Anaerobic treatment of poultry mortality in a temperature-phased leachbed–UASB system

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Abstract

Anaerobic digestion has been proposed as an alternative to the conventional disposal methods of burial, incineration, rendering and aerobic composting. A temperature-phased system consisting of one UASB (at 55 °C) and three leach-bed reactors (at ambient temperatures) was tested for its efficiencies in treating poultry mortality. The thermophilic UASB was difficult to start-up. It also showed signs of inhibited methanogenesis. Chemical parameters such as long chain fatty acids, volatile fatty acids and ammonia concentrations were all very high for the thermophilic UASB. Lowering its temperature to 35 °C enhanced its stability and improved its performances. Lowering the pH of the 55 °C UASB also improved its chemical oxygen demand (COD) reduction efficiency as well as its methane production rate. The results were compared to that of another similar system where the UASB reactor was maintained at 35 °C instead of at 55 °C.

Keywords: Poultry mortality; Anaerobic digestion; Leachbed; Up-flow anaerobic sludge blanket; Temperature-phased

1. Introduction

Proper disposal of mortality is crucial to sustaining animal industries, improving public health and protecting the environment. Anaerobic digestion has been proposed as an alternative to the conventional disposal methods of burial, incineration, rendering and aerobic composting. A closed-loop pair consisting of a leachbed (LB) and an up-flow anaerobic sludge blanket (UASB) completed treatment of one batch of dead chicken in 118 days (Chen and Wang, 1998). Due to the adverse environments for methanogenesis in the LB within the first month after start-up, the pair initially functioned as a two-stage system; the LB served as the hydrolytic/acidogenic stage while the UASB the methanogenic stage. Effluent from the UASB continuously provided un-granulated biomass to the LB. Methanogens and associated enzymes thus carried-over from the UASB were essential to the maturation of the LB (Chen and Wang, 1998). The faster the LB entered its methanogenic stage the sooner digestion of the mortality was completed. Therefore, if liquid transfer rates from the UASB to the LB could be increased, the overall treatment efficiency of the pair could be improved. Higher liquid transfer rates could be brought about by higher loading rates (LR) to the UASB. Since thermophilic operation offers the potential advantage of higher LRs (Dinsdale et al., 1997a), maintaining the pair at thermophilic temperatures might improve their efficiencies. However, treatment efficiency of a 55 °C LB–UASB pair was poor (Chen and Shyu, 1998). The 55 °C UASB performed poorly when its LR exceeded 2 kg COD/m³/day. Although rapid paces of LR increments could have caused the problem, higher leachate concentration at
55 °C than at 35 °C as a result of faster hydrolysis/acidification rates could also have played a role; higher leachate concentrations, in turn, necessitated lower liquid transfer rates in order to maintain proper LRs to the UASB. Thus, build-up of carried-over methanogenic population in the LB was slower, hence, slower maturation of the LB and poorer overall treatment efficiency for the system. When the LB–UASB pair was operated in a batch mode, the UASB became idle once the connecting LB entered active methanogenesis stage (Chen and Wang, 1998). Thereafter, when the next batch of LB was started, LR to the UASB had to be kept to a low level again to avoid shock loading the granular sludge and be built up gradually to higher levels. This periodic idling of the UASB and then gradual re-building of its LR must have contributed to the inefficiency of the system. By sequentially connecting three LBs, maintained at ambient temperatures, to a 35 °C UASB, and using 80% COD reduction as a condition for raising LRs, Chen (2000) was able to maintain high LRs to the UASB between succeeding batches of mortality. It thus appeared that keeping the UASB at a higher temperature than the LBs allowed better coupling between the methane- and acid-forming stages of this treatment system. Consequently, it might be possible to further improve the system’s efficiency by keeping the UASB at even higher temperatures. Therefore, the objective of this study was to investigate the effectiveness of a temperature-phased LB–UASB system in treating poultry mortality, with the UASB at 55 °C and the LBs at ambient temperatures.

2. Methods

2.1. Poultry mortalities

Dead chickens were obtained from the poultry farm at the National Chung Hsing University or a nearby layers operation.

2.2. Reactors

The anaerobic digestion system used consisted of one UASB reactor and three LBs. The reactors were made of Plexiglas. The LBs were 240 mm (i.d.) and 400 mm in height, with a working volume of 10 l. The UASB was 90 mm (i.d.) by 600 mm in height, with a working volume of 3 l.

2.3. Experimental set-up

Only one LB was paired to the UASB at any one time. The reactors were connected with Tygon tubing. Experimental set-up of the LB–UASB pair is shown in Fig. 1. The UASB reactor was incubated in a temperature-controlled chamber maintained at 35 or 55 ± 1 °C, whereas the LB was kept at ambient temperatures. Biogas from each reactor was collected in a water displacement system, filled with solution containing 25% NaCl and 0.5% citric acid. Duplicate sets of system were set up (hereafter designated systems A and B).

2.4. Start-up

The UASBs were started with about 50% (by volume) of granular sludge obtained from a mesophilic UASB at I-Lan Brewery Plant (Taiwan Tobacco and Wine Board, I-Lan). Each LB was started with 10 l of tap water and a whole dead chicken wrapped in a No. 32 mesh nylon bag. After closure, the LB was flushed with O₂-free N₂ gas.

2.5. Operation

Liquid in both LB and UASB were circulated by peristaltic pumps six times daily for 30 min each. Since the dead chicken floated to the surface of the liquid, liquid in the LB was circulated downwards to promote degradation of the chicken. Liquid in the UASB was circulated upwards. Leachate from the LB was fed to the UASB six times per day. During feeding, appropriate volume of the leachate was pumped to the UASB as influent while effluent from the UASB overflowed to the LB to maintain constant liquid volumes in both reactors. Loading rates to the UASB determined the volume of liquid to be pumped. Filtered COD (through Supor®-450 filters, Gelman Sci.) of the leachate was monitored frequently and the data used for calculating LRs to the UASB. Liquid lost through sampling and evaporation was replaced with tap water. The UASB was started at a LR of 0.5 kg COD/m³/day to avoid shock loading the inoculum. The LR to the UASB was raised in steps of about 0.1 kg COD/m³/day if it could maintain a COD reduction efficiency of about 80%. As the mortality in the LB was continuously being degraded, leachate concentration eventually started to drop. When the leachate concentration became so low that it could no longer sustain the UASB at high LRs, the LB was replaced with a second LB containing another dead chicken. Operation of the new LB–UASB pair followed the same procedure as described above. Meanwhile, digestion continued in the off-lined LB, without leachate circulation, until its methane production rate dropped below 0.5 l/day. The UASB moved on to a third LB when the second LB entered its accelerated methanogenic stage and leachate from it could no longer support active methanogenesis in the UASB.

2.6. Sampling and analyses

Leachate from the LBs and effluent from the UASB were sampled periodically for pH, alkalinity,
COD, ammonia, volatile fatty acids (VFA) and long-chained fatty acids (LCFA) analyses. The pH was determined with a pH electrode. The alkalinity was measured according to Standard Methods (American Public Health Association, 1992). The CODs of total and filtered samples were determined colorimetrically according to Standard Methods (American Public Health Association, 1992). The difference between total and filtered COD was taken to be sludge. Ammonia nitrogen was determined with the distillation method (Bremner and Keeney, 1965). Free-ammonia concentration was calculated as described by Kayhanian (1999). The VFAs and the LCFAs were determined with a flame ionization detector on a GC (Hitachi 5000A) as previously described (Chen and Wang, 1998).

Biogas production from the LBs and the UASBs was recorded daily. Gas composition was analyzed with a thermal conductivity detector on a gas chromatograph (GC, Shimadzu GC-14A) as previously described (Chen and Wang, 1998).

**3. Results**

The chickens used for the first, second and third sets of LBs for systems A and B weighed 1.8 and 1.5; 1.78 and 1.76; and 1.46 and 1.76 kg, respectively. Calculated total solids concentrations in the LBs ranged from 4.5% to 5.7%.

**3.1. Leachbed characteristics**

Fig. 2 shows the operating characteristics of LB-As. Leachate COD rose quickly after start-up, peaking at 25 g/l. When the ambient temperatures dropped below 25 °C, peak leachate COD dropped as well. VFAs of the leachate followed similar pattern. Leachate pH initially declined to as low as 5.7, then gradually rose to above 7.5. Total alkalinity was as high as 15 g/l as CaCO₃. Operating characteristics of LB-Bs were similar to those of the LB-As (data not shown).

The LBs were connected to their respective 55 °C UASBs within 12 days, at the latest, after their start-ups.
Fig. 2. Leachbed operating characteristics for system A. Leachbeds 1 (LB-1A), 2 and 3 were sequentially started on days 0, 148 and 222, respectively. They were connected to UASB-A on days 0, 158 and 226, respectively.
Table 1

Summary of LB operation

<table>
<thead>
<tr>
<th>Reactor ID</th>
<th>Started day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CH&lt;sub&gt;4&lt;/sub&gt; appeared on day&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time to maturation, on day&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Connected to UASB&lt;sup&gt;c&lt;/sup&gt;, on day&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Period connected, days</th>
<th>Period operated, days</th>
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<tr>
<td>LB-1A</td>
<td>0</td>
<td>2</td>
<td>151</td>
<td>0</td>
<td>158</td>
<td>261</td>
</tr>
<tr>
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<td>2</td>
<td>126</td>
<td>0</td>
<td>126</td>
<td>242</td>
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<tr>
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<td>6</td>
<td>53</td>
<td>10</td>
<td>72</td>
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</tr>
<tr>
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<td>17</td>
<td>101</td>
<td>12</td>
<td>94</td>
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<td>6</td>
<td>53</td>
<td>8</td>
<td>51</td>
<td>264</td>
</tr>
<tr>
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<td>13</td>
<td>113</td>
<td>7</td>
<td>81</td>
<td>358</td>
</tr>
</tbody>
</table>

<sup>a</sup> Counting from the time when the 55 °C UASBs were started.
<sup>b</sup> Counting from the time the indicated LB was started. Maturation is defined as the point where methane content in the biogas from a LB rose beyond 75% (Chen, 1999).
<sup>c</sup> Efforts were made to maintain the pH of UASB-B at 7.5 by daily injecting 0.2 N HCl into this reactor between days 113 and 229.

(Tables 1). The LB-As started producing methane within 7 days after their start-ups. Methane was found in the biogas from LB-2A and LB-3A even before they were connected to UASB-A. The LB-Bs started producing methane within 6 days after being connected to UASB-B. Methane content in biogas from each LB gradually increased thereafter. Upon termination, each LB had been operated for at least 232 days up to a year.

3.2. Characteristics of the 55 °C UASB

Fig. 3 shows performances of the UASBs when they were connected to their respective first LBs. As COD reduction efficiencies approached 80%, LR to the UASBs was increased from 0.5 to 1 kg COD/m<sup>3</sup>/day between days 24 and 33 (Fig. 3e). Unfortunately, COD reduction efficiencies started to decrease and effluent CODs began to increase. Effluent CODs reached 14 g/l around day 70 (Fig. 3a) while COD reduction efficiencies dropped below 40% (Fig. 3b). VFAs for UASB-A reached 3.7 g/l as acetate on day 57 and that for UASB-B reached 4.2 g/l on day 66 (Fig. 3c). Methane production rates dropped below 0.1 kg COD/m<sup>3</sup>/day, apparently unable to keep pace with the LR (Fig. 3e and f). The UASBs appeared severely stressed. Thus, we decided to suspend feeding on day 70 and replaced the granular sludge on day 89. The replacement sludge had been obtained from the same source as the inoculum and kept at ambient temperatures for 2 years with occasional feeding. COD reduction efficiencies for the re-started UASBs rose above 80% between days 89 and 112. Influent CODs started to decline around day 97 (Fig. 3a), thus, the hydraulic retention time of the UASB was shortened in order to maintain a LR of around 1 kg COD/m<sup>3</sup>/day. Unfortunately, COD reduction efficiencies started to deteriorate. Consequently, LR to the UASBs was not raised beyond 1 kg COD/m<sup>3</sup>/day during the remaining time they were connected to their respective first LBs.

We suspected that either the seed sludge or the 55 °C temperature had been the problem. To resolve this issue, we started two other pairs of LB-UASB on day 167. The new UASBs were inoculated with the same replacement sludge and operated following similar procedures as mentioned above except that they were maintained at 35 °C. The 35 °C UASBs were operated for 136 days, during which each was sequentially connected to two LBs. These two LBs were started approximately when the second and third sets of LBs for the 55 °C UASB were started. With comparable influent CODs ranging from about 25 g/l to around 2 g/l (Fig. 4b), the 55 °C UASB had much higher effluent CODs (Fig. 4c) than the 35 °C UASBs did. Effluent CODs of the 55 °C UASB rose to 12 g/l on day 191 whereas the maximum COD concentration for the 55 °C UASBs was 1.6 g/l. Maximum COD reduction efficiency for the 55 °C UASB reached about 70%. In contrast, the 35 °C UASBs were able to maintain mostly above 80% COD reduction. Loading rate to the 55 °C UASB barely reached 2 kg COD/m<sup>3</sup>/day while that to the 35 °C UASBs reached 3 kg COD/m<sup>3</sup>/day between days 239 and 258 (Fig. 4f). The 55 °C UASB also had much higher VFAs concentrations than the 35 °C UASBs did (Fig. 4d). After day 150, VFAs of the 55 °C UASB-A were typically above 1.5 g/l as acetate, peaking at 5.5 g/l on day 195. VFAs for the 35 °C UASBs were generally below 0.5 g/l as acetate, with 0.8 g/l being the maximum. Propionate to acetate ratio for the 35 °C UASBs was generally very low except during the initial stage after their start-ups (Fig. 4e). Those ratios for the 55 °C UASB were typically above 1. Apparently, the 55 °C temperature, rather than the seed sludge itself, had been the problem.

We also suspected free-ammonia inhibition to be the cause of the problem for the 55 °C UASBs. Following an initial drop to around 7, the pH of the 55 °C UASBs rose and leveled off at around 8 before feeding was suspended (Fig. 5a). Total ammonia reached 2.6 g/l and free-ammonia concentration reached 910 mg/l. Even after being re-started with replacement sludge, total ammonia concentrations reached as high as 2.9 g/l (Fig. 5f), with a free-ammonia concentration of 671 mg/l. In contrast, the maximum total and free-ammonia
concentrations for the 35 °C UASBs were 2.4 g/l and 138 mg/l, respectively (data not shown). To further study this problem, we tried to maintain the pH of one of the 55 °C UASBs (i.e., UASB-B) at 7.5 by injecting appropriate volume of 0.2 N HCl solution into UASB-B once daily between days 113 and 229.

Fig. 3. Performance of the 55 °C UASBs when they were connected to their respective first LBs. Thick lines represent system A while thin lines represent system B.
Although high alkalinity and ammonia probably caused its pH to rise again when pH measurement was made the next day, these adjustments resulted in lower VFAs, LCFAs, effluent COD, alkalinity and ammonia (Fig. 5), and generally higher COD reduction efficiency and methane production rate (data not shown).
Operation of the UASBs was terminated when they were separated from LB-3s. In total, the UASB-A was operated for 281 days while the UASB-B was operated for 301 days.

Fig. 5. Comparing operating characteristics (pH, VFAs, LCFAs, effluent COD, alkalinity and ammonia) of the pH-adjusted and non-adjusted 55 °C UASBs. UASB-B received daily dosage of 0.2 N HCl between days 113 and 229.
3.3. System methane production

The 55 °C UASB–LB systems were operated for up to 571 days. Systems A and B produced a total of 1107 and 1366 l of CH\textsubscript{4}, with respective methane yields of 0.219 and 0.272 m\textsuperscript{3}/kg wet weight. The LBs contributed an average of 77.7% to the system CH\textsubscript{4}.

4. Discussion

Methane from the LBs of this study typically appeared earlier than from those of previous studies (Chen, 1999, 2000), where the connecting UASBs were maintained at 35 °C, although the fermentation patterns in both studies were comparable. Since the LBs relied on their connecting UASBs for carried-over methanogens, earlier methane production indicates earlier establishment of methanogenic community in the LBs. However, maturation of the first set of LBs in this study came about 60 days later than comparable LBs in the previous study (Chen, 1999), even though the LBs in this study were maintained at warmer temperatures (Fig. 6a). The first set of LBs in the present study took an average of 138 days to mature. Since maturation of LBs depended on accumulation of threshold amounts of un-granulated sludge (Chen and Wang, 1998), slower maturation indicates slower attainment of that threshold.

Fig. 6. Comparing performances of the 55 °C UASB-A from the present study to that of the 35 °C UASBs from previous study (Chen, 2000). Thick lines represent data from the present study while thin lines represent data from the previous study.
Indeed, sludge accumulation in the first set of LBs of the present study was much slower than that of corresponding LBs in the previous study (Fig. 7a), probably due to the much smaller liquid transfer rates of the present study (Fig. 8). Maturation of the second and third sets of LBs in both studies took about the same time after being connected to their respective UASBs. This is consistent with their rates of sludge accumulation being comparable (Fig. 7b and c). Average methane yields for both systems were comparable, although it is apparent that the system with 35 °C UASB was more efficient. It took the system with 35 °C UASB about 210 days to reach its final yield whereas the system with 55 °C UASB took over 500 days to reach its final yield (data not shown).

For the first sets of LBs of the present study, LB-1B matured faster than LB-1A did. However, for the second and third sets of LBs, the LB-Bs took more than twice the time to mature than did the LB-As (Table 1). This seems to contradict the observations that the connecting UASB-B was performing much better than UASB-A after day 114 (Fig. 5). However, this paradox could be resolved by understanding the way maturation was defined. Maturation of a LB was somewhat arbitrarily defined as the point when methane content in biogas from the LB rose beyond 75% (Chen, 1999). Therefore, if the pH of a near mature LB is lowered by adding an acid reagent, thus causing more CO₂ to be released into its biogas, the LB might appear to be immature even when it has entered accelerated methanogenic stage. Indeed, methane production rates from the LB-Bs were mostly slightly higher than that from comparable LB-As. However, methane content of LB-2B and LB-3B rose to beyond 75% later than that of comparable LB-As (Fig. 9), probably due to the formers' slightly lower pH. The pH of the LB-Bs was slightly lower because effluent being transferred from the UASB-B had lower pH (Fig. 5a) as a result of daily dosage of 0.2 N HCl.

The 55 °C UASBs were hard to start-up. Chen (2000) was able to raise 35 °C UASBs’ LRs from 0.5 to 2 kg COD/m³/day in 48 days after start-up. The LRs were further successfully increased to 4 kg COD/m³/day in another 60 days, and to above 5 kg COD/m³/day in yet another 55 days. In comparison, the 55 °C UASBs in the present study could only achieve a LR of
2 kg COD/m³/day during their entire period of operation. One probable cause for this difficulty was the abrupt temperature change experienced by the inoculum. We started the 55 °C UASBs by directly placing the reactors containing mesophilic inoculum into the 55 °C chamber. Although reports varied on susceptibility of thermophilic anaerobic sludge to temperature fluctuations (van Lier et al., 1993; Dinsdale et al., 1997b; Fang and Lau, 1996), several researchers have shown that thermophilic UASBs started up with mesophilic granules performed poorly immediately after the temperature increase (Fang and Lau, 1996; van Lier et al., 1992). Fang and Lau (1996) found step-raising reactor temperature from 37 to 55 °C caused washout, disintegrated granules and deteriorated COD removal efficiency. van Lier et al. (1992) found step-raising reactor temperature from 38 to 55 °C immediately led to a sharp drop in methane production rate. Even though they were able to re-establish stable methanogenesis within 2 weeks, however, after 2–3 months of operation, the granules’ structure became spongy and finally fell apart, causing significant washout. The mesophilic granular sludge used as inoculum in the present study had been stored for several days at ambient temperatures of about 22 °C prior to start-up. Thus, the abrupt temperature increase to 55 °C upon start-up could have caused significant disintegration of the granules. Effluent sludge concentrations for the 55 °C UASBs of this study, especially during the time when they were connected to their first LBs, were much higher than those for the 35 °C UASBs in the previous study (Chen, 2000) (Fig. 10). Consequently, it is possible that significant amount of
the disintegrated granules was being washed out from the 55 ºC UASBs. This might have prevented the 55 ºC UASBs from experiencing a smooth start-up and achieving higher loading rates.

The 55 ºC UASBs also showed signs of inhibition. They consistently had higher effluent VFAs and CODs (Fig. 6b and c) than did the 35 ºC UASBs of the previous study (Chen, 2000). Although thermophilic systems can be operated stably at higher VFA levels (Angelidaki and Ahring, 1994; Dinsdale et al., 1997b), especially higher propionate fraction (Wiegant et al., 1985), lower COD reduction rate and lower methane production rate (Fig. 6d and e) clearly indicate inhibited methanogenesis.

5. Conclusions

The 55 ºC UASB of this LB–UASB system treating poultry mortality was difficult to start-up. It also showed signs of inhibited methanogenesis. Overall, the LB–UASB system with its UASB maintained at 55 ºC was not as efficient as another similar system where the UASB was kept at 35 ºC. In view of these difficulties, it is not recommended that the UASB of a temperature-phased LB–UASB system treating poultry mortality be kept at 55 ºC.

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References