CHAPTER 3

MATERIALS AND METHODS

3.1 GENERAL

Materials, experimental setup, and methodology are discussed in detail in this chapter. Studies were conducted using the lab, bench, and pilot scale reactors at Environmental Technology Laboratory of Central Leather Research Institute (CLRI), Chennai.

3.2 MATERIALS

3.2.1 Limed Fleshings

LF samples from different tannery clusters were collected, stored at 4°C for a maximum period of 3 days.

3.2.2 Tannery Effluent

TE was collected from Common Effluent Treatment Plant (CETP) and stored in a refrigerator at 4°C for maximum period of 3 days.

3.2.3 Chemicals

Analytical grade alkalis NaOH, KOH, Ca(OH)$_2$ and Na$_2$CO$_3$ from Merck chemicals were used for thermo-chemical studies.
3.2.4 Inoculum

Inoculums from aerobic and anaerobic sources were used for liquefaction of LF. For this purpose, inoculum from supernatant of anaerobic reactor (UASB), aerobic reactor (ASP) and anaerobic sludge digesters were collected.

3.3 INSTRUMENTS AND EQUIPMENT USED

3.3.1 Gas Chromatography (GC)

Volatile fatty acids were quantified using gas chromatography CHEMITO 800 model fitted with Flame Ionization Detector (FID) and column BP 21, 60 m x 0.32 mm ID. Temperature of injector, detector and column were 180ºC, 250ºC, and 110ºC respectively. Argon was used as a carrier gas at the flow rate of 2 mL/min at pressure 22 psi.

3.3.2 Particle Size Analyzer

LF samples after pretreatment studies were analysed using laser scattering particle size distribution analyzer Model LA - 950 for particle size distribution.

3.3.3 Elemental (C H N) Analyzer

The percentage of carbon, hydrogen and nitrogen were determined using Elemental Analyzer for CHNS-O (Model- Euro EA 3000, Euro Vector SpA, Via Tortona, Milan, Italy).
3.4 EXPERIMENTAL SETUP

3.4.1 Biochemical Methane Potential (BMP) Reactor (Laboratory Scale)

Experimental set up of laboratory scale BMP reactor is shown in Figure 3.1. A double-jacketed 2-Litre capacity reactor was used to assess the BMP of LLF. The reactor was provided with airtight lid, a stirrer, a gas outlet and a sample port. A variable speed stirrer was connected to a timer so that stirring interval frequency and duration of stirring can be controlled. To maintain constant temperature, water circulation was maintained through a thermostat. Gas outlet from the reactor was connected to a Marriot flask containing 6 N NaOH solution with thymol blue as indicator. Sodium hydroxide solution absorbs CO$_2$ and H$_2$S from the biogas. Volume of NaOH equivalent to methane generated was displaced into a measuring cylinder.

![Figure 3.1 Biochemical Methane Potential Reactor (Laboratory Scale)](image-url)
3.4.2 Experimental Setup of UASB Reactors (Bench Scale)

To study the biomethanization of combined treatment of TE and liquefied limed fleshings (LLF), UASB reactors were fabricated. The schematic diagram and experimental setup of bench scale UASB reactor is shown in Figures 3.2 -3.4. The UASB reactor consists of three main parts: bottom, middle and top section. The bottom and middle sections are provided with a jacket. The reactor elements are connected onto each other with stainless steel clamps. The top section, without temperature control, is provided with Gas- Liquid- Solid (GLS) phase separator. A magnetic joint is connected to the tube of the separator for a gas tight connection between the stirrer and the stirrer motor. The speed of the stirrer is 1 rpm. The reactor is provided with a stirrer composed of motor – magnetic joint, glass connector, stainless steel axis, stirrer blades. The top is covered with a stainless steel top plate, which is attached to the top with stainless clamp. The reactor is installed on a special table and rack with PVC clamps. The volume of reactor is five-litre capacity. The inlets are the ends of a stainless pipe with a diameter of 10mm. For an equal distribution of the wastewater this pipe is provided with holes. To prevent clogging the holes are in a downward position. The feed was fed into the reactor using a peristaltic pump controlled by timers. The treated effluent was recycled from the top through another peristaltic pump with adjustable speed controller. Biogas was passed through soda lime pellets and then through wet gas flow meter to measure the methane generated. Two such reactors were setup for the studies. One reactor was fed with TE and another one with LLF and TE. Temperature of both reactors was maintained at 30°C±1 by water circulation through thermostat.
Figure 3.2 Schematic flow Diagram of Bench Scale Studies
Figure 3.3 Schematic Diagram of the UASB Reactor (Bench scale)
3.4.3 Experimental Setup of Modified Bench Scale UASB Reactor

To study the effect of simultaneous liquefaction and biomethanization of LF the reactor was modified and schematic diagram of modified bench scale UASB reactor is shown in Figure 3.5. For liquefaction of LF an additional liquefaction reactor (LR) was introduced with provision to feed LF and re-circulate the part of the supernatant from UASB reactor through LR using a peristaltic pump. Supernatant from LR was fed at the bottom of the reactor along with TE through a peristaltic pump. Experimental setup of modified bench scale UASB reactor is depicted in Figure 3.6.
Figure 3.5 Schematic Diagram for Modified Bench Scale Studies
3.4.4 UASB Reactor (Pilot Scale)

Based on the outcome of the bench scale studies using the concept kinetics of substrate utilization and mathematical modeling described elsewhere in location 2.7.8. Kinetic constants half velocity constant, rate of substrate utilization, yield coefficient, decay coefficient, maximum specific growth rate, specific utilization rate were arrived at by operating the reactor on continuous basis and the same were used to design the pilot scale reactor.

Volume of pilot scale reactor was designed based on the results obtained from the bench scale studies and the effective volume of UASB reactor was arrived at as 8.5 m$^3$ and considering a GLS separator, total volume of the reactor was arrived at as 12.5 m$^3$. 
Pilot scale studies were carried out in UASB reactor (pilot scale) of capacity 12.5 m$^3$, with 1.8 m diameter and 5 m liquid depth. Reactor was provided with three inlets to maintain uniform flow. The reactor is also provided with sampling ports at every 0.5 m height. Top of the reactor is provided with a GLS separator. Overflow from the reactor passes through a launder of 2.8 m length. Size of the GLS separator is 1.4 m x 1.4 m. Gas outlet pipe from the top of the reactor is connected to a condensate pot. Sampling port for sampling effluent and sludge is provided at the top of the reactor. Gas flow was measured using wet gas flow meter. Pilot scale UASB reactor is depicted in Figure 3.7 and wet gas flow meter is depicted in Figure 3.8.
3.5 METHODOLOGY

The experimental methodology adopted in the study is depicted in Figure 3.9. LF from different tannery clusters were characterized and mechanical, thermo-chemical and biological pretreatment studies were carried out on liquefaction of LF. BMP studies were carried out for liquefied limed fleshings (LLF). Based on the outcome of the BMP studies, bench scale and pilot scale biomethanization studies were carried out for combined treatment of TE and LLF using UASB reactor. Techno-economic analysis was carried out for treatment of TE using anaerobic lagoon and disposal of fleshings in landfills versus the combined treatment of TE and LLF using UASB reactor based on the outcome of the present study. Financial benefits due to electrical energy generation and CDM benefits through carbon trading were also considered for techno-economic analysis.
Figure 3.9 Experimental Methodology Adopted in the Study

**Studies on liquefaction of limed fleshings and enhancement of biomethenization from tannery waste**

**Characterization of Limed Fleshings**

**Mechanical pre-treatment**
- Mincing time: 30, 60, and 90 sec.
  - Effect of mincing
  - LLF

**Thermo-chemical pre-treatment**
- Temp: 70°C, Time: 15 min
  - Effect of NaOH
  - Effect of KOH
  - Control (distilled water)
  - LLF

**Effect of NaOH**
- BMP (lab scale) studies
- CH₄ yield

**Effect of KOH**
- BMP (lab scale) studies
- CH₄ yield

**Control (distilled water)**
- BMP (lab scale) studies
- CH₄ yield

**Biological pre-treatment**
- Temp: 120°C, Pressure: 1 bar
- Time: 14 days
  - Effect of anaerobic inoculum (effluent)
  - Effect of anaerobic inoculum (sludge)
  - Effect of aerobic inoculum (effluent)
  - Control (distilled water)
  - LLF

**Bench scale BM studies (Liquefied limed fleshings with Tannery effluent)**
- Temp: 30°C
- Duration: 14 months
- Effect of OLR
- Effect of HRT
- CH₄ yield

**Enhancement of BM studies**
- Effect of OLR
- Duration: 14 months
- CH₄ yield & Kinetics

**Pilot scale BM studies (Liquefied limed fleshings with Tannery effluent)**
- Temp: 30°C
- Duration: 3 months
- Effect of OLR
- Effect of HRT
- CH₄ yield
- Techno-economic studies

BMP – Biochemical Methane Potential
BM – Biomethenization
LLF – Liquefied limed fleshings
TE – Tannery effluent
OLR – Organic loading rate
HRT – Hydraulic retention time
3.5.1 Characterization of Limed Fleshings

LF samples from different clusters were collected and stored at 4°C for a maximum period of 3 days. Samples were characterized for pH, moisture content, volatile solids, non-volatile solids, COD, CODs, TKN, oil & grease, ammonical nitrogen and phosphorus as per Standard Methods (APHA, 1998). Elemental composition of fleshings was arrived at using CHN analyzer.

3.5.2 Characterization of Tannery Effluent

TE was collected from CETP and stored in a refrigerator at 4°C. Samples were characterized for pH, TS, SS, COD, BOD, TDS, sulphide, sulphate, chloride, volatile solids and chromium as per Standard Methods (APHA, 1998).

3.5.3 Effect of Mechanical Pretreatment on Liquefaction

To study the effect of mechanical pretreatment on liquefaction of LF, one kg of LF was mixed with one litre of distilled water and temperature was raised to 70°C and it was maintained at 70°C for 15 minutes. Samples were cooled and minced using commercial blender for 30, 60 and 90 seconds. Minced samples were passed through ISS 3.35 mm sieve to assess the percentage of particle size reduction.

3.5.4 Effect of Thermo-chemical Pretreatment using Alkalis on Liquefaction

To study the effect of thermo-chemical pretreatment on liquefaction of LF, one kg of LF was mixed with one litre of different chemical solutions such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)₂) and sodium carbonate (Na₂CO₃) in the order of 1,2,3,4 and 5% solutions. Samples were subjected to a pressure of 1 kg/cm² at a
temperature of 120ºC for 15 minutes and a control was also kept with distilled water alone. Liquefaction of LF was estimated by analyzing the COD and COD$_s$ before and after pretreatment. In addition, particle size reduction and VFA production were also measured for assessing the liquefaction of LF. After pretreatment, liquefied LF was characterized and BMP studies were carried out after adjusting pH to neutral using hydrochloric acid. Experiments were carried out in triplicate. The experiments were repeated for five times.

3.5.4.1 Effect of Pretreatment on Particle Size Reduction

Effect of pretreatment on LF in terms of particle size reduction was estimated by screening with ISS 3.35 mm sieve. The percentage particle size reduction was calculated based on mass of LF retained in sieve on weight basis.

\[
\text{Particle size reduction (\%) } = \frac{(\text{Initial wt of LF} - \text{wt of LF retained in ISS 3.35mm sieve})}{\text{Initial wt of LF}} \times 100 \tag{3.1}
\]

3.5.4.2 Effect of Pretreatment on Liquefaction (COD$_s$)

Liquefaction of LF in terms of soluble organics (COD$_s$) was estimated by analyzing the COD and COD$_s$ before and after pretreatment. The percentage COD$_s$ was calculated based on the equation 3.2.

\[
\text{COD$_s$ (\%) } = \frac{\text{COD$_s$ after pretreatment}}{\text{Initial COD before pretreatment}} \times 100 \tag{3.2}
\]

3.5.4.3 Effect of Pretreatment on VFA Production

VFA was estimated as per procedure adopted in Wageningen University, The Netherlands (TNO report). In addition, acidification
efficiency was estimated as ratio between the VFA present in the reactor to theoretical VFA of the substrate (Shin et al 2001). Theoretical VFA of the substrate could be expressed as 1.49 gm COD/gm VFA (Tembhukar and Mhaisalkar 2008). Acidification yield was arrived at based on the total VFA in the digester and total COD input using Equation 3.4 (Raynal et al 1998).

\[
\text{Acidification (\%)} = \frac{\text{VFA produced}}{\text{VFA theoretical}} \times 100
\]  
(3.3)

Where in

- \(\text{VFA theoretical}\) = the theoretical VFA of substrate added.
- \(\text{VFA produced}\) = Total VFA produced from the liquefaction process
  \(\text{(VFA conc x liquid volume in the reactor)}\)

### 3.5.5 Effect of Biological Pretreatment using Inoculums on Liquefaction

Effect of biological pretreatment on liquefaction of LF was carried out using anaerobic and aerobic inoculums, for this purpose, inoculum from supernatant of anaerobic reactor (UASB), aerobic reactor (ASP) and anaerobic sludge digesters were collected and experiments were carried out. Experiments were carried out in triplicate. The experiments were repeated for five times.

#### 3.5.5.1 Effect of Biological Pretreatment (Anaerobically Treated Tannery Effluent as Inoculum)

To study the effect of biological pretreatment with inoculum from anaerobically treated tannery effluent on liquefaction of LF, Volatile solids (VS) concentration of inoculum and LF was estimated. Liquefaction of LF was estimated by analyzing COD and COD\(_s\) before and after pretreatment. LF (1 kg; moisture, 85.5 ± 6.3%; VS, 0.67 ± 0.06 w/w) were mixed with
anaerobic inoculum in the VS percentage of 0.75, 1.12, 1.5, 1.8 and 2.25 for trials I to V. The weight of VS in inoculum taken for the study were 0.6 ± 0.06 g (Trial I), 0.9 ± 0.09 g (Trial II), 1.2 ± 0.12 g (Trial III), 1.5 ± 0.15 g (Trial IV) and 1.8 ± 0.18 g (Trial V) respectively. Concentration of VS in inoculum was 550 ± 110 mg/L. A control was also taken up for study without inoculum but with distilled water. During the study, pH, VFA, COD and COD$_s$ concentrations were monitored. Liquefaction of LF is reported in terms of COD$_s$. LLF were characterized and BMP studies were carried out without pH correction.

3.5.5.2  Effect of Biological Pretreatment (Aerobically Treated Tannery Effluent as Inoculum)

Effect of pretreatment with aerobic inoculum on liquefaction of LF was carried out. Liquefaction of LF was estimated by analyzing the COD and COD$_s$ before and after pretreatment. One kg of LF, with 85.5 ± 6.3% of moisture and volatile solids in the range of 0.67 ± 0.06 % (w/w) were taken for the studies. One kg of LF was mixed with aerobic inoculum, containing VS of 0.6 ± 0.06 g (sample I), 0.9 ± 0.09 g (sample II), 1.2 ± 0.12 g (sample III), 1.5 ± 0.15 g (sample IV) and 1.8 ± 0.18 g (sample V) from an ASP reactor outlet. Percentages of VS in aerobic inoculum taken for the studies, per kg of LF, were 0.75, 1.12, 1.5, 1.8 and 2.25 respectively. A control was also taken up for study without inoculum but with distilled water. Samples were kept under anaerobic condition. During the study period pH, VFA, COD and COD$_s$ concentrations were monitored. Liquefaction of LF was reported in terms of soluble organics (COD$_s$).

3.5.5.3  Effect of Biological Pretreatment (Anaerobic Sludge as Inoculum)

Effect of pretreatment with anaerobic sludge as inoculum on liquefaction of LF was carried out. One kg of LF, with 85.5 ± 6.3% of
moisture and volatile solids in the range of 0.67 ± 0.06 % (w/w) were taken for the studies. One kg of LF was mixed with anaerobic sludge, containing VS of 8 ±0.1 g (Trial I), 16 ± 0.1 g (Trial II), 24 ± 0.1 g (Trial III), from the sludge digester. Percentages of VS in anaerobic sludge taken for the studies, per kg of LF, were 6.6, 13.3 and 20 respectively. A control was also taken up for study without anaerobic sludge but with distilled water. Samples were kept under anaerobic condition. During the study period pH, VFA, COD and COD_s concentrations were monitored. Liquefaction of LF is reported in terms of soluble organic (COD_s).

3.5.6 Kinetics of Liquefaction

Kinetics of liquefaction was determined based on kinetic relationship using equation 3.4 and 3.5. (Jash and Ghosh 1996 and Jiang 2005).

\[- \frac{dC}{dt} = kC \quad (3.4)\]

\[t = -\frac{1}{k} \ln \left( \frac{C_t}{C_o} \right) \quad (3.5)\]

\[k = \text{First order specific rate constant (day}^{-1})\]

\[C_t = \text{COD concentration at time } t\]

\[C_o = \text{Intial COD concentration of particles at zero time}\]

3.5.7 Studies on Biochemical Methane Potential (BMP) of LLF

Laboratory scale studies on BMP of LF were carried out after pretreatment by (i) Biological pretreatment with anaerobically treated tannery effluent as inoculum and (ii) Thermo-chemically pretreated with NaOH, and KOH. The reactor was seeded with sludge from UASB reactor treating TE. Assessment of biochemical methane potential involves incubating LLF after
inoculating with anaerobic sludge for 30 days at 30°C and methane gas production was monitored.

3.5.8 Biomethanization Studies of TE (Bench Scale)

UASB reactor treating TE was seeded with sludge from UASB reactor and the temperature of the reactor was maintained at 30°C ± 1°C. Total suspended solids (TSS) concentration of seed sludge was 50000 mg/L and volatile suspended solids (VSS) concentration was 29000 mg/L. VSS/TSS ratio was 0.58. Reactor was fed with TE alone. OLR was increased to the reactor by increasing the pumping rate of peristaltic pump. TE collected from a CETP was used. Recirculation was maintained to keep the upward velocity constant.

3.5.9 Biomethanization Studies of TE and LLF (Bench Scale)

UASB reactor treating LLF with TE was seeded with sludge from UASB reactor and temperature of the reactor was maintained at 30°C ± 1°C. TSS concentration of seed sludge was 50000 mg/L and VSS concentration was 29000 mg/L. VSS/TSS ratio was 0.58.

Initially 35 g LF was liquefied and mixed with 5 L of TE and was fed into the UASB reactor. OLR was increased to the reactor by increasing the COD of influent concentration by gradually increasing quantity of LLF with TE. TE was collected from a CETP. Recirculation was maintained to keep the upward velocity constant.

3.5.10 Biomethanization Studies of TE and LLF (Modified Bench Scale)

Initially liquefaction reactor (LR) was filled with effluent from UASB reactor. LF (150 g) was introduced into LR. Part of effluent from
UASB reactor was circulated through LR to facilitate liquefaction of LF. Supernatant from LR was re-circulated through UASB reactor along with TE. Effect of liquefaction of LF on recirculation rates of 3.0 L/day, 3.2 L/day, 6.5 L/day, 10.6 L/day and 11 L/day were studied. Biogas from LR and UASB reactor was passed through soda lime pellets and then through wet gas flow meter to measure the methane generated from the reactors separately.

3.5.11 Mathematical Modeling and Kinetics of Biomethanization

Mathematical modeling of biomethanization was developed by arriving at the kinetics of biomethanization. Influent, effluent, MLVSS concentration in the reactor and flow data collected by operating bench scale reactor is used to arrive at kinetic constants and to predict the effluent soluble substrate concentration, reactor biomass and volume of the reactor. To evaluate the kinetic constants for TE and combined treatment of TE with LLF, experiments were carried out in continuous UASB reactor by varying HRT, OLR and \( \theta_c \). The following modified Monod’s equations were used to develop kinetic coefficient (Ghangrekar 2006; Bal and Dhagat 2001; Metcalf and Eddy 2003).

\[
\frac{1}{\theta_c} = \frac{Y k S}{K_s + S} - k_d \tag{3.6}
\]

\[
\frac{1}{\theta_c} = Y U - k_d \tag{3.7}
\]

\[
\frac{1}{U} = \left( \frac{K_s}{k} \right) \left( \frac{1}{S} \right) + \left( \frac{1}{k} \right) \tag{3.8}
\]

where

\[
U = \frac{S_0 - S}{\theta X} \tag{3.9}
\]

where \( K_s \) - half velocity constant (mg/L)
Y - yield coefficient, \((\text{mgVSS/mg COD})\)
\(k\) - rate of substrate utilization (day\(^{-1}\))
\(k_d\) - decay coefficient (day\(^{-1}\))
\(\theta_c\) - mean cell residence time (day)
\(U\) - specific utilization rate (mg COD applied / mg MLVSS/day)

Using the equations 3.8 and 3.10 kinetic coefficient \(K_s\), \(k\), \(Y\) and \(k_d\) were arrived at by plotting the data collected from bench scale studies. Effluent soluble substrate concentration for a biological process is a function of SRT and kinetic coefficient for growth and decay. By applying the kinetic constants in the equation 3.10 volume of the reactor can be arrived at

\[
VX = \frac{YQ(S_o - S)\theta_c}{1 + (k_d)\theta_c}
\]  
(3.10)

By applying equation 3.11 substrate concentration of the effluent can be arrived at.

\[
S = \frac{K_s[1 + (k_d)\theta_c]}{\theta_c (Yk - k_d) - 1}
\]  
(3.11)

### 3.5.12 Biomethanization Studies of TE and LLF (Pilot Scale)

Pilot scale biomethanization studies were conducted in UASB reactor for treating TE and LLF. The studies were conducted for a period of 3 months. TE were transported daily through tankers and stored in the equalization tank. LF from tanneries was collected and liquefied in liquefaction tank. TE was pumped into the UASB reactor through a conditioning tank. LF of 200 kg was liquefied by adding inoculum in the ratio of 1.5% of VS, LLF was mixed with 30 m\(^3\) of TE in the conditioning tank. Sludge samples in the reactor were collected through sample ports for analysis of TS and VS. Volume of gas produced from the reactor was
corrected to temperature of 30°C and atmospheric pressure. Reactor influent and effluent were monitored for COD and VFA on regular time intervals.

3.5.13 Design Methodology for Full Scale Plant

A full scale plant was designed considering the results obtained from bench and pilot scale studies. Kinetic constants arrived from bench scale studies were applied for full scale plant. In addition, organic loading rate and hydraulic retention time were also considered. Equation 3.10 was rearranged to arrive at the volume of the reactor. Design criteria adopted is given in Appendix 3.