BIOLOGICAL NUTRIENTS AND GROWTH

IN

ANAEROBIC DIGESTION

by

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Abstract

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Submitted to the Department of Civil and Sanitary Engineering on May 18, 1961, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The anaerobic digestion process has proven to be an efficient method for the treatment of many organic wastes. Some wastes are of a heterogenous nature and contain all of the nutrients required to support the biological life responsible for its stabilization. However, many industrial wastes are very specialized, both in constituency of the organic content and in biological nutrients. To successfully treat a waste biologically, sufficient nutrients must be present in the waste or be supplemented. It is important, therefore, to know the type and quantity of nutrients required for a particular waste in order to base process design on a solid engineering basis. Also since all organic material in an anaerobic digester must eventually be converted to methane and carbon dioxide by the methane bacteria, the activity of these bacteria can govern the overall rate of digestion. A knowledge of the factors which stimulate the activity of the methane bacteria would be most helpful and these were studied in this investigation.

In order to determine the nitrogen and phosphorous requirements and growth during the anaerobic digestion of wastes, a number of studies were made using a series of digesters and feeding substrates in the classes of fats, carbohydrates, and protein. All digesters had been purged of essentially all of the original contents through feeding and withdrawal of uniformly mixed samples. In this manner, analyses of the digester contents were not affected by the initial seed. Individual substrates were fed to digesters which were operated at different sludge retention times on a batch feed system. Inorganic nitrogen was supplied in excess. The nitrogen and phosphorous requirements, solids accumulation, gas production, and removal efficiency were determined. In order to study other factors which stimulate methane bacterial activity, continuous feed digesters which had been purged of the original seed were operated on a feed consisting of acetate and the normal nutrient salts. Stimulation was noted upon the addition of trace quantities of various pure compounds. The rate of acetate utilization was taken as the basis of stimulation.

From this study it was found that both the type of substrate and the sludge retention time were very important factors controlling the nitrogen and phosphorous requirements for anaerobic digestion. The carbohydrates were found to exhibit exceptionally high nitrogen requirements with accompanying high solids accumulation, and a resulting decreased methane production during anaerobic digestion at short sludge retention times. Net synthesis of carbohydrates into biological solids was found to be approximately 35% at sludge retention times of 5 days as compared with only approximately 6% for the fats and protein class under the same conditions. The effect of sludge retention time was much more pronounced with carbohydrates than with the fats and protein class. The growth equations for starch and glucose were evaluated to be: $A = 0.46F - 0.088M$, while the evaluation for the amino and fatty acids was: $A = 0.054F - 0.038M$. For nutrient broth: $A = 0.08F - 0.02M$. The nitrogen requirement was equal to the solids accumulation, A, divided by 9.4, while the phosphorous requirement was approximately one-seventh of the nitrogen requirement.

Stimulation of the rates of acetate utilization by methane bacteria was found to be produced by many different pure compounds. Rates of 6300 mg/l/day were possible upon addition of mixtures of the stimulatory compounds: iron, cobalt, thiamine, and components of vitamin B_{12}. Low constant acetate utilization rates of 0.06 #/ft^3/day however were possible with only the normal inorganic nutrient salts in the feed.

From the results of this study it is now possible to predict the operating characteristics of an anaerobic waste treatment process knowing only the composition of the waste in the categories of fats, carbohydrates, and proteins. Thus, the nutrient requirements and biological growth for the process can be predicted without making pilot studies on each and every particular waste. The methane bacteria can maintain normal low digestion rates with only inorganic nutrients required in the feed. However, high rates of digestion can be maintained only by additions of trace growth stimulants required by the methane bacteria.

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I. INTRODUCTION

The anaerobic decomposition of strong organic wastes (10,000 mg/l BOD or greater) has generally been favored over aerobic decomposition for three reasons: 1) the difficult problem of sufficient oxygen transfer in the aeration tank is eliminated; 2) synthesis of bacterial cells has been assumed to be lower and thus nutrient requirements would be correspondingly lower; and 3) an economic return is realized from the methane production.

In an aerobic system, about 50% of the utilized organics may be synthesized into new cells. This merely changes 50% of the organics from the less stable influent form into the more stable, more difficult to degrade bacterial cell form, which yet remains to be stabilized. However, this does serve the very important function of converting soluble and colloidal BOD into an insoluble form which can be concentrated by gravity. In this respect, anaerobic digestion is assumed to hold an advantage over aerobic treatment by having a lower sludge production based on pounds of BOD treated. Also, nutrient requirements are proportional to sludge production. Thus, the two-fold advantage of lower synthesis emerges; 1) greater efficiency of waste stabilization per unit weight of organisms, and 2) less nutrients required per pound of BOD treated.

Since many industrial wastes are deficient in nutrients, these must be added in order to successfully carry out the biological treatment. Nitrogen is the chief nutrient required and its cost might constitute the major part of the total nutrient costs for an anaerobic waste treatment process.

No extensive research has been carried out to determine the actual sludge production or nitrogen and phosphorous requirements for anaerobic decomposition of pure compounds in the separate categories of fats, carbohydrates, and proteins, taking detention time into consideration. It has been shown that the approximate BOD/N ratio is 20 for aerobic decomposition of a waste as compared with Buswell's (5) approximation of 300 for anaerobic digestion of acetate. If this BOD/N ratio of 300 does not hold for a particular waste, it may have considerable influence on the design chosen for that treatment facility. For this reason, it is important to know the effect of organic substrate and detention time on the nitrogen and phosphorous requirement. It is also important to know the effect of these variables on net synthesis, because synthesized material does not represent stabilization of organic wastes, but only a change in its form. Knowing the effect of these variables, engineering design for waste treatment could be carried out on a firmer basis.

Knowledge of the methane fermentation phase in anaerobic digestion is still in its infancy. There are only a few scattered claims of successful pure culture isolation of methane bacteria, and even then, difficulty in maintaining the pure culture is reported. Since the organic material is broken down to the volatile acids by the acid formers and then converted to methane and carbon dioxide by the methane formers, the methane bacteria constitute the "backbone" of the anaerobic digestion process. Yet, they seem to be adversely affected more readily than the acid formers. "Stuck" digesters are

usually characterized by high volatile acids and malfunction of the methane bacteria is assumed to be responsible for such conditions.

Complicated wastes such as sewage sludge and slaughterhouse wastes contain abundant trace elements and growth factors. These wastes have a long history of generally successful anaerobic treatment. However, with less complicated wastes, e.g., winery and acetate wastes, difficulty has been experienced in maintaining digestion over long periods. McCarty (31) found that acetate digestion rates dropped off with time in laboratory digesters seeded with well digested sludge and fed only acetate, dilution tap water, and the normal nutrient salts. Upon addition of supernatant liquor from a domestic sewage sludge digester, utilization rates could be increased. This indicated that a bacterial requirement not found in the acetate or nutrient salts was present in the supernatant liquor. The identification of the factors causing stimulation would be a significant contribution to our knowledge of the biochemistry in methane bacteria. Also addition of such stimulants may help increase the possible rates of digestion.

Thus, the objectives of this thesis fall into two categories: 1) a study of the nitrogen and phosphorous requirement for digestion of pure compounds at varying detention times; and 2) identification of the factors causing stimulation of acetate utilization by methane bacteria.

II. LITERATURE REVIEW

Anaerobic digestion or methane fermentation as known by the sanitary engineer is a unique and hardy process. Practically any type of organic matter can be decomposed to carbon dioxide and methane as the principal end products. Decomposition of more complicated organic compounds passes through the lower aliphatic acids, which are the immediate precursors of carbon dioxide and methane. Very little in the way of toxic by products are produced. As Buswell (7) has mentioned, methane fermentation can be carried on in a mixed or enriched culture and hence it is possible to maintain the process on a large scale continuously for apparently an indefinite period. This is in contrast to many other types of fermentation which require sterile feed and pure culture inoculation. Barker (2) stated that the ability to form methane is not a common or widely distributed property of anaerobic bacteria. Buswell went on to say that in the majority of cases the reaction is quantitative, converting all the substrate to methane and carbon dioxide. There are a few fundamental limitations, however, such as the requirement for necessary ions and salts and proper carbon to nitrogen ratio.

Gould (14) stated that the rate of activity of anaerobic biology under controlled conditions is rapid and comparable to aerobic organisms. In the past, this has been masked by the nature of complex organics provided for sludge digestion. Ordinarily the settled solids from the settling tanks and excess sludge production from the aeration tank or trickling filter are sent to the digester for decomposition. This class of organics is much more difficult to decompose biologically than

soluble or simple colloidal material. Since bacteria are the organisms involved, all of their food source must be soluble, and so all the settled solids and excess sludge must be solubilized by extra cellular enzymes before passing into the bacteria for processing to methane and carbon dioxide. Sawyer and Grumbling (49) pointed out that sludge differs uniquely from sewage in being able to be concentrated under the influence of gravity. This enables the organic load to be increased without decrease in detention time. For a given detention time, the digested sludge is essentially the same quality, regardless of the concentration of sludge fed.

Rudolfs (47) stated there seemed to be no reason to believe that the time required for decomposition and partial stabilization of putrescible organic matter cannot be materially reduced. However, he considered addition of certain trace elements, to stimulate the digestion process, a dangerous practice as long as fundamental knowledge was lacking.

A. Biological Nitrogen and Phosphorous Requirements and Growth in Anaerobic Digestion.

There is an interdependence between biological growth and the nitrogen and phosphorous required to sustain such growth. In fact, Bennett and Williams (4) have stated that the most widely used indirect chemical procedure for determining both mass and multiplication of bacteria is total cell nitrogen.

For unrestricted growth, bacteria requires carbon, hydrogen, oxygen, nitrogen, phosphorous, and sulfur as the major nutrients, trace amounts of other nutrients, an energy source, and a satisfactory environment. Carbon, hydrogen, and oxygen are constituents of cellular organic matter in general. Nitrogen and sulfur are required for snythesis of amino acids which make up the cell protein and phosphorous and nitrogen are necessary for nucleic acid synthesis. On a dry weight basis, the bacterial cell is approximately 50% protein and 20% nucleic acids.

In dealing with bacterial populations, one fundamental fact must be borne in mind - the bacteria exist primarily for their own benefit. That is, all of their processes are ultimately concerned with and directed toward maintenance and propagation of their cells. This can be achieved only on media which already contains, or to which has been added, an adequate supply of nutrients. Otherwise, they are hindered in metabolism of the media. Under favorable conditions they will thrive and multiply. Such would be the case with a domestic sewage in a satisfactory environment, since all major and trace nutrients along with an energy source would be present.

In biological waste treatment the sanitary engineer endeavors to capitalize upon this natural attribute of bacteria to propagate themselves by creating favorable conditions for growth. He is confronted with an organic waste which requires stabilization or conversion into innocuous products. If the waste is deficient in nitrogen or phosphorous, as may well be the case with the effluent from an industry, supplemental addition of the limiting nutrients must be made prior to biological treatment. Under such favorable conditions, the bacteria can thrive and will metabolize the waste - a fraction being oxidized to provide energy for synthesis of the remainder. Thus, in the process, two ends are achieved: 1) the bacteria are able to maintain and propagate themselves, and 2) the organic waste stream becomes stabilized after removal of the bacterial cells. However, in order to carry out waste treatment as economically as possible, the nitrogen and phosphorous requirements for the waste in question must be known so that deficient or excess additions of nutrients will be avoided. The cost of nitrogen usually exceeds that of phosphorous in treating an industrial waste, because the bacterial cell contains more nitrogen than phosphorous. For aerobic treatment of a waste, Sawyer (48) said the, BOD/N should be 20 while the BOD/P should be 100. Buswell (5) said the corresponding BOD/N for anaerobic digestion of acetate was 300. It is readily apparent that wastes which yield high rates of net synthesis require proportionally high amounts of nitrogen and phosphorous. From the standpoint of the sanitary engineer, high net synthesis rates exhibit two disadvantages: 1) the synthesized material

still needs to be stabilized, and 2) treatment costs are increased for nutrient deficient wastes.

Symons (59) conducted an extensive study on the anaerobic bacterial hydrolysis of carbohydrates. He fed a wide variety of organic substrates to an anaerobic digester which was operated with no wasting of the contents. He observed the fraction of substrate which was decomposed to methane and carbon dioxide. The increase in organic nitrogen of the digester contents was noted after the substrate was utilized and he calculated the mg N required/gm substrate fed. Values of his nitrogen requirements with no wasting are shown in Table I. In these studies, he also determined the C/N ratio of the cells responsible for the anaerobic decomposition of the substrates. The values he obtained yielded an average C/N = 5. He estimated that nitrogen accounted for 9.5% of the total weight of the bacteria.

Mylroie and Hungate (35) fed formate in experiments on the methane bacteria in sludge. They found the energy stored in cell material to be 11% of the energy released in the fermentation, and the carbon assimilated to be 7% of the carbon metabolized. They concluded that the efficiencies of energy conversion were of the same order of magnitude as those reported for various aerobic autotrophs. Gould (14) has also stated that the rate of activity of anaerobic biology under controlled conditions is comparable to aerobic organisms.

Table I

Nitrogen Requirements in Methane Fermentation of Carbohydrates after Symons (59)

Substrate	Grams Fed	Mg N required gm substrate fed	Org. N. Volatile Matter
Dextrose	1004.0	8.6	1:10.3
Xylose	474.0	6.5	1:10.0
Dextrose	682.0	5.7	1:12.0
Xylose	242.5	4.1	1:10.1
Mannitol	79.6	10.3	1: 8.0
Lactose	258.6	5.7	1:10.0
Sucrose	70.8	6.8	1: 9.0
Cellulose	10.0	20.2	1:12.1
Levulose	29.5	9.1	1:11.5
Galactose	9.9	16.0	1:10.5
Ethylene Glycol	38.7	9.1	1:11.5
Maltose	10.0	15.8	1:11.5
Raffinose	21.0	9.4	1:10.2

Biological Growth in Aerobic Systems

A great deal of investigation has been conducted on synthesis in the activated sludge process. Since there is much in common between aerobic and anaerobic systems, the results of some of the aerobic studies will be discussed. However, McKinney and Conway (33A) pointed out that the available energy will be less for an anaerobic decomposition, than if the degradation were carried out aerobically. They illustrated this as follows:

Aerobic $CH_3COONa + 2O_2 \longrightarrow NaHCO_3 + H_2O + CO_2 - \Delta F$

$-152.3 \quad 0 \quad -203.9 \quad -56.7 \quad -94.3 - \Delta F$

$\Delta F = 202.6$ Kcal/mole acetate

Anaerobic $- CH_3COONa + H_2O \longrightarrow CH_4 + NaHCO_3 - \Delta F$

$-152.3 \quad -56.7 \quad -12.1 \quad -203.9 - \Delta F$

$\Delta F = 7.0$ Kcal/mole acetate

Monod (34) said that bacterial growth experiments generally give cell yields proportional to the amount of substrate available. With a particular organism and substrate, the yield was constant for all growth rates and substrate concentrations, and varied only slightly with temperature. Hoover and Porges (23) also concluded, after studying the assimilation of dairy wastes by activated sludge that there existed a definite relationship between removal of substrate for respiration and for synthesis. They believed that for a given set of biological conditions, the amount of substrate removed by synthesis was always a constant proportion of the amount of substrate removed by synthesis plus respiration.

$$\frac{\text{Synthesis}}{\text{Synthesis + Respiration}} = \text{constant.}$$

For skim milk Hoover and Porges found:

$$\frac{S}{S + R} = 0.625$$

For glucose and nitrate, Hoover and Allison (20) found:

$$\frac{S}{S + R} = 0.75$$

Placak and Ruchhoft (45) compared O_2 uptake with the corresponding substrate removed. They found there was a correlation between the per cent oxidation and the compound classification, as carbohydrate, alcohol, amino acid, or organic acid. No nitrogen was supplied in their experiments. Their findings are tabulated in Table II.

Table II

Aerobic Synthesis of Organics

Class	% Oxidized		% Synthesized
	Range	Mean	
Carbohydrates	5-25	13	65-85
Alcohol	24-38	30	52-66
Amino Acids	22-58	42	32-68
Organic Acids	30-80	50	10-60

They concluded that the organic acids produce the smallest yield of activated sludge and carbohydrates produce the largest amount. They believed this explained why there is such a stimulation in sludge production when large quantities of carbohydrates are added to a sewage being treated by the activated sludge process.

Ruchhoft, Kachmar, and Placak (46) found that only 4-24% of glucose was concurrently oxidized by a sludge. They stated that since only such a small part of the glucose was oxidized and practically no soluble organic compounds were produced during glucose removal by activated sludge, that a very significant increase in sludge solids was to be expected.

Endogenous Respiration

Stewart (58) cited Hoover et al as defining endogenous respiration of organisms as the digestion of their own cellular tissue by their own metabolic processes for energy production. He also pointed out that synthesized cell tissue may apparently disappear due to endogenous metabolism or be measurable as net growth of organisms. He stated that it is probable that an organism's own cellular constituents are used as a source of materials for synthesis. In accordance with this concept the process involving synthesis takes place concurrently with endogenous respiration and can be thought of as a recycling of cell material.

Hoover and Allison (20) studied the endogenous respiration of bacterial cells. They found the endogenous rate to be a constant proportion of the weight of cells present. This rate continued unchanged at a rate proportional to cell weight regardless of the substrate concentration present. Cochrane and Gibbs (9) used C^{14} labelled glucose in endogenous respiration studies and confirmed the results of Hoover and Allison.

Hoover, Jasewicz, Pepinsky and Porges (21) fed skim milk to pilot plant activated sludge units on detention times of 20, 13.5, and 9.5 hours. The removal was complete in all units and the suspended solids concentration remained the same. They concluded that the endogenous respiration rate of the sludge must have been low, since the suspended solids concentration was nearly the same in all three units.

Hoover, Jasewicz, and Porges (22) stated that since only 3/8 of the oxygen requirement for complete oxidation of lactose and casein by activated sludge was consumed during the growth of the bacteria, the remaining 5/8 of the total oxygen requirement was consumed by the organisms in endogenous respiration. However, it has been reported by Kountz et al (25A) that the so-called "complete oxidation" units operating in the endogenous phase have a build-up of inert polysaccharide amounting to 20% of the BOD fed. This inert fraction would not be oxidized.

Formulation of Cell Composition

A mixed population of bacteria is responsible for the biological stabilization of wastes. However, it has been of interest to determine an empirical chemical formulation of cell population. Hoover and Porges (23) determined the carbon, hydrogen, oxygen, nitrogen and ash content of activated sludge in one of their studies to establish the empirical composition. The formulation was calculated to be $C_5H_7O_2N$. Symons and McKinney (60) also analyzed bacteria cells

responsible for aerobic waste stabilization and found the formula to be $C_5H_8O_2N$. In nitrogen deficient systems they found the C/N ratio was higher than in corresponding systems fed adequate amounts of nitrogen. They attributed this to be due to build-up of polysaccharide material.

Growth Equation

In biological treatment, a constant build-up of solids composed of bacterial cells occurs. In the activated sludge process, the solids level in the aeration tank is held at a constant level by pumping the excess sludge production to the sludge digester. A convenient expression for solids accumulation which in the past has described the experimental data is equation (1)

$$A = cF - kM \qquad (1)$$

Where: A = sludge accumulation #/day.

c = constant - fraction of substrate synthesized.

F = substrate #BOD / day.

k = constant - endogenous respiration rate per day.

M = # volatile solids present in system.

The validity of this equation has not been established, however, it has proved to be a reliable expedient for engineering design. Until more information is available, it is not feasible to use a more refined form of growth equation to predict solids accumulation. A convenient feature of equation (1) is that it can be expressed in the form of a straight line equation for evaluation of the constants c and k. Instances are given in the following paragraphs of investigators who have found equation (1) to be a satisfactory expression of solids accumulation.

Heulelekian, Orford, and Manganelli (19) took laboratory data from activated sludge plants treating domestic sewage. From this data, they derived the familiar equation (2) for activated sludge production:

$$A = 0.5\ F - 0.055\ M$$

Gram (15) transformed equation (1) into the form $y = mx + b$, a straight line equation. His equation (3) follows:

$$1/T' = cM^1 - k \qquad (3)$$

where T' = detention time of solids

M^1 = weight of substrate removed per day per unit weight of sludge in the system.

Heukelekian, et al (19) equation (2) in this form is shown in equation (4):

$$A/M = 0.5\ F/M - 0.055 \qquad (4)$$

Gram verified equation (3) as accurately describing the growth of activated sludge fed skim milk.

Stewart (58) made a thorough study of the reaction kinetics of continuous flow anaerobic processes. He set up an anaerobic system and fed a substrate consisting of:

Bacto-Tryptone	20 g/l
Dextrose	10 g/l
Bacto-Beef extract	6 g/l

The rates of feed were varied and he observed the sludge accumulation which resulted. He was able to evaluate the constants of equation (3) for his system as follows:

$C = 0.18$ #VSM/#COD

$k = 0.0247$/day

Other work was done by Weston and Eckenfelder (64) who analyzed data of other investigators and plotting A/M vs F/M to show the

relationship between BOD removed and activated sludge produced. They thought a definite ratio should exist between BOD removed, portion oxidized and the portion synthesized for any given set of conditions, but that in practice it is difficult to determine these relationships exactly because cell material is oxidized concurrently with that of the waste.

B. Stimulation of Acetate Utilization in Anaerobic Digestion

In a domestic sewage treatment plant, the sludge coming into the digester contains a wide variety of growth stimulating substances as well as an abundance of trace metals. With an industrial waste the distribution of stimulants and metals would probably be less. Conceivably, a waste could be encountered which lacked a growth factor vital to the life processes of a strain of bacteria responsible for its decomposition. In such a case, the growth factor could possibly be produced by another class of bacteria also present. However, if no source were available, the lack of a growth factor would bring about some malfunction in the biochemical processes of the bacteria in question. Prolonged deprivation may result in death of the bacteria or loss or diminution of an otherwise normal function.

In order to determine the growth factors and trace metals necessary for a strain of bacteria, pure culture isolation is normally carried out and selective substrates fed. Successive elimination of the growth factors from the media can be correlated with growth to establish those substances which the bacteria are unable to synthesize from the nutrients fed and which must be supplied intact. Essential trace metals can be determined in the same way. Due to the difficulty encountered in development of methane bacteria in pure culture, the growth factors and trace metals have not been established. Yet the importance of this group of methane forming bacteria is emphasized in that they must decompose all the volatile acids formed in the digestion process. It is quite probable to expect that a build-up of volatile

acids in a digester is the result of a malfunction of the biochemical processes of the methane forming bacteria.

Growth Factors Present in Anaerobic Digestion

McCarty (31) operated laboratory digesters, originally seeded with well digested domestic sewage sludge, on acetate as a substrate to isolate the methane stage of digestion. He fed nutrient salts and used Cambridge tap water for dilution of the daily feed. As the digesters were gradually purged of the initial contents through daily feeding and withdrawal, the rate of acetate utilization dropped off. He then reasoned that since digestion rates remain constant in a domestic sewage sludge digester, some growth stimulating substance must be lacking in his daily feed. Supernatant liquor from a domestic sewage sludge digester was added to the feed and acetate utilization rates resumed their normal value. Thus, the supernatant liquor supplied some growth promoting substance required by the methane forming bacteria utilizing acetate.

Leary (27) found that small amounts of powdered Milorganite (.01-.25% by wt) added to a carbohydrate containing mash accelerated the fermentation rates. Reductions of 10-20% in fermentation time and higher yields were noted. Since Milorganite is made from dried digester sludge of a treatment plant using the activated sludge process, this again points to the presence of growth stimulating substances in a digester.

Stander (54) studied the possibility of treating winery wastes by anaerobic digestion. His digester was originally started with well

digested sewage sludge and then fed only the winery waste. After a period of time, he noticed the volatile acids concentration increasing. He attributed this to an imbalance in the bacterial population, since in feeding raw sludge to an active sludge digester, a heavy inoculum of bacteria is added each time. Acting on this basis, he set up a 1½ liter inocula digester which was fed 5-10 ml raw sewage sludge daily along with the feed. Whenever a rise in volatile acids occurred in an experimental digester, an inoculum from the inocula digester would drop the volatile acids concentration within a day. (The amount of inoculum was one-fifth of the volume of the experimental digester.) He then considered the possibility of dispensing with the inoculum digester and feeding raw sludge directly to the experimental digester. Volatile acids increased rapidly and therefore he concluded that this practice did not fulfill the same function as the separately maintained inoculum.

A series of experiments were conducted by Heukelekian and Heinemann (17) on the seeding of digesters. They enumerated the methane forming bacteria in raw sludge and well digested sludge. The results showed that the number of methane bacteria in digested sludge was only slightly higher than in raw sludge. They concluded that the chief value of digested sludge lies in the production of favorable environmental conditions; the slow rate of digestion of fresh solids not being due primarily to paucity of methane fermenting organisms. This observation might also indicate the presence of some growth stimulating substance in digested sludge, which is required by methane

bacteria. Heukelekian and Heinemann (18) also pointed out that from a laboratory viewpoint, the use of growth stimulating substances such as yeast extract has been of great practical value by reducing the time necessary for incubation. They add that it is doubtful such artificial addition of substances to digesting sludge would similarly reduce time, since in such a complex material, it would be reasonable to expect small quantities of a number of growth stimulating substances would be either present originally in the solids or produced by bacterial action.

Heukelekian and Berger (16) stated that the important consideration for good digestion was the multiplication of bacteria with the resulting biochemical activity rather than the existance of a lot of bacteria. Symons (59) stated that a pH of 4 is bacteriostatic and not bacteriocidal for methane bacteria. Thus, the bacteria in a "stuck" digester would still be viable.

Another indication of malfunctioning of the biochemical processes is brought out by Stander and Hide (55). They reasoned that a sudden steep rise in volatile acids in an acclimated digester held at a fixed feed rate and at low volatile acids concentration was certainly not attributable to excessive feeding. Reduction or stoppage of the feed was found to have very little effect in reducing the volatile acids level.

Heukelekian and Heinemann (18) also "seeded" septic solids with digested sludge. Their conclusion was that liquid from septic solids was not as good a media for development of methane bacteria as fresh

sludge. They suggested the deleterious effect to be due to production of growth inhibiting substances during acid digestion.

Potential Acetate Utilization by Methane Bacteria

As mentioned previously, the supernatant liquor from a municipal digester probably contains practically all known grown factors and trace metals. McCarty and Vath (33) investigated the stimulation potential of this supernatant liquor on acetate utilization by methane bacteria. They found that the liquor could be evaporated to dryness with the growth stimulant remaining in the solids. The stimulant could be extracted from the dried solids by water. The increase in acetate utilization appeared to be proportional to the amount of dried supernatant solids added to the digester. In an attempt to determine the maximum potential rate at which the methane bacteria could utilize acetate, they added excess dried supernatant solids to a digester. Continuous feeding was provided and the volatile acids concentration was kept above 1000 mg/l. They were able to achieve the exceptional rate of 21 gm/l/day. This rate is equivalent to 1.35 #/ft^3/day of volatile matter. This gives an indication of the capability of methane forming bacteria to decompose acetate when sufficient growth factors and trace metals are present to allow the biochemical processes to proceed unhindered.

Bacterial Requirements for Growth Factors

Growth factors are a group of organic compounds of relatively simple structure. They are required to carry out the biochemical processes which sustain and propagate bacterial growth. Some bacteria

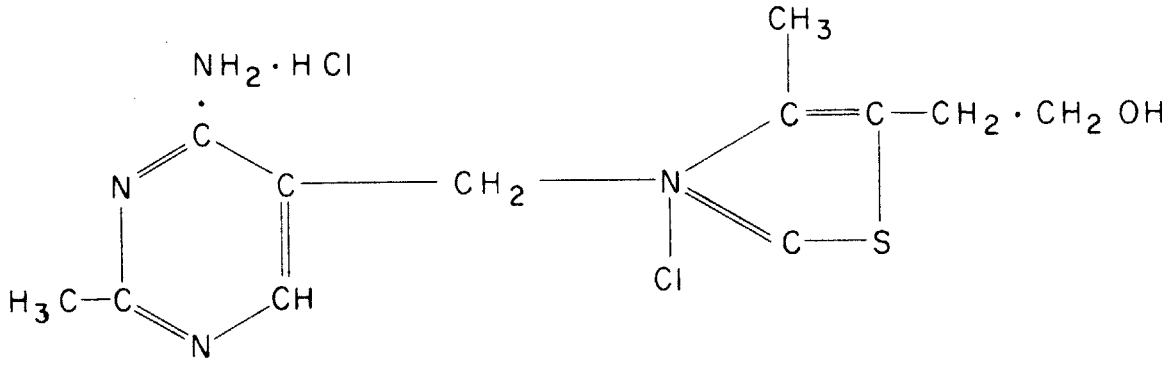
are unable to synthesize one or more growth factors from the nutrients fed. Thus the bacteria must be supplied such growth factors intact if they are to carry on their life processes unhindered. The main group of growth factors are either vitamins or amino acids. Nutritional Data (44) lists some growth factors as follows:

Vitamins	Amino Acids
Fat Soluble	Arginine
Vitamin A	Histidine
Vitamin D	Threonine
Vitamin E	Valine
Vitamin K	Leucine
Lipoic Acid	Isoleucine
Water Soluble	Lysine
Vitamin B_1	Methionine
Vitamin B_2	Phenylalanine
Nicotinic Acid	Tryptophan
Folic Acid	
Pantothenic Acid	
Biotin	
Vitamin B_6	
Inositol	
Para-Aminobenzoic Acid	
Vitamin B_{12}	
Vitamin C	

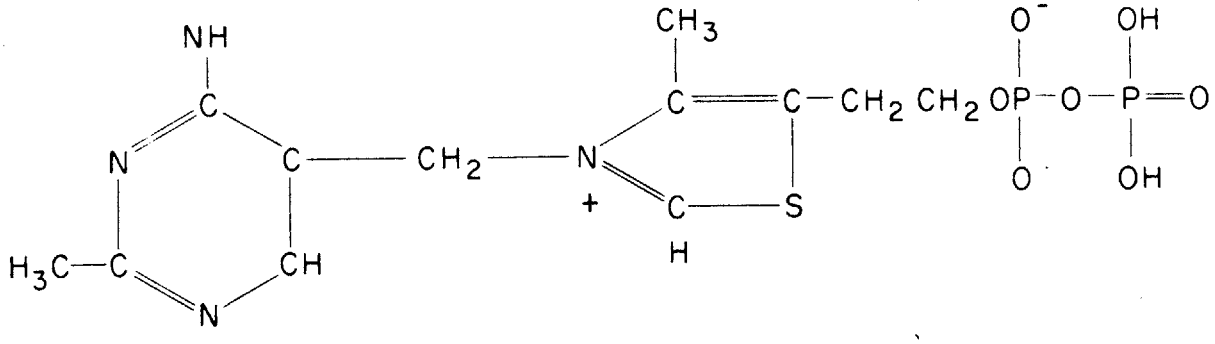
Stanier, Doudoroff, and Adelberg (56) stated that vitamins are a group of growth factors required by different organisms. They said that in all cases in which the nutritional function of the vitamins had been clarified, it had been found that these compounds served as building blocks for the biosynthesis of coenzymes. The coenzymes are special organic molecules which act with the larger protein apoenzymes to catalyze enzymatic reactions and are dissociable prosthetic groups.

As an example of the function of a vitamin, thiamine (vitamin B_1, Fig. 1), as pointed out by Stanier, et al (56) is required by some microorganisms which are unable to synthesize it from their principal nutrients. Other microbes, given the two halves of the molecule as nutrients, can couple them together. Others need only the pyrimidine portion or thiazole portion because they can synthesize the other half. The minimal growth factor requirements are different for each type of organism, but the entire thiamine molecule is eventually needed by all of the types described. If thiamine is provided as a nutrient, it can be used as the growth factor by all types. However, even the entire thiamine molecule is not the compound which is eventually an essential component of living matter. The functional compound is the coenzyme, cocarboxylase. This acts as the prosthetic group in several enzymatic reactions, including the decarboxylation of pyruvic acid. Cocarboxylase, also called thiamine pyrophosphate (TPP) is an essential cellular component of every organism, even of those that show no nutritional requirement for thiamine or one of its derivatives.

Amino acids are the structural units of proteins, which are of vital importance in the maintenance of the structural and functional integrity of all biological forms as stated by Fruton and Simmonds (11). Some bacteria are unable to synthesize certain amino acids required for their cellular protein. These must be supplied intact to allow the bacteria to maintain their integrity. By way of illustration, Langley (26) found that Salmonella typhosa require tryptophan as a growth factor.



THIAMINE HCl



THIAMINE PYROPHOSPHATE

Figure 1 Structure of Thiamine and TPP

Vath (63) added most of the growth factors to an anaerobic digester utilizing acetate and obtained no stimulation. Barker (2) said that addition of extracts containing amino acids, growth factors, and other nutritional substances to synthetic media did not have a beneficial effect on the rate of magnitude of growth of the species that had been studied in that respect.

Bacterial Requirements for Trace Inorganics

The role of inorganic ions in metabolism is treated by Fruton and Simmonds (11). Enzymes, flavo proteins, and electron transport systems utilize these ions. These so-called "trace elements" of living matter may play very important roles despite their minute concentrations as pointed out by Stanier, et al (56). Nutritional Data (44) listed the catalytic function of some trace elements in enzyme systems of plants and animals as follows:

Enzyme	Metal(s) Required
Arginine phosphopherase	Ca, Mn, Mg
Cytochrome	Fe
Oxaloacetic decarboxylase	Mg, Co, Zn
Lecithinase	Ca, Mg, Co, Zn, Mn
Yeast phosphatase	Mg, Mn, Co, Fe, Ni
Laccase	Cu
Pectinpolygalacturonase	Ca, Na, Al

Growth Factors in Digested Sludge

In a domestic sewage, a wide spectrum of growth factors is present. Proteins constitute a significant portion of the organics. This is evidenced by the fact that ammonium bicarbonate is produced and serves as the major buffer in a domestic sludge digester, the ammonia coming from protein degradation. As the protein is hydrolysed,

amino acids are released into solution to be further degraded or utilized as growth factors. Study on nutritional requirements of the human body has determined that vitamins are excreted from the body and thus would be a potential source of growth factors in a domestic treatment plant. To illustrate this point, Gortner and Gortner (13) stated that microorganisms in the digestive trace of some mammals supply the thiamine requirements of the host.

Vitamin B_{12} Fig. 2 has been found in digested sewage sludge. So much so, that the economic recovery of vitamin B_{12} by extraction from Milorganite at the Milwaukee treatment plant is practiced. Neujahr and coworkers 36, 37, 38, 39, 40, 41, 42, 51 carried out an extensive investigation on the vitamins in sewage sludge. Much of their work centered around vitamin B_{12} and they also realized that it was produced in such quantities as to suggest a possible source for commercial production. Therefore, they directed their efforts toward determination of those factors which encouraged maximum vitamin B_{12} production. Initially investigations were attemped on a pure culture of Methanobacterium omelianskii. Difficulty in maintaining sufficient growth caused the workers to switch to an enriched culture. They found that addition of $CoCl_2$ to fermenting sewage sludge caused stimulation of vitamin B_{12} synthesis in the initial stages of digestion. However, the high contents of vitamin B_{12} in sewage sludge could not be explained solely by the action of Methanobacterium omelianskii. Next, they grew enriched methane cultures on methanol, acetate, butyrate, and

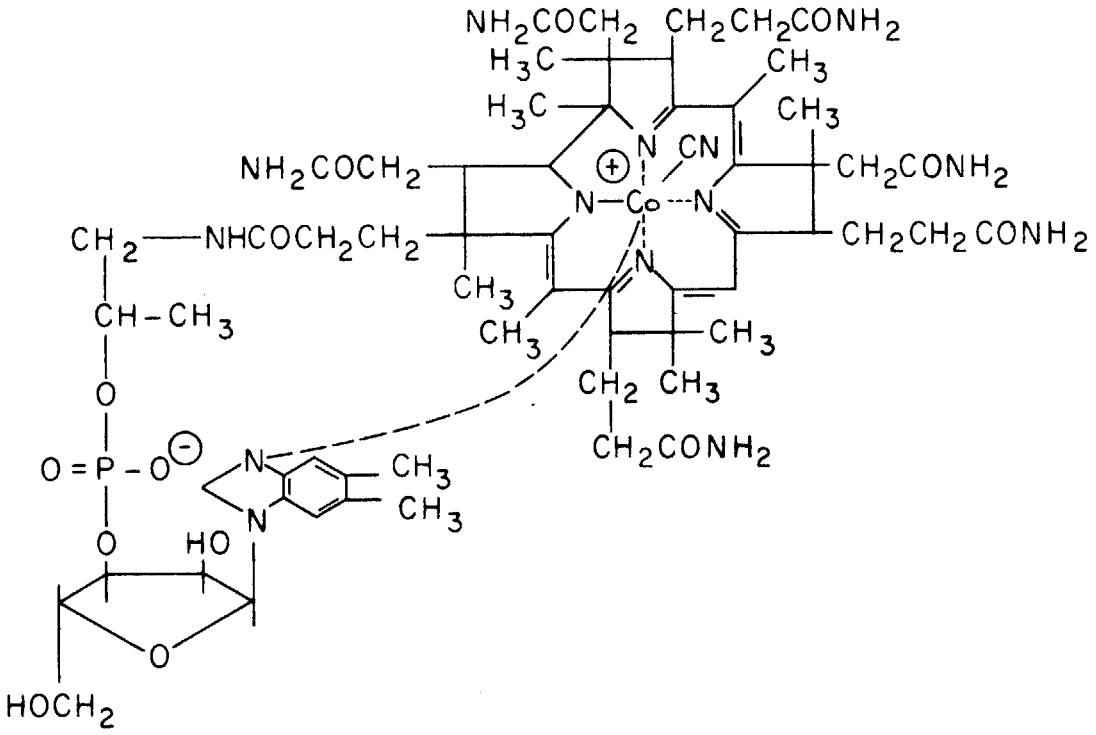


Figure 2 Structure of Vitamin B_{12}

other simple organic substrates. When supplied cobalt, 5,6-dimethyl benzimidazole, and Bacto Peptone the methanol culture produced B_{12} activities of 80-300 times greater than produced on the simple organic substrates, excepting acetate and butyrate. This methanol culture yield appeared higher than that of Streptomyces olivaceous, which is used commercially as a producer of vitamin B_{12}. The yield from cultures grown on methanol, acetate, and butyrate were all sufficiently high to explain the high vitamin B_{12} activity of digested sewage sludge. The addition of cobalt seemed essential for production of any appreciable growth.

Leviton and Hargrove (28) also stated that cobalt ions have been shown to be a limiting factor in synthesis of vitamin B_{12}. They observed that development of B_{12} activity followed only roughly the protoplasm development and that the activity resided entirely within the cell. Neujahr (40) found that raw sewage sludge contained cobalt ions in an amount about ten times as large as the amount of cobalt found in vitamin B_{12} factors. Sjostrom, Neujahr, and Lundin (51) determined that 82% of the vitamin B_{12} content of digested sludge was produced during the digestion process. Neujahr and Callieri (41) suggested that the microorganisms were unable to synthesize the specific base of vitamin B_{12} - 5,6-dimethyl benziminazole. However, all other parts of the molecule could be synthesized and then the parts coupled together. They noted that since this was not a pure culture, only tentative conclusions could be drawn.

Methane Fermentation of Acetate

In anaerobic digestion the biological transformation of the volatile acids into methane and carbon dioxide is a basic operation. Yet, our knowledge of the mechanism by which it occurs is scant.

Biological transformations in general are carried out with the aid of enzyme catalysts, which are continually regenerated within the bacterial cell. For each particular transformation, there exists a specific combination of enzymes required for its execution. The identification of the particular enzyme responsible for each step in some of the well established pathways of intermediary metabolism has been accomplished. The Gilson Medical Electronics charts (12) are a compilation of such information. However, the enzymes involved in many biological transformations are still obscure, as are the metabolic pathways. The methane fermentation of acetate is in this obscure category.

Barker (3) has worked extensively on the methane fermentation of acetate and made the following comment:

> "The methane bacteria have not been studied as extensively as most other groups of bacteria of comparable scientific and practical importance. The reason for this is readily apparent. In order to study the biology and biochemistry of bacteria most effectively it is necessary to use pure cultures. Unfortunately, with the methane bacteria, this elementary but basic requirement has been difficult and in many instances impossible to achieve."

Because of the difficulties encountered in isolation and maintenance of pure culture methane bacteria, only a rather limited amount of information has been obtained concerning the biological and physiological characteristics of the group as a whole. Barker also stated that for some obscure reason, all methane bacteria, which have been studied in pure culture with various substrates, are unable to decompose the more usual substrates for bacteria such as carbohydrates and amino acids. In the chemistry of the methane fermentation, he found a variety of compounds could be more or less quantitatively converted to CH_4 and CO_2. The remarkable facet of this result was that the nature of products (CH_4 and CO_2) was independent of the structure of the substrate.

Stadtman and Barker (53) performed a convincing experiment on the transformation of acetate to CH_4 and CO_2. By means of the tracer method they showed:

$$C\,{}^{*}H_3\,\overset{\circ}{C}OOH \longrightarrow C\,{}^{*}H_4 \text{ and } C^{\circ}O_2$$

Pine and Barker (44A) continued this experiment and found:

$$CD_3\,COOH \xrightarrow{H_2O} CD_3H \text{ and } CO_2$$

$$CH_3\,COOH \xrightarrow{D_2O} CH_3D \text{ and } CO_2$$

The pathway of carbon in methane formation has not been established. Barker (3) postulated a possible pathway for acetate decarboxylation, but said that the most important point at present was to determine the nature of the enzyme, or enzymes, which effected this decarboxylation.

III. THEORETICAL CONSIDERATIONS

A. Bacterial Synthesis in Anaerobic Digestion

Synthesis and energy are inseparably related. Monod (34) studied the growth of three bacteria. They were grown anaerobically on a mineral media with a wide range of carbohydrates as the energy source and as long as the energy source was the factor limiting growth, the dry weight of organisms produced was proportional to the energy source added. Since the available energy governs synthesis, anaerobic metabolism would probably yield less synthesis than the corresponding aerobic metabolism of a substrate.

Next, the effect of substrate enters. Assuming identical conditions, Wilson and Peterson (65) pointed out that synthesis of bacterial protoplasm from beef extract can be accomplished with much less expenditure of energy than is necessary when the substrate is glucose and nitrates. They gave two reasons: 1) energy must be expended to reduce the nitrates to ammonia before use, and 2) the beef extract contains many amino acids which could be incorporated into protoplasm upon hydrolysis. Therefore, it is possible to conceive of a relatively low energy substrate, composed of a heterogenous mixture of compounds required for cell synthesis, which would give comparable protoplasm yields to a higher energy substrate from which all cell material must be synthesized from scratch.

Another consideration in bacterial synthesis is the type of organism responsible for metabolism of the substrate. DeMoss, Bard and Gunsalus (10) grew Streptococcus faecalis and Leuconostoc mesenteroides under similar conditions on a glucose substrate. They

found there was a linear relationship between the dry weight of organisms produced and the glucose added in both cases. However, the yield of S. faecalis was significantly greater than that of L. mesenteroides. They concluded from the results that because both organisms used glucose mainly as an energy source and because growth was proportional to the amount of energy added, that S. faecalis obtained more energy per mole of glucose. Also L. mesenteroides was thought to have fermented glucose by a mechanism other than the Embden-Meyerhof pathway.

From the preceeding discussion, four factors governing biological growth are summarized:

1. The energy available in the substrate
2. The conditions under which the substrate is metabolized, i.e., aerobically or anaerobically
3. The composition of the substrate and readiness with which it can be incorporated into cell protoplasm.
4. The type of organism responsible for metabolism.

Substrate Energy

Klotz (25) has considered the matter of energy in biochemical reactions. He pointed out that there are processes which occur spontaneously despite the fact that the internal energy at the end is greater than at the beginning of the reaction. Thus, he signified that reactions may occur spontaneously when E is + - or 0.

He went on to say that free energy has proved to be powerful in solving many thermodynamic problems as well as providing a conventional criterion of feasibility. Changes in free energy provide: 1) a measure of the maximum amount of energy which may be obtained as useful work during the course of a material transformation, and 2) a quantitative indication of the potential ability of a substance to undergo a chemical or physical transformation. It needs to be emphasized, however, that changes in free energy are dependent on the initial and final states of the substances undergoing change. The path taken by the reaction has no effect. Whereas, the energy obtained by bacteria does depend upon the path of the reaction. The fact that some bacteria are more efficient than others in capturing the available energy has already been related. Thus free energy changes should provide only qualitative indications of the bacterial growth resulting from the transformation.

The comparison is given of yeast production under aerobic and anaerobic conditions on glucose. The cell production aerobically was only three times the anaerobic production even though the corresponding free energy change ratio was about 13. Stanier, Doudoroff, and Adelberg (56) stated that over 30 high energy phosphate bonds (ATP) can be regenerated in the respiration of one mole of glucose, whereas, only 2 are made available to the cell in the alcoholic fermentation of glucose. This is an energy ratio of 15. Looking at it from a different point of view, however, the seeming discrepancy between an energy yield ratio of 13-15 and a cell production ratio of 3 for

aerobic vs anaerobic conditions can be explained. Assuming 85% synthesis aerobically, then the anaerobic synthesis would be 1/3 of 85% or 28%.

Aerobic $\frac{0.85 \text{ synthesized}}{0.15 \text{ catabolized}} = 5.7 \frac{\text{\# synthesized aerobically}}{\text{\# catabolized}}$

Anaerobic $\frac{0.28 \text{ synthesized}}{0.72 \text{ catabolized}} = 0.39 \frac{\text{\# synthesized anaerobically}}{\text{\# catabolized}}$

Therefore $5.7/0.39 = 14.6 \frac{\text{aerobic synthesis}}{\text{anaerobic synthesis}}$ per unit weight catabolized

This synthesis ratio per unit weight substrate catabolism can be made to agree with the energy ratio of 13 - 15.

Wilson and Peterson (65) stated that changes in free energy must be considered for concentrations other than standard state (1M). Dilution of a substance lowers the free energy of formation. Equation 10 gives the relation between concentration and change in free energy of formation. Free energy values are from Lange (25B).

$$\Delta F = \Delta F^{\circ} + RT\ 2.3 \log_{10} \left(\frac{\text{dilute conc.}}{\text{standard conc}}\right) \qquad (10)$$

Assuming acetic acid of 670 mg/l in the digester:

$$\Delta F = -89.7 + 1.987(298)(2.3) \log_{10} \left(\frac{1}{100}\right)$$

$\Delta F = -92.5$ k cal/mole at 670 mg/l and 298° K

With glucose at 670 mg/l ;in the digester:

$\Delta F = -217.0 - 3.4$

$= -220.4$ k cal/mole at 670 mg/l and 298°K

The variation of CO_2 and CH_4 free energies of formation will not be appreciable between 0.25 and 1.0 atmospheres.

A comparison will now be made between the free energy changes of glucose and acetate in anaerobic decomposition. Based on free energy values from Lange (25B), the glucose free energy is taken to be - 220.4 k cal/mole at a concentration of 670 mg/l. Likewise, for acetate the corresponding free energy change is - 92.5 k cal/mole at a concentration of 670 mg/l.

Glucose

$$C_6H_{12}O_6 = 3\ CO_2 + 3\ CH_4$$

$$\triangle F \quad -220.4 \qquad 3(-94.6) \qquad 3(-12.1)$$

$$\triangle F_{net} = -99.7 \text{ k cal/mole}$$

Acetate

$$CH_3COO^- + H_2O = HCO_3^- + CH_4$$

$$\triangle F \quad -92.5 \qquad -56.7 \qquad -142.3 \qquad -12.1$$

$$\triangle F_{net} = -5.2 \text{ k cal/mole}$$

On the basis of energy per gram of substrate decomposed:

Glucose -0.55 k cal/gm

Acetic acid -0.087 k cal/gm

If the substrates are fed on the basis of equal COD amounts, the equivalent COD per gram is shown below:

1 gm Glucose equivalent to 1.06 gm COD

1 gm Acetic Acid equivalent to 1.06 gm COD

Free energy per gm COD is:

Glucose -0.52 k cal/gm COD

Acetic Acid -0.082 k cal/gm COD

Temperature and pressure will both remain constant for all practical purposes in the anaerobic studies under consideration.

To illustrate the inadequacy of COD alone as an indication of the energy available and synthesis resulting from anaerobic treatment of a compound, the following simplified possible reaction is given for glucose:

$$\begin{array}{rll} & C_6H_{12}O_6 & = 3\ CH_3COOH \\ \Delta F & -220.4 & 3\ (-96.6) \end{array}$$

$$\Delta F_{net} = -69.4 \text{ k cal/mole glucose}$$

$$\begin{array}{rlll} & CH_3COOH & = CO_2 & + CH_4 \\ \Delta F & -96.6 & -94.6 & -12.1 \end{array}$$

$$\Delta F_{net} = 10.1 \text{ k cal/mole acetic acid}$$

The free energy change which results from one mole of glucose being broken down into three moles of acetic acid is -69.4 k cal/mole. The free energy change which occurs when three moles of acetic acid are broken down to three moles of carbon dioxide and three moles of methane is - 30.3 k cal/3 moles of acetic acid. Now observe that even though a free energy change occurred in the breakdown of glucose to acetic acid, there was no net COD reduction.

COD of Reactants and Products

$C_6H_{12}O_6$ = 192 gm COD/mole glucose

3 CH_3COOH = 192 gm COD/3 moles of acetic acid

3 CH_4 = 192 gm COD/3 moles of methane.

Here, it has been shown that COD is not always a reliable indication of the energy which is potentially available to the bacteria for growth. The transformation of glucose into acetic acid is a constant COD stage, however, much energy is available for growth. The next stage, the transformation of acetic acid to carbon dioxide and methane, is a COD reduction stage since methane separates from the waste as a gas. Yet only about one-third of the amount of free energy available in the overall reaction is available in this COD reduction stage.

Therefore, anaerobic digestion is shown to be composed of two stages, one in which the COD or in effect ultimate BOD of the waste remains constant and the other in which the COD of the waste is decreased. In the constant COD stage, there may be considerable energy for biological synthesis or growth, but the desirable reduction in oxygen demand of the waste is not achieved. This stage, although not accomplishing waste treatment, serves the necessary function of transforming the complex organics into a form which can be used by the methane bacteria. It is then in the second stage of methane fermentation that the desired decrease in waste COD is accomplished.

Conditions of Metabolism

The available energy from complete oxidation of an organic compound as compared to methane fermentation is clearly shown in Table III compiled by Symons (59).

TABLE III

Per cent of the Total Energy Available from Complete Oxidation Liberated By the Methane Fermentation.

Substance	Per cent
Acetic Acid	5.07
Acetone	5.85
Butyl Alcohol	4.75
Glucose	12.50
Ethanol	6.94
Glycerol	12.70
Mannitol	13.50
Succinic Acid	8.9
Sucrose	14.4

From this information, it is certainly reasonable to expect that synthesis under anaerobic conditions would be considerably lower than under aerobic conditions.

Substrate Composition

Within a bacterial cell are a multitude of enzymes, amino acids incorporated into protein, and other organics required to sustain life. In a growing population of bacteria, duplication of all these constituents is continuous. They must be synthesized from the nutrients present. With a culture fed only acetate and nutrient salts, this means that for the bacteria to propagate, all new cell materials, sugars, proteins, nucleic acids, and other compounds are synthesized with the acetate molecule as the starting point. On the other hand with a heterogenous waste containing many compounds used in the cell and in a form available to the bacteria, the feed-back mechanism decreases the production of those adaptive enzymes responsible for

production of the compounds already in solution. The net effect is to decrease the energy required for synthesis of each cell and results in greater overall cell production. For this reason, it would be anticipated that a substrate consisting of a mixture of all amino acids would yield greater cell production than with any one amino acid as the sole substrate.

Type of Organisms Responsible for Stabilization

The predominant organisms in an anaerobic digestion process are determined by the nature of the substrate, under comparable physical conditions. For instance, it is known that the organisms responsible for the decomposition of cellulose are different from the organisms which decompose glucose, even though cellulose is a polymer of glucose. Therefore, a difference in nitrogen requirements might be expected for anaerobic digestion of the two substrates.

Summary

It would therefore be anticipated that the biological nutrient requirements and growth in a waste treatment process would depend upon the amount of energy in the substrate which was available to the organisms in the process of decomposition. The conditions under which the decomposition took place would govern this available energy for a particular waste, since only a fraction of the energy available in a substrate under aerobic conditions can be obtained under anaerobic conditions. Also the COD or BOD of a waste does not provide a reliable indication of the energy available anaerobically to the organisms. This is evidenced by the fact that biological transformations

are sustained in the anaerobic digestion process for which there is no accompanying change in COD. Another aspect to consider is that substrates whose chemical structure requires less alteration by the bacteria for synthesis into cellular protoplasm would be expected to result in greater net synthesis. Lastly, the nutrient requirements and growth of a bacterial population would be directly proportional to their efficiency in capturing the energy available from a biological transformation. Therefore, predominance of different organisms in a process may be expected to result in different nutrient requirements and growth.

IV. EXPERIMENTAL PROCEDURES

A. Description of Apparatus

Batch-Feed Digesters

This study was conducted to ascertain the nitrogen and phosphorous requirements and resulting biological growth for the anaerobic digestion of certain organic compounds. Each substrate was fed to a digester for at least two detention times, with feed administered to all digesters once a day. The digesters were purged of the initial seed sludge by the daily withdrawal of mixed contents and feeding of substrate and nutrient salts. When equilibrium conditions were attained, final data was taken on each digester. Routine analyses of the digester effluent were made for control purposes all along. The conditions of the study were chosen to simulate actual digestion practice.

While the size of a laboratory digester must of necessity be smaller than field installations, the method of operation can be patterned after field practices. Conventional digestion in the mesophilic range is carried out with the tanks heated to about 35°C. A closed atmosphere of gases produced in the digestion process is maintained to eliminate the toxic effect of oxygen from the air. The contents are usually mixed at least once a day and some installations provide periodic mixing throughout the day. Preferably, sludge should be added periodically during the day, but daily addition is common. Detention times range from about 10 days for high rate digestion to about 30 days or more in conventional digesters.

These field conditions were used as the pattern in setting up the laboratory digesters. Fourteen one liter narrow mouth bottle

fitted as shown in Fig. 3 were used throughout this study. A three-holed rubber stopper was used to seal the bottle contents from the atmosphere. Through one hole was inserted a curved piece of glass tubing which extended almost to the bottom of the bottle. Withdrawals were made through this tube. The open end of this tube was fitted with a short piece of rubber tubing and clamped with a spring pinch clamp. In the second hole, a short piece of glass tubing was placed with the exposed end also fitted with a short piece of rubber tubing and sealed with a spring pinch clamp. Feeding and gas expulsion was accomplished through this tube. The third hole in the stopper was also fitted with a short piece of glass tubing, with a rubber hose connecting the open end with a 1200 ml glass gas collection tube used for measuring gas production. The gas collection tube was connected with 3/8" tygon tubing to a manifold. This served to connect all gas collection tubes to a common 20 liter reservoir for the gas confining fluid. This confining fluid consisted of a saturated sodium chloride solution acidified with 15 per cent sulfuric acid. Methyl orange was added to give it color, so as to facilitate gas readings. The 20 liter confining fluid reservoir consisted of a narrow mouth bottle fitted with a two hole rubber stopper. Through one hole passed an extension of the manifold leader to the bottom of the bottle. The second hole was fitted with a short piece of glass tubing and connected to a pressure reducing mechanism through a three-way control valve. This air line pressure of 35 feet of water was reduced to about 4 feet of water and could be introduced into the 20 liter reservoir through the

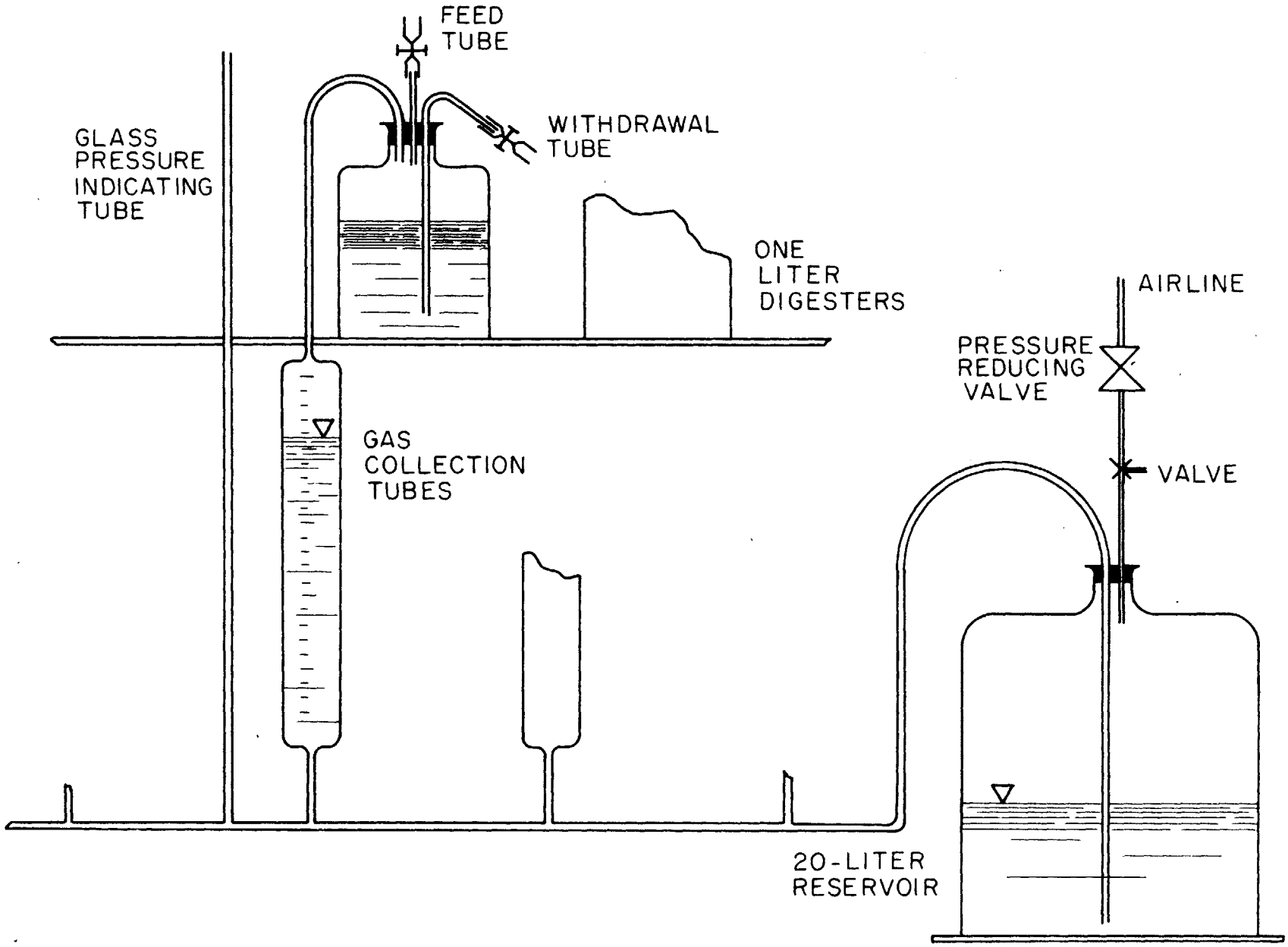


Figure 3 Batch Feed Digester System

three-way control valve. By applying this pressure to the reservoir, gas or liquid could be expelled from the digesters. Releasing the pressure placed a vacuum of about 2 feet of water on the digesters and feed could thus be introduced.

These digesters were kept in an incubation room in which the temperature was maintained at $34.5^{\circ} \pm 0.5^{\circ}C$. An electric mercury thermoregulator controlled a small electric heater, while a circulating fan on the floor operated continually to prevent temperature stratification.

Continuous Feed Digesters

In the study conducted to ascertain factors which result in stimulation of acetate digestion, rates in excess of 5 gm/l/day were encountered. At these high rates, it was desirable to add the feed on a continuous basis, since batch feeding could easily result in overtaxing the alkalinity with a resultant drop in pH. The system shown in Fig. 4 was used. Four eight liter narrow mouth bottles were fitted with three hole rubber stoppers to keep a closed atmosphere. Feed was introduced through one hole. A glass tube was fitted through the second hole and extended to the bottom of the bottle. This line was connected to the pressure side of a gas recirculating pump for continual mixing of the contents by recirculating the gases produced in digestion. The third stopper hole was connected to the vacuum side of the recirculation pump. This pump was a Fischer No. 1-092-10 air pump having a capacity of 430 cubic inches per minute at a maximum pressure of 14 psi. A condensate trap was placed in the gas return line.

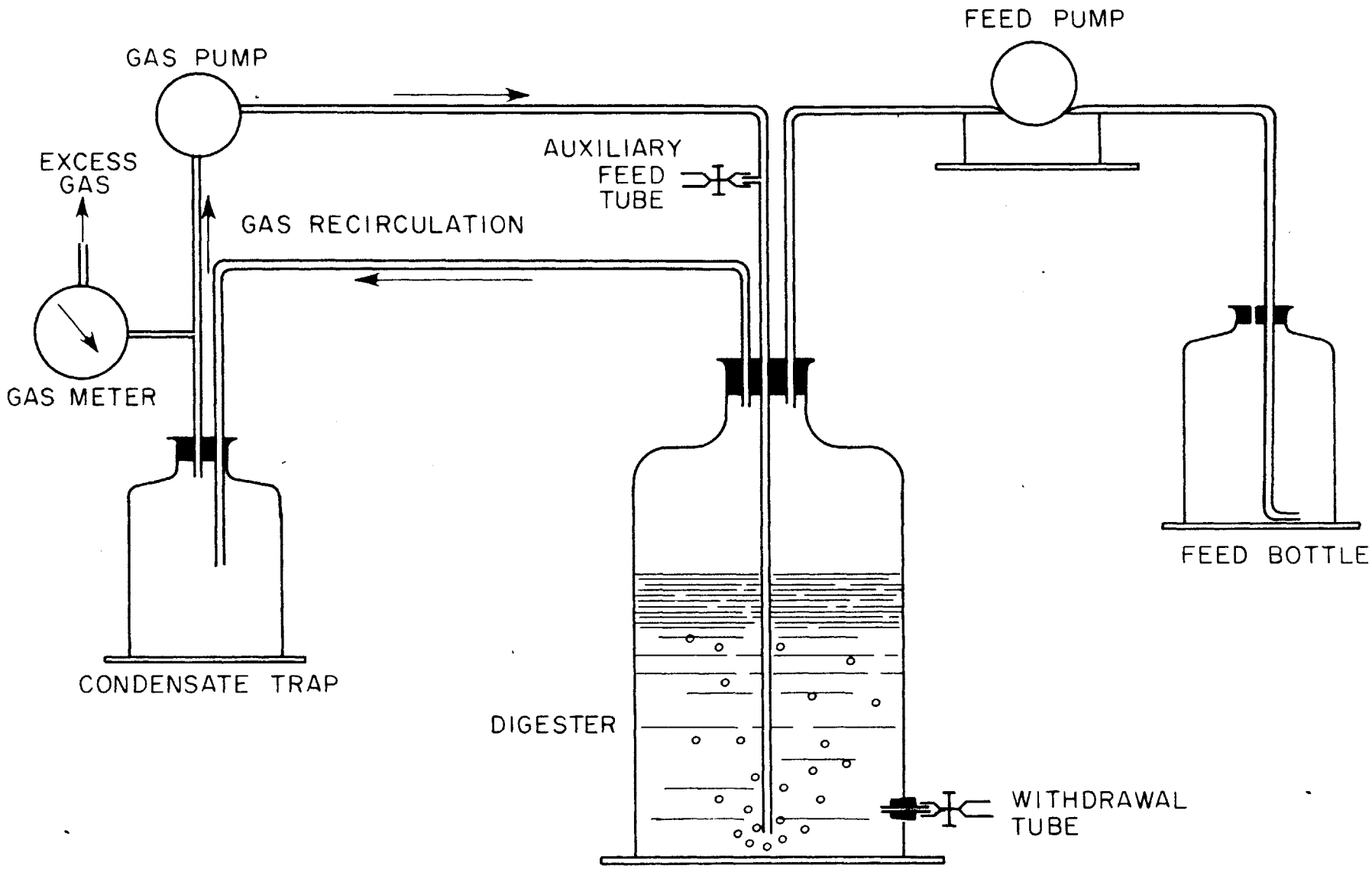


Figure 4 Continuous Feed Digester System

The removal of condensate enabled longer periods of pump operation between overhauls. A tee was also placed in the gas return line to bleed off the gas produced by the digester. This excess gas was passed through a Precision Scientific Company TS-63110-1 wet test meter with a capacity of 680 liters per hour.

Continuous feeding was accomplished by a low capacity positive displacement Harvard-type pump drawing feed from a one liter bottle. Direct additions of liquid could also be made through a tee in the pressure gas line by pinching off the rubber tubing between the tee and the pump. Withdrawals from the digester were made through a hole cut through the side of the digester and fitted with a rubber stopper. A short piece of glass tubing was placed in the stopper and the open end fitted with a piece of rubber tubing held closed by a pinch clamp.

B. Experimental Procedures

1. Nitrogen and Phosphorous Requirements and Biological Growth-Study

The procedures followed in this study were designed to yield reliable data for each substrate. All digesters were started using well digested sludge from Boston's Nut Island treatment plant. They were then placed on a schedule of daily withdrawal of a 50 ml mixed sample of the digester contents and fed substrate and nutrient salts in 50 ml of water. This procedure effectively purged the digester of practically all the original contents, since it was carried on for seven months. Adequate additions of nutrients A and E provided excess nitrogen which along with the organic substrate enabled the bacteria to synthesize cellular products. The organic nitrogen in these products served as the basis for the nitrogen requirement calculations. In the case of the substrates containing soluble organic nitrogen, the effluent was centrifuged and organic nitrogen was determined only on the solids, thus excluding unused portions of the substrate. Phosphorous requirements were determined by microchemical analysis of the effluent solids, excepting the cellulose and oleate units.

Per cent utilization of the substrate was based on volatile acids in the effluent for acetate. COD served as the basis for utilization calculations in all other substrates. COD was run on both the mixed effluent and clarified liquor.

The procedures generally followed through the study will be described with exceptions taken up under each individual run.

Selection of Substrates

Fats, carbohydrates, and proteins are the three classes of organic compounds generally encountered in biological waste treatment processes. Therefore, representative compounds were chosen for this study. Fatty acids were chosen to represent the fats category since they are a step in the breakdown of fats. Acetate, octanoate, and oleate were chosen as representatives of short, medium, and long chain fatty acids. For the carbohydrate substrates, glucose, starch and cellulose served as representative compounds. These are quite commonly encountered. Starch and cellulose are polymers of glucose. However, the bacteria which break down starch and cellulose are quite different. The protein class presented a problem. Due to the complexity of proteins, it is difficult to choose "representative" compounds. Since proteins are composed of amino acids, it was decided to choose two amino acids and a common complex protein. Glycine, leucine, and nutrient broth were thus chosen. No precipitation was found to occur in the digesters using any of these three compounds. This was important for precipitation of an organic feed would obscure cellular organic nitrogen data in the effluent by adding non-cellular solids containing organic nitrogen. Here again, glycine represents short chain amino acids, leucine is a medium length amino acid, and nutrient broth is a representative protein mixture sometimes used as a component in laboratory synthetic sewage.

Inorganic nutrients essential to biological growth were added with the daily feed. Additions were made from two stock solutions which were modifications of those used by Barker (2) in studies of methane-

producing bacteria. During the course of these studies, iron was found to be essential, so it was added daily as ferric chloride. Also, a sulfur source was desired and magnesium sulfate was placed in the stock solution. The composition of these stock solutions was as follows:

Nutrient A	Nutrient E
11.4 gm $(NH_4)_2 HPO_4$	14.0 gm NH_4Cl
1.0 gm $Mg SO_4 \ 6H_2O$	2.0 gm KCl
	5.0 gm $MgCl_2 \ 6H_2O$
diluted to 1 liter with distilled water	5.0 gm $FeCl_3$
	diluted to 1 liter with distilled water

Start-Up and Purging of Digesters

The initial seed for these digesters was primary digested sludge from the Nut Island Treatment Plant. It was passed through 1/8" screen to remove coarse material and paper. This resulted in a homogeneous fluid. One fourth liter was transferred to each digester and 500 ml of warm tap water was added to dilute the contents to the required 750 ml volume for each digester.

All digesters were operated on a 15-day detention time for at least 8 detention periods. This purged the units of essentially all the original contents and thus analyses of digester contents would not be affected by the original seed sludge.

Operation of Batch Feed Digesters

Initially, much difficulty was encountered in maintaining satisfactory digestion in these units. As the original contents were

gradually purged, digestion rates fell off. This indicated that some essential materials were in the seed sludge, the lack of which disrupted digestion. The nutrient salts added with the daily feed were unable to maintain proper digestion. In the course of time, it was found that iron and some trace metals were able to sustain digestion at the rates chosen for this study. The feed rate to each 750 ml unit was 500 mg COD/day. This converts to 667 mg COD/l/day or 0.04 #COD/ft^3/day, corresponding to the loading rate of conventional digesters.

A set procedure was followed for the care of all digesters when final data was being taken on each run. Thus a reliable comparison of the various runs was possible due to the similar conditions prevailing. The digesters were fed at the same time each day $\pm$ 2 hours. First, the withdrawal tube on each digester was emptied of liquid. A vacuum on the digester sucked this liquid in when the pinch clamp on the outlet was barely opened. This admitted 2 or 3 ml of air into the digester, but assured that the effluent sample would be representative of the uniformly mixed contents. The withdrawal tubes had a volume of about 10 ml. Even though the digester contents were thoroughly shaken, the liquid trapped in the withdrawal tube could not mix with the main contents of the unit. This would cause appreciable error in the 30 day detention time digesters, where the daily effluent volume was only 25 ml. After draining of the withdrawal tubes, air pressure was applied to the gas confining fluid reservoir so that atmospheric pressure plus no more than 1/2 psi prevailed inside the digesters. Under these conditions gas production was recorded for the previous 24 hour period. Gas

production was used only as a qualitative indication of digester operation and therefore was not corrected to its absolute volume.

Next, pressure in the gas confining fluid reservoir was raised so that pressure inside the digester was about 1 psi above atmospheric. This enabled expulsion of a sample through the withdrawal tube. The digester was shaken in a swirling motion 20 times to completely mix the contents and then a sample was drawn off according to the digester detention time. With this digester pressure maintained, the gas produced was expelled to the atmosphere. The digester was then placed under a vacuum to draw in the feed. The feed consisted of 500 mg COD of each particular substrate taken from a stock solution and nutrient salts. In the units which could not produce their own buffer from the substrate, sufficient sodium bicarbonate was added to maintain the alkalinity at 4000 mg/l as calcium carbonate. In those units producing excess buffer from the substrate, hydrochloric acid was added to maintain the pH near 7.0. Nutrient salt solutions A and E were each added to all units at a feed concentration of 40 ml/liter. Thus, on an absolute basis, a 5-day digester received six times as much as a 30-day unit. The feed was then diluted to the proper volume, which corresponded to the digester detention time and fed to the digester by pipette. A separate pipette was used to feed each substrate. The digester contents were then shaken 20 times in a swirling manner to mix the feed and contents evenly.

During the period when the digesters were being purged, unsatisfactory digestion on many occasions necessitated feed stoppage until proper digestion could be restored. The feeding schedule was not as rigidly adhered to during this purging period as when final data was being taken.

Routine analyses of the digester effluent consisted of the following: pH, alkalinity, volatile acids, ammonia and organic nitrogen, COD on the mixed effluent and also on the centrifuged liquor, total and volatile solids, and analysis of the digester gas for nitrogen, methane, and carbon dioxide. The frequency of these analyses can be noted in Appendix B.

Criteria for Equilibrium Conditions

These digesters were operated for a period of many months. Wide fluctuations existed in the efficiency of removal and at times gas production ceased. Various remedies, listed under their respective runs, were used to reinitiate and promote digestion to an equilibrium condition which would yield reliable data.

Organic nitrogen served as the factor upon which equilibrium conditions were based. In the case of digesters fed with a soluble substrate containing organic nitrogen, the organic nitrogen analyses were based on centrifuged solids and not mixed liquor. Organic nitrogen is commonly used as an indication of bacterial cell mass according to Bennett and Williams (4). Of course, the constancy of effluent COD and gas production values would also indicate equilibrium conditions. It was decided that consistent organic nitrogen values must be obtained

for an interval of not less than seven days of satisfactory digestion in order to consider such data reliable and representative. A week's period would allow any undue fluctuations to be noticed.

Microchemical Analysis of Bacterial Cells

An empirical chemical formation of the bacterial cells produced during the digestion of each respective substrate was obtained for two reasons: 1) a significant change in chemical formulation would be an indication of change in the digestion process, and 2) the organic phosphorous content of the cell could be established. It would be interesting to compare the long and short detention time chemical formulations for a particular substrate.

No microchemical analyses were made of the cells present in the oleate and cellulose digesters as these two substrates were insoluble under the digestion conditions and could not be separated from the cells.

Preparation of samples was accomplished by centrifuging a daily effluent sample for three minutes at 18,000 times the force of gravity. The clear liquor was poured off and the solids were resuspended in wash water buffered at pH1 with HCl. A small wire beater was used to mix the acid wash water and solids to insure dispersion. (This procedure served to dissolve the precipitated sulfide and ammonium salts. The odor of H_2S could be detected during this process. Analysis of the washed solids for NH_3-N revealed none was present. The microchemical analyses of the cells showed 1-3% phosphorous, which indicates that phosphate salts were also dissolved.) The solids were then centrifuged

as before, decanted, and washed into a 3 dram vial. The dry weight of the solids ranged from about 10-100 mg per sample and were washed in a minimum of 25 ml acid wash water. After drying overnight at 103°C, the solids were taken to the Microchemical Analysis Laboratory. Samples were analyzed for carbon, hydrogen, nitrogen, phosphorus, and inert material. The per cent oxygen was taken to be the difference between 100% and the sum of the percentages of carbon, hydrogen, nitrogen, and inert material. An empirical chemical formula for the cells was then calculated.

Variation in Procedures Followed in the Various Runs

The exceptions to the general procedure heretofore described will be discussed for each respective run in the following section.

Run I - Acetate Feed

Due to difficulty experienced during this run in maintaining satisfactory digestion, a preparation of digester supernatant was added. The preparation was made as follows: Supernatant liquor from a well operating domestic sewage sludge digester at the Nut Island Treatment Plant was evaporated to dryness on a steam bath. The dried solids were then pulverized in a Waring Blender. Since the supernatant liquor usually contained about 0.3% solids, 100 grams of dried solids represented the solids from approximately 30 liters of supernatant liquor solution. The pulverized solids were then boiled in 1 liter of water for 30 minutes in order to extract the stimulant, discovered by Vath (63), from the solids. Next the solids were separated by centrifuge and the resultant liquor served as the stimulant source for acetate digestion.

The organic nitrogen of the solution was approximately 100 mg/l and 1 ml of the solution was added daily with each feed. This would result in an equilibrium concentration of supplemental organic nitrogen in the 5-day detention time digester of 0.7 mg/l and 4.0 mg/l in the 30-day digester.

It was later shown that acetate digestion could proceed satisfactorily up to rates of 1000 mg/l/day with additions of nutrient solutions A and E only. Since the feed rates in this study were only 670 mg/l/day, it was later concluded that the additions of supernatant preparation would not have been necessary. No sulfur was included in nutrient solution A during this run.

Detention times of 5, 10, 15, 20, 25, and 30 days were chosen for this run. The withdrawal tubes on those digesters from which more than a 50 ml sample was expelled, were not emptied prior to expulsion.

The organic nitrogen content of each daily sample was approximately 1 mg for all detention times. Therefore nesslerization of the samples after distillation was followed in order to obtain more accurate analyses. However, no noticeable difference was found between organic nitrogen contents obtained by titration and those obtained by nesslerization.

Run II - Octanoate Feed

These units were started from previously purged digesters fed acetate as the substrate. It required about two weeks for the units to acclimate to octanoate. Two units were used and were operated at 5 to 30 day detention times.

Run III - Oleate Feed

The precipitation of oleate by the magnesium in the nutrient solutions precluded the use of solids data and microchemical analyses.

The digestion process gradually deteriorated when placed on a 5-day detention time. Satisfactory digestion could not be maintained with a 7.5 day detention time either. Therefore, final data was collected on only the 30 day detention time.

Run IV - Glycine Feed

Glycine contains organic nitrogen, and so it became necessary to separate the bacterial cells from solution in order to analyze for the bacterial nitrogen. Thus, the effluent was centrifuged for solids separation and the organic nitrogen content of the solids was determined. Sufficient HCl was added to the feed of the 30-day unit to lower the digester pH to 7.0 whenever it approached 7.4.

Digesters were operated on 5 and 30 day detention times.

Run V - Leucine Feed

These digesters were started from purged units fed acetate as the substrate. Preliminary data was obtained on a detention period of 15 days, with final data obtained on $7\frac{1}{2}$ and 30 day detention times. Satisfactory digestion could not be maintained on a 5 day detention time.

As with the glycine digester, the solids in the leucine fed digester were separated by centrifuge for analysis of biological nutrient requirements.

Due to the ammonium bicarbonate alkalinity produced upon degradation of the substrate only 500 mg $NaHCO_3$ was added daily to the 5 day unit and none to the 30 day unit.

Run VI - Nutrient Broth

Laboratory experience had shown that nutrient broth would not precipitate at the pH range in which the digester operated - pH 6.8 to 7.4. This substrate is very complex and contains many

minerals as shown by its composition in Appendix A. For this reason supplemental additions of nutrient solutions A and E were cut to 1/3 the quantity generally added. No sodium bicarbonate was added to the 30 day digester and 500 mg was added every third day to the 5 day digester.

Run VII - Glucose Feed

Four digesters previously purged while being fed acetate as a substrate were converted to a glucose substrate with 5, 10, 20 and 30 day detention times.

Run VIII - Starch Feed

For this run two digesters were changed from an acetate feed to a starch feed and operated on 5 and 30 day detention times respectively. Preliminary data was obtained on 15 day detention period also.

Run IX - Cellulose Feed

This substrate was prepared by weighing 20 grams of S and S 589 filter paper. A pint of water was then placed in a Waring Blender. The filter papers were then added to the blender one at a time and shredded into a slurry. When the slurry became too viscous, it was poured into a beaker and another pint of tap water placed in the blender. Two pints of water were sufficient to shred the total 20 grams of filter paper. The paper slurry was then diluted to one liter and passed through the blender twice to insure uniform mixing. Even after this treatment in the blender, it was necessary to use an open tip pipette to withdraw it from the stock solution bottle. It would not

stratify upon standing, however. This heterogenous mixture resulted in larger variations in feed COD than normally encountered.

Since 100% liquifaction of the daily cellulose feed could not be assured, the analyses for volatile solids, solids COD, and microchemical composition were not run.

2. Stimulation of Acetate Utilization

In this aspect of the research, it was desired to determine those substances which could stimulate the methane phase of digestion. To isolate this phase, acetate was fed as the only organic substrate. Substances which were assumed to possess growth stimulating properties were added to the digesters for assay. Continuous feed digesters were used and operated to obtain maximum acetate utilization rates. Volatile acid concentration in the digesters were maintained above 1200 mg/l except when an occasional rapid stimulation resulted in a temporary reduction. By operating the digesters in this fashion, they served to assay the stimulation potential of materials added to them.

Selection of Substrate

Acetic acid and sodium acetate served as the substrates during this study. The reasons for this choice have been pointed out by McCarty (31) as follows: Under strictly anaerobic conditions, this acid can be utilized only by methane bacteria and so its use permits the separation of the methane phase of digestion. Acetic acid is one of the intermediate products from the breakdown of almost all proteins, carbohydrates, and fats. Thus, conditions affecting the fermentation of this acid will affect the anaerobic decomposition of practically all

organic wastes. Also, one of the first indications of trouble in the digestion process, according to Kaplovsky (24), is a build-up of acetic acid. This is a strong indication that the organisms responsible for the degradation of this acid are probably most sensitive to environmental change.

Sufficient sodium acetate was fed daily to maintain the alkalinity between 4000 and 6000 mg/l as $CaCO_3$. Nutrient solutions A and E were originally fed at a concentration of 20 ml/l of feed and later increased to 60 ml/l of feed.

Operation of Continuous Feed Digesters

It was expedient to use a continuous feed unit since the rates achieved during this study exceeded 5 g/l/day. Thus, feed would have to be administered in increments over a prolonged period throughout the day or continuously in order not to exceed the buffer capacity.

The digesters consisted of 8 liter narrow mouth pyrex bottles containing 6 liters of liquid. The liquid volume was first drawn down to the 6 liter mark. This involved drawing off about 200 ml, since the units were operated on a 30 day detention time. The gas meter readings were then recorded to $\pm$ 0.1 liter. A 50 ml portion of the effluent was analyzed for volatile acids concentration. It was desired to maintain the volatile acids concentration in the digester above 1200 mg/l and below 2000 mg/l, with 1500 mg/l as the desired level. The amount of acetic acid fed per day was then calculated as follows:

$$\left[1500 \text{ mg/l} - (\text{Volatile Acids Concentration mg/l}) + \text{Rate of acetate utilization on previous day mg/l}\right] \times 6 \text{ l.}$$

The nutrient salt solutions A and E were then each added to the feed at the rate of 2 ml/day/liter of digester capacity. This amount of the nutrient solutions maintained the total nitrogen concentration in excess of 500 mg/l and provided ample ammonia nitrogen for bacterial synthesis. The feed bottle contents were then diluted to 200 ml with tap water. The pump would feed this volume in approximately 21 to 23 hours. It was necessary to rinse the feed bottle with 50 ml of tap water once or twice a week as some of the ferric chloride in nutrient solution E would flocculate and form a deposit which would not be drawn through the feed tube. This rinse water containing the ferric chloride was then pipetted directly into the respective digester.

On many occasions, sufficient feed was placed in the feed bottle to last for several days. Likewise, the liquid was allowed to build up for a day or two before drawing the level back down to 6 liters.

Periodically the digester effluent was analyzed to determine pH, alkalinity, ammonia nitrogen, and organic nitrogen. On one occasion, the effluent from Digester II was centrifuged, the solids dried and then analyzed microchemically. This digester was operating at 5000 mg/l/day and was exceptionally free from extraneous material.

In several instances, toxic concentrations of materials were added to a digester, with the result that digestion abruptly stopped. Two procedures were used to restore digestion. The first involved draining the digester of up to 90% of its mixed contents. Carbon dioxide from a cylinder was then connected to the gas effluent line

during this operation to insure that no oxygen would be drawn into the digester. The digester was then filled back up to the 6 liter volume with tap water to which was added the same concentration of nutrients and buffer as that originally in the digester. Effectively, this diluted the toxic constituents. The second procedure involved precipitation of the toxic substance with sulfide. Minute amounts of copper and zinc ions (approximately 1 mg/l of each) were introduced on one occasion with digestion being seriously hindered. Addition of 25 mg/l sulfide took these ions from solution and enabled restoration of the digestion process.

The substances which were added to the digesters for assay of stimulation potential, were introduced by pipette directly into the auxiliary feed tube of the digester and not through the feed pump.

The amount of acetic acid utilized each day was determined by subtracting the change in volatile acids concentration from the amount fed the previous day. This rate varied depending on the time at which the sample was drawn, fluctuations in the volatile acids determination, and changes in the mixed bacterial culture. The magnitude of such variations was observed to be less than 10% of the rate of utilization in practically all cases. It was considered a significant stimulation if the increase was at least 20% of the past rate.

The digesters were operated until the utilization rate reached an equilibrium. Then a substance would be added to the digester for assay of its stimulation potential. By using a plot of utilization rate vs time, changes in rate could easily be observed. After the unit

had again reached an equilibrium rate, whether it was higher or lower than the previous, another assay of material could be made.

The stimulation resulting from addition of a substance to the digester may be due solely to the substance added, or it may be the result of a synergistic effect of the material added and a compound already in the digester.

C. Analytical Techniques

The procedures set forth in the 10th edition of Standard Methods (53A) were followed in general. The page number of the analysis followed in Standard Methods and any modifications used are described below.

pH Determination

This determination was made with a Beckman Zeromatic model pH meter and glass electrode. The procedure followed is described on pages 161 and 352 of Standard Methods. When standardizing the instrument, the electrodes were dipped into an acid solution of about pH=1 and then immersed in a basic solution of pH 10. The electrodes were then rinsed with distilled water, wiped off with soft cleansing tissue and placed in buffer solution of pH = 7.0 for standardization. This procedure decreased the response time of the instrument.

Values of pH determinations were recorded $\pm$ 0.05 to signify that the pH meter had been standardized and no appreciable time lapse had occurred between sample withdrawal and pH determination. Prompt analysis prevented the loss of carbon dioxide and resultant pH increase.

Alkalinity

A modification of the procedure described on page 228 of Standard Methods was followed. The strength of H_2SO_4 used in the titration was 1.0N instead of the recommended 0.02N. Mixing of the sludge during titration was accomplished by a magnetic stirrer. The sample size used for the titration was 50 ml where possible with 25 ml as the smallest sample used.

Volatile Acids

The tentative method outlined on page 347 of Standard Methods was followed with the following variations. Fifty ml samples were used except when the total sample withdrawn was less; 25 ml was the least volume analyzed. On volumes less than 50 ml, distilled water was added to bring the volume up to 50 ml. After addition of the sample plus 150 ml tap water and 2.5 ml concentrated H_2SO_4 to the distilling flask a small chip of paraffin, was placed in the flask to control foaming. About 1 gram of NaCl was placed in each of the collection flask to enable the distillate to conduct electricity. Two electrodes were suspended in each flask so as to make contact with the distillate when its volume reached 150 ml. Thus the electrodes would be shorted and a relay in the system would actuate an alarm. This facilitated the distillation of exactly 150 ml, while the NaCl did not affect the titration. McCarty (31) has shown that for acetic acid, 65% is recovered in the distillate. So the factor of 0.70, recommended in Standard Methods, was replaced by 0.65 in the calculation of volatile acid concentrations for the acid.

Ammonia Nitrogen

This determination was based on the distillation procedure described on page 241 of Standard Methods. Modifications were as follows: 1) Sample size varied from 10 to 150 ml, 2) Distillation was into 50 ml of boric acid solution (20 grams/liter), 3) 3) N/14 H_2SO_4 was used to titrate the sample to the methyl orange end point. The end-point was also determined electrometrically at pH 4.3 at times,

4) A small piece of paraffin was added to some samples to prevent foaming. 5) Nesslerization of the distillate was also used when samples contained below 1 mg of nitrogen. Experimentally, it was found that 20mg NH_3-N could be distilled and collected in 50 ml boric acid solution with no loss.

Organic Nitrogen

On samples which required organic nitrogen analysis of the solids, separation of such solids was by centrifuge. After pouring off the clear supernatant, the solids were washed in acid water buffered at pH 1 with HCl. This served to dissolve any salts precipitated along with the solids. The acid wash water was decanted and the solids were washed into a 3 dram vial. This vial was then placed in the 800 ml Kjehldahl flask and appropriate chemicals were added for digestion. On other samples, the residue from the ammonia nitrogen determination was used for analysis of organic nitrogen. The procedure then followed was the Kjehldahl method for sewage described on page 247 of Standard Methods. Modifications were as follows: 1) Prior to digestion, 20 ml of concentrated sulfuric acid was added along with the copper and selenium calatysts and potassium sulfate, 2) 100 ml of water was also added to those flasks containing solids in the 3-dram vials. This allowed the vials to boil immersed in the strong acid solution for a period and insured breakdown of the organic matter. The flasks were shaken after digestion was complete so that sulfuric acid swirled inside the vial. Boiling was then continued for another 10 minutes. After digestion, 350 ml of distilled water was added to each flask and the

pH raised to 10 with sodium hydroxide. Distillation and titration were then carried out as described in the ammonia nitrogen section.

Chemical Oxygen Demand

COD was determined by the Dichromate Reflux Method for industrial wastes described on page 333 of Standard Methods. The one modification was on the distilled water blank. It was made up at the same time as the other samples and not boiled. No significant difference was noted on 24 replicate blanks, 12 of which were boiled with the remaining 12 not boiled.

Total and Volatile Solids

This analysis was run to determine the weight of bacterial cells in a sample. All the units except cellulose and oleate had been purged of extraneous organic material so that the solids consisted of only bacterial cells and precipitated salts. The samples were centrifuged for 3 minutes at a force of 18,000 times gravity. The supernatant liquor was decanted and the solids were washed in acid wash water, buffered at pH 1 with HCl, to dissolve certain precipitated salts. After centrifuging under the same conditions as before, the wash water was decanted and the solids washed into a previously fired and tared crucible. Upon drying overnight in a 103°C oven, the crucibles were cooled and weighed. Next, they were fired for at least 15 minutes in a 600°C muffle furnace. Again, they were cooled and weighed so that no moisture was absorbed. Weights were recorded $\pm$ 1 mg on a Mettler analytical balance.

D. Determination of Rate of Feed Utilization

The rate of feed utilization was based upon one of three different analyses of the digester effluent, depending on the substrate fed. All three methods placed the efficiency on the basis of the amount of substrate utilized by the bacteria. This enabled the reaction kinetics to be calculated.

For the acetate run, the efficiency was based on volatile acids concentration in the effluent. In all other runs, excepting cellulose and oleate, the COD of the effluent filtrate served as the basis of efficiency calculations. The effluent was centrifuged at 18,000 x g for 3 minutes. COD was then determined on the clarified supernatant liquor. Intermediate breakdown products were thus taken into consideration.

In the oleate and cellulose digesters, a different method had to be employed for efficiency calculation. There was no way of insuring that both of these substrates would be 100% removed each day. In both cases, the unused portion of substrate was not in solution. For this reason a third method of efficiency calculation was used. The uniformly mixed effluent from these units was analyzed for COD. To exclude that portion of the COD contributed by bacterial cells, a factor of 11.4 times the organic nitrogen concentration in the effluent was substracted from the COD of the mixed effluent. The factor of 11.4 was established after observing that the COD/N ratio of all bacterial cells microchemically analyzed grouped about this value.

E. Analytical Errors

Substrate Concentration

The substrates were made up into stock solutions from which an amount, equivalent to 500 mg COD, was taken for the daily feed of the respective digesters. The COD of the stock solution was determined and the volume required to provide 500 mg COD was calculated on the basis of stock COD concentration. The variation in stock COD for all substrates except acetate and glucose are shown below.

		COD
Octanoic Acid	45 ml/l	93,000 mg/l
		95,000 "
		101,000 "
		102,000 "
Sodium Oleate	54 g/l	140,000 mg/l
		137,000 mg/l
		134,000 "
		138,000 "
Glycine	100 g/l	61,000 mg/l
		64,000 "
		63,000 "
		82,000 "
		78,000 "
Leucine	13.6 g/l	25,500 mg/l
		25,700 "
		25,000 "
Nutrient Broth	88.5 g/l	97,000 mg/l
		92,000 "
		94,000 "
Starch	46.9 g/l	49,000 mg/l
		50,000 "
Cellulose	20 g/l	21,600 mg/l
		23,000 "
		27,000 "
		18,000 "
		24,000 "
		23,000 "
		26,000 "

The COD variation with acetic acid and glucose stock solutions was ± 2%. There is an unusual spread in the glycine COD, which probably reflects a weighing error. Cellulose was the only insoluble substrate fed. Even after passing through the Waring blender several times, it was difficult to pipette accurately. This is reflected in the wide spread of stock COD values. A small percentage of the feed was always lost due to adherence to the sides of the graduated cylinder or pipette.

Chemical Oxygen Demand

Standard Methods (53A) reported the standard deviation of the back titration was 0.07 ml on distillery wastes and 0.095 ml on miscellaneous wastes with a COD ranging from 350 to 57,500 mg/l. It is stated that for most organic compounds the oxidation is 95-100% of theory. With the silver catalyst, short chain alcohols and acids were oxidized 85 to 95% or better. Generally speaking, the digester effluents would all have contained some short chain acids produced in the digestion process.

Ammonia Nitrogen

The recovery of 99 to 100% is reported by Standard Methods for the distillation and titration procedure. The precision over a range from 5 to 50 mg/l was expressed as a standard deviation of 0.18 ml of 0.02 N H_2SO_4 or 0.5 mg/l for a 100 ml sample. N/14 H_2SO_4 was used during these experiments with sample sizes ranging from 25 to 150 ml.

Total and Volatile Solids

Standard Methods reported a standard deviation of 1.5 mg, but considered this value statistically unreliable. Weights were recorded to the nearest mg in this study so that 2 mg would probably be the standard deviation. For solids from a 25 ml sample this would be 80 mg/l.

Volatile Acids

The tentative method described in Standard Methods with a recovery factor of 0.65 for acetic acid as suggested by McCarty (31) was used. The samples on which the volatile acids concentration was analyzed were very low in solids, approximately 0.1%, and not characteristic of domestic digested sludge.

This analysis was used only for control of all digesters, except those fed acetate as a substrate and then utilization was based on this test. Five standard samples of acetic acid of known concentration were diluted to 200 ml with distilled water, distilled over and yielded the following results:

Actual Concentration	Calculated Concentration
3000 mg/l	3100 mg/l
2400 "	2410 "
1800 "	1800 "
1200 "	1200 "
600 "	570 "

Organic Nitrogen

The cellular nitrogen for substrates in the proteins class was determined by centrifuging a sample of the digester effluent. The supernatant was poured off and the solids were washed in acid water buffered at pH 1. After centrifuging again, the acid wash water was poured off and the solids were washed into a three dram vial. This vial was then placed in an 800 ml Kjehdahl flask, catalysts and 20 ml of concentrated sulfuric acid were added. Distilled water was added in the amount of about 100 ml to raise the liquid level so that the vial was submerged. With this procedure, the contents of the vial were always turned colorless, there were no solids clinging to the sides of the vial, and complete digestion of the solids was insured.

Symons (67) found good agreement between the data obtained by the Kjehdahl method and the data supplied by the Microchemical Analysis Laboratoyy on samples of bacterial cells. Standard Methods reported the accuracy of recovery to be 99 - 100% of the organic nitrogen also. Thus, it was assumed in this study that approximately 100% of the organic nitrogen in bacterial cells was recovered by the Kjehdahl method.

Gas Analysis

Gas samples were analyzed by a gas chromatography apparatus. A glass gas collection tube was used to collect samples. Samples were either analyzed immediately or if not, a large enough sample was taken to expel all retaining fluid from the tube and the ends were clamped to avoid carbon dioxide absorption by the retaining fluid. Lawrence (26A) found the gas chromatography apparatus gave results of $\pm$ 2% of values obtained by analysis with the Burrell apparatus. McCarty (31) found reproducibility of the Burrell to be $\pm$ 1%. As the adsorption column became fouled with continuous use, the accuracy of results may have dropped to $\pm$ 2%.

pH

Standard Methods reports the accuracy of line operated pH meters with glass electrodes to be 0.1 of a pH unit under normal conditions.

Alkalinity

The alkalinity of sludges is determined on the liquor that separates from them as stated by Standard Methods. In these studies, the mixed liquor of the digesters was very low in solids, 0.3% at the highest, and not characteristic of domestic digested sludge. Therefore, alkalinity analyses were determined on the mixed liquor sample as it was taken from the digester.

Standard Methods specifies N/50 H_2SO_4 with titration to pH 4.0 as the methyl orange end point. The precision expressed as standard deviation when using methyl orange as the indicator in settled sewage was 0.07 ml. Due to the high alkalinities encountered, 1N H_2SO_4 was used in these studies.

V. EXPERIMENTAL RESULTS

A. Nitrogen and Phosphorous Requirements and Growth in Anaerobic Digestion

A study was made of the biological requirements for nitrogen and Phosphorous in the anaerobic digestion of organic compounds. The substrates and the category to which they belonged were:

Fats	:	Acetate Octanoate Oleate
Proteins	:	Glycine Leucine Nutrient Broth
Carbohydrates	:	Glucose Starch Cellulose

Each substrate was batch fed on an equal COD basis of 670 mg COD/l of digester capacity/day or 500 mg COD/digester/day. All substrates except oleate were studied on at least two different retention times. With oleate, satisfactory digestion could be achieved neither on a 5 day nor 7.5 day retention time, only on a 30 day retention time. Due to unsatisfactory digestion with leucine and cellulose at a 5 day retention time, operation was switched to 7.5 days where successful digestion was maintained.

It was discovered that addition of ferric chloride along with the other nutrient salts stimulated digestion, and its addition was adopted as standard procedure with all substrates. Excess nitrogen as ammonium salts was added with all substrates.

All of the units were operated for an extended period prior to the final data period. This served three purposes: 1) the daily feeding and withdrawal effectively purged the digester of the original sludge

used to initiate digestion; 2) the biological population became acclimated to the substrate; and 3) steady state conditions of operation were established for the respective detention times chosen.

Due to the limited number of digesters used, all runs could not be conducted simultaneously. Therefore, some digesters which had originally been "seeded" with digested sludge and had been thoroughly purged of this original "seed" while operating on an acetate substrate, were changed to other substrates when the acetate run was completed. After an acclimation period, which varied for each of the new substrates, all units developed satisfactory digestion. Figure 5 gives the relation between the percentage of original contents remaining in a digester and the number of detention times for which it has been operating. This is based on the assumption that the effluent is a completely mixed sample of the digester contents as was the case in these studies.

The gas production values reported are volumes at atmospheric pressure, but are not corrected for barometric changes.

The removal efficiencies were based upon the COD concentration in the feed and on the weighted average of the bacterial cell-free effluent. One exception to this was acetate where efficiencies were based upon volatile acid concentrations in the feed and in the effluent. Since the same amount of substrate was fed to each digester daily in varying quantities of dilution water, the feed concentrations for the various detention times were as follows:

Detention Time	Feed Concentration
5 days	3330 mg/l
7.5 "	5000 "
10 "	6670 "
15 "	10000 "
20 "	13330 "
25 "	16670 "
30 "	20000 "

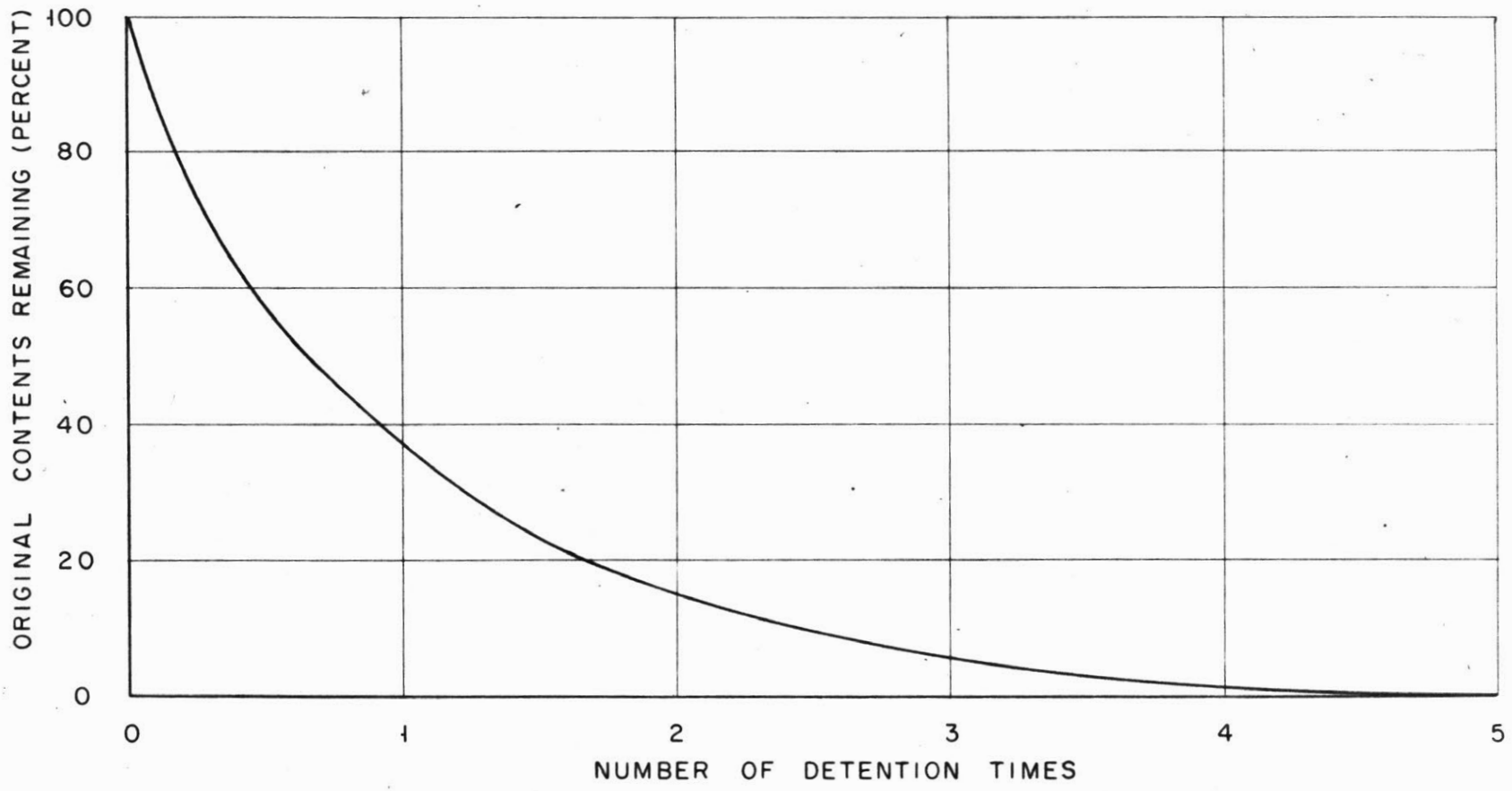


Figure 5 Per Cent of Original Digester Contents vs Time

The COD analyses of the effluent were not made daily or at regular intervals, so that a weighted average was taken which took into consideration the time interval over which the analysis was representative. In effect, the average height of the COD vs time curve was obtained.

The weighted results for cellular nitrogen shown in the summary table in each run were obtained by arithmetic average of the values recorded during the interval over which final data was recorded. Those values which fell outside the limits of $\pm$ 3 standard deviations from the mean were disregarded as due to sampling and analysis errors.

Microchemical analyses of the biological solids from all digesters except oleate and cellulose were made to formulate the average chemical constituency of the protoplasm. The phosphorous requirement was determined from the microchemical analyses and the COD/N ratio for the bacterial cells was calculated also. This information is tabulated in Table XI.

The nitrogen requirement in all cases was calculated as follows:

$$\text{Nitrogen requirement} = \frac{\text{Cellular Nitrogen mg/l}}{(\text{Retention Time-days})(\text{Feed Rate gmCOD/day})(\text{Efficiency})}$$

For the 5-day acetate unit, a sample calculation is given:

$$\text{Nitrogen requirement} = \frac{12 \text{ mg/l}}{(5 \text{ days})(.670 \text{ gm COD/day})(.67)}$$

$$= 5.3 \text{ mgN/gm COD utilized}$$

As a check on the reliability of the data, a balance was made on the COD input to the system and the COD output from the system. The feed was the only source of COD input while methane and the effluent sample were the two sources of COD output. Some methane was undoubtedly lost due to diffusion through the rubber connections. McCarty (31) made a study of the methane loss which occurred in a system similar to the system used in this study and reported approximately 5 ml of methane was lost per day. The total gas production was recorded and the methane composition determined. The volume of methane at 35°C which is equivalent to 1 gram of COD is as follows:

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2\,H_2O$$

1 mole CH_4 is equivalent to 64 grams of COD

1/64 mole CH_4 equivalent to 1 gram of COD.

At 0°C and 1 atmosphere pressure 1 mole of a gas occupies 22.4 l. According to Charles' Law, the volume of one mole at 35°C (308°k) is

$$V_2 = \frac{T_2}{T_1} V_1$$

$$V_2 = \frac{308}{273} (22.4\ l)$$

$$V_2 = 25.2\ l$$

Therefore 1/64 mole $CH_4 = \frac{25.2}{64} = 395$ ml at 35°C and 1 atm.pressure or 395 ml CH_4 is equivalent to 1 gram COD.

The COD and volume of the mixed effluent were known. The COD balance was made for the entire final data period and is shown in each summary table.

Run I - Acetate Substrate

Digestion was initiated in these units with 250 ml of screened, digested sludge. Tap water was added to dilute the contents to 750 ml. All units were operated for a total of 170 days before final data was collected from them. During most of this time, operation of all units was on a 15 day retention time. The period of operation on each respective retention time before the final data was collected was as follows:

5	day	digester	7	days	operation
10	"	"	11	"	"
15	"	"	170	"	"
20	"	"	30	"	"
25	"	"	30	"	"
30	"	"	27	"	"

The cellular nitrogen concentration had reached an equilibrium value in all cases by the end of the interval of operation shown above.

Final data was collected for a minimum of 43 days for each of the digesters.

Results

Results of this run are shown in Figure 6, Table IV and Appendix Tables B-I to B-VI.

The removal efficiency was based upon the volatile acids concentration in the effluent for all of the acetate digesters. The volatile solids analyses were not run on any of these units, since this run was completed several months before any of the other runs and it had not been decided at that time to record volatile solids.

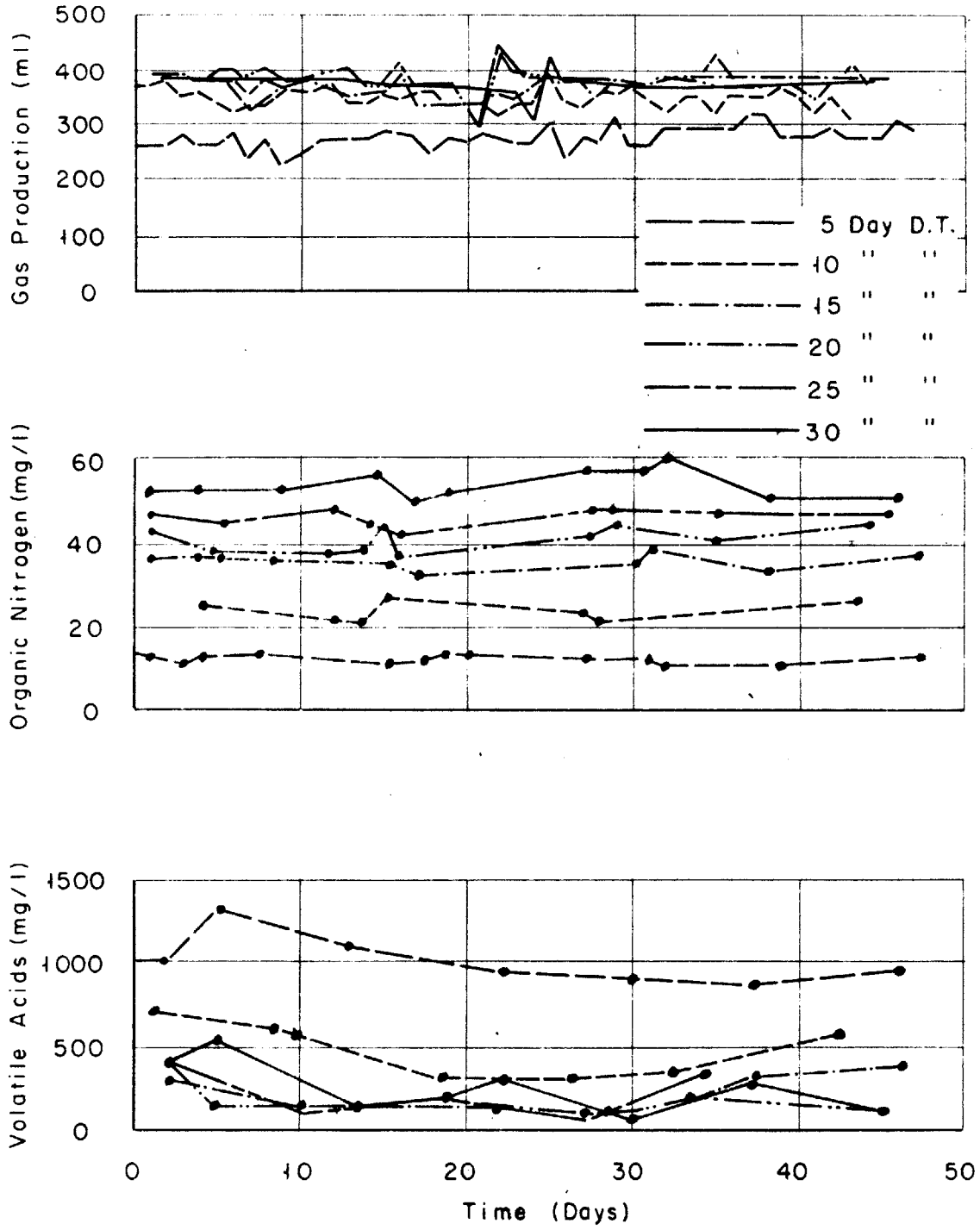


Figure 6 Acetate Digester Operation

A preparation, made by hot water extraction of the solids remaining after evaporation of supernatant liquor from a domestic sewage sludge digester, was added with the daily feed to stimulate digestion. One ml of the preparation, which contained 100 mg/l organic nitrogen, was added daily to each unit. The organic nitrogen concentration in the feed due to this source would range from 0.7 mg/l for the 5 day detention time to 4 mg/l for 30 days. The effect of this organic nitrogen in the feed was neglected in determining the nitrogen requirements.

TABLE IV

SUMMARY OF ACETATE DIGESTION DATA

Detention Time Day	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
5	143	12	67	53	111
10	186	23	93	37	106
15	205	36	98	37	110
20	202	40	99	30	106
25	206	46	99	28	108
30	198	54	99	27	104

Run II - Octanoate Substrate

Digestion was initiated in these units by a sludge acclimated to an acetate substrate. The organic nitrogen concentration at the time when the octanoate feed was started was 12 mg/l. A total period of 80 days elapsed from the time at which the substrate was changed from acetate to octanoate and the time at which the collection of final data began. The acclimation period took approximately 45 days before the substrate was readily metabolized.

Data was recorded on 5 and 30 day retention times. Each digester was operated on its respective retention time for 20 days prior to the collection of final data. After this period, the equilibrium of the organic nitrogen concentration was established in both cases.

Final data was collected over a period of 35 to 26 days for the 5 and 30 day units respectively.

Results

A summary of results from this run are shown in Table V and Figure 7 along with data from the following run on sodium oleate. Appendix Tables B-VII and B-VIII contain the operating data for this run. Approximately twice as much nitrogen was required to digest octanoate at a 5-day retention time as compared to a 30 day retention period, the values being 6.1 and 2.8 mgN/gm COD respectively.

TABLE V

SUMMARY OF OCTANOATE AND OLEATE DIGESTION DATA

Detention Time Days	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
Octanoate 5	115	11	65	61	101
Octanoate 30	171	53	96	28	94
Oleate 30	175	51	95	27	94

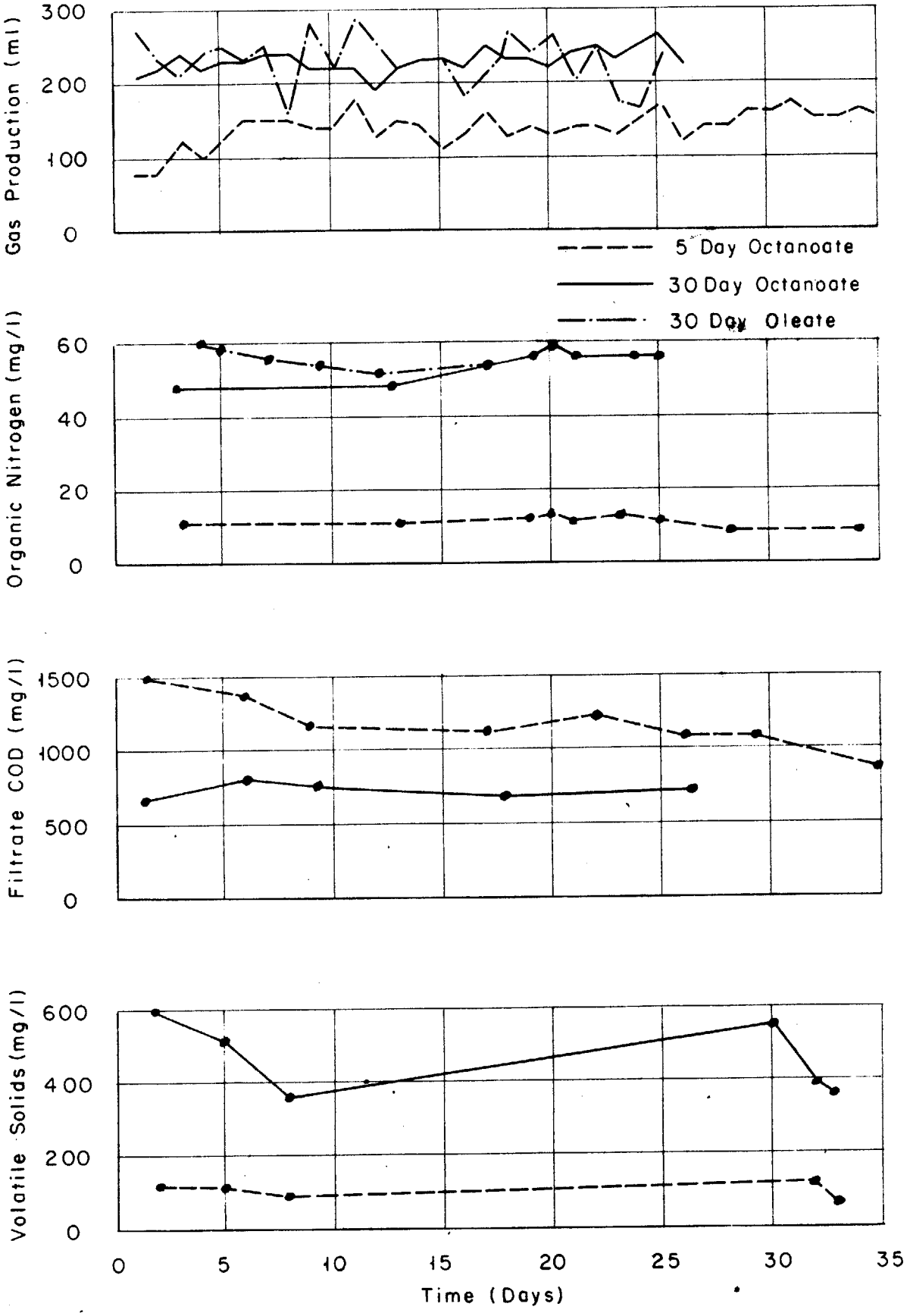


Figure 7 Octanoate and Oleate Digester Operation

Run III - Oleate Substrate

Oleate proved to be very difficult to digest under the conditions of this study. Digested sludge in the amount of 250 ml was used to initiate digestion. With the feeding of only oleate and nutrient salts and with withdrawal of mixed liquor, the growth factors and trace elements present in the initial digested sludge-seed were washed out. It appeared these were helpful in oleate digestion, since, as time progressed, the rate of digestion in the 5-day detention time unit decreased. Feeding was stopped for a period and when digestion resumed, feeding was continued on a 7.5 day detention time. Even on a 7.5 day detention period, satisfactory digestion could not be maintained, and the unit finally died. However, satisfactory digestion could be maintained on a 30 day detention time and final data was collected from this unit.

A total of 320 days elapsed from initiation of digestion until final data was taken from the 30 day unit. Most of this time, however, operation was on a 15 day detention time and digestion was poor. It was switched from a 15 to 30 day detention period and in 15 days an equilibrium organic nitrogen concentration was established.

Since a portion of the feed was precipitated by the magnesium ions contained in the nutrient salts, the centrifuged effluent solids contained not only biological solids but insoluble magnesium oleate. For this reason, neither the volatile solids nor microchemical analyses were determined.

Results

The results of this run are shown in Figure 7, Table V, and Appendix Table B-IX. Due to the insolubility of the feed, it was necessary to base the efficiency on the COD of the mixed effluent minus the COD of the biological solids. In order to evaluate the COD of the biological solids, it was necessary to determine a factor which would relate cellular nitrogen to cellular COD. Due to the presence of reduced salts in the effluent, it was not possible to determine the COD/N ratio directly. Therefore, a COD/N ratio based on the average chemical formulation of all biological solids for all substrates was used. This is shown on page 104. The average chemical formulation was $C_5H_9O_3N$ and the COD/N ratio of this formula is 11.4 and was taken as the average for all biological solids. Thus. the COD of the biological solids was calculated as follows;

COD of biological solids = 11.4 x average organic nitrogen conc.

= 11.4 x 53

= 600 mg/l

Run IV - Glycine Substrate

Both glycine digesters were started by "seeding" with 250 ml digested sludge. They were operated for a period of 310 days before final data was recorded from them. During most of this period operation was on a 15-day retention time. The 5-day unit reached equilibrium cellular nitrogen concentration 9 days after being placed on that retention time. Thirteen days were required for equilibrium to be established after changing from 15 to 30 day retention times.

The 5-day detention time enabled maintenance of the ammonium bicarbonate alkalinity, produced during the degradation of glycine, at approximately 2800 mg/l as $CaCO_3$. Thus, the pH was also maintained at approximately 7.2. However, in the digester operating on a 30 day detention time, the ammonium bicarbonate alkalinity tended to increase the pH to over 7.5. At this high pH, the digestion process was adversely hindered due to the concentration of free ammonia in solution. Therefore, hydrochloric acid was added at irregular intervals to the 30 day digester to maintain a neutral pH. The gas production curve in Figure 8 reflects the excess carbon dioxide released after addition of HCl to neutralize the NH_4HCO_3. The ammonia nitrogen concentration was approximately 3600 mg/l. This is exceptionally high. It was necessary to centrifuge the effluent and analyze the solids for organic nitrogen to arrive at the cellular nitrogen concentration since complete decomposition of the organic nitrogen in the feed could not be assured.

Results

Table VI, Appendix Tables B-X and B-XI and Figure 8 shows the results of this run.

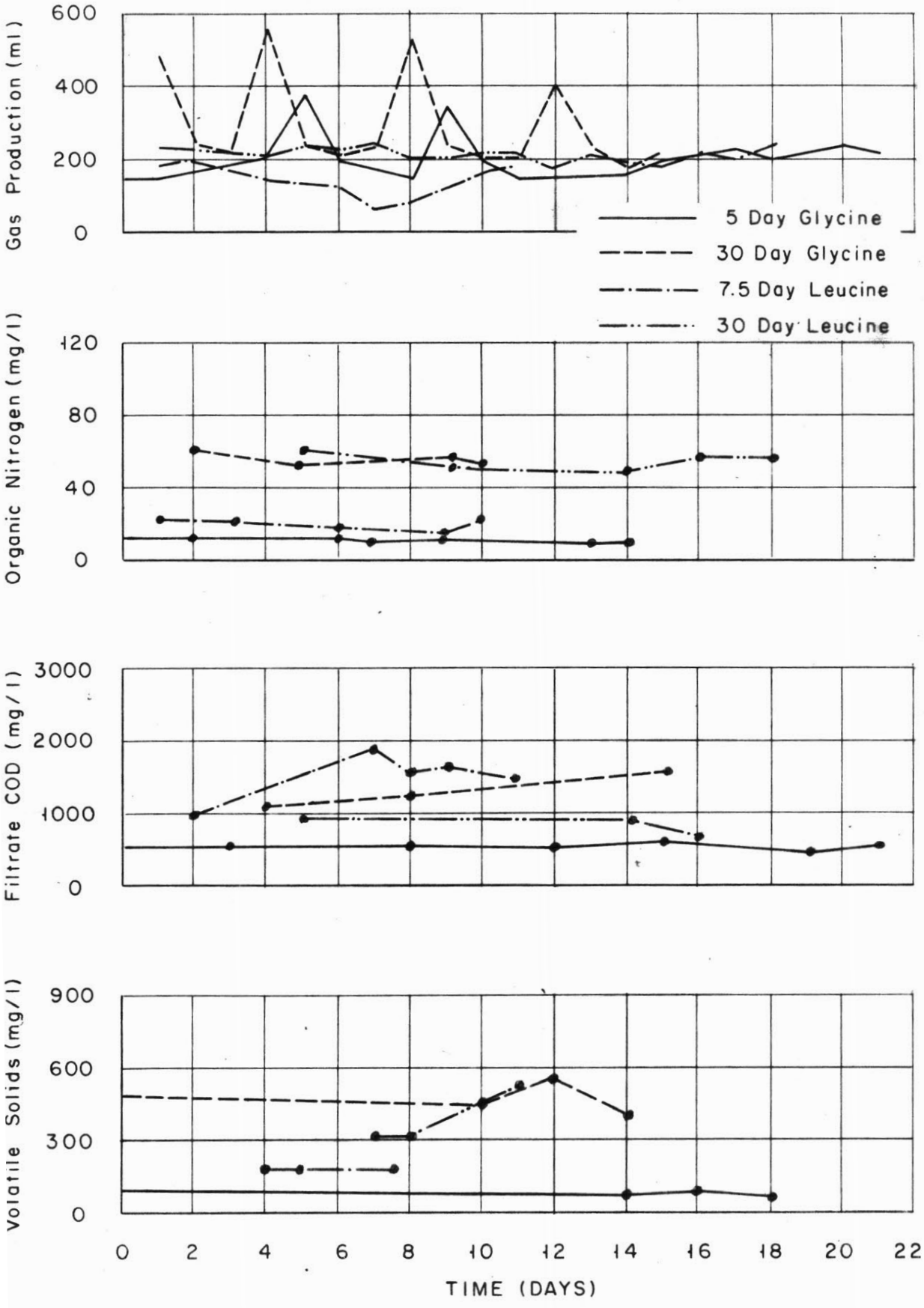


Figure 8 Glycine and Leucine Digester Operation

TABLE VI

SUMMARY OF GLYCINE AND LEUCINE DIGESTION DATA

Detention Time Days	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
Glycine-5	137	11	83	40	108
Glycine-30	156	55	93	29	88
Leucine-7.5	105	20	70	56	88
Leucine-30	156	54	95	28	86

Run V - Leucine Substrate

Digestion was initiated in these units by "seeding" with mixed liquor from a unit which had been operating on acetate and nutrient salts for many months. Thirty days were required for each unit to acclimate to the leucine substrate.

The 7.5 day digester operated for 22 days on that detention time before final data was recorded. The 30 day digester operated for 15 days on that detention time before final data was recorded.

The reason 7.5 days was chosen as one of the detention times was that the digestion process deteriorated on a 5 day detention time.

The concentration of organic nitrogen in the leucine feed was low enough so that alkalinity remained at approximately 2800 mg/l in the 30 day unit, and it was not necessary to lower the alkalinity with hydrochloric acid, as was the case with glycine on a 30 day detention time.

Results

A temporary decrease in digestion rate caused the gas production to drop and the filtrate COD to rise, as shown in Figure 8, for 7.5 days. The cause, low alkalinity, was corrected and in two days operation was back to normal. The cellular nitrogen did not change significantly as noted from Figure 8. The removal efficiency was therefore based upon the filtrate COD concentration before the decrease in digester activity occurred instead of taking the weighted average of all filtrate COD values as done with the other cases.

Run VI - Nutrient Broth Substrate

Two units had been operating satisfactorily while being fed a substrate of proteose peptone on 5 and 30 day detention times. The digesters had been purged of foreign matter during previous operation of over 200 days. It was observed that a slight amount of precipitation of the proteose peptone took place in the digester. For this reason the substrate was changed to nutrient broth, which was determined to be soluble at the pH range found in a digester. Nutrient broth is very much like proteose peotone in composition. In fact, nutrient broth contains a large fraction of peptone (40%). No difficulty or slowdown occurred in digestion when the change from proteose peptone to nutrient broth occurred. Both digesters achieved equilibrium cellular nitrogen concentrations within eleven days after nutrient broth was first fed.

It became necessary to add HCl to maintain a neutral pH in the 30 day unit due to the build-up of ammonium bicarbonate alkalinity.

This resulted from the degradation of organic nitrogen present in the feed. The gas production curve reflects gas production surges after each addition of HCl.

Since nutrient broth contains a nitrogen source, and some of the essential trace elements, the addition of nutrient salts A and E was cut to one-third of the amount normally fed with all of the other substrates.

Results

The results of this run are shown in Table VII, Appendix Tables B-XIV, B-XV and Figure 9. A significant increase in the nitrogen requirement of nutrient broth was found over the amino acid substrates glycine and leucine. The variation of nitrogen requirement with detention period was only slight. For 5 and 30 day detention times, the corresponding nitrogen requirements were 7.5 and 5.8 mgN/gm COD.

TABLE VII

SUMMARY NUTRIENT BROTH DIGESTION DATA

Detention Time Days	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
5	130	22	87	75	90
30	126	104	89	58	82

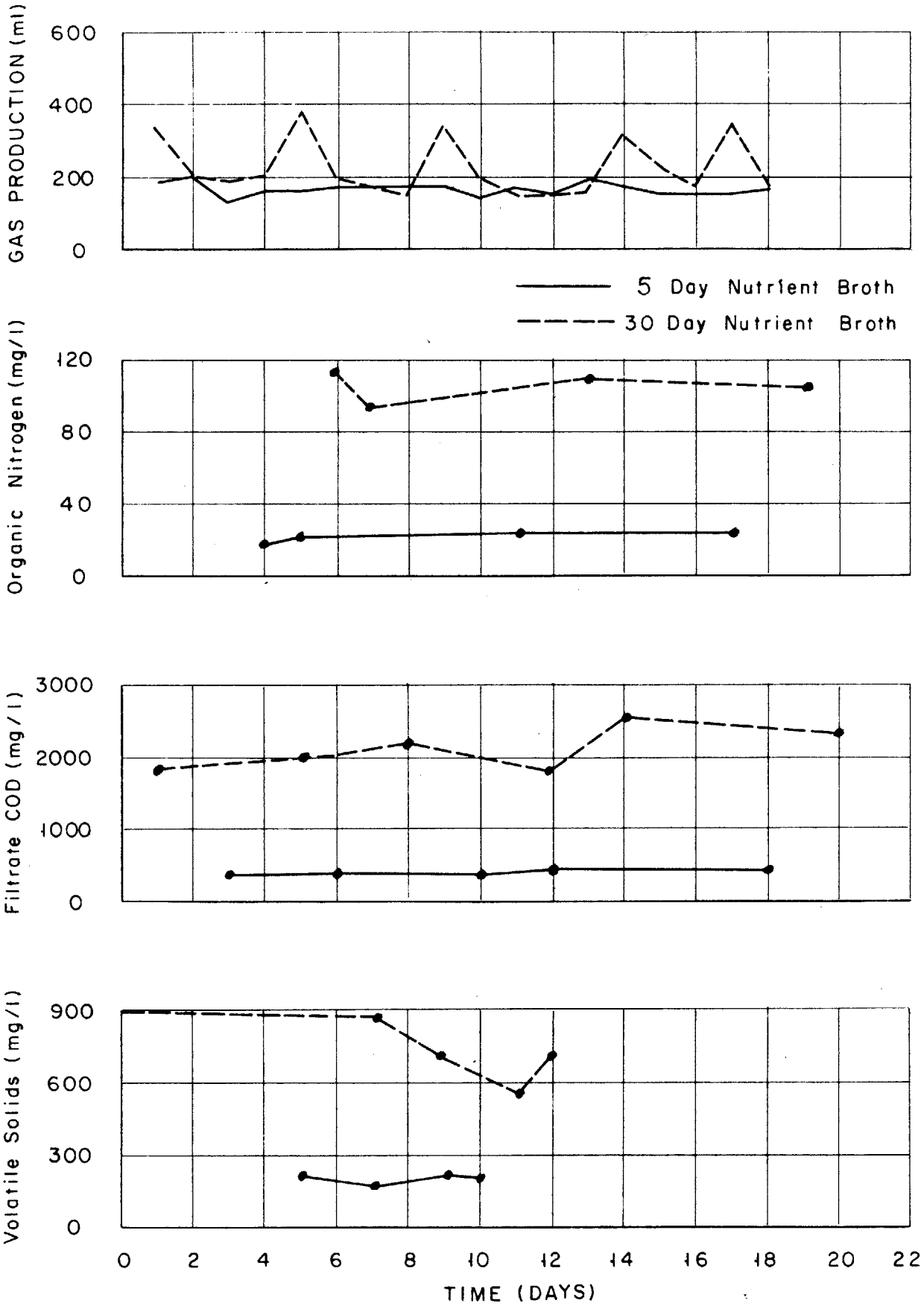


Figure 9 Nutrient Broth Digester Operation

Run VII - Glucose Substrate

Four digesters which had previously operated for 200 days on acetate were converted to a glucose substrate. The organic nitrogen concentrations in all digesters were approximately 50 mg/l when the glucose substrate feed was initiated. Acclimation required only four days in each case. All units were operated on 15 day detention periods for fifty days. They were then changed to 5, 10, 20 and 30 day detention periods. After 7 days, the 5 and 10 day detention period digesters reached equilibrium. A longer period of 14 and 25 days were required for steady state conditions to develop in the 20 and 30 day units respectively.

Results

Figure 10, Table VIII, and Appendix Tables B-XVI to B-XIX show the results of this run. An exceptionally high nitrogen requirement was shown by glucose. All of the detention times yielded values from 4 to 6 times as great as the corresponding nitrogen requirements for the amino and fatty acid substrates. It was found that approximately 40% of the glucose COD was converted into biological solids COD at the 5 day detention time. At the 30 day detention period, approximately 13% net conversion of the substrate COD to bacterial protoplasm as COD occurred.

The decrease in nitrogen requirements with increasing detention times was very marked as seen in Table VIII. With such a high rate of conversion of substrate to bacterial cells the resulting methane production was accordingly diminished.

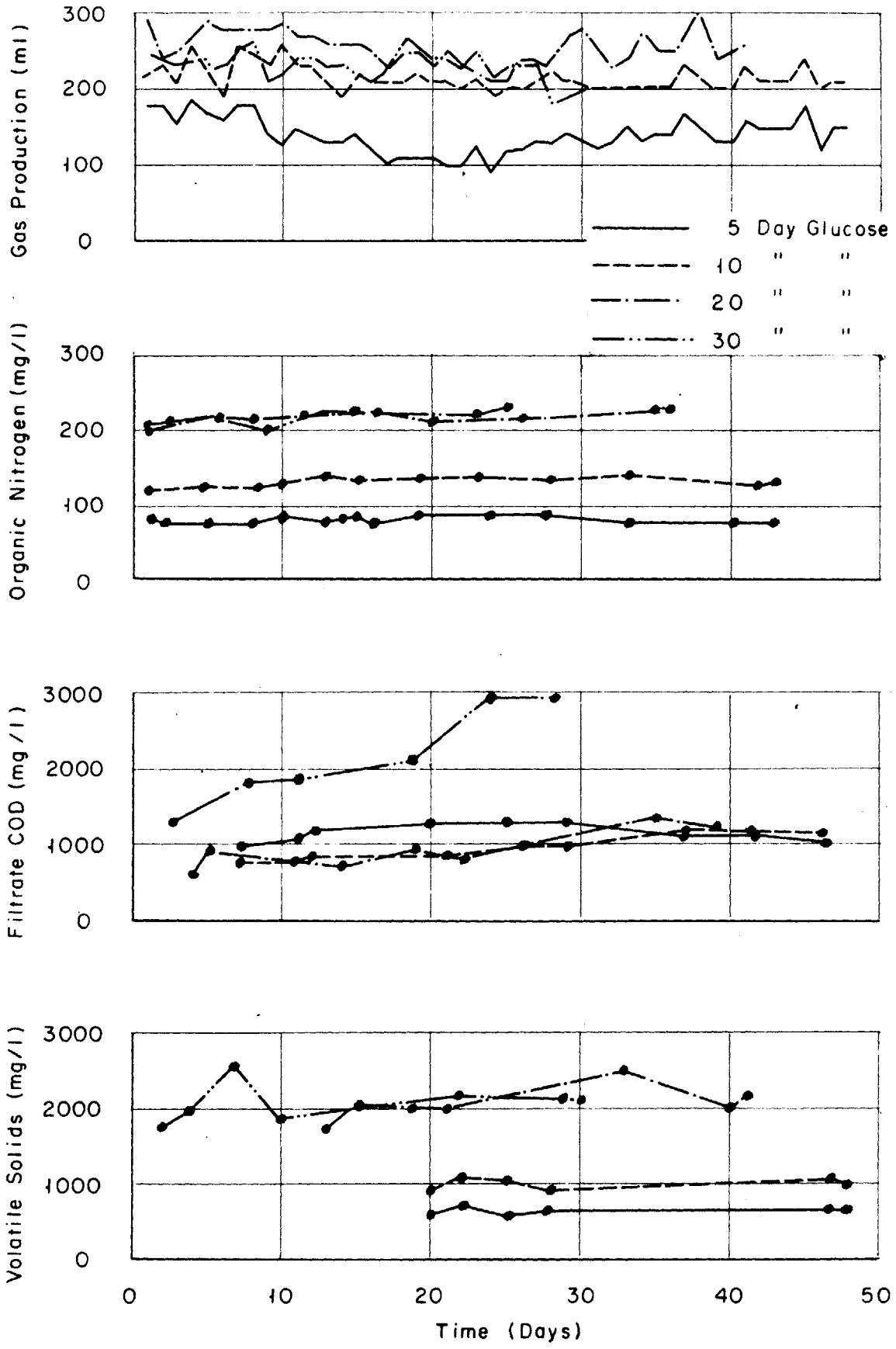


Figure 10 Glucose Digester Operation

TABLE VIII

SUMMARY OF GLUCOSE DIGESTION DATA

Detention Time Days	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
5	77	81	65	37	112
10	114	130	86	22	99
20	135	216	93	17	96
30	117	215	89	12	84

Run VIII - Starch Substrate

Digestion was initiated in these units by a mixed liquor previously fed acetate. All foreign material had been washed out while on the acetate feed and the organic nitrogen concentration was approximately 25 mg/l in both digesters. It required 60 days for acclimation of the organisms to the starch substrate. Both the 5 and 30 day detention time digesters were operated for 40 days on their respective detention periods before final data was recorded.

Starch is not readily soluble in water and tended to precipitate in the stock solution. This made it difficult to measure the proper amount of COD for the daily feed. In order to circumvent this trouble, a stock solution was prepared by heating the water to solubilize the starch. The proper feed strength was then made up for each digester in large quantities from the warm stock solution which was completely soluble. The ready-made feed solution was stored under refrigeration and no trouble was encountered with starch precipitation in the digesters.

Results

The results of this run are shown in Table IX, Figure 11 and Appendix B-XX and B-XXI. The assumption that anaerobic digestion necessarily yields low net synthesis was again disproved in the case of starch, as with glucose.

TABLE IX

SUMMARY OF STARCH DIGESTION DATA

Detention Time Days	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
5	49	60	50	36	101
30	152	293	97	15	98

Run IX - Cellulose Substrate

Digested sludge in the amount of 250 ml was used to initiate digestion. Operation of each digester on a 15 day detention time was practiced for 300 days. The digesters were then placed on 5 and 30 day detention periods. Only low rates of digestion could be maintained with intermittent feeding at the 5 day detention time. This was then extended to 7.5 days and equilibrium conditions were quickly achieved. Steady state conditions were achieved in the 30 day detention time unit in 14 days.

As in the case of oleate, the cellulose was insoluble and therefore volatile solids and microchemical analyses were not determined on the biological solids. The efficiency was based upon the COD of the mixed

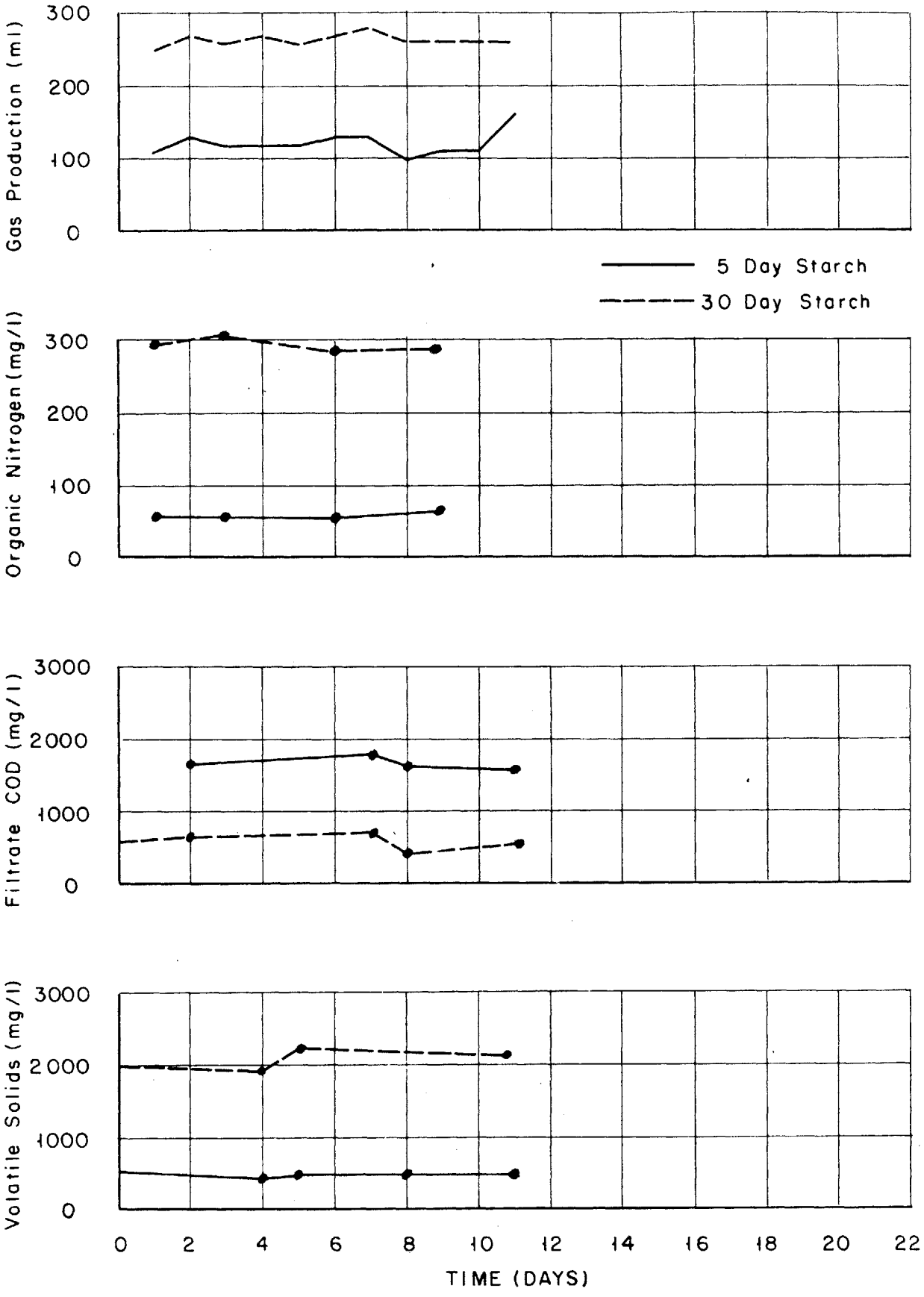


Figure 11 Starch Digester Operation

effluent minus the biological solids. Again, as with oleate, the COD of the biological solids was calculated to be 11.4 times the organic nitrogen concentration.

Results

The results of this run are shown in Figure 12, Table X, and Appendix Tables B-XXII and B-XXIII. The cellulose nitrogen requirements were much greater than the corresponding amino and fatty acid requirements, but not quite as great as those of glucose and starch. The slope of the nitrogen requirement curve vs retention time is comparable to the glucose and starch values.

The COD recovery for the 30 day unit was only 76% of the theoretical input. This can probably be traced to the inability to accurately measure and feed the cellulose slurry.

TABLE X

SUMMARY OF CELLULOSE DIGESTION DATA

Detention Time Day	Daily Methane Production ml	Cellular Nitrogen mg/l	Removal Efficiency %	Nitrogen Requirement mgN/gm COD	COD Recovery %
7.5	90	79	68	23	92
30	137	162	94	9	76

Summary of Results From All Runs

Figure 13 illustrates the effect of substrate and detention time on the nitrogen requirements for anaerobic digestion. From Figure 13, it is observed that some of the substrates exhibit similar nitrogen requirements. Glucose and starch which lie close together on the nitrogen

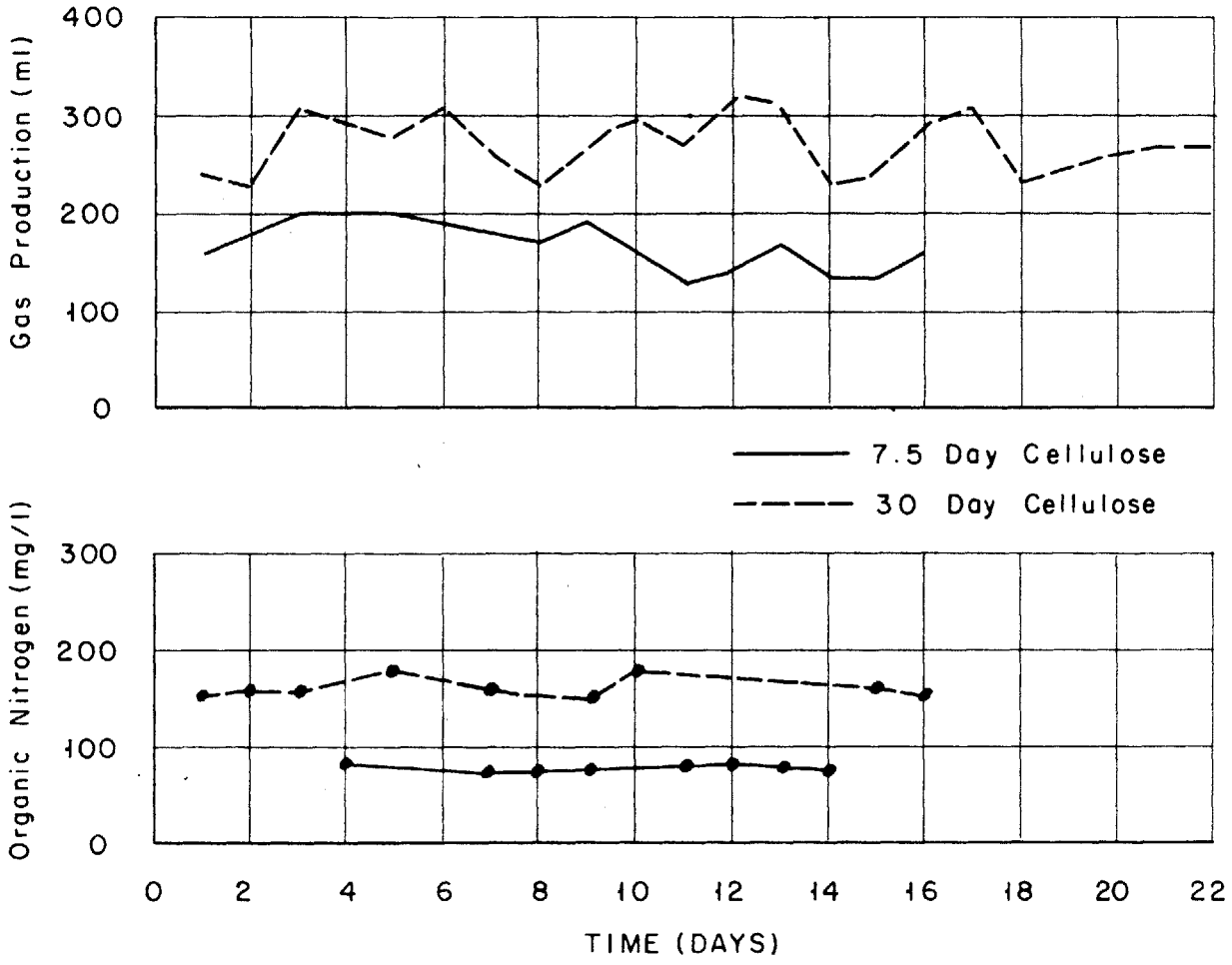


Figure 12 Cellulose Digester Operation

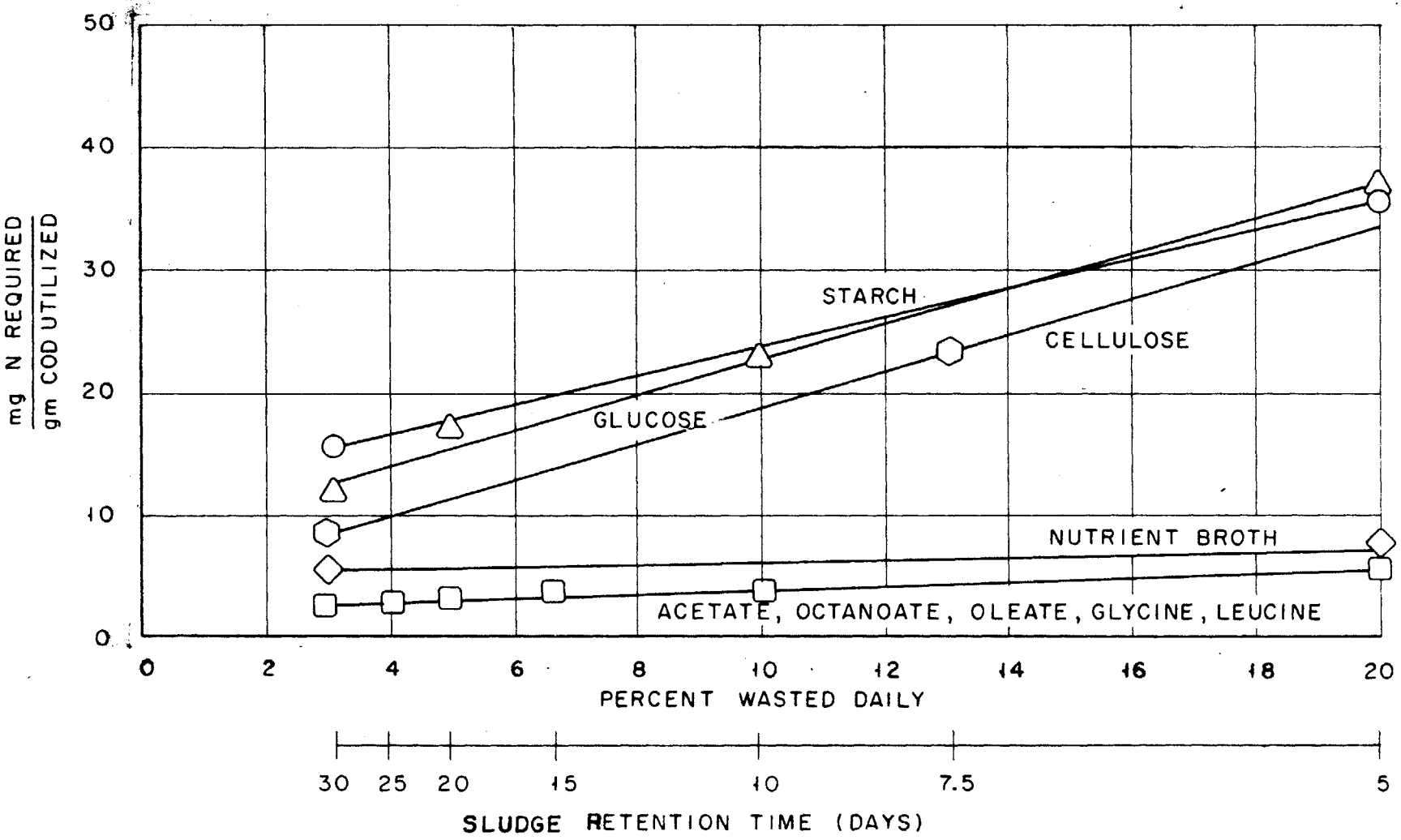


Figure 13. Nitrogen Requirements

requirement curve, also maintained approximately the same volatile solids and cellular nitrogen concentrations for corresponding detention periods. Likewise acetate, octanoate, oleate, glycine, and leucine exhibit similar nitrogen requirements, volatile solids concentrations, and cellular nitrogen concentrations in anaerobic digestion.

Chemical Formulation of Bacterial Protoplasm

Microchemical analyses of the biological solids responsible for the anaerobic digestion of all substrates, except the insoluble oleate and cellulose, were determined. The percentage composition of carbon, hydrogen, nitrogen, phosphorous, and ash are shown in Appendix D. An empirical chemical formulation was then calculated for the biological solids. Table XI is a compilation of all of these chemical formulations. The N/P ratio in the biological solids based on weight is given. Also, the COD/N ratio for the cell formulation is listed.

The average of all the empirical formulations in Table XI yields the formula $C_{4.8}H_{8.8}O_{2.9}N$. For simplicity the subscripts will be rounded off to the nearest whole number to yield the general empirical formula of anaerobic biological solids as found in this study:

$$C_5H_9O_3N$$

The COD/N ratio for this formulation is:

$$COD/N = 11.4$$

The cell weight/N ratio for this formula is:

$$\text{Cell weight}/N = 9.4$$

TABLE XI

CHEMICAL FORMULATION OF BACTERIAL PROTOPLASM
RESPONSIBLE FOR ANAEROBIC DIGESTION OF SUBSTRATES

Substrate	Detention Time Day	Empirical Formula C	H	O	N	P	Ash(%)	N/P Wt.Basis	COD/N
Acetate	5	4.9	11.3	3.5	1	0.03*	55.5	7	12.0
"	10	5.1	10.4	3.1	1	0.03*	35.4	7	12.4
"	15	4.9	9.4	2.9	1	0.03*	36.9	7	11.6
"	20	4.3	8.3	2.2	1	0.03*	32.4	7	10.4
"	25	5.4	9.4	3.4	1	0.03*	28.0	7	12.2
	30	5.0	11.0	2.7	1	0.03*	24.4	7	12.9
Octanoate	5	4.6	6.9	2.4	1	0.05	9.4	9	10.1
"	30	4.7	7.7	2.1	1	0.04	4.8	12	11.1
Oleate	30								
Glycine	5	4.8	7.6	3.0	1	0.06	7.7	8	10.2
"	30	4.9	9.0	3.0	1	0.11	10.9	4	11.2
Leucine	7.5	5.0	8.8	3.2	1	0.05	9.2	9	11.1
"	30	4.1	7.6	3.3	1	0.05	5.3	9	8.0
Nut.Broth	5	4.1	6.8	2.2	1	0.04	9.8	12	9.1
"	30	3.8	7.0	2.8	1	0.05	16.4	9	7.8
Glucose	5	4.8	8.3	2.5	1	0.17	20.1	3	11.6
"	10	5.1	8.5	2.5	1	0.14	14.0	3	12.0
"	20	5.9	10.1	3.2	1	0.18	11.1	2	13.8
"	30	4.9	10.2	3.1	1	0.21	26.1	2	11.8
Starch	5	5.0	8.7	2.9	1	0.09	6.2	5	11.5
"	30	5.3	9.1	2.5	1	0.06	5.7	8	12.6
Cellulose	7.5								
"	30								

*Phosphorous Values Determined on Solids From Separate Acetate Digester Utilizing 4800 mgCOD/l/day.

All of the values averaged together for the general empirical formula fell with ± 2 standard deviations of the mean. There was no statistical difference in any single formula.

The nitrogen to phosphorous ratio for the cells appears to be approximately 7. About this ratio is assumed in aerobic processes also. The low values for glucose may be attributed to the fact that these solids were washed in an acid water buffered at pH 3, while the others, excluding acetate, were washed in acid water buffered at pH 1. At pH3, perhaps not all of the precipitated phosphate salts were dissolved and thus a higher phosphorous concentration in the solids was obtained.

Biological Growth

The net conversion of substrate to biological solids is calculated in Table XII. The values in the column marked A/F, net synthesis in mg of solids/gm COD, are based upon the measured volatile solids concentration. The values in the last column, marked Net Synthesis Based on Nitrogen Requirement, are calculated from the nitrogen requirement data using the factor of 9.4 to convert cellular nitrogen to volatile solids by weight. Column A_n/F in Table XIII contains the values of cellular nitrogen accumulation per gm COD utilized which were multiplied by the factor of 9.4 to obtain volatile solids. The comparison of solids data calculated by these two methods reveals a relatively close agreement.

Since the nitrogen requirement and biological growth are related and show close agreement, the growth equation constants, c and k, can

TABLE XII

CONVERSION OF SUBSTRATE TO VOLATILE SOLIDS

Substrate	Detention Time Days	Volatile Solids mg/l	A Vol.Solids Accumulation per day mg	Removal Efficiency %	F COD Utilized per day gm	A/F Net Synthesis mg solids/ gmCOD	Net Synthesis Based on Nitrogen Requirement mg solids / gm COD
Octanoate	5	104	16	65	.325	49	48
"	30	492	12	96	.480	25	26
Glycine	5	78	12	83	.415	29	37
"	30	468	12	93	.465	26	27
Leucine	7.5	180	18	70	.350	52	54
"	30	400	10	95	.475	21	26
Nut.Broth	5	190	28	87	.435	65	71
"	30	700	18	89	.445	41	55
Glucose	5	620	93	65	.325	290	360
"	10	990	74	86	.430	170	220
"	20	1970	73	93	.465	160	160
"	30	2080	52	89	.445	120	110
Starch	5	500	75	50	.250	300	340
	30	2200	55	97	.485	110	140

be evaluated on the basis of either nitrogen requirements of volatile solids. There is less fluctuation in the nitrogen analysis than in the volatile solids analysis, so the growth equation constants were evaluated on the basis of nitrogen requirements as shown in Table XIII and Figures 14 and 15. The value of k was based on total cellular nitrogen. This is analogous to total mass. In aerobic studies, sometimes k is based on the active mass, or that fraction of total mass which is considered to be subject to endogenous respiration. The data are plotted in the form $A_n/F = c_n - kM_n/F$, which is the straight line equation form of the growth equation. The subscript, n, denotes nitrogen quantities instead of the usual volatile solids quantities. A_n is the cellular nitrogen wasted from the system each day, F is the COD utilized, and M_n is the cellular nitrogen present in the system. These three quantities are measurable. The value of c_n is the y intercept and k is the slope of such a plot.

The value of c_n thus obtained is in terms of mg nitrogen systhesized per mg COD utilized and must be multiplied by the cell weight/N factor of 9.4 to convert it to mg volatile solids synthesized/mg COD utilized. The value of k, endogenous respiration rate, has units of day^{-1} and applies equally to cellular nitrogen or volatile solids quantities.

From Figure 13, the amino and fatty acids appear to show similar nitrogen requirements. Glucose and starch appear to form a common group also. The growth constants for the amino and fatty acids are evaluated in Figure 14, while the glucose and starch constants are evaluated in Figure 15. The line of best fit in both cases was determined by the method of least squares.

TABLE XIII

EVALUATION OF GROWTH EQUATION CONSTANTS

Substrate	Detention Time (Days)	M_n Cellular Nitrogen (mg/l)	A_n Cellar Nitrogen Accumulation per day (mg)	Removal Efficiency	F COD Utilized per day (mg/l)	A_n/F Cellular Nitrogen Accumulation per COD Utilized	M_n/F Cellular Nitrogen per COD Utilized
Acetate	5	12	2.4	67	450	.0053	.027
"	10	23	2.3	93	620	.0037	.036
"	15	36	2.4	98	650	.0037	.055
"	20	40	2.0	99	660	.0030	.061
"	25	46	1.8	99	660	.0027	.070
"	30	53	1.8	99	660	.0027	.080
Octanoate	5	11	2.2	65	430	.0051	.026
"	30	54	1.8	96	640	.0028	.084
Oleate	30	53	1.8	95	640	.0028	.083
Glycine	5	11	2.2	83	560	.0039	.020
"	30	55	1.8	93	620	.0029	.089
Leucine	7.5	20	2.7	70	470	.0057	.042
"	30	54	1.8	95	640	.0028	.084
Nut.Broth	5	22	4.4	87	580	.0076	.038
"	30	104	3.5	89	600	.0058	.174
Glucose	5	81	16.2	65	430	.038	.188
"	10	131	13.1	86	580	.023	.225
"	20	216	10.8	93	620	.017	.350
"	30	215	7.2	89	600	.012	.360
Starch	5	60	12.0	50	330	.036	.182
"	30	293	9.8	97	650	.015	.451
Cellulose	7.5	79	10.6	68	460	.023	.172
"	30	162	5.4	94	630	.009	.257

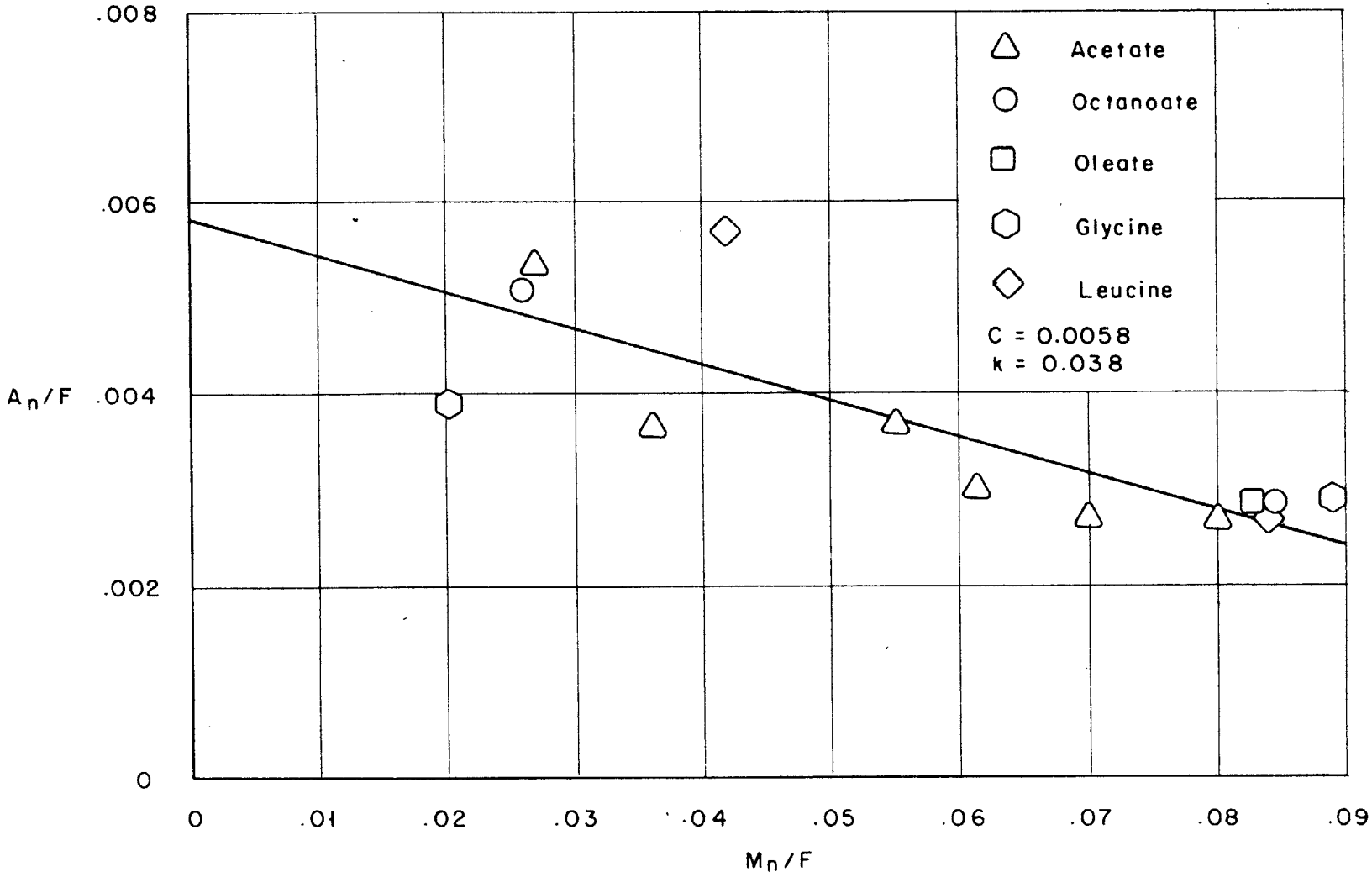


Figure 14 Evaluation of Amino and Fatty Acid Growth Constants

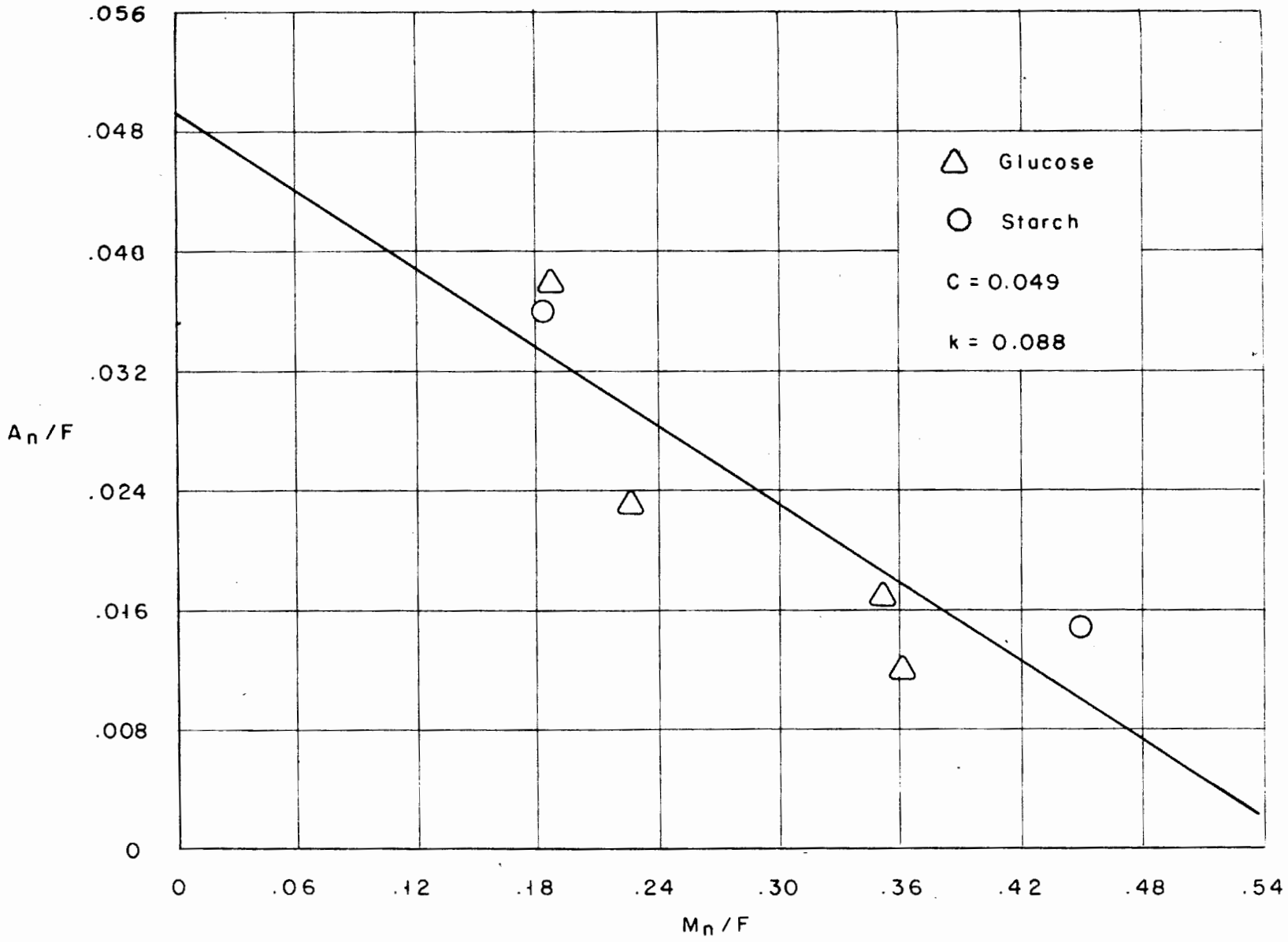


Figure 15 Evaluation of Glucose and Starch Growth Constants

The values for the amino and fatty acids were:

c_n = 0.0058 mg N/mg COD

c = 9.4 c_n = 0.054 # volatile solids/#BOD

k = 0.038/day

Growth Equation A = 0.054F - 0.038M

The values for glucose and starch were:

c_n = 0.049 mg N/mg COD

c = 9.4 c_n = 0.46 # volatile solids/# BOD

k = 0.088/day

Growth Equation A = 0.46F - 0.088M

Limited, but reliable data were available to make an evaluation of the growth equations for cellulose and nutrient broth. A tentative equation is given below for the two substrates.

Cellulose A = 0.4F - 0.1M

Nutrient broth A =.0.08F -.0.02M

B. Stimulation of Acetate Utilization in Anaerobic Digestion

In this phase of the investigation, four-six liter assay digesters operated on a 30 day detention time were used. All four units had been operating for one year on acetate or butyrate substrate. The contents were mixed continuously by recirculation of the digester gases through the liquid. The feed consisting of acetic acid, nutrient salts, as recommended by the literature, and a sufficient amount of sodium acetate to maintain the alkalinity at approximately 4000 mg/l as $CaCO_3$, was applied on a continuous basis.

The volatile acids concentration was maintained above 1000 mg/l, except for an occasional instance when increased stimulation would decrease it temporarily. Nutrient salts were added in excess of the amounts actually needed. Here again, there were occasions when it was found that nutrient salts were deficient.

By operating the digesters with excess substrate and nutrients, only growth factors were limiting. Thus, any stimulation could be attributed to the compounds added to the digesters.

A great number of compounds were tested. At the start, the compounds to be assayed were chosen at random. After several successes, it became possible to choose more reasonably those compounds likely to stimulate acetate utilization.

Figures 16, 17, 18, and 19 graphically show the effects of additions of the many compounds to each of the four digesters for the period of operation in which results, pertinent to this thesis, have been obtained. Appendix C contains the data from which the figures were plotted.

Record of Digester I

The addition of various substances to the digester and the resulting effect on acetate utilization, as shown in Fig.16 is treated here. On day 2, the addition of 200 mg $CoCl_2 \cdot 6\ H_2O$ did not affect acetate utilization. However, the subsequent addition of 100 mg l(-)proline and 100 mg l-hydroxyproline on day 8 increased the digestion rate from 700-1600 mg/l/day. An additional 200 mg of proline on day 20 brought the rate to 2000 mg/l/day. The effect of 40 mg hydroxyproline on day 31 was nil. The organic nitrogen concentration on day 9 was 36 mg/l with 313 mg/l ammonia nitrogen.

On day 36, 100 mg potassium iodide showed no effect.

Nutrients proved to be limiting at this stage, since additions of nutrient A and/or E on days 38, 52, and 57 brought the rate of acetate utilization to 3500 mg/l/day. On the 55th day, organic nitrogen was 96 mg/l and volatile solids were 680 mg/l.

Benzimidazole was added in the amount of 75 mg on the 62nd day. An increase from 3500-4300 mg/l/day in the digestion rate resulted in four days. However, an overfeeding of acetate increased the volatile acid concentration to 2800 mg/l and dropped the pH to 6.60. One g/l of sodium bicarbonate was added as $CaCO_3$ to raise the pH to 6.80.

Record of Digester II

Figure 17 shows the acetate utilization for the digester. The acetate utilization rate had fallen to 300 mg/l/day in this unit. On the 9th day, the daily addition of 200 mg $FeCl_3$ was initiated. The rate had risen to 900 mg/l/day by the 38th day. Mixture A, added

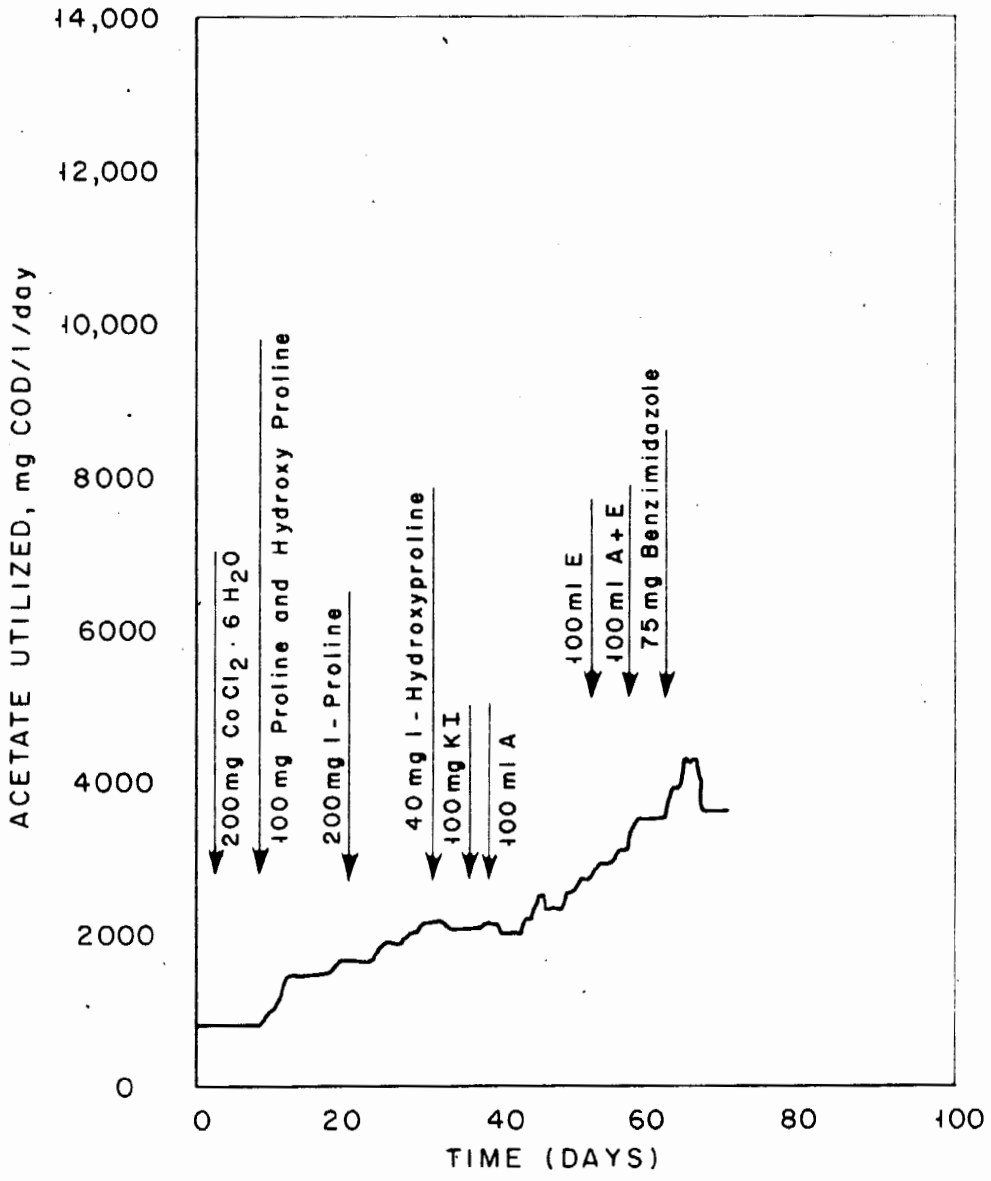


Figure 16 Acetate Utilization - Digester I

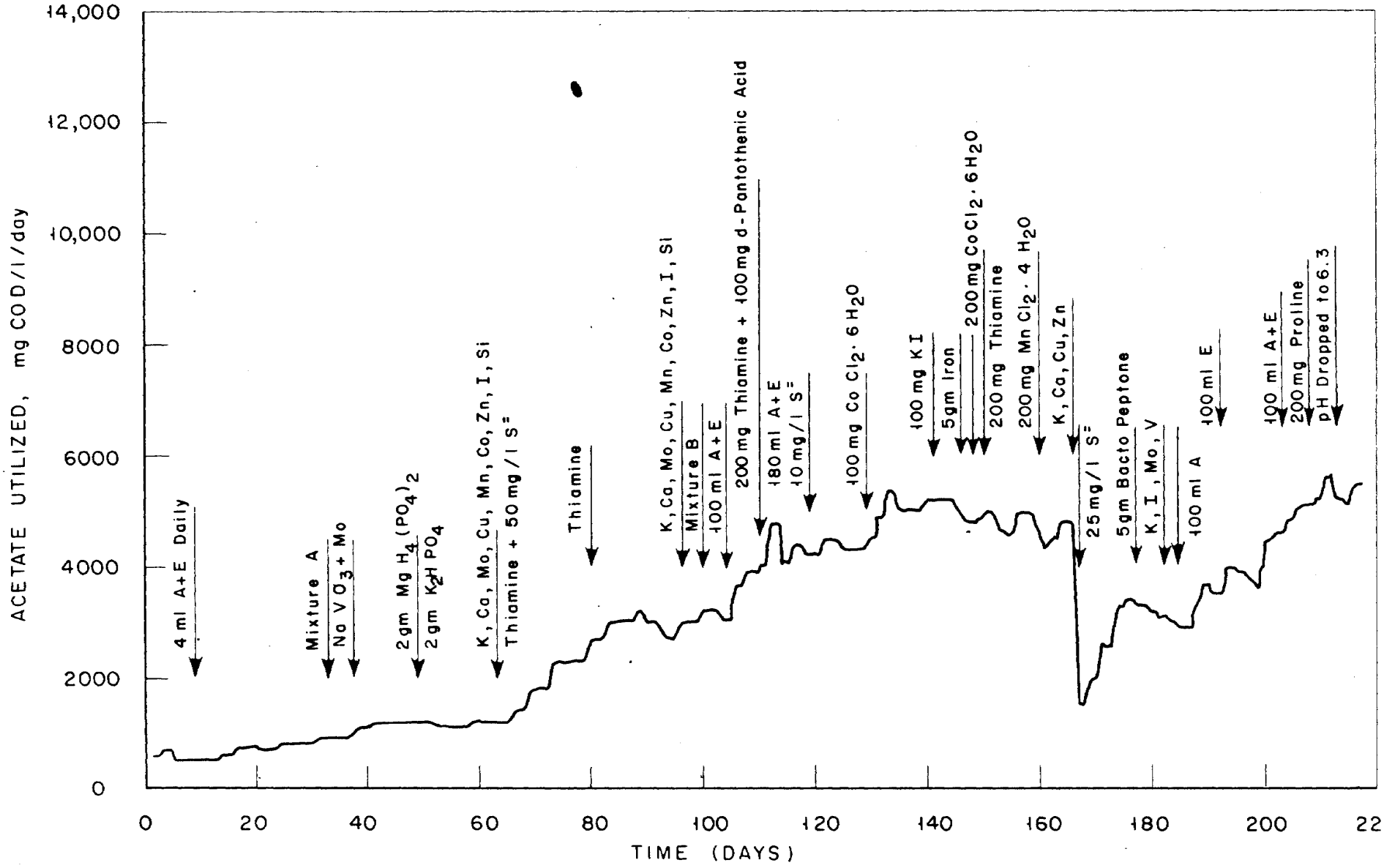


Figure 17 Acetate Utilization – Digester II

on day 33, was a trace amount (1-5 mg) of various organics listed on page 236 in Appendix C. No stimulation resulted from Mixture A.

Sodium vanadate and molybdenum powder in trace amounts added on the 38th day appeared to increase acetate utilization slightly from 900 - 1200 mg/l/day.

The addition of 2 grams each of $MgH_4(PO_4)_2 \cdot 3H_2O$ and K_2HPO_4 on the 49th day showed no stimulation.

A significant addition was made on day 63. Trace amounts of K^+, Ca^{++}, Mo^{++}, Cu^{++}, Mn^{++}, Co^{++}, Zn^{++}, I^-, and Si^{++} along with thiamine and 50 mg/l S^{--} were added to the digester. The acetate utilization rate was raised from 1200 to 2700 mg/l/day. The organic nitrogen concentration was 48 mg/l and the ammonia nitrogen concentration was 120 mg/l at the time of this addition on day 63. An additional trace of thiamine on day 80 failed to increase the digestion rate. One to five mg of the metallic ions which were added on the 63rd day were again added on the 96th day with no significant increase.

Mixture B, composed of trace quantities of a great number of organics shown on page 237 in Appendix C, showed no stimulation when added on the 100th day.

The addition of nutrient salts A and E was increased from 2/3 ml/l/day of both A and E to 1 1/3 ml/l/day when the ammonia nitrogen present in solution was found to be zero on day 104. The organic nitrogen content was 150 mg/l at this time. Slug additions of a total of 280 ml of A and E were made in the next six days to supply sufficient nutrient salts.

On the 106th day 200 mg thiamine and 100 mg d-pantothenic acid-calcium salt were added. The acetate utilization rose abruptly from 3900 to 4800 mg/l/day within three days. A temporary drop in volatile acid concentration to 380 mg/l caused a corresponding drop in digestion rate. However, the rate eventually reached 4500 mg/l/day.

Sulfide addition - 10 mg/l - on the 124th day effected no stimulation. The organic nitrogen concentration was 188 mg/l and the ammonia nitrogen concentration was 320 mg/l at this time.

On the 129th day, 100 mg $CoCl_2 \cdot 6\ H_2O$ raised the digestion rate from 4300 to 5300 mg/l/day.

From day 141 to 165 the addition of the following compounds resulted in no stimulated digestion: 5 g iron powder, 200 mg $CoCl_2 \cdot 6\ H_2O$, 200 mg thiamine, and 200 mg $MnCl_2 \cdot 4\ H_2O$. Nutrients A & E were added at 2 ml/l/day starting on the 142nd day.

Digestion was vitally hindered by addition of approximately 1-3 mg/l of each of four metallic ions - K^+, Ca^{++}, Cu^{++}, and Zn^{++} on day 166. Twenty five mg/l S^{--} was added the next day to precipitate any heavy metals left in solution.

By the 177th day acetate utilization was back up to 3300 mg/l/day. Addition of 5 gm Bacto Peptone on this day had no effect.

One to five mg of K^+, I^-, Mo^{++}, and V^{++} on the 182nd day had no stimulatory effect.

Nutrient salts were found to be limiting at this time, since addition of 200 ml each of A & E increased digestion rates from 3200 to 5200 mg/l/day in the period from day 184 to 207. The organic nitrogen concentration was 198 mg/l and the volatile solids concentration was 1300 mg/l on the 201st day.

Proline in the amount of 200 mg brought the digestion rate up slightly from 5200 to 5700 mg/l/day. A drop in pH to 6.30 due to overfeeding of volatile acids temporarily hindered digestion. One g/l of sodium bicarbonate alkalinity as $CaCO_3$ raised the pH from 6.30 to 6.70. The rate then again reached 5500 mg/l/day.

Record of Digester III

The inclusion of $FeCl_3$ with the daily additions of nutrient salts to the digester was practiced for the entire period shown in Figure 18. The addition of 100 mg of thiamine on the 3rd day slowly increased the digestion rate from 700 to 1200 mg/l/day. Whereupon, 200 mg $CoCl_2 \cdot 6H_2O$ on day 20 brought the rate to 3600 mg/l/day. Two ml/l of both nutrients A & E were being added daily.

The period from the 47th day to the 60th day gave no stimulation upon addition of: 200 mg thiamine, 200 mg $CoCl_2 \cdot 6H_2O$, and 200 mg d-pantothenic acid.

Nutrients were found to be limiting on day 60, and an additional 300 ml of both A & E were added in the next 20 days to supplement the daily addition of 2 ml/l/day. This supplementation of nutrients A & E increased acetate utilization rates from 3300 to 5500 mg/l/day.

On the 86th day, 200 mg of glycine abruptly increased the digestion rate from 5500 to 6500 mg/l/day. The pH dropped to 6.30, due to overfeeding of acetate, and curtailed digestion slightly. Sodium bicarbonate was added in sufficient quantity to increase the alkalinity 1000 mg/l as $CaCO_3$ and raise the pH to 6.70. The rate then again reached 6300 mg/l/day.

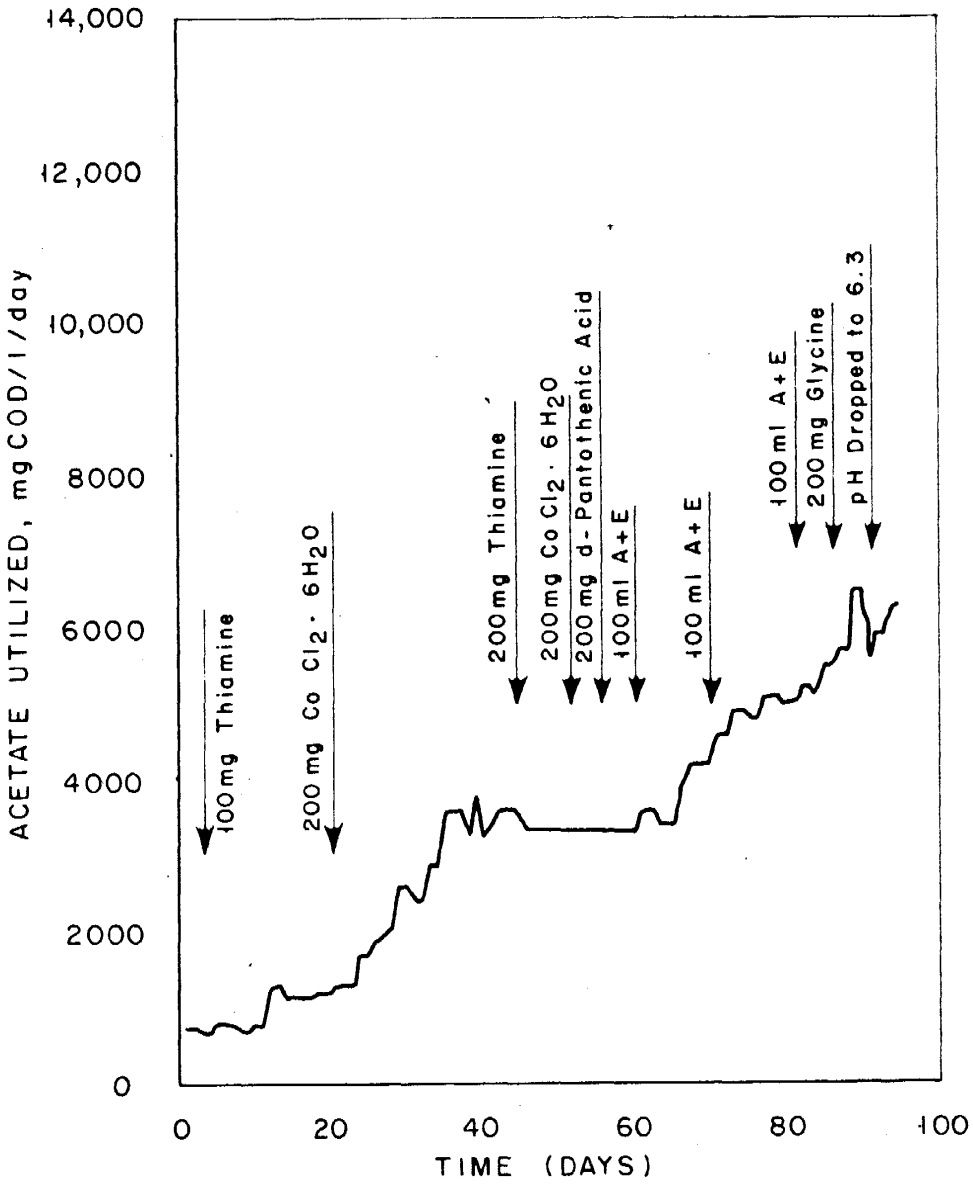


Figure 18 Acetate Utilization – Digester III

Record of Digester IV

The record of acetate utilization for this unit is shown in Figure 19. An excess of iron powder was added to this unit and enabled the maintenance of digestion rates at about 1000 mg/l/day continuously. The addition of 2g each of $MgH_4(PO_4)_2 \cdot 3H_2O$ and K_2HPO_4 had no effect when added on the 31st day. On the 46th day, the organic and ammonia nitrogen concentrations were 51 and 85 mg/l respectively.

Additional iron powder and 50 mg/l $S^=$ on the 53rd day did not appreciably affect digestion. Nor did the addition 1 to 5 mg of the metallic ions K^+, Ga^{++}, Mo^{++}, Cu^{++}, Mn^{++}, Co^{++}, Zn^{++}, I^-, and Si^{++} have any effect when added on day 79.

Thiamine was added on the 83rd and 86th days to stimulate digestion from 1200 to 2600 mg/l/day. At the same period 280 ml of both A & E were added in addition to the daily supplementation of 8 ml/digester/day in order to insure sufficient nutrients. The organic and ammonia nitrogen concentrations were 82 and 300 mg/l on the 98th day.

The digestion rate abruptly fell from 2600 to 400 mg/l/day on the 108th day by the addition of trace quantities of Ca^{++}, Mo^{++}, Cu^{++}, and Mn^{++}. Four liters of the mixed digester contents were wasted and the contents diluted to six liters again with tap water and nutrients. This procedure was to dilute concentrations of toxic substances in the digester. No oxygen was admitted to the digester when the wasting of four liters of the contents took place.

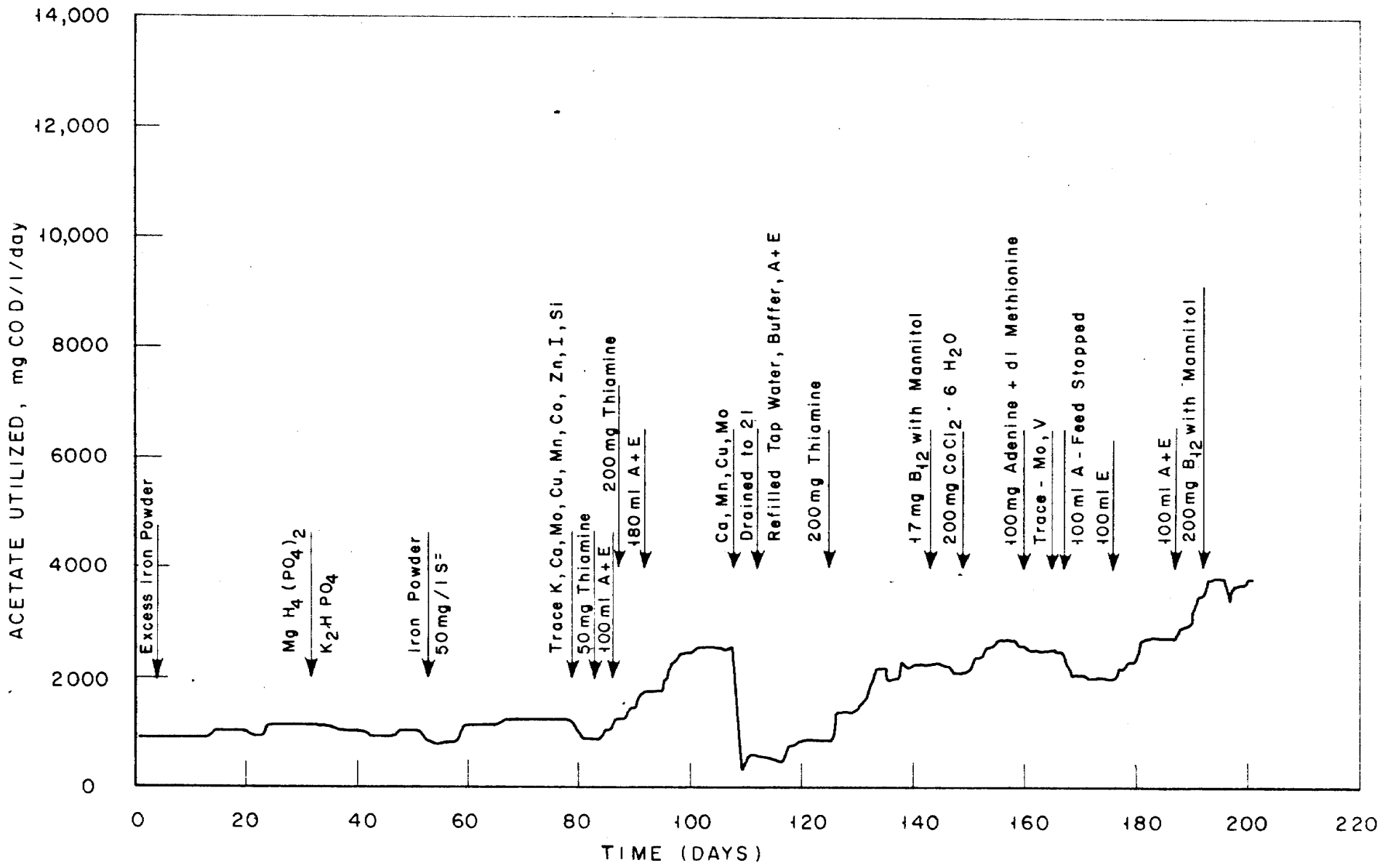


Figure 19 Acetate Utilization – Digester IV

On the 125th day, daily additions of nutrients A & E were increased from 1 1/3-2 ml/l/day. The organic and ammonia nitrogen were 68 and 235 mg/l respectively on the 138th day.

Vitamin B_{12} with an intrinsic factor - mannitol - was added in the amount of 17 mg on the 144th day. No stimulation resulted.

Stimulation was noted upon addition of 200 mg $CoCl_2 \cdot 6H_2O$ on the 150 day. The utilization rate went from 2200 to 2800 mg/l/day

No effect was noted upon addition of 100 mg adenine hydrochloride and 100 mg d l methionine on the 161st day. Nor did a trace of Mo^{++} and V^{++} and 100 ml A increase the acetate utilization rate when added on days 165 and 167. However, 100 ml E added on the 176th day and another supplementation of 100 ml A & E on day 187 increased the rate from 1900 to 3500 mg/l/day. The volatile solids on day 186 were 500 mg/l.

Vitamin B_{12} with mannitol-200 mg - was added on the 192nd day, but produced no stimulation in the acetate utilization rate.

Results of Preliminary Studies on the Effect of Iron on Acetate Digestion

Iron exhibited a very pronounced stimulation in some of these studies. In one instance 5 one liter digesters were being operated identically on an acetate substrate, nutrient salts recommended in the literature, and tap water for dilution to the proper volume for feed at 15 day detention times. Nine detention times had passed since initiation of digestion by 250 ml of digested sludge, thus leaving only minute amounts of the original seed in the digesters. Acetate

utilization had dropped to rates less than 100 mg/l/day and the volatile acid concentrations were approximately 2000 mg/l in all units. The volatile acid concentration would not appreciably decline even when the daily feed was omitted. Addition of iron powder immediately increased acetate utilization and the volatile acids concentration decreased to about 400 mg/l in all digesters.

Three one liter digesters were batch fed on 15 day detention times. Nutrient salts were added with the feed and the volatile acids concentration maintained above 1000 mg/l. The contents were mixed once a day. The concentration of iron in the feed was 7, 21, and 35 mg/l as iron. Acetate utilization rates in the units fed 21 and 35 mg/l were relatively constant at 1000 mg/l/day, while the unit receiving 7 mg/l of iron maintained only 600 mg/l/day digestion rates. It appeared that for digestion rates of 1 g/l/day, the required iron concentration in the feed was 7 to 21 mg/l.

Stimulatory Compounds

The synergistic effect of combinations of stimulatory substances is noted in all digesters, but chiefly in Digester III, Figure 18 where a maximum acetate utilization rate of 6500 mgCOD/l/day was achieved.

The following six substances were found to be stimulatory to acetate digestion. An instance is given in which addition of each compound by itself to one of the digesters resulted in stimulation.

Iron - enabled digestion rates to be maintained at approximately 1 g/l/day continually as noted in Figure 19 of Digester IV. Otherwise, the utilization rate would drop off to zero as the contents were washed out by daily feeding and withdrawal. Iron powder or $FeCl_3$ were both able to produce this stimulation.

Thiamine - addition of 200 mg to Digester IV, Fig. 19 on day 125 increased acetate utilization from 900-2300 mg/l/day.

Cobalt - stimulation from 1200-3600 mg/l/day of acetate resulted after addition of 200 mg $CoCl_2 \cdot H_2O$ to Digester III, Fig. 18 on day 20.

Proline - Digester I, Fig. 16 showed increase from 700-2000 mg/l/day after adding 100 mg on day 9.

Glycine - addition of 200 mg on day 86 of Digester III, Fig. 18 increased acetate utilization rate from 5500 to 6500 mg/l/day.

Benzimidazole - 75 mg added to Digester I, Fig. 16 on day 62 resulted in stimulation from 3500 to 4300 mg/l/day.

VI. DISCUSSION OF RESULTS

A. Biological Nitrogen and Phosphorous Requirements and Growth in Anaerobic Digestion

The results of this phase of the investigation have elucidated the actual biological response to the anaerobic decomposition of each of the three classes of substrates studied. The most significant finding during this phase of the research was the discovery of the exceptionally high net synthesis of carbohydrates which occurs at short sludge retention periods of 5 and 7.5 days. It was found that 30 to 40% of the carbohydrate COD was converted to biological solids COD during anaerobic digestion at short sludge retention times. The corresponding conversion of substrate COD to cellular COD for the fats and proteins class was only 6-8% at 5 days.

In the past, this information on net synthesis and related nitrogen and phosphorous requirements has been obscured by the heterogeniety of the wastes usually encountered. In the case of domestic sewage sludge, digestion of the solids is only approximately 60% complete due to the presence of a multitude of complex organics which are very difficult to degrade. The residue of organics, not decomposed masks the effect of any increase in biological solids. Excess nitrogen is also present to maintain adequate growth, so nitrogen supplementation of domestic sewage is not a point of consideration. These conditions, plus the fact that net synthesis rates for anaerobic decomposition of substrates in the fats and proteins class have been shown to be very low might have led to Buswell's (7) statement that in

the majority of cases the substrate is entirely converted to methane and carbon dioxide.

Another barrier to obtaining this information in the past has been the difficulty experienced in sustaining satisfactory digestion with a very simple substrate, for example winery wastes (54). The requirements of the methane bacteria have not been clearly defined and so most cases of satisfactory digestion have been on complex substrates which contained practically all growth factors and trace elements. Satisfactory digestion was achieved on the simple and clearly defined feeds used in this study.

Nitrogen Requirements

The effect of substrate and detention time is shown graphically in Figure 13. It is observed that the carbohydrate substrates exert a much greater nitrogen requirement for anaerobic decomposition than substrates in the fats and proteins class. This observation has not been reported in the Sanitary Engineering literature and should elicit a note of caution from those responsible for process design of waste treatment facilities. Four disadvantages result from high nitrogen requirements:

1. Cost of nitrogen supplementation to nitrogen deficient wastes is increased.
2. A higher conversion of COD from substrate to biological solids results instead of the desired conversion of substrate to methane and carbon dioxide.
3. Additional sludge disposal facilities must be maintained to handle the increased sludge production.

4. Lower methane production is concomitant with higher net synthesis of substrate.

Thus, the designer must realize the additional operating and capital costs involved in treatment of a waste with a high nitrogen requirement.

Figure 13 reveals a very marked decrease in nitrogen requirements for carbohydrates as the retention time is increased, due to conservation of nutrients as a result of endogenous respiration. The designer can exploit this characteristic by providing for sludge recovery and recycle in order to obtain a greater sludge retention time. In these studies, the hydraulic detention time and the sludge retention time were identical since the effluent was a completely mixed sample of the digester contents. However, it is possible to maintain a short hydraulic detention time and a long sludge retention by retaining the biological solids in the digester through their recovery from the effluent and recycle back to the digester. Two advantages are thus achieved:

1. Short hydraulic detention times result in smaller digestion tank volumes which require less capital investment.

2. Long sludge retention times result in lower nitrogen requirements with the lower capital and operating costs involved therewith.

The nitrogen requirement for cellulose appears to be less than that for glucose and starch at all retention times. It is a known fact that the organisms responsible for cellulose decomposition are unique from the class of organisns which decompose starch and glucose to acids. This may be the reason for the difference in nitrogen requirements for cellulose as opposed to starch and glucose. The same rate of decrease in nitrogen

requirement with increase in detention period is noted for cellulose, starch, and glucose.

Nutrient broth exhibited a nitrogen requirement less than cellulose and greater than the amino and fatty acids. The hypothesis is given that this additional synthesis occuring with nutrient broth above the synthesis resulting from the pure amino acids, glycine and leucine, is mainly attributed to the ready availability of many compounds present in nutrient broth for incorporation into bacterial protoplasm. Many amino acids required for cell protein are present in nutrient broth and require little modification and energy input for cell synthesis. Thus a given amount of biological solids can be synthesized with less expenditure of energy from a substrate which contains many partially synthesized raw materials, than if all materials must be synthesized from a pure substrate. The net result is greater synthesis per unit substrate fed for such a feed of varied organics as nutrient broth.

Another possibility exists when two or more different amino acids are used as substrates. One amino acid can act as a hydrogen donor and become oxidized, while the other can act as a hydrogen acceptor and becomes reduced. Perhaps a similar phenomena occurred with nutrient broth and an additional energy yield was obtained by the bacteria.

Another consideration with nutrient broth is that there would be amino acids present which contained ring structures. The energy obtained from the decomposition of these amino acids was not determined in this study, only the energy from the decomposition of the straight chain amino acids, glycine and leucine.

All of the nitrogen requirement values for the amino and fatty acids appear to be grouped closely to a common curve as shown by Figure 13. The structure of the amino acid compounds is similar to that of the fatty acids after deamination of the amine group from the amino acids. This would tend to yield comparable nitrogen requirements for the two groups. The effect of sludge retention time on the anaerobic decomposition of the amino and fatty acids was to reduce the nitrogen requirement of approximately 5 mg N/gm COD at a 5 day sludge retention time to 2.8 mg N/ gm COD at a 30 day sludge retention time.

It is of note that nitrogen requirements obtained from continuously fed systems operating on an acetate substrate at a 30 day retention time were significantly less than corresponding values from a 30 day batch fed system. Ten values were calculated from the data in Appendix Tables C-I to C-IV. These values ranged from 1.1 to 1.7 mg N/gm COD as compared to 2.8 mg N/ gm COD for the batch fed 30 day digesters. This is probably due to the "feed and starve" cycle upon which the batch fed systems were operated.

The Constant COD and the COD Reduction Stages

It was shown in the theoretical considerations that theoretically one mole of glucose can be assumed to break down to three moles of acetic acid with no net COD reduction. Likewise, when one mole of glucose is broken down to two moles of ethanol, no COD reduction is accomplished. The stage in which the COD is reduced is when methane is formed and leaves the solution as a gas.

The ultimate BOD of the waste is correspondingly reduced only in the methane production stage or COD reduction stage of digestion. However, the 5-day BOD may appear to increase during the constant COD stage when the complex organics are decomposed to the simple organics, since these simple organics are oxidized more readily than the complex organics.

The essence of biological waste treatment is reduction in the oxygen demand of the waste. For this reason, no direct benefit is gained from the constant COD stage of digestion. Yet, this is the stage which appears to exhibit the greatest nitrogen demand, with certain wastes such as carbohydrates. Also, with carbohydrates, the greatest fraction of sludge production results from the constant COD stage which likewise is responsible for

the decrease in methane production.

Bacteria receive the energy to maintain their life processes by oxidation-reduction reactions. Aerobically, oxygen is reduced while serving as a hydrogen acceptor; anaerobically, organic matter can serve as the hydrogen acceptor. The unique characteristic of the COD reduction stage of digestion is that carbon dioxide can serve as a hydrogen acceptor, in which case it is reduced to methane. This methane goes off as a gas with a resultant decrease in the COD in the waste.

In the constant COD stage, a portion of the substrate is oxidized to provide energy for reduction of the remaining fraction. For example, with glucose, one anaerobic reaction which may occur during the constant COD stage is as follows:

$$C_6H_{12}O_6 = 2CO_2 + 2CH_3CH_2OH$$

Here, one portion of the molecule is oxidized to carbon dioxide, while the other portion is reduced to ethyl alchohol.

In the constant COD stage, the bacteria responsible are not able to reduce some compounds further than their present state. For instance, acetate can not be reduced further in the constant COD stage. Only in the COD reduction stage can the volatile acids successfully be degraded.

Since the complex material is degraded to the simpler organics such as the volatile acids with no net COD reduction in this constant COD stage, these simpler organics remain to be degraded further. In the COD reduction stage, a separate population of methane producing bacteria, which is apparently relatively proportional to the COD of the waste, degrades these simpler organics. Since all substrates in this study were fed at the same rate per

day, based on COD, all digesters should have thus contained comparable populations of methane organisms required in the COD reduction stage.

Attention is now drawn to Figure 13 again. The nitrogen requirement for the anaerobic decomposition of acetate is essentially the same as for octanoate, oleate, glycine, and leucine. From this observation, the hypothesis is drawn that the population of the COD reduction stage of digestion is responsible for approximately the entire decomposition of all these substrates.

Next, the carbohydrates would likewise require the same population in the COD reduction stage as acetate since the feed COD's are equal. The hypothesis is then drawn that the difference between the carbohydrate nitrogen requirement minus the acetate nitrogen requirement for any one retention time is attributed to the population in the constant COD stage.

It would not be feasible to starve a system treating carbohydrates anaerobically of nutrients in an attempt to restrict the large amount of synthesis due to the population in the constant COD stage, since the population in the COD reduction stage would be restricted at the same time and thus digestion would be restricted.

Synthesis and Free Energy

The glucose and starch substrates yielded approximately 7 times the amount of synthesis as acetate at a 5 day retention time. At the 30 day retention time, the ratio was reduced to 5 due to the higher rate of endogenous respiration of carbohydrates as compared to acetate. The corresponding ratio of the change in free energy per gram of COD for glucose and acetate, with both substrates going to methane and carbon dioxide, is as follows as

given in the theoretical considerations:

Glucose -0.52 k cal/gm COD

Acetate -0.082 k cal/gm COD

Free Energy Ratio = -0.52/-0.082

= 6.4

Thus, the synthesis ratio of 5 to 7 is compared to the free energy ratio of 6.4. The close agreement of these two ratios is perhaps misleading. A more exact comparison is made on the basis of net synthesis per gram of COD catabolized. At 5 days retention time, the glucose and starch convert approximately 0.37 mg N/mg COD removed x 11.4 mg cell COD/mg N = 42% of the substrate COD to COD in biological solids. Accordingly, the acetate yields 0.053 mg N/mg COD removed x 11.4 mg cell COD/mg N = 6% conversion at 5 days. The yield of net synthesis ratio per unit of COD catabolized is:

42/58 = .73 mg synthesized/mg glucose COD catabolized

6/94 = 0.064 mg synthesized/mg acetate COD catabolized

Therefore, the ratio of glucose synthesized to acetate synthesized per gram of COD catabolized is:

0.73/0.064 = 11.4

At a 5 day retention period 11.4 times as much synthesis of glucose per gram of substrate catabolized results as compared to acetate with the corresponding change in free energy ratio of only 6.4.

At 30 days retention time, the yield of net synthesis ratio is as follows:

Glucose 0.140 mg N/mg COD removed x 11.4 mg cell COD/mg N = 16%

Acetate 0.028 mg N/mg COD removed x 11.4 mg cell COD/mg N = 3.2%

Glucose 0.16/.84 = 0.19 mg synthesized/mg COD catabolized

Acetate 0.032/.968 = 0.033 mg synthesized/mg COD catabolized

Therefore the ratio of glucose synthesized to acetate synthesized per gram of COD catabolized is:

$$.19/.033 = 5.8$$

This ratio of 5.8 at 30 days retention time compares well with the free energy ratio of 6.4.

Thus, the free energy available in a reaction yielded qualitative, but not quantitative indications of the net synthesis to be anticipated in these studies. De Moss, **et. al.** (10) reported also that free energy is not a reliable indicator of growth.

Phosphorous Requirements

The results of the microchemical analyses reveal a N/P ratio which varies from 2 to 14. The low values were obtained for samples which were washed in acid water buffered at pH 3 with phosphoric acid. After centrifuging, the acid water was decanted and the cells were washed in distilled water, centrifuged, and decanted. It was decided that the low values were due to two factors. One was that the pH of 3 was not sufficiently low enough to dissolve all phosphate salts which may have precipitated. The second factor was that the possibility existed that not all phosphates from the phosphoric acid wash water were washed out in the distilled wash water.

In subsequent preparations acid water buffered at pH 1 with hydrochloric acid was used in the washing of cells. It was observed that no ammonia nitrogen was distilled from the solids washed in the acid water buffered at pH 1, while small quantities, about 1 mg were distilled over from the solids samples washed in acid water buffered at pH 3.

The average N/P ratio as shown in Table XI may be taken as approximately 7. This is close to the N/P ratio suggested for aerobic treatment processes by Sawyer (48).

Endogenous Respiration Rates

Endogenous respiration has been defined as the destructive metabolism, by bacteria, of their own protoplasm for energy. It has often been considered to occur at a rate proportional to the amount of protoplasm present regardless of the presence of an oxidizable substrate. The older the sludge retention time, the greater is the degree of endogenous respiration. This would account for the decrease in nitrogen requirements for all substrates with increasing retention time.

The endogenous respiration value, k, for glucose and starch was found to be 0.088/day, while k for the amino and fatty acids was 0.038/day. Considering the hypothesis that a comparable population of methane bacteria is requiredfor the COD reduction stage for the acetate, glucose, and starch systems, with an additional population required for the constant COD stage of glucose and starch, the following statements can be made. The endogenous respiration rate,k, for glucose and starch would be a composited value which was the weighted average of an individual endogenous respiration rate,k, for the population of the constant COD stage and the endogenous respiration rate k = 0.038/day for the bacterial population of the COD reduction stage. Evaluation of the endogenous respiration rate, k, for the

constant COD population will now be evaluated. The growth equation curve can be written:

$$A = cF - (k_c M_c + k_r M_r)$$

where $(k_c M_c + k_r M_r) = kM$

k = .088/day composite endogenous respiration rate for starch and glucose

M = total volatile solids in glucose or starch units

k_c = endogenous respiration rate for bacteria of the constant COD stage

M_c = weight of bacteria present from the COD reduction reduction stage

k_r = .038/day endogenous respiration rate of bacteria from the COD reduction stage

M_r = weight of bacteria present from the COD reduction stage

Only k_c and M_c are unknown. The values of volatile solids present in the various retention time digesters for acetate are calculated from Table XIII by multiplying the cellular nitrogen concentrations by the conversion factor, 9.4 mg volatile solids/mg N. The values obtained are:

5 day - 114 mg/l
10 day - 218 mg/l
20 day - 380 mg/l
30 day - 500 mg/l

Table XIV contains the information used in evaluating the endogenous respiration rate for the bacterial population of the constant COD stage.

Table XIV

Evaluation of Endogenous Respiration Rate Constant for Constant COD Stage

Retention Time	M	M_r	M_c $(M-M_r)$	.088M	.038 M_r	W	k_c
Glucose 5	620	114	506	54	4	50	.101
Glucose 10	990	218	772	87	8	79	.091
Glucose 20	1970	380	1590	173	14	159	.091
Glucose 30	2080	500	1580	183	19	164	.090
Starch 5	500	114	386	44	4	40	.091
Starch 30	2200	500	1700	194	19	175	.090

Column W is equal to the difference:

$$W = .088\ M - .038\ M_r$$

Column k_c was derived as follows:

$$k = W/M_c = (.088M - .038M_r)/(M - M_r)$$

The value of k_c = .091/day appears most reasonable.

Since the value of the factor F in the growth equation is the same for the constant COD stage as for the COD reduction stage, the constant C_c for the bacterial population of the constant COD stage equals:

$$C_c = C - C_r$$
$$C = .46 - .054$$
$$C_c = .406 \quad \#\ \text{volatile solids}/\#\text{COD}$$

The growth equation for the constant COD stage for glucose and starch alone then becomes:

$$A_c = 0.406F - 0.091\ M_c$$

where A_c = volatile solids accumulation due to the constant COD stage alone.

A very interesting observation was made from the glucose digesters. After the final data was recorded, all of the units were left standing with

no feeding of substrate for a period of nine weeks. The volatile solids concentrations in the units were analyzed at the end of the final data period, six weeks later, and nine weeks after the final data period. Table XV shows the results of the analyses at these intervals.

Table XV

Endogenous Respiration of Biological Solids in Glucose Digesters after Stoppage of Feed

Time Weeks	Volatile Solids Concentration mg/l Retention Time - days			
	5	10	20	30
0	620	990	1970	2080
6	540	900	1540	1580
9	500	800	1540	1560

Thus, after 60 days with no feeding, from 75 to 81% of the volatile solids were still present. This represents an endogenous respiration rate of less than 0.005/day as compared to 0.088/day computed for the case when feed was being administered.

It is hypothesized that just as the bacterial population in the constant COD stage appeared not to be able to derive energy from the amino and fatty acid group due to the lack of a hydrogen acceptor, so also, in the absence of the reducible substrate, endogenous metabolism is considerably diminished. The bacteria appear not to be able to attack their own protoplasm without a hydrogen acceptor present.

Chemical Formulation of Biological Solids

The average empirical chemical formulation of biological solids from all retention times and substrates was:

$$C_5H_9O_3N$$

This compares very closely to empirical formulations of biological solids produced aerobically. Symons and McKinney (60) reported a formula of $C_5H_8O_2N$. Hoover and Porges (23) reported $C_5H_7O_2N$. There is only a difference of 1 H_2O between the formula obtained in this study and that obtained by Hoover and Porges, while the only difference is an extra OH between Symons and McKinney's value.

The average of the C/N values for all 5 day retention times was 4.7. Likewise, the average of all 30 day retention values for C/N was 4.7 also. Thus, the C/N ratio is not altered with changing retention times.

B. Stimulation of Acetate Utilization in Anaerobic Digestion

The results of this investigation have considerably enhanced our knowledge of the growth stimulants for methane bacteria. Prior to this study, it was realized that methane bacteria were capable of metabolizing acetate at exceptionally high rates. However, the factors responsible for this stimulation were unknown. In this study, a group of pure compounds has been isolated, which have been shown to be capable of stimulating very high rates of acetate utilization. It only remains now to try various combinations of these compounds to isolate their maximal effect.

Since the methane forming phase of anaerobic digestion is reported to be the most sensitive to adverse effects, it becomes a vital importance to the sanitary engineer to understand as fully as possible the biochemical processes involved. Only in this way, can the excellent potentialities of the process be exploited for waste treatment and intelligent, remedial measures be taken when the process encounters difficulty. Establishment of the growth stimulants is one of the first steps in this endeavor to become familiar with the life processes of the methane bacteria.

These studies were conducted on mixed cultures of methane bacteria. For practical purposes, this would be the situation encountered in waste treatment. However, from the biochemical point of view, it introduces a degree of uncertainty in predicting the role played by a stimulatory substance.

The acetate utilization rates achieved in this study were exceptional considering the fact that only a fraction of one per cent of any supernatant solids or digested sludge was present in the system. Essentially all of the extraneous and unknown material had been washed out. Rates in some cases had dropped off to 200 mg/l/day of acetate before addition of iron increased the rate to a steady 1 g/l/day. McCarty (31) started up a digester with 100% well digested sludge and

found the maximum acetate utilization was approximately 5 g/l/day even with all the trace nutrients available. Schulze and Raju (50) investigated the maximum rate of acetate utilization by anaerobic digestion and attained only 1 g/l/day. The rate of 6300 mg/l/day achieved in this study is considerably higher.

Iron

Inclusion of $FeCl_3$ in the nutrient salts enabled digestion rates of 1 g/l/day continuously without inclusion of any organic stimulants. On such a feed, the digester was continually purged of any original trace organics in the seed sludge by daily feeding and withdrawal of a mixed effluent. The fact that digestion rates remained constant signifies that all organic compounds vital to the life processes of the methane bacteria were being synthesized from the basic acetate molecule. The observation is then made that methane bacteria require no organic growth factors for subsistence.

Upon examination it is noted that if the organisms only required electrons, such that $Fe^{o}-3e^{-}=Fe^{+++}$, $FeCl_3$ would not have stimulated digestion. On the other hand if only a reducible substance was required, such that $Fe^{+++}-e^{-}=Fe^{++}$, then iron powder would not have produced stimulation. Therefore, the function of iron must have been more specific in nature. It is reasonable to assume that it was involved in an electron transport system. Strict anaerobes do not possess a cytochrome system. However, there are other electron transport systems in obligate anaerobes which utilize iron. Peck and Gest (44B)

studied hydrogenase from the strict anaerobe Clostridium butylicum. They stated that the hydrogenase was a ferriflavoprotein basically similar to the hydrogenase of other strict anaerobes.

Next, it is noted from the acetate utilization rate of no more than 1 g/l/day with only inorganic additions that one or more rate limiting steps must occur in the biochemical processes of the methane bacteria. The synthesis of one or more required organic compounds thus appears to govern the overall activity.

Thiamine

The fact that thiamine produced a stimulation in the digestion rate indicated a deficiency of this substance in the bacteria. This could possibly be one of the rate-limiting processes in methane bacterial activity. Thiamine is used by bacteria to synthesize the coenzyme thiamine pyrophosphate (TPP). Stanier, et al (56) stated that all organisms require this coenzyme whether they exhibit a need for thiamine as a unit or any part of the molecule thereof. Among the known functions of TPP are pyruvate decarboxylation and pentose synthesis. Pentoses are required in the nucleic acids. As mentioned before, since acetate is the sole organic source, all of the components of the cell-sugars, nucleic acids, proteins, etc. - must be synthesized from the basic acetate molecule.

Since the metabolic pathway of acetate and the enzymes involved has not been determined, the possibility must be left open that TPP is involved directly in acetate decarboxylation.

Vitamin B_{12}

The combination of cobalt, glycine, proline, and benzimidazole all yielding increased digestion rates tends to implicate vitamin B_{12} as playing a fundamental role in acetate metabolism under anaerobic conditions. Fig. 2 shows the structure of vitamin B_{12}. A cobalt ion is bound in the center of the 4 pyrrole rings. The structure which is composed of the 4 pyrrole rings connected by methene bridges and containing short side chains, is referred to as protoporphyrin IX. The Gilson Medical Electronics (12) intermediary metabolism pathway chart shows protoporphyrin IX as being synthesized from glycine as the base. Upon addition of glycine to Digester III, which contained cobalt, stimulation resulted. However, since there is a similarity between the chemical structure of a pyrrole ring and the amino acid proline, it was decided to observe the effect of proline on acetate utilization in a digester which contained cobalt ions. Stimulation from 700-2000 mg/l/day resulted.

If the proline actually did serve as the base for synthesis of the protoporphyrin IX structure, this would be a hitherto unreported pathway.

From the structure of vitamin B_{12} in Fig. 2, it is noted that a double ring structure - 5, 6, -dimethyl benzimidazole - is attached to the cobalt ion in the center of the porphyrin ring. Benzimidazole was the compound most nearly alike 5,6-dimethyl benzimidazole which was available. Addition to a digester in which proline and cobalt was present stimulated digestion from 3500-4300 mg/l/day.

Fruton and Simmonds (11) made the following statement about the function of vitamin B_{12}:

> "The biochemical activity of the vitamins of the B_{12} group has been associated with the biosynthesis of methyl groups from C_1 precursors and with the biosynthesis of thymidine and of other deoxyriboses. In this respect, the functions of the cobalamin derivatives are closely associated with those of folic acid vitamins. Although vitamin B_{12} appears to be essential for the biosynthesis of the DNA deoxyribose in Lactobacillus leishmannii, the mechanism of this effect has not been elucidated."

It is well known that digested sludge contains an exceptionally high vitamin B_{12} content. The results of this study give evidence of the synthesis of appreciable quantities of vitamin B_{12} also. The action of vitamin B_{12} is not clear and only postulations can be given of possible functions. It may be involved directly in acetate decarboxylation for the derivation of energy to maintain the life processes or it could be involved in the synthesis of one or more compounds required in the anabolic processes of the bacteria.

The fact that the addition of vitamin B_{12} with mannitol to the digester failed to produce stimulation may be due to the large size of the molecule. With such large molecules, it may be difficult for it to diffuse through the cell wall. Another factor in favor of this assumption is the observation that vitamin B_{12} is found only inside the bacterial cell. The bacteria may not have been able to utilize the molecule in the form it was added even if it was possible for it to pass through the cell wall.

Quantities of Stimulants Required

Relatively large quantities of materials were used in these studies to stimulate acetate utilization. This may be the reason why Vath (63) and Barker (2) reported no stimulation after addition of growth factors to a methane culture.

Tentative quantities will be given for iron, cobalt, and thiamine. Two batch fed digesters with excess acetate maintained in solution and which were mixed once a day maintained acetate utilization rates of 1 g/l/day with $FeCl_3$ concentrations of 21 and 35 mg/l as iron in the feed. A third digester operated in similar fashion had 7 mg/l of $FeCl_3$ as iron in the feed and achieved only 600 mg/l/day digestion rates. Neglecting the precipitation of iron in solution by sulfides, from 7 to 21 mg/l $FeCl_3$ as iron was thus required to maintain digestion at the rate of 1 g/l/day. It is interesting to note that comparable digestion rates were achieved in this batch fed system with mixing occurring once a day as in a continuously mixed and continuously fed system.

The addition of 200 mg or 33mg/l of thiamine hydrochloride enabled acetate utilization rates of approximately 5 g/l/day even after one detention period had passed. Nutrients were found to have been limiting also at this rate. After one detention period, only one-third or 11 mg/l of the thiamine would have been present. This would be the maximum concentration required for acetate digestion rates of 5 g/l/day. The actual requirement may be much less. Even so, this is a rather high concentration when dealing with growth factors.

For cobalt, an addition of 200 mg or 33 mg/l of $CoCl_2 \cdot 6\ H_2O$, which is equivalent to 8 mg/l as cobalt, resulted in stimulation of Digester III to approximately 4 g/l/day when nutrients again appeared to be limiting. This also occurred about one detention period after addition. Therefore, 1 or 2 mg/l of cobalt as cobalt appeared to be the necessary feed concentration to achieve high digestion rates.

No estimation is made of the quantities of glycine, proline, and benzimidazole which were required for stimulation.

C. Significance of Nutrient Requirements and Growth Stimulants in Engineering Practice.

The treatment of industrial wastes has assumed a very important role with the industrialization of our society and the resulting pollutional load imposed on the water courses receiving the wastes. As opposed to a domestic sewage, an industrial waste can be very specific in its constituency and concentrated in its pollutional load.

For successful biological treatment of an organic waste, the waste must either contain or be supplemented with the nutrients required for the maintenance of the flora responsible for its decomposition. Nitrogen and phosphorous are the main nutrient requirements.

The anaerobic digestion process exhibits a great potential for the treatment of strong industrial wastes in an efficient manner. Solid engineering design for a waste treatment facility has to be based on the operational characteristics of that waste during treatment. However, it is time consuming and burdensome to run pilot plant studies on each and every particular waste to determine the operational characteristics.

The desirable situation would be to have in possession that information which would allow the calculation of the following design criteria, knowing only the composition of the waste:

Nitrogen requirement
Sludge accumulation
Volatile solids accumulation
Methane production
Optimum sludge detention time
Alkalinity produced in the decomposition
Supplemental additions of nutrients.

A simple laboratory analysis would indicate the percentages of fats, proteins, and carbohydrates in the waste.

Design Considerations

Nitrogen Requirements, Sludge Accumulation, Methane Production and Detention Time

As a result of this study, it is now possible to rationally design anaerobic treatment facilities knowing only the waste concentration, volume, and composition. To illustrate the pertinence of the results of this study, an example is given of a waste which was thoroughly investigated by Stewart (58).

The waste composition was stated to be:

Bacto-Tryptone	-	20 g/l
Dextrose	-	10 g/l
Bacto-beef extract	-	6 g/l
		36 g/l

If this composition had not been known, a laboratory analysis would have shown the composition to be:

Carbohydrates	-	10 g/l
Proteins	-	26 g/l

The COD of the waste was found to be 37,000 mg/l. Glucose exhibits 1.06 gm COD/gm glucose, so that 10,600 mg/l of the waste COD is exerted by the carbohydrate fraction and all the remaining 26,400 mg/l of COD is represented by the protein fraction.

The growth equations evaluated in this study for these classes of compounds were:

Glucose and Starch $A = 0.46F - 0.088M$

Nutrient Broth $A = 0.08F - 0.02M$

The fraction of the total COD exerted by the carbohydrate is:

$$10{,}600 \text{ mg/l} \div 37{,}000 \text{ mg/l} = 29\%$$

The fraction of the total COD exerted by the protein is:

$$26{,}400 \text{ mg/l} \div 37{,}000 \text{ mg/l} = 71\%.$$

Evaluation of this constant, c, for the waste is based upon these fractions of total COD. The carbohydrate fraction will exert a value of $c = 0.46$ #volatile solids/#COD, while the protein fraction will exert a value of $c = 0.08$ #volatile solids/#COD. Therefore, the composited value of c, for the waste is:

Carbohydrate $- (.29)(.46) = 0.132$

Protein $- (.71)(.08) = 0.056$

c for composite waste $= 0.188$ #volatile solids/#COD

This compares with the value obtained experimentally by Stewart (58) of $c = 0.18$ #volatile solids/#COD.

It is emphasized at this point that a great amount of experimental effort was required for Stewart to obtain this value for this particular waste and it can not be used to predict the c value of a waste of any different composition. For that matter not even this same waste could be evaluated if the percentage composition were altered. Notice that the carbohydrate represents $0.132/0.188 = 70\%$ of the composite value of c, while only 29% of the waste is represented by carbohydrates.

The endogenous respiration rate, $\underline{k}$, for a waste is related to retention time for a mixed substrate. At any retention time, the endogenous respiration rate for the composite waste would be influenced by the total weight of solids present in each fraction at that time, and the rate at which each fraction was respiring. For example, at short retention times, the large volume of solids produced from carbohydrate metabolism and the high rate at which they respire, would control. At long retention times, the low respiration rate of the solids produced in protein metabolism would control.

The endogenous respiration rate will be evaluated at 5 and at 30 days retention time. At equilibrium: M = A x Retention Time.

For the protein fraction at 5 days retention time:

$$A = (0.71)\ 0.08F - 0.02M$$

$$M/F = 50A/F$$

$$M_p/F = 0.260 \text{ gm volatile solids/gm COD}$$

For the carbohydrate fraction at 5 days retention time:

$$A = (0.29)\ 0.46F - 0.088M$$

$$Mc = 0.455 \text{ gm volatile solids/gm COD}$$

The total volatile solids M = 715 mg volatile solids/gm COD

The accumulation A = M/5 = 143 mg volatile solids/day/gm COD

The endogenous respiration rate for the composite waste at 5 days:

$$A = 0.188 - kM$$

$$0.143 = 0.188 - k(0.715)$$

$$k = 0.063/\text{day}$$

Similar calculations for a 30 day retention time for the composite waste yields:

$$k = 0.054/day$$

Stewart (58) evaluated one value for $k = 0.0247/day$.

Continuing the design on the basis of two values of the endogenous respiration rate:

5 day $k = 0.063/day$

30 day $k = 0.054/day$

the design criteria are evaluated. The solids due to both the carbohydrate component and the protein component are computed as well as the total composite solids. At a 5 day sludge retention time, the composite growth equation is:

$$A = 0.188F - 0.063M$$

$$M = 700 \text{ mg/gm COD}$$

$$A = 140 \text{ mg/day/gm COD}$$

At a 30 day sludge retention time, the composite growth equation is:

$$A = 0.188F - 0.054M$$

$$M = 2160 \text{ mg/gm COD}$$

$$A = 72 \text{ mg/day/COD}$$

A comparison of values obtained by the growth equations calculated from the results of this study and the values computed from Stewart's equation is given.

5 Day R.T.	M	A	N Requirement	%	CH_4
Theoretical	(mg/gmCOD)	(mg/day/gmCOD)	mg N/gm COD	Synthesis	ml/gmCOD
Carbohydrate	450	90			
Protein	250	50			
Composite	700	140	15	17	327
Stewart	800	160			
30 Day R.T.					
Theoretical					
Carbohydrate	1095	37			
Protein	1065	35			
Composite	2160	72	66	7.5	365
Stewart	3100	103			

It is possible to calculate the nitrogen requirement, per cent synthesis, and methane production from these results also.

When it is considered that the theoretical values are within 30% of the experimental values, this is exceptionally good agreement, considering that no pilot studies were required. In an actual waste stream the composition would probably change by more than 30% at times. Before these studies were made, it would have been possible to be off by a factor of 500% if carbohydrates would have been assumed to exhibit the same nitrogen requirements as acetate.

Alkalinity

The nitrogen content of protein is close to 17%. Therefore since protein comprised 72% of the waste COD, the concentration of nitrogen in the waste stream was:

$$(0.17)(0.72)(37{,}000 \text{ mg COD/l}) = 4500 \text{ mg N/l}$$

The nitrogen requirement for a 5 day sludge retention time was 15 mgN/gm COD. Therefore, with a waste strength of 37 g/l COD, the total nitrogen requirement would be:

$$15 \text{ mg N/gmCOD} \times 37 \text{ COD/l} = 550 \text{ mgN/l}$$

For 30 days sludge retention time:

$$\text{Nitrogen requirement} = 6.6 \times 37 = 250 \text{ mgN/l}$$

Therefore the waste carries sufficient nitrogen for nutrient purposes.

The ammonium bicarbonate alkalinity at the 5 day sludge retention time would be based on the total organic nitrogen in the waste minus the nitrogen required for synthesis: 4500 mgN/l - 550 mgN/l = 3950 mgN/l. The alkalinity would then be 13,000 mg/l as $CaCO_3$. With this high alkalinity production, provision would have to be made to add hydrochloric acid in order to destroy a fraction of the alkalinity and maintain a neutral pH. For a 30 day sludge retention time, the alkalinity would be calculated in a similar fashion and yields a concentration of 14,200 mg/l $CaCO_3$. Again, acid addition must be practiced to maintain a neutral pH.

Recovery and Recycle of Biological Solids

The results of this study have shown that reduced nutrient requirements result from increased sludge retention times. Carbohydrate nutrient requirements are considerably reduced in this way. Short hydraulic detention times are desirable from the standpoint of requiring smaller digestion tank volumes. Both desirable ends can be achieved by maintaining a short hydraulic detention time and

recovering and recycling the biological solids. Thus the long sludge retention time is achieved in a small digestion tank.

In some cases, it is unfeasible to recover the solids due to a large undegradable fraction of solids in the incoming waste stream of a highly concentrated waste. Recovery of effluent solids in such a case would result in loss of digester capacity due to build-up of the relatively inert organic material. In this case the desirable solution would be to pass the influent waste stream through a settling tank. Here the solid fraction could be settled out, concentrated and pumped to one digester operating on a long hydraulic and sludge retention times for treatment of that fraction. The soluble and colloidal part of the concentrated waste could then be sent to another digester, separate from the solids digester, and treated on a short hydraulic detention time with the recovery and recycle of biological solids to reduce the nutrient requirements.

Digestion Rate

Conventional digestion design rates have been approximately 0.05 #volatile solids/ft^3/day, with high rate loadings of 0.20#/ft^3/day. Of course, it is most desirable to utilize the digester capacity to the greatest possible extent. Since almost all organic material passes through the volatile acids stage, one of the determining factors of the maximum loading rate will be the rate at which the methane bacteria can utilize the volatile acids.

Other investigators have shown that exceptionally high rates of acetate utilization were possible with an adequate supply of nutrients.

However, it was not known which pure compounds produced the stimulation. These compounds have now been determined, and the maximum design rate for digester loading does not appear to be limited by the capacity of methane bacteria to utilize the volatile acids produced, provided the proper environment is maintained.

Rates of at least 0.36#/ft^3/day can be assured by the addition of the following nutrients:

Iron
Cobalt
Thiamine
Proline
Glycine
Benzimidazole

Without these nutrients, only low rates were possible, with them very high rates are possible.

The addition of iron to the substrate enabled constant normal digestion rates. Therefore, conventional rates of digestion could be maintained with no organic growth stimulants required.

Digester Start-Up

Sometimes it is not feasible to transport large volumes of digested sludge to a treatment plant site in order to initiate digestion. In such cases, the digester contents are filled with the waste and allowed to stand until methane production is initiated. The results of this study have shown that the activity of methane bacteria is stimulated by certain compounds. It might therefore be advantageous to add these substances in concentrations of approximately 5 mg/l to the digester

in such cases to make conditions as favorable as possible for the methane bacterial population to develop. Of course, the pure organic compounds would not have to be added. Products, such as brewer's yeast and gelatin would be very inexpensive and provide such a source.

VII. CONCLUSIONS

From this study on the biological nutrient requirements and growth in anaerobic digestion, the following conclusions have been drawn.

1. Using the results of this study, it is possible to predict for a waste, the nitrogen requirements, sludge production, net synthesis and methane production which will occur during anaerobic digestion for any sludge retention time.
2. Two stages exist in the anaerobic decomposition of a complex waste, one in which the COD remains constant and another in which the COD is reduced due to the production of methane.
3. In the case of carbohydrates, the constant COD stage exhibits the greatest nutrient requirement, results in the greatest sludge production, and results in no direct COD or ultimate BOD reduction.
4. The growth equation for the anaerobic digestion of glucose and starch is: $A = 0.46\ F - 0.088\ M$.
5. The growth equation for the anaerobic digestion of amino and fatty acids is: $A = 0.054\ F - 0.038\ M$.
6. The growth equation for anaerobic digestion of proteins, based on limited data, is: $A = 0.08\ F - 0.02\ M$.
7. The nitrogen requirements for all substrates were equal to A/9.4.
8. The phosphorous requirement for all substrates was approximately one-seventh of the nitrogen requirements for each particular substrate.
9. Exceptionally high rates of acetate utilization, 0.36 #/ft^3/day, under anaerobic conditions can be achieved by addition of combinations of iron, cobalt, thiamine, and components of vitamin B_{12}.
10. Low normal digestion rates can be maintained by additions of only inorganic salts to the organic feed.

VIII. SUGGESTIONS FOR FUTURE WORK

1. Further studies should be conducted to determine the nutrient requirements and biological growth for anaerobic digestion of various classes of substrates when added on a continuous feed basis.

2. The ability of facultative bacteria to respire in the absence of a hydrogen acceptor under anaerobic conditions should be determined.

3. The parameter which predicts the amount of biological growth in the constant COD stage should be defined.

4. An investigation should be conducted to isolate the cause which limited the digestion rates achieved in this study to 6 g/l/day.

5. An investigation of the function of the stimulatory compounds for methane bacteria found in this study should be conducted to determine their role in the biochemical processes.

6. A study should be made of the possibility of cultivating a population of bacteria in an anaerobic digester, which would excrete compounds required for stimulation of the methane bacteria.

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APPENDIX A

MISCELLANEOUS INFORMATION

Composition of Nutrient Broth

Nutrient broth is a combination of 3/8 Bacto-Beef Extrace and 5/8 Bacto-Peptone. The Difco Manual (10A) lists the analyses of Bacto-Peptone as follows:

	% Composition
Total Nitrogen	16.16
Primary Protease N	0.06
Secondary Protease N	0.68
Peptone N	15.38
Ammonia N	0.04
Free Ammonia N (vanSlyke)	3.20
Amide N	0.49
Mono-amino N	9.42
Di-amino N	4.07
Tryptophane	0.29
Tyrosene	0.98
Cystine (Sullivan)	0.22
Organic Sulfur	0.33
Inorganic Sulfur	0.29
Phosphorous	0.22
Chlorine	0.27
Sodium	1.08
Potassium	0.22
Calcium	0.058
Magnesium	0.056
Manganese	nil
Iron	0.0033
Ash	3.53
Ether Soluble Extract	0.37
Reaction pH	7.0

APPENDIX A

Standard Free Energies of Formation at 298°k

Compound	State	F^{o} k cal/mole
Acetic Acid	Gas	-91.2
Acetic Acid	Liquid	-93.8
Acetate Ion	Aqueous	-89.7
Ammonium Bicarbonate	Aqueous	-159.3
Ammonium Ion	Aqueous	-19.0
Ammonia	Aqueous	-6.4
Bicarbonate Ion	Aqueous	-140.3
Carbon Dioxide	Aqueous	-92.3
Carbon Dioxide	Gas	-94.6
Ethanol	Liquid	-41.8
Glucose-D	Aqueous	-217.0
Glycine	Crystalline	-88.6
Sodium Ion	Aqueous	-62.6
Sodium Acetate	Aqueous	-152.3
Sodium Bicarbonate	Crystalline	-203.6
Sodium Bicarbonate	Aqueous	-203.6
Water	Gas	-54.6
Water	Liquid	-56.7

APPENDIX B

STEADY STATE OPERATIONAL CHARACTERISTICS DURING FINAL DATA PERIOD FOR FAT, PROTEIN, AND CARBONHYDRATE SUBSTRATES

TABLE B - I

HISTORY OF ACETATE DIGESTER - 5 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids	Gas Comp. % CH_4	Remarks
1	260				204	13					
2	260			1030				1320			
3	280				206	11					
4	260				210	13					
5	260			1340				1520			
6	280										
7	240										
8	270				192	14					
9	220	6.30									
10	230										
11	260									49	
12	270									48	
13	270	6.65	2200	1200							
14	270										
15	210	6.80				11					
16	290										
17	280				206	11					
18	250					13					
19	270					13					
20	260										
21	280										
22	270	7.00	2600	950							
23	260										

TABLE B - I (Continued)
HISTORY OF ACETATE DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
24	260										
25	300									51	
26	240						1160				
27	270					12					
28	260							1180			
29	310										
30	250	6.80	2500	900							
31	250					12					
32	290					10					
33	290										
34	290										
35	290										
36	290										
37	310			850							
38	310					10				51	
39	270										
40	270										
41	270										
42	290										
43	270										
44	270										
45	260										
46	300			970							
47	280					12	1350				

TABLE B - II

HISTORY OF ACETATE DIGESTER - 10 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	370			710				1100			
2	380										
3	350										
4	360				180	25					
5	340										
6	320									55	
7	330									57	
8	330										
9	350	6.80	2500	600							
10	350										
11	350	6.90									
12	370										
13	340				190	21					
14	330					22					
15	350					27					
16	340										
17	360										
18	360	7.00	2500	300							
19	330										
20	330										
21	340									54	
22	310						520				

TABLE B - II (Continued)
HISTORY OF ACETATE DIGESTER - 10 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
23	340										
24	330										
25	390							780			
26	340	6.90	2400	300							
27	320					23					
28	350					21					
29	340										
30	350										
31	340										
32	310										
33	340			420							
34	340									54	
35	310										
36	350										
37	340										
38	340										
39	360										
40	340										
41	310										
42	340			550							
43	300					25		830			

TABLE B - III

HISTORY OF ACETATE DIGESTER - 15 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	Solids NH_3-N (mg/l)	Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	390				172	36					
2	390			440				790			
3	390				174						
4	380				168	37					
5	370			160				770			
6	370										
7	340										
8	390				170	36					
9	370										
10	350									54	
11	380									53	
12	340										
13	350	6.75	2700	150							
14	350										
15	350	7.00				36					
16	380										
17	330				188	32					
18	340										
19	350										
20	350										
21	350										
22	360	7.00	2600	200							
23	350										

TABLE B - III (Continued)
HISTORY OF ACETATE DIGESTER - 15 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml | Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
24	340										
25	390									54	
26	380						310				
27	370										
28	380										
29	390							740			
30	380	7.00	2500	70							
31	360					35					
32	380					38					
33	370										
34	380										
35	370										
36	380										
37	370			300							
38	380					33				53	
39	370										
40	380										
41	370										
42	380										
43	370										
44	360										
45	370										
46	390			350							
48	370					37		730			

TABLE B - IV

HISTORY OF ACETATE DIGESTER - 20 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced	pH	Alk.	Vol. Acids	NH_3-N	Solids Org.N	Eff. COD Filtrate	COD Mixed	Vol. Solids	Gas Comp. % CH_4	Remarks
	(ml)		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		
1	390				170	43					
2	380			300				1030			
3	400										
4	360										
5	410				170	37					
6	410										
7	380									54	
8	400									55	
9	380										
10	370	6.90	2800	130							
11	370										
12	390	7.10				37					
13	400										
14	360				200	38					
15	370					44					
16	390					36					
17	370										
18	370										
19	370	7.10	2500	170							
20	360										
21	290										
22	430									54	

TABLE B - IV (Continued)
HISTORY OF ACETATE DIGESTER - 20 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
23	380										
24	370										
25	380										
26	390							810			
27	380	7.00	2400	50							
28	340					42					
29	380					44					
30	370										
31	380										
32	360										
33	380										
34	380			220							
35	360					40					
36	360									52	
37	370										
38	370										
39	370										
40	370										
41	350										
42	360										
43	380			130							
44	370					44		850			

TABLE B - V

HISTORY OF ACETATE DIGESTER - 25 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced	pH	Alk.	Vol. Acids	NH_3-N	Solids Org.N	Eff. COD Filtrate	Eff. COD Mixed	Vol. Solids	Gas Comp. % CH_4	Remarks
	(ml)		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		
1	400				170	47					
2	380			410				860			
3	380										
4	370										
5	390				166	45					
6	370										
7	370									53	
8	380									54	
9	380										
10	380	7.00	3000	130							
11	370										
12	370	7.10				48					
13	370										
14	350				178	44					
15	360										
16	410					42					
17	360										
18	360										
19	350	7.20	3000	180							
20	360										
21	290										
22	450									54	
23	370						290				

TABLE B - V (Continued)
HISTORY OF ACETATE DIGESTER - 25 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
24	360										
25	380										
26	390							850			
27	380	7.10	2700	70							
28	360					48					
29	370					44					
30	390										
31	390										
32	390										
33	390										
34	390			310							
35	430					47				52	
36	390										
37	390										
38	380										
39	390										
40	390										
41	390										
42	340										
43	410										
44	390										
45	390					46		940			

TABLE B - VI

HISTORY OF ACETATE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	420				180	52					
2	400			370				1020			
3	390				184						
4	380				180	52					
5	380			550				960			
6	400										
7	350										
8	400				172	52					
9	360										
10	340									52	
11	380									54	
12	350										
13	360	7.10	3200	130							
14	350										
15	360	7.10				56					
16	370										
17	350				180	50					
18	350										
19	390					52					
20	350										
21	340										
22	360	7.20	3200	300							
23	340										

TABLE B - VI (Continued)
HISTORY OF ACETATE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml | Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
24	300										
25	430									54	
26	380										
27	360					57					
28	370										
29	370							1030			
30	380		2800	50							
31	340					57					
32	370					60					
33	370										
34	390										
35	360										
36	370										
37	370			290							
38	390					50				51	
39	360										
40	370										
41	370										
42	390										
43	360										
44	370										
45	370			100							
46	370					50					

TABLE B - VII

HISTORY OF OCTANOATE DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced	pH	Alk.	Vol. Acids	NH_3-N	Solids Org.N	Eff. COD Filtrate	Eff. COD Mixed	Vol. Solids	Gas Comp. % CH_4	Remarks
	(ml)		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		
1	80										
2	80						1580	1890			
3	120				500	11			120		
4	100										No A & E
5	130										
6	150								120		
7	150				240		1380	1720			
8	150	7.25	2000								
9	140								90	75	
10	140						1170	1460			
11	180										
12	130										
13	150				200	11					
14	140			980							
15	110	7.20	2100	700							
16	130										
17	160						1130	1420			

TABLE B - VII (Continued)
HISTORY OF OCTANOATE DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
18	130										
19	140				210	12					
20	130				220	13				77	
21	140				220	12					
22	140						1240	1570			
23	130				220	13					
24	150	7.25	2100	700							
25	170					11					
26	120						1110	1210			
27	140										
28	140					8					
29	160						1060				
30	160										
31	170										
32	150								130		
33	150								70		
34	160					8				77	
35	150						880	1060			

TABLE B - VIII

HISTORY OF OCTANOATE DIGESTER - 30 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	210						660	1340			
2	220								600		
3	240				440	48					No A & E
4	220										"
5	230								520		
6	230						800	1460			
7	240				440						
8	240	7.30	3800						360	77	
9	220						750	1460			
10	2220										
11	220										
12	190										
13	220				440	48					
14	230			500							
15	230	7.45	2200	500							
16	220										
17	250						700	1280			
18	230										
19	230				312	56					
20	220				320	60				78	
21	240				300	56					
22	250										
23	230				295	56					
24	250	7.60	4000	220							
25	260					56					
26	220						730	1240			

TABLE B - IX

HISTORY OF OLEATE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	270	7.05	2800					1510			
2	230										
3	210				160						
4	240				200	60				76	
5	250				192	48					
6	230						640				
7	250				156	56					
8	160										
9	280					48					
10	220						610	1700			
11	290										
12	250					52					
13	220						700	1760			
14	230										
15	230										
16	180										
17	210										
18	270					56				76	
19	240						710	2060			
20	260										
21	200										
22	250										
23	170										
24	160										
25	230			220							

TABLE B - X

HISTORY OF GLYCINE DIGESTER - 5 DAY DETENTION TIME

STEADY STATE OPERATION OF CHARACTERISTICS DURING FINAL DATE PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	150	7.25	2800	480							
2	170					13					
3	190						540	1080			
4	170										
5	170										
6	170					13				73	
7	170	7.20	2800			11					
8	170						550	1410			
9	170					10					
10	180	7.35	2600	400							
11	190										
12	150						540				
13	160					9					
14	160					10			73		
15	200						610				
16	220								87		
17	230										
18	200								73		
19	220						480				
20	240									74	
21	220				1100		580	1210			

TABLE B-XI

HISTORY OF GLYCINE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	480									65	
2	250					60				65	
3	210	7.40	6800								2nl HCl
4	560						1060	1470			
5	240					52					
6	210	7.45	6500	300							
7	230										2nl HCl
8	530						1250	1490			
9	240					56					
10	200					52			440		
11	200										1nl HCl
12	400								560		
13	240										
14	170								400		
15	210						1580				

TABLE B - XII

HISTORY OF LEUCINE DIGESTER - 7.5 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	180					22					
2	190						990	1450			
3	170					22					
4	140								180		
5	130								180		
6	120					18					
7	60	6.70	1000	470			1930				1200 mg Alk.
8	80						1590		180		
9	120					17	1640			74	
10	170				430	21				76	
11	190						1480	1850			

TABLE B - XIII

HISTORY OF LEUCINE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	230	7.00	2600								
2	230										
3	210										
4	210										
5	240					60	950	1250			
6	220										
7	240								320		
8	200								320		
9	200					52					
10	210										
11	210				1040			1330	520		
12	170										
13	210	7.25	2800	100						71	
14	190					48	920	1510			
15	180										
16	210					56	690	1620			
17	200										
18	240					56		1570			

TABLE B - XIV

HISTORY OF NUTRIENT BROTH DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	180	7.10	1500	150							
2	200										
3	130						380	730			
4	160					19					
5	160					21			187		
6	170						400				
7	170								173		
8	170										
9	170								213		
10	150						380		193		
11	170					23				74	
12	150						400	870			
13	190				550						
14	170	7.05	1800								
15	150										
16	150										
17	150					23					
18	160						480	740			

TABLE B - XV

HISTORY OF NUTRIENT BROTH DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	340						1820				
2	210										
3	190	7.20	3300	150							
4	200										1 ml HCl
5	380						1970	3060			
6	200					112					
7	170					92			880		
8	160						2250				1 ml HCl
9	360								680		
10	210										
11	160								520		
12	170						1850		720		
13	180					108				63	1 ml HCl
14	320						2550	3900			
15	230				2000						
16	170	6.95	3400		1800						1 ml HCl
17	345										
18	185										
19	150					104					
20	180						2350	4000			

TABLE B - XVI

HISTORY OF GLUCOSE DIGESTER - 5 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	180				352	87				57	
2	180					79					
3	160										
4	190	7.10	3700	550							
5	170				380	75					
6	160							2210			
7	180						980				
8	180				515	75					
9	140			1000							3 A
10	130					86					
11	150						1050				
12	140						1180	2430		48	
13	130				490	79					
14	130										
15	140					82					No A & E
16	120					78					"
17	100										"
18	110										"
19	110					87					
20	1110								590		
21	100						1300	2560			
22	100								710		
23	130				446	87					No A & E
24	90										"

TABLE B - XVI (Continued)
HISTORY OF GLUCOSE DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
25	120								570		
26	120						1300	2480			
27	130				185	87					
28	130	6.70	2600						650	49	
29	140						1270	2440			
30	130										
31	120										
32	130										
33	150				166	77					
34	130			1000							
35	140	6.95	3300	920							
36	140										
37	170						1100	2300			
38	150										
39	130										
40	130									51	
41	160					72					
42	150						1150	2900			
43	150					77					
44	150	7.25	3400	820							
45	180										
46	120						980	1900			
47	150								600		
48	150								580		

TABLE B - XVII

HISTORY OF GLUCOSE DIGESTER - 10 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	220				210	120				55	
2	230										
3	210										
4	260	7.05	3000	370							
5	220				324	125					
6	230							2570			
7	250						750				
8	260				374	124					
9	210			510							3 A
10	220					129					
11	240						715				
12	240						820	2570		52	
13	230				470	138					
14	230										
15	220					131					No A & E
16	210										No A & E
17	210										No A & E
18	210										No A & E
19	220				261	136					
20	210								900		
21	210						850	2760			
22	200								1100		
23	210				346	136					No A & E
24	190										No A & E

TABLE B - XVII (Continued)
HISTORY OF GLUCOSE DIGESTER - 10 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced	pH	Alk.	Vol. Acids	NH_3-N	Solids Org.N	Eff. COD Filtrate	Eff. COD Mixed	Vol. Solids	Gas Comp. % CH_4	Remarks
	(ml)		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		
25	200								1030		
26	200						960	2660			
27	210				230	136					
28	220	7.05	3000						900		
29	210						980	2830			
30	210										
31	200										
32	200										
33	200				186	141					
34	200			780							
35	200	6.90	2800	740							
36	200										
37	230						1100	2920			
38	210										
39	200										
40	200									54	
41	230					128					
42	210						1150				
43	210					131					
44	210	7.10	3200	820							
45	240										
46	200						1150	2740			
47	210								1030		
48	210								970		

TABLE XVIII

HISTORY OF GLUCOSE DIGESTER - 20 DAY DETENTION TIME

STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	Solids NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	290				227	200					
2	240			500							3 A
3	250					205					
4	270						620				
5	290						920	3430		50	
6	280				370	218					
7	280										
8	280					213					No A & E
9	280										"
10	290										"
11	270										"
12	270				300	221					
13	260								1720		
14	260						680	3360			
15	260								2040		
16	250				454	226					No A & E
17	230										"
18	250								2000		
19	250						920	3380			
20	230				350	210					
21	250	6.95	3600						2000	54	

TABLE XVIII (Continued)
HISTORY OF GLUCOSE DIGESTER - 20 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
22	230						840	3890			
23	220										
24	210										
25	210										
26	240				295	215					
27	240			900							
28	230	6.80	3600	800							
29	270										
30	280						1180	4100			
31	260										
32	230										
33	240								2500	51	
34	280					223					
35	250						1330				
36	250					228					
37	280	7.05	3400	820							
38	300										
39	240						1200	3870			
40	250								1970		
41	260								2130		

TABLE B - XIX

HISTORY OF GLUCOSE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	250				335	204					
2	240								1760		
3	230						1330	4040			
4	240								2040		
5	240				400	216					No A & E
6	190										No A & E
7	260								2640		A & E
8	250						1820	4420			
9	230				320	200					
10	260	7.00	4200						1880	50	
11	230						1870	4750			
12	230										
13	210										
14	190										
15	220				280	224					

Table B - XIX (Continued)
HISTORY OF GLUCOSE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
16	240			1800							
17	220	6.95	4000	1500							
18	260										
19	260						2060	4980			
20	230										
21	240										
22	230								2160	48	
23	250					216					
24	210						2960	5890			
25	220					228					
26	220	7.10	3400	2020							
27	230										
28	180						3080				
29	190								2040		
30	200								2080		

TABLE B - XX

HISTORY OF STARCH DIGESTER - 5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume -750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Eff. COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	110					59					
2	130						1690	2500			
3	120					58					
4	120								480		
5	120								500		
6	130					54					
7	130						1800	2610			
8	100						1590		500		
9	110				150	67					
10	110									43	
11	160	6.90	3300	1300			1550	2620	510	44	

TABLE B - XXI

HISTORY OF STARCH DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml | Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3-N (mg/l)	Solids Org. N (mg/l)	Eff. COD Filtrate (mg/l)	COD Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	250					292					
2	270						660	3190			
3	260					304					
4	270								1920		
5	260								2280		
6	270					288					
7	280						690	4100			
8	260						400				
9	260				152	288					
10	260									62	
11	260	6.95	3100	180			530	2970	2120	60	

TABLE B - XXII

HISTORY OF CELLULOSE DIGESTER - 7.5 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced (ml)	pH	Alk. (mg/l)	Vol. Acids (mg/l)	NH_3N (mg/l)	Solids Org.N (mg/l)	Eff. COD Filtrate (mg/l)	Mixed (mg/l)	Vol. Solids (mg/l)	Gas Comp. % CH_4	Remarks
1	160									56	
2	180						390	2150			
3	200										
4	200				135	83					
5	200										
6	190										
7	180					71					
8	170					76					
9	190					77					
10	160							2470			
11	130				122	80					
12	140					86					
13	170				123	81					
14	130				129	78		3170			
15	130										
16	160	6.95	2700	750						50	

TABLE B - XXIII

HISTORY OF CELLULOSE DIGESTER - 30 DAY DETENTION TIME
STEADY STATE OPERATION CHARACTERISTICS DURING FINAL DATA PERIOD

Digester Volume - 750 ml

Daily Feed - 500 mg COD

Day	Gas Produced	pH	Alk.	Vol. Acids	NH_3-N	Solids Org.N	Eff. COD Filtrate	Mixed	Vol. Solids	Gas Comp. % CH_4	Remarks
	(ml)		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)		
1	240				136	152					
2	230				140	160				57	
3	310				128	156					
4	290						750	2160			
5	280				124	180					
6	310	7.05	3000								
7	260					164					
8	230						650	3110			
9	270					148					
10	300					180					
11	270						570	3290			
12	320										
13	310										
14	230										
15	240					164					
16	240					152				51	
17	310						610	3470			
18	230										
19	250										
20	260										
21	270										
22	270										
23	290			640							

APPENDIX C

DATA FROM STIMULATION STUDIES

TABLE C-I

ASSAY DIGESTER I
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
1	573.5	1400	830	400	500	
2	575.6	1050	830	400		20 mg $CoCl_2$ 6 H_2O
3	578.0	1100	830	500		
4	582.5	1100	830	500		
5	584.8		830	500		
6	584.8	1000	830	500		
7	587.0	1100	800	500		
8	589.5	1000	800	500		100 mg l(-)Proline 100 mg l-Hydroxproline
9	592.0	660	1050	1500		NH_3-313 mg/l OrgN 36 gm/l
10	595.7	1520	1050	1000		
11			1440	1000		
12	605.5		1440	1000		
13	611.5	1100	1440	1500		
14	617.0	1450	1450	1500		
15	622.8	1650	1600	1500		
16	629.3	2050	1400	1000		
17		1850	1500	1000		
18			1670	1000		
19			1670	1000		
20	652.7	740	1670	2000		200 mg l(-)Proline
21	658.8	1300	1700	1500		
22	665.0		1630	1500		
23	672.2	1650	1630	1500		
24	680.1		1900	1500		
25	686.9	1470	1900	2000		

TABLE C-I (Continued)

ASSAY DIGESTER I
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
26	694.8		1950	2000		
27	704.0	2200	1950	1500		
28	710.5		2100	1500		
29	720.0	1550	2100	2000		pH 720 Alk 5100
30	728.2		2100	2000		
31	737.1	1900	2100	1500		40 mg l-hydroxyproline
32	745.3		2000	1500		
33	752.5		2000	1500		
34		1200	2000	2000		
35	769.4		2000	2000		
36	779.5	1840	2000	1500		100 mg KI
37	787.0		2100	1500		
38	795.4	1250	2100	2000		100 ml A
39	801.7		1900	2000		pH 7.05 Alk 5000 Feed stopped
40	701.4		1900	2000		
41	820.7	2400	1900	1000		
42	828.0	1840	1900	1500		
43	836.0		2270	1500		
44	844.4	900	2270	2500		
45	854.4		2550	2500		
46	864.0	1400	2550	2500		
47	874.5		2400	2500		
48	886.8	2200	2400	2000		
49	895.5		2750	2000		
50	907.0	1470	2750	2500		

TABLE C-I (Continued)

ASSAY DIGESTER I
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
51	918.2	1300	2800	2500		
52	930.7	1450	2800	2500		100 ml E pH 7.05 Alk. 5200
53	941.0		3000	2500		
54	952.8		3000	2500		
55	967.0	900	3000	3500		Vol. solids 680 mg/l OrgN 96 mg/l
56	982.3	1650	3200	3500		
57	997.2	2150	3200	3000		100 ml A & E & Trace Co, Mo, V, Mn
58	1111.0	1450	3500	3500		
59	1026.8	2050	3500	3500		
60	1044.3	2400	3500	3000		
61			3600	3000		
62	1073.5	1750	3600	4000		
63			4000	4000		
64	1109.0	2400	4000	4000		
65	1128.		4400	4000		
66	1146.8	3200	4400	4000		pH 6.80 Alk - 3000
67	1164.7	2800	3700	3500		pH 6.60, 6 g Alk. added pH after 6.80
68			3700	3500		
69	1195.8	3100	4000	3000		
70			4000	3000		
71	1228.0	2200	4000	3000		
72			4200	3500		
73	1258.7	1600	4200	4000		pH 7.00, Alk 5300, 200 mg B
74	1276.	2400	3500	3000		
75			4000	3500		
76	1309.4	1450	4000	4000		

TABLE C-II

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Con. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
1	91.3		600	400	330	
2	93.2	1670	600	400		
3	94.4		680	400		
4	95.7		680	400		
5	97.5	1420	680	500		
6	99.1		500	500		
7	101.4		500	500		
8			500	500		
9	103.6	2200	500	300		4ml A & E daily & NaAc
10	104.6		500	300		
11			500	300		
12	107.0		500	300		
13	108.5	2200	500	300		
14	110.0		550	300		
15	111.6		550	300		
16	113.1	2050	550	300		
17			690	300		
18	116.4		690	300		
19	119.2		690	350		
20			690	350		
21	123.6		690	350		
22	125.4		690	350		
23	127.2	900	690	600		
24	130.1		780	600		
25			780	600		

TABLE C-II (Continued)

ASSAY DIGESTER -
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
26	136.0		780	600		
27	138.3		780	600		
28	140.5		780	400		
29			780	400		
30		640	780	700		
31	148.2		860	700		
32	150.8		860	700		
33	153.7		860	700		Mixture A
34	156.7		860	700		
35	160.3		860	700		
36	164.3		860	700		
37	168.0	900	860	1000		$NaVO_3$ & Mo powder
38	171.6		1100	1000		
39	176.1		1100	1000		
40	180.8	1250	1100	1000		
41	184.7		1200	1000		
42	189.2		1200	1000		
43	194.0		1200	1000		
44	198.2	1100	1200	1200		
45	203.6		1200	1200		
46	209.0		1200	1200		
47			1200	1200		
48	217.8		1200	1200		
49	223.6		1200	1200		$2gMgH_4(PO_4)_2$ $3H_2O$ & 2g K_3HPO_4
50	227.4		1200	1200		

TABLE C-II (Continued)

ASSAY DIGESTER 1
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Conc. mg/1	Utilization Rate mg/1/day	HAc Feed Rate mg/1/day	NaAC Feed Rate mg/1/day	Remarks
51	233.0	2440	1200	700		
52	236.8		1070	700		
53			1070	700		
54	246.9		1070	1000		
55			1070	1000		
56	255.0		1070	1000		
57			1070	1000		
58		2460	1070	800		
59			1150	800		
60			1150	800		
61	277.2		1150	900		
62	281.6		1150	900		
63	287.0		1150	900		k, Ca, Mo, Cm, Mn, Co, Zn, I, Si
64	288.7		1150	900		Thiamine & 50 mg/1 S = NH_3-N=120 mg/1
65	291.8	1900	1150	900		Org N = C8 mg/1
66	296.4		1370	900		
67			1370	900		
68	309.0	1100	1370	1500		
69	314.0		1780	1500		
70	321.7		1780	2000		
71	328.6		1780	2000		
72	339.6	1770	1780	1800		
73	344.3		2280	1800		
74			2280	1800		
75	368.7	920	2280	2000		

TABLE C-II (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
76	378.6		2280	2000		
77	387.0	770	2280	2500		
78	398.0		2300	2500		
79	410.0	1600	2300	2100		
80	419.3		2700	2100		Trace of Thiamine
81			2700	2100		
82	443.0	370	2700	4000		
83	456.5	2850	1700	1000		
84	466.7	1150	2900	3000		
85			2950	3000		
86	494.2	1619	2950	2500		
87			3000	2500		
88			3000	2500		
89	533.1	681	3000	3000		
90			3100	3000		
91	558.3	920	3100	3000		
92			2900	3000		
93	588.3	1500	2900	2800		
94	599.6		2650	2000		
95			2650	2000		pinch
96	625.8	950	2650	3000		K, Ca, Mo, Cu, Mn, Co, Zn, I, Si
97	638.4		3000	3000		
98	652.3	1340	3000	3000		
99	665.9		3070	3000		
100	681.6	1600	3070	3000		Mixture B pH 690 Alk 3600

TABLE C-II (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
101	694.0	1550	3200	3000		
102	710.6		3200	3000		
103	726.5	1520	3200	3000		
104	738.5	1250	2950	3000		NH_3-N=0 mg/l 100 ml A & E NH_3=90 mg/l
105	754.1	2020	2950	3000		
106	769.0		3700	3000		100 ml A & E NH_3 = 90 mg/l
107	786.9	1050	3700	4000		
108	821.8		3900	4000		NH_3-N-84 mg/l OrgN-150 mg/l
109	821.8		3900	4000		
110	843.3	2100	3900	3500		180 ml each A & E 200 mg Thiamine & 100 mg d-Pantotheme Acid
111	860.0	1300	4000	3500		
112	879.5	1500	4000	4000		
113	900.0		4750	4000		
114	921.4	380	4750	6000		
115	943.6	2570	4000	4000		NH_3-220 mg/l Org-N - 158 mg/l
116	963.5		4400	4000		
117	986.4	2160	4400	4000		
118	1006.5	2000	4400	4000		
119	1026.3	2100	4100	3700		
120	1051.1		4170	3700		
121	1063.0	1560	4170	4000		
122	1082.4	1300	4500	4000		
123	1104.7		4500	4000		
124	1123.2	500	4500	5000		10 mg/l 7.00 Alk. 4000
125	1142.5	2400	3200	2000		

TABLE C-II (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
126	1151.3	400	4200	4500		
127	1174.2	1000	4300	4500		
128	1196.7	1200	4300	4200		
129	1217.5	1390	4200	4200		100 mg $CoCl_2$ 6 H_2O NH_3-N=320 OrgN=188
130	1239.0	1170	4500	4200		
131	1259.0	1170	4500	4500		
132	1277.3		4850	4500		
133	1301.0	850	4850	5000		
134	1322.3	1380	5400	5000		
135	1350.0	400	5400	5000		
136			4950	5000		
137	1381.8	1470	4950	5000		
138			4950	5000		
139	1445.5	1470	4950	5000		
140	1469.0		5200	5000		
141	1495.2	1840	5200	5000		100 mg KI
142	1518.0	1380	5200	5000	500	2 ml A & E/l/d
143	1543.0	1650	5200	5000		
144	1563.5		5200	5000		
145	1589.0	1700	5200	5000		
146	1610.7	2000	4900	4500		5 gm Iron Powder
147	1634.0	2100	4600	4000		
148	1655.5	1470	4800	5000		200 mg $CoCl_2$ 6 H_3O
149	1677.8	2130	4550	4000		
150	1699.5	2020	4300	4000		20 mg Thiamine

TABLE C-II (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
151	1720.7		5000	4000		
152	1742.7	650	5000	5000		
153	1764.2		4600	4000		
154	1784.0	1000	4600	5000		
155	1807.0	1840	4400	4000		NH_3-230 mg/l OrgN - 218 mg/l
156	1830.0	1070	5000	4500		
157			5000	4500		
158	1873.3		5000	4500		
159	1897.0	550	5000	5000		
160	1918.0	1520	4300	5000		200 mg $MnCl_2 4H_2O$
161	1941	1420	5400	6000		
162	1964.6	3200	4500	2000		
163		1050	4500	4500		
164			4730	4500		
165			4730	4500		
166	2048.0	1260	4730	4500		trace of k, Ca, Cu, Zn
167	2060.0	4600				25 mg/l S =
168	2064.0		1500			
169	2069.2	2200	1500			
170	2075.0		2000	2000		
171	2082.0	800	2000	3000		NH_3-226 mg/l OrgN - 190 mg/l
172	2092.7		2550	3000		[illegible]
173	2106.8	2300	2550	2500		
174	2119.8		3300	2500		
175	2133.3	1420	3300	3500		pH 7.15 Alk. 5200

TABLE C-II (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
176	2148.0		3400	3500		
177	2164.0	2200	3400	3000		5 g Bacto Peptone
178	2180.8		3300	3000		
179	2195.6		3300	3000		
180		2350	3300	2500		
181	2226.5		3200	2500		
182	2240.8	1500	3200	3000		Trace k, I, Mo, V
183	2254.0		3100	3000		
184	2268.8	1840	3100	3000		100 ml
185	2276.7		3030	3000		pH 6.70 Alk 4500 Feed stopped
186	2292.0		3030	3000		
187	2313.5	2600	3030	1500		
188	2326.2	900	3500	3500		
189	2542.2		3800	3500		
190	2359.4	940	3800	4000		
191	2378.0		3600	4000		
192	2396.0	2300	3600	3000		100 ml E & traces of Co,Mo,V,Mn
193	2410.8		4100	3000		
194	2427.0	740	4100	4500		
195	2445.8		4000	4500		
196	2465.4	2300	4000	4000		
197	2490.7	2660	3900	4000		
198	2503.3	4780	2200			pH 6.45 Alk 3300
199	2517.3	1300	3800	4000		
200	2534.4		4700	4000		
201	2556.6	600	4700	5000		Vol.solid 1360 OrgN 198 mg/l

TABLE C-II (Continued)

ASSAY DIGESTER I
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
202	2577.7	900	4750	5500		
203	2602.5	2200	4750	4000		100 ml & E & trace Co,Mo,V, Mn
204	2634.0	1300	5000	5000		
205	2648.2	1840	5000	5500		
206	2673.0	2400	5200	5000		
207			5200	5000		
208	2718.5	2600	5200	5000		200 mg Proline
209			5300	5000		
210	2766.5	2650	5300	5000		
211	2794.		5800	5500		
212	2819.4	2050	5800	6000		pH 6.80 Alk. 3000
213	2845.0	3100	5300	5000		pH 6.30 6gAlk.added pH 6.70
214			5200	5000		
215	2891.6	3300	5200	5000		
216			5900	5000		
217	2942.0	2700	5900	5000		
218			5600	5000		
219	2992.6	2600	5600	4500		pH 6.75 Alk. 4600 200 mg $CoCl_2$
220	15	2200	5200	5000		
221			5700	5000		
222	66.3	1450	5700	5500		

TABLE C-III

ASSAY DIGESTER I
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Con. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
1	293.4		700	500	300	
2	296.0	1500	700	500		pH 6.90 Alk. 3400
3	298.0		650	500		100 mg Thiamine
4	300.7	1600	650	500		
5	303.5	1650	700	500		
6	306.3	1650	700	500		
7	308.0	1650	700	500		NH_3-N=340 OrgN = 12
8	310.8		620	500		
9	313.5	1800	620	500		
10	315.3		700	500		
11	318.0	1770	700	500		
12	321.5		1250	500		
13	326.0	670	1250	800		
14			1070	800		
15	333.5		1070	800		
16			1070	800		
17	342.6	350	1070	1000		
18	346.8		1120	1000		
19	352.1	530	1120	1000		
20	357.0	600	1230	1000	500	200 mg $CoCl_2 \cdot 6H_2O$ 2ml A&E/l/day
21	362.3		1360	1000		
22	367.1		1360	1000		
23	372.2	400	1360	1500		
24	378.4		1800	1500		
25	386.4	400	1800	2000		

TABLE C-III (Continued)

ASSAY DIGESTER

RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Con, (l)	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
26	394.8	700	2000	2000		
27	404.6	900	2100	2000		
28	414.5	1030	2170	2000		
29	425.0		2700	2000		
30	436.5	250	2700	3000		
31	447.0		2500	2000		
32	458.0	850	2500	3000		
33	571.0	1170	3000	3000		NH_3 275 mg/l OrgN 115 mg/l
34	485.4	1450	3000	3000		
35			3580	3000		
36	515.7		3580	3000		
37	532.0	600	3580	4000		
38	547.3	1550	3300	4000		
39	564.0	1970	3900	4000		
40	580.7	3000	3300	1500		
41		1300	3500	3500		
42			3680	3500		
43			3780	3500		
44	643.6	1650	3780	3500		200 mg Thiamine
45	658.5	1940	3500	3000		
46	674.2		3400	3500		
47	690.6	2300	3400	3000		
48	706.5		3450	3000		
49	720.5	2000	3450	3000		
50	734.6		3500	3000		

TABLE C-III (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Con. (l)	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
51	750.3	1600	3500	3000		200 mg $CoCl_2+6H_2O$
52	763.0		3300	3000		
53	777.8	1630	3300	3000		pH 7.10 Alk. 4800
54	792.6		3300	3000		
55	807.5	1550	3300	3000		
56	822.4		3450	3000		
57	837.0		3450	3000		
58		1100	3450	3500		
59	868.0		3400	3500		
60	886.1	1950	3400	3000		100 ml A & E
61	901.2		3430	3000		
62	917.8	1240	3430	3500		
63	928.0		3400	3500		pH 6.70 alk. 4200 Feed stopped
64	942.0		3400	4000		
65	966.7	3700	3400	1000		
66	979.4	1150	3850	4000		
67	998.6		4200	4000		
68	1017.2	1370	4200	4000		
69	1038.0		4200	4500		
70	1057.0	2040	4200	4000		100 ml A & E & trace Co,Mo,V,Mn
71	1076.3		4700	4000		
72	1098.3	1470	4700	4500		
73	1120.2		4900	4500		
74	1143.8	1270	4900	5000		
75	1165.5	1740	4800	5000		

TABLE C-III (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Con. (l)	Utilization Rate mg/l/day	HAc Feed Rate Mg/l/day	NaAC Feed Rate mg/l/day	Remarks
76	1190.8	2200	4800	4500		pH 7.00 Alk. 4200
77	1210.5		5100	5000		
78	1234.0		5100	5000		
79	1261.3	2100	5100	5000		
80	1283.0	2000	5000	5000		
81	1308.0	2700	5000	4000		100 ml A & E & trace Co,Mo,V, Mn
82	1329.0	2250	5200	4500		
83	1356.0	1400	5200	5500		
84	1377.3	2100	5100	5000		
85			5550	5000		
86	1425.0	1600	5550	6000		200 mg Glycine
87			5800	6000		
88	1480.0	2650	5800	5500		
89	1509		6600	6000		
90	1537.3	1560	6600	6500		pH 680 Alk. 3000
91	1565.0	2750	5600	5500		pH 6.30 6g Alk.added pH 6.70
92			6300	5500		
93	1618.0	2600	6300	5500		
94			6600	6000		
95	1672.5	2200	6600	6000		
96			6400	6000		
97	1728.0	2600	6400	5500		200 mg B_1,$CoCl_2$ benzimidozole 100 ml A&E
98	1750.8	3350	5100	4000		pH 6.70 Alk 3900 trace Mo,V
99			5700	4500		
100	1801.4	950	5700	6000		

TABLE C-IV
ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. (mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
1	150.3		1000	800	330	
2	149.0		1000	800		
3			1000	800		
4	158.0		1000	800		
5	161.7		1000	800		Iron Filings (5 ccvol.)
6	164.2	1500	1000	800		
7	169.3		1040	800		
8			1040	800		
9	178.1		1040	1200		
10	182.6		1040	1200		
11	186.8		1040	800		
12			1040	800		
13		2050	1040	700		
14	199.6		1100	700		
15	208.5		1100	700		
16	207.8		1100	800		
17	211.8		1100	800		
18	216.4		1100	800		
19	221.3		1100	800		
20	223.5	1000	1100	1000		
21	226.5		1050	1000		
22	231.5		1050	1000		
23	236.3	1800	1050	700		
24	239.4		1120	700		
25	243.6		1120	700		

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
26	247.7		1120	700		
27	251.1	940	1120	1200		
28	255.4		1160	1200		
29	260.8		1160	1200		
30			1160	1000		
31	269.8		1160	1000		
32	275.6		1160	1000		2g $MgH_4(PO_4)$ $3H_2O$ & $2gk_2HPO_4$
33	279.7		1160	1000		
34	285.0	2000	1160	800		
35	288.7		1100	800		
36			1100	800		
37	298.7		1100	900		
38			1100	900		
39	306.0		1100	900		
40			1100	900		
41		1800	1100	900		
42			1120	900		
43			1020	900		
44	326.6		1020	900		
45	330.6		1020	900		
46	336.0	2200	1020	900		NH_3=85 mg/l OrgN = 51 mg/l
47	339.1		1100	900		
48	343.0	2200	1100	900		
49	347.0		1050	900		
50			1050	900		

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
51	356.2	2350	1050	600		
52	359.0		800	600		
53	363.0		800	600		Iron Powder & 50 mg/l $S^{=}$
54	365.0		800	600		
55	367.8	2350	800	800		
56	371.5		850	800		
57			850	800		
58	378.9	2800	850	800		
59	384.1		1200	800		
60	388.6		1200	800		
61	392.5		1200	800		
62	396.3	1970	1200	1000		
63	400.2		1200	1000		
64			1200	1000		
65	410.0	2100	1200	1000		
66	414.1		1300	1000		
67	419.0	1850	1300	900		
68			1250	900		
69	428.1		1250	900		
70			1250	900		
71			1250	900		
72	441.8		1250	900		
73			1250	900		
74	450.7		1250	900		
75			1250	900		

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
76	459.0	550	1250	2000		
77	464.0	2000	760	0		
78			900	0		
79	470.0	570	900	1000		k,Ca,Mo,Cu,Mn,Co,Zn,I,Si
80	473.0		900	1000		
81	476.6	1200	900	1000		
82	481.0		1100	1000		
83	486.5	1380	1100	1000		50 mg 7 liamine pH 7.10 Alk. 4000
84	489.8	1450	1280	1000		
85	495.0		1280	1000		
86	500.8	1200	1280	1200		100 ml A & E NH_3-N=60 mg/l
87	506.5		1500	1200		200 mg Thiamine
88	513.4	1000	1500	1500		
89	520.3		1780	1500		
90	528.9	830	1780	2000		
91	536.8	1250	1800	2000		NH_3 N 166 mg/l OrgN - 74 mg/l
92	544.7		1800	2000		
93	555.6	2480	1800	1500		180 ml each of A & E
94	563.3	1300	2300	1500		
95	572.6	1200	2300	2500		
96	583.1		2450	2500		
97	595.2	1700	2450	2500		
98	606.3	1650	2520	2500		NH_3 300 mg/l OrgN 82 mg/l
99	617.0		2520	2500		
100	629.2	2240	2520	2000		

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
101	639.9	1470	2600	2000		
102	650.2	1400	2600	2500		
103	665.7		2500	2500		
104	672.8	1750	2500	2200		
105	684.2	1650	2570	2200		
106	696.0		2570	2200		
107	707.2	1250	2570	2500		Trace Ca,Mo,Cu,Mn, pH 7.00, Alk. 3700
108	715.5	2100	1800			
109	717.0	2020	300	0		
110	718.1	1600	600	0		
111	719.1	1200	600	500		
112	721.0	1550		500		Drained to 2 l, refilled 150 ml 150 ml E &
113	721.7			500		16 g alk. NH_3-N=340 OrgN = 84
114	723.7	1200		0		
115	704.7		500	0		
116	726.0	650	500	500		
117	727.4		740	500		
118	729.7	570	740	800		
119			850	800		
120	735.1		850	800		
121			850	800		
122	743.0	1200	850	800		
123	747.5		850	800		
124	751.8	900	850	800		
125	756.2	1650	850	700	500	500 mg/l/day Ac & 2 ml A&E/l/day 200 mg Thiamine

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
126	761.0		1450	700		
127	764.1	870	1450	700		
128	768.2	280	1450	2000		
129	774.7	1150	1450	1500		
130	782.0	1400	1550	1500		
131	789.1	1470	1700	2000		
132	797.4	1800	1970	2000		
133	806.5	1840	2300	2000		
134	816.0		2300	2000		
135	826.0	1900	2300	2000		
136	836.0		2100	1000		
137	843.7	1300	2100	2000		
138	852.4	1100	2500	2000		NH_3 - 235 mg/l OrgN 68 mg/l
139	861.8	1100	2300	2000		
140			2380	2000		
141	880.8		2380	2000		
142	891.8	850	2380	2500		
143	901.6	1380	2300	2500		17 mg B_{12} with mannitol
144	911.6	1740	2400	2000		
145	921.4	2200	1800	1000		
146		1200	2300	2000		
147			2200	2000		
148			2200	2000		
149	957.4	1500	2200	2000		
150	966.6	1500	2300	2000		

TABLE C-IV(Continued

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (l)	Vol. Acid Conc. mg/l	Utilization Rate mg/l/day	HAc Feed Rate mg/l/day	NaAC Feed Rate mg/l/day	Remarks
151	976.5		2470	2000		
152	987.8	1150	2470	2500		
153	1000.0		2680	2500		
154	1010.4	1400	2680	2500		
155	1023.5		2850	2500		
156	1086.6	1300	2850	2500		
157	1047.4		2800	2500		
158	1059.6	1290	2800	2500		pH 7.05 Alk. 4900
159	1071.6		2700	2500		
160	1083.1	1550	2700	2500		100 mg Ademine HCl 100 mg dl methiomine
161	1095.0		2550	2500		
162	1105.7		2550	2500		
163		2300	2550	2000		
164	1130.2		2550	2000		
165	1141.3	1800	2550	2000		Trace Mo,V
166	1151.0		2550	2000		
167	1161.5	1290	2550	2500		100 ml A
168	1169.0		2100	2500		pH 6.80 Alk. 5000 Feed stopped
169	1178.0		2100	2500		
170	1192.6	3350	2100			
171	1199.5	1900	1750	1500		
172	1207.7		2050	1500		
173	1216.0	1420	2050	2000		
174	1225.8		2000	2000		
175	1234.5	2000	2000	1500		100 ml E & trace Co,Mo,V,Mn
176	1243.3		2300	1500		
177	1253.0	900	2300	2500		

TABLE C-IV (Continued)

ASSAY DIGESTER
RECORD OF OPERATION ON ACETATE SUBSTRATES

Day	Gas Reading (1)	Vol. Acid Conc. mg/1	Utilization Rate mg/1/day	HAc Feed Rate mg/1/day	NaAC Feed Rate mg/1/day	Remarks
178	1263.7		2400	2500		
179	1275.5	1700	2400	2500		
180	1286.3	1470	2750	2000		
181	1298.5	1300	2750	2500		pH Alk.5000
182	1309.5		2800	2500		
183	1321.6		2800	2500		
185	1347.7	950	2800	3000		vol. alk 1150 mg/1 total N-500 mg/1 Org.N 100 mg/1
186	1362.0	1700	2800	2500		100 ml A & E & trace Co,Mo,V, Mn
187	1373.0	1450	3000	2500		
188	1385.5	1250	3000	3000		
189	1401.0	1400	3100	3000		
190			3550	3000		
191	1429.0	900	3550	4000		200 mg B_{12} with mannitol
192			3900	4500		
193	1464.6	2200	3900	4000		
194	1484		3900	4000		
195	1501.3	3100	3900	2500		pH 6.80 Alk. 3000
196	1517.6	2400	3500	3500		pH 6.70 6g Alk. added pH 7.00
199			3800	3500		
198	1549.0	2500	3800	3500		
199			3900	3500		
200	1584.3	2250	3900	3000		
201			4000	3500		
202	1618.6	1400	4000	4000		pH 6.85 Alk. 5400 200 mg B_1
203	1636.2	1600	4100	4000		
204			4500	4000		
205	1677.0	1300	4500	4500		

APPENDIX C

Components of Mixture A fed to Digester II on day 33.
Trace amounts - 1 to 5 mg - of each of the following:

Vitamin B_{12} with mannitol
Adenine HCl
Protease
Guanine HCl
Thiamine HCl
Cytidine SO_4
Xanthine
Uracil
Powdered catalase 15
Pepsin
Thymine
Hemicellulose
Lipase

APPENDIX C

Components of Mixture B added to Digester II on day 100.
Trace amounts of each of the following:

dl-B-Phenylalanine
Papain powder
Thymine
d-Pantothenic acid-calcium salt
Glutathione
Pyridoxine HCl
Guanine HCl
Cytidine SO_4
Xanthine
l-(-)-Leucine
Vitamin B_{12} with mannitol
Uracil
Adenine HCl
l-Valine
i-Inositol
l-Cystine
l-Aspartic
dl-Methionine
d-Arginine mono HCl
l-Histidine mono HCl
l(-) Proline
l(+) Lysine mono HCl
dl-a-Alanine
dl-Serine
dl-Ornithine HCl
l-Threonine
l-Phenylalanine
dl-Tryptophan
Ficin
l-Cysteine
dl-Isoleucine
Glycine
Para aminobenzoic acid
Nicotinic acid
Ascorbic acid
Choline chloride

APPENDIX D

RESULTS OF MICROCHEMICAL ANALYSES

MICROCHEMICAL ANALYSIS OF BIOLOGICAL SOLIDS

Digester	Detention Time	% Carbon	% Hydrogen	% Nitrogen	% Phosphorous	% Ash
Acetate	5	19.62	4.34	4.64		47.47
Acetate	10	29.01	5.05	6.70		35.37
Acetate	15	28.87	4.52	6.85		36.93
Acetate	20	31.86	5.13	8.69		32.41
Acetate	25	32.62	4.72	7.05		28.03
Acetate	30	35.23	6.46	8.28		24.39
Acetate*	30	50.31	6.85	13.35	0.80	2.26
Octanoate	5	43.99	5.43	11.18	1.33	9.36
"	30	47.21	6.45	11.99	1.07	4.82
Glycine	5	41.37	5.53	10.09	1.26	7.70
"	30	41.15	6.15	9.52	2.34	10.88
Leucine	7.5	40.31	5.88	9.43	1.03	9.22
"	30	37.49	5.81	10.74	1.12	5.29
Nut.Broth	5	42.30	5.72	11.91	1.04	9.75
" "	30	34.09	5.23	10.46	1.16	16.40
Glucose	5	38.26	5.50	9.32	3.51	20.10
"	10	42.59	5.91	9.77	3.10	13.98
"	20	43.10	6.17	8.46	3.39	11.12
"	30	32.97	5.68	7.85	3.72	26.09
Starch	5	44.09	6.28	10.05	1.71**	6.19
"	30	47.85	6.76	10.43	1.23**	5.66

* Solids taken from a Continuous Feed Digester
** Values obtained from a separate sample.

BIOGRAPHY

Name: Richard Eugene Speece

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Secondary: Whetstone High School
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College: Fenn College
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Professional Experience:

1952 Draftsman
Galion Iron Works
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1953 Engineer
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1954 Inspector
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