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SOME BIOCHEMICAL AND
BACTERIOLOGICAL STUDIES OF
ACTIVATED SLUDGE

THESIS FOR THE DEGREE OF M. S.
George Harold Robinson
1933

THESIS

Refuse + refuse
disposal

Title: Activated sludge

Title: Sludge

Bacteriology

SOME BIOCHEMICAL AND BACTERIOLOGICAL
STUDIES
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OF ACTIVATED SLUDGE

A THESIS

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at the
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THESIS

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INTRODUCTION

During the past eighteen years considerable work has been done on the disposal of wastes by aeration—a method commonly called the "Activated Sludge" process. This means of waste disposal is a distinct improvement over the anaerobic and aerobic process-Imhoff system using filters for secondary treatment. Without doubt the two major improvements over the anaerobic processes are (1) the almost complete absence of noxious odors and, (2) the retention and synthesis of the nitrogen, otherwise lost, in a form which increases the fertilizer value of the dried sludge. Although many studies of both chemical and bacteriological nature have been made on the process, no correlation studies stressing the relationship of these two procedures have been conducted. Naturally, studies undertaken separately by chemists and bacteriologists would offer a wide variance in results as well as an expression of great divergence of opinions regarding the most intricate of the biological methods of sewage purification.

The mechanism is not clearly understood either chemically or biologically, or surely someone would have discovered most of the important factors governing that most troublesome feature of the process-"bulking". It is very true that many theories have been advanced along with much data bearing out the contentions, but 95 per cent of the data has been collected at a plant in operation which for one reason or another has gone through a period of bulking.

It has been the author's contention, that the laboratory was the best place to carry out experimental procedures. Here, one could vary the influencing factors and once getting consistent results could later apply them to a large scale plant in an effort to secure the practical effects. This conception of research has been ably proven after watching the increase in numbers of research laboratories in large industrial plants, together with the increase of small commercial testing laboratories in the larger communities throughout the United States during the past fifteen or twenty years.

The work embodied in this paper was undertaken with these views in mind: (1) to combine chemical and bacteriological studies, (2) to use laboratory facilities to the greatest extent possible, (3) to apply them to a small sized plant in order to get practical results and, (4) to visualize any practical application to a full size plant.

This work will, in many cases, duplicate previous findings. However, in some instances different results were obtained, also different methods of arriving at the same conclusions were used, and a few new factors were determined which have influence on bulking.

SCOPE OF PROBLEM

This problem is a study on some mechanics of the activated sludge process with particular emphasis on bulking. Bulking is a situation encountered when the sludge remains suspended and does not settle. All of the methods used revolve around food requirements and overloading. The methods of creating and later correcting a bulking activated sludge were governed by food types, their utilization, and effect upon pH. The hydrogenion concentration is very important in the maintenance of a rapidly settling sludge as well as being an important factor in the efficiency of the purification process by the sludge.

Variations in conditions affecting pH and load studies were accomplished by carbohydrate and protein additions, separately at first, and then together. A brief review of the progress of experimentation follows, listing the treatments in chronological sequence:

1. For carbohydrate utilization lactose was selected, due primarily to the fact that dairy wastes were being studied at the time. The exact amounts used and reasons for such concentrations are given under the "Experimental". The carbohydrate effect on pH, suspended solids,

settleable solids, carbonate and bicarbonate production, were considered and also utilization as food by sludge.

2. Protein utilization was determined by the use of peptone additions in measured amounts. Peptone was used because of its solubility in water, also because of the absence of other impurities or constituents which would tend to influence results. The effect of protein matter upon pH, suspended solids, settleable solids, carbonate and bicarbonate production, and in addition the conversion of NH_3 through nitrite formation to nitrates was carefully considered.

3. Mixtures of lactose and peptone were also used and the same observations made as on these substances when used singly. This addition of carbohydrate and protein mixture should theoretically maintain the sludge characteristics more or less uniformly. A condition which was not maintained when they were used singly.

4. Fat content determinations were made at periods on sludges from the large plant along with settling rates to determine the effect of various fat concentrations upon the settling property of the sludge.

5. Lactic acid was used to determine its availability for sludge organisms. An increase in pH was taken as a measure of this utilization.

6. On all of these aforementioned studies bacteriological samples were taken for total counts on plain and milk powder agar, the number of acid and alkali producers, nitrate reducers, and liquefiers were made. In addition the relationship between plankton and bacteria were determined. Later, some data were obtained stressing possible carbonate and bicarbonate production in various media by organisms isolated from the sludges. This work was undertaken in an effort to secure, if possible, an explanation of the behavior of pH results during the periods of sludge treatment with synthetic food wastes. From a survey of literature it was found that no previous work had been done on alkali production in association with the aerobic treatment of sewage. This phase of the problem seemed quite important.

REVIEW OF LITERATURE

About 1883 Angus Smith (8) found that jars of sewage, in which considerable algae growths were found, failed to putrefy as readily as jars of sewage containing no algae. He inferred that oxygen was produced by the growths. Following his first observations, he placed variable amounts of algae in large jugs into which he later placed raw sewage. Similar jugs were set up using no algae. These were used for controls. He was able by this set-up to prove that the presence of oxygen aided in sewage decomposition and that less odor was produced, than when no oxygen was present. Other workers as Arden, Lockett, Fowler, Clark, Bartow, and others, (8) took up the study of oxygen as an agent for rapid decomposition of sewage. The bottle tests were not in use to any extent after 1905. Small aeration plants were installed and at Lawrence, Massachusetts H. W. Clark (5) installed the first activated sludge system in this country.

The studies at Lawrence were started during 1911. Dr. Clark's experiments were carried out in carboys and gallon bottles using a 24 hour aeration period. He found that algal growths produced oxygen in bottles containing weak sewage. In this point he substantiates

the work done previously by Smith. The sewage was purified in 24 hours in both open and sealed bottles. Shorter periods of aeration gave good clarification but nitrates were not given a chance to develop, where as with 24 hours aeration about 15p.p.m. nitrates were produced. He also found that the settled material, sludge remaining in the carboys and bottles, contained considerable nitrogen. This observation indicates the increased agricultural value of the sludge as fertilizer.

Arden and Lockett (6) confirmed Clark's work at Manchester; and by drawing off the supernatant liquor leaving the settled sludge in the bottom of the tank as a starter, were able to completely purify raw sewage with 6 to 9 hours simple aeration. A large accumulation of sludge could be obtained by allowing the sludge to settle, drawing off the supernatant, and again filling the bottle with raw sewage and aerating for 6 to 9 hours. By repeating this process a number of times a large quantity of activated sludge was produced. This was the first work reported on the "starter" idea, e. g. using a developed activated sludge as a starter for future plant operations. Clark did not use a starter in his work and as a result six weeks were required to develop an activated sludge. Cramer (7)

points out that sludge protozoa occur commonly in soil and in ground water, but not in human and animal wastes. This might account for the long period of time required by Clark for sludge development.

The work of Ardern, Lockett, and Clark was, without doubt, the most important when considering the origin and development of the activated sludge practice. Others have had important places in the actual mechanical and other engineering improvements, but to those three must go the honor of being the real founders of the new biological method of sewage treatment. A. J. Martin (8) in his fine book covering the development of the process up to the year 1927, gives credit to many who have introduced improvements and also to those who have contributed materially to a plausible explanation of the finer features of the system. A complete historical resume has been given by him and at present is the finest record ever compiled covering the complete activated sludge process.

It is needless to give a complete historical summary when such an excellent reference is at hand. Suffice it to give credit only to those men who have really built up the system, either by research or discovery.

Copeland (9) of the Milwaukee Sewage Commission gives the finest definition of activated sludge. He states, "The sludge embodied in sewage and consisting of suspended organic solids, including those of colloidal nature, when agitated with air for a sufficient period, assumes a flocculent appearance very similar to small pieces of sponge. Aerobic and facultative aerobic bacteria are present to the extent of 12 to 14 million per cc. some having been strained from the sewage and others by natural growth. Among the latter are those which possess power to decompose organic matter especially of an albuminoid nature-others (meaning bacteria) absorbing this free nitrogen liberated from the decomposition, convert it to nitrites and nitrates. This takes time, air, and favorable environment such as suitable temperature, food supply, and sufficient agitation to distribute them to all parts of the sewage.

"The process preserves the aerobic bacteria by keeping the sludge, which is their natural food in intimate contact with air at all times and keeps them thoroughly supplied at all times with fresh food from the raw sewage, throughout the whole body of which

they are in intimate contact." He also mentions that the process should not be confused with the practices in which air alone was used in attempts to purify sewage, but which made no use of sludge as an aid to oxidation.

The activated sludge process was defined in 1916 by a committee appointed by the American Society Civil Engineers as "A biochemical process by which the purification of sewage is accomplished by passing it through tanks in which the sewage sludge is artificially agitated and intimately mixed with sewage, and is supplied with the requisite oxygen for the optimum development of countless numbers of nitrifying organisms incorporated in and adhering to the sludge, the final settling of which causes a distinct clarification of the oxidized sewage".

As to some of the finer points of the process, air, agitation, type of sewage treated, per cent sludge return, and load per day are very important factors to be taken into consideration when best operating conditions are to be obtained. Streander (10) listed common methods which are in general use today. They are listed as occurring under two main divisions, (A) aeration and agitation using compressed air, and (B) aeration and agitation by mechanical means. Ridge

and Furrow aerators, Spiral flow aerators, and the air lift aerators make use of diffused air. The Link-Belt aerator, Simplex aerator, and an experimental aerator now being used by the American Well Water Works Association make use of the mechanical system. The Dorcco aerator is a combination of both mechanical and compressed air agitation. Surface aeration using agitation currents for air bubble distribution is the basis for all mechanical methods. These methods were the result of observations made from the economic side of sewage treatment; also from the findings of chemists and bacteriologists relative to the possibility of bulking conditions being caused by too great an agitation by mechanical means or by too much air being forced through the sludge suspension.

Haworth (11) in 1919 described mechanical aerators, the designs of which are only being improved upon today. Aeration in contact beds and trickling filters was probably the first use of air in plant operation. These observations were made by Fort (12) in 1915, and previously by Clark (5) and Fowler (8) at the same place (Lawrence, Mass.)

Buswell, Shire, and Neave (13) in 1928 theorized that air bubbles were surrounded by a shell which hinders aeration. By opposing the water flow the shell was disrupted and increased diffusion was noted. They found that simple mechanical aeration caused fairly rapid absorption to take place and only 0.01 cu. ft. of air actually was required for one gallon of sewage.

Lockett (14) as early as 1917, found no advantage in quantities of air above that required for circulation. Good effluents in 3 hours with 20 per cent sludge content and 1.5 hours with 40 per cent sludge content were obtained. He used 1.5 cu. ft. of air per gallon with 20 per cent sludge. A given quantity of air introduced intermittently produced consistently better results than continuous aeration. This latter treatment caused a removal of 99 per cent of the bacteria. Buckworth (15) in 1915, found 2 hour agitation satisfactory.

At Milwaukee during 1914 and 1915, Fuller (16) reporting on the year's operation at the first full scale plant in the United States, observed that 2 to 3 hour aeration removed 96 to 99 per cent of the bacteria present; also the nitrites were completely removed. In 1916 after another year's experience T. C. Hatton (17) reported that

1.75 cubic feet of air per gallon with 20 per cent sludge using a 4 hour contact period was sufficient to treat sewage.

Burn (18) reported that sufficient agitation is ample to keep a developed activated sludge in excellent condition. This supposition, which has been proven many times in the laboratory, also suggests the possibility that excessive air being blown through the sludge mixture might cause a breaking up of the clumps to cause them to form colloidal particles with markedly reduced specific gravity. This causes bulking. He found that when air was turned off for 4 hours and then turned on again bacterial conditions in the sludge returned to normal after 6 hours aeration. He also found that free ammonia, nitrite, and nitrate conditions were normal after 7 hours aeration while only 3 hours aeration was required to cause normal albuminoid ammonia content. While these determinations demonstrated that normal dissolved oxygen conditions existed after only two hours aeration. Air evidently had considerable effect on the nitrogen status in the sludge.

Buckworth (19) reported 90 per cent purification on oxygen absorption and 76 per cent purification on albuminoid ammonia.

Bartow and Mohlman (20) found 15.0 P.P.M. nitrates were formed in 15 days by blowing 4830 cu. ft. of air through fresh sewage. The sludge was accumulated and after the 31st treatment 3 cu. ft. of air per gallon for 5 hours caused complete nitrification, i. e. complete conversion of nitrogen to nitrates. These studies indicated that one hour aeration appeared to be ample for practical treatment. The worm life was active during these studies. There were many Vorticella and Rotifera.

The nitrogen curves during the first blowing show that nitrates are formed from nitrites almost quantitatively. In all cases the removal of the supernatant caused decreased time in the purification on the succeeding batch. Bartow and Mohlman also observed during these nitrification studies that the dried sludge from this experiment had a fertilizer value of \$29.00 per ton. The growths of flowers in pots gave better results when this dried sludge was used than when an equivalent amount of nitrogen was furnished from dried blood. In 1916, Bartow and Molhman (21) used the free ammonia and nitrite content as indicators for aeration.

H. P. Eddy (22) in 1916, observed that different types of wastes necessitated variations in amounts of air used.

Tannery wastes required 10 cu. ft. of air per gallon with a 12 hour aeration period. In tannery waste treatment it was found that over 88 per cent of the fats were digested.

The action of activated sludge on the nitrogen compounds was found to be marked by Carel (23) in 1920. He found that the ammonia content of sewage, when aerated, remained constant, but when activated sludge was added the ammonia was converted to nitrates within 24 hours. These results show the necessity of bacteria plus air rather than air alone in the nitrogen conversions. This study was extended by Dienert and Girault (24) who experimented with allonges in a water bath whose temperature was held at 25°C. A 450 cc. quantity of activated sludge mixture containing about 12 gm. dry material was added to 1200 and 1500 cc. of sewage respectively and to ordinary water. The air was bubbled through until ammonia disappeared, then the time was noted, nitrites and nitrates were estimated.

This work was continued for 9 months. They concluded that the free ammonia conversion is practically the same in all sewage concentrations studied as well as in ordinary water to which ammonia had been added. The same men (25) then attempted to find the effect of various concentrations of sludge upon the rate of nitrification. One liter samples

of Seine river was used and to these 50, 100, 200, and 400 cc. sludge mixtures were added. These sludge mixtures corresponded to 1.5, 3.0, 6.0, and 12.0 gms. dry matter. The air was bubbled through at the rate of 50 liters per hour. The free ammonia, nitrite, and nitrate nitrogen determinations were made. Observations indicated that all sludge mixtures readily gave an effluent free of ammonia and non-putrescible. Due to the great variation in bacteria in the sludge and in the different samples of water, the reduction in bacteria was very inconsistent.

McVea and Fugate (26) reporting on the Houston, Texas plants found that as long as 1 p.p.m. nitrate was present about 99 per cent relative stability was experienced. Ammonia content of over 8 p.p.m. correlated with poor plant operation.

Arden (27) in 1917, discussed the relationship of temperature and nitrification. The question of temperature is not serious in considering the extent of nitrification. They also concluded that the maintenance of activity of sludge was not dependent on the stage to which nitrification was carried. Copeland (28) and Ledener (29) found temperature to have but little effect upon bacterial removal and clarification. The work done from 1920 to 1928 covers only the necessary research incidental to improved plant design. This work has nothing in common

with this particular paper so no effort was made to record the literature.

Buswell (34), Heukelekian (31), Rudolfs (32), and Levine (3) and (4) as well as Slaughter (33) and Eldridge (35)(36) and (37) have done considerable work on aerobic and anaerobic processes of sewage purification, particularly on dairy wastes. Their findings have been used to a considerable extent in the general discussions appearing in this thesis.

After this time, research work on the activated sludge process is progressing rapidly at present with the advent of many small installations in some of the smaller towns and cities throughout the country.

EXPERIMENTAL

Description of Plants

In this work the mechanical aeration plant was the one use by Eldridge in his studies on the application of the activated sludge process to milk wastes.

This plant consisted of a large cylindrical tank of approximately 2300 gallons capacity to the water line, as aerator unit furnished by the courtesy of the Simplex Ejector and Aerator Corporation of Chicago, a settling tank of 600 gallons capacity, and two centrifugal pumps. The necessary piping, orifice box, and electrical equipment was furnished by the Michigan Engineering Experiment Station.

The plant, as set up for the treatment of the wastes from the college dairy, is shown in figure 1. The descriptive points are indicated. A sewer line from the dairy building was accessible through a manhole located about midway between the Kedzie Chemical Laboratory and the Red Cedar river. Here a 3 ft. sump was constructed some 8 feet below the ground surface. It was of concrete construction and afforded an excellent location from which to obtain dairy waste.

The waste was taken from the sump by means of a pipe (A) and pumped by the first of the centrifugal pumps (B) to the orifice box (C) from where the flow to the large aeration tank (D) could be governed. The photograph

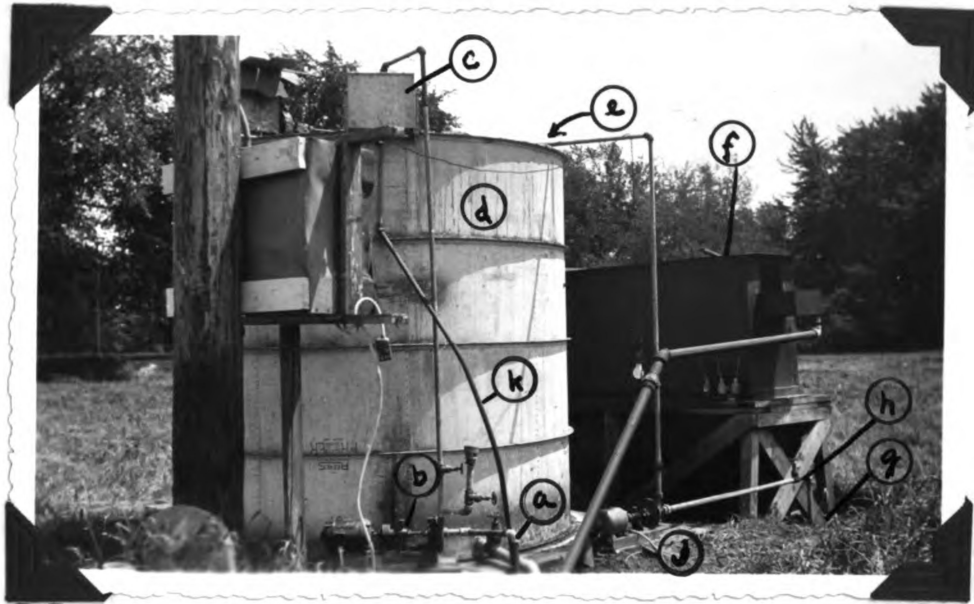
shows the overflow line from the orifice box returning to the sump basin. The flow of raw waste to the aeration tank entered behind a baffle plate some 3 feet in depth, thus reducing to a minimum, the possibility of any untreated sewage reaching the effluent. This effluent pipe (E) lead from the large cylindrical tank on the opposite side (shown in figure 3) and can be seen emptying into the hopper-bottomed settling tank (F). There was also a baffle plate so constructed in this tank, that the aerated waste entered in the enclosed portion, thereby eliminating the churning action on the settling sludge in the large central portion of the tank necessarily caused by the continuous flow from the aeration tank. A weir was constructed in the outflow end of the settling tank to decrease the possibility of floating sludge making its way to the final effluent pipe (K). This tank was also provided with a 2 inch drain pipe (G). This pipe was tapped by another smaller pipe, fitted with a valve (H), which furnished a means of returning certain amounts of settled sludge to the aeration tank for seeding purposes. The second centrifugal pump (J) served to pump the

sludge from the settling tank over into the aeration tank. This pump was not operated continuously as was the first one, but periodically according to the amount of return sludge desired. These periods were varied throughout the work and are given in the paper.

At Mason, Michigan the plant set-up was the same as that used at East Lansing, (fig. 2) except that an automatic time switch was installed to operate the return sludge pump. By this means an accurate amount of sludge could be returned each hour. Also, the necessity of personal attention each hour was eliminated.

A small laboratory plant was used to aerate the aeration tank contents of the larger plant after the development of a good sludge. This small set-up enabled the writer to study mixtures of sludge and raw waste during various periods in the operation of the larger plant. The laboratory set-up was extremely simple in construction as is seen in Fig. 4. It consisted of two galvanized iron tanks, cylindrical in shape, of about twenty-five liters capacity. Each tank was fitted with 6 inch filtrose plate, in turn connected to a compressed air line. Light in construction and easy to operate, they formed a very useful piece of apparatus for the study of sludge mixtures as carried out in this work. It can readily be seen that in using a laboratory set-up of this design, a synthetic waste can be added and the steps in its utilization could

Figure I.



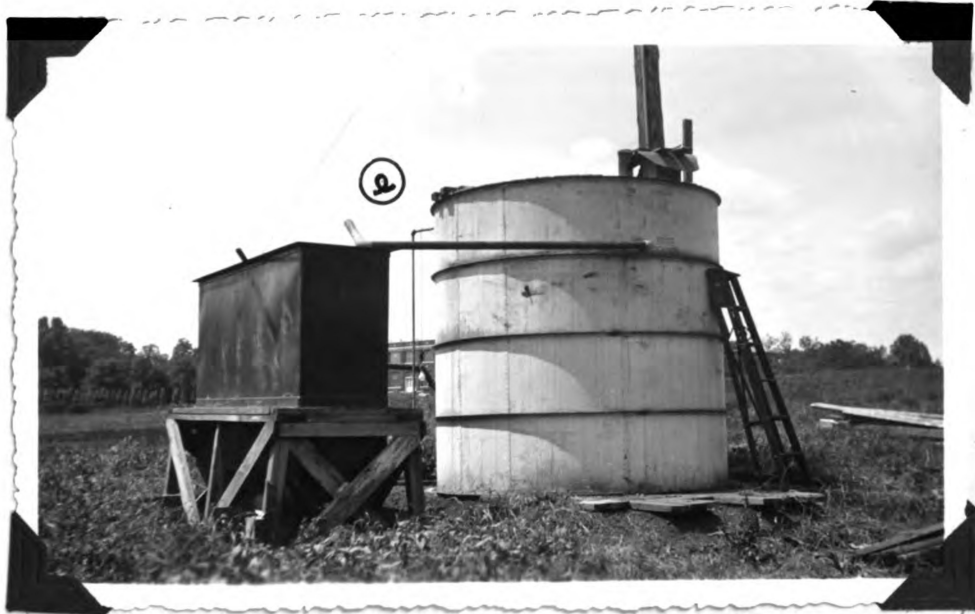
- A. Pipe from sump basin.
- B. First centrifugal pump.
- C. Orifice box.
- D. Aeration tank.
- E. Effluent pipe from aeration tank (Shown in Fig.3)
- F. Hopper bottomed settling tank.
- G. Drain pipe from settling tank.
- H. Valve in pipe allowing a return of settled sludge.
- J. Second centrifugal pump.
- K. Overflow pipe from orifice box.

Figure II.



Plant set-up at Mason, Michigan.

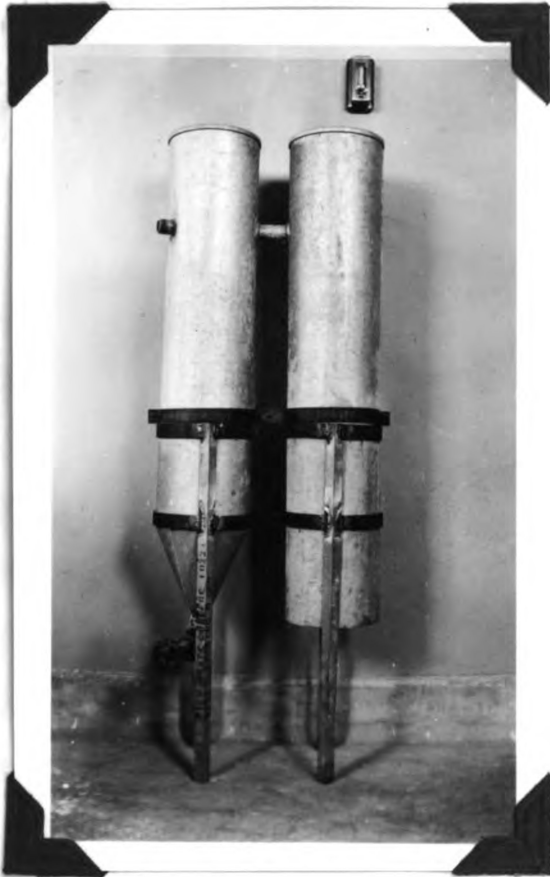
Figure III.



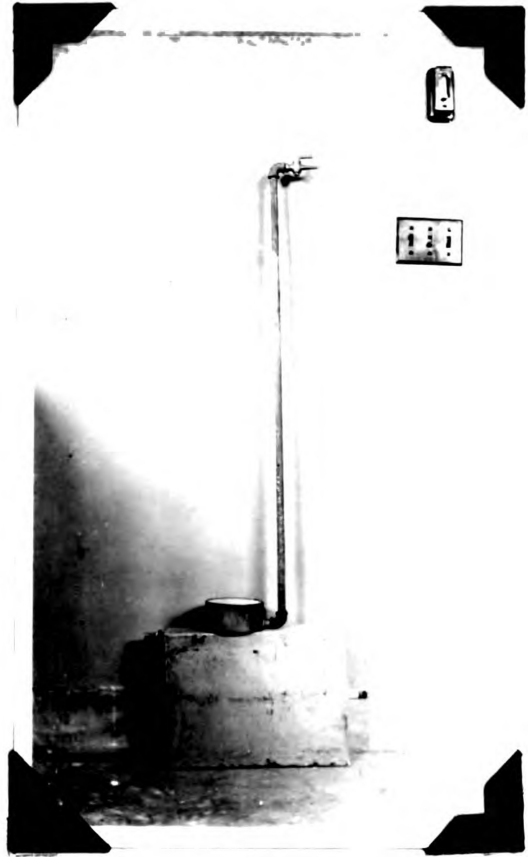
Rear view of plant set-up at East Lansing, Michigan

E. Effluent pipe from aeration tank

Figure IV.



A.



B.

A. View of tanks used for the laboratory plant.

B. View of diffuser unit as used in the laboratory plant.

be easily observed along with the correlated effect on the tank contents.

The amount of air used in the laboratory set-up was not measured; only enough was allowed to bubble through to cause a boiling appearance on the surface.

In the larger plant two types of waste were studied. The wastes from the College dairy consisted almost entirely of common dairy waste as can washings, bottle washings, floor washings, churn scourings, and, at times, old cottage cheese. There was only one toilet connected with the sewer line.

The wastes did not have a constant pH because of the cleaning and scouring powders used. Some were very caustic while others were slightly acid because of the sulphuric acid which at times was used for cleansing purposes. In general, the wastes were quite milky in appearance and had the distinct odor of slightly soured milk. The sump had to be cleaned out from time to time. The materials removed, consisted largely of settled milk solids smelling very strongly of butyric acid and of protein cleavage products characteristic of anaerobic decomposition.

The wastes encountered at Mason, consisted primarily

of the final effluent of the Mason Sewage Treatment plant. The Mason plant used primary sedimentation and separate sludge digestion. Link-Belt equipment is used on the sedimentation tanks. The final effluent had an average biochemical oxygen demand of 120 to 130 p.p.m. The pH value ranged from pH 7.4 to pH 7.6.

The raw waste encountered at Mason was relatively strong in the B. O. D. and solids content. It was composed of about 50 per cent domestic sewage and about 50 per cent waste from a large commercial milk products plant. Such sewage is fairly characteristic of a number of smaller cities and towns throughout the country. The milk waste in question consisted of washings from cans, large storage tanks, coolers, vacuum pans, and from casein and casein vats. As a great percentage of the work at the milk plant consisted of powdered milk preparations, the solids content of the raw milk waste was very high. Eldridge (36) in 1929 found them to be 1160.0 p.p.m. The average taken at the time of opening operations at the Mason plant showed about 1030.0 p.p.m. These results showed hardly any variation and can be considered as a normal average. Aside from the powdered milk product, the plant manufactures condensed milk, casein, and milk sugar. The plant normally handles approximately 120,000 lbs. of milk per day, but during the

time of operation of the mechanical aeration system at Mason a decrease in production occurred so an accurate report cannot be given as to the number of pounds of milk handled per day. It probably did not fall below 75,000 pounds per day at any time. The volume of waste from the milk plant was about 225,000 gallons per day.

In addition, the domestic wastes of the town which has about 1500 inhabitants connected with the sewage system, increased the volume to a combined total of 450,000 gallons per day with an average oxygen demand of 600 p.p.m.

Methods of Laboratory Plant Operation

In operating the laboratory plant it was not necessary to keep check upon the time periods as much as when dealing with the larger set-up. The aeration was continuous and no settling tank was used. Continuous reaeration conditions existed enabling a study of the action of various sludges under continuous activation. The samples of about 25 liters were taken from the larger tank, placed into the small tanks and the air turned on. Initial determinations were made in all cases. It was not considered necessary to record the analysis in a few cases especially if no abnormal condition of the sludge existed. The food material (synthetic waste) was added in measured amounts i.e., lactose in sufficient

quantity to make 400 p.p.m. concentration, while peptone was added in amounts necessary to form a concentration of 300 p.p.m. In all cases amounts were added which corresponded as closely as possible to amounts found naturally in wastes of the types studied. Woodman's "Food Analysis" was used as the reference text in this respect.

Chemical Analyses and Methods

As far as possible the methods outlined in the "Standard Methods of Water Analysis" published in 1925 by the American Public Health Association were followed. At times modifications of these methods were made to conform with the heavier concentrations of the various compounds sought in strong wastes and sludges. These are listed in full as follows:

1. Total Solids

One hundred cubic centimeters of the sludge was placed in a tared porcelain dish and evaporated to dryness over a water bath. It was then placed in an oven at 103°C and dried for one hour, cooled in a desiccator for 15 minutes and then weighed. The weight in milligrams multiplied by 10 gives parts per million total solids.

2. Ash (Fixed residue)

This determination was made on the same sludge sample as was used in the total solids determination.

After weighing for total solids, the dish was ignited for one hour in a muffle furnace at 700°C . The dish was then placed in the 103°C . oven for 15 minutes, cooled in the desiccator and then weighed. The weight in milligrams multiplied by 10 gives parts per million ash.

3. Volatile Matter (Loss on ignition)

This result was obtained by the difference between the total solids in p.p.m. and the ash content in p.p.m.

4. Suspended Solids

A 25 cc sample was taken for this determination. It was passed through a weighed Gooch crucible containing a matrix of asbestos. After filtration the mass was washed with two 10 cc portions of distilled water. The increase in weight multiplied by 40 gives the suspended solids in p.p.m.

5. Settleable Solids and Settling Rate

Use of 1 liter graduates instead of Imhoff cones was made in this determination. A liter of aeration tank contents was placed in the cylinder. At 15, 30, 45 and 60 minute intervals readings were taken of the settled sludge in cubic centimeters. The 60 minute interval was

considered as final in this determination and was recorded as the total quantity of settleable solids. The readings were made in cubic centimeters rather than in parts per million.

Graduates were used because of the decreased friction caused by the slanting sides of an Imhoff cone. One hour settling periods were selected, although for general work 2 or 3 hours should be taken as the standard.

6. NH₃ determination

The new Hellige' Aqua Tester was used for this determination. Amounts were taken according to the "Standard Methods" and clarified with CuSO₄ and KOH. Aliquots were then taken and the comparisons made using the comparator. Calculations were made accordingly.

7. NO₂ determinations

The Hellige' apparatus was used in this determination and also in the nitrate estimation. The samples were taken (aliquots) and diluted to the mark. One cc. sulphanilic acid and 1 cc. alpha-naththylamine reagent were added and the comparisons were made at the end of 10 minutes. Calculations were made accordingly.

8. NO₃ - Nitrate determination.

The supernatant liquid was used for this analysis. Amounts from 1 cc. to 25 cc. were used during the course of the studies. These were evaporated to dryness over the water bath. After cooling, 1 cc. of phenoldisulphonic acid was used to moisten the residue. Stirring with a glass rod insured intimate contact of the acid with the residue. The material was diluted with nitrate-free water and strong KOH added until a dark yellow color was produced. The solution was filtered through a filter paper and diluted to the mark in the comparison tube. Comparisons were made using the comparator. Calculations were made according to the amount of sample evaporated.

9. Total Nitrogen

The Kjeldahl method was used and the final filtrate titrated with standard HCl solution. Results were reported in p.p.m. rather than in per cent.

The results for the total nitrogen determination do not include nitrite and nitrate nitrogen. It includes only that nitrogen which is decomposed by H₂SO₄. Therefore, as the organic nitrogen not including the ammonia nitrogen is assumed to be combined in the protein molecule, the protein is calculated as (total nitrogen in p.p.m.-ammonia nitrogen in p.p.m.) x 6.38 = protein in p.p.m.

10. Carbohydrate Determination

A modification of the method used by Munson and Walker as outlined in Woodman's "Food Analysis" was used in this estimation. The modification is given in detail by Eldridge (38) and is repeated here, in part:

- a. A Gooch crucible was prepared and dried to constant weight.
- b. A 450 cc. sample of the material studied was placed in a 500 cc. volumetric flask.
- c. 10 cc. of CuSO_4 solution (69.28 gms./ 1 liter) and 35 cc. of 0.1N NaOH or its equivalent were added.
- d. The contents were diluted to the mark, mixed well, and filtered through a dry filter.
- e. 25 cc. Fehling's solution B(173 gms. potassium sodium tartrate - 50 gms. sodium hydrate in 500 cc. solution) were added to a 400 cc. pyrex beaker.
- f. Then 50 cc. of the filtered sample from (d) were added to the mixture and the beaker covered with a watch glass.
- g. This solution was boiled exactly 2 minutes over a burner so regulated as to bring 100 cc. of solution to boiling in exactly 4 minutes.
- h. The mixture was then filtered hot through the Gooch crucible. Residue of Cu_2O was washed with

- i. The precipitate was then washed with 10 cc. of alcohol and by 10 cc. of ether.
- j. The Gooch filter was then removed to the 103°C. oven and dried for 30 minutes, cooled in a desiccator and weighed.
- k. The weight of Cu_2O in milligrams x the factor 14.44 gives the amount of reducing sugar present, in p.p.m.

11. pH determination

In the chemical studies a Yoden⁴ electrometric device was used for the determination of pH values. The apparatus used was manufactured by the William Welch Company.

In bacteriological studies, however, a different apparatus was used. This apparatus used the colorimetric principle (Hellige' Comparator) and was manufactured by the Fischer Scientific Company of Pittsburgh. Fairly close similarity was obtained on standard solutions of known pH when run on both pieces of apparatus. The alkalinity produced by some bacteria was too great to be determined by the electrometric method, otherwise that method would have been used throughout the course of the studies. The colorimetric method was not used universally because of the error introduced by the sludge particles particularly while studying the chemical behavior.

12. Alkalinity Determination

Alkalinity was determined according to "Standard

Methods" with the exception of a few cases where 0.2N acid was used in titrating instead of 0.02N. This occurred in the bacteriological studies and facilitated end point readings in presence of minute quantities of phosphates, which had considerable effect on them. The calculations were made on 50 cc. samples and results are given in p.p.m. bicarbonate and p.p.m. carbonate.

13. Bacteriological Methods

The methods varied during the course of the determinations so they are described as they are discussed.

RESULTS

It was assumed in this work that a multiplicity of factors were responsible for any and all bulking characteristics of activated sludge. Morgan and Beck (1) in 1928, observed that filamentous organisms increased in number in sludges which were treated with carbohydrate wastes. Scott (2) in 1928, observed that the types of waste had considerable effect on bulking of activated sludge, particularly milk, starch, and brewery wastes. These are naturally very high in carbohydrate content. Scott also pointed out that the damage done to activated sludge by milk wastes was irreparable. Buswell, (30) in 1931, emphasized the appearance of filamentous organisms of the Sphaerotilus type in wastes of high starch content. Bach, (39) also concluded that "Carbohydrates were the chief causes of bulking" in that they promote growth of organisms of the Sphaerotilus genus. All of the Cladothrix organisms, however, have a dense interwoven structure and therefore are very inducive to bulking.

Smit (40), in 1932, working independently corroborates considerable work covered in this paper. He attacked the problem from a different angle however using a "Continuous operating" experimental set-up, while the small laboratory set-up used by the author was primarily one of reaeration. The cylinders used by Smit in the determination of settling rates and of settleable solids were of 250 cc. capacity,

while 1 liter capacity containers were used in this work. The time periods of 190 minutes duration were used while 60 minute periods were used here. However, most of the results obtained at Amsterdam, were compatible with those we obtained. The exceptions will be discussed more completely in the "Discussion".

With these views in mind, the author undertook the work from the food and load standpoint.

Dairy wastes have been studied at length by Levine (3) & (4), Slaughter (33), and Eldridge (35), (36), and (37). Because of the work done here by the latter two men and also because of the interest still shown at this institution regarding dairy waste disposal the primary studies in this paper were made on wastes from the College dairy, which has been stated previously.

By filling the large cylindrical tank with the raw waste and aerating from 4 to 6 days a fairly good sludge was developed. Within 65 hours from the start of aeration a decrease of 64 per cent in B.O.D. in the aeration tank was observed. At this time new waste was admitted for 8 hours and then a 48 hour period in which no new waste was admitted ensued. On April 16 the actual operation of the plant was begun. After continued operation until May 4, 1932 the first samples were removed from the aeration tank.

A dark brown, rapidly settling sludge had developed by this time and operation of the plant had been progressing in a most satisfactory manner.

In Table IA are recorded the results of a preliminary study to determine the relationship of the amounts of carbohydrates, fats, and proteins in the raw influent, aeration tank effluent, and final effluent.

The results of 6 samplings from the orifice box overflow, aeration tank effluent, and final effluent are averaged. The averages showed 100 per cent utilization of carbohydrate in the aeration tank and an increase of 294 per cent in the protein content. The protein content then decreased markedly in the final effluent. The fat content slowly diminished throughout the plant showing a gradual utilization.

As these samples were collected over different periods of the day variations in the raw waste were to be expected. This may be due to the hourly changes encountered during the course of their operating day. If buttermilk happened to have been discharged quite different fat content occurred in the waste than with skim milk.

These averages, however, do indicate the food content in the raw waste in an 8 hour day. They also served as foundation work on which the later experiments were carried out.

TABLE I a

**RESULTS OF ANALYSES ON INFLUENT, AERATION TANK, AND FINAL EFFLUENT
FOR CARBOHYDRATES, PROTEINS, AND FATS.**

Results in P.P.M.

SAMPLE	INFLUENT			AERATION TANK EFFLUENT			FINAL EFFLUENT		
	CARBO- HYDRATE	PROTEIN	FATS	CARBO- HYDRATES	PRO- TEINS	FATS	CARBO- HYDRATES	PRO- TEINS	FATS
1.	152	128	212	NONE	247	104	NONE	81	24
2.	282	288	120	"	396	67	"	124	27
3.	190	190	125	"	638	89	"	76	21
4.	250	242	102	"	715	94	"	78	16
5.	292	166	96	"	795	82	"	95	17
6.	260	217	108	"	835	65	"	64	12
AVG.	237.6	205.1	127.1	NONE	604.3	83.5	NONE	86.3	19.5

Knowing that carbohydrates (lactose) were completely utilized in the aeration tank under an 8 hour retention period, the actual rate of lactose utilization by the sludge mixture in the tank was determined by use of the laboratory plant which, as previously stated, operated with continuous reaeration.

Pure lactose was added after the initial pH and lactose determinations were made on the sludge mixtures. This lactose was added in amounts which made a final concentration of 400 p.p.m. At the periods shown in Table I, the amount of sugar still remaining was measured by the aforementioned modification of the Munson and Walker method for reducing sugars.

No sugar was found to be present and a pH of 6.52 was also found to be fairly constant, even after 12 hours aeration in the small tanks.

Graph #1 was made using pH and percentage utilization columns in Table I. This clearly shows the fluctuation in pH as the lactose was being used. Graph #1 also indicates the same results obtained on the second day of operation. These charts show a tendency of phase activity in the utilization of sugar. This was concluded from the seeming tendency to diminish in rate of utilization after the second hour. This tendency was also encountered in

the work which followed.

During the first 4 hours the pH increased from 6.52 to 8.21, a marked increase when considering no change had taken place during the previous 12 hours before starting the experiment. This was the first encounter with reverse pH.

Table II and Graph 2 show results of tests run the same as before only on a new sludge sample. Checks were made on free ammonia and suspended solids in addition to the lactose and pH determinations. Results from the second sludge sample show in general the same trends as were observed on the first sludge mixture. The fluctuations in per cent and pH loci were not as marked as on the first attempt but they did bear out the results as found at first. Lactose shows about 98 per cent utilization in 8 hours during the first day of operation, but only about 90 per cent utilization during the second day over the same period of time. On this day the fluctuation of pH was not pronounced and gradually diminished from 8.21 and 2.12 to 7.87 and 7.50 respectively, during two days operation. At the same time the per cent lactose utilized had reached upwards of 80 per cent utilization at the end of 3 hours continued aeration. Table III shows a pH fluctuation nearly at all

TABLE I.

SLUDGE FROM MILK WASTE

SHOWING CARBOHYDRATE (LACTOSE) UTILIZATION AND

CONSEQUENT EFFECT ON pH

1st SAMPLE

TIME IN HOURS	1st DAY			2nd DAY		
	pH	LACTOSE UTILIZATION		pH	LACTOSE UTILIZATION	
		P.P.M. Present	% Used		P.P.M. Present	% Used
INITIAL	6.52	404.0*	-----	7.78	402.0*	-----
0.5	6.91	317.0	21.5	7.95	350.0	12.9
1.0	7.37	231.0	42.8	8.04	326.0	18.9
2.0	7.61	150.0	62.9	7.87	279.0	30.6
3.0	7.95	61.0	84.9	7.78	250.0	37.8
4.0	8.12	26.0	93.5	7.87	234.0	41.8
24.0	7.78	2.0	99.0	7.53	160.0	60.2
48.0	7.78	-----	100.0	7.05	119.8	70.2

* 4 parts per million reducing sugar was present when the lactose was added. This number represents the sum total of both, lactose added and lactose present.

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GRAPH - I. (TABLE I)

P. H.

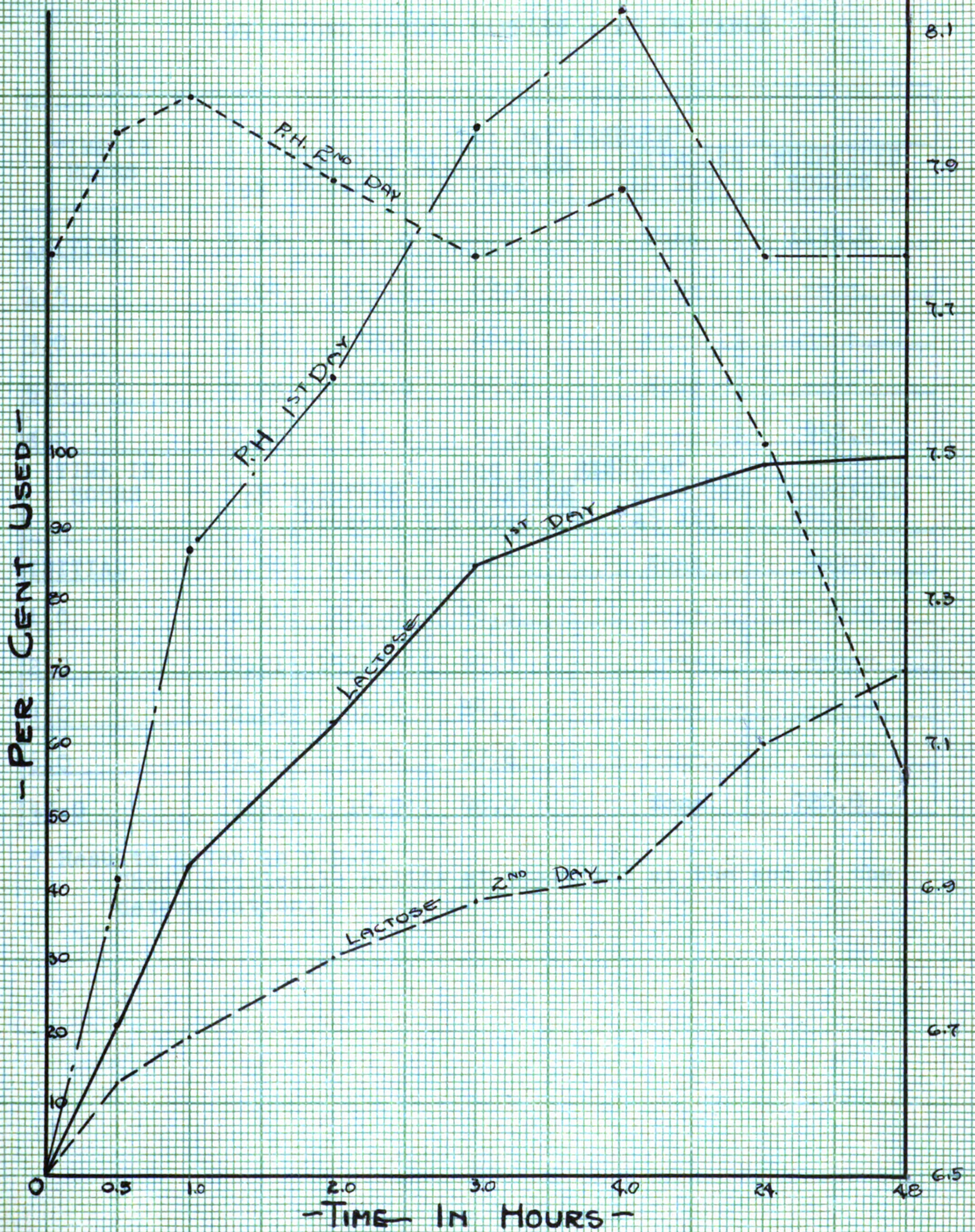
LACTOSE UTILIZATION AND P.H. CURVES OVER 2 DAY PERIOD ON MILK WASTE SLUDGE
SAMPLE NO. 1

TABLE II.

SLUDGE FROM MILK WASTE
SHOWING CARBOHYDRATE (LACTOSE) UTILIZATION
ON SECOND SLUDGE SAMPLES - AS IN TABLE I

TIME IN HOURS	1st DAY RESULTS*				
	pH	LACTOSE UTILIZATION		NITROGEN AS NH ₃	SUSPENDED SOLIDS
		Present	% Used		
INITIAL	7.23	417.0	----	None	1133.0
1.5	8.04	127.0	69.5	----	----
3.0	8.12	58.0	86.1	----	----
5.0	7.95	20.0	95.2	None	----
8.0	7.63	10.0	97.6	----	952.0
TIME IN HOURS	2nd DAY RESULTS*				
	pH	LACTOSE UTILIZATION		NITROGEN AS NH ₃	SUSPENDED SOLIDS
		Present	% Used		
INITIAL	7.50	400.0	----	None	960.0
1.0	8.04	215.0	46.3	----	-----
2.0	8.21	159.0	60.3	----	-----
4.0	8.04	61.0	84.3	----	-----
6.0	7.95	49.0	87.8	----	-----
48.0	7.87	16.0	96.0	None	720.0

* Results given in p.p.m.

Check settleable solids at start and at the end of the determination.

GRAPH-2 (TABLE II)

LACTOSE AND P.H. CURVES OVER 2 DAY PERIOD ON MILK WASTE SLUDGE
SAMPLE No. 2

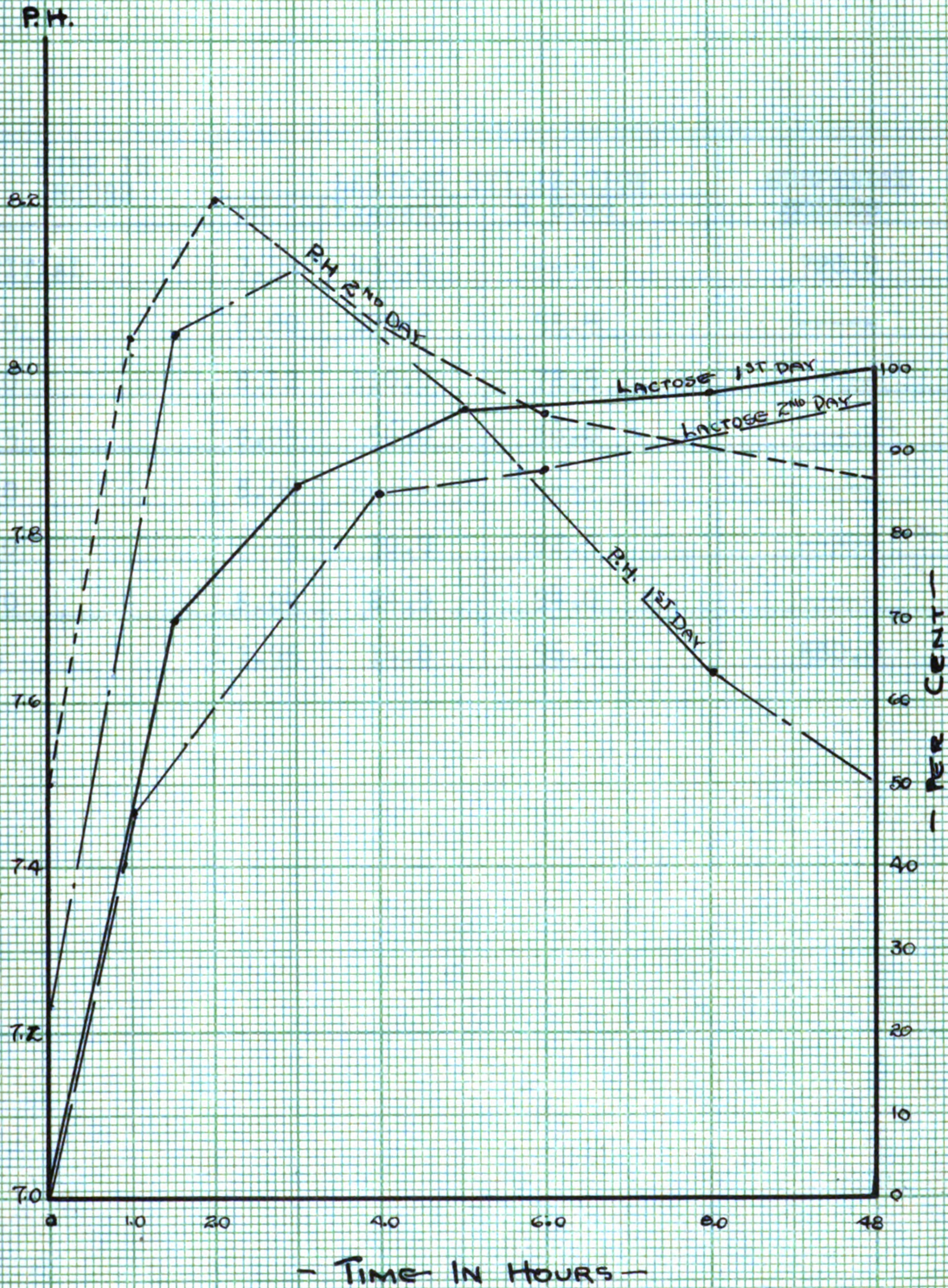
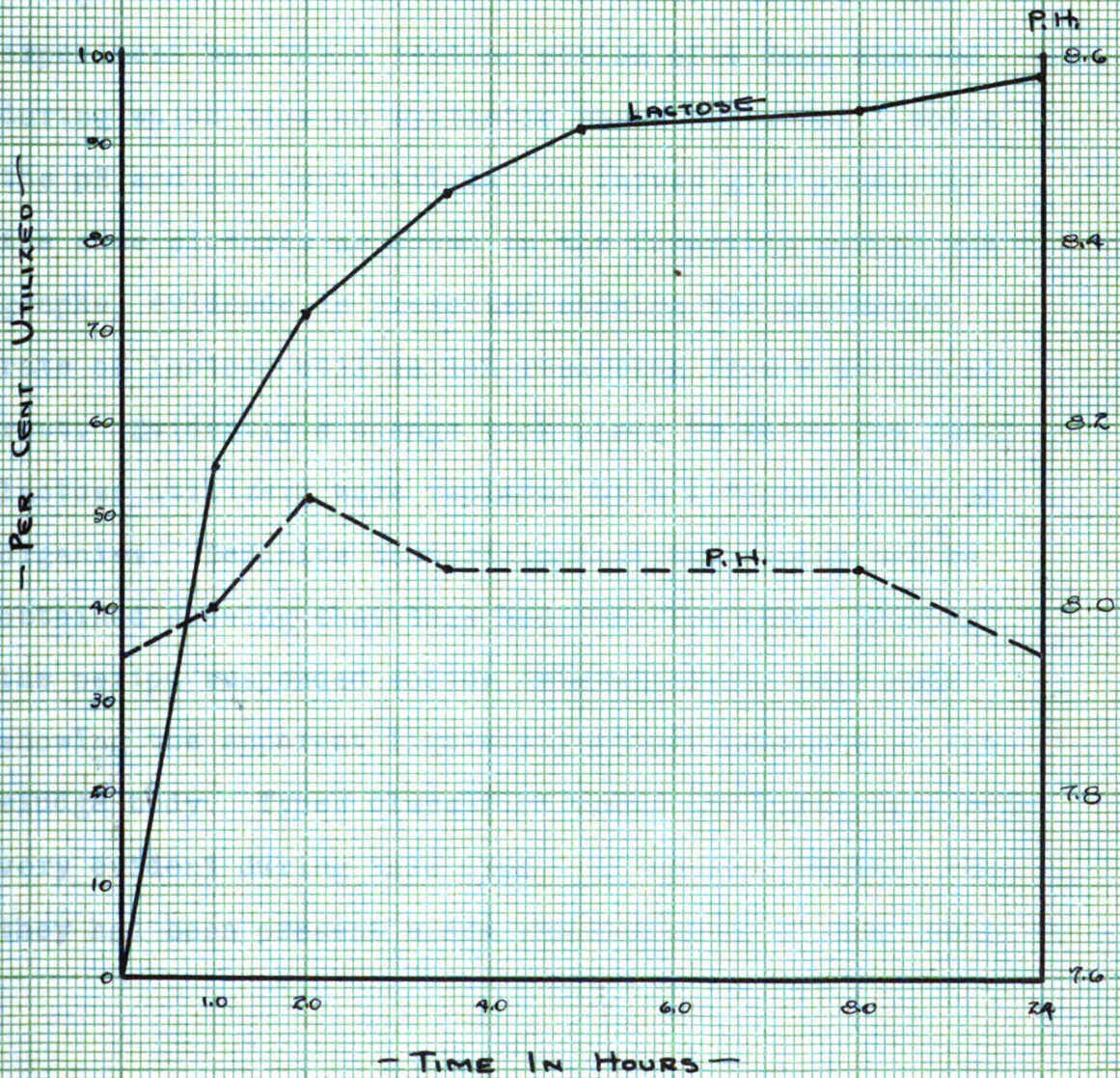


TABLE III. SLUDGE FROM MILK WASTE
RESULTS OF VERIFICATION RUN ON SECOND SAMPLE

TIME IN HOURS	LACTOSE UTILIZATION			SUSPENDED SOLIDS
	pH	Present	% Used	
INITIAL	7.95	400.0	-----	720.0
1.0	8.00	178.0	55.5	-----
2.0	8.12	110.0	72.5	-----
3.5	8.04	56.0	86.0	-----
4.0	8.04	-----	-----	-----
5.0	-----	30.0	92.5	-----
8.0	8.04	24.0	94.0	-----
24	7.95	14.0	96.5	580.0

GRAPH 3.

LACTOSE UTILIZATION AND PH CURVES ON MILK WASTE AFTER ONE WEEK
WITHOUT LACTOSE TREATMENT.



times above pH 8.0. Only at the initial period and after 24 hours was there any particularly low pH value. This was at 7.95. All during this period lactose was being utilized at the rate shown on Graph 3. On this "verification" run evidence is clearly shown concerning the marked effect which lactose exerts on the pH while being utilized.

The next table (table IV) and graph (4) show the change over a four day period of time. Before this experiment was run a resting period of 4 days elapsed during which time no synthetic waste was added. While the pH and per cent utilization curves give generally the same pictures as shown in the foregoing tables and graphs, evidence is presented showing the decreased rate of lactose utilization resulting from a period of rest. Evidently a change took place in which the rapid lactose utilizers were replaced by a different type of organism. Definite bacteriological data could not be obtained showing that this had been the case. During the period suspended solids determinations were made showing the apparent lightening effect inherent in the sludge body. Types classified under the Cladothrix were very evident during this time. It is also evident that they had been present since the beginning of the labor-

atory experiments. There was no possible way for them to have made their way into the tanks afterwards because of the fact that only a laboratory-made waste was used.

A fact evidenced from the Table, which could not be shown on the graph, was the relatively high pH value throughout the 4 day period. This stability of the acid - alkaline ration can only be described as a period in which no material change occurred in the flora of the sludge itself. At this point it might again be mentioned that duplicity in results are extremely difficult to obtain. Consideration must be made of the "living equation" which is in evidence at all times. No two periods are exactly the same. The flora of an activated sludge is changing constantly. The flora today varies from that of yesterday or tomorrow. Some certain organisms or group of organisms are continually reaching the peak of their existence. They can be largely responsible for the difference in characteristics of a sludge taken at different intervals. It is the living bodies themselves that constitute a major portion of the sludge floc.

A rest period of three weeks was then given the sludge before additional determinations were made. By this means the actual utilization rate change caused by

three weeks period without food could be found.

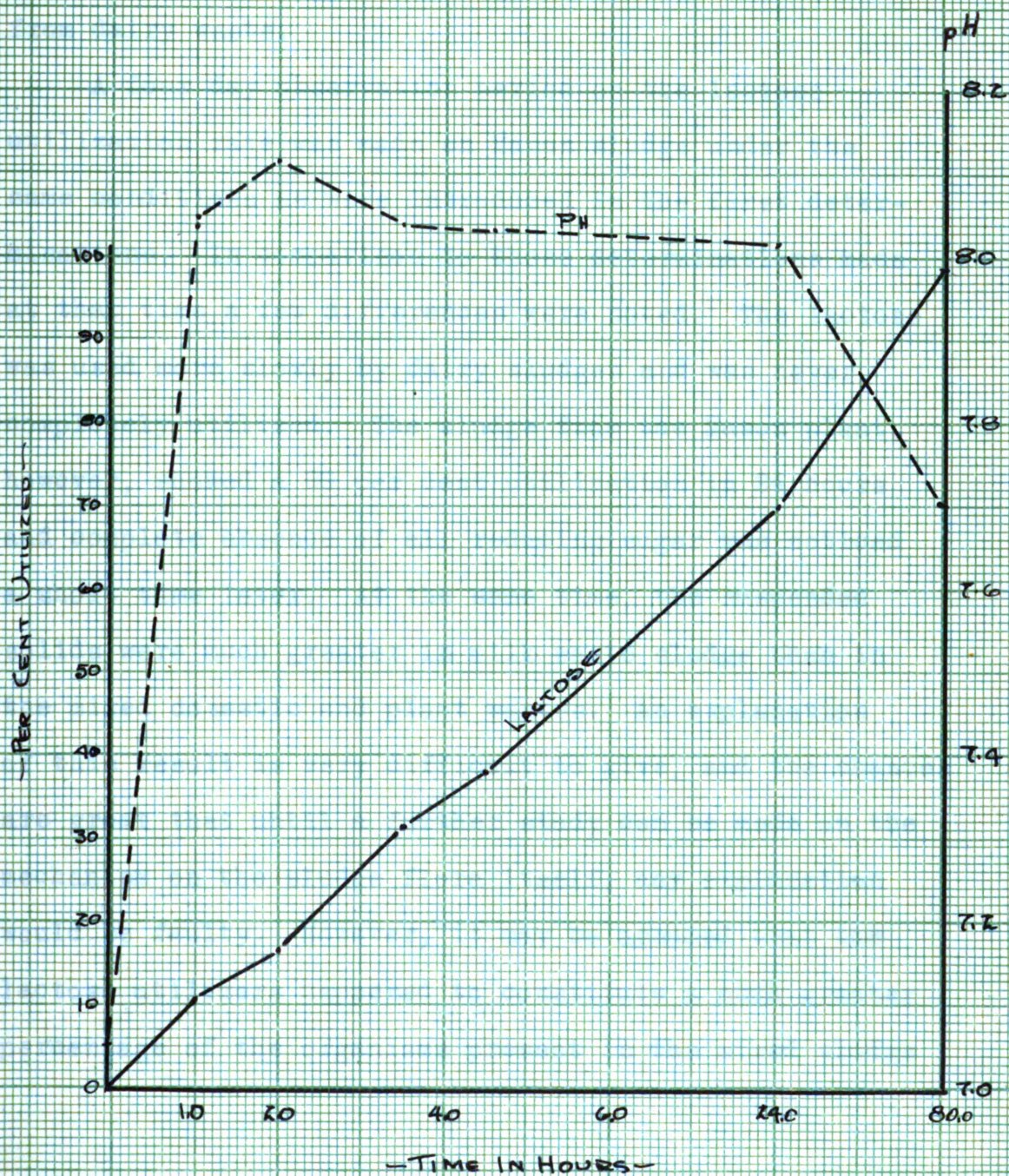
The results as shown in Table V and Graph 5 show this effect. In addition the bicarbonate activity was recorded. This seemed to have but little effect on the pH of the liquor. No parallelism whatsoever could be noticed. The lactose utilization curve, however, shows a marked decrease in rate of utilization. No carbonate was present. The suspended solids again showed a decrease in volume but to a much less extent than in the previous runs. Throughout these determinations the suspended solids decreased in weight while the settleable solids increased in volume. These features play a very prominent part in the experiment and are of notable importance when reference is made to bulking conditions. Otherwise the work clearly substantiates that of the other lactose treatments.

The same set-up using aeration tank contents developed at Mason, gave in a general way the same results as obtained at East Lansing. An increase in alkalinity with the corresponding decrease in lactose and in suspended solids was noted. However, in this case the sludge mixture did not use lactose as rapidly as did the sludge developed from milk waste alone. This was to be expected in consideration of the types of bacteria which would normally be found in

TABLE IV. SLUDGE FROM MILK WASTE
SHOWING CARBOHYDRATE (LACTOSE) UTILIZATION OVER
A FOUR DAY PERIOD, ALSO pH FLUCTUATIONS DURING
THIS PERIOD

All results in P.P.M.

TIME IN HOURS	pH	LACTOSE		NH ₃ Nitrogen	Suspended Solids
		Present	% Used		
INITIAL	7.05	520.0	-----	None	1106.0
1.0	8.04	465.0	10.6	-----	-----
2.0	8.12	433.0	16.7	-----	-----
3.5	8.04	355.0	31.7	-----	-----
4.5	8.04	318.0	38.8	-----	-----
24.0	8.02	156.0	70.0	None	780.0
25.5	8.04	-----	-----	-----	-----
26.5	8.12	-----	-----	-----	-----
30.5	8.04	-----	-----	-----	-----
32.5	8.04	-----	-----	-----	-----
48.0	7.95	-----	-----	-----	-----
49.0	8.04	-----	-----	-----	-----
50.0	8.12	-----	-----	-----	-----
52.0	8.12	-----	-----	-----	-----
53.0	8.04	-----	-----	-----	-----
54.0	8.04	-----	-----	-----	-----
72.0	7.78	-----	-----	None	710.0
78.0	7.61	-----	-----	-----	-----
80.0	7.70	5.0	99.0	None	690.0

GRAPH-4 (TABLE IV)LACTOSE UTILIZATION AND PH CURVES ON MILK WASTE SLUDGE OVER 4 DAY PERIODSAMPLE No. 2

the two different types of wastes. The rate changes on the same samples when treated on successive days did show a conditioning effect, that is, the rate of lactose utilization increased as the additions were made. The pH ranges were slightly lower than those encountered from dairy waste.

A four day observation period on the lactose utilization rate was made on Mason sludge under the same conditions as were introduced in the first determinations. In comparison to those determinations made at that time a few differences were noted. The lactose was used more slowly by dairy sludges and was never completely utilized. The sludge from the Mason plant, however, showed a 99 per cent utilization in 24 hours and complete absence of all reducing sugar was noticed a short time later. At the same time, the suspended solids were much greater in milk waste sludge than in the mixed domestic and plant wastes. An explanation of this result is very difficult to give, and the chances are great that until a further study can be made on the mechanism on the sludge flora itself, any explanation would be faulty. However, it may be assumed that the lactose utilizers happened to constitute a much greater percentage of the organisms present in the mixed sludge

TABLE V. SLUDGE FROM MILK WASTE

SHOWING EFFECT OF LACK OF CARBOHYDRATE DIET

OVER THREE WEEK PERIOD ON RATE OF LACTOSE UTILIZATION

TIME IN HOURS	pH	NAS Nitrate N	Lactose Utilization		Alkalinity		Susp. Solids
			Present p.p.m.	% Used	CO ₃	HCO ₃	
0.0	6.69	80.0	400.0	---	0.0	106.0	808.0
1.5	7.20	---	374.0	6.5	0.0	---	---
3.0	7.28	---	364.0	9.0	0.0	66.0	---
4.5	7.70	80.0	361.0	9.7	0.0	86.0	---
6.0	8.04	---	352.0	12.0	0.0	86.0	---
24.0	6.95	80.0	170.0	57.5	0.0	102.0	704.0
72.0	7.20	80.0	130.0	67.5	0.0	104.0	616.0

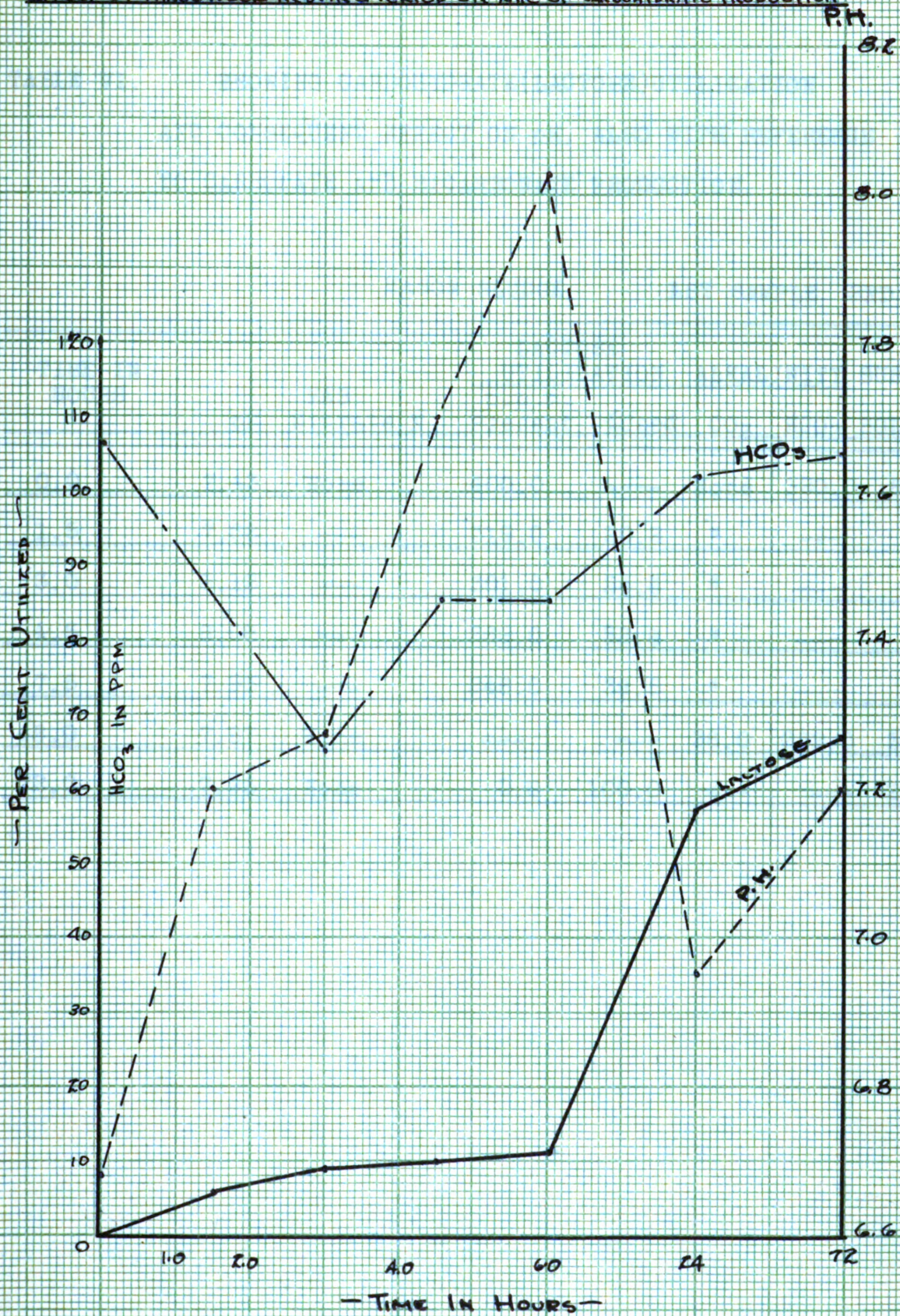
GRAPH-5 (TABLE V)EFFECT OF THREE WEEK RESTING PERIOD ON RATE OF CARBOHYDRATE PRODUCTION

TABLE VI. SHOWING CARBOHYDRATE(LACTOSE)UTILIZATION
AND CONSEQUENT EFFECT ON pH, ALSO WITH REFERENCE
TO CARBONATE AND BICARBONATE PRODUCTION.

MASON SLUDGE				1ST SAMPLE 2ND DAY			
TIME IN HOURS	pH	NAS Nitrate N	LACTOSE UTILIZATION		ALKALINITY		SUSP.
			p.p.m. Present	%Used	CO ₃	HCO ₃	SOLIDS
0.0	7.43	None	400.0	----	None	320.0	552.0
1.5	7.68	---	319.0	20.2	"	---	---
3.0	8.12	---	301.0	24.8	"	---	---
4.5	8.08	---	269.0	32.8	"	---	---
7.5	7.95	---	246.0	38.5	"	---	---
48.0	7.87	None	---	---	36.0	272.0	380.0
72.0	7.95	None	0.0	100.0	---	---	280.0

GRAPH-G (TABLE VI)

LACTOSE UTILIZATION AND PH CURVES OVER THREE DAY PERIOD ON MASON SLUDGE
SAMPLE NO. 1

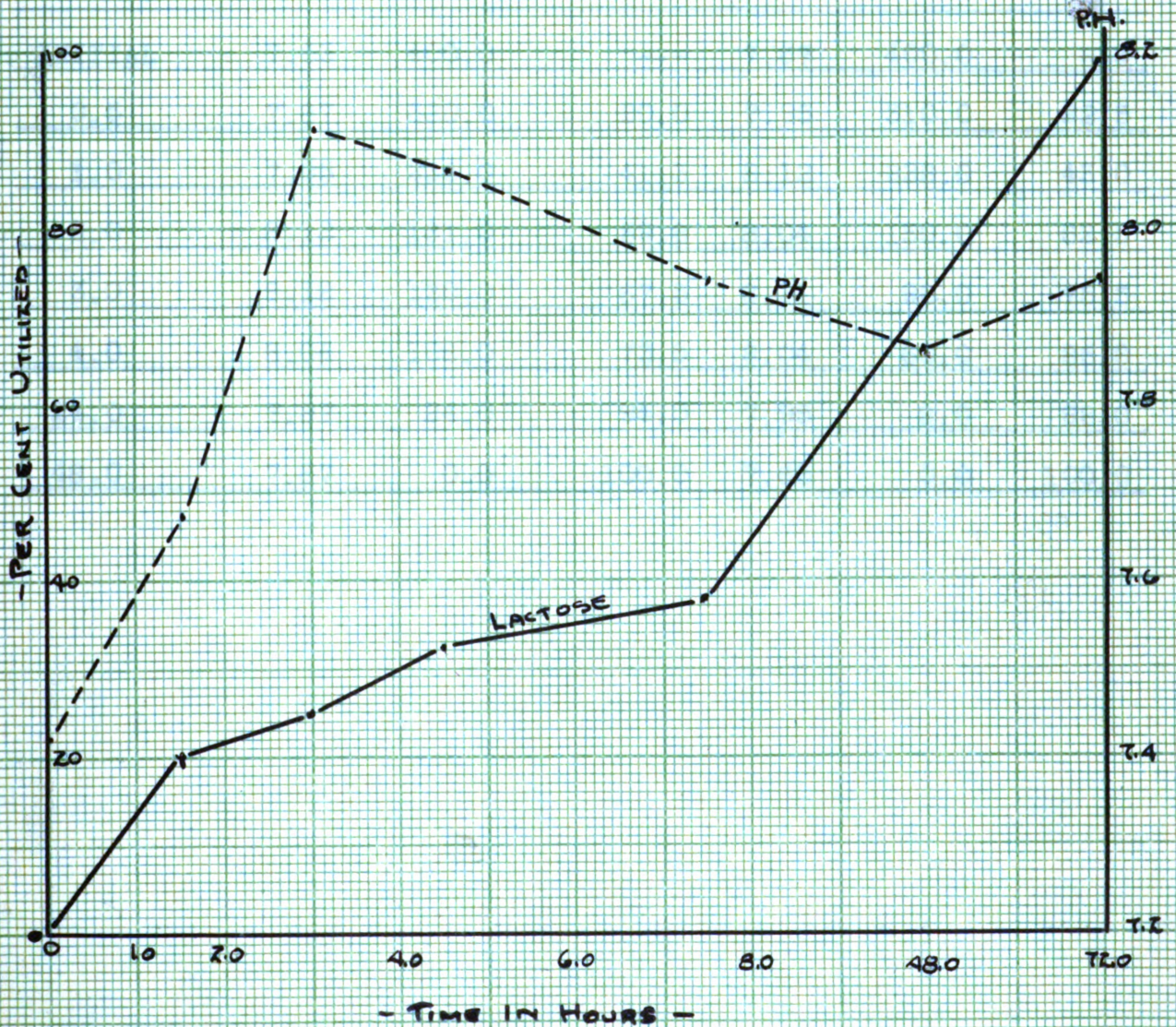


TABLE VII. SLUDGE FROM MIXED WASTE (2ND SAMPLE)
 SHOWING CARBOHYDRATE (LACTOSE) UTILIZATION
 AND CONSEQUENT EFFECT ON pH.

TIME IN HOURS	1ST DAY			2ND DAY		
	LACTOSE UTILIZATION			LACTOSE UTILIZATION		
	pH	p.p.m. Present	%Used	pH	p.p.m. Present	%Used
INITIAL	7.63	400.0	---	7.67	400.0	---
0.5	7.87	320.0	20.0	7.95	310.0	23.5
1.0	7.95	235.0	41.3	8.12	228.0	43.0
2.0	8.12	161.0	59.8	8.04	150.0	62.5
3.0	8.12	130.0	67.5	8.21	98.0	75.5
4.0	8.04	106.0	73.5	8.04	60.0	85.0
24.0	7.87	24.0	94.0	7.95	8.0	98.0
48.0	7.67	0.0	100.0	7.63	0.0	100.0

GRAPH 7 (TABLE VII)

LACTOSE UTILIZATION AND PH CURVES OVER 2 DAY PERIOD ON MASON SLUDGE
SAMPLE NO. 2

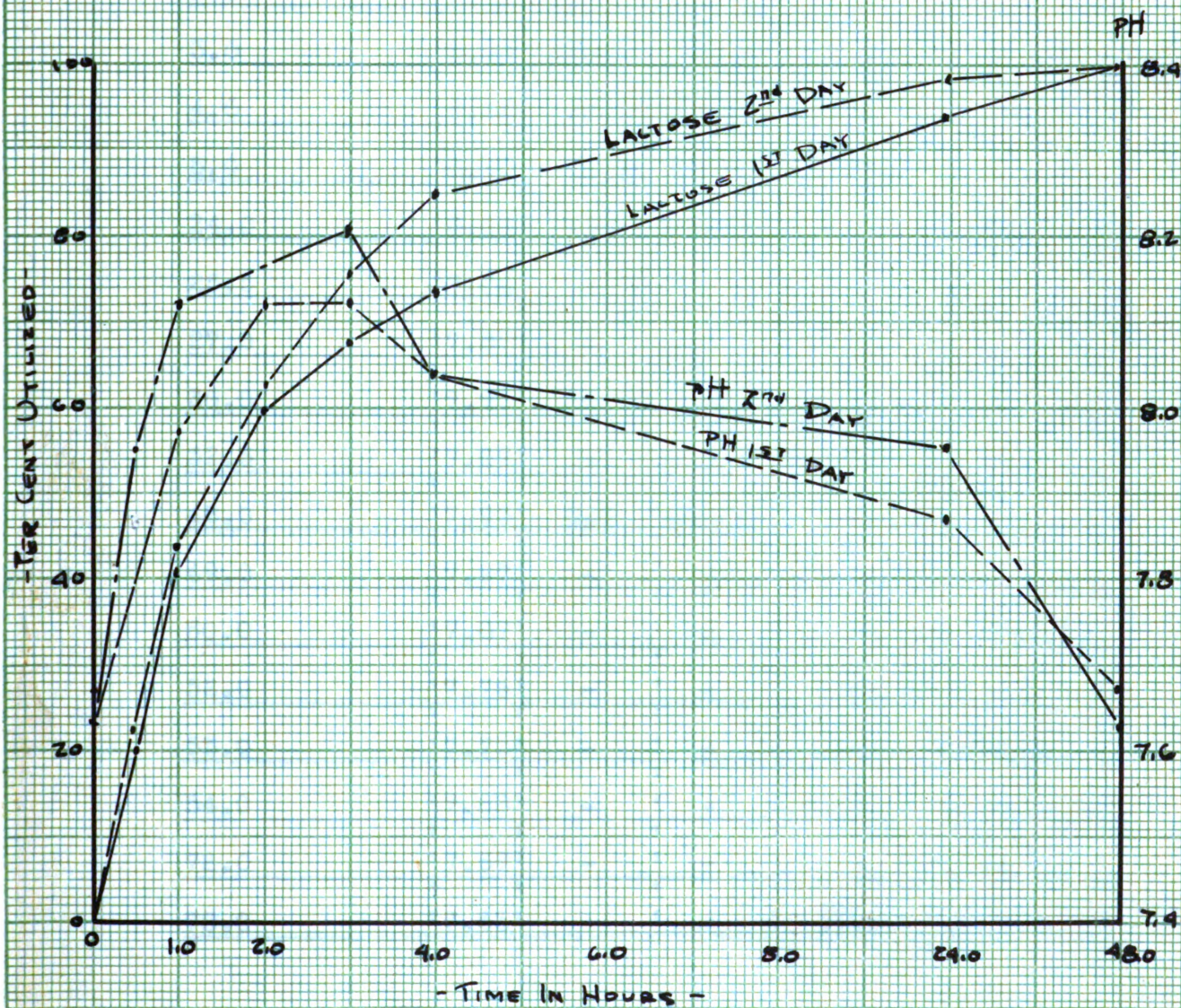


Table VIII. Sludge from Mason Showing Carbohydrate (Lactose) Utilization over a four day Period, also pH Fluctuations During this Period.

Time in Hours	pH	2nd sample 3rd day.			
		Lactose	NH ₃ Nitrogen	Suspended Solids	
		ppm Present % Used			
Initial	7.72	400.0	-	0.1	560.0
1.0	8.12	320.0	20.0	-	-
2.0	8.21	260.0	35.0	-	-
3.0	8.21	210.0	47.5	-	-
4.0	8.12	95.0	76.3	-	-
5.0	8.12	30.0	92.5	None	-
24.0	8.04	2.0	99.5	-	480.0
25.5	8.00	-	-	-	-
27.0	7.87	-	-	-	-
30.0	7.95	-	-	-	-
32.5	7.87	-	-	-	-
48.0	7.93	0.0	100.0	-	-
49.0	7.97	-	-	-	-
50.0	7.87	-	-	-	-
52.0	7.76	-	-	-	-
53.0	7.75	-	-	None	430.0
54.0	7.68	-	-	-	-
72.0	7.57	-	-	-	-
78.0	7.63	-	-	None	415.0

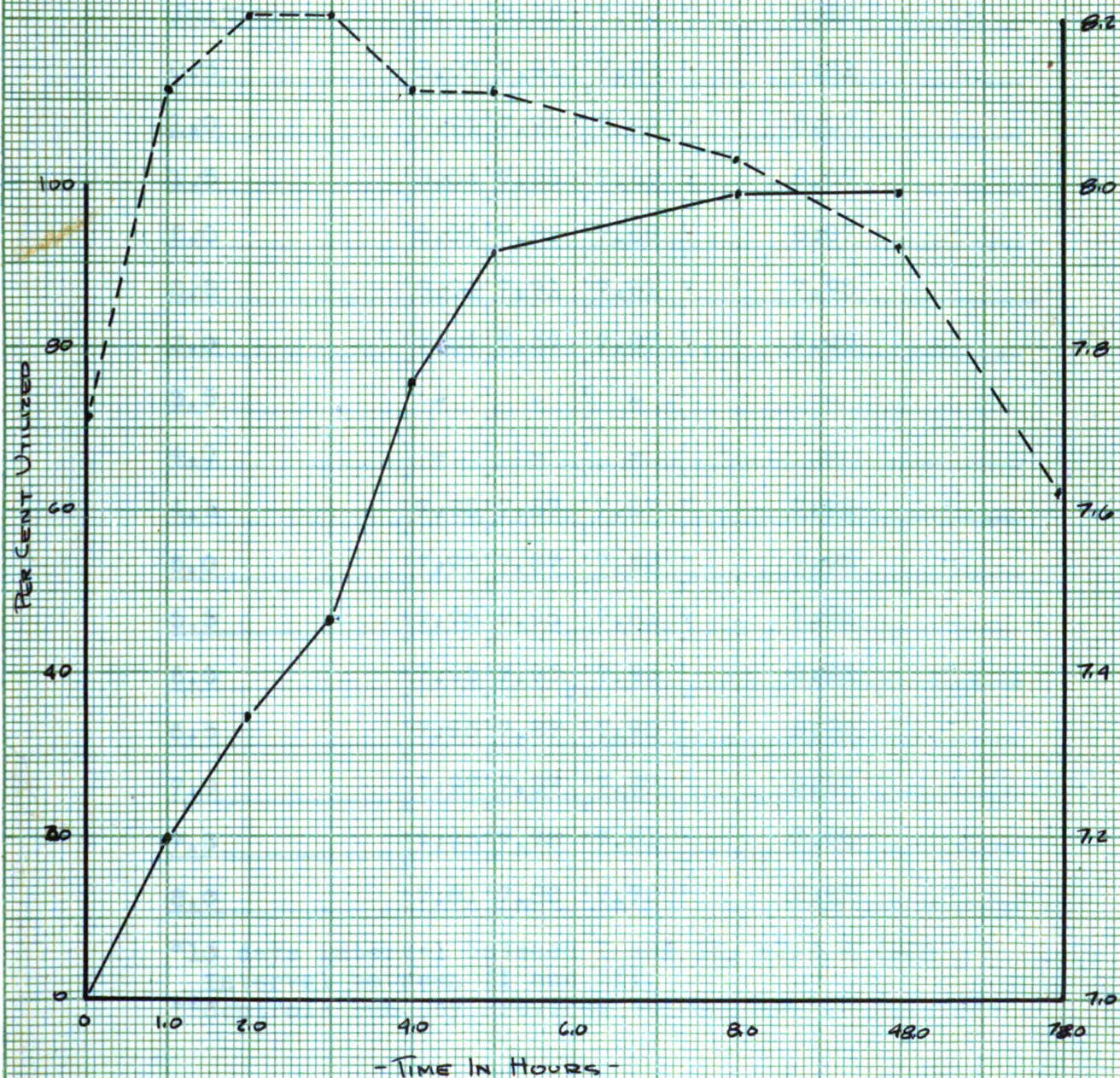
GRAPH-8 (TABLE VIII)LACTOSE UTILIZATION CURVE AND RELATIONSHIP TO PH VARIATION OVER 4 DAY PERIODSLUDGE FROM MASON

Table IX. Sludge from Milk Waste Showing Effect of Carbohydrate (Lactose) Presence on pH Under Conditions of no Aeration.

Time in Hours	'400 ppm Lactose added			Control** pH Values
	pH Trial			
	1	2	3*	
Initial	'8.21	'8.12	'7.95	8.12
0.5	'8.00	'7.91	'7.63	8.21
1.0	'7.95	'7.95	'7.69	8.07
1.5	'8.04	'7.95	'7.69	8.07
2.0	'8.04	'7.87	'7.69	8.07
2.5	'8.04	'7.91	'7.81	7.95
3.0	'7.87	'7.67	'7.87	7.95
3.5	' -	' -	' -	-
4.0	' -	' -	' -	-
4.5	'7.95	'7.87	'7.87	7.98
5.0	'7.95	'7.87	'7.68	7.98
5.5	'7.78	'7.95	'7.81	7.95
6.0	'7.95	'7.95	'7.95	8.04
6.5	'7.95	'7.87	'7.95	8.04
7.0	'7.97	'7.87	'7.87	8.04
7.5	'7.95	'7.78	'7.81	7.95
8.0	'7.81	'7.80	'7.78	7.95

*10 days interval between I and II, also 5 days interval between II and III.

**Controls Run Day Previous to Addition of Lactose.

GRAPH-2 (TABLE IX)

pH FLUCTUATION CAUSED BY ADDITION OF LACTOSE WITHOUT AERATION USING MILK WASTE SUDGE MIXTURE

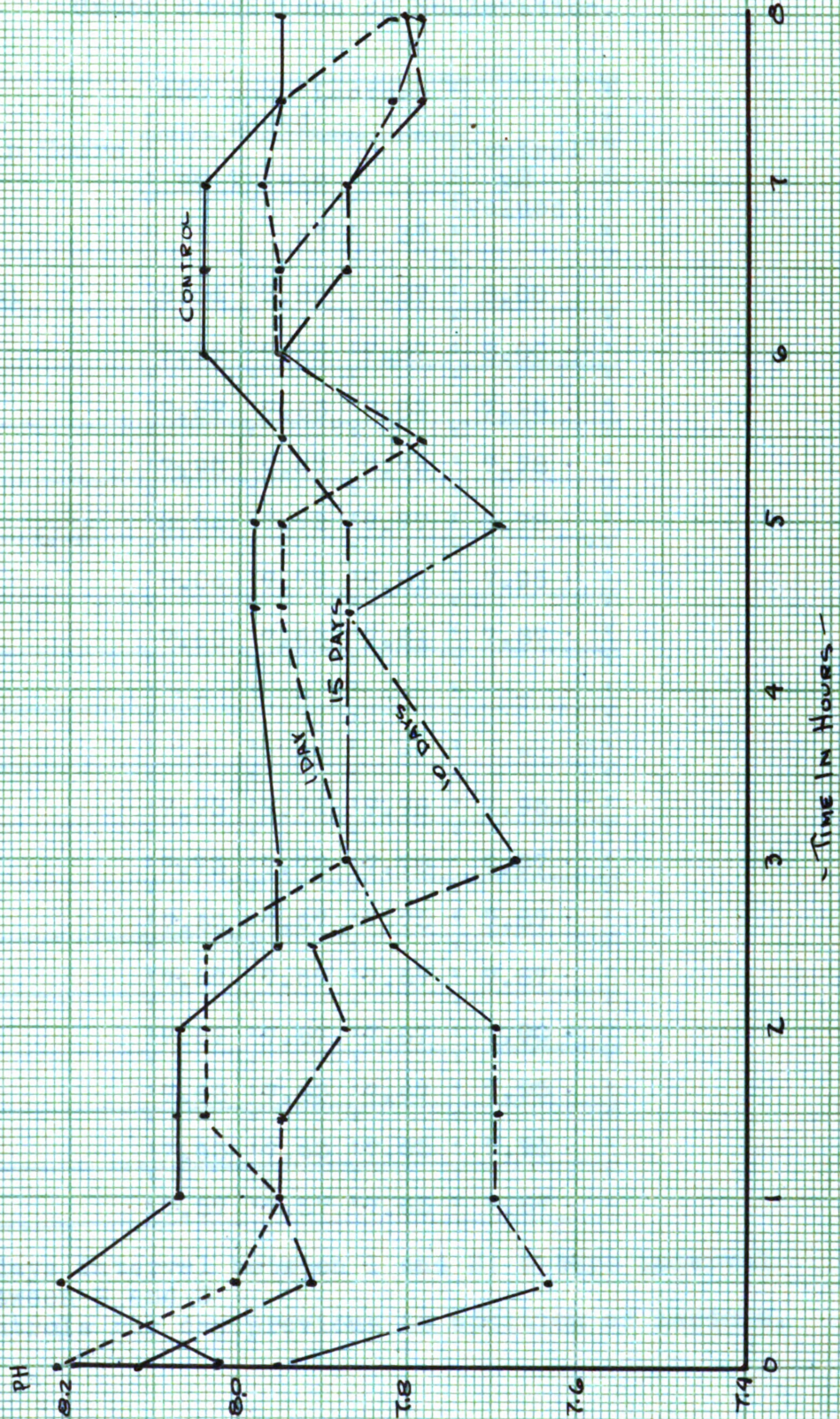


Table X. Sludge from Mason Set-Up Showing Effect of Carbohydrate (Lactose) Presence on pH Under Conditions of No-Aeration.

Time in Hours	400 ppm. Lactose Added			Control** pH Values
	pH Trials			
	1	2*	3*	
Initial	8.04	7.87	7.87	8.00
0.5	7.95	7.63	7.87	8.00
1.0	7.87	7.63	7.82	7.95
1.5	7.95	7.87	7.73	7.78
2.0	8.04	7.72	7.77	7.78
2.5	8.04	7.72	7.81	7.95
3.0	7.63	7.63	7.81	7.87
3.5	-	-	-	-
4.0	-	-	-	-
4.5	7.73	7.72	7.78	7.95
5.0	7.73	7.72	7.78	7.87
5.5	7.63	7.67	7.75	7.87
6.0	7.57	7.67	7.67	7.87
6.5	7.57	7.57	7.67	7.90
7.0	7.63	7.63	7.67	7.95
7.5	7.63	7.63	7.63	7.90
8.0	7.57	7.57	7.63	7.87

* 10 days interval between 1 and 2; 5 days interval between 2 and 3

** Controls run day previous to addition of lactose.

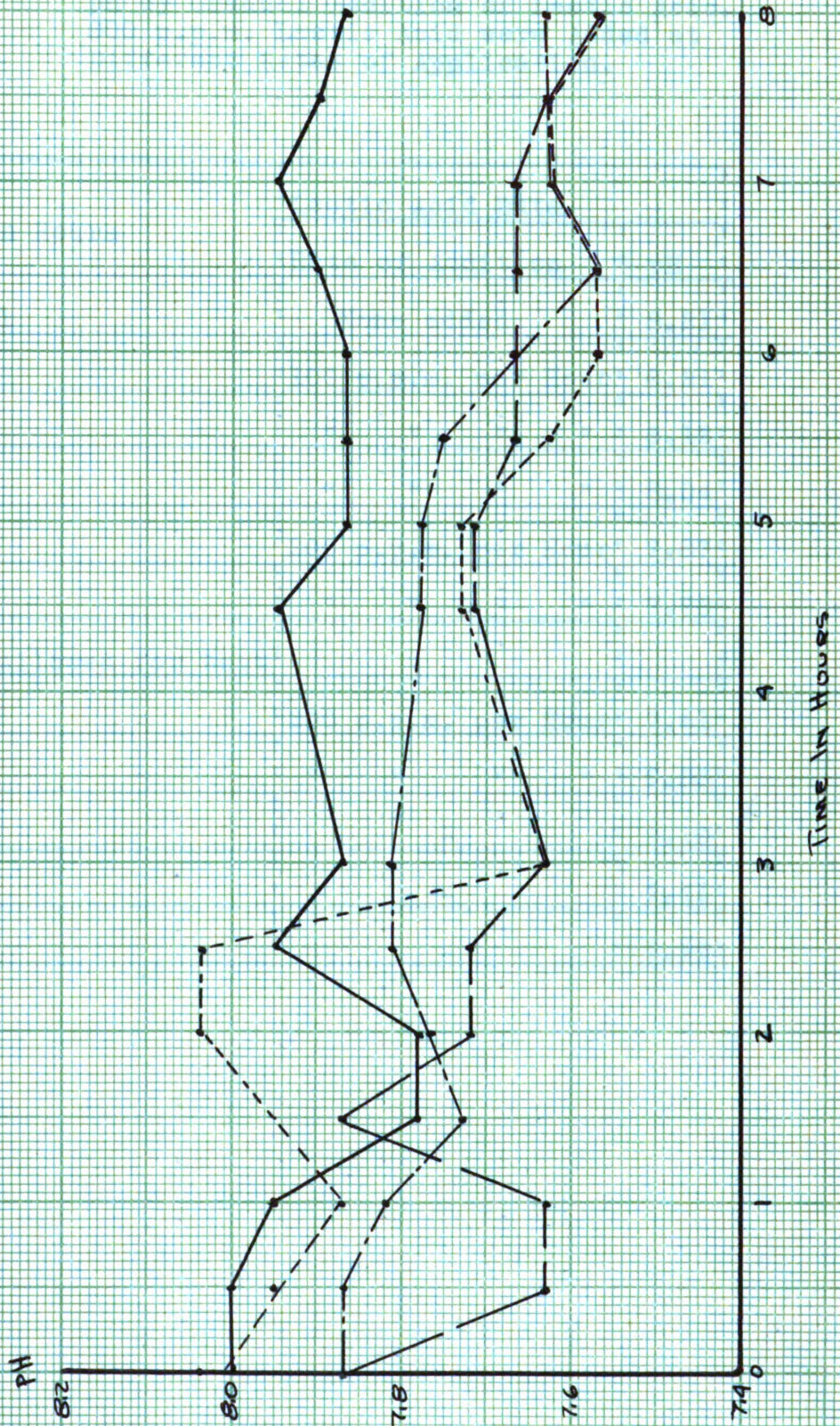
GRAPH - 10 (TABLE X)PH FLUCTUATION CAUSED BY ADDITION OF LACTOSE WITHOUT AERATION ON MASON SLUDGE MATURE

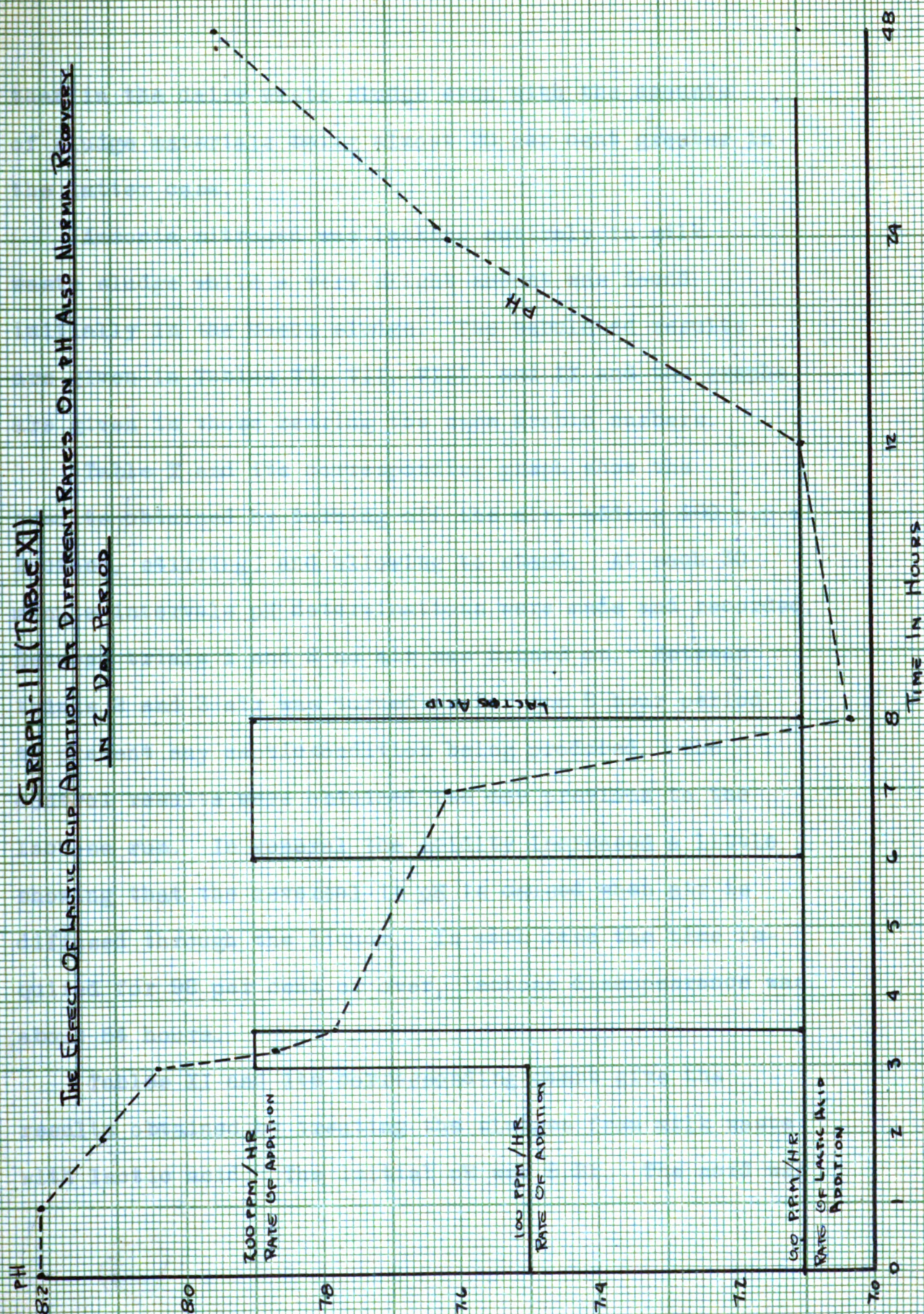
Table XI. Sludge from Milk Waste Showing Effect on pH by Gradual Addition of Lactic Acid. 800 p.p.m. Lactic Acid were Added at Different Rates of Application.

Time in Hours	Lactic Acid Data	
	Rate of Addition	pH
Initial	None added	8.21
1.0	100 p.p.m. per Hour	8.21
2.0	" " " "	8.12
3.0	" " " "	8.04
3.25	200 p.p.m. per Hour	7.87
3.5	" " " "	7.78
1 Hr. Allowed for Rest		
7.0	200 p.p.m. per Hour	7.61
8.0	" " " "	7.03
12.0	-	7.10
24.0	-	7.61
48.0	-	7.95

GRAPH-11 (TABLE XI)

THE EFFECT OF LACTIC ACID ADDITION AT DIFFERENT RATES ON PH ALSO NORMAL RECOVERY

IN 2 DAY PERIOD



than in the dairy waste sludge even with the amounts of sludge materials being almost 70 per cent greater in the latter case.

Ammonia nitrogen was lacking entirely in milk waste sludge while a very slight amount was found initially in the Mason sludge. The tabulated results are given in Tables VI, VII, VIII, and IX and the graphs are given in those bearing the same arabic numbers.

Table X and its corresponding graph show the results obtained by turning off the air, adding 400 p.p.m. lactose, stirring, and allowing to stand. At each 30 minute intervals pH determinations were made and recorded. Between trials 1 and 2 no air was turned on. Between trials 2 and 3 air was turned on for one 8 hour period to prevent any septic condition being produced. The control results were obtained the day previous to the lactose run. In general the acidity increased a little showing that the reversal in pH is caused when air is diffused through the liquor. In each case the time required for 98 per cent or over, lactose disappearance was about 54 hours.

Tables XI and its corresponding chart give the results obtained by treating the sludges from milk waste with lactic acid. The initial pH was 8.21. The acid was

added at the rate of 100 p.p.m. per hour for 3 hours when no noticeable change was noted in the pH. For the next half hour, acid was added at the rate of 300 p.p.m. per hour. A decided drop was noticed. For a 2 hour period in the afternoon lactic acid was added at the rate of 200 p.p.m. per hour. The pH at the end of this treating stood at 7.03 or almost exact neutrality. The administration of 800 p.p.m. lactic acid over an eight hour period was necessary to decrease the alkalinity from 8.21 to 7.03. These findings show that lactic acid was used by the sludge organisms as a direct source of energy. The pH had regained normality at the end of 48 hours without the use of any additional food. Aeration was continuous during the course of the experiment.

PROTEIN UTILIZATION

Peptone additions were made using amounts that would produce a concentration of 300 p.p.m. in the small aeration tanks. Usually the peptone was added at 8:00 A.M. on the days when additions were to be made and the determinations made daily over a twenty day period in one instance and a thirty day period in another. Table XII gives the results obtained from protein addition to sludge developed from milk waste

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and Table XIII shows the results obtained from the mixed domestic and dairy milk plant wastes. Graphs 12 and 13 show the trends respectively.

A slight acidity is noticed throughout the peptone experiments. This was not as pronounced in mixed waste sludge as in milk waste sludge. The period of time necessary for complete nitrification was taken as the time length of the experiment. In other words when the free ammonia and nitrite were oxidized through the steps to nitrate and also when the nitrate remained constant the experiment was assumed to be finished.

The nitrogen conversion could not be determined quantitatively at any one time, that is, the nitrogen equivalent in nitrate when the experiment was finished might not be equivalent to that nitrogen equivalent shown in free ammonia nitrite at any time previous to the complete conversion. Anaerobically this is true as the nitrogen is not used to such a great extent in building up the protozoon bodies as is true in the activated sludge process.

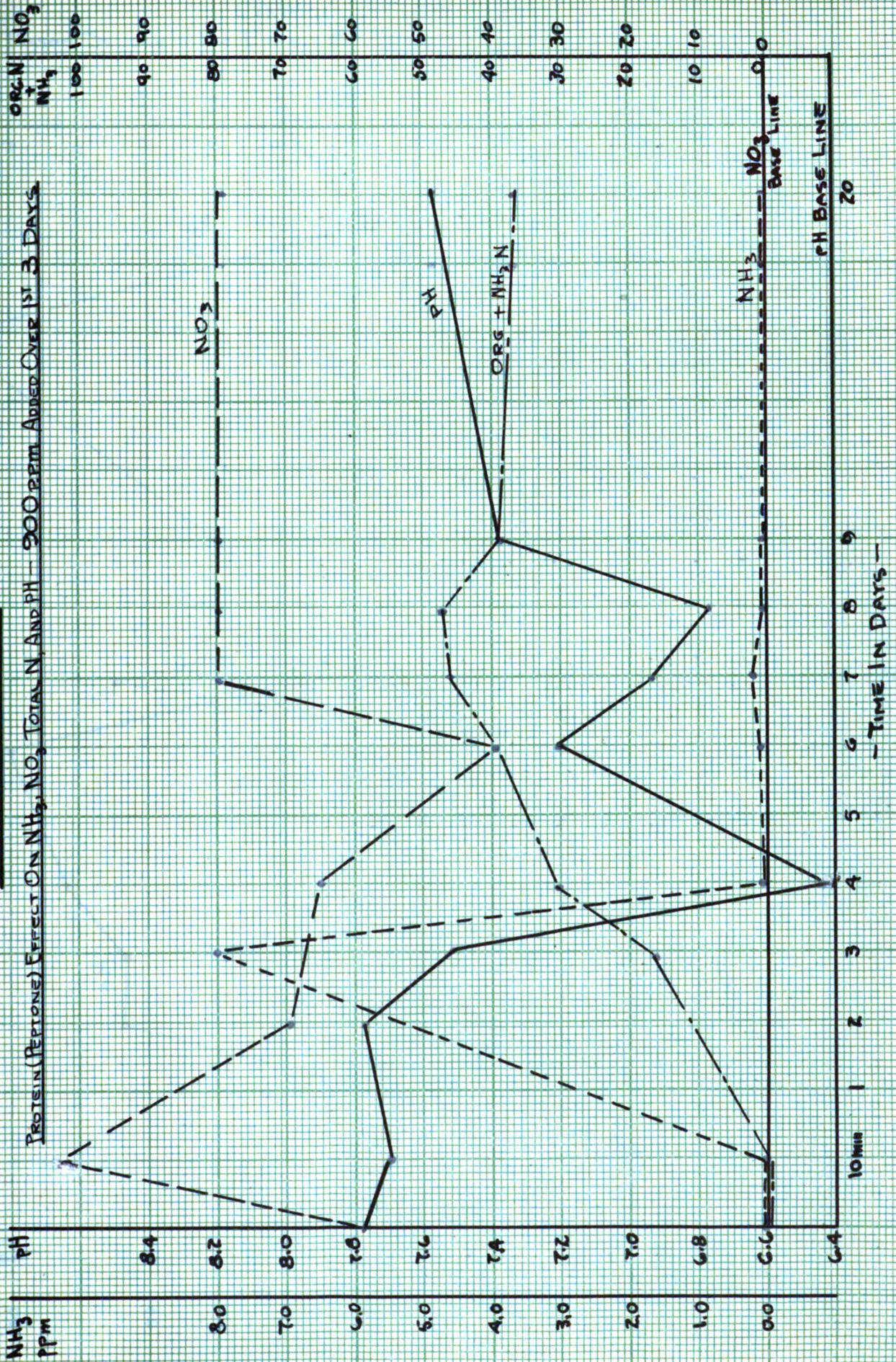
The most important results of these experiments are the suspended solids and settleable solids values. In case of milk waste sludge the suspended solids are given in Table XVII along with the settling rates and settleable

Table XII. The Effect of Protein (peptone) on Sludge Developed from Milk Waste.

Time in Days	'Peptone' Added	pH	Nitrogen As			'Org.N' Present	'Protein' Calculated	'Suspended Solids'
			NH ₃	NO ₂	NO ₃			
Initial Day	300.0	7.78	0.00	0.00	0.00	53.7	342.5	752.0
10 min. after addition	-	7.70	0.00	0.00	0.00	103.2	658.3	-
2.0	300	7.78	Error in Method Caused Incorrect results.					
3.0	300	7.53	8.00	1.5	16.0	69.6	393.0	672.0
4.0	None	6.43	0.00	0.75	30.0	65.6	418.0	656.0
6.0	"	7.20	0.01	0.01	40.0	39.8	271.7	536.0
7.0	"	6.93	0.02	0.01	80.0	46.0	292.0	528.0
8.0	"	6.77	0.00	0.005	80.0	47.6	303.8	584.0
9.0	"	7.37	0.00	0.00	80.0	39.0	248.8	535.0
20.0	"	7.57	0.00	0.00	80.0	37.0	236.0	621.0

GRAPH-12 (TABLE XII)

PROTEIN (PEPTONE) EFFECT ON NH_3 , NO_3 , TOTAL N, AND PH - 200 PPM ADDED OVER 1ST 3 DAYS.



GRAPH-13 (TABLE XIII)

MASON SLUDGE - THE EFFECT OF PEPTONE ADDITION AS ON GRAPH 12

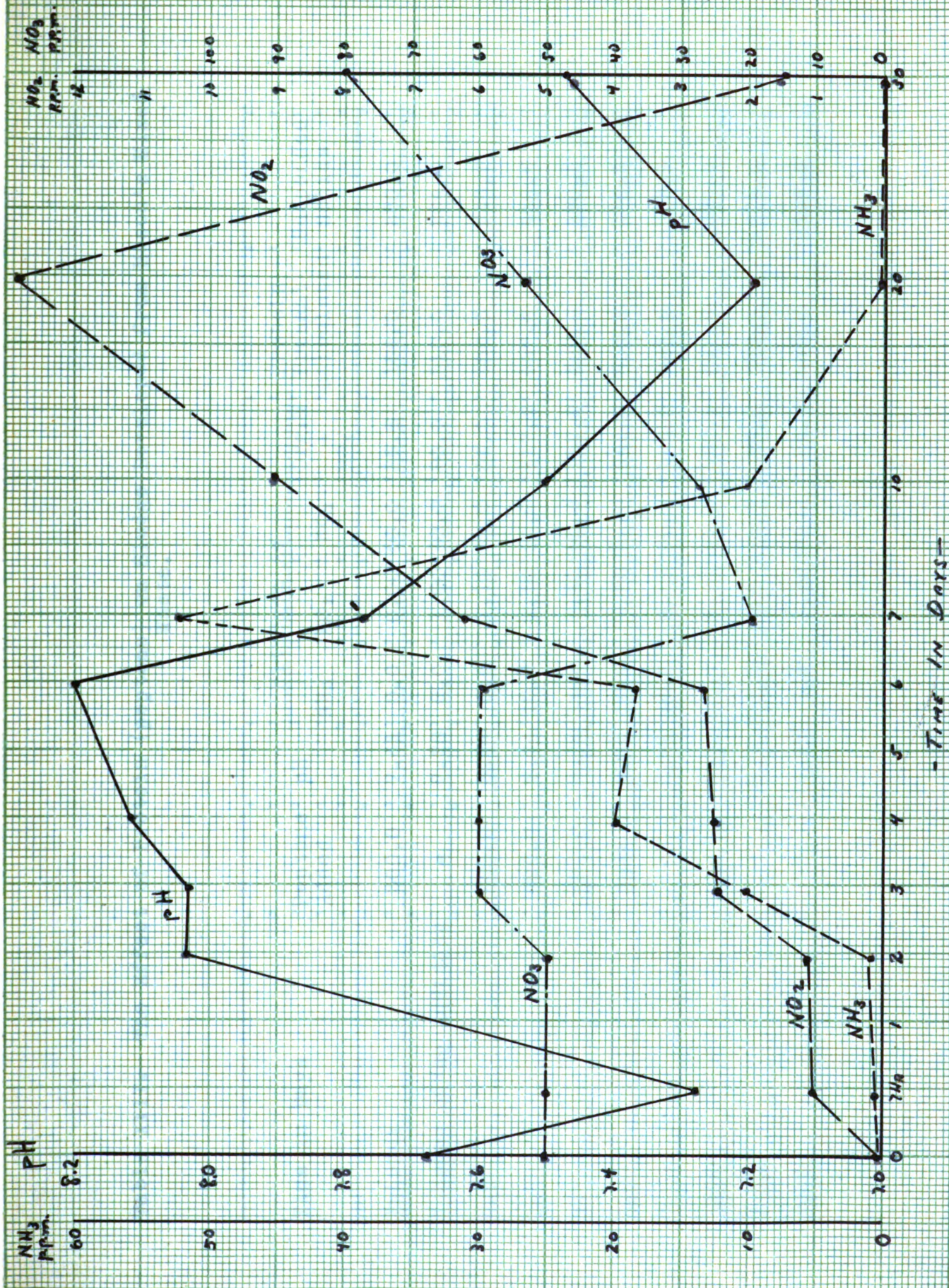


Table XIII. The Effect of Protein (peptone) on Sludge Developed from Mason's Mixed Waste.

Time in Days	Peptone Added	pH	Nitrogen As			Org.N. + NH ₃	Protein Present Calculated	Suspended Solids
			NH ₃	NO ₂	NO ₃			
Initial	300.0	7.61	0.0	0.00	50.0	25.8	164.0	280.0
7 Hours After Addition	-	7.28	0.0	1.00	50.0	80.0	510.0	224.0
2.0	300.0	8.04	0.6	1.10	50.0	64.0	404.5	248.0
3.0	300.0	8.04	10.5	2.50	60.0	112.0	647.6	456.0
4.0	300.0	8.12	20.0	2.50	60.0	150.0	829.4	480.0
6.0	None	8.21	17.0	2.70	60.0	146.0	823.0	320.0
7.0	"	7.78	56.0	6.25	20.0	145.0	567.8	362.0
10.0	"	7.51	20.7	9.15	27.6	137.0	741.9	1080.0
20.0	"	7.20	0.6	13.0	54.0	98.0	621.4	1624.0
30.0	"	7.47	0.0	1.60	80.0	72.0	459.4	1136.0

TABLE XIV. SETTLING RATES OF MILK WASTE SLUDGE
DURING CARBOHYDRATE ADMINISTRATION.

SAMPLE NUMBER	SUSP. SOLIDS	SETTLING RATE (CCS.-MIN.)				
		0	15	30	45	60
1	---	1000	130	100	90	90
2	---	1000	112	90	90	85
3	395.0	1000	140	140	130	125
4	370.0	1000	110	100	93	90
5	380.0	1000	125	100	100	92
6	268.0	1000	106	97	93	93
7	428.0	1000	131	103	94	91
AVERAGE	368.2	1000	122	104	98	95

TABLE XV. SETTLING RATES OF SLUDGE DEVELOPED AT
MASON SET-UP DURING CARBOHYDRATE ADMINISTRATION

SAMPLE NUMBER	SUSP. SOLIDS	SETTLING RATE (CCS.-MIN.)				
		0	15	30	45	60
1	552.0	1000	250	195	180	175
2	280.0	1000	240	190	185	178
3	560.0	1000	260	210	190	185
4	480.0	1000	245	205	190	190
5	430.0	1000	210	170	165	165
6	415.0	1000	235	210	198	195
7	295.0	1000	230	195	190	187
AVERAGE	430.3	1000	239	196	185	182

TABLE XVI. SETTLING RATES OF SLUDGE DEVELOPED AT
MASON SET-UP DURING PROTEIN UTILIZATION

CONFORMS TO DATA IN TABLE X						
TIME IN	SUSP.	SETTLING RATE (CCS.-MIN.)				
DAYS	SOLIDS	0	15	30	45	60
INITIAL	280.0	1000	200	170	130	120
2.0	248.0	1000	163	135	128	120
3.0	456.0	1000	120	112	100	80
4.0	480.0	1000	80	70	65	60
6.0	320.0	1000	50	44	42	40
7.0	362.0	1000	60	55	50	50
10.0	1080.0	1000	120	100	90	88
20.0	1624.0	1000	230	170	140	120
30.0	1136.0	1000	190	140	115	105
AVERAGE	733.0	1000	135	111	96	87

TABLE XVII CONFORMS WITH TABLE IX

THE SETTLING RATES OF SLUDGES DEVELOPED FROM
MILK WASTE(OBTAINED FROM OPERATION DATA ON PROTEIN
UTILIZATION) AS COMPARED TO SUSPENDED SOLIDS.

TIME IN DAYS	SUSP. SOLIDS	SETTLING RATE (CCS. - MIN.)				
		0	15	30	45	60
INITIAL	752.0	1000	75	74	72	70
2.0	---	1000	72	70	68	66
3.0	672.0	1000	72	62	61	61
4.0	656.0	1000	80	73	71	70
6.0	536.0	1000	62	56	55	55
7.0	528.0	1000	68	60	60	60
8.0	584.0	1000	64	60	60	60
9.0	535.0	1000	70	60	58	58
20.0	621.0	1000	72	63	62	62
AVERAGE	610.0	1000	70	64	63	62

solids results. Briefly, the suspended solids only decrease some 200 p.p.m. while in the case of Mason's mixed waste sludge the amount steadily increased some 900 p.p.m. Tables XIV, XV, XVI and XVII show these relationships of suspended solids to settleable solids in carbohydrate treatment and in peptone treatment to both mixed domestic sludges from Mason and to dairy waste sludges from the College Dairy. These relationships are very important.

During the peptone treatment on mixed wastes an instance of nitrate reduction was noticed. Bacteriological examinations made during this time indicated that nitrate reducers were present in great numbers. Ten out of 12 cultures isolated nitrate reduction was noticed. The ammonia content at this time caused an alkaline pH. The fluctuations are indicated on Graphs 12 and 13.

COMBINED CARBOHYDRATES AND PROTEIN ADDITION

In order to determine if possible, any effect one class of foods had upon the other, combined protein and carbohydrate materials were added. As in the previous experiments lactose was used as the carbohydrate and peptone as the protein material.

The addition amounted to 500 p.p.m. lactose and 300 p.p.m. peptone. Both were dissolved in separate beakers in water and added immediately after a sample had been taken for the initial determinations. Lactose

utilization, pH, nitrogen as ammonia, nitrites, and nitrates, also the suspended solids and settling rates including settleable solids were recorded. Only one addition of peptone was made because of the time consumed in complete nitrification.

The results in Table XVIII, show an initial pH of 7.95 and a suspended solids content of 652 p.p.m. As has been stated these two values were most important when considering the dual effect of food ratios upon bulking. According to expectations the pH immediately after the experiment started rose slightly. Possibly this was due to the reversal effect of lactose metabolism or to the very small amount of ammonia that was produced immediately from the peptone. However, at the end of 4 or 5 hours the pH had dropped to 7.15 while the ammonia in solution had increased to 0.4 p.p.m. About 15.0 p.p.m. of the lactose had been used. On this basis it is safe to conclude that neither foods have the same effect when used together that they do when added separately. Until more is known about the actual construction of the protein and carbohydrate molecules the effect one has upon the other will not be definitely known.

Throughout the course of the experiment the pH became slightly acid and then became increasingly alkaline at the end of 75 hours. At the end of 72 hours a slight reduction

of nitrates took place. At this time all lactose had been used. By the time the experiment was completed, however, the nitrates had increased slightly once more and all of the ammonia had disappeared. The suspended solids during this period fluctuated slightly and ended with 593 p.p.m. This could not be considered as a material change in the face of the variations noticed on individual lactose and peptone additions.

A check run was made using the same amounts and similar results were obtained. This time it was noticed that the pH remained rather, constant, only a slight tendency toward neutrality being noticed.

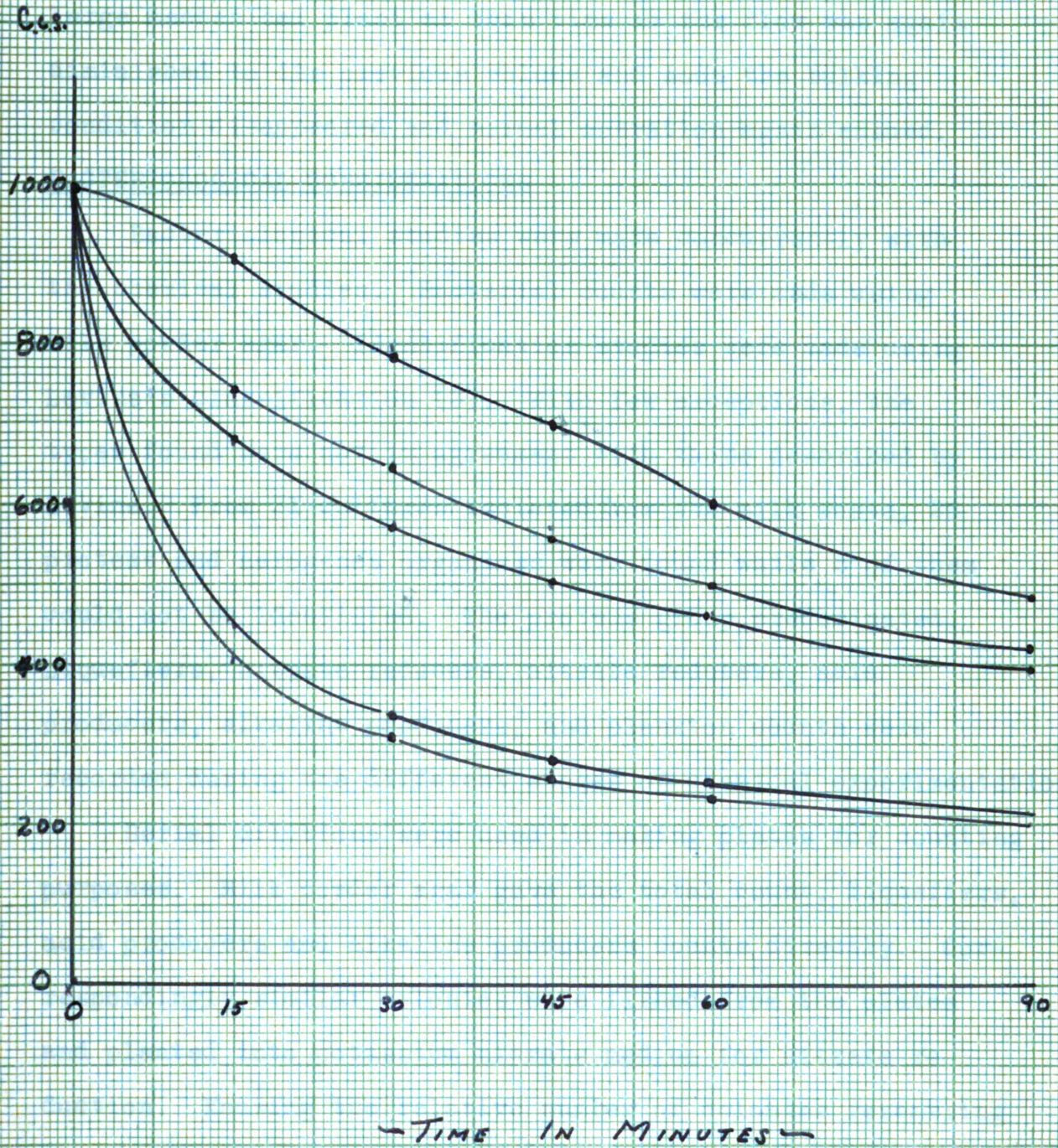
FAT DETERMINATIONS

To determine the effect of fat upon the settling characteristics, an attempt was made to add a known amount of pure butter fat prepared from sweet butter to the small aeration tank. The material was emulsified but was extremely unstable. It formed a thin scum over the tanks. This phase of the work was abandoned.

It was possible to get increased fat content in the main aeration system by increasing the flow from the orifice box. The settling rates and fat content are shown on graph 18.

GRAPH 14

RELATION OF FAT CONTENT TO SETTLING RATES



An almost perfect correlation was observed showing the effect of fat content upon settling rates in general. Incidentally a severe bulking condition existed in the large mechanical plant at this time so the flow was again diminished to its previous volume and soon the bulking condition disappeared and normal operation again ensued.

BACTERIOLOGICAL CONSIDERATIONS

Bacteriological studies were made concurrently with the chemical studies. Bacterial counts were made using milk powder agar. Identification studies were made using Levine's classification (3). With few exceptions, the types found were not in agreement with those described by Levine. From 31 cultures isolated from the milk waste, 26 proteus and aerogenes organisms were found while 5 of them could not be identified. The proteus group predominated. Liquefiers, nitrate reducers, nitrite reducers, acid producers, alkaline producers, and plankton counts were recorded during the different stages of chemical experimentation.

Serial dilutions were used in making plates for counting purposes. Milk powder agar gave the total count, liquefiers, acid producers and alkaline producers counts directly. Correspondingly similar colonies were fished from the plates and planted into potassium nitrate medium for nitrate reduction. Knowing the relative number of colonies resembling

Table XVIII. The Effect of Combined Carbohydrate and Protein Diet upon pH and Solids in Activated Sludge.

Trial 1.							
Time in Hours	pH	Lactose Utilization		Nitrogen As			Suspended Solids
		ppm Pres.	% Used	NH ₃	NO ₂	NO ₃	
Initial	7.95	500 added	None	0.00	0.025	60.0	652.0
1.0	8.12	501.0	0.0	0.12	0.025	60.0	-
2.0	-	445.0	11.0	-	-	-	-
2.5	7.78	-	-	0.12	0.025	60.0	-
4.0	-	426.0	14.8	-	-	-	-
4.5	7.15	-	-	0.40	0.050	70.0	-
6.5	7.48	-	-	0.40	0.075	70.0	-
7.0	-	414.0	17.2	-	-	-	-
8.0	7.61	-	-	0.40	0.075	80.0	-
24.0	6.52	-	-	4.00	0.190	120.0	530.0
24.5	-	352.0	29.6	-	-	-	-
26.0	6.90	-	-	5.00	0.200	120.0	-
30.0	-	350.0	30.0	-	-	-	-
48.0	6.60	104.0	79.2	3.65	0.350	120.0	584.0
54.0	6.95	-	-	2.80	0.270	125.0	-
72.0	-	0.0	100.0	-	-	-	-
75.0	7.50	-	-	2.00	0.200	100.0	620.0
125.0	7.53	-	-	0.00	0.080	110.0	593.0

TRIAL II. SLUDGES FROM MASON & COLLEGE MIXED-NOT GRAPHED.

TABLE XIX. THE EFFECT OF COMBINED CARBOHYDRATE AND PROTEIN
DIET UPON pH AND SOLIDS IN ACTIVATED SLUDGE.

TIME IN HOURS	pH	LACTOSE UTILIZATION		NITROGEN AS			SUSP. SOLIDS	
		ppm Pres.	% Used	NH ₃	NO ₂	NO ₃		
INITIAL	7.53	500.0	Added	None	0.0	0.08	120.0	590.0
1.0	7.11	506.0		---	0.0	0.15	120.0	---
1.5	7.10	494.0		1.2	---	---	---	---
4.5	7.03	479.0		4.2	0.30	0.25	120.0	---
11.0	7.37	375.0		25.0	---	---	---	---
24.0	7.53	108.0		78.4	1.90	1.00	120.0	470.0
30.0	--	0.0		100.0	---	---	---	---
48.0	7.53	###		---	0.20	1.10	140.0	525.0
72.0	7.53	---		---	0.00	0.50	160.0	565.0

these types on a plate a rough estimate was made of the nitrate and nitrite reducers. Special media were not used for this purpose.

Table XX shows the counts made on raw waste entering the plant. This table gives the number of organisms appearing on milk powder agar. The number is slightly higher than counts obtained using plain nutrient agar(A.P.H.A. standard medium). The nutrient agar gave fewer chromogenic organisms as well as a total reduced count. Organisms producing red, yellow, brown, black, pink, orange, blue, and green colonies were isolated from milk powder agar but were not identified. Ability to induce carbonate and bicarbonate production was extremely limited.

Table XXI gives the counts made on the sludges in the small aeration tank during lactose addition.

The nitrate reducers were prominent. The plankton counts show a great prevalence of the filamentous organisms belonging to the Cladothrix group. Infusoria are prominent also. A large amount of inert sludge particles was noticed in all lactose treatments.

A comparison of the results of Table XXI with those of Table XXII, which were compiled from data taken during peptone treatment, shows a marked diminution of plankton organisms while the bacteriological counts were increased.

TABLE XX. COUNTS MADE ON RAW MILK WASTE ENTERING PLANT.

SAMPLE NUMBER	TOTAL COUNT	LIQUEFIERS %	NO ₃ REDUCED %	NO ₂ REDUCED %	B.1.a. %
1.	1,280,000	2	None	None	60
2.	2,040,000	0	4	2	72
3.	1,600,000	1.4	3	None	66
4.	972,000	3.0	5	None	57

TABLE XXI. BACTERIOLOGICAL COUNTS MADE DURING LACTOSE
ADDITION .

SAMPLE NUMBER	TOTAL COUNT	PROTOZOA	ACID PROD. %	ALK.PROD. %	LIQ. %
1	12,300,000	19,000	62	2	7
2	14,050,000	17,000	65	13	10
3	10,010,000	17,000	66.7	11	13
4	9,060,000	18,700	61	12.1	9
5	11,120,000	16,900	56.7	4.2	11

TABLE XXII. BACTERIOLOGICAL COUNTS MADE DURING PROTEIN
ADDITION.

SAMPLE NUMBER	TOTAL COUNT	PLANKTON	ACID PROD. %	ALK.PROD. %	LIQ. %
1	10,010,000	13,000	23.0	71.0	2.0
2	8,370,000	12,000	19.0	64.0	4.0
3	7,970,000	11,380	15.0	74.0	6.5
4	14,700,000	12,320	21.0	66.0	3.8
5	13,270,000	11,210	34.0	54.0	6.2

The acid producers decrease in numbers during protein addition while the alkali producers increased. Strangely, the nitrate reducers are a little less in number than when carbohydrates were added.

That the decrease in plankton organisms offers an explanation of bulking has been known for some time or at least has been referred to. Not merely an increase in the protozoan count is significant but also the increase of filamentous organisms stimulated by carbohydrate supply. All through this work it was noticed that any increase in lactose addition caused a great increase in *Sphaerotilus* organisms. This has been observed by others many times. Nevertheless, the mere significance attached to bulking by increased carbohydrate supply does not necessarily indicate that *Sphaerotilus* group organisms represent the only cause of bulking. Observations show that these filamentous forms are present at all times and their counts cannot readily be determined because of their interwoven structure. It is not possible at this time to state that plankton organisms have no effect upon bulking because too much data is given to indicate that they do have some effect. However, it is rational to believe that there is a multiplicity of factors involved in creating bulking conditions rather than merely a difference in ratio of total plankton to bacterial counts.

It is more logical to believe that the balance between the types of organisms has been changed rather abruptly. It is conceivable that this change can be influenced by pH change, food supply, and other more obscure changes which might easily be induced by different types of waste.

As pH change has just been mentioned as an agent in causing bulking features and has been found in the foregoing work to be radically different than was expected, experiments using synthetic media using different initial pH values were carried out. These experiments allowed us to observe the rapidity in pH fluctuation under aerobic conditions also the production of alkali should any have been produced.

A set-up consisting of 3 sets of graduates were used. Each set consisted of one graduate with sugar solution and another with a control solution. Each set used different initial pH values. The values used were pH 6.0, pH 7.0, and pH 8.0 respectively. The synthetic sugar waste solution was made according to the following formula:

10.0 gm.	dextrose
10.0 "	Peptone
10.0 "	monobasic potassium phosphate
5.0 "	sodium chloride
1,000.0 cc.	distilled water

The control solution consisted of all those constituents named above with the exception of sugar.

Each of the six graduates was seeded with 1.0 cc of activated sludge taken from the small laboratory plant while running on sludges from milk waste. Air was bubbled through the graduates by means of glass capillary tubes extending nearly to the bottoms.

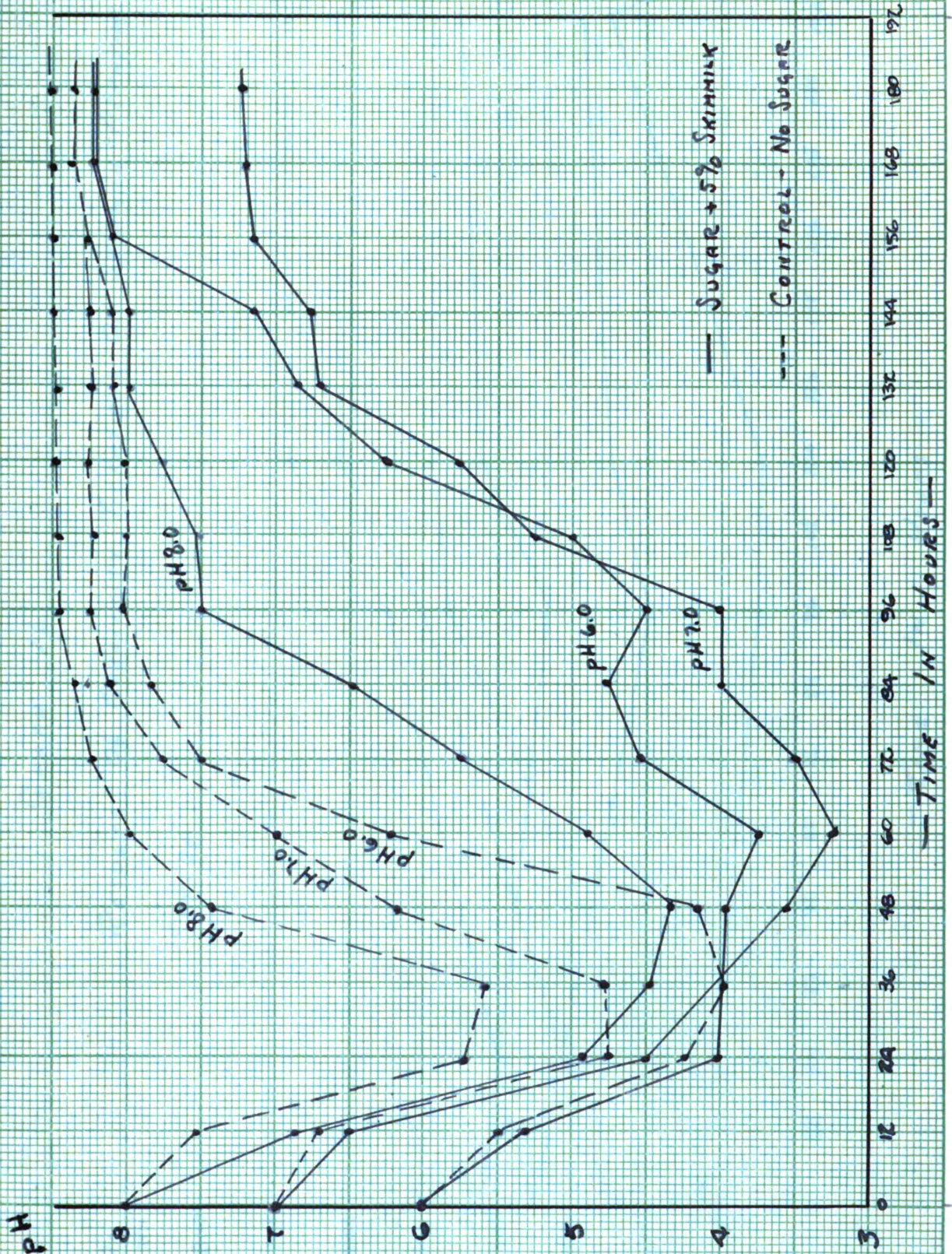
In the first trial the pH of all the sugar solutions dropped so rapidly that it was thought advisable to try a different solution. The synthetic sugar waste was abandoned in favor of a 5 per cent skim milk solution with sugar added and adjusted to pH 6.0, pH 7.0, and pH 8.0. The pH was recorded at the end of 12 hour intervals. Graphs 14 and 15 show the results obtained from 5 per cent skim milk solutions and sugar broth solutions respectively.

The sugar waste with an initial pH of 6.0 dropped as rapidly as before but did not become as acid as when using the broth. The pH reversed and started to go alkaline after 72 hours of aeration. At the end of 180 hours the pH of all solutions was considered constant and each gave a higher pH reading than initially.

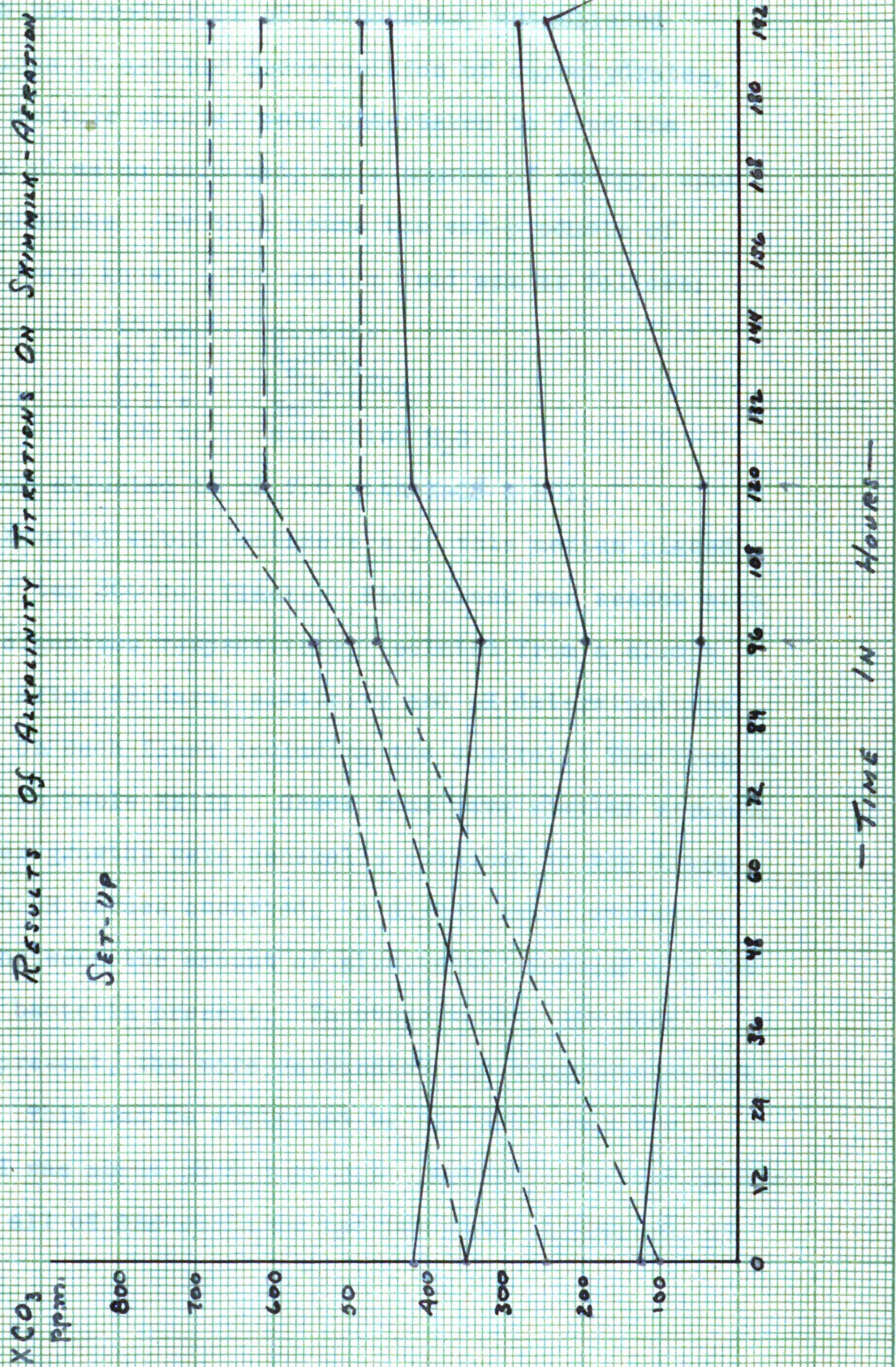
Alkalinity titrations were made at various intervals during the 216 hours period. The carbonate curve parallels the alkalinity curve. No bicarbonate alkalinity was noticed at any time during this study.

GRAPH 15

pH FLUCTUATION OVER A LONG PERIOD UNDER FORCED AERATION. 5% SKIMMILK + DEXTROSE USED AS MEDIUM.



GRAPH 16.
RESULTS OF ALKALINITY TITRATIONS ON SWIMMING-POOL
SET-UP



With the production of carbonates, established end-products in aerobic decomposition of carbohydrates, the next culture studies were undertaken to find the influence an amino acid had, as a source of energy, upon growth abundance. By this means the end-products of digestion were known. The medium used was as follows:

10 gm.	glycine
0.1 "	K_2HPO_4
0.1 "	$(NH_4)_2SO_4$
5.0 "	NaCl
1000 cc.	distilled H_2O

Medium adjusted to pH 7.0 with NaOH & HCl.

One hundred cubic centimeters of this medium placed in each of ten 200 cc. flasks. Each flask was seeded with one of 9 organisms respectively, isolated from a sludge mixture. After 28 days, alkalinity and pH determinations were made on each flask including the control. The results are given in Table XXIII. Check runs gave similar results.

Better growths were generally obtained in the flasks where high pH values occurred. A direct ratio evidently occurred between the amount of alkalinity produced and the amount of bacteria present. In other words, the more bacteria present, the more carbonates and bicarbonates are produced. This can be explained by the fact that the optimum pH was about pH 8.0 or 8.5. However, in view of the fact that all of the cultures had the same initial pH, it

was assumed that the organisms produced the alkalinity from the medium. All of the organisms except one grew abundantly so the food requirements of the organisms and the available food supply in the medium were of primary importance when consideration of abundance of growth was made.

For further studies on alkali production, a medium was used consisting of a base medium to which the desired salts were added in the concentrations thought necessary for good growths.

The base medium consisted of:

0.1 gm.	$(\text{NH}_4)_2\text{SO}_4$
5.0 "	NaCl
1000 cc.	distilled H_2O

The salts added were:

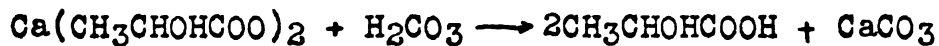
Medium A.	Calcium lactate in amounts sufficient to produce a 1.0% and a 0.5% concentration.
Medium B.	Sodium lactate in amounts sufficient to produce a 1.0% and a 0.5% concentration.
Medium C.	Sodium asparaginate in amounts sufficient to produce a 1.0% and 0.5% concentration.

Each medium was adjusted to a pH of 7.0. The ammonium salt was added to supply a convenient source of nitrogen. After tubing and sterilizing at 15 pounds pressure for 20 minutes, the media were seeded with 9 cultures isolated from a sludge

mixture (as in previous experiment) and allowed to incubate at 21°C. for 28 days. The results are recorded in Table XXIV.

In most instances it was found that growths were heavy with development of acid pH. Sodium asparaginate medium produces most growth, followed closely by calcium lactate medium. An unexplainable result was the inhibitory effect of sodium lactate upon the organisms which grew abundantly in calcium lactate medium.

It is conceivable that calcium lactate is broken down into lactic acid and calcium carbonate according to the reaction:



This being the case the calcium would be removed from solution leaving an accumulation of lactic acid which exerts the acid effect upon the pH. The pH determinations were not made upon the sodium lactate or sodium asparaginate. The pH determinations were made upon 1 per cent calcium lactate when growth was present, otherwise upon 0.5 per cent calcium lactate.

TABLE XXIV. RESULTS OF TWENTY-EIGHT DAY INCUBATION
OF ISOLATED ORGANISMS ON SODIUM AND CALCIUM SALTS.

CULTURE NUMBER	FINAL pH	Ca LACTATE		Na LACTATE		Na Asparaginate	
		1%	0.5%	1.0%	0.5%	1.0%	0.5%
1	5.585	+++	++	+++	++	++	++
2	6.960	-	-	+	+	+	+
3	6.010	++	++	+++	++	++++	++++
4	6.095	+	+	-	-	++	++
5	6.180	++++	++	-	-	+	+
6	6.984	-	-	-	-	-	-
7	6.010	+++	+	-	-	+	+
8	6.080	+	+	-	+	+++	+++
9	6.975	-	+	-	-	+	+
Cont.	7.025	-	-	-x	-	-	-
E.Coli	6.945	++++	+++	++++	++++	+++	+++
B.Subtilis	7.20	++++	++++	++++	++++	++++	++++

DISCUSSION

It has been the practice of physiological bacteriologists in the past to assume that the complicated processes attributed to bacterial growths were caused by enzymes. The enzymic theory as known at present offers a very secure haven for those who attempt to explain difficult biological reactions. It also seems that with the present knowledge concerning enzymes the arguments presented by one versed in enzymology are too forceful to be easily destroyed. Biological equations are impossible to balance, therefore the enzymic theory seems at least, to be the most logical when considering all angles. It is the writer's idea that enzymes do play an important part in all physiological phenomena and that they are largely responsible for the myriad of phenomena relative to life.

In considering an activated sludge we are not only dealing with single celled organisms but also with many metazoa. Of course the relationships existing between the bacteria and the protozoa have been the most important facts previously used by chemists and bacteriologists in explaining bulking. This is true, but further study was necessary to definitely establish the cause of changes in these relationships and to study the changes themselves.

Cramer (7) has observed that vigorous protozoan growths are necessary for good sludge operation and to maintain power to carry down bacteria, protozoa and finely divided organic materials. It is shown here that the types of protozoa invigorated by different classes of food are very important. It is shown that organisms of the Cladothrix types are very undesirable when good settling is desired. The condition could be varied with considerable success merely by changing the diet. It was possible to change the whole sludge picture also by this simple practice. Rudolfs and Heukelekian (32) in discussing the digestion of sludge concentrates both aerobically and anaerobically, stress a "phase" action in protein utilization and nitrite and nitrate production. They found a peculiar effect upon the rates of nitrification and protein utilization in the presence of fats, which of course occur to some extent in the primary stages of digestion. The rate of nitrification progresses at a fairly rapid rate for about 30 days at which time analyses show a period in which the nitrification remains stationary, also there was a total disappearance of fat. At the end of 40 or 45 days, however, the nitrogen conversion again progresses slowly at first, then more rapidly until

complete nitrification is obtained. They thus conclude that presence of fat is important in the food balance and that when it is all used, the organisms must become accustomed to its absence before normal nitrification again takes place.

At the time this work was in progress no observations were made daily to determine whether fats were present in sufficient amounts in the sludges to cause an effect of this kind although it was noticed that "phase" activity was present to a small degree in nearly all of the experiments. It was thought that this was due to changes in the flora rather than to the presence or absence of any food constituent. The work of **Rudolf** and Heukelekian seems more logical perhaps and does seem to partially explain the results obtained when protein and carbohydrates were added concurrently.

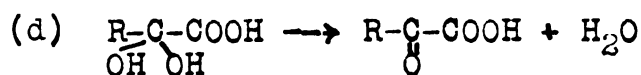
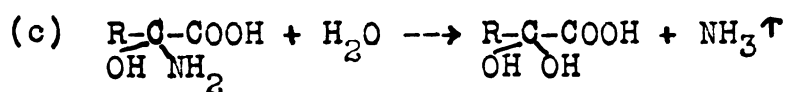
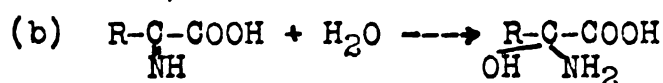
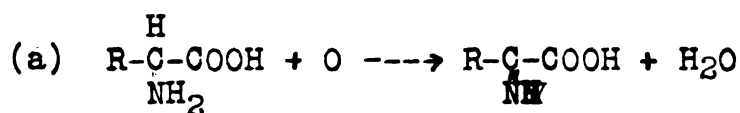
In considering the variations observed in pH during the food addition it was found that during lactose addition a tendency toward alkalinity existed and in peptone feeding the reverse was noticed. No great variation in pH was noticed when combined lactose and peptone additions were made after the sludge became conditioned.

It is difficult to give an explanation for alkaline production from a carbohydrate source. There is a

possibility that there is normally an acid condition existing in the sludge suspension and as soon as carbohydrate is added and aeration started, the alkali producers are developed more rapidly than the acid producers, or another assumption is, that acid utilizers start to work immediately. Cramer (7) has shown that sludge organisms have their origin in the soil and are therefore primarily soil organisms, thus it can be assumed that either or both of these assumptions may be correct. We know that the soil contains many potential alkali producers that probably obtain their energy and growth from carbohydrate sources. Still another possibility is that when lactose or any other carbohydrate is broken down we find that various acids are produced as intermediate steps. It may be true that in the presence of either sodium or potassium salts, probably the chlorides, a formation of a slightly basic salt may be formed. The H_2CO_3 produced prior to the final end product may form Na_2CO_3 in very minute amounts. A very small amount of sodium carbonate could produce all of the variation toward the alkaline side that was noticed. The literature carries no results of previous work done along this line of experimentation, except in test tubes. In these we find an entirely different environment existing and although

no anaerobic condition exists, still they are not subjected to a continuous supply of atmospheric oxygen. Facilities for aeration were not available at that time.

When protein was added it was found that the pH values did not follow the free ammonia produced but proceeded in an acid direction. It was assumed that the liberation of a fatty acid during the process of metabolism accounted for this occurrence. Buchanan and Fulmer (41) show that an amino acid undergoing oxidative dissimilation probably follows this reaction:



The keto acid is then decarboxylated and the aldehyde forms an intermediate product. This leaves a fatty acid which in the case of glycine would be formic acid. The amounts of formic acid are without doubt great enough to neutralize the ammonia produced by deaminization and possibly are great enough to leave a small excess.

However, the reversal in pH was an incidental occurrence and is merely brought out in the discussion because of the previous theories that all carbohydrate decomposition produced acid in sufficient quantity to cause a pronounced acid environment and that protein decomposition produces enough ammonia to cause a decided alkaline pH. This is probably true under the conditions existing in test tubes but certainly is not true under continuous aeration with diffused air.

It has been briefly mentioned before that the ratio of suspended solids to the settleable solids seem to be very important in considering bulking sludges. This is true regardless of the buoying effect that fat has upon sludges. We have seen that upon observation of Tables XIV, XV, XVI & XVII that lactose administration alone causes a marked decrease in the weight of suspended materials in the sludge, while causing a decided increase in settleable solids. In other words the sludge volume increases and also tends to lose weight. Bulking sludges are very voluminous and light in weight, the specific gravity being lighter than that of water. During peptone administration however we find that the opposite takes place. The suspended solids increase in weight while the volume of sludge decreases in amount. This treatment,

therefore, gave us a relatively small volume of sludge very heavy in weight. Good settling ^{was} demonstrated in contrast to that obtained during lactose administration. Smit's observations made upon lactose administration correlated with those found in this paper. By discontinuing the treatment and taking settling rates each day until normal recovery took place, he found that in most cases an eight day period was sufficient to restore satisfactory settling.

During the combined carbohydrate and peptone treatment the pH varied enormously but the suspended solids remained rather constant. The settleable solids also remained fairly constant. This was a feature that was more or less expected after individual treatment.

Bacteriologically it was found that the ratio of bacteria to plankton was about 638 to 1 in case of lactose addition, whereas in the case of peptone treatment we find a ratio of approximately 900 to 1. These data indicate that good settling results with a high bacterial ratio to plankton, with certain reservations as to type of plankton. It is evident from this work that limits of this ratio lies between 900 to 1 and 600 to 1 for good settling rates. This is only a difference amounting to 33.3 per cent but indicates a possible basis for future study upon bulking conditions.

The acid producers during lactose addition were far greater in number than when peptone was administered. Alkaline producers are very much more prominent during peptone administration than during lactose treatment while the liquefiers seem to be slightly more in excess during lactose feeding. The relationships of acid and alkali producers during the two kinds of treatment do not vary greatly from previous test tube experiments, therefore, continuous aeration during growth evidently influences the reaction to a great extent.

Bacterial examinations were not made on the chemical treatment in which both peptone and carbohydrates were added as no great fluctuation, positive or negative, took place in the suspended solids determination. It was concluded that mere changes in types of bacteria as recorded on the medium used could possibly offer an explanation to the great pH variation. During the second trial no great pH change was observed while the solids determinations remained about as constant as during the first trial.

During our study of bicarbonate and carbonate production by the method, which was described, using graduates fitted with air diffuser tubes and seeded with skim milk etc., we noticed a rather sharp turn of the pH toward acidity and after a short time a gradual trend toward alkalinity in all

cases including the controls. Levine (42) states that when applying skimmilk waste to an experimental lathe filter pH determinations show that from the upper layers of the filter the samples are more acid than initially and that those from the lower layers are more alkaline than initially. He attempted an explanation stating "Skimmilk solution contains not only the lactose (whose decomposition readily accounts for the initial increase in acidity) but other carbonaceous substances as casein, which is a compound high in nitrogen, phosphorous, and lime. It is conceivable that the increased alkalinity might be due to decomposition of the casein with the production of ammonia which might act as a neutralizing agent, or again, it might be brought about by the actual destruction of the organic acids produced in the fermentation of the milk sugar and the liberation of alkaline calcium salts by the destruction or oxidation of the carbonaceous portion of the casein present in the skimmilk. The latter seems the more plausible view".

In the face of results obtained upon aerated skimmilk in the graduates, this assumption could be taken as being true particularly the latter part of his explanation, but this drop did not take place when pure lactose was added to the small laboratory activated sludge set-up. Therefore

the writer is influenced by the fact that in a closed system in which lactose was added from day to day an accumulation of carbon dioxide and water in form of carbonic acid occurred, while lactic acid, an intermediate product, remained fairly constant. It is thought that the concentration of H_2CO_3 could become great enough to form sodium carbonate or potassium carbonate and these in very small amounts could cause a very marked change toward alkalinity without any tendency for acid formation at the beginning of the experiment. This is shown on the graphs. We can see that no acid pH values existed immediately after lactose addition, at least, during the first three hours. The writer offers an explanation to Levine's findings in that during the travels of the waste through the top few layers of the filter an oxidative process does not occur and that a hydrolysis exists until it reaches the lower levels in which aeration actually takes place. Here, the pH goes alkaline and remains so. The work of Ayers, Rupp and Johnson (43) has also been reviewed and their results used to bear out this contention as well as Levine's.

No bacteriological data were taken during fat treatment. The results desired being obtained immediately. It has been pointed out previously that fats showed a very marked effect upon settleability of the sludge.

It has also been shown that bicarbonate and carbonate formation takes place under cultural conditions using a glycine medium and offers another possibility as to an explanation to reversal in pH. The balance between bicarbonate and carbonate usually present biologically can be easily upset which might cause more than normal XCO_3 production thereby upsetting the average balance. A marked change toward alkalinity would take place if this were the case.

During the time in which this work was being carried out in the laboratory and in the field considerable data were taken upon the adaptability of the process to treatment of milk waste. This work has been published by the Engineering Exp. Station in Bulletin No. 48 therefore it will not be repeated in this thesis. We found, however, that mechanical aeration by use of Simplex aerators is applicable to milk waste treatment and small installations will probably be in order during the next few years.

THE SUMMARY

1. Evidence of alkaline salts being formed during carbohydrate metabolism, under conditions of forced aeration, by activated sludge was indicated.
2. Peptone utilization is shown to have a slight acid effect upon the fluctuation of pH.
3. Lactose administration caused an increase in volume of settleable material in the sludge body and a decrease in the weight of the suspended solids.
4. Peptone administration caused a decrease in volume of settleable material in the sludge body and a definite increase in weight of the suspended solids, in other words exerting the opposite effect of lactose administration.
5. Bacteriologically, the work indicated a definite bacterial ratio to plankton being necessary for good settling. Excellent settling rates were obtained with bacteria/plankton ratios of 900 /1 and poor settling rates were obtained with bacteria / plankton ratios of 600 /1.
6. That Levine's method of determining nitrate reducers liberating free ammonia could not be used for minute quantities(as high as 55.0 p.p.m.) of ammonia in

attempting to determine nitrate reducers, bacteriologically.

7. Carbonates and bicarbonates have been produced experimentally in culture flasks, when glycine was the only source of food.
8. Results also indicate that no initial drop in pH was necessary before a final alkaline pH value was obtained. It is assumed that during lactose addition an accumulation of H_2CO_3 strong enough to form XCO_3 and XHCO_3 with no periodic increase in lactic acid.
9. By the same process it is thought that peptone (protein) decomposition causes an accumulation of a fatty acid strong enough to neutralize all of the ammonia formed and still have a surplus left to cause an acid reaction to pH.
10. The mere mechanical presence of fat caused bulking conditions to exist.

The work disclosed in this thesis really amounts to a preliminary study of bulking conditions. It indicates that more studies of food and loading relationships to bulking should be carried out. Trends of chemists and bacteriologists are to take up the more detailed study of individual processes of the system at present and with the completion of the work

now being carried out by Rudolfs, Henkelekian, Smit, Levine, Buswell and others, the writer predicts many heretofore unexplained features will be clarified, allowing future possible explanation of the whole process.

LITERATURE CITED

1. Morgan, E. H., and A. J. Beck. Carbohydrate wastes stimulate growth of undesirable filamentous organisms in activated sludge. Sewage Wks. Jour. 1:46-51, 1928.
2. Scott, W. The Bulking of Activated Sludge. Surveyer 73:345, 1928.
3. Levine, Max and Lulu Soppeland. Bacteria in Creamery Wastes. Iowa Eng. Exp. Sta. Bulletin 77, 1926.
4. Levine, Max, G. W. Burke, and J. H. Watkins. Removal of Milk Constituents by Filtration. Iowa Eng. Exp. Sta. Bull. 95, 1929.
5. Clark, H. W. Development of Purification of Sewage by Aeration and Growth at Lawrence, Mass. Ind. and Eng. Chemistry 8:653, 1916.
6. Ardern, E. and W. T. Lockett. Experiments on the oxidation of Sewage without Filters. Surveyer 45: 610, 1914.
7. Cramer, Robert. The Role of Protozoa in Activated Sludge. Ind. and Eng. Chemistry 23:3, 1931.
8. Martin, A. J. The Activated Sludge Process. Published in London 1927.
9. Hatton, T. C. Activated Sludge Defined. Presented by Copeland. Eng. News Record. 75: 503, 1916.
10. Streander, P. B. Activated Sludge Practice. Sewage Works Jour. IV(5): 865, 1932.
11. Howorth, J. Activated Sludge without Compressed Air. Mun. Jour. Pub. Wks. 46: 356, 1919.
12. Fort, E. J. Brooklyn Sewage-Aeration and Activated Sludge Experiments. Eng. News Record. 74: 214, 1915.
13. Buswell, A. M., R. A. Shire, and Neave. Bio-precipitation Studies. Ill. State Water Survey 25, 1928.

14. Lockett, W. T. Experiments Relating to Activated Sludge Process of Sewage Purification. Jour. Soc. Chem. Ind. 36: 264, 1917.
15. Buckworth, W. H. The Activated Sludge Experiments at Salford. Surveyer 48:648, 1915.
16. Fuller, G. W. A Year of Activated Sludge at Milwaukee. Eng. News Record 74:1146, 1915.
17. Hatton, T. C., and G. D. Holmes. Activated Sludge at Milwaukee. Mun. Jour. Pub. Works 40:785-824-830, 1916.
18. Burn, G. A. H. Effect of Non-aeration on the Activated Sludge Process. 39th Annual Report Provincial Bd. of Health, Ontario, Canada. 1926.
19. Buckworth, W. H. Aeration Experiments with Activated Sludge. Surveyer 46:681, 1914.
20. Bartow, E., and F. W. Mohlman. Sewage Treatment Experiments with Aeration and Activated Sludge. Ind. and Eng. Chemistry 7:318, 1915.
21. Bartow, E., and F. W. Mohlman. Recent Results of Experiments on Purification of Sewage by Aeration in Presence of Activated Sludge at U. of Ill. Ind. and Eng. Chemistry 8:15, 1916.
22. Eddy, H. P. Digestion of Activated Sludge. Canadian Engineer 31:353, 1916.
23. Carel, Lucien. Notes on Purification of Sewage by the Activated Sludge Process. Compt. rend. Soc.d.Biol. 171:1406-7, 1920.
24. Dienert, F., and Girault. Action of Activated Sludge upon Ammonia of Sewage and of Ordinary Water. Compt. rend. Soc. d.Biol. 170:899-901, 1920.
25. Dienert, F., et.al. Action of Activated Sludges. Compt. rend. Soc. d. Biol. 170:1089-92, 1920.
26. McVea, J. C., and G. L. Fugate. Notes on Operation of Plants at Houston, Texas. Eng. News Record 83:1003, 1919.
27. Ardern, E. The Activated Sludge Process of Sewage Purification. Jour. Soc. Chem. Ind. 36:65, 1917.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	12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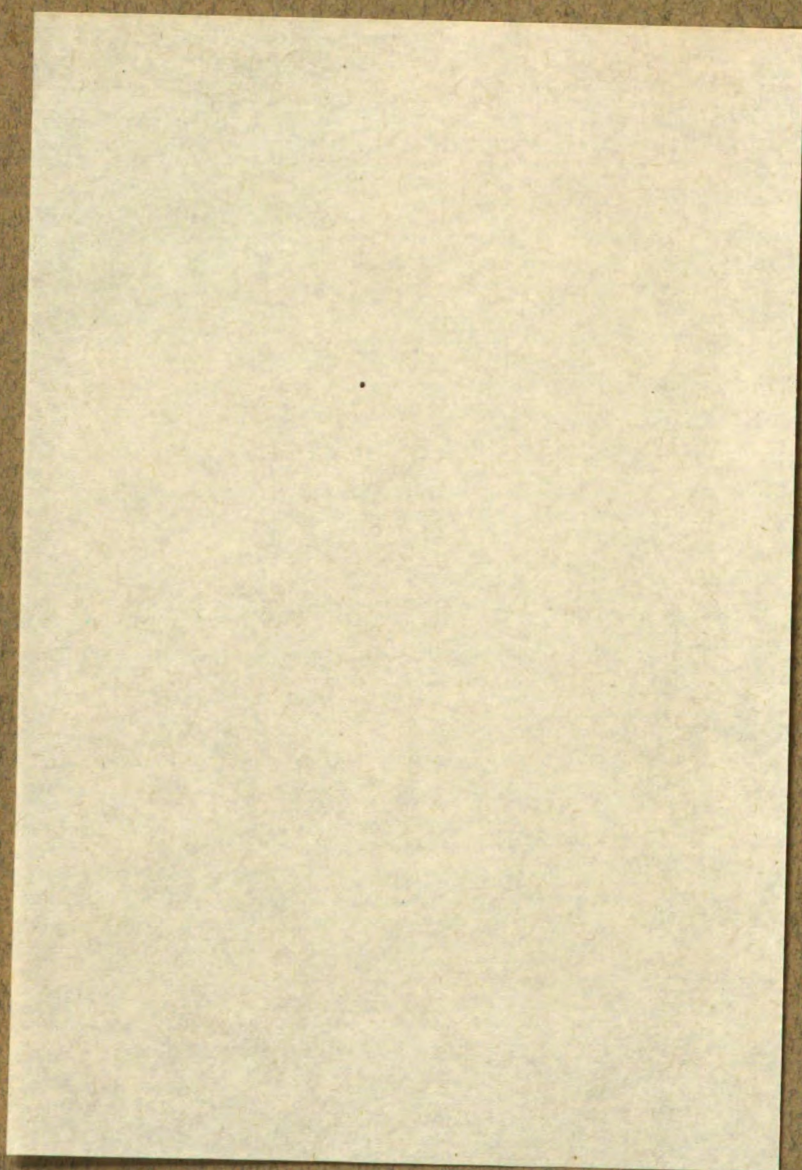
28. Copeland, W. R. Purification of Sewage by Activated Sludge in Winter at Milwaukee, Wis. Ind. and Eng. Chemistry 8:642, 1916.
29. Lederer, A. Chemical Observations on the Activated Sludge Process. Ind. and Eng. Chemistry 8:652, 1916.
30. Buswell, A.M. The Biology of Activated Sludge - An Historical Review. Sewage Works Journal III(3):362, 1931.
31. Heukelekian, H. Partial and Complete Sterilization of Activated Sludge and the Effect on Purification. Sewage Works Jour. III(3):369, 1931.
32. Rudolfs, W., and H. Heukelekian. Aerobic and Anaerobic Decomposition of Sewage Solids. Ind. and Eng. Chem. 24(11): 1312, 1932.
33. Slaughter, Clare E. A Study of the Disposal of Creamery Wastes. Mich. Eng. Exp. Sta. Bull. 18. July, 1928.
34. Buswell, A.M., C.S. Boruff, and C.K. Wiesman. Anaerobic Stabilization of Milk Wastes. Ind. & Eng. Chem. 24:1423, 1932.
35. Eldridge, E.F. Milk Products Waste Treatment-Report No.1 Mich. Eng. Exp. Sta. Bull. 24. July 1929.
36. Eldridge, E. F. Milk Products Waste Treatment-Report No.2 Mich. Eng. Exp. Sta. Bull. 28. March, 1930.
37. Eldridge, E.F. Milk Products Waste Treatment-Report No.3 Mich. Eng. Exp. Sta. Bull. 36. April, 1931.
38. Eldridge, E. F., and G. H. Robinson. Studies of the Activated Sludge Process. Mich. Eng. Exp. Sta. Bull. 46. May, 1932.
39. Bach, H. Original reference impossible to be obtained. Cited verbatim in (40).
40. Smit, Jan. A Study of the Conditions Favoring "Bulking" of Activated Sludge. Sewage Works Jour. IV(6):960, 1932
41. Buchanan, R.E. and E. I. Fulmer-Physiology and Biochemistry of Bacteria. Williams and Wilkins Co. Baltimore 1930.
42. Levine, Max, and J. H. Watkins. Destruction of Carbohydrates and Organic Acids by Bacteria From a Trickling Filter. Iowa Eng. Exp. Sta. Bulletin #110: 1932.

43. Ayers, S.H., Philip Rupp, and W.T.Johnson, Jr.
A Study of the Alkali-Forming Bacteria Found in Milk.
U.S.D.A. Bulletin #782: 1919.
44. Eldridge, E.F. Milk Products Waste Treatment-Report
No.4. Application of the Activated Sludge Process.
Mich. Eng. Exp. Sta. Bull. No.48. November 1932.

TEXT BOOKS CITED

1. Metcalf, L., and H.P.Eddy. American Sewerage Practice
Volume III. Disposal of Sewage. McGraw-Hill Book
Company, Inc. New York, 1915.
2. American Public Health Association. Standard Methods of
Water Analysis. New York, 1925.
3. Woodman, A.G. Food Analysis. McGraw-Hill Book Company,
Inc. New York, 1931.

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