CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL

In order to plan the present study, a comprehensive and pertinent literature available were collected and reviewed. The literature reviewed included research articles published in national and international journals and technical reports prepared by the government departments and research agencies. The present study focuses on

(i) Anaerobic digestion
(ii) Co-digestion of solid wastes
(iii) Lipid degradation
(iv) Pre-treatment of sludge, and
(v) Analysis of digestate.

And therefore, the review of the pertinent literature was also done as per the different areas of focus for the present study and the same is presented in this chapter.

2.2 ANAEROBIC DIGESTION

2.2.1 Historical Background

Historical evidence indicates that, the anaerobic digestion (AD) process is one of the oldest technologies. Biogas was used for heating bath
water in Assyria during the 10th century BC. Advancement in AD with scientific research was developed in 17th century. Jan Baptista Van Helmont established that flammable gases will be evolved from decaying organic matter. Also, Count Alessandro Volta in 1776 showed that, there was a relationship between the amount of decaying organic matter and the amount of flammable gas produced. In 1808, Sir Humphry Davy demonstrated the production of methane by the anaerobic digestion of cattle manure. Industrial applications of AD began in the year 1859 with the first anaerobic digestion plant in Bombay, India. Buswell and others in 1930’s identified anaerobic bacteria and the conditions that promote methane production. Anaerobic digestion process has been popular in the waste treatment field because it has many advantages such as high treatment efficiency and ability to produce methane gas.

China and India have now adopted a trend towards larger, more sophisticated farm based systems with better process control to generate electricity from biogas. The technology is now being applied for municipal waste treatment as well as for industrial waste. Taiwan has cut down river pollution, caused by direct discharge of waste generated from animal husbandry by adopting standard AD systems. In recent times, Europe came under pressure to explore AD market because of two significant reasons: (i) high energy prices and (ii) stringent environmental regulations especially to have a control on organic matter entering into landfills.

2.2.2 Process

Anaerobic digestion is the breakdown of complex organic matter by the microorganisms in the absence of free oxygen producing methane, carbon dioxide, and ammonia, traces of other gases and organic acids of low molecular weight as end products of the process (Polprasert 1989). The process has been employed in several developed countries with the objective
of biostabilization of fermentable organic waste produced by rural and urban activities (Van Lier et al 2001).

During anaerobic digestion, complex organic materials are first hydrolyzed and fermented by rapidly growing and pH-insensitive acidogenic bacteria into volatile fatty acids (VFAs) (Li and Noike 1992; Siegrist et al 1993). The VFAs are then oxidized by slowly growing acetogenic bacteria into acetate (HAc), molecular hydrogen and carbon dioxide that are suitable as substrates for the methanogenic bacteria (Denac et al 1988; Pavlostathis and Geraldo-Gomez 1991; Ozturk et al 1993). Four major steps involved in AD are hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 2.1.

![Figure 2.1 Processes of Anaerobic Digestion](Source: Speece 1996)

The VFAs are important intermediate compounds in the metabolic pathway of methane fermentation, if present in high concentrations, resulting in decrease of pH and ultimately leading to failure of the digester. Therefore, the concentration of VFA is an important consideration for good performance
of a digester. It is widely known and accepted that, the occurrence of VFAs during anaerobic digestion of sludge decreases as the chain length increases. In addition, the presence of formate is usually quite limited due to its rapid conversion to other compounds (e.g. methane and carbon dioxide).

Anaerobic digestion offers significant advantages over aerobic process, like low energy consumption, reduced solids formation, low nutrient requirement and potential energy recovery from the methane produced (Stewart et al 1995). Additionally, the use of the anaerobic digestion reduces the volume of waste and the digestate could be used as soil amendment. This process is now widely used in many environmental applications, in different configurations and modes of operation.

Both the total ammonia nitrogen (TAN) and volatile fatty acids (VFAs) are important intermediates and potential inhibitors in the anaerobic digestion process (Parkin and Owen 1986). Environmental conditions like pH, temperature, type and quality of substrate, mixing factors and process inhibitory parameters like high organic loading, formation of high volatile fatty acids and inadequate alkalinity affect the anaerobic digestion (Molnar and Bartha 1989).

Anaerobic digestion processes have been widely recognized as a way to control greenhouse gas emissions and for bio-energy generation (Lettinga 1995 and 2001; Barton et al 2008). Anaerobic digestion of organic waste resulted in production of energy in the form of biogas is arguably the most likely option as a commercial interest and also in light of the emission reductions agreed at the Kyoto protocol, where environmental considerations are accorded greater significance than economics. The European Union (EU) countries have agreed on supporting the production of biogas as a renewable
energy source to decrease the greenhouse gas emissions according to the Kyoto protocol (CEC 2001).

Studies were carried out in a full-scale plant located at Verona- Ca del Bue, Italy for anaerobic digestion of the organic fraction of municipal solid waste (OFMSW) in semi-dry thermophilic conditions to reduce the digestion period. It was reported that, for the organic loading rate of 0.135 kg TVS_{feed}/ kg TVS_{reactor}/day, the specific gas production of 0.23 m^{3}/kg TVS_{feed} and a gas production rate of 2.1 m^{3}/m^{3}/ day was achieved within a digestion period of 30 days (Bolzonella et al 2006).

The methane potential of solid organic waste was developed by Hansen et al (2004). The anaerobic biogas potential from biologically treated municipal solid waste can be predicted by using dynamic respiration index (Barbara Scaglia et al 2010). The successful application of AD process depends on the nature of organic substrate which in turn influences the biodegradation process and methane yield (Forster-Carneiro et al 2008, 2008a). Not only that but also from biochemical characterization (carbohydrates, lipids and proteins) and ratio of chemical oxygen demand to total organic carbon, the biodegradability indicators were developed for waste activated sludge (Alexis Mottet et al 2010). High solids and ligno-cellulosic fiber components are recalcitrant to conventional anaerobic digestion process and to overcome this problem a super blue box recycling (SUBBOR) process for anaerobic digestion of mixed municipal solid waste (MSW) and other biomass from feedstock materials was developed by Vogt et al (2002).

Many agricultural and industrial wastes are ideal substrates for anaerobic digestion because they contain high levels of biodegradable materials. Full scale AD plants are in operation using energy crops with organic fraction of municipal solid waste for biogas generation (Pognani et al 2009).
2.2.3 Operational Parameters

2.2.3.1 Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT)

The effects of hydraulic retention time and organic loading rate were investigated by Salminen and Rintala (2002) during semi-continuous anaerobic digestion of solid poultry slaughter house waste. It was reported from the studies that, upto an OLR of 0.8 kg volatile solids (VS)/m$^3$ d and an HRT of 50–100 days, the specific methane yield was high from 0.52 to 0.55 m$^3$/kg VS$_{added}$ whereas, by increasing the OLR range from 1.0 to 2.1 kg VS/m$^3$ day and shortening the HRT from 25 days to 13 days, process was inhibited due to overloading of the reactors.

The performance of the temperature based anaerobic digestion process has been evaluated for the digestion of livestock wastes with organic loading rates of 1.87 to 5.82 g VS/L/day. Volatile solids (VS) reduction in the range of 36–41% and biological conversion efficiency of 0.52– 0.62 L methane / gram of VS destroyed were reported by Harikishan and Sung (2003). The impact of mode of mixing on anaerobic digestion of animal waste was investigated by Khursheed et al (2005) and it was reported that mixing did not improve the performance of the digesters fed with more dilute (5% solids) manure. However, the effect of mixing and the mode of mixing became prominent when digesters were fed with thicker manure slurry (10% solids).

The influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue was investigated by Rincon et al (2008) and it was reported that OLRs higher than 9.2 g COD/L day and HRTs lower than 17 days favored process failure, decreasing the pH
and COD removal efficiency and the maximum methane production rate obtained was 1.7 L CH$_4$ /L day.

The effect of organic load and mixing intensities on biogas generation was investigated by Kaparaju et al (2008) and it was stated that, for low substrate to inoculum ratio, gentle or minimal mixing is required whereas for high substrate to inoculum ratio, gentle mixing resulted in higher methane production. The most critical step in an anaerobic methane reactor is the start-up and restart phase of the reactors. A stepwise increase of OLR despite high VFA and pH > 6.8 are beneficial for stable performance of the methane reactor (Gallert and Winter 2008).

2.2.3.2 Solids Retention Time (SRT) and Hydraulic Retention Time (HRT)

During the anaerobic digestion of poultry slaughter house wastes digested at a temperature of 31$^\circ$C, at an organic loading rate of 0.8 kg VS/m$^3$ day and an HRT of 50–100 days, methane production was in the range of 0.52–0.55 m$^3$/ kg VS$_{added}$ as reported by Salminen and Rintala (2002). When the organic load was increased i.e. 1.0 to 2.1 kg VS/m$^3$ day, and a shorter HRT in the range from 25 to 13 day was adopted, the process was inhibited due to accumulation of volatile fatty acids and long-chain fatty acids and the decline in the methane yield was observed.

The influence of the solid retention time in mesophilic anaerobic digestion of waste activated sludge was investigated by Bolzonella et al (2005). The biogas production was in the range of 0.07–0.18 m$^3$/kg VS$_{fed}$ or the specific gas production per kilogram of volatile solids destroyed was in the range 0.5–0.9 m$^3$/kg VS$_{destroyed}$ with a hydraulic retention time in a range of 20–40 days and an organic loading rate of 1 kg VS/m$^3$ reactor day. With a typical hydraulic retention time (HRT) of 15–30 d, only 50–70 % of organic
matter was converted into biogas with an average methane yield of 0.20–0.25 m$^3$/kg volatile solids (VS)\textsubscript{added} (Hartmann et al 2000). The effect of solids retention time (SRT) on thermophilic anaerobic digestion of sewage sludge was studied by Rubia et al (2006). At VS and COD loading rate of 2.2 kg VS/m$^3$/day and 3.9 kg COD/m$^3$/day, greater than 50% VS and 42% COD reductions were observed.

Anaerobic digestion of food waste at a hydraulic retention time (HRT) of 8 to 10 days and operating temperature of 30°C to 50°C was studied by Kim et al (2006). In this study, maximum biogas production occurred at 50°C with a 10 day HRT, the methane yield was highest (223 L CH$_4$/kg SCOD$_{degraded}$). The distinct advantage in considering the treatment of food waste by anaerobic digestion in the thermophilic range with a 10 day HRT, was emphasized.

### 2.2.3.3 Mode of Operation

About 90% of the full scale plants, currently in use in Europe, for the anaerobic digestion of biomass rely on continuous single-stage systems (Lissens et al 2001). Another type of digestion in operation is batch mode where digesters are filled once with fresh biomass, and allowed to go through all degradation steps sequentially. Other systems work in two steps, the first is the continuous digestion and the second step is batch digestion of the digested biomass. The hallmark of batch systems is the sharp separation between a first phase, where acidification proceeds much faster than methanogenesis and a second phase, where acids are transformed into biogas (De Baere 2000).

Two-phased hyper-thermophilic anaerobic co-digestion of waste activated sludge with kitchen garbage was investigated by Lee et al (2009). Acidogenesis at 70°C and methanogenesis at 55°C were stable and well-functioned in terms of treatment performance and low ammonium nitrogen
concentrations. The effect of reactor configuration on biogas production from wheat straw hydrolysate was investigated by Kaparaju et al (2009a). A better process performance and methane yield were observed in continuous stirred tank reactor (CSTR) when compared with upflow anaerobic sludge blanket (UASB) reactor.

Two-phase anaerobic co-digestion of olive mill wastes in semi-continuous digesters at mesophilic temperature was investigated by Fezzani and Cheikh (2010). It was reported that, two-phase anaerobic digestion system gave the best performances concerning methane productivity, soluble COD and phenol removal efficiencies and effluent quality compared to those given by conventional single-phase anaerobic digestion reactors.

Elena Comino et al (2009) conducted studies on anaerobic digestion of cow manure with whey mix under batch and fed-batch conditions for a period of 56 days. The total methane yield was in the order of 211.4 L CH$_4$/kg VS. Also the electricity generation from this process was accounted to be 8.86 kwh/tonne/day. The two-phase acidogenic-thermophilic and methanogenic-mesophilic anaerobic digestion process had an efficient removal of fecal coliforms, salmonella and helminth eggs, achieving values lower than those specified for Class A biosolids according to the Mexican Standard NOM-004-SEMARNAT-2002 (1000 MPN/g TS, 3 MPN/gTS and 1 HE$_{larval}$/g TS, respectively) (Rubio-Loza and Noyola 2010).

A summary of important studies on significance of operating parameters in anaerobic digestion is presented below in Table 2.1.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Substrate(s)</th>
<th>Investigator(s)</th>
<th>Year</th>
<th>Operating Conditions</th>
<th>Methane/Biogas Generation</th>
<th>Remarks</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Livestock wastes</td>
<td></td>
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<tr>
<td>a</td>
<td>Solid poultry slaughter house waste</td>
<td>Salminen and Rintala</td>
<td>2002</td>
<td>OLR- 0.8 kg volatile solids (VS)/m³ d; HRT- 50–100 days and OLR- 1.0 to 2.1 kg VS/m³ day, HRT shortening from 25 days to 13 days</td>
<td>specific methane yield - 0.52 to 0.55 m³/kg VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td>Process was inhibited due to overloading of the reactors.</td>
</tr>
<tr>
<td>b</td>
<td>Livestock wastes</td>
<td>Harikrishan and Sung</td>
<td>2003</td>
<td>OLR- 1.87 to 5.82 g VS/L/day; VS reduction</td>
<td>0.52– 0.62 L methane / gram of VS destroyed; VS reduction - 36–41%</td>
<td></td>
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<tr>
<td>c</td>
<td>Cow manure with whey mix</td>
<td>Elena Comino</td>
<td>2009</td>
<td>HRT- 56 days</td>
<td>methan yield: 211.4 L CH&lt;sub&gt;4&lt;/sub&gt;/kg VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Waste activated sludge</td>
<td>Bolzonella et al</td>
<td>2005</td>
<td>OLR:1kg VS/m³&lt;sub&gt;reactor&lt;/sub&gt; and HRT: 20–40 days</td>
<td>biogas production: 0.07–0.18 m³/kg VS&lt;sub&gt;fed&lt;/sub&gt;; specific gas production: 0.5– 0.9 m³/kg VS&lt;sub&gt;destroyed&lt;/sub&gt;;</td>
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</table>
2.3 CO-DIGESTION OF SOLID WASTES

Co-digestion is nothing but anaerobic digestion of two or more substrates simultaneously. For treatment and disposal of organic fraction of solid waste, co-digestion with sludge in anaerobic reactors is the best available option. Sometimes the use of a co-substrate can also help to establish the required moisture content of the digester feed. Other advantages are ease in handling mixed wastes and the use of common access facilities. In recent years, anaerobic co-digestion studies are gaining momentum due to the following benefits:

- Dilution of potential toxic compounds, if any
- Improved balance of nutrients
- Synergistic effects of microorganisms
- Better biogas yield
- Generation of organic fertilizer

The anaerobic co-digestion of organic wastes has the potential to contribute significantly to the reduction of the landfill disposal as well as to the renewable energy budget. In co-digestion process, different organic substrates combines to generate a homogeneous mixture as input to the anaerobic reactor in order to increase process performance (Viotti et al 2004; Zhang and Banks 2008) and avoid nutrient addition when a co-digested waste contains nutrients in excess (Pavan et al 2005 and Neves et al 2009b). Process requirements for anaerobic co-digestion are optimum mixing ratio of substrate and co-substrates, presence of macro and micronutrients, C/N ratio, pH, absence of inhibiting substances, availability of biodegradable organic matter, alkalinity, temperature and operational parameters such as hydraulic retention time (HRT), solids loading rate (SLR).
Many types of organic wastes such as sewage sludge, industrial waste, slaughter house waste, fruit and vegetable waste, manure and agricultural biomass have been digested anaerobically in a successful way either separately and or in co-digestion processes (Murto et al 2004).

The biomethanation potential of the waste depends on the relative amounts of the four main components – proteins, lipids, carbohydrates and cellulose. The significance of composition of restaurant waste on the biomethanation potential at mesophilic temperature was investigated by Neves et al (2008).

2.3.1 Organic Fraction of Municipal Solid Waste

The co-digestion of OFMSW with primary sewage sludge, industrial organic sludge and agricultural slurries has been investigated and it was an operational process at full scale in several centralized co-digestion plants in Denmark (Kiely et al 1997). Kiely et al (1997) and Sosnowski et al (2002) stated that anaerobic co-digestion of the organic food fraction of the municipal solid wastes with primary sewage sludge is an attractive method for environmental protection and energy savings point of view.

Raynal et al (1998) carried out studies on vegetable substrates using two-stage fermentation process i.e. multiple liquefaction reactors and a central methanizer at 35ºC. In the two-stage process, high hydrolytic yield was observed. This efficiency was achieved by recycling total suspended solids into the first-stage digesters and by the adaptation of microorganisms.

The co-digestion of OFMSW with manure at thermophilic temperature of 55ºC, hydraulic retention time of 14-18 days and an organic loading rate (OLR) of 3.3-4.0 g-VS/L/d was investigated by Hartmann and Ahring (2005) and biogas yield in the range of 180-220 m³/tonne of MSW was reported.
Capela et al (2008) studied the feasibility of anaerobic co-digestion of OFMSW, industrial sludge (IS) and cattle manure (CM). The increase of OFMSW in mixture resulted in higher methane production and solids reduction. The co-digestion of OFMSW with BS and CM, significantly improved the stability and the process performance, with an effective decrease on the need for buffer addition. The mixture with 77% of OFMSW and 23% of IS in composition had shown a better performance in terms of methane production and solids reduction.

Anaerobic co-digestion of a simulated organic fraction of MSW and fats of animal and vegetable origin was investigated by Fernandez et al (2005) and it was found that, both fats from animal and vegetable origin were almost degradable and suitable for treatment of fat containing wastes. Co-digestion of industrial sludge with municipal solid wastes in anaerobic simulated landfilling reactors was carried out by Agdag and Sponza (2005). A decrease in methane gas productions was observed due to toxicity effect of addition of industrial sludge.

In a full scale plant the anaerobic co-digestion of organic waste from domestic refuse and municipal sludge was conducted by Zupancic et al (2008) with an organic loading rate of 1.0 kg/m$^3$/day of volatile suspended solids. Specific biogas production increased from 0.39 m$^3$/kg volatile suspended solids to 0.89 m$^3$/kg volatile suspended solids.

### 2.3.2 Vegetable and Food Wastes

Callaghan et al (2002) conducted studies on continuous co-digestion of cattle slurry (CS) with fruit and vegetable wastes (FVW) and chicken manure (CM) and reported that, CM is not suitable as co-substrate. As the amount of CM in the feed and the organic loading was increased, the
VS reduction deteriorated and the methane yield decreased due to the presence of free ammonia in the liquor.

Two-phase anaerobic digestion of a mixture of fruit and vegetable wastes was studied using anaerobic sequencing batch reactors operated at mesophilic temperature by Bouallagui et al (2004). It was reported that 81% of hydrolysis yield was achieved at an OLR of 7.5 g COD/L.d. Volatile fatty acids concentration reached a maximum value of 13.3 g/L at a loading rate of 10.1 g COD/L.d. Kaparaju and Rintala (2005) have carried out co-digestion studies using organic residues from potato tuber with pig manure at a loading rate of 2 kg VS/ m$^3$/ day in CSTR at 35$^0$C and they observed that, the methane yield and process performance for potato tuber is similar to that of its industrial residues.

Dearman and Bentham (2007) carried out studies on anaerobic digestion of food waste and the comparing leachate exchange rates in sequential batch systems for digestion of food waste and bio-solids. The studies revealed that, by increasing the leachate volume between mature and start-up reactors, the time to degrade feedstock decreased, but total methane generation yield did not markedly differ, being 229 L CH$_4$/ kg VS$_{added}$ and 214 L CH$_4$/ kg VS$_{added}$. From the analysis of biogas composition, the changes in carbon-di-oxide and methane content in mature reactors were observed due to stress caused by the addition of leachate with high VFA concentrations from the start-up reactors.

Tembhurkar and Mhaisalkar (2007) carried out studies on hydrolysis and acidogenesis of kitchen wastes in two phase anaerobic digester and the specific rate constant (k) during hydrolysis and acidification of kitchen waste was 0.155 d$^{-1}$. Anaerobic co-digestion of vegetable market waste and sewage sludge seems to be an attractive method for waste
management, environmental protection, and energy savings (Anhuradha et al 2007).

Anaerobic co-digestion of kitchen waste (KW) with cattle manure (CM) for biogas production was investigated by Li et al (2009). The co-digestion of KW with CM increased the methane yield by 44% as compared to the single digestion of KW alone. The reason for enhanced methane generation was the synergistic effect in the co-digestion process.

The effect of abattoir wastewater (AW), fish waste, waste activated sludge (WAS) addition as co-substrates for the fruit and vegetable waste on anaerobic digestion performance was investigated in mesophilic condition using anaerobic sequential batch reactors by Bouallagui et al (2009) at an organic loading rate of 2.46–2.51 g volatile solids (VS)/L/day. It was observed from the studies that, the AW and WAS addition at a ratio of 10% VS, enhanced the biogas yield by 51.5% and 43.8% and total volatile solids removal by 10% and 11.7% respectively. However FW addition led to improvement of the process stability, as indicated by the low VFAs/Alkalinity ratio of 0.28.

The behavior of co-digestion of cow manure with food waste by applying increasing concentrations of intermittent pulses of residual-oil generated from a canned fish processing industry was investigated by Neves et al (2009b). Considering the mixture of lipids present in the oily waste, addition of oily waste at 12 g COD_{oil} /L_{reactor} enhanced the methane production in the co-digestion of cow manure and food waste. But further addition of oily waste to 18 g COD_{oil} /L_{reactor} induced a persistent inhibition of the process, detected by the decrease in pH to a minimum of 6.5 and an increase in effluent soluble COD and VFA.
Co-digestion of different biodegradable waste streams from the food and agriculture industry sectors in Cyprus was investigated by Monou et al (2009) and a rapid screening procedure to optimize the co-digestion process was established. The role of trace elements in anaerobic co-digestion was investigated by Zhang et al (2011). The co-digestion of the food waste with the piggery wastewater showed a high methane production rate without VFA accumulation. The reason for enhancement of methane production was due to trace elements supplemented from piggery wastewater during co-digestion with food waste.

2.3.3 Agro and Other Wastes

Calcium addition to swine wastewater is essential to improve the performance of anaerobic digestion. The performance of anaerobic digestion improved with increasing concentration of calcium and reached to a maximum concentration of 3 g/L, and then decreased at higher concentrations 5–7 g/L. However, an over dosage of calcium was found to inhibit anaerobic digestion (Johng-Hwa Ahn et al 2006).

Yen and Brune (2007) investigated on co-digestion of algal sludge and waste paper, for optimizing the C/N ratio and to increase in cellulose activity. Increasing the cellulose activity was helpful for the biodegradation of algal sludge, which provided nutrients in the digester and also the methane production rate. Thermophilic anaerobic co-digestion of olive mill wastewater with olive mill solid wastes in a tubular digester was carried out by Fezzani and Cheikh (2007, 2008) and it was concluded that, without dilution and addition of chemical nitrogen substances, methane productivity and soluble chemical oxygen demand (SCOD) removal efficiency were 461 CH\textsubscript{4}/L and 68.97% respectively.
Studies were carried out in anaerobic mixed biofilm reactor for co-digestion of onion juice and wastewater sludge and a C/N ratio of 15 was recommended for treating the mixture of onion juice and sludge (Romano and Zhang et al 2008). Feng et al (2008) reported that, co-digestion of swine wastes and garbage was a promising method for the recovery of bio-energy. From the studies conducted in a pilot plant operated in two-phase anaerobic digestion system, the methane yield obtained was 865–930 L / kg VS$_{added}$ at an OLR of 5.0–5.3 kg-VS/ m$^3$ / day and a HRT of 9 days. Co-digestion of olive mill effluent with agro-industrial residue streams such as cheese whey and laying hen litter enhanced the biogas generation (Nuri Azbar et al 2008).

Agricultural wastes and aquatic flora are good co-substrates for anaerobic co-digestion processes and mixing manure with crop residues and aquatic biomass enhanced the biogas generation (Alvarez and Liden 2008).

Co-digestion of swine manure with wheat straw in a continuously operated system and pre-treatment of wheat straw with a wet explosion method were evaluated for increasing the methane yield. However, pre-treatment of wheat straw was not effective in increasing the methane yield (Wanga et al 2009).

Cavinato et al (2010) carried out a pilot and full scale anaerobic co-digestion studies using cattle manure with agro-wastes and energy crops under thermophilic temperature of 55°C instead of a ‘reduced’ thermophilic temperature of 47°C which improved the biogas generation from 0.45 to 0.62 m$^3$/kg VS.

2.3.4 Sludge

Cecchi et al (1996) studied the possibilities of co-digestion of sewage sludge (SS) with macro-algae to contribute to the solution of the final disposal of macro-algae and concluded that the mesophilic co-digestion
process is applicable with a potential utilization of around 30% of SS. Thermophilic digestion was not possible, because of the inhibition of methanogens probably due to the activity of sulphate-reducers.

Lafitte and Forster (2000) carried out studies on anaerobic co-digestion of sewage sludge and confectionery waste in a CSTR; first stage was operated at 55°C and second stage was operated at 35°C and it was found that a HRT of 12 days was the most effective in terms of specific methane yield production. Cinar et al (2004) investigated co-disposal alternatives for various municipal wastewater treatment plant sludges with refuse under mesophilic conditions and found that the addition of primary sludge plus waste activated sludge to the solid waste had shown beneficial effect for stabilization of solid waste.

Gomez et al (2006) studied the anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the MSW in different modes of mixing conditions in digesters at an OLR of 2.5-3.6 g VS/d for primary sludge and 2.5-4.3 g VS/d for primary sludge plus MSW under mesophilic conditions. They found that the absence of agitation resulted in reduction of specific gas production (SGP). Hence good contact between the substrate and the microbes was needed. Feasibility studies were conducted for co-digestion of sewage sludge with OFMSW of wastewater treatment plants in Germany and the technical, economic and ecological aspects of co-digestion process were investigated (Krupp et al 2005).

Arnaiz et al (2006) carried out studies on volatile solids reduction and biomass stabilization in the anaerobic digestion of different urban sludges and it was found that the biodegradability of primary sludge was around 87% whereas, in case of secondary sludge biodegradability was only 43%. From kinetic studies, the biomass in secondary sludge decayed much faster than the biomass in primary sludge.
Anaerobic co-digestion of sludge from grease trap and sewage sludge was successfully performed both in laboratory batch tests and in continuous pilot-scale digestion tests. Single-substrate digestion of grease trap sludge gave high methane potentials in batch tests (845–928 mL/g VS\text{in}), but could not reach stable methane production in the continuous digestion tests. Addition of grease trap sludge with sewage sludge increased the methane potential (Davidson et al 2008).

The overall performance in bioreactor landfills was increased during co-digestion of septic tank sludge with MSW (Valencia et al 2009) and the anaerobic co-digestion of olive mill wastewater with olive mill solid waste was investigated by Fezzani and Cheikh (2008). The co-digestion of waste sludge generated in the wastewater treatment plant of a meat industry with organic wastes generated from the same industry was an economical and environmentally sound option because those wastes could be treated in the same facility, improving the energy balance at the plant (Buendia et al 2009).

The feasibility of anaerobic co-digestion of sewage sludge and grease trap sludge from a meat-processing plant by addition of 46% of feed VS with HRT of 16 d and OLR of up to 3.46 kg VS/m$^3$d at 35°C was investigated by Luostarinen et al (2009). The high methane production potential of grease trap sludge increased specific methane production of 463 m$^3$/ tonne of VS\text{added} when compared to digestion of sewage sludge alone i.e 278 m$^3$/ tonne VS\text{added}).

Kabouris et al (2009) investigated co-digestion of municipal primary sludge (PS), thickened waste activated sludge (TWAS), fat, oil, and grease (FOG) in a laboratory-scale anaerobic digester operated at mesophilic (35°C) and thermophilic (52°C) temperatures in semi-continuous mode. It was observed from the studies that the addition of 48% of FOG fraction to
the total VS load 2.95 times more methane yield at mesophilic conditions and 2.6 times more methane yield at thermophilic conditions.

### 2.3.5 Slaughter House and Tannery Waste

Tritt and Schuchardt (1992) and Banks (1994) studied anaerobic digestion of solid slaughter house waste. Banks (1994) studied the performance of anaerobic digester treating cattle and lamb paunch contents, blood and process wastewaters with an organic loading rate of 0.36 kg COD/m$^3$.d, HRT of 43 days and observed that the methane gas of 0.18 m$^3$/kg COD$_{added}$ was generated and noticed the ammonia inhibition also. Sosnowski et al (2002) and Dohanyos et al (2004) stated that addition of easily degradable substrate improves the degradation on the molecular level which could couple with the degradation pathways bringing to a higher energetic yield and better degradation of problematic compounds.

Salminen et al (2000) studied anaerobic degradation of solid slaughter house waste in continuously stirred tank reactor with an organic loading rate of 0.8 kg VS/m$^3$.d, HRT of 50 days and a methane yield of 0.52-0.55 m$^3$/kg VS was reported. Anaerobic digestion of organic solid poultry slaughter house waste was reviewed by Salminen and Rintala (2002). Cuetos et al (2008) carried out studies on anaerobic digestion of slaughter house waste and the influence of co-digestion with organic fraction of municipal solid waste at an organic loading rate of 3.7 kg VS/ m$^3$/ day with an HRT of 25 days and found that, the co-digestion was suitable technology for treatment of lipid and protein rich wastes.

Anaerobic digestion of slaughter house by-products was carried out by Hejnfelt and Angelidaki (2009). The animal wastes were good substrates for biogas production with a methane potential of 619 m$^3$ / kg, which was
much higher than the methane potential of manures (20–30 m³/kg). However due to high ammonia loads, co-digestion was a suitable process for dilution.

Biogas production was improved by the saponification of fatty wastes generated from slaughter houses (Battimelli et al 2009). The co-digestion of swine manure and dairy cattle manure with garbage of limited quantity was a prospective method to treat them simultaneously and effectively as reported by Liu et al (2009). During anaerobic co-digestion of hazardous tannery solid waste and primary sludge, specific gas production between 0.419 and 0.635 L/g volatile solids feed was reported by Thangamani et al (2010).

Shanmugam and Horan (2009a and 2009b) have optimized the biogas production from leather fleshings co-digested with municipal solid waste (MSW) in order to minimize ammonia toxicity and to maximize biogas yield. They also developed a rapid technique to evaluate the methane potential and biomass yield of solid wastes considering the empirical formula based on specific methanogenic activity (SMP) together with ATP measurement in order to augment the traditional biochemical methane potential (BMP) and volatile suspended solids (VSS) measurements.

Bacterial composting of animal fleshings, the major solid waste generated from leather manufacturing industry was an attractive and alternative methodology to the existing chemical and thermal methods for the disposal of solid waste (Ravindran and Sekaran 2010). The use of fermented tannery fleshings for utilization as feed ingredients in animal feed formulations was reported by Amit et al (2010). A new technology to treat chrome tanned based leather wastes was developed by Kolomaznik et al (2008) and the technology was tested under laboratory, pilot-scale and industrial conditions.
Anaerobic digestion of skin trimmings, fleshings and tannery wastewater sludge was evaluated by Zupancic and Jemec (2010) in semi-continuous mode in anaerobic sequential batch reactors (ASBR) with an OLR of 4.0 kg/m$^3$/VSS for successful and economic operation. In the ASBR process with OLR of 3.96 kg/m$^3$/day resulted in a specific methane production (SMP) of 0.596 m$^3$/kg and a VSS removal of 71.4% was reported at thermophilic temperature of 55$^0$ C.

2.3.6 Summary

A summary of important studies on co-digestion of different solid wastes is presented in Table 2.2.
### Table 2.2 A Summary of Important Studies on Co-Digestion

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Substrate(s)</th>
<th>Investigator(s)</th>
<th>Year</th>
<th>Operating Conditions</th>
<th>Methane/Biogas Generation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OFMSW with manure</td>
<td>Hartmann and Ahring</td>
<td>2005</td>
<td>Temp.– Thermophilic range i.e. 55°C HRT- 14 to 18 days</td>
<td>Biogas yield - 180-220 m³/tonne of MSW</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Organic waste from domestic refuse and municipal sludge</td>
<td>Zupancic</td>
<td>2008</td>
<td>OLR- 1.0 kg/m³/day of volatile suspended solids</td>
<td>Specific biogas production - increased from 0.39 to 0.89 m³/kg volatile suspended solids.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Swine wastes and garbage</td>
<td>Feng et al</td>
<td>2008</td>
<td>OLR of 5.0–5.3 kg-VS/ m³/ day; HRT- 9 days</td>
<td>Methane yield-865–930 L / kg VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mixture of fruit and vegetable wastes</td>
<td>Bouallagui et al</td>
<td>2004</td>
<td>Temperature-mesophilic; OLR of 7.5 g COD/L.d</td>
<td>81% of hydrolysis yield was achieved</td>
<td>VFA&gt; 13.3 g/L</td>
</tr>
<tr>
<td>5</td>
<td>Fruit and vegetable waste, fish waste, abattoir wastewater (AW) and waste activated sludge (WAS)</td>
<td>Bouallagui et al</td>
<td>2009</td>
<td>Temperature-mesophilic; OLR: 2.46–2.51 g volatile solids (VS)/L/d; HRT- 10 days</td>
<td>Enhanced the biogas yield - 51.5% and 43.8% respectively</td>
<td>VS removal- 10% and 11.7% respectively</td>
</tr>
<tr>
<td>6</td>
<td>Primary sludge and the fruit and vegetable fraction of the MSW</td>
<td>Gomez et al</td>
<td>2006</td>
<td>Temperature-mesophilic; OLR of 2.5-3.6 g VS/d for</td>
<td>Absence of agitation resulted in</td>
<td></td>
</tr>
<tr>
<td>Sl. No</td>
<td>Substrate(s)</td>
<td>Investigator(s)</td>
<td>Year</td>
<td>Operating Conditions</td>
<td>Methane/ Biogas Generation</td>
<td>Remarks</td>
</tr>
<tr>
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</tr>
<tr>
<td>7</td>
<td>Cattle manure with agro-wastes and energy crops</td>
<td>Cavinato et al</td>
<td>2010</td>
<td>Temperature - Thermophilic range i.e. 55°C instead of 47°C</td>
<td>Increase in biogas generation- 0.45 to 0.62 m³/kg VS</td>
<td>reduction of specific gas production</td>
</tr>
<tr>
<td>8</td>
<td>Cattle and lamb paunch contents, blood and process wastewaters from slaughter house</td>
<td>Banks</td>
<td>1994</td>
<td>OLR- 0.36 kg COD/m³.d; HRT- 43 days</td>
<td>methane gas- 0.18 m³/kg COD&lt;sub&gt;added&lt;/sub&gt;</td>
<td>Noticed the ammonia inhibition also.</td>
</tr>
<tr>
<td>9</td>
<td>Solid slaughter house waste</td>
<td>Salminen et al</td>
<td>2000</td>
<td>OLR-0.8 kg VS/m³.d; HRT- 50 days</td>
<td>methane yield- 0.52-0.55 m³/kg VS</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Slaughter house by-products</td>
<td>Hejnfelt and Angelidaki</td>
<td>2009</td>
<td></td>
<td>methane potential- 619 m³/kg</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Tannery solid waste and primary sludge</td>
<td>Thangamani et al</td>
<td>2010</td>
<td></td>
<td>specific gas production- 0.419 and 0.635 l/g volatile solids feed</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Skin trimmings, fleshings and tannery wastewater sludge</td>
<td>Zupancic and Jemec</td>
<td>2010</td>
<td>Temperature: Thermophilic range of 55°C; OLR: 3.96 kg/m³.day</td>
<td>specific methane production- 0.596 m³/kg; VSS removal - 71.4%</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Co-digestion of sewage sludge and grease trap sludge</td>
<td>Luostarinen et al</td>
<td>2009</td>
<td></td>
<td>Addition of grease trap sludge increased the specific methane production from 278 to 463 m³/tonne of VS&lt;sub&gt;added&lt;/sub&gt;</td>
<td></td>
</tr>
</tbody>
</table>
2.3.7 Performance Evaluation of Co-Digestion Process

2.3.7.1 Mix Proportion of Substrates

Callaghan et al (2002) conducted studies in continuously stirred tank reactor in mesophilic conditions for co-digestion of cattle slurry with fruit and vegetable wastes (FVW) and chicken manure at an OLR in the range of 3.19–5.01 kg VS/m³/day and HRT of 18 days. It was reported from the studies that, increasing the proportion of FVW from 20% to 50%, improved the methane yield from 0.23 to 0.45 m³ CH₄/kg VS added whereas, increasing the proportion of chicken manure in the feed caused a steady deterioration due to ammonia inhibition.

Cinar et al (2004) investigated co-disposal of various municipal wastewater treatment plant sludges viz., primary sludge (PS), waste activated sludge (WAS) and a mixture of PS plus WAS with refuse at sludge to solid waste ratio of 1:7, the stabilization of solid waste was faster due to addition of PS and WAS. Agdag and Sponza (2007) carried out studies on co-digestion of mixed industrial sludge with municipal solid wastes in anaerobic simulated landfilling bioreactors. Co-digestion of MSW with an industrial sludge in the ratio of 1:2 gave the highest methane yield of 8.5 g CH₄ COD removed/kg VS added. This indicated that, co-digestion processed at a higher efficiency than that of MSW alone.

Anaerobic co-digestion of cattle manure (CM)) with NaOH-treated corn stover (CS) was investigated by Li et al (2009) with CM:CS ratios of 1:1, 1:2, 1:3, and 1:4 with feed concentrations of 50, 65, and 80 g/L and with CM:CS ratio of 1:3, methane yield of 194 mL/g VS was reported. Anaerobic co-digestion of fruit and vegetable waste (FVW) and activated sludge (AS) was investigated using anaerobic sequencing batch reactors at different AS:FVW ratios and reported that reactor fed with a 30:70 ratio; the
highest VS removal and biogas production yield of 88% and 0.57 L / g VS$_{added}$ was observed (Habiba et al 2009).

Co-digestion of by-products from meat-processing (MP) industry and sewage sludge (SS) with MP: SS ratio of 1:3 gave maximum methane yield at a HRT of 20 days and also observed that when HRT decreased to 14 days, methane production decreased due to high OLR (Luste and Luostarinen 2010). Alvarez et al (2010) optimized a methodology for maximizing the methane production by anaerobic co-digestion of pig manure, fish waste and biodiesel waste, and found that the highest biodegradation potential of 321 L CH$_4$/kg COD was found with a mixture composed of 84% pig manure, 5% fish waste and 11% biodiesel waste, while the highest methane production rate of 16.4 L CH$_4$/kg COD d was obtained by a mixture containing 88% pig manure, 4% fish waste and 8% biodiesel waste.

### 2.3.7.2 Carbon to Nitrogen (C/N) Ratio

Lettinga et al (1996) reported that the carbon to nitrogen ratio of 26:66 was required for biomass with high and low yield coefficients respectively. High C/N ratio causes an increase in acid formation which retarded the methanogenic activity and on the other hand, at low C/N ratio, nitrogen will be converted into ammonium-N which is toxic to methanogens. An appropriate carbon-to-nitrogen ratio (C: N) is a prerequisite for the continued successful functioning of a digester (Gerardi 2003).

For readily degradable substrates, the optimum C/N ratio was of the order of 20 to 25. However, for materials that were resistant to microbial degradation, the C/N ratio could be as high as 40 (UNEP IETC 2005). For nitrogen-rich waste, lower values of C/N ratio in the range was suitable for anaerobic digestion as reported by Mshandete et al (2004). The co-digestion of biosolids and organic fraction of municipal solid waste were compared with
the direct digestion of biosolids. Addition of organic fraction of municipal solid waste improved the carbon-to-nitrogen ratio and enhanced the biogas generation (Zhang et al 2008).

Meat industry wastes have high organic content and low C/N ratio when compared to domestic or vegetable waste. For enhancement of biogas yield, anaerobic co-digestion was the feasible option (Carucci et al 2005, Macias et al 2008).

2.3.7.3 Inoculum to Substrate (I/S) Ratio

For the optimization of the anaerobic digestion process, the selection of inoculum source and the inoculum to substrate (I/S) ratio are important for the assessment of anaerobic biodegradability of solid wastes (Sanchez et al 2001, Lopes et al 2004). It is always better to use active anaerobic inoculum or inoculum from animal waste such as bovine manure in order to reduce digestion period and digester volume (Obaja et al 2003; Callaghan et al 2002; Sosnowski et al 2003).

Forster-Carneiro et al (2008a) carried out experiments on anaerobic thermophilic digestion of MSW using the various inoculum sources such as corn silage, restaurant digested waste mixed with rice hulls, cattle excrement, swine excrement, digested sludge and swine excrement mixed with sludge. They found that digested sludge and swine excrement mixed with sludge are the better sources of inoculum. As reported by Forster-Carneiro et al (2008), only limited information is available on total solids content and the proportion of the inoculum required during start–up period of anaerobic digestion of solid wastes.

For the anaerobic solid-state fermentation, the inoculum with high methanogenic activity and low biodegradability were necessary and the
findings were validated using a mathematical model (Kalyuzhnyi et al 2000). The use of the granular sludge and suspended sludge as inoculum for the biomethanation of kitchen waste with 0.5–2.3 waste/inoculum ratio was investigated by Neves et al (2004). When composition of waste fluctuated it was better to use granular sludge to prevent acidification. Raposo et al (2006) carried out studies on the influence of the inoculum to substrate ratio on anaerobic digestion of maize waste.

Influence of bovine rumen fluid inoculum during anaerobic treatment of the organic fraction of municipal solid waste (MSW) was studied by Lopes et al (2004) and affirmed that the bovine rumen fluid inoculum substantially improved the performance of the process. The better performance of the inoculated reactors was related to the potential increase in number of indigenous anaerobic microorganisms of rumen that contributed substantially to degradation of the organic material in the reactor. Also in relation to the TVS reduction percentage, no substantial difference was in evidence when 5% and 10% of the inoculum were used in preparation of the substrate.

2.3.7.4 pH

Methane yield is strongly dependent on pH. For selective production of various organic acids from organic wastes pH control was effective (Horiuchi et al 2002). The optimum pH range for methanogens is 6.5 to 8.2 (Anderson and Yang 1992, Speece 1996).

2.3.7.5 Temperature

The effect of temperature on the performance of an anaerobic tubular reactor treating fruit and vegetable waste was evaluated by Bouallagui et al (2004). The higher degradation efficiency in terms of higher specific
biogas production and an improvement of the energy balance of the process were observed in thermophilic conditions when compared with psychrophilic and mesophilic conditions.

Biologically pre-treated abattoir wastewater was anaerobically digested in an upflow anaerobic filter under mesophilic and thermophilic conditions. Under mesophilic conditions pathogens had been destroyed partially, whereas in thermophilic condition, the process was more efficient in removal of indicator microorganisms and pathogenic bacteria at different organic loading rates (Gannoun et al 2009).

2.3.7.6 Oxidation Reduction Potential (ORP)

During anaerobic digestion, the microorganisms reduce the ORP of the medium to a level suitable for volatile fatty acids and methane to form. Hydrolysis stage is characterized by oxidation reduction potential (ORP) values of around \(-300\text{mv}\), while methanogenesis takes place at ORP values lower than \(-300\text{mv}\) and close to \(-500\text{mv}\) (Fannin 1987; Wheatley 1990). Cheng-Nan Chang et al (2002) demonstrated ORP as a controlling parameter in waste activated sludge hydrolysis process and a relation between change of ORP value and increase in SCOD was developed with the help of a model. At the start of anaerobic digestion, the ORP values for the sludge samples ranged from \(-250\) to \(-200\text{mv}\) and at the end of anaerobic digestion process ORP values dropped to \(-320\text{ to }-330\text{ mv}\) (Chu et al 2003). The ORP influenced the hydraulic retention time and ORP decreases as the HRT increases favouring the methanogenic stage (Colmenarejo et al 2004).

2.3.7.7 Alkalinity

If the acid concentrations (\(\text{H}_2\text{CO}_3\) and VFA) exceed the available alkalinity, sour will takes place during anaerobic digestion and it will inhibit
the methanogenic microbial activity and the methane production will cease. The alkalinity will be consumed through NH$_4$-N conversion of organic nitrogen, the buffering of H$_2$CO$_3$ acidity by CO$_2$ and VFA generation by the acidogenic microorganisms.

During methanogenesis, pH will be raised and will be controlled by bicarbonate buffering. Hence a balance between acid production and acid consumption is essential for a stable anaerobic digestion process. The minimum buffer/substrate ratio of 0.06 kg / kg TS is required in order to control the pH (Plaza et al 1996). An alkalinity level ranging from 1000 to 5000 mg CaCO$_3$/ liter was recommended by Ren et al (2004). The effect of alkalinity on the performance of a simulated landfill bioreactor digesting organic solid wastes was investigated by Agdag and Sponza (2005). The alkalinity addition reduced the waste quantity, the organic content of the solid waste and the biodegradation time.

2.3.7.8 Volatile Fatty Acid (VFA) and VFA to Alkalinity Ratio

Volatile fatty acid (VFA) is an important intermediate compound in the anaerobic digestion process and conversion of the VFA through the acetogenic and acetoclastic step into methane and carbon dioxide is the most important conversion process. Volatile fatty acids are the indicators for process imbalance in anaerobic digestion process. The iso- butyrate and isovalerate are the reliable indicators of changes in the process balance (Ahring et al 1995, Cobb and Hill 1991, Hill and Bolte 1989, Hill and Holmberg 1988). Pind et al (2003) developed a new sensor technique to monitor VFA online in one of the most difficult medium i.e. in animal slurry or in manure.

Acetate concentration more than 13mM, iso-butyrate and isovalerate concentrations in between 0.06 to 0.17 mM are the indicators for process imbalance as reported by Hill et al (1987); Hill and Holmberg (1988).
The toxicity of VFA concentration on anaerobic digestion has been reported by Arhing and Westermann (1988) and Gorris et al (1989). That pH drop was the main reason for VFA accumulation has been reported by Hill et al (1987) and Gourdon and Vermande (1987). The degradation of propionate and butyrate was inhibited by acetate as reported by Arhing and Westermann (1988). However the specific role of individual VFA is still under debate. The VFA was evaluated as a process indicator by imposing different types of perturbation imbalance in continuously stirred tank reactors fed with manure under thermophilic conditions by Arhing et al (1995).

To assess the digester imbalance, the ratio between propionic acid to acetic acid could be used (Norstedt and Thomas 1985, Marchaim and Krause 1993). If an individual acetic acid concentration exceeds 800 mg/L or the ratio of propionic acid to acetic acid exceeds 1.4, digester failure can occur (Hill et al 1987). The VFA/alkalinity ratio is more appropriate for the assessment of the stability of reactors in transient conditions instead of the organic loading rate as the design parameter (Schoen et al 2009). For stable performance of anaerobic digestion process the VFA/alkalinity ratio should be between 0.4 and 0.8 (Behling et al 1997).

Volatile fatty acids production by anaerobic fermentation of sewage in fixed-bed and suspended biomass reactors were assessed. The fixed bed reactors were more efficient than suspended biomass reactors (Colmenarejo et al 2004). The volatile fatty acid formation in an anaerobic hybrid reactor was investigated by Buyukkamaci and Filibeli (2004) and the increase in VFA concentrations were observed from top to the bottom of the reactor indicating higher methanogenic activity in the upper part and higher acetogenic activity in the bottom of the reactor.

The maximum methane production rates were obtained with 125mM for acetate, 100mM for propionate and butyrate but only 50mM for
valerate, after which methane production rate decreased immediately. A concentration between 150 and 175 mM of valerate inhibited methane production completely during anaerobic digestion of Olive mill wastewaters (Mechichi and Sayadi 2005).

The co-digestion of the food waste with piggery wastewater showed a high methane production rate without VFA accumulation. By adding a trace element solution instead of the piggery wastewater to the food waste, a superior performance was reproduced in terms of the high methane yield and there was no VFA accumulation (Zhang et al 2011).

2.3.7.9 Ammonia Inhibition

Nitrogen plays an important role in anaerobic digestion and is essential for formation of new biomass. Nitrogen may cause problems in anaerobic digestion because of its metabolic products like ammonia (NH₃), ammonium NH₄⁺, dinitrous oxide (N₂O), nitrite NO₂⁻ and nitrate NO₃⁻. Ammonia nitrogen is one of the most common toxic substances encountered during anaerobic digestion of protein containing wastes. Although it is an important buffer in the process and an essential nutrient for microorganisms, high concentrations can be a major cause of operational failure. Operational problems in anaerobic digestion plants resulting from nitrogen in MSW was reported by Fricke et al (2007). Free ammonia inhibits the anaerobic digestion process rather than total ammonia.

Organic fraction of municipal solid waste, waste activated sludge, wastewaters originating from potato starch, dairy, seafood processing industries, young landfill leachate and animal manures contains high ammonia concentrations near or above inhibitory level for anaerobic digestion.
In literature, ammonia inhibition was reported to occur at a pH above 7.4 and total ammonia nitrogen in the range of 1500–3000 mg/L reported by Koster and Lettinga (1988) and Van Velsen AFM (1979). Baris Calli et al (2005) reported that inoculum acclimatized to free ammonia concentrations could be able to tolerate elevated free ammonia concentrations upto 800 mg/L. However, propionate accumulation affected the free ammonia concentration of 200 mg/L and indicated that propionate degradation of acetogenic bacteria is more sensitive to free ammonia than methanogenic archaea.

In general, co-substrates such as agro-industrial residues benefit more from the association of manure (Molnar and Bartha 1998), as manures can be excellent base substrates due to their inherent high buffering capacity, high ammonia content and a wide range of nutrients needed by the methanogens (Angelidaki and Ahring 1993 and 1997a and b; Wilkie et al 1986). The co-digestion of manures with agro-residues will also aid in overcoming ammonia inhibition related to pure manure digestion.

Methanogen acetoclastic species are more sensitive than hydrogen utilizing species to free ammonia. Free ammonia inhibition thresholds were reported as 700 and 1200 mg/L for acetoclastic and hydrogenotrophic methanogens respectively by Angelidaki and Ahring (1993) and Hansen et al (1998). Since two-thirds of the methane will be produced in an anaerobic reactor derived from acetate, a decrease in the activity of acetoclastic methanogens severely affected the anaerobic degradation process. Chronic inhibition of aceticlastic methanogens were reported by Sung and Liu (2003) when total ammonia nitrogen (TAN) concentrations of 4.92 and 5.77 g/L caused 39% and 64% reduction in specific methanogenic activity respectively.
The ammonia-induced inhibition occurs primarily during the anaerobic digestion of organic waste materials, which are rich in proteins, as ammonia nitrogen is released through the mineralization of organic nitrogen compounds. Ammonium is released during the anaerobic hydrolysis of organic nitrogen compounds, causing an increase of the pH value. The ammonification thus counteracts the reduction of the pH value resulting from the acidification step of anaerobic digestion. Fricke et al (2007) reported the inhibiting concentrations of ammonia was in the range of 30 and 100 mg/L at pH ≤ 7.0 and temperature ≤ 30°C whereas for ammonium ion was in the range of 4000 and 6000 mg/L.

Ammonia inhibition could be contributed during anaerobic digestion of animal wastes and protein rich slaughter house wastes (Nielsen and Angelidaki 2008; Salminen and Rintala 1999; Chen et al 2008). The effect of toxic or inhibitory compounds on the process may be minimized by using different substrates as co-substrates due to sufficient buffering capacity (Capela et al 2008). Not only that but also digestion of poorly biodegradable wastes, which cannot be digested alone (e.g. fat or protein wastes) can be digested by addition of co-substrates (Alatriste et al 2006).

2.3.7.10 Heavy Metals

Heavy metals are present in significant concentrations in some industrial wastewaters and municipal sludge. Heavy metals can be stimulatory, inhibitory or even toxic to biochemical reactions depending on their concentrations. A trace level of many metals is required for activation or function of many enzymes and co-enzymes. Heavy metals could potentially have a negative impact on methane-producing anaerobic granular sludge.

The mechanism for heavy metal toxicity was investigated by Harrison et al (2007) and they stated that the reasons for metal toxicity in
microorganisms are due to (i) substitutive ligand binding, (2) redox reactions with sulfur groups, (3) Fenton type reactions, (4) inhibition of membrane-transport processes and (5) electron siphoning.

Li and Fang (2007) carried out studies on toxicity of heavy metals on H₂-producing activity of a granular sludge. The IC₅₀ at which the bioactivity of the sludge was reduced to 50 % of the control, for individual heavy metals were in the order of Cu 30 mg/L, Ni and Zn 1600 mg/L, Cr 3000 mg/L, Cd 3500 mg/L and Pb > 5000 mg/L. Overall it was observed from the literature that H₂-producing sludge exhibited in general higher resistance to metal toxicity than methanogenic granular sludge due to higher concentrations of extracellular polymeric substances (EPS) in the H₂-producing sludge and their dense distribution at the outer layer of the granular sludge.

The effect of metal concentrations on simulated aerobic and anaerobic landfill bioreactors were evaluated by Bilgili et al (2007). It was reported that the leachate recirculation enhanced the rate of biodegradation and also lowered the metal concentrations well below the regulatory limits in the case of anaerobic landfill bioreactors rather than in aerobic reactors.

Inhibitory effect of heavy metals such as zinc (II), chromium (VI), nickel (II), and cadmium (II) with a concentration upto 128 mg/L on methane-producing anaerobic granular sludge was investigated by Levent Altas (2009) and it was found that methane production was influenced by the type and concentration of heavy metal. The inhibitory effect of the four heavy metals examined was in the order of Zn > Cr > Ni > Cd with IC₅₀ values for the individual heavy metals being Zn- 7.5 mg/L, Cr(VI) -27 mg/L, Ni-35 mg/L, and Cd-36 mg/L.
The trace elements such as iron and cobalt are necessary for fermentation and methane (CH$_4$) production during anaerobic digestion process. Therefore the lack of these elements also hinders the digestion process. The role of iron, nickel and cobalt in the production of biogas during anaerobic digestion of sludge was investigated by Aresta et al (2003). Iron is the part of carbon monoxide dehydrogenase (CODH) enzyme required for formation of acetic acid by anaerobic bacteria (Aresta et al 1998). Similarly cobalt catalyses the transfer of methyl-groups thereby, improves the methane production (Hippler and Thauer 1999). It was reported by Aresta et al (2003) that CH$_4$ to CO$_2$ ratio around 3.0, addition of Fe improved the CH$_4$ to CO$_2$ ratio. For good quality biogas to be generated the ratio of CH$_4$ to CO$_2$ is to be around 4.5 (Aresta et al 2003). Also addition of Fe improves the availability of H$_2$ for CO$_2$ methentaion through the Fe$_4$S$_4$. To avoid accumulation of acetate in anaerobic granular sludge reactor, addition of 10 mM iron almost doubled the methane formation as reported by Zandvoort et al (2003).

### 2.3.7.11 Summary

A summary of important studies on performance evaluation of co-digestion process is presented in Table 2.3.
Table 2.3 Performance Evaluation of Co-Digestion Process

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Substrate(s)</th>
<th>Investigator(s)</th>
<th>Year</th>
<th>Operating Conditions</th>
<th>Methane/ Biogas Generation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cattle slurry with fruit and vegetable wastes (FVW) and chicken manure</td>
<td>Callaghan et al</td>
<td>2002</td>
<td>OLR range- 3.19–5:01 kg VS / m³/day; HRT- 18 days</td>
<td>methane yield improved from 0.23 to 0.45 m³ CH₄ / kg VS added when FVW increased from 20 to 50%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Meat-processing (MP) industry and sewage sludge (SS)</td>
<td>Luste and Luostarinen</td>
<td>2010</td>
<td>MP:SS ratio- 1:3; HRT- 20 days</td>
<td>Observed maximum methane yield</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pig manure, fish waste and biodiesel waste</td>
<td>Alvarez et al</td>
<td>2010</td>
<td>88% pig manure, 4% fish waste and 8% biodiesel waste</td>
<td>methane production rate- 16.4 L CH₄/kg COD d</td>
<td></td>
</tr>
</tbody>
</table>

It was observed from Tables 2.1, 2.2 and 2.3 the nature of substrate and operating conditions plays significant role in generation of methane. It was concluded from the above cited literature that for slaughterhouse waste with OLR of 0.36 kg COD/m³.d the methane gas yield was 0.18 m³/kg COD added whereas by increasing the OLR to 0.8 kg VS/m³.d the methane gas yield was 0.52-0.55 m³/kg VS. Further increasing the OLR upto 5.82 g VS/L/day the observed methane yield was 0.62 L methane / gram of VS destroyed. Also a minimum hydraulic retention time (HRT) of 50 days was reported.
2.4 LIPOS DESGRADATION

Lipids are one of the major organic matters found in food waste and industrial wastewaters such as slaughter houses, dairy industries or fat refineries (Li et al 2002) and theoretically lipids will produce more methane when compared with proteins and carbohydrates (Pereira et al 2003). Primarily lipid rich wastes contain low content of nutrients, low alkalinity (Angelidaki and Ahring 1997a, b) and show toxicity towards the anaerobic digestion process (Hanaki et al 1981, Hwu et al 1996, Rinzema et al 1994). The long chain fatty acids (LCFA) are the inhibiting compounds during anaerobic digestion of lipid rich wastes. Inhibition of the long chain fatty acids of lipid-rich wastes in anaerobic reactors fed in continuous mode was studied by Hwu et al (1998); Rinzema et al (1989); Pereira et al (2005) and in batch mode by Lalman and Bagley (2000, 2001, 2002) and Shin et al (2003).

Lipids, typically the tri-glyceride esters of long chain fatty acids (LCFAs), represent an important fraction of the organic matter in dairy wastewater, fish waste, ice-cream wastes, oil/ fat wastewater, slaughter house wastewater and from food processing industries. During anaerobic digestion, the tri-glyceride esters are hydrolyzed into glycerol and LCFA by a combination of hydrolytic, fermentative, syntrophic acetogenic (SAB) and methanogenic microorganisms (Cuetos et al 2008). The hydrolysis mechanism of LCFA was demonstrated by Vavilin et al (1996).

During degradation, LCFAs convert into acetate via short chain fatty acids (β-oxidation) and then methanogenic bacteria remove inhibitory levels of acetate, formate and hydrogen produced as reported by Novak and Carlson (1970) and Broughton et al (1998). Long chain fatty acids degradation in anaerobic digester and thermodynamic equilibrium consideration were reported by Oh and Martin (2010).
2.4.1 Effect of LCFA during Anaerobic Digestion of Solid Waste

Theoretically 1.0 L of methane at standard temperature and pressure (STP) can be produced from, for instance, 1 g of oleate (unsaturated LCFA, C18:1), whereas only 0.37 L can be produced from 1 g of glucose (Kim et al 2004). Sludge flotation and washout, transport limitation phenomena caused by LCFA accumulation and deposition over the sludge are the important contributors for lag phase in methane production (Pereira et al 2005). Adaptation of microorganisms to high loads of LCFA to degrade concentrations well above the inhibition limits are reported by Alves et al (2001) and Broughton et al (1998). After acclimatization, microbial consortium could be able to biodegrade oleate-rich wastewaters without LCFA accumulation in the process (Cavaleiro et al 2007).

It has been reported that LCFA are inhibiting anaerobic microorganisms at very low concentrations, with IC\textsubscript{50} values for oleate over 50 and 75 mg/ L (Alves et al 2001 ; Hwu et al 1996), palmitate over 1100 mg/l (Pereira et al 2005) or stearate over 1500 mg/L (Shin et al 2003) at mesophilic temperature range. Methanogens were reported to be more susceptible to LCFA inhibition compared to acidogens (Lalman and Bagley 2002; Mykhaylovin et al 2005; Pereira et al 2003). The inhibition is a reversible process also.

In order to achieve methane yield at higher rate, under steady-state conditions discontinuous mode of application of LCFA based effluents are necessary (Cavaleiro et al 2008). Co-digestion of different types of waste with lipids, added in a discontinuous way is a common practice in many full scale biogas plants. Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids were discussed by Neves et al (2009a). The conversion of oleic acid, the
main LCFA fed to the reactor, by the adapted biomass became faster and more effective along the successive pulses.

The strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors were recommended by Palatsi et al (2009). Dilution with active inoculum for increasing the biomass/LCFA ratio or addition of adsorbents for adsorbing the LCFA and reducing the bioavailable LCFA concentration were found to be the best recovery strategies for improving the recovery time from 10 to 2 days in semi-continuously fed systems.

2.4.2 Enzymatic Treatment of Lipid Rich Waste

Lipids contribute major portion in the biomass from various sources. At present most of the enzymes are produced from bio-based materials. Lipolytic enzymes are in application for waste treatment processes. During lipolytic enzyme treatment of lipid rich waste, breakdown and mobilization of lipids within the cells of individual organisms as well as transfer of lipids from one organism to another take place (Beisson et al 2000). Microorganisms also release certain enzymes and solubilize lipids and are useful than enzymes derived from plant and animal sources.

Lipids are mainly oil, grease, fat and free long chain fatty acids. Triglycerides and oleate is one of the most abundant compounds present in LCFAs. Though Lipids are attractive substrates for anaerobic digestion due to the higher methane yield, it is difficult to degrade when compared with carbohydrates and proteins (Hansen et al 1999). Hydrolysis of lipids was investigated by Rollon (1999) and Masse et al (2002) and found they that the surface area available and type of lipid are the rate limiting factors. Hydrolysis constants for various substrates i.e. lipids 0.005 to 0.01 day⁻¹, 0.015 to 0.075 day⁻¹ for proteins and 0.025 to 0.20 day⁻¹ carbohydrates were

Lipase is an enzyme and the application of the various enzymes for biochemical conversions were investigated by Haki and Rakshit (2003) and the various advantages have been described by Hasan et al (2006). Lipases extracted from the microbial origin are thermostable and perform biochemical conversions like hydrolysis, esterification, alcoholysis, acidolysis and aminolysis (Jaeger et al 1994; Pandey et al 1999; Nagao et al 2001). Lipases derived from different sources have a wide range of properties i.e., positional specificity, fatty acid specificity, thermo stability and pH optimum (Huang 1984).

The enzyme assisted effluent treatment of abattoirs, food processing, leather and poultry effluents were investigated by Godfrey and Reichelt (1983). Application of lipase for the removal of subcutaneous fat in the leather industry is in practice (Pandey et al 1999, Traore and Buschle-Diller 2000). The application of lipase on anaerobic digestion of source-separated household solid waste was investigated by Rintala and Ahring (1994). Enzyme addition on anaerobic digestion of Jose Tall Wheat Grass was investigated by Romano et al (2009) in a single stage anaerobic digester. Enzymatic pretreatment on the hydrolysis and size reduction of fat particles in slaughter house wastewater were investigated by Masse et al (2001). Enzymatic pretreatment on acid fermentation of food waste was studied by Kim et al (2006) and dairy wastewater by Leal et al (2006). Lipase was prepared from animal source and enzymatic hydrolysis of lipid-rich dairy wastewater was investigated by Mendes et al (2006). The pretreatment was optimized for 12 h hydrolysis time, which facilitated enhanced biogas generation of 445±29 mL whereas 354±34 mL biogas generation was observed without pretreatment.
A study carried out by Masse et al (2001) revealed that enzymatic pretreatment with pancreatic lipase PL-250 effectively reduced the pork fat particles and hydrolyzed some triglycerides in slaughter house wastewater. The effects of pH and acetate on the enzymatic hydrolysis of a potato sample that contains both carbohydrate and protein were investigated by He et al (2006). The effects of pH and acetate concentration on the hydrolysis of carbohydrate were different from the hydrolysis of protein.

Hydrolytic pretreatment of oily wastewater by immobilized lipase was studied by Jeganathan et al (2007). Although application of lipase has significant benefits, its application to full-scale treatment units is limited due to the economic constraints. Ayol 2005 and Ayol and Dentel 2005a reported that enzymatic pre-treatment of anaerobically digested bio-solids improved the de-waterability of sludge. Cirne et al (2006) investigated the effect of bioaugmenting lipolytic strain, Clostridium lundense (DSM 17049T) on aerobic digestion of lipid rich waste and found a higher methane production rate, 27.7 cm$^3$ CH$_4$ (STP)/g VS$_{added}$/day was observed bioaugmenting lipolytic strain under methanogenic conditions. A summary of important studies on application of enzymatic treatment including conditions maintained are presented in Table 2.4.
<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Details of Enzyme</th>
<th>Nature of Waste/substrate</th>
<th>Conditions</th>
<th>Investigator (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pancreatic Lipase 250 (PL-250)</td>
<td>Pieces of pork, beef fat</td>
<td>Initial Particle Size 105 to 383 µm Experimental Temperature range 21 to 24 º C pH – 6.5 to 9.0</td>
<td>Masse et al (2001)</td>
</tr>
<tr>
<td></td>
<td>Enzymatic Activity – 250 units/ g Lipase Dose-125 to 1000 mg dm⁻³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Enzyme Jose Tall Wheat Grass</td>
<td></td>
<td>1. Novozyme 342 (N342) – derived from derived from Humicola Insolens, Activity - 90 EGU/g (EGU, endoglucanase units), 470 FXU/g (farvet xylan units), and 45 FBG/g (fungal β-glucanase units), Optimum pH-7.0 2. Mixture of 85% Celluclast 1.5L (C15L) by weight and 15% Novozyme 188 (N188) by Weight and</td>
<td>Romano et al (2009)</td>
</tr>
</tbody>
</table>
Table 2.4 (Contd…)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Details of Enzyme</th>
<th>Nature of Waste/substrate</th>
<th>Conditions</th>
<th>Investigator (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Palatase (Lipase); Viscozyme (Carbohydrase); Flavourzyme (Protease)</td>
<td>Lipase for Dairy industry; cheese flavor enhancement</td>
<td>Lipase activity - Liquid 20 000 lipase units/g; Carbohydrase activity- 100 fungal β-glucanase units/g; Protease activity - 500 leucine amino peptidase units/g; pH requirement- 6.5 ± 0.2</td>
<td>Kim et al (2006)</td>
</tr>
</tbody>
</table>

2.5 PRE-TREATMENT PROCESSES FOR SLUDGE

Anaerobic digestion is one of the options for generation of biogas from sludge and to reduce sludge volume. However, due to the high solids
content and the low biodegradability, long retention times of the order of 20 to 30 days are required to reach even moderate efficiencies, i.e. 30 to 50 percent (Pavlostathis and Gossett 1986). In wastewater treatment plants, anaerobic digestion is generally applied to a mixture of primary and secondary (waste activated) sludge. But waste activated sludge is known to be more difficult to digest than primary sludge. Kepp and Solheim (2000) stated that methane production during anaerobic digestion was 306 L / kgVS\text{feed} for a primary sludge against 146–217 L / kgVS\text{feed} for WAS. In order to improve hydrolysis and anaerobic digestion performance, one possibility was to use lysis pre-treatments.

Lysis refers to the breakdown of cell. For lysis and extraction of cells homogenization, sonication, bead beating and molecular grinding resin treatments are the available pre-treatment processes. Cell lysis using mechanical treatment (High Pressure Homogenizer) was investigated on sludge generated from activated sludge process and recycling of mechanical treated sludge to the aeration tank improved the sludge setting characteristics (Camacho et al 2002). The combination of two pretreatments such as partial enzymatic lysis by Zymolyase followed by mechanical disruption in a Microfluidizer high-pressure homogenizer was carried out for enhancement of disruption of a native strain of Candida utilis (ATCC 9226) and stated that the cell disruption was 95 % by partial enzymatic followed by mechanical disruption whereas it was only 65 % in case of mechanical disruption (Baldwin and Robinson 1994).

In order to make anaerobic digestion more feasible, pretreatment of sludge prior to anaerobic digestion is the recent technological advancement. Pretreatment process accelerates the hydrolysis of sludge thereby reducing the retention time requirement in anaerobic digestion process. Feasibilities of most of these pretreatment technologies have been demonstrated using
municipal activated sludges. The benefits of sludge solubilization prior to anaerobic treatment are:

- Increase in the amount of released soluble substrate significantly increases VFA generation for subsequent improved gas production during anaerobic digestion.

- Pretreatment reduces the viscosity of the sludge, enabling greater solids concentration to enter the anaerobic reactor.

In order to reduce residence time and to enhance biogas generation, several pretreatment methods such as ozonation, ultrasonication, alkaline treatment, alkaline followed by thermal treatment have been investigated by several researchers on waste activated sludge (Chiu et al 1997; Kim et al 2003; Eastman and Ferguson 1981; Weemaes and Verstraete 1998). The effect of an oxidative pretreatment of sewage sludge was reported by Weemaes et al (2000). Tanaka et al (1997) investigated the effect of thermo-chemical pretreatment on anaerobic digestion of waste activated sludge. Li and Noike (1992) and Dichtl et al (1997) investigated ultrasonication of sewage sludge. Elliott and Manhood (2007) reviewed the various pretreatment techniques to enhance anaerobic digestion of pulp and paper mill bio-sludge. The purpose of these pretreatments is to solubilize and/or reduce the size of organic compounds especially refractory compounds, in order to make them biodegradable (Lehne et al 2001; Weemaes et al 2000). The final quantity of residual sludge and the time of digestion can thus be reduced and the biogas generation can also be enhanced (Nah et al 2000, Tanaka et al 1997; Goel et al 2003). Mechanically/biologically treated and municipal waste was amenable to anaerobic digestion with sewage sludge (Pahl et al 2008).
The techno-economic evaluation of thermal treatment, ozonation and sonication for the reduction of wastewater biomass volume before aerobic or anaerobic digestion was investigated by Salsabil et al (2010). Ozone and ultrasonic treatment before anaerobic digestion led to the best improvement of TSS removal. Ultrasonic treatment was costly but the digestion time could be reduced. Ozone treatment was less costly but the length of the digestion largely contributed to sludge reduction.

2.5.1 Ozonation

Anaerobic digestion of sludge is hampered due to the rigid structure of the microbial cell walls, protecting the inner cell products. Hence, hydrolysis of sludge requires longer retention time. Cell solubilization and hydrolysis of the sludge cells prior to anaerobic digestion is therefore a logical approach to improve the anaerobic digestion process.

The cell solubilization and hydrolysis of the sludge can be accomplished with ozone to rupture the cell wall and release SCOD (Goel et al 2003a, Weemaes et al 2000, Yasui and Shibata 1994). During this process, ozone reacts with polysaccharides, proteins and lipids (components of cell membranes), transforming them into compounds of smaller molecular weight (Bablon et al 1991, Cesbron et al 2003). Goel et al (2003) found the optimum ozone dose to range from 0.05 to 0.5 g O₃/g of total solids (TS). If the ozone dose is sufficiently high, mineralization of the released cellular components could also occur. The ozone dosage for the mineralization phenomenon has been observed to be more than 0.18 g O₃/g TS (Bougrier et al 2005).
2.5.2 Ultrasonication

Ultrasonication has been considered as an environmentally and economically sound pretreatment strategy. During this process, pressure waves propagate through the wastewater and the sludge disintegration is accomplished by exposure to high frequency sound waves generated by a vibrating probe, commonly known as a “horn”. Localized temperature and pressure gradients rupture the cell membrane and release the SCOD (Monnier et al 1999; Atchley and Crum 1988). The sonication process also produces hydro-mechanical shear forces through cavitation (Tiehm et al 2001) and the degree of cavitation produced by sonication has been demonstrated by de Silva (2005). The use of sludge sonication has been demonstrated in the laboratory and in full scale systems treating municipal wastewaters (Muller et al 2005; Sandino et al 2005). However, only limited information is available on the disintegration of sludges produced from the treatment of industrial wastewaters. Rai et al (2004) stated that sonication is an effective pretreatment for solubilizing tannery wastewater sludge.

As pre-treatment to anaerobic digestion of waste activated sludge, ultrasound enhanced the biogas production by more than 40% at low specific energy-inputs (SE) and approximately 15% at higher SE-values as reported by Appels et al (2008).

2.5.3 Peroxide Treatment

Dewil et al (2007) conducted studies to enhance biogas generation in the anaerobic digestion of biosolids using peroxidation as one of the pretreatment methods and found that a significant increase in biogas production was observed. The reason for increased biogas generation was the disintegration and solubilisation of organic matter during the treatment of the
sludge thereby more organic matter was readily available for the anaerobic micro-organisms and could thus participate in the digestion.

### 2.5.4 Alkaline Treatment

The performance of an anaerobic digester fed with waste activated sludge (WAS) pretreated with NaOH was examined by Jih-Gaw Lin et al (1997) and found that, the reactors fed with WAS (1% TS) pretreated with 20 and 40 meq/L of NaOH, WAS (2% TS) pretreated with 20 meq/L NaOH, the biogas generation was increased by 33, 30 and 163% over that of reactor fed with untreated WAS.

Jih-Gaw Lin et al (1998) conducted alkaline pretreatment studies on sludge generated during treatment of a petrochemical product to enhance the efficiency of biological hydrolysis process. It was observed from the studies that alkaline pretreatment method was useful in biodegradability enhancement. The effect of alkaline pre-treatment has been studied by Hu et al (2009) on waste activated sludge from a wastewater treatment plant in Shanghai, China. At pH 12, COD solubilization achieved was about 50%, which induced 40 times increase in SCOD whereas at pH ≤ 9, the degree of COD and protein solubilization was not significant.

Yunqin (2009) investigated alkali pre-treatment of pulp and paper sludge prior to anaerobic digestion and stated that with 8 g NaOH/100 g TS_{sludge}, methane yield was 0.32 m^3 CH_4/kgVS_{removed} and the increase in biogas generation was 183.5% of the control. The thermo-chemical pretreatment of meat and bone meal (MBM) and its effect on the CH_4 production potential was investigated by Wu et al (2009) and reported that, methane production potential of pretreated MBM was in the range of 389 to 503 mL CH_4/g VS MBM and 464 to 555 mL CH_4/g VS MBM at 55°C and
131°C respectively. Paper tube residuals were pretreated with sodium hydroxide and the pre-treatment improved the methane yield by 70–107% from 238 to 403–493 mL/g VS (Teghammar et al 2010).

2.5.5 Thermal Treatment

In a study conducted by Vlyssides and Karlis (2004) on WAS from the food industry, it was observed that at moderate temperatures (50–90°C), effective solubilization of sludge was observed. Also in another study with and without alkaline addition to the sludge, it was found that pretreatment at 90°C (at pH 11) reduced VSS by 46% and improved methane production by 0.28 L/kg VSS loading.

Low-temperature thermal pretreatment is advantageous as compared with high-temperature pretreatment, in terms of gas production from thermophilic anaerobic digestion (Ferrer et al 2006). The degree of WAS solubilization during thermal hydrolysis depended on pretreatment temperature when compared to thermal treatment time (Valo et al 2004).

Gavala et al (2003) reported that the temperature and duration of thermal sludge pretreatment required to achieve acceptable solubilization depends on the nature of sludge i.e. primary to secondary sludge ratio. Greater is the intensity of pretreatment required for achieving the satisfactory solubilization in case of biological sludge, due to difficulty in hydrolyzing.

Eskicioglu et al (2007) conducted studies on thermal microwave effect on enhancing digestibility of waste activated sludge and found that WAS solubilization results did not indicate any thermal effect. Microwave (MW) pretreated WAS samples consistently produced higher biogas than
conventional heating (CH) samples at pretreatment temperatures of 50, 75 and 96°C, indicating that a MW thermal effect was more effective.

Bougrier et al (2007) conducted studies on impact of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge and found that thermal treatment at 190°C was more efficient than treatment at 135°C in terms of total COD, lipids, carbohydrates and protein removals and an increase in methane production of 25% was observed. Gavala et al (2003) conducted studies on the effect of the pretreatment at 70°C on mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Pretreatment has a significant effect on the methane potential and production rate upon subsequent thermophilic digestion of primary sludge whereas the methane potential was effective when mesophilic digestion followed in case of secondary sludge.

Luste and Luostarinen (2010) conducted studies on effect of pre-treatments i.e. thermal, ultrasound, acid, base and bacterial product on hydrolysis and methane production potentials of by-products from meat-processing industry. The ultrasound pretreatment was the most effective pretreatment when compared with other pretreatments. The effect of a low temperature pre-treatment at 70°C on the efficiency of thermophilic anaerobic digestion of primary and secondary waste sludge was investigated by Ferrer et al (2008). Low temperature pre-treatment enhanced the methane production through thermophilic anaerobic digestion of sludge. A summary of pretreatment processes are presented in Table 2.5.
Table 2.5  A Summary of Pre-Treatment Processes

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Nature Waste of Waste/Substrate</th>
<th>Conditions &amp; Observations</th>
<th>Investigator (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozonation</td>
<td>Waste Activated Sludge</td>
<td>Dose - 0.05 to 0.5 g O₃/g of total solids (TS)</td>
<td>Goel et al (2003)</td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>Anaerobic Digestion of Waste Activated Sludge</td>
<td>Enhancement of Biogas - 40% using at low specific energy-inputs (SE) and 15% using at higher SE-values</td>
<td>Appels et al (2008).</td>
</tr>
<tr>
<td>Peroxide Treatment</td>
<td>biosolids</td>
<td>biogas generation enhance</td>
<td>Dewil et al (2007)</td>
</tr>
<tr>
<td>Alkaline Treatment</td>
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<td>At pH 12 about 40 times increase in SCOD</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Alkaline Treatment</td>
<td>Waste Activated Sludge</td>
<td>8 g NaOH/100 g TS&lt;sub&gt;sludge&lt;/sub&gt;, the increase in biogas generation was 183.5% of the control</td>
<td>Yunqin (2009)</td>
</tr>
<tr>
<td>Thermal Treatment</td>
<td>Waste Activated Sludge from food industry</td>
<td>effective solubilisation temperature range 50–90°C</td>
<td>Vlyssides and Karlis (2004)</td>
</tr>
</tbody>
</table>

2.6 ANALYSIS OF DIGESTATE

Gomez et al (2005) investigated anaerobic digestion process using thermal analysis to determine the stability of the digestate, using thermogravimetric analysis for ascertaining the degree of stabilization reached
by the organic matter under the anaerobic digestion process. Acidogenic fermentation of proteinaceous solid waste and characterization of different bioconversion stages and extracellular products were investigated by Kumar et al (2008). The degradation of animal fleshings started with non-fibrillar proteins and proceeded with fibrillar proteins. The bioconversions were evidenced by the releases of aliphatic amino acid in the early stages of hydrolysis followed by aromatic amino acids.

Anaerobic treatment of poultry blood was carried out in a semi-continuous anaerobic co-digestion reactor with OFMSW working at an HRT of 36 days and an organic loading rate of 1.5 kg VSS$_{feed}$/ m$^3$/ day. The specific gas production was 0.33 m$^3$ per kg VSS$_{feed}$. The stability of the end product after anaerobic digestion was evaluated using Fourier Transform Infrared Spectroscopy and Thermo-gravimetry Analysis (TG–DTG) (Cuetos et al 2009). Fourier Transform Infrared Spectroscopy (FTIR) along with Thermo-gravimetric Analysis together with mass spectrometry (TG–MS analysis) were employed to study the organic matter transformation attained under anaerobic digestion of slaughter house waste in order to establish the stability of the digestates obtained when compared with fresh wastes (Cuetos et al 2009).

Maria et al (2010) investigated the transformation of organic fraction of slaughter house wastewater during anaerobic digestion using Fourier Transform Infrared Spectroscopy along with Thermo-gravimetric Analysis together with mass spectrometry (TG–MS analysis). After completion of the digestion process an increase in aromaticity degree and a reduction of volatile compounds after stabilization were observed.

Digestate obtained from different bio-wastes were evaluated for their stability with the help of thermo-gravimetric analysis. The thermal
methods were the appropriate tools for assessing the degree of stabilization attained by organic matter under anaerobic digestion (Gomez et al. 2007).

The significance of thermal methods for monitoring the degree of stabilization of municipal solid wastes was investigated by Smidt and Lechner (2005) and it was reported that stabilization was indicated by the shift of TG/DTG peaks towards higher temperatures. The interaction between digestion conditions, sludge physical characteristics and behaviour was investigated for anaerobically digested primary sludge in completely stirred tank reactors by Mahmoud et al. (2006) and reported substantial reduction in floc sizes with improved digestion conditions.

2.7 SUMMARY OF THE REVIEW OF LITERATURE

The literature review discusses the various studies on co-digestion of solid wastes and the advantage of co-digestion process. The mechanisms of lipid degradation in anaerobic digestion process and operational problems associated with the process have been summarized. Apart from that, various pretreatment technologies for waste activated sludge to increase the soluble COD and thereby enhance biogas generation have been summarized. Enzymatic applications to hasten the rate, limiting step of hydrolysis process and its significance in anaerobic digestion process have been summarized from the literature review. The significance of process parameters such as mix proportions of substrates, seed to substrate ratio, organic loading rate, hydraulic retention time and C/N ratio have been summarized. The studies on factors such as pH, oxidation and reduction potential, alkalinity, volatile fatty acids (VFA), VFA/alkalinity ratio, ammonia and heavy metals on anaerobic digestion process has been summarized from the literature and reported. The significance of instrumental analysis such as FT-IR, Thermo-gravimetric analysis, SEM and particle size analysis on digestate samples has been summarized.