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Improved Fermentation Process for Producing Methane from Cheese Whey

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IMPROVED FERMENTATION PROCESS FOR PRODUCING

METHANE FROM CHEESE WHEY

by

AbdulRaman.Y.Awad

A thesis submitted in partial fulfillment

of the requirement for the degree

of

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in

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1982

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<div align="right">AbdulRahman.Y.Awad</div>

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Improved Fermentation Process for Producing

Methane from Cheese Whey

by

AbdulRahman.Y.Awad, Master of Science

Utah State University, 1982

Major Professor: Dr. C.A. Ernstrom
Department: Nutrition and Food Science

Two methods of single-stage fermentation were used to produce
methane from whey. The first method involved batch fermentation in
which the pH was automatically controlled at 7.0. In addition to pH
control, the second method was characterized by introducing the
substrate continuously into the fermenter along with dilution water to
keep the organic acids at a non-toxic level.

The first method did not improve the production of methane compared
to the batch fermentation in which a pH control system was not used (41).
However, the second method showed that the concentration of organic
acids has a major effect on limiting the production of methane from
whey, and that by diluting the substrate with water the concentration of
these acids was kept at levels not toxic to methanogenic bacteria until
the population of methanogenic bacteria was such that they could convert
organic acids to methane as rapidly as the acids were produced by

non-methanogenic bacteria in a continuous process. This continuous

process was capable of producing .179 m^3 methane per kilogram of lactose

in cheese whey.

(79 pages)

INTRODUCTION

The production of methane gas from organic material is not a new process (41). Volta (44) discovered methane in marsh gas in the eighteenth century, and ideas and experiments on how the process can be used have been going on for the past hundred years. Much work has been reported on the application of anaerobic digestion to produce methane from wastes (45). As the energy crisis worsens, bioconversion of agricultural, animal, and industrial wastes have become increasingly attractive. Anaerobic fermentation is a biological process in which an organic substrate is first metabolized by non-methanogenic organisms to produce mainly organic acids and carbon dioxide. These acids are then degraded by methanogenic organism to produce methane and carbon dioxide (2, 4, 5, 7, 8, 36).

Initial work involved the design of single stage (batch) digesters. They did not allow for very efficient digestion, since acid forming and methane-forming bacteria differ in their optimum environmental conditions.

The growth rate of methane-forming bacteria is very low compared to the growth rate of acid-forming bacteria. Thus, acid production is usually much faster than the degradation of the acids to methane and carbon dioxide. As a result, the concentration of these acids in the fermenter will increase to levels which inhibit methanogenic bacteria. There are two different explanations of this inhibition (7, 37). One is

that the high concentration of acids in the medium results in a low pH which is toxic to the methanogenic bacteria (37). The other is that the organic acids themselves are inhibitory to methanogenic bacteria (7).

As a result, two stage anaerobic digestion has been used in which the acidification phase and methane fermentation were carried out in separate digesters to provide optimum conditions for each group of bacteria (12, 22). However the use of two separate fermenters increased the capital outlay and required much greater instrumentation and control to maintain optimum conditions (55).

The purpose of this study was to improve the single stage method for producing methane from whey to make it comparable in efficiency to the typical two-stage fermentation. One approach was to use automatic pH control during fermentation. Another was to use automatic pH control plus dilution of the substrate, during the initial stage of fermentation to develop an optimum ratio of substrate to methanogenic bacteria.

LITERATURE REVIEW

Whey is the serum or watery part of milk that separates from curd during cheese making. It is the largest by-product of the huge U.S. dairy industry, and is one of the most troublesome produced by any industry. It contains roughly half the solids of whole milk and most of the water soluble vitamins and minerals (23). A typical analysis of whey might be: total solids 6.9% which includes Lactose 68-72%, protein 12-13%, minerals 8-9%, fat 4-5%, and lactic acid 2-3% (31).

In 1980, total whey production in the United States was over 30 billion pounds (66), of which 60% was utilized compared to only 53% of the 28 billion pounds produced in 1972 (67). The amount of whey available for processing is bound to increase as the proportion, and the absolute volume of milk used in cheese continues to increase. If whey is unused, its organic nutrients make it a costly pollutant in the nations sewage systems and waterways.

Gillies (23) reported in 1974 that by 1980 pressures to use whey in an economical manner and not treat it as waste or sewage will increase. This will take the form of more rigid regulations for sewage disposal and more research for new whey products both for industrial and food use.

Whey fermentation

One of the tools available in the effort to utilize whey is the manufacture of useful products through the growth of microorganisms. Many microorganisms ferment whey, and many metabolic products are formed as they grow.

The fermentation industry is characterized by a certain degree of flexibility with respect to raw materials. Since this is a highly competitive industry which must operate with maximum production efficiency, the substrate which will produce end products at the lowest cost will be the one that is used. If a fermentation process can use whey, and it provides a cheaper substrate than some other raw materials which also would be suitable, it will probably be used. The use of whey in the manufacture of vitamin B_{12}, riboflavin, lactic acid, and ethyl alcohol has been tried, but whey does not provide the cheapest substrate for these fermentations (23). Yeast production from whey was tried by several workers during past years. Gillies (23) reported that 0-55 kilograms of cells can be obtained from a kilogram of lactose. Friend and Shahani (17) predicted that acid whey would be an ideal medium for citric acid production since most organisms prefer an acidic environment for citric acid fermentation.

One of the most promising suggestions in whey fermentation has been the production of ethanol as a beverage alcohol or as an alternative for liquid fuel. The manufacture of ethanol is accomplished by specially selected yeast fermentation of lactose via the glycolytic pathway.

Strains of <u>Kluyveromyces</u> <u>fragilis</u> and <u>Torula</u> <u>cremoris</u> are the most important microorganisms since they have faster fermentation rates and produce a higher yield of ethanol than other yeasts (31). Each 40 cubic meters of whey containing 5% lactose produces one cubic meter of 100% alcohol (46, 68) with an 80% efficiency. The Department of Energy (62) estimated that there are approximately 0.9 million tons of whey solid available to produce 0.34 million cubic meters of ethanol per year at a cost of 58 dollars per cubic meter.

The development of ethanol as an energy fuel has recently been considered (31). Amundson (1) reported that the major limitations to the conversion of whey to ethanol for energy are the cost and energy required. He estimated that a cubic meter of alcohol produced from 2000 kg of lactose, contains approximately 22,000,000 Btu and it may require as many as 43,000,000 Btu to produce. He estimated also, that if all the whey in the United States were converted into ethanol, it would represent only 0.5-1% of annual gasoline consumption.

During the past few years, an anaerobic biological treatment was used to produce methane gas from whey. Reesen and Strube (46) studied the production of methane from permeate, and indicated that the anaerobic process works most efficiently at a steady temperature in the range of 34° C to 38° C.

Amundson (1) reported that Danish Cooperative Dairies and Danish Distillers produce beverage alcohol and methane from whey permeate. He also reported that methane production during anaerobic fermentation replaced 17-20% of the energy required in the plant.

Anaerobic digestion

The dissimilatory process involved in the break down of organic materials has been observed and noted for centuries (54). Gas production from decomposing animal and vegetable remains was noticed by early scientists such as Boyle in 1682 (44). The identification of a specific gas from the anaerobic digestive process was first made in 1776 by Volta (44,54), while the first known description of methane formation was in 1867 when Bechamp ascribed the process to microorganisms with ethanol as the substrate (54). In 1887 Hoppe-Seyler (55) produced methane directly from acetate, and in 1906 methane was formed directly from CO_2 and H_2 (54). As early as the nineteenth century, when the industrial revolution meant the rapid growth of towns and the concentration of people and factories into small areas, domestic waste disposal became a major problem. Therefore, anaerobic digestion plants were used to treat town sewage. In 1911 (57) Birmingham sewage works built an anaerobic digester plant to stabilize the sewage sludge. This was the first of its kind in the world, followed by many plants in several locations to treat agricultural, animal, and industrial wastes.

Today, many of the larger sewage works run successful digesters for treating waste water, fruit and vegetable waste, fermentation plant waste (57), molasses waste (54), and human and animal waste (43).

Methane fermentation process

Anaerobic fermentation of complex organic materials is normally a
two stage process as shown below.

Acid forming bacteria   Methane forming bacteria

```
                    ↓                        ↓
┌──────────────────┐      ┌──────────────┐      ┌──────────────┐
│ complex organics │ ──►  │ organic acids│ ──►  │  CH₄ + CO₂   │
└──────────────────┘      └──────────────┘      └──────────────┘
```

$$\text{complex organics} \rightarrow \text{organic acids} \rightarrow CH_4 + CO_2$$

first stage   second stage
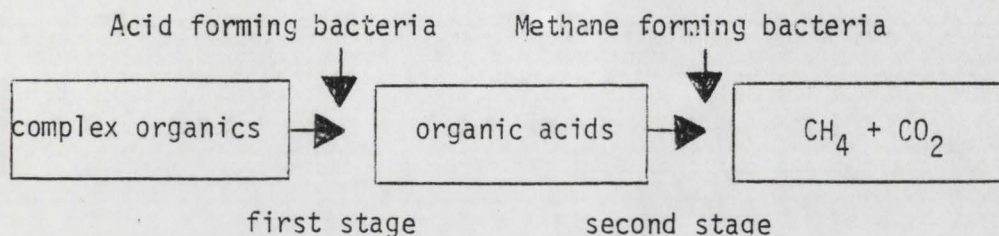
First stage fermentation

In the first stage, there is no methane production and hence no
waste stabilization. The complex organics are changed by a group of
facultative and anaerobic bacteria commonly termed acid formers.
Complex materials such as fats, proteins, and carbohydrates are
converted to simple organic fatty acids (3, 13, 15, 26, 36). Acid
forming bacteria use small amounts of energy for growth, and only a
small portion of the organic waste is converted to cellular material.

Few studies on the nature of the acid forming bacteria have been
carried out. Gaub (20) isolated 16 anaerobic and 5 facultative
anaerobic bacteria (mostly intestinal types) from anaerobic digested
sludge. Hotchkiss (28) indicated the presence of denitrifying, albumin
digesting, and H_2S producing bacteria in the sludge. Ruchhoft and Keller (49)

indicated the presence of different types of proteolytic bacteria.
Roediger (61) suggested that the acid forming bacteria were facultative.
McKinney (40) commented that the acid formers are made up predominantly
of facultative bacteria, with a few strict anaerobes. The ease of
growth of the facultative bacteria give them an edge over the strict
anaerobes. Toerien et. al. (60) indicated that anaerobic and
facultative anaerobic bacteria formed only a minor part of the acid
forming bacterial population. On the other hand, obligate anaerobes
formed a major part. He (60) reported 39×10^7 to 15×10^9 obligate
anaerobic non-methanogenic and 8×10^5 to 1×10^8 aerobic and
facultative anaerobic bacteric per milliliter in several digesters.
This estimate was supported by Gaudy and Gaudy (21). The end products
of these acid forming bacteria are CO_2 and short chain organic acids
(Table 1) which constitute the major intermediates produced by first
stage conversion of sewage to methane.

The second stage

In the second stage, the organic acids are converted by a special
group of bacteria termed the methane formers into the gaseous end
products, carbon dioxide and methane (36, 60).

There are several different groups of methane formers, each group
characterized by its ability to ferment a relatively limited number of
organic compounds. Therefore, in the complete methane fermentation of
complex materials, several different methane bacteria are required (36).
These organisms are rods or cocci, gram-positive or gram-negative.

Table 1. Common organic acids produced by non-methanogenic bacteria.

Acids	Chemical Formula
Formic Acid	$HCOOH$
Acetic Acid	CH_3COOH
Propionic Acid	CH_3CH_2COOH
Butyric Acid	$CH_3(CH_2)_2COOH$
Isovoleric Acid	$(CH_3)_2CH - CH_2COOH$
Voleric Acid	$CH_3(CH_2)_3COOH$
Caproic Acid	$CH_3(CH_2)_4COOH$

Table 2. Methanogenic bacteria and the products metabolized by them.

Organisms	Substrates
Methanobacterium M.O.H. Mebact.thermoautotrophicum	$H_2 + CO_2$
Mbact.ruminantium Mbact.formicium Mbact.mobilis Methanospirillum hungati Methanococcus Vanniclii	$H_2 + CO_2$ or $HCOOH$
Methanosarcina-barkeri	$H_2 + CO_2$ or CH_3OH or CH_3COOH

Spores have not been found in any pure culture (4, 6). These organisms obtain energy for growth by using electrons generated in the oxidation of compounds such as hydrogen and formate (reduction of CO_2), or via fermentation of compounds such as acetate and methanol with the formation of methane and CO_2.

Table 2 shows the substrate metabolized by some species of methanobacterium.

Methanogenic bacteria have proven very difficult to isolate because they are strictly anaerobic and even small quantities of oxygen are harmful to them (36). Optimum growth occurs at neutral pH values. One species show optimum growth in the thermophilic temperature range, but most other species are mesophilic (6). Toerien et. al. (61) showed that the number of methanogenic bacteria present in anaerobic digestion is 10^6 to 10^8 per milliliter. These organisms develop at a relatively slow rate and require several weeks after innoculation for the development of an active culture.

The biochemistry of methanogenisis

Toerien et. al. (61) reported that short fatty acids could be quantitatively converted to methane and carbon dioxide. He also reported that a mixture of hydrogen and carbon dioxide could be utilized to produce methane. There is some doubt as to what end-products of anaerobic metabolism the methane producers can utilize.

Those compounds that in 1956 were thought to be substrates of the methanogenic bacteria were listed by Barker (3) (See Table 3).

However, it is now apparent that the cultures used in earlier studies were not pure and that the formation of methane from complex organic molecules involves the conversion of these molecules by other bacteria to a few simple substrates that can be used by the methanogenic bacteria (54, 49, 69).

Toerien et. al. (61) concluded that methanobacterium are not able to utilize substrates other than formate, acetate, methyl alcohol and carbon dioxide. McCarty (36) indicated that the two major organic intermediates formed in anaerobic treatment are acetic acid and propionic acid. Figure 1 shows the pathways by which mixed complex organic materials are converted to methane gas and carbon dioxide (37). From the figure we see that the most important acid converted to methane is acetic acid, most of which is formed by the oxidation of other intermediate compounds by the action of various methane producing bacteria which ferment these intermediates to acetic acid and methane (3, 24, 36, 44, 69) as shown in the following reactions:

$$2CH_3CH_2OH + CO_2 \longrightarrow 2CH_3COOH + CH_4$$

$$2CH_3CH_2CH_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4$$

$$4CH_3CH_2COOH + 2H_2O \longrightarrow 4CH_3COOH + CO_2 + 3CH_4$$
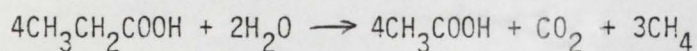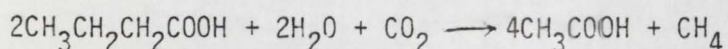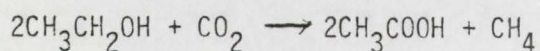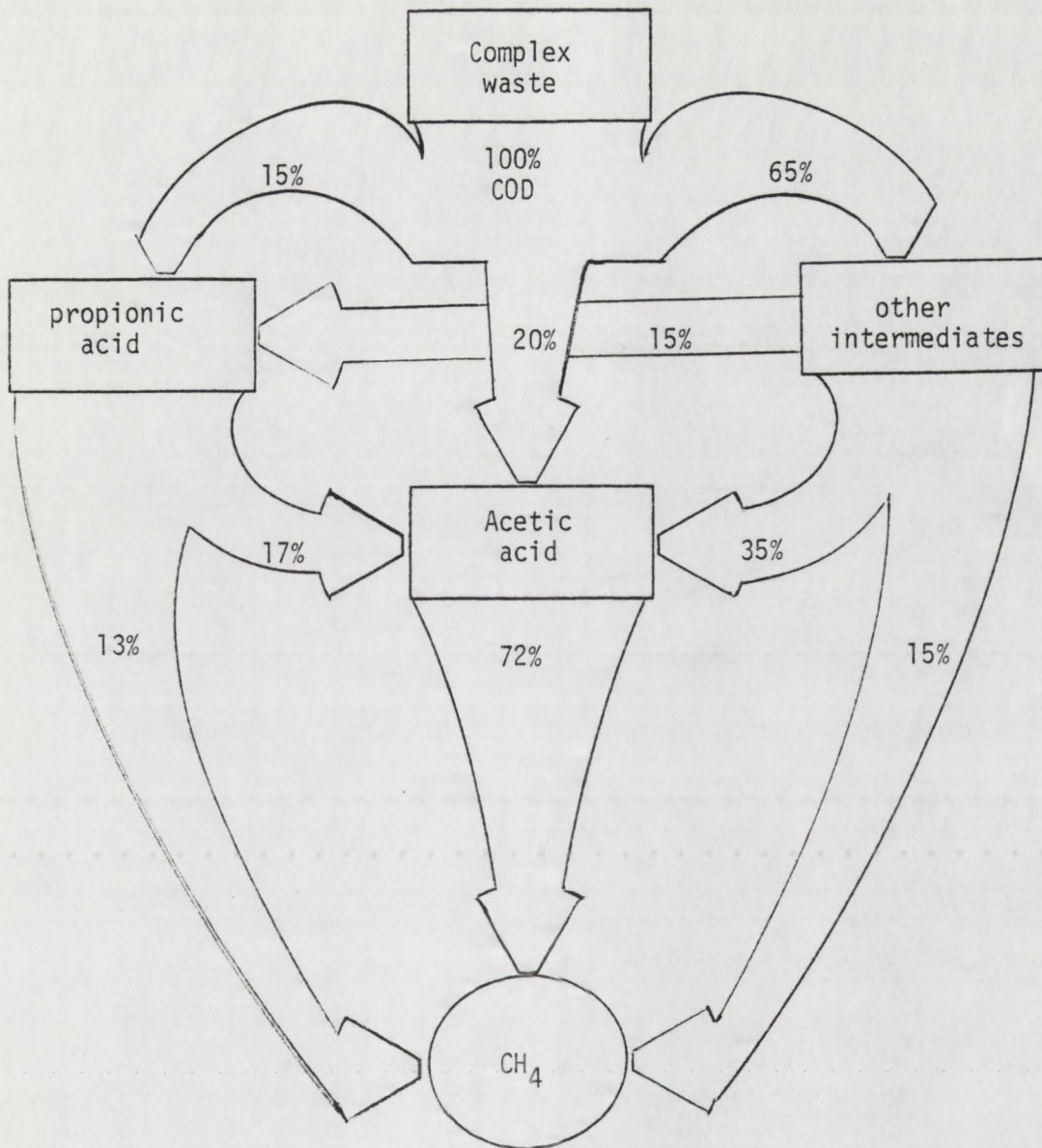
Figure 1. Pathways in methane fermentation of complex wastes.

Then, acetic acid, methyl alcohol, formic acid and CO_2 are reduced to CH_4 as in the following reactions:

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

$$4HCOOH \longrightarrow 4H_2 + 4CO_2$$

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$
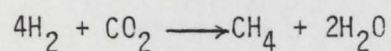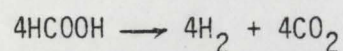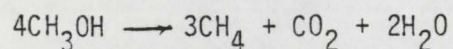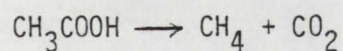
The formation of methane from CO_2 and H_2 was studied by several workers and it was found that hydrogen was removed from organic compounds by reducing CO_2 to methane gas (32, 36, 44, 55) according to the following reactions:

$$2CH_3CH_2OH + 2H_2O \longrightarrow 2CH_3COOH + 4H_2$$

$$4H_2 + CO_2 \longrightarrow 2H_2O + CH_4$$

$$2CH_3CH_2OH + CO_2 \longrightarrow 2CH_3COOH + CH_4$$

$$4CH_3CH_2COOH + 8H_2O \longrightarrow 4CH_3COOH + 4CO_2 + 24H$$

$$3CO_2 + 24H \longrightarrow 3CH_4 + 6H_2O$$

$$4CH_3CH_2COOH + 2H_2O \longrightarrow 4CH_3COOH + CO_2 + 3CH_4$$
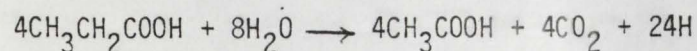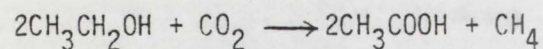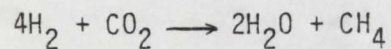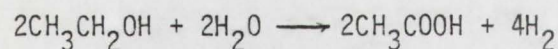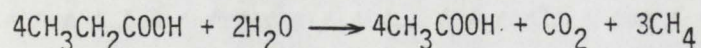
Figure 2 shows how these intermediate compounds break down to produce methane and carbon dioxide (15).

McCarty (36) concluded that the major source of methane in various digesters was acetic acid and carbon dioxide. He also concluded that approximately 70% of the methane originated from acetate and the rest originated from carbon dioxide and hydrogen.

Factors influencing growth of methanobacterium

The methane bacteria which are responsible for the majority of waste stabilization in anaerobic treatment grow quite slowly compared to acid forming organisms. Therefore a longer time is required for them to adjust to changes in organic loading, temperature, composition of the substrate, and other environmental conditions (61). For this reason, it is usually desirable in design and operation to strive for optimum environmental conditions so that more efficient and rapid treatment might be obtained.

Composition of substrate

The composition of the substrate is one of the major factors which determines the characteristics of the ecosystem in an anaerobic fermentation. The organic and inorganic components in the substrate
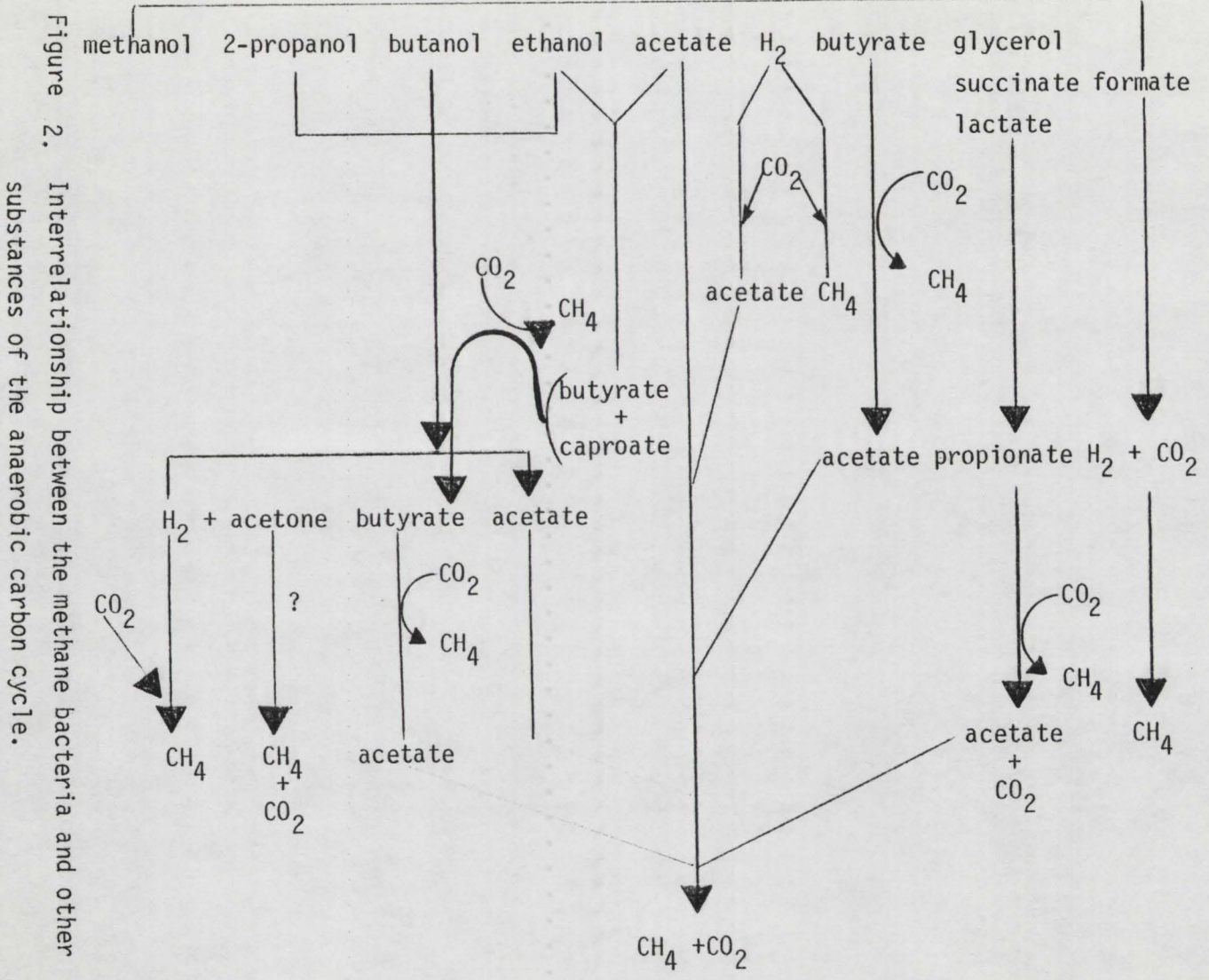
Figure 2. Interrelationship between the methane bacteria and other substances of the anaerobic carbon cycle.

lead to a selection of those bacteria which are able to metabolize these compounds.

Biological wastes are mainly made up of varying ratios of the following compounds: carbohydrates, amino acids, proteins, fatty acids, lipids, alcohols, and a group of nitrogenous compounds originating from living cells (61).

The carbohydrates in sewage and industrial effluents may consist of a large variety of compounds including hexoses, pentoses, aldoses, ketoses, disaccharides, polysaccharides such as starch, glycogen, cellulose and pectin.

The complex compounds of the substrate such as cellulose and starch are hydrolyzed by non-methanogenic organisms by two major biochemical reactions (22, 36). First, these complex molecules are hydrolyzed to simpler soluble molecules with the aid of extracellular enzymes such as cellobiase and amylase. The product of hydrolysis is glucose which is next catabolised to form C_1 - C_6 fatty acids, aldehydes, alcohols, carbon dioxide and hydrogen that are utilized as substrates by the methane bacteria (22, 27).

Malik (33) reported that the carbohydrates are the most forward substrates for complete anaerobic conversion to CH_4 and CO_2.

$$C_6H_{12}O_6 \longrightarrow 3CH_4 + 3CO_2 + 70 \text{ kcal}$$
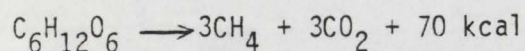
These organic materials are the main source of nutrients for the bacteria involved in anaerobic fermentation.

In addition to these compounds, the waste also contains phosphate, sulfur, and other inorganic compounds which are required as cofactors in the enzymatic reaction.

In anaerobic fermentation, a portion of the organic substrate is converted to microbial cells, while most of the remainder is stabilized by conversion to methane and carbon dioxide (13, 18, 41).

To maintain satisfactory digestion it is necessary to ensure that optimum biological growth is maintained at all times. For optimum biological growth, magnesium, iron, potassium and cobalt are required (41). However, the major nutrients are nitrogen, carbon, sulfur and phosphorus (29).

Earlier studies on a few pure cultures established that methanogenic bacteria were restricted in energy sources utilized for growth and suggested that their nutritional requirements were quite simple (3). The species studied could be grown in defined media with ammonia as a nitrogen source and sulfide as a sulfur source. Vitamins and other organic growth factors were not required. Carbon and energy sources could be supplied by CO_2 and H_2 or a simple organic compound such as methanol or formate.

Further studies on the nutritional requirements of methanobacteria in biological waste showed that these organisms can use ammonia, which is produced from organic nitrogen compounds by non-methane bacteria as a main nitrogen source in the first stage (45). This study indicated also that much of the cellular carbon compounds of methanobacterium are probably synthesized from acetate and carbon dioxide.

Temperature

The temperature ranges for optimal growth of microorganisms can conveniently be divided into three regions, the psychrophilic (less than 20° C), Mesophilic (20-45° C) and the thermophilic (greater than 45° C) (29).

Buswell (8) reported that the production of methane could be achieved from 0° C to 55° C. McCarty (37) found the rate of reaction much faster at higher temperatures resulting in a more efficient operation.

Two optimum temperature levels for anaerobic treatment have been reported (14,19,29,37,41). One in the mesophilic range (34-37° C), and the other in the thermophilic range (60° C).

Even though greater gas production can be expected if a digester is operated in the thermophilic range, it is·very rarely done, because the energy required to maintain the digester at a suitable temperature more than outweighs the increased gas produced. Also thermophilic bacteria are more sensitive to change in environmental conditions than mesophilic bacteria (29). Therefore most treatment systems are designed to operate in the mesophilic range.

pH and organic acid concentration

One of the most important environmental requirements is that of a proper pH. The internal environment of living cells is thought to be at a pH near neutrality and, since living cells are relatively impermeable to hydrogen and hydroxyl ions, the pH value inside the cell remains

fairly constant despite wide variations in the concentration of hydrogen ions in the medium surrounding the cell (29). However, with nearly all microorganisms, there is a fairly narrow range of pH which is most favorable for growth. Outside this range growth is restricted (48).

The pH of the medium probably influences the behavior of the microorganism as a result of an interaction between hydrogen ions and enzymes on the cell surface (48). Stanier (29) indicated that undissociated molecules can penetrate more rapidly into bacterial cells than the ionic forms of the organic acids, therefore, weak acids have a greater effect than strong acids in changing the internal pH.

The optimal pH for growth varies with each kind of organism. Barker (4) concluded that the pH range of 6.4-7.2 was most effective for methane production and below pH 6.0 and above 8.0, gas production was rapidly reduced. McCarty (37) indicated that the anaerobic process can proceed quite well with a pH varying from about 7.0-7.2. For this reason it is important that the pH not be allowed to drop below 6.2 for significant periods of time to avoid toxic effects. Simpson (53) reported that the main buffering substrate in most digesters is NH_4HCO_3 whose ions NH_4^+ and HCO_3^- remain in solution and contribute to the alkalinity of the substrate. Greater NH_4^+ and HCO_3^- production has been associated with thermophilic digestion due to the more complete breakdown of protein (29). When the organic acids begin to increase in concentration, they are neutralized by the bicarbonate alkalinity (37, 39). If an increase in organic acid concentration decreases the bicarbonate concentration too much, and a serious drop in pH threatens,

then the bicarbonate alkalinity should be controlled. This may be done
by the addition of alkaline materials. Calcium hydroxide is the most
widely used material for controlling pH in anaerobic treatment (11, 37,
41). When the $Ca(OH)_2$ is added, it initially increases the bicarbonate
alkalinity by combination with the carbon dioxide present as follows:

$$Ca(OH)_2 + 2CO_2 \longrightarrow Ca(HCO_3)_2$$
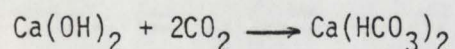
When the bicarbonate alkalinity reaches some point between 500 and
1000 mg/L, additional lime additions result in the formation of
insoluble calcium carbonate as follows (11, 37).

$$Ca(OH)_2 + CO_2 \longrightarrow CaCO_3 + H_2O$$
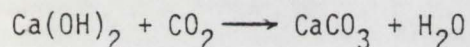
Lime addition beyond this point reduces the CO_2 concentration to
less than 10%. The pH then suddenly increases above 7 and approaches 8
largely as a result of a decrease in CO_2. A better substance is thought
to be sodium bicarbonate (37, 39). Although it is more expensive than
calcium hydroxide it has significant advantages over other materials.
It does not react with carbon dioxide to create a vacuum in the
digester. It is quite soluble and can be dissolved prior to addition,

and there is little danger that it will raise the pH to undesirable levels.

Other agents may be used as buffering agents too, such as potassium hydroxide and ammonium hydroxide, but care must be exercised since excess ammonia can be toxic (37). Even so it is important as a source of nutrient nitrogen (29).

Under normal conditions anaerobic processes may become unbalanced. The unbalanced digester is one which is operating at less than normal efficiency. The efficiency may decrease to almost zero in which case a stuck digester results (37). Many studies have been made to determine the cause of digester failure which indicates that the prominent factor influencing methanogenic bacteria are high organic acid concentrations and pH (2). Although most workers agree that a pH less than 6.5 is detrimental to the process, there have been considerable differences of opinion concerning the effect of high concentrations of organic acids. One group, as exemplified by McCarty (37), feels that organic acids are inhibitory to the methane bacteria only in an indirect way through a reduction in pH and that this inhibition can be relieved by maintaining the pH near neutrality. Another group, exemplified by Buswell (7,8), feels that organic acid concentrations above 2000 to 3000 mg per l are inhibitory regardless of pH and that this inhibition can be relieved by reducing the organic load so that the acids will be fermented to CH_4 and CO_2 as rapidly as they are formed from the raw substrate.

Buswell (7) indicated that many of the early failures were due to batch types of fermentation in which too much substrate was present.

Cassel and Sawyer (9) concluded that the methane bacteria may be divided into two groups. The first group is relatively resistant to high organic acid content, and under favorable pH conditions may increase rapidly enough to produce a significant amount of methane in 10 to 15 days. The second group is affected by high concentrations of organic acids which when reduced to normal levels by the first group, will start to grow rapidly.

Schulz et. al. (52) noted that even when the pH was maintained within permissible limits, fermentation was inhibited when the concentration of organic fatty acids was between 2000 and 3000 mg/L.

Sarma (50) in his study on the fermentation process of converting plant material into methane indicated that inhibition of methane fermentation would not occur even when the concentration of organic acid in the digester reached a value of 7440 mg/L.

Vaseen (65) showed that methane production is more stable when acid by-products are absorbed into inert fluid media down to 3000 mg/L.

Toxic materials

There are many materials, both organic and inorganic, that may be toxic or inhibitory to anaerobic microorganisms. The methanobacteria form the key group in the process since they are more sensitive to environmental conditions than other groups of bacteria (28).

The toxicity of any material is dependent on the concentration in the substrate. In general, when a substance is present in low concentration, it may be stimulatory. As the concentration increases

above the stimulatory concentrations, it may become toxic and the biological activity may approach zero (28, 41, 43, 45).

The concentration of alkali and alkaline earth metal salts such as sodium, potassium, calcium, or magnesium may be quite high in industrial wastes. These may be either stimulatory or inhibitory depending on their concentration. Toxicity is normally associated with the cation rather than the anion of the salt. The stimulatory and inhibitory ranges of these salt cations are shown in Table 4. When combinations of these cations are present, the nature of the effect becomes more complex as some of the cations act antagonistically or synergistically.

Antagonism is a reduction of the toxic effect of one substance by the presence of another. It has been found that 7,000 mg/L of sodium may significantly retard anaerobic treatment. If 300 mg/L of potassium is added, this retardation may be reduced by 80% (38).

Synergism is an increase in the apparent toxicity of one substance caused by the presence of the second substance in the environment, and it has been found if calcium is added to a process which initially has magnesium cations the inhibition may be increased.

McCarty (38) reported that the concentration of soluble sulfide varying from 50 to 100 mg/L can be tolerated in anaerobic treatment with little or no acclimation requirement. As the concentration exceeds 200 mg/L the soluble sulfide will be toxic to methanobacterium. The soluble sulfide concentration in a digester is a function of the incoming sources of sulfur, the pH, the availability of heavy metals to act as precipitants and the rate of gas production. The higher the gas

Table 3.   Compounds thought in 1956 to be substrates for
           methanogenic bacteria.

Fatty Acids	Alcohols	Gases
formic	methanol	hydrogen
acetic	ethanol	carbon monoxide
propionic	n-propanol	carbon dioxide
b-butynic	iso-propanol	
n-valeric	n-butanol	
n-caprioc	iso-butanol	
	n-pentanol	

Table 4.   The stimulatory and inhibitory concentration of alkaline
           earth metal.

	Stimulatory	Moderately Inhibitory	Strongly Inhibitory
	(mg/ml)	(mg/ml)	
sodium	100-200	3,500-5,500	8,000
potasium	200-400	2,500-4,500	12,000
calcium	100-200	2,500-4,500	8,000
magnesium	75-150	1,000-1,500	3,000

production per liter of waste, the higher will be the amount of sulfides driven from solution as a gas, and the lower the concentration remaining in solution (38, 41).

McCarty and McKinney (35) reported that pH played a significant role in the toxicity of ammonium ion. Ammonia may be present during treatment either in the form of ammomium ion (NH_4^+) or as dissolved ammonia gas (NH_3). These two forms are in equilibrium with each other and the relative concentration of each depends upon the pH as indicated below.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$
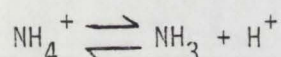
When the hydrogen ion concentration is sufficiently high (pH 7.2 or lower) the equilibrium is shifted to the left so that inhibition is related to the ammonium ion concentration. At higher pH levels, the equilibrium shifts to the right and the ammonia gas concentration may become inhibitory.

McCarty (38) indicated that the ammonia gas is inhibitory at a much lower concentration than the ammonium ion. Toxicity occurs at ammonia concentrations above 1.500 mg/L above pH 7.4, while the toxicity occurs at ammonium ion concentrations above 3,000 mg/L at any pH (63).

Heavy metal ions such as copper, zinc and nickel are toxic (38, 41). Moon (42) reported that chromium can also be toxic to anaerobic processes. He stated that this metal ion is normally reduced to the

trivalent form which is relatively insoluble at normal digestion pH levels and consequently is not very toxic.

These metals, to be toxic, should be present in the soluble form. Therefore, if sufficient sulfide is present, a high concentration of these metals can be tolerated (30).

Chin (10) reported that the concentration of less than 1 mg/L of all heavy metals except iron are extremely toxic, but higher concentrations can be tolerated if sufficient sulfide is present to at as a precipitant.

Farnin and Chynoweth (16) reported that the heavy metal toxicity of methane producing bacteria decreases in the order: $Cr > Cu > Zn > Cd > Ni$.

McCarty and McKinney (34) showed that iron and aluminum salts are not toxic because of their low solubility. There are also many organic materials which may inhibit the digestion process. These include alcohols, long-chain fatty acids, methanol and high concentrations of organic fatty acids.

Buswell (7) indicated that the concentration of organic acids above 3000 mg/L will be toxic to methane bacteria but not to acid formers.

Mixing

Mixing of anaerobic sludge accomplishes three major objectives. Firstly, the organisms are kept continuously in contact with the substrate. Secondly, the substrate is uniformly distributed and made available, and thirdly the concentration of inhibitory biological

intermediates and end-products are maintained at minimum local concentrations (29). Mixing is carried out by either mechanical agitation, digester content recirculation, or gas recirculation.

MATERIALS AND METHODS

Whey

Dry sweet whey was obtained from Gossner Cheese Company, Logan, Utah.

Sludge

Sludge from a waste water treatment plant in Preston, Idaho was used as a source of inoculation material.

Chemicals

Analytical regeant grade of the following chemicals were used:

1. Concentrated ammonium hydroxide
2. Potassium hydroxide
3. Sodium bicarbonate

pH controlled batch fermentation

A schematic diagram of the experimental set-up for the anaerobic batch fermentation of whey is shown in Figure 3. Two and one half liters of whey containing 2.5 or 5.0% lactose were incubated with 2% sludge in a 3 L Erlenmeyer flask as a fermenter. The flask was sealed with a #9 stopper into which was placed an Ingold electrode (#18513 Ingolds Electrodes Inc.), gas collection tube, and sampling tube. The contents were heated and maintained at the desired temperature (37° C and 60° C) using hot plate with a magnetic sterrier. The pH was controlled by connecting the pH-electrode to a pH-controller that recorded a pattern indicating acid production and provided injector of liquid ammonium hydroxide through a plastic tube to hold the pH to 7. The ammonium hydroxide pump was connected to the pH-controller through a masterflex controller to give the desired ammonium hydroxide injection speed. Another pump connected to the pH controller was used to agitate the substrate during ammonium hydroxide injection.

pH controlled continuous fermentation

Since the growth of methanobacterium was so slow in the batch fermentation process, a new technique was tried in which 300 ml of whey and 2% inoculum were placed in the fermenter. Both pH and temperature were controlled. Organic acid concentration was determined throughout the fermentation using the procedure outlined in the Standard Methods for Examination of Water and Waste Water (56). Water was added to keep

Figure 3. Schematic diagram of experimental set-up for the anaerobic batch fermentation.
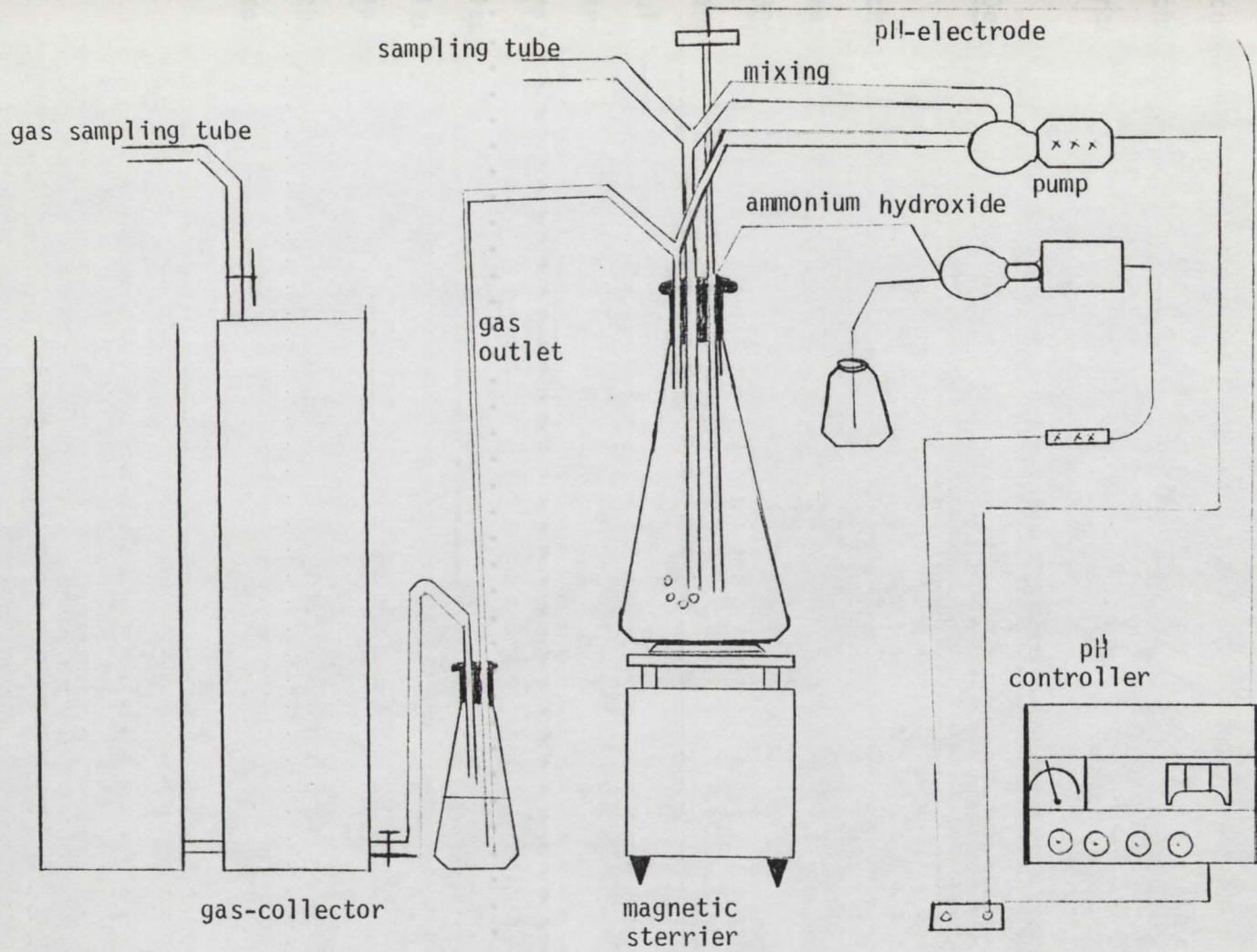
the ratio between the methane-bacteria and the toxic substrae
concentration closely constant. Gas chromatography analysis was run to
check for the production of methane which when its production increased,
raw substrate was added continuously without dilution.

Determination of lactose concentration

Lactose concentration was determined by high pressure liquid
chromatography. A standard solution of 0.1, 0.5 and 1% wt/wt of lactose
was prepared. Fermented whey samples were centrifuged for 15 minutes at
3000 rpm. The supernatant was filtered through a 0.45 µ syringe filter
and diluted to contain not more than 1% wt/wt lactose. A sample of 175
µl each of standard and fermented whey sample was applied by syringe
injection to an analytical acid analysis column (Aminex HPX-87) using
(Perkin-Elmer series 2) high pressure liquid chromatography and a
laboratory data control refrectromoniter detector. Separation of the
lactose peak was achieved on the chromatography data station
(Perkin-Elmer Sigma 10B). Lactose was then quantified by comparison of
the peak area with those of corresponding concentrations of the standard
solutions.

Gas collection apparatus

Fermenter gases were collected in   15.24 cm diameter and 50.80 cm
long plexi-glass cylinders. These cylinders were initially filled with
water which was subsequently displaced by the fermented gases. Figure 4
illustrates the gas collection system. To fill the gas cylinder, valve
1 was closed and valve 2 was opened. As the water entered the bottom of
the gas collecting cylinder, the gas was displaced and discharged into
the atmosphere through the gas sampling tube. When the cylinder was
completely filled valve 2 was closed and valve 1 opened to allow the
gases to displace the water in the cylinder. Therefore, the volume of
gas produced was obtained by reading the gas level in the gas collecting
cylinder and standardizing it to standard temperature and pressure for
natural gas, which is one standard atmosphere and 15.5^{o} C.

Analysis of fermentation gases

A Varian model 2740 gas chromatograph was employed for analyzing
the digester gases. Methane gas in the fermenter gases was indicated by
the chromatograph qualitatively by the position of the peak on the time
scale and quantatively by the area of the peak above the chromatogram
base line which was estimated by a CDS 101 chromatography data system.
The program of operation for the gas chromatography is indicated in
Table 5. The gas sampling outlet was a 100 ml round bottom boiling
flask. The flask initially was filled with water which was subsequently
displaced by the fermenter gases coming from the gas-sampling tube. A

water reservoir $\longrightarrow$

valve 2

gas-sampling

to the
fermenter

gas collection

valve 1
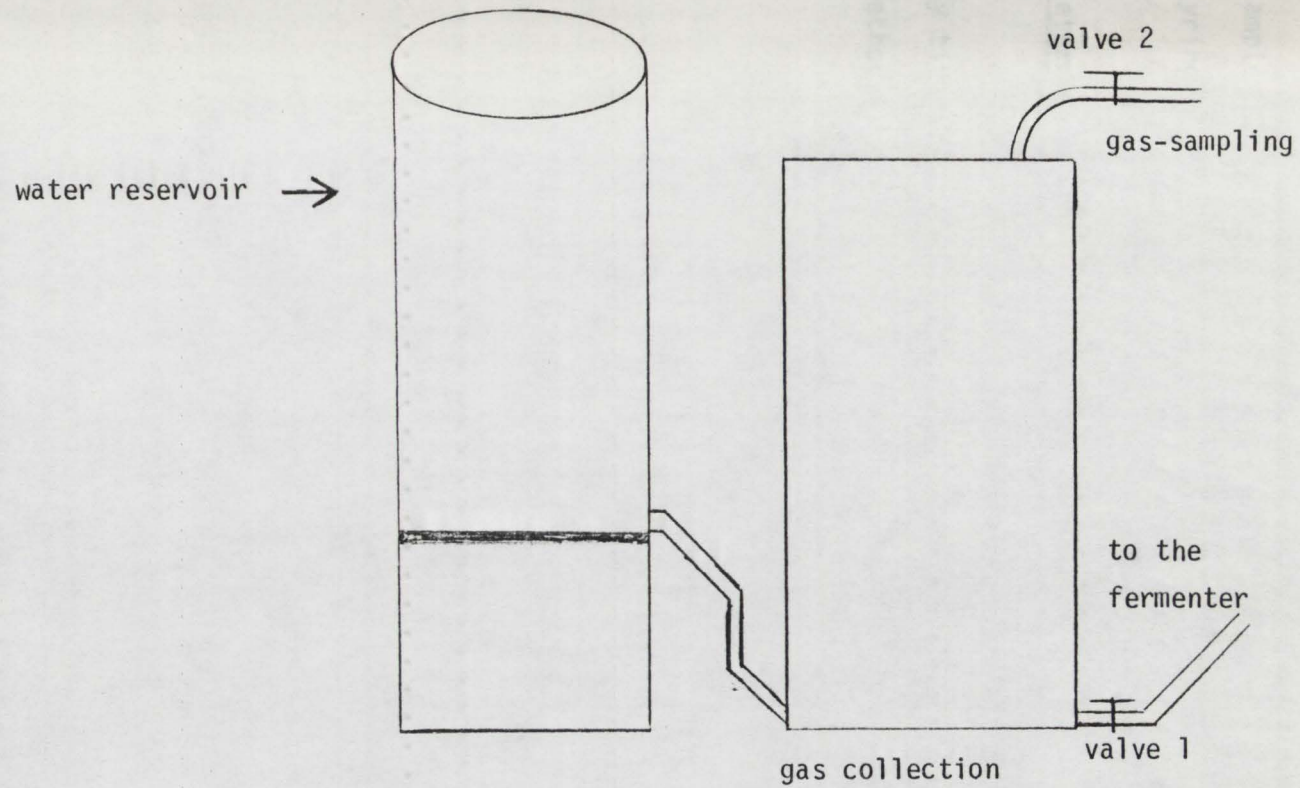
Figure 4. Gas collection system.

sample from the gas sampling flask was obtained with a 25 ml gas-tight syringe and introduced into the gas chromatography system.

Determination of alkalinity

Alkalinity concentration was determined throughout the fermentation by titration with 0.02 N HCl using the procedure outlined in Standard Methods for Examination of Water and Waste Water (56).

RESULTS

pH controlled batch fermentation

Whey containing 2.5% and 5.0% lactose was fermented at 37° C and 60 $^\circ$ C with the pH automatically controlled at pH 7.0. Appendix Tables A1, A2, A3, A4, A5, A6 and A7 show the overall results of these fermentation experiments.

Lactose concentration. Lactose concentration in the fermented whey was determined quantitatively during the fermentation process. Table 6 shows the utilization of lactose by acid forming bacteria during the fermentation of whey containing 2.5 and 5.0% lactose at 37° C and 60° C. More than 50% of the lactose was utilized within the first 20, while the rest was completely utilized after 30. The fermenters run at 37° C show faster lactose utilization than those run at 60° C.

Gas production. Two groups of organisms should be present in the methane fermentation batch. One group converts organic matter to organic acids and CO_2, and the other group converts these acids to methane and CO_2. In these experiments gas production started after 20 hours and continued to increase quantitatively. Gas volumes in m^3 are listed in Appendix Tables A1, A2, A3, A4, A5, A6 and A7. No methane was present in the gas during the first step of fermentation, but gas

continued to be produced even after all the lactose was utilized. In the later stages, methane started to be generated and continued to increase until the fermentation was completed.

Fermentation time. Time is one of the important factors that should be considered for the most economic production of methane. Table 7 shows the minimum time required to initiate methane production. Analysis of variance (Table 8) shows a significant (α=0.01) effect of temperature on time needed to start gas production.

It also shows no significant effect of lactose concentration and its interaction with temperature on the time of initial gas production. Table 9 shows that at 60° C a shorter time was required to initiate methane production than that at 37° C in both lactose concentrations.

Methane production. Methane is the main purpose of this study, and its volume is the critical point in the production. Table 10 shows the volume of 100% methane produced per kilogram of lactose in each fermenter. This table shows that whey containing 2.5% lactose produces more methane per gram of lactose than the fermenter which contains 5% lactose. It also shows that at 37° C more methane was produced than at 60° C.

Analysis of variance (Table 11) shows a significant (α=0.01) effect of temperature on the methane production, but there was no effect of lactose and its interaction on methane production. Table 12 shows that the production of methane was better at 37° C than 60° C.

Table 5. Program of operation of gas chromatograph for
         analysis of fermenter gases.

Item	Details
chromatograph	Varian model 2740 with CDS 101 chromatograph data system
column support	Porpack S
column	6 feet long, 1/8 inch diameter stainless tubing
column temperature	80 C
injector temperature	100 C
detector temperature	260 C
carrier gas	Nitrogen

Table 6. Utilization of lactose during fermentation of whey
         containing 2.5% and 5.0% lactose at 37° C and 60° C.

Time	2.5 Lactose		5.0 Lactose	
	37°C	60°C	37°C	60°C
(h)	(%)	(%)	(%)	(%)
0	2.510	2.509	5.142	5.110
20	0.913	1.357	2.714	4.320
30	0.00	0.00	0.00	3.651
42	0.00	0.00	0.00	2.501
50	0.00	0.00	0.00	1.483
61	0.00	0.00	0.00	0.90
90	0.00	0.00	0.00	0.00

Table 7.   Minimum time required for initiating methane production
           at 37° C and 60° C in whey containing 2.5% and 5.0%
           lactose.

Temperature	Lactose	Minimum time
(°C)	(%)	(h)
37	2.5	432
27	2.5	432
37	5.0	480
37	5.0	432
60	2.5	408
60	5.0	384
60	5.0	408

Table 8.   Analysis of variance of the effect of temperature
           and lactose concentration and their interaction on
           time for initiating methane production.

Source	S.S.	df	M.S.	f	α
Temperature	2822.40	1	2822.4	5.88	xx
Lactose %	57-60	1	57.6	0.12	--
LT	518.40	1	518.4	1.080	--
Error	1440	3	480	--	--

Table 9. Effect of temperature on the time required to initiate
methane production in whey containing 2.5% and 5%
lactose.

Temperature	Rep	Mean Time	Standard Deviation
(°C)		(h)	(h)
37	4.0	444	±11
60	3.0	402	±13

Table 10. Volume of methane per kilogram lactose produced by
fermentation of whey at 37° C and 60° C.

Temperature	Lactose	Methane produced per kg of lactose
(°C)	(%)	(m^3)
37	2.5	0.1264
37	2.5	0.1513
37	5.0	0.1182
37	5.0	0.1303
60	2.5	0.1039
60	60	0.0922
60	60	0.0943

Effect of ammonium hydroxide, sodium bicarbonate and potassium
hydroxide on neutralizing whey. Methane gas production did not start
during the early stages of fermentation, but required more than two
weeks to generate. This suggested that some conditions were not
favorable for methanogenic bacteria during the first stage of
fermentation. Since pH and temperature were kept constant at optimum
levels all the time, other factors should be considered. Since a high
alkalinity value was noted as a result of adding NH_4OH as a neutralizing
base, it was assumed that the ammonium ion might have an inhibitory
effect on methanogenic bacteria. Another experiment was run using a
mixture of sodium bicarbonate and potassium hydroxide as a base for
neutralizing the acids during fermentation. Table 13 shows the result
of 10 days fermentation without production of methane.

pH controlled continuous fermentation

Effect of organic acid concentration on the production of methane.
As noticed by many investigations, when the pH was kept constant, salts
of organic acids continued to increase in concentration until they
inhibited the methanogenic bacteria. Two things can be done to reduce
the concentration of organic acids or their salts. Reduce the
concentration of the substrate, or dilute the substrate throughout the
fermentation. Tables 14 and 15 show the results of such an experiment.
A mixture of sodium bicarbonate and potassium hydroxide was used to

Table 11. Analysis of variance of the effect of temperature and lactose concentration on the volume of methane produced by fermentation of whey.

Source	S.S.	df	M.S.	f	α
T	0.00174	1	0.00174	13.5419	**
L %	0.000255	1	0.000255	1.9850	--
TL	0.0006	1	0.000006	0.04875	--
Error	0.000385	3	0.000128		

Table 12. Effect of temperature on the volume of methane produced from fermentation of whey containing 2.5 and 5.0% lactose.

Temperature	Rep	Mean	Standard Deviation
(°C)		(m^3)	(m^3)
37	4	0.131	±0.006
60	3	0.098	±0.006

Table 13. A fermentation of whey containing 5% lactose at 37° C by using a mixture of sodium bicarbonate and potassium hydroxide.

Time	gas volume	CH_4	Alkalinity as $CaCO_3$
(h)	($m^3 \times 10^{-2}$)	(%)	(mg/L)
0	0.00	0.00	1790
20	0.00	0.00	1920
30		0.00	2080
42	0.2814	0.00	2450
50	0.4003	0.00	3060
61	0.6469	0.00	3870
90	0.8441	0.00	4430
102	0.9747	0.00	5110
120	1.0211	0.00	5520
144	1.0762	0.00	5930
168	1.0936	0.00	6320
192	1.0936	0.00	6730
216	1.0936	0.00	6880
240	1.0936	0.00	6970

neutralize 300 ml of whey containing 5% lactose as an initial substrate. Water was added gradually to reduce the concentration of organic acids below its inhibition value. This procedure improved the ability of methanogenic bacteria to generate methane gas after a few hours. As the growth rate increased, more whey was added and less dilution was required until the acids were fermented to CH_4 and CO_2 as rapidly as they were formed. Table 16 shows the result of a similar procedure by using NH_4OH for neutralization during a 7 day fermentation. No methane was generated from this experiment which explains that ammonium hydroxide had an effect on methanogenic bacteria.

Effect of dilution of the production of methane. How much water should be added, and when are serious questions to consider in the generation of a significant amount of methane in a short time. Using equal amounts of water (200 ml) in each time interval did not improve the production of methane as shown in Table 17. This result shows that the rate of acid production is much more than the rate of dilution especially at the beginning of the fermentation. This table shows also that when the concentration of organic acids was reduced below 5000 mg/L as acetic acid the production of methane went up. Table 18 shows that a minimum amount of water required to dilute whey containing 5% lactose should be enough to keep the concentration of organic acid below 4000 mg/l as acetic acid. This table also shows that when

Table 14. Cumulative production of methane from whey containing 5% lactose by the continuous process at 37° C. (First experiment.)

Time	Gas volume	CH_4	CH_4 volume	Whey volume added
(h)	($m^3 \times 10^{-2}$)	(%)	($m^2 \times 10^{-2}$)	(ml)
0	0.00	0.00	0.00	300
3	0.0095	0.00	0.00	
6	0.0176	0.00	0.00	
9	0.0249	0.00	0.00	
12	0.0350	0.051	0.00001	
24	0.0473	0.124	0.00005	100
36	0.0623	0.186	0.00011	
48	0.1008	0.486	0.00049	100
72	0.1761	1.550	0.00273	100
96	0.3094	13.81	0.04278	100
108	0.4309	21.161	0.09118	100
120	0.5997	26.551	0.15922	200
132	0.7500	28.71	0.21539	200
144	1.8252	43.636	0.79646	

Table 15. Cumulative production of methane from whey containing
5% whey by the continuous process at 37° C.
(Second experiment.)

Time	Gas volume	CH_4	CH_4 volume	Whey volume added
(h)	(m^3 x 10^{-2})	(%)	(m^2 x 10^{-2})	(ml)
0	0.00	0.00	0.00	300
3	0.0085	0.00	0.00	000
6	0.0194	0.061	0.00001	
9	0.0275	0.109	0.0003	
12	0.0336	0.126	0.00004	
24	0.0426	0.167	0.00007	100
36	0.0710	0.319	0.00022	
48	0.1328	1.102	0.00146	100
72	0.1946	2.017	0.00392	100
96	0.3234	5.052	0.01633	100
108	0.6414	13.513	0.08589	100
120	0.6936	14.610	0.10126	100
132	0.8766	17.742	0.15552	100
144	1.1270	20.782	0.23421	100
156	1.4328	25.017	0.35843	200
168	1.7159	28.104	0.48223	200
180	2.0523	32.6112	0.66924	

Table 16. Methane fermentation of whey containing 5% lactose
using NH_4OH as a neutralizing base by the continuous
process at 37° C.

Time	gas volume	CH_4	Whey
(h)	($m^3 \times 10^{-2}$)	(%)	(ml)
0	0.00	0.00	300
3	0.00	0.00	
6	0.0118	0.00	
9	0.0211	0.00	
12	0.0272	0.00	
24	0.0379	0.00	100
36	0.0484	0.00	
48	0.0946	0.00	100
72	0.1402	0.00	100
96	0.2632	0.00	100
108	0.3556	0.00	100
120	0.3864	0.00	200
132	0.4452	0.00	200
144	0.5394	0.00	

Table 17. Cumulative production of methane from whey containing
5% lactose at 37° C. Whey and dilution water added
in increments as shown.

Time	gas volume	CH_4	Volatile acid CH_3COOH	H_2O	Whey added
(h)	($m^3 \times 10^{-2}$)	(%)	(mg/1)	(ml)	(ml)
0	0.00	0.00	816	0.0	300
3	0.00	0.00	864	200	
6	0.00	0.00	2832	200	
9	0.0609	0.00	4224	200	
12	0.1363	0.00	5400	200	
24	0.2349	0.00	5304	200	
36	0.2958	0.037	5472	200	
48	0.3277	0.034	5352	200	
72	0.3683	0.091	4800	200	100
96	0.4234	0.366	4584	200	
108	0.4553	0.912	4632	200	
120	0.5481	2.3429	4296	200	100
132	0.6612	7.599	3888	200	100
138	0.6873	8.377	4100	200	100
144	0.7308	8.9373	4560	200	100
150	0.7859	9.6679	4776	00	

Table 18. The minimum amount of water required to maintain
a low level of toxic material for the production
of methane from whey containing 5% lactose at
37° C.

Time	gas volume	CH$_4$	CH$_4$ volume	volatile acid	alkalinity	H$_2$O	Whey
(h)	(m^3 x 10^{-2})	(%)	(m^3 x 10^{-2})	(mg/liter)	(mg/liter)	(ml)	(ml)
0	0.00	0.00	0.00	720	1810	0.0	300
3	0.0229	0.00		672	1850	400	
6	0.0426	0.00		2006	2130	400	
9	0.0759	0.00		2640	2490	400	
12	0.1186	0.077	0.00009	3168	2570	300	
24	0.1896	0.216	0.00041	3552	2620	200	
36	0.2253	2.142	0.00482	3984	2840	100	100
48	0.2563	3.801	0.00974	3648	3170	300	
72	0.3558	13.513	0.04879	3720	3260	75	100
96	0.4506	19.462	0.08769	3096	3410	50	100
108	0.5101	22.744	0.11601	2784	3490	0.00	100
120	0.6406	27.689	0.17737	2496	3670	0.00	100
132	0.7235	30.103	0.21779	2184	3980	0.00	200
138	1.0712	37.251	0.39904	1896	4360	0.00	500
144	1.6680						500
150	2.7808	46.540	1.29420				

CH$_4$/lactose (kg)   = 0.1790 m^3

the production of methane increases, only short time (12 hr) is required to convert one liter of whey containing 5% lactose to a substantial volume of 100% methane equivalent.

Effect of substrate concentration on the production of methane. One way to reduce the rate of acid production in a fermenter is to reduce the initial concentration of lactose in the substrate. Table 19 shows the result of using whey containing 2.5% lactose in the substrate, while Table 20 shows the result of using whey containing 2.5% lactose in the substrate at the starting time and later increasing it to 5.0% lactose. It is very important to start the fermentation with low concentrations of organic acids. As the activity of the bacteria increase, more lactose can be added to produce more methane. This procedure reduced the fermentation time from 7.0 to 5.5 days with almost the same volume of methane produced.

Table 19. Cumulative production of methane from whey containing 2.5% lactose by the continuous process at 37° C.

Time	gas volume	CH_4	CH_4 volume	volatile acid	alkalinity	H_2O	Whey
(h)	($m^3 \times 10^{-2}$)	(%)	($m^3 \times 10^{-2}$)	(mg/L)	(mg/L)	(ml)	(ml)
0	0.00	0.00	0.00	648	1000	zero	300
3	0.00	0.00	0.00	1200	2150	250	
6	0.00	0.00	0.00	1920	2096	200	
9	0.00	0.00	0.00	2160	2010	150	
12	0.0188	1.780	0.00334	2640	2120	100	100
24	0.0617	3.941	0.00243	3280	2160	100	
36	0.1281	7.279	0.09324	2952	2330	75	100
48	0.2276	10.933	0.02487	2976	2790	50	100
72	0.3369	17.702	0.05963	2616	2810	0.00	100
96	0.5266	25.621	0.13480	2784	33-0	0.00	100
108	0.6643	28.453	0.18901	2472	3430	0.00	200
120	0.8844	33.523	0.29648	1680	3650	0.00	
132	1.2465	33.625	0.71208				
138	2.1177						

CH_4/lactose (kg) = 0.1662 m^3.

Table 20. Methane production during fermentation of whey containing 2.5% lactose at the starting time and later increasing to 5%.

Time (h)	gas volume $(m^3 \times 10^{-2})$	CH_4 (%)	CH_4 volume $(m^3 \times 10^{-2})$	volatile acid (mg/L)	alkalinity (mg/L)	H_2O (ml)	Whey (ml)
0	0.00	0.00	0.00	480	970	0.00	300
3	0.0336	0.00	0.00	840	1870	400	0.00
6	0.0539	0.00	0.00	1774	2090	300	0.00
9	0.0803	0.00	0.00	2496	2020	250	0.00
12	0.1044	1.082	0.00112	2986	2040	200	100
24	0.1370	4.033	0.00525	3888	2200	150	
36	0.2088	7.814	0.01631	3096	2590	100	100
48	0.2468	12.642	0.03120	2910	3070	100	100
72	0.2941	18.996	0.05586	3360	3580	50	100
96	0.3893	25.751	0.10024	3408	3870	0.00	100
108	0.6373	39.012	0.24862	3024	4220	0.00	200
120	0.9445	42.784	0.40410	2592	4050	0.00	500
126	1.6680			1896	3570	0.00	500
132	2.5957	49.280	1.27920	1488	3163	0.00	

$$CH_4/lactose \ (kg) = 0.1750 \ m^3.$$

DISCUSSION

An automatic pH control was used in this study to maintain the pH of whey at an optimum level (pH=7) during fermentation by methanogenic bacteria. The fermentation was run in a single stage rather than a two stage fermenter. The pH control did not enable the methanogenic bacteria to use organic acids as rapidly as they were produced by the non-methanogenic bacteria. This failure indicates that some factor other than pH inhibited methanogenic bacteria during the early stages of fermentation.

The fast assimilation of lactose indicated that the rate of lactose metabolism by the non-methanogenic organisms was much faster than the utilization of these acids by the methanogenic bacteria since no methane was detected during the first two weeks. Analysis of variance ($=0.01$) of the effect of temperature and lactose concentration on fermentation time and total amount of methane produced indicated that the temperature has a major effect on both fermentation time and quantities of methane produced. The fermentation time was shorter at 60° C but with lower methane production, while at 37 degrees C more methane was produced, but required a longer time. This differs from the results obtained by Rorick et. al. (47) and Varel et. al. (64), who showed that in the fermentation of cattle wastes, more methane was produced in a shorter time when they used thermophilic temperatures. However, other studies showed that optimum temperatures for methane production were 35-38° C (4, 5, 13, 25, 29, 37, 59).

Reesen and Strube (46) showed that the optimum temperature for methane production from whey was at 37° C. On the other hand, Bryant (6) indicated that most methanogenic species are mesophilic rather than thermophilic. The difference in these optimum temperatures is not clearly known, and might be due to differences in the substrates. On the other hand, lactose concentrations used in this study did not affect the quantity of methane produced (Table 10). Even the specific volume of methane produced was higher at 2.5% than at 5.0% lactose.

pH due to accumulation of organic acids did not have a direct effect on inhibition of methane production as McCarty believed (37) since an automatic pH control did not reduce the time needed for initiation of methane production. The time used was the same as that required by others in which a pH control system was not used (3, 13, 18, 27, 37, 41, 51, 58). This means that the low activity of methanogenic bacteria in a single stage fermenter was not the result of improper pH, but of some other factor which was minimized in two stage fermentations. When ammonium hydroxide was replaced by sodium bicarbonate and potassium hydroxide for pH control, there was still no methanogenic activity. Sodium bicarbonate was not toxic to methanogenic bacteria (35). Therefore some factor other than the neutralization inhibited the fermentation.

Buswell (7,8) indicated that organic acid concentrations above 3000 mg/liter are inhibitory regardless of pH. This inhibition can be

relieved by reducing the organic loading or diluting the fermenter contents.

Diluting and reducing the amount of whey initially added to the fermenter greatly improved methane production from whey as shown in Tables 14 and 15. The time required to get 50% CH_4 in the digester gas was less than on third the time in a batch fermenter with much more gas production. Reducing and diluting a substrate reduced the concentration of toxic material to a safe level and kept the activity of methanogenic bacteria rising to ferment acids to CH_4 and Co_2 as rapidly as they were formed. The inhibitory factor was organic acid concentration as shown in Tables 17 and 18, which when its production rate was faster than the growth rate of methane bacteria, the production of methane was reduced rapidly. On the other hand, ammonium hydroxide showed the same effect even with the dilution of substrate which indicated that a mixture of sodium and potassium hydroxide had a better effect on production of methane as suggested by McCarty (37).

The results in Table 18 suggested the best dilution to maintain a high activity for methanogenic bacteria. When the activity of the organisms were low, adding additional substrate did not help, since the utilization of the organic acids was very slow. However, as the activity of these organisms increased, no water was required to dilute the substrate since the organisms can utilize these acids as rapidly as they are produced. Thus a continuous fermentation can run in a very short time.

From this table we can also conclude that more methane gas was produced as the ratio of the organic acid concentration to number of methanogenic bacteria in the fermenter was kept constant. This rejects Buswell's conclusion (8) since these bacteria can be inhibited at the start of fermentation by concentrations of organic acid as low as 1000 mg/l. He believed that a level of 3000 mg/l of organic acid was toxic to methanogenic bacteria.

Table 19 shows that 2.5% lactose in the fermenter was better than 5% lactose as measured by activity of methanogenic bacteria, but the specific volume of methane produced was lower than that produced from whey containing 5% lactose. In a subsequent experiment fermentation was started with 2.5% lactose then increased to 5.0% after about 3 days. This increased the activity of methanogenic bacteria. More methane was produced in only 5.5 days compared to the fermentation which originally had a 5.0% lactose.

The main factor influencing methanogenic bacteria was the concentration of organic acids. This concentration was reduced by reducing and diluting the substrate. This enabled methane production to be achieved within a few days rather than a few weeks as in batch fermentation. Thus, a continuous fermentation can run without adding water since the population of methanogenic baacteria reached a level that they could convert organic acids to methane and carbon dioxide as rapidly as they were produced by non-methanogenic bacteria. This method was also better than a two-stage fermentation since no gas escaped during the fermentation as happens during the acidification phase in a

two-stage fermentation. This gas is responsible for part of the methane

produced by the continuous methods (3, 37, 55). A two stage

fermentation is a method in which substrate loading is controlled for

high activity of methanogenic bacteria. The same thing was accomplished

in a continuous method with no need to separate the fermentation phases.

Zall (68) expected that 3.785 liters of whey yields about 0.140 m^3

of digester gas containing 50-60% methane, assuming whey contains 5%

lactose. Practically, a very much lower volume was obtained. Reesen

and Strube (46) reported that only a 17-20% of fuel oil required in his

distillation plant was replaced by the gas produced from a fermentation

of whey. In this study, Cache Valley Cheese factory was taken as a

standard plant to calculate the efficiency of gas production. This

plant produces an average of 295,000 kg of whey, which contains about

14,755 kg lactose, each day. If this were fermented to methane gas it

would provide about 2,582 m^3/day of methane. This amount of methane

could replace 44% of the plant's fuel requirements.

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APPENDIX

Table 21. Methane production during fermentation of whey containing 2.5% lactose at 37° C by pH controlled batch fermentation.

Time	Lactose	gas-volume	gas-rate	CH_4	CH_4-volume	Alkalinity
(h)	(%)	$(m^3 \times 10^{-2})$	$(m^3 \times 10^{-2}/h)$	(%)	$(m^3 \times 10^{-2})$	(mg/L)
0	2.510	0.00	0.00	0.00	0.00	990
20	0.913	0.1239	0.0061	0.00	0.00	3240
30	0.00	0.5785	0.0578	0.00	0.00	3910
42	0.00	0.7049	0.0587	0.00	0.00	4670
50	0.00	0.3945	0.0493	0.00	0.00	5090
61	0.00	0.6431	0.0584	0.00	0.00	5330
90	0.00	0.8969	0.0309	0.00	0.00	5680
102	0.00	0.1172	0.0097	0.00	0.00	5840
120	0.00	0.0281	0.0015	0.00	0.00	6020
144	0.00	0.00	0.00	0.00	0.00	6120
168	0.00	0.00	0.00	0.00	0.00	6190
192	0.00	0.00	0.00	0.00	0.00	6220
216	0.00	0.00	0.00	0.00	0.00	6200
240	0.00	0.00	0.00	0.00	0.00	6240
264	0.00	0.00	0.00	0.00	0.00	6240
288	0.00	0.00	0.00	0.00	0.00	6230
312	0.00	0.00	0.00	0.00	0.00	6240
336	0.00	0.00	0.00	0.00	0.00	6230
360	0.00	0.00	0.00	0.00	0.00	6250
384	0.00	0.00	0.00	0.00	0.00	6280
408	0.00	0.00	0.00	0.00	0.00	6280
432	0.00	0.0451	0.0018	0.952	0.0004	6270
480	0.00	0.1803	0.0037	6.633	0.0189	6340
504	0.00	0.3148	0.0131	25.874	0.0814	6370
552	0.00	1.252	0.0260	55.152	0.6898	6490
					0.7905	

Table 22. Methane production during fermentation of whey
         containing 2.5% lactose at 37° C by a pH
         controlled batch fermentation.

Time	gas-volume	gas-rate	CH_4	CH_4 volume	Alkalinity
(h)	($m^3 \times 10^{-2}$)	($m^3 \times 10^{-2}$/h)	(%)	($m^3 \times 10^{-2}$)	(mg)
0	0.00	0.00	0.00	0.00	930
20	0.142	0.00	0.00	0.00	2980
30	0.3945	0.00	0.00	0.00	3860
42	0.5224	0.0043	0.00	0.00	4690
50	0.6382	0.0797	0.00	0.00	5010
61	0.8006	0.0727	0.00	0.00	5280
90	1.2184	0.0420	0.00	0.00	5530
102	0.4148	0.0345	0.00	0.00	5770
120	0.2286	0.0127	0.00	0.00	5980
144	0.0551	0.0023	0.00	0.00	6110
168	0.00	0.00	0.00	0.00	6200
192	0.00	0.00	0.00	0.00	6240
216	0.00	0.00	0.00	0.00	6270
240	0.00	0.00	0.00	0.00	6260
264	0.00	0.00	0.00	0.00	6260
288	0.00	0.00	0.00	0.00	6260
312	0.00	0.00	0.00	0.00	6260
336	0.00	0.00	0.00	0.00	6260
360	0.00	0.00	0.00	0.00	6260
384	0.00	0.00	0.00	0.00	6260
408	0.00	0.00	0.00	0.00	6290
422	0.2262	0.0094	4.800	0.0187	6330
480	0.6605	0.0137	27.932	0.1842	6380
504	1.2416	0.0517	59.954	0.7431	6510
				0.9458	

Table 23. Methane production during fermentation of whey
containing 5% lactose at 37° C by pH controlled
batch fermentation.

Time	gas-volume	gas-rate	CH_4	CH_4 volume	Alkalinity
(h)	$(m^3 \times 10^{-2})$	$(m^3 \times 10^{-2}/h)$	(%)	$(m^3 \times 10^{-2})$	(mg/L)
0	0.00	0.00	0.00	0.00	1820
20	0.2059	0.00	0.00	0.00	3390
30	0.4139	0.0414	0.00	0.00	4570
42	0.8064	0.0672	0.00	0.00	5890
50	1.1139	0.1392	0.00	0.00	6910
61	0.5018	0.0456	0.00	0.00	7540
90	1.4127	0.0487	0.00	0.00	8220
102	0.6585	0.0548	0.00	0.00	8530
120	0.3133	0.0174	0.00	0.00	9190
144	0.1769	0.0073	0.00	0.00	10080
168	0.0754	0.0031	0.00	0.00	10330
192	0.0261	0.0010	0.00	0.00	10570
216	0.00	0.00	0.00	0.00	10730
240	0.00	0.00	0.00	0.00	10810
264	0.00	0.00	0.00	0.00	10830
288	0.00	0.00	0.00	0.00	10830
312	0.00	0.00	0.00	0.00	10830
336	0.00	0.00	0.00	0.00	10820
360	0.00	0.00	0.00	0.00	10820
384	0.00	0.00	0.00	0.00	10820
408	0.00	0.00	0.00	0.00	10850
432	0.1392	0.002	4.132	0.0057	10890
480	0.3133	0.00225	17.824	0.0557	10970
504	0.7223	0.01038	43.806	0.3163	11080
552	1.6216	0.0116	77.227	1.2518	11160
				1.6295	

Table 24. Methane production during fermentation of whey containing 5% lactose at 37° C by pH controlled batch fermentation.

Time (h)	Lactose (%)	gas-volume ($m^3 \times 10^{-2}$)	gas-rate ($m^3 \times 10^{-2}/h$)	Ch_4 (%)	CH_4-volume ($m^3 \times 10^{-2}$)	Alkalinity (mg/L)
0	5.142	0.00	0.00	0.00	0.00	1800
20	2.714	0.4612	0.0230	0.00	0.00	3650
30	0.00	1.0211	0.1021	0.00	0.00	4870
43	0.00	1.1749	0.0903	0.00	0.00	5980
50	0.00	0.5543	0.0792	0.00	0.00	7020
90	0.00	1.1321	0.0330	0.00	0.00	7990
100	0.00	0.3078	0.0307	0.00	0.00	8370
106	0.00	0.3560	0.0223	0.00	0.00	8810
120	0.00	0.2680	0.0191	0.00	0.00	9660
144	0.00	0.0760	0.0031	0.00	0.00	10480
168	0.00	0.0087	0.0003	0.00	0.00	11130
192	0.00	0.00	0.00	0.00	0.00	11480
210	0.00	0.00	0.00	0.00	0.00	11590
216	0.00	0.00	0.00	0.00	0.00	11630
222	0.00	0.00	0.00	0.00	0.00	11640
240	0.00	0.00	0.00	0.00	0.00	11640
264	0.00	0.00	0.00	0.00	0.00	11650
288	0.00	0.00	0.00	0.00	0.00	11660
312	0.00	0.00	0.00	0.00	0.00	11650
320	0.00	0.00	0.00	0.00	0.00	11650
336	0.00	0.00	0.00	0.00	0.00	11640
360	0.00	0.00	0.00	0.00	0.00	11650
384	0.00	0.00	0.00	0.00	0.00	11640
408	0.00	0.00	0.00	0.00	0.00	11640
432	0.00	0.00	0.00	0.00	0.00	11680
480	0.00	0.2773	0.0115	1.909	0.0052	11730
504	0.00	0.3246	0.0135	13.123	0.0425	11790
552	0.00	0.5921	0.0123	25.418	0.1504	11870
600	0.00	2.3411	0.0487	54.703	1.2805	12030

Table 25. Methane production during fermentation of whey containing 2.5% lactose at 60° C by pH controlled batch fermentation.

Time	Lactose	gas-volume	gas-rate	CH_4	CH_4 volume	Alkalinity
(h)	(%)	$(m^3 \times 10^{-2})$	$(m^3 \times 10^{-2}/h)$	(%)	$(m^3 \times 10^{-2})$	(mg/L)
0	2.509	0.00	0.00	0.00	0.00	1020
20	1.357	0.0943	0.0047	0.00	0.00	3340
30	0.00	0.4469	0.0446	0.00	0.00	4100
42	0.00	0.6638	0.0553	0.00	0.00	4920
50	0.00	0.4250	0.0531	0.00	0.00	5730
61	0.00	0.3245	0.0295	0.00	0.00	6150
90	0.00	0.9691	0.0334	0.00	0.00	6990
102	0.00	0.4049	0.0337	0.00	0.00	7120
120	0.00	0.2142	0.0119	0.00	0.00	7610
144	0.00	0.1097	0.0045	0.00	0.00	7880
168	0.00	0.0456	0.0019	0.00	0.00	8180
192	0.00	0.00	0.00	0.00	0.00	8240
216	0.00	0.00	0.00	0.00	0.00	8260
223	0.00	0.00	0.00	0.00	0.00	8270
240	0.00	0.00	0.00	0.00	0.00	8290
264	0.00	0.00	0.00	0.00	0.00	8300
288	0.00	0.00	0.00	0.00	0.00	8290
312	0.00	0.00	0.00	0.00	0.00	8300
836	0.00	0.00	0.00	0.00	0.00	8310
384	0.00	0.00	0.00	0.00	0.00	8340
408	0.00	0.0760	0.0031	3.202	0.0024	8390
432	0.00	0.2189	0.0091	13.039	0.0284	8480
480	0.00	0.3964	0.0082	27.928	0.1106	8610
504	0.00	0.2862	0.0119	52.950	0.1515	8780
552	0.00	0.6422	0.0133	55.545	0.3564	
					0.6494	

Table 26. Methane production during fermentation of whey containing
5% lactose whey at 60° C by pH controlled batch fermentation.

Time	Lactose	gas-volume	gas-rate	CH$_4$%	CH$_4$-volume	Alkalinity
(h)	(%)	(m^3 x 10^{-2})	(m^{3}x10^{-2}/h)	(%)	(m^{3}x10^{-2})	(mg/L)
0	5.110	0.00	0.00	0.00	0.00	1460
20	4.320	0.00	0.00	0.00	0.00	3860
30	3.65	0.0957	0.0031	0.00	0.00	4030
42	2.503	0.5062	0.0421	1.25	0.0002	5800
50	1.483	0.6467	0.0808	0.00	0.00	7300
61	0.917	0.5761	0.0523	0.00	0.00	9440
90	0.00	1.0320	0.0355	0.00	0.00	13100
102	0.00	0.5221	0.0435	0.00	0.00	14270
120	0.00	0.2814	0.0156	0.00	0.00	14540
144	0.00	0.1160	0.0048	0.00	0.00	14610
168	0.00	0.00	0.00	0.00	0.00	14630
192	0.00	0.00	0.00	0.00	0.00	14630
216	0.00	0.00	0.00	0.00	0.00	14620
240	0.00	0.00	0.00	0.00	0.00	14640
264	0.00	0.00	0.00	0.00	0.00	14630
288	0.00	0.00	0.00	0.00	0.00	14630
312	0.00	0.00	0.00	0.00	0.00	14630
336	0.00	0.00	0.00	0.00	0.00	14620
360	0.00	0.00	0.00	0.00	0.00	14620
384	0.00	0.1335	0.0055	0.9237	0.00122	14710
408	0.00	0.2114	0.0088	6.745	0.0141	14770
432	0.00	0.2149	0.0089	10.441	0.0234	14800
480	0.00	0.4597	0.0095	30.038	0.1379	15010
504	0.00	0.8729	0.0363	51.691	0.4512	15220
552	0.00	0.9807	0.0202	51.333	0.5034	15280
					1.1302	

Table 27. Methane production during fermentation of whey containing
5% lactose at 60° C by pH controlled batch fermentation.

Time	gas-volume	gas-rate	CH_4	CH_4-volume	Alkalinity
(h)	$(m^3 \times 10^{-2})$	$(m^3 \times 10^{-2}/h)$	(%)	$(m^3 \times 10^{-2})$	(mg/L)
0	0.00	0.00	0.00	0.00	1780
20	0.00	0.00	0.00	0.00	3220
30	0.1201	0.0120	0.00	0.00	3980
42	0.4051	0.0337	0.00	0.00	5600
50	0.5362	0.0670	0.00	0.00	6860
61	0.3945	0.0358	0.00	0.00	8870
90	0.7971	0.02748	0.00	0.00	12540
102	0.5053	0.0421	0.00	0.00	13750
120	0.3278	0.01821	0.00	0.00	13990
144	0.1798	0.0074	0.00	0.00	14040
168	0.0754	0.0031	0.00	0.00	14060
192	0.00	0.00	0.00	0.00	14060
216	0.00	0.00	0.00	0.00	14030
240	0.00	0.00	0.00	0.00	14050
264	0.00	0.00	0.00	0.00	14050
288	0.00	0.00	0.00	0.00	14050
312	0.00	0.00	0.00	0.00	14050
336	0.00	0.00	0.00	0.00	14050
360	0.00	0.00	0.00	0.00	14050
384	0.00	0.00	0.00	0.00	14100
408	0.084	0.0035	0.392	0.0003	14160
432	0.1421	0.0059	2.580	0.0036	14280
480	0.5018	0.0104	20.518	0.1028	14420
504	0.8035	0.0334	38.528	0.3093	14650
552	1.2329	0.0256	61.883	0.7629	14810
				1.1788	