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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

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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

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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 V*ibrio 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 (Zhang *et al.* 2005; Jiang *et al.* 2013; Wang *et al.* 2014; Riungu *et al.* 2018). However, during the digestion of sewage sludge the high buffer capacity limits pH changes (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 micro-organisms 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_{am}) comprising a hydrolysis/acidogenic reactor (R_a) 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_a'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)

	UDDT-F		OMW	
	Value	**STDEV**	**Value**	**STDEV**
TS (% wgt)	24.5	3.8	17.9	1.6
Moisture content	75.5	3.8	80.7	4.1
VS (% wgt)	20.1	3.5	16.9	4.4
TOC (g C/g TS)	64.4	7.7	54	4.3
COD_{Total} (g COD/g TS)	195.3	5.9	139.6	10.1
E. coli (CFU/g TS)	1.7×10^9	5.3×10^8	2.7×10^5	7.4×10^4
Ascaris eggs	Not detected		Not detected	

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 D_1, D_2, D_3, D_4, D_5 and D_6, were divided into two groups (D_1, D_3 and D_5, and D_2, D_4 and D_6), 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 (R_s) and two-stage (R_{am}) anaerobic digestion processes. Three replications of two treatments groups R_{am} and R_s were set, with D_1, D_3 and D_5 being R_{am}'s and D_2, D_4 and D_6 being R_s'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 R_s reactors. In the R_{am} reactor setup, effluent from R_a acted as influent to the R_m. Details on the design of R_a are provided below under 'Total solids and volatile solids'. Two R_a'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 R_m reactors. Thereafter, 6 L of the mix was fed to each of the three R_m's (D_1, D_3 and D_5) every morning.

Finally, the concentration of the feed into R_{am} was reduced to 10% TS. Thereafter, 100 mL of R_s, R_a and R_m'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.* 1993; Lahav & Morgan 2004). Therefore,

$$\frac{((H^+).(A^-))}{(HA)} = Ka \tag{1}$$

$$pH = pKa + {}^{10}log\left(\frac{A^-}{HA}\right) \tag{2}$$

$$A_T = (HA) + (A^-) \tag{3}$$

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):

$$\ln(C_t/C_0) = -kt \quad (4)$$

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 (R_s) system

ND-VFA profiles

An evaluation of the performance of single stage plug flow reactor (R_s) was carried out using a UDDT-F:OMW ratio 4:1, 12% TS ($R_{s-4:1,\ 12\%}$), UDDT-F: OMW ratio 1:0, 12% TS ($R_{s-1:0,\ 12\%}$) and UDDT-F: OMW ratio 1:0, 10% TS ($R_{s-1:0,\ 10\%}$) systems, with results shown in Figure 2(a)–2(c). Among the tested substrates, co-digestion ($R_{s-4:1,\ 12\%}$) 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 (SP_1) and SP_2 sampling points. However, in $R_{s-1:0,\ 12\%}$ and $R_{s-1:0,\ 10\%}$, a 6 and 6.5-fold decline in ND-VFA concentration was observed between sampling points SP_1 and SP_2 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 SP_2 and SP_3, 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 $R_{s-1:0,\ 12\%}$ and $R_{s-1:0,\ 10\%}$ reactors along the reactor length may be attributed to the high buffer capacity of UDDT-F

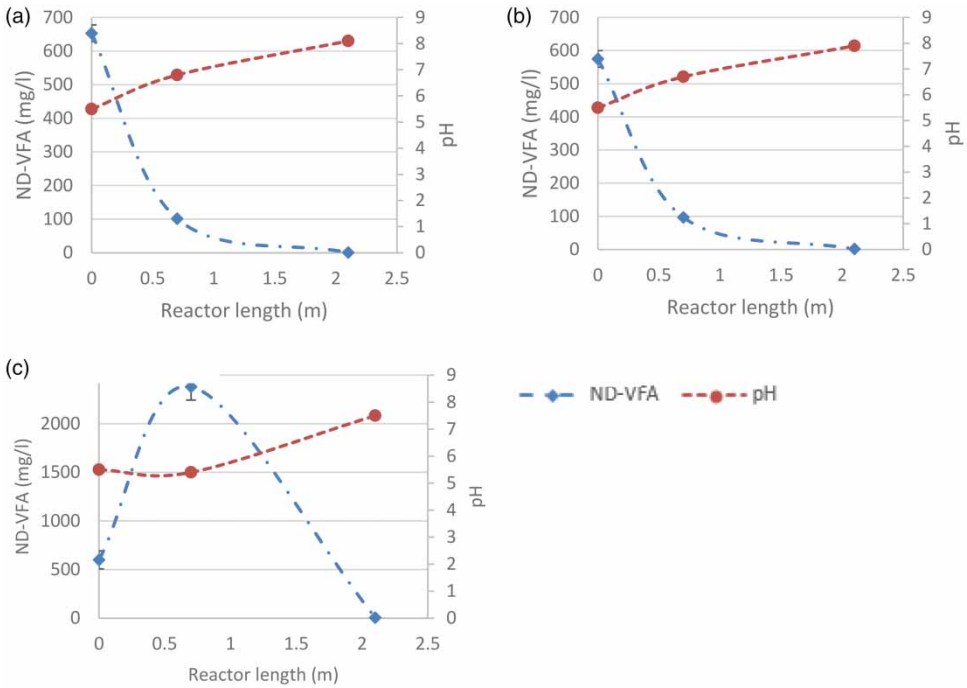

Figure 2 | Development of ND-VFA (blue) and pH (red) along the reactor length. (a) $R_{s-1:0,\ 12\%}$ (b) $R_{s-1:0,\ 10\%}$ (c) $R_{s-4:1,\ 12\%}$. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/washdev.2018.160.

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 SP_2 and SP_3, whereas in single substrate reactors a gradual increase in pH was observed, the co-digestion reactor ($R_{s-4:1,\ 12\%}$) showed similar pH in the influent (SP_1), and the first sampling point (SP_2) followed by a sharp increase between SP_2 and SP_3 (see Figure 2(c)). The low pH at SP_2 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 $R_{s-4:1,\ 12}$, $R_{s-1:0,12}$ and $R_{s-1:0,10}$ at the three sampling points SP_1, SP_2 and SP_3, located at 0, 0.7 and 2.1 m of the reactor length, respectively. The higher pathogen inactivation shown in $R_{s-4:1,\ 12\%}$ (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 SP_1 and SP_2 in $R_{s-1:0,\ 12\%}$ and $R_{s-1:0,\ 10\%}$, an increase was observed in the $R_{s-4:1,\ 12\%}$ 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

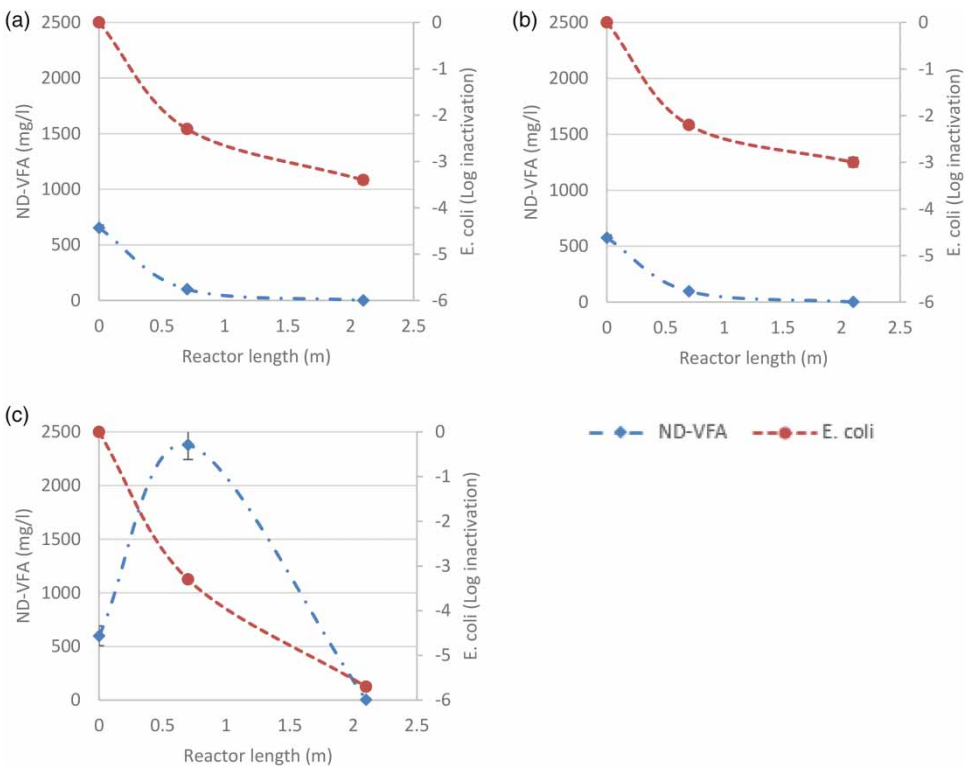

Figure 3 | *E. coli* log inactivation (red) and the production of non-dissociated VFAs (blue) in single stage anaerobic digestion of UDDT-F at 10% and co-digestion of UDDT-F and OMW at 12% TS. (a) $R_{S-1:0,\ 12\%}$ (b) $R_{S-1:0,\ 10\%}$ (c) $R_{S-4:1,\ 12\%}$.

(discussed below under '*E. coli* inactivation along reactor profile in R_{am-10} and R_{am-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 (R_{am}) system

Co-digestion of UDDT-F and OMW ratio 4:1 was evaluated in a two-stage reactor (R_{am}) system, at 10 and 12% TS concentration. Two reactors: hydrolysis (R_a) and methanogenic (R_m), 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.

$R_{m-4:1,\ 12\%}$ and $R_{m-4:1,\ 10\%}$ 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 $R_{m-4:1,\ 12\%}$) compared to R_a 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 (SP_2) TVFA and ND-VFAs concentrations in $R_{m-4:1,\ 12\%}$ were 10.5 and 0.3 g/L respectively, distinctly lower than its corresponding influent (SP_1) 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 R_a reactor, in the single stage plug flow reactor hydrolysis/acidogenesis prevailed between sampling points SP_1 and SP_2.

E. coli inactivation along reactor profile in R_{am-10} and R_{am-12}

$R_{am-4:1,12\%}$ depicted 8.0 log *E. coli* inactivation, slightly higher than the corresponding value of 7.3 log attained in the $R_{am-4:1,\ 10\%}$ system (Figure 4(a) and 4(b)).

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

Table 2 | Variation in TVFA, ND-VFA and pH in R_m and R_s reactors

	Reactor	Parameter	SP_1	SP_2	SP_3
Co-digestion UDDT-F:OMW ratio 4:1	$R_{m-4:1,\ 12\%}$	TVFA (mg/L)	15,685 ± 1,772	10,526 ± 844	1,575 ± 607
		ND-VFA (mg/L)	800 ± 112	286 ± 68	1.7 ± 0.2
		ND-VFA (%)	5.1 ± 0.6	2.7 ± 0.6	0.1
		pH	6.4 ± 0.1	6.4 ± 0.1	7.8 ± 0.1
	$R_{m-4:1,\ 10\%}$	TVFA (mg/L)	12,347 ± 887	8,702 ± 72	1,744 ± 101
		ND-VFA (mg/L)	660 ± 311	281 ± 49	1.6 ± 0.3
		ND-VFA (%)	3.5 ± 2	3.2 ± 0.6	0.1
		pH	6.3 ± 0.1	6.2 ± 0.1	7.8 ± 0.1
	$R_{s-4:1,12\%}$	TVFA (mg/L)	3,844 ± 679	12,121 ± 1153	2,629 ± 326
		ND-VFA (mg/L)	599.4 ± 150	2,379 ± 409	5 ± 1.2
		ND-VFA (%)	15.8 ± 3.4	19.6 ± 2.8	0.2
		pH	5.4 ± 0.1	5.4 ± 0.1	7.5 ± 0.1
		Temperature (°C)		30.1 ± 0.3	

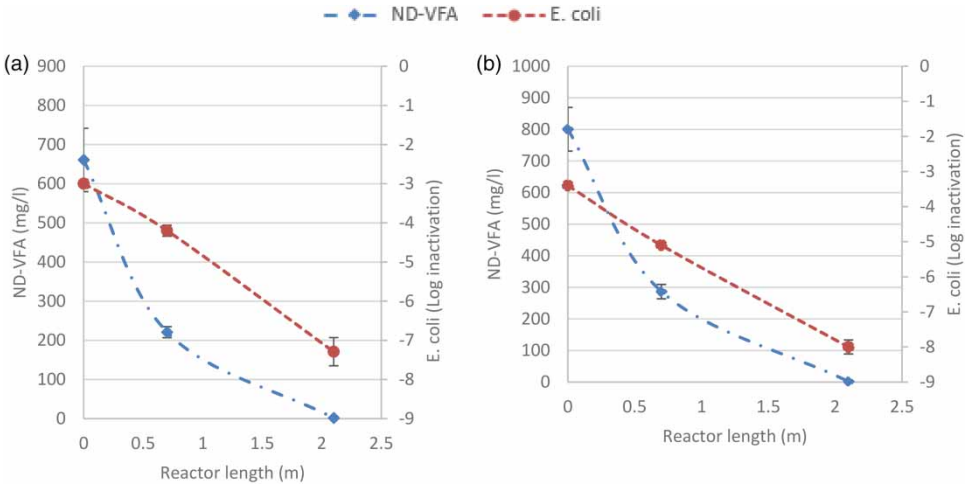

Figure 4 | ND-VFA concentrations and *E. coli* inactivation variation along the reactor profile: (a) $R_{am-10\%}$ and (b) $R_{am-12\%}$.

E. coli inactivation progressed along the reactor length, with highest inactivation being achieved within the first one-third of methanogenic reactor length. Between SP_1 and SP_2, a 4.2 and 5.1 *E. coli* log inactivation was achieved in $R_{am-4:1,\ 10\%}$ and in $R_{am-4:1,\ 12\%}$ respectively, corresponding to decay rates of 1.1 and 0.9/day respectively. Overall, in the entire $R_{am-4:1,\ 12\%}$ and $R_{am-4:1,\ 10\%}$ system, an 8.0 and 7.2 *E. coli* log inactivation was achieved at SP_3 (effluent), corresponding to a decay rate of 0.6 in both cases. Moreover, ND-VFA calculated concentration in $R_{am-4:1,\ 12\%}$ and $R_{am-4:1,\ 10\%}$ 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

Substrate	Temp (°C)	SRT (days)	Log inactivation	k_d (/d)	Digestion method	Ref
UDDT-F &OMW (12% TS)	30	4	3.4	0.9	CSTR fed	This study
UDDT-F &OMW (10% TS)	30	4	3.0	0.8	CSTR fed	This study
UDDT-F (12% TS)	30	29	3.4	0.1	Single stage plug flow	This study
UDDT-F (10% TS)	30	29	3	0.1	Single stage plug flow	This study
UDDT-F&OMW (12% TS)	30	29	8	0.3	Two-stage plug flow	This study
UDDT-F&OMW (10% TS)	30	29	7.2	0.3	Two-stage plug flow	This study
UDDT-F &OMW	30	29	5.7	0.2	Single stage plug flow	This study
Cattle slurry	35	15	6.5	0.4	Batch	Steffen *et al.* (1998)
Liquid sewage sludge	35	15–20	0.5–2.0	0.0–0.1	Batch	Smith *et al.* (2005)
Sewage sludge	35	21	1.5–1.7	0.1	Semi-continuous	Horan *et al.* (2004)
Beef cattle slurry	35	7	6.5	0.9	Batch	Kearney *et al.* (1993)
Beef cattle slurry	35	8	4.25	0.5	Semi-continuous	Kearney *et al.* (1993)
Sewage sludge	37	21	1–2	0.1	Batch	Higgins *et al.* (2007)
Sewage sludge	37	21	2.0	0.1	Batch	Higgins *et al.* (2007)
Swine manure	24	7	2.8–2.9	0.4	SBR	Massé *et al.* (2011)
Cattle slurry	18–25	25	6.5	0.3	Batch	Santha *et al.* (2006)
Swine slurry	20	20	2–5	0.1–0.2	SBR	Côté *et al.* (2006)

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 R_a stage of the R_{am} 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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