Chapter 2

Theoretical Consideration

2.1 Biochemistry of Anaerobic Digestion Process

In anaerobic system, microorganisms obtain energy for their life processes from biochemical reactions of both organic and inorganic materials. The most significant reactions are : (1) degradation of organic matters, (2) denitrification and (3) sulfate-reduction. The details of these reactions are as follow :

2.1.1 Degradation of Organic Matters

The total degradation of organic matters consists of three stages, usually described as follows (see also Fig. 2.1) :

2.1.1.1 Hydrolysis

In the initial stage, complex molecules such as carbohydrates, polysaccharides, lipids and proteins are hydrolyzed into utilizable substrates by extracellular enzymes. For example, carbohydrates are hydrolyzed into glucose by amylase, proteins are hydrolyzed into amino acids by protease. As for fats and lipids, with the result of glyceral and fatty acids, they are hydrolyzed very slowly by lipase and enterase respectively. Hence the hydrolysis might be regarded as a rate limiting step (including methane production) for a waste containing considerable amount of lipids and other slowly hydrolyzing compounds.

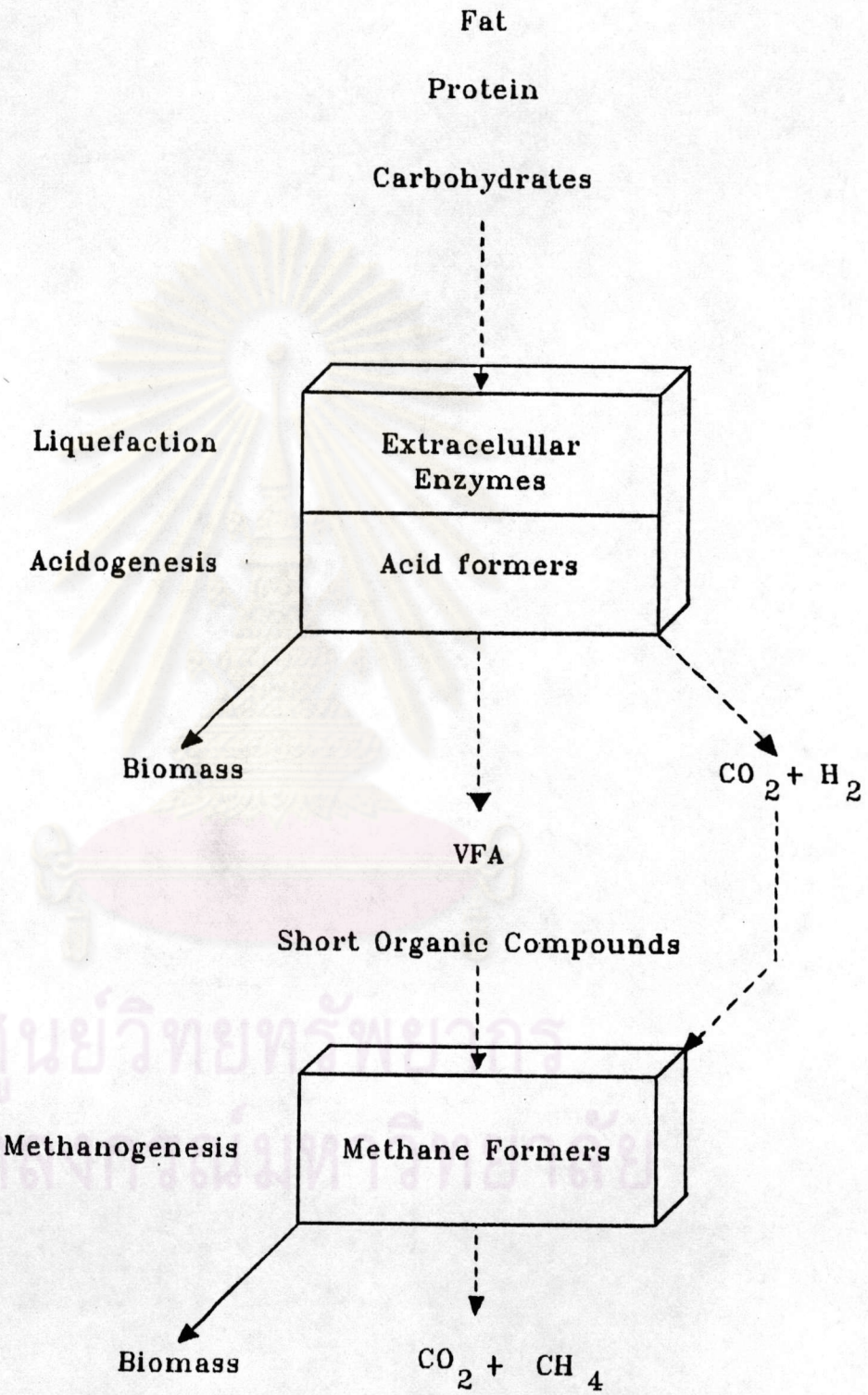


Fig.2.1 Scheme of anaerobic conversion (Zeevalkint & Maaskant, 1984).

2.1.1.2 Acid Formation

These hydrolysis products are further fermented into various intermediates, mainly volatile fatty acids (VFA) such as acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), and butyric acid ($CH_3CH_2CH_2COOH$). The other intermediates, i.e., organic acids, alcohols, hydrogen (H_2), carbon dioxide (CO_2), ammonia (NH_3) and sulfide ion (S^{2-}) are also found in this stage (see Fig. 2.2). This results from the reaction of a hardly separable group of fast growing facultative and obligately anaerobic acidifying bacteria or "acid formers." The acid production rate is high compared to the methane production rate, which may lead to accumulation of acids and a subsequence drop in pH-value.

2.1.1.3 Methane Production

During this stage, methane (CH_4) is produced by slowly growing obligately anaerobic bacteria or "methane formers." McCarty (1964) suggested that most of the methane is produced from propionic and acetic acid. This stage is a very slow process which is a rate-limiting step of anaerobic digestion. Methane formers are generally known as very sensitive to disturbances. They account for the main COD reduction of the effluent, and work well at a pH of about 7. So it is a cause to the instability if there are considerable increase in intermediate products in the digesters; thus good pH-control is absolutely necessary.

We now have an alternative three-stage scheme which is best suited to describe the present knowledge of anaerobic digestion. This is shown in Fig. 2.3. The complete degradation of

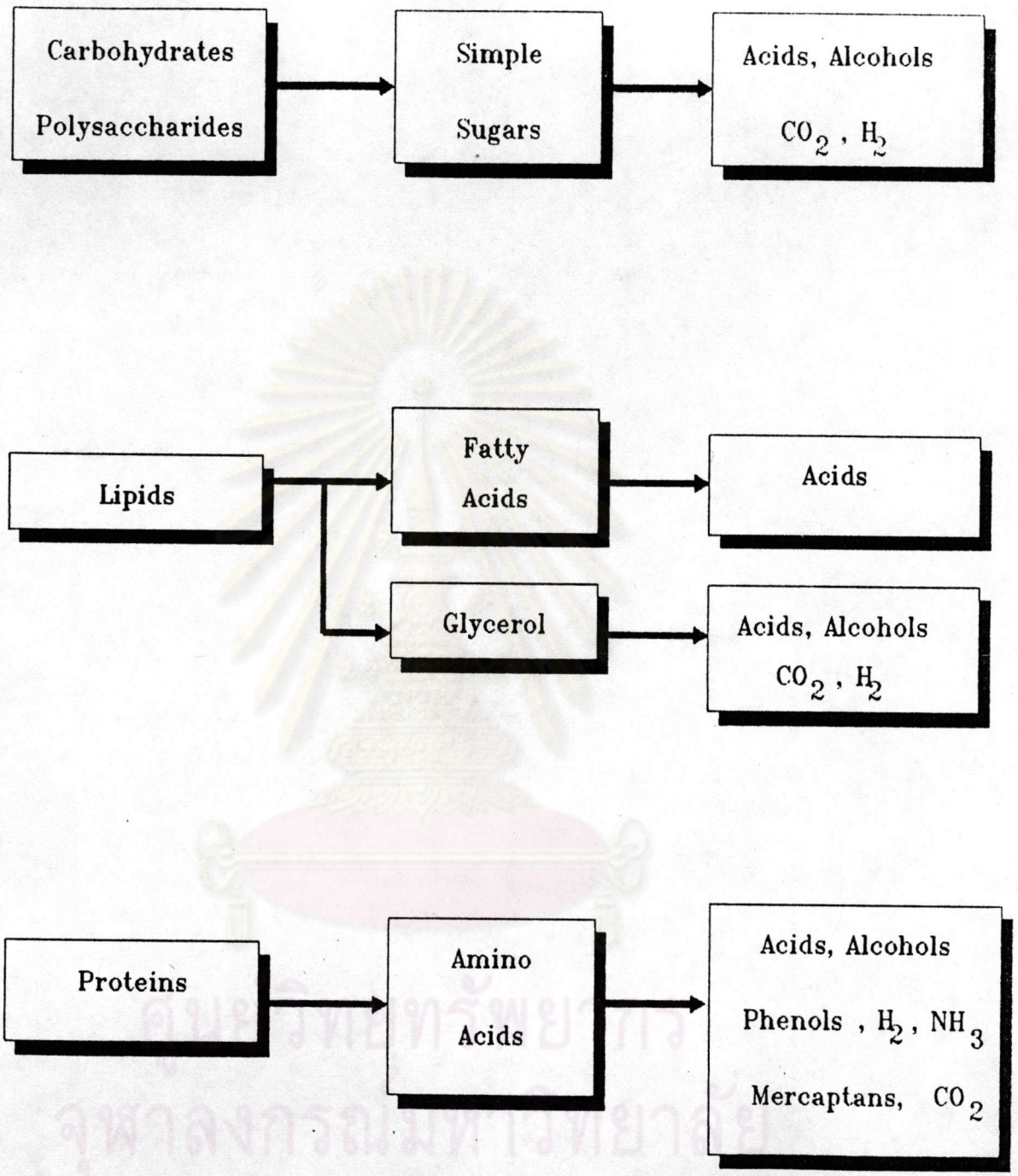
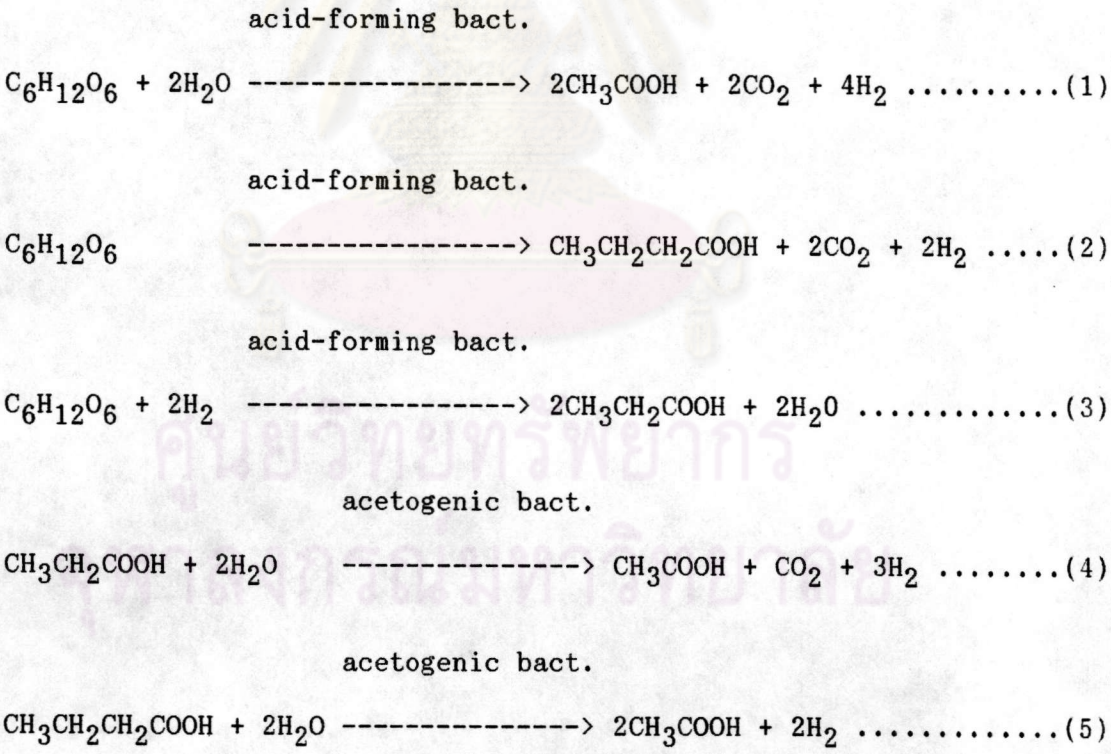


Fig.2.2 Anaerobic decomposition of organic matter in hydrolysis and acidogenesis.

organic matter to carbon dioxide and methane involves four, not two, groups of bacteria. The new concept of syntropism in anaerobic cultures enables better understanding of the way these organisms are metabolically dependent upon each other for survival. Namely, the fast growing acid-forming group (minimum doubling time of 2-3 hours at 35°C) accounts for converting complex organic matter to intermediate products, primarily acetic, propionic, and butyric acids. Then the second microbial group, the acetogenic bacteria, takes hold of these fatty acids and other compound producing acetic acid, hydrogen and carbon dioxide. These reactions can be illustrated by the simpliest form of sugar, glucose, as follow :



The terminal methane group comprises the methanogens effectively utilizing acetic acids and those utilizing the mixture of hydrogen and carbon dioxide. Namely, the slow growing

acetoclastic methane bacteria (minimum doubling times of 2-3 days at 35°C) are responsible for converting acetic acid into methane and carbon dioxide according to the reaction :

$$CH_3COOH \longrightarrow CH_4 + CO_2 \quad \ldots\ldots\ldots\ldots\ldots(6)$$

And the hydrogen-utilizing methane bacteria are responsible for converting the mixture of hydrogen and carbon dioxide into methane gas according to the reaction :

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \quad \ldots\ldots\ldots\ldots\ldots(7)$$

It can be seen that almost all of hydrogen is utilized by this latter methane group. Regarding the trace concentration of hydrogen availability in the system, we have recently known that these forms of control, as described above, are regulated by the trace of hydrogen through the bacteria in the digester. The reactions drawn in Fig. 2.3 as : $\xrightarrow{H_2}$ are obviously sped up by high concentrations of hydrogen, but notice also the reactions drawn as : $\overset{H_2}{\dashrightarrow}$. Usually the reactions are the conversion of hydrolysis products into acetic acid (see example from Eq. (1)). If the concentration of hydrogen is increased, e.g. during surge loads, it can slow down the overall rate of acid production. Moreover, it can further reduce the acid load by diverting some part of the acid products towards butyric acid (by producing one mole of butyric acid instead of two moles of acetic acid, see Eq. (2)). Sometimes events proceed further and trigger accumulation of hydrogen; the bacteria will response by putting the hydrogen into formation of propionic acid (see Eq. (3)). The hydrogen also controls the rates at which propionic and butyric acids are subsequently

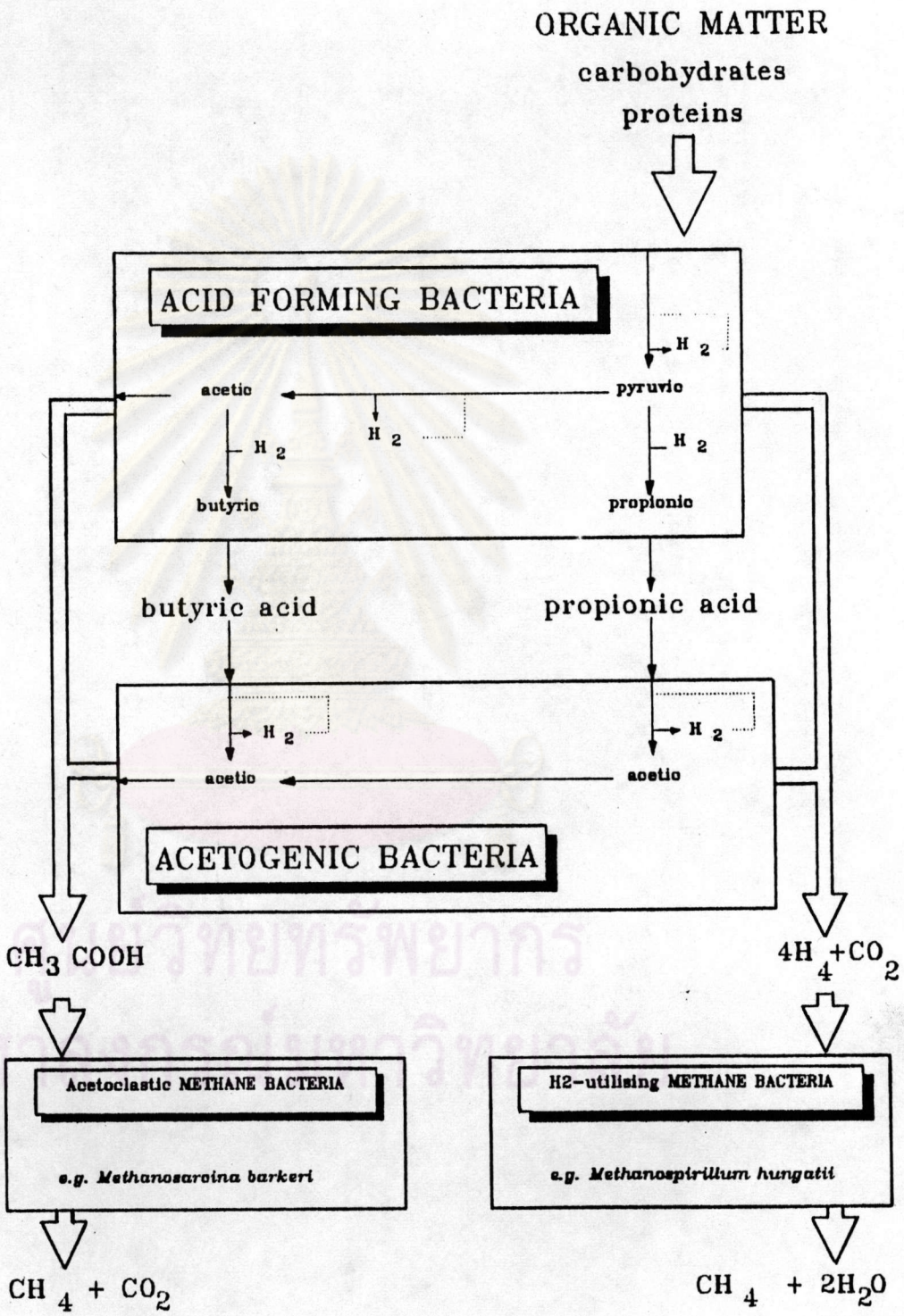


Fig.2.3 Regulation of metabolism in the anaerobic process (Mosey, 1982).

reversed into acetic acid. This provide the methanogenic bacteria with the prime substrate for methane production. Thus, we can say that the trace concentration of hydrogen regulates not only the fatty acid productions but also the rate of methane formation in the digestion control (Mc Inerney, Bryant and Stafford, 1980; Mosey, 1982).

2.1.2 Denitrification

Nitrates in wastewater are reduced by facultative denitrifying bacteria under anaerobic conditions provided a source of carbon is available. This group of bacteria is heterotrophic organisms i.e., *Pseudomonas, Achromobacter, Bacillus* and *Micrococcus.* They obtain energy for growth from the biochemical reaction between nitrates and organic matter. Namely,nitrates (NO_3^-) are presumably reduced to nitrites (NO_2^-), and then reduction of nitrites occurs. Reduction of nitrite carried all the way to ammonia (NH_3) by a few bacteria, but most of them carry the reduction to nitrogen (N_2) gas which escape with the digester gas produced (Sawyer & Mccarty, 1978). For example, when methanol is used as the carbon source, nitrate is converted to nitrogen as the following equation :

$$NO_3^- + \frac{5}{6}CH_3OH \text{ ------> } \frac{1}{2}N_2 + \frac{5}{6}CO_2 + \frac{7}{6}H_2O + OH^- + \text{Energy...(8)}$$

2.1.3 Sulfate Reduction

In the absence of dissolved oxygen and nitrates, sulfates can be served as a source of oxygen (or electron acceptor) for anaerobes called sulfate-reducing bacteria. This group of bacteria is also routinely found in the digester i.e., *Desulfovibrio,*

Desulfotomaculum. The biochemical reaction of sulfate are shown in the following equation :

$$SO_4^{2-} + \text{organic matter} \text{ ----------> } S^{2-} + H_2O + CO_2 \text{.....(9)}$$

Sulfides produced from the reaction may exist in a soluble or insoluble form, depending upon the cation with which they become associated. At the normal digester pH level, metal sulfides can precipitate to form in a black colloid in solution. The remaining soluble sulfides form hydrogen sulfide (H_2S) that some extent can be released as a gas called "rotten egg gas" to the biogas production.

2.1.4 Microbial Aspects

The microorganisms responsible for acidogenesis are well known as the acid formers or sometimes called "non-methanogens." These groups of bacteria always consist of both facultative and obligately anaerobes. We can commonly find them in natural environment such as sewage sludge, organic sediments and the rumen of cud chewing animals. Table 2.1 shows various kinds of the non-methanogens collected by Toerien & Hattingh (1969). They are found in gram-negative facultative rods, gram-positive cocci, endospore-forming rods and gram-positive asperogenous rods (Zeikus, 1980). This is similar to the result from the experiment with the separated upflow anaerobic reactor by Cohen, Zoetemeyer, Deursen & Andel (1979). The microorganisms observed in the acid reactor were reported to be white in appearance, consisting of rod shape bacteria in variable lengths; and young cells (just after division) were gram-negative, whereas older cells were gram-positive.

The methanogenic microorganisms are fastidious anaerobes which have strictly requirements for redox potential and oxygen absence. The methanogens are unusual in that they are composed of many species with very different cell morphologies. They are reported in various types of cocci, rods, or even filamentous shapes, found in both gram negative and gram positive. Moreover, it can not locate single colonies of methanogens in purified agar due to the fact that they often grow with the non-methanogen contaminants (William and Clawford, 1985). They are in black and smell of hydrogen sulfide after treating with 1 N HCl (Cohen *et al.*, 1979). Table 2.2 show a number of revised grouping of the methanogens (McInerney *et al.*, 1979).

2.2 Methane Production

The end products obtained from organic destruction in anaerobic process is "biogas." This is often referred to as a mixture of methane (CH_4) and carbon dioxide (CO_2) plus traces of other gases, i.e., hydrogen (H_2), nitrogen (N_2), ammonia (NH_3), and hydrogen sulfide (H_2S), etc. Table 2.3 shows the typical gases which are always measured in the stable digester. It can be seen that methane is the major portion in the composition of product gas. Jeris and McCarty (1965) estimated that approximately 70% of methane came from acetic acid formation. At present, the two theoretical methane production are accepted as follow :

1. Acetic acid cleavage : (McCarty, 1964)

$$CH_3COOH \text{ ----------> } C^*H_4 + CO_2 \quad \text{.....(10a)}$$

Table 2.1 The non-methanogens that has been found in anaerobic digesters (Toerien & Hattingh, 1969)

Genus	Bacterial species	Reference
Aerobacter	*A. aerogenes*	Toerien (1967a)
Aeromonas	*Aeromonas sp.*	Kotze et al. (1968)
Alcaligenes	*A. bookerii*	Toerien (1967b)
	A. faccalis	McCarty et al. (1962), Toerien (1967b)
	A. viscolactis	McCarty et al. (1962)
	Alcaligenes sp.	Korze et al. (1968)
Bacillus	*B. cereus*	Hattingh et al.(1967), Toerien (1967a,b)
	B. cereus var mycoides	Hattingh et al.(1967), Toerien (1967a,b)
	B. circulans	Toerien (1967a,b)
	B. endorhythmos	Buck et al. (1954)
	B. firnus	Toerien (1967b)
	B. knefelkampi	Cookson & Burbank (1965), Burbank et al. (1966)
	B. megaterium	Hattingh et al.(1967), Toerien (1967a,b)
	B. pantothenticus	Hattingh et al.(1967)
	B. pumilis	Hattingh et al.(1967), Toerien (1967b)
	B. sphaericus	Toerien (1967b)
	B. subtilis	Toerien (1967a)
	Bacillus sp.	Toerien (1967a)
Bacteroides	*Bacteroides sp.*	Post et al.(1967)
Clostridium	*C. aminovalericum*	Hardman & Atadtman (1960)
	C. carmofoetidum	Cookson & Burbank (1965), Burbank et al. (1966)
Escherichia	*E. coli*	McCarty et al. (1962), Cookson & Burbank (1965) Burbank et al. (1966), Toerien (1967b)
	E. intermedia	Toerien (1967a)
	Escherichia sp.	Kotze et al.(1968)
Klebsiella	*Klebsiella sp.*	Burbank et al.(1966)
Leptospira	*L. bitlexa*	Toerien (1967b)
	Leptospira sp.	Maki (1954)
Micrococcus	*M. candidus*	Toerien (1967a, b)
	M. luteus	Toerien (1967b)
	M. varians	McCarty et al. (1962), Toerien (1967a, b)
	M. ureae	Toerien (1967a, b)
	Micrococcus sp.	Kotze et al.(1968)
Neisseria	*N. catarrhalis*	McCarty et al.(1962)
Paracolobacirum	*P. intermedium*	Toerien (1967b)
	P. coliforme	Toerien (1967b)
Proteus	*P.vulgaris*	Toerien (1967b)
Pseudonionas	*P. acruginosa*	Toerien (1967a)
	P. ambigua	Toerien (1967a)
	P. denitrificans	Burbank et al.(1966)
	P. oleovorans	Toerien (1967a)
	P. perolens	Toerien (1967b)
	P. pseudomallei	Toerien (1967a)
	P. reptilivora	McCarty et al. (1962), Toerien (1967b)
	P. riboflavina	Toerien (1967b)
	Pseudonionas spp.	Burbank et al.(1966), Hatting et al.(1967) Kotze et al. (1968), Toerien(1967b)
Rhodopscudomonas	*R. pulusiris*	Toerien (1967b)
Sarcina	*S. cooksonli*	Cookson & Burbank (1965), Burbank et al.(1966)
	S. lutea	McCarty et al.(1962)
Serratia	*S. indicans*	Burbank et al.(1966)
Streptococcus	*S. diploidus*	Buck et al.(1953)
Streptomyces	*S. bikiniesis*	Toerien (1967b)

Table 2.2

A revised grouping of the methanogen classified by using the 165 ribosomal RNA sequences and substrates (McInerney et al., 1979)

	Former designation	Substrates for growth and CH_4 production
Order I. *Methanobacteriales* (type order)		
Family I. *Methanobacteriaceae*		
Genus I. *Methanobacterium* (type genus)		
1. *Methanobacterium formicicum* (neotype species)	*Methanobacterium formicicum*	H_2, formate
2. *Methanobacterium bryantii*	*Methanobacterium* sp. strain M.o.H.	H_2
Methanobacterium bryantii strain M.o.H.G.	*Methanobacterium* sp. strain M.o.H.G.	H_2
3. *Methanobacterium thermoautotrophicum*	*Methanobacterium thermoautotrophicum*	H_2
Genus II. *Methanobrevibacter*		
1. *Methanobrevibacter ruminantium* (type species)	*Methanobacterium ruminantium* strain M1	H_2, formate
2. *Methanobrevibacter arboriphilus*	*Methanobacterium arbophilicum*	H_2
Methanobrevibacter arboriphilus strain AZ	*Methanobacterium* sp. strain AZ	H_2
Methanobrevibacter arboriphilus strain DC	*Methanobacterium* strain DC	H_2
3. *Methanobrevibacter smithii*	*Methanobacterium ruminantium* strain PS	H_2, formate
Order II. *Methanococcales*		
Family I. *Methanococcaceae*		
Genus I. *Methanococcus*		
1. *Methanococcus vannielii* (neotype species)	*Methanococcus vannielii*	H_2, formate
2. *Methanococcus voltae*	*Methanococcus* sp. strain PS	H_2, formate
Order III. *Methanomicrobiales*		
Family I. *Methanomicrobiaceae* (type family)		
Genus I. *Methanomicrobium* (type genus)		
1. *Methanomicrobium mobile* (type species)	*Methanobacterium mobile*	H_2, formate
Genus II. *Methanogenium*		
1. *Methanogenium cariaci* (type species)	Cariaco isolate JR1	H_2, formate
2. *Methanogenium marisnigri*	Black Sea isolate JR1	H_2, formate
Genus III. *Methanospirillum*		
1. *Methanospirillum hungatii*	*Methanospirillum hungatii*	H_2, formate
Family II. *Methanosarcinaceae*		
Genus II. *Methanosarcina* (type genus)		
1. *Methanosarcina barkeri* (type species)	*Methanosarcina barkeri*	H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain 227	*Methanosarcina barkeri* strain 227	H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain W	*Methanosarcina barkeri* strain W	H_2, CH_3OH, CH_3NH_2, acetate

2. Carbon dioxide reduction : (McCarty, 1964)

$$CO_2 + 8H \text{ ----------> } CH_4 + 2H_2O \text{.....(10b)}$$

Table 2.3

Chemical and physical properties of gas production (Wheatley, 1980)

Property	CH_4	CO_2	H_2S	H_2	Typical biogas (60%CH_4/40%CO_2)
% by volume	54-80	20-45	1/10	0.0-10	100
Energy value (Kcal/litre)	9.0	-	-	2.9	5.4
Explosive range (% by vol.with air)	5-15	-	4-46	6.71	6-12
Density (g/litre) C 760 mm	0.72	1.98	1.54	0.99	1.22
Specific gravity (relative to air)	0.55	1.5	1.2	0.07	0.93
Critical temperature (°C)	-82.5	+31.1	+100.4	-239.9	
Critical pressure (Atm.)	45.8	73.0	88.9	12.8	
Odour	none	none	rotten egg	none	

Buswell and Mueller (1952) developed the following equation to predict the quantity of methane from a knowledge of chemical composition of the waste:

$$C_nH_aO_b + (n-\frac{a}{4}-\frac{b}{2})\ H_2O \text{ ----------> } (\frac{n}{2}-\frac{a}{8}+\frac{b}{4})\ CO_2 + (\frac{n}{2}+\frac{a}{8}-\frac{b}{4})\ CH_4$$

McCarty (1964) proposed that the theoretical methane production from complete stabilisation of 1 Kg of ultimate BOD or COD was 0.351 m^3 at STP.

2.3 Digester Control Parameter

2.3.1 Temperature

Temperature is a very important parameter in anaerobic fermentation processes. Generally, at higher temperature, rates of reaction proceed much faster resulting in more efficient operation and smaller digester size. There are two optimum temperature levels, i.e., the mesophilic level range from 35° to 40°C and the thermophilic level range from 55° to 60°C (see Fig. 2.4). Although the rates of reaction in the thermophilic level are much faster than those in the mesophilic level, the resulting methane production is insufficiently high to make the operation economically attractive (Pfeffer, 1974).

Similar trends were reported by Tebbutt (1971), who found that there was improvement in gas production and substrate utilization with increasing temperature, and remarked that when the optimum temperature in mesophilic range was 40°C, gas production at 45°C was not much better than at 35°C and, while the gas production increased in the transition from 40°C to 45°C, until reaching 43°C the gas production decreased, as shown in Fig. 2.5.

Van Den Berg (1977) studied the effect of temperature on growth and activity of a methanogenic culture utilising acetate. He reported that the culture had an optimum growth

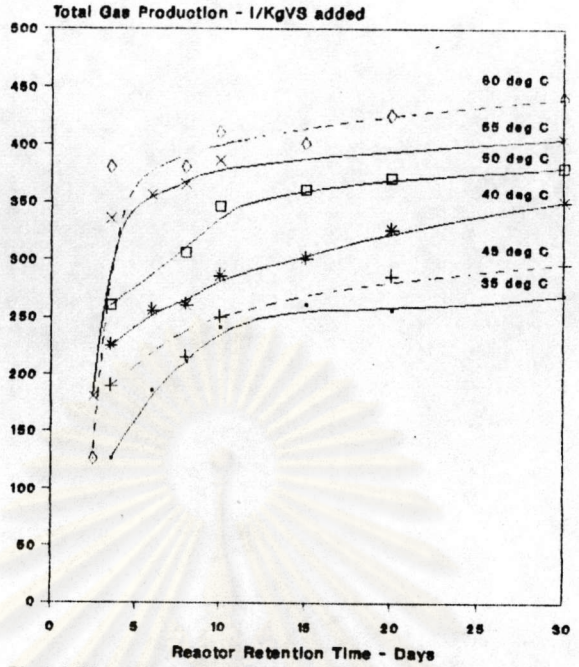


Fig.2.4 The effect of temperature and retention time on the production of gas from urban refuse (Pfeffer,1974).

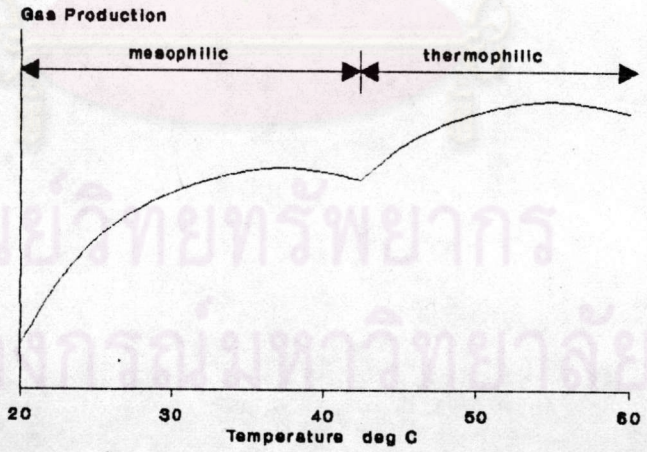


Fig.2.5 Effect of temperature on gas production (Tebbutt,1971).

temperature of 35°C, with only small differences for other temperatures between 30°C and 40°C. The maximum temperature for growth was 42° - 44°C; the minimum was below 15°C.

The internal temperature of the anaerobic digester is around 2°C to 3°C higher than the ambient temperature (Polprasart & Thanh,1979). According to Kotze', Thiel & Hattingh (1969), the methane bacteria is rather sensitive to sudden transition of the temperature just exceeding 2°C to 3°C; the resulting methane production is changeable. In order to gain the stability process, the temperature should be kept at a narrow range. The maximum acceptable variation value is +2.8°C for mesophilic condition. Those for the thermophilic conditions at 49°C and 52°C were +0.8°C and +0.3°C, respectively.

2.3.2 CO_2 Production

Change in CO_2 content can forecast difficulties in anaerobic digestion process. In normal digester, CO_2 is released due to decomposition of the organic matter, a combination of it and ammonia, also produced by biological activity, provides a source of alkalinity. As the process becomes retarded and volatile acids begin to accumulate, the acids tend to react with the available alkalinity to form salts of the acids and release CO_2 from the system, as shown by the following equation :

$$R\text{-}COOH + NH_4HCO_3 \text{ ---------> } R\text{-}COO\cdot NH_4 + CO_2 + H_2O \quad \ldots\ldots\ldots (12)$$

Therefore, because the methane-producing mechanism is inhibited during the accumulation of volatile acids, the

percentage of CO_2 released is increased. This is accompanied by a decrease in CH_4 content of the digester gas (Pohland & Bloodgood, 1963).

It is recommended that, under proper condition, the CO_2 and the CH_4 in the gas should range from about 30 to 35 percent and 65 to 70 percent, respectively (WPCF's manual of practice No.16).

2.3.3 pH and Alkalinity

Total alkalinity in a digester is normally composed of bicarbonate alkalinity, such as ammonium bicarbonate, and volatile acid salts which also provide another source of alkalinity, as shown by Eq. (12). The same equation also shows that total alkalinity has less importance as a control parameter than the bicarbonate alkalinity. The reason is that, as volatile acids increase, the bicarbonate are reduced and replaced by volatile acid alkalinity, with a result that there is an only slightly decrease in the total alkalinity value (DiLallo & Albertson, 1961).

The bicarbonate alkalinity can be approximated by the formula that was derived by McCarty (1964) :

$$BA = TA - (0.85)(0.833)\ TVA \quad \text{.....(13)}$$

where :

BA = bicarbonate alkalinity, mg/l as $CaCO_3$

TA = total alkalinity, mg/l as $CaCO_3$

TVA = total volatile acid conc., mg/l as HAc.

Regarding pH, it is normally considered as a function of the bicarbonate alkalinity. According to DiLallo &

Albertson (1961), it is difficult to differentiate between pH and alkalinity, and they are best considered as one effect. With this reason, the pH will change only slightly until the excess volatile acids exhaust the available bicarbonate alkalinity. Thereafter, free volatile acids are present and the rapid decrease in pH are obtained.

McCarty (1964) illustrated the relationship between pH, bicarbonate alkalinity, and CO_2 content in digester gas produced in Fig. 2.6. He recommended that anaerobic treatment proceed reasonably well with a pH range of 6.6 to 7.6 with an optimum of about 7.0 to 7.2. He further indicated that a significant inhibition of methanogenic bacteria occurred when pH values dropped below 6.2. The desirable range of alkalinity to buffer against pH drop should not be below 1,000 mg/l as $CaCO_3$.

Kirsch and Sykes (1971) reported that sodium bicarbonate ($NaHCO_3$) was the best chemical reagent for controlling pH of anaerobic digestion. This was because it had a high solubility and performed directly as a function of bicarbonate alkalinity to the system.

A more recent work by Lettinga and Vinken (1980a) showed that alkalinity was an eminent and important factor in operating high-rate anaerobic processes. They found that the substrate solids were accumulated if the supply of alkalinity was not resumed in time. Consequently, the pH would reach critical levels; this obviously resulted in a serious reduction of the sludge age and the methane production.

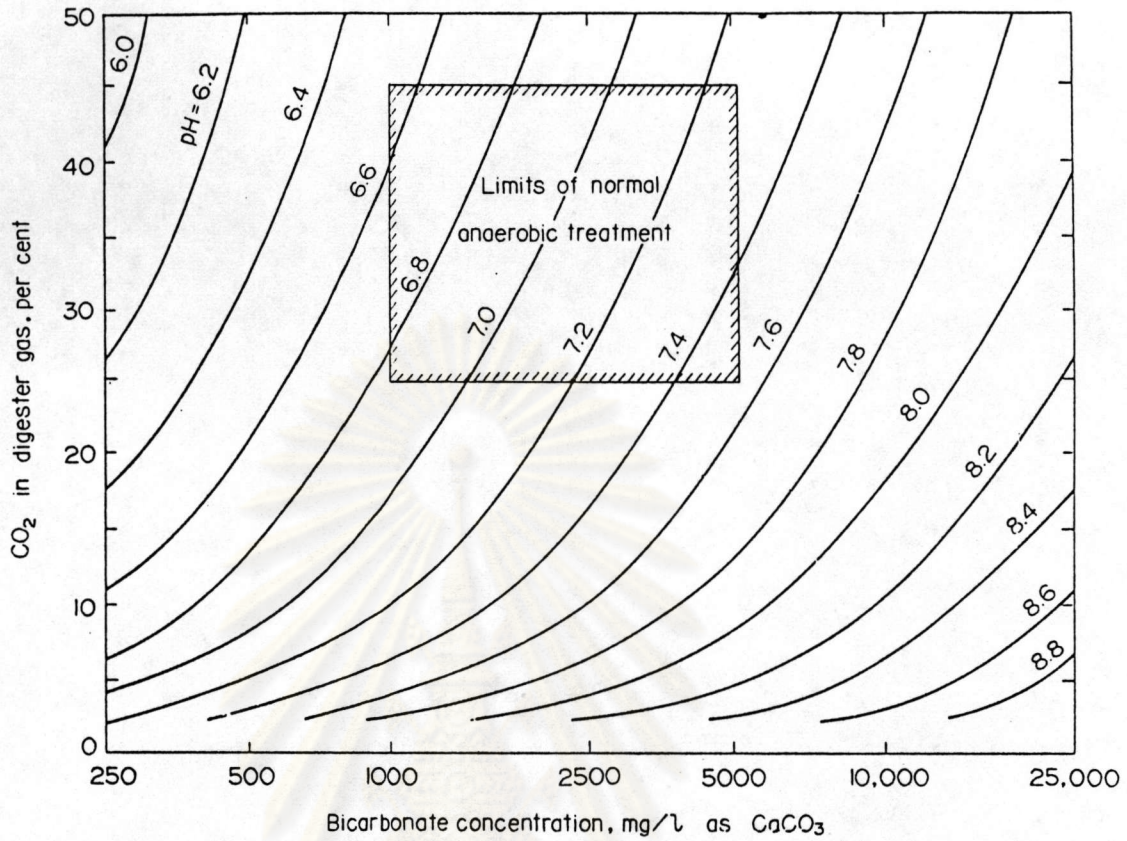


Fig.2.6 Relationship between pH, bicarbonate concentration, and carbon dioxide concentration at 35°C. (McCarty, 1964).

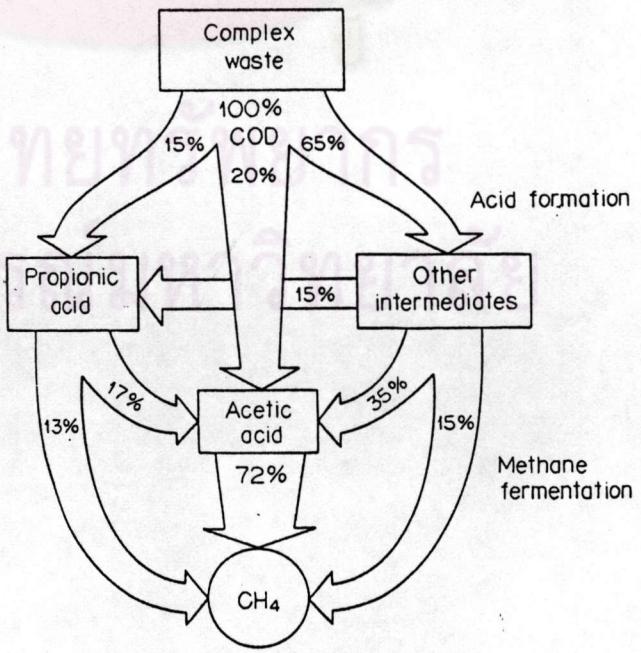


Fig.2.7 Pathways in methane fermention of complex wastes such as domestic waste sludge. Percentages represent conversion of waste COD by various routes. (McCarty, 1964).

2.3.4 Volatile Fatty Acid (VFA)

Volatile fatty acids (VFA's) are the intermediate in the anaerobic digestion process. Fig. 2.7 shows just a few of the many steps through which a complex waste such as domestic waste sludge must pass during its conversion to methane gas. About 72 percent of the organic matter is converted to acetic acid before finally being changed into methane gas. The remaining of the methane gas results from fermentations of other VFAs such as formic, butyric, and propionic acids.

VFA at high concentrations is attributed either to toxicity or to decrease in pH value. During retardation process, VFAs begin to accumulate in the digester. This is accompanied by the slight decrease in pH value until the excess VFAs exhaust the available bicarbonate alkalinity. When the pH reaches the danger-point, it causes the digester to stop producing gas.

Buswell (1952) recommended that the concentration of acetic acid should not exceed 2,000 or 3,000 mg/l, in order to prevent an inadmissible reduction of the methane ferment capacity.

Tebbutt (1971) explained that excess production of volatile acids should destroy the buffering capacity of alkalinity in the sludge, lower the pH, and reduce gas production (see Fig. 2.8). Furthermore, he recommended that the normal volatile acids should be 250-1,000 mg/l; if they exceed 2,000 mg/l, the system would be in trouble.

According to Andrews, the undissociated VFA was

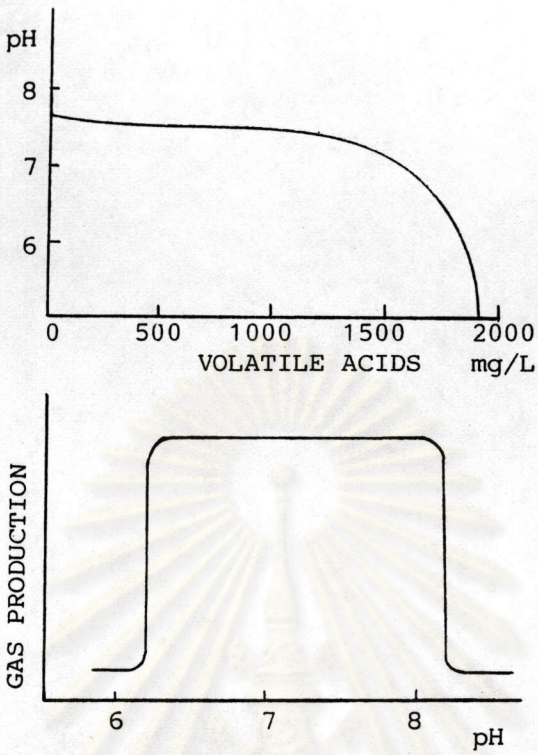


Fig.2.8 Effect of volatile acids production on digestion (Tebbutt, 1971).

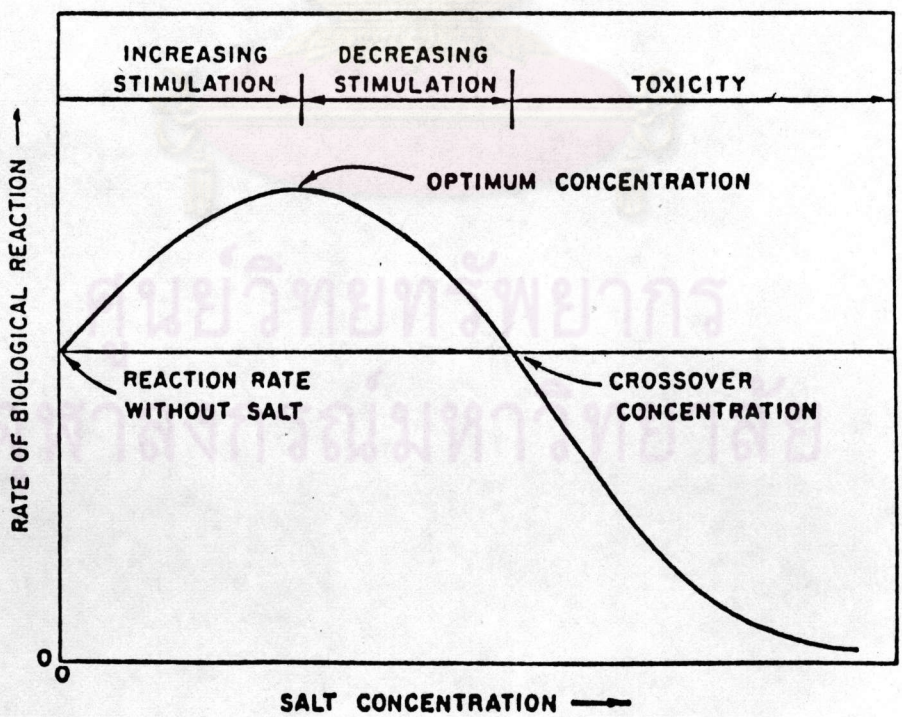


Fig.2.9 General effect of salts or other materials on biological reactions (McCarty, 1964).

inhibitory, since the bacterial cell wall was far more permeable to undissociated molecules in comparison with their ionised state. Therefore, more VFA would be taken up by the organisms at low pH values. Once taken up, the VFA's dissociate within the cell and cause a decrease of the pH, which would adversely affect the enzyme activity (reffered to by van Velsen and Lettinga, 1980).

Lettinga and Vinken (1980) reported that the VFA increased concentration in the effluence directly after an increase in the loading rate and after a period in which an inadequate quantity of $NaHCO_3$ had been supplied to the feed solution.

According to WPCF's Manual of Practice No. 16, the ratio of VFA/alkalinity may be used as a sensitive indicator. In any event, the ratio above 0.3 to 0.4 indicates stress to the digestion process. If this ratio reached 0.8, the process failure will be anticipated.

2.3.5 Nutrient Requirements

Nutrient is one of the important factors for bacterial growth. In anaerobic system, the nutrient requirement is low because of the low yield of excess sludge existing.

Speece & McCarty (1964) calculated the nitrogen and phosphorus requirements based on an average chemical formulation of biological cells of $C_5H_9O_3N$. Nutrient must be adequate to satisfy the cell growth requirements. The nitrogen requirement was about 11% of the cell volatile solid weight and the requirement for phosphorus was equal to about one-fifth of the nitrogen requirement. McCarty

(1964) suggested that the nutrients requirement for anaerobic treatment process should be kept such that at least the ratio of ultimate BOD or COD : N : P equal to 100 : 1.1 : 0.2.

Lettinga et al. (1980c) reported that, although the nutrient requirement was low, all essential nutrients had been presented in an available form above the minimum requirements; if not, poor COD removal may result.

As far as the trace elements are concerned, it is known that they also play an eminent role in the growth of anaerobic organisms. Especially Fe, Ni, and Co seem to be important. However, so far very little information is available as to the questions of which elements are essential and in which concentrations they should be present (Lettinga *et al.*, 1985).

2.3.6 Toxic Materials

Many materials, both organic and inorganic, may be toxic or inhibitory to the anaerobic waste treatment process. The term "toxic" is relative and the concentration at which a material becomes toxic or inhibitory may vary from a fraction of an mg/l to several thousand mg/l. Fig. 2.9 indicates the general effect resulting from the addition of most substances to biological systems. At some very low concentration, stimulation of activity is usually achieved. This stimulatory concentration may range from only a fraction of an mg/l for heavy metal salts to over one hundred mg/l for sodium or calcium salts. As the concentration is increased above the stimulatory concentration, the rate of biological activity begins to decrease and in some cases stop methane production.

McCarty (1964) classified toxicants in anaerobic digester into 4 major groups as follow :

(a) Alkali and Alkaline-Earth Salt Toxicity

It has been found that toxicity is normally associated with the cation, rather than the anion portion of the salt. Table 2.4 shows concentration of the cations of these salts which may be stimulatory and those which may be inhibitory to anaerobic treatment.

And when combinations of these cations are present, the nature of the effect becomes more complex as some of the cations act antagonistically, reducing the toxicity of other cations, while others act synergistically, increasing the toxicity of the other cations. For example, it has been found that 7,000 mg/l of sodium may significantly retard anaerobic treatment. If 300 mg/l of potassium is added, this retardation may be reduced by 80%. If 150 mg/l of calcium is then added, the inhibition may be completely eliminated. However, if calcium is added in the absence of potassium, no beneficial effect at all would be achieved (McCarty, 1964).

(b) Ammonia and Sulfide Toxicity

Ammonia is usually formed in anaerobic processes from deamination of wastes containing proteins or urea. Inhibitory concentrations may be approached in industrial wastes containing high concentrations of these materials. Ammonia may be present either in the form of ammonium ion (NH_4^+) or as dissolved

Table 2.4

The cation of inorganic salts which have influences on the anaerobic system (McCarty, 1964).

Cation	Concentration range (mg/l)		
	Stimulatory	Moderately Inhibitory	Strongly Inhibitory
Sodium (Na^+)	100 - 200	3,500 - 5,500	8,000
Potassium (K^+)	200 - 400	2,500 - 4,500	12,000
Calcium (Ca^{+2})	100 - 200	2,500 - 4,500	8,000
Magnesium (Mg^{+2})	75 - 150	1,000 - 1,500	3,000

ammonia (NH_3) gas; the latter form is inhibitory at a much lower concentration than the former form. At concentrations between 1500 and 3000 mg/l of total ammonia nitrogen and a pH greater than 7.4, NH_3 concentration can become inhibitory. At concentrations about 3000 mg/l, the NH_4^+ itself becomes quite toxic regardless of pH. (McCarty, 1964).

About sulfide, it is generally produced by sulfate reducing bacteria from reduction of organic sulfur compound and other sulfur-containing inorganic compounds, as well as from anaerobic protein degradation. Sulfates (SO_4^{2-}) usually represent the major precursors of sulfide (S^{2-}) in numerous industrial wastes such as wastewater from starch and fermentation industries, yeast production industries, and the pulp and paper industries, etc. Moreover sulfides are also derived from the sulfate in the "hard"

water. Sulfate itself can inhibit methanogenesis from acetate to a distinct extent at a concentration exceeding 5 g SO_4^{2-}/l (Lettinga *et al.*, 1985). Sulfide, particularly undissociated H_2S, is highly toxic in common anaerobic processes. Recently, the experimental result obtained has been reported that 50% acetoclastic methanogen was inhibited at 250 mg/l H_2S, at 30°C and pH range 6.5-7.2 (this is referred to by Lettinga, *et al.*, 1985). However, modern high rate anaerobic treatment, with higher sludge retention time, i.e., UASB and anaerobic filter (AF), seem to be relatively insensitive to inhibition by sulfide (Parkin & Speece, 1983).

(c) Heavy Metal Toxicity

Free heavy metal ions, even at low concentrations but soluble, have a toxic effect on anaerobic digestion processes. McCarty (1964) reported that concentrations of copper, zinc, and nickel salts were quite toxic and these salts were associated with most of the problems of heavy metal toxicity in anaerobic treatment. However, this metal ion was normally reduced to the trivalent which was relatively insoluble at normal digester pH levels and consequently was not very toxic. Iron and aluminum salts were also not toxic because of their low solubility. Concentrations of the more toxic heavy metals (copper, zinc, and nickel) which could be tolerated were related to the concentration of sulfides available to combine with the heavy metals to form the very insoluble sulfide salts.

d) Toxic Organic Materials

There are many organic materials which may inhibit and may be toxic to the digestion process of such substances as formaldehyde, alcohol, long-chain fatty acids, chlorinated compounds, and aromatic compounds, etc. For example, methanol is reported to be detrimental to anaerobic treatment at concentrations between 1000 to 2000 mg/l. Sodium oleate is found to be inhibitor at concentrations exceeding 500 mg/l (McCarty, 1964). Noack stated that phenol concentration between 0.1 - 0.4% of the total solid (TS) can be inhibitor in the digester, and it will be toxic if the content is exceeding this range (referred to by Van Velsen & Lettinga, 1980).

Chlorinated compounds such as CCl_4, $CHCl_3$, and CH_2Cl_2 are widely known to be toxic at a low concentration. However, a recent experiment indicated that the digestion process can be restored from a $CHCl_3$ inhibition by stripping it from the system with N_2, provided the $CHCl_3$ concentration is not exceeding 2 to 3 mg/l (Lettinga *et al.*, 1979).

Speece (1985) conducted an experimental observation with cyanide, chloroform, formaldehyde and other toxicants, he found that the methanogens had the ability to recover from and acclimate to relatively high concentration of these toxicants. Nevertheless, the information available in this field is not yet sufficient; the more comprehensive assays are still needed.

Recently Koster and Cramer (1986) reported the effect of four saturated long-chain fatty acids (caprylic, capric, lauric, and myristic) and one unsaturated long-chain fatty acid

(oleic) on microbial formation of methane in UASB reactor. They found that lauric acid appeared to be the most versatile inhibitor. Moreover, it was able to act synergistically, increasing the toxicity of capric acid and myristic acid.

2.3.7 Organic Loading Rate

Loading rate can be defined in various terms as hydraulic loading, which is controlled by influent flow rate (or detention time), and organic loading, which uses organic matter input to the system (BOD or COD) as a controller. Another parameter, solid loading, employs the amount of influent volatile solids (VS) to control the unit function. Thereby, organic loading rate is varied with hydraulic retention, volatile solids and COD concentration of substrate (according to the equation below), but also affected by temperature.

$$\text{Organic Loading} = \frac{(\text{VS or BOD or COD}) * Q}{V}$$

where Q is the rate of influent and V is the effective volume of the digester.

At the overloading rate, it causes the digester to accumulate volatile acids which are known as "sour condition." This may result in reducing both pH-value and the treatment efficiency. On the contrary, in the underloading circumstances, the rate of gas production obtained is very low. (Polprasert and Thanh, 1979).

2.3.8 Hydraulic Retention Time (HRT)

The resident time of the feed in the digester before it is replaced with new material is theoretically calculated by dividing the effective digester volume (V) by the rate of material input as follows:

$$HRT = \frac{V}{Q}$$

Retention time is equal to the reciprocal of the microbial growth rate when it is long enough to allow the maintenance of the microbial population. There is a minimum HRT below which efficient fermentation ceases, due to the wash-out of the microbial population.

2.3.9 Solid Retention Time (SRT)

The relationship between microorganisms growth rate and SRT is given by : $1/\mu = SRT$, where μ is the specific biological growth rate (day^{-1}). To prevent the washout of slowly-growing anaerobic bacteria, a minimum SRT must be maintained so that microorganism growth rate exceeds the washout rate determined by HRT. These conditions require large reactor volumes with their associated capital costs.

New anaerobic technologies have incorporated changes which allow the HRT and SRT to be varied independently. Typically, the solids in the reactor effluent are allowed to settle and are recycled back to the reactor influence. This modification, called high-rate anaerobic process, allows long SRTs to be maintained even with large hydraulic (short HRT) throughput and, as a result,

the size of the reactor and associated cost have been reduced significantly. Recycling the solids allows a faster development of a biological population, thereby reducing start-up time. (Christensen Gerick & Eblen, 1984).

2.4 The Upflow Anaerobic Sludge Blanket (UASB) - Concept

This upflow reactor has been developed by Lettinga ever since 1971 (Lettinga, Velsen, Hobma, Zeeuw & Klapwijk, 1980b). The basic idea underlying the concept is that a high sludge concentration can be maintained by mounting the settler in the upper part of the upflow reactor. Lettinga, who has improved a more effective settler, and called it the Upflow Anaerobic Sludge Blanket (UASB) process. The basic operation of the UASB reactor is that the wastewater is fed into the reactor from below and leaves it at the top settler for separation of gas, sludge, and liquid. The sludge particles are then settled back towards the digesting zone, thus ensuring adequate sludge resident times and high anaerobic sludge concentrations in the reactor.

We can say that the main components of the UASB reactor are the feed-inlet distribution system and the settler or Gas Solid Separator (GSS)-device. The schematic diagram of UASB reactors is shown in Fig. 2.10. One of the main feature of the UASB process is a so-called granular sludge in the reactor. The anaerobic sludge can be flocculated and formed into granules, which have excellent settling properties, under proper physical and chemical conditions (Lettinga & Vinken, 1980a). In contrast to other anaerobic high rate processes,

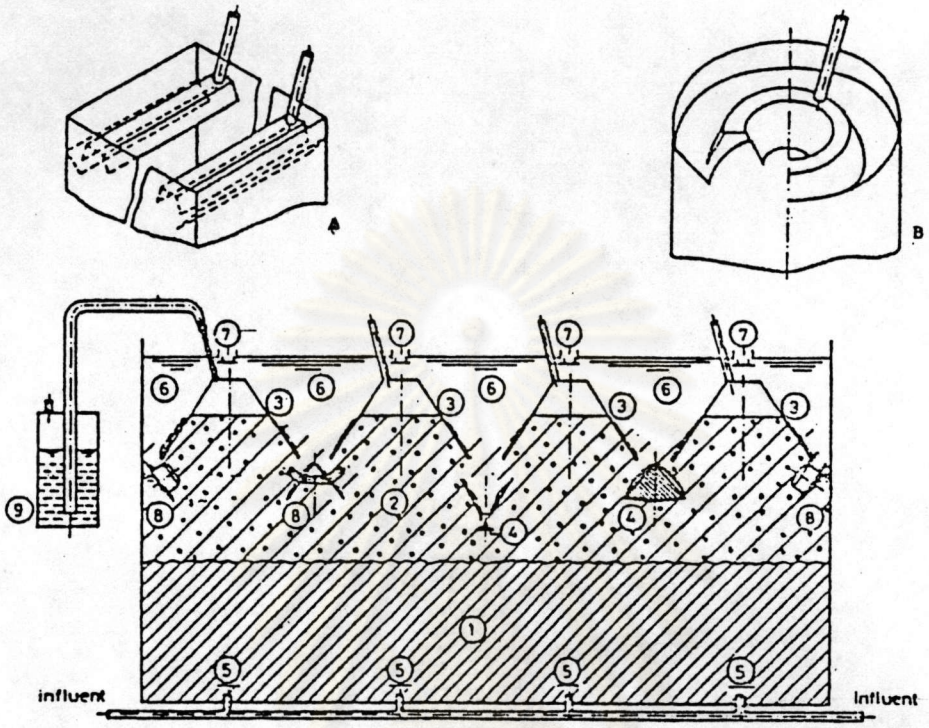


Fig.2.10 Schematic diagrams of a large scale UASB plant (ZEVALKINK, 1982)
A. Rectangular reactor
B. Cylindrical reactor

(1) sludge bed,
(2) bulk of liquid with suspended sludge,
(3) gas collector,
(4) gas seal,
(5) feed inlet,
(6) settler compartment,
(7) launderer,
(8) gas collector with gas outlet pipe to (3)
(9) water seal

the UASB is operated entirely as a concentration biological growth and consequently uses no packing materials to support itself. Mechanical mixing and/or sludge recirculation are kept at a minimum (or even completely omitted) to improve the settleability of the sludge.

2.4.1 Granulation of Anaerobic Sludge in UASB Reactor

As mentioned above, the sludge in the UASB reactor can be formed in spherical flocs with a quite consistent structure referred to as granular sludge. The granular type of methane anaerobic sludge has a high methanogenic activity and excellent settleability. Then reactors can be operated stably at a high volumetric COD loading rate even when the hydraulic retention time is very short. Many satisfactory results are obtained from various types of UASB reactors, both from laboratory and pilot-plant scale, as well as the full-scale experiments. This can be seen in part of the literature data of previous experiments concerning the UASB process (item 3.4, Chapter 3).

So far the mechanism underlying the granulation process has not yet been clear; it is still one of the main studies for several researchers, e.g., Zeeuw & Lettinga (1981), Hulshoff Pol, Zeeuw, Velzeboer & Lettinga (1983) and Weigant & Lettinga (1985), etc. However, the granulation or pelletization process is not restricted to methane anaerobic sludge. Pellet-formation can occur in an upflow sludge bed (USB) reactor serving for denitrifying nitrified final effluent mixed with settled domestic sewage. These pellets are formed only at high surface loads (8 m/h) and contained up to 50%

$CaCO_3$, with pellet diameter up to 5 mm, but are rather unstable. Including the granulations in the acidification of glucose, with diameter of about 1 mm, undertaken by the residence times shorter than 6 hours at a glucose concentration of 50 Kg/m^3, are also reported to be unstable under unfed conditions (quoted by Hulshoff Pol *et al.*, 1983). Comparatively, the "methanogenic" granulations take place at higher residence times and have far more stable granules. These are the evidences that the mechanism of the formation of methanogenic granules differs from those of denitrifying and acidifying bacteria (Hulshoff Pol *et al.*, 1983). With referrence to existing literature, the data concerned has been obtained that the methanogenic granular sludge can remain stably in the UASB reactor for several years under unfed conditions (Lettinga *et al.*, 1980, Hulshoff Pol *et al.*, 1983, Wu *et al.*, 1985).

2.4.2 Microbiology of Granular Sludge

Lettinga, Hulshoff Pol, Koster & Wiegant (1984) reported that the different types of granulation sludge may develop on the nature of the seed sludge, the composition of substrate, and the conditions applied during the start up.

"Sarcina" granules developed when a high concentration of acetic acid was maintained in the reactor, i.e., methanosarcina with diameter of approximately 0.5 mm.

"Rod" granules consisted predorminantly of rod-shaped bacteria in fragments of approximately five cells. It developed not only on the potato-processing waste and sugar-beet wastes in full scale plants, but also on VFA substrate when the

digested sewage had been enriched with a small amount of (crushed) granular sludge of the "rod" type, i.e., methanothrix with diameter of approximately 2 mm.

"Filamentous" granules mainly consisted of long multicellular rod-shaped bacteria. These granules developed on pure VFA substrate and digested sewage sludge of a relatively high specific methanogenic activity (0.12 $KgCH_4$-COD/KgVSS-d), i.e., Methanothrix soehngenii with diameter of approximately 5 mm.

"Spiky" granules, which were very uniform in shape and size, contained up to 60% $CaCO_3$. These granules were up to 1 mm long and less than 0.5 mm thick, and developed on maize-starch waste in a 900 m^3 full scale UASB.

Regarding a high-rate anaerobic reactor with low strength wastes, Lettinga *et al.* (1985) concluded that a sarcina type granule was less desirable because it exerted a rather poor activity at lower acetate concentrations and consequently provided an unsatisfactory treatment efficiency unless the process was operated at low sludge loading rates. Another reason to prevent the development of sarcina was that they generally remained smaller in size ($\phi < 0.5$ mm), so they easily washed out from the reactor. In this case the rod type and the filamentous type were preferred in a UASB process. And a filamentous type was referred to by Cail and Barford (1985) that, although it was undesirable from the sludge "bulking" aspect, under proper condition, this filamentous type could form the granules and had a high methanogenic activity.

Recently, an experimental result obtained by cultivating granular sludge with glucose and brewery wastewater revealed that most of the granules consist of Methanothrix, Methanobacterium formicium, and Methanosarcina. And the biofilms of such granules were also observed and referred to as methanogenic bacterial protection against unfavourable impact such as shock loading and low pH in the short term (Wu *et al.*, 1985).

2.4.3 Requirements for Granulation

As stated earlier, although the granulation process has not yet been clearly understood at present, it can be summarized by the beneficial growth factors for granulation as follows:

(a) Types of The Seed Sludge

The reports discussed by Lettinga *et al.* (1985) revealed two types of digested sewage sludge as proper seed materials: thicker types (approximately 60 KgDS/m^3) and thinner types (< 40 KgDS/m^3). The thicker types are preferred because of better settleability. Although they are generally lower in specific methanogenic activity (in terms of $KgCH_4$-COD/KgVSS-d), it is recommended that 12-15 KgVSS/m^3 suffice for mesophilic reactor start-up. And they further described the "inert carrier material" that it played an important role in sludge granulation. The rate of granulation may be enhanced by supplying crushed inert granular carrier material (hydro-anthracite or gravel) to the sludge. Similar observations have been made by other researchers (referred to by Lettinga *et al.*, 1985).

(b) Types of Wastewater

Several evidents indicated that granulation can be made to occur by cultivating various types of wastewater, viz., high, low, or medium strength wastewater, i.e., sugarbeet waste (Pette & Vletter, 1980), potato processing waste (Pette & Versprille, 1981), brewery waste (Wu *et al.*, 1985), slaughter house waste (Sayed, Zeeuw & Lettinga, 1984), maize starch waste (Zeevalkink & Maaskant, 1984), and domestic waste (Fernandes, Cantwell & Mosey, 1985), etc. According to the experimental results obtained in mesophilic UASB-reactor start-up by Lettinga *et al.* (1985), they found that granulation proceed faster for lower strength wastes. They described that it related to the selection pressure effects, namely, the wash-out of finely dispersed matter, whereas the heavier fraction would be retained in the reactor. As a consequence, they recommended application of effluent recycle or dilution when UASB-reactors must be started up for higher strength wastes (COD > approximately 5000 mg/l).

The presence of higher concentration of inert dispersed solid in the wastewater was detrimental for the development of granular sludge because a significant bacterial attachment of newly formed bacterial matter to the continuously supplied "fresh" surfaces would retard or even stop the development of granules (Lettinga *et al.*, 1985).

(c) Physical and Chemical Condition

-Temperature : has a major effect on the bacterial growth rate and activity; the mesophilic temperature (35° -

40°C) is regarded as the optimum condition, based on the activity and economy as well as on the operation aspects (Lettinga *et al.*, 1979).

-pH and Alkalinity : pH should be maintained between 6.5-7.8; however within the pH range of 6.3-8.0, no difference could be observed in the conversion of the digesters (Pette *et al.*, 1980). It is necessary to add a buffering agent to the influence to keep pH at a desirable level. The experiment with brewery wastewater reported by Wu *et al.* (1985) has shown that alkalinity of 800 mg/l as $CaCO_3$ in their feeding solution was sufficient for granulation.

-Effect of Ca^{2+} : It is well-known fact that divalent cations exert a positive effect on the flocculation of anaerobic sludge. Several experiments concerned are reported that the wash-out of sludge during the initial phase of start-up can be reduced by increasing the Ca^{2+} concentration in the feed solution. It is presumable that small $CaCO_3$-crystals act as a carrier for bacterial attachment (Hulshoff Pol *et al.*, 1983). Nevertheless, a serious effect may arise because of high Ca^{2+}-concentration in a wastewater because a considerable fraction of the active bio-mass present in the granule may be lost due to severe $CaCO_3$-scaling at the outer borders of the granule. Another problem is the increase in the specific density of the granule; as a result, not only high sludge concentration gradually develops in the lower part of the reactor but also it becomes impossible to make contact between the wastewater and the sludge sufficiently (Lettinga *et al.*, 1985). It is not yet possible to provide more exact figures concerning the effect of Ca^{2+}

concentration; however experiments with very low and high concentrations will be carried out in the near future (Cail and Barford, 1985).

-Effect of NH_4^+ : A compound of ammonia is often considered as an important effect because it can be occurred in various types of wastewater. The adverse effect of ammonia is found when NH_4^+-concentrations are as high as 1000 mg/l. Cail and Barford (1985) reported that concentrations of NH_4^+ greater than 1000 mg/l are inhibitory to granulation while Hulshoff Pol *et al.* (1983) found that there was no real granulation in high NH_4^+ concentration.

-Inhibitors and Toxic Compound : These substances, including formaldehyde, cyanide, etc., should be absent in the feeding, if possible, especially during the initial stage of granulation, because they can kill the bacterial population in the digester (Lettinga *et al.*, 1985).

(d) Nutrient

The growth factor of macro nutrients, i.e., N, P and S must be present in sufficient concentrations and in available form. The requirement of COD : N : P for the cultivation of granular sludge is the same as in general anaerobic processes. As an application for S, the experimental results obtained in batch fed experiment with VFA-feeds reveal the positive effect on growth of methanogenic bacteria upon addition of 0.1 mM/l S^{2-} (Lettinga *et al.*, 1985). This includes trace elements which also have significant effect on the growth of bacteria. It should be supplemented to the system, especially to the one treating wastes which often do not have

enough of these elements. Addition of trace elements such as Ni, Co, Mo, and $ZnSO_4$ into the digester results in positive effects (Lettinga *et al.*, 1985).

(e) Operational factors

In the past, anaerobic process was known as a very slow start-up digester. But at the present time, this disadvantageous problem seems to disappear because of technological advances and the experiences in the experiments concerned. Although the mechanism of granulation in the first start-up is still unclear, many reports show a rapid start-up of the UASB reactor. Based on the available experiences, so far, we can conclude that frequently the granular sludge appear provided the conditions for growth (as described above) and the operational factors are proper. These operations are especially the required selection mechanisms between heavier and lighter sludge fractions. The most significant operation factors are stable organic loading and higher hydraulic loading rate; namely, during surge loads, under-loading may lead to development of voluminous sludges whereas over-loading lead to higher gas production which hampers the settlement of sludge in the settler (Hulshoff Pol *et al.*, 1983). As for the higher hydraulic loading rate, the lighter sludge will move upward easily whereas the heavier sludge will move downward. Thereby, the bacteria in the granules at the bottom of the reactor obtain the influence food first; so they grow quickly (Lettinga *et al.*, 1985), while bulking floc sludges are discharged from the reactor much more easily.

Wu *et al.* (1985) recommended that the hydraulic loading rate at 0.25-0.4 m^3/m^2-h was high enough for granulation. Excellent results had been obtained in a 1400 m^3 full scale UASB plant treating wastewater of the Bavaria Brewery in Lieshout. Wastewater consisting of fractions of discharges from a malting plant, the brewery, and a soft drink plant was fairly dilute (COD approximately 1.5 g/l) and low in temperature (15° - 23°C). It was reported that the first start-up was accomplished within one week (this is referred to by Lettinga *et al.*, 1985).

The tentative guidelines for the first start-up of UASB reactor using digested sewage sludge are suggested by Lettinga *et al.* (1983) in Table 2.5 as follows:

Table 2.5

Tentative guidelines for the first start-up of an UASB-plant

1.	Amount of seed sludge : 10-20 KgVSS/m^3
2.	Initial sludge load : 0.05-0.1 KgCOD/KgVSS·d
3.	The space load should not be increased unless all VFA's are well degraded (> approx.80% for medium strength wastes))
4.	Voluminous sludge should be allowed to wash-out, the heavy part of the seed sludge should be retained.

2.4.4 Characteristic Pattern of Granulation

According to the forms of the sludge in the reactors and COD loading rates achieved, the granulation processes can be divided into three or more stages. The experimental results of brewery wastewater are shown in Fig. 2.11 (Wu *et al.*, 1985).

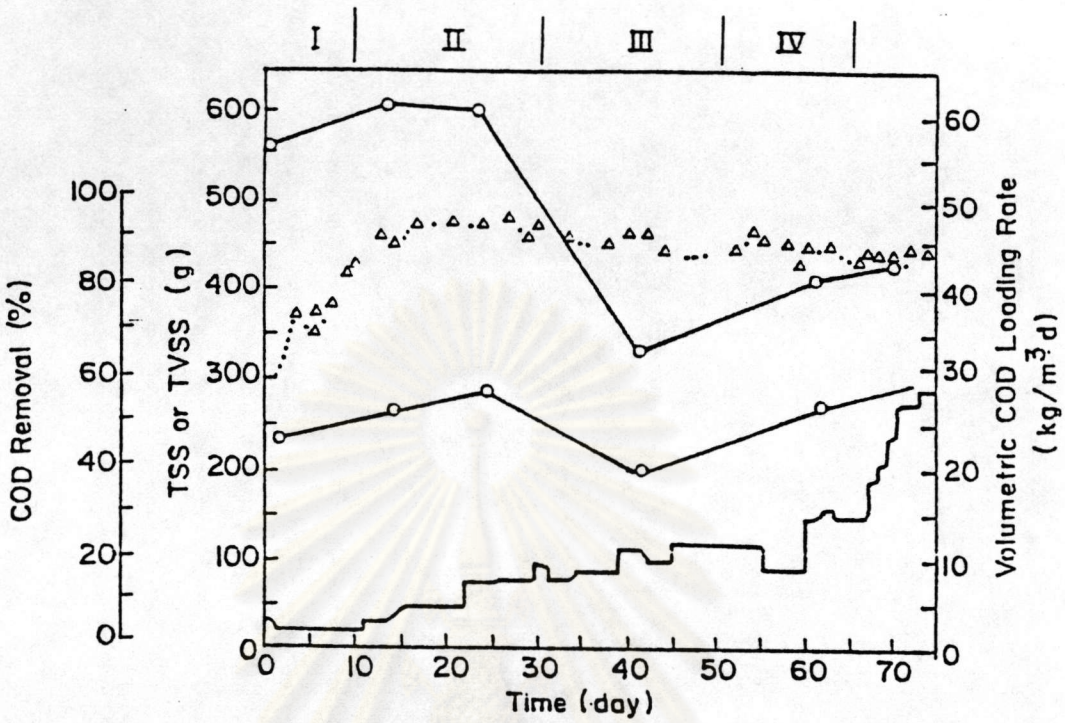


Fig.2.11 The stage of start - up (Wu et al., 1985).

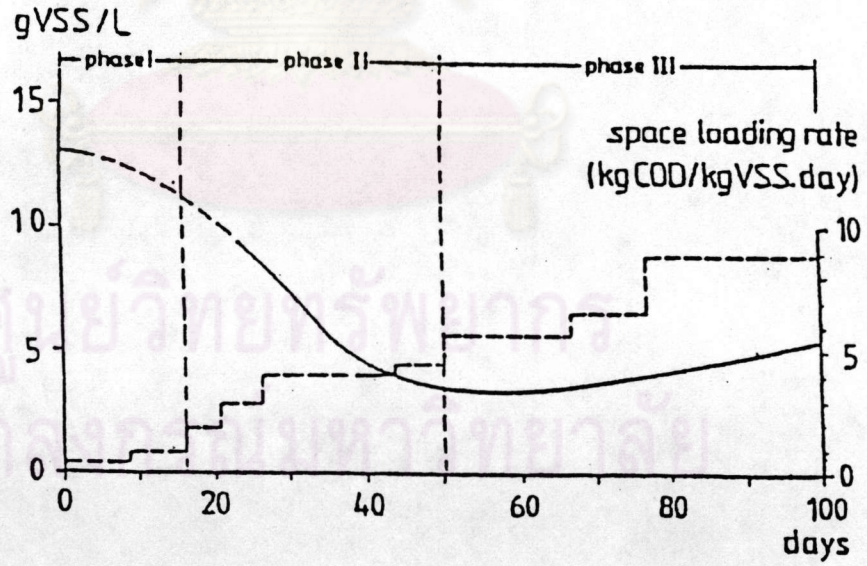


Fig.2.12 Development of the sludge-concentration and organic space loading rate during the granulation-process in reactor. (Hulshoff, Zeeuw and Lettinga 1983).
——— VSS-concentration
- - - - organic space loading rate

Stage I - The stage of start-up

This period begins with the addition of seed sludge and ended with the volumetric organic loading rate up to 5 Kg/m^3-d. In this stage the sludge bed will markedly expand of a result the initiation of gas production.

Stage II - Appearance of the granules

In this period, granular sludge begins to appear and floc-forming sludge is wash-out or lost. Finally, the amount of the heavier sludge is concentrated in the lower part of the reactor. Apparently the UASB-system promotes a selection between the sludge ingredient such that heavier particles are retained. Growth will be concentrated at these particles, which ultimately result in the formation of distinct granules with diameters up to 5 mm.

Stage III - Formation of granules

In this period, granules formed very fast, the sludge bed is filled with granules, and the volumetric COD loading rate is increased to 16 Kg/m^3-d, or higher. The concentrated granular growth now exceeds the sludge wash-out. After a period of stagnation (Stage III), the organic loading rate can be further increased to its maximum value, which may be as high as 30 KgCOD/m^3-d at 30°C.

Stage IV - Steadiness after granulation

In this period, the sludge

settleability has improved steadily, the wash-out will hardly occur.

Hulshoff Pol *et al.* (1983) operated a granulation process experiment. In almost all experiments, a very similar characteristic pattern was observed concerning the behavior of the system with respect to the sludge retention (or wash-out) as illustrated in Fig. 2.12.

Phase I : In this phase the sludge bed markedly expands as a result of the initiation of the gas production and of the increasing surface load applied. Growth of filamentous organisms will cause a deterioration of the sludge settleability.

Phase II : The heavier particles are retained. Growth will increasingly be concentrated at these particles which ultimately results in the formation of distinct granules with diameters up to 5 mm and the color is black or dark brown.

Phase III : The concentrated granular growth now exceeds the sludge wash-out. After a period of stagnation the organic loading rate can be further increased to its maximum value which may be as high as 50 KgCOD/m^3-d at 30°C.

The main characteristics at the three stages of granulation by Wu *et al.* (1985) are listed in Table 2.6.

2.4.5 Design Criteria

1. Feed Inlet Distribution System

Many relevant results indicate that only one feed inlet nozzle per every 5-10 m^2 is sufficient for the UASB

reactor applied with low-strength wastewater (1-2 KgCOD/m^3-d) under moderate climatic conditions (referred to by Lettinga, 1984). But in the case of achieving higher loading rate or treating cold and/or dilute wastewater, it often poses risk to channelling in the sludge bed. This is due to that 1) sludge exerts a high settleability 2) gas production is not high enough for mixing or even 3) height of

Table 2.6

Characteristics at different stages of granulation (Wu *et al.*, 1985)

Stage	I	II	III
Form of sludge	dispersed and/or flocculent	flocculent	granular
Interface between blanket and bed	clear	not clear	clear
SS in blanket (g/l)	10	18 - 30	5 - 16
Sludge washout or loss	did not appear	appeared	did not appear
Discharge of sludge from blanket	not needed	needed	not needed
COD loading rate (Kg/KgVSS-d)	0.3	0.3 - 0.6	0.6
Protozoa	a lot	a few	few

sludge bed is too low. These problems can be solved by applying more sophisticated feed-inlet distributors. And some rough guidelines for required number of nozzles are present in Table 2.7 by Lettinga *et al.* (1984) as follows :

Table 2.7

Rough guidelines for the number of feed-inlet nozzles required in a UASB reactor

Type of sludge	Area (m^2) per nozzle
1. Dense flocculant sludge (exceeding 40 KgDS/m^3)	One at loads less than 1-2 Kg COD/m^3-d
2. Thin flocculant sludge (less than 40 KgDS/m^3)	Five at loads exceeding approximately 3 KgCOD/m^3-d
3. Thick granular sludge	One at loads of approximately1-2 COD/m^3-d

2. The Gas-Solids Separators (GSS)

As we have known, a significant characteristic of anaerobic is very slow growing of (methanogenic) bacteria. Therefore washing-out of sludge can easily lead to decrease in the amount existing. To prevent this matter, the settler must be mounted in the upper part of the reactor. The settler, or a so-called Gas-Solid Separator (GSS) device, actually has several functions, unlike its name, as follows:- (Lettinga *et al.*, 1984)

1. Separation of the biogas from the mixed liquor and from floating sludge particles.

2. Separation of dispersed sludge particles or flocs by settling, flocculation and/or entrapment in a sludge blanket (if) present in a settler compartment.

3. Enabling the separated sludge to slide back (e.g., as large aggregates) into the digestive compartment.

4. Restricting excessive expansion of the sludge blanket.

The performance of the settler or GSS-device is shown in a cross-section of schematic diagram (in Fig. 2.13) by Van der Meer and Vlettler (1982). We can see that the influent, or entering stream, the effluent, and the recirculation stream are separated from one another. After separation of the gas, the suspension of solid matter and liquid enters a compartment where dissolved gas that is released by expansion can escape, and where turbulence originating from the reactor can be damped. Finally, the sludge is separated from the effluent in the settling compartment where laminar liquid flow exists, and where (in the lower part) a sludge blanket is formed. From this compartment the thickened sludge can sink back into the reactor.

So far stringent design criteria for a more sophisticated GSS-device is not yet provided; however, Lettinga has suggested a number of guidelines collected from his experimental experiences for designing this device as follows: (Lettinga *et al.*, 1980, 1983 a,b).

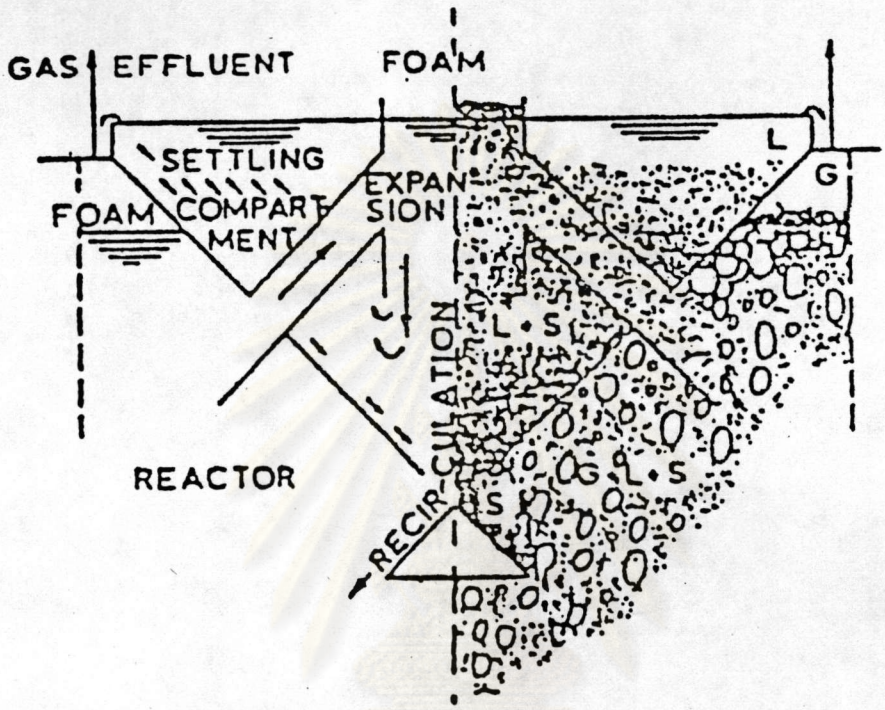


Fig.2.13 Schematic generalized picture of the cross section of settlers used in the pilot plants and the full-scale reactor. At the left side of this figure, the flow patterns of the fluid and sludge are shown. At the right are indicated the presence and distribution of sludge (S), gas (G), and liquid (L) In the compartments in and below the settler. (Meer & Vletter, 1982).

a) The inclined wall of the settler should be at one-angle of approximately 50°.

b) The surface load of the settler should be kept below about 0.7 m/hr, and the average flow through the aperture between the gas collectors below about 2 m/hr.

c) The development and maintenance of a well settling sludge should be enhanced by adequate measures.

d) The surface area of the liquid-gas interface in the gas collector should not be too small (because it might lead to severe scumming), and the sludge present in this gas collector should be kept well immersed.

e) The height of the settler compartment should not be lower than 1.5-2 m (in order to keep blanket well below in preventing sludge wash-out).

3. Organic Loading Rate

Most of the full-scale UASB reactors built so far are designed at a loading rate of 5-18 KgCOD/m^3-d. Despite that, the higher organic loading rate can potentially be applied from several evidences, both large pilot-scale and small scale as well as bench scale UASB experiments. For example, the experimental results by Hulshoff Pol *et al.* and Lettinga *et al.* indicated that the loading rate up to 40-60 KgCOD/m^3-d at 30°C could be achieved, for mainly soluble wastes. The experiment by Wiegant and de Man 1986 was reported that under thermophilic condition even loading up to 162 KgCOD/m^3-d could be accommodated, etc. Lettinga *et al.* (1985).

referred to the main reason for this conservative design criteria of loading rate as the lack of full-scale experience of UASB operating reactor.

4. Hydraulic Retention Time

From the numerous experimental results in part of the literature dealing with the UASB process (item 3.4, Chapter 3), very short hydraulic retention time (3-8 hrs) could be applied with low and medium concentrated wastewater (1-3 Kg/m^3 of soluble COD). With more concentrated wastewater (10 - 50 Kg/m^3 of soluable COD) hydraulic retention time of approximately 1 day have to be applied. In both cases we can see that a reduction of 80-98% of a soluble COD could be obtained.

5. Temperature

A number of the experimental results observed from various types of UASB experiments applied with variety of wastewater indicate that the temperature has a crucial effect on the system. Most of the high efficiency treatment (COD reduction > 90%) is obtained from the system operated under mesophilic temperature. We can see that the more temperature increased, the higher loading can be applied. Notwithstanding, Lettinga *et al.* (1979b) found the reverse effect, under the mesophilic condition, that a temperature exceeding 45°C was detrimental particulary if the temperature "shock" lasts longer than one day.

Lettinga *et al.* (1982) provided guidelines for designing the capacity of UASB reactors treating mainly soluble wastes related to temperature in Table 2.8.

Table 2.8

Tentative rough design-capacities for UASB-reactors in relation to the temperature (Lettinga *et al.*, 1983).

Temperature (°C)	Design-capacity ($KgCOD/m^3$-d)
40	15 - 25
30	10 - 15
20	5 - 10
15	2 - 5
10	1 - 3

6. Sludge Hold-up and Sludge Bed Height

So far the maximum amount of granular sludge in UASB reactor has not been provided because of a limited number of relevant experimental data concerned, especially the sludge bed height. However, we has already known that this matter is closely connected to the loading potential applied, namely, the more amount of sludge the reactor contains, the higher is the loading of the system. For example, the experiment carried out in a 6 m^3 pilot plant in which a dense-mainly granular sludge bed occuppied the lower 1-2 m of the reactor (with a more flocculant sludge blanket above it) has shown that space loads up to 45 $KgCOD/m^3$-d with potato processing waste and 30 $KgCOD/m^3$-d with sugar beet waste can be well accommodated (Lettinga *et al.*, 1982).

The basic design criteria for reactors of 30 m^3 and 800 m^3, treating liquid sugar wastes and beet sugar wastes respectively, are given in Table 2.9.

Table 2.9

Basic Design Future of the Anaerobic Treatment Plants in Operation in 1979.

	30-m^3 Plant	800-m^3 Plant
Tank configuration	Cylindrical	Rectangular
Building material	Steel	Concrete
Height (m)	6	4.5
Bottom surface (m^2)	5	178
Depth of digesting zone (m)	4.9	3.3
Depth of settling zone (m)	1.1	1.2
Type of wastewater	Liquid sugar	Beet sugar
Organic loading (KgCOD/d)	400	13,000
(KgCOD/m^3-d)	13.3	16.25
Influent concentration (mgCOD/l)	17,000	3,000
Purification efficiency (%)	94	88
Average hydraulic flow (m^3/h)	1	180
Peak flow (m^3/h)	1.5	240
Hydraulic Settler Surface load (m^3/m^2-h)	0.5	1.5
Gas production (m^3/m2-h)	1.7	1.2