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Review

Review of synthetic human faeces and faecal sludge for sanitation and wastewater research

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Investigations involving human faeces and faecal sludge are of great importance for urban sanitation, such as operation and maintenance of sewer systems, or implementation of faecal sludge management. However, working with real faecal matter is difficult as it not only involves working with a pathogenic, malodorous material but also individual faeces and faecal sludge samples are highly variable, making it difficult to execute repeatable experiments. Synthetic faeces and faecal sludge can provide consistently reproducible substrate and alleviate these challenges. A critical literature review of simulants developed for various wastewater and faecal sludge related research is provided. Most individual studies sought to develop a simulant representative of specific physical, chemical, or thermal properties depending on their research objectives. Based on the review, a suitable simulant can be chosen and used or further developed according to the research needs. As an example, the authors present such a modification for the development of a simulant that can be used for investigating the motion (movement, settling and sedimentation) of faeces and their physical and biological disintegration in sewers and in on-site sanitation systems.

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1. Introduction

Investigations involving human faeces are of great importance in many fields of research, such as medicine (Lewis and Heaton, 1997; Bekkali et al., 2009), sanitary product development (such as diapers, toilets etc.) (Stern and Holtman, 1987; Palumbo and D'acchioli, 2001), operation and maintenance of sewer systems (Butler et al., 2003; Penn et al., 2017), and implementation of faecal sludge collection and treatment for onsite sanitation systems (Wignarajah et al., 2006; Bassan et al., 2014; Colón et al., 2015). Development of synthetic faeces and faecal sludge is a challenging task due to their high variability depending on diet, lifestyle and geographical location (Rose et al., 2015). In this paper, we focus on synthetic faeces and faecal sludge developed for sanitation research, hence resembling human stool and faecal sludge in specific physical and chemical properties. The high variability of faeces (Rose et al., 2015) and faecal sludge collected from onsite systems (Strande et al., 2014) makes it difficult to obtain consistent samples and therefore execute repeatable experiments. Moreover, due to the potentially pathogenic content of human excreta, working with real faecal matter involves special safety precautions. Working with synthetic faecal matter can alleviate these challenges.

Faeces and faecal sludge are different. Faecal sludge is the faecal waste stored within onsite sanitation technologies. In addition to faeces it includes everything that goes into the toilet, for example, urine, flush water, greywater, anal cleansing materials and municipal solid waste (Strande et al., 2014). Faecal sludge differs significantly from fresh faeces alone; it is typically much more dilute due to the addition of liquids. Additionally, its characteristics are highly variable due to differences in storage duration, storage temperature and storage technology, and can range from fresh, to partially degraded, to completely stabilized (Strande et al., 2014). Synthetic faeces have been developed to address many sanitation related research questions. Most of the developed simulants mimic specific physical, chemical or thermal characteristics of human faeces important to the research objectives for which they are developed. Physical properties such as shape, size, density and rheology are of importance for simulating phenomena such as faeces settling, transport in sewer pipes, dewatering, viscous heating for pathogen destruction, and physical disintegration (e.g., Butler et al., 2003; Veritec Consulting Inc. & Koeller and Company, 2010; Podichetty et al., 2014). Chemical properties including chemical and biological oxygen demand, nutrient concentration, pH and conductivity are of importance for simulating biological disintegration, treatment of faeces and biogas production (e.g., Kaba et al., 1989; Wignarajah et al., 2006; Miller et al., 2015). Heating properties and elemental composition (C,H,N,O) are of importance for analysing energy recovery and for using the faeces for soil amendment e.g., biochar or compost production (e.g., Ward et al., 2014; Colón et al., 2015; Onabanjo et al., 2016a). Studies on the fate of faeces in sewers and in onsite sanitation systems include their movement, settling and physical disintegration together with biochemical disintegration. For these kinds of investigations the simulant is required to obtain a combination of chemical, biological and physical properties. Such a faeces simulant is still missing in the literature.

Three distinct recipes for synthetic faecal sludge have been reported in the literature. Their intended purposes include research into anaerobic digestion (Zuma, 2013; Colón et al., 2015) and pit latrine emptying (Radford et al., 2015). Together with the synthetic faeces recipes presented in this review, they could be used as a basis for the development of improved faecal sludge simulants in the future.

In this article, we provide a critical literature review of synthetic faeces and faecal sludge used for human waste related research. Based on this overview we present a modified simulant recipe that is applicable to be used for studying the fate of faeces in sewers and in onsite sanitation systems. A series of experimental results showing how these properties can be selectively manipulated by making changes in the recipe and an explicit preparation procedure can be found in the appendix of this paper.

2. Characteristics of human faeces and faecal sludge

2.1. Faeces

Faecal solids are composed of proteins, fats, fibre, bacterial biomass, inorganic materials and carbohydrates. Their chemical and physical characteristics vary widely depending on person's health and diet, as presented in Table 1. The average number of stools produced by adults is one per day (Ciba− Geigy, 1977). The median daily wet mass of faeces produced per person is 128 g (Rose et al., 2015), which falls within the reported full range of 35–796 g reported by Ciba − Geigy (1977) and Rose et al. (2015). Wyman et al. (1978) compared average stool sizes of 20 people (average of 10 samples from each individual). They identified that 250 g/stool and 111.3 g/stool were the maximum of these averaged weights of the men and women participants, respectively, in the study. In their review of faeces characteristics Rose et al. (2015) further report that live and dead bacteria comprise between 25 and 54% of the dry weight of faeces. The median water content in faeces is 75%, with a range of 63–86% across mean values of studies. Variations in water content and faecal mass are attributed to differences in fibre intake, as non-degradable fibre absorbs more water in the colon and degradable fibre stimulates growth of bacterial biomass (Eastwood, 1973; Garrow et al., 1993; Reddy et al., 1998). Rose et al. (2015) report that volatile solids comprise 92% of the total solids (TS) fraction of faeces. Faeces pH ranges between 5.3 and 7.5, with biological oxygen demand (BOD) between 14 and 33.5 g/cap/day and chemical oxygen demand (COD) between 46 and 96 g/cap/day (Rose et al., 2015).

Faeces are also highly variable in their physical structure. This variability can be characterized by the “Bristol Stool Form Scale” introduced by Lewis and Heaton (1997) for assessing intestinal

Table 1
Chemical and physical properties of faeces found in the literature.

		Range	Range	Median
		amount/cap/d	Other units	
Chemical properties	Wet mass	35–796 g[a,f]		128 g/cap/d[f]
	Water content		63–86 wt%	75 wt%[f]
	Protein		2–25 wt% of solids weight (+50% of bacterial biomass)[f]	
	Fibre	0.5–24.8 g[f]		6 g/cap/d [f]
	Carbohydrates	4–24 g [f]	25 wt% of solids weight [f]	9 g/cap/d [f]
	Fats	1.9–6.4 g	8.7–16 wt% of solids weight [f]	4.1 g/cap/d [f]
	Bacteria content		25–54 wt% of solids weight [f] 100–2200 10^{12} cells/kg [b]	
	BOD	14–33.5 g[f]		
	COD	46–96 g[f]		
	TN	0.9–4 g[f]	5–7% wt% of solids weight[f]	1.8 g/cap/d[f]
	VS		92 wt% of TS[f]	
	pH[e]		5.3–7.5[f]	6.6[f], 7.15 (average)[b]
	Calorific value	0.21–1.45 MJ[f]		0.55 MJ/cap/d[f]
Physical properties	Shape		Type 1 (hard lumps) – type 7 (watery diarrhoea) [e]	3.6 (average)[f]
	Viscosity [c]		3500–5500 cPs	
	Density'		<1 g/ml for 10–15% of healthy humans [a]	1.06–1.09 (average) [b,d]

[a]Levitt and Duane, 1972 ; [b]Ciba-Geigy, 1977; [c]Yeo and Welchel 1994[b]; [d]Brown et al., 1996[c]; [e]Lewis and Heaton 1997[d]; [f]Rose et al., 2015[e].

transit rate. The scale categorizes stools into one of seven types, ranging from type 1 (hard lumps) to type 7 (watery diarrhoea). Types 3 and 4 (“hard, lumpy sausage” and “loose, smooth snake”) are classified as normal stool forms. Onabanjo et al. (2016a) identified the moisture content of each stool classification ranging from ~50% (type 1) to >80% (type 7). The Bristol Scale has been used to assess stool form in the study of gastrointestinal disorders (e.g., Garsed et al., 2014; Nolan et al., 2015). Woolley et al. (2014a) measured the rheological properties of fresh human faeces. They showed that with increasing shear rate the apparent viscosity measurements of the samples decreased. For any given shear rate, higher apparent viscosities were associated with lower moisture contents. Viscosity measurements of runny to solid faeces were found to be in the ranges of 3500–5500 cP (Yeo and Welchel, 1994). According to the US National Bureau of Standards (NBS) faeces are characterized by density of 1.06 g/ml (Brown et al., 1996). 10–15% of healthy humans produce stool that floats (has a density less than 1 g/ml) due to trapped gas in the faeces (Levitt and Duane, 1972).

2.2. *Faecal sludge*

Faecal sludge originates from onsite sanitation technologies, and has not been transported through a sewer. It is raw or partially digested, a slurry or semisolid, and results from the collection, storage or treatment of combinations of excreta and blackwater, with or without greywater (Strande et al., 2014). Blackwater is defined as wastewater generated by the toilet, and includes excreta as well as flush water and anal cleansing water and/or dry anal cleansing materials (Tilley et al., 2014). Greywater contain all other domestic wastewater flows including bathing, washing, laundry and kitchen (Gross et al., 2015).

Typical quantities and qualities of faecal sludge are difficult to determine due the variety of onsite sanitation technologies in use, such as pit latrines, septic tanks, aqua privies, and dry toilets. They further depend on the design and construction of the sanitation technology, how the technology is used, how the faecal sludge is collected, and the frequency of collection (Strande et al., 2014). Recent findings have indicated that faecal sludge characteristics are correlated to the containment technology, but that there is no discernible difference between faecal sludge from public toilets and households (Strande et al., in preparation). Lack of standardized methods for the characterization of faecal sludge further contribute to the variability in the measured parameters (Velkushanova et al., in preparation).

The important parameters to be considered for faecal sludge characteristics are similar to those of faeces, they are presented in Table 2.

3. Synthetic faeces and faecal sludge found in the literature

Appropriate simulants for faeces and faecal sludge should be able to reflect the range of physical, chemical, biological and thermal characteristics relevant for the research objective. This specifically includes:

- physical characteristics e.g. represented by the Bristol stool scale (for faeces simulants),
- shapability into the characteristic faeces cylinder, and can be made to float or sink (for faeces simulants),
- viscosity and dewatering properties,
- chemical and biological properties including COD, BOD, TN, pH, EC, TS, VS, elemental composition, biogas potential,
- thermal properties, such as calorific value and ash content,
- ability to physicality disintegrate with a resulting aqueous suspension having similar chemical properties as real disintegrated faeces (for faeces simulants) and biologically degrade in a representative way (for faecal sludge simulants).

This wide variety of faecal and faecal sludge properties pose a substantial challenge for creating a universal synthetic replacement. Indeed, such an optimal simulant has not yet been developed. Simulants found in the literature were developed to reproduce specific characteristics of human stool or faecal sludge, depending on the research objectives, with varying degrees of success. All developments were successful in producing a simulant that is safe to use and does not represent any biohazard.

3.1. *Physical parameters*

The following simulants are designed to reflect specific physical properties of human faeces and faecal sludge such as shape, rheology or density. As faeces are distinct from faecal sludge we discuss each type of simulant separately.

Table 2
Physical and chemical properties of human faeces and faecal sludge compared with simulants.

Properties	Faeces	Synthetic faeces	Faecal sludge	Synthetic faecal sludge
Shape	From "hard lump" to "watery diarrhoea", "hard lump sausage" and "loose smooth snake" are normal forms[c]	Cylinder[i]		
Length (cm)		8–10[i,u]		
Diameter (cm)		2.5–3.4[i,u]		
Volume (ml)	90-169 (for women) 82-196 (for men)[c]			
Density (kg/l)	1.06–1.09[c,h]	1.02–1.06[i,u]	1–2.2[ag]	0.8–2.2[ag]
Viscosity (cP)	3500–5500[h]	1000–40,000[h,ab]	8.9×10^{-1} - 6×10^{9}-[ac]	NF*
Dewatering rate (g/m^2/min)	350-400 (for regular stool, very high for runny faeces)[f]	50–400[h]	11 (% of TS in the dewatered cake)[ao,ap]	4.5 (% of TS in the dewatered cake)[ao,ap]
Shear strength (Pa)			<1760[ag]	9-10,000[ag]
COD_{total}	0.6–1.5% of TS [q,af,ah]	1.3% of TS [ad,af]	7000–106,000 mg/l[x,z]	73[ad] 12,500–72,800 mg/l[y,ad]
$COD_{soluble}$	0.38% of TS [ad]			1000–48,300 mg/l[y,ad]
BOD	14–33.5 g/cap/d [ah]		600–40,000 mg/l [m,z]	
TN	2-7% of TS [q,t,af,ah]	2.8% of TS [ad,af]	50–1500 mg/l[u,ab]	880–7200 mg/l[y,ad]
$N\text{-}NH_3$ (% of N_{total})	<7[af]	<3.02[af]		
C/N	5–16[af]	17.3[af]		
pH	4.6–8.4 [e,ad,af,ah]	5.3[ad,af]	6.7–8.5[s,z]	5.5–7.73[y,ad]
EC (mS/cm)		5.7[ad]	2.2–14.6[am]	14.4[ad]
TS (%)	14–37[r,af,ah]	18.4[p,af]	0.5–40[am,w]	1.7–85[y,ad,ag]
VS	80-92% of TS[a,d,e,af, ah,ak]	86.8–88.5% of TS [ad,af,ak]	7000–52,000 mg/l[x,z]	78.9–79.9[ad] 1600–1800 mg/l[y]
C (% of TS)	44–55[p,ae,ak,al]	43.4–47.3 [k,aj,ak,af,al]	27.8–28.8[an]	
H (% of TS)	7.0–7.6[ae,ak,al]	6.2–7.2 [k,aj,ak,af,al]	4.2[an]	
N (% of TS)	1.1–18 [e,p,ae,ak,al]	2.1–7.2 [k,aj,ak,af,al]	3.0–3.2[an]	
O (% of TS)	21–32[ae,ak,al]	30–42 [k,aj,ak,af,al]		
Fe (μg/kgTS)	72,381–1,230,769[e]	59,950 [ad]		
Zn	64,762–660,256μg/kgTS [e]	46,210 μg/kgTS [ad]	646-918 ppm[an]	
Ni	1016–34,615μg/kgTS [e]	1289 [ad]	24-30 ppm[an]	
Co (μg/kgTS)	254–3,846[e]	642 [ad]		
Mn (μg/kgTS)	46,857–236,539 [e]	6251 [ad]		
Mo (μg/kgTS)	1148–12,180 [e]	1555 [ad]		
Cu (μg/kgTS)	24,889–125,641 [e]	5654 [ad]	114-216 ppm[an]	
B (μg/kgTS)		3524 [ad]		
S	0.5–1.6% of TS [e,ae]	0.06–0.19% of TS [k,v,af]		388-1300 mg/l[y]
P (% of TS)	0.39–4.93 [e]	0.28 [k]	1.5–0.95[an]	
Calorific value (MJ/kg)	17.2–25.1[b,e,j,l,ae,ah,ak]	17.5–22.36[ai,ak]	11–19[aa,am]	
Ash (% of TS)	9.7–14.6[ae,ak]	13.15[ak]	47–59[an]	
Biogas yield	0.16–0.53 NLbiogas/gCOD* [a,e,o]	0.44 NLbiogas/gCOD* [ad]		0.24 NLbiogas/gVS [y] 0.12–0.37 NLbiogas/gCOD*[ad]
Average methane (% vol)		63[ad]		38–60[ad]
$Sulphate_{soluble}$ (mg/l)				88–392[y]
Total protein	3.2–16.2 g/cap/d[ah]			2874–8835 mg/l[y]
$Protein_{soluble}$ (mg/l)				497–1,723[y]
Total carbohydrates (mg/l)	4-24 g/cap/d[ah]			660–3,812[y]
Lipids	0.09–0.16 g/gTS [ah] 4.2 g/d[e]			0.03–0.3 g/gTS[y]
Total fiber (g/gTS)	0.25 [ah]			0.33–0.79[y]
Hemicellulose (g/gTS)				0.15–0.31[y]
Cellulose (g/gTS)				0.03–0.34[y]
Lignin (g/gTS)				0.03–0.16[y]

[a]Snell 1943; [b]Lovelady and Stork 1970; [c]Levitt and Duane, 1972; [d]Fry 1973; [e]Ciba-Geigy, 1977; [f]Wyman et al., 1978; [g]Meher et al., 1994; [h]Yeo and Welchel 1994; [i]Brown et al., 1996; [j]Girovich 1996; [k]Tennakoon et al., 1996; [l]Spellman, 1997; [m]Heinss et al., 1999; [n]Koottatep et al., 2001; [o]Park et al., 2001; [p]Eawag, 2002; [q]Jönsson et al., 2005; [r]Wignarajah et al., 2006; [s]Henze et al., 2008; [t]Barman et al., 2009; [u]Veritec Consulting Inc. & Koeller and Company, 2010; [v]Serio et al., 2012; [w]Still and Foxon 2012; [x]Bassan et al., 2013; [y]Zuma 2013; [z]Appiah-Effah et al., 2014; [aa]Muspratt et al., 2014; [ab]Podichetty et al., 2014; [ac]Strande et al., 2014; [ad]Colón et al., 2015; [ae]Monhol and Martins, 2015; [af]Miller et al., 2015; [ag]Radford et al., 2015; [ah]Rose et al., 2015; [ai]Yermán et al., 2015; [aj]Ilango and Lefebvre 2016; [ak]Onabanjo et al., 2016a; [al]Onabanjo et al., 2016b; [am]Gold et al., 2017a; [an]Gold et al., 2017b, [ao]Ward et al., 2017a; [ap]Ward et al., 2017b.
* NLbiogas/gCOD – normal liter (volume of gas at 273 K and 1 atm) of biogas to gCOD added.

3.1.1. Faeces simulants

Butler et al. (2003) prepared artificial faeces for laboratory investigation of gross solids movement in sewers (referred to here as simulant #1). Solids were represented with plastic cylinders with a diameter of 3.4 cm, length of 8 cm and density of 1.06 g/ml, following the US NBS solid (Swaffield and Galowin, 1992). Penn et al., (Submitted) implemented similar solids for examining their movement in real sewers. Two techniques for tracking the gross solids were developed; using light sticks tracked by computerized light detector and RFID (radio frequency identification) based tracking. They further analysed the effect of reduced sewer flows on the movement of the solids (Penn et al., 2017).

Maximum Performance (Map) in the USA (Veritec Consulting Inc. & Koeller and Company, 2010) developed a media for testing toilet flush performance (simulant #2). In a 'Toilet Fixture Performance Testing Protocol', they define a test media (i.e., synthetic faeces) to comprise the following: "*one or more 50 ± 4 g test specimen consisting of one of the following (i) soybean paste contained in latex casing (cased media), tied at each end forming a 'sausage' or (ii) same quantity consisting of extruded soybean paste (uncased raw media), and four loosely crumpled balls of toilet paper. Each test specimen shall be approximately 100 ± 13 mm in length and 25 ± 6 mm in diameter.*" A similar media was developed by DIN (German Industrial Norm/European Norm, 2006). The U.S. Environmental Protection Agency's (EPA's) WaterSense program (EPA WaterSence, 2014) adopted MaPs protocol and indicated that a "high efficiency" toilet should successfully and completely clear 350 g of the test specimen from the fixture in a single flush in at least four of five attempts.

All the above inert simulants were developed to reflect shape, size and density of real faeces. A summary of their physical properties can be found in Table 2. These simulants were mainly used for investigating solids movement in sewers and in drainage pipes of buildings and for investigating flush performance of toilet user interfaces. Simulant faeces with varying densities and shapes as described in the Bristol stool chart (Lewis and Heaton, 1997) can be produced by modification of these physical simulants. Simulants can be further modified to represent other type of solids found in sewers such as FOGs (fats, oils and greases) by producing them from materials with various densities. With the rising uptake of in-sink food waste disposals the discharge of FOGs to sewers is widely increasing (Thyberg et al., 2015) and hence investigating their transport in sewers is of significance. These simulants do not disintegrate and therefore are not impacted by the shear stress present in the system and obviously, the chemical properties are not reflected at all. It is also important to realise that the rheological properties of these simulants differ significantly from the real material.

Podichetty et al. (2014) evaluated the application of viscous heating for the destruction of pathogens in faeces. Heat was generated within faecal simulants by applying shear stress with an extruder. They found, based on a literature review, several alternative materials displaying the same shear thinning behaviour as human stool, and demonstrating similar viscosity profiles with changing shear rate. Viscosity profiles of human stools were taken from Woolley et al. (2013). The alternatives included contents from pig caecum (a section of the pig lower intestine) (Takahashi and Sakata, 2002), content from chicken caecum (Takahashi et al., 2004), wheat flour (Podichetty et al., 2014), different types of mashed potatoes (Podichetty et al., 2014) and simulant stool (simulant based on Susana.org.2008 (SusanA, 2008), simulant #13 presented later in Table 3, viscosity profile of the simulant was made by Podichetty et al., 2014). While wheat flour had the closest match to the rheological behaviour of human faeces, they selected red potato mash since it had a higher resemblance in terms of moisture content (simulant #3). Their choice of red potato mash as a faecal simulant was confirmed by its structural, thermal and viscoelastic properties (Singh et al., 2008). simulant #13 (Table 3) showed poor rheological resemblance to human stool. Rheological characteristics of the various simulants is presented in Fig. 1.

Viscous heating of the red potato mash simulant was not compared to viscous heating performance of real human stool. Further, this simulant was not tested for its density or whether it could be representative of faeces shape. It can reasonably be assumed that this simulant will poorly represent the chemical characteristics of human faeces, as it lacks important components such as bacterial content, fibre, proteins and inorganic matter.

Yeo and Welchel (1994) patented a synthetic faeces for simulating the dewatering rate of human stool. It was developed to be used as a substitute for real faeces in the testing and development of diapers. They examined 32 formulations using different components. Many of their attempts were based on a commercially available synthetic faeces, FECLONE®BFPS -4 (simulant #4, Table 3) powder from Silicone Studio of Vallez Forge, Pa. FECLONE®BFPS -4 was reported to have a viscosity of 2276–4032 cP which is comparable to human stool, but a substantially higher dewatering rate of 524–535 $g_{water}/m^2_{simulant}/min$. In comparison, viscosity and dewatering values of human stool were reported as 3500–5500 cp and 350–400 $g_{water}/m^2_{faeces}/min$ for regular stool (Tables 1 and 2). The units of the dewatering rate ($g/m^2/min$) include m^2 of material, which is determined according to the measurements procedure reported in Yeo and Welchel (1994). Since such a unit is not applicable to be used easily for other research purposes, the authors of this paper converted the unit to $g_{water}/l_{material\ (simulant\ or\ real\ stool)}/min$ according to the methods described in Yeo and Welchel (1994). The converted results were found to be 110.1–125.9 $g_{water}/l_{faeces}/min$ and 164.9–168 $g_{water}/l_{simulant}/min$ for regular stool and simulant respectively. The viscosity was measured in centipoise at 50 revolutions per minute using a model RVT viscometer manufactured by Brookfield Engineering Laboratories, Inc., Stoughton, Mass. The shear rate at which the viscosity was measured was not given.

Yeo and Welchel (1994)'s best-performing simulant (simulant #5, Table 3) was composed of 15% polyvinylpyrrolidone (PVP), 5% psyllium mucilloid and 80% water. By varying the weight percent of the soluble to insoluble components, the molecular weight of the soluble component (PVP) and the water content, the viscosity of the simulant could be varied along the Bristol stool scale. Therefore, the viscosity can be adjusted between 1000 and 40,000 cP covering the range of real human stool (Tables 1 and 2). When the simulant was adjusted to a viscosity range of 3500–5500 cp (similar to that of human stool) a dewatering rate of 50–400 $g/m^2/min$ (15.73–125.9 g/l/min after conversion) was reported. The simulant was found to bind water to a better extent than other commercially used alternatives. The alternatives included mashed potatoes, brownie mix, peanut butter and pumpkin filling, and were reported to have a dewatering rate of over 500 $g/m^2/min$ (157.3 g/l/min after conversion), much higher than human faeces. A proper mixture of both water soluble (84 wt% of total solids) and water insoluble (16 wt% of total solids) components was necessary to achieve low dewatering rates while keeping the water content relatively constant at 70–90% of the total weight. The authors also found that water-soluble components which had an average molecular weight of over 10,000 g appeared to provide lower dewatering rates. They further reported that adding saturated fat to the solids portion at less than 2 wt% of total simulant weight, resulted in reduction of both the surface tension and dewatering rate of the compound.

According to Wignarajah et al. (2006) the shortfall of the simulant developed by Yeo and Welchel (1994) is its inability to act as a faeces-like substrate for microbial activity. Furthermore, the addition of PVP resulted in much higher nitrogen levels than are typically found in faeces.

3.1.2. Faecal sludge simulants

The physical properties of faecal sludge are different from faeces. Hence, investigations making use of faecal sludge require different simulants from those used for faeces. However, as faeces are an essential ingredient in faecal sludge, some of the simulants described above can be a base for the development of faecal sludge simulants.

Radford and Fenner (2013) developed a synthetic faecal sludge to represent the physical characteristics of pit latrine sludge (simulant #15, Table 3). It was developed for studying pit emptying performance by vacuum trucks, specifically for systems in southern

Table 3
Recipes for faeces and faeces sludge simulants.

Component	Composition of solid content (wt%)																			
	Faeces simulants												Faecal sludge simulants							
Simulant number	#4	#5	#6	#7	#8	#9	#10	#11	#12^{+}	#13^{++}	#14 (a)	#14 (b)	15	16	17	18	19	20	21	22
source	b	b	a,c	g	e	m	k	I,j	l	h	n		f	i	g					n
Cellulose	65.1*		33	15	37.5	12.4	15	10			10°	10°								
wheat	11																			
psyllium	6.6**	25⁻																		
Poly (oxyethylene)	11																			
polyvinylpyrrolidone		75																		
Potassium sorbate	0.7																			
Burnt sienna***	2.8																			
Yellow ocher***	1.4																			
Raw umber****	1.4																			
Torpulina			25																	
E.coli			7	30																
Baker's yeast					37.5	32.8	10	30			0	3								
Yeast extract											30	27								
Casein			10																	
Oleic acid			20					20			20	20								
KCl			2		4			2			2	2								
NaCl			2					2			2	2								
$CaCl_2$			1			11.3		1			1	1								
Polyethylene glycol				20																
Psyllium husk				5		24.3	15	17.5			17.5	17.5								
Peanut oil				20	20	17.5	5													
Miso paste				5		10.95	30	17.5			17.5	17.5								
Inorganics				5																
Dried coarsely ground vegetable matter (mg)				50																
$Ca_3(PO_4)_2$							5													
CaH_2PO_4					1															
Baker's yeast					37.5	32.8	10	30												
Propylene glycol						10.95	20													
Soybean paste									62.6	52.2										
Rice									34.4	28.5										
Salt									0	19.3										
Ethanol									3	0										
Walnuts															0	20.23	39.08	62.83	77.6	
Hayflour															79.4	60.56	39.08	20.94	0	
$Na_2HPO_4 \cdot 12H_2O$															6.35	6.71	6.14	7.41	6.29	
NH_4HCO_3															14.25	12.49	15.71	8.82	16.11	
kaolin clay (ultra-fine particle size)													67							
compost (by dry mass)													33							
1 ml Synthetic urine (#IV Table 5)+ 0.4 g simulant #11														100						
1 ml synthetic urine (#IV Table 5) + 12.9 ml tap water + 0.58 g simulant #14a°°																				100

Kaba et al., 1989[a]; Yeo and Welchel 1994[b]; Tennakoon et al., 1996[c]; Wignarajah et al., 2006[d]; Danso-Boateng et al., 2012[e]; Radford and Fenner 2013[f]; Zuma 2013[g]; Podichetty et al. 2014[h]; Colón et al., 2015[i]; Miller et al., 2015[j]; Yermán et al., 2015[k]; Ilango and Lefebvre 2016[l]; Onabanjo et al., 2016a[m]; This paper.[n].
The components composition is of the dry solids composition. Water can be added in different amounts to adjust to various TS concentrations.
⁻psyllium mucilloid.
*powder.
**fibrall® psyllium hydrophilic muccilloid
***reddish brown and yellowish pigments.
****hydrous silicates and oxides of iron and manganese.
$^{+}$Water was added as 39.8% of total ingredients.
$^{++}$based on www.Susana.org (SusanA 2008), Water was added as 35.5% of total ingredients.
°Microcrystalline cellulose.
°°This is assuming the liquid portion of the faecal sludge is 7% urine, rest is flushwater.

Africa. It was composed of a mixture of compost, kaolin clay, and water. The authors calculated the shear strength of faecal sludge as <400 Pa from a previous study of sludge densities in pit latrines (Bösch and Schertenleib, 1985). The simulant could be tuned to have a shear strength from 60 to 900 Pa, which replicated and exceeded the full range of shear strengths found in faecal sludge. The simulant densities were in the range of some faecal sludges (800–1200 kg/m^3) but were not representative of sludge with elevated sand content, which has a much higher density (up to 2200 kg/m^3).

Radford et al. (2015) expanded the recipe developed by Radford and Fenner (2013) by proposing two simulants to cover the entire range of faecal sludge densities and shear strenths. The simulants were further developed to be used for research on emptying various types of containment systems (e.g. septic tanks, pour-flush systems, pit latrines, and urine diverting dry toilets). Detailed recipes for these simulants were not described in the literature, however their compenets were given. Simulant *a*: replaced the compost in simulant # 15 with topsoil, it further included (like simulant #15) kaolin clay and range of water contents. Their second simulant (simulant *b*) contained milorganite organic fertilizer derived from sewage sludge, salt, vinegar and range of water

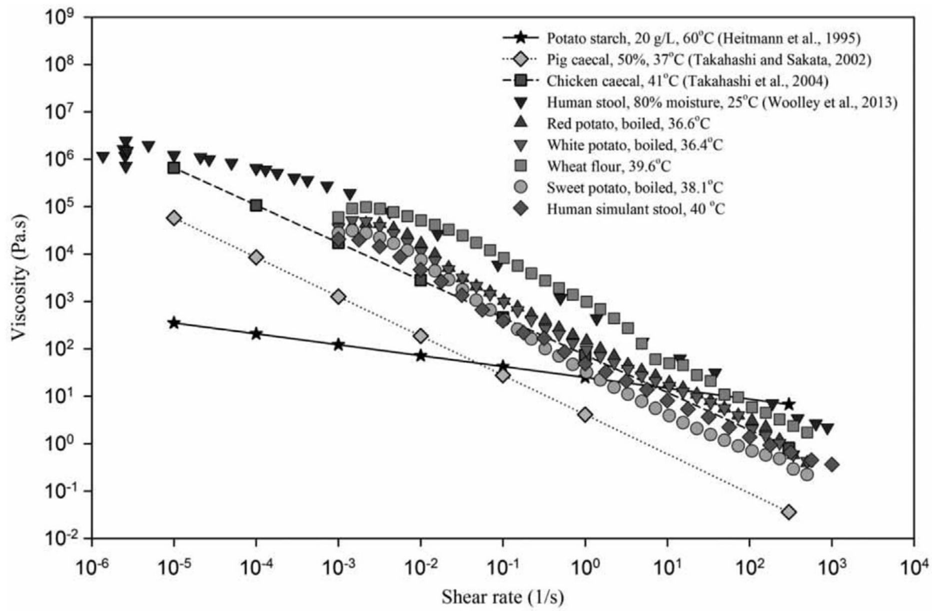

Fig. 1. Rheological behaviour of the various simulants (*Reprinted from* Journal of Water, Sanitation and Hygiene for Development, 2014. *volume* 4, *pages* 32–71, *with permission from the copyright holders, IWA Publishing*).

contents. Both simulants were found to represent the full range of shear strengths reported for faecal sludge, but had different densities of 1400 kg/m^3 and 980 kg/m^3 for simulants *a* and *b* respectively. Simulant *a* was used for a Water for People led research project in Uganda as those materials were locally available. Milorganite was recommended for faecal sludge processing technology development testing in the USA because it is a standardisable and easy to obtain material in that country. Thorough validation of the faecal sludge simulants was imposible because there has been limited characterization studies of the rheological properties and 'pumpability' of actual faecal sludge.

3.2. Chemical, biological, and thermal parameters

The previously discussed simulants were developed to mimic specific physical properties of human stool and faecal sludge, and are unlikely to reflect their chemical properties. Various simulants reflecting specific chemical, biological, and thermal properties of human stool and faecal sludge also have been developed. These chemical and biological properties are mostly defined as chemical oxygen demand (COD), total nitrogen (TN), pH, electrical conductivity (EC), total solids (TS), volatile solids (VS), elemental composition, and biogas potential. Important thermal properties are calorific value and ash content. Some of these simulants provide very high chemical, biological, and/or thermal resemblance to human stool and faecal sludge. However, many lack physical resemblance to faeces and faecal sludge.

3.2.1. Faeces simulants

First attempts to simulate the chemical composition of faeces were made by Kaba et al. (1989) for investigating faeces treatment by onsite oxidation (simulant #6, Table 3). The treatment was carried out by electrochemical incineration of waste. Bhardwaj et al. (1990) reported that oxidation of real faeces and oxidation of this simulant, with urine serving as an electrolyte, occurred at the same potential. Their simulant was developed based on the assumption that faeces solids are made up of one third microorganisms from the intestinal flora, one third undigested fibre and the rest lipids and inorganic material. Tennakoon et al. (1996) made use of this simulant for investigating electrochemical treatment of human wastes in a packed bed reactor.

Based on the simulants developed by Kaba et al. (1989) (simulant #6 Table 3) and Yeo and Welchel (1994) (simulant #5 Table 3) Wignarajah et al. (2006) developed synthetic faeces formulations for NASA's development of onsite waste processing for its space missions (simulant #7, Table 3). These recipes focus primarily on representing the water-holding capacity, rheology and the chemical composition of real faeces. They replaced the oleic acid suggested by Kaba et al. (1989) with peanut oil (Table 3). They justified this replacement referencing peanut oil's high fraction of oleic acid (50–80%). Additionally, they replaced the casein (protein) in the original recipe with Miso paste, composed of 38% proteins, 21% fats, 20% fibre and 4% minerals. *E. coli* was the only organism used. In their simulant, Wignarajah et al. (2006) opted to use the nitrogen-free polyethylene glycol to represent the water holding capacity instead of PVP based on lessons learned from the high nitrogen content of simulant #5 (Table 3). The resulting product was reported to be more chemically similar to faeces than the previously developed simulants #5 and #6 (Table 3). Wignarajah et al. (2006) produced 5 different versions to represent different aspect of faeces: water holding capacity, rheology and chemical composition. They indicated that each version may be best used for different studies. Table 4 presents the function of the different components in the basic recipe proposed by Wignarajah et al. (2006).

Simulant #7 (see Table 3) is the basis of the synthetic faeces used by a number of research groups focusing on the energy recovery from faeces and its treatment in onsite sanitation systems. Ward et al. (2014) and Danso-Boateng et al. (2012) used it to simulate the energy content of carbonized faeces. Danso-Boateng et al. (2012) modified this simulant for investigating converting biomass within faeces into char using hydrothermal carbonisation (HTC). Their modified recipe is presented as simulant #8 in Table 3 (further description on their modification can be found below). No information on the purpose of their modification or the simulant's resemblance to faeces was reported. Ward et al. (2014) evaluated solid fuel char briquettes produced from faeces. They found that although both the faeces and the simulant (simulant #7 as in Table 3) had similar calorific values, the char produced from synthetic faeces had a higher calorific value compared to char produced from real faeces. They attributed this difference to the low inorganic content of the simulant in comparison with real faeces. They further showed that the faecal char had a comparable calorific value to wood char. The energy content was reported as 25.57 MJ/kg and 29.53 MJ/kg for chars produced from faeces and synthetic faeces respectively at pyrolysis temperature of 300 °C. Increasing the pyrolysis temperature to 750 °C decreased the energy content of the chars to 13.83 MJ/kg and 18.92 MJ/kg for faeces and synthetic faeces respectively. Onabanjo et al. (2016a) and Yermán et al. (2015) adapted simulant #7 (Table 3) to investigate combustion performances of faeces. Their modifications can be found in as simulants #9 and #10 in Table 3. Further description on their modification can be found below. The result presented by Onabanjo et al. (2016a) showed good representation of human faeces regarding parameters effecting combustion including calorific value, VS, ash content and element chemical composition (Table 2). Yermán et al. (2015) validated the combustion performance of the simulants with the performance of dog faeces.

Colón et al. (2015) modified simulant #7 to investigate anaerobic digestion of undiluted synthetic faeces and urine, and Miller et al. (2015) looked at supercritical oxidation of a similar simulant to treat faecal sludge. Their simulant is listed in Table 3 (simulant #11), and shows high chemical and biological resemblance to human faeces (Table 6). Colón et al. (2015) further adjusted the simulant for trace metal contents since trace metals play an important role in the growth of methanogens and methane formation. The adjustment was made by adding a trace element solution with the following composition: $FeCl_2 \cdot 4H_2O$, 28.6; H_3BO_3, 1.14; $MnCl_2 \cdot 4H_2O$, 1.91; $CoCl_2 \cdot 6H_2O$, 2.29; $ZnCl_2$,1.34; $NiCl_2 \cdot 6H_2O$, 0.48; $CuCl_2 \cdot 2H_2O$,0.29; $NaMoO_4 \cdot 2H_2O$, 0.48; $FeCl_2 \cdot 4H_2O$, 28.6; H_3BO_3, 1.14 mg/kgTS (further description on their modification can be found in the following paragraph). The results shown by Colón et al. (2015) demonstrated that anaerobic digestion of undiluted human simulant excreta in simple unmixed digesters was feasible and yields biogas, which is a valuable by product of treatment. As it was not relevant to their studies, no attempt was made to match the physical properties of their simulant to that of real human stool.

Of the previously addressed modifications to simulant #7, four of them use active baker's yeast instead of *E. coli* to represent microbial material (see Table 3). The inorganic fraction was supplied by various salts including calcium phosphate (Ward et al., 2014), a mixture of calcium phosphate and potassium chloride (simulant #8, Table 3), or a mixture of calcium chloride, sodium chloride, and potassium chloride (simulant #11, Table 3). The quantities of the other components of simulant #7 were only slightly modified (Table 3), no further information was given for those modifications.

Simulant #11, developed by Colón et al. (2015), was the only one thoroughly analysed for chemical properties important for wastewater treatment (including COD_{total}, $COD_{soluble}$ TN, pH, EC, TS VS and elemental composition). It showed high chemical resemblance to human faeces (Table 2). It further showed adequate potential for production of biogas. However, based on personal experience of the authors of this article (presented later in the discussion part of this paper), the large amount of baker's yeast included in this recipe makes it physically very different from real human stool as it inflates like bread dough, and yields a sticky, unshapable slime.

Ilango and Lefebvre (2016) used Miso paste (a mixture of soybean paste, rice, salt, ethanol and water) as a chemical approximation of faeces for a study of biochar production from faeces (simulant #12, Table 3). This simulant was found to have a similar elemental composition to faeces (Table 2) along with comparable moisture content and calorific value (Table 2). While this recipe produced a successful simulant for pyrolysis studies, a similar simulant was also evaluated by Podichetty et al. (2014) (simulant #13, Table 3) in the previously discussed rheology studies and deemed to be a poor physical representation of human faeces. Both studies provide similar compositions for miso paste based simulant.

Simulant # 11 and simulant # 12 (Table 3) appear to provide good approximations of faeces in terms of the chemical properties (Table 2). Simulant # 11 (Table 3) showed good resemblance to the chemical and properties important for wastewater treatment. It further showed high compatibility in terms of its elemental content important for energy and nutrient recovery and similar biogas production as stool. Simulant # 12 had similar elemental composition and heating properties as of faeces, both important for energy recovery from faeces. However, as described above, they both proved to poorly resemble the physical properties of faeces.

3.2.2. Faecal sludge simulants

Fresh faecal sludge can be represented as a combination of faeces and urine with the option to include flush water, greywater, anal cleansing material, municipal solid waste, or other constituents depending on the system. Faecal sludge emptied from onsite containment or arriving at a treatment facility undergoes biological degradation, contributing to the various chemical and physical characteristics that a simulant will need to address. Two simulants were found in the literature intended to represent the chemical and biological properties of faecal sludge for anaerobic digestion research (Zuma, 2013; Colón et al., 2015). In addition, a recipe for synthetic urine (Colón et al., 2015) and a few recipes for synthetic greywater were developed (Gross et al., 2015). These can be combined with synthetic faeces for the preparation of synthetic faecal sludge. Examples for these simulants are presented below (Tables 5 and 6).

Colón et al. (2015) mixed 300 ml of a modified urine simulant developed by Putnam (1971) with 120 g of wet simulant #11 in their studies of onsite anaerobic digestion of undiluted fresh faecal sludge (simulant #16, Table 13). Their simulant was required to have chemical similarity to facilitate growth of anaerobic bacteria (specifically, COD_{total}, $COD_{soluble}$, N, N-NH_3, C:N, pH, EC, P, Fe, Zn, Ni, Co, Mn, Mo, B, Cu). Their recipe for the synthetic urine can be found in Table 5 (simulant #IV). For adjusting the simulant to contain missing trace elements (important for methanogen growth), the same trace element solution described with the discussion of their faeces simulant (simulant #11, Table 3), was added. The simulant had specific gas production of 0.12–0.37 NL biogas/gCOD (gas volume at 237 K and 1atm). A comparison with real faecal sludge could not be made, as there are not currently any biogas potential numbers reported for faecal sludge in the literature.

Zuma (2013) developed synthetic faecal sludge for representing the chemical and biological properties of faecal sludge for anaerobic digestion testing. Five different recipes were developed by varying the proportions of hayflour, ground walnuts, sodium phosphate ($Na_2HPO_4 \cdot 12H_2O$), and ammonium bicarbonate (NH_4HCO_3) (simulants #17–21, Table 3). This simulant was found to have a comparable biomethane potential to dairy manure, with

Table 4
Functions of the components in the synthetic faeces #7 (Wignarajah et al., 2006).

Component	Function
E.coli	Bacteria debris
Cellulose	Fibre/Carbohydrate
Polyethylene glycol	Water retention
Psyllium husk	Dietary Fibre/Carbohydrate
Peanut oil	Fat
Miso paste	Proteins/Fats/Fibre/Minerals
Inorganics	Minerals
Dried coarsely ground vegetable matter	Undigested vegetable matter

0.237 $NLCH_4$/g VS after 24 days and 0.24 $NLCH_4$/g VS after 40 days at 37 °C for the simulant and the dairy manure respectively. Sludge parameters TS, VS, TSS, and VSS were easily adjusted for the entire ranges present in faecal sludge by varying ingredients ratios. COD could be varied with hayflour content. Nutrients could be adjusted with sodium phosphate and ammonium carbonate, and sulphate content was adjustable by varying walnut content. Recipes with more hayflour had higher lignin and cellulose, and recipes with more walnut had higher lipid levels. The range of values achievable for these simulants is presented in Table 2. This simulant needs further development to be able to model a broader range of faecal sludge characteristics. The authors found that they were unable to replicate sludge with a VS/TS ratio lower than 0.85, which seriously limits applicability in the case of more stabilized faecal sludge. VS/TS ratio for faecal sludge samples collected during discharge at treatment facilities typically range between 0.43 and 0.73 (Gold et al., accepted). The physical properties of this simulant were not reported.

3.2.2.1. Synthetic urine and greywater. In order to facilitate future development of faecal sludge simulants synthetic versions of the various components of faecal sludge can be combined. These components include excreta (i.e., faeces and urine) and sometimes greywater. Simulants for urine and greywater are presented below.

3.2.2.1.1. Synthetic urine. Like with faeces the quantity and quality of urine produced daily can vary significantly. These variations can depend on environmental conditions and on person's diet, health, physical activity, and consumption of liquids, salts and proteins (Strande et al., 2014; Rose et al., 2015). The majority of nitrogen, phosphorus and potassium that is consumed by food is excreted in the urine with the proportions of 80–90%, 59–65% and 50–80% of the total consumption, respectively. Recipes for synthetic urine should contain these elements and should enable to alternate the composition of its various components.

Table 5 presents recipes for synthetic urine found in the literature. Griffith et al. (1976) (simulant #I) developed a recipe for the study of formation of urinary stones. It is the basis for many synthetic urine recipes used in nutrient recovery research (e.g. Lind et al., 2001; Wilsenach et al., 2007; Tilley et al., 2008). Udert and Wächter (2012) developed an alternate recipe for synthetic nitrified urine for research on nutrient recovery from source-separated urine (simulant #II). Their recipe is based on the theoretical concentrations in stored urine according to Udert et al. (2006) The recipe by Pronk et al. (2006) (simulant #III) was based on Ciba-Geigy, 1977 and Burns and Finlayson, 1980. Their simulant was developed for studying separation of micropollutants from source-separated urine. They spiked their solution with a representative set of micropollutants, containing propanolol, diclofenac, ethinylestradiol (EE2), ibuprofen and carbemazapine. As discussed above the recipe presented by Colón et al. (2015) (simulant #IV) was used for studying on-site anaerobic digestion of faecal sludge.

Table 5
Recipes for synthetic urine.

Component	Quantity (g/l)			
Simulant #	I	II	III	IV
Reference	a	b	c	d
Urea	25		16.2	14.2
Creatinine	1.1			3
Ammonium citrate				2
NaCl	4.6	3.6	6.2	8
KCl	1.6	3.4	4.7	1.65
$KHSO_4$				0.5
$MgSO_4$				0.2
KH_2PO_4	2.8			1.75
$KHCO_3$		1.1		0.5
$CaCl_2 \cdot 2H_2O$	0.65			
$MgCl_2 \cdot 6H_2O$	0.651			
Na_2SO_4	2.3	2.3	2.8	
Na_3 citrate · $2H_2O$	0.65			
Na_2 –$(COO)_2$	0.020			
NH_4Cl	1		1.8	
NaH_2PO_4			3.9	
$NaH_2PO_4 \cdot 2H_2O$		2.7		
NH_4NO_3		19.2		

Griffith et al., 1976[a]; Pronk et al., 2006[b]; Udert and Wächter, 2012[c]; Colón et al., 2015[d].

3.2.2.1.2. Synthetic greywater. In addition to faeces and urine, greywater is an important component of some faecal sludge, especially within higher economic brackets that are likely to have piped-water and septic tanks (Strande et al., 2014; Schoebitz et al., 2016). Recipes for synthetic greywater contain ingredients typically found in real greywater such as a variety of personal hygiene products, chemicals used in the home and bacteria. The mixture of these substances should yield the concentrations of pH, COD, BOD_5, TSS and surfactants usually found in greywater. Greywater characteristics are influenced by the type of flows contained within the greywater (e.g., kitchen, showers, sinks, laundries etc.), cultural and socioeconomic variables and characteristics of the occupants, climate and geographical variables and quality of the source water (Gross et al., 2015). Recipes for synthetic greywater found in the literature and in government standards is presented in Table 6.

4. Discussion

There have been successful simulants mimicking specific physical and chemical properties of human faeces and faecal sludge. A summary of the reviewed simulants and their similarity to human faeces is presented in Table 7 and Table 8. The differences in the simulant properties are readily apparent in Table 8, since each was developed to mimic specific faeces and faecal sludge characteristics, but ignore most others. A clear distinction can be made between the physical (simulants #1–5 and #15, #22) and chemical, biological, and thermal simulants (#6–14, #16–21). Almost none of the simulants adequately represent both chemical and physical properties.

The information provided by the table can support in choosing the adequate simulant to be used or to be further developed for any intendant research. For example, in wastewater research of sewer systems and onsite sanitation systems a combination of some of these properties is of importance. Such investigations include faeces movement and faeces and faecal sludge settling, dewatering and physical and biochemical disintegration. A first attempt to combine these properties in one faecal simulant is made by modifying one of the identified simulants (simulants #14a, 14b and 22) described in section 4.1 below.

To date, constituents of interest, such as comprehensive COD fractionation, odor, pathogens, pharmaceuticals and hormones, have hardly been included in the simulants. The development of simulants including COD fractionation (e.g., inert and slowly and readily biodegradable fractions of COD) will be very important for the study of biochemical properties of faecal sludge during onsite storage and treatment. Odours can be simulated by real or synthetic components, such as hydrogen sulphides, methyl sulphides and benzopyrrole derivaties (Moore et al., 1987). Sato et al (2001a,b)., found that sulphur-containing components were 2.2% of the total gaseous fraction, while the nitrogenous benzopyrrole compounds were only about 0.3%. Ammonia occurred at 6.3%. Faeces simulants #6 and #7 (Table 3) used *E. Coli* and *Torpulina* to mimic the microbial content of faeces, which could be a base for faecal sludge

Table 6
Recipes for synthetic greywater (taken from Gross et al., 2015).

Type	Greywater	Greywater	Bath	Laundry	Laundry and Bath
Reference	b	a	d	d	c
Unit	mg/l	g/100 l	g/100 l	Amount/100 l	Amount/100 l
Ammonium chlorine	75				
Soluble starch	55				
Potassium sulphate	4.5				
Sodium sulphate Na_2SO_4		3.5		4 g	
Na_2PO_4				4 g	
Sodium dihydrogen phosphate	11.4	3.9			
Sodium bicarbonate $NaHCO_3$		2.5		2 g	
Boric acid		0.14			
Lactic acid		2.8	3		
Synesthetic soap					
Body wash with moisturizer			30		
Conditioner			21		
Shampoo	0.022	72	19		86 ml
Liquid hand soap			23		
Bath cleaner			10		
Liquid laundry fabric softener				21 ml	
Liquid laundry detergent				40 ml	
Laundry		15			At recommended concentrations for hard water
Kaolin	25				
Clay		5			
Test dust			10	10 g	
Sunscreen/moisturizer		1/1.5			
Toothpaste		3.25	3		
Deodorant		1	2		
Vegetable oil		0.7			1 ml
Secondary effluent		2l	2l	2 l	To give final concentration of 10^5-10^6 cfu* of total coliforms

*cfu - Colony forming unit.
[a]Diaper et al. (2008); [b]Friedler et al. (2008); [c]BSI 2010; [d]NSF 2011.

Table 7
Summary description of all the simulants.

	Simulant #	reference	description	Investigation
Faeces simulants	1	d,q	Plastic cylinders with detecting device	Investigating gross solids movement in sewers
	2	e,g,k	Soybean paste in a latex casing	Testing toilet performance (connected to sewers and off grid)
	3	l	Red potato mash	Viscous heating of faeces for pathogen destruction
	4	b	Composed of water soluble polymer (for water	For testing personal care products serve to collect and contain faecal
	5	b	holding capacity), fiber and water	matter
	6	a,c	Variations in a recipe containing bacteria,	Electrochemical oxidation for treatment of faeces
	7	f	water, retention component, fiber, fat, proteins and minerals	Waste-water treatment in space vehicles (f) Production of char briquettes from faeces (k)
	8	h		Production of char briquettes from faeces through hydrothermal carbonisation
	9	s		Combustion performances of human faeces
	10	q		
	11	n,o		Anaerobic digestion of faeces and urine (l) Supercritical oxidation to treat faecal sludge (m)
	14	u		Physical disintegraion of faeces under sewer flow conditions, biological disintegration of faeces in onsite systems and optimization of faecal sludge treatment
	12	r	Mixture of soybean paste, rice, salt, ethanol and	Biochar production from faeces
	13	k	water	
Faecal sludge simulants	15	i,p	Mixture of compost, kaolin clay and water	For studying pit emptying procedure
	16	n	Same as simulant 11 + addition of synthetic urine	Anaerobic digestion of faecal sludge
	17–21	j	Mixture of hayflour, ground walnuts, sodium phosphate and ammonium carbonate	
	22	u	Same as simulant 14 + addition of synthetic urine	Dewatering studies of faecal sludge

Kaba et al., 1989[a]; Yeo and Welchel 1994[b]; Tennakoon et al., 1996[c]; Butler et al., 2003[d]; German Industrial Norm/European Norm 2006[e]; Wignarajah et al., 2006[f]; Veritec Consulting Inc. & Koeller and Company, 2010[g]; Danso-Boateng et al., 2012[h]; Radford and Fenner 2013[i]; Zuma 2013[j]; EPA WaterSense.

simulants. Pharmaceuticals (Diclofenac, Ibuprofen, Propranolol and Carbamazepine) as well as ethinylestradiol (EE2, synthetic hormone) were added to synthetic urine #II (Table 5). Further research is needed on the addition of synthetic or real pathogens and on the addition of synthetic or natural pharmaceuticals and hormones to the simulants.

An alternative to creating simulants, there are also attempts in the literature to create reproducible samples from real faeces and faecal sludge. Different techniques are used to overcome the challenges in using the high variable, non-stable and non-homogenised

Table 8
Summary comparison of human faeces simulants.

	Faeces simulants													Faecal sludge simulants			
Simulant #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17–21	22
Reference	d,t	e,g,k	e	b	b	a,c	f	h	s	q	n,o	r	l	u	i,p	n	j	u
Shape	+	+									−			+				
Density	+	+												+	+			
Physical disintegration	−	−												+				
Viscosity	−	−	+	+	+		*					−	−	+	+			+
Dewatering	−	−		−	+		*											−
Water content	−	−	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+
COD_{total}	−	−					*				+			+		+	+	+
$COD_{soluble}$	−	−									●			●		●	●	●
TN	−	−					*				+			+		+	+	+
NH_3-N and NH_4-N	−	−					*				+			+		+	+	+
C/N	−	−									+					+		
BOD	−	−																
pH	−	−					*				+			+		+	+	+
EC	−	−					*				●			●		●		●
TS	−	−	+	+	+	+	*	+	+	+	+			+		+	+	+
VS	−	−					*		+		+			+		+	−	+
Elemental composition	−	−				+	+		+		+	+	+					
S	−	−				+					+							
P	−	−															+	
Fe	−	−									+					+		
Zn	−	−									+					+		
Ni	−	−									+					+		
Co	−	−									+					+		
Mn	−	−									+					+		
Mo	−	−									+					+		
Cu	−	−									+					+		
B	−	−									+					+		
Calorific value	−	−					+	+	+			+	+					
Ash content	−	−							+				+					
Biogas yield											+					+		
Odor	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−
Pathogens	−	−	−	−		+	+	−	−	−	−	−	−	−	−	−	−	−

Kaba et al., 1989[a]; Yeo and Welchel 1994[b]; Tennakoon et al., 1996[c]; Butler et al., 2003[d]; German Industrial Norm/European Norm 2006[e]; Wignarajah et al., 2006[f]; Veritec Consulting Inc. & Koeller and Company, 2010[g]; Danso-Boateng et al., 2012[h]; Radford and Fenner 2013[i]; Zuma 2013[j]; EPA WaterSence, 2014[k]; Podichetty et al. 2014[l]; Ward et al., 2014[m]; Colón et al., 2015[n]; Miller et al., 2015[o]; Radford et al., 2015[p]Yermán et al., 2015[q]; Ilango and Lefebvre 2016[r]; Onabanjo et al., 2016a[s]; Penn et al., 2017[t]; This paper.[u].
+ validated with real faeces or faecal sludge.
● reported value for synthetic, but no available data to compare to real faeces or faecal sludge.
-not expected to be comparable to real faeces or faecal sludge (based on reported literature, other literature values, and experiences of authors).
Blank box: not enough data to make a conclusion.
*reported to be comparable to faeces or faecal sludge but no results provided (Simulants # 7–10 and simulant # 13 are based on this recipe).

real faeces and faecal sludge. For example, some experiments are conducted with controlled characterization of faeces donors or of faecal sludge production storage and collection to cover specific conditions (e.g., Woolley et al., 2014b); others use uncontrolled characteristics to cover the high variability (e.g., Stachler et al., 2017). Making batch experiments with homogenised samples averages the highly variable parameters (e.g., Ward et al., 2014). All samples are used within a short period of time from production or immediately frozen and thawed just before usage (e.g., Cammarota et al., 2017). This is in order to keep the bacterial content of the samples stable and prevent degradation (e.g., Allegretti et al., 2018). Taking photos of the stools enables to analyse some of their physical characteristics (e.g., size, color and shape) also long after its production (e.g., Barman et al., 2009). Repeating experiments with different collecting methods enables to take into consideration the effect of these methods on the characteristics of faecal sludge (e.g., Bassan et al., 2014). These are all attempts to manage the intrinsic variability in faeces and faecal sludge. Ideally, real faeces and faecal sludge can be managed using these standardization methods, and used to validate optimal simulants. In reality, the high variability can only be standardized to a certain extent. For example, efforts to standardize sampling methods for faecal sludge can only partially reduce the variability in the samples, since much of the variability is caused by external factors that are beyond the control of the sample collectors. This means that very large sample sizes are required to obtain statistically significant data, and that is often prohibitively expensive.

It is important to note that faecal sludge is highly variable and it differs significantly from fresh faeces. As seen in this review, the development of faecal sludge simulants is in its preliminary stages. The importance of faecal sludge management has only been acknowledged relatively recently (Moe and Rheingans, 2006; WHO, 2017), which is one reason for the comparative lag in simulant development. One reason for the complexity of developing representative simulants is due to the lack of comprehensive characterization data for faecal sludge. Although, with the increasing awareness of the importance of faecal sludge management, this data is becoming more available (Gold et al., 2017a). The lack of available information on faecal sludge characteristics makes it difficult to validate simulant performance. Faeces is obviously an important constituent of faecal sludge, which typically also includes additional components such as urine, greywater, flush water, and/or solid waste, and with varying levels of biological and physical degradation. Therefore, the comprehensive review of faeces, urine, and greywater simulants presented in this paper will support the further development of faecal sludge simulants. This will be valuable for conducting research to understand what is occurring during onsite storage of faecal sludge, to develop treatment technologies, and to enhance potential for resource recovery (Diener et al., 2014: Muspratt et al., 2014; Gold et al., 2017a, 2017b).

Also important for the discussion of faeces and faecal sludge simulant development, is that average values are often targeted for desired simulant characteristics. However, in reality, the characteristics of faeces vary widely depending on health and diet. Variability in excreta in addition to influences of storage time, containment technology, and usage patterns make faecal sludge properties extremely variable. Further research is necessary prior to the development of simulants that reflect regional and dietary dependent variations. To achieve this, it will be important to identify which parameters are most sensitive to such effects and how much impact they have on the purpose of the simulant.

Another note of importance is that there are not standard methods for analysing the different parameters within faeces and faecal sludge. This makes it difficult to ascertain what level of variability is innate, and what level is due to different analytical methods. Methods are typically adapted from those used for analysing wastewater, sludge, and drinking water (Apha, 2005) and soil (Dinauer, 1982). However, faeces and faecal sludge differ significantly. Hence, there is a need for standardization of the methods, for example currently in preparation for faecal sludge Velkushanova et al., (in preparation).

4.1. Making use of Table 8 for development of a new simulant

For research into the fate of excreta in urban sewers and in onsite sanitation systems, both the chemical/biological aspects of faeces and faecal sludge and their physical properties are important. Investigations of their fate include their physical motion (movement, settling, sedimentation, and dewatering) and their physical and biochemical disintegration in sewer pipes and in onsite sanitation systems. We would like to show, how based on the information provided in this review such an adapted simulant with mixed physical and chemical properties can be successfully created. The extensive description of materials, methods, extended results, detailed instructions on the simulant preparation and recommended storage practices can be found in the appendix.

4.1.1. New faeces simulant

It is required that the new simulant represents a range of physical characteristics based on the Bristol stool scale. It should be able to be tuned from soft to hard by adding different amounts of water, should be shapable into the characteristic faeces cylinder, sausage, or snake, and whether it floats or sinks should be controllable. The desired simulant should also possess a similar viscosity and dewatering rate to real faeces. Additionally, it should have similar chemical composition to stool including COD, TN, pH, EC, TS, VS and elemental composition. It should be able to disintegrate in water and the resulting aqueous suspension should have similar chemical properties to disintegrated faeces.

By looking at Table 8, one can see that both simulant #11 (Table 3) and simulant # 12 (Table 3) showed high chemical and biological resemblance in their elemental composition but poor physical resemblance in their shape and rheological properties. Indeed, none of the simulants with proper physical parameters has a representative chemical composition. The modification of the physically related simulants to represent additional chemical properties was found to be impracticable. Simulants #11 and #12 were the best candidates for further development. Simulant # 11 shows high compatibility in its chemical properties important for wastewater related research, including COD, TN, TS, and VS. Baker's yeast is used to represent microbial biomass and to produce floating stool (due to gas produced by the yeast). However, the quantity included in this recipe creates an unfavourable physical structure. It produced a gassy and sticky material that floated when added to water, but was too sticky to be shaped into a cylinder. Fig. 2, (a) illustrates the high gas production, shown by the many bubbles in the beaker. The stickiness of the material is shown on part *b* of Fig. 2. Use of active yeast also contributes to quick biological changes within the synthetic material, which is undesirable if reduced sample variability is a priority. An ideal simulant would be storable and resistant to physical or biological change over a span of at least several days in order to maximize reproducibility of experiments. We hypothesised that by adapting the baker's yeast content of simulant # 11 (Table 3), a physically representative simulant could be produced, while still maintaining its chemical and biological resemblance.

Simulant # 12 also looks like a good candidate for further development. However, as simulant # 11 showed good results (see appendix) we did not investigate simulant # 12 further.

Two substitutions for the bacterial content (i.e., baker's yeast) of the adapted simulant were evaluated for shape formation (i.e., whether it could be shaped into a cylinder) and density. These substitutions include yeast extract and baking soda. The resulting optimal recipe was then analysed for its chemical and physical properties.

Replacing baker's yeast with yeast extract resulted in a simulant with representative physical properties (shape formation, viscosity, density) and chemical properties (COD, TN, ES, pH, TS and VS) (Table 9). Compared to simulant #11 the physical properties of the modified simulant were improved while the well-represented chemical properties were not affected. In addition, the disintegration of the modified simulant in turbulent flow revealed a disintegration mode similar to that of human faeces, with a similar time span (Penn et al., in preparation).

The density of this modified simulant was found to be 1.07 g/ml. Since faeces densities can be < 1 (Table 2) quantities of two rising agents were tested whether they could be used to manipulate the density without losing the shapable capabilities. The two rising agents include baker's yeast, which generates gas through fermentation, and sodium bicarbonate, which produces gas through a chemical reaction with acids in the mixture. The optimum quantity of baker's yeast was identified as 3 wt% of solids content. This amount of baker's yeast produced faeces with roughly the same buoyancy as water, with an average density of 0.99 g/ml. A range of water contents can be added to represent the span present in human faeces – from 65 to 80% moisture. When lower than 80 wt% water content is required, the portion of baker's yeast can be increased to a maximum of 5 wt% of solids content (in case of a solid containing 65 wt% water) in order to facilitate quicker gas production. The density and viscosity of the modified simulant could be tuned with varied yeast extract and water content fractions respectively.

Replacing baker's yeast with sodium bicarbonate did not provide satisfying results. The minimum quantity of bicarbonate required for sufficient gas production to yield floating was 3 wt% of solids content in the recipe. However, the resulting product had an undesirable fluffy, sticky structure, and did not pass the shape formatting test.

4.1.2. New faecal sludge simulant

The synthetic faeces developed by the authors (simulant #14(a), Table 3) was combined with synthetic urine (simulant #IV Table 5) and water to produce a synthetic faecal sludge for dewatering studies (simulant #22, Table 3). The simulant was chemically very similar to simulant #16 (Table 3) and to fresh faecal sludge, however it displayed a 60% reduced dewaterability compared to real fresh faecal sludge. In this case, dewaterability is defined as the percent of dry solids in the dewatered cake after centrifugation and

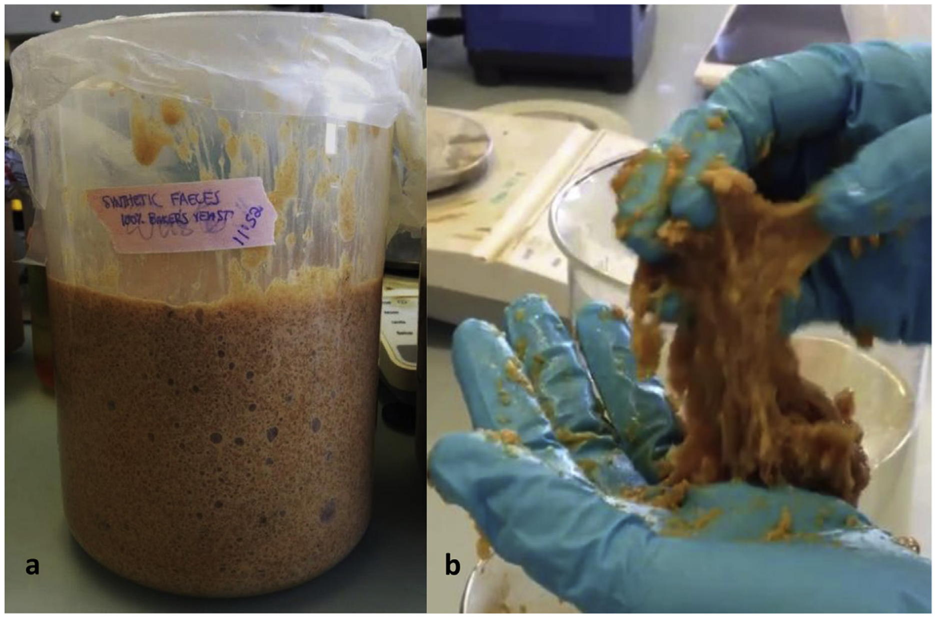

Fig. 2. Synthetic faeces containing 30 wt% of solids content baker's yeast. Left (a): mixture after standing at room temperature for 1.5 h; Right (b): sample of the corresponding mixture.

was 11% and 4.5% for fresh faecal sludge and synthetic fresh faecal sludge respectively (Ward et al., 2017a, 2017b). This is likely due to the high water-binding affinity of the psyllium husk included in the simulant. For further dewatering experiments, a faeces simulant with a reduced proportion of water-binding components could be evaluated.

5. Conclusions

The use of synthetic faeces and synthetic faecal sludge enables replicable experimentation, while simultaneously reducing health risks. There are multitude simulants for faeces in the literature, however, they are still relatively scarce for synthetic faecal sludge. At this stage, simulants have for the most part been developed to resemble real faeces and faecal sludge with specific characteristics, depending on the objective of application. Some simulants were found to highly resemble the real matter in the specific characteristics. For other simulants a poor resemblance was found. Perfect simulants that are mutually representative of physical, chemical, biological and thermal properties are still lacking. It will be important to develop recipes including COD fractionations for detailed biochemical process, and potentially other properties such as pharmaceuticals and hormones, pathogens and odours. The compilation of existing simulants in this paper has been valuable for the identification of strengths and weaknesses of simulants, and areas for further research.

A critical analysis of the literature yields the following conclusions:

Table 9
Comparison of chemical and physical properties of the new synthetic faeces from this study, simulants #14 (a) and #14 (b) (Table 3) with real and artificial faeces from the literature.

	Parameters	This study		Literature	
		Simulant #14 (a)	Simulant #14 (b)	Human faeces	Simulant faeces
Chemical properties	COD_{total} (gCOD/gTS)	1.117 ± 0.056	1.194 ± 0.162	0.567–1.450[j] 1.24[f]	1.33[i]
	$COD_{soluble}$ (gCOD/gTS)	0.624 ± 0.017	0.551 ± 0.048		
	TN (% of TS)	3.56 ± 0.13	4.05 ± 0.22	5–7[j] 2–3[f,h]	2.75[i]
	pH	5.4	5.2	5.0–8.0 (median 6)[j] 4.6–8.4[i]	5.3[i]
	EC	6.06 ± 0.17	6.40 ± 0.25		5.7[i]
	TS (%)	20.65 ± 0.29	20.79 ± 0.30	14–37[j] 15–35[g]	18.4[i]
	VS (% of TS)	87.61 ± 0.13	87.93 ± 0.07	92[j] 80–92[a,b,c]	88.5[i]
Physical properties	Viscosity (cPs at 50 rpm)	6360	4640	3500–5,500[d]	
	Density (g/ml)	1.07 ± 0.02	0.98 ± 0.05		1.06[e]

[a]Snell 1943; [b]Fry 1973; [c]Meher et al., 1994; [d]Yeo and Welchel, 1994; [e]Brown et al., 1996; [f]Jönsson et al., 2005; [g]Wignarajah et al., 2006; [h]Barman et al., 2009; [i]Colón et al., 2015; [j]Rose et al., 2015.
*Average ± standard deviation calculated from three replicates.
**Results are for synthetic faeces containing 80 wt% water.

- Synthetic faeces and faecal sludge are very useful, but can only partly replace research with real faeces.
- To date benefits of having a simulant with a stable composition and safe handling is traded off by limited resemblance.
- As with any surrogate, the results have to be validated with real faeces and faecal sludge.
- Newly emerging fields like faecal sludge research greatly benefit from the use of simulants for scientific purposes
- Standardization and validation of others results can be greatly increased through the use of standard methods for the characterization of faeces and faecal sludge.

Appendix I. Development of new simulant

For research into the fate of excreta in urban sewers and in onsite sanitation systems, both the chemical/biological aspects of faeces and its physical properties are important. Investigations of its fate include its physical motion (movement, settling and sedimentation) and its physical and biochemical disintegration in sewer pipes and in on site sanitation systems. Such investigations are conducted by the authors of this paper and an adequate simulant was in need. Both types of chemically related simulants simulant #11 (Table 3) and the simulant # 12 (Table 3) showed poor physical resemblance, as discussed above (Table 8 in the main text). Similarly, none of the simulants with proper physical parameters have an adequate chemical composition.

In the following experimental sections, substitutions for the bacterial content (i.e., baker's yeast) of the adapted simulant were evaluated for shapeable capability and density. These substitutions include yeast extract and baking soda. The resulting optimal recipe was then analysed for its chemical and physical properties.

1. Material and methods

1.1. Chemicals and materials used

For preparation of the simulant the following materials and chemicals were used (Table 10):

Table 10
Chemicals and materials used for recipe preparation

Component	Chemical/material	CAS number
Yeast Extract	Sigma Aldrich	8013-01-2
Cellulose	Sigma Aldrich	9004-34-6
Oleic acid	MP Biomedicals LLC	112-80-1
NaCl	Merck KGaA	7647-14-5
KCl	Fluka Chemika GmbH	7447-40-7
$CaCl_2 \cdot 2H_2O$	E. Merck	10035-04-8
Baker's yeast	Dry, Betty Bossi, COOP, Switzerland	
Psyllium husk	Govinda Nature GmbH	
Miso paste	Seasoned Soybean Paste HACCP, TS content ~48%	

1.2. Measurement methods

Total chemical oxygen demand (COD_{total}), soluble COD ($COD_{soluble}$), total nitrogen (TN), ammonium nitrogen (NH4-N), total solids (TS), volatile solids (VS), pH, and electrical conductivity (EC) were determined based on standard methods (Apha, 2005). Hach LCK test kits were used to measure COD_{total} and $COD_{soluble}$, TN, and NH_4-N with a Hach DR 6000 spectrophotometer. EC and pH were measured using a WTW Multi 3320 following the procedure described in Colón et al. (2015), by diluting synthetic faeces in DI water at a 1:5 w:v ratio. Viscosity was measured with a Brookfield DVII-LV viscometer using a #64 spindle at 50 rpm with 30 s measurement time.

Physical structure of the synthetic faeces, i.e., its shapable capabilities were evaluated by attempting to shape it into a cylinder, following the normal stool form according to the Bristol stool chart (Lewis and Heaton, 1997). Approximately 100 g of synthetic faeces was handled and rolled gently into a cylinder, while wearing wetted nitrile gloves. If the material was too sticky, gooey, or liquid to form a cylinder, it failed the shape test.

Buoyancy of the synthetic faeces was evaluated by placing a piece of prepared substance in a beaker filled with water. Floating or sinking performance of the faeces was recorded.

The estimated density was measured by weighing a 40 g portion of simulant and placing it in a test tube filled with 600 ml of deionized water. The increase in volume was measured, and the density was calculated. In order to reduce the uncertainty in this measurement, a pycnometer could be used in future experiments. An average and standard deviation from three repetitions was calculated.

1.3. Base synthetic faeces recipe

The range of recipes for preparation of 1 kg of synthetic faeces is presented in Table 11. Explicit preparation procedure is presented in the appendix.

Table 11
Ingredients for basic recipe of the simulants S80 and S65, all quantities are in grams.

Water content (%TS)[a]	80% (S80)		65% (S65)	
	SB80[c]	SE80[b]	SB65[c]	SE65[b]
Yeast extract	65.06	72.29	105.42	126.51
Baker's yeast	7.23	0.00	21.08	0.00
Microcrystalline cellulose	24.10	24.10	42.17	42.17
Psyllium	42.17	42.17	73.80	73.80
Miso paste	42.17	42.17	73.80	73.80
Oleic acid	48.19	48.19	84.34	84.34
NaCl	4.82	4.82	8.43	8.43
KCl	4.82	4.82	8.43	8.43
$CaCl_2 \cdot H_2O$	2.75	2.41	4.81	4.81
DI Water	758.7	758.7	577.72	577.72
Final mass “Feces”	1000.00	1000.00	1000.00	1000.00

[a] The water content was determined by TS measurements.
[b] Simulants starting with SE contain only yeast extract.
[c] Simulants starting with SB contain baker's yeast and yeast extract.

1.4. Experiments

1.4.1. Identification of the base recipe. With the goal of producing a simulant to be used for investigating the fate of faeces in sewer systems and in onsite sanitation systems, which will resemble human faeces in both its physical and chemical properties, we attempted to adapt one of the above reviewed simulants. Modifying the physical simulants to represent also the chemical properties of human stool was found to be impracticable. Both simulant # 11 (Table 3) and simulant #12 (Table 3) showed high compatibility in terms of elemental content but poor physical resemblance in terms of shapable capabilities and rheology (Table 8).

Simulant # 11 shows high compatibility in its chemical properties important for wastewater related research, including COD, TN, TS, and VS. Baker's yeast is used to represent microbial biomass and to produce floating stool (due to gas produced by the yeast). However, the quantity included in this recipe creates an unfavourable physical structure, as explained above and later demonstrated in the results. We had the hypothesis that by adapting the baker's yeast content of simulant # 11 (Table 3), a physical representative simulant can be produced, while still maintaining its chemical resemblance.

In order to consider simulant # 12 (Table 3) as a good base for further development, its additional wastewater related chemical properties (i.e., COD, TN, TS, VS) would first need to be analysed. Only if the analysis provides a close resemblance to human faeces, its shapable capabilities and density would then need to be further adjusted to human faeces. We therefore started with simulant #11 and as the results showed good chemical and physical resemblance and we did not investigate simulant #12 further.

1.4.2. Density adjustments. After identifying the base recipe, a series of experiments were performed to adjust the density of the simulants. For each formulation of yeast and baking soda shapable capability and floating tests were conducted. The time required for the simulant to float was recorded.

Quantities of two rising agents, baker's yeast, which generates gas through fermentation, and sodium bicarbonate, which produces gas through a chemical reaction with acids in the mixture, were tested to determine whether they could be used to manipulate the density without losing the shapable capabilities. These tests were conducted on simulants containing 80% and 65% water, S80 and S65 respectively, corresponding to the reported maximum and minimum water content expected in human faeces. The corresponding ingredients are listed in Table 11. The water content was determined by TS measurements and not only by the water added, since miso paste also contains water.

Different formulations of baker's yeast and yeast extract were tested. The total yeast content was held constant at 30% (dry weight by dry weight), but the ratio of these two forms of yeast were varied. Reduced quantities of baker's yeast were replaced by respective quantities of yeast extract. Quantities of baker's yeast examined were 0, 0.9, 1.4, 3, 5, 10, 15, 30 wt% of the recipe's solids content. The activity of the yeast depends on the temperature, amount of yeast added and substrate availability. The optimal quantity of baker's yeast was determined when a simulant obtained the desired cylinder shape and buoyancy properties after waiting around 1.5 h at room temperature (23 °C). 1.5 h is the minimum time required for the psyllium husks to gel. It further should enable a relatively "comfortable" time range (not less than an hour with preference to longer) in which the simulant maintains its physical structure.

Replacing baker's yeast with sodium bicarbonate as an alternative to the biological gas production was further examined. Quantities of bicarbonate examined were 0.4, 1, 3, 5 and 15 wt% of the recipe's solids content.

1.4.3. Physical and chemical properties. Once the optimum formulation was obtained, chemical properties and viscosity of two types of simulant S80 were evaluated. These simulants include SB80, made with baker's yeast and yeast extract, and SE80, made with only yeast extract. As addition of bicarbonate showed poor results, simulants containing bicarbonate were not analysed further for their chemical properties and viscosity. Density was evaluated for these two types and for SB65 and SE65, i.e., simulant S65 made with baker's yeast and yeast extract, and only yeast extract, respectively.

2. Results and discussion

2.1. Physical structure

Synthetic faeces SE80 and SE65, i.e., both simulants not containing baker's yeast, immediately sank when added to water, with an average density of 1.07 g/ml and standard deviation (sd) of 0.02 for SE80 and 1.12 g/ml with sd of 0.05 for SE65. Densities resemble the density of an NBS solid (Swaffield and Galowin, 1992). These simulants were easily shaped (Fig. 3) and sank when placed in standing water.

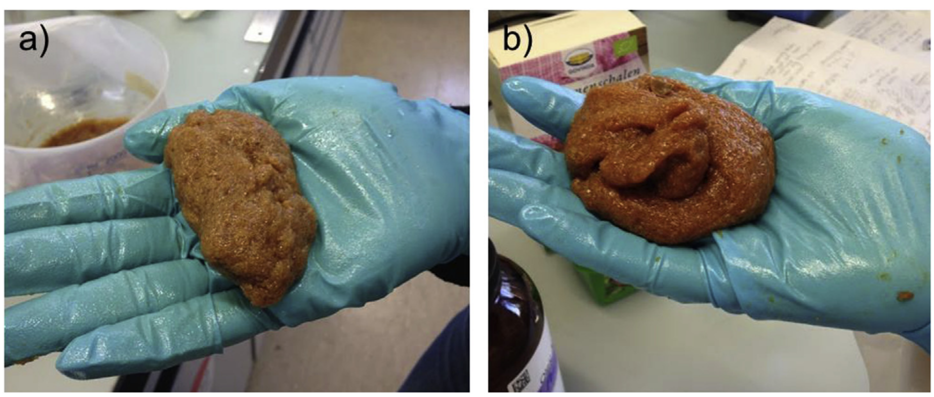

Fig. 3. Simulants with 30 wt% of solids content yeast extract and no baker's yeast a) SE65; b) SE80.

A summary of the physical characteristics of synthetic faeces made with the different amounts of rising agents (baker's yeast and sodium bicarbonate) is shown in Table 12. The results presented are for simulants S80. Adding baker's yeast contents of more than 3 wt% created a gassy and sticky material that floated when added to water, but was too sticky to be shaped into a cylinder. The resultant simulant didn't represent the physical structure of human faeces. An extreme example can be depicted in Fig. 4 where one can observe high gas quantities, shown by the many bubbles in the beaker (Fig. 4, left) and a very sticky material that could not be shaped into a cylinder (Fig. 4, right). Addition of smaller quantities of baker's yeast (1.4 wt% of solids content or lower) resulted in a long delay in yeast activation. These simulants eventually floated in water, but only after a long time standing at room temperature (3 h–2 days).

The optimum quantity of baker's yeast was therefore identified as 3 wt% of solids content, i.e., simulant SB80 (the shaded area in Table 12). This amount of baker's yeast produced faeces with roughly the same buoyancy as water, with an average density of 0.99 g/ml and sd of 0.05 (Fig. 5). Simulants SB65 required a longer period of 4 h (compared to the 1.5 h mentioned above) for the yeast to produce sufficient gas to enable floating of the stool. Increasing baker's yeast quantity to 5 wt% of solids content enabled floating of the simulant, while maintaining its physical properties, in a shorter period of 2 h. The average density of this simulant was found to be 0.96 g/ml with sd of 0.005.

Replacing baker's yeast with sodium bicarbonate did not provide satisfying results. For simulant S80 the minimum quantity of bicarbonate required for sufficient gas production to yield floating was 3 wt% of solids content in the recipe. However, the resulting product had an undesirable fluffy, sticky structure, and did not pass the shapable capability test.

Table 12
Results of physical testing for synthetic faeces S80 with different quantities of rising agents (baker's yeast and sodium bicarbonate).

Rising agent	Amount of rising agent added (wt% of solids content in recipe[a])	Shapable?[b]	Floats?[b]	Waiting time (h)[c]
None	0%	yes	no	1.5
Baker's yeast	30%[a]	no	yes	1.5
	15%	no	yes	1.5
	10%	no	yes	1.5
	5%	no	yes	1.5
	3%	yes	yes	1.5
	1.4%	yes	yes	3
	0.9%	yes	yes	2d
Baking soda	15%	no	yes	1.5
	3%	no	yes	1.5
	1%	no	no	1.5
	0.4%	yes	no	1.5

[a] Original recipe from (Colón et al., 2015).
[b] Results are from synthetic faeces made with 80% water (actual water content obtained from TS measurements of the simulant). In each recipe, wt% rising agent + wt% yeast extract = 30 wt% of solids content.
[c] waiting time – time needed for the mixture to stand at room temperature.

Fig. 4. Synthetic faeces S80 containing 30 wt% of solids content baker's yeast. Left: mixture after standing at room temperature for 1.5 h; Right: sample of the corresponding mixture.

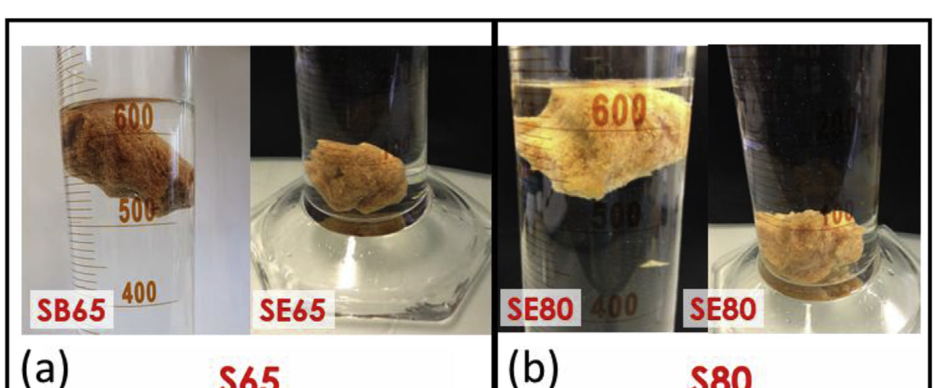

Fig. 5. Density tests for synthetic faeces (a) S65 (b) S80.

Table 13
Properties of the two simulants identified to most closely represent the range of human faeces.

Simulant		Density (g/ml)	
		average	sd
Sinking stool (baker's yeast is not added)	SE80	1.07	0.02
	SE65	1.12	0.05
Floating stool (baker's yeast is added)	SB80	0.99	0.05
	SB65	0.96	0.005

As a result of these physical tests, two recipes were identified to most closely represent the range of human faeces, according to Table 13.

The addition of baker's yeast resulted in a simulant with a weaker structure, corresponding to the lower viscosity measured. Simulant made with baker's yeast was less robust to handle, and disintegrated more rapidly upon immersion in water than simulant made without baker's yeast. Higher water content also resulted in a simulant with decreased structural strength. Ongoing research conducted by the authors of this paper includes examination of physical disintegration of faeces in turbulent flow conditions. The experiments are conducted on the reported simulant and verified by real human stool.

2.2. Chemical composition

Chemical properties of interest to wastewater treatment were analysed for the modified simulant and compared to properties found in the literature (Table 14, Snell, 1943; Fry, 1973; Meher et al., 1994; Jönsson et al., 2005; Wignarajah et al., 2006; Barman et al., 2009; Colón et al., 2015; Rose et al., 2015). Results are presented only for simulants S80 (Table 14, footnote * and **). The synthetic faeces developed in this study provide compatible chemical and physical properties resembling real human faeces. The simulants are appropriate candidates for replacing human faeces in investigations into faeces physical and biochemical disintegration in sewer systems and in onsite sanitation systems.

Table 14
Comparison of chemical and physical properties of synthetic faeces from this study SE80 and SB80 (Table 10) with real and artificial faeces from the literature

	Parameters	This study		Literature	
		SE80 (Table 11)	SB80 (Table 11)	Human faeces	Simulant faeces
Chemical properties	COD_{total} (gCOD/gTS)	1.117 ± 0.056	1.194 ± 0.162	0.567–1.450[j] 1.24[f]	1.33[i]
	$COD_{soluble}$ (gCOD/gTS)	0.624 ± 0.017	0.551 ± 0.048		
	TN (% of TS)	3.56 ± 0.13	4.05 ± 0.22	5–7[j] 2–3[f,h]	2.75[i]
	pH	5.4	5.2	5.0–8.0 (median 6)[j] 4.6–8.4[i]	5.3[i]
	EC	6.06 ± 0.17	6.40 ± 0.25		5.7[i]
	TS (%)	20.65 ± 0.29	20.79 ± 0.30	14–37[j] 15–35[g]	18.4[i]
	VS (% of TS)	87.61 ± 0.13	87.93 ± 0.07	92[j] 80–92[a,b,c]	88.5[i]
Physical properties	Viscosity (cP)	6360	4640	3500–5500[d]	
	Density (g/ml)	1.07 ± 0.02	0.98 ± 0.05		1.06[e]

[a] Snell 1943; [b]Fry 1973; [c]Meher et al., 1994; [d] Yeo and Welchel, 1994; [e] Brown et al., 1996; [f] Jönsson et al., 2005; [g] Wignarajah et al., 2006; [h] Barman et al., 2009; [i] Colón et al., 2015; [j] Rose et al., 2015.
*Average ± standard deviation calculated from three replicates.
**Results are for synthetic faeces S80.

2.3. Recommendation for recipes

Based on detailed chemical and physical characterization, two most suitable recipes have been selected for providing good chemical and physical similarity to human stool. Recommended recipes are presented in Table 15. A range of water contents can be added to represent the span present in human faeces – from 65 to 80% moisture. Baker's yeast should be added if floating faeces is desired. When lower than 80 wt% water content is required, the portion of baker's yeast can be increased to a maximum of 5 wt% of solids content in order to facilitate quicker gas production.

Table 15
Recommended recipes for synthetic faeces solids. Detailed instructions can be found in the appendix.

Component	Composition of solids content (wt%)	
	Yeast Extract	Baker's Yeast + Yeast Extract
Baker's yeast	0	3
Yeast extract	30	27
Microcrystalline cellulose	10	10
Psyllium husk	17.5	17.5
Miso paste	17.5	17.5
Oleic acid	20	20
NaCl	2	2
KCl	2	2
$CaCl_2$	1	1

2.4. Recommended storage practices

Baker's yeast produces gas via a biological process which is time and temperature sensitive. It was observed that the mixture should be held at room temperature for at least 1.5 h but not more than 4 h in order to produce the required amount of gas for floating synthetic faeces. Results were obtained at room temperature of ~23 °C, higher temperatures will shorten the time interval, and lower temperatures will lengthen it. The synthetic faeces can be refrigerated for a period of not more than 24 h if they contain baker's yeast or one week if they do not contain baker's yeast. Additionally, both mixtures can be held in the freezer for longer period of time (not evaluated for more than one month). The frozen synthetic faeces containing baker's yeast should be allowed to reach room temperature, until the point at which the yeast will again become active. Activity can be confirmed by examining the floating of the simulant. Further investigations are needed to verify that chemical and physical properties of the simulant will not change due to it freezing. This is since freezing and thawing may change the properties of the recipe material.

Appendix II. Synthetic faeces production

There is a lack of detailed published instructions for the production of synthetic faeces in the literature, so the authors thought it helpful to provide a thorough procedure for the manufacture of this material. Fig. 6 lists the detailed procedure for making synthetic faeces.

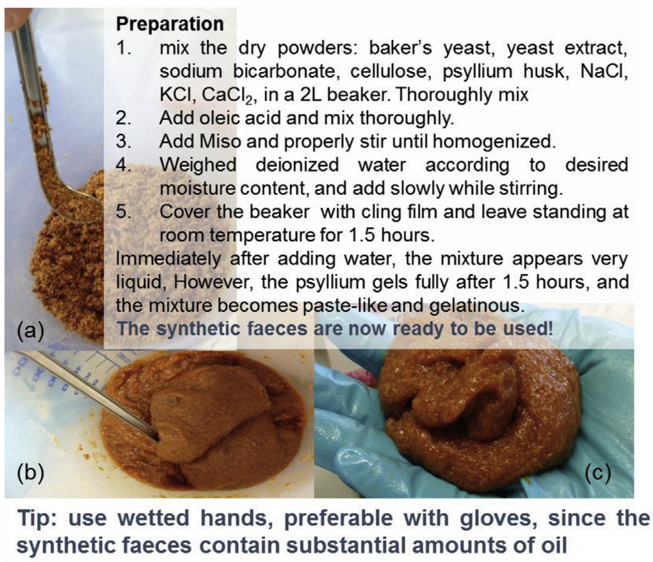

Fig. 6. Procedure for preparation of synthetic feces; (a) mixture of synthetic faeces prior to addition of water; (b and c) mixture of prepared synthetic faeces containing ~80% water (b)prepared mixture after standing for 1.5 h; (c). structured faeces.

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