CHAPTER 2

REVIEW OF LITERATURE

2.1 GENERAL

Managing limed fleshings (LF) generated from tannery is one of the major problems in the tanning industry. From the studies carried out earlier, it is observed that more than 95% of the fleshings generated are disposed off unscientifically in open dumps and landfills, creating nuisance to public health and environment. Considering the above, a new process was developed for efficient anaerobic digestion of limed fleshings. Various pretreatment methods were studied to liquefy the limed fleshings and treat liquefied limed fleshings (LLF) along with tannery effluent (TE) in anaerobic reactor. The various investigations in this area of research has been reviewed and presented in this chapter.

2.2 LEATHER PROCESSING

"Leather tanning" is a general term for the numerous processing steps involved in converting animal hides or skins into finished leather. Chrome tanning accounts for approximately 90% leather production in India. Trimming, soaking, liming, fleshing, unhairing, deliming and bating processes are referred as the beam house operations. Further processes like pickling, tanning, saddling, splitting and shaving are referred to as tanyard operations. Retaining, dyeing and fat-liquoring processes constitute wet finishing. Finishing processes include setting, dyeing, conditioning, staking,
toggling, dry milling, buffing, spray finishing, and plating (Buljan et al 1997).

Process flow diagram of leather processing is given in Figure 2.1.

**Figure 2.1 Process flow Diagram of Leather Processing**

Description of each unit in leather processing is given below:

- **Soaking:** The skins or hides are soaked in water to restore moisture removed during preservation and to remove salt.

- **Un-hairing and Liming:** In this process, skins and hides are treated with lime and reducing agents like sulphide or enzymes to remove hair and other keratin matter as well as opening of fibre structure of hides through osmotic swelling.
and plumping and also unharing flesh is loosened and natural fat is removed. Mechanical process is used to remove hair.

- **Fleshing:** By mechanical process, excess flesh and fatty substances are removed.

- **Deliming and Bating:** The hides are drum-washed with water and treated with ammonium salt and other deliming agents to remove any residual un-hairing chemicals and then enzymes (i.e., "bates") are added to remove undesirable hair roots, pigments, etc.

- **Pickling:** The hides are soaked in a salt and acid solution to prepare them for tanning.

- **Tanning:** In large revolving drums, the hides are "chrome tanned" (i.e. tanned with basic chromium salts) which results in a more stable, resistant and flexible leather product.

- **Samming and Sorting:** The hides are put through rollers to remove excess moisture and are sorted for thickness.

- **Splitting and Shaving:** The flesh side underneath the grain is cut off on a splitting machine and shaved to achieve desired thickness.

- **Re-tanning, Dyeing and Fat liquoring:** The hides are tanned again with other chemicals to achieve certain desired properties (e.g., softness, fullness, etc.) and then are drum-dyed for the desired color and finally are "fat liquored" (with oil-in-water emulsion) to lubricate the fibers and make them soft.

- **Setting Out and Drying:** The hides are mechanically smoothed, stretched, compressed and then dried. Drying
methods include hanging or pasting the hides on boards and passing them through an oven or vacuum drying.

- Conditioning: Hides are kept covered in moist saw dust or water is sprayed on the hides to rewet them to the desired moisture level.
- Milling: The hides are put onto a dry drum where they are tumbled for various lengths of time to achieve the desired softness, texture and grain pattern. In some cases, water may be added to the milling drum to render the hides softer and more flexible.
- Leather finishing: Leather may be finished in a variety of ways: buffed with fine abrasives to produce a suede finish; spray coated with wax, or treated with pigments, dyes, and resins to achieve a smooth, polished surface and the desired color; or lacquered with urethane for a glossy patent leather. Water-based or solvent-based finishes may also be applied to the leather. Plating is then used to smooth the surface of the coating materials and bond them to the grain. Hides may also be embossed.

2.3 SOLID WASTE FROM TANNERIES

It has been estimated that one ton of wet salted hide results in 800kg of solid waste which includes raw trimmings, fleshings, shavings, trimmings and buffing dust, out of which fleshings is about 200 kg (Ratna 2003). Fleshings is a thin layer appended to the corium consisting of proteineous loose connective tissue or adipose tissue; it is a loose connective tissue lying between the hide or skins and the actual body of the animal (Sarkar 1997). Major solid waste generated during the pre-tanning process is
about 50-60% of total waste generated in leather industry (Kanagaraj et al 2006). Tanneries all over the world face problems due to disposal of solid waste by dumping. The disposal cost is also high and large land area is require for disposal. Odour nuisance is also one of the issues (Roberto 2006).

2.3.1 Characteristics of Limed Fleshings

It was reported by Tarlea (2006) that moisture content of limed bovine fleshings were 80-85%, ash content 13-15%, fatty matter 9-15% and pH was in the range of 10.5-12.5. However, the results reported were for bovine of Romanian origin where due to climatic conditions, fat content is higher compared to Indian bovine. In order to protect the ecosystem, LF is to be neutralized before they are disposed to the environment (Ozgunay 2007).

2.3.2 Characteristics of Tannery Effluent

Wastewater generated from tanneries are treated in Common Effluent Treatment Plants (CETPs) or Effluent Treatment Plant (ETP) with primary treatment followed by two stage biological system. First stage biological systems is provided with anaerobic lagoon followed by second stage extended aeration system in most of the CETPs. Typical characteristics of TE reported were pH: 7-9; BOD: 1230-1700 mg/L; COD: 5900-6300 mg/L; sulphide: 130-150 mg/L; sulphate: 1400-1600 mg/L; chlorides: 5000-6000 mg/L; TS: 20000-25000 mg/; TDS: 16000-21000 mg/L; SS: 2000 - 4000 mg/L; Total chromium: 20-100 mg/L (NEERI 1997). Similar results were also reported by Krishnamoorthy et al (2009) and Haydar et al (2007).

2.4 TREATMENT TECHNOLOGIES FOR SOLID WASTE

In this section, literatures reported on various technological options for solid waste have been reviewed.
2.4.1 Composting

Composting is another biotechnological process by which different microbial communities convert organic waste into a stabilized form. During the composting process, temperatures increase because of the heat released due to biological activity. Composting is an aerobic process that requires oxygen, optimal moisture and enough free air space and C/N ratio within certain limits (Haug 1993).

Limed Fleshings (LF) generated as solid waste from tanning industries was subjected to vermi-composting using the epigeic earthworm Eisenia fetida. It was reported that fleshing was washed in large quantities of water to remove lime. The washed fleshing was then treated with 10 mL/L hydrochloric acid (HCl) solution to remove sulphide and calcium salts (Annapurna et al 1997), followed by chopping them into smaller pieces. These pieces were again washed thoroughly in tap water and then mixed in the ratio of 3:1:1 with fleshing, cow dung and agricultural waste respectively (Ravindran 2008). In this reported process, wastewater generated from washing was to be further treated by adding HCl which reacts with lime and generate harmful fumes and smell.

The concept of Sequential Batch Anaerobic Composting (SBAC) developed at the Agricultural Engineering Department, University of Florida, USA has been used to overcome the limitations of most designs for anaerobic digestion, such as the requirement for heavy inoculation, mixing, possibility of instability etc (Kayhanian et al 1992). The plant was used to treat two fractions of Municipal Solid Waste (MSW), the organic fraction of the processed MSW and yard waste. The sequential batch anaerobic composting of the two primary organic fractions has been reported to be stable, reliable and effective.
It is also reported that composting of fleshing along with other wood based organic waste needs a longer time for fermentative exothermic processes, necessary for the stabilization of the composting. Therefore, it was reported that this process cannot be implemented in full scale treatment (Roberto 2006).

2.4.2 Incineration / Pyrolysis

Animal waste has been traditionally disposed of by incineration or land-filling, which are not sustainable alternatives since they only allow for low recovery of resources (Raquel et al 2009). Due to high moisture content in fleshing i.e higher than 70%, incineration is unviable. (Roberto 2006). Meat and bone meal, similar to fleshing, is treated and disposed by incineration, landfill and anaerobic digestion. In the European Union (EU) and the USA, incineration has been intensively investigated and widely used for meat and bone meal disposal with the aim of energy recovery (Conesa et al 2005). However, dioxins and toxic gases such as sulfur dioxide (SO$_2$) and nitrous oxide (N$_2$O) are released during incineration causing air pollution (Goeran et al 2002; Conesa et al 2005). In addition, investment cost for this process are also high and hence it could not be implemented in full scale in developing countries like India.

2.4.3 Anaerobic Co-digestion

Another method considered for disposal of fleshings and other organic solid waste is anaerobic co-digestion. Other methods for improving yields of anaerobic digestion of solid wastes are co-digestion i.e. the use of a co-substrate, which in most cases improves the biogas yields due to positive synergy established in the digestion medium and the supply of missing nutrients by the co-substrates (Mata-Alvarez et al 2000).
Pilot scale studies have been reported for recovery of energy from fleshing by co-digestion with primary sludge collected from tannery wastewater treatment plants. In this study, fleshings were cut into 6 mm pieces and fed into the anaerobic reactor along with primary sludge. It was observed that the cut fleshings floated and scum layer was formed on the liquid top of the digester. Therefore, fleshings (food) to microbes contact was very much limited and reduction in the efficiency of the digester was reported. Moreover, due to the presence of lime and gritty matter in the fleshings the cutting knife was frequently damaged (Thangamani et al 2009b). Digestion of fleshings was carried out under mesophilic condition for 5 weeks (Thangamani et al 2009a). This process also required longer retention time and a separate reactor for digestion.

Vasudevan and Ravindran (2007) studied the efficiency of LF and sludge in combination with cow dung for biogas production for a period of 30 days. Shanmugam and Horan (2009) studied the co-digestion of LF with municipal solid waste. It was reported in both cases that the process required longer retention times. In addition, various studies have been reported for co-digestion of organic solid wastes (Rene and Liden 2008). It includes anaerobic co-digestion of different organic waste such as slaughterhouse waste with manure, fruit and vegetable waste and reported that a yield of 270-350 mLCH$_4$/g VS.

It was also reported that anaerobic co-digestion treatment is one of the technologies considered as a solution for biodegradable fraction of organic matter in recovery of energy (Chittibabu et al 2009). A full-scale simulation study was reported for co-digestion in thermophilic condition for wastes coming from kitchens, slaughterhouses and meat-processing industries (Brinkman 1999).
2.4.4 Liquefaction

Freeman and Sawhill (1984) had developed a hydrolyzation process to liquefy protein rich waste like fish, poultry, pork and beef as well as single cell microorganism. The process involves increasing the pH of the waste to 12 and above and heating the waste at the temperatures of 48°C to 76°C (120°F to 170°F) to produce amino acids and lipids. During this process cell rupture and denaturation occur and this facilitates the enzymes to rapidly breakdown the intact protein to smaller more soluble molecules.

Stephenson et al 2000 had developed a process for liquefying waste activated sludge generated from municipal or industrial wastewater treatment plants. The process involves passing the sludge at a high pressure through a nozzle having a restricted flow area to cause liquefaction of sludge as they are discharged from nozzle. The liquefied sludge is anaerobically converted to methane and carbon dioxide.

Thermochemical liquidization as a pretreatment for anaerobic digestion of food waste was studied using a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor for a period of 82 days. Model food waste (approximately 90 wt% moisture content) was thermochemically liquidized at 175°C for 1 h. The liquidized food waste was separated into a solid phase and a liquid phase. The diluted liquid phase was treated by anaerobic digestion using a UASB reactor at 35°C (Tsukahara et al 1999).

2.4.4.1 Mechanism of Anaerobic Degradation of LF

Shanmugam and Horan 2009 have reported an empirical formula for leather fleshings as \( C_4H_{13}NO_2 \) in term of volatile solids and average value of 1.4 g COD/g of VS. The mechanism of anaerobic degradation of typical LF is given below.
Hydrolysis

3 C₄H₈NO₃ + 3 OH → C₆H₁₂O₆ + 3 CH₂NH₂COOH (2.1)

Acidogenisis

C₆H₁₂O₆ → 2 CH₃CH₂OH + 2 CO₂ (2.2)

C₆H₁₂O₆ + 2 H₂ → 2 CH₃CH₂COOH + 2 H₂O (2.3)

C₆H₁₂O₆ → 3 CH₃COOH (2.4)

Acetogenisis

CH₃CH₂COO⁻ + 3H₂O → CH₃COO⁻ + H⁺ + HCO₃⁻ + 3H₂ (2.5)

C₆H₁₂O₆ + 2H₂O → 2CH₃COOH + 2CO₂ + 4H₂ (2.6)

CH₃CH₂OH + 2H₂O → CH₃COO⁻ + 2H₂ +H⁺ (2.7)

CH₂NH₂COOH + 3 H → CH₃COOH + NH₄ (2.8)

Methanogenesis

CO₂ + 4H₂ → CH₄ + 2H₂O (2.9)

2C₂H₅OH + CO₂ → CH₄ + 2CH₃COOH (2.10)

CH₃COOH → CH₄ + CO₂ (2.11)

2.5 ANAEROBIC TREATMENT PROCESS AND REACTORS

2.5.1 Mechanism of Anaerobic Treatment Process

The decomposition of biowaste occurs in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. During hydrolysis, the first stage, bacteria transform the particulate organic substrate into liquefied monomers and polymers i.e. proteins, carbohydrates and fats are transformed to amino acids, monosaccharides and fatty acids respectively (Ostrem, 2004).
\[ C_xH_yO_z + H_2O \rightarrow C_6H_{12}O_6 + H_2 \]  

(2.12)

Acidogenesis is the second stage in the anaerobic process, where acidogenic bacteria transform the products of the first reaction into short chain volatile acids, ketones, alcohols, hydrogen and carbon dioxide. The principal acidogenesis stage products are propionic acid (CH\(_3\)CH\(_2\)COOH), butyric acid (CH\(_3\)CH\(_2\)CH\(_2\)COOH), acetic acid (CH\(_3\)COOH), formic acid (HCOOH), lactic acid (C\(_3\)H\(_6\)O\(_3\)), ethanol (C\(_2\)H\(_5\)OH) and methanol (CH\(_3\)OH), among other. From these products, the hydrogen, carbon dioxide and acetic acid will skip the third stage, acetogenesis, and be utilized directly by the methanogenic bacteria in the final stage (Bilitewski et al 1997).

\[ C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \]  

(2.13)

\[ C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \]  

(2.14)

\[ C_6H_{12}O_6 \rightarrow 3CH_3COOH \]  

(2.15)

In the third acetogenesis stage, the rest of the acidogenesis products, i.e. the propionic acid, butyric acid and alcohols are transformed by acetogenic bacteria into hydrogen, carbon dioxide and acetic acid. Hydrogen plays an important intermediary role in this process, as the reaction will only occur if the hydrogen partial pressure is low enough to thermodynamically allow the conversion of all the acids. Such lowering of the partial pressure is carried out by hydrogen scavenging bacteria, thus the hydrogen concentration of a digester is an indicator of its health (Mata-Alvarez, 2003).

\[ CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \]  

(2.16)

\[ C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \]  

(2.17)

\[ CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 +H^+ \]  

(2.18)

The fourth and final stage is called methanogenesis. During this stage, microorganisms convert the hydrogen and acetic acid formed by the
acid formers to methane gas and carbon dioxide (Verma 2002). The bacteria responsible for this conversion are called methanogens and are strict anaerobes. Typical anaerobic process is shown in Figure 2.2

\[
\begin{align*}
\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \\
2\text{C}_2\text{H}_5\text{OH} + \text{CO}_2 & \rightarrow \text{CH}_4 + 2\text{CH}_3\text{COOH} \\
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2
\end{align*}
\]

(2.19) (2.20) (2.21)

In the anaerobic process, sulphates (SO\text{4}^{2-}) are converted to sulphide (S\text{2}^-); sulphide formed equals to 1/3\text{rd} of sulphates removed. Sulphide combines with H\text{2} to form H\text{2}S. Sulphate reducing bacteria consume COD and to that extent, lesser COD is available for gas production (Bal and Dhagat 2001).
Biological processes like composting and anaerobic digestion (AD) provide advantage due to their natural treatment processes over other technologies. AD has unique and integrative potential, simultaneously acting as waste treatment and resource process. AD also showed an excellent life cycle analysis performance when compared to other treatment technologies like composting or incineration as it can improve the energy balance. In addition, the residues are stable and hence a compost potential for agriculture (Mata-Alverez 2003).

Biomethanation is one of the processes adopted for the treatment and recycling of municipal solid wastes. Biomethanation is an anaerobic fermentation process in which organic waste is converted into $\text{CH}_4$ and $\text{CO}_2$. Microbiological conversion has dual advantage of waste treatment and energy generation. These include increased residence time and the inability of microorganisms to completely ferment all the organics in the municipal solid wastes (Chandran et al 2006).

Anaerobic digestion of solid organic waste has gained increased attention as a means for producing energy-rich biogas, destructing pathogenic organisms and reducing problems associated with the disposal of organic waste (Sonakya et al 2001). In terms of global warming, which is often used as a reference value for ecological balance, anaerobic digestion scores much better than other treatment options (Baldasano and Soriano 1999; Riggle, 1998).

Solid waste from the slaughterhouse contains high amounts of different types of proteins and lipids. Fermantative bacteria, particularly the proteolytic Clostridium species, hydrolyse proteins to polypeptides and amino acids, while lipids are hydrolysed via $\beta$-oxidation to long-chain fatty acids, glycerol (Koster 1989; Mc Inerney 1988; Zinder 1984) and polycarbohydrates to sugars and alcohols (Koster 1989; Pavlostatins and Giraldo-Gomez 1991;
Zinder 1984). Manure and slaughterhouse waste, including blood, fat, stomach, visceral contents and residues from a rendering plant, are also being treated (Ling 1997). Rosenwinkel and Meyer (1999) successfully treated slaughterhouse waste, hog and cow stomach contents with sewage sludge in a pilot-scale mesophilic digester.

Anaerobic treatment of wastewater from slaughter house at temperature between 20°C to 30°C slowed down or impaired due to high concentration of suspended solids, particularly fats (Martinez et al 1995). Anaerobic digestion is preferentially suited for high water- containing liquid or pasty waste materials. Biodegradation tests carried out with semisolid and pasty proteins and lipids containing byproducts from slaughterhouses, pharmaceutical, food, beverage industries, distilleries and municipal bio-wastes showed biogas yield ranging from 0.3 to 1.36 L/g of volatile solids added. In continuous fermentation test, Hydraulic Retention Times (HRTs) ranging between 12 and 60 days, at a fermentation temperature of 35°C, were required for stable operation and maximum gas yield (Braun et al 2003).

Although cellulose materials are the most abundant biomass resources, the rates of their degradation and methane production are much lower than those of other biomasses. Naomichi and Yutaka 2007 reported a need for basic study of an artificial microbial consortium.

### 2.5.2 Anaerobic Digesters

Significant effort has been dedicated in recent years to find ways of improving the performance of anaerobic digestion, especially when treating solid wastes, characterized by a high degree of particulate material. With these substrates, both accessibility of hydrolytic microorganisms to the solid matter and hydrolysis of complex polymeric components constitute the rate-limiting step (Eastman and Fergusson 1981; Noike et al 1985; Pavlostathis
et al 1988). For treating solid waste from swine, diary, beef and poultry industries different kinds of digesters are in use. The retention time reported were varying from 13 to 22 days (Barker 2001).

The applied solid content in association with the substrate loading rate is critical to the cost, performance and stability of anaerobic solid waste digesters (Lissens et al 2001; Rene and Liden 2008). However, the maximum CH₄ production rate decreased with increasing solid contents in the range of 1–10%. The maximum production rates were 39.7 mL CH₄/g VS.d, 25.6 mL CH₄/g VS.day and 13.5 mL CH₄/g VS.d at solid contents of 1%, 2% and 5%, respectively. The specific CH₄ yield was 351–381 mL CH₄/g VS.day, which is similar to 270–350 mL CH₄/g VS in anaerobic digestion of other types of slaughterhouse waste or co-digestion of slaughterhouse waste with manure, fruit and vegetable waste. The less CH₄ production at the solid content of 10% than at the solid content of 5% was reported due to overloading or insufficient buffering capacity (Rene and Liden 2008).

2.5.3 Anaerobic Lagoon

The anaerobic lagoon is the simplest form of anaerobic treatment device, which is based on a natural ecosystem. Low strength wastewater with BOD concentrations about 500 mg/L can successfully be treated by an anaerobic lagoon. Studies on biogas production using anaerobic lagoons, have been conducted by Safley and Westerman (1988). The requirements of large land area, odour nuisance, chances of ground water pollution etc are main drawbacks of the system.

2.5.4 Anaerobic Contact Process

The anaerobic contact process comprises of completely mixed digester tank unit followed by a settling tank unit (clarifier). The process is
suitable for the treatment of wastes with intermediate strength (2000 - 10000 mg/L COD). Comparatively low investment costs and the capability of handling relatively high concentrations of suspended solids are the principle advantages, whereas the limited loading capacity and the poor settleability of the biomass are the major drawbacks of the contact process. The anaerobic contact process, developed in the 1950s was the first high rate anaerobic treatment system. This system was suitable for treating effluents containing high concentration of suspended solids (Allen and Liu 1998; Habets 1999).

### 2.5.5 Up-flow Anaerobic Filter (UAF)

Anaerobic filters have been used for variety of industrial wastes. The anaerobic filter is essentially a tall reactor with height to diameter ratio of 8 to 10 provided with a fixed media matrix over which retention of anaerobic biological sludge is achieved to maintain a longer solid retention time. This reactor could accept variable and severe shock loads and restarted within a short period, even after a prolonged period of shut down. The up-flow anaerobic filter actually performs as a combination of fixed-film and up-flow sludge blanket processes, where the biological activities are mainly associated with the unattached sludge present in the voids. The potential problem of an up-flow anaerobic filter is clogging of the voids due to sludge build-up, which causes short-circuiting of the waste flow.

Song (2004) reported up-flow anaerobic fixed bio-film reactor (UAFBR) for retention of high concentration of accumulated biomass in the form of bio-film supported by a carrier for treatment of tannery wastewater. Effects of major process variables such as HRT, OLR, temperature on Chemical Oxygen Demand (COD) removal, and methane yield performances of the reactor have been evaluated. It reported that COD removal of 60-75% and methane yield of 0.36 m³ CH₄/kg COD removed remained stable across a range of OLRs and under conditions of temperature shock. Main problems
intrinsic to UAF i.e clogging and poor efficiency in the upper zone were investigated by Punal et al (1998) using a new feeding strategy consisting of distributing the influent flow through several entrances along the system with synthetic substrate at an OLR of 10 to 32 kg COD/m$^3$.day. It was concluded that after more than 1 year of operation, the Multiple Fed Reactor strategy proved to be more efficient than the single fed reactor, in terms of COD removal.

2.5.6 Anaerobic Rotating Biological Contactor (AnRBC)

The Anaerobic rotating biological contactor is the same as that used for an aerobic system, except that it is completely closed. The circular discs, made of plastic mesh, PVC, polystyrene foams or just plain asbestos, are placed in a closed tank filled with wastewater. The discs are rotated at a speed of about 3 to 6 rpm. The micro-organisms are attached to the disc surfaces, thereby providing larger surface areas that are exposed for contact with the waste. An et al (1997) studied the performance of an AnRBC for the treatment of high-strength synthetic wastewater at different flow rates and influent organic strengths. Overall COD removal efficiencies ranged from 74 to 82% at an HRT of 32 h, from 56 to 61% at an HRT of 16 h and from 44 to 53% at an HRT of 8 h. It was concluded from this study that the AnRBC reactor is an effective treatment process at an HRT of 32 h and influent COD between 3248 and 12150 mg/L. Igor et al (2002) studied the effect of temperature and HRT on the start-up and steady-state performance of Upflow Anaerobic Filter (UAF) with mixture of synthetic substrate (glucose and sodium acetate) and real municipal wastewater at temperature in the range 9–23°C and HRT in the range 6–46 h. It was reported that average removal efficiency of COD was 46–92% (UAF) depending on used temperature and HRTs.
2.5.7 Anaerobic Baffled Reactor (ABR)

The Anaerobic Baffled Reactor is a modification of the Upflow Anaerobic Sludge Blanket reactor. This reactor consists of a simple rectangular tank divided into five or six compartments of equal volumes by means of walls running from the roof to the bottom of the tank (Bachman et al 1985). The liquid flows upwards and downwards between the walls. On its upward passage, the waste flows through an anaerobic sludge blanket. Hence, the waste is in intimate contact with the active biomass, but because of the baffled nature of the design, most of the biomass is retained within the reactor, even with large hydraulic shocks. This type of reactor appears to be able to treat wastes with high solid content, and hence, it may be a viable alternative in certain situations. The design offers the advantages of reactors in series, i.e. high efficiency, low bypass, resistance to shock loading together with high biomass retention capacity and high specific activity of the methanogenic acetoclastic biomass. However, the necessity to build shallower reactors, relatively higher building complexity and difficulties to distribute the influent evenly are the main drawbacks of the design.

Rongrong (2009) critically reviewed Anaerobic Baffled Reactor treating wastewater on the development and application, performance and characteristics, modeling of the ABR for wastewater treatment and the combination of ABR with other processes during the last decade. It was reported that ABR had become a promising alternative for wastewater treatment with better development potential. Malakahmad (2008) studied application of ABR for the production of biogas from kitchen waste with different proportions of kitchen waste and activated sewage sludge and reported that the combination of 75% of kitchen waste and 25% of activated sewage sludge resulted in 74.1% of methane gas. In addition, it was reported that the sludge in the reactor showed its potential for future use in composting with N, P and K values of 0.95, 0.80 and 0.45% respectively.
2.5.8 Hybrid Bioreactors

Several different designs of hybrid reactors have been proposed. The majority of the laboratory and full scale examples of hybrid reactors have been realized following a simpler design, where the filter is located in the upper part of the reactor without any gas, solid, and liquid separation device. Studies have been undertaken on anaerobic digestion of the liquid fraction of beef cattle waste using this type of reactor. The reactor had a suspended growth zone at the bottom and an upflow filter at the top.

2.5.9 Semi-dry Anaerobic Digestion Process of Organic Solid waste

Based on semi-dry (20% dry matter) anaerobic process, two full-scale industrial plants have been reported to be installed in Italy under thermophilic condition. From the experiments conducted, it was reported that the plant was able to produce nearly 2,500 Nm$^3$/day of biogas and 39 tons/d of digested sludge (10% dry matter) from 500 tons of MSW. The dewatered digested sludge is then co-composted with parts of the fresh organic fraction to produce rich comports (55% dry matter) (Cecchi et al 1989; Cozzolino et al 1992).

2.5.10 Upflow Anaerobic Sludge Blanket (UASB) Reactor

2.5.10.1 UASB Process

In UASB process, wastewater enters the reactor from the bottom at low velocities and flows upwards through relatively dense sludge bed and a blanket of sludge particles. The substrate comes in contact with a sludge suspension (biomass) and eventually gets digested. The gas produced bubbles through the bed and brings about an excellent mixing so that an adequate contact is made between the biomass and substrate. A gas-solid separation device is placed at the top of the UASB reactor. It allows the rising gas
bubbles to get separated from the liquid. Liquid flowing beyond the gas-solid-liquid (GLS) separator is free from gas bubbles and a quiescent zone is created. This zone acts as a separation compartment where sludge particles carried along with upward flow of effluent leaves the settling compartment.

The anaerobic reactor inherently processes good settling properties, for this reason mechanical mixing is generally omitted in UASB reactors. At high OLR, the biogas production guarantees sufficient contact between substrate and biomass. With respect to dynamic behavior of the water phase UASB reactor approaches the completely mixed reactor. For achieving the sufficient contact between sludge and wastewater, the UASB system relies on the agitation brought about by the natural gas production and on an even feed inlet distribution at the bottom of the reactor.

Well setting sludge aggregates, being dispersed under the influence of the biogas production, are retained in the reactor by separating (collecting) the biogas in a gas collector system placed in the upper part of the reactor and releasing the biogas via the device from the reactor. By separating the biogas in this way, a settler is created in the uppermost part of the reactor. Sludge particles can coalesce and settle out here (Lettinga et al 1980a).

Although UASB reactor is essentially a suspended growth reactor, it can be considered as a fixed biomass process (Forster 1985). The essential feature of the system is the development of a sludge blanket in which the component particles are sufficiently aggregated to withstand the hydraulic shear of the upward flowing liquid without being carried upwards and out of the reactor. In addition, these shear forces must not cause particles to break up into smaller units that could be washed out of the reactor. In other words, the sludge flocs must be structurally stable and have good settlement properties. Specific report regarding the anaerobic wastewater treatment based on biomass retention with emphasis on the UASB process has been reported by Lettinga et al (1985).
The inlet system for the wastewater in UASB is designed to spread the wastewater uniformly over the bed of the reactor. The lower part of the reactor is called reaction compartment. It contains a layer of active sludge. When the wastewater comes in contact with the sludge, the unstable organic matter present in the wastewater is digested anaerobically resulting in end product which mainly consists of methane and CO$_2$. The part above the reaction compartment is called solid-liquid-gas separator or three phase separator. In this part, the gas generated in reaction compartment is separated from the liquid and collected in the gas hood. The water flows into the settler zone where solids are made to settle down and return to reaction compartment. The treated effluent is then collected in the effluent channels and conveyed out of the reactor.

2.5.10.2 Essential Features of UASB Reactor System

Typically the Upflow Anaerobic Sludge Blanket reactor is divided into three distinct zones, namely sludge bed, sludge blanket and solid liquid gas separator (Figure 2.3).

![Figure 2.3 Main Component of UASB Reactor](image_url)
The sludge in this reactor is kept in dynamic motion by two factors namely: (a) controlling the wastewater flow in upward direction from the bottom, and (b) produced biogas in sludge bed will be able to push sludge particles in upward direction. The flow of the wastewater entering the reactor is always let in through the distributor in order to avoid the short circuiting of flow. The wastewater initially fed into the reactor by ensuring that the incoming flow is uniformly distributed across the entire plan area of the reactor in order to promote efficient substrate utilization by the microbial population, and to prevent short-circulating through the sludge bed zone. The biological sludge is formed by the combination of active biological solids along with the suspended particles of waste that are retained in the reactor due to the settling and thickening properties of the anaerobic (active biological solids) sludge. Waste stabilization occurs as the waste passes up through the sludge bed (Pette 1979).

The sludge bed zone has been described as a perfectly mixed region (Heertjes and Meer 1978), which can actually be divided into smaller sub regions. The first sub region encountered in the sludge bed is the area around the influent port, which is considered to be a perfectly mixed region. The rest of the sludge bed is considered a transition region between the initial bed zone and the sludge blanket zone. Gas production by the biological solids results in gas bubbles, which creates mixing throughout the bed. The sludge bed zone is responsible for 80 to 90% of the waste degradation occurring in the reactor while occupying approximately 30% of the reactor volume.

The next zone encountered by the waste stream is the sludge blanket section which occupies about 70% of the total reactor volume and contains solid concentration in an order of magnitude lower than that in the sludge bed. As gas bubbles rise through the sludge bed and blanket, biomass particles become attached and are carried up along with the gas. If long SRT
values are to be achieved, these rising solid particles must be separated from the gas bubbles. The separation process is carried out by the introduction of a combination solids settler/gas collector device at the top of the reactor. The main purpose of the settling device is to effectively separate and carry the rising gas and solids particles towards the gas collector. At this point, a swirling action occurs as the gas goes into the collector, and the solids settle back down into the reactor. The effluent flows through the available space between the gas collector and settler towards the effluent weir. Solids that escape with the effluent in most cases settle back down on the settler and drop back down into the reactor (Obayashi and Gorgan 1985).

Torkian et al 2003 have studied the effect of OLR in performance of UASB reactor treating effluent from slaughter house and reported removal efficiency in the range of 75-90% when operated with OLR between 13 and 39 kg soluble COD /m$^3$.day and HRT of 2-7 h. Treatment of wastewater from sugar industry by UASB with a capacity of 800 m$^3$ plant was reported by Pette et al (1980). The UASB technology has been successfully applied to treat a variety of wastewater from pulp/paper industry, food industry, breweries, distilleries and chemical industries (Driessen et al 1994; Zoutberg and de Been 1997). Ruiz et al (1997) studied treatment of wastewater from slaughter house in an UASB reactor.

Over the past two decades, UASB technology has been employed for wastewater treatment. More than 200 units are currently being operated all over the world. It exhibits positive features such as high organic loadings, low energy demand, short HRT and easy reactor construction. Important parameters affecting the treatment efficiency of UASB reactor include the granulation process in the reactor, the characteristics of the wastewater to be treated, the selection of inoculum material, the influence of nutrient and several other environmental factor (Sundar and Manjunath 2007).
Lefebvre (2006) studied soak liquor from tanneries with high organic load and high salinity using a UASB and reported COD removal of 78% at an OLR of 0.5 kg COD/m$^3$.day, HRT of 5 days and TDS concentration of 71 g/L. In addition, it was reported that COD removal efficiency 96% with combined anaerobic/aerobic treatment system. Chuphal et al (2007) studied UASB reactor for treatment of pulp and paper mill effluent and evaluated the effect of major process variables i.e COD, colour, lignin removal and methane yield from the reactors. The biogas production was 0.210 L/L.d with an average 8.6% of methane. It was also observed that the UASB reactor was stable, easy to operate with low maintenance and operational cost.

2.5.11 Single and Two-Stage Anaerobic Reactors

In single stage reactor, the hydrolysis of the polymeric organic compounds are converted into intermediary metabolic end products like VFA and subsequently in to CH$_4$, H$_2$S, NH$_3$ and CO$_2$ by methanogenesis in the same reactor. Whereas in the two-stage reactor, the production of VFA by acetogenic bacteria is expected to take place in the hydrolyser (first reactor) and the consumption of VFA by methenogenesis is expected to take place in the methaniser(second reactor). The separation of digesters as hydrolysers and methanisers changes the process dynamics of the digesters as it segregates the anaerobic degradation of the individual bacterial species separately to perform the hydrolysis and methanogenesis. It was reported that the two-stage anaerobic digesters perform more efficiently than the single stage digesters (Bhattacharya et al 1996; Borja et al 2003; Ghosh and Pholand 1974; Alverez 1987; Kuba et al 1990; Shimizu et al (1993); Miron et al (2000); Nozhevnikova et al (1999); Inanc et al (1999); Ochao et al (1999). Whereas, other researchers (Ong et al 2000; Viturtia et al 1995; Bujoczek et al 2000; Bhattacharya 1996; Veeken et al 2000; Stephen et al 1986; Vavilin et al 1996;
Breure and Andel (1984; Borja et al. 2003) reported that single phase are more advantageous than the two phase system, particularly for proteinaceous waste. Production of $\text{NH}_3$ neutralizes the VFA during its accumulation and becomes ammonium acetate or ammonium bicarbonate so as to maintain favorable pH but to avoid digester failure. Nevertheless, this will neutralize all the CH$_4$ precursors and reduces the CH$_4$ content in biogas.

Trouque and Forster (2000) reported that in terms of reduction of VS in the three dual digestion configurations were similar but more effective than single stage and two-stage and the VS reduction was 60%, whereas in single stage the same was low. Stephen et al (1986) stated that the necessary interspecies hydrogen transfer functions were disturbed which will obstruct the syntrophic relation between H$_2$ production and consuming bacteria in the two phase system and attributed that the overall digester performance in a fermentor increased in a two-phase system. Supporting this study, Stephen et al (1986); Costello et al (1991a, b) reported that the two-stage reactors achieved optimum growth conditions for hydrolytic acidogens at pH 6 and methanogenic bacteria (II stage) at pH 7, which makes it obvious that the two-phase system is essential for the separation of specific pH for two different species of bacteria which can survive at different pH. Kim et al (2002) stated that the two phase system has advantage on single phase as slow growing methanogens were not affected by any change in environmental conditions in the methaniser. The authors have expressed that methanogenesis was affected by lower temperature which might be due to the unbalanced reaction rate between acetogens and methanogens and concluded that the two phase systems was more advantageous than the single-phase system. However, it has been found that the VS reduction efficiency was slightly higher in two phase than in single phase.
Held et al (2002) reported that separation of organic waste in two fraction and two stage anaerobic fermentation of liquid fraction in a Upflow Anaerobic Filter (UAF) is a promising alternative to existing processes. Further it is expressed that the CH$_4$ content was higher in the second stage than the first stage and this was also supported by the Ince (1995) and Sosnowski et al (2002) who reported that the single stage system requires 37 days HRT whereas the two-stage requires 30 days HRT for maximum COD removal efficiency of 80%. Guerrero et al (1999) recommended that the two-phase system is optimum for proteinous waste like fish and meat and reported that NH$_3$ toxicity was observed to be the rate-limiting step of dissociation of NH$_3$ at high temperature. In addition, it was concluded that the methenogenic reactor was not advisable to operate at thermophilic condition for a proteineous waste as the dissociation of NH$_4^+$ to NH$_3$ is high at higher temperatures. Hansen et al (1998) reported that the single phase system is more advantageous than the two phase system as denitrification and methanogenesis in a single reactor would be optimum for the neutralizing capacity of VFA and further prevent from the drop in pH and VFA toxicity.

Miron et al (2000) reported that the two-phase system was considered to be more advantageous than the single stage under climatic condition of low summer and high winter and further stated that the hydrolysis of protein is the rate-limiting step for methogenesis. It was also reported that the hydrolysis of lipids and carbohydrates increased with increase in HRT, whereas protein hydrolysis occurred only in methanogenic condition and further revealed that hydrolysis was the rate limiting step for conversion of carbohydrates, while both hydrolysis and acidification were the rate limiting step for conversion of protein, hence two phase system was suggested for the protein enriched wastes. Dinsdale et al (2000) have studied the suitability of the two-phase system for fruit and vegetable waste and the acetogenic and methanogenic reactors were found to operate successfully.
The researchers have further reported that the stable two-phase system was achieved for vegetable and fruit waste with HRT of three days for acetogenic reactor and 10 days for methanogenic digestion, which is again supporting the hydrolyser to methaniser.

Yu et al (2002) have reported that the efficient degradation of organic matter is dependent on coordinate metabolism of acid forming and CH₄ forming bacteria. It was emphasized that separating the optimum condition for each bacterial group can increase the anaerobic process stability and overall degradation rate and hence the two-phase system was considered more efficient than single-phase system. Kuba et al (1990) has reported that the methanogenic phase outlet gives better quality in two phase than in the single phase anaerobic digestion for synthetic waste, which was also supported by Nozhemikova et al (1999). Shimizu et al (1993) have reported that the overall gas production rate of two phase process was four times greater than single phase process.

Kaul and Nandy (1992) reported that the CH₄ production rate from two-phase digesters was 7 times higher than that of the conventional single stage digester. Parkin and Owen (1986) stated that the phase separation of digester would only be feasible for the substrate where hydrolysis step is clearly the overall rate-limiting step which was also confirmed by Miron et al (2000) who stated that hydrolysis of lipids and carbohydrates increased with increasing SRT, whereas protein hydrolysis only occurred under methanogenic conditions. Under methanogenic conditions, hydrolysis was the rate-limiting step in the whole digestion process. Tembhurkar and Mhaisalkar (2008) studied biomethanation of kitchen waste suitably adopting two phase anaerobic treatment using anaerobic fixed film fixed bed reactor (AFFFBR). The acidogenic process (first phase) was suitably incorporated in the new
approach, wherein the kitchen waste was appropriately kept in submerged conditions in the acidogenic reactor to obtain leachates.

2.6 PERFORMANCE INDICATORS IN THE ANAEROBIC DIGESTERS

Andrews (1969) reported that the concentrations of unionized acids were the growth limiting substance in an anaerobic digestion, which occurred at very low pH attributed due to organic shock loading. Torre and Stephanopoulos (1986) revealed that the ionic equilibrium is assumed for \( \text{NH}_4^+ \), \( \text{NH}_3 \), VFA and \( \text{HCO}_3^- \) and pH could be calculated from the right or left side of the equations and also suggested that \( \text{Na}^+ \) (or \( \text{NH}_4^+ \)), \( \text{HCO}_3^- \) and hydroxide performed better than lime in control of pH in the digester whereas Soto (1993) reported that \( \text{Na}_2\text{CO}_3 \) could be used as better pH control agent because neutralizing material will not be precipitated.

Yang and Okas (1987) reported that the optimum growth of methanogens was found to be slightly different for each species and stated that Methanococcus mazei was proliferated well in the pH between 6 and 8 whereas Methanococcus barkeri was grown at pH between 6 to 7.4. Dinopoulou et al (1988 a, b) have reported that the concentration of \( \text{CH}_3\text{COOH} \) in the digester was affected by the pH of the system much more than the propionic acid and further stated that the percentage of \( \text{CH}_3\text{COOH} \) as a proportion of total VFA seem to increase with increase in pH. In addition, it was reported that at pH 7, the acetic and propionic acids were 50% and 39% of total VFA. But at lower pH, the acetic and propionic acids were 25% and 42% respectively. A similar observation was also reported by Nozhevnikova et al. (1999) wherein the accumulation of VFA and the subsequent drop in pH to less than 6.0 was attributed to inhibition of methonogenisis.
Andrews (1969); Ghosh and Pohland (1974); Fang et al (1999); Braun and Huss (1982); Parkin and Owen (1986); Yang and Okos (1987); Rintala and Lepsisto (1997); Yang and Anderson (1994); Pfeffer et al (1976); Varaldo (1997); Kaul and Nandy et al (1992); Trouque and Forster (2000) reported that the optimum pH for anaerobic digester was between 6.2 to 7.2. Miron et al (2000) reported that although VFA was found to accumulate at low pH values in the continuous stirred tank reactor, it is not so in UASB reactor due to dilution in sludge bed with influent with high buffering capacity, whereas Dinsdale et al (2000) reported that the pH control was not necessary for the acetogenic (Hydrolyser) reactor. In addition, it was emphasized that the pH of acetogenic digester was 4.4 to 6.2, whereas in the methanogenic reactor, the pH was 7.7 attributed to VFA production in acetogenic reactor and VFA consumption takes place in methanogenic reactor.

Qing and Fang (2003) reported that the gelatin degradation in the anaerobic digester increased with increase in pH as the degree of acidification was increased from 32% at pH 4 to 71.6% at pH 6.5, while the acidification has dropped to 66.8% when the pH was increased to 7 and further stated that the pH between 7 to 8 favors the acetate and butyrate formation whereas pH 4 to 5 favored the propionate and hydrogen production. Similar trends have also been reported by Dinopoulou et al (1988 a, b). Breure and Andel (1984) reported that the optimum pH for hydrolysis and acidogenisis were different for gelatin (7-8) and carbohydrate (5.5-6.5), attributed to high ammonia content in the gelatin waste which contributes to a highly alkaline pH than the carbohydrate waste. Noike et al (1985) reported that single-phase egg shaped digester for sewage sludge operated at pH between 7.0 - 7.7, the alkalinity was found to be 3460 - 3820 mg/L. Bill et al (1995) reported that the experimental biogas production rate deviate from model prediction and the degree of deviation decreased with decrease in alkalinity and pH attributed to
increase in VFA and decrease in buffering capacity. Yang and Anderson (1994) investigated the characteristics of wastewater treated and the biological efficiencies of the reactors and reported that the model was able to predict the pH, concentration of HCO$_3^-$ in the effluent and percentages of CH$_4$, CO$_2$ and H$_2$S in the biogas from several laboratory scale reactors of different configurations treating different types of effluent (Merkel and Krauth 1999; Munch et al 1998).

Yang and Anderson (1994) reported that the pH was decreased from 7.2 to 6 accompanied by accumulation of VFA and there was sharp decrease in gas production when the OLR was increased. Further, it was also reported that the performance of acetate fed reactor was not significantly affected by increase in OLR which emphasized the fact that the two phase system is more viable than single phase system, Varaldo et al (1997) investigated the cattle manure with activated sludge as co-digester and concluded that the reactor could be operated successfully with OLR of 8.2 kg/m$^3$·day. In addition, it was further reported that CO$_2$ would be 40 times more soluble than CH$_4$ and the amount of CO$_2$ absorption in the reactor was influenced by a number of factors such as temperature, partial pressure, pH and ionic strength which reflects the level of CO$_2$ in biogas. Similarly Parkin and Owen (1986) reported that NaHCO$_3$, KOH, lime and NH$_3$ were generally tested as pH control agents but the NaHCO$_3$ was preferred to be effective pH control agent. Mata-Alvarez et al (2000) reported that the pH for the protein waste digester should be less than 6.5 and emphasized that at pH between 6.5-8.2, the methanogenic activity decrease with increase in NH$_3$-N concentration. It was also reported that rapid dissociation of ammonium into ammonia took place at pH between 7.5 and 8.2. However, the lag phase of digester depended on NH$_3$ level and not on NH$_4^+$ which was also consistent with the digester pH as greater than 6.5 as the dissociation of NH$_4^+$ to NH$_3$ would be more prominent at pH between 7.5-9.
Feitkenhauer (2002) and Guerrero et al (1999) reported that CH$_3$COOH was the main acid during protein fermentation. Dinsdale et al (2000) reported that VFA yield for waste activated sludge in acidification reactor was varying from from 0.112 to 0.59 gm/gm of VS added and further emphasized that the pH was correlating well with HCO$_3^-$ alkalinity indicating good process stability. Similarly, Parkin and Owen (1986) reported that HCO$_3^-$ ion is the major alkalinity contributing anion for the digester buffering capacity (alkalinity) in an anaerobic system for the optimum pH range of 6.5 - 7.6. Bujoczek et al (2000) reported that free NH$_3$ and unionized form of VFA could also be toxic at higher levels and the toxicity would increase with increase in pH of solution between 8-9 favoring the dissociation of ammonium to ammonia.

Qing and Fang (2003) investigated the biomethenization of wastewater and reported that the VFA profile showed 64% acetate, 12% propionate, 13% butyrate and 2% pyruvate. Ghosh and Pohland (1974) reported that the optimum VFA should be in the range of 2000 – 3000 mg/L for an efficient anaerobic degradation of wastewater. Yang and Anderson (1994) revealed that the digester overloading always leads to accumulation of VFA and further stated that acetogens grow faster and are less sensitive to the environmental changes than methanogens. This could be ascribed to the conversion of CH$_4$-precursors acetate, to ammonium acetate resulting in increase of digester buffering capacity and decrease in CH$_4$ composition in biogas. Rao et al (2000) expressed that the pH was reduced during the first few days of start-up due to high VFA accumulation and thereafter it was maintained between 7.2 to 7.9 by 0.5 N NaOH solution during which the VFA was reduced and thereafter maintained around 3800 mg/L.
2.7 FACTORS AFFECTING ANAEROBIC PROCESS

2.7.1 Temperature

Anaerobic process is more sensitive to temperature variation. Acetate to methane conversion is more temperature dependent than the acetate forming biomass. Temperature has a major influence on the effectiveness of biological systems, affecting the metabolic rate, ionization equilibria, solubility of substrates, fats and bioavailability of iron (Speece 1996). Research carried out earlier showed that anaerobic microorganisms function effectively in the mesophilic range of 29°C to 38°C (Eckenfelder 1999). Anaerobic digester was developed for different temperature ranges including mesophilic temperatures (35°C) and thermophilic temperatures ranging from 55°C to 60°C (Sanchez et al 2001). Anaerobic digestion is a function of temperature, where the rate of decomposition increases with temperature increase until the optimum growth temperature is reached. At temperatures above and below the optimum growth temperature, metabolic activity decreases, resulting in a decrease in reactor performance. Full-scale anaerobic treatment systems typically operate in the mesophilic range since maintaining higher operating temperatures is usually not economically justifiable. An important characteristic of anaerobic bacteria is that their decay rate is very low. Thus, it is possible to preserve the anaerobic sludge for long periods without losing much of its activity. This is especially useful in the anaerobic treatment of wastewater, where frequent failure of power occur (Saleh and Mahmood 2004).

2.7.2 pH

The pH within the reactor of a system affects the anaerobic digestion and overall performance of the digestion process. Methane producing microbes function effectively between pH range of 6.5 and 8.2,
with an optimum near pH 7.0 (Eckenfelder 1999; Speece 1996). In an anaerobic system, the acidogenic bacteria convert organic matter to organic acids, possibly decreasing the pH and reducing the rate of methane production and thus the overall anaerobic process of the acids are not quickly consumed by methanogens, at pH levels above 8.2 and below 6.5. Therefore, maintaining pH level is important for the successful operation of anaerobic systems. The optimum pH for anaerobic digestion was reported as 6.3-7.8 (Haandel and Lettinga 1994).

2.7.3 Volatile Fatty Acids

The presence of increasing concentrations of VFA in a batch anaerobic reactor system were shown to have a differential effect on the metabolically distinct phases of hydrolysis, acidogenesis and biogas production associated with the anaerobic digestion process. Independent of the system pH, VFA caused the inhibition of the cellulolytic activity at concentrations \( \geq 2 \) g/L and therefore of the rate of cellulose hydrolysis. The fermentation of glucose was slightly inhibited at VFA concentrations above 4 g/L. Inhibitory effect on the production of biogas and also on the methane to carbon dioxide ratio was more evident above 6 g/L VFA in the initial mixture used as sole substrate. In combination with paper as primary substrate, biogas production due to paper was more than halved above 1 g/L initial VFA, indicating inhibition of the hydrolysis process, where glucose was the primary substrate. Biogas production was more than halved above 8 g/L which indicated that the fermentation was less sensitive to inhibition caused by VFA (Siegert and Banks 2005). VFA accumulation results in unbalanced microbial consortia, which is detrimental to the anaerobic process operation leading to the total system failure (Venkatamohan et al 2005; Komisar et al 1998). In two-phase anaerobic digestion, some portion of the acid produced in acid digester was transferred to methane digester and it is a substrate for
methanogenic bacteria and simultaneously reduce product (VFAs) inhibition (Sandhya et al 2004).

Callaghan et al (2000) expressed that one of the criteria for judging the stability of the digester is VFA/Alkalinity ratio. If the VFA/Alkalinity ratio were <0.4, the reactor is considered to be stable; between 0.4-0.8, some instability will occur and ≥0.8 significant instability.

Acidification efficiency of the reactor was expressed as a function of VFA concentration in the influent and the effluent by Shin et al (2001) as

\[
\text{Percentage acidification} = \left( \frac{\text{VFA}_p}{\text{VFA}_t} \right) \times 100 \quad (2.22)
\]

Where in

\[
\text{VFA}_t = \text{the theoretical VFA of substrate (g COD) which could be expressed as } (g\text{VSx0.79 g acid/g VS x 1.49g COD/g acid})
\]

\[
\text{VFA}_p = \text{actual VFA produced in the acidogenic fermentation of the substrate at a time (g COD), expressed as } (\text{VFA g/L x 1.067})
\]

2.7.4 Organic Loading Rate and Hydraulic Retention Time

The effect of OLR and HRT with different type of wastes reported in previous studies are summarized in Table 2.1.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of wastewater</th>
<th>Influent COD (mg/L)</th>
<th>Volumetric loading rate (kg COD/m³.day)</th>
<th>F/M (kg COD/kg MLVSS day)</th>
<th>SRT (day)</th>
<th>Biogas Yield (m³/kg COD removed)</th>
<th>CH₄ % in the gas</th>
<th>COD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciftci and Ozturk (1995)</td>
<td>Bakers yeast mfg.</td>
<td>17000-20000</td>
<td>8-13</td>
<td>0.2-0.3</td>
<td>&gt;100</td>
<td>0.65</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>Gohil and Nakhla (2006)</td>
<td>Tomato processing</td>
<td>4000-10000</td>
<td>2.5-10</td>
<td>0.2-0.5</td>
<td>100</td>
<td>0.50</td>
<td>78-82</td>
<td>96</td>
</tr>
<tr>
<td>Lapisto and Rintala (1997b)</td>
<td>Carrot blanching</td>
<td>9500-27600</td>
<td>24</td>
<td>NS</td>
<td>NS</td>
<td>0.64</td>
<td>49</td>
<td>90</td>
</tr>
<tr>
<td>Lapisto and Rintala (1997a)</td>
<td>Potato blanching</td>
<td>10500-11800</td>
<td>3.5-7.6</td>
<td>NS</td>
<td>NS</td>
<td>0.71</td>
<td>49</td>
<td>68-85</td>
</tr>
<tr>
<td>Wahaab and Hamdy el-Awady (1999)</td>
<td>Meat processing</td>
<td>1544</td>
<td>4.63</td>
<td>0.1</td>
<td>NS</td>
<td>0.010</td>
<td>NS</td>
<td>56</td>
</tr>
<tr>
<td>Chernicharo and Nascimento (2001)</td>
<td>Domestic sewage</td>
<td>634</td>
<td>3.8</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>65-81</td>
</tr>
<tr>
<td>Kalyuzhnyi et al. (1997)</td>
<td>Whey</td>
<td>5000-77000</td>
<td>1-28.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>95-99</td>
</tr>
<tr>
<td>Kalyuzhnyi et al. (1997)</td>
<td>Whey</td>
<td>47000-55000</td>
<td>7-9.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>90-94</td>
</tr>
<tr>
<td>Schroder and DeHaast (1989)</td>
<td>Whey</td>
<td>11000</td>
<td>7.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>94</td>
</tr>
<tr>
<td>Lefebvre et al. (2006)</td>
<td>Tannery soak liquor</td>
<td>0.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>78</td>
</tr>
<tr>
<td>Banu and Kalliappan (2007)</td>
<td>Tannery wastewater</td>
<td>2.74-3.14</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Oktem and Tuekci (2006)</td>
<td>Brewery wastewater</td>
<td>7</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>95</td>
</tr>
</tbody>
</table>
Rintala and Lapisto (1997) reported that the optimum OLR under mesophilic condition is 4 kg COD/m$^3$.d, which was also supported by Hills and Dykstra (1980) where the optimum OLR was 5 kg COD/m$^3$.d. Stevens and Schute (1979) stated that OLR of 0.61 g of VS/L.d and 1.80 g of VS/L.d at low temperature anaerobic digestion of swine manure was found to proceed satisfactorily and further emphasized that the biogas production was low in the high rate digester than in the low rate digester. It was reported that the OLR of 0.8 kg VS/m$^3$.d to 2.1 kg VS/m$^3$.d was feasible with stable methane production (Salminen and Rintala (2002); Grobicki and Stuckey (1991)). Kuba et al (1990) stated that the methanogenic anaerobic fluidized bed reactor achieved a good efficiency up to the operated OLR of 6 g COD/L.d with HRT of 12 hours and the system pH was also in the optimum range of 6.8 - 7.5. Manjunath et al (2000) reported that the UASB reactor with slaughterhouse wastewater at the OLR of 1.2 kg COD/m$^3$.d with HRT of 24 hours was operated efficiently. In contrast to this study, Yang and Anderson (1994) reported that the COD reduction efficiency of 86% with CH$_4$ yield of 0.32 m$^3$/kg COD was removed at OLR of 26 kg COD/m$^3$.d. Parkin and Owen (1986) reported that the favorable conditions for an optimum digestion performance are optimum retention time in addition to adequate mixing (bacteria and substrate contact), optimum pH, optimum temperature control, adequate concentration of nutrients, absence (assimilative) of toxic materials, proper feed characteristics, proper design of anaerobic reactors, optimum VFA/Alkalinity ratio and optimum C/N ratio of feed stock.

Ince et al (1995) reported that the OLR in the reactor was 0.7 kg/m$^3$.day with COD removal efficiency of 65%. However, Lettinga et al (1980) stated that the maximum achievable OLR in most of the reactors was 10 - 14 kg COD/m$^3$.d.
Speece (1988) has stated that the diluted raw feed sludge was the major cause for several digester failures. Lettinga et al (1983) and Ubay and Ozturk (1997) reported that the COD removal efficiency was 75% at an OLR of 5 -18 kg COD/m$^3$.d with an HRT of 24 hours but Dinsdale et al (2000) stated that 90% of COD removal was achieved within 12 hours of HRT with OLR of 1.8 kg COD/m$^3$.d. Further, it was reported that the methanogenic reactor needed 10 -14 days of HRT and the VFA in the methanogenic reactor reduced to 1300 - 1400 mg/L and the overall VS reduction efficiency increased from 40 to 44%. Noike et al (1985) revealed that the in mesophilic egg shaped tank with HRT of 30 days, the removal efficiency of carbohydrates, lipids, protein and VFA reached 75%, 50.6%, 46% and 90% respectively. Miron et al (2000) reported that the SRT greater than 8 days was required for acidogenic reactors and hydrolysis is the rate limiting step for the anaerobic degradation of proteins and lipid. Owen et al (1972) stated that to prevent the washout of the bacteria sufficient HRT is required to build the bacterial population inside the digester. However, Miron et al (2000) reported that SRT of less than 8 days for acidogenic conditions and greater than 10 days for methanogenic conditions was required. Schober et al (1999) reported that the HRT of 11 days was required for 72% VS removal efficiency. Grobicki and Stuckey (1991); McLeod (1990); Stewart et al (1995) stated that the hydrolyser needs 2.2 hours of HRT and methogenizer needs 5.2 hours of HRT which is again corroborating well with the concept of Dinsdale et al (2000). Miron et al (2000) stated that Methenogenic reactor need more HRT than the acidogenic reactor.

It was reported that slaughterhouse wastewater contains high amounts of organic matter with a soluble fraction in the range of 40–60%. The suspended and colloidal components in the form of fats, proteins, and cellulose could have an adverse impact on the performance of UASB reactors, leading to deterioration of the microbial activity and washout of active
biomass (Nunez and Martinez 1999). This would limit the OLRs to 4–6 kg COD/m³.d (Lettinga and Hulshoff Pol 1991). Ruiz et al (1997) reported sludge floatation and increased effluent solids concentration at OLR values higher than 5 kg COD/m³.d. Others researchers (Sayed et al 1988; Sayed and De Zeeuw 1988) have shown satisfactory treatment of slaughterhouse effluent with OLR values as high as 11 kg COD/m³.d at a process temperature of 30°C. Rosenwinkel and Meyer (1999) showed a successful treatment of slaughterhouse waste containing hog and cow stomach contents with sewage sludge in a pilot-scale mesophilic digester at a loading rate of 2.9 kg TS/m³.d and an HRT of 17 days with a methane production of 0.23 m³/kg TS. It was reported that attachment of gas bubbles to the biomass is a usual problem of UASB systems at high OLR values leading to biomass suspension and cell washout as methane production rate increases (Torkian et al 2003).

The growth of Sulphide Reducing Bacteria was dependent on the carbon and sulphate concentration, whereas the growth of Methanogenic Bacteria was solely dependent on the concentration of acetate as carbon source (Vossoughi et al 2003). At low sulphate concentration, growth of SRB would be sulphate limiting and enables MB to out compete SRB. Although thermodynamic and kinetic considerations favour sulphate reduction over methanogenesis, it was often observed that MB was able to effectively out compete SRB for acetate (Isa et al 1986; Yoda et al 1987).

It was reported that at high COD/SO₄⁻² ratios (>6), methanogenic bacteria (MB) predominated while at lower COD/SO₄⁻² ratios (<1.5), sulphate reducing bacteria (SRB) were more competitive. The increase in COD/SO₄⁻² ratio favored the anaerobic fermentation by the effective performance of the reactor (COD removal) along with increase in biogas yield (Venkatamohan et al 2005). For reduction of sulphate to sulphide for the SRBs, 0.67 kg of COD is required per kg of sulphate. Methane gas production is directly related to
organic matter removal by anaerobic bacteria, its drop with increasing sulphate level implies the decrease in methanogenic activity as both SRBs and MB compete for the same organic sources (Dilek et al 2007).

### 2.7.5 Stoichiometry of Methane Conversion

Anaerobic fermentation and oxidation process are used primarily for the treatment of waste sludge and high strength organic wastes. However, applications for dilute waste stream have also been demonstrated and are becoming more common. Organic substrates can be converted into methane. A limited number of substrate are used by the methanogenic organisms and reactions defined as CO$_2$ and methyl group type reaction are shown as follows (Madigan et al 1997), involving the oxidation of hydrogen, formic acid, carbon monoxide, methanol, methylamine and acetate respectively.

\[
4H_2 + CO_2 = CH_4 + 2H_2O \quad (2.23)
\]

\[
4HCOO^- + 4 H^+ = CH_4 + 3CO_2 + 2H_2O \quad (2.24)
\]

\[
4CO + 2H_2O = CH_4 + 3CO_2 \quad (2.25)
\]

\[
4CH_3OH = 3CH_4 + CO_2 + 2H_2O \quad (2.26)
\]

\[
4(CH_3)_3N + H_2O = 9 CH_4 + 3CO_2 + 6H_2O + 4 NH_3 \quad (2.27)
\]

\[
CH_3COOH = CH_4 + CO_2 \quad (2.28)
\]

On the reaction for the aceticlastic methanogens as given by equation 2.28, the acetate is cleaved to form methane and carbon dioxide.

A COD balance can be used to account for the changes in COD during fermentation. Instead of oxygen accounting for the changes in COD, the COD loss in the anaerobic reactor is accounted for by methane production. By stoichiometry the COD equivalent of methane can be
determined. The COD of methane is the amount of oxygen needed to oxidize methane to carbon dioxide and water.

\[
\text{CH}_4 + 2\text{O}_2 = \text{CO}_2 + 2 \text{H}_2\text{O} \quad (2.29)
\]

From the above, the COD per mole of methane is 2 \((32\text{g O}_2/\text{mole}) = 64 \text{ g O}_2/\text{mole } \text{CH}_4\). The volume of methane per mole at standard condition (0°C and 1 atm) is 22.414 L, so the \text{CH}_4 equivalent of COD converted under anaerobic condition is 22.414/64 = 0.35 L CH\textsubscript{4} / g COD.

Sanchez et al (1996) reported that the specific CH\textsubscript{4} yield obtained from the anaerobic biomethenization process of sugar mill waste was 0.25 L/kg of COD removed. Hills and Dykstra (1980) reported that the specific biogas production for tomato cannery waste was in the range of 0.262-0.52 m\textsuperscript{3} of CH\textsubscript{4} /kg of COD removed and the percentage of CH\textsubscript{4} in the tomato cannery waste biomethenization plant was 61%.

Stevens and Schulte (1979) reported that CH\textsubscript{4} and CO\textsubscript{2} in the biomethanation plant in pig manure was 65% and 35% respectively, which was very low compared to the Ubay and Ozturk (1997) studies on Olive mill waste. Sosnowski et al (2002) concluded that the specific biogas production for Organic Fractions of Municipal Solid Wastes (OFMSW) was 0.4 - 0.6 m\textsuperscript{3}/kg of VS added. Qing and Fang (2003) reported that at pH 4 in the digester, the biogas composition was 30% of CO\textsubscript{2} and 56% of H\textsubscript{2} but no CH\textsubscript{4}, whereas the CH\textsubscript{4} fraction has increased at pH 7 and no H\textsubscript{2} was detected. Hence it is imperative that the pH beyond 5.5 the CH\textsubscript{4} producing bacteria were significantly active indicating the influence of pH on methane production. Iglesias et al (2000) reported that the biogas contains 52% of CH\textsubscript{4} from MSW. Lin et al (1999) concluded that the specific biogas production was 0.349 m\textsuperscript{3}/kg of COD destroyed. Cheubarn and Pagilla (2000) has reported that the specific gas production was 0.61 m\textsuperscript{3}/kg of VS destroyed, but Parkin et al
(1980) reported that 0.70 m$^3$/kg of VS destroyed was observed with high carbohydrate content than the protein waste. Sankar Ganesh (2007) reported that the Upflow anaerobic filters (UAF) attached with solid waste produced 2 m$^3$ of biogas per m$^3$ of reactor volume per day, whereas the conventional biogas digester produced 0.1 m$^3$ to 0.2 m$^3$ of biogas per m$^3$ volume of digester volume per day. Eliyan et al (2007) investigated the anaerobic digestion of OFMSW in a two phase reactor with continuous operation. During the reactor’s startup period, the process was stable and there was no occurrence of inhibition as methane composition leveled off at 66% with higher rate of biogas production. The reactor was fed in continuous mode and methane content of the biogas reduced to 30 - 40%. Ghangrekar (2005) evaluated the effect of different biogas production rates on UASB reactor performance and on the characteristics of the sludge produced and observed that biogas yield was higher than 0.7 m$^3$/m$^3$.d which was sufficient to carry out natural mixing inside the reactor. However, very high biogas yield, greater than 2.3 m$^3$/ m$^3$.d was observed to be unfavorable for determining the requisite sludge age and necessary strength of granules.

It has been reported that theoretically 0.38 m$^3$ of biogas will be produced for every kg of COD removed at 25°C and 1 atom. COD is balanced in UASB reactor, considering COD influent against COD effluent, COD methane (recovered and dissolved methane), COD formation of new cell and COD sulphate production.

In anaerobic treatment of sulphate containing wastewater, sulphate reducing bacteria (SRB) will compete with methanogenic bacteria (MB) and acetogenic bacteria (AB) for the available substrate such as hydrogen, acetate, propionate and butyrate. The outcome of this competition will determine the end product of the anaerobic mineralization process: methane or sulfide. The occurrence of the sulfate reduction process is often considered unwanted due
to the problem associated with the sulfide formed in the process. These problems are: malodour, corrosion, toxicity, reduced removal of COD, reduced methane formation and higher levels of $\text{H}_2\text{S}$ in the biogas (Visser 1995).

In terms of COD, sulphate reduction also gives some COD removal. When sulphate is reduced to sulphide, some organic materials (COD) acts as donor of electron and receptor of oxygen. In other words, COD is removed by sulphate reduction. But this COD is neither converted into methane, nor into sludge nor does it leave with the effluent and must be accounted for separately in a materials balance at 1g of COD per 1.5 g of sulphate reduced. COD removed as sludge production is measured as volatile solids in sludge in over the period and it is accounted in a COD balance at 1kg of VS contain 1.5 kg of COD (Arceivala and Asolekar 2007). Considering the sulphate reduction, COD balance becomes

$$
\text{COD}_i = \text{COD}_e + \text{COD}_{\text{CH}_4} + \text{COD}_{\text{SO}_4,R} + \text{COD}_{\text{new cells}} \tag{2.30}
$$

Where in

- $\text{COD}_i$ = COD in the influent
- $\text{COD}_e$ = COD in the effluent
- $\text{COD}_{\text{CH}_4}$ = COD of methane ($1 \text{ m}^3 \text{CH}_4 = 2.57 \text{ kg COD}$)
- $\text{COD}_{\text{SO}_4,R}$ = COD consumed for sulphate reduction ($1\text{g COD per 1.5 g sulphate reduced}$)
- $\text{COD}_{\text{new cells}}$ = COD consumed for new cells formation ($1 \text{ kg of VS = 1.42 kg of COD}$)
Yang and Xu 2007 have reported that the methane solubility increases with temperature and pressure, and decreases as the salinity increases. It was reported that the solubility of gas is also lowered as salinity increases (Zatsepina and Buffett 1998).

2.7.6 Nutrient Concentration

To ensure the optimal digestion of organic matter, trace of inorganic elements such as iron, nickel, cobalt, and zinc are required in low concentrations to stimulate fermentation and aid in the metabolism of the organic matter. Some wastes are deficient in these nutrients and then inorganic elements have to be added to the system for it to function properly. However, determining the specific nutrient requirements for a biological system can be difficult and it depends on the waste characteristics, availability of nutrients within the system, the design of the system and other parameters (Malaspina et al 1995). The essential nutrients for the anaerobic digestion of a specific wastewater can be determined by conducting a Biochemical Methane Potential (BMP) assay to evaluate the effect of addition of different nutrient solutions on the methane production rate.

2.7.7 Effect of Calcium on Anaerobic Process

Methanogenic microorganisms are susceptible to the minute changes in the pH values. Optimum pH range of 6.9 -7.2 has been reported to be favourable for the methane bacteria (Dugan 1977; Haug 1977). The pH, maintained inside the reactor, due to the process results, from the interaction of the carbon dioxide – bicarbonate buffering system and volatile acids – ammonia formed by the process (Bisselli 1975). It is necessary to prevent the accumulation of acids to a level, which may reduce the pH below optimum level. For this purpose, it is important that there should be sufficient buffering present in the reactor, which may prevent the reactor souring (Capri and Marias 1975)
The equations for the process of buffer destruction and formation are

\[
\text{C}_6\text{H}_{12}\text{O}_6^{\text{Acid}_{\text{Formers}}} \rightarrow 3\text{CH}_3\text{COOH} \quad (2.31)
\]

\[
3\text{CH}_3\text{COOH} + 3\text{NH}_4\text{HCO}_3 \rightarrow 3\text{CH}_3\text{COONH}_4 + 3\text{H}_2\text{O} + 3\text{CO}_2 \quad (2.32)
\]

\[
3\text{CH}_3\text{COONH}_4 + 3\text{H}_2\text{O} \xrightarrow{\text{Methane}_{\text{Bacteria}}} 3\text{CH}_4 + 3\text{NH}_4\text{HCO}_3 \quad (2.33)
\]

The reaction shown in the equation 2.31 represents the breakdown of glucose to acetic acid by acid forming bacteria. The acid formed is then neutralized, as shown in equation 2.32, by the bicarbonate buffer. If insufficient buffer is present, the pH will drop, and conversion of the acetate to methane, as shown in equation 2.33, would be inhibited. Although the carbonate and bicarbonates of sodium and calcium are required to be added to the digester to provide buffering action. Lime (calcium hydroxide) is most commonly used for this purpose, thus presence of lime helps in the efficient working of the reactor (Bal and Dhagat 2001)

2.7.8 Modeling of wastewater treatment process kinetics

All biological treatment reactor designs are based on using mass balance across a defined volume for each specific constituent of interest (i.e biomass, substrate, etc). The mass balance includes the flow rates for the mass of the constituent entering and/ or leaving the system and appropriate reaction rate terms for the depletion or production of the constituent within the system. The units for a mass balance are usually given in mass per volume per time. A reactor can be used in a laboratory study to assess wastewater treatability and to obtain model kinetic coefficient and develop a mathematical modeling of biological process for the prediction of effluent soluble substrate concentration and reactor biomass and volume of the reactor follow.
The substrate utilization rate in the biological system can be modeled with the following expression for soluble substrates. Mass of substrate is decreasing with time due to substrate utilization. Equation is used for substrate mass balance is.

$$r_{su} = \frac{kXS}{K_s+S}$$  \hspace{1cm} (2.34)

Where

- $r_{su}$ = rate of substrate concentration change due to utilization, g/m$^3$.day
- $k$ = maximum specific substrate utilization rate g / g .day
- $X$ = biomass (microorganism) concentration, g /m$^3$
- $S$ = growth- limiting substrate concentration in solution, g/m$^3$

When the substrate is being used at its maximum rate, the bacteria are also growing at their maximum rate. The maximum specific growth rate of the bacteria is thus related to the maximum specific substrate utilization rate as follows

$$\mu_m = kY$$  \hspace{1cm} (2.35)

and

$$k = \frac{\mu_m}{Y}$$  \hspace{1cm} (2.36)

Where $\mu_m$ = maximum specific bacterial growth rate, g new cells/g cells.

$Y$ = true yield coefficient, g/g
Using the definition for the maximum specific substrate utilization rate given by equation 2.34, the substrate utilization rate is also reported in the literature as

$$r_{su} = -\frac{\mu_m X S}{Y (K_s + S)}$$  \hspace{1cm} (2.37)

In reviewing kinetic expressions used to describe substrate utilization and biomass growth rate, it is very important to remember that the expression used to model biological process are all empirical, based on experimentally determined coefficient values. Besides the substrate limited relationship presented above, other expressions that have been used to describe substrate utilization rates include the followings

$$r_{su} = -k$$  \hspace{1cm} (2.38)

$$r_{su} = -k S$$  \hspace{1cm} (2.39)

$$r_{su} = -k X S$$  \hspace{1cm} (2.40)

$$r_{su} = -k X \frac{s}{s_0}$$  \hspace{1cm} (2.41)

The particular rate expression used to define the kinetics of substrate utilization depends mainly on the experimental data available to fit the kinetic equation and the application of the kinetic model.

A model can be developed considering biomass and substrate balances as follows.
Rate of accumulation of microorganism within the system boundary = Rate of flow of microorganism into the system boundary - Rate of flow of microorganism out of the system boundary + Net growth of microorganism within the boundary

\[
\frac{dx}{dt} V = QX_o - [Q - Q_w]X_e + Q_wX_R + r_g V
\] (2.42)

Where in

\[
\frac{dx}{dt} = \text{rate of change of biomass concentration in reactor, g VSS/m}^3.d
\]

\[
V = \text{reactor volume, m}^3
\]

\[
Q = \text{influent flow rate, m}^3/d
\]

\[
X_o = \text{concentration of biomass in influent, g VSS/m}^3
\]

\[
Q_w = \text{waste sludge flow rate, m}^3/d
\]

\[
X_e = \text{concentration of biomass in effluent, g VSS/m}^3
\]

\[
X_R = \text{concentration of biomass in return line from clarifier, g VSS/m}^3
\]

\[
r_g = \text{net rate of biomass production, g VSS/m}^3.d
\]

If it is assumed that the concentration of microorganisms in the effluent can be neglected and that steady-state conditions prevail \( (dX/dt = 0) \)

\[
(Q - Q_w)X_e + Q_wX_R = r_g V
\] (2.43)

Whereas \( r_g = Yr_{su} - K_dX \) (2.44)

By substituting for \( r_g \) the following equation was obtained
\[
\frac{(Q-Q_w)X_e + Q_wX_R}{VX} = \frac{r_{su}V}{X} = Y \frac{r_{su}}{X} - K_d
\] (2.45)

Where \( X \) = concentration of biomass in the reactor

The inverse of the term on left hand side of the above equation is defined as the average solid retention time (\( \theta_c \)) as given below

\[
\theta_c = \frac{VX}{(Q-Q_w)X_e + Q_wX_R}
\] (2.46)

Equation 2.46 can be rewritten as

\[
\frac{1}{\theta_c} = -Y \frac{r_{su}}{X} - K_d
\] (2.47)

The term \( 1/SRT \) is also related to \( \mu \), the specific biomass growth rate as given in equation 2.48,

\[
\frac{1}{\theta_c} = \mu
\] (2.48)

The term \( (-r_{su}/X) \), specific substrate utilization rate, is calculated as follows

\[
U = \frac{r_{su}}{X} = \frac{Q(S_o-S)}{VX} = \frac{S_o-S}{V/Q.X}
\] (2.49)

Where

\( U \) = Specific substrate utilization rate, g COD/gVSS.d

\( Q \) = wastewater flow rate, m\(^3\)/d

\( S_o \) = influent soluble substrate concentration, g COD / m\(^3\)

\( S \) = effluent soluble substrate concentration, g COD/m\(^3\)

\( V \) = Volume of the reactor, m\(^3\)

\( X \) = Biomass concentration, g/m\(^3\)
Substituting equation 2.37 into equation 2.47 yields

\[
\frac{1}{\theta_c} = \frac{YkS}{K_s + S} - K_d
\]  

(2.50)

By rearranging the equation 2.50, the following equation is arrived

\[
\frac{1}{U} = \left( \frac{K_s}{k} \right) \left( \frac{1}{S} \right) + \left( \frac{1}{k} \right)
\]

(2.51)

The solid retention time is an important design and operating parameter for the biological process. The SRT is the average time the biological sludge is retained in the system. Wasting is accomplished by removing biological sludge from the reactor from various depths. Solving equation 2.50 for the effluent substrate concentration \(S\) can be predicted as follows

\[
S = \frac{K_s[1 + (k_d)\theta_c]}{\theta_c (Yk - k_d) - 1}
\]  

(2.52)

The mass balance for substrate utilization in the reactor is

Accumulation = inflow – out flow + generation

\[
\frac{ds}{dt} V = QS_o - QS + r_{su} V
\]  

(2.53)

Substituting value from \(r_{su}\) under steady state condition equation can be rewritten as

\[
S_o - S = \left[ \frac{V}{Q} \right] \left[ kXS \right] \left[ \frac{K_s + S}{K_s} \right]
\]  

(2.54)

If equation 2.50 is solved for the term \(S/(K_s + S)\) and substituting into equation 2.54,

\[
X = \frac{\theta_c}{V/Q} \left[ \frac{V(S_o - S)}{1 + (k_d)\theta_c} \right]
\]  

(2.55)
Thus, the reactor biomass concentration is a function of the system SRT (Metcalf and Eddy 2003).

Influent, effluent, MLVSS concentration in the reactor and flow data collected by operating bench scale reactor is used to arrive at kinetic constants and to predict the effluent soluble substrate concentration, reactor biomass and volume of the reactor.

2.8 PRETREATMENT PROCESS

Size reduction of particles and the resulting increase in the specific surface available to the medium improves the biological process. First, if the substrate has a high fibre content and low degradability, their comminution leads to improved gas production and size reduction can lead to more rapid digestion (Palmowski and Muller 1999 a, b). It was reported that increased degradation rate of substrates could be obtained with pretreatment by mechanical, thermal, chemical, or enzymatic processes (Muller et al 2003). The decomposition process is faster with decreasing particle size but does not necessarily increase the methane yield (Mshandete et al 2006).

Fruit and vegetable wastes were chopped and then reduced to a size of 1-2 mm to obtain a homogeneous suspension with the primary sludge and results showed an acceleration of the digestion process as well as an increase in the degree of anaerobic digestion of the sludge. In general, the smaller the fibres of the substrates, the higher the biogas potential. The best results showed an increase of about 20% with fibres smaller than 0.35 mm. The chemical treatment of the fibres with NaOH, NH₄OH or a combination also led to increased methane potential and thermo-chemical pretreatment based on sodium hydroxide addition was used to enhance COD solubilization (Mata-Alvarez et al 2000).
Various sludge disintegration methods have been studied earliest as a pretreatment method to achieve a significant result in a lysis or disintegration of sludge cell which would have the potential to enhance the biogas production (Lin et al 2009).

It is suggested from research carried out that the application of a pretreatment to hydrolyse part of the fat particles could accelerate the anaerobic treatment of slaughterhouse wastewater. In the anaerobic digestion the biological hydrolysis is identified as the rate-limiting step. To reduce the impact of the rate limiting step, pretreatment of waste activated sludge is required such as thermal, alkaline, ultrasonic or mechanical disintegration. These treatment can accelerate the solubilization (hydrolysis) of WAS and reduce the particle size, which subsequently improves the anaerobic digestion (Tiehm et al 2001; Tanaka et al 1997). In general, hydrolysis is the rate limiting step if the substrate is in particulate form (Bouallagui et al 2005).

2.8.1 Mechanical Pretreatment

In order to increase the solubilization of organic matter and reduce the size of the fleshings, various pretreatment methods were reported to increase the biogas recovery and to reduce retention time in the reactor. One of the methods studied was to mince the fleshings with mincer and further feeding it into conventional anaerobic digester. Similar studies were reported by Shanmugam and Horan (2009). Pretreatment of the substrate by mechanical disintegration (size reduction of particles) had positive effects on the anaerobic biodegradability of the substrate. The obvious reason is the increase of the available specific surface to the medium. (Mace et al 2001)

LFs were reduced to the size of 6 mm diameter and mixed with primary sludge in the ratio of 1:1 to be fed into the anaerobic reactor for biomethanization process with HRT of 5 weeks (Thangamani 2009a). Waste
fleshings were pre-processed using an industrial meat cutter to size of 1-4 mm and subjected to anaerobic digestion in anaerobic sequential batch reactor (ASBR) and reported specific methane production potential of 0.649 m$^3$/kg of fleshing (Gregor and Jemec 2010).

Waste from tanneries were macerated, flash dried, densified and gasified to generate clean syngas for reuse in boilers (Booth et al 2006). The anaerobic co-digestion of vegetable waste, after chopping, sieving, grinding to a size less than 1 mm and hydrolyzing in combination with sewage sludge and cow dung in batch reactor was reported to give relatively more biogas generation for the ratio of 1:2 when compared to the ratio of 1:1 (Chittibabu et al 2009).

Hartmann et al (1999) found an increase of up to 25% in biogas from fibres in manure feedstock, after pretreatment of the whole feed in a macerator before digestion. No significant difference in the biogas potential was found from fibres in the 5-20 mm range (Mace et al 2001). Salminen and Rintala (2002) reviewed various pretreatment methods for increasing methane yield of feather by combined thermal and enzymatic treatments and reported an increase in methane yield of 37-51% whereas thermal (70ºC to 120ºC), chemical (NaOH: 2-10 g/L, 2-24 h) and enzymatic treatments were less effective in yielding methane which increased only in the range of 5-32%.

### 2.8.2 Thermo-Chemical Pretreatment

Thermo-chemical pretreatment was used to enhance COD solubilization and increase the biodegradability through various reactions such as saponification or uronic acids and acetyl esters, reactions occurring with free carboxylic groups and neutralization of various acids (Jeongsik kim et al 2003). Thermo-chemical pretreatment based on sodium hydroxide addition was used to enhance COD solubilization using the following optimal
conditions: pH – 12 and temperature - 140º C for 30 minutes where 70% solubilization was reported. However, anaerobic biodegradability of the pretreated substrate did not improve, remaining near 40% (Mace et al 2001). It was reported that alkaline pretreatment increased the biodegradability of the sewage sludge/Organic Fraction Municipal solid waste mixture over that of the untreated control in lab-scale studies (Mata-Alvarez et al 2000).

Thermo-chemical pretreatment has been studied to improve the anaerobic digestibility properties (Sawayama et al 1996). Alkaline pretreatment had also been investigated to solubilize various substrates such as Waste Activated Sludge (WAS) and was reported to give enhancement in gas production and reduction in COD and volatile solids when WAS was pretreated with NaOH (Penaud et al 1999; Lin et al 1997; Jeongsik Kim et al 2003). Tacker and Kastner (2004) reported that carcass could be hydrolyzed using of NaOH and KOH with heat treatment. Fleshings were delimed and hydrolyzed using mild organic acids and steam-cooked for extraction of protein (Amit et al 2009). Fleshings from tanneries were heated to remove fat and conversion into biodiesel (Colak et al 2006). Waste from food processing industries like fish, poultry, pork and beef were hydrolysed to provide liquid products containing amino acids and lipids. This process involved alkaline treatment with heat (120-170ºC and pH above 12) which facilitates liquefaction (US patent 4,473,589).

Concentrations of VFA of around 7500 mg/L was reported with an alkaline pretreatment of 24 h, followed by a fermentation of 5 days at mesophilic temperatures, which represents an improvement of around 45% with respect to the control fermenter without pretreatment. This pretreatment introduces a foreign anion into the system (Mace et al 2001). When the barley waste was subjected to alkaline hydrolysis pretreatment before co-digestion with activated sludge, the methane production increased from 0.025 to 0.222
m³ CH₄/kgVS initial. The total and volatile solids reduction increased from 31 to 67% and 67 to 84% respectively (Neves et al 2006).

Four pretreatments to hydrolyse and/or reduce the size of fat particles in slaughterhouse wastewater were tested which included sodium hydroxide and three lipases of plant, bacterial and animal (pancreatic) origin. Hydrolysing agents and SHW containing between 2.5 and 3 g/l of fat particles were mixed at room temperature for 4 h. Addition of 5 - 400 meq NaOH/l did not increase soluble COD in SHW, but the average particle size was reduced to 73% ± 7% of the initial average particle size (Dₐᵣ) at NaOH concentrations ranging from 150 to 300 meq/l. Pretreatment with pancreatic lipase (PL-250) reduced the average particle size to a maximum of 60% ± 3% of Dₐᵣ. As Dₐᵣ was decreased from 359 to 68 μm, the enzyme concentration required to obtain the maximum particle size reduction increased from 200 to 1000 mg/l (Masse et al 2001).

Alkaline pretreatment was performed for Waste Activated Sludge (WAS) at pH 12 with alkaline agents NaOH, KOH, Mg(OH)₂ and Ca(OH)₂. At ambient temperature, the COD solubilization of WAS was reported to be 39.8%, 36.6%, 10.8 and 15.3% respectively. Similarly, it was reported that after treatment at 121°C for 30 min, NaOH addition resulted in 51.8% COD solubilization with values, with other alkaline agents being 47.8, 18.3 and 17.7% respectively.

Singh et al (2007) investigated biogas production potential of flower waste coming out from temples, in laboratory scale digester of 1.5 L capacity. The waste was digested for retention periods of 35 days under batch fed system at constant temperature of 30°C ± 2°C. The quantity of inoculum fed was 10% of total volume of slurry in each digester. It was concluded that fresh flowers are not suitable for biomethanation while pretreated flowers with NaOH give good gas production and waste digestion.
Batch mesophilic BMP tests were carried out for rapeseed and sunflower meal and biogas production of 0.45 m$^3$/kg and 0.481 m$^3$/kg was reported. Various pretreatment methods like thermal, chemical and combination of above methods were investigated for enhancement of methane production but methane enhancement could not be achieved due to inhibitory compounds which could have been released during pretreatment (Antonopoulou et al. 2010). The poor anaerobic biodegradability performances were attributed to the soluble molecules generated being inhibitory to anaerobic micro organism.

2.8.3 Biological Pretreatment

Raynal et al. 1998 have reported that hydrolysis (Liquefaction) rate was evaluated from the COD removal measured in the digester per unit time and expressed in g COD/L.day. Nevertheless, some organic solid wastes present a low biodegradability in spite of the high COD content and, therefore further studies needs to be investigated to enhance the biomethenization process of such wastes (Neves et al. 2006). Chulhwan Park et al. (2005) have reported biological hydrolysis using aerobic bacteria of waste activated sludge and has reported that rate of the biological hydrolysis is relatively low but it is cheaper and has recommended for biological hydrolysis for large scale application.

2.9 CLEAN DEVELOPMENT MECHANISM (CDM)

The Clean Development Mechanism (CDM) allows emission-reduction projects to earn Certified Emission Reduction (CER) credits, each equivalent to one ton of CO$_2$ through Kyoto Protocol of United Nations Framework Climate Change (UNFCCC). These CER can be traded and sold, and used by industrialized and developed countries to meet a part of their emission reduction targets under the Kyoto Protocol (IPCC 2007). The
mechanism stimulates sustainable development and emission reductions, while giving industrialized countries some flexibility in how they meet their emission reduction limitation targets of UNFCCC. The methodology has been applied for project activities that avoid methane emission reductions.

2.9.1 Certified Emission Reduction (CER)

Methane is the main greenhouse gas from wastewater treatment, mostly produced by methanogens in anaerobic conditions (Yang et al 2003), while NO\textsubscript{2} is a byproduct of nitrification and de-nitrification generated during advanced wastewater treatment process (Brown et al 2008; Lin et al 2008). The life span of methane in the atmosphere is only 9 to 15 years, which is shorter than that of CO\textsubscript{2} (50 to 200 years), so the reduction in methane emissions could lead to slowing of short-term climate change.

Developing countries have been taking part in the carbon market to get investment and financial benefit for their own development as well as for sustainable development. China and India were the largest sellers of carbon credits with 61% and 12% respectively of the total CDM market in 2006, while the EU countries are the biggest buyers. Emission trading has been steadily increasing in recent years. The CER exchangers were 78, 110 and 374 million metric tons of CO\textsubscript{2} in 2003, 2004 and 2005 respectively in 2009 (Wong et al 2009). However, 8.2 billion metric tons of CO\textsubscript{2} transferred from developing countries to developing countries worldwide, up 68% from 2008 (Sweet 2010).

Anaerobic wastewater treatment facility based on Upflow Anaerobic Sludge Blanket (UASB) technology at an existing starch manufacturing plant has been installed in the place of the present wastewater treatment facility based on anaerobic lagoon to treat the wastewater before it is discharged into the water at Sima Interproduct Co., Ltd, Thailand. The
methane (GHG) produced in lagoons from anaerobic digestion of the organic content in the wastewater is released into the atmosphere. The use of UASB to treat the wastewater generated in the plant would enable the capture of methane produced in the process while meeting the effluent standards which would reduce the emission of GHG. With a total production capacity of 430 tonnes of native starch per day generating 2.8 million liters of wastewater per day, it was estimated that 21,733 CER per year would be generated (UNFCCC-SIMA 2004).

Tamilnadu Newsprint and Papers Limited (TNPL) installed a closed anaerobic system to produce and collect consistently high quality methane-rich biogas from bagasse wastewater (BWW) generated at the TNPL. The BWW is diverted from TNPL’s existing wastewater treatment system i.e. open lagoon under anaerobic conditions into a latest closed anaerobic granular sludge bed technology, which is a special kind of reactor concept for the "high rate" anaerobic treatment of wastewater called Upflow Anaerobic Sludge Blanket (UASB) reactor. The project activity also includes a system for consumption of collected biogas as a fuel in a lime kiln, which has been using furnace oil (fossil fuel), by extracting and capturing biogas in a closed digester. The project reduced the CH₄ emission that would have otherwise been emitted to atmosphere from existing open lagoons. In addition, the use of collected biogas as a fuel in limekiln displaces fossil fuel and its associated GHG emissions. The plant was designed to handle 12,000 m³/day of bagasse wash wastewater with average 6000 mg/L COD concentration with a HRT of 20 hours and maximum OLR of around 5.75 kg COD/m³.day. It was estimated that 35,966 CER per year would be generated (UNFCCC-TNPL 2004).
2.10 ECONOMIC ANALYSIS OF EFFlUENT TREATMENT

Comparative assessment of the cost and quality of treatment of tannery wastewater in India by two CETPs constructed for two tannery clusters, at Jajmau (Kanpur) and at Unnao in the state of Uttar Pradesh, India were studied. The Jajmau plant is upflow anaerobic sludge blanket (UASB) process-based, while the Unnao plant is activated sludge process (ASP)-based. Investigations indicated that the ASP-based plant was superior in all respects. The results of this study are at variance with the conventional wisdom of the superiority of anaerobic processes for tannery wastewater treatment in tropical developing countries like India (Tare et al 2003).

The total annual cost including capital and operation and maintenance (O&M) costs for the up-flow anaerobic sludge blanket (UASB) and waste stabilization pond (WSP) systems operated in India was evaluated. Comparison between UASB and WSP systems with the activated sludge process (ASP) and biological aerated filter (BAF) systems in terms of total annual cost and chemical oxygen demand (COD) removal cost by assuming various annual interest rates and land prices. It was concluded that the relationship between capital and O&M costs per unit size of a UASB or WSP system and its treatment capacity followed first-order equation. The relation between the cost of organic removal and capital and O&M cost for various sewage treatment systems at various annual interest rates revealed that in the Indian context, UASB was the most suitable option in terms of expenses and treatment efficiency (Nobuyuki et al 2007).

2.11 SUMMARY

It has been reported elsewhere that when a ton of wet salted hides is processed 800 kg of solid waste is generated out which about 200 kg is fleshings (moisture content : 80-85 %, ash content : 13-15 % and remaining
are volatile solids). At present fleshings is disposed along with municipal solids and decomposed in open dump yards, although some quantity is used for chicken feed and glue manufacturing. Various studies were carried out to recover energy in the form of methane by digesting the fleshings in digesters. The retention time reported for such digesters vary from 30-60 days. Considering the pH of 12 for LF, it is required to be neutralized before it is fed into the digesters. Neutralizing lime requires huge quantity of acid. Addition of acid in lime will produce effervescence. Decomposition of LF in open yards leads to release of CO₂.

Various pretreatment studies were carried out to accelerate the digestion process and to enhance the methane production. Some of the techniques reported were chopping of waste to the size of 1 - 2 mm (Mata-Alvarez et al 2000), passing the sludge at high pressure through a nozzle (Stephenson et al 2000), Limed fleshings were reduced to the size of 6 mm diameter using an industrial meat cutter (Thangamani 2009a), similar pretreatment was carried out to reduce the size of fleshings to 1-4 mm (Gregor and Jemec 2010). Chulhwan Park et al (2005) has reported that during biological hydrolysis 90% of the particles were reduced to 230 µm of size or less. It has been widely reported in the literature that reduction of size enhances biodegradation.

Thermo-chemical pretreatment has been studied to improve the anaerobic digestibility properties by COD solubilization and size reduction. Alkaline pretreatment (NaOH, KOH, Ca(OH)₂) had also been investigated to solubilize various substrates (Sawayama et al 1996; Penaud et al 1999; Lin et al 1997; Jeongsik Kim et al 2003; Mace et al 2001) these processes involve addition of chemical, heating under pressure.

Chulhwan Park et al (2005) has reported biological hydrolysis using aerobic bacteria of waste activated sludge has also been reported and
the rate of biological hydrolysis is relatively low but it is cheaper and has recommended for biological hydrolysis for large scale application. Both thermochemical and biological hydrolysis pretreatment decreased the particle size and increased the level of soluble protein and anaerobic digestion efficiency also increased.

Biogas is a product of anaerobic degradation of organic substrates, which is one of the oldest processes used for the treatment of industrial wastes and stabilization of sludge (Yadvika et al 2004). Several tests at laboratory scale were carried out to study different pretreatment prior to its anaerobic treatment. Results show that digestibility is improved by means of pretreatment (Mace et al 2001). Higher anaerobic digestion efficiency were obtained through thermochemical pretreatment of sludge (Jeongik kim 2003).

For liquefaction of tannery fleshings, effect of mechanical pretreatment, effect of thermo-chemial pretreatment and effect of biological pretreatment are to be carried out and a suitable pretreatment needs to be identified based on the above pretreatment studies to reduce particle size and to solubilize organic matter, and then the liquefied LF can be mixed with TE for recovery of biogas in the anaerobic reactor which avoids separate digester needed for anaerobic digestion of fleshings for recovery of energy.

Performances of the anaerobic reactor are influenced by pH, temperature, VFA, OLR, HRT. The pH affects the anaerobic digestion and overall performance of the digestion process. Methane producing microbes function effectively between pH range of 6.5 and 8.2 (Eckenfelder 1999; Speece 1996 ; Medhat et al 2004). The anaerobic process is more sensitive to temperature variation. Temperature has a major influence on the effectiveness of biological systems, affecting the metabolic rate and solubility of substrates(Speece 1996). Literature review carried out showed that anaerobic microorganisms function effectively in the mesophilic range of 29°C to 38°C.
The required optimum C:N: P ratio for enhanced methane production has been reported to be 100:2.5:0.5 (Hulshoff Pol and Lettinga 1986). UASB reactors have been operated for slaughter house waste at OLR ranging from 5-40 kg/m$^3$.day (John 1995). In anaerobic biomethanization of heterogeneous solid waste organic residues, the complex organic substances are first solubilized and hydrolyzed due to metabolic activity of non methanogenic bacteria to produce fermentation intermediates, mainly volatile fatty acids (VFA) by utilizing a range of extra cellular enzymes. These intermediates are then degraded by methanogenic bacteria to methane and carbon dioxide (Xu et al 2002; Horiuchi et al 2002).

Hence, there is a need to conduct bench scale and pilot scale studies on biomethanization of liquefied limed fleshings with tannery effluent to ascertain the feasibility of such process for industrial applications.

It is necessary to estimate the benefits obtained from treatment options like energy generation and Clean Development Mechanism (CDM) benefits. The CDM is an arrangement under the Kyoto Protocol allowing industrialized countries with a greenhouse gas (GHG) reduction commitment to invest in projects that reduce emissions in developing countries as an alternative to more expensive emission reductions.

Hence, there is also a need to study the techno-economic viability of liquefaction of LF by appropriate pretreatment options and enhance biomethanization process and also to arrive at the carbon credits for the above process based on CDM.