Journal of
Water, Sanitation & Hygiene for Development

 Journal of Water, Sanitation and Hygiene for Development Vol 14 No 3, 277 doi: 10.2166/washdev.2024.319

Research Paper

Relation of organic fractions in fresh and stored fecal sludge and foodwaste to biogas production

Nida Maqbool[a,b], Stanley Sam[a], Sher Jamal Khan[b] and Linda Strande[a,*]

[a] Sandec: Department of Water, Sanitation and Solid Waste for Development, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse, 133, CH-8600 Dübendorf, Switzerland
[b] Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST), Islamabad, Pakistan
*Corresponding author. E-mail: linda.strande@eawag.ch

LS, 0000-0003-4477-6268

ABSTRACT

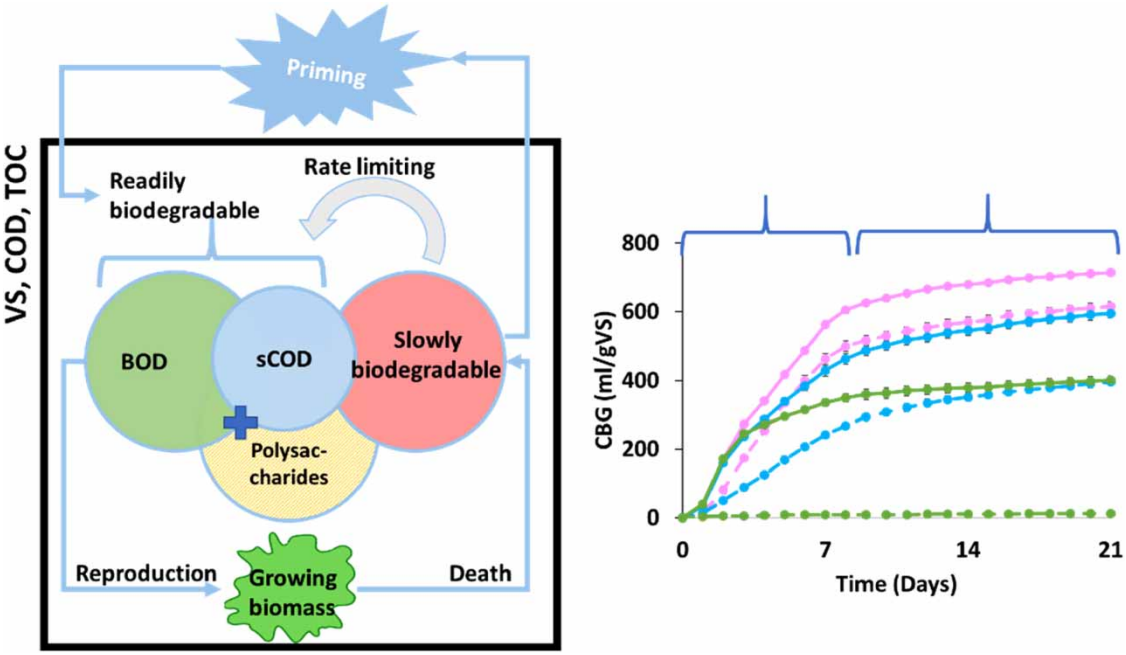
There is limited understanding of the potential for anaerobic digestion and biogas production from fecal sludge. In this study, biomethane potential (BMP) tests from fresh, stored, and dewatered fecal sludge, together with co-digestion with fresh foodwaste, revealed that fresh fecal sludge produced similar cumulative biogas (CBG) to fresh foodwaste (615–627 mL/gVS), while stored fecal sludge showed a wide range of gas production (13–449 mL/gVS). Co-digestion significantly enhanced the CBG production of fresh (1.2×), dewatered (1.5×), and stored (29–36×) fecal sludge. In BMP tests with the higher range of gas production, a biphasic CBG production was observed, with degradation of readily biodegradable organics occurring during the first week. The first-order rate coefficients indicated hydrolysis limitation, which was also confirmed by the presence of slow-growing methanogens (Halobacterota). Priming with co-digestion significantly enhanced CBG from stored fecal sludge. The physical–chemical metrics VS/TS and TOC/TN were not predictors of biogas production, while BOD/COD and sCOD were better indicators, suggesting that metrics of stabilization representing biologically available fractions are more representative than metrics of entire pools of organic matter. This study suggests that biogas production from anaerobic digestion is viable for fresh fecal sludge, whereas for stored fecal sludge it requires co-treatment or pretreatment.

Key words: anaerobic digestion, co-digestion, dewaterability, hydrolysis, microbial community, stabilization

HIGHLIGHTS

- Fecal sludge is not as degradable in anaerobic conditions as wastewater sludges.
- Physical–chemical metrics (i.e. VS/TS, TOC/TN) are not reliable predictors of biogas.
- There is an initial improvement in dewatering following 1 week of digestion.
- Anaerobic digestion is recommended for fecal sludge that has been stored for less than 1 week.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The sanitation needs of one-third of the world's population are met through non-sewered sanitation (NSS) (WHO 2018), and NSS provides the majority of sanitation in low- and middle-income countries. However, NSS, in general, lacks adequate management; for example, in South Asia, less than 6% of fecal sludge undergoes any form of treatment, and its improper disposal poses a significant risk to public health and safety (Maqbool *et al.* 2022). Fecal sludge is defined as what accumulates during storage in onsite containments with NSS. A range of constructions are utilized for onsite containment, including unlined, partially lined, fully lined, and with or without overflows, and are commonly referred to as pit latrines, septic tanks, or cesspits (Gold *et al.* 2018). Fecal sludge consists of everything going into the containment, including excreta, flush water, cleansing material, gray water, and solid waste (Ahmed *et al.* 2018). The characteristics of fecal sludge arriving at treatment facilities are highly variable with up to two orders of magnitude higher organic strength (as chemical oxygen demand (COD) or volatile solids (VS)) as compared to municipal sewer-based wastewater. This is due to the wide range of inputs, management practices, and batch-wise delivery to treatment (Strande *et al.* 2018). The water content in fecal sludge is more than 70–80%, which is linked to challenges in transport, effective dewatering, and subsequent treatment (Gold *et al.* 2018). The difficulty in transporting fecal sludge through congested urban areas to treatment plants, together with highly variable characteristics, makes decentralized treatment plants an attractive option (Semiyaga *et al.* 2022). However, currently established technologies for the treatment of fecal sludge rely on passive technologies (e.g. settling-thickening tanks, or drying beds) and have large footprints (Tayler 2018; WHO 2018), making them unsuitable for densely populated urban areas. There is an urgent need for tenable solutions with low footprints for dense urban areas.

Anaerobic digestion is an extensively applied treatment process for the stabilization of wastewater sludge with the added benefit of biogas production (Tchobanoglus *et al.* 2014); however, to date, there is a lack of controlled experimental data on fecal sludge. Part of the problem is that it is not understood how much degradation fecal sludge undergoes during storage in containment. Existing studies report a range of biogas and biomethane production, including no biogas production from pit latrine sludge (PLC) (Madikizela *et al.* 2017), 32 mL of biomethane from pit latrine sludge (Cui *et al.* 2023), and 57–811 mL biogas/gVS from fresh fecal sludge (Sam *et al.* 2022). Based on the literature on anaerobic digestion of wastewater sludge, biogas production, and stabilization of fecal sludge have been investigated in relation to COD (Colón *et al.* 2015; Le Phuong & Thai 2018; van Eekert *et al.* 2019) and VS (Kilucha *et al.* 2022). However, based on the low and unpredictable performance of anaerobic digestion, there is a need for further understanding of the level of degradation of fecal sludge

during storage, and the fractions of bioavailable organic matter remaining for treatment (Levira *et al.* 2023). Due to the lack of controlled experimental results, biomethane potential (BMP) tests are needed to understand the biogas potential and anaerobic biodegradability of fecal sludge (Filer *et al.* 2019). In addition, there is limited knowledge of microbial communities present in fecal sludge, and the role they play is not clear (Sam *et al.* 2022; Ward *et al.* 2023).

The objective of this study was to evaluate the kinetics of anaerobic degradation with BMP tests for a range of fecal sludge, in order to gain an understanding of whether different organic fractions reflecting different levels of stabilization can predict biogas potential. The experimental setup included mono-digestion of fresh, stored, and dewatered fecal sludge, and co-digestion with fresh food waste. Dewatering performance and particle size distribution were evaluated during the BMP tests, and microbial communities were evaluated. The analysis included volatile solids to total solids (VS/TS), total organic carbon to total nitrogen (TOC/TN), BOD/COD as metrics of stabilization, and hydrolysis rate coefficients for COD (K_{COD}) soluble COD (K_{sCOD}), polysaccharides (K_{PS}), and degradability extent (f_d).

2. MATERIALS AND METHODS

2.1. Inoculum, feed, and co-feed

Eight fecal sludge samples were collected during January 2022 from household onsite containments located in Islamabad, Pakistan, four from pit latrines and four from septic tanks. The collected four pit latrine sludges (PL_1, PL_2, PL_3, and PL_4) were mixed in equal proportions to produce a composite pit latrine sludge (PL_C). Other than PL_C, pit latrine sludge 1 (PL_1), pit latrine sludge 2 (PL_2), and septic tank sludge 1 (ST_1) were used. The selection of fecal sludge samples for BMP tests was based on source (residential and commercial), time in containment (<1, <10, and >10 years), and TS concentration (<10, 10–50, and >50 g/L). One sample was collected from a restaurant septic tank sludge (ST_R) in Jalpaiguri, India in January 2022. One sample was collected from a household septic tank in Switzerland in April 2022, was dewatered as described in Shaw *et al.* (2022) and referred to as dewatered septic tank sludge (ST_D). The grab composite sampling method was used to collect samples (Velkushanova *et al.* 2021). Fresh fecal sludge (FS_F) was prepared by mixing feces and urine (1:2.5) collected in Eawag, Switzerland in May 2022 and diluted to 5% as described in Sam *et al.* (2022). In this study, fresh was defined as what would be coming directly from a toilet and not stored in containment. PL_1, PL_2, PL_C, ST_1, ST_D, ST_R, and FS_F were used as feed during BMP tests. All fecal sludge samples were stored at 4 °C and shipped (excluding FS_F, which was obtained from Eawag) to Eawag for characterization and subsequent use in BMP experiments. Inoculum was collected from an anaerobic digester at the Neugut wastewater treatment plant in Zurich, Switzerland. Readily available organic content in waste from green markets and restaurants makes it an ideal co-substrate for decentralized anaerobic digestion. Synthetic green market waste (GW_S) and synthetic restaurant waste (RW_S) were prepared according to Song *et al.* (2020) and Carmona-Cabello *et al.* (2020), respectively, and their recipes are included in the supplemental information (Tables S1 and S2). Restaurant food waste (RW_R) was collected from the restaurant at Eawag and comprised rice, pasta, vegetables, and meat. The RW_R was manually shredded and blended with a Phillips Avance Collection Blender (HR2096, The Netherlands). GW_S, RW_S, and RW_R were used as co-feed during BMP. Table 1 presents the characterization of feed, co-feed, and inoculum used in the study.

2.2. BMP test experimental setup

BMP tests were carried out in 250 mL bottles with 70% working volume. Three sets of BMP tests were conducted: (i) mono-digestion of fresh (FS_F), stored (PL_1, PL_2, PL_C, ST_1, ST_R), and dewatered fecal sludge (ST_D), (ii) co-digestion of fresh (FS_F), dewatered (ST_D), and stored (PL_C) with RW_R individually and stored (PL_C) fecal sludge with GW_S and RW_S, respectively; and (iii) effect of micronutrients addition on mono-digestion (in 1× and 2× concentrations represented as $PL_{C\text{-}MN1}$ and $PL_{C\text{-}MN2}$, respectively) and co-digestion of fecal sludge ($PL_{C\text{-}MN1+GWS}$ and $PL_{C\text{-}MN1+RWS}$). The inoculum-to-feed (I/F) ratio was maintained at two for all the experiments. BMP tests for positive and negative controls were run to ensure the reliability and accuracy of the results obtained from experimental samples. The positive control was inoculum and microcrystalline cellulose (control substrate) and the negative control was inoculum only with no substrate added. BMP bottles were purged with N_2 gas to maintain the anaerobic environment and placed at 37 °C and 100 rpm in a 5,000 L orbital shaking incubator (VWR, Avantor, USA). Triplicates of BMP bottles were used, and two sets of extra sacrificial bottles were used for weekly analysis during the BMP tests. Micronutrients (NH_4Cl, 1,000 mg/L; NaCl, 100 mg/L; $MgCl_2{\cdot}6H_2O$, 100 mg/L; $CaCl_2{\cdot}2H_2O$, 50 mg/L; $K_2HPO_4{\cdot}3H_2O$, 400 mg/L; $FeCl_2{\cdot}4H_2O$, 2 mg/L; H_3BO_3, 0.05 mg/L; $CuCl_2{\cdot}2H_2O$, 0.038 mg/L; $ZnCl_2$, 0.05 mg/L; $MnCl_2{\cdot}4H_2O$, 0.05 mg/L; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.05 mg/L; $AlCl_3$, 0.05 mg/L; $CoCl_2{\cdot}6H_2O$, 0.05 mg/L; $NiCl_2{\cdot}6H_2O$,

Table 1 | Characterization of feeds pit latrine sludges (PL_1, PL_2), composite pit latrine sludge (PL_C), septic tank sludge (ST_1), restaurant septic tank sludge (ST_R), fresh fecal sludge (FS_F), dewatered septic tank sludge (ST_D), restaurant food waste (RW_R), synthetic green market waste (GW_S), synthetic restaurant food waste (RW_S), and anaerobic digester inoculum

Sample	Time since the last emptying	TS (g/L)	VS (g/L)	VS/TS	NH_4^+ – N (mg/L)	sCOD (g/L)	COD (g/L)
PL_1	7 years	4.53 ± 0.06	1.71 ± 0.07	0.38	547 ± 5.77	0.78 ± 0.009	79.67 ± 5.42
PL_2	14 years	5.81 ± 0.08	2.43 ± 0.13	0.42	295 ± 5.13	0.75 ± 0.002	79.95 ± 6.58
PL_C	–	19.07 ± 0.23	8.57 ± 0.06	0.45	430 ± 5	0.33 ± 0.001	14.78 ± 0.38
ST_1	1 year	0.99 ± 0.11	0.37 ± 0.18	0.38	105 ± 2.89	0.71 ± 0.027	5.78 ± 0.23
ST_R	3 months	54.45 ± 4.61	40.06 ± 2.25	0.74	170 ± 2.08	5.66 ± 0.045	66.77 ± 0.76
FS_F	0 years	64.98 ± 0.29	55.15 ± 0.25	0.85	2,130 ± 42	28.85 ± 0.3	118.75 ± 8.13
ST_D	2 years	139.31 ± 0.56	111.34 ± 0.08	0.80	164 ± 3	5.50 ± 0.022	252.75 ± 3.18
RW_R	0 years	336.93 ± 8.80	323.19 ± 8.6	0.96	61 ± 1	72.50 ± 3.13	513.00 ± 23.81
GW_S	–	0.98 ± 0.002	0.97 ± 0.05	0.96	–	–	–
RW_S	–	0.99 ± 0.005	0.98 ± 0.03	0.98	–	–	–
Inoculum	–	21.94 ± 0.87	14.19 ± 0.41	0.65	938 ± 31.82	0.68 ± 0.01	18.43 ± 0.70

0.092 mg/L, ethylenediaminetetraacetate, 0.5 mg/L; $Na_2SeO_3{\cdot}5H_2O$, 0.1 mg/L; HCl conc. 0.001 mL/L) were added (Angelidaki *et al.* 2009) to determine the effect of their addition on anaerobic digestion and biogas production. Biogas was measured daily with the water displacement method (Filer *et al.* 2019), and gas volume produced by negative control was subtracted and normalized to VS (g/L) in the BMP bottle. The BMP test was stopped when the daily biogas volume reduced to <1% of the cumulative biogas (CBG) for 3 consecutive days (Filer *et al.* 2019). CBG produced from the negative control and positive control was 28.67 ± 1 and 892 ± 17 mL/gVS, respectively.

2.3. Analytical methods

pH and electrical conductivity (EC) were determined using a multi-parameter portable meter (WTW ProfiLine pH/Cond 3320, Germany). Total solids (TS) were determined volumetrically by drying the sample at 105 °C and VS by combusting the residue obtained from the TS at 550 °C until a constant weight was achieved (Velkushanova *et al.* 2021). COD, soluble chemical oxygen demand (sCOD), TN, and ammonium nitrogen (NH_4^+–N) were determined using Hach Lange test kits according to the manufacturer's instructions based on standard methods in American Public Health Association (APHA) standard methods 5220 D, 4500-N C, and 4500-NH3 F, respectively (APHA 2017). The 5-day biological oxygen demand (BOD_5) was determined using APHA standard 5210–B at 20 °C (APHA 2017). Alkalinity was determined by titration and volatile fatty acids (VFA) were analyzed using an ion chromatograph (Shimadzu 881 compact IC pro, Japan). TOC was analyzed using a TOC analyzer (TOC-LCPH Shimadzu, Japan). Polysaccharide concentrations were determined using the colorimetric method with the anthrone assay method as described by Loewus (1952). pH, EC, VFA, alkalinity, TN, and NH_4^+–N were used to evaluate process inhibition. TS was used as an indicator of total organic and inorganic matter, while VS, COD, polysaccharides, and TOC for pools of organic matter, BOD_5 for biologically degradable organic matter and sCOD for soluble organic matter only. pH, TS, VS, COD, sCOD, NH_4^+–N, and polysaccharides were measured weekly and TN, BOD_5, and TOC were measured at the start and end of experiments.

To evaluate the dewatering performance of fecal sludge, supernatant turbidity following centrifugation and capillary suction time (CST) were monitored on a weekly basis. Samples were centrifuged at 3,300 × *g* for 20 min and decanted and supernatant turbidity was quantified with a turbidity meter (Hach TL 2300, USA) (Ward *et al.* 2021). CST was measured in quadruplicate with a CST apparatus (Triton 319, Canada) as described in Velkushanova *et al.* (2021). Particle size distribution was analyzed with the laser light scattering method with a laser diffraction particle analyzer (Beckman Coulter LS 13 320, USA) using universal liquid module (ulm) to determine how particle size changes during anaerobic digestion. In this study, the common stabilization indicators VS/TS, TOC/TN, and BOD/COD were used to evaluate stabilization. Relative decreases in VS and BOD indicate that degradation is occurring, and TOC/TN indicates nitrogen availability for the growth of microbes with an ideal range of 20–30 to utilize carbon for energy and nitrogen for cellular structure (Tayler 2018).

2.4. Hydrolysis rate coefficient (K) and degradability extent (f_d)

The hydrolysis rate coefficients for COD, sCOD, and polysaccharides were calculated with a first-order model (Abubakar *et al.* 2017):

$$X_t = X_i e^{-kt} \tag{1}$$

where X_i represents the initial concentration (mg/L) for COD, sCOD, and polysaccharides, X_t represents the concentration (mg/L) at time t, and k (day^{-1}) represents the hydrolysis rate coefficient. The degradability extent (f_D) was calculated as conversion of substrate into methane (Jensen *et al.* 2011) and was calculated by:

$$f_D = \frac{CBG * \%CH_4 * F}{COD_i} \tag{2}$$

where CBG is cumulative biogas (mL) produced, F is the fraction of feed converted to methane, and COD_i is initial COD (g). To estimate the anaerobic biodegradability of organic matter, it is important to consider that even if the organic matter is 100% biodegradable, only 90% will be converted into methane with the remaining 10% being utilized for microbial biomass production, thus, the fraction F was assumed as 90% and methane content was assumed as 70% in the biogas (Filer *et al.* 2019).

2.5. Microbial community investigation

To evaluate the microbial community in BMP tests, samples were taken from selected BMP bottles (FS_F, PL_1, PL_2, $PL_{C\text{-}MN1}$, ST_D, and ST_R) at time 0 days and anaerobic digester sludge inoculum. 2 mL of sample was centrifuged at 6,000 × g for 10 min. The supernatant was discarded and 1 mL of RNAlater was added to each sample. The samples were stored at −20 °C until DNA extraction. DNA extraction was performed according to the modified method by Sam *et al.* (2022). 16S rRNA gene amplicon sequencing was carried out by Novogene as described in Sam *et al.* (2022). The plots of dominant microorganisms at the phylum and genus level were plotted for each sample.

3. RESULTS

3.1. CBG production from BMP tests

All characterization results for BMP tests at time 0 and time 21 are presented in Table S3 (Supplemental Information). CBG production of fecal sludge samples in mono-digestion BMP tests is reported in Figure 1(a). The results were variable, ranging from 13 ± 5 mL/gVS for a composite pit latrine sample (PL_C) to 615 ± 13 mL/gVS with a fresh (FS_F) that had not been stored in containment. In comparison, 50.6 ± 19.4 mL/gVS has been reported for pit latrine sludge that had not been emptied from 2 to 10 years, and 276 ± 151 mL/gVS with fecal sludge stored in portable toilets for 4 days (Rose 2015), 493 ± 37 mL/gVS for fresh fecal sludge (not stored) with pit latrine sludge inoculum, and 811 ± 4 mL/gVS for fresh fecal sludge with anaerobic digester sludge inoculum (Sam *et al.* 2022). Presented in Figure 1(b) are mono-digestion results for fresh (not stored) restaurant waste (RW_R), which had a similar biogas production of 627 ± 20 mL/gVS to that of FS_F. In comparison, a similar CBG of 619 mL/gVS has previously been reported for food waste (Kim *et al.* 2019). Presented in Figure 1(c) are the CBG results from the co-digestion of fecal sludge and food waste. Co-digestion resulted in higher levels of CBG for all the fecal sludge. For example, co-digestion with the fresh wastes ($FS_F + RW_R$) resulted in a CBG of 715 ± 13 mL/gVS, and CBG from ST_D increased from 396 ± 11 to 595 ± 4 mL/gVS, and PL_C increased from 13 ± 5 mL/gVS to 466 ± 33, 401 ± 15, and 383 ± 11 mL/gVS, respectively, with co-digestion of RW_S, RW_R, and GW_S. As PL_C produced the lowest CBG (Figure 1(c)), micronutrients were added to evaluate if they were limiting the mono-digestion and co-digestion of PL_C (Figure 1(d)). However, aliquots of micronutrients did not significantly increase the CBG, indicating that micronutrients were not limiting.

The observed differences in CBG in this study resulted from feeds and not the inoculum, as the same inoculum and I/F were used throughout the study. Based on the results of pH, EC, alkalinity, VFA, and NH_4^+–N (Table S3), no inhibition was observed. The pH was always in the acceptable range of 7.04–8.08. The alkalinity was adequate and between 1,500 and 3,500 mg/L for all BMP tests other than ST_1. The VFA to alkalinity ratio was always <0.4, including for ST_1, which indicates stable BMP performance (Filer *et al.* 2019). The VFAs were never high enough to be inhibitory and were even as low as zero, which indicates their production and consumption at the same time. Low VFA production with rapid by methanogens is

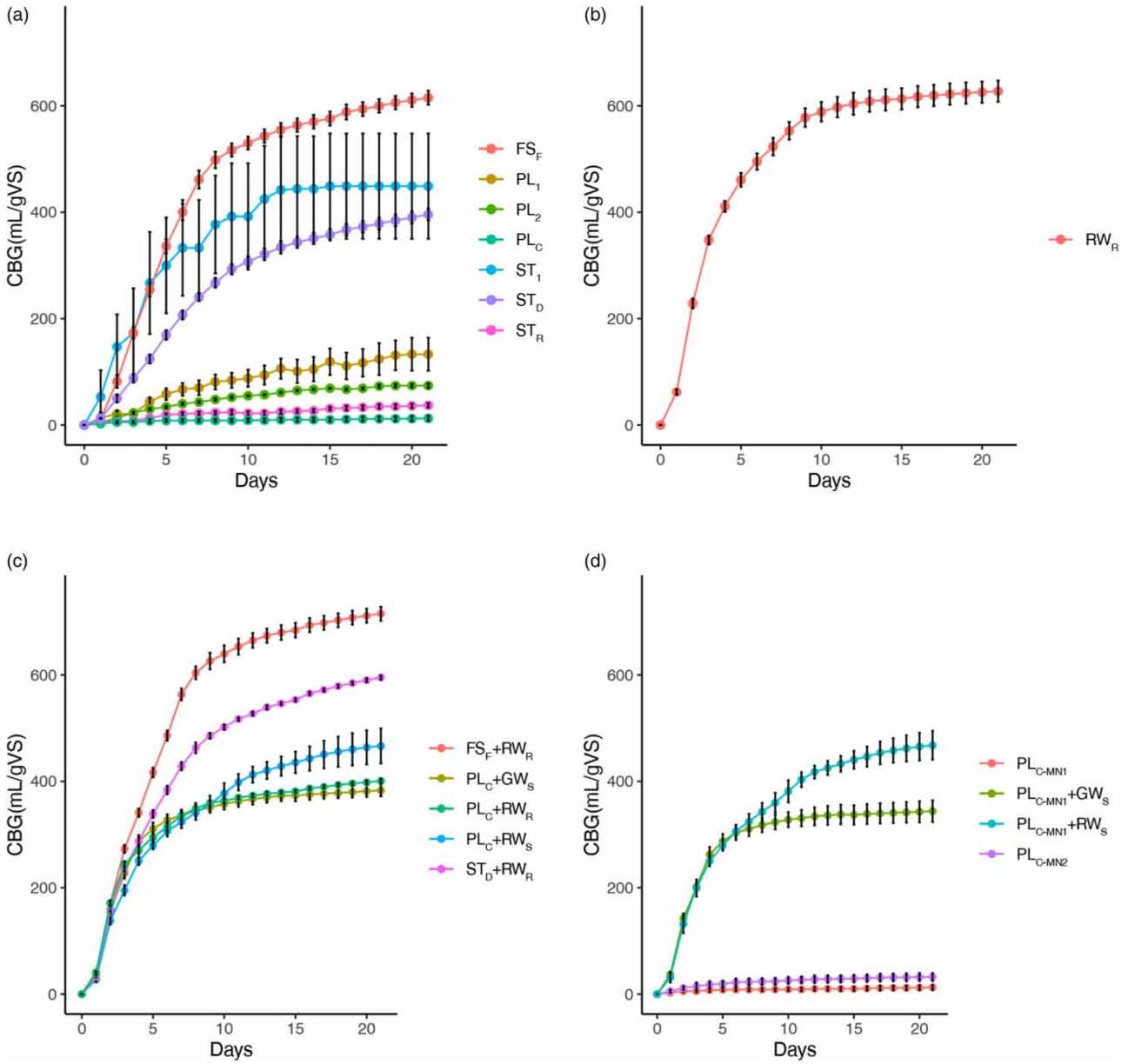


Figure 1 | Cumulative biogas production (CBG) from: (a) mono-digestion of fecal sludge (PL_1, PL_2, PL_C, ST_1, ST_R, ST_D, FS_F); (b) mono-digestion of foodwaste (RW_R); (c) co-digestion of fecal sludge and foodwaste ($FS_F + RW_R$, $PL_C + GW_S$, $PL_C + RW_R$, $PL_C + RW_S$, $ST_D + RW_R$); and (d) mono-digestion of fecal sludge with the addition of micronutrients (single aliquot $PL_{C\text{-}MN1}$, two aliquots $PL_{C\text{-}MN2}$) and co-digestion with addition of micronutrients ($PL_{C\text{-}MN1} + GW_S$, $PL_{C\text{-}MN1} + RW_S$).

also observed during anaerobic digestion of blackwater due to a low hydrolysis rate (Elmitwalli *et al.* 2011). Overall, there was an increase in NH_4^+–N, EC, and alkalinity from time 0 to time 21. As organic nitrogen in proteins and urea are broken down, NH_4^+–N is released, which subsequently increases alkalinity (Adou *et al.* 2020). However, the concentration of NH_4^+–N was never high enough to cause inhibition (Colón *et al.* 2015).

3.2. Metrics of stabilization

Metrics of stabilization for BMP tests at time 0 are reported in Table 2. The VS/TS in BMP tests at time 0 with an I/F ratio of two was relatively consistent (VS/TS of feeds are presented in Table 1). It was higher than 0.49 reported for fecal sludge alone (Andriessen *et al.* 2023), and lower than primary wastewater sludge (0.60–0.80), or waste-activated sludge (0.59–0.88)

Table 2 | Metrics of stabilization (VS/TS, TOC/TN, BOD/COD) for BMP tests time 0, hydrolysis rate coefficients for COD (K_{COD}), sCOD (K_{sCOD}) PS (K_{PS}), and degradability extent (f_d) during mono- and co-digestion of fecal sludge

Substrate	VS/TS	TOC/TN	BOD/COD	K_{COD}	R^2	K_{sCOD}	R^2	K_{PS}	R^2	f_d
	time 0	time 0	time 0	d^{-1}		d^{-1}		d^{-1}		$(\frac{L_{CH4}}{g_{COD}})$
Section 1: Mono-digestion fecal sludge following storage in containment										
ST_1	0.64	0.83	0.06	0.013	0.62	0.077	0.86	NA	-	0.017
PL_C	0.63	1.42	0.05	0.002	0.23	0.004	0.59	0.014	0.80	0.002
$PL_{C\text{-}MN1}$	0.63	1.23	0.05	0.001	0.23	0.004	0.59	0.014	0.79	0.002
$PL_{C\text{-}MN2}$	0.55	1.39	0.05	0.013	0.93	0.000	0.08	0.018	0.55	0.007
PL_1	0.49	1.35	0.03	0.008	0.53	0.016	0.78	NA	-	0.013
ST_R	0.68	2.49	0.04	-0.002	0.05	0.027	0.85	NA	-	0.006
PL_2	0.62	1.82	0.05	-0.014	0.69	0.013	0.75	NA	-	0.016
ST_D	0.67	2.31	0.07	0.006	0.45	0.000	0.07	0.006	0.69	0.050
Section 2: Mono-digestion of fresh fecal sludge and fresh food waste										
FS_F	0.68	1.58	0.09	0.013	0.82	0.045	0.73	0.010	0.69	0.082
RW_R	0.67	1.10	0.10	0.007	0.97	0.047	0.73	0.008	0.84	0.112
Section 3: Co-digestion of fresh and stored fecal sludge with fresh food waste										
$PL_{C\text{-}MN1}$+GW_S	0.65	1.82	0.09	0.005	0.36	0.058	0.81	0.045	0.87	0.059
PL_C+RW_R	0.59	1.76	0.07	0.005	0.29	0.009	0.15	0.010	0.71	0.052
PL_C+GW_S	0.66	2.00	0.07	0.002	0.06	0.050	0.73	0.046	0.88	0.063
PL_C+RW_S	0.64	2.13	0.10	0.004	0.29	0.047	0.75	0.042	0.93	0.075
$PL_{C\text{-}MN1}$+RW_S	0.63	2.08	0.11	-0.001	0.01	0.052	0.75	0.038	0.95	0.084
ST_D+RW_R	0.67	1.80	0.06	0.020	0.80	0.035	0.71	0.007	0.76	0.067
FS_F+RW_R	0.68	1.98	0.10	0.012	0.98	0.057	0.75	0.011	0.68	0.103

The color scale from darker to lighter shading illustrates higher to lower values.

(Tchobanoglus *et al.* 2014; Odirile *et al.* 2021). The observed TOC/TN values of the BMP tests at time 0 were consistent with literature values for fecal sludge of 1.5–6 (Meher *et al.* 1994; Manga *et al.* 2022), and lower than primary (13.6) and waste-activated sludge (9.2) (Sakaveli *et al.* 2021). The fecal sludge that had been stored had relatively lower BOD/COD values (0.03–0.07) as compared to the fresh waste streams FS_F and RW_R (0.09–0.10). A wide range of BOD/COD values have been reported for fecal sludge, including 0.18–0.62 in Argentina, 0.08–0.44 in Ghana, 0.35 in Palestine, 0.16 in Burkina Faso, and 0.10 in the Philippines (Tayler 2018). A BOD/COD of 0.5 is typical for domestic wastewater, with 0.31 reported for primary sludge and 0.11 for waste-activated sludge (Metcalf *et al.* 2014).

3.3. Hydrolysis and degradation coefficients

The first-order hydrolysis rate coefficients were calculated for COD (K_{COD}), sCOD (K_{sCOD}), and polysaccharides (K_{PS}) with Equation (1) and are shown in Table 2. K_{sCOD} had the highest values, followed by K_{PS} and K_{COD}. All the observed rate coefficients reported in Table 2 were toward the lower end of the values reported in the literature for wastewater 0.09–0.12 day^{-1} (Elmitwalli *et al.* 2011), wastewater sludge 0.077–0.15 day^{-1} (Pavlostathis & Giraldo-Gomez 1991), cellulose 0.29–0.42 day^{-1} (Jensen *et al.* 2011), and primary 0.13 day^{-1} and waste activated sludge 0.11 day^{-1} (Abubakar *et al.* 2017), indicating that hydrolysis was limiting fecal sludge degradation, and that fecal sludge is less digestible than wastewater sludges. The degradation extent (f_D) increased from stored fecal sludge (0.002–0.050), to fresh fecal sludge (0.082), to co-digestion of stored fecal sludge and food waste (0.052–0.084) and fresh fecal sludge and food waste (0.103) (Table 2). These values were all low in comparison to the theoretical degradation extent (yield) of 0.35 L_{CH4}/g_{COD} (Filer *et al.* 2019), and 0.28 L_{CH4}/g_{COD} that has been reported from a simulant feces (Colón *et al.* 2015).

3.4. Dewatering metrics and particle size distribution

The dewatering metrics over time 0–21 days are presented in Table S3 and Figure S1. After the first week of the BMP test, there was a discernible improvement in CST and turbidity with no significant changes afterward. A range of CST

(1.88–11.68 sL/gTS) was observed at time 0 of the BMP test with a 42–79% reduction over 21 days. All samples had improved dewatering performance during the first 7 days (other than $PL_{C\text{-}MN1}$), whereas from time 7 to time 21, changes in dewatering performance did not follow consistent trends, from no overall change to continued improvement. In addition, there were no significant differences in overall improved dewatering for fresh (43–45%) and stored (32–63%) waste streams upon anaerobic digestion.

At time 0, supernatant turbidity values were 24-656 Nephelometric Turbidity Units (NTU) with a 28–79% reduction over 21 days. Supernatant turbidity also had a clear decrease during the first week, with no significant overall changes from time 7 to time 21. There were clear improvements in turbidity for fresh (49–60%) and stored (32–60%) fecal sludge during anaerobic digestion. In comparison, Sam *et al.* (2023) observed that stored fecal sludge had lower turbidity and CST than fresh fecal sludge (not stored), which could be likely due to partial stabilization during storage in containments. In comparison, anaerobic digestion of activated sludge typically worsens dewaterability (Houghton *et al.* 2000; Christensen *et al.* 2015) and anaerobic digestion of primary wastewater sludge shows inconsistent results.

Particle size distribution (PSD) over time 0–21 days is presented in Figure S2. The peaks were mainly unimodal, and at time 0, the peak of the curve was between 84 and 110 μm, comprising 3.9–5.1% of the sample volume. Based on a comparison of the peaks from time 0 to time 21, no major differences were observed (other than ST_D); therefore, no strong relation was seen between PSD and dewatering metrics. In comparison, Sam *et al.* (2022) reported an increase in supracolloidal particles (1–100 μm) and a decrease in larger particles during anaerobic storage of fecal sludge; however, no relationship between supracolloidal particles and turbidity or CST was observed.

3.5. Microbial community

The taxonomic classifications at the phylum and genus level of samples at time 0 of the anaerobic digestion (AD) inoculum together with FS_F, PL_1, PL_2, $PL_{C\text{-}MN1}$, ST_D, and ST_R are presented in Figure 2. In this study, the most abundant phyla in AD inoculum alone were *Actinobacteriota* 14%, *Halobacterota* 14%, *Bacteroidota* 14%, *Chloroflexi* 13%, *Proteobacteria* 11%, *and Firmicutes* 10%. *Actinobacteriota and Bacteroidota* are prevalent during hydrolysis and degrade cellulose, polysaccharides, and proteins, *Chloroflexi* and *Proteobacteria* during acidogenesis and degrade carbohydrates, *Firmicutes* during acetogenesis for the conversion of VFAs to acetate and hydrogen and *Halobacterota* during methanogenesis. In comparison, the most abundant phyla in the sludge of 98 mesophilic ADs were *Cloacimonadota* (0–14.80%), *Chloroflexi* (0–11.29%), *Firmicutes* (0–9.87%), *Bacteroidota* (0–9.30%), and *Spirochaetota* (0–8.75%) (Dueholm *et al.* 2023). The three BMP tests with PL_1, PL_2, and ST_D were the most similar, with *Proteobacteria* (17 ± 7%), *Bacteroidetes* (14 ± 5%), *Halobacterota* (13 ± 4%), and *Firmicutes* (13 ± 4%), whereas in tests with ST_R and $PLc_{\text{-}MN1}$, *Halobacterota* (30 ± 8%) were most abundant. Sam *et al.* (2022) also reported *Firmicutes* (18–35%), *Proteobacteria* (12–21%), *Bacteroidetes* (6–25%), and *Halobacterota* (3–30%) in fecal sludge from pit latrines and septic tanks. The phylum *Chloroflexi* was most abundant in FS_F (43%), the one FS sample that had not undergone any storage in containment, followed by *Bacteroidota* (18%), *Halobacterota* (13%),

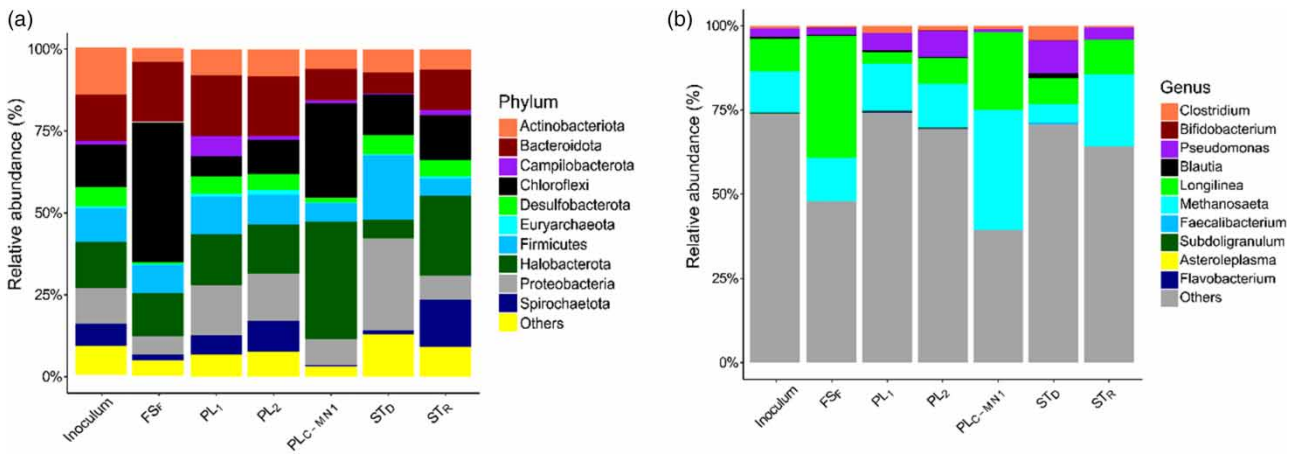


Figure 2 | Relative abundance of microbial communities in BMP bottles (inoculum to feed ratio of two) at time zero at (a) phylum level and (b) genus level.

Firmicutes (9%), and *Proteobacteria* (6%). Sam *et al.* (2022) reported a 19% abundance of *Chloroflexi* in septic tank sludge, whereas Torondel *et al.* (2016) reported a lower abundance in pit latrine sludge from Vietnam and Tanzania (relative quantity but not number reported). Archaea was dominated by the *Halobacterota* with 35.80% in $PL_{C\text{-}MN1}$ and 24.46% ST_R, indicating the presence of methanogens.

In this study at the genus level, *Methanosaeta* (*Halobacterota* phylum) had the highest relative abundance of 36% in $PL_{C\text{-}MN1}$ and 21% in ST_R. The highest abundance of *Methanosaeta* (*Halobacterota* phylum) was observed in AD inoculum (12%), PL_1 (14%), and PL_2 (13%), while their lowest abundance was found in ST_D (6%). *Longilinea (Chloroflexi* phylum) had the highest relative abundance of 36% in FS_F.

4. DISCUSSION

Based on the results of this study, it appears that fecal sludge is, in general, less biodegradable in anaerobic conditions than wastewater sludge. An average reduction of total VS over 21 days of 26% was observed for fresh fecal sludge, 15% for co-digested fecal sludge with food waste, and 11% for mono-digestion of fecal sludge that had been stored in containments (Table S3). Similar trends with fresh fecal sludge have been reported by Sam *et al.* (2022) (20% VS removal) and Ward *et al.* (2023) (20% VSS removal). In contrast, a 30–50% reduction in VS (due to limiting hydrolysis) is considered low for wastewater sludge (Appels *et al.* 2008). In contrast to fecal sludge, primary wastewater sludge is mainly composed of settled solids from the transport of fresh excreta, and waste-activated sludge is biomass that has been growing in aerobic conditions. As demonstrated with PLc, the limited degradation does not appear to be due to a limitation of micronutrients, but rather the overall degradability; however, this should be further verified by comparing the effect of different inoculums (Koch *et al.* 2020). This is also supported by the degradation extent (f_D), which was quite low in comparison to the theoretical yield.

In tests with greater CBG, a biphasic biogas production was observed, which can be attributed to the sequential consumption of readily and then slowly biodegradable organic components, as supported by sCOD, BOD, and polysaccharides and their K values (Table 2). A 66% sCOD removal was observed for both FS_F and RW_R, which had initial sCOD concentration three to six times higher than fecal sludge that had been stored in containments. The fresh waste streams with higher sCOD and polysaccharides had higher K values, whereas mono-digestion of stored fecal sludge had the lowest K values. The lower K values indicate that hydrolysis is limiting the biogas production after the readily degradable components are used up (Pavlostathis & Giraldo-Gomez 1991). The results do not fit well into the ADM1 anaerobic digestion model due to the high variability of fecal sludge and varying hydrolysis rates of slowly and readily biodegradable fractions (Batstone *et al.* 2002). The ADM1 model uses fixed fractions of proteins (30%), lipids (30%), carbohydrates (30%), and particulate inert material (10%), which are not established for highly heterogeneous fecal sludge samples. The development of models for the anaerobic digestion of fecal sludge will first require a more detailed understanding of the readily and slowly biodegradable particulate and dissolved fractions of organic matter including lipids, proteins and carbohydrates in these pools.

The greatest degradation of sCOD was observed with co-digestion of fecal sludge and foodwaste, with a 74% sCOD removal with co-digestion of $FS_F + RW_R$. The synergistic benefit of increased CBG was not explained by the concentration of the sCOD, BOD, and polysaccharides (Table S3) in the two feeds, and CBG was higher than either when digested alone (Koch *et al.* 2020), indicating a priming effect due to addition of readily degradable organic matter (Insam & Markt 2016). The concept of priming is not commonly reported in anaerobic digestion; however, it has been reported with slowly biodegradable organic matter becoming readily bioavailable upon co-digestion (Vivekanand *et al.* 2018). The priming effect in anaerobic reactors enhances microbial enzyme production with the consumption of readily degradable organic matter, allowing for the decomposition of slowly biodegradable compounds and an increase in the total production of biogas (graphical abstract & Figure 1(c)) (Insam & Markt 2016). This has also been observed with BMP tests of septic tank sludge and food waste at ambient temperatures (Le Phuong & Thai 2018), and wastewater sludge and municipal solid waste (Nielfa *et al.* (2015). Further research is needed to identify the enzymes involved and the underlying mechanism in order to quantify the effect.

It is commonly perceived that fecal sludge steadily undergoes anaerobic digestion and stabilization with storage time (or time since last emptied) in onsite containments (Cofie *et al.* 2006). However, the effect of longer-term storage on stabilization of fecal sludge remains unclear, following the first week of storage or digestion where readily degradable organic matter is used up (Ward *et al.* 2023). Rose (2015) had similar results, with a CBG (276 ± 151 mL/gVS) from fecal sludge that had been stored less than 1 week (i.e. 4 days), in comparison to samples that had been stored in containment with time since

last emptied of 2–10 years (50.6 ± 19.4 mL/gVS), with the majority of methane production ceasing within 10 days. Fresh food waste is also known to undergo rapid degradation during storage at ambient conditions, and is therefore not recommended prior to AD (Påledal *et al.* 2018). In our study, storage time in containment after more than 1 week also did not show any clear pattern with CBG, for example with 3 months since last emptied (ST_R, 37 ± 4 mL/gVS), compared to 14 years (PL_2, 74 ± 4 mL/gVS). This has also been observed with controlled anaerobic storage of fecal sludge in laboratory reactors, revealing no clear trend for stabilization based on storage time in reactors after the first week (Sam *et al.* 2022; Ward *et al.* 2023). In the field, time since last emptied does not indicate the actual storage time or age in containment of the sludge, as there are continual fresh inputs, variable emptying patterns, and storage conditions that are not analogous to controlled anaerobic digestion (Shaw & Dorea 2021). Further confirming this, there is an analysis of fecal sludge from 450 onsite containments in three countries, which found no relation to time since emptied and level of stabilization (Ward *et al.* 2021; Ward *et al.* 2023).

As time since last emptied is not a reliable predictor of stabilization, we need more dependable metrics for process control to predict the treatment performance of AD. Conventional wastewater parameters such as TS, VS, TOC, and COD are used to predict the stabilization and biogas potential of wastewater sludge (Odirile *et al.* 2021), but are not adequate to predict the biogas production from fecal sludge. As illustrated in the graphical abstract, these indicators measure overlapping pools of organic matter that have a direct impact on the production of biogas. In this study, the VS/TS values in the BMP tests at time 0, which were relatively consistent, were not predictors of biogas production, as shown by the lower f_d values for fecal sludge in comparison to fresh waste streams and co-digestion (Table 2). The feeds alone had differing VS/TS that were more reflective of biogas potential, however only at the level of fresh waste streams versus stored fecal sludge, and VS/TS of the stored fecal sludge feed alone did not have clear patterns in relation to f_d (Table 1). TOC/TN was also not a predictor of biogas production from fecal sludge. Comparative metrics like VS/TS and TOC/TN are more useful for AD of municipal wastewater, as both primary (settled solids) and waste-activated sludge (biomass) generated during wastewater treatment are more similar to each other than fecal sludge. Fecal sludge in contrast to municipal wastewater treatment processes has a wide range of inputs and storage conditions, resulting in fractions of readily and slowly biodegradable organic matter making up pools of VS, COD, and TOC also being highly variable. In addition, TS of fecal sludge contains varying amounts of inert material from soil or non-biodegradable wastes (Ahmed *et al.* 2018), whereas wastewater commonly undergoes grit removal. Because TOC measures both readily and slowly degradable organic carbon, the TOC/TN of wastewater also does not always correlate with stabilization or biogas production (Manga *et al.* 2022).

In contrast, metrics that are based on the potential for biological activity will be more reliable indicators of stabilization and biogas potential. BOD measures biodegradable organic matter, sCOD measures the soluble organic fraction, and polysaccharides include more readily degradable organic content (Płuciennik-Koropczuk & Myszograj 2019) (Table S3). In this study, the BOD/COD ratio of BMP tests at time 0 was a better indicator of f_D values (Table 2) (Adou *et al.* 2020), and the higher the ratio, the greater the biodegradability (Tchobanoglus *et al.* 2014). Similarly, Levira *et al.* (2023) also observed that the treatment performance of two similar mesophilic anaerobic fecal sludge digesters in Tanzania was significantly different and could not be predicted based only on the chemical parameters COD, total suspended solids, and free ammonium nitrogen. One anomaly in this study was ST_R, which had a high sCOD but a low f_d (0.006), the reasons for the low CBG with this sample are not known. Since the sample was from a restaurant, it is possible that the fractions of sCOD were comprised of less biodegradable fractions such as high contents of oil and grease, or inhibition could be due to cleaning products (Krueger *et al.* 2021). It is important to keep in mind that non-household sources of fecal sludge can account for half of the fecal sludge in a city and can have different characteristics than household sludge (Strande *et al.* 2018).

The dewatering metrics followed the same pattern as stabilization, with dewatering performance initially improving the first week as readily biodegradable organic matter was used up and then plateauing, with any further hydrolysis of particulate organic matter having no significant relation to dewaterability. This is similar to the findings of Shahid *et al.* (2022), who observed improved CST of simulant fecal sludge under anaerobic conditions. Semiyaga *et al.* (2017) also observed that during the early stages of anaerobic digestion, fine colloidal particles were degraded which slightly improved dewatering.

Regardless of the high presence of methanogens *Halobacterota* at the phylum level and *Methanosaeta* at the genus level in $PL_{C\text{-}MN1}$ and ST_R, the biogas production was still low. The presence of *Halobacterota* being a slow-growing organism (Lyu *et al.* 2018) also suggests that the limiting step in biogas production during anaerobic digestion of fecal sludge that had been stored in containment is not methanogenesis but rather the breakdown of organic material through hydrolysis.

4.1. Implications

Based on the results of this study, anaerobic digestion can be a viable treatment option for fresh fecal sludge with little or no retention time in onsite containments, such as container-based sanitation, or public toilets if the NH_4^+–N is not too high. Although in this study the stored fecal sludge still had significant organic matter, the readily biodegradable fraction was low and biogas production was limited by hydrolysis, indicating that anaerobic mono-digestion is not an optimal technology for biogas production or the stabilization of stored fecal sludge. However, the co-digestion of fecal sludge with other waste streams that have more bioavailable organic matter such as fresh food waste could result in a priming effect with increased biogas production (Insam & Markt 2016). Co-digestion also has the potential to buffer the variability of fecal sludge coming into the treatment (Levira *et al.* 2023). Alternatively, thermal pretreatment, or pretreatment with strong oxidants and alkalis resulting in membrane disruption, could potentially enhance the hydrolysis of fecal sludge by converting the slowly biodegradable organic matter into readily bioavailable fractions (Cui *et al.* 2023). Further knowledge of the pools of organic fractions arriving at fecal sludge treatment facilities, including aggregates and bulk solutions, will lead to an understanding of how it performs with treatment technologies. This includes identifying reliable metrics in order to predict stabilization, biogas production and dewatering performance instead of relying on VS, COD and TOC as metrics of total organic matter, versus what is available for biological degradation (BOD and sCOD). With implementation, it is important to consider that fecal sludge is highly variable, and anomalies observed as ST_R in this study are to be expected.

5. CONCLUSIONS

Key conclusions of this study include:

- Anaerobic digestion of fecal sludge for biogas production is not a recommended treatment option for fecal sludge that has undergone storage in containment due to hydrolysis limitation. However, it could be applied with short retention times to improve dewatering, or in co-digestion with fresh foodwaste.
- Anaerobic digestion for biogas production could be viable for feces, excreta, or blackwater that has not been stored or has been stored for less than 1 week.
- Following 1–2 weeks of storage in containment, the time since last emptied is not a predictor of the level of stabilization of fecal sludge.
- Physical–chemical metrics of stabilization (i.e. VS/TS, TOC/TN) are not reliable predictors of biogas production from fecal sludge, and instead, metrics based on the potential for biological degradation should be used (e.g. BOD, sCOD).
- Dewatering performance (filtration and turbidity) improves following 1 week of anaerobic digestion, but then levels off.

ACKNOWLEDGEMENTS

The authors appreciate Anik Dutta, Helena Verloo, and Michael Vogel for providing ST_R and ST_D samples, Sylvia Richter and Kelsey Shaw for laboratory assistance, and Barbara Jeanne Ward for critical feedback.

FUNDING

This work received support from Swiss National Science Foundation (SNF), Eawag Partnership Program (EPP), ETH for Development (ETH4D) and International Research Support Initiative Program (IRSIP), Higher Education Commission (HEC), Pakistan.

AUTHOR CONTRIBUTIONS

N. M. conceptualized the research, supported in funding acquisition, developed the methodology, conducted the research, analyzed data, and wrote the original draft. S. S. developed the methodology, contributed to the research, reviewed, and edited the article. S. J. K. reviewed and edited the article. L. S. conceptualized the research, supervised the work, supported in funding acquisition, validated the data, and wrote the original draft.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Abubakar, U. A., Ajibike, M. A. & Adie, D. B. 2017 Hydrolysis rates of domestic wastewater sludge using biochemical methane potential tests. *Nigerian Journal of Technology* **36** (4), 1315–1323.

Adou, K. E., Alle, O. A., Kouakou, A. R., Adouby, K., Drogui, P. & Tyagi, R. D. 2020 Anaerobic mono-digestion of wastewater from the main slaughterhouse in Yamoussoukro (Côte d'Ivoire): Evaluation of biogas potential and removal of organic pollution. *Journal of Environmental Chemical Engineering* **8** (3), 103770.

Ahmed, I., Quarshie, A. M., Ofori-Amanfo, D., Cobbold, F., Amofa-Sarkodie, E. S. & Awuah, E. 2018 Assessment of foreign material load in the management of faecal sludge in the Greater Accra Region of Ghana. *International Journal of Energy and Environmental Science* **3** (1), 27–36.

Andriessen, N., Appiah-Effah, E., Browne, S. J. I., al Jahjah, R., Kabika, J., Kinobe, J. R., Korir, N., Nishimwe, P., Niwagaba, C. B., Pradeep, R., Prasad, P., William, A., Tembo, J. M., Ambuehl, B. & Strande, L. 2023 Quantities and qualities of faecal sludge: Experiences from field implementation with a Volaser in 7 countries during a pandemic. *Frontiers in Water* **5**, 35.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P. & Van Lier, J. B. 2009 Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Science and Technology* **59** (5), 927–934.

APHA 2017 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), Washington, DC, USA.

Appels, L., Baeyens, J., Degrève, J. & Dewil, R. 2008 Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science* **34** (6), 755–781.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H. A. & Vavilin, V. A. 2002 The IWA anaerobic digestion model no 1 (ADM1). *Water Science and Technology* **45** (10), 65–73.

Carmona-Cabello, M., García, I. L., Sáez-Bastante, J., Pinzi, S., Koutinas, A. A. & Dorado, M. P. 2020 Food waste from restaurant sector – characterization for biorefinery approach. *Bioresource Technology* **301**, 122779.

Christensen, M. L., Keiding, K., Nielsen, P. H. & Jørgensen, M. K. 2015 Dewatering in biological wastewater treatment: A review. *Water Research* **82**, 14–24.

Cofie, O. O., Agbottah, S., Strauss, M., Esseku, H., Montangero, A., Awuah, E. & Kone, D. 2006 Solid–liquid separation of faecal sludge using drying beds in Ghana: Implications for nutrient recycling in urban agriculture. *Water Research* **40** (1), 75–82.

Colón, J., Forbis-Stokes, A. A. & Deshusses, M. A. 2015 Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries. *Energy for Sustainable Development* **29**, 57–64.

Cui, H., Wang, J., Cai, X., Feng, K., Xie, G.-J., Liu, B.-F. & Xing, D. 2023 Chemical pretreatments and anaerobic digestion shape the virome and functional microbiome in fecal sludge. *Environmental Science & Technology* **57** (14), 6008–6020.

Dueholm, M. K. D., Andersen, K. S., Petersen, A.-K. C. & Rudkjoebing, V. MiDAS Global Consortium for Anaerobic Digesters & Nielsen,, P. H. 2023 MiDAS 5: Global diversity of bacteria and archaea in anaerobic digesters. *bioRxiv* 2023-2008.

Elmitwalli, T., Zeeman, G. & Otterpohl, R. 2011 Modelling anaerobic digestion of concentrated black water and faecal matter in accumulation system. *Water Science and Technology* **63** (9), 2039–2045.

Filer, J., Ding, H. H. & Chang, S. 2019 Biochemical methane potential (BMP) assay method for anaerobic digestion research. *Water* **11** (5), 921.

Gold, M., Harada, H., Therrien, J.-D., Nishida, T., Cunningham, M., Semiyaga, S., Fujii, S., Dorea, C., Nguyen, V.-A. & Strande, L. 2018 Cross-country analysis of faecal sludge dewatering. *Environmental Technology* **39** (23), 3077–3087.

Houghton, J. I., Quarmby, J. & Stephenson, T. 2000 The impact of digestion on sludge dewaterability. *Process Safety and Environmental Protection* **78** (2), 153–159.

Insam, H. & Markt, R. 2016 Comment on "Synergistic co-digestion of solid-organic-waste and municipal-sewage-sludge: 1 plus 1 equals more than 2 in terms of biogas production and solids reduction" [Water research 87, 416–423]. *Water Research* **95**, 392–393.

Jensen, P. D., Ge, H. & Batstone, D. J. 2011 Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent. *Water Science and Technology* **64** (4), 880–886.

Kilucha, M., Cheng, S., Minza, S., Nasiruddin, S. M., Velempini, K., Li, X., Wang, X., Mokeira Doroth, K. & Li, Z. 2022 Insights into the anaerobic digestion of fecal sludge and food waste in Tanzania. *Frontiers in Environmental Science* **10**, 1294.

Kim, J., Kim, J. & Lee, C. 2019 Anaerobic co-digestion of food waste, human feces, and toilet paper: Methane potential and synergistic effect. *Fuel* **248**, 189–195.

Koch, K., Hafner, S. D., Weinrich, S., Astals, S. & Holliger, C. 2020 Power and limitations of biochemical methane potential (BMP) tests. *Frontiers in Energy Research* **8**, 63.

Krueger, B. C., Fowler, G. D., Templeton, M. R. & Septien, S. 2021 Faecal sludge pyrolysis: Understanding the relationships between organic composition and thermal decomposition. *Journal of Environmental Management* **298**, 113456.

Le Phuong, H. & Thai, N. T. K. 2018 The potential of biogas recovery from anaerobic co-digestion of fecal sludge and organic waste. In: *Utilization and Management of Bioresources* (Ghosh, S. K., ed.). Springer, Singapore, pp. 113–121.

Levira, B., Bright-Davies, L., Carmargo, J., Duma, L., Fettback, T., Lazaro, A., Mahundo, C., Matwewe, F., Mrimi, E. & Schmidt, A. 2023 Decentralised treatment solutions for on-site faecal sludge: Quantifying the removal efficiencies of two novel systems in an East African city. *Environmental Science: Water Research & Technology* **9** (2), 603–619.

Loewus, F. A. 1952 Improvement in anthrone method for determination of carbohydrates. *Analytical Chemistry* **24** (1), 219–219.

Lyu, Z., Shao, N., Akinyemi, T. & Whitman, W. B. 2018 Methanogenesis. *Current Biology* **28** (13), R727–R732.

Madikizela, P., Laubscher, R., Tandlich, R. & Ngqwala, N. 2017 Optimizing biogas recovery from pit latrine faecal sludge. In: *5th International Conference on Sustainable Solid Waste Management*. Athens, Greece, 21–24 June 2017.

Manga, M., Evans, B. E., Ngasala, T. M. & Camargo-Valero, M. A. 2022 Recycling of faecal sludge: Nitrogen, carbon and organic matter transformation during co-composting of faecal sludge with different bulking agents. *International Journal of Environmental Research and Public Health* **19** (17), 10592.

Maqbool, N., Shahid, M. A. & Khan, S. J. 2022 Situational assessment for fecal sludge management in major cities of Pakistan. *Environmental Science and Pollution Research* **30** (44), 98869–98880.

Meher, K. K., Murthy, M. V. S. & Gollakota, K. G. 1994 Psychrophilic anaerobic digestion of human waste. *Bioresource Technology* **50** (2), 103–106.

Metcalf and I. Eddy 2014 *Wastewater Engineering: Treatment, Disposal, Reuse*. Tata McGraw-Hill, New York.

Nielfa, A., Cano, R. & Fdz-Polanco, M. 2015 Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports* **5**, 14–21.

Odirile, P. T., Marumoloa, P. M., Manali, A. & Gikas, P. 2021 Anaerobic digestion for biogas production from municipal sewage sludge: A comparative study between fine mesh sieved primary sludge and sedimented primary sludge. *Water* **13** (24), 3532.

Påledal, S. N., Hellman, E. & Moestedt, J. 2018 The effect of temperature, storage time and collection method on biomethane potential of source separated household food waste. *Waste Management* **71**, 636–643.

Pavlostathis, S. G. & Giraldo-Gomez, E. 1991 Kinetics of anaerobic treatment: A critical review. *Critical Reviews in Environmental Science and Technology* **21** (5–6), 411–490.

Płuciennik-Koropczuk, E. & Myszograj, S. 2019 New approach in COD fractionation methods. *Water* **11** (7), 1484.

Rose, C. 2015 Developing a Nutrient Recovery Process for Recovering Nutrients in Anaerobic Digestate in Low Income Countries. PhD Thesis, Cranfield University, Cranfield, UK.

Sakaveli, F., Petala, M., Tsiridis, V. & Darakas, E. 2021 Enhanced mesophilic anaerobic digestion of primary sewage sludge. *Water* **13** (3), 348.

Sam, S. B., Ward, B. J., Niederdorfer, R., Morgenroth, E. & Strande, L. 2022 Elucidating the role of extracellular polymeric substances (EPS) in dewaterability of fecal sludge from onsite sanitation systems, and changes during anaerobic storage. *Water Research* **222**, 118915.

Sam, S. B., Morgenroth, E. & Strande, L. 2023 Changes in organic fractions, cations, and stabilization from feces to fecal sludge: Implications for dewatering performance and management solutions. *Journal of Water. Sanitation and Hygiene for Development* **13** (9), 699–710.

Semiyaga, S., Okure, M. A. E., Niwagaba, C. B., Nyenje, P. M. & Kansiime, F. 2017 Dewaterability of faecal sludge and its implications on faecal sludge management in urban slums. *International Journal of Environmental Science and Technology* **14** (1), 151–164.

Semiyaga, S., Nakagiri, A., Niwagaba, C. B. & Manga, M. 2022 Application of anaerobic digestion in decentralized faecal sludge treatment plants. In: *Anaerobic Biodigesters for Human Waste Treatment* (Meghvansi, M. K. & Geol, A. K., eds.), Springer, Singapore, pp. 263–281.

Shahid, M. A., Maqbool, N. & Khan, S. J. 2022 An integrated investigation on anaerobic membrane-based thickening of fecal sludge and the role of extracellular polymeric substances (EPS) in solid-liquid separation. *Journal of Environmental Management* **305**, 114350.

Shaw, K. & Dorea, C. C. 2021 Biodegradation mechanisms and functional microbiology in conventional septic tanks: A systematic review and meta-analysis. *Environmental Science: Water Research & Technology* **7** (1), 144–155.

Shaw, K., Vogel, M., Andriessen, N., Hardeman, T., Dorea, C. C. & Strande, L. 2022 Towards globally relevant, small-footprint dewatering solutions: Optimal conditioner dose for highly variable blackwater from non-sewered sanitation. *Journal of Environmental Management* **321**, 115961.

Song, K. H., Woo, J. S., Kim, J. R., Ryu, G. L., Baek, Y. C., Oh, Y. K., Kwak, W. S. & Park, K. K. 2020 Nutritional value and in situ degradability of fruit-vegetable byproducts and their feeding effects on performance of growing Hanwoo steers. *Asian-Australasian Journal of Animal Sciences* **33** (6), 973–980.

Strande, L., Schoebitz, L., Bischoff, F., Ddiba, D., Okello, F., Englund, M., Ward, B. J. & Niwagaba, C. B. 2018 Methods to reliably estimate faecal sludge quantities and qualities for the design of treatment technologies and management solutions. *Journal of Environmental Management* **223**, 898–907.

Tayler, K. 2018 *Faecal Sludge and Septage Treatment: A Guide for Low-and Middle-Income Countries*. Practical Action Publishing, Warwickshire, UK.

Tchobanoglus, G., Stensel, H. D., Tsuchihashi, R. & Burton, F. 2014 *Wastewater Engineering: Treatment and Reuse*. McGraw-Hill.

Torondel, B., Ensink, J. H. J., Gundogdu, O., Ijaz, U. Z., Parkhill, J., Abdelahi, F., Nguyen, V. A., Sudgen, S., Gibson, W. & Walker, A. W. 2016 Assessment of the influence of intrinsic environmental and geographical factors on the bacterial ecology of pit latrines. *Microbial Biotechnology* **9** (2), 209–223.

van Eekert, M. H. A., Gibson, W. T., Torondel, B., Abilahi, F., Liseki, B., Schuman, E., Sumpter, C. & Ensink, J. H. J. 2019 Anaerobic digestion is the dominant pathway for pit latrine decomposition and is limited by intrinsic factors. *Water Science and Technology* **79** (12), 2242–2250.

Velkushanova, K., Brdjanovic, D., Koottatep, T., Strande, L., Buckley, C. & Ronteltap, M. 2021 *Methods for Faecal Sludge Analysis*. IWA Publishing, London.

Vivekanand, V., Mulat, D. G., Eijsink, V. G. H. & Horn, S. J. 2018 Synergistic effects of anaerobic co-digestion of whey, manure and fish ensilage. *Bioresource Technology* **249**, 35–41.

Ward, B. J., Andriessen, N., Tembo, J. M., Kabika, J., Grau, M., Scheidegger, A., Morgenroth, E. & Strande, L. 2021 Predictive models using "cheap and easy" field measurements: Can they fill a gap in planning, monitoring, and implementing fecal sludge management solutions? *Water Research* **196**, 116997.

Ward, B. J., Nguyen, M. T., Sam, S. B., Korir, N., Niwagaba, C. B., Morgenroth, E. & Strande, L. 2023 Particle size as a driver of dewatering performance and its relationship to stabilization in fecal sludge. *Journal of Environmental Management* **326**, 116801.

WHO 2018 *Guidelines on Sanitation and Health*. World Health Organization, Geneva.

First received 23 December 2023; accepted in revised form 26 February 2024. Available online 6 March 2024