CHAPTER 6
STUDIES ON TWO STAGE FED BATCH SYSTEM

6.1 Introduction

The rationale of two- and multi-stage systems is that the overall conversion process of tannery solid and liquid waste to biogas is mediated by a sequence of biochemical reactions, which do not necessarily share the same optimal environmental conditions. Optimising these reactions separately in different stages or reactors may lead to a larger overall reaction rate and biogas yield (Ghosh et al., 1999). Typically, two stages are used where the first one harbours the liquefaction-acidification reactions; with a rate limited by the hydrolysis, and the second one harbours the acetogenesis and methanogenesis, with a rate limited by the slow microbial growth rate (Liu and Ghosh, 1997; Palmowski and Müller, 1999). With these two steps occurring in distinct reactors, it becomes possible to increase the rate of methanogenesis by designing the second reactor with a biomass retention scheme or other means (Weiland, 1992; Kübler and Wild, 1992). In parallel, it is possible to increase the rate of hydrolysis in the first stage by using microaerophilic conditions or other means (Capela et al., 1999; Wellinger et al., 1999). The application of these principles has led to a great variety of two-stage designs.

The increased technical complexity of two-stage relative to single-stage systems has not however always been translated in the expected higher rates and yields (Weiland, 1992). In fact, the main advantage of two-stage systems is not a putative higher reaction rate, but rather a greater biological reliability for wastes, which cause unstable performance in one-stage systems. It should be noted however that, in the context of industrial applications, even for the challenging treatment of highly degradable biowastes, preference is given to technically simpler one-stage plants. Biological reliability is then achieved by adequate buffering and mixing of incoming wastes, by precisely controlled feeding rate and, if possible, by resorting to co-digestion with other types of wastes (Weiland, 2000). Industrial applications have up to now display little acceptance for two-stage systems as these represent only 10 % of the current treatment capacity (De Baere, 1999).
A distinction is made in this chapter between two-stage systems with different organic loading rate. The reason for using this criterion is to determine the biological stability of the digester. Unstable performance can be caused either by fluctuations of OLR, due to wastes heterogeneity or discontinuous feeding, or by wastes excessively charged with inhibiting substances such as nitrogen. All types of two-stage systems, provide some protection against the fluctuations of OLR. However, only those two-stage systems with biomass retention schemes display stable performance with wastes excessively charged with nitrogen or other inhibitors (Weiland, 1992). Most commercial two-stage designs propose a biomass retention scheme in the second stage.

TABLE - 6.1 ADVANTAGES AND DISADVANTAGES OF TWO-STAGE SYSTEMS

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Technical</td>
<td>Design flexibility</td>
<td>Complex</td>
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<td>Biological</td>
<td>More reliable for cellulose-poor kitchen waste.</td>
<td>Smaller biogas yield.</td>
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<td>Only reliable design for C/N &lt;20.</td>
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<tr>
<td>Economical &amp; Environmental</td>
<td>Less heavy metal in compost</td>
<td>Large investment</td>
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6.2 Technical Evaluation

In order to increase rates and resistance to shock loads or inhibiting substances, it is desirable to achieve high cell densities of the slowly-growing methanogenic consortium in the second stage. There are two basic ways to achieve this.

The first method to increase the concentration of methanogens in the second stage is to uncouple the hydraulic and solids retention time, thereby raising the solid content in the methanogenic reactor. These accumulated solids represent active biomass only in the case of wastes leaving no more than 5-15% of their original solid content as residual suspended solids inside the reactor. This design will therefore be
effective only for highly hydrolyzable kitchen or market wastes (Weiland, 1992; Madokoro et al., 1999). One way to uncouple the solid and hydraulic retention times is to use a contact reactor with internal clarifier (Weiland, 1992). Another way is to filter the effluent of the second stage on a membrane and return the concentrate in the reactor in order to retain the bacteria (Madokoro et al., 1999). Plugging of the microfiltration membranes can be avoided using a high cross-flow velocity achieved via reinjection of biogas. Excessive biomass was purged in a separate outlet line. Further upscaling of these two interesting designs, which up to now could only be tested in small pilot plants, may face technical challenges such as the crushing of the feed down to 0.7 mm. Another method to increase the concentration of slowly growing methanogens in the second stage is to design the latter with support material allowing attached growth, high cell densities and long sludge age. The prerequisite of this design avenue is however that the feed to the attached growth reactor be very little charged with suspended particles, which means that the suspended solids remaining after the hydrolysis (first) stage should be removed. Two industrial processes, the BTA and Biopercolat designs, are based on these principles.

In the BTA 'wet-wet' process, the 10 % TS pulp exiting the pasteurization step is dewatered and the liquor directly sent to the methanogenic reactor (Kübler and Wild, 1992). The solid cake is resuspended in process water and hydrolyzed in a complete mix reactor under mesophilic conditions (HRT 2-3 d). The pH within the hydrolysis reactor is maintained in the range 6-7 by recirculating process water from the methanogenic reactor. The output stream of the hydrolysis reactor is once more dewatered and the liquor fed to the methanogenic reactor. The latter, receiving only liquid effluents, is designed as a fixed film loop reactor in order to increase biomass concentration and age. From a technical point of view, this design shares the same limitations as the one-stage 'wet' system, i.e. short-circuiting, foaming, sinking of heavies, fouling of the impeller blades with plastic foils, obstruction of pipes with long objects such as sticks, and loss of 10-30 % of the incoming VS caused by the removal of the rake fraction in the hydropulper (Kübler and Wild, 1992). The major drawback of the 'wet-wet' system remains however its technical complexity as four reactors are necessary to achieve what other systems achieve in a single reactor. The Biopercolat follows the same principles as the BTA process, with the difference that the first stage is carried out under 'dry' and microaerophilic conditions and is
continuously percolated with process water to accelerate the liquefaction reaction (Edelmann et al., 1999; Wellinger et al., 1999). The flush water, containing up to 100 g COD/l, is fed to an anaerobic plug-flow filter filled with a support material. The separate optimization of the first stage, via aeration, and of the second stage, via biofilm growth, allows the system to run at the exceedingly low overall retention time of 7 days. The Biopercolat system is quite innovative from a technical point of view. In order to prevent the channeling and clogging typically occurring in 'dry' percolated systems, percolation occurs in large slowly-rotating (1 rpm) sieve drums with 1 mm mesh openings. In the methanogenic filter, a pulsating motion is imparted to the horizontal plug flow.

6.3 Biological Performance of Two-Stage System

The main advantage of the two-stage system is the greater biological stability it affords for very rapidly degradable wastes like fruits and vegetables (Pavan et al., 1999). The reason commonly invoked is that the slower metabolism of methanogens relative to acidogens would lead to inhibiting accumulation of acids. Theoretically, however, this reasoning seems illogical as it would suffice to adjust the OLR of a one-stage system to the rate which can be handled by the methanogens to avoid any risk of acid accumulation. The OLR chosen in this manner for a one-stage system would not be inferior to that of a two-stage system.

In the practice, however, the greater reliability of two-stage systems has indeed at times been observed, at least in discontinuously-fed laboratory set-ups. For example, Pavan et al. (1999) compared the performances of the one- and two-stage systems, using pilot complete mix reactors fed with very rapidly hydrolysable bio wastes from fruit and vegetable markets. While the one-stage system failed at 3.3 kg VS/m³.d, the performance of the two-stage plant remained stable at an overall system OLR of 7 kg VS/m³.d. This departure from theoretical predictions can be explained by the fact that actually applied OLR vary a great deal with time and space due to the heterogeneity of wastes and due to the discontinuous feeding. In cases where special care is taken to mix the feed thoroughly and dose it at constant OLR, and performance of the two stage systems well suited for highly degradable agroindustrial wastes, provided these have a C/N above 20 (Weiland, 1992).
The short-lived fluctuations of the actually applied OLR may lead to short-lived overloading in the one-stage system. In a two-stage system, however, these OLR fluctuations are somewhat buffered by the first stage, so that the OLR applied to the second stage is more uniform in time and space. In fact, this buffering of OLR in the first stage is somewhat similar to the effect of the plug flow pattern often used in the one-stage 'dry' systems because a plug flow with external mixing leaves large zones in the digester unexposed to transient high concentrations of inhibitors. Highly biodegradable kitchen wastes can indeed be digested in single-stage reactors provided these are thoroughly mixed before feeding and provided feeding occurs continuously, or at least five days per week as in the one-stage 'dry' Dranco plant in Salzburg, Austria. This plant, which treats kitchen wastes, achieves a mean OLR of 5.0 kg VS/m$^3$.d with 80% VS destruction.

As pointed out by Edelman et al. (1999), the OLR buffering taking place in a pre-digester is beneficial and useful only for the treatment of cellulose-poor wastes for which methanogenesis rather than hydrolysis-acidification is the rate-limiting step. For the majority of wastes, however, hydrolysis of cellulose is the rate-limiting step (Noike et al., 1985), and shock loads are not conducive to inhibition.

The second type of inhibition, resulting from unbalanced average composition of feed rather than from transient shock load, is, however, as deleterious to two-stage systems as it is to one-stage systems, except in cases where two-stage systems are equipped with a biomass retention scheme in the second stage, e.g. via attached growth on a fixed bed.

In terms of biogas yields and OLRmax, little difference can be noted between one- and two-stage systems, at least for these two-stage systems without biomass retention discussed in this section. For example, the BRV plant in Heppenheim is designed with an OLR of 8.0 kg VS/m$^3$.d while the Schwarting-Uhde process seems to sustain an OLRmax up to 6 kg VS/m$^3$.d (Trösch and Niemann, 1999).
6.4 High Rate Systems and Retentions of Biomass Concentration

As a consequence of the higher biomass concentration in two-stage designs with attached growth, greater resistance toward inhibiting chemicals is achieved. Weiland (1992) compared one- and two-stage 'wet' pilot plants for the treatment of highly biodegradable agro-industrial wastes. While the one-stage system failed at OLR of 4 kg VS/m$^3$.d for those wastes, which yielded 5 g NH$_4^+$/l due to ammonium inhibition, the same wastes could be processed in the two stage system at OLR of 8 kg VS/m$^3$.d without impairment of methanogenesis.

The stability of the methanogenesis at such elevated ammonium concentration was attributed to the higher bacterial concentration and age which could be obtained in the contact reactor with internal clarifier used in the second stage.

Another consequence of two-stage systems with biomass retention is the possibility of applying higher OLR in the methanogenic reactor, with values up to 10 and 15 kg VS/m$^3$.d reported for the BTA and Biopercolat processes, respectively (Kübler and Wild, 1992; Wellinger et al., 1999). These relatively high rates were however only achieved at the cost of 20-30 % lower biogas yields, due to the fact that the coarse solid particles remaining after the short hydrolysis stage, which still contain residual biodegradable polymers, are not fed to the methanogenic digester (Kübler and Wild, 1992; Garcia and Schalk, 1999).

6.5 MATERIALS AND METHODS

6.5.1 Experimental Protocol and Analysis

Tannery and effluent treatment plant solid waste samples were analyzed at least once a week or once a new sample was received. Samples from the acidogenic and methanogenic reactors of the two-stage system were collected twice a week. The samples were collected from an effluent sampling port, located on the top for hydrolysis reactor and two ports at 7 and 23 centimeters below the liquid surface in methanogenic reactor. The feed and effluent samples from hydrolysis and methanogenesis reactors were analyzed for pH, Total Solids (TS), Volatile Solids (VS), Volatile Fatty Acids (total VFA), in accordance with Standard Methods (APHA
et. al. 1998). The volume of biogas produced by each reactor was measured daily by water displacement technique and biogas methane content was analysed by both alkali scrubbing technique and Gas chromatography (GC) procedure periodically.

6.6 Results and Discussion

6.6.1 Two-Stage System

The two stage system was operated to evaluate the effect of phase separation on the treatment of Tannery solid and liquid wastes. In this section the data collected from the acidogenic and methanogenic reactors of the two stage system are reported and performance of the each stage of the system has been correlated with the overall performance of the two stage system.

6.6.1a Acidogenic Reactor

The objective of the acidogenic reactor was to acidify the tannery solid and liquid wastes in an effort to improve the performance of the methanogenic reactor by increasing the TS and VS removal efficiencies and methane yield. In addition, it was hypothesized that the acidogenic reactor may also reduce the effect of shock loadings to the methanogenic reactor, increasing the stability of the two stage system. The acidogenic reactor was batch fed with tannery solid and liquid wastes and was operated on a 10-day cycle for a total period of 123 days.

6.6.1b Reactor Operation

Operating conditions for the acidogenic reactor were set based on several studies discussed in the literature evaluating the performance of acid-phase reactors treating various synthetic and raw dairy wastewaters (Fang et. al. 2000; Ince, 1998; Kasapgil et. al. 1995; Yilmazer et. al. 1999). These studies evaluated the effects of pH, HRT and temperature on substrate degradation, degree of acidogenic conversion from COD to VFAs, changes in the distribution of major VFAs produced, methane and hydrogen yield.

It was suggested that the degree of acidification and the biodegradability of the carbohydrates, proteins and lipids in industrial solid wastes increased with HRT (Fang et. al. 2000).
The acidogenic reactor in this study was operated at a HRT of 10 days and the feed volume was determined based on the desired HRT. A HRT of 10 days was chosen due to the high organic content (average VS was 10.4 g/l) of the raw waste and wastewater compared to the studies reported in the literature. The pH in the hydrolysis reactor was 7 ± 1. The pH of the reactor contents was maintained between 7 and 8 as suggested by Kasapgil et. al. (1995).

6.6.1c Removal Efficiencies

The total solids and volatile solids contents profile in the feed and the drain of the hydrolysis process are shown in Fig. 6.1 and Fig. 6.2. The average TS and VS concentrations in the feed were 17.68 g/l and 10.4 g/l, respectively and the average drain TS and VS concentrations were 12.84 g/l and 7.10 g/l, respectively. Due to the low SRT maintained in the acidogenic reactor, little solids destruction has occurred in the reactor. The low SRT was maintained to promote the washout of the active methanogenic microorganisms to the methanogenic reactor, otherwise at a higher SRT, greater solids destruction and methane production could take place.

6.6.1d VFA Production

The six main VFAs measured in the effluent were acetic, propionic, and butyric (n, iso), and valeric (n, iso) accounting for more than 95% of the total VFA concentration. Acetic, propionic, and butyric acids are reportedly the main VFAs identified in acid-phase reactors treating dairy wastewaters (Fang et. al. 2000; Ince, 1998; Kasapgil et. al. 1995; Yilmazer et. al. 1999). Although these VFAs are detected in varying concentrations depending on operating conditions, they are consistently detected at a more significant concentration than any other VFA.

Fang et. al. (2000) discussed the products of two common types of acidogens typically found in the acid-phase of anaerobic digestion systems; the first produces butyrate, acetate, carbon dioxide and hydrogen, and the second produces propionate, acetate, and some valerate with no significant biogas production. Neither of these two acidogenic microorganisms has been found to produce ethanol. Later Fang et. al. (2000) also described a third type of acidogenesis that produces ethanol, acetate,
hydrogen and carbon dioxide at pH of less than 4.5. Based on the average VFA distribution in the acidogenic reactor, it appears that all three types of acidogens were present due to the identification of acetic, propionic, butyric and valeric acid. Since the biogas was not analyzed for carbon dioxide and hydrogen content, and the effluent concentration of alcohols (i.e., methanol, ethanol, etc.) were not quantified; it is difficult to rule out any one of the three types of acidogenic microorganisms. Therefore, it is possible that all three acidogenic microorganisms were present in the acidogenic reactor in this study.

Figure 6.3 shows the OLR and corresponding total VFA concentration prevailed in the acidogenic reactor effluent over the duration of the study. It appears that the effluent total VFA concentration was sufficiently high to use as feed to the second stage and suggests that the acidogenesis of tannery solid and liquid wastes was rapid for HRT of 10 days. The maximum concentration of total VFA observed in the acidogenic reactor was 18225mg/l. The average VFA concentration was 10574 mg/l. Although the goal of the acidogenic reactor was to hydrolyze the tannery solid and liquid wastes and generate significantly high VFA concentration in a short HRT, it appears that the acidogenic reactor performed more like an equalization tank than an acid-phase reactor since the fraction of easily biodegradable matter present in the feed is low.

6.6.1e Biogas and Methane Production

The daily and cumulative gas production are shown in Figure 6.4 and Figure 6.5 The acidogenic reactor produced an average of approximately 184 ml of biogas per day, of which the methane content was insignificant (less than 10%). It was expected that the acidogenic reactor would not create a significant amount of methane, but in the literature methane percentages of 5 to 15 and 7 to 27 in the biogas generated from acidogenic reactor have been reported (Ince, 1998; Kasapgil et. al. 1995). The biogas created by the acidogenic reactor, in this study, is found to contain methane in the range of 5 to 10 % and the balance composed of mainly carbon dioxide and very low concentration of hydrogen. The ammonia concentration in the acidogenic reactor was well below the toxic limits (5000 mg/L) and does not
contribute in the biogas composition and the performance of the found to be better. The average specific gas production is 0.02 l/g VS fed.

6.6.2 Methanogenic Reactor

The acidogenic reactor was operated to promote the growth of acid-forming microorganisms, while the methanogenic reactor was operated to promote the growth of methane-forming microorganisms. The methanogenic reactor was batch fed with acidified Tannery solid wastes from the acidogenic reactor and was operated on a 20 day cycle for a total of 123 days.

6.6.2a Reactor Operation

The methanogenic reactor was operated at various OLRs and the feed volume was set based on the concentration of VS in the drain from acetogenic reactor and the desired OLR. The methanogenic reactor HRT was an average of 20 days, but the HRT varied depending on the OLR. To acclimate the biomass to the tannery solid waste, the OLR was changed gradually and steadily to avoid shock loading. The operation of the methanogenic reactor can be divided into three periods with fairly consistent OLRs for the purposes of analyzing the data and generating relationships between various parameters and the OLR.

The pH of the reactor contents ranged from 7.6 to 8.3 and with an average pH of 7.7± 0.5 over the duration of the study. Since the pH of the methanogenic reactor was above 7.5 and below 8.5 throughout the entire study, pH control was not necessary.

6.6.2b Removal Efficiencies

The influent and effluent concentrations, and performance, in terms of TS, and VS percent removal over the duration of the study is presented in Figure 6.6 and Figure 6.7. It appears that reasonably high TS and VS removal rates were achieved.
At the beginning of the study, the average OLR was 1.25 kg VS/m³-day, the average TS and VS removal rates both increased at this OLR at an average of 36.79% and 20.22%, indicating that as OLR decreases, the TS and VS removal efficiencies increase. On day 76, the OLR was maintained to an average of 1.23 kg VS/m³-day for approximately 7 days. On day 109, the TS and VS removals decreased to 28.77% and 35.63%, respectively. This decrease in TS and VS removal may be due to increase in OLR. The TS and VS removal efficiencies of TS and VS are 40% and 46% respectively despite the changing OLR.

6.6.2c VFA Conversion

Figure 6.8 shows the variation of VFA concentration with in the reactor for various OLRs. Initially VFA was gradually increasing and reached a steady state after 40 days of operation. The average concentration of VFA in the reactor during the study period was 8987 mg/l. There is a steady stage observed beyond 40 days indicating a dynamic balance between VFA conversion and biogas generation and stabilization of the methanogenesis process.

6.6.2d Biogas and Methane Production

Methane production rates for methanogenic reactors in two stage systems digesting synthetic, dilute or raw industrial solid waste can be found in the literature (Ghaly et. al. 2000; Lo et. al. 1986; Strydom et. al. 1997; Yilmazer et. al. 1999). Ghaly et.al. (2000) observed a biogas yield of 0.05 and 0.1 m³/kg VSS added from a methanogenic reactor of a two stage anaerobic digester fed with a raw cheese whey wastewater. Yilmazer et. al. (1999) treated a synthetic whey substrate in an upflow anaerobic filter and reported a biogas production rate of 0.55 m³/kg COD removed. Evaluating the performance of a two stage anaerobic hybrid reactor treating cheese, milk and butter factory wastewaters, Strydom et. al. (1997) reported methane production rates of 0.36, 0.33, and 0.29 m³/kg COD removed, respectively. And Lo et al. (1986) observed a methane yield of 0.15 and 0.17 m³/kg VS added treating a industrial wastewater in an anaerobic rotating biological contactor (ARBC) reactor. In addition to methane production rates, methanogenic reactor biogas methane percentages of 52%, 67%, and 75% to 80% have been reported in the literature (Ghaly
et. al. 2000; Ince 1998; Strydom et. al. 1997). It was also found in methanogenic reactor, where the ammonia concentration was well below 5000 mg/L and does not contribute in the biogas composition and the composition of biogas produced was comparatively much better than that of acidogenic reactor.

The daily gas production and cumulative gas production for the entire study period are shown in Figure 6.9 and Figure 6.10. The methanogenic reactor in this study produced an average of 1.152 liters of biogas per day, with an average methane content of 66%. The reactor pH was between 7.6 and 8.2 over the duration of the study. The average methane production rate over the entire study period was 0.31 m$^3$ CH$_4$/kg VS destroyed and 0.15 m$^3$ CH$_4$/kg VS fed at an overall average OLR of 1.05±0.05 kg VS/m$^3$-day. This methane production rate is comparable to the value in published report (Strydom et. al. 1997).

6.6.3 Overall Performance of the Two stage System

The goal of the two stage system was to increase the anaerobic biodegradation of the tannery solid and liquid wastes by separating the hydrolysis and acidogenic reactions to within the first phase of the system and the methanogenic reactions to within the second phase of the system. The tannery solid and liquid wastes were fed to the acidogenic reactor and the drain from this reactor was fed to the methanogenic reactor. It was believed that the acidogenic reactor would also reduce shock loadings to the methanogenic reactor since it would also act as an equalization tank.

The variations in TS and VS in the feed to first stage and the drain from the second stage are shown in Figure 6.11 and Figure 6.12. The average TS and VS removal efficiencies are 65% and 67% respectively. The daily and cumulative gas production for the entire study period are shown in Figure 6.13 and Figure 6.14. The specific gas production is found to be 0.14 m$^3$/kg VS fed and 0.21 m$^3$/kg VS destroyed.
6.7 Conclusion

It is observed that the specific gas production obtained in the second stage (0.23 m³/kg VS added) in the present study is in reasonable agreement with performance reported by Ghaly et al (2000) and Lo et al (1986). The methane content of 66% in the biogas is also in good agreement with the values reported by Ghaly et al (2000), and Ince (1998). The higher methane content in the second stage indicates the presence of highly active methanogens in the reactor and the dynamic balance prevailing between VFA concentration and biogas generation rate at an OLR of 1.05 ± 0.05 kg VS/m³.day

According to Weiland (1993) protein rich residues with C:N ratio below 10 can be treated only in the two step process. In the present study also the fleshing material rich in protein and C/N ratio of 3 has been anaerobically digested in two step process with a OLR of 3.47 gm VS/l.d and the overall VS conversion efficiency of 67% has been achieved. The co-digestion of protein rich solid wastes having C/N ratio of 3 with liquid waste used as diluent minimises ammonia toxicity in two stage process as the bacteria cultures in the two stage process are more robust against high ammonia concentration. Hence it is concluded that the two-stage digestion system can be operated with protein rich substrates at higher loading rates without any process instabilities of fatty acid accumulation.
FIG. 6.1 - TOTAL SOLIDS CONTENT IN FEED AND DRAIN (Hydrolysis)
FIG. 6.3 - VARIATION OF OLR AND VFA CONCENTRATION (Hydrolysis)
FIG. 6.6 - TOTAL SOLIDS CONTENT IN FEED AND DRAIN (Methanogenesis)
FIG. 6.7 - VOLATILE SOLIDS IN FEED AND DRAIN (Methanogenesis)
FIG. 6.8 - VARIATION OF OLR AND VFA CONCENTRATION (Methanogenesis)
FIG. 6.10 - CUMULATIVE GAS PRODUCTION (Methanogenesis)
FIG. 6.11 - TOTAL SOLIDS CONTENT IN FEED (Hydrolysis) AND DRAIN (Methanogenesis)
FIG. 6.14 - CUMULATIVE GAS PRODUCTION (Hydrolysis + Methanogenesis)