Improved Anaerobic Acidification of Unscreened Dairy Manure

Vedat Yılmaz and Göksel N. Demirer*

Department of Environmental Engineering
Middle East Technical University
Inönü Bulvari, 06531, Ankara, Turkey

ABSTRACT

Even though several aspects of two-phase configuration might be very significant for efficient anaerobic digestion (AD) of dairy manure, its application has very been limited. Two sets of experiments daily-fed continuously mixed acidogenic anaerobic reactors with no recycle were conducted to investigate the optimum conditions on anaerobic acidification of unscreened dairy manure in terms of maximum acidification and VS reduction. Effect of solids and hydraulic retention time (SRT/HRT), organic loading rate (OLR), and pH control on anaerobic acidification in terms of volatile fatty acid (VFA) formation and volatile solids (VS) reduction were investigated. Results indicated that SRT/HRT and OLR of 2 days and 15 g VS/L · day, respectively, yielded maximum acidification. The pH control was not effective on the acidification of dairy manure. The degree of acidification increases as HRT decreases, and also higher efficiency was determined in an uncontrolled reactor.

Key words: anaerobic; digestion; acidification; manure

INTRODUCTION

The rapid growth in the size of dairy operations has resulted in new laws and regulations governing the handling and disposal of manure. Requirements for nutrient management plans, manure solids disposal, and odor control make it necessary that new manure management approaches be considered. One of the more promising methods is anaerobic digestion (Burke, 2001). Once the material is viewed as a resource rather than a waste and is properly managed, it will be easier to meet government regulatory standards on air and water quality.

Anaerobic digestion (AD) of organic matter is a process used for treatment of organic waste and production of energy. This technology became more attractive in the recent past because new reactor designs significantly improved the reactor performance (Mladenovska and Ahring, 2000). AD is also an effective way to convert organic wastes including animal manure into profitable byproducts as well as to reduce the pollution of water, air, and soil caused by these wastes (Demirer and Chen, 2004a).

The growing interest in the technology can be attributed to anaerobic digestion’s potential for reducing pathogen levels and weed seeds, controlling odour, improving fertilizer value, and producing value-added products such as energy-rich methane; all of which can help remedy or alleviate many of the economic and environmental issues facing the growers and society. Extensive research has been conducted and

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*Corresponding author: Department of Environmental Engineering, Middle East Technical University, Inönü Bulvari, 06531, Ankara, Turkey. Phone: +90-312-210 58 67; Fax: +90-312 210 26 46; E-mail: goksel@metu.edu.tr
well documented on the feasibility of anaerobic treatment of farm animal manure and its advantages, reactor types used, performance, etc. (Hobson and Wheatley, 1993; Demirer et al., 2000; Wilkie, 2000; Demirer and Chen, 2004a, 2004b, 2005; Gungor-Demirci and Demirer, 2004).

In AD, complex organics, such as polysaccharides, proteins, and lipids, are first hydrolyzed by enzymes, forming sugars, amino acids, and fatty acids. These intermediate products are then degraded by acidogens, forming volatile fatty acids (VFAs), which are further degraded by acetogens, forming acetate, carbon dioxide, and hydrogen. Last, both acetate and H₂/CO₂, are converted by methanogens to methane (Harper and Pohland, 1986). Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens. This usually results in the accumulation of organic acids and lowering of pH, leading to the suppression of methanogenic activities and, in some cases, even process failure (Zoetemeyer et al., 1982).

Conventional one-phase slurry digestion is not an effective system for wastes containing high solids (>10%), because they require the manure to be capable of being pumped, which in itself necessitates a concentration below 10% solids. This, in turn, results in a significant increase in fluid and digester volume which results in increased capital and operating costs (Demirer and Chen, 2004a). Instability or failure of single-phase methanogenic digesters has been widely reported for a variety of wastewaters, especially under high loading conditions (Fox and Pohland, 1994; Ghosh et al., 1995). Problems encountered with stability and control in conventional design applications have led researchers to new solutions (Demirel and Yenigun, 2002).

To improve the process stability and efficiency, the concept of two-phase reactor was thus proposed. In a two-phase reactor system, acidification is conducted in the first reactor followed by acetogenesis and methanogenesis in the second (Pohland and Ghosh, 1971; Massey and Pohland, 1978). Although the proper operational conditions for the acetogenic/methanogenic phase have been extensively studied, little information is available for the aci-
dogenic phase (Elefsiniotis and Oldham, 1994; Fox and Pohland, 1994). The lack of such knowledge is one of the major barriers for the widespread of the two-phase process (Ghosh et al., 1995).

About 90% of the full-scale plants currently in use in Europe for AD of solid wastes and biowastes rely 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, multistage, or batch systems as on one-stage systems. A likely reason for this discrepancy is that two- and multistage 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.

There are several important advantages that are intrinsic to the two-phase digestion process design. First, because each stage is optimized for maximizing the performance of the two distinctly different microbial communities, the digestion system is substantially more stable in performance than a conventional single-stage process that must be sub-
optional for one or both populations of bacteria. The stability of two-phase digestion over conventional (single stage) digestion has been documented in the literature for the conversion of solid waste and sewage sludge to biogas (Ghosh, 1985; Ghosh et al., 1995).

Even though several aspects of two-phase configuration including liquification might be very significant for efficient AD of dairy manure, its application has very been limited. Burke (2001) also pointed out the fact that phased digestion has not been applied to dairy waste. Our recent research on the subject indicates a strong potential of enhancing AD of animal manure (Demirer and Chen, 2004a, 2005). In recognition of these facts and in support of their needed application to high solids containing dairy manure, it was aimed in this study to investigate the optimum conditions for anaer-
obic acidification of unscreened dairy manure in daily-fed continuously mixed reactors with no recycle leading to max-
imum acidification and volatile solids (VS) reduction.

**MATERIALS AND METHODS**

**Dairy manure and anaerobic seed cultures**

Wet manure was collected from a private Dairy around Gölbapi, Ankara, and stored at 4°C prior to use. The composition of the dairy manure used in this study had the following characteristics; total solids (TS) 21 ± 1.5%, total volatile solids, 65 ± 3.9% of TS and density, 1,042 ± 0.04 g/L. The raw manure was diluted with water to decrease the

<table>
<thead>
<tr>
<th>Reactor</th>
<th>SRT (days)</th>
<th>OLR (g VS/L day)</th>
<th>Volume of feeding/wasting (mL)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>5</td>
<td>25</td>
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<tr>
<td>2</td>
<td>4</td>
<td>10</td>
<td>25</td>
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<td>15</td>
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<td>6</td>
<td>2</td>
<td>15</td>
<td>50</td>
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<td>7</td>
<td>1.25</td>
<td>5</td>
<td>80</td>
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<tr>
<td>8</td>
<td>1.25</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>1.25</td>
<td>15</td>
<td>80</td>
</tr>
</tbody>
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SRT, sludge retention times; OLR, organic loading rate.
solids content to achieve a slurry with 5, 10, and 15 g VS/L. The relationship between chemical oxygen demand (COD) and VS of this manure was found as 1.04.

The mixed anaerobic culture used as seed was obtained from the anaerobic sludge digesters at the Ankara wastewater treatment plant, which has a sludge retention time (SRT) of 14 days. The mixed anaerobic culture was concentrated by settling before being used as inoculum. The volatile suspended solids (VSS) concentration of the concentrated seed cultures used was 23,930 ± 3,162 mg/L.

**Experimental setup**

In the first part of the study, the optimum retention time and organic loading rate (OLR) values leading to maximum acidification and VS reduction were investigated. Thus, nine daily-fed continuously mixed acidogenic anaerobic reactors with no recycle were operated as duplicates. The total volume of reactors was 250 mL. Reactor operation involved daily feeding of wet dairy manure and wasting of the corresponding reactor contents as indicated in Table 1. Solids and hydraulic retention times (SRT/HRT) applied to each reactor was the same, because no recycle of the effluent was practiced. Initially, each reactor was seeded with 100 mL of concentrated anaerobic seed cultures. The reactors were flushed with N₂/CO₂ gas mixture for 3 min and maintained in an incubator shaker at 35 ± 1°C and 165 rpm. Eight stock solutions for nine reactors were prepared prior to reactor feeding for targeted VS concentration.

**Figure 1.** pH, T-VFA, VS, CH₄ percentage, biogas production, and sCOD values observed during the first part of the experiment.

**Figure 2.** Theoretical no VS reduction and experimental VS concentrations in the reactors at steady state.
centrations. The next day dairy manure (25 mL to reactors 1–4, 50 mL to reactors 5–8, and 80 mL to reactors 9–12) were added to each reactor. Daily feeding and wasting were conducted as seen in Table 1.

In the second part of the study, the effect of the pH control on acidification of dairy manure was investigated. Two similar reactors with a working volume of 800 mL were fed with 400 mL mixed anaerobic culture and 400 mL of wet dairy manure. Both reactors were operated at an HRT/SRT of 2 days and OLR of 15 g VS/L·day. To determine the effect of pH control, pH of one of the reactors was not controlled, while the other one was set to a constant value in the range of 5.0–5.5 using a pH-stat unit.

**Analytical methods**

The daily pH, daily gas production, methane percentage, TS, VS, total volatile fatty acids (TVFA) and effluent soluble chemical oxygen demand (sCOD) contents of dairy waste of each reactor were periodically measured in each reactor. pH, sCOD, TS, and VS analysis were performed using Standard Methods (APHA, 1995).

For VFA analyses a gas chromatography (GC) (Thermo Electron Co., Manorville, NY) equipped with a flame ionization detector and ZB-FFAP column (30 m, 0.25 mm i.d.) was used. The column was set to 100°C with a 2-min holding time and then increased to 250°C with 8°C/min ramping. The injector/detector temperature was kept at 200/350°C. Helium was used as the carrier gas with a flow rate of 30 mL/min. The gas flow rates were gauged at 350 mL/min for air and 35 mL/min for hydrogen. Reactor effluents were initially centrifuged for 20 min at 4,000 rpm and then the supernatant was filtered by a 0.22 mm glass fiber filter (Whatman Co., Florham Park, NJ). The filtered samples were acidified with 98% formic acid to a pH less than 3 to convert the fatty acids to their undissociated forms (i.e., acid forms) before injection to GC. The concentration of each VFA species (i.e., acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) was expressed in terms of acetic acid equivalents by dividing the concentration value by its molecular weight and multiplying by the molecular weight of acetic acid. TVFA was determined by the sum of the concentrations of all VFA species expressed in terms of acetic acid equivalents.

Total gas produced in the reactors was measured by connecting the reactor headspace to a water displacement column filled with distilled water and recording the volume of displaced solution. Gas samples for gas composition analysis were periodically withdrawn from the reactors by a 100 μL Hamilton gas-tight glass syringe. Gas composition was determined by a GC (Shimadzu 8A) equipped with thermal conductivity detector. CH4, N2, and CO2 were separated through a Porapak Q column (2 m, 5 mm i.d.). Column was operated with helium as the carrier gas at a constant pres-

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**Table 2. Comparison of the reactors with selected parameters.**

<table>
<thead>
<tr>
<th>Reactor</th>
<th>VS Reduction (%)</th>
<th>TVFA (mg/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.4</td>
<td>806</td>
<td>6.53</td>
</tr>
<tr>
<td>2</td>
<td>14.5</td>
<td>1,444</td>
<td>6.38</td>
</tr>
<tr>
<td>3</td>
<td>19.5</td>
<td>2,236</td>
<td>6.29</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>399</td>
<td>6.54</td>
</tr>
<tr>
<td>5</td>
<td>8.9</td>
<td>476</td>
<td>6.42</td>
</tr>
<tr>
<td>6</td>
<td>14.8</td>
<td>1,300</td>
<td>6.24</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>412</td>
<td>6.57</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>400</td>
<td>6.52</td>
</tr>
<tr>
<td>9</td>
<td>2.3</td>
<td>647</td>
<td>6.45</td>
</tr>
</tbody>
</table>

VS, volatile solids; TVFA, total volatile fatty acids.

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**Figure 3.** VFA production per gram VS fed and SRT at different SRTs
sure of 20 kPa at 40°C. The injector and detector temperatures were set to 100°C.

RESULTS AND DISCUSSION

SRT and OLR effect on acidification

Nine acidogenic anaerobic reactors were operated for 57 days to determine the optimum SRT and OLR values resulting in maximum acidification, and in turn, VS reduction. Three different OLRs (5, 10, and 15 g VS/L · day) were applied to the reactors. For each OLR value, three SRTs (1.25, 2, and 4 days) were studied (Table 1). The results are given in Fig. 1 in terms of the change in the operating parameters [pH, TVFA, VS, cumulative gas production (CGP), methane content, and scCOD] with respect to the combination of OLR and SRT values. Figure 1 does not include the data points within the first “3 × SRT” days (12 days for R1–R3, 6 days for R4–R6, and 4 days for R7–R9), which is the theoretical time to reach to steady-state conditions in a continuous reactor.

As seen in Fig. 1a, pH drop was inversely proportional with the increase in the SRT for each OLR studied. Similarly, for each SRT studied, as the OLR increased, pH decreased. It was observed that the extent of pH drop increased with the increase in the OLR being smallest for the lowest OLR of 5 g VS/L · day. Besides, it should be noted that the extent of pH drop was also affected by the SRT. For all the OLRs studied, the extent of pH drop for the SRT increase from 1.25 to 2 days was greater than that observed for SRT of 2 to 4 days. It is a well-known fact that low retention times and high loading rates lead to higher acidification in two-phase systems. However, as seen in Figure 1a, average pH values observed in the reactors were within 6.2–6.6 and the extent of pH drops was lower relative to acidification of other high solid substrates such as organic fraction of municipal solid wastes. Han et al. (2002) operated the MUS-TAC (multistep sequential batch two-phase anaerobic composting) process to recover methane and composted material from food waste, where the pH ranged between 6.5 and 7.0 during the acidogenic fermentation step. In another research, Kübler and Schertler (1994) demonstrated that favorable pH condition was 6.7 in the three-phase anaerobic degradation of solid waste. Verrier et al. (1987) stated that both mesophilic and thermophilic liquefaction and acidogenesis of vegetable solid wastes were found to be maximal when the pH was maintained at approximately 6.5 in the hydrolysis reactor. The relatively high pH values observed in our study can be explained by the alkalinity generated by the anaerobic biodegradation of nitrogenous organic compounds contained in the dairy manure used in this study (Ghosh, 1987; Speece, 1996; Wang et al., 2003). The similar self-buffering capacity of the manure was also observed in other acidification studies (Demirer and Chen, 2004a, 2005).

As expected, the increase in the OLR resulted in the increase in the TVFA production (Fig. 1b). In addition, the extent of TVFA production for the SRT increase from 1.25 to 2 days was greater than that observed for SRT increase from 2 to 4 days, especially for OLRs of 10 and 15 g VS/L · day. This observation was also verified by the extent of pH drop (being greater for SRT increase from 1.25 to 2
days). These TVFA production trends for all reactors coincided with the sCOD productions (Fig. 1c) which increased with the increased OLR and SRT.

The effect of SRT and OLR on TVFA production was also observed for CGP data. As the OLR and SRT increased the CGP in the reactors increased (Fig. 1d). It is well known that in addition to VFAs and alcohols both \( \text{H}_2 \) and \( \text{CO}_2 \) are produced through acidification. However, GC analyses unexpectedly indicated that methane was produced in all of the reactors studied at varied OLRs and SRTs (Fig. 1e). Especially, methane percent of the biogas increased from 5 to 15–27% when SRTs and OLRs were increased to greater values than 1.25 days and 5 g VS/L·day, respectively. Although the pH conditions were close to the optimum operating conditions of highly organic wastes required for acetogenesis. The applied SRT values (1.25 to 4 days) were not favorable for the most sensitive anaerobic bacteria type known as methanogens. The methane production at such low SRTs could be explained by unintentional extended retention times of microorganisms in the reactors due to very high solids concentration and thus lack of homogeneity during daily wasting of sludge. GC analyses also indicated a sig-

\[ \text{Figure 5. T-VFA, VS, sCOD, and gas composition values observed during the second part of the experiment.} \]
nificant amount of N\textsubscript{2} in the biogas of all reactors changing from 35 to 90\% (data was not shown). As expected, denitrification was more dominant at the higher oxidation-reduction potential at the beginning of the experiment. Denitrification might occur during the acidogenic phase, so as to achieve simultaneous VFA production and nitrate elimination, a system could be applied to organic carbon and nitrogen removal from the wastes (Rustrian et al., 1999; Vigneron et al., 2006).

Better hydrolysis in acidification process means higher VS reduction. Therefore, in addition to pH and TVFA production, VS is among the critical parameters in determination of the acidification extent of dairy manure known with its high solids content. The average VS concentrations observed in the reactors at varied SRT and OLR combinations were given in Fig. 1e. It was observed that increasing the OLR and SRT resulted in the VS accumulation. However, due to the continuous feeding and wasting process, such an accumulation may not clearly indicate the possible VS reduction in the reactors. Therefore, a completely stirred tank reactor (CSTR) system modeling was performed to observe the change in the VS content of the reactors at steady-state conditions. In this CSTR model, each reactor was accepted as reactors, which were operated under feeding and wasting process without any destruction/degradation of the feeding. The results or the change in the VS content of the reactors studied under varied SRTs and OLRs are depicted as theoretical VS concentration in Fig. 2. Experimentally found average VS concentrations of the reactors (Fig. 1f) were also depicted in Fig. 2 to assess the VS reduction at each OLR and corresponding SRT value studied. For better comparison, percent VS reduction in each reactor was calculated by considering the theoretical and experimental VS concentrations and given in Table 2.

As seen in Fig. 2 and Table 2, as OLR and SRT increased the percent VS reduction increased. The greatest VS removal was seen at R3 with 19.5\%, which is followed by R6 with 14.8\%. The third highest VS removal was observed in R2 with 14.5\%. The rest of the reactors did not display a significant VS reduction performance. Especially in the reactors operated at SRT of 1.25 days (R7–R9) almost no VS destruction was observed (Table 2).


degree of acidification

Reactors and their acidification performances were also compared in terms of acidification extent and the rate of product formation. The degree of acidification can be quantified using the percentage of the initial substrate concentration converted to VFAs (Dinopoulou et al., 1988). The initial substrate concentration (S\textsubscript{i}) was measured in mg COD/L and the quantity of VFAs was converted to the theoretical equivalent in mg COD/L (S\textsubscript{p}), using the COD equivalents for each VFA (Demirel and Yenigun, 2004). The following formula was used to express the degree of acidification in this work:

\[
\text{Degree of acidification (\%)} = \left( \frac{S_p}{S_i} \right) \times 100
\]

The COD equivalents of each volatile acids for the conversion were taken as follows: acetic acid, 1.066; propionic acid, 1.512; butyric acid, 1.816; valeric, 2.036; caproic acid, 2.204.

Higher organic loadings and shorter HRTs (or SRTs) were previously reported to provide the optimum conditions for the acid-forming bacteria (Gosh et al., 1985; Elefsiniotis ad Oldham, 1994). When the VFA production per gram VS fed at different SRTs is considered (Fig. 3a), it is seen that the OLR did not affect the VFA yield. However, if the VFA production per SRT is considered, it will be seen that the maximum VFA production yield is obtained at SRT of 2 days both at 10 and 15 g/L-day OLRs (Fig. 3b). Note that normalizing VFA production with the SRT of the reactor (Fig. 3b) is not conventional. However, it provides information on the extent of acidification, which can be obtained in unit reactor size (volume) simply because SRT determines the reactor size.

As previously mentioned, R6 yielded the second highest VS reduction (14.8\%) and the lowest pH among all the reactors operated (Table 2). Moreover, TVFA concentration and degree of acidification in R6 was greater than most of the reactors (Fig. 4). It is known that the increase in HRT (or SRT) value results in the increase in the investment and capital costs of the treatment systems. Therefore, considering both from the economical point of view and acidification performance in terms of TVFA production and VS reduction, SRT of 2 days and OLR of 15 g VS/L·day were selected as the optimum operational conditions for acidification of dairy manure (R\textsuperscript{*}). The selected conditions were used in the second part of the study where the optimum pH or pH effect on acidification of dairy manure was investi-
gated in two reactors. The selected operating parameters were also harmonious with the relevant literature (Demirer and Chen, 2004a).

The pH effect on acidification

In one of the reactors (R*-pH) pH was set to a constant value around 5.0–5.5 using a pH-stat unit. In the other reactor (R*) pH was not controlled. Both reactors were operated for 42 days. During this period, pH varied between 6.2–6.4 in R* (uncontrolled reactor) while it was 5.3–5.5 in R*-pH. The optimum pH range of acidogenic bacteria is 5.2–6.5, while it is 6.6–8.5 for acetogenic/methanogenic bacteria (Demirer and Chen, 2004b). Thus, it was thought that methanogenesis that was experienced in the first part of the study could be eliminated, and optimum acidification conditions, and in turn maximum acidification, could be achieved in R*-pH at a lower pH of 5.3–5.5. As expected, methanogenesis was inhibited in R*-pH, while methane percent of the biogas content varied around 5–7% in R* (Fig. 5a). However, acidification efficiency was just the opposite of the expectations. In terms of TVFA production, uncontrolled reactor (R*) displayed a better performance. Peak TVFA concentration observed in R* reached up to 2,300 mg/L (as TVFA), while it was only 980 mg/L in R*-pH (Fig. 5b–c). Better hydrolysis, and in turn acidification conditions, were also verified by the sCOD analyses where the concentrations of R* were equal and greater than that of R*-pH most of the time (Fig. 5d). In both of the reactors, acetic and propionic acids were the main VFA products, whereas butyric, i-butyric, valeric, i-valeric and caproic acids were also present, but in substantially lower quantities. The main fermentation pathway was found as acetic acid fermentation, which was mainly suppressed in the R*-pH, and thus resulted in lower TVFA production. This is relevant with the literature reporting the favorable pH conditions for acetic acid production as 6.0–6.5 (Yu and Fang, 2002).

As in the first part of the study, a similar approach was used to compare the VS removal efficiency of the reactors. The CSTR system model was performed to calculate the theoretical VS concentrations in the reactors under the operational case of feeding/wasting but no degradation/destruction. The theoretical VS and experimental VS concentration of the reactors is depicted in Fig. 5e. Also, the pH controlled reactor had a lower acidification degree than the uncontrolled reactor (Fig. 6).

CONCLUSIONS

This study investigated the two-phase AD of dairy manure with particular emphasis on the effects of retention time and OLR on anaerobic acidification of unscreened dairy manure. The results indicated that:

- Preacidification of dairy manure in daily-fed continuously mixed reactors with no recycle led to significant VFA production.
- The main acidification products of the dairy manure studied were acetate and propionate corresponding to the carbohydrate acidification. The higher molecular weight of VFAs, including valeric and i-valeric acid were at low concentrations.
- Based on both minimizing the reactor volumes and the acidification performance in terms of TVFA production and VS reduction, SRT of 2 days and OLR of 15 g VS/L · day were selected as the optimum operational conditions for the anaerobic acidification phase.
- The pH control between 5.3 and 5.5 did not improve the extent of acidification.

ACKNOWLEDGMENTS

This study was funded by The Scientific and Technological Research Council of Turkey through Grant Number 1041127.

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