Waterlines, 37:3, 207–228
<http://dx.doi.org/10.3362/1756-3488.18-00011>

# Efficiency of additives and internal physical chemical factors for pit latrine lifetime extension

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**Abstract:** *Pit latrines are the most common form of on-site sanitation, but are blighted by the problem of pit fill-up. Little is known about what factors and conditions affect decomposition of pit content and thus govern pit filling, but the liquid–mass balance is the key factor. Under laboratory conditions the effect of inorganic and biological additives and the effect of physical chemical factors on solids hydrolysis of black water and human faeces were investigated to establish the potential of these to extend pit latrine lifetime. Additives did little or nothing to enhance net solids hydrolysis in batch tests or to reduce pit fill height in miniature simulated pit latrines. Physical chemical factors such as redox condition and initial pH increased solids hydrolysis, whereas temperature and substrate moisture did little. Since additives need contact with the substrate to act, measurements on faeces crust formation speed and strength were performed and showed that crusts formed within three hours and persisted after covering with fresh faeces or water.*

**Keywords:** additives, solids hydrolysis, human faeces, pit latrines

## Introduction

An estimated 1.77 billion people globally use pit latrines for daily containment of 2.1 billion kilograms of urine and faeces (Graham and Polizzotto, 2013). The design and size of the pit latrine aim to service a certain number of people for a certain amount of time: the pit lifetime (Buckley et al., 2008; WHO n.d.). When the pit is full it needs emptying or a new pit is required, which comes at a cost of money, health issues, and possibly land requirement.

Pit lifetime is determined by in- and out-flows in the latrine and by pit content conversions. This 'working' of pit latrines is difficult to define and to investigate, which is partially due to the variation in influent composition and loading, and conditions in the pits (Buckley et al., 2008; Nwaneri et al., 2008; Torondel, 2010). Furthermore, defining whether a latrine is 'good' or 'efficient' or 'bad' is more or less determined by the amount of human resources needed to prevent it from becoming a health, smell,

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 *ISSN: 0262-8104/1756-3488*

or financial nuisance (Still, 2002; Buckley et al., 2008; Graham and Polizzotto, 2013). On that basis, a 'good' pit latrine can be defined as a latrine that requires little or no human intervention to fulfil its function. An ideal working pit latrine would convert all incoming waste into pathogen-free liquid with soluble harmless minerals, exiting the pit at such a rate that a correctly dimensioned pit never fully fills (WHO n.d.) For that, the incoming waste material needs to be fully degraded and liquid needs to be able to exit the pit via percolation and/or evaporation. Such ideal pit latrines do not exist since not all incoming pit waste is biologically degradable; however, the waste that is degradable should be stabilized as much as possible. Physical chemical factors such as temperature and the oxygen concentration of the pit content and the conditions surrounding a pit, e.g. soil drainage characteristics and groundwater table level, are important variables in the physical, chemical, and biological dynamics of a pit latrine.

Without human intervention, material can exit pit latrines as gas, but mainly does so as liquid since the main constituent of human excrements is water: 93–99 per cent water in urine and 66–85 per cent water in faeces (Torondel, 2010). The outflow potential of pit content is related to hydrolysis of solids into dissolved components, conversion of solids into biogas, and the ability of the liquid to percolate or evaporate. It should be noted that the exiting pit latrine liquid can be a health hazard if it pollutes surface or groundwater sources (Cave and Kolsky, 1999; Abay, 2010; Graham and Polizzotto, 2013). However, pit emptying also poses a health hazard to the pit-emptying personnel and via spillage during transport from the pit to a waste disposal or treatment site (Still, 2002; Buckley et al., 2008).

Pit latrine additives can be inorganic or organic and act as conditioners for enhancing conversion processes inside a pit, or increasing the potential beneficial biomass inside the pit. Pit latrine additives should enhance the degree, and, if possible, also the rate, of organic matter degradation, and enhance the dewatering capacity of the pit contents. The added additive quantity should not exceed pit content reduction. In practice, additives are used as a curative measure in 'bad' latrines or are applied on a maintenance basis with the objective of avoiding problems.

Biological additives need contact time with substrate to work and mobility within the substrate matrix to continue working. The pit content consistency (viscosity and yield stress) will influence the contact, contact time, and mobility of additives. The pit content consistency is mainly determined by the moisture levels of substrate. An obstacle in additive contact with the substrate can originate from crust formation on substrate.

The objective of this research is to investigate the effect of pit latrine additives and physical chemical aspects on the conversion of human faeces, aiming to extend the lifetime of pit latrines by analysing:

- degradation (COD (chemical oxygen demand) hydrolysis) of faecal solids into soluble or gaseous products with and without additives;
- degradation (COD hydrolysis) of faecal solids into soluble or gaseous products under different physical chemical conditions such as pH, redox condition, water content, and temperature; and
- pit fill height reduction or air space increase and COD reduction of faeces-loaded miniature simulation pit latrines with and without additives and varied redox conditions.

## Material and methods

In total, 47 different pit latrine additives were screened for their efficiency regarding solubilization of faecal solids in batch tests. Based on the results, the most promising additives were used for creating three additive mixes. The selected additive mixes, a commercial product, and aeration were all tested in standardized simulation pit latrines for their effectiveness regarding pit fill height reduction and COD conversion. The effect of physical chemical aspects such as pH, temperature, water content, and gas phase composition on the solubilization of solids was tested in batch tests.

### *Materials*

As substrates, black water and human faeces were used. The black water was collected from a demonstration facility in Sneek (Lemmerweg, the Netherlands) and was stored at 4°C. This black water was collected with vacuum toilets with a 1 litre (L) flush (de Graaff et al., 2011). Before use in tests, the black water was sieved through a kitchen sieve (mesh size 2 mm) to remove large particles, mainly toilet paper, to facilitate easy sampling. Eleven batches of black water were used over the whole experimental period. The COD concentration variation of the used black water batches is given in Table 1. The total solids (TS) content was around 5 g/L (± 0.08).

The substrate used for investigating the effect of substrate moisture levels on solids solubilization and for pit latrine simulation was human faeces. Two batches of faeces were used, produced by at least 17 adults, who were kindly requested to chew well and to avoid consuming peanuts and sweetcorn. The faeces were immediately stored at 4°C after production and used for experiments within four weeks. Before use, the faeces were blended and mixed well in an anaerobic cabinet using a kitchen mixer. The original TS was 203 g/kg (± 1.2). Varying the moisture level was done by adding tap water. The COD values are given in Table 1.

Several types of additives were screened in batch tests for their faecal solids hydrolysis capacity: two soils (including soil bacteria); three inorganic conditioners; fifteen pure strain bacteria species spores; three bacterial spore mixes; one fungus spore mix; four commercial bacterial spores products; two live bacteria consortia; six enzyme concentrates; plus one mix and ten faeces extracts of herbivores. The faeces extracts were prepared as follows: 5 g natural fresh faeces mixed in 45 ml basal bacterial medium (Lindeboom, 2014), followed by two minutes of

**Table 1** The average total, soluble, and calculated suspended solids (ss) COD values of the 11 black water batches (analysed in triplicate or 12-fold, standard deviation between brackets) and of the two faeces batches (analysed as single or in triplicate, standard deviation between brackets)

| COD (mg COD/L) | $COD_{total}$ | $COD_{soluble}$ | $COD_{ss}$ |
|---|---|---|---|
| **Black water (11 batches)** | 7,125 (822) | 2,876 (798) | 4,249 (502) |
| COD (g COD/kg) | $COD_{total}$ | $COD_{soluble}$ | $COD_{ss}$ |
| **Faeces (batch 1 )** | 358 | 81 (0.75) | 277 (0.75) |
| **Faeces (batch 2)** | 328 | 51.5 | 276.5 |

ultrasonic treatment (Branson 510, USA, at 47 kHz and 185 W), one night shaking at 30°C, and again two minutes of ultrasonic treatment. The filtrate from sieving the mix through a sieve mesh size 1 mm is the faeces extract.

The advised commercial bio-additive dosages in real pit latrines is 100 g dry material in a pit of approximately 3,000 L (0.03 g/L), whereas the application of dry additives in our screening experiments is 1 g/L to establish the ultimate solids solubilization within a relatively short testing time. This applied amount of solid organic additive is a considerable addition to the faecal substrate solids. Assuming 1 g of organic additive solids is 1 g COD, then this additive quantity equals 23.5 per cent of the average black water solids COD (4,249 mg $COD_{ss}$/L). The dry additive material was added to the testing bottles and mixed well with the black water. For the moisture level testing, human faeces were used as substrate, which is too viscous to mix by swirling. Here, the additives were blended into the substrate by stirring before inserting the substrate with the additives in the testing bottles.

The effect of additives on solids hydrolysis was assessed from the difference between hydrolysis of substrate with and without an additive (natural biological activity) using the same batch of substrate.

In the simulation pit latrines, the bacterial mixes A–C (see Table 2) and a bacterial commercial product were tested. Besides the bacterial mixes, the application of air into the substrate was also investigated.

For the four-month-long pit simulations the additive dosage was 0.1 g dry material in 10 ml lukewarm water added on top of 280–550 g faeces (= 0.2–0.36 g/L) per month. The content of the aerated simulation pit latrine was injected close to the bottom with 50–100 ml air daily.

### *Experimental set-up*

The batch bottles were mixed at 100–125 rpm on rotational shakers (Innova 400, USA). The test temperature used was 30°C, except for the temperature variation tests. The choice of gas phase condition depends on the type of additive under investigation. Aerobic microorganisms were tested under aerobic conditions, and anaerobic microorganisms under anaerobic conditions. Non-specific additives were subjected to a more realistic latrine scenario: initially aerobic (~2 days), followed by anaerobic conditions. Correction of pH was maintained between pH 6.5 and 7.8. If required, the pH was adjusted to pH 7 ± 0.1 with HCl or NaOH. For aerobic

**Table 2** Simulation pit latrine experimental set-up, additive mix pure strain bacteria components, and mix ratios

| *Pit no.* | *Additive type* | *Additive mix components and ratio* |
|---|---|---|
| – | None = Blank, 4°C | |
| 1, 2 | None = Blank, 30°C × 2 | |
| 3 | Mix A, 30°C | 1 *P. pantothrophus* + 1 *B. megaterium* |
| 4 | Mix B, 30°C | 1 *E. dissolvens* + 1 *B. megaterium* + 1 *R. pyrdinivorans* |
| 5 | Mix C, 30°C | 1 *B. megaterium* + 1 *R. pyrdinivorans* |
| 6 | Commercial product 4, 30°C | |
| 7 | Air, 30°C | |

conditions, the gas phase was replenished with fresh air to guarantee sufficient $O_2$. No additional buffering or nutrients were added to the black water.

Aerobic experiments were performed in 500 ml Schott bottles closed with a manometer or airtight cap and a rubber liner with NaOH pellets after filling with 100 ml black water and additive, with air as gas phase. The NaOH absorbs the produced $CO_2$. As a consequence, gas pressure change is due to $O_2$ consumption as long as denitrification does not proceed which should not be the case in aerobic circumstances. Nitrification was allowed to proceed, since nitrification would probably proceed in a real pit at sufficient $O_2$ levels. The concentrations of $NO_3^-$ and $NO_2^-$ were measured at the end of the experiment to correct for $O_2$ consumption for the nitrification and to correct the $COD_{soluble}$ measurement.

Aerobic-anaerobic experiments were performed in 245 ml serum bottles closed with butyl stoppers and crimp caps after filling with 100 ml black water and additive, with air as gas phase. The gas pressure was measured with a handheld pressure meter. The gas composition ($CH_4$, $CO_2$, and $H_2$) was measured by gas chromatograph (GC). Anaerobic experiments were performed in 245 ml serum bottles closed with butyl stoppers and crimp caps after filling with 100 ml black water and additive, and flushing the gas phase for five seconds with $N_2$ gas. The gas pressure was measured with a handheld pressure meter. The gas composition ($CH_4$, $CO_2$, and $H_2$) was measured by GC.

Standardized small-scale simulation pit latrines were used for measuring the effect of additive mixes in human faeces on pit fill height (Figure 1).

The bottom of a two-litre PET (polyethylene terephthalate) water bottle was removed. This set-up has a maximum internal surface area for pit content to the air of 69.4 $cm^2$. The cut bottom was punctured and used as a lid on the simulation pit, allowing air exchange and evaporation and to keep out insects (Figure 1). The bottle top opening, with a diameter of 25 mm, was covered with stocking fabric fixed by an elastic band and string. The stocking-covered opening was the bottom of the simulation pit, keeping the faeces inside the bottle and allowing for the drainage of liquid. To generate liquid suction out of the pit, tissue paper in a plastic bag was attached to the bottom of the simulation pit and held in place by an elastic band; this also prevented evaporation from the tissue paper. The tissue paper was in contact with the pit bottom opening. The amount of drained water could easily be determined by weighing the tissue paper, including the bag, before and after loading. A measuring tape was attached to the side of the bottle to measure the height of pit fill. The simulation pit latrine was placed in a beaker to keep it upright.

In this pit latrine simulation, the volume, drainage surface area, and air contact area are scaled down in a standardized way. However, the penetration depth of atmospheric oxygen into the substrate cannot be scaled down. As a consequence, the simulation pit will have a relatively larger aerobic zone than a real pit latrine.

The simulation pit latrines were operated at 30°C; no additional buffering or nutrients were added. Substrate was loaded in the pits once or twice a month with larger amounts of faeces in preference to loading small daily amounts. This reduced the effect of air oxygen penetration. The experiment was started with 185 g faeces in each pit. After two weeks all pits had received a load of 280–375 g faeces per month.

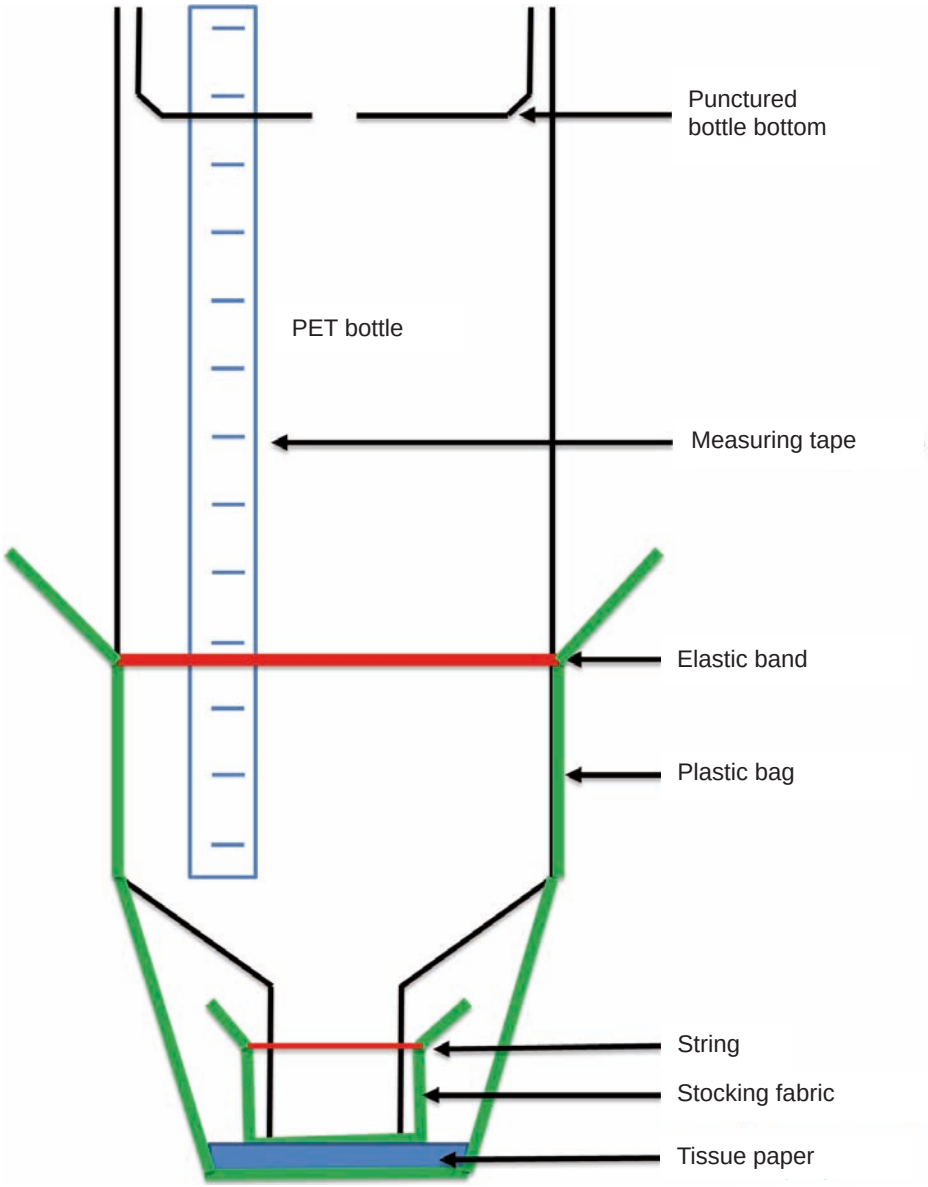


**Figure 1** Schematic drawing of two-litre PET bottle used as miniature simulation pit latrine set-up

### *Methods*

Batch experiments were sampled for analysis at the start of the experiment (t = 0) and after 15 (t = 1) and 20 (t = end) days for the enzyme additives. The anaerobic (an), aerobic-anaerobic (ae-an), aerobic (ae), and extended aerobic (ext. ae) tests with other additives were sampled at the start of the experiment and at least twice after

20–120 days, to establish if the ultimate hydrolytic conversion was reached. The batch tests were performed in triplicate (black water samples), four-fold (faecal samples), or 12-fold (pH 7 at 30°C) depending on ease of sampling. All determinations of components were performed as singular determinations, with the exception of the start substrate solids COD analysis which was determined in triplicate.

The simulation pit latrine experimental set-up consisted of one blank substrate collection pot and seven simulation pit latrines. The blank substrate collection pot received the same amounts and samples of substrate without additive and was stored over the running period at 4°C. This sample was used as the untreated substrate sample (input). Two of the simulation pits were used as blanks for determination of the natural activity of the substrate, and the remaining five simulation pits were loaded with substrate and additives or air, running at 30°C.

Throughout the duration of the experiment, well-mixed substrate was loaded into the simulation pits and also into the untreated sample pot. At the end of the experiment the blank simulation pits without additives gave the natural activity of the substrate and were compared with the simulation pits with additives.

The mass loading of the substrate was recorded via weight and the pit fill height was read from the measuring tape fixed to the simulation pits. The mass of liquid drained was recorded via weight. An airtight plastic bag with tissue paper was weighed before use (dry) and after use, with the mass difference being the amount of drained liquid. The liquid evaporation from the simulation pit was measured by weighing the whole set-up. Sample preparation for analysis at the end of the pit latrine simulation involved emptying the entire simulation pit content into a mixing bowl and mixing with a kitchen mixer to ensure a representative sample for the whole pit. The contents of one of the blank pits was scooped out in layers to collect vertical profile samples.

## Analysis and measurements

The analysis of substrate total and soluble COD fractions, and volatile fatty acids (VFAs), the total solids (TS), and volatile solids (VS), and the preparation of the soluble liquid fraction were performed using methods described by Fernandes et al. (2012) and APHA (2005). The gas pressure of the batch bottles was measured using a handheld pressure meter (GMH 3150, Greisinger Electronic GmbH, Germany). The gas phase $CH_4$, $CO_2$, and $H_2$ composition was measured according to the method described by Fernandes et al. (2012). The COD of the gas phase was calculated using the ideal gas law discounting dissolved methane in the liquid.

$N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were determined from the soluble fraction of the liquid with kits (Hach Lange, Germany) and the results were used for correcting the $O_2$ consumption from the gas phase due to nitrification. The COD determination was also corrected for the presence of $NO_2^-$.

The pH of faeces was measured in samples diluted two times with demineralized water by means of a pH meter (MeterLab® Radiometer Analytical, France); black water was measured undiluted. The pH was determined directly after opening batch bottles.

To quantify crust formation on faeces, puncture tests were performed using an XT Plus Texture Analyzer (Texture Technologies Corp. Hamilton, MA, USA) mounted with a 500 g load cell. An Allen key with a tip diameter of 6.16 mm was used as a puncturing probe. Ten disposable plastic 125 ml cups (Bellaplast AG, Altstätten, Switzerland) were filled with 60 g well-mixed faeces (~20 per cent TS), the surface smoothed with a spatula. The puncture force of the sample faeces–air interface was measured over time during 0.89 days (relative humidity during the day was 75–80 per cent). The puncture speed was 5 mm/s. Crust persistence after a fresh donation of substrate was measured as follows. On the 0.89-day-old crust in five of the cups, 10 ml water was poured and left for 15 minutes. The puncture force of the intermediate crust (crust 1; see Figure 2) was determined five times. To the other five 0.89-day-old cups, 15 g of fresh faeces was added and the surface smoothed carefully. In these cups, the intermediate crust 1 was measured after approximately five hours.

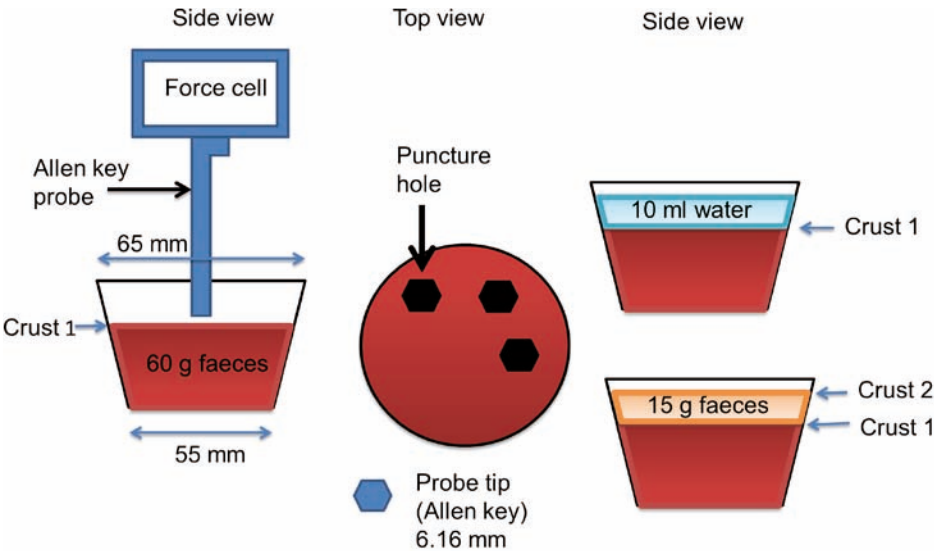


**Figure 2** Set-up for measuring the puncture force of crusts

### *Calculations*

In batch experiments, the substrate with additive was compared with substrate without additives to assess the effect of the additives in relation to their ability to hydrolyse solids, since black water and faeces have a natural capability to do so. The equations used are as follows:

Substrate natural solids hydrolysis percentage at ultimate conversion (t = x) =

$$\begin{aligned} &\mathrm{COD}_{\text{suspended solids hydrolysed natural}}\,(\%) \\ &= 100 \times \mathrm{COD}_{\text{hydrolysed natural t=x}}/\mathrm{COD}_{\text{suspended solids t=0}} \end{aligned} \quad (1)$$

The natural hydrolysis COD at t = x

$$\begin{aligned} &COD_{\text{suspended solids hydrolysed natural t=x}}\,(\text{mg/L}) \\ &= COD_{\text{soluble t=x}} + COD_{CH_4\text{ produced} + O_2\text{consumed t=x}} - COD_{\text{soluble t=0}} \end{aligned} \tag{2}$$

The suspended solids COD at t = 0

$$COD_{\text{suspended solids t=0}}\,(\text{mg/L}) = COD_{\text{total t=0}} - COD_{\text{soluble t=0}} \tag{3}$$

Substrate with additive hydrolysis percentage at ultimate conversion (t = x)

$$\begin{aligned} &COD_{\text{suspended solids hydrolysed natural + additive}}\,(\%) \\ &= 100 \times COD_{\text{hydrolysed natural + additive t=x}}/COD_{\text{suspended solids + additive t=0}} \end{aligned} \tag{4}$$

The natural + additive hydrolysis COD at t = x

$$\begin{aligned} &COD_{\text{suspended solids hydrolysed natural + additive t=x}}\,(\text{mg/L}) \\ &= COD_{\text{soluble t=x}} + COD_{CH_4\text{ produced} + O_2\text{consumed t=x}} - COD_{\text{soluble t=0}} \end{aligned} \tag{5}$$

The suspended solids COD at t = 0

$$COD_{\text{suspended solids + additive t=0}}\,(\text{mg/L}) = COD_{\text{total + additive t=0}} - COD_{\text{soluble t=0}} \tag{6}$$

where the $COD_{\text{suspended solids additive}}$ is estimated at 1 g COD/g dry organic solids added. The additives net solubilization of solids COD percentage (ANS%) at t = x =

$$\begin{aligned} \text{ANS}\,(\%) = {} & COD_{\text{suspended solids hydrolysed natural + additive}}\,(\%) \\ & - COD_{\text{suspended solids hydrolysed natural}}\,(\%) \end{aligned} \tag{7}$$

The ANS% of the substrate without additive is thus normalized (= 0). The ANS% when additives are used is positive if the additive has a positive influence on solids hydrolysis, or negative when the activity of the additive does not remove more solids than by natural activity plus what was added as an additive. The ANS% of an additive is considered significant when it exceeds the sum of the standard deviations of ANS% of the substrate without additive and the ANS% of the substrate with additive.

In batch experiments, the effect of physical chemical aspects on solids hydrolysis at ultimate conversion (t=x) was compared with other conditions. The solids hydrolysis percentage was calculated as follows:

$$COD_{\text{suspended solids hydrolysed t=x}}\,(\%) = COD_{\text{hydrolysed t=x}}\,/COD_{\text{suspended solids t=0}} \tag{8}$$

The hydrolysis COD at t = x

$$\begin{aligned} &COD_{\text{suspended solids hydrolysed natural t=x}}\,(\text{mg/L}) \\ &= COD_{\text{soluble t=x}} + COD_{CH_4\text{ produced} + O_2\text{ consumed t=x}} - COD_{\text{soluble t=0}} \end{aligned} \tag{9}$$

The suspended solids COD at t = 0

$$COD_{\text{suspended solids t=0}}\,(\text{mg/L}) = COD_{\text{total t=0}} - COD_{\text{soluble t=0}} \tag{10}$$

The total COD removal percentage at t = x was calculated as follows:

$$COD_{\text{total removed t=x}}(\%) = 100 \times COD_{\text{total t=x}}/COD_{\text{total untreated}} \quad (11)$$

## Results and discussion

The ANS% from these batch tests are presented in Table 3 (see Appendix A for the raw data).

Of all the tested additives, a significant positive ANS% was found for *Rhodococcus pyridinivorans* with an ANS% of +5% (2) at 21 days aerobic condition, which is not a likely scenario for a pit latrine, and with llama and ring tail lemur faeces extracts at aerobic-anaerobic conditions with an ANS% of 4% (0) and +2% (1) respectively. All other additives did not enhance or hinder hydrolysis of solids COD.

The effect of physical chemical conditions such as redox condition, pH, temperature, and water content on faecal solids hydrolysis was tested with black water or faeces as substrate without any additives. The results are presented as solids hydrolysis percentage on COD basis according to equation 8 (Table 4), comparing the start amount of solids to the ultimate (t = x) solids.

The applied redox condition shows that ultimate faecal solids hydrolysis percentage at aerobic conditions is 82 per cent and 26 per cent higher than at anaerobic conditions.

The initial pH has a marked effect on ultimate solids solubilization of black water. A pH of 5 yielded the lowest and pH 9 the highest $COD_{ss}$ hydrolysis. The bottles with an initial pH of 9 ended with an average pH of 7.4. A higher pH tends to solubilize substrate by increasing the solubility of proteins and ionizing VFAs, making it easier to convert biologically as long as the biomass is active at the given pH (Lin Lin et al., 2013).

Both temperature and moisture content given in weight–weight ratios have little impact in the tested range on the hydrolysis of solids.

To assess pit fill height and COD reduction in pit latrines, miniature simulation pits were loaded with faeces approximately once a month, and the additives suspended in water were added on top of the freshly added faecal matter. After 129 days, the pit content was sampled and analysed. The results are given in Table 5 (see also Appendix B).

As the pits were emptied, crust formation on the bottom of the pits was noticed. Drainage from the pits into the tissue paper did not proceed. For future experiments, wetted tissue paper could avoid this phenomenon. Evaporation was highest in the pit with aeration. Aeration proved the strongest driver for pit height reduction and COD removal compared with the blanks. This agrees with the findings of Nwaneri et al. (2008), who found that the main COD removal occurs in the aerobic zone of the pit latrine. Bacterial commercial product 4 did slightly enhance $COD_{total}$ removal in comparison to the blanks, but did not reduce pit fill height. The bacterial mixes A, B, and C did not enhance $COD_{total}$ removal or reduce pit fill height compared with the blanks.

Simulation pit 1 (no additive) was sampled at the end of the experiment at several heights. The results are presented in Table 6. The solubilization ($COD_{soluble}$)

**Table 3** Ultimate additive net solids solubilization (ANS%) on COD basis of black water solids at given conditions (standard deviation of substrate with additive plus standard deviation of the substrate without additive between brackets; significant ANS% in bold)

| *Additive* | *ANS% (st. dev. %)* | *Redox condition* | *Additive material type and dosage* |
|---|---|---|---|
| Inorganic additives: | | | |
| Tanzanian roadside dirt | –11 (1 + 2) | ae-an | Mainly inorganic solids |
| Perlite EMP1 | 0 (7 + 6) | ae-an | Dosage 1 g/L black water |
| Dutch roadside dirt | –8 (2 + 2) | ae-an | |
| Talcum 30/80 | +3 (0 + 6) | ae-an | |
| Diatobon TGM | +3 (2 + 6) | ae-an | |
| Commercial bacterial: | | | |
| Product 1 | –7 (4 + 2) | ae-an | Mainly organic solids (spores/bran) |
| Product 2 | –9 (4 + 2) | ae-an | Dosage 1 g/L black water |
| Product 3 | –6 (1 + 6) | ae-an | |
| Product 4 | –9 (4 + 3) | ae-an | |
| Enzymes: | | | |
| Amylase | –4 (1 + 0) | an | Mainly organic liquid |
| Protease | –4 (0 + 0) | an | Dosage 0.1 ml/L black water |
| Hemicellulase | –2 (2 + 0) | an | |
| Lipase | –3 (1 + 0) | an | |
| Cellulase complex | –1 (3 + 0) | an | |
| Pectate lyase | –2 (3 + 0) | an | |
| Mix of above enzymes | –4 (1 + 0) | an | Dosage 0.5 ml/L black water |
| Fungi: | | | |
| Blend of 4 fungi | –2 (9 + 5) | ae | Mainly organic solids (spores) Dosage 1 g/L black water |
| Pure strain microorganisms: | | | |
| *R. erythropolis* | –23 (5 + 1) | ae | Mainly organic solids |
| *P. monteilii* | –20 (5 + 1) | ae | (spores/bran) |
| *B. amyloliquefaciens* | –4 (3 + 1) | ae | Dosage 1 g/L black water |
| | –12 (2 + 5) | ext. ae | |
| *B. amyloliquefaciens* | –45 (3 + 1) | ae | |
| *P. pantotrophus* | +6 (7 + 1) | ae | |
| | +3 (8 + 5) | ext. ae | |
| | +3 (1 + 3) | ae-an | |
| *B. subtilis* | –10 (2 + 1) | ae | |
| | –16 (3 + 5) | ext. ae | |
| *B. pumilus* | –32 (5 + 3) | ae | |
| *E. dissolvens* | –8 (5 + 3) | ae | |
| | +1 (2 + 3) | ae-an | |
| *B. amyloliquefaciens* | –39 (2 + 1) | ae | |
| *B. licheniformis* | –8 (10 + 1) | ae | |
| | –10 (11 + 5) | ext. ae | |
| | –1 (1 + 6) | ae-an | |
| *B. megaterium* | –2 (6 + 1) | ae | |
| | –9 (8 + 5) | ext. ae | |
| | **–18** (1 + 3) | ae-an | |
| *R. pyridinivorans* | **+5** (2 + 1) | ae | |
| | –10 | ext. ae | |
| | –4 (9 + 3) | ae-an | |
| *B. amyloliquefaciens* | –12 (2 + 1) | ae | |
| | –11 (9 + 5) | ext. ae | |
| | –5 (0 + 6) | ae-an | |

*(continued)*

**Table 3** *(continued)*

| *Additive* | *ANS% (st. dev. %)* | *Redox condition* | *Additive material type and dosage* |
|---|---|---|---|
| *P. denitrificans* | –17 (7 + 1) | ae | |
| | +4 (0 + 6) | ae-an | |
| *B. pumilus* | –11 (2 + 1) | ae | |
| | –16 (3 + 5) | ext. ae | |
| | –6 (1 + 6) | ae-an | |
| Microorganisms live: | | | |
| Eerbeek granular sludge + digested cow manure | –25 | an | Mainly organic solids (live) Dosage 12 g VSS + 1.2 g VS/L black water |
| Bennekom MWTP activated sludge | –25 | ae | Mainly organic solids (live) Dosage 450 mg VSS/L black water |
| Microorganisms mixes: | | | |
| Mix A | –13 (6 + 3) | ae-an | Mainly organic solids (spores/bran) |
| Mix B | –2 (1 + 3) | ae-an | Dosage 1 g/L black water |
| Mix C | +2 (1 + 3) | ae-an | |
| Faeces extracts: | | | |
| domestic goat | +1 (1 + 0) | ae-an | Mainly organic liquid |
| ring tail lemur | **+2** (1 + 0) | ae-an | Dosage 10 ml faeces extract/L black |
| African elephant | –1 (1 + 0) | ae-an | water |
| Bennett's wallaby | 0 (0 + 0) | ae-an | |
| Bactrian camel | +1 (1 + 0) | ae-an | |
| Chapman's zebra | 0 (1 + 0) | ae-an | |
| Rothschild's giraffe | –1 (0 + 0) | ae-an | |
| domestic yak | –7 (0 + 0) | ae-an | |
| llama | **+4** (0 + 0) | ae-an | |
| red panda | –6 (1 + 0) | ae-an | |

**Table 4** Solids hydrolysis percentage at different gas phase redox conditions, initial pH, temperature, or water content in batch (standard deviation between brackets)

| *Substrate type Applied condition* | *Varied condition* | *Run time (days)* | *% solids solubilization* |
|---|---|---|---|
| Black water | Aerobic | 21 | 82.3 |
| At 30°C, pH 7 | Aerobic + anaerobic | 169 | 55.8 (3) |
| Black water | pH 5 | 169 | 25.3 (1) |
| Aerobic + anaerobic | pH 7 | 169 | 55.8 (3) |
| at 30°C | pH 9 | 169 | 65.3 (2) |
| Black water | 20°C | 160 | 62.7 (0) |
| Aerobic + anaerobic, pH 7 | 37°C | 169 | 66.6 (1) |
| Faeces and water | Faeces 1 : Water 0 | 158 | 8.60 (9) |
| Aerobic + anaerobic, pH 7, 30°C | Faeces 1 : Water 1 | 158 | 10.9 (2) |
| | Faeces 1 : Water 3 | 158 | 6.11 (4) |

**Table 5** End pit fill heights and $COD_{removed}$ of simulation pit latrines

| *Additive type* | *Blank* | *Mix A* | *Mix B* | *Mix C* | *Product 4* | *Air* |
|---|---|---|---|---|---|---|
| *Pit no.* | *1/2* | *3* | *4* | *5* | *6* | *7* |
| End pit height (cm) | 19.5/18.5 | 20.5 | 19.5 | 19.5 | 18.5 | 14.0 |
| COD removed (%) | 4.4/5.8 | 2.2 | 0.8 | 3.2 | 7.6 | 10.5 |

**Table 6** $COD_{total}$, $COD_{soluble}$, $COD_{VFA}$, and pH in blank pit latrine at different heights from the bottom at day 129

| *Sample height (cm)* | *$COD_{total}$ (g/kg)* | *$COD_{soluble}$ (g/kg)* | *$COD_{VFA}$ (g/kg)* | *pH (–)* |
|---|---|---|---|---|
| 14.6 | 369 | 119 | 64.2 | 6.50 |
| 10 | 355 | 118 | 65.8 | 6.48 |
| 7 | 351 | 124 | 70.5 | 6.35 |
| Total mixed sample | 359 | 119 | | |

and acidification ($COD_{VFA}$) were higher and the pH decreased slightly, whereas the $COD_{total}$ decreased as the sample came from lower down in the simulation pit holding the first loaded material. This indicates that hydrolysis and further degradation proceed over time within the pit latrine when the substrate is covered with new substrate.

In the simulation pit latrines, crusts on the surface of the faeces were formed. In the aerated pit the crusts did not disappear when a new layer of faeces was added. This will likely hamper the mobility of bacteria, degassing, and mass transfer. Therefore, the speed and strength of crust formation were measured on faeces. After crust formation, the faeces was covered with water or new faeces and the stability of the intermediate crust was measured (Table 7).

The formation of crusts on faecal matter takes place within three to five hours. The crust is dry to the touch (tested with gloves on) after four hours. The crusts show a few shrinkage cracks. Crusts covered with water for 15 minutes softened the previously formed crust by 47 per cent. This simulates faeces crusts coming into contact with urine. It is unlikely that urine will cover faeces for 15 minutes; more probably it will run off and seep through fissures and cracks in a real pit latrine. The covering with fresh faeces for 4.75 hours softened the previously formed crust by 8.5 per cent. This is a simulation of faeces crusts covered with new faeces. The speed of formation and persistence of crusts indicate that the mixing and contact possibilities of additives in a real pit latrine would not be positive unless the additive is motile with some force and it does not run off the substrate.

The mixing and temperature conditions in the batch tests were applied for fast conversions. The liquid nature of the black water substrate allowed easy mass and additive transport. The conditions in a real pit latrine are likely to lead to slower conversion rates and may result in a lower ultimate extent of conversion.

Different types of microorganisms are involved in the complete anaerobic digestion of converting biodegradable solids into biogas. In our experiments, the degree of solids hydrolysis and ANS% was consistently found not to be dependent on methane production (see results in Appendix A). In other words, accumulation of hydrolysis (waste) products such as mono- and oligomers or VFAs did not influence the extent of the hydrolysis. The pH in our experiments remained between 6.8 and 7.2. The effect of pH and VFAs on the hydrolysis of organic solid waste was investigated by Veeken et al. (2000). They found that a VFA concentration of up to 30 g/L, dissociated or not, did not affect the hydrolysis rate, but the pH, tested between 5 and 7, did.

**Table 7** Average puncture force (crust strength) of faeces over time, measured 10 times, and after coverage with water or new faeces, measured in five fold (standard deviation (%) in brackets)

| *Time (hours)* | *Puncture force (mN)* | *Puncture force of intermediate crust after $H_2O$ coverage (mN)* | *Puncture force of intermediate crust after faeces coverage (mN)* |
|---|---|---|---|
| 0 | 0 | | |
| 1.4 | 0 | | |
| 3 | 126 (39.8) | | |
| 5 | 154 (13.4) | | |
| 21.3 | 639 (48.6) | 338 (29.3) | 585 (26.1) |

The tested additives and additive mixes either had no effect or had only a very small effect on the hydrolysis of solids in batch experiments, nor did they reduce COD and pit fill height in simulation pit latrines compared with the natural activity of the waste under fairly favourable controlled laboratory conditions. Aeration did enhance COD removal and evaporation and reduced pit fill height in a simulation pit latrine. These findings are in line with the results of Buckley et al. (2008) regarding COD removal and pit fill height reduction by bio-additives, both in lab experiments and in field trials.

## Conclusions

The negative or meagre solid hydrolysis (ANS%) results, the lack of height reduction and the resulting filling space increase in simulation pit latrines, and the crust formation on the substrate under favourable lab conditions compared with conditions in real pit latrines make additives unlikely candidates for solving pit fill problems. Our results show that additives mainly act as extra substrate in the pit or even hinder solids hydrolysis.

Physical chemical environment control of the substrate offers greater promise in terms of enhancing faecal solids breakdown, in particular extended aeration compared with anaerobic redox conditions, and raising the initial pH to around 9 compared with the naturally occurring pH.

## Acknowledgments

This paper is dedicated to the memory of Dr Jeroen Ensink, our friend and colleague.

This research was supported by a grant to the London School of Hygiene and Tropical Medicine from the Bill & Melinda Gates Foundation.

The additives were donated by several companies, Ouwehand Zoo (Rhenen, the Netherlands), and Frederick Salukele, who collected and transported Tanzanian roadside dirt. The black water substrate was donated by the DESAR test facility site in Sneek (the Netherlands) and the faeces by volunteers.

## Appendix A

Average $COD_{solids}$, $COD_{soluble}$, $COD_{CH_4}$, and $COD_{O_2}$ results in mg $O_2$/L and ultimate ANS% (*unless stated differently) of the screening tests with black water solids under given conditions, with and without additives (standard deviation between brackets)

| *Additive* | *Redox condition* | *$COD_{soluble}$ $mgO_2/L$* | *Time = 0* | | | *$COD_{O_2}$ $mgO_2/L$* | *$COD_{CH_4}$ $mgO_2/L$* | *$COD_{soluble}$ + $COD_{O_2}$ + $COD_{CH_4}$ $mgO_2/L$* | *Time = x* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *$COD_{solids}$ $mgO_2/L$* | *Run Days* | *$COD_{soluble}$ $mgO_2/L$* | | | | *$COD_{hydrolized}$ $mgO_2/L$* | *$COD_{hydrolized}$ of $COD_{solids}$ t = 0 %* | *ANS%[&]* |
| | | *A* | *B* | | *C* | *D* | *E* | *F* | *G* | *H* | *I* |
| None | ae-an[†] | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| Tanzanian roadside | ae-an | 3663 (81) | 4001 (60) | 87 | 735 | 409 | 4090 | 5234 (48) | 1571 | 39 | –11 (1) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Perlite EMP1 | ae-an | 1786 (44) | 4524 (55) | 93 | 1014 | 425 | 2597 | 4036 (300) | 2250 | 50 | 0 (7) |
| None | ae-an | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| Dutch roadside | ae-an | 3621 (17) | 4001 (60) | 87 | 784 | 408 | 4105 | 5297 (91) | 1676 | 42 | –8 (2) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Talcum 30/80 | ae-an | 1820 (96) | 4524 (55) | 93 | 1035 | 436 | 2714 | 4185 (16) | 2365 | 52 | +3 (0) ns |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Diatobon TGM | ae-an | 1766 (37) | 4524 (55) | 93 | 978 | 431 | 2725 | 4134 (100) | 2368 | 52 | +3 (2) ns |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Product 1 | ae-an | 1815 (19) | 5524 (55) | 93 | 1383 | 425 | 3094 | 4902 (122) | 3087 | 56 | +6 (2) ns |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Product 2 | ae-an | 2023 (74) | 5524 (55) | 93 | 1522 | 433 | 3088 | 5043 (61) | 3020 | 55 | +5 (1) ns |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| Product 3 | ae-an | 1790 (44) | 5524 (55) | 93 | 1029 | 440 | 2749 | 4218 (24) | 2428 | 44 | –6 (1) |
| None | ae-an | 3397 (42) | 3637 (183) | 169 | 1138 | 399 | 3889 | 5426 (160) | 2029 | 56 | 0 (3) |
| Product 4 | ae-an | 3822 (59) | 4637 (183) | 169 | 1100 | 396 | 4483 | 5979 (211) | 2157 | 47 | –9 (4) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Amylase | an | 2351 (17) | 4589 (46) | 21* | 3437 | 0 | 264 | 3701 (21) | 1350 | 29 | –4 (1) |

*(continued)*

## Appendix A (*continued*)

| *Additive* | *Redox condition* | $COD_{soluble}$ $mgO_2/L$ | *Time = 0* | | | $COD_{O_2}$ $mgO_2/L$ | $COD_{CH_4}$ $mgO_2/L$ | $COD_{soluble} + COD_{O_2} + COD_{CH_4}$ $mgO_2/L$ | *Time = x* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $COD_{solids}$ $mgO_2/L$ | *Run Days* | $COD_{soluble}$ $mgO_2/L$ | | | | $COD_{hydrolized}$ $mgO_2/L$ | $COD_{hydrolized}$ *of* $COD_{solids}$ *t = 0 %* | *ANS%&* |
| | | *A* | *B* | | *C* | *D* | *E* | *F* | *G* | *H* | *I* |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Protease | an | 2361 (15) | 4589 (46) | 21* | 3484 | 0 | 214 | 3698 (0) | 1337 | 29 | –4 (0) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Hemicellulase | an | 2371 (24) | 4589 (46) | 21* | 3511 | 0 | 272 | 3783 (74) | 1412 | 31 | –2 (2) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Lipase | an | 2301 (5) | 4589 (46) | 21* | 3437 | 0 | 259 | 3696 (25) | 1395 | 30 | –3 (1) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Cellulase complex | an | 2359 (50) | 4589 (46) | 21* | 3560 | 0 | 278 | 3838 (126) | 1479 | 32 | –1 (3) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| Pectate lyase | an | 2292 (16) | 4589 (46) | 21* | 3465 | 0 | 265 | 3730 (95) | 1438 | 31 | –2 (3) |
| None | an | 2264 (16) | 4589 (46) | 21* | 3521 | 0 | 260 | 3781 (19) | 1517 | 33 | 0 (1) |
| mix of the above enzymes | an | 2550 (21) | 4589 (46) | 21* | 3681 | 0 | 214 | 3895 (24) | 1345 | 29 | –4 (1) |
| None | ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| Blend of 4 fungi | ae | 1672 (71) | 5819 (200) | 70 | 1153 | 4877 | 0 | 6030 (537) | 4358 | 75 | –2 (9) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *R. erythropolis* | ae | 2303 (16) | 5410 (109) | 21 | 728 | 4194 | 0 | 4922 (227) | 2619 | 48 | –23 (5) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *P. monteilii* | ae | 2352 (39) | 5410 (109) | 21 | 652 | 4463 | 0 | 5115 (248) | 2763 | 51 | –20 (5) |
| None | ae | 1577 (78) | 4819 (200) | 23 | 695 | 4040 | 0 | 4735 (66) | 3158 | 66 | 0 (1) |
| *B. amyloliquefaciens* | ae | 1948 (104) | 5819 (200) | 23 | 843 | 4689 | 0 | 5532 (154) | 3584 | 62 | –4 (3) |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. amyloliquefaciens* | ext.ae | 1948 (104) | 5819 (200) | 70 | 820 | 4906 | 0 | 5726 (80) | 3778 | 65 | –12 (2) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. amyloliquefaciens* | ae | 2823 (70) | 5410 (109) | 21 | 1237 | 3012 | 0 | 4249 (124) | 1426 | 26 | –45 (3) |

*(continued)*

## Appendix A (*continued*)

| *Additive* | *Redox condition* | $COD_{soluble}$ $mgO_2/L$ | *Time = 0* | | | $COD_{O_2}$ $mgO_2/L$ | $COD_{CH_4}$ $mgO_2/L$ | $COD_{soluble} + COD_{O_2} + COD_{CH_4}$ $mgO_2/L$ | *Time = x* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $COD_{solids}$ $mgO_2/L$ | *Run Days* | $COD_{soluble}$ $mgO_2/L$ | | | | $COD_{hydrolized}$ $mgO_2/L$ | $COD_{hydrolized}$ *of* $COD_{solids}$ *t = 0 %* | *ANS%&* |
| | | *A* | *B* | | *C* | *D* | *E* | *F* | *G* | *H* | *I* |
| None | ae | 1577 (78) | 4819 (200) | 23 | 695 | 4040 | 0 | 4735 (66) | 3158 | 66 | 0 (1) |
| *P. pantotrophus* | ae | 1609 (52) | 5819 (200) | 23 | 769 | 5022 | 0 | 5791 (394) | 4182 | 72 | +6 (7) ns |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *P. pantotrophus* | ext.ae | 1609 (52) | 5819 (200) | 70 | 785 | 5477 | 0 | 6262 (529) | 4653 | 80 | +3 (8) ns |
| None | ae-an | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| *P. pantotrophus* | ae-an | 3709 (21) | 5001 (60) | 87 | 1169 | 415 | 4666 | 6199 (84) | 2490 | 50 | 0 (1) |
| None | ae | 1577 (78) | 4819 (200) | 23 | 695 | 4040 | 0 | 4735 (66) | 3158 | 66 | 0 (1) |
| *B. subtilis* | ae | 2027 (105) | 5819 (200) | 23 | 951 | 4323 | 0 | 5274 (127) | 3247 | 56 | −10 (2) |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. subtilis* | ext.ae | 2027 (105) | 5819 (200) | 70 | 887 | 4713 | 0 | 5600 (163) | 3573 | 61 | −16 (3) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. pumilus* | ae | 2863 (31) | 5410 (109) | 21 | 1306 | 3698 | 0 | 5004 (241) | 2141 | 40 | −32 (5) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *E. dissolvens* | ae | 2324 (21) | 5410 (109) | 21 | 717 | 5050 | 0 | 5767 (295) | 3443 | 64 | −8 (5) |
| None | ae-an | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| *E. dissolvens* | ae-an | 3784 (28) | 5001 (60) | 87 | 1986 | 407 | 3902 | 6295 (125) | 2511 | 50 | 0 (2) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. amyloliquefaciens* | ae | 2752 (63) | 5410 (109) | 21 | 1125 | 5050 | 0 | 4519 (100) | 1767 | 33 | −39 (2) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. licheniformis* | ae | 2782 (57) | 5410 (109) | 21 | 979 | 5670 | 0 | 6649 (357) | 3867 | 71 | 0 (5) |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. licheniformis* | ext.ae | 2120 (74) | 5819 (200) | 70 | 885 | 5143 | 0 | 6028 (669) | 3908 | 67 | −10 (11) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *B. licheniformis* | ae-an | 2259 (80) | 5524 (55) | 93 | 2315 | 440 | 2189 | 4944 (25) | 2685 | 49 | −1 (1) |

*(continued)*

## Appendix A (*continued*)

| Additive | Redox condition | $COD_{soluble}$ $mgO_2/L$ | Time = 0 | | | $COD_{O_2}$ $mgO_2/L$ | $COD_{CH_4}$ $mgO_2/L$ | $COD_{soluble} + COD_{O_2} + COD_{CH_4}$ $mgO_2/L$ | Time = x | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $COD_{solids}$ $mgO_2/L$ | Run Days | $COD_{soluble}$ $mgO_2/L$ | | | | $COD_{hydrolized}$ $mgO_2/L$ | $COD_{hydrolized}$ of $COD_{solids}$ t = 0 % | ANS%[&] |
| | | A | B | | C | D | E | F | G | H | I |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. megaterium* | ae | 2626 (24) | 5410 (109) | 21 | 778 | 5561 | 0 | 6339 (291) | 3713 | 69 | −3 (5) |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. megaterium* | ext.ae | 1996 (109) | 5819 (200) | 70 | 750 | 5199 | 0 | 5949 (502) | 3953 | 68 | −9 (8) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *B. megaterium* | ae-an | 2084 (61) | 5524 (55) | 93 | 1505 | 436 | 3015 | 4956 (47) | 2872 | 52 | +2 (1) ns |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *R. pyridinivorans* | ae | 2353 (22) | 5410 (109) | 21 | 885 | 5608 | 0 | 6493 (140) | 4140 | 77 | +5 (2) s |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *R. pyridinivorans* | ext.ae | 1709 (45) | 5819 (200) | 70 | 684 | 4921 | 0 | 5605 | 3896 | 67 | −10 |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *R. pyridinivorans* | ae-an | 1981 (63) | 5524 (55) | 93 | 1650 | 442 | 2879 | 4971 (186) | 2990 | 54 | +4 (4) ns |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *B. amyloliquefaciens* | ae | 2481 (25) | 5410 (109) | 21 | 941 | 5219 | 0 | 6160 (40) | 3679 | 68 | −3 (1) |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. amyloliquefaciens* | ext.ae | 1837 (50) | 5819 (200) | 70 | 1209 | 4445 | 0 | 5654 (498) | 3817 | 66 | −11 (9) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *B. amyloliquefaciens* | ae-an | 2115 (33) | 5524 (55) | 93 | 2364 | 433 | 1791 | 4588 (18) | 2473 | 45 | −5 (0) |
| None | ae | 2244 (16) | 4410 (109) | 21 | 653 | 4731 | 0 | 5384 (80) | 3140 | 71 | 0 (1) |
| *P. denitrificans* | ae | 2796 (125) | 5410 (109) | 21 | 1098 | 4631 | 0 | 5729 (405) | 2933 | 54 | −17 (7) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *P. denitrificans* | ae-an | 2175 (53) | 5524 (55) | 93 | 4441 | 434 | 240 | 5115 (13) | 2940 | 53 | +4 (0) ns |
| None | ae | 1577 (78) | 4819 (200) | 23 | 695 | 4040 | 0 | 4735 (66) | 3158 | 66 | 0 (1) |
| *B. pumilus* | ae | 2240 (48) | 5819 (200) | 23 | 1003 | 4388 | 0 | 5391 (119) | 3151 | 54 | −11 (2) |

*(continued)*

## Appendix A (*continued*)

| *Additive* | *Redox condition* | $COD_{soluble}$ $mgO_2/L$ | *Time = 0* | | | $COD_{O_2}$ $mgO_2/L$ | $COD_{CH_4}$ $mgO_2/L$ | $COD_{soluble} + COD_{O_2} + COD_{CH_4}$ $mgO_2/L$ | *Time = x* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $COD_{solids}$ $mgO_2/L$ | *Run Days* | $COD_{soluble}$ $mgO_2/L$ | | | | $COD_{hydrolized}$ $mgO_2/L$ | $COD_{hydrolized}$ *of* $COD_{solids}$ *t = 0 %* | *ANS%[&]* |
| | | *A* | *B* | | *C* | *D* | *E* | *F* | *G* | *H* | *I* |
| None | ext.ae | 1577 (78) | 4819 (200) | 70 | 995 | 4294 | 0 | 5289 (255) | 3712 | 77 | 0 (5) |
| *B. pumilus* | ext.ae | 2240 (48) | 5819 (200) | 70 | 983 | 4824 | 0 | 5807 (176) | 3567 | 61 | −16 (3) |
| None | ae-an | 1858 (32) | 4524 (55) | 93 | 1585 | 442 | 2080 | 4107 (242) | 2249 | 50 | 0 (6) |
| *B. pumilus* | ae-an | 2462 (70) | 5524 (55) | 93 | 2167 | 441 | 2255 | 4863 (33) | 2410 | 43 | −6 (1) |
| None | an | 2830 | 3192 | 39 | 4179 | 0 | 556 | 4735 | 1905 | 60 | 0 |
| Live anaerobe | an | 3320 | 4432 | 35 | 993 | 0 | 3857 | 4850 | 1530 | 35 | −25 |
| None | ae | 3561 | 2947 | 14 | 1131 | 5054 | 0 | 6185 | 2624 | 89 | 0 |
| Live aerobe | ae | 3389 | 3397 | 14 | 667 | 4900 | 0 | 5567 | 2178 | 64 | −25 |
| None | ae-an | 3397 (42) | 3637 (183) | 169 | 1138 | 399 | 3889 | 5426 (160) | 2029 | 56 | 0 (3) |
| Mix A | ae-an | 3717 (56) | 4637 (183) | 169 | 1176 | 412 | 4111 | 5699 (335) | 1982 | 43 | −13 (6) |
| None | ae-an | 3397 (42) | 3637 (183) | 169 | 1138 | 399 | 3889 | 5426 (160) | 2029 | 56 | 0 (3) |
| Mix B | ae-an | 3613 (48) | 4637 (183) | 169 | 1197 | 400 | 4502 | 6099 (76) | 2486 | 54 | −2 (1) |
| None | ae-an | 3397 (42) | 3637 (183) | 169 | 1138 | 399 | 3889 | 5426 (160) | 2029 | 56 | 0 (3) |
| Mix C | ae-an | 3749 (46) | 4637 (183) | 169 | 1206 | 397 | 4805 | 6408 (55) | 2659 | 57 | +2 (1) ns |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| goat | ae-an | 2703 (34) | 4149 (103) | 89 | 884 | 399 | 3197 | 4480 (43) | 1777 | 43 | +1 (1) |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| ring tail lemur | ae-an | 2666 (13) | 4149 (103) | 89 | 858 | 402 | 3309 | 4569 (45) | 1903 | 46 | +2 (1) s |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| African elephant | ae-an | 2696 (33) | 4149 (103) | 89 | 861 | 400 | 3141 | 4402 (49) | 1706 | 41 | −1 (1) |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| wallaby | ae-an | 2696 (60) | 4149 (103) | 89 | 865 | 392 | 3202 | 4459 (15) | 1763 | 42 | 0 (0) |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| Bactrian camel | ae-an | 2771 (6) | 4149 (103) | 89 | 892 | 397 | 3269 | 4558 (36) | 1787 | 43 | +1 (1) |

*(continued)*

## Appendix A (*continued*)

| *Additive* | *Redox condition* | $COD_{soluble}$ $mgO_2/L$ | *Time = 0* | | | $COD_{O_2}$ $mgO_2/L$ | $COD_{CH_4}$ $mgO_2/L$ | $COD_{soluble} + COD_{O_2} + COD_{CH_4}$ $mgO_2/L$ | *Time = x* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $COD_{solids}$ $mgO_2/L$ | *Run Days* | $COD_{soluble}$ $mgO_2/L$ | | | | $COD_{hydrolized}$ $mgO_2/L$ | $COD_{hydrolized}$ *of* $COD_{solids}$ *t = 0 %* | *ANS%*[&] |
| | | *A* | *B* | | *C* | *D* | *E* | *F* | *G* | *H* | *I* |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| zebra | ae-an | 2722 (69) | 4149 (103) | 89 | 868 | 410 | 3190 | 4468 (57) | 1746 | 42 | 0 (1) |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| giraffe | ae-an | 2766 (53) | 4149 (103) | 89 | 873 | 404 | 3191 | 4468 (16) | 1702 | 41 | –1 (0) |
| None | ae-an | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| yak | ae-an | 3694 (85) | 4001 (60) | 87 | 865 | 402 | 4121 | 5398 (25) | 1704 | 43 | –7 (0) |
| None | ae-an | 2780 (63) | 4149 (103) | 89 | 1038 | 406 | 3081 | 4525 (9) | 1745 | 42 | 0 (0) |
| llama | ae-an | 2720 (17) | 4149 (103) | 89 | 931 | 410 | 3318 | 4659 (20) | 1939 | 47 | +4 (0) s |
| None | ae-an | 3686 (44) | 4001 (60) | 87 | 4930 | 414 | 337 | 5681 (129) | 1995 | 50 | 0 (2) |
| red panda | ae-an | 3694 (54) | 4001 (60) | 87 | 868 | 412 | 4158 | 5438 (60) | 1744 | 44 | –6 (1) |

Notes: [†] Redox condition: ae = aerobic; an = anaerobic; ae-an = initially aerobic ± days followed by anaerobic; ext.ae = aerobic ultimate conversion.
* Conversion not ultimate but limited to ±20 days. The extended aerobic tests were left to reach the ultimate conversion.
[&] A positive ANS% of an additive is significant when it exceeds the sum of the standard deviation of the 'None' (blank) ANS% and the additive ANS% (results marked with s). If not, it is considered 'not significant' (ns). Negative ANS results are not taken into account since they did not demonstrate usefulness.
Live anaerobe = Eerbeek paper factory anaerobic granular sludge + digested cow manure.
Live aerobe = Bennekom MWTP activated aerobic sludge.
Column A = 0.45 µm pore size membrane filtrate COD measurement of batch bottle content t = 0
Column B = $COD_{solids}$ = $COD_{total}$ of substrate – 0.45 µm pore size membrane filtrate COD of substrate, t = 0
Column C = 0.45 µm pore size membrane filtrate COD measurement of batch bottle content t = x
Column D = $O_2$ consumed of the gas phase of the batch bottle t = x, gas pressure measured, calculation $n_{O2}$ via gas law, t = x
Column E = $CH_4$ produced in the gas phase of the batch bottle t = x, gas pressure and gas composition measured, calculation $n_{CH4}$ via gas law, t = x
Column F = C + D + E
Column G = F – A
Column H = 100 × (G/B)
Column I = ANS% = (H of bottle with additive) – (H of bottle with no additive)

## Appendix B

Input and end COD total and soluble, TS, and VS concentrations, COD removal, weights, and pit heights of simulation pit latrines (no standard deviation – all pits were run as singles, except for the blanks, pits 1 and 2)

| *Additive type* | *Blank/blank* | *Mix A* | *Mix B* | *Mix C* | *Commercial product 4* | *Air* |
|---|---|---|---|---|---|---|
| Cum. load faeces (g) | 1158/1156 | 1163 | 1159 | 1162 | 1158 | 1157 |
| Additive donations | 0/0 | 3 x 0.1 g | 3 x 0.1 g | 3 x 0.1 g | 3 x 0.1 g | 60 ml air/d |
| (Weight loss =) | | | | | | |
| Cum. evaporation (g) | 147/183 | 155 | 153 | 170 | 204 | 365 |
| **End pit height (cm)** | **19.5/18.5** | **20.5** | **19.5** | **19.5** | **18.5** | **14** |
| Input TS (g/kg) | 203/203 | 203 | 203 | 203 | 203 | 203 |
| Input VS (g/kg) | 173/173 | 173 | 173 | 173 | 173 | 173 |
| End TS (g/kg) | 197/199 | 199 | 197 | 204 | 198 | 296 |
| End VS (g/kg) | 157/154 | 158 | 162 | 164 | 162 | 236 |
| End VS/TS | 0.80/0.77 | 0.79 | 0.82 | 0.80 | 0.82 | 0.80 |
| Input $COD_{total}$ (g/kg) | 328/328 | 328 | 328 | 328 | 328 | 328 |
| Input $COD_{soluble}$ (g/kg) | 81/81 | 81 | 81 | 81 | 81 | 81 |
| End $COD_{total}$ (g/kg) | 359/367 | 370 | 375 | 372 | 368 | 429 |
| End $COD_{soluble}$ (g/kg) | 119/115 | 117 | 111 | 100 | 109 | 79 |
| COD mass balance | | | | | | |
| $COD_{total}$ input (g/pit) | 380/379 | 382 | 380 | 381 | 380 | 380 |
| $COD_{total}$ end (g/pit) | 363/357 | 373 | 377 | 369 | 351 | 340 |
| COD removed (g/pit) | 17/22 | 9 | 3 | 12 | 29 | 40 |
| **COD removed (%)** | **4.4/5.8** | **2.2** | **0.8** | **3.2** | **7.6** | **10.5** |

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