CHEMICAL CHARACTERIZATION OF ANAEROBIC DIGESTION TREATMENT OF POULTRY MORTALITIES*

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Abstract
Proper disposal of poultry mortalities is of major concern for the poultry industry. A preliminary investigation was conducted to evaluate an anaerobic digestion system as an alternative for poultry mortality disposal. The system consisted of a leachbed (LB) and an upflow anaerobic sludge blanket (UASB), connected in a closed loop. The LB served as the hydrolysis/acidification phase, while the UASB served as the methanogenesis phase. Effluent from the LB served as influent to the UASB while effluent from the UASB overflowed to the LB to continuously inoculate the LB with methanogens. Three experiments were conducted, two at 55°C and one at 35°C. In one of the 55°C experiments, different moisture contents in the LB were tested to evaluate the rate and extent of digestion of the mortalities. Higher initial moisture content in the LB was beneficial to methane production. The mortality was solubilized quickly and converted into a very concentrated effluent. The UASB was able to generate methane efficiently at loading rates below 2 g COD L⁻¹ day⁻¹. The UASB at 55°C performed poorly when the loading rate exceeded 2 g COD L⁻¹ day⁻¹. Digestion at 35°C looks promising. However, the process needs to be optimized to be competitive with other biological disposal alternatives. © 1998 Published by Elsevier Science Ltd.

Key words: poultry mortalities, anaerobic digestion, leachbed, upflow anaerobic sludge blanket, liquid recirculation, long-chained fatty acids.

INTRODUCTION

Chicken population in Taiwan was 97.8 million in 1994 (Agriculture Yearbook, 1995). At an average mortality rate of about 9.3% (Shih, 1994), about 8.6 million birds need to be disposed of annually. Being easily putrefiable, potential of causing sanitary problems, spreading of diseases and, in the worst scenario, smuggling of tainted meat into the market are of great concerns. To dispose of mortalities properly is crucial to improving public health, sustaining animal industry and protecting the environment.

In addition to the conventional disposal methods of burial, incineration and rendering, aerobic composting is becoming popular in America lately (Donald and Blake, 1992) and accepted by many states (Proctor, 1992). This research examined anaerobic digestion as yet another alternative for mortality disposal. The advantage of anaerobic treatment is that it couples waste treatment with methane production. Additionally, the process kills pathogens (Lee and Shih, 1988; Shih, 1987; Turner et al., 1983).

Although there are publications on anaerobic digestion of protein- and fat-containing wastewater, such as slaughterhouse wastewater (e.g. Sayed et al., 1984; 1987), no published information was found on anaerobic digestion of poultry mortalities. However, being similar in nature to slaughterhouse wastes, poultry mortalities could be a suitable substrate for anaerobic digestion. The specific objective of this paper is to document the operating characteristics and treatment efficiencies of an anaerobic digestion system in treating poultry mortalities.

METHODS

Poultry mortalities
Chickens were purchased from a local market as needed. The chickens were slaughtered, their blood drained and collected and their feathers plucked. Each dead bird, simulating mortalities, was divided into four components for the experiments: carcass, blood, viscera and feathers.
Experimental set-up
The anaerobic digestion system used consisted of a leachbed (LB) and an UASB reactor connected in a closed loop (Fig. 1). The reactors were incubated in temperature-controlled chambers maintained at either 55 ± 1°C or 35 ± 1°C. Biogas was collected in a water displacement system, filled with solution containing 25% NaCl and 0.5% citric acid. Operational parameters of the reactors are summarized in Table 1.

![Schematics of experimental setup of an LB-UASB system.](image)

Fig. 1. Schematics of experimental setup of an LB-UASB system.

<table>
<thead>
<tr>
<th>Table 1. Operational parameters of the reactors</th>
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<tbody>
<tr>
<td>Experiment</td>
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<tr>
<td>Reactor</td>
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<tr>
<td>Dimension</td>
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<tr>
<td>Diameter (mm)</td>
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<td>Height (mm)</td>
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<td>Liquid volume (dm³)</td>
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<td>Recycle ratio</td>
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<tr>
<td>Liquid recycle flow rate (dm³ h⁻¹)</td>
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<td>Liquid upflow velocity (m h⁻¹)</td>
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<td>Temperature (°C)</td>
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Operation
Three experiments were conducted, each with two sets of LB-UASB combination. At the start of each experiment, chicken components, each in a separate No. 32 mesh nylon bag, were placed in the LBs. One chicken for each LB-UASB set. Three liters of water was added to the LBs, except in one of the LBs in experiment 2 where 10 liters of water was added. Liquid was recirculated by pumping from the bottom to distribute over chicken components from the top to promote leaching. The UASBs were started with about 25% (by volume) of granular sludge from existing reactors. Liquid in both the LB and the UASB was continuously recirculated except during feeding of the UASB. During feeding, liquid was transferred between the LB and the UASB. An appropriate volume of the leachate was pumped, with a peristaltic pump, to the UASB as influent from the UASB overflowed to the LB. Chemical oxygen demand (COD) of the leachate was monitored frequently and the data used for controlling loading rates to the UASBs. Loading rates normally expressed in the same unit. Loading rates to the UASBs determined the volume of liquid to be transferred.

Sampling and analyses
Daily biogas production was determined from the volume of solution displaced. Gas samples for composition analyses were taken through a septum on top of the liquid displacement system (Fig. 1). Gas composition was analyzed with a thermal conductivity detector on a gas chromatograph (GC, Shimadzu GC-14A) as described previously (Chen and Shyu, 1996). Volume of methane was calculated as COD equivalent (ie., 1 g COD = 350 ml CH4) and volumetric methane production rates were expressed in g COD L−1 day−1 to facilitate comparison to loading rates normally expressed in the same unit.

The initial dry weights (TS) of the chicken components were determined according to the Standard Methods (APHA, 1989). A separate chicken was divided into five components for COD determinations: bones, meats, blood, viscera and feathers. The components were dried and ground through a 0.5 mm screen in a Cyclone Sample Mill (UDY Corp.). All components except the blood were freeze dried. The blood component was dried in a 70°C oven. The COD was determined with a colorimeter (Hach DR/700, Hach Co.).

Effluent from the LB and the UASB were periodically analyzed for TS, volatile solids (VS), pH, alkalinity, COD, volatile fatty acids (VFA) and long-chained fatty acids (LCFA, C14−C24). The TS, VS and alkalinity were analyzed according to the Standard Methods. The pH was determined with a pH electrode. The VFA and LCFA were determined with a flame ionization detector on the GC. The column and GC conditions for VFA analyses were as previously described (Chen and Shyu, 1996). Samples for LCFA analyses were prepared in a two-hour one-step extraction-transmethylation procedure (Sukhija and Palmquist, 1988) and frozen (−20°C) until analyzed. A 3 mm by 2 m column packed with 100/120 Chromosorb WAW, GP 10% SP-2330 was used for the LCFA analyses. Nitrogen gas at a flow rate of 20 ml min−1 was the carrier gas. The injector, column and detector temperatures were 220, 200 and 250°C, respectively. Standard (Catalog no. O 7756) for the LCFA analyses came from Sigma (Sigma Chem. Co.). Individual LCFA were calculated by comparing the peak areas of the samples to those of the standards.

RESULTS AND DISCUSSION
The average live weight of the chickens was 2279 ± 63 g (n = 6). The average dry weight of the chickens was 853 ± 38 g (n = 6). Addition of 3 and 10 liters of water resulted in initial TS contents of 22% and 8%, respectively. The dry weight composition of the carcass, collected blood, viscera and feathers were 79.54%, 0.83%, 8.49% and 11.14%, respectively. Bones constituted about 41% of the dry weight of the carcass. The total COD of the chicken was 1016 grams.

Leachbed reactors
Figure 2 compares profiles of leachate CODs from all experiments. Leachate CODs reached high levels within 10 days after start-up, as a result of solubilization of the blood, the meat and the viscera components. At 55°C and with 3 liters of water added, leachate COD reached a maximum of 90 g L−1. With 10 liters of water added, COD concentration reached 30 g L−1, consistent with the dilution due to the added water. The COD concentration in the 35°C experiment did not reach as high as in the 55°C experiments, indicating slower solubilization at 35°C. Volatile fatty acids concentrations were high (Fig. 3). The VFAs made up between 65% and 75% of the leachate CODs. Fortunately, with high alkalinity levels, comparable to that found in the digestion of poultry manure (Webb and Hawkes, 1985), the pH of the LB was well buffered at above 6.0 (Fig. 4).

There were fatty deposits on the nylon bags as well as a layer of floating oils and fats on the surface of the fermentation liquid, indications that the chicken components were decomposing and adipose tissues being released. These fats and oils probably did not contribute to the CODs of the leachate since leachate samples were taken from the bottom of the LB. The leachate contained low levels of LCFA, indicative of limited lipids hydrolysis since lipids are hydrolyzed to LCFA and glycerol (Hanaki et al., 1981). Total concentrations of the LCFA were around 2 g COD equivalent per liter. Myristate (C14:0) constituted over 70% of the LCFA while
oleate (C18:1) and palmitate (C16:0) made up about 20% and 10%, respectively. Traces of stearate (C18:0) also detected. Long-chained fatty acids are beta-oxidized to acetate, carbon dioxide and hydrogen in anaerobic digestion (Weng and Jeris, 1976), which are then converted to biogas. However, there was no methane production from the leachbed before day 10. Obviously, the methanogen population there was still small, if any.

As methane production slowly built up, leachate COD concentrations gradually decreased to the range of 10–20 g L⁻¹ over the next 60 days. The alkalinity and VFAs also decreased while the pH increased and they all levelled off after day 70. Leachate COD concentrations rose again after day 80, accompanied by a sharp rise in LCFA concentrations (Fig. 5). Thus, the rise in leachate COD probably resulted from hydrolysis of the floating layer of fats and oils, through which, what had been floating on the top of the fermentation liquid was made part of the leachate. The LCFA made up over 50% of the leachate CODs. Palmitate concentration as high as 4.3 g L⁻¹ and stearate as high as 2.2 g L⁻¹ were detected.

**UASB reactors**

Because of a break in the feed line on day 20, all liquid in one of the UASBs in experiment 1 was lost. Granular sludge agglomerated and part of the aggregates floated when refilled with water. Normal operation of the UASBs was interrupted. Thus, data from this run will not be discussed.

Figures 6 and 7 show the operating conditions and performances of the UASBs in experiment 2. The UASBs were started at a loading rate of 1 g COD L⁻¹ day⁻¹ to avoid shock-loading the granular sludge. The loading rate was raised to 2 g COD L⁻¹ day⁻¹ on day 30. It was further raised to 5 g COD
Chemical characterization of anaerobic digestion treatment of poultry mortalities

L⁻¹ day⁻¹ over a 15-day period between days 60 and 75. To achieve these loading rates under changing influent COD concentrations required adjusting feeding rates and hydraulic retention times (HRTs) frequently because leachate from the LBs was fed to the UASBs without dilution. Since leachate from the LB was very concentrated, feeding undiluted leachate to the UASB at these low loading rates resulted in very low feeding rates (16–900 mL day⁻¹) and very long HRTs for the UASBs. At the shortest, the HRT was around 2.6 days, much longer than many UASB applications (e.g., Sayed et al., 1984).

The effluent COD for the UASB connected to the LB with 10 liters of water stabilized at about 6 g L⁻¹ after the loading rate was raised to 2 g COD L⁻¹ day⁻¹ (Fig. 6). Methane production rates followed loading rates closely between days 35 and 60. However, COD reduction efficiencies decreased from above 70% to about 55%. The apparent discrepancy between methane production and COD reduction could be attributed to increased amount of flocculent sludge in the effluent; since the influent COD concentration was decreasing (Fig. 2), increasingly higher flow rates and shorter HRTs were used to maintain the desired loading rates. These conditions caused increased wash-out of flocculent sludge. However, it is important to note that as long as the wash-out rate did not exceed growth rate, the methane production rate of the UASB should not be affected. Furthermore, because of the closed-loop operation, washed-out sludge from the UASBs was useful to the LBs since it provided the latter with needed inocula for methanogenesis. The effluent COD for the UASB connected to the LB with 3 liters of water stabilized at about 12 g L⁻¹. Effluent CODs for both UASBs started to rise on day 80, accompanied by rising ICFAs (Fig. 8) after the loading rate was raised to 5 g COD L⁻¹ day⁻¹. Excessive wash-out of the granules occurred due to heavy sludge floatation, probably caused by the ICFAs as was the case in the digestion of mixtures of sodium laurate and acetate (Rinzema et al., 1993). Methane production rates dropped far below their corresponding loading rates. The operation was terminated on day 117 due to the poor performances. After 117 days of fermentation, 192 and 142

liters of methane were produced from the LB-UASB systems that started with 10 and 3 liters of water in the LB, respectively (Fig. 9).

Low methane production rates and poor treatment efficiencies, beyond day 80, could probably be attributed to LCFAs, since LCFAs may cause severe inhibition of the anaerobic micro-organisms (Hanaki et al., 1981). The UASBs contained stearate as high as 2.2 g L\(^{-1}\), higher than the 1.0 g L\(^{-1}\) that was found to stop methane production from VFAs immediately (Angelidaki and Ahring, 1992). The UASBs also contained as much as 3.5 g L\(^{-1}\) of palmitate and small amounts of other LCFAs, thus, synergism of toxicity could have further inhibited methanogenesis (Koster and Cramer, 1987). Additionally, since LCFAs constituted over 50% of the influent CODs, their digestion in the UASB reactors would require sufficient mixing of the liquid and contact between substrate and all the biomass (Rinzema et al., 1993). Unfortunately, the low flow rates during feeding operations in this study provided little mixing of the liquid. Even though a relatively high upflow velocity of 0.63 m h\(^{-1}\) and high recycle ratios of 126–4000 were used, some white lumpy precipitates and floating sludge aggregates were observed in the UASBs, probably contributing to the poor performances. Perhaps higher upflow velocities should be used to ensure sufficient mixing and good contact between the lipids-rich substrate and all the biomass. Additionally, a smaller recycle ratio may be desirable since frequent feeding line breakage due to prolonged recirculation was causing operational problems.

Figure 10 shows the operating conditions and performances of the UASBs in experiment 3. At a loading rate of 0.5 g COD L\(^{-1}\) day\(^{-1}\), COD reduction was almost 100%. However, methane production did not match up until after day 10. The discrepancy between methane production and COD reduction might be attributed to retention of COD by the granular sludge (Sayed et al., 1987). The LCFAs (mostly myristate) concentration was always below 0.5 g L\(^{-1}\). The VFAs started to rise on day 35 when loading rate was increased to 2 g COD L\(^{-1}\) day\(^{-1}\) (Fig. 11). However, the COD reduction efficiencies remained above 80% until the loading rate was increased beyond 2 g COD L\(^{-1}\) day\(^{-1}\). It briefly dropped below 30% at loading rates above 5 g COD L\(^{-1}\) day\(^{-1}\). However, COD reduction efficiency and methane production rate seemed to be improving.

![Fig. 5. Profiles of long-chained fatty acids in the leachate: (a) with 3 liters of water added, (b) with 10 liters of water added.](image-url)
when the experiment was interrupted. The VFAs were decreasing as well. The experiment was interrupted on day 79 when one of the UASBs lost most of its liquid due to a broken feed line. Feeding to the other UASB stopped and VFAs dropped to lower than 0.5 g L\(^{-1}\). In a 79-day period, more methane was produced from the 35°C system than the 55°C system (Fig. 9). The highest methane production rate achieved in this run was 4 g COD L\(^{-1}\) day\(^{-1}\), slightly higher than the 3.6 g COD L\(^{-1}\) day\(^{-1}\) achieved at 55°C.

**LB-UASB system performance**

The high leachate COD and VFA concentrations (Figs 2 and 3) and the lack of methane production from the leachbed indicate an unbalanced fermentation in the LB during the first few weeks of each experiment. In contrast, biogas with over 80% methane indicates stable methanogenesis in the UASB. Thus, the LB-UASB system initially behaved like a two-phase system, with LB serving as the hydrolysis/acidification phase while the UASB the methanogenesis phase.

Since effluent from the UASB contained un-granulated methanogens and associated enzymes, the liquid transfer from the UASB to the LB during feeding operation served to dilute as well as to repeatedly inoculate the LB with methanogens. Repeated inoculation helped the LB overcome the inhibitory concentration of acids and establish methanogenesis there. The LBs started producing methane several weeks after start-up (an average of 13 days for the 55°C experiments and 27 days for the 35°C experiments). Thereafter, methane content of the biogas from the LB continued to rise to above 60% (Fig. 10), a sign that the LB had become a single-phase methane reactor. Continued periodic transfer of liquid between the LB and the UASB eventually homogenized the liquid phase of both reactors.

The slow start of methanogenesis in the LBs could be attributed to its inhibitory leachate concen-

![Graph](image-url)  
**Fig. 6.** Operating conditions and performance of the UASB for experiment 2, with 10 liters of water and at 55°C.
trations and light inoculation due to low liquid transfer rates from the UASB to the LB. Both were affected by the initial moisture contents in the LB. A higher initial moisture content resulted in a lower leachate concentration which should have provided a better LB environment for efficient methanogenesis. In addition, a less concentrated leachate would allow higher flow rates through the UASB while maintaining the same loading rate. Since effluent from the UASB was inoculating the LB with methanogens, higher liquid transfer rates from the UASB to the LB should bring more methanogens to the LB and allow methanogenesis there to begin sooner. Results from experiment 2 supported this contention; of the two LBs in experiment 2, the one that began with 10 liters of water started producing methane on day 9 while the one with 3 liters of water on day 15. Thus, the optimum amount of water to be added deserved further investigations.

At the end of each experiment, all nylon bags were retrieved from the LB, rinsed with water and dried to determine weight losses. Figure 12 shows weight losses from the carcass, feathers and viscera components. The blood completely disappeared from the bags. On average, 76% of the carcasses was degraded. The remains were mostly bones and it is estimated that 42% of the bones was degraded. The feathers lost 71.4% and the viscera lost 80.4% weight. Overall, 24% of the mortality remained to be treated. In addition, a layer of oils and fats persisted on the top of the fermentation liquid at the end of the experiments. Although more complete degradation might be possible after an even longer period of digestion, the process already took too long to be competitive with another biological disposal alternative—the composting process (Donald and Blake, 1992). So in practice, the remaining solid materials will have to be disposed of through other

Fig. 7. Operating conditions and performance of the UASB for experiment 2, with 3 liters of water and at 55°C.
Fig. 8. Profiles of long-chained fatty acids in the UASB for experiment 2: (a) with 3 litres of water, (b) with 10 liters of water.

Fig. 9. Comparison of cumulative methane productions from different experiments.
Fig. 10. Operating conditions and performance of the UASB for experiment 3, with 3 liters of water and at 35°C.

Fig. 11. Profiles of individual volatile fatty acids in the UASB for experiment 3.
methods. Alternatively, if the mortalities were mechanically chopped up before entering the digestor system, faster and more complete degradation might be possible. As for the oils and fats, in view of their tendency to cause scum and relatively poor stabilization, they should be skimmed off and digested in a separate sludge digestor as suggested for the digestion of slaughterhouse wastes (Sayed et al., 1984).

The final COD of the fermentation liquid was also determined. Table 2 shows mass balance for the experiments on a COD basis. Samples for various analyses removed significant amounts of CODs due to the high leachate concentrations (Fig. 2). The many samples, final residual CODs and methane production accounted for 17.5%, 18.6% and 47.2%, respectively of the initial COD. Presumably, the leftover bones, viscera and feathers accounted for the rest. Based on the cumulative methane production (Fig. 9), methane yields ranged from 0.14 to 0.189 m$^3$ (kg COD)$^{-1}$. Since the LB still contained significant amounts of residual CODs, more complete conversion to biogas and higher methane yields seem possible had the experiments not been interrupted. However, treatment efficiency needs to be improved. Treatment can only be considered complete when bioconversion results in methane production. Towards this end, the effects of initial moisture contents in the LB, upflow velocity through the UASB and recycle ratio on system performances deserve further investigations.

### CONCLUSIONS

After two and a half to four months of anaerobic digestion in the LB-UASB system, about 76% of the poultry mortality was degraded. Treatment of the remaining solid materials through other methods is necessary. Alternatively, the mortalities could be mechanically chopped up before entering the diges-
tor system to effect faster and more complete degradation. A layer of fats and oils remained. In view of their poor stabilization and tendency to cause scum, provision to skim off this layer to be digested in a separate sludge digester is recommended. The mortality was solubilized quickly and converted into a very concentrated leachate. The leachate was bioconverted to methane efficiently at loading rates below 2 g COD L\(^{-1}\) day\(^{-1}\). The UASB at 55°C performed poorly when the loading rate exceeded 2 g COD L\(^{-1}\) day\(^{-1}\). Digestion at 35°C looks promising. However, the process needs to be optimized to be competitive with other biological treatment alternatives. Towards this end, the effects of initial moisture contents in the LB, upflow velocity through the UASB and recycle ratio on system performances deserve further investigations.

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