



# Elucidating the role of extracellular polymeric substances (EPS) in dewaterability of fecal sludge from onsite sanitation systems, and changes during anaerobic storage.

Stanley B. Sam<sup>a,b,\*</sup>, Barbara J. Ward<sup>a,b</sup>, Robert Niederdorfer<sup>a</sup>, Eberhard Morgenroth<sup>a,b</sup>, Linda Strande<sup>a</sup>

<sup>a</sup> Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

<sup>b</sup> ETH Zurich, Institute of Environmental Engineering, 8093 Zurich, Switzerland

## ARTICLE INFO

### Keywords:

Blackwater  
Sludge filtration  
Biomethane potential test  
Microbial community analysis  
Particle size distribution

## ABSTRACT

As the importance of fecal sludge management (FSM) is increasingly being realized, the need for adequately designed and functioning fecal sludge (FS) treatment plants is also increasing. Research to fill this gap is only emerging and dewatering is a key challenge for developing sustainable treatment solutions. This study evaluated the effect of extracellular polymeric substances (EPS) on dewaterability of FS, and how EPS and dewaterability change during anaerobic storage (as a proxy for time in onsite containment). EPS was extracted from FS and activated sludge using Na<sub>2</sub>CO<sub>3</sub> and sonication and added to sludge samples to determine the effect on dewaterability. The results confirmed that an increase in EPS had a direct impact of decreasing FS dewaterability (as capillary suction time). In this context, we evaluated FS degradation during anaerobic storage, the effect of anaerobic storage time on EPS, EPS fractions and particle size distribution, and the effect of variations in these factors on FS dewaterability. Variations in EPS, EPS fraction and particle size distribution during anaerobic storage were less than expected and average VS reduction of 20% was recorded over 7 weeks. Although anaerobic digestion was verified (biogas production), the results indicate that kinetics of degradation of FS is different from wastewater sludges. Comparatively, EPS fractions in FS were 70 – 75% lower and with higher fractions of humic-like substances than wastewater sludges. Although EPS significantly affects FS dewaterability, anaerobic storage time is not a predictor of dewaterability.

## 1. Introduction

Adequate fecal sludge management (FSM) is a key aspect of meeting the sustainable development goal (SDG) 6.2 (United nations, 2015), which aims to safely manage sanitation and hygiene services. Similar to wastewater sludge treatment, the critical step in FSM is the separation of liquids and solids in fecal sludge (FS) by dewatering (Strande et al., 2014). FS typically consists of more than 95% water (Gold et al., 2017) and improvement in dewatering can reduce the cost of transportation and facilitate further treatment processes. Unlike FS, dewatering is widely implemented and studied in wastewater sludges, however, a direct application of dewatering technologies from wastewater to FS is not feasible due to the marked differences between FS and wastewater, and the high variability of FS characteristics and composition (Ward et al., 2019). The variability of FS stems from the different types of

containments, differences in emptying practices, usage patterns (e.g. flush toilet), and the duration of storage in onsite containment, which affects the level of stabilization (Ward et al., 2019). Hence, FS arriving at treatment plants, has much more variable characteristics than wastewater, which is relatively more homogenized as it travels in the sewer network, and is one to two orders of magnitude more variable in COD and TS concentrations (Strande et al., 2018). For example COD of FS has been reported to be about 8000–127,000 mg/L compared to 500–2500 mg/L for wastewater sludges (primary and secondary sludge) (Junglen et al., 2020; Niwagaba et al., 2014). Dewatering performance of FS is more variable than wastewater sludges (Gold et al., 2017) with differences occurring among distinct onsite sanitation systems, different cities or even similar onsite systems such as lined and unlined pit latrines (Strande et al., 2018).

Based on extensive knowledge of dewatering of wastewater, floc

\* Corresponding author at: Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland.

E-mail address: [stanley.sam@eawag.ch](mailto:stanley.sam@eawag.ch) (S.B. Sam).

<https://doi.org/10.1016/j.watres.2022.118915>

Received 14 April 2022; Received in revised form 25 July 2022; Accepted 26 July 2022

Available online 28 July 2022

0043-1354/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

**Table 1**

Operating conditions and characteristics of the BMP tests at time 0, reported as average and standard deviation. Feed for run A and B had feces to urine ratios of 1:2.5 and 1:3.75 respectively. For each inoculum, test samples (i.e. the inoculum with feed) and controls (positive and negative control) were performed in triplicate and laboratory analysis of the samples were conducted in duplicate.

RUN	Inoculum	Incubation Time (days)	Temp °C	Inoc. TS (g/L)	Inoc. VS (g/L)	Inoc. NH <sub>4</sub> <sup>+</sup> -N (mg/L)	Feed TS (g/L)	Feed. VS (g/L)	Feed NH <sub>4</sub> <sup>+</sup> -N (mg/L)	BMP bottle TS (g/L)	BMP bottle VS (g/L)	BMP bottle NH <sub>4</sub> <sup>+</sup> -N (mg/L)
RUN A												
A*	AD	47	20	27.6 ± 0.3	12.3 ± 0.1	765 ± 21	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	27.8 ± 0.1	13.78 ± 0.2	975 ± 14
A*	AD	47	20	27.6 ± 0.3	12.3 ± 0.1	765 ± 21	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	27.8 ± 0.1	13.78 ± 0.2	975 ± 14
A	CM	47	20	32.0 ± 0.3	20.0 ± 0.3	1845 ± 21	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	25.8 ± 0.4	16.85 ± 0.1	1135 ± 7
A	ST	47	20	29.0 ± 0.7	19.5 ± 0.0	896 ± 56	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	28.3 ± 0.7	19.78 ± 0.2	605 ± 7
A	PL	47	20	39.8 ± 0.5	21.6 ± 0.4	1920 ± 7	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	33.3 ± 1.0	20.28 ± 0.3	1745 ± 7
A	AD	47	37	27.6 ± 0.3	12.3 ± 0.1	765 ± 21	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	27.8 ± 0.1	13.78 ± 0.2	975 ± 14
A	PL	47	37	39.8 ± 0.5	21.6 ± 0.4	1920 ± 7	27.4 ± 0.2	22.5 ± 0.6	849 ± 17	33.3 ± 1.0	20.28 ± 0.3	1745 ± 7
RUN B												
B	AD	40	20	23.7 ± 0.3	11.17 ± 0.1	565 ± 4	15.87 ± 0.4	11.9 ± 0.1	1245 ± 7	NA	NA	NA
B	CM	40	20	19.5 ± 0.2	11.51 ± 0.0	1170 ± 21	15.87 ± 0.4	11.9 ± 0.1	1245 ± 7	NA	NA	NA
B	ST	40	20	24.2 ± 0.3	17.11 ± 0.0	1330 ± 21	15.87 ± 0.4	11.9 ± 0.1	1245 ± 7	NA	NA	NA
B	PL	40	20	26.2 ± 0.1	15.66 ± 0.0	1185 ± 14	15.87 ± 0.4	11.9 ± 0.1	1245 ± 7	NA	NA	NA

\*These operating conditions were run in duplicate. AD – Anaerobic digested sludge, CM – Cow manure sludge, ST – Septic tank sludge, PL – Pit latrine sludge, Inoc. – Inoculum.

properties such as microorganisms, extracellular polymeric substances (EPS), organic debris and inorganic particles (Christensen et al., 2015) are major factors that influence dewaterability of activated sludges. Additionally, particle size distribution affects dewaterability because higher concentrations of small particles can contribute to clogging of filter media and reduce settleability (Christensen et al., 2015; Houghton and Stephenson, 2002; Lawler et al., 1986). The role of EPS and particle size distribution on dewaterability of wastewater are well known. Dewaterability is enhanced by soluble and loosely-bound EPS, which contribute to increased bioflocculation resulting in improved filtration (Christensen et al., 2015). However, floc disintegration and the release of EPS into solution worsens dewaterability (Lei et al., 2007; Novak et al., 2003). In addition, floc disintegration generates suspended fine flocs and individual particles which further reduce dewaterability (Houghton and Stephenson, 2002). Anaerobic conditions can either worsen or improve dewaterability in wastewater sludges. Under anaerobic conditions, flocs disintegrate, which releases suspended organic matter and worsens dewatering (Novak et al., 2003). However, suspended organic matter including EPS and particles are also ultimately degraded, which improves dewaterability (Mikkelsen and Keiding, 2002). It is not clear if these relationships exist in FS because research on FS is very limited in comparison to wastewater, with over a hundred year gap in research knowledge (Jenkins and Wanner, 2014). Furthermore, processes occurring in FS containments are not well understood. There is a common perception that a thin micro-aerophilic layer exist on the surface of the FS in containments while the bulk of the FS containment is predominantly anaerobic (Bakare et al., 2012; Brouckaert et al., 2013).

Current research on FS dewaterability has identified that physical and chemical characteristics such as pH, conductivity, total solids, EPS, particle size distribution and microbiology have statistical correlations with FS dewaterability (Gold et al., 2017; Ward et al., 2019). It is commonly accepted that stabilization of FS with time in containment, which affects these factors, will influence the sludge dewaterability. To the best of our knowledge, there has only been one study characterizing

EPS in FS, where a correlation was observed between EPS and dewatering of FS based on 20 field samples (Ward et al., 2019). However, it has never been demonstrated or verified that EPS is controlling dewaterability of FS. The study by Ward et al. (2019) observed that FS that appeared to be more stabilized had lower EPS and better dewatering performance compared to unstabilized sludge. However, these were grab samples taken at one time point in the field. The effect of time on EPS concentrations during storage of FS in anaerobic containments, and the relation to dewaterability, is not known. Therefore, the objective of this study was to validate the role of EPS in dewaterability of FS, and conduct controlled anaerobic stabilization experiments to gain an understanding of changes in EPS and dewaterability that take place with time during anaerobic storage reflective of onsite containments. Laboratory based anaerobic batch reactors and anaerobic biomethane potential (BMP) test were used to mimic conditions in onsite containments.

## 2. Materials and methods

### 2.1. Source of inoculum and feed

Four different inocula were used in this study; anaerobic digester sludge (AD), cow manure (CM), septic tank sludge (ST) and pit latrine sludge (PL). The AD sludge was obtained from a pilot scale anaerobic reactor at Eawag in Dübendorf, Switzerland, which was being fed with waste activated sludge and operating under mesophilic conditions (35°C). CM samples were obtained from a farm in Dübendorf. ST sludge was collected from vacuum trucks during discharge at a FS treatment plant in Accra, Ghana. PL sludge was collected from pit latrines in Kampala, Uganda. Both ST and PL samples were immediately stored in cooling boxes with ice before being airfreighted to Switzerland with ice packs to maintain the temperature.

At Eawag, the inocula were homogenized using a kitchen blender at the highest speed and stored at 4 °C. Feed for anaerobic reactors and BMP tests consisted of feces and urine, collected with urine separating dry toilets at Eawag. The feed was prepared by mixing freshly collected

**Table 2**

Overview of analysis done on the batch reactors and serum bottle test for evaluating the influence of EPS concentrations, EPS fractions, and particle size distribution on dewaterability. Runs 1–5 and the serum bottle test in run 6 were all fed once with feed, which had feces to urine ratio of 1:2.5. In addition, Run 4 included a replicate fed with synthetic wastewater (positive control for reactors). EPS fractions include humic-like substances (Humic substances and building blocks of humic substances) and protein-like substances. The biopolymer fraction of the organic carbon was assumed to be composed of mainly proteins due to the low carbon/nitrogen ratio (2–7). AD\* indicates the run fed with synthetic wastewater.

Run	Inoculum	Duration (week)	Vol (L)	Temp °C	pH	TS	VS	TSS	VSS	TCOD	SCOD	EPS	NH <sub>4</sub> -N	TN	FTIR	PSD	CST	EPS/EPS fractions	Turb	Biogas
Batch reactors																				
1	AD	5	12	20	x	x	x	x	x	x	x	X					x	x		x
2	AD	7	12	20	x	x	x	x	x	x	x	x	x	x			X	x		x
3	PL	7	12	20	x	x	x	x	x	x	x	x	x	x			X	x		x
4	AD	8	12	20	x	x	x	x	x	x	x	x	x	x			X	x		x
5	AD*	6	12	35	x	x	x	x	x	x	x	x	x	x			X	x		x
6	AD*	6	12	35	x	x	x	x	x	x	x	x	x	x			X	x		x
7	AD	7	5	35	x	x	x	x	x	x	x	x	x	x			X	x		x
8	PL	7	2	35	x	x	x	x	x	x	x	x	x	x			X	x		x
9	AD	7	0.175	35	x	x	x	x	x	x	x	x	x	x			X	x		x
10	PL	7	0.175	35	x	x	x	x	x	x	x	x	x	x			X	x		x

feces and urine to obtain a feces to urine ratio of 2.5 or 3.75 by wet weight and subsequently diluted to less than 5% TS with tap water to be in the range of reported TS concentrations of FS (Velkushanova et al., 2021). In addition to the TS, other characteristics (i.e. VS, VSS, TSS and COD) were confirmed to be in the range of characteristics reported for FS. The feed was stored at 4 °C and brought to room temperature prior to use. Characterization data of all the inocula and the feeds is provided in the Supplementary information (Table S1). To make a slurry for the BMP tests and batch reactors, CM which originally had a TS of 18.6% was diluted by adding deionized water to achieve a TS concentration of 4%, which is a standard value when using CM as inoculum for anaerobic digestion systems (Sunada et al., 2018).

## 2.2. Biomethane potential tests

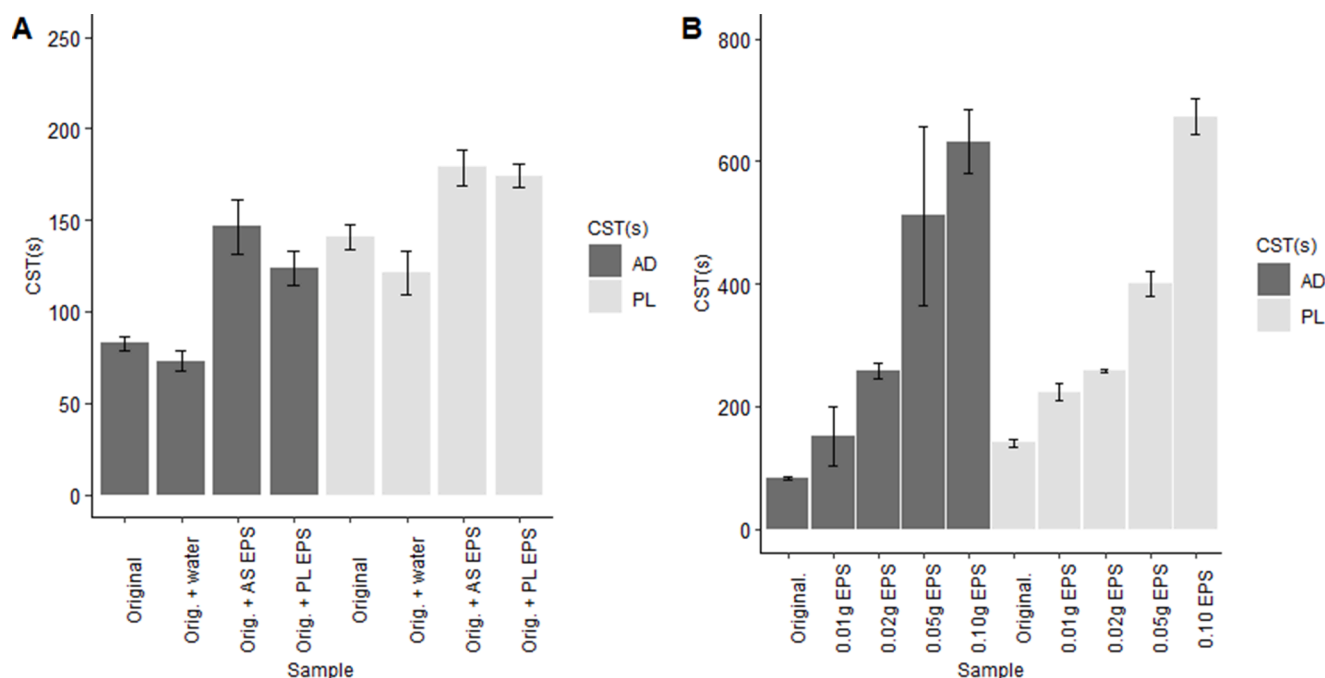
BMP tests were conducted to evaluate the ability of the four inocula to degrade FS under laboratory conditions. The tests were conducted as described by Holliger et al. (2016) with two modifications including operating temperatures of 20 °C and 37 °C, and the feed composition of urine and feces. Two sets of BMP tests were conducted (run A and run B), with varying feed composition and temperatures. The feed for run A had feces to urine ratio of 1:2.5 by wet weight, and for run B feces to urine ratio of 1:3.75. The ratios of feces to urine was based on the daily per capita production of feces and urine reported in the literature with an average of 400 g feces and 1 L urine (1:2.5) (Colón et al., 2015), or 400 g feces and 1.5 L urine (Vögeli et al., 2014). Although higher daily per capita production of urine has been reported it was taken into account that not all urine is captured in onsite containment especially in low-income countries where there is limited access to sanitation (Colón et al., 2015).

An inoculum to feed ratio of four based on the volatile solids (VS) concentration was selected due to unknown degradation characteristics of the feed, as described in Angelidaki et al. (2009). The BMP tests were carried out in triplicate in 200 ml glass serum bottles with 70% active volume. The headspace of reaction bottles were flushed with N<sub>2</sub> gas to provide anaerobic conditions and incubated in a VWR 5000 L shaking incubator at 100 rpm. Following collection of the gas measurement, bottles were shaken by hand to ensure complete mixing of the floating layer that formed immediately after biogas is released. The performance of the BMP tests were measured by the biogas volume using a water displacement setup described by Filer et al. (2019) and normalized with the initial volatile solids (VS (g/L)) added. Methane content was measured periodically using gas chromatography, GC 9350 with flame ionization detector and ion capture detector (Agilent Technologies, US). Microcrystalline cellulose (Avicel® pH – 101) and Nanopure water were used as positive and negative controls respectively and the BMP tests were stopped when biogas production for 3 consecutive days was less than one percent of the cumulative gas production (Filer et al., 2019). The microbial community of the different inocula were also determined to understand the differences and similarities between the inocula in relation to biogas production. Presented in Table 1 are the characteristics of the all the BMP tests at the start of the experiments.

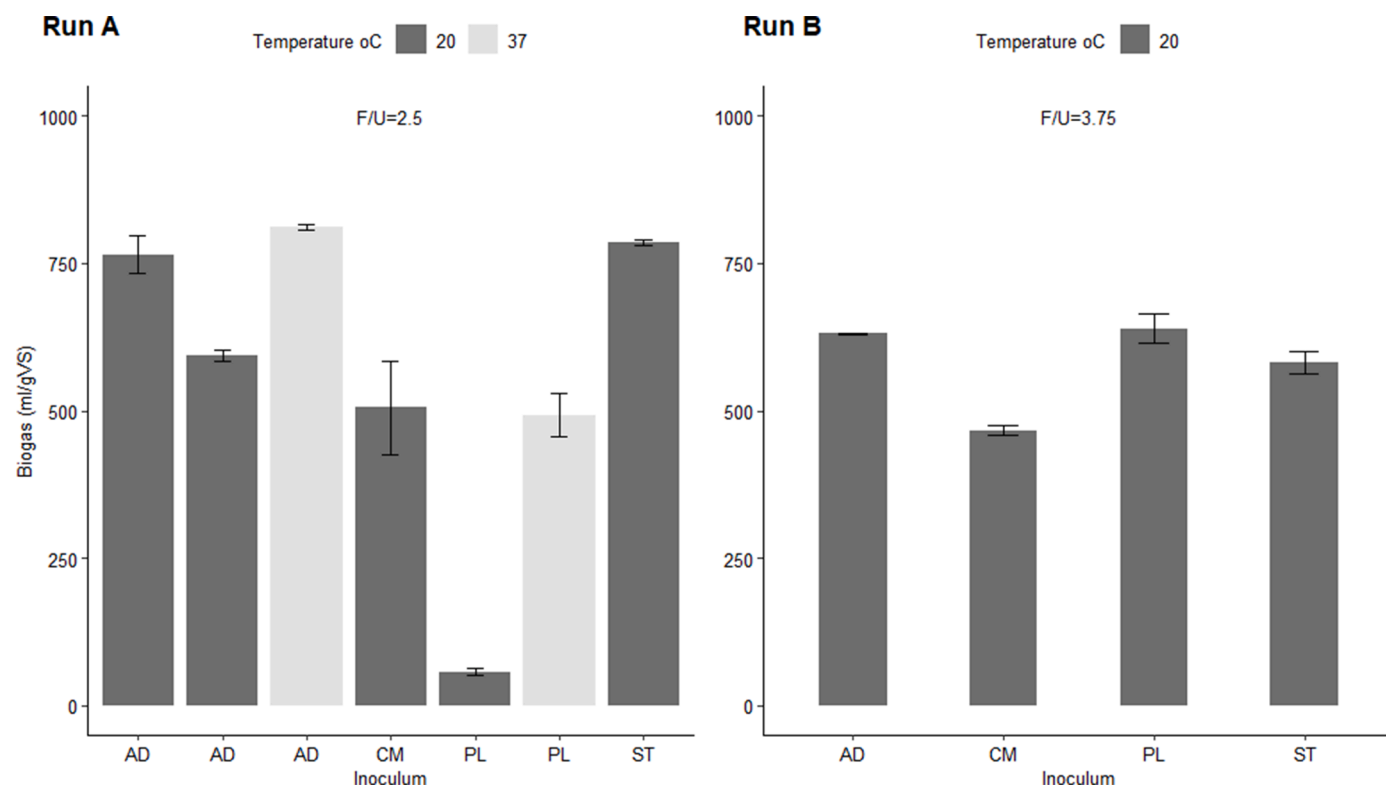
## 2.3. Anaerobic batch reactors and serum bottle tests

As summarized in Table 2, a series of batch reactor runs were conducted using AD and PL as inoculum (designated as run 1 to run 6), to evaluate the influence of EPS concentrations, EPS fractions, and particle size distribution, with time on the dewaterability of FS. The selection of inoculum and feed ratios for the batch reactors in this study were based on results of the BMP tests. After confirming that both AD and PL sludges were capable of degrading the feed in BMP tests, AD was selected to ensure that the results were comparable to literature, and PL sludge was selected as it was considered to be the most representative of FS in Sub-Saharan Africa.

AD sludge was used as inoculum in 12 L reactors in runs 1–3 at 20 °C



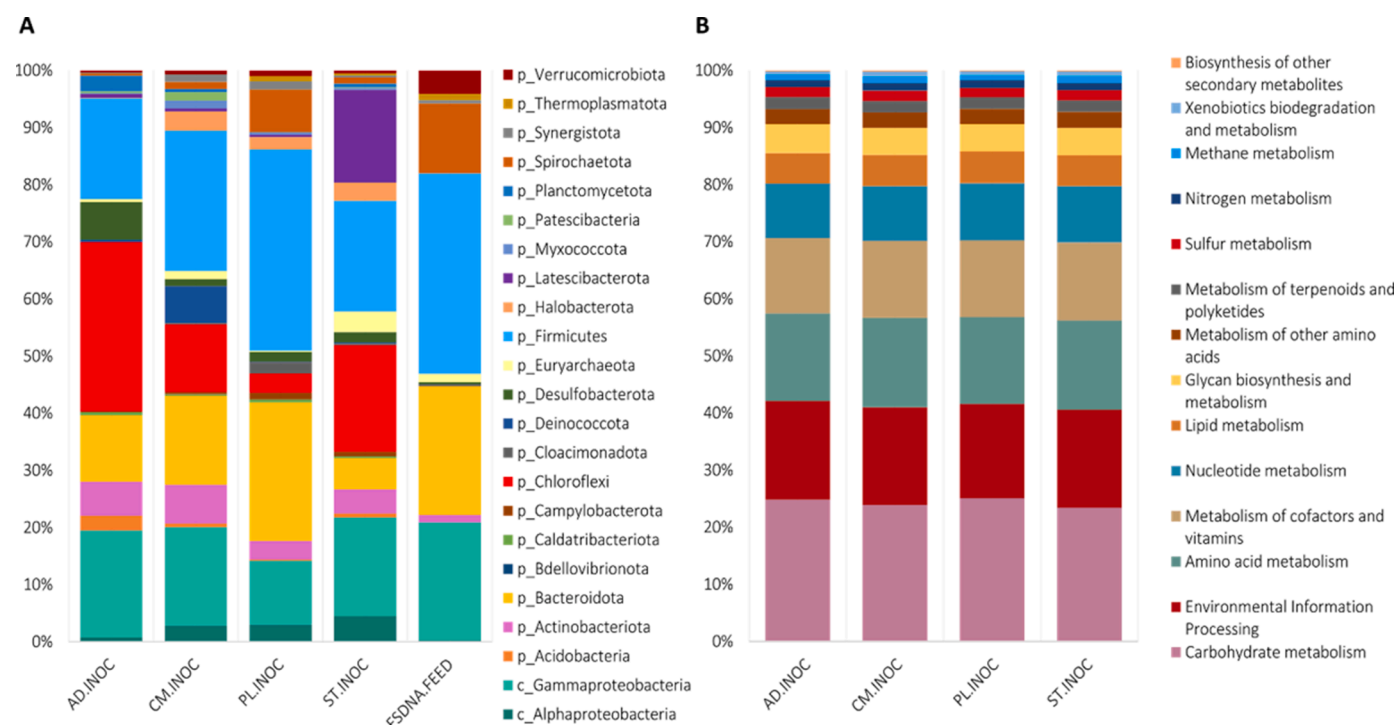
**Fig. 1.** Changes in dewaterability of fecal sludge (PL) and anaerobic digested sludge (AD) as measured by CST, with addition of extracted EPS. (A) Addition of soluble/loosely bound EPS from activated sludge (AS EPS), pit latrine sludge (PL EPS) and water (blank) to original AD and PL sludge samples. (B) Addition of freeze-dried EPS to AD and PL sludges.



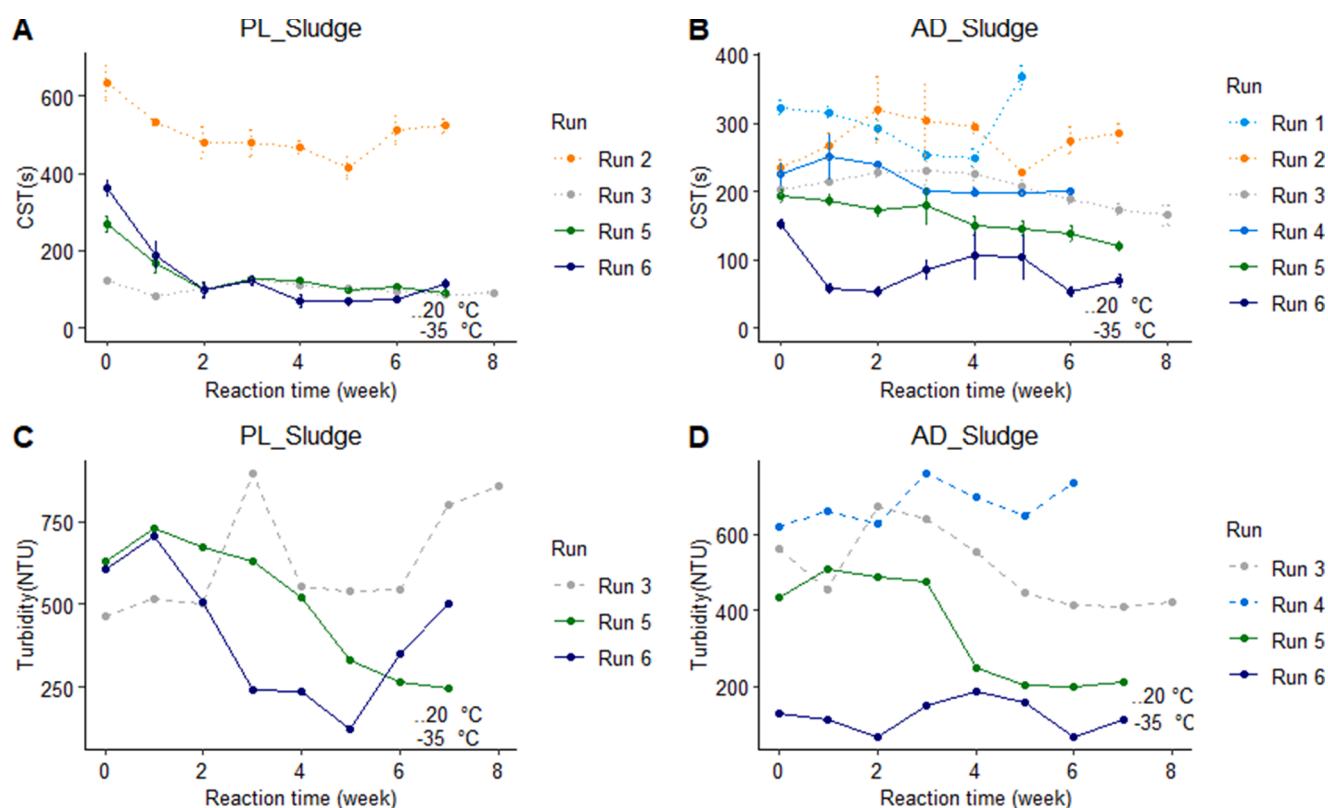
**Fig. 2.** Total biogas production of BMP runs A (at 20 °C and 37 °C) and B (at 20 °C). Inocula were anaerobic digester sludge (AD), pit latrine sludge (PL), cow manure (CM), and septic tank sludge (ST). Runs A and B were fed with FS recipes that had feces to urine of ratio 1:2.5 and 1:3.75 respectively.

and run 4 at 35 °C whiles PL sludge was used in runs 2 and 3. In runs 2, and 4, two reactors were operated in parallel fed with the 1:2.5 feces: urine feed, with one reactor in run 4 fed with synthetic wastewater as a control reactor. Although the reaction conditions and inocula were the same for runs 2 and 3, the two runs differed in the reaction time. In run

4, 35 °C reaction temperature was selected for comparison with the literature and the control reactor with synthetic wastewater was used to control for the interference of other substances in FS and to verify that the FS feed had adequate nutrients for growth. The reactor contents were mixed continuously using Heidolph R2R2020 mechanical stirrers



**Fig. 3.** (A) Relative abundance of the microbial community composition of the different inocula; anaerobic digested sludge (AD), cow manure (CM), pit latrine sludge (PL), septic tank sludge (ST) and feed based on the amplicon sequence variants. (B) Predicted functional potential based on the least common ancestor.

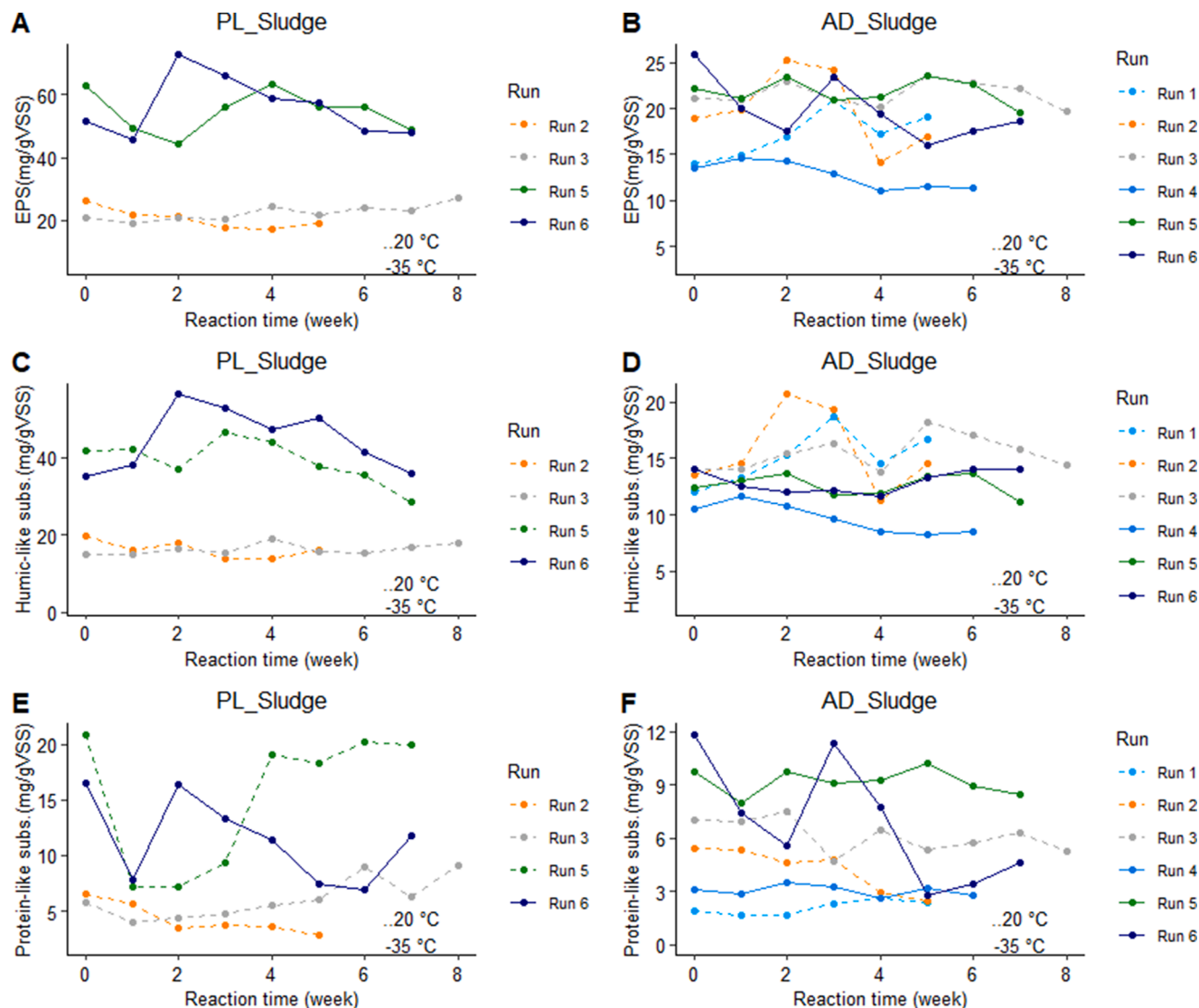


**Fig. 4.** Line plots for CST and turbidity during anaerobic storage of FS with PL sludge and AD sludge as inoculum. (4A) and (4B) are CST for PL and AD runs over time. (4C) and (4D) are turbidity measurements for PL and AD sludge runs.

at speed 7. Biogas was collected in 10 L plastic biogas collection bags and the volume and methane content determined by a gas sensor (Ritter, drum type TG-series). Runs 5 and 6 were operated in parallel with

similar conditions and inocula but in different setups to evaluate mixing in the reactors. Run 5 consisted of 5 L and 2 L glass batch reactors for AD and PL sludge respectively and the operational temperature was 35 °C.





**Fig. 5.** Line plot showing total EPS concentrations and fractions of EPS during anaerobic storage. (5A) and (5B) are total EPS concentrations for PL and AD sludge runs respectively. (5C) and (5D) are the concentrations of humic-like substances fraction of PL and AD sludge runs (5E) and (5F) are the protein-like substance fraction of EPS in PL and AD sludge runs.

Glass batch reactors were used to visually verify adequate mixing which was achieved with a magnetic stirring rod and biogas was collected in 2 L biogas. Run 6, hereafter referred to as the “serum bottle test” was conducted in serum bottles with the same setup as described in Section 2.2. This verification of mixing in glass reactors was conducted because the degradation of organic matter in runs 1–4 was less than expected in the anaerobic batch reactors which were opaque and did not allow for visual observation of mixing.

## 2.4. Physicochemical analysis

### 2.4.1. Sample analysis

The inoculum, feed and content of the reactors were analyzed for total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS), using methods for FS analysis (Velkushanova et al., 2021). COD and soluble COD were determined with HACH Lange test kits according to the manufacturer’s instructions which is based on the American Public Health Association (APHA) standard methods 5220 D. Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and total nitrogen (TN) were measured with HACH Lange test kits based on APHA standards 5400N-C and 4500NH3-F respectively. Alkalinity was

determined using the titration method and volatile fatty acids (VFA) were analysed using a Shimadzu 881 compact IC pro ion Chromatograph.

### 2.4.2. Extracellular polymeric substances

EPS measurements were performed by the method described by Ward et al. (2019). The EPS concentration and fractions were measured with the size-exclusion chromatography organic carbon detection-organic nitrogen detection (LC—OCD-OND) and fiffikus software (DOC-Labor Dr. Huber,

Germany) was used for the analysis of the different fractions. The LC OCD OND chromatogram presents the fraction of the sample according to their molecular weight in the dissolved phase. Five peaks are generated, representing biopolymers ( $20,000\text{--}7.5 \times 10^{11}$  g/mol), humic substances ( $\sim 1000$  g/mol), building blocks ( $\sim 300\text{--}500$  g/mol), low molecular weight organics ( $<350$  g/mol), and neutrals including aldehydes and ketones ( $<350$  g/mol) (Huber et al., 2011; Jacquin et al., 2017). The biopolymer fraction of EPS in this study had a low C/N ratio (2–7) which indicates that the biopolymers are composed mainly of proteins. Thus the EPS was therefore categorized into protein-like (biopolymer peak) and humic-like substances (humic acids and

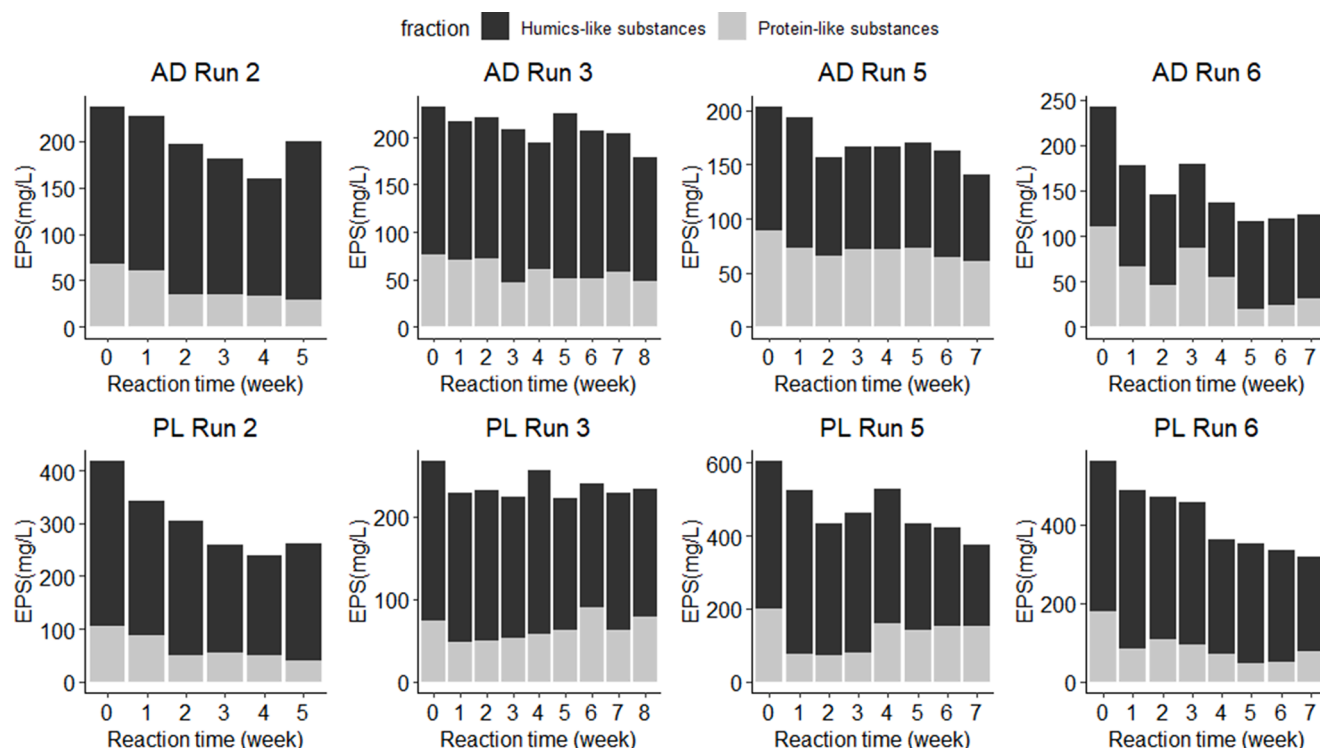


Fig. 6. Bar plots showing total EPS concentrations broken down into specific fractions comprised of protein and humic-like substances of EPS (mg/L) over the reaction time for runs 2, 3, 5 and 6 where AD and PL sludges were used for anaerobic storage of FS.

building block peak) according to Jacquin et al. (2017) and Ward et al. (2019) and the total EPS was calculated as the sum of the two.

#### 2.4.3. EPS extraction

EPS (soluble/loosely-bound) was extracted from activated sludge obtained from Eawag and from pit latrine FS samples using the  $\text{Na}_2\text{CO}_3$  method described by Shambeck et al. (2020) and sonication as described in detail by Ward et al. (2019), followed by centrifugation at 3500 g for 20 min. To determine the effect of EPS on dewaterability in terms of CST, two tests were performed. In test A, 2 ml of soluble/loosely-bound EPS was added to 10 ml of AD sludge and PL sludge samples and compared to addition of 2 ml of water as a control. Samples were vortexed for 1 min and CST was measured using the setup described in Section 2.4.4. In test B, different weights (0.01, 0.02, 0.05 and 0.1 gs) of freeze-dried EPS was added to 10 ml of AD sludge and PL sludge samples, vortexed and CST measured accordingly. CST of AD sludge and PL sludge samples were measured prior to and after EPS addition.

#### 2.4.4. Dewaterability

Sludge dewaterability was assessed by measuring the capillary suction time (CST) and turbidity of supernatant following centrifugation. CST measures the time required for water to pass through a filter paper (filterability), whereas supernatant turbidity indicates the extent of settleability of the sludge. CST was measured in quadruplicates according to Methods for Fecal Sludge Analysis (Velkushanova et al., 2021) using the 319 Multi-CST apparatus instrument from Triton Electronics Ltd, UK with an 18 mm funnel. Supernatant turbidity of centrifuged sludge was measured using 30 ml of sludge and centrifuging at 3000 x g for 20 min in a 50 ml falcon tube. The turbidity of the supernatant after centrifugation was determined with a HACH TL 2300 turbidity meter.

#### 2.4.5. Particle size distribution

Particle size distribution was analyzed using the static light scattering measurement according to AHPA standard method 2560D using a

Beckman Coulter LS 13 320-Laser Diffraction Particle Size Analyzer. Liquid samples were gently mixed by pipetting with a Pasteur pipette to homogenize the sample without breaking up aggregates, and then dispersed for measurement using the Universal Liquid Module, which is capable of suspending and analyzing samples in the 0.017 – 2000  $\mu\text{m}$  size range.

#### 2.4.6. Microbial community analysis

To assess the influence of the microbial community of the different inocula on the BMP test, 2 ml sample of each inoculum was centrifuged at 6000 rcf for 10 min. The supernatant was discarded, and 1 ml of RNA later was added to the pellets and stored at  $-20^\circ\text{C}$  until DNA extraction. DNA was extracted following a modified method by Griffiths et al. (2000). To each inoculum pellet, 0.5 ml of hexadecyltrimethylammonium bromide buffer was added and gently mixed with the sample. The mixture was transferred to a 2 ml lysing matrix tube. 0.5 ml of phenol: chloroform isoamylalcohol (PCI) (25:24:1, pH 6.8) was added after which a FastPrep equipment is used to lyse the sludge samples. Further extraction was carried out with the addition of 0.5 ml of Chloroform Isoamylalcohol (CI) 24:1 to each sample. Nucleic acids were precipitated with Polyethylene glycol 6000 on ice and further washed with 70% ethanol before being dissolved in 100  $\mu\text{l}$  of molecular grade water.

Nucleic acid quality and quantity was determined with a Nanodrop ND-2000c. 16S rRNA gene amplicon sequencing was carried out by Novogene on an illumina MiSeq platform based on bacterial and archaeal V4 region. Raw sequences were analysed within the QIIME2 framework. Taxonomical assignment of the amplicon sequence variants (ASVs) was performed within QIIME2 environment with the MIDAS database ((Nierychlo et al., 2020), accessed October 2021). Based on the relative abundances, we plotted the top bacterial phyla for each sample. Picrust2 (Douglas et al., 2020) was used to predict the functional potential of the microbial communities based on the lowest common ancestor approach where the 16S rRNA genes are mapped against a public available genome reference database that allows for prediction of

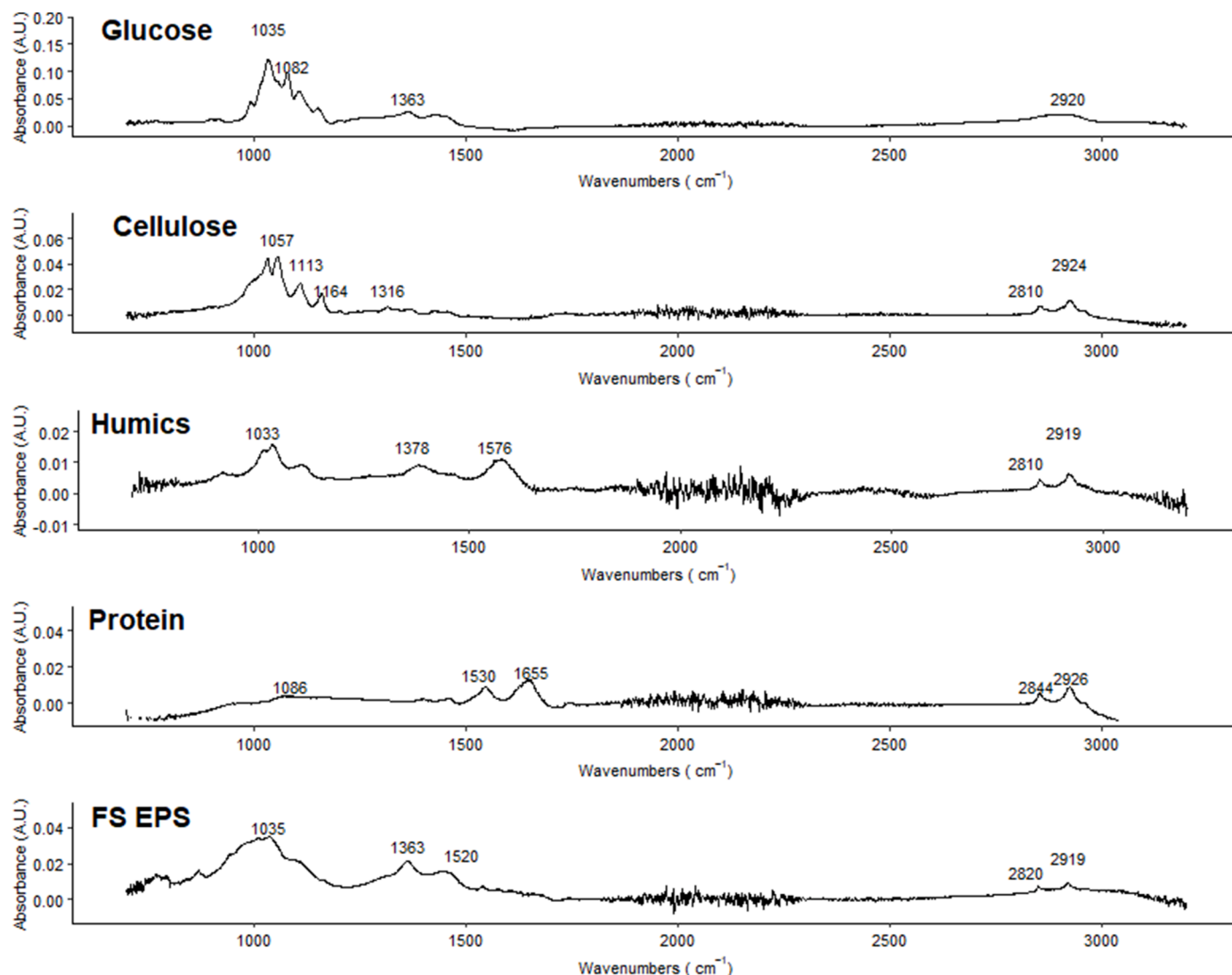


Fig. 7. FTIR spectra of the glucose, cellulose, humic acid, and protein standards and EPS extracted from FS.

the functional potential.

#### 2.4.7. Statistical analysis

The Kendall rank correlation was used to assess the potentially non-linear dependencies between the measured parameters and anaerobic storage time. A p-value below 0.05 was considered statistically significant. The same approach was used to determine the correlation between factors that affect dewaterability (EPS and particle size) and the metrics of dewaterability (CST and turbidity).

The data for this study are openly available in eawag repository [<https://doi.org/10.25678/0006FP>].

### 3. Results and discussion

#### 3.1. Effect of EPS on dewaterability of fecal sludge and wastewater sludge

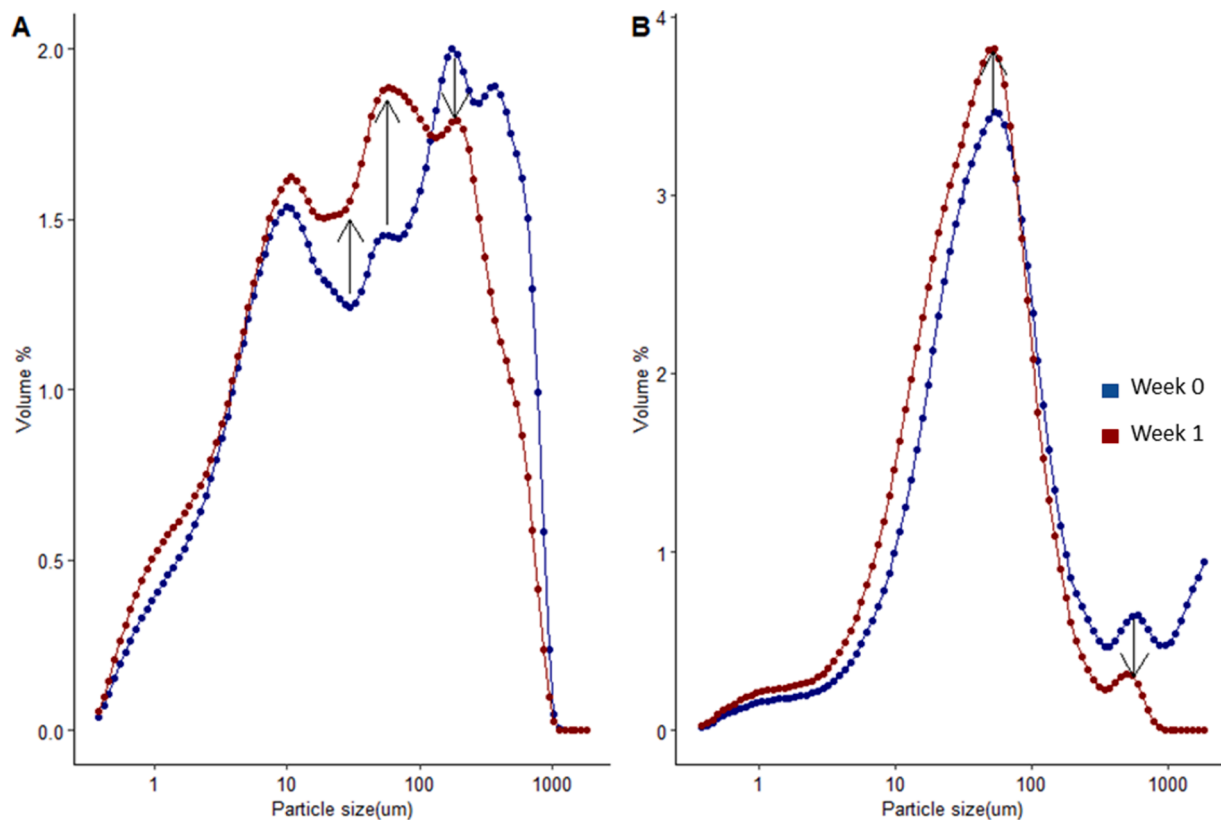
As illustrated in Fig. 1, the influence of EPS on the dewaterability of FS and AD was evaluated by measuring changes in CST with the addition of aliquots of soluble/loosely-bound (Fig 1A) and freeze-dried form of the same soluble/loosely-bound EPS (Fig 1B), that was extracted from FS and activated sludge with two different methods of extraction (Sonication and  $\text{Na}_2\text{CO}_3$ ). Addition of EPS extracted from both FS and activated sludge had the same effect on dewaterability, and increasing amounts of EPS continued to decrease dewaterability indicated by a

high CST. This confirms that concentrations of EPS can govern dewaterability in FS, and that EPS from FS and activated sludge have a similar effect. Ward et al. (2019) also reported that FS samples collected in Senegal and Tanzania had lower dewaterability with higher concentrations of EPS. An increase in EPS results in increased clogging of the filter media, thus reducing the dewatering performance. As suggested by Novak et al. (2003), the release of bound EPS into solution, is expected to decrease sludge dewaterability. Based on experiences in municipal wastewater where EPS increases during activated sludge (Jia et al., 1996; Liu and Fang, 2003), and decreases during anaerobic digestion (Lei et al., 2007; Nielsen et al., 1996). If EPS is the main controller of dewaterability of FS, it is expected that anaerobic storage will result in degradation of biopolymers resulting in improved dewaterability.

#### 3.2. Preliminary BMP tests

Presented in Fig. 2 are results of the total biogas production for BMP Runs A and B, which were conducted to validate FS degradation under anaerobic storage with different inocula. Results of biogas from the positive controls are presented in table S2. Biogas from the AD sludge, which is a conventional inoculum for BMP tests, was within 86–98% of the theoretical biogas production. ST sludge had 97% of the theoretical biogas from microcrystalline cellulose, CM 59–94%, and PL 8–53%. A range of field temperatures have been reported for onsite containments,





**Fig. 8.** Line plot showing the particle size distribution of FS during anaerobic storage. (7A) and (7B) represents the size distribution of the initial and final sludge samples of the PL and AD inoculated runs respectively.

for example 22.3 - 30.7 °C for pit latrines in Kampala (Nakagiri et al., 2017), 30 °C for septic tanks in Hanoi (Huynh et al., 2021), 30–32 °C for pit latrines in Morogoro, Tanzania (van Eekert et al., 2019), 19–32 °C pit latrines in Tanzania and Vietnam (Torondel et al., 2016). The selection of 20 °C and 37 °C for the BMP test therefore covers the range of reported temperatures. Using a feed with 1:2.5 feces to urine ratio, the AD inoculum produced similar volumes of biogas at 20 °C and 37 °C, whereas the PL inoculum had higher biogas production at 37 °C than 20 °C. However, TS and ammonium concentration may have contributed more to the lower biogas production than temperature, as PL sludge had a higher concentration of TS (see Table 1) that could have resulted in a mass transfer limitation and ammonia inhibition due to concentrations > 1.5 g/l (Zuo et al., 2021). The BMP test demonstrated that anaerobic degradation of FS was possible irrespective of the inoculum, temperature and  $\text{NH}_4^+\text{-N}$  concentration.

### 3.3. Microbial community analysis

Varying biogas production in the BMP tests could also be associated with microbial community, as illustrated in Fig. 3. Dominant bacterial phyla observed in all inocula included *Proteobacteria* (12–21%), *Firmicutes* (18–35%) and *Bacteroidetes* (6–25%). While members of *Chloroflexi* were highly abundant in AD (30%), ST (19%) and CM (12%) they only made up a small fraction in the PL inoculum (3%). This is in agreement with members of *Proteobacteria*, *Firmicutes* and *Bacteroidetes* being reported as most abundant in FS samples (Ward et al., 2019). While phylum *Latescibacteria* which is present in animal intestines and sediments (Farag et al., 2017) appeared as a dominant phylum in the ST inoculum, it was absent in all other inocula. Similarly, the phylum *Deinococcota* found mainly in animal feces and intestines (Murray, 2004) was also unique to the cow manure inoculum. Besides the differences in community composition at the phylum level, the different inocula presented significant differences in diversity within the group of

methane-producing bacteria (Figure S2). A prediction of the functional potential of the community via a lowest common ancestor approach (Douglas et al., 2020) as illustrated in Fig. 3B indicates a high degree of functional redundancy despite the differences in community composition. It is therefore not surprising that irrespective of the inoculum used, FS was degraded to some extent as indicated by the biogas production.

### 3.4. Effect of anaerobic storage on dewaterability

Illustrated in Fig. 4A and 4B are the changes in CST(s) with anaerobic storage time for PL and AD inoculated runs. Using the Kendall rank correlation with a 95% confidence level to test the correlation between CST(s) and storage time, we observed that CST(s) for both PL and AD inoculated runs generally decreased with anaerobic storage (table S3). However, the decrease in CST(s) were only statistically significant ( $p < 0.05$ ) for run 5 inoculated with AD and runs 2 and 5 inoculated with PL. Variations in supernatant turbidity, illustrated in Fig. 4C and 4D for PL and AD inoculated runs, showed both a decrease and increase in supernatant turbidity with anaerobic storage. Reduction in turbidity was only statistically significant for run 5 for PL sludge and runs 3 and 5 for AD sludge. A more clear trend in decreasing CST and supernatant turbidity was expected based on the literature (Sakaveli et al., 2021).

### 3.5. Influence of anaerobic storage on EPS and EPS fractions

Reported in Fig. 5A and 5B are EPS/VSS concentration for PL and AD inoculated runs with anaerobic storage time. It was observed that for PL inoculated runs, in three out of four runs (2, 5, and 6) there was decreasing EPS/VSS and in AD inoculum runs five out of six runs (2, 3, 4, 5, and 6). However, the reductions were not statistically significant, which indicates that there is no preferential degradation of EPS over other VSS components. Also illustrated in Fig. 5C, 5D, 5E and 5F are the humic-like and protein-like fractions of the extracted EPS with time

during anaerobic storage. There were no clear trends, and changes in humic-like substances were statistically significant for only run 2 in the PL inoculated runs, and for protein-like substances only run 2 in both PL and AD inoculated runs.

However, EPS (mg/L) for runs involving both AD and PL shown in Fig. 6 shows a decrease with time. Decreasing EPS with time in anaerobic storage was expected and agrees with observations by Nielsen et al. (1996) where anaerobic storage resulted in a reduction in EPS. However, the total reduction was less than expected, possibly due to the lower initial concentrations of EPS in FS.

Illustrated in Fig. 6 are the total concentrations of EPS as mg/L, and the specific fractions comprised of protein and humic-like substances of EPS (mg/L) over the reaction time for runs 2, 3, 5, and 6 for comparison between AD and PL runs. The figure indicates that the fractions of EPS were generally degraded with anaerobic storage time with an average reduction of 40% for protein-like and 22% for humic-like fraction in AD inoculated runs. In runs inoculated with PL on the other hand, an average of 47% and 33% degradation was observed for protein- and humic-like substances respectively. The decrease in total EPS (mg/L) and EPS fractions generally did not have a significant correlation with metrics of dewaterability (CST) using the Kendall rank correlation (table S5). Although degradation is seen in the total EPS (mg/L), the extent of degradation is lower relative to anaerobic storage or digestion of activated sludge, where a higher reduction of EPS is observed. However, of interest, humic-like substances in all samples were about twice the concentration of protein-like fractions. This observation is similar to FS field samples from Senegal and Tanzania (Ward et al., 2019) but is contrary to wastewater sludges where proteins and carbohydrates constitute a higher fraction of EPS (Neyens et al., 2004), indicating that EPS from FS is comprised of greater fractions of humics.

To verify the legitimacy of our observations of high humic-like substances in FS EPS, FTIR was performed as a qualitative analysis on the extracted EPS together with standard samples of glucose, cellulose, humic acids, and proteins. As illustrated in Fig. 7, peaks representing, carboxylic or hydrocarbon containing compounds ( $1500\text{--}1300\text{ cm}^{-1}$ ) and carbohydrates ( $1200\text{--}900\text{ cm}^{-1}$ ) were clearly evident (Badireddy et al., 2010). A major peak between  $1033$  and  $1086\text{ cm}^{-1}$ , which is attributed to C–O stretching of polysaccharides or polysaccharide-like substances, was seen in all samples except proteins. Two prominent peaks around  $2850\text{ cm}^{-1}$  and  $2919\text{ cm}^{-1}$  which are aliphatic C–H group stretching of fatty acids and long chain structures were seen in all samples (Zhu and Zhao, 2011). The spectrum of the extracted EPS showed more similarities in peaks with the humic acid and cellulose standard, which supports the measurement of higher humic acid concentrations in the extracted EPS from FS.

### 3.6. Performance of BMP tests and anaerobic batch reactors

All inocula in BMP tests were monitored for pH, VFA, alkalinity and  $\text{NH}_4^+\text{-N}$  concentrations to ensure adequate operating conditions. The inocula all had a pH between 7.2 to 8.0, VFA between 0.1 and 1.6 g/L acetate,  $\text{NH}_4^+\text{-N}$  between 0.8 and 3.3 g/L and alkalinity between 2.8 and 9.4 g/L  $\text{CaCO}_3$ . Recommendations for BMP test are  $\text{pH} > 7.0$  and  $< 8.5$ ,  $\text{VFA} < 1\text{ g/L}$  acetate,  $\text{NH}_4^+\text{-N} < 2.5\text{ g/L}$   $\text{NH}_4^+\text{-N}$  and alkalinity  $> 3\text{ gCaCO}_3\text{ L}^{-1}$  (Holliger et al., 2016). The percentage of  $\text{CH}_4$  in biogas was 58–68%. Anaerobic batch reactors and serum bottle tests were monitored for biogas, methane, VFA, pH, alkalinity and reduction in VS and COD. Biogas was produced in all reactors with methane of 56–70%. The VFA/alkalinity ratio (g/L: g/L  $\text{CaCO}_3$ ) was between 0.09 and 0.31 and pH between 6.85–7.92. Average VS and COD reductions were 20% and 30% respectively. VS reduction was low (expected 50–65% (Tchobanoglous et al., 2014)), however the control synthetic wastewater showed a comparable VS reduction of 24% indicating no inhibition in the reactors.

### 3.7. Influence of particle size distribution

Fig. 8 illustrates the particle size distribution of samples from the start and end times of reactors inoculated with PL and AD sludge in run 6, and the percentage of particles by volume from  $0.4\text{--}2000\text{ }\mu\text{m}$ . Particle size distribution was performed in run 6 to see if it could help explain the dewaterability results in runs 1–5. While PL sludge shows multiple peaks at 30, 52, and  $185\text{ }\mu\text{m}$ , AD sludge had a unimodal distribution with a peak at  $52\text{ }\mu\text{m}$  and a shoulder at  $560\text{ }\mu\text{m}$  in the higher particle size range. The results indicate that, in both AD and PL reactors, anaerobic storage resulted in an increase in supracolloidal particles ( $1\text{--}100\text{ }\mu\text{m}$ ) and a decrease of larger particles. During anaerobic storage of wastewater sludge the breakdown of organic matter generates more supracolloidal particles, which if not completely utilized are known to have a negative effect on dewaterability (higher CST) (Rudolf and Heukelekian, 1934). However, in this study there was no significant correlation between the supracolloidal particles and the CST or turbidity for both AD and PL inoculated runs (Table S3). In wastewater sludges, tightly bound EPS provides binding sites for flocculation, which improves dewaterability (Lin et al., 2019), whereas extracted soluble and loosely bound EPS in this study, represents colloidal and suspended organic substances that affect dewaterability by clogging of filter media (Ward et al., 2019), and not flocculation. If extracted soluble and loosely bound EPS is being degraded at the same time that small particles are generated, it is difficult to know which is contributing more to dewaterability. It is possible that the influence of EPS on CST is more pronounced than the effect of small particle generation and this is an area for further research.

### 3.8. Elucidation the role of EPS in dewaterability of FS

In this study, it was empirically demonstrated that the addition of extracted EPS decreases dewaterability. However, the degradation of EPS during anaerobic storage was not as great as expected, and the relation to dewaterability was not clear. Improvement in sludge dewaterability has been reported with both an increase or a decrease in EPS (Liu and Fang, 2003; Shahid et al., 2022). Houghton and Stephenson (2002) argued that an initial increase in EPS increases dewaterability, but beyond a certain EPS threshold, dewaterability decreases. Fractions of EPS (mainly proteins and carbohydrates) have also been reported as having different effects on sludge dewaterability (Cetin and Erdinciler, 2004; Sheng et al., 2010). EPS concentrations in this study ( $9\text{--}72\text{ mgEPS/gVSS}$ ) were lower than reported EPS concentrations in wastewater sludges using similar methods of extraction ( $30$  and  $290\text{ mg/gVSS}$ ) (Caudan et al., 2012; Comte et al., 2006; Pellicer-Nacher et al., 2013). The EPS concentration in this study is below the  $100\text{ mgEPS/gTSS}$  that has been suggested as a minimum for floc formation (Jørgensen et al., 2017), and rather fits the description of colloidal and suspended organic substances that affect dewaterability by clogging of filter media (Ward et al., 2019). In addition, humic-like and protein-like fractions of EPS did not follow clear trends during anaerobic storage, or have significant relations to sludge dewaterability, but the humic-like fractions of EPS were consistently greater than for wastewater sludges.

## 4. Implications to fecal sludge treatment

In this study, anaerobic storage of FS did not fit into the well-known anaerobic digestion model (Batstone et al., 2002), which predicts 50–60% VS reduction. The lower average VS reduction (20%) observed in this study, indicates that anaerobic degradation of FS follows different kinetics than for wastewater sludges. This could be due to inhibitory factors or organic fractions in feces (Rose et al., 2015), which are difficult to degrade and different from biological (secondary) or blended (primary and secondary) wastewater sludges. Further studies should consider the behavior of the less biodegradable organic compounds such as cellulose and lignin during anaerobic storage. In addition,

experiments in this study were based on the general consensus that predominantly anaerobic conditions are present in onsite containments. As research in FS increases, these conventions are being questioned, and purely anaerobic conditions may or may not be totally reflective of onsite containments, especially in the surface or border regions (Shaw and Dorea, 2021). The presence of anaerobic, aerobic and anoxic zones in onsite containments needs to be further investigated, in order to understand the degradation kinetics of organics in different layers, and predict what is occurring throughout storage in containment with time (López-Vázquez et al., 2021).

## 5. Conclusions

To the best of our knowledge, this was the first study to evaluate EPS concentrations in FS during anaerobic storage in the laboratory, and to assess the influence of changing total EPS concentrations and fractions on FS dewaterability, conclusions from this research include:

- EPS has a significant effect on FS dewaterability. Irrespective of the extraction method or the source of sludge used in this study, an increase in EPS concentration of the sludges decreased dewaterability.
- FS is different from wastewater sludges meaning knowledge of wastewater treatment performance cannot be directly transferred. Fundamental differences include lower overall concentrations of EPS and greater concentrations of humic-like fractions of EPS.
- It is clear that EPS plays a role in dewaterability of FS. However, due to the lower than predicted degradation during anaerobic storage, the fate of tightly bound, loosely bound, and humic, protein, and carbohydrate fractions of EPS needs to be further investigated, in addition to the contribution of other compounds with water holding capacities.
- Anaerobic storage time is not a predictor of particle size distribution, other physical properties such as charge density that play a role in dewaterability need to be further investigated.
- Differences in degradation of EPS and changes in particle size distribution are likely related to variations in microbial community, there remains a lack of knowledge of pathways of digestion of FS during storage in containment.

## Author contribution

Sam S.B., Ward B.J., Strande L. and Morgenroth E. contributed to study design. All authors provided helpful feedback and suggestions throughout the study. Sam S.B. was responsible for experimental design, reactor setup, collection and analysis of sample. Ward B.J. performed particle size distribution analysis. Niederdorfer R. performed the data analysis on microbial community. Sam S.B. took the lead in writing the manuscript, Strande L. contributed to data analysis and writing with critical and helpful reviews from all authors. Strande L. conceived the idea, obtained funding and supervised the project.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

The data for this study is openly available in eawag data repository [https://doi.org/10.25678/0006FP]

## Acknowledgments

Financial support for this study was provided by the Swiss National

Foundation for Scientific Research (SNSF). We acknowledge Jacqueline Traber for helping with EPS extraction procedure, Karin Beck and Patrick Kathriner for helping with DNA extraction methods and gas measurement respectively, as well as Helmut Bergmann for the expert advice on the DNA extraction. The authors would like to thank Andreas Scheidegger and Damian Hausherr for the helpful scientific discussions and all the technical staff of the experimental hall, EAWAG in Dübendorf.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118915.

## References

- 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 biometane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* 59 (5), 927–934. <https://doi.org/10.2166/wst.2009.040>. *NLM*.
- Badireddy, A.R., Chellam, S., Gassman, P.L., Engelhard, M.H., Lea, A.S., Rosso, K.M., 2010. Role of extracellular polymeric substances in bioflocculation of activated sludge microorganisms under glucose-controlled conditions. *Water Res.* 44 (15), 4505–4516. <https://doi.org/10.1016/j.watres.2010.06.024>. *ScienceDirect*.
- Bakare, B.F., Foxon, K.M., Brouckaert, C.J., Buckley, C.A., 2012. Variation in VIP latrine sludge contents. *Water SA* 38 (4), 479–486. <https://doi.org/10.4314/wsa.v38i4.2>.
- Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. The IWA anaerobic digestion model No 1 (ADM1). *Water Sci. Technol.* 45 (10), 65–73. <https://doi.org/10.2166/wst.2002.0292>.
- Brouckaert, C.J., Foxon, K.M., Wood, K., 2013. Modelling the filling rate of pit latrines. *Water SA* 39 (4), 555–562. <https://doi.org/10.4314/wsa.v39i4.15>.
- Caudan, C., Filali, A., Lefebvre, D., Spérandio, M., Girbal-Neuhausser, E., 2012. Extracellular polymeric substances (EPS) from aerobic granular sludges: extraction, fractionation, and anionic properties. *Appl. Biochem. Biotechnol.* 166 (7), 1685–1702. <https://doi.org/10.1007/s12010-012-9569-z>. *Springer Link*.
- Cetin, S., Erdinciler, A., 2004. The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. *Water Sci. Technol.* 50 (9), 49–56.
- Christensen, M.L., Keiding, K., Nielsen, P.H., Jorgensen, M.K., 2015. Dewatering in biological wastewater treatment: a review. *Water Res.* 82, 14–24. <https://doi.org/10.1016/j.watres.2015.04.019>.
- 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 Sustain. Dev.* 29, 57–64. <https://doi.org/10.1016/j.esd.2015.09.005>.
- Comte, S., Guibaud, G., Baudu, M., 2006. Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: part I. Comparison of the efficiency of eight EPS extraction methods. *Enzyme Microb. Technol.* 38 (1), 237–245. <https://doi.org/10.1016/j.enzmictec.2005.06.016>. *ScienceDirect*.
- Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.L., 2020. PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* 38 (6), 685–688. <https://doi.org/10.1038/s41587-020-0548-6>.
- Farag, I., Youssef, N., Elshahed, M., 2017. Global distribution patterns and pangenomic diversity of the candidate phylum lateisobacteria (WS3). *Appl. Environ. Microbiol.* 83 <https://doi.org/10.1128/AEM.00521-17>. *AEM.00521-17*.
- Filer, J., Ding, H.H., Chang, S., 2019. Biochemical Methane Potential (BMP) Assay Method For Anaerobic Digestion Research. *Water*, p. 11. <https://doi.org/10.3390/w11050921>.
- Gold, M., Harada, H., Therrien, J.D., Nishida, T., Cunningham, M., Semiyaga, S., Fujii, S., Dorea, C., Nguyen, V.A., Strande, L., 2017. Cross-country analysis of faecal sludge dewatering. *Environ. Technol.* 1–11. <https://doi.org/10.1080/09593330.2017.1374472>.
- Griffiths, R.L., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* 66 (12), 5488–5491.
- Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., de Wilde, V., Ebertseder, F., Fernández, B., Ficarra, E., Fotidis, I., Frigon, J.-C., de Lacroix, H.F., Ghasimi, D.S.M., Hack, G., Hartel, M., Wierinck, I., 2016. Towards a standardization of biometane potential tests. *Water Sci. Technol.* 74 (11), 2515–2522. <https://doi.org/10.2166/wst.2016.336>.
- Houghton, J.L., Stephenson, T., 2002. Effect of influent organic content on digested sludge extracellular polymer content and dewaterability. *Water Res.* 36 (14), 3620–3628. [https://doi.org/10.1016/S0043-1354\(02\)00055-6](https://doi.org/10.1016/S0043-1354(02)00055-6).
- Huber, S.A., Balz, A., Abert, M., Pronk, W., 2011. Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography – organic carbon detection –

- organic nitrogen detection (LC-OCD-OND). *Water Res.* 45 (2), 879–885. <https://doi.org/10.1016/j.watres.2010.09.023>.
- Huynh, L.T., Harada, H., Fujii, S., Nguyen, L.P.H., Hoang, T.-H.T., Huynh, H.T., 2021. Greenhouse gas emissions from blackwater septic systems. *Environ. Sci. Technol.* 55 (2), 1209–1217. <https://doi.org/10.1021/acs.est.0c03418>.
- Jacquin, C., Lesage, G., Traber, J., Pronk, W., Heran, M., 2017. Three-dimensional excitation and emission matrix fluorescence (3DEEM) for quick and pseudo-quantitative determination of protein- and humic-like substances in full-scale membrane bioreactor (MBR). *Water Res.* 118, 82–92. <https://doi.org/10.1016/j.watres.2017.04.009>.
- Jenkins, D., Wanner, J., 2014. *Activated Sludge—100 Years and Counting*. IWA Publishing, London, UK.
- Jia, X.S., Furumai, H., Fang, H.H.P., 1996. Extracellular polymers of hydrogen-utilizing methanogenic and sulfate-reducing sludges. *Water Res.* 30 (6), 1439–1444. [https://doi.org/10.1016/0043-1354\(96\)00028-0](https://doi.org/10.1016/0043-1354(96)00028-0).
- Jørgensen, M.K., Nierychlo, M., Nielsen, A.H., Larsen, P., Christensen, M.L., Nielsen, P. H., 2017. Unified understanding of physico-chemical properties of activated sludge and fouling propensity. *Water Res.* 120, 117–132.
- Junglen, K., Rhodes-Dicker, L., Ward, B.J., Gitau, E., Mwalugongo, W., Stradley, L., Thomas, E., 2020. Characterization and prediction of fecal sludge parameters and settling behavior in informal settlements in Nairobi, Kenya. *Sustainability* 12 (21), 9040. <https://doi.org/10.3390/su12219040>.
- Lawler, D.F., Chung, Y.J., Hwang, S.-J., Hull, B.A., 1986. Anaerobic digestion: effects on particle size and dewaterability. *J. (Water Pollut. Control Federation)* 58 (12), 1107–1117.
- Lei, Z., Luo, X., Zhang, Z., Sugiura, N., 2007. Effects of variations of extracellular polymeric substances and soluble microbial products on activated sludge properties during anaerobic storage. *Environ. Technol.* 28 (5), 529–544. <https://doi.org/10.1080/09593330.2007.9619781>.
- Lin, F., Zhu, X., Li, J., Yu, P., Luo, Y., Liu, M., 2019. Effect of extracellular polymeric substances (EPS) conditioned by combined lysozyme and cationic polyacrylamide on the dewatering performance of activated sludge. *Chemosphere* 235, 679–689. <https://doi.org/10.1016/j.chemosphere.2019.06.220>.
- Liu, Y., Fang, H.H.P., 2003. Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge. *Crit. Rev. Environ. Sci. Technol.* 33 (3), 237–273. <https://doi.org/10.1080/10643380390814479>. Taylor and Francis+NEJM.
- López-Vázquez, C.M., Rincon, Rubio, Francisco, J., Brdjanovic, Damir, 2021. Towards city-wide inclusive sanitation (CWIS) modelling—modelling of faecal sludge containment/treatment processes. *Methods For Faecal Sludge Analysis*. IWA Publishing, pp. 145–185.
- Mikkelsen, L.H., Keiding, K., 2002. Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Res.* 36 (10), 2451–2462. [https://doi.org/10.1016/S0043-1354\(01\)00477-8](https://doi.org/10.1016/S0043-1354(01)00477-8). ScienceDirect.
- Murray, R., 2004. *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. Springer-Verlag.
- Nakagiri, A., Niwagaba, C.B., Nyenje, P.M., Kulabako, R.K., Tumuhairwe, J.B., Kansime, F., 2017. Assessing ambient and internal environmental conditions of pit latrines in urban slums of Kampala, Uganda: effect on performance. *J. Water, Sanitation Hygiene Develop.* 7 (1), 92–101.
- Neyens, E., Baeyens, J., Dewil, R., De heyder, B., 2004. Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *J. Hazard. Mater.* 106 (2), 83–92. <https://doi.org/10.1016/j.jhazmat.2003.11.014>. ScienceDirect.
- Nielsen, P.H., Frølund, B., Keiding, K., 1996. Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage. *Appl. Microbiol. Biotechnol.* 44 (6), 823–830. <https://doi.org/10.1007/BF00178625>. Springer Link.
- Nierychlo, M., Andersen, K.S., Xu, Y., Green, N., Jiang, C., Albertsen, M., Dueholm, M.S., Nielsen, P.H., 2020. MiDAS 3: an ecosystem-specific reference database, taxonomy and knowledge platform for activated sludge and anaerobic digesters reveals species-level microbiome composition of activated sludge. *Water Res.* 182, 115955.
- Niwagaba, C.M., Mbéguéré, M., Strande, L., 2014. Faecal sludge quantification, characterization and treatment objectives. *Faecal Sludge Management: Systems Approach for Implementation and Operation*. IWA Publishing, pp. 9–44.
- Novak, J.T., Sadler, M.E., Murthy, S.N., 2003. Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Res.* 37 (13), 3136–3144.
- Pellicier-Nächer, C., Domingo-Félez, C., Mutlu, A.G., Smets, B.F., 2013. Critical assessment of extracellular polymeric substances extraction methods from mixed culture biomass. *Water Res.* 47 (15), 5564–5574. <https://doi.org/10.1016/j.watres.2013.06.026>. ScienceDirect.
- Rose, C., Parker, A., Jefferson, B., Cartmell, E., 2015. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Crit. Rev. Environ. Sci. Technol.* 45 (17), 1827–1879. <https://doi.org/10.1080/10643389.2014.1000761>.
- Rudolfs, W., Heukelekian, H., 1934. Relation between drainability of sludge and degree of digestion. *Sewage Work J.* 6 (6), 1073–1081.
- Sakaveli, F., Petala, M., Tsiroidis, V., Darakas, E., 2021. Enhanced mesophilic anaerobic digestion of primary sewage sludge. *Water (Basel)* 13 (3), 348.
- Schambeck, C.M., Girbal-Neuhauser, E., Böni, L., Fischer, P., Bessière, Y., Paul, E., da Costa, R.H.R., Derlon, N., 2020. Chemical and physical properties of alginate-like exopolymers of aerobic granules and flocs produced from different wastewaters. *Bioresour. Technol.* 312, 123632. <https://doi.org/10.1016/j.biortech.2020.123632>.
- 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. *J. Environ. Manage.* 305, 114350. <https://doi.org/10.1016/j.jenvman.2021.114350>.
- Shaw, K., Dorea, C.C., 2021. Biodegradation mechanisms and functional microbiology in conventional septic tanks: a systematic review and meta-analysis. *Environ. Sci.* 7 (1), 144–155. <https://doi.org/10.1039/D0EW00795A>.
- Sheng, G.-P., Yu, H.-Q., Li, X.-Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnol. Adv.* 28 (6), 882–894. <https://doi.org/10.1016/j.biotechadv.2010.08.001>.
- Strande, L., Ronteltap, M., Brdjanovic, D., 2014. *Faecal Sludge management: Systems approach for Implementation and Operation*. IWA Publishing.
- 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. *J. Environ. Manage.* 223, 898–907. <https://doi.org/10.1016/j.jenvman.2018.06.100>.
- Sunada, N., da, S., Orrico, A.C.A., Orrico Junior, M.A.P., Lucas Junior, J.de, Lopes, W.R. T., Schwingel, A.W., 2018. Anaerobic co-digestion of animal manure at different waste cooking oil concentrations. *Ciência Rural* (7), 48.
- Tchobanoglous, G., Burton, F.L., Stensel, H.D., 2014. *Wastewater Engineering: Treatment and Resource Recovery*. McGraw-Hill Higher Education; /z-wcorg/.
- Torondel, B., Ensink, J.H., Gundogdu, O., Ijaz, U.Z., Parkhill, J., Abdelahi, F., Nguyen, V. A., Sudgen, S., Gibson, W., Walker, A.W., Quince, C., 2016. Assessment of the influence of intrinsic environmental and geographical factors on the bacterial ecology of pit latrines. *Microb. Biotechnol.* 9 (2), 209–223. <https://doi.org/10.1111/1751-7915.12334>. NLM.
- United Nations, G. A., 2015. Resolution Adopted by the General Assembly on 11 September 2015. New York: United Nations. A/RES/69/315 15 September 2015.
- 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 Sci. Technol.* 79 (12), 2242–2250. <https://doi.org/10.2166/wst.2019.220>.
- Velkushanova, K., Brdjanovic, D., Koottatep, T., Strande, L., Buckley, C., Ronteltap, M., 2021. *Methods for Faecal Sludge Analysis*. IWA Publishing.
- Vögel, Y., Lohri, C., Gallardo, A., Diener, S., & Zurbrugg, C. (2014). *Anaerobic digestion of biowaste in developing countries—practical information and case studies*. <https://doi.org/10.13140/2.1.2663.1045>.
- Ward, B.J., Traber, J., Gueye, A., Diop, B., Morgenroth, E., Strande, L., 2019. Evaluation of conceptual model and predictors of faecal sludge dewatering performance in Senegal and Tanzania. *Water Res.* 167, 115101. <https://doi.org/10.1016/j.watres.2019.115101>.
- Zhu, Y., Zhao, Y., 2011. Stabilization process within a sewage sludge landfill determined through both particle size distribution and content of humic substances as well as by FT-IR analysis. *Waste Manage. Res.* 29 (4), 379–385. <https://doi.org/10.1177/0734242X10384309>. PubMed.
- Zuo, S., Zhou, X., Li, Z., Wang, X., Yu, L., 2021. Investigation on Recycling Dry Toilet Generated Blackwater by Anaerobic Digestion: From Energy Recovery to Sanitation. *Sustainability*, p. 13. <https://doi.org/10.3390/su13084090>.