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Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries

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ABSTRACT

Improving access to sanitation is one of the most effective means to improve public health. Anaerobic digestion of high-strength undiluted human simulant excreta was investigated in laboratory systems. The focus was on demonstrating the suitability of using simple unmixed anaerobic digesters for the treatment of a simulant high-strength undiluted human excreta and to quantify the effects of high ammonia concentration on the biogas yield. A maximum biogas yield of 0.44 $NL_{biogas}\ g^{-1}_{COD}$ was obtained in batch experiments, while yields of 0.38 and 0.24 $NL_{biogas}\ g^{-1}_{COD}$ were obtained at 5 and 8 g total ammonia nitrogen (TAN) L^{-1}, respectively. Using an inoculum acclimated to high ammonia concentrations was critical to successful biogas production at these high TAN concentrations. Stable long-term anaerobic digestion of simulant human excreta at ammonia concentrations ranging from 5.20 to 7.15 g-N L^{-1} was obtained in a scaled-down mimic of a low cost floating dome anaerobic digester. Overall, the results demonstrate that anaerobic digestion of undiluted human simulant excreta in simple unmixed digesters is feasible and yields biogas, which is a valuable commodity. When combined with proper hygienization of its effluent, anaerobic digestion could contribute to effective sanitation in developing countries with limited water availability.

Acronyms–Notation

AD	anaerobic digestion
AI	acclimated inoculum
COD	chemical oxygen demand (t, diss, ss subscripts refer to total, dissolved or suspended, respectively)
dw	dry weight
FA	free ammonia
HRT	hydraulic retention time
NAI	non-acclimated inoculum
NL	normal liter (volume of gas at 273 K and 1 atm)
OLR	organic loading rate
RE	removal efficiency
STP	standard temperature and pressure
TAN	total ammonia nitrogen
TS	total solid
VFA	volatile fatty acids
VS	volatile solid

Introduction

Improving global access to clean drinking water and safe sanitation is one of the least expensive and most effective means to improve public health and save lives (Montgomery and Elimelech, 2007). In 2014, an estimated 2.5 billion people were still without improved sanitation, of which about 1 billion people practiced open defecation (WHO-UNICEF, 2014). The United Nations World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002, articulated a number of targets for the coming decade, among these targets was to "halve by the year 2015, the proportion of people who do not have access to basic sanitation" (Dellström, 2005). Sanitation coverage by region shows marked differences. While in developed countries the coverage rate is >95%, many countries are not on track in meeting the ≥75% coverage Millennium Development Goals for sanitation. Sub-Saharan Africa, Oceania and Southern Asia are the three regions with the lowest sanitation coverage (30%, 35% and 42%, respectively) (WHO-UNICEF, 2014).

The impacts of poor sanitation are staggering. Fecal–oral contamination is an underlying factor in more than 50% of child deaths in the developing world. Every year, food and water tainted with fecal matter cause up to 2.5 billion cases of diarrhea among children, resulting in

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600,000 child deaths (BMGF, 2011). Furthermore, the WEHAB estimated that in China, India and Indonesia, twice as many people are dying from diarrheal diseases as from HIV/AIDS (WEHAB, 2002).

One of the major challenges with sanitation is developing and implementing innovative, user-friendly, low-cost systems. The centralized sewer-based collection and treatment systems existing in developed nations are too costly, too complex and use too much energy to implement in poor and less-developed countries (Lalander et al., 2013; Mara, 2013). Even in developed countries, the connection of dispersed human settlements such as remote houses, summerhouses, farms and some recreation facilities to sewerage systems is often too costly. Definitely, decentralized wastewater management is inevitable for comprehensive wastewater treatment and environmental protection worldwide. Decentralized sanitation technologies have the potential to convert urine and feces to safe end-products with fertilizing value for agricultural purposes (Dellström, 2005; Mara, 2013). Nitrogen and phosphorous have the greatest value in this context, while the organic matter offers possible energy recovery potential. The amount of feces and urine excreted daily by individuals varies considerably depending on water consumption, climate, diet and occupation. While the wet mass of feces excreted daily ranges between 70 and 520 g per person per day (g p^{-1} d^{-1}), an amount of 350–400 g p^{-1} d^{-1} is generally considered as a reasonable average (Torondel, 2010; Wignarajah et al., 2006; Franceys et al., 1992; Fry, 1973). Similarly, the urine volume produced daily ranges between 0.6 and 1.1 L p^{-1} d^{-1}, and an average of 1 L p^{-1} d^{-1} is suggested (Putnam, 1971; Franceys et al., 1992). These average excreta values correspond to a total of about 70–80 g_{dry} p^{-1} d^{-1} 1 or about 100–110 g chemical oxygen demand (COD) p^{-1} d^{-1}, almost all of it coming from the feces, a total of 7–10 g-N p^{-1} d^{-1} (with 80–90% of the nitrogen coming from the urine) and about 1 g-P p^{-1} d^{-1}.

Anaerobic digestion (AD) is a well-established process in which bacteria convert organic wastes to a methane and CO_2 gas mixture (generally about 60% methane and 40% CO_2) called biogas. This is the process occurring naturally in septic tanks, although in that case, the methane is released to the environment. Methane emissions are a lost opportunity and an environmental liability: methane is a valuable source of energy (about 36 kJ L^{-1} for methane at STP) and is a greenhouse gas generally agreed to be about 25 times more potent than CO_2 (on a mass basis) over a 100-year time frame.

There is very little reliable data on AD of undiluted human excreta. Snell (1943) published the first study on AD of human excreta: 0.5 m^3_{biogas} kg^{-1}_{VS} was produced during the anaerobic digestion of human feces. However, when feces were mixed with urine, the anaerobic digestion process was completely inhibited (Snell, 1943). Park et al. (2001) reported a biogas production of up to 0.21 m^3_{biogas} kg^{-1}_{COD} (or roughly 0.30 m^3_{biogas} kg^{-1}_{VS}) using an anaerobic sequencing batch reactor (ASBR) fed night soil and working at an organic loading rate (OLR) of 3.1 kg_{COD} $m^{-3}_{reactor}$ day^{-1}, a temperature of 35 °C and a hydraulic retention time (HRT) of 10 days. They found a large increase in biogas production after implementing a thickening scheme, which allowed to concentrate solids in their bioreactor. Meher et al. (1994) reported a biogas production of 0.16 m^3_{biogas} kg^{-1}_{VS} for AD of slightly diluted human waste (i.e., water consumption of 2.5 L p^{-1} d^{-1}) at psycrophilic temperatures (15 ± 1 °C) using a fixed dome anaerobic digester designed for a HRT of 30 days. Recently, Rajagopal et al. (2014) studied the co-digestion of brown water and food waste. They specifically separated feces from urine to increase the hydrolytic and acidogenic potential of co-digestion of food waste and feces. Additionally, co-digestion of excreta with other organics improves process efficiencies that are inhibited by excreta characteristics as seen in a similar study (Panyadee et al., 2013).

There is more information about treatment performance in septic tanks (Luostarinen et al., 2007; Canter and Knox, 1985) but usually the feedstock characteristics are very different compared to high-strength undiluted human excreta. Moreover septic tanks studies are generally focused on the removal of chemical oxygen demand (COD) and little or no information is given about methane or biogas production.

The main objective of the present study was to demonstrate the suitability of using anaerobic digestion in simple unmixed anaerobic digesters for the treatment of a simulant high-strength undiluted human excreta and to quantify the effects of high ammonia concentration on the biogas yield. Ultimately, these studies would support our field research on using anaerobic digesters for the treatment of high-strength undiluted human excreta in developing countries.

Material and methods

Simulant human excreta

The use of real human wastes in laboratory studies can pose health and safety concerns and thus a suitable simulant was developed and used in this study. While using a simulant may not fully represent actual waste, it avoids logistical issues and provides a consistent, well-characterized feedstock.

A modification of the recipes developed by Wignarajah et al. (2006) and Putnam (1971) was developed to prepare the simulated feces and simulated urine, respectively. The major components of feces are fats (5–25%$_{dw}$), carbohydrates (10–30%$_{dw}$), nitrogenous materials (2–3%$_{dw}$), bacterial debris (10–30%$_{dw}$) and inorganic matter (10–20%$_{dw}$) (Barman et al., 2009). Urine is mainly composed of inorganic salts (38%$_{dw}$), urea (36%$_{dw}$), organic compounds (13%$_{dw}$) and organic ammonium salts (13%$_{dw}$) (Putnam, 1971).

Table 1 shows the composition of simulant feces and urine used in this study. Feces simulant composition in % dry weight (dw) was as follows: baker's yeast (30%$_{dw}$) was used as bacterial debris, microcrystalline cellulose (10%$_{dw}$) and psyllium (17.5%$_{dw}$) were used as a carbohydrate/fiber simulant, oleic acid (20%$_{dw}$) was used for fats and 17.5%$_{dw}$ of miso was used to adjust nitrogen content as well as other chemical properties. The miso paste composition is given as 38% proteins, 21% fats, 20% fiber and 4% minerals. All chemicals were supplied by VWR (Radnor, Pennsylvania) except miso and psyllium that were purchased at a local grocery store (365 psyllium husk from Whole Foods, and miso was either Miso Master Organic from Whole Foods, or Shirakiku Miso, from Amazon.com). The simulant formulation was adjusted for trace metal contents after day 200 (see Results section for details) by adding a trace element solution to the simulant feces so that the composition was as follows: $FeCl_2 \cdot 4H_2O$, 28.6 mg kg^{-1}_{TS}; H_3BO_3, 1.14 mg kg^{-1}_{TS}; $MnCl_2 \cdot 4H_2O$, 1.91 mg kg^{-1}_{TS}; $CoCl_2 \cdot 6H_2O$, 2.29 mg kg^{-1}_{TS}; $ZnCl_2$, 1.34 mg kg^{-1}_{TS}; $NiCl_2 \cdot 6H_2O$, 0.48 mg kg^{-1}_{TS}; $CuCl_2 \cdot 2H_2O$, 0.29 mg kg^{-1}_{TS}; $NaMoO_4 \cdot 2H_2O$, 0.48 mg kg^{-1}_{TS}. The adequacy of the simulant formulation and how it matches real fecal waste is discussed in the Results section.

Table 1
Chemical composition of simulated feces and simulated urine. Stages 3 and 4 refer to different phases during the experiments (see text for details).

Simulant feces		Simulant urine		
Compound	Amount (g kg^{-1})	Compound	Amount (g L^{-1})	
			Stage 3	Stage 4
Water	800	Urea	9.3	14.2
Baker's yeast (dry)	60	Creatinine	2.0	3.0
Microcrystalline cellulose	20	Ammonium citrate	1.0	2.0
Psyllium	35	NaCl	8.0	8.0
Miso paste	35	KCl	1.65	1.65
Oleic acid	40	$KHSO_4$	0.5	0.5
NaCl	4	$MgSO_4$	0.2	0.2
KCl	4	KH_2PO_4	1.75	1.75
$CaCl_2$	2	$KHCO_3$	0.5	0.5

Biogas potential assays

The biogas potential and methane production from the anaerobic digestion of simulant feces were determined using a modification of the procedure described by the German Institute for Standardization (Germany, 2001). Three grams of wet feces was mixed with an inoculum (60 mL so that the VS feces:inoculum ratio was 1:2) coming from a mesophilic anaerobic digester treating sludge at a local sewage treatment plant. That ratio was used to avoid acidification and inhibition caused by volatile fatty acids accumulation. The mixtures (63 mL) were incubated on a shaker at 30 °C in 120 mL gas-tight bottles until no significant biogas production was observed. Before sealing each bottle, they were purged with nitrogen gas to ensure anaerobic conditions.

The tests were carried out in triplicate and the results were expressed as biogas volume produced at normal conditions (in NL at T = 293 K, P = 1 bar) per gram of COD. As is the norm in the field, the COD used to calculate the biogas yield is the COD added and not the COD consumed. The biogas generated was measured periodically. A triplicate measure of the biogas production of the inoculum was carried out as a blank test and subtracted from the biogas production obtained with the fecal waste samples. A control test was conducted to verify that the inoculum had adequate biological activity according to the German Institute for Standardization. This test states that biogas production should be at least 0.4 L_{biogas} $kg^{-1}{}_{TS}$ to validate the activity of the anaerobic inoculum used, which was the case here.

In order to test the importance of acclimation to high total ammonia nitrogen (TAN) concentrations, different biogas and methane potential runs were conducted at 3, 5, 8 and 10 g TAN L^{-1} using non-acclimated and acclimated inoculum. These concentrations were selected considering that 3 g TAN L^{-1} is the expected maximum concentration for diluted excreta in low-flush toilets, 5 g TAN L^{-1} would be a medium value for undiluted human excreta, and 8 g TAN L^{-1} should be considered as a high value for undiluted excreta. For these experiments, the non-acclimated inoculum was routinely exposed to 1.1 g TAN L^{-1} while the acclimated inoculum was exposed to a TAN maximum concentration of 4.3 g TAN L^{-1}. The acclimation process was done by four repeated-batch runs (3 weeks each) in 1 L sealed bottles in which NH_4Cl was supplied at the desired concentration; the first batch run was performed at a concentration of 1 g TAN L^{-1} and at each new batch run the concentration was increased by 1.1 g TAN L^{-1}. Additionally, in order to provide a carbon source, 2 g of microcrystalline cellulose was added at each batch run.

Anaerobic digester configuration and operation

A semi-continuous floating dome anaerobic digester with a working volume of 17 L was used in these experiments (Fig. 1). The digester is cuboid-shaped (25 cm × 25 cm sides by 40.5 cm high), and fitted with a dome (30.5 cm × 30.5 cm × 28 cm) holding the gas and floating in a water jacket surrounding the reactor. Feeding is accomplished via a 2 cm (ID) tube leading to the bottom of the digester distributing the waste at 6 cm from the bottom. In order to simulate operation of a full-scale digester in a less-developed country, no mixing was provided and the temperature was maintained at 30 °C by heating the water jacket with an aquarium heater. The digester was started with an inoculum (17 L with a total solid content of 1.75%) obtained from an anaerobic reactor treating sludge at a local sewage treatment plant. Synthetic fecal waste (120 g wet feces and 300 mL urine, corresponding roughly to the waste produced by a third of one person) was fed manually once a day. During the start-up, the organic loading rate was increased progressively from 0.5 to 1.8 kg_{COD} m^{-3} d^{-1} and the total nitrogen inlet concentration (N_{in}) was increased from 1.0 g-N L^{-1} to 7.15 g-N L^{-1} during the entire experiment. The feed rate corresponded to a hydraulic retention time of 40 days.

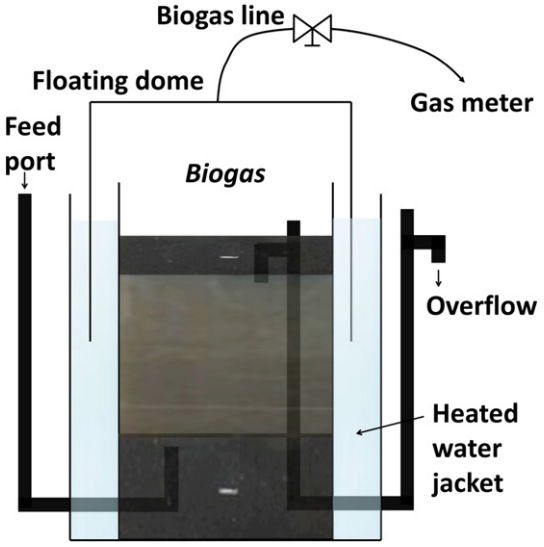

Fig. 1. Schematic diagram of the lab-scale floating dome anaerobic digester. The dome is floating in a water-filled jacket surrounding the anaerobic digester.

Analytical methods

Unless noted otherwise, the feed and digester effluent were analyzed once a week. Total solids (TS), volatile solids (VS), pH, and electrical conductivity were determined according to standard methods (APHA, 1998). COD, total nitrogen content and ammonia content were analyzed using Hach kits (Hach, Loveland, CO), and a spectrophotometer. Unfiltered, unprocessed samples were used for measuring total COD (COD_t) and 0.45 μm membrane-filtered samples for dissolved COD (COD_{dis}); suspended COD (COD_{ss}) was calculated by subtracting COD_{dis} to COD_t.

Free ammonia was calculated from the following equation (proposed by Østergaard and quoted by Hansen et al., 1998):

$$\frac{[NH_3]}{[TNH_3]} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(k)}\right)}}\right)^{-1} \quad (1)$$

where $[NH_3]$ is the concentration of free ammonia, $[TNH_3]$ is the total ammonia concentration and T(K) is the temperature (Kelvin).

The biogas produced in the digester was collected in the floating dome and its volume was measured periodically using a wet tip gas flow meter (wettipgasmeter.com). CH_4 and CO_2 content were analyzed by gas chromatography (SRI Instruments, Menlo Park, CA) with a thermal conductivity detector (TCD) and using a Hayesep column (1.8 m × 3.2 mm × 2.1 mm). The gas chromatography operating conditions were as follows: (a) oven temperature isothermal at 60 °C; (b) injector temperature 60 °C; (c) TCD temperature 150 °C; and (d) carrier gas He at 14 psi pressure. The GC was calibrated with gas standards of known concentration.

Volatile fatty acid (VFA) analysis followed the method of Fernández et al. (2005): 50 μL of sulfuric acid (98%) was added to each 0.6 mL of sample. The acidified sample was then centrifuged (30 min, 3500 × *g*) and the resulting supernatant filtered through a 0.2 μm syringe filter. This sample was used for VFA determination by gas chromatography (Shimadzu GC, Kyoto, Japan) with a flame ionization detector (FID) and using a HP-FFAP 25 m × 0.32 mm × 0. 5 μm column. The analysis conditions were as follows: (a) an initial oven temperature of 80 °C was maintained for 1 min, then it was increased to 120 °C at 20 °C min^{-1} and then to 205 °C at 6 °C min^{-1} and maintained at that temperature for 2 min; (b) injector temperature 260 °C; (c) FID temperature 260 °C; and (d) carrier gas He at 10.8 psi pressure. The system was

calibrated with different dilutions of a standard mixture of VFAs (including acetic, propionic, isobutyric, butyric, valeric and isovaleric acids, from Supelco (Bellefonte, PA)).

Trace element samples were digested with trace metal grade HNO_3 at 90 °C for 6 h and diluted with an acid solution that contains 2% of trace metal grade HNO_3 and 0.5% of trace metal grade HCl. This sample was used for trace elements determination by Agilent model 7700X ICP-MS (Agilent, Santa Clara, CA).

Results and discussion

Simulant excreta composition and properties

Table 2 shows the physicochemical properties of the simulant feces and urine used in this work and compares them with published data from the analysis of real excreta. The formulation of the simulant human feces used in this study was designed to mimic the water-holding capacity, the consistency and the chemical composition of human feces. Thus moisture content, VS, TS and COD:VS ratio as well as the nitrogen content and other important parameters were closely matching the values reported for real feces. Note that while the simulant feces had a consistency of a thick paste, no attempt was made to match the rheological properties of fecal matter, as this was deemed irrelevant in the present studies.

Nitrogen content is one of the most important parameters when treating undiluted human excreta biologically because of the well-known inhibition of anaerobic digestion at high ammonia concentrations (Rajagopal et al., 2013). Urine contains about 75% of the total daily nitrogen excretion. In order to cover the typical range of nitrogen human excretion, two different urine concentrations were tested in the lab-scale reactor, the first one containing 5.20 g-N L^{-1} and the second one containing 8.04 g-N L^{-1}. The nitrogen concentration of the final human excreta (mixture of feces + urine) had 5.2 and 7.15 g-N L^{-1} which corresponds to a total daily excretion of 7.25 and 10.0 g-N p^{-1} d^{-1} respectively. Thus, Table 2 contains information for these two nitrogen concentrations.

Many researchers have indicated that trace metals play an important role in the growth of methanogens and methane formation (Qiang et al., 2012; Facchin et al., 2013; Dong et al., 2013; Schmidt et al., 2014). In particular, it has been demonstrated that the growth of methanogenic bacteria is dependent on Fe, Co and Ni among others during enzyme synthesis (Gustavsson et al., 2011; Qiang et al., 2012). The content of metals and heavy metals is generally low in human excreta, and depends on the amounts present in consumed products (Jönsson et al., 2004). Although very large variability has been observed in the trace metal daily excretion, a range for some key trace elements can be established (Jönsson et al., 2005; Clemente et al., 1977; Biego et al., 1998; Herring et al., 1960): 5–38 mg Fe p^{-1} d^{-1}, <0.4 mg Ni p^{-1} d^{-1}, 0.006–0.08 mg Co p^{-1} d^{-1}, 0.25–10 mg Zn p^{-1} d^{-1}. A closer analysis of the simulant excreta revealed that among the main required trace elements, cobalt was underestimated in the first formulation and could affect the overall performance (see further details in Performance of the unmixed anaerobic digester). Thus a trace element solution was added to overcome the potential lack of Co so that the corresponding daily value in the excreta was adjusted from 0.006 to 0.05 mg Co p^{-1} d^{-1} (i.e., an increase from 75 to 641 μg Co kg^{-1}_{TS}). The addition of the trace elements solution did not significantly increase the other trace elements (13.4, 1.3, 9.1, 8.4, 13.4, 5.6 and 1.9% increase of the total composition of Fe, Zn, Ni, Mn, Mo, B and Cu, respectively).

Biogas potential assays

The results of the biogas potential assays conducted with the fecal simulant are shown in Fig. 2a, b and Table 3. For the non-acclimated inoculum (NAI) (initial concentration of 1 g TAN L^{-1}), biogas potential assays resulted in a biogas production of 0.437 L_{biogas} g^{-1}_{COD}. Note that the COD used to calculate the biogas yield is the COD added and not the COD consumed. No statistically significant differences were found

Table 2
Physicochemical properties of simulated feces and simulated urine and comparison with data obtained from real samples (trace elements in simulant feces is the sum of feces values plus the added trace element solution). Values are given on a per total solids (TS) basis, for calculation of daily personal value, a conversion factor of 0.08–0.10 kg_{TS} p^{-1} d^{-1} can be used. Stages 3 and 4 refer to different phases during the experiments (see text for details).

Physicochemical properties								
Feces			Urine				Excreta (feces + urine)	
Properties	Simulant feces	Real feces (reference)	Properties	Simulant urine (stage 3)	Simulant urine (stage 4)	Real urine (reference)	Simulant excreta (stage 3)	Simulant excreta (stage 4)
Moisture (%)	81.6	65–85 (Wignarajah et al., 2006)	Moisture (%)	97.6	96.5	95–98 (Putnam, 1971)	93.0	92.3
TS (%)	18.4	15–35 (Wignarajah et al., 2006)	TS (%)	2.4	3.5	2.5–3.7 (Putnam, 1971)	6.96	7.75
VS (% TS)	88.5	80–92 (Fry, 1973; Meher et al., 1994; Snell, 1943)	VS (% TS)	49.5	62.5	60–75 (Putnam, 1971; Fry, 1973)	78.9	79.9
COD_t:VS	1.51	1.56 (Jönsson et al., 2005)						
COD_t (g_{COD} g^{-1}_{TS})	1.33	1.24 (Jönsson et al., 2005)	COD_t (g_{COD} L^{-1})	2.9	3.9	3.8–8.2 (Jönsson et al., 2005)	72.1	72.8
COD_{SS} (g_{COD} g^{-1}_{TS})	0.85	–	COD_{SS} (g_{COD} L^{-1})	0	0	–	23.8	24.5
COD_{dis} (g_{COD} g^{-1}_{TS})	0.38	–	COD_{dis} (g_{COD} L^{-1})	2.9	3.9	–	48.3	48.3
N_{tot} (% dry matter)	2.75	2–3 (Jönsson et al., 2005; Barman et al., 2009)	N_{tot} (mg-N L^{-1})	5200	8040	5000–8000 (Putnam, 1971)	5190	7200
$N–NH_3$ (% N_{tot})	3.0	<7 (Jönsson et al., 2005)	$N–NH_3$ (mg-N L^{-1})	197	403	<100 (Jönsson et al., 2005)	246	295
C/N	17.3	5–16 (Jenkins, 2005)	C/N	0.58	0.59	0.8 (Rodale, 2000)	5.2	3.9
pH (1:5 w:v)	5.3	4.6–8.4	pH	6.0	6.0	6–8.2 (Putnam, 1971)	5.5	5.5
Conduct. (1:5 w:v, mS cm^{-1})	5.7	–	CE (mS cm^{-1})	23	24	16–22 (Putnam, 1971)	14.3	14.4
			P-total (mg-P L^{-1})	400	400	400–1000 (Putnam, 1971)		
Fe (μg kg^{-1}_{TS})	59,950		Fe (μg L^{-1})	654	654	240	51,900	
Zn (μg kg^{-1}_{TS})	46,210		Zn (μg L^{-1})	240	240		37,300	
Ni (μg kg^{-1}_{TS})	1289		Ni (μg L^{-1})	8.2	8.2		1060	
Co (μg kg^{-1}_{TS})	642		Co (μg L^{-1})	1.3	1.3		497	
Mn (μg kg^{-1}_{TS})	6251		Mn (μg L^{-1})	nd	nd		4700	
Mo (μg kg^{-1}_{TS}))	1555		Mo (μg L^{-1})	15.5	15.5		1330	
B (μg kg^{-1}_{TS})	3524		B (μg L^{-1})	89.4	89.4		3580	
Cu (μg kg^{-1}_{TS})	5654		Cu (μg L^{-1})	4.7	4.7		4300	

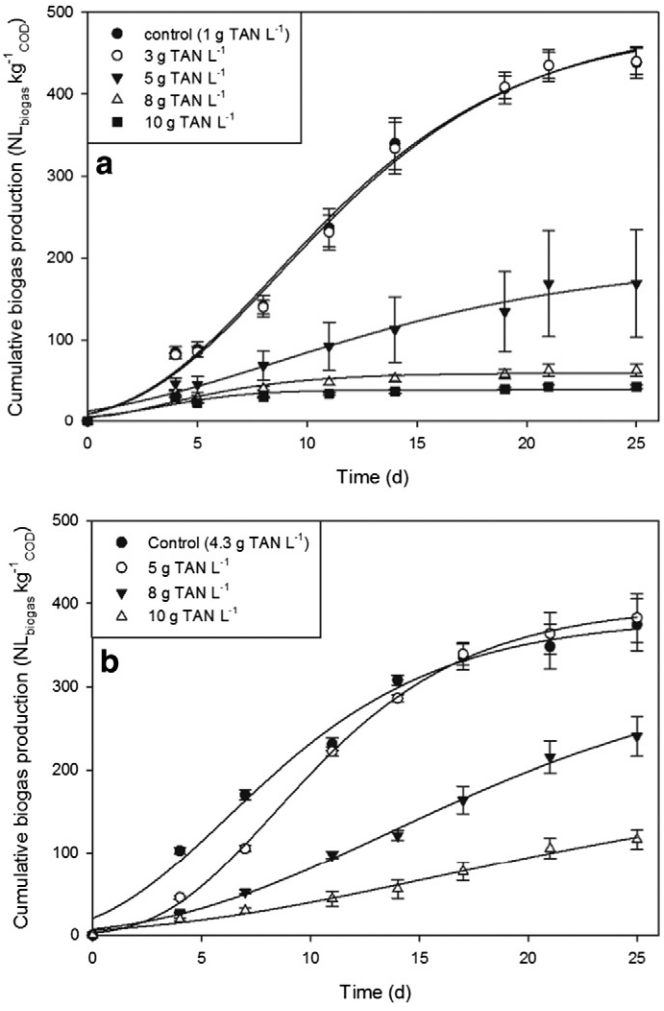

Fig. 2. Cumulative biogas production during biomethane potential tests with a) non-acclimated inoculum (NAI) and b) high TAN acclimated inoculum (AI). The lines show fitting using Gomperz model (details not shown).

between the control and the sample adjusted to 3 g TAN L^{-1} ($p > 0.05$). On the contrary, strong inhibition of biogas production was found for the rest of the samples, as a decrease in the biogas production of 66, 86 and 90% was measured for samples adjusted to 5, 8 and 10 g TAN L^{-1} respectively. No significant difference was found between samples adjusted to 8 and 10 g TAN L^{-1} ($p > 0.05$).

For the acclimated inoculum (AI) which had an initial concentration of 4.3 g TAN L^{-1}, biogas potential assays with simulant feces resulted in a biogas yield of 0.374 L_{biogas} $g^{-1}{}_{COD}$. No statistical differences were found between the acclimated control and the sample adjusted to 5 g TAN L^{-1} ($p > 0.05$). A decrease of the biogas production of 35 and 68% was measured for samples adjusted to 8 and 10 g TAN L^{-1}, respectively compared to the acclimated control (4.3 g TAN L^{-1}).

The comparison of biogas yields obtained from different inocula (AI vs. NAI) highlights the importance of microorganism acclimation for anaerobic digestion at high TAN concentrations. This is a phenomenon that was reported in previous studies (Chen et al., 2008; Zeshan et al., 2012; Hansen et al., 1998). Here, for example, the biogas yield increased 125% and 285% at 5 and 8 g TAN L^{-1}, respectively, in flasks with the AI compared to those with the NAI. Moreover, the average methane concentration remained stable in AI flasks with a methane concentration close to 60% in flasks with 4 to 8 g TAN L^{-1}, while flasks with the NAI showed a constant decrease in methane concentration from 60 to 20% (vol.) for flasks that had TAN ranging from 3 to 8 g L^{-1}. These findings are consistent with previous studies (Koster and Lettinga, 1988; Kayhanian, 1994), conducted at 8 g TAN L^{-1} and support the conclusion that the methanogens are the organisms least tolerant to elevated TAN and the most likely to be inhibited by high free ammonia concentrations. Ammonia has been shown to mainly affect acetate-utilizing methanogenic archaea, and to a lesser degree, hydrogen-utilizing methanogens and syntrophic bacteria (Ahring et al., 2003).

Table 3
Cumulative biogas production for non-acclimated and acclimated inoculum at different TAN concentrations.

	Non-acclimated inoculum		Acclimated inoculum	
g TAN L^{-1}	Biogas production (NL_{biogas} $g^{-1}{}_{COD}$)	Average methane (% vol.)	Biogas production (NL_{biogas} $g^{-1}{}_{COD}$)	Average methane (% vol.)
1*	0.437 ± 0.019	63.5 ± 1.1	ne	ne
3	0.433 ± 0.016	63.0 ± 0.5	ne	ne
4.3**	ne	ne	0.374 ± 0.020	60.1 ± 0.6
5	0.169 ± 0.065	43.4 ± 4.6	0.382 ± 0.029	60.0 ± 0.4
8	0.062 ± 0.007	20.5 ± 1.2	0.240 ± 0.023	57.8 ± 4.2
10	0.042 ± 0.006	16.7 ± 0.6	0.116 ± 0.012	38.4 ± 0.6

ne: no experiment.
* Initial TAN concentration for non-acclimated inoculum.
** Initial TAN concentration for acclimated inoculum.

Performance of the unmixed anaerobic digester

Fig. 3 shows the biogas and methane production during the entire experiment along with the OLR and the nitrogen inlet (N_{in}) concentration. The reactor was started slowly and acclimated (during stage 1 and 2) until working conditions of 1.8 g_{COD} $L^{-1}{}_{reactor}$ d^{-1} and 3.7 g-N_{in} L^{-1} were established; the reactor was then maintained for 40 days at these conditions. Then, for stage 3, the N_{in} concentration was increased up to 5.2 g-N_{in} L^{-1}. At these conditions, the nitrogen content of the simulant excreta is equivalent to 7.25 g-N p^{-1} d^{-1} which matches reported values for developing countries ranging from 5.2 to 8.2 g-N p^{-1} d^{-1} for countries such as Uganda, Haiti, India or South Africa (Richert et al., 2010). Later in the experiment (stage 4), the N_{in} concentration was increased up to 7.15 g-N_{in} L^{-1} which corresponds to a daily excretion of 10 g-N p^{-1} d^{-1}. This value is above the normal range for developing countries (Richert et al., 2010) and in the upper range for developed countries (Putnam, 1971; Jönsson et al., 2005). It should be noted that such high N concentrations imply that all urinating is conducted in latrines, which is often not the case where access to improved sanitation is limited, and where urinating in the open is often preferred (Cofey et al., 2014).

After a short lag phase during stage 1, the biogas production increased exponentially over time and with successive loading increases, until an OLR of 1.8 g_{COD} $L^{-1}{}_{reactor}$ d^{-1} was established. From day 60 to day 100 (stage 2) the reactor was fed with simulant feces and urine at a 2:1 urine:water ratio, i.e., in the low range of nitrogen concentration for human excreta (5.2 g-N p^{-1} d^{-1}). During the last 25 days for stage 2, the biogas yield was 0.41 NL_{biogas} $g^{-1}{}_{COD}$ with a methane content of 65%. Fig. 4 shows the COD_t removal efficiency (RE), which during this period was high with an average total COD removal value close to 80%. Detailed examination of COD data reveals that most of the remaining COD (>80%) was in form of dissolved COD. During that stage the VFA concentration ranged between 2.5 and 3.3 g VFA L^{-1}, with acetic acid (0.7–2.1 g L^{-1}) and propionic acid (1.0–1.4 g L^{-1}) being the dominant VFAs (Fig. 5) and constituting about 30–40% of the residual soluble COD. The fact that a majority of the residual COD was easily biodegradable is somewhat surprising. Biomethane potential tests conducted with the digester effluent revealed that significant biogas potential was indeed remaining in the effluent. This is likely because the floating dome anaerobic digester was not stirred and thus the majority of biomass settled at the bottom of the digester resulting in poor contact

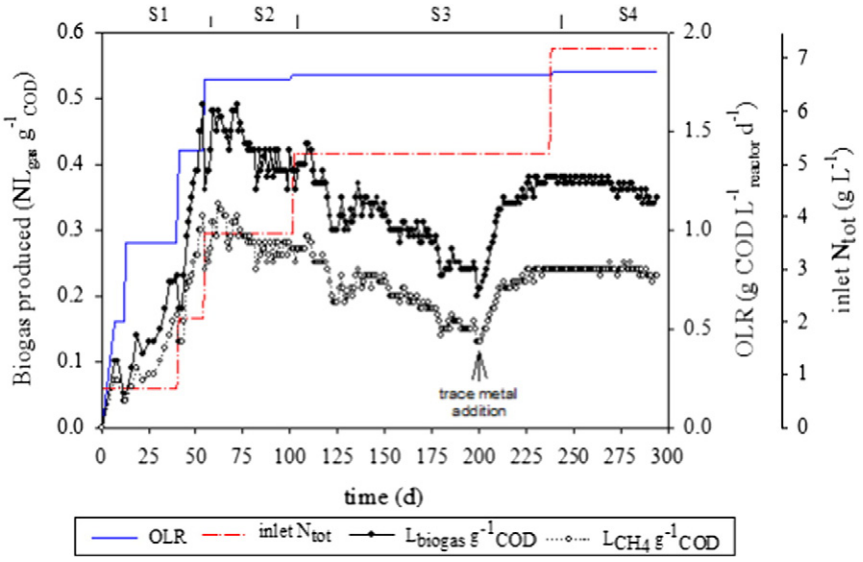

Fig. 3. Biogas and methane production from the lab-scale floating dome digester. S1 through S4 at the top of the graph denotes different stages (N loading) during the experiment (see text for details).

between methanogenic bacteria and the waste and dissolved COD undergoing treatment.

After day 100, during stage 3, the N_{in} concentration was increased up to 5.2 g-N_{in} L^{-1} (no urine dilution) and was maintained until day 240. After a few days of steady operation, the biogas production gradually decreased from day 120 to day 200, with a simultaneous increase in the residual VFA content. At the end of this period, the biogas yield had decreased to 0.25 ± 0.02 NL_{biogas} g^{-1}_{COD} and a methane content of 60%; the VFA concentration increased up to 14 g VFA L^{-1} (Fig. 5). The overall COD removal also decreased down to 60% (Fig. 4), mainly due to accumulation of dissolved COD and washout of biomass from the reactor (as represented by COD_{SS}). The combination of lower biogas yield and higher VFA concentrations clearly indicated that the activity of methanogenic bacteria was rate-limiting during that period. The fact that residual VFA was mostly acetate (with concentrations exceeding 10 $g_{acetate}$ L^{-1}), indicated that it was primarily the acetate-utilizing methanogenic bacteria which were inhibited. Two hypotheses were proposed to explain the drop in biogas production.

The first hypothesis was ammonia inhibiting methanogenesis as observed during biomethane potential tests presented earlier (Fig. 2). As discussed, several authors have shown an inhibition of anaerobic digestion due to high ammonia concentrations. Fig. 6 shows TAN (total ammonia nitrogen) and FA (free ammonia) in the reactor during the entire experiment. FA concentrations ranging from 80 to 150 mg-N L^{-1} have been reported to cause inhibition by several authors (Rittmann and McCarty, 2001; Braun et al., 1981; De Baere et al., 1984), on the contrary, total ammonia nitrogen was only found (Rittmann and McCarty, 2001) to cause inhibition at much higher concentrations, i.e., at about 3000 mg-N L^{-1}. A decrease of close to 50% of the methanogenic activity has been reported for ammonia concentrations in the range of 4000–5500 mg TAN L^{-1} (Angelidaki and Ahring, 1993; Hashimoto, 1986; Hansen et al., 1998; Koster and Lettinga, 1988). Further, process instabilities due to high ammonia concentrations often result in VFA accumulation, which leads to a detrimental decrease in pH but also a lower concentration of FA. The interaction between FA, VFAs and pH may lead to an "inhibited steady state", a condition where the process is somewhat stable but operates with a lower methane yield (Rittmann and McCarty, 2001). The conditions observed during stage 3 (>4000 mg TAN L^{-1} and 80–150 mg FA L^{-1}) were consistent with previous published data, but no steady state performance was achieved. Methane yield continued to decrease and thus a new hypothesis was proposed to explain the declining performance.

The second hypothesis was that a trace elements deficiency was affecting the anaerobic digester. Given the long HRT, trace elements

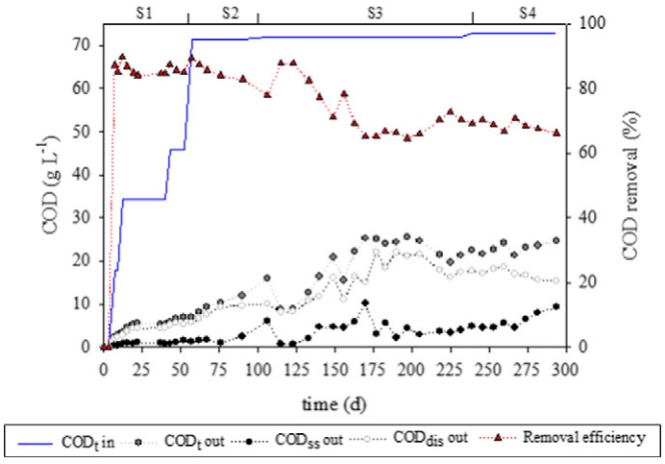

Fig. 4. COD concentrations and removal efficiency over time in the lab-scale floating dome digester.

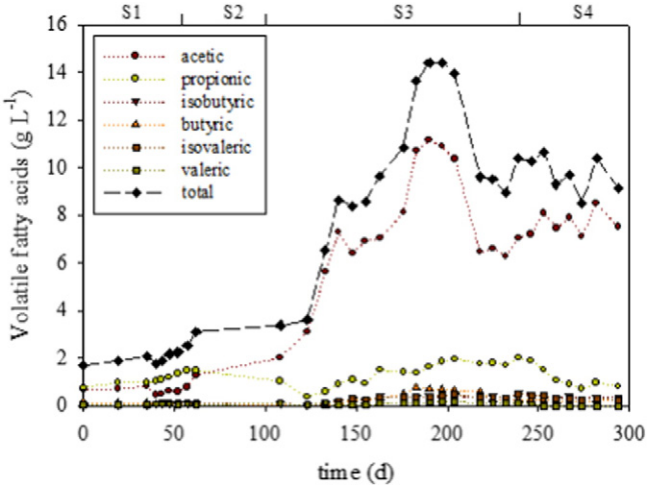

Fig. 5. VFA concentrations over time in the lab-scale floating dome digester.

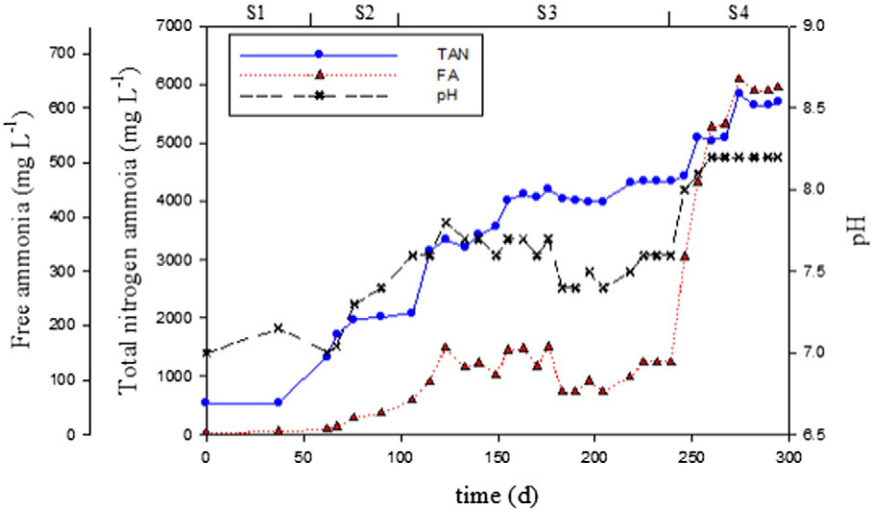

Fig. 6. pH, total ammonia nitrogen and free ammonia over time in the lab-scale floating dome digester.

present initially (added e.g., with the inoculum) but deficient in the feed could slowly become limiting and in the biogas production pattern reported in Fig. 3. As explained in Simulant excreta composition and properties, a lack of cobalt in the simulant excreta was most likely among all trace elements. Thus a trace metal solution was supplemented from day 200 on. As shown in Figs. 3–5, the biogas production immediately increased thereafter and reached a steady state performance around day 210, while a simultaneous decrease in the VFA concentration and an increase in COD removal were observed. When steady performance was re-established, performance was comparable or slightly lower than when stage 3 was initiated. The average biogas yield was 0.37 ± 0.02 $NL_{biogas}\ g^{-1}{}_{COD}$, the residual VFA concentration was close to 9 g L^{-1} with acetate as the main component. The COD removal recovered to 70% mostly due to the reduction of COD_{diss}. Some biomass and solids washout was observed from the reactor (as indicated by COD_{SS}) and promoted by scum accumulation during this stage.

On day 240, the inlet nitrogen concentration was increased up to 7.15 g-N L^{-1} (stage 4) and it was maintained at that level until the end of the experiment. As a consequence of higher ammonia concentration, the pH increased to 8.2 and thus FA concentration also increased up to values close to 650 mg-N-NH_3 L^{-1}. Several authors have reported a 50% decrease in methane production when FA ranges between 220 and 750 mg-N L^{-1}. However, during stage 4, the reactor did not show signs of major inhibition as the main parameters remained constant during this stage. The average biogas yield was 0.36 ± 0.02 $NL_{biogas}\ g^{-1}{}_{COD}$ with an average methane content of 66%, the average VFA concentration was 9.8 g VFA L^{-1} with acetate as the dominant species. As in the previous stages, the COD removal was maintained close to 70%. Comparison of these values with those obtained in stage 2 indicates reduced (but not complete loss of) performance. In particular means to increase VFA conversion to reduce VFA concentration in the effluent should be researched, e.g., by optimizing methanogenesis or developing a simple post-treatment for digestate effluent.

It is relevant to conduct a COD balance over the anaerobic digester. All the COD entering the digester should end up in either in the end-product (methane), leave the digester in the liquid effluent, or be incorporated in new bacterial mass. Since both methane and COD in the reactor effluent streams were monitored, the COD balance can be used to estimate the amount of biomass formed and infer a desludging frequency. The COD balance was conducted over a period of 65 days (from day 211 to 275) when the biogas production and the total COD removal were relatively stable. Calculations (not shown) reveal that 66.4% of the influent COD was recovered as CH_4, 29.9% of the influent COD was found in the effluent liquid (as VFA and suspended COD) and thus the balance (3.7%) was incorporated as new bacterial mass. With the OLR of 1.8 kg COD $m^{-3}{}_{reactor}\ day^{-1}$ and an average COD of cells of 1.4 $kg_{COD}\ kg^{-1}{}_{VSS}$ (van Lier, 2008), this represents roughly a dry biomass production of 0.05 kg VSS $m^{-3}{}_{reactor}\ d^{-1}$. This low biomass accumulation rate is related to the naturally low biomass yield of anaerobic digestion combined with some biomass washout (in form of COD_{SS}) in the outlet effluent. For example, some days, the accumulated biomass was 0 or even negative due to sludge washout. As a result, the anaerobic digester has not been desludged for over 2 years (except for minor sludge sampling for analysis or to conduct specific experiments). The desludging time is an important parameter for a successful low-cost sanitation system as it directly relates to maintenance requirements and costs. More research, in particular with actual feces and urineand full-scale systems, is needed to understand the true rate of biomass accumulation under actual field conditions.

Conclusions

The results of these laboratory studies show that anaerobic digestion of high-strength undiluted human simulant excreta in simple unmixed anaerobic digesters is feasible and effective. This approach could be part of a sanitation alternative for developing countries where water availability is limited. In fact, we have used data obtained in this study to support the design, start-up and operation of five full-scale (2 m^3) anaerobic digesters treating essentially undiluted human excreta in Kenya, India and the Philippines (Forbis-Stokes et al., 2015). Additionally, this work shows that anaerobic digestion of human simulant excreta can provide a meaningful source of energy. Biogas yields ranging from 0.24 to 0.44 $NL_{biogas}\ g^{-1}{}_{COD}$ were obtained in the lab, which correspond to about 24 to 44 NL biogas per person per day. The COD removal reached about 80%. While further studies are needed to assess the value of the digestate as fertilizer, it is anticipated that the digestate retains most of the nitrogen and phosphorous content of the feed, and thus could be used locally if sanitized. Both biogas and locally produced fertilizer have been shown by others to have potential for significant economic and environmental impacts at the household level (Laramee and Davis, 2013; San et al., 2012). However, mesophilic anaerobic digestion does not by itself produce effluent of suitable hygienic quality for safe use as fertilizer, and thus post-treatment is required to meet guidelines for reuse. Consequently, for biogas digesters to deliver improved sanitation, designs incorporating additional sterilization stages and possibly post-treatment to reduce VFAs to lower concentrations are required. A biogas-powered heat sterilization system is being developed in our group (Colón et al., 2013) which combined with the anaerobic digester offers the potential of self-contained sanitation.

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