CHAPTER 5

BIO CHEMICAL METHANE POTENTIAL (BMP), ANAEROBIC TOXICITY ASSAY (ATA) AND BATCH STUDIES OF ANAEROBIC CODIGESTION OF TANNERY SOLID WASTE AND EFFULENT TREATMENT PLANT SLUDGE

5.1 Introduction

The biomethanisation of organic wastes is accomplished by a series of biochemical transformations, which can be roughly separated into a first step where hydrolysis, acidification and liquefaction take place and a second step where acetate, hydrogen and carbon dioxide are transformed into methane. In one-stage systems, all these reactions take place simultaneously in a single reactor, while in two- or multi-stage systems; the reactions take place sequentially in at least two reactors. About 90% of the full-scale plants currently in use for anaerobic digestion of industrial biowastes relies on one-stage systems and these are approximately evenly split between 'wet' and 'dry' operating conditions (De Baere, 1999). This industrial trend is not mirrored by the scientific literature, which reports as many investigations on two-, multi-stage or batch systems as on one-stage systems. A likely reason for this discrepancy is that two- and multi-stage systems afford more possibilities to the researcher to control and investigate the intermediate steps of the digestion process. Industrialists, on the other hand, prefer one-stage systems because simpler designs suffer less frequent technical failures and have smaller investment costs. Biological performance of one-stage systems is, for most organic wastes, as high as that of two-stage systems, provided the reactor is well-designed and operating conditions carefully chosen (Weiland, 1992). Consistency of organic solid wastes is made to resemble that of biosolids, via grinding and slurrying to less than 15 % TS with dilution primary sludge, so that a classical complete mix reactor may be used.
TABLE 5.1 - POSSIBLE UNIT PROCESSES, PRODUCTS AND QUALITY STANDARDS INVOLVED IN ANAEROBIC DIGESTION PLANT FOR ORGANICS SOLIDS

<table>
<thead>
<tr>
<th>Unit Process</th>
<th>Reusable Products</th>
<th>Standards or Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRE TREATMENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnetic separation</td>
<td>Ferrous metals</td>
<td>Organic impurities</td>
</tr>
<tr>
<td>Size reduction (Drum or shredder)</td>
<td></td>
<td>Communion of paper, cardboards and bags</td>
</tr>
<tr>
<td>Pulping with gravity separation</td>
<td>Heavy inerts reused as construction material</td>
<td>Organic impurities</td>
</tr>
<tr>
<td>Drum screening</td>
<td>Coarse fraction, plastics</td>
<td>Calorific value</td>
</tr>
<tr>
<td>Pasteurisation</td>
<td></td>
<td>Germs kill off</td>
</tr>
<tr>
<td><strong>DIGESTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanogenesis</td>
<td>Biogas</td>
<td>Norms nitrogen, sulfur</td>
</tr>
<tr>
<td>Biogas valorisation</td>
<td>Electricity</td>
<td>150-300 kwe/ton</td>
</tr>
<tr>
<td></td>
<td>Heat (steam)</td>
<td>250-500 kwe/ton</td>
</tr>
<tr>
<td><strong>POST TREATMENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical dewatering</td>
<td>Compost</td>
<td>Load on water treatment</td>
</tr>
<tr>
<td>Aerobic stabilisation or biological dewatering</td>
<td>Water</td>
<td>Norms soil amendments</td>
</tr>
<tr>
<td>Water treatment</td>
<td></td>
<td>Disposal norms</td>
</tr>
<tr>
<td>Biological dewatering</td>
<td>Compost</td>
<td>Norms soil amendments</td>
</tr>
<tr>
<td>Wet separation</td>
<td>Sand fibres (peat) sludge</td>
<td>Organic impurities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>calorific value</td>
</tr>
</tbody>
</table>

In contrast with the apparent simplicity of such one-stage wet process, many technical aspects need actually be taken into account and solved in order to guarantee a satisfactory process performance (Westergard and Teir, 1999; Farneti et al., 1999) (Table 5.1). The pre-treatment necessary to condition the wastes in slurry of adequate consistency and devoid of coarse or heavy contaminants can be very complex, especially in the case of tannery solid and liquid waste. To achieve the objective of
removing these contaminants while at the same time keeping as much biodegradable wastes within the main stream requires a complicated plant involving screens, pulpers, drums, presses, breakers, and flotation units (Farneti et al., 1999). These pretreatment steps inevitably incur a 15 - 25 % loss of volatile solids, with a proportional drop in biogas yield (Farneti et al., 1999). Slurried wastes do not keep a homogenous consistency because heavier fractions and contaminants sink and a floating scum layer forms during the digestion process, resulting in the formation of three layers of distinct densities, or phases, in the reactor. The heavies accumulate at the bottom of the reactor and more over may damage the propellers while the floating layer, several meters thick, accumulates at the top of the reactor and will hamper effective mixing. It is therefore necessary to foresee means to extract periodically the light and heavy fractions from the reactor. Another technical drawback of the complete mix reactor is the occurrence of short-circuiting, i.e. the passage of a fraction of the feed through the reactor with a shorter retention time than the average retention time of the bulk stream. Not only does short-circuiting diminish the biogas yield, most importantly it impairs the proper hygienization of the wastes, i.e. the kill-off of microbial pathogens that requires a minimum retention time to complete.

**TABLE 5.2 - ADVANTAGES AND DISADVANTAGES OF ONE-STAGE 'WET SYSTEMS'**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical</td>
<td>Inspired from known process</td>
<td>Short-circuiting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sink and float phases</td>
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<tr>
<td></td>
<td></td>
<td>Abrasion with sand</td>
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<tr>
<td></td>
<td></td>
<td>Complicated pre-treatment</td>
</tr>
<tr>
<td>Biological</td>
<td>Dilution of inhibitors with fresh water</td>
<td>Particularly sensitive to shock loads as inhibitors spread immediately in reactor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VS lost with inerts and plastics</td>
</tr>
<tr>
<td>Economic</td>
<td>Equipment to handle slurries is cheaper</td>
<td>High consumption of water</td>
</tr>
<tr>
<td></td>
<td>(compensated by additional pretreatment steps and large reactor vol)</td>
<td>Higher energy consumption for heating large volume</td>
</tr>
</tbody>
</table>
There exists a great variety of means to ensure adequate stirring of the digesting slurry within the reactor. For example, Weiland (1992) describes a pilot reactor with mechanical mixing ensured by downward movement in a centrally located draft tube enclosing a screw (loop reactor). An interesting advantage of this mixing mode is that it prevents the build-up of a floating scum layer. Since moving parts within a sealed reactor are technically challenging, several designs were developed that ensure adequate mixing without any mechanical moving parts within the reactor. Mixing modes using a combination of propellers and gas recirculation are also sometimes used (Cozzolino et al., 1992).

5.2 Biological process

The three most important indicators of biological performance are the rate of digestion process, the degree of completion, and the stability of the biochemical reactions. The degree of completion is quantified by comparing the biogas yield obtained in the reactor per unit mass substrate fed with the maximum biogas yield obtained in lab-scale batch reactors operated under optimal conditions. While this comparison is perhaps the most important test used in the industry, published reports almost invariably fail to mention what the maximum yield amounts to. Instead, publications refer simply to the biogas yield or alternatively to the % VS removal from the waste stream to assess the degree of completion of the methanisation process. Biogas yield as such is however of very little use because it is much more dependent on waste composition than on process performance. For example, the methane yield in one full-scale plant varied between 170 and 320 m$^3$ CH4/kg VS fed (40 and 75 % VS reduction) during the summer and winter months.

A more useful criterion of biological performance is the maximum sustainable reaction rate which is expressed as a rate of substrate addition, i.e. the maximum organic loading rate OLRmax (kg VS/m$^3$ reactor.d), or as a rate of product formation, i.e. the volume of dry biogas or, better, of methane (under standard conditions of pressure and temperature) produced per unit time per unit reactor volume (Nm$^3$ CH4/m3 reactor.d). These indicators are more useful than the biogas yield or % VS reduction. Another parameter of use to quantify the rate is the retention time, which is
roughly the inverse of the OLR when the OLR is expressed as mass wet substrate instead of mass substrate VS. The \( \text{OLR}_{\text{max}} \) indicates the degradative capacity of the system and the biogas yield its conversion efficiency determined under optimal conditions in the laboratory. Performing Bio chemical methane potential (BMP) and Anaerobic Toxicity Assay (ATA) experiments can evaluate all this collective informations in small serum bottles.

5.3 Biochemical Methane Potential (BMP) Assay

McCarty's group at Stanford developed an anaerobic biotransformation assay termed the Biochemical Methane Potential or BMP (Owen et al 1979). Just as the BOD assay indicates how much organic pollution can be degraded in an aerobic process, the BMP is the correlative measure in the anaerobic process. Since most organic pollutants can be biodegraded under both anaerobic and aerobic conditions, the BMP and BOD assay will often measure similar amounts. Regulatory requirements sometimes specify BOD analysis of the effluent, but a BMP assay should be determined in addition to BOD.

Table 5.3 summarizes the purposes of the BMP assay. Table 5.4 details the three principle reasons why the BMP should be part of the normal analyses performed on the effluent of any anaerobic treatment process.

**TABLE 5.3 - USES OF THE BMP ASSAY**

- Assaying the concentration of organic pollutants in a wastewater which can be anaerobically converted to \( \text{CH}_4 \).
- Evaluating potential anaerobic process efficiency
- Measuring residual organic pollution amenable to further anaerobic treatment
- Testing for non-biodegradables remaining after treatment
TABLE 5.4 - ADVANTAGES OF THE BMP ASSAY

The BMP realistically measures anaerobic biodegradability and can be, used to identify aerobic non-biodegradable components, which are subject to anaerobic biodegradation.

The BMP is a realistic measure of residual organic pollution amenable to further anaerobic treatment and gives a more realistic measure of potential process efficiency than a BOD assay.

The BMP requires minimal labour to set up and monitor and considerably less time than a BOD.

5.3.1 Biochemical Methane Potential (BMP)

The procedure for implementing the BMP assay involves placing an aliquot of the effluent sample, 50-100 ml, in a 125 ml serum bottle with an anaerobic inoculum. In many cases the reactor effluent already contains an adequate inoculum. In other cases an acclimated inoculum can be taken directly from the anaerobic reactor.

The headspace in the serum bottle should be purged with anaerobic mixed gas (70% N₂, 25%CO₂ and 5%H₂). The serum bottle is then incubated at 35°C and CH₄ production recorded after a prescribed number of days (usually five days). The gas production is measured by inserting a hypodermic needle connected to a calibrated fluid reservoir, through the serum cap. At this temperature 395 ml of CH₄ production is equivalent to 1gm COD reduction, a stoichiometric relationship which allows calculation of the COD reduction in the liquid phase.

It is important that CO₂ production be excluded because CO₂ does not represent COD reduction under anaerobic conditions. For example, if 2000 mg/L (COD equivalent) of biodegradable organic pollutant remains in the effluent, a BMP assay would indicate that after a period of time 39.5 ml of CH₄ net gas production would result from a 50 ml sample of effluent.

A cardinal rule of the BMP assay, as with the BOD, is that the biomass must be acclimated to the pollutants. Care must be exercised 1) to run a control with only the anaerobic inoculum and 2) to insure adequate time/acclimation for the biomass to metabolize the pollutant. Whereas a 20 day BOD is considered to represent the ultimate demand aerobically, the BMP may be extended to 30 or 60 days to
accommodate acclimation of the biomass to toxic and/or unusual pollutants occurring in some industrial wastewaters.

Since COD conversion is normally proportional to the product of biomass x time, the relative amount of biomass inoculum will affect the rate of conversion but not the net ultimate value. This rule holds for both the BOD and BMP assays, since a control blank containing only the inoculum is run in both cases and subtracted from the samples to yield the net results of the sample.

5.4 Anaerobic Toxicity Assay (ATA) Procedure

McCarty's group at Stanford (Owen et al. 1979) also developed a very useful and simple assay procedure to evaluate the potential toxicity of a wastewater sample to the anaerobic biomass, the anaerobic toxicity assay (ATA). The biomass to be evaluated is placed in a serum bottle and gassed with 50 % CO2 and 50 % CH4; then the wastewater sample is injected in increasing volumes into successive bottles. This procedure results in a range of dilution of the wastewater with the initial inocula of biomass. Excess substrate is also added initially to the serum bottles to avoid substrate limitation. If there is toxicity in the wastewater sample, it will be reflected in a reduced initial rate of gas production in proportion to the volume of wastewater added. Because the aceticlastic methanogens are commonly the most sensitive to toxicity in the consortium, this characteristic can be assayed by adding a surplus of acetate (10,000 mg/L of calcium acetate salt is recommended). More complex substrates such as glucose, ethanol, propionate, or other complex substrates, can be added in excess to assay toxicity to members in the consortia other than methanogens.

The significant difference between the BMP and the ATA assays is that the ATA is flooded with acetate (or other simple substrate noted above) as well as the waste water sample whereas the BMP is not. Also it must be borne in mind that in the ATA assay, the initial rate of gas production is of primary interest, while in the BMP it is the total amount of gas production, which is important. Acclimation phenomena can be observed in both assays as the biomass demonstrates the ability to acclimate to the toxicity. In the BMP if gas production rate (corrected for the control) per unit
volume of wastewater decreases as the amount injected into the bottles increases, this change is also an indication of inherent toxicity in the wastewater.

5.5 Overview

The scope of this chapter is limited to feedstocks consisting mainly the organic fraction of tannery solid and liquid wastes. They are referred to here as industrial biowaste. While this chapter specifically addresses the design of the biomethanization reactor, it should be kept in mind that the latter has many important implications on the need for specific pre and post treatment unit processes. Necessary pre treatment steps may include buffering and diluting the waste with primary sludge / treated waste water from effluent treatment plant. As post treatment steps, the typical sequence involves mechanical dewatering, aerobic maturation, and water treatment but possible alternatives exist such as biological dewatering or wet mechanical separation schemes wherein value added products may be recovered.

A plant treating tannery solids anaerobically is therefore best seen as a complex train of unit processes whereby wastes are transformed into a various intermediate products. Appropriate rating of reactor designs should therefore also address the quantity and quality of these products as well as the need for additional pre and post treatments. These considerations are often decisive factors for the selection of a technology for an actual project. The two main parameters chosen in this chapter to classify the realm of reactor designs are the number of stages and the concentration of total and volatile solids (%TS and VS) in the fermenter because these parameters have a great impact on the cost, performance and reliability of the digestion process.
5.6 Reactors systems based on feeding type and different stages for laboratory scale studies

5.6.1 Batch Systems

In batch systems, digesters are filled once with fresh wastes, with or without addition of seed material, and allowed to go through all degradation steps sequentially in the dry mode, i.e. at 30 – 40% TS. Though batch systems may appear as nothing more than a landfill-in-a-box, they in fact achieve 50 to 100 fold higher biogas production rates than those observed in landfills because of two basic features. The first is that the leachate is continuously recirculated, which allows the dispersion of inoculant, nutrients, and acids, and in fact is the equivalent of partial mixing. The second is that batch systems are run at higher temperatures than that normally observed in landfills.

Batch systems have up to now not succeeded in taking a substantial market share. However, the specific features of batch processes (Table 5.5), such as a simple design and process control, robustness towards coarse and heavy contaminants, and lower investment cost make them particularly attractive for developing countries (Ouedraogo, 1999).

5.6.2 Technical evaluation

The hallmark of batch systems is the clear separation between a first phase where acidification proceeds much faster than methanogenesis and a second phase where acids are transformed into biogas. Three basic batch designs may be recognized, which differ in the respective locations of the acidification and methanogenesis phases.

In the single-stage batch design, the leachate is recirculated to the top of the same reactor where it is produced. This is the principle of the Biocel process, which is implemented in a full-scale plant in Lelystad, The Netherlands, treating 35,000 Ton/yr source-sorted biowaste (ten Brummeler, 1999). The waste is loaded with a shovel in fourteen concrete reactors, each of 480 m³ effective capacity and run in parallel. The leachates collected in chambers under the reactors, are sprayed on the top surface of the fermenting wastes. One technical shortcoming of this and other
batch systems is the plugging of the perforated floor, resulting in the blockage of the leaching process. This problem is alleviated by limiting the thickness of the fermenting wastes to four meters in order to limit compaction and by mixing the fresh wastes with bulking material (one Ton dewatered digested wastes and 0.1 Ton wood chips added per Ton fresh wastes) (ten Brummeler, 1992). The addition of dewatered digested wastes, aside from acting as bulking material, also serves the purpose of inoculation and dilution of the fresh wastes. Safety measures need to be closely observed during the opening and emptying of the batches, as explosive conditions can occur.

### TABLE 5.6 - ADVANTAGES AND DISADVANTAGES OF BATCH SYSTEMS

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical</td>
<td>• Simple</td>
<td>• Clogging</td>
</tr>
<tr>
<td></td>
<td>• Low tech</td>
<td>• Need for bulking agent</td>
</tr>
<tr>
<td></td>
<td>• Robust (no hindrance from bulky items)</td>
<td>• Risk explosion during emptying of reactors</td>
</tr>
<tr>
<td>Biological</td>
<td>Reliable process due to niches and use of several reactors</td>
<td>• Poor biogas yield due to channelling of percolate</td>
</tr>
<tr>
<td>Economical &amp; Environmental</td>
<td>• Cheap, applicable to developing countries</td>
<td>• Small OLR</td>
</tr>
<tr>
<td></td>
<td>• Small water consumption</td>
<td>Very large land acreage required</td>
</tr>
</tbody>
</table>

In the sequential batch design, the leachate of a freshly-filled reactor, containing high levels of organic acids, is recirculated to another more mature reactor where methanogenesis takes place. The leachate of the latter reactor, freed of acids and loaded with pH buffering bicarbonates, is pumped back to the new reactor. This configuration also ensures crossinoculation between new and mature reactors which eliminates the need to mix the fresh wastes with seed material. The technical features of the sequential batch design are similar to those of the single-stage design. Finally, in the hybrid batch UASB design, the mature reactor where the bulk of the methanogenesis takes place is replaced by an upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor, wherein anaerobic microflora accumulates as granules, is well suited to treat liquid effluents with high levels of organic acids at high loading rates (Anderson and Saw, 1992; Chen, 1999). This design is in fact very similar to the two-stage systems with biomass retention such as the Biopercolat
system discussed above, with the difference that the first stage is a simple fill-and-draw (batch) instead of fully mixed design.

5.6.3 Biological performance

In the sequential batch design, the conversion of the acids in a separate mature reactor ensures the rapid depletion of the produced acids, thus a more reliable process performance and less variable biogas composition (O'Keefe et al., 1992; Silvey et al. 1999). At OLR of 3.2 kg VS/m³.d, biogas yields equivalent to 80 - 90% of the maximal yield could be obtained in pilot reactors at 55°C, which is considerably more than the yield reported in the Biocel plant. While the Biocel data were obtained from a full-scale plant treating compacted poorly-structured source-sorted biowaste at 40% TS, the impressive biogas yields reported for the sequential batch design were obtained in pilot plants treating industrial solid waste at 60% TS with high levels of paper and cardboard and low bulk density (280 kg/m³). The coarser structure and lesser degree of compaction of these wastes render these less conducive to the channeling and plugging phenomena responsible for poor biogas yields.

5.6.4 Treatability Protocol

Acclimation of the anaerobic biomass to unfamiliar substrates toxicity has been repeatedly demonstrated. However such a high percentage of success with so many compounds is dependent upon having provided an adequate acclimation period of 30 - 60 days before assaying. Any conclusions drawn concerning anaerobic treatability, before such a time increment has transpired may produce a premature judgement against anaerobic treatability.

The two screenings assays, the BMP and the ATA, are employed to give a preliminary estimate of the potential amount of COD, which can be biotransformed to CH₄ and a preliminary estimate of any toxicity inherent in a wastewater. If unacclimated biomass is assayed, however, the results will not be conclusive and therefore one's interpretation of BMP and ATA data should be tempered with considerable caution. No treatability assay should be curtailed because of negative results from the BMP and ATA.
The BMP and ATA assays yield preliminary results and are not intended to develop design criteria for the prototype. Design criteria should be developed from the operation of a pilot plant, which is as large as possible to minimize scale-up problems. Continuous feeding and even load variations should be simulated. Prime concern is that the reactor configuration of the pilot plant must simulate that of the anticipated prototype. Since the ultimate choice of reactor configuration is often not known, it may be necessary to operate multiple pilot plants to develop the data to make this decision.

In order to establish the appropriateness of anaerobic biotechnology for the treatment of a given industrial wastes, all of the following questions must be answered:

- Is the wastes sample used representative?
- What is the concentration of volatile solids amenable to conversion into CH₄?
- How rapidly can volatile solids be converted to CH₄?
- What are the effluent quality requirements?
- What is the desired temperature of the prototype reactor?
- Does the wastes contain toxicants?
- If so, can the biomass acclimate to the toxicants?
- If so, can the toxicants be biodegraded?
- What is the net yield of biomass production per g volatile solids converted?

5.7 ASSAY TECHNIQUES

5.7.1 Biochemical Methane Potential (BMP)

The 30 day BMP is a first-cut evaluation of the amount of organic pollutant in a waste and wastewater which potentially can be converted to methane. Only the final process design will determine the fraction, which is actually converted in the process. A conventional BMP evaluation of less than 30 days normally will not indicate key considerations such as biofilm or granule development, net biomass yield, kinetic coefficients, trace metal supplementation requirements, and perhaps not even full acclimation characteristics of the biomass. Nevertheless an assay of such short duration can be a valuable piece of data when considering the suitability of anaerobic treatment for a given wastewater.
The BMP assays the amount of the organic contaminant in the wastewater, which can potentially be converted to methane. Whereas the BOD measures the depletion of an oxidized product (dissolved oxygen or D.O.), the BMP measures the production of a reduced product (methane gas). It is important that CO₂ production be excluded, because CO₂ does not represent COD reduction under anaerobic conditions.

Since 395 ml of methane at 35°C is equivalent to 1g of COD removed from the Wastewater, there is a stoichiometric relationship, which allows calculation of the COD reduction in the liquid phase. The anaerobic BMP assay involves placing an measures the aliquot of the candidate wastewater in a 175ml serum bottle which contains approximately 20 - 50 ml of an the inoculum of the anaerobic biomass. If no other source product of anaerobic biomass is available, an inoculum from an anaerobic digester of a municipal wastewater treatment plant can be used.

If the waste and wastewater components are toxic and or unusual, an initial BMP of brief duration will not be valid because an important principle will have been violated in the assay, i.e. the biomass must be acted to the pollutants. In such cases care must be exercised to insure adequate time/acclimation for the biomass to metabolize the pollutant. Whereas a 20-day BOD is considered sufficient time for measurement aerobically, due to the lower growth rates of anaerobic microbes the BMP may necessitate being extended to 30, 60 or even in some cases 90 days, to accommodate acclimation of the biomass to toxic and/or unusual pollutants in the industrial wastewaters.

5.7.2 Anaerobic Toxicity Assay (ATA)

Some organic pollutants such as acrylic acid, acrolein, lipids, and chlorinated organics are biodegradable but will manifest inhibition of the biomass above certain concentrations.

The ATA is conducted in the presence of excess substrate for the class of microorganisms in the consortia, which potentially may be the most inhibited by any inherent toxicity in the wastewater sample. Usually this class is found to be the propionate and/or acetate utilizing class of microbes in the anaerobic consortia. Therefore acetate and propionate are initially supplemented at concentrations which are approximately 10 times their respective Ks which would be approximately 4000 and 2000 mg/L respectively. These concentrations are also below the toxicity threshold for unionized HAc and HPr at pH = 7.0.
Since the biomass activity is at the maximum rate and is independent of substrate concentration under these conditions, any reduction in the rate of gas production would be related to inherent toxicity in the wastewater. The full impact of toxicity in the wastewater is usually muted by the dilution of the sample with the inoculum. This fact must be considered in any interpretation of the results; however if the inoculum should be dewatered granules, there would be no dilution of toxicity.

In order to properly evaluate inhibition, ATA assays can be conducted on a range of ratios of wastewater for a given inoculum of biomass. If inhibition is occurring it will be manifested by a reduced rate of initial gas production becoming progressively lower as the ratio of the aliquot of wastewater per fixed amount of biomass inoculum increases. (Normally the ultimate BMP will not be affected, but the time requirement for the biomass to reach the ultimate BMP can become excessive). Consequently when initially characterizing the anaerobic treatability of wastewaters, care needs to be exercised to avoid the possibility of overlooking inhibition; this caution should also be exercised for a BOD assay.

Differentiating between the fare and the total volume of gas production is necessary because only the volume is indicative of the BMP, whereas the rate is the more critical parameter in the ATA which is supplemented with unlimited substrate concentrations.

5.8 MATERIALS AND METHODS

5.8.1 Experimental Setup

A simple methanogenic activity test procedure was adopted (Isa, et al, 1993; Jawed, M. and Tare, V., 1996; McHugh, S. et al, 2004) with suitable modifications to the requirement of this study. The experimental set-up is shown in Fig.4.14. A known amount of substrates containing mixture of wastes was transferred into a 130 ml (control, BMP1, and BMP2) and 200 ml (BMP3) serum bottles (with working volume of 70, 90, 110 and 130 ml). Appropriate quantities of wastes were mixed and added to the serum bottle so as to obtain an initial VS load in the range of 1.5 - 3.5 g in each reactor (Table 5.7).

Substrate being sourced from animal tissue, no additional nutrients were supplemented to enhance the growth of biomass during the test period (Dolfing and Bloemen, 1985; Soto et al, 1993; John D. Coates, et al, 1996). Total gas production was measured by means of the liquid displacement method at an interval of 24 h after
3 – 5 days of startup period. Contents of the serum bottle were mixed manually, after every gas measurement. Daily gas production was recorded. The entire test was conducted at a temperature 30 ± 3°C for a period of 8 weeks.

5.8.2 Substrate

The substrate used was tannery solid waste (limed fleshing). The fleshing was grounded to less than 6mm diameter using a meat-grinding machine (Make: Wolfking). To this, primary sludge from the wastewater treatment plant of a tannery was added as diluent in the ratio of 1:1 (weight basis) to maintain the fleshing solids in suspension and to achieve the desired level of flowability in the feed mixture. Primary sludge would also act as a source of various microorganisms required for anaerobic digestion process.

5.8.3 Inoculum

Pre-digested material consisting of all the essential microbes (hydrolyzing, fermentative, acetogenic and methanogenic bacterial consortium) for the anaerobic digestion process has been chosen for the study. This predigested material was synthesized in the laboratory using cow dung, limed fleshing and primary sludge in equal weight. After cessation of gas production the predigested material was tested for its activity with known quantity of sodium acetate as substrate for gas production and later the digested residue was used as Inoculum for the study.

5.8.4 Analyses

Several parameters have been suggested in the literature as stress indicators of the anaerobic process. Some of the most commonly used indicators include pH, Chemical Oxygen Demand destruction, Volatile Solids (VS) destruction, Volatile Fatty Acids (VFA) concentration, gas production and gas composition. The early detections of deteriorating condition of the system could not be made by monitoring pH, Volatile Solids reductions and gas composition (Angelidaki and Ahring 1994, Benjamin S. Magbanua, et al 2001). Kinetic studies on raw domestic primary sludge have reported acid phase process in terms of destruction of chemical oxygen demand (COD) of the substrates (Eastman and Ferguson. 1981; Held, C. et al 2002). But in practice, it is actually very difficult to obtain a representative sample from a heterogenous mixture for COD estimation (Jash and Ghosh, 1996; Nopharatana, A., et al, 2003). However, most of these indicators are reported as appropriate for
evaluating the effect of organic loading rate on the bioconversion of the organic substrates and these parameters have been chosen for the substrate investigated.

Total solids (TS), Volatile solids (VS), and Total Volatile fatty acids (steam distillation method) were estimated according to the procedures recommended in the Standard methods for examination of water and waste water (APHA 1992) VFA concentration was determined by Gas Chromatography technique as described by Levet P N et al (1991). Assay bottles were periodically analysed for the above-mentioned parameters for 8 weeks. Daily gas production from reactors was monitored by means of water displacement method. The volume of water displaced from the bottle was equivalent to volume of gas generated at the temperature and pressure prevailed during the study period. The total methane content present in the gas was evaluated by both gas chromatography (Levet P N et al 1991) and alkali scrubbing method, where a known quantity of gas drawn out through a sterile syringe was injected back in liquid displacement system containing strong potassium hydroxide solution. The methane gas volume present in biogas mixture was determined by volume of alkali displaced against the known quantity of biogas injected.

5.8.5 Microscopy

Optical phase contrast, epifluorescence and scanning electron microscopy were performed by applying standard procedures for sample preparation, observation and photographic documentation (Zellner et al, 1993). Total cell counts were estimated using an improved Neubaur chamber (Visser et al, 1991; McHugh, S. et al, 2003).

5.8.6 Experimental procedures and Sampling schedules

Measured quantity of both solid and liquid wastes corresponding to each volatile organic load were added in eight bottles after evaluating the substrate composition (Table 5.7). At the end of every week one bottle for each VS load and its corresponding control bottle were analyzed for various parameters. Thus test reactors for three different organic loading and corresponding Control (pre digested sample) reactors were constructed. The initial VS concentrations in the control and test reactors are given in Table 5.8 A-C.
Table 5.7 - The average composition of Limed fleshing, Primary sludge and Inoculum used in the batch experiments

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Limed Fleshing</th>
<th>Primary Sludge</th>
<th>Pre Digested Sample (Inoculum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>12.10</td>
<td>7.41</td>
<td>8.00</td>
</tr>
<tr>
<td>Total Solids (TS)</td>
<td>13.37%</td>
<td>6.98%</td>
<td>2.45%</td>
</tr>
<tr>
<td>Volatile Solids (VS)</td>
<td>55.60%</td>
<td>35.24%</td>
<td>35.57%</td>
</tr>
<tr>
<td>Oil and grease (crude lipid)</td>
<td>4.79%</td>
<td>2.57%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Protein (crude)</td>
<td>56.5%</td>
<td>28.39%</td>
<td>34.47%</td>
</tr>
<tr>
<td>Volatile Fatty Acid (as acetic acid equivalent)</td>
<td>-</td>
<td>1845 mg/l</td>
<td>360 mg/l</td>
</tr>
</tbody>
</table>

**TABLE 5.8 - VOLATILE SOLIDS PRESENT IN THE REACTORS**

**TABLE 5.8A - BMP STUDIES STUDY PERIOD 54 DAYS** (8 bottles for each VS load, one each form every VS load assayed on every 7th day)

<table>
<thead>
<tr>
<th>Reactors Name</th>
<th>Reactor Volume, ml</th>
<th>Initial VS concentration, g/l</th>
<th>Predigested sample (Inoculum), ml</th>
<th>Lime Fleshing, g</th>
<th>Primary sludge, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>130 ml</td>
<td>8.7</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMP1</td>
<td>130 ml</td>
<td>17.2</td>
<td>70</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>BMP2</td>
<td>130 ml</td>
<td>21.2</td>
<td>70</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>BMP 3</td>
<td>200 ml</td>
<td>26.7</td>
<td>70</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

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TABLE 5.8 B ATA 1 Study period 35 days (5 bottles for each VS load, one each form every VS load assayed on every 7th day)

<table>
<thead>
<tr>
<th>Reactors Name</th>
<th>Reactor Volume, ml</th>
<th>Initial VS concentration, g/l</th>
<th>Predigested sample (Inoculum), ml</th>
<th>Lime Flesching, g</th>
<th>Primary sludge, g</th>
<th>Distilled water, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>130 ml</td>
<td>17.61</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>ATA 2</td>
<td>130 ml</td>
<td>51.96</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.8 C ATA 2 Study period 35 days (5 bottles for each VS load, one each form every VS load assayed on every 7th day)

<table>
<thead>
<tr>
<th>Reactors Name</th>
<th>Reactor Volume, ml</th>
<th>Initial VS concentration, g/l</th>
<th>Predigested sample (Inoculum), ml</th>
<th>Lime Flesching, g</th>
<th>Primary sludge, g</th>
<th>Distilled water, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>130 ml</td>
<td>4.4</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>ATA 1</td>
<td>130 ml</td>
<td>15.8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>70</td>
</tr>
</tbody>
</table>

5.9 RESULTS

5.9.1 Microscopy

Fig. 2 shows the epifluorescence photomicrograph exhibit of the pre digested sample used as inoculum. The study demonstrates the use of fluorescent probes for determinative microscopy of methanogenic archaea (Stahl, D. A., et al. 1995). The total methanogenic population present in pre digested sample was enumerated using a Neuabaur chamber (Chin, K J., et al. 1999; Kuk-Jeong Chin and Peter H. Janssen, 2002). The same sample was plated on simple methanogenic medium under strict anaerobic condition and enumerated for methanogenic population. The sample was found to contain an average of $2.1 \times 10^9$ cells/ml. These counts illustrate that the active methanogenic biomass present in the pre digested sample are sufficient for batch study (Kuk-Jeong Chin and Peter H. Janssen 2002).
5.9.2 BMP STUDY 1, 2 AND 3

5.9.2a Gas Production

The cumulative gas production from each of the test reactors operating at various organic loading of tannery solid wastes and primary sludge is shown in Fig.5.2. The cumulative gas production from test reactor BMP1 (VS concentration of 17.2 g/l) was 648 ml during the 8 week study period and the peak gas production of 34 ml was observed on 32\textsuperscript{nd} day. The gas production almost ceased during the eighth week.

The cumulative gas production from the test reactor BMP2 (VS concentration of 21.2 g/l) was 1,484 ml and a peak gas production of 133 ml was observed on 35\textsuperscript{th} day.

A cumulative gas production of 1860 ml was observed in test reactor BMP3 (VS concentration of 26.7 g/l) during the study period and the peak gas production of 150 ml observed on 46\textsuperscript{th} day.

A gradual shift was observed in the period of peak gas production with increasing initial VS concentration. The specific gas production in terms of volatile solids fed is ranging between 0.419 – 0.635 l/g VS\textsubscript{in}. The methane content in the biogas of the test reactors is shown in Table 5.9. The specific gas production and biogas composition obtained was comparable and show similar trends with published resources (Gavala, H.N.et al. 2003).

<table>
<thead>
<tr>
<th>Reactors</th>
<th>Methane %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP1</td>
<td>71 – 76</td>
</tr>
<tr>
<td>BMP2</td>
<td>75 – 77</td>
</tr>
<tr>
<td>BMP3</td>
<td>72 – 77</td>
</tr>
</tbody>
</table>
Table 5.10 pH and VS concentration of digester contents

<table>
<thead>
<tr>
<th>Reactors</th>
<th>pH</th>
<th>VS concentration, g/l</th>
<th>VS reduction%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Control (C)</td>
<td>8.0</td>
<td>8.0</td>
<td>8.7</td>
</tr>
<tr>
<td>BMP1</td>
<td>8.42</td>
<td>7.78</td>
<td>17.2</td>
</tr>
<tr>
<td>BMP2</td>
<td>8.63</td>
<td>7.83</td>
<td>21.2</td>
</tr>
<tr>
<td>BMP3</td>
<td>8.92</td>
<td>7.79</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Table 5.11 Concentration of Volatile fatty acids (VFA) present in the reactors

<table>
<thead>
<tr>
<th>Reactors</th>
<th>Initial, mg/l</th>
<th>Maximum observed and period, mg/l</th>
<th>Final, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>360</td>
<td>2115 (1-2 week)</td>
<td>1800</td>
</tr>
<tr>
<td>BMP1</td>
<td>2790</td>
<td>4320 (1-2 week)</td>
<td>1800</td>
</tr>
<tr>
<td>BMP2</td>
<td>2880</td>
<td>6210 (1-2 week)</td>
<td>1530</td>
</tr>
<tr>
<td>BMP3</td>
<td>2835</td>
<td>7740 (1-2 week)</td>
<td>1440</td>
</tr>
</tbody>
</table>

5.9.2b VS destruction in BMP reactors

In anaerobic digestion process of solid wastes biogas generation is more specifically related to reduction of biodegradable fraction of VS in the digester. The variation of VS reduction and the cumulative gas production with respect to time are shown in Figs.5.2 – 5.4. Table 5.10 shows initial and final values of pH, VS concentration and VS destruction in the control and BMP test reactors. VS reduction in the BMP reactors was observed in the range of 41 - 52%. These values are comparable with the VS reductions reported in the literature for various substrates (Poggi-Varaldo, et al, 1997, Maibaum and Kuehn, 1999, Mackie and Bryant, 1995, Gavala, H.N.et al, 2003;Song, Y.C. et al, 2004).

The values of VS destruction indicate that the initial VS concentration are high for the reactors BMP 2 and BMP 3 and these reactors achieved only 44.33% and 41.20% respectively when compared to reactor BMP1, which achieved 51.97%.
5.9.2c Volatile fatty Acid Production in the batch reactors

In order to monitor the anaerobic digestion of tannery solid waste the concentration of total volatile fatty acids (VFA) present in the BMP test reactors were estimated at various organic loads. The total VFA concentration in each test reactor is shown in Table 5. The maximum concentration was observed in all reactors during second assay period (14th day), which indicates that the organic acid producers have demonstrated their activity during this period (Gavala, H.N.et al, 2003). The variation of VFA with respect to time for each test reactor is shown in Fig.5.6. It is observed that the concentration of the volatile fatty acids present in the reactors at the end of the study period is in the range of 1440 – 1800 mg/l. The reduction of concentrations of VFA beyond second assay period and simultaneous increase in gas production beyond this period have confirmed the onset of methanogenic activity in the system during second and third week of the study period (Figs.5.7-5.9) (Gavala, H.N.et al, 2003).

5.9.2d Biodegradability of Feed mixture Kinetic Analysis

The refractory fraction in the feed mixture is an indicator of the extent of biodegradability of substrates. It is the portion of the initial VS that remained in the digester as solid retention time (SRT) approached infinity (Morris, et al, 1977; Borja, R. et al, 1995). It was determined graphically from the intercept of the plot drawn between $(S/S_0)$ and $(S_0 \cdot HRT)^{-1}$ where $S =$ substrate concentration (g/l), $S_0 =$ initial VS concentration (g/l), HRT = hydraulic retention time (d). The biodegradability potential of feed mixture was determined from the intercept. The amount of biodegradable present in the feed was in the range of 34 – 43% of the influent volatile solids concentrations (Table 5.12). The biodegradability factor indicates the presence of resistant volatile matter in the major portion of volatile solids in the digester. Higher the concentration of refractory material lower is the destruction efficiency. This is reasonably conformed by the experimentally determined VS destruction efficiency of 41 – 52% in Table 5.12, (Parawira, W.et al., 2004.). Hence, it is essential to monitor the biodegradable fraction of VS in the feed to have better operational control over the process.

The anaerobic digestion process is generally described by first order kinetic model, which is based on the following two factors. (Mahmoud, N. et al, 2004; Parker, W.J., 2005).

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(i) The rate of substrate conversion to biogas is directly proportional to the substrate concentration and

(ii) Volume of gas generated is proportional to the mass of substrate destroyed

\[
dS/dt = -kS \quad \text{--- (1)}
\]

\[
G = C.V(S_0 - S) \quad \text{--- (2)}
\]

\[
S = \text{Final substrate concentration, g/l}
\]

\[
S_0 = \text{Initial substrate concentration, g/l}
\]

\[
G = \text{Cumulative gas production, l}
\]

\[
V = \text{Volume of reactor, l}
\]

\[
C = \text{Yield constant, l/g}
\]

\[
k = \text{Rate constant, d}^{-1}
\]

For a batch reactor the substrate remaining in the digester is given by integrating (1)

\[
S = S_0 \exp(-k(t-t_0)) \quad \text{for } t > t_0 \quad \text{--- (3)}
\]

\[
t_0 = \text{lag time, d}
\]

This model (Fulford, 1988; Edeline F, 1980; Tong X., et al, 1990; McCarty P.L. and Mosey F.E., 1991) describes the average reactor behaviour at a longer retention time. Substituting \(S\) from Equation (3) in Equation (2), the cumulative gas production can be predicted by

\[
G = CVS_0 [1-\exp(-k(t-t_0))] \quad \text{--- (4)}
\]

Rearranging by taking natural logarithm gives

\[
\ln(1 - G/(C.V.S_0)) = -kt + kt_0 \quad \text{--- (5)}
\]

Knowing the yield constant (C), plot of \(\ln(1 - G/(C.V.S_0))\) vs time gives slope of \(-kt\) and intercept of \(kt_0\) from which the rate constant and lag time can be calculated. An example of the graphical analysis of the data for the reactor BMP1 is shown in
Fig. 5.10. The yield constant, rate constant and 'lag time' for various initial VS concentrations are given in Table 5.13. The lag time actually observed for the onset of maximum gas generation was more or less in the same period as predicted by using equation (5) (Table 5.13). The day on which onset of maximum gas generation observed for the reactor BMP1 is shown in Fig. 11.

5.10 Discussion of BMP Studies

The performance of anaerobic co-digestion of tannery fleshing and primary sludge has been evaluated in terms of VS destruction, biogas yield and methane content. Based on the experimental data the following conclusions have been drawn in support of biomethanation potential of the selected solid wastes and substrate specific kinetics of the process.

1. As the inoculum was acclimatized with the substrates taken for the study and sufficient active biomass was present in the inoculum the start-up of the reactor has been achieved easily. It is confirmed based on VS destruction efficiencies and specific gas production in terms of quantity of VS fed that fleshing and primary sludge are amenable for anaerobic treatment for the recovery of biogas with high methane content.

The batch reactors were operated with initial VS concentrations of 17.2, 21.2, and 26.7 g/l, and the corresponding specific gas production obtained in terms of volatile solids fed was 0.419, 0.635 and 0.535 l/g VS\textsubscript{f}. The VS destruction efficiency was 51.97%, 44.33% and 41.19%. Methane content in the biogas was varying between 71 – 77% (Poggi-Varaldo, et al, 1997, Maibaun and Kuehn, 1999, Mackie and Bryant, 1995, Gavala, H.N. et al, 2003; Song, Y.C. et al, 2004).

2. The maximum concentrations of VFA observed during first two weeks indicate that the organic acid producers in the inoculum were sufficiently active. The rapid consumption of VFA observed in the subsequent period, confirms the adequacy of methanogenic activity of methanogens present in the reactor (Poggi-Varaldo, et al, 1997, Maibaun and Kuehn, 1999, Mackie and Bryant, 1995, Gavala, H.N. et al, 2003; Song, Y.C. et al, 2004).

3. The kinetic analysis of the data fit in first order reaction mechanism. First order kinetic model is adequate to describe the anaerobic co-digestion of tannery solid waste and primary sludge (Edeline F, 1980, Eastman and Ferguson, 1981, Fulford,

4. The composition of substrates indicates that the major constituents of volatile solids of the feed mixture are fat and protein. Hence, the efficiency of anaerobic co-digestion of limed fleshing and primary sludge depends on the biodegradability nature of fats and proteins present in the substrates. It is observed that the larger the refractory fraction present in the feed mixture longer the lag time for the onset of biogas generation. The ranges of lag time obtained from the equation (5) for various initial VS concentrations fit into the actually observed time period of onset of maximum gas generation (Morris, et al, 1977; Borja, R. et al, 1995 Parawira, W. et al., 2004).

**Table 5.12 - Biodegradability of VS**

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Refractory fraction of VS</th>
<th>Biodegradable fraction of VS</th>
<th>% VS destruction based on total VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP1</td>
<td>0.5719</td>
<td>0.4281</td>
<td>52</td>
</tr>
<tr>
<td>BMP 2</td>
<td>0.6597</td>
<td>0.3403</td>
<td>44</td>
</tr>
<tr>
<td>BMP 3</td>
<td>0.623</td>
<td>0.3770</td>
<td>41</td>
</tr>
</tbody>
</table>

**Table 5.13 - Kinetic constants**

<table>
<thead>
<tr>
<th>Reactors</th>
<th>C, ml/kg</th>
<th>k, d⁻¹</th>
<th>R²</th>
<th>t₀, d (from Eqn.5)</th>
<th>t₀, d (actually observed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP1</td>
<td>711</td>
<td>0.0193</td>
<td>0.986</td>
<td>5.4</td>
<td>4</td>
</tr>
<tr>
<td>BMP 2</td>
<td>1160</td>
<td>0.0204</td>
<td>0.953</td>
<td>16.3</td>
<td>13</td>
</tr>
<tr>
<td>BMP 3</td>
<td>765</td>
<td>0.0207</td>
<td>0.936</td>
<td>6.8</td>
<td>6</td>
</tr>
</tbody>
</table>
FIG 5.1 EPIFLUOROSCENCE MICROGRAPH OF METHANOGENS PRESENT IN THE DIGESTED SAMPLE
FIG.5.4 - BMP 2 - VARIATION OF VS DESTRUCTION AND CUMULATIVE GAS PRODUCTION WITH TIME

![Graph showing variation of VS destruction and cumulative gas production with time.](image-url)
FIG. 5.5 - BMP 3 - VARIATION OF VS DESTRUCTION AND CUMULATIVE GAS PRODUCTION WITH TIME

![Graph showing the variation of VS destruction and cumulative gas production with time.](image-url)
FIG. 5.6 - VARIATION OF VFA CONCENTRATION VS. TIME

VFA concentration, mg/L

Time, d
FIG. 5.7 - BMP 1 - WEEKLY VARIATION OF CUMULATIVE GAS PRODUCTION AND VFA
FIG. 5.8 - BMP 2 - WEEKLY VARIATION OF CUMULATIVE GAS PRODUCTION AND VFA

![Graph showing weekly variation of cumulative gas production and volatile fatty acids. The x-axis represents time in days (0 to 54), and the y-axes represent cumulative gas production in ml (0 to 1600) and volatile fatty acids in mg/l (0 to 7000). The graph includes two lines: one for gas production and another for volatile fatty acids (VFA).]
FIG. 5.9 - BMP 3: WEEKLY VARIATION OF CUMULATIVE GAS PRODUCTION AND VF-A

Volatile fatty acids, mg/l

Cumulative gas production

Time, d

Gas production

VF-A
FIG. 5.10 - BMP1 - PLOT OF LOGARITHMIC GAS PRODUCTION VS. TIME

\[ y = -0.0193x + 0.1042 \]

\[ R^2 = 0.8889 \]
5.11 ATA STUDY 1 & 2
Gas production and its composition

The cumulative gas production for a period of five weeks was 145 ml in the
ATA 1 and 35 ml in the ATA 2 (Figure 5.12). The specific gas production was 27.9
l/kg VS_in in ATA 1 and 22 l/kg VS_in in ATA 2 (Table 5.13). VS destruction was 35%
in test reactor 1 and 23% in ATA 2. Total gas production per kg of VS destroyed was
80 l in test reactor 1 and 95 l in ATA 2 (Table 5.13). The weekly variation of VS
destruction and cumulative gas production are shown in Fig.5.12. Methane content
was in the ranges of 71 - 77% in both the test reactors during the study period. The
biogas composition obtained were comparable with the published resources (Gavala,
et al, 2003).

5.11.1 VS Conversion Efficiency in ATA reactors
Biogas generation is more specifically related to reduction of biodegradable fraction
of VS in the digester. The variation of VS reduction and the cumulative gas
production with respect to time are shown in Figs.5.14 Table 5.13 shows initial VS
concentration and % VS destruction in the ATA 1 and 2 reactors. VS reduction in the
ATA 1 and 2 was observed 23 and 35 % respectively.

The lower concentration of biodegradable fraction present in the feed mixture,
lower VS destruction efficiency and lower specific gas production for the detention
time of five weeks indicate that the rate appears to be defined by rate of conversion of
simple molecules in the feed mixture into methane and methanogenesis seems to be
the rate limiting step under the specified experimental conditions.

5.11.2 Concentration of VFA

The toxic effects of high VFA concentration on the anaerobic digestion
process have been reported by several authors (Ahling and Westermann 1988; Gorris
et al 1989). The VFA concentration was 20,385 and 7,425 mg/l in ATA 1 and 2 respectively after one week and decreased to 14,355 and 5,310 mg/l at the end of five weeks. The weekly variation of VFA and cumulative gas production are shown in Fig.5.14. In the ATA 1 with higher loading rate (51.9g/l), VFA concentration was very high whereas in the ATA 2 (15.8g/l), the VFA level was relatively lower (Table 5.14).

From the many different levels of VFA concentrations reported in the literature (Young-Chae et al, 2004; Chulhwan Park, et al 2005) for different substrates, it could be concluded that it is not feasible to define an absolute VFA level indicating the state of the process. Stable anaerobic systems have their own “normal” levels of VFA, according to the nature of the constituents of the substrates digested and the operating conditions (Angelidaki et al 1993).

Time required for the conversion of biodegradable organic fraction of substrates into products of hydrolysis and fermentation and subsequently into that of acetogeneis varies from one substrate to another. During methanogenesis, conversion of certain volatile fatty acids (propionate, butyrate) is very slow, resulting in accumulation of fatty acids in the digester.

The composition of methanogenic population in an anaerobic digester and its response to changes in the organic loading and physical and chemical factors have been reported (Visser et al, 1991). However, the microbial interactions and the mechanism of growth of microbial consortia are still not completely known.

5.11.3 Evaluation of biodegradability nature and kinetic constant

The experimental results were evaluated in terms of biodegradability of mixture of wastes and performance of ATA. In the biodegradability estimation of the mixture of wastes, the refractory fraction was determined from the portions of VS that
remained in the reactor as HRT approached infinity. The refractory fraction was
determined graphically as the intercept of the plot of \((S/S_0)\) vs \((S_0\cdot HRT)^{-1}\) for the two
substrate concentrations studied. The refractory fraction in the ATA 1 was found as
0.64 and in the test reactor 2 was 0.75. The biodegradable fraction present in the
mixture of wastes studied was ranging between 25 - 35% of the influent VS
concentration.

Regression analysis of the experimental data with first order kinetic model described
earlier gives \(k\) & \(t_0\) for the mixture of waste used in the study. The first order rate
constant \(k\) and lag time \(t_0\) for the mixture of wastes determined from the Figures 5.5 &
5.6 are shown in Table 5.4. There seems to be good agreement of the first order
kinetic rate constant obtained in the present study with the values reported for pig
dung and cattle dung using first order kinetic model (Fulford, 1988).

5.12 Conclusions

The anaerobic digestion of mixture of fleshing and primary sludge reasonably
fits in first order kinetic model. The mixture of fleshing and primary sludge is found
to contain very low concentration of biodegradable volatile solids. This is confirmed
by the rather low observed VS destruction efficiency of 23% and 35% and very low
specific gas yields. The rapid increase of VFA in ATA 1 is attributed to higher VS
load applied in the reactor. However, the presence of higher fraction of biodegradable
VS in the ATA 1 contributes towards higher gas production as compared to ATA 2.
It is also observed that higher the refractory matter content in the substrate, longer is
the lag time observed for the onset of maximum gas production. The predicted and
observed lag time are found to be closely related to biodegradable fraction present in
the substrates.
Hence, it is more appropriate to consider available biodegradable fraction of VS rather than total VS for evaluating biomethanation potential of the substrates containing predominantly lipids.

Table 5.14 – Performance of test reactors

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>VS load, g/l</th>
<th>Cumulative gas, ml</th>
<th>VFA, l week, mg/l</th>
<th>VFA, V week, mg/l</th>
<th>VS&lt;sub&gt;a&lt;/sub&gt;, %</th>
<th>Sp. gas production, l/kg VS&lt;sub&gt;in&lt;/sub&gt;</th>
<th>Sp. gas production, l/kg VS&lt;sub&gt;d&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATA 1</td>
<td>51.9</td>
<td>145</td>
<td>20385</td>
<td>14355</td>
<td>35</td>
<td>27.9</td>
<td>80</td>
</tr>
<tr>
<td>ATA 2</td>
<td>15.9</td>
<td>35</td>
<td>7425</td>
<td>5310</td>
<td>23</td>
<td>22.0</td>
<td>95</td>
</tr>
</tbody>
</table>

VS<sub>in</sub> : Volatile solids in the feed, VS<sub>d</sub> : Volatile solids destroyed

Table 5.15 - Refractory and Biodegradable fraction in the feed and kinetic constant

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Refractory fraction of VS</th>
<th>Biodegradable fraction of VS</th>
<th>C, ml/kg VS</th>
<th>k, d⁻¹</th>
<th>R²</th>
<th>t₀, d (predicted by Eqn.5)</th>
<th>t₀, d (actually observed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATA 1</td>
<td>0.64</td>
<td>0.35</td>
<td>52</td>
<td>0.023</td>
<td>0.967</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>ATA 2</td>
<td>0.74</td>
<td>0.25</td>
<td>54</td>
<td>0.018</td>
<td>0.984</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>
FIG. 5.12 - CUMULATIVE GAS PRODUCTION

Gas production, ml

Time, d

ATA 1
ATA 2
FIG. 5.13 - WEEKLY VARIATION OF VFA AND CUMULATIVE GAS PRODUCTION
FIG. 5.14 - WEEKLY VARIATION IN VS DESTRUCTION AND CUMULATIVE GAS PRODUCTION

- VS - ATA 1
- VS - ATA 2
- Gas - ATA 1
- Gas - ATA 2
FIG. 5.15 - PLOT OF LOGARITHMIC GAS PRODUCTION VS. TIME

VS load = 51.9 g/l

$y = -0.0239x + 0.08$

$R^2 = 0.9676$
FIG 5.16 - PLOT OF LOGARITHMIC GAS PRODUCTION VS. TIME

\[ y = 0.0186x + 0.102 \]

\[ R^2 = 0.9945 \]

\( V/S \) load = 15.8 g/l