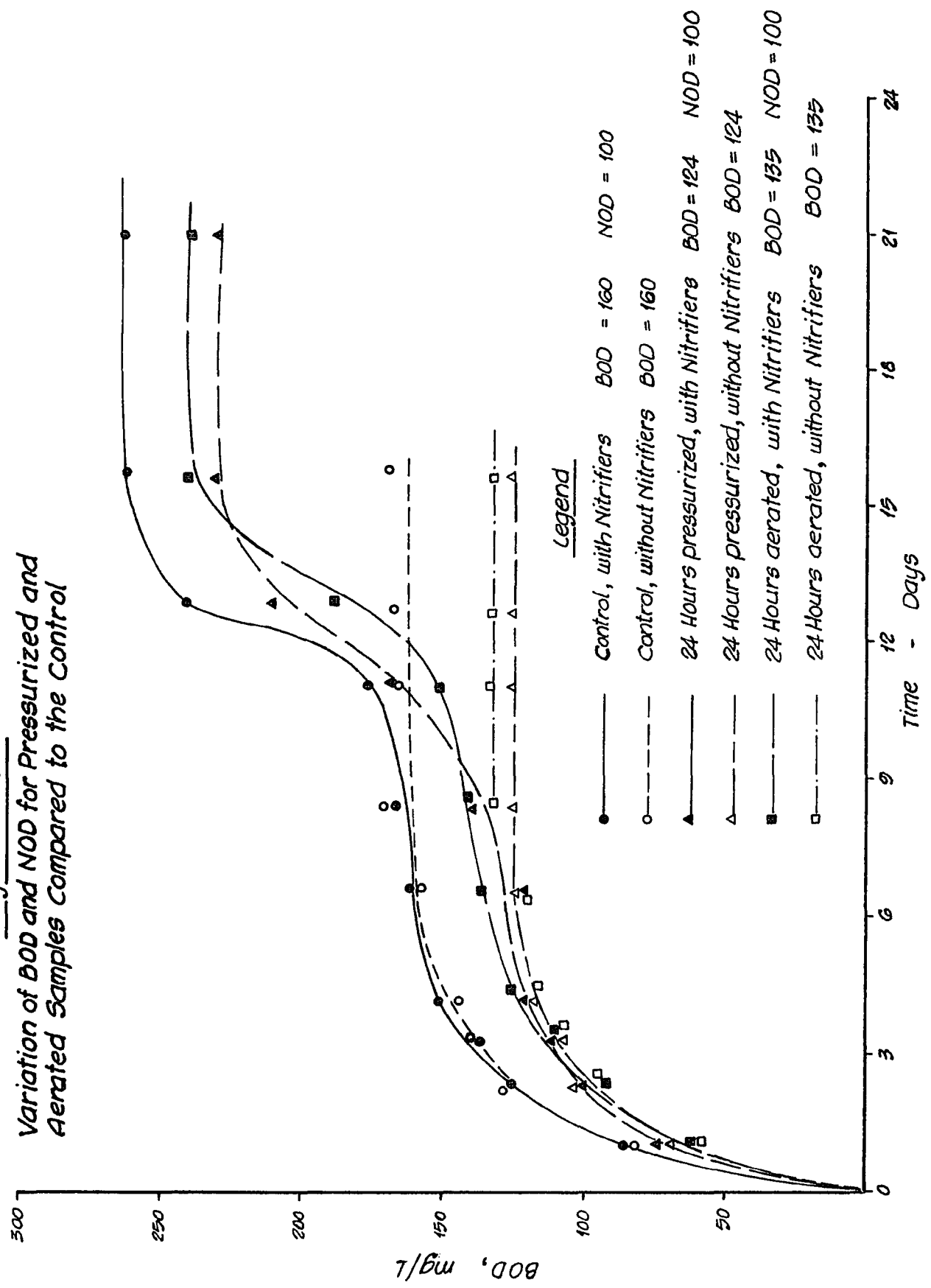


TABLE NO. 5

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 nitrified 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.

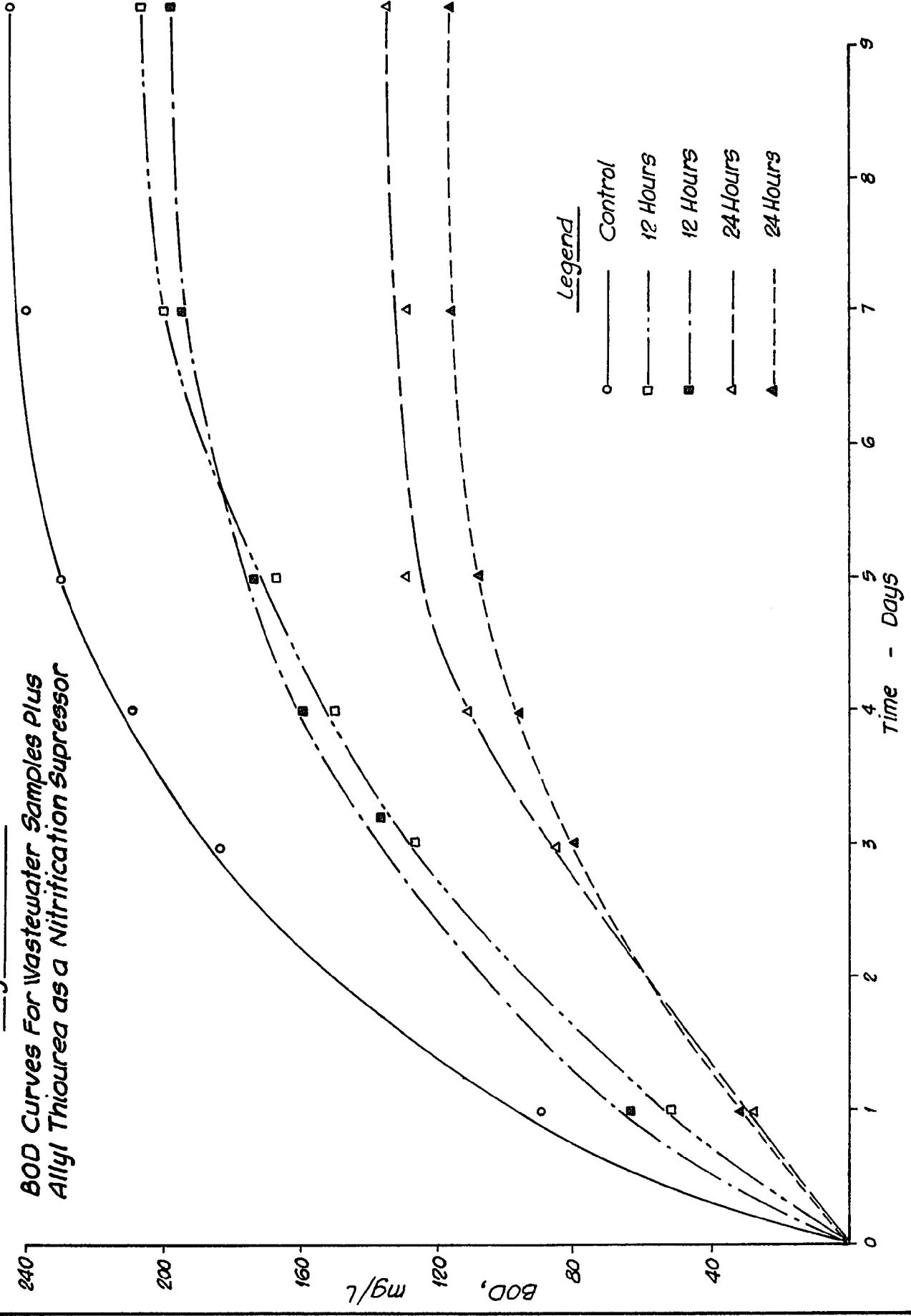
5. The NOD was approximately 120 mg/l and this was the same for both the aerated, pressurized, and the control, meaning there was no loss in the nitrification potential of the sample and the control as a result of pressurization.

The third investigation involved adding a nitrification suppressor, 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 Suppressor



Legend

- Control
- 12 Hours
- 12 Hours
- 24 Hours
- 24 Hours

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:

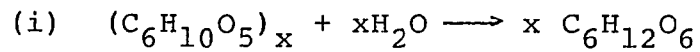
- 1) Detention time in the pressure vessel.
- 2) The concentration of microorganisms as effected by pressure.
- 3) Chemical oxidation.

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.

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).



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 monosaccharide 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.

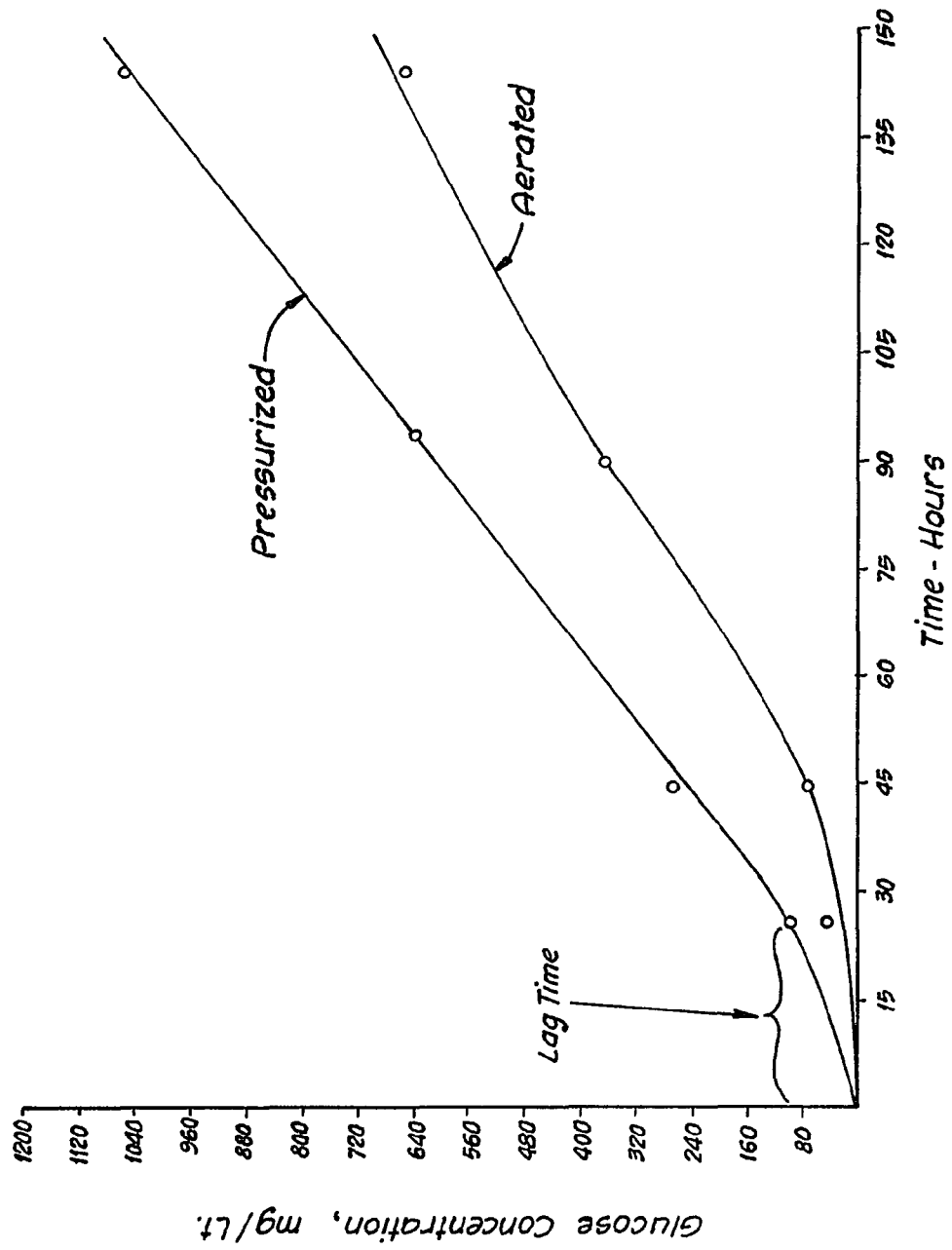
Amylglucosidase enzyme was then selected and obtained and this enzyme is able to hydrolyze starch only to glucose. Two starch solutions of pH 7.0 and 4.5 were prepared and the amyglucosidase was added and the experiment proceeded. The results indicated that the hydrolysis was successful and proceeded 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.

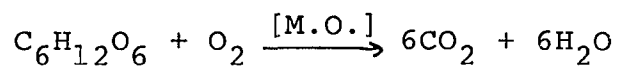
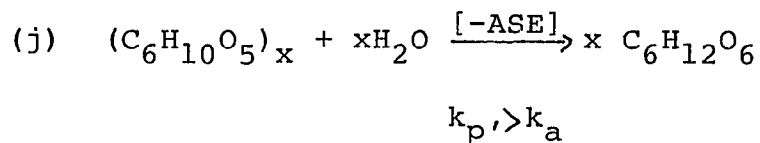
A 500 mg/l starch solution plus 2.4 ml of amyl-glucosidase was prepared and divided into two parts. One was pressurized at 40 psig and the other aerated at atmospheric pressure. Glucose readings were obtained from the calibration curve and these results are shown in Figure 18. Apparently, an initial 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):

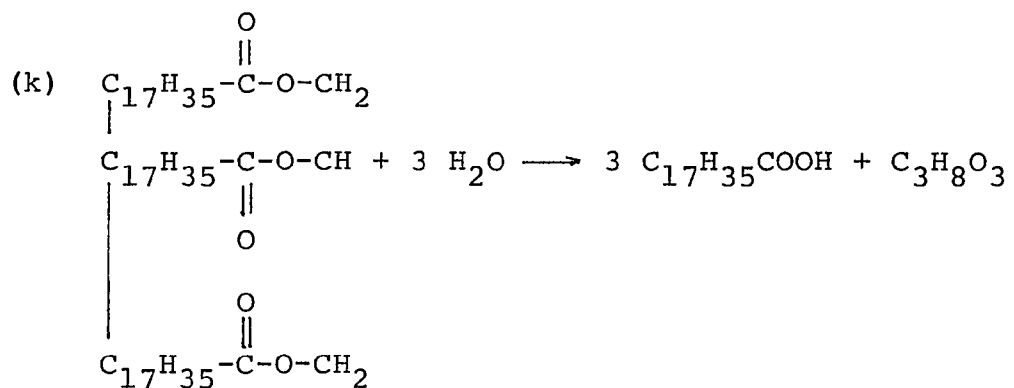
Figure No. 18
The Rate Of Hydrolysis Of Starch Catalyzed By Amylglucosidase
Enzyme For Pressurized And Non - Pressurized Samples





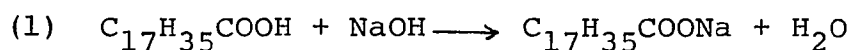
FATS

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:



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/tetrahydrofuran 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).



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 pressurization at 40 psig and the other sample aerated at atmospheric 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 0.009m HCl
At The Start Of The Experiment*

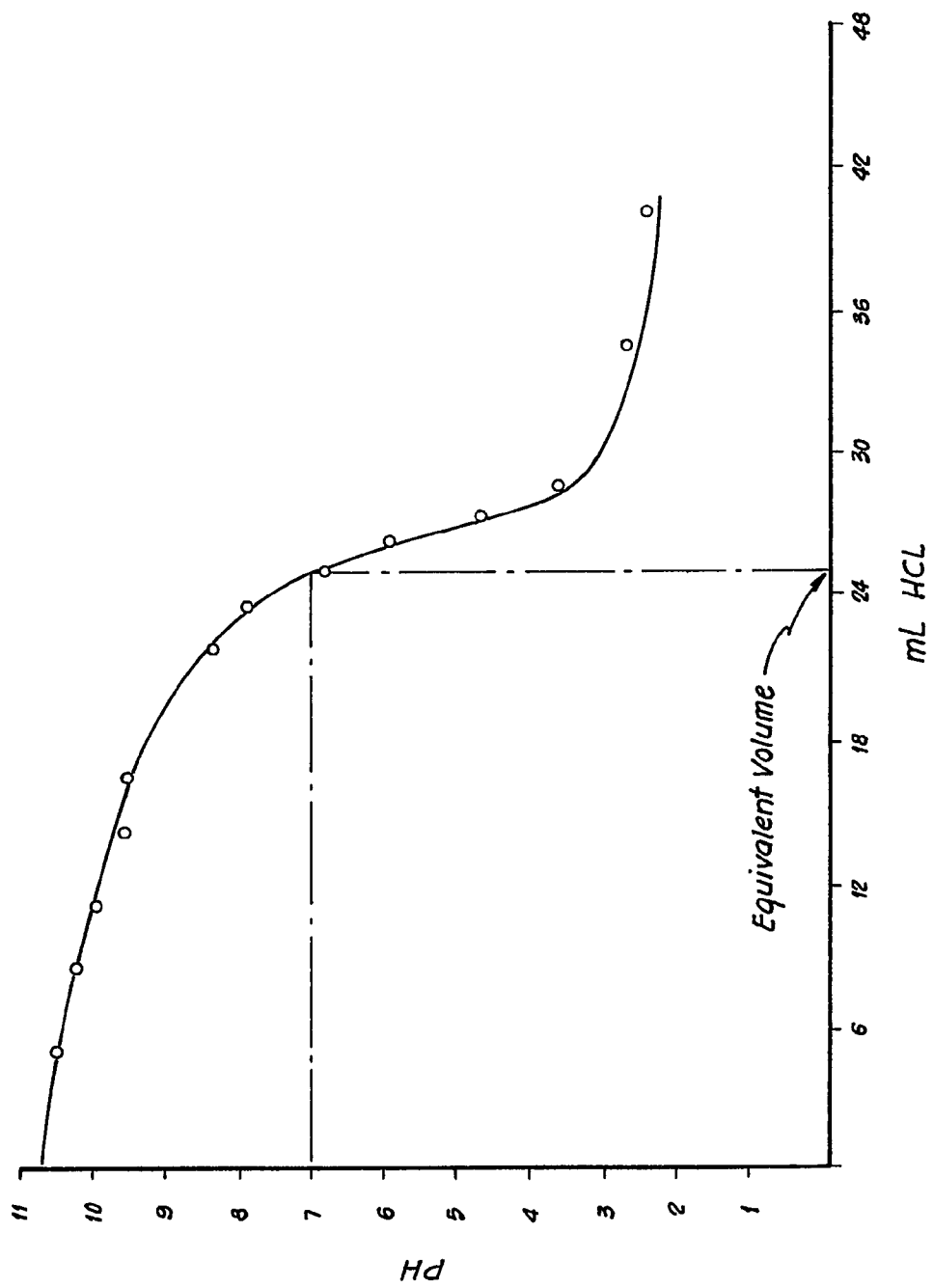


Figure No. 19A
Titration Of Sample Pressurized For 50 Hours

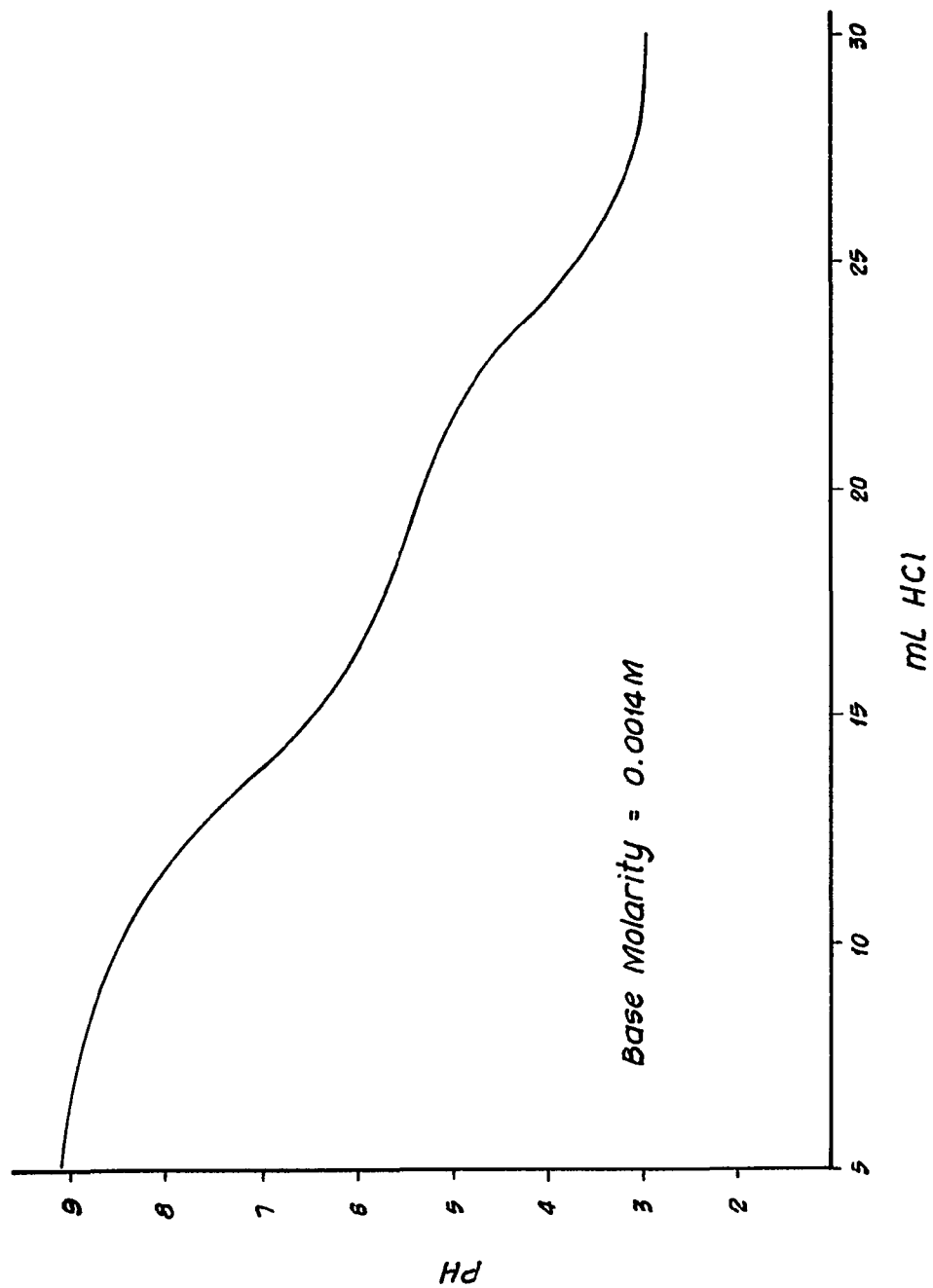


Figure No. 19B

Titration of Quiescent Tristearin Sample After 50 Hours

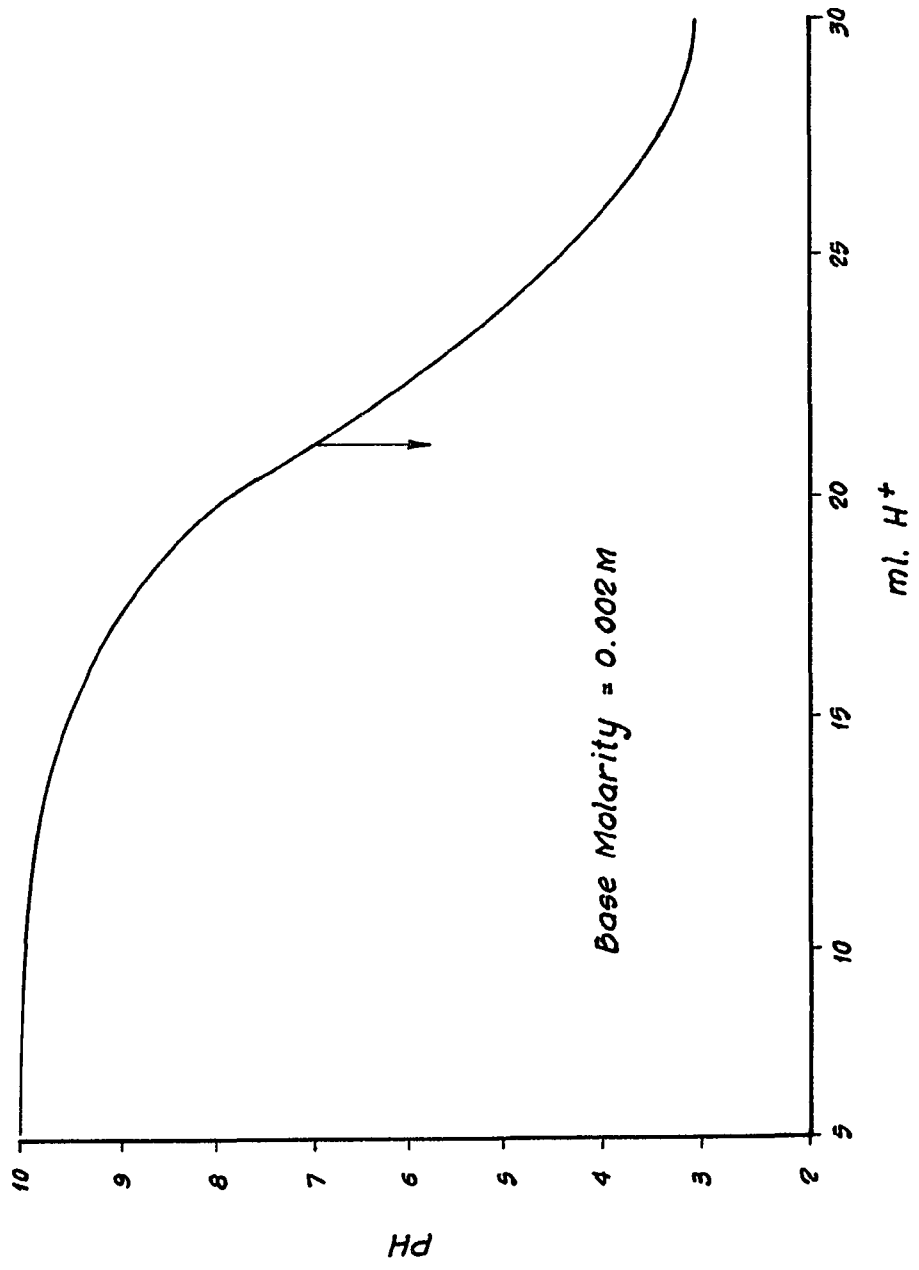


Figure No. 19 C
Titration of Pressurized Tristearin After 100 Hours Of Pressure

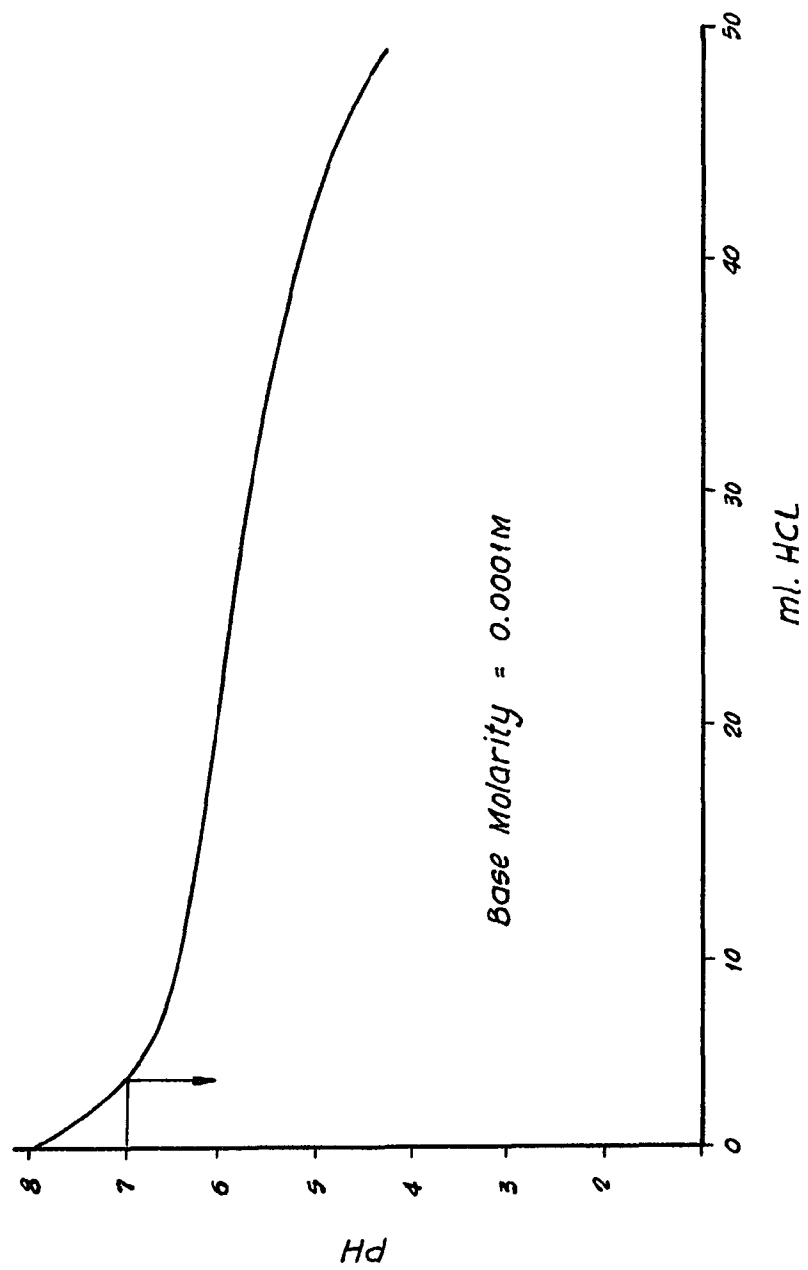
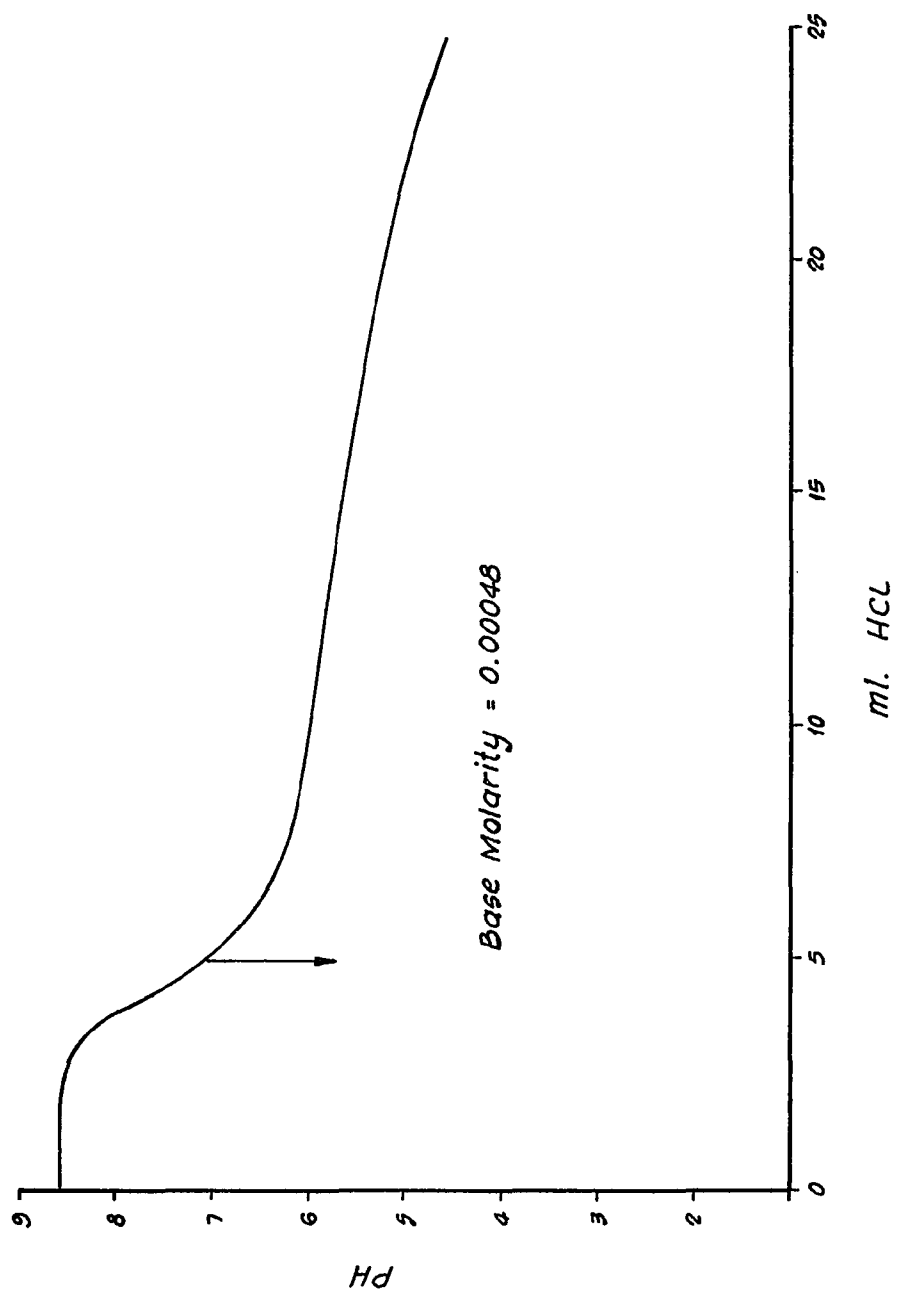


Figure No. 19 D
Titration of Quiescent Tristearin Sample After 100 Hours



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.

$$(50\text{ml NaOH/TS}) (M) = (0.005\text{M HCl}) (20\text{ml})$$

$$M \text{ NaOH} = 0.002\text{M}$$

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.

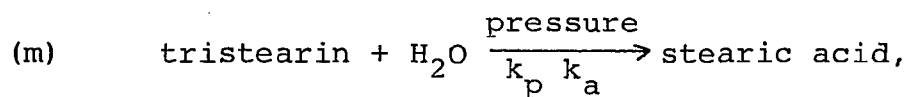
TABLE NO. 7
BOD_L AND SPECIFIC RATE CONSTANTS FOR PRESSURIZED
AND NON-PRESSURIZED STEARIC ACID SOLUTIONS

SAMPLE	PRESSURIZED				AERATED			
	BOD	ΔBOD	% 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

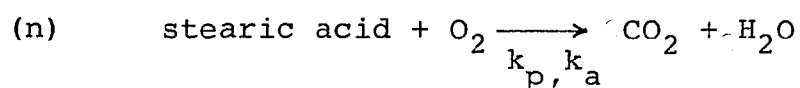
The BOD_L results show that the samples responded exactly the same, indicating no pressure effect. Also, the kinetic rate constants were almost identical suggesting no changes in the oxidation conditions for both the pressurized and non-pressurized samples.

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:



where $k_p > k_a$



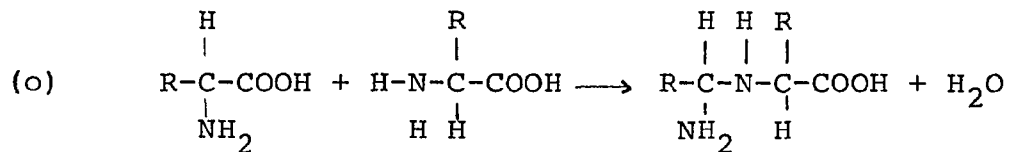
where $k_p = k_a$

AMINO ACIDS AND PROTEINS

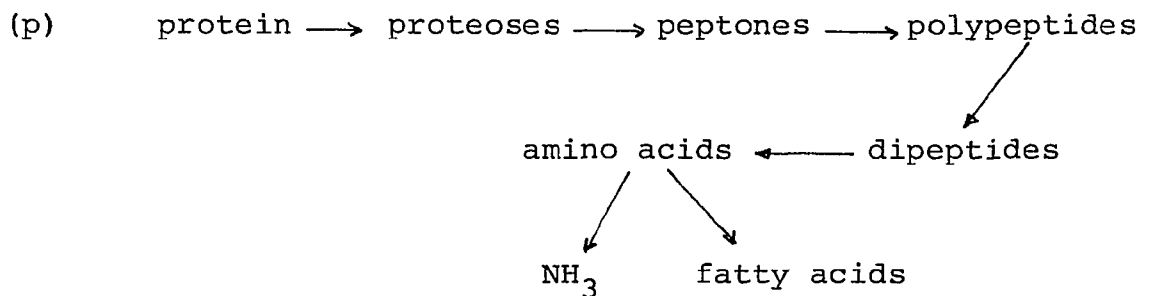
The last series of compounds to be analyzed is the protein/amino acid series. Proteins are complex combinations of the twenty or so amino acids and are a vital constituent for life processes of plants and animals.

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:



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



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.

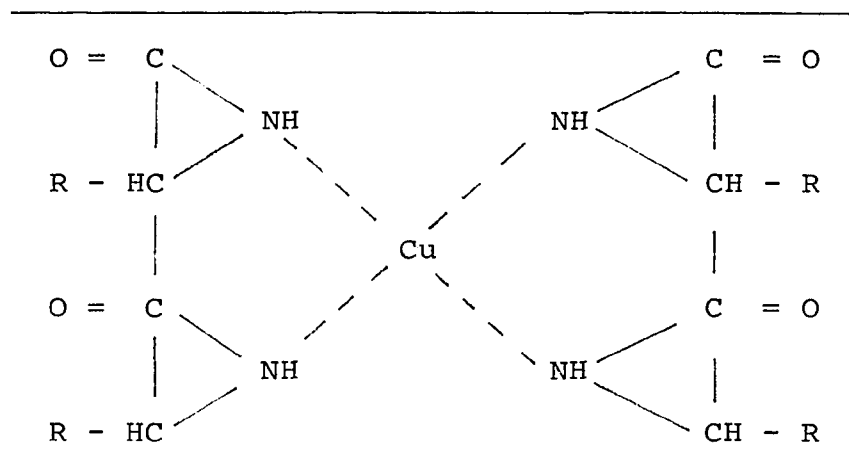
The method of protein analysis most commonly used is the Kjeldahl method which involves digestion of organic samples followed by distillation and measurement of

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, $\text{NH}_2\text{-CO-NH-CONH}_2$, 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, $\text{C}_5\text{H}_9\text{NO}_4$, with a molecular weight of 147 and the following structure:

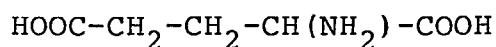
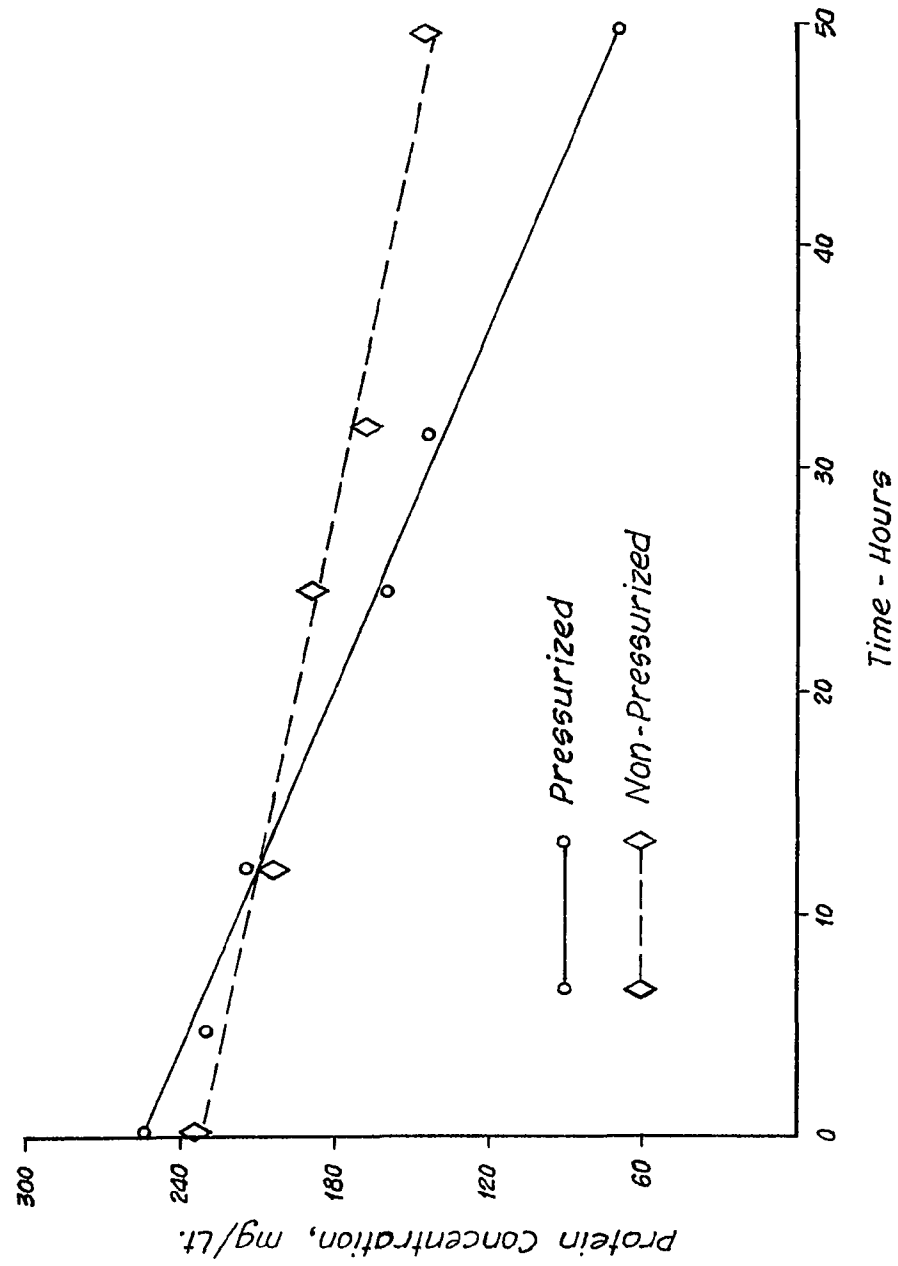


Figure No. 21
The Effect of Pressure On The Hydrolysis Rate Of Protein



A solution containing 6.12 grams of glutamic acid and 2.12 grams of ammonium chloride 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	Δ BOD	% BOD REDUC.	BOD _L	Δ BOD	% BOD REDUC.	BOD _L	Δ BOD	% 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

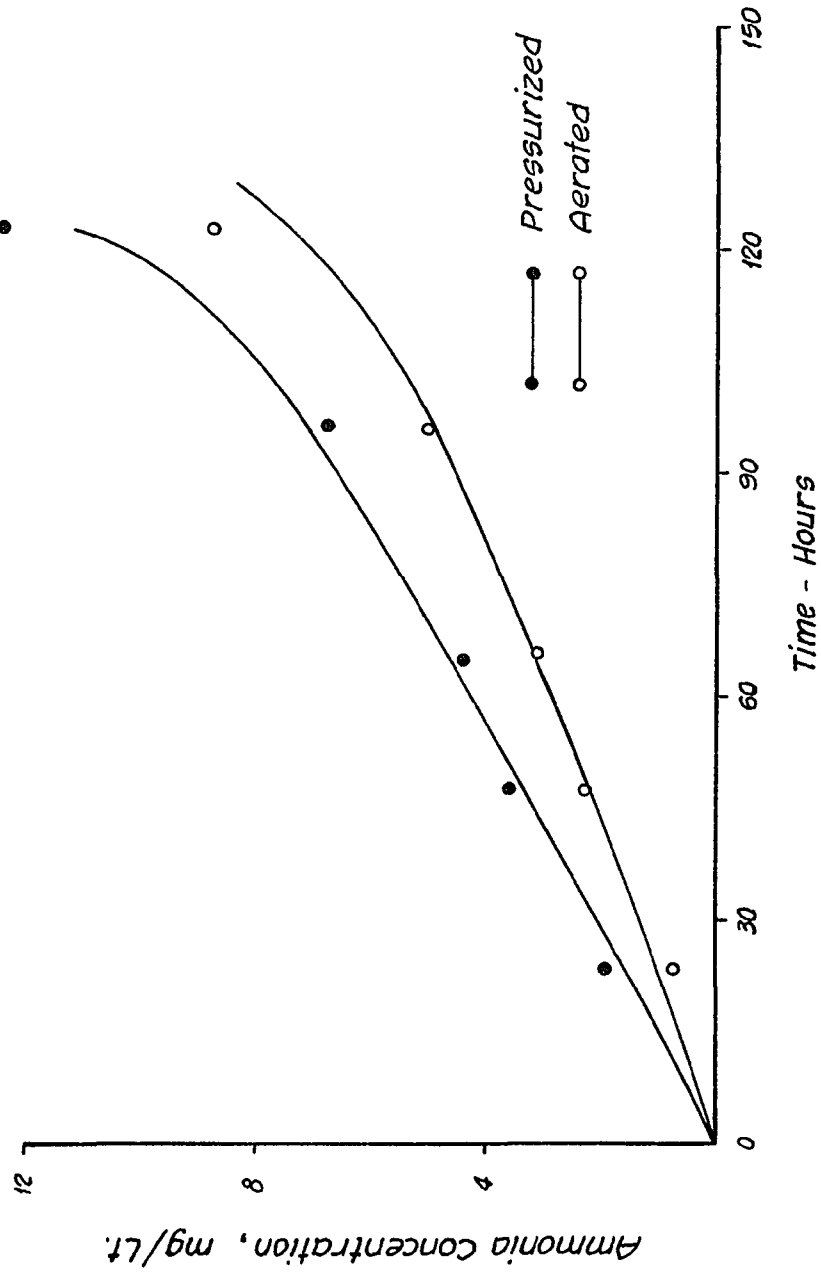
SAMPLE	PRESSURIZED			AERATED			QUIESCENT		
	COD	Δ COD	% COD REDUC.	COD	Δ COD	% COD REDUC.	COD	Δ COD	% COD REDUC.
C	110	--	--	108	--	--	99	--	--
4	87	23	21	81	27	35	90	9	8
8	77	33	30	79	29	26	74	25	23
24	55	55	50	62	46	42	78	21	21

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 spectrophotometrically (23). Initially, a calibration curve

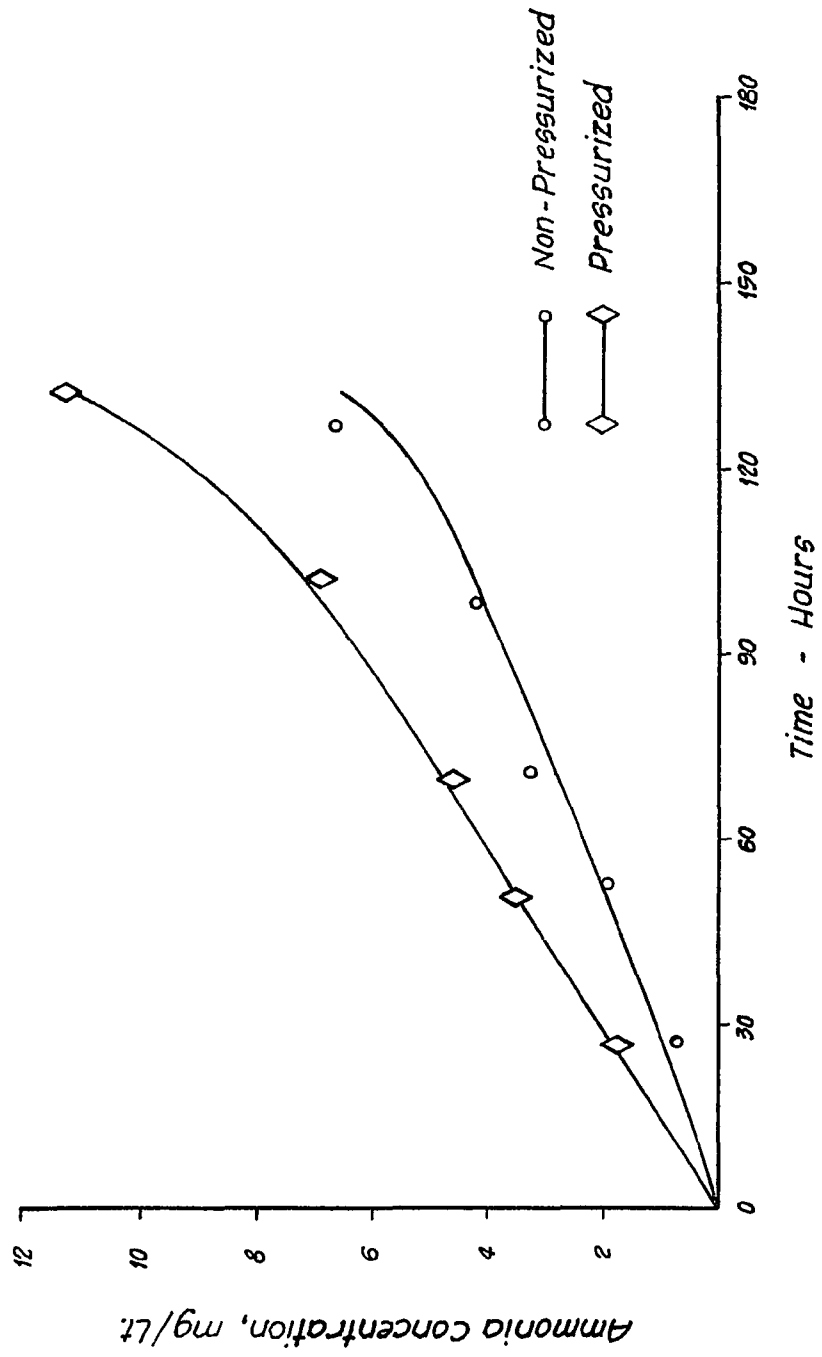
was prepared from standard ammonia solutions and the Nessler reagent and this is shown in Appendix D. The results of this trial is shown in Figure No. 44 where the pressurized sample shows a greater concentration of ammonia as compared to the aerated sample.

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 nitrifier population was higher in the pressurized samples and correlated the increase in nitrifier 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 nitrifiers 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, experimental 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

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 \longrightarrow Monosaccharides
(starches) (sugars)

- 2) Fats \longrightarrow Fatty Acids

- 3) Proteins \longrightarrow 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 pressurization 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-pressurized. 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.

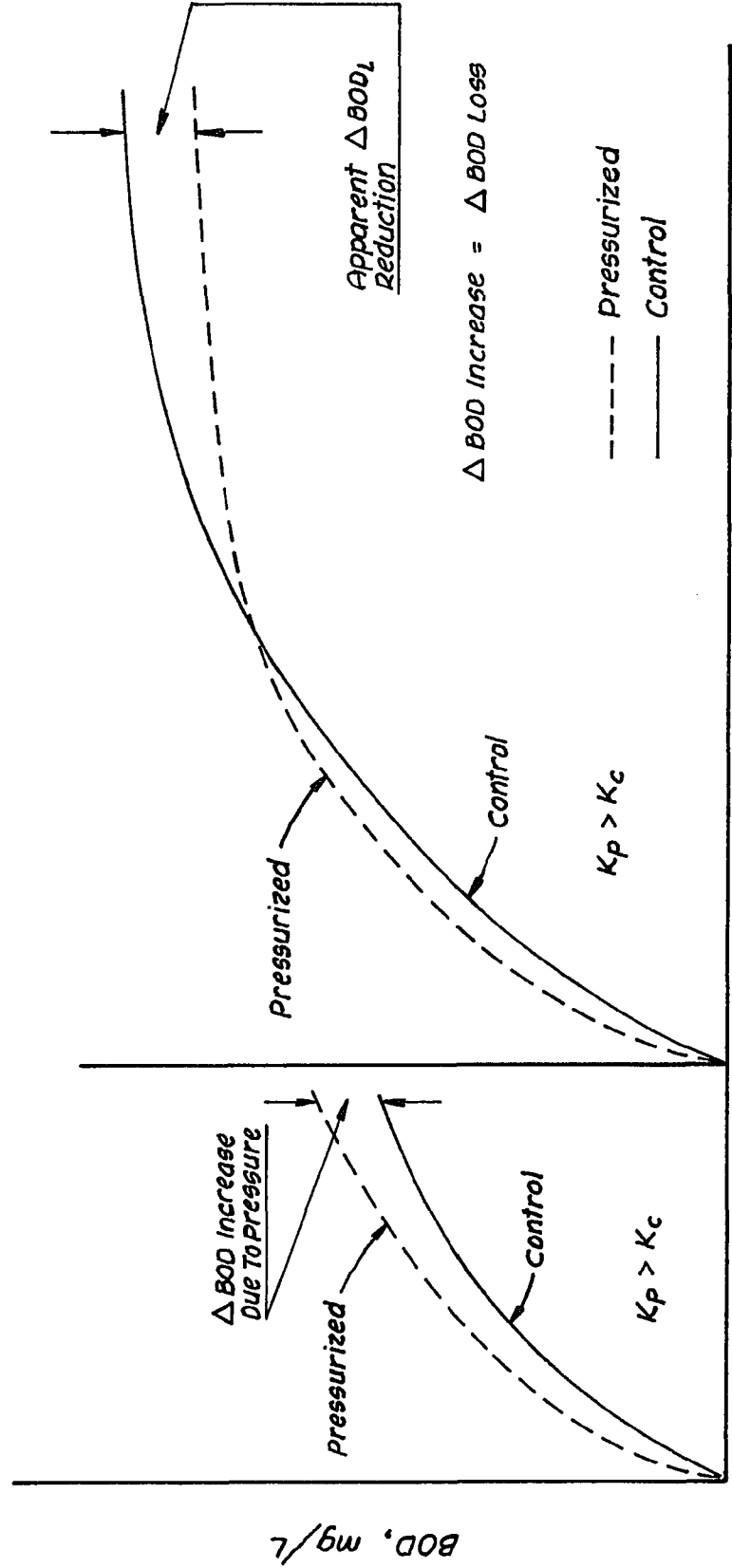
After pressurization for a specified duration is completed, samples are drawn off and BOD's performed. BOD's also were performed for non-pressurized samples which acted as the control. The resulting BOD profiles are shown in Figure 24, "Part B" which shows two important facts; the 1st stage BOD, BOD_L , 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



Time - Days

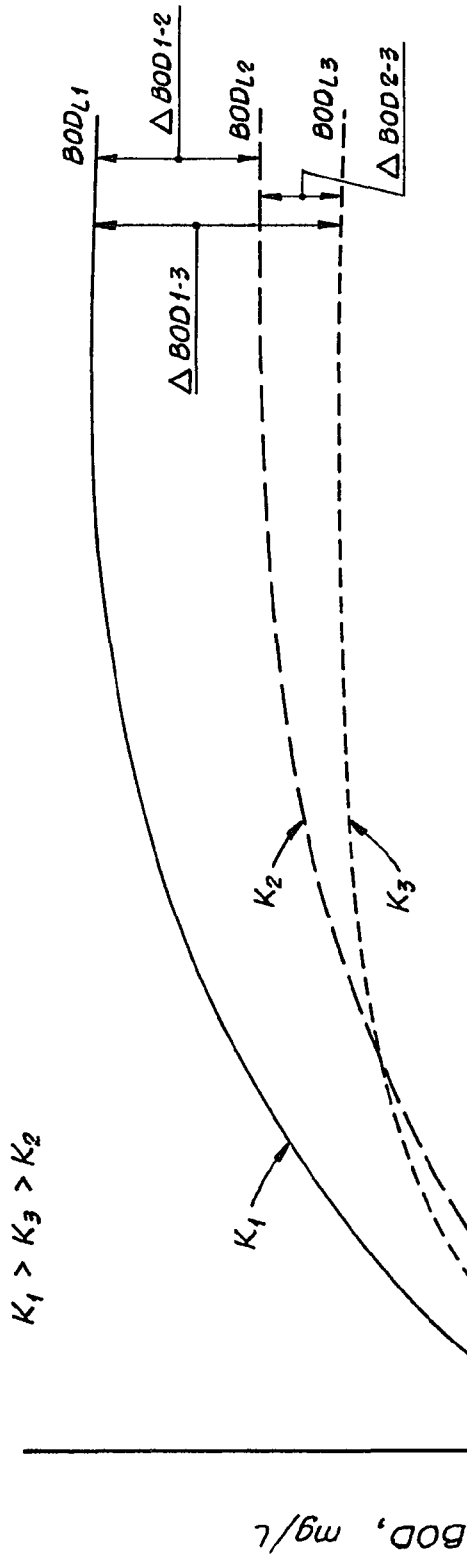
Time - Days

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 concentration 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 $t = T_1$



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

Time, Days

BOD, mg/L

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 Δ 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 wastewater 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 concentration 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 displaced higher than the control BOD curve. Another consideration 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 autotrophic organisms 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 occurring 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 suppressor, allyl thiourea, ATU, was added. This chemical has the ability to suppress the growth of nitrifiers 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 nitrifiers. A synthetic sample had to be used for two reasons. The first was consistency; the pressurized samples, with and without nitrifiers 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 nitrifiers 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 nitrifiers were identical for the first stage BOD.

3) Aerated samples with and without nitrifiers 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

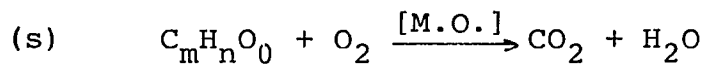
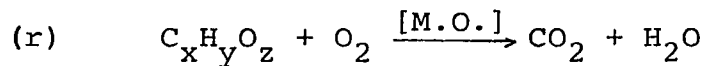
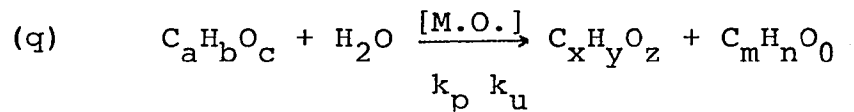
the BOD oxidation process. Two different dilution waters were prepared, one seeded with microorganisms, and the other, non-seeded. BOD analyses were performed and the BOD curves drawn were identical for the control and pressurized samples. Organism counts were also conducted up to 48 hours pressure time. There was a significant drop in microorganism population ($10^{14} \longrightarrow 10^8/100 \text{ ml}$) 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 nitrifiers. 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:



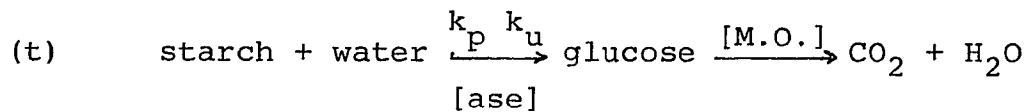
where subscripts a, b, c > subscripts x, y, z., m,
n, o.

k_u = rate of unpressurized sample

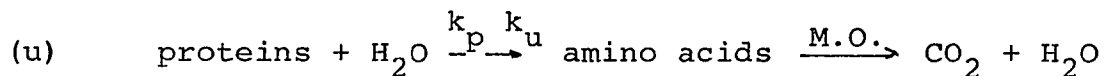
k_p = rate of pressurized sample

$k_p > k_u$

Specific examples of these reactions applicable to this
research are:



where [ase] = Enzyme concentration

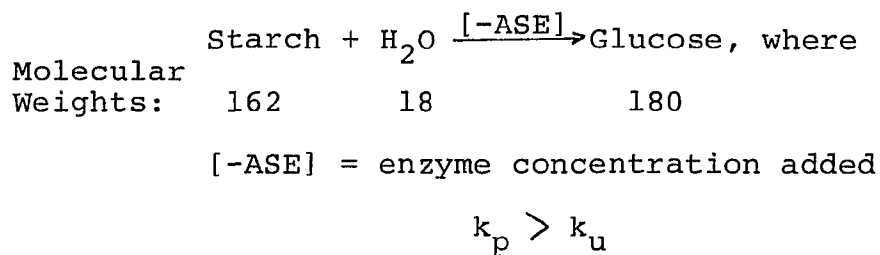


where it has been shown that $k_p > k_u$.

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 occurred 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 pressurization, 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:

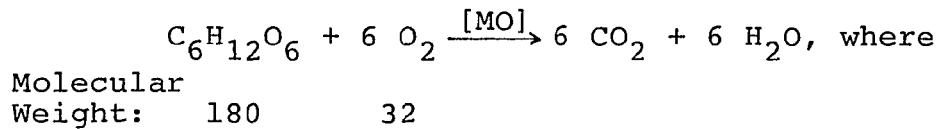


This would produce 111.1 mg/l glucose for the pressurized sample and 55.5 mg/l glucose for the control, as shown in the following stoichiometric relationships:

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

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

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



[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:

$$\frac{111}{180} = \frac{X \text{ mg/l O}_2}{(6) (32)}, X = 118 \text{ mg/l}$$

$$\frac{55.5}{180} = \frac{Y \text{ mg/l O}_2}{(6) (32)}, Y = 59 \text{ mg/l}$$

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 pressurization to be lower by the additional oxidation in the pressure vessel.

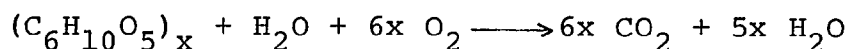
To show if this hypothesis is viable, the following experiment was conducted. Various combinations of starch and glucose were prepared and their BOD_L determined to ascertain any change in BOD_L and the rate of decomposition. As an example, a 75 percent starch-25 percent glucose solution was compared 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:

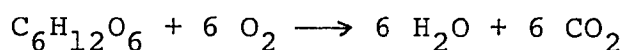
- 1) 100 percent starch - 0 percent glucose
- 2) 75 percent starch - 25 percent glucose
- 3) 50 percent starch - 50 percent glucose
- 4) 25 percent starch - 75 percent glucose
- 5) 0 percent starch - 100 percent glucose

The chemical reactions are as follows:

(v) Starch



(w) Glucose



Stoichiometric amounts of sugar and starch were weighed out to obtain the proper proportions. Also, amyloglucosidase 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 metabolized. 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

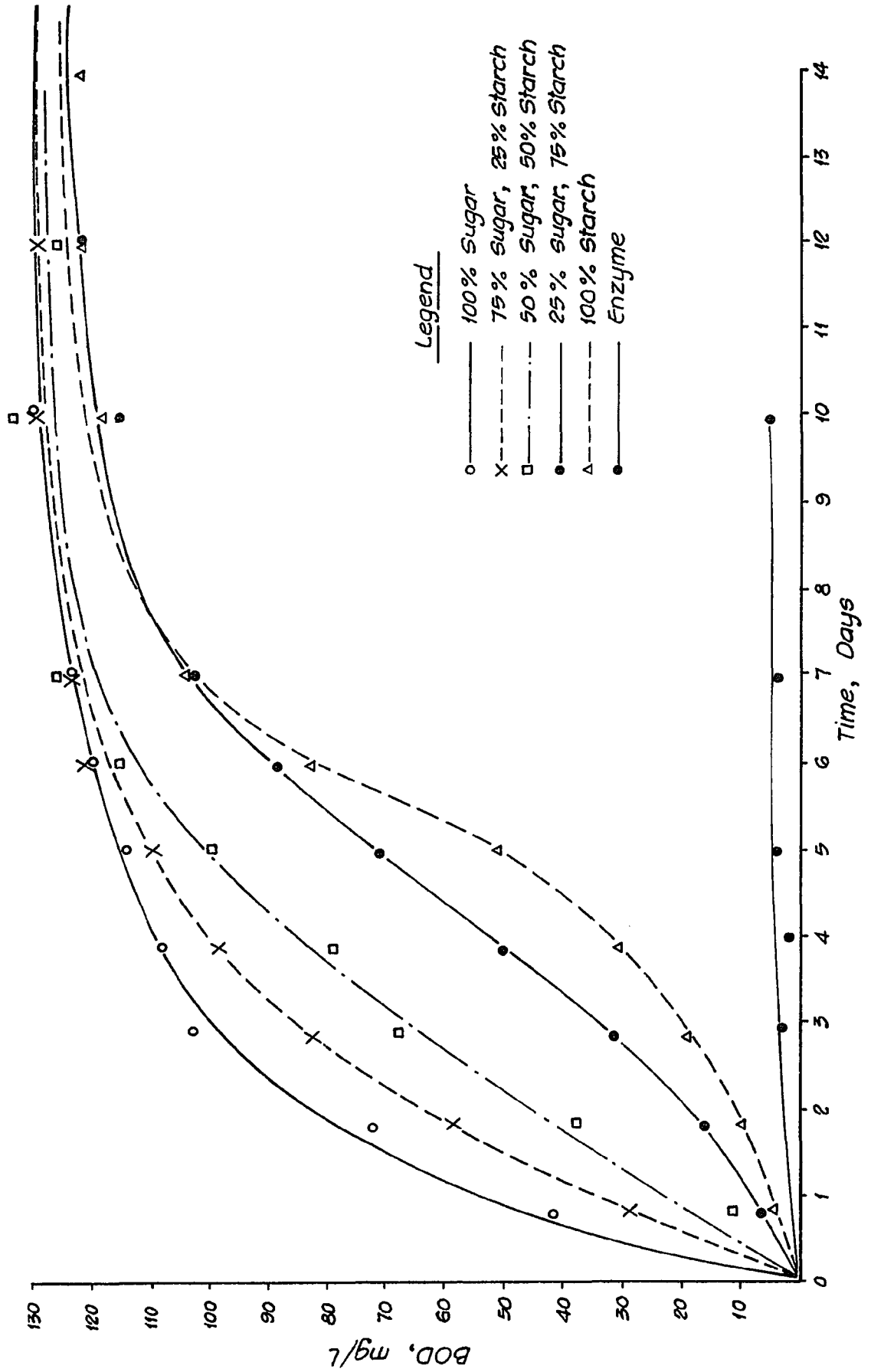
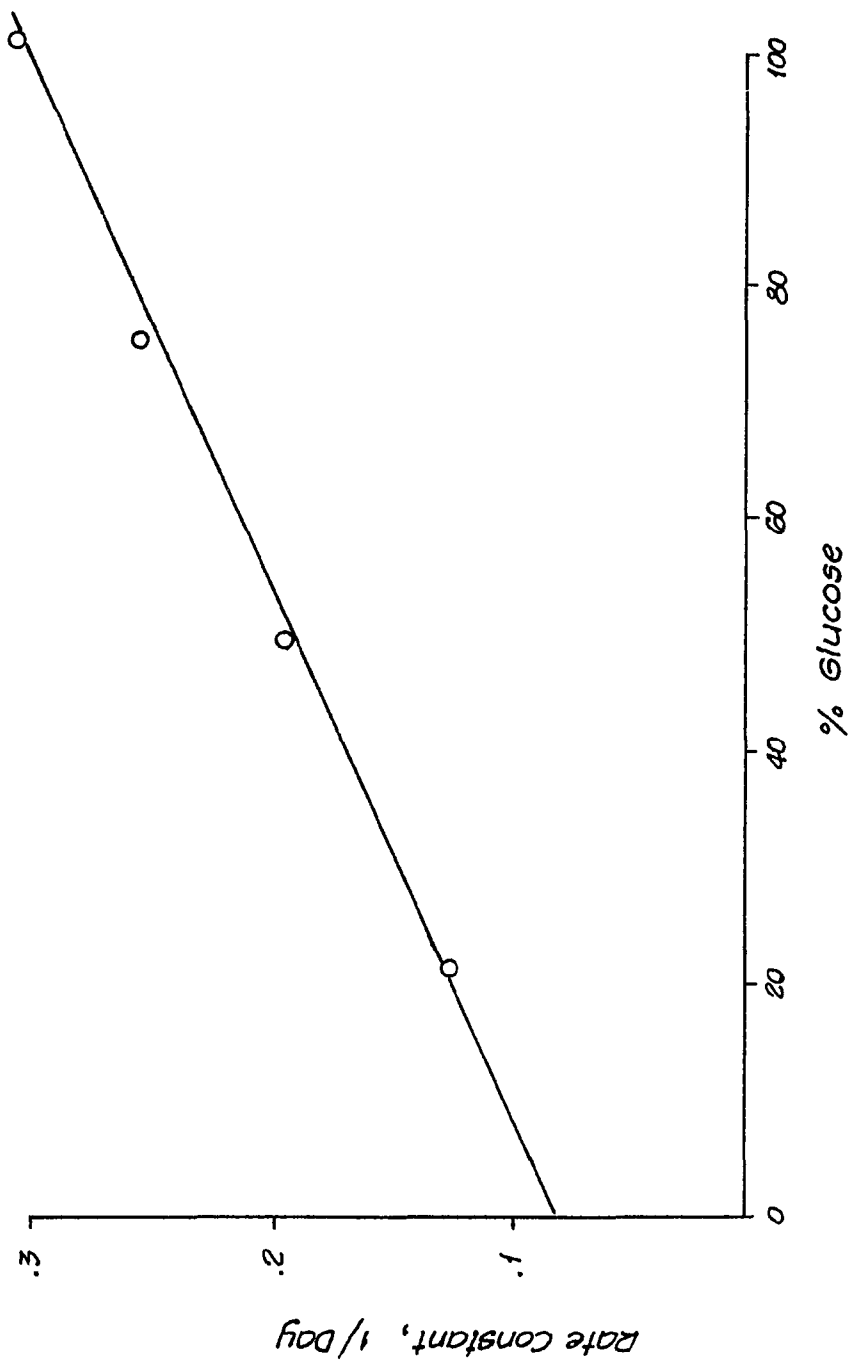


Figure No. 27
*Variation of the Specific Rate Constant as a Function of
the Amount of Glucose in a Glucose/ Starch Mixture*



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-pressurized 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*

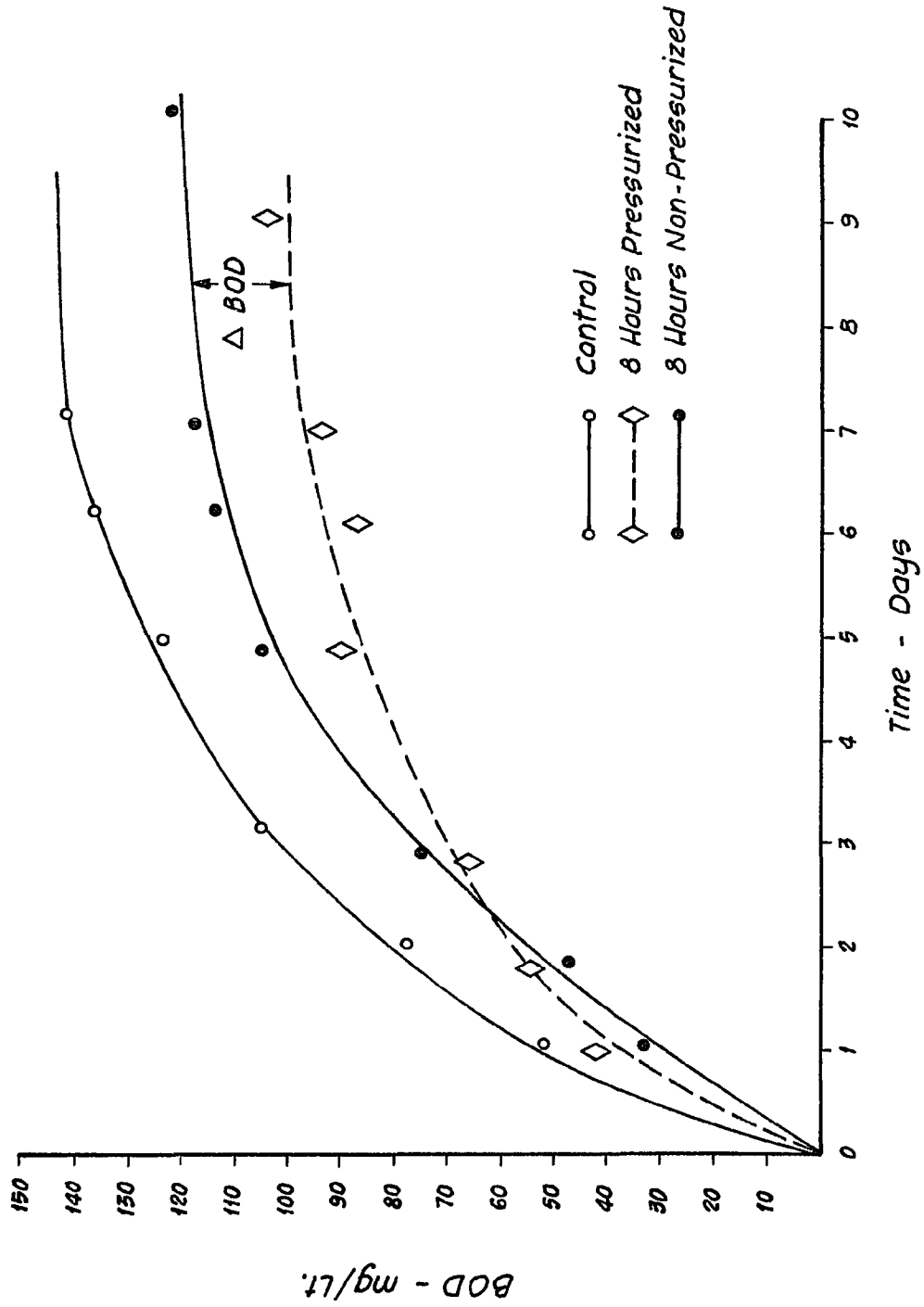
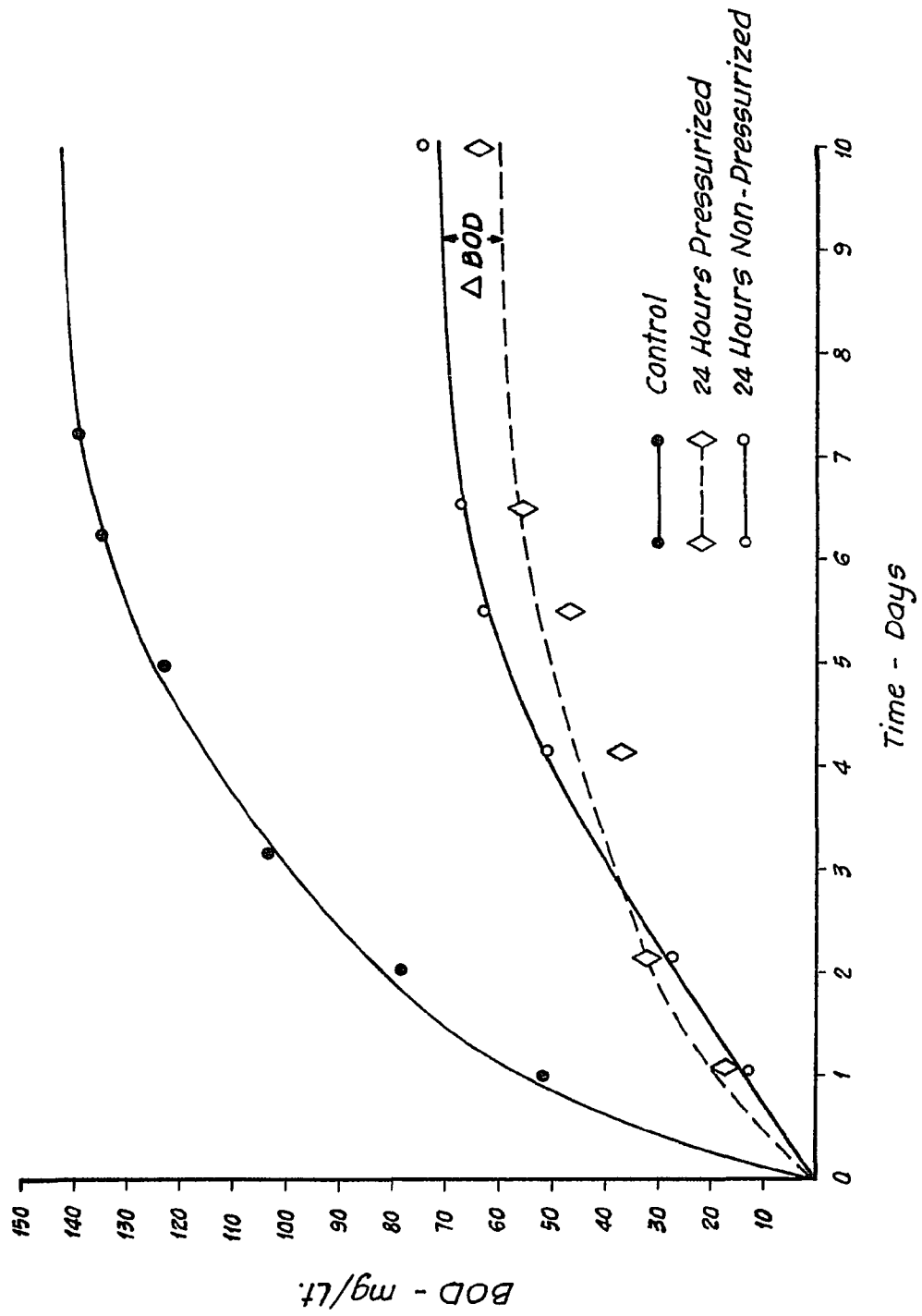


Figure No. 29
*BOD Variations Of Synthetic Wastewater Samples
 Pressurized And Non-Pressurized*



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. Obviously, 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

<u>MATERIAL</u>	<u>PERCENTAGE</u>	<u>GRAMS</u>
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 advantages. These are:

- 1) It is saturated with dissolved oxygen with concentration 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.

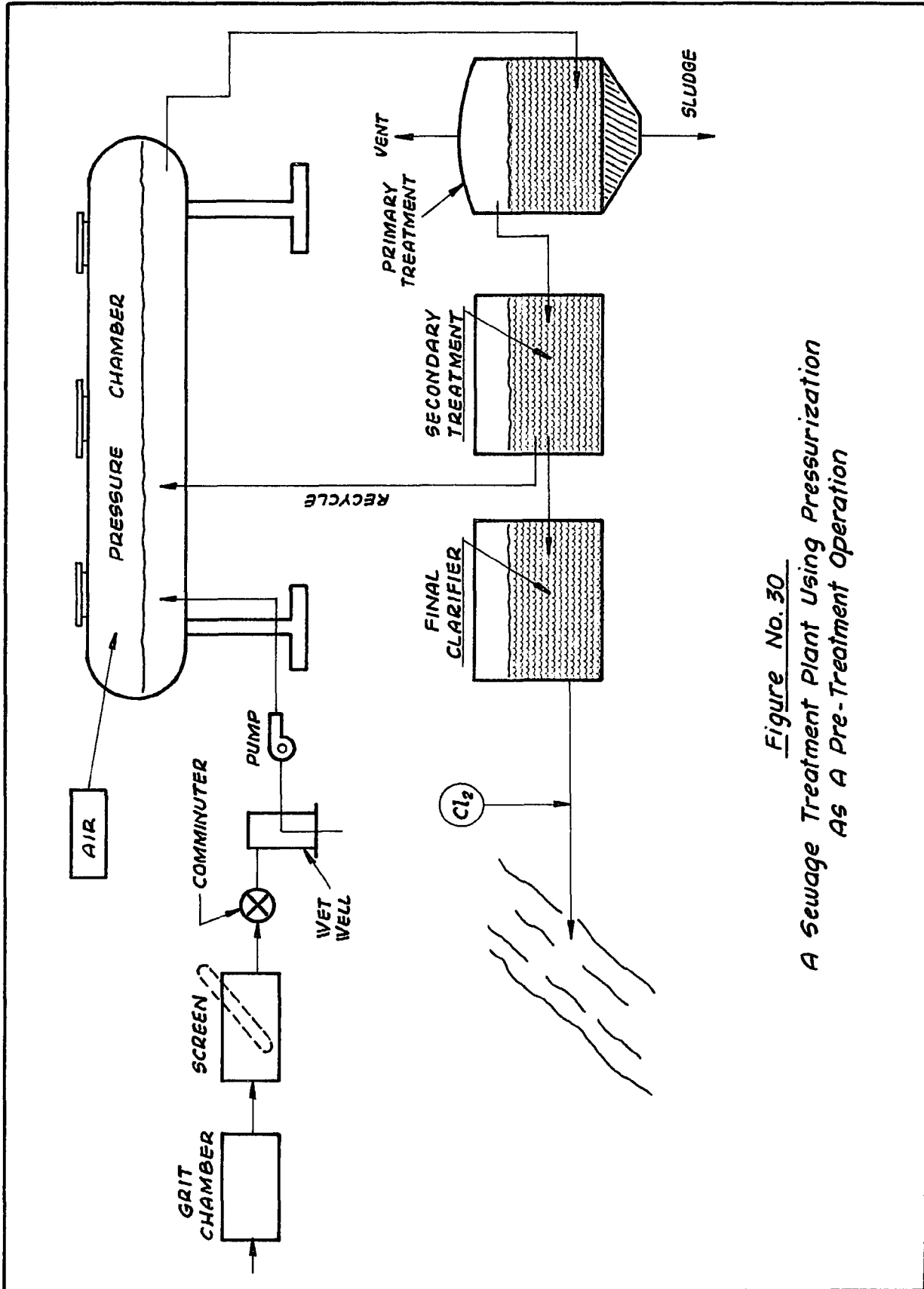


Figure No. 30
A Sewage Treatment Plant Using Pressurization
As A Pre-Treatment Operation

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 practiced 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 supernatant.
- 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 pressurizing conventional primary sludge and this process is shown in Figure 31.

Another digestion alternative would be to add sulfuric 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

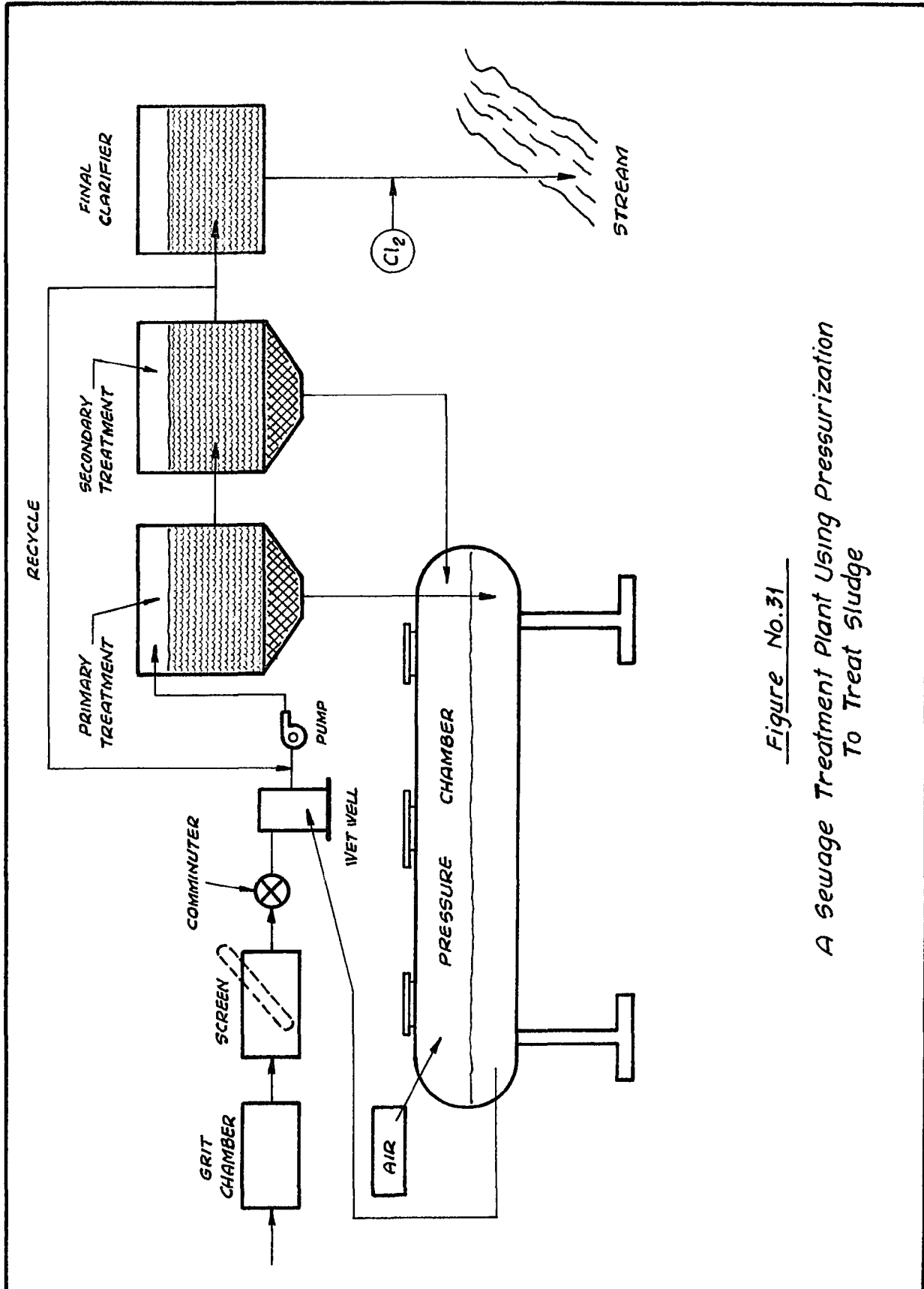


Figure No.31
*A Sewage Treatment Plant Using Pressurization
To Treat Sludge*

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

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 point 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 signalling 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-vitro 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

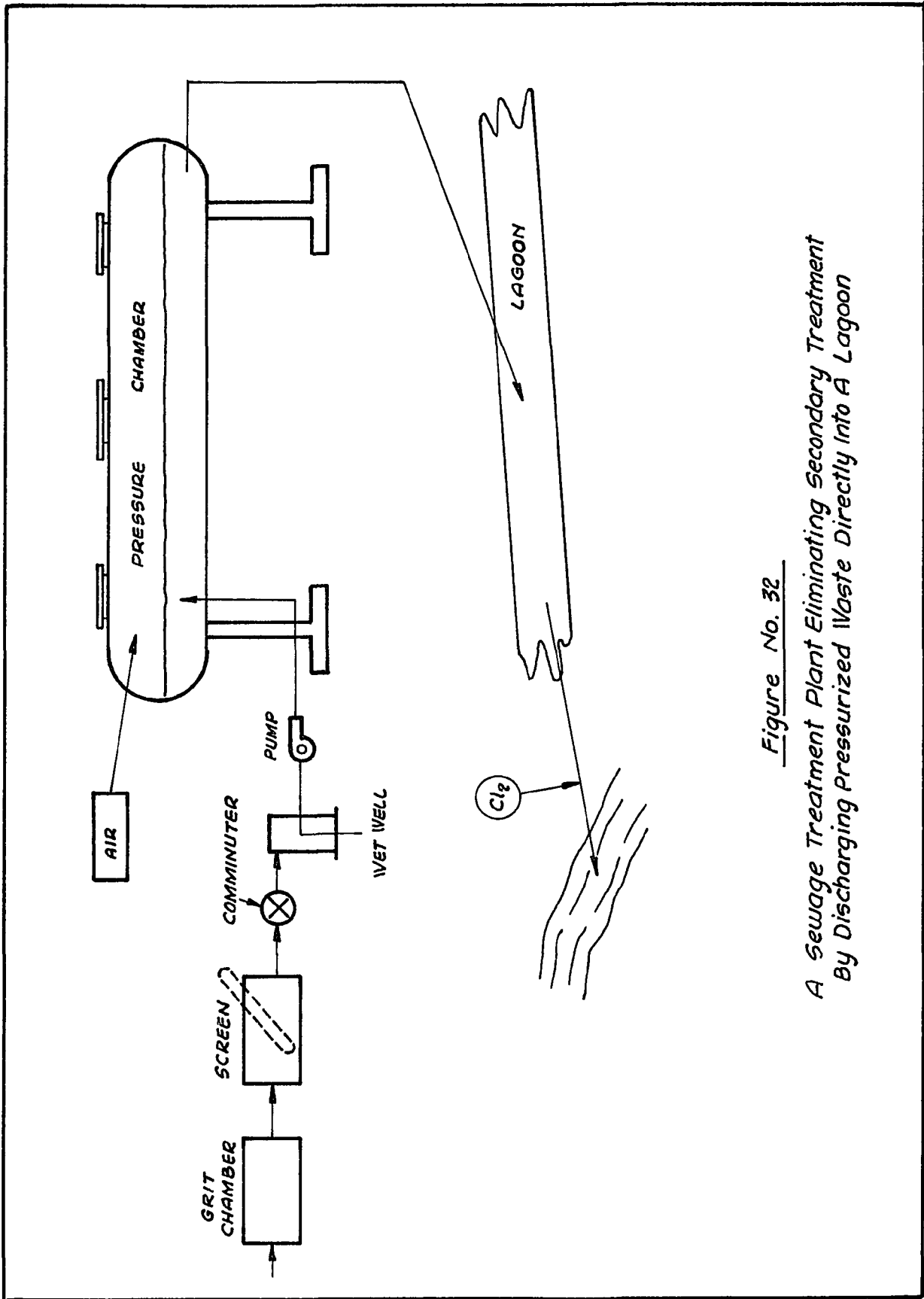


Figure No. 32
*A Sewage Treatment Plant Eliminating Secondary Treatment
By Discharging Pressurized Waste Directly Into A Lagoon*

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 resistant to chlorination than the coliforms. Studies have shown (52) that at a pH of 6 and chlorine dose of 30 mg/l 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 inactivation 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.

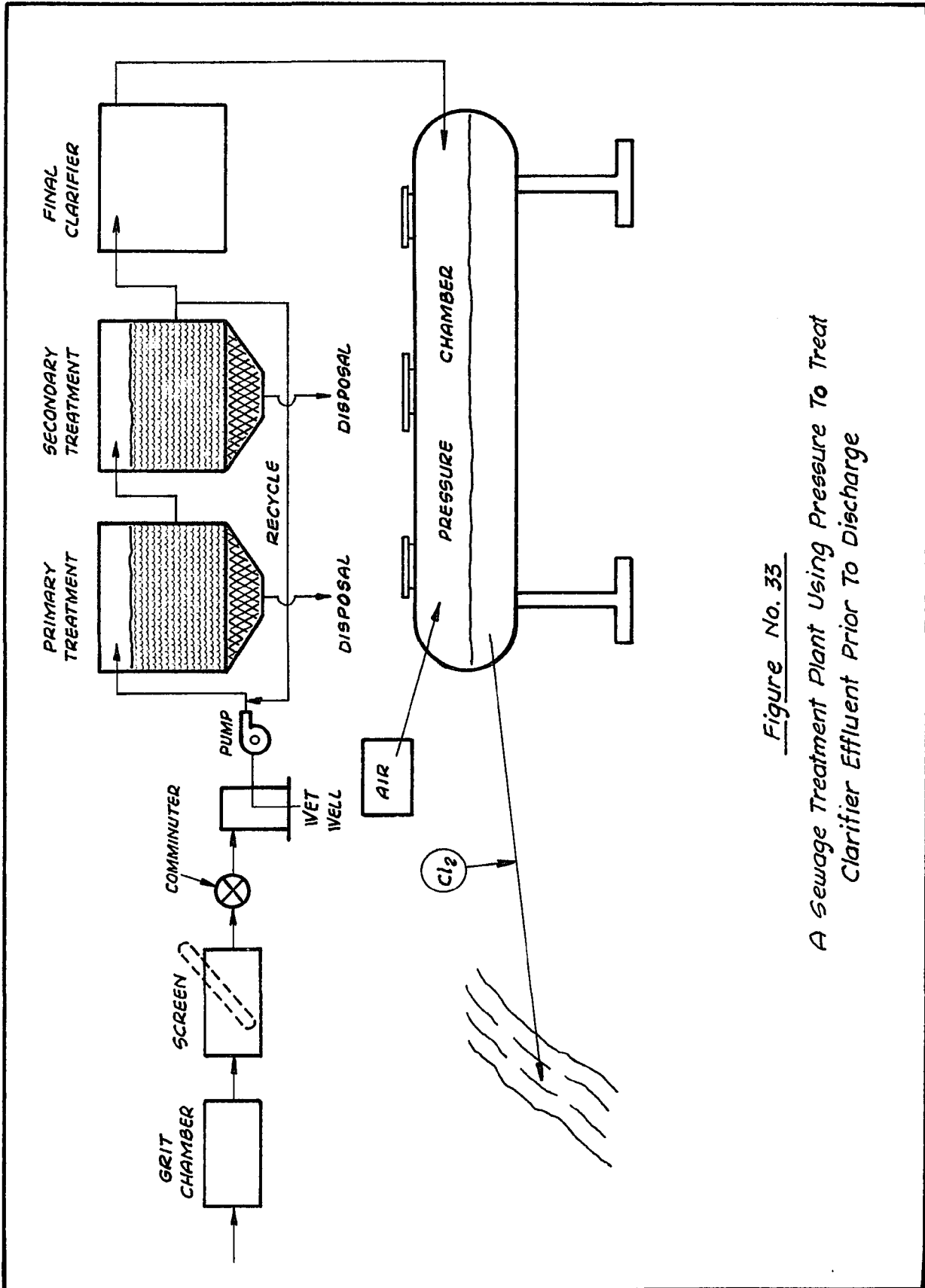


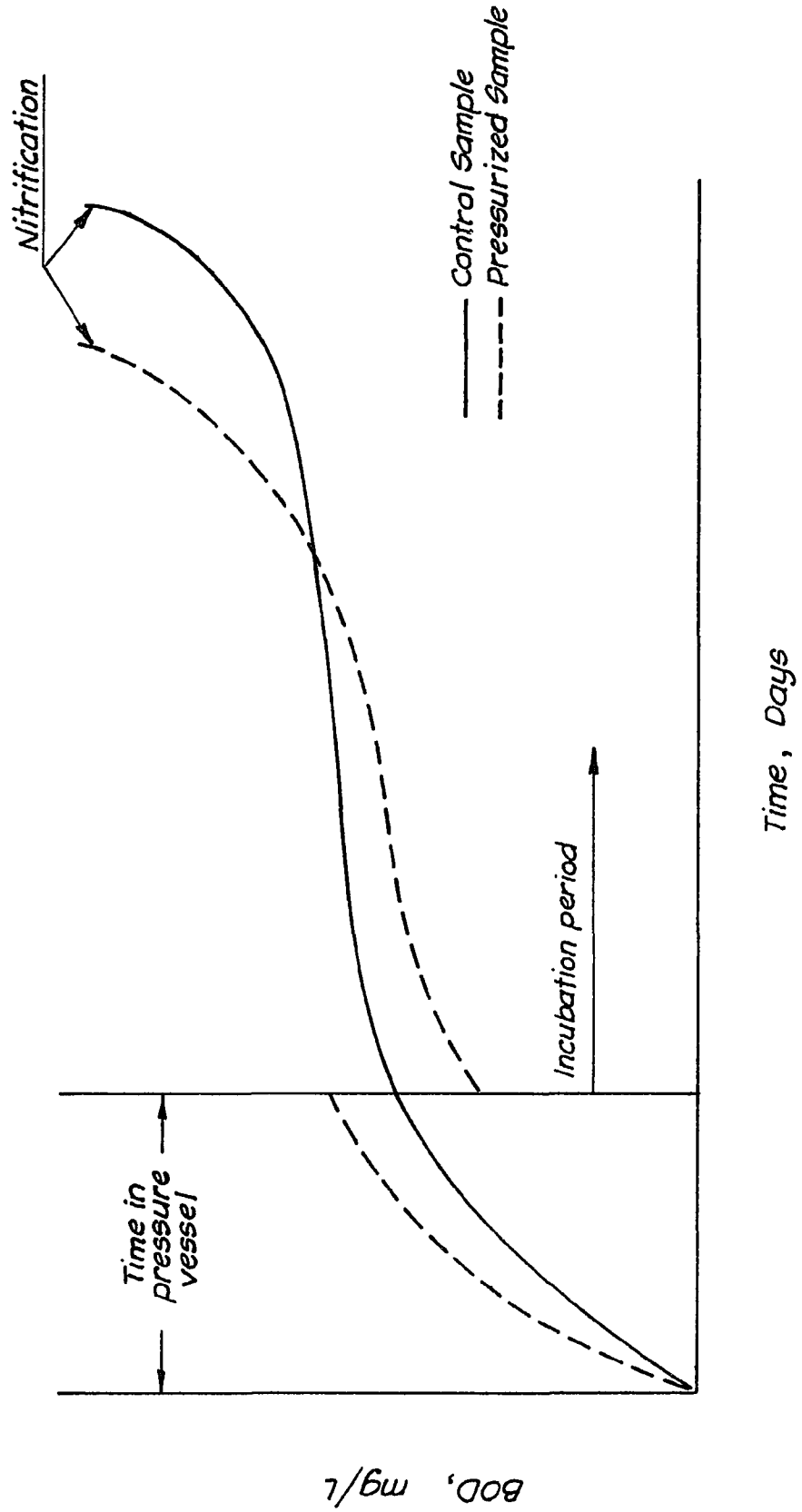
Figure No. 33
A Sewage Treatment Plant Using Pressure To Treat Clarifier Effluent Prior To Discharge

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

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 adjustment 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 extinction 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 understanding can contribute to useful applications of this process to existing wastewater facilities.

APPENDIX A

DEMAND CURVES OF WASTEWATER SAMPLES RESULTING
FROM ENVIRONMENTAL STRESSES

Figure No. 35
 Variation of The BOD of Pure Sewage And Diluted
 Samples After Standing For One Day

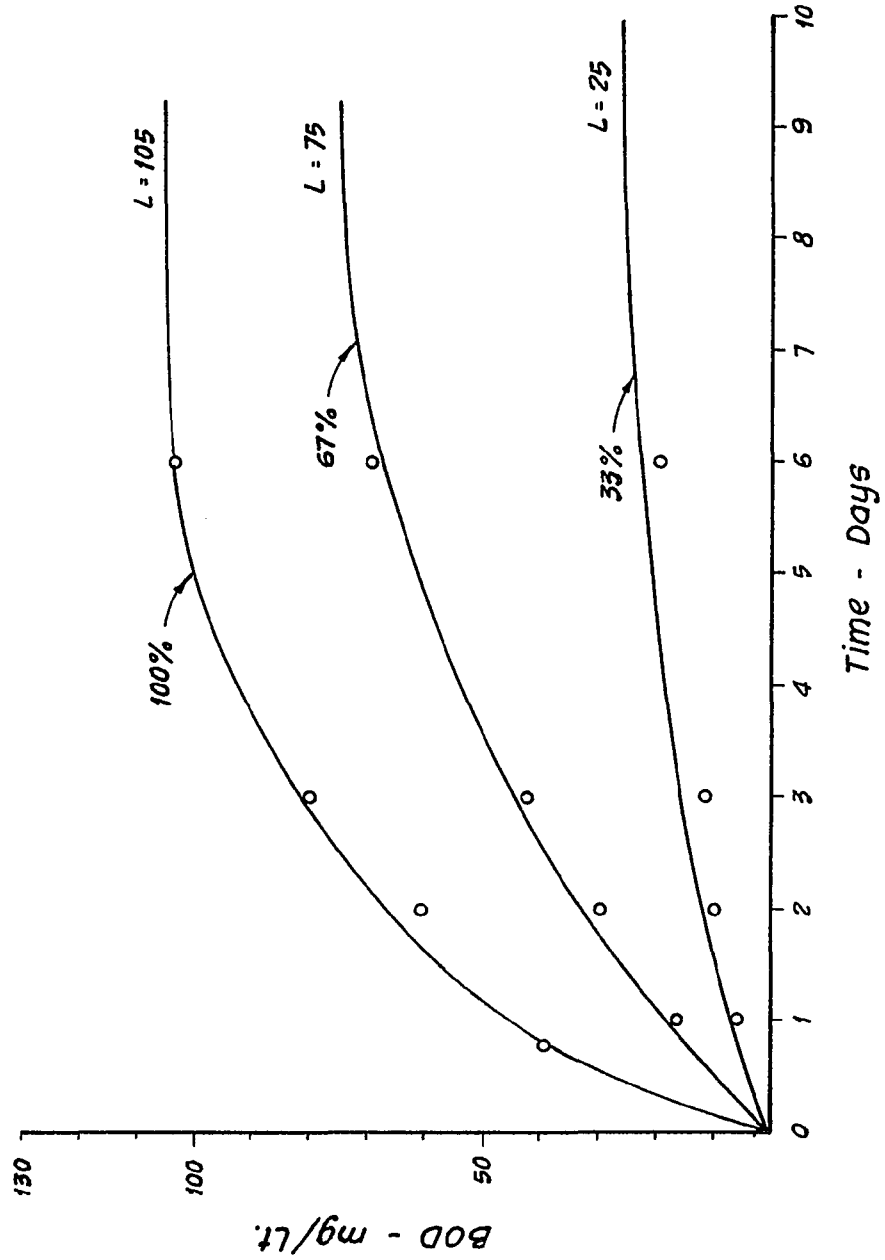


Figure No. 36

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

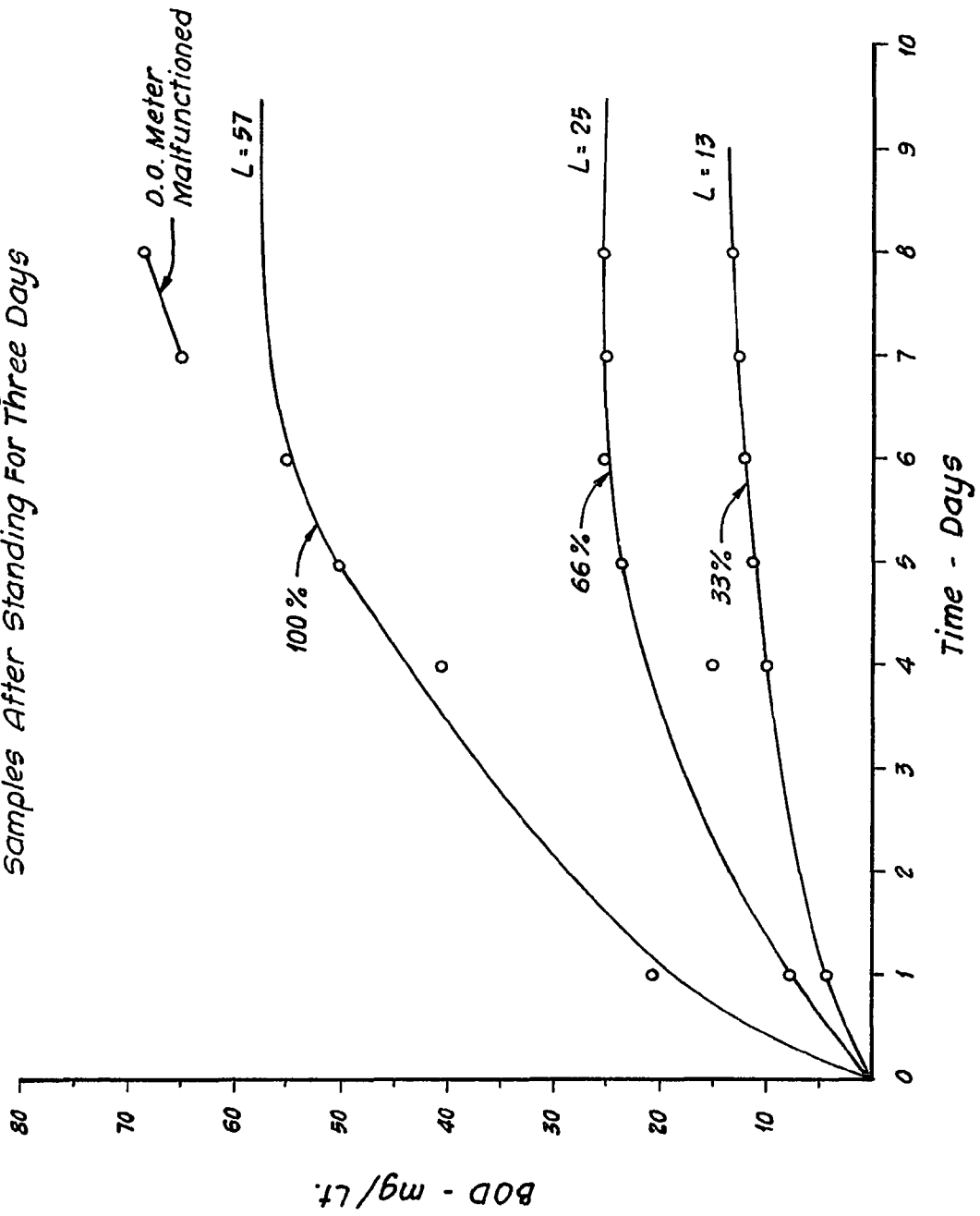


Figure No. 37
 Variation of The BOD Of Pure Sewage And Diluted
 Samples After Standing For Two Days

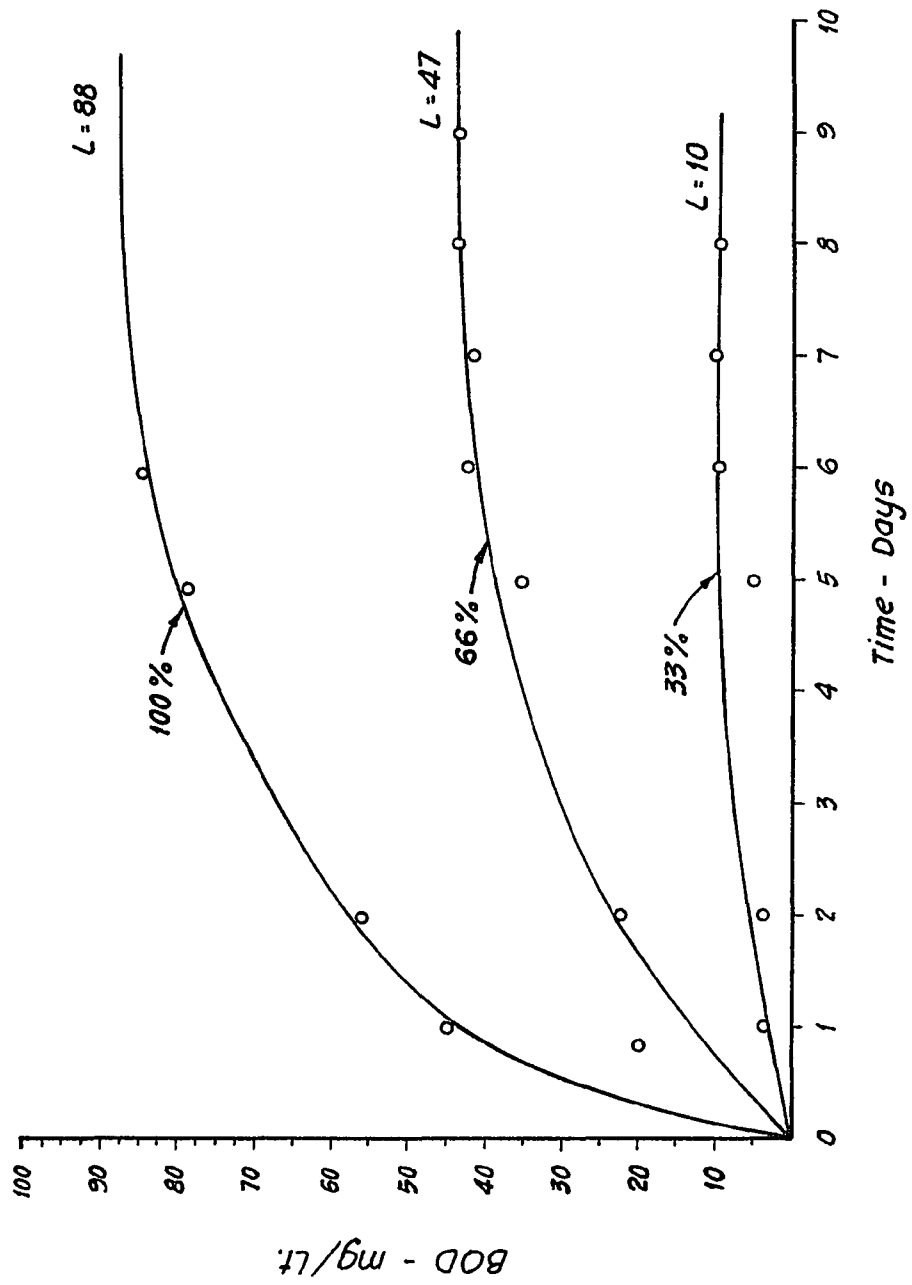


Figure No. 38
*Variation of The BOD of Pure Sewage And Diluted
Samples After Standing For Four Days*

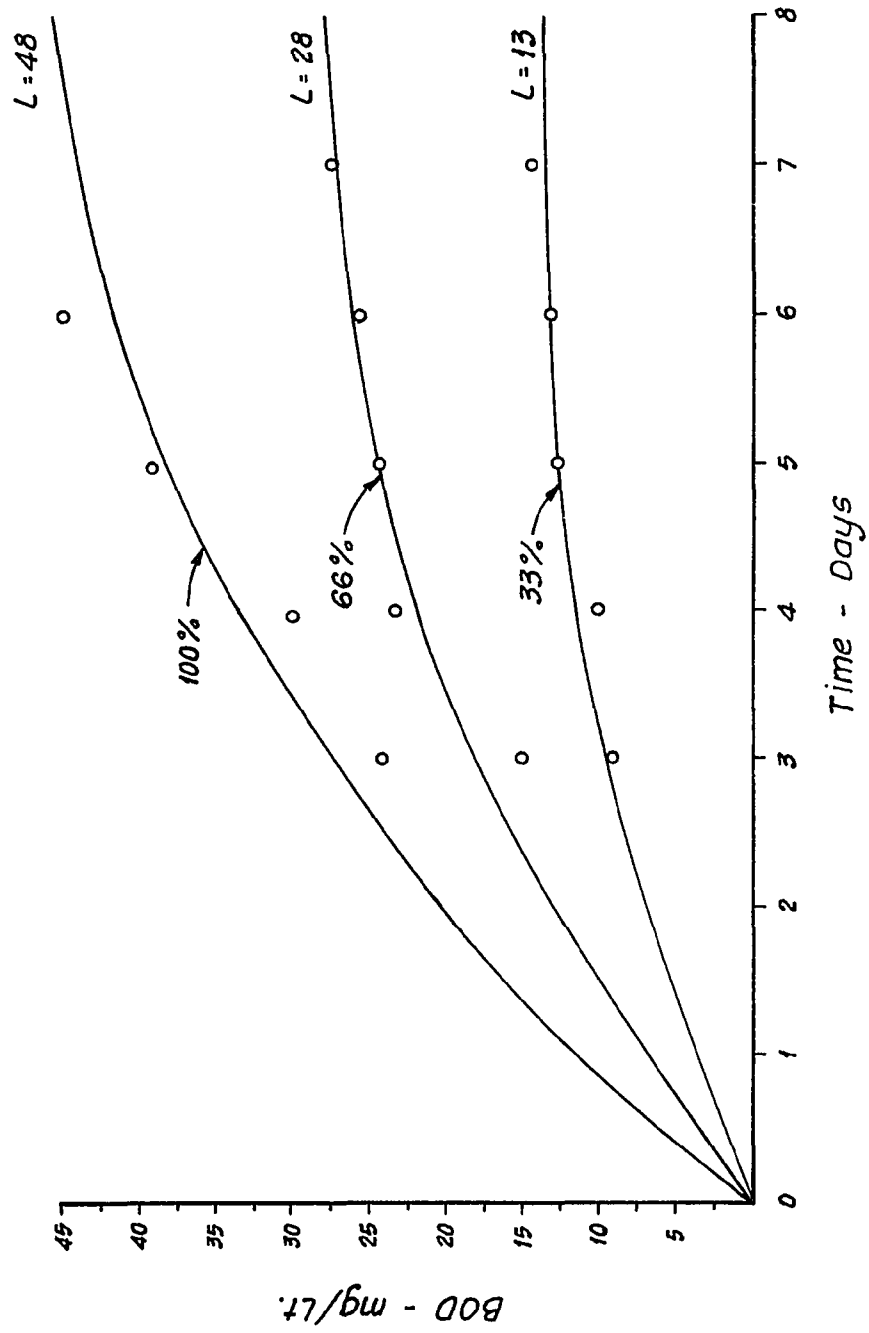


Figure No. 39
*Decrease Of BOD_L Values For Samples Allowed
To Stand Under Quiescent Conditions*

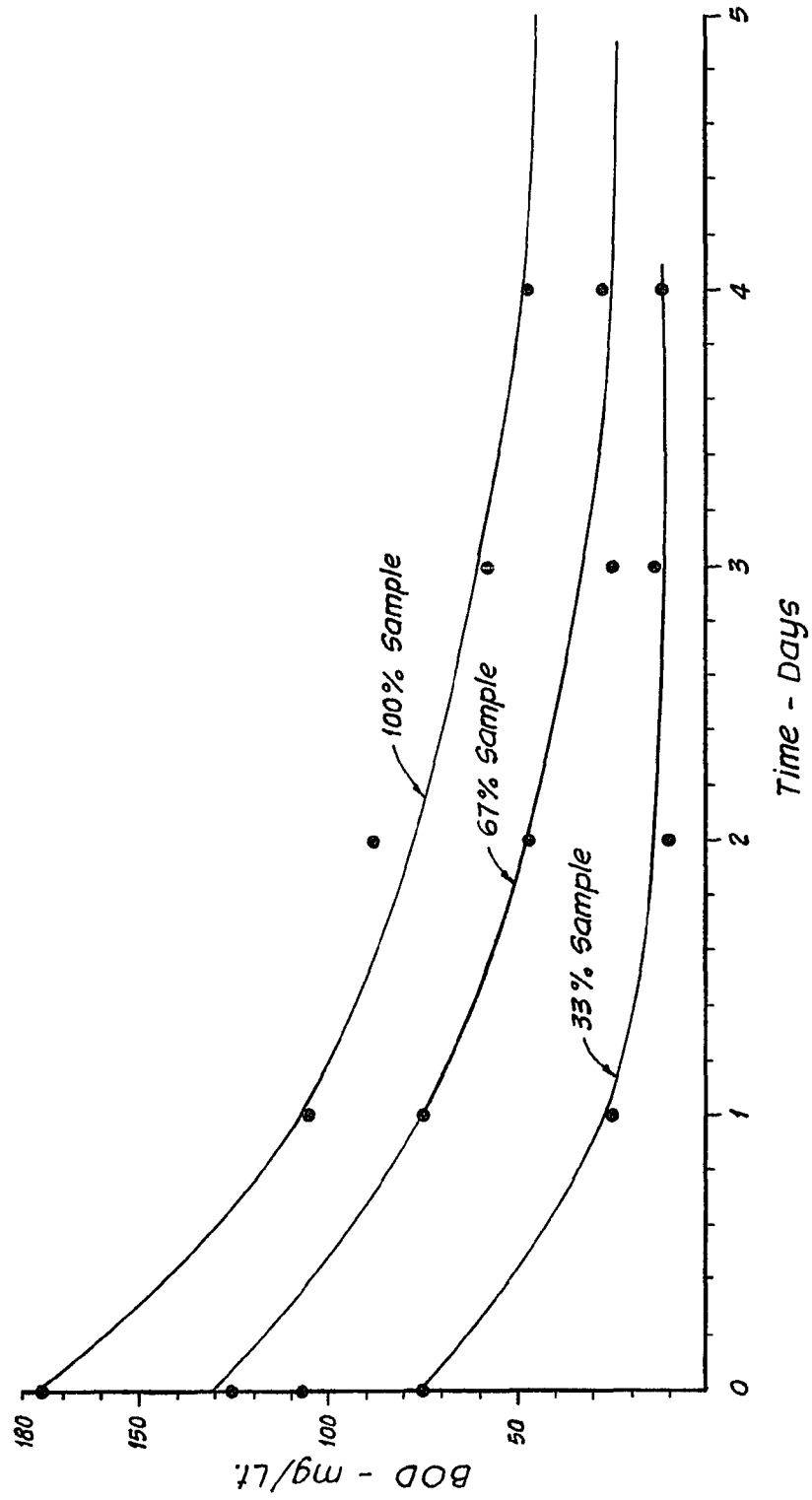


Figure No. 40
*Variation Of The Specific Rate Constant In The BOD Equation
 As A Function Of The Time The Sample Is Allowed To Stand*

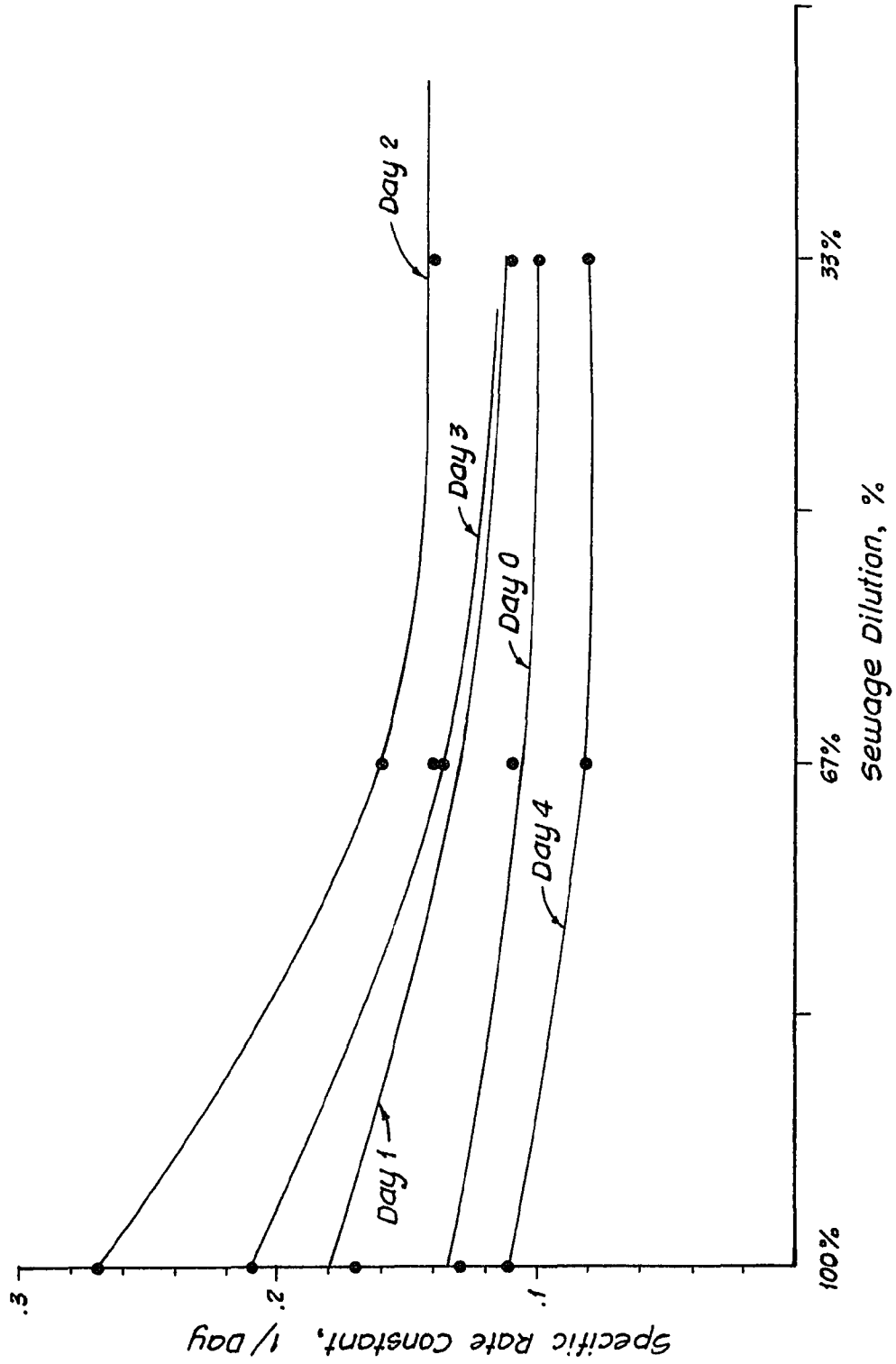
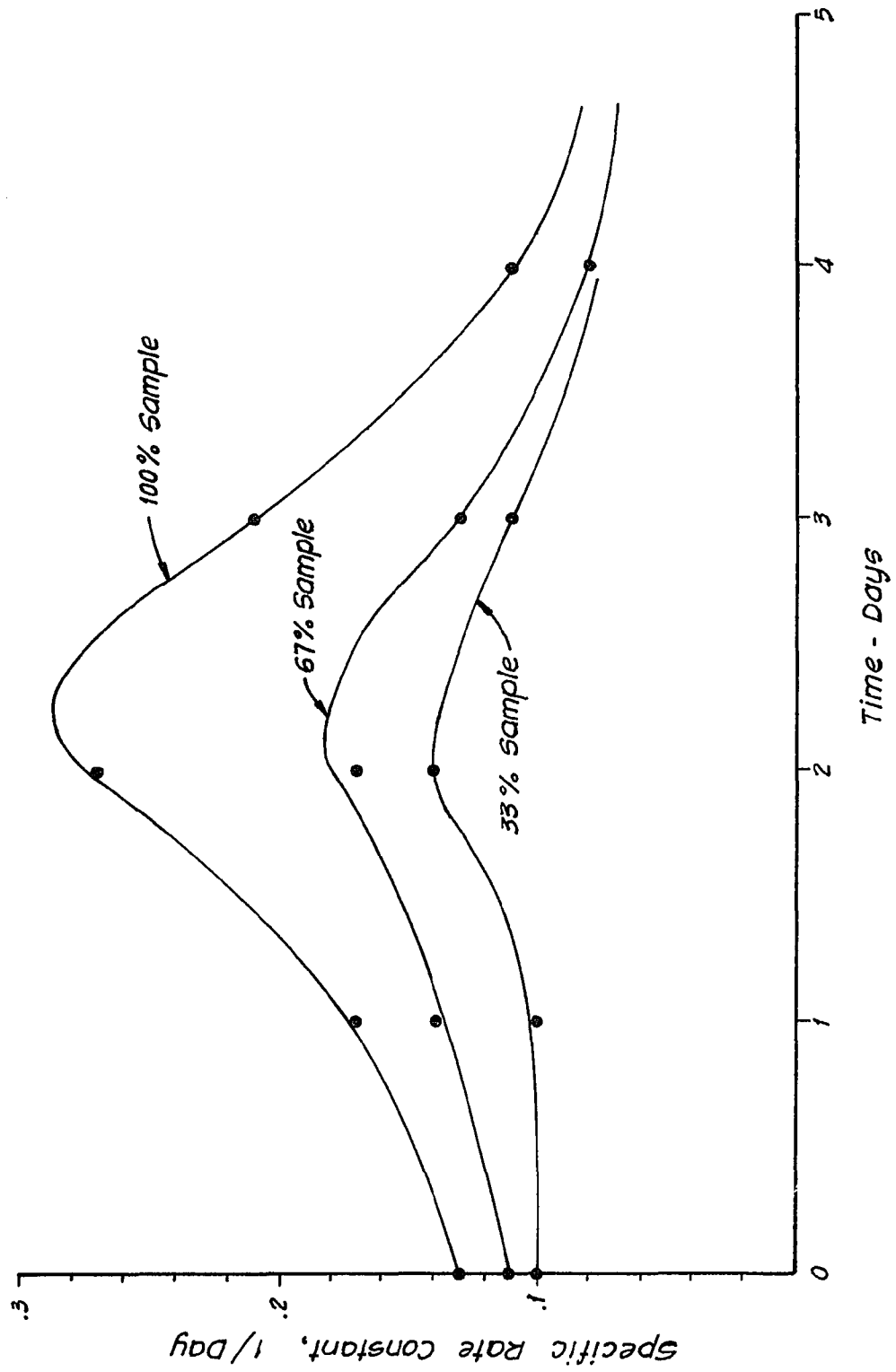


Figure No. 41

*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

Day 0	100%									67%										
	12/9	12/10	12/11	12/12	12/15	12/16	12/17				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	4.2												
B ₂	7.5	6.6	5.7	5.0	4.3	4.1	4.1	4.2												
10	5.8	4.0	4.6	2.1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
8	5.6	4.2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
6	6.6	5.8	4.0	2.5	0.8	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
5	6.6	5.4	3.8	3.3	1.2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4	6.8	5.7	4.6	3.4	2.1	1.8	1.8	2.0												
Ave	53	72	100	122	178	169	172	165												
B ₁	7.5	7.0	6.9	6.6	6.0	5.9	5.9													
B ₂	7.6	7.1	6.9	6.0	5.9	5.9	5.9													
12	6.5	5.4	4.5	3.7	1.3	1.2	1.2													
10	5.4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
8	6.8	6.3	5.6	4.9	2.8	2.4	2.4													
6	6.9	6.6	6.2	5.1	3.2	2.8	2.8													
5	7.1	X	X	X	X	X	X	X	X	X	X	X	X	X	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	12/11	12/12	12/15	12/16	12/17	
B ₁	7.3	6.8	6.3	5.9	5.2	5.2	5.2	5.2
B ₂	7.2	7.0	6.7	6.4	5.3	5.2	5.2	5.3
	1	2	3	4	7	8	9	10
20	5.8	4.8	3.4	2.0	0.4	.2	X	X
	22	31	46	62	73	75	X	X
18	6.0	4.9	3.4	1.9	0.5	0.4	X	X
	21	33	51	71	79	80	X	X
15	6.2	5.2	3.7	3.2	X	X	X	X
	21	34	56	60	X	X	X	X
12	7.1	5.7	4.5	4.3	2.3	2.3	2.3	2.3
	X	30	50	46	75	72	72	72
10	7.2	6.0	5.2	4.5	2.8	2.9	2.7	2.8
	X	27	39	49	73	69	75	74
Ave	21	29	49	57	75	75	75	73

33%

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

Day 1		12/10	12/11	12/12	12/15	12/16
100%	B ₁	5.8	5.2	4.5	3.8	3.6
	12 ml	4.1	2.5	1.3	0.3	X
	10 ml	4.5	3.0	1.7	0.4	X
	8	4.9	3.8	2.5	0.5	X
	6	5.0	X	X	X	X
	5	5.2	4.4	X	X	X
	Ave	38	61	80	104	X
	B ₁	6.0	5.6	5.1	4.2	
	B ₂	5.8	5.3	4.6	3.6	
	15	5.0	3.8	2.5	0.5	
67%	14	5.0	4.1	X	X	
	12	5.2	X	X	X	
	10	5.4	4.5	3.3	1.7	
	8	5.6	4.7	3.9	1.8	
	Ave	16	29	43	71	

BOD DATA FOR DILUTED SAMPLES AFTER
STANDING TIME OF 1 DAY

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

33%

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

Day 2	12/11	12/12	12/15	12/16	12/17	12/18	12/19
	1	2	5	6	7	8	9
B ₁	5.9	5.8	5.5	5.5	5.5	5.8	5.5
B ₂	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	0.9	X
14	4.0	3.0	2.0	1.8	1.0	X	X
12	4.2	3.6	2.4	1.9	1.5	1.3	1.2
10	4.4	3.9	2.7	2.5	2.1	2.2	2.2
8	X	X	X	X	X	X	X
Ave	46	57	79	84	97	99	103
B ₁	5.9	5.6	5.2	5.3	5.3	5.5	5.2
B ₂	5.9	5.7	5.3	5.4	5.4	5.5	5.5
25	4.3	3.6	2.3	1.8	1.5	1.4	1.4
20	4.6	4.1	2.8	2.2	2.4	2.3	2.2
15	4.9	4.6	3.6	3.5	3.4	3.5	X
12	5.0	4.7	3.7	3.5	3.5	3.4	X
10	X	X	X	X	X	X	X
Ave	20	23	36	45	44	47	47

67%

100%

BOD DATA FOR DILUTED SAMPLES
AFTER STANDING TIME OF 2 DAYS

Day 2	12/11	12/12	12/15	12/16	12/17	12/18	12/19
B ₁	5.8	5.6	5.2	5.2	5.2	5.2	5.0
B ₂	1	2	5	6	7	8	9
30	5.4 4	5.2 4	4.4 6.5	4.4 7.5	4.2 10	4.2 10	
25	5.8 0	5.4 2.4	4.7 4.2	4.6 7	4.6 7	4.7 8	
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	5.5	4.7	4.8	4.7	4.8	
Ave	3	2	5	8	10	10	

33%

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

Day 3	100%								67%									
	12/12 1	12/15 4	12/16 5	12/17 6	12/18 7	12/19 8	12/12 1	12/15 4	12/16 5	12/17 6	12/18 7	12/19 8	12/12 1	12/15 4	12/16 5	12/17 6	12/18 7	12/19 8
B ₁	6.0	5.5	5.6	5.6	5.8	5.7	6.0	5.5	5.6	5.8	5.7	6.0	5.5	5.6	5.8	5.7	6.0	5.7
B ₂	6.1	5.7	5.8	5.8	6.0	6.0	5.7	5.7	5.8	6.0	6.0	5.7	5.7	5.7	5.8	6.0	5.7	6.0
25 ml	4.5	2.3	1.8	1.2	0.8	X	4.5	2.3	1.8	1.2	X	4.5	2.3	1.8	1.2	X	4.5	2.3
	18	39	47	54	61	X	18	39	47	54	X	18	39	47	54	X	18	39
20	4.7	2.9	2.6	2.0	1.6	1.5	4.7	2.9	2.6	2.0	1.6	1.5	4.7	2.9	2.6	2.0	1.6	1.5
	20	40	47	55	64	65	20	40	47	55	64	65	20	40	47	55	64	65
15	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12	5.0	3.8	X	X	X	X	5.0	3.8	X	X	X	5.0	3.8	X	X	X	5.0	3.8
	26	45	X	X	X	X	26	45	X	X	X	26	45	X	X	X	26	45
10	5.3	4.2	3.7	3.6	3.5	3.4	5.3	4.2	3.7	3.6	3.5	3.4	5.3	4.2	3.7	3.6	3.5	3.4
	22	42	60	63	72	73	22	42	60	63	72	73	22	42	60	63	72	73
Ave	21	41	51	57	66	69	21	41	51	57	66	69	21	41	51	57	66	69
B ₁	6.2	5.8	5.8	5.8	6.0	5.8	6.2	5.8	5.8	6.0	5.8	6.2	5.8	5.8	6.0	5.8	6.2	5.8
B ₂	5.7	5.2	5.2	5.0	7	8	5.7	5.2	5.2	5.0	7	8	5.7	5.2	5.2	5.0	7	8
	1	4	5	6	7	8	1	4	5	6	7	8	1	4	5	6	7	8
25	5.7	4.8	4.7	4.1	4.0	4.0	5.7	4.8	4.7	4.1	4.0	4.0	5.7	4.8	4.7	4.1	4.0	4.0
	6	12	13	20	24	22	6	12	13	20	24	22	6	12	13	20	24	22
20	5.7	4.8	4.5	4.2	4.3	4.1	5.7	4.8	4.5	4.2	4.3	4.1	5.7	4.8	4.5	4.2	4.3	4.1
	8	15	19	24	26	26	8	15	19	24	26	26	8	15	19	24	26	26
18	5.9	4.8	4.5	4.3	4.4	4.2	5.9	4.8	4.5	4.3	4.4	4.2	5.9	4.8	4.5	4.3	4.4	4.2
	5	16	26	25	26	27	5	16	26	25	26	27	5	16	26	25	26	27
15	5.9	5.0	4.7	4.6	4.7	4.6	5.9	5.0	4.7	4.6	4.7	4.6	5.9	5.0	4.7	4.6	4.7	4.6
	6	16	22	24	19	18	6	16	22	24	19	18	6	16	22	24	19	18
12	5.9	5.1	4.7	4.4	X	X	5.9	5.1	4.7	4.4	X	X	5.9	5.1	4.7	4.4	X	X
	7	17	26	X	X	X	7	17	26	X	X	X	7	17	26	X	X	X
Ave	7	15	23	26	25	26	7	15	23	26	25	26	7	15	23	26	25	26

BOD DATA FOR DILUTED SAMPLES
AFTER STANDING TIME OF 3 DAYS

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

33%

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

Day 4	100%							67%						
	12/15	12/16	12/17	12/18	12/19			12/15	12/16	12/17	12/18	12/19		
B ₁	5.7	5.7	5.5	5.8	5.7			X	X	X	X	X		
B ₂	5.7	5.6	5.5	5.8	5.6			X	X	X	X	X		
25 ml	4.0	3.7	2.6	2.2	1.9			20	24	35	43	45		
20	X	X	X	X	X			X	X	X	X	X		
18	4.2	3.9	3.1	2.9	2.8			25	29	40	48	47		
15	4.4	3.8	3.4	3.5	3.2			26	X	42	46	49		
12	4.7	4.3	3.9	4.0	3.8			25	34	40	45	46		
Ave	24	31	39	45	47									
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	2.6	2.2	1.7	1.4			12	20	21	26	27		
30	3.6	2.9	2.4	2.0	1.6			15	21	23	28	30		
25	32	26	2.1	X	X			23	28	31	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	12/15	12/16	12/17	12/18	12/19
	3	4	5	6	7
B ₁	7.4	7.2	7.3	7.6	7.5
B ₂					
40	5.4 X	X	X	X	X
35	6.4 9	6.0 10	5.9 13	6.1 13	5.8 14
30	6.6 8	6.1 11	6.1 12	6.3 13	6.0 15
25	6.7 8	6.5 9	6.3 12	6.6 12	6.4 13
20	7.0 6	6.7 8	X X	X X	X X
Ave	8	10	12	13	14

33%

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
57	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		

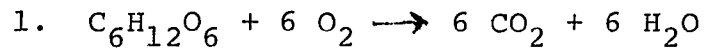
100%, L=48			67%, L=28			33%, L=13		
y	TIME	k	y	TIME	k	y	TIME	k
1	1	.01	4	1	.07	2	1	.07
17	2	.09	9	2	.08	4	2	.08
25	3	.10	14	3	.10	6	3	.09
31	4	.11	21	4	X	8	4	.1
38	5	X	24	5	X	10	5	.11
Ave .1			Ave .083			Ave :09		

APPENDIX C

DERIVATION OF THE COD FORMULA

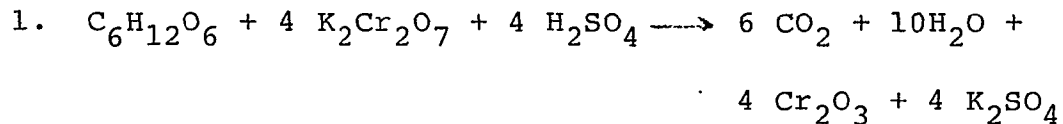
DERIVATION OF COD FORMULA

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

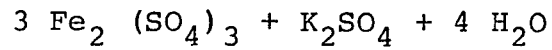
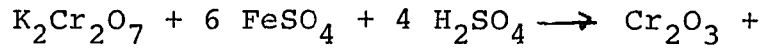


- a. There is a 6 to 1 mole ratio of sugar to oxygen.
- b. The time for completion is 10-20 days.

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



- 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.



- 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. $V_S M = \text{mole Ferrous Sulfate}$
2. $V_S M / 6 = \text{mole XS PDC remaining}$

3. Moles of Dichromate which reacted

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

4. Moles of organic present

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

5. Moles of oxygen required

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

6. Milligrams of oxygen required

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

7. Final form after simplification

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

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.

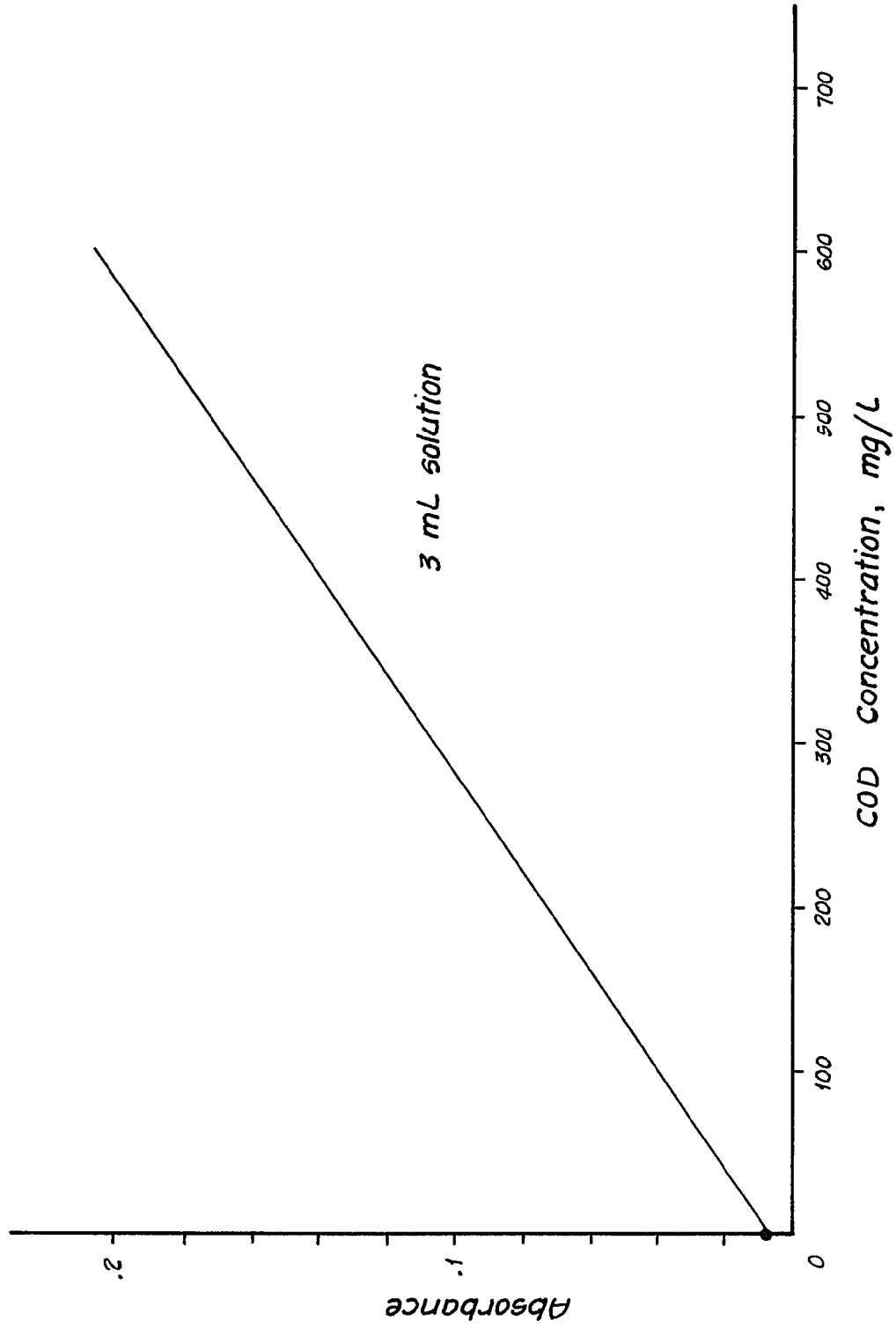


Figure No. 43
*Calibration Curve For Glucose Determination
Using O-Toluidine Method @ 635 NM.*

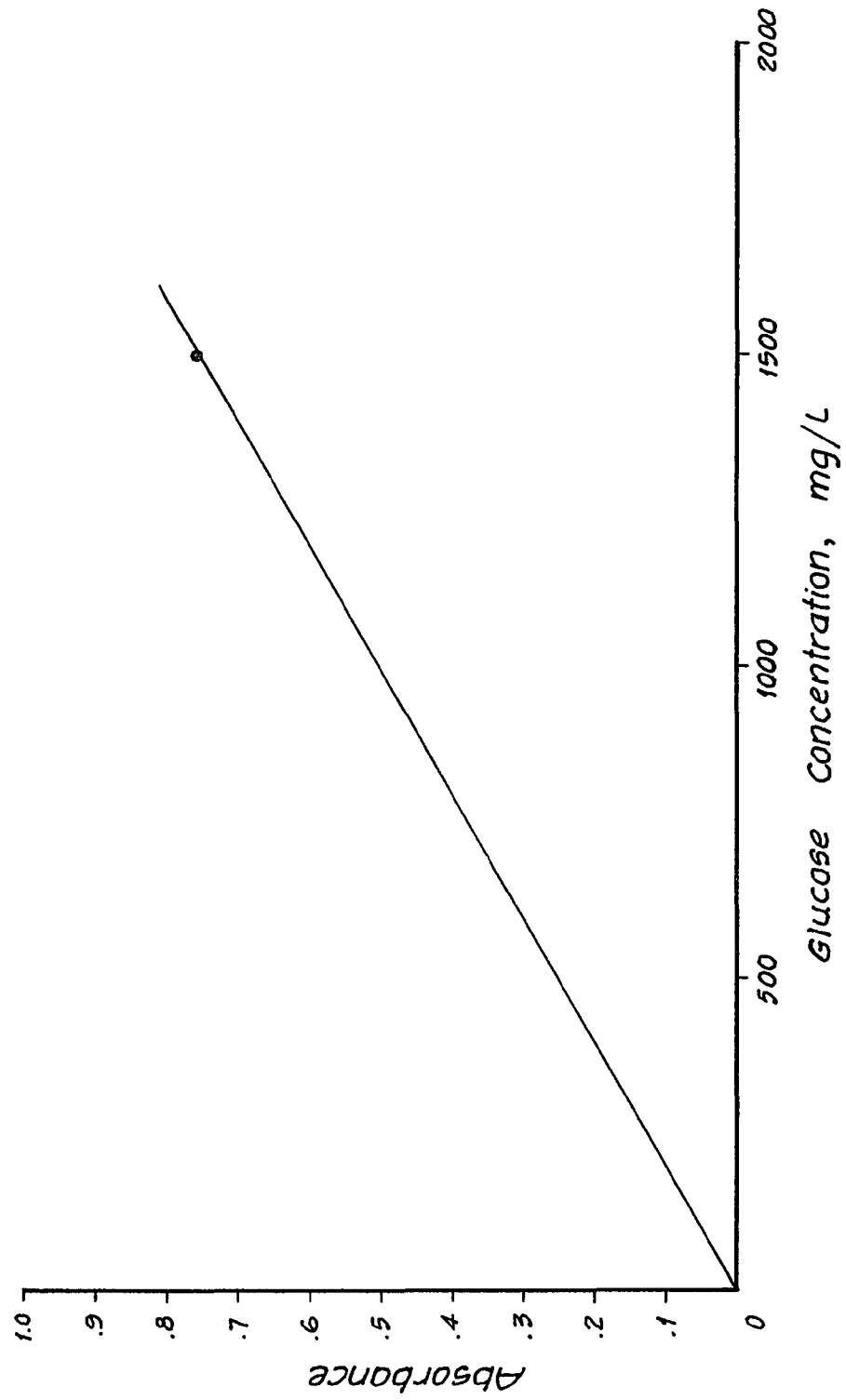


Figure No. 44
*Calibration Curve For Ammonia
Determination By Nessler Procedure*

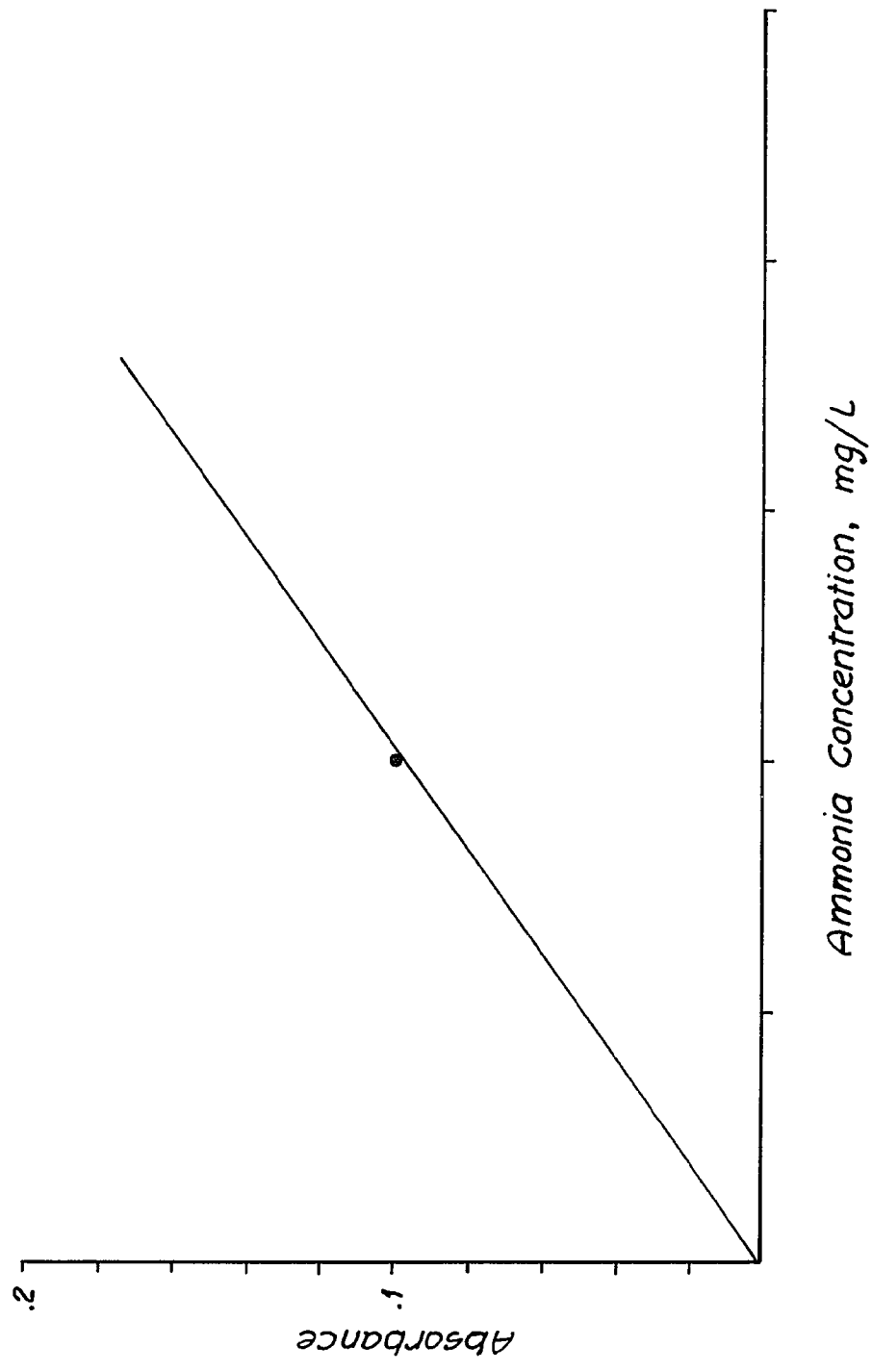
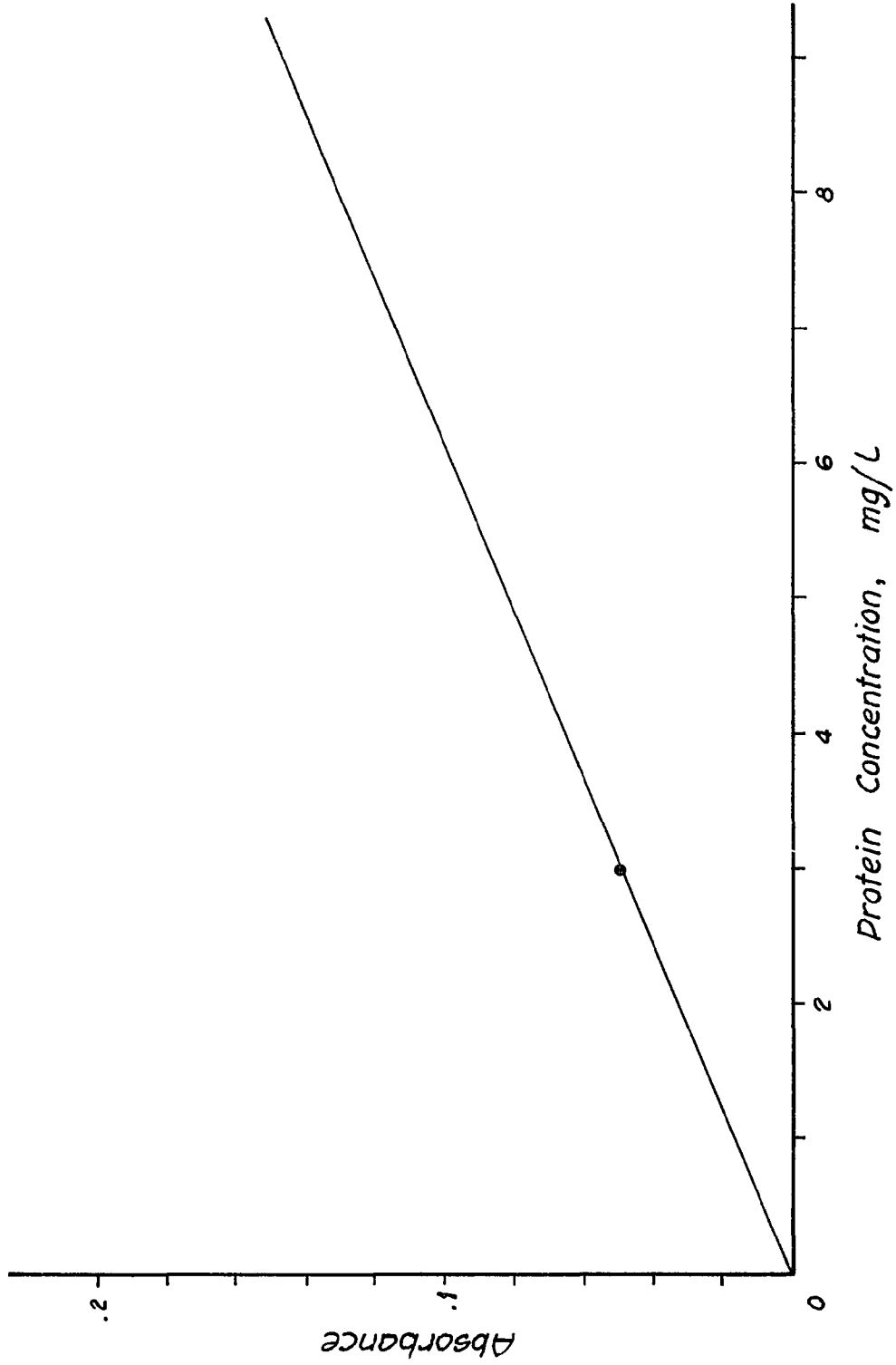


Figure No. 45
*Calibration Curve For Protein Using The Biuret
Reaction @ 540 NM.*



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

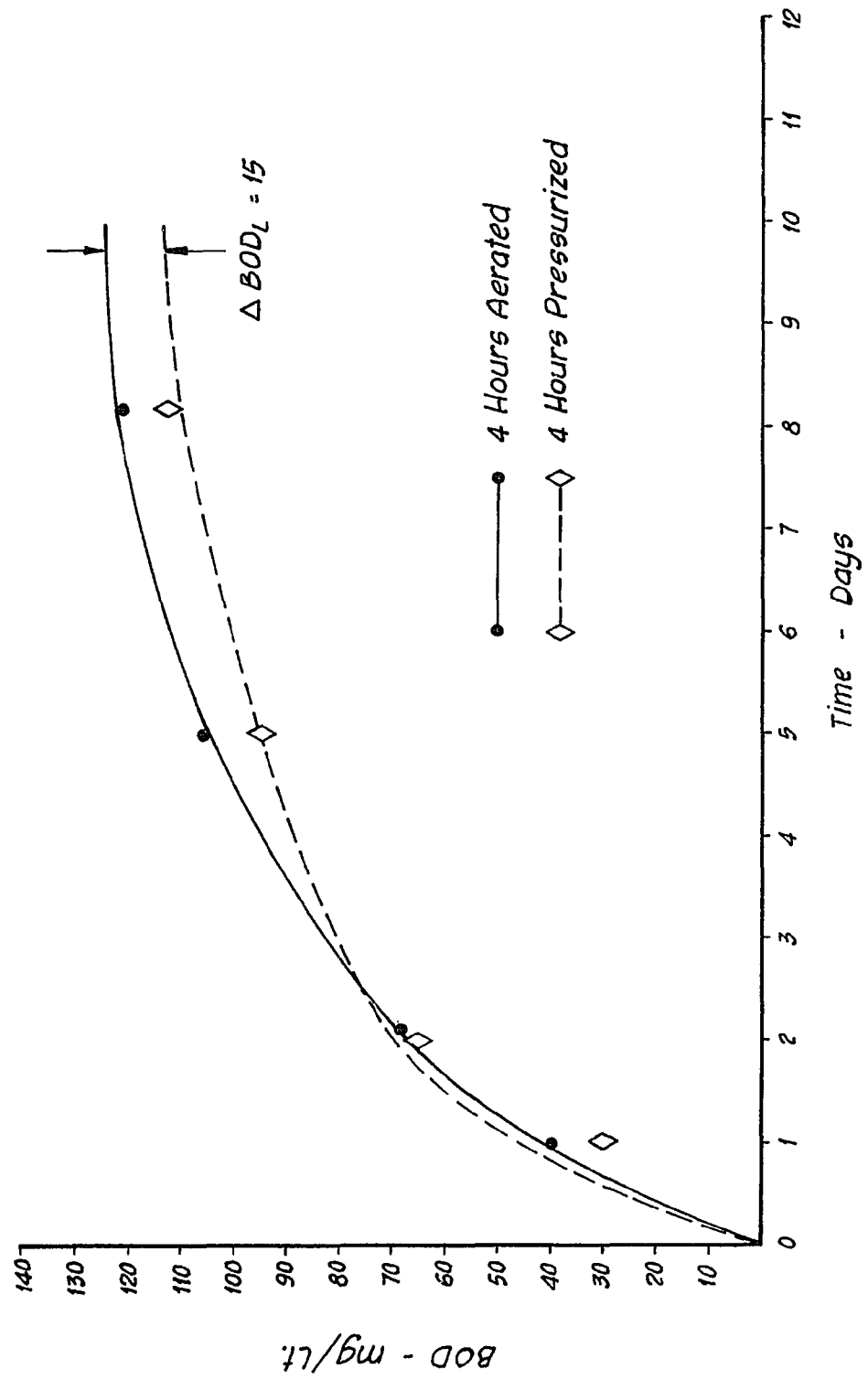


Figure No. 47
 Variation of BOD For Pressurized And Non-Pressurized
 Wastewater Samples For The Same Detention Time

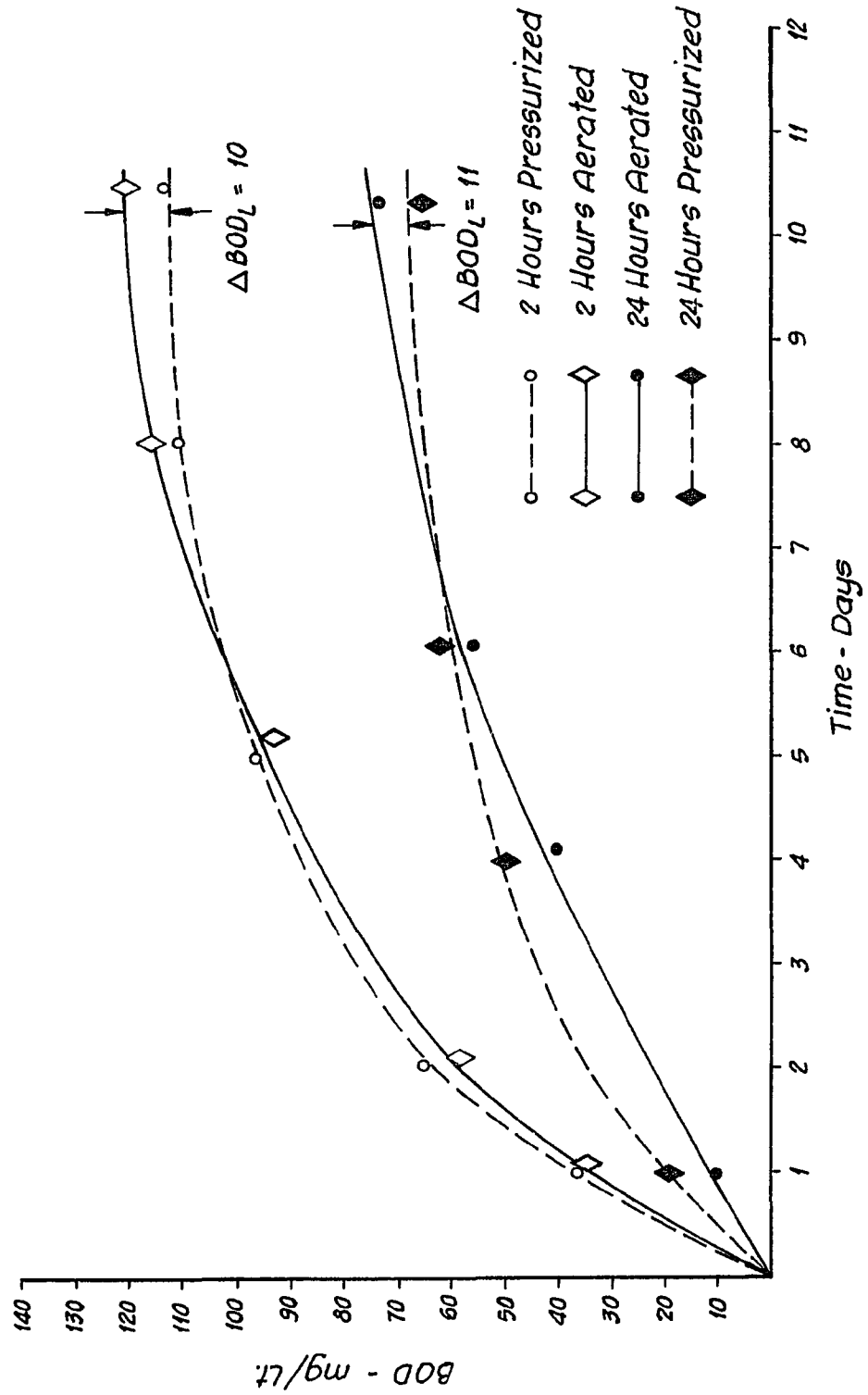
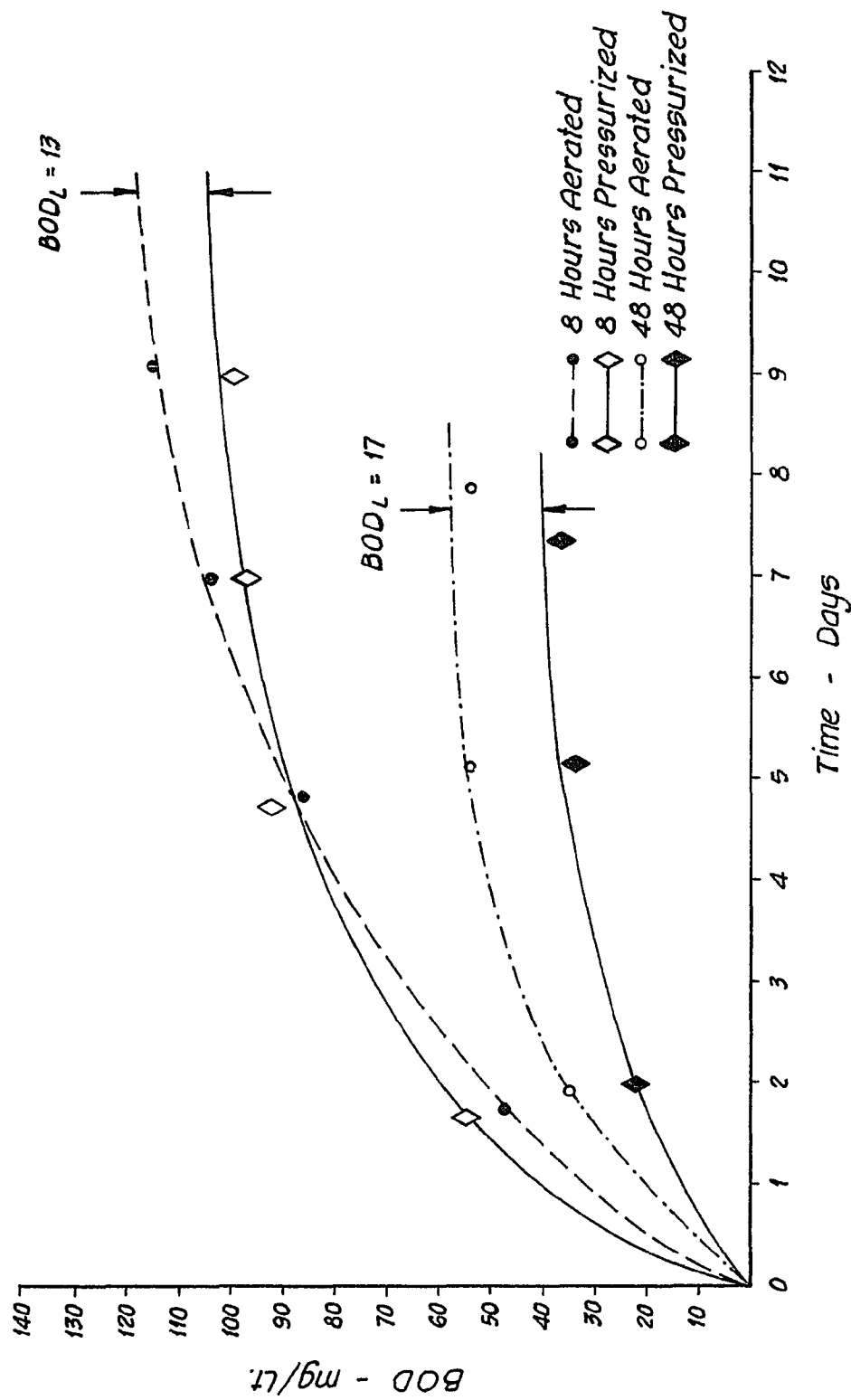


Figure No. 48
 Variation of BOD For Pressurized And Non-Pressurized
 Wastewater Samples For The Same Detention Time



APPENDIX F

DEMAND CURVES OBTAINED FROM SEEDED AND
UNSEDED DILUTION WATER

Figure No. 49
 Variation of BOD For Wastewater Samples Prepared In Seeded And
 Unseeded Dilution Water Pressurized For Different Detention Times

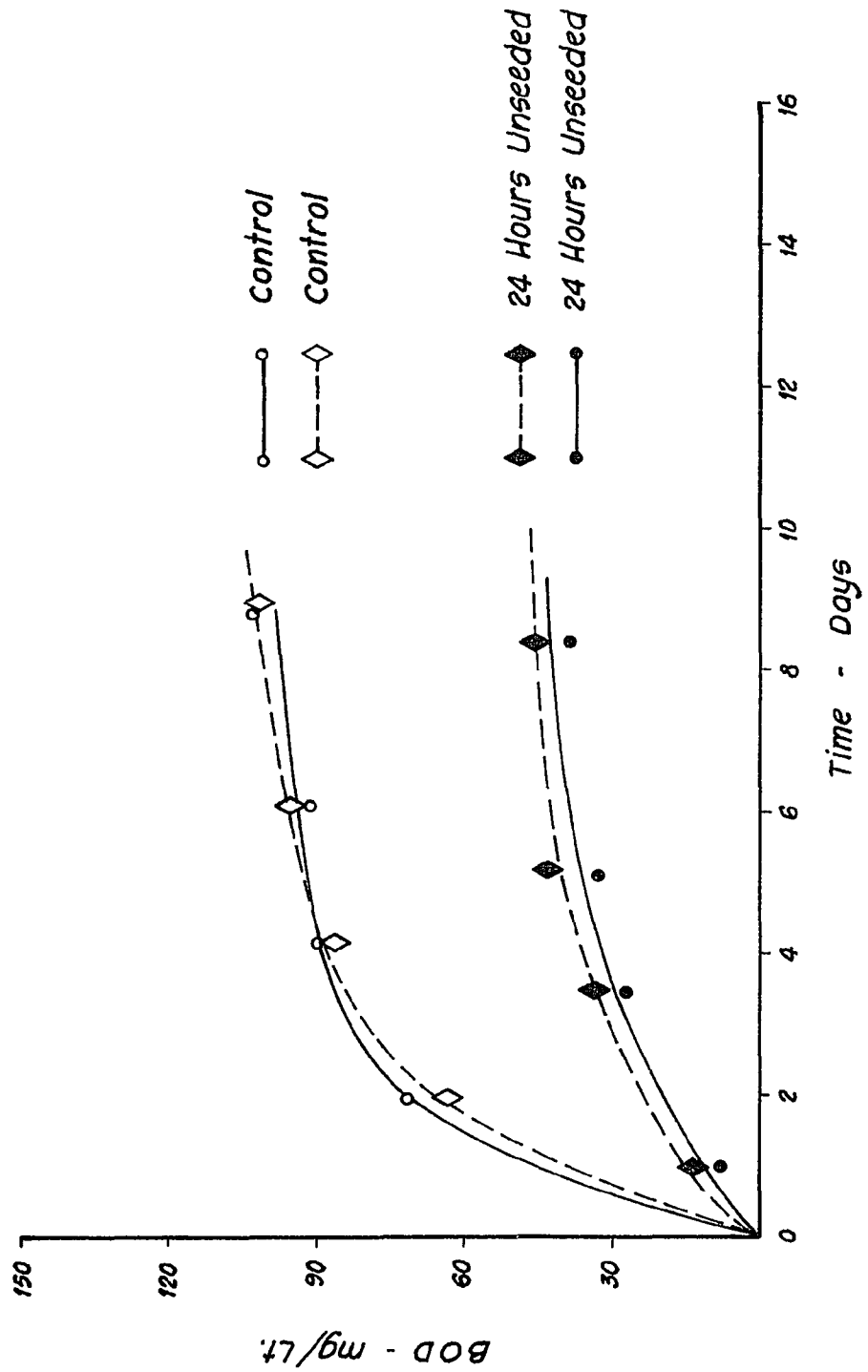


Figure No. 50
 Variation Of BOD For Wastewater Samples Prepared In Seeded And Unseeded Dilution Water Pressurized For Different Detention Times

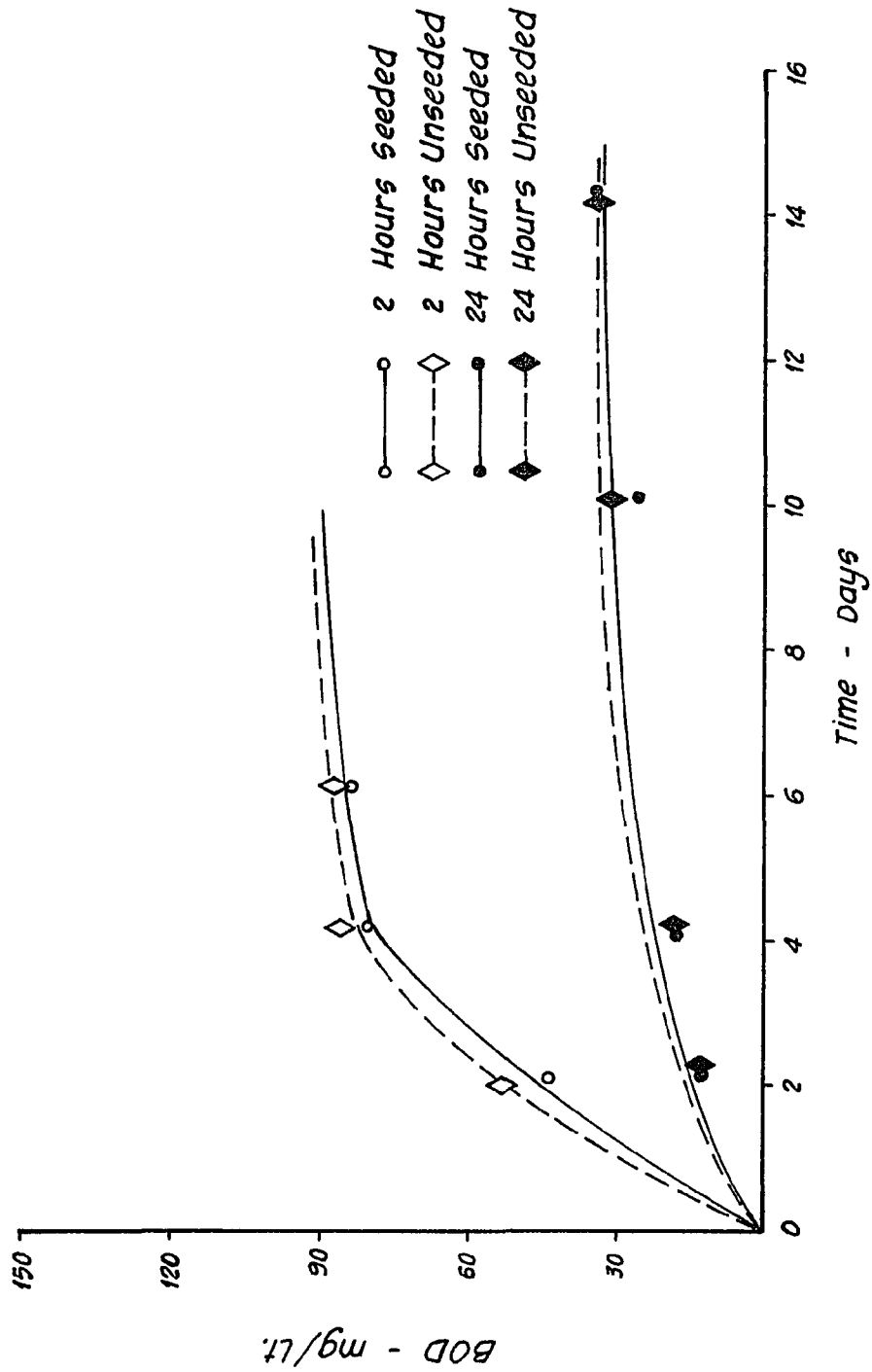


Figure No. 51
 Variation of BOD For Wastewater Samples Prepared In Seeded And
 Unseeded Dilution Water Pressurized For Different Detention Times

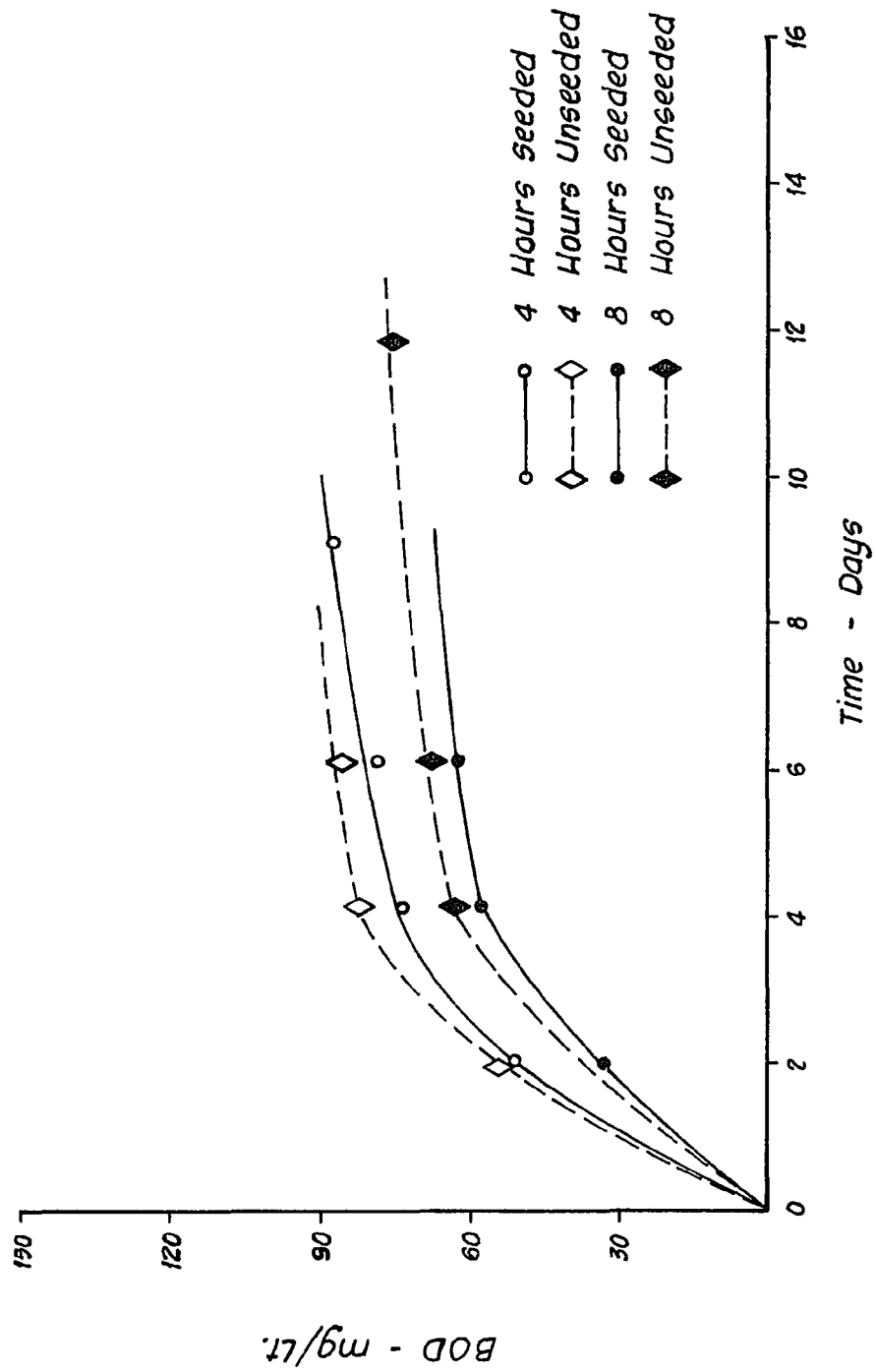


Figure No. 52
Variation of COD of Pressurized And
Non-Pressurized Wastewater Samples

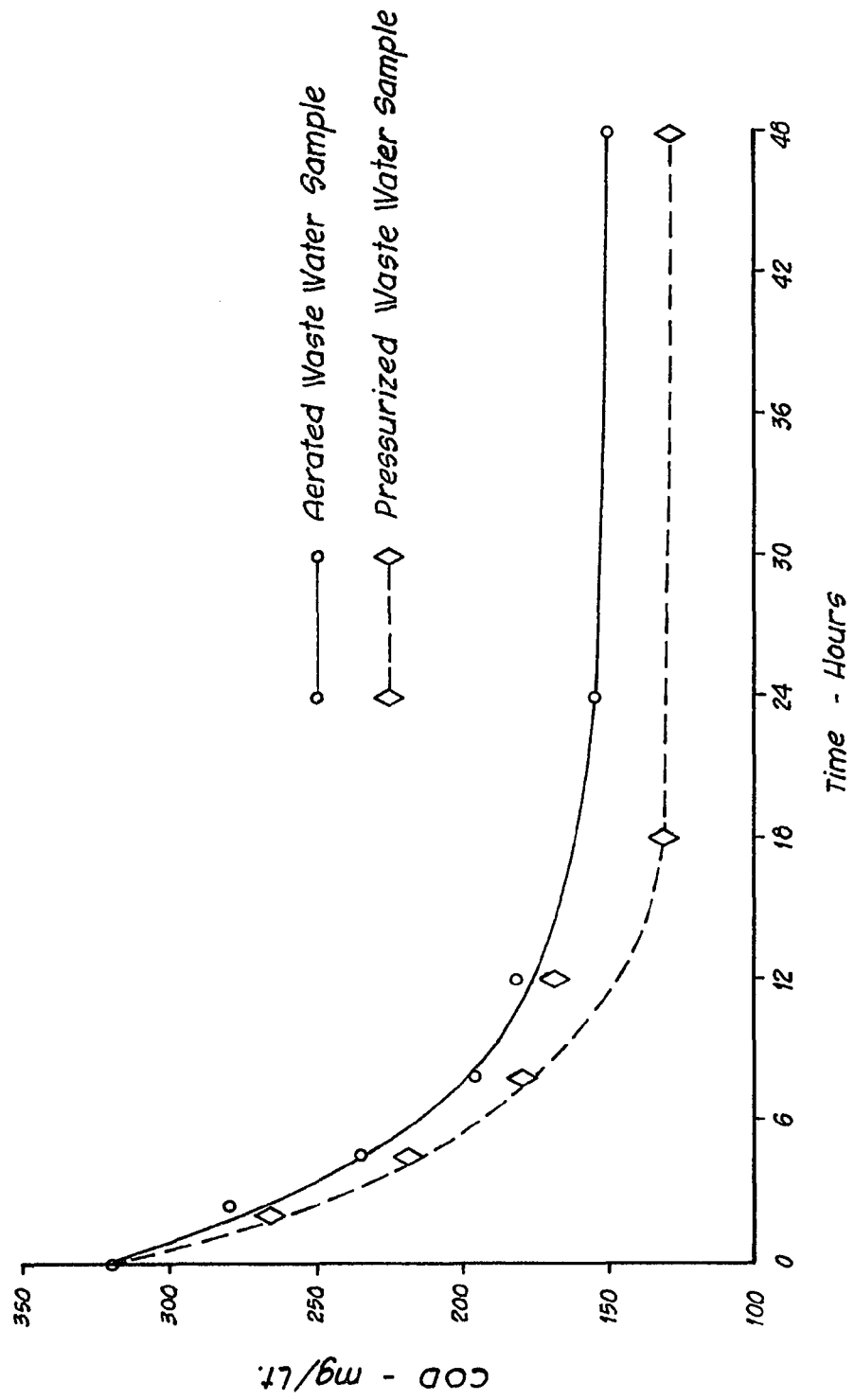
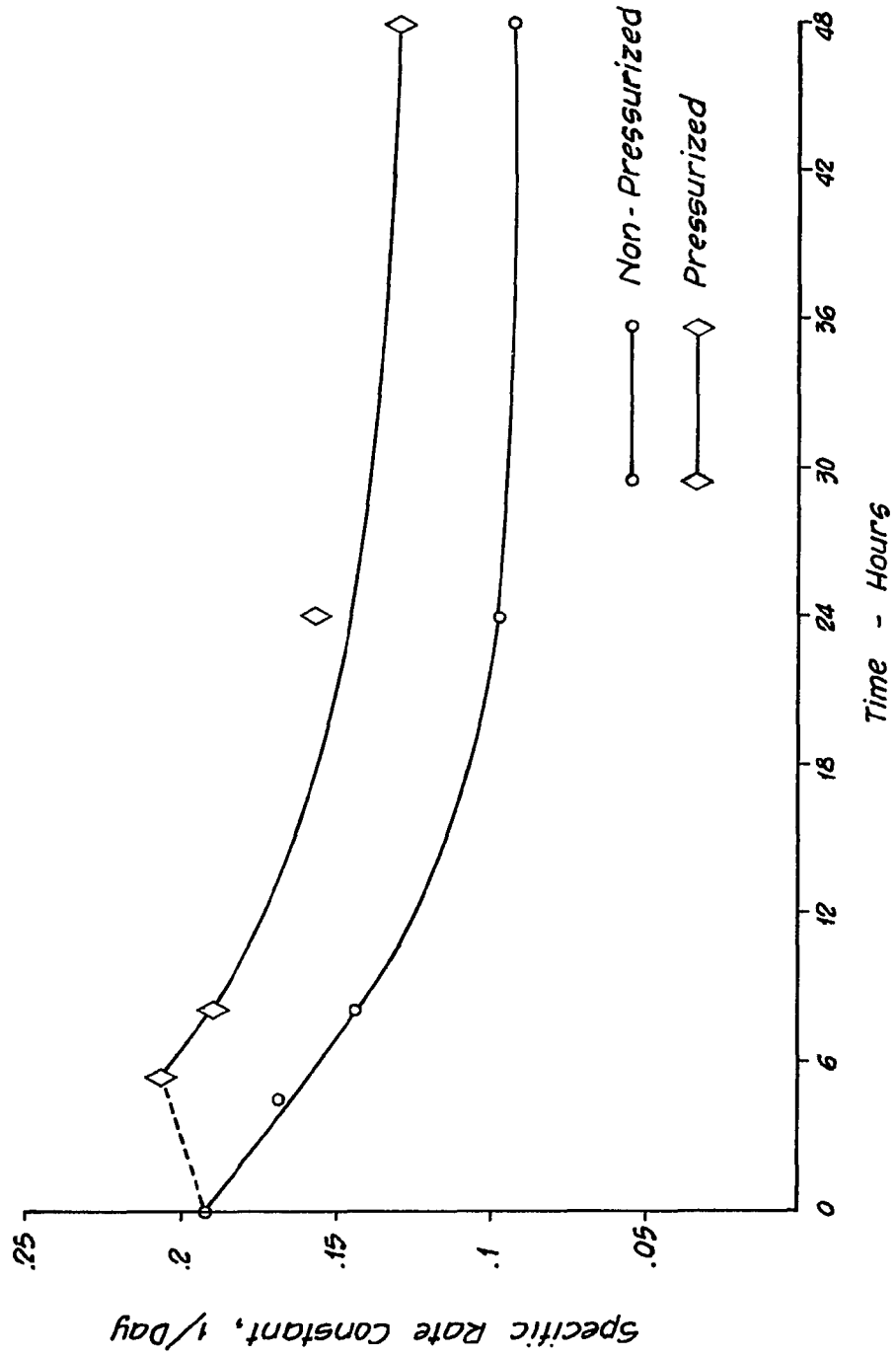


Figure No. 53
Variation Of Specific Rate Constant For The Decomposition Of Wastewater For Pressurized And Non-Pressurized Samples



APPENDIX G

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

Figure No. 54
 Variation of BOD For samples Aerated And
 Pressurized For 24 Hours Versus The Control

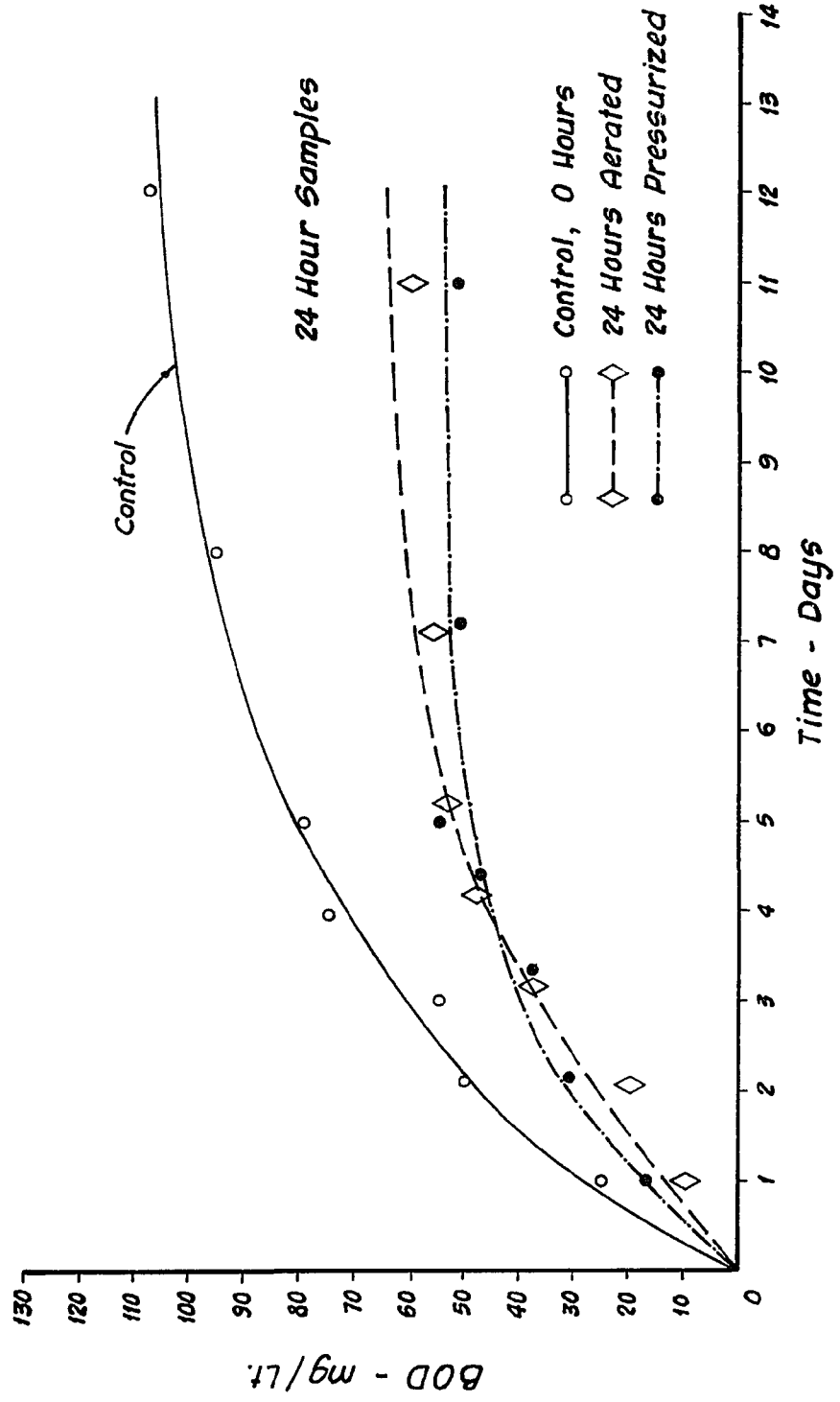
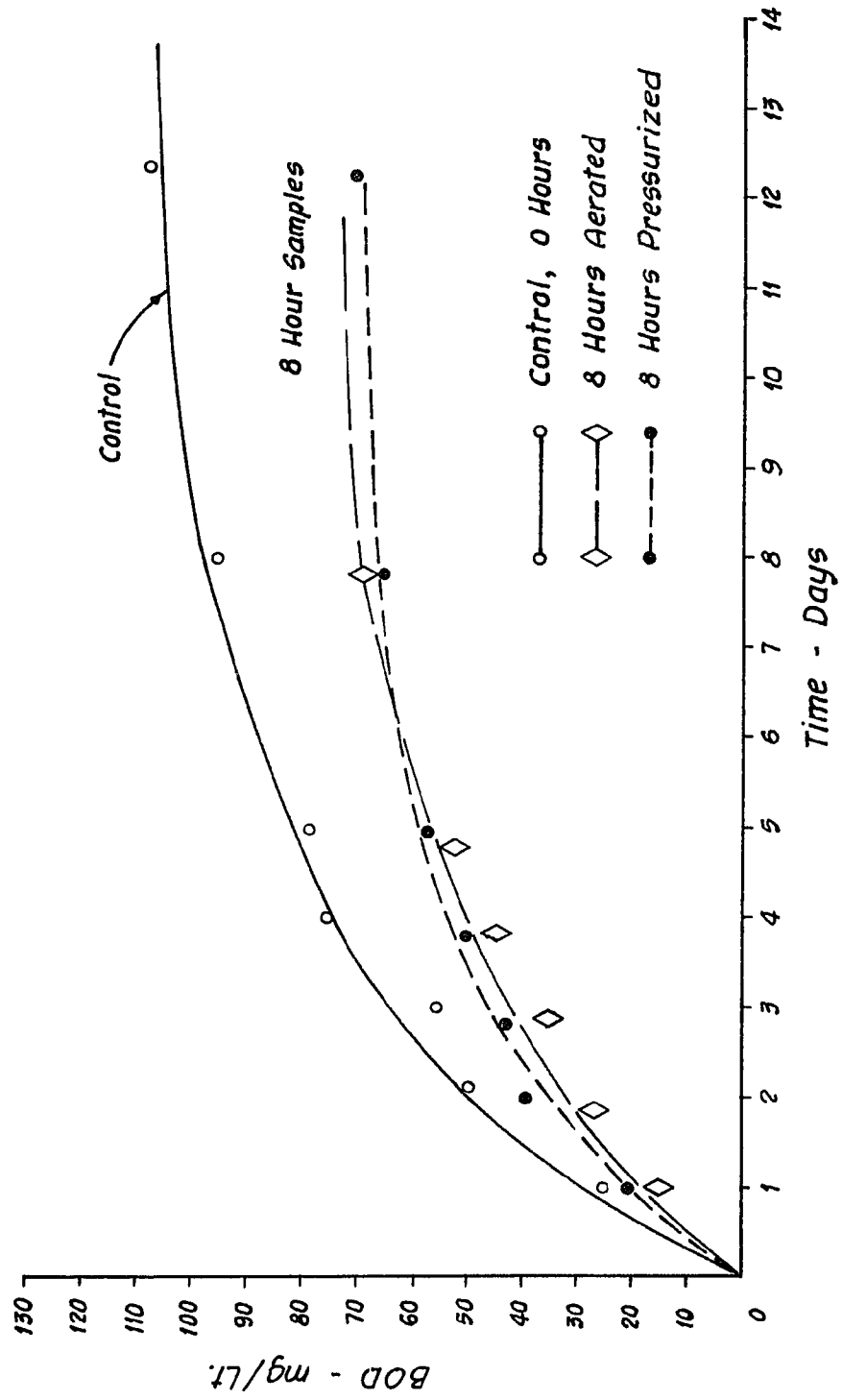


Figure No. 55
 Variation Of BOD For Samples Aerated And
 Pressurized For 8 Hours Versus The Control



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

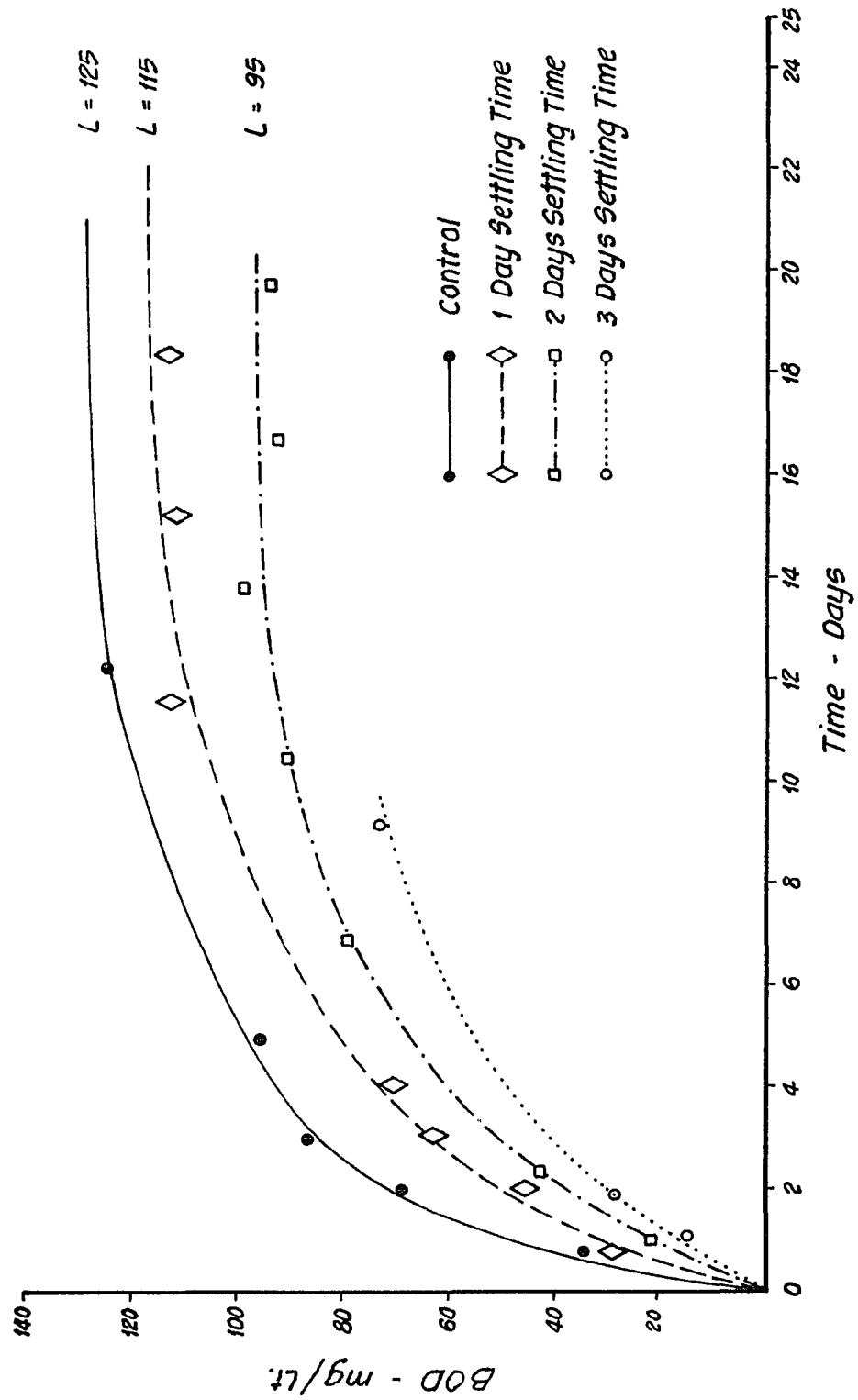


Figure No. 57
 Variation of BOD With Time For Samples Pressurized For
 Half Hour And Allowed To Settle For A Period Of Three Days

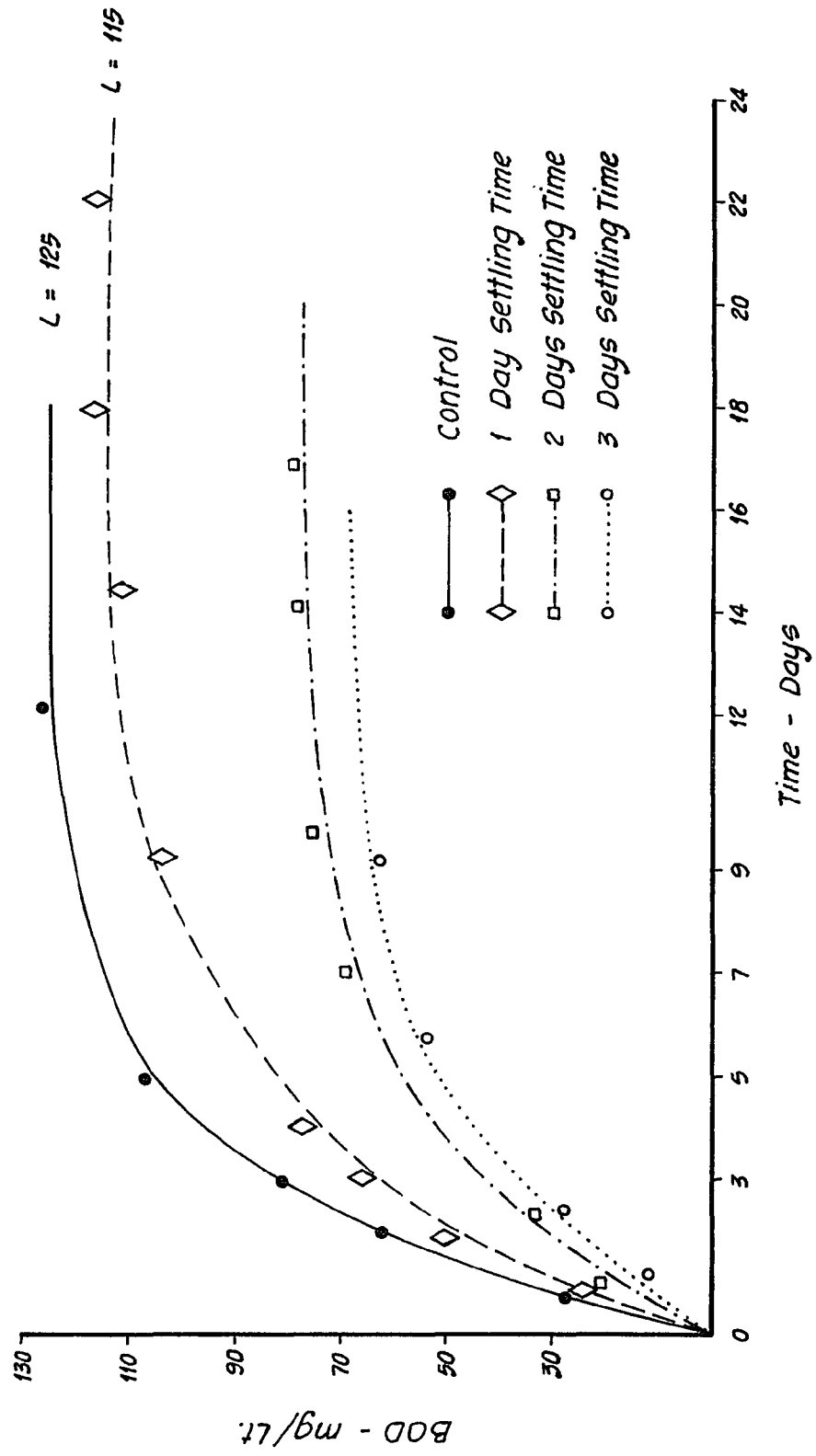


Figure No. 58
 Variation of BOD With Time For Samples Pressurized For
 1.5 Hours And Allowed To Settle For A Period Of Three Days

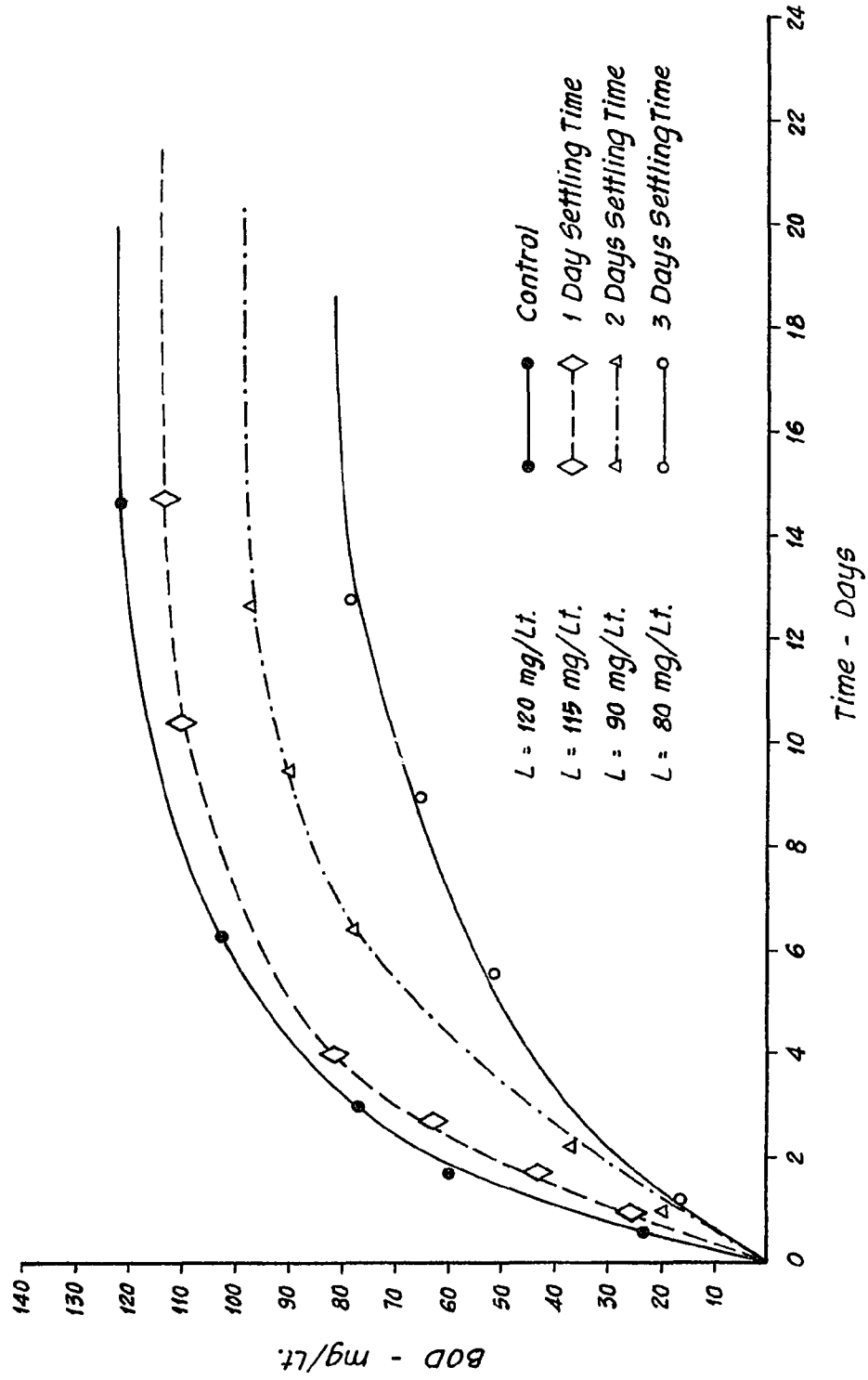


Figure No. 59
 Variation Of BOD With Time For Samples Pressurized For
 Three Hours And Allowed To Settle For Up To Three Days

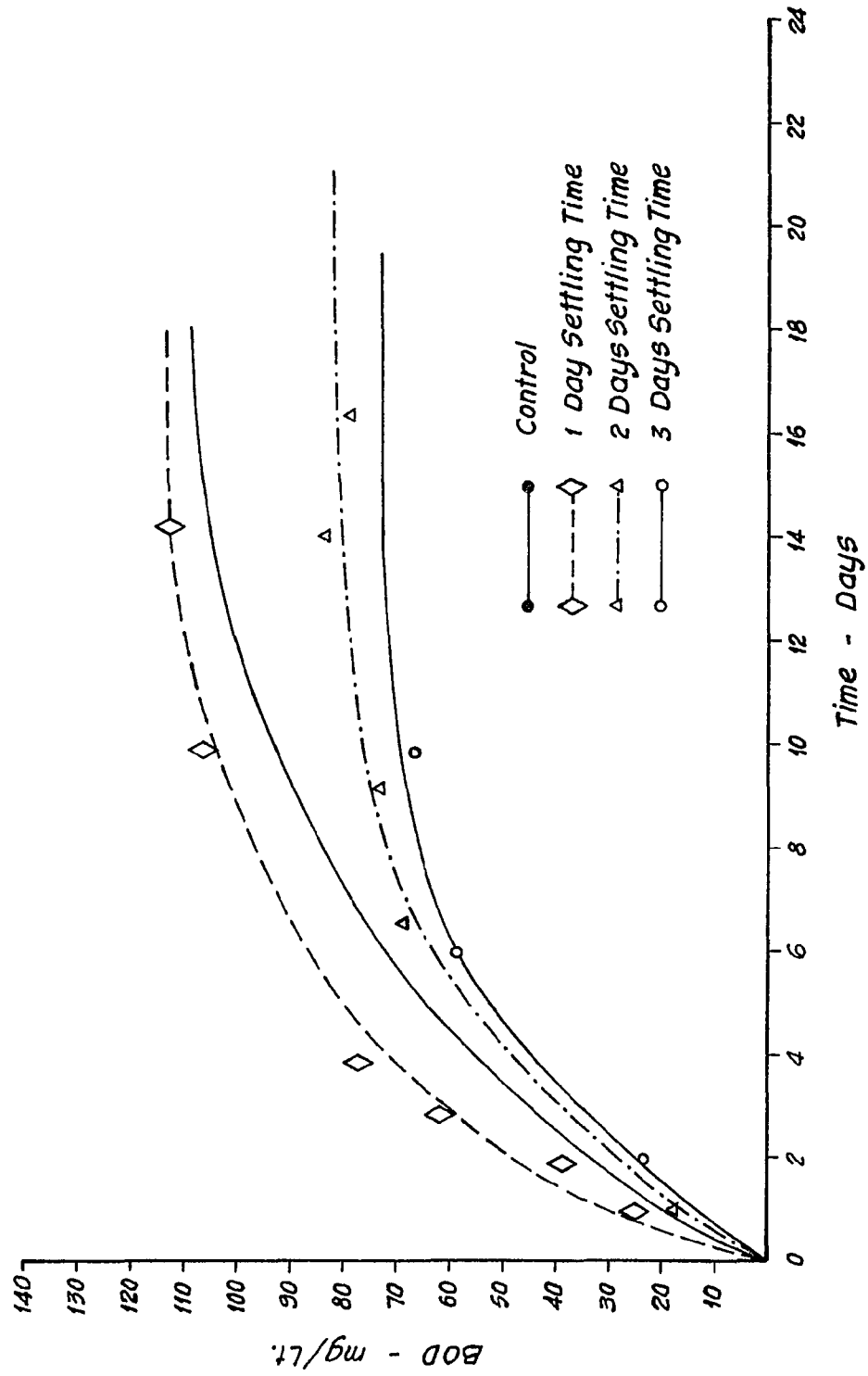
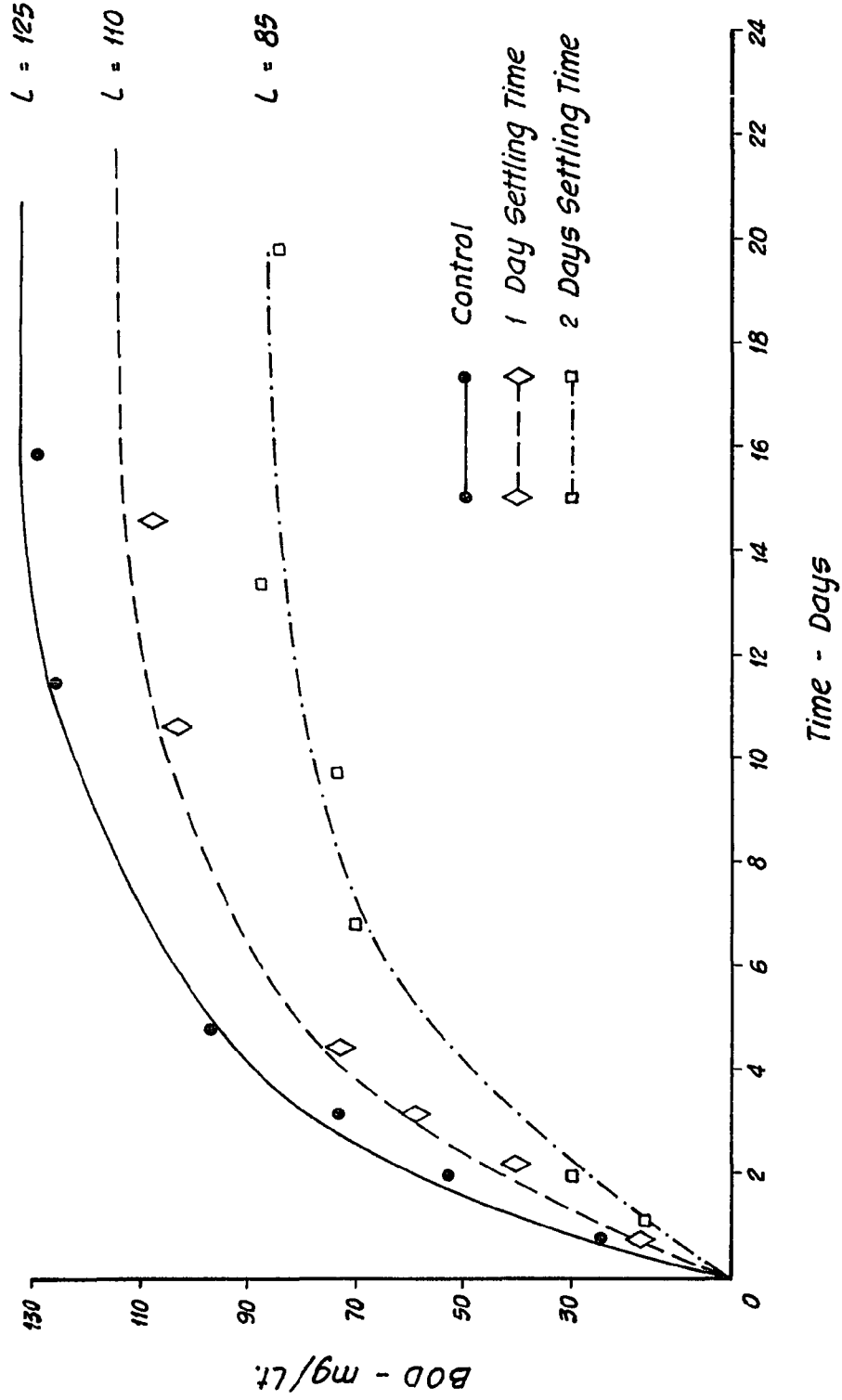


Figure No. 60
 Variation of BOD With Time For Samples Pressurized For
 One Hour And Allowed To Settle For A Period Of Three Days



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

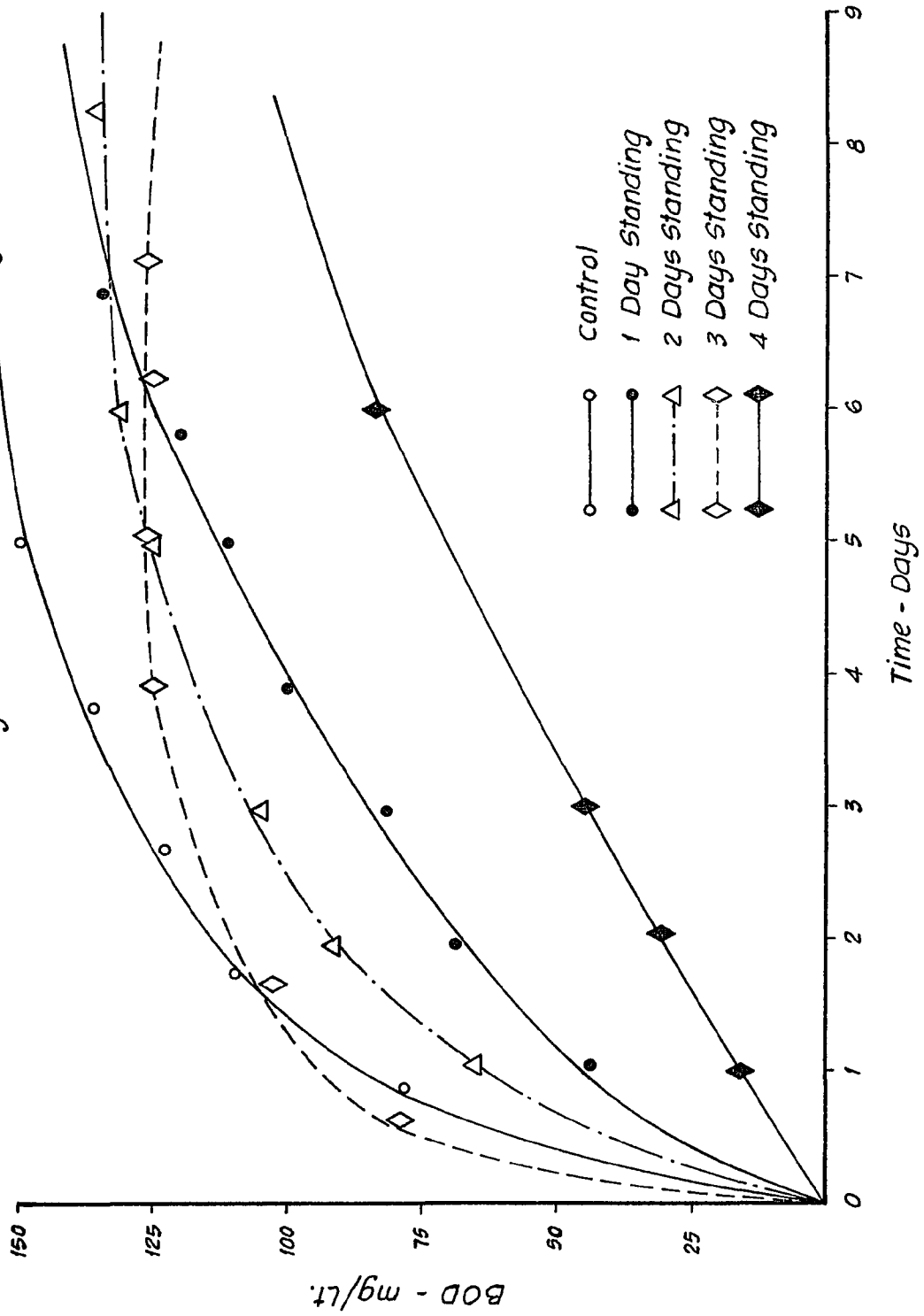


Figure No. 62
Variation Of The Specific Rate Constant For A Pressurized Sample Allowed To Stand For A Period Of Three Days

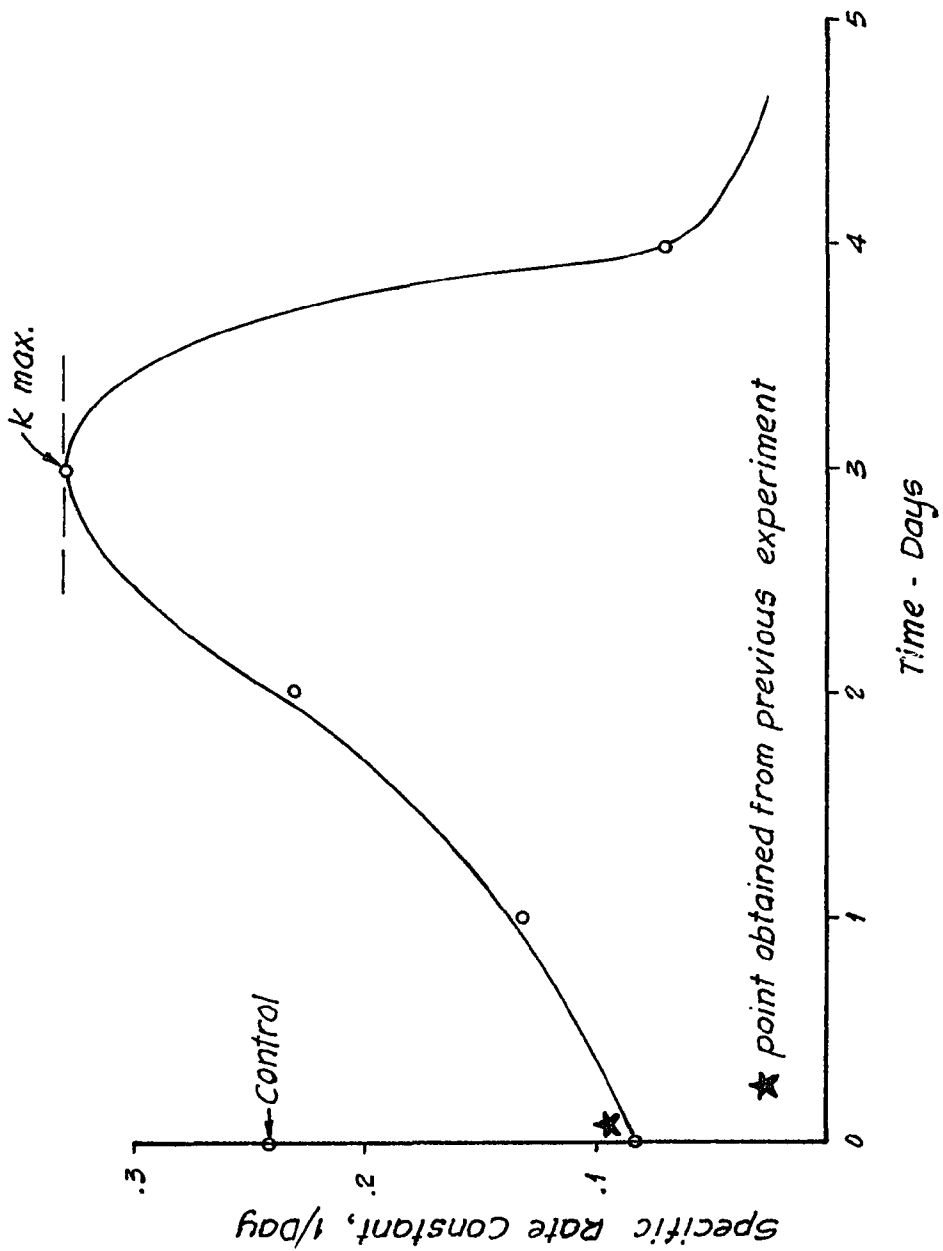
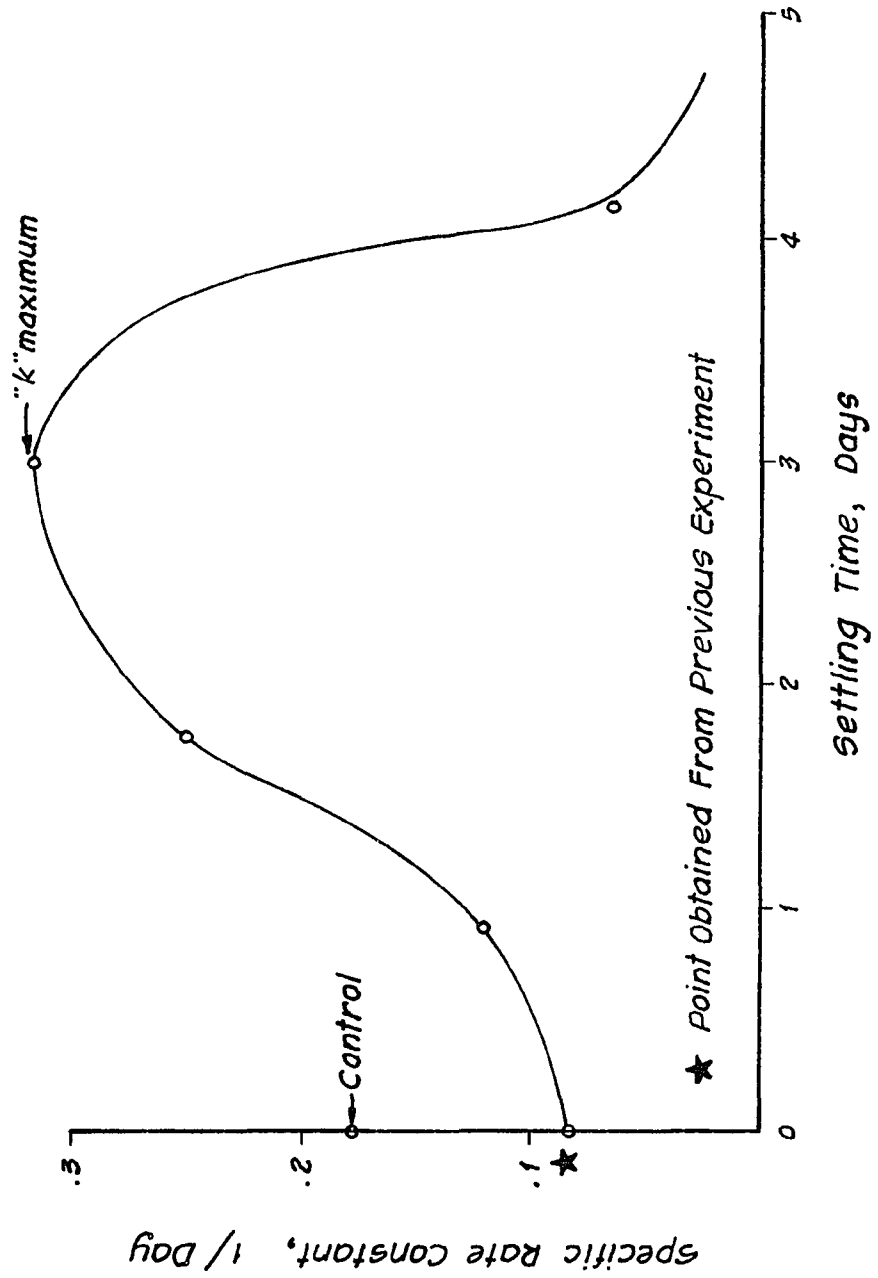


Figure No. 63
*Variation Of The Specific Rate Constant For A Pressurized
Sample Allowed To Stand For A Period Of Four Days*



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-Dimethylaminoantipyrine, 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

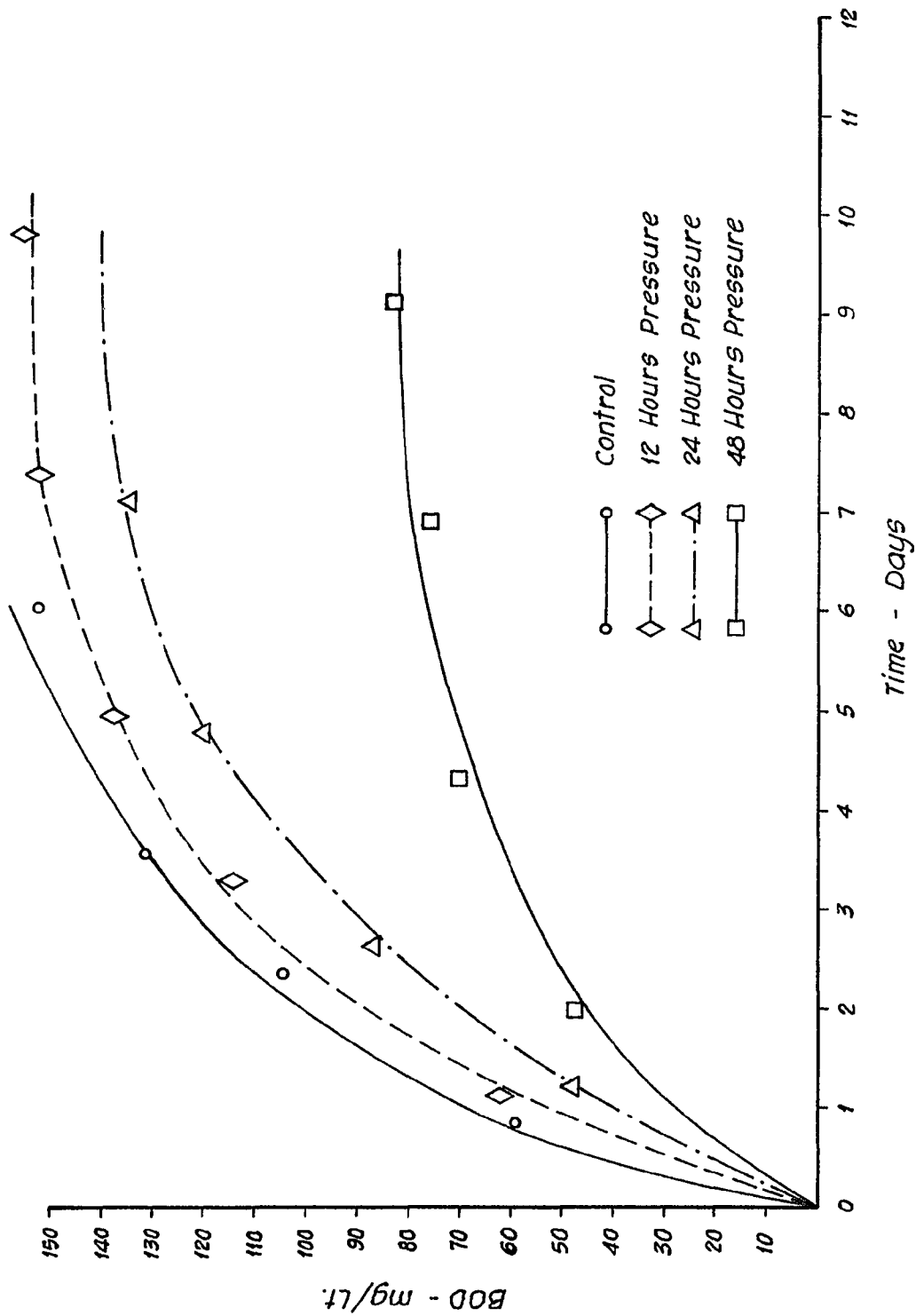
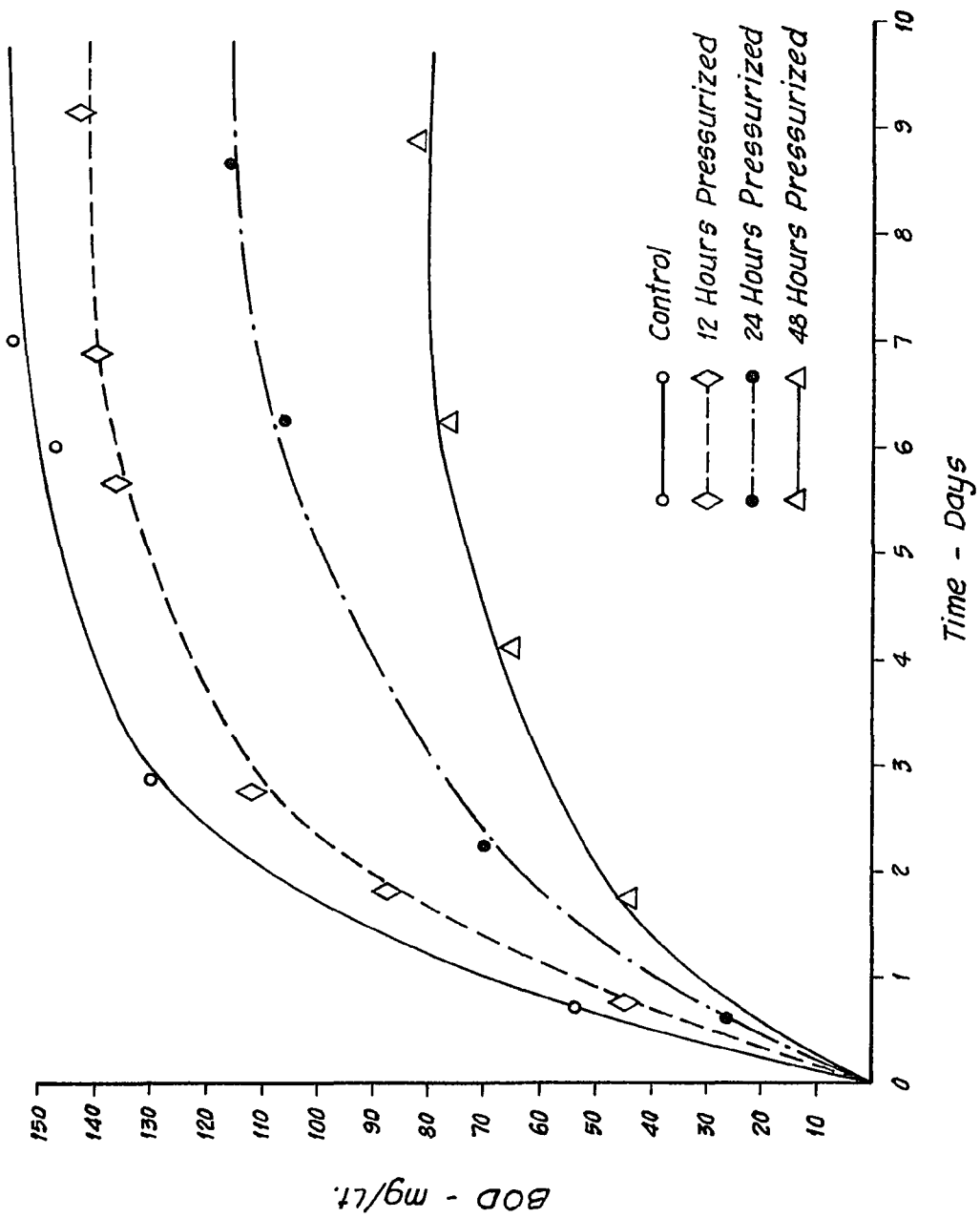


Figure No. 65
 Variation of BOD Values of Stearic Acid For Aerated Samples



APPENDIX L

THE EFFECTS OF PRESSURE ON THE HYDROLYSIS
RATE OF PROTEINS TO PRODUCE AMMONIA

Figure No. 66
Variation Of The Specific Rate Constant As A Function
Of The Amount Of Glucose In A Glucose/Starch Mixture

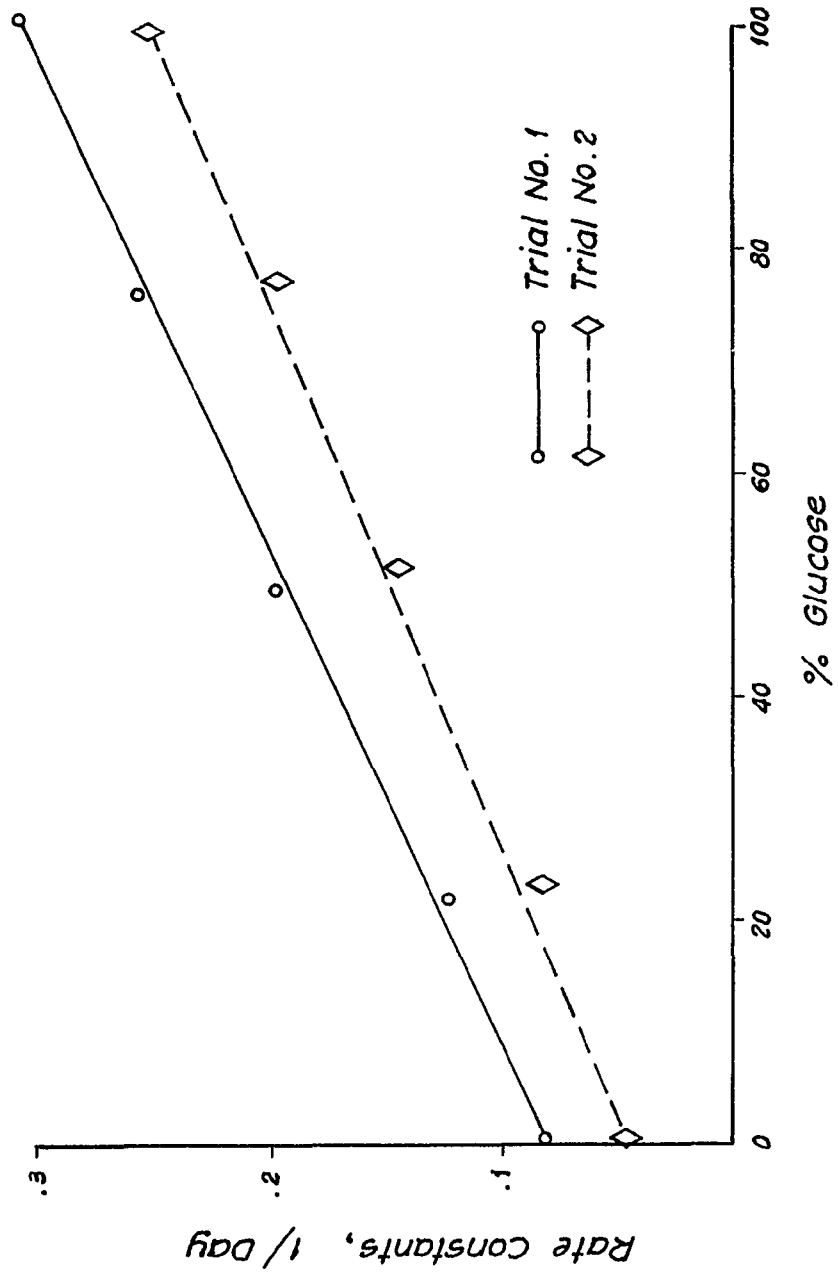


Figure No. 67
*The Effect Of Pressure On The Hydrolysis
Rate Of Protein Versus Non - Pressurization*

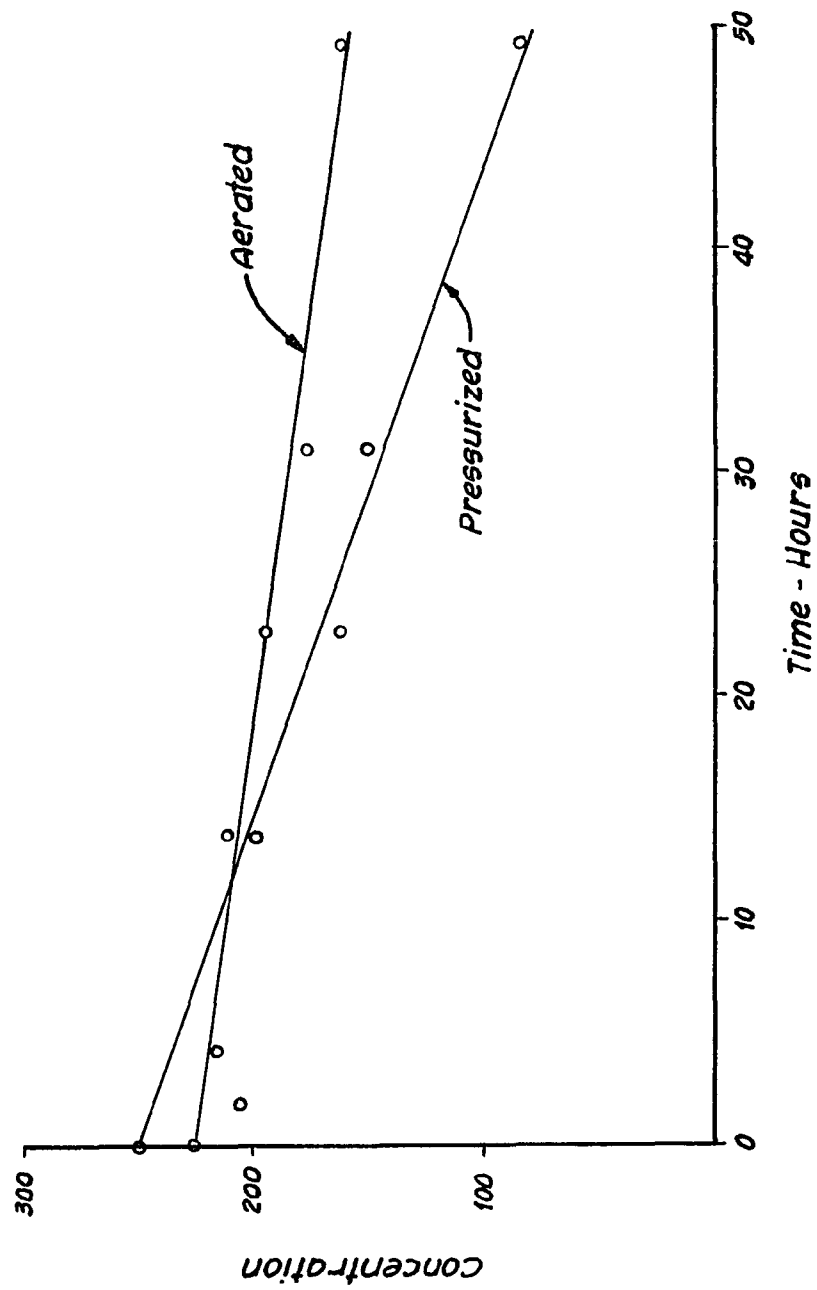


Figure No. 68
*Variation In The Ammonia Concentration For Pressurized
And Non-Pressurized Protein Solutions*

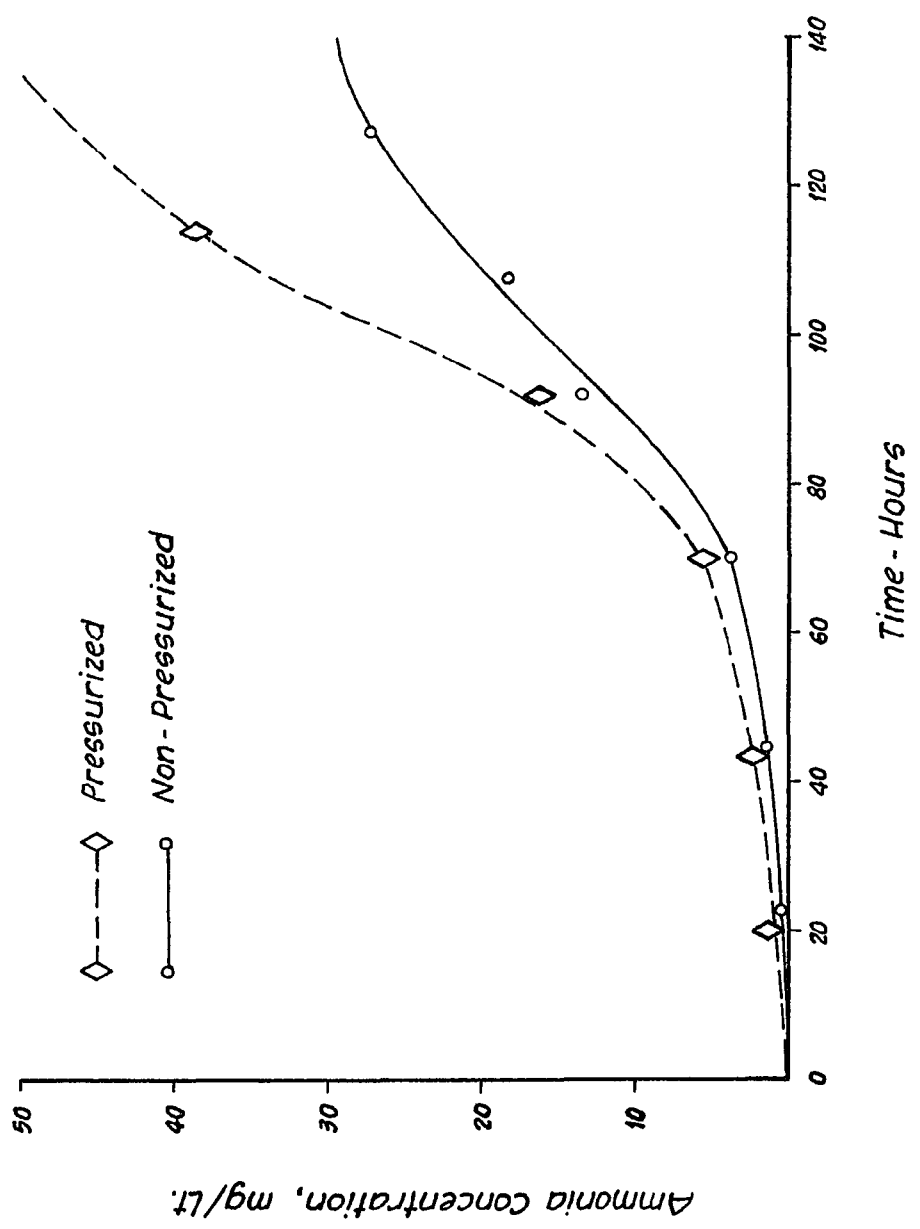


Figure No. 69

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

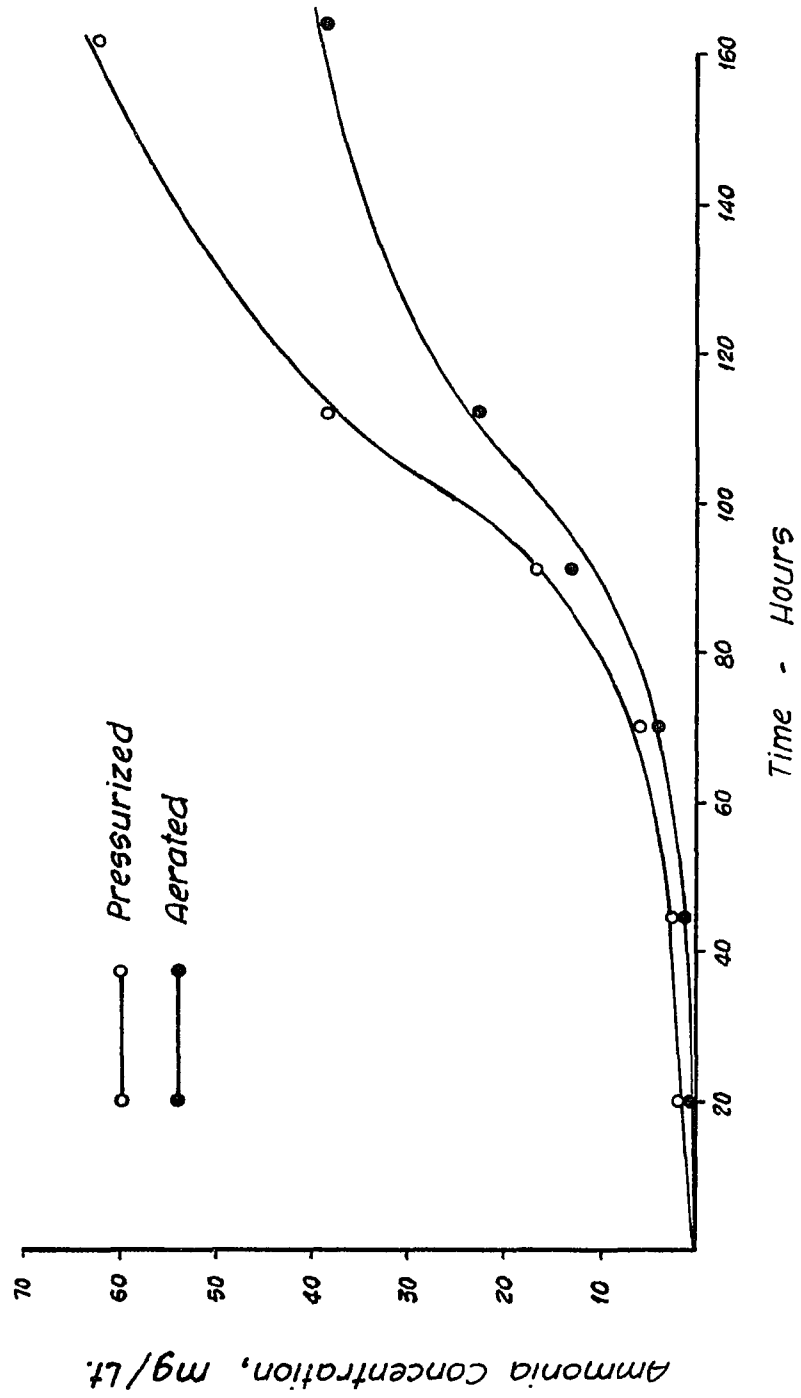


Figure No. 70
Variations of BOD Values For Glutamic Acid
For An Aerated Sample

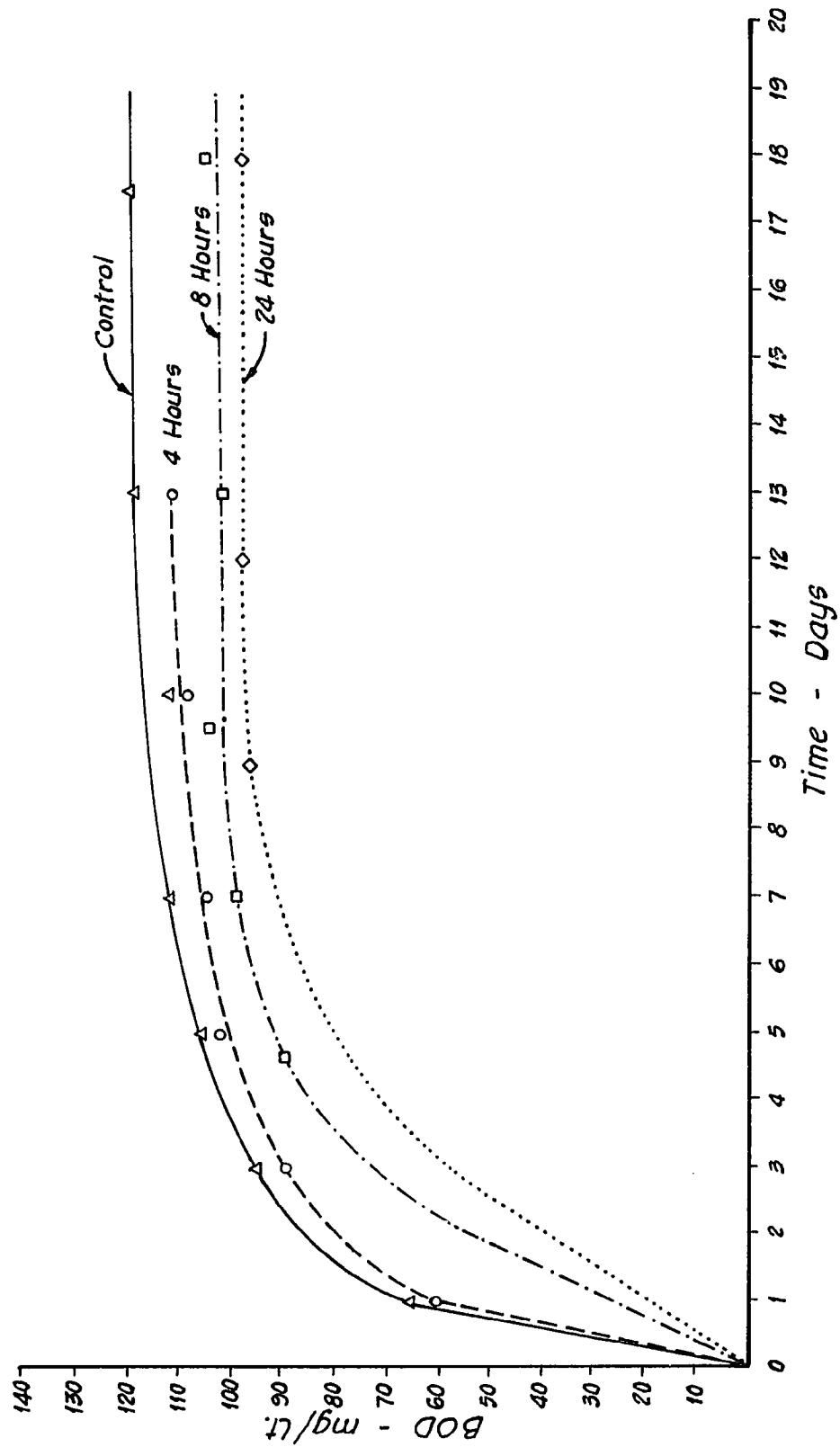


Figure No. 71
Variation of BOD Values For Glutamic Acid
For A Quiescent Sample

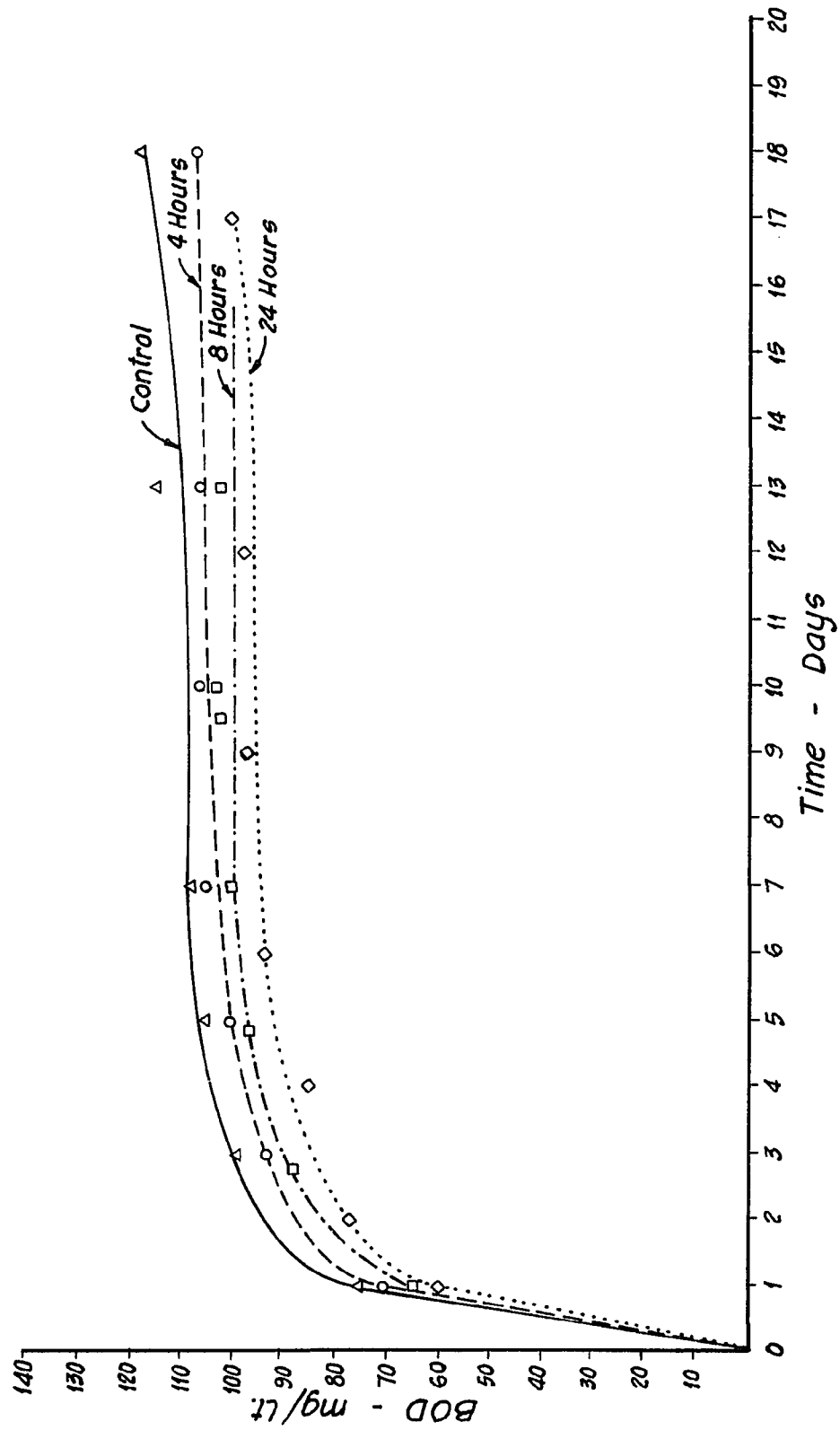
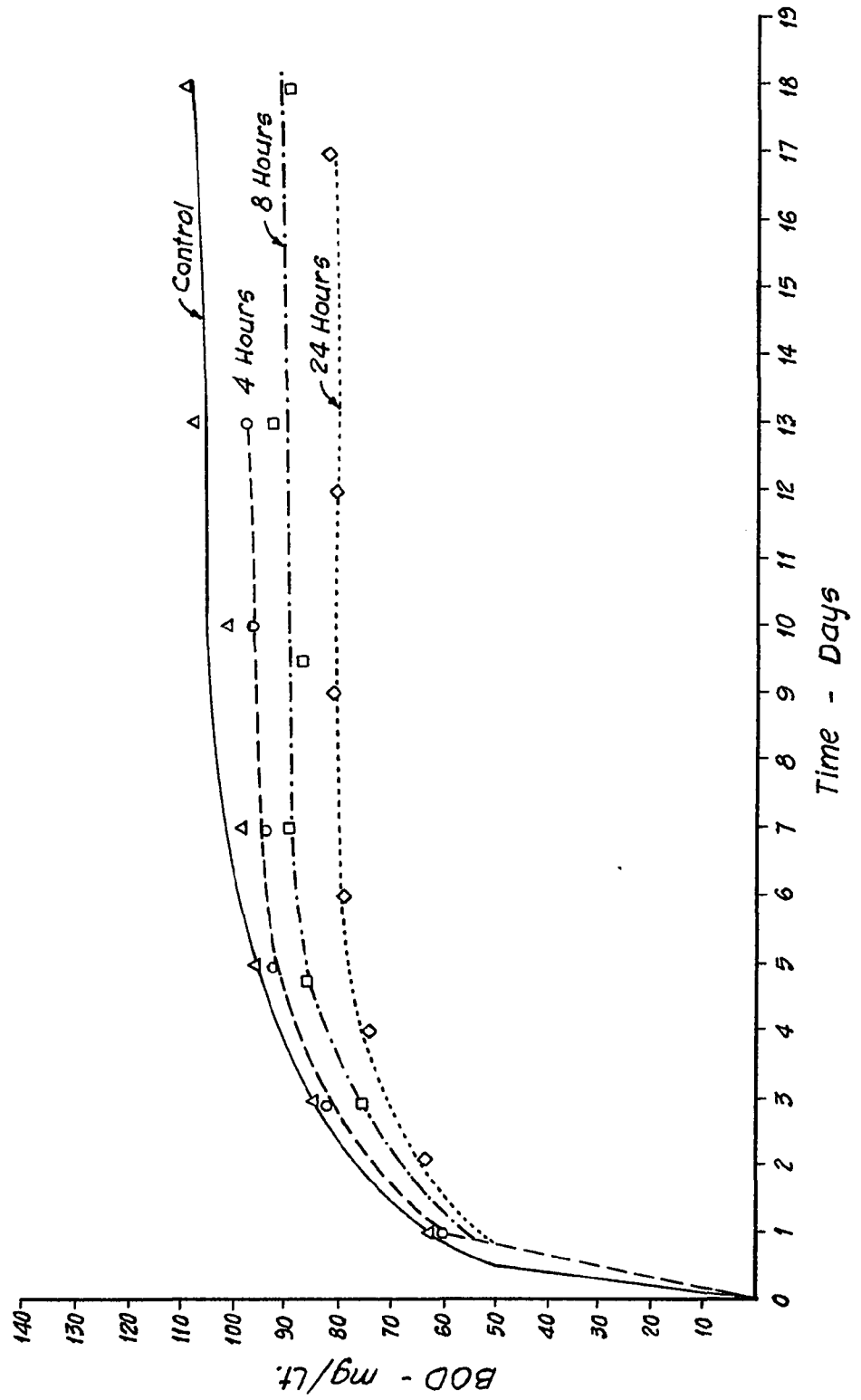


Figure No. 72
Variation of BOD Values For Glutamic Acid
For Pressurized Samples



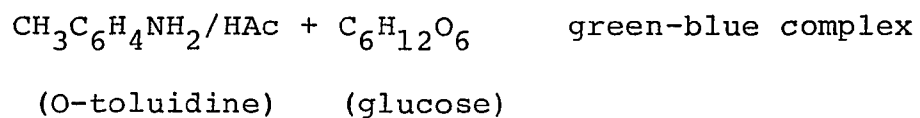
APPENDIX M

EXPERIMENTAL PROCEDURE

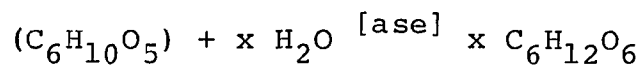
1. GLUCOSE ANALYSIS
2. PROTEIN ANALYSIS

APPENDIX MGLUCOSE ANALYSIS

<u>REAGENTS</u>	<u>EQUIPMENT</u>
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

The reaction involved preparing a 1,000 mg/l starch solution $(\text{C}_6\text{H}_{10}\text{O}_5)_x$ and adding glucosidase enzyme to aid in the hydrolysis of starch to glucose. The hydrolysis is shown as follows:



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 ANALYSISREAGENTS

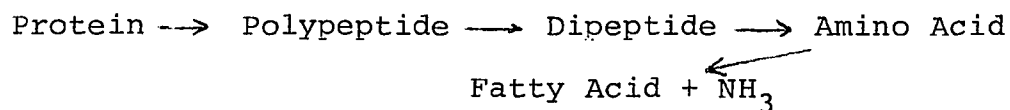
1. Biuret reagent
 (1.5 g $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$)
 (6 g $\text{NaK}_2\text{C}_4\text{O}_6 \cdot 4 \text{H}_2\text{O}$)
 (300 ml of 10% NaOH
 diluted to 1 L)
2. NaCl solution, 0.9%
3. Sodium Deoxycholate
4. Albumin; 10,000 mg/l

EQUIPMENT

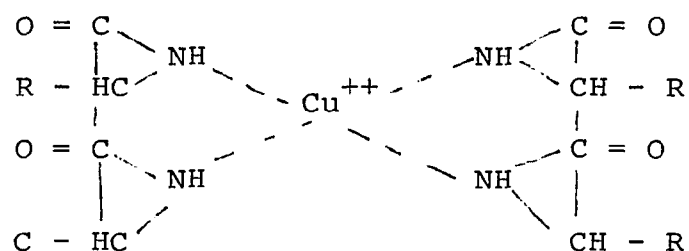
1. Spectrophotometer, set
 at 540 nm.

REACTION

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



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	0.00
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 Environmental 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 Technology, 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 Biodegradability of Sanitary Sewage in a Model Activated Sludge Reactor", Master's Thesis, New Jersey Institute of Technology, 1977.
25. Mazzei, Dennis, "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 Confidential 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 Composition 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.