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APPLICATION OF PROCESS KINETICS FOR PHASE SEPARATION OF THE ANAEROBIC STABILIZATION PROCESS

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APPLICATION OF PROCESS KINETICS FOR PHASE SEPARATION OF THE ANAEROBIC STABILIZATION PROCESS

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SUMMARY

The anaerobic stabilization process has long been used as a biological treatment method for wastes containing substantial amounts of organic material. The process has true utility due to its ability to convert waste material to a useful by-product, methane. However, due to the past difficulties in controlling the process and the large capital expenditures often required, this potential has not been realized and the process has been usually relegated to a secondary position as a means of sludge treatment prior to ultimate disposal.

The process is frequently considered to occur in a biphasic mode, with complex organic compounds being converted to simpler intermediates such as the volatile fatty acids in an "acid fermentation" phase followed by further conversion to methane and other end products in a "methane fermentation" phase. These biochemical conversions are correspondingly considered to be attributable to the activities of two rather distinct populations of microorganisms that must exist in a symbiotic relationship for optimum process efficiency to occur. If this rather delicate balance between acid production and utilization in the anaerobic process is upset, the faster growing acid bacteria may overwhelm the more sensitive methane bacteria to the point that the environment

has been adversely altered and the entire process fails.

It has been suggested by some researchers [3,4] that a more logical means of structuring and controlling the process to achieve maximum stability and efficiency is to separate the activities of the two major microbial population groups in order to protect the more sensitive methane forming bacteria from potential upsets by optimizing requisite environmental conditions. Pohland and Ghosh [3] have proposed that this could be accomplished by taking advantage of the different growth rates of the two populations and exerting kinetic controls to achieve the phase separation.

Some research has been initiated to provide such control by operating processes in this fashion. Ghosh, et al. [8] have reported on the operation of a two-phase anaerobic process utilizing sewage sludge as the primary substrate. However, difficulties in the measurement of active organism concentration presented problems in estimating kinetic parameters for the two phases. Ghosh and Pohland [9] and Pohland and Massey [10] have succeeded in estimating kinetic parameters for the two anaerobic phases by successively decreasing hydraulic retention time in a single anaerobic reactor to a point where acid production predominated.

These studies have been extended by current research devised to investigate the separation of both the acidogenic and methanogenic phases by kinetic means. Accordingly, the study reported herein was formulated to pursue the following

major objectives:

(1) Demonstration that phase separation could be achieved by the exertion of kinetic controls on a two-stage biological reactor system;

(2) Observation of the effect of cell recycle on the operation of both phases of the anaerobic stabilization process and the practicality of gravity clarification for solids concentration and recycle;

(3) Demonstration of the utility of mathematical models based on Monod bacterial growth kinetics for describing both the acidogenic and methanogenic phases of the process; and,

(4) Estimation of the values of kinetic parameters for each phase when applied to simple and complex substrates.

To achieve these objectives, a series of steady state experiments were conducted with a simple soluble synthetic substrate and a complex industrial waste. The experimental apparatus used during the studies consisted of two ten-liter biological reactors operated in series so that the effluent from the first phase could serve as the influent to the second phase. Each reactor had an associated clarifier to permit biomass capture and recycle. The total system was fed continuously until steady state conditions were attained as indicated by organism and COD concentrations; a usual minimum of three retention times. COD, suspended solids, pH, volatile acids, alkalinity, and gas production and quality were

routinely monitored across the system.

For the simple substrate (glucose), the system was operated both with and without cell recycle over a seven-month period. Phase separation was achieved by operating the first reactor at a dilution rate that exceeded the maximum specific growth rate of the methane formers. Acid production was maximized in the first reactor with virtually complete conversion of the substrate to volatile acids and biomass. Methane generation was encouraged by environmental control in the second reactor where acidogenic bacterial growth was minimized due to a lack of acceptable substrate.

The data was analyzed to determine the utility of a mathematical model using Monod bacterial growth kinetics for process prediction and design. The model eventually selected and presented is a refinement of that proposed by Ramanathan and Gaudy [83] and includes the recycle organism concentration as a variable, rather than reliance on a concentration factor as used by Herbert [82].

After completion of studies with the simple soluble substrate, additional data were obtained on the treatment of an industrial waste (candy manufacturing effluent) by the two-phase anaerobic stabilization process. These data served to substantiate the two-phase anaerobic stabilization concept and the utility of phase separation and control for efficient application of the anaerobic stabilization process in conventional practice. A design and operational procedure

is presented to permit realization of optimum capacity in both the acid and production phases and kinetic parameters are presented and compared for both the simple and complex substrates.

CHAPTER I

INTRODUCTION

The heightened public awareness of the steady decline in quality of the national water resources led to enactment of stringent new effluent standards for wastewater discharges. To meet these new standards, the Environmental Engineer must be more innovative in selecting treatment processes that are capable of economically meeting effluent quality parameters. The rapidly escalating cost of energy needed to fuel these highly efficient processes has led to increased interest in processes that are not energy intensive. The impetus of these pressures has resulted in research directed toward minimizing energy requirements of existing processes and the development of new pollution control strategies. Much of this research effort has been directed toward improvements in biological process techniques for treatment of wastewaters.

Biological Processes

Biological processes have long been routinely applied to the treatment of domestic, industrial and agricultural wastewaters. The conversion of waste organic material into acceptable end products such as carbon dioxide, methane and nitrogen is the prime objective of biological processes. This is accomplished by making use of the ability of a

microbial population to utilize dilute organic wastes as an energy source for growth and proliferation.

The biological conversion of organic matter by microbial cells is the result of a series of coupled biochemical reactions, each reaction being mediated by biological catalysts termed enzymes. The major portion of the energy derived from these reactions is stored within the microbial cell in the energy rich phosphate bonds of compounds such as adenosine triphosphate (ATP). The stored energy within the microbial cell is subsequently utilized for synthesis processes such as cell maintenance or cell reproduction. The end result is that only a fraction of the organic wastes can be converted to end products such as CO_2 or CH_4; a substantial portion ends up as biomass which must be subsequently removed and properly discarded. Therefore, successful treatment of a soluble organic waste creates a solid residue to which further treatment and ultimate disposal techniques must be applied.

A successful harnessing of the ability of a microbial cell to mediate the desirable destruction of waste organic material calls for careful control of the microbial environment. In most every case facing the Environmental Engineer, the wastewater contains a vast array of complex organic compounds, with the composition of the wastewater continually changing. Thus the microbial population present is not a pure culture, but of a heterogeneous nature, with the dominant

culture continually changing to respond to fluctuating conditions. By proper manipulation of the microbial environment, selection of microorganisms that are well suited for the task at hand can be accomplished.

Thus biological processes should be designed to carefully create and control an artificial ecosystem for the sheltering and proliferation of the appropriate microbial complement that is capable of providing the desired results. Physical and chemical factors are manipulated within the relatively narrow ranges necessary for maximum attainable growth rates of the appropriate population.

One important aspect of the microbial environment is the presence or absence of dissolved oxygen in the aqueous medium. Thus, biological processes may be classified as either aerobic or anaerobic depending on their dependence or independence of the presence of free oxygen. Organisms having inefficient processes for synthesis of macromolecular cellular constituents require more energy from the oxidation of organic molecules to provide this energy requirement. Due to the existence of the respiratory chain in aerobic organisms and their capacity for the use of oxygen as a terminal electron acceptor, energy production is a more efficient process in them than in those species (e.g., the anaerobes) not endowed with this capacity. Thus, the anaerobes require proportionately more oxidation of organic molecules for energy per unit of biomass than the aerobes;

an advantage in terms of dealing with the problem of ultimate biomass disposal from biological processes.

Aerobic biological processes have predominated in recent years due partly to the less rigid environmental requirements of the hardy aerobic microorganisms. The increased energy available from the respiratory process also leads to higher growth rates than for the slower growing anaerobes. Thus, the length of time required to achieve a particular reduction in organic concentration is shorter and capital investment in treatment facilities is minimized. However, certain unique advantages of anaerobic biological processes have impelled researchers to reconsider this treatment alternative.

Anaerobic Biological Processes

Among the biological treatment alternatives, anaerobic stabilization processes offer several significant advantages including: (1) a high degree of conversion of available organic carbon to gaseous end products; (2) low production of biomass due to the lack of the respiratory pathway for the anaerobic bacteria; and, (3) generation of product gases high in recoverable methane content. With increasing costs of energy, this latter advantage becomes particularly significant and the anaerobic stabilization process is receiving renewed attention for energy recovery from a wide variety of waste organic materials.

The most frequent application of the anaerobic stabilization process has been to sludges produced during primary and secondary treatment of domestic wastewater where it has served a most useful function despite occasional problems with instability and control. The control problems that have arisen, however, have led to the characterization of the process as being difficult to operate thereby causing the process to fall in some disfavor among treatment plant designers and operators. These problems may be attributed to the multiphase nature of the process wherein complex organic compounds are sequentially converted through simpler intermediates eventually to gas and comparatively inert residues. Because of the consistent appearance of volatile organic acids as measurable intermediates, it has become convenient to simplify this sequential conversion pattern into two phases; the first or "acid fermentation" phase leading to the production of intermediate products predominated by the volatile organic acids, and the second or "methane fermentation" phase resulting in the conversion of these intermediates to stable end products, principally methane. According to current practice, the biochemical conversions occurring during the process may be considered attributable to the activities of at least two rather distinct populations of microorganisms that must exist in a symbiotic relationship to ensure consistent process efficiency.

As has been described previously [1,2,3], if a rather delicate balance between volatile acid production and utilization is upset, the more rapid growing acid forming bacteria may overwhelm the sensitive methane forming bacteria to a point where the environment has been adversely altered and the process fails to satisfy its intended purpose. Moreover, normal operating procedure has called for both of these phases to coexist in a single reactor, operated so as to provide suitable environmental conditions for the slower growing and sensitive methane forming bacteria. Even with the advent of stage digestion, the second stage has served mainly as a holding and sludge concentrating system. Therefore, in the first or actively digesting stage, the temperature, pH and organic loading are operationally controlled to optimize environmental conditions for the methane forming bacteria, possibly at the expense of the acid forming bacteria and process efficiency as a whole. Accordingly, present designs usually provide relatively long hydraulic retention times to minimize the possibility of process upset and to ensure an acceptable degree of stabilization. Such anaerobic digestion installations are thereby often capital intensive and require careful monitoring of process behavior in order to forestall possible failures.

Phase Separation

Problems with stability and control have suggested an evaluation of the feasibility of separating the acid and

methane fermentation phases by employing a two reactor system; the first receiving raw or preconditioned wastes, and the second receiving the effluent from the first with or without interphase adjustment. With the process so structured, more attention could then be directed toward determining and providing optimal environmental conditions for each separate microbial community, and organic loading and recycle requirements could thereby be controlled individually to enhance overall process efficiency. Of particular significance, the slower growing and more sensitive methane forming bacteria would be effectively sheltered from potential upsets by close monitoring of the acid fermentation reactor effluent and elimination of potential problems before the methane forming bacteria were subjected to the impending stress.

A variety of methods could be conceived to provide physical phase separation including the dialysis techniques suggested by Hammer and Borchardt [4] and Schaumburg and Kirsch [5] and the addition of inhibitors such as oxygen, nitrates, sulfates or metals, or oxidation-reduction potential poising or control [6,7] to either preclude or encourage the growth and proliferation of the methane forming bacteria. In contrast, the method reported by Pohland and Ghosh [2,3], and extended herein, uses the difference in growth rates of the microbial populations responsible for acid and methane production to provide population selection through manipulation of the hydraulic retention time and recycle. Figure I-1

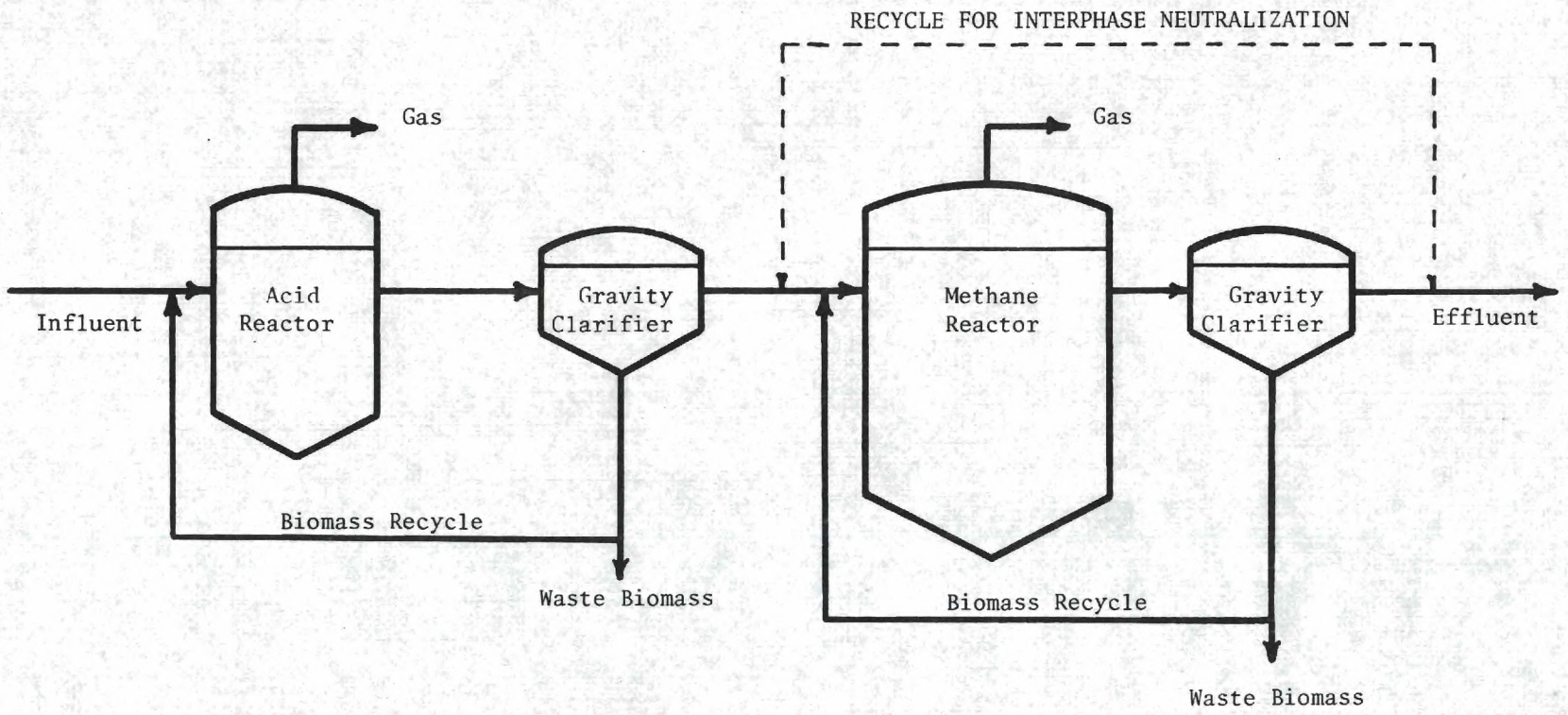


Figure I-1. Proposed Two Phase Anaerobic Digestion Process

illustrates a possible flow configuration for a two phase reactor system capable of providing such separation and optimization of acid and methane formation during anaerobic stabilization of waste organic substrates. With this system, appropriate selection of the hydraulic retention time in the first or acid reactor as determined by kinetic analysis would tend to preclude the development of significant populations of methane forming bacteria and thus encourages volatile acid accumulation. The effluent from the reactor thus becomes available to the second or methane reactor where the hydraulic retention time and recycle may be adjusted to provide for optimum growth of a methane producing population directly responsive to the substrate loading. Such a technique also provides opportunity for external regulation of the substrate pH with either acid or base additions in order to counteract the possibility of pH sensitivity. Moreover, growth of acid forming bacteria in the second reactor is suppressed due to a lack of acceptable substrate particularly when soluble-type wastewaters are being treated. Opportunities for further enrichment of the respective bacterial populations would also be provided by optimization of biomass separation and recycle.

Research Objectives

Some preliminary research has been conducted to provide phase separation of the anaerobic stabilization process by kinetic control. Ghosh, et al. [8] have reported on the

operation of a two-phase anaerobic process utilizing sewage sludge as the primary substrate. However, difficulties in measurement of active organism concentrations presented problems in estimating kinetic parameters for both of the phases and the reactor system did not include provisions for biomass separation and recycle. Ghosh and Pohland [9] and Pohland and Massey [10] have estimated kinetic parameters for the two phases by employing simple soluble substrates and by successively decreasing hydraulic retention time in a single, continuous flow and completely mixed reactor to a point where volatile acid production predominated. These initial studies have provided a basis for extended research efforts into the potential for separation of both the acidogenic and the methanogenic phases of the anaerobic stabilization process by kinetic control. The effort reported herein was focused on the following objectives:

(1) Demonstration that phase separation can be achieved by exertion of kinetic controls on a two-stage biological reactor system operated under anaerobic conditions;

(2) Determination of the effect of biomass recycle on operation of both phases of the anaerobic process and the practicality of gravity clarification for biomass separation;

(3) Demonstration of the utility of mathematical models based on bacterial growth kinetics for describing

both the acidogenic and methanogenic phases of the anaerobic stabilization process;

(4) Determination of appropriate kinetic parameters for each phase when operated with simple and complex soluble substrates; and,

(5) Development of control strategies for application of phase separation to anaerobic stabilization in conventional practice.

CHAPTER II

REVIEW OF LITERATURE

The decomposition of organic matter by microorganisms existing under anaerobic conditions occurs widely in natural environments. The degradation of organic deposits in the anaerobic strata of lake, river and ocean bottoms with the subsequent release of carbon dioxide and methane is commonly reported and is responsible for destruction of large quantities of organic material. Early researchers discovered methane in the neighborhood of decomposing vegetation in bodies of water and in soil and established its origin in microbial metabolism [11]. Anaerobic microbial metabolism also occurs in the alimentary tracts of herbivorous animals for digesting vegetable foodstuffs and much of the research performed on the bacteria and reactions that take place in the rumen is also applicable to the study of anaerobic stabilization processes.

The use of anaerobic stabilization processes for the treatment of waste organic matter prior to final disposal dates back to the 19th century. Buswell [12] has traced the evolution of anaerobic treatment processes from their early beginnings to the advent of controlled digestion of waste sludges, as is common practice at this time. Initial

application of the process was to capitalize on the ability of anaerobic bacteria to solubilize organic matter. Subsequent advances in the application of the process were made to achieve stabilization of the waste organic solids with concomitant generation of useful product gases, especially methane. Design advances were made to optimize rates of decomposition and improve operational stability, with the following being the more important: (1) initial separation of the sedimentation and digestion processes; (2) provisions for heating to provide optimum temperatures for growth of the anaerobes; (3) control of feed rates to provide improved stability; (4) improved mixing to insure intimate microorganism-substrate contact; and (5) development of system modifications such as the anaerobic contact process to facilitate increases in microorganism concentration within the process.

Although these advances have imparted significant process improvements, the continued use of anaerobic digestion has fallen into disfavor in recent years primarily because of problems associated with control of the process during periods of upset and the large digester volumes and long retention times normally associated with the process. Clearly, additional design advances are required to take advantage of the unique properties of the anaerobic process.

Nature of the Anaerobic Stabilization Process

The anaerobic stabilization process is generally considered to involve sequential conversion of complex organic compounds to recognizable intermediates, primarily volatile organic acids, which are further converted to gaseous end-products, methane and carbon dioxide. At least two large, physiologically diverse microbial populations must be present to mediate the conversion of complex organics to methane and carbon dioxide. In the initial stage, a heterogeneous group of microorganisms convert complex soluble and insoluble organic compounds into primarily the volatile organic acids by hydrolysis and fermentation. In the final stage, methane and carbon dioxide are produced by a unique group of strict anaerobes classified as the methane bacteria. It is important that the stages of volatile acid production and utilization be kept closely balanced to prevent process upset.

The following review will deal with a discussion of literature concerning the acid fermentation and methane fermentation phases of the anaerobic stabilization process. Also included will be a discussion of the environmental requirements, operating and control variables and strategies, and a description of process configurations that may be used.

Acid Fermentation Phase

The conversion of complex soluble and insoluble organic compounds during anaerobic stabilization into

intermediate products suitable for the metabolic activities of methane bacteria has been termed the acid fermentation phase. During this conversion, large solid particles of organic matter are converted to soluble forms by an enzymatic process that has been termed liquefaction [13,14]. Further microbial processes then convert these soluble organic compounds into simpler organic molecules, primarily the volatile organic acids, as well as hydrogen and carbon dioxide.

In the acid fermentation phase, energy for bacterial processes is obtained through oxidation-reduction reactions; however, unlike aerobic processes, dissolved oxygen is not present as an electron acceptor. Thus, electrons are either transferred from one organic compound to another, resulting in a more reduced species, or released as hydrogen by a hydrogenase system. Only for a small number of bacteria can an inorganic ion, such as nitrate or sulfate, act as an electron acceptor [94]. Thus, true stabilization in the form of a reduction in oxidizable organic concentration is not normally achieved in the acid fermentation phase.

The preparatory stage of the acid fermentation phase is termed liquefaction. The process proceeds by enzymatic attack which hydrolyzes complex polysacchrides to simple sugars, proteins to peptides and amino acids, and fats to glycerol and fatty acids. Thus, the organic compounds are rendered soluble so that they may be transported through the

bacterial cell wall and made available for intracellular metabolism.

The decomposition of large molecules into simpler components by microbial action has been reviewed by Rogers [15] with the following methods of attack postulated: (1) by extracellular enzymes from bacterial secretions into the surrounding medium; (2) by intracellular enzymes in the medium due to cell lysis; and (3) by cell contact with the solid particles and the probable action of highly active surface enzymes. There is some evidence of direct contact between the bacterial population and organic solids in sewage sludge anaerobic digestion [14]. By this means of attack, the extracellular enzymes are not diluted by the surrounding medium and the products of hydrolysis are diffused directly into the cell.

The observation that digestion of primarily soluble wastes proceeds more quickly than for sewage sludges has led to speculation that liquefaction may be rate limiting in the anaerobic stabilization process. However, Kotze, et al. [16] have concluded that hydrolytic activity catalyzed by extracellular enzymes did not appear to be a rate limiting step during digestion, though the surface area of the organic particle exposed to enzyme activity might limit the rate of liquefaction. Furthermore, Ghosh and Klass [8] have demonstrated that liquefaction and acid fermentation can take place in less than two days retention time during two-phase

anaerobic stabilization of a sewage sludge, whereas methane fermentation required 2 to 7 days to proceed efficiently. It seems apparent also that substrate composition would have a major effect on the rate of liquefaction with cellulose being very slow to degrade anaerobically [94]. The use of heat and chemical pretreatment is sometimes considered to accelerate this step in the stabilization of substrates that are difficult to degrade [117].

The liquefaction stage results in the formation of simple sugars, amino acids, glycerol and long-chain fatty acids from the complex organics originally present. These are further degraded by microbial metabolism to simpler intermediates, most noticeably the volatile fatty acids. The importance of the volatile acids as intermediates and their importance as an indicator of digestion efficiency has long been recognized [17,18]. These observations have been further reinforced by later studies as detailed by Pohland [19] in his initial review of literature on anaerobic sludge digestion.

The fermentation of carbohydrates such as glucose proceeds by both the Embden-Meyerhof pathway and the hexose monophosphate shunt, as demonstrated by tracer studies performed by Jeris and McCarty [20]. Of the two, the Embden-Meyerhof pathway shown in Figure II-1, appears to be the more important [16]. Wood [21] has illustrated the production of various volatile acids from pyruvate, the common

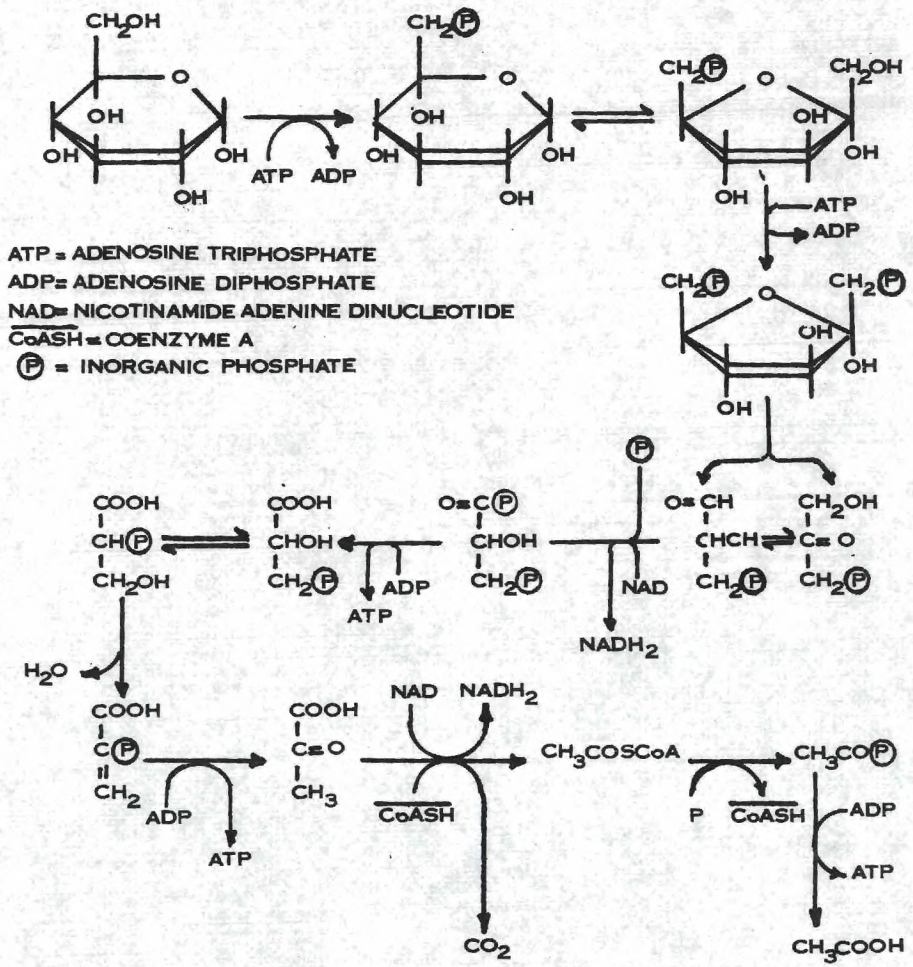


Figure II-1. Embden-Meyerhof Pathway for Degradation of Carbohydrates. After Mosey [35]

intermediate product of the fermentation of carbohydrates, glycerol and several amino acids [21].

The degradation of liquids proceeds by the initial hydrolysis to glycerol and fatty acids by the enzyme lipase. The long chain fatty acids are then degraded by β-oxidation, as demonstrated with tracer studies using octanoic and palmitic acids [20]. The usual pathway of β-oxidation is shown in Figure II-2. The even-carbon fatty acids are converted to acetate fragments and the odd-carbon fatty acids are oxidized to acetate with the final three carbon fragment being converted to propionate.

The degradation of proteins is initiated by hydrolysis of the protein into polypeptides and finally into amino acids. The subsequent fermentation of the amino acids may follow several pathways with the primary products being the volatile fatty acids and ammonium ions [23]. Additional information concerning the pathways of fermentation is available in reviews by Pohland [19], Kotze, et al. [24] and Torien and Hattingh [25].

The major volatile acids usually detected in quantity during the anaerobic stabilization process have been acetic, propionic and butyric acids. In addition, formic, isobutyric, valeric, isovaleric and caproic acids have also been identified in digesting sludge [26,27] and compounds other than volatile acids may be formed as a result of the fermentation process. Ethanol, methanol and acetone were detected by

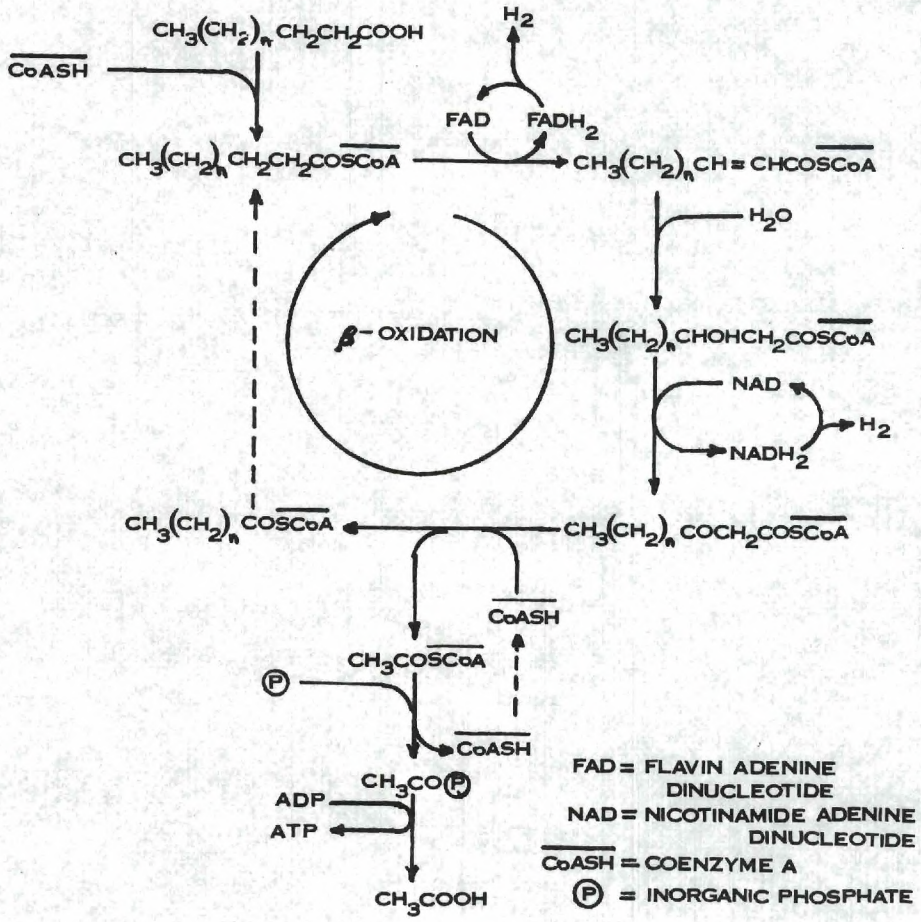


Figure II-2. β-Oxidation of Long-Chain Fatty Acids After Mosey [35]

Hindin, et al. [28] in a digester where an accumulation of volatile acids led to a distressed condition. Willimon [47] detected ethanol at times in anaerobic reactors operated on a glucose substrate at low retention times.

Acetic acid was judged to be the most important volatile acid precursor to methane during anaerobic digestion studies by Pohland and Bloodgood [29]. Radical changes in volatile acid concentrations during retarded digestion occurred especially for the acetic and propionic acid concentrations. Similar observations have also been reported by McCarty, et al. [30]. For the digestion of mixed wastes, McCarty [13] has also estimated that 70 percent of the methane formed is derived from acetate, with the balance resulting from the reduction of carbon dioxide.

The precise composition of the microbiological population responsible for the acid fermentation phase has not been well defined. Due to the complexity and changing composition of substrate introduced to most digesters, it is likely that due to population dynamics, the prominent species are constantly shifting to accommodate substrate variations. The make-up of this constantly shifting population can affect the intermediate products of fermentation during the non-methanogenic phase. For instance, many of the anaerobic and facultatively anaerobic bacteria that have been isolated from active digesters are known to produce a variety of intermediate products when studied under pure culture

conditions [94]. Among these intermediate products are hydrogen, carbon dioxide, ethanol and formic, acetic, propionic, butyric, valeric, caproic, succinic and lactic acids. However, as pointed out previously, the primary products detected in active digesters are the volatile acids and hydrogen is seldom detected in the off-gas of digesters. Thus, conditions within the digester apparently preclude the formation of these other products or they are utilized subsequently by the bacterial complement present. It seems likely that a combination of these two events do occur during digestion processes.

It has been demonstrated [95,96] that bacterial growth rate and pH affect the fermentation products resulting during continuous culture. Although anaerobic digesters normally operate around a neutral pH, it is possible that environmental conditions could be manipulated to obtain the most favorable product mix from the non-methanogenic phase and thereby also optimize subsequent methane formation. Additional work is needed to identify the non-methanogenic bacterial population as well as their response to different environmental conditions in order to determine possible control strategies for this portion two-phase anaerobic stabilization processes.

Methane Fermentation Phase

The final phase of the anaerobic stabilization process is the utilization of the intermediate products created in the acid fermentation phase by a population of sensitive and

unique microorganisms, the methane bacteria. The end products of their metabolic activities are principally carbon dioxide and methane. It is in this phase that true stabilization of oxidizable organic matter is achieved by the generation of the insoluble gas, methane. Thus, the removal of oxidizable organic matter is directly related to the quantity of methane generated.

The methane bacteria are characterized by their common ability to produce methane as a product of metabolism. They occur as sarcinae, rods and cocci and were initially considered to be non-motile, non-spore forming and gram negative [31]. However, other research [32] casts doubt on these characteristics being common for all the methane bacteria, with Methanococcus vanniellii being highly motile [11]. The methane bacteria do share many similar attributes. They are obligate anaerobes with great sensitivity to oxygen. Their substrate requirements are simple and very narrow, with some uncertainty as to the exact compounds being utilized as will be discussed later. They can grow over a wide range of temperature but apparently at a pH only near neutrality [11].

Table II-1 lists the methane bacteria that are presently maintained in pure culture. Other species not presently in pure culture include: Methanococcus mazei, Methanobacterium soehngenii, and Methanosarcina methanica [97]. These species have been lost or were never obtained

Table II-1. Properties of Taxonomically Described Methanogenic Species in Pure Culture (1977) After Zeikus [93]

Species Name	Substrates that serve as sole electron donor for both methanogenesis and growth	Autotrophic growth	Taxonomic Description
Methanobacterium[a] arbophilicum	Hydrogen	Yes	Zeikus and Henning [100]
Methanobacterium formicium	Hydrogen or formate	Yes	Schnellen [101]
Methanobacterium ruminantium	Hydrogen or formate	No	Smith and Hungate [102]
Methanobacterium mobile	Hydrogen or formate	No	Paynter and Hungate [103]
Methanobacterium[a] thermoautotrophicum	Hydrogen	Yes	Zeikus and Wolfe [104]
Methanococcus vannieli	Hydrogen or formate	Not Determined[b]	Stadtman and Barker [105]
Methanosarcina barkeri	Hydrogen or methanol	Yes	Schnellen [101]
Methanospirillum[a] hungatii	Hydrogen or formate	Not Determined[b]	Ferry et al. [106]

[a]Type strain deposited in American Type Culture Collection.

[b]Growth occurred in mineral salts medium that contained H_2 or formate and an organic reducing agent (cysteine or sodium thioglycolate). These species may be capable of autotrophy.

in well-documented pure cultures. Other strains have been isolated but remain to be described in more detail before taxonomic assignment is established. These strains include Methanobacterium strain MOH isolated from the Methanobacillus omelianskii symbiosis, and a recently obtained Methanobacterium strain [99] that metabolizes acetate in a complex medium [93].

Of the simple organic compounds utilized by the methane bacteria, McCarty [13] has estimated that about 70 percent of the methane produced by sewage-sludge digesters derives from acetate and most of the remainder from carbon dioxide and hydrogen. Additionally, Smith and Mah [98] determined that 73 percent of the methane came from acetate in sludge. However, as illustrated in Table II-1, acetate has not been demonstrated to serve as sole electron donor for the methane bacteria maintained in pure culture. Isotopic labeling studies by Stadtman and Barker [32] were conducted with "highly purified" cultures of M. barkeri and Methanococcus species. This work indicated that the methyl group of acetic acid is transferred to methane intact, as in the following reaction:

$$C^{14}H_3COOH \longrightarrow C^{14}H_4 + CO_2$$

$$CH_3C^{14}OOH \longrightarrow CH_4 + C^{14}O_2$$

The work of Pine and Barker [33], using crude enrichment cultures again demonstrated that the intact methyl group of acetate was fermented to methane, as illustrated in

the following reactions:

$$CH_3COOH \xrightarrow{D_2O} CH_3D + CO_2$$

$$CD_3COOH \xrightarrow{H_2O} CD_3H + CO_2$$

Conservation of the protons in the methyl moiety strongly suggests that CH_4 production from acetate was attained via a single reductive step by a single organism.

Additional research cited by Zeikus [93] indicates that acetate conversion has been demonstrated in both pure and mixed cultures; however, in all cases, growth was very slow. The free energy available from the metabolism of hydrogen and formate is almost four times as great as the free energy available from acetate metabolism. Thus, there is still considerable uncertainty as to the significance of acetate as an energy source of growth and the mechanism for its microbial conversion to methane.

The one unifying characteristic of the known methane bacteria is their common ability to utilize hydrogen as a substrate and produce methane according to the following reaction:

$$CO_2 + 4H_2 \longrightarrow CH_4 + H_2O$$

The use of hydrogen by those methane bacteria in pure culture is well documented [94]. It seems that these bacteria are

the easiest to isolate and probably have the fastest growth rate of the methane bacteria. Very little hydrogen appears in digester gas [34], though this may be caused by either a lack of the appropriate bacteria complement which produces hydrogen or its rapid utilization by the methane bacteria.

The role of hydrogen in the methane fermentation phase has long been considered, as reported by Buswell and Mueller [41]. The initial discovery resulted from the observation that enrichment flora growing anaerobically on cellulose normally produce CH_4 as an end-product but then usually produce H_2 after pasteurization. Isolated methane bacteria rarely sporulate and of the major flora that initially attack cellulose anaerobically, only the clostridia would have survived pasteurization. Thus it was reasoned that the H_2 and carbonate present in the growth media were the immediate sources of methane and the reaction was demonstrated. Observations on anaerobic digestion of acetic acid indicated that hydrogen, however, was not present in detectable amounts and led Buswell [31] to doubt the scheme as the main mechanism involved. However, the common trait of many of the methane bacteria is their ability to reduce carbon dioxide. Thus the generation of H_2 in the acid fermentation phase may be of substantive importance in the overall process [42].

Smith and Shuba [37] reported that propionate metabolism was a hydrogenic process that resulted in the formation of free molecular hydrogen. Propionic acid

enrichments contained large numbers of hydrogen oxidizing methane bacteria which were incapable of metabolizing propionate. Isotope dilution experiments showed that the ecological role of these methane formers was to maintain a hydrogen concentration low enough to prevent the inhibition of propionate metabolism and the concurrent cessation of fermentation. It was suggested that the same may be true for the metabolism of other fatty acids. Evidence was provided to support the hypothesis that organic substances were digested in four stages: hydrolysis, acidification, hydrogenesis and methane formation.

The biochemical pathways for methane formation are only partially understood although both tetrahydrofolate and vitamin B_{12} coenzyme are known to be involved [35]. A summation of the possible pathway that unifies the disparate observations has been proposed by Barker [36] and is shown in Figure II-3.

As discussed previously, the methane bacteria are very specific in terms of their substrate requirements. Pine [11] has detailed the methane fermentations by individual species of the methane bacteria or possibly in some cases by closely dependent symbiotes as shown in Figure II-4. The heavy arrows indicate methane fermentations and the remaining reactions are catalyzed by propionibacteria, clostridia, butyribacteria, and other anaerobes.

According to the preceding, the utilization of hydrogen,

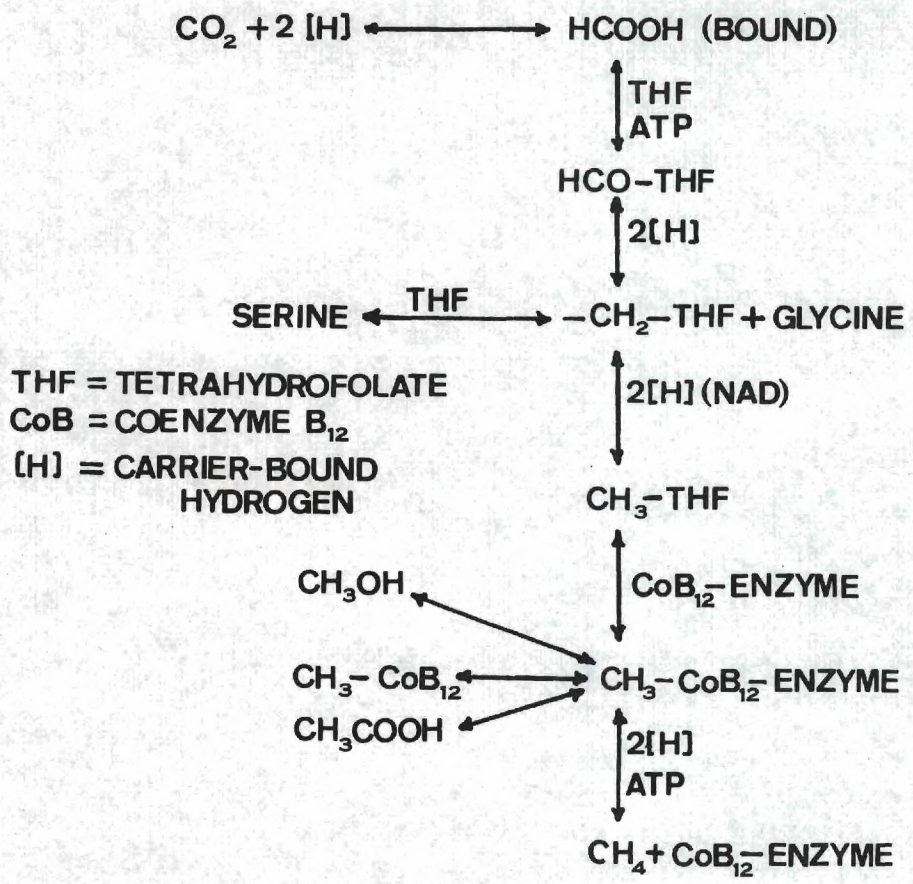


Figure II-3. Pathways of Methane Formation After Barker [23]

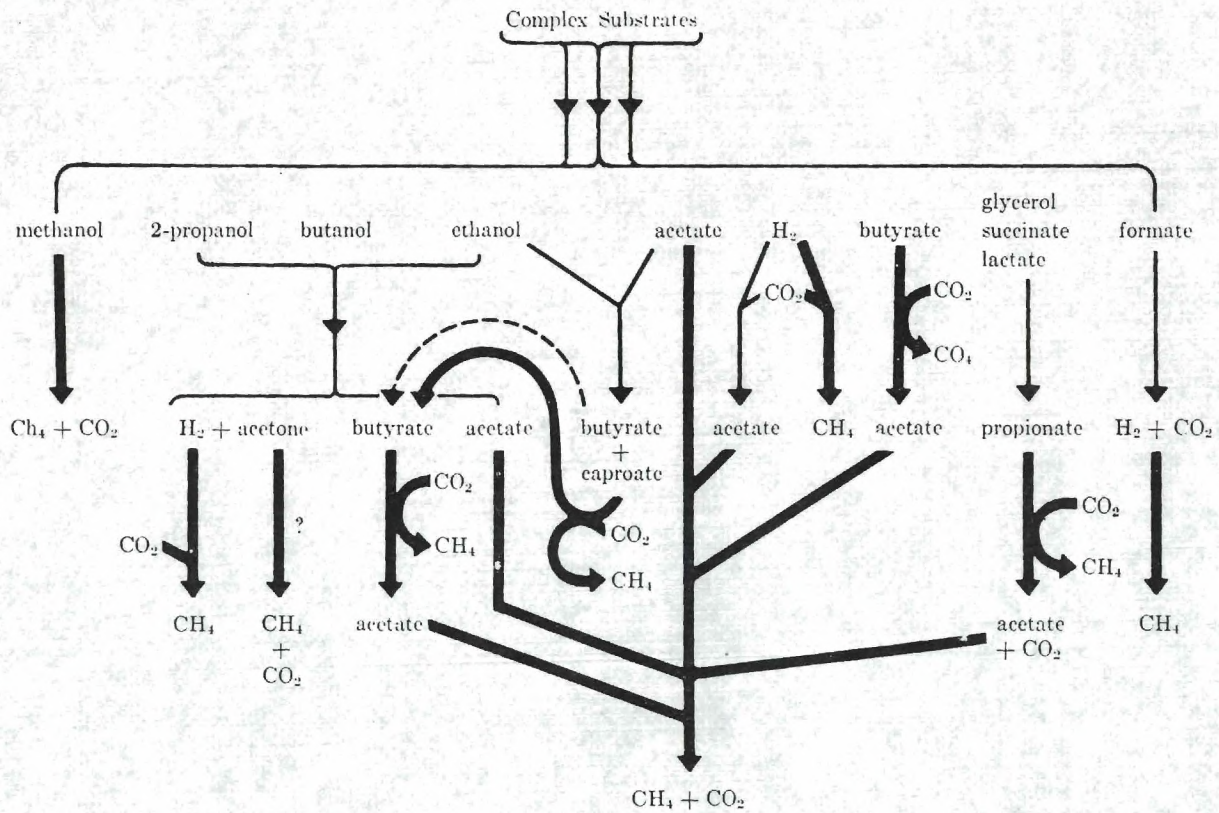


Figure II-4. Interrelationship Between Methane Bacteria and Other Substances of the Anaerobic Carbon Cycle. After Pine [11]

formate, acetate and methanol by the methane bacteria has been demonstrated. The utilization of the other volatile acids is not so clear but there seems to be overwhelming evidence for the existence of some bacteria or symbiotic associations of bacteria which will prodice methane from C_2 to C_5 volatile fatty acids [94].

The nutritional requirements of the methane bacteria appear to be relatively simple. They grow well in media containing the usual nutritive salts, carbon dioxide, a reducing agent, a simple oxidizable compound suitable for the organism and are able to use ammonia as a source of nitrogen [38]. Speece and McCarty [39] have reported more complex nutritional requirements for studies performed using soluble synthetic substrates. Iron, cobalt, thiamine and components of vitamin B_{12} were found to accelerate the digestion of acetate. Studies by Bryant, et al. [40] indicate that some of the more numerous methanogenic bacteria depend strongly on other bacteria in the ecosystem which supply essential sources of nutrients in addition to energy sources. Therefore, it is obvious that a good understanding of the nutritional requirements for the methane bacteria and the potential sources of these nutrients is necessary to forestall poor results due to deficiencies.

Environmental Requirements for the Anaerobic Stabilization Process

The successful operation of a biological waste

treatment process calls for maintenance of environmental conditions within limits required by the microbiological community present. It is necessary to maintain conditions not only within the tolerable range but also to manipulate the environment to provide as near to optimum conditions as can be attained in order to achieve maximum treatment efficiency.

Standard design procedures have called for structuring the anaerobic stabilization process so that both the methane and acid fermentation phases occur concurrently in a single reactor. Thus the environment within this reactor has been regulated to provide conditions for the process as a whole. It is generally conceded that the methane bacteria are the slower growing and more sensitive of the microbial population responsible for anaerobic stabilization [94]; thus the environmental conditions have normally been manipulated to satisfy their particular needs. It should be considered, therefore, that the majority of the work reported has been concerned with optimization of environmental conditions for the overall process. There is a dearth of available information on conditions required for the optimization of growth in the non-methanogenic phase.

Temperature

Temperature exerts an important influence on the anaerobic process just as it does for any other biological or chemical reaction. As early as 1934, Fair and Moore [43]

summarized work from several sources and upon analysis found three or possibly four temperature zones of activity for sludge digestion. These zones were identified as thermophilic (above 42°C), intermediate (28°-42°C), temperature (below 28°C and possibly above 10°C) and a cryophilic zone (below 10°C). General practice today divides the temperature zone for the optimal growth of microorganisms into three ranges, the psychrophilic (<20°C), the mesophilic (20°-45°C) and the thermophilic (<45°C) [44]. A particular bacterial species can then be described as a psychrophilic, mesophilic or thermophilic bacterium depending upon the range that optimal growth is attained.

Pohland [19] has reviewed the early literature detailing studies performed at both the mesophilic and thermophilic ranges. Optimum mesophilic temperature is suggested to be about 37°C, with the temperature range generally adopted in conventional practice to be within the range of 32-35°C. Thermophilic-operating temperatures are within the 50-60°C range.

Kotze, et al. [24], in their review of the literature, report of controversy concerning the advantage and disadvantages of thermophilic versus mesophilic digestion, with conflicting information concerning the rate of digestion at various temperatures. Although thermophilic digestion is generally considered more efficient for sewage sludge, the development of foul odors, extra heating requirement and

poor sludge dewatering characteristics usually rule against its use.

However, it has been recently reported [107] that thermophilic digestion will be employed by the City of Los Angeles in order to achieve better dewaterability of digested sludge and reduced concentrations of pathogenic bacteria and virus as compared to the same sludge digested under mesophilic conditions. Among the disadvantages reported was a higher volatile acid concentration, trace metals and a lower volatile solids reduction. It was also noted that thermophilic digester organisms were more sensitive than mesophilic organisms to temperature fluctuations.

Brown and Kinchusky [46] concluded that a digester normally operated at 32°C could be upset by a temperature change to 40°C, as the mesophilic conditions were drastically changed to thermophilic conditions and fatty acids accumulated. Thus, both the established temperature and the minimization of temperature fluctuations are important to overall process stability. Additonally, Therkelsen and Carlson [77] report increased reaction rates and improved gas yields with thermophilic digestion when compared to mesophilic.

pH, Alkalinity and Volatile Acid Concentration

The pH, alkalinity and volatile acid concentration are commonly accepted parameters for practical control of anaerobic processes. Because of their interdependence, their effect on the process will be discussed commonly.

The anaerobic stabilization process can be operated successfully at any pH value between 6.0 and 8.0, however, the optimum is usually considered to be around neutral for the proliferation of the methane bacteria and the overall process is generally operated at this value to accommodate their sensitivity. Barker [46] asserted that the pH range 6.4-7.2 was most effective for methane production and that below 6.0 and above 8.0, gas production declined rapidly. Willimon [47] investigated the operation of a two-stage system with limited aeration in the first stage followed by an anaerobic stage. Maintenance of the anaerobic stage pH at 6.5 resulted in increased treatment efficiency over that obtained at pH 7.0. Therkelsen and Carlson [77] reported satisfactory operation of an acid phase reactor utilizing an insoluble synthetic substrate at a pH of 4. Ghosh, et al. [8] have reported operating at a pH of 5.7 in the acid phase of a two phase anaerobic process. Therefore, additional work is required to investigate the effect of pH on volatile acid production and product mix in acid phase reactors.

The pH alone is not a necessarily sensitive parameter for evaluation of the acid-base conditions that may be present at any given moment within the digester environment. Additional factors that must also be taken into consideration are the buffering capacity and alkalinity of the system and the volatile acid concentration. The alkalinity reflects the results of an internal neutralization of acid. Production

of high volatile acid concentrations tend to decrease the alkalinity and depress the pH as the normal bicarbonate buffering capacity is exhausted. However, considerable increases in volatile acid concentration and decreases in alkalinity may be a forewarning of problems before the pH of the digester is seriously affected.

In a properly operating digester, a dynamic equilibrium is maintained between buffer formation and destruction. Both alkalinity and volatile acids are derived primarily from the decomposition of organic material by the biochemical processes occurring during digestion. The dominating acid-base equilibria for the natural buffering system during digestion are shown in Figure II-5, where acetic acid as the most plentiful organic acid is chosen to represent this group. The graph illustrates the difficulty in choosing an end point for the alkalinity titration, since variations in total concentrations of inorganic carbon and organic acids greatly influence the contribution of the ionized and unionized constituents that are measured.

The major chemical system controlling pH in an anaerobic digester during normal conditions is the carbon dioxide-bicarbonate buffer system. Thus the gas atmosphere above an anaerobic reactor will have a marked effect on pH of the system. External pH control for a digester consists of adding bicarbonate alkalinity in the form of bicarbonate or to add a base which traps CO_2 and converts it to bicarbonate.

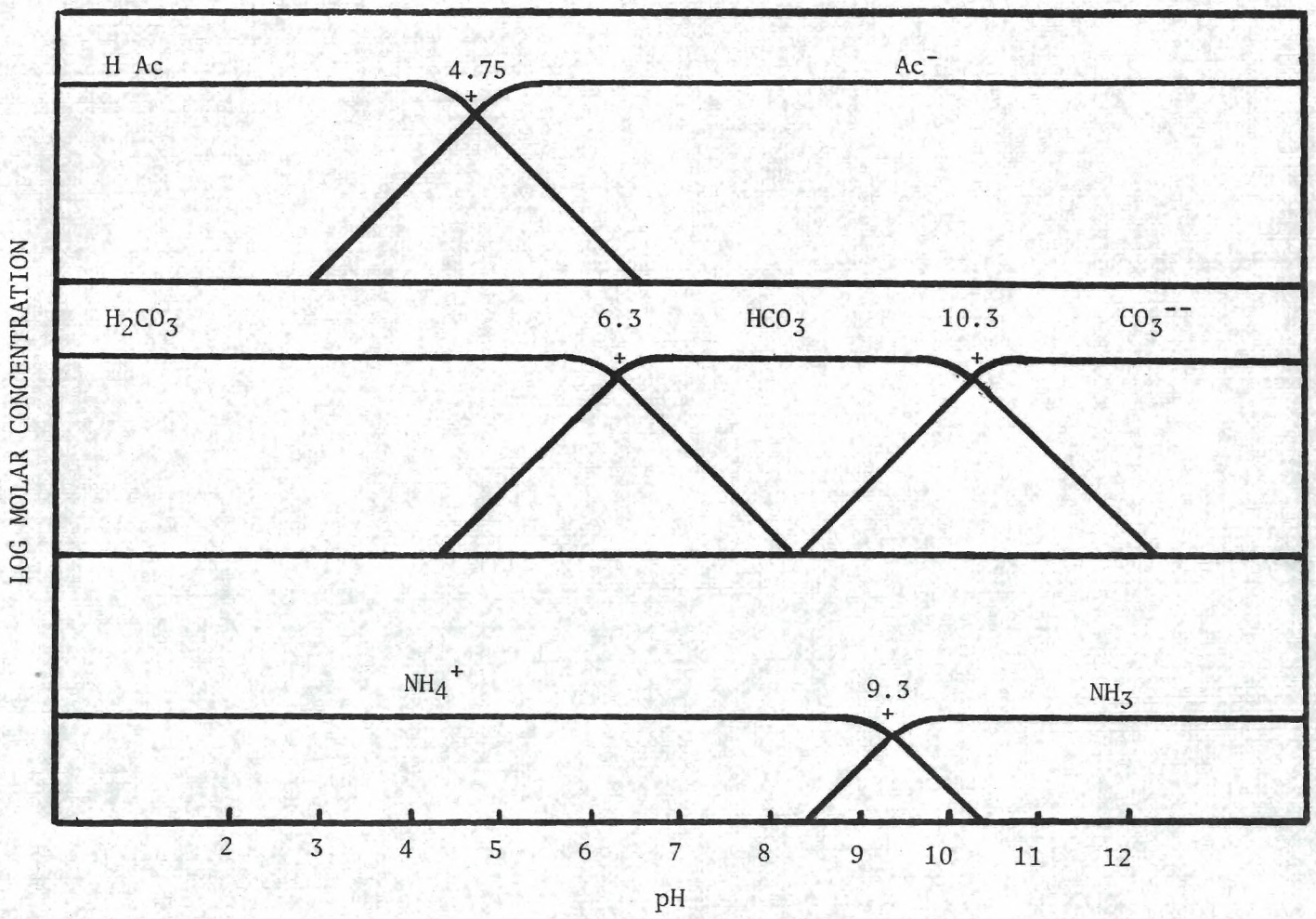


Figure II-5. Dominating Acid Base Equilibria in an Anaerobic System

A controversy develops as to the best method for external pH control if reducing the rate of volatile acid formation is not a logical alternative. It has been pointed out by Pohland [48] that lime, which is sometimes added for external pH control, reacts with CO_2 initially to yield calcium bicarbonate, which is not very soluble. After the bicarbonate alkalinity exceeds 500-1000 mg/l, introduction of additional lime results in the formation of insoluble calcium carbonate which will precipitate and have little direct effect on pH. This CO_2-HCO_3 equilibrium will maintain the pH between 6.5 and 7.0 until most of the CO_2 is removed. At this point, the buffer capacity of the system is depleted and the pH can rise rapidly with the addition of more lime.

Other CO_2 consuming chemicals such as ammonium hydroxide, gaseous ammonia or sodium carbonate have been used to make pH adjustments in "sour" digesters. Pohland [48] defined the limits of total alkalinity for normal digestion as between 1500-5000 mg/l as $CaCO_3$, with total alkalinity for distressed digestion as between 1000-3000 mg/l as $CaCO_3$. Therefore, it is obvious that alkalinity alone cannot be used as an indicator of satisfactory digestion.

The third consideration for description of the acid-base chemical environment within the digester is the volatile acids present in solution. Buswell, et al. [17] were among the first to recognize the importance of volatile acids as a control parameter. The setting of a permissible concentration

has, however, been a source of controversy.

Pohland [19] and Kotze, et al. [24] reviewed the work of several earlier investigators concerning permissible concentrations. Many of these studies indicated that concentrations above 2000-3000 mg/l, expressed as acetic acid, were indicative of unbalanced digestion conditions; others cited in these reviews stated that 200-300 mg/l was the maximum concentration for normal digestion. Whatever the resolution of the controversy, a sudden rise in volatile acid concentration is an indication that volatile acid production is outstripping utilization by the methanogenic bacteria. This condition must be remedied through changes in operational techniques to prevent a permanently unbalanced condition.

A source of controversy concerning the effect of volatile acids on the digestion process is whether a high volatile acid concentration is the cause or simply the end result of an unbalanced condition. Advocates of the cause theory argue that the high concentrations of volatile acids, regardless of pH, are inhibitory to the methane bacteria and that reduction in organic load or dilution can remedy the situation [49,50]. Proponents of the effect theory reason that the pH effect of the volatile acid formation is toxic to the methane formers. If this is true, then high concentrations of volatile acids that are buffered to a neutral pH should not be detrimental to the digestion process. Several

studies [29,51] have demonstrated the ability of anaerobic digesters to operate satisfactorily at high concentrations of volatile acids.

Mueller et al. [52] and Pohland and Bloodgood [29] have emphasized the need for a balance between alkalinity and volatile acid concentration for normal digester operation. It is apparent that consideration be given to pH, total alkalinity and volatile acid concentration as a whole to anticipate problems associated with the uncoupling of the acid fermentation-acid utilization balance that must be maintained during normal digestion.

Gas Production

The production of gas by the anaerobic digestion process is an important control parameter and one that is regulated to a great extent by the characteristics of the substrate supplied. Not only the rate of gas production, but also the gas composition needs to be considered when analyzing data concerning gas generation by the digester.

Gas productions vary, however, an average of about 7 cu ft/lb volatile solids added seems to be reasonable for domestic and farm wastes [94]. Griffiths [108] lists gas production values between 5 and 10 cu ft/lb of volatile matter added or 9-24 cu ft/lb of volatile matter destroyed, with gas compositions of 65-70 percent methane and 30-35 percent carbon dioxide.

The CO_2 content of digester gas can fluctuate

substantially and has been used as an indicator in digestion studies [53]. This fluctuation is due to the solubility of carbon dioxide and the sensitivity of its solubility to pH effects. The CO_2 produced in the digestion process either escapes to the off gas or remains in solution as dissolved carbon dioxide and carbonic acid or it reacts with a base such as ammonia to form bicarbonate ions. The solubility of CO_2 is dependent upon its partial pressure; however, the quantity of CO_2 converted to carbonate and bicarbonate ions is dependent upon its partial pressure as well as the pH and ammonia concentration of the solution. A decrease in partial pressure or pH will release CO_2 from solution and thus alter the CO_2 fraction of the digester off gas.

Buswell [17] and others have reported the detection of small quantities of hydrogen during digestion. Heukelekian [14] attributed the relative lack of hydrogen to the absence of fermentable carbohydrates and to the reaction of hydrogen with hydrogen acceptors such as sulfate and carbon dioxide. The ability of large numbers of anaerobes and facultative anaerobes to produce H_2 [54], and the ability of the methane bacteria to utilize it as a substrate, leads to speculation of the importance of hydrogen utilization in the methane phase.

It has been demonstrated [109] that the fermentation of undiluted piggery waste initially led to a gas composition of 81 percent carbon dioxide, 10 percent methane and 9 percent

hydrogen after 6 days. Also the onset of methane production in a piggery waste digestion being built up by slow addition of piggery waste to water was accompanied by an increase in counts of hydrogen utilizing methane bacteria from zero to greater than 2×10^4/ml [110]. As previously mentioned, Smith and Shuba [37] attribute the formation of hydrogen as an essential mechanism of the digestion process.

Toxicity and Inhibition

An important environmental condition for the anaerobic stabilization process is that the substrate be free of inhibitory concentrations of toxic materials. The effect of any substance on the metabolism of an organism is concentration dependent. The toxic range is defined as a concentration above the peak range of metabolic stimulation for a given substance and a given microbial population [55]. The magnitude of the toxic effect generated by a substance can often be reduced significantly if its concentration is increased slowly and acclimation, or adjustment of the biological population to the toxin, occurs. Toxic effects on the anaerobic system are further complicated by consideration of antagonistic or synergistic effects and complex formations of toxic substances in the waste.

There are many materials, both organic and inorganic, which may be toxic or inhibitory to the overall anaerobic stabilization process. These include the light metal cations such as sodium, potassium, calcium and magnesium.

When combinations of these cations are present, the nature of the toxic effect becomes more complex as some of the cations act antagonistically, reducing the toxicity of other cations, while others act synergistically, increasing the toxicity of the other cations [56]. The methane bacteria are generally assumed to be the more sensitive to environmental toxins in the anaerobic stabilization process since most process failures attributed to toxic upsets are manifested by volatile acid accumulation, although Lawrence and McCarty [57] report cases where digesters were inhibited by a toxic material that seemingly affected both bacterial populations equally.

Evaluation of the toxicity of light metal cations is complicated by the presence of antagonistic and synergistic effects as well as the possibility that low concentrations of the cations can be essential as nutrients and exert stimulatory effects. Much of the earlier work on toxicity of the light metal cations suffered from the fact that the ionic constitution of the medium was not well defined or controlled. Kugelman and McCarty [56] have investigated the effects of light metal cations on an anaerobic reactor utilizing acetate as a substrate. Acetate was chosen because about 70 percent of the methane produced from the digestion of municipal waste is judged to result from acetate fermentation [20]. The results, shown in Figure II-6, generally agree with those reported from the traditional

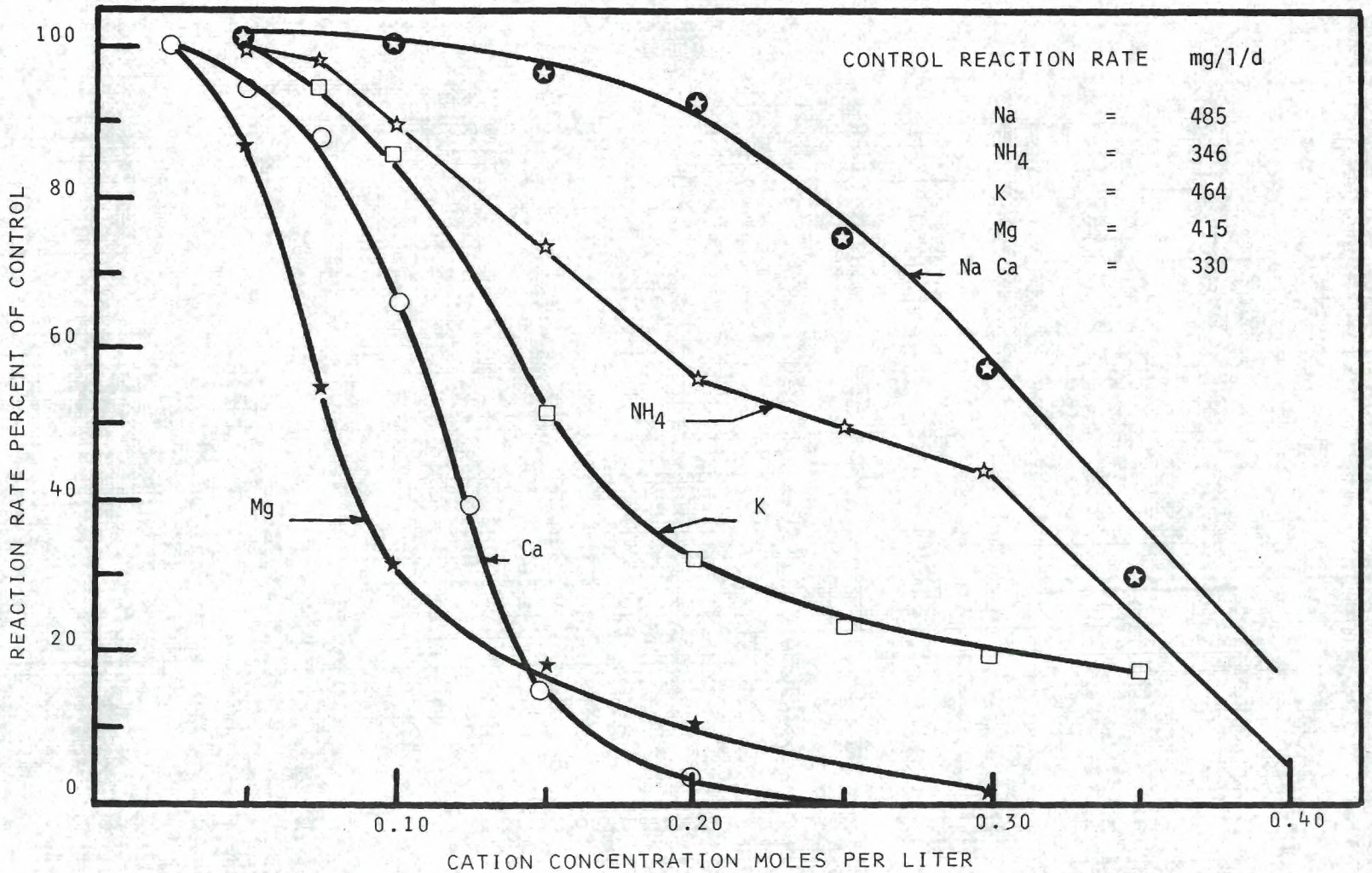


Figure II-6. Effect of Individual Cation Concentration on the Rate of Acetate Utilization. After Kugelman and McCarty [56]

biology studies, i.e., the divalent cations are more toxic than the monovalent cations. Additionally, it was found in dual cation systems that either antagonism or synergism was exhibited. Based on the results of their studies, Kugelman and McCarty [56] suggested design values for the maximum cation concentration which could be tolerated in anaerobic waste treatment as shown in Table II-2.

Table II-2. Upper Limit of Cation Concentration in Anaerobic Waste Treatment

Cation	Slug Feed		Daily Feed	
	Single Cation,M	Antagonists Present,M	Single Cation,M	Antagonists Present,M
Sodium	0.2	0.3-0.35	0.3	70.35
Potassium	0.09	0.15-0.2	0.13	0.35
Calcium	0.07	0.125-0.15	0.15	0.20
Magnesium	0.05	0.125	0.065	0.14
Ammonium	0.1	0.25	Not Measured	Not Measured

Additional work by Chin, Kugelman and Molof [58] on continuously fed anaerobic systems utilizing acetate indicated that the maximum specific growth rate and maximum specific substrate utilization rate were affected by increases in potassium but this could be reversed by additions of sodium. Sodium increases alone decreased organism yields and decay rates but not the maximum specific

growth rate. Again these conditions could be reversed by potassium addition.

Heavy metal toxicity is a significant factor in digester failures due to their extreme toxicity. Since only the soluble fraction of an inhibitor affects the process, precipitation of heavy metals by the reactions with sulfides or complex-type reactions causes wide variations in reported tolerance levels [55]. Sulfides have been demonstrated very effective in controlling heavy metal toxicity problems due to the low solubility of heavy metal sulfides [57].

Mosey and Hughes [111] have investigated the relationship between sulfide ion concentration and the degree of inhibition by the heavy metals, Cu, Ni, Pb, Hg, Zn, Cd, Fe and Ag. pS values greater than 14, as determined from millivolt readings measured by an Ag/Ag_2S electrode, indicated the presence of inhibitory concentrations of Zn^{2+}, Cd^{2+}, Fe^{2+}, Cu^{+} or Cu^{2+}. During anaerobic digestion, Fe^{3+} was reduced to Fe^{2+} and Cu^{2+} to Cu^{+}. The toxicity of Cr added as the hexavalent salt was similar to that of the trivalent salt.

Hayes and Theis [112] evaluated the removal mechanisms of heavy metals and their distribution among the soluble, precipitated, extracellular and intracellular components as well as their effect on the digestion process. The order of toxicity on a molar basis was Ni>Cu>Pb>Cr>Zn. Control strategies to minimize the impact of heavy metals were the

addition of more precipitating ligands, such as sulfide, and operation at the maximum of more precipitating ligands, such as sulfide, and operation at the maximum pH allowable to bring about increased tolerance to heavy metal additions.

Considerable controversy has developed regarding the toxicity of the volatile acid intermediates to the methane bacteria [19]. The question posed was whether the toxic effects resulted from the volatile acids present, the associated pH drop or the corresponding cations present. The work of McCarty and McKinney [59] indicated that the problem was cation toxicity, based on work performed on anaerobic reactors utilizing acetate as the substrate. Thus the use of alkaline substances to maintain an adequate buffer capacity is a valid procedure if care is exercised in the selection of the alkaline material.

Ammonia, although formed during the anaerobic process, may be found in inhibitory concentrations in industrial wastes or highly concentrated municipal sludges. Ammonia may be present either in the form of the ammonium ion or as dissolved ammonia gas, the relative concentration of each depending on the pH. At pH ranges normally encountered in anaerobic processes, 7.2 or lower, inhibition is related to the ammonium ion [59,60]. In the presence of carbon dioxide, therefore, ammonium bicarbonate is produced as an end-product of decomposition. Normally this compound is a beneficial part of the natural buffering system, but high concentrations

may possibly create ammonium ion toxicity [59]. McCarty and McKinney [59] reported that pH is significant in the toxicity of ammonium ion. Significant quantities of free ammonia, however, are not present in the pH range of 7.0.

Sulfides can be toxic to the anaerobic process, whether introduced as sulfides or produced by the biological reduction of sulfates during anaerobic degradation. Sulfides may exist in soluble or insoluble form depending upon the cations with which they become associated. Sulfides may also be distributed as gaseous hydrogen sulfide, depending upon the pH. According to Lawrence and McCarty [57], concentrations of soluble sulfides in the range 50-100 mg/l can be tolerated in anaerobic digesters with little or no acclimation required, concentrations up to 200 mg/l can be tolerated with acclimation, but above 200 mg/l, soluble sulfides are quite toxic. However, precipitation of soluble sulfides with heavy metals to form insoluble sulfide salts effectively removes the inhibitory effects of the sulfide.

Organic materials may also inhibit the anaerobic process. These organic toxins range from organic solvents to many common materials such as alcohols and long chain fatty acids. Recent work in England has shown that as little as one 55-gallon drum of methyl chloroform (1,1,1,trichloroethane), a solvent which is used as a degreasing and cleaning agent, can upset 40 million gallons of digesting sludge, at a concentration of about 1.4 mg/l [61]. Organic materials

which are toxic at high concentrations, but which can be anaerobically treated at low concentrations, can be handled by feeding modes that prevent the buildup of toxic concentrations in the anaerobic system [62].

McCarty [63] has discussed ways of eliminating toxic materials: including iron addition for sulfide toxicity; long chain amine addition to ameliorate the effects of LAS; sulfide addition for heavy metal toxicity; and, potential toxic organic compounds by extraction with a non-toxic substance such as methanol. Beyond these procedures, an investigation to pinpoint the source of the toxin is required.

Process Configuration and Application

A number of advances have been instituted in the application of the anaerobic stabilization process to treatment of waste organic material. The initial applications, such as the septic tank, took advantage of the liquefaction phase of the process without consideration to methane generation. In these types of systems, no attempt was made to separate the sedimentation and digestion phases of the system. A major problem encountered with this type of process was the lifting of solids into the clarification zone by rising gas bubbles generated from the biological phase.

The two-story or Imhoff tank solved this problem by providing a design such that the sedimentation chamber was

separated from the digester chamber by baffles. The separation of functions in the Imhoff tank increased effluent quality; however, no control was exerted over process variables. As Buswell [12] has described, by the mid or late twenties, the trend was to provide separate sedimentation and digestion facilities. This trend was furthered by development of the floating cover to facilitate sludge entry or withdrawal from the process.

The conventional process configuration is a single reactor design that may or may not have a second stage tank that acts generally as a quescent settling basin for digested sludge concentration. Digesters falling into this conventional design pattern are categorized as standard or high-rate systems depending upon the quantity of organic matter applied per unit time per unit volume of digester capacity. The standard-rate system was used for many years and were designed generally with a 30 to 60 day theoretical hydraulic retention time and a sludge loading of 30-100 pounds of volatile solids per 1000 cubic feet of digester volume per day [42]. These digesters usually had no provisions for mixing and are generally as represented in Figure II-7. High rate systems included provisions for mixing by mechanical mixers, gas recirculation or pumped recirculation. Provisions for heating to near optimum temperatures for the mesophilic range were also incorporated. With this system, significant increases in organic loading rate were

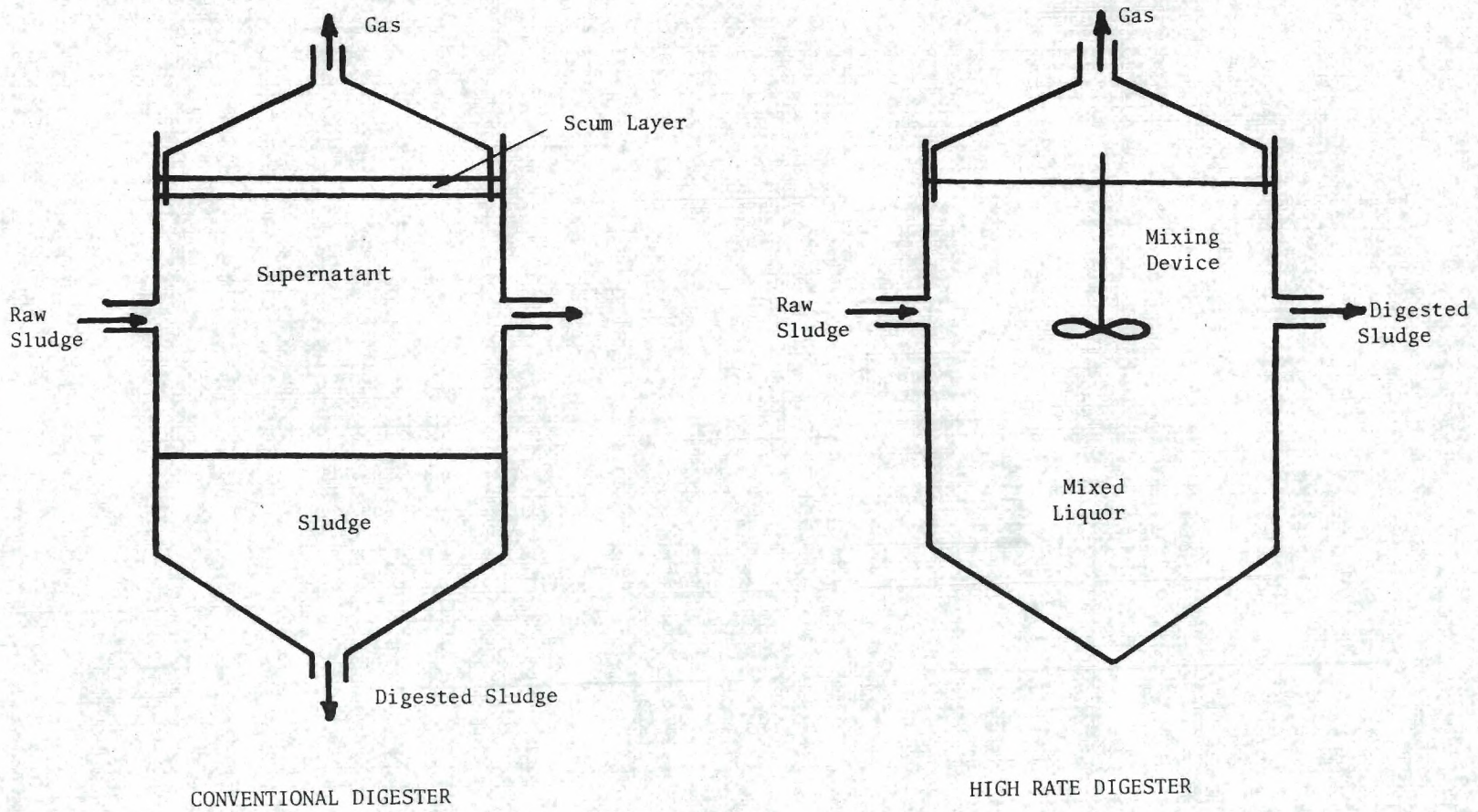


Figure II-7. Schematic Diagram of Conventional and High Rate Anaerobic Digester

demonstrated by Morgan [64] and Torpey [65]. By these modifications, the theoretical hydraulic retention time was reduced to 15 days or less and the organic leading to 100-500 pounds of volatile solids per 1000 cubic feet of digester volume per day [42]. A high-rate digestion system is also shown in Figure II-7.

Another modification of the anaerobic stabilization process is the anaerobic contact process or the anaerobic activated sludge process, diagrammed in Figure II-8. In this process, the slower growing methane bacteria may be retained in the system and recycled to provide - large concentration of suitable microorganisms for the digester. Schroepfer et al. [66] applied the process to packinghouse wastes and reported 95 percent BOD removal at hydraulic retention times of 12 hours for a waste with an influent BOD of 800-1800 mg/1. One problem with the application of this process to dilute wastes is the necessity of heating the waste to optimum mesophilic temperatures. Another problem is the erratic behavior of biomass settling due to entrapped gas generated by biological action. A vacuum degasifier was installed between the digester and sedimentation basin to eliminate the problem [66]. Dague [71], in a study of the process using a low strength, synthetic waste reported that a hydraulic retention time of two days at 35°C gave 85 percent reduction in influent COD as long as sufficient biomass was recycled to the system. Good settling

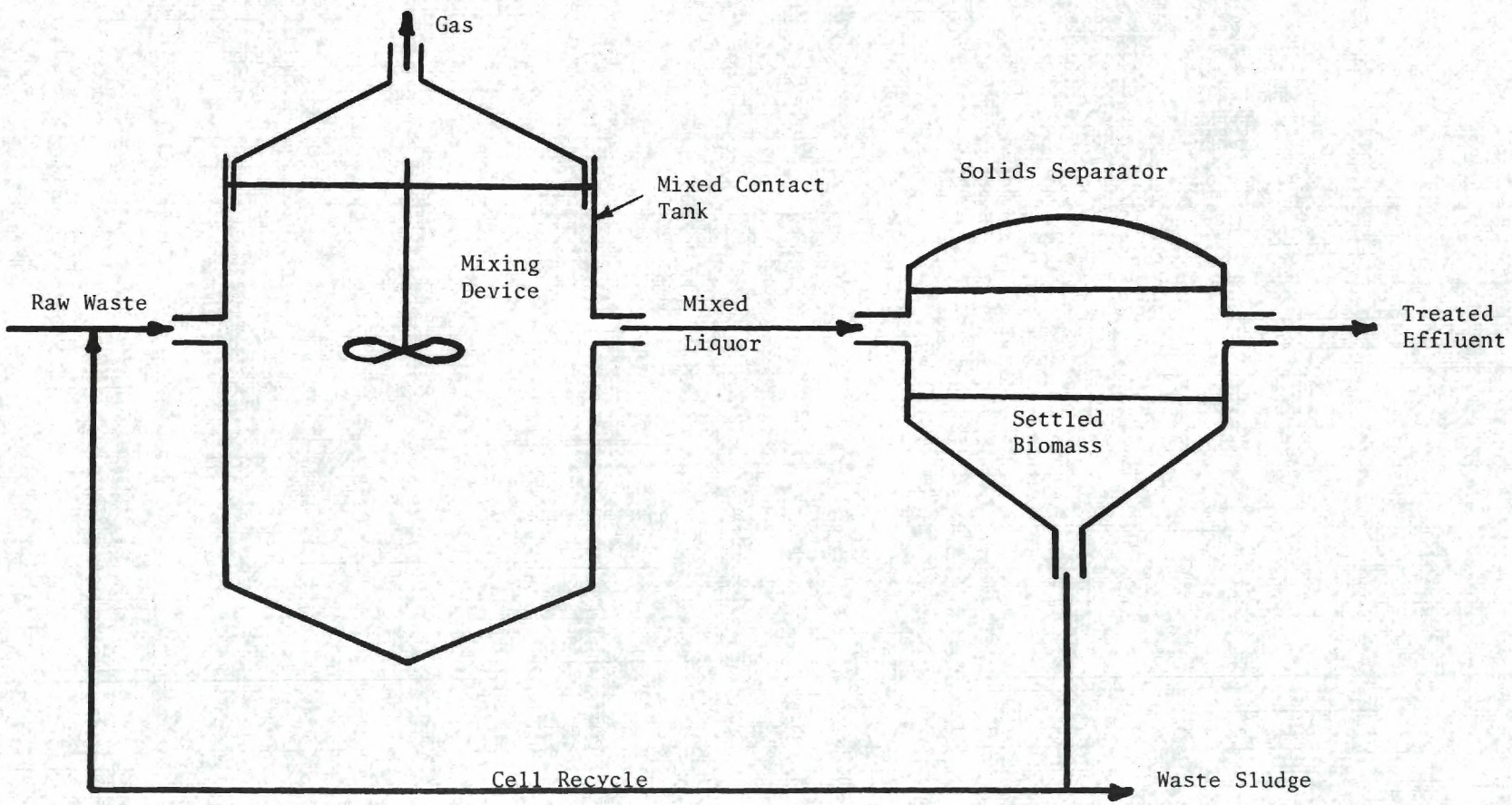
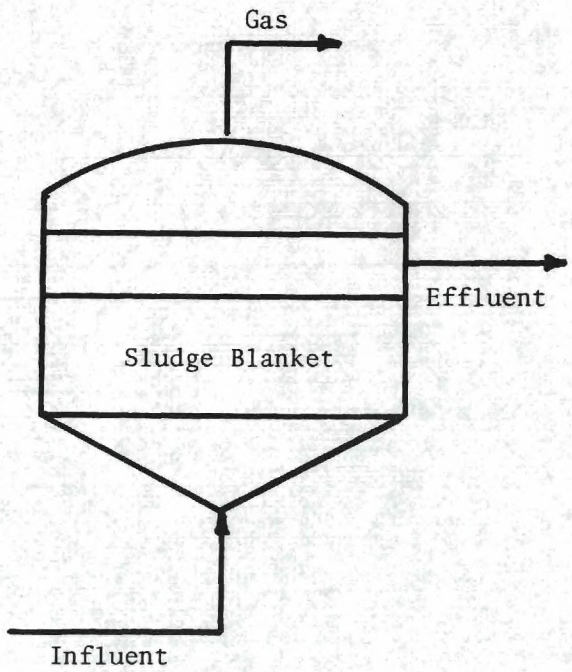


Figure II-8. Schematic Diagram of Anaerobic Contact Process

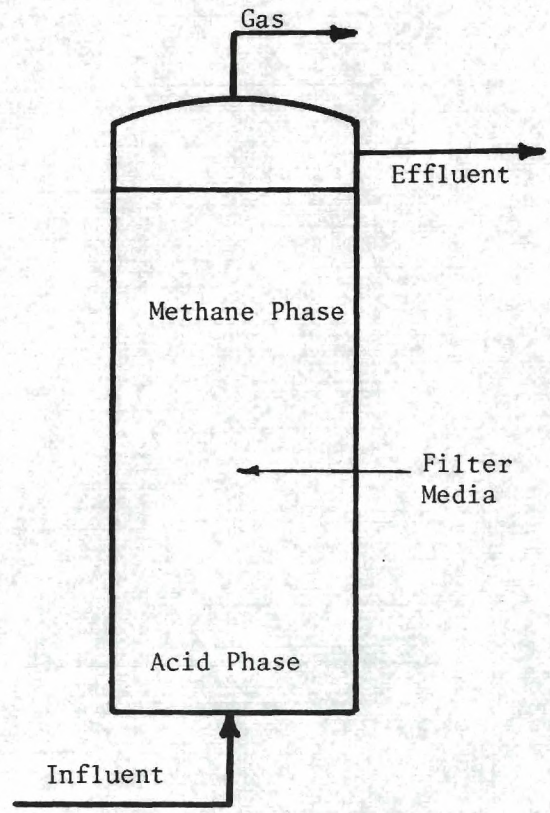
of the biomass was observed and gas formation in the clarifier did not hamper settling. This system was operated at mixed liquor suspended solids concentration up to 5000 mg/l. It is likely that higher solids levels that may be obtained when treating high strength soluble wastes or sludges may aggravate the settling problem by gas entrapment and subsequent floating of the solids.

An upflow anaerobic contact process based on the movement of waste upward through a sludge blanket maintained in a biological reactor, as shown in Figure II-9, has been evaluated by Coulter et al. [68], Stander [69] and more recently by Van der Meer [70]. The sludge blanket acts as a filter as well as provides sites for fixed film growth of the anaerobic bacteria which are effectively retained within the system.

The early studies by Coulter et al. [68] were concerned with the treatment of dilute domestic waste from small clusters of homes not serviced by existing sewer systems. The system was effective in suspended solids reduction while BOD reduction was only 50 to 65 percent. Stander [69] demonstrated the viability of the system on wine distillery wastes with an average strength of 12,000 to 18,000 mg/l BOD. The digester obtained better than 95 percent BOD and COD reduction with a hydraulic retention time of 7.2 days and a suspended solids concentration of 2.5 percent. More recently, Van der Meer [70] investigated the



UPFLOW ANAEROBIC CONTACT



ANAEROBIC UPFLOW FILTER

Figure II-9. Schematic Diagrams of Anaerobic Fixed Film Processes

efficiency of upflow reactors of various design on the treatment of dilute wastewaters such as acetate, beet sugar wastes and domestic wastewater. He reported COD reductions up to 90 percent with influent COD concentrations from 500-1500 mg/1.

An additional development in anaerobic process configuration is the upflow anaerobic filter, a fixed film reactor which again maximizes the retention of the slower growing methane bacteria by encouraging their growth on a support media such as rock or plastic. The process, shown in Figure II-9, is analogous to a trickling filter except that the flow is upward for submergence and maintenance of anaerobic conditions. Because of the plug flow configuration of the process, the acid and methane phases of the anaerobic stabilization process are effectively separated, with the lower portion of the filter acting as the acid phase followed by volatile acid utilization in the methane phase. However, with this configuration, some of the control advantages inherent in two reactor systems are lost since interphase neutralization cannot be practiced as easily. Also the adjustment and optimization of environmental conditions for each phase is made much more difficult.

Young and McCarty [71] have reported extensive work with the anaerobic filter on low and intermediate strength synthetic wastes with COD concentrations ranging from 375 to 12,000 mg/1 and hydraulic retention times between 2.25

and 72 hours. At hydraulic retention times less than 4.5 hours, treatment efficiencies began to decrease. At waste strengths greater than 750 mg/1 and with hydraulic retention times of 12 to 72 hours, COD reductions from 60 to 90 percent were attained. The work of Jeris, et al. [72] suggests that another possible configuration for anaerobic treatment would utilize a fluidized granular bed with attached anaerobic growth. This treatment scheme overcomes the problem of high head loss and possible clogging associated with packed beds. This expanded upflow bed is simply an extension of the anaerobic filter concept as a fixed film reactor; however, it may possess operational advantages in comparison to the fixed bed anaerobic filter.

Anaerobic Phase Separation

As outlined previously, the problems encountered with stability and control of the anaerobic process have suggested an evaluation of the feasibility of separating the acid and methane fermentation phases. With the process so structured, more attention could then be directed toward determining and providing optimal environmental conditions for each microbial community, and organic loading and recycle requirements could thereby be controlled individually to enhance overall process efficiency. Of particular significance, the slower growing and more sensitive methane forming bacteria would be effectively sheltered from potential upsets by close monitoring

of the acid fermentation reactor effluent and elimination of the potential problems before the methane bacteria were subjected to the impending stress.

The idea of multistage digestion processes has been existent for many years. Buswell [31] reported on research where two-stage digestion was utilized; however, the bulk of the reaction occurred in the first reactor, with the second reactor serving primarily as a storage tank and sludge concentrator to prepare the digested sludge for ultimate disposal. It should be emphasized that two-phase digestion is inherently different from conventional two-stage digestion, since this type of process does not encourage separation of the acid and methane phases.

The use of membrane techniques to separate the acid and methane phases has been reported by several investigators [4,5]. Hammer and Borchardt [4] utilized a system consisting of two fermentation vessels connected by a dialysis unit. The study was performed on sewage sludge and an attempt was made to separate and optimize the acid and methane phases. Based upon these studies, it was concluded that it was possible to isolate an enrichment culture of acid producing bacteria in the initial stage followed by a strong population of methane bacteria. Optimum conditions for the acid phase was measured in the electrode potential, E_c, range of -508 mv to -516 mv at a pH of 6.9-7.0. Optimum conditions for the methane phase were in the E_c range of -520 to -527 mv

and a pH range of 7.05-7.20. It was also concluded from their experiments that hydrolysis and acid fermentation were the rate limiting steps in the digestion process. Significant gas production was measured from both the acid and methane fermentors. Borchardt [74] reviewed the application of dialysis to anaerobic phase separation and concluded that electrode potential measurements and the dialysis technique have great potential in research on basic mechanisms in sludge digestion. Schaumburg and Kirsch [5] investigated the use of membranes to separate pure cultures of the obligate anaerobe methane bacteria, M. Omelianskii, from a nutrient medium and to demonstrate the feasibility of the use of membranes to study microbial interactions. Although useful for the study of basic mechanisms in the laboratory, it is doubtful that membrane technology is adequate for full-scale systems due to associated problems with fouling.

A more practical method for phase separation is the manipulation of kinetic controls to segregate the acid and methane phases into two reactors. Ghosh and Pohland [2,3] have demonstrated procedures necessary for the control of the required growth rates to restrict methane production in the acid fermentation phase. Using a synthetic carbohydrate substrate and a single reaction vessel, kinetic parameters were estimated for both the acid and methane cultures by successively decreasing hydraulic retention time in the continuous flow reactor until volatile acid production

predominated and washout of the methane bacteria was achieved. Methane production was observed, even at retention times less than 12 hours. Biomass recycle techniques were not practiced in these studies. Pohland and Massey [10] have further analyzed the data obtained in the studies reported by Pohland and Ghosh [2,3] and have demonstrated its applicability to the design of a multi-phase anaerobic stabilization process.

Ghosh, et al. [8] have investigated the effects of phase separation for an anaerobic digestion process using sewage sludge as a substrate, with special emphasis on the acid fermentation phase. Successful separation of the phases was reported with the acid fermentation phase operating satisfactorily at a pH of 5.7 and an electrode potential referenced to the calomel electrode of -240 mv. This was significantly more positive than the values reported by Hammer and Borchardt [4]. Detention times between 10 to 24 hours appeared satisfactory for good conversion of substrate to volatile acids. Kinetic parameters were estimated for the acid phase, as will be discussed in the following chapter.

Keenan [75] reported the operation of a two-stage system at both mesophilic and thermophilic temperatures using dog food at 5 percent concentration as the substrate. During thermophilic operation, end products of the first stage were 98 percent carbon dioxide and 2 percent hydrogen and an aqueous effluent of 13 g/l of volatile acids. The

methane phase produced a gas containing 80 percent methane. Although no advantage in overall treatment efficiency was observed, better process stability was demonstrated in the two-phase system.

Maier and Fredrickson [76] demonstrated the use of phase separation in the anaerobic digestion of ground cornstalk residue using a 2.3-liter acid phase reactor followed by a 90-liter methane phase reactor. Acetate conversion to methane and carbon dioxide could be increased by maintenance of constant temperatures, high concentrations of acetate and an active population of appropriate microorganisms.

Therkelson, et al. [77] investigated the two-phase system for thermophilic digestion of a ground dog food slurry. Process configuration included a plug flow acid phase reactor followed by a completely mixed methane phase reactor. Volatile acid concentrations as high as 8000 mg/l as acetic acid were observed from the acid phase reactor and it performed satisfactorily at both mesophilic and thermophilic temperatures. Only marginal improvements in solids destruction were noted for two-phase operation in comparison to single stage performance. Smith, et al. [78] reported investigation of a two-stage process used for the digestion of poultry waste using an anaerobic filter as the methane phase. Volatile acids were obtained from a holding tank, screened of solids, and then introduced to an anaerobic filter. Good volatile acid reduction was obtained at

hydraulic retention times ranging from 1 to 40 days. Kinetic constants were reported; however, confusion existed as to the difference between slurry and fixed-film reactors so that values reported may be unreliable.

The review of literature reveals that limited investigations of the two-phase anaerobic stabilization process have been undertaken. However, the data obtained have not been representative of a system where true separation of the acid and methane phases has been achieved. Because of the use of sludges or simulated sludges as substrate, complete conversion to volatile acids in the acid reactor has not been demonstrated within the time frame required to achieve washout of the methane bacteria, thus acid production and utilization occurs in the methane reactor. Additionally, no work has been reported on the use of gravity clarifiers for concentration and recycle of biomass back to the respective reactors.

CHAPTER III

DEVELOPMENT OF KINETIC MODEL

The anaerobic stabilization process is similar to other biological treatment processes in that it is dependent upon the growth and metabolic activities of a given set of microorganisms. Thus it should be possible to describe the process mathematically if the microbial growth and metabolic rates are predictable. The following chapter deals with the development of mathematical expressions that may be used to describe and design the acid and methane fermentation phases of the process.

A simplistic representation of the complex scheme that develops in the anaerobic stabilization of a complex substrate may be written as shown in Figure III-1. Thus, a mathematical model of the process must account for both the activities of the microorganisms responsible for acid fermentation as well as for methane fermentation. Since it is assumed that these two activities are brought about by two different sets of microorganisms, the kinetic parameters describing their growth should also be different. It is this difference in growth rates that is exploited in the separation of the phases by kinetic means.

Growth kinetic models have been successfully used to

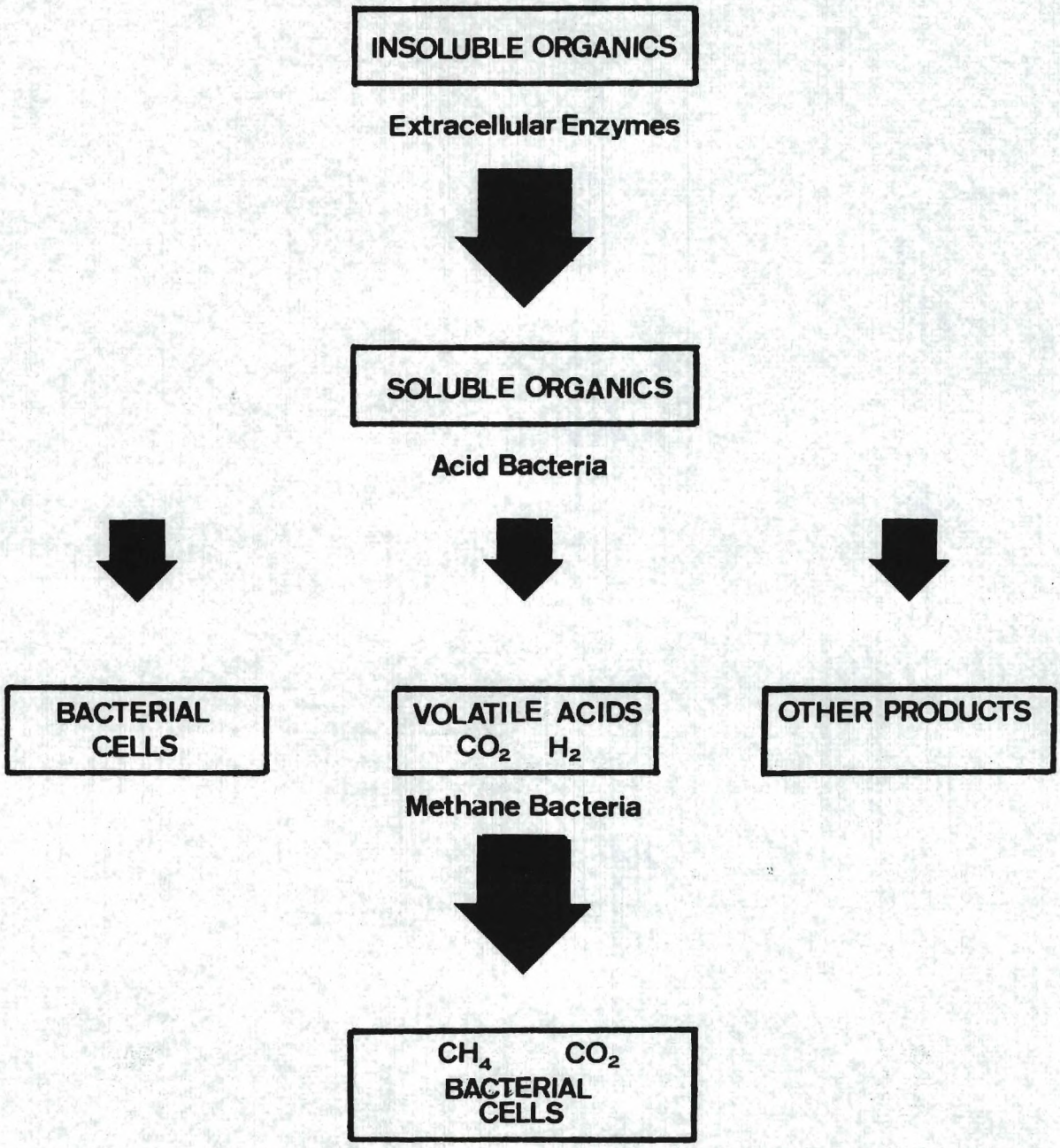


Figure III-1. Anaerobic Stabilization Process

describe both aerobic and anaerobic processes. The models applied to anaerobic processes have generally been applied to single-stage systems [68,79], however, models have been derived for two-phase systems [3,47]. Application of these models to anaerobic systems presupposes the fact that the methane generated by the methane bacteria is directly related to their growth rate, since this is the primary means of substrate removal from solution in the process.

Since the use of kinetic models has most often been applied to anaerobic systems containing a mixed population of acidogenic and methanogenic bacteria and the methane phase is generally assumed to be rate limiting, these kinetic models describe the activities of the methanogenic bacteria. Very little work has been performed in an investigation of kinetic parameters for the bacteria responsible for acid fermentation.

Continuous Culture Theory

The application of microbial growth kinetics to biological waste treatment processes has been well documented. Although the available literature describing kinetics of anaerobic processes is not as extensive as that for aerobic systems, the work of Andrews [73], Lawrence and McCarty [79], Pohland and Ghosh [3] and Ghosh, et al. [8] has demonstrated the applicability of kinetic models for anaerobic systems. All of the preceding investigators made use of the work of

Monod [80] to describe the rate of microbial growth. Although the equation of Monod is, strictly speaking, empirical, it does have some rational basis from the standpoint of its similarity to the familiar Michaelis-Menten [22] expression commonly used in enzyme kinetics.

The development of a mathematical model to describe substrate and organism concentration in a biological reactor can be performed by application of principles of mass balance to a biological reactor. The process configuration chosen for this model was two completely mixed reactors in series with provisions for cell separation and recycle following each reactor. The reactor contents are assumed to be uniform and the effluent from each reactor has the same composition as the reactor contents [81].

The function of the first reactor is to provide optimal environmental conditions for the growth of the acid bacteria. It is assumed that methane generation will not take place in the first reactor, or at least be greatly restricted by the kinetic controls exerted by the mode of operation, since the hydraulic retention time will always be less than the generation time determined for the methane bacteria. Thus, the effluent from the acid fermentation phase should be low in acceptable substrate for the acid bacteria but high in volatile acids, the principal organic substrate for the methane bacteria.

The methane fermentation stage will be accommodated

in the second completely mixed reactor in the train. This reactor will be operated to provide favorable environmental conditions for the methane bacteria and the bulk of waste stabilization should occur in this phase. Figure III-2 shows a schematic representation of the intended process configuration. (This is by no means intended as the only possible scheme, other process forms and possible advantages will be considered later.)

With the type of configuration illustrated in Figure III-2, an analysis of the substrate and organisms mass balances can be made around the reactor system for both the acid and methane fermentation phases. An organism balance for the acid reactor may be written as:

$$\left\{\begin{matrix}\text{Rate of change of organism}\\ \text{concentration in acid reactor}\end{matrix}\right\} = \left\{\begin{matrix}\text{Rate of}\\ \text{input}\end{matrix}\right\} + \left\{\begin{matrix}\text{Rate of Growth}\\ \text{in reactor}\end{matrix}\right\} - \left\{\begin{matrix}\text{Rate of}\\ \text{output}\end{matrix}\right\}$$

Expressed mathematically,

$$V_1\left(\frac{dx_1^A}{dt}\right)_{net} = QX_0^A + RQX_R^A + V_1\left(\frac{dx}{dt}\right)_{growth} - Q(1+R)X_1^A \qquad (1)$$

where:

V_1^A = acid reactor volume

X_0^A = concentration of acid formers in influent

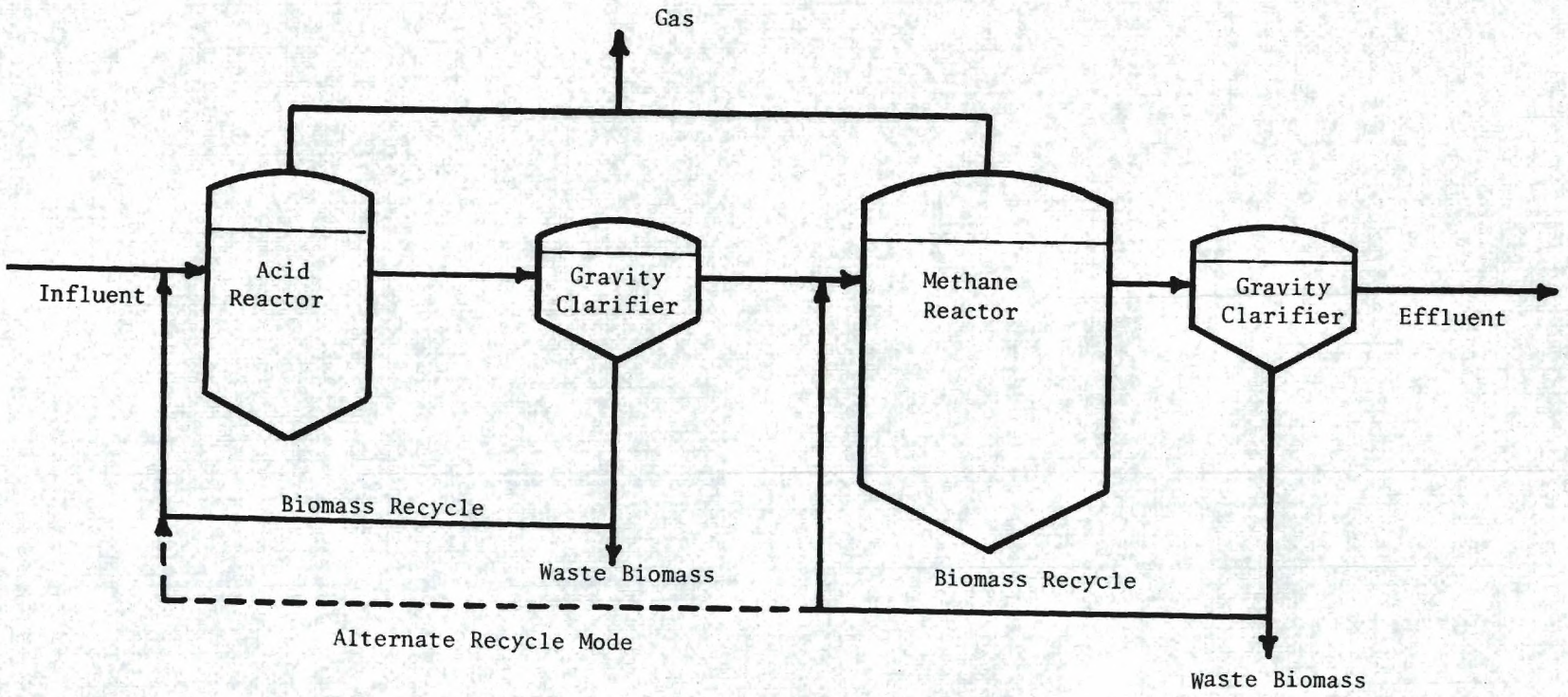


Figure III-2. Proposed Two Phase Anaerobic Digestion Process

X_1^A = concentration of acid formers in effluent

X_R^A = concentration of acid formers in recycle flow

Q = influent flow rate

R^A = fraction of Q recycled to acid reactor

Assuming steady state conditions and an influent free of acid, Equation 1 can be reduced and rearranged to:

$$\left(\frac{dx^A}{dt}\right)_{growth} = \frac{(1+R)X_1^A}{\theta_1^A} - \frac{RX_R^A}{\theta_1^A} \qquad (2)$$

where:

$\theta_1^A = \frac{V_1^A}{Q}$ = hydraulic retention time.

Assuming that minimal biological activity occurs in the clarifier, a substrate balance around the acid reactor may be made in the same manner, or

$$\left\{\begin{matrix}\text{Rate of change of substrate}\\ \text{concentration in acid reactor}\end{matrix}\right\} = \left\{\begin{matrix}\text{Rate of}\\ \text{input}\end{matrix}\right\} - \left\{\begin{matrix}\text{Rate of substrate}\\ \text{utilization}\end{matrix}\right\}$$

$$- \left\{\begin{matrix}\text{Rate of}\\ \text{output}\end{matrix}\right\}$$

Again expressed mathematically,

$$\left(\frac{dS^A}{dt}\right)_{net} = QS_o^A + RQS_1^A - V_1^A \left(\frac{dS^A}{dt}\right)_{\substack{\text{growth and}\\ \text{maintenance}}} - Q(1+R)S_1^A \qquad (3)$$

where:

S_0^A = substrate concentration for acid formers in influent

S_1^A = substrate concentration for acid formers in effluent

Again, assuming steady state conditions and inserting the expression for the hydraulic retention time, Equation 3 reduces to:

$$\left(\frac{dS^A}{dt}\right)_{\substack{\text{growth and}\\ \text{maintenance}}} = \frac{S_o^A}{\theta_1^A} - \frac{S_1^A}{\theta_1^A} \tag{4}$$

In order to develop the equations beyond this point, it is necessary to define some relationships for growth, decay and substrate utilization. The fundamental theory for these relationships was pioneered by Monod [80]. It is assumed that the rate of organism growth neglecting decay can be defined as:

$$\frac{dx}{dt}_{\text{growth}} = \mu X \tag{5}$$

where:

μ = specific growth rate

It is also assumed that the yield of microorganisms is a constant proportion of the substrate utilized, or

$$\left(\frac{dx}{dt}\right)_{\text{growth}} = -Y\left(\frac{dS}{dt}\right)_{\text{growth and maintenance}} \tag{6}$$

where:

Y = yield of microorganisms produced per mass of substrate utilized

Finally, Monod [80] observed that the change in specific growth rate for continuous cultures of bacteria could be described by a hyperbolic function of substrate concentration. This function, similar in form to the Michaelis-Menten relationship often used in enzyme kinetics, was shown by Monod to fit observed data very well. The Monod equation is of the form:

$$\mu = \mu_m \frac{S_1}{K_g + S_1} \tag{7}$$

where:

μ_m = maximum specific growth rate

K_g = saturation constant = the substrate concentration where $\mu = 0.5\ \mu_m$

Making use of these relationships, the organism and substrate balances can be extended to give expressions for organism and substrate concentrations for a given set of conditions. To illustrate, combination and rearrangement of the growth expression derived from Equation 2 and the expression for organism growth given in Equation 5 gives the following as an expression for the specific growth rate for the acid bacteria:

$$\mu^A = \frac{(1+R^A)}{\theta_1^A} - \frac{R^A}{\theta_1^A}\frac{X_R^A}{X_1^A} \tag{8}$$

Utilizing the concentration factor as proposed by Herbert [82], Equation 8 can be restated as:

$$\mu^A = \frac{1+R^A(1-C)}{\theta_1^A} \tag{9}$$

where:

$C = X_R^A/X_1^A$ = concentration factor

By then combining Equation 7 with this expression for μ^A, a relationship for substrate concentration can be developed. Accordingly,

$$S_1^A = \frac{K_g^A[1+R^A(1-C^A)]}{\theta_1^A\mu_m^A - [1+R^A(1-C^A)]} \tag{10}$$

The steady state expression for organism concentration can be developed by the substitution of Equations 5, 6 and 9 into Equation 4 and rearrangement to give:

$$X_1^A = \frac{Y^A(S_0^A-S_1^A)}{1+R^A(1-C^A)} \tag{11}$$

By analogous methods, expressions for the steady state effluent substrate concentration and reactor organism concentration from the methane reactor can be derived to yield:

$$S_1^{Me} = \frac{K_g^{Me}[1+R^{Me}(1-C^{Me})]}{\theta_2^{Me}\mu_M^{Me} - [1+R^{Me}(1-C^{Me})]} \tag{12}$$

and,

$$X_1^{Me} = \frac{Y^{Me}(S_o^{Me} - S_1^{Me})}{1 + R^{Me}(1-C^{Me})} \tag{13}$$

These equations have been developed previously to describe, with good agreement with experimental data, the kinetics of a two-phase anaerobic process [3].

As Ramanathan and Gaudy [83,84] have observed, the use of the concentration factor as a system constant was extremely difficult experimentally and resulted in severe fluctuations in the steady state values of organism and substrate concentration when employed with heterogeneous populations. Although the use of the concentration factor does simplify the derived mathematical expressions, the equations not incorporating it are better adapted to actual evaluation of experimental results. Using the organism concentration of the recycle flow more nearly approximates actual operating conditions. The effect of influent substrate concentration and recycle organism concentration on effluent parameters also becomes more readily apparent in the derived relationships.

Derivation of equations using organism recycle concentration as a system parameter may be addressed in one of two

ways. The substitution of the expression X_R/X_1, for c in Equations 10 and 11 followed by rearrangement will provide the final equations. The second approach, which will be used here, is to return to the basic mass balance and redevelop the equations.

Equation 8 gives the relationship of the specific growth rate without the use of the concentration factor, or

$$\mu^A = \frac{(1+R^A)}{\theta_1^A} - \frac{R^A}{\theta_1^A}\frac{X_R^A}{X_1^A} \tag{8}$$

Combination of Equations 4, 5 and 6 gives the following expression:

$$\frac{S_o^A}{\theta_1^A} - \frac{S_1^A}{\theta_1^A} = \frac{\mu^A}{Y^A} X_1^A \tag{14}$$

By combining Equations 8 and 14, a relationship for organism concentration can be derived:

$$X_1^A = \frac{Y^A(S_0^A - S_1^A) + R^A X_R^A}{1+R^A} \tag{15}$$

This expression is in variance with that derived by Ramanathan and Gaudy [83] for a completely mixed reactor with organism recycle. In their derivation, the substrate concentration of the recycle flow was assumed to be negligible in the substrate balance. Although this may be satisfactory

for specialized situations, it is not an adequate description for all cases and unnecessarily restricts the usefulness of the equations without greatly simplifying the final expression.

The development of the expression for substrate concentration is somewhat more complex. Equations 7 and 8 can be combined to give:

$$\mu_m^A \left(\frac{S_1^A}{K_g^A+S_1^A}\right) = \frac{(1+R^A)}{\theta_1^A} - \frac{R^A}{\theta_1^A}\frac{X_R^A}{X_1^A} \tag{16}$$

By substituting Equation 15 into Equation 16 and collecting terms, a quadratic equation for effluent substrate concentration can be obtained:

$$S_1^A = \frac{-b\pm\sqrt{b^2-4ac}}{2a} \tag{17}$$

where:

$$a = \mu_m^A - \frac{(1+R^A)}{\theta_1^A}$$

$$b = \frac{1+R^A}{\theta_1^A}(S_0^A-K_g^A) - \mu_m^A\left(S_o^A + \frac{R^A X_R^A}{Y^A}\right)$$

$$c = \frac{K_g^A\ S_o^A\ (1+R^A)}{\theta_1^A}$$

The equations for the methane reactor are derived in the same manner and are as follows:

$$X_1^{Me} = \frac{Y^{Me}(S_0^{Me} - S_1^{Me}) + R^{Me}X_R^{Me}}{1 + R^{Me}} \tag{18}$$

and,

$$S_1^{Me} = \frac{-e \pm \sqrt{e^2 - 4df}}{2d} \tag{19}$$

where:

$$d = \mu_m^{Me} - \frac{(1+R^{Me})}{\theta_2^{Me}}$$

$$e = \frac{1+R^{Me}}{\theta_2^{Me}} (S_o^{Me} - K_g^{Me}) - \mu_m^{Me} \left(S_o^{Me} + \frac{R^{Me}X_R^{Me}}{Y^{Me}}\right)$$

$$f = \frac{K_g^{Me} \; S_o^{Me} \; (1+R^{Me})}{\theta_2^{Me}}$$

Table III-1 shows a comparison of the steady state equations using the concentration factor, c, with those using the organism recycle concentration for the acid reactor.

Organism Decay

As Kirsch and Sykes [42] have pointed out, the expressions developed using the Monod relationships as previously stated are derived on the assumption of constant yield. Herbert [82] proposed a method of handling variable yields by incorporating a decay constant, k_d, which has had wide application in the environmental engineering field. Since most anaerobic systems are operated at long hydraulic retention times, a condition that leads to variable yields,

Table III-1. Comparison of Steady-State Equations for the Acid Fermentation Stage

Ghosh and Pohland [3]	
$X_1^A = \dfrac{Y^A(S_0^A - S_1^A)}{1+R^A(1-C^A)}$	$X_1^A = \dfrac{Y^A(S_0^A - S_1^A) + R^A(X_R^A)}{1+R^A}$
$S_1^A = \dfrac{K_g^A\ [1+R^A(1-C^A)]}{\theta_1^A \mu_m^A - [1+R^A(1-C^A)]}$	$S_1^A = \dfrac{-b \pm \sqrt{b^2-4ac}}{2a}$ $a = \mu_m^A - \dfrac{(1+R^A)}{\theta_1^A}$ $b = \dfrac{1+R^A}{\theta_1^A}\ [S_0^A - K_g^A] - \mu_m^A \left(\dfrac{S_o^A + R^A X_R^A}{Y}\right)$ $c = \dfrac{K_g^A\ S_o^A}{\theta_1^A}\ (1+R^A)$
$\mu^A = \dfrac{[1+R^A(1-C^A)]}{\theta_1^A}$	$\mu^A = \dfrac{1+R^A}{\theta_1^A} - \dfrac{R^A}{\theta_1^A}\ \dfrac{X_R}{X_1^A}$
$C = \dfrac{X_R^A}{X_1^A}$	

the derivation of equations that incorporate organism decay was considered justified.

The reduced yields for long hydraulic retention times are apparently caused by the existence of an organism maintenance energy requirement. Postgate and Hunter [85] lent credence to this observation by their work describing the phenomenon of endogenous metabolism. This is defined as the process that occurs when growing cells are placed in an environment devoid of exogenous substrate. During this period of population decay, components of the cell are consumed for energy and, in part, resynthesized. The viable population decreases and cell lysis may occur.

The growth rate equation of Herbert [82] included a term for endogenous metabolism or so-called negative growth, or

$$\left(\frac{dx}{dt}\right)_{growth} = (\mu - k_d)X \qquad (20)$$

where:

k_d = organism decay constant

Pirt [86] approached the problem by stating that the total rate of substrate removal is equal to that utilized for cell growth and cell maintenance. Ghosh and Pohland [9] have made use of this approach in their derivation of kinetic equations for a two-phase anaerobic system.

As shown by Kirsch and Sykes [42], the method of Pirt [86] is similar to that of Herbert [82]. Because that of

Herbert [82] has been historically most often utilized in the environmental engineering literature, it will be used here. Using this approach and the same method of derivation as described previously, equations may be developed that incorporate the effects of organism decay into the mathematical model.

Referring to Figure II-11, the acid reactor organism balance may be refined to add the effects of organism decay as follows:

$$V_1^A\left(\frac{dX^A}{dt}\right)_{net} = QX_o^A + RQX_R^A + V_1^A + V_1^A\left(\frac{dX^A}{dt}\right)_{growth} - V_1^A\left(\frac{dX^A}{dt}\right)_{decay} - Q(1+R)X_1^A \qquad (21)$$

Assuming steady state conditions and negligible concentration of acid formers in the influent, Equation 21 can be simplified to:

$$\left(\frac{dX^A}{dt}\right)_{growth} - \left(\frac{dX^A}{dt}\right)_{decay} = \frac{(1+R^A)X_1^A}{\theta_1^A} - \frac{RX_R^A}{\theta_1^A} \qquad (22)$$

The right side of the equation can be replaced to yield:

$$\left(\frac{dx}{dt}\right)_{growth} - \left(\frac{dx}{dt}\right)_{decay} = (\mu^A - k_d^A)X_1^A \qquad (23)$$

Therefore, Equation 22 can be restated and rearranged to give:

$$\mu^A = \frac{(1+R^A)}{\theta_1^A} - \frac{R^A X_1^A}{\theta_1^A X_1^A} + K_d^A \tag{24}$$

The substrate balance remains unchanged. However, substitution of Equation 24 into Equation 14 yields the following relationship for organism concentration:

$$X_1^A = \frac{Y^A(S_0^A - S_1^A) + R^A X_R^A}{(1+R^A+k_d^A\theta_1^A)} \tag{25}$$

By substitution of Equation 25 into Equation 16 and rearrangement, an expression for substrate concentration can be obtained, or

$$S_1^A = \frac{-n \pm \sqrt{n^2 - 4mo}}{2m} \tag{26}$$

where:

$$m = \mu_m^A - \frac{(1+R^A)}{\theta_1^A} - k_d^A$$

$$n = \left(\frac{1+R^A}{\theta_1^A} + k_d^A\right) [S_o^A - K_g^A] - \mu_m^A\left(S_o^A + \frac{R^A X_R^A}{Y^A}\right)$$

$$o = S_o^A K_g^A \left[\frac{1+R}{\theta_1^A} + k_d^A\right]$$

As before, analogous expressions can be derived for the methane reactor, or

$$X_1^{Me} = \frac{Y^{Me}(S_o^{Me} - S_1^{Me}) + R^{Me}X_R^{Me}}{1 + R^{Me} + k_d^{Me}\,{}^{Me}_{2}} \tag{27}$$

$$S_1^{Me} = \frac{-s \pm \sqrt{s^2 - 4rt}}{2r}$$

where:

$$r = \mu_m^{Me} - \frac{(1+R^{Me})}{\theta_2^{Me}} - k_d^{Me} \tag{28}$$

$$s = \left(\frac{1+R^{Me}}{\theta_2^{Me}} + k_d^{Me}\right)[S_o^{Me} - k_g^{Me}] - \mu_m^{Me}\left(S_o^{Me} + \frac{R^{Me}X_R^{Me}}{Y^{Me}}\right)$$

$$t = S_o^{Me}k_g^{Me}\left[\frac{1+R^{Me}}{\theta_2^{Me}} + k_d^{Me}\right]$$

Table III-2 shows the final equations for both the acid and methane phases with and without organism decay.

Estimation of Kinetic Parameters

In order to utilize the preceding equations for design purposes, it is necessary to predict the values of the kinetic parameters, μ_{max}, K_g, Y and k_d. These values are assumed to be constant only for a waste stream of a particular organic composition; therefore, these constants must be experimentally determined for each application. These values are generally estimated from data obtained during continuous flow pilot plant studies which are conducted

Table III-2. Kinetic Expressions for Organism and Substrate Concentration for Two-Phase Anaerobic System

Acid Reactor

Without Organism Decay	Including Organism Decay
$X_1^A = \dfrac{Y^A(S_0^A - S_1^A) + R^A X_R^A}{1+R^A}$	$X_1^A = \dfrac{Y^A(S_0^A - S_1^A) + R^A X_R^A}{1+R^A+k_d^A\theta_1^A}$
$S_1^A = \dfrac{-b \pm \sqrt{b^2-4ac}}{2a}$	$S_1^A = \dfrac{-n \pm \sqrt{n^2-4mo}}{2m}$
$a = \mu_m^A - \dfrac{(1+R^A)}{\theta_1^A}$	$m = \mu_m^A - \dfrac{(1+R^A)}{\theta_1^A} - k_d^A$
$b = \dfrac{1+R^A}{\theta_1^A}[S_o^A - k_g^A] - \mu_m^A[S_o^A + \dfrac{R^A X_2^A}{Y^A}]$	$n = (\dfrac{1+R^A}{\theta_1^A} + k_d^A)[S_o^A - K_g^A] - \mu_m^A[S_o^A + \dfrac{R^A X_2^A}{Y^A}]$
$c = \dfrac{K_g^A S_o^A}{\theta_1^A}(1+R^A)$	$o = K_g^A[\dfrac{S_o^A + R^A S_o^A}{\theta_1^A} + S_o^A k_d^A]$
$\mu^A = \dfrac{1+R^A}{\theta_1^A} - \dfrac{R^A X_R^A}{\theta_1^A X_1^A}$	$\mu^A = \dfrac{1+R^A}{\theta_1^A} - \dfrac{R^A X_R^A}{\theta_1^A X_1^A} + k_d^A$

Methane Reactor

Without Organism Decay	Including Organism Decay
$X_1^{Me} = \dfrac{Y^{Me}(S_0^{Me}-S_1^{Me})+R^{Me}X_R^{Me}}{1+R^{Me}}$	$X_1^{Me} = \dfrac{Y^{Me}(S_0^{Me}-S_1^{Me})+R^{Me}X_R^{Me}}{1+R^{Me}+k_d^{Me}\theta_2^{Me}}$
$S_1^{Me} = \dfrac{-e\pm\sqrt{e^2-4df}}{2d}$	$S_1^{Me} = \dfrac{-s\pm\sqrt{s^2\pm 4rt}}{2r}$
$d = \mu_m^{Me} - \dfrac{(1+R^{Me})}{\theta_2^{Me}}$	$r = \mu_m^{Me} - \dfrac{(1+R^{Me})}{\theta_2^{Me}} - k_d^{Me}$
$e = \dfrac{1+R^{Me}}{\theta_2^{ME}}[S_o^{Me}-K_g^{Me}]-\mu_m^{Me}$ $[S_o^{Me} + \dfrac{R^{Me}X_R^{Me}}{Y^{Me}}]$	$s = (\dfrac{1+R^{Me}}{\theta_2^{ME}} + k_d^{Me})[S_o^{Me}-K_g^{Me}]$ $- \mu_m^{Me}[S_o^{Me} + \dfrac{R^{Me}X_R^{Me}}{Y^{Me}}]$
$f = \dfrac{K_g^{Me}\ S_o^{Me}}{\theta_2^{Me}}(1+R^{Me})$	$t = K_g^{Me}[\dfrac{S_o^{Me}+R^{Me}S_o^{Me}}{\theta_2^{Me}} +$ $S_o^{Me} + k_d^{Me}]$
$\mu^{Me} = \dfrac{1+R^{Me}}{\theta_2^{Me}} - \dfrac{R^{Me}X_R^{Me}}{\theta_2^{Me}X_1^{Me}}$	$\mu^{Me} = \dfrac{1+R^{Me}}{\theta_2^{Me}} - \dfrac{R^{Me}X_R^{Me}}{\theta_2^{Me}X_1^{Me}} + k_d^{Me}$

under varying conditions of hydraulic retention time and recycle.

The technique for determination of the values of these parameters generally involves manipulation of the mathematical model derived to describe the biological system so that a plot of the observed data will result in a straight line with a specified slope and intercept. Accordingly the Monod growth expression can be rewritten in the following forms to give a linearized equation of the form, y = ax + b [42]:

1. Lineweaver--Burke [42]

$$\frac{1}{\mu} = \frac{k}{\mu_m}\left(\frac{1}{s}\right) + \frac{1}{\mu_m} \tag{29}$$

2. Hofstee [42]

$$\mu = \mu_m - k\left(\frac{\mu}{s}\right) \tag{30}$$

3. Eadie [42]

$$\frac{s}{\mu} = \frac{1}{\mu_m}(s) + \frac{k}{\mu_m} \tag{31}$$

The Lineweaver-Burke plot utilizes the plotting of $1/\mu$ and $1/S$, the Hofstee form requires a plot μ versus μ/S, and the Eadie form uses S/μ versus S [42]. The Lineweaver-

Burke form has been most often utilized in the environmental engineering literature and will be used hereafter for data analysis.

Many times, the kinetic constants are determined by treatability studies that do not utilize organism recycle. In this case, the equations derived for effluent substrate and organism concentration which include the effects of organism decay can be reduced to the following:

$$S_1 = \frac{K_g(1+k_d\theta_2)}{\theta\mu_{max}-(1+k_d\theta)} \tag{32}$$

and

$$X_1 = \frac{Y(S_0-S_1)}{(1+k_d\theta)} \tag{33}$$

To determine the kinetic constants for these type of data, these equations may be properly rearranged to a $y = ax + b$ form. Accordingly, Equation 32 may be rearranged to give:

$$\frac{\theta}{1+k_d\theta} = \frac{k_g}{\mu_{max}}\frac{1}{s} + \frac{1}{\mu_{max}} \tag{34}$$

Similarly, Equation 33 will reduce to the form:

$$\frac{S_0-S_1}{X_1} = \frac{k_d\theta}{Y} + \frac{1}{Y} \tag{35}$$

Both equations should provide linear plots. By plotting $(S_0-S_1)/X$ against θ, a straight line with an intercept on the y-axis of $1/Y$ and a slope of k_d/Y is obtained. Then the organism decay may be used to obtain a plot of $\theta/(1+kd\theta)$ versus $1/S_1$, to produce a line with the intercept $1/\mu_{max}$ and slope K_g/μ_{max}.

For the equations developed that do not include the effects of organism decay, a value for specific growth rate can be calculated using Equation 8. Then the Lineweaver-Burke form of the Monod growth expression (Equation 31) can be utilized by plotting $1/\mu$ versus μ. The organism yield can be calculated directly through use of Equation 15.

For those equations incorporating the effects of organism decay, the procedure is necessarily more complex. By rearrangement of Equation 25, the following expression can be obtained:

$$\frac{1+R}{\theta} - \frac{R}{\theta}\frac{X_R}{X_1} = \frac{Y(S_0-S_1)}{X_1\theta} - k_d \qquad (36)$$

This is of the form $y = ax + b$ with $y = \frac{1+R}{\theta_1} - \frac{R}{\theta}\frac{X_R}{X_1}$ and $X = \frac{S_0-S_1}{X_1\theta}$. A plot of these variables should yield a straight line with a slope of Y and a y-intercept of -kd. Once these parameters have been obtained, a value for μ can be calculated and the Eadie form of the Monod growth expression (Equation 31) can be applied.

Experimental Justification

There is substantial support within the literature to justify the use of microbial growth kinetics, similar to those presented, to describe biological waste treatment processes [83,84,87]. This approach has been used with success to model both aerobic and anaerobic treatment systems.

Although the bulk of the experimental data recorded to date deals with aerobic treatment processes, there have also been significant advances in the description of the kinetics of anaerobic bacterial growth. The kinetic studies that have been performed on anaerobic suspended growth processes can generally be classified into two categories:

(a) Those studies that view the kinetics of anaerobic digestion as a single-stage process; and,

(b) Those investigations that have explored the kinetics of the separate phases of the anaerobic stabilization process.

Since anaerobic stabilization is viewed from a kinetic viewpoint as occurring in a stepwise fashion as shown in Figure III-1, evaluation of single-stage process data requires the assumption of a rate-limiting step. Lawrence [87] has defined the rate-limiting step as that step in the process which will cause process failure to occur under imposed conditions of kinetic stress. In anaerobic treatment, failure of this type is usually characterized by cessation of methane production and decreased COD removal. Several

investigators [29,73,88] have reported that kinetic failure is also characterized by a build-up in the concentration of the volatile fatty acids, substrate for the methane bacteria. Thus, it is reasonable to conclude that the rate-limiting step and the key to kinetics in the anaerobic treatment process is the utilization of the volatile acids by the methane bacteria.

Since most of the work reported in the literature has been concerned with evaluation of the kinetics of single-stage processes or the study of the utilization by the methane bacteria of the volatile organic acids, these data will be reported initially. There are very little data available on acid phase kinetics.

The most meaningful information available for evaluating the kinetics of the methane phase is that obtained from continuous culture studies on the anaerobic stabilization of the volatile organic acids. Lawrence and McCarty [79] extensively investigated the fermentation to methane and carbon dioxide of three volatile acids, acetic, propionic and butyric acids, precursors to the great majority of the methane produced from the digestion of a complex waste [62].

According to Barker [38] acetic acid is fermented to methane and carbon dioxide in a single step while both propionic and butyric require two steps, as shown in the following stoichiometric equations:

Acetic Acid

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$$

Propionic Acid

First Step -

$$CH_3CH_2COO^- + 1/2H_2O \rightarrow CH_3COO^- + 3/4\ CH_4 + 1/4\ CO_2$$

Second Step -

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$$

Overall -

$$CH_3CH_2COO^- + 3/2\ H_2O \rightarrow 7/4\ CH_4 + 1/4\ CO_2 + HCO_3^-$$

Butyric Acid

First Step

$$CH_3CH_2CH_2COO^- + HCO_3^- \rightarrow 2CH_3COO^- + 1/2\ CH_4 + 1/2\ CO_2$$

Second Step

$$2CH_3COO^- + 2H_2O \rightarrow 2CH_4 + 2HCO_3^-$$

Overall

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 5/2\ CH_4 + 1/2\ CO_2 + HCO_3^-$$

The validity of these stoichiometric relationships for propionic and butyric acid may be in doubt in light of the postulate [25,37] that the fermentation to methane of propionic, butyric and long chain fatty acids may involve hydrogen oxidizing methanogenic bacteria. However, regardless of the mechanism, the work of Lawrence and McCarty [79]

demonstrated the feasibility of operating an anaerobic reactor fed solely with the simple volatile acids and gave estimates on the kinetic parameters for the methane phase of a two-phase system.

Lawrence [87] summarized the values of yield coefficient, Y, and organism decay coefficient, k_d, as a range and average for all the volatile acids investigated. The yield was computed on the basis of mg of biological solids produced per mg of substrate COD converted for energy--i.e., to methane. The reactors were maintained at 35°C and buffered to a neutral pH throughout the study. The values obtained are indicated in Table III-3.

Table III-4 indicates the average values obtained for the balance of the kinetic parameters necessary to completely define the model, based upon substrate utilization at 35°C.

Additional results have been reported by Kugelman, Chin and Molof [58] on the results of cation concentrations on the kinetic parameters of a continuous culture anaerobic reactor utilizing acetate at a temperature of 35°C and a neutral pH. The values of the base parameters before the addition of large concentrations of potassium are shown in Table III-5.

The values in Table III-5 correlate very well with the values reported by Lawrence and McCarty [79]. Both of these studies indicate that the kinetic expressions based upon the work of Monod [80] can be used to describe the kinetics of volatile acid utilization by the methane bacteria

Table III-3 [87]. Range and Average Values of Y and k_d in Methane Fermentation of Volatile Acids [79]

Parameter	Range	Average
Y(mg/mg)	0.040-0.054	0.044
$k_d(day^{-1})$	0.010-0.040	0.019

Table III-4 [79]. Average Values of Kinetic Parameters for Substrate Utilization [79]

Volatile Acid Substrate	μ_m* $(days^{-1})$	K_g (mg/l)
Acetic	0.5-0.24	154 as HA_c
Propionic	0.37-0.25	39 as HP_r
Butyric	0.37	7 as HB_u

*Calculated as $\frac{1}{\text{Minimum Solids Retention Time}} = \mu_m$

Table III-5 [58]. Kinetic Parameters for Anaerobic Stabilitation of Acetate [58]

Y--0.041 mg biological solids/mg of substrate converted

k_d--0.0356/day

k_s--161.4 mg/l as HA_c

μ_{max}--0.35/day

as well as to indicate that digesters can exist solely on volatile acids as a substrate, thus lending additional credence to the two-phase approach of separation of the acid and methane phases.

Ghosh and Pohland [3] have made use of the Monod expressions to estimate kinetic parameters for both the acidogenic and methanogenic phases of a two-phase system. A single glucose-fed anaerobic reactor was operated at 37°C and a pH near neutral. Residence time was decreased in successive steps until washout of the methane organisms occurred and the reactor was operating as an acidogenic reactor. Kinetic parameters estimated for both the acidogenic and methanogenic phases were:

	Acidogenic Phase	Methanogenic Phase
μ_{max}(days^{-1})	30.0	3.34
K_s(mg/l)	22.5 as glucose	600 as HA

The reported values for the methanogenic phase are an order of magnitude higher than the two previously discussed studies [58,79]. It is probable that the presence of attached growth within the reactor contributed to the persistence of acetate utilization and the unexpectedly high μ_{max}. The study was conducted at volatile acid concentrations of only 300-600 mg/l where attached growth metabolism would be more significant than the studies of Lawrence and McCarty [79] and Kugelman, Chin and Molof [58] where

volatile acid concentrations were several times higher. The concentration of attached methane bacteria is surface limited, thus restricting their metabolic activities. At lower acid concentrations, the net effect on equilibrium volatile acid concentration would become more significant. (The data for the acidogenic phase will be subsequently discussed and analyzed in the chapter dealing with experimental results.)

There have been other attempts to characterize the kinetics of the acid phase of the anaerobic process. Andrews and Pearson [89] investigated the rates of volatile acid production in an anaerobic reactor being fed a high strength soluble waste composed primarily of Tryptone and glucose. They estimated the acid phase kinetic parameters as follows: μ_{max} - 1.33/day; Y = 0.54 mg VSS/mg COD utilized; and k_d = 0.87/day.

Ghosh, et al. [8] extended their investigation of two-phase systems to include a process using wastewater sludge as a substrate. The two-reactor system was operated so as to optimize acid production in the first reactor while the second reactor was operated in such a manner as to encourage methane production. The difficult problem of organism mass measurements in a substrate of sewage sludge was confronted by the measurement of dehydrogenase activity and the correlation of this data with active biomass concentration. Because of lack of knowledge concerning this

relationship, the absolute values of the active biomass determinations must be somewhat suspect. Furthermore, much of the acid formation had occurred prior to introduction of the sludge to the acid reactor while the sludge was in storage. Nonetheless, kinetic parameters for the acid phase were estimated at μ_{max} = 3.84 day^{-1} and K_s = 26.0 g/l as volatile solids.

Although some inconsistencies in the published data are apparent, the studies cited do provide ample justification for the use of Monod kinetics to predict kinetic characteristics of the acid and methane phases of a two-phase anaerobic system. Additional information on kinetic theory for single phase systems may be obtained from the excellent reviews prepared by Kirsch and Sykes [42], Pretorius [90] and Lawrence [87].

CHAPTER IV

EXPERIMENTAL PROCEDURES

Experimental methods and procedures were developed to provide information in support of the research objectives. The necessary equipment and procedures are described in the following sections.

Description of Experimental Apparatus

A two-phase laboratory reactor system with facilities for gravity clarification and biomass recycle for each phase was designed and constructed as indicated in Figure IV-1. The system provided the following functional requirements:

(1) A reactor and clarifier system capable of being maintained under strict anaerobic conditions;

(2) Provisions for adequate mixing of each reactor to provide near ideal complete mixing;

(3) Temperature control at a system optimum of 37°C;

(4) A reliable substrate delivery system with adequate controls to accommodate variable organic loadings and hydraulic retention times;

(5) Gas collection and monitoring capabilities; and,

(6) Gravity clarification and biomass recycle capabilities that could be maintained under anaerobic conditions.

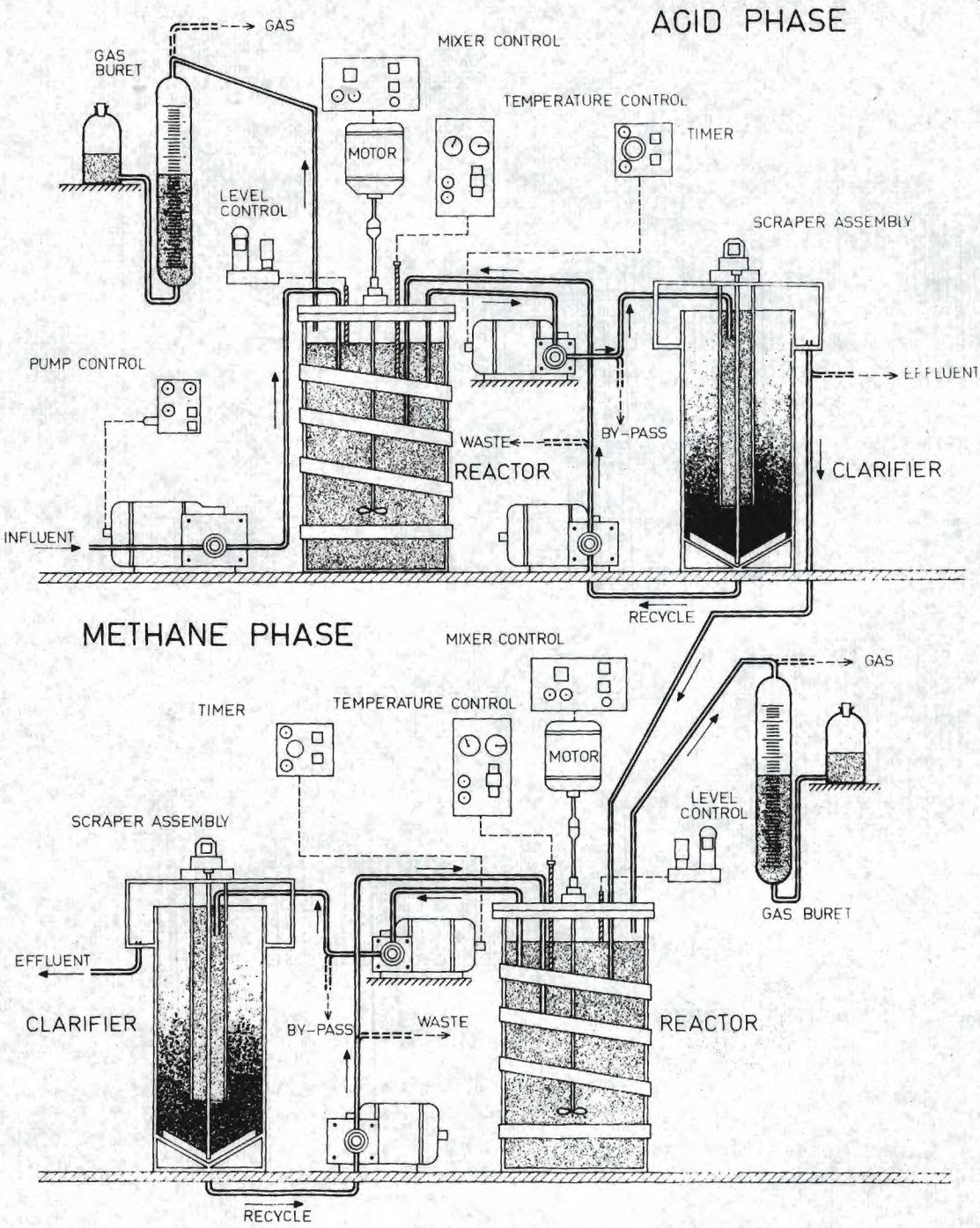


Figure IV-1. Reactor and Clarifier Systems for Phase Separation of the Anaerobic Stabilization Process

Each reactor was constructed from 8-inch diameter Plexiglas pipe with a 1/4-inch wall thickness. The operating volume of each reactor was 10 liters, which produced a liquid depth in each reactor of approximately 14 inches. The reactors were mounted on a frame constructed of Unistrut and plywood for easy access to all components and for operational convenience. The top and bottom plates of each reactor were fabricated from 1/2-inch thick Plexiglas sheets and machined to provide a gas-tight seal. Each reactor top was provided with 10 drilled and tapped 1/2-inch openings which were used to facilitate sampling access, influent and effluent tubing and various process control probes. Each top also was equipped with a bearing and seal housing at the mixer shaft entrance into the reactor.

Each reactor was mechanically mixed by a 3-inch stainless steel impeller located 4 inches above the reactor bottom. The impellers were mounted on a 1/2-inch stainless steel shaft that was driven by a 1/8-horsepower Bodine Electric Company motor, Type NSH-54. Mixing speed was controlled through use of a Bodine Electric Company DC Motor Speed Controller, Type ASH-500, which was capable of controlling mixing speed across the full range of motor speed up to 1200 rpm and also allowed the reversal of the rotational direction. At the entrance point of the shaft into the reactor top, a 3-inch cast acrylic machined rod accommodated three roller bearings and two oil seals for shaft support and a gas-tight seal.

Dye tests were conducted on a virtually identical reactor to determine the effect of the mixing apparatus on the residence time distribution within the reactor. A C-curve [81] resulted from a plot of effluent dye concentration versus time, plotted in reduced units, after a pulse input of dye was added to the reactor influent. This curve, shown in Figure IV-2, is compared to the expected theoretical curve for a completely mixed reactor. Good agreement between the two curves indicated that virtual ideal completely mixed conditions were obtained at a impeller rotational speed of about 600 rpm.

The anaerobic reactors were operated at a mixing speed of about 500 rpm and thus were assumed to be at essentially completely mixed conditions. Assuming turbulent conditions and a k value of 1.0 for a propeller type impeller with three blades and a pitch of 2 [114], a power input of 0.338 ft/lb force/second can be calculated. Using this power input, a mean velocity gradient of 82/second can be calculated to describe conditions within the reactor. Typical values for the mean velocity gradient are from 20/second to 75/second. Reported values of Gt_d vary from 10^4 to 10^5 [114].

The desired reactor temperature of 37° ± 0.5°C was maintained constant throughout the course of the study. Effective temperature control was achieved through the use of YSI Thermistemp Temperature Controllers, model 63RC. The associated control probe was inserted into the reactor through

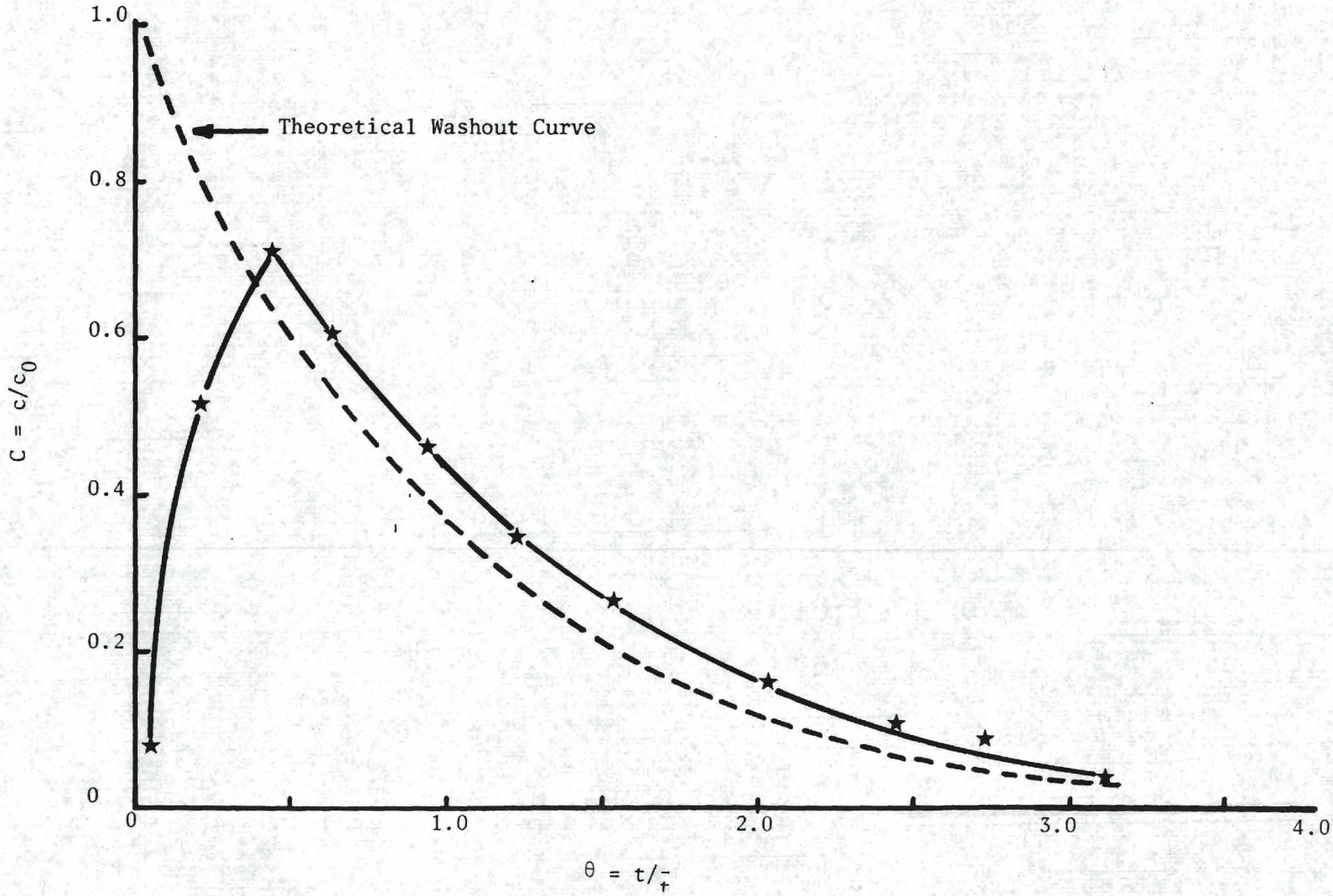


Figure IV-2. Reactor Response to Pulsed Dye Injection

1/2-inch openings at the reactor top. The probe was encased in Tygon tubing at the point of entry and a gas-tight seal was provided by using a Swagelock tube fitting. The controller actuated a 1/2-inch heating tape, 48-inches long, which provided 192 watts of heat energy. The heating tape was wrapped around each reactor and was sandwiched between two layers of insulation to avoid direct heat on the Plexiglas surface.

The substrate delivery system for each reactor consisted of influent and effluent pumps with appropriate controls for each. The influent pump for each reactor was equipped with controls to provide for either continuous or intermittent substrate introduction into the reactor. This flexibility was necessary in order to achieve accurate control of hydraulic retention times over a broad range of values. The principal components of the influent system were an influent pump with a variable output DC power supply and a repeating cycle timer with an associated electrical relay device. The Industrial Timer Corporation Model CM-5 timer operated on a 3-minute cycle for the acidogenic reactor and a 9-minute cycle for the methanogenic reactor. Each timer could be adjusted to give continuous operation of the influent pump or to operate the influent pump for a timed interval during each cycle. The timers activated a control relay which provided power to the influent pumps. Each influent pump was a Cole-Parmer Masterflex Tubing Pump

outfitted with Masterflex 7014 pump heads. The pumps were driven by G. K. Heller Corporation electric motors, model GT21-18, with a speed range of 0-333 rpm. The motor speed was controlled by a G. K. Heller Corporation Motor Controller, model GT-21. With this type of feed arrangement, hydraulic retention times from 4-50 hours could be reliably controlled in the acidogenic reactor and from 12-150 hours in the methanogenic reactor.

The substrate for the acidogenic reactor was stored in a large cooler adjacent to the reactor system. Storage temperature was maintained at 2-5°C to minimize substrate decomposition prior to use. The suction line for the acidogenic reactor influent pump was connected to the substrate reservoir in the cooler by 3/8-inch plastic tubing. The suction line for the methanogenic reactor was connected either to the acidogenic reactor, 6 inches below the liquid surface or 2 inches below the liquid surface of the clarifier for the acidogenic reactor. The influent line entering each reactor extended 10 inches below the liquid surface.

The effluent removal system for each reactor consisted of a liquid level control system with associated effluent pump. The heart of the control system was a Cole-Parmer Dyna-Sense Electronic Liquid Level Controller, model 7186. The controller had a high level and low level probe which were inserted into the reactor through 1/2-inch openings at the reactor top. A gas-tight fit around each probe was

provided by the use of 1/4-inch Swagelock tube fittings with 1/2-inch pipe threads. The ground connection of the controller was attached to the influent tube of each reactor. The high level probe was adjusted to activate the effluent pump when the liquid volume reached 10.15 liters and the low level probe deactivated the effluent pump at a liquid capacity of 9.85 liters. The effluent pumps were Cole-Parmer Masterflex Tubing Pumps, model 7540 with model 7014 Masterflex Pump Heads. The pumps were powered by a fixed speed motor at 575 rpm.

Fermentation gases were collected with 2.5-liter graduated burets mounted adjacent to each reactor on the support frame. Each buret was partially filled with an aqueous solution of sulfuric acid and sodium sulfate as described in Standard Methods [91]. The head space at the top of each reactor was connected by 3/8-inch Tygon tubing to the top of each buret. The aqueous solution was displaced into 3-liter reservoirs as gas was produced. The connecting tubing at the top of each reactor was equipped with a sample tee to provide easy access to off-gas samples.

The entire reactor system was tested for gas leaks after construction by pressurization with nitrogen to the maximum extent possible for the displacement-type gas collection system (about 13 inches of water). The system was then observed for a 48-hour period for possible leaks and also periodically soap tested during test operations to

insure system integrity. No loss of pressure was observed during the 48-hour test period.

A gravity clarification system was designed for use with the two-phase reactor system. The system included provisions for intermittent organism recycle to the appropriate reactor. The gravity clarifiers were constructed of 8-inch diameter Plexiglass pipe with a liquid volume of 11.5 liters. Each clarifier was constructed to include a 2-inch diameter central stilling chamber extending 12 inches below the liquid surface. Effluent from the reactor was introduced into the stilling chamber. The overflow chamber was constructed of 11-inch diameter Plexiglas pipe. The cone bottom of the clarifier was machined from built-up laminated plexiglass plates and had a slope of 38°. A 1/2-inch diameter opening was provided at the bottom for sludge withdrawal. A rotary sludge scraper was provided for each clarifier to insure a consistent underflow sludge concentration. The scraper was powered by a small Bodine Electric Company motor mounted on the clarifier top and rated at 0.9 rpm.

The clarifier was covered by a 1/4-inch Plexiglas cover. Because gas-tight seals were not provided for the scraper shaft and incoming lines, a flow of nitrogen was maintained into the overflow chamber of each clarifier to provide a slight positive pressure and minimize oxygen intrusion. It was assumed that methane production in the clarifier was minimal and no attempts were made to capture this gas.

The solids recycle system consisted of a pump with a variable speed controller and a repeating timer. The recycle pumps were Cole-Parmer Masterflex 7015 Pump Heads. An Industrial Timer Corporation Model CM-5 repeating cycle timer was used to control the recycle pump. The timer had a 60-minute cycle so that sludge was recycled to the reactors one time each hour at a predetermined volume.

In summary, the reactor system was specifically designed to accommodate a two-phase anaerobic process with the necessary controls considered necessary to insure accurate and reliable data acquisition.

Development of Experimentation Plan

A plan was developed to utilize the two-stage reactor system previously described to achieve the objectives of the two-phase research envisioned. The study was divided into a sequence of several stages with each succeeding stage designed to utilize previous results obtained. The research investigations thus consisted of the following stages:

Stage I--Two-phase Operation with Simple Substrate without Biomass Recycle.

Stage II--Two-phase Operation with Simple Substrate and Biomass Recycle.

Stage III--Two-phase Operation with Industrial Wastewater and Biomass Recycle.

Stage I of the plan was designed to demonstrate the

feasibility of kinetic control for separation of the anaerobic phases. Thus a simple soluble substrate, glucose, was used to facilitate reactor system operation. At the same time that phase separation was being achieved by control of hydraulic retention time, data suitable for estimating the kinetic parameters of the acid and methane phases would be collected. Additionally, biomass for recycle during Stage II would be generated.

Stage II of the study was developed to include the additional complication of biomass concentration and recycle by use of gravity clarification. Thus the applicability of gravity clarification and biomass recycle for process enhancement would be evaluated. At the same time, the data collected during Stage II could be used to evaluate the reliability of the kinetic data accumulated in Stage I.

The Stage III segment of the study was designed to demonstrate the applicability of the process for treatment of soluble wastes more complex in make-up than the synthetic substrate used in the initial stages of the study.

Reactor Start-Up Procedures

Each reactor was prepared for use by filling to the 9-liter level with a phosphate buffered solution containing 1000 mg/l of glucose. The reactor system was then purged of oxygen by bubbling pure nitrogen through the reactor and allowing it to escape through the gas collection system for

a minimum period of 15 minutes. A gas sample was then taken from the reactor and analyzed for oxygen content. If the reactor was devoid of oxygen, seeding operations were commenced once the reactors reached operating temperature.

Each reactor was seeded with one liter of supernatant from actively digesting sludge obtained from the R. M. Clayton Municipal Wastewater Treatment Plant in Atlanta, Georgia. Five liters of digester sludge were obtained from the plant and allowed to settle for several hours. The digester supernatant was then pumped directly into each reactor. Both the acid and methane phase reactors were seeded by the same procedure.

The reactors were allowed to acclimate and began to produce small amounts of gas within the first 24 hours. Daily feed with 1000 mg of concentrated glucose was commenced and continued until gas production was well established and detectable concentrations of methane were observed. This condition was reached within five days of operation. At this point, continuous feeding operations were initiated.

Steady-State Operation

Once continuous feeding operations were initiated, sampling and data acquisition were commenced. A series of hydraulic retention times were scheduled for the two reactors in the system. Flexibility in the operation of the acid reactor was somewhat restricted since it was desired to minimize substrate carryover to the methane reactor in

order to effect true phase separation. Thus, relatively long retention times were scheduled in order to achieve good substrate conversion to volatile acids. This was not a consideration in the selection of hydraulic retention times for the methane reactor and the only limits were those imposed by equipment design.

During each stage of the study, a complete series of hydraulic retention times were scheduled. Changes in hydraulic retention time were generally made in a stepwise fashion from the longest to the shortest during the stage of study. No changes were made in substrate concentration; therefore, organic loading to the reactors increased as the hydraulic retention time decreased.

Since the mathematical model developed was for steady-state conditions, the attainment of steady-state in the system during data acquisition was of prime importance. Due to the series nature of the reactor system used, the advent of steady-state conditions was more difficult to achieve and maintain than for single flowthrough reactors. The use of clarifiers in Stages II and III provided additional complications.

Because substrate utilization is closely tied to the biomass concentration and the use of clarifiers for biomass recycle was to be studied, it was decided that suspended solids concentrations within each reactor would be of prime consideration in the judgement of whether the reactor system

as a whole was at steady-state. As discussed by Levenspiel [81], the washout curve for a completely mixed reactor can be described by a negative exponential function, similar in form to a radioactive decay function. It was assumed that attainment of steady-state conditions was achieved by the washout of the original reactor contents after a change in conditions had been implemented. Three retention times will provide for 95 percent removal of the original reactor contents after a change has been made [81]. Therefore, a period of time equal to three hydraulic retention times was the minimum guideline used before steady-state conditions were assumed. Moreover, the total system was not considered to be steady-state until the methane reactor was operated for a minimum of three hydraulic retention times beyond the time that the acid reactor was deemed to be at steady-state.

The addition of the clarification and biomass recycle system during Stage II and III presented special problems in the achievement of steady-state conditions. Here again, particular emphasis was placed on the suspended solids concentration within each reactor. When the measured suspended solids concentration within the acid reactor had stabilized and the three retention time guidelines had been satisfied, steady-state conditions were assumed to be satisfied for this reactor. The methane reactor was then operated for three retention times and the suspended solids concentration monitored. Steady-state conditions were assumed

to be satisfied and data collected for analysis only after the operation of the reactor system as a whole was fully stabilized. Figure IV-3 typifies the variation in TSS concentration during Test II-A. Starting at the fourteenth day, the TSS concentration appeared to stabilize and steady-state conditions were assumed to be attained. The balance period was started on the fifteenth day and the final sample was obtained on the seventeenth day. Operation in this manner generally provided for the passage of time equivalent to at least three sludge ages for each reactor.

In addition to these criteria, each reactor was sampled twice weekly and full testing was performed. Thus, routine monitoring of reactor performance was carried out and was also used to evaluate the achievement of stabilization of reactor system performance.

Substrate Composition

The substrate used in Stages I and II of the study was a carbohydrate media. The choice of synthetic substrate used during the initial phases of the experimental studies was based upon the following considerations:

(1) A synthetic substrate provides a constant and non-varying source of organic carbon and nutrients of known composition and characteristics, thus facilitating material balances to determine analytical accuracy;

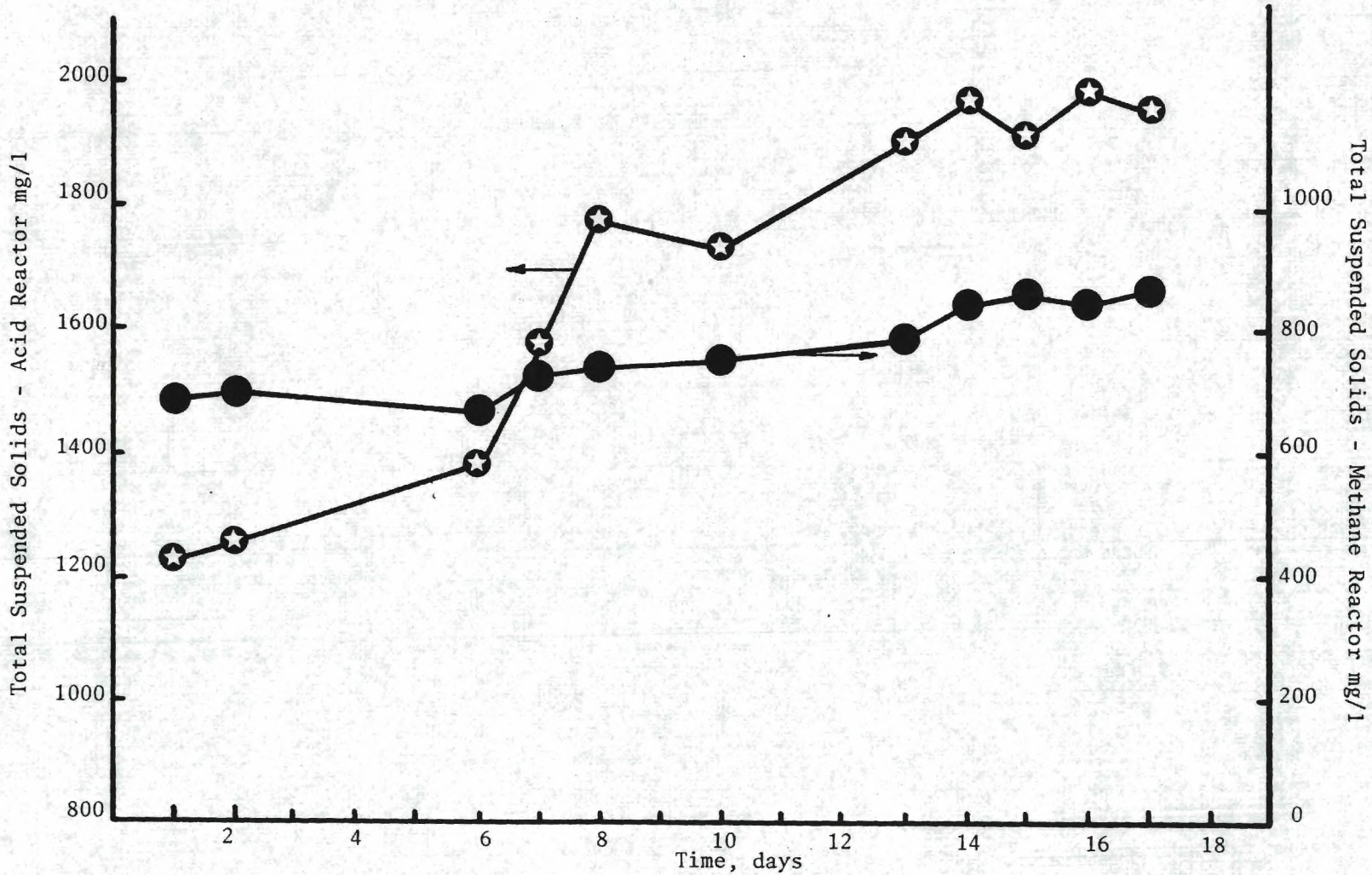


Figure IV-3. Variation in Total Suspended Solids Concentration During Test II-A--Stage II

(2) It is easily formulated and assigned under laboratory conditions; and,

(3) It has been used in other studies, thereby providing a source of data for comparison of its characteristics in single-stage anaerobic stabilization processes.

The glucose substrate was prepared as needed in a concentrated solution that included the necessary inorganic nutrients. The procedure used for preparation of the concentrated substrate and nutrient solution is presented in Appendix A.

The influent substrate concentration for Stages I and II was selected by balancing difficulties anticipated with very high and very low concentrations. High concentrations of glucose result in high microorganism concentrations and volatile acid concentrations in the effluent from the acidogenic reactor. This necessitates heavily buffering the substrate to keep pH values in a range between 6.5-7.0 in the methane reactor. Measurement of substrate concentration becomes less accurate as the tests must be performed on diluted samples.

On the other hand, low substrate concentrations are undesirable due to the low microorganism concentrations that are present. The low density leads to inaccuracies in measurement of solids concentrations and also presents problems in generating enough biomass to sustain a system incorporating

cell recycle.

After initiating continuous feed with a substrate concentration of 1000 mg/l as glucose, a decision was made to increase the substrate concentration to 3000 mg/l as glucose. This decision was based on generating enough biomass to sustain a system with organism recycle. All steady-state values obtained during Stages I and II were made with a nominal influent substrate concentration of 3000 mg/l as glucose.

Buffer Composition

A phosphate buffer was selected for use during the study. The buffer was prepared at a concentration of 1.5 M phosphates by use of sodium dibasic phosphate and sodium monobasic phosphate and added to the diluted substrate. The 1.5 M concentrated phosphate buffer solution was added in the ratio of 1.5 liters of concentrate to 20 liters of dilute substrate.

The concentration of phosphate buffer was adequate to maintain the pH in the acidogenic reactor at 6.5 or higher and to maintain the pH throughout the reactor system within the generally accepted values of 6.5-7.0 for good fermentation. The addition of this concentration of phosphate buffer to the substrate solution resulted in a sodium concentration in the influent to the reactor system of 4100 mg/l or 0.18 M, less than the upper limit for the single cation concentration in

anaerobic waste treatment that Kugelman and McCarty [56] have suggested.

Sampling and Analysis

The reactor system was serviced daily to insure proper operation of the control systems and to provide required maintenance. The daily servicing schedule included the following provisions:

(1) Preparation of fresh substrate for refrigerated storage and maintenance of an adequate supply.

(2) Adjustment of substrate flowrate to each reactor if required. The flowrate to each reactor was measured by observation of the volume delivered through three timed cycles of the substrate influent pump. Adjustments were made as required.

(3) Measurement of gas production and removal of accumulated gas from the collection system.

(4) Visual inspection of the components in the reactor control systems for proper functioning.

(5) Daily sampling and analysis for pH, temperature and gas production and quality.

(6) Twice weekly sampling and analysis for:

(a) Total and volatile suspended solids concentration in each reactor,

(b) Unfiltered and filtered influent and effluent COD concentrations,

(c) Volatile acid concentrations.

Once the reactor system was adjudged at steady-state, a 48-hour balance period was begun. During this period of time, accurate measurements of substrate feed to the acidogenic reactor and effluent flow from the methanogenic reactor were made. Gas production rates were also closely monitored. Influent and effluent substrate flow measurements were made by weighing the amount of dilute substrate pumped to the acidogenic reactor and wasted from the methanogenic reactor, respectively. Since both reactors were operated with the same volume, a substantial fraction of the substrate fed to the acidogenic reactor was wasted from the system.

After the end of the balance period, samples were removed from the reactor system for prompt analysis. All analyses were performed in triplicate and the values reported are the averages of the three determinations. The analyses conducted on the samples were:

(1) COD of mixed liquor and filtrate;

(2) Total and volatile suspended solids;

(3) Volatile acid distribution and concentration;

(4) pH and alkalinity; and,

(5) Fermentation gas quality.

All analyses were performed in accordance with the procedures described in the following section.

During Stage I of the study, a carbon-hydrogen-nitrogen analysis was conducted on the suspended solids present in the acid reactor. These data were used only to

estimate a carbon balance around the reactor systems and was not repeated routinely.

Analytical Techniques

pH

pH was determined immediately upon a 50 ml sample of the mixed liquor drawn from the respective reactor. The immediate determination was necessary to minimize the effect of carbon dioxide loss to the atmosphere after withdrawal. The procedure used was the Glass Electrode Method as described in Standard Methods [91]. The analysis was conducted with a Leeds and Northrup Model 7411 pH meter that was standardized daily with a commercially prepared buffer solution with a pH of 6.86 at 25°C. The pH meter was equipped with a temperature calibration dial that was adjusted to 37°C after standardization.

Alkalinity

Alkalinity of the mixed liquor from each reactor was determined in accordance with the procedure described for anaerobic digester supernatants in Standard Methods [91]. A 50 ml sample was withdrawn from the reactor and immediately titrated to a pH of 4.0 using 0.1 N H_2SO_4. The sample was mixed utilizing a magnetic stirrer which was operated at the lowest possible speed in order to minimize carbon dioxide stripping yet still provide adequate mixing.

Suspended Solids

The mixed liquor suspended solids and volatile suspended solids concentrations were determined by filtration through a glass fiber filter pad as described in Section 224 C and D of Standard Methods [91]. The glass fiber filer pads were washed in distilled water and dried to a constant weight in a 103°C oven prior to use in the analysis. The solids determination of the clarifier underflow were determined in the same manner except that the sample was diluted with distilled water before the analysis was performed. The precision of the technique is said [91] to vary directly with the concentration of suspended matter in the sample with a standard deviation of ±24 mg/l at 242 mg/l and ±13 mg/l at 1707 mg/l.

Chemical Oxygen Demand

The chemical oxygen demand (COD) of influent solutions, mixed liquors and reactor filtrate was determined by the dichromate reflux method described in Section 220 of Standard Methods [91]. The samples were diluted in the ratio of one part sample to two parts distilled water. Suspended solids were removed from the mixed liquor by filtration through a glass fiber filter pad prior to filtrate analysis. Precision for this test is reported [91] as a standard deviation of ±13 mg/l for 200 mg/l COD solution of potassium acid phthalate. For most organic compounds, the oxidation is 95 to 100 percent complete [114]. A series of tests were

performed on a volatile acid standard containing 500 mg/l each of acetic, propionic and butyric acid to determine the oxidation of these compounds. The samples were diluted by addition of 20 ml of distilled water to 10 ml samples. Results of the test are shown in Table IV-1.

Table IV-1. COD Analysis of Volatile Acid Standard

Sample Number	COD (mg/l)	Percent Recovery
1	2192	99.7
2	2158	98.1
3	2249	102.3
4	2147	97.6
5	2090	95.0

The recovery averaged 98.54 percent with a standard deviation of 52.5 mg/l. Thus, it was assumed that oxidation of the volatile acids with dichromate was virtually complete.

Volatile Organic Acids

The filtrate from each reactor was analyzed both quantitatively and qualitatively for the individual volatile acids using a gas-liquid chromatographic procedure similar to that described by van Huyssteen [92]. Table IV-2 records the equipment and operational procedures utilized.

The chromatograph was standardized prior to use by injection of an aqueous solution containing 1000 mg/l each

Table IV-2. Equipment and Operational Procedures for Volatile Acid Determination

Chromatograph	F & M Scientific Series 700 Laboratory Chromatograph manufactured by Hewlett-Packard Corporation. Equipped with Dual Hydrogen Flane Ionization Detector
Recorder	Hewlett-Packard Model 7127A Strip Chart Recorder
Column	1/8" O.D. x 6' stainless steel with 20% Carbowax 4000 TPR on 60/80 mesh chromosorb W, acid washed, treated with dimethyldichlorosilane
Column Temperature	155°C
Injection Port Temperature	200°C
Carrier Gas	Nitrogen
Carrier Gas Flow Rate	30 cc/min
Hydrogen Flow Rate	20-25 cc/min
Sample Size	5 µl

of acetic, propionic, n-butyric and n-valeric acids by the same procedure used for the unknown sample analysis. The samples were injected into the chromatograph with a 10 µl Hamilton syringe. To minimize "ghosting," 0.1 N HCl was injected into the chromatograph after sample resolution. The injector port septum was also replaced prior to use each day to minimize the "ghosting" problem.

Standard operating procedure for the analysis of steady-state samples was to standardize the instrument using the 1000 mg/l standard volatile acid solution. Because of the variability of the response from the chromatograph at times, the standard solution was analyzed until three consecutive injections gave essentially the same response.

The filtrate samples were prepared by acidification with N H_2SO_4 to a pH between 1 and 2. The samples were injected into the chromatograph until three consecutive injections gave essentially the same response.

Ideally, the area under a peak of the chromatogram is proportional to the concentration of a compound. Because the area of a triangle is directly related to its height, the concentration is proportional to the peak height if the base width does not change appreciably.

Base widths were observed to be uniform when chromatographic conditions were closely controlled; therefore, concentrations were determined by comparison of peak heights from the unknown with those obtained from the standard solution.

Gas Analysis

Gas composition both for individual identification and quantitative measurement of carbon dioxide, oxygen, nitrogen and methane concentration was determined by the use of a Fisher Model 25V Gas Partitioner using helium as a carrier gas. Gas analyses were made at isothermal conditions, with a column oven and detector temperature of 33°C. Helium, at a rate of 80 cc/min served as the carrier gas. The columns for the instrument were as follows:

Column 1--30" x 1/4" aluminum packed with 30% HMPA on 60/80 mesh Chromosorb P

Column 2--6-1/2' x 3/16" aluminum packed with 40/60 mesh molecular sieve 13X

Sample peaks recorded on a Coleman Hitachi 165 Recorder were compared against the peaks obtained by analysis of a standard gas mixture supplied by Matheson Company, Inc. The standard gas consisted of known mixtures of carbon dioxide, oxygen, nitrogen and methane in approximately the same proportions as those anticipated from the anaerobic system. Sample gas was introduced into the instrument using a syringe injection technique. Concentrations were determined by comparison of the peak heights obtained from the unknown with those obtained from the standard samples. The instrument was calibrated prior to each use.

The instrument set-up as described above did not provide for adequate determination of hydrogen concentrations

since the thermal conductivity of helium, the carrier gas, and hydrogen is very similar. The substitution of nitrogen for the carrier gas can provide for hydrogen detection.

Instrument reproducibility is listed by the manufacturer as 0.5%. Typical detection limits are cited as follows [118].

Gas	Typical Detection Limit (Volume percent)
Carbon Dioxide	0.0077
Oxygen	0.0083
Nitrogen	0.0113
Methane	0.0180
Hydrogen	0.0075

Carbon-Hydrogen-Nitrogen Analysis

The carbon-hydrogen-nitrogen (CHN) concentration of a volatile suspended solids sample was determined by a chromatographic analysis using a Model 185 Carbon Hydrogen Nitrogen Analyzer manufactured by the F&M Scientific Corporation. The sample analyzed was taken from the acid reactor and analyzed in the following manner:

1. The suspended solids were conducted by use of an International Clinical Centrifuge, Model CL manufactured by Will Corporation. The cells were washed three times with distilled water and then reconcentrated.

2. The concentrated and washed solids were freeze-dried using a Virtis Manifold Freeze-Dryer.

3. The dried solids were then analyzed in the F&M CHN analyzer to determine carbon concentration in the biological solids. The results were compared with a standard of cyclohexanone-2, 4 dinitrophenyl hydrazone containing 51.99 percent carbon, 5.07 percent hydrogen, 21.14 percent nitrogen and the balance being oxygen.

CHAPTER V

PRESENTATION AND DISCUSSION OF EXPERIMENTAL DATA

The intent of the research project was to focus on the objectives outlined in Chapter I. These objectives were as follow:

(1) Demonstration that phase separation can be achieved by exertion of kinetic controls on a two stage biological reactor system operated under anaerobic conditions;

(2) Determination of the effect of biomass recycle on the operation of both phases of the anaerobic process and the practicality of gravity clarification for biomass separation;

(3) Demonstration of the utility of mathematical models based on bacterial growth kinetics for describing both the acidogenic and methanogenic phases of the anaerobic stabilization process;

(4) Determination of appropriate kinetic parameters for each phase when operated with simple and complex soluble substrates; and,

(5) Development of control strategies for application of phase separation to anaerobic stabilization in concentional practice.

The following sections describe the result of the

study designed to achieve these objectives. This study was organized into separate stages and performed in a step-wise fashion. The results of each stage and a kinetic analysis will be reported herein.

Stage I

The plan of experimentation for Stage I called for the confirmation of kinetic control as a means for phase separation of the anaerobic process before the additional complication of biomass separation and recycle was introduced. Thus, the reactor system was operated in such a manner as to provide effective phase separation, while at the same time, operating conditions were adjusted to provide data for the estimation of kinetic parameters for both the acid and methane fermentation phases. The experimentation plan for Stage I also included the refinement of control and operating techniques to provide reliable functioning of the reactor system. However, no attempt was made during this stage of the study to determine optional conditions for overall treatment efficiency. The steady-state operating data is summarized in Table V-1 and carbon balances are included in Table V-2.

In order to determine the extent of conversion of the influent organic carbon to the metabolic by-products and to judge the adequacy of analytical techniques being utilized, carbon balances around the reactor systems were computed using the data from the influent and effluent analyses.

Table V-1. Stage I Data Summary: Two-Phase Stabilization of a Simple Soluble Substrate without Biomass Recycle

Test Designation	I-A		I-B		I-C	
Phase	Acid	Methane	Acid	Methane	Acid	Methane
Hydraulic Retention Time, hours	16.11	68.16	19.57	81.96	24.31	96.72
Influent Analysis						
Total COD, mg/l	4080	3876	3946	3824	3808	3298
Glucose, mg/l	3824	--	3698	--	3569	--
Total Alkalinity, mg/l as $CaCO_3$	3648	--	3594	--	3622	--
Effluent Analysis						
Total COD, mg/l	3876	3264	3824	2950	3298	2754
Soluble COD*, mg/l	2856	2448	2920	2201	2482	2040
Total Suspended Solids, mg/l	862	491	915	730	832	646
Volatile Suspended Solids, mg/l	688	401	685	529	586	478
Acetic Acid, mg/l	1463	1170	1426	954	832	483
Propionic Acid, mg/l	455	455	526	349	877	862
Butyric Acid, mg/l	319	277	323	258	196	214
Valeric Acid, mg/l	0	0	0	0	0	0
Volatile Acid COD**, mg/l	2829	2440	2904	2015	2571	2208
pH	6.35	6.50	6.34	6.62	6.50	6.55
Total Alkalinity, mg/l as $CaCO_3$	2996	3052	2980	3116	3114	3228

Table V-1 (concluded)

Test Designation	I-A		I-B		I-C	
Phase	Acid	Methane	Acid	Methane	Acid	Methane
Gas Production						
Production Rate, ml/day	198	860	720	793	1493***	
Carbon Dioxide, ml/day	161	230	414	224	431***	
Carbon Dioxide, %	22	26	27	27.5	26***	
Methane, ml/day	37	630	306	569	1062***	
Methane, %	5	71	20	70	64***	
Methane, ml/g COD applied	0.61	46.16	6.32	50.18		

*After filtration through glass fiber filter.

**Calculated value based on COD equivalence of individual volatile acids.

***Common gas phase.

Table V-2. Stage I Data: Two-Phase Stabilization of a Simple Soluble Substrate without Biomass Recycle

Test Designation	I-A		I-B		I-C	
Phase	Acid	Methane	Acid	Methane	Acid	Methane
General						
Reactor Volume, l	10	10	10	10	10	10
Influent Flow, l/day	14.898		12.264		9.873	
Effluent Flow, l/day		3.521		2.928		2.482
Hydraulic Retention Time, hours	16.11	68.16	19.57	81.96	24.31	96.72
Gas Production, ml/day	198	860	720	793	1493***	
Influent Analysis						
Total COD, mg/l	4080	3876	3946	3824	3808	3298
Glucose, mg/l	3824	--	3698	--	3569	--
Total Alkalinity, mg/l as $CaCO_3$	3648	2996	3594	2980	3622	3114
Effluent Analysis						
Total COD, mg/l	3876	3264	3824	2950	3298	2754
Soluble COD, mg/l*	2856	2448	2920	2201	2482	2040
Total suspended solids, mg/l	862	491	915	730	832	646
Volatile suspended solids, mg/l	688	401	685	529	586	478
Acetic acid, mg/l	1463	1170	1426	954	832	483

Table V-2 (continued)

Test Designation	I-A		I-B		I-C	
Phase	Acid	Methane	Acid	Methane	Acid	Methane
Propionic Acid, mg/1	455	455	526	349	877	862
Butyric Acid, mg/1	319	277	323	258	196	214
Valeric Acid, mg/1	0	0	0	0	0	0
Volatile Acid, COD, mg/1**	2829	2440	2904	2015	2571	2208
Volatile Acid, mg/1 as hectic acid	2050	1728	2073	1413	1677	1528
pH	6.35	6.50	6.34	6.62	6.50	6.55
Total Alkalinity, mg/1 as Ca CO_3	2996	3052	2980	3116	3114	3228
Gas Production						
Production Rate, ml/day	198	860	720	793	1493***	
Carbon Dioxide, ml/day	161	230	414	224	431***	
Carbon Dioxide, %	22	26	27	27.5	26***	
Methane, ml/day	37	630	306	569	1062***	
Methane, %	5	71	20	70	64***	
Methane, ml/g COD applied	0.61	46.16	6.32	50.18	--	

*After filtration through glass fiber filter.

**Calculated value based on COD equivalence of individual volatile acids.

***Common gas phase.

Table V-2 (Concluded)

Test Designation	I-A		I-B		I-C	
Phase	Acid	Methane	Acid	Methane	Acid	Methane
Carbon Balance						
Total Carbon In, mgC/day	22788	5364	18141	4449	14095	
Carbon as $(CO_2)_2 + (HCO_3^-)$, mgC/day	2062	699	2059	733	1960	529
% of carbon In	9.0	17.0	11.3	17.3	13.9	15.7
Carbon in Effluent Gas, mgC/day	94	409	342	377	710***	
% of carbon In	94	76	19	69	61***	
Carbon in V.S.S., mgC/day	4951	682	4057	748	2794	573
% of Carbon In	21.7	12.7	22.4	17.6	19.8	
Carbon in Acetic Acid, mgC/day	8718	1648	6995	1117	3286	480
% of carbon In	38.3		38.6		23.3	14.3
Carbon in Propionic Acid, mgC/day	3298	779	3138	497	4212	1041
% of carbon In	14.5	14.5	17.3	11.7	29.9	
Carbon in Butyric Acid, mgC/day	2592	532	2161	412	1056	290
% of carbon In	11.4	9.9	11.9	1.7	7.5	8.6
Total Carbon Out, mgC/day	21715	4749	18752	3884	13308****	3623*****
% of carbon In	95.3	86.5	103.4	91.4	94.4	107.7

**** Does not include carbon in effluent gas.

***** Includes all carbon in effluent gas.

These carbon balances are included in Table V-2.

The influent carbon concentration was estimated by assuming that all chemical oxygen demand (COD) in the influent was exerted as a result of the oxidation of glucose to carbon dioxide and water. The carbon contained in the liquid and gas effluent steams from each reactor was estimated from the data obtained through the analytical efforts previously discussed. Due to the length of retention times used for the acid reactor, the assumption was made for the purpose of the carbon balance only that the effluent substrate concentration was negligible and the great majority of organic carbon in the effluent would be present in the form of the volatile organic acids.

Figure V-1 shows the validity of these assumptions by illustrating the extent of conversion of influent glucose substrate into volatile organic acids (VA), biomass (VSS), and product gases in the acid reactor. The carbon balances indicate that essentially all influent organic carbon was being accounted for by the analytical techniques employed, with 95.3 and 103.4 percent recovery being calculated for the 16.11 and 19.57-hour retention times, respectively. An inspection of Figure V-1 also reveals that the proportions of influent organic carbon converted to the acid reactor products were uniform.

The 24.31-hour retention time shown in Figure V-1 does not include the amount of carbon contributed by the product

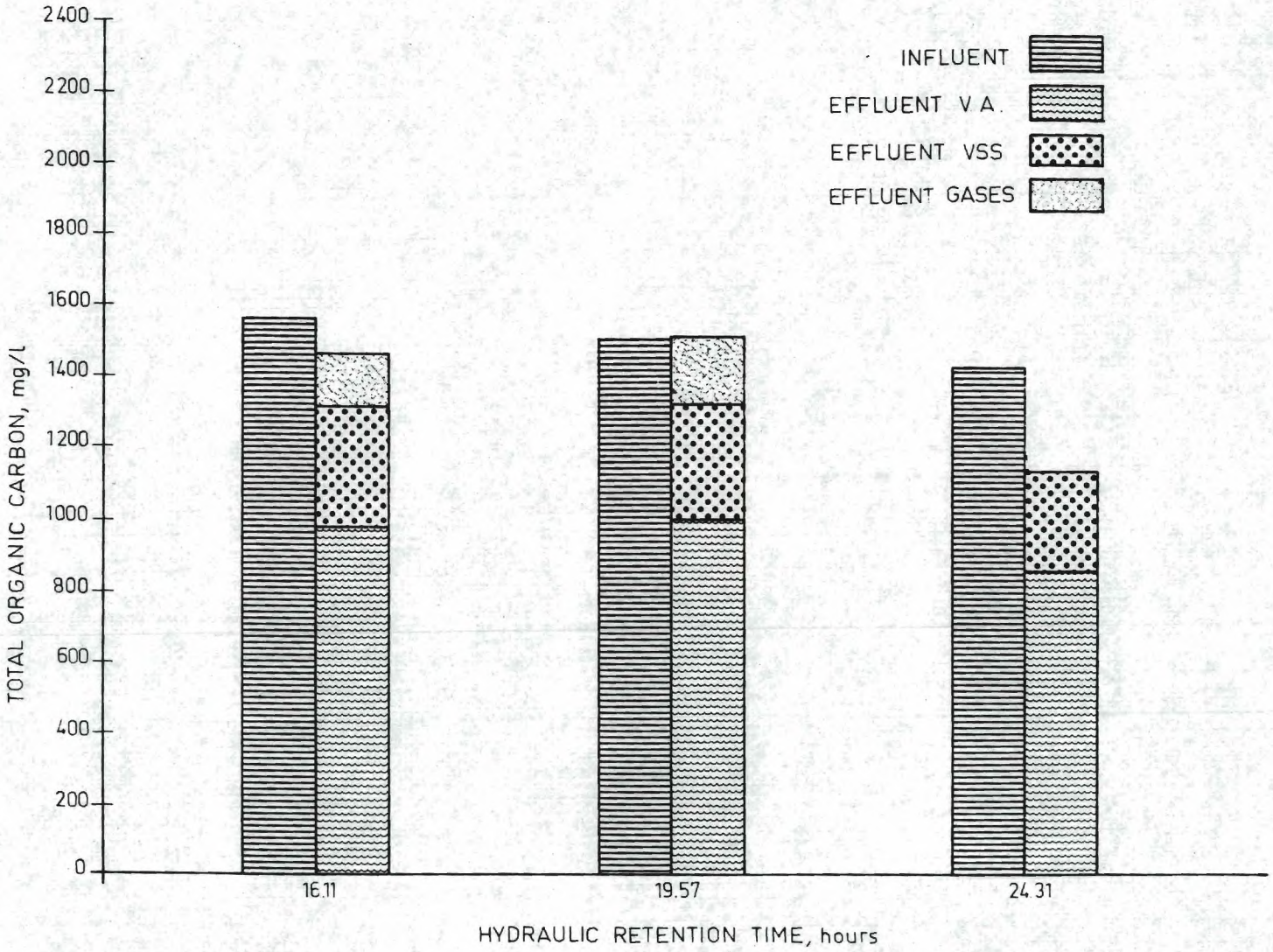


Figure V-1. Influent Carbon Conversion in Acid Phase Reactor--Stage I

gases due to a change in process configuration which provided for a common gas phase for both reactors. The result of this combination of gas phases will be discussed later; however, the change made it impossible to measure gas produced in the acid reactor separately. However, the results obtained from volatile acid and biomass analysis during that retention time are similar to those obtained at the lower retention times. At the 24.31-hour retention time, 60.7 percent of the influent carbon was converted to volatile acid carbon and 19.8 percent to biomass carbon. This compares favorably with the averages of 66 percent and 22 percent obtained for the first two retention times. For the 24.31-hour retention time, 94.4 percent of the influent carbon was recovered, neglecting the amount of carbon lost in the gas effluent stream from the acid reactor.

During Stage I of the study, the mixed liquor from the acid reactor was pumped directly to the methane reactor. Since both reactors were operated at fixed volumes of 10 liters but at greatly different retention times, a large portion of the acid reactor liquid effluent was pumped to waste. In order to estimate the influent carbon introduced to the methane reactor, it was assumed that the total carbon to the methane reactor was in direct proportion to the ratio of the methane influent flow and the total flow from the acid reactor. Thus the assumption of negligible substrate effluent concentration in the acid reactor was compounded in

the methane reactor carbon balance due to the uncertainty as to the exact carbon concentration in the acid reactor effluent. However, the carbon balances appear adequate, with 88.5 and 91.4 percent of the carbon feed being recovered for the 68.16 and 81.96-hour retention times, respectively. Again, the final retention time was performed with a common gas phase. The recovery for the 96.72-hour retention time was 107.7 percent based upon credit for all product gas stream carbon being placed in the methane reactor carbon balance.

The adequacy of the carbon recoveries demonstrated in Stage I lends confidence to the accuracy of the analytical techniques employed and demonstrates that the major constituents present as by-products of the anaerobic process were being detected.

The types and concentrations of the volatile organic acids detected in the acid reactor are depicted in Figure V-2. As was anticipated from previous investigations [3,10], acetic acid (HAc) and propionic acid (HPr) predominated; smaller concentrations of buturic acid (HBu) were measured, but valeric acid was not detected. For the 16.11 and 19.57-hour retention times, the distribution of volatile acids was similar; however, at the 24.31-hour retention time, propionic acid increased to a greater concentration than that for acetic acid. Although the distribution was changed, the amount of influent carbon converted to volatile

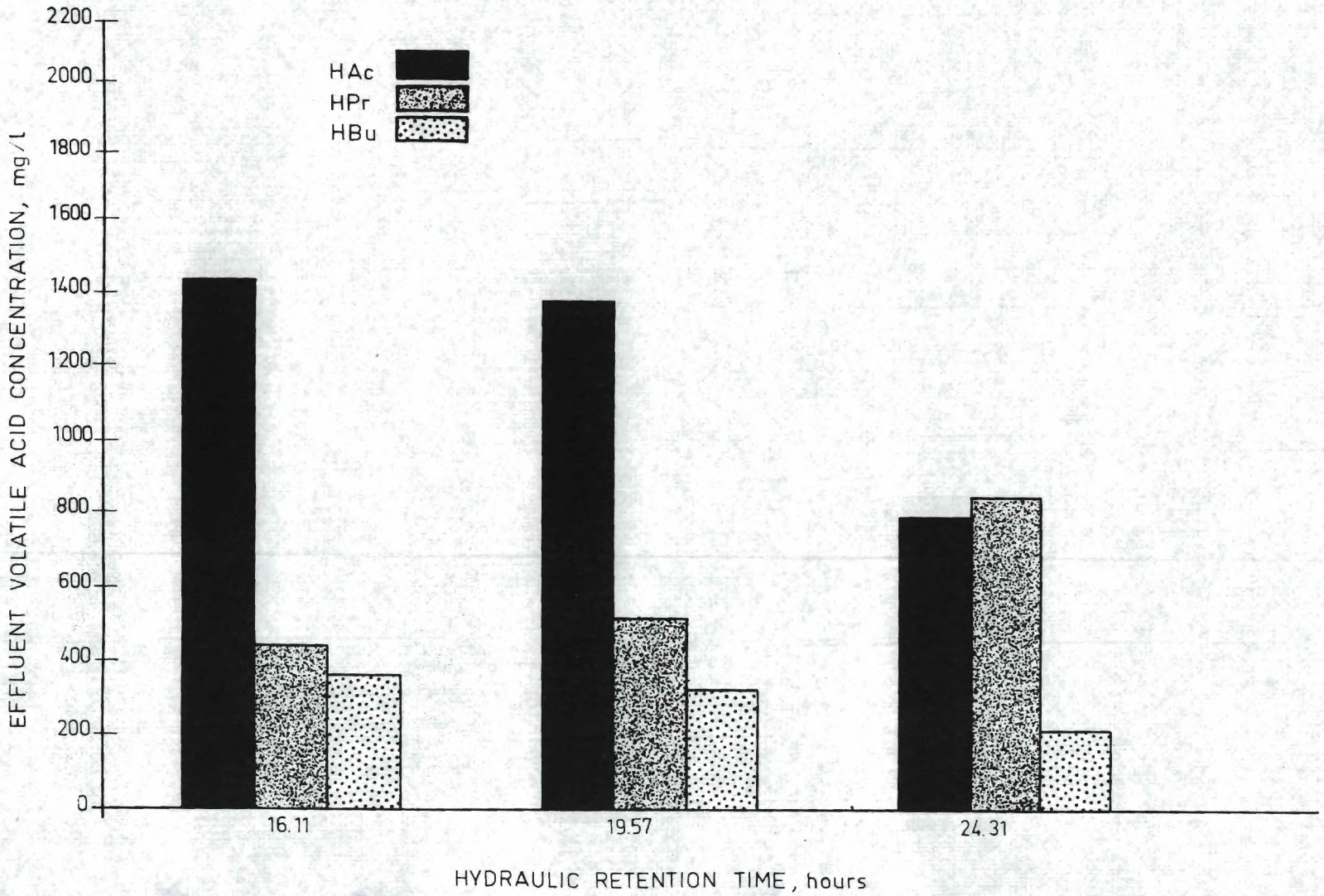


Figure V-2. Volatile Acids Distribution in Acid Phase Reactor--Stage I

acids was approximately the same as for the lower hydraulic retention times. As will be discussed later, the change in process configuration to provide a common gas phase for the reactor system may have contributed to these unexpected conditions.

Figure V-3 presents a comparison of the influent and effluent volatile acids concentrations for the methane reactor. It is evident that the acetic acid was noticeably reduced in the methane reactor at all retention times. The propionic acid concentration remained essentially unchanged except at the 81.96-hour retention time. Little change was observed in the butyric acid concentrations. Based upon the work of Lawrence and McCarty [79], the minimum solids retention time for propionate utilization in an anaerobic process is of the same order as for acetate utilization. Thus, it would appear that the retention times used during Stage I should have been adequate for the proliferation of both acetate and propionate utilizing organisms. However, the environmental conditions were apparently not suitable for the organisms responsible for propionate metabolism. During the three months of Stage I, no appreciable amount of propionate utilization was ever noted.

A review of the solids data summarized in Table V-1 indicates substantial biomass production in the acid reactor, with 20-22 percent of the influent organic carbon being converted to VSS. Concentrations of VSS in the range of

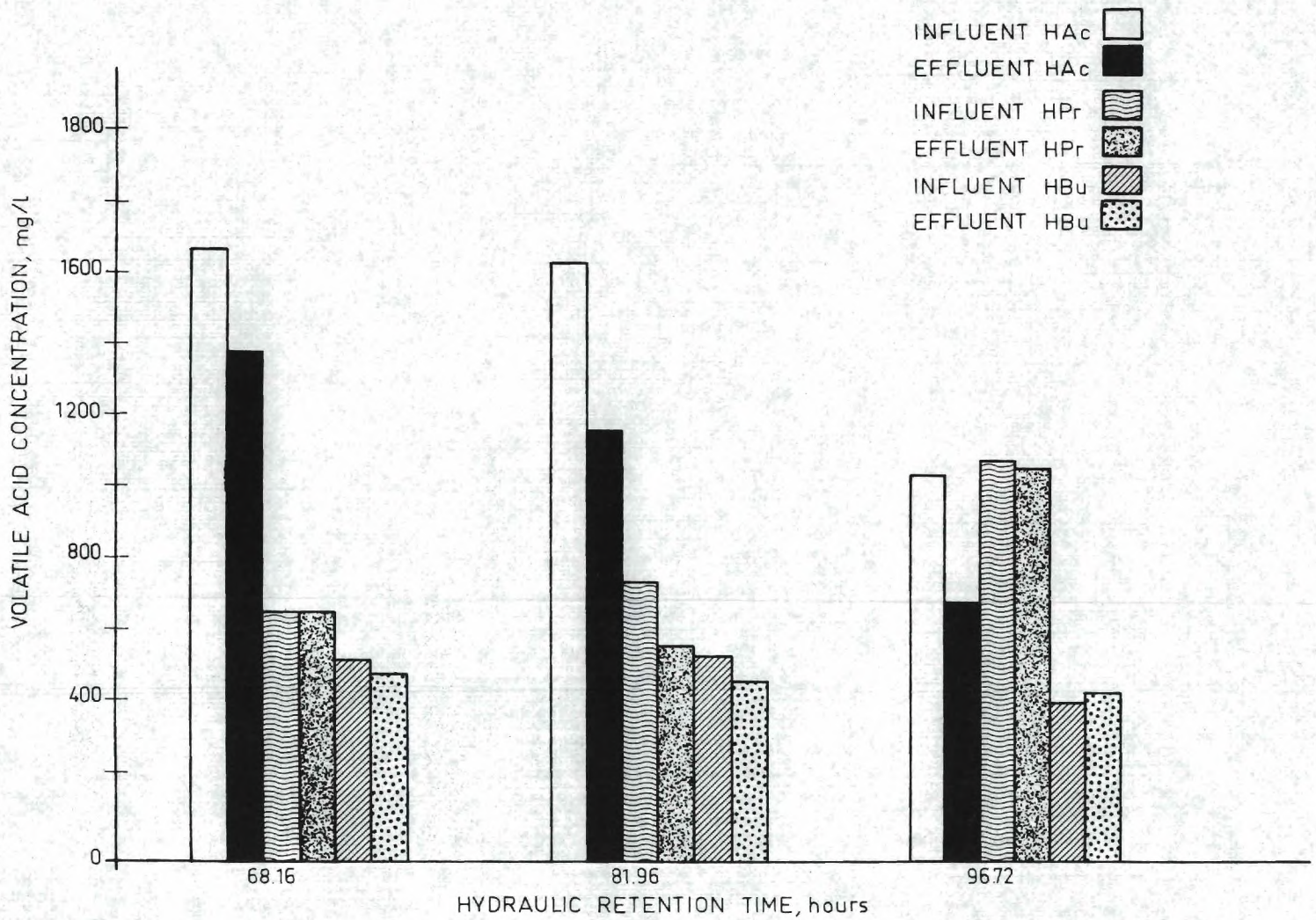


Figure V-3. Comparison of Influent and Effluent Volatile Acid Concentrations for Methane Phase Reactor--Stage I

586-688 mg/l were noted for the acid reactor during Stage I. Since no biomass separation was practiced during this stage, the concentration of VSS in the methane reactor is not exclusively representative of the concentration of methane bacteria. A substantial reduction of VSS occurred in the methane phase which was expected due to organism decay. The long retention times coupled with a lack of acceptable substrate for the acid-forming bacteria which carried over from the acid reactor contributed to substantial reductions in VSS concentration through the methane reactor.

The reactor system was operated in such a manner as to minimize methane production in the acid reactor by operation at hydraulic retention times well below the minimum solids retention time of 2.0-4.2 days reported by Lawrence and McCarty [79] for methane bacteria utilizing acetate and propionate. As indicated by the data in Table V-1, methane production persisted in the acid reactor at all hydraulic retention times indicating that complete washout of the methane bacteria from the acid reactor was not achieved. This was not unanticipated, based on prior work by Lawrence and McCarty [79], Willimon [47], and Ghosh and Pohland [3]. Since the methane bacteria could not be completely washed out of the acid reactor, it was assumed that continued methane production must be a result of a fixed film attachment to the walls of the acid reactor.

To verify this assumption, substrate feed was

discontinued to the reactor system and the contents of the acid reactor removed to a temporary container. Strict anaerobic conditions were maintained during this period by blanketing the temporary container with nitrogen gas to prevent oxygen intrusion. The acid reactor was then thoroughly cleaned of a thin black slime growth by use of a hypochlorite cleaning solution followed by thorough rinsing. The reactor and gas collection system were then purged of oxygen by the use of nitrogen gas, and the mixed liquor was pumped back into the reactor. Substrate feed was then resumed to the reactor.

The gas production from the acid reactor immediately decreased after the fixed film growth was removed. Figure V-4 shows the gas production from the acid reactor and the percent methane in the gas phase both before and after the removal of the fixed film growth. As can be seen, the methane bacteria reestablished themselves in the acid reactor as evidenced by the detection of methane within three days. Gas production also began to rise after an initial decrease to near zero. Although this verified that significant gas production occurred as a result of fixed film growth, it was not possible to keep the acid reactor completely free of methane bacteria by frequent cleaning. The growth would be reestablished before steady-state conditions could be attained.

As shown in Figure V-5, the bulk of methane produced

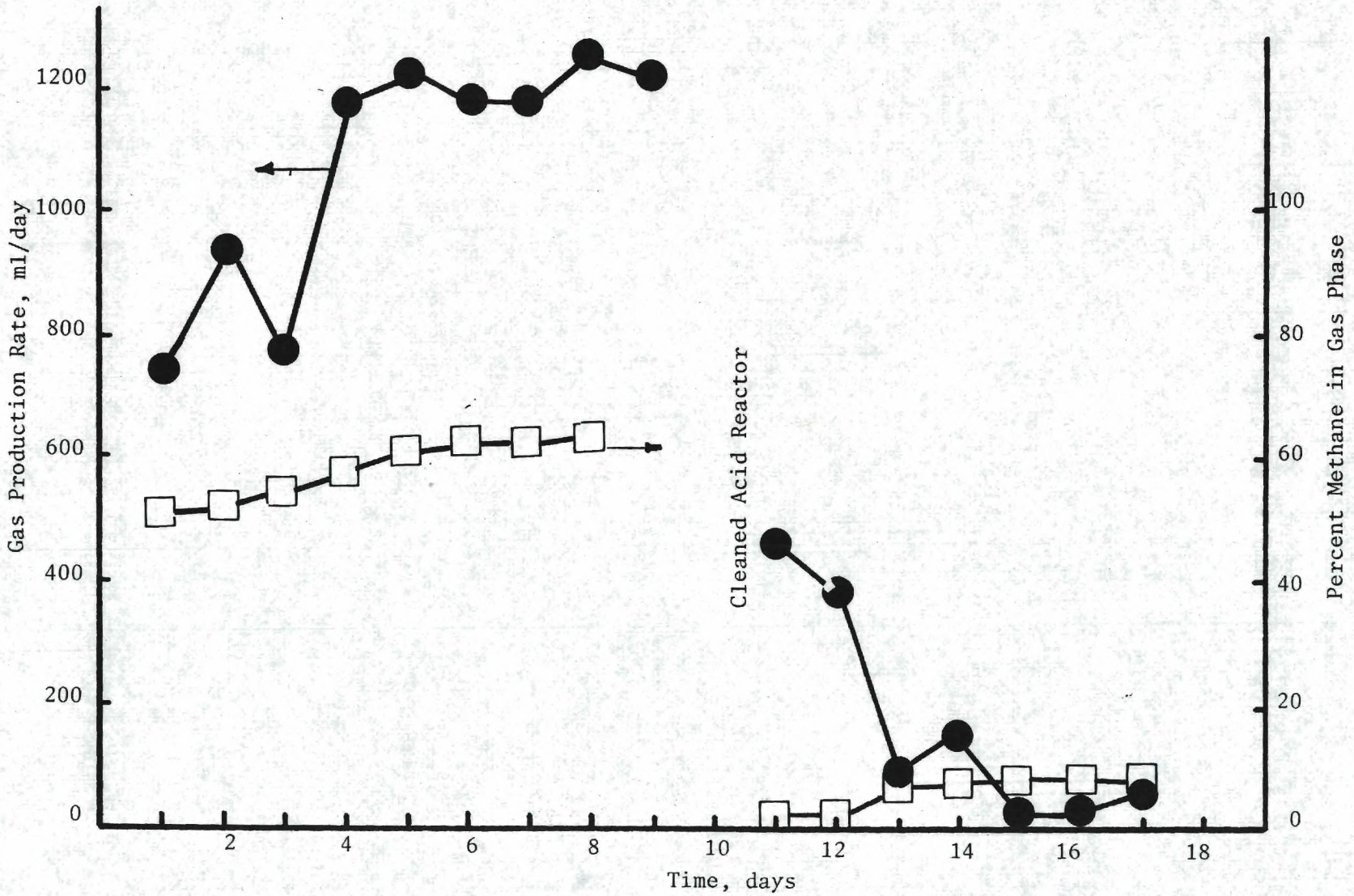


Figure V-4. Gas Production and Quality in the Acid Reactor

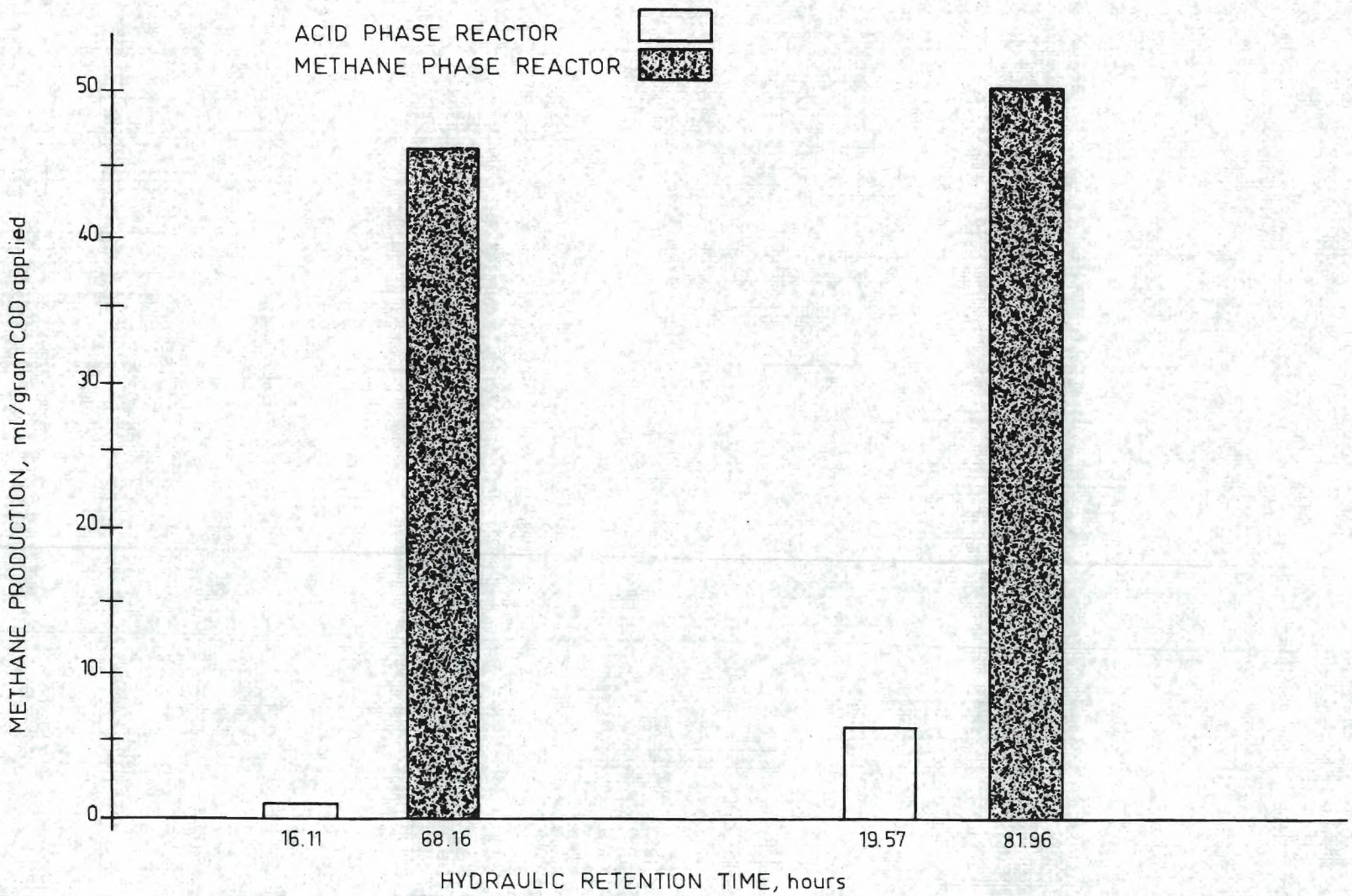


Figure V-5. Comparison of Methane Production in the Acid and Methane Phase Reactors--Stage I

per weight of COD applied occurred in the methane phase reactor for both tests where the gas phases were separated. Although the volumes of methane produced do not show this clear-cut difference, it must be considered that, since both reactors were operated at a fixed volume of 10 liters, much of the substrate introduced to the acid reactor was wasted in order to accommodate the much longer retention times in the methane reactor. Thus methane production per weight of substrate introduded to the reactor is a more meaningful parameter for comparison of methane production in the acid and methane reactor.

The methane bacteria that have been identified and maintained in pure culture all share the common characteristic of being able to utilize hydrogen as a substrate [93]. It has been suggested [37] that this may be the most important process for methane production. Gray and Gest [54] have shown that the ability of both facultative and strict anaerobes to produce molecular hydrogen is widespread. A decision was made, therefore, to evaluate the effect of connecting the atmospheres of the acid and methane phases in terms of process efficiency and methane production. If hydrogen was produced in the acid fermentation phase, it could be subsequently utilized in the methane phase by provision of a common gas phase.

A series of tests were conducted to determine the effect of a common gas atmosphere on process treatment

efficiency and gas production. Table V-3 contains a summary of the data obtained during this period of testing. The tests were performed under equivalent hydraulic and substrate conditions; the only variable during the program was the alternation of the reactor gas phases between a common and separate mode. Test I-C was part of the Stage I testing program and thus was conducted to achieve steady-state conditions. The series of tests were performed to validate the common gas phase assumption and were operated so as to detect trends in the data. The tests were generally conducted over week long periods.

Figure V-6 shows the effect of gas phase separation on the total COD of the methane reactor effluent. The trend seems to indicate that a common gas phase did improve effluent quality, with a slight deterioration noted when the atmospheres were separated.

As indicated before, the combination of the atmospheres induced a stress upon the reactor system which resulted in the propionic acid concentration increasing to a value greater than that of the acetic acid. This was still the case after the atmospheres were separated again in test I-D, with propionic acid still predominating. During tests I-E and I-F, this condition again reversed and acetic acid was the predominant volatile acid constituent. The carry-over of the high concentrations of propionic acid through the reactor system is reflected in the data which shows

Table V-3. Stage I Data Summary--Gas Phase Separation

General								
Test Number	I-C		I-D		I-E		I-F	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Hydraulic Residence Time (hrs)	24.31	96.72	24.31	92.24	23.34	94.56	23.75	97.60
Gas Phase	Common		Separate		Common		Common	
Influent Analysis								
Chemical Oxygen Demand (mg/1)	3808	3298	3360	3168	3460	2940	3340	2960
Glucose Concentration (mg/1)	3569	--	3149	--	3243	--	3130	--
Alkalinity (mg/1 as $CaCO_3$)	3622	3114						
Effluent Analysis								
Total COD (mg/1)	3298	2754	3168	2856	2940	2660	2960	2380
Filtrate COD (mg/1)	2482	2040	2384	2128	2300	2080	2150	1710
Total Suspended Solids (mg/1)	832	646	788	604	822	658	858	660
Volatile Suspended Solids (mg/1)	586	478	560	454	594	470	620	488
Acetic Acid (mg/1)	832	483	900	432	690	376	1062	571
Propionic Acid (mg/1)	877	862	1008	954	477	621	227	294
Butyric Acid (mg/1)	196	214	207	153	307	171	274	121
Valeric Acid (mg/1)	--	--	--	--	--	--	--	--
Volatile Acid (mg/1 as HAc)	1676	1328	1858	1310	1286	996	1433	892
pH	6.50	6.55	6.25	6.35	6.40	6.50	6.45	6.60
Alkalinity (mg/1 as $CaCO_3$)	3114	3228						

Table V-3 (concluded)

	Acid Methane I-C	Acid Methane I-D		Acid Methane I-E	Acid Methane I-F
Digester Gas Production					
Gas Production Rate (ml/day)	1493	883	598	2213	2398
Carbon Dioxide (ml/day)	431	265	181	669	694
Methane (ml/day)	1062	618	417	1514	1704
Digester Gas Phase Composition					
% Carbon Dioxide	26	25.7	28.8	30.0	27.2
% Methane	64	59.8	66.2	65.0	66.8
% Nitrogen	10	14.5	5.0	5.0	6.0

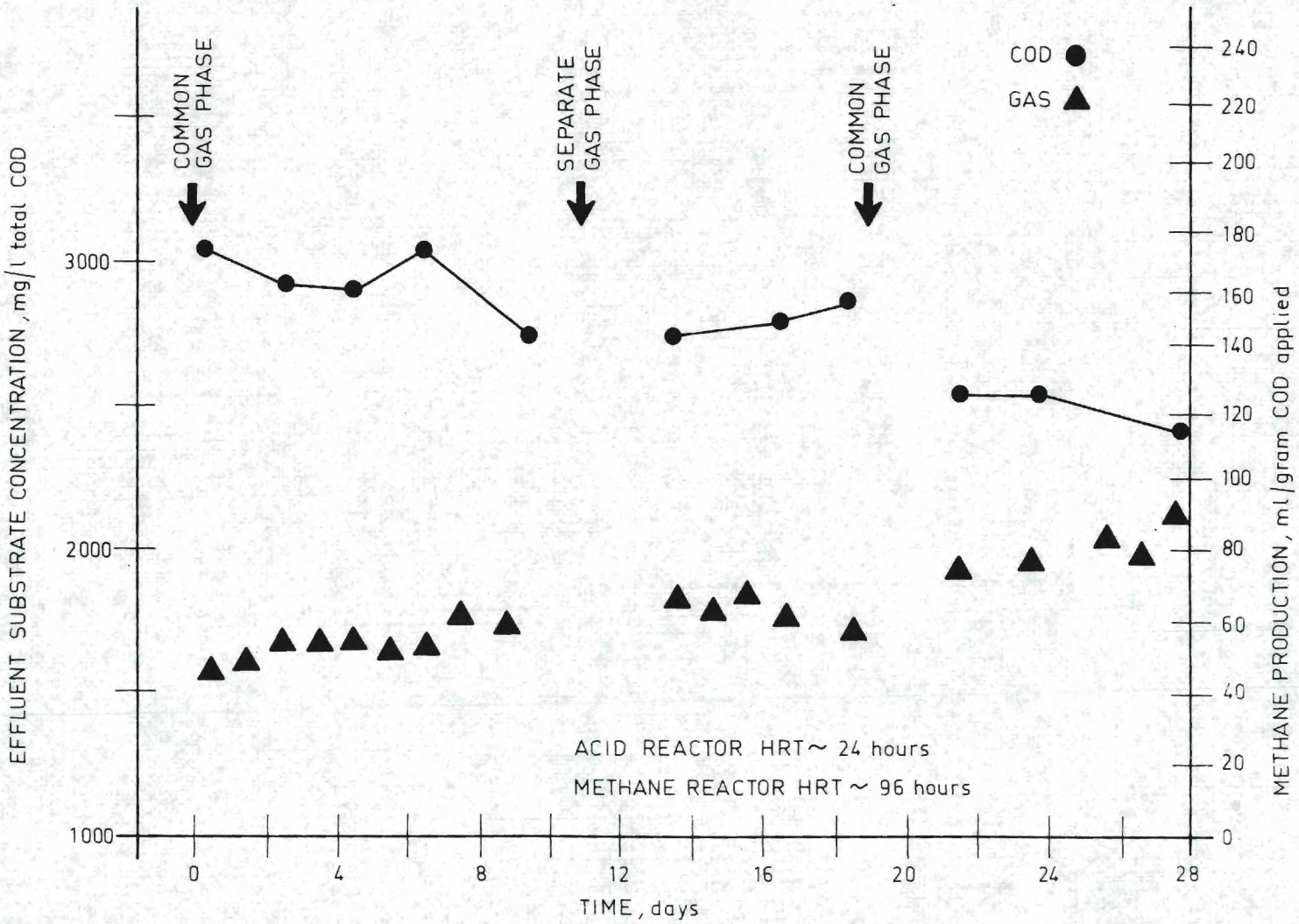


Figure V-6. Effect of Gas Phase Changes on Methane Reactor Performance--Stage I

higher propionic acid concentrations in the methane reactor effluent during tests I-E and I-F than that in the influent.

No significant changes in suspended solids concentration were noted during the test sequence. Though the VSS did decrease when the phases were separated, it is doubtful that the marginal increase in treatment efficiency was due to significantly higher biomass concentration in the methane reactor.

As would be expected by the improved treatment efficiency evidenced during the common phase testing, the methane production rate also increased somewhat. This rise is illustrated in Figure V-6. The gas produced from each reactor was analyzed for the presence of hydrogen during this period. The Fisher Model 25V Gas Partitioner was modified so that hydrogen analyses could be undertaken. No hydrogen was detected in either the acid or methane reactor atmospheres or when the common gas phase configuration was used.

Although hydrogen was not detected, it is possible that concentrations were present below the 0.0075 percent limit of detection for which the instrument is sensitive. Since the demonstrated improvement in effluent quality from the methane reactor was small, the amount of hydrogen necessary to produce this effect was also necessarily small. It is also possible that the rate of utilization of hydrogen is rapid; therefore, very little is released to the gas phase

after formation. The presence of methane bacteria in the wall growth found within the acid reactor could account for this immediate utilization.

The improvements in reactor system performance were attributed to the establishment of a common gas phase. The data are supportive of this conclusion although the improvement in process efficiency could conceivably be attributed to a change in the volatile acid mix. Higher propionic acid concentrations were evident during the initial portion of the common gas phase study. The propionate was not readily utilized in the methane reactor. Reestablishment of acetic acid as the dominant by-product of the acid fermentation stage provided a readily metabolized substrate for the methane reactor. This could possibly account for the improved efficiency noted. Whatever the reason, the reactor system demonstrated improved performance and the decision was made to operate the balance of the studies in the common gas phase mode.

Based on the results presented to this point, phase separation was demonstrated feasible by kinetic control of the two-reactor system. Conversion of organic carbon to volatile acids and biomass was achieved in the acid reactor with a minimum production of methane. Volatile acid reduction was demonstrated on the methane reactor, with acetic acid being the primary volatile acid utilized by the methane forming bacteria. Moreover, based upon COD analysis,

Figure V-7 illustrates that little change in total COD was observed between the acid phase influent and effluent; an indication that methane production was retarded in this phase. In addition, although no effort was made to optimize either phase and increase process efficiency, the soluble COD reductions of 40 to 46 percent were comparable to values reported by Willimon [47] which were obtained in single-stage systems with the same total retention time and using the same substrate. These comparative data are shown in Table V-4.

Stage II

After providing confirmation of the validity of kinetic control for anaerobic phase separation during Stage I, emphasis was placed on improving overall treatment efficiency in both phases with biomass separation and subsequent recycle. The maintenance of high concentrations of the appropriate bacterial populations is of prime importance in optimization of a biological process. Since gravity clarifiers are routinely employed for this purpose in standard practice, investigation into the feasibility of such an operation was considered a logical step toward overall process development. Therefore, the two closed gravity clarifiers illustrated in Figure IV-1, were inserted into the treatment train and biomass concentration and recycle were initiated. A summary of the steady-state data accumulated during Stage II is presented in Table V-5, with

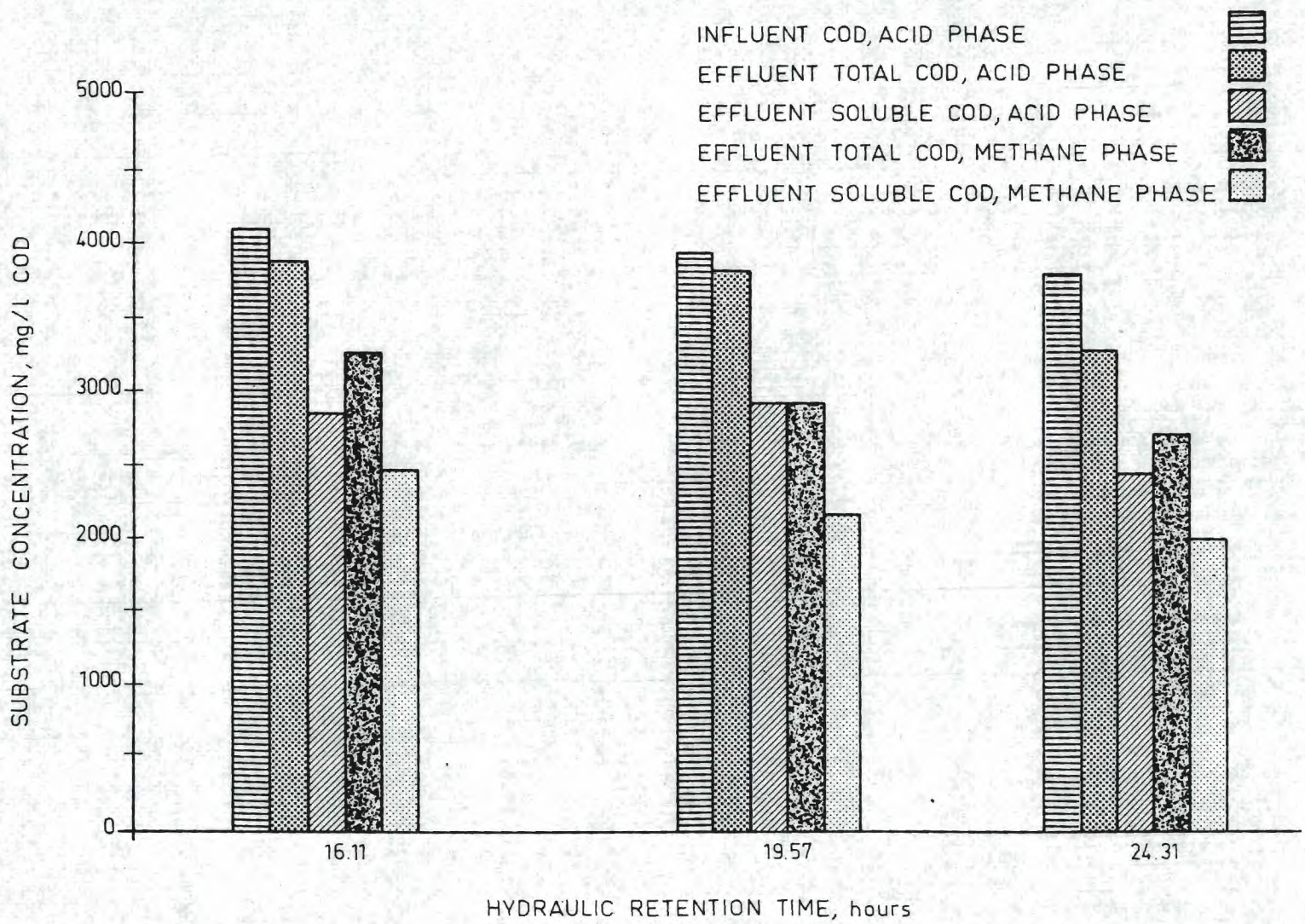


Figure V-7. COD Conversions in the Acid and Methane Phase Reactors--Stage I

Table V-4. COD Treatment Efficiency in Anaerobic Stabilization Systems

Hydraulic Retention Time (Hours)			Chemical Oxygen Demand (mg/l)			COD Removal (Percent)	
Acidogenic	Methanogenic	Total	Influent	Mixed Liquor Effluent*	Filtrate	Total	Filtrate
Two-Phase System--Stage I							
16.11	68.16	84.27	4080	3264	2448	20.0	40.0
19.57	81.96	101.53	3946	2950	2201	25.2	44.2
24.31	96.72	121.83	3808	2754	2040	27.7	46.4
Single Stage System [47]							
--	--	80	4050	2828	1824	30.2	55.0
--	--	104	3940	2817	1999	28.5	49.3
--	--	128	3680	2552	1845	30.7	49.9

*Methane phase

Table V-5. Stage II Data Summary: Two Phase Stabilization of Simple Soluble Substrate with Biomass Recycle

Test Designation	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Hydraulic Retention Time, hrs	23.12	116.8	15.2	96.8	15.9	46.9	10.9	47.6
Influent Analysis								
Total COD, mg/l	3460	--	3760	--	3667	--	3440	--
Glucose, mg/l	3243	--	3524	--	3437	--	3224	--
Total Alkalinity, mg/l as $CaCO_3$	3637	--	3371	--	--	--	3257	--
Effluent Analysis								
Total COD, mg/l	3770	2340	--	1940	--	2464	--	2442
Soluble COD*, mg/l	2180	1120	2380	1520	2289	1900	2362	2100
Total Suspended Solids, mg/l	1964	876	3514	646	5100	654	5210	632
Volatile Suspended Solids, mg/l	1290	607	2398	412	3589	430	3570	390
Acetic Acid, mg/l	951	388	932	403	884	698	646	602
Propionic Acid, mg/l	194	184	331	229	280	286	278	278
Butyric Acid, mg/l	358	52	473	77	703	269	474	233
Valeric Acid, mg/l	0	0	0	0	0	0	0	0
Volatile Acid COD**, mg/l	1959	787	2355	916	2644	1659	1972	1982
pH	6.50	6.70	6.50	6.65	6.58	6.67	6.49	6.66
Total Alkalinity, mg/l as $CaCO_3$	3155	3275	2996	3189	2933	3175	2951	3012

*After filtration through glass fiber filter.
**Calculated value based on COD equivalence.

Table V-5 (concluded)

Test Designation	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Clarifier Supernatant Analysis								
Total COD, mg/l	2820	--	2720	--	2754	--	2809	--
Soluble COD, mg/l								
Total Suspended Solids, mg/l	634	696	604	484	1330	464	574	486
Volatile Suspended Solids, mg/l	434	463	360	291	925	276	330	252
Acetic Acid, mg/l	902	--	912	--	913	--	631	--
Propionic Acid, mg/l	177	--	308	--	296	--	293	--
Butyric Acid, mg/l	362	--	426	--	625	--	534	--
Valeric Acid, mg/l	0	--	0	--	0	--	0	--
Clarifier Underflow Analysis								
Recycle Ratio	0.22	0.68	0.15	0.60	0.16	0.28	0.15	0.29
Total Suspended Solids, mg/l	7230	1170	17,170	864	37,400	958	35,500	1022
Volatile Suspended Solids, mg/l	4350	840	13,450	596	25,900	653	22,300	690
Gas Production***								
Production Rate, ml/day	3555		4200		5255		5955	
Carbon Dioxide, ml/day	976		1282		1709		1933	
Carbon Dioxide, %	26.5		29.0		31.7		31.0	
Methane, ml/day	2579		2918		3546		4022	
Methane, %	70		66.0		65.8		64.5	

***Common gas phase.

the complete set of data shown in Table V-6.

A gravity clarifier was successfully employed to concentrate biomass from the acid phase reactor for subsequent recycle back to the system. Concentrations from 1300 to 3600 mg/l VSS were obtained in the acid reactor by solids recycle as compared to 586 to 686 mg/l for the Stage I operation without recycle. Thus VSS concentrations equivalent to those obtained in aerobic activated sludge systems could be easily maintained in the acid phase reactor being fed an intermediate strength substrate. The volatile portion of the suspended solids averaged 68.2 percent, somewhat lower than the 75 percent observed in Stage I.

The acid phase clarifier achieved VSS removal efficiencies from 66 to 91 percent during Stage II. The underflow concentrations in the acid phase clarifier ranged from 0.4 to 2.6 percent VSS, with the improved settling characteristics being noted during the shorter hydraulic retention times. Moreover, the anticipated settling problems arising from gas formation in the clarifier failed to materialize and the solids concentration and recycle process was a stable one.

In contrast to the favorable results obtained in the acid phase, the biomass from the methane phase could not be concentrated in a gravity clarifier. The solids produced would not settle readily, and microscopic examination revealed them to be highly dispersed and nonflocculent. The

Table V-6. Stage II Data: Two Phase Stabilization of Simple Soluble Substrate with Biomass Recycle

Test Designation	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
General								
Reactor Volume, l	10	10	10	10	10	10	10	10
Influent Flow, l/day	10.376		15.779		15.117		21.995	
Effluent Flow, l/day		2.055		2.479		5.115		5.033
Nominal HRT, hours	23.12	116.8	15.2	96.8	15.9	46.9	10.9	47.6
Recycle Ratio (R) Flow, l/day	2.28	1.392	2.376	1.488	2.424	1.440	3.288	1.464
Actual HRT, hours	19.0	69.5	13.2	60.5	13.7	36.6	4.5	36.9
Sludge Age, days	2.86	6.38	4.22	5.71	2.57	3.05	4.92	3.07
Influent Analysis								
Total COD, mg/l	3460	282.0	3760	2720	3667	2759	3440	2509
Glucose, mg/l	3243		3524		3437		3224	
Total Alkalinity, mg/l as $CaCO_3$	3637		3371		--		3257	
Effluent Analysis								
Total COD, mg/l	3770	2340	--	1940	--	2464	--	2446
Soluble COD*, mg/l	2180	1120	2380	1520	2289	1800	2362	2100
Total Suspended Solids, mg/l	1964	876	3514	646	5100	354	5210	632
Volatile Suspended Solids, mg/l	1290	607	2398	412	3589	430	3570	340

Table V-6 (continued)

Test Designation	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Acetic Acid, mg/1	951	388	932	403	884	698	646	692
Propionic Acid, mg/1	194	184	331	229	280	286	278	278
Butyric Acid, mg/1	358	52	473	77	703	265	474	233
Valeric Acid, mg/1	0	0	0	0	0	0	0	0
Volatile Acid COD**, mg/1	1959	787	2355	916	2644	1659	1972	1582
pH	6.50	6.70	6.50	6.65	6.58	6.67	6.49	6.55
Total Alkalinity, mg/1 as $CaCO_3$	3155	3275	2996	3189	2933	3175	2951	3012
Clarifier Supernatant Analysis								
Total COD, mg/1	2820	--	2720	--	2754	--	2809	--
Total Suspended Solids, mg/1	634	696	604	484	1330	464	574	486
Volatile Suspended Solids, mg/1	434	463	360	291	925	276	330	252
Acetic Acid, mg/1	902	--	912	--	913	--	631	--
Propionic Acid, mg/1	177	--	308	--	296	--	293	--
Butyric Acid, mg/1	362	--	426	--	625	--	534	--
Valeric Acid, mg/1	0	--	0	--	0	--	0	--
Clarifier Underflow Analysis								
Recycle Ratio	0.22	0.68	0.15	0.60	0.16	0.28	0.15	0.29
Total Suspended Solids, mg/1	7230	1170	17.170	864	37.400	958	35.500	1022
Volatile Suspended Solids, mg/1	4350	840	13,450	596	25.900	653	22.300	690

Table V-6 (continued)

Test Description	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Gas Production***								
Production Rate, ml/day	3555		4200		5255		5955	
Carbon Dioxide, ml/day	976		1282		1709		1933	
Carbon Dioxide, %	26.5		29.0		31.7		31.0	
Methane, ml/day	2579		2918		3546		4022	
Methane, %	70.0		66.0		65.8		64.5	
Carbon Balance								
Influent Flow to System, l/day	10.376		15.779		15.117		21.995	
Effluent Flow from Methane Clarifier		2.055		2.479		5.115		5.033
Waste Effluent from Acid Clarifier	8.321		13.300		10.002		16.962	
Total Carbon In, Mg C/day	13.460		22242		20783		28365	
Carbon in Effluent Gas, mg C/day		1680		1997		2498		2831
Carbon as $(CO_2)_d$ + (HCO_2^-), mg C/day								
Acid Clarifier Waste	1632		2852		2630		3835	
Methane Clarifer Overflow		545		664		1544		1239
Carbon in VSS, mg C/day								
Acid Clarifier Waste	1744		2313		4469		2704	
Methane Clarifer Overflow		460		348		682		613

Table V-6 (concluded)

Test Description	II-A		II-B		II-C		II-D	
Phase	Acid	Methane	Acid	Methane	Acid	Methane	Acid	Methane
Carbon in Acetic Acid, mg C/day								
Acid Clarifier Waste	3002		4852		3653		4281	
Methane Clarifier Overflow		319		400		1428		1393
Carbon in Propionic Acid, mg C/day								
Acid Clarifier Waste	717		1993		1440		2418	
Methane Clarifier Overflow		184		276		712		681
Carbon in Butyric Acid, mg C/day								
Acid Clarifier Waste	1643		3090		3410		4941	
Methane Clarifier Overflow		58		104		739		640
Total Carbon Out, mg C/day		11994		18889		23205		25576
% of Carbon In		89.1		84.9		111.7		90.2

*After filtration through glass fiber filter.

**Calculated value based on COD equivalence.

***Common gas phase.

VSS concentration in the methane reactor was not appreciably increased over that observed in Stage I. The volatile fraction of the suspended solids averaged 65.1 percent, lower than the 76 percent observed in Stage I.

A possible reason for the poor settling experienced in the methane reactor was the intensity of mixing within the reactors. An analysis reveals a G value of about 84/second and a Gt_d value of 3.5×10^7 for the longest retention time. Reported values of Gt_d vary from 10^4 to 10^9 [116]. Moreover, the same mixing speed was used in both the acid and methane reactors and good settling was experienced in the acid reactor clarifier.

A comprehensive test program was undertaken to rectify the poor settling situation in the methane phase clarifier. Dague, et al. [69] have reported the chemical coagulation of anaerobic biological solids derived from the single-stage digestion of a synthetic substrate with a COD of 600 mg/l. The coagulant used was ferrous chloride at a pH of 8.3. Although successful, the maintenance of a pH of 8.3 in the clarifier did not seem to be a practical alternative.

A series of jar tests were undertaken to evaluate the impact of inorganic coagulant and polymer additions on settling characteristics of the mixed liquor from the methane reactor. The tests were conducted on 200 ml samples at the pH of the sample as obtained from the reactor. The samples

were flash mixed for 2 minutes after chemical addition and then slow mixed for 10 minutes. The samples were then allowed to settle, and a visual determination of the settling characteristics was noted. Table V-7 indicates the coagulants and polymers tested and the results obtained. A wide range of polymers produced no discernible improvement in settling. The inorganic coagulants were also not effective in the dosage ranges and pH that were tested.

In a further attempt to promote good settling in the methane reactor, biological solids from the acid clarifier were introduced to the methane reactor over a 12-hour period to provide a seed population that might induce continued good settling. Figure V-8 shows the solids concentration in the methane reactor during this period of time. Within 10 days after the introduction of acid reactor solids, the VSS had again stabilized at approximately the same level as when the test began due to washout. No improvement in effluent quality was noted during this time.

During Stage II, the methane clarifier was closely observed to determine if gasification in the clarifier was responsible for poor settling. No evidence of gas formation or bubbles was observed.

Since satisfactory acid production during the Stage I studies was obtained at hydraulic retention times from 16 to 24 hours, the hydraulic retention time during Stage II was varied from 23 to 11 hours to observe the effects of solids

Table V-7. Evaluation of Inorganic Coagulants and Polymers for Improved Settling of MLVSS from Methane Reactor

Chemical	Type	Dosage Range (mg/1)	Initial pH	Results
Ferrous Chloride	Inorganic coagulant	0-25	6.6	No observable improvement
Ferric chloride	Inorganic coagulant	0-25	6.6	No improvement
Magnifloc 530C	Cationic, High Molecular Weight Polyelectrolyte Polymer	0-5	6.7	No improvement
Hercofloc 810	Cationic, High Molecular Weight Polyelectrolyte Polymer	0-5	6.6	No improvement
Magnifloc 836A	Anionic, Polyacrylamide, Synthetic High Molecular Weight Polyelectrolyte Polymer	0-5	6.8	No improvement
Magnifloc 521-C	Cationic, High Molecular Weight Organic Polymer	0-5	6.6	No improvement

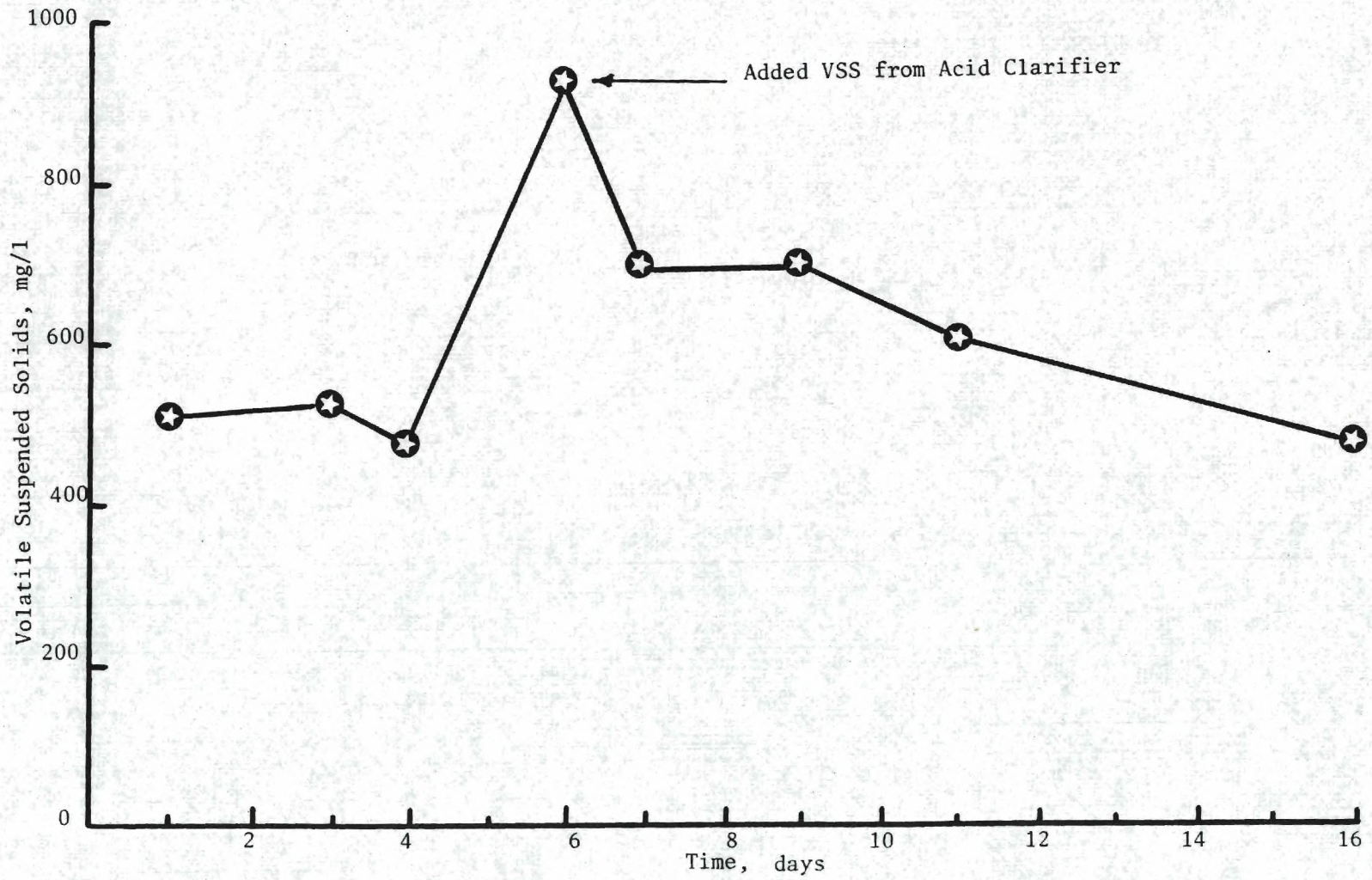


Figure V-8. Response of Methane Reactor to Introduction of Volatile Suspended Solids from the Acid Reactor

recycle. As indicated in Figure V-9, the acid phase reactor again produced an effluent high in volatile acids with acetic acid predominating. It is interesting to note that the use of biomass recycle produced significantly higher butyric acid concentrations in the acid reactor effluent. This could be possibly explained by a population selection process whereby organisms that produce butyric acid are permitted ascendancy or those that further convert it are suppressed by the solids concentration process.

The COD data for the acid phase of Stage II are presented in Figure V-10. A substantial reduction in total COD was observed between the influent and the supernatant from the acid phase clarifier due to biomass removal. The soluble COD from the acid reactor effluent was also at a lower level than that observed in Stage I. This indicated that methane fermentation was occurring to some extent in the acid reactor sufficient for the growth of methane reactor.

At the 15.9-hour retention time, the calculated COD of the volatile acids present exceeds the soluble effluent COD, an obvious impossibility, The COD data of Figure V-10 do indicate, however, that the majority of soluble COD in the acid reactor effluent can be attributed to the volatile acids, even at a retention time as low as 10.9 hours.

The observed utilization of volatile acids in the methane reactor is shown in Figure V-11. The most striking difference between Stage I and Stage II is the utilization

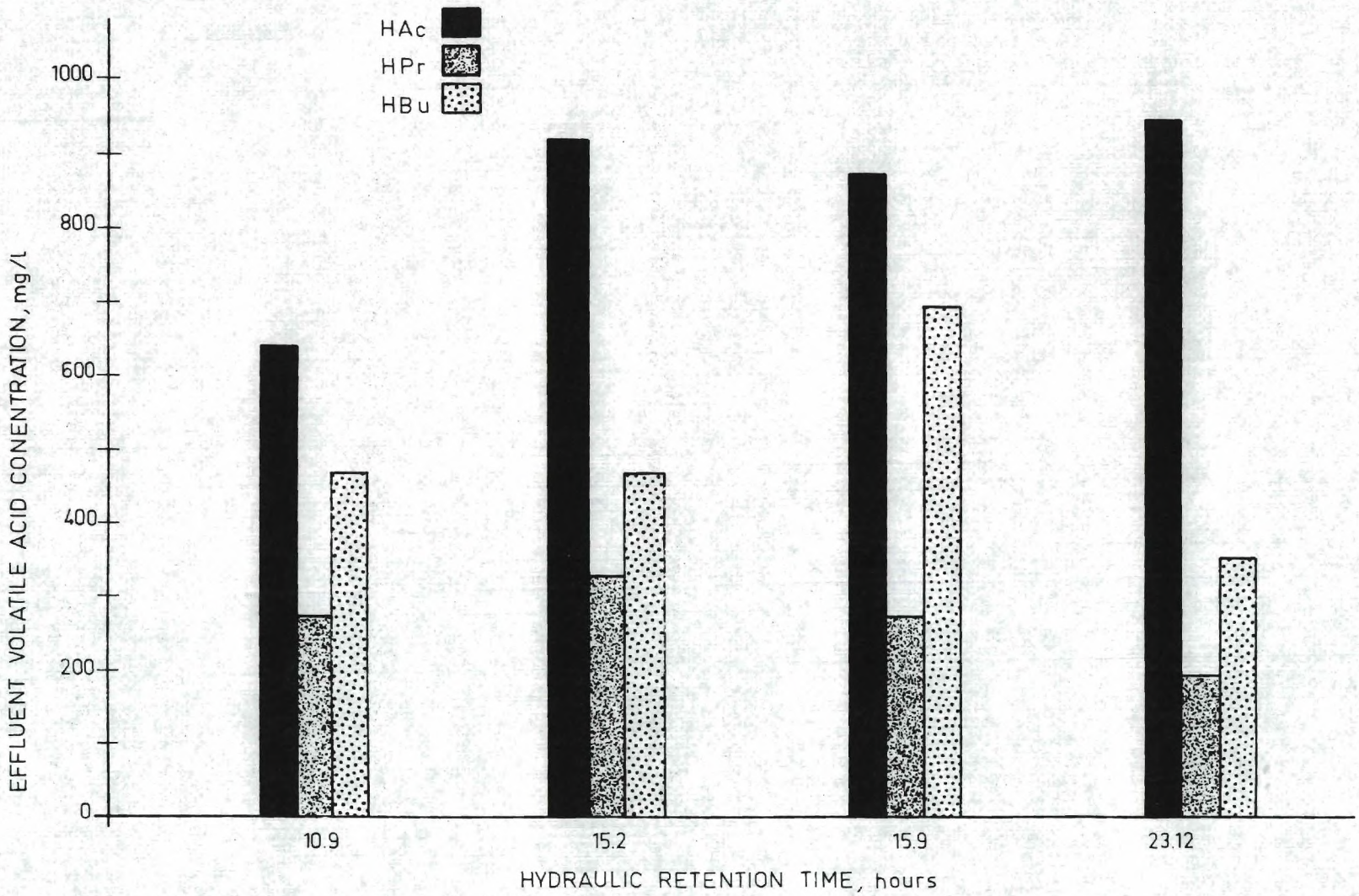


Figure V-9. Volatile Acids Distribution in the Acid Phase Reactor--Stage II

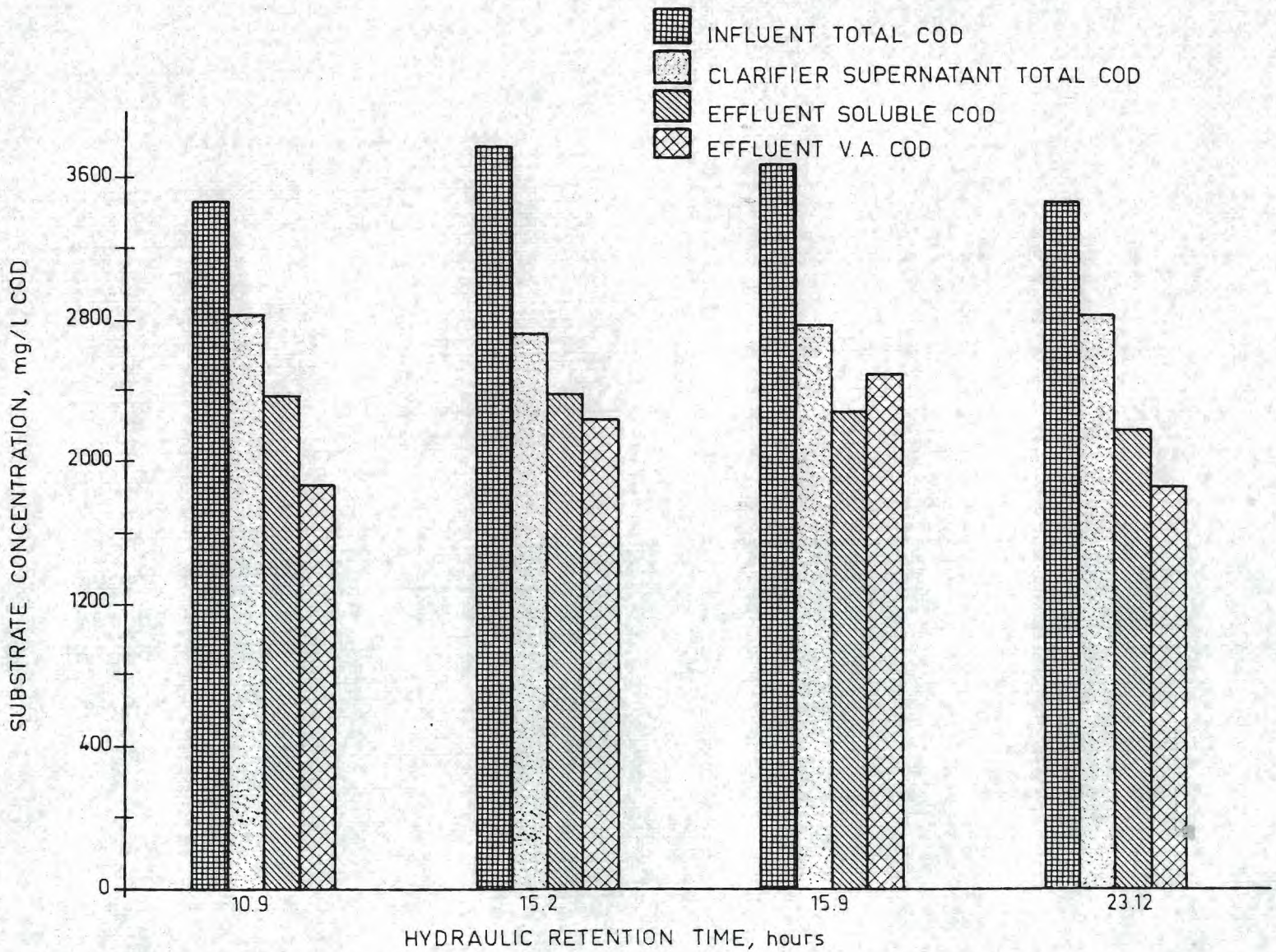


Figure V-10. COD Conversions in the Acid Phase Reactor--Stage II

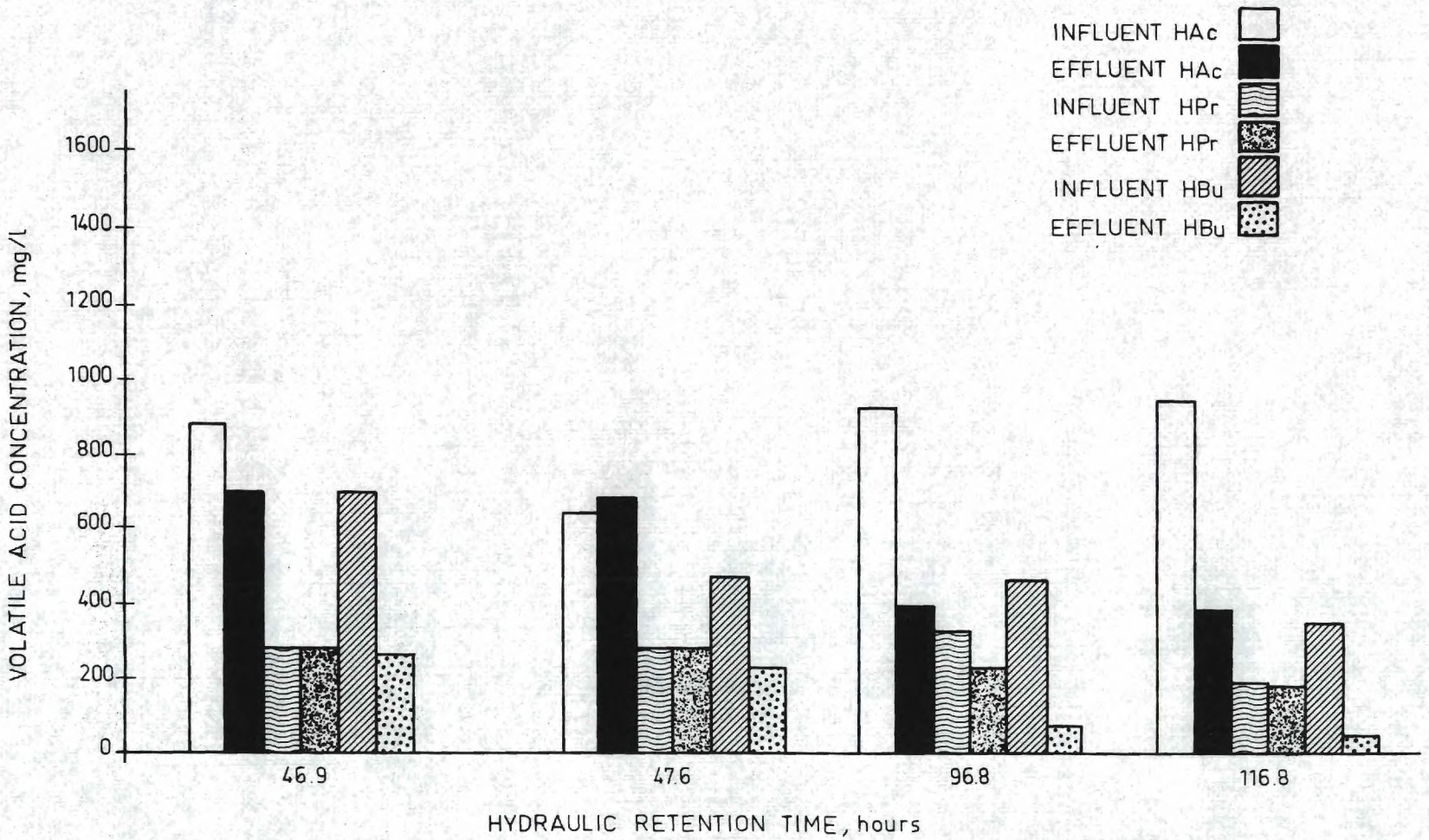


Figure V-11. Volatile Acids Utilization in the Methane Phase Reactor--Stage II

of butyric acid, even at the shorter retention times. As before, acetic acid was noticeably reduced, but propionic acid utilization was not noted.

Although the concentration of biomass from the methane reactor was only marginal, some increase in overall treatment efficiency was achieved during Stage II. Soluble COD reduction up to 67.6 percent was noted with a total retention time of 140 hours as shown in Table V-8. The soluble COD in the acid reactor was consistently lower during recycle operations, indicating that methane generation was also occurring in the acid reactor due to the sludge ages that were observed as well as possibly being due to the higher solids concentration and concomitant greater surface area for the attachment and persistence of methane formers. The increase in efficiency for the methane reactor can also possibly be attributed to significant utilization of butyric acid which was converted even at low hydraulic retention times.

Stage III

To accommodate the final objective of the research investigations, the two-phase system was used for the treatment of a wastewater effluent from a confectionary manufacterer. This wastewater was selected to demonstrate the applicability of the process to a complex substrate and was well-suited for this portion of the study since it was of the same approximate organic strength as the previously used synthetic substrate. The wastewater also contained few

Table V-8. COD Treatment Efficiency in Anaerobic Stabilization System--Stage II

Two-Phase System--Stage II

Hydraulic Retention Time (Hours)			Chemical Oxygen Demand (mg/l)		COD Removal (Percent)
Acidogenic	Methanogenic	Total	Influent	Filtrate	
10.9	47.6	58.5	3440	2100	39.0
15.9	46.9	62.8	3667	1900	48.2
15.2	96.8	112.0	3760	1520	59.6
23.1	116.8	139.9	3460	1120	67.6

suspended solids, making it compatible with the experimental equipment available for the investigation.

The wastewater was pumped from the final process sewer of the plant into 55-gallon drums and transported to the laboratory. The wastewater was immediately refrigerated and kept chilled until introduction to the reactor. The few settleable solids present in the wastewater were allowed to settle to the bottom of the drums.

The characteristics of this wastewater are shown in Table V-9. Prior to use, the wastewater was supplemented with 135 mg/l of nitrogen in the form of ammonium chloride to provide inorganic nutrient sufficiency, i.e., COD:N of 20:1. Concentrated phosphate buffer was also added for pH control and to adjust the total alkalinity to about 3600 mg/l as $CaCO_3$. The soluble COD of the wastewater was 3300 mg/l.

Table V-10 summarizes the data obtained during Stage III of the investigations. As before, the process results exhibited the characteristics of a two-phase system with acid production in the first reactor followed by acid utilization in the second reactor. The acid distribution was similar to that observed previously with the synthetic substrate, acetic acid being the predominant volatile acid produced. The higher concentrations of butyric acid produced during Stage II were not observed for this substrate even though organism recycle was again practiced. In addition, small concentrations of valeric acid were detected in the effluent from the

Table V-9. Stage III Wastewater Characteristics

Parameter	Analysis
COD, mg/l	3,300
pH	6.3
Total Alkalinity, mg/l as $CaCO_3$	1,960
Total Ejeldahl Nitrogen, mg/l as N	28
Ammonia Nitrogen, mg/l as N	11
Total Phosphorus, mg/l as P	42
Total Suspended Solids, mg/l	22
Volatile Suspended Solids, mg/l	17

Table V-10. Stage III Data Summary: Two Phase Stabilization of a Soluble-Type Industrial Waste with Biomass Recycle

Test Designation	III-A		III-B	
Phase	Acid	Methane	Acid	Methane
Hydraulic Retention Time, hrs	23.26	96.8	32.7	133.9
Influent Analysis				
Total COD, mg/l	3275	2850	3356	2610
pH		6.3		6.5
Total Alkalinity, mg/l as $CaCO_3$	1951	1795	1983	1626
Effluent Analysis				
Total COD, mg/l	3916	1869	3718	1826
Soluble COD*, mg/l	2429	1584	2152	1414
Total Suspended Solids, mg/l	1464	410	1628	432
Volatile Suspended Solids, mg/l	1122	309	1218	339
Acetic Acid, mg/l	840	457	759	259
Propionic Acid, mg/l	475	443	348	326
Butyric Acid, mg/l	364	57	322	46
Valeric Acid, mg/l	83	35	94	23
Volatile Acid COD**, mg/l	2447	1333	2115	900
pH	6.30	6.52	6.5	6.66
Total Alkalinity, mg/l as $CaCO_3$	1795	--	1626	--
Clarifier Supernatant Analysis				
Total COD, mg/l	2850	--	2610	--
Soluble COD/ mg/l				
Total Suspended Solids, mg/l	382	380	438	406
Volatile Suspended Solids, mg/l	271	292	350	304
Acetic Acid, mg/l	813	--	771	--
Propionic Acid, mg/l	468	--	340	--
Butyric Acid, mg/l	371	--	308	--
Valeric Acid, mg/l	68	--	101	--

Table V-10 (concluded)

Test Designation	III-A		III-B	
Phase	Acid	Methane	Acid	Methane
Clarifier Underflow Analysis				
Recycle Ratio	0.34	0.30	0.31	0.32
Total Suspended Solids, mg/l	4050	365	4870	497
Volatile Suspended Solids, mg/l	2758	270	3506	353
Gas Production***				
Production Rate, ml/day	139		122	
Carbon Dioxide, ml/day	40		30	
Carbon Dioxide, %	27.6		23.5	
Methane, ml/day	99		92	
Methane, %	68.2		73.4	

*After filtration through glass fiber filter.

**Calculated value based on COD equivalence of individual volatile acids.

***Common gas phase.

acid reactor probably due to the presence of the more complex substrate.

As observed in Stage II, acetic and butyric acids were readily utilized in the methane reactor with little reduction in propionic acid again being noted. The valeric acid produced in the acid reactor was also utilized in the methane reactor.

The solids produced in the acid reactor during Stage III did not settle as readily as those produced from the synthetic substrate. Underflow concentrations of 0.27 and 0.35 percent in the acid phase clarifier resulted in VSS concentrations of only 1100-1200 mg/l in the acid reactor. The solids concentration from the methane phase reactor were of the same nature as those observed in Stage II with no significant solids concentration being provided by gravity clarification. Again no gas formation that might hinder gravity settling was noted during the investigation.

Although the substrate used in Stage III was an actual wastewater and presumably more complex than the synthetic substrate used previously, no significant difference in system performance was noted. Soluble COD reductions of greater than 50 percent were obtained with total system retention times up to 166 hours. Figure V-12 illustrates, moreover, that the soluble COD in the effluent from the acid reactor was composed primarily of the COD equivalent of the volatile acids, thereby indicating good substrate conversion

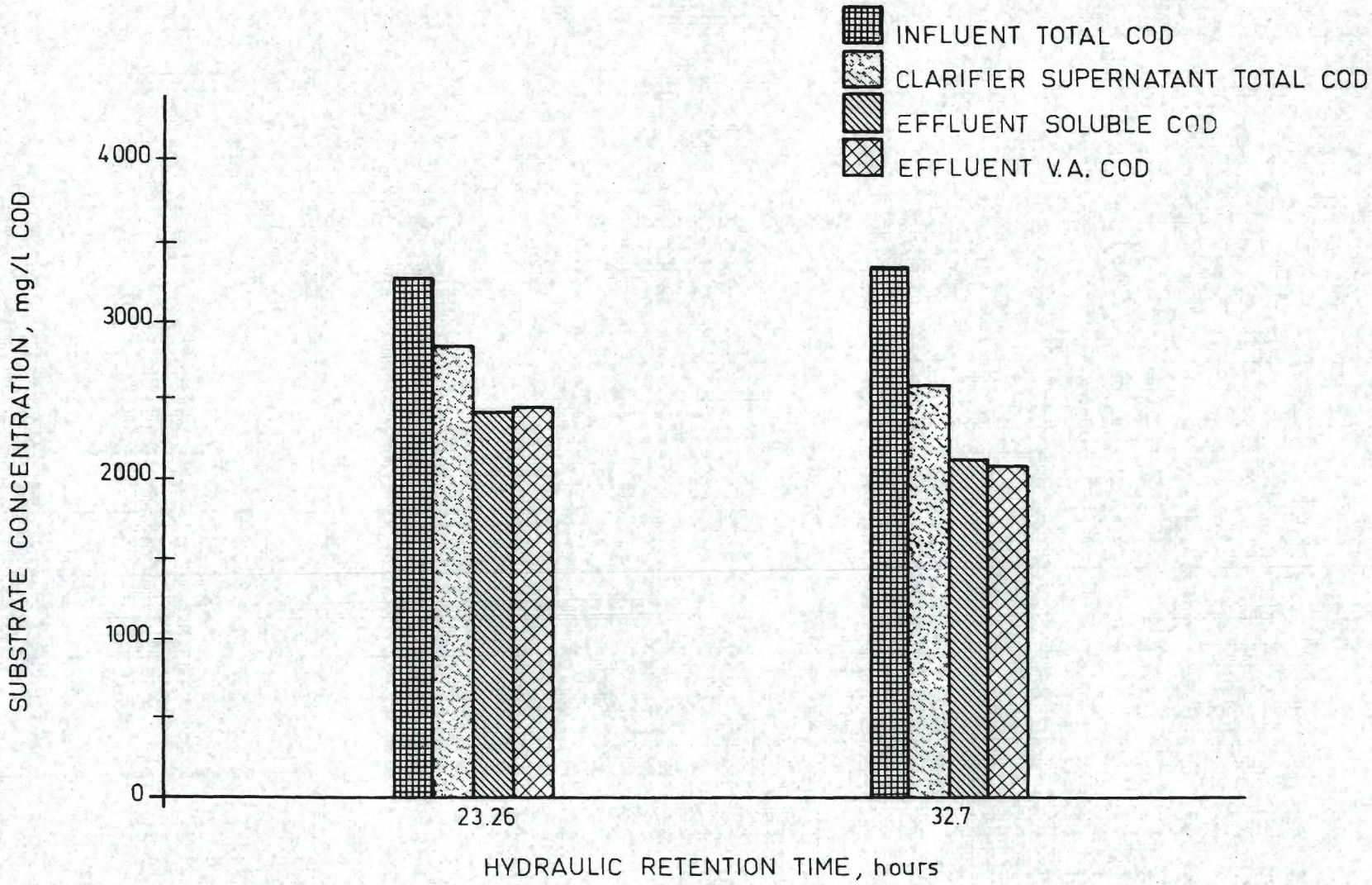


Figure V-12. COD Conversions in the Acid Phase Reactor--Stage III

to acids in this reactor. Subsequent utilization of these acids in the methane reactor illustrates that the two phase process is applicable to complex substrates.

Kinetic Evaluation

Mathematical models were developed in Chapter III to describe steady-state conditions for the two phase anaerobic stabilization process. Although the difficulty in attaining steady-state with the complicated reactor system utilized was anticipated, kinetic parameters were estimated. These parameters were used to evaluate the usefulness of the model developed and also as a basis of comparison with other studies.

In the study described herein, the acid phase reactor was operated in a manner to maintain phase separation and thus minimize original substrate carryover to the methane reactor. In order to achieve the primary objective of phase separation, it was necessary to use sufficiently long retention times in the acid reactor to insure the conversion of the carbohydrate substrate to volatile acids. Thus an accurate estimation of the small amount of substrate acceptable to the acid bacteria that remained in the reactor effluent was made difficult. Since substrate carryover was not a constraint on the operation of the methane phase reactor and its retention time could be manipulated without consequence to the overall reactor system operation and the substrate remaining was a direct measurement, data acquisition and

analysis were facilitated.

To permit kinetic analysis based on the previously presented models for the acid phase, it was necessary to estimate the substrate concentration remaining in the reactor effluent. Direct measurement of the remaining glucose concentration by the enzymatic-dye technique described by Jeris and Cardenas [115] was considered for this determination. However, results of their experiments with an anaerobic digester indicated that a slug of approximately 1000 mg/l glucose persisted in the digester for only 30 to 40 minutes. It seems likely that the molecular structure of glucose was changed sufficiently to make it undetectable by the analytical technique. Since the work of Ghosh and Pohland [9] indicated that significantly longer times were necessary to produce good glucose conversion to volatile acids, other analytical techniques for the measurement of substrate concentration in the acid reactor were considered.

The method used was based upon the difference in the soluble COD of the effluent and the calculated COD associated with the measured volatile acid concentrations in the effluent (ΔCOD). Since COD determinations on a mixed sample of volatile acids indicated approximately 100 percent recovery, no corrections were applied to the calculated COD values. It was thus assumed that all soluble COD in the effluent, with the exception of that resulting from the volatile acids, would be available for substrate for acid-forming bacteria. It was

recognized that the data from this study would be difficult to use for kinetic analysis due to the mode of operation for the acid phase reactor. Determination of the ΔCOD term would be subject to the inaccuracies associated with the determination of small differences between relatively large numbers as well as the restrictions imposed by associated analytical uncertainties.

It was decided to avoid these problems by reevaluating selected data previously obtained by Ghosh and Pohland [9] for a similar glucose substrate. These data, shown in Table V-11, were obtained at much lower hydraulic retention times (where volatile acids were being produced but not utilized by methane bacgeria to any degree), and analyzed using the ΔCOD concept. These data were then plotted as shown in Figures V-13 and V-14 to permit evaluation of the kinetic parameters included in the models previously presented. Using a least squares analysis, the estimated value of the kinetic parameters for the acid phase were:

Y^A = 0.31 mg VSS/mg Δ COD utilized

μ_m^A = 2.7/hour

k_d^A = 0.065/hour

K_s^A = 2583 mg COD/1

Using these values and an average influent substrate concentration from Table V-11 of 3950 mg COD/1, curves predicting effluent substrate (ΔCOD) and organism (VSS)

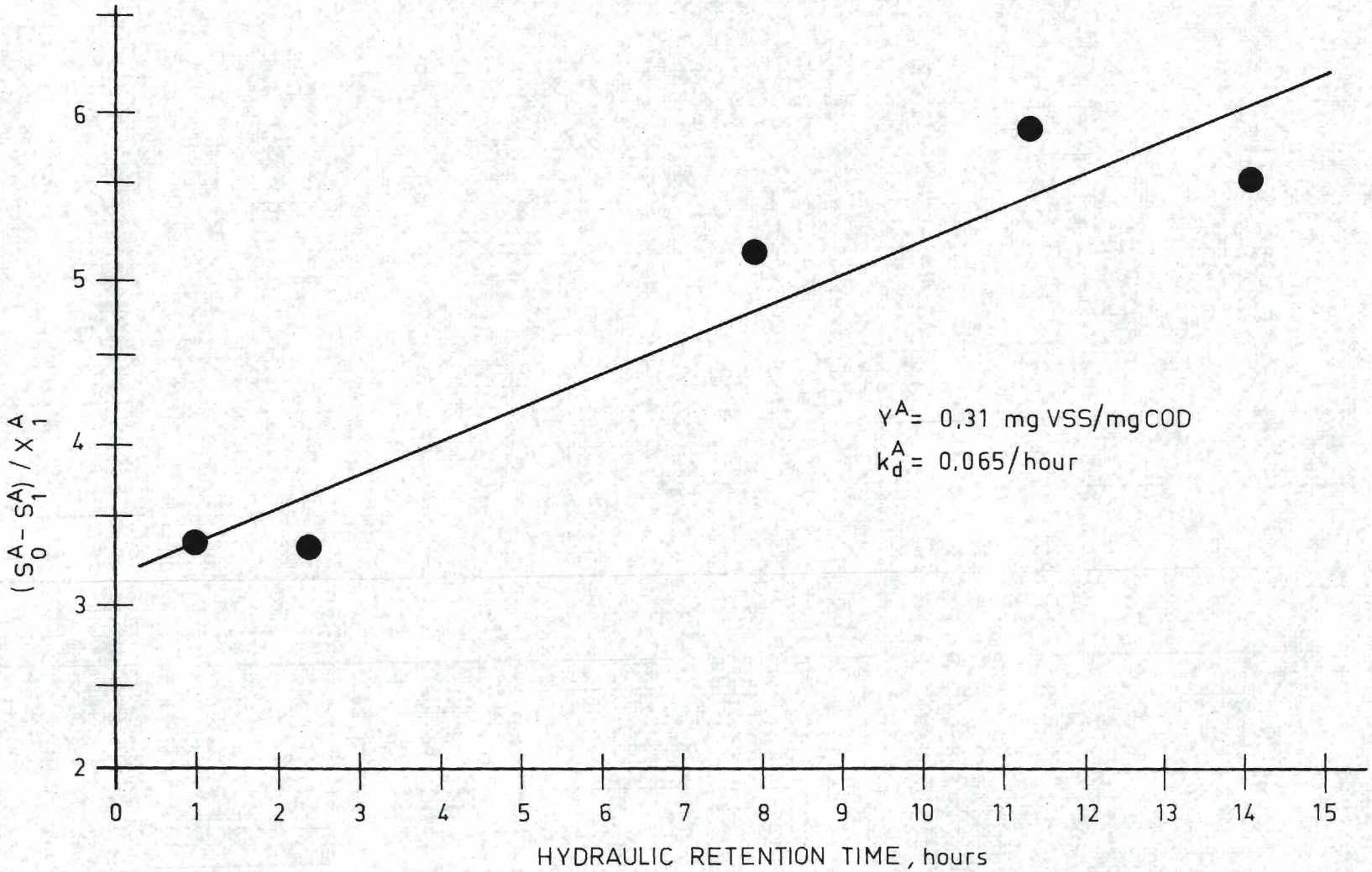


Figure V-13. Estimation of Kinetic Parameters for Y and k_d, for Acid Phase (After Ghosh and Pohland [9])

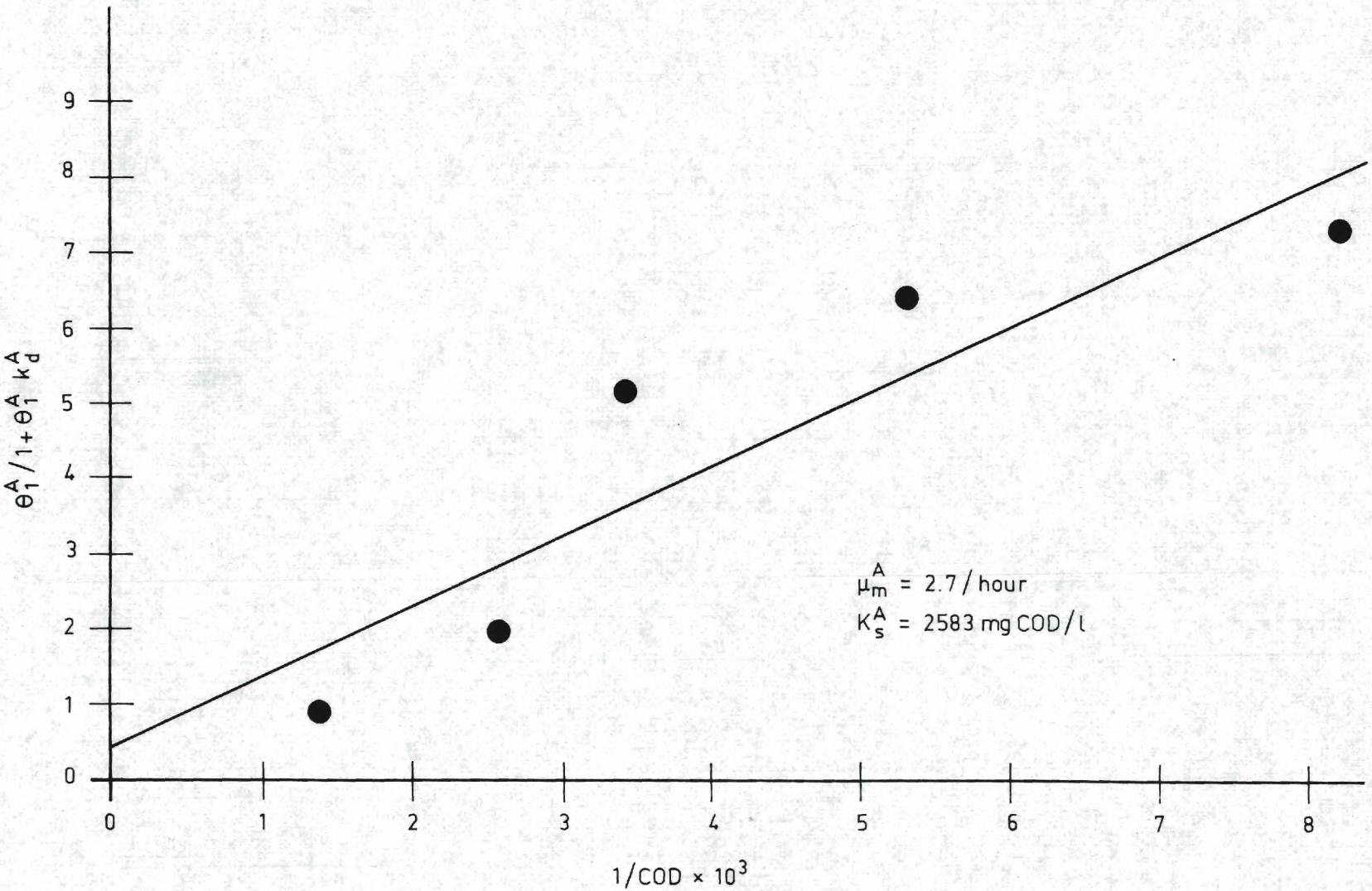


Figure V-14. Estimation of Kinetic Parameters, μ_m and K_s, for Acid Phase (After Ghosh and Pohland [9])

Table V-11. Selected Data From Anaerobic Stabilization of Simple Sojuble Substrate*

Hydraulic Retention Time, Hours	Influent Soluble COD**, mg/l	Effluent COD, mg/l			Effluent Volatile Suspended Solids, mg/l
		Measured Soluble	Volatile Acids**	ΔCOD	
0.93	1,094	984	252	732	106
2.28	1,094	730	333	397	208
7.72	1,216	894	599	295	178***
11.24	1,205	1,010	820	190	170
13.90	1,256	904	781	123	200

*After Ghosh and Pohland [9].

**Influent soluble COD and effluent soluble volatile acid COD are calculated COD equivalence of measured glucose substrate and individual volatile acids, respectively.

***Extrapolated value.

concentrations were calculated from Equations 25 and 26 and plotted in Figures V-15 and V-16, respectively. The actual experimental data obtained from Stage I were then compared with the predicted values. Inspection of Figures V-15 and V-16 indicates that the VSS and COD observed in Stage I generally agree with the predicted values which, in view of the difficulty in attaining accurate substrate measurements, were considered sufficient to provide acceptable correlation. Estimation of kinetic parameters for the methane phase of Stage I was based upon measured concentrations of acetic acid (HAc) since it was the only acid being utilized consistently. Since adequate biomass data were not obtainable for the methane phase due to acid phase biomass carry-over and decrease due to cell decay, only values of μ_m^M and K_s^M could be estimated. As shown in Figure V-17, a Lineweaver-Burke plot for the methane phase provided the following estimates for these two kinetic parameters:

$$\mu_m^M = 0.43/\text{day} \qquad K_s^M = 369 \text{ mg HAc/l}$$

Lawrence and McCarty [79] have reported comparative values with μ_m of 0.5/day and K_s of 207 mg HAc/l at a similar influent substrate concentration. As indicated in Figure V-18, these parameters could then be used to predict anticipated effluent acetic acid concentrations at the various hydraulic retention times.

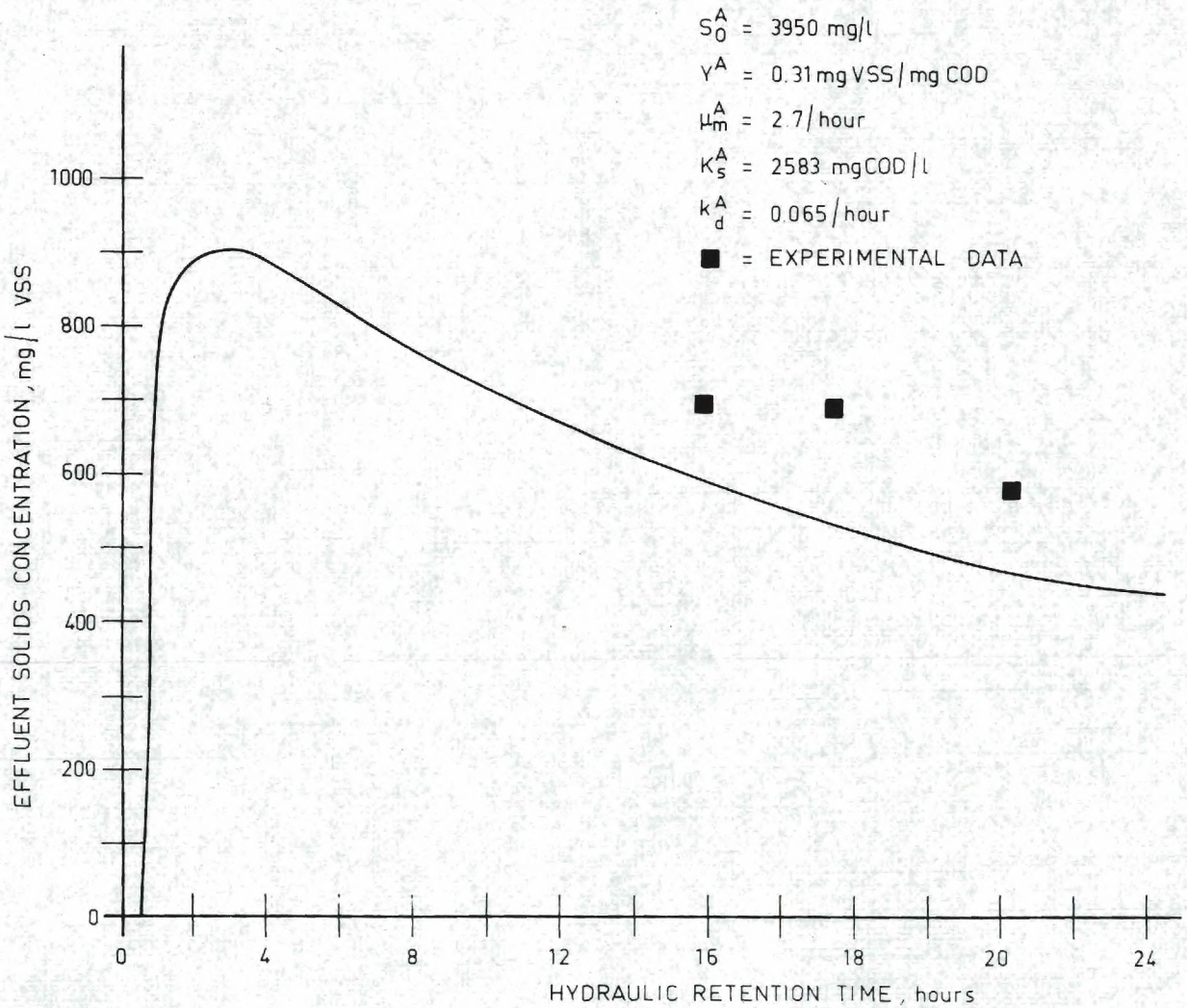


Figure V-15. Volatile Suspended Solids Concentration of Acid Phase--Stage I

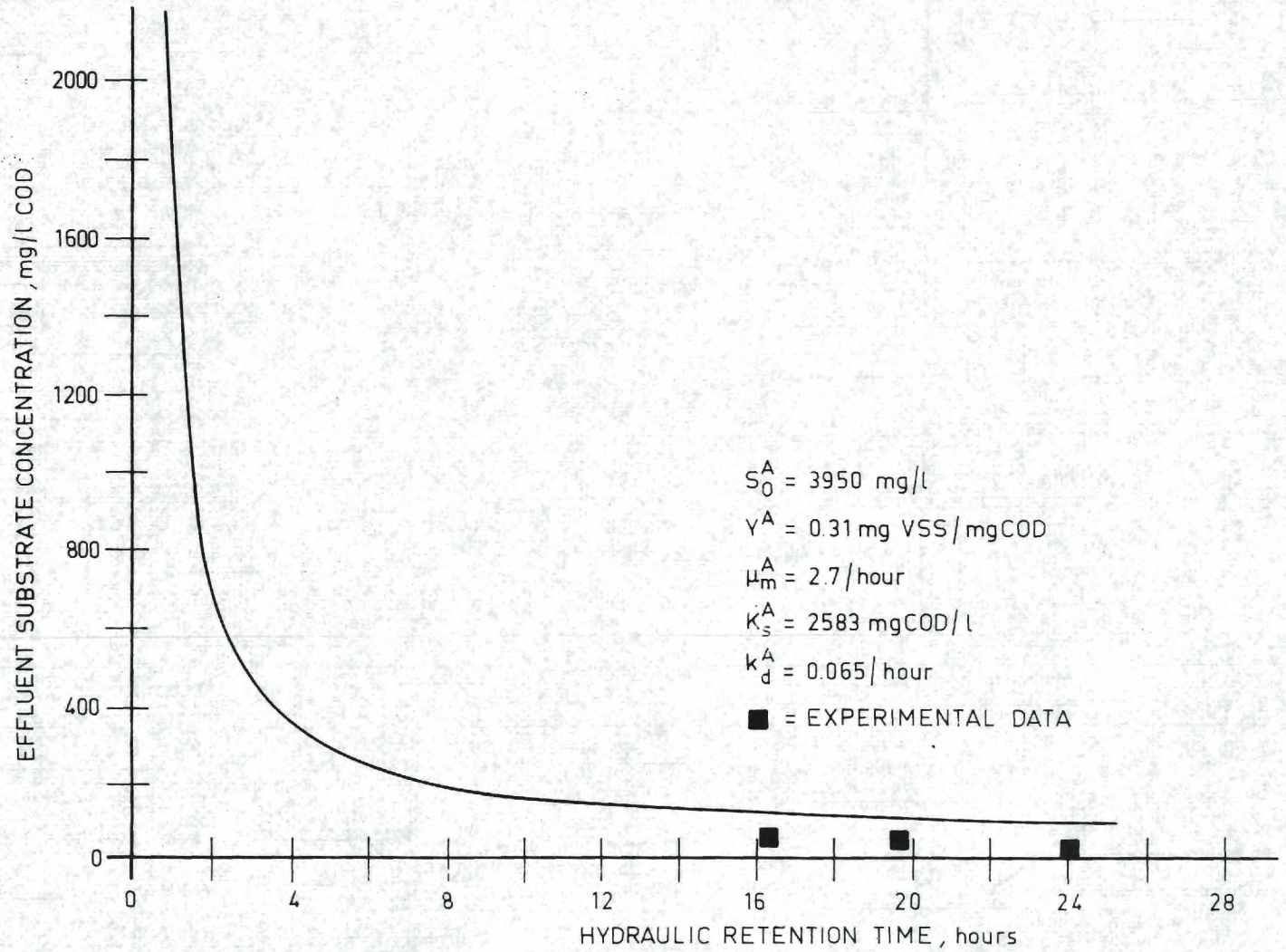


Figure V-16. Substrate Concentration of Acid Phase--Stage I

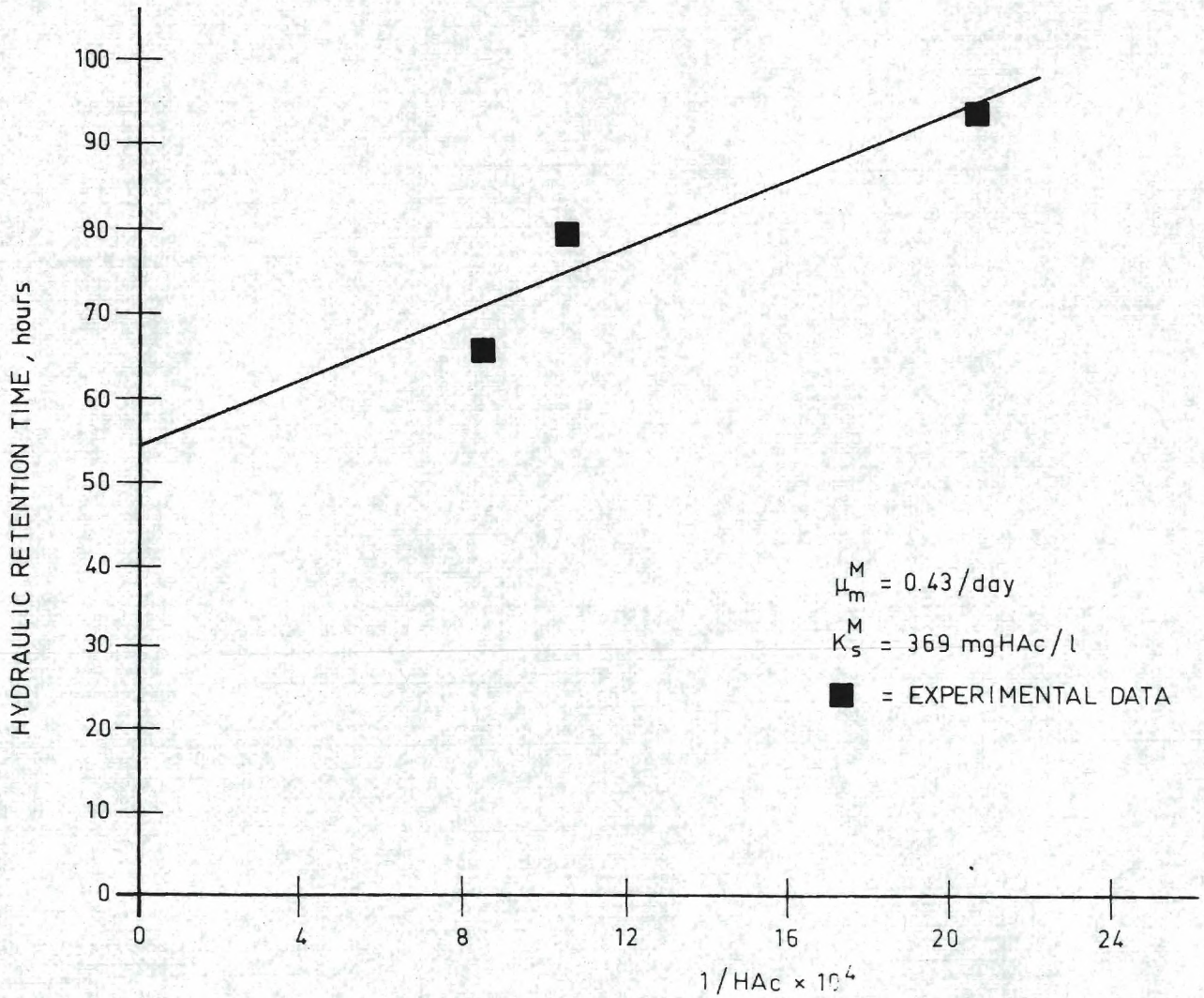


Figure V-17. Estimation of Kinetic Parameters, μ_m and K_s, for Methane Phase Using Acetic Acid Substrate--Stage I

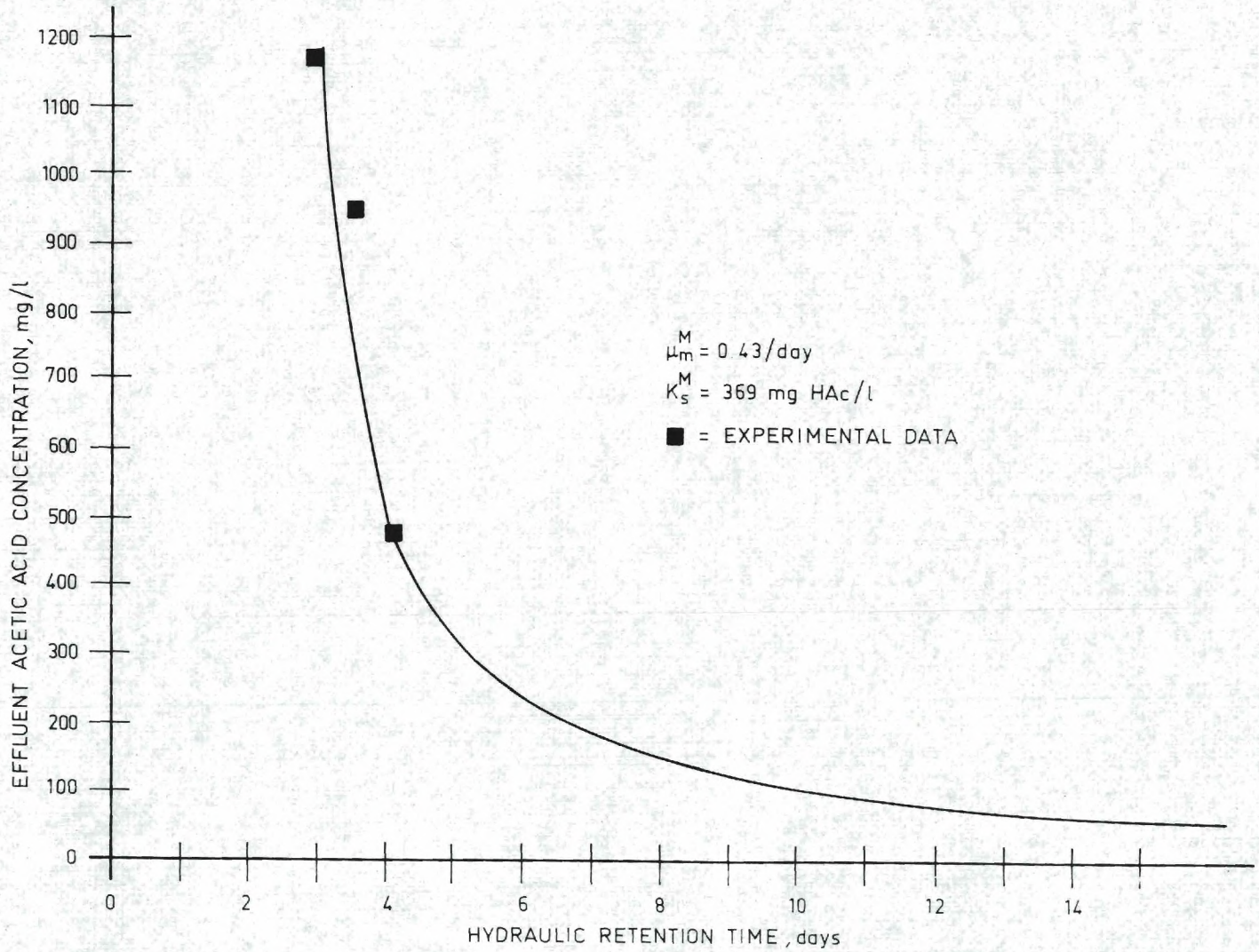


Figure V-18. Acetic Acid Concentration of Methane Phase--Stage I

Since the same substrate was used during Stage II, the kinetic parameters obtained during Stage I could be used to compare the experimental data from Stage II with predicted values and thereby determine the adequacy of the estimates. Figure V-19 shows the comparison between the VSS observed in the acid phase reactor during Stage II and those calculated using Equation 25 and the kinetic parameters previously obtained. As shown in Figure V-19, the VSS concentration calculated is consistently less than the values observed during recycle operations. Although the recycle ratio varied for each retention time, it was kept constant during each test period. Thus, it should have no effect on predicted results. However, this deviation could logically occur if the organism yield is underestimated or if the decay constant is overestimated. Since the k_d^A of 0.065/hour is unusually high when compared to values obtained in other anaerobic systems [58,87] the data can be reevaluated by adjusting the decay constant downward. If k_d^A is adjusted to 0.012/hour, then the fit is greatly improved.

Figure V-20 reflects the problems encountered with the method of using ΔCOD values for estimating substrate concentration remaining in the acid phase. No consistent correlation between predicted and observed substrate concentrations was noted at the indicated hydraulic retention times, thereby precluding rational adjustment of the kinetic constants necessary to predict changes when recycle

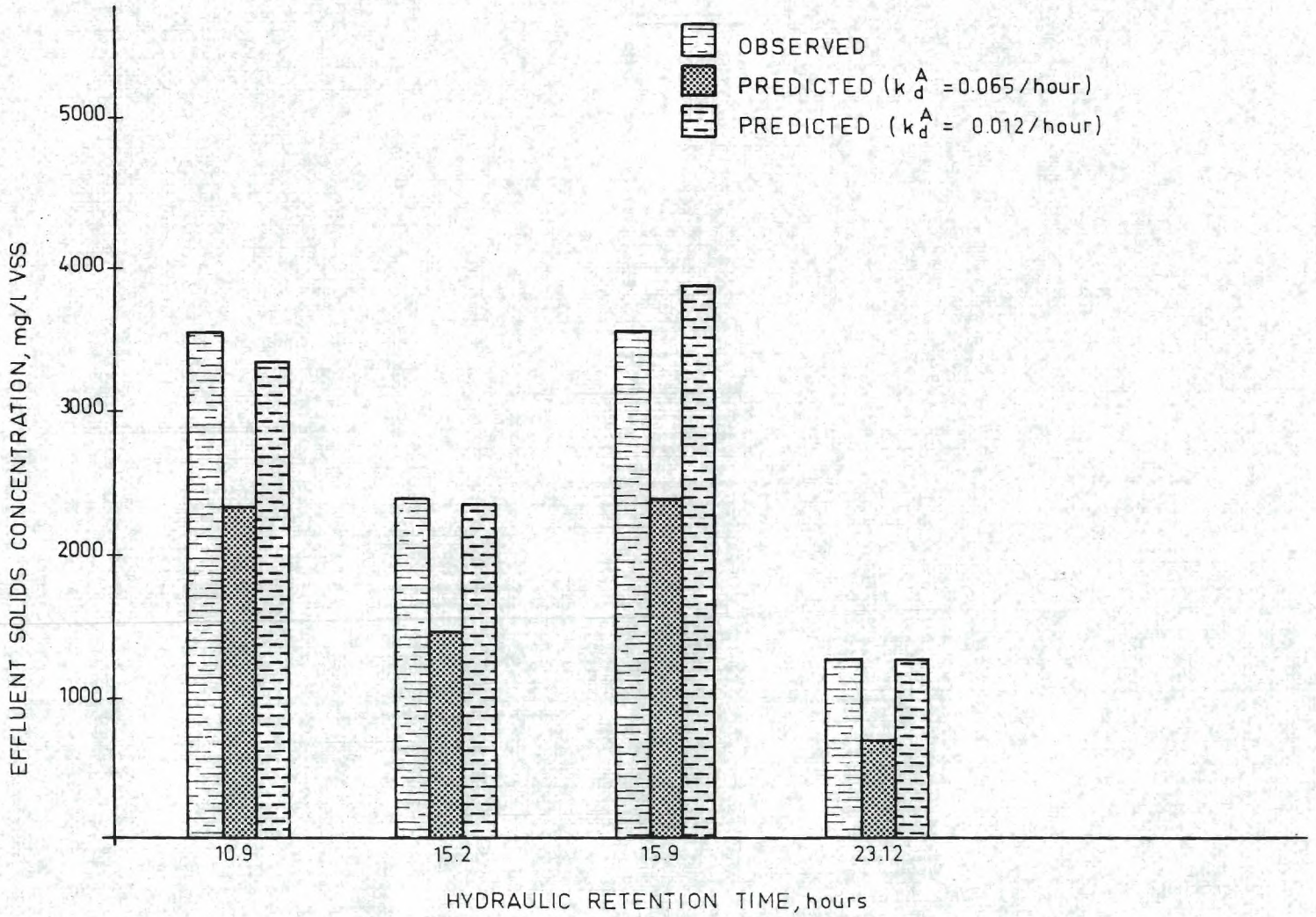


Figure V-19. Comparison of Observed and Predicted Volatile Suspended Solids Concentrations in Acid Phase Reactor--Stage II

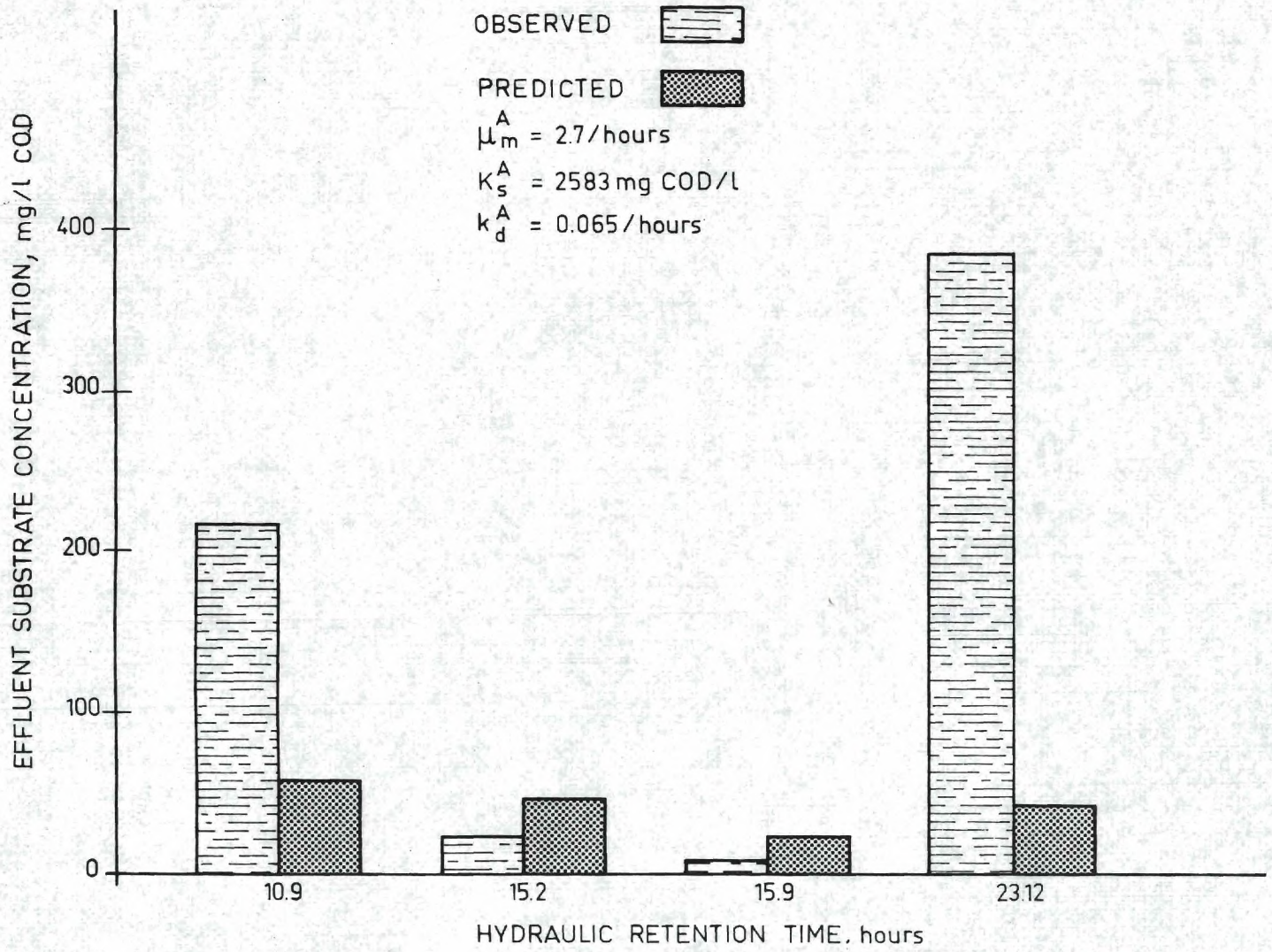


Figure V-20. Observed and Predicted Substrate Concentrations of Acid Phase--Stage II

was employed.

If no net concentration of methane-forming bacteria is assumed to have occurred during Stages II and III of the studies, then the equations describing the substrate concentration reduce to those for a single-stage system without recycle. As a consequence, the effluent acetic acid concentrations observed during Stages II and III should then closely follow the predicted curve shown previously in Figure V-17. Figure V-21 illustrates the good correlation obtained with this assumption thereby lending credence to the assumption that little or no concentration of methane-forming bacteria occurred in the methane phase clarifier. It also shows that the utilization of acetic acid during Stages II and III followed essentially the same kinetics as exhibited in Stage I. Thus, the utilization of acetic acid in the methane phase follows the same pattern whether the acetic acid is derived from glucose or a more complex industrial effluent.

Since butyric acid appeared in significant quantities in the effluent of the acid phase reactor and was then utilized in the methane phase reactor during Stages II and III of the studies, kinetic constants could also be estimated for the methane-forming bacteria in the methane phase clarifier, of $X_1 = X_r$, a Lineweaver-Burke plot of the butyric acid data from Stages II and III show on Figure V-22 a good model fit with $\mu_m^M = 0.86$/day and $K_s^M = 164$ mg HBu/l. This is

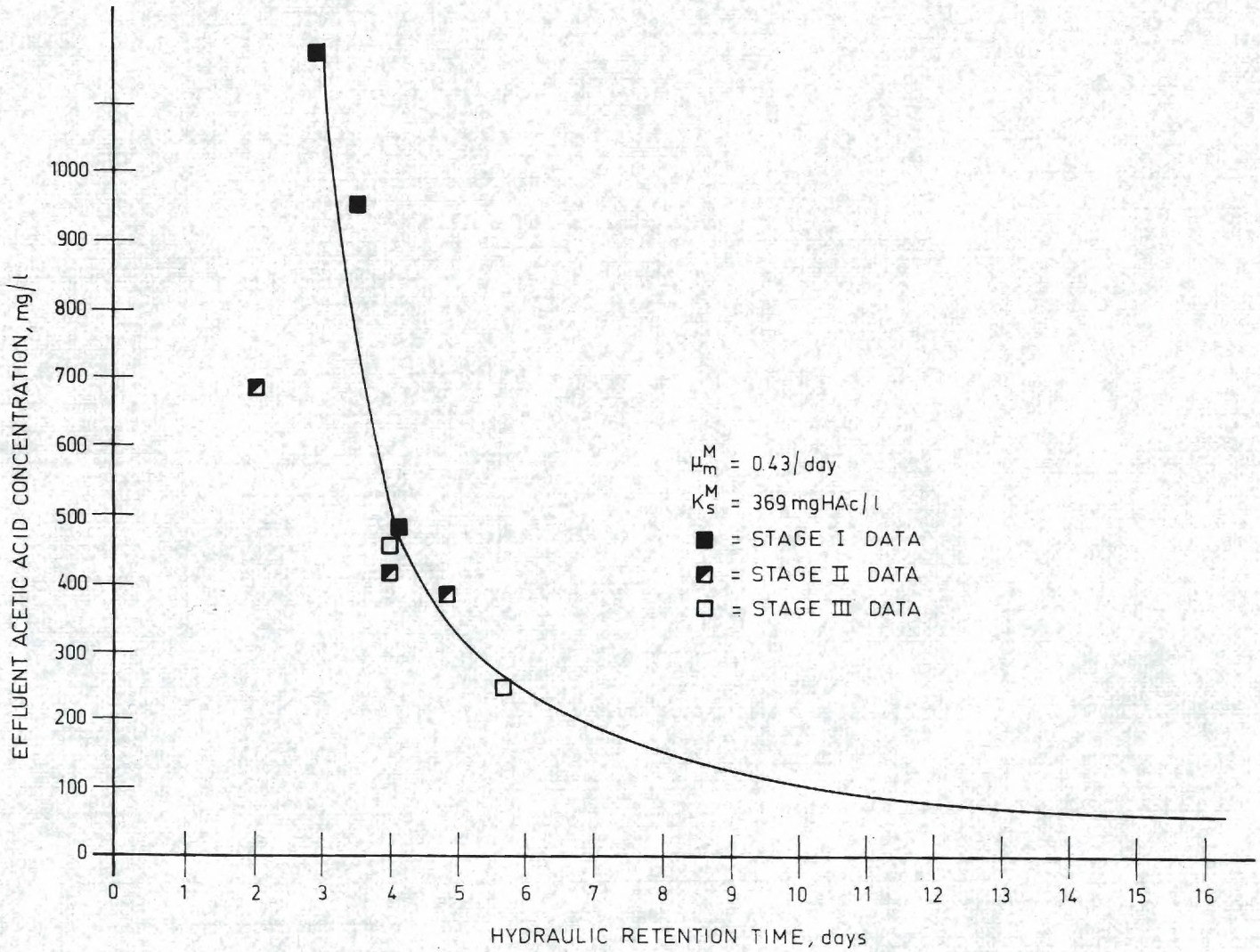


Figure V-21. Comparison of Predicted and Observed Acetic Acid Concentrations of Methane Phase--Stages II and III

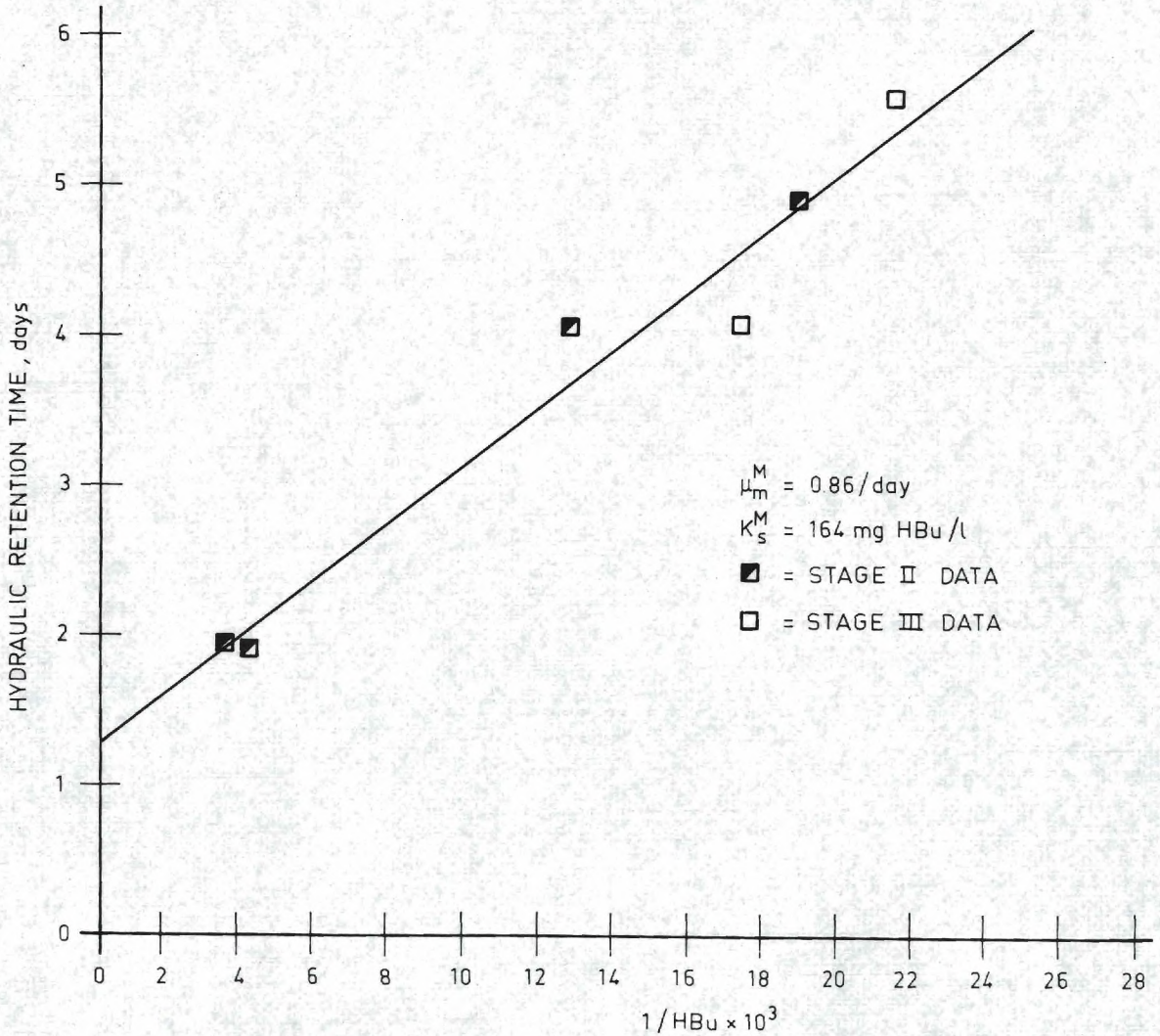


Figure V-22. Estimation of Kinetic Parameters, μ_m and K_s, for Methane Phase Using Butyric Acid Substrate--Stages II and III

at variance with the results obtained by Lawrence and McCarty [79], i.e., μ_m^M of 0.37/day and K_s of 7 mg HBu/l.

CHAPTER VI

CONCLUSIONS AND ENGINEERING SIGNIFICANCE

Conclusions

The objectives set forth for the research study were attained and demonstrated by the data reported in the previous chapters. Specifically, the study confirmed the following:

1. In contrast with other research efforts, the acid and methane forming phases of the anaerobic stabilization process were isolated in separate reactors by appropriate manipulation of the hydraulic retention times, with the available organic carbon being converted primarily to volatile organic acids and biomass in the acid phase reactor. Other efforts in two-phase digestion did not achieve true separation of the phases due to the use of sludges or simulated sludges as substrate. Complete conversion of these solids to volatile acids was not achieved in the acid reactor and thus volatile acid production as well as utilization occurred in the methane reactor.

2. Gravity sedimentation and recycle of biological solids originating in the competely mixed acid phase reactor is a feasible and practical method of improving process operation; however, biological solids from the methane phase reactor settle poorly, and gravity sedimentation

followed by biomass recycle does not appear to be a feasible procedure, even with chemical pretreatment.

3. Mathematical models were successfully employed for description of the acid and methane forming phases of the anaerobic stabilization process. A more sophisticated analytical technique must be developed to estimate the effluent substrate concentration for the acid reactor.

4. Kinetic parameters were estimated for both the acid and methane forming phases with analysis of the data from the acid phase yielding a maximum specific growth rate, μ_m^A = 2.7/hour, a saturation constant, K_s^A = 2583 mg COD/l, a yield, Y^A = 0.31 mg VSS/mg COD utilized and a decay constant, k_d^A = 0.065/hour. Similar analysis of the data obtained from the methane phase indicated a maximum specific growth rate, of 0.43/day and 0.86/day and saturation constants of 369 mg HAc/l and 164 mg HBu/l for organisms utilizing acetic acid and butyric acid, respectively.

5. The provision of a common gas phase for the acid and methane reactor appeared to contribute to an improved overall process efficiency.

Engineering Significance

The engineering significance of this study lies in the development of a more rational system configuration for the anaerobic stabilization process. The study has demonstrated that the imposition of suitable kinetic controls on

a two-reactor system can lead to phase isolation, thus providing opportunity for better overall control of the reactor environments.

Additionally, the successful use of kinetic models to describe system performance provides a rational basis on which to design a full scale two-phase anaerobic system. Thus, acceptable conversion of soluble carbohydrates to volatile organic acids can be accomplished in one day or less in the acid reactor, and gravity clarification can be applied successfully. The methane reactor requires 10-20 days to achieve an acceptable effluent without biomass concentration and recycle. Gravity clarification does not appear to be an acceptable method for biomass separation.

The two-phase concept also provides a valuable tool for separating the two distinct phases of the anaerobic stabilization process in order to better determine the limiting kinetic parameters of the process and to study possible improvements in overall treatment rate by control of environmental conditions.

The improved kinetics obtained by the utilization of butyric acid suggest that better treatment efficiency might be obtained by adjustment of acid reactor conditions to favor butyric acid production. However, Barker [38] indicates that fermentation of butyric acid is a two-step process. The first step results in 1/2 mole of methane and 1 mole of acetate for each mole of butyric acid degraded. Thus, the degradation of acetic acid would be the rate limiting step.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE STUDY

The study reported herein indicated the feasibility of the two-phase concept to the anaerobic stabilization process. However, several areas were uncovered which held promise for future development of the two-phase process for anaerobic treatment. Specifically, these include the following:

1. The evaluation of alternate process configurations for the methane phase reactor. This study has indicated that improved means of enlarging the methane bacteria population within the methane reactor is essential to develop reasonable treatment efficiencies. The limited success obtained by gravity clarification in this phase clearly illustrates the necessity to investigate other configurations. The possible alternatives may be fixed film systems as described in Chapter II. The anaerobic filter, upflow anaerobic contact system and anaerobic fluidized bed process would all seem to possess potential, particularly in light of the reported propensity [31] of methane bacteria to grow well on surfaces. Another possibility to be considered is the addition of suitable attachment surfaces for the methane bacteria by introduction of particles, such as pulverized

coal or powdered activated carbon that can be readily removed by clarification.

2. The evaluation of different environmental conditions on the product mix obtained from the acid reactor. Potential would seem to exist to control product mix from the acid reactor as was seen by the high concentrations of butyric acid noted during Stage II. Additional research is called for to evaluate what control is possible and what product mix would contribute most to overall treatment efficiency and stability.

3. Additional investigations into the impact of provision of a common gas phase and the mechanisms resulting in improved treatment efficiency are required.

4. More intensive investigation into the microbiology of the separate bacterial complements in each reactor, the mechanisms for substrate conversion to desired end products and the possibilities of process improvement by culture enrichment should be undertaken.

APPENDIX A

CONCENTRATED SUBSTRATE PREPARATION

The concentrated substrate is prepared by procedure given below. The procedure must be carefully followed in the sequence given in order to avoid precipitation of salts. The following instructions are for the preparation of one liter of concentrated substrate.

1. Tare container
2. Add 33.30 ml of trace salt solution prepared below
3. Add 550 ml of water
4. Add each of the following with mixing. Do not add compound until previous compound is in solution
   (a) KH_2PO_4 . . . . . 13.61 gm
   (b) $NaH_2PO_4H_2O$ . . . . . 8.28 gm
   (c) $(NH_4)2SO_4$. . . . . 5.28 gm
   (d) NH_4Cl. . . . . 49.2 gm
   (e) $CaCl_2$. . . . . 4.44 gm
   (f) $MgCl_2$ $6H_2O$ . . . . . 8.13 gm
   (g) Glucose. . . . . .144.0 gm
5. Dissolve 3.60 gm yeast extract in 100 ml of water, with heating and stirring. Do not allow to boil. When cool, add to substrate container.

6. Add water to solution to make 1000 grams. The concentrated substrate must be diluted 1:40 by mass to give proper elemental composition.

a. Trace Salt Solution

The trace salts and the complexing agent, sodium citrate, are prepared in a solution 240 times their strength in the diluted substrate.

The trace salts are added to distilled water in amounts given below and made to two liters with distilled water. The solution must be stirred until all salts are in solution.

Compound	Mass, grams
$FeCl_3$	38.88
$MnCl_2$, $4H_2O$	9.48
$ZnCl_2$	6.54
$CuCl_2$, $2H_2O$	4.10
$CoCl_2$, $6H_2O$	5.71
$(NH_4)6Mo_7O_{24}$,$4H_2O$	4.15
$Na_2B_4O_7.10H_2O$	2.29
Na_3Citrate	353.0

Note: When prepared as above, salt solution will have very dark color, however, with adequate stirring all salts will go into solution.

Compounds should be added in the order shown above, and each compound should not be added until previous compound is in solution.

When prepared in the concentrated form and diluted

1:40 with water the substrate will have the following composition:

Element	Concentration, m moles/l	Concentration, mg/l
C	120.0	1440.0
N	25.0	350.0
P	4.0	123.9
*Na	3.0	69.00
K	2.5	97.80
S	1.0	32.10
Ca	1.0	40.10
Mg	1.0	24.30
Fe	0.10	5.59
Mn	0.02	0.496
Zn	0.02	1.308
Cu	0.01	0.635
Co	0.01	0.589
Mo	0.01	0.960
B	0.01	0.108
Sodium Citrate	0.50	147.1
Yeast Extract		90.0

*Note: Sodium concentration includes that sodium added as sodium citrate.

These tests will be run at room conditions and checks will be made to insure that the D.O does not fall below 3.0 mg/l and the pH remains in the range of 6.0-7.0. If necessary for pH control add a 1.5 M phosphate buffer (pH = 7.0) at a flow rate of approximately 2.0 ml/min.

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VITA

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Mr. Massey entered the U. S. Army as a Second Lieutenant in 1963. In 1965 he was employed as a project engineer at Rayonier, Incorporated at Jesup, Georgia. He accepted a position as project engineer in 1967 with Westvaco Corporation at Covington, Virginia. He was employed there until 1970, when he resigned to attend graduate school at Georgia Tech. In 1971 he received a Master of Science degree in Sanitary Engineering. In 1975 he again accepted a position with Westvaco Corporation. Currently he is product manager of water and wastewater treatment for the Carbon Department of Westvaco.

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Mr. Massey was married in 1962 to the former Mary Suzanne Harper. They have two children, Michael and Jennifer. They currently reside in Covington, Virginia.