CHAPTER 1
INTRODUCTION
History and Present Status of Anaerobic Digestion

In the past, anaerobic technologies emphasized energy production from animal manure and organic waste in combination with animal manure. Simple digesters run by owner-operators produced biogas for heat, light and electricity. Worldwide, results were mixed with as many failures as successes in attempts to economically produce energy. In recent years, the utilization of anaerobic digestion has been broadened as other benefits of anaerobic digestion have been realized. Today, energy production and recovery are still important, but recognition of anaerobic digestion as an inexpensive technology to stabilize organic waste, reduce biological oxygen demand, suspended solids with minimal sludge production, and reduce odor etc., are almost as important as energy production.

As the understanding of anaerobic digestion process control and process benefits has increased, new devices have evolved in design and operational technique. Improved devices have yielded benefits of higher economic value, and the evolution continues as anaerobic digestion process benefits are recognized (Lusk and Moser, 1996).

Anaerobic decomposition started in the primordial times. There is little of note in the historical record, other than stinky rivers and cesspools, to support the notion that there was any development of anaerobic digestion as a process for practical use. However, cesspools or digestive pools could be viewed as development. One can only guess that an episode of smoking in the outhouse led to the discovery of the energy-producing potential of anaerobic digestion. Occasional individuals probably proceeded to use biogas for heat and light.
The taming of the anaerobic digestion process began in the late 19th century and early 20th century prompted by the sanitation concerns of individuals and municipalities. The wily English were fueling street lamps in the 1890's. The 1900’s used anaerobic digestion in many parts of the world, mostly in anaerobic ponds. The development of microbiology as a science led to research by (Buswell and Hatfield, 1936) to identify anaerobic bacteria and the conditions that promoted methane production.

**Developments in Years (1920-1973)**

The benefits of heating and mixing waste in closed tanks to enhance and control anaerobic digestion were introduced in 1920's-1930's and waste stabilization became a goal, which led to a basic municipal sludge digester design that spread throughout the world. The basic design has changed today. Only occasional use was made of methane beyond digester heating, because coal and petroleum-based energy was readily available. While most of the world wasted biogas, China began using it for heat, light and cooking.

During World War II, petroleum shortages in Europe led to the rediscovery of biogas as a fuel and anaerobic digestion systems were developed in Europe for this purpose. However, this energy production goal was dropped after the end of the war with the advent of cheap, plentiful oil.

From the 1940's until the 1970's, anaerobic digestion was largely ignored in North America and Europe except as a sludge solids stabilization technique. Cheap energy and an engineering infatuation with aerobic systems limited further use of anaerobic digestion. The 1950s-70s saw the slow spread of small-scale
anaerobic digestion systems in India, Southeast Asia and China for sanitation and energy production purposes

Ancient history in terms of waste treatment ends at a time of 1960's industrial expansion, when discharge of high-strength organic wastes and water pollution became politically unacceptable. In the industrialized parts of the world, government programs and goals pushed rapid development of aerobic wastewater treatment plants, with cheap electricity driving the development of aerobic processes. Anaerobic digestion was only included in wastewater treatment plants for sludge digestion. Often, high-strength wastes were land applied or landfilled.

**Developments in Years (1973-1985)**

The period from 1973-1985 saw the rapid and worldwide development of simple anaerobic digestion systems for methane production as an energy source. The energy crisis of 1973 and subsequent higher energy costs increased the interest in anaerobic digestion to produce methane for fuel. India, China and Southeast Asia rapidly and massively expanded their anaerobic digestion unit-base to counter their increased energy costs. Millions of small digesters were constructed throughout the region. They were designed to use combinations of animal dung, human waste and kitchen refuse. Europe, North America and the Soviet Union responded to the crisis with some limited research in anaerobic digestion for methane production from animal manure. The US established several renewable energy programs, emphasizing anaerobic digestion of biomass for energy production.

The energy crisis of 1979 triggered another round of digester building aimed at energy production. China and India expanded the number of small family digesters and experimented with
community digesters to produce larger biogas volumes for village electrification and mobile fuels. The Philippines built hundreds of small digesters and many larger digesters on animal production facilities.

Unfortunately, the available knowledge on anaerobic digestion was not as widespread, and failures were more common than successes. China, India and Thailand reported 50% failure rates. The US had farm digester failure rates approaching 80%. Europe also experienced a high rate of farm digester failures. Soviet collective animal farms built substantial numbers of digesters that performed poorly. However, research and development of new anaerobic digester configurations for treatment of high-strength organic industrial waste showed some success. Pilot and full-scale industrial units were constructed.

**Developments in Years (1985-1996)**

Anaerobic digestion failures became pathfinders of successful systems and recognition of the non-energy benefits of anaerobic digestion. Those designs and equipment that did succeed yesterday are being recognized and new and improved anaerobic digestion technologies are being developed. Today, energy production and recovery are still important, but recognition of anaerobic digestion as an inexpensive technology to stabilize organic waste, reduce BOD and suspended solids with minimal sludge production, and reduce odor is almost as important as energy production. Recent goals of some anaerobic digestion systems include odor reduction and nutrient recovery. The types of waste treated with anaerobic digestion have expanded widely. Some anaerobic digestion systems have become more complex as industrial applications become more prevalent.
Around 1985, there was an epiphany in the anaerobic digestion world. China, Taiwan, India and Thailand formed government boards to investigate the reasons for success of digesters and to promote those successes. Research and training programs were developed and put into place to encourage small-scale energy and sanitation systems. Within China and India, there is a trend toward employing larger, more sophisticated farm-based systems with better process control that generate electricity (RAP Bulletin, 1995).

In Taiwan, the desire to reduce pollution of rivers by direct discharge from the animal production industry led to the development of a standard anaerobic digestion system now in use at over 5,000 farms. The Taiwan system utilizes ambient temperature tanks with inflatable rubber covers. Standard-size digesters are built in series until adequate capacity is achieved. Anaerobic digestion serves as pretreatment for aerobic systems. The goal is waste treatment and most biogas is flared.

Europe returned to the drawing board because energy prices were high and provided tempting targets. Also, European governmental actions to reduce agricultural and industrial pollution, and control municipal solid waste, landfill expansion raised costs for organic waste producers. Brazil started a program of energy self-sufficiency, including the promotion of anaerobic digestion systems. Waste treatment engineers, now familiar with anaerobic digestion's low operating costs and efficient reduction of BOD in high-strength waste, concentrated on developing high-rate anaerobic digestion processes.

In Europe, Germany led the way in small on-farm digesters for odor control. Italy developed a series of farm anaerobic digestion systems. European determination resulted in
construction of over 150 new anaerobic digestion plants between 1987 and 1995. Denmark and The Netherlands decided that small individual plants were not economically efficient and moved forward with large systems for groups of farms. Most experience with large centralized digestion facilities has been in Denmark, where 15 plants are now operating. More than 30 large, centralized digesters are operating in Europe, with another 30 under construction or being planned. Some of these facilities have been in operation for more than 10 years. The goal of the centralized plants is to provide waste management and to redistribute nutrients in odorless liquids/solids to farms (Danish Energy Agency Report, 1995).

The use of the anaerobic digestion process for treating industrial waste waters has grown tremendously during the past decade. Industry realized that pollution reduction from high-strength organics in industrial wastewater was very costly if done aerobically. The older model anaerobic digestion tanks could achieve 80-90% BOD reductions, but occupied valuable space. Systems such as up anaerobic sludge blanket, anaerobic filters and other systems that immobilize bacteria were developed to treat these waste flows on-site in smaller units.

As with farm-based digesters, the economic advantage of recovering and using biogas from industrial digesters becomes more attractive in larger facilities. Over 35 industries that use digesters have been identified, including processors of chemicals, fiber, food, meat, milk, and pharmaceuticals, among others. Many of these industries use anaerobic digestion as a pretreatment step that lowers sludge disposal costs, controls odors, and reduces the costs of final treatment onsite or at a municipal wastewater treatment facility. From the perspective of the municipal facility,
pretreatment effectively expands existing treatment capacity (NFFO/SRO Review, 1995).

**Anaerobic Digestion Tomorrow**

For tomorrow and the future, the driving forces for the use of anaerobic digestion to continue to drift away from energy production. Organic stabilization and pathogen reduction, in addition to energy production will be important reasons to use anaerobic digestion in developing countries. Energy savings in operation and minimal sludge production from anaerobic digestion versus aerobic treatment will become more important in energy and landfill deficient areas of Europe and Asia. The use of more complex anaerobic digestion processes for industrial waste treatment will increase. Anaerobic digestion can decompose some organic toxic and hazardous materials in co-digestion schemes and this potential will be realized. Odor control and recovery of nutrients for export to farms away from livestock concentrations will be other important functions of animal manure anaerobic digestion (Lusk et al., 1996).

A future application in many new developments is digestion of municipal solid waste (MSW). Biological treatment processes such as anaerobic digestion and composting offer the only route for recycling organic matter and nutrients from the putrifiable fraction of MSW. However, MSW composting technologies require energy inputs of 30-35 kWh per ton of MSW input, whereas anaerobic digestion is a net energy producing process that can generate from 100-150 kWh per ton of MSW input.

MSW digestion poses many technical problems. A number of anaerobic digestion systems have been developed for low-or high-solids content waste, operated at medium or high temperature. High solids allow a higher loading rate of organic
materials, while high temperatures allow shorter retention times. Higher temperatures also increase the destruction of pathogens present in MSW.

Co-products produced by anaerobic digestion of MSW include compost and liquid leachate that have value as soil conditioner and fertilizer. The amount and quality of these products depend entirely upon the quality of the MSW feedstock, the method of digestion, and the extent of any post-treatment refining processes. Many countries have developed standards for MSW co-product use. Any use of these co-products must strictly comply with consumer quality standards (IEA Bioenergy, 1996).

ANAEROBIC DIGESTION

Introduction

Anaerobic digestion is the process that occurs when various kinds of bacteria consume plant or animal material in an airtight container called a digester. Temperatures between 30°C and 60°C favor bacteria that release biogas (50 to 70 percent methane with most of the remainder as carbon dioxide) (Wolis, 1974). The bacteria may be present in the original material when charged (as is the case with cattle manure) or may be placed in the digester when it is initially charged. The gas has the heat value of its methane component, 500 to 700 Btu/sqft. and can be used directly as a heat fuel or in internal combustion engines. In some cases there is enough hydrogen sulfide (H2S) present to cause corrosion problems, particularly in engines. H2S can be removed by a simple, inexpensive, existing technology. CO2 can be removed by a somewhat more complex and expensive technology, which would need to be employed if the gas is to be fed into a natural gas pipeline.
The anaerobic digestion process is especially well adapted to slurry-type wastes and has environmental benefits in the form of treating wastes to reduce pollution hazards and to reduce odor nuisances. Furthermore, the residual from the process can be returned to land, either directly or through animal refeeding technologies, and thus retain nitrogen and organic levels of soil. Most other biomass energy conversion processes more nearly totally destroy the input material.

**Broad Aspects of Anaerobic Digestion**

The anaerobic digestion process involves a number of different bacteria. The digester performance depends on a large number of variables. Here the basic process is considered first and then the feedstocks and byproducts of the process.

**Basic Process of Anaerobic Digestion:**

Not all of the bacteria involved in anaerobic digestion have been cultivated and/or identified and the exact biochemical processes are not fully well understood. Basically the process consists of three steps (Clausen and Gaddy, 1977):

1. Decomposition and break down (hydrolysis) of the plant or animal matter to usable molecules such as sugars and aminoacids.
2. Conversion of the decomposed matter to organic acids, carbon dioxide and hydrogen.
3. Conversion of the organic acids to methane.

The rate at which the biogas forms is depend on the temperature (higher temperature usually gives a faster rate) and the nature of the substrate to be digested. Cellulosic materials, such as crop residues and municipal solid waste, produce biogas more slowly than sewage sludge and animal manure. Disturbances of the digester system due to changes in temperature, pH,
feedstock composition, toxins in feedstock etc., can lead to a buildup of acids that inhibit the methane-producing bacteria. Generally, anaerobic digestion systems work best when a constant temperature and a uniform feedstock are maintained. When a digester is started, the bacterial composition is seldom at the optimum. But if the feedstock and operating conditions are held constant a process of natural selection takes place until the bacteria best able to metabolize the feedstock (and thus grow) dominate. Biogas production begins within a day or so, but complete stabilization sometimes takes months.

Numerous sources for good anaerobic bacteria have been tried, though the process is basically one of hit and miss type. Future developments could produce superior genetic strains of bacteria, but too little is known about the process to judge if or when this can be accomplished. It is quite possible that if such strains are to be effective, the input material may first require pasteurization. Biogas yields vary considerably with feedstock and operating conditions. Operating a digester at high temperatures usually increases the rate at which the biogas is formed, but raising the temperature can actually decrease the net fuel yield, as more energy is required to heat the digester (Pfeffer, 1978). The optimum conditions for biogas yields have to be determined separately for each feedstock or combination of feedstocks.

**Bacteriology of Anaerobic Digestion:**

The anaerobic digestion process requires strict anaerobic conditions, it is a bacterial fermentation which involves at least four groups of bacteria which co-operate to provide complete breakdown of complex organic bio-polymers into CO\textsubscript{2} and CH\textsubscript{4}. The generation of energy for bacterial growth generally requires the
biochemical oxidation of substrates in the complete absence of air. This is normally achieved by complete removal of oxygen followed by utilization of surplus hydrogen. Anaerobic digestion is always dominated by need of suitable hydrogen acceptor. The methanogenic bacteria act as terminal hydrogen acceptor for the other bacteria and in doing so they provide a regulatory system for over all fermentation (Grainer and Lynch, 1984).

**Hydrogen Transfer Reactions in Anaerobic Bacteriology**

Bacteria that oxidize substrate to provide energy for growth accomplish oxidation by a series of dehydrogenase reactions that produce a stepwise transfer of H from the substrate to carrier molecule, usually nicotinamide adenine dinucleotide (NAD).

\[
\text{NAD}^+ + 2\text{H} \rightarrow \text{NADH} + \text{H}^+ \quad (1)
\]

Aerobic bacteria have a mechanism of reoxidizing NADH to \( \text{NAD}^+ \) using the cytochrome chain and dissolved \( \text{O}_2 \). Anaerobic bacteria have no such mechanism and the products of fermentation are decided by the availability of suitable acceptor molecule to absorb the surplus hydrogen generated by the energy yielding reactions.

**The Role of Acid Forming Bacteria**

The dominant acid forming bacterial digestion process use the Embeden-Meyerhof pathway and the preferred product is acetic acid e.g.

\[
\text{C}_6\text{H}_12\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2 \text{CO}_2 + 4\text{H}_2 \quad (2)
\]

This reaction provides the bacteria with their largest yield of energy (4mol ATP/mol glucose) but requires the simultaneous production of largest quantities of \( \text{H}_2 \) and is possible only in environments containing very small quantities of \( \text{H}_2 \) (< 1000 ppm in the gas phase). One response of these bacteria to large
concentration of H₂ or low pH values is the production of butyric acid instead of acetic acid, i.e.

\[ C_6H_{12}O_6 \rightarrow CH_3 CH_2CH_2COOH + 2 CO_2 + 4H_2 \]  

(3)

This reduces the production of H₂ and acid in the growth medium. Another response is the production of propionic acid, which consumes H₂, i.e.

\[ C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3 CH_2COOH + 2H_2O \]  

(4)

The conversion of glucose to propionic acid is used by the bacteria as a method of removing surplus H₂ from the system.

**The Role of H₂ – Utilizing Bacteria**

Hydrogen utilizing bacteria are the “H₂ -Scavengers” of the anaerobic digestion process. They normally control the redox potential of the fermentation by conversion of H₂ and CO₂ to CH₄

\[ 4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \]  

(5)

Carbon dioxide is normally present in excess and some of the surplus unreacted CO₂ dissolves in the growth medium to form bicarbonate ions, which provide the major constituents of the pH buffer. Hydrogen is the rate limiting substrate for these bacteria and is almost completely consumed but the traces that remain exert a strong regulatory effect on the metabolism of acid forming bacteria.

**The Role of Acetoclastic Methane Bacteria**

Acetoclastic methane bacteria are the most important and least studied bacteria in anaerobic digestion process. They are responsible for most of the CH₄ produced in the fermentation, which they form exclusively from acetate:

\[ CH_3COOH \rightarrow CH_4 + CO_2 \]  

(6)
They control the pH value of the growth medium by preventing an accumulation of acetic acid. Their slow growth rate (Minimum doubling time of 2-3 days) is often rate-limiting step in the fermentation.

**The Role of Acetogenic Bacteria**

Acetogenic bacteria ferment higher fatty acids, notably propionic and butyric acid, to produce acetic acid:

\[
\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2 \quad (7)
\]

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2 \quad (8)
\]

They do not compete well with other bacteria, as they are only able to grow in the presence of higher acids and in absence of H₂. The very small initial number of these bacteria is largely responsible for the long-term persistence of propionic acid in batch reactors.

**Feedstocks**

A wide range of plant and animal organic matter can be anaerobically digested. Both the gas yields and rates of digestion vary. Generally materials that are higher in lignin (e.g., wood and crop residues) are poor feedstocks because the lignin protects the cellulose from bacterial attack (McCarty et al., 1979). Pretreatment could increase their susceptibility to digestion. However, even then digestion energy efficiencies generally do not exceed 50 to 75 percent. Thus, more usable energy can generally be obtained through combustion or thermal gasification of these feedstocks.

The best feedstocks for anaerobic digestion usually are wet biomass such as fresh animal manure, various aquatic plants, and wet food-processing wastes such as those that occur in the cheese, potato, tomato, and fruit-processing industries.
**Byproducts**

The digester effluent contains bacteria as well as the undigested material of the feedstock (mostly lignocellulosic material) and the solubilized nutrients. The process has the potential for killing most disease causing bacteria, volatile losses of ammonia may increase with anaerobic digestion (Moore et al., 1980).

The most generally accepted technology for disposal of the effluent is to use it as a soil conditioner (low-grade fertilizer). Animal manure is already used widely for this but there is some controversy over whether the digester effluent is a better source of nitrogen than the undigested manure. The actual added value (if any) as a fertilizer, however, will have to be determined experimentally and is likely to be highly feedstock specific. The effluent may also be used as fertilizer for aquatic plant systems. In one case the effluent is dewatered and used as animal bedding in place of sawdust (John et al., 1980).

Another potential use of the effluent is as an animal feed. It has been claimed that the protein mix in the cake obtained from dewatering the effluent is superior to that of undigested manure (Hashimoto et al., 1978).

Although most of the disease-causing bacteria are killed by digestion of the manure, several questions about refeeding of digester effluents need to be resolved. Buildup of toxic materials, development of resistance to antibiotics by organisms in the cake, permissible quantities of cake in the diet, storage, and product quality are all issues that have been raised. There is no firm evidence that these will present significant problems, however. To avoid some of these problems, the Food and Drug Administration
has generally favored cross-species feeding, but has not sanctioned its use as a feed or feed ingredient (Abeles, 1977).

**Biomass Composition and Methane Production**

The relationship between the composition of the organic substrate being degraded and the amount of methane formed is given by:

\[
C_nH_{2a}O_b + \left( n - \frac{a}{2} - \frac{b}{2} \right) H_2 O \rightarrow \left( \frac{n}{2} - \frac{a}{8} + \frac{b}{4} \right) CO_2 + \left( \frac{n}{2} + \frac{a}{8} - \frac{b}{4} \right) CH_4
\]  

(9)

It is clear from equation (9) that organics that are high in oxygen like poly-acids and sugars produce less methane than organics that are low in oxygen such as fats. As a result, the parameter of total volatile solids (TVS), which is used extensively in waste water treatment, does not relate well to the amount of methane produced (Henze and Harreemoes, 1983). Moreover, the chemical oxygen demand (COD) value of a substance or mixture of substances relates stoichiometry to methane production.

The relation between COD and methane can be derived from the COD value of methane as determined by complete oxidation.

\[
CH_4 + 2O_2 \rightarrow CO_2 + 2H_2 O
\]  

(10)

In other words, 16.04 grams of methane requires 64.00 grams of oxygen for complete oxidation. Hence, at 273.15 K and 101.325 kPa (STP-standard temperature and pressure), 0.35 M³ methane is produced per kg COD removed. The same result is obtained when the breakdown of organic compounds such as glucose is studied:

\[
C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2
\]  

(11)
**Anaerobic Digesters**

The use of digester effluents as an animal feed, however, would greatly improve the economics of manure digestion. Consequently, the value, use, and restrictions on using digester effluents as animal feeds should be thoroughly investigated. Moreover, the animal feed value of effluents from the digestion of feedstocks other than manure should also be investigated.

There are numerous possible designs for anaerobic digesters, depending on the feedstock, the availability of cheap labor, and the purpose of the digestion. The most complex and expensive systems are for municipal sewage sludge digestion, but the primary purpose of these has been to stabilize the sludge and not to produce biogas. Digester processes have been classified into three types, depending on the operating temperature:

1. Psychrophilic (under 20°C),
2. Mesophilic (20 to 45°C),
3. Thermophilic (45 to 65.5°C).

The cost, complexity, and energy use of the systems increase with the temperature, as does the rate of gas production. The amount of gas produced per pound of feedstock, however, can either increase or decrease with temperature. Retention time is also an important consideration, wherein maximum gas production per pound of feedstock is sacrificed for reduced size and cost of the digester.

Anaerobic digesters in the mesophilic and thermophilic ranges have used agricultural wastes, residues, and grasses, to produce biogas. The optimum temperature appears to be both site and feedstock specific. There are still unresolved technical questions about the tradeoffs between mesophilic and thermophilic
digesters, but most on farm systems have been mesophilic. Other design parameters include continuous versus batch processes, mixed versus unmixed reactors, and other features. Some of the major types are summarized here.

### Table 1.1: Summary of Various Digester Designs and Their Applications

<table>
<thead>
<tr>
<th>Type of System</th>
<th>Application and Inputs</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landfill</td>
<td>Existing and planned landfills, municipal solid wastes, sewage sludge, warm climates.</td>
<td>Low cost, tanks not required, high loading rates possible, no moving parts.</td>
<td>Gas generation may last only 10 years, gas usage on site may present problems</td>
</tr>
<tr>
<td>Single tank plug flow</td>
<td>All types of organics, farm and feedlot operations,</td>
<td>Low cost, simple design can run, high solids waste, can have gravity feed and discharge.</td>
<td>Low solid waste may stratify.</td>
</tr>
<tr>
<td>Multi tank batch system</td>
<td>Can accept all types of waste, limited application crop residues, grasses, chicken broilers, turkeys</td>
<td>Simple, low maintenance, low cost, complete digestion of materials</td>
<td>Gas generation not continuous, labor intensive feed and discharge, low gas production per day</td>
</tr>
<tr>
<td>Single-tank complete mix</td>
<td>All types of organics sewage treatment, farm and feed lot, municipal solid waste</td>
<td>Proven reliability, works well on all types of wastes.</td>
<td>Greater input energy to run mixers, higher cost than plug flow.</td>
</tr>
<tr>
<td>Anaerobic contact</td>
<td>Sewage sludge and other organics, limited applications</td>
<td>Smaller tank sizes, operation not overly critical</td>
<td>Two tanks necessary</td>
</tr>
<tr>
<td>Two or Three phase</td>
<td>Cellulosic feed stocks</td>
<td>Allow s more complete decomposition, greater gas yields,</td>
<td>Feed rates vary with feed stocks, have not been attempted full</td>
</tr>
</tbody>
</table>
**Introduction**

greater loading rates, lower retention times.  
scale, require type controls and management of the operation

<table>
<thead>
<tr>
<th>Packed Bed</th>
<th>Dilute organics-sewage, food processing waste, very dilute animal wastes-industrial and commercial</th>
<th>High loading rates possible, short retention times.</th>
<th>Tends to clog with organic particles, limited to dilute wastes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expanded Bed</td>
<td>Dilute organics-sewage, food processing waste, very dilute animal wastes</td>
<td>High loading rates, low temperature digestion, high quality gas, short retention times.</td>
<td>Not developed, high energy input to operate pumps, no operating data.</td>
</tr>
<tr>
<td>Mixed Bed</td>
<td>Sewage sludge, food processing waste, animal wastes, fairly dilute mixtures</td>
<td>Fast throughput, times High loading rates, higher solids input than packed beds.</td>
<td>Tends to clog, high pumping energy input no operating data.</td>
</tr>
<tr>
<td>Variable Bed</td>
<td>All types of organics, farms and feed lots</td>
<td>Allows seasonal peaking of gas production, preserves nutrient value of material, low cost</td>
<td>Feed-discharge may require extra pump.</td>
</tr>
</tbody>
</table>

**Reactor Designs**

1) **Conventional Types of Reactors**

As an industrial process, anaerobic digestion came into its own as a result of the industrial revolution, when centralized treatment of sewage became a necessity. Aerobic treatment of sewage resulted in the production of a fairly concentrated sludge (1 to 5% organic solids) which had a pollution load equivalent from two-thirds to three-fourths of the original sewage. This sludge when left to itself, without exposure to air, would turn into a sour, foul and unhealthy liquid, which in some instances would start to
produce methane. Anaerobic digesters were therefore designed to accelerate and control the process, and to eliminate as far as possible, the odoriferous intermediate stage.

These digesters, as originally developed, are sealed tanks designed to eliminate oxygen and to trap the methane produced. The process was controlled by the rate of addition of raw sludge. By adding 1/50 to 1/100 of the digester volume each day it was found that methane production was maintained and that the liquid removed from the digester was quite innocuous, it smelled relatively odorless, black, contains considerably less pathogens than the raw sludge, and it is a good fertilizer. Further improvements in control were obtained by maintaining the temperature at about 35°C and provisions as much mixing were possible from a technical and economic point of view.

**Single-Tank Plug Flow**

This system is the simplest adaptation of Asian anaerobic digester technology. The feedstock is pumped or allowed to flow into one end of a digester tank and removed at the other. Biogas is drawn off from the top of the digester tank. The feed rate is chosen to maintain the proper residence time (the time the feedstock remains in the digester) in the digester and the feed or digester contents can be heated as needed (Jewell et al., 1979). Depending on the placement of the heating pipes, some convective mixing can also occur.

**Multi-Tank Batch System**

This system consists of a series of tanks or chambers, which are filled sequentially with biomass and sealed. As each unit completes the digestion process, it is emptied and recharged. This
type of reactor is best suited to operations where the feedstock arrives in batches, for example, grass or crop residues that are collected only at certain times of the year or turkey or broiler operations that are cleaned only when the flocks are changed. This digester system, however, is relatively labor intensive.

**Single-Tank Complete Mix**

The single-tank complete mix system has a single rigid digester tank, which is heated and mixed several times a day. It has been argued that mixing enhances the contact of bacteria with the feedstock and inhibits scum formation, which can interfere with digester operation. Theoretical calculations, however, indicate that the mixing does not improve bacterial contact, and these calculations have been confirmed experimentally in one case. Single-tank complete-mixed digesters are used to treat municipal sewage sludge and have been used in the larger anaerobic digester systems (exclusive of landfills) (Augenstein, 1978).

**ii) Advanced Types of Reactors**

The slow growth of the microorganisms involved in the production of methane is the main reason why biomass retention in advanced reactors is so important for performance. Methanogenic bacteria may have a mass doubling time of over 80 days in anaerobic reactors, and this makes it very difficult to obtain fast acting reactors without retaining most of the biomass normally washed out with the effluent. Also, a high biomass content is essential for operation at very dilute waste strengths and to obtain a reasonably high conversion efficiency (Jewell, 1982). Most of the methane formed, originates from acetic acid, and the methanogens responsible for this conversion are especially slow-
growing. Fortunately, they attach themselves readily to surfaces or to other bacteria, and this characteristic has made it possible to develop the advanced reactors known today.

It is likely that some anaerobic reactors unintentionally, become retained biomass reactors. Intermittently or poorly stirred reactors for example, could readily retain flocculent biomass. In fact, the upflow sludge bed reactor appears to have existed this way before being recognized as such. Horizontal plugflow reactors may also become retained biomass reactors because the biomass tends to stay in the bottom of the reactor.

Retained biomass reactors for anaerobic waste treatments were first systematically studied with the development of the anaerobic contact process in the 1950s. This process was followed by the development of the anaerobic filter. The other processes were developed in the 1970s.

**Anaerobic Contact Reactor**

This was the first retained biomass reactor to be studied and developed systematically (Schroepfer et al., 1955). The principle involved is the same as in the activated sludge process: the settling of microbiological floc and other suspended solids and contacting the raw waste with the anaerobic sludge. The single-tank complete-mix system effluent can be transferred to a second unmixed and unheated storage tank. Here the biomass undergoes further digestion and solids settle out. The reactor's performance depends markedly therefore, on the efficiency with which the microorganisms and suspended solids settle. An additional factor is the degree to which sludge and raw wastes are mixed in the reactor. Good mixing is required, yet it is essential that the settling characteristics are not adversely affected (van den
Berg and Lentz, 1977). These systems have been used extensively in sewage treatment and may receive wide application where preservation of the effluents nutrient value requires covered lagoons or in short throughput systems. The anaerobic contact process has been applied commercially in several countries including the U.S.A., Sweden, France and Canada (Hall, 1983).

Two or Three Phase

As mentioned previously under “Broad Aspects of Anaerobic Digestion,” the basic process consists of a series of biochemical steps involving different bacteria. The idea behind the multi-tank systems is to have a series of digester tanks each of that is separately optimized for one of the successive digestion steps (Ghosh and Klass, 1977). The motivation behind such system is the hypothesis that they: 1) can accept higher feed-stock concentrations without inhibiting the reactions in successive stages, 2) have greater process stability, 3) produce higher methane concentrations in the biogas, and 4) require lower retention times in the digester than with most single-phase digesters. The majority of the work on this approach has been on municipal sewage sludge, although the Institute for Gas Technology hopes to eventually transfer the technique to kelp digestion. The need for uniform feed rates and controls may limit the use of two or multi-phased systems to larger or extremely well managed operations; but this type of reactor should be carefully examined for other anaerobic digestion applications because of its potentially high efficiencies.
Anaerobic Filter Reactor

This reactor was developed by Young and McCarthy (1967), and resembles an upflow trickling filter. Waste enters at the bottom and flows upwards through the packing composed of rocks or plastic media, with biomass collecting in void spaces and on surfaces. Most of the biomass is present in a suspended form in the void spaces with a relatively small portion attached to the surface (van den Berg and Lentz, 1979). Since the suspended growth tends to collect at the bottom of the reactor, that is where most of the activity is centered. The growth on the surfaces of the packing provides a polishing action. The packing itself serves to separate the gas and to provide quiescent areas for the settling of suspended growth. Since the reactor is largely a suspended growth reactor, the liquid distribution system at the bottom of the reactor is critical in obtaining optimum performance (Dahab and Young, 1982). The process is particularly suitable for dilute soluble wastes. The process has been applied widely in the Netherlands, Belgium, the U.S.A. and Cuba (Lettinga et al., 1983).

Anaerobic Fluidized and Expanded Bed Reactors

These reactors are similar to suspended growth reactors in that the active biomass is present in the form of a bed of aggregates prone to settle readily. These aggregates are obtained by having the biomass grown on small, inert particles such as fine sand or alumina. A rapid and even flow of liquid is used to keep the particles in suspension. The rate of liquid flow and the resulting degree of expansion of the bed (10-25%) determine whether the reactor is called a fluidized or an expanded (less expansion) bed reactor. Reactor performance depends greatly on the evenness of the flow of the liquid and therefore, the system of
liquid distribution is very critical (Jewell, 1982). The process has been found to be quite stable with high organic inputs, short residence times in the digester, and relatively low temperatures (10 to 21°C) (Genung and Scott, 1979). The study did indicate, however, that the process would not be a net energy producer due to the energy required to expand the bed.

**Variable Feed**

The idea behind variable feed system is to store undigested manure in times of low gas demand for use during periods of high demands. The key is to be able to store the manure for long periods (e.g. 6 months) without excessive deterioration. The effect of long-term storage is being investigated (Jewell, 1978), but the systems may be limited to areas with cool summers or to operations in which the gas is used to generate electricity for export during the peak electric demand periods in summer.

**Downflow Stationary Fixed Film Reactor**

This reactor was also developed from the anaerobic filter to avoid plugging problems. In this case the packing was left in, and the suspended growth in the reactor was removed by operating the reactor in the downflow mode. Suspended growth is thereby removed with the effluent and so are indigestible waste suspended solids. The need for an elaborate distribution system is also eliminated because waste entering at the top of the reactor is readily dispersed by the gas escaping from the packing. The important factor in this reactor is the formation and stability of an active biomass film on the surface provided (Murray and van den Berg, 1981). To avoid the accumulation of non-active suspended material in the packing, the architecture of the packing must be
chosen correctly. The downflow stationary fixed film (DSFF) reactor is capable of handling a wide variety of wastes because of its configuration and the addition of the waste at the top of the reactor. Suspended solids, such as those present in some food processing wastes and in manures, are readily accommodated, although their degradation depends on the time they spend in the reactor in contact with active biomass.

Loading rates of the reactor are limited by the amount of active biomass that can be retained in the reactor. Effective film thickness is limited by diffusion and hence the amount of biomass is a function of the specific surface area available for film formation as well as the type of surface (its roughness). The specific surface area is limited to less than 100 M²/M³ because the channels in the packing have to be a minimum size to prevent them from filling up with film. The DSFF reactor is not suitable for the treatment of very dilute waste streams (e.g. less than 500 mg COD/l). To obtain reasonable high loading rates with dilute wastes, the hydraulic retention time has to be short resulting in low COD removals. Because the channels have a minimum dimension, the probability of contact between waste organics and film decreases with decreasing hydraulic retention time. The process has been applied commercially in Puerto Rico and in Canada (Hall, 1983).

**Hybrid or Combination Reactors**

Recently, a number of hybrid or combination reactors have been studied such as anaerobic contact expanded bed reactors (Martensson, and Frostell, 1983) and sludge bed/filter reactors (Guiot and van den Berg, 1985). These are designed to combine the strong points of both original reactors and to avoid some of the weak points. In the former, settling is greatly facilitated by adding
carrier particles around which bacteria flocculate. The latter is an attempt to make the sludge bed reactor more independent of the sludge quality (granulation). In both cases, the result is increased retention of active biomass.

The increased retention of biomass results in increased maximum COD loading rates and increased COD removal rates. These reactors are also expected to be more stable against adverse conditions (overloading) because of the increased biomass retention, and because of the more efficient way of retaining biomass when changed to a disperse form under such adverse conditions.

**Methanogenesis**

The fermentative microorganisms are capable of converting biomass to methane (CH$_4$), or to molecular hydrogen, which has been proposed as a gaseous fuel for large-scale use. The process that yields methane is called methane fermentation, or anaerobic digestion. It takes place in the absence of oxygen, and the microorganisms that perform the process are mixed populations of anaerobic bacteria. Methane fermentation occurs naturally in many ecosystems such as river mud, lake sediments, sewage, marshes, and swamps. It is most conspicuous where plants die and decompose underwater. The water layer acts as a blanket to exclude oxygen and promote the growth of many different anaerobes. Methane fermentation also occurs in the digestive tracts of ruminants. The rumen is supplied with ample quantities of food, is well buffered, has a nearly neutral pH, and is almost free of oxygen.

The basic method of generating hydrogen using microorganisms is well known. One is fermentation with certain
intermediate pyruvic acid is converted to hydrogen and other products. Some organisms use fermentation intermediates from biomass as hydrogen donors.

Methane fermentation is used worldwide, either alone or in combination with other processes, for the stabilization and disposal of waste such as domestic, municipal, agricultural, and industrial. During digestion, the amount of organic material, its biological oxygen demand (BOD), and the pathogenic organisms present in the waste are reduced. Many virgin biomass species can also be gasified in the same manner. The gas produced by anaerobic digestion of biomass (biogas) is basically a two-component gas composed of methane and carbon dioxide, although minor amounts of other gases such as hydrogen sulfide and hydrogen may be present. An anaerobic digester (fermenter) operating in a stable mode yields biogas that has a methane content on a dry basis ranging from about 40 to 75 mol %, depending on the operating conditions and a higher heating value of 15.7 to 29.5 MJ/ml(n). Dry natural gas and pure methane have higher heating values of about 39.3 MJ/M³(n) (1000 Btu/SCF). Thus, biogas is a medium-energy gas. Because of these characteristics, biogas obtained by anaerobic digestion of animal manure and human wastes has been used as a fuel for cooking, heating, and lighting for decades in many developing countries. In urban communities, the anaerobic digestion process is often used, frequently in combination with the activated sludge process, to treat municipal sewage (biosolids). Anaerobic digestion is also used for the stabilization and volume reduction of municipal solid waste (MSW) and in industry for the treatment of wastes from meat packing plants, breweries, canneries, and other food processing
plants. One of the oldest applications of anaerobic digestion is the stabilization of human wastes in septic tanks.

Wastewater treatment plants in urban communities where municipal biosolids are treated by anaerobic digestion frequently use biogas on-site. Biogas combustion for heat, steam, and electric power generation at municipal waste-water treatment plants is almost universal in many countries. Sanitary landfills are the equivalent of large-scale, batch digesters for MSW and emit biogas (landfill gas or LFG). Sanitary landfills are used worldwide to dispose of solid wastes.

Anaerobic digestion has been used for over 100 years for waste biomass treatment and disposal and as a source of fuel gas. The effort to apply the process to virgin biomass grown specifically for microbial conversion to pipeline-quality gas (substitute natural gas, SNG) is a relatively recent development that started in the early 1970s (Klass, 1974).

Methane fermentation is a multistage process. The complex polymers and compounds in biomass are degraded to lower molecular weight intermediates which are converted to methane and carbon dioxide. It involves complicated microbiological transformations, it is useful in explaining some of the characteristics of anaerobic digestion such as the effect of pH and acid buildup. The process can be maintained on a large scale for an indefinite period as long as the important fermentation parameters are kept within an acceptable range and fermentable material is available. Almost any kind of biomass feedstock mixture is suitable, with the possible exception of the lignin and keratin, which have low biodegradabilities.
Methane Fermentation

Early Work on Microbial Methane

Natural gas wells were known in Asia as early as 615. The Chinese reported the transport of natural gas through bamboo tubes for lighting in 900. In 1691, the English researcher Robert Boyle reported that a combustible gas is produced when coal is heated. In 1775, General George Washington described a gas well in West Virginia adjacent to a tract of land granted to him and General Andrew Lewis as a "burning spring." In 1806, the first gas mains laid in a public street were constructed in London. In 1819, the first gas company was formed in France to light the city of Paris.

The biological origin of methane, however, was recognized by Van Helmont, Volta, and Davy long before Berthollet's studies (McCarty, 1982). In 1630, Van Helmont found that flammable gases can be emitted from decaying organic matter. Volta observed in 1776 that there is a direct correlation between the amount of flammable gas emitted and the amount of decaying matter. In 1808, Davy found that during the anaerobic digestion of cattle manure, methane is present in the gas. Direct experimental evidence of the origin of biogas was reported in 1875 (Popoff, 1875). Popoff was able to account for the microbial decomposition of cellulose by the formation of methane. In 1886 and 1887, Hoppe-Seyler found that microorganisms in river mud cause the formation of methane from cellulose and the salts of fatty acids (Hoppe-Seyler, 1886; Hoppe-Seyler, 1889). About 20 years later, Omelianski reported that the decomposition of cellulose and the simultaneous formation of methane are caused by bacteria (Omelianski, 1904).
Introduction

The early work of these investigators established that methane can have a biological origin. Sohngen later substantiated Hoppe-Seyter's observations that fatty acids can yield methane and showed that under certain conditions, hydrogen and carbon dioxide combine in molar ratios of 4:1 to form methane and water (Sohngen, 1906; Sohngen, 1910). After these observations, many researchers studied the microbial formation of methane in relation to such applications as biosolids treatment and the utilization of animal wastes. The position of methane in the carbon cycle was largely determined by observation and analysis of material from river mud and soils. Bacteria that produce methane in an anaerobic environment, from a microbial standpoint, are analogous to those that produce carbon dioxide from methane in an aerobic environment.

Biogas was recognized as a useful fuel gas from this early work. In 1896, biosolids digestion supplied fuel for street lamps in England. In 1897, a waste-disposal tank serving a leper colony in Bombay, India, was equipped with gas collectors and the biogas was used to drive gas engines. In 1925, biogas was found to be satisfactory for general municipal use and was distributed through city mains in Essen, Germany. Millions of low-cost digesters have been operated for many years in China and India on farms and in cooperative village systems to generate biogas from animal manure and human wastes for local use. Anaerobic digestion is a valuable tool for waste biomass treatment and disposal, and biogas is a valuable, renewable fuel that can be recovered and used.

Microbiology of Methanogenic Bacteria

Methanogenic bacteria are unicellular, Gram-variable, strict anaerobes that do not form endospores. Their morphology,
structure, and biochemical makeup are quite diverse. More than ten different genera have been described (Zeikus, 1977; Zeikus et al., 1985). All genera have been assigned to the kingdom Archaebacteria, which comprises a group of bacteria typically found in unusual environments, and is distinguished from the rest of the prokaryotes by several criteria, including the number of ribosomal proteins, the lack of muramic acid in the cell walls, membrane lipids that contain isoprenoid side chains bound by ether linkages instead of ester-linked hydrocarbons, and the absence of ribothymine in transfer ribonucleic acid (tRNA). The methanogens have been divided into three groups based on the fingerprinting of their 16S ribosomal RNA (rRNA) and the substrates used for growth and methanogenesis (Woese and Fox, 1977; Balch et al., 1979). The methanogens were found to be unexpectedly divergent from other bacteria.

A revised taxonomic order was developed based on this work. Group I contains the genera Methanobacterium and Methanobrevibacter, Group II contains the genus Methanococcus; and Group III contains several genera, including Methanomicrobium, Methanogenium, Methanospirillum, and Methanosarcina. Species classified as Methanobacterium are generally rod-shaped organisms that are sometimes curved and that vary in size and arrangement of the cells; the cells may or may not be motile. Species classified in the genus Methanococcus are small spherical organisms whose cells occur singly or in irregular masses; some are motile. Methanogens in Group III having large spherical cells that occur in packets and are non-motile have been classified in the genus Methanosarcina. The long, helical, rod-shaped methanogens with polar flagella have been classified in the genus Methanospirillum and are also in Group III. The analysis of
the 16S rRNA allowed recognition of the archaebacteria as a distinctive group of bacteria that includes the methanogens as well as the halophiles and thermoacidophiles.

Methanogenic bacteria have not been studied as extensively as most other groups of bacteria. Until 1936, all attempts to isolate pure cultures or even to grow colonies on solid media were unsuccessful (Barker, 1936). Taxonomic classification was difficult because mixed cultures were employed in much of the early work. Essentially all of the early work and many recent studies have been carried out with enrichment cultures in which substrates and environmental conditions are chosen to selectively promote the growth of certain microbial species (Klass, 1984). By enrichment culture techniques, it is possible to obtain considerable information and data about the morphology of methanogens that have been identified, the conditions that favor their development, and the types of substrates utilized. Other methanogens have been isolated, but remain to be described in more detail before their taxonomic assignment is established. All species that have been studied in pure culture are strictly anaerobic and grow only in the absence of oxygen and in the presence of a suitable reducing agent. Methanogens are much more sensitive to oxygen than most other anaerobes. For this reason, it is much easier to grow methanogenic bacteria in liquid or semisolid media than on the surface of an agar plate. Even in liquid media not fully protected from air, sufficient oxygen may leak into the system to inhibit growth. A roll-tube method (Hungate technique) has been shown to be the most successful method for isolating pure cultures of methanogens (Zeikus, 1977).

Several species have been isolated, studied in pure culture, and taxonomically identified and classified. Some of the notable
species are *Methanobacterium formicicum*, *M. bryantii*, *M. thermoautotrophicum*, *Methanobrevibacter ruminantium*, *M. arborophilus*, *M. Smith*; *Methanococcus vannielii*, *M. voltae*, *Methanomicrobium mobile*; *Methanogenium catiaci*, *M. marisnigri*, *Methanospirillum hungatei* and *Methanosarcina barkeri* (Zeikus, 1977; Zeikus et al., 1985; Balch et al., 1979; Macario, 1982;). Most, if not all, methanogens can use hydrogen and carbon dioxide for methanogenesis and growth. Hydrogen is the electron donor and carbon dioxide is the electron acceptor that is reduced to methane. Thus, most of methanogens are facultative autotrophs. In addition, some species can use formate for growth and methane production (e.g., *M. vannielii*); others can use methanol, methylamines, or acetate (e.g., *M. barkeri*). Pure cultures generally grow well in media containing the usual mineral nutrients needed for growth of microorganisms, a reducing agent, and ammonium ion as the nitrogen source. The addition of extracts containing amino acids, growth factors, and other nutritional supplements to synthetic media may not have a beneficial effect, although some species of methanogenic bacteria require complex media for growth (*M. mobile*, *M. voltae*, *M. ruminantium*, and *M. smithii*). Several species need large amounts of carbon dioxide because it is used as the primary carbon source. Generally, growth is best in the pH range 6.4 to 7.4. Inhibition may occur at higher pH. But there are exceptions, *M. vannielii* grows best between pH 7 and 9. Despite their diverse morphology, which consists of many different cell shapes and structures, all methanogenic bacteria are unique in that all use simple substrates for energy and growth and all are specialized in their ability to produce methane as a major end product. A few microorganisms that are not classified as methanogens can be
induced to produce methane under certain conditions. *Clostridium perfringens*, which normally does not produce methane, can be induced to do so in a peptone-formate medium by addition of a small amount of iodine (Laigret, 1945).

Even though only a few species of methanogenic bacteria are believed to be capable of utilizing acetate as a substrate (McInerney and Bryant, 1981), about 70% of the methane formed in anaerobic biosolids digesters and from lake sediments is derived from the methyl group of acetate (Stadtman and Barker, 1951; Jerris and McCarty, 1965; Smith and Mah, 1965; Cappenberg and Prins, 1974). The carboxyl group yields carbon dioxide. Because of the large number of anaerobes in these systems as well as in other methane fermentation systems, it is probable that there are many yet-to-be identified methanogens that utilize acetate. The relatively simple compounds that serve as carbon and energy sources for methanogenic bacteria are clearly limited, and each methanogenic species is characteristically limited to the use of a few compounds. These compounds are generally supplied as intermediate fermentation products by other anaerobes present in methane fermentation systems. Indeed, it is apparent that several species of fermentative, acetogenic, and methanogenic bacteria are necessary to anaerobically digest the complex substrates in waste and virgin biomass. Mixed cultures are required for complete fermentation. Methane fermentation under sterile condition is not possible or not desirable in many systems.

**Biochemistry of Methane Formation**

Some of the early studies of the biochemical mechanisms of methane formation by methanogens were carried out with mixed
cultures and a single substrate. It was observed that some of the pure substrates can sometimes be converted almost quantitatively to methane and carbon dioxide. The yields of new cellular biomass during methanogenesis are small. The stoichiometries of several of the observed reactions are as follows:

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2 \\
4\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} & \rightarrow 7\text{CH}_4 + 5\text{CO}_2 \\
2\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} & \rightarrow 5\text{CH}_4 + 3\text{CO}_2 \\
2\text{CH}_3\text{CH}_2\text{OOH} & \rightarrow 3\text{CH}_4 + \text{CO}_2 \\
\text{CH}_3\text{COCH}_3 + \text{H}_2\text{O} & \rightarrow 2\text{CH}_4 + \text{CO}_2 
\end{align*}
\]

These equations indicate that the fermentation of acetic acid, propionic acid, butyric acid, ethanol, and acetone all yield the same products, but the ratio of methane to carbon dioxide changes with the oxidation state of the substrate. It is remarkable that the products are independent of substrate structure.

Indirect evidence of the mechanism of methane formation was reported in the early part of the twentieth century, and in the 1930s. (Sohngen, 1910; Stephenson and Strickland, 1931, 33; Fischer et al., 1931, 32) Sohngen found that enrichment cultures can couple the oxidation of hydrogen with the reduction of carbon dioxide according to:

\[
4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (17)
\]

These observations were later confirmed with pure cultures. It was found that methane is formed by the reduction of carbon dioxide by hydrogen supplied by the various substrates utilized by the bacteria or, in the case of *Methanobacillus omelianskii*, by uncombined hydrogen itself (Barker, 1936, 40, 41, 43).
Much of the early work done on the biochemistry of methane formation supported the position that methane is formed almost exclusively by reduction of carbon dioxide. However, it was shown with methanol and a species of *Methanosarcina* that less than 1% of the methane is derived from carbon dioxide (Schnellen, 1947; Stadtman and Barker, 1949). According to the mechanism proposed by van Neil for catabolism of acetic acid, all of the acid should be oxidized to carbon dioxide, half of which should be reduced to methane (Barker 1936).

\[
\text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 4\text{H}_2 \quad (18)
\]
\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (19)
\]
Net: \[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \quad (20)
\]

This mechanism was tested by use of $^{14}$C-labeled carbon dioxide (Barker, 1943; Buswell and Sollo, 1948; Stadtman and Barker, 1949, 51; Pine and Barker, 1956; Baresi et al., 1978).

Essentially none of the methane was found to be derived from carbon dioxide. Methane is derived entirely from the methyl carbon atoms and carbon dioxide is derived exclusively from carboxyl carbon atoms. Van Neil's mechanism is clearly not valid because the methyl carbon atom is not oxidized to carbon dioxide. Other work has been done to ascertain whether hydrogen atoms are removed during the fermentation of acetic acid, and whether the methyl group is incorporated intact into methane (Pine and Barker, 1954). Water and heavy water were used with deuterated and non-deuterated acetic acid. Acetic acid labeled in the methyl group, when used as the substrate, showed that the isotopic content of acetic acid and methane are the same. Unlabeled acetic acid fermented in the presence of heavy water indicated that about one atom of deuterium per molecule of methane formed is derived
from heavy water. It was concluded that the methyl group is transferred from acetic acid to methane as a unit without the loss of attached hydrogen or deuterium atoms.

The fermentation of butyric acid by *Methanobacterium suboxydans* is represented by (Stadtman and Barker, 1951)

$$2\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} + \text{CO}_2 \rightarrow 4\text{CH}_3\text{COOH} + \text{CH}_4$$  \hspace{1cm} (21)

The oxidation of 2 mole butyric acid to 4 mole acetic acid is coupled with the reduction of 1 mole of carbon dioxide to methane. Tracer experiments showed that 98% of the methane is derived from carbon dioxide. In these examples of methane fermentation involving carbon dioxide reduction, no carbon dioxide is formed in the oxidation of the substrate. The fermentation of propionic acid by *M. propionicum* is more complicated because it involves both carbon dioxide formation and consumption (Stadtman and Barker, 1951):

$$4\text{CH}_3\text{CH}_2\text{COOH} + 8\text{H}_2\text{O} \rightarrow 4\text{CH}_3\text{COOH} + 4\text{CO}_2 + 12\text{H}_2$$ \hspace{1cm} (22)

$$3\text{CO}_2 + 12\text{H}_2 \rightarrow 3\text{CH}_4 + 3\text{CH}_4 + 6\text{H}_2\text{O}$$ \hspace{1cm} (23)

Net: $$4\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 4\text{CH}_3\text{COOH} + 3\text{CH}_4 + \text{CO}_2$$ \hspace{1cm} (24)

Tracer experiments with $^{14}$C-labeled carbon dioxide or propionic acid indicate that approximately 1 mole of carbon dioxide is formed per mole of propionic acid consumed and that carbon dioxide is the precursor of most of the methane. Tracer experiments were also conducted with propionic acid using enrichment cultures (Buswell et al., 1951). These cultures use carbon dioxide and apparently convert all three carbon atoms of propionic acid to both methane and carbon dioxide in varying amounts.
The simplest biochemical mechanism proposed for the conversion of carbon dioxide to methane is a sequential reduction involving formic acid, formaldehyde, and methanol as intermediates. But it has been found that several methanogens cannot use these postulated intermediates as substitutes for carbon dioxide when hydrogen is used as the reductant (Kluyver and Schnellen, 1947). Subsequent experiments with cell-free extracts of methanogens, however, established that methane can be formed from individual one-carbon compounds such as CO₂, CH₃OH, HCHO, and HCOOH. For the three-carbon compounds pyruvic acid (CH₃COCOOH) and serine (CH₂OHCHNH₂COOH), the carboxyl carbon atom of pyruvic acid and the hydroxylated carbon atom of serine are converted to methane.

The experimental product distributions and selectivity suggest that methanogenesis is highly efficient for production of methane and carbon dioxide. Ignoring the small amount of substrate that is used to produce new cells and to provide cellular maintenance energy, the gross stoichiometry of the methane fermentation of glucose can be represented by

\[ \text{C}_6\text{H}_{12}\text{O}_6 \text{(aq)} \rightarrow 3\text{CH}_4 \text{(g)} + 3\text{CO}_2 \text{(g)} \quad (25) \]

The standard Gibbs free energy and enthalpy changes for this conversion under physiological conditions (pH 7, 25°C, unit activities) per mole of glucose fermented are about -418 and -131 kJ, and the mass and energy contents of the methane expressed as fractions of the glucose converted are about 27 and 95%. Thus, the thermodynamic driving force is large; the exothermic energy loss is small; the energy in the glucose is transferred at a higher energy density to a simple gaseous hydrocarbon; methane is easily separated from the aqueous system, and if desired, from the co-product carbon dioxide; methane and carbon dioxide selectivities
are high; and the mass of substrate is significantly reduced, which is important if a waste is disposed of or stabilized by anaerobic digestion.

**Fermentative and Acetogenic Bacteria in Methane Fermentation**

Because of the wide variety of complex substrates in biomass, many different bacterial species are necessary to facilitate degradation. The limited number of substrates catabolized by methanogens also requires that other types of organisms be present to implement the overall process. It is apparent that mixed cultures are necessary to convert complex substrates to methane and carbon dioxide. Methane fermentation is a three-stage and possibly a four-stage process that involves, in addition to methanogenic bacteria in the last stage, at least two other groups of organisms that implement the initial stages (McInerney and Bryant, 1981). In the first stage, fermentative bacteria convert the complex polysaccharides, proteins, and lipids in biomass to lower molecular weight fragments and acetate, carbon dioxide, and hydrogen. Another group of bacteria, the obligate, hydrogen-producing acetogenic bacteria, catabolize the longer-chain organic acids, alcohols, and possibly other degradation products formed in the first stage to yield additional acetate, carbon dioxide, and hydrogen. It is probable that some carbon dioxide and hydrogen are also converted to acetate by the acetogens. In the last stage, methanogenic bacteria convert intermediate acetate to methane and carbon dioxide by decarboxylation, and the intermediate carbon dioxide and hydrogen to additional methane. Thus, at least three groups of bacteria are necessary for methane fermentation to
proceed namely fermentative, acetogenic and methanogenic bacteria.

The fermentative bacteria found in operating methane fermentations supplied with complex substrates are usually obligate anaerobes in genera such as *Bacteroides*, *Bifidobacterium*, *Butyrovibrio*, *Eubacterium*, and *Lactobacillus*. Many are enteric bacteria, which include the coliform bacteria. The coliform bacteria, classically represented by the pathogen *Escherichia coli* in the genus *Escherichia* and pathogens in the genera *Salmonella* and *Shigella*, are probably the most common fermentative bacteria in methane fermentation because the feedstocks are often biosolids and animal wastes, or the mixed cultures used are derived from active methane fermentations grown on these wastes. The enteric bacteria also include those in the genera *Enterobacter*, *Serratia*, and *Proteus*, which occur primarily in soil and water, and plant pathogens of the genus *Erwina*.

The first step in the fermentation of complex substrates by fermentative bacteria is the hydrolysis of polysaccharides to oligosaccharides and monosaccharides, of proteins to peptides and amino acids, of triglycerides to fatty acids and glycerol, and of nucleic acids to heterocyclic nitrogen compounds, ribose, and inorganic phosphate. The sugars are degraded by the Embden-Meyerhof pathway, in the case of fermentative metabolism with enteric bacteria, to intermediate pyruvic acid, which is converted to acetate, fatty acids, carbon dioxide, and hydrogen. At low partial pressures of hydrogen, acetate is favored. At higher partial pressures, propionate, butyrate, ethanol, and lactate are favored, generally in that order (McInerney and Bryant, 1981). There is also a special mode of cleavage of intermediate pyruvic acid to formic
acid by enteric bacteria that is not found in other bacterial fermentations (Stanier et al., 1986). Some of these bacteria possess the enzyme systems that cleave formic acid by

\[ \text{CH}_3\text{COCOO} + \text{CoA} \rightarrow \text{CH}_3\text{COCoA} + \text{HCO}_2^- \quad (26) \]

\[ \text{HCO}_2^- + \text{H}^+ \rightarrow \text{CO}_2 + \text{H}_2 \quad (27) \]

members of the genera *Escherichia* and *Enterobacter* contain this enzyme system while those in the genera *Serratia* and *Shigelia* and *Salmonella typhi* do not. The amino acids and glycerol are also degraded by the glycolysis pathway to the same products and by other routes. After hydrolysis and glycolysis, some of the fermentation products are suitable substrates for methanogens, others are not.

Further degradation of unsuitable substrates is caused by another group of anaerobes, collectively called acetogenic bacteria. This group is known to exist on the basis of experimental data collected with several co-cultures containing one hydrogen-utilizing species such as a methanogen. The acetogens convert the alcohols and higher acids produced on glycolysis to acetate, carbon dioxide, and hydrogen. The isolation of "S" organism from *Methanobacterium omelianskii" is the first documented evidence of species of the acetogenic group. Originally, *M. omelianskii* was thought to be a methanogen that catabolized ethanol by (Barker, 1941)

\[ 2\text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow 2\text{CH}_3\text{COOH} + \text{CH}_4 \quad (28) \]

This stoichiometry represented the experimental data and indicated that acetic acid is derived from ethanol and methane is derived from carbon dioxide. To support this interpretation, unlabeled ethanol was incubated with $^{14}$C-labeled carbon dioxide; the $^{14}$C content of methane was approximately equal to that of the
carbon dioxide at the end of the fermentation (Stadtman and Barker, 1949). Later, this result was shown to be caused by the syntrophic association of two strict anaerobes, the unidentified S organism, which converts ethanol to acetate and hydrogen, and a methanogen, which uses the hydrogen to reduce carbon dioxide to methane (Bryant et al., 1967). Neither bacterium alone can grow on ethanol or carbon dioxide, and the growth of S organism is inhibited by the accumulation of hydrogen. Thus, the two organisms have a true symbiotic relationship and are maintained as a mixed culture. The biochemical reactions are

\[
2\text{CH}_3\text{CH}_2\text{OH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 4\text{H}_2 \quad (29)
\]

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (30)
\]

It has been established that propionate and the longer fatty acids are catabolized by similar syntrophic associations (Boone and Bryant, 1980).

**Biochemical Pathways to Methane**

From a biochemical standpoint, methanogens contain cofactors not found in other bacteria, including the carriers of the carbon dioxide during its reduction to methane-methanopterin, methanofuran, and CoM (Stanier et al., 1986). Other factors are believed to function as hydrogen carriers during these reductions. The oxidation of hydrogen is presumed to occur on the outside of the cytoplasmic membrane, while carbon dioxide (or bicarbonate) reduction occurs inside the cell. The net result is the equivalent of transporting two protons out of the cell for each hydrogen oxidized. Because so many different bacteria are involved in methanogenesis and the fact that most of the methane is derived from acetate and
not carbon dioxide, multiple pathways are undoubtedly involved, even for carbon dioxide reduction.

The biochemical pathway by which carbon dioxide is incorporated as cellular carbon and the mechanism of coupling methanogenesis to ATP synthesis has not been established with certainty, although knowledge of this and related pathways is about to expand greatly. Classical electron transport pathways are probably not operative, but metabolic pathways have been proposed for methanogenic bacteria that synthesize methane during growth on single-carbon substrates and hydrogen (Zeikus et al., 1985). All methanogens seem to be universally capable of using hydrogen as an electron donor and carbon dioxide as an electron acceptor. Many are also capable of using sulfur, sulfate, and nitrate as electron acceptors. Most methane fermentation systems reduce sulfate and other sulfur compounds that may be present to hydrogen sulfide, which forms insoluble sulfides with heavy metals in the fermentation broth. Methanogenesis ceases when methanogens of Groups I and II are grown in the presence of sulfur, whereas those in Group III continue methanogenesis simultaneously with sulfur reduction (Stanier et al., 1986).

Several factors make the biochemical pathways for methane production from pure substrates difficult to elucidate. One is that some of the early work was carried out with mixed cultures, so the experimental data may be questionable. Another is that the observations made with one pure methanogen do not necessarily apply to another. The third is that complex substrates complicate matters further. Although the basic carbon flows to products in acetogenic and methanogenic bacteria are predictable, a better understanding of the exact biochemistry (enzymes, coenzymes, electron carriers, and their cellular localization) is needed even for
single-carbon substrates to test the various proposed models for carbon and electron flow and energy conservation during growth (Zeikus et al., 1985).

The catabolism of ethanol by acetogenic S organism to form acetate is inhibited by hydrogen and proceeds at good growth rates only when a hydrogen utilizer is present. This can be explained by use of the standard Gibbs free energy changes for the dominant reactions of the major groups of bacteria in methane fermentation (Table 1.1). Ethanol conversion to acetic acid by acetogens has a slightly positive free energy change, so coupling of this reaction with a methanogenic reaction that has a strongly negative free energy change is thermodynamically favorable. Other trends can also be perceived from the free energy changes. The thermodynamic driving force for a few of the acetogenic reactions in which the higher acids are converted to acetate are positive, whereas that for direct conversion of glucose to acetate is strongly negative. For fermentative bacteria, the free energy changes listed in the table are negative, but cellulose hydrolysis is the least favorable reaction.
TABLE 1.1: Estimated Gibbs Free Energy Changes of Selected Biological Reactions in Methane Fermentation under Physiological Conditions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fermentative Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>((C_6H_{10}O_5) + H_2O \rightarrow C_6H_{12}O_6)</td>
<td>-18</td>
</tr>
<tr>
<td>(C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12 H_2O)</td>
<td>-26</td>
</tr>
<tr>
<td>(C_6H_{12}O_6 \rightarrow 2CH_3COCOO^- + 2H^+ + 2H_2)</td>
<td>-112</td>
</tr>
<tr>
<td>(C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2COO^- + H^+ + 3CO_2 + 5H_2)</td>
<td>-192</td>
</tr>
<tr>
<td>(C_6H_{12}O_6 \rightarrow CH_2CH_2CH_2COO^- + H^+ + 2CO_2 + 2H_2)</td>
<td>-264</td>
</tr>
<tr>
<td><strong>Acetogenic Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>(CH_3CH_2COO^- + H^+ + 2H_2O \rightarrow CH_3COO^- + H^+ + CO_2 + 3H_2)</td>
<td>+72</td>
</tr>
<tr>
<td>(CH_3CH_2CH_2COO^- + H^+ + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2H_2)</td>
<td>+48</td>
</tr>
<tr>
<td>(CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2)</td>
<td>+10</td>
</tr>
<tr>
<td>COOH((CH_2)_2 CH(NH_2)COO^- + 3H_2O \rightarrow 2CH_3COO^- + HCO_3^- + H^+ + H_2 + NH_4^+)</td>
<td></td>
</tr>
<tr>
<td>(CH_3COOO^- + H_2O \rightarrow CH_3COO^- + CO_2 + H_2)</td>
<td>-34</td>
</tr>
<tr>
<td>HOCH_2 CH(OH)CH_2OH + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + 2H^+ + 3H_2)</td>
<td></td>
</tr>
<tr>
<td><strong>Methanogenic Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>(CH_3COO^- + H^+ \rightarrow CH_4 + CO_2)</td>
<td>-36</td>
</tr>
<tr>
<td>(CH_3OH + H_2 \rightarrow CH_4 + H_2O)</td>
<td>-113</td>
</tr>
<tr>
<td>(CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O)</td>
<td>-131</td>
</tr>
<tr>
<td>(HCOO^- + H^+ + 3H_2 \rightarrow CH_4 + 2H_2O)</td>
<td>-134</td>
</tr>
<tr>
<td>(HCOO^- + H^+ + 4H_2 \rightarrow CH_4 + 3H_2O)</td>
<td>-136</td>
</tr>
<tr>
<td><strong>Inorganic reducing Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>(S + H_2 \rightarrow HS^- + H^+)</td>
<td>-28</td>
</tr>
<tr>
<td>(SO_4^{2-} + H^+ + 4H_2 \rightarrow HS^- + 4H_2O)</td>
<td>-152</td>
</tr>
<tr>
<td>(SO_3^{2-} + 2H^+ + 3H_2 \rightarrow H_2S + 3H_2O)</td>
<td>-173</td>
</tr>
<tr>
<td>(NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O)</td>
<td>-600</td>
</tr>
<tr>
<td>(2NO_2^- + 2H^+ + 3H_2 \rightarrow N_2 + 4H_2O)</td>
<td>-794</td>
</tr>
</tbody>
</table>
The free energy changes were calculated from the standard Gibbs free energies of formation in Thauer, Jungermann, and Decker (1977) or are from the reference. The conditions are 25°C, pH 7, and aqueous solutions at unit activity where possible. Methane, H\(_2\), and CO\(_2\) are in the gaseous state. Cellulose is assumed to have the same standard free energy of formation per unit of glucose as glycogen, and the hydrolysate is assumed to be \(\alpha\)-D-glucose.

Complete conversion of glucose to carbon dioxide and hydrogen in dark fermentations, having slightly negative free energy change, has not been considered an efficient process for the production of hydrogen, since fermentation reaction must be coupled with the synthesis of ATP and inorganic phosphate. Under physiological conditions ATP requires about 42 to 50 Kj/mol ATP formed; the glucose to hydrogen reaction supplies only about 26 Kj/mol glucose converted. Note that each of the methanogenic reactions in table exhibits a negative free energy change. Methanation of carbon dioxide and bicarbonate is more favored than direct conversion of acetate, which produces about 70% of methane fermentation.

Thermodynamic data are very useful for making predictions and explaining methane fermentation, but judgment should be exercised in interpreting them.

Information accumulated from the examination of pure compounds and natural products as substrates for methane fermentation and the characterization of anaerobic organisms indicate that actions of the three major groups of bacteria in the process and the sources of methane and carbon dioxide (Zehnder et al., 1982). The fermentative bacteria accomplish hydrolysis and conversion of the complex substrates to intermediates in yields of about 4% carbon dioxide and hydrogen, 20% acetate, and 76%
about 4% carbon dioxide and hydrogen, 20% acetate, and 76% intermediate higher acids and other lower molecular weight compounds. The acetogenic bacteria convert about one-third of the higher acids and lower molecular weight compounds to additional carbon dioxide and hydrogen, and two-thirds to additional acetate. About 70% of the methane and carbon dioxide is produced by methanogenic bacteria from acetate, and 30% is produced from carbon dioxide and hydrogen. For example, when a steady-state fermentation is upset by an undesirable change in environmental conditions or an operating parameter that reduces gas production, the pH decreases while the volatile acids in the fermentation broth and carbon dioxide evolution increase.

The phasic or stepwise nature of methane fermentation is also supported by many observations of the behavior of individual substrates. For example, when pure glucose was fermented in the batch mode with an inoculum from an active sewage sludge digester, almost all the glucose was assimilated in the first 30 h of fermentation; the product gas during this period contained 70 to 100% carbon dioxide (Ghosh and Klass, 1978). No methane was detected for the first 6 h. Most of it was collected after about 95% of the glucose had been consumed. The gas production data indicate that methanogenic bacteria function at a much lower rate than the fermentative and acetogenic bacteria, which rapidly catabolize glucose. Other observations that support stepwise methane fermentation have been made with *Macrocystis pyrifera* (giant brown kelp), a complex substrate (Ghosh and Klass, 1977). Some denitrification occurred in the first few hours, as indicated by the nitrogen peak at a concentration of about 70 mol% of the product gas. Maxima in the concentrations of hydrogen and carbon dioxide in the biogas were observed at 13 h (about 28
mol%) and 103 h (about 89 mol%) during the early portion of the process when methane production was low. Methane production rapidly increased from about 100 to 300 h. Its concentration reached a plateau of about 85 to 89 mol % while carbon dioxide concentration rapidly decreased and stabilized at about 10 to 15 mol %. The production of hydrogen and nitrogen fell to zero during this period.

The microbial transformations and stages in anaerobic digestion are supported by experimental data accumulated over many years. The overall process is perhaps the simplest chemical representation of the hydrolysis, acid-formation, and methane formation stages. This information led to the development of what has been called two-phase methane fermentation or digestion in which methanogenesis is physically separated from hydrolysis and acid formation. This resulted in significant improvements in process performance that can easily be obtained at low cost.

Factors Affecting the Anaerobic Process:

Wide spread application of the anaerobic process has been hampered somewhat by its reputation as being easily upset and unreliable. The development of anaerobic process technology is dependent on a better understanding of the factors associated with the stability of the biological process involved.

Process in stability is usually indicated by a rapid increase in the concentration of the volatile acids with a concurrent decrease in methane gas production, indicating that the more fastidious methanogens are the most susceptible to upset optimum conditions and range for anaerobic digestions have been studied by many investigators are not always in agreement. One reason for this may be that their studies are conducted using different feed
materials and different methodologies. The nature and composition of the substrate material dictate the microbial regime present, and it appears that single sets of parameters are not valid for all situations.

Many process failures may be the result of insufficient acclimatization periods. Acclimation of the microorganisms to a substrate has been reported to take more than 5 weeks but sufficiently acclimated bacteria have shown considerable stability towards stress inducing events.

**Temperature:** Digestion and gas production can occur over a wide range of temperature as long as the temperature is relatively constant. Once a temperature range is established and the microorganisms have become adapted, fluctuations can result in process upset. Rates of gas production VS temperature are shown.

Although the most sludge digesters are operated in the mesophilic range (30-40°C), methanogenesis can occur as low as 4°C. While decomposition is considerably slower at lower temperature, most of the degradable material will eventually be destroyed. Complex materials such as lipids and long chain carbohydrates are the most resistant.

Thermophilic digestion (45-60°C) has the advantages of shorter solid retention times, increased digestion efficiency, better sludge dewatering characteristics and increased destruction of pathogenic organisms. Its disadvantages are the thermophilic bacteria are more susceptible to upset and they require more careful buffering. Thermophilic operations must also provide superior mixing to ensure better heat distribution and more uniform feeding. The increased energy requirements for heating may be of additional disadvantage.
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Denitrification was found to occur rapidly at 25°C, and 15°C but at 4°C it began only after prolonged lag time. The optimum temperature for yeast fermentation process is generally considered to be approx. 35°C.

**pH Acidity and Alkalinity**: The optimum pH ranges of 6.9-7.2, 4.6-7.2 and 6.6-7.6 have been reported for the methane bacteria. The non-methanogenic organisms are not merely as sensitive and are able to function in a range of pH 5-8.5. Process pH results from the interaction of CO₂ Bicarbonate buffering system present with the volatile fatty acids and ammonia formed by the process. It is important that there be sufficient buffering capacity for the acids produced in order that they do not lower the pH to a level upsetting to the methane bacteria.

There has been considerable debate concerning the toxicity of the ammonia and volatile acids themselves, independent of pH. Current thinking is that only the unionized volatile acids in the concentration range 30-60 mg/lit are toxic and process inhibition by ammonia results from excessive concentration of free ammonia rather than ammonium ions.

**Factors Affecting Rate and Extant of Methanogenesis**

**Rate Limiting Reactions**

For anaerobic digestion of waste materials it is hydrolysis of polymers, which limits the extent of methanogenesis from organic wastes. With a properly designed and operated digester, the theoretical potential of methane production represented by the soluble organic matter in the effluent is usually very small compared with total amount of methane produced. When viewed in this way, the potential for improvement of conventional digestion
lies in increase the extent of hydrolysis of polymers. However, the fermentative methanogenic reactions can limit methanogenesis in two ways: Instabilities in the fermentation can cause digesters to go sour and, the slow growth of bacteria accomplishing the conversion of volatile organic acids to methane and CO₂ require long solids retention times in order to maintain adequate population in digesters.

When digesters are operated at high loading rates, there is a rapid turnover of Volatile organic acids, so that they accumulate rapidly if their degradation is inhibited. When their levels begin to increase, halting substrate addition some times allows the acetogenic and methanogenic microflora to recover. However, the continued fermentation slowly degrading substrates may cause the increase to continue. The ultimate result of volatile acid accumulation is a drop in pH, which further inhibits methanogenesis. This situation is known as "sour digestion", and it can be expensive and inconvenient. When very dilute or soluble compounds form the substrate, they are rapidly fermented, and it is often the growth rate of the slow-growing methanogens and acetogens this limits methanogenesis. Various methods have been used to increase the hydraulic loading rate of digesters while maintaining solids (i.e., bacteria) retention time at a level high enough to maintain adequate populations of slow-growing bacteria. The traditional technique is to recycle solids, but more novel techniques are now receiving increased attention. The ability of some bacteria to flocculate or adhere to inert surfaces can be exploited to keep them within the fermenter. The anaerobic filter and related techniques rely on this ability, allowing liquid to flow through the system rapidly while organisms remain within the reactor. This can give hydraulic retention times as short as a few
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hours while maintaining solids retention times long enough for methanogens and acetogens to flourish. The principle of the downflow system is different, but its operating characteristics are similar.

**Temperature:**

There is some controversy as to whether there exist two temperature optima (i.e., mesophilic and thermophilic) for anaerobic digestion. An anaerobic digestion of domestic refuse at temperature between 35 and 60°C at 5°C intervals and found 40°C consistently more favorable at 35 or 45°C when the retention time was between 4 and 30 days. With the same retention times it was found a second optimum at 60°C even more favorable than at 40°C digesters perform more satisfactorily at 60°C than at 55 or 65°C there are only small differences in gas production of completely mixed anaerobic digesters at temperatures between 30 and 60°C when the loading rate is not high (less than 7 Gms/lit) and the retention time is atleast 6 days. However, when loading rates are high or retention times short, thermophilic digesters usually out performed digesters operated at low temperatures.

Buhr and Endrews designed a dynamic process model to describe effects of temperature. It predicts that the temperature which gives minimum volatile organic acids concentrations increases with decreasing retention time so that at very low retention times (3.5 days) the optimum temperature is high (about 50°C) it also predicts greater maximum methanogenic rates at increasing temperature upto 60°C. These predictions generally consistent with the studies which tested shorter retention times. The digesters were always allowed a period of acclimation to the temperatures at which test are done. To obtain thermophilic
digestion, digesters can be started up to desired temperatures with low initial loading rate or temperatures of mesophilic digesters can be shifted slowly (about 1°C per week) when the temperatures is raised gradually there is a reproducible decrease in gas production. More rapid changes in temperature may not cause ill effects to digester is not already operated near maximum loading rate or minimum retention time. Little is known about effects of sudden decrease in temperature of digestion but the above model predicts that a drop from 50°C to 40°C would cause digester failure within two to three days.

Thermophilic digestion has the advantage of its ability to operate at shorter retention time and higher loading rates, include better destruction of bacterial and viral pathogens and better dewaterability of sludge. Its chief disadvantage is the heat requirement to attain and maintain higher digester temperature. Temperatures higher than 60°C have consistently been found to give less favorable fermentation. Volatile acids concentration were higher at 65°C than 60°C. It was possible that because the complex nature of anaerobic digestion require many interacting groups, it is unable to function normally at very high temperatures. In extreme environment such as high temperatures, microbial diversity is known to be limited.
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