Research Paper

Volatile fatty acids build-up and its effect on *E. coli* inactivation during excreta stabilisation in single-stage and two-stage systems

Joy Riungu, Mariska Ronteltap and Jules B. van Lier

ABSTRACT

Digestion and co-digestion of faecal matter collected from urine diverting dehydrating toilet faeces (UDDT-F) and mixed organic market waste (OMW) was studied in single stage pilot scale mesophilic plug-flow anaerobic reactors at UDDT-F:OMW ratios 4:1 and 1:0. *Escherichia coli* inactivation and volatile fatty acids (VFA) build-up was monitored at sampling points located along the reactor profile. When applying UDDT-F:OMW ratio of 4:1 at 12% total solids (TS), *E. coli* inactivation achieved was 2.3 log times higher than that achieved in UDDT-F:OMW ratio of 1:0. In subsequent trials, a two-stage reactor was researched, applying a UDDT-F:OMW ratio of 4:1 and 10 or 12% TS slurry concentrations. Highest VFA concentrations of 16.3 ± 1.3 g/L were obtained at a pH of 4.9 in the hydrolysis/acidogenesis reactor, applying a UDDT-F:OMW ratio of 4:1 and 12% TS, corresponding to a non-dissociated (ND)-VFA concentration of 6.9 ± 2.0 g/L. The corresponding decay rate reached a value of 1.6 per day. In the subsequent methanogenic plug-flow reactor, a decay rate of 1.1 per day was attained within the first third part of the reactor length, which declined to 0.6 per day within the last third part of the reactor length. Results show that a two-stage system is an efficient way to enhance pathogen inactivation during anaerobic digestion.

Key words | anaerobic (CO) digestion, non-dissociated volatile fatty acids, pathogen inactivation, UDDT faeces

INTRODUCTION

Ecological sanitation concepts have been developed due to the growing need for improved onsite sanitation systems aimed at the protection of human and environmental health (Esrey 2001; Niwagaba et al. 2009). Urine Diverting Dehydrating Toilets (UDDTs) fit well into this concept, especially in densely populated, low lying settlements (Niwagaba et al. 2009; Schouten & Mathenge 2010; Katukiza et al. 2012). The technology has been adopted by Sanergy (Nairobi, Kenya), a company working on sanitation in informal slum settlements. Currently, from Mukuru Kwa Njenga and Mukuru Kwa Reuben, informal slum settlements, approximately 700 kg UDDT-faeces (UDDT-F) are delivered per day to the central treatment plant, located 50 km from the city center. A key concern is stabilisation and sanitisation of the waste as the addition of ash and sawdust after toilet use is insufficient for pathogen inactivation (Niwagaba et al. 2009).

Anaerobic digestion (AD) provides a cost effective and energy saving alternative for waste treatment (Nallathambi Gunaseelan 1997; Avery et al. 2014; Romero-Güiza et al. 2014; Fonoll et al. 2015). Anaerobic systems can be applied at any scale and almost any place, whereas the effluent is
stabilised with good fertiliser value for agriculture use (Van Lier et al. 2008; Pabón-Pereira et al. 2014). A key reported drawback, however, is insufficient pathogen inactivation (Chaggu 2004; Horan et al. 2004; Kunte et al. 2000; Massé et al. 2011; Chen et al. 2012; Fagbohungbe et al. 2015), with solid and liquid digestate containing high levels of pathogenic bacteria such as Salmonella, Shigella and Vibrio cholerae (Kunte et al. 1998, 2000; Fagbohungbe et al. 2015). As such, the poor microbial quality of the digested solids may lead to transmission of enteric diseases when applied to agricultural land (Pennington 2001; Smith et al. 2005).

During anaerobic digestion, temperature and time play a key role in pathogen inactivation (Olsen et al. 1985; Olsen & Larsen 1987; Gibbs et al. 1995; Smith et al. 2005), as does reactor configuration (Olsen et al. 1985; Kearney et al. 1993). In addition, pH and volatile fatty acids (VFA) concentration in the reactor broth are an indication for bacterial survival (Sahlström et al. 2008). At a low reactor pH, the same amount of VFAs lead to a higher fraction of non-dissociated VFAs (ND-VFAs), which may result in higher microbial decay: ND-VFAs pass freely bacterial cell walls by passive diffusion and affect the internal pH (Gallert et al. 1998; Murto et al. 2004; Fonoll et al. 2015; Franke-Whittle et al. 2014) and hence reduces the options of using ND-VFAs for pathogen inactivation. By co-digesting human waste (UDDT-F) with mixed organic market waste (OMW), acid formation is enhanced, since OMW is carbohydrate rich and easily hydrolysable (Gómez et al. 2006; Lim et al. 2008).

Enhanced build-up of total VFA (TVFA) concentrations during co-digestion of sewage sludge and other organic waste can be achieved by inhibition of methanogenesis (Wang et al. 2014), through use of a two-stage reactor system, where hydrolysis/acidogenesis and methanogenesis are separated. The different species of microorganisms involved in the AD process can be divided into two main groups of bacteria, namely organic acid producing and organic acid consuming or methane forming microorganisms (Rincón et al. 2008). They operate under different pH conditions: whereas the optimal pH for acidogenic bacteria activity ranges between 5 and 7 (Fang & Liu 2002; Noike et al. 2005; Liu et al. 2006; Guo et al. 2010), methanogenic activity requires a minimum pH of 6.5 (Yuan et al. 2006; Wang et al. 2014). A key drawback in the two-stage reactor is the high VFA concentration in the acidogenic reactor, which requires pH correction for stable methanogenesis (Zuo et al. 2014). Yet, the low pH and high VFA concentrations create very good pathogen inactivating conditions. Hence, an optimum must be found between good hygienisation and well-functioning methanogenic stabilisation. In practice, the latter can be achieved by recycling part of the digestate upfront to be mixed with the acidified UDDT-F-OMW.

In our recent study, we evaluated the effect of UDDT-F and OMW mix ratios on VFA build-up and Escherichia coli inactivation in laboratory scale batch anaerobic reactors, within a retention time of 4 days. E. coli inactivation was a function of the OMW fraction in the substrate, increasing as the fraction increased (Riungu et al. 2018). The ratio appropriateness depends on the required degree of sanitisation, final pH values in the final digestate, and obviously the availability of OMW.

This study evaluates the potential for pathogen inactivation in anaerobic digestion, co-digesting UDDT-F and OMW, using pilot scale plug-flow reactors. In particular, the study results will give a comparison of E. coli inactivation from single and two-stage plug-flow reactors.

MATERIALS AND METHODS

Materials

UDDT-faeces (UDDT-F) waste samples

UDDT-F samples used for this study were obtained from the Fresh Life© urine diverting dry (UDDT) toilets within Mukuru Kwa Njenga/Mukuru Kwa Reuben informal slum settlement, Kenya. The Fresh Life© toilets are fabricated and installed by a social enterprise, Sanergy, in collaboration with entrepreneurs in the slums who maintain them. The toilets are provided on a pay and use basis, charging approximately 0.05 euro/use and have an average user load of 50 persons/day. Within each toilet facility, a 30 L container is used for waste collection, with approximately
10 g sawdust added after every toilet use. The toilets are emptied on a daily basis, where used containers are replaced with clean ones.

From a batch consisting of about 60 containers, ten containers were randomly selected after which mixing of the contents was done in order to obtain a homogeneous mix. Fifteen kg UDDT-F was then drawn and further mixing was carried out in order to homogenise the sample.

Mixed organic market waste samples (OMW)

Mixed OMW was obtained from Mukuru Kwa Njenga and Mukuru Kwa Reuben informal slum settlements. About 20 kg of the waste was collected daily and contained food, vegetable and fruit waste, in about equal proportions. Size reduction of OMW substrates for pilot scale tests was achieved by manual chopping to about 1 cm size. Table 1 shows the characteristics of the materials used in the study.

Experimental method

Pilot scale AD experiments

Two sets of reactors were used, namely a single stage reactor ($R_s$) and a two-stage reactor ($R_{sam}$) comprising a hydrolysis/acidogenic reactor ($R_h$) and a methanogenic reactor ($R_m$).

Experiments were conducted at a UDDT-F:OMW ratio 4:1, at 10 and 12% total solids (TS) concentrations. Substrate concentration selection was based on a series of laboratory scale batch-tests derived experimental data on the effect of substrate concentration on pathogen inactivation (Riungu et al. 2018). Research was aimed at treating the highest possible TS concentration that can freely flow through the plug-flow reactor without the necessity of using pumps.

Hydrolysis reactor design

The single stage reactors $R_s$’s were fabricated from 30 L plastic containers, with a working volume of 20 L. These reactors were equipped with a cover, incorporated with two separate ports, i.e. a feeding port and a port fixed with a manual stirring mechanism, whereas the bottom of each reactor was equipped with a discharge/effluent valve.

Plug flow reactor design

Six plug flow digesters (Figure 1) were constructed using 175 L tubular polyethylene bags. Each of the bags had a diameter of 30 cm and a length of 2.1 m and the polyethylene material had a thickness of 0.2 mm. Produced biogas flowed by pressure to a 175 L biogas storage bag that was installed directly above each reactor. In addition, three separate ports were incorporated onto each bag namely: inlet port (SP$_1$); a sampling port (SP$_2$) at 0.7 m digester length; a gas discharge port at 1.4 m digester length; and an effluent/discharge port (SP$_3$) at 2.1 m digester length. A total solids retention time (SRT) of 29 days was maintained for the anaerobic digestion process.

Table 1 Characterisation of UDDT-F and OMW used in the study (adopted from Riungu et al. 2018)

<p>| | | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>UDDT-F</td>
<td>OMW</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Value</td>
<td>STDEV</td>
<td>Value</td>
<td>STDEV</td>
</tr>
<tr>
<td>TS (% wgt)</td>
<td>24.5</td>
<td>3.8</td>
<td>17.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Moisture content</td>
<td>75.5</td>
<td>3.8</td>
<td>80.7</td>
<td>4.1</td>
</tr>
<tr>
<td>VS (% wgt)</td>
<td>20.1</td>
<td>3.5</td>
<td>16.9</td>
<td>4.4</td>
</tr>
<tr>
<td>TOC (g C/g TS)</td>
<td>64.4</td>
<td>7.7</td>
<td>54</td>
<td>4.3</td>
</tr>
<tr>
<td>COD$_{Total}$</td>
<td>195.3</td>
<td>5.9</td>
<td>139.6</td>
<td>10.1</td>
</tr>
<tr>
<td>$E. coli$ (CFU/g TS)</td>
<td>$1.7 	imes 10^9$</td>
<td>$5.3 	imes 10^8$</td>
<td>$2.7 	imes 10^5$</td>
<td>$7.4 	imes 10^4$</td>
</tr>
<tr>
<td>Ascaris eggs</td>
<td>Not detected</td>
<td>Not detected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Plug flow digester layout; reactors on the floor, biogas collection bags directly above; sampling points are indicated (SP1, 2 and 3).
Plug flow reactor start-up and operation in single substrate and co-digestion experiments

For smooth start-up, reactors were inoculated using inoculum obtained from fixed dome anaerobic digesters (operated by Umande Trust, Nairobi, Kenya, https://umande.org/). The inoculum upon collection was incubated for 1 week to methanise any organic matter before use.

The six plug flow reactors D1, D2, D3, D4, D5 and D6, were divided into two groups (D1, D3 and D5, and D2, D4 and D6), representing two treatment groups in single substrate digestion of a UDDT-F:OMW ratio 1:0 at 12% TS and 10% TS respectively. About 5 L/day of the appropriate substrate was fed to each respective digester every morning. Stabilisation of the digesters was achieved after 1.5 months, and sample collection and analysis commenced and continued for a further 9 weeks.

Co-digestion experiments with UDDT-F:OMW ratio 4:1 at 12% TS concentration commenced 15 weeks after the start-up. The experiments were aimed at comparing pathogen inactivation in single (Rs) and two-stage (Ram) anaerobic digestion processes. Three replications of two treatments groups Ram and Rs were set, with D1, D3 and D5 being Ram’s and D2, D4 and D6 being Rs’s. Each morning, a UDDT-F:OMW ratio of 4:1, 12% TS concentration was prepared after which 5 L of the substrate was fed into the Rs reactors. In the Ram reactor setup, effluent from Ra acted as influent to the Rm. Details on the design of Ra are provided below under ‘Total solids and volatile solids’. Two Ra’s were operated in parallel and every morning 5 L of effluent was drawn from each and mixed. pH of the mixture was adjusted to the range of 5.8–6.2 using effluent from Rm reactors. Thereafter, 6 L of the mix was fed to each of the three Rm’s (D1, D3 and D5) every morning.

Finally, the concentration of the feed into Rm was reduced to 10% TS. Thereafter, 100 mL of Ra, Rm and Rm’s influent and effluent were sampled for analysis of moisture content, total solids, volatile solids (VS), E. coli and VFA.

Analytical procedures

Total solids and volatile solids

Total solids and volatile solids analysis were conducted according to the gravimetric method (SM-2540D and SM-2540E), as outlined in Standard Methods for the Examination of Water and Wastewater (APHA 1995). pH measurement was carried out using a calibrated analogue pH/ORP meter (model HI8314-S/N 08586318).

VFA measurements

The method used is based on esterification of the carboxylic acids present in the sample and subsequent determination of the esters by the ferric hydroxamate reaction (DR 2800 Hach, June 2007 edition). The method has a measuring range of 27–2,800 mL/L. As such, homogenised samples were serially diluted (10−1–10−6) with de-ionised water to obtain the correct measuring range.

From the TVFA concentration, the fraction of ND-VFAs was calculated. VFAs are commonly considered to constitute a single weak-acid system with a single equilibrium constant Ka because of the similarity of their pK values (Moosbrugger et al. 1995; Lahav & Morgan 2004). Therefore,

\[\frac{\left[(H^+)(A^-)\right]}{(HA)} = Ka\]  

\[pH = pK_a + 10\log\left(\frac{A^-}{HA}\right)\]  

\[A_T = (HA) + (A^-)\]

where \(A_T\) = total VFA species concentration (mg/L), \(HA\) represents the acidic, protonated species and \(A^-\) is the ionised form of each acid.

Similarly, total organic carbon (TOC) and chemical oxygen demand (COD) measurements were carried out using protocols adopted from Hach spectrophotometer, DR 2800.

E. coli enumeration

E. coli, one of the indicator organisms for possible use of digestate coming from faecal matter in agriculture, was used as an indicator organism for pathogen inactivation. Its enumeration was carried out using a surface plate technique with Chromocult Coliform Agar (Chromocult: Merck, Darmstadt, Germany) (Byamukama et al. 2000; Mawioo et al. 2016).

The first order reaction coefficients for E. coli inactivation
were calculated using the Chick–Watson model that expresses the rate of inactivation of micro-organisms by a first order chemical reaction (Gerba 2008):

\[ \ln\left(\frac{C_t}{C_0}\right) = -kt \]  

(4)

where \( C_t \) = number of micro-organisms at time \( t \), \( C_0 \) = number of micro-organisms at time 0, \( k \) = decay rate, and \( t \) = time.

Using the results, \( E. \ coli \) inactivation (-ln(ct/co)) was plotted against time.

RESULTS AND DISCUSSION

Evaluation of the performance of single stage reactor (Rs) system

ND-VFA profiles

An evaluation of the performance of single stage plug flow reactor (Rs) was carried out using a UDDT-F:OMW ratio 4:1, 12% TS (Rs-4:1, 12%), UDDT-F: OMW ratio 1:0, 12% TS (Rs-1:0, 12%) and UDDT-F: OMW ratio 1:0, 10% TS (Rs-1:0, 10%) systems, with results shown in Figure 2(a)–2(c). Among the tested substrates, co-digestion (Rs-4:1, 12%) showed highest TVFA and ND-VFA build-up, with a 4-fold increase in ND-VFA and 3.2-fold increase in TVFA build-up being observed between influent (SP1) and SP2 sampling points. However, in Rs-1:0, 12% and Rs-1:0, 10%, a 6 and 6.5-fold decline in ND-VFA concentration was observed between sampling points SP1 and SP2 respectively, owing to an increase in the local pH. OMW, associated with rapid hydrolysis (Zhang et al. 2005, 2008; Riungu et al. 2018), enhanced the VFA build up in the digestion medium when used as co-substrate (Riungu et al. 2018), and thus increased the ND-VFA concentration, particularly when a concomitant pH drop is observed. However, a sharp decline in TVFA and ND-VFA concentration was observed between SP2 and SP3, which indicated proper methanogenic conditions in the final stages of the plug-flow reactor reaching pH values of 7.5. A decline in ND-VFA concentration in Rs-1:0, 12% and Rs-1:0, 10% reactors along the reactor length may be attributed to the high buffer capacity of UDDT-F.
substrate and prevailing methanogenic conditions. The high buffer capacity of the UDDT-F substrate may be attributed to the occasional wrong toilet use, collecting both urine and faeces in the same vessel, resulting in increased ammonium bicarbonate concentrations.

The effluent pH in single-stage single substrate and co-digestion experiments reactor set-ups was comparable and within the optimal range for methanogenic bacteria, i.e. 7.5–8.1 (Figure 2(a)–2(c)). pH control along these reactor profiles was self-regulatory. Between SP2 and SP3, whereas in single substrate reactors a gradual increase in pH was observed, the co-digestion reactor (Rs-4:1, 12%) showed similar pH in the influent (SP3), and the first sampling point (SP2) followed by a sharp increase between SP2 and SP3 (see Figure 2(c)). The low pH at SP2 resulted from OMW hydrolysis/acidification, which emphasises the importance of a proper UDDT-F:OMW ratio, avoiding full system acidification and potential failure. In full-scale systems, recycle flows may be used for pH regulations preserving methanogenic conditions in the final stage. Use of the recycle stream for pH adjustment in the two-stage reactor system was sufficient to guarantee methanogenic conditions in the plug-flow reactors.

**E. coli log inactivation in single substrate digestion**

Figure 3 depicts *E. coli* log inactivation trends in Rs-4:1, 12%, Rs-1:0,12 and Rs-1:0,10 at the three sampling points SP1, SP2 and SP3, located at 0, 0.7 and 2.1 m of the reactor length, respectively. The higher pathogen inactivation shown in Rs-4:1, 12% (Figure 3(c)) coincides with the prevailing higher maximum ND-VFA concentrations as a consequence of increased OMW hydrolysis/acidification. Whereas a decline in ND-VFA was observed between sampling points SP1 and SP2 in Rs-1:0, 12% and Rs-1:0, 10%, an increase was observed in the Rs-4:1, 12% system. The increase in ND-VFA allowed more contact time of the pathogens to the high ND-VFA concentrations, consequently leading to higher inactivation.

The *E. coli* removal in the two stage co-digestion reactors, applying a UDDT-F:OMW ratio of 4:1 and 12% TS

![Figure 3](https://iwaponline.com/washdev/article-pdf/8/2/257/224264/washdev0080257.pdf)
(discussed below under ‘E. coli inactivation along reactor profile in Ram–10 and Ram–12’), showed an 8.0 log inactivation, whereas only a 5.7 log inactivation was achieved in the single stage co-digestion reactor at 12% TS. Results indicate that the two-stage reactor is about 200 times more effective in removing the E. coli indicator organism.

Co-digestion of UDDT-F and OMW in a two-stage reactor (Ram) system

Co-digestion of UDDT-F and OMW ratio 4:1 was evaluated in a two-stage reactor (Ram) system, at 10 and 12% TS concentration. Two reactors: hydrolysis (Rₐ) and methanogenic (Rₘ), were used to separate the hydrolysis and methanogenic stages. Details on the design and operation of the reactors have been discussed above under ‘Hydrolysis reactor design’ and ‘Plug flow reactor design’.

Volatile fatty acids and pH changes

Table 2 shows the trend in TVFA, ND-VFA and pH in the single stage and two-stage co-digestion reactor systems.

Rₘ–4:1, 12% and Rₘ–4:1, 10% showed similar trends in TVFA, ND-VFA and pH during the entire experimental period. The influent to the methanogenic reactors showed high TVFA concentrations, attributed to biomass pre-hydrolysis/acidification in the acidogenic reactors (Table 2). However, the ND-VFA concentration in two-stage reactors was low (e.g. 0.8 g/L for Rₘ–4:1, 12%) compared to Rₐ effluent (6.9 ± 2.0 g/L), due to the buffer effect of the recycle stream used for pH adjustment. A declining trend was observed in both TVFA and ND-VFA along the reactor length. For example, at the mid sampling point (SP₂) TVFA and ND-VFAs concentrations in Rₘ–4:1,12% were 10.5 and 0.3 g/L respectively, distinctly lower than its corresponding influent (SP₁) concentration of 15.6 and 0.8 g/L, respectively. With an average pH of 6.4 at the first two sampling points, the distinct drop in TVFA can be attributed to prevailing methanogenesis (Goepfert & Hicks 1969). Whereas in the two-stage system hydrolysis/acidogenesis was clearly located in the separate Rₐ reactor, in the single stage plug flow reactor hydrolysis/acidogenesis prevailed between sampling points SP₁ and SP₂.

E. coli inactivation along reactor profile in Ram–10 and Ram–12

Rₘ–4:1,12% depicted 8.0 log E. coli inactivation, slightly higher than the corresponding value of 7.3 log attained in the Ram–4:1, 10% system (Figure 4(a) and 4(b)).

The observed improved inactivation can likely be attributed to the initial hydrolytic/acidogenic phase of the Rₐ reactor that depicted an average of 3.4 and 3.0 E. coli log inactivation in Ram–4:1, 12% and Ram–4:1, 10%, respectively, corresponding to decay rates of 1.6 and 1.7/day respectively (using Equation (4)). E. coli log inactivation in Ram–4:1, 12% and Ram–4:1, 10% systems depicted a similar trend along the reactor length.

Table 2 | Variation in TVFA, ND-VFA and pH in Rₐ and Rₘ reactors

<table>
<thead>
<tr>
<th>Reactor Parameters</th>
<th>Parameter</th>
<th>SP₁</th>
<th>SP₂</th>
<th>SP₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-digestion UDDT-F:OMW ratio 4:1</td>
<td>TVFA (mg/L)</td>
<td>15,685 ± 1,772</td>
<td>10,526 ± 844</td>
<td>1,575 ± 607</td>
</tr>
<tr>
<td></td>
<td>ND-VFA (mg/L)</td>
<td>800 ± 112</td>
<td>286 ± 68</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>ND-VFA (%)</td>
<td>5.1 ± 0.6</td>
<td>2.7 ± 0.6</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.4 ± 0.1</td>
<td>6.4 ± 0.1</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>Rₘ–4:1, 12%</td>
<td>TVFA (mg/L)</td>
<td>12,547 ± 887</td>
<td>8,702 ± 72</td>
<td>1,744 ± 101</td>
</tr>
<tr>
<td></td>
<td>ND-VFA (mg/L)</td>
<td>660 ± 311</td>
<td>281 ± 49</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>ND-VFA (%)</td>
<td>3.5 ± 2</td>
<td>3.2 ± 0.6</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.3 ± 0.1</td>
<td>6.2 ± 0.1</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>Rₘ–4:1, 10%</td>
<td>TVFA (mg/L)</td>
<td>3,844 ± 679</td>
<td>12,121 ± 1153</td>
<td>2,629 ± 326</td>
</tr>
<tr>
<td></td>
<td>ND-VFA (mg/L)</td>
<td>599.4 ± 150</td>
<td>2,379 ± 409</td>
<td>5 ± 12</td>
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<tr>
<td></td>
<td>ND-VFA (%)</td>
<td>15.8 ± 3.4</td>
<td>19.6 ± 2.8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>5.4 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td></td>
<td>30.1 ± 0.3</td>
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</table>
E. coli inactivation progressed along the reactor length, with highest inactivation being achieved within the first one-third of the methanogenic reactor length. Between SP1 and SP2, a 4.2 and 5.1 E. coli log inactivation was achieved in Ram/C0 4:1, 10% and in Ram/C0 4:1, 12% respectively, corresponding to decay rates of 1.1 and 0.9/day respectively. Overall, in the entire Ram/C0 4:1, 12% and Ram/C0 4:1, 10% system, an 8.0 and 7.2 E. coli log inactivation was achieved at SP3 (effluent), corresponding to a decay rate of 0.6 in both cases. Moreover, ND-VFA calculated concentration in Ram/C0 4:1, 12% and Ram/C0 4:1, 10% systems showed a declining trend along the reactor length (see Figure 4). Apparently, the decay rate ‘k’ is highest under high ND-VFA conditions and levels off when ND-VFA drops and/or pH increases. Under

### Table 3 | Comparing E. coli inactivation with related literature results

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Temp (°C)</th>
<th>SRT (days)</th>
<th>Log inactivation</th>
<th>k_d (/d)</th>
<th>Digestion method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDDT-F &amp;OMW (12% TS)</td>
<td>30</td>
<td>4</td>
<td>3.4</td>
<td>0.9</td>
<td>CSTR fed</td>
<td>This study</td>
</tr>
<tr>
<td>UDDT-F &amp;OMW (10% TS)</td>
<td>30</td>
<td>4</td>
<td>3.0</td>
<td>0.8</td>
<td>CSTR fed</td>
<td>This study</td>
</tr>
<tr>
<td>UDDT-F (12% TS)</td>
<td>30</td>
<td>29</td>
<td>3.4</td>
<td>0.1</td>
<td>Single stage plug flow</td>
<td>This study</td>
</tr>
<tr>
<td>UDDT-F (10% TS)</td>
<td>30</td>
<td>29</td>
<td>3</td>
<td>0.1</td>
<td>Single stage plug flow</td>
<td>This study</td>
</tr>
<tr>
<td>UDDT-F &amp;OMW (12% TS)</td>
<td>30</td>
<td>29</td>
<td>8</td>
<td>0.3</td>
<td>Two-stage plug flow</td>
<td>This study</td>
</tr>
<tr>
<td>UDDT-F &amp;OMW (10% TS)</td>
<td>30</td>
<td>29</td>
<td>7.2</td>
<td>0.3</td>
<td>Two-stage plug flow</td>
<td>This study</td>
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<tr>
<td>UDDT-F &amp; OMW</td>
<td>30</td>
<td>29</td>
<td>5.7</td>
<td>0.2</td>
<td>Single stage plug flow</td>
<td>This study</td>
</tr>
<tr>
<td>Cattle slurry</td>
<td>35</td>
<td>15</td>
<td>6.5</td>
<td>0.4</td>
<td>Batch</td>
<td>Steffen et al. (1998)</td>
</tr>
<tr>
<td>Liquid sewage sludge</td>
<td>35</td>
<td>15–20</td>
<td>0.5–2.0</td>
<td>0.0–0.1</td>
<td>Batch</td>
<td>Smith et al. (2005)</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>35</td>
<td>21</td>
<td>1.5–1.7</td>
<td>0.1</td>
<td>Semi-continuous</td>
<td>Horan et al. (2004)</td>
</tr>
<tr>
<td>Beef cattle slurry</td>
<td>35</td>
<td>7</td>
<td>6.5</td>
<td>0.9</td>
<td>Batch</td>
<td>Kearney et al. (1995)</td>
</tr>
<tr>
<td>Beef cattle slurry</td>
<td>35</td>
<td>8</td>
<td>4.25</td>
<td>0.5</td>
<td>Semi-continuous</td>
<td>Kearney et al. (1995)</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>37</td>
<td>21</td>
<td>1–2</td>
<td>0.1</td>
<td>Batch</td>
<td>Higgins et al. (2007)</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>37</td>
<td>21</td>
<td>2.0</td>
<td>0.1</td>
<td>Batch</td>
<td>Higgins et al. (2007)</td>
</tr>
<tr>
<td>Swine manure</td>
<td>24</td>
<td>7</td>
<td>2.8–2.9</td>
<td>0.4</td>
<td>SBR</td>
<td>Massé et al. (2011)</td>
</tr>
<tr>
<td>Cattle slurry</td>
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<td>25</td>
<td>6.5</td>
<td>0.3</td>
<td>Batch</td>
<td>Santha et al. (2006)</td>
</tr>
<tr>
<td>Swine slurry</td>
<td>20</td>
<td>20</td>
<td>2–5</td>
<td>0.1–0.2</td>
<td>SBR</td>
<td>Côté et al. (2006)</td>
</tr>
</tbody>
</table>

Figure 4 | ND-VFA concentrations and E. coli inactivation variation along the reactor profile: (a) Ram/C0 4:1, 10% and (b) Ram/C0 4:1, 12%.
methanogenic conditions the k-value may be governed by microbial ‘predation’ and chemical interactions, reported to play a role in pathogen inactivation (Smith et al. 2005).

The E. coli decay rates obtained in this study are comparable to related studies as shown in Table 3.

The observed high Kd values observed in our present study may be attributed to two factors: (1) optimal activity of hydrolytic/acidogenic bacteria and concomitantly suppressing alkalinity regeneration by methanogenesis in the R4 stage of the Rsm system, resulting in high levels of ND-VFA; (2) OMW contains appreciable amounts of fats that are easily hydrolysable to long chain fatty acids, which may impose additional toxic effects on micro-organisms involved in the AD process (Silva et al. 2014; Angeriz-Campoy et al. 2015).

CONCLUSIONS

This study evaluated the technical feasibility of pathogen inactivation during digestion and co-digestion of UDDT-F and UDDT-F-OMW mixtures. All waste substrates were obtained from Mukuru Kwa Njenga and Mukuru Kwa Reuben informal slum settlements, Nairobi, Kenya. Results showed that co-digesting UDDT-F and OMW at a ratio of 4:1 in a two-stage reactor enhances sanitisation as shown by assessing E. coli levels along the reactor length. E. coli inactivation of 8.0 log units was achieved within 29 days SRT. Rapid ND-VFA build-up was achieved from the mixed waste substrate, especially within the separate completely mixed hydrolysis reactor, where ND-VFA build-up between 5,200 and 6,500 mg/L achieved 3.4 E. coli log inactivation in 4 days. An up to 5.1 log inactivation was achieved within the first one-third of reactor length of the plug-flow reactor, agreeing with an SRT of 11 days.

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