40th WEDC International Conference, Loughborough, UK, 2017

LOCAL ACTION WITH INTERNATIONAL COOPERATION TO IMPROVE AND SUSTAIN WATER, SANITATION AND HYGIENE SERVICES

What happens inside a pour-flush pit? insights from comprehensive characterization

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PAPER 2823

The pour-flush toilet is extensively used in many countries, but the biodegradation within pour-flush leach pits has not been fully characterised. We present a comprehensive physical, chemical, and microbiological analysis of pour-flush active and standing leach pits in South Africa. Four household toilet sites were sampled four times over 11 months. The pour-flush pit filling rate was estimated to be 0.11m3/y, which is lower than those of other sanitation technologies. Faecal sludge in active leach pits had similar ash, VS, CODT and TKN as other onsite technologies, but higher moisture content. The CODT in pour-flush sludge decreased 85% in 27 days in a short-term laboratory test. Microbial DNA sequencing showed that both aerobic and anaerobic degradation occurred in active and standing pits. Specific microorganisms were identified and differences in microbial communities in active, standing, and single pits were described, providing important insights into processes occurring within pour-flush pits.

Background

The pour-flush (PF) toilet is a common onsite sanitation technology in regions where anal cleansing with water is common, and is seen as bridging the gap between basic on-site sanitation technologies and water-borne sewerage that people aspire to. In South Africa, the PF toilet, adapted from traditional Indian design, was tested on the outskirts of the Pietermaritzburg area by Partners in Development (PID). A toilet pedestal rather than a squatting pan was developed, with the capacity to flush anal cleansing material such as toilet paper or newspaper with only 1.5 litres of water or greywater. The pedestal is connected to either single or twin leach pits on-site by underground pipes. If twin leach pits are constructed, the underground pipe is connected to one leach pit until it is full; the pipe connection is then diverted to the second leach pit, which begins filling. The first pit is removed from use and the faecal sludge degrades.

The filling rate was monitored as well as some aspects of user behaviour, such as user number, quantity of flush water used, use of toilet paper versus newspaper and user satisfaction (Still and Louton, 2012). The trial for 20 households was deemed a success. However this is the extent of data available on the performance of PF toilets, and the characteristics of PF sludge is documented in neither South Africa nor India. In this paper, we present the results of chemical, physical and biological characterization of the faecal sludge stored in the PF leach pits. The difference between the active and standing leach pits was examined. Biodegradation studies of the FS removed from PF pits were conducted. Molecular microbial analysis of the pit contents using Illumina sequencing of the 16S rRNA gene was used to characterize the microbial populations. The combined data produces a more complete picture of the biodegradation that occurs inside standing, active, and single PF leach pits.

Methods

Site selection and sampling

To determine the chemical, physical and biological properties of FS in PF leach pits, four sites were selected for sampling and analysis from the PID pilot scheme. The sites were located in the areas of Azalea and

France on the outskirts of Pietermaritzburg, South Africa. All participants signed a letter of consent prior to participation in the study and answered a questionnaire to provide additional information about the household and hygiene behaviour. Samples were collected from the leach pits on four separate occasions over a period of eleven months. Samples were taken at four month intervals after ascertaining that change in depth over two months was insufficient to measure easily. Samples were stored in a 2.5 litre bucket with a lid and lined with a plastic bag. The samples were transported to University of KwaZulu-Natal and stored in a cold room below 4°C in the Pollution Research Group laboratory. For DNA analysis, samples were immediately processed for DNA extraction using a modified aluminium sulphate method (Staley et al., 2011).

Compositional analysis and biodegradability tests

The PF sludge samples were analysed for total solids (TS), moisture content (MC), volatile solids (VS), ash content, total suspended solids (TSS), total COD (COD_T), soluble COD (COD_S), particulate COD (COD_P), nitrogen species (TN, TKN, ammonia, nitrate), phosphates (total phosphate and ortho-phosphate), sodium and potassium according to Standard Methods (APHA et al., 1995). Biodegradability of PF sludge was quantified using a short-term and a long-term test. The short-term test involved repeated COD analysis over time. The long-term test involved operating a completely stirred tank reactor (CSTR) and monitoring gas production over time.

DNA sequencing and analysis

Amplification of the 16S rRNA gene of Bacteria and Archaea was performed using forward and reverse primer pair sequences, modified 341F and modified 806R, respectively (Yu et al., 2005; Sundberg et al., 2013). Amplicon libraries were run on an Illumina MiSeq platform for paired-end read sequencing at the Gsenomic Sciences Laboratory, North Carolina State University, NC. Amplicon sequences were processed using QIIME pipeline (Caporaso et al., 2010b) for pair merging, quality filtering, taxonomic assignment, chimera removal, and phylogeny analysis. The final table contained 406 356 sequences with an average of 33 863 sequences per sample. Chemical and physical characteristics corresponding to each PF sample were used as environmental variables for canonical correspondence analysis (CCA) to evaluate the effect of environment on the community structure. Community analysis was conducted using the R vegan package (Oksanen et al., 2015) and visualized using the R ggplot2 package (Wickham, 2009).

Results and discussion

Filling rates

The filling rates for the leach pits were determined using depth data over time. The average filling rate of the leach pits monitored in this study was 109 l/y, or 0.11 m3/y, in line with the value determined earlier by PID. PF pits that were commissioned at the beginning of the study showed an initial increase in volume followed by a decrease, which was interpreted as a ‘start-up’ phase, as microorganisms started to build up in the pits. The volume of FS in standing (inactive) leach pits decreased over time, while the volume in active leach pits increased over time, as the rate of material being added to the pit was greater than the rate of biodegradation.

Further analysis shows that the filling rate for PF toilets (0.11 m^3/y) is at least two orders of magnitude lower than in theoretically closed systems. This indicates the liquid fraction of the faecal sludge in the leach pit is percolating into the surrounding soil. The filling rate of the PF toilet is approximately half an order of magnitude less than that of a theoretically open system. This difference could be caused by user diet, as the values assumed for calculating the theoretical open system are based on the Swedish diet and hence the South African diet might result in less excreta being produced (Jönsson et al., 2004). The difference may also be a result of the faecal sludge undergoing degradation by the microorganisms present in the leach pit, which is not accounted for in the theoretical open system. A comparison of FS accumulation for different sanitation technologies (Figure 1) shows that the PF toilet has the slowest filling rate, less than half of the other technologies. This is most likely because minimal non-faecal material was observed in the leach pits of PF toilets. This has a significant effect in reducing the filling rate because household waste can contribute up to 25% of simple pit latrines and ventilated improved pit latrines (VIP) contents (Wood, 2013).

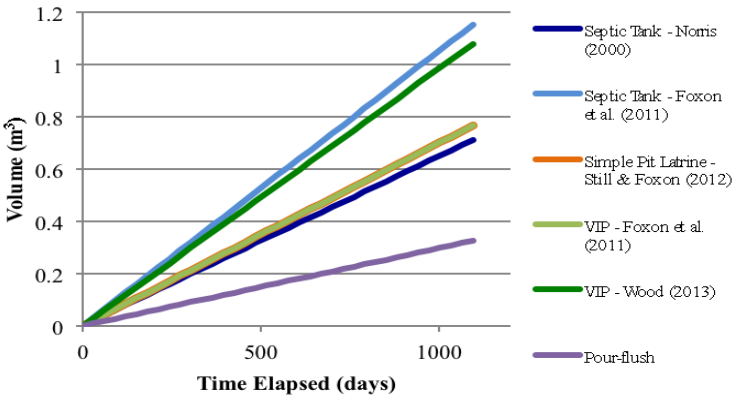

Figure 1. Estimated FS accumulation for different sanitation technologies

Overall comparison of PF toilets to other sanitation technologies

Wood (2013), Zuma et al. (2013) and Bakare et al. (2012) provided data for VIPs, Irish et al. (2013) contained data for pit latrines and Nwaneri (2009) contained data for fresh faeces. Velkushanova (2014) provided data for multiple sanitation technologies covering pit latrines, dry VIP, wet VIP, urine diversion toilets, community ablution block (CAB) solid, CAB liquid, school toilet, unimproved pit and fresh faeces. Finally, data for septage was sourced from Koottatep et al. (2002).

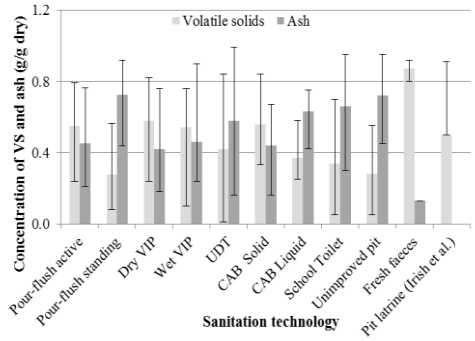

Figure 2. VS and ash comparison

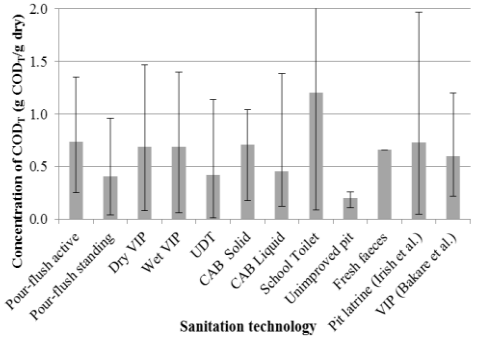

Figure 3.Total COD comparison

In terms of ash, VS, COD_T and TKN (Figure 2), the active PF sludge has similar concentrations to FS from wet VIPs, dry VIPs and the solids from community ablution blocks (CAB). This indicates that the sludge in the PF leach pit is undergoing similar processes to those in the VIP and CAB pits. The PF leach pits however have a higher MC suggesting that they could be emptied mechanically more easily. The absence of non-faecal material in the PF leach pits would make mechanical emptying easier again by avoiding blockages in the emptying equipment. The standing leach pits have similar concentrations to unimproved pits in terms of MC, ash, VS, COD and TKN (Figure 3). Unimproved leach pits are unlined and usually old. The PF leach pits are lined with open face block work. It is likely that the similar concentrations in the two pits are a result of both technologies allowing liquids to easily leach from the pits and the age of the contents. The MC of the standing PF leach pits is the second lowest of all the sanitation technologies, after unimproved pits. It is unlikely that the sludge could be emptied mechanically. However the PF toilet is designed such that the standing leach pit can be safely emptied manually due to the inactivation of pathogens by the time the active leach pit is full (Still and Louton, 2012). Overall, the density of the PF sludge is more consistent then the sludge from the other technologies, most likely due to the negligible presence of non-faecal material.

Biodegradation studies

The total COD decrease over time for PF sludge was similar at three dilution factors, showing an 85% decrease in COD_T over 27 days (Figure 4). The CSTR experiment showed 85% of the gas production after 48 days, and 70% gas production after 27 days (Figure 5). This suggests that the initial rapid biodegradation is aerobic, followed by slower anaerobic degradation.

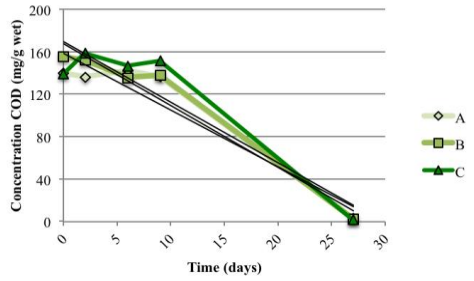

Figure 4. COD decrease over time of PF sludge

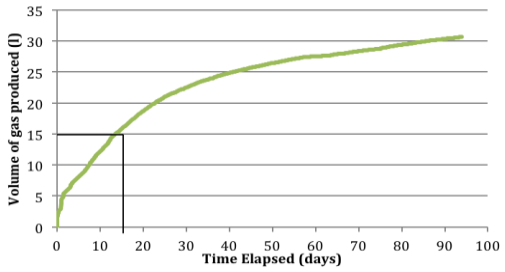

Figure 5.Total gas production over time in CSTR biodegradation test

Molecular microbial analysis

Four sites were sampled for DNA sequencing analysis. Sites 1 and 2 have an active pit and a standing pit; Sites 3 and 4 have only a single pit. Front (where the pipe entered) and back samples were collected. Twelve samples were collected and analysed separately: PF1 from Site 1, Active-Front; PF2 from Site 1, Active-back; PF3 from Site 1, Standing-Front; PF4 from Site 1, Standing-Back; PF5 from Site 2, Active-Front; PF6 from Site 2, Active-Back; PF7 from Site 2, Standing-Front; PF8 from Site 2, Standing-Back; PF9 from Site 3, Single-Front; PF10 from Site 3, Single-Back; PF11 from Site 4, Single-Front; and PF 12 from Site 4, Single-Back.

Overall microbial community differences

Canonical correspondence analysis (CCA) (Figures 6 and 7) shows the differences in the overall microbial communities in 2-dimensional space, and the correlations of various physico-chemical parameters on these communities. Whether the sample is collected near the front (closest to the input pipe) or the back of the pit does not appear to make an appreciable difference. However, there is a clear grouping of the microbial communities, depending on the pit status (active, single, standing), showing that shifts in microbial populations are significant in active vs. standing pits, and that the community is different in single pits. Interestingly, the communities in single pits appear to be "in between" active and standing pits. Clearly, as biodegradation occurs in standing pits, the microbial community shifts to populations that are presumably active in degradation. Active pits are likely to be influenced more by the incoming material. The difference between standing pits and other samples appear to be correlated to potassium and nitrate levels. Potassium was found to be lower in standing pits than in active pits since no urine is added in standing pits. Nitrate was also low in PF pits, but lower in standing pits than in active pits.

Specific microbial populations

The dominant Archaeal and Bacterial species (greater than 5% of the sequences) are shown in Figure 8. Several insights can be gleaned from these bar diagrams. First, the same major populations appear in all PF pits, and standing pits (e.g., PF3, PF4) are different in levels of species compared to active pits (PF1, PF2). Second, there are varying communities at the different sites (PF5, PF6 vs. PF1, PF2) and the community changes differently at the different sites (PF7, PF8 vs. PF3, PF4).

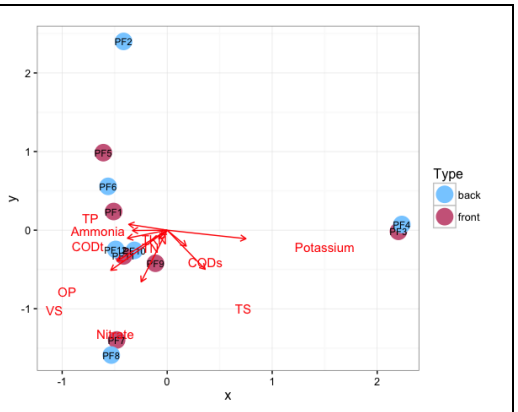

Figure 6. CCA plot showing differences in microbial communities in the front and back of PF pits

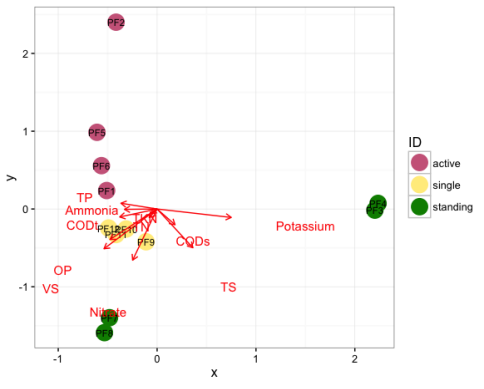

Figure 7. CCA plot showing differences in microbial communities in active, standing, and single PF pits

The main populations in PF pits are: (1) Bacteroidales, which have been identified as the main potential metabolizers of carbohydrate-based substrates in various types of microbiomes; (2) *Porphyromonodaceae*, which are obligatory anaerobic, asaccharolytic (growth not significantly affected by carbohydrates but is enhanced by protein hydrolysates), and produce fermentation products mainly n-butyric acid and acetic acid; (3) *Paludibacter*, another strictly anaerobic glucose fermenter that produces propionate and acetate; and (4) *Ruminococcaceae*, known polysaccharide degraders. *Chitinophagaceae* (a chitin-degrader) appears in standing pits, indicating increased degradation. Other populations are pathogenic, such as *Arcobacter*, which causes enteritis and septicemia, and *Treponema* (found in Site 2) a causative agent of syphilis.

Sulphur transformation is also indicated by the presence of *Sulfurimonas*, obligate chemolithoautotrophic bacteria that use sulfide, thiosulfate and elemental sulphur as electron donors, and CO_2 as a carbon source, and reduce nitrate. Methanogenesis is primarily through H_2/CO_2, as evidenced by the archaeal dominance of *Methanocorpusculum*, a member of the Methanomicrobiales.

Summary

- PF toilets have low filling rates due to lower trash input, and occurrence of aerobic and anaerobic degradation.
- Chemical characteristics of PF leach pit contents were compared to other onsite sanitation technologies.
- Biodegradation tests show 85% COD reduction over 27 days, and provide further evidence for both aerobic and anaerobic degradation.
- The microbial communities varied among active, standing, and single pits, and shifts in populations can be discerned using DNA sequencing.
- Aerobic and anaerobic degraders were detected and quantified. Methane production in PF pits is primarily through the H2/CO2 pathway.

Acknowledgements

The authors would like to extend thanks to Partners In Development (Pty) Ltd for granting access to the pour-flush pilot scheme. The Water Research Commission of South Africa funded the project.

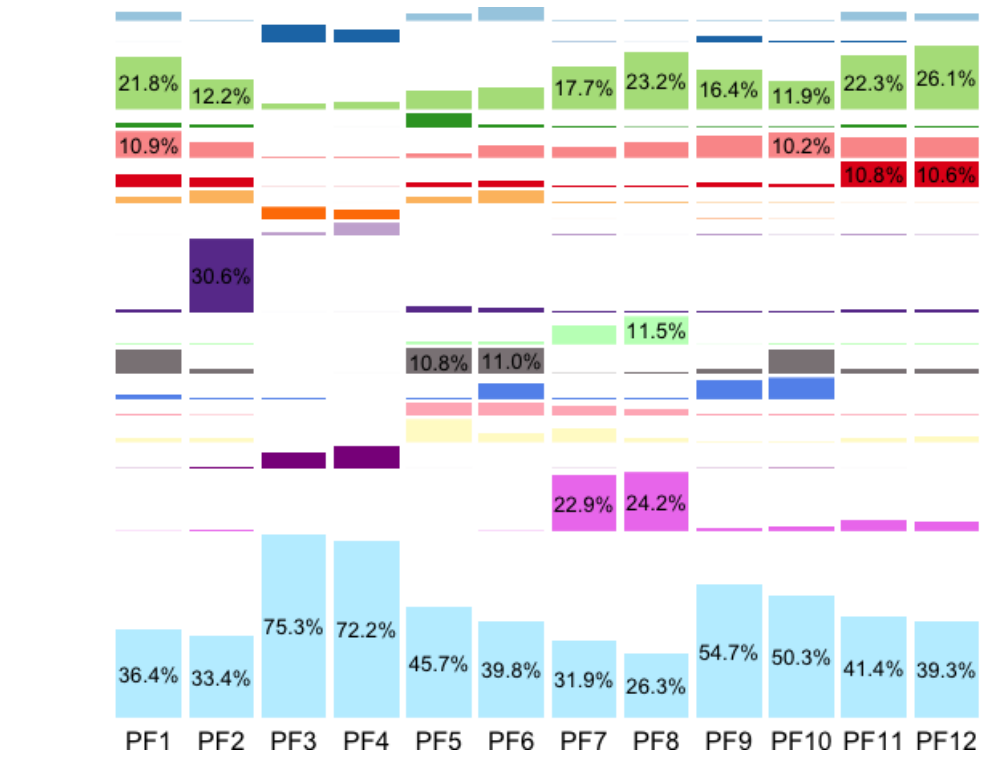

Figure 8. Microbial populations in PF latrine samples

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