ANAEROBIC DIGESTION OF CHEESE WHEY IN AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR

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Dedication

To my parents for their love, encouragement and sacrifice.

ABSTRACT

The anaerobic digestion of cheese whey was studied in an upflow anaerobic sludge blanket reactor for its start-up characteristics, the effects of various process parameters, the effect of sulfate addition and the determination of optimal operating conditions.

Start-up of an UASB reactor treating cheese whey was extremely difficult due to its tendency to acidify. Various start-up strategies were tested to facilitate start-up and to ensure stable operation. Among the operating parameters, sludge loading rate was the most critical for proper start-up of the UASB reactor. The initial sludge loading rate during start-up period should not exceed 0.25 g COD/g VSS.

The response of whey digestion to several process parameters was investigated. Without pH-control, over 97% COD removal was obtained for influent concentrations from 5 to 28.8 g COD/l and HRT of 5 days. However, instability was observed when the influent concentration was increased to 38.1 g COD/l.

Gas production from whey is affected by organic loading rate (OLR). At an OLR less than 4 g COD/l-d, higher influent strength resulted in a higher methane production rate. When the OLR was greater than 6, higher strength feed or shorter hydraulic retention time (HRT) produced less methane.

From the profiles of substrate concentration measured at various levels above the bottom of the reactor, two reaction stages, acidogenesis and methanogenesis were distinguished. It was experimentally illustrated that the rate of acidogenesis is much faster than the rate of methanogenesis in a whey anaerobic digestion system. The accumulation of VFAs in the first stage being faster than its assimilation in the second stage creates a distinct acidogenic phase in the bottom of the reactor. The instability caused

by high influent concentration could be attributed to the accumulation of VFAs beyond the assimilative capacity of the methanogenic stage.

A set of empirical models for accumulation and degradation of VFAs was developed using linear regression analysis. The requirement for maintaining this system in a dynamic balance was that the degradation capacity for VFA in the second stage be greater than the accumulation of VFA in the first stage. Based on this idea, the optimal influent concentration was given as between 25 to 30 g COD/l for system stability.

A hypothesis was proposed in this study that a proper amount of sulfate may be applied to moderate the detrimental influence of excess hydrogen on a stressed anaerobic reactor. The effect of sulfate was tested to study the biochemical mechanism. The permissible influent COD concentration was increased from 30 g COD/l to 50 g COD/l by using sulfate addition. The pH in the reactor was on the average 0.8 units higher and the concentration of butyric acid in the acidogenic phase much lower with added sulfate than without sulfate addition. The significant improvement of process stability and treatment efficiency made by the addition of sulfate clearly illustrated that sulfate acted like a stimulator which helped to maintain conditions favorable to methanogenesis. The mechanism of this stimulation is explained according to thermodynamics and hydrogen regulation which suggested that sulfate is able to promote the β-oxidation of VFAs by consuming hydrogen.

A two-stage inhibition mechanism was proposed to explain the inhibition of high VFA concentrations and the stimulation of sulfate. Higher hydrogen pressure is the cause of preliminary inhibition, resulting in the accumulation of VFAs, which subsequently inhibit the activity and growth of methanogens in the second inhibition stage. The mechanism of inhibition of methanogens from VFAs was interpreted as being caused by the acidification of the internal cytoplasm and destruction of the pH gradient by non-ionized acids based on the theory of bacterial membrane transport. A new control strategy for stabilization

of an anaerobic system is recommended.

Under the optimal operating conditions based on the results in the first three steps, over 97% reduction of COD was achieved when the influent COD was 30 g /l using an HRT of 2 days, an OLR of 16.61 g COD/l-d and sulfate concentration of 0.2 g/l.

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ATP	adenosine triphosphate
ADP	adenosine diphosphate
BOD	biochemical oxygen demand
COD	chemical oxygen demand
HOA	hydrogen oxidizing acetotrophs
HOM	hydrogen oxidizing methanogens
HRT	hydraulic retention time
MPB	methane producing bacteria
NAD	nictinamide adenine dinucleoticide
NHOA	non-hydrogen oxidizing acetotrophs
NRB	nitrogen reducing bacteria
OHPA	obligate hydrogen-producing acetogens
OLR	organic loading rate
Pi	phosphate
pmf	proton motive force
SLR	sludge loading rate
SRB	sulfate reducing bacteria
SRT	solids retention time
TSS	total suspended solid
UASB	upflow anaerobic sludge blanket reactor
VFAs	volatile fatty acids
VSS	volatile suspended solid

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Chapter 1

INTRODUCTION

1.1 DISPOSAL/UTILIZATION OF CHEESE WHEY

Cheese whey is a by-product of cheese production. Each pound of cheese produced results in five to ten pounds of fluid whey. In the U.S.A., approximately $27x10^9$ tonnes of whey are produced each year, while 2 million tonnes of whey are generated annually in Canada. With the increasing cheese demand in North America, fluid whey production is tending to increase.

Cheese whey contains about 5% lactose, 1% protein, 0.3% fat and 0.6% ash (Loehr,1977). The COD of cheese whey ranges from 60,000 to 70,000 mg COD/liter, depending on the type of cheese process. Every 1000 gallons per day of raw whey discharged into a sewage treatment plant can impose a load equivalent to that from 1800 people. Every 1000 gallons of raw whey discharged into a stream requires for its oxidation the dissolved oxygen in over 4,500,000 gallons of unpolluted water. The high organic content of whey, the trend towards increasing production of cheese whey and stricter pollution control standards have led to an expensive and difficult waste disposal problem for the cheese manufacturers.

Cheese whey represents about 90% of the milk used in the cheese manufacturing process. A number of solutions have been proposed to recover nutrients and reduce the pollution level resulting from whey. These rely on converting the whey or various components of it to marketable products. Considering that whey has a high content of

lactose and protein, several investigators have developed new schemes for whey treatment with an emphasis on product recovery and new product development (Castillo et al. 1982; Friend et al. 1982). These efforts include fermentation of whey to ethanol for beverage production (Palmer 1978, 1979) or gasohol production; drying of whey into powder which may be used as animal feed or as a supplement in human foods (Muller, 1979; Modler, 1980); separation of whey components by membrane technology (Teixeira, 1982) or fermentation of whey for protein production. Many of these schemes, however, are limited to larger producers due to economic constraints. But even so, they don't completely solve the final waste disposal problem of whey. Further treatment is needed to meet the requirement for waste discharge.

Biological waste treatment systems, either aerobic, anaerobic or combinations of them can be used to treat a wide variety of waste streams and are capable of reducing the levels of pollutants to meet even the most stringent requirements. However, the activated sludge process, which is one of the most commonly applied methods in waste treatment, is unsuitable for the treatment of very high strength wastes such as whey due to its large energy consumption for aeration, which leads to high operating costs. In contrast, anaerobic systems have much lower operating due to the low yield of cells and low energy consumption (no aeration) and produce methane which can be used as an energy source.

1.2 ANAEROBIC METHANE FERMENTATION OF WHEY

As a process to convert the organic materials contained in biomass into useful energy (methane) and to reduce the emission of pollutants from industrial and agricultural wastes, anaerobic fermentation has already exhibited its potential. The growing realization of the potential of anaerobic treatment is evident from the large number of the

research reports published on this process each year. Significant advances have been made in extending this process for the treatment of a variety of wastes .

With regard to cheese whey treatment, these advantages are pronounced because cheese whey constitutes a high strength organic waste. Several studies have shown that anaerobic treatment can achieve an adequate removal of chemical and biological oxygen demand from cheese whey and that the methane production is close to the theoretical yield. It has been estimated that 1 liter of whey can generate about 45 liters of gas with a CH_4 content of around 55%, given that an expected COD removal efficiency of 80% is achievable. For every liter of whey, 20 liters of CH_4 can be generated, which is equivalent to an energy production of 700 Btu's. In Canada, 2 x 10^6 tonnes of whey are produced every year. This means 1.4 x 10^{12} Btu's of energy could be obtained by anaerobic methanogenesis of whey. According to the results of a survey among cheese producers in New York state, up to 46% of the oil and gas needs of a cheese plant could be supplied by methane generated from whey (Switzenbaun, 1982).

In spite of its present significance and its optimistic future potential, the anaerobic process has not had a favorable reputation because the conventional process usually suffers from a long start-up period, a slow digestion rate and unstable process performance, all largely due to the low growth rate of the anaerobic bacteria. These drawbacks have prevented the anaerobic process from having wide application. In the last decade, advances in the microbiology and biochemistry of the anaerobic process, along with the advances in high-rate digestor technology have led to a great improvement in treatment efficiency and process control.

One of the major advantages of anaerobic digestion is the relatively low yield of microbial cells (sludge) in comparison with the aerobic process. However, this becomes a disadvantage with respect to its longer period of start-up and its poor capability for tolerating load shock and other drastic changes in environmental conditions. It is now

well understood that the efficiency of the anaerobic process is strongly dependent on the solid retention time (SRT). SRT requires considerably more attention in anaerobic digestion than in the aerobic process.

Most of the new anaerobic reactor designs attempt to maximize SRT, and in turn the concentration of biomass in the reactor to permit a reduction in the required hydraulic retention time (HRT). The anaerobic filter was the first design which allowed the SRT to be independent from HRT. Since then, a series of high-rate digestors haveeen developed. The development of novel high SRT designs for biological reactors has resulted in many new anaerobic systems which are suitable for the treatment of concentrated organic wastewaters. These new anaerobic digestors include the upflow and downflow anaerobic filter, the fluidized bed reactor,the expanded bed reactor and the sludge blanket reactor.

The Upflow Anaerobic Sludge Blanket (UASB) reactor is one of these innovative reactor designs (Lettinga et al.1980) . Two distinguishing features of the UASB reactor are the installation of the 3-phase separator in the upper part of the reactor and the ability to cultivate sludge in granules. These two features permit the maintenance of a high concentration of biomass in the digestor. The UASB reactor has been widely used to successfully treat a variety of wastewaters (Wang et al. 1985; Lettinga et al. 1985). However, little research has been done on the use of this reactor for treating cheese whey. Few studies on treating acidic substrates, such as monosodium glutamate wastewater and cheese whey, with an UASB reactor are available so far (Wu and Zhang 1983; Samson et al. 1984). It has been suggested that the development of an anaerobic sludge with high activity and settleability is associated with the proper start-up procedures (Lettinga et al. 1979). Therefore, an assessment of the technical feasibility of treating cheese whey in an UASB reactor, concentrating on the start-up procedures, is necessary.

1.3 PROBLEMS ASSOCIATED WITH THE ANAEROBIC DIGESTION OF CHEESE WHEY

The anaerobic digestion of cheese whey has been investigated by using different digestor configurations (Hakansson, 1977; Clanton et al., 1980; Boening and Larsen, 1982; Switzenbaun, 1979; Hickey and Owens, 1981; Sutton and Li, 1981). Treatment efficiency was affected by reactor type, experimental method, pH-control, nutritional supplement and waste strength. Unlike the other substrates which are usually used as feed for anaerobic digestion, the treatment of cheese whey is much more difficult . Unsuccessful experiments have been reported, apparently due to its high organic strength and its tendency towards rapid acidification. A primary obstacle is a lack of fundamental understanding of the process. The problems encountered in this process can be attributed to inadequate buffering capacity and micronutrient deficiency. It was reported that stable operation of an anaerobic fermentation reactor with cheese whey could not be maintained unless pH control or additional nutrients (or mixture with manure) were applied.

1.3.1 Inadequate Buffering Capacity

Examining the effects of medium and inoculum on the treatment of whey and cellulose in an anaerobic fixed-bed reactor, Nordstedt and Thomas (1984) found that the reactor could not achieve stable operation within 30 days without pH control. Treating full strength whey in a fixed-film reactor, Marshall and Timbers (1982) reported that addition of NaOH was needed to maintain stability. Dehaast et al. (1983) used dilute whey as feed substrate and neutralized the acids to avoid a rapid pH drop in their experiment. In the study conducted by Follmann and Markl (1979), a pH-static process was used in which the pH value was the control signal for whey feed. When the pH increased beyond 7.0, a

pump was automatically triggered to add substrate until the pH fell to 6.95. Wildenauer and Winter also used a pH-titrate control unit in their fixed-film system to treat high strength acidic whey.

Using an expanded bed reactor Switzenbaum and Danskin (1982) found that when the influent strength was increased from 5 to 20 g COD/liter, the COD removal decreased from 83% to 58%. It was suggested that the physiological balance between the methane-producing organism and the hydrogen- and acid-producing organisms was more easily upset because of the great difference in the rates of acidogenesis and methanogenesis for an easily biodegradable carbon source, such as whey. It seems that a certain level of influent concentration represents a kind of inhibition to the system.

1.3.2 Micronutrient Deficiency

In their study of the effects of temperature on an anaerobic film expanded bed reactor (AFEB) treating cheese whey, Kelly and Switzenbaun (1984) noticed that in the absence of nutrient supplement, the COD removal efficiency was much lower as compared with Switzenbaun and Danskin's results (1982) for the same loading rate and influent concentration. It was assumed that the poor removal efficiency was due to nutritional limitations. Some necessary nutrients, that were present in the tap water used by Switzenbaun and Dankin, were absent from the tap water used for dilution by Kelly and Switzenbaun.

The results of experiments on nutrient requirements indicated that trace nutrients had a significant influence on reactor performance. Operation of the AFEB reactor improved after the addition of the nutrients. Gas production increased within 24 hrs.. COD removal efficiencies increased from 60.3% for the nutrient-limited experiment to 80% for the nutrient-supplied experiment. The volatile organic acid (VFA) concentration in the effluent of the nutrient-limited experiment was at least three times higher than the VFA

concentration of the nutrient-supplied experiment.

Based on the theoretical value of the C/N ratio, DeHaast et al.(1983) performed a study in a downflow-fixed film reactor treating deproteinated cheese whey with different C/N-ratios ranging from 7.5 to 73. The results indicated that no decrease in efficiency and stability occurred even at the highest C/N ratio. It is known from the literature (Henze 1982) that the nutrient requirement is a function of organic loading rate (OLR). Since the OLR used by Haast and his co-workers was only 2.6 g COD/liter day, their conclusions could not be regarded as being universal.

Treating both whole and diluted cheese whey in an anaerobic rotating biological contact reactor, Lo and Liao(1986) noticed that the system could not be maintained at steady-state when the HRT was decreased below 5 days. However, a significant improvement in efficiency and stability was obtained by using a mixture of cheese whey and screened dairy manure instead of cheese whey alone as the feed substrate (Lo and Liao 1987). In this case, the reactor could be operated successfully at a HRT of only 2 days without the addition of buffering and nutritional reagents. This study showed that something which existed in the manure was necessary for maintaining the stability of the system. The question remains as to what nutrients contained in manure help to restore the system and maintain it's stability.

Chapter 2

LITERATURE REVIEW

2.1 DEVELOPMENT OF REACTOR DESIGN TECHNOLOGY/UASB REACTOR

In traditional anaerobic sludge treatment the solid retention time (SRT) and the hydraulic retention time (HRT) were almost identical. More recent studies have defined SRT as the crucial design and operational parameter because of the very low growth rate of anaerobic microbes. Proper SRT control provides sufficient acclimation time to allow efficient treatment. Young and McCarty (1969) were among the first to recognize this "SRT effect" when they introduced the concept of the anaerobic filter, in which the ratio of SRT and HRT could be increased to 100 (Henze 1982). The independence of SRT from HRT has proven to be a turning point in the study of the anaerobic process and has made anaerobic wastewater treatment economically interesting as compared to the aerobic process. Since then, the development of the anaerobic process has focused on the maintenance and accumulation of a high concentration of biomass in the reactor. A variety of high-rate reactors has been developed (Callander 1983). These high-rate, anaerobic reactors differ in the way in which biomass is retained. They can be broadly divided into two systems: attached growth system and suspended growth systems (Stronach 1986). The former includes upflow and downflow fixed-film reactors, expanded bed reactors and fluidized bed reactors, in which a high SRT is achieved by retaining the biomass as a film on the inert support media, packing material in a fixed-film reactor or particle material in

fluidized bed reactor. The latter is called an Upflow Anaerobic Sludge Blanket (UASB) reactor in which high biomass levels are accumulated and retained in the digestor by means of an interior gas-liquid-solid separator that relies for its effectiveness on the design of the separator as well as on sludge settleability.

The UASB reactor, which was initially developed by Lettinga and his co-workers, has already been recognized as one of the most promising methods for anaerobic treatment of organic waste (Lettinga et al 1984). Instead of using a packing material to support and concentrate biological growth, the upflow sludge blanket operates as a suspended growth system. Two distinguishing features of the UASB reactor permit it to maintain a high concentration of biomass. These two features are a 3-phase separator and the quality of the cultivated granular sludge. The gas-liquid-solid separator (Lettinga et al 1980; Van der Meer 1982) is mounted in the upper part of the reactor. This arrangement has the following advantages:

- The bottom plates of the settler can serve as gas separator.
- No additional space is required.
- The sludge separated in the settler can flow directly back into the reactor without any mechanical means, such as a pump or scrapers, being required.
- The sludge is not exposed to "strange" conditions, it remains within the system.

In order to enhance the return of the sludge from the settler, the following conditions have to be fulfilled:

- Gas bubbles must be separated before the mixture of water and sludge enters the settling compartment.

- To avoid gas production in the settling compartment, the retention time of the sludge in the settler must be short.
- The inclined wall of the settler should be at an angle of approximately 50°.
- The surface load on the settler should be kept below about 2 to 2.5 m/hr.
- The sludge present at the liquid-gas interface in the gas collector should be kept well immersed.

The key to successful operation of the UASB system is that the hydraulic loading rate and upflow velocities of fluid must not exceed the sludge particle settling rate.

The second principal feature of the UASB reactor is its ability to cultivate granular sludge with good settleability and high activity. The development of this granular sludge, in terms of both specific activity and settleability in the UASB reactor, is associated with the composition of the wastewater, initial seeding and environmental conditions, including a temperature of 30 to 35°C and a pH of 6.5 to 7.2 (Wu 1985).

Calcium ions play a positive effect on the flocculating ability of sludge, presumably due to the improvement of the mechanical strength of the flocs (Lettinga 1980a; Hulshoff 1982).

It has been found, using yeast, sugar beet or potato waste as substrates, that granulation of sludge proceeded satisfactorily, whereas problems arose with distillery, corn starch and rendering wastes (Hulsoft pol et.al,1983). Granule characteristics may be detrimentally affected by significant amounts of suspended solid in the influent.

The properties of the initial seed have an effect on sludge aggregation. Lettinga et al. (1985) suggested that thicker types of digested sewage sludge (approximately 60 kg TS/m^3) are preferred because the thicker sludge has better settleability, although it is

generally lower in specific methanogenic activity than thinner types of digested sewage sludge (i.e. 40 kg TS/m^3).

The microorganisms themselves could function as a filtering medium (Lettinga 1981; Frostell 1981). To facilitate the filtration, the feed inlet system should introduce the influent wastewater homogeneously by using a distributor at the base of the reactor column.

One of the most serious limitations of the UASB process is the considerable time (4-8 weeks or longer) for start-up. The washout of sludge during the initial phases of operation is significant. It has been noticed that the development of a sludge with high specific activity and settleability could be highly dependent on the start-up procedure. Both sludge loading rate and hydraulic loading rate are the most important factors which affect the properties of anaerobic sludge (Wu 1985). Considerable attention has already been paid to the start up procedure (De Zeeuw 1985; Hulshoff et al 1983, 1984; De Zeeuw and Lettinga 1980; Lettinga 1979, 1985; Dubourguier et al 1983; Ross 1983). It was indicated that the initial sludge loading should not exceed 0.2-0.4 kg COD/kg VSS day until the volatile fatty acids were well removed. Sludge granules formed rapidly only at loading rates in excess of 0.6 kg COD/kg VSS day (Lettinga et al. 1979).

In the UASB system, agitation is provided by both hydraulic upflow and rising gas bubbles. A proper upflow velocity and the agitation due to rising gas bubbles are necessary for sludge aggregation. Higher hydraulic loading rates, ranging between 0.25-0.4m^3/m^2 hr, are favorable to the granulation of sludge because at these higher loading rates light floc sludge will move upward and be discharged from the reactor, whereas heavy granular sludge will move downward and grow quickly.

The ability of the 3-phase separator to maintain a high concentration of biomass in the reactor makes the UASB reactor capable of attaining high rates of methane production, high rates of conversion for dilute and mostly soluble wastes, and of accepting organic and

hydraulic shock loads and temperature fluctuations. Since there is no need for a recycle pump, mechanical mixer, nor support media, the UASB reactor is simple in construction and operation, has low energy consumption and is cheap to run and to maintain.

In order to mathematically describe and optimize the UASB process, various kinetic and dynamic models have been proposed , which include the fluid-flow pattern , the kinetics of substrate conversion and bacterial growth and the sludge distribution and behavior (Bolle et al 1986a, 1986b; Buijs et al 1980, 1982; Heertjes and Ven der Meer 1978, 1982,). A model based on a mass balance for the sludge in the blanket has been developed and experimentally checked for the physical behavior of the sludge in the blanket. Also, using stimulus response experiments with a Li^+ tracer, the dynamics of the fluid flow in a UASB reactor were studied (Heertjes 1978). From the model, three distinct parts of the sludge could be distinguished. Both sludge bed and blanket can be described as perfectly mixed tank reactors with short-circuiting flows; the settler volume acts like a plug-flow region. Bolle and his co-workers proposed a dynamic model of a continuous working UASB reactor which included the integration of the fluid flow pattern in the reactor, the kinetic behavior of the bacteria and the mass transport phenomena between different compartments and different phases. The mathematic equations are able to predict the various observable, nonobservable or difficult to observe state variables and were prepared for computation and simulation

The wastewaters that have been treated include: sugar beet, potato starch, maize starch, alcohol, yeast, brewery, slaughter house, dairy, paper mill, distiller's grain supernatant, synthetic fatty acid, sewage and bean production wastes

Although the application of the UASB reactor to the treatment of industrial wastewater has been widely reported (Lettinga et al 1985; Wang et al 1985), few studies on treating acidic substrates so far are available. Using an UASB reactor in the anaerobic treatment of monosodium glutamate wastewater with a pH of 2.0 and a chemical oxygen

demand (COD) concentration of 3 to 4.5 g/liter, Wu and Zhang (1983) reported a rapid decrease in pH in the reactor resulting in instability and a complete loss of bacterial activity. Lettinga(1979) suggested that improper procedures used in a UASB start-up period could lead to the development of sludge with low specific activity and poor settleability. Treating cheese factory effluent with an average strength of 2 g COD/liter, Samson et al (1984) found that the granular sludge in the UASB reactor tended to float, and a large amount of sludge was washed out. It might have occurred in the acidic environment because a massive growth of filamentous bacteria created a bulky sludge with poor settleability.

2.2 ANAEROBIC TREATMENT OF CHEESE WHEY IN DIFFERENT REACTOR CONFIGURATIONS

The anaerobic digestion of cheese whey using different reactor configurations has been reported. These reports show that anaerobic treatment can achieve significant removal of chemical oxygen demand (COD) from whey. It is evident that treatment efficiency is affected by reactor type, waste strength and the experimental method used. Without the addition of nutrients and pH-control, a fixed-film reactor was able to treat higher strength substrates and had higher COD removal efficiency than a fluidized bed. However, a longer HRT was needed in the fixed-film reactor than in the fluidized bed. It was reported by several authors that at least 5 days of HRT were needed in the fixed-film reactor system (Hakansson 1977; Claton et al 1980; Boening and Larsen 1982; Switzenbaun 1979; Hickey and Owens 1981; Sutton and Li 1981); whereas the fluidized bed reactor could be operated at much shorter HRTs. In a study of the anaerobic digestion of high strength, acidic whey using a pH-controlled, up-flow fixed-film loop reactor, Wildenauer and Winter

(1985) noticed that re-circulation of the reactor liquid helped to significantly improve the process efficiency. They suggested that the circulation of liquid was essential for fast gas expulsion. In addition, according to Monteith's studies (1981), liquid circulation could improve the mixing behavior of the reactor contents, in turn, reducing any dead volume and facilitating the contact between substrate and biomass . Furthermore, liquid circulation could minimize pH gradients along the reactor column and maintain a good pH environment for methane producing bacteria. These considerations help to explain why fluidized bed and expanded bed reactors are able to be operated at much lower HRT. Therefore, liquid circulation would be recommended to improve process operation in a stationary system.

2.3 ENVIRONMENTAL FACTORS IN ANAEROBIC DIGESTION

2.3.1 pH Control in the Anaerobic Process

The successful control of the anaerobic treatment process depends upon a knowledge of the various environmental factors which affect the microorganisms responsible for waste degradation. Of the various control factors, pH is one of the most important. Under anaerobic conditions, pH is controlled by the interaction of the carbonic acid system and a net strong base, the latter being the net result of the activities of the VFAs, ammonia and any other strong acids and bases present. This control depends on the maintenance of an adequate bicarbonate buffer system to counteract any acidity due to the carbon dioxide and organic acids produced during the anaerobic treatment.

Alkalinity is a measure of the buffering capacity of the digestor contents. It consists of bicarbonate, carbonate, ammonia and hydroxide components. VFA, hydrosulphuric and orthophosphoric acids should also be considered. McCarty (1964) indicated that a

bicarbonate alkalinity in the range of 2.5 to 5.0 g $CaCO_3$/liter (25 to 50 mM) provided a safe buffering capacity for anaerobic treatment of waste. Hydrosulphuric and orthophosphoric acid systems under anaerobic conditions were reported to provide very limited buffering capacity as they existed in extremely low concentrations. The dissociation constants of acetic and propionic acids allow them to be considered as weak acids (Capri and Marnis,1975; Stronach et al. 1986). Between pH values of 6.0-7.7, the buffering function of ammonia and the VFAs could be negligible.

In an anaerobic system with a substrate which has a rapid acidification rate, the accumulation of intermediate fatty acid products might reach a concentration that exceeds the system's buffering capacity (Mah 1969; Chynoweth and Mah 1977). Cheese whey is a substrate in which a majority of its contents are easily acidified. An instability in the pH of an anaerobic system of cheese whey was often observed. Although high VFA concentrations with low pH values are particularly detrimental to methanogenic activity through the toxic action of the unionized VFA (Andrews et al 1971; Kroeker 1979), a high cation concentration caused by neutralizing agents, added to restore the pH, may also inhibit methanogenic activity (McCarty 1964; Kugelman 1971). Therefore, neutralization by base addition is not the best way to control pH.

Recent studies, based on thermodynamic considerations and the hydrogen regulation function, have suggested that the accumulation of intermediate organic acids, during periods of overloading or other process stress is, due to hydrogen sensitivities moderated by syntrophic associations between hydrogen-producing bacteria (acidogens) and hydrogen consuming bacteria (methanogens, sulfate reducing bacteria or SRB and nitrate reducing bacteria or NRB). Propionate, lactate and ethanol are produced when the accumulation of hydrogen is beyond the collective assimilative capacity of these hydrogenotrophs including methanogens, SRB and NRB . Since methanogens cannot use those end products directly as substrate, their accumulation leads to a depression in pH.

Considering that the group of acid and methane forming bacteria each display different requirements with respect to both environmental factors and nutrients for optimal growth, the concept of phase separation was proposed (Babbitt and Baumann 1958; Andrews and Pearson 1965; Pohland and Ghosh 1971; Fan et al. 1973). However, it should be realized that an one-phase anaerobic process can not be regarded simply as the sum of the acidogenic and the methanogenic phases of the two-phase process (Cohen 1980) because of the role of interspecies hydrogen transfer which is an important interaction that occurs between non-methanogens and methanogens in an anaerobic environment (Wolin 1974; Ianotti et al 1973; Hungate 1967; Scheifinger et al 1975; Mah et al 1977; Zeikus 1977; Patel and Roth 1978; Winfrey et al 1977). It might be argued that the physical separation of methanogens and acidogens (two-phase system) can not enhance the anaerobic bioconversion rate for some wastes since the necessary interspecies hydrogen transfer function is disturbed

2.3.2 Nutrient Requirement

The anaerobic process is dependent on bacteria which require certain nutrients for growth. In addition to the fundamental requirements for macronutrients such as carbon and nitrogen, the inability of a great number of anaerobes to synthesise some essential vitamins or amino acids necessitates their supplementation with such micronutrients for growth and activity. Since municipal waste sludge normally contains a variety of these nutrients, it usually can provide an ideal environment for growth. However, industrial wastes are frequently more specific in composition, micronutrients must be added to optimize the process.

Our lack of knowledge of the nutritional requirements of the methanogens has hindered the development of the anaerobic process. One of the earliest studies of the nutritional needs of the anaerobic process was conducted by Speece and McCarty (1964). Since then, attention to nutritional requirements has been paid by only few researchers. Such research has indicated that the microbial regeneration time is a function of the nutrients present. The rate of substrate metabolism may also be limited by nutrient limitations. Specific substrate utilization rates can be increased several fold when all the required nutrients are in excess (Speece 1985).

Generally, nitrogen is the major nutrient, other than an energy source, for microbial systems. Speece and McCarty (1964) determined that the nitrogen requirements for the anaerobic digestion of fatty acids, carbohydrates and protein obeyed the following growth equations:

for Glucose and Starch

$$A = 0.46F - 0.088M \tag{2.1}$$

for Amino and Fatty Acids

$$A = 0.054F - 0.03M \tag{2.2}$$

for Nutrient Broth

$$A = 0.076F - 0.014M \tag{2.3}$$

Where

A=substrate synthesized into biomass

F=substrate metabolized by biomass

M=mass of biomass in system

The nitrogen requirements for all substrates were equal to A/9.4.

It was noted that microbial synthesis and, thus the nitrogen requirement, for carbohydrate digestion is about six times greater than for proteins and fatty acids (Speece 1985). This has a significant impact on some nitrogen-deficient biomass feedstocks. Therefore, anaerobic conversion of high carbohydrate content feedstocks to methane gas deserves special consideration because of the relatively high ratio of microbial synthesis to substrate consumption. Speece (1964) also found that two stages exist in the anaerobic digestion of complex substrates, one in which the BOD remains constant and another in which the BOD is reduced due to production of methane. In the case of carbohydrates the major nutrient requirements and major biological solids accumulation result from the first or constant BOD stage of digestion.

2.3.3 Sulfate Effect

Among the nutrient studies, the function of sulfate has received much less attention than other nutritional compounds. Therefore, the sulfur requirement for methanogens has not been extensively documented. However, many industrial wastes contain sulfate, and the role of sulfate and the sulfate-reducing bacteria (SRB) in anaerobic digestion cannot be underestimated. Species of the genera Desulfovibrio and Desulfotomaculum are routinely isolated from digestors.

The reason why this family of organisms must be considered in anaerobic digestion processes is shown by the examination of the similarity and complementary and competitive relationship between them and the other native groups of microorganisms, i.e.,

mainly acetogens and methanogens.

It is known that methanogenesis and sulfate reduction are the two main terminal processes in the complete anaerobic mineralization of organic matter. As all of the methane-producing bacteria (MPB) are known to be able to couple the oxidation of molecular H_2 with the reduction of its electron acceptor CO_2 to yield the electron sink product CH_4, all of the SBR are capable of coupling the oxidation of molecular H_2 with the reduction of its electron acceptor $SO_4^=$ to yield the electron sink product H_2S. The electron donor in either of these cases may be supplied as a dissolved gas from an exogenous source or through a syntrophic association with an obligate proton reducing or hydrogen-producing acetogen (OHPA). Certain VFAs are metabolized by the SRB to acetate, CO_2, and H_2 in the presence of a methanogen, thus switching roles in mutualistic pairing. This type of intimate association or mutualism, is an example of interspecies hydrogen transport and plays an important role in this fermentation (Postgate 1984).

In anaerobic digestion, sulfate reduction is usually considered undesirable not only because of the very toxic and corrosive gas, H_2S, produced from the reduction of sulfate, (therefore creating a problem with regarded its removal from the biogas) but also because of the inhibition of methanogenesis. This inhibition has been explained in terms of the level of sulfide produced and the resultant cytotoxicity. Very recently this inhibition has been subjected to reaction rate kinetics and thermodynamic analysis.

Kristjanson (1982) found that methane formation is generally absent in marine sediments when the sulfate concentration is high. He attributed the apparent inhibition of methane formation by sulfate to a variety of factors including hydrogen sulfide inhibition (Cappenberg, 1974), kinetic competition for substrate (Abram and Nedwell 1978 a,b; Bryant et al.1977; Mountfort 1980; Winfry and Zeikus 1977), and thermodynamic considerations (Zehnder 1978).

On theoretical grounds, the higher free energy change associated with sulfate reduction to H_2S as compared to CO_2 reduction to CH_4 cannot in principle explain the inhibition of sulfide to methanogenesis (McCarty 1972; Thauer et al. 1977). The free energy change of sulfate reduction to H_2S with H_2 is 151 KJ/mol sulfate whereas that of CO_2 reduction to CH_4 with H_2 is only 135 KJ/mol carbon dioxide. Therefore, the most likely mechanism is probably competition for substrates.

Inhibition caused by the activity of sulfate reducers has been attributed to competition for the common substrates, since in the presence of excess substrates both processes can take place (Mountfort et al. 1980; Oremland and Taylor 1978; Winfrey and Zeikus 1977).

The competition between sulfate reducers and methanogens in sediments or mixed cultures for H_2 and acetate has been investigated by several workers. Qualitatively, their data indicate that sulfate reducers effectively compete with methanogens for both substrates. The fact that the addition of sulfate inhibits methanogenesis in anaerobic digestion can be attributed to the finding that the coupled sulfate reducing reactions are thermodynamically more favorable than are the methanogenic reactions. Sulfate reducers have demonstrated a higher affinity for hydrogen than methanogens in marine sediments (Ormaland et al. 1978 and 1982, Martens et al.1974, Abram et al. 1978a, 1978b, Sorensen et al. 1981), freshwater sediments (Winfrey et al.1977, Strayer et al. 1978, Cappenberg 1974 and 1975) and in pure or mixed culture with methanogens (Kristjiansson et al. 1982). Schonheit et al.(1982) has shown that in the case of H_2 the Ks (affinity constant) value of a typical sulfate reducer was found to be about 5-fold lower than that of a methanogen isolated from a similar habitat. They also reported that the apparent Ks value of D.postgatei for acetate is lower than that of M. barkeri by a factor of 15. The explanation for the sulfate-reducing bacteria in general having a lower Ks for acetate than the methanogens might be the different mechanisms of acetate utilization. D.postgatei oxidizes acetate via the citric acid cycle (Thauer 1982) and Methanosarcina

cleaves acetate by a still unknown mechanism (Zehnder and Brock 1979). The most straightforward explanation as to why SRB has a lower Ks for H_2 might be the different topology of the common hydrogenase enzyme system. The hydrogenase reaction is a specific control mechanism in strict and facultative anaerobes which governs the flow of electrons. In the MPB, hydrogenase is free in the cytoplasm, while in the SRB it is located within the periplasmic space (Kristjiansson et. al 1983, Stronach et al 1986). Such a location presents less of an osmotic barrier. Another possibility is that thermodynamics indirectly puts a constraint on the kinetic parameters of the biological reactions involved. As mentioned previously, the free energy change of sulfate reduction is higher than that of CO_2 reduction. The Haldane equation (Fresht 1977) predicts that the Ks of an enzyme is partly determined by the free energy change associated with the particular reaction. Their experimental results show that CH_4 production and sulfate reduction are not mutually exclusive and in the presence of excess H_2 they have no effect on each other. When the H_2 supply becomes rate limiting, however, competition does take place. The methanogenic bacteria are not inhibited by the activity of the sulfate reducing bacteria but have a lower affinity for the common substrate which results in suppression. In other words, the difference in substrate affinities accounts for the inhibition of methanogenesis from H_2 and CO_2 in sulfate rich environments where the H_2 concentration is well below 5 M.

Complete conversion of organic waste is possible by SRB even with total methanogenic inhibition (Stephen, 1986). A comparison of the kinetics of hydrogen and acetate uptake by MPB and SRB (Table 2-1) suggests that higher organic waste conversion rates may be available through sulfate reduction than through methanogenesis. Moreover, SRB are not limited to one or two-carbon substrates, as are methanogens. This approach therefore may hold possibilities for reducing propionic acid and hydrogen in a stressed reactor, in order to reestablish equilibrium with the hydrogen removal system. However,

such an approach has major disadvantages, including the loss of energy available from methane and the production of sulfide.

Limited references in recent literature do reveal that the sulfur requirement is part of a complex picture. Observations have shown that a special relationship exists between the H_2 utilizing methanogens and the sulfate reducing bacteria. It has been suggested that the sulfate reducing bacteria might help to maintain the anaerobic conditions required for the growth of methanogens (Stephen 1985). In addition, the methanogens are dependent on the production of sulfide for growth.

Ronnow and Gunnersson (1981) reported that a thermophilic methanogenic bacterium has a specific sulfide requirement for methane production and growth. Scherer and Sahm (1981) stated that optimal growth of M. barkeri occurred on a defined medium containing methanol when 2.5 to 4.0 mM sodium sulfide was added. They also reported that iron sulfide, zinc sulfide or L-methionine could also act as sulfur sources. However, the addition of sodium sulfide to a sulfide depleted media failed to restore growth. Mountfort and Asher (1979) found that a part of the sulfide requirement for growth is used as a precursor of HS-CoM which is 40% sulfide by weight. According to Ronnow and Gunnarsson's studies, 2.6% of the cell mass of M. thermoautotrophicum is sulfur. At sulfide levels below 0.1 mM, growth of M. thermoautotrophicum was poor and the methane production rate decreased. After injection of sulfide, a large increase in methane production was noted within 30 minutes. They noticed a linear relation between growth, methane production and sulfide concentration. They also noted that sulfate or thiosulfate could not replace the sulfide requirement.

In subsequent work Ronnow and Gunnarsson (1982) noted that when sulfide was added in increments of 20 mg/l, the methane production rate increased until sulfide was completely depleted in the medium (as determined by sulfide analysis) and that the methane production rate then decreased. This observation provides further indication of a

Table 2.1: **Kinetic Characteristics of Sulfate Reducers and Methanogens**

Reference	Culture	Substrate	μ_{max} (day^{-1})	Ks (mg COD/l)	Y (g VSS/g COD)
Ghosh	methane phase	acetate	0.49	4200	
Massey	methane phase	acidified glucose	0.43	395	
Smith	Methanosarcina barkeri	acetate	0.60	320	0.04
Zhender	Methanothrix soehngenii	acetate	0.11	30	0.03
Huser	Methanothrix soehngenii	acetate	0.16	45	0.02
Hungate	Rumen bacteria	H_2/CO_2	5.4	$2*10^{-4}$	
Shea	digesting sludge	H_2/CO_2	1.1	0.75atm	
Badziong	Desulfovibrio vulgaris	$H_2/SO_4^=$	3.6		0.09
Badziong	Desulfovibrio vulgaris	$H_2/SO_4^=$	5.0		0.15
Lovley	Lake sediment	$H_2/SO_4^=$		0.001atm	
Middleton	digester sludge	acetate/$SO_4^=$	8.3		0.05
Liu	Desulfovibrio vulgaris	lactate/$SO_4^=$			0.12
Schonheit	Desulfovibrio postgatei	acetate/$SO_4^=$		13	
Liu	Desulfovibrio oreintis	lactate/$SO_4^=$			0.04

sulfur compound requirement for high-rate methane formation rates. They further stated that although methanogenic bacteria contain large amounts of coenzyme M, between 0.3 to 16 $\mu mole/g$ dry weight (Balch and Wolfe 1979), coenzyme M cannot account for a large part of the sulfur absorbed. A large pool of sulfur compounds, possibly low molecular weight compounds, still remains to be discovered. Methionine and cysteine obviously account for a large part of the sulfur. Zehnder (1980) noted the effects of various sulfide concentrations on the growth and specific methane production rate of M. arboriphilus at pH 7.0. Optimal growth and specific rate of methane production required the presence of between 10^{-6} and 10^{-3}M sulfide. A sulfur source of about 0.85 mM was found to be essential for degradation of cellulose to methane (Khan and Trottier 1978). At 9 mM, all inorganic sulfur compounds other than sulfate inhibited both cellulose degradation and methane formation. The inhibition increased in the following order: thiosulfate $\ll$ sulfite $\ll$ sulfide $\ll H_2$. Sulfide was found to act as a sulfur source rather than a reducing agent (Wellinger and Wuhrman 1977). The optimum sulfide concentration was found to be 10^{-4}M, which coincides with the sulfide concentration in the rumen. In the absence of sulfide, cysteine was the only compound which stimulated methanogenesis.According to Speece's results, the total sulfide requirement for optimum anaerobic digestion is essentially equivalent to the nitrogen requirement if a CSTR is used.

Besides the nutritional effect, other beneficial effects of sulfate on anaerobic digestion include the prevention of the biotoxicity of heavy metals through precipitation. Also, there is a report in the literature that sulfate promotes the biodegradation of the normally recalcitrant celluloses (Khan A.W. and Trottier 1978) through a shift in interspecies hydrogen transport.

It should be mentioned that the stimulation effect of sulfate on methanogenesis has not been studied. Since anaerobic β oxidation of long chain fatty acids is considered to be the rate-limiting step for the fermentation of soluble substrates, there are reasons to

suggest that the rate of this digestion can be enhanced through interspecies hydrogen transport caused by the presence of sulfate at a concentration below that which may be inhibitory to methanogenesis

Finally, an interesting new technology has been developed that will permit an anaerobic digestor to be able to process higher sulfate wastes effectively while simultaneously enriching the off-gases in methane by passing them through a secondary, smaller anaerobic photobioreactor (Cork et al. 1982, Cork 1985, Maka and Cork 1988). In this process, driven by light photons, CO_2 is converted to biomass, H_2S is oxidized to economically valuable elemental sulfur, and CH_4 is free to pass through.

2.4 MICROBIOLOGY OF ANAEROBIC DIGESTION

2.4.1 Anaerobic Microorganisms

The anaerobic biological conversion of organic wastes to methane is a complex process involving directly and indirectly a number of microbial populations linked by their individual substrates and product specificities. Nine recognizable steps, each mediated by a specific group of microorganisms, can be identified (Stephen et al.1986). In general, four steps are considered to be essential (Figure 2.1). They are hydrolysis of carbohydrates, proteins and fat, fermentation of sugar and amino acids, β-oxidation of intermediate and long chain fatty acids, and methane formation from acetate CO_2 and H_2. For soluble substrates the first step can be neglected.

The bacteria which are responsible for the fermentation of pyruvic acid to a mixture of acetic, propionic and butyric acids are the acid forming bacteria. They are fast-growing bacteria (minimum doubling time is around 30 mins) (Mosey 1983). The reactions involved in this step are:

$$CH_3COCOOH + H_2O = CH_3COOH + CO_2 + H_2 \quad (2.4)$$

$$CH_3COCOOH + 2H_2O = CH_3CH_2COOH + CO_2 \quad (2.5)$$

$$6CH_3COCOOH + 4H_2O = 4CH_3CH_2CH_2COOH + 2CO_2 \quad (2.6)$$

The preferable reaction is the first one, the conversion of pyruvate to acetic acid. The other reactions, the fermentation of butyric and propionic acid are carried out by the bacteria response for the accumulations of hydrogen during surge loads.

The obligate hydrogen-producing acetogenic bacteria (OHPA) are those that convert propionic and butyric acids into acetate according to the equations:

$$CH_3CH_2COOH + 2H_2O = CH_3COOH + CO_2 + H_2 \quad (2.7)$$

$$CH_3CH_2CH_2COOH + 2H_2O = 2CH_3COOH + 2H_2 \quad (2.8)$$

These bacteria grow relatively slowly, even under optimum conditions of low concentration of dissolved hydrogen, with a minimum doubling time of from 1.5-4.0 days. Table 2.2 shows that these reactions are energetically very difficult and can easily be stopped by the accumulation of hydrogen. They can catabolize those substrates into acetate only when the hydrogen pressure is at an extremely low level. In other words, when they are placed in syntrophic association with H_2-utilizing organisms, such as methanogens and desulfovibrio, the reactions become energetically favorable enough to proceed (Table 2.2).

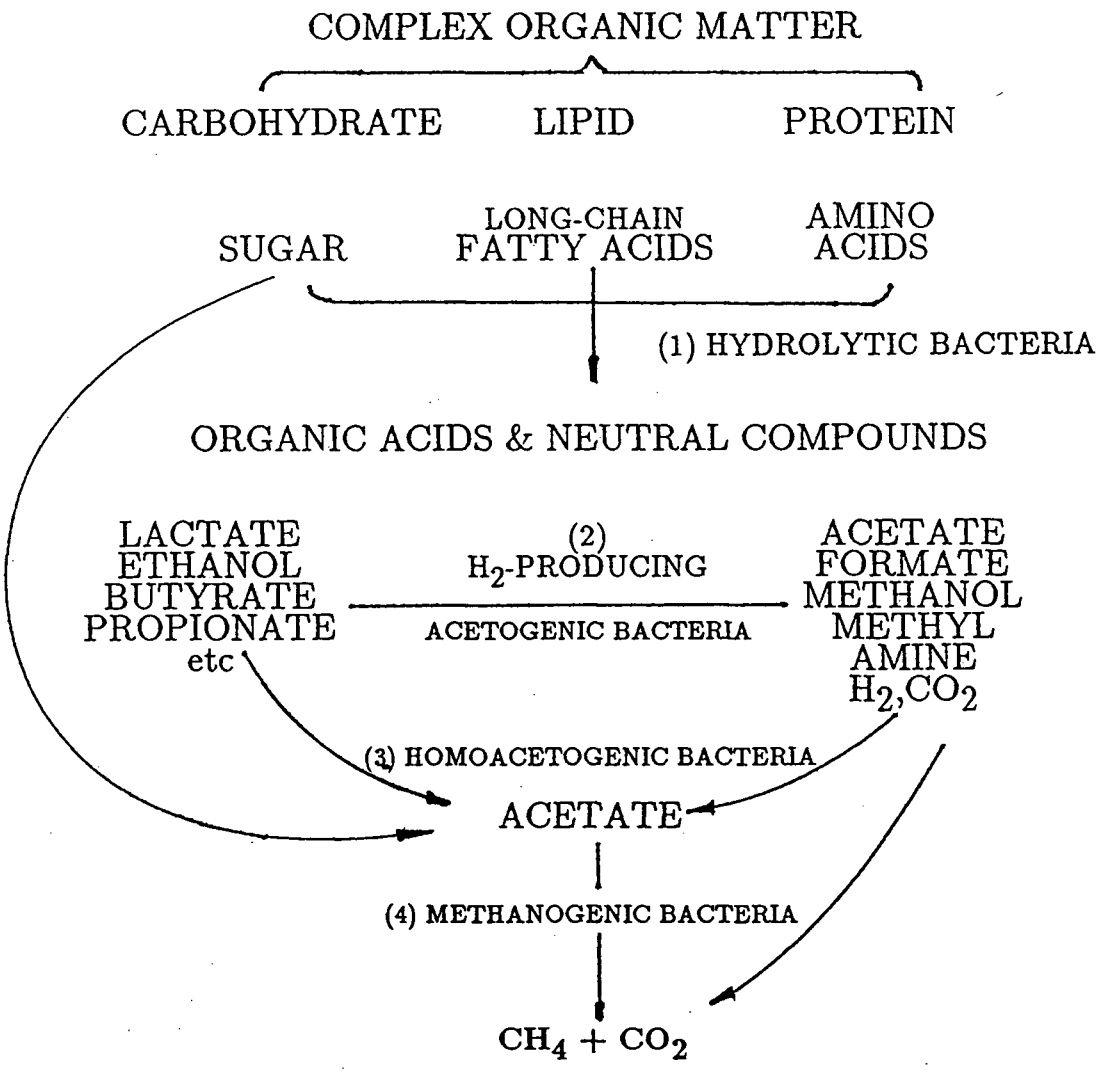


Figure 2.1: Metabolic Distinction of the Microbial Population in Anaerobic Digestion

Table 2.2: **Stoichiometry and Change in Free Energy of Reaction**

REACTIONS	ΔG^{o}(Kcal)
A. Single culture of H_2-producing acetogenic bacteria :	
$CH_3CH_2COO^- + 3H_2O$ ----> $CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+18.2
$CH_3CH_2CH_2COO^- + 2H_2O$ ----> $2CH_3COO^- + H^+ + 2H_2$	+11.5
$2CH_3CHOHCOO^- + 4H_2O$ ----> $2CH_3COO^- + 2HCO_3^- + 2H^+ + 4H_2$	-1.9
$CH_3CH_2OH + H_2O$ ----> $CH_3COO^- + H^+ + 2H_2$	+2.3
B. H2-utilizing methanogens and desulfovibrios :	
$4H_2 + HCO_3^- + H^+$ ----> $2CH_4 + 3H_2O$	-32.4
C. Syntrophic association of coculture (A+B):	
$4CH_3CH_2COO^- + 3H_2O$ --->$4CH_3COO^- + HCO_3^- + H^+ + 3CH_4$	-24.4
$2CH_3CH_2CH_2COO^- + HCO_3 + H_2O$--->$4CH_3COO^- + CH_4 + H^+$	-9.4
$2CH_3CHOHCOO^- + H_2O$--->$2CH_3COO^- + HCO_3^- + H^+ + CH_4$	-34.3
$2CH_3CH_2OH + HCO_3^-$--->$2CH_3COO^- + CH_4 + H_2O + H^+$	-27.3

There is another group of bacteria, the homoacetogenic bacteria, which can ferment a very wide spectrum of multi or one carbon compounds (e.g. sugars, acids, CO_2, CO, H_2, etc) to acetic acid (Yang, 1984). As a consequence of consuming hydrogen rather than producing it, homoacetogens may lower the hydrogen partial pressure during anaerobic digestion. However, in a normal anaerobic digestion system, methanogens appear to successfully out-compete homoacetogenic bacteria for hydrogen since H_2 is mainly used for reducing CO_2 to CH_4. So far, little is known about the functional importance of homoacetogenic metabolism in anaerobic digestion or the metabolic interaction of homoacetogens and methanogens (Zeikus 1981).

Acetate, CO_2 and H_2 are catabolized to the terminal products by methanogens (Figure 2.1). The methanogens are a unique but diverse group of organisms. Methanogenic bacteria isolated to date are limited to catabolism of one- and two carbon compounds (Table 2.3). Most of them utilize hydrogen and one-carbon compounds such as carbon monoxide, carbon dioxide, and formate as substrates for methane production. There are two known genera of methanogens, Methanosarcina and Methanothrix, which can utilize the two-carbon compound, acetate. Methanosarcina, which can utilize H_2/CO_2 as well as acetate and other one-carbon compounds, are classified as hydrogen-oxidizing-acetotrophs (HOA). Since methanothrix are unable to use hydrogen in combination with carbon dioxide, they are classified as non-hydrogen-oxidizing acetotrophs (NHOA). Those which can utilize H_2/CO_2 and other one- carbon compounds, but do not cleave acetate are named hydrogen-oxidizing methanogens (HOM).

Acetoclastic methanogens convert acetic acid into end productions: carbon dioxide and methane according to the reaction

$$CH_3COOH = CH_4 + CO_2 \tag{2.9}$$

The conversion of acetate accounts for 60% to 75% of the methane formed and is of critical importance in maintaining a relatively stable fermentation and in determining the maximum volatile solids loading rate at a given hydraulic retention time. However, up to date, only few microbes have been found which can produce CH_4 from acetic acid so far. Therefore they most likely do not play a major role in acetate utilization in methanogenic fermentations. Perhaps, since they are difficult to isolate due to a close symbiotic relationship with other bacteria, they grow slowly (minimum doubling time of 2-3 days), and they are extremely sensitive to oxygen, the acetate utilizating methanogenic bacteria of major important have not yet been isolated.

The hydrogen-utilizing bacteria (hydrogen-oxidation methanogens) are responsible for removing almost all of the hydrogen from the system. They grow quite quickly with minimum doubling times of around 6 hours and control the redox potential of the digestion process. The traces of hydrogen that they leave behind are believed to regulate both the total rates of acid production and acid oxidation. They are the autopilot of the anaerobic digestion process.

2.4.2 Microbial Interaction in Anaerobic Digestion

A microbial ecological population distribution is determined when a fragile compromise is forged among the various species present and their environment; and it is this compromise, involving synergistic and antagonistic interrelationships, that makes anaerobic digestion both possible and limited.

It has been accepted that sugar is fermented mainly via the Embden-Meyerhof-Parnas pathway to pyruvate, which is then further catabolized to fatty acids, alcohols, hydrogen and CO_2. NADH generated in glycosis must be reoxidized to NAD before the degradation

Table 2.3: **Methanogenic Substrates and Reactions**

Substrate	Reaction	ΔG^o(Kcal)
1. H_2-CO_2:	$H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	- 32.4
2. Formate:	$HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^-$	- 31.2
3. Acetate:	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-7.4
4. Methanol:	$4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H^+ + H_2O$	-75.2
5. Methyl amines:	$4CH_3NH_3^+ + 3H_2O \rightarrow 3CH_4 + HCO_3^- + 4NH_4^+ + H^+$	-53.8
	$2(CH_3)_2NH_2^+ + 3H_2O \rightarrow 3CH_4 + HCO_3^- + 2NH_4^+ + H^+$	-52.5
	$4(CH_3)_3NH^+ + 9H_2O \rightarrow 9CH_4 + 3HCO_3^- + 4NH_4^+ + 3H^+$	-159.8
6. CO:	$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	-43.1

of organic matter can continue (Figure 2.2).

In single-culture system, electrons from pyruvate are used for the production of ethanol, and H_2 is not a significant product. Regeneration of NAD for glycolysis is dependent on ethanol formation. This is due to the fact that the evolution of H_2 from reduced nicotinamide adenine dinucleoticide (NADH) is thermodynamically unfavorable under standard conditions:

$$NADH + H^+ = H_2 + NAD^+ \tag{2.10}$$

$$\triangle G^o = +4.3 \text{ Kcals}$$

When the H_2-utilizing methanogens are cocultured with fermentative bacteria, the metabolic activities of the latter are shifted to generate more oxidized end products, e.g. acetate. Wolin (1976) illustrated this phenomenon by a hypothetical, but not unrealistic, fermentation pathway for the formation of ethanol, acetate, CO_2 and H_2 from glucose (Table 2.4). When the partial pressure of H_2 is reduced, the free energy change becomes increasingly negative. Therefore, with methanogens, NAD is regenerated by formation of H_2, which is removed by methanogens. Thus electrons from NADH are used for H_2 formation and methanogenesis in the mixed system rather than for ethanol formation. Through the methanogenic H_2 removal, the cocultures not only alter the fermentation, but also provide an additional mole of ATP per mole of glucose fermented (Table 2.4). The effect of the interspecies H_2-transport reaction on fermentation has been demonstrated by several experiments (Zeikus 1982; Wolin 1974; Chung 1976; Weimer 1977)

The H_2-producing acetogenic bacteria catabolize the products of the hydrolysis stage, mainly propionate, butyrate, long-chain fatty acids, alcohols, and probably aromatic and other organic acids to acetate, H_2 and CO_2. However, these organisms cannot catabolize

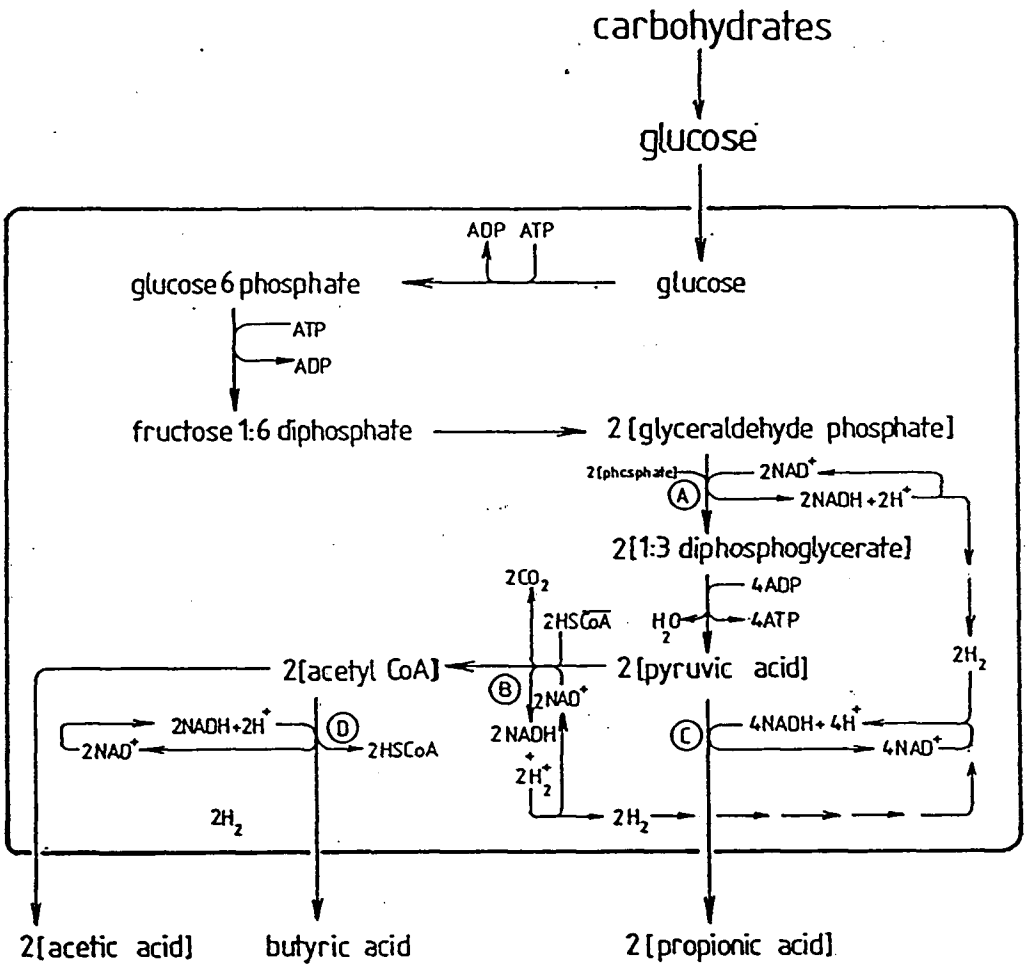


Figure 2.2: **Metabolic Pathway Inside Acid-forming Bacteria**

Table 2.4: **Partial Reaction for a Hypothetical Glucose Fermentation Without and With a Methanogen**

Glucose + 2 ATP ---> 2 Glyceraldehyde-3-p +2 ADP

Glyceraldehyde-3-p + 2 NAD^+ +4 ADP + 2 pi --->
2 Pyruvate + 2 NADH + 4 ATP + 2 H^+

2 Pyruvate + 2 CoASH ---> 2 Acetyl-SCoA + 2 H_2 + 2 CO_2

A. Glucose-fermenting organism alone:

Acetyl-SCoA + pi + ADP ---> Acetate +CoASH + ATP

Acetyl-SCoA + NADH + H^+ ---> Acetyldehyde + NAD^+ + CoASH

Acetyldehyde + NADH + H^+ ---> Ethanol + NAD^+

Sum:

Glucose + 3 ADP + 3 pi --->
Ethanol + Acetate + 2 H_2 + 2 CO_2 + 3 ATP

B. Glucose-fermenting organic plus methanogen :

2 Acetyl-SCoA + 2 pi + 2 ADP --->
2 Acetate + 2 CoASH + 3 ATP

2 NADH + 2 H^+ ---> 2 NAD^+ + 2 H_2

4 H_2 + CO_2 ---> CH_4 + 2 H_2O

sum: Glucose + 4 ADP + 4 pi ---> 2 Acetate + CH_4 + CO_2 + 4 ATP

these substrates to acetate when H_2 in the environment is not at an extremely low level. As is illustrated in Table 2.2 , only when acetogenic bacteria are placed in syntrophic association with H_2-utilizing organisms, such as methanogens and desulfovibrio, do the reactions become energetically favorable to proceed. (McInerney 1979).

Although microbial interactions among diverse trophic groups of microbes in methane fermentation are not clear, methanogens have been considered as effective bio-regulators of anaerobic digestion through proton and electron exchange. By removing the metabolite of the acid-formers, acetate, methanogens provide a constantly favorable environment for the fermentative bacteria. Otherwise most of them would be inhibited by their own metabolite. Also, electron transfer (or H_2 transfer) between methanogens and H_2-producers creates favorable conditions for metabolism of certain metabolites. The fermentation pathway of fermentative bacteria may be altered to gain more energy.

On the other hand, the accumulation of intermediate organics, which are metabolites of acid-formers and have a detrimental effect on methanogenesis during periods of overloading or other process stress, is due to hydrogen sensitivity moderated by syntrophic associations between hydrogen-producing bacteria (acidogens) and hydrogen-consuming bacteria (methanogens, SRB, NRB). The physical separation of methanogenesis and acidogenesis (two-phase) in an attempt to optimize growth conditions for each group of bacteria theoretically cannot enhance anaerobic bioconversion rates since the necessary interspecies hydrogen transfer function is disturbed.

2.4.3 Hydrogen Regulations

General Perspective

Hydrogen in relatively low concentration appears to regulate the overall conversion process by throttling the acidogenic reactions at several points in the glucolytic pathway. Accumulation of hydrogen obviously needs an alternate method of electron disposal for NAD regeneration. This need drives the fermentation of pyruvate to propionate, lactate and ethanol and/or the fermentation of acetyl-CoA to butyrate. Since the methanogens cannot use these end products as substrates directly, their accumulation leads to a problematic depression in pH.

The subsequent degradation of these acids to acetate is also hydrogen dependent and is mediated by obligate hydrogen producing acetogens (OHPA) linked to the various hydrogen oxidizers. In addition to their hydrogen sensitivities, the number of OHPA and associated HOM (hydrogen oxidation methanogens) bacteria existing in an anaerobic process may vary, depending on its stability history. Under a process upset, the OHPA-HOM must increase to accommodate the accumulated hydrogen and propionate. If a long period of stable operation ensues, the relative proportion of propionate may decrease, and the number of OHPA will decrease accordingly. This dynamic balance is considered central to the overall stability and efficiency of many anaerobic processes and is regarded as the key to stabilizing and improving anaerobic treatments.

In single-phase processes, the proportion of OHPA and methanogenic bacteria responds to the relatively uncontrolled production of acidogenic products, which may alternately reach inhibitory and substrate-limiting proportions. A number of researchers have also demonstrated that the environmental requirements of acidogenic and methanogenic enrichments are quite different from each other as reflected in terms of pH and ORP (Hammer et al. 1969, Dirasian 1963, Niktin 1968, Blanc 1973). The two-phase system

was originally proposed to address these issues. However, the role of hydrogen was not emphasized as the primary control factor in earlier investigations. Although the two-phase process seems best suited for the treatment of soluble-type wastewaters, which present a high potential for volatile acids accumulation,(Ghosh et al. 1981, 1983a) superior performance has also been demonstrated for particulate-type substrates such as sewage sludge and agricultural wastes. (Ghosh 1983b, Keenan 1976, Normann et al. 1977). However, these studies failed to show definitive reasons for process improvements.

Thermodynamic Quantification of Hydrogen Effects

In view of the reported differences in hydrogen effects, and the traditional difficulties in adequately defining the redox nature of the wastewater substrate, at hydrogen levels of 10^{-6} to 10^{-10} atm, the regulatory effects of hydrogen on OHPA and HOM have been illustrated using thermodynamic models and equilibrium assumptions by several authors (McCarty 1971,1981, McInerney 1981, Gujer 1983). Thermodynamic calculations associated with those half reactions during anaerobic stabilization of organic wastes to methane indicated that propionic acid oxidation to acetate becomes favorable only at a hydrogen partial pressure below 10^{-4} atm, while butyric acid oxidation becomes favorable at or below 10^{-3} atm (Figure 2.3). Figure 2.3 provides insight into the product formation patterns to be expected in a two-phase process. Evident from Figure 2.3 is the preference of sulfate reduction over bicarbonate respiration at all hydrogen partial pressures and the preference of acetate cleavage by SRB over methanogens.

The Role of Hydrogen in Regulation of Methanogenesis

Standard free energy levels associated with the methanation reaction, listed in Table 2.3, indicate that the favorability of acetate cleavage is an order of magnitude lower than H_2/CO_2 and methanol conversions (at standard state of 1 atm of H_2 in gaseous phase). However, this high level of hydrogen is uncommon in well-operating anaerobic wastewater treatment systems. At more realistic hydrogen concentrations, acetate cleavage competes more favorably with the methanogenic respiration of bicarbonate, as shown in Figure 2.3

In addition, hydrogen regulation in an anaerobic reactor is a result of the integrated effects of the specific capabilities of OHPA, HOA, NOHA, and HOM, and may be dependent on the cultures selected by seeding, substrates and operational procedures. For example, species of Methanosarcina (HOA) which utilize both acetate and H_2/CO_2 as substrates may be subject to catabolic repression of acetate cleavage by low levels of hydrogen. However, other investigators have reported a complete and rapid inhibition of acetate cleavage in the presence of H_2/CO_2 (Baresi et al.). H_2/CO_2 is preferentially utilized over acetate in mixtures of these substrates by Methanosarcona species to the extent that acetate cleavage is inhibited until H_2 is exhausted (McInerney 1981, Ferguson 1983, Mah 1978)

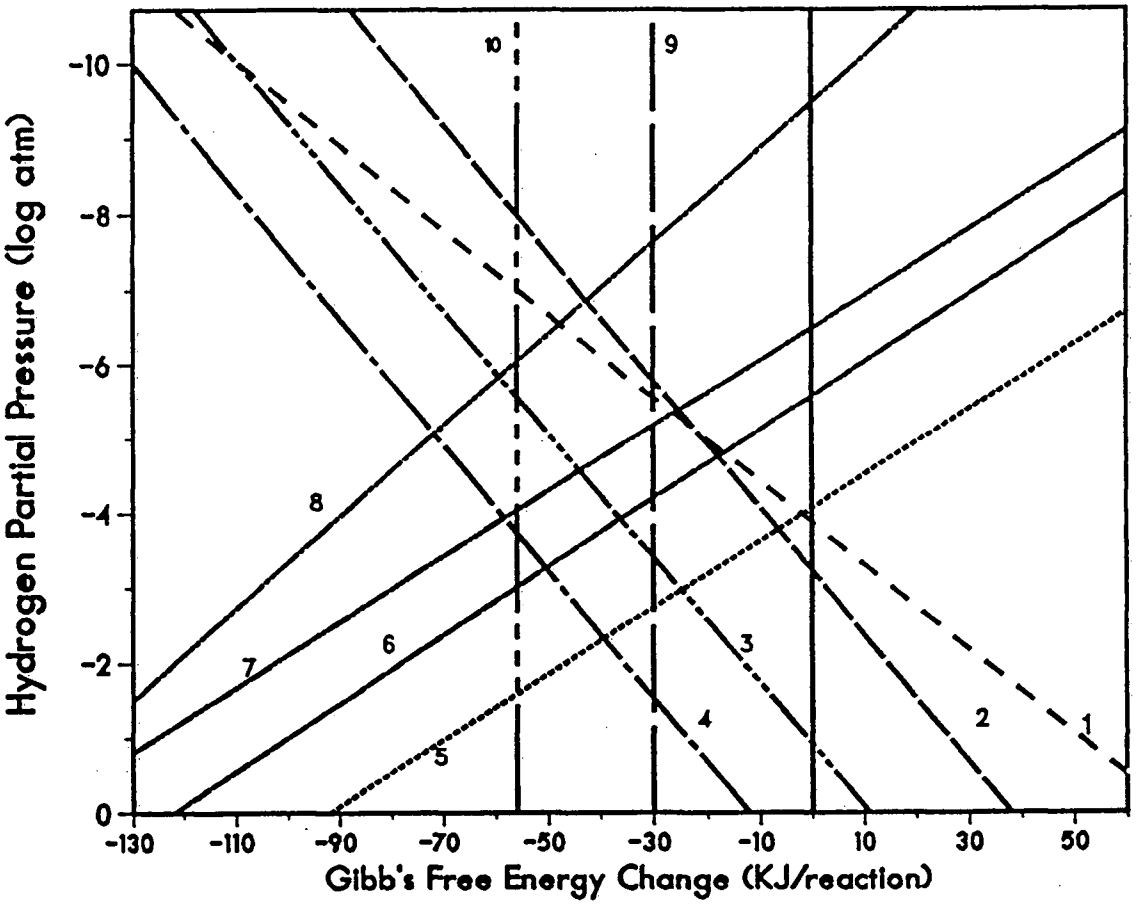


Figure 2.3: **Graphical Representation of the Hydrogen-dependent Thermodynamic Favorability of Acetogenic Oxidations and Inorganic Respirations Associated with the Anaerobic Degradation of Waste Organics:** 1 Propionic acid oxidation to acetic acid, 2 Butyric acid oxidation to acetic acid, 3 Ethanol to acetic acid, 4 Lactic acid to acetic acid, 5 Acetogenic respiration of bicarbonate (CO_2), 6 Methanogenic respiration of bicarbonate, 7 Respiration of sulfate to sulfite, 8 Respiration of sulfite to sulfide, 9 Methanogenic cleavage of acetic acid, 10 SRB-mediated cleavage of acetic acid (from Harper, 1986)

Chapter 3

RESEARCH OBJECTIVES

A considerable amount of research has been directed over the years at using different reactor configurations to establish the development of an industrial anaerobic digestor for cheese whey. However, most of the previous studies were feasibility assessments of the process and were generally preliminary in nature and incomplete. Unsuccessful fermentation experiments have often been reported apparently due to the high organic concentration of cheese whey and its tendency toward rapid acidification. pH quickly droped and gas production and COD reduction decreased. However, surprisingly little work has been done so far to explain the reason for this system instability. In addition, although the high organic strength of cheese whey can supply a useful source of energy for cheese manufacturers by means of the anaerobic fermentation, industrial applications have been very limited due to the unreliability of the process.

It is therefore necessary to carry out a study to increase the fundamental understanding of the process, to find an explanation for its instability, to efficiently control such instability and to optimize the process.

The special objectives include:

(1) To assess the technical feasibility of treating cheese whey in a UASB reactor, and to determine the effects of start-up procedures on reactor performance and treatment efficiency.

(2)To determine the effects of influent concentration, HRT and OLR on the treatment efficiency, and to determine the inhibitory effects of influent concentration and organic

loading rate.

Previous studies have shown that, when influent concentration was increased to a certain level, the system became unstable. This was attributed to an upset in the physiological balance between the methane-producers and hydrogen-and acid-producers because of the great difference in the rates of acidogenesis and methanogenesis. This research will explore the difference in the rates between these two stages, the threshold level of the influent concentration and will try to obtain the optimal influent concentration for the particular system.

(3)To assess the effect of sulfate addition on the buffering capacity and stability of the anaerobic system, and to investigate the mechanisms of inhibition.

As has been discussed in earlier sections, knowing the importance of the nutritional requirements of methanogens is important to the overall knowledge of an anaerobic processes. Among these required nutrients, sulfur should be given more attention for special substrates such as whey. A limited number of literature references, which were cited in section 2 of the literature review, revealed that a sulfur requirement is part of a complex picture. On one hand, sulfate reducing bacteria help to maintain the anaerobic conditions required for the growth of methanogens, while on the other hand the addition of sulfate inhibits methanogenesis because sulfate reducers have demonstrated a higher affinity for hydrogen than methanogens. Methanogens and sulfate reducers are not mutually exclusive. In the presence of excess hydrogen, they have no effect on each other. When the hydrogen supply becomes rate limiting, competition does take place.

From the point of view of thermodynamics and hydrogen regulation, the hydrogenotrophic association would help to maintain the reaction condition favorable for methanogenesis. It is expected that the results of this research will yield the proper sulfate concentration needed to moderate the detrimental influences of excess hydrogen on a stressed anaerobic reactor.

Obviously, the practice of sulfate addition holds numerous potential challenges, including the control of the inhibitory and corrosive hydrogen sulfide gas. However, such a study might yield valuable information on the utility of manipulating hydrogen to control acidogenesis and methanogenesis. Moreover, a number of industrial wastewaters contain significant levels of sulfates. The successful anaerobic treatment of these wastewaters requires an understanding of the competition for methanogenic precursors by SRB as well as of the associated microbiology and substrate conversion kinetics.

(4)To explore maximum treatment efficiency by using optimal control parameters based on all the above studies .

Chapter 4

EXPERIMENTAL METHOD

4.1 REACTOR SET-UP

The flow sheet of the UASB system is presented in Figure 4.1. The reactor was made of acrylic pipe with an inner diameter of 11.5 cm (4.5 in.) and a height of 168 cm (60 in.). The total volume and working volume of the reactor were 17.5 and 14.3 liters, respectively. A series of sampling ports were fitted at intervals on one side of the reactor to permit sampling for the analysis of the reactor contents. The reactor was operated in an upflow, continuous mode.

A distinguishing feature of this UASB reactor design is a conical three-phase separator located at the top of the reactor. The three phases refer to gas, liquid and solid (sludge). The basic principle of separation by the separator is explained as follows. Gas bubbles produced by the bacteria rise to the top of the reactor where they are first separated from the liquid and collected in the gas chamber between the column and the top cone. The biogas passes through a water seal column outside the reactor, which is used to regulate the liquid level in the separator, then goes to a wet gasmeter for measurement. The lower baffle, a small cone suspended below the larger,top cone is used to minimize the possibility of gas entering the separator by blocking the uprising gas bubbles from the open tip of the larger cone.

The separation of sludge is accomplished by fluid dynamics. The flocs of sludge with poorer settleability rise through buoyancy provided by rising gas bubbles, and travel with

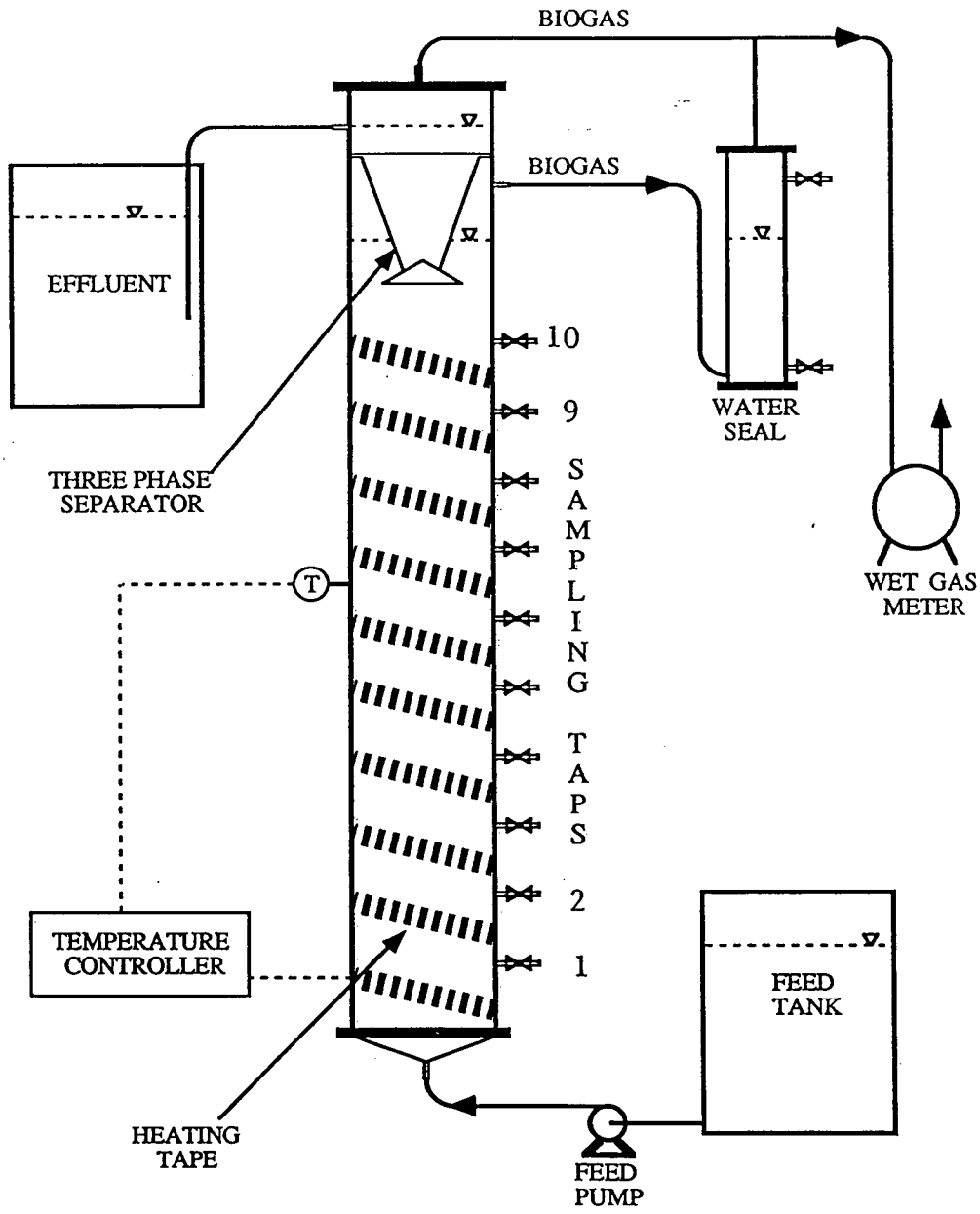


Figure 4.1: Schematic Diagram of UASB System

the liquid into the upper cone. The upflow velocity of the fluid decreases as the cross sectional area increases. When the upflow velocity of fluid decreases to a value equal to downflow velocity of the sludge, the rising particles slow down, drop and return back to the reactor. This design was efficient in retaining a large percentage of the bacteria in the reactor.

The whey, stored in a refrigerator at 4°C, was introduced continuously into the bottom of the reactor by a peristaltic pump through a copper-alloy coil immersed in a water bath to bring the feed temperature to about 34° C. The effluent left the reactor from the top of the settlling chamber and was collected in a plastic container.

Fermentation temperature was maintained at $33 \pm 1°$ C using a feedback controller and a number of external electric heater pads wrapped around the reactor. In this set-up, there was no pH control system.

4.2 FEED SUBSTRATE

Cheddar cheese whey used in this study was obtained from the Fraser Valley Milk Producers Association's cheese producing plant at Abbotsford, British Columbia, Canada. Typical whey composition is presented in Table 4-1.

The manufacture of cheese from either whole or skim milk produces, in addition to cheese itself, a greenish-yellow fluid known as whey. Whole milk is used to produce natural or processed cheeses such as cheddar, and the resulting whey has a pH in the range of 4.5 to 6. The lower pH is the result of the acid developed during coagulation.

As indicated in Table 4.1, although the basic nutritional requirement of nitrogen in whey for bacterial growth seems to be adequate according to the criteria of COD:N:P being 100:5:1, the extremely low ammonium nitrogen is perhaps the main cause of the

Table 4.1: **Characteristics of Chedder Cheese Whey**

Total solid (TS)	5.66-5.88 %
Volatile solid (VS)	4.52-4.70 %
Total Chemical Oxygen Demand (TCOD)	64-67 g/l
Soluble Chemical Oxygen Demand (SCOD)	62-65.5 g/l
SCOD as percent of TCOD	96-97 %
Biological Oxygen Demand (BOD)	60-62 g/l
BOD as percent of TCOD	95-96 %
Total Kjeldahl nitrogen (TKN)	2.75-3.05 g/l
Ammonium Nitrogen	2.8-3.0 mg/l
Nitrate	0.45-0.70 mg/l
Total Phosphorus	0.34-0.37 g/l
pH	4-5.5

lower buffering capacity of whey. In addition, a shortage of phosphorus is apparent.

Two characteristics of raw whey are its unusually high SCOD to TCOD ratio and extremely low pH. These can create difficulties during anaerobic processing.

Fresh cheese whey was contained in 5 50-liter tanks and transported from the cheese plant to the large freezer of the bio-resource lab at UBC once every 3 months, and stored there at -30° C. About one week before it was needed, a portion of the frozen whey was moved into a cold room at 4° C for defrosting. Then it was prepared to the desired COD concentration by mixing with cool tap water, adjusted to a pH of about 7 by using 5% NaOH, and transferred to the small scale feed tank, where it was kept refrigerated at 4° C and ready for use.

4.3 SEED SLUDGE

The seed sludge was originally obtained from the effluent of an anaerobic rotating biological reactor (AnRBC) in the laboratory and had been stored in a plastic container in a cool room at 4° C for several months. The bacterial concentration in the effluent from the AnRBC was very low, even in the dense portion in the bottom. To obtain the necessary concentration and activity of seed sludge to ensure a successful start-up of a UASB reactor, it had to be incubated. First, the dense portion of the effluent in the container bottom was moved to a glass jar at a room temperature of 22° C. The sludge was acclimated using 200 ml of raw cheese whey daily for more than 30 days before it was put into the reactor. At the time of seeding, the sludge concentration was 3% TS and 2.1% VS, respectively. Four liters of this effluent were used as seed .

4.4 EXPERIMENT DESIGN

It was the author's intention to attempt to achieve a high treatment efficiency initially by using a UASB reactor without pH-control or nutritional addition, and to optimize the process through experimental investigations.

This research was carried out in 18 months in 4 steps, in which a variety of influent concentrations and HRTs were used as shown in Table 4.2. For each step, the reactor was started using 4 liters of the seed sludge and 6 liters of diluted cheese whey of 5 g COD /l. The remaining 7 liters (the digestor capacity) were filled by continuously adding whey at the rate of 2.5 liters/day.

The experiment was started with a very dilute influent concentration of about 5 g COD/l in order to observe the effect of a wide range of substrate strengths on treatment efficiency and gas production. After 40 days of operation, the reactor was stabilized at a constant gas production and effluent properties, including COD, VFA, TSS and pH. Then the influent concentration was increased stepwise from approximately 10 to 40 g COD/l at a constant HRT of 5 days during the preliminary assessment of technical feasibility. The stepwise increase of feed strength not only allowed the microbes to gradually acclimate themselves to the new environment with a minimum negative impact, but also permitted a full view of the performance profile over the entire range of the operational settings with the objective of locating the optimum performance conditions.

The tests on the effects of sulfate ions were first carried out in batch experiments to get a basic sense of sulfate behavior in the anaerobic process. A 3-factor experimental design was used in the batch experiment. The details are given in Appendix A. On the basis of the results of the batch experiments, continuous flow experiments were designed to gain more accurate information about the sulfate effects. Two reactors were operated simultaneously at the same temperature with the same seed, but with different operating

Table 4.2: **Experimental Design**

Experiment	Parameters		
	HRT (days)	Inf.Conc. (g COD/l)	Sulfate (g/l)
Start-up	5	5-9.9	0
Effect of Inf.Conc.	5	5-38	0
Effect of HRT	24-5	20 40	0 0
Effect of Sulfate	5	15-50	0.2 0.3 0.5
Optimal Operation	7.5 3.8 2.0	26-32	0.2 0.2 0.2

conditions.

Finally, an experiment was designed using the optimal operating conditions found from the previous experimental results to achieve the optimum treatment efficiency.

4.5 REACTOR OPERATION

The reactors were continuously fed and operated at 33° C. The feed rate was monitored and recorded every 3 hours to ensure a constant daily feed.

The production of biogas was measured daily. Samples were taken daily of the influent and effluent for analysis of COD, VFA, TSS, VSS and pH. The analysis of the gas composition was also performed every day. During the time of changing loading rate, the biogas was sampled up to three or four times daily. When the gas production rate and the effluent COD were stationary again (3 to 5 days for gas production and 6 to 10 days for COD) the reactor was at steady state. Samples of the mixture of liquid and solid in amount of 200 to 300 ml (just enough for chemical analyses) were then taken from the 10 sampling ports mounted in the reactor wall for measurement of the sludge, COD, butyric, propionic, acetic acids and pH for each operating condition. The amount of sample taken from each sample port was recorded for the calculation of sludge growth in the reactor. To obtain reliable results of reactor profiles, only one port was sampled every hour from sample port 10 (top) to port 1 (bottom). For each subsequent increment of influent concentration, an operating period of 2 to 3 HRTs was maintained to ensure stable operation.

4.6 ANALYSIS

Analyses conducted on the influent and effluent were as follows: total solids (TS), total suspended solids (TSS), volatile suspended solid (VSS) and ash according to the "standard methods" (APHA, 1975). Chemical oxygen demand (COD) was determined by a colorimetric method using an optical fiber instrument (Knechtal 1978). Gas production was measured by a wet gas meter and then corrected to the standard temperature and pressure (STP). Both gas composition and volatile fatty acids (VFAs) were analyzed on a Hewlett Packard 5890a gas chromatograph, using an external standard. The GC was equipped with both a flame ionization detector and a thermal conductivity detector with separate columns; a Porapak column was used for gas analysis and a carbopack C column for VFAs. Total Kjeldahl nitrogen (TKN), and ammonia nitrogen ($NH_3 - N$) were determined using a block digestor and a Technicon Auto Analyzer II (Schulmann et al 1973).

Chapter 5

START-UP AND EFFECTS OF PROCESS PARAMETERS

5.1 INTRODUCTION

Cheese whey acidifies easily and frequently causes problems when treated biologically. Some frustrating, unsuccessful experiments have led to the suggestion that the system was hard to maintain stable without pH control. The necessity of some micronutrients was also seriously considered. In view of the obvious difficulties in start-up of a cheese whey anaerobic system and maintenance of its stability, this part of the dissertation centers on the feasability of anaerobic digestion of cheese whey by using a new reactor configuration, a UASB reactor, in an effort to increase VSS concentration in the reactor.

The other major differences of this experiment from previous research are that no pH control or nutriential addition were applied in order to find the mechanism of the reported inhibition.

The UASB process has been described in the literature as one of the innovative reactor designs which permits efficient and economical treatment because of its ability to retain higher VSS concentrations in the reactor. However, the application of a UASB reactor to treat high strength acidic wastes has not been established. This could be due to improper start-up procedures and/or a poor understanding of the importance of the control parameters during the start-up period.

This study included the following investigations:

1. Proper start-up procedures for a cheese whey UASB system.

2. Determination of the quality of effluent and treatment efficiency when the reactor was operated over a wide range of influent concentrations. Various operating parameters were to be used to assess the effectiveness of the process.

3. Effect of influent concentration on gas production and system performance.

5.2 STUDY IN START-UP OF THE REACTOR

5.2.1 Difficulties in Start-up

Use of the UASB process is dependent on good sludge floc formation. The procedure of start-up is important for the development of active sludge with both high specific activity and settleability. Considerable attention has been directed to the start-up of the UASB reactor because of the very slow growth of anaerobic microbes. It was found in this experiment, that for cheese whey, the start-up of a UASB reactor was even more arduous. This might be attributed to the inherent characteristic of the substrate of being extremely easily acidified.

Various start-up strategies were tried. The parameters applied in the period of start-up are presented in Table 5.1. The results have shown that sludge loading rate was the most critical parameter and must be carefully controlled.

The reactor 1 which was fed with the lowest strength whey of 5 g COD/l operated very well. The other two (reactor 2 and reactor 3), which were started with higher strength whey, experienced difficulties. Over the first 3 days, reactor 2 was fed at a rate of 2.7, 2.4 and 2.6 liters daily with an influent concentration of 30 g COD/l. The methane content in the biogas was extremely low, only 19.8% on the third day. The reactor finally failed to start-up because the sludge loading rate (SLR) reached 0.38 to 0.43 g COD/g VSS.d. Then, 5 liters of seed sludge with a VSS of 24.2 g /l was added.

Table 5.1: **Sludge Loading in Start-up**

Reactor	Influent (g COD/l)	Feed (l/d)	SLR (g COD/g VSS)	Performance
R_1	5	2.8	0.162	stable
R_2	30[a]	2.6	0.300	failure
	28[b]	2.6	0.490	failure
	20	1.0	0.190	stable
R_3	63	1.0	0.214	stable
R_4	20	0.5	0.256	stable

Note: In a & b, the reactor was seeded in different amount of sludge

The total amount of VSS in the reactor was 121 g VSS. The daily feed of 2.1 liters of influent with a concentration of 28 g COD/l caused the methane content to drop from 51.9% to 44.3%, and further to 25.2%, since the SLR was as high as 0.49 g COD/g VSS.d at that point. An attempt to recover the reactor was made by reducing the feed to 0.9 liters daily. Immediately, biogas composition rose to 44.1% methane by the next day. 2.2 l/d feed was tried again. However, the reactor was entirely unstable which was indicated by drastic drop in methane content of the biogas from 44% to 34%.

A number of attempts to start-up the cheese whey UASB reactor indicated that a successful start-up was strongly related to the SLR. Table 5.1 clearly shows this relationship between the performance of the reactor and SLR. No matter how strong the influent concentration, up to 63 g COD/l for reactor 3, or how large the feed rate, up to 2.8 l/d for reactor 1, if the SLR was lower than 0.26 g COD/g VSS-d, the reactors were able to start without any problem. The reactor start-up was destined to fail, on the other hand, if the SLR employed was beyond 0.26 to 0.3 g COD/g VSS-d.

From this study it can be concluded that SLR is the most important parameter to be controlled during the start-up of an UASB reactor. The permissible SLR for a cheese whey anaerobic system was 0.25 g COD/g VSS-d.

5.2.2 Start-up Performance

This part of the study presents the behavior of the reactor which was initially fed the low strength of 5 g COD/l at HRT of 5 days.

The first 48 days were considered as the start-up period during which two influent concentrations (5 and 9.93 g COD/l) were used.

The UASB reactor performance during the start-up period is shown in Figures 5.1 to 5.8. Figure 5.3 shows the effluent COD concentration vs time of reactor operation. The

COD concentration decreased significantly with the length of time of operation. On day 40, in spite of high influent concentration of 17.7 g COD/l, effluent COD was reduced from 1500 to 110 mg/l (Figure 5.3). The same trend of increasing efficiency in terms of COD removal and gas production was also observed, as shown in Figures 5.2 and 5.3. Within the first 40 days, the COD removal efficiency increased from 70 to 97%. In the meantime, biogas composition increased from 48 to 57% methane and the gas production rate reached a value of 2.5 liters CH_4 per liter feed per day within 15 days and remained at approximately the same level under the same loading rate. Comparing COD and VFA in the effluent and gas production, it could be noticed that the lowest effluent VFA, the highest methane content in the produced biogas and the highest COD removal were reached after 40 days of operation. However, the highest gas production rate was reached much earlier at day 15 (Figure 5.9).

The effluent VSS concentrations are shown in Figure 5.8. Due to the poor settling quality of the seed sludge, a large amount of sludge left the reactor at the beginning of the operation. After 15 days of operation, the amount of sludge remaining in the reactor was reduced from 86 to 60 g VSS. At day 15, 67.2 g VSS of seed sludge were added to the reactor to maintain the specific activity of the sludge. As a result of natural selection and sludge particle growth, the sludge settleability improved gradually. At day 30, the TSS content in the effluent was as low as 0.1 g/l.

Many factors have been shown to be associated with the start-up of the UASB reactor and its later performance. Attention has also been directed to the use of different seed sludge in order to shorten the start-up period. Starting with a poor quality digested sewage sludge, De Zeeuw (1985) was able to cultivate a highly active biomass (specific activity of 0.75 g COD/g VSS d) within a period of 6 weeks (organic loading rate of 7.5 g COD/l d).

Compared to the sewage sludge used by De Zeeuw , the seed used in this study had a

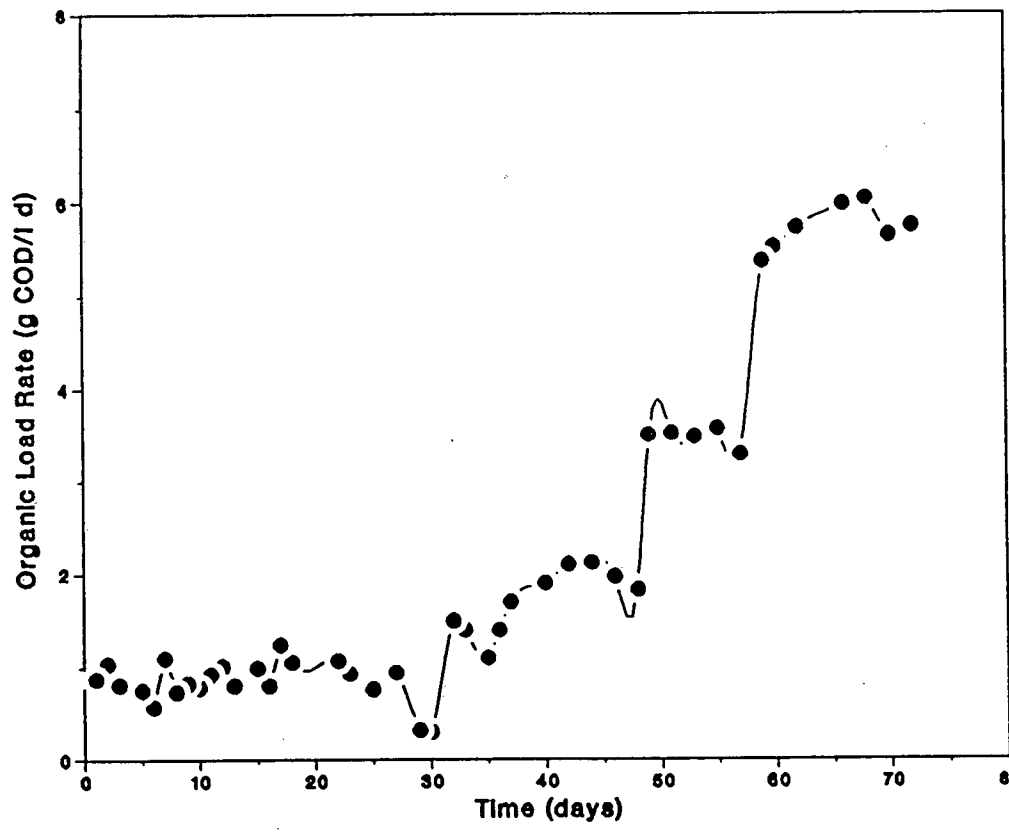


Figure 5.1: **Organic Loading Rate versus Time**

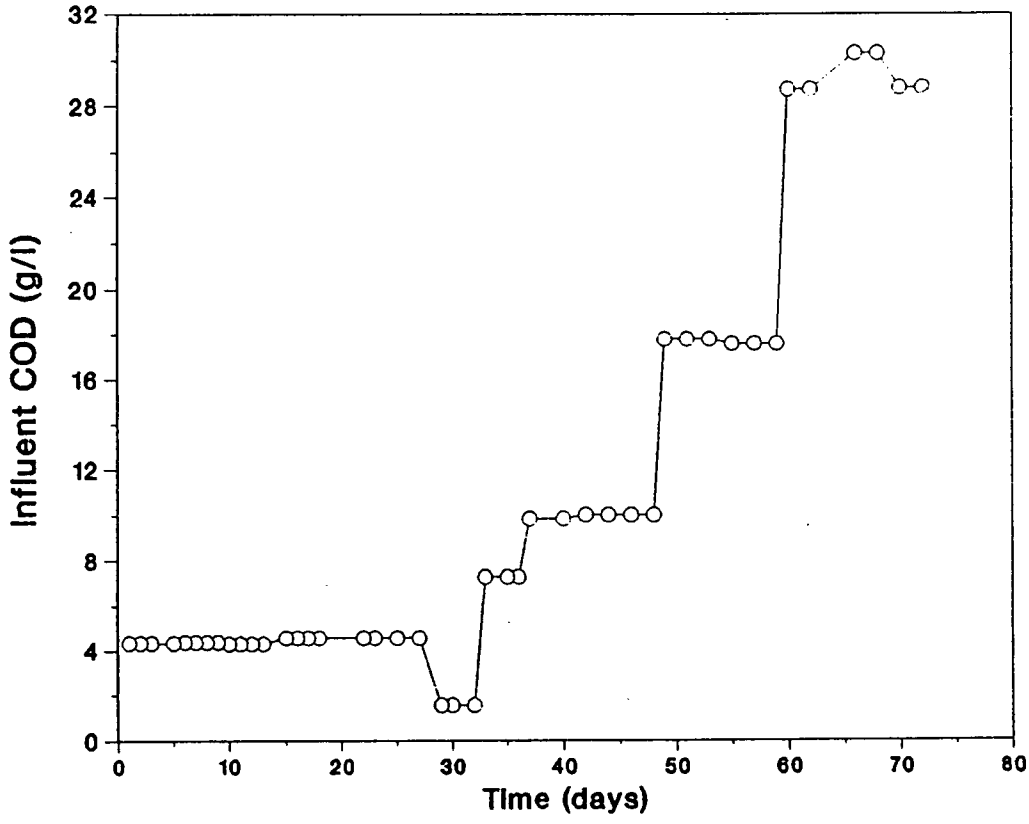


Figure 5.2: **Influent COD versus Time**

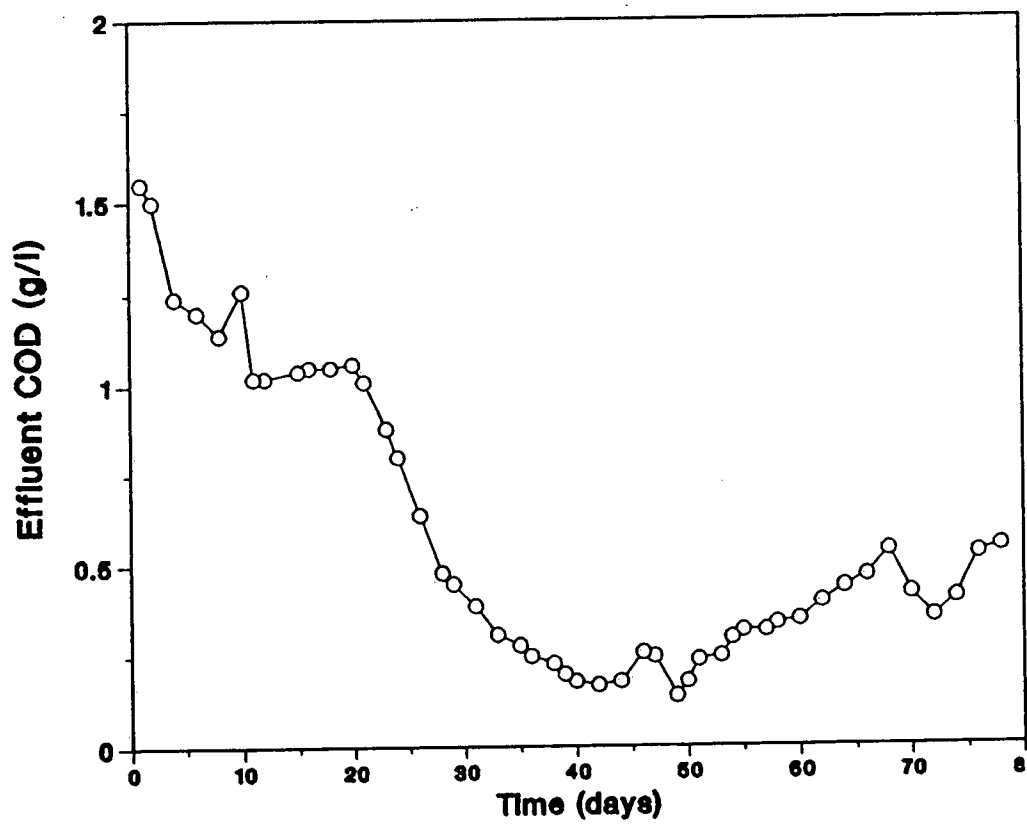


Figure 5.3: Effluent COD versus Time

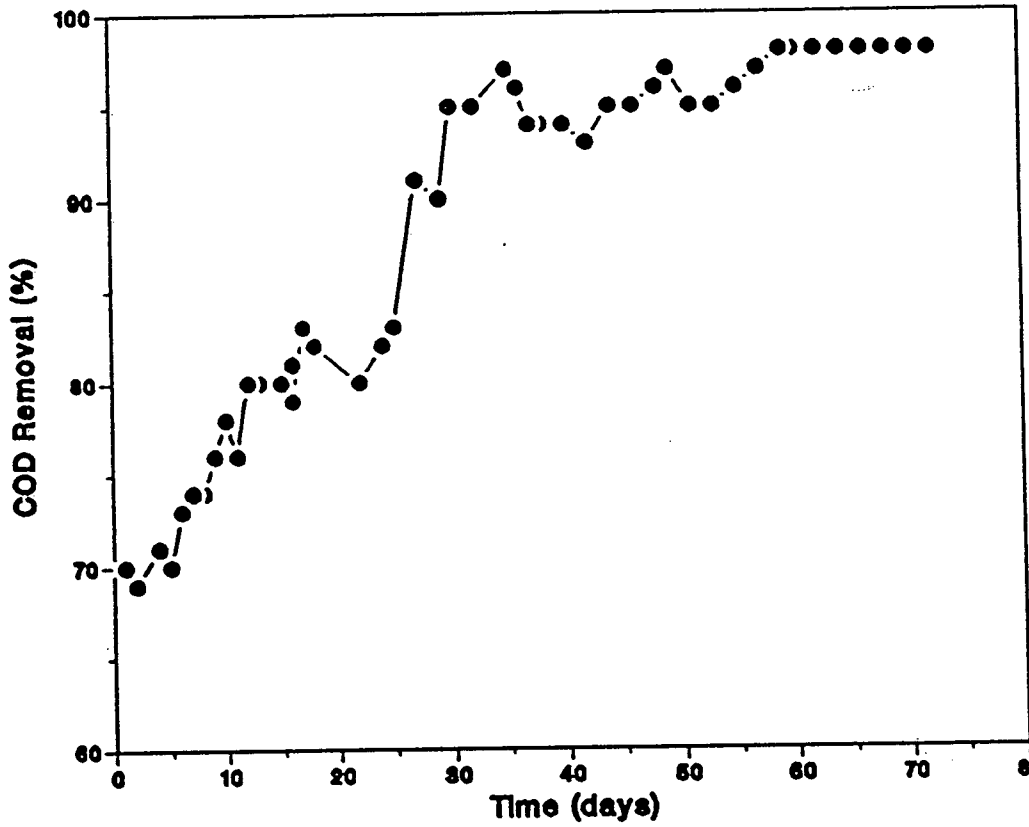


Figure 5.4: **COD Removal versus Time**

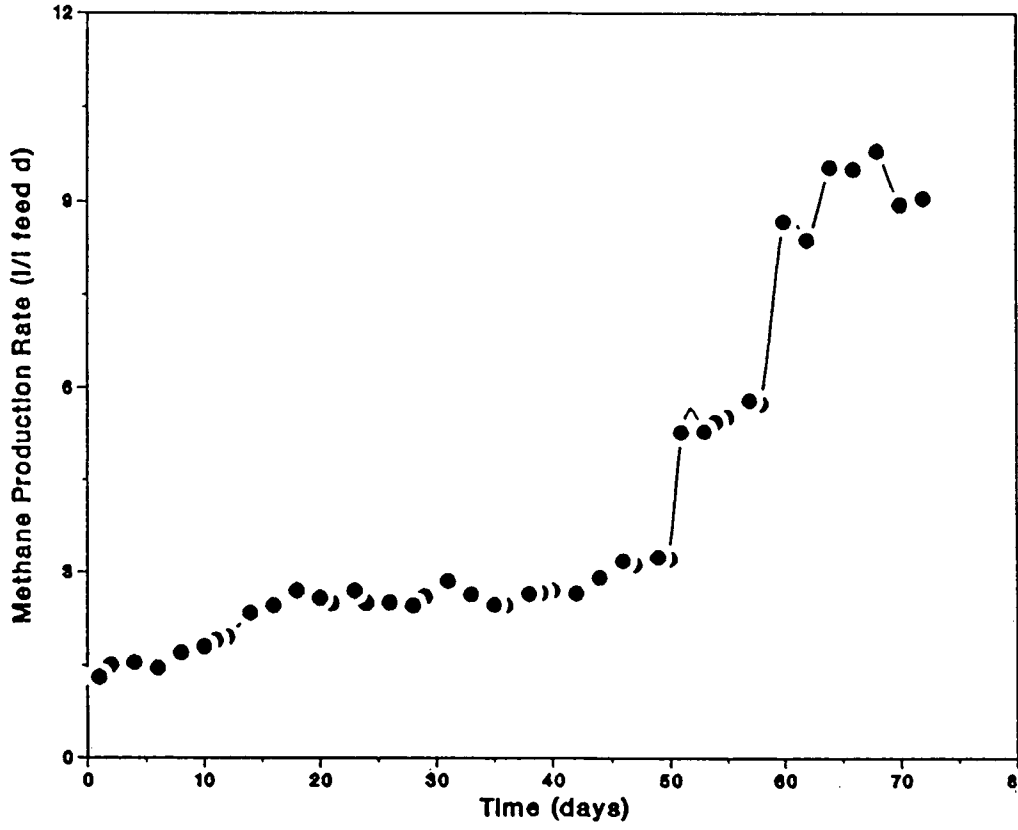


Figure 5.5: Methane Production Rate versus Time

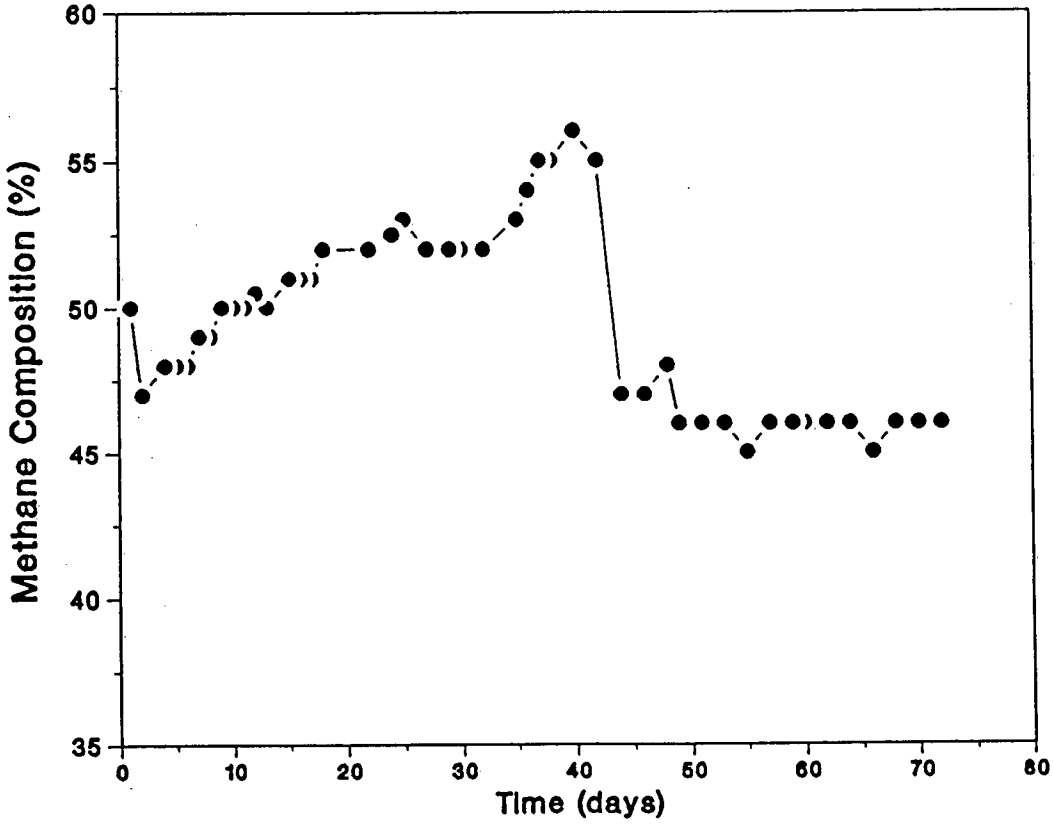


Figure 5.6: **Biogas Composition versus Time**

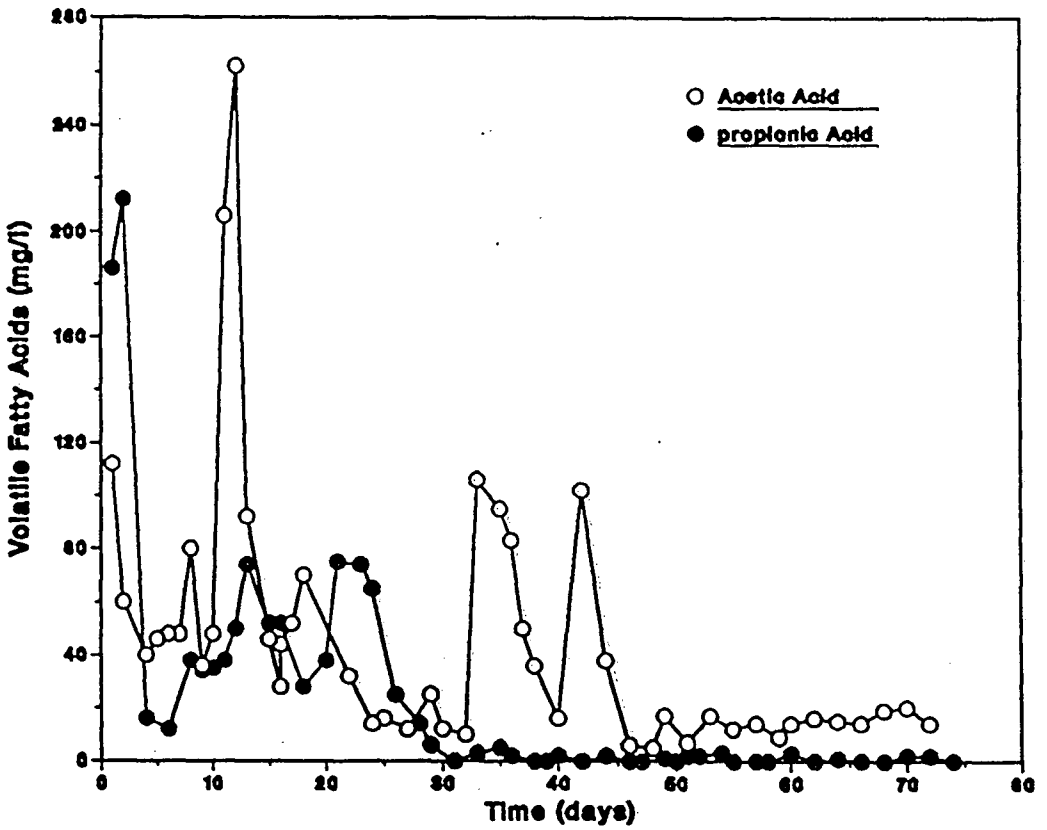


Figure 5.7: Effluent Volatile Fatty Acids versus Time

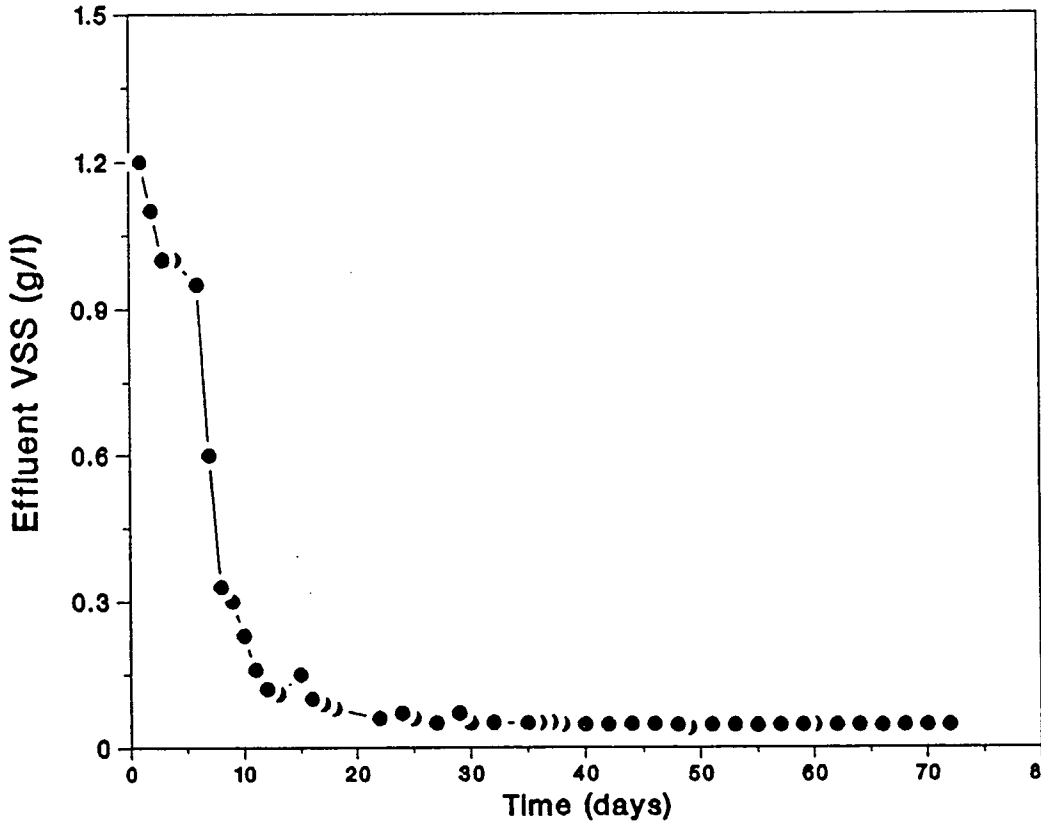


Figure 5.8: **Volatile Suspended Solid (VSS) in the Effluent**

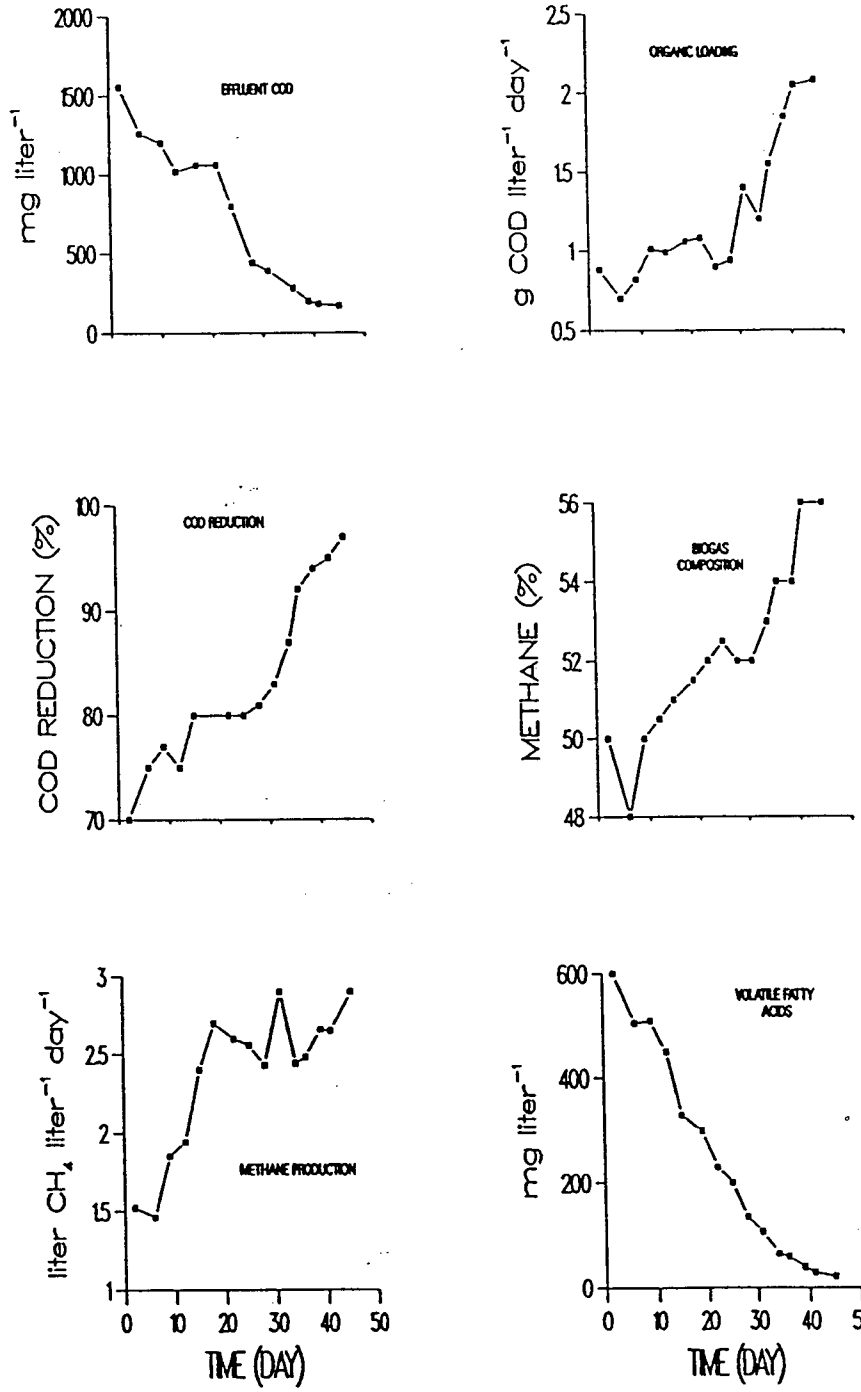


Figure 5.9: **Comparison of COD, VFA and Biogas Production**

lower VSS content and poorer settleability. This was demonstrated by the loss of a large amount of sludge (Figure 5.8), high VFA concentrations and low gas production at the beginning of the start-up. However, the seed was able to degrade acetate and propionate resulting in very low effluent VFA concentrations within a period of 40 days, reaching 0.71 g COD/g VSS-d of specific activity after an operation period of 70 days.

5.2.3 VFA Behavior in Reactor Start-up

Figure 5.7 shows the daily VFAs concentrations in the effluent of the reactor. It was found that VFAs were sensitive parameters which responded to loading change and reactor performance. At the very beginning of the reactor operation, VFAs in the effluent were fairly high due to the lower activity and concentration of the sludge at that time. Then they were gradually decreased.

An increase in loading rate increased the VFA concentration. At day 30, the peak in acetic acid concentration was a response to the increase of influent concentration from 4.56 to 9.9 g COD/l. As long as the sludge was acclimated and had built up enough concentration and achieved a high specific activity, the VFA in the effluent would decrease to a very low level. In this case, after 45 days from start, the total VFA in the effluent was as low as about 40. Although at days 45 and 60 when the influent concentration was increased from 10 to 18 g COD/l and from 18 to 28 g COD/l respectively, there was no peak observed.

It is very important during the start-up of a UASB reactor that the OLR be increased only after the majority of VFA is removed. At day 12, when an attempt was made to decrease the HRT from 5 days to 4, a sudden increase in VFA was detected. By using VFA concentration as a sensitive indicator while varying the start-up parameters it has been shown that 0.2-0.25 g COD/g VSS would be recommended for start-up of the reactor.

5.3 STEADY STATE OPERATION

The steady-state performance of whey digestion as a function of influent concentration is summarized in Table 5.2. A COD removal efficiency of over 97% was maintained after 45 days of reactor operation. A 98% COD reduction with a gas production rate of 9.57 l CH_4 /l feed-d was achieved at a loading rate of 5.96 g COD/l d and an influent concentration of 28.8 g COD/l.

Figure 5-10 illustrates the quality of effluent as a function of influent concentration. In spite of the high influent COD concentration of 28.8 g COD/l, the effluent COD concentrations were between 400 and 500 mg/l. Effluent pH increased with an increase in influent concentration. VFA contents of the effluent were in the range of 76-16 mg/l.

A comparison of the behavior of a UASB reactor with a fixed-film reactor for cheese whey treatment showed that the growth rate and bacterial activity were somewhat different for the suspended-growth system (UASB system) and the attached-growth system (fixed-film) (Lettinga et al 1979, Kelly and Switzenbaum 1984, Nordstedt and Thomas 1984, Wildenauer and Winter 1985, LO and Lioa 1986). Lower effluent VFA concentrations were reported for the UASB reactor than for the fixed-film system. The effluent VFA concentration has proven to be a good indicator for the condition of an anaerobic reactor. Such a comparison revealed that a higher activity of biomass was developed in the UASB reactor than in the attached growth system with respect to the degradation of VFA.

COD balances were determined for the four experimental conditions. A mass balance of the system enables the sludge formed in the reactor to be calculated (Table 5.3). The observed yields calculated from the COD balance over the whole experimental period were in the range of 0.098 to 0.166 g TSS formed/g COD removed. This appears to be within a reasonable range for this type of waste which is primarily carbohydrate

Table 5.2: **Experimental Results in Steady State Performance**

Influent	OLR	Effluent			COD	GAS		
g COD/l	g COD/l d	COD mg/l	pH	VFA mg/l	removal %	l CH_4/ l reactor d	l CH_4/ g COD d	CH_4%
4.56	0.91	120	6.75	35	98	1.37	0.30	56.5
7.30	1.36	215	6.92	56	97	2.35	0.32	51.5
9.93	1.97	191	7.08	16	98	3.25	0.33	48.2
17.70	3.54	286	7.05	18	99	5.80	0.33	47.0
21.50	4.23	348	7.10	20	99	7.14	0.34	46.5
28.80	5.96	457	7.18	18	99	9.57	0.33	46.1
38.10	7.77	505	7.26	20	99	11.20	0.30	42.5

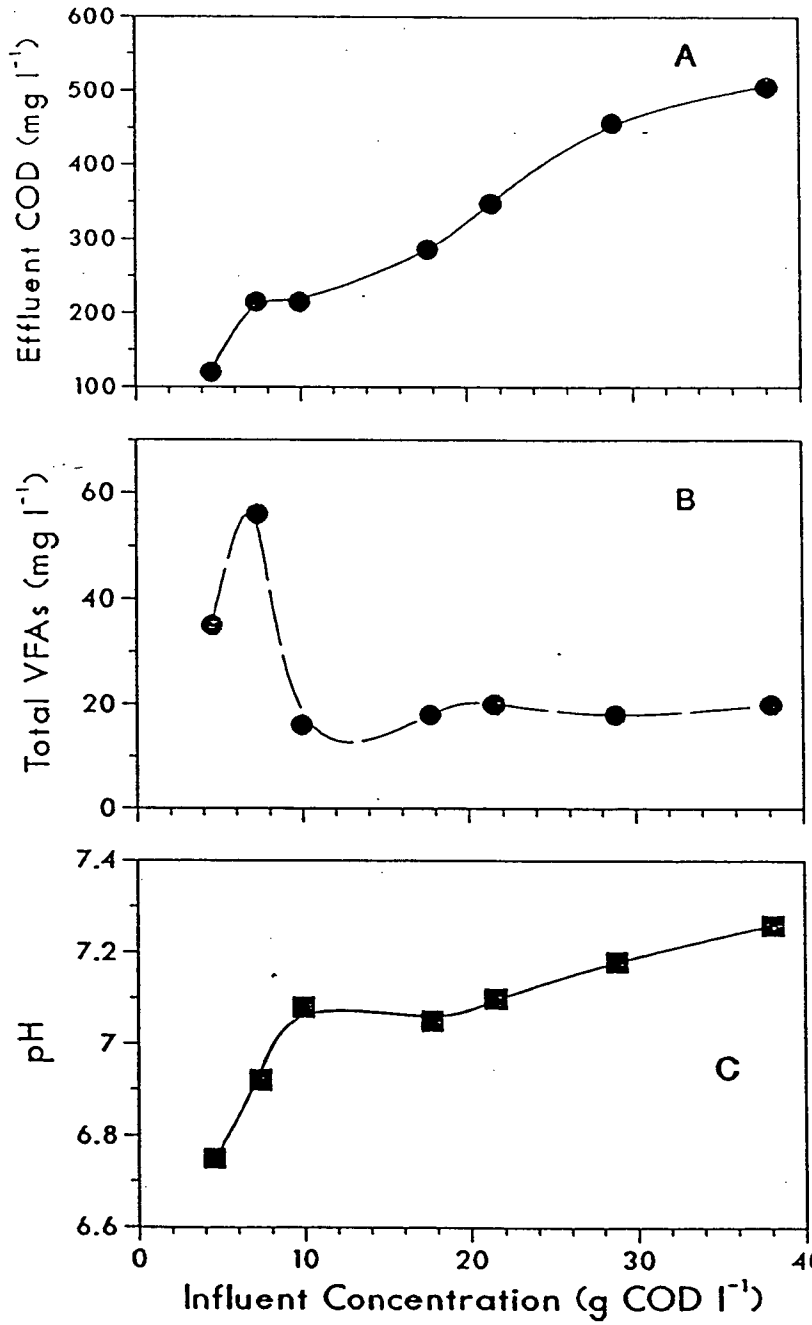


Figure 5.10: **The Quality of Effluent as a Function of Influent Concentration (at HRT = 5 days)**

(Switzenbaum and Danskin 1982, Henze and Harremoes 1982).

5.4 UNSTEADY-STATE PERFORMANCE

The process became unstable 14 days after the reactor was fed influent at a concentration of 38.1 g COD/l. This was first noticed by a decrease in gas production from 70 to 61 l/d and an increase in effluent acetic acid and propionic acid concentrations to 80 and 64 mg/l, respectively, following an increase of effluent COD from 500 to 643 mg/l. The influent concentration was decreased to 28.5 g COD/l until steady-state was re-established in 20 days and then increased to 41.1 g COD/l. An instability appeared again and finally the reactor was entirely upset. This apparently indicated that the system was overloaded and the influent strength of about 38 g COD/l appears to be a barrier or threshold for this system. Similar findings were reported by Switzenbaum and Danskin (1982) when they treated cheese whey by using an expanded bed reactor. The COD reduction was decreased from 83% to 58 % and the process became unstable when they increased the influent concentration from 5 to 20 g COD/l (Switzenbaum et al 1982). It was suggested that in treating high strength whey the physical balance between the methanogens and the hydrogen and acid-producing bacteria was more easily upset in this system. This will be the main topic of this thesis and will receive further consideration in a later chapter.

5.5 GAS PRODUCTION

5.5.1 The Effect of Influent Concentration

In this part of the study, the reactor was operated at a constant HRT of 5 days, and the influent COD concentration, and therefore OLR, was varied. Biogas production rate and

Table 5.3

COD Balance

(1) *Influent concentration* (*mg COD l⁻¹*)	(2) *Influent* (*mg COD d⁻¹*)	(3) *Effluent concentration* (*mg COD l⁻¹*)	(4) *Effluent* (*mg COD d⁻¹*)	(5) *CH_4 production* (*mg COD d⁻¹*)	(6) *CH_4 in effluent* (*mg COD d⁻¹*)	(7) *Total COD out* (*mg COD d⁻¹*)	(8) *Sludge formed* (*mg COD d⁻¹*)	(9) *Sludge accumulation factor* (*mg VSS formed/ mg COD removed*)
4 780	13 623	132	376	9 885	235	10 496	3 127	0·166
9 934	28 312	191	544	23 447	235	24 228	4 084	0·104
17 725	50 515	286	815	41 848	235	42 898	7 618	0·108
28 700	81 795	457	1 304	69 049	235	70 587	17 208	0·098

The above parameters were calculated as follows:

(2) = 2·85 * (1), 2·85 (l.d) is the amount of feed
(4) = 2·85 * (3)
(5) 1 g COD = 395 ml CH_4 at 32°C
(6) assume effluent is saturated with CH_4 at 32·8 ml CH.L
(7) = (4) + (5) + (6)
(8) = (2) − (7)
(9) = (8)/[(2) − (4)]. Assume 1·0 mg solid = 1·42 mg COD

methane composition are presented in Table 5.4.

A plot of gas production rate in terms of liters of methane produced per gram COD added per day as a function of the influent concentration is shown in Figure 5.11. The production rate of methane was increased with increasing influent COD concentration up to a concentration of 28.8 g COD/l, then the trend reversed. The methane production rates ranged between 0.219 and 0.313 l/g COD d.

Figure 5.12 demonstrates the effect of influent concentration on the methane composition. It has been believed that gas composition is a function of the nature of the biodegradable portion of the feed. It would be an interesting study to relate the biodegradable portion of substrates to the methane composition. Degradation of carbohydrates yields CO_2 and CH_4 in equal quantities. A higher proportion of CH_4 is generated from proteins and fatty substances, and this may reach a level as high as 75% methane and 25% carbon dioxide. A high CO_2 content in the gas phase in this system (up to 45% and higher) indicates the domination of acidogenic fermentation over methanogenesis.

In general, a lower influent COD concentration yields a higher methane composition in the produced biogas. This implies that the dominance of acidogenesis over methanogenesis is enhanced by an increase in substrate strength in the cheese whey anaerobic process. Therefore, it could also be possible to develop a useful technique based on this phenomena for quickly determining stability or to monitor any stress in an anaerobic system from the spectrum of produced biogas composition since the methane composition is such a sensitive parameter which responds to any environmental change and reflects the reactor operation.

The methane in the produced biogas rapidly diminished from 56% down to 48% with an increase in influent concentration up to a certain level (9.9 g COD/l). There was little change in biogas methane composition with a further increase in influent concentration

Table 5.4

Gas Production Rate and Composition for Different Influent Concentration at 5 days HRT

Influent concentration (g COD litre^{-1})	*Organic loading rate (g COD litre^{-1} day^{-1})*	*Gas composition (CH_4%)*	*Gas production*		
			(litres biogas litre^{-1} day^{-1})	*(litres biogas g^{-1} COD day^{-1})*	*(litres CH_4 g^{-1} COD day^{-1})*
4·56	0·91	56·1	2·45	0·537	0·282
7·30	1·36	51·5	4·60	0·630	0·302
9·94	1·97	48·2	6·73	0·677	0·304
17·7	3·56	47·0	12·4	0·647	0·305
21·5	4·20	46·6	15·3	0·714	0·310
28·8	5·96	46·1	20·8	0·721	0·311
38·1	7·77	42·5	26·5	0·705	0·300
41·1	8·14	41·0	23·9	0·581	0·222

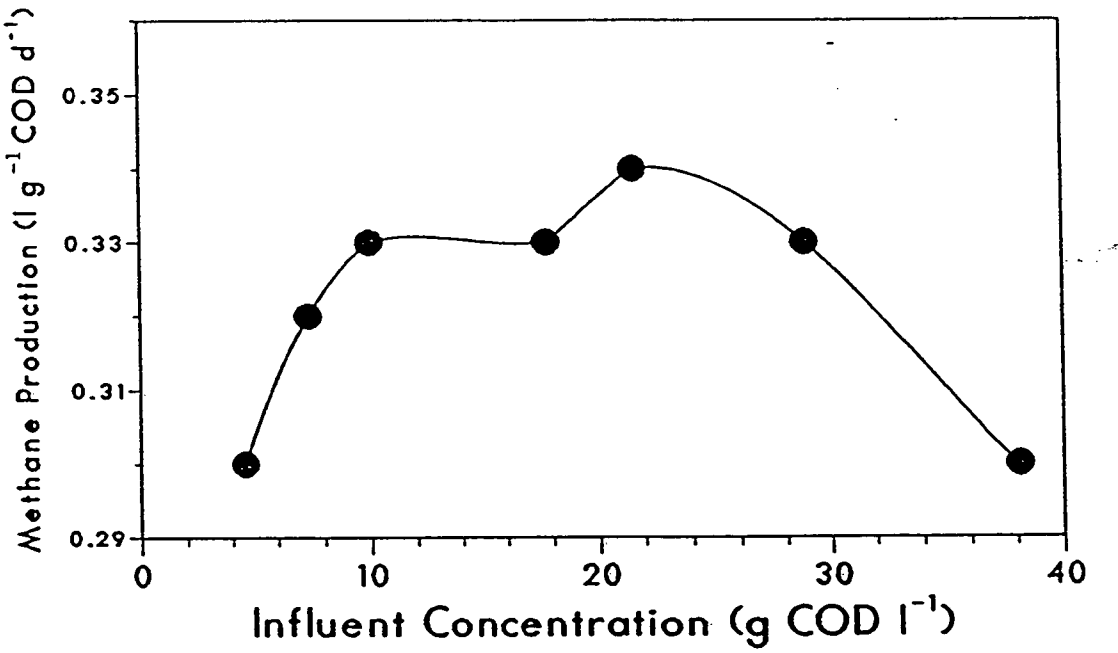


Figure 5.11: **Effect of Influent Concentration on Methane Production**

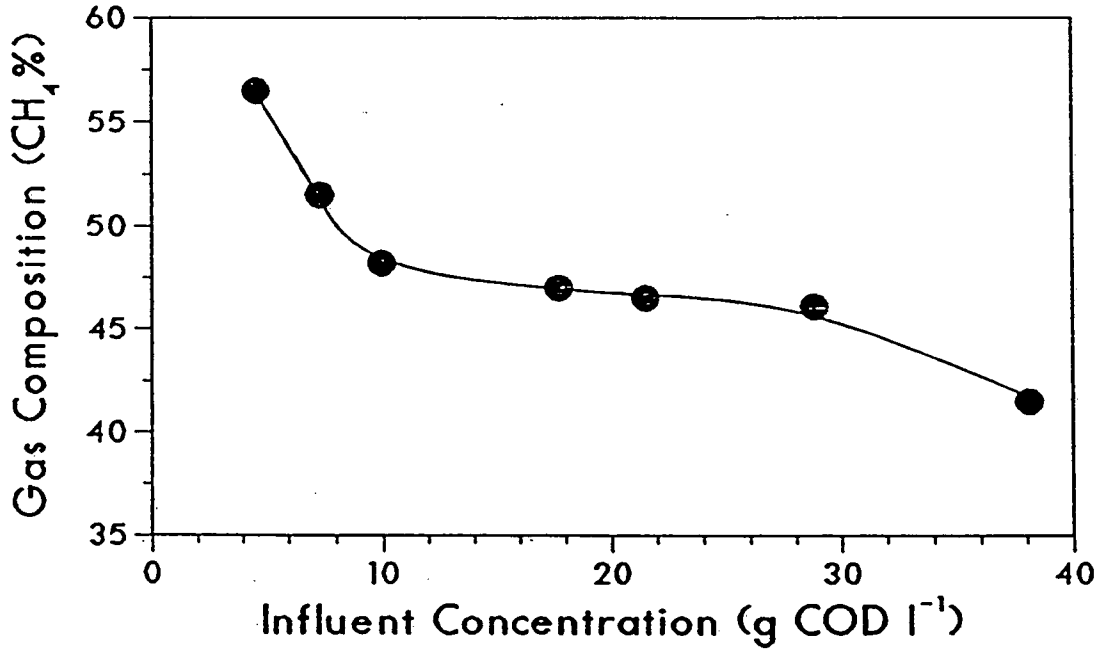


Figure 5.12: **Effect of Influent Concentration on Biogas Composition**

to 28.8 g COD/l. The methane composition decreased when the influent COD was raised to more than 28.8 g COD/l. It showed that no simple linear relationship exists between the gas composition and the influent concentration.

The average methane production rate was about 0.32 l/g COD-d. The methane compositions decreased after the influent concentration was raised to more than 28.8 g COD/l (loading rate great than 6 g COD/l-d). At this loading rate, instability was observed. The highest methane production rate of 9.57 l CH_4 /l feed d was obtained at a loading rate of 6 g COD/l d and an influent concentration of 28.8 g COD/l.

5.5.2 The Effect of Hydraulic Retention Time (HRT)

In this part of the study, the UASB reactor was first tested at an influent concentration of 20.5 g COD/l, while the operating HRT was varied from 24.7 to 4.9 days. The experiment was then conducted over the same range of HRTs with a higher feed concentration (41.1 g COD/l). The methane production rates are summarized in Tables 5.5 and 5.6.

The methane production rate was increased with increasing HRT, which agrees with previous research (Preffer, 1974). Figure 5.13 shows the relationship between methane production rate and HRT. This study gave almost the same results as were reported by Eckenfelder and Ford that at 22.5 days HRT, the methane production rate was almost twice that at 8.8 days HRT.

A more interesting finding was that there is an interaction between HRT and feed strength.. In other words, the effect of HRT was related to influent concentration. At HRTs longer than 10 days, the methane production rate was similar for two influent concentrations as shown in Figure 5.13. At short HRTs, the effect of HRT on methane production rate was more pronounced for the higher influent concentration (41.1 g COD/l) than for the lower influent concentration (20.5 g COD/l). The methane production rate

decreased slightly from 0.356 to 0.341 l CH_4/g COD-d at an influent concentration of 20.5 g COD/l when HRT was decreased from 10.1 to 6.8 days. However, under the same range of operating HRT, a sharp decrease in methane production rate from 0.376 to 0.249 l CH_4/g COD-d was observed for tests with the influent concentration of 41.1 g COD/l. The results also indicated that for a given HRT, a higher methane production rate (l CH_4/g COD d) was obtained at a lower influent COD concentration at HRTs shorter than 10 days.

The effect of HRT on methane production rate can be represented by the slopes of the linear equations.

for Co=20.5 g COD/l

$$Gp = 0.009\theta + 0.292 \tag{5.1}$$

for Co=41.1 g COD/l, $\theta > 10$ days

$$Gp = 0.010\theta + 0.286 \tag{5.2}$$

for Co=41.1 g COD/l, $\theta < 10$ days

$$Gp = 0.027\theta + 0.107 \tag{5.3}$$

where

θ=hydraulic retention time, days

Co=influent concentration, g COD/l

Gp=methane production rate, l CH_4/g COD d

Equations (5.1) and (5.2) showed similar slopes and intercepts at HRTs longer than 10 days for both influent concentrations. Equation (5.3) has a slope 2.7 times greater than

Table 5.5

Methane Production Rate at an Influent Concentration of 20·5 g COD litre^{-1}

HRT (days)	*Influent concentration (g COD litre^{-1})*	*Organic loading rate (g COD litre^{-1} day^{-1})*	*Gas composition (CH_4%)*	*Gas production*		
				(litres gas litre^{-1} feed day^{-1})	*(litres gas g^{-1} COD day^{-1})*	*(litres CH_4 g^{-1} COD day^{-1})*
24·7	18·2	0·74	50·6	19·0	1·021	0·492
23·1	18·2	0·79	50·6	19·2	1·054	0·497
16·5	20·2	1·23	48·5	18·4	0·909	0·411
10·1	20·0	1·98	47·4	15·9	0·807	0·356
6·8	20·5	3·01	47·0	16·0	0·780	0·341
5·2	21·0	4·08	46·5	15·4	0·731	0·317

Table 5.6

Methane Production Rate at an Influent Concentration of 41·1 g COD litre^{-1}

HRT (days)	*Influent concentration (g COD litre^{-1})*	*Organic loading rate (g COD litre^{-1} day^{-1})*	*Gas composition (CH_4%)*	*Gas production*		
				(litres gas litre^{-1} feed day^{-1})	*(litres gas g^{-1} COD day^{-1})*	*(litres CH_4 g^{-1} COD day^{-1})*
23·9	41·1	1·73	49·7	43·3	1·053	0·490
10·8	41·1	3·82	46·5	34·5	0·846	0·367
7·1	41·1	5·84	44·7	27·1	0·658	0·274
6·0	41·1	6·91	43·2	25·2	0·618	0·249
5·0	41·1	8·21	41·0	23·9	0·581	0·222

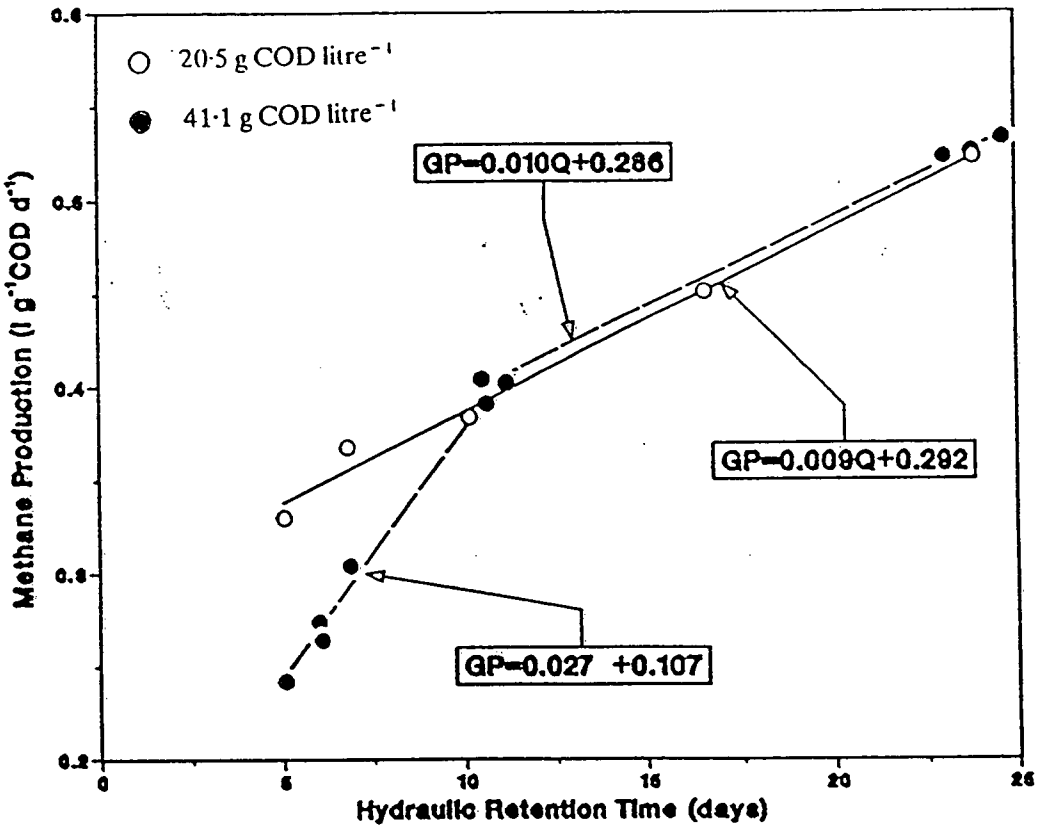


Figure 5.13: Effect of HRT on Methane Production

that of equation (5.2), and the methane production rates decreased rapidly when the reactor was fed with an influent concentration of 41.1 g COD/l. This dramatic decrease in the methane production rate indicates some inhibition of the methanogenesis, a fact supported by the VFAs data in Table 5.7. The concentration of VFAs generally increases with a decrease in HRT or an increase in organic loading rate. There was a surge of propionic acid when the HRT was decreased from 10 to 7 days. As the HRT was further decreased, propionic acid concentration increased, followed by an increase in acetic acid.

The effect of HRT on methane composition is presented in Figure 5.14. As with biogas production, the methane in the biogas decreased as the HRT decreased.

5.5.3 The Effect of Organic Loading Rate (OLR)

Figure 5.15 shows that at a constant influent COD concentration the methane production rate decreased with an increase in organic loading rate (a decrease of HRT). At an OLR less than 4 g COD/l d, a higher influent concentration resulted in a higher methane production rate. Longer HRTs led to higher methane production rates at a similar OLR (Table 5.8). When the OLR was greater than 4 g COD/l d, an increase in influent concentration or a decrease in HRT resulted in a decrease in the methane production rate. The observed effects of OLR on biogas production were in good agreement with the findings of a number of investigators. For example, Varel et al had quite similar experimental results. Studying methane production from beef cattle waste, Varel et al (1980) found that for a given VS loading rate, higher methane production rates are possible at higher influent VS concentrations and longer HRT up to an influent VS concentration of about 8%.

At a constant HRT of 5 days, a change in influent concentration resulted in a small change of methane production rate, which remained relatively constant up to an influent

Table 5.7

VFA in the Reactor and Effluent at Differential HRT

Influent COD (g COD litre^{-1})	*HRT (days)*	*VFA at 60 cm in the reactor (mg litre^{-1})*				*VFA in the effluent (mg litre^{-1})*				*COD Removal (%)*
		Acetic	*Propionic*	*Iso-Butyric*	*Butyric*	*Acetic*	*Propionic*	*Iso-Butyric*	*Butyric*	
20·5	23·8	18	0	0	0	10	0	0	0	99
	16·4	20	0	0	0	5	0	0	0	99
	10·1	23	0	0	0	15	0	0	0	98
	6·8	22	2	0	0	26	0	0	0	98
	5·8	45	14	0	0	10	0	0	0	98
41·1	23·8	29	20	4	0	10	3	0	0	99
	10·4	278	762	0	0	6	0	0	0	95
	6·8	266	1204	0	0	54	838	0	0	92
	5·9	993	1636	168	809	398	1252	111	0	86
	5·0	556	2231	76	158	208	1793	57	0	81

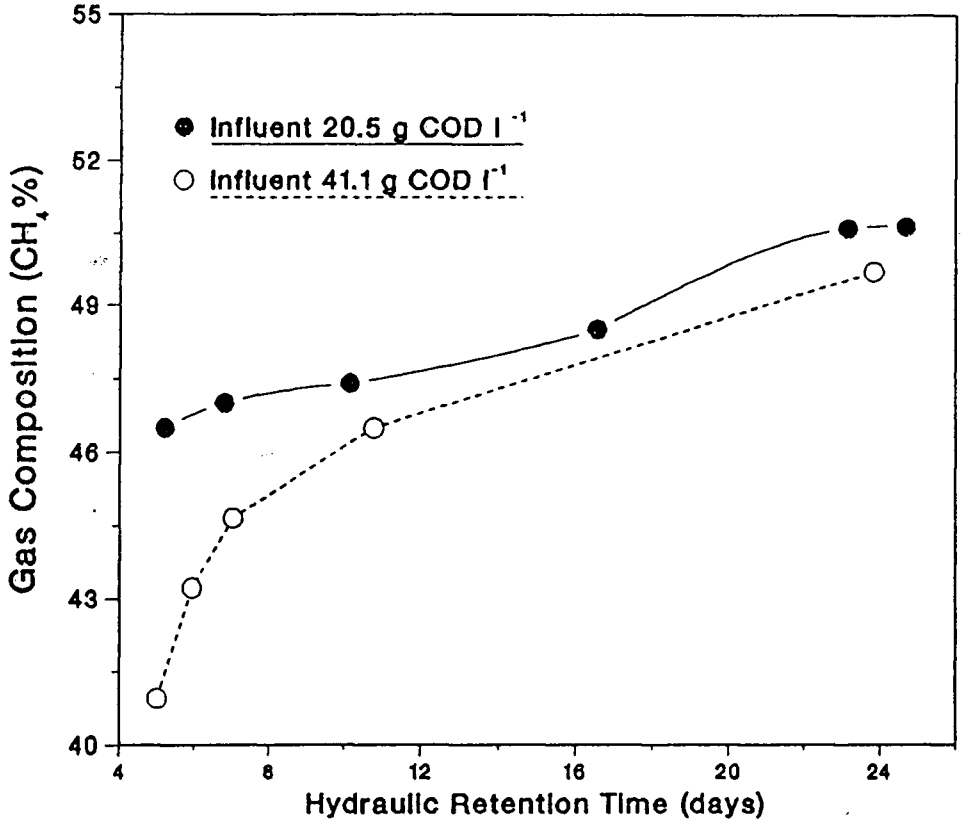


Figure 5.14: Effect of HRT on Biogas Composition

Table 5.8

Effects of Influent Concentration and HRT on Gas Production Rate for Similar Organic Loading Rate

Organic loading rate (g COD litre^{-1} day^{-1})	*Influent concentration (g COD litre^{-1})*	*HRT (days)*	*Gas composition (CH_4%)*	*Gas production*	
				(litres gas g^{-1} COD day^{-1})	*(litres CH_4 g^{-1} COD day^{-1})*
0·90	4·56	5·0	56·3	0·537	0·302
0·79	18·2	23·0	50·6	1·054	0·533
1·36	7·30	5·0	51·5	0·630	0·324
1·23	20·2	16·4	48·5	0·888	0·431
1·92	9·85	5·0	48·2	0·678	0·327
1·97	20·0	10·1	47·4	0·806	0·382
1·73	41·1	23·9	49·7	1·053	0·524
4·08	21·0	5·1	46·5	0·731	0·340
3·82	41·1	10·8	46·6	0·846	0·394
5·96	28·1	5·0	46·1	0·723	0·333
5·84	41·1	7·1	44·7	0·658	0·294

concentration of 28.8 g COD/l, then decreased (see Table 5.5 and Figure 5.16). The highest methane production rate of 9.57 l CH_4/l feed-d (0.33 l CH_4/g COD) was obtained at an OLR of 5.96 g COD/l-d and influent concentration of 28.8 g COD/l (Table 5.2). These results imply that the reactor can be operated at short HRTs, as long as the OLR does not exceed 6 g COD/l-d.

A simple linear relationship between OLR and methane production in terms of liters CH_4 per liter reactor per day was found in OLR range of 2 to 8 g COD/l-d (Figure 5.16)

5.6 TREATMENT EFFICIENCY

The highest COD removal efficiency for raw whey treated in a chemostat (Clanton et al. 1985) was only 58% for a completely-mixed anaerobic reactor. Wildenauer and Winter (1985) achieved a COD removal of 95%, close to the results of this study with the help of pH-control, which maintained the pH at about 6.7. Without pH control in the whey digestion process, lower organic loading rates were usually applied to the reactor and relatively lower COD reductions were obtained as indicated in Table 8-2. In this study, the UASB process accommodated fairly well to whey strength up to 28.8 g COD/l and maintained a COD reduction of over 97% . These results indicated that the UASB reactor can tolerate a higher wastewater strength although this type reactor was supposed to be best for dilute wastes, typically under 20 g COD/l and at moderate OLR.

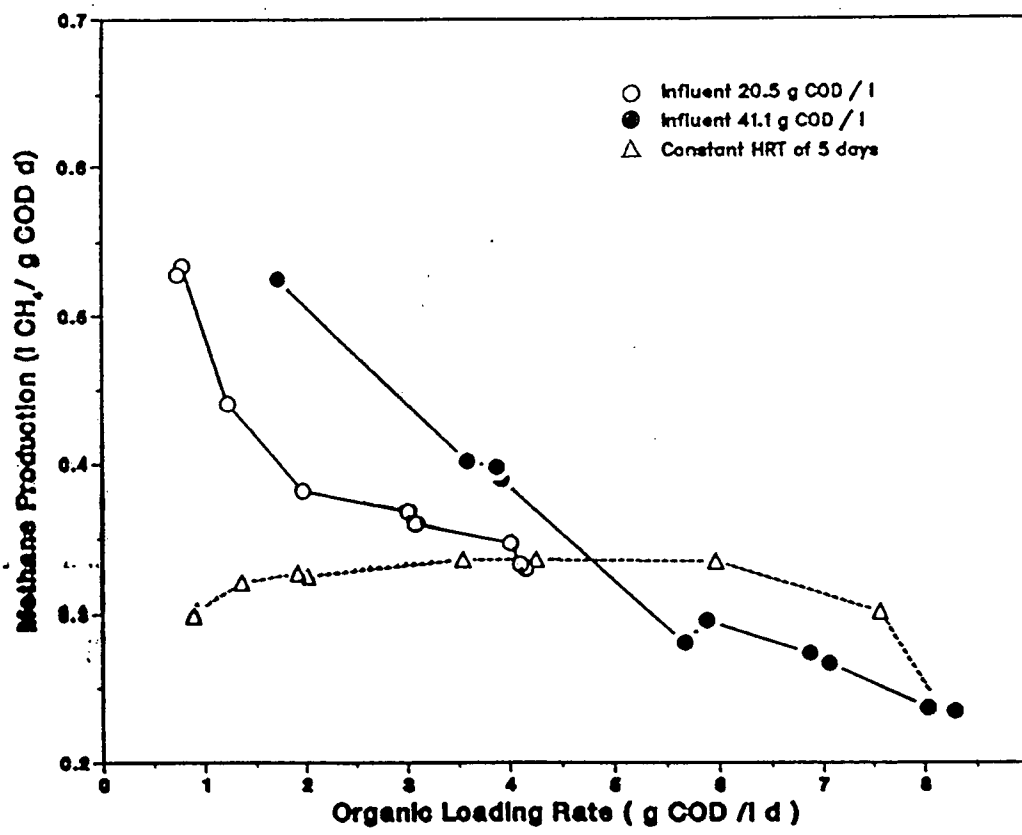


Figure 5.15: Effect of Organic Loading Rate on Methane Production

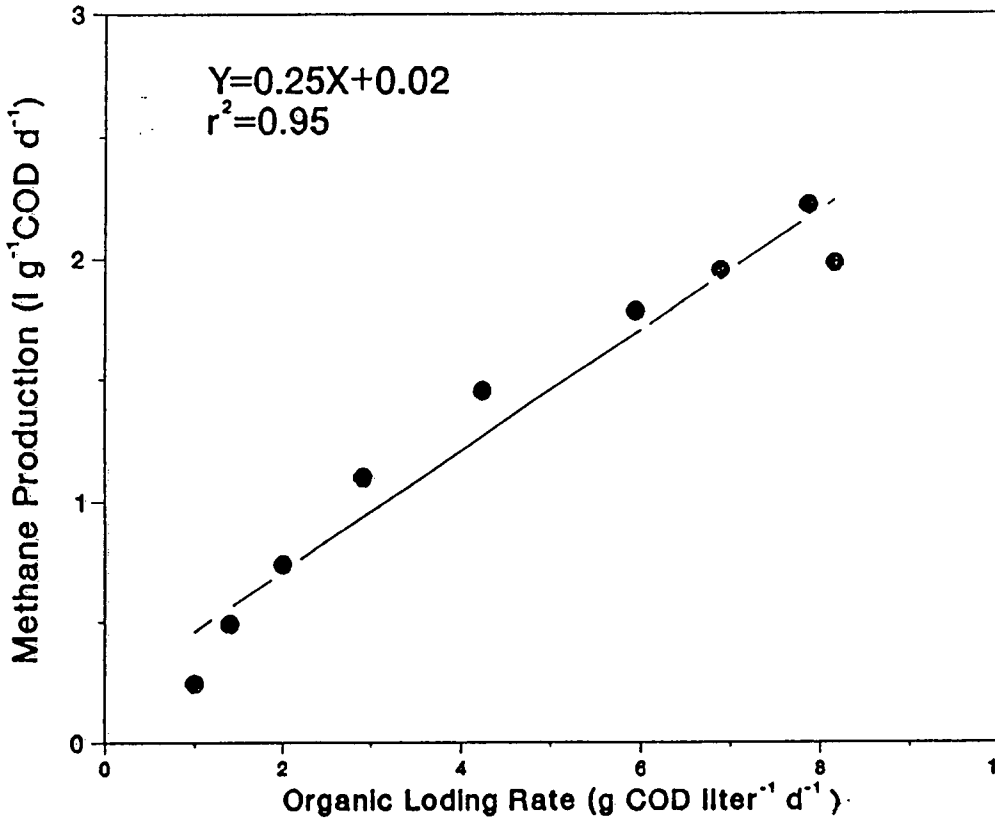


Figure 5.16: **Relation Between Methane Production and OLR**

5.7 SUMMARY

1. The performance of the UASB reactor was evaluated in the range of 4.5 to 38.8 g COD/l of cheese whey influent concentrations at a 5 days hydraulic retention time. The results of the present experiments have shown that anaerobic digestion using a UASB reactor can be an efficient treatment method for diluted cheese whey. Over 97% COD removal was achieved. Without pH control and nutritional addition, the system could treat cheese whey successfully up to a concentration of about 29 g COD/l.

2. It is known that reactor performance strongly depends on the start-up procedure. Various start-up strategies were used to facilitate the start-up of an UASB reactor and ensure stable operation. Among the operating parameters, sludge loading rate was the most important during start-up. In the very beginning, the initial sludge loading should not exceed 0.25 g COD/g VSS d.

3. VFAs in the effluent were found to be a very useful indicator for primary adaptation of the sludge and for monitoring the system's stability. The loading rate should be increased only after the VFA concentrations are greatly reduced . Too rapid an increase of OLR may result in the loss of activity of the sludge.

4. The methane production rate increased with increasing HRT. At HRTs longer than 10 days, the methane production rate was similar for two influent concentrations. At short HRTs, the effect of HRT on methane production rate was more pronounced for the higher influent concentration (41.1 g COD/l) than for the lower influent concentration (20.5 g COD/l). For a given HRT, a higher methane production rate (l CH_4/g COD-d) was obtained at a lower influent COD concentration and at those HRTs shorter than 10 days. From the point of view of fuel gas production, the organic loading rate was a critical parameter. At an OLR less than 4 g COD/l-d, the reactor fed with a higher influent concentration yielded a higher methane production rate. When OLR was greater

than 6 g COD/l-d, the higher influent concentration or shorter HRT produced a lower methane production rate. Therefore, the optimal OLR for this particular system with regard to methane production would be between 4-6 g COD/l-d. For a HRT of 5 days, the optimal influent concentration should be between 20-30 g COD/l.

5. When the influent concentration was increased to 38 g COD/l, instability was observed. For this system the influent concentration should be maintained below 30 g COD/l at HRT of 5 days.

Further studies were emphasize a search for the reasons for the inhibition caused by high influent concentrations and to seek an efficient way to prevent instabilities.

Chapter 6

DISTRIBUTIONS OF SLUDGE AND SUBSTRATES

6.1 INTRODUCTION

The UASB process has been widely investigated since it was developed. A great number of research papers which have been published each year reveal that it is an effective process for anaerobic wastewater treatment.(Lettinga et al.1985, 1984, 1983, 1979; Wang et al. 1985; Wu et al. 1985; Sayed et al.1984). The treatment of cheese whey in a UASB reactor has further demonstrated that the easily acidified substrates, such as cheese whey, can be treated by using an UASB reactor, if the proper start-up procedure is used. Satisfactory treatment efficiencies have been obtained (Yan et al. 1989).

In order to describe and optimize the UASB process, various models have been proposed to explain the kinetics of anaerobic digestion in a UASB reactor, which include the fluid flow pattern, the kinetics of substrate conversion and bacterial growth, and the sludge distribution and behavior in the reactor (Bolle et al.1986a, 1986b; Buijs et al.1982, 1980; Heertjes et al.1978,1982; Ven der Meer et al. 1983). However, the substrate distributions in the reactor have not been reported, in spite of their obvious importance for better understanding and for optimizing the process. Such a procedure is time and effort consuming and can be tedious because of the huge number of experiments and analyses that must be performed to obtain the details of the profiles. This study was motivated by the surprising shortage of data about the substrate distribution which is needed for modelling and optimization.

In this part of the research, therefore, an extensive study of the profiles in a UASB reactor, i.e.the distribution of the sludge, COD, VFAs and pH under a wide spectrum of operating conditions was conducted. There were 5 different operating conditions, in which the influent COD was increased stepwise from 5 to 40 g COD/l at a HRT of 5 days. For each operating condition, samples were taken from the influent, the corresponding effluent and 10 sampling ports mounted along the column of the reactor.

This study was originally designed, as was stated above, to gain detailed information about the sludge and substrate distributions in the UASB reactor and to serve as the basis of future modelling research, and to increase the understanding of the UASB process. It was also expected to provide information about the cause of the instability of the process which can result in a rapid upset. As these experiments progressed, it was found that the profiles of the substrates truly did provide much information about the instability mechanisms, which will be discussed in this chapter. The results which are summarized in Table 6.1 have shown definitively that two reaction stages, i.e. acidogenesis and methanogenesis could be distinguished in different regions in the same reactor.

6.2 RESULTS

6.2.1 Distribution and Behaviour of the Sludge

The profiles of sludge concentration at different influent concentrations are presented in Figure 6.1. The curves show that two sludge regions exist in the reactor. The dense sludge phase was retained in the lowest part, below 30 cm from the bottom, constituting a sludge bed with VSS of 18 to 56 g/l. In this zone, the sludge concentration varied with the location. For example, at 4 cm height, the sludge content was 35 g VSS/l for an influent of 28.8 g COD/l, while the sludge concentration was 25 g VSS/l at 25 cm height at the same influent concentration. Above 37 cm height (sampling port 4), there was

a sludge blanket. The average VSS concentration in this area ranged from 2 to 10 g/l, depending on the loading rate. Unlike the sludge in the bed, the sludge concentration in the blanket was constant and quite homogeneously distributed. As indicated in Fig.6.1, the plots of sludge concentrations in the blanket (above 38 cm) vs. the reactor height were horizontal lines parallel to the x axis. The results indicated that, in the course of the experiment, the sludge in the blanket was completely mixed, but the sludge in the bed was not well mixed. The mixing, which was brought about by gas evolution, might be insufficient under these experimental conditions in the lower zone, although the biogas production was as high as 75 l/d which equals 0.30 m^3/m^2-h.

Generally, when the organic loading was increased, the sludge bed and blanket both expanded upward, as the result of gas lift and sludge settleability deterioration. As loading was being increased, more gas was produced, creating more flotation to lift the sludge upward. At the lowest influent concentration of 4.5 g COD/l, the sludge bed occupied as little as one eighth of the total working volume of the reactor (below 13 cm from the bottom). With an increase in the influent concentration, mainly as the result of the increase in the gas production, the sludge bed extended to the upper part of the reactor. The sludge bed reached a height of 25 cm when the influent concentration was increased to 9.93 g COD/l. When the loading rate was increased to such an extent that it exceeded the sludge digestive capacity, the sludge would not be able to maintain its vitality. In effect, its settleability deteriorated. Hereafter, poorly settled sludge tended to be suspended. It was particularly true in the case of an influent concentration of 38.1 g COD/l. A large amount of floating sludge was collected in the effluent at this time.

It was common when the reactor was subjected to a new higher organic loading rate, that the increase of gas production was followed by a surge of sludge wash out due to the sudden increase in floatation. If the new operating conditions were within the capacity of the reactor, a new adaptation and stabilization would be re-established, resulting in a

Table 6.1: the Distribution of Sludge and Substrates

Influent g COD/l	Height cm	VSS g/l	COD mg/l	pH	AA mg/l	PA mg/l	BA mg/l	COD Removal
4.56	4.0	26.18	3390		382	92	480	0.26
	12.5	19.04	800		152	60	58	0.82
	25.0	5.42	570		162	100	0	0.88
	37.5	1.96	410		50	56	0	0.91
	50.0	1.69	400		45	52	0	0.91
	62.5	1.51	370		48	56	0	0.92
	87.5	1.42	370		120	60	0	0.92
	112.5	1.39	330		112	44	0	0.93
9.93	4.0	35.92	7520	4.52	688	134	1140	0.24
	12.5	18.18	330	6.40	96	14	14	0.97
	25.0	14.38	290	6.48	60	10	0	0.97
	37.5	5.23	240	6.68	194	0	0	0.97
	50.0	3.96	230	6.65	96	0	0	0.98
	62.5	3.16	220	6.69	338	0	0	0.98
	87.5	2.76	220	6.68	82	0	0	0.98
	112.5	2.65	220	6.66	30	0	0	0.98
17.70	4.0	31.12	10940	4.40	876	290	1454	0.38
	12.5	20.50	560	6.50	20	3	0	0.97
	25.0	18.55	590	6.54	22	3	0	0.97
	37.5	6.19	350	6.72	32	0	0	0.98
	50.0	4.96	380	6.72	20	0	0	0.98
	62.5	4.74	400	6.70	24	0	0	0.98
	87.5	4.73	370	6.71	16	0	0	0.98
	112.5	4.59	340	6.68	18	0	0	0.98
28.80	4.0	35.39	15300	4.50	1166	748		0.49
	12.5	32.11	970	6.90	40	36		0.97
	25.0	25.07	700	7.05	18	34		0.97
	37.5	7.62	520	7.10	20	0		0.98
	50.0	9.35	500	7.10	18	0		0.98
	62.5	7.77	480	7.10	16	0		0.98
	87.5	7.60	480	7.10	22	0		0.98
	112.5	7.87	440	7.15	18	0		0.98
38.10	4.0	56.56	31500	2.89	2685	231	243	0.17
	12.5	38.20	35250	3.12	2895	468	255	0.07
	25.0	39.77	33240	3.12	2883	537	495	0.13
	37.5	31.43	2640	6.70	702	1336	108	0.93
	50.0	3.38	2760	6.70	596	1192	160	0.93
	62.5	4.84	2760	6.70	396	846	44	0.93
	87.5	3.26	2790	6.75	376	722	2	0.91
	112.5	2.69	2690	7.20	262	694	20	0.91

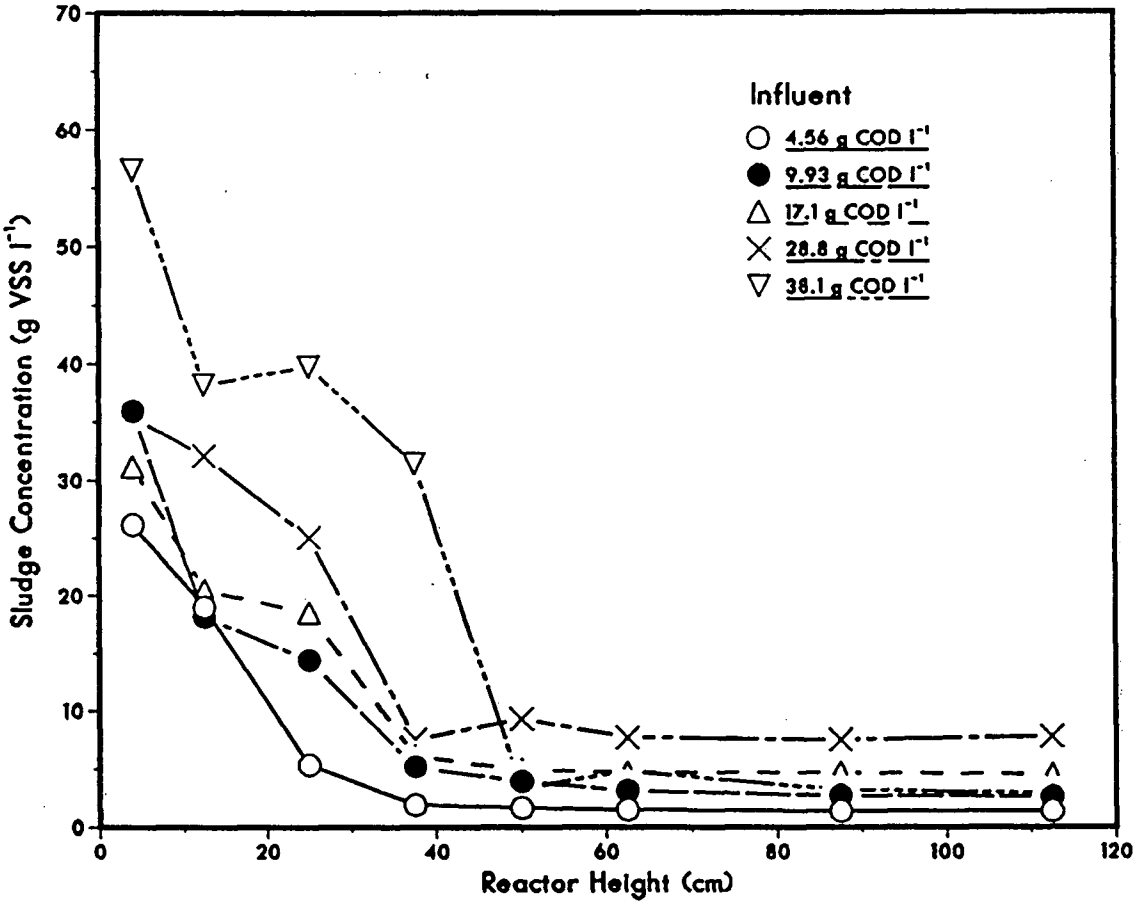


Figure 6.1: **Profiles of Sludge at Different Influent Concentrations**

new balance between sludge and substrates.

Figures 6.2 and 6.3, which show the concentration of the sludge at each location for different influent concentrations, illustrate that the sludge moved upward with an increase in the influent concentration. For example, the portion of sludge at 4 cm height decreased from 41% for the influent concentration of 4.56 g COD/l to 29% for an influent COD of 28.8 g/l, which is different from the higher location of the reactor, at 25 cm height, where the sludge increased from 9% to 19% for the same range of influent concentration change.

The relation between the sludge distribution and the gas production is shown in Figure 6.4. and Table 6.2. The sludge concentration in the bed did not change significantly with gas production, which is indicated by the VSS concentration at sampling ports 1 and 2 in Figure 6.4a, while the sludge concentration in the blanket varied with the gas production (Figure 6.4c). The most significant effect of gas production on the sludge distribution was observed in the area of sampling port 3, which is at the junction between the bed and the blanket. Good linear relations between the sludge and gas production in the blanket, which are shown in Figure 6-4c, were fitted with the following equations:

for the area between the bed and the blanket (Figure 6.4b)

$$X = 8.71\theta + 5.30 \tag{6.1}$$

for the blanket (Figure 6.4c)

$$X = 3.5\theta + 0.7 \tag{6.2}$$

where X is the sludge concentration in g VSS/l and θ is the biogas production l/d. The greater slope of equation 6.1 compared to equation 6.2 indicates the more significant

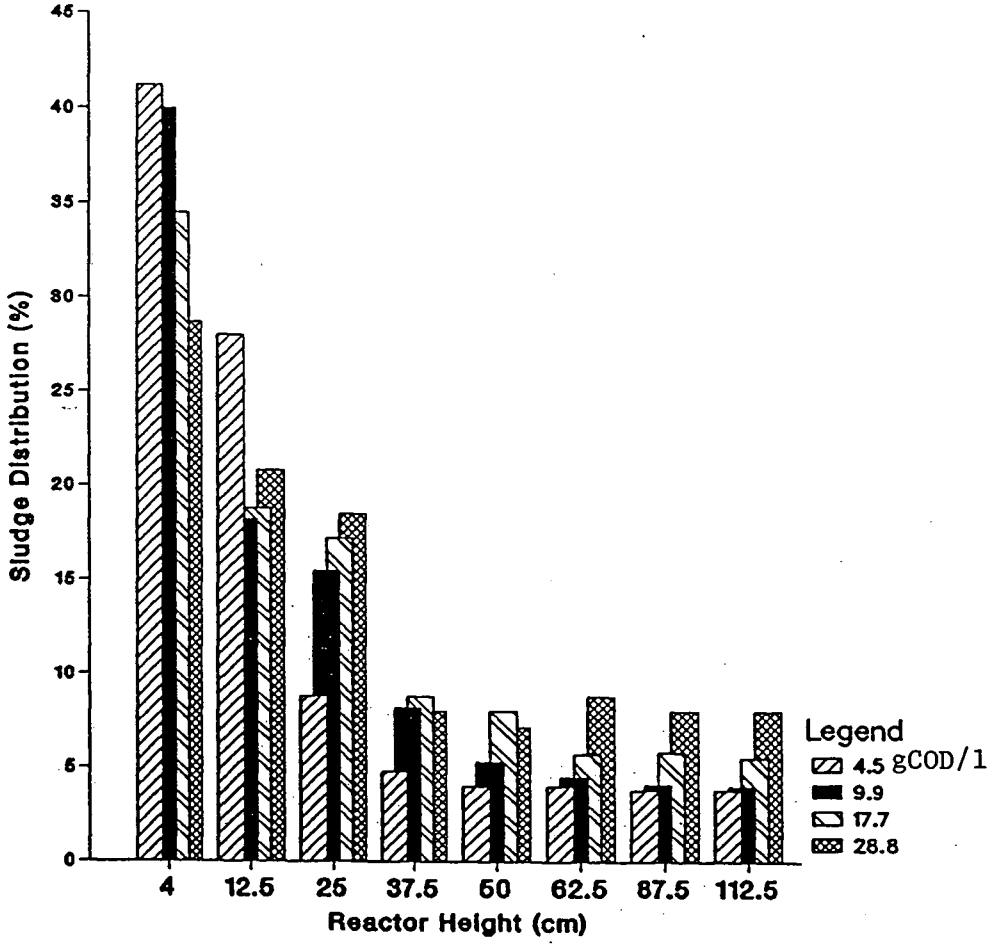


Figure 6.2: **Sludge Distribution in the UASB Reactor**

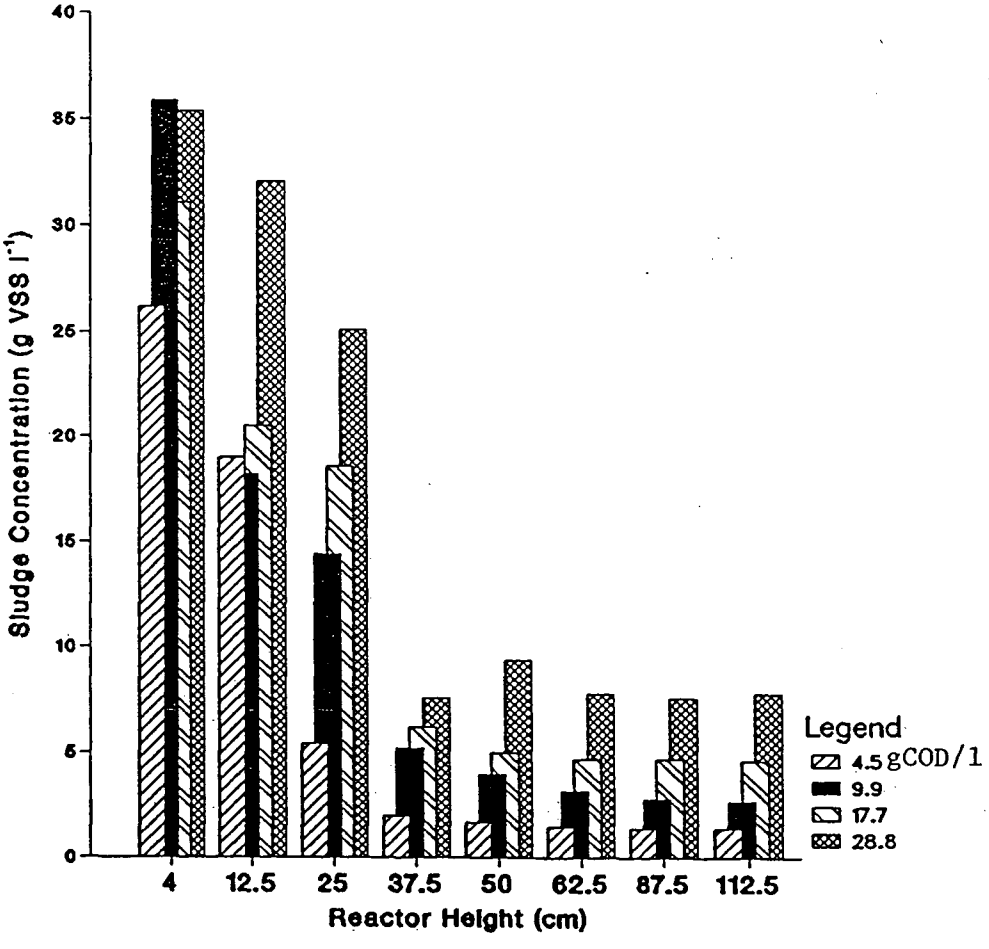


Figure 6.3: **Sludge Concentration in the UASB Reactor**

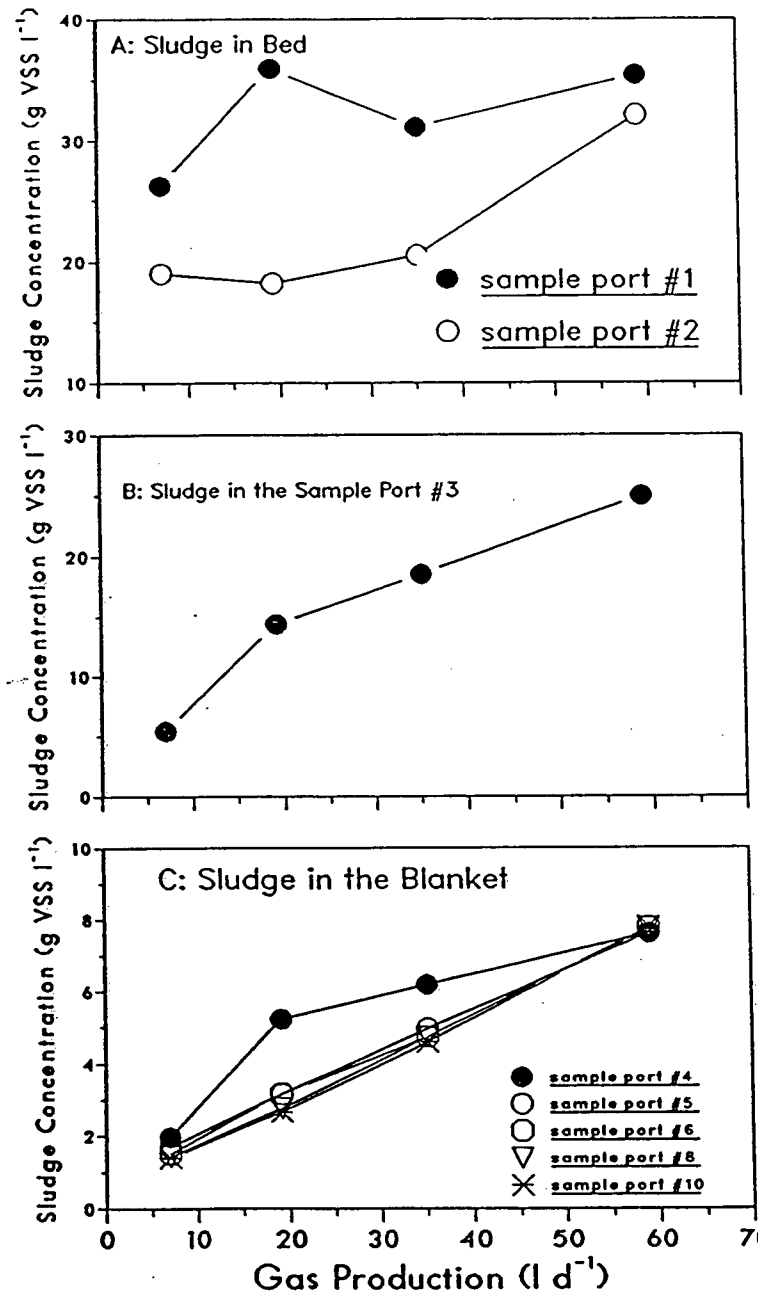


Figure 6.4: **Sludge Distribition versus the Gas Production**

Table 6.2: **Relation between Sludge Distribution and the Biogas**

Input	Biogas	SludgeConcentration g VSS/l							
g COd/l	l/d	1#	2#	3#	4#	5#	6#	8#	10#
4.56	6.96	26.18	19.40	5.43	1.96	1.69	1.51	1.42	1.39
9.93	19.11	35.92	18.18	14.38	5.23	3.96	3.16	2.75	2.65
17.73	35.10	34.58	20.50	18.55	6.19	4.96	4.74	4.73	4.59
28.70	59.02	35.39	32.11	25.07	7.62	9.35	7.77	7.77	7.80

effect of gas flow rate on the sludge distribution in the area between the bed and the blanket than in the blanket.

6.2.2 Growth of Sludge

The total amount of sludge in the reactor was determined by sludge profiles over the height of the reactor for each experimental condition. There was a net increase in VSS concentration in the reactor with loading rate as is shown in Table 6.3. The small amount of biomass formation at the beginning was attributed to the low food/sludge ratio of 0.164 g COD/g VSS d. A significant increase in VSS in the lower regions of the reactor took place only after the influent concentration was increased to 17.7 g COD/l, corresponding to a sludge loading rate of 0.547 g COD/g VSS. These results together with the process operating conditions, which are presented in Table 6.3, provided the possibility of calculating the biomass yield coefficient Y and the decay constant Kd using the following model:

$$\frac{dX}{dt} = Y\frac{dF}{dt} - K_d X \tag{6.3}$$

where Y is the yield coefficient in terms of g VSS/g COD, X is the sludge concentration in the reactor in term of g VSS/l and Kd is the decay constant of the biomass.

A plot of (dX/dt)/X against (dF/dt)/X gave a straight line with Rsq of 0.8 (Figure 6.5), from which the yield coefficient Y and the decay constant Kd can be derived (see Table E.1). They are 0.058 g VSS/g COD and 0.02 d^{-1}, respectively.

The observed yields calculated from the COD balance (see chapter 5) over the whole

experimental period (0.098 to 0.166 g TSS formed/g COD removed) agree quite well with the sludge growth yield of 0.058 g VSS/g COD as estimated from the sludge growth kinetics.(The ratio of VSS to TSS is about 0.49).

6.2.3 Profiles of COD, VFA and pH

COD, VFA and pH were monitored at influent concentrations of 4.56, 9.93, 17.1, 28.8 and 38.1 g COD/l, (pH was not measured at an influent concentration of 4.56 g COD/l). The pH, acetic acid, propionic acid and COD profiles are presented in Figures 6.6, 6.7, 6.8, 6.9 and 6.10, respectively. Each curve represents the effect of different influent concentration at steady state except for a concentration of 38.1 g COD/l.

Below 4 cm, the pH was in the range for 4 to 5, VFA concentrations were high (up to 2895 mg/l of acetic acid) and COD reduction was between 17 to 49%, depending on the activity of the sludge and the influent concentration. Above 12 cm, the pH increased to 6.4 and the volatile fatty acid decreased to 100 mg/l or less. More than 60% of total COD reduction occurred between 4 cm and 12.5 cm above the reactor bottom except for an influent concentration of 38.1 g COD/ l. The two completely different sets of pH COD and VFAs values demonstrate that two separate reaction phases: acidogenesis and methanogenesis were established in the reactor. The acidogenic phase was in the bottom below 4 cm, which was indicated by lower pH, higher VFA and lower COD reduction (Figure 6.11). Above 4 cm, methanogenesis took place. This was demonstrated by higher pH, lower VFAs and high COD removal.

So far, in anaerobic digestion studies, phase separation has been accomplished in two separate reactors by controlling the pH and dilution rate. It is believed that this is the first time that the two phases were reported in the same reactor. It was thought that this would be true for all substrates in a plug flow reactor. Interestingly enough, when using a UASB reactor to treat baker's yeast wastewater in the same lab, two distinct

Table 6.3: **Sludge in the UASB Reactor**

OLR	Input	Sludge	Ratio	Sludge Lost in		Sludge
				Effluent	Sample	growth
g COD/l d	g COD/l	g VSS	g COD/g VSS	g VSS	g VSS	g VSS
	5.00	86.5	0.164	-	-	-
0.91	4.56	133.6*	0.099	22.36	-	3.06
1.97	9.93	107.4	0.262	20.11	13.58	7.37
3.54	17.7	91.7	0.547	4.87	17.87	7.05
5.96	28.7	114.2	0.711	2.32	13.72	38.46
7.77	38.1	164.9	0.654	2.52	13.92	67.14

* Add 67.23 g VSS of sludge to digester at day 15.

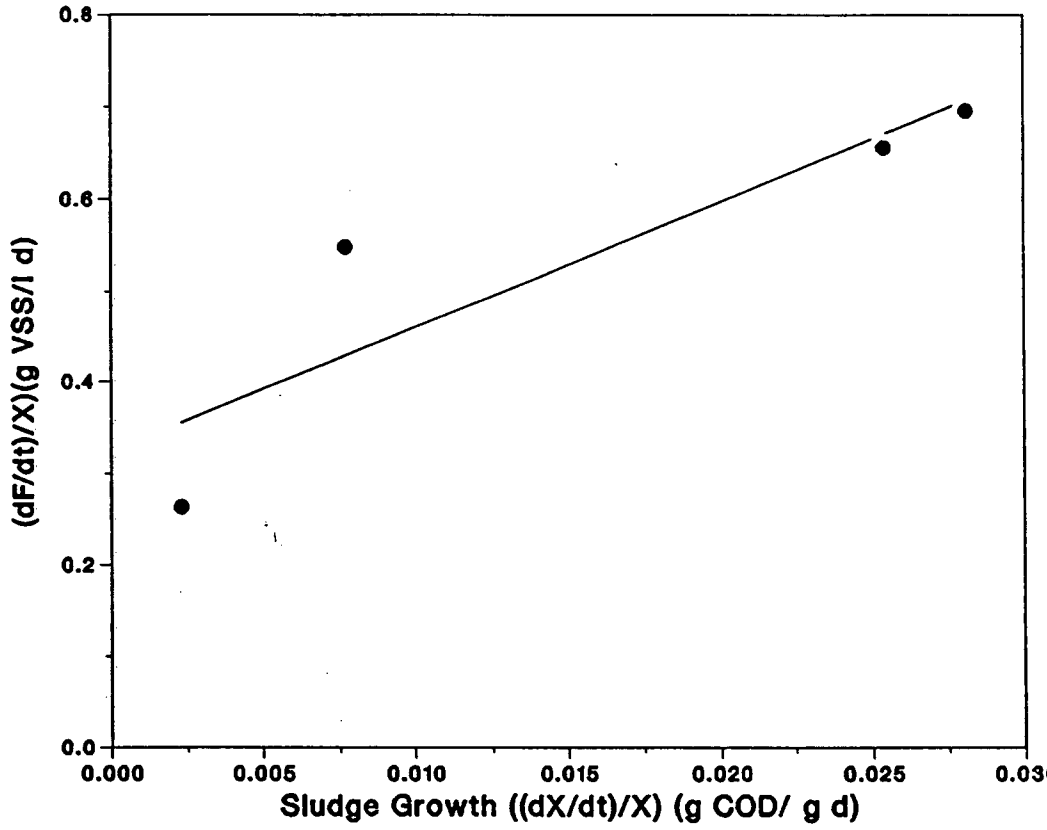


Figure 6.5: Sludge Growth

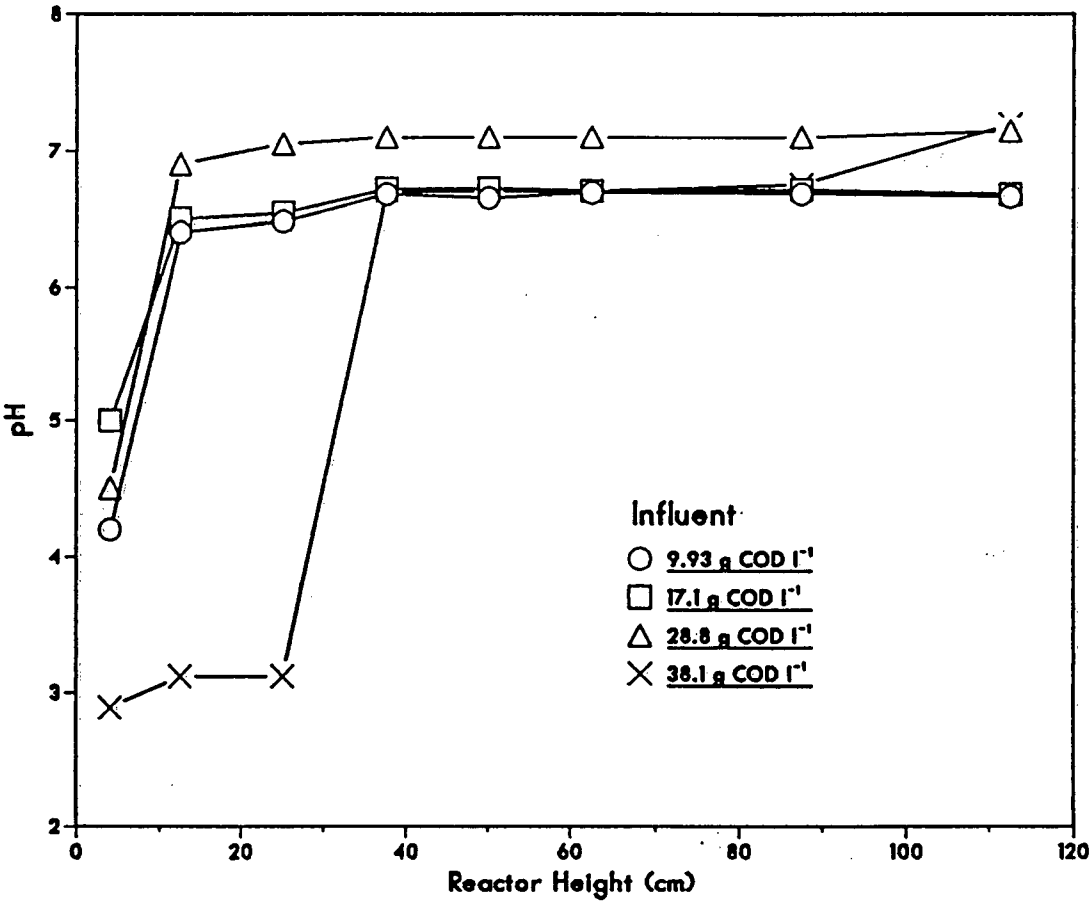


Figure 6.6: pH Profile

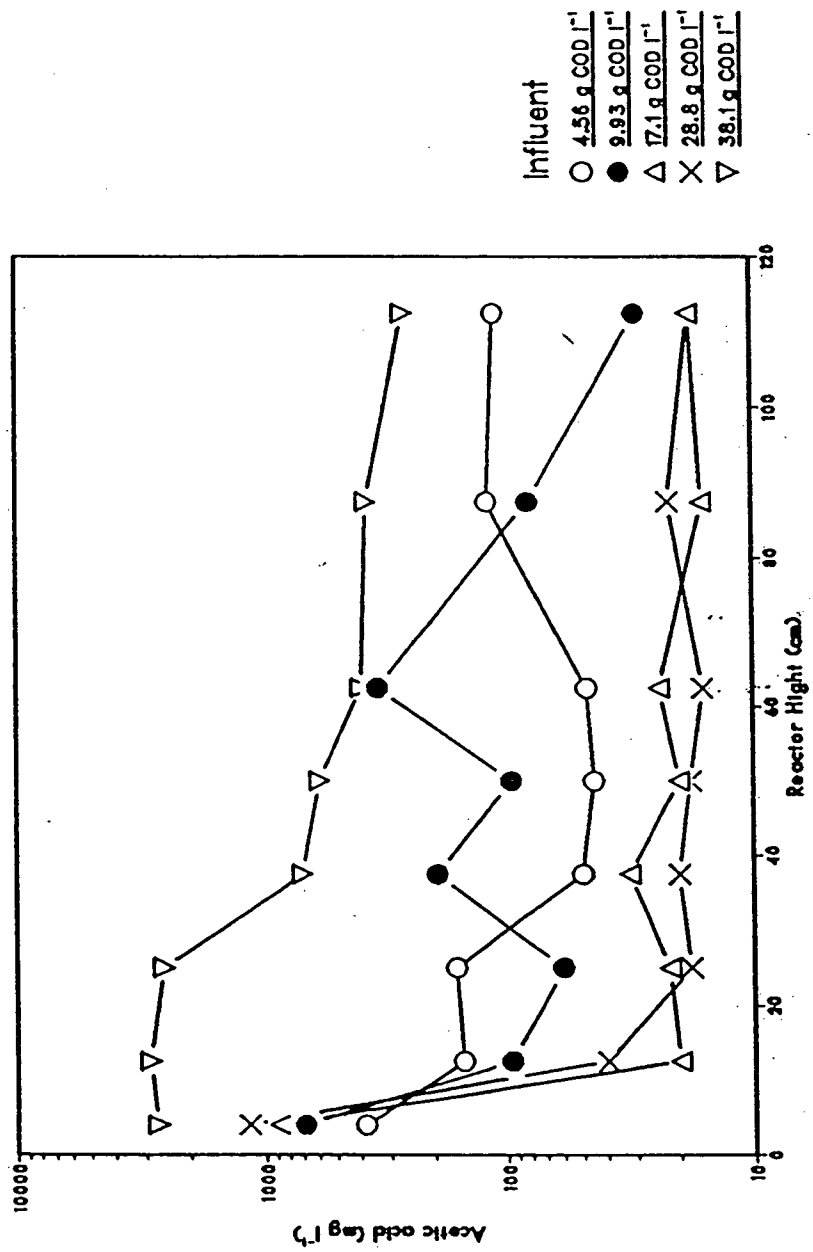


Figure 6.7: Profile of Acetic Acid

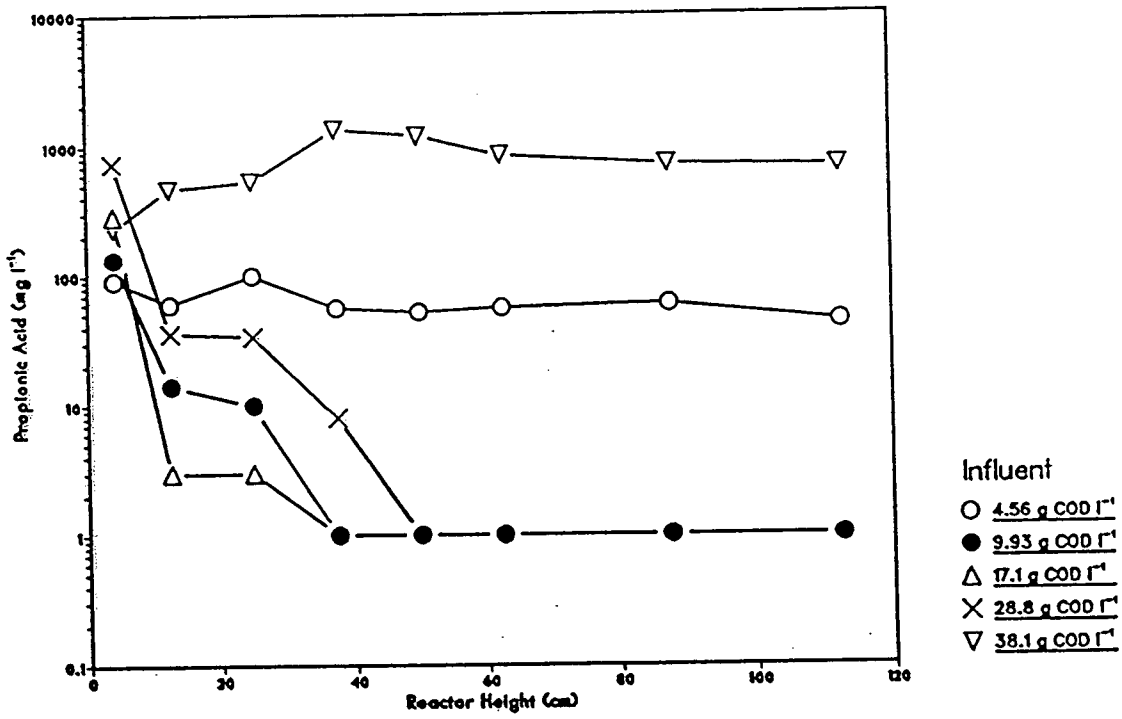


Figure 6.8: **Profile of Propionic Acid**

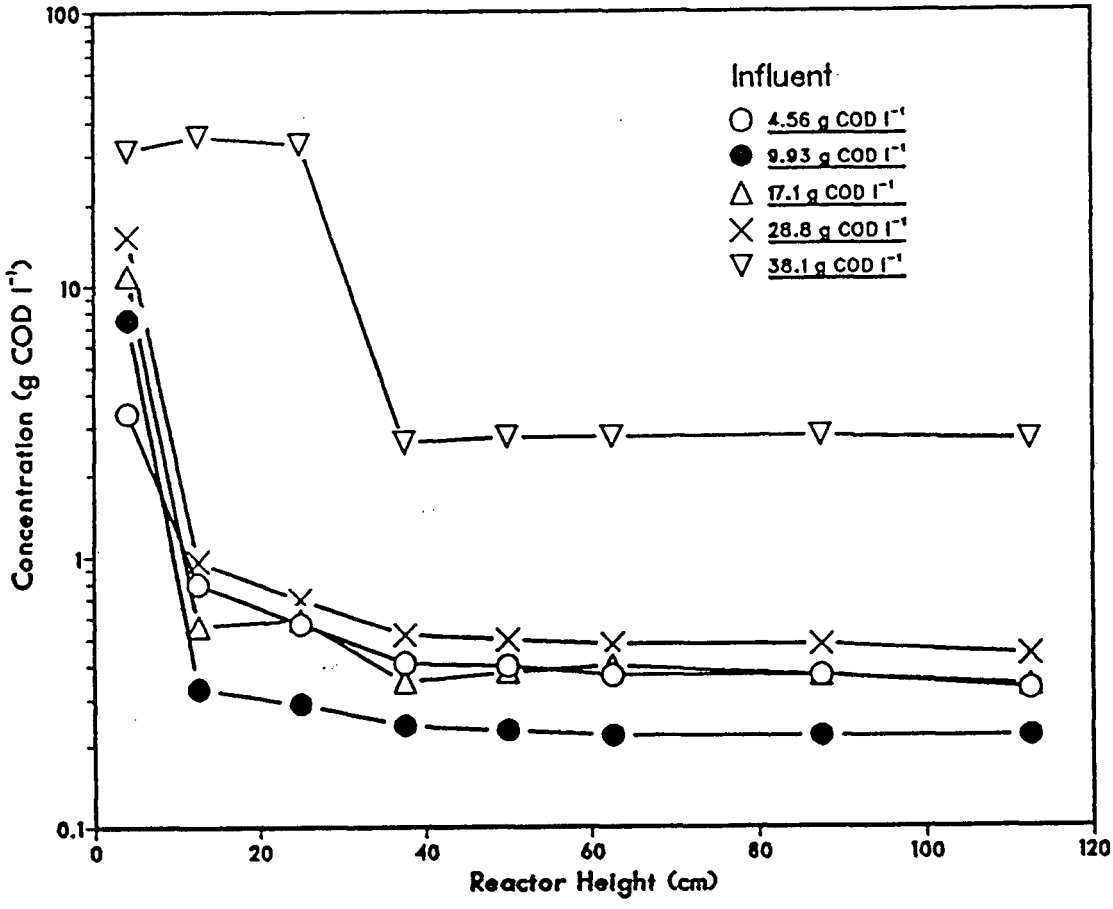


Figure 6.9: **Profile of COD**

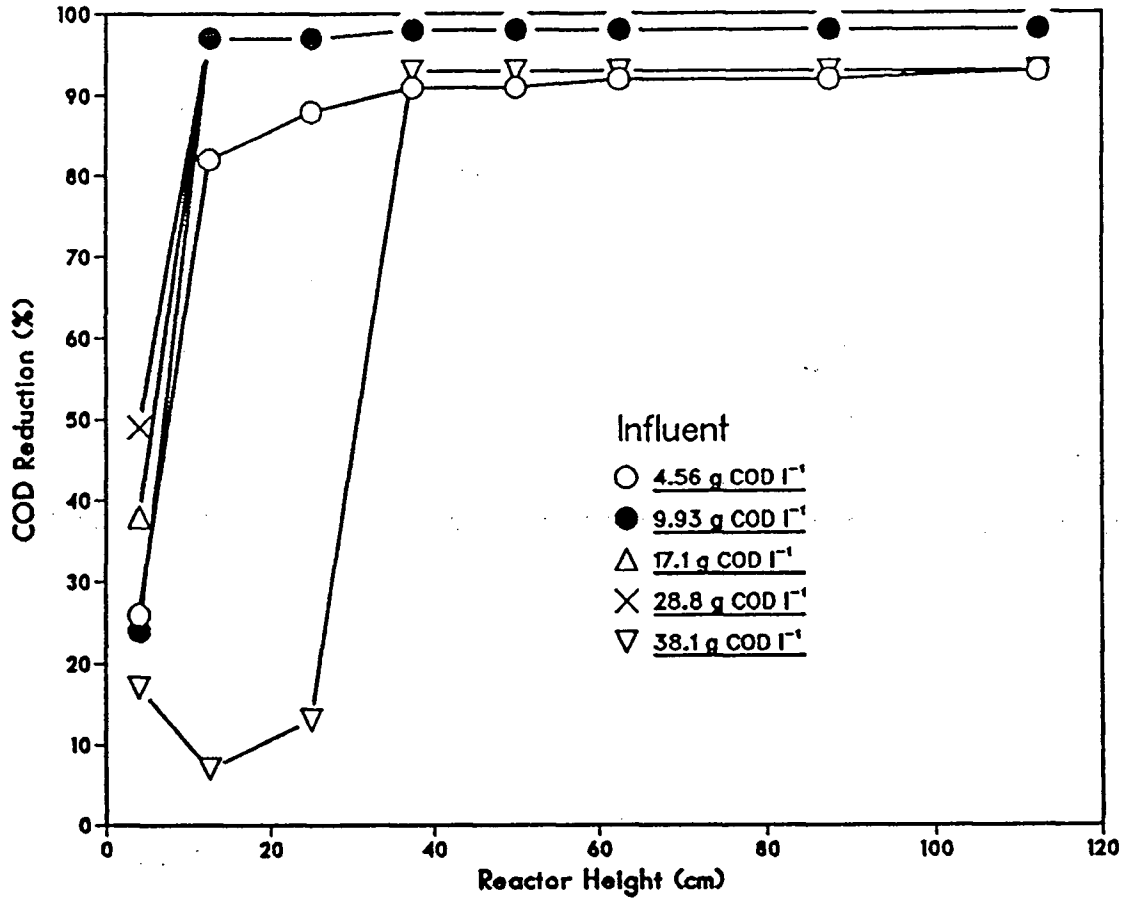


Figure 6.10: **Profile of COD Reduction**

phases were not observed. The different results could be due to the inherent chemical characteristics of the substrates. Anaerobic digestion is a biological process in which a series of parallel and consecutive reactions take place. From an oversimplified point of view, it has been accepted that only two major steps, acidogenesis and methanogensis, are considered essential, and generally, the second one is extremely slow and therefore is the rate-controlling step. If the two major steps remain in balance, the intermediate products, i.e. VFAs, would not be detected. Therefore, two phases wouldn't be seen in one reactor. It is only possible to observe the two phases in cases in which the reaction rates of the two steps are very different. In other words, the observation of two phases in one reactor is only possible for some particular substrates, such as cheese whey which is easily converted into short chain acids by acidogenic bacteria. When more fatty acids are formed than can be converted, VFAs accumulation occurs and the pH drops. The accumulation of VFAs in the first step being faster than the assimilative capacity in the second step creates a distinct acidogenic phase. The backer's yeast wastewater contained high concentrations of hard-to-degrade organic material that could not be easily acidified. In contrast, whey has a tendency of rapid acidification. The observation of two phases indicates that the anaerobic system using whey was not maintained in dynamic balance even at very low organic loading rates even though the overall system, from the effluent analyses, appears to be very stable at influent concentrations below 28 g COD/l.

With an increase in influent concentration, the VFAs and COD in the reactor gradually increased. The curves for COD, acetic acid, propionic acid and pH distribution as a function of the height didn't change markedly until an influent concentration of 38.1 g COD/l was applied . At this condition, the acidogenic as well as the methanogenic zones extended upward. Much higher COD, acetic and propionic acid concentrations were accumulated at the bottom. These high concentrations also extended to a height of 37.5 cm above the reactor bottom. For example, the pH value remained around 3 at a height

of 37.5 cm. In particular, propionic acid concentrations remained high throughout the reactor (Figure 6.11). Low pH values (below 3.2) were also observed in this region. Consequently, the overall reactor performance was affected. The process became unstable 14 days after the reactor was initially fed at this loading. This was indicated by a decrease in gas production from 67 to 61 liters/ day, and an increase in effluent COD from 55 to 643 mg COD/ l and also an increase in effluent acetic and propionic acid concentrations to 80 and 64 mg/l, respectively.

The upward extension of the acidogenic as well as the methanogenic zones causes the intrusion of the acidogenic phase into the region previously occupied by methanogens. The methanogens, previously highly active, in this region could be rendered inactive under the acidic environment. Moreover, the methanogens could not be easily replenished in a newly established methanogenic phase to counteract the accumulation of VFA concentrations due to the very slow growth rate of the anaerobes. It can be predicted that the acidogenic region will extend into the upper portion of the reactor as the substrate loading is increased until the whole region is occupied by the acidogenic reaction and fermentation fails. This is the bottleneck of a suspended growth system. In general, the maximum influent concentration accepted in the UASB process has been 30 g COD/l to the best knowledge of author based on a literature search, even for those substrates which were not quickly acidified.

With an increase in influent concentration, VFA and COD in the reactor increased. In other words, more of the VFAs produced in the first step accumulated. This could indicate that the rate of acidogenesis increased with the increase in influent COD.

Let the acetic acid concentration of sample port # 1 represent the accumulation of VFA in the first phase. The difference in acetic acid between # 1 and # 2 represents the degradation capacity of VFA in the second phase (Table 6.4). The requirement for maintaining the anaerobic system in a dynamic balance is that the degradation capacity

of VFA in the second phase is greater than the accumulation of VFA in the first phase. Based on this idea, the best influent concentration can be found with regard to the system stability.

Using linear regression analysis, a set of empirical models for accumulation of acetic acid and propionic acid with increase of influent concentration has been developed which is the best fit to the experimental data (see Appendix A) .

$$AA = -0.27 + 0.183Co - 0.0102Co^2 + 0.000197Co^3 \tag{6.4}$$

$$PA = 0.112 - 0.00893Co + 0.00108Co^2 \tag{6.5}$$

The accumulation and degradation of acetic acid are shown in Figure 6.12. The degradation rate first increased until the influent concentration reached 20 g COD/l, then declined. Between 15-28 g COD/l, the degradation curve is above the accumulation curve , which means that in this region the degradation capacity exceeds the accumulation capacity. Therefore, this would be the optimal influent concentration for a cheese whey anaerobic fermentation system. This conclusion agrees with the experimental results.

The experimental results indicated that the majority of the COD was reduced below a height of 13 cm in the reactor (more than 80%). The COD concentration decreased from 3.4 g/l at 4 cm to 0.8 g/l at 13 cm for an influent COD of 4.56 g/l, and from 15.3 g/l to 0.97 g/l for an influent COD of 28.1 g/l. The rest of volume of the reactor functions as a settler so that the height of the reactor could effectively be reduced from 140 cm to 30 cm.

From these results the suggested operating conditions and reactor size can be described as follows :

- The optimum influent concentration would be 25 to 30 g COD/l
- The reactor height should be reduced to 40 cm or less for the reactor with a diameter of 12 cm.

6.3 SUMMARY

Profiles of the sludge concentration showed that two sludge regions, a sludge bed with high density VSS and a sludge blanket, exist in the UASB reactor. The distribution of the sludge was strongly dependent on the process conditions. With an increase of the loading rate, the sludge bed expanded so that the sludge concentration in the blanket and also in the area between the bed and blanket, varied because of gas production.

The two reaction stages: acidogenesis and methanogenesis were distinguished in the UASB reactor by the profiles of the substrates, which indicated that cheese whey is very easily converted to short chain fatty acids and that the rate of the first step is much faster than the second step. The appearance of two stages in the same reactor was associated with either the nature of the substrate or process stress and could be attributed to the fact that the rate of VFAs production exceeded the rate of their utilization.

The optimum influent concentration would be between 25-30 g COD/l. The reactor height could be reduced to 40 cm or less.

There is an upper influent concentration threshold of cheese whey for stable operation of the UASB system. If the feed strength is in excess of this threshold value, such as 30 g COD/l at HRT of 5 days, instability occurs because the accumulation of VFAs from the first phase exceeds the degradation capacity of the second phase.

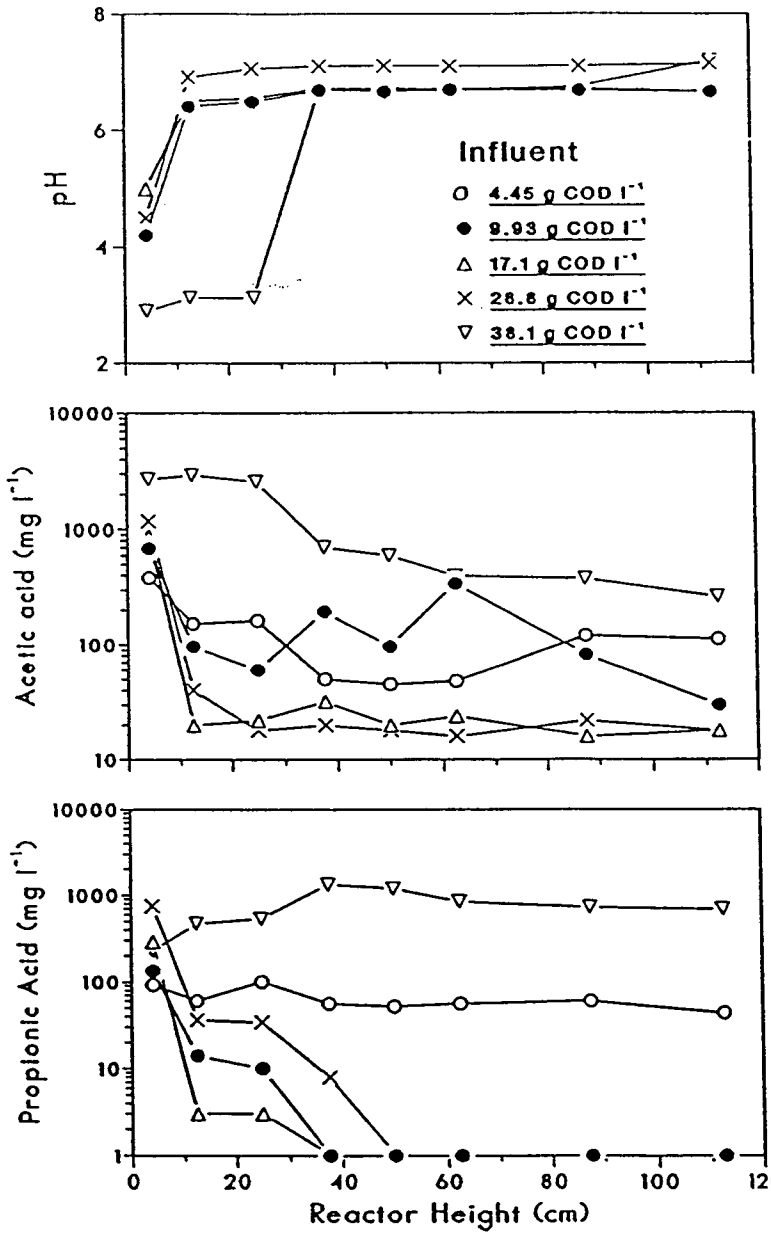


Figure 6.11: **Profiles of pH and VFAs**

Table 6.4: **Acetic Acid (AA)**

Input (g COD/l)	AA at 1# (g/l)	AA at 2# (g/l)	AA_1-AA_2 (g/l)
4.56	0.382	0.152	0.23
9.93	0.688	0.096	0.59
17.7	0.876	0.02	0.856
28.8	1.166	0.04	1.126
38.1	2.895	2.553	0.342

1# sample port 1
2# sample port 2

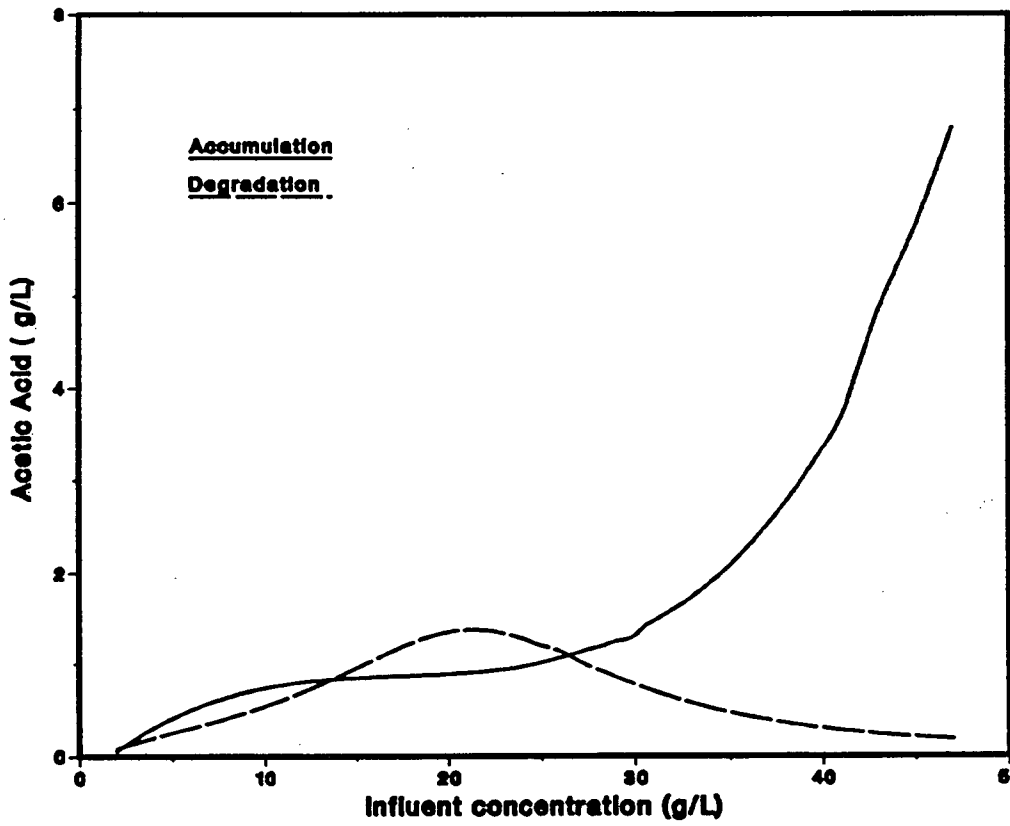


Figure 6.12: Accumulation and Degradation of Acetic Acid

Chapter 7

EFFECTS OF SULFATE ON ANAEROBIC DIGESTION OF WHEY

7.1 GENERAL REMARKS

High concentrations of sulfate have been thought to be inhibitory to methanogenic bacteria. The inhibition of the highly fastidious methane producing bacteria(MPB), due to the presence of sulfate, is usually interpreted in relation to the levels of sulfide produced via the SRB (Lawrence et al 1966 and 1965, Cappenberg 1974, Kroiss et al 1983, Speece et al 1983) . Previous studies have paid more attention to the inhibition caused by sulfate on the anaerobic digestion and several mechanisms have been proposed to explain the inhibitory effect. The kinetics of competition for the available electron donors between sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB) have received considerable attention,and it has been concluded that the SRB apparently have a higher affinity (lower Ks) for hydrogen and acetate, which are the major methane precursors relative to the MPB (Abrum et al 1978, Kristjiansson et al 1982, Schonheit et al 1982). The reason might be the periplasmic location of the hydrogenase of sulfate reducing bacteria (Badziong and Thauer 1980; Bell et al. 1974). Besides, the toxicity of sulfide or free H_2S produced by microbial reduction of sulfate is also thought to be a factor of primary importance.

On the other hand, from the point of view of thermodynamics and hydrogen regulation (Stephen et al 1986, Jack et al 1989), the presence of sulfate appears to help maintain the anaerobic conditions required for the growth of methanogens. Surprising little research

has been done on this aspect, but rather, to provide information which supports this idea theoretically. Motivated by a need to explore the inhibition mechanism caused by high substrate strength and a desire to improve the stability and efficiency of the system as well, an effort was made to examine the effect of sulfate on system performance. The sulfate function in the cheese whey anaerobic fermentation will be the subject in this chapter.

7.2 HYPOTHESIS

It is well known that high organic loading results in an inhibitory effect in the anaerobic digestion of cheese whey. As the preliminary results of Chapter 5 indicated, when influent concentration was increased to 38.1 g COD/l system instability occurred. The distribution of substrates in the reactor given in Chapter 6 further provided evidence that the instability was the result of the ease of conversion of cheese whey into short chain VFAs. The two major steps, acidogenesis and methanogenesis in anaerobic fermentation of whey, have been shown to have very different rates of metabolism. When the accumulation of VFA in the first step exceeds the capacity of the methanogenesis as influent COD approaches a threshold, such as 38 g COD/l, catabolism leads to the system failure. An optimal influent concentration as being found in Chapter 6 can be chosen to avoid the problem. When higher influent concentrations are desirable to achieve the satisfaction of treatment efficiency, however, the question then arises as to how to enhance and control the process stability. Theoretically, the system stability can be enhanced by increasing the activity or concentration of the methanogens, or by inhibiting acidogenic activity, or creating a microbial association which can help to degrade VFAs .

pH control was chosen as a reliable method to neutralize acids for maintaining stability

of an anaerobic reactor. However, the link between pH and system stability has not been clearly shown. In addition, other possible strategies have not been explored for the improvement of the stability of control.

Recent studies have shown that molecular H_2 and interspecies hydrogen transport play an important role in anaerobic digestion since anaerobic β-oxidation of long chain fatty acids is considered to be the rate-limiting step for the digestion of soluble substrates (Figure 7.1, Stephen 1986). As previously described (in the literature review), the free energy changes of β-oxidation are feasible only when the reaction can be "pulled" to the right by the continuous removal of H_2 (Table 7.1, Stephen 1986). In terms of control strategies, then it is apparent that operation of the anaerobic reactor at the lowest H_2 pressure will minimize the accumulation of fatty acids.

Inhibition of the MPB by the SRB has been recognized in relation to the value of K_S, which suggests a higher affinity of the SRB toward H_2 (Kristjiansson et al 1982, 1983). The free energy change for the oxidation of reduced pyridine dinucleotides becomes ever more favorable as the H_2 partial pressure diminishes. The H_2 partial pressures generated by the OHPA (obligate hydrogen-producing acetogens) can be kept at a low enough value by associating them with a H_2-utilizing organism, either a MPB or a SRB.

Therefore, a hypothesis was proposed that the rate-limiting step (for soluble substrates) for anaerobic digestion, β-oxidation, or degradation of fatty acids can be enhanced through the presence of sulfate. In other words, a proper amount of sulfate may be applied to moderate the detrimental influence of excess H_2 on a stressed anaerobic reactor.

A study of the effect of sulfate ions (sodium sulfate) in anaerobic fermentation was conducted to determine whether or not they help maintain a favorable environment for methanogens and how they effect the anaerobic digestion system in terms of methane production, COD reduction, pH, VFA and stability of the system.

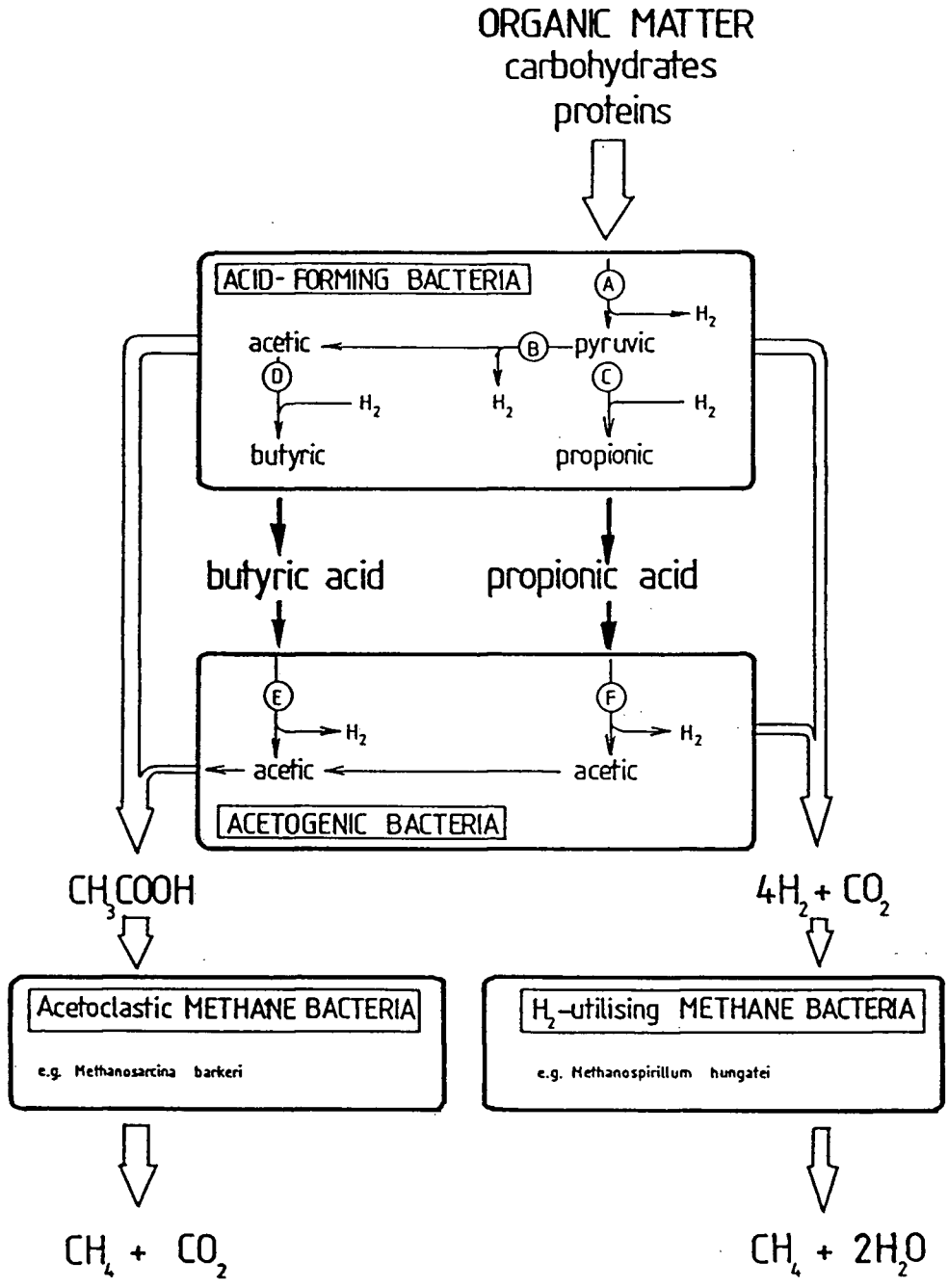


Figure 7.1: **The Microbial Ecology for the Anaerobic Digestion Process** (Stephen 1986)

Table 7.1: **Free Energy Changes of Reactions Involved in Metabolism of Some Organic Matter** (Stephen 1986)

REACTIONS	ΔG^{o}(Kcal)
1. Single culture of H2-producing acetogenic bacteria :	
A. $CH_3CH_2COO^- + 3H_2O$ ----> $CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+18.2
B. $CH_3CH_2CH_2COO^- + 2H_2O$ ----> $2CH_3COO^- + H^+ + 2H_2$	+11.5
C. $2CH_3CHOHCOO^- + 4H_2O$ ----> $2CH_3COO^- + 2HCO_3^- + 2H^+ + 4H_2$	-1.9
D. $CH_3CH_2OH + H_2O$ ----> $CH_3COO^- + H^+ + 2H_2$	+2.3
2. H2-utilizing methanogens and desulfovibrios :	
E. $4H_2 + HCO_3^- + H^+$ ----> $2CH_4 + 3H_2O$	-32.4
F. $4H_2 + SO_4^= + H^+$ ----> $HS^- + 4H_2O$	-36.3
3. Acetate-utilizing methanogens	
G. $2CH_3COO^- + 2H_2O$ ----> $2CH_4 + 2HCO_3^-$	-14.8
4. Syntrophic association of coculture :	
(A+E) $4CH_3CH_2COO^- + 3H_2O$ --->$4CH_3COO^- + HCO_3^- + H^+ + 3CH_4$	-24.4
(B+E) $2CH3CH_2CH_2COO^- + HCO_3 + H_2O$--->$4CH_3COO^- + CH_4 + H^+$	-9.4
(C+E) $2CH_3CHOHCOO^- + H_2O$--->$2CH_3COO^- + HCO_3^- + H^+ + CH_4$	-34.3
(D+E) $2CH_3CH_2OH + HCO_3^-$--->$2CH_3COO^- + CH_4 + H_2O + H^+$	-27.4
(A+F) $4CH_3CH_2COO^- + 3SO_4^=$--->$4CH_3COO^- + 4HCO_3^- + H^+ + 3HS^-$	-36.1
(B+F) $2CH_3CH_2CH_2COO^- + SO_4^=$--->$4CH_3COO^- + H^+ + HS^-$	-13.3
(C+F) $2CH_3CHOHCOO^- + SO_4^=$--->$2CH_3COO^- + 2HCO_3^- + H_2S$	-38.2
(C+E+G) $2CH_3CHOHCOO^- + 3H_2O$--->$3CH_4 + 3HCO3^- + H^+$	-49.1

7.3 RESULTS IN BATCH EXPERIMENTS

A preliminary experiment was first conducted in batch reactors. The details are presented in Appendix B.

An interesting finding was that the effect of sulfate on the gas composition was related to the feed strength. At lower feed strengths, the $CH_4\%$ decreased when sulfate was added. However, no difference in gas composition was observed at higher feed strengths for both continuous and batch experiments. It would seem that the SRB competed with methane bacteria more effectively at lower substrate concentration.

No significant inhibition was observed even when the ratio of COD to sulfate was as low as 5 and the sodium sulfate concentration was as high as 60 mM/l. This can be attributed to the fact that this substrate had higher solubility and higher feed strength than that used in the previous studies (compared to 0.5 - 1 g COD/l). Using higher solubility substrates as feed, such as cheese whey, the inhibition threshold concentration might be higher than that of other substrates which have lower solubility since excess hydrogen exists in such systems. Therefore, the critical inhibition value of sulfate varies from one substrate to another.

The effect of sulfate on pH is very significant. The pH was higher when sulfate was applied. This implies that a proper concentration of sulfate might be able to increases the pH stability of an anaerobic process by competition for hydrogen and fatty acids between the sulfate reducing bacteria and the methane bacteria, helping to maintain stable operation.

Batch experiments could not indicate a meaningful relationships between sulfate concentration and other responses, such as COD and VFAs concentration. A continuous experimental mode was therefore chosen to determine these effects. It turned out that the effect of sulfate addition was far more noticeable in continuous operation than in

batch experiments.

7.4 RESULTS IN CONTINUOUS EXPERIMENTS

7.4.1 Effect of Sulfate on Reactor Operation: Time Profile

During the 300 days of operation, the loading rate of the reactor was increased through 7 steps from 1.2 (the sludge loading rate of 0.2 g COD/g VSS-d) to 10 g COD/l-d. The first 3 load changes were accomplished by the reduction of the HRT from 15 to 5 days at a constant influent concentration of 15 g COD/l with an addition of 0.2 g/l sodium sulfate. Following that, the change of load was done by changing the influent concentration at a HRT of about 5 days. For each subsequent increment of influent concentration, an operating period of 6 to 10 HRTs was maintained to ensure stable operation.

The results are graphically presented in Figure 7.2 to 7.10. Several stages could be identified from the time profile of the reactor performance.

The first 45 days could be considered the start-up period. The effluent concentration decreased from 1.95 to 0.35 g COD/l, while COD reduction increased from 87% to 98%. The reactor start-up was also indicated by other process parameters. Methane content in the biogas increased from 40% to 51%, then stabilized at about 48%. pH of the effluent increased from an average of 7.5 to a value of about 8.2. The content of VFA in the effluent significantly decreased during this period of time. After 45 days operation, the total VFA in the effluent was maintained below 0.3 g/l. Note that there was no difference between the results with sulfate addition and with no-addition. (in comparison with the results in Chapter 5).

From day 46 to 134, in the first 4 increases of organic loading rate (OLR) of 1.2, 1.8, 2.8 and 4 g COD/l d, the system was stable. The effluent COD, VFA and gas composition

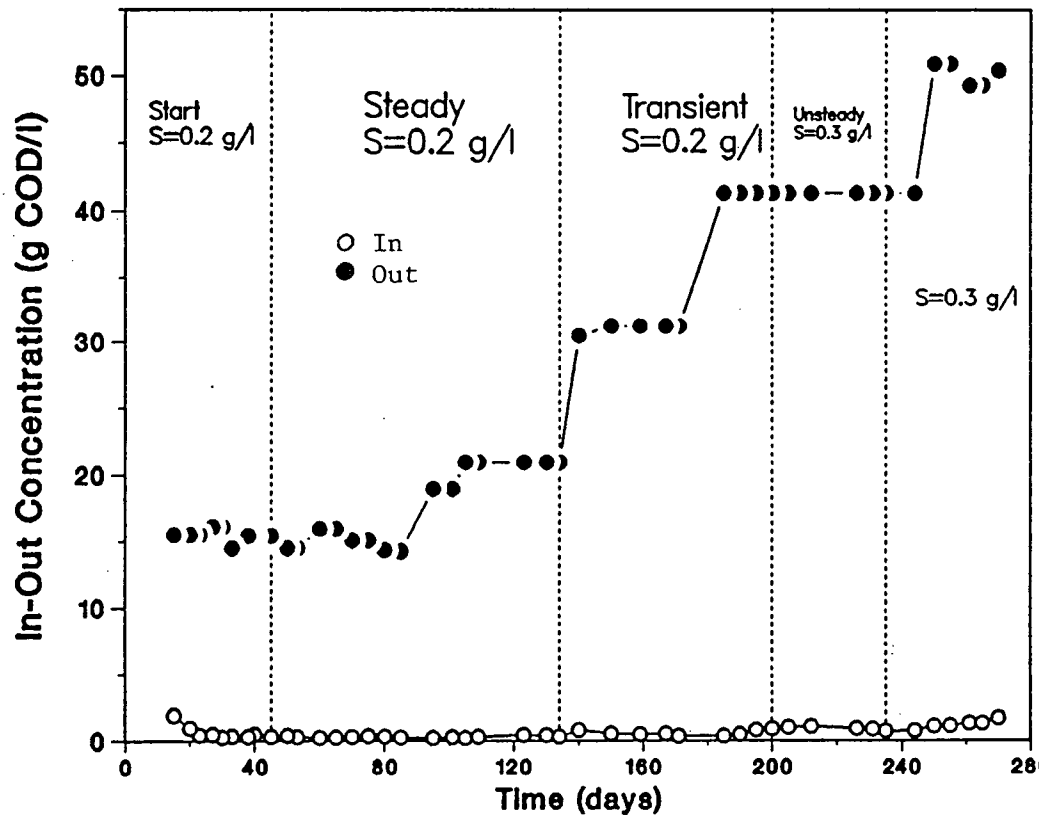


Figure 7.2: **COD Concentration versus Time**

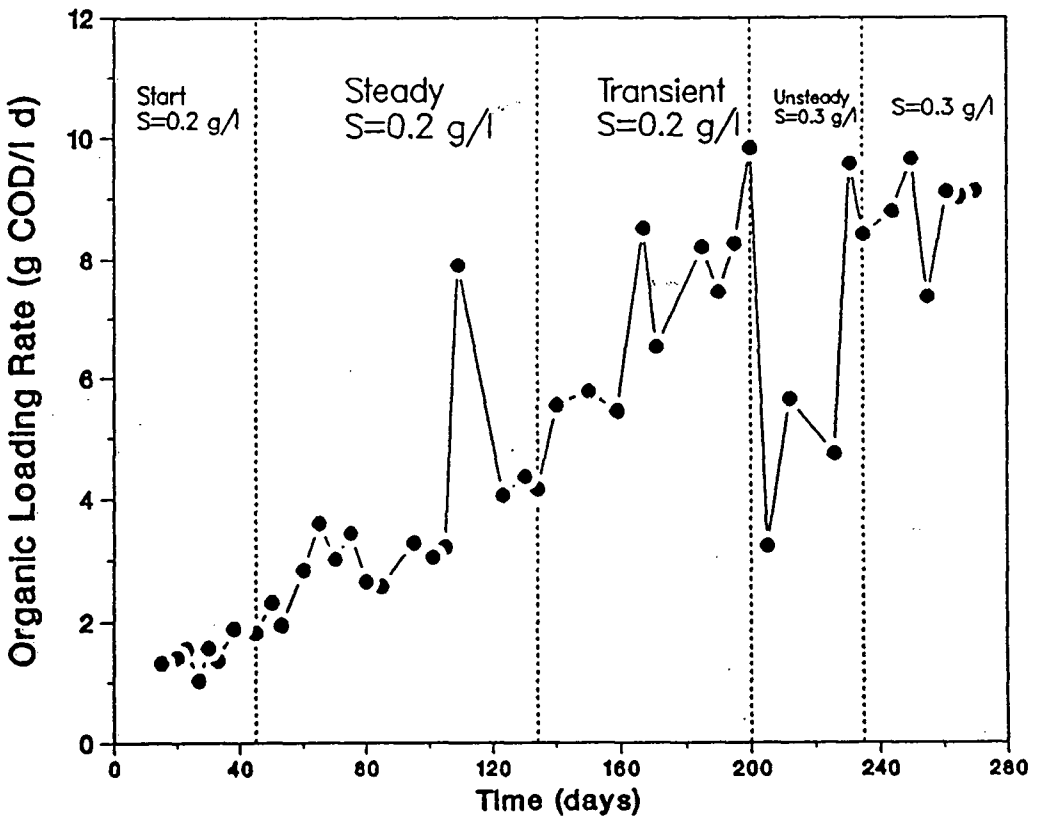


Figure 7.3: Organic Loading Rate versus Time

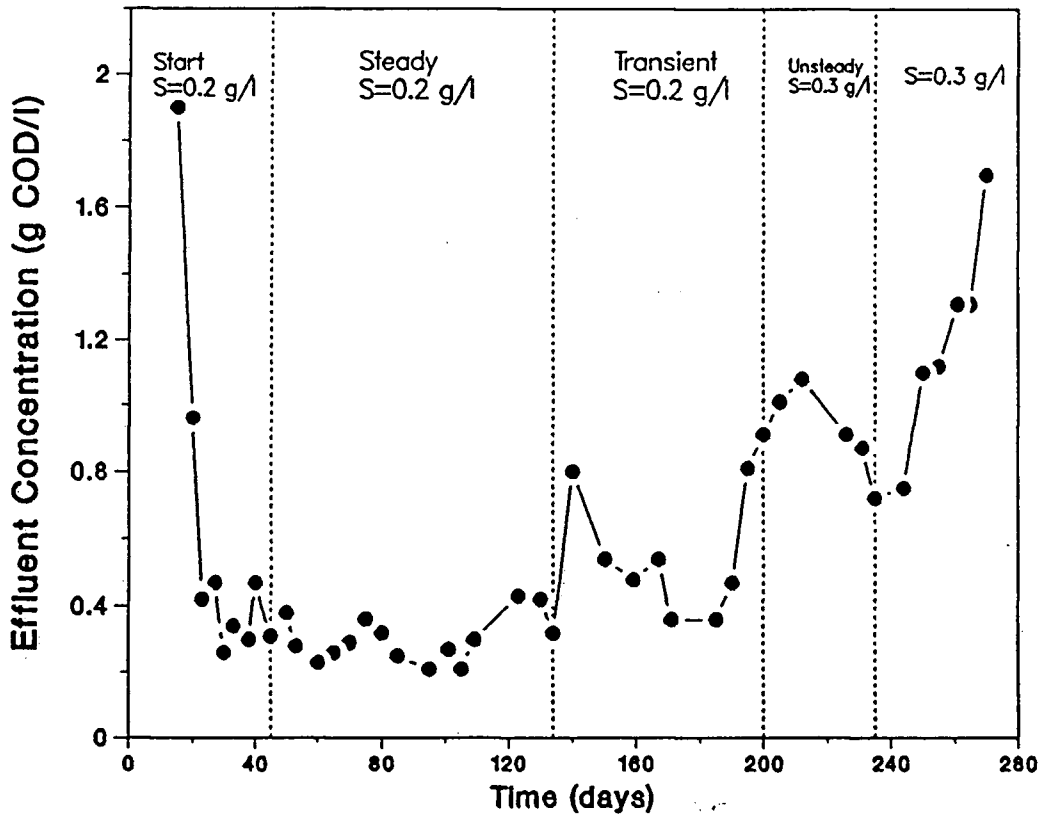


Figure 7.4: **Effluent COD versus Time**

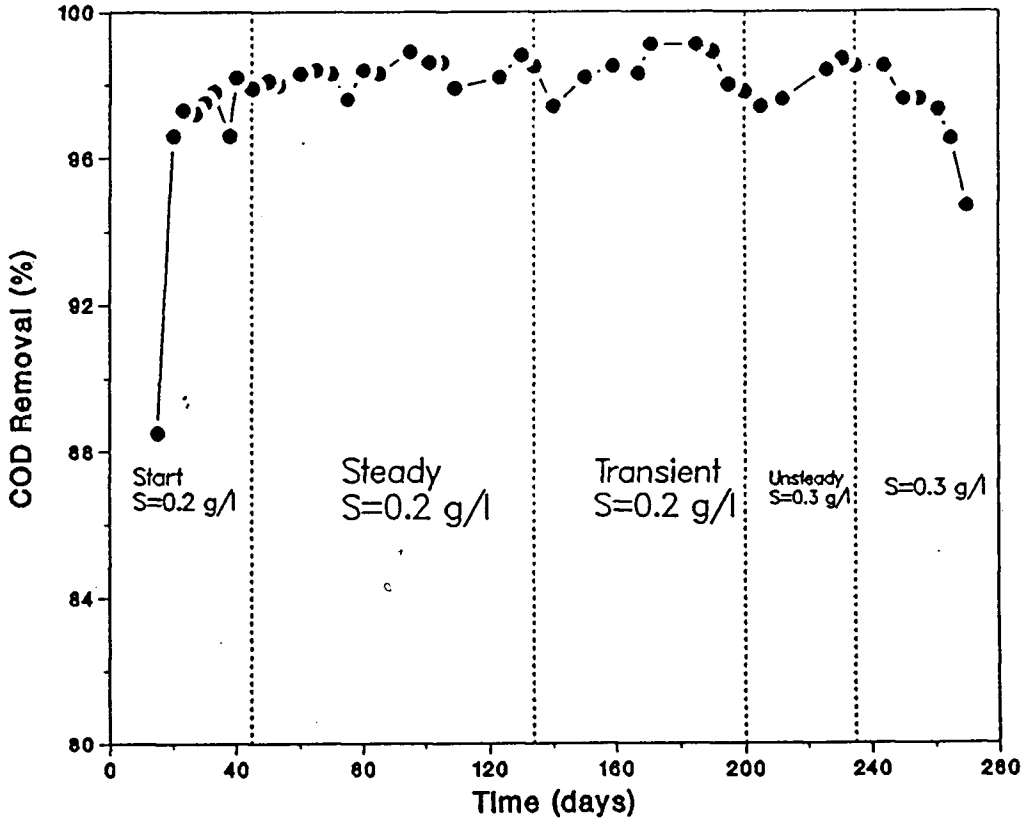


Figure 7.5: **COD Removal versus Time**

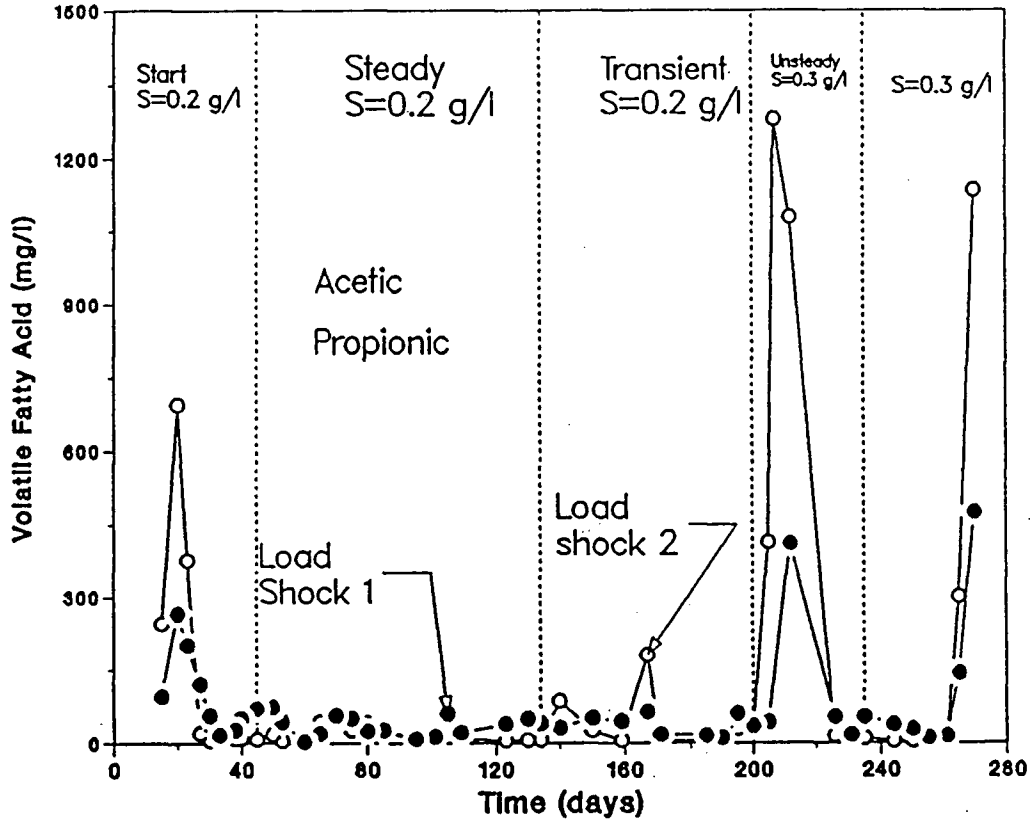


Figure 7.6: **Effluent Volatile Fatty Acid Versus Time**

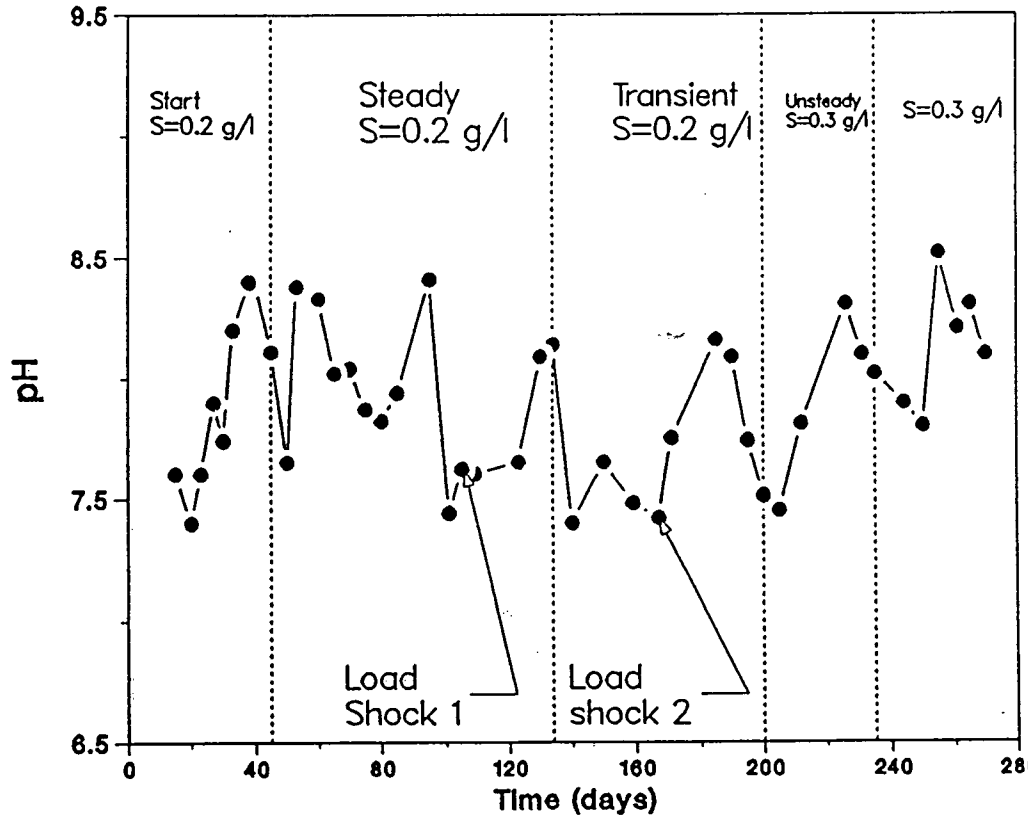


Figure 7.7: Effluent pH versus Time

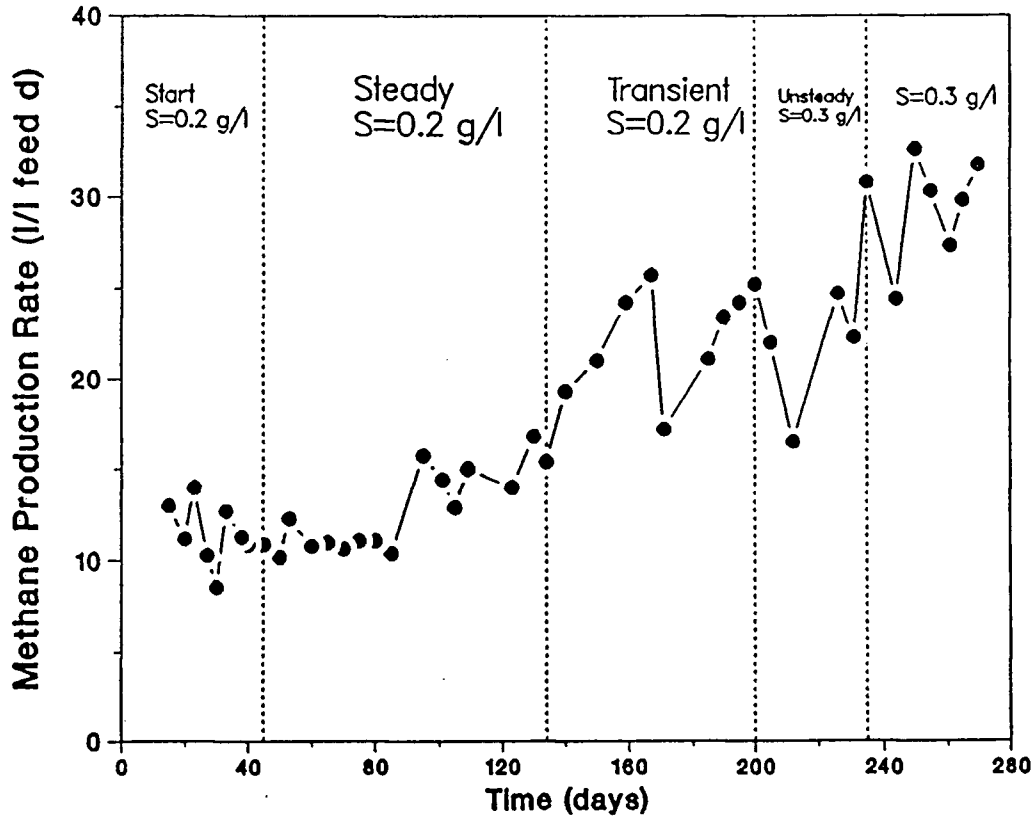


Figure 7.8: **Methane Production Rate versus Time**

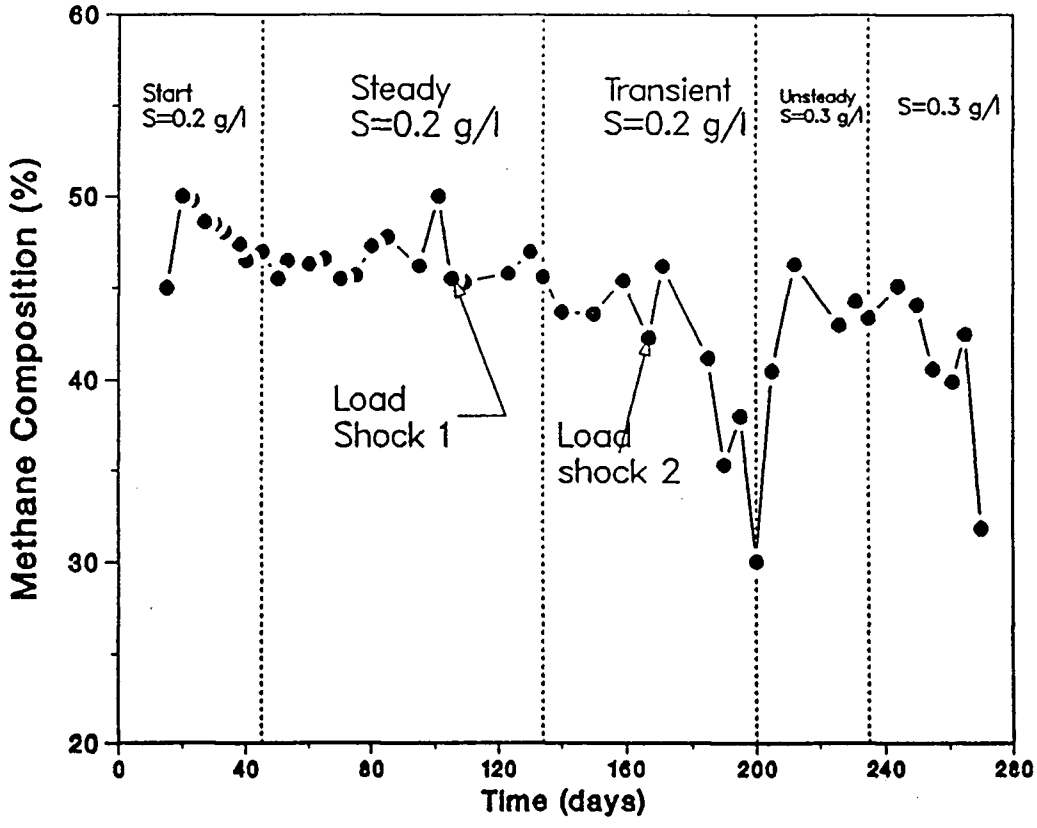


Figure 7.9: Biogas Composition versus Time

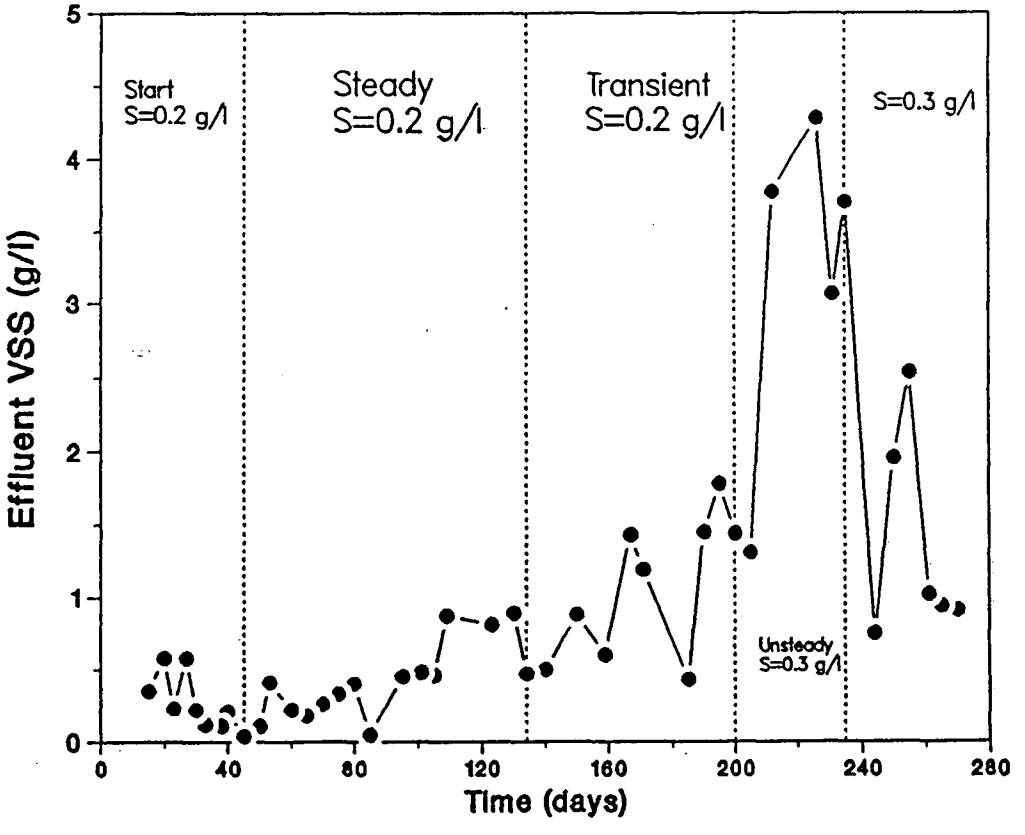


Figure 7.10: **VSS in the Effluent versus Time**

were constant, which indicated that the reactor was in a very active and underloaded conditions without any stress up to an OLR of 4 g COD/l d. The gas production, in terms of liters of methane per liter of feed per day, had not changed as the ORL increased stepwise to 4 g COD/l d. From Figure7.2 we can see that the same influent COD of 15 g/l was applied for the first 3 steps. The OLR was changed by changing the HRT during this period of time. Independent evidence (Yan et al 1988), which showed that the effect of HRT on gas production was not significant for low influent concentrations, agrees with this result.

Similar to the previous results without the addition of sulfate (Yan et al 1989), when an influent concentration of 30 g COD/l (OLR of 5.5 g COD/l d) was applied on the 138th day, the system experienced a non-steady state condition. Only the sensitive parameters, such as VFA, pH and gas composition showed a respond to a "transient state". Gas composition immediately declined to 40% methane. Four days after the new load was applied, VFA, especially propionic acid increased to 500 mg/l and pH dropped to 7.3. The transient state returned to steady state within 1.5 HRTs.

The results of this experiment demonstrated once again that an influent concentration of 40 g COD/l is the threshold concentration for the stability of this system, as is shown for the case without sulfate addition. The instability of the system appeared on day 15 after the load change (day 200). It was first detected by a drop in methane content in the biogas from 47% to 30%, pH fell and VSS in the effluent rose. A large amount of sludge, up to 4.3 g VSS/l, left the reactor with the effluent. The total VFA in the effluent accumulated to 2000 mg/l. The ratio of propionic to acetic acid increased from 0.5-1 at steady state to 1.5, and continued up to 2.5-5.

The results under these conditions with the addition of sulfate, were less favorable than the author expected, based on the concept of interspecies hydrogen transfer and the functions of hydrogenotrophic association of SRB with MPB in the fermentation process.

It was assumed that 0.2 g/l sulfate was not enough to create a favorable environment for methane fermentation of high strength cheese whey up to 40 g COD/l, which represents a ratio of COD to sulfate of 200. Further tests were then made with higher sulfate concentration in the hope that this would enhance the activity of the SRB.

It was first necessary to return the reactor operation to a stable steady state. Various strategies have been tried to bring the reactor back to normal operation. Maintaining the feed concentration at 40 g COD/l, the OLR was decreased from 8.5 to 3 g COD/l d (daily feed of 1.1 liters), then increased step wise, until it again reached 8 g COD/l d. It took 30 days (day 204-233) to re-establish the full activity of the methane bacteria. Both acetic acid and propionic acid decreased well below 200 mg/l. VSS in the effluent decreased, remaining below 1-2 g/l.

After day 235, a feed of 50 g COD/l with 0.3 g/l sulfate concentration was used. The reactor remained stable until day 262, 15 days later the influent concentration was increased to 50 g COD/l. The gas composition suddenly dropped to 40% CH_4 and further declined to 37% the next day.

To avoid an entire upset of the reactor, more sulfate was added into the feed substrate to rise the sulfate concentration to 0.5 g/l in the feed. However, the addition of sulfate was not able to immediately reverse the decline of reactor performance. Methane content in the biogas remained about 39% for a while (day 263-267). Both acetic and propionic acids kept increasing from day 265 to 272, which showed that the high concentration of cheese whey had already upset the reactor. 5 days later, the gas composition return to about 43% CH_4, and both acetic and propionic acids dropped below 0.2 g/l. A much lower butyric acid concentration than found during the previous stressed period indicated that the bacteria were less severely inhibited this time.

A high concentration cheese whey of 69 g COD/l with 0.5 g/l sulfate was used during the last stage from day 282 on. A low methane content of only 31.8% in biogas on the

third day of the last stage indicated that the reactor was subjected to stress. Further addition of sulfate up to 1 g/l no longer improved the reactor stability.

7.4.2 Effect of Sulfate on Steady State Performance

Steady state was defined as the condition in which the system parameters remained constant within $\pm 10\%$ over the period of operation. For each operating condition, at least two HRTs were needed to reach a new steady-state. The results for whey digestion at steady state as a function of influent concentration are summarized in Table 7.2. In comparison with no-sulfate addition, the results at steady state were quite similar in terms of gas production and COD removal with the exception that with sulfate addition the reactor could treat a higher allowable influent concentration and organic loading rate, which shows that no inhibition occurred at these operating conditions. The results show little effect of sulfate on gas composition or methane production rate for continuous operation in the UASB reactor (Figure 7.11 a and b). The presence of sulfate also appeared to have no influence on the relationship between methane production and organic loading (Figure 7.12). The addition of sulfate resulted in similar volumes of biogas produced in terms of l CH_4 /l reactor per day as those in the absence of sulfate. With the increase of influent concentration, the percentage of CH_4 in the produced biogas gradually diminished which indicated that the domination of acidogenesis in the digestion of whey increased with an increase in influent concentration. Gas production in terms of liters CH_4 per gram COD per day also declined when influent concentration was increased.

On the other hand, the presence of sulfate did affect the yield of methane in the batch experiments (Appendix B). The studies on the inhibitory effects of sulfate on the activity of methanogens led Isa et al (1986) to conclude that the competitive nature of SRB relative to MRB was dependent on the nature of the feed substrate. The effect of

Table 7.2: **Experimental Results in Steady State**

		Effluent				
Influent g COD/l	OLR g COD/l.d	COD g/l	pH	COD Removal%	Gas l/l d	CH_4%
15.50	2.03	0.33	7.95	97	5.2	47.25
	3.13	0.28	7.87	98	5.5	47.41
20.96	4.21	0.32	7.73	98	7.2	45.50
30.50	5.58	0.63	7.56	98	9.1	43.92
41.30	7.52	0.68	7.56	98	10.9	41.07
50.91	11.41	1.16	7.77	97	13.9	40.10

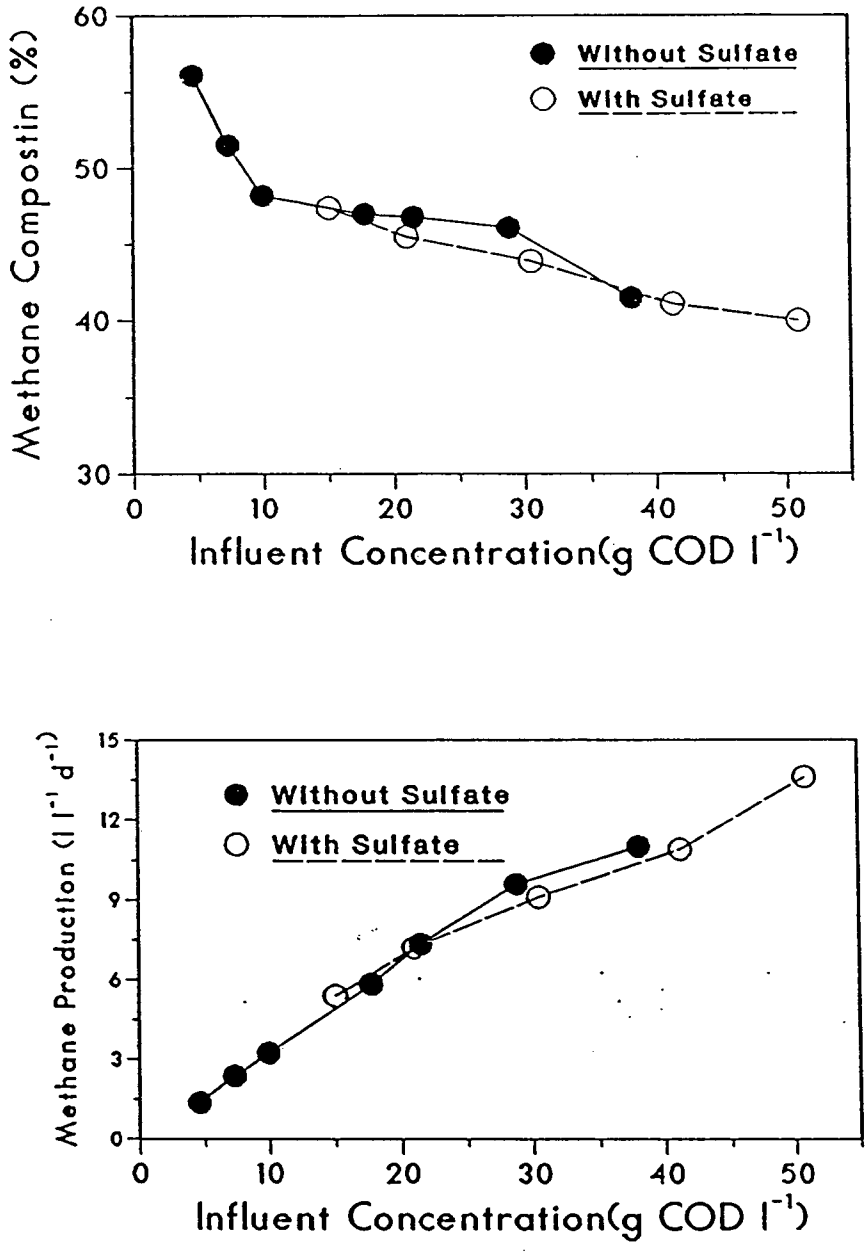


Figure 7.11: **Effect of Influent Concentration on Methane Production and Composition**

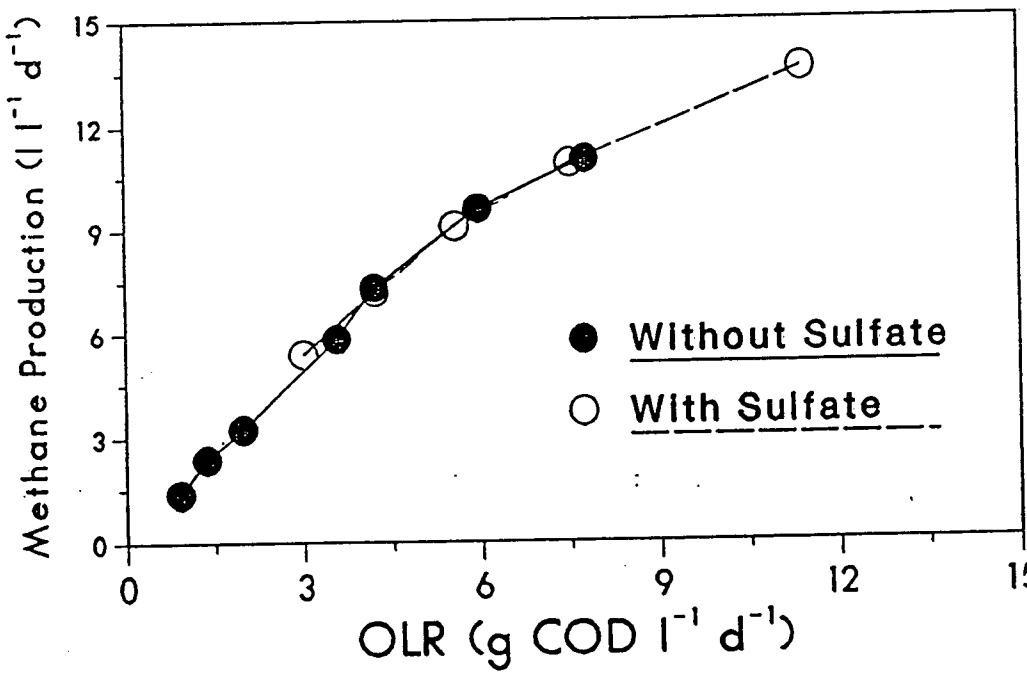


Figure 7.12: Effect of OLR on Methane Production

sulfate on biogas composition can be attributed to the different substrate mix used in two experiments, basically the ratio of substrate to sulfate. In the batch experiments, the concentration of whey and sulfate were compatible, since the ratio of COD to sulfate ranged between 5 to 10. Only small amounts of sulfate were employed in the continuous experiments, a range of ratios from 100 to 200. The substrate concentration used (0.5-5 g COD/l) by Isa was also much lower than those used in the continuous experiments of this study. In addition, the different types of substrate used in the two cases should be also taken into account. Using acetate as a feed material for digestion, Isa found that both specific methane production and CH_4 increased as the feed concentration was increased. This was interpreted as meaning that the SRB became more competitive at lower substrate strengths. Since acetate was the only carbon source and cleavage of acetate therefore was the only major reaction involved in the digestor, it is reasonable to believe that the methanogenic cleavage of acetate was inhibited by SRB at a very low H_2 partial pressure. When whey, which mainly consists of lactose, was used as substrate for anaerobic digestion, the reactions involved in interspecies H_2 transport were more complicated. H_2 as an interspecies plays many roles in whey fermentation. In this case, SRB could be competing for H_2 with H_2-using methanogens and could inhibit both H_2-using methanogens and acetoclastic bacteria. On the other hand SRB might also promote and stimulate the degradation of fatty acids by removing excess hydrogen. The final result would be the overall effect of these two opposing mechanism.

The amount of sludge was monitored by measuring the VSS profile in the reactor and the VSS content in the effluent. These results are presented in Table 7.3. A plot of (dX/dt)/X vs (dS/dt)/dX provides the means for determining Y (yield coefficient) and K_d (decay coefficient), which were 0.053 g VSS/g COD and 0.00047 day_{-1}. The sludge yield was the same, while the decay rate was much lower than in the process without sulfate.

Despite the fact that methane production, effluent COD and COD reduction at steady state for the two cases (with sulfate addition and without sulfate addition) were virtually the same, several remarks can be made about the difference in the two operating conditions.

7.4.3 The Improvement of Reactor Stability

The impressive improvement in the reactor stability with the addition of sulfate was a significant result. During the first 180 days, the reactor experienced a number of load shocks. The first load shock appeared on day 106 when the reactor was fed 3.4 l/d of cheese whey with a concentration of 20 g COD/l daily. Two days later, 5.4 l of the same concentration were pumped into the reactor again. Surprisingly, except for gas production and gas composition, which drastically decreased then rapidly recovered, there was no other evidence that the reactor was becoming unstable. This is seen by considering the constant VFAs and COD concentration in the effluent. The second load shock was applied on day 167. 3.9 l/d of 31 g COD/l cheese whey was used. At this time, both the gas production and effluent VFA decreased immediately, then returned to their normal values shortly afterwards. The reactor was so stable that it was expected that an influent concentration of 40 g COD/l could be applied.

Although 0.2 g/l of sodium sulfate concentration in the feed did improve the bacterial resistance to shock loads, it could not help with further increases in the loading rate. As in the earlier studies, the influent concentration of 40 g COD/l or OLR of about 8 g COD/l-d caused instability. Even though the VSS profiles show that the total amount of sludge was greater than that without sulfate, it could not help to improve the OLR, nor could the 0.2 g/l of sulfate added. It was interesting to find that no H_2S was detected when only 0.2 g/l sulfate was added to feed, while 0.3-0.6% H_2S was observed for 0.3 g/l sulfate addition. It could be explained that sulfate was first used as nutrient for MPB

Table 7.3: **Sludge in the reactor**

OLR	Inf.COD	Sludge	F/S	lost in Eff.	lost in Sample	Net Growth
g COD/l-d	g COD/l	g VSS		g VSS	g VSS	g VSS
		128.95	0.250	-	-	-
2.96-3.13	14.4	142.21	0.254	-51.75	-	65.01
4.03	20.96	206.13	0.254	64.26	14.48	142.66
5.50	31.24	253.34	0.308	115.83	33.59	185.53
7.56	41.36	152.52		198.24	24.1	
9-11.	50.42	224.52	0.56	130.56	18.57	220.86

and SRB rather than hydrogen utilizing reagent.

7.4.4 The Effect of Sulfate on Buffer Capacity

Figures 7.13 and 7.14 show the effect of sulfate on pH by comparing the results with and without sulfate addition. A statistical analysis of the data using Minitab t-test is presented in Appendix D. It was evident that there was a substantial increase in pH with sulfate addition, an average of 0.6 (from 0.48 to 0.82) pH units higher than without sulfate.

7.4.5 The Improvement of Treatment Efficiency

The improvement in the treatment efficiency can be seen from the higher allowable influent concentration with further sulfate addition of 0.3 - 0.5 g/l (Table 7.2). Instead of 30-38 g COD/l without sulfate (Table 5.2), the highest permissible feed concentration reached 50 g COD/l in the experiments with sulfate of 0.5 g/l. Accordingly, the organic loading rate rose to 11.41 g COD/l-d, which is higher than without sulfate, 7 g COD/l d.

7.4.6 The Effect of Sulfate on Profiles of pH, Sludge and VFAs

Profiles of pH, sludge and VFAs as a function of influent concentration are showed in Figure 7.15 to 7.19 and Table 7.4.

In general, it can be said that sulfate addition in the amount applied here did not change the shapes of the profiles. Two different stages were distinguished again for the first 4 operating conditions, as was noticed in earlier experiments. Acetic acid, propionic acid and COD were well reduced below a height of 12 cm, and the border between the two stages appeared clearly. Also, two sludge regions, a sludge bed and sludge blanket,

were observed as shown in Figure 7.15. However, the sludge was darker and thicker in the reactor with sulfate addition than without sulfate.

The profiles of the VFAs showed a change when the influent concentration was increased to 50 g COD/l, presumably due to the fact that the organic loading rate of the reactor approached its upper limit. Under this circumstance, concentrations of acetic acid and propionic acid drastically increased and the acidogenic stage extended upward. At a height of 50 cm, 1100 mg/l of acetic acid and 1400 mg/l of propionic acid were detected. Acetic acid concentration was essentially zero at a height of 80 cm, while propionic acid maintained a high concentration of 1600-2000 mg/l. In spite of the high propionic acid concentration in the reactor, the system was fairly stable.

The establishment of biomass activity and the fermenter performance can largely be interpreted by its VFA values. Total VFA concentrations greater than 2000 mg/l, or acetic acid alone being greater than 800 mg/l and a P/A ratio greater than 1.4 are the predictions of anaerobic process failure, as suggested in the literature (Hill et al,1987). The extremely high propionic acid concentration observed in the reactor with faily stable operation showed that the reactor performance was superior due to the sulfate addition. Figures 20 and 21 show the difference in acetic and propionic acids in the reactor for the absence and presence of sulfate. When the influent concentration was increased to 38.1 g COD/l without the addition of sulfate, acetic acid and propionic acid were as high as 3000 and 1000 mg/l, respectively. However, acetic acid and propionic acid were only 30 and 100 mg/l at the influent concentration of 40 g COD/l/ when sulfate was added.

A substantial decrease in butyric acid concentration in the acidogenic stage caused by sulfate addition was observed. Figure 7.22 shows this effect. In the absence of sulfate, a considerable concentration of butyric acid was accumulated in the first stage (acidogenesis), but was hardly detectable in the presence of sulfate.

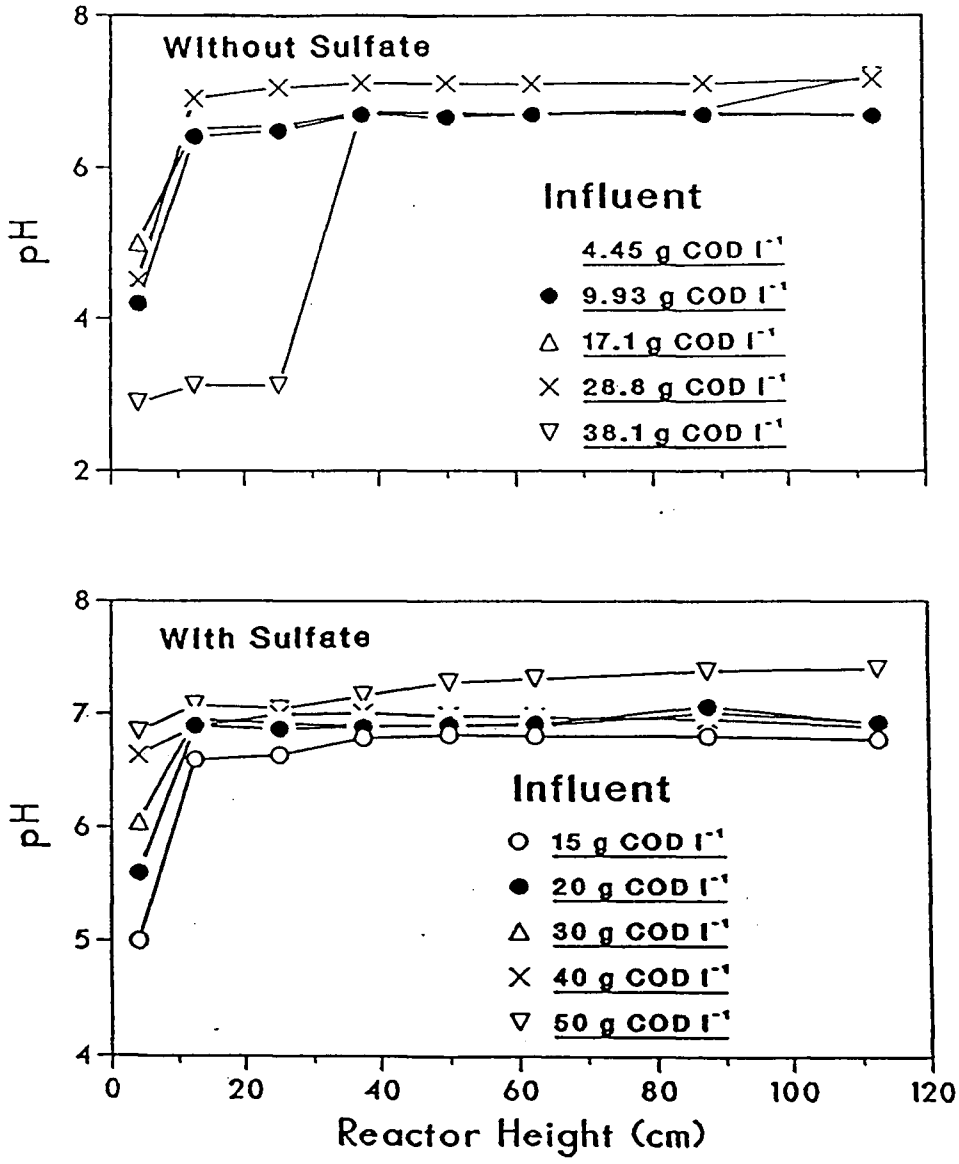


Figure 7.13: **Effect of Sulfate on pH Profiles**

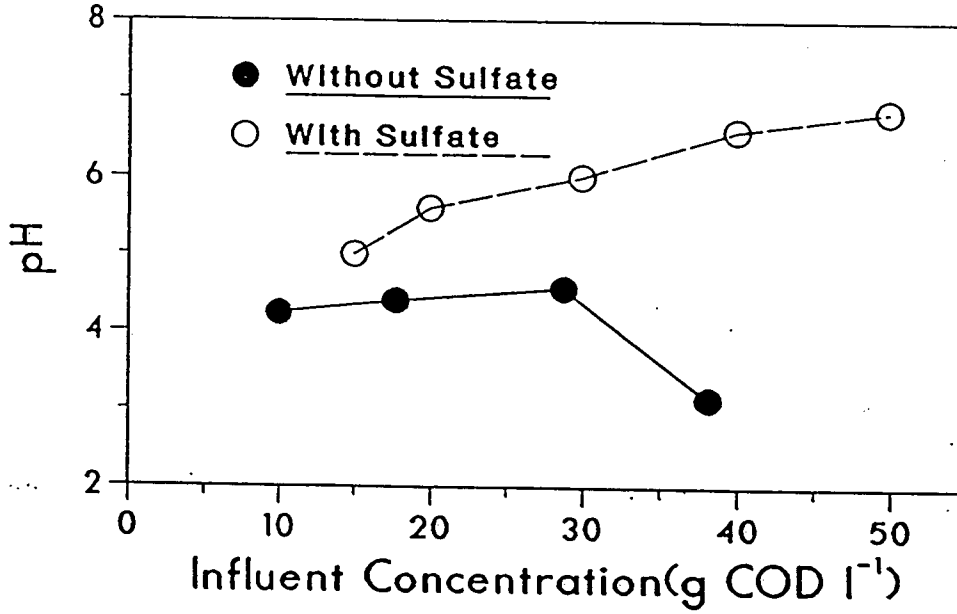


Figure 7.14: **Comparison of pH without Sulfate and with Sulfate**

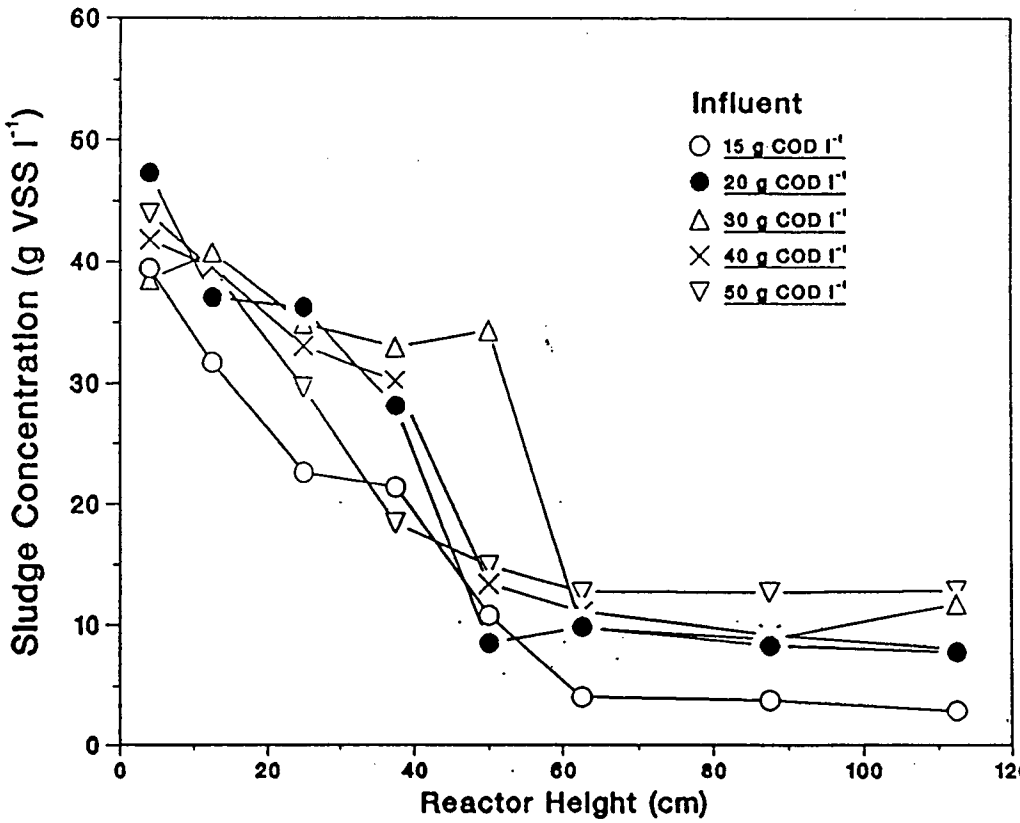


Figure 7.15: **Profile of Sludge**

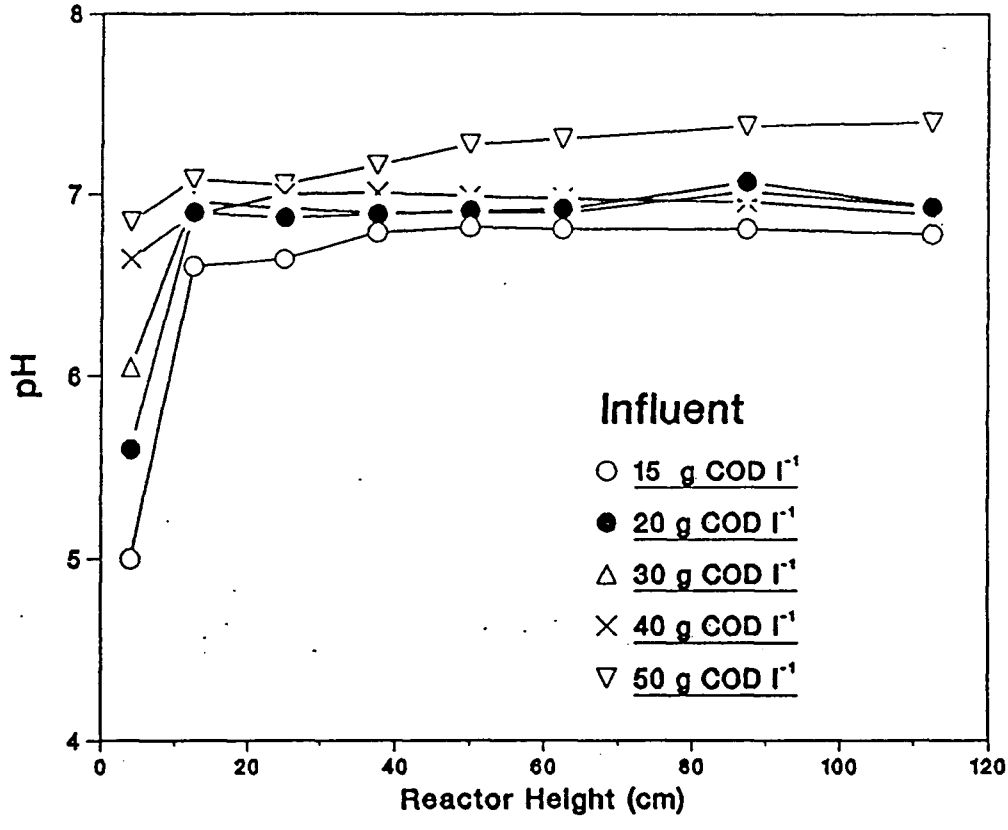


Figure 7.16: **Profile of pH**

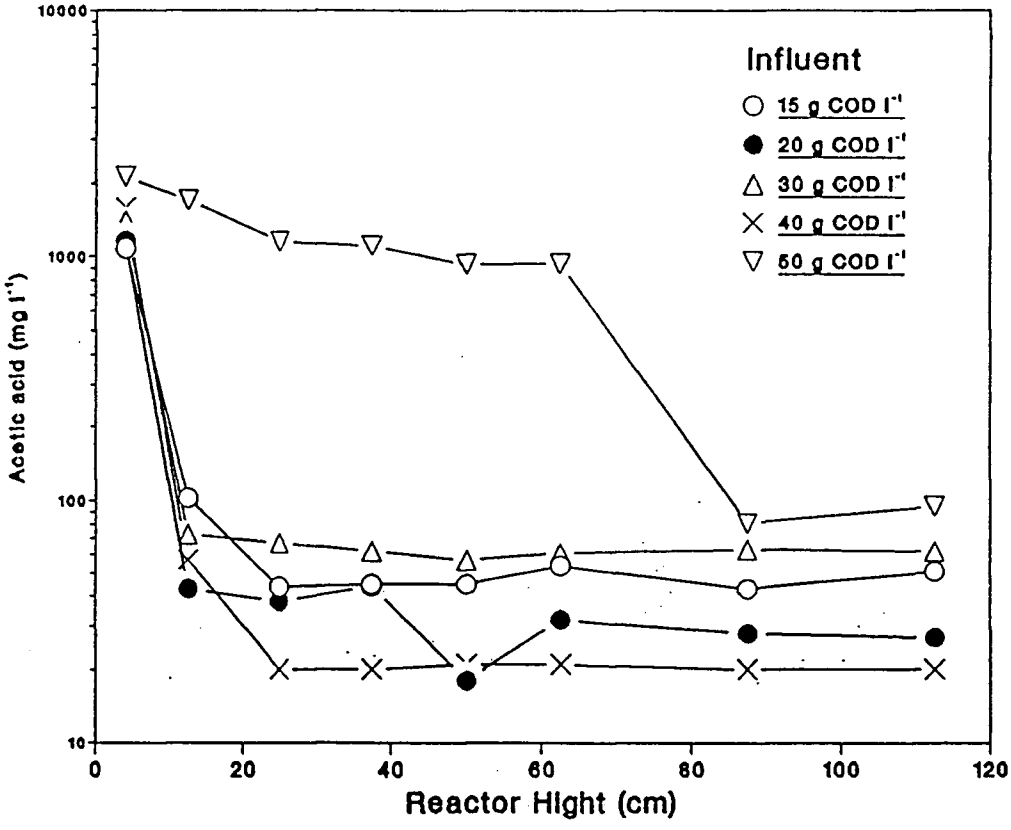


Figure 7.17: **Profile of Acetic Acid**

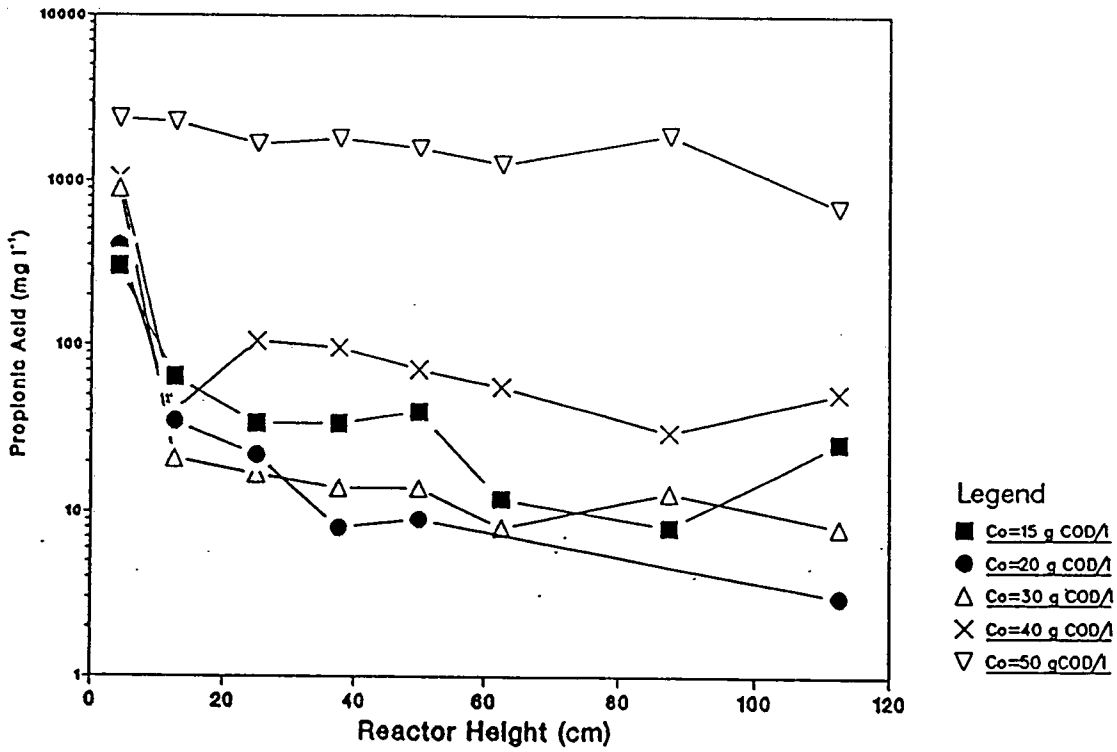


Figure 7.18: **Profile of Propionic Acid**

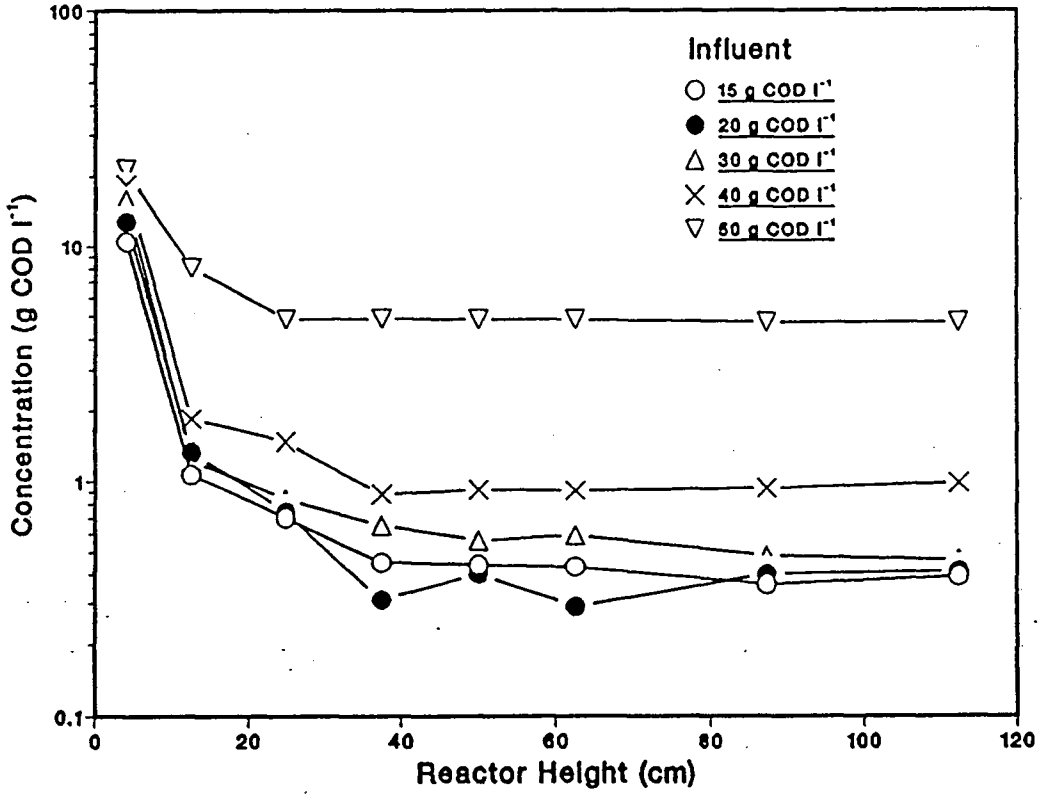


Figure 7.19: **Profile of COD**

Table 7.4: **Profiles of the UASB Reactor with Sulfate Addition**

Influent g COD/l	Height cm	VSS g/l	COD mg/l	pH	AA mg/l	PA mg/l	BA mg/l	IBA mg/l	COD Removal
14.40	4.0	39.42	10478	5.05	1038	297	36	941	0.31
15.11	12.5	31.72	1071	6.64	103	64	20	0	0.93
	25.0	22.63	696	6.60	44	34	0	0	0.95
	37.5	21.42	456	6.79	45	34	0	0	0.97
	50.0	10.84	437	6.82	45	40	0	0	0.97
	62.5	4.11	430	6.81	54	12	0	0	0.97
	87.5	3.85	363	6.81	43	8	0	0	0.98
	112.5	2.98	388	6.78	51	26	0	0	0.97
18.51	4.0	47.25	12835	5.56	1163	399	63	687	0.36
20.96	12.5	37.05	1339	6.87	43	35	4	14	0.94
	25.0	36.26	739	6.89	38	22	1	0	0.96
	37.5	28.16	308	6.89	44	8	1	0	0.99
	50.0	8.52	398	6.91	18	9	2	0	0.98
	62.5	9.82	289	6.92	32	0	3	0	0.99
	87.5	8.28	399	7.07	28	0	0	0	0.98
	112.5	7.77	407	6.93	27	3	0	0	0.98
30.08	4.0	38.47	15985	6.05	1413	901	75	364	0.47
31.24	12.5	40.77	1232	6.96	73	21	1	0	0.96
28.73	25.0	34.92	842	6.92	67	17	1	0	0.97
	37.5	33.01	654	6.90	62	14	2	0	0.98
	50.0	34.36	556	6.90	57	14	1	0	0.98
	62.5	9.77	590	6.96	61	8	1	0	0.98
	87.5	8.86	479	7.02	63	13	2	0	0.98
	112.5	11.70	455	6.93	62	8	2	0	0.98
40.48	4.0	32.82	18422	6.64	1600	1050			0.55
	12.5	31.50	1848	6.89	57	40			0.95
	25.0	12.94	1466	7.00	20	105			0.96
	37.5	12.17	880	7.01	20	96			0.98
	50.0	13.24	919	6.99	21	71			0.98
	62.5	11.10	910	6.98	21	56			0.98
	87.5	9.21	929	6.96	20	30			0.98
	112.5	8.02	980	6.89	20	51			0.98
50.91	4.0	43.98	21449	6.85	2127	2377	163	421	0.57
47.05	12.5	39.38	8067	7.08	1731	2268	349	173	0.84
	25.0	29.64	4836	7.05	1154	1685	123	145	0.91
	37.5	18.40	4869	7.16	1109	1815	119	73	0.90
	50.0	14.94	4834	7.28	935	1586	193	50	0.91
	62.5	12.74	4821	7.31	941	1268	99	46	0.91
	87.5	12.70	4690	7.38	81	1902	131	41	0.91
	112.5	12.82	4692	7.40	95	1700	125	41	0.91

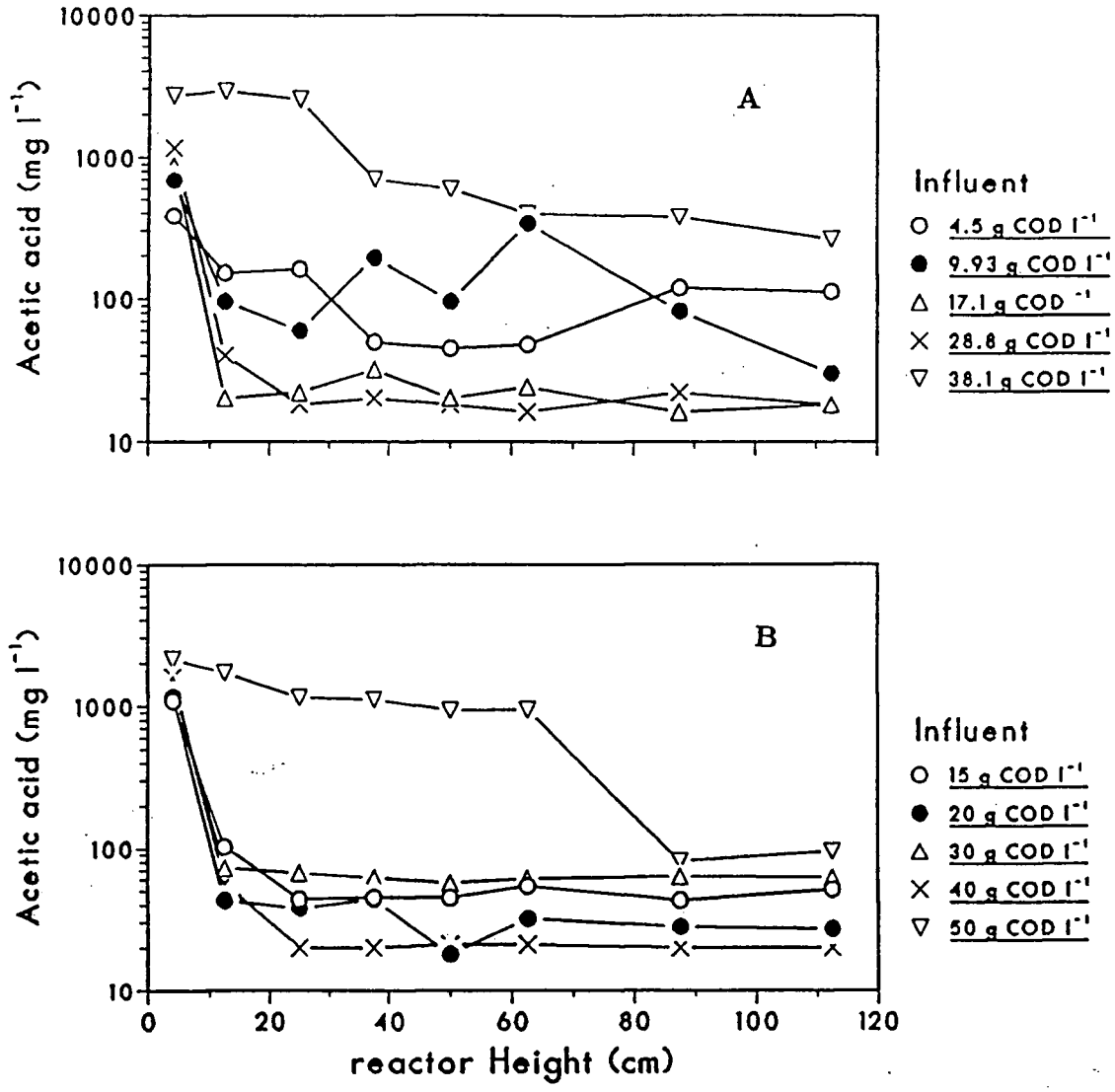


Figure 7.20: **Effect of Sulfate on the Profile of Acetic Acid: (A) Without S, (B) With S**

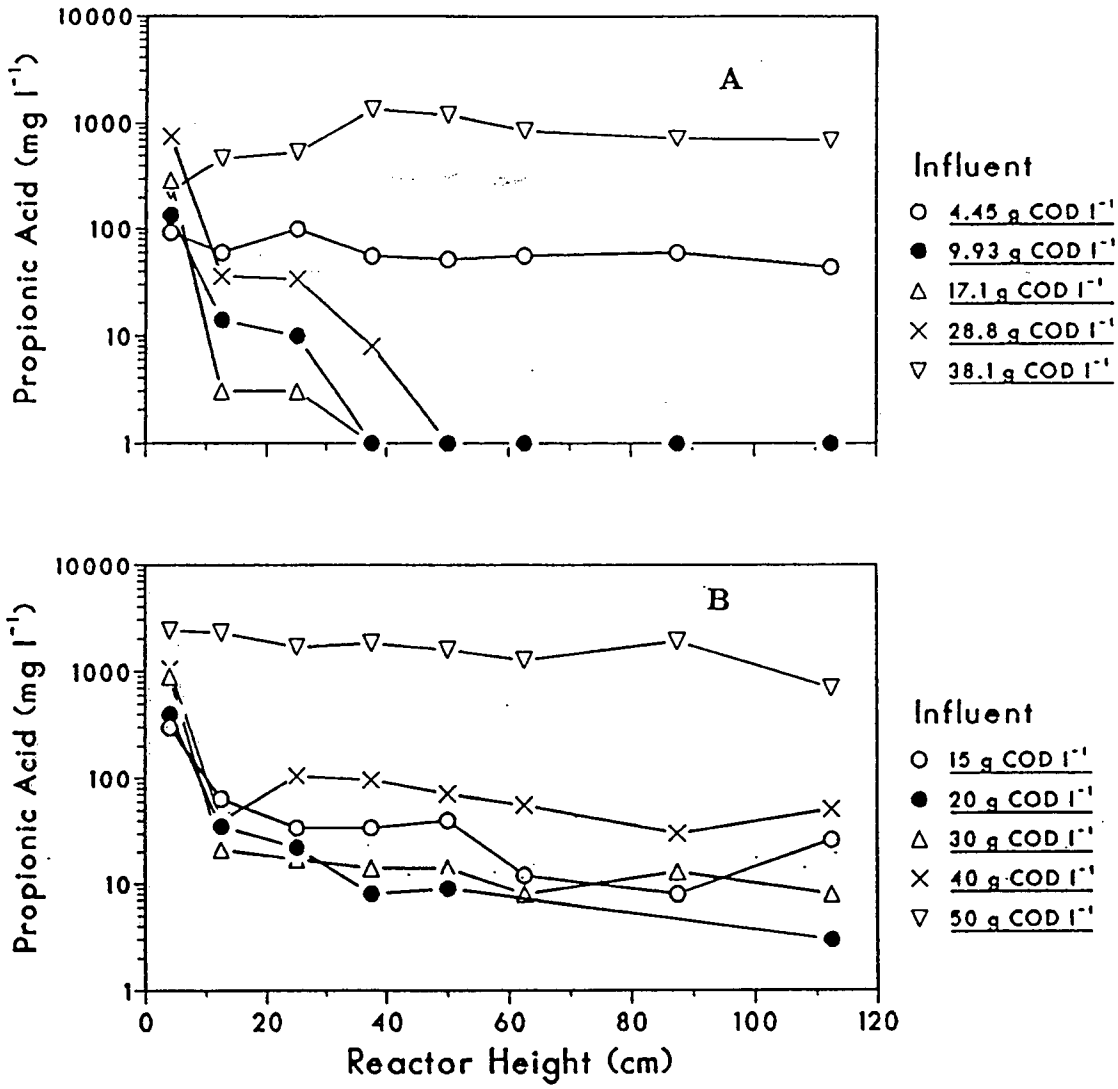


Figure 7.21: **Effect of Sulfate on the Profile of Propionic Acid: (A) Without S, (B) With S**

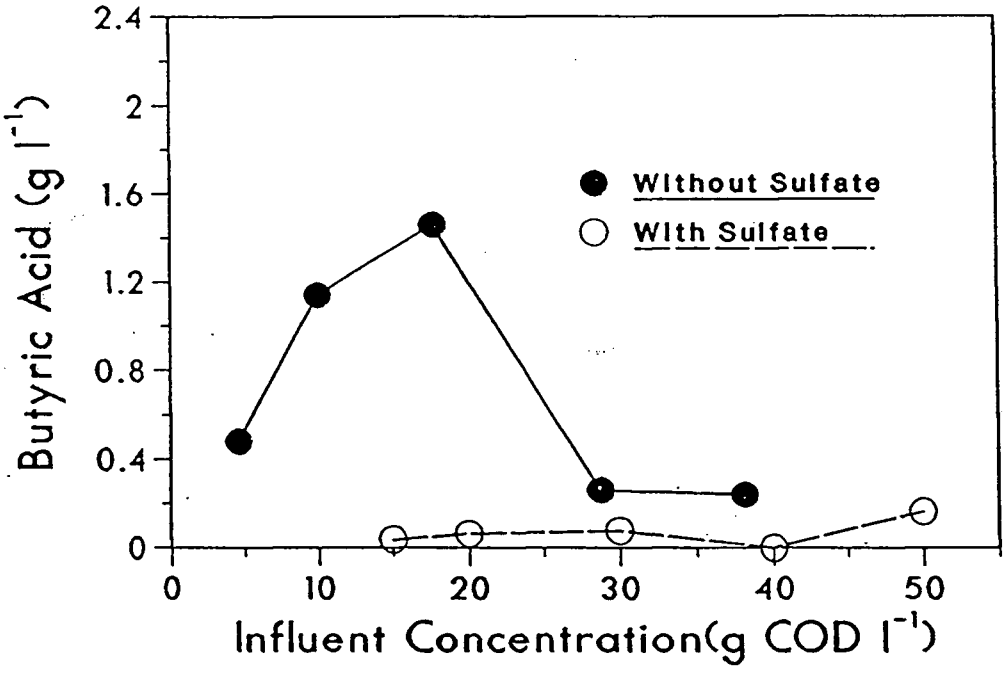


Figure 7.22: Effect of Sulfate on Butyric Acid Concentration in the Acidogenic Phase

7.5 MECHANISM OF INHIBITION OF HIGH CONCENTRATION OF VFAs AND LOW pH

Inhibition and toxicity in anaerobic digestion are subjects which have received considerable attention due to the important role they play in digester failure and also because they must be allowed for in the correct design and operation of reactors. Much information can be found about the effects of various chemicals and environmental factors on anaerobic digestion, but the data tend to be dispersed and without any basic unifying theory.

With highly soluble carbohydrate substrates, anaerobic digestion systems are always found to be easily upset. Under loading stress or impending failure conditions, low pHs and high VFAs concentrations are commonly observed, particularly for whey anaerobic digestion systems. pH control has been demonstrated to be crucial for maintaining stability. Inhibition modeling of digestors has been mainly restricted to the apparent effects of high concentrations of VFAs on methanogenic bacteria (Andrews 1968). However, the inhibition mechanism is not fully understood.

It has been accepted that non-ionized VFAs are inhibitors for methanogens and suggested that the methanogenic bacteria are inhibited either by hydrogen ions or by their substrate, the volatile acid (Andrews 1965, 1968)). The relationship between non-ionized VFAs and pH is that when pH decreases, the non-ionized VFAs increase. Calculated concentrations of non-ionized VFAs (Appendix D) for cases with and without sulfate are presented in Table 7.5, which shows how pH affects the concentration of non-ionized acid. A dramatic decrease in the concentration of non-ionized acids was obtained due to the increase in pH when sulfate was added.

A theoretical explanation for why non-ionized acid acts as inhibitors can be given, combining bacterial membrane transport and the Mitchell chemiosmotic hypothesis. The

Table 7.5: **Calculated Concentration of Non-ionized VFAs**

CH_4%	pH	Non-ionized Acids AA(mg/l)	PA(mg/l)
		Without Sulfate	
56	4.0	324	78
51	4.2	510	105
48	4.4	612	202
45	4.5	753	483
39	3.9	2355	1336
		With Sulfate	
47	5.05	263	100
46	5.56	155	55
44	6.05	69	44
41	6.64	21	14
40	6.85	17	19

accumulation of non-ionized acids leads to the acidification of the internal cytoplasmic pH and destruction of the pH gradient which is necessary for ATP synthesis.

Bacteria have a cytoplasmic membrane that acts as a permeability barrier for hydrophilic and charged molecules. A peptidoglycan layer, that surrounds the cytoplasmic membrane, confers rigidity and shape on the bacteria. In gram-negative bacteria, an additional outer membrane serves as a barrier to large hydrophilic and to hydrophobic molecules (Hancock 1984). Three different kinds of bacterial membrane transport: passive diffusion, facilitated diffusion and active transport, were elucidated by Harold and Brock et al (Harold 1977, Brock 1984). Passive diffusion is a transport mechanism by which neutral molecules tend to equilibrate across the membrane. The driving force for transport is a concentration gradient. Water, oxygen and carbon dioxide are transported by passive diffusion across the cytoplasmic membrane. In the case of facilitated diffusion, the permeating molecule combines with a membrane carrier and is transported inside the cell along its concentration gradient. For active transport, a specific carrier is generally required for each solute. Three categories of active transport: ATP-dependent, group translocation and transport coupled to the pmf (proton motive force), are recognized. The pmf is a chemiosmotic gradient across the bacterial cytoplasmic membrane that can be considered to have two distinct components: an electrical potential (interior negative) and a pH gradient (interior alkaline). Translocation of protons outside the cell membrane thus increases both components of the pmf. Major roles of the pmf are in the production of ATP by the membrane-bound ATPase enzyme complex, and for the transport of substrates. In ATP-dependent transport, the hydrolysis of ATP drives the internal accumulation of solutes such as negatively charged amino acids. In group translocation, the solute is modified during its transport (e.g. sugars by phosphoenol pyruvate). In transport coupled to the pmf, cations, anions or neutral molecules can be co-transported with protons or other cations such that the molecule is neutral or carries a net positive

charge when it crosses the membrane. For neutral molecules, such as sugars or amino acids, the carrier proteins effectively transfer a positively charged molecule where protons are bound to the carrier for its activation.

According to the Mitchell chemiosmotic hypothesis (Mitchell 1966), a trans-membrane pH gradient is generated between electron transport and phosphorylation of ADP. Electron transport causes H^+ ions to be pumped outward across the bacterial membrane. This proton expulsion leads to an increase in the pH gradient and the membrane potential, and concomitantly an increase in the proton motive force. This pH gradient (interior more alkaline) is the high-energy intermediate required for ATP synthesis.

When the pH of the medium decreases, the non-ionized VFAs consequently accumulated. The acetic, propionic and butyric acids in their non-ionized form will penetrat the bacterial membrane without any resistance based on the principle of the passive diffusion of neutral compounds across the bacterial membrane. A dissociation of the acids inside the cytoplasm is then provoked by the higher cytoplasmic internal pH. Thereby, protons are released and the cytoplasm is acidified, leading to the pH gradient dissipation. As a consequence, less energy will be available for the synthesis of bacteria and the attainable growth rate will be lowered. The acetic and propionic acids act as uncoupling agents for pH gradient destruction and potential membrane modification. Hence, the undissociated forms of VFAs are the inhibitors.

Different bacteria have different systems and capacity to maintain their pH gradient. Aerobic neutrophilic bacteria, like E.Coli, are capable of maintaining an internal pH near 7.6 for an external pH range of 5.5 to 8.5 (Padan et al., 1981). Some anaerobic bacteria, such as Clostridium pasteurian and Closdium thermoaceticum also show a similar system of pH gradient. However, methanogenic bacteria have only a limited capacity to maintain a constant internal pH. It has been explained that the H^+-ATPase system, combined with an antiport cation, could be responsible for the maintenance of a high pH gradient at a

low external pH (Kobayashi et al 1982).

Not all researchers share in the same belief that high VFA concentration are the inhibitors which cause failure. An argument has existed for years that accumulation of VFAs are the result of unfavorable conditions for anaerobic process rather than the cause of inhibition. A further discussion on this topic will be given in next section.

7.6 MECHANISM OF STIMULATION BY SULFATE.

Microbial sulfate reduction is a process in which certain bacteria use sulfate as the electron acceptor during metabolism of organic matter. The kinetics of competition for available electron donors (acetate and hydrogen) by MPB and SRB have received attention in the literature. Although acetate oxidizing SRB have been isolated and identified (Desulfobacter postgatei), they do not seem to be found among digester bioflora (Hocks et al 1984, Mulder 1984). As previously mentioned, all SRB are able to perform the lithotrophic reduction of SO_4^{-2}:

$$4H_2 + H^+ + SO_4^{-2} = HS^- + 4H_2O \tag{7.6}$$

Therefore, this common modality for H_2 utilization sets up a situation of competition between the MPB and the SRB.

Molecular H_2 is generated in two distinct steps of the anaerobic digestion process, i.e. fermentation and β oxidation. The fermentation of the hydrolytic products – amino acid and glucose – is performed by a number of acidogenic Clostridial species native to anaerobic reactors. Hydrogen gas is evolved when pyruvate, the end product of glycolysis, is decarboxylated and dehydrogenated to acetate via the phosphoroclastic reaction. H_2 is also generated in the anaerobic oxidation of volatile and long chain acids. This is performed by a number of native obligate syntrophic bacteria (usually referred to as

obligate proton reducing or hydrogen-producing acetogen [OHPA]). In this process acetate units are split off endwise from the chain with molecular H_2 being the main sink for electrons. The stoichiometry is as follows (Gujer and Zehnder 1983):

$$(-CH_2CH_2-) + 2H_2O = CH_3CO_2H + 2H_2 \tag{7.7}$$

This reaction, mediated by pyridine dinucleotides, is believed to be inhibited by increasing partial pressure of H_2, as its ORP, -0.32 V, is higher than the standard hydrogen electrode. Oxidation of fatty acids is feasible only when the reaction can be "pulled" to the right by the continuous removal of H_2.

The argument is, therefore, whether or not the fermentation of pyruvate to acetate is H_2-sensitive. It was thought that this reaction seemed not be inhibited by increasing partial pressures of H_2 (Gottschalk 1986, Cohen 1979), considering its oxidation/reduction potential (ORP) of -0.68 V, which is lower than the standard hydrogen electrode potential of -0.42 V. On the other hand, it has been long believed that fermentation of acetic, propionic and butyric acids from sugar via the EMP pathway is regulated by the availability of H_2. Figure 2.2 (from Mosey) provides the main route – the Embden Meyerholf pathway for conversion of sugar to organic acid. The acid-forming bacteria use this pathway to obtain energy from the oxidation of glucose to acetate. During the course of this oxidation, hydrogen atoms removed from the glucose are transferred first to the carrier molecule NAD^+, converting it to NADH + H^+ and then released into solution as dissolved hydrogen gas. In order for catabolism to proceed continuously, given a constant pool of NAD, the NADH produced during substrate-level phosphorylation of glyceraldehyde-3-phosphate and the oxidative decarboxylation of pyruvic acid to acetyl-CoA must be regenerated. This function is accomplished by the reduction of protons to form hydrogen gas, which is subsequently removed by the hydrogenotrophs such as

methanogens, SRB and NRB through interspecies hydrogen transfer. Accumulations of hydrogen need an alternate method of electron disposal for NADH regeneration. This need is fulfilled by the fermentation of pyruvic to propionate, lactate, and ethanol and/or by the fermentation of acetyl-CoA to butyric acid.

Accumulation of H_2 during the formation of acetic acid from pyruvate pushes the reaction in the directions which will release the stress from a high pressure of H_2 (see line D in Figure 2.3) by oxidation of acetic acid to butyric acid. Further accumulation of H_2 would shift the fermentation of pyruvate from producing acetic acid to butyric and propionic acids. The evidence that the concentrations of butyric acid as well as propionic acid decreased due to the presence of sulfate led to a new interpretation of hydrogen regulation of the overall conversion process by throttling the acidogenic reaction at several points in the glycolytic pathway. First, SRB, which have the capacity to utilize H_2 might be able to maintain the H_2 pressure low enough (say well below 10^{-4} atm), allowing the fermentation of pyruvate to continue going to acetate. Secondly, as can be seen from Figure 7-1, the removal of H_2 can move the metabolism to that of an OHPA. The oxidation of butyric acid can be motivated when H_2 pressure drops to below 10^{-3} atm according to thermodynamic calculations, so can be the oxidation of propionic acid at H_2 pressure below 10^{-4}(Figure 2.3). No matter what the fate of butyric and propionic acids might be, the results indicated that fermentation is H_2 sensitive and is regulated by H_2 pressure.

A question remains whether SRB are able to change the pathway or promote β-oxidation by providing a H_2 sink under the conditions of the present study. Taking a closer look at Figure 7.21 (also Tables 6.1 and 7.4) it was easy to notice that the concentrations of propionic acid in the acidogenic stage (sample port 1) were the same for two experiments (in the presence of sulfate and in the absence of sulfate), while in the methanogenic stage (sample port 2) the propionic concentrations were much lower

with the addition of sulfate than without sulfate addition. A possible explanation for this observation is that pyruvic originally was fermented to propionic and butyric acids, perhaps acetic acid as well. Then propionic acid was further broken down to acetic acid. The lower the H_2 pressure, the more propionic acid was oxidized, thus the lower the concentration of propionic acid would be. The results from these experiment suggest that SRB are more likely to promote β-oxidation by providing a H_2 sink, due to the level of H_2 pressure to which the sulfate could reduce.

A reason for the decrease of butyric acid being far more noticeable than that of propionic acid after the addition of sulfate can be explained by the effect of the different H_2 partial pressure.

The regulatory effects of hydrogen on acetogenic and methanogenic bacteria have been conveniently illustrated using thermodynamic models and equilibrium assumptions by several authors (Zhender et al.1980, Gujer 1983)). Thermodynamic calculations (see Figure 2.3) indicate that a partial pressure of hydrogen of 10^{-3} atm would favor the oxidation of butyric acid, while propionic acid oxidation to acetate becomes favorable only at H_2 partial pressure below 10^{-4} atm. If H_2 is maintained at sufficiently low levels (10^{-4}atm), the production of propionic acid needs never occur.

The high concentration of butyric acid in the experiment without sulfate addition indicated that the hydrogen pressure must have been greater than 10^{-3} atm, which inhibited the oxidation of butyric acid. The decline of H_2 pressure to below 10^{-3} by SRB due to the addition of sulfate then promoted the further oxidation of butyric acid to acetic acid. However, the level of H_2 pressure must be higher than 10^{-4} even with the addition of sulfate since the concentration of propionic acid remained very high in acidogenic stage.

More evidence were found to support the stimulation function of sulfate from Figures 7.23 to 7.26. They were made based on the equations developed in Appendix A, which

were statistically fitted to the experimental data.

Figure 7.23B shows that the degradation of propionic acid in the absence of sulfate was much lower than that with sulfate, although the accumulation of propionic acid was similar for both experiments (Figure 7.23A). These results indicate that oxidation of propionic acid in the absence of sulfate was inhibited. Supporting evidence was also found that the permissible concentration determined by utilization of propionic acid was much lower in the absence of sulfate (Figure 7.24).

Figure 7.25 shows the accumulation and degradation of acetic acid, propionic acid and total VFAs in the absence of sulfate. Figure 7.26 is for the case when sulfate addition was applied. It is interesting to note that in the absence of sulfate, the permissible influent concentration determined by propionic acid concentration was 22 g COD/l, which was much lower than the value given by acetic acid and total VFAs. These were 28 and 30 g COD/l respectively. The permissible concentration was defined as the highest influent concentration at which the degradation of VFAs was greater than their accumulation (as described in Chapter 6). With sulfate added, the permissible influent concentration calculated from propionic acid was 39 g COD/l, higher than the one determined by VFAs, 35 g COD/l (Figure 7.26). These results show that in the absence of sulfate, degradation of propionic acid is the rate-limiting step. When sulfate was applied to the feed stream, oxidation of propionic acid was no longer the controlling step for the overall process due to the stimulation function of the SRB's consumption of hydrogen. No single parameters could be identified as being responsible for the control of the overall process in the presence of sulfate.

It is postulated that the rate-limiting step of the complicated reactions involved in anaerobic fermentation can be changed and that hydrogen pressure plays a central role in causing the shift between those different controlling steps. Acetoclastic activity could be the limiting steps at hydrogen pressures smaller than 10^{-4} atm. Once the hydrogen

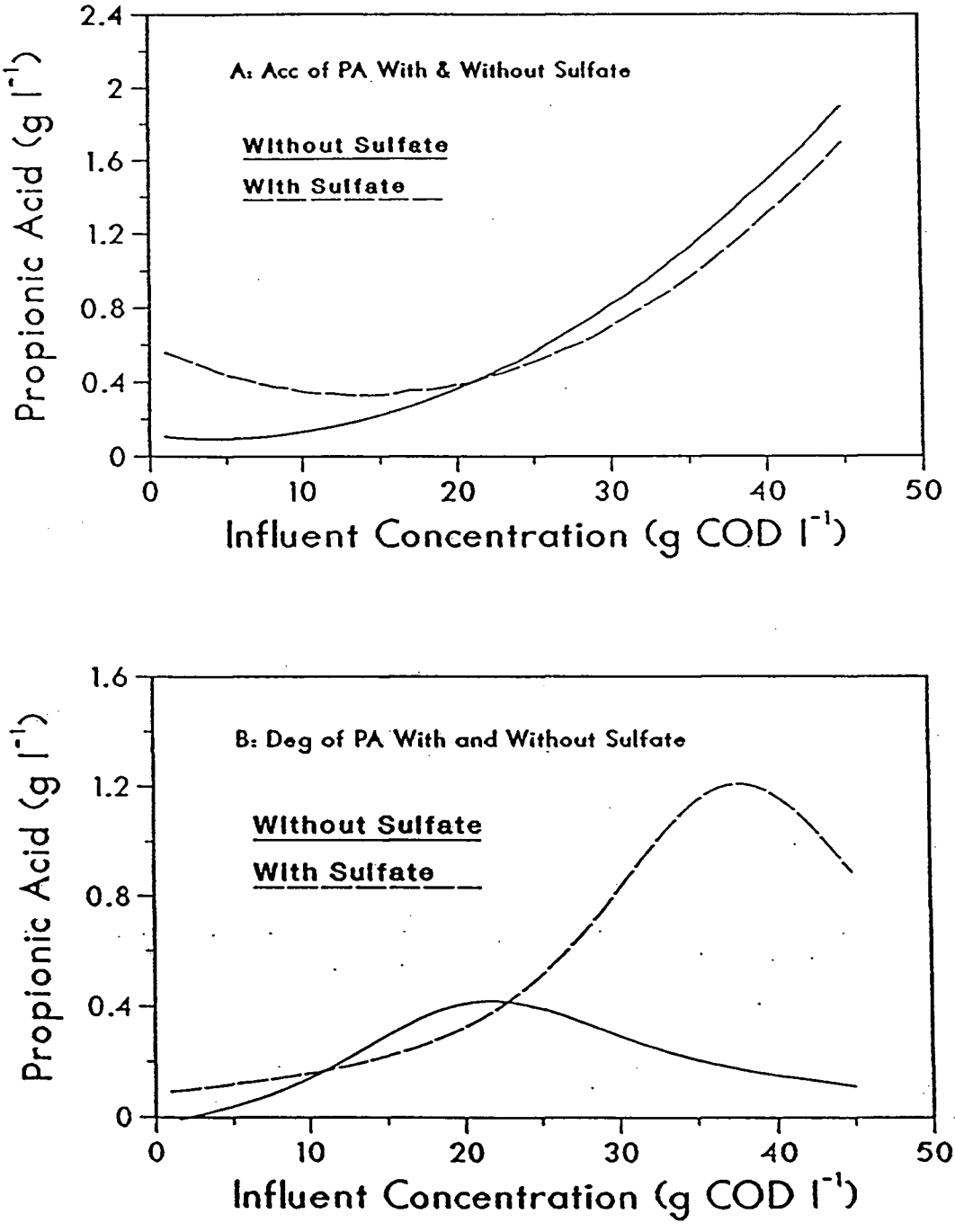


Figure 7.23: **Effect of Sulfate on the Accumulation(A) and the Degradation (B) of Propionic Acid**

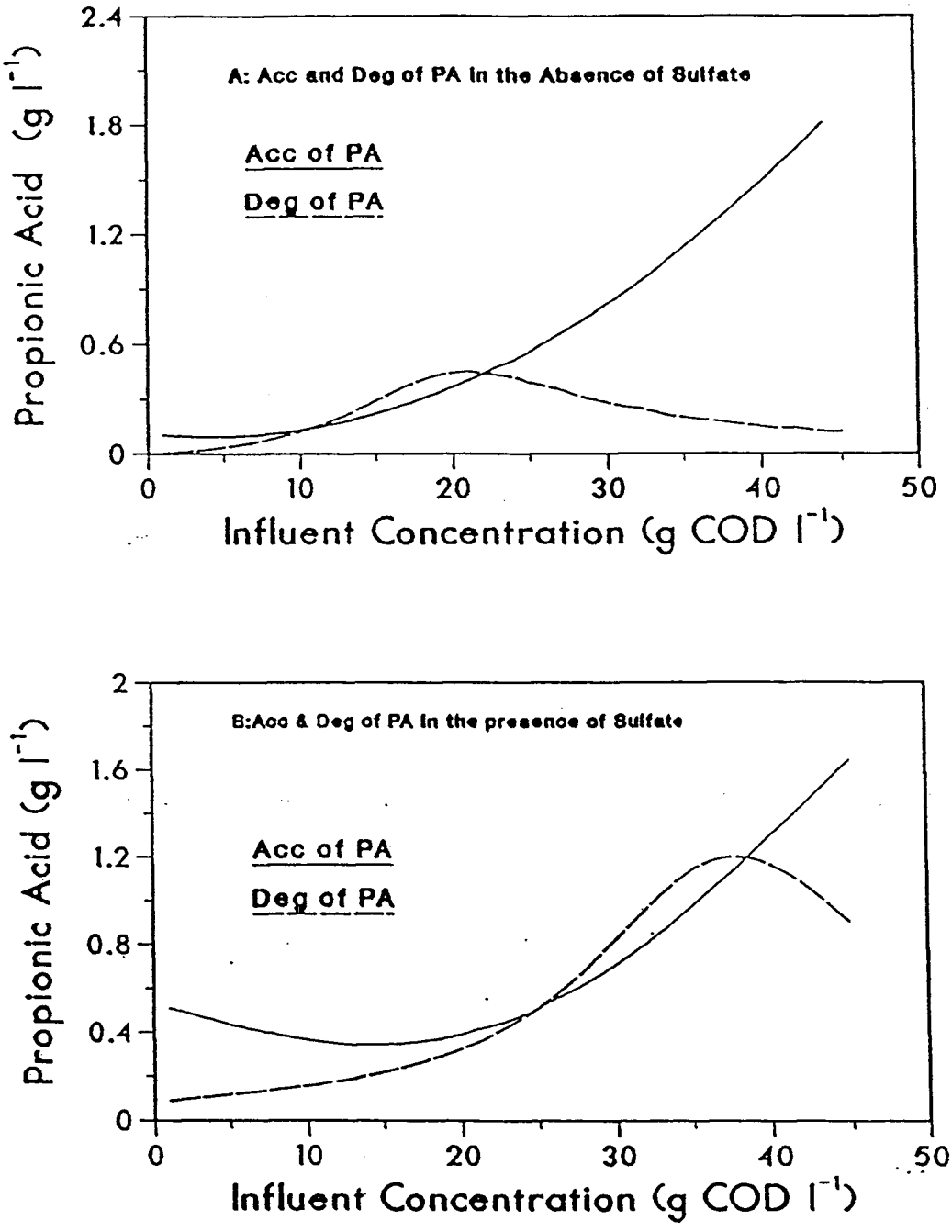


Figure 7.24: Effect of Sulfate on the Permissible Concentration Determined by Accumulation and the Degradation of Propionic Acid. (A) in the Absence of Sulfate; (B) in the Presence of Sulfate

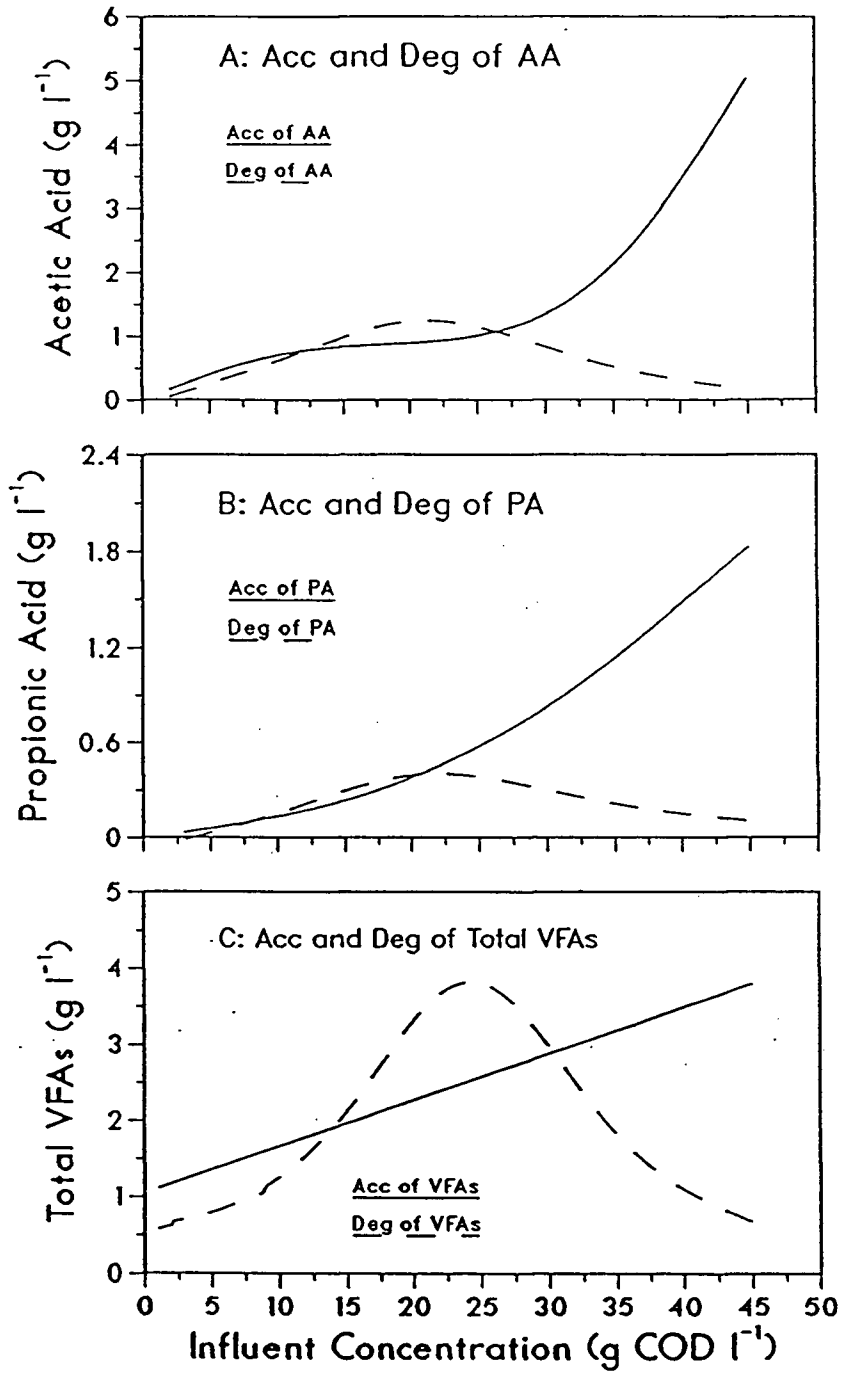


Figure 7.25: **Accumulation and Degradation of Acetic Acid, Propionic Acid and total VFAs in the Absence of Sulfate**

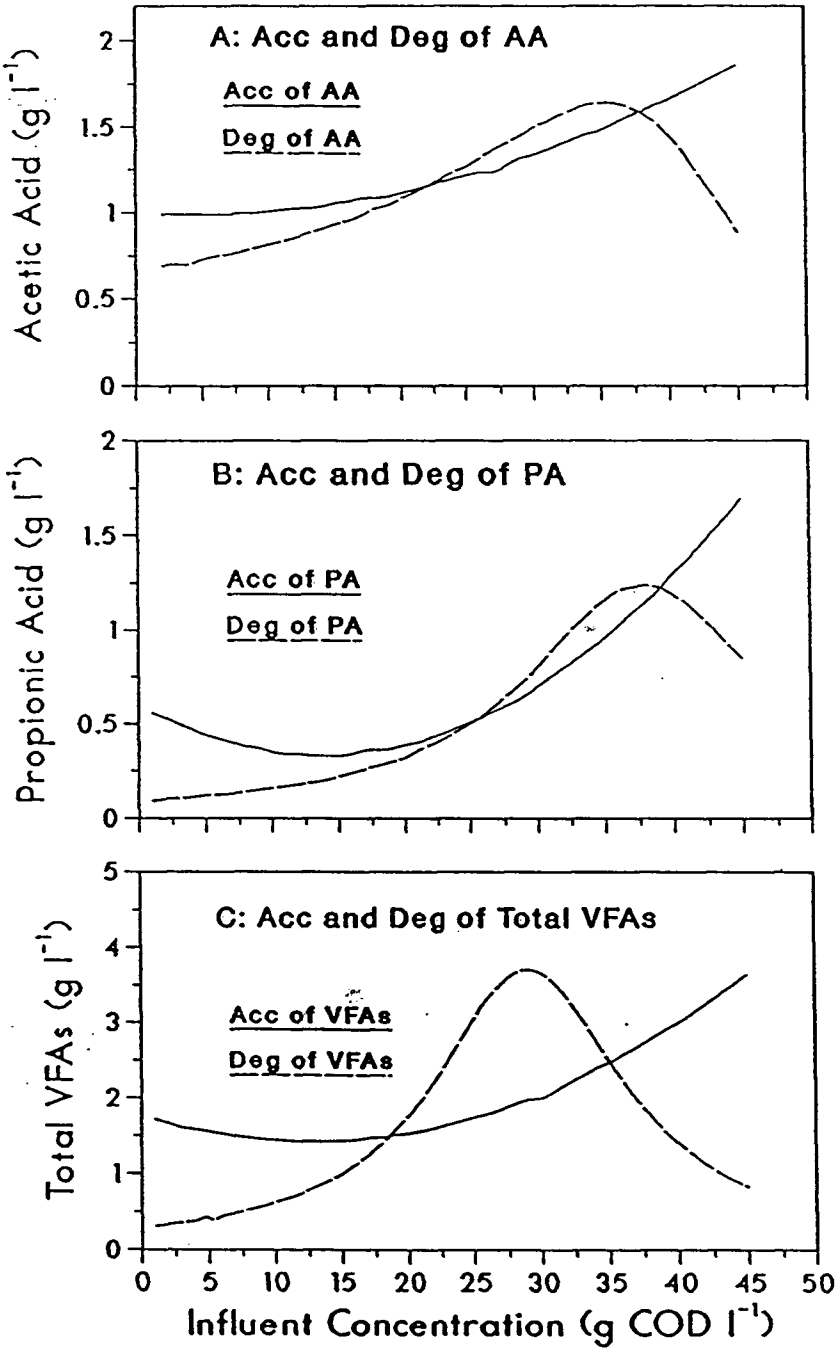


Figure 7.26: Accumulation and Degradation of Acetic Acid, Propionic Acid and total VFAs in the Presence of Sulfate

pressure is greater than 10^{-4}, the overall reaction rate might be determined by the rate of oxidation of propionic acid as well as butyric acid.

The similarity in the shape of the acetic acid accumulation curves for the two experiments (with sulfate and without sulfate) implies that SRB and MPB do not appear to compete for acetic acid.

As a result of the above discussion, a new inhibition scheme, two-stage inhibition in anaerobic fermentation can be suggested, which could answer the question of whether VFAs are the inhibitor or the results of inhibition (Figure 7.27). Inhibition of an anaerobic system occurs in the following two stages. First, high hydrogen pressure drives the pyruvate fermentation to produce propionic and butyric acids rather than acetate, which is called preliminary inhibition. In this preliminary inhibition, higher hydrogen pressure is the cause and the accumulation of VFAs is the result. The accumulation of VFAs in the system subsequently predominates and the consequence of this accumulation causes the direct inhibition of the activity of methane bacteria in the second inhibition stage. High VFA concentrations are the result of unfavorable conditions for the anaerobic process, and also the cause of failure due to the second inhibition. Once a high concentration of VFAs or a low pH is detected, the system has already suffered the second inhibition.

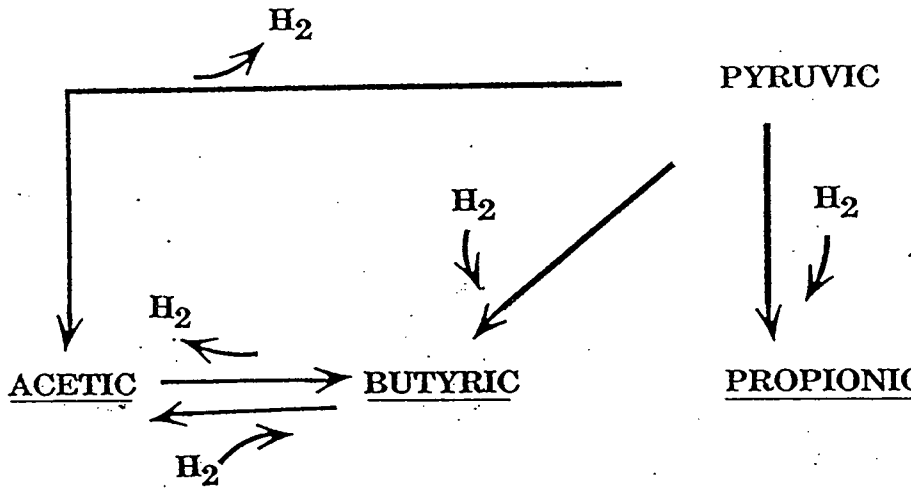
Obviously, there is a more effective way to control the process prior to the observation of an apparent accumulation of VFAs, which is to maintain the hydrogen pressure low enough by using hydrogenotrophic association. The advantage of the new control strategy over the old pH control system is that it prevents the system from the first inhibition at the very beginning. Moreover, hydrogenotrophic association is able to promote β-oxidation of butyric and propionic acids, in turn, to increase the conversion of acetate. Theoretically, it would increase the production of methane.

7.7 SUMMARY

The significant improvement in process stability and treatment efficiency made by adding sulfate has clearly illustrated that sulfate acts like a stimulator which helps to maintain a favorable condition for methanogenesis. The mechanism of the stimulation can be explained according to thermodynamics and hydrogen regulation. It is that sulfate is able to promote the β-oxidation of VFAs by consuming hydrogen.

The results showing the profiles of pH and substrate concentrations elucidated the fact that the rate-limiting step of the complicated reactions involved in anaerobic fermentation is changed and interspecies hydrogen transfer plays a central role. The significant decrease of butyric acid in the first stage indicated that sulfate serves as a hydrogen sink. The conversion of pyruvate to acetic acid offers a solution for the removal of excess hydrogen, improving the overall stability.

A two-stage inhibition mechanism in anaerobic fermentation was proposed. Higher hydrogen pressure is the cause of preliminary inhibition, resulting in the accumulation of VFAs which subsequently inhibited the activity and growth of methanogens in the second inhibition stage. The mechanism of inhibition of methanogens from VFAs was interpreted as being caused by the acidification of the internal cytoplasmic pH and the destruction of pH gradient by non-ionized acids based on the theory of bacteria membrane transport. A new control strategy for anaerobic system stability was recommended. That is to maintain the hydrogen presure at low level through hydrogenotrophic association.



FIRST	High H_2 pressure	INHIBITOR
	VFAs	RESULTS
SECOND	VFAs	INHIBITOR

Figure 7.27: Two-Stage Inhibition Mechanism

Chapter 8

TREATMENT EFFICIENCY IN OPTIMAL OPERATION

An experiment was conducted in a 3.04 l UASB reactor under optimal operating conditions. The reactor configuration was the same as before except that the reactor height was reduced from 168 cm to 30 cm.

The optimal operating conditions were selected from the results found in the first three sets of experiments and gave highest treatment efficiency and reliable system stability. These conditions included optimal influent concentration of about 30 g COD/l, reactor height of 30 to 40 cm (for the same diameter) and amount of sulfate addition of 0.2 g/l. The reactor was seeded with 1.5 l of sludge with a VSS concentration of 35.48 g/l. The total amount of VSS in the reactor was 53.12 g/l.

The start-up procedure strictly followed the recommendations from the start-up experiment, being certain that the sludge loading was less than 0.25 g COD/g VSS. In the first 2 weeks the reactor was fed 0.4 l of whey daily, which corresponds to a specific sludge loading of 0.22 g COD/g VSS. The daily feed was increased to 0.8 l after the 3rd week. The gradual increase in feed rate was continued until it finally reached 1.5 l/d after a month. The influent concentrations were in the range from 26 to 32 g COD/l and sulfate was added at a concentration of 0.2 g/l.

The experimental results are presented in Table 8-1. A very high treatment efficiency was obtained in this study. Over 97% COD reduction was achieved with an influent concentration of 32.6 g COD/l, HRT of 2 days and an organic loading rate of 16.61 g COD/l d.

By comparing the results from this work to other treatment systems given in Table 8-2, it can be seen that the highest COD reduction for high values of organic loading were obtained using the UASB reactor.

Table 8.1: **Results in the Optimal Operation**

Input g COD/l	Feed l	OLR g COD/l d	pH	Output g COD /l	Gas l/d	CH_4%	Reduction COD %
29.46	0.84	8.05	8.09	0.70	14.07	42.51	97
	1.16	11.39	7.79	0.69	18.12	46.09	97
	1.39	13.65	7.96	0.63	20.59	43.67	98
32.59	1.47	15.96	7.74	0.80	20.36	42.38	97
	1.53	16.61	7.69	0.80	21.42	42.44	97

Influent Concentration: 29-32 g COD/l
Volume Of Reactor: 3 littres
Sulfate Concentration: 0.2 g/l

Table 8.2: Comparison of Anaerobic Treatment Process for Cheese Whey

Reactor	Waste	HRT	Temp (°C)	pH control	Raw Waste g COD/l	Loading g COD/l d	Removal %	Reference
CMR_1	raw	14-70	38	7.5	69	-	18-58	Wildenauer (1985)
Fixed	sour	5 days	35	6.7	79	14.0	95	Boening (1982)
film	whey							
$AAFEB_2$	whey	8.9-27hrs	28-31	No	10	8.9-27	77-93	Switzenbaum (1982)
	powder	14-16hrs	35		5-15	8.2-22	61-92	
$AnRBC_3$	raw	5 days	35	No	64	10.2	76	Lo & Liao (1986)
	whey	6-11days	35		61-70	6.3-10	82-93	
$UASB_4$	raw	5 days	33	No	5-28.7	0.91-6	97-99	this
	whey	5 days	33	No	41	7.9-8.2	81-86	study
		2 days			32.6	16.61	96-98	

1 CMR completely mixed reactor.
2 AAFEB Anaerobic attached film expanded reactor.
3. AnRBC Anaerobic rotating biological reactor.
4. UASB Upflow anaerobic sludge blanket reactor.

Chapter 9

CONCLUSIONS, CONTRIBUTIONS AND RECOMMENDATIONS

9.1 CONTRIBUTIONS AND CONCLUSIONS

Cheese manufacture, one of the biggest food industries in North America,is facing the challenge of a difficult waste disposal problem. Despite the fact that a number of studies on the anaerobic treatment of cheese whey during last decade have proved that anaerobic fermentation of cheese whey could be an alternative solution for waste disposal, the system was suspect due to many unsuccessful experiments and the difficulties frequently encountered in maintaining a stable operation. This study attempted to overcome these difficulties by solving some of the major problems which existed in previous studies by increasing the basic knowledge of this process; to in turn, facilitate the operation of anaerobic reactors and to lead to its better application by industries.

Concentration profiles of various parameters in a UASB reactor are necessary for the development of a dynamic model for the process which can be used for its optimization. From the measurement of these profiles, several interesting conclusions have been drawn. It is believed that this study is the first to show in anaerobic fermentation studies that the two stages, acidogenesis and methanogenesis, occur separately in the same reactor. The significance of this observation is the realization that two major steps in the whey anaerobic fermentation process are not necessarily in dynamic balance since the two reaction rates are very different. This explains why this system is easily upset. This observation also gave a better understanding of how to efficiently control the system's

stability by preventing the accumulation of the products in the first stage, e.g. VFAs. This in turn has led to the experimental study of sulfate effects.

Sulfate ions have long been believed to be toxic to anaerobic bacteria. The previous studies on the effect of sulfate have been limited to its inhibition effects. These experimental tests first illustrated the stimulation function of sulfate ions, which greatly supported the fundamental concept of a hydrogen regulation theory. As a result of the stimulation, a significant improvement in process stability and treatment efficiency was achieved. The knowledge gained from the study on sulfate stimulation provided a better understanding of the inhibition mechanism of anaerobic digestion and had significant implications for the handling of other high strength soluble carbohydrate wastes.

Based on this experimental work, the major conclusions are summarized as follows.

1. The results from the preliminary feasibility assessment experiments have shown that the anaerobic digestion of cheese whey using a UASB reactor can be an efficient treatment method for diluted cheese whey. Without pH control and nutrient addition, the system could successfully treat cheese whey up to a concentration of about 29 g COD/l. Over 97% COD removal was achieved.

2. The start-up procedure is very important for successful reactor performance. Various start-up strategies were tested in the present studies to facilitate start-up of the UASB reactor and to ensure stable operation. Among the operating parameters, sludge loading rate was the most critical for proper start-up of the UASB reactor. The initial sludge loading during the start-up period should not exceed 0.25 g COD/g VSS-d.

3. VFAs were found to be a very useful indicator for primary adaptation of sludge and for monitoring the system stability. The loading rate could be increased only after the VFA concentrations in the reactor were at a low value. A rapid increase of OLR may result in the loss of activity of the sludge.

4. The methane production rate was a function of OLR. At an OLR less than 4 g

COD/l-d, the reactor fed with a higher influent concentration yielded a higher methane production rate. When the OLR was greater than 6 g COD/l-d, a higher influent concentration or shorter HRT led to a lower methane production rate. The optimal OLR for this particular system was between 4-6 g COD/l-d. If we choose a HRT of 5 days, the optimal influent concentration should be between 20-30 g COD/l.

5. The profile of the sludge showed that two sludge regions, sludge bed with high density VSS and sludge blanket, existed in the UASB reactor. The distribution of the sludge was dependent on the process parameters. With an increase in the loading rate the sludge bed expanded, mainly due to the gas production, so that the sludge concentration in the blanket, especially in the area between the bed and blanket, varied with gas flow rate.

6. For highly soluble and easily acidified substrates, such as cheese whey, difficulties existed in maintaining stable operation. The results from this study provided a better understanding of the cause of the instability. The observation that two reaction stages, acidogenesis and methanogenesis, were distinguished in the UASB reactor clearly indicated that cheese whey is very easily converted to short chain fatty acids and that the rate of the first step is much higher than that of the second step. The appearance of two stages in the same reactor was caused by the excessive accumulation of VFAs from the acidogenesis stage which was the result of either the nature of the substrates or process stress.

7. There is a threshold of influent concentration of cheese whey at a given HRT for stable operation of a UASB system. If the feed strength exceeds this threshold, instability occurs. For this system the influent concentration should be maintained below 30 g COD/l at a HRT of 5 days. The optimum influent concentration would be 25 to 30 g COD/l based on the finding that system stability can be maintained if the degradation capacity for VFAs is greater than their accumulation.

8. All measured substrate profiles at different operating conditions showed that more than 80% of the COD reduction took place below a height of 12 cm in the reactor and the sludge concentration was as low as 5 to 10 g VSS/l above a height of 30 cm. It is therefore recommended that the reactor height should be reduced to 40 cm or less for this reactor with a diameter of 12 cm. 9. The significant improvement of process stability and treatment efficiency made by the addition of sulfate clearly illustrated that sulfate acted like a stimulator which helps to maintain favorable conditions for methanogenesis. The mechanism of this stimulation is explained according to thermodynamics and hydrogen regulation which suggested that sulfate is able to promote the β-oxidation of VFAs by consuming hydrogen.

10. A two-stage inhibition mechanism in anaerobic fermentation was proposed. Higher hydrogen pressure is the cause of preliminary inhibition, resulting in the accumulation of VFAs which subsequently inhibit the activity and growth of methanogens in the second inhibition stage. The mechanism of inhibition of methanogens from VFAs was interpreted as being caused by the acidification of the internal cytoplasmic pH and destruction of the pH gradient by non-ionized acids based on the theory of bacterial membrane transport. A new control strategy for anaerobic system stability by using hydrogenotrophic association was recommended.

11. Under the optimal operating conditions, over 97% COD reduction has been achieved at an influent concentration of 32.6 g COD/l , an HRT of 2 days and an organic loading of 16.61 g COD/l d. This is much higher than what has previously been reported in the literature.

9.2 RECOMMENDATIONS FOR FUTURE RESEARCH

The following areas are recommended for future investigation based on the findings made in this research:

1. It would be instructive to conduct research on the quantitative relationship between hydrogen pressure and the concentration or accumulation rate of each volatile fatty acids using different substrates as well as different amounts of sulfate or other hydrogen utilizating reagents. It is known that the conversion of pyruvate to butyric, propionic and acetic acids is regulated by hydrogen pressure. The significant reduction of butyric acid shown by profiles of the VFAs indicated that sulfate serves as a hydrogen sink. The conversion of pyruvate to acetic acid offers a solution for the removal of excess hydrogen, improving the overall stability and the process efficiency. A quantitative relationship is definitely needed for the development of a mathematical model to describe and predict the possible products and their concentrations in the acidogenic step and their effect on system stability. These models would be very useful for further development of a more efficient control strategy for the anaerobic fermentation processes. They would be based on hydrogen partial pressure control as it is especially important according to the two-stage inhibition mechanism.

2. It is recommended that an extensive study on inhibition kinetics in anaerobic processes could be made following the two-stage inhibition mechanism proposed in this dissertation. The discovery of new inhibition kinetics could be made on the concept that unexpected high hydrogen pressure is the inhibitor for preliminary inhibition and VFAs are both the result of preliminary inhibition and the inhibitor for the secondary inhibition.

3. This research provided the information on the concentration profiles in the UASB reactor, which has extended our understanding of the anaerobic process and the causes

of instability. However, information about the substrate concentrations between sample port 1 and 2 is lacking. Interesting information, related to inhibition and instability, might be presented if there were more sample ports located between port 1 and 2. An extensive spectrum of substrate concentrations along the reactor column could be attained by constructing the column with a smaller diameter and a larger number of sampling ports. Improved technology such as on-line analytic equipment should also be considered due to the large number of samples and the necessity for simultaneous monitoring at all levels.

4. The positive effects of sulfate on the anaerobic process as a stimulator have been experimentally demonstrated in the present research. However, the cause of the higher concentration of iso-butyric acid in the presence of sulfate remains unknown. Furthermore, an explanation of the different behaviors shown by butyric and propionic acids could be made according to thermodynamics and hydrogen regulation. This needs to be verified before a complete conclusion can be drawn. Finally, determination of the optimum sulfate addition and the critical value with respect to system stability are recommended.

5. It was found that no H_2S was detected when only 0.2 g/l sulfate was added to feed, while 0.3-0.6% H_2S was observed for 0.3 g/l sulfate addition. This could be explained that sulfate was first used as nutrient for MPB and SRB rather than hydrogen utilizing reagent. Only when extra sulfate was provided, such as in the case of 0.3 g/l sulfate added, might a portion of sulfate act as hydrogen utilizing reagent. A further study is needed to determine the quantitative relationship between the amount of hydrogen reduced and the amount sulfate applied.

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Appendix A

EMPIRICAL MODEL

In such circumstances when the mechanism underlying a process is not understood sufficiently well, or is too complicated to allow an exact model to be postulated from theory, an empirical model may be useful. Particularly if it is desired to approximate the response only over limited ranges of the variables.

A.1 THE PROGRAM OF MINITAB

Minitab's regression is used to investigate the relationship among variables, particularly those relationships that enable the experimenter to predict a variable from one or more others. Minitab's regression has three features:

1. It applies simple linear regression to investigate the form of a linear relationship between two variables

2. Multiple regression is for the investigation of the form of the linear relationship between one variable and several other variables

3. Stepwise regression allows one to explore the relative importance of various predictor variables.

To invoke the regression command, the necessary information, including the dependent variable and its location (column number where it is stored) and independent variables as well as their locations, must be provided.

After performing a simple linear regression by REGRESS, MINITAB first gives the default output. The first line is the estimated regression equation. The second line is

the estimate for each of the regression coefficients, their estimated standard deviations, the t-statistics and p-values for testing whether the coefficient is different from zero. The third line is the the estimated standard deviation of the regression, the R -squared value, or coefficient of determination and the adjusted R-squared. The final part of the output is the significance test for the regression in an ANOVA form, with a F-statistic and p-value as before.

An additional output can be got by issuing the "BRIEF LEVEL 3" Command in conjunction with the "REGRESS" COMMAND as show in Table A-6. Additional information is the independent value, the dependent (response) value, the fitted or predicted value (the estimated mean value of the dependent variable from the independent value and the regression equation), the standard error of the fitted value, the residual and the standardized residual.

Use of STEPWISE command allows one to select from a large set of candidates the independent variables that best predict the dependent variable.

A.2 ANALYSIS OF THE ACCUMULATION OF ACETIC ACID

A.2.1 Determination of the Equation

Using the data in Table A-1 and running the program of Minitab's regression, we have following results:

Due to the shortage of data from the original experimental design, we can not completely go through the Stepwise procedure, as shows in Table A.2. But, through step by step of addition of each parameter, it was found that the inclusion of the squared terms increased the value of R-squared and decreased the S value. Here S is called "the standard deviation of y about the regression line" or "the standard error of estimate". The value S can be thought of as a measure of how much the observed y values differ

Table A.1: **Accumulation of Acetic Acid**

In the Absence of Sulfate

Influent (g COD/l)	AA at 1# (g/l)	AA at 2# (g/l)	AA_1-AA_2 (g/l)
4.56	0.382	0.152	0.23
9.93	0.688	0.096	0.59
17.7	0.876	0.02	0.856
28.8	1.166	0.04	1.126
38.1	2.895	2.553	0.342

1# sample port 1
2# sample port 2

In the presence of Sulfate

Influent (g COD/l)	AA at 1# (g/l)	AA at 2# (g/l)	AA_1-AA_2 (g/l)
15	1.038	0.103	0.935
20	1.163	0.043	1.120
30	1.403	0.073	1.330
40	1.600	0.057	1.543
50	2.127	1.714	0.413

Table A.2: Stepwise Analysis for Accumulation of Acetic Acid

```
MTB >
MTB > stepwise y in c2, x in c1 c3-c6

 STEPWISE REGRESSION OF    AA    ON  5 PREDICTORS, WITH N =     5

    STEP         1
CONSTANT    0.5143

Co**3       0.00004
T-RATIO       8.50

S             0.207
R-SQ          96.01
 MORE? (YES, NO, SUBCOMMAND, OR HELP)
SUBC>
SUBC>
SUBC>
```

from the corresponding average y value

If a cubic term was included, the R-squared value went further up to 96 and the S value went down. It was obvious that the best equation was one which included influent concentration, its square and cubic terms as follow:

$$AA = -0.27 + 0.183Co - 0.0102Co^2 + 0.000197Co^3 \qquad \text{(A.1)}$$

A.2.2 Analysis of the Adequacy of the Equation

The evaluation of the residuals and fitted values can be used to examine the model adequacy. Residual is the difference between the observation and the fitted value determined by regression equation. Thus residuals tell us how the model missed in fitting the data.

Table A.3: **Stepwise Analysis for Accumulation of Acetic Acid**

The regression equation is
AA = - 0.032 + 0.0601 Co

Predictor	Coef	Stdev	t-ratio	p
Constant	-0.0317	0.3560	-0.09	0.935
Co	0.06010	0.01528	3.93	0.029

s = 0.4185 R-sq = 83.8% R-sq(adj) = 78.3%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	2.7089	2.7089	15.47	0.029
Error	3	0.5255	0.1752		
Total	4	3.2344			

Obs.	Co	AA	Fit	Stdev.Fit	Residual	St.Resid
1	4.6	0.382	0.242	0.299	0.140	0.48
2	9.9	0.688	0.565	0.241	0.123	0.36
3	17.7	0.876	1.032	0.190	-0.156	-0.42
4	28.8	1.166	1.699	0.232	-0.533	-1.53
5	38.1	2.685	2.258	0.336	0.427	1.71

Table A.4: Stepwise Analysis for Accumulation of Acetic Acid

```
MTB >
MTB > regr c1 2 c2 c6

The regression equation is
AA = - 0.058 + 0.0597 Co + 0.171 Co**1.5

Predictor        Coef       Stdev    t-ratio        p
Constant      -0.0577      0.3745      -0.15    0.892
Co            0.05969     0.01603       3.72    0.065
Co**1.5        0.1711      0.2004       0.85    0.483

s = 0.4388      R-sq = 88.1%     R-sq(adj) = 76.2%

Analysis of Variance

SOURCE        DF          SS          MS         F        p
Regression     2      2.8494      1.4247      7.40    0.119
Error          2      0.3850      0.1925
Total          4      3.2344

SOURCE        DF      SEQ SS
Co             1      2.7089
Co**1.5        1      0.1405

CONTINUE?

Obs.       Co         AA        Fit  Stdev.Fit  Residual   St.Resid
  1       4.6      0.382      0.386      0.355    -0.004      -0.01
  2       9.9      0.688      0.364      0.345     0.324       1.20
  3      17.7      0.876      1.170      0.256    -0.294      -0.83
  4      28.8      1.166      1.490      0.345    -0.324      -1.20
  5      38.1      2.685      2.387      0.384     0.298       1.40
```

Table A.5: **Stepwise Analysis for Accumulation of Acetic Acid**

```
MTB > regr c1 2 c2 c5

The regression equation is
AA = 1.87 + 0.180 Co - 1.02 Co**0.5

Predictor        Coef       Stdev    t-ratio        p
Constant        1.870       1.739       1.08    0.395
Co             0.1799      0.1084       1.66    0.239
Co**0.5       -1.0164      0.9113      -1.12    0.381

s = 0.4025      R-sq = 90.0%     R-sq(adj) = 80.0%

Analysis of Variance

SOURCE        DF          SS          MS         F        p
Regression     2      2.9104      1.4552      8.98    0.100
Error          2      0.3240      0.1620
Total          4      3.2344

SOURCE        DF      SEQ SS
Co             1      2.7089
Co**0.5        1      0.2015

CONTINUE?

Obs.       Co         AA        Fit  Stdev.Fit  Residual   St.Resid
  1       4.6      0.382      0.520      0.380    -0.138     -1.04
  2       9.9      0.688      0.453      0.252     0.235      0.75
  3      17.7      0.876      0.778      0.292     0.098      0.35
  4      28.8      1.166      1.596      0.242    -0.430     -1.34
  5      38.1      2.685      2.450      0.366     0.235      1.41
```

Table A.6: Stepwise Analysis for Accumulation of Acetic Acid

```
MTB > brief output level 3
MTB > regr c1 2 c2 c3

The regression equation is
AA = 0.666 - 0.0381 Co + 0.00230 Co**2

Predictor        Coef       Stdev    t-ratio        p
Constant       0.6665      0.4642       1.44    0.288
Co          -0.03808     0.05469      -0.70    0.558
Co**2       0.002299    0.001252       1.84    0.208

s = 0.3128      R-sq = 93.9%     R-sq(adj) = 87.9%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     2      3.0387      1.5193      15.53    0.061
Error          2      0.1957      0.0979
Total          4      3.2344

SOURCE        DF      SEQ SS
Co             1      2.7089
Co**2          1      0.3298

CONTINUE?

Obs.       Co         AA         Fit  Stdev.Fit   Residual   St.Resid
  1       4.6      0.382      0.541      0.276     -0.159      -1.08
  2       9.9      0.688      0.515      0.182      0.173       0.68
  3      17.7      0.876      0.713      0.225      0.163       0.75
  4      28.8      1.166      1.476      0.212     -0.310      -1.35
  5      38.1      2.685      2.552      0.298      0.133       1.40
```

Table A.7: Stepwise Analysis for Accumulation of Acetic Acid

```
MTB > brief output level 3
MTB > regr c1 2 c2 c4

The regression equation is
AA = 0.540 - 0.0026 Co +0.000039 Co**3

Predictor        Coef        Stdev    t-ratio        p
Constant       0.5400       0.3161       1.71    0.230
Co          -0.00260      0.02695      -0.10    0.932
Co**3     0.00003910   0.00001578       2.48    0.132

s = 0.2541      R-sq = 96.0%     R-sq(adj) = 92.0%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     2      3.1053      1.5526      24.05    0.040
Error          2      0.1291      0.0646
Total          4      3.2344

SOURCE        DF      SEQ SS
Co             1      2.7089
Co**3          1      0.3964

CONTINUE?


CONTINUE?
Obs.       Co         AA         Fit  Stdev.Fit   Residual   St.Resid
  1       4.6      0.382       0.532      0.216     -0.150      -1.12
  2       9.9      0.688       0.552      0.146      0.136       0.65
  3      17.7      0.876       0.711      0.174      0.165       0.89
  4      28.8      1.166       1.399      0.186     -0.233      -1.34
  5      38.1      2.685       2.603      0.247      0.082       1.39
```

Table A.8: Stepwise Analysis for Accumulation of Acetic Acid

```
MTB > brief output level 3
MTB > regr c1 3 c2-c4
* NOTE *       Co is highly correlated with other  predictor variables
* NOTE *    Co**2 is highly correlated with other  predictor variables
* NOTE *    Co**3 is highly correlated with other  predictor variables

The regression equation is
AA = - 0.270 + 0.183 Co - 0.0102 Co**2 +0.000197 Co**3

Predictor       Coef       Stdev    t-ratio        p
Constant     -0.2703      0.1449      -1.87    0.313
Co            0.18258     0.02967      6.15    0.103
Co**2       -0.010247    0.001609      -6.37    0.099
Co**3      0.00019653  0.00002496       7.88    0.080

s = 0.05573     R-sq = 99.9%     R-sq(adj) = 99.6%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    3      3.2313      1.0771     346.81    0.039
Error         1      0.0031      0.0031
Total         4      3.2344

CONTINUE?

SOURCE       DF      SEQ SS
Co            1      2.7089
Co**2         1      0.3298
Co**3         1      0.1926

Obs.       Co        AA       Fit  Stdev.Fit  Residual   St.Resid
  1       4.6    0.3820    0.3678     0.0539    0.0142       1.00
  2       9.9    0.6880    0.7247     0.0419   -0.0367      -1.00
  3      17.7    0.8760    0.8408     0.0432    0.0352       1.00
  4      28.8    1.1660    1.1832     0.0530   -0.0172      -1.00
  5      38.1    2.6850    2.6805     0.0555    0.0045       1.00 X

X denotes an obs. whose X value gives it large influence.
```

Figure A-1 is a plot of the fitted values vs the actual values. This plot shows a very good fit between the model and the data. In addition, plots of the residuals vs fit, actual values and independent variables were all made (Figures A-2 to A-5). Strong pattern or tendency in the residual plot indicates that we probably have a poor model. If the model is serious wrong, the residuals would tend to more positive for some parts of x values, and more negtive for others. However, none of them showed up any problem regarding the model adequacy.

A.2.3 The Comparison between the Experimental Data and the Model

Figure A-6 presents the experimental data (points) and the model (curve). It shows a good fit.

A.3 SUMMARY OF EMPIRICAL MODELS

Following exactly the same procedure as described in the above section, the models for degradation of acetic acid and models for accumulation and degradation of propionic acid and total VFAs in both the absence and presence of sulfate were developed as fo

A.3.1 In the Absence of Sulfate

Accumulation of AA (acetic acid)

$$AA = -0.27 + 0.183Co + -0.0102Co^2 + 0.000197Co^3 \qquad \text{(A.2)}$$

Degradation of AA Ra (Table A.9)

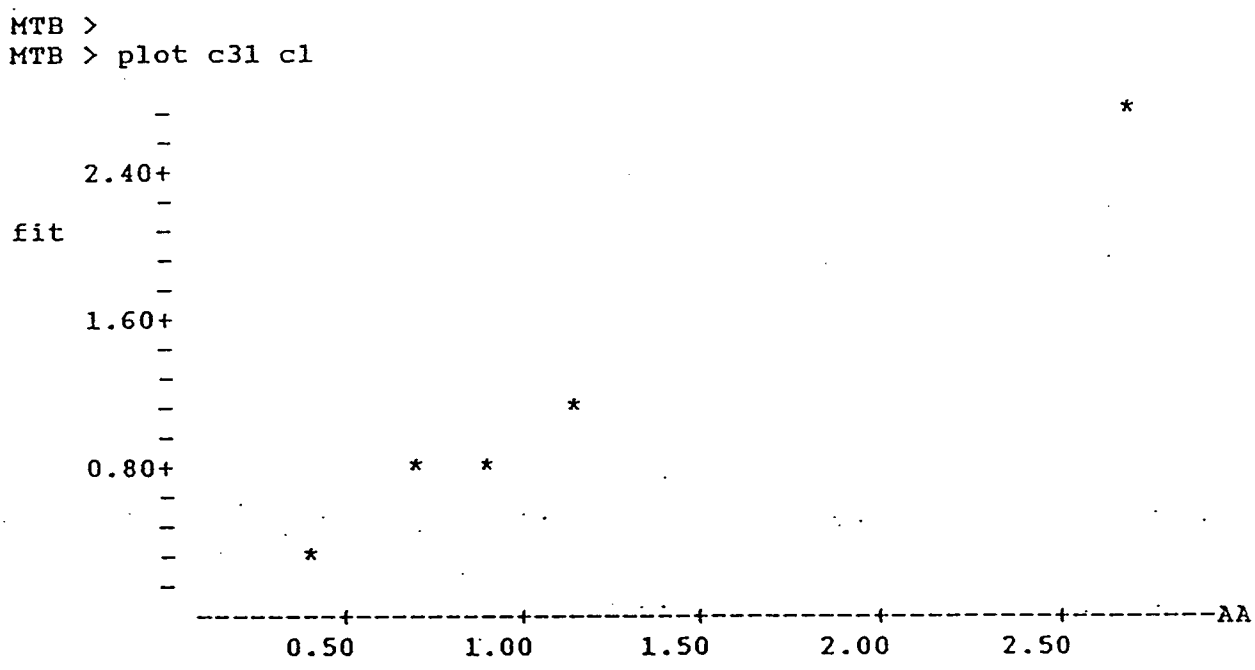


Figure A.1: **Fitted Values versus the Actual Values**

```
MTB > print c1 c31 c22

 ROW       AA       fit     residual

   1    0.382    0.3678    0.0141891
   2    0.688    0.7247   -0.0367000
   3    0.876    0.8408    0.0352088
   4    1.166    1.1832   -0.0172389
   5    2.685    2.6805    0.0045412

MTB >
MTB >
MTB >
```

```
MTB > plot c22 c31

          -                 *
          -
    0.025+
          -
residual-       *
          -
          -                                                  *
    0.000+
          -
          -
          -                         *
          -
   -0.025+
          -
          -              *
          -
          -
            --------+---------+---------+---------+---------+--------fit
                 0.50      1.00      1.50      2.00      2.50

MTB >
MTB >
```

Figure A.2: Analysis of residuals

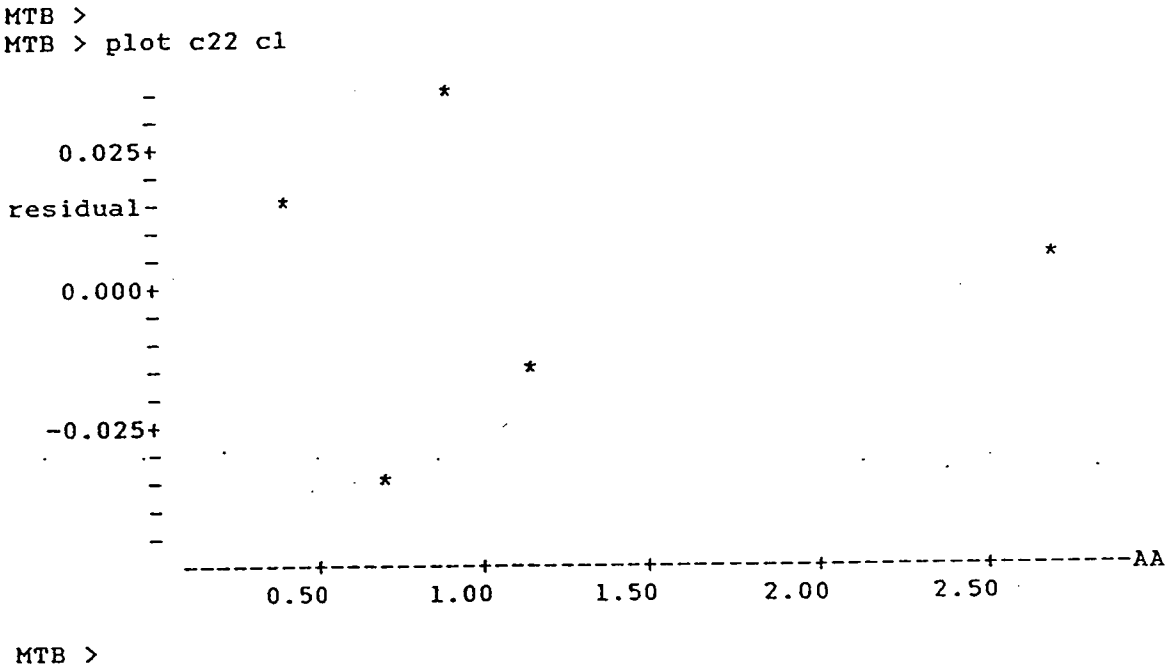


Figure A.3: Analysis of residuals

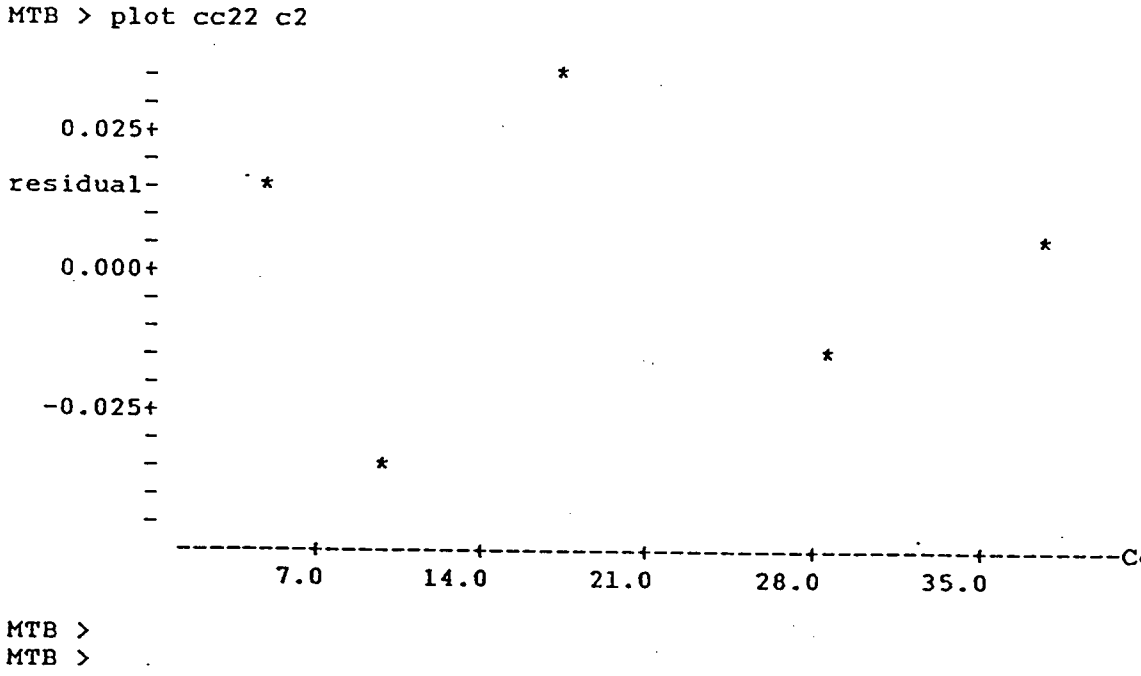


Figure A.4: Analysis of residuals

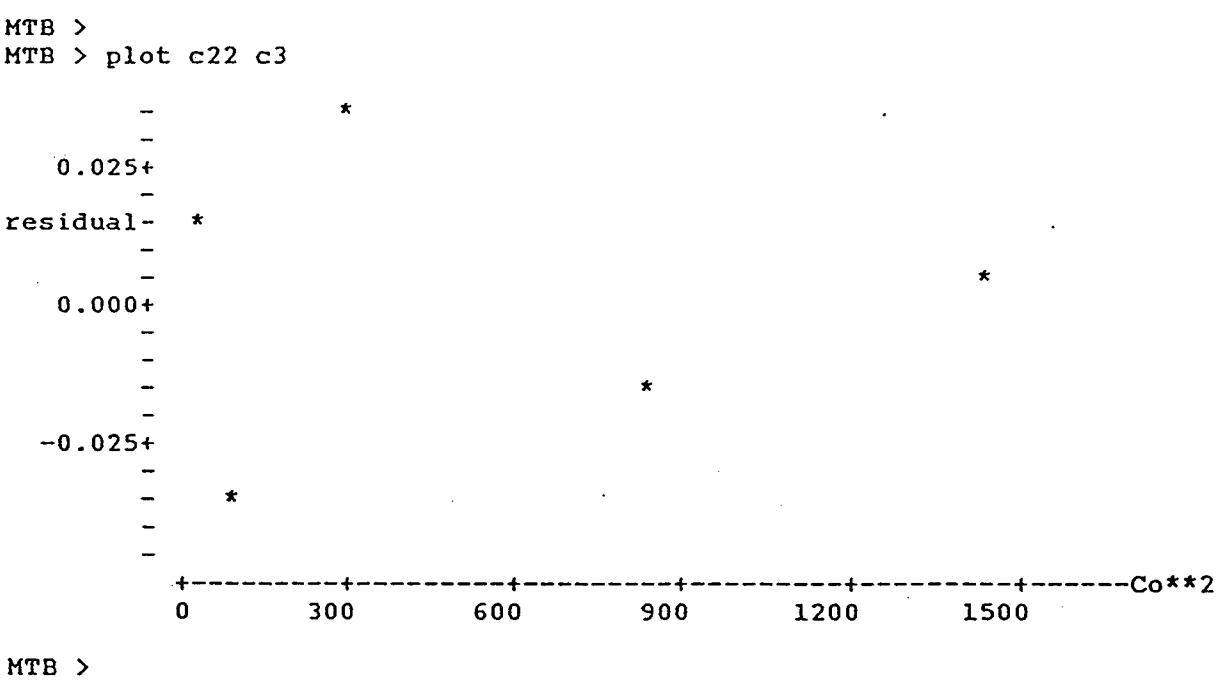


Figure A.5: **Analysis of residuals**

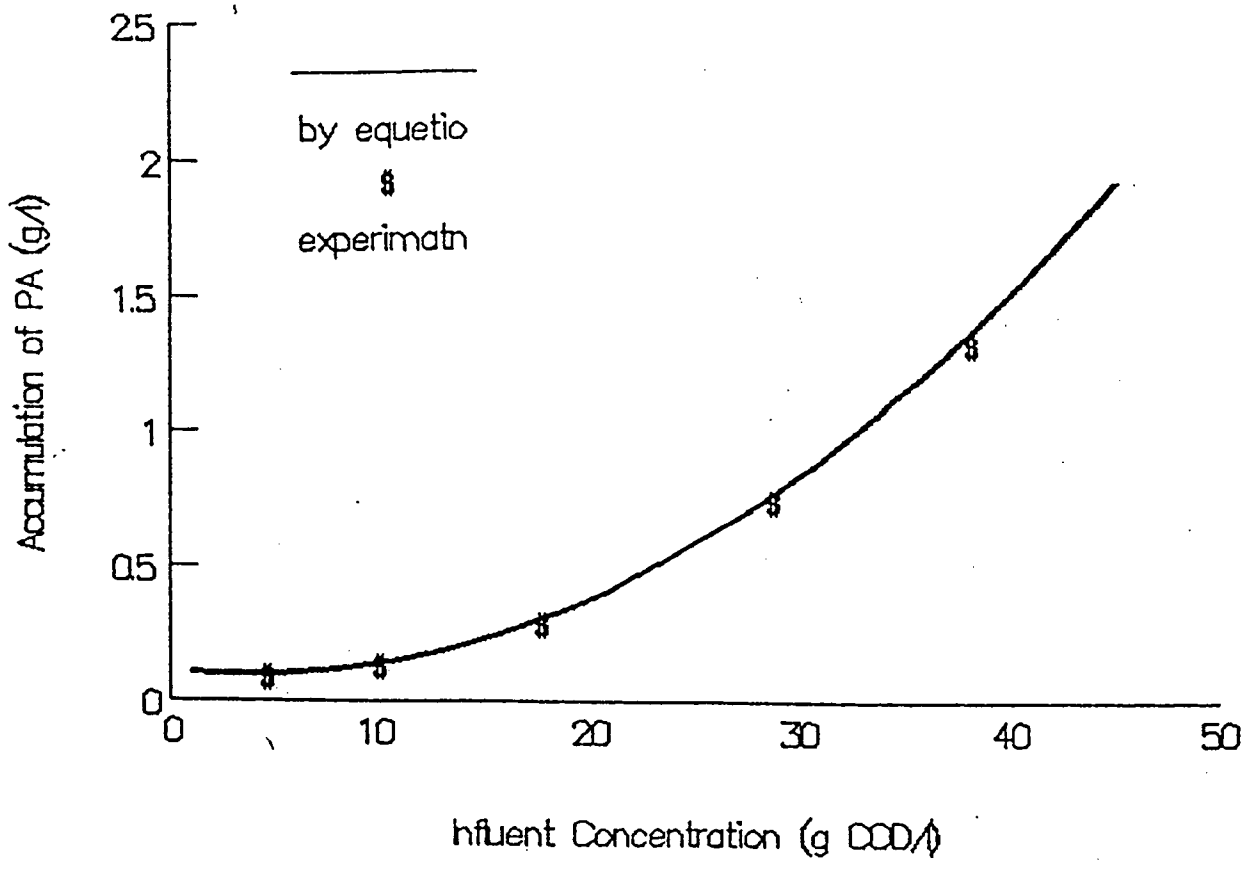


Figure A.6: **Comparison of Experimental Data with Model**

Table A.9: Regression Analysis for Degradation of Acetic Acid

```
MTB > print c12-c15

 ROW        1/r         C       C**2      C**(-2)

   1    4.34783      4.56      20.79   0.0480917
   2    1.68919      9.93      98.60   0.0101415
   3    1.16822     17.70     313.29   0.0031919
   4    0.88810     28.80     829.44   0.0012056
   5    2.92398     38.10    1451.61   0.0006889

MTB >


The regression equation is
1/r = 3.68 - 0.293 C + 0.00707 C**2 + 38.1 C**(-2)

Predictor        Coef        Stdev    t-ratio        p
Constant        3.682        1.993       1.85    0.316
C             -0.2927       0.1703      -1.72    0.335
C**2         0.007075     0.003305       2.14    0.278
C**(-2)         38.14        30.45       1.25    0.429

s = 0.4739      R-sq = 97.3%     R-sq(adj) = 89.0%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     3      7.9592      2.6531      11.81    0.210
Error          1      0.2246      0.2246
Total          4      8.1838

SOURCE        DF      SEQ SS
C              1      0.7734
C**2           1      6.8336
C**(-2)        1      0.3523

CONTINUE?

Obs.         C         1/r         Fit  Stdev.Fit    Residual    St.Resid
  1        4.6       4.348       4.328       0.474       0.020        1.00 X
  2        9.9       1.689       1.859       0.442      -0.170       -1.00
  3       17.7       1.168       0.839       0.341       0.329        1.00
  4       28.8       0.888       1.166       0.384      -0.277       -1.00
  5       38.1       2.924       2.825       0.464       0.099        1.00

X denotes an obs. whose X value gives it large influence.
```

$$\frac{1}{Ra} = 3.68 - 0.293Co + 0.00707Co^2 + 38.1Co^{-2} \quad \text{(A.3)}$$

Accumulation of propionic acid (PA)

$$PA = 0.112 - 0.00893Co + 0.00108Co^2 \quad \text{(A.4)}$$

Degradation of PA Rp

$$\frac{1}{Rp} = -18.3 + 0.488Co + 216Co^{-1} \quad \text{(A.5)}$$

Accumulation of VFA

$$VFA = 2.12 - 0.0325Co - 5.76Co^{-1} \quad \text{(A.6)}$$

Degradation of VFA Rv

$$\frac{1}{Rv} = -0.648 + 0.0288Co + 9.02Co^{-1} \quad \text{(A.7)}$$

A.3.2 In the Presence of Sulfate

Accumulation of AA

$$AAs = 0.504 + 0.0590Co - 0.00172Co^2 + 0.000024Co^3 \tag{A.8}$$

$$\frac{1}{Rs} = 15.6 - 0.63Co + 0.00815Co^2 - 104Co^{-1} \tag{A.9}$$

Accumulation of PA

$$PAs = 0.603 - 0.0391Co + 0.00141Co^2 \tag{A.10}$$

$$\frac{1}{Rs} = 11.0 - 0.54Co + 0.00715Co^2 \tag{A.11}$$

Accumulation of VFA

$$VFA = 1.77 - 0.0552Co + 0.00215Co^2 \tag{A.12}$$

Degradation of VFA

$$\frac{1}{Rs} = 3.36 - 0.213Co + 0.00367Co^2 \tag{A.13}$$

Appendix B

EFFECTS OF SULFATE IN BATCH EXPERIMENTS

B.1 INTRODUCTION

When wastewater is subjected to anaerobic digestion, a high concentration of sulfate has been thought to be toxic for methanogenic bacteria (see literature review). Recent research has greatly increase the knowledge about the function of sulfate in the anaerobic process. It is known that the sulfate requirement of methanogens in anaerobic digestion is a complex function. On one hand, sulfate reducing bacteria help to maintain the anaerobic condition required for the growth of methanogens. Sulfur, which is the reduced product of sulfate in the anaerobic process, is also a nutrient necessary for methanogens. On the other hand, the addition of sulfate inhibits methanogenesis. The inhibition from sulfate is attributed to that methanogens and sulfate reducing bacteria competing for the common substrates, acetic acid and hydrogen. In the presence of excess hydrogen, they have no effects on each other. When the substrates supply becomes rate limiting, however, competition does take place.

As described in earlier chapters, unsuccessful experiments on anaerobic digestion of cheese whey have been reported by a number of researchers. Little research, however, has been done so far to solve or to explain these failures. The problems encountered in previous studies has been generally attributed to inadequate buffering capacity and micronutrient deficiency of cheese whey. According to the mechanisms considered in Chapter 7, it can be assumed that sulfate, in the proper concentration, may be applied

Table B.1: **Independent Variables in Batch Experiment**

Variables	Levels	
	+	-
Sulfate (g/l)	0.6	0
Cheese Whey Concentration (g COD/l)	6	3
Sludge Concentration (g/l)	1.5	0.75

to moderate the detrimental influences of excess hydrogen on a stressed anaerobic reactor.

The practical question is what sulfate concentration in an industrial wastewater of a given composition, such as cheese whey, makes anaerobic digestion possible and successful. This preliminary experiment, therefore, was carried out to find how sulfate affects the anaerobic digestion system

B.2 PRELIMINARY EXPERIMENTAL DESIGN

B.2.1 Variables

The independent variables are presented in Table B-1.

The concentration of cheese whey was chosen based on consideration of the possible organic loading rate during the start-up period for successful treatment. According to previous studies for a cheese whey anaerobic process, the organic loading rate cannot

Table B.2: **Experimental Design of Batch Run**

Table B-2 Experimental Design of Batch Run

Run	S	Co	A
1	-	-	-
2	+	-	-
3	-	+	-
4	+	+	-
5	-	-	+
6	+	-	+
7	-	+	+
8	+	+	+

exceed 0.3 g COD/g VSS at the start-up. The maximum concentration of sludge available in the lab is about 20 g VSS/l. Therefore, the upper limit of cheese whey concentration was 6 g COD/l. A 3 g COD/l difference would make the response variables, such as gas composition and treatment efficiency, different. So 3 g COD/l was the lower limit. The possible lower limit of sludge concentration might be 10 g/l based on the same consideration of organic loading rate.

The concentration of sulfate was chosen based on the literature review. The toxic levels of sulfate for methanogens varied from one study to another. Generally, the optimal concentration of sulfate for methanogens growth in the anaerobic process was reported in the range of 0.1-1 mM, which is equal to 0.014-0.14 g Na_2SO_4/l. Based on the mechanism of competition of both methanogens and sulfate reducing bacteria for common substrates, it would be reasonable to consider the ratio of COD concentration and sulfate in the experiment. The only information that could be had from literature indicated that when the ratio of COD to sulfate decreased below 10, sulfate reduction would occur and methanogenesis would be inhibited. The upper limit concentration of sulfate was set in such a way that the lowest ratio of COD/Na_2SO_4 was about 5. Therefore, the sulfate concentration should be 0.6 g/l at its upper limit. The lower limit was set at zero to maximize the difference.

B.2.2 Experimental Design

A full 2^3 factorial design with duplicates was used in this experiment. The layout is shown in Table B-2

B.3 EXECUTION OF THE EXPERIMENT

B.3.1 Substrate

Cheddar cheese whey was used in this study. The COD concentration of the original substrate was as high as 65 g COD/l and pH as low as 4. The cheese whey was dilute to the desired concentrations, 6 gCOD/l and 3 g COD/l. Then potassium hydroxide was used to adjust the pH to 7

B.3.2 Seed Sludge (Bacteria)

The seed sludge was obtained from the effluent of a laboratory-scale, upflow anaerobic sludge blanket (UASB) reactor and had a concentration of about 20 g VSS/l.

B.3.3 Reactor Operation

16 flasks with volume of 250 ml were used to as the batch reactors in this experiment. Each flask was seeded 75 ml sludge with two different VSS concentrations as desired in experimental design. They were sampled and fed once a week. In the first week each of flasks was fed 100 ml substrate liquid containing different concentrations of sulfate and cheese whey according to the experimental design. In the subsequent week, from each of them was drawn a 60 ml sample for analysis and fed 60 ml of the substrates which ware exactly the same composition as the first time. All the flasks were randomly located in the incubator under a constant temperature of 35°C.

B.3.4 Chemical Analyses

Gas production was collected daily for each reactor. Gas composition was analyzed every other day. The liquid in the flasks was withdrawn once a week for analysis of COD (Chemical Oxygen Demand), VFA (Volatile Fatty Acids), pH and residual sulfate. Both gas composition and VFA were analyzed on a Hewlett Packard 5890A gas chromatography. COD was determined by a colorimetric method. Sulfate analysis was conducted according to the "standard methods".

B.4 RESULTS AND DISCUSSION

B.4.1 Effects of Sulfate (S), Feed Strength (Co) and Seed Sludge Amount (A) on the Methane Production (CH_4%)

An anaerobic process is considered to be a step-wise process which proceeds in different successive stages. Anaerobic conversion of the acid products into methane and carbon dioxide, also called "methanogenesis", is the terminal and rate-limiting step of the whole sequence. Methane formation is accomplished by the methane bacteria. Methane content in the produced gas is one of the indicators of activity of the methane bacteria as well as of the system's performance. The experimental results are summarized in Table B-3. Subscripts a, b and c stand for sampling time, the first, the third and the fourth week from the start of the experiment respectively. Using the JASS program, the main effects of S, Co and A and their interactions were estimated, which are presented in Table B-4. During the first week of the experiment, the effect of Co was the most significant one. As we can see from the cube plots (Figure B-1) and the data analysis, CH_4% increased an average of about 7.4 units when the influent concentration decreased from 6 g COD/l to 3 g COD/l. In comparison with the effect of Co, the effects of S and A were less

Table B.3: **Experimental Results**

Row	Sulfate	COD	Sludge	CH_4%-a	CH_4%-b	CH_4%-c	pH
1	-1	1	-1	60.15	47.65	48.48	6.500
2	1	-1	-1	59.29	45.26	48.41	6.725
3	-1	-1	-1	52.99	49.30	45.47	6.445
4	1	1	-1	52.09	49.25	46.81	6.615
5	-1	1	1	60.30	48.06	49.88	6.770
6	1	-1	1	57.70	47.08	46.65	6.805
7	-1	-1	1	52.25	46.61	54.02	6.500
8	1	1	1	50.11	46.92	48.12	6.750
9	-1	1	-1	61.13	48.12	49.00	6.500
10	1	-1	-1	59.66	44.74	46.48	6.715
11	-1	-1	-1	54.82	50.06	44.84	6.430
12	1	1	-1	51.75	49.70	47.12	6.605
13	-1	1	1	60.10	53.36	51.48	6.785
14	1	-1	1	58.62	49.05	47.56	6.830
15	-1	-1	1	51.92	47.00	47.28	6.490
16	1	1	1	51.55	47.19	46.87	6.740

significant during the first week. The less significant effect of S might be attributed to two reasons. On one hand, sulfate reducing bacteria (SRB) might not have built up and less competitive in the first week. It could also be due to the fact that growth of SRB was limited by the low concentration of sulfate in the culture. A higher concentration of sulfate addition might have led to a statistically significant effect of sulfate on the gas composition.

As time passed, it became clear that the effect of S on the gas composition was somehow related to the feed strength. Look at the upper surface (higher strength) and the lower surface (lower strength) of the cube plot (Figure B-2, CH_4% in the third week). For lower feed strength of 3 g COD/l, the CH_4% in the generated gas changed from 47.9% in the absence of sulfate to 45% in the present of sulfate, or from 50.7% to 48.1%. While

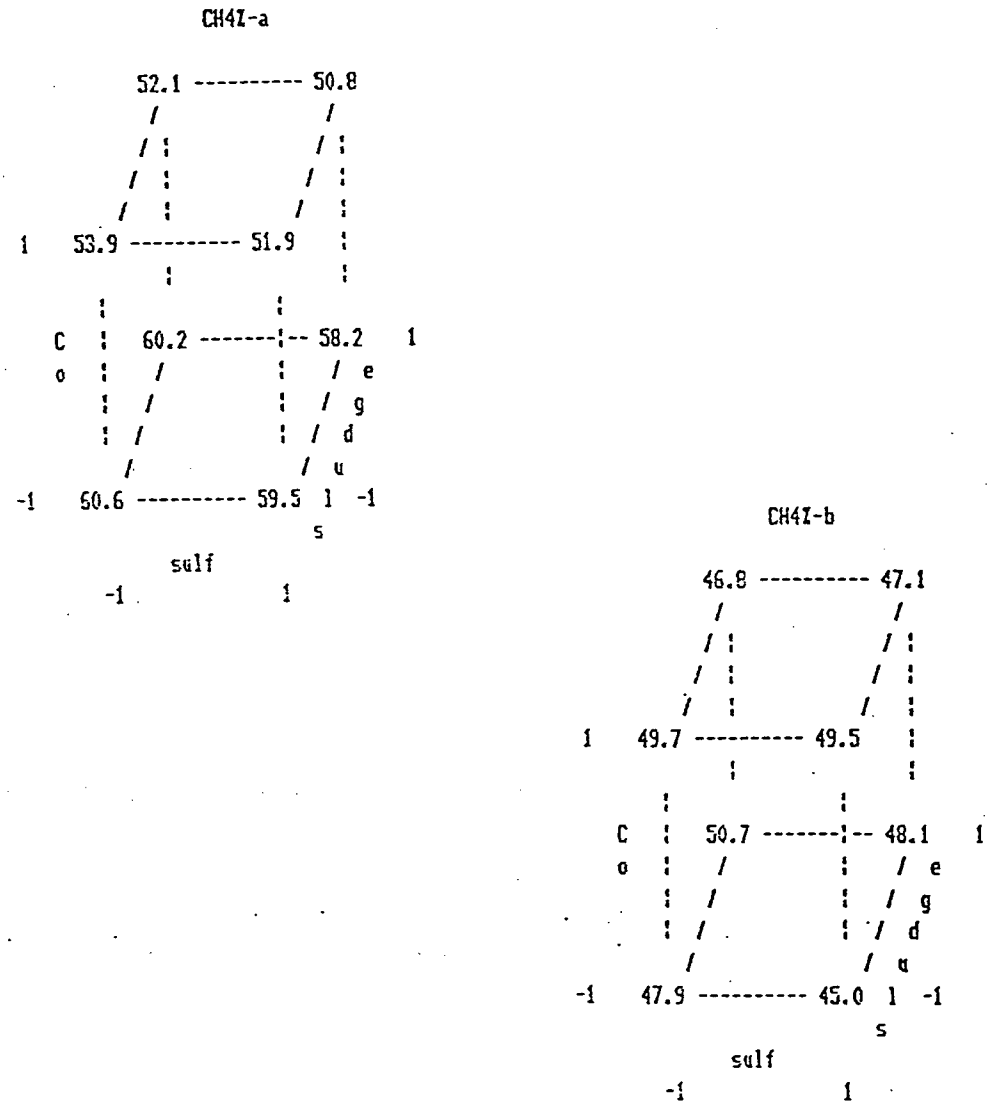


Figure B.1: **Effect of sulfate on Biogas Composition**

Table B.4: **Yates Analysis**

	CH_4%-a	CH_4%-b	CH_4%-c	pH
Average	55.90	48.08	48.03	6.64
S	-1.61	-1.37	-1.55	0.17
Co	-7.43	0.33	-0.92	-0.13
A	-1.12	0.15	1.91	0.14
SCo	-0.01	1.39	0.88	0.04
SA	-0.04	0.17	-1.18	-0.03
CoA	-0.29	-2.80	1.16	-0.05
CoAS	0.40	0.05	-0.67	0.06

$CH_4\%$ was almost the same for both cases of absence and presence of sulfate when higher COD concentration was applied. This result demonstrated that the SRB competed more effectively at lower substrate level. In contrast to sulfate, the effects of Co became less significant after 3 weeks running. This is because the microbes in the reactor had been acclimatized and their concentration increased.

The interaction effects of S and Co, Co and A were not negligible compared to the main effect of S although they didn't cause inhibition. The interaction plot of $CH_4\%$ (Figure B-3) shows that the interactions between sulfate and feed strength (the ratio of COD/$NaSO_4$), and feed strength and sludge concentration (sludge loading) are more significant than the interaction of sulfate and sludge concentration. It has been reported that the inhibition of sulfate on the activity of methanogens is related to the ratio of feed strength and sulfate concentration in terms of COD/Na_2SO_4. When the ratio is smaller than 10, inhibition could occur. In this study, the ratio was even as low as 5, but no inhibition was noticed. We would suggest that the critical value of sulfate causing inhibition to an anaerobic process may vary with substrates. For a highly soluble feed, such as cheese whey, a higher sulfate concentration can be tolerated since excess hydrogen and fatty acid exist in this system.

An unusual observation was made that the effect of sludge on the gas composition was negative in the first week. That meant that the more sludge in the reactor, the less methane that was produced in the experiment. Generally, a higher concentration of sludge should result in higher methane production since the sludge loading is lower. The only explanation for this observation was that the lower concentration of sludge was made in such a way that 35 ml sludge was mixed with 35 ml of effluent from an anaerobic digester, which contained bacteria and a higher pH (about 8.0), which might have increased the buffer capacity of the system. Therefore, in spite of a low concentration of sludge, it could have higher activity and buffer capacity than that of the original sludge.

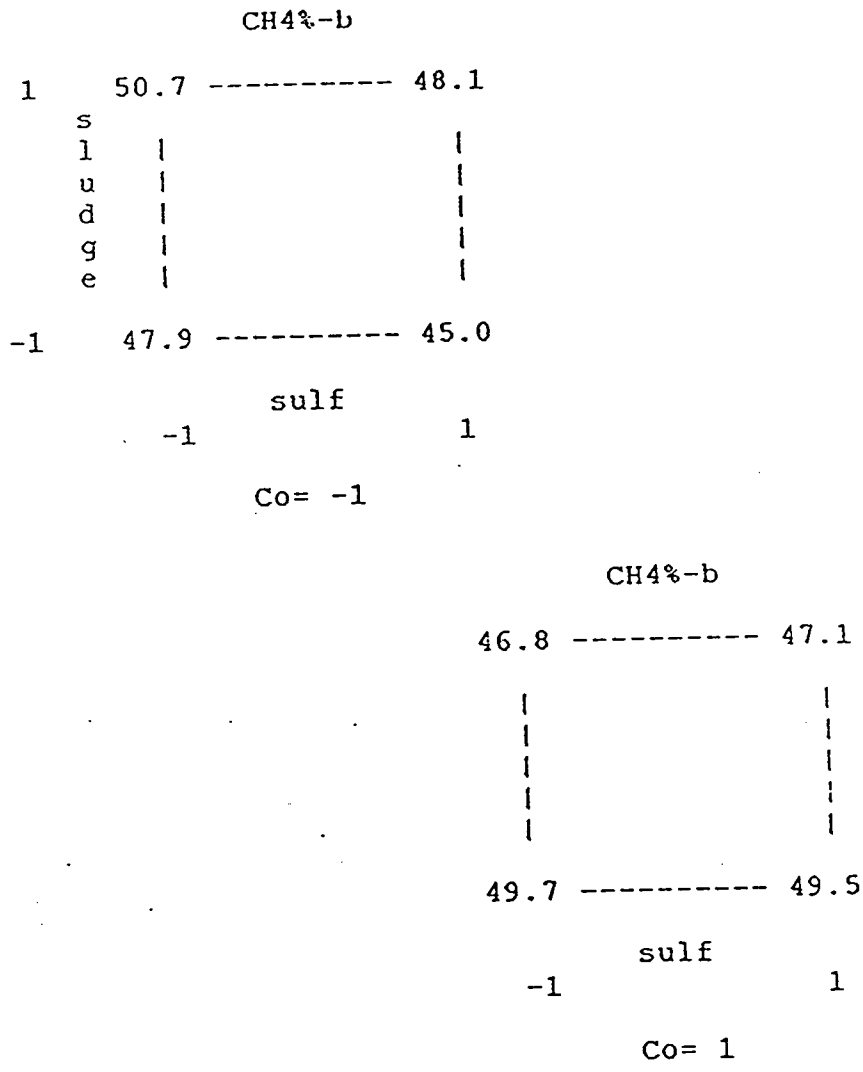
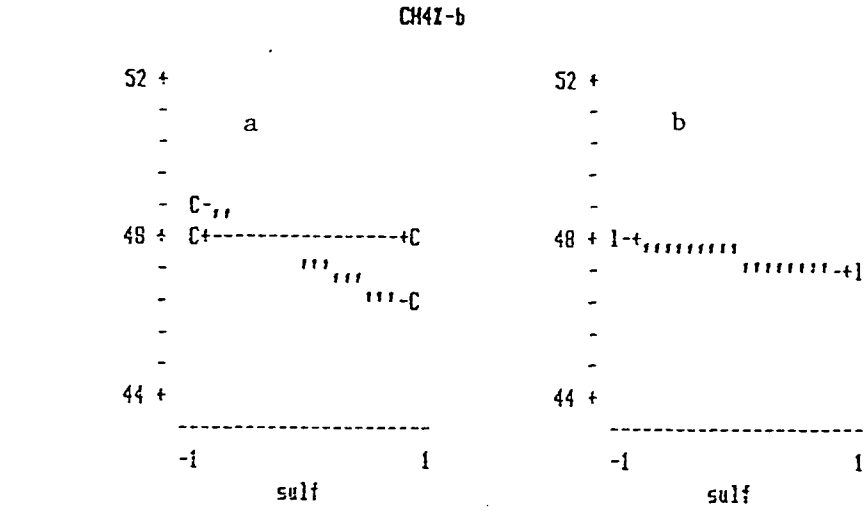


Figure B.2: **Effect of Sulfate on the Biogas Composition**

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tvol>
tvol>


tvol>
tvol>
tvol> intplot Cl
```

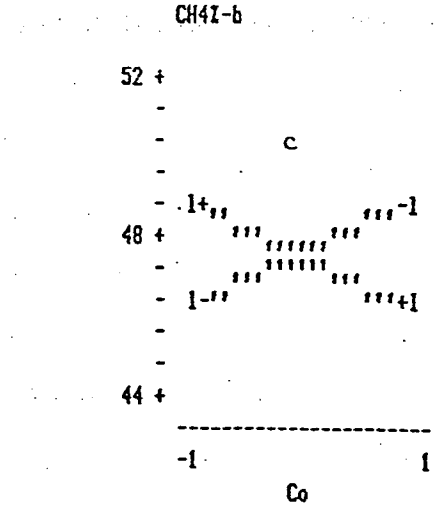

Figure B.3: **Interaction Effects of Sulfate (S), Influent COD (C), and Sludge (A): (a) S and C, (b) S and A, (c) C and A**

Table B.5: ANOVA Table

Source	DF	SS	MS	F	Fcrit
Block	2	656.49	328.25	42.07	2.80
Treat	15	149.54	9.97	1.28	1.88
Exp.E	30	234.09	7.80		
Total	47	1040.12			

Where DF=degreee of freedom, SS=sum of square, MS=mean square.

The unusual phenomenon disappeared and the effect of sludge on the methane production could be ignored in subsequent weeks experiments because the sludge in both cases was acclimatized.

It was noticed in this experiment that there was a time effect in batch culture. Considering time effect as the block effect (the first week and the third week), we constructed the ANOVA table (Table B-5) to find how significant the block effect was.

From the F values in Table B-5 we can see that the block effect was very significant. This indicated that the effects of feed strength and sludge concentration changed with time.

None of the main effects was clearly statistically significant from this experiment except the feed strength in the first week based on the calculation of a 95 % confidence

interval. Therefore, the scales of the factors should be made larger to increase the discrimination, especially the sulfate concentration. The upper limit of sulfate concentration was based on the previous studies, but it still didn't cause any significant effects, which also revealed that the critical inhibition value of sulfate on cheese whey is higher than that of other substrates which have lower solubility.

B.4.2 Effects of S, Co and A on pH of the System

pH is one of the important parameters for an anaerobic system. The optimum pH for methanogenesis is between 7.0.to 8.0 units. Below 6.0, methanogenesis will be inhibited. Therefore, pH value could be an indicator of the system stability and its performance. The higher the pH, the more stable the system is.

From the results of yates analysis we can see that the effect of sulfate on pH was very significant. By using feed containing sulfate, pHs averaged 0.2 units higher than using feed without sulfate. This indicated that, at these experimental conditions, the addition of sulfate helped to maintain the stability of the system since it can increase the buffer capacity of a cheese whey anaerobic digestion system.

The effects of Co and A were also significant. pH decreased with the increase of feed strength and increased with the increase of the amount of the seed sludge. There was no unusual observation as in the case of methane production since the pH data were taken after 3 weeks operation.

The effects on pH had an estimated standard deviation of 0.0096 with 8 degree of freedom. To determine the confidence interval from the N=16-run

$$\frac{ts}{\frac{N}{4}} = \frac{2.306 \times 0.0096}{2} + 0.011 \qquad \text{(B.1)}$$

Table B.6: **95% Confidence Interval**

S	Ø.17±Ø.Ø1	or	Ø.18	to	Ø.16
Co	-Ø.13±Ø.Ø1	or	-Ø.12	to	-Ø.14
A	Ø.14±Ø.Ø1	or	Ø.15	to	Ø.13
SCo	Ø.Ø4±Ø.Ø1	or	Ø.Ø5	to	Ø.Ø3
SA	-Ø.Ø3±Ø.Ø1	or	-Ø.Ø2	to	-Ø Ø4
CoA	-Ø.Ø5±Ø.Ø1	or	-Ø.Ø4	to	-Ø.Ø6
SCoA	Ø.Ø6±Ø.Ø1	or	Ø.Ø7	to	Ø.Ø5

The 95% confidence intervals are shown in Table B-6.

Since none of these effects include zero in the confidence interval, it can be stated that all of these effects are significant at the 95% confidence level. However, the intplot of Fig.B-5 shows that the interactions are not important in these operating conditions. The predominant effects are basically the first order terms. Figure B-6 is the plot of fitted value vs the actual pH value with a linear model. Note the fit is fairly good. So the interaction effects on pH can be negligible. This allows one to use the method of steepest ascent in the next experimental step. The different results between the two responses, $\%CH_4$ and pH, show that pH is more sensitive to the process parameters than $\%CH_4$.

As a general procedure, the residuals were plotted. None of those plots appeared anything unusual. Figure B-7 demonstrates that residuals basically center at zero.

```
two1>
two1> effects
                Effect  Std Error   t-Ratio
                        Effect

  Average      6.63781    .00240 .27653E+4
  sulf (S)      .17063    .00480  35.54180
  Co (C)       -.13188    .00480 -27.47010
  sludge (1)    .14188    .00480  29.55300
  SC            .04062    .00480   8.46230
  S1           -.02563    .00480  -5.33778
  C1           -.04563    .00480  -9.50384
  SC1           .06438    .00480  13.40960

S =  .00960 with  8 d.f.
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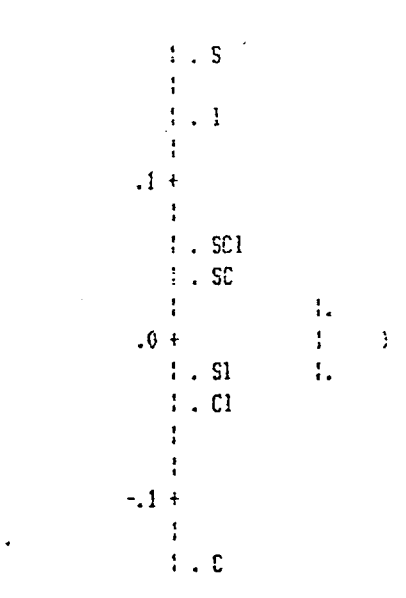


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two1> cube
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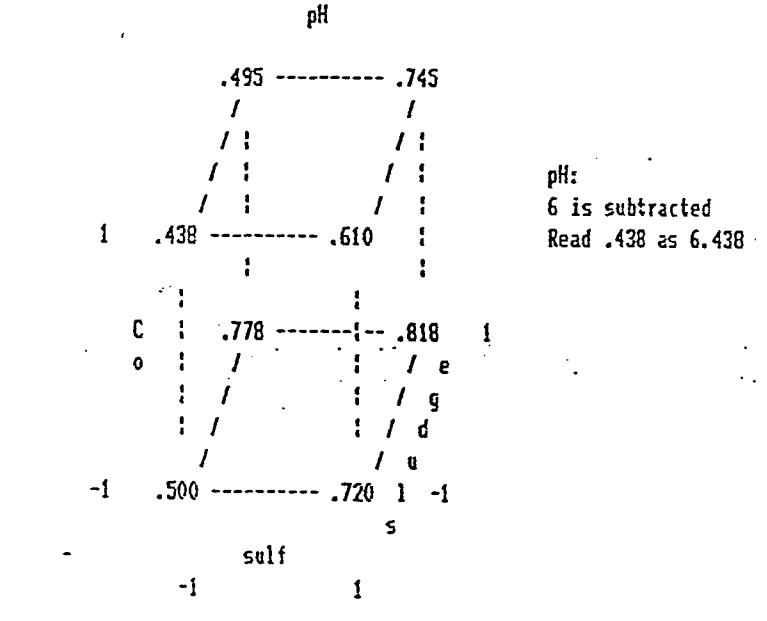
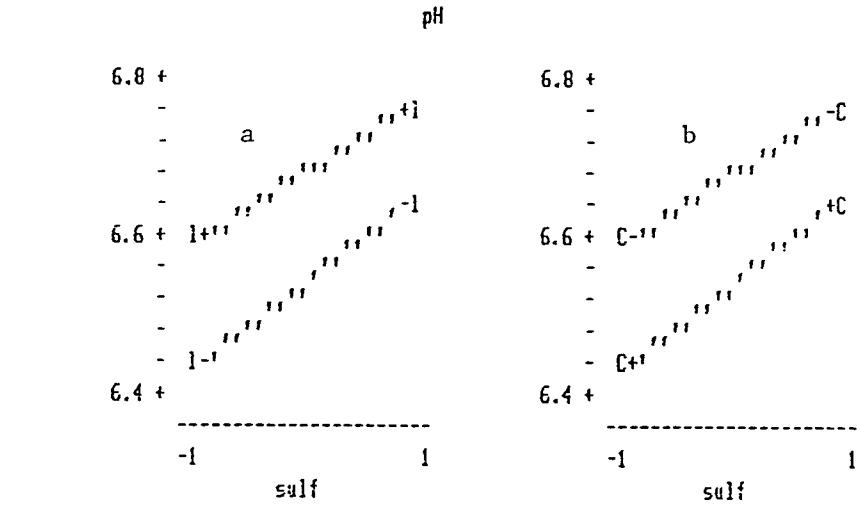


Figure B.4: Effect of Sulfate on pH

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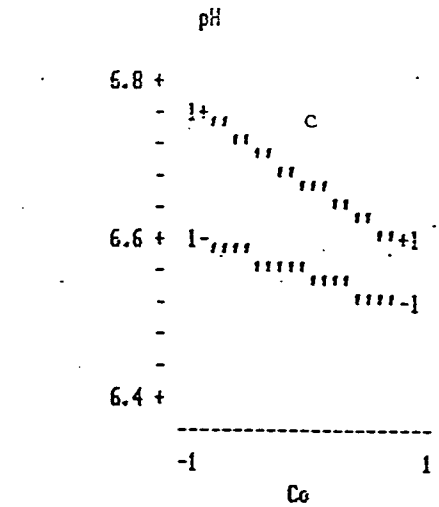


Figure B.5: **Interaction Effects of Sulfate (S), Influent COD (C), and Sludge (A): (a) S and A, (b) S and C, (c) C and A**

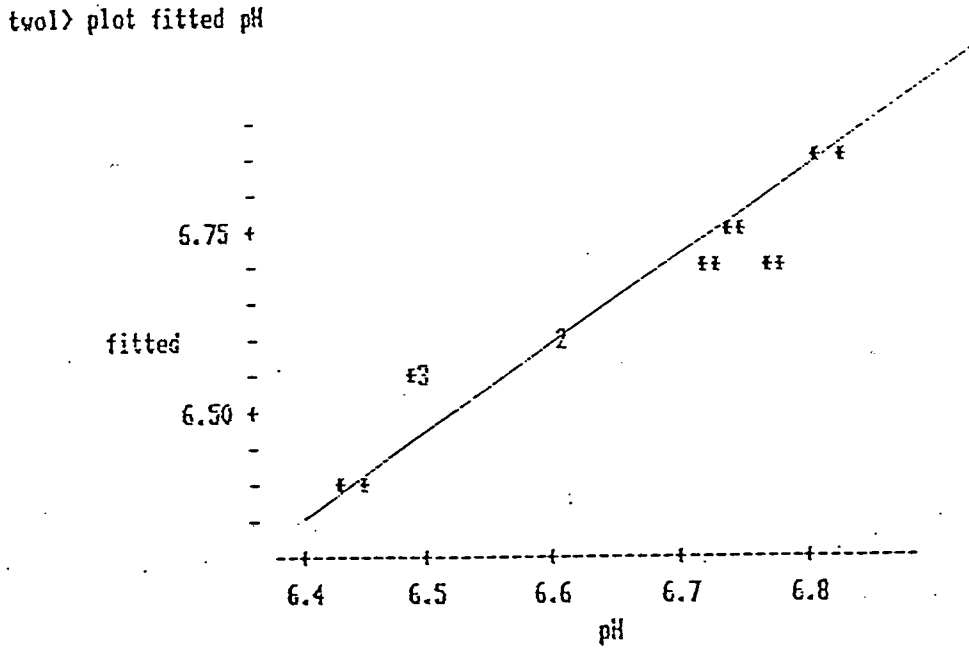


Figure B.6: **Fitted pH versus Actual Value**

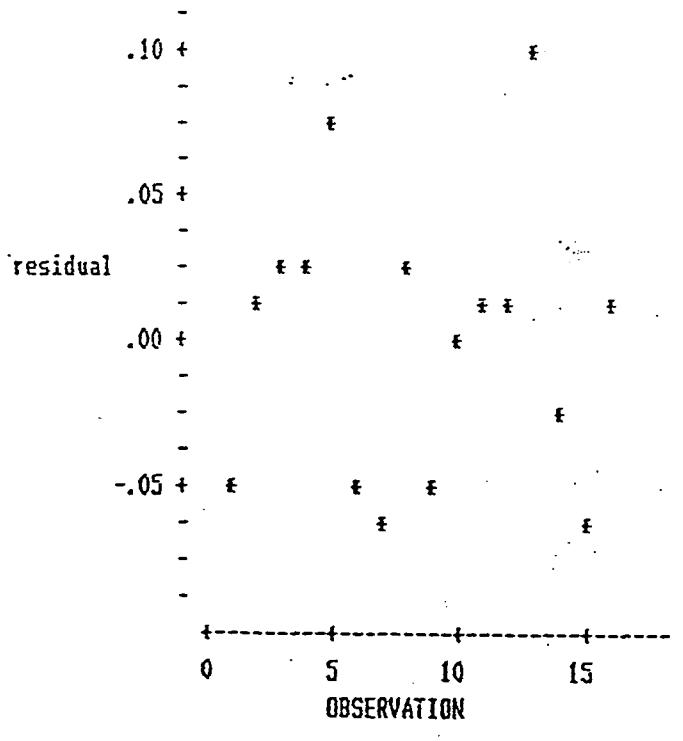


Figure B.7: **Analysis of Residuals**

B.5 CONCLUSIONS

1. An interesting finding is that the effects of sulfate on the gas composition was related to the feed strength. At lower feed strength, $\%CH_4$ decreased when sulfate was used. However, no difference in gas composition between sulfate used and the control case was observed for higher feed strength. This illustrated that SRB competed with methane bacteria more effectively at lower substrate levels.

2. Contrary to reports in the literature, no inhibition was observed even when the ratio of COD and sulfate was as low as 5 and the sulfate concentration was as high as 60 mM/l. This can be attributed contributed to cheese whey characteristics of high solubility , and high feed strength (compared to 0.5 -1 g COD/l in the previous studies). Therefore, the critical inhibition value of sulfate varies from one substrate to another. Using higher solubility substrates as feed, such as cheese whey, the inhibition value of sulfate is higher than that of other substrates which have lower solubility since excess hydrogen exists in such a system.

3. The effect of sulfate on pH is significant. pH is higher when sulfate was applied. This indicates that a proper concentration of sulfate can help to maintain stability since sulfate in a proper concentration increases the buffering capacity of an anaerobic process by competing for excess hydrogen and fatty acids with methane bacteria.

4. pH significantly decreased with the increase of feed strength and increased with the increase of sludge amount.

B.6 CRITIQUE OF THE EXPERIMENT AND FUTURE PLAN

The upper limitation of sulfate was set based on a literature review. This sulfate concentration could cause inhibition in some anaerobic treatment of wastewaters, but it seemed too low to produce a significant effect on the gas composition for cheese whey. Much

Table B.7: Experimental Design Based on Steepest Ascent

	S g/l	Co g COD/l	A g VSS
Coding increment	Ø.3	1.5	Ø.5
Coefficients of coded unit	Ø.Ø85	-Ø.Ø66	Ø.Ø71
Coefficients of original	Ø.Ø255	-Ø.Ø99	Ø.Ø36
Adjusting coefficients	Ø.25	-1	Ø.36
Design centre point	Ø.3	4.5	1.13
Possible steepest path	Ø.55	3.5	1.5
	Ø.8	2.5	1.86
	1.Ø5	1.5	2.22
	1.3	Ø.5	2.56

more sulfate should be used in order to obtain a possibly significant effect. So far, we don't know what the possible inhibition value of sulfate for a cheese whey anaerobic process is because we believe that the value varies with the substrates. Since a linear relationship was observed between pH and the operation variables (no interactions), it would be useful to use a steepest ascent strategy to search optimum operating conditions. Table B -7 below shows values for the three operating variables along the direction of steepest ascent.

It was originally proposed to test more response behaviors, such as COD, volatile fatty acids and so on. However, these responses could not give meaningful information, perhaps due to the disadvantages of batch experimentation and the short period used for a biological process. A continuous experimental mode will be set up to gain more

accurate determination of these effects.

One may choose 6 different levels of sulfate ranging between 0.5 to 10 g/l for two levels of 5 and 20 g COD/l cheese whey feed. For a long run, the initial seed amount is not a very important parameter. So, a 6×2 experimental design will be carried out.

Hence the interaction effects may have some effects even though they didn't play significant roles in the effects on the CH_4 %. Future studies would be necessary to interpret the interaction effects

Appendix C

THE CALCULATION OF NON-IONIZED ACID

C.1 THEORY

A weak acid or base is a weak electrolyte, which is only partially ionized in an aqueous solution, and as a result equilibrium is established between the ionized and unionized chemical forms.

According to the Bronsted theory, an acid is a proton donor, whereas a base is a proton acceptor. A chemical equation representing the ionization of a weak monoprotic acid may written as

$$HA \longrightarrow H^+ + A^- \tag{C.1}$$

the equilibrium constant expression for equation C-1 has the form

$$Ka = \frac{[H^+][A^-]}{[HA]} \tag{C.2}$$

Where Ka represents the thermodynamic equilibrium constant for the acid reaction. By introducing the definition of pH and pKa, this equation becomes

$$pH = pKa + Log\frac{[A^-]}{[HA]} \tag{C.3}$$

This equation is known as the Henderson-Hasselbalch equation and is useful in calculation of pH of a solution.

C.2 CALCULATION OF NON-IONIZED ACID

Let X represent the fraction of the initial acid concentration which is ionized, from equation (C-1)

$$\begin{array}{ccccc} HA & = & H^+ & + & A^- \\ \text{Co-X} & & \text{X} & & \text{X} \end{array} \tag{C.4}$$

where Co is termed the initial concentration of the weak acid, Co-X represents the non-ionized acid. Substituting x for A^- and Co-X fo HA, we have the results in Table 7-5

Appendix D

STATISTICAL ANALYSIS OF pH

Comparison of pH in the absence and presence of sulfate was carried out by using a statistical program Minitab. Minitab is a general purpose statistical computing system which can be used to solve various statistical problems.

Before carrying out any statistical test, some assumptions should be made, ie the error is independent, identically and normally distributed (IIND (0.0)) and the samples are representive.

To compare two sets of independent data , the standard deviations and other statistics are calculated. Independent data means the members of the two sets are not related, thus two groups of pH measurements are not linked to the same influent concentration, even though the substrate and operation temperature are the same. The results are given in Table D-1 .

The important difference between the two sets of pH data can be seen clearly from the dotplot. The pH in the absence of sulfate is much lower than that observed in the presence of sulfate. The pH in the absence of sulfate ranges from 6.75 to 7.18, giving a average of 7.037. The pH in the presence of sulfate has a range of 7.55 to 7.87, resulting in a mean value of 7.687.

Next we need to know how much difference there is between these two means. That can be done by the t-test using the TWOSAMPLE command as shown in Table D-2. The 95% confidence interval means that if these 7 pH values were a random sample from a large population of pH sampling, then a 95% confidence interval for the average change

due to the addition of sulfate in that population would be 0.485 to 0.815. Thus, we would be fairly certain that pH is increased, on the average, by at least 0.48 and maybe by as much as 0.82 pH units.

As shows in Table D-2, the confidence interval is from 0.485 to 0.815, which does not include zero, thus it can be stated that the pH without sulfate is significantly different from the pH with sulfate.

It should be mentioned that if the two populations have the same standard deviation, a subcommand of POOLED can follow TWOSAMPLE, allowing one to get smaller confidence interval and slightly more likelihood to reject a true null hypothesis. The POOLED means the standard deviation from the two samples are pooled to get an estimate of the common standard deviation. This difference essentially disappears with moderately large sample size. It was recommended that it's better not to use the POOLED subcommand. If the standard deviations are equal, you have lost little as shows in Table D-3. The use of POOLED did not make very much difference. If they are unequal, you may have gained a lot. If you use the pooled procedure when it is not appropriate, such as, when the standard deviation of the two population are not equal, you could be seriously misled. For example, you might falsely claim to have evidence that the two populations differ when they really do not.

A t-test can be further made to test the statistic significance.

T=8.68 > t(0.025, 12)= 2.179

here 0.025=a/2, a=0.05 and 12=$n_1 + n_2$-2=7+7-2

The critical T for 95% confidence and 12 degrees of freedom is 2.179 which is smaller than the calculated t=8.68. The conclusion is that the pH of the effluent was significantly increased by adding sulfate.

Table D.1: Calculation of Standard Deviations

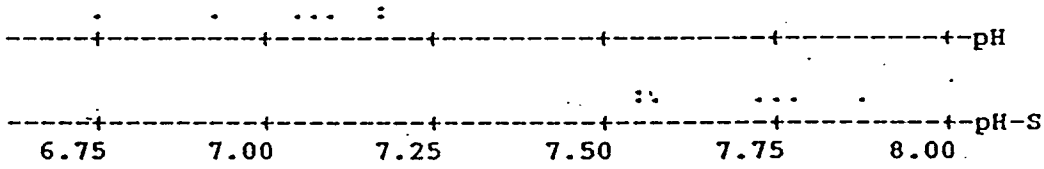
```
MTB > print c1 c2

 ROW      pH    pH-S

   1    6.75    7.75
   2    6.92    7.87
   3    7.08    7.73
   4    7.05    7.58
   5    7.10    7.56
   6    7.18    7.55
   7    7.18    7.77

MTB >
MTB >
MTB >

MTB > dotplot c1 c2;
SUBC> same.
```




```
MTB >

MTB > describe c1 c2

                N     MEAN   MEDIAN   TRMEAN    STDEV   SEMEAN
pH              7   7.0371   7.0800   7.0371   0.1543   0.0583
pH-S            7   7.6871   7.7300   7.6871   0.1242   0.0469

               MIN      MAX       Q1       Q3
pH          6.7500   7.1800   6.9200   7.1800
pH-S        7.5500   7.8700   7.5600   7.7700
```

Table D.2: t-test

```
MTB > twosample c2 c1

TWOSAMPLE T FOR pH-S VS pH
       N      MEAN     STDEV   SE MEAN
pH-S   7     7.687     0.124     0.047
pH     7     7.037     0.154     0.058

95 PCT CI FOR MU pH-S - MU pH: (0.485, 0.815)

TTEST MU pH-S = MU pH (VS NE): T= 8.68  P=0.0000  DF=  11

MTB >
```

Table D.3: t-test

```
MTB >
MTB > twosample c1 c2;
SUBC> pooled.

TWOSAMPLE T FOR pH VS pH-S
       N      MEAN     STDEV   SE MEAN
pH     7     7.037     0.154     0.058
pH-S   7     7.687     0.124     0.047

95 PCT CI FOR MU pH - MU pH-S: (-0.813, -0.487)

TTEST MU pH = MU pH-S (VS NE): T= -8.68  P=0.0000  DF=  12

POOLED STDEV =      0.140
```

Appendix E

DATA TABLES

Table E.1: **Sludge Growth**

KINETICS ANALYSIS

S1 IN. COD g/l	LOADIG g/l d	EFF.COD g/l	X SLUDGE g VSS	(dF/dt)/X RATIO OF F/S g/g d	dX g VSS	dt day	(dX/dt)/X
5.000			86.540				
4.560	0.910	0.132	133.600	0.097	3.060	17.000	0.0013
9.930	1.970	0.191	107.400	0.263	7.370	30.000	0.0023
17.700	3.540	0.286	91.700	0.548	7.050	10.000	0.0077
28.000	5.960	0.457	114.200	0.696	38.460	12.000	0.0281
38.100	7.770	4.877	164.900	0.656	67.140	16.000	0.0254

FOR (dX/dt)/X=Y*(dF/dt)/X-Kd

Regression Output:

Constant	Kd=-0.015742
Std Err of Y Est	0.007
R Squared	0.799
No. of Observations	4.000
Degrees of Freedom	2.000
X Coefficient(s)	Y=0.0584564447
Std Err of Coef.	0.0208

Table E.2: **The Propionic Acid (PA)**

In the Absence of Sulfate

Influent (g COD/l)	PA at 1# (g/l)	PA at 2# (g/l)	PA_1-PA_2 (g/l)
4.56	0.092	0.060	0.032
9.93	0.134	0.014	0.120
17.7	0.290	0.330	-0.040
28.8	0.748	0.036	0.712
38.1	1.336	1.192	0.144

In the Presence of Sulfate

Influent (g COD/l)	PA at 1# (g/l)	PA at 2# (g/l)	PA_1-PA_2 (g/l)
15	0.279	0.064	0.215
20	0.399	0.035	0.364
30	0.901	0.021	0.880
40	1.050	0.040	1.010
50	2.268	1.685	0.583

Table E.3: **The Total Volatile Fatty Acid (TVFA)**

In the Absence of Sulfate

Influent (g COD/l)	TVFA at 1# (g/l)	TVFA at 2# (g/l)	$TVFA_1$-$TVFA_2$ (g/l)
4.56	0.958	0.274	0.684
9.93	1.962	0.124	1.838
17.7	2.624	0.023	2.601
28.8	2.174	0.076	2.098
38.1	3.585	2.148	1.437

In the Presence of Sulfate

Influent (g COD/l)	AA at 1# (g/l)	AA at 2# (g/l)	AA_1-AA_2 (g/l)
15	1.335	0167	1.168
20	1.562	0.078	1.484
30	2.314	0.094	2.220
40	2.650	0.097	2.553
50	4.504	3.999	0.505

Table E.4: Analysis for Accumulation and Degradation of VFA

ROW	AAs	Co	Co**2	Co**3	Co**0.5
1	1.103	15	225	3375	3.87298
2	1.136	20	400	8000	4.47214
3	1.413	30	900	27000	5.47723
4	1.600	40	1600	64000	6.32456
5	2.127	50	2500	125000	7.07107

```
MTB >

*  Co**0.5 is highly correlated with other X variables
*  Co**0.5 has been removed from the equation

* NOTE *       Co is highly correlated with other  predictor variables
* NOTE *    Co**2 is highly correlated with other  predictor variables
* NOTE *    Co**3 is highly correlated with other  predictor variables

The regression equation is
AAs = 0.504 + 0.0590 Co - 0.00172 Co**2 +0.000024 Co**3

Predictor        Coef       Stdev    t-ratio        p
Constant       0.5036      0.9074       0.55    0.677
Co            0.05899     0.09825       0.60    0.656
Co**2       -0.001724    0.003221      -0.54    0.687
Co**3      0.00002382  0.00003267       0.73    0.599

s = 0.07773     R-sq = 99.1%     R-sq(adj) = 96.5%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    3     0.69183     0.23061     38.16    0.118
Error         1     0.00604     0.00604
CONTINUE?

CONTINUE?
Total         4     0.69787

SOURCE       DF      SEQ SS
Co            1     0.65956
Co**2         1     0.02906
Co**3         1     0.00321

Obs.      Co       AAs       Fit  Stdev.Fit  Residual   St.Resid
  1     15.0    1.1030    1.0809     0.0745    0.0221       1.00
  2     20.0    1.1360    1.1843     0.0609   -0.0483      -1.00
  3     30.0    1.4130    1.3647     0.0609    0.0483       1.00
  4     40.0    1.6000    1.6290     0.0721   -0.0290      -1.00
  5     50.0    2.1270    2.1201     0.0774    0.0069       1.00 X

X denotes an obs. whose X value gives it large influence.
```

Table E.5: Analysis for Accumulation and Degradation of VFA

ROW	A1	A2	1/Rs	C	C**2	C**(-1)	C**0.5
1	1.038	0.103	1.06952	15	225	0.0666667	3.87298
2	1.163	0.043	0.89286	20	400	0.0500000	4.47214
3	1.403	0.073	0.75188	30	900	0.0333333	5.47723
4	1.600	0.057	0.64809	40	1600	0.0250000	6.32456
5	2.127	1.714	2.42131	50	2500	0.0200000	7.07107

```
MTB >

*   C**0.5 is highly correlated with other X variables
*   C**0.5 has been removed from the equation

* NOTE *         C is highly correlated with other  predictor variables
* NOTE *      C**2 is highly correlated with other  predictor variables
* NOTE *   C**(-1) is highly correlated with other  predictor variables

The regression equation is
1/Rs = 15.6 - 0.630 C + 0.00815 C**2 - 104 C**(-1)

Predictor       Coef      Stdev    t-ratio       p
Constant      15.569      9.801       1.59   0.358
C            -0.6297     0.3424      -1.84   0.317
C**2        0.008148   0.003674       2.22   0.270
C**(-1)       -104.01      84.95      -1.22   0.436

s = 0.3100      R-sq = 95.4%     R-sq(adj) = 81.7%

Analysis of Variance

SOURCE       DF          SS          MS         F       p
Regression    3     2.00290     0.66763      6.95   0.270
Error         1     0.09611     0.09611
CONTINUE?

Total         4     2.09901

SOURCE       DF      SEQ SS
C             1     0.71137
C**2          1     1.14746
C**(-1)       1     0.14408

Obs.        C       1/Rs        Fit  Stdev.Fit  Residual  St.Resid
  1      15.0      1.070      1.022      0.306     0.048      1.00
  2      20.0      0.893      1.032      0.277    -0.140     -1.00
  3      30.0      0.752      0.543      0.229     0.209      1.00
  4      40.0      0.648      0.816      0.261    -0.167     -1.00
  5      50.0      2.421      2.371      0.306     0.050      1.00


MTB >
MTB >
```

Table E.6: **Analysis for Accumulation and Degradation of VFA**

```
 ROW      Co      PA1      PA2       1/r      Co**2      Co**3  Co**(-1)

   1    4.56    0.092    0.060   31.2500      20.79       94.8  -1.00000
   2    9.93    0.134    0.014    8.3333      98.60      979.1   1.00000
   3   17.70    0.290    0.003    3.4843     313.29     5545.2  -1.00000
   4   28.80    0.748    0.036    1.4045     829.44    23887.9   1.00002
   5   38.10    1.336    1.192    6.9444    1451.61    55306.3  -1.00014

MTB >
MTB >
MTB >
MTB > regr c2 4 c1 c5-c8
* NOTE *       Co is highly correlated with other  predictor variables
* NOTE *    Co**2 is highly correlated with other  predictor variables
* NOTE *    Co**3 is highly correlated with other  predictor variables

The regression equation is
PA1 = 0.108 - 0.00749 Co +0.000984 Co**2 +0.000002 Co**3 + 0.00213 Co**(-1)

Predictor       Coef        Stdev    t-ratio        p
Constant    0.107665     0.000000           *        *
Co        -0.00748717   0.00000000           *        *
Co**2      0.00098372   0.00000000           *        *
Co**3      0.00000159   0.00000000           *        *
Co**(-1)   0.00212939   0.00000000           *        *

s = *
Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    4    1.102920    0.275730          *        *
Error         0           *           *
Total         4    1.102920

CONTINUE?

SOURCE       DF      SEQ SS
Co            1    1.030516
Co**2         1    0.072380
Co**3         1    0.000007
Co**(-1)      1    0.000017

Obs.      Co       PA1       Fit  Stdev.Fit  Residual   St.Resid
  1      4.6   0.09200   0.09200          *   0.00000          *
  2      9.9   0.13400   0.13400          *   0.00000          *
  3     17.7   0.29000   0.29000          *   0.00000          *
  4     28.8   0.74800   0.74800          *   0.00000          *
  5     38.1   1.33600   1.33600          *   0.00000          *


MTB >
MTB >
```

Table E.7: **Analysis for Accumulation and Degradation of VFA**

```
MTB > stepwise y in c2 x in c1 c5 c 6 c7
* ERROR * ARGUMENT IS A CONSTANT OR MATRIX, BUT A COLUMN WAS EXPECTED

MTB > stepwise c2 4 c1 c5 c6 c7 c8
* ERROR * ILLEGAL ARGUMENT

MTB > stepwise c2 4 c1 c5-c7

 STEPWISE REGRESSION OF   PA1    ON  4 PREDICTORS, WITH N =     5

    STEP         1         2
CONSTANT   0.04401   0.11244

Co**2      0.00088   0.00108
T-RATIO      35.41     76.93

Co                  -0.00893
T-RATIO               -14.60

S           0.0296   0.00350
R-SQ         99.76    100.00
 MORE? (YES, NO, SUBCOMMAND, OR HELP)
SUBC>
SUBC>
```

Table E.8: **Analysis for Accumulation and Degradation of VFA**

```
MTB > stepwise c2 4 c1 c5-c7

 STEPWISE REGRESSION OF   PA1    ON  4 PREDICTORS, WITH N =    5

    STEP         1         2
CONSTANT  0.04401   0.11244

Co**2     0.00088   0.00108
T-RATIO     35.41     76.93

Co                 -0.00893
T-RATIO              -14.60

S          0.0296   0.00350
R-SQ        99.76    100.00
 MORE? (YES, NO, SUBCOMMAND, OR HELP)
SUBC>
SUB

MTB > regr c2 2 c1 c5

The regression equation is
PA1 = 0.112 - 0.00893 Co + 0.00108 Co**2

Predictor       Coef        Stdev    t-ratio        p
Constant    0.112443    0.005189      21.67    0.002
Co         -0.0089276   0.0006114    -14.60    0.005
Co**2      0.00107690  0.00001400      76.93    0.000

s = 0.003497    R-sq = 100.0%    R-sq(adj) = 100.0%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    2     1.10290     0.55145   45084.95    0.000
Error         2     0.00002     0.00001
Total         4     1.10292

SOURCE       DF      SEQ SS
Co            1     1.03052
Co**2         1     0.07238

CONTINUE?

Obs.      Co       PA1       Fit  Stdev.Fit  Residual   St.Resid
  1      4.6   0.09200   0.09413    0.00309  -0.00213     -1.30
  2      9.9   0.13400   0.12998    0.00203   0.00402      1.41
  3     17.7   0.29000   0.29181    0.00251  -0.00181     -0.74
  4     28.8   0.74800   0.74855    0.00237  -0.00055     -0.21
  5     38.1   1.33600   1.33554    0.00333   0.00046      0.44
```

Table E.9: **Analysis for Accumulation and Degradation of VFA**

ROW	Co	PA1	PA2	1/r	Co**2	Co**3	Co**(-1)
1	4.56	0.092	0.060	31.2500	20.79	94.8	0.219298
2	9.93	0.134	0.014	8.3333	98.60	979.1	0.100705
3	17.70	0.290	0.003	3.4843	313.29	5545.2	0.056497
4	28.80	0.748	0.036	1.4045	829.44	23887.9	0.034722
5	38.10	1.336	1.192	6.9444	1451.61	55306.3	0.026247

```
MTB > stepwise c4 4 c1 c5-c7

 STEPWISE REGRESSION OF   1/r    ON  4 PREDICTORS, WITH N =     5

    STEP         1         2
CONSTANT    -2.369    -9.280

Co**(-1)       145       184
T-RATIO       5.30     17.67

Co**3                0.00020
T-RATIO                 5.80

S             4.32      1.25
R-SQ         90.35     99.46
 MORE? (YES, NO, SUBCOMMAND, OR HELP)
SUBC>

MTB > regr c4 4 c1 c5-c7
* NOTE *       Co is highly correlated with other  predictor variables

*     Co**2 is highly correlated with other X variables
*     Co**2 has been removed from the equation


The regression equation is
1/r = - 11.4 + 0.112 Co +0.000160 Co**3 + 192 Co**(-1)

Predictor       Coef        Stdev    t-ratio        p
Constant     -11.438        8.721      -1.31    0.415
Co             0.1119       0.4396      0.25    0.841
Co**3      0.0001597    0.0001797      0.89    0.537
Co**(-1)       191.59        34.37      5.57    0.113

s = 1.717       R-sq = 99.5%     R-sq(adj) = 98.0%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    3      576.66      192.22      65.19    0.091
Error         1        2.95        2.95
Total         4      579.61
CONTINUE?
```

Table E.10: **Analysis for Accumulation and Degradation of VFA**

```
SOURCE          DF       SEQ SS
Co               1       243.14
Co**3            1       241.92
Co**(-1)         1        91.61

Obs.       Co        1/r        Fit Stdev.Fit  Residual    St.Resid
  1       4.6     31.250     31.101     1.711     0.149        1.00 X
  2       9.9      8.333      9.123     1.525    -0.789       -1.00
  3      17.7      3.484      2.252     1.196     1.232        1.00
  4      28.8      1.404      2.252     1.493    -0.848       -1.00
  5      38.1      6.944      6.688     1.698     0.256        1.00

X denotes an obs. whose X value gives it large influence.

MTB >
MTB >
```

Table E.11: **Analysis for Accumulation and Degradation of VFA**

```
MTB >
MTB > regr c4 2 c1 c7

The regression equation is
1/r = - 18.3 + 0.488 Co + 216 Co**(-1)

Predictor        Coef       Stdev    t-ratio        p
Constant      -18.311       3.819      -4.79    0.041
Co             0.4884      0.1115       4.38    0.048
Co**(-1)       216.18       19.30      11.20    0.008

s = 1.625        R-sq = 99.1%     R-sq(adj) = 98.2%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     2      574.33      287.17     108.80    0.009
Error          2        5.28        2.64
Total          4      579.61

SOURCE        DF      SEQ SS
Co             1      243.14
Co**(-1)       1      331.20

CONTINUE?

Obs.       Co        1/r        Fit Stdev.Fit  Residual   St.Resid
  1       4.6     31.250     31.324     1.601    -0.074      -0.27
  2       9.9      8.333      8.310     1.154     0.024       0.02
  3      17.7      3.484      2.548     1.087     0.936       0.78
  4      28.8      1.404      3.262     0.917    -1.858      -1.39
  5      38.1      6.944      5.972     1.414     0.972       1.22


MTB >
```

Table E.12: **Analysis for Accumulation and Degradation of VFA**

```
The regression equation is
1/r = - 2.37 + 145 Co**(-1)

Predictor       Coef       Stdev    t-ratio        p
Constant      -2.369       3.070      -0.77    0.497
Co**(-1)      144.61       27.28       5.30    0.013

s = 4.317       R-sq = 90.4%     R-sq(adj) = 87.1%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    1      523.70      523.70      28.10    0.013
Error         3       55.91       18.64
Total         4      579.61

Obs.Co**(-1)       1/r       Fit Stdev.Fit  Residual   St.Resid
   1     0.219     31.25     29.34      4.08      1.91       1.35
   2     0.101      8.33     12.19      1.96     -3.86      -1.00
   3     0.056      3.48      5.80      2.11     -2.32      -0.61
   4     0.035      1.40      2.65      2.41     -1.25      -0.35
   5     0.026      6.94      1.43      2.55      5.52       1.59

MTB >
```

Table E.13: **Analysis for Accumulation and Degradation of VFA**

```
The regression equation is
PAs1 = 0.275 - 0.0160 Cso + 0.00105 Cso**2 + 0.117 1/Cos

Predictor       Coef        Stdev    t-ratio        p
Constant      0.2746       0.8816       0.31    0.808
Cso          -0.01595      0.06160     -0.26    0.839
Cso**2     0.0010453    0.0009478       1.10    0.469
1/Cos         0.1168       0.1202       0.97    0.509

s = 0.2411      R-sq = 97.7%     R-sq(adj) = 90.7%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     3     2.44093     0.81364      14.00    0.193
Error          1     0.05811     0.05811
Total          4     2.49904

SOURCE        DF      SEQ SS
Cso            1     2.23270
Cso**2         1     0.15334
1/Cos          1     0.05489

CONTINUE?


CONTINUE?
Obs.      Cso       PAs1        Fit  Stdev.Fit   Residual   St.Resid
  1      15.0      0.279      0.387      0.215     -0.108      -1.00
  2      20.0      0.399      0.257      0.195      0.142       1.00
  3      30.0      0.901      0.854      0.236      0.047       1.00
  4      40.0      1.050      1.192      0.195     -0.142      -1.00
  5      50.0      2.268      2.207      0.233      0.061       1.00


t
```

Table E.14: **Analysis for Accumulation and Degradation of VFA**

```
MTB >
MTB > regr c22 2 c21 c25

The regression equation is
PAs1 = 0.603 - 0.0391 Cso + 0.00141 Cso**2

Predictor        Coef       Stdev    t-ratio        p
Constant       0.6026      0.8030       0.75    0.531
Cso          -0.03910     0.05602      -0.70    0.557
Cso**2     0.0014122   0.0008572       1.65    0.241

s = 0.2377      R-sq = 95.5%     R-sq(adj) = 91.0%

Analysis of Variance

SOURCE        DF          SS          MS          F        p
Regression     2      2.3860      1.1930      21.11    0.045
Error          2      0.1130      0.0565
Total          4      2.4990

SOURCE        DF      SEQ SS
Cso            1      2.2327
Cso**2         1      0.1533


Obs.      Cso       PAs1        Fit  Stdev.Fit  Residual   St.Resid
  1      15.0      0.279      0.334      0.205    -0.055      -0.46
  2      20.0      0.399      0.386      0.141     0.013       0.07
  3      30.0      0.901      0.701      0.174     0.200       1.24
  4      40.0      1.050      1.298      0.159    -0.248      -1.40
  5      50.0      2.268      2.178      0.228     0.090       1.34


MTB >
MTB >
MTB >
```

Table E.15: **Analysis for Accumulation and Degradation of VFA**

```
MTB >
MTB > regr c12 2 c11 c15

The regression equation is
C12 = 1.77 - 0.0552 Cso + 0.00215 Cso**2

Predictor       Coef        Stdev    t-ratio        p
Constant       1.768        1.115       1.59    0.254
Cso          -0.05523      0.07776      -0.71    0.551
Cso**2       0.002148     0.001190       1.81    0.213

s = 0.3299      R-sq = 96.5%     R-sq(adj) = 93.1%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    2      6.0888      3.0444      27.97    0.035
Error         2      0.2177      0.1089
Total         4      6.3065

SOURCE       DF      SEQ SS
Cso           1      5.7340
Cso**2        1      0.3549

CONTINUE?

Obs.      Cso        C12        Fit  Stdev.Fit   Residual   St.Resid
  1      15.0      1.335      1.423      0.285     -0.088      -0.53
  2      20.0      1.562      1.523      0.195      0.039       0.15
  3      30.0      2.314      2.045      0.241      0.269       1.20
  4      40.0      2.650      2.996      0.221     -0.346      -1.41
  5      50.0      4.504      4.378      0.317      0.126       1.36


MTB >
MTB >
MTB >
```

Table E.16: **Analysis for Accumulation and Degradation of VFA**

```
MTB > print c21-c28

 ROW    Cso     PAs1     PAs2       1/rs   Cso**2   Cso**3  Ps1-Ps2

   1     15    0.279    0.064    4.65116      225     3375    0.215
   2     20    0.399    0.035    2.74725      400     8000    0.364
   3     30    0.901    0.021    1.13636      900    27000    0.880
   4     40    1.050    0.040    0.99010     1600    64000    1.010
   5     50    2.268    1.685    1.71527     2500   125000    0.583

MTB
```

```
MTB > stepwise c24 4 c21 c25 c26 c28

 STEPWISE REGRESSION OF   1/rs    ON   4 PREDICTORS, WITH N =     5

    STEP         1         2
CONSTANT   -0.5539   -3.4673

1/Cos         71.84    120.65
T-RATIO        3.89    129.91

Cso**3                0.00002
T-RATIO                 62.83

S             0.709    0.0196
R-SQ          83.46     99.99
 MORE? (YES, NO, SUBCOMMAND, OR HELP)
```

Table E.17: **Analysis for Accumulation and Degradation of VFA**

```
MTB > regr c24 2 c21 c25

The regression equation is
1/rs = 11.0 - 0.540 Cso + 0.00715 Cso**2

Predictor       Coef        Stdev    t-ratio        p
Constant     10.9582       0.9559      11.46    0.008
Cso         -0.54036      0.06669      -8.10    0.015
Cso**2      0.007148     0.001020       7.00    0.020

s = 0.2830      R-sq = 98.2%     R-sq(adj) = 96.5%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    2      8.9662      4.4831     56.00    0.018
Error         2      0.1601      0.0801
Total         4      9.1263

SOURCE       DF      SEQ SS
Cso           1      5.0380
Cso**2        1      3.9282

CONTINUE?

Obs.      Cso       1/rs        Fit  Stdev.Fit  Residual   St.Resid
  1      15.0      4.651      4.461      0.244     0.190       1.33
  2      20.0      2.747      3.010      0.167    -0.263      -1.15
  3      30.0      1.136      1.180      0.207    -0.044      -0.23
  4      40.0      0.990      0.780      0.189     0.210       1.00
  5      50.0      1.715      1.809      0.272    -0.094      -1.18

MTB
```

Table E.18: **Analysis for Accumulation and Degradation of VFA**

```
MTB > print c1-c8

 ROW      Co     VFA1     VFA2      1/rr      co**2       1/Co

   1    4.56    0.958    0.274   1.46199      20.79   0.219298
   2    9.93    1.962    0.124   0.54407      98.60   0.100705
   3   17.70    2.624    0.023   0.38447     313.29   0.056497
   4   28.80    2.174    0.076   0.47664     829.44   0.034722
   5   38.10    3.585    2.148   0.69589    1451.61   0.026247

MTB >
MTB >
MTB >

*  Co**0.5 is highly correlated with other X variables
*  Co**0.5 has been removed from the equation

* NOTE *       Co is highly correlated with other  predictor variables

The regression equation is
VFA1 = 5.91 - 0.236 Co + 0.00484 co**2 - 18.3 1/Co

Predictor       Coef       Stdev    t-ratio        p
Constant       5.908       4.018       1.47    0.380
Co           -0.2357      0.2704      -0.87    0.544
co**2       0.004839    0.004825       1.00    0.499
1/Co          -18.32       14.32      -1.28    0.422

s = 0.5842      R-sq = 90.7%     R-sq(adj) = 62.9%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    3      3.3382      1.1127      3.26    0.382
Error         1      0.3413      0.3413
Total         4      3.6795

CONTINUE?

SOURCE       DF      SEQ SS
Co            1      2.7596
co**2         1      0.0197
1/Co          1      0.5589

Obs.       Co       VFA1        Fit Stdev.Fit   Residual    St.Resid
  1       4.6      0.958      0.916     0.583      0.042        1.00 X
  2       9.9      1.962      2.200     0.534     -0.238       -1.00
  3      17.7      2.624      2.217     0.420      0.407        1.00
  4      28.8      2.174      2.498     0.486     -0.324       -1.00
  5      38.1      3.585      3.472     0.573      0.113        1.00

X denotes an obs. whose X value gives it large influence.
```

Table E.19: **Analysis for Accumulation and Degradation of VFA**

```
MTB > regr c2 2 c1 c6

The regression equation is
VFA1 = 2.12 + 0.0325 Co - 5.76 1/Co

Predictor       Coef        Stdev    t-ratio        p
Constant       2.121        1.375       1.54    0.263
Co           0.03246      0.04016       0.81    0.504
1/Co          -5.762        6.950      -0.83    0.494

s = 0.5851      R-sq = 81.4%     R-sq(adj) = 62.8%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    2      2.9949      1.4974      4.37    0.186
Error         2      0.6846      0.3423
Total         4      3.6795

SOURCE       DF      SEQ SS
Co            1      2.7596
1/Co          1      0.2353

CONTINUE?

Obs.        Co        VFA1         Fit   Stdev.Fit    Residual    St.Resid
  1        4.6       0.958       1.006       0.577      -0.048       -0.48
  2        9.9       1.962       1.863       0.416       0.099        0.24
  3       17.7       2.624       2.370       0.391       0.254        0.58
  4       28.8       2.174       2.856       0.330      -0.682       -1.41
  5       38.1       3.585       3.207       0.509       0.378        1.31
```

Table E.20: **Analysis for Accumulation and Degradation of VFA**

```
*  Co**0.5 is highly correlated with other X variables
*  Co**0.5 has been removed from the equation

* NOTE *        Co is highly correlated with other  predictor variables

The regression equation is
1/rr = - 0.579 + 0.0239 Co +0.000088 co**2 + 8.79 1/Co

Predictor        Coef       Stdev    t-ratio        p
Constant      -0.5789      0.1511      -3.83    0.163
Co            0.02394     0.01017       2.35    0.256
co**2      0.0000880   0.0001814       0.48    0.713
1/Co          8.7929      0.5385      16.33    0.039

s = 0.02197     R-sq = 99.9%     R-sq(adj) = 99.7%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    3     0.75313     0.25104     520.17    0.032
Error         1     0.00048     0.00048
Total         4     0.75361

CONTINUE?

SOURCE       DF      SEQ SS
Co            1     0.17626
co**2         1     0.44817
1/Co          1     0.12869

Obs.       Co       1/rr        Fit  Stdev.Fit   Residual   St.Resid
  1       4.6    1.46199    1.46040    0.02191    0.00159       1.00 X
  2       9.9    0.54407    0.55301    0.02007   -0.00894      -1.00
  3      17.7    0.38447    0.36918    0.01577    0.01529       1.00
  4      28.8    0.47664    0.48883    0.01828   -0.01218      -1.00
  5      38.1    0.69589    0.69165    0.02155    0.00424       1.00
```

Table E.21: **Analysis for Accumulation and Degradation of VFA**

```
MTB >
MTB > regr c4 2 c1 c6

The regression equation is
1/rr = - 0.648 + 0.0288 Co + 9.02 1/Co

Predictor       Coef       Stdev    t-ratio         p
Constant    -0.64769     0.04058     -15.96     0.004
Co          0.028812    0.001185      24.31     0.002
1/Co          9.0212      0.2051      43.99     0.001

s = 0.01726     R-sq = 99.9%     R-sq(adj) = 99.8%

Analysis of Variance

SOURCE        DF          SS          MS          F         p
Regression     2     0.75301     0.37651    1263.40     0.001
Error          2     0.00060     0.00030
Total          4     0.75361

SOURCE        DF      SEQ SS
Co             1     0.17626
1/Co           1     0.57675

CONTINUE?

Obs.       Co       1/rr         Fit  Stdev.Fit   Residual   St.Resid
  1       4.6    1.46199     1.46204    0.01701   -0.00005      -0.02
  2       9.9    0.54407     0.54690    0.01227   -0.00283      -0.23
  3      17.7    0.38447     0.37196    0.01155    0.01251       0.97
  4      28.8    0.47664     0.49534    0.00974   -0.01869      -1.31
  5      38.1    0.69589     0.68683    0.01503    0.00906       1.07


MTB >
```

Table E.22: **Analysis for Accumulation and Degradation of VFA**

ROW	Cso	C12	C13	1/Rs	Cso**2	tf1-tf2	1/Cos
1	15	1.335	0.167	0.85616	225	1.168	0.0666667
2	20	1.562	0.078	0.67385	400	1.484	0.0500000
3	30	2.314	0.094	0.45045	900	2.220	0.0333333
4	40	2.650	0.097	0.39170	1600	2.553	0.0250000
5	50	4.504	3.999	1.98020	2500	0.505	0.0200000

```
MTB >
   1 >

SUBC> n
MTB > regr c12 3 c11 c15 c17
* NOTE *      Cso is highly correlated with other  predictor variables
* NOTE *   Cso**2 is highly correlated with other  predictor variables
* NOTE *    1/Cos is highly correlated with other  predictor variables

The regression equation is
C12 = 11.2 - 0.378 Cso + 0.00553 Cso**2 - 82.0 1/Cos

Predictor        Coef       Stdev    t-ratio        p
Constant        11.17       11.32       0.99    0.504
Cso           -0.3781      0.3953      -0.96    0.514
Cso**2       0.005526    0.004243       1.30    0.417
1/Cos         -82.00       98.10      -0.84    0.557

s = 0.3580      R-sq = 98.0%     R-sq(adj) = 91.9%

Analysis of Variance

SOURCE       DF          SS          MS          F        p
Regression    3      6.1784      2.0595      16.07    0.181
Error         1      0.1282      0.1282
Total         4      6.3065

CONTINUE?

SOURCE       DF      SEQ SS
Cso           1      5.7340
Cso**2        1      0.3549
1/Cos         1      0.0895

Obs.      Cso         C12         Fit  Stdev.Fit   Residual   St.Resid
  1      15.0       1.335       1.280      0.354      0.055       1.00
  2      20.0       1.562       1.723      0.320     -0.161      -1.00
  3      30.0       2.314       2.072      0.264      0.242       1.00
  4      40.0       2.650       2.843      0.301     -0.193      -1.00
  5      50.0       4.504       4.446      0.353      0.058       1.00
```

Table E.23: Analysis for Accumulation and Degradation of VFA

```
MTB >
MTB > regr c14 3 c11 c15 c17
* NOTE *      Cso is highly correlated with other  predictor variables
* NOTE *   Cso**2 is highly correlated with other  predictor variables
* NOTE *    1/Cos is highly correlated with other  predictor variables

The regression equation is
1/Rs = 14.0 - 0.577 Cso + 0.00748 Cso**2 - 92.4 1/Cos

Predictor       Coef       Stdev    t-ratio        p
Constant      13.958       7.578       1.84    0.317
Cso          -0.5773      0.2647      -2.18    0.274
Cso**2      0.007480    0.002841       2.63    0.231
1/Cos         -92.42       65.69      -1.41    0.393

s = 0.2397      R-sq = 96.6%     R-sq(adj) = 86.3%

Analysis of Variance

SOURCE       DF          SS          MS         F        p
Regression    3     1.61853     0.53951      9.39    0.234
Error         1     0.05747     0.05747
Total         4     1.67600

CONTINUE?

SOURCE       DF      SEQ SS
Cso           1     0.46789
Cso**2        1     1.03689
1/Cos         1     0.11375

Obs.      Cso      1/Rs       Fit  Stdev.Fit  Residual   St.Resid
  1      15.0     0.856     0.819      0.237     0.037       1.00
  2      20.0     0.674     0.782      0.214    -0.108      -1.00
  3      30.0     0.450     0.289      0.177     0.162       1.00
  4      40.0     0.392     0.521      0.202    -0.129      -1.00
  5      50.0     1.980     1.942      0.237     0.039       1.00


MTB >
MTB >
```

Table E.24: Analysis for Accumulation and Degradation of VFA

```
MTB > regr c14 2 c11 c15

The regression equation is
1/Rs = 3.36 - 0.213 Cso + 0.00367 Cso**2

Predictor       Coef      Stdev    t-ratio       p
Constant      3.3566     0.9884       3.40   0.077
Cso          -0.21347    0.06896     -3.10   0.090
Cso**2       0.003672   0.001055       3.48   0.074

s = 0.2926      R-sq = 89.8%     R-sq(adj) = 79.6%

Analysis of Variance

SOURCE       DF          SS          MS         F       p
Regression    2     1.50479     0.75239      8.79   0.102
Error         2     0.17121     0.08561
Total         4     1.67600

SOURCE       DF      SEQ SS
Cso           1     0.46789
Cso**2        1     1.03689

CONTINUE?

Obs.      Cso       1/Rs        Fit  Stdev.Fit  Residual   St.Resid
  1      15.0      0.856      0.981      0.253    -0.125      -0.85
  2      20.0      0.674      0.556      0.173     0.118       0.50
  3      30.0      0.450      0.258      0.214     0.193       0.97
  4      40.0      0.392      0.694      0.196    -0.302      -1.39
  5      50.0      1.980      1.864      0.281     0.116       1.41


MTB >
```

Appendix 1

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