Build-up and impact of volatile fatty acids on *E. coli* and *A. lumbricoides* during co-digestion of urine diverting dehydrating toilet (UDDT-F) Faeces

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Abstract

This study examined the potential of *Escherichia coli* (*E. coli)* and *Ascaris lumbricoides (A. lumbricoides)* eggs inactivation in faecal matter coming from urine diverting dehydrating toilets (UDDT-F) by applying high concentrations of volatile fatty acids (VFAs) during anaerobic stabilization. The impact of individual VFAs on *E. coli* and *A. lumbricoides* eggs inactivation in UDDT-F was assessed by applying various concentrations of store-bought acetate, propionate and butyrate. High VFA concentrations were also obtained by performing co-digestion of UDDT-F with organic market waste (OMW) using various mixing ratios. All experiments were performed under anaerobic conditions in laboratory scale batch assays at 35±1^{o}C.

A correlation was observed between *E. coli* log inactivation and VFA concentration. Store bought VFA spiked UDDT-F substrates achieved *E. coli* inactivation up to 4.7 log units/ day compared to UDDT-F control sample that achieved 0.6 log units/ day. In co-digesting UDDT-F and organic market waste (OMW), a ND-VFA concentration of 4800-6000 mg/L was needed to achieve *E. coli* log inactivation to below detectable levels and complete *A. lumbricoides* egg inactivation in less than four days. *E. coli* and *A. lumbricoides* egg inactivation was found to be related to the concentration of non-dissociated VFA (ND-VFA), increasing with an increase in the OMW fraction in the feed substrate. Highest ND-VFA

concentration of 6500 mg/L was obtained at a UDDT-F:OMW ratio 1:1, below which there was a decline, attributed to product inhibition of acidogenic bacteria. Results of our present research showed the potential for *E. coli* and *A. lumbricoides* inactivation from UDDT-F up to WHO standards by allowing VFA build-up during anaerobic stabilization of faecal matter.

Keywords:

Anaerobic co digestion

UDDT faeces

Non-dissociated volatile fatty acids

E.coli inactivation

1. Introduction

As an innovative solution for enhancing sanitation in low income urban areas, urine diverting dehydrating toilets (UDDTs) can be offered on a pay-and-use basis in the form of serviced shared facilities. The UDDT principle involves separate collection of faeces and urine (Austin, 2001; Austin & Cloete, 2008; Niwagaba *et al.,* 2009a; Sherpa *et al.,* 2009). The above is the set-up of Sanergy, Kenya, a company working on sanitation improvement within Mukuru Kwa Njenga and Mukuru Kwa Reuben informal slum settlements, Kenya. After every use, sawdust is sprinkled on separated faeces mainly for odour and flies elimination (Austin & Cloete, 2008; Niwagaba *et al.,* 2009a). However, addition of saw dust or ash is not sufficient to kill pathogens (Niwagaba *et al.,* 2009a). Thus, an extra pathogen inactivation step is required after waste collection, especially when the faecal matter will be valorised for agricultural purposes. Some of the treatment options associated with source separated human waste include anaerobic digestion, composting, ash addition, chemical treatment and storage (Fagbohungbe *et al.,* 2015; Larsen & Maurer, 2011; Niwagaba *et al.,* 2009b; Rajagopal *et al.,* 2013; Vinnerås, 2007).

Anaerobic digestion (AD) offers an attractive approach in human waste treatment (Rajagopal *et al.,* 2013). It provides organic waste treatment by avoiding volatile organic compounds emissions, stabilization of organic matter, build-up of an effluent with good fertilizing qualities in addition to energy recovery through methane build-up (Avery *et al.,* 2014; Fonoll *et al.,* 2015; Nallathambi Gunaseelan, 1997; Romero-Güiza *et al.,* 2014). However, studies have reported unsatisfactory pathogen inactivation in AD (Chaggu, 2004; Dudley *et al.,* 1980; Foliguet & Doncoeur, 1972; Leclerc & Brouzes, 1973; McKinney *et al.,* 1958; Pramer *et al.,* 1950). Thus, sludge produced requires a post-treatment step, which can be expensive, time consuming, or may create pathways for disease transmission. As such, more research is needed on enhancing pathogen inactivation during the overall anaerobic digestion process.

Some key factors influencing pathogen inactivation during AD include: temperature and time (Gibbs *et al.,* 1995; Olsen *et al.,* 1985; Olsen & Larsen, 1987), reactor configuration (Kearney *et al.,* 1993b; Olsen *et al.,* 1985), pH and VFA concentration (Abdul & Lloyd, 1985; Farrah & Bitton, 1983; Sahlström, 2003).

Basically, AD is a biological process where organic matter (carbohydrates, lipids, proteins) except lignin components, is degraded in the absence of oxygen, producing methane and carbon dioxide (Jankowska *et al.,* 2015; Van Lier *et al.,* 2008), with main processes involved being hydrolysis, acetogenesis, acidogenesis and methanogenesis. During the treatment of solids-rich waste streams, hydrolysis is the rate determining step (Van Lier *et al.,* 2008) with pH, temperature, C/N ratio and hydraulic retention time (HRT) being reported as the key factors controlling the VFA build-up (Chen *et al.,* 2007; Lee *et al.,* 2014; Wang *et al.,* 2014), and its subsequent conversion into methane.

VFAs are commonly produced during the hydrolysis/ acidogenesis stage of anaerobic digestion. This stage has widely received attention within various studies focusing on either enhancing methane build-up, bio-hydrogen generation, pathogen inactivation or VFA production (Battimelli *et al.,* 2009; Ghimire *et al.,* 2015; Ghosh *et al.,* 1985; Kim *et al.,* 2011; Mata-Alvarez, 1987; Palmowski *et al.,* 2006). In

addition, hydrolysis increases solubilization of the particulate organic fraction in the feed mixture (Rajagopal *et al.,* 2013).

The degree of disinfection achieved in anaerobic digestion is influenced by a variety of interacting operational variables and conditions (Smith *et al.,* 2005). Reactor configuration, hydraulic retention time (HRT), organic loading rate (OLR), temperature, VFA and pH have all emerged as critical variables affecting pathogen inactivation. Bacteria inactivation due to temperature is related to time, with digestion under thermophilic conditions requiring less time for bacterial inactivation than under mesophilic conditions. *M. paratuberculosis* and *Salmonella* were inactivated within 24 hours under thermophilic conditions compared to months under mesophilic anaerobic digestion (Olsen *et al.*., 1985).

In addition, pH and VFA concentrations in the feed substrate may determine bacterial survival during anaerobic digestion (Abdul & Lloyd, 1985; Farrah & Bitton, 1983; Sahlström, 2003). VFA toxicity is associated with the dissociation of the acid molecule: non-dissociated VFAs are able to pass through the cell membrane of microbes by passive diffusion and will dissociate internally, disturbing internal pH, impacting protein's tertiary structure, and inhibiting microbial growth (Jiang *et al.,* 2013; Wang *et al.,* 2014; Zhang *et al.,* 2005). Additionally, non-dissociated (ND)-VFAs can make the cell membrane permeable, which allows leaching of the cell content and disintegration of the microbes. The antibacterial effects of ND-VFA have been demonstrated in treatment of enteric *E. coli* infections of rabbits and pigs, where a rise in caecal pH in diarrhoeic condition over the normal pH was cited as the main infection cause (Prohászka, 1980; Prohászka, 1986): at higher pH, less ND-VFA was present to inactivate pathogens.

As much as this technique is promising, adjusting reactor settings to maximize the ND-VFA fraction is not yet a standard practice; more research is needed in order to understand the possible sanitizing effect of VFAs during the anaerobic fermentation of source separated human faeces.

As such, this study assesses the potential of pathogen inactivation by VFAs in an acidogenic reactor. The research is part of a bigger research project investigating ways to enhance pathogen inactivation and biogas production from faecal matter coming from urine diverting dehydrating toilets (UDDT-F), at Sanergy Kenya. Provided in this paper are laboratory batch scale results of:

i. Comparing *E .coli* inactivation in VFA spiked and non-spiked UDDT waste samples

ii. Assessing effect of initial pH and UDDT-F:OMW mix ratio on VFA build-up and pathogen inactivation.

2. Experimental setup

2.1 Materials

2.1.1 UDDT-F waste samples

UDDT-F samples used for this study were obtained from the Fresh Life® urine diverting dry toilets (UDDT) 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. 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 daily basis, where used containers are replaced with clean ones. Every day, approximately 7 tons of UDDT-F are collected and transported to an offsite central treatment location (in Machakos County Government approximately 30 km from the city of Nairobi).

Five containers with UDDT-F were randomly selected after which mixing of the contents was done in order to obtain a homogeneous mix. From each of the ten containers, 1 kg UDDT-F was sampled and transferred into a plastic container. Further mixing of this waste was done in order to homogenize the sample.

2.1.2 Organic market waste samples

OMW was collected from vegetable vendors, eating points and waste disposal points within Mukuru Kwa Njenga and Mukuru Kwa Reuben informal slum settlements. 15 kg of the waste was collected and contained food waste, vegetable waste and fruit waste, in equal proportions. After collection of the waste, 2 kg of each waste was sampled out and further separately homogenized by use of a domestic blender for one minute.

In readiness for the experiments, the waste was then mixed in three different UDDT-F:OMW ratios (by weight): 4:1, 1:1 and 1:0, and refrigerated at 4^{o}. Table 1 shows the characteristics of UDDT-F and OMW waste used in the study.

Table 1: Characterization of urine diverting dehydrating toilets waste and mixed organic market waste used in study

	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
COD_{Total} (g COD /g TS)	195.3	5.9	139.6	10.1
***E. coli* (CFU/g TS)**	1.7E+09	5.3E+08	2.7E+05	7.4E+04
Ascaris eggs	Not detected		Not detected	

2.1.3 Fertilized *A. lumbricoides* eggs

These were purchased from Mahidol University School of Tropical Diseases, Bangkok. They were packed in 50 ml tubes, each with 100,000 eggs suspended in 0.1% formalin. A viability test conducted on the eggs showed that they were 100% viable. Eggs were stored at 4^{o}C before use.

2.2 Experimental set-up

This section describes the methodology used in the three different experiments. Other than the substrate preparation, same test conditions applied. Waste collection and preparation is described in section 2.1.2.

The experiments were conducted in 0.1 L serum bottles, maintaining a working volume of 80 ml. After substrate addition to the bottles, they were tightly closed with rubber stoppers and flushed with argon gas in order to create anaerobic conditions (Jensen *et al.,* 2011). The bottles were then incubated at 35±1^{o}C. Mixing of the serum bottles was done manually.

2.2.1 *E. coli* inactivation in VFA spiked versus VFA un-spiked waste samples

VFA concentration effect was tested by separately spiking serum bottles containing UDDT-F substrate, 15 g TS/L concentration with store bought acetic, propionic and butyric acids (supplied by Sigma Aldrich, (ACS reagent, ≥99%)), each at three different concentrations; 2000, 3000 and 4000 mg/L, and adjusting the pH of the serum bottle contents to 4.8 ($pH_{4.8}$) with 0.1 M HCl. By adjusting the initial pH to 4.8, which is the average pK_a value of the acids, the concentration of non-dissociated acids and dissociated acids was set to a 1:1 ratio. In addition, three controls were set in: 1) UDDT-F with a fixed pH at 4.8 ($pH_{4.8}$), and 2) UDDT-F without pH adjustment (pH_{un}), and 3) organic market waste (OMW) without pH adjustment (pH_{un}). For each of these experiments, a total of three serum bottles was set, so that one was sacrificed each day for analysis of *E. coli*, pH and VFA. The serum bottles were then closed with rubber stoppers and the procedure described in section 2.2 was followed.

2.2.2 UDDT-F:OMW mix ratio on VFA build-up and pathogen inactivation

Each serum bottle contained on average 4 g VS/100 mL of the waste substrate. Experiments were conducted at two initial pH levels: 4.8 ($pH_{4.8}$), (adjusted using 0.1M HCl or NaOH solution) and unaltered substrate pH (pH_{un}). UDDT-F:OMW ratios and the initial substrate pH used in the experiments are as shown on Table 2. For each experiment, five serum bottles were set, three for VFA, *E. coli* and pH and two for *A. lumbricoides* eggs analysis. For VFA, *E. coli* and pH, one was sacrificed on day 1, 3 and 4 for analysis. The two serum bottles set for helminth egg analysis were spiked with fertilized *A. lumbricoides* eggs at a concentration of 20 eggs/ml in order to evaluate efficiency of the treatment process in their inactivation. Extraction of *A. lumbricoides* eggs for viability checks was done on day 2 and 4.

2.3 Analytical procedures

2.3.1 Total solids and volatile solids

Total solids (TS) and volatile solids (VS) analysis were conducted according to the gravimetric method (SM-2540D and SM-2540E), as outlined in the Standard Methods for the Examination of Water and

Wastewater (APHA, 1995). TS was analyzed by drying 10 g sample weight in an oven at 105 ^{o}C for 24 hours, after which they were cooled and weighed. For VS determination, the samples were further dried in a muffle furnace at 550^{o}C for 2 hours. pH measuring was done using an analogue pH/ORP meter (model HI8314-S/N 08586318) calibrated with buffer solution at pH 4 and pH 7.

2.3.2 Chemical oxygen demand (COD)

COD measurement on samples was measured according to the closed reflux method (SM 5220 C) (APHA, 1995) and expressed in mg COD per g TS added (mg COD/g TS added).

2.3.3 VFA measurements

Volatile fatty acids (VFA) in wastewater samples can be measured using gas chromatographical methods for organic acids or by titrimetric method (Jobling Purser *et al.,* 2014; Lützhøft *et al.,* 2014). In this work, the titrimetric method as described in analytical methods for waste water characterization and evaluation of reactor performance during anaerobic treatment (AGROIWATECH, DeliverableD2) was adopted. The sample, placed in centrifuge tubes was centrifuged for 5 minutes at 5,000 revolutions per minute (rpm). 50 ml of the supernant was put in a beaker. The pH of the solution was adjusted to 6.5 using either 0.1M HCl or NaOH solution, and thereafter it was titrated with 0.1M HCl to pH 3. This volume of acid was recorded. The samples was transferred to a digestion flask with glass beads and connected to a condenser. Flask was heated until liquid began to boil and then allowed for three minutes boiling. The heater was then switched off and two minutes were allowed for cooling. The sample was then titrated immediately to pH 6.5 and volume of base recorded. The total VFA values in meq/ l added were then calculated using the formula (AGROIWATECH, DeliverableD2);

$$\left(\frac{B\times 0.1\,\frac{meq}{ml}}{C}\right)\times 1000 \ldots\ldots\ldots (1)$$

Where; *B*- volume of 0.1 M sodium hydroxide required to titrate from pH 3-6.5, **C**-Total volume of titrated sample (ml), 0.1-meq conversion factor, 1000- ml to l conversion.

In addition, background acidity correction was carried out to correct for proton acceptors present in the waste water that are not volatile, e.g. humic acids. For this, the prescribed procedure was carried out on fresh UDDT-F and OMW samples and obtained VFA values subtracted from incubated sample values. The procedure also accounted for actual VFAs in the sample before treatment, so as to set a baseline for VFA build-up after treatment.

From the Total Volatile Fatty Acid (TVFA) concentration, the fraction of ND-VFA was calculated. VFAs are commonly considered to constitute a single weak-acid system with equilibrium constant Ka because of the similarity of their pK values (Lahav & Morgan, 2004; Moosbrugger R. E. *et al.,* 1993). Therefore:

$$((H^+) \times (H^-))/(HA) = Ka \ldots\ldots (2)$$

$$pH = pKa + Log_{10}(\frac{A^-}{HA}) \ldots\ldots (3)$$

$$A_T = (HA) + (A^-) \ldots\ldots\ldots (4)$$

Where: *A*T = total VFA species concentration (mg/L), HA represents the acidic, protonated species and A^- the ionized form of each acid.

2.3.4 E. coli enumeration

E. coli enumeration was done using the chromocult coliform agar (CCA) technique, which was proved applicable for use in temperate regions (Buckley *et al.,* 2008; Byamukama *et al.,* 2000; Frampton *et al.,* 1988; Manafi & Kneifel, 1989). The CCA (Chromocult; Merck, Darmstadt, Germany) was prepared following manufacturer's instructions. Homogenized samples were serially diluted (10^{-1} to 10^{-6}) with the peptone buffered water. For each sample dilution, 0.1 ml was spread on prepared chromocult agar plates in duplicate. The prepared plates were then incubated for 24 hours at a temperature of 36±1 ^{0}C, after which colony counting was facilitated by use of a colony counter (IUL magnifying glass colony counter, IUL, S.A., Barcelona, Spain). The criteria used for identification were able to identify dark blue- to violet-coloured colonies as *E. coli* (Byamukama *et al.,* 2000; Finney *et al.,* 2003). The average

numbers of colonies were used to calculate the *E. coli* concentrations in the samples, expressed in CFU/ g 100 ml of the test sample. In the method, the lowest detection limit is 1000 CFU/L.

The first order reaction coefficients for *E. coli* removal were calculated using the Chick-Watson model that expresses the rate of inactivation of micro-organisms by a first order reaction.

$$\ln\left({}^{C_t}/_{C_0}\right) = -kt \quad \ldots\ldots(5)$$

Where:

$C_t = Number\ of\ micro-organisms\ at\ time\ t$
$C_0 = Number\ of\ micro-organisms\ at\ time\ 0$
$k = decay\ constant$
$t = time$

2.3.5 *A. lumbricoides* egg recovery

A. lumbricoides egg recovery was performed according to method developed by Moodley *et al.,* (2008) and modified by Pebsworth *et al.,* (2012). Ammonium bicarbonate solution was added to 80 ml of sample in order to wash and dissociate the eggs attached on the particles. It was then passed through a 100 µm sieve onto a 20 µm sieve. Sieve contents were well washed and all material held on 100-µm discarded. Material held on the 20 µm sieve was washed and collected onto autoclaved 15 ml centrifuge tubes, and centrifuged using a bench top centrifuge (EBA 20, Andreas Hettich GmbH &CO. KG, Germany) at 3000 rpm for 5 minutes. Supernatant was discarded and remaining pellets re-suspended in $ZnSO_4$ (specific gravity 1.3), while vortexing until the 14 ml level. The samples were then centrifuged again at 2000 rpm for 5 minutes. Supernatant was then poured through a small 20-µm filter, and washed off into an autoclaved plastic test tube. It was then centrifuged again at 3000 rpm for 5 minutes. The supernatant was discarded and the egg pellets were transferred into a 50 mL Falcon tube, with de-ionized water added to the 45 ml mark. The Falcon tube was covered with parafilm that was pricked (to allow air exchange within the sample) and then incubated at 28±1 ^{o}C for 28 days. Regular checks with de-ionized water additions were conducted in order to account for water lost through evaporation.

After the 28 days incubation, the samples were divided into 15 ml centrifuge tubes and centrifuged for 5 min. at 3000 rpm. The supernatant was discarded and the remaining pellet containing eggs was well mixed using a pipette. 1 ml of sample was transferred on a Sedgewick-rafter counting cell (from Wildlife Supply Company®). The slide was observed under the microscope (AmScope, California, USA) at a magnification of 10 and 40.

Eggs developed to the larval stage, with motile larvae, were considered viable, while all eggs that stopped under any other developmental stage and eggs that presented some kind of deterioration with no motile larvae inside were considered non-viable.

2.4 Data analysis

Data analysis was done using Microsoft Excel software. Data obtained from each batch was first analyzed by computing the averages of the three trials conducted per batch. Average values of the three batches were then combined by computing their average values, standard deviations and standard errors. The average values of the three batches were then presented in either table of figure form.

3. Results and discussion

3.1 VFA concentration effect on *E. coli* inactivation

Volatile fatty acids are produced when larger organic molecules are hydrolyzed and anaerobically oxidized to carboxylic acids. Depending on the alkalinity of the solution, this will affect the pH value. With a decrease in pH, the fraction of acids present in the non-dissociated form will increase, which are reported to support bacteria inactivation. In order to study the relationship between non-dissociated VFAs and *E.coli* inactivation, batch experiments were conducted with store-bought VFAs (acetate, propionate and butyrate at 2,000, 3,000 and 4,000 mg/L) and spiked in to 15 g/L TS UDDT-F samples. Acetate, propionate or butyrate VFA concentration of 4000 mg/l achieved *E. coli* inactivation to below detectable levels after two days of incubation.

Increasing the VFA concentration has a direct effect on *E. coli* inactivation (Fig. 1): for butyrate, the *E. coli* log inactivation increases from 3 to 6 by increasing the spiking concentration from 2,000 to 4,000 mg/L. A similar trend was observed for the acetic acid and propionic acid spiked bottles. Increasing concentration leads to increase in ND-VFA fraction which increases the inhibitory effect.

The VFA chain length apparently had slight impact on *E. coli* inactivation as indicated by the calculated log inactivation rates (log inactivation per ND-VFA (meq/g TS added)). After one day treatment, with acetic, propionic and butyric acids, the achieved log inactivation was 2.15, 2.13 and 2.11, respectively, showing a slight decrease with increasing chain length of the fatty (Fig. 2). Related studies investigating the effect of VFA chain length on *Salmonella typhimurium* and *Vibrio cholera* inactivation reported that inactivation efficiency decreased with increasing chain length (Goepfert & Hicks, 1969; Kunte *et al.,* 2000; Salsali *et al.,* 2006), which agrees with the trend observed in our present study for *E. coli* inactivation. Acetic acid, due to its lower molecular weight, diffuses faster across the bacterial membrane compared to propionic and butyric acid under the same conditions of temperature and pH. Upon passage of ND-VFA's through the cell membrane of microbes they dissociate internally thus disturbing internal pH, impacting protein's tertiary structure, and inhibiting microbial growth (Jiang *et al.,* 2013; Wang *et al.,* 2014; Zhang *et al.,* 2005), thus inactivating pathogens. Under neutral pH conditions, increased VFA toxicity with increasing chain length on methanogenesis has been described previously (Van Lier *et al.,* 1993). The increased toxicity was ascribed to the presence of a longer a-polar aliphatic tail that more easily interferes with the bacterial or archaeal membrane.

Experimental results applying a set VFA concentration showed that the *E. coli* inactivation rate increased in time during the incubations: higher *E. coli* inactivation was achieved on the second day of treatment (Figure 2). The *E. coli* log inactivation achieved at 3000 mg/L butyric acid, after day 1 and day 2 of treatment was 4.2 and 6.0, respectively. The increased inactivation rate coincided with a drop in pH from 4.8-4.3 between day 0 and day 2 and a corresponding increase in ND-VFA concentration from 21.6-33.9

meq/l. The pH drop from 4.8-4.3 increased protonation of the organic acids thus higher ND-VFA fraction in the waste sample; with overall effect being increased inhibitory effect. In addition, increased incubation time leads to longer exposure time of *E. coli* to toxic effect of the ND-VFA.

The control sample with UDDT-F also showed a higher log inactivation rate at lower pH: at 4.8, the *E. coli* log inactivation was 1.2 log/d, while at pH 6.2 the inactivation did not exceed 0.6 log/d. The total amount of ND-VFA increased up to 0.5 meq/ g TS added in UDDT-F, $pH_{4.8}$ and 0.2 meq/ g TS added in UDDT-F, pH_{un}. In the VFA spiked incubations, a lower pH was achieved at higher initial VFA concentration.

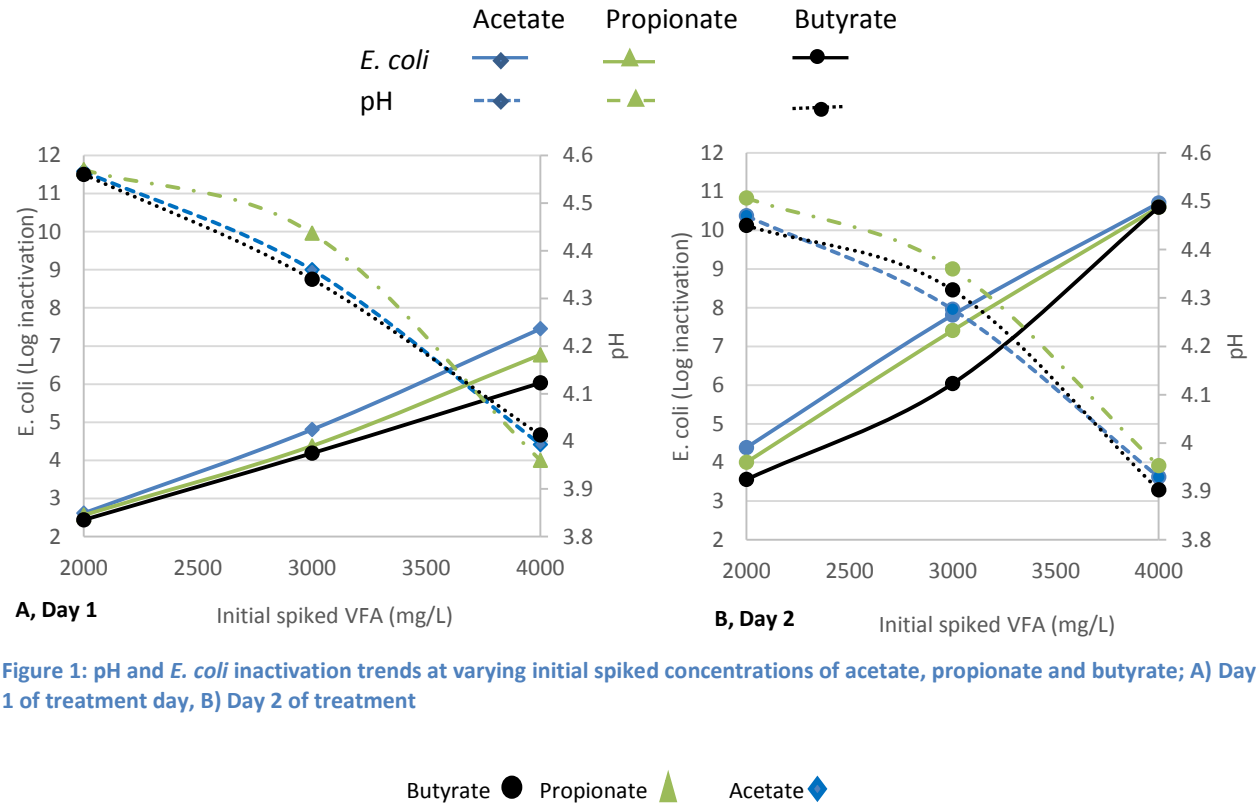


Figure 1: pH and *E. coli* inactivation trends at varying initial spiked concentrations of acetate, propionate and butyrate; A) Day 1 of treatment day, B) Day 2 of treatment

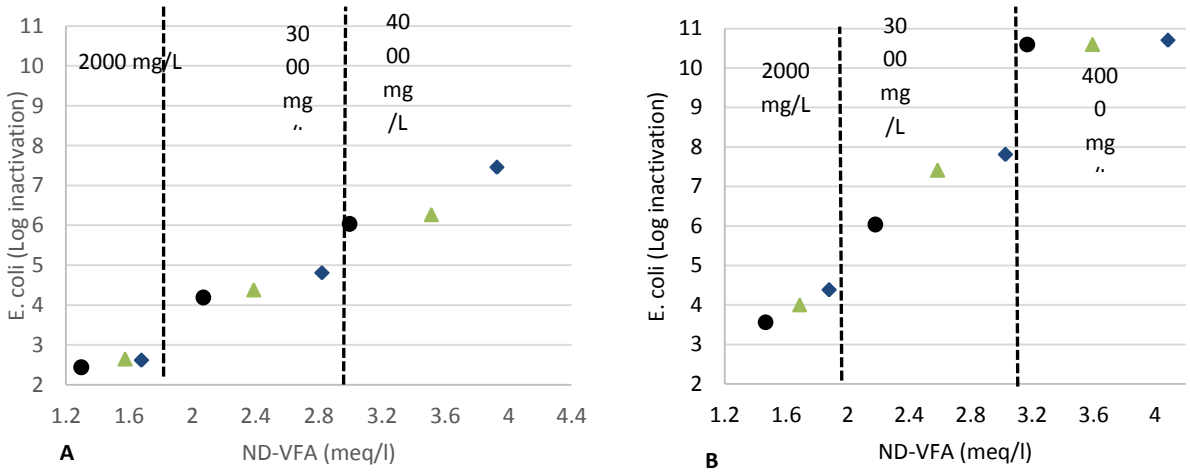


Figure 2: Build up and effect of ND-VFA on *E. coli* inactivation at various concentrations of acetate, propionate and butyrate on; A) Day 1 and B) Day 2 of treatment

OMW hydrolysis: The used OMW hydrolyzed rapidly, leading to a pH of 3.3 after 24 hours incubation. From an average initial *E. coli* count of 1.75E+07 ±1.3E+07, values were already below detectable levels after the first day. At the measured pH, over 97% of the total VFA was present in non-dissociated form, leading to a ND-VFA concentration of 36.4 meq/l. For OMW, the presence of readily degradable fractions of organics caused a rapid acidification leading to a high concentration of volatile fatty acids and a low pH. The latter is of interest for co-digestion with dried faecal matter since it will accelerate pathogen inactivation, particularly in two-stage or plug flow digestion systems. Further experiments were conducted to assess the potential of OMW co-digestion for UDDT-F stabilization.

3.2 Co-digestion of UDDT Waste and Mixed OMW

3.2.1 Effect of mix ratio on pH and VFA build-up

The experiments were conducted at various UDDT-F:OMW mix ratios (1:0 4:1, 2:1, 1:1, 1:2, 1:4 and 0:1) at an average total TS concentration of 40 g/L. Table 2 shows the initial and final pH values of the tested substrates.

Table 2: UDDT-F:OMW mix ratios and respective pH levels as used in the experiments

pH level	UDDT F: OMW mix ratio	1:0	4:1	2:1	1:1	1:2	1:4	0:1
Adjusted	Initial pH	4.8	4.8	4.8	4.8	4.8	4.8	4.8
	Final pH	5.1	4.4	4	3.7	3.5	3.3	3.1
Unaltered	Initial pH	5.8	5.7	5.7	5.6	5.6	5.5	5.3
	Final	5.4	4.9	4.4	3.8	3.6	3.4	3.2

Fig. 3 shows the ND-VFA and dissociated-VFA (D-VFA) build-up at different UDDT-F:OMW ratios. Co-digesting UDDT waste with OMW led to a decline in pH, with a stronger decline in samples with a higher OMW fraction (Fig. 3), which was already observed during the first day of treatment. After incubating the UDDT-F:OMW ratios of 1:1, 1:2, 1:4 and 0:1, the pH declined within 24 hours to the range 3.1-3.7 for the bottles with an initial adjusted pH of 4.8 and to the range 3.2-3.8 for the unadjusted substrates. Apparently, no pH adjustments are needed for reaching a sufficiently low pH at these ratios. In the experiments with a higher content of UDDT-F, a more clear difference was observed between the samples with and without adjusted pH. For example, at a UDDT-F:OMW ratio of 4:1, the final pH was 4.4 and 4.9 in the bottles with initial $pH_{4.8}$ and pH respectively.

Results clearly show that the VFA build-up was dependent on the OMW fraction in the substrate, which subsequently impacted the final pH of the batch incubations. Maximum VFA build-up was achieved at an UDDT-F:OMW ratio of 1:1, below which a decline was observed both in VFA and ND-VFA build-up, while the pH dropped to below 4.

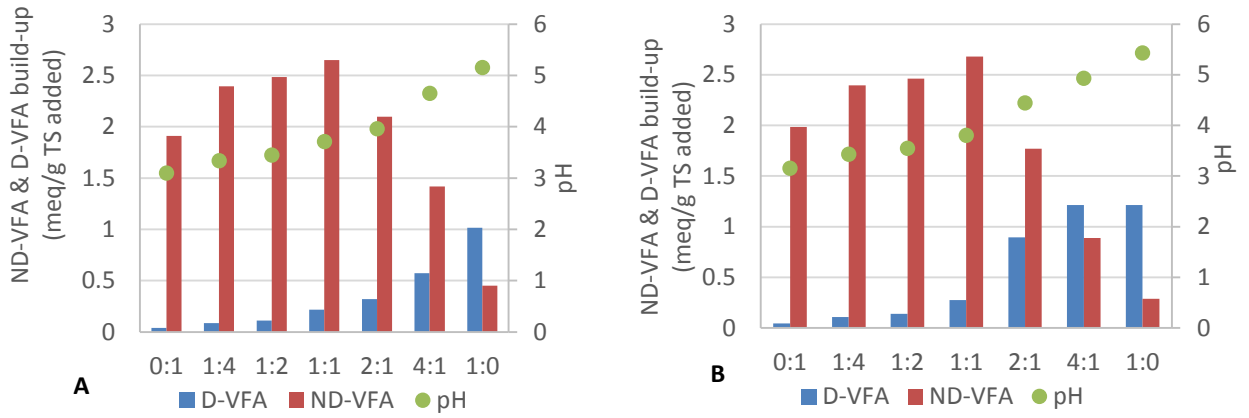


Figure 3: ND-VFA and D-VFA build-up in co-digestion experiments at the end of incubation period: a) Initial $pH_{4.8}$, b) Initial pH_{un}, at UDDT-F:OMW ratios 0:1, 1:4, 1:2, 1:1, 2:1, 4:1 and 1:0

Moreover, it was observed that the VFA build-up was not proportional to the amount of OMW added (Fig. 3). Results show highest ND-VFA build-up (2.7 meq/ g total TS added) at UDDT-F:OMW=1:1, below which a decline was observed both in D-VFA and ND-VFA. In the latter incubations, the final pH recorded was below 4. VFA toxicity is associated with the degree of dissociation of the carboxylic acid group, which is governed by a pKa of about 4.8. Increasing the OMW fraction in the feed substrate leads to rapid acidification thereby lowering the pH and increasing the ND-VFA concentration. The increased concentration of ND-VFAs has a toxic effect not only on pathogens but also on the anaerobic bacterial population including the VFA producers and methanogenic Archaea. The latter calls for an optimization of OMW dosing.

3.3 E. coli inactivation *in single substrate and co-digestion experiments*

UDDT-F:OMW mix ratio: *E. coli* inactivation increased with an increase in OMW fraction in the feed substrate (Fig 5). UDDT-F:OMW=1:4, 1:2 and 1:1, achieved *E. coli* inactivation to below detectable limits between day 1 and day 2, with corresponding decay rates (k values) in the range of 7.4-13 /day (Fig. 4), whereas OMW alone showed inactivation to below detectable limits in one day, all meeting WHO standards of $<1*10^3$ CFU/ 100 ml (WHO, 2006). UDDT-F:OMW ratio 2:1 showed *E. coli* inactivation to below detectable levels in 3 days, whereas UDDT-F:OMW ratio 4:1 showed inactivation to $2.0*10^3$ ±

$1.35*10^3$ and $5.6*10^4 \pm 6.5*10^5$ CFU/100 ml at initial pH 4.8 and unadjusted initial pH respectively. Results clearly showed that *E. coli* decay rates (k value) increased with increase in OMW in the waste substrate (Fig. 4). Below UDDT-F:OMW ratio 1:1, an increase in decay rate was observed despite a decline in ND-VFA concentration. As discussed in section 3.2.1, aiming at highest k-values is not considered the best strategy, since increased toxic effect of ND-VFA not only affects pathogens but all anaerobic bacterial population, thus process failure.

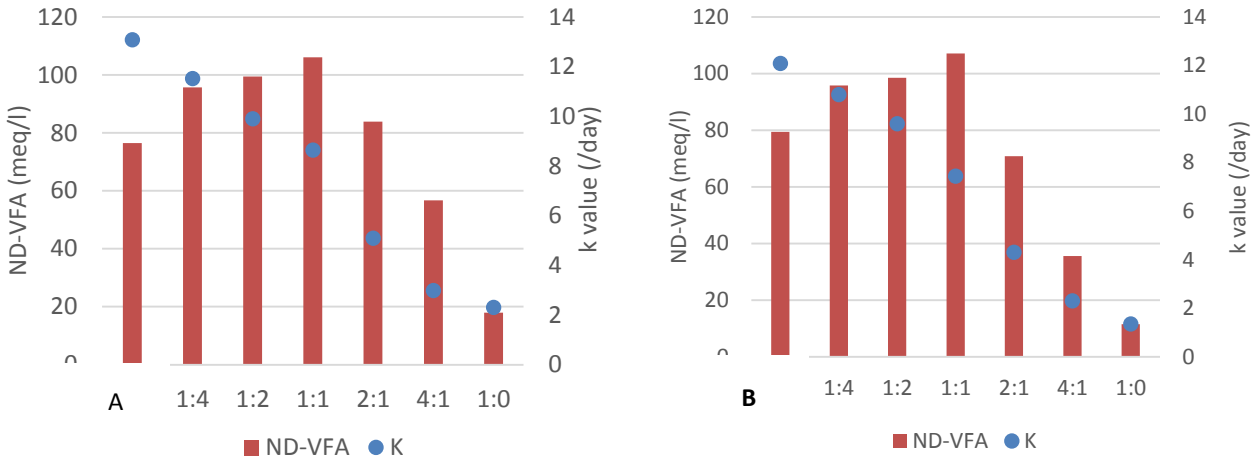
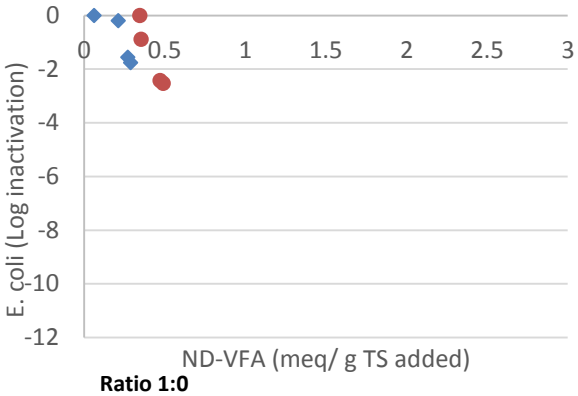


Figure 4: ND-VFA concentration and k value trend at UDDT-F:OMW mix ratios 1:4, 1:2, 1;1, 21, 4:1 and 1:0; a) Initial $pH_{4.8}$, b) Initial pH_{un}

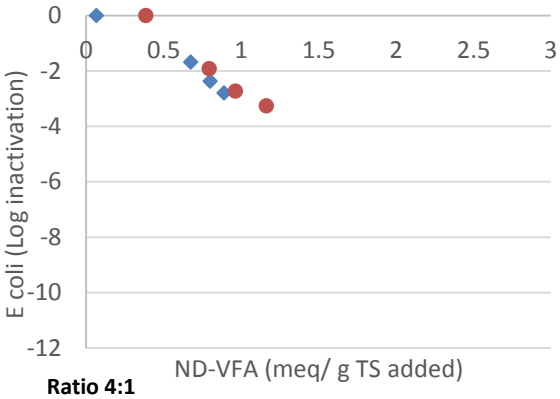
It was observed that total ND-VFA concentration in the range of 80-100 meq/l was needed to achieve *E. coli* log inactivation to below detectable levels in four days. Similarly, ND-VFA concentration in the range of 48-72 meq/l caused between 3-5 *E. coli* log inactivation in four days.

pH: Initial substrate pH did not affect *E. coli* inactivation in substrates with high OMW fraction, owing to prevailing VFA production. The case was observed at UDDT-F:OMW ratios 0:1, 1:4, 1:2 and 1:1, where *E. coli* inactivation to below detectable limits was achieved within the same period of time. However, a reduced *E. coli* inactivation was observed at decreasing OMW fractions in feed substrate, i.e. UDDT-F:OMW ratios 1:0, 4:1, 2:1. The ratio 2:1, $pH_{4.8}$ achieved *E. coli* inactivation to below detectable limits in

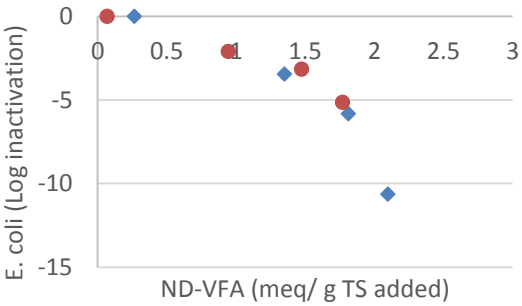
four days, whereas at pH_{un}, the achieved log inactivation was 5.1 log. At lower OMW fractions, adjusting pH led to an increase in ND-VFA concentrations in the feed substrate.



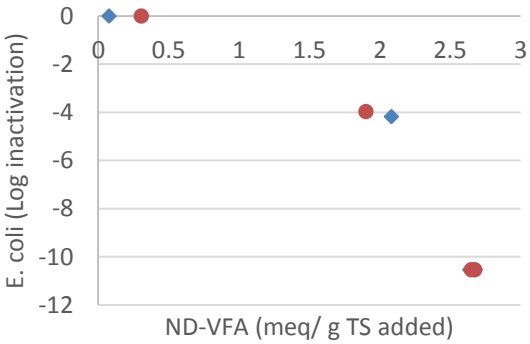
Ratio 1:0



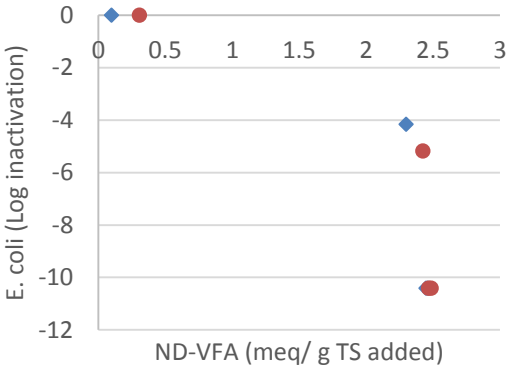
Ratio 4:1

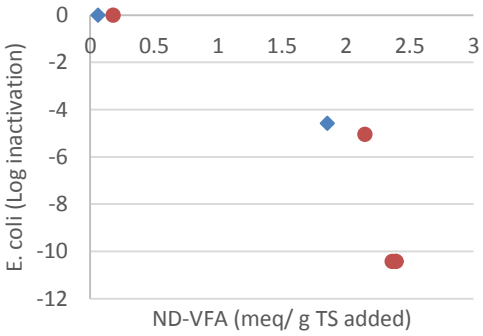


Ratio 2:1



Ratio 1:1





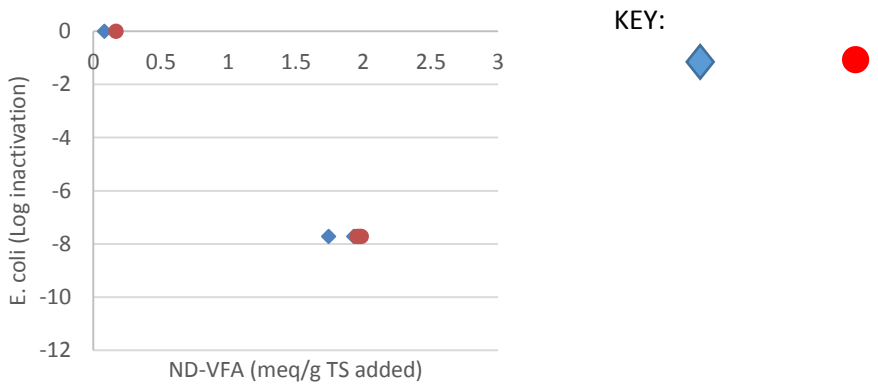


Figure 5: ND-VFA build-up (meq/g total TS added) versus *E. coli* log inactivation profiles at various UDDT-F:OMW mix ratios

3.3.2 *A. lumbricoides* egg inactivation

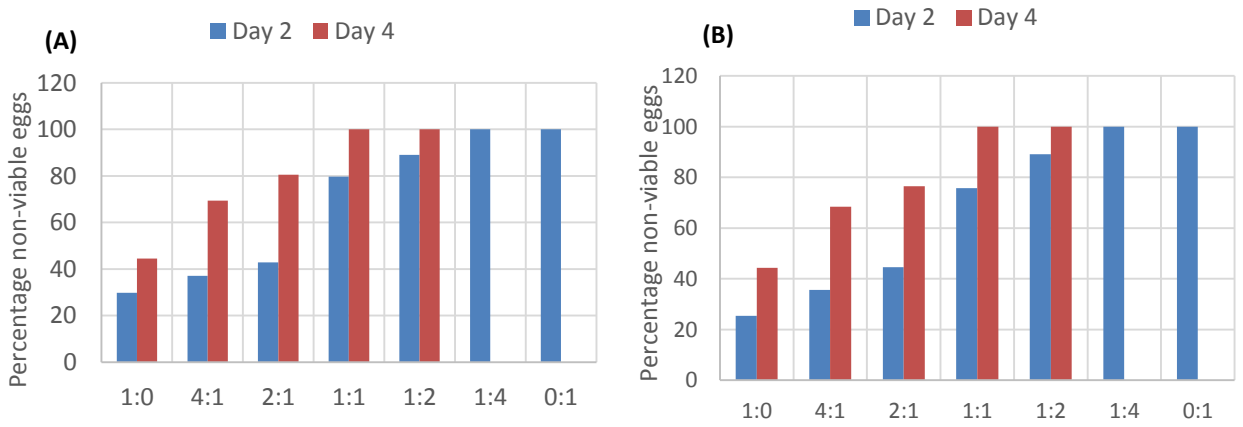


Figure 6: *A. lumbricoides* egg inactivation with time for **UDDT-F:OMW mix ratios 1:4, 1:2, 1;1, 21, 4:1 and 1:0**; A. pH 4.8, B. pH_{un}

The higher the OMW fraction in the substrate, the higher was the *A. lumbricoides* egg inactivation achieved. Complete *A. lumbricoides* egg inactivation was achieved within two days in OMW and UDDT-F:OMW ratio 1:4 substrates at both initial pH values (Fig. 6). Similarly, complete *A. lumbricoides* eggs inactivation was achieved in OMW and UDDT-F:OMW ratio 1:2 and 1:1 after four days of treatment, and more than four days are required in UDDT-F:OMW ratios 2:1, 4:1 and 1:0. UDDT-F:OMW=4:1 pH 4.8, UDDT-F:OMW=4:1 pH 5.82, UDDT-F pH 4.8, UDDT-F pH 5.74 and UDDT-F pH 7 recorded low inactivation levels of 73, 70, 65, 68 and 16 % respectively after four days treatment. Overall, our current experimental results showed a rapid inactivation of helminth ova, which depended on the OMW fraction in the feed substrate. Results are in contrast to studies that reported high resistance of helminth ova to

inactivation (Jimenez-Cisneros, 2007). The persistence of helminth ova are ascribed to their 3-4 protective layers that increase their resistance to treatment against desiccation, strong acids/ bases, oxidants, reductive agents, detergents and proteolytic compounds (Jimenez-Cisneros, 2007). Fig. 7 shows microscopic images of *A. lumbricoides* eggs after various treatments in this study.

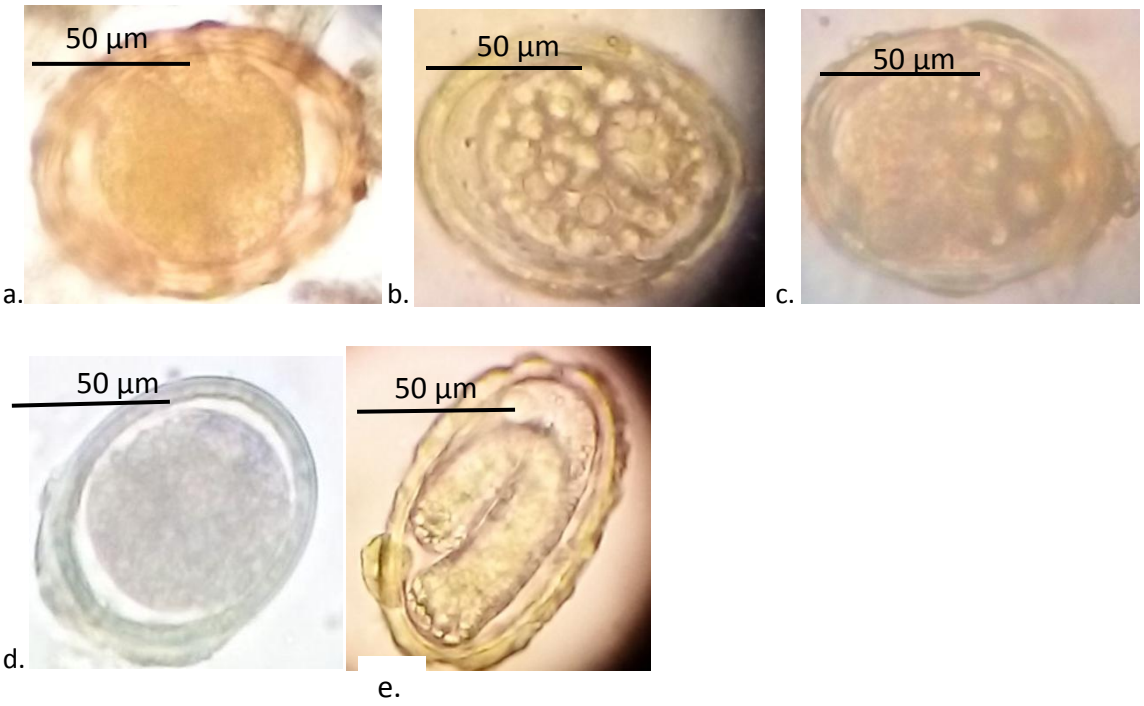


Figure 7: Microscopic images of *A. lumbricoides* eggs after two days of incubation at 35°C: a) Fertilized untreated *A. lumbricoides* egg, b) non-viable egg, OMW, pH 4.8; c) Non-viable *A. lumbricoides* egg, UDDT:OMW=1:4, pH 5.48; c) Non-Viable *A. lumbricoides* egg, UDDT-F**, pH 5.7 e) Embroyonated *A. lumbricoides* egg,** UDDT-F **pH 7.**

3.4 Practical application in enhancing UDDT-F sanitization

Study results showed increasing *E. coli* inactivation at increasing OMW fraction in the feed substrate until an optimum dosage in a ratio of 1:1.

Results indicate that ND-VFA build-up of 2-2.5 meq/ g total TS was required to achieve *E. coli* inactivation to below detectable level in less than four days. The VFA build-up agrees with a total VFA concentration of approximately 4800-6000 mg/L, taking the molar mass differences of acetate, propionate and butyrate into account. The required build-up was achieved at UDDT-F:OMW ratios 0:1, 1:4, 1:2 and 1:1 at both pH levels. However, the application of high OMW fractions is disadvantageous due to: 1) Logistic

concerns due to collection, sorting and transportation costs of the waste from the slums to the treatment site, 2) At high OMW fraction e.g. UDDT-F:OMW ratios 1:4, 1:2 and 1:1, pH declines to very low levels, which may be toxic to *E. coli* as well all other microbial population. For practical purposes the build-up of ND-VFA in the range of 1.2-1.8 meq/g total TS added seems to be sufficient, which agrees with a ND-VFA concentration of approximately 2800-4300 mg/L, causing between 3-5 *E. coli* log inactivation in four days. This was achieved by an UDDT-F:OMW ratio 2:1, pH_{un} and a ratio of 4:1, at both pH levels.

This treatment can be beneficial in various situations including emergency cases where waste is anaerobically hydrolyzed for sanitization before disposal.

4 Conclusion

Experiments were conducted to investigate the potentials for UDDT-F waste sanitization by VFA build-up. Results showed a correlation between OMW fraction in the substrate and *E. coli* inactivation, with an increasing trend in *E. coli* inactivation being observed at increasing OMW fraction.

In co-digestion, maximum ND-VFA build-up was achieved at UDDT-F:OMW ratio 1:1, where over 90% of the measured TVFA existed in their non-dissociated form, achieving *E. coli* inactivation to below detectable levels in 3 days. A decline in ND-VFA fraction at UDDT-F:OMW ratios 1:2, 1:4 was attributed to an increased toxicity effect on all bacterial species, including the acidifying organisms.

ND-VFA build-up in the range of 2.0-2.5 meq/g total TS added, leading to a NF-VFA concentration of approximately 4800-6000 mg/L, was needed to achieve 10 *E. coli* log inactivation and complete *A. lumbricoides* eggs inactivation in four days. An ND-VFA build-up in the range of 1.2-1.8 meq/g TS added, agreeing with a ND-VFA concentration of approximately 2800-4300 mg/L, was needed to achieve *E. coli* log inactivation in the range of 3-5 in four days. As such, the degree of sanitization of UDDT-F, depends on the OMW fraction applied.

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