Chapter V

DISCUSSION

Anaerobic digestion for treatment of organic waste and biogas production is an environmentally attractive technology. It has environmental benefits with regard to waste treatment, pollution reduction and converting organic carbon to its most oxidized (CO₂) and most reduced (CH₄) (Tafdrup, 1995). Tannery solid wastes (fleshing and biological sludge) have the potential to contaminate surface water and ground water causing environmental problems. Techniques available for recycling of sludge are thickening, conditioning, anaerobic digestion for methane generation, incineration, agricultural utilization, brick production, light weight aggregation, cement production and heal drying for palletizing and composting (Kotaseck, 1997). However, anaerobic digestion of tannery solid waste by biomethanation seems to be a better proposition, which has been undertaken in this study. This discussion will focus on the interpretation of the various studies conducted on the recycling the biological tannery sludge and fleshing.

The physico-chemical characteristics of tannery biosludge and fleshing were analyzed. Major component includes proteins and minerals were determined. The protein content in fleshing is 7.2% and the fat content (11 %) is high because of the presence of fat as an important part of the hide.

The chemical composition of the tannery sludge indicates a very balanced medium, ideal for the growth of organisms involved in methanogenesis. The present study results indicated that the initial COD of tannery biosludge is 2485 mg/l. Similar, analysis of tannery waste has been reported by Rajesh et al. (1999). Due to increasing demand for energy the use of various industrial wastes with nutrients suitable for methane production
and minimization of pollution has been attempted by various authors (Vimal and Tyagi, 1984; Hobson, 1984).

In the present study, the primary screening of proteolytic bacteria was done by the hydrolysis of skimmed milk casein, gelatin and egg albumin. Two strains to be screened for the hydrolysis of fleshing has been done by Shumi et al. (2004). The isolated strains showing higher proteolytic activity were selected and screened using standard biochemical procedures. In the present study the strains confirmed as proteolytic was based on a similar study conducted by Ganesh Kumar and Sekaran, (2006). Bacillus strains are ubiquitous microorganism, which can grow on natural media without any special requirements for nutrients (Raju et al., 1996; Henner, 1990; Rao et al. 1990).

Several workers have worked in proteases from Bacillus and Pseudomonas sp. (Chakraborty and Srinivasan, 1993; Dayanandan et al, 2003). In the present study results indicated that the two isolates selected showing higher proteolytic ability were identified as Bacillus subtilis and Pseudomonas fluorescense. Muhammad Nauman Alla I' et al. (2006) recorded similar findings viz., biodegradation of solid leather waste by Bacillus subtilis. Zerdani et al. (2004) also attempted similar research viz., digestion of solid tannery wastes by strains of Bacillus sp.

Lipolytic bacterial isolates were isolated from different locations such as tannery polluted soil, agricultural soil and sludge stored soil. Watanabe et al (1979) conducted an extensive screening for alkaline lipase producing microorganisms from soil. In the present study results showed that two isolates selected showing higher lipolytic activity were identified as Bacillus cereus and Pseudomonas aeruginosa. Stanier et al. (1966)
reported that *Pseudomonas* *sp.* is known for its lipolytic property. Several *Bacillus* *sp.* were reported to be the main source of lipolytic enzymes (Luisa et al., 1997).

Svendsen et al. (1997) studied bacterial lipases and their study focused on particular classes of enzymes such as lipases from the genus *Pseudomonas*, which are of interest due to biotechnological application. Production of proteinase by *Pseudomonas aeruginosa* Morihara, 1963. Mohammad Hasannzzaman et al. (2004) studied on isolation, identification and characterization of a novel, oil degrading bacterium *Pseudomonas aeruginosa* when growth in minimal salt medium containing 1% triacylglycerol and the hydrolysis products were free fatty acids and monoacylglycerol.

The ability of the proteolytic bacterial strains was evaluated based on the ability to release free amino acid. The present study result indicated that the cultural conditions for higher proteolytic activity was at pH 8.0 and 9.0 subjected to a temperature of 37°C. The results are in line with results obtained by Shumi et al. (2004) studied the cultural conditions determination for maximum proteolytic activity under pH range and they observed that maximum proteolytic activity was at 37°C. The results arrived regarding the optimum pH 8.0 and 9.0 and temperature with 42°C agreed with results obtained by a wide variety of authors who found that the majority of bacterial lipases examined showed highest activity in a neutral to alkaline pH range (Adams and Brawley, 1981; Jorisson and Suygg, 1974). Lawrence et al. (1967) reported similar findings and the maximum lipolytic activity was found at 42°C. The present study results indicated that the lipase activity has been observed in a wide range of temperature (25 to 42°C) and the results are in line with the findings of Papon and Talon (1988).
Growth was determined to assess the nutritional need for the strains and their capabilities to release the free fatty acid and other nutrients from the solid leather wastes by hydrolyzing the lipid. In the present study that the cell growth of lipolytic bacterial isolates was determined OD at 600 nm. Recent research done by Heung-Chae Jung et al (2006) indicated the production of thermostable lipase by *Pseudomonas putida* during the log phase of growth and maximum production correlated with cell growth.

For solid organic wastes, the common pretreatment method is to use hydrolysis reactor to liquefy the substrate before feeding into a methanogenic reactor (Scherer *et al*, 2008). The objective of the present investigation is to accelerate the fleshing digestion process after which it could be subjected to biomethanation and to compare it with raw fleshing. Interest in microbial lipase production has increased in the last decades, because of its large potential in industrial applications particularly tannery industries (Kumar *et al*, 2005). Biological liquefaction of tannery fleshing was carried out in the present study by inoculating the proteolytic and lipolytic bacteria. Ravindranath (1998) carried out similar work on biological liquefaction of limed fleshing and methane generation. Karmaraguru *et al* (1997) also made similar observation. A similar study was carried out on enzymatic degreasing of a solid waste from the leather industry by lipases (Fernando *et al*, 2001). Earlier attempts reported by Zerdani *et al* (2004) that digestion of solid tannery wastes by strains of *Bacillus sp.* isolated from compost in Morocco. A novel process for liquefaction of solid organic matter by biological method, envisages enormous application in tanning industry for effective disposal of various solid wastes, which otherwise add to the pollution load (Rengasamy Sunthararajan *et al*, 2007)
The possibilities of utilizing tannery solid wastes such as fleshing and biological sludge for biomethanation was investigated in batch system using in the present investigation. Earlier attempts to convert calfskin collagenous solid waste into methane through biomethanation have been reported by Lalitha et al. (1994). The configuration of the biogas reactor in the present study is similar to that designed by Manilal et al. (1990). Laboratory batch digesters were done in vials of 150 ml capacity and the feedstock was subjected to anaerobic digestion at 37°C for biomethanation. Similar studies using such digester has been tried by (Chakraborthy et al., 2002 and Pawinee Chaiprasert et al. 2001).

The biogas production was quantified and the volume of gas produced in vials was measured by the downward displacement of water in an inverted burette, which was described earlier by Chanakya, (2006). Results reveals that the average gas production and cumulative gas production, the best performance was found with a 3:2 mixture of liquefied fleshing: tannery biological sludge ratio anaerobically digested in a batch digester. Similar kind of results was obtained by (Kalia and Kanwar, 1995). In the present study, experiments were designed to compare gas production from raw and liquefied fleshing and it has been found that gas production was higher for pretreated fleshing than raw fleshing. The results were in accordance with Ghose and Bhattacharyya, (1999).

Analysis of acetic acid concentration revealed that there is utilization of acetate produced by the breakdown and hydrolysis of polymers which inturn are utilized by methanogens (Chakraborthy et al., 2002). It was observed in the present investigation there is an initial increase in the concentration of acetic acid during biomethanation using raw fleshing (2.0g/l) and liquefied fleshing (2.4g/l). A similar observation has been made
by Varei et al. (1977). According to Smith et al. (1966), acetate and H₂-CO₂ are the major precursors in sludge digesters. Van den berg et al. (1976) concluded that concentration upto 6.0 g/l are not toxic to the methanogenic bacteria. A rapid buildup of VFA is established in the reactor before methanogenesis occurs (Kalpana et al, 1985).

Methane production is a direct result of COD reduction within the anaerobic digester. Analysis revealed that in control and other treatments, COD reduction increases with retention time, which was in accordance with the findings of Ghose and Bhattacharyya, (1999). Anaerobic treatment is often used to treat low strength waste water with a COD less than 200 mg/l (Kato et al, 1999). Significant COD reduction was observed in both raw and liquefied fleshing. A similar finding was observed on COD reduction in organic solid waste generated from tanneries (Muthukrishnan, 1997), methanogenesis of rice straw subjected to white and brown rot fungus showed better reduction in COD (Ghose and Bhattacharyya, 1999) and COD and BOD removal in anaerobic treatment of vegetable tannery waste water (Routh, 2000).

Total Solids denotes organic as well as inorganic matter in the feedstock. Initial TS/VS of the feedstock was determined before feeding into the reactor. After the biomethanation TS/VS was determined and result indicated 75% Volatile Solid reduction as in the case of liquefied fleshing and 68% in the case of raw fleshing. A similar observation has been made by Chulhwan et al. (2005). Earlier attempts to convert calfskin collagenous solid waste into methane through biomethanation, was subjected to 80days retention time and the overall decrease in volatile solid level was 65% (Lalitha et al, 1994). Gujer and Zehnder, (1983) reported that more reduction in the carbon content resulted in the higher
the ratio of methane to carbon dioxide. Similar findings were observed in the present investigation.

After biomethanation, the digested slurry can be used as organic manure. In the present course of investigation, an analysis of NPK was undertaken. The results indicated that there is manurial value of the digested slurry obtained after biomethanation. Thorstensen et al. (1979) reported on the tannery sludge having value as a fertilizer based on its nutrient content. Effect of different concentrations of tannery effluent on seed germination and early seedling growth of *Cajanus cajan* L. and *Oryza saliva* L. were studied by Bera et al. (1998).

In the present studies, the results indicated the fact that the tannery biosludge and fleshing can be efficiently recycled through biomethanation. Proteolytic and lipolytic microorganisms used played an important role in enzymatic hydrolysis of fleshing. The biologically liquefied tannery fleshing facilitate higher biodegradability of organic matter, and was found suitable for biomethanation. After biomethanation, the digested slurry can be used as an organic manure. This relative simple treatment of tannery wastes may provide a practical and economical solution for the tannery industries.