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Solid-Liquid Separation of Fecal Sludge: Understanding the Governing Mechanisms for Improved Global Sanitation

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Summary

Non-sewered sanitation relies on at-source containment and storage of fecal sludge, which is then collected and transported to treatment. This is a relatively new field that has started to gain attention. Contrary to the initial perception that non-sewered sanitation only serves rural areas, it plays a crucial role in fulfilling sanitation needs for over one-third of the world's population. In urban areas of sub-Saharan Africa, onsite sanitation caters for 65-100 % of the sanitation needs. Despite the progress made in enhancing access to sanitation facilities over the years, there is a persistent discharge of untreated fecal sludge into the environment, which is due to the absence of adequate fecal sludge treatment facilities. The consequences of this is the increase in diarrhoea diseases especially in low and middle-income countries. Treatment of fecal sludge is thus essential for public and environmental health protection. Since fecal sludge consist of more than 95% water, dewatering is indispensable for adequate fecal sludge management. However, a key challenge in fecal sludge treatment is the unpredictable dewatering performance, which stems from the high variability in fecal sludge characteristics, and a lack of knowledge on the components of fecal sludge that could influence dewatering performance.

Developing consistent treatment solutions for fecal sludge necessitates a deeper understanding of feces and fecal sludge composition, as well as the factors and mechanisms that control dewatering performance. In addition, changes that occur in feces or fecal sludge during anaerobic storage provides information on the characteristics of fecal sludge is arriving at the treatment. The main objective of this thesis was to elucidate the composition of feces and fecal sludge, and to understand the governing mechanisms of solid-liquid separation or dewatering of fecal sludge. To achieve this objective, we investigated in this thesis: (i) the general composition of feces and fecal sludge with emphasis on the concentrations of extracellular polymeric substances (EPS), proteins, polysaccharides, lipids, fibres and cations, to gain a mechanistic understanding of how these constituents affect the dewatering properties of fecal sludge, (ii) the role of EPS in the dewatering of fecal sludge under anaerobic storage, focusing on how EPS changes with time under anaerobic storage and the effect on dewatering behavior, (iii) the role of stabilization, or level of biochemical degradation, on dewatering performance of fecal sludge, and (iv) the association between fecal sludge microbiome to fecal sludge characteristics and factors that affect fecal sludge qualities and quantities (demographic, environmental, and technical data).

Summary

This study showed that feces and fecal sludge contains EPS, proteins, polysaccharides, fibres, lipids and cations that are known to influence fecal sludge dewatering performance. However, the concentrations of EPS are lower compared to what is found in activated sludges. Cellulose was found to increase dewatering performance by decreasing supernatant turbidity and capillary suction time whiles increasing the sludge cake solids. On the contrary, EPS was detrimental to dewatering performance by increasing the capillary suction time and supernatant turbidity. Similarly, lipids had a negative effect on dewatering performance through an increase in supernatant turbidity. Interestingly, cations did not have a significant effect on fecal sludge dewatering performance. In addition to the role of components on dewatering performance, what was compelling in this thesis was that fecal sludge that had been stored in containments had better dewatering performance than fresh fecal sludge that had not undergone storage, which is likely due to the role of stabilization.

By using anaerobic batch reactors as a proxy for storage in containment and stabilization, the roles of EPS and particle size distribution on dewatering performance were evaluated in this thesis. While the addition of extracted EPS (from fecal sludge and activated sludge) generally decreased dewatering performance, the effect of anaerobic storage on EPS, EPS fractions and particle size distribution were not substantial, owing to the minimal reduction of the total volatile solids concentration. The kinetics of degradation in fecal sludge appeared to be different from that of wastewater sludges, and anaerobic storage was neither analogous to process controlled anaerobic digestion nor a predictor of stabilization. What was clear in this study was that fecal sludge had a lower concentration of EPS and a higher EPS fraction of humic-like substances compared to wastewater sludges.

The different degradation kinetics in fecal sludge compared to wastewater, coupled with variation in levels of stabilization pointed to probable differences in microbial communities in fecal sludge. This thesis observed similarities in the microbial composition among fecal sludge from eight countries but with different proportional abundances, which helps to explain the possible contribution of microbial communities to the varying levels of stabilization. Since the microbial community in fecal sludge has not been thoroughly investigated, this thesis further explored the microbial community structure of 135 fecal sludge samples obtained from Lusaka, Zambia, with emphasis on the associations between microbial communities and demographic environmental factors, characteristics of fecal sludge and treatment performance such as dewatering and stabilization. Generally, fecal sludge had similar microbial communities at higher taxonomic levels; however, differences in community structure occurred at lower

taxonomic levels, which was dependent on the differences in containment type, which is dictated mainly by differences in water usage, ammonia, total solids concentration and total organic content. This variation in microbial communities at lower taxonomic levels may arise from environmental conditions selecting for different microorganisms or different microbial communities themselves creating different environmental conditions. On the contrary, the similarity at the higher taxonomic levels stems from the dominant metabolic pathways for microorganisms that select for communities like firmicutes and Bacteroides that mainly fermentative organisms.

This thesis provides fundamental knowledge of the composition of feces or fecal sludge and how these components influence dewatering performance. Overall, EPS, cellulose and lipids influenced dewatering performance, which sheds light on governing mechanisms of fecal sludge dewaterability. Furthermore, differences in dewatering between fresh and stored fecal sludge are attributed to the levels of stabilization. This is informative for practitioners on selection of appropriate management and treatment solutions as fresh feces are likely to cause clogging in drying beds due to the higher concentrations of EPS and cellulose than stabilized fecal sludge. These outcomes suggest that to improve dewatering performance, fresh fecal sludge that has not been stored in containments, should undergo some level of stabilization, which can be achieved through anaerobic pretreatment.

Zusammenfassung

Sanitärversorgung ohne Kanalisation, z.B. in Klärgruben, beruht auf der Lagerung von Fäkalschlamm an der Quelle, der dann gesammelt und zur Behandlung transportiert wird. Diese Art der Sanitärversorgung ist relativ neu, gewinnt aber allmählich mehr Aufmerksamkeit. Entgegen der allgemeinen Meinung, dass die nicht kanalisierte Abwasserentsorgung nur in ländlichen Gebieten zum Einsatz kommt, spielt sie eine entscheidende Rolle bei der Deckung des Sanitärversorgung von mehr als einem Drittel der Weltbevölkerung. In den Städten von Sub-Sahara Afrika sind zwischen 65 bis 100 % der Sanitärversorgung Klärgruben. Trotz der Fortschritte, die im Laufe der Jahre bei der Verbesserung des Zugangs zu Sanitärversorgung erzielt wurden, wird weiterhin unbehandelter Fäkalschlamm aus Klärgruben in die Umwelt entsorgt, was oft auf das Fehlen geeigneter Fäkalschlammbehandlungsanlagen zurückzuführen ist. Die Folge davon ist die Zunahme von Durchfallerkrankungen, insbesondere in Ländern mit niedrigem und mittlerem Einkommen. Die Behandlung von Fäkalschlamm ist daher für den Schutz der Gesundheit und der Umwelt von wesentlicher Bedeutung. Eine zentrale Herausforderung bei der Fäkalschlammbehandlung ist die unvorhersehbare Entwässerbarkeit des Fäkalschlamms, welche auf die Variabilität der Eigenschaften und das mangelnde Wissen über die Bestandteile des Fäkalschlamms zurückzuführen ist, welche die Entwässerungseigenschaften beeinflussen.

Die Entwicklung konsistenter Behandlungslösungen für Fäkalschlamm erfordert ein tieferes Verständnis der Zusammensetzung von Fäkalschlamm und Fäkalien, sowie der Faktoren und Mechanismen, die die Schlammentwässerbarkeit steuern. Darüber hinaus geben die Veränderungen, die während der anaeroben Lagerung von Fäkalien und Fäkalschlamm auftreten Aufschluss über die Eigenschaften des Fäkalschlamms, der die Kläranlage erreicht. Diese Arbeit untersuchte die Zusammensetzung von Fäkalien und Fäkalschlamm und die wichtigsten Mechanismen welche die Fest-Flüssig-Trennung und Entwässerung von Fäkalschlamm beeinflussen. Dabei wurde spezifisch folgendes untersucht: (i) Die Zusammensetzung von Fäkalien und Fäkalschlamm mit Schwerpunkt auf die Konzentrationen von extrazellulären polymeren Substanzen (EPS), Proteinen, Polysacchariden, Lipiden, Fasern und Kationen, um ein mechanistisches Verständnis dafür zu gewinnen, wie diese Bestandteile die Entwässerungseigenschaften von Fäkalschlamm beeinflussen. (ii) Die Rolle von EPS bei der Entwässerung von anaerob gelagertem Fäkalschlamm. (ii) Die Rolle von EPS bei der Entwässerung von anaerob gelagertem Fäkalschlamm, wobei der Schwerpunkt darauf lag, wie sich EPS im Laufe der Zeit verändern wenn Fäkalschlamm anaerob gelagert wird und wie es

sich dies auf das Entwässerungsverhalten auswirkt. (iii) Die Rolle der Stabilisierung von Fäkalschlamm auf die Entwässerbarkeit und (iv) die Verbindung zwischen mikrobiologischen Gemeinschaften in Fäkalschlamm, den Fäkalschlamm Eigenschaften sowie Faktoren, die die Fäkalschlamm Zusammensetzung und Menge beeinflussen (demografische, umweltbezogene und technische Daten).

Diese Studie zeigte, dass Fäkalien und Fäkalschlamm EPS, Proteine, Polysaccharide, Fasern, Lipide und Kationen enthalten, von denen bekannt ist, dass sie die Entwässerbarkeit beeinflussen. Die EPS-Konzentrationen sind jedoch niedriger als in Belebtschlämmen aus konventionellen Kläranlagen. Es wurde festgestellt, dass Zellulose die Entwässerungsleistung erhöht, da es die Trübung des Überstandes verringert und die Entwässerungsgeschwichtigkeit und den Feststoffgehalt im Schlammkuchen erhöht. Im Gegensatz dazu wirkten sich EPS negativ auf die Entwässerbarkeit aus, da sie die Entwässerungsgeschwichtigkeit verringerten und die Trübung des Überstands erhöhten. In ähnlicher Weise wirkten sich Lipide negativ auf die Entwässerbarkeit aus, da sie die Trübung des Überstandes erhöhten. Interessanterweise hatten Kationen keinen signifikanten Einfluss auf die Entwässerbarkeit. Neben der Rolle von Fäkalschlammbestandteilen auf die Entwässerbarkeit war in dieser Arbeit besonders auffällig, dass in Behältern gelagerter Fäkalschlamm bessere Entwässerungsleistung aufwies als frischer Fäkalschlamm, der nicht gelagert wurde, was wahrscheinlich auf die Schlammstabilisierung zurückzuführen ist.

Anaerobe Batch-Reaktoren wurden in dieser Studie genutzt um die Lagerung und Stabilisierung von Fäkalschlamm in Klärgruppen zu simulieren und den Einfluss von EPS und der Partikelgrößenverteilung auf die Entwässerungsleistung zu untersuchen. Während die extrahierten EPS im Allgemeinen die Entwässerungsleistung verringerten, hatte die anaerobe Lagerung aufgrund der minimalen Verringerung der Gesamtkonzentration flüchtiger Feststoffe keinen wesentlich Einfluss auf EPS, EPS-Fraktionen und Partikelgrößenverteilung. Die Abbaukinetik von Fäkalschlämmen scheint sich von der von Klärschlamm zu unterscheiden, und die anaerobe Lagerung von Fäkalschlamm ist weder mit der prozessgesteuerten anaeroben Faulung vergleichbar noch ein Anzeichen für den Stabilisierungsgrad. In dieser Studie wurde deutlich, dass Fäkalschlamm im Vergleich zu Klärschlämmen eine geringere EPS-Konzentration und einen höheren EPS-Anteil mit humusähnlichen Stoffen aufweist.

Die unterschiedliche Abbaukinetik von Fäkalschlamm im Vergleich zu Klärschlamm in Verbindung mit unterschiedlichen Stabilisierungsgraden deutet auf Unterschiede in der Zusammensetzung von mikrobiologischen Gemeinschaften in Fäkalschlamm hin. In dieser

Arbeit wurden Ähnlichkeiten in der Zusammensetzung von mikrobiologischen Gemeinschaften in Fäkalschlämmen aus acht Ländern festgestellt, jedoch mit unterschiedlichen Häufigkeiten, was dazu beitragen könnte den möglichen Beitrag von mikrobiologischen Gemeinschaften zu den unterschiedlichen Stabilisierungsgraden zu erklären. Da mikrobiologischen Gemeinschaften in Fäkalschlamm noch nicht gründlich untersucht worden ist, wurde in dieser Arbeit mikrobiologischen Gemeinschaften von 135 Fäkalschlammproben aus Lusaka, Sambia, weiter erforscht, wobei der Schwerpunkt auf den Zusammenhängen zwischen der Zusammensetzung von mikrobiologischen Gemeinschaften und den demografischen und Umweltfaktoren, den Eigenschaften des Fäkalschlamms und der Behandlungsleistung wie Entwässerung und Stabilisierung lag. Im Allgemeinen wiesen Fäkalschlämme auf höheren taxonomischen Ebenen ähnliche mikrobiologische Gemeinschaften auf; auf niedrigeren taxonomischen Ebenen traten jedoch Unterschiede in der Gemeinschaftsstruktur auf, die von den Unterschieden in der Art der Klärgrube abhingen, die hauptsächlich von Unterschieden in der Wassernutzung, dem Ammoniakgehalt, der Gesamtfeststoffkonzentration und dem gesamten organischen Gehalt bestimmt werden. Diese Unterschiede in den mikrobiologischen Gemeinschaften von Fäkalschlamm auf niedrigeren taxonomischen Ebenen könnte darauf zurückzuführen sein, dass die Umweltbedingungen für unterschiedliche Mikroorganismen selektieren oder dass unterschiedliche mikrobiologischen Gemeinschaften selbst unterschiedliche Umweltbedingungen schaffen. Im Gegensatz dazu ist die Ähnlichkeit auf den höheren taxonomischen Ebenen auf die vorherrschenden Stoffwechselwege für Mikroorganismen zurückzuführen, die für Gemeinschaften wie Firmicutes und Bacteroides selektieren welche typische fermentative Organismen sind.

Diese Arbeit liefert grundlegende Erkenntnisse über die Zusammensetzung von Fäkalien und Fäkalschlamm und darüber, wie diese Komponenten die Entwässerbarkeit beeinflussen. Insgesamt beeinflussten EPS, Zellulose und Lipide die Entwässerbarkeit, was Aufschluss über die maßgeblichen Mechanismen der Entwässerbarkeit von Fäkalschlamm gibt. Darüber hinaus werden die Unterschiede in der Entwässerbarkeit zwischen frischem und gelagertem Fäkalschlamm auf den Grad der Stabilisierung zurückgeführt. Dies ist für Praktiker bei der Auswahl geeigneter Management- und Behandlungslösungen aufschlussreich, da frische Fäkalien aufgrund der höheren EPS- und Zellulosekonzentrationen als stabilisierter Fäkalschlamm wahrscheinlich zu Verstopfungen in Trocknungsbetten führen. Diese Ergebnisse legen nahe, dass frischer Fäkalschlamm, der nicht gelagert wurde, zur Verbesserung der Entwässerbarkeit zu einem gewissem Maße stabilisiert werden sollte, was durch anaerobe Prozesse erreicht werden kann.

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Chapter 1 : Introduction

1.1 General Introduction

1.1.1 Global relevance of fecal sludge treatment

Over a third of the world's population is estimated to rely on onsite sanitation and in Sub-Saharan Africa, the sanitation needs of 65-100 % of the population are provided by these systems (Koottatep et al., 2020). Over the years, onsite sanitation technologies, which include pit latrines, septic tanks, aqua privies, bucket latrines, public toilets and dry toilets, were considered as temporary solutions until the construction of sewers. However, these technologies have just recently been recognised as offering long term solutions in the provision of sustainable and affordable sanitation to rural and urban populations (Koné, 2010). This is primarily because the costs of constructing sewerage systems are high and they, concomitantly, require piped water supply to transport wastewater to treatment. Unlike a community drinking water supply, the management of the excreta that accumulates in onsite containments has garnered relatively little awareness or attention. There is a lack of adequate waste disposal or treatment facilities especially in low and middle-income countries for the fecal sludge that accumulates in onsite sanitation systems and this has led to considerable human health and environmental problems (Peal et al., 2020).

According to the WHO (2021), diarrhoeal diseases alone are responsible for about 1.8 million deaths each year worldwide and this is largely attributed to unsafe water supply, sanitation and hygiene. The lack of adequate sanitation facilities in low and middle-income countries is a result of the huge economic burden that sanitation places on local governments and households, and the fact that there seem to be no financially viable options in the sanitation service chain that can offset some of the costs involved in the management of excreta. Additionally, there is a lack of willingness to pay realistic tariffs for sanitation services by governments and households. Despite improvements in the coverage of onsite sanitation systems over the years (WHO and UNICEF, 2017), indiscriminate disposal of excreta in the open environment continues to abound in low- and middle-income countries. What is worse is that there is limited knowledge about the effective treatment technologies that could be implemented for the safe management of excreta. Additionally, there is limited research on the composition of fecal sludge and how these components influence treatment performance.

1.1.2 Fecal sludge management (FSM) – characteristics and variability

Fecal sludge is composed of excreta (feces and urine) and additional inputs, which may consist of toilet paper, greywater, flush water, garbage and municipal solid waste (Strande et al., 2014) that has been stored in onsite containments and has not been transported through a sewer. It consists primarily of about 95 % water, which makes it heavy and expensive to transport or to safely dispose of (Gold et al., 2017) . Additionally, fecal sludge exhibits considerable variability in its characteristics due to a multitude of influential factors in addition to its composition. Factors, such as the type of containment, manner of usage of the onsite system, duration of storage of fecal sludge, quality of construction, local climate, collection practices, availability of water, and level of stabilization, are known to affect the characteristics of fecal sludge significantly. For instance, the total solids (TS) concentration of fecal sludge is influenced by the use of dry or flush toilets, volume of water used, type of cleansing material (water, wipes, toilet paper), addition of greywater and the introduction of soil (unlined pit latrines) into the containment (Andriessen et al., 2023; Eliyan et al., 2021; Strande et al., 2014). These factors cause the high variability in fecal sludge characteristics, which is up to 1-2 orders of magnitude higher when compared to domestic wastewater. An example of this variability is seen in the COD concentrations of fecal sludge and wastewater determined by Ward et al. (2019). The COD concentrations range from 10,000 – 50,000 mg/L for fecal sludge from septic tanks and public toilets, and 500-2,500 mg/L for primary and secondary wastewater sludges (Heinss et al., 1998; Niwagaba et al., 2014). This difference in variability between fecal sludge and wastewater sludge stems from the distinct methods of collection employed. Unlike wastewater, which undergoes mixing as it travels through a sewer network and becomes relatively homogenised, fecal sludge is collected in separate batches, each batch exhibiting unique and completely different characteristics.

In addition to fecal sludge characteristics, stabilization processes in fecal sludge appears to be different from what occurs in wastewater sludges. Fecal sludge stabilization had long been associated with the duration of storage in the containment and the filling rate (Chaggu et al., 2007; Strande et al., 2014; van Eekert et al., 2019a). For instance, public toilets that experience a significant influx of users, requiring frequent emptying, are expected to exhibit less stability and higher levels of organic content (BOD or COD) and ammonia concentrations. In contrast, household septic tanks, which can store fecal sludge for extended periods (years), allow for greater stabilization of sludge over time. However, recent studies have shown that the stabilization in fecal sludges is more complicated than previously imagined. Studies by Ward et al. (2019, 2021a) and Sam et al. (2022a) have indicated that the duration of storage in a

containment is not a predictor of the level of stabilization, and that during process-controlled anaerobic stabilization of fecal sludge (which has mixing, sludge retention time and temperature control), degradation occurs only in the first few days, after which no further degradation occurs.

Because fecal sludge is a relatively new area of research, much about it is not yet fully understood, in contrast to wastewater treatment, which has been extensively studied for over a hundred years, resulting in a vast wealth of knowledge (Stensel and Makinia, 2014). Very often, fecal sludge technologies are based on the knowledge gained from wastewater research; however, this knowledge cannot be directly applied to fecal sludge because of the differences in its composition and characteristics. Yet, fecal sludge treatment can borrow from and adapt the learnings of wastewater research.

1.1.3 Composition of fecal sludge and relevance of components

1.1.3.1 Feces

Feces is a vital component of fecal sludge that accumulates in a containment. It is probably possible to say that the term fecal sludge exists because feces are a component of it. Although the term feces is often been used in medical research, there is a general lack of knowledge of what constitutes feces and how it translates into fecal sludge. Additionally, the role that the constituents of feces have on the fecal sludge treatment process or fecal sludge management is not clear. Because feces form a critical component of fecal sludge, it is essential to understand its constituents and their functions in order to understand treatment processes, such as dewatering and stabilization. This knowledge is important because it could help in the development of sustainable low footprint technologies.

Currently, information on feces is scarce and scattered across different fields. Most of the information available is from mass balance reports from NASA on the chemical composition of feces during the development of the space programme (Goldblith and Wick, 1961). Medical studies have also looked at the composition of feces, but usually in relation to the influence of macromolecules, such as lipids and proteins on inflammatory bowel disease and steatorrhea, respectively (Goldblith and Wick, 1961; Holscher, 2017; Hopkins et al., 2002; Stephen and Cummings, 1980). There have also been studies that examined the gut microbiome in which the composition of feces has also been examined. Although these studies provide some information on the composition of feces, knowledge of the complete composition of human feces and how fecal constituents could be related to fecal sludge properties is still lacking.

As illustrated in **Figure 1-1**, human feces consist of about 75 % water and 25 % solid material with a pH range of 5.4 – 7.5. The solid content of feces consist of macromolecules, including proteins, fats and carbohydrates (fibres), which occur in nearly equal proportions and could make up to 75 % of the solid content (Rose et al., 2015; Stephen and Cummings, 1980). The solids in feces may also include metabolites and secretions, which form part of the protein content and inorganic materials. The inorganics compounds in feces make up only a small fraction of the total solids and as such the organic fraction of the solids constitute between 84-93 %. Recent studies by Sam et al. (2023) on the composition of feces confirmed that the organic fraction of feces is between 85-89 %. This fraction will contribute to the COD and determine the strength of the fecal sludge. Additionally, the biodegradability or bioavailability of the organic content will influence the rate of fecal sludge degradation and stabilization. Bacterial biomass seem to be variable in feces samples. While Rose et al. (2015) suggest that bacterial biomass contributes about 25-54 % of the solids in feces, Stephen and Cummings (1980) indicate that fecal total solids are comprised of about 55 % bacteria. Microorganisms, mainly bacteria in are responsible for the utilisation of readily degradable organics in fecal sludge and mineralisation, resulting in a stabilized product (Ijaz et al., 2022). Additionally, extracellular polymers produced by bacteria have functions related to the dewatering properties of fecal sludge (Sheng et al., 2010).

Feces forms part of fecal sludge that accumulates in onsite containments. Although feces has often been used in medical research, there is a general lack of knowledge of what constitutes feces and how it translates into fecal sludge. Additionally, the role of essential constituents of feces (EPS, cellulose, lipids, lignin, hemicellulose, cations) on fecal sludge treatment processes or fecal sludge management is not clear. It is therefore essential to understand the constituents of feces and their functions in order to understand treatment processes, such as dewatering and stabilization. This knowledge is crucial in the development of sustainable and low footprint technologies for fecal sludge management.
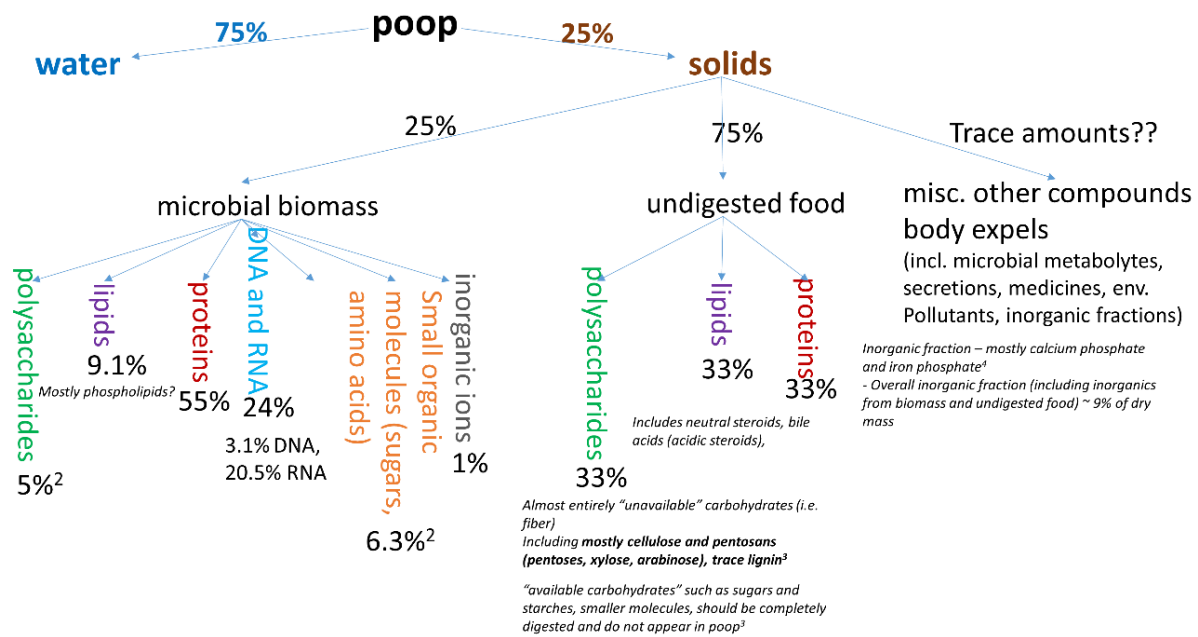
Figure 1-1: Composition of feces, the percentage distribution of components were compiled from values in Tchobanoglous et al. (2014) , Rose et al. (2015), Volk and Rummel. (1987), Southgate and Durnin. (1970) (Diagram by Barbara Jeanne Ward)

1.1.3.2 Urine

Urine is a by-product secreted by the kidneys after extracting soluble waste from the bloodstream, excess water, sugars and other organic compounds (Niwagaba, 2009). It consists mainly of water (91-96 %), and has a high amounts of nutrients in water-soluble form (Rose et al., 2015; Vinnerås et al., 2006). Urine has a high concentration of organic nitrogen in the form of urea, which breaks down to form substantial quantities of ammonia. Additionally urine contains inorganic salts, such as chloride, sodium, and potassium, organic acids, and water-soluble toxins. Studies have shown that the quantities of urine excreted by an individual is associated with the quantities of fluids ingested, sweat, diet, physical activity and existing climatic conditions (Feachem et al., 1983; Niwagaba, 2009), and it is estimated that most adults generate between 0.6-2.6 L/cap/day with a median volume of 1.4 L/cap/day. In onsite sanitation systems, urine is usually not separated from feces and it contributes a majority of the ammonia found in fecal sludge. The contribution of ammonia and nutrients from urine to fecal sludge make them important components that must be considered during fecal sludge treatment. Since ammonia is a potent inhibitor in anaerobic degradation systems, it is likely that high urine concentration in fecal sludge could hinder the degradation of fecal sludge in containments, as observed by Colon et al. (2015).

In addition to feces and urine, fecal sludge may have other components resulting from usage patterns of the onsite sanitation system. For instance, fecal sludge may contain varying levels of fats, oils, grease, and detergents as a result of greywater addition. Additionally, the use of toilet paper as a cleansing material and solid waste introduces fibers into the fecal sludge. In certain cases, such as dry toilets, materials like sawdust, wood shavings, or sand are added to the fecal sludge to mitigate odors. Moreover, chemicals are sometimes introduced during the emptying process to prevent odor and flies (Strande et al., 2014).

1.1.4 FSM-current status and bottlenecks

The prevailing fecal sludge service chain is expensive and complicated with many challenges, ranging from high emptying costs due to long haulage distances, to emptying equipment having limited access due to the high density of housing units. As it stands now, most fecal sludge that accumulates in these containments is not properly managed. For instance, in urban areas of low-income countries, 60 % of the fecal sludge is not safely managed, which places a huge burden on public and environmental health (Peal et al., 2020). In places where treatment plants exist, large footprint technologies, such as drying beds, are often implemented, which are unsustainable especially in urban areas in low- and middle-income countries because of their large space requirements. In order to reduce the costs involved in the fecal sludge service chain, there is the need for a solid-liquid separation step (dewatering) to reduce the volume of the sludge leading to the development of low footprint technologies.

1.1.5 Fecal sludge dewatering: an overview

Dewatering is the process of separating the solid matter and liquid in wastewater sludge or fecal sludge, resulting in a liquid stream and a solid content stream (cake) with a total solids (TS) concentration of up to 20 % (Tchobanoglous et al., 2014). Further removal of water following dewatering is achieved by drying. Dewatering mainly occurs through particle settling or by filtration of the water content in the sludge. This solid-liquid separation process ensures better handling, storage and transportation of the sludge. Additionally, the reduction in sludge volume is important for the development of low footprint treatment technologies, increases the capacity of treatment plants and generates a suitable precursor for resource recovery (Strande et al., 2014). The development of low footprint technologies also translates into lower operation and maintenance costs. Thus, the primary step in developing an efficient fecal sludge management is the settling and dewatering process.

Dewatering is a commonly applied process in wastewater sludge treatment, and technologies such as screw presses, belt presses and centrifuges, are widely used for wastewater sludge

dewatering. Metrics including the capillary suction time (CST), sludge resistance to filtration, supernatant turbidity after centrifugation and total solids (percentage TS) of centrifuged sludge are used to measure dewatering performance. The capillary suction time evaluates the rate of dewatering, while the total solids (% TS) of centrifuged sludge measures the extent of dewaterability.

Despite its importance, the process of dewatering fecal sludge remains poorly understood and under-researched. This is likely due to the fact that the broader field of fecal sludge research is still relatively new and emerging, with many knowledge gaps yet to be addressed. Settling and thickening tanks and drying beds are the common technologies currently in use for dewatering at fecal sludge treatment plants. However, their solid-liquid separation performance is very unpredictable. This is mainly due to the variability in fecal sludge characteristics and composition. Moreover, there is a lack of knowledge of the mechanisms governing solid-liquid separation in fecal sludge. Information from wastewater treatment indicates that during dewatering, the free water and loosely bound water that are trapped in the pores and interstitial spaces are removed by the dewatering technologies, such as filtration and settling (Novak, 2006). Unlike bound water, which consists of interstitial, surface, and intracellular water, free water in wastewater sludge constitutes water that is not attached to the sludge particles and makes up 55-97 % of the water in the sludge (Kopp and Dichtl, 2001). The mechanisms of dewatering (settling and filtration) in wastewater sludges are driven by floc formation and disintegration, and it has been observed that the formation of large and compact flocs in activated sludges results in better settling, while the disintegration of the flocs into smaller particles results in slow filtration and higher supernatant turbidity after centrifugation (Christensen et al., 2015; Lawler et al., 1986).

In the context of fecal sludge from onsite sanitation systems, there is a significant variation in dewatering rates across different systems and cities (Gold et al., 2017), and even for similar systems, e.g. pit latrines that are lined or unlined (Semiyaga et al., 2017) because it is not yet understood what specifically controls dewatering in fecal sludge. Because of this, we contend that understanding the mechanisms driving the dewatering of wastewater serves as a good starting point for dewatering research on fecal sludge.

1.1.5.1 The quest to understanding fecal sludge dewatering

Sludge management is a costly process with an operating cost that could exceed 50% of the total operating cost of municipal wastewater treatment plants (Nowak, 2006). In view of that, dewatering in wastewater treatment has been well researched and factors and mechanisms that control dewatering performance of wastewater sludges are well understood. Such factors as extracellular polymeric substances (EPS), microbial community structure, particle size distribution, cations, and non-EPS organic fraction, i.e. fibres, have been observed to be associated with dewatering properties (**Figure 1-2**). Recent studies have suggested that the variation of fecal sludge dewatering performance are dependent on such factors as the degree of stabilization, the particle size distribution and the concentration or fractions of EPS (Sam et al., 2022; Semiyaga et al., 2017; Ward et al., 2019, 2023). Studies by Mercer et al (2021a) and Ward et al. (2019, 2021a), indicate that EPS concentrations and particle size distribution are related to the dewatering performance of fecal sludge. By analysing fecal sludge samples from Tanzania and Senegal, Ward et al. (2019) observed that fecal sludge with high EPS concentration appears to be less stabilized and had a low dewatering performance. On the other hand, fecal sludge, which appeared stabilized, with low EPS concentrations, were associated with good dewatering performance. Further studies by Ward et al. (2023) on the role of particle size on dewatering indicate that an increase in the concentration of particles smaller than 10 µm was associated with high capillary suction time (CST) and supernatant turbidity in field samples from Naivasha, Kenya and Kampala, Uganda, whereas larger particle sizes correlated with low CST and turbidity. These revelations by Ward et al. (2023, 2019) shed light on some of the factors that are influencing fecal sludge dewatering performance. However, these factors alone do not seem to explain the observed dewatering performance of fecal sludge field samples. This suggests that in addition to these factors, the dewatering of fecal sludge could be influenced by elements that have not yet been explored. Based on the literature from wastewater and the preliminary work by Ward et al. (2019), it is expected that the dewatering performance of fecal sludge will also be dependent on such factors as EPS and EPS fractions, non-EPS organic matter, inorganic substances, particle size distribution, the level of stabilization, surface properties and microbial communities.
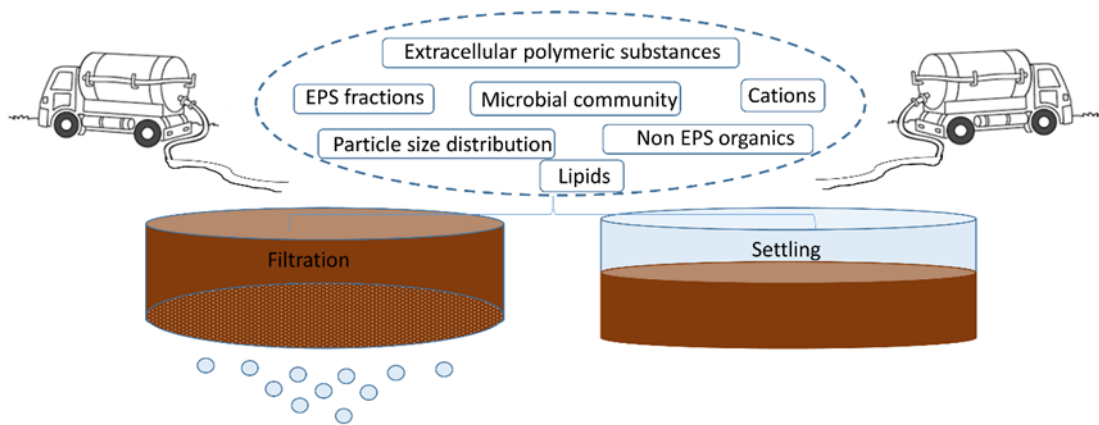
Figure 1-2: Schematic depicting the factors that potentially influence fecal sludge dewatering performance either through particle settling or by filtration of the water in the sludge through a filter medium

1.1.5.2 Role of extracellular polymeric substances (EPS) on dewatering

Microbial aggregates in biological wastewater treatment are known to be held together by a slime and highly hydrated matrix of EPS, which form a 3-dimensional gel-like structure (Huang et al., 2022; Melo et al., 2022). The EPS results from active secretions, cell lysis, shedding of cell surface material and adsorption from the environment (Pal and Paul, 2008). In activated sludge, although EPS can be found on the inside of microbial aggregates, they are generally on the exterior of cells, and function as a protective lining against external environmental factors, such as biocides and changes in pH (Wingender et al., 1999). Additionally, EPS serves as carbon and energy sources for the microorganisms in the absence of substrates. EPS consist mainly of proteins, polysaccharides, humic acid substances, nucleic acids, lipids, and extracellular DNA and its production is controlled by several factors, including the availability of nutrients, carbon source, temperature, shear stress and stressor compounds (Sheng et al., 2010). Two forms of EPS exist and they are the soluble EPS, which comprise of polymers that can move in between the sludge flocs and in solution and the bound EPS (Huang et al., 2022). Bound EPS have a double-layered structure and are intimately attached to the exterior of microbial cells due to the peripheral functional groups. The inner layer of the double-layered structure forms the tightly bound EPS, while the outer layer, which is diffused in the surrounding fluid, comprises the loosely bound EPS (Huang et al., 2022). The loosely bound EPS, therefore, tend to form a highly hydrated slime layer and recent studies have shown that

the soluble and loosely bound EPS are associated with sludge flocculation, dewatering and settling.

EPS is regarded as one of the most critical factors that influence sludge dewatering performance and its effect has been linked to the ability of negatively charged EPS to bind to the water molecules in the sludge floc, which affects the charge and stability of the floc (Sheng et al., 2010). The interaction between EPS and water molecules is known to occur through two mechanisms: an electrostatic interaction between the charged functional groups on the EPS and the permanent dipole of the water molecules, and hydrogen bonding, which results from interaction between the hydroxyl groups on both EPS and water molecules. In activated sludge systems, the presence or increase in EPS results in increased water retention in the sludge floc and in interstitial spaces of the flocs (Neyens et al., 2004), and EPS has been found to hinder sludge dewatering performance. More so, the gel forming ability of EPS appears to be detrimental to sludge dewatering performance since the formation of gels prevents the flow of water through the sludge flocs. However, other studies have suggested rather contrasting effects of EPS on dewatering performance. Mikkelsen and Kidding. (2002) opine that high concentrations of EPS improve dewatering performance by reducing the stress sensitivity and the degree dispersion. Because EPS holds flocs together, the interaction between EPS and solution properties, such as divalent cations, increases the floc resistance to stress and the floc size. Studies by Houghton et al. (2002), also suggest that the role of EPS on dewatering performance is related to the concentration of EPS in the sludge. Low concentrations of EPS have been observed to be beneficial to sludge dewatering, while a high EPS beyond a certain threshold was seen to reduce dewatering performance. This observation implied that there exists an EPS concentration threshold, below which dewatering performance is enhanced and above which dewatering performance is impeded due to the ability of the EPS to bind more water with increased concentration. It has also been suggested that the composition of EPS rather than the concentration determines the effect of EPS on dewatering properties.

Soluble and loosely bound EPS have been measured in fecal sludge with concentrations lower than what has been measured in wastewater sludges using similar extraction procedures (Sam et al., 2022a; Ward et al., 2019). Even though soluble and loosely bound EPS have been observed to be associated with dewatering performance in fecal sludge, the exact mechanism seems to be different from activated sludges. The low concentration of EPS in fecal sludge does not permit the formation of sludge flocs (Jørgensen et al., 2017) and, thus, the EPS in fecal sludge tend to behave as colloidal and suspended organic substances that clog the pores of a

filter media during filtration as indicated in **Figure 1-3**. These colloidal suspensions also do not promote settling and reduce the settleability of the sludge. Hence, the effect of EPS on fecal sludge dewatering performance seems to be detrimental rather than beneficial. This is supported by observations by Ward et al. (2019), where EPS concentration in fecal sludge field samples had a positive correlation with filtration time. Sam et al. (2022a) also observed that adding extracted EPS to fecal sludge causes an increase in filtration time and decreases settling performance. Despite some suggestions that the fractions of EPS affect dewatering rather than the total EPS (Shao et al., 2009), Sam et al. (2022a) observed that the protein-like and humic-like substances of EPS in fecal sludge did not have a substantial effect on the dewatering performance of fecal sludge. Although our empirical studies have shown the effect of EPS on the dewatering properties of fecal sludge, what is not clear is the effect of fecal sludge storage conditions on EPS and EPS fractions, and how that affects dewatering performance.
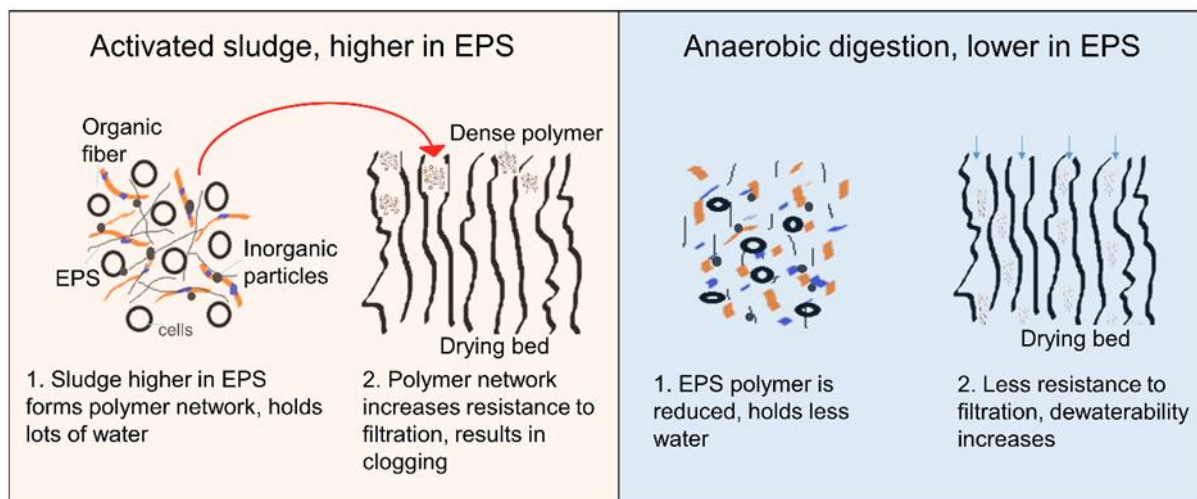

Figure 1-3: Schematic illustrating the role of EPS in dewatering. The left side of the Figure (pink) shows how EPS affects filtration in activated sludge systems and the right side shows the effect of anaerobic digestion on EPS and how it affects filtration

1.1.5.3 How do proteins and polysaccharides affect dewatering?

Proteins and polysaccharides are major components of EPS, but are also part of the fecal content that become part of the fecal sludge. In activated sludge, proteins and polysaccharides contribute between 70 % and 80 % of the extracellular organic carbon (Dignac et al., 1998). Both polysaccharides and proteins are known to play a role in flocculation and settling through the bridging of divalent cations and negatively charged groups in the proteins and polysaccharides. However, the literature is diverse on whether these factors enhance or deteriorate the dewatering performance of sludge and whether proteins, polysaccharides or

protein/carbohydrate ratio are key factors that influence dewatering performance. Due to the higher concentrations of proteins in activated sludges than polysaccharides, which is attributed to large quantities of exoenzymes in the sludge floc (Frølund et al., 1996), proteins are expected to play a major role in flocculation through the formation of electrostatic bonds with multivalent cations. Proteins consist mainly of hydrophobic amino acids and have been acknowledged to have strong positive correlations with negative surface charge and flocculating ability (Shao et al., 2009). An increase in hydrophobicity and negative surface charge will promote cell to cell interaction that improves the stability of flocs (Wilén et al., 2003). Furthermore, an increase in flocculation ability will also mean that the amount of small particles that have the propensity to cause clogging of the filter pores during filtration will be reduced. On the other hand, the polysaccharides in EPS have been linked to the gel forming ability of EPS and, Sam and Dulekgurgen. (2016) have evaluated the gel-forming abilities of exopolysaccharides extracted from granular sludge treating brewery wastewater. However, the polysaccharide content in EPS from fecal sludge is insignificant (Ward et al., 2019) and its role on dewatering has not yet been clearly established. The role of proteins and polysaccharides in fecal sludge dewatering is still inconclusive. This is because EPS in fecal sludge seem to be playing a different role on dewatering compared to activated sludges. Studies by Sam et al. (2022a) observed no significant relationship between protein-like substances (which included proteins and polysaccharides) with dewatering metrics.

1.1.5.4 Relevance of lipids in fecal sludge dewatering performance

The role of lipids, which consist of oils, greases, fats and long-chain fatty acids on sludge settling properties have been observed in wastewater treatment (Chipasa and Mędrzycka, 2006). Lipids are polar molecules with lower densities than water and, therefore, do not mix with water. In wastewater treatment, they are removed through biodegradation and separation of sludge biomass since lipids adsorb onto bacteria biomass (Chipasa and Mędrzycka, 2006). The adsorption of lipids onto bacteria biomass causes a decrease in specific gravity and the ability of the sludge particles to settle, resulting in a reduced solid-liquid separation. In other industries, such as the food and cosmetics sectors, lipids are known to form an oil-in-water emulsion that presents cloudiness or turbidity due to the scattering of light by dispersed oil droplets (Linke and Drusch, 2016). It seems obvious that lipids are detrimental to settleability. However, the role of lipids in fecal sludge dewatering has not been investigated. Comparatively, fecal sludge has a higher lipids concentration than wastewater and, thus, it is expected that lipids will contribute to lower settling properties in fecal sludge. Studies by Sam et al. (Submitted) on the

effect of fecal constituents on fecal sludge dewatering performance confirmed that an increase in lipid concentration in fecal sludge led to reduced settling in the sludge. The concentration of lipids in fecal sludge generally depends on the source of the sludge as sludge from restaurants and households where heavy greywater is added to the fecal sludge containment will have higher FOG concentration than from households or dry toilets without greywater addition. This implies that fecal sludge from restaurants and sources with heavy greywater addition will have low settling performance.

1.1.5.5 Relevance of fibres in fecal sludge dewatering performance

Fibres are portions of plant-derived food that cannot be completely broken down by human digestive enzymes. They comprise primarily of degradable and non-degradable fibre and play a major role in the variation of fecal composition and generation (Rose et al., 2015). A survey of the literature indicates that few studies have investigated the effect of fibres on the dewatering properties of wastewater sludges. Indeed Zhang et al. (2020) investigated the role of fibres on the filtration time and settling performance of wastewater from the pulp and paper industry, which has considerable concentrations of fibres. Their study suggested that fibres in general have a positive influence on sludge filtration and settling. An increase in cellulose fibre content of a simulated pulp and paper mill sludge resulted in a low specific resistance to filtration, which implies an improvement in sludge dewatering performance. Nittami et al. (2015) in their investigation to understand the role of synthetic fibres in improving dewatering performance of activated sludges concluded that the synthetic fibres they tested have compaction properties that seem to aid their role as skeleton builders or filtration aids to improve dewatering performance. In another study by Mäkinen et al. (2013), contrasting results were observed where fibres were seen to have a negative effect on sludge dewatering. An increase in fibres and fine materials were associated with a decrease in sludge filtration rates and high filtration rates occurred when fibres were decreased.

With respect to the water holding ability of sludge, fibres are expected to increase the water holding capacity due to the presence of a large number of peripheral hydroxyl groups that is expected to interact with the water molecules through hydrogen bonding. However, some studies have also shown that fibres are able to increase the compressibility of sludge, resulting in reduced water holding capacity and increased sludge cake (Zhang et al., 2020). The role of fibre on the filtration and settling properties of sludge is associated with the ability of fibre to provide surfaces of attachment for particles, thereby, reducing the concentration of suspended particles and increasing the settleability of floating particles (Mäkinen et al., 2013; Zhang et al.,

2020). The role of fibres on dewatering properties is also related to the length of the fibre molecules. As the fibre length decreases, it provides a greater surface area for the adhesion of sludge particles and increases floc formation. Hence, the shorter the fibre length, the better the dewatering properties of the sludge (Zhang et al., 2020). However, short fibre length improves dewatering performance to a limit. Zhang et al. (2020) observed that below a threshold of 0.303 mm, fibres tend to cause blinding effect of the sludge cake, resulting in an increase in sludge resistance to filtration (poor dewatering performance). Even though the role of fibres on dewatering properties seems to be well understood, research on the effect of the individual fibre components, such as cellulose, hemicellulose and lignin, on the dewatering performance of wastewater sludges seems scarce and appears to be non-existent in fecal sludge research.

Cellulose, which is the most abundant polymer on the planet, possesses a large number of peripheral hydroxyl groups of glucose per unit weight that interact with water through hydrogen bonding (Boulos et al., 2000). This confers a high water holding capacity to cellulose. Based on the structure alone, cellulose is expected to be detrimental to dewatering performance, since the hydroxyl groups are expected to interact with water, conferring on it a high water holding capacity. However, the ability of cellulose to reduce filtration resistance and turbidity in wastewater sludges indicates that perhaps the effect of the adhesive properties supersedes that of the water holding capacity. Hemicellulose, which is similar to cellulose, but with more branching in its structure also possesses hydroxyl groups and is expected to behave like cellulose (Boulos et al., 2000). Another component of fibres is lignin, which is a multiaromatic ring compound with both hydrophobic nonpolar frameworks of phenyl groups and hydrophilic carboxylic and hydroxyl groups. Lignin is used in many industries as a surfactant due to its adhesive, surface active and complexation properties. Based on these properties, it is expected that lignin will also improve filtration and settling through the adhesion of particles. Fibre composition of fecal sludge has not been widely studied and it is not known how the fibres will influence fecal sludge dewatering performance. Only a few studies have assessed the concentration of fibres in fecal sludge even though it was in relation to thermal decomposition of fecal sludge and utilization by black soldier fly larvae (Gold et al., 2020; Krueger et al., 2021).

Fibres are expected to contribute to the high variability in fecal sludge, as different sources of fecal sludge will poses varying concentration and types of fibres. Despite the possibility of fibres influencing the dewatering properties in fecal sludge, there is a lack of knowledge regarding the concentration ranges in fecal sludge and it is also not clear how they will influence

the dewatering performance of fecal sludge. Recent studies by Sam et al (submitted) indicates that the different fibre components seem to have varying effects on fecal sludge dewatering performance. An increase in the cellulose concentration in fecal sludge resulted in an increase in the sludge filtration rate and a decrease in the supernatant turbidity after centrifugation. These observations are in agreement with observations by Zhang et al. (2020) and Nittami et al. (2015) on the effect of fibres on dewatering performance. However, the effects of hemicellulose and lignin on the dewatering performance of fecal sludge were not consistent. Lignin increased sludge cake solids (good dewatering performance) and at the same time decreased the sludge filtration rate (worse dewatering performance). These discrepancies reveal the complexity of mechanisms in fecal sludge dewatering and suggest that in addition to these fibre components, other factors could be controlling the dewatering properties of fecal sludge.

1.1.5.6 Impact of microbial communities on dewatering performance

The microbial community composition of fecal sludge has not been studied comprehensively and only a few studies have characterised the microbial communities of fecal sludge (Beukes, 2019; Byrne et al., 2017; Connelly et al., 2019; Ijaz et al., 2022; Naphtali et al., 2022; Torondel et al., 2016). Ijaz et al. (2022) had investigated microbial communities in pit latrines sludge in relation to pit filling rates, while Torondel et al. (2016) looked at the effect of geographic factors on microbial community composition. Ward et al. (2019), after identifying the microbial community composition in fecal sludge from Senegal and Tanzania, further evaluated correlations between microorganisms in fecal sludge and dewatering performance. To have a better appreciation of the general microbial community structure in fecal sludge, Sam et al. (submitted) made a scoping study of the microbial community composition of 135 fecal sludge samples from Lusaka, Zambia, and observed that the most dominant phyla were Firmicutes ($60 \pm 5\%$), Bacteroidota (14 ± 3 %), Proteobacteria (8 ± 3 %), Actinobacteria (4 ± 1 %) and Desulfobacterota (3 ± 1 %). This was similar to observations in the literature (Beukes, 2019; Ijaz et al., 2022; Ward et al., 2019). What was interesting in the study by Sam et al. (submitted) was that microbial communities in fecal sludge differed with respect to water usage which was manifested in the type of containment (pit latrine or septic tank), and ammonia concentration of the sludge, but not the type of building use (**Figure 1.4**).
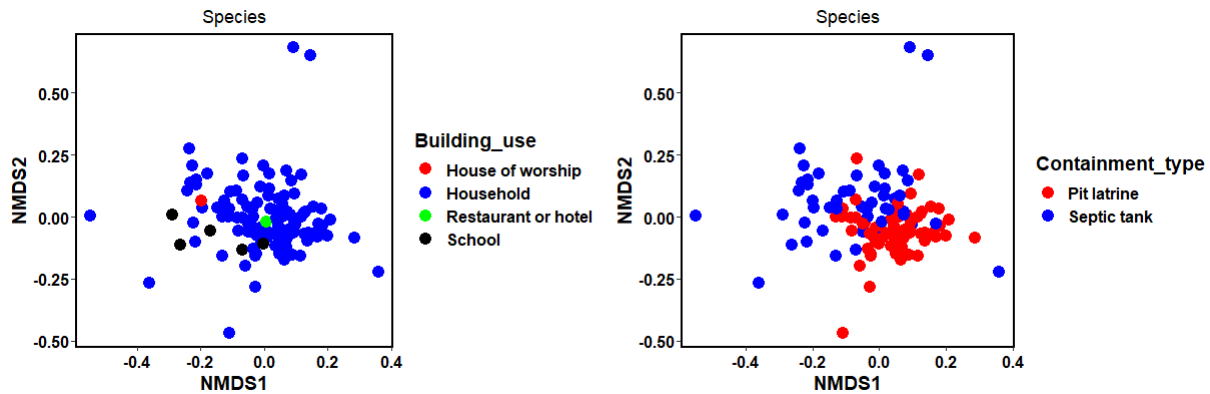
Figure 1-4: Comparison of microbial structure among different usage patterns and water usage at the species level. On the left is the microbial community distribution with respect to the type of building use and on the right is the microbial community distribution with respect to the type of containment (Sam et al. (accepted)).

Association between microbial communities and dewaterability

Generally, dewatering performance is dependent on sludge floc properties, such as the strength of the sludge flocs, compressibility of flocs, particle size distribution and the surface area of the particles (Christensen et al., 2015; Lawler et al., 1986; Neyens et al., 2004). These properties are also subject to the growth of distinct microbial communities or the secretions of specific communities. Additionally, the morphology of some microorganisms contribute to their influence on settling. For example, filamentous bacteria, which grow in long strands, have a high surface to volume ratio and provides a backbone for activated sludge flocs to attach during aggregate formation (Liang et al., 2022). However, the excessive growth of these filamentous organisms causes sludge bulking or foaming reducing settling performance (Sam et al., 2022). Even though, fecal sludge have been shown not to contain flocs (Ward et al., 2019), the role of filamentous organisms could be essential to its dewatering performance. Besides filamentous organisms, microorganisms that produce EPS are expected to influence the dewatering performance of fecal sludge due to the water retention capability of EPS and the ability to enhance settling through binding of sludge particles together due to its slimy or gluey nature. Thus, the presence or absence of EPS producing organisms could dictate the dewatering performance of the sludge. A number of microorganisms, which have the ability to produce EPS, have been documented in the literature (Bala Subramanian et al., 2010; Klai, 2016). However, the quantity and characteristics of EPS produced have been described as being specific to the microbial communities, where under the same conditions; different bacteria strains produce different amounts of EPS. For example, in the study by Klai et al. (2016) on activated sludges, the bacterial strains Cloacibacterium normanense and Brevibacillus

parabrevus produced the highest concentrations of EPS amongst all the bacteria considered. Sabramanian et al. (2010) also reported that the genera bacillus, Serratia and Pantoea produced the highest concentrations of EPS, ranging from 25 – 30 g/L, and that the genus Serratia produced slime EPS with the best sludge settling performance. Bacteria belonging to the genus Zoogloea have also been identified as producing copious amounts of highly water-containing gels, which strongly affect dewatering performance (Lajoie et al., 2000). Since the interaction between EPS and sludge flocs is due to the electrostatic forces on the surface of the flocs, microbial mediated processes that affect the functional groups of EPS will also change the dewatering performance of the sludge. For example, variations in pH will affect surface properties of EPS and therefore microbial communities whose activities alter the pH will influence the dewatering properties of the sludge. In addition to environmental factors that can impact the charges on EPS, variations in the concentration and chemical composition of EPS, which are influenced by the specific bacterial species producing the EPS, can also exert an influence on the surface charge of EPS(Bala Subramanian et al., 2010).

A few studies have looked at the microbial community structure in fecal sludge in relation to dewatering properties. Studies by Ward et al. (2019) suggest that the abundance of specific phyla of bacteria in fecal sludge correlate with some metrics of dewatering performance. A high abundance of phylum Proteobacteria was associated with a low supernatant turbidity and low CST, whilst the abundance of Euryarchaeota was associated with high supernatant turbidity and high CST. The genera Gammaproteobacteria Pseudomonas, Aeromondales, and Tolumonas, and the Bacteroidetes Porphyromonadaceae, Bacteroides, and Macellibacteroides had a high relative abundance in samples with low turbidity, whereas turbid samples had a high relative abundance of the Firmicutes Family XI, and Ruminococcaceae. Sam et al. (submitted) also observed a significantly negative correlation between the class Syntrophia and CST or turbidity, which seems to suggest that the high abundance of this class could enhance dewatering performance by reducing filtration time and reducing the supernatant turbidity after filtration. Surprisingly, the study by Sam et al. (Submitted) did not identify significant correlations between organisms, such as Pseudomonas, which were previously identified to be correlated with CST in fecal sludge samples from Tanzania and Senegal.

The role of stabilization on dewaterability

Sludge stabilization reduces the organic content of the sludge to a level where further reduction is not feasible, resulting in sludge with less odor and reduced pathogen content (Strande et al., 2014). The activated sludge process and anaerobic digestion are prominent methods of the

biological stabilization of municipal wastewater and waste activated sludge, respectively, which reduces the organic matter concentration and generates energy in the form of biogas in the case of anaerobic digestion. Both processes have been observed to influence the dewatering properties of sludge. The effect of anaerobic stabilization on dewatering properties is attributed to its effect on EPS (Novak et al., 2003a) or on small particles that clog the pores and interstitial spaces in sludge cake (Lawler et al., 1986). During anaerobic stabilization, EPS that bind sludge flocs together are degraded. Similarly, the breakdown of larger organic molecules results in the release of small particles, which were held in the flocs, into the bulk solution, thus increasing supernatant turbidity. Particle sizes in the supracolloidal range (1–100 μm) has been observed as being responsible for the differences in filtration (Karr and Keinath, 1978). Therefore, it is widely acknowledged that anaerobic stabilization typically hampers dewatering performance.

However, other studies on the anaerobic stabilization of primary sludge have reported conflicting results, indicating that anaerobic stabilization could improve or worsen dewatering performance (Christensen et al., 2015; Lawler et al., 1986). This indicates that the relationship between stabilization and dewatering performance remains inconclusive. The role of stabilization on the dewatering performance of fecal sludge has not been thoroughly investigated, but based on observations from practitioners, the general perception is that sludge that appears to "stabilized" has better dewatering performance than "unstabilized" sludge. This assertion was confirmed by Ward et al. (2019), where fecal sludge samples from Tanzania and Senegal, which appeared to be more stabilized, with a dark brown to black color and smelling like anaerobic digested sludge, were found to have better dewatering performance than fecal sludge that appeared less stabilized with a light brown color and smelling like fresh feces. A similar observation was also made by Sam et al. (2022a) on fecal sludge samples taken from eight different countries. Although stabilized sludge has long been associated with the long storage of fecal sludge in onsite containments, recent studies have proven that the time in containments is not a predictor of stabilization. Hence, there are still challenges with predicting the levels of stabilization, especially when the metrics of stabilization are not agreed upon. The VS/TS, C/N and the BOD/COD ratios are common metrics that have been applied in wastewater sludges to determine the level of stabilization. However, recent studies by Maqbool et al. (submitted) suggest that high VS concentrations do not correspond to high degradation, which implies that the VS content in fecal sludge comprise less biodegradable or bioavailable organics and more slowly biodegradable organics. Additionally, fecal sludge TS could also comprise grit, sand and stones, which influences the VS/TS ratio. It is, therefore, inaccurate to use the VS/TS or BOD/COD as metrics of stabilization for fecal sludge due to its complex

composition. These challenges continue to complicate efforts to relate the level of stabilization to dewatering performance.

Despite the wealth of knowledge that exists on dewatering in wastewater sludges, only a few studies have focused on identifying the mechanisms that control the dewatering properties in fecal sludge. Our current research efforts have initiated the development of an understanding of the role of such factors as EPS (Sam et al., 2022a), particle size distribution (PSD) (Ward et al., 2023) and cations (Ward et al., 2019), on the dewatering performance of fecal sludge. While we have made progress in identifying the aforementioned factors as playing a role, there is still a shortage of evidence of other factors that could be controlling dewatering performance in fecal sludge, and the effect of complex interactions among these factors that are yet to be investigated.

1.2 Objectives and Research Questions

The overall objective of this thesis was to understand the governing mechanisms of solid-liquid separation (dewatering) of fecal sludge. Specific objectives included:

1) To validate the role of EPS in the dewatering of fecal sludge under anaerobic storage, which helps to understand the changes in EPS and dewatering behaviour of fecal sludge in onsite containments.

2) To evaluate the concentrations of EPS, proteins, polysaccharides, lipids, fibres and cations in feces, and to gain a mechanistic understanding of how these constituents affect the dewatering properties of fecal sludge

3) To evaluate the role of stabilization on dewatering performance, by assessing different levels of stabilization from feces to fecal sludge

4) To evaluate the association of the fecal sludge microbiome to fecal sludge characteristics and factors that affect fecal sludge qualities and quantities (demographic, environmental, and technical data)

The following research questions were asked to address these objectives

- **Chapter 2**: What influence does the amount and composition of EPS (i.e., protein-like and humic-like substances) have on the dewatering performance of fecal sludge, and how do EPS and dewatering performance change with time in containments?

- **Chapter 3:** What role do non-EPS organic and inorganic components of feces have on the dewatering performance of fecal sludge?

- **Chapter 4:** What is the association between the microbial community structure of fecal sludge and its characteristics, demographic, environmental and technical factors, the level of stabilization and the dewatering performance of fecal sludge?

1.3 Thesis Outline

This PhD thesis is structured in five chapters. **Chapter 1** is the introduction, which provides the general context for research into dewatering in fecal sludge. **Chapter 2** focuses on the role of EPS and the fraction of EPS on dewatering performance. We evaluate the effect of EPS on dewatering performance by introducing EPS into fecal sludge, and how the changes in EPS during anaerobic storage of fecal sludge affects dewatering performance. **Chapter 3** investigates mechanistically the effect of constituents expected to be in feces on dewatering performance of fecal sludge. Organic and inorganic constituents in feces are identified, and evaluated for their effect in fresh and field fecal sludge. **Chapter 4** presents a scoping study on the microbial communities in fecal sludge, and how communities are associated with fecal sludge properties. Furthermore, other extrinsic factors that influence fecal sludge characteristics and treatment performance are investigated. 135 fecal sludge samples from Lusaka Zambia are evaluated. **Chapter 5** presents the overall conclusions of this study, while highlighting the practical implication for practitioners and giving some recommendations for future research.

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Chapter 2 : Elucidating the role of extracellular polymeric substances (EPS) in dewaterability of fecal sludge from onsite sanitation systems, and changes during anaerobic storage.

Graphical Abstract
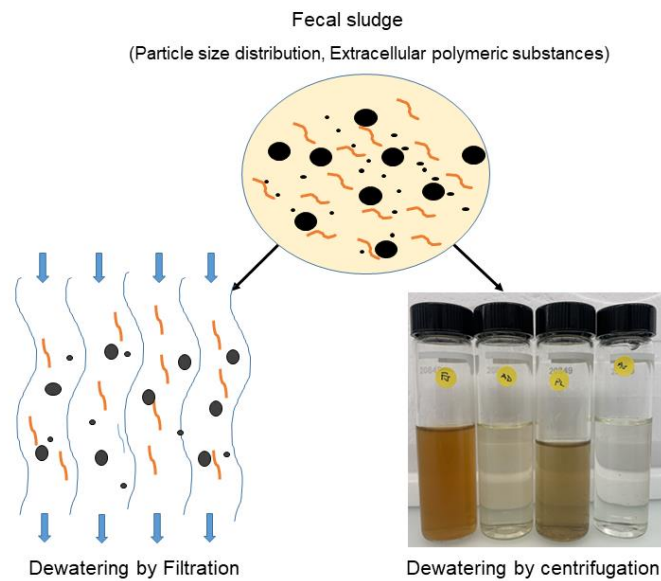
Highlights:

- Fecal sludge can be degraded during anaerobic storage using an inoculum

- Extracted EPS has a significant effect on fecal sludge dewatering time

- EPS concentrations are lower in fecal sludge than wastewater sludges

- Fecal sludge has higher humic-like fraction of EPS compared to wastewater sludges

- Time in anaerobic storage is not a predictor of fecal sludge dewaterability.

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_2CO_3 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.

Keywords: Blackwater, sludge filtration, biomethane potential test, microbial community analysis, particle size distribution

2.1 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 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., 2003b). 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., 2003b). 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.

2.2 Materials and Methods

2.2.1 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 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 S2.1** in **Appendix A**). 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).

2.2.2 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_2 gas to provide anaerobic conditions and incubated in a VWR 5000L 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 2-1** are the characteristics of the all the BMP tests at the start of the experiments.

Table 2-1: 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 ^oC	Inoc. TS(g/L)	Inoc. VS(g/L)	Inoc. NH_4^+-N (mg/L)	Feed TS (g/L)	Feed. VS (g/L)	Feed NH_4^+-N (mg/L)	BMP bottle TS (g/L)	BMP bottle VS (g/L)	BMP bottle NH_4^+-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

2.2.3 Anaerobic batch reactors and serum bottle tests

As summarized in **Table 2-2**, 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 ^{o}C and run 4 at 35 ^{o}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 ^{o}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 R2R 2020 mechanical stirrers 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 5L and 2L glass batch reactors for AD and PL sludge respectively and the operational temperature was 35 ^{o}C. 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.

Table 2-2 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 ºC	pH	TS	VS	TSS	VSS	TCOD	SCOD	EPS	NH4+ -N	TN	FT IR	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
		PL	7	12	20	x	x	x	x	x	x	x	x	x	x			X	x		x
	3	AD	8	12	20	x	x	x	x	x	x	x	x	x	x			X	x	X	x
		PL	8	12	20	x	x	x	x	x	x	x	x	x	x			X	x	X	x
	4	AD	6	12	35	x	x	x	x	x	x	x	x	x	x			X	x	x	x
		AD*	6	12	35	x	x	x	x	x	x	x	x	x	x			x	x	x	x
Glass batch reactors	5	AD	7	5	35	x	x	x	x	x	x	x	x	x		x		X	x	x	x
		PL	7	2	35	x	x	x	x	x	x	x	x	x		x		X	x	X	x
Serum bottle	6	AD	7	0.175	35	x	x	x	x	x	x	x	x	x		x	x	X	x	X	x
		PL	7	0.175	35	x	x	x	x	x	x	x	x	x		x	x	X	x	X	x

2.2.4 Physicochemical analysis

2.2.4.1 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 (NH_4^+-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 analyzed using a Shimadzu 881 compact IC pro ion Chromatograph.

2.2.4.2 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 (20,000-7.5x10^{11} g/mol), humic substances (~1000 g/mol), building blocks (~300-500 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 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.

2.2.4.3 EPS extraction

EPS (soluble/loosely-bound) was extracted from activated sludge obtained from Eawag and from pit latrine FS samples using the Na_2CO_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 minutes. 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 minute 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 grams) 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.

2.2.4.4 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 minutes in a 50 ml falcon tube. The turbidity of the supernatant after centrifugation was determined with a HACH TL 2300 turbidity meter.

2.2.4.5 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 $0.017 – 2000$ µm size range.

2.2.4.6 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 minutes. The supernatant was discarded, and 1 ml of RNA later was added to the pellets and stored at -20 $^{\circ}$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 ul 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 analyzed 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 the functional potential.

2.2.4.7 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].

2.3 Results and Discussion

2.3.1 Effect of EPS on dewaterability of fecal sludge and wastewater sludge

As illustrated in **Figure 2-1**, 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 Na_2CO_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. (2003b), 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.
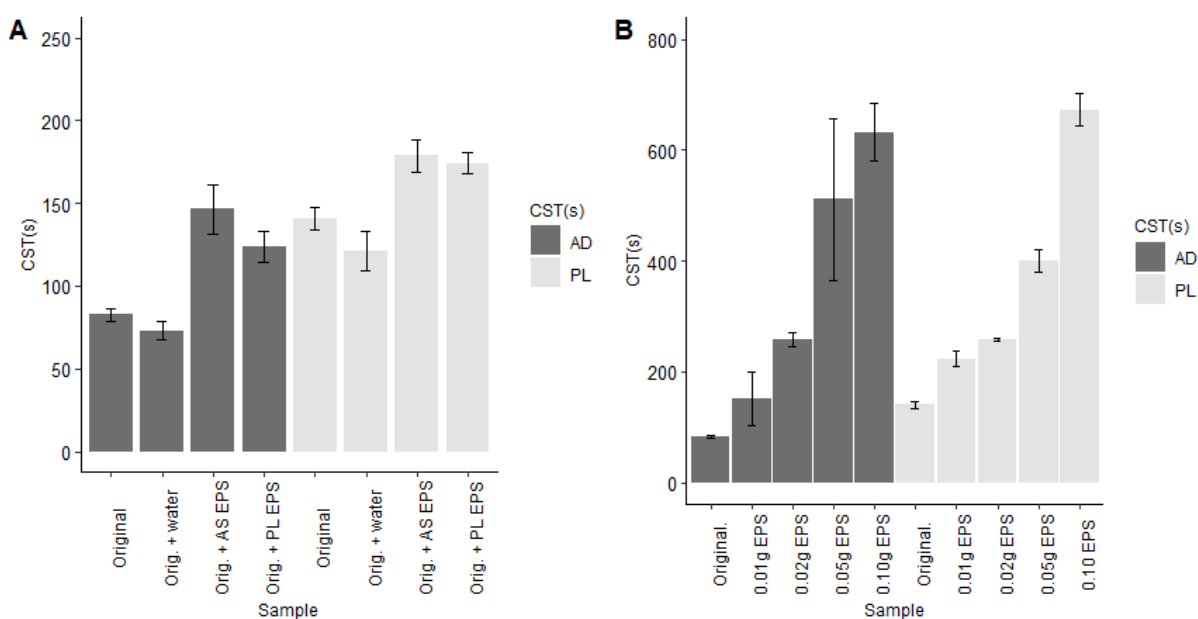

Figure 2-1: Changes in dewaterability of fecal sludge (PL) and anaerobic digested sludge (AD) as measured by CST, with addition of extracted EPS. (A) Addition of soluble/loosely bound EPS from activated sludge (AS EPS), pit latrine sludge (PL EPS) and water (blank) to original AD and PL sludge samples. (B) Addition of freeze-dried EPS to AD and PL sludges.

2.3.2 Preliminary BMP tests

Presented in **Figure 2-2** 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 S2-2** in **Appendix A**. 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, 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., 2019b), 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 faeces to urine ratio, the AD inoculum produced similar volumes of biogas at 20 ℃ and 37 ℃, whereas the PL inoculum had higher biogas production at 37 ℃ than 20 ℃. 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 2-1**) 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 NH_4^+-N concentration.
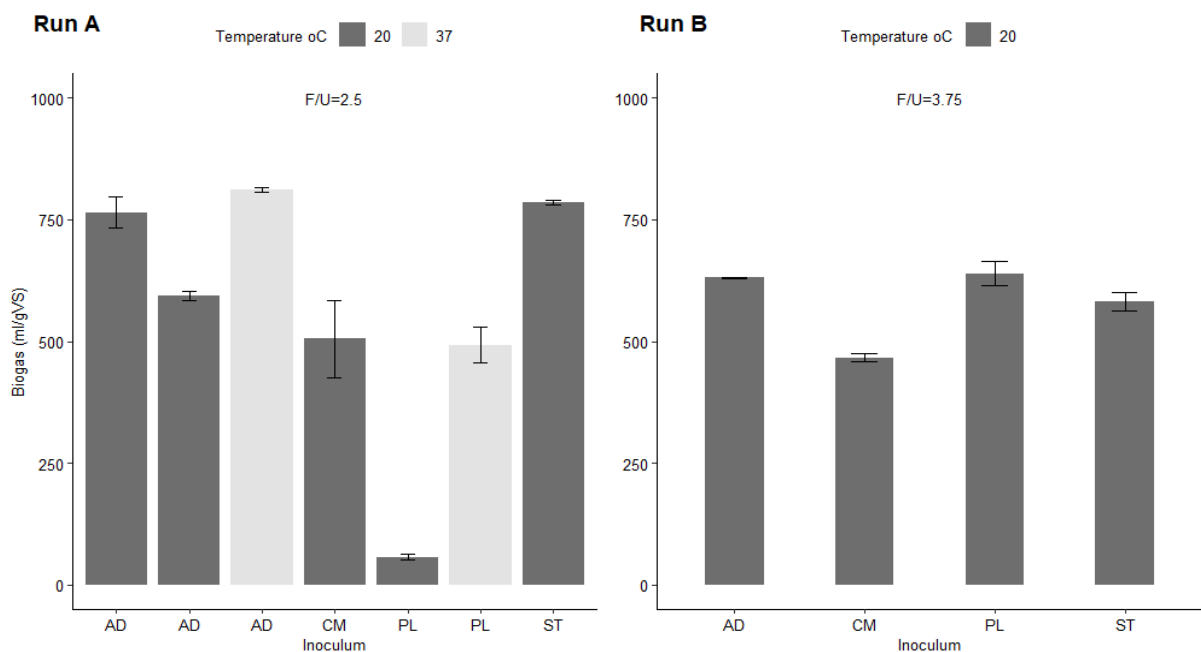

Figure 2-2: Total biogas production of BMP runs A (at 20 oC and 37 oC) and B (at 20 oC). Inocula were anaerobic digester sludge (AD), pit latrine sludge (PL), cow manure (CM), and septic tank sludge (ST). Runs A and B were fed with FS recipes that had feces to urine to feces ratio 1:2.5 and 1:3.75 respectively.

2.3.3 Microbial community analysis

Varying biogas production in the BMP tests could also be associated with microbial community, as illustrated in **Figure 2-3**. 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 Bacteriodes* 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 S2-2** in **Appendix A**). A prediction of the functional potential of the community via a lowest common ancestor approach (Douglas et al., 2020) as illustrated in **Figure 2-3B** 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.
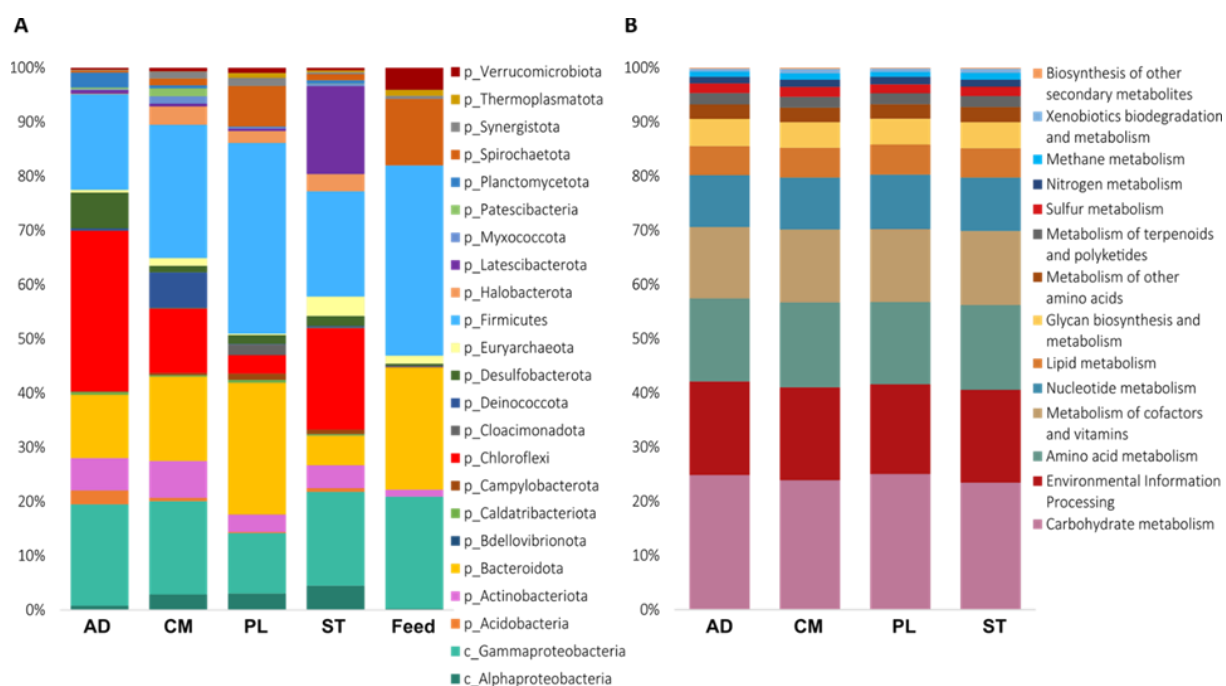

Figure 2-3: Relative abundance of the microbial community composition of the different inocula; anaerobic digested sludge (AD), cow manure (CM), pit latrine sludge (PL), septic tank sludge (ST) and feed based on the amplicon sequence variants. (B) Predicted functional potential based on the least common ancestor.

2.3.4 Effect of anaerobic storage on dewaterability

Illustrated in **Figure 2-4A** and **2-4B** 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 S2-3** in **Appendix A**). 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 **Figure 2-4C** and **2-4D** 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).
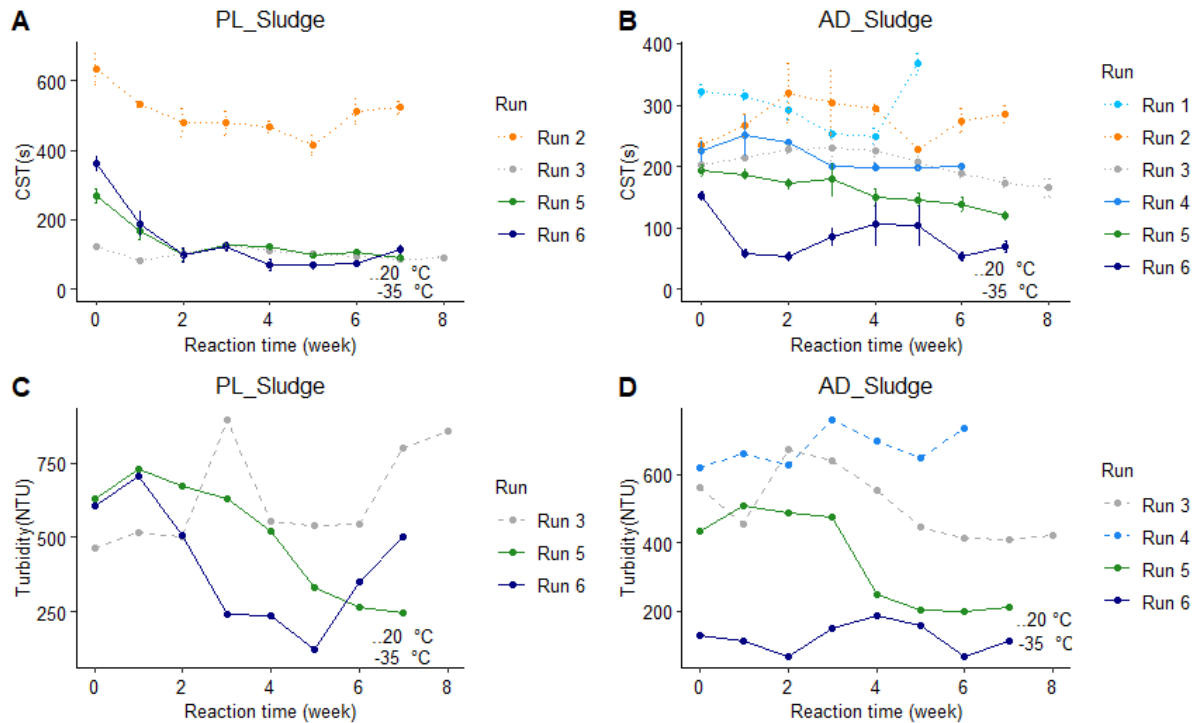

Figure 2-4: Line plots for CST and turbidity during anaerobic storage of FS with PL sludge and AD sludge as inoculum. (4A) and (4B) are CST for PL and AD runs over time. (4C) and (4D) are turbidity measurements for PL and AD sludge runs

2.3.5 Influence of anaerobic storage on EPS and EPS fractions

Reported in **Figure 2-5A** and **2-5B** 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 **Figure 2-5C, 2-5D, 2-5E** and **2-5F** 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 **Figure 2-6** 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.
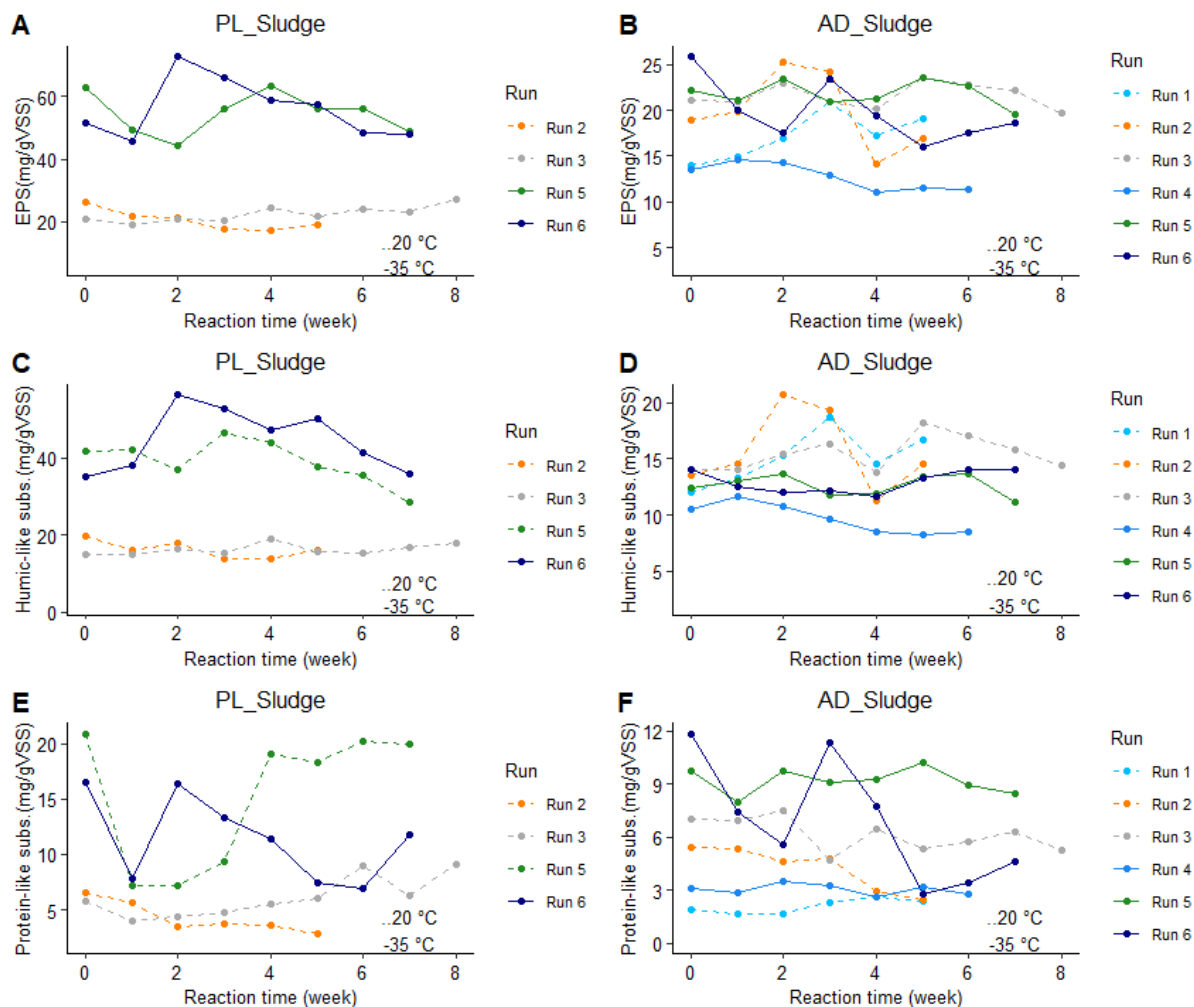

Figure 2-5: Line plot showing total EPS concentrations and fractions of EPS during anaerobic storage. (5A) and (5B) are total EPS concentrations for PL and AD sludge runs respectively. (5C) and (5D) are the concentrations of humic-like substances fraction of PL and AD sludge runs (5E) and (5F) are the protein-like substance fraction of EPS in PL and AD sludge runs

Illustrated in **Figure 2-6** 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 S2-5** in **Appendix A**). 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.
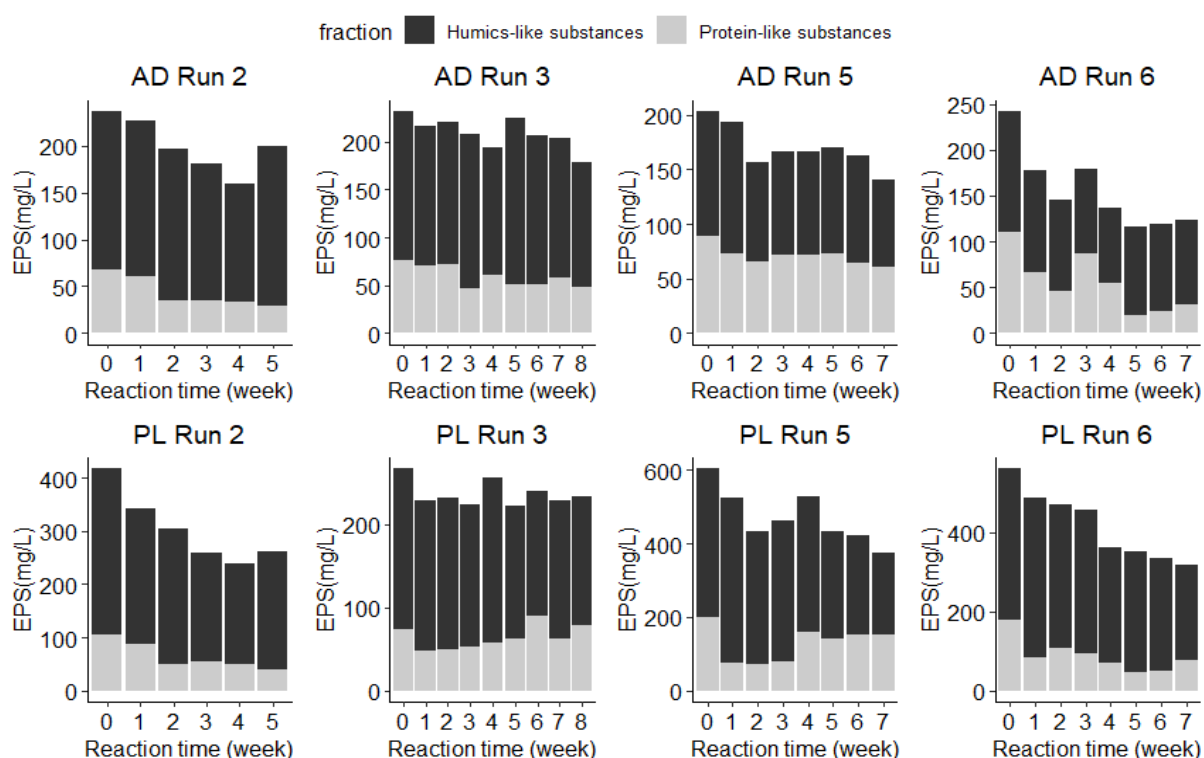

Figure 2-6: Bar plots showing total EPS concentrations broken down into specific fractions comprised of protein and humic-like substances of EPS (mg/L) over the reaction time for runs 2, 3, 5 and 6 where AD and PL sludges were used for anaerobic storage of FS

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 **Figure 2-7**, peaks representing, carboxylic or hydrocarbon containing compounds (1,500-1,300 cm-1) and carbohydrates (1,200-900 cm-1) were clearly evident (Badireddy et al., 2010). A major peak between 1,033-1086 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 2,850 cm-1 and 2,919 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.
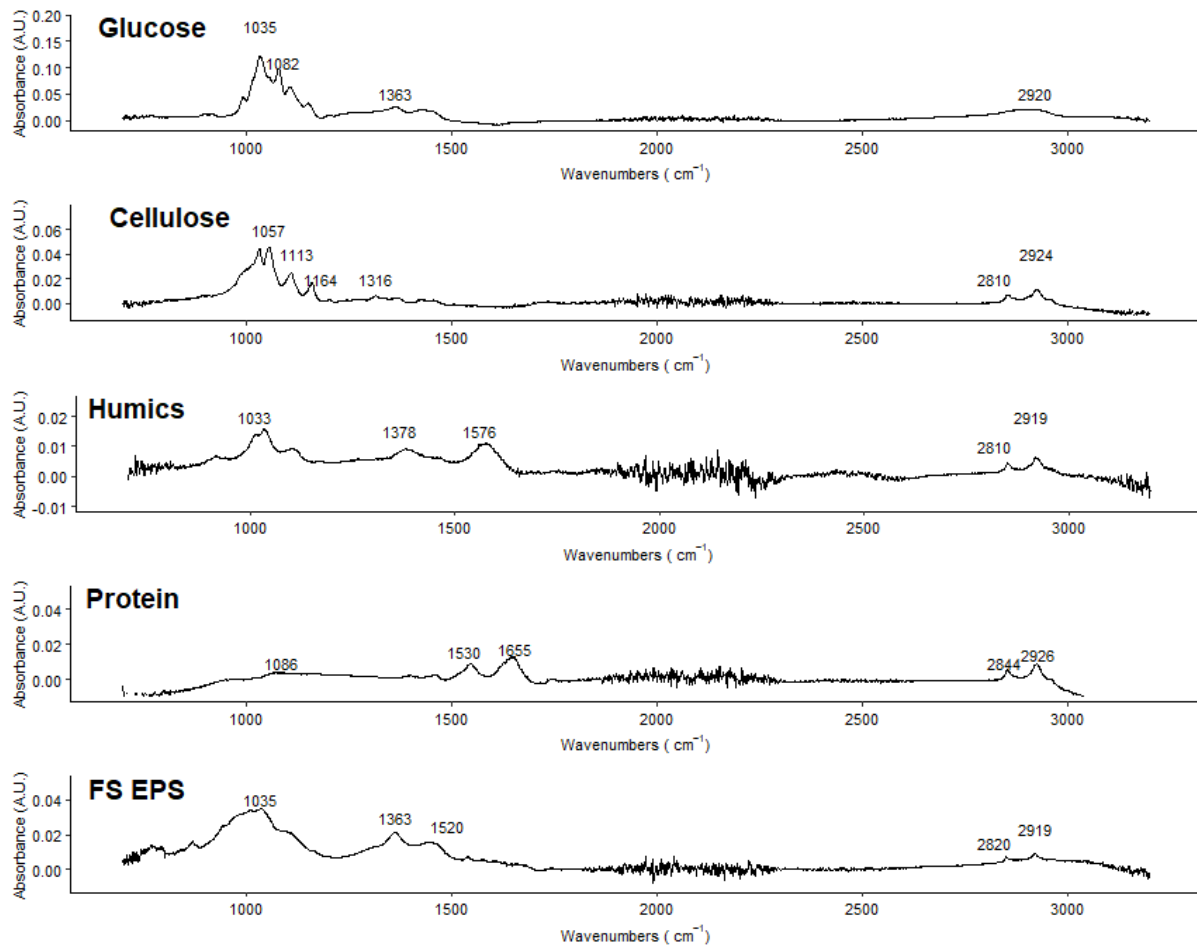

Figure 2-7: FTIR spectra of the glucose, cellulose, humic acid, and protein standards and EPS extracted from FS

2.3.6 Performance of BMP tests and anaerobic batch reactors

All inocula in BMP tests were monitored for pH, VFA, alkalinity and NH_4^+-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, NH_4^+-N between 0.8 and 3.3 g/L and alkalinity between 2.8 and 9.4 g/L $CaCO_3$. Recommendations for BMP test are pH > 7.0 and < 8.5, VFA < 1 g/L acetate, NH_4^+-N < 2.5 g/L NH_4^+-N and alkalinity > 3 $gCaCO_3$ L^{-1} (Holliger et al., 2016). The percentage of CH4 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 $CaCO_3$) 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.

2.3.7 Influence of particle size distribution

Figure 2-8 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 -2000 µm. Particle size distribution was performed in run 6 to see if it could help explain the dewaterability results in runs 1-5. Whiles PL sludge shows multiple peaks at 30, 52, and 185 um, AD sludge had a unimodal distribution with a peak at 52 µm and a shoulder at 560 µm 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 (1-100 µm) 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) (Rudolfs 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 S2-3** in **Appendix A**). 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.
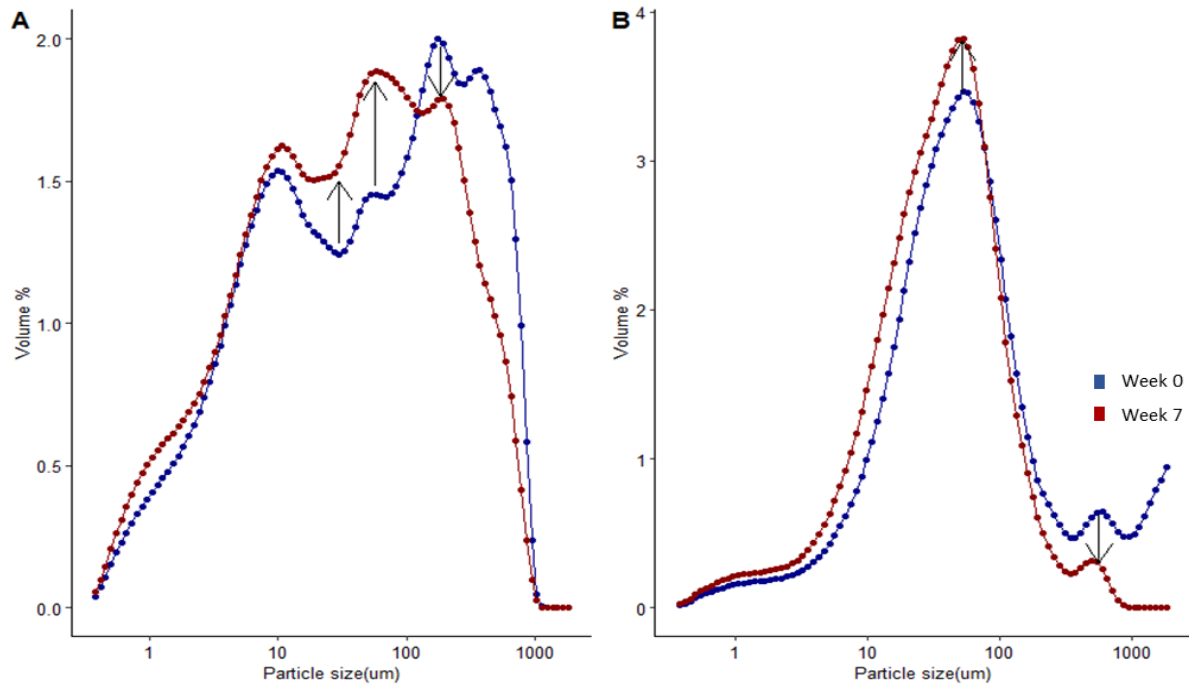
Figure 2-8: Line plots showing the particle size distribution of FS during anaerobic storage. (7A) and (7B) represents the size distribution of the initial and final sludge samples of the PL and AD inoculated runs respectively.

2.3.8 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 Erdincler, 2004; Sheng et al., 2010). EPS concentrations in this study (9-72 mgEPS/gVSS) were lower than reported EPS concentrations in wastewater sludges using similar methods of extraction (30 and 290 mg/gVSS (Caudan et al., 2012; Comte et al., 2006; Pellicer-Nàcher et al., 2013). The EPS concentration in this study is below the 100 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

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significant relations to sludge dewaterability, but the humic-like fractions of EPS were consistently greater than for wastewater sludges.

2.3.9 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 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 , C. M. et al., 2021).

2.4 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.

2.5 Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this publication.

2.6 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.

2.7 Acknowledgements

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Chapter 3 : Changes in organic fractions, cations, and stabilization from feces to fecal sludge: implications for dewatering performance and management solutions.

Published manuscript

Graphical Abstract
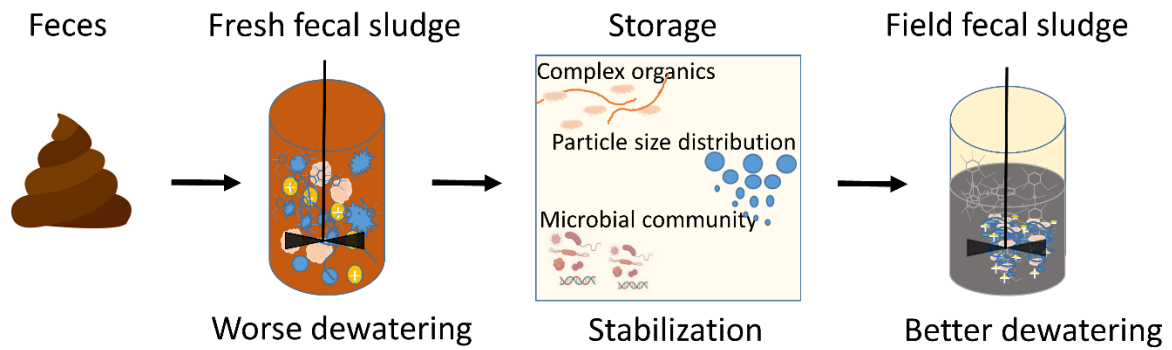
supplement

Highlights:

- Fibers, EPS and lipids influence dewatering performance of fecal sludge
- Stabilization plays a key role in improving dewaterability of fecal sludge
- Management practices play a larger role than diet in accumulated fecal sludge
- Variations in FS microbial community could explain differences in dewaterability

Abstract

Reliable dewatering performance remains a key challenge in fecal sludge management, and the controlling factors or mechanisms are not well understood. There remain limited studies on constituents in feces and fecal sludge and how they affect the dewaterability of fecal sludge. This study aimed at evaluating a range of constituents in feces, and to gain empirical knowledge towards a mechanistic understanding of how they influence dewaterability. In this study cellulose reduced capillary suction time, decreased supernatant turbidity and increased cake solids. While hemicellulose decreased supernatant turbidity, lignin increased supernatant turbidity, capillary suction time and cake solids. Extracellular polymeric substances (EPS) increased both capillary suction time and supernatant turbidity and decreased cake solids, whereas lipids increased turbidity. Cations had no significant effect on dewatering properties. Overall, fecal sludge stored in containments had better dewatering performance than "fresh" fecal sludge, which was attributed to stabilization. Field fecal sludge had higher relative abundance of Pseudomonas, which is associated with better aggregation, and less small particles (<10 µm) that clog filters to reduce dewatering performance. Further understanding of stabilization, and developing agreed upon metrics of stabilization, is essential for predicting fecal sludge dewatering performance, and developing smaller footprint dewatering treatment technologies.

Keywords: microbial community, organic fibers, water-holding capacity, settling performance, stabilization

3.1 Introduction

Globally, 31 % of sanitation needs in urban areas are met through non-sewered sanitation (WHO, 2021). Fecal sludge (FS) is defined as what accumulates during storage in onsite containments, and can include feces, urine, flush water, greywater (e.g. kitchen, bathing), food waste and rubbish (Strande et al., 2014). In urban areas of low-income countries, at least 60 % of FS is not safely managed, placing a huge burden on public and environmental health (Peal et al., 2020). Typically FS consists of more than 95 % water (Gold et al., 2017) making it expensive and difficult to transport. Dewatering has proven to be inconsistent and unpredictable, making effective dewatering one of the greatest knowledge gaps for sustainable management of FS (Ward et al., 2019), and a barrier for implementing low footprint technologies (Mercer et al., 2021a). Unlike wastewater sludges, dewaterability of fresh feces, FS, nor FS stored in onsite containments is well understood. FS is different from waste activated or anaerobically digested sludges, and has variable solids, organic and nutrient concentrations, which can be up to two orders of magnitude greater than municipal wastewater (Ward et al., 2019). We have started to develop an understanding of the role of properties like extracellular polymeric substances (EPS) (Sam et al., 2022), particle size distribution (PSD) (Ward et al., 2023) and cations (Ward et al., 2019) in dewaterability of FS, but they on their own do not yet explain observed dewatering performances.

Feces is a component of FS, but there remains a general lack of knowledge of constituents and the role they play in dewatering behavior of FS. Examples of existing knowledge include mass balance reports on the chemical composition of feces, mainly conducted by NASA during the early stages of the space program (Goldblith and Wick, 1961) and studies on gut microbiome starting around the turn of the 21st century (Hopkins et al., 2002). Other examples include soluble material and fiber content in feces in relation to their effect on fecal weight and transit time in the intestines (Stephen and Cummings, 1980), and proteins and lipids in relation to inflammatory bowel disease and steatorrhea (Hidaka et al., 2000; Wenzl et al., 1995). Research initiated by the reinvent the toilet challenge (RTTC), a program initiated by the Bill and Melinda Gates foundation for 'back end' treatment at the source of production (no transport) with mainly thermal or chemical treatment at source (Bhagwan et al., 2019), has also investigated the rheological properties, energy recovery, microwave treatment technologies and hydrothermal liquefaction of human feces (Mercer et al., 2021a; Watson et al., 2017). A review by Rose et al. (2015) summarizes reported ranges of microbial biomass, unabsorbed macromolecules (proteins, polysaccharides and lipids), trace constituents such as secretions and inorganic

fractions. Gold et al. (2017) and Krueger et al. (2021) also analyzed the fiber content of feces in relation to fecal bioconversion by black soldier fly larvae and thermal decomposition. Penn et al. (2018) attempted to develop synthetic feces and FS recipes for research purposes. However, the properties related to dewaterability could not be replicated and the synthetic FS recipe had 60 % reduced dewaterability in comparison to FS. Diet is often considered to have an influence on total fecal output or generation rate (Rose et al., 2015), however, it is not clear the role on fecal composition in terms of organic fractions and microbial communities and if that translates into FS characteristics or dewatering properties.

Research in centralized wastewater treatment spans over a 100 years (Stensel and Makinia, 2014), and based on that we know EPS (proteins, polysaccharides), lipids, fibers, cations and microorganisms play an important role in dewatering performance. EPS are highly charged and incorporates up to 99 % water in a cross-linked hydrogel through electrostatic interactions, hydrogen bonding or Van de Waal forces (Pfaff et al., 2021), and promote floc formation by bridging particles together which enhances settling performance (Christensen et al., 2015). Lipids adsorb onto bacterial biomass, decreases the specific gravity and settling of sludge particles (Chipasa et al., (Chipasa and Mędrzycka, 2006). Fibers, (cellulose hemicellulose and lignin) increase water holding capacity due to peripheral hydroxyl groups of glucose that interact with water through hydrogen bonding. Divalent cations form strong and compact flocs in activated sludge through their interaction with extracellular polymers, whereas monovalent cations have detrimental effect on dewatering (Christensen et al., 2015). Due to the importance of these constituents in governing dewatering performance of wastewater sludges, they were selected for investigation of the role they play in governing dewaterability of fresh FS and field FS. Fresh FS is defined here as FS containing feces, urine, flushwater and cleansing water, that has not yet been stored in a containment.

The objectives of this study were to: (1) evaluate the concentrations of constituents in feces that have been identified as governing dewaterability in wastewater sludges (i.e. EPS, proteins, polysaccharides, lipids, fibers, cations); (2) gain empirical knowledge towards a mechanistic understanding of how these constituents affect dewatering properties of FS; (3) compare these constituents and dewaterability of fresh FS to FS collected from onsite containments in eight different countries; (4) and to evaluate the role of stabilization from feces to FS in dewaterability. In this study, dewaterability is defined as the process of separating solid matter and liquid fractions, employing well-established metrics for filtration quantified by capillary

suction time (CST), settling measured by the supernatant turbidity following centrifugation, and water holding capacity as measured by the dewatered cake solids.

3.2 Materials and Methods

3.2.1 Source of feces, fresh FS, and field FS

Feces were collected from 20 anonymous volunteers (from 11 different countries) at Eawag in Dübendorf, Switzerland, using a double blind system so no information could be traced back to individuals. Participants completed a questionnaire including diet and use of medications for the 24 hour period prior to sample collection. The questionnaire is provided in the supplemental information (**Appendix B**) and the responses in the published data package. Feces were stored at -20°C and brought to room temperature before analysis were performed. Ten samples of FS from containments (field FS) were obtained from eight countries: Ghana, Senegal, Uganda, Kenya, Lebanon, India, Canada and Guatemala. Samples from Ghana and Senegal were septic tank sludges obtained from vacuum tracks during discharge at a FS treatment plants, and all other samples were obtained by direct sampling from onsite containments as described in Shaw et al. (2022). Previous studies by Ward et al. (2019, 2021a) have established that the time since last emptied for FS in containment is not a predictor of dewaterability, hence it was not considered in the samples collection. Characteristics of the 20 feces, 10 field FS and one fresh FS samples used in the study are provided in the supplemental information (**Appendix B**). Samples were stored immediately in cooling boxes with ice packs before being airfreighted to Switzerland under refrigerated storage. For a more consistent FS characteristics, Fresh FS was prepared by mixing freshly collected feces and urine from a urine separating dry toilet at Eawag to obtain a feces to urine ratio of 1:2.5 by wet weight and diluted with water to 2 % TS according to Sam et al. (2022a). The ratio of feces to urine is according to the daily per capita production of feces and urine, with an average of 400 g feces and 1 L urine (1:2.5) (Colón et al., 2015) and it was diluted to a TS of 2 % which is within the range of reported TS concentrations in FS (Velkushanova et al., 2021). Although the fresh FS recipe used in this study established a baseline for understanding dewatering performance, the results should be further validated with a range of fresh feces. Field FS and fresh FS were stored at 4 °C prior to experiments.

3.2.2 Analytical methods

Analysis of physical-chemical characteristics including total and volatile solids (TS and VS) were performed gravimetrically according to methods for FS analysis (Velkushanova et al., 2021). pH and electrical conductivity (EC) were quantified with a WTW pH/conductivity 3320

meter (Xylem Analytics Germany GmbH). Total and soluble chemical oxygen demand (COD and sCOD) (Method 5220), total nitrogen (TN) and total phosphorus (TP) (Method 4500-N C and 4500-P E) were quantified with HACH Lange test kits, as described in Velkushanova et al. (2021). Analysis on feces samples involved diluting 0.1 g of feces in 50 ml nanopure water.

Total organic carbon (TOC) was analyzed using Shimadzu TOC-VCPN Total Organic Carbon Analyzer according to Velkushanova et al. (2021). Mono- and divalent cations Ca^{2+}, Na^+, Mg^{2+} and K^+ were quantified with an ICP-OES (Perkin Elmer, Waltham, MA, USA) on the supernatant after centrifuging samples at 3,000 g for 20 minutes. The supernatant was filtered through a 0.45 µm filter and acidified with 65 % nitric acid in a 1:100 dilution (Park et al., 2006). Cations were reported in mg/L since cations will also be part of the liquid fraction of feces and FS.

PSD of the FS samples were analyzed using the static light scattering measurement according to AHPA standard method 2560D (Velkushanova et al., 2021) using a Beckman Coulter LS 13 320-Laser Diffraction Particle Size Analyzer.

Total and intact cells were measured for both feces and field FS by flow cytometry using the CytoFLEX (Beckman Coulter, Brea, California, USA) at a flow rate of 60 µL/min for 60 s. SYBR Green I stain was used to determine total cells whiles the intact cells were determined with the aid of the Sybr Green I/Propidium Iodide (SG/PI) stain. Total and intact cells helped to quantify dead and live cells (Van Nevel et al., 2017).

Sludge dewatering properties filterability, settling measured by supernatant turbidity (after centrifugation) and water holding capacity were quantified with well-established metrics that are accepted and widely applied in wastewater and fecal sludge research, including capillary suction time (CST), supernatant turbidity after centrifugation and dewatered cake solids (Christensen et al., 2015; Velkushanova et al., 2021; Ward et al., 2019). CST, supernatant turbidity and dewatered cake solids (per gram wet weight) were determined according to the methods for FS analysis (Velkushanova et al., 2021).

The extraction and quantification of the soluble and loosely bound EPS in both feces and FS was carried out by sonication and size exclusion chromatography (SEC) respectively according to Ward et al. (2019), with dilution of the feces samples prior to analysis. Proteins and

polysaccharides were determined on diluted feces and FS samples using colorimetric methods; the BCA protein assay with bovine serum albumin (BSA) as the standard and the Anthrone assay with glucose as the standard respectively (Raunkjær et al., 1994). Lipids were extracted with the soxhlet extraction method using petroleum ether (Lenaerts et al., 2018) in a Gerhardt classic soxhlet apparatus at 40–65 °C (Gerhardt, Königswinter, Germany). The extraction was carried out for 7-8 hours (about 24 cycles). Fiber content of feces and FS were analyzed according to the Van Soest fiber analysis methods (Van Soest et al., 1991) using the Fibretherm FT 12 (Gerhardt, Königswinter, Germany; AOAC index no. 973.18).

3.2.3 Effect of representative constituents on dewatering properties

To gain an understanding of the effect of constituents that govern dewaterability (as described in the introduction) specifically on fresh FS, materials identified as representatives for each of the constituents were used in a series of jar tests as described in methods of FS analysis (Velkushanova et al., 2021). The representative materials identified from the literature for cellulose, hemicellulose, lignin, and lipids were cellulose fiber (CAS Number: 9004-34-6), xylan (CAS 9014-63-5), lignin alkali (CAS 8068-05-1) and sunflower oil (Migros, Switzerland), which is a standard material to represent food lipids (Krueger et al., 2021). NaCl, KCl, CaCl$_2$, and MgCl$_2$ salts were used as sources to provide Na$^+$, K$^+$, Ca^{2+} and Mg^{2+} in solution and freeze-dried EPS extracted from activated sludge with the Na$_2$CO$_3$ method (Schambeck et al., 2020) was used for EPS. Jar tests were conducted in quadruplicate with one control using 400 mL fresh FS. A higher dose (10X) of each constituent based on baseline concentration (1X) (Supplemental information in **Appendix B**) in fresh FS were added to the fresh FS and system was stirred at a uniform speed of 60 rpm for 2 min (Shaw et al., 2022) after which metrics of dewaterability were quantified.

3.2.4 Microbial community analysis

DNA of feces and field FS were extracted following a modified method by Griffiths et al. (2000) described in Sam et al. (2022a). 16S rRNA gene amplicon sequencing was carried out by Novogene on an illumina MiSeq platform based on bacterial and archaeal V3-V4 region. Raw sequences were analyzed within the QIIME2 environment with the MIDAS database (Nierychlo et al., 2020). One FS sample, FS10 from Lebanon failed sequencing test and is therefore not included in the microbial community analysis.

3.2.5 Data analysis and statistics

R software and R Studio version 4.1.1 (R Studio Inc., Boston, MA, USA) were used for the analysis of the data. Significance of differences in mean organic and inorganic constituents of the different food groups were tested with analysis of variance (ANOVA) single test. Note: the data for this study will be openly available in Eawag Research Data institutional Collection (ERIC) at https://doi.org/10.25678/0007MP when this paper is published.

3.3 Results and Discussion

3.3.1 Characteristics of feces

Feces collected from 20 study volunteers had an average TS concentration of 0.23 ± 0.04 (g/g wet wt) and VS of 0.87 ± 0.02 (g/gTS), and were within the range of 0.14-0.37 (g/gTS) and 0.84-0.93 (g/gTS) respectively reported by Rose et al. (2015). Feces contained a TP concentration of 0.02 ± 0.01 (g/gTS), which is the same as 0.02 (g/g) reported by Vinneras et al. (2006). The TN of 0.07 ± 0.03 (g/gTS) was comparable to the 0.05 ± 0.02 (g/gTS) in feces reported by Stephen et al. (1980), and is thought to come from undigested proteins or the protein content of microbial biomass. The total cells in feces were between 6.7×10^{11} - 16.1×10^{11} cells/g dry weight, which is in the range of $5 \times 10^{11} \pm 4 \times 10^{11}$ bacterial cells per gram dry weight of feces reported by Suau et al. (1999). The total cells in feces accounted for 8 - 19 % of the TS, which is less than 25-54 % of dry solids reported in the review by Rose et al. (2015). However, only 5 % of the total cells were viable (Supplemental information in **Appendix B**) in contrast to 40-57 % reported by Ben-Amor et al. (2005), which could be due to freezing of samples.

The results by constituents in feces that are likely to be governing the dewaterability of FS are illustrated in **Figure 3-1**. Overall, it was observed that the fiber content was predominantly lignin, as also observed by Krueger et al. (2021). Lignin fractions remain in feces due to a low digestibility and bioavailability, resulting from limited enzymatic hydrolysis (Pérez et al., 2002). The concentration of cellulose was lower than the 0.17 (g/g TS) reported by Gold et al. (2017). In this study, soluble and loosely bound EPS accounted for about 3 % of the TS, which is higher than previously observed in FS from septic tanks (0.7 -1.0 %), but comparable to EPS from pit latrine FS (2-3 %) (Sam et al., 2022). The concentrations of proteins, polysaccharides and lipids in feces were comparable to each other, and proteins and polysaccharides were within the range of 0.11-0.56 and 0.14-0.85 (g/gTS) reported by Rose et al. (2015), although lipids were slightly higher than the reported 0.087–0.16 (g/gTS). Although soluble and loosely bound EPS contains proteins and polysaccharides, the proteins and polysaccharides quantified by colorimetric methods are greater, as they are the total amount contained in the feces and can include undigested food matter, biomass, and EPS (Rose et al., 2015). Cations in feces reported by Rose et al. (2015) were within 0.08-0.72 %, which is lower than in this study. By analyzing all of these constituents for the same sample set, it allows for a direct comparison of the individual constituents that was previously difficult. A comparison of the characteristics of feces and fecal sludge samples is provided in the supplemental information (**Appendix B**).
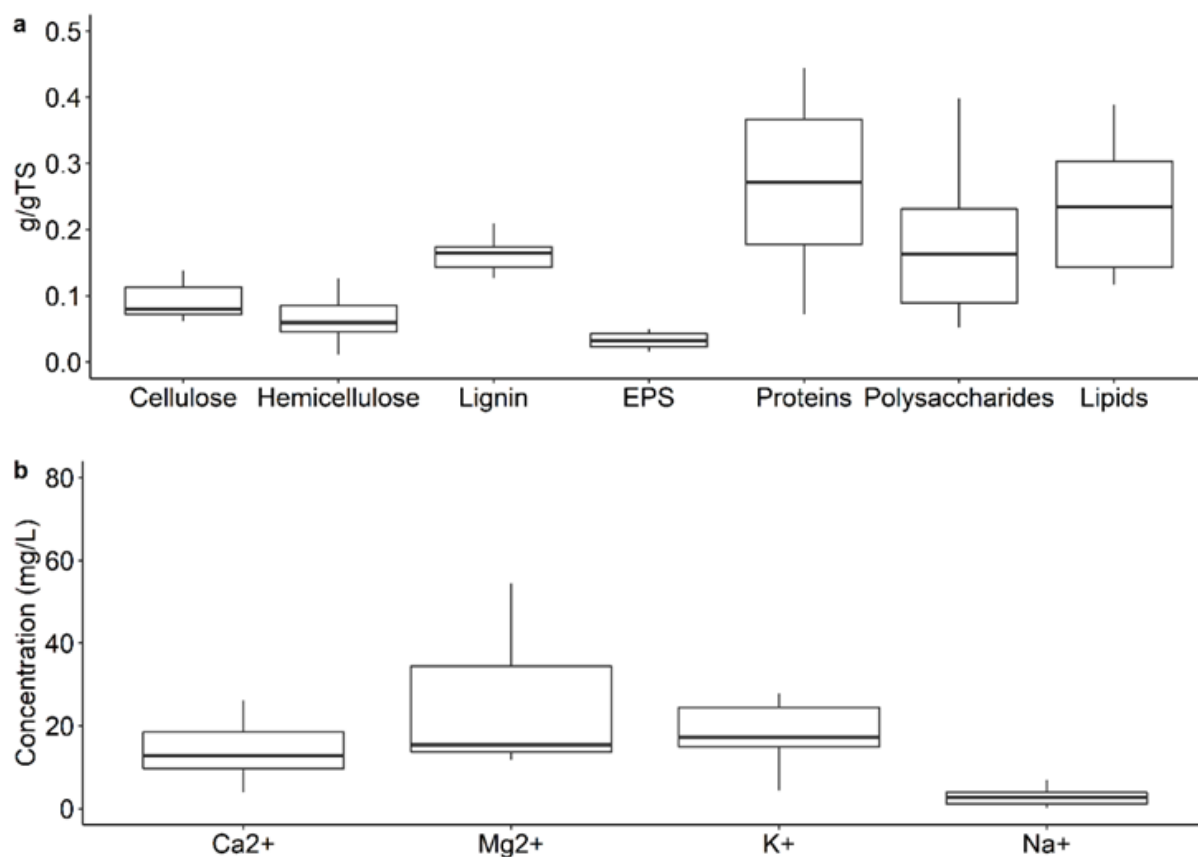
Figure 3-1: Boxplots showing (a) concentrations of fibers (cellulose, hemicellulose and lignin), EPS, and the organic content (proteins, polysaccharides and lipids) per gram TS of feces and (b) mono- and divalent cation concentrations in mg/L in feces

3.3.2 Effect of diet on feces characteristics

Feces were compared for differences in organic fractions reported in **Figure S3-2** in **Appendix B**, by reported consumption of dairy, fruits, proteins and vegetables 24 hours prior to the collection of feces. (Kolodziejczyk et al., 2012). Five people reported consuming fruits and vegetables and two not, six people reported consuming proteins and dairy and six not. None of the participants reported taking antibiotics or probiotics, and grains were not included as they were reported as consumed by all volunteers. Overall, there were no distinct differences in the organic constituents of feces based on diet in this study (based on ANOVA single test), although it is in general difficult to draw conclusions on the effect of diet on feces. One factor is that transit time in intestines is quite variable, reported from 24-48 h (de Vries et al., 2016) to 40-60 h (Degen and Phillips, 1996), which is thought to also vary by gender, physical activity, age and body mass index (Procházková et al., 2023). Another is the role of diet on total mass, where it is worthy to note that even in controlled diet studies where participants are given the same

food, the characteristics and total production of feces is variable (Attebery et al., 1972). Rose et al. (2015) concludes greater feces production by vegetarians, however, the cited studies actually observed the same total production (Silvester et al., 1997), or methods of data collection that are not culturally relevant based on purely observational data (e.g., "white" versus "Indian" women in London (Reddy et al., 1998). Additionally, differences in the microbial communities in feces (section 3.4.2) did not vary by diet (**Figure S3-3- Figure S3-6 in Appendix B**). This is in agreement with Ferrocino et al. (2015), who observed that environmental factors due to geographic location are stronger factors than diet. In conclusion, although the effect of diet or differences between individual stools is maybe of interest for technologies that are treating feces at the individual level (Mercer et al., 2021b), it is most likely not of relevance for community- or semi-centralized management following storage as FS in containments.

3.3.3 Dewaterability of fresh and field FS and the role of representative constituents

In order to gain an understanding of which constituents are governing dewaterability of FS, the influence of individual constituents on dewatering properties was evaluated with fresh FS, and compared to the range of constituents observed in field FS. Using the concentrations in fresh FS as the baseline, dewatering performance was evaluated following the addition of 10x aliquots of proxy constituents for cellulose, hemicellulose, lignin, lipids, EPS and mono- and divalent cations.

3.3.3.1 Role of representative constituents on filtration time (CST)

As illustrated in **Figure 3-2a**, addition of cellulose fiber decreased CST for fresh FS (red circle) over the baseline case of fresh FS alone (green squares), and addition of lignin alkali and EPS increased CST over the baseline. All of the constituents in field FS were similar to those in the fresh FS, however, the CST was always lower. The effect of xylan/hemicellulose, oil/lipids and mono- and divalent cations were not substantial (**Figures 3-2a** and **Figure S3-6**). Cellulose has been observed to improve filtration in wastewater sludges by providing surfaces for attachment of small particles and microorganisms, thus preventing clogging of filter media or reducing resistance to filtration (Zhang et al., 2020). The role of free and bound water plays a role in dewatering of wastewater sludges, with bound water more difficult to remove (Chen et al., 2002). Alternatively, filtration is hindered by cross-linked polymeric networks of cellular and particulate components can form an impermeable layer (Skinner et al., 2015). This appears to be the case for FS, where EPS behaves like colloidal and suspended organic substances that clogs pores of filter media, and is more relevant than bound and free water (Ward et al., 2019).
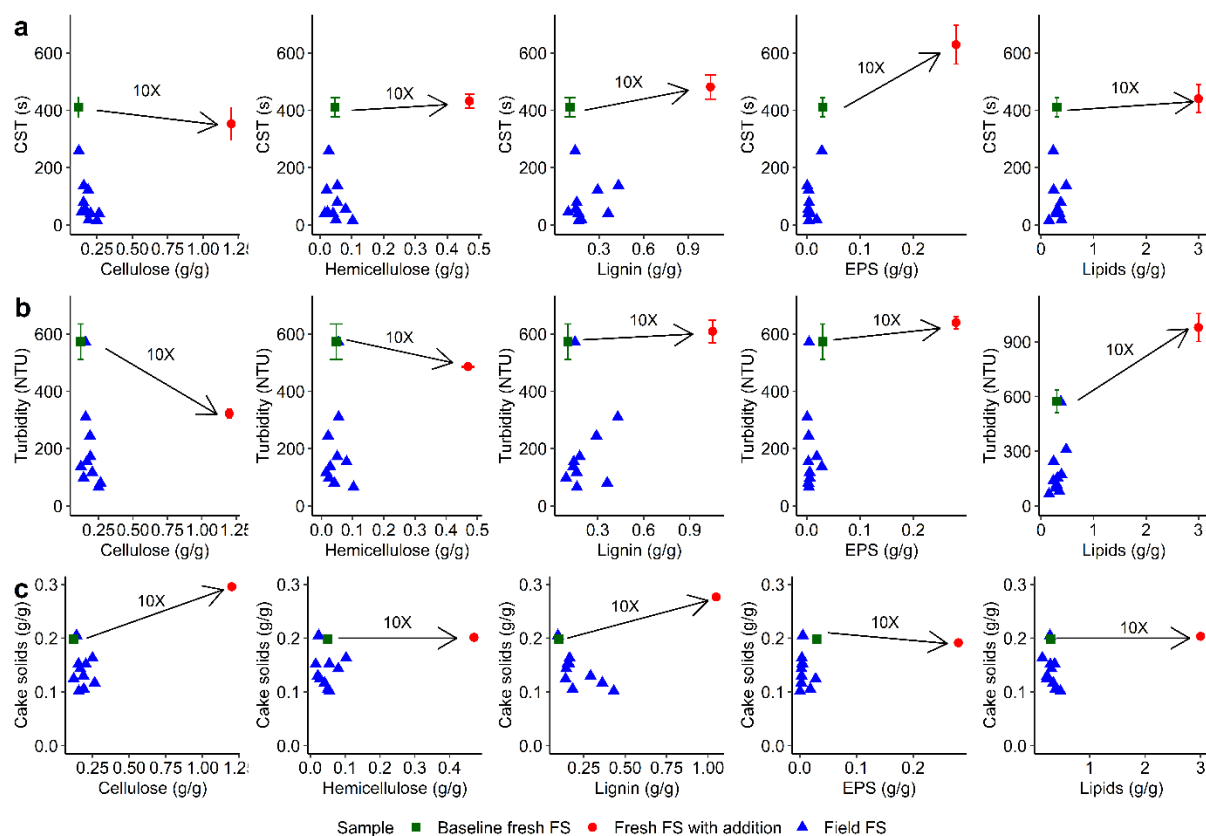
Figure 3-2: Dewaterability of fresh and field FS showing the effect of representative constituents on dewatering properties (a) Change in filtration time (CST) in fresh FS and CST of field FS; (b) supernatant turbidity(after centrifugation; and (c) water holding capacity (dewatered cake solids). The green squares represent the baseline dewaterability of fresh FS, the red circles represent addition of aliquots to the fresh FS and the blue triangles represent the field FS.

3.3.3.2 Role of representative constituents on supernatant turbidity after centrifugation

Addition of cellulose fiber and xylan/hemicellulose decreased supernatant turbidity of fresh FS (**Figure 3-2b**), elevated lipid/oils, EPS and Ca^{2+} increased supernatant turbidity, and monovalent cations did not have an effect (**Figures 3-2b** and **Figure S3-6**). The turbidity of field FS was always lower than fresh FS, indicating the field FS contained less fine, unbound particles. Fibers are reported to reduce supernatant turbidity in wastewater sludges by reducing suspended residual colloids or small particles (Huang et al., 2019). In contrast, Hofgen et al., (2019) report that fibers reduce gel point TS by inhibiting the compressional effects of gravity, making fibers detrimental to settling performance through increased turbidity. Mercer et al. (2021a, 2021b) also predicts better settling performance in fresh feces than field FS, but it is difficult to compare the results as the feces were macerated and turbidity was not measured in their study. In this study compression yield stress was also not measured. In contrast to

wastewater sludges where Ca^{2+} is reported to decrease supernatant turbidity based on the cation bridging theory (Higgins and Novak, 1997), it does not appear to hold true for FS, which was also observed by (Ward et al., 2019), and is interesting, as it is marked difference to wastewater sludges. Based on these results, the cation bridging theory proposed for wastewater sludges does not apply in FS (Ward et al., 2019). One potential explanation for this is that the theory of divalent cation bridging is based on the premise that divalent cations drive bioflocculation by bridging EPS to negatively charged sites on cell surfaces or by interlinking EPS molecules. However, the interactions between divalent cations and EPS have been observed to be significant for floc formation at high EPS concentrations of the sludge (activated sludges) (>100 mg/gTSS).

3.3.3.3 Role of representative constituents on water holding capacity (dewatered cake solids)

The addition of cellulose fiber and lignin alkali increased the dewatered cake solids but EPS, lipids and cations did not have a substantial effect. Elevated cake solids with higher fiber concentration is thought to be due to the chelating properties of fibers (Zhang et al., 2020). Potentially EPS from feces or fresh FS have different properties and water holding capacities than the EPS in activated sludge which is built up during growth in an aerobic environment, as it did not affect water holding capacity in FS (Guo et al., 2020).

3.3.3.4 Interdependence of dewatering metrics

In this study as a first step we evaluated one by one whether organic constituents and cations have an effect on dewaterability. More extensive research is needed in order to fully understand mechanisms and complex interactions in order to be able to reliably model or predict behavior. In addition, the reported metrics of dewaterability are interrelated. High filtration rates correlate with low supernatant turbidity because the small particles responsible for clogging pores are also responsible for turbidity. Greater sludge cake solids means more particles are captured, which also translates to lower supernatant turbidity. Our results largely corroborate this interrelatedness. However, there were some discrepancies, for example, an increase in supernatant turbidity due to the addition of oil, was expected to increase CST and decrease cake solids but this was not observed. Methods based on the rheological properties of FS and the use of filtration models could potentially provide further insight into these observations (Skinner et al., 2015).

3.3.4 The role of stabilization on dewaterability

Field FS had better dewaterability than fresh FS for all the dewatering metrics, which is most likely due to differences in level of stabilization. In this study, field FS had a VS/TS of 0.51-0.77, and a dark gray to black color, appearing to be more stabilized than fresh FS which had a VS/TS of 0.85 with a light brown color (Ward et al., 2021a). Additionally, the VS concentration of fresh FS (86 %) was greater than field FS (66 %), which also suggests that the fresh FS has a high water holding capacity, if VS follows similar relationships to wastewater sludges (Skinner et al., 2015). During stabilization the breakdown of organic substrates results in changes in the physical properties of the sludge, for example the breakdown of alginate-like exopolysaccharides (ALE) forms of EPS, which are gel forming agents with higher water holding abilities (Lin et al., 2010). Insoluble fibers that contribute to water holding capacity and gel formation and are poorly digested by gut microbiota, are more likely to be present in fresh FS (Wenzl et al., 1995). As observed in this study, the soluble and loosely bound EPS in fresh feces and fresh FS were higher than the field FS. In general, during the first week of anaerobic storage, fresh FS has been observed to have a reduction in readily biodegradable organic matter and an improvement in the dewatering performance, which then levels off after one week (Sam et al., 2022a; Ward et al., 2023). Lignin, hemicellulose and cellulose were higher in field FS than fresh FS (32, 12, and 10.7 %), which could also be due to relative increase as other constituent are degraded, in addition to fiber inputs from toilet paper and food waste (i.e. fruits, vegetables).

In the particle size distribution analysis, fresh FS had more small particles (<1 µm =2.0 Vol %, <10 µm = 18.1 Vol %) compared to median values of field FS (<1 µm =1.0 Vol %, <10 µm= 13.1 Vol %) (**Figure S3-3 to Figure S3-10**), which is also expected with increased stabilization reducing small particles of <10 um (Lawler et al., 1986). This reduction reduces clogging of filtration (decreased CST) and supernatant turbidity, which has been previously observed for FS (Ward et al., 2023).

The distribution of organisms in feces and field FS are quite different (**Figure 3-3**), which should be expected as the environment in the intestines is quite different from within onsite storage. The presence of common microorganisms such as *Firmicutes*, indicates the persistence of some fecal-associated microbiota that are able to survive outside the human body. The microbial community in feces had commonly reported gut microbiota such as *Blautia* (17 ± 8 %) and *Subdoligranulum* (13 ± 7 %), while field FS had a high relative abundance of

Pseudomonas (21 ± 22 %) and *Clostridium* (14 ± 16 %) (Cai et al., 2014). This was also observed in pit latrines in Tanzania, where a shift from gut microbiota to environmental and wastewater-related communities was seen with depth (Ijaz et al., 2022). Differences in microbial communities also help explain the differences in stabilization and dewaterability in fresh FS and field FS. For example, *Proteobacteria* are known versatile consumers of organic substrates and are largely responsible for organic matter removal in wastewater systems (Belila et al., 2013), and *Pseudomonas* has been associated with larger aggregate formation and faster filtration in FS (Ward et al., 2019, 2023). Additionally, the methane-producing *Methanosaeta* (Euyarchaeota), was observed in a higher abundance in fecal sludge (11 %) compared to feces (0.03 %), indicating the biomass degradation (stabilization) in field fecal sludge samples.
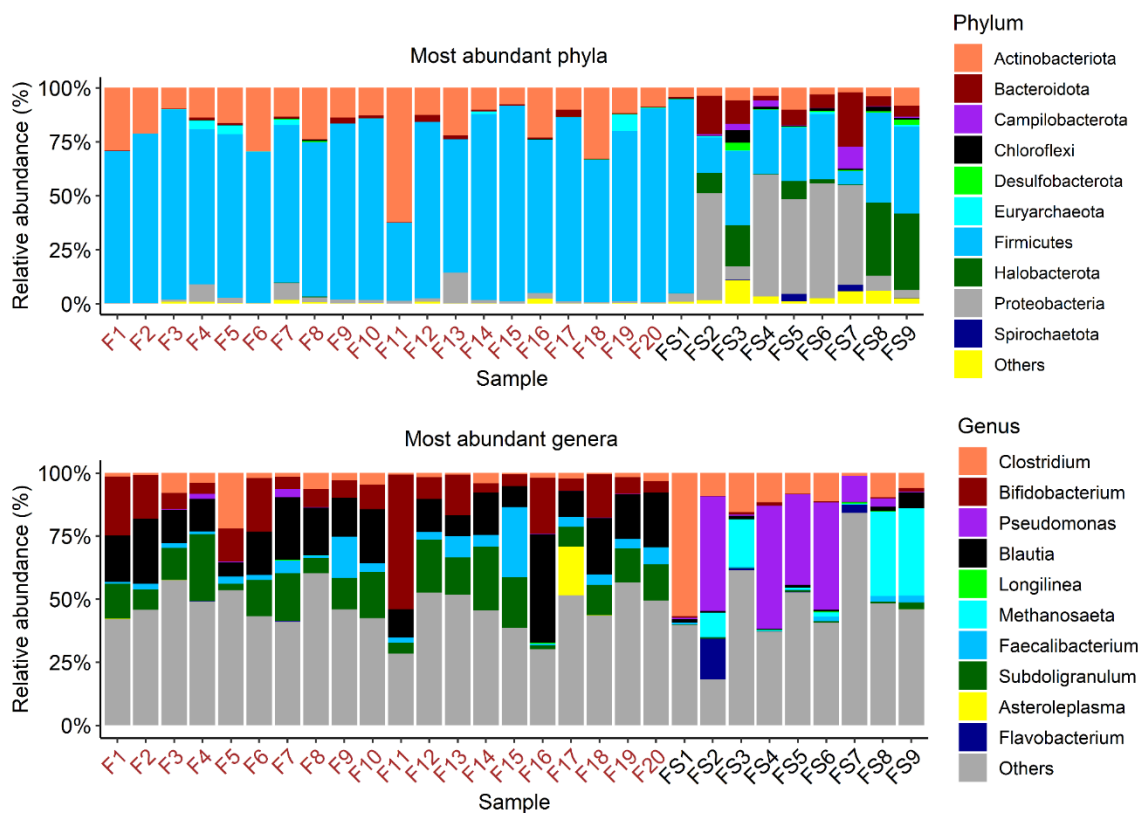
Figure 3-3: Relative abundance of most abundant phyla and genera in feces (F1- F20) and field FS (FS1-FS9) in this study.

3.3.4.1 Implications

This study clearly demonstrates that fresh feces and fresh FS that have not been stored in containment are different from field FS, and the observed differences are accredited to the overall changes that take place during stabilization. A further understanding of the role of stabilization during storage in containment will lead to better understanding and control over treatment performance. The results of this study shed light on governing mechanisms of FS

dewaterability due to fibers, EPS, lipids and cations, however our findings suggest that conventional characterization parameters (e.g. VS. COD) or individual organic content (e.g. cellulose, lipids, and lignin) will not adequately capture important differences in characteristics or properties that mediate dewatering performance. Furthermore, the relationship between dewatering and stabilization and how to best predict dewatering performance requires more study. Currently, there are not yet agreed upon metrics for quantifying stabilization of FS, or how metrics of stabilization relate to dewaterability. It is commonly considered that time since last emptied is equivalent to overall storage, and is a predictor of stabilization (Mercer et al., 2021a, 2021b). However, time since last emptied does not equal total storage time as there are continual fresh inputs to the containment, and anaerobic storage is not the same as anaerobic digestion which is process controlled for optimum degradation (Shaw and Dorea, 2021). Furthermore, time since last emptied has no statistical relation to stabilization (Ward et al., 2023), and we have confirmed with our laboratory research where following the initial first week of storage, additional storage time in containment does not result in a continual degradation or stabilization (Sam et al 2022, Ward et al., 2023). Hence, even though field fecal sludge that has been stored in containments is in general more stabilized than fresh fecal sludge prior to storage, and has better dewatering properties, time since last emptied does not predict the level of stabilization. Based on 1,206 data points from 13 countries, a VS/TS of 0.49 ($R2=0.88$) has been observed for field FS (Andriessen et al., 2023), which is lower than 0.6-0.8 for primary wastewater sludge (Tchobanoglous, 2014). However, VS/TS relationships of stabilization observed in municipal wastewater are not directly transferable to FS, due to organic fractions of VS in FS being comprised of less readily biodegradable organics (Krueger et al., 2021). In addition, there is a greater concentrations of inert TS of FS from soil intrusion and non-biodegradable garbage, whereas sewerage typically undergoes grit removal. It is also clear that other common indicators such as mono-/divalent cations and dewatering performance in wastewater treatment processes, are not directly transferable to FS. Metrics of bioavailability that appear more promising for FS include color (Ward et al., 2021a) and metrics of biological activity such as biochemical oxygen demand (BOD) and biomethane potential (BMP).

Knowledge on stabilization and relation to treatment performance will be useful for the design of non-sewered treatment solutions that treat excreta closer to the source of production, reducing reliance on trucking large volumes of water through cities to treatment. To improve dewatering of feces or fresh FS, they should first undergo some level of stabilization. For instance, fresh FS from no-flush container based sanitation (CBS) solutions could employ anaerobic digestion

for the conversion of readily biodegradable fractions in feces to biogas before dewatering on a drying bed. The variation between individual feces are not relevant for community-scale to semi-centralized treatment of FS that has undergone storage and transport to treatment, as the complexity of individual variation is averaged out. However, differences in individual feces may be relevant for the development of emerging RTTC technologies that combine the front-end (user interface) with back end (containment) for simultaneous onsite containment and treatment.

3.4 Conclusions

The specific conclusions from this research include:

- Feces, fresh FS and field FS have distinct characteristics. Although FS includes feces, there are many additional inputs, differences in microbial communities, level of stabilization and particle size distribution, all of which contribute to properties of dewaterability.

- Fresh FS has worse dewatering performance than field FS that has been stored in containment, which is likely due to the role of stabilization, although stabilization processes during storage are still not well understood.

- Fibers, EPS, and lipids appear to be key factors that control dewaterability of FS.
    - Filtration was reduced by EPS and lignin, and improved by cellulose.
    - Supernatant turbidity was reduced by cellulose and hemicellulose, and decreased by lipids.
    - Cellulose and lignin increased cake solids, and decreased by EPS.
    - Mono- and divalent cations did not have an effect on dewatering performance in FS.

3.5 Declaration of Competing Interests

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.

3.6 Author contribution

Author contribution: Sam S.B. was responsible for experimental design, collection and analysis of samples and took the lead in writing the manuscript. Eberhard Morgenroth provided critical feedback and helped shape the manuscript structure. Strande L. conceived the project idea, contributed to writing, obtained funding and supervised the project.

3.7 Acknowledgements
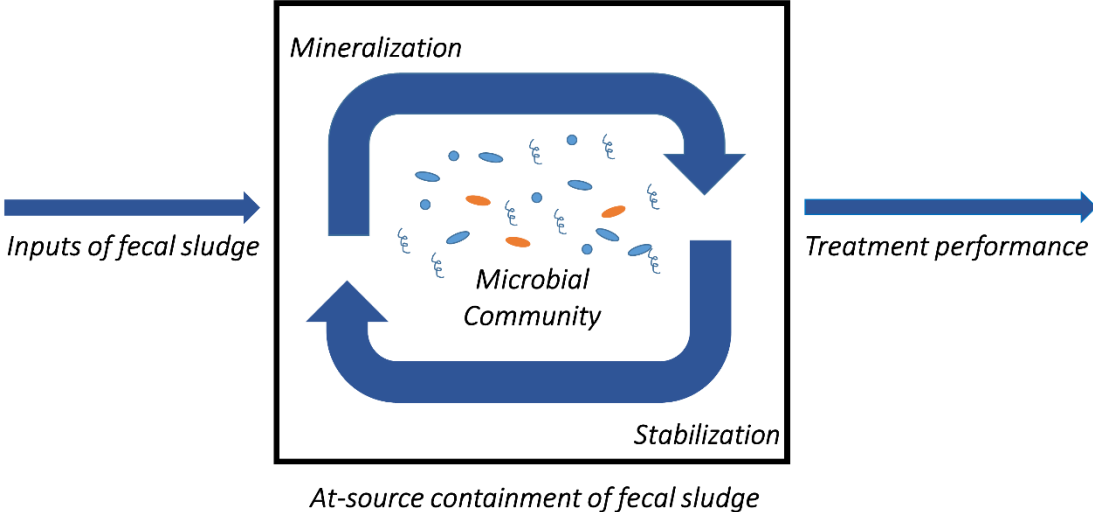
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Chapter 4 : Microbial communities in fecal sludge at city-wide scale in Lusaka, Zambia: relation to usage patterns, accumulation in containments, and treatment performance

Submitted Manuscript

This chapter is submitted in Water Research as

Graphical Abstract

Abstract

Characteristics of fecal sludge are highly variable with unpredictable treatment performance, leading to untreated discharges to the environment. An understanding of the stabilization processes that occur during storage in at-source containments in dense urban areas (e.g. 'pit latrine', 'septic tank') is required to overcome this. In this study, we evaluated 16s rRNA sequencing for 135 containments in Lusaka, Zambia, which were collected in a statistically representative fashion, together with existing metadata. We evaluated relative abundance, diversity metrics and metabolic potential, together with research questions based on the current state of knowledge. Across Lusaka, at higher taxonomic levels communities were relatively similar, as indicated by *Firmicutes, Proteobacteria,* and *Bacteroidota*, constituting 80 % abundance and a core community comprised of 48.4 ± 8.8 % of the relative abundance. At lower taxonomic levels, pit latrine and septic tank were predictors of microbial community, based on differences in moisture content, total organic carbon, total solids, and ammonia. These differences resulted in inhibition in pit latrines and greater carbon and nitrogen metabolism in septic tanks. On the contrary, building use, time since emptied, TKN, pH, and metrics of stabilization and dewatering performance exhibited no clustering with microbial communities. Microbial communities are resilient to disturbance, as measured by time since last emptied. With this study and others, the most abundant phyla in fecal sludge are becoming established, but the role of less abundant or specialist (niche) organisms remains mainly unknown. Based on 59 taxa that were uniquely identified as specialists, these roles will be important to tease out.

4.1 Introduction

One-third of the world's population relies on sanitation solutions that do not include sewers for conveyance to treatment, and almost half do not have access to safely managed sanitation services (WHO, 2021). This results in indiscriminate disposal or leakage of human waste into the environment, with direct consequences on public and environmental health (Peal et al., 2020). Treatment solutions for the wastewater, or 'fecal sludge', that accumulates during at-source storage in containments are urgently needed. A huge gap, is that microbially mediated processes that occur during the storage of fecal sludge, such as mineralization and stabilization, remain poorly understood (von Lützow and Kögel-Knabner, 2009). Only a few studies have evaluated microbial communities in septic tanks (Connelly et al., 2019; Naphtali et al., 2022), pit latrines (Beukes, 2019; Byrne et al., 2017; Ijaz et al., 2022; Torondel et al., 2016), or both septic tanks and pit latrines (Sam et al., In press; Ward et al., 2023, 2019). These studies have focused on differences in community in relation to geographic location (Torondel et al., 2016), pit latrine filling rates (Ijaz et al., 2022), mode of septic tank operation (Naphtali et al., 2022), efficiency of solar treatment (Connelly et al., 2019) and dewatering performance (Ward et al., 2023, 2019). These studies focused more on labels (e.g. 'septic tank', 'pit latrine'), and not the underlying casual factors such as redox conditions, fecal sludge properties or characteristics, and have not fully reported statistics on abundance. Additionally, the existing studies had limited number of samples (n < 35), which leaves an uncertainty regarding their representativeness.

Fecal sludge differs considerably from well-studied municipal wastewater and exhibits a much higher variability in properties and concentrations of characteristics (Deering et al., 2018; Doglas et al., 2021; Zewde et al., 2021). Recent studies have statistically grouped ranges of fecal sludge based on types of demographic, environmental and technical (DET) data in an empirical fashion, in order to make more accurate estimates of what is accumulating in containments (Strande et al., 2018; Tembo et al., 2019). However, this does not provide an understanding of the fundamental reasons for variations in fecal sludge characteristics. Additionally, research into treatment also mainly relies on empirical observations (Akumuntu et al., 2017; Ganesa Pillai et al., 2022).

The goal of this study was to evaluate microbial communities at a citywide scale based on 16S RNA analysis of 135 onsite containments, with samples that were collected in a statistically representative fashion. Samples were collected together with a large set of metadata consisting of demographic and technical data about the containments, physicochemical characteristics of

the fecal sludge and metrics of treatment performance (Ward et al., 2021a, 2021b). In order to focus our statistical analysis of this extensive data inventory, we evaluated relative abundance, diversity metrics and metabolism, together with the following set of research questions based on the current state of knowledge.

Is microbial community structure associated with waste streams going into onsite containment? In the previous analysis of this metadata, in the questionnaire 97 % of pit latrines were reported as dry toilets, and 93 % of septic tanks as flush toilets (Ward et al., 2021a). Moisture content of fecal sludge should be expected to play a role in microbial community (Chen et al., 2007; Drenovsky et al., 2004), and so containment type was selected as a proxy for evaluation. Non-household sources can represent 50% of municipal wastewater, for example coming from offices, restaurants, markets, malls, small-scale manufacturing, and hotels (Tchobanoglous et al., 2014). Different usage patterns could be expected to affect characteristics and microbial community structure. For example, higher NH_4^+-N concentrations have been reported with public toilets (Koné and Strauss, 2004; Ward et al., 2019), greater fats, oils and grease with restaurants (Velkushanova and Strande, 2021) and higher moisture content in sludge from ablution blocks (Somorin et al., 2021). In this study, household, school, restaurant, and houses of worship were selected for analysis.

Is microbial community structure associated with storage conditions within onsite containment? Disturbances could be expected to result in differences in microbial community composition (Nguyen et al., 2021). Thus, the time since fecal sludge was last emptied was selected as a metric of disruption, in comparison to containments that had never been emptied. Furthermore, the chemical characteristics of fecal sludge that accumulate during storage conditions in containments are expected to have a relation (Furtak and Gałązka, 2019), as different microbial taxa exhibit varying sensitivities and growth patterns in relation to content of ammonia and organic matter (Drenovsky et al., 2004; Furtak and Gałązka, 2019). Hence, TOC, NH_4^+-N, TKN and pH were evaluated in relation to microbial community structure.

Is microbial community structure in onsite containments related to treatment performance at treatment facilities? Microbial communities are known to influence the level of stabilization of organic matter (Lützow et al., 2006), and hence dewatering performance in different types of sludges (Ward et al., 2019). Dewatering is also influenced by filamentous organisms or the production of extracellular polymeric substances (EPS) (Bala Subramanian et al., 2010). Metrics of stabilization (C/N, VS/TS and color) and dewatering performance (CST and supernatant turbidity) were evaluated with microbial community structure.

4.2 Materials and Methods

4.2.1 Sample and data collection and processing

Samples for the microbial analysis in this study were collected at the same time as the results presented in Ward et al. 2021a, Andriessen et al. 2020, and the open data package (Ward et al., 2021b) (https://doi.org/10.25678/00037X). Sample collection was randomized based on a one kilometer grid, and 465 fecal sludge samples were collected from 421 onsite containments. A subset of 135 of the containments were collected for the extraction of DNA and microbial community analysis in this study. Representative composite samples were collected from the bottom, middle and top layers of containments. Sampling procedure, complete characterization and metrics of dewatering performance are described in Ward et al. (2021a). In addition to sample collection, questionnaire data included demographic (e.g. building use, income levels, occupation), environmental (e.g. seasonal changes), and technical (e.g. containment type, emptying frequency). The characteristics of subset of 135 microbial samples are provide in Table S1 (supplemental information) and they follow the same distribution as the 465 samples, as demonstrated through boxplots (**Figure S4-1 in Appendix C**). The open data package for all microbial analysis in this study is available at https://doi.org/10.25678/0008ZW and will be accessible when the paper is published. During collection, samples were thoroughly homogenized by shaking/stirring and a 2 ml subsample of each sample was centrifuged at 6000 g for 10 min. The pellets were retained after centrifugation and 1 ml of RNAlater® (Sigma Aldrich, Steinheim, Germany) was added. Samples were stored at - 20 ∘C and then airfreighted to Switzerland on dry ice.

4.2.2 DNA extraction

Prior to DNA extraction, samples were rinsed three times with 1X phosphate buffer to remove the RNAlater. DNA was extracted from all samples following the modified method of Griffiths et al. (2000). Briefly, 0.5 ml of hexadecyltrimethylammonium bromide buffer was added to each sample, mixed and transferred to a 2 ml lysing matrix tube. Lysing was done using a FastPrep®-24 (M.P. Biomedicals, Irvine, CA, USA) after adding 0.5 ml of phenol:chloroform:isoamylalcohol (PCI) (25:24:1, pH 6.8) (Sigma Aldrich) to the samples and bead-beating at 45 sec (4.5 m/s). A second bead-beating step was performed after cooling samples on ice for 5 minutes. Cell debris was removed by centrifugation at 14,000 g for 10 minutes. Supernatants were extracted again with 0.5 ml of chloroform: isoamylalcohol (CI) 24:1 and centrifuged. Nucleic acids were precipitated from the supernatant with polyethylene

glycol 6000 (Sigma Aldrich) for 2 hours on ice followed by centrifugation for 60 min at 13000 g and 4°C. Pellets were washed with ethanol, dried, and then dissolved in 100 µl of molecular grade water. DNA quantity (1.2-1200 ng) was determined on a NanoDrop™ One/One (Thermo Fisher Scientific, USA) and purity was assessed by the 260/280 nm and 260/230 nm absorption ratios.

4.2.3 Sequencing and Microbial community analysis

Isolated and purified DNA samples were shipped to Novogene UK where the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform using 2x300 paired-end sequencing with primers F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACVSGGGTATCTAAT-3') and Phusion® High-Fidelity PCR Master Mix (New England Biolabs). This generated raw reads of the V4 hypervariable region.

Raw sequences were rarefied to an even sequencing depth (37521 reads/sample) using the QIIME2 framework and amplicon sequence variants (ASVs) were generated using the DADA2 pipeline (Callahan et al., 2016). The taxonomic assignment of the amplicon sequence variants (ASVs) was performed based on the microbial database for activated sludge (MIDAS4 database, accessed October 2022 (Nierychlo et al., 2020)). From the sequence table generated, biostatistics were carried out using QIIME2 and the vegan, Phyloseq and ggplot2 packages in R software (version 2.4.1). A non-metric multidimensional scaling (NMDS) analysis based on the Bray–Curtis dissimilarity was performed using the vegan package in R software (Oksanen et al., 2016) to visualize the dissimilarities in community composition in relation to demographic and technical variables as well as the fecal sludge characteristics. Significance of the differences were determined by ANOSIM. Variables that could explain variation in community composition were further analyzed: relationships between fecal sludge characteristics and relative abundance of microbial communities were quantified using the Spearman rank correlation test, and p-values were corrected for multiple comparisons with the Bonferroni correction; differences in communities relative to demographic and technical factors were assessed using Wilcoxon rank-sum or ANOVA tests; alpha diversity indices (Shannon, Inverse Simpson, Chao1 and evenness) were quantified from the rarefied datasets; and the functional potentials of microbial communities were predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) based on the least common ancestor approach (Douglas et al., 2020).

4.3 Results and Discussion

4.3.1 Overview of fecal sludge microbial communities

4.3.1.1 Relative abundance of phylum and class

We obtained 7,742 high-quality 16S rRNA Amplicon Sequence Variants (ASVs) (Callahan et al., 2019) after quality filtering and removal of chimeric sequences and singletons. On average, there were $1,216.9 \pm 229.2$ ASVs per sample. Presented in **Figure S4-2 in Appendix C** are the most abundant phyla, class, and genera. The five most abundant phyla across all samples were: *Firmicutes* (57 ± 4 %), *Bacteroidota* (14 ± 2 %), *Proteobacteria* (9 ± 2 %), *Actinobacteria* (4 ± 1 %) and *Desulfobacterota* (3 ± 1 %). At the class level, the most abundant were: *Clostridia* (47 ± 4 %) (*Firmicutes*), *Bacteroidia* (14 ± 2 %) (*Bacteroidota*), *Bacilli* (8 ± 2 %) (*Firmicutes*), *Gammaproteobacteria* (8 ± 1.5 %) (*Proteobacteria*) and *Synergistia* (2 ± 1%) (*Synergistota*). The most abundant genera were all within Firmicutes, and were: *Clostridium_sensu_stricto_1* (7 ± 3 %), *Subdoligranulum* (5 ± 3 %), *Turicibacter* ($3 + 2$ %), *Blautia* (3 ± 1.7 %) and *Romboutsia* (3 ± 1 %).

As reported in Table 1, we found nine other studies reporting on the microbial community composition of fecal sludge. None of the studies reported the actual percent relative abundance, but provided a ranking of highest to lowest. Across all studies, the three most abundant phyla are *Firmicutes, Proteobacteria* and *Bacteroidota,* which together constituted 80 % of the abundance in this study. *Firmicutes* alone represented 57 % of the microbial composition of fecal sludge in this study, and indicates they play a key role in fermentation (Ley et al., 2006). A similar overall abundance of 46.4 ± 11.8 % *Firmicutes* was reported in anaerobic digesters with manure, agricultural waste, activated sludge and feces, but much lower of 9.5 ± 2.1% in anaerobic digesters at municipal wastewater treatment plants (Calusinska et al., 2018), and 1.8% in soils (Janssen, 2006). *Proteobacteria* and *Bacteriodota* have also been reported as most abundant in activated sludge, anaerobic digesters, soil, human feces, gut microbiome and compost (Arumugam et al., 2011; Calusinska et al., 2018; Dube et al., 2019; Eckburg et al., 2005; Hu et al., 2012; Janssen, 2006; Li et al., 2021; Nguyen et al., 2021; Sam et al., In press; Wan et al., 2011). *Actinobacteria and Synergistota* are also abundant in the human gut microbiome (Arumugam et al., 2011; Eckburg et al., 2005)(Ottman et al., 2012; Rajilić-Stojanović et al., 2009), *Proteobacteria, Chloroflexi* and *Actinobacteria* in anaerobic digesters

(Calusinska et al., 2018; De Vrieze, 2020), and *Acidobacteria and Actinobacteria* in soils (Janssen, 2006; Lauber et al., 2009; Prober et al., 2015).

Table 4-1: Top five phyla as relative abundance reported in fecal sludge studies. The green color gradient represents the most abundant (dark green) to the least abundant (light green) as reported qualitatively in the respective studies (actual values not reported). ST = septic tanks and PL = pit latrines.

Study	PL/ST	Country	Abundant phyla (Actinobacteria · Bacteroidota · Chloroflexi · Cloacimonetes · Desulfobacterota · Euryarchaeota · Firmicutes · Halobacterota · Proteobacteria · Spirochaetota · Synergistota · Unknown · Verrucomicrobia)
Beukes et al. 2019 (n=1, pooled)	PL	South Africa	
Bryne et al. 2017 (n=12)[1]	PL	South Africa	
Connely et al. 2019 (n=NA)	ST	Thailand	
Ijaz et al. 2022 (n= 35)	PL	Tanzania	
Naphtali et al. 2020 (n=12)	ST	Canada	
Sam et al. In press (n=9)	PL/ST	8 countries[2]	
Sam et al. This study (n=135)	ST/PL	Zambia	
Torondel et al. 2016 (n=55)	PL	Tanzania/Vietnam	
Ward et al. 2019 (n=25)	ST/PL	Tanzania/Senegal	
Ward et al. 2023 (n=20)	PL/ST	Uganda	

[1] Byrne et al. 2017 only reported the top two most abundant
[2] The eight countries were Canada, Ghana, Guatemala, India, Kenya, Lebanon, Senegal, and Uganda

4.3.1.2 Classification of core, generalist, and specialist by class

Reported in **Figure 4-1** are the 20 most abundant classes grouped by core, generalist and specialist groups. The core, defined here as ASVs at the class level present in ≥ 90 % of the samples, comprise 48.4 ± 8.8 % of the relative abundance. Generalists (present in > 60 to < 90 % of samples) comprise 27.3 ± 4.8 %, whereas specialists (present in < 60 % of samples) comprise 24.3 ± 8.2 %. In this analysis, no classes were unique to the core grouping, and only one was unique to the generalist grouping (**Figure S4-3** in **Appendix C**). Core microorganisms can be considered to be consistently present in a particular habitat, and if the conditions are highly variable, the core may also be considered to be generalists (Kokou et al., 2019). Generalists can adapt to conditions and specialize in exploiting a specific niche (Nordberg and Schwarzkopf, 2019). The class *Clostridia* (*Firmicutes*) and *Bacteroidia*, which are obligate anaerobic spore forming bacteria, were dominant within the core, generalist and specialist

groups (Guo et al., 2015). *Clostridia* are versatile with a range of metabolic processes from organic substrate hydrolysis to acidification or acetate oxidation (Yang et al., 2016). *Bacteroidia* are also abundant in the human gut microbiome, and can withstand oxygen even as obligate anaerobes (Wexler and Goodman, 2017). In contrast, 59 taxa were unique to the specialist grouping, indicating less abundant organisms that are able to survive in restricted environments and/or due to dispersal (Kokou et al., 2019).

A comparison of core, generalist and specialist groupings in fecal sludge to other systems is difficult, as there is no consistent definition in the literature. As a result, reported percent of microbial community in core groups includes: 12.4% to 84.8% in activated sludge (Hou et al., 2019; Wu et al., 2019) , 36.4 to 70.3% in anaerobic digesters (Lee et al., 2018), 21.7 % to 82.8 % in soil (Hugoni et al., 2021), 0 to 15% in human feces (Huse et al., 2012), and 8 to 93% in gut microbiome (Cheng et al., 2016; Claesson et al., 2011; Durban et al., 2012; Salonen et al., 2012; Shetty et al., 2022). In general, *Proteobacteria* and *Bacteroidota* are commonly reported in core groups of these communities, whereas *Chloroflexi* is common in anaerobic digesters but not fecal sludge (Calusinska et al., 2018; Lee et al., 2018), *Planctomycetes* and *Cyanobacteria* in soil but not in fecal sludge (Hugoni et al., 2021) and *Nitrospirae* in activated sludge but not fecal sludge (Wu et al., 2011).
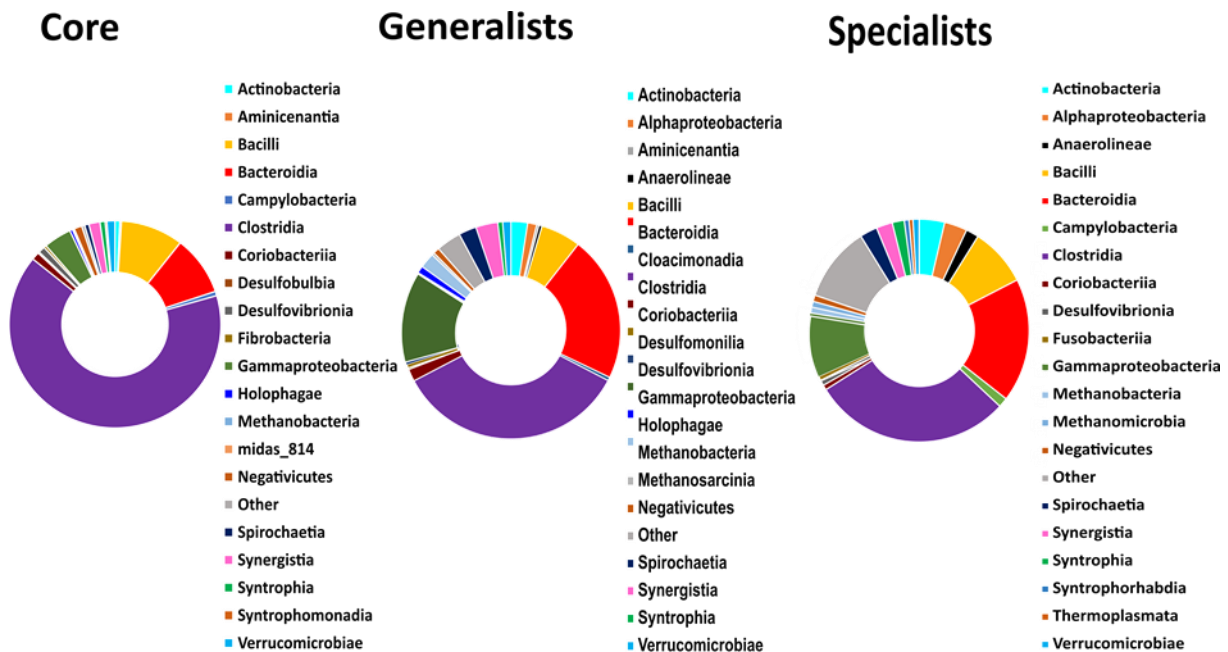

Figure 4-1: Pie charts showing the distribution of the 20 most abundant microbial classes belonging to the core, generalist and specialist groups in fecal sludge samples. Organisms that were not classified are referred to as "other".

4.3.2 Assessment of formulated hypothesis

4.3.2.1 Microbial community structure with containment inputs (flush volume, building usage)

NMDS graphical ordination representation at the phyla, class and species level for flush versus dry toilet was evaluated, with species level results presented in **Figure 4-2**, which were significantly different (analysis of similarities, R=0.36, Sig=0.0001). Differences were not significant at class or phylum level, but followed a similar clustering at the genus level (**Figure S4-4 in Appendix C**). Pit latrine and septic tank were used as proxies for moisture, as 97 % of pit latrines were reported in questionnaires as dry toilets, and 93 % of septic tanks as pour- or cistern-flush toilets (Ward et al., 2021a). The reported differences were also validated with the actual measured fecal sludge moisture content, with the median for pit latrine fecal being 85.4 % and septic tanks 98.1 %. These long-term water usage patterns are likely drivers of microbial communities, as is also observed in soils and anaerobic digesters (Banerjee et al., 2016; Yi et al., 2014). In addition, the Wilcox test indicated that the genera *Bacilli, Negativicutes, Syntrophia* and *Coriobacteriia* had significantly different relative abundance between septic tanks (9.1 %, 0.7 %, 1.2 % and 1.0 %) and pit latrines (6.9 %, 1.3 %, 0.8 % and 1.4 %).

The categorization of data in dense urban areas based on labels like 'septic tank' and 'pit latrine' is problematic (Strande et al., 2023). For example, the fact that 7 % of septic tanks were reported as dry toilets, clearly indicates that they were not operating as classically defined 'septic tanks' (Isunju et al., 2013; Tilley et al., 2014). Additionally, as shown by the similarity in moisture content, pit latrines that are commonly classified as dry toilets typically also include inflows of water. This is important, as the clustering of microbial communities based on containment types as a proxy for flush or total moisture in this study are specific to Lusaka, and should not be directly translated to other locations as results by septic tank or pit latrine. What is more important than these labels is the actual pattern of water usage, for instance where pit latrines are connected to flush toilets, or public ablution blocks with larger volumes of water. As discussed below, the complex interaction of NH_4^+-N, TOC, TS and soil intrusion are also likely contributors.
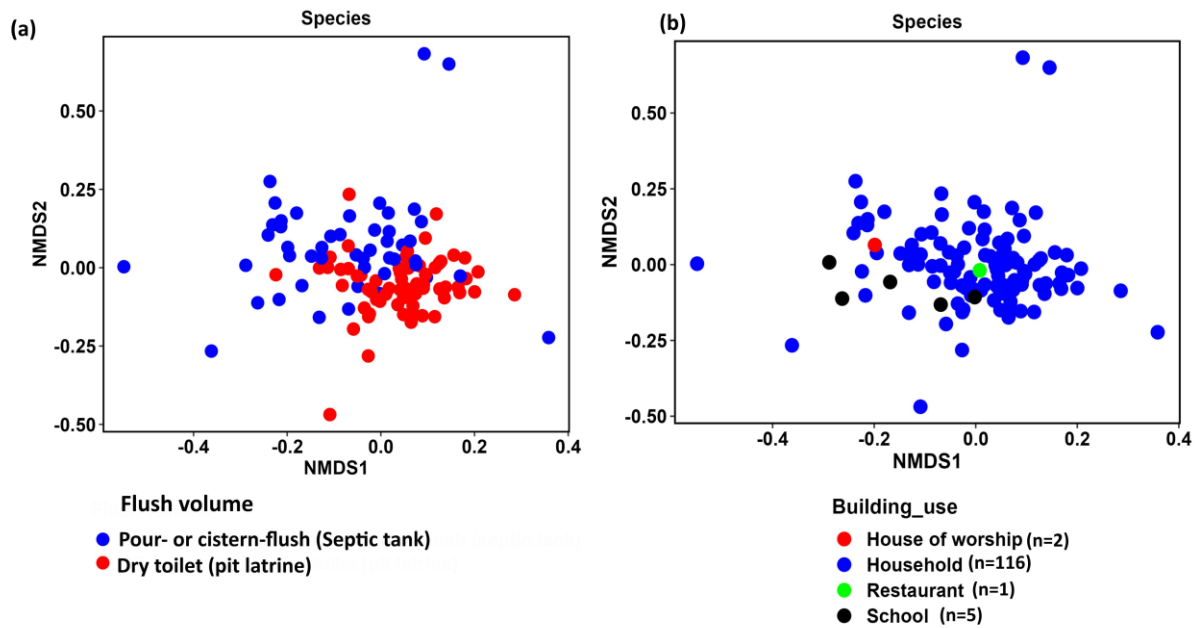
Figure 4-2: NMDS ordination plots comparing (a) flush volume (water usage) and (b) building use at the species level.

As reported in **Figure 4-2b**, NMDS ordination representation did not reveal clear clustering at the species level based on building use. While analysis of similarities (ANOSIM) was not significant, the Anova test indicated differences of relative abundance at the class level: *Deferribacteres, Deinococci, Acidobacteriae and Acidimicrobiia*, and genus level: *midas_g_6223 (class Chloroflexia), Moheibacter (Flavobacteria), midas_g_32455 (Bacilli)* and *midas_g_4324 (Aminicenantia). Deferribacteres* was highest (0.059 %) for schools, which also had a median NH_4^+-N concentration of 2,477 mg/L, whereas *Acidobacteriae* (0.025%) were more abundant for households, with a median NH_4^+-N concentration of 1,760 mg/L. *Deferribacteres* are anoxic denitrifying organisms using nitrate as an electron accepter (Alauzet and Jumas-Bilak, 2014), while *Acidobacteriae* are nitrite reducers (Kielak et al., 2016). The results suggest that the label 'building use' itself is not necessarily a useful grouping, unless usage patterns result in clear differences in characteristics, such as public market toilets (expect high NH_4^+-N) versus ablution blocks (expect higher moisture) (Somorin et al., 2021; Ward et al., 2019).

4.3.2.2 Microbial community structure with containment conditions (time since emptied)

NDMS at the class, genus and species level showed no obvious clustering with respect to the time since the containment was last emptied (species results shown in **Figure 4-3**), indicating disruptions did not influence the community structure, possible due to the large core and generalist communities. However, the Anova comparison showed that the abundance of

Babeliae, Deinococci, Deferribacteres and Caldatribacteriia did vary based on time since emptied. As we evaluated composite samples, it is plausible that pockets of different environmental conditions remain, for example, *Deferribacteres* is an anoxic (nitrate reducing) bacteria, and *Deinococci* thrives in high temperatures (Cann et al., 2001; Speirs et al., 2019; Yu et al., 2014). Ijaz et al. (2022) did observe differences in microbial composition in pit latrines from the top layer in comparison to depths of 20, 40 and 80 cm. The families *Rikenellaceae* (*Bacteroidota*), *Lactobacillaceae* (*Firmicutes*) and *Burkholderiaceae* (*Proteobacteria*) associated with gut microbiomes were abundant families in the top, in comparison to *Gammaproteobacteria_incertae_sedis, Thermomonosporaceae,* and *Caldilineaceae* in lower layers.
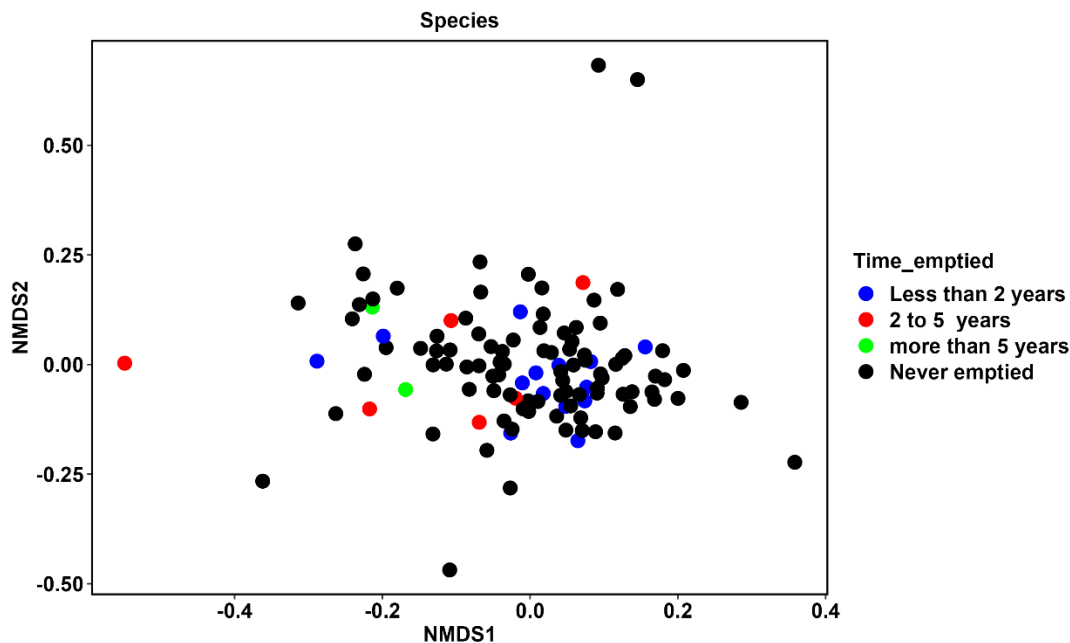
Figure 4-3: NMDS ordination plots comparing the microbial community structure among different times since containments were last emptied

4.3.2.3 Microbial community structure with containment conditions (NH4$^+$-N, TKN, TOC, pH)

As shown in the NMDS ordination plot in **Figure 4-4,** microbial communities at the species level clustered differently by low and high NH$_4^+$-N and TOC concentrations, but not pH or TKN. Correlation analysis at the class level showed that *Syntrophia* (R= -0.40, p=0.0001) *Leptospirae* (R= -0.37, p=0.0004), *Thermacetogenia* (R=0.35, p=0.0013) and *Coriobacteriia* (R= 0.33, p=0.0021) correlated with varying NH$_4^+$-N, whereas *Midas_c_814* (R= 0.37, p=0.006) was the only class that correlated with TKN. The top four microorganisms correlated

with NH_4^+-N are not directly involved in nitrogen cycling. However, *Syntrophia* are associated with protein metabolism which releases ammonia during proteolysis (Griffin and Bradshaw, 2019; McInerney et al., 1981). Ammonia concentrations in this study were significantly higher in pit latrines (median of 2843 mg/L) than septic tanks (median of 415 mg/L) (Kruskal Wallis, $p < 0.05$). Ammonia inhibition could partially explain the overall differences in community structure, as concentrations above 1700–1800 mg/L are known to be inhibitory (e.g. methanogens) (Yenigün and Demirel, 2013). The correlation analysis showed that *Desulfovibrionia* (R=0.36, p=0.014) and *Midas_c_814* (R= 0.32, p=0.036) were also positively associated to TOC (**Figure S4-5 in Appendix C**), which is also reflective of the expected differences with TS, as TOC was always a certain percentage of TS (indicated in the linear model in **figure S4-9 in Appendix C**.

The correlation analysis based on pH revealed that the top four abundant classes *Desulfovibrionia (R= -0.41, p=0.0001), Methanosarcinia (R= -0.36, p= 0.0010), Aminicenantia (R= -0.33, p= 0.0034), Syntrophomonadia (R= -0.28, p=0.0265)*, had negative associations with pH. Although exact roles cannot be determined by statistical correlation, the results fit within what is reported in the literature. For example, *Desulfovibrionia, Aminicenantia*, and *Syntrophomonadia* oxidize simple organic molecules to produce short chain fatty acids (Galushko and Kuever, 2020; Sekiguchi, 2015), *Methanosarcinia* utilizes hydrogen and CO_2 for methanogenesis, *Desulfovibrio* is a sulfate reducer known to thrive in low pH environments (Tran et al., 2021), and *Desulfotomaculia* is a spore-former and resistant to low pH (Nierychlo et al., 2020).
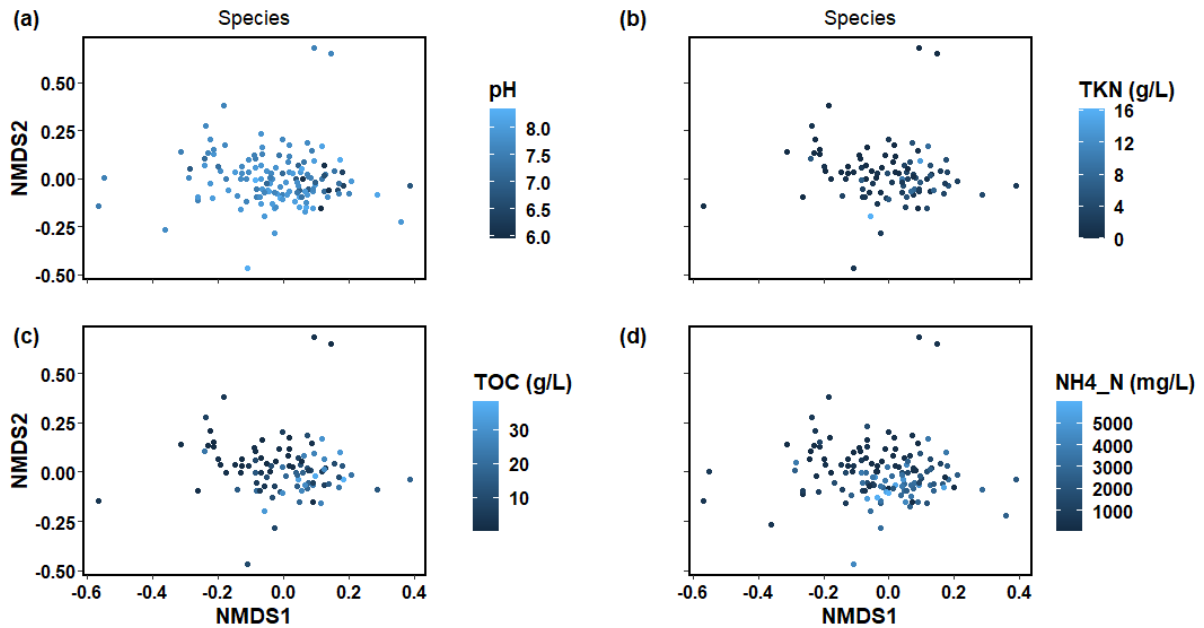
Figure 4-4: NMDS ordination plots comparing microbial structure for different fecal sludge characteristics at the species level. The plots shows distribution of microbial community due to (a) varying pH (n=134), (b) TKN (n=94), (c) TOC (n=94), and (d) NH_4^+-N (n=134).

4.3.2.4 Microbial community structure with treatment performance (dewaterability, stabilization)

NMDS analysis at the class, species and genus level revealed no distinct clustering of microbial communities based on metrics of dewaterability (CST, supernatant turbidity) (**Figure S4-6 in Appendix C**). As depicted in Figure 5, correlation analysis for potential relationships at the class level indicated weakly significant associations with CST and supernatant turbidity between the abundance of classes *Midas_c_796* (R= 0.36, p=0.05)*, Syntrophia (R=-0.53, p=0.0001)* and *Polyangia* (R=0.44, p=0.0028). These organisms all had relative abundance of < 1%, potentially indicating niche roles within the community. NMDS analysis based on metrics of stabilization (VS/TS, C/N, color) also showed no clear trends (**Figure S4-7 in Appendix C**), and had a weak correlation between C/N and the genera *Midas_g_8364* and *DMER64*, which is a syntrophic group that facilitates electron transfer within methanogenic archaea (Lee et al., 2019). The relationships between dewatering and stabilization were not significant enough to come to any conclusions.
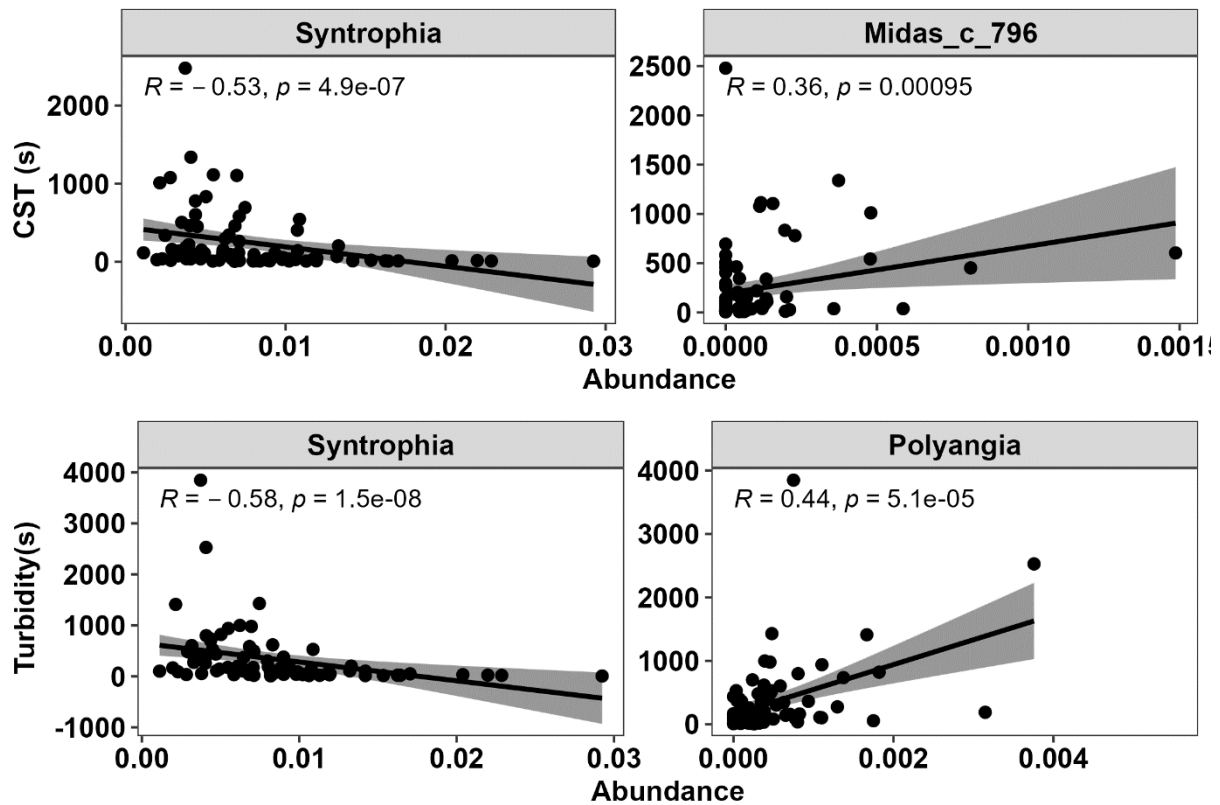
Figure 4-5: Correlation between microbial classes and metrics of dewaterability, the capillary suction time (CST) and supernatant turbidity

4.4 Metabolic functions

As reported in **Figure 4-6**, potential nitrogen and methane metabolism derived from 16S rRNA gene data (PICRUSt2), were evaluated by the groupings 'septic tank' and 'pit latrine', with a higher potential of methane and nitrogen metabolism in septic tanks over pit latrines (P<0.05). This analysis is a predictive tool, that refers cumulatively to biosynthesis and/or assimilation pathways (Douglas et al., 2020). In addition to NH_4^+-N, TOC was also higher in pit latrines than septic tanks (**Figure S4-8** in **Appendix C**). The higher potential for methane metabolism in septic tanks also aligns with inhibition of methanogenesis, and estimates that septic tanks produce more greenhouse gas (GHG) emissions (211 Mt CO_2e) than pit latrines (166 Mt CO_2e) (Cheng et al., 2022; Johnson et al., 2022). This also fits with reported ORP for septic tanks of -310 to -339 mV (Philip et al., 1993) and -230 to -489 mV (Huynh et al., 2021), which are more conducive to methane production than -199 to 59 for pit latrines (Nakagiri et al., 2017).

(a) Methane metabolism   (b) Nitrogen metabolism
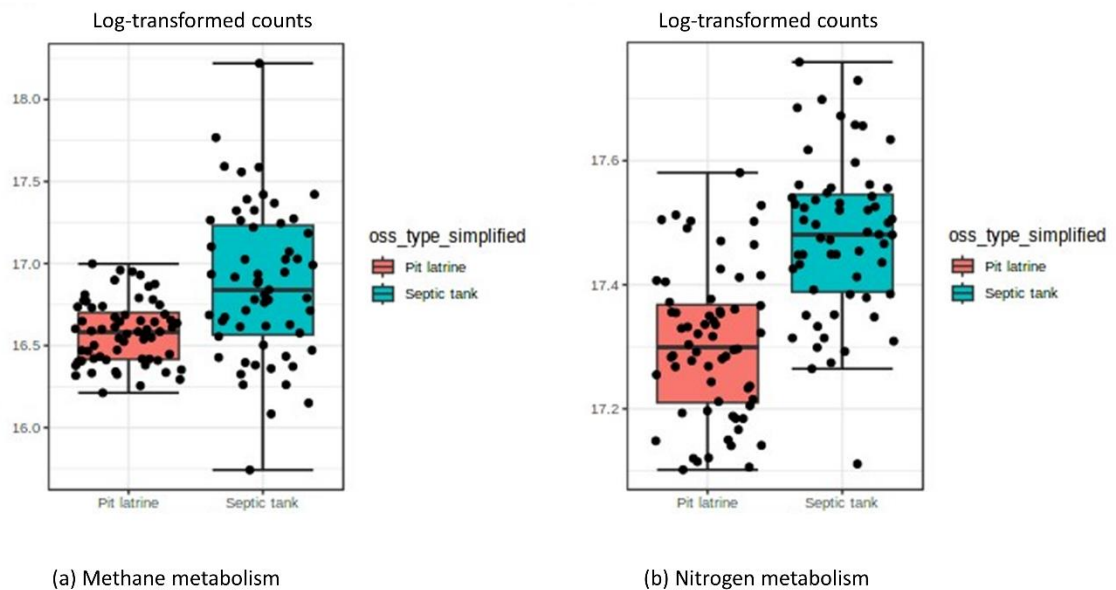
Figure 4-6: Boxplots showing the potential biochemical processes occurring in fecal sludge from septic tanks and pit latrines based on 16S rRNA gene data (PICRUSt2); (a) methane metabolism potential, and (b) nitrogen metabolism potential.

4.4.1 Microbial diversity

As reported in Table 2, there was no difference in diversity indices based by containment type or level of lining (separation from soil and groundwater). A similar Chao value of 1,354 has been reported for anaerobic digesters (Lee et al., 2018). Similarly, although Ijaz et al. (2022) observed differences in communities with depth, there were not significant difference in alpha diversity in pit latrine by depth. Studies of human feces and gut microbiomes, have also observed no significant differences in alpha diversity (Cheng et al., 2016; Li et al., 2021) or Simpson and Chao-1 (Pan et al., 2020). In contrast, soil environments are observed to be more diverse and variable (Lauber et al., 2009; Prober et al., 2015; Zhi Qi and Zhan Xi, 2021). For example, Qi et al. (2021) reported a Chao-1 value of 1,641 for soils with no grass in comparison to 3,241 with grass.

Table 4-2 :Summary of bacterial diversity of the 16S rRNA gene libraries for the different types of containments (pit latrines and septic tanks)

Diversity index	All samples		Pit latrines by lining / unlined		
	Septic tank (n=61)	Pit latrine (n=64)	Lined pit Latrine (n=25)	Partially lined pit latrine (n=31)	Unlined pit latrine (n=8)
Simpson_1-D	0.98 ± 0.02	0.99 ± 0.01	0.98 ± 0.01	0.99 ± 0.01	0.99 ± 0.004
Shannon_H	5.51 ± 0.51	5.74 ± 0.25	5.71 ± 0.22	5.68 ± 0.27	5.76 ± 0.16
Evenness_e^H/S	0.23 ± 0.07	0.26 ± 0.05	0.27 ± 0.04	0.25 ± 0.06	0.27 ± 0.04
Chao-1	1181 ± 251	1245 ± 203	1285.81 ± 237	1218.23 ± 168	1217.71 ± 137

4.4.2 Implications

This study is unique, in that the samples were collected in a statistically robust fashion to be representative of fecal sludge quantities and qualities for an entire city (Andriessen et al., 2020; Ward et al., 2021a, Ward et al., 2021b). Although the results are representative of the city of Lusaka, at the phyla level they are similar to relative abundance of fecal sludge from nine other studies in 13 cities. At the level of phyla and class, differences in community were not observed based on usage and management practices (i.e. what is going into and conditions within containments, and dewatering metrics). The large relative abundance (48.4 ± 8.8 %) of the core microbiome at the class level, suggests a lot of similarity among microbial communities at the city-scale, which also seems to provide resilience to fluctuations and disturbances.

NDMS ordination clustering at the level of genus and species, shows more clear separation at the lower taxonomic levels of genus and species, and indicate that usage and management patterns that affect moisture, total solids, organic matter and ammonia concentrations influence microbial communities due to differences in local environments and functional redundancies (Fetzer et al., 2015). Similarities between the core and generalist groupings also indicate highly variable conditions, which we know to be the case with characteristics of fecal sludge. Although abundant taxa are recognized as important for ecosystem function, this illustrates the importance of overall community structure, and the role of less abundant microbial populations, which also have a wide range of functional roles (Zhang et al., 2021).

In this study, differences in microbial communities between 'septic tanks' and 'pit latrines' were significant. However, it is clear that these differences are explained by usage patterns that affect moisture, ammonia and organic matter (Ward et al., 2021a), which can then shape communities through metabolism and inhibition. Prior to using labels for statistical

comparisons, it must be kept in mind they are not necessarily reflective of real-life operating conditions, and not in themselves explanatory of the fundamental reasons for the observed differences (Strande et al. 2023). These results based on characteristics are globally relevant, and can help to predict microbial community structure and greenhouse gas emissions from fecal sludge in other cities. This analysis was done on composite samples, and did not specifically evaluate differences in microbial community with depth, or by measuring different pockets of environmental conditions within containments, or comparing to surrounding soils. This is a need for further research, along with representative sampling across multiple cities to validate the findings.

It should also be considered that the bacteria and archaea examined in this study are not solely responsible for biological activity and differences in stabilization processes. Although studying microbial communities will bring insight, onsite containments are not analogous to wastewater treatment plants with process-controlled environments that specifically encourage the growth of certain organisms and stabilization processes. In addition to microorganisms that carry out enzymatic reactions, invertebrates, worms and fungi are contributing to the cycling of nutrients and organic matter in fecal sludge. Although it is not practical to assume we can manipulate microbial communities within existing onsite containments, a deeper understanding of the microbes that are present with varying conditions, and the roles that they play, could lead to improved management practices for onsite storage of fecal sludge.

4.5 Conclusions

- Moisture content, organic carbon, and ammonia seem to be the strongest predictors of microbial community and metabolic function.

- Time since emptied was again shown to not be a useful indicator, and did not have a relation to microbial community structure.

- Microbial communities in fecal sludge are relatively similar and stable following disturbance at the level of phyla and class, with differences occurring at lower taxonomic levels.

- The most abundant phyla in fecal sludge are becoming established, but the role of less abundant or specialist (niche) organisms remains mainly unknown.

Groupings such as 'septic tank', 'pit latrine' and 'building usage' could be useful in simplifying further analysis of microbial communities, but only if the usage patterns result in significantly different moisture content, organic carbon, or ammonia within containments.

4.6 Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this publication.

4.7 Author contribution

Sam S.B. was responsible for DNA extraction, analysis of data and took the lead in writing the manuscript. Sam SB, Niederdorfer R, Scheidegger A, Johnson D, Burgmann H, Morgenroth E, Strande L contributed to the data analysis and interpretation of results... Strande L., conceived the idea, obtained funding, supervised the project and contributed to co-writing of the manuscript.

4.8 Acknowledgements

Chapter 5 : Conclusions and Outlook

5.1 General Conclusions

The high variability in fecal sludge characteristics and composition, coupled with the lack of knowledge of the constituents of feces and fecal sludge, makes it challenging to understand fundamentally how properties of fecal sludge are related to treatment performance (e.g. dewatering and stabilization). The goal of this thesis was to provide a better understanding of the role of feces and fecal sludge constituents and stabilization on dewatering performance, and to identify the microbial community structure in fecal sludge in relation to management practices and fecal sludge characteristics. The Key findings are presented here.

(1) *This study identified that soluble and loosely bound EPS, cellulose, and lipids, which form part of the organic components in fecal sludge, influence dewatering performance.* These organic molecules have the potential to influence dewaterability due to properties such as hydrophobicity, water-holding capacity, interaction with divalent ions and clogging due to particle size distribution. The addition of EPS to fecal sludge increases the sludge filtration time and the supernatant turbidity after centrifugation, whereas lipids increases the supernatant turbidity. Cellulose however improves fecal sludge dewatering performance by reducing the sludge filtration time and reducing the water-holding capacity. Clear effect of EPS, cellulose and lipids are seen when added to fresh fecal sludge. However, observable trends between these constituents and dewatering performance are not statistically significant in the field fecal sludge samples that were analyzed in this study.

(2) *Cations have no substantial effect on dewatering of fresh fecal sludge, and observable trends do not exist in field fecal sludge, contrary to what is observed in wastewater sludges.* The cation bridging theory that occurs in wastewater was not observed to occur in fecal sludge, possibly due to the low concentration of EPS in fecal sludge. Divalent cations drive bioflocculation by bridging EPS to negatively charged sites on cell surfaces or by interlinking EPS molecules, which improves settling. The absence of a substantial effect of effect of cations on dewatering indicates that the mono-/divalent (M/D) ratio that is used to predict settling in wastewater sludges is not applicable treatment of fecal sludge.

(3) *Dewatering performance is associated with the level of stabilization of fecal sludge, and "stabilized" fecal sludge has better dewatering than "unstabilized" fecal sludge. .* Fresh fecal sludge that has not been stored in containments has worse dewatering performance than field fecal sludge that has been stored in containment for a period, which is likely due to the role of stabilization. Fresh feces or less stabilized fecal sludge will likely have higher

concentrations organic matter such as alginate-like exopolysaccharides (ALE), which are gel forming agents with higher water holding abilities that decreases dewatering performance.

(4) ***Time during anaerobic storage is not a predictor of stabilization. Thus, the perception that dewaterability of fecal sludge will improve with increasing time under anaerobic conditions does not withstand scientific scrutiny.*** Anaerobic storage of fecal sludge is not the same as anaerobic digestion which is process controlled for optimum degradation and results in a lower reduction of volatile solids (about 20 % reduction) compared to waste activated sludges (50-60 % reduction). Time since last emptied also does not equate total storage time as fresh inputs are continually added to the containment. The degradation of organics in fecal sludge thus proceeds only up to one week after which further degradation is not observed.

(5) ***The microbial community structure in fecal sludge from Lusaka (Zambia) is associated with differences in types of containment, which is due to differences in water usage, ammonia, organic matter or whether containments are exposed to migration from surrounding soil organism.*** Water content has a strong association with microbial community structure, because onsite containments with more water content such as septic tanks have (median moisture = 95 %) different community structure compared to pit latrines with less water (median moisture = 87 %). Microbial communities in fecal sludge (form Lusaka) were relatively similar at higher taxonomic levels, but differences in community structure based on variation in moisture content, total organic carbon, total solids, and ammonia occurred at the lower taxonomic levels. Significant associations between fecal sludge characteristics or the level of stabilization and microbial communities were not observed. However, microbial classes Syntrophia and Polyangia were identified as having a relationship with dewatering metrics even though their exact roles are not well understood.

5.2 Implications

EPS are known to play a key role in dewatering of wastewater sludges. However, this study revealed that the overall concentration of EPS and the concentration of individual EPS fractions in fecal sludge are low compared to wastewater sludges. Furthermore, unlike in wastewater sludges, fecal sludge have a higher fraction of humic-like substances than protein-like substances. These disparities between EPS in fecal sludge and wastewater implies that EPS plays a different role in fecal sludge compared to wastewater. Additionally, during anaerobic stabilization of fecal sludge, degradation of EPS and EPS fractions is limited as observed in the VS reduction, leading to the absence of significant relationships between EPS or EPS fractions and the dewatering performance.

The outcome of this study highlights the role of fibers and lipids on fecal sludge dewaterability. The influence of fecal sludge constituents on dewatering performance present a clear indication that usage practices that affect fiber and lipid content will influence dewatering in fecal sludge. For instance fecal sludge with paper materials or from sources where toilet paper is used in the containment will have different characteristics and dewatering performance compared to where water is used as cleansing material. Similarly, fecal sludge from restaurants will likely have higher levels of fats oils and grease, which will affect the dewatering performance. This information together with information on the level of stabilization is useful for practitioners to in deciding on the selection of appropriate treatment solutions.

Fresh feces and field fecal sludge showed different characteristics mainly due to changes that occurred during stabilization, and demonstrated differences in dewatering performance. This is informative for practitioners on selection of appropriate management and treatment solutions. Fresh feces are likely to cause clogging in drying beds due to the higher concentrations of EPS and cellulose than stabilized fecal sludge. Thus, anaerobic digestion could be used as pretreatment step before fresh feces or fecal sludge is loaded onto drying bed. Knowledge on stabilization and relation to treatment performance is essential for the design of fecal sludge treatment solution closer to the source of production, thus reducing issues with fecal sludge transportation.

The type of fecal sludge containment (septic tank or pit latrine) appears to be associated with microbial community structure. However, differences in containment are due to differences in moisture, ammonia, total solids and organic matter. Labels such as "Pit latrines and "Septic

tanks do not reflect the characteristics or conditions in the containment but could only be useful if the usage patterns affect the concentrations of moisture, organic carbon, or ammonia in containments Therefore, sludge characterization should be made with less emphasis on labels or terminologies but rather on the actual characteristic differences between containments. Based on the association between microbial community and FS dewatering performance, specific microbial organisms could be used for diagnostic purposes to predict dewatering performance or some other properties of fecal sludge in a containment

5.3 Future Research

In this thesis, we elucidated the organic, inorganic and microbial composition of feces and fecal sludge in relation to their potential influence on treatment performance. However, there are still open questions regarding the fate of organic compounds during anaerobic storage, how stabilization occurs in fecal sludge and the role of microbial communities in fecal sludge.

5.3.1 The fate of loose and tightly bound EPS during storage and effect on dewatering

We observed in this study that soluble and loosely bound EPS extracted from activated sludge or from fecal sludge decreased the dewatering performance (filtration) when introduced to fecal sludge samples. However, monitoring the fecal sludge concentration of loosely bound EPS, EPS fractions and dewatering metrics during anaerobic storage did not show clear associations between EPS and dewatering performance. There is a hypothesis that the loosely bound EPS and tightly bound EPS have contrasting effect on activated sludge settling properties (Huang et al., 2022), however only the influence of the loosely bound EPS with a higher fractions of humic-like and protein-like substances than in activated sludge EPS was evaluated in this study. Future research should investigate the aforementioned hypothesis to elucidate to what extent the different forms of EPS affect fecal sludge dewaterability. A relevant approach could be the use of a combination of centrifugation and heating to obtain the tightly bound EPS, which could be added in aliquots to fecal sludge to determine its effect on dewatering properties. Secondly, the fate of both loosely and tightly bound EPS during anaerobic storage and the effect on fecal sludge dewatering performance should be assessed through anaerobic batch reactors with continuous monitoring of these EPS forms. Such an approach will provide a better understanding of how storage affect EPS and which form of EPS truly affect fecal sludge dewaterability.

5.3.2 The fate of cellulose, lipids and cations during storage of fecal sludge

We showed in this thesis that representatives of cellulose and lipids affected the dewatering performance of fresh fecal sludge. Nevertheless, the concentrations of cellulose, which was higher in field fecal sludge, and lipids (lower in field fecal sludge), did not show substantial associations with dewatering performance. This call into question the effect of anaerobic storage on cellulose and lipids, and how that transformation affects dewatering properties. Future research should focus on establishing how these constituents change over time under anaerobic storage, and at the same time monitor dewatering metrics for direct relationships. This thesis evaluated mechanistically the role of each fecal sludge constituent (EPS, cellulose, lipids, lignin, hemicellulose, cations) one at a time. However, it will be interesting to evaluate the effect of complex interactions between constituents. A better understanding of the interplay among these constituents during anaerobic storage, together with other physical and chemical properties of the sludge could help to develop an empirical model, which could be used to predict the effect of different interactions on dewatering performance at the fecal sludge treatment plant.

5.3.3 Taxa identification and monitoring of organism associated with dewatering properties

To advance our understanding of the organic and nutrient cycles and transformation of fecal sludge constituents during anaerobic storage, we need to know the microbial community composition and dynamics in fecal sludge in relation to fecal sludge properties. This thesis included a scoping study of communities in fecal sludge and the association with FS characteristics or dewatering properties. Despite the associations observed, for instances between classes Syntrophia and Polyangia and CST or turbidity, their exact roles were out of the scope of this thesis. Future research should dive into identifying the role of specific organisms that are associated with fecal sludge properties through metagenomics and metatranscriptomics. For example identifying organisms that function in particle aggregation or EPS production will advance our understanding of dewatering behaviour in fecal sludge containments, leading to a more informed and rational approach in designing effective treatment solutions.

5.3.4 Better understanding of stabilization and the bioavailability of organic substrates (hydrolysis)

This thesis focused on elucidating the roles of fecal sludge constituents on dewatering performance, together with identifying the microbial community structure in fecal sludge. The level of stabilization has been associated with dewatering performance even though this is not fully understood. It is unclear why degradation is observed to cease after a week of anaerobic

storage. Moreover, metrics of stabilization for fecal sludge are not agreed upon based on the characteristics of fecal sludge, the conventional metrics of stabilization for wastewater may not apply for fecal sludge. For instance, the total solids in fecal sludge are very complex, could include sand silt or grit or recalcitrant organic substances, which are not readily degradable, and therefore distorts metrics such as VS/TS. Future research should focus on establishing stabilization metrics that are specific to fecal sludge. Respirometric methods with relatively short run time such as the specific oxygen utilization rate (SOUR) and other microbial activity based methods such as the biomethane potential test have recently been tested for their application in fecal sludge. Though the results seems promising, the BMP test takes a longer time to perform whereas the SOUR test has just been tested once on a few fecal sludge samples. Thus, further testing and optimization of these methods for fecal sludge are required. Methods with rapid results can be used for process control decisions, whereas longer-term methods could be used for general waste flow characterization.

Chapter 6 : References

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Appendix A

Supplementary information for Chapter 2

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

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Supplementary Information for chapter 2

Table S2.1: Characteristics of inoculum and feed used for the BMP test

Sample	pH	TS (g/L)	VS (g/L)	TSS (g/L)	VSS (g/L)	TCOD (mg/L)	SCOD (mg/L)	NH4_N (mg/L)	VFA (mg/L)	ALK (mg/L)
AD	7.23	27.64	12.32	25.50	9.50	19,675	768	765	196	2,843
CM	7.33	31.97	20.04	23.20	16.40	65,300	22,740	1,136	2,235	6,743
ST	7.89	29.04	19.45	28.40	14.70	35,400	2,090	896	1,027	2,973
PL	7.67	39.77	21.62	29.60	20.80	86,050	28,680	3,320	2,338	9,438
Feed	7.41	27.40	22.53	20.8	17.8	60,350	28,520	849	450	2,942
FS	7-8[a]	8-52[a]	43-77 %[a]	5-30[a]	3-19[a]	8400 -67500[b]		500-2500[b]		

Sample: AD – Anaerobic digested sludge, CM – Cow manure sludge, ST – Septic tank sludge, PL – Pit latrine sludge. FS – Fecal sludge recipe, Ward et al 2019[a], Cofie et al 2006[b]

Table S2.2: Produced and theoretical methane from microcrystalline cellulose

Inoculum	MCC biogas (ml/gVS)	% CH4 of MCC	CH4 (ml/gVS)	Theor. CH4 (ml/gVS)	% of Theor CH4	Inoculum	MCC biogas (ml/gVS)	% CH4	CH4 (ml/gVS)	Theor CH4 (ml/gVS)	% of Theor CH4
		Run A				Run B					
AD_20	533	0.67	357	414	0.86	AD	668	0.61	408	414	0.98
AD_20b	592	0.65	385	414	0.93	CM	571	0.68	388	414	0.94
AD_35	653	0.62	405	414	0.98	ST	692	0.58	402	414	0.97
PL_20	110	0.67	74	414	0.18	PL	326	0.67	219	414	0.53
PL_35	49	0.67	33	414	0.08						
CM_20	358	0.68	243	414	0.59						
ST_20	668	0.60	401	414	0.97						

MCC- microcrystalline cellulose, Theor. CH4 – theoretical methane.

Table S2.3: Kendall rank correlation (Tau) for the change in EPS, EPS fractions and metrics of dewatering with anaerobic storage time.

Run		AD inoculated runs		PL inoculated runs	
Run		Tau	p-value	Tau	p-value
Run 1		-0.07	1.00		
Run 2		0.38	0.28	-0.21	0.55
Run 3	CST and Time	-0.22	0.48	-0.22	0.48
Run 4		0.14	0.77		
Run 5		-0.71	0.01	-0.79	0.01
Run 6		0.00	1.00	-0.50	0.1
Run 1		0.73	0.06		
Run 2		-0.20	0.72	-0.73	0.06
Run 3	EPS/VSS - Time	-0.11	0.76	0.78	0.00
Run 4		-0.62	0.07		
Run 5		-0.07	0.90	-0.57	0.06
Run 6		-0.50	0.11	-0.29	0.40
Run 1		0.60	0.13		
Run 2		-0.07	1.00	-0.68	0.03
Run 3	Humic/VSS - Time	0.25	0.35	0.50	0.08
Run 4		-0.62	0.07		
Run 5		0.00	1.0	-0.43	0.18
Run 6		0.11	0.71	-0.07	0.90
Run 1		0.60	0.13		
Run 2		-0.87	0.02	-0.81	0.01
Run 3	Protein/VSS - Time	-0.44	0.12	0.72	0.01
Run 4		-0.24	0.56		
Run 5		-0.14	0.72	0.36	0.28
Run 6		-0.50	0.12	-0.50	0.12
Run 3		-0.61	0.02	0.55	0.04
Run 4	Turbidity and Time	0.43	0.24		
Run 5		-0.64	0.03	-0.84	0.004
Run 6		0.00	1.00	-0.43	0.18

Table S2.4: Kendall rank correlation for the relationship between EPS, EPS fractions or particle size distribution (PSD) and metrics of dewaterability (tau-correlation coefficient and p-values).

		Tau	p-value	Tau	p-value
Run 1		-0.33	0.47		
Run 2		0.33	0.47	0.47	0.27
Run 3	EPS/VSS and	0.11	0.76	0.00	1.00
Run 4	CST(s)	0.23	0.56		
Run 5		-0.21	0.55	0.64	0.03
Run 6		0.21	0.55	-0.07	0.90
Run 1		-0.20	0.72		
Run 2		0.47	0.27	0.28	0.44
Run 3		0.03	0.92	0.05	0.92
Run 4	Humics and CST(s)	0.24	0.56		
Run 5		-0.14	0.72	0.56	0.11
Run 6		0.25	0.38	-0.43	0.18
Run 1		-0.20	0.72		
Run 2		-0.33	0.45	1.00	0.00
Run 3		0.00	1.00	-0.17	0.61
Run 4	Proteins and CST (s)	0.05	1.00		
Run 5		0.00	1.0	-0.14	0.72
Run 6		0.21	0.55	0.43	0.18
Run 3		-0.19	0.46	0.44	0.12
Run 4	Humics and	-0.33	0.38		
Run 5	Turbidity	0.00	1.00	0.43	0.18
Run 6		-0.18	0.53	-0.14	0.18
Run 3		0.17	0.61	0.33	0.26
Run 4		-0.14	0.77		
Run 5	Proteins and	0.00	1.00	-0.50	0.11
Run 6	Turbidity	0.07	0.90	0.14	0.72
Run 3		-0.06	0.90	0.61	0.02
Run 4	EPS/VSS and	-0.52	0.13		
Run 5	Turbidity	-0.07	0.90	0.29	0.40
Run 6		0.07	0.90	-0.36	0.28
Run 6	PSD and CST(s)	-0.14	0.72	-0.71	0.01
Run 6	PSD and Turbidity	0.07	0.80	-0.79	0.01

Table S2.5: Kendall rank correlation for the relationship between EPS (mg/L), EPS fractions metrics of dewaterability (tau-correlation coefficient and p-values).

		Tau	p-value	Tau	p-value
Run 1		0.07	1.00		
Run 2		-0.33	0.47	0.73	0.06
Run 3	EPS (mg/L) and	0.22	0.48	0.17	0.61
Run 4	CST(s)	0.33	0.38		
Run 5		0.62	0.03	0.71	0.01
Run 6		0.29	0.40	050	0.11
Run 1		-0.06	1.00		
Run 2		-0.60	0.14	0.73	0.06
Run 3	Humics-like	0.22	0.48	0.22	0.48
Run 4	substances (mg/L)	0.52	0.13		
Run 5	and CST(s)	0.33	0.26	0.78	0.01
Run 6		-0.07	0.90	0.36	0.28
Run 1		0.33	0.46		
Run 2		0.67	1.00	0.87	0.01
Run 3	Proteins-like	0.11	0.76	0.00	1.00
Run 4	substances (mg/L)	0.05	1.00		
Run 5	and CST (s)	0.60	0.03	0.07	0.90
Run 6		0.54	0.06	0.64	0.03
Run 3	Humics -like	0.00	1.00	-0.39	0.18
Run 4	substances (mg/L)	-0.52	0.13		
Run 5	and Turbidity	-0.04	0.90	0.64	0.90
Run 6		-0.43	0.18	0.21	0.03
Run 3		0.11	0.76	0.17	0.61
Run 4	Proteins-like	-0.24	0.56		
Run 5	substances (mg/L)	0.11	0.71	-0.0	0.11
Run 6	and Turbidity	0.18	0.53	0.36	0.28
Run 3		0.33	0.26	-0.28	0.35
Run 4	EPS (mg/L) and	-0.43	0.23		
Run 5	Turbidity	0.18	0.53	0.55	0.06
Run 6		0.07	0.80	0.43	0.18

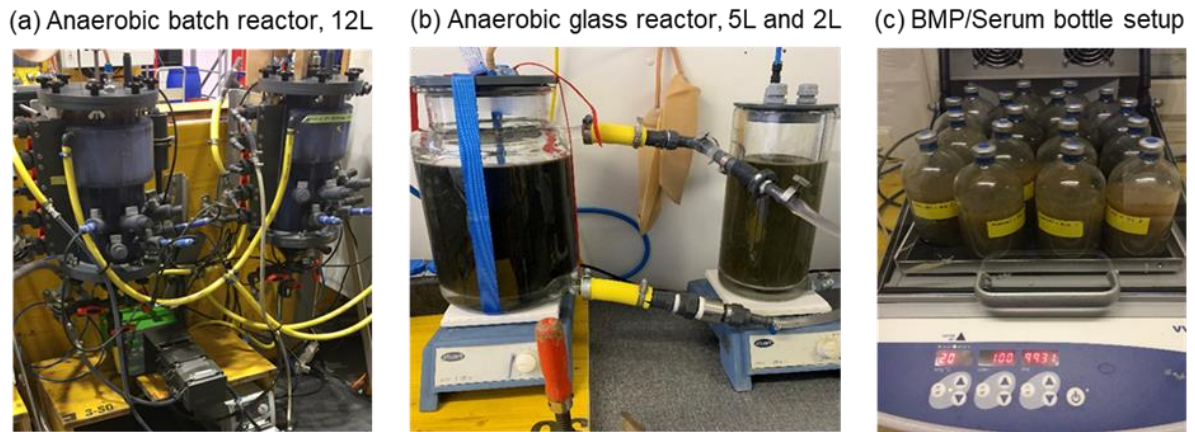**Figure S2. 1**: Setup for the series of batch reactors. (a) Anaerobic batch reactors for runs 1 through to 4 using 12 L reactors (b) 5L and 2L anaerobic glass reactors for run 5 (c) Serum bottle setup with 250ml serum bottles and an incubator for run 6.
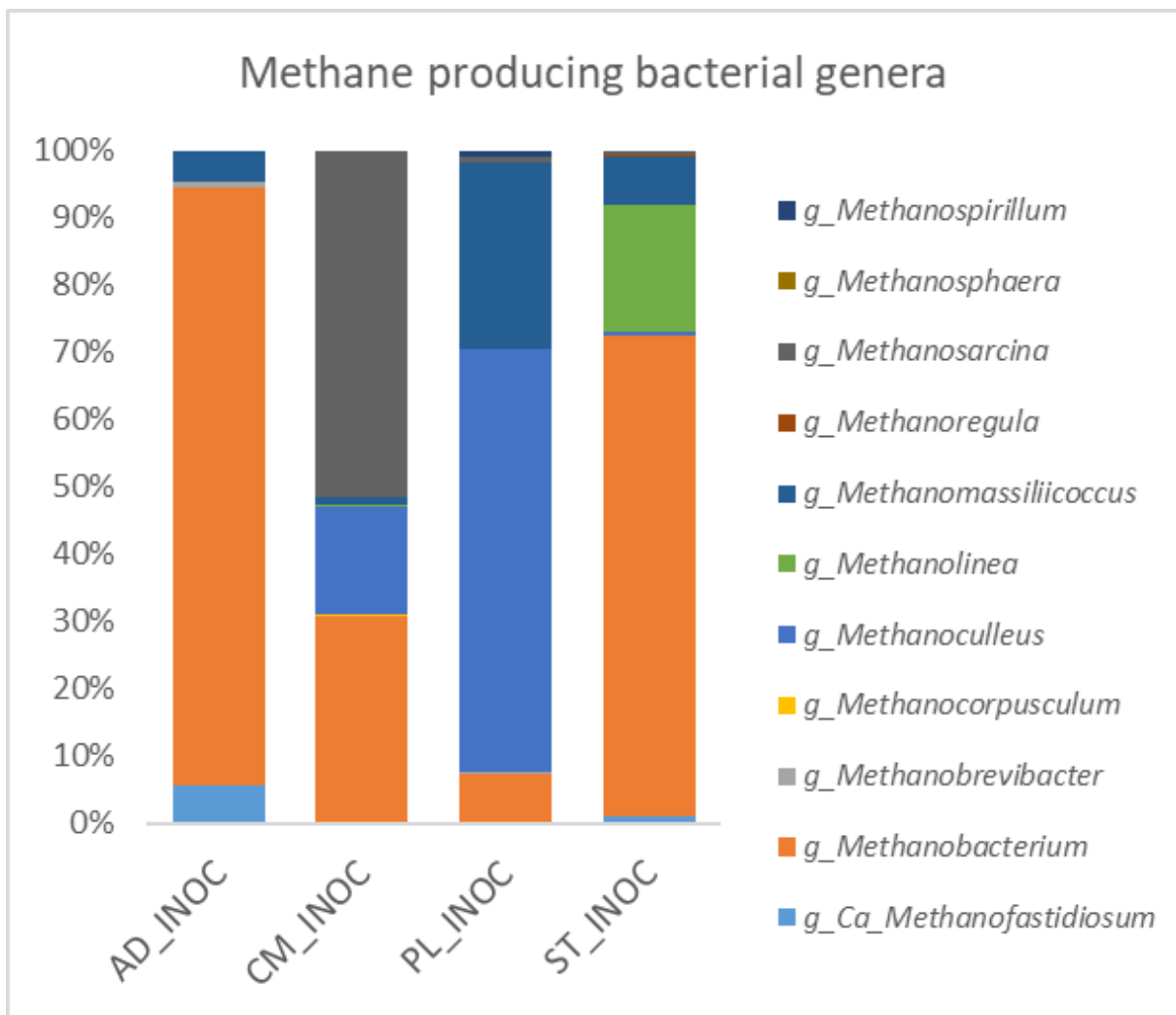
Figure S2. 2: Microbial diversity within the group of methane-producing bacteria for the different inocula and feed used in the BMP test

Appendix B

Supplementary Information

Changes in organic fractions, cations, and stabilization from feces to fecal sludge: implications for dewatering performance and management solutions

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The data for this study are openly available in eawag repository:
https://doi.org/10.25678/0007MP

Supplemental information for chapter 3
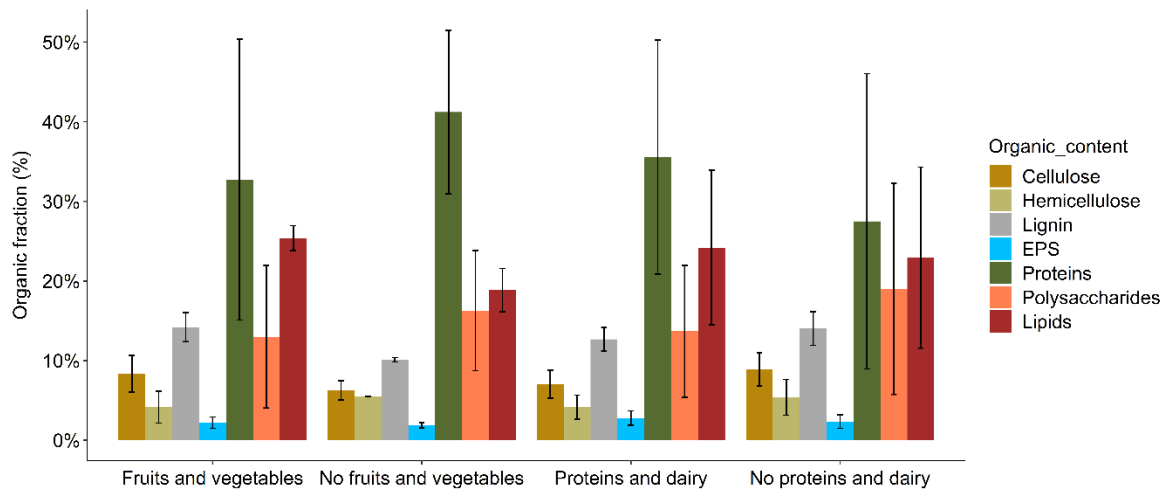
Figure S3. 1: Organic contents of feces grouped by whether or not volunteers reported eating categories of 'fruits and vegetables' or 'proteins and dairy' in the past 24 hours prior to sample collection.
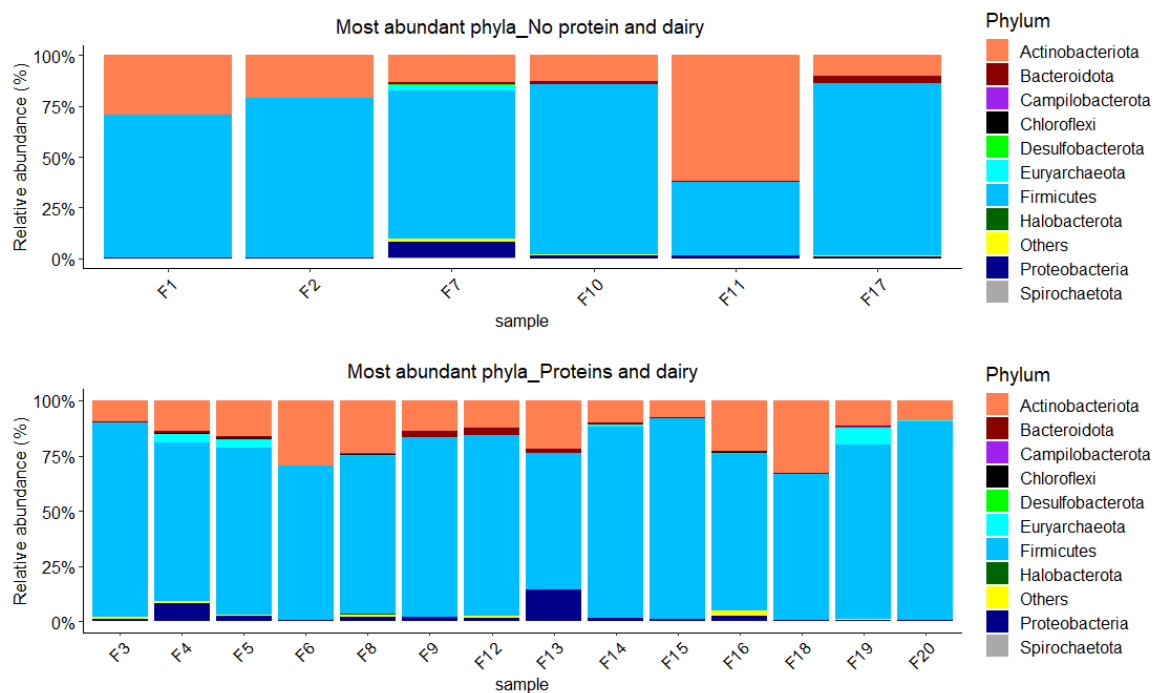
Figure S3. 2: Relative abundance of most abundant phyla in feces grouped according to participants who consumed no proteins or dairy and those that consumed proteins and dairy.

Figure S3. 3: Relative abundance of most abundant phyla in feces grouped according to participants who consumed no fruits or vegetables and those that consumed fruits and vegetables.
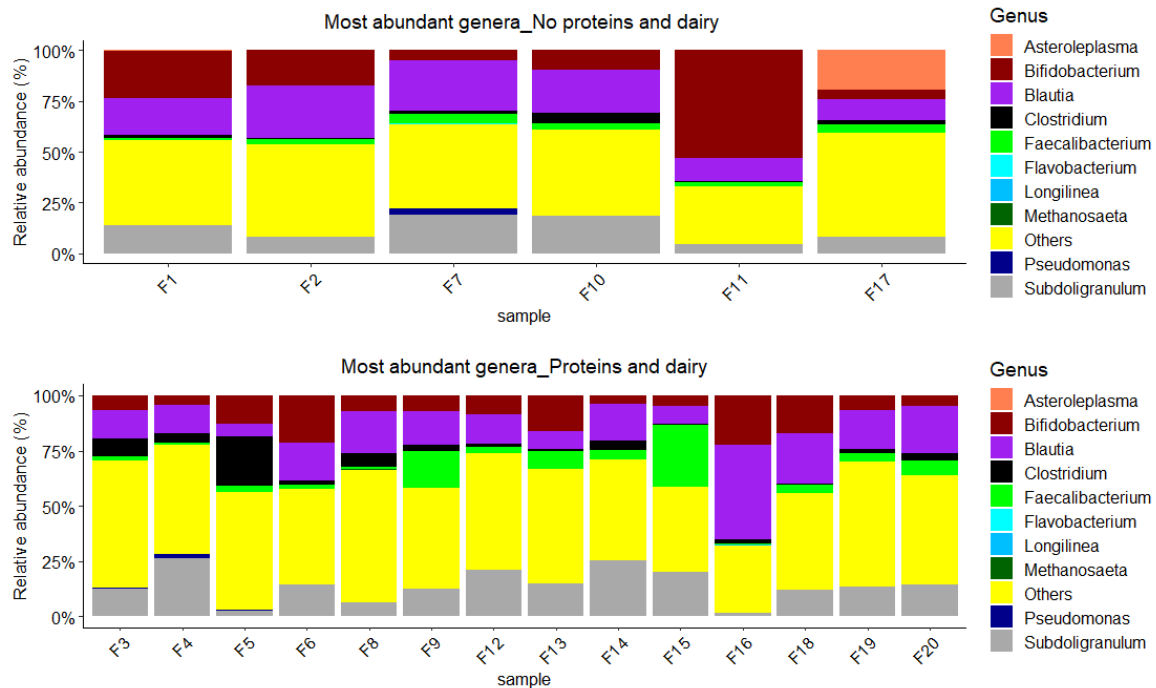
Figure S3. 4: Relative abundance of most abundant genera in feces grouped according to participants who consumed no proteins or dairy and those that consumed proteins and dairy.
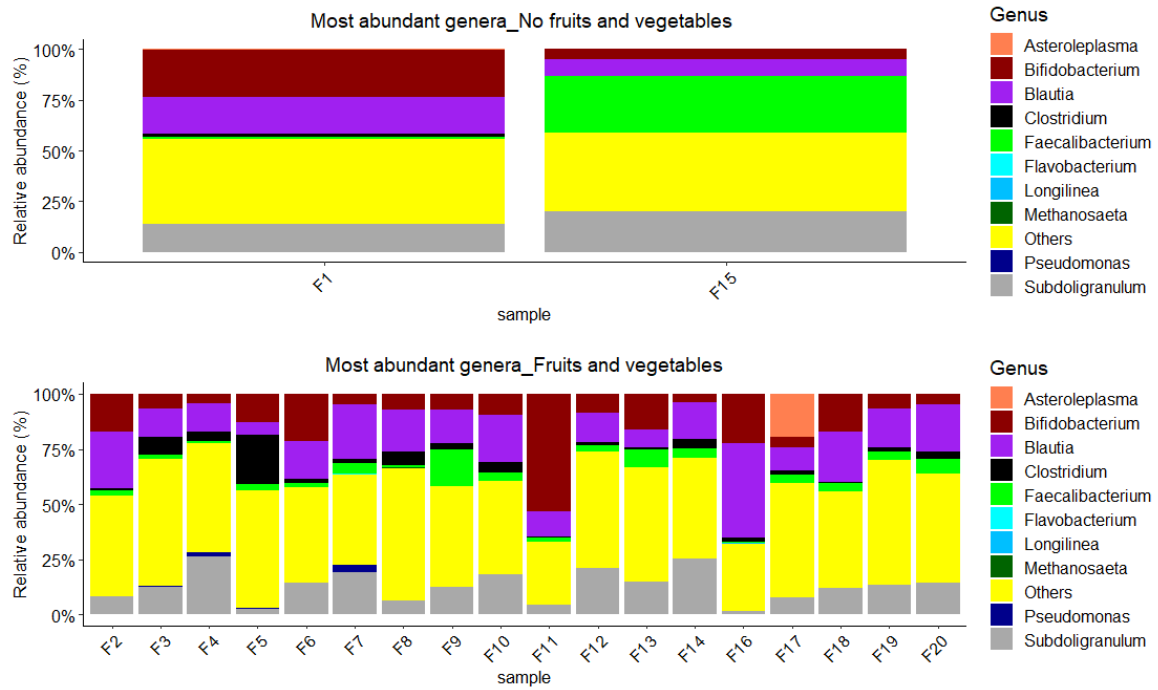
Figure S3. 5: Relative abundance of most abundant genera in feces grouped according to participants who consumed no fruits or vegetables and those that consumed fruits and vegetables.
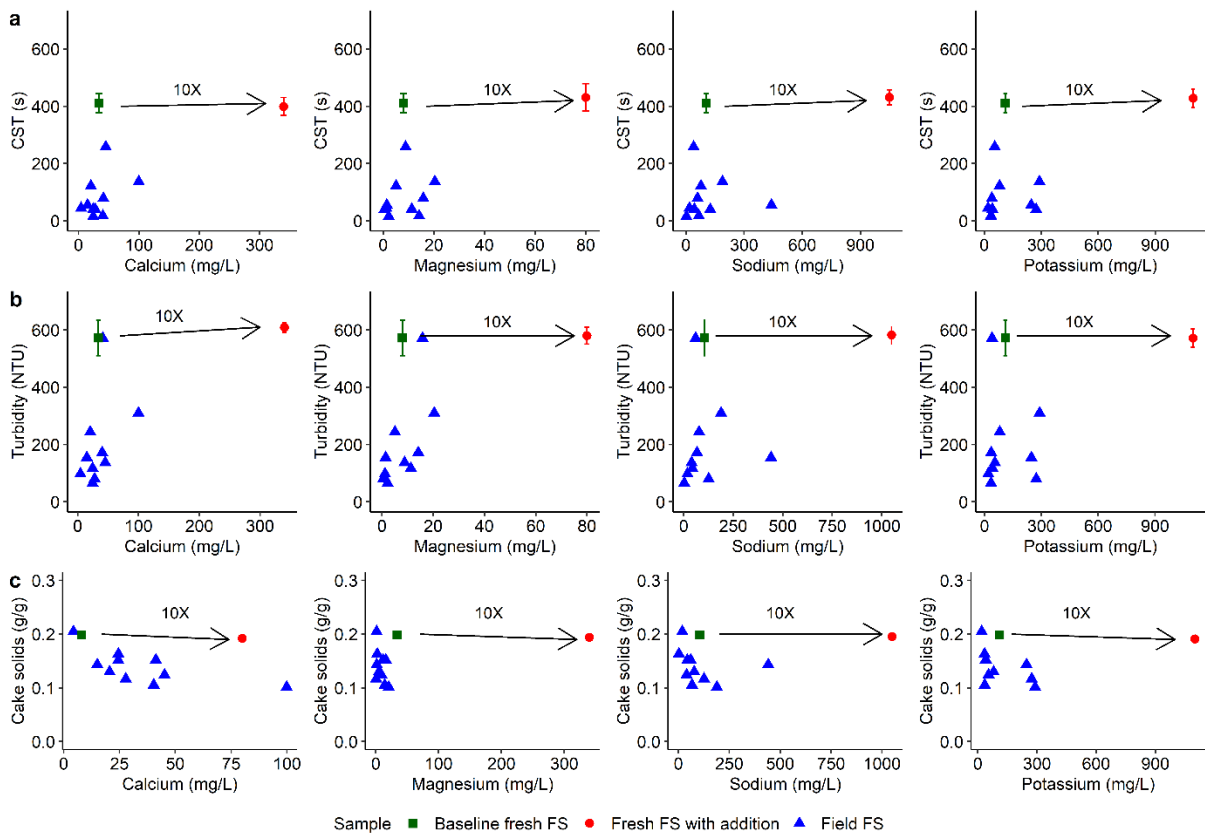
Figure S3. 6:Dewaterability of fresh and field FS showing the effect of cations on dewatering properties (a) Change in filtration time (CST) in fresh FS and field FS; (b) supernatant turbidity (after centrifugation); and (c) water holding capacity (dewatered cake so solids). The green squares represent the baseline dewaterability of fresh FS, the red circles represent addition of aliquots to the fresh FS and the blue triangles represent the field FS.
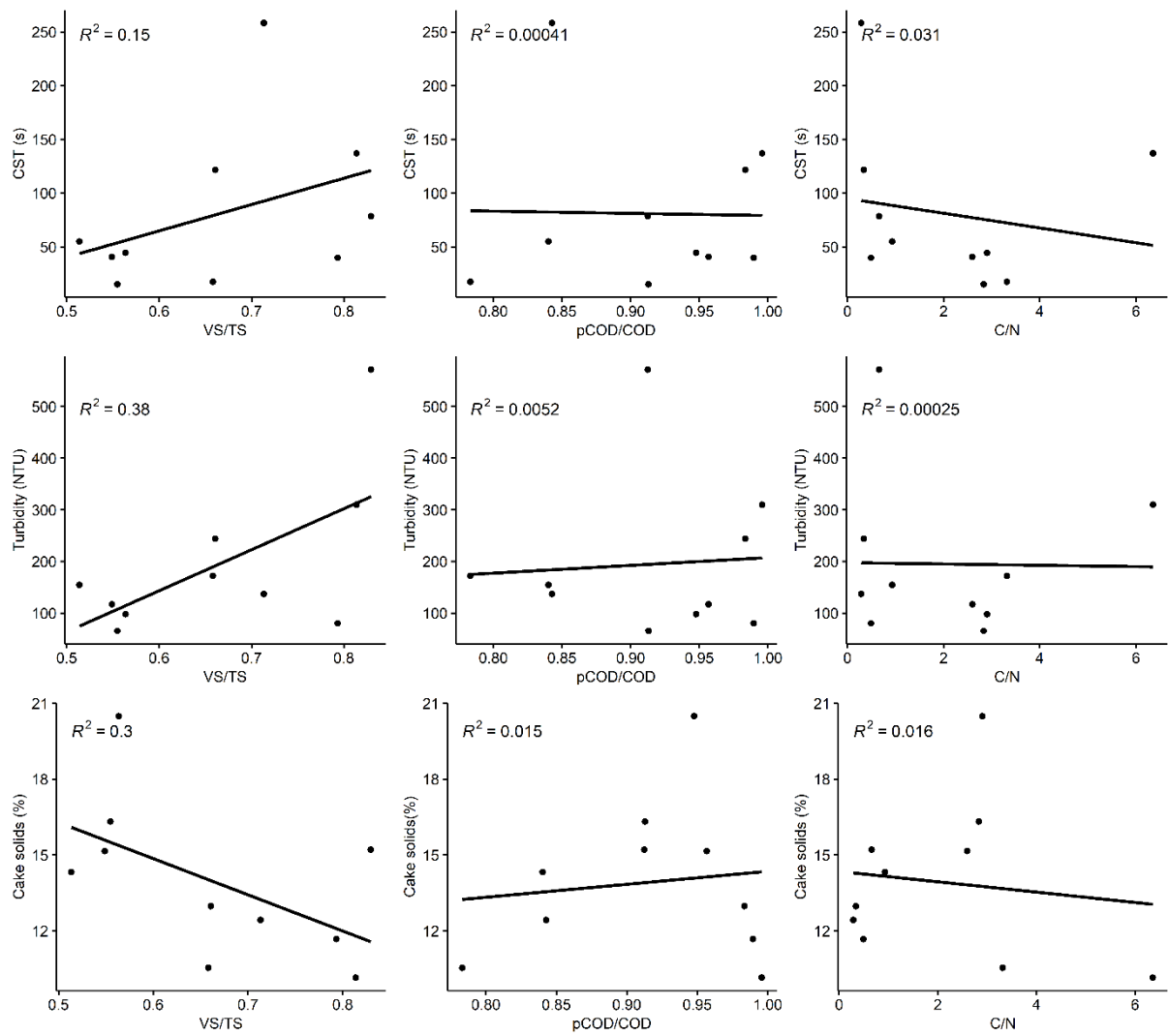
Figure S3. 7: Scatter plot illustrating the relationship between the level of stabilization indicated by VS/TS, PCOD/COD and CN and metrics of dewaterability.
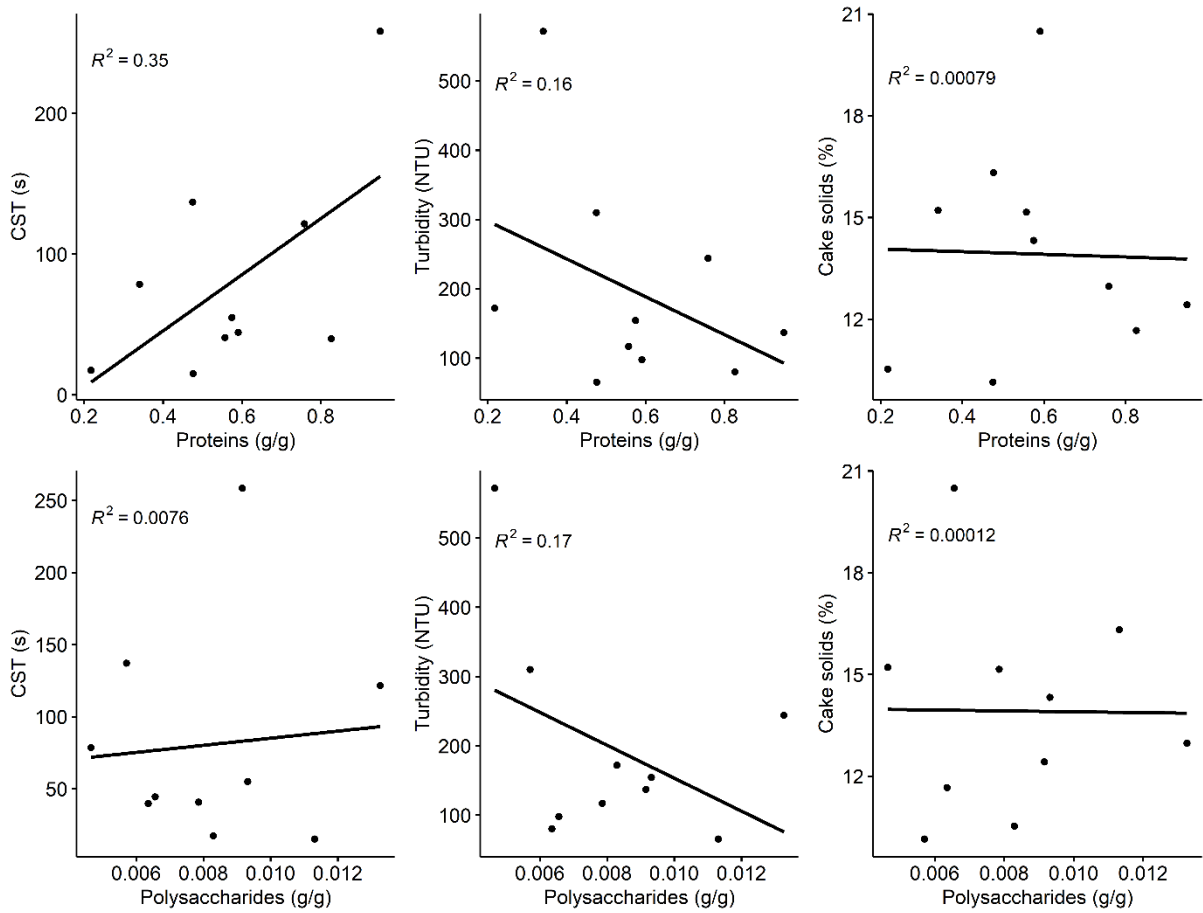
Figure S3. 8: Scatter plot illustrating the relationship between organic content of field FS and dewatering properties
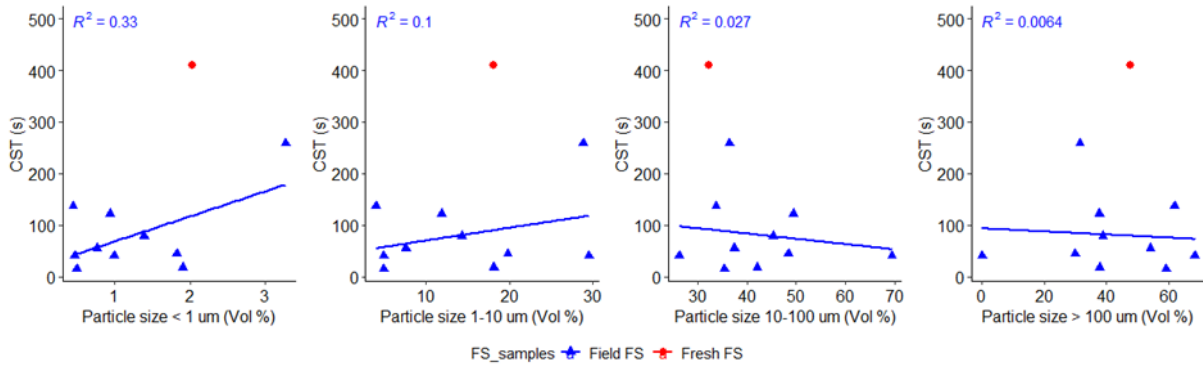
Figure S3. 9:Influence of amount of particles in specific size range (in volume %) on dewaterability indicated by CST (s) for fresh and field FS. The blue triangles are for field FS samples and the red circle is for fresh FS.
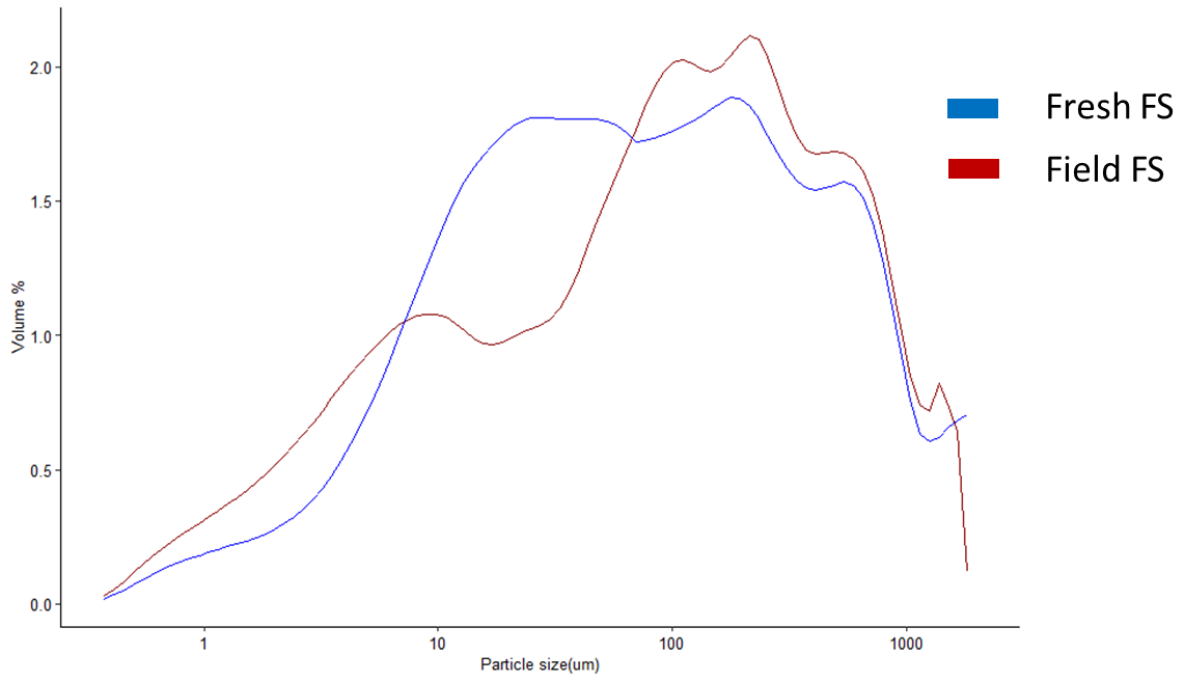
Figure S3. 10: Particle size distribution of fresh FS recipe and field FS samples.
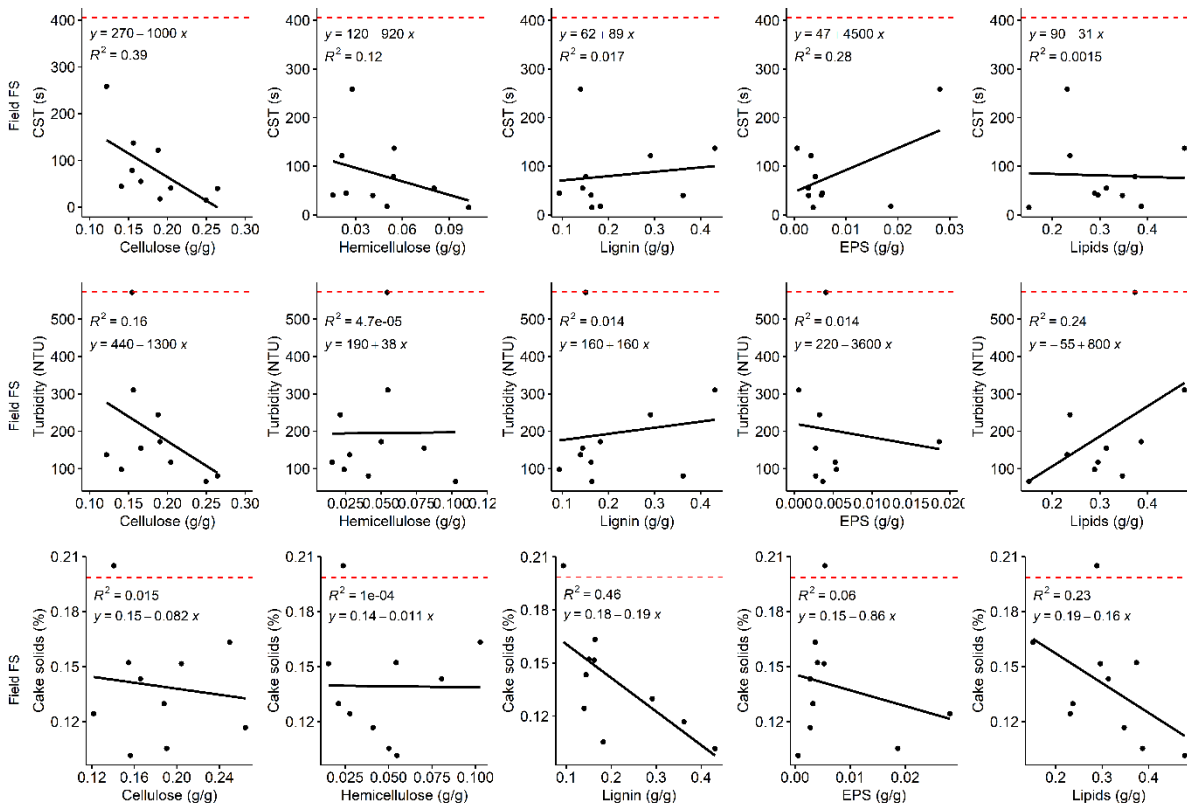
Figure S3. 11: Scatter plot illustrating the relationship between the organic constituents in field FS and dewaterability
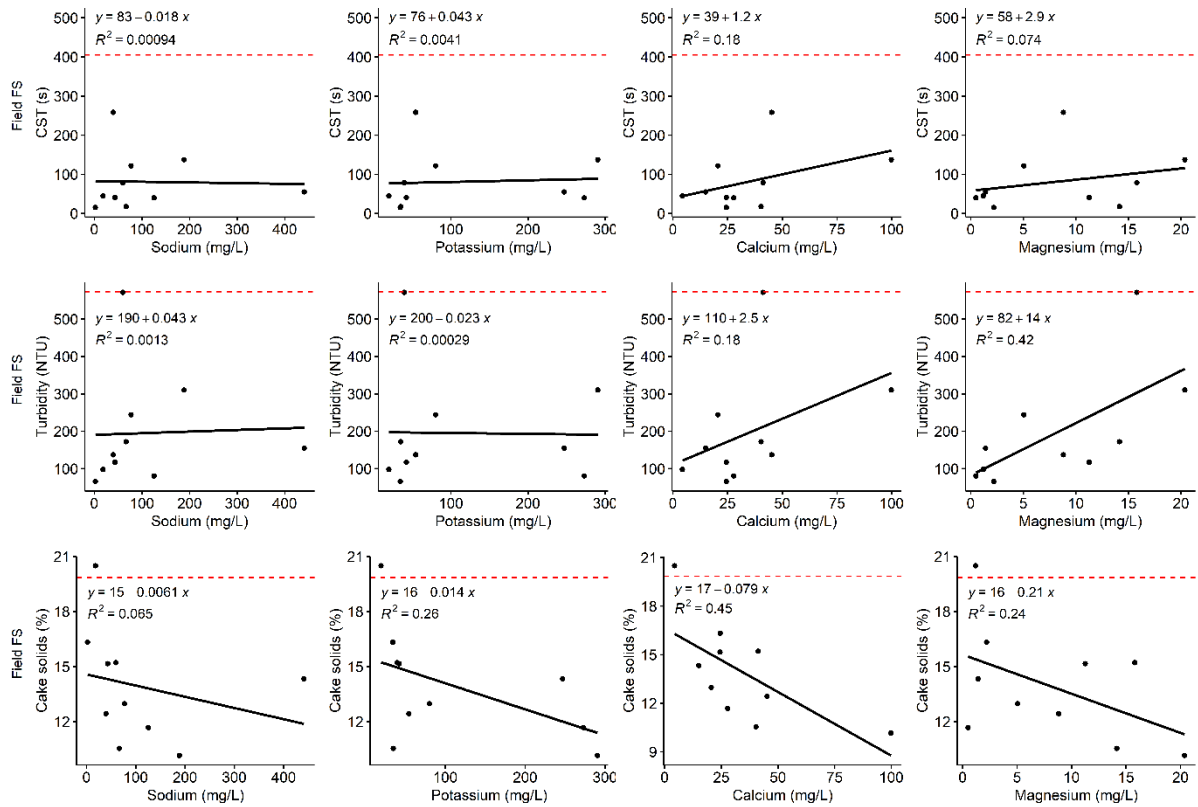
Figure S3. 12:Scatter plot illustrating the relationship between the organic constituents in field FS and dewaterability

Table S3. 1: Organic constituents in feces based on food groups of the food frequency table.

Food groups	Statistics	Cellulose (g/g)	Hemice llulose (g/g)	Lignin (g/g)	EPS (g/g)	Protein (g/g)	Polysa ccharid es (g/g)	Lipids (g/g)
Fruits	Average	0.10	0.06	0.17	0.03	0.46	0.25	0.32
	Medium	0.10	0.06	0.17	0.03	0.46	0.17	0.30
	Stdev	0.03	0.04	0.03	0.008	0.23	0.20	0.12
Vegetables	Average	0.09	0.06	0.17	0.03	0.38	0.19	0.31
	Medium	0.08	0.05	0.17	0.03	0.33	0.17	0.28
	Stdev	0.02	0.02	0.03	0.01	0.23	0.12	0.17
Proteins	Average	0.09	0.06	0.165	0.03	0.37	0.16	0.26
	Medium	0.08	0.05	0.16	0.03	0.29	0.16	0.26
	Stdev	0.02	0.02	0.03	0.01	0.19	0.10	0.12
Dairy	Average	0.08	0.06	0.16	0.03	0.50	0.22	0.34
	Medium	0.08	0.06	0.16	0.03	0.50	0.20	0.32
	Stdev	0.02	0.03	0.02	0.01	0.22	0.16	0.17
Fruits and vegetables	Average	0.10	0.05	0.18	0.03	0.41	0.16	0.32
	Medium	0.11	0.05	0.18	0.03	0.40	0.13	0.30
	Stdev	0.03	0.03	0.02	0.009	0.22	0.11	0.13
Proteins and dairy	Average	0.09	0.05	0.15	0.03	0.43	0.17	0.29
	Medium	0.08	0.05	0.15	0.03	0.43	0.17	0.30
	Stdev	0.02	0.02	0.02	0.01	0.18	0.10	0.12
No fruits and vegetables	Average	0.09	0.08	0.15	0.03	0.61	0.24	0.28
	Medium	0.09	0.08	0.15	0.03	0.61	0.24	0.28
	Stdev	0.02	0.00	0.00	0.005	0.15	0.11	0.04
No proteins and dairy	Average	0.11	0.07	0.17	0.03	0.33	0.23	0.28
	Medium	0.11	0.08	0.17	0.03	0.33	0.17	0.27
	Stdev	0.03	0.03	0.03	0.01	0.23	0.16	0.14

Appendix

Table S3. 2: Concentration of cations in feces based on food groups of the food frequency table.

Food groups	Statistics	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Na (mg/L)
Fruits	Average	11.50	3.48	14.69	4.45
	Medium	12.38	2.12	14.26	4.45
	Stdev	2.11	4.37	1.81	1.49
Vegetables	Average	13.60	3.13	15.02	3.31
	Medium	12.91	2.83	14.87	3.22
	Stdev	6.13	3.14	1.60	2.02
Proteins	Average	12.25	2.18	14.79	3.27
	Medium	10.53	2.18	14.79	3.27
	Stdev	5.52	1.53	1.12	2.09
Dairy	Average	15.48	2.47	14.85	2.58
	Medium	15.48	2.12	14.81	2.78
	Stdev	6.67	1.95	1.42	1.74
Fruits and vegetables	Average	11.25	3.75	15.18	4.93
	Medium	11.82	2.14	14.72	4.88
	Stdev	2.20	4.69	1.61	1.22
Proteins and dairy	Average	13.62	2.03	14.74	3.12
	Medium	13.62	1.71	14.74	3.12
	Stdev	5.63	1.71	1.12	2.04
No fruits and vegetables	Average	16.79	7.57	17.82	24.48
	Medium	16.79	7.57	17.82	24.48
	Stdev	3.30	4.79	3.26	16.10
No proteins and dairy	Average	12.57	5.94	15.86	11.49
	Medium	12.75	3.86	15.86	5.88
	Stdev	2.34	5.18	3.11	14.18

Table S3. 3: Volume percent of the particle sizes ranges in fecal sludge samples from onsite sanitations systems

Samples	Particles < 1um (% vol)	Particles < 10 um (% vol)	Particles < 100 um (% vol)	Particles > 100 um (% vol)
FS 1	1.39	14.33	45.36	38.92
FS 2	0.77	7.57	37.45	54.21
FS 3	0.49	4.89	35.39	59.23
FS 4	0.44	4.01	33.69	61.86
FS 5	1.82	19.80	48.40	29.98
FS 6	3.27	28.88	36.41	31.44
FS 7	0.99	29.54	69.47	0.001
FS 8	1.90	18.14	42.12	37.84
FS 9	0.94	11.87	49.50	37.69
FS 10	0.47	4.92	26.20	68.42
Fresh FS	2.03	18.11	32.30	47.57

Table S3. 4: Statistical analysis of the variation in food groups. Variation in all food groups (Fruits and vegetables, Proteins and dairy, No fruits and vegetables, No proteins and dairy) analyzed with ANOVA single factor.

Cellulose	Source of Variation	SS	df	MS	F	P-value	F crit
	Between Groups	0.002	3	0.00062	0.7408	0.54408	3.2873821
	Within Groups	0.013	15	0.00084			
	Total	0.015	18				
Hemicellulose	Between Groups	0.002	3	0.00060	0.8576	0.48431	3.2873821
	Within Groups	0.011	15	0.00070			
	Total	0.012	18				
Lignin	Between Groups	0.002	3	0.00078	1.2268	0.33448	3.2873821
	Within Groups	0.009	15	0.00063			
	Total	0.012	18				
Proteins	Between Groups	0.115	3	0.03819	0.6102	0.61869	3.2873821
	Within Groups	0.939	15	0.06259			
	Total	1.053	18				
Polysaccharides	Between Groups	0.021	3	0.00715	0.2983	0.82610	3.2873821
	Within Groups	0.360	15	0.02397			
	Total	0.381	18				
Lipids	Between Groups	0.005	3	0.00173	0.0767	0.97164	3.2873821
	Within Groups	0.339	15	0.02259			
	Total	0.344	18				

Table S3. 5: Organic and inorganic constituents of FS samples from onsite sanitation systems

Sample	Country	Ca	K	Mg	Na	Polysaccharide (g/g)	Proteins (g/g)	Cellulose (g/g)	Hemicellulose (g/g)	Lignin (g/g)	EPS (g/g)	Intact cells	Total cells
Sample FS1	Guatemala	41.28	39.63	15.81	59.69	0.0047	0.34	0.166	0.080	0.144	0.003	20586.67	302766.7
Sample FS2	Kenya	15.04	246.89	1.42	440.94	0.0093	0.57	0.250	0.103	0.164	0.004	207350	345366.7
Sample FS3	Kenya	24.48	34.25	2.19	1.99	0.0113	0.48	0.156	0.055	0.431	0.001	771400	2049233
Sample FS4	India	99.86	290.42	20.38	188.28	0.0057	0.48	0.141	0.024	0.094	0.005	73033.33	343083.3
Sample FS5	Senegal	4.37	19.52	1.19	18.30	0.0066	0.59	0.122	0.028	0.140	0.028	1262067	5166683
Sample FS6	Ghana	45.18	54.42	8.82	39.57	0.0092	0.95	0.204	0.015	0.162	0.005	89800	444250
Sample FS7	Ghana	24.46	42.22	11.27	43.29	0.0079	0.56	0.190	0.050	0.182	0.019	162383.3	1747300
Sample FS8	Canada	40.33	34.94	14.16	66.609	0.0083	0.22	0.188	0.021	0.291	0.003	639066.7	2497367
Sample FS9	Uganda	20.57	80.12	5.05	77.12	0.0133	0.76	0.264	0.041	0.362	0.003	394000	1178067
Sample FS10	Lebanon	27.80	272.83	0.50	125.38	0.0064	0.83	0.155	0.054	0.151	0.004	21500	1799733

Table S3. 6: Physical and chemical characteristics of FS samples from onsite sanitation systems

sample	Country	pH	Conductivity	TS (g/L)	VS (g/L)	COD (mg/L)	SCOD (mg/L)	pCOD	CST (s)	Turbidity (NTU)	TS_dew (%wt)
Sample 1	Guatemala	5.80	2.65	40.11	33.27	85150	7459	77691	78.55	571.5	15.20
Sample 2	Kenya	7.66	12.75	12.22	6.28	6900	1103	5797	54.93	154.5	14.32
Sample 3	Kenya	7.05	2.81	14.08	7.81	16300	1418.5	14881.5	15.10	65.45	16.32
Sample 4	India	7.55	1.93	42.88	34.89	57900	245	57655	137.03	310	10.14
Sample 5	Senegal	7.43	3.01	6.78	3.82	9900	517	9383	44.40	97.8	20.48
Sample 6	Ghana	7.32	12.22	16.38	11.68	37850	5952	31898	258.33	137	12.42
Sample 7	Ghana	7.82	4.62	11.63	6.38	15350	664.5	14685.5	40.68	117	15.15
Sample 8	Canada	6.16	12.49	2.14	1.41	4100	889	3211	17.43	172	10.53
Sample 9	Uganda	8.29	8.82	29.86	19.73	45250	743	44507	121.55	244	12.97
Sample 10	Lebanon	8.03	3.31	16.29	12.93	29150	304	28846	39.80	80.2	11.66

Table S3. 7: Baseline concentrations of organic and inorganic constituents in fresh feces

	Cellulose (g/g)	Hemicellulose (g/g)	Lignin (g/g)	EPS (g/g)	Lipids/oils (g/g)	Sodium (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)
Baseline conc.	0.12	0.047	0.105	0.028	0.3	105	110	34	8

Questionnaire

Sample ID_____

Gender
☐ Male
☐ Female

Nationality _____

1. Provide information about what you ate for your meals the last 24 hours:
   _____
   _____
   _____
   _____

2. Have you taken any medication in the last 24 hours? If so, please provide name of product, brand name or active ingredient.
   ☐ Yes
   ☐ No

   _____

3. If yes, what is the dosage of the medication that was taken?
   _____mg

4. Have you taken any antibiotics in the 24 hours? If so, what antibiotic?

   ☐ Yes
   ☐ No

   _____

5. Have you taken any probiotics the past 24 hours? If so, please provide the name of the product.

   ☐ Yes
   ☐ No

   _____

6. What has been your diet for the past one year?

   ☐ Meat eater
   ☐ vegetarian
   ☐ other

7. If you are a vegetarian, what type of vegetarian are you?

   ☐ Lacto (consume milk product but not eggs)
   ☐ Ovo (Eat eggs but not milk products)
   ☐ Lacto-ovo (Eat dairy products including milk and eggs)
   ☐ Vegan (Does not eat product of animal origin)

☐ Semi (Eat poultry or fish occasionally, but no red meat)

8. Can provide more information of your diet (what portions of your diet consist of grains, pulses, nuts, seeds, vegetables and fruits)

      _____

      _____

      _____

      _____

To be filled by Researcher

9. Wet Weight of sample

 _____kg

Information For volunteers

Samples collection and storage procedure

- Pick a labelled sample container with sample ID and a questionnaire from designated locations at eawag (FC F-floor washroom or BU B-floor washroom).
- Collect poo directly into the sample container or in a clean plastic bag and transfer into the sample container.
- Ensure that the sample container with poo is tightly sealed
- Put the sealed container in a plastic zip lock bag and seal it
- wash your hands thoroughly with soap and warm running water

Note
- Sample should be collected in the morning and delivered on the same day to the designated locations (same as where containers were picked from)
- If samples are collected before the day of delivery, the sample must be kept in a fridge before delivered
- Samples must be delivered within 24 hours after collection.

Appendix C

Supplementary Information

Microbial communities in fecal sludge at city-wide scale in Lusaka, Zambia: relation to usage patterns, accumulation in containments, and treatment performance

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Supplemental information for chapter 4
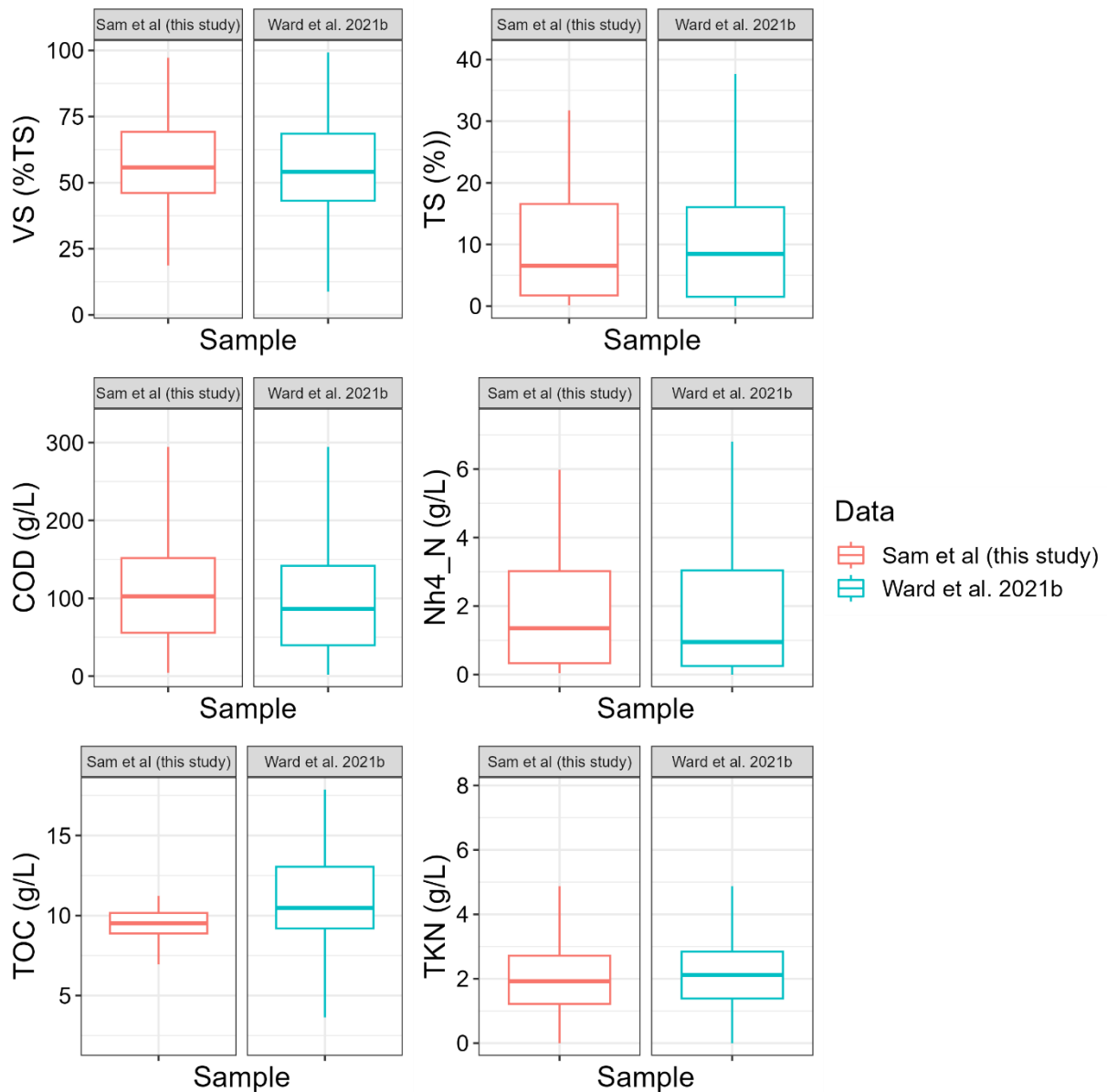
Figure S4. 1: Boxplots illustrating the comparison between fecal sludge characteristics of the main data set as obtained from Ward et al 2021 and the subset data in this study

(A) Relative abundance at phylum level

(B) Relative abundance at class level

(C) Relative abundance at genus level
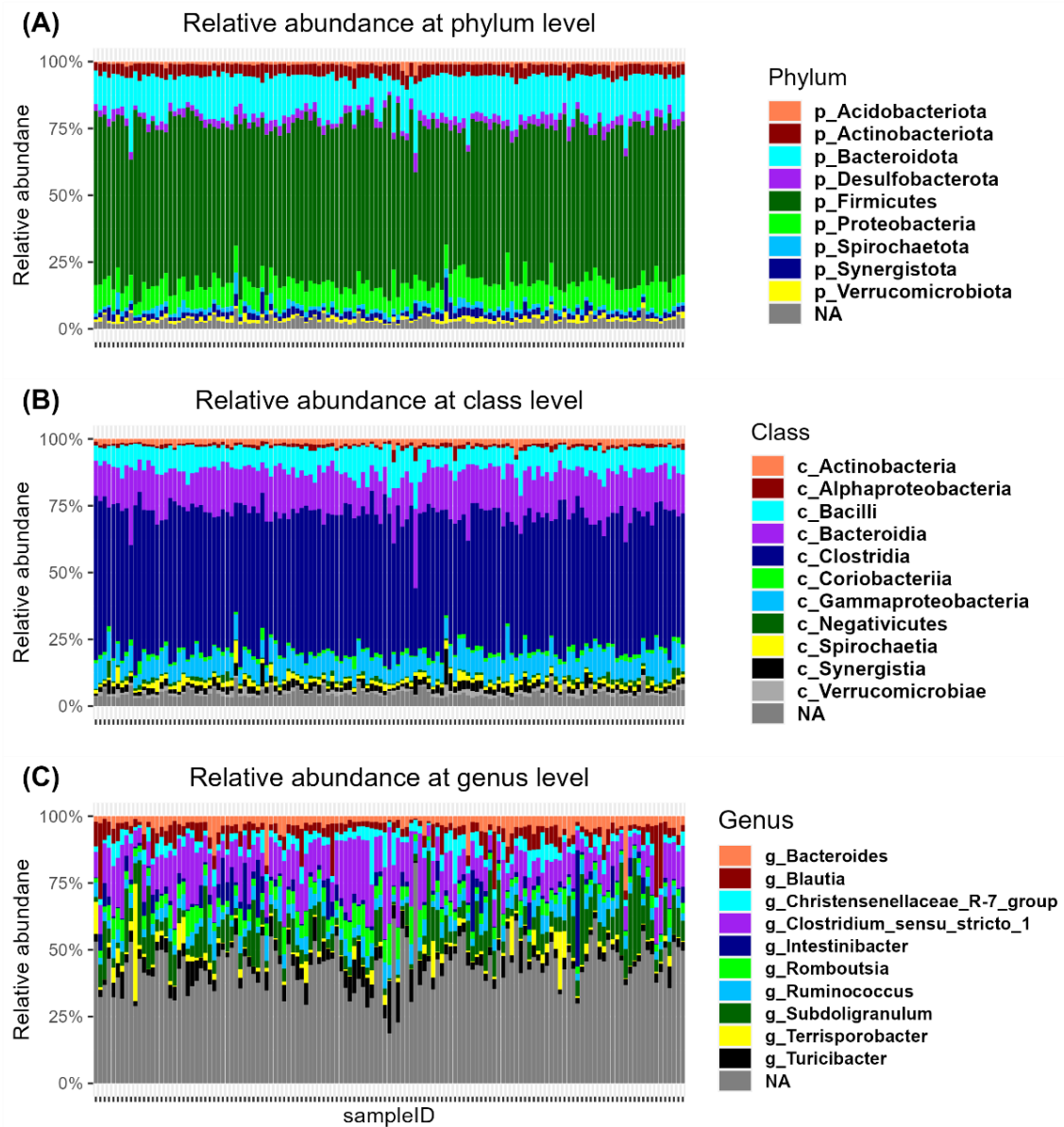
Figure S4. 2: Bar plots showing the relative abundance (>1 %) of organisms at the phyla, class and genus levels. Taxa with less than 1% relative abundance are classified as NA
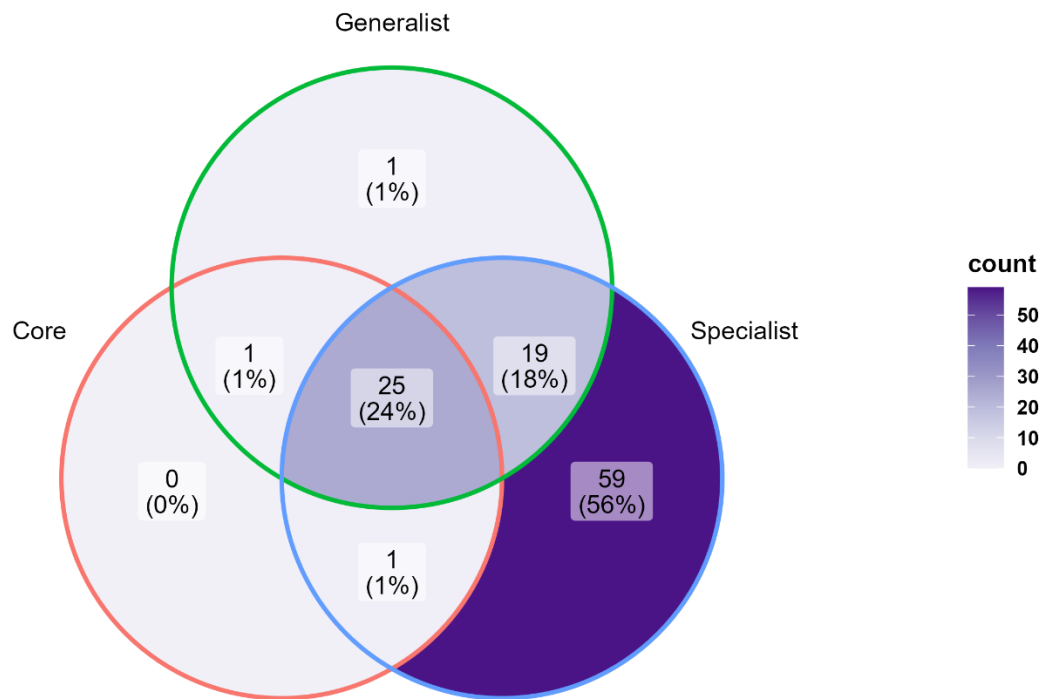
Figure S4. 3: Venn diagram showing the distribution of the core generalist and specialist groups and the interrelatedness between the groups
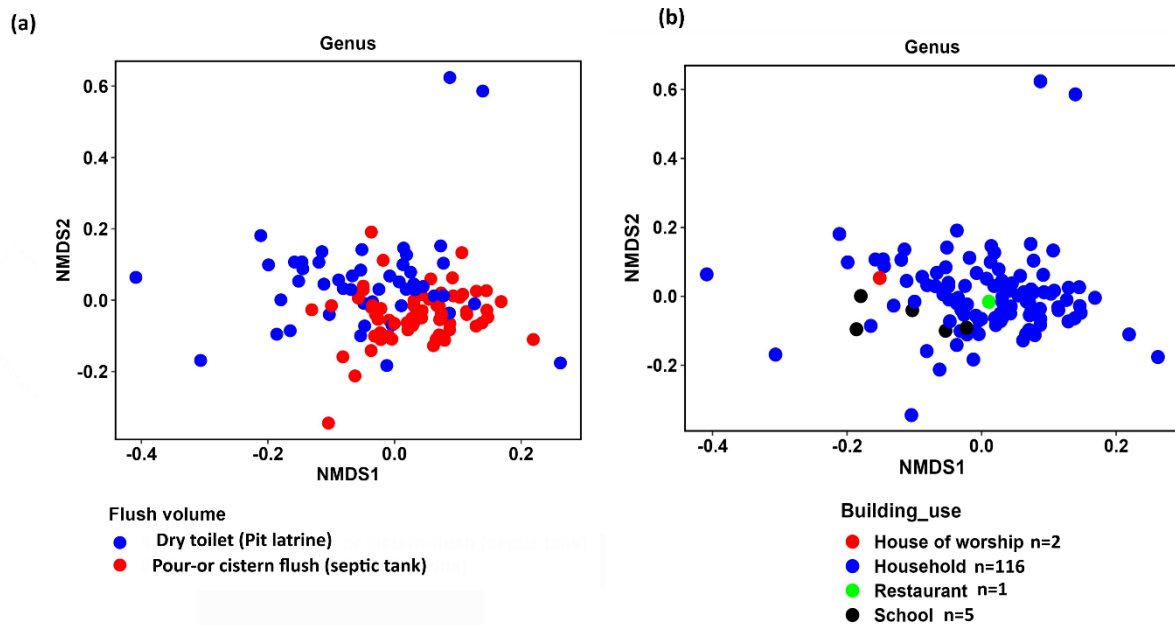
Figure S4. 4 NMDS ordination plots comparing (a) flush volume (water usage) and (b) building use at the genus level.
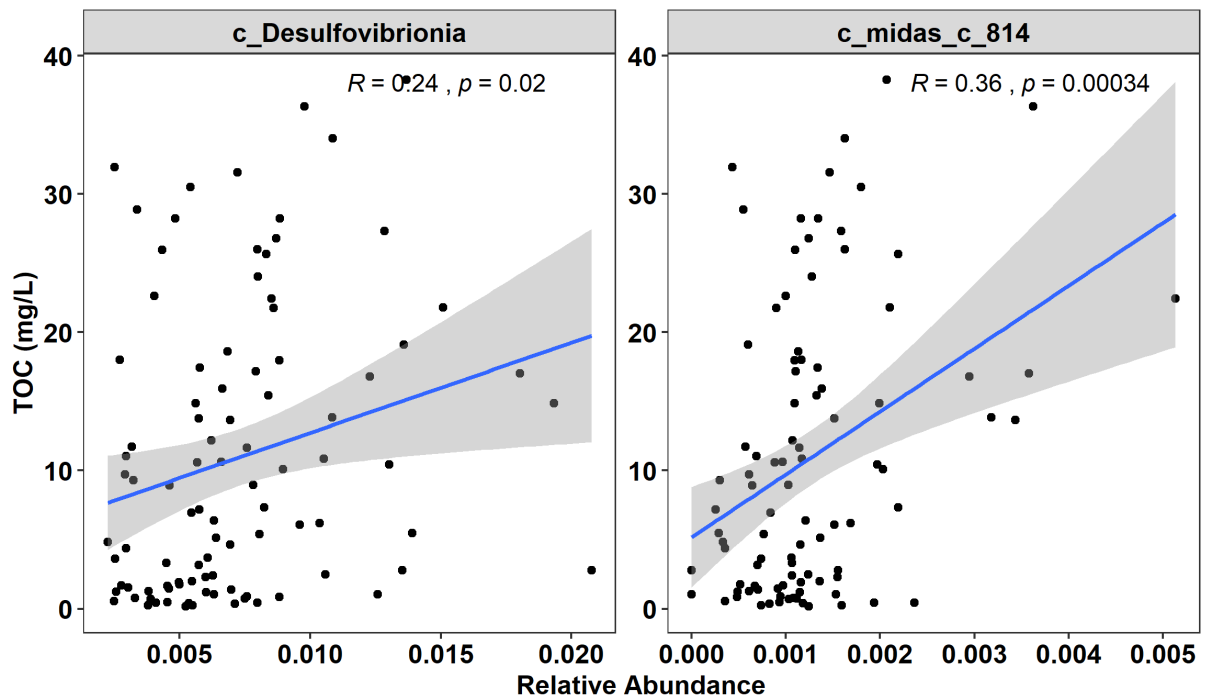
Figure S4. 5: Scatter plots illustrating the correlation between microorganisms at the class level and the total organic carbon concentration (TOC)
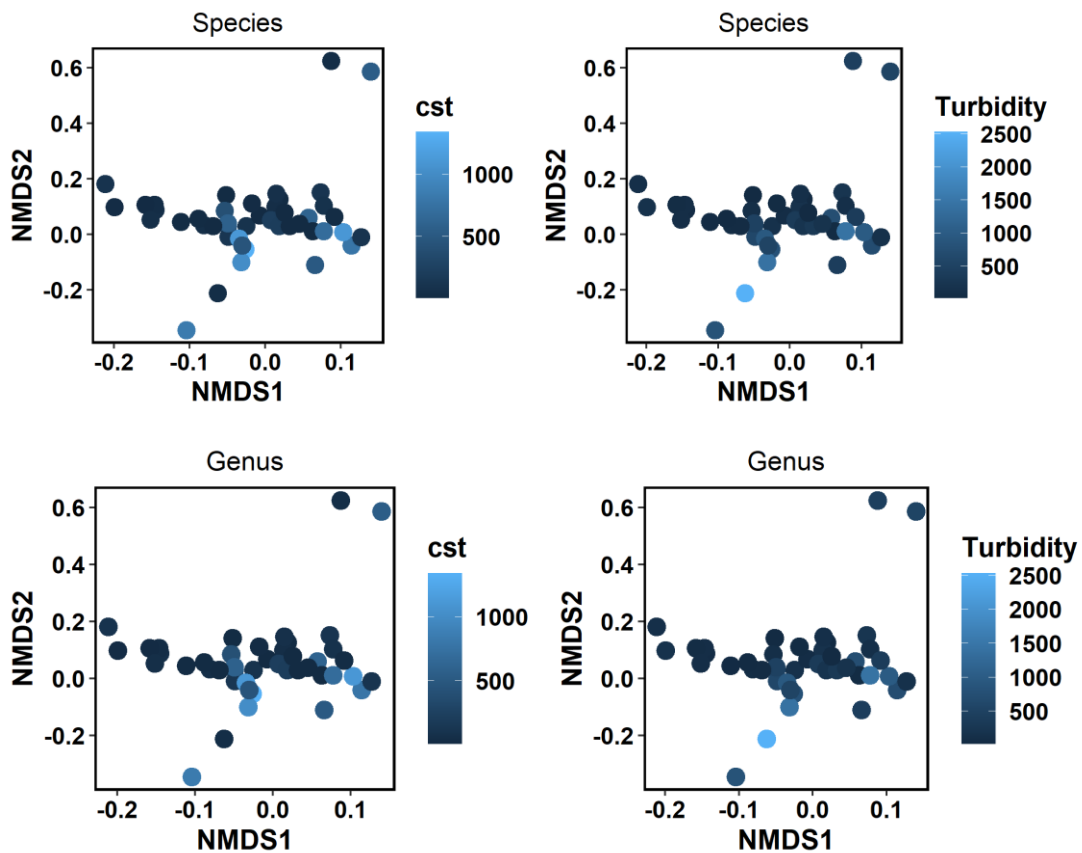
Figure S4. 6: Comparison of microbial structure with respect to metrics of dewaterability at the species level. Metrics of dewaterability used in this study are the capillary suction time (CST) and turbidity after centrifugation
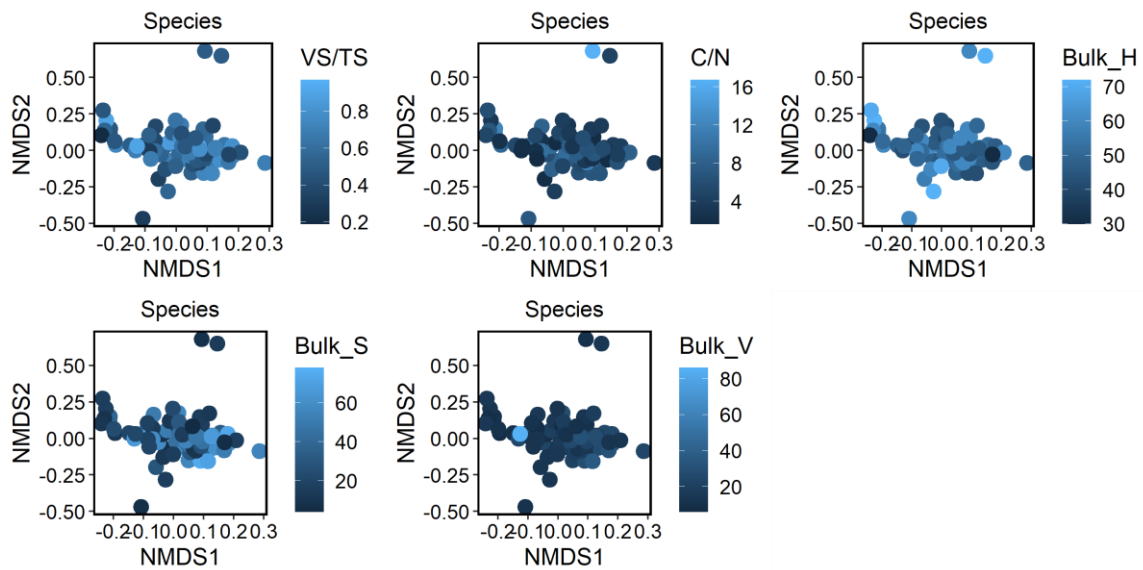
Figure S4. 7: Comparison of microbial structure with respect to metrics of stabilization at the species level. Metrics of stabilization used in this study included the volatile to total solids ratio (VS/TS) , carbon to nitrogen ratio (C/N).and Color of the bulk fecal sludge indicated by the hue (Bulk_hue), saturation (bulk_S) and value (Bulk_V)
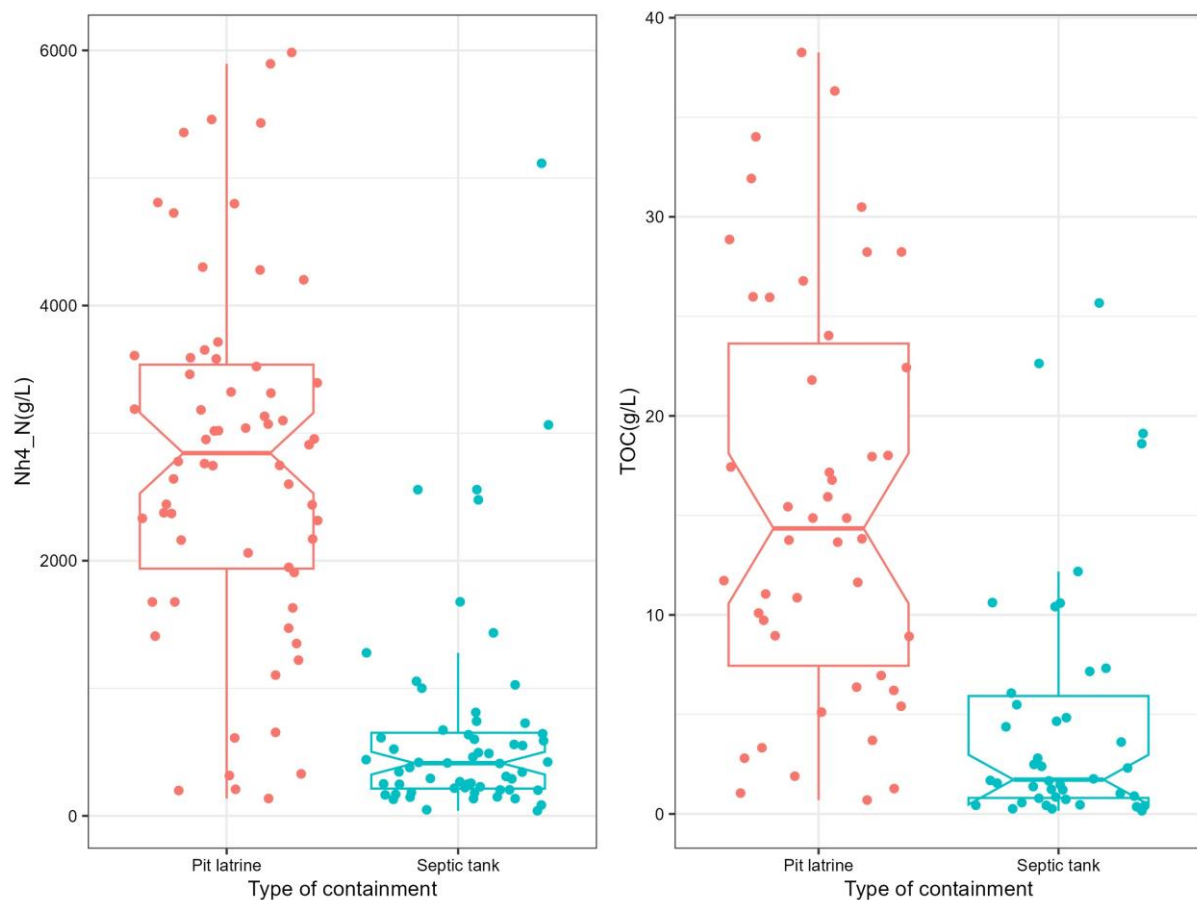
Figure S4. 8: Boxplots illustrating the comparison between fecal sludge characteristics for different types of containment. Figure S13 (a) shows the differences in ammonium nitrogen concentration and (b) the total organic carbon (TOC)
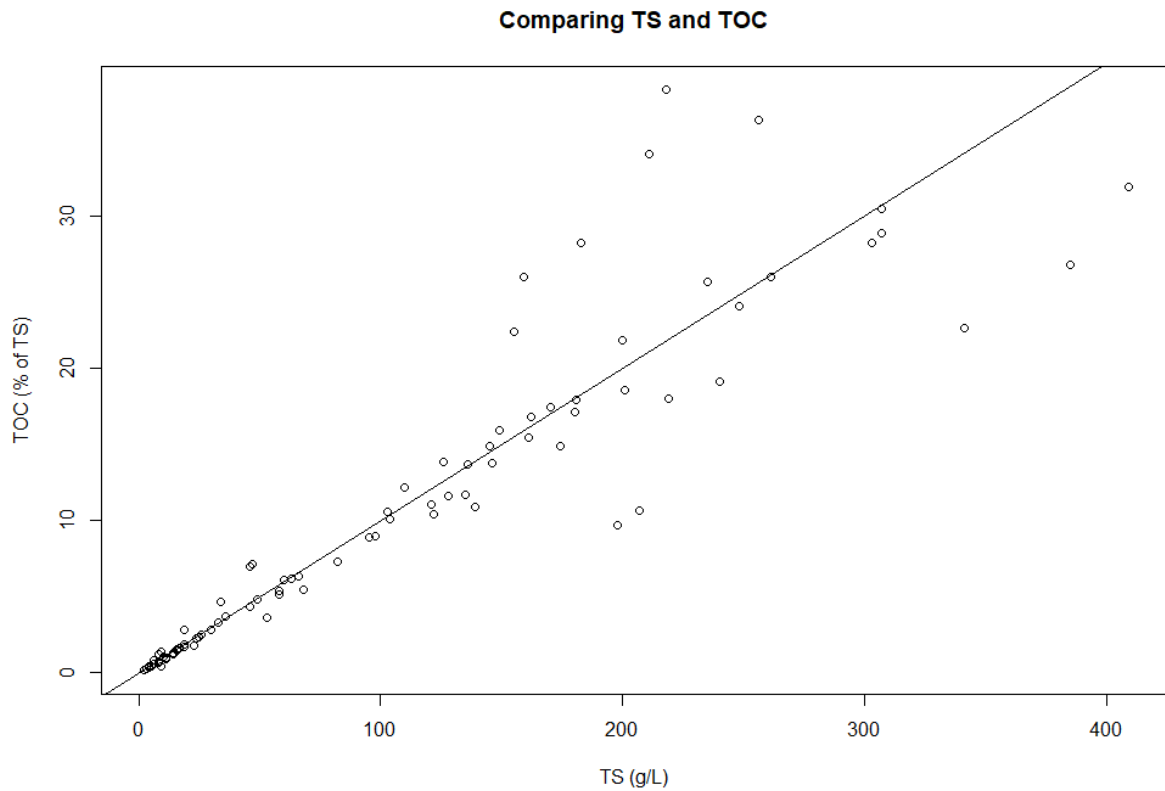
Figure S4. 9: Comparing the relationship between total solids and total organic carbon (which is always a percentage of total solids
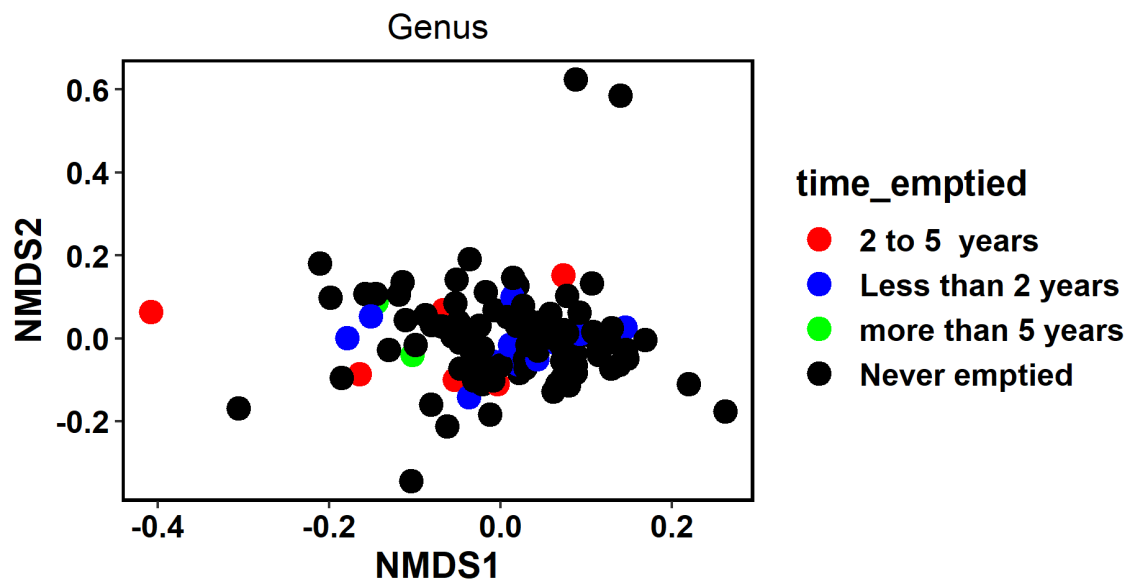
Figure S4. 10: Comparison of microbial community structure with respect to time since containment at the genus level. The time since emptied is categorized into 2-5 years, less than 2 years, more than 5 years and never emptied
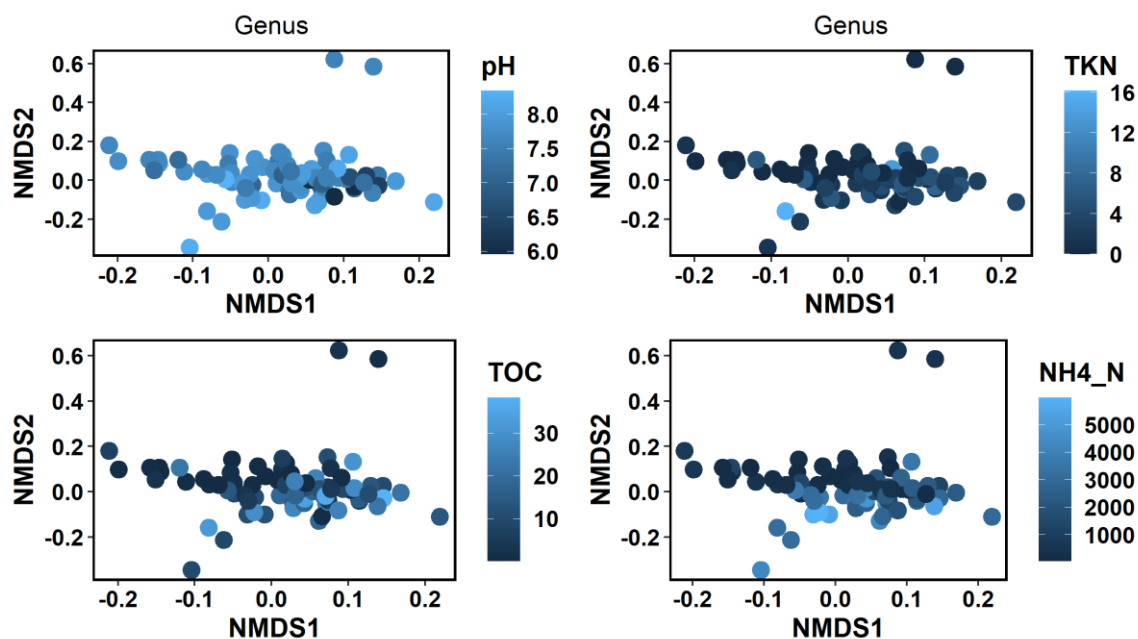
Figure S4. 11: Comparison of microbial community of fecal sludge characteristics at the genus level. The plot shows the distribution of microbial communities at different levels of pH, total Kjeldahl nitrogen (TKN), total organic carbon (TOC) and ammonium nitrogen (NH_4^+-N)
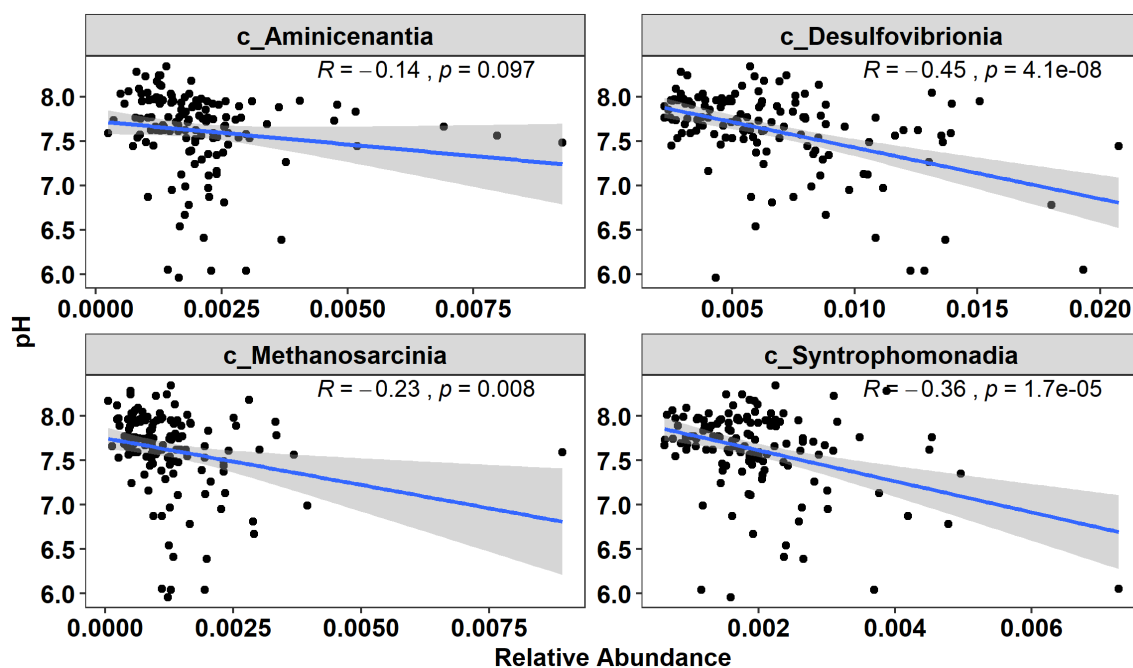
Figure S4. 12: Scatter plots showing the correlation between top four microorganisms and pH at the class level.
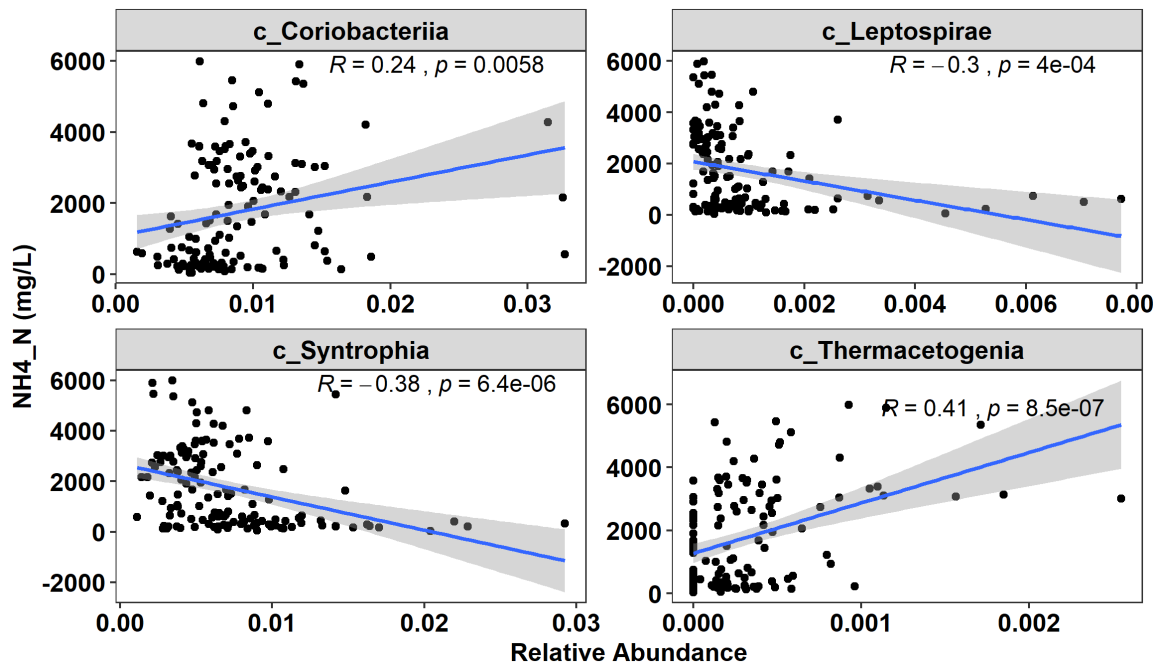
Figure S4. 13: Scatter plots showing the correlation between top four microorganisms and NH4-N at the class level
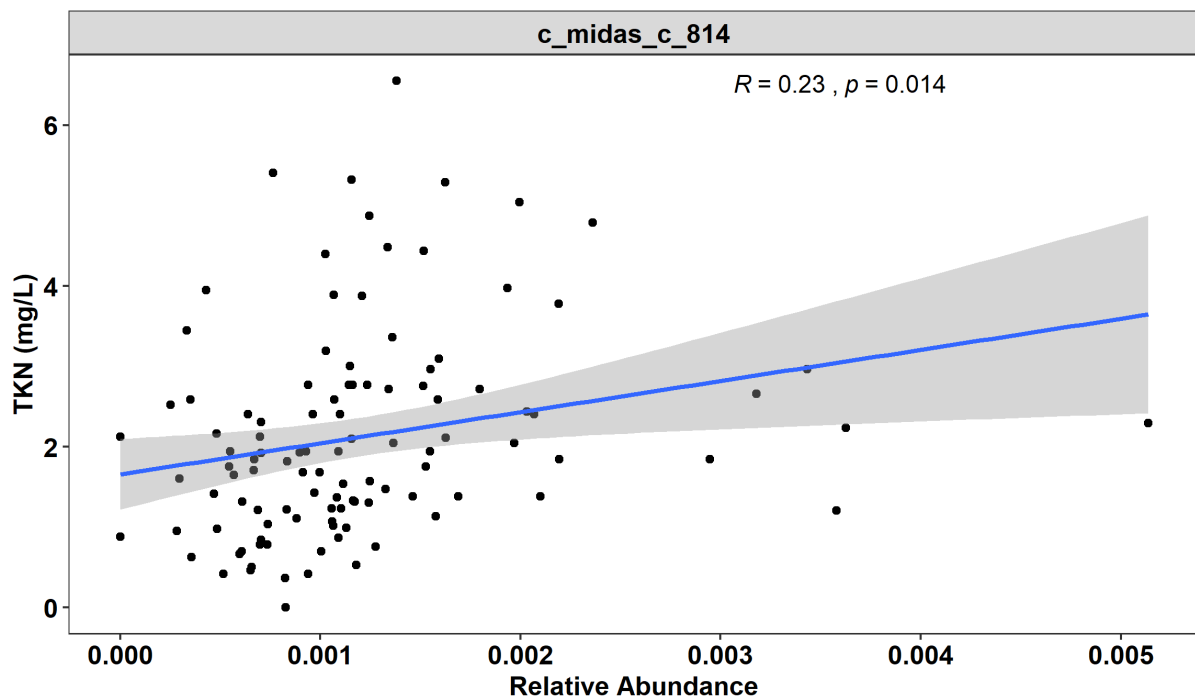
Figure S4. 14: Scatter plots showing the correlation between microbial classes and total Kjeldahl nitrogen (TKN)
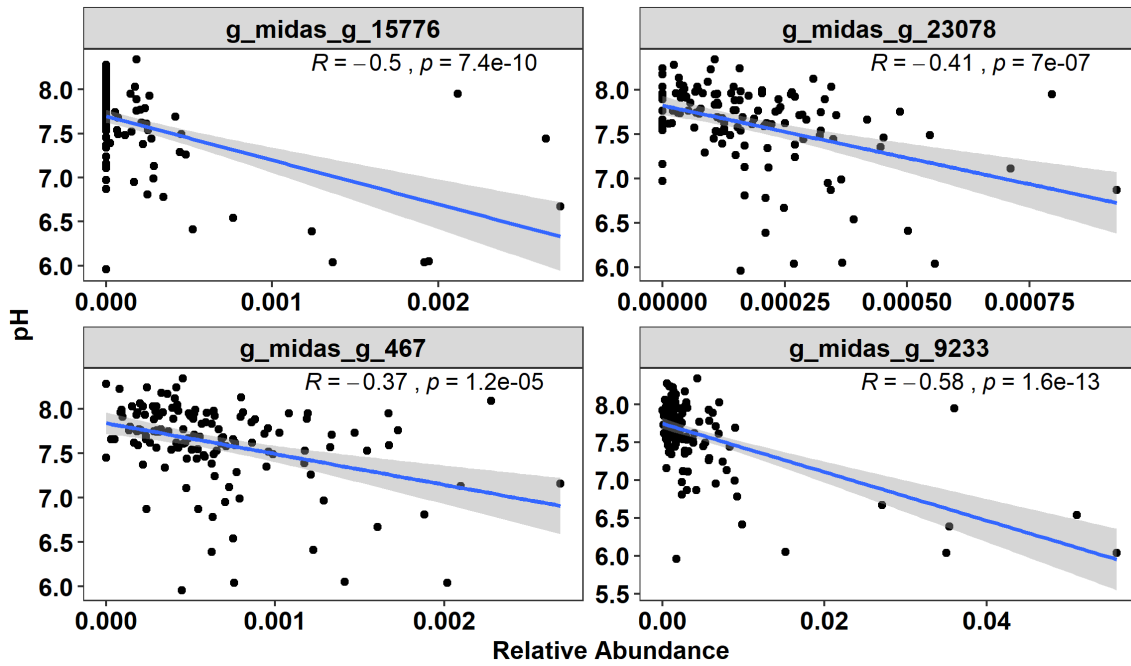
Figure S4. 15: Scatter plots showing the correlation between top four microorganisms and pH at the genera level.
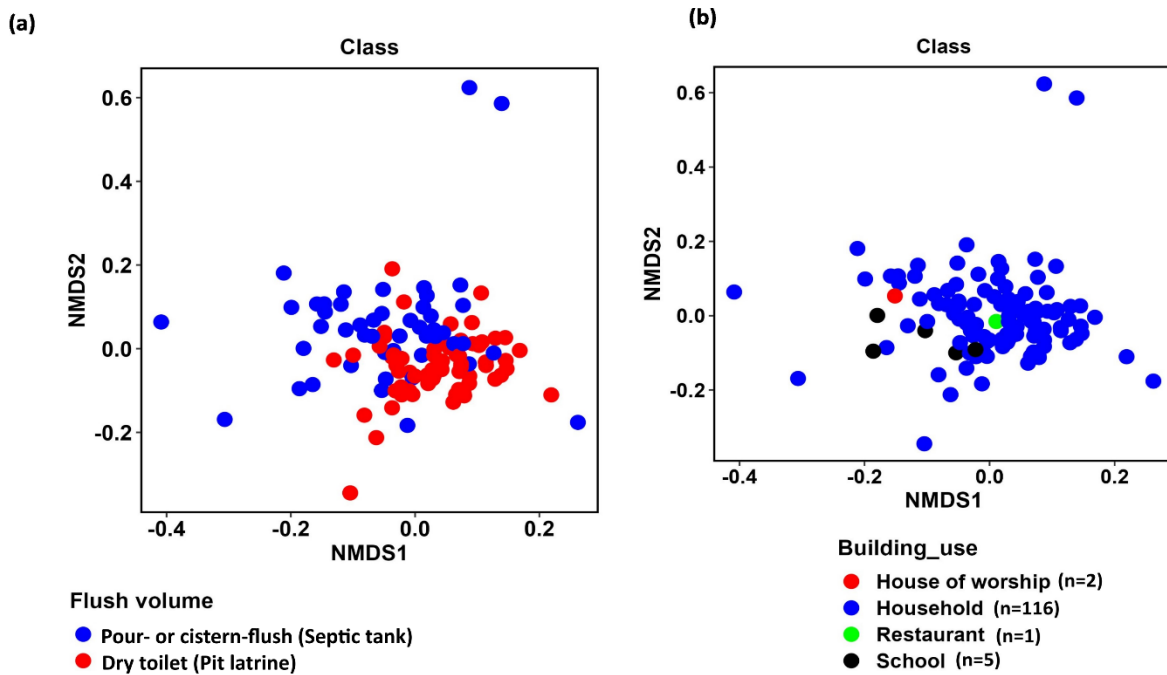
Figure S4. 16: Comparison of microbial community structure among different building uses and flush volume (water usage) at the class level. Building use is categorized into house of worship, households, restaurants and schools. The main types of containment are septic tanks and pit latrines
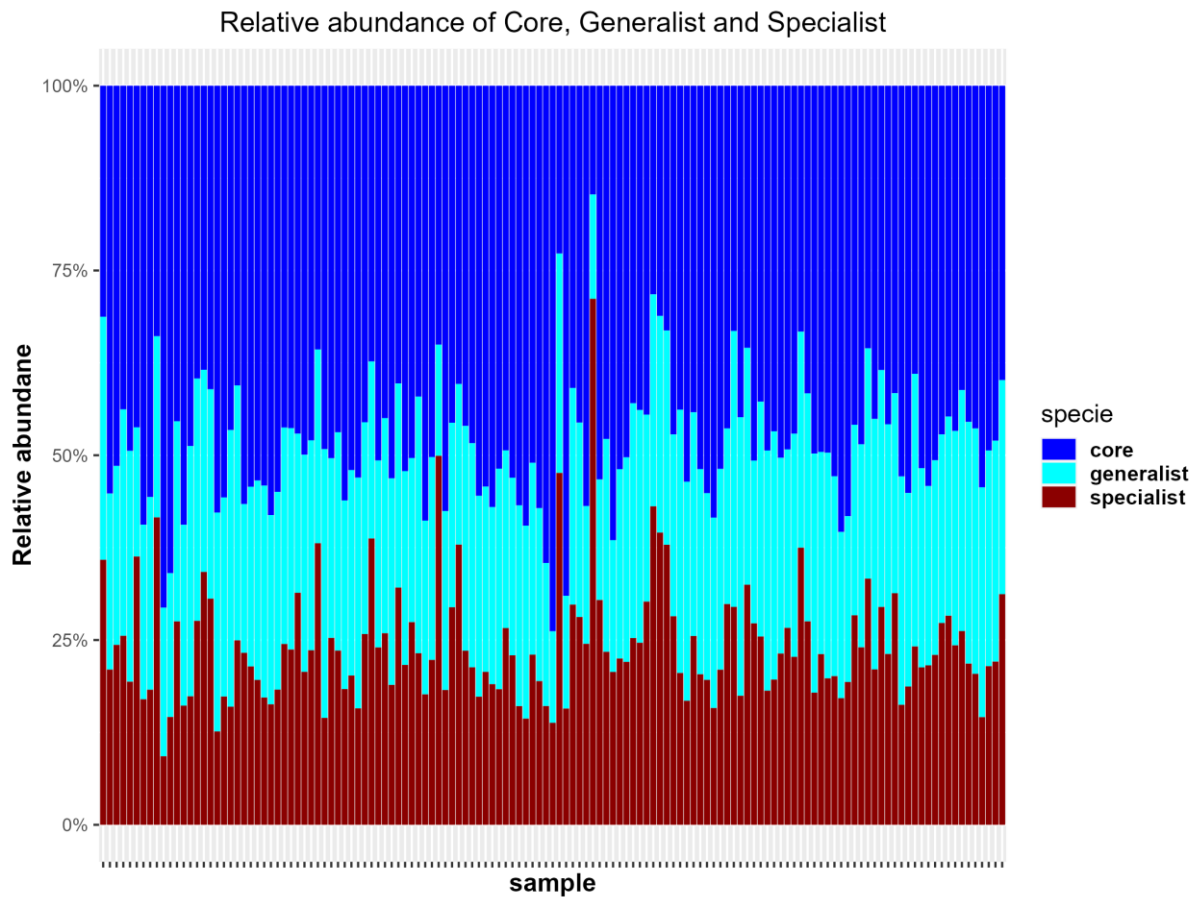
Figure S4. 17: Relative abundance plots of the core, generalist and specialist groups. The core: has ASV in 90% of samples, the generalists 89% - 60% of the samples and the specialist <60% of samples
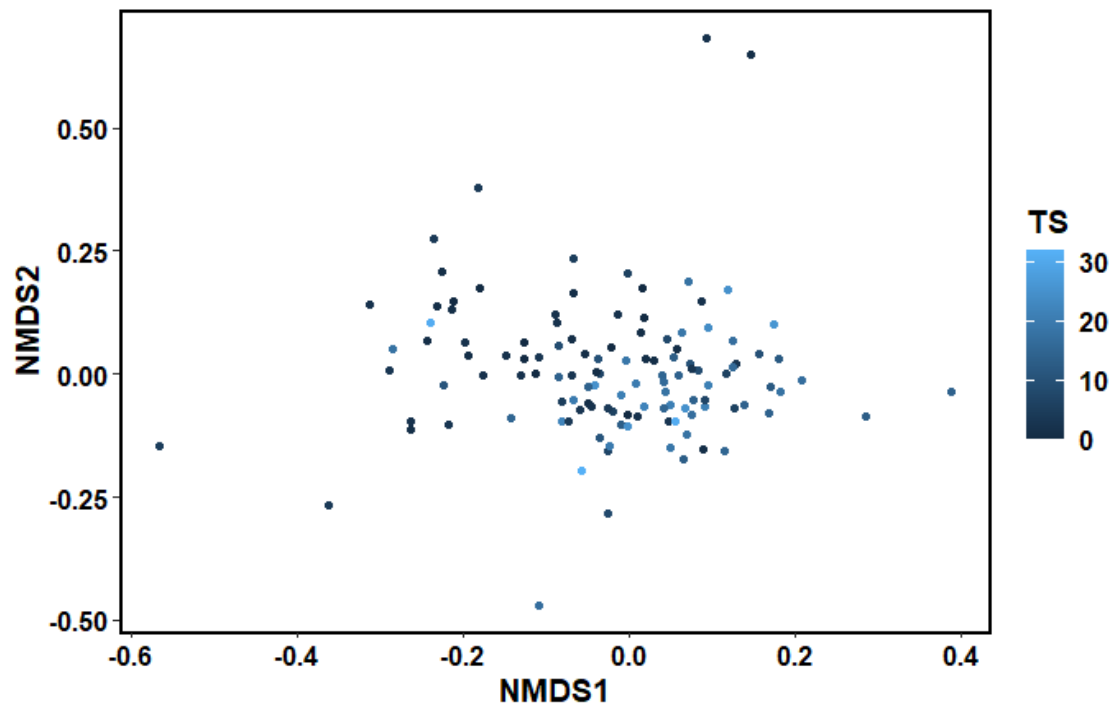
Figure S4. 18: Comparison of microbial structure with respect total solids concentrations at the species level

Table S4: 1 Summary of characteristics of FS samples

		Fecal sludge Characteristics				Metrics of stabilization					Metrics of dewatering	
All data (n=135)		pH	TOC (g/L)	nh4_mg (mg/L)	TKN (g/L)	CN	VS/TS	Bulk_H (Â°)	Bulk_S (%)	Bulk_V (%)	CST (s)	Turbidity (NTU)
	Range (min)	5.96	0.16	40	0	1.63	0.19	0	0	3	6	7
	Range (max.)	8.34	38.26	5984	6.552	26.15	0.97	90	81	86	2479	3848
	Median	7.71	21.23	1409	1.92	6	0.56	50	32	15	82	160.5
Septic tank (n = 61)	Range (min)	6.87	0.16	40	0	1.88	0.19	0	0	3	6	10
	Range (max.)	8.03	25.67	5115	4.872	18.59	0.97	72	78	86	694	1429
	Median	7.66	1.72	415	1.92	5.5	0.54	53	15	13	40	100
Pit latrines (n = 64)	Range (min)	5.96	0.7	136	0.36	1.63	0.26	30	6	4	8	7
	Range (max.)	8.34	38.26	5984	6.55	26.15	0.8	72	80	49	2479	3848
	Median	7.77	14.35	2843.5	1.85	6	0.63	48	50	19	543.5	618
Household (n = 116)	Range (min)	5.96	0.16	40	0	1.63	0.19	0	0	3	6	7
	Range (max.)	8.34	38.28	5895	6.552	26.15	0.97	72	80	86	2479	3848
	Median	7.73	7.32	1351	1.85	6	0.56	49.5	32.7	15	82	170.5
Non household* (n = 8)	Range (min.)	7.24	1.21	165	0.994	2.47	0.38	40	11	7	9	10
	Range (max.)	7.961	28.86	5984	3.892	9.41	0.79	72	59	24	149	140
	Median	7.69	2.39	1678	1.94	5	0.55	60	15	15	20	39

*Samples specifically came from school (n=5), restaurant (n = 1), house of worship (n =2)

Table S4: 2: Relative abundance of the 20 most abundant microbial classes belonging to the core, generalist and specialist groups in fecal sludge samples. Organisms that were not classified are referred to as "other

Core	Abundance (%)	Generalist	Abundance (%)	Specialist	Abundance (%)
Actinobacteria	0.34	Actinobacteria	0.64	Actinobacteria	0.85
Aminicenantia	0.12	Alphaproteobacteria	0.35	Alphaproteobacteria	0.76
Bacilli	4.52	Aminicenantia	0.09	Anaerolineae	0.42
Bacteroidia	4.38	Anaerolineae	0.12	Bacilli	1.94
Campylobacteria	0.32	Bacilli	1.55	Bacteroidia	4.09
Clostridia	30.83	Bacteroidia	5.67	Campylobacteria	0.32
Coriobacteriia	0.57	Cloacimonadia	0.14	Clostridia	6.64
Desulfobulbia	0.10	Clostridia	9.09	Coriobacteriia	0.16
Desulfovibrionia	0.48	Coriobacteriia	0.48	Desulfovibrionia	0.18
Fibrobacteria	0.19	Desulfomonilia	0.21	Fusobacteriia	0.14
Gammaproteobacteria	2.01	Desulfovibrionia	0.24	Gammaproteobacteria	2.11
Holophagae	0.20	Gammaproteobacteria	3.43	Methanobacteria	0.19
Methanobacteria	0.07	Holophagae	0.28	Methanomicrobia	0.19
midas_814	0.07	Methanobacteria	0.60	Negativicutes	0.21
Negativicutes	0.57	Methanosarcinia	0.10	Other	2.49
Other	0.21	Negativicutes	0.24	Spirochaetia	0.58
Spirochaetia	0.31	Other	0.97	Synergistia	0.54
Synergistia	0.81	Spirochaetia	0.68	Syntrophia	0.40
Syntrophia	0.37	Synergistia	0.85	Syntrophorhabdia	0.16
Syntrophomonadia	0.13	Syntrophia	0.19	Thermoplasmata	0.13
Verrucomicrobiae	0.58	Verrucomicrobiae	0.32	Verrucomicrobiae	0.20

Table S4: 3 Relative abundance of phyla that differ between pit latrines and septic tanks as obtained from the Wilcox test

Pit latrines	Abundance	Relative abundance (%)	Septic tanks	Abundance	Relative abundance (%)
p_Verrucomicrobiota	30711.9	1.3	p_Verrucomicrobiota	27163.7	1.2
p_Thermoplasmatota	6004.8	0.3	p_Thermoplasmatota	3702.7	0.2
p_Synergistota	52966.8	2.2	p_Synergistota	50847.8	2.2
p_Spirochaetota	35558.5	1.5	p_Spirochaetota	45137.5	2.0
p_SAR324_cladeMarine_group_B	4423.8	0.2	p_SAR324_cladeMarine_group_B	3123.2	0.1
p_Proteobacteria	205281.2	8.6	p_Proteobacteria	200057.9	8.7
p_Planctomycetota	5830.1	0.2	p_Planctomycetota	6433.4	0.3
p_Myxococcota	2287.7	0.1	p_Halobacterota	10019	0.4
p_Halobacterota	8609.2	0.4	p_Fusobacteriota	4721.6	0.2
p_Fusobacteriota	4005.4	0.2	p_Firmicutes	1288206.6	56.3
p_Firmicutes	1356704.1	56.5	p_Fibrobacterota	4044	0.2
p_Fibrobacterota	7854	0.3	p_Euryarchaeota	15881.9	0.7
p_Euryarchaeota	23736.5	1.0	p_Desulfobacterota	74660.9	3.3
p_Desulfobacterota	66034.2	2.8	p_Cyanobacteria	2674.8	0.1
p_Cloacimonadota	6287.9	0.3	p_Cloacimonadota	5677	0.2
p_Chloroflexi	15678.7	0.7	p_Chloroflexi	13002.4	0.6
p_Campylobacterota	12169.8	0.5	p_Campylobacterota	18547.4	0.8
p_Bacteroidota	355030.1	14.8	p_Bacteroidota	315686.2	13.8
p_Actinobacteriota	101592.6	4.2	p_Actinobacteriota	95356.7	4.2
p_Acidobacteriota	27261.3	1.1	p_Acidobacteriota	24281.9	1.1
NA	63727.7	2.7	NA	68846.7	3.0
Other	9181.7	0.4	Other	10298.7	0.5

Table S4: 4 Relative abundance of classes that are different between types of containment

Pit Latrine	Abundance	Relative abundance (%)	Septic tanks	Abundance	Relative abundance (%)
c_Actinobacteria	46610.8	1.9	c_Actinobacteria	39908.1	1.7
c_Alphaproteobacteria	23905.3	1.0	c_Alphaproteobacteria	29012	1.3
c_Anaerolineae	14749.2	0.6	c_Aminicenantia	7135.7	0.3
c_Bacilli	166704.2	6.9	c_Anaerolineae	11985.4	0.5
c_Bacteroidia	353593.9	14.7	c_Bacilli	208509.6	9.1
c_Campylobacteria	12169.8	0.5	c_Bacteroidia	314294.1	13.7
c_Cloacimonadia	6287.9	0.3	c_Campylobacteria	18547.4	0.8
c_Clostridia	1135820	47.3	c_Clostridia	1043371	45.6
c_Coriobacteriia	33695.5	1.4	c_Coriobacteriia	23725.6	1.0
c_Desulfovibrionia	23297.4	1.0	c_Desulfomonilia	7842.4	0.3
c_Fibrobacteria	7719.3	0.3	c_Desulfovibrionia	18684.7	0.8
c_Gammaproteobacteria	180860.3	7.5	c_Gammaproteobacteria	170452.8	7.4
c_Holophagae	15095.1	0.6	c_Holophagae	12092.6	0.5
c_Methanobacteria	23486.1	1.0	c_Methanobacteria	15474.7	0.7
c_Negativicutes	30457.4	1.3	c_Methanomicrobia	5899.2	0.3
c_Spirochaetia	33323.2	1.4	c_Negativicutes	16573	0.7
c_Synergistia	52966.8	2.2	c_Spirochaetia	40256	1.8
c_Syntrophia	18167.2	0.8	c_Synergistia	50847.8	2.2
c_Syntrophomonadia	7169.2	0.3	c_Syntrophia	27164.8	1.2
c_Verrucomicrobiae	28862.1	1.2	c_Verrucomicrobiae	23581.7	1.0
NA	88717.7	3.7	NA	103780.7	4.5
Other	97280	4.1	Other	99232.7	4.3

Table S4: 5 Relative abundance of genera that are different between types of containment

Pit latrines	Abundance	Relative abundance (%)	Septic tanks	Abundance	Relative abundance (%)
g_Bacteroides	47199.7	2.0	g_Acholeplasma	19272.8	0.8
g_Blautia	68342.5	2.8	g_Arcobacter	13152.8	0.6
g_Christensenellaceae_R-7_group	38959	1.6	g_Bacteroides	54858.7	2.4
g_Clostridium_sensu_stricto_1	135875.5	5.7	g_Blautia	48840.9	2.1
g_Coprococcus	16541.7	0.7	g_CAG-352	17002.1	0.7
g_Eggerthella	18686.9	0.8	g_Christensenellaceae_R-7_group	62926.6	2.7
g_Fusicatenibacter	20678.2	0.9	g_Clostridium_sensu_stricto_1	207659.6	9.1
g_Intestinibacter	65150.1	2.7	g_Erysipelotrichaceae_UCG-003	14622.8	0.6
g_midas_g_249	16744.8	0.7	g_Eubacterium_ruminantium_group	13012.2	0.6
g_midas_g_9233	18670.8	0.8	g_Intestinibacter	16964.9	0.7
g_Parabacteroides	16403.2	0.7	g_midas_g_4579	23875.1	1.0
g_Proteiniphilum	22200.6	0.9	g_Peptostreptococcus	20254.1	0.9
g_Romboutsia	42067.3	1.8	g_Romboutsia	70999	3.1
g_Ruminococcus	52510.6	2.2	g_Ruminococcus	66589.3	2.9
g_Sarcina	15751.2	0.7	g_Smithella	14343.4	0.6
g_Subdoligranulum	160722.6	6.7	g_Subdoligranulum	64509.9	2.8
g_Terrisporobacter	17667.4	0.7	g_Terrisporobacter	42859.5	1.9
g_Thiopseudomonas	22499.9	0.9	g_Thiopseudomonas	14698	0.6
g_Turicibacter	42436.9	1.8	g_Turicibacter	78775.2	3.4
g_UCG-002	22144	0.9	g_UCG-002	15354.7	0.7
NA	542469	22.6	NA	513040	22.4
Other	997216.1	41.5	Other	894760.4	39.1

Table S4: 6 Five most correlated organisms to fecal sludge characteristics (pH, NH4-N, CST, and CN)

Parameter	Class	R	P-value	Genus	R	P-value
pH	c_Desulfovibrionia	-0.4135	0.0001	g_midas_g_15776	-0.4192	0.0004
	c_Methanosarcinia	-0.3583	0.0010	g_midas_g_23078	-0.3987	0.0005
	c_Aminicenantia	-0.3289	0.0034	g_midas_g_467	-0.3953	0.0005
	c_Syntrophomonadia	-0.2799	0.0265	g_midas_g_9233	-0.4031	0.0005
	c_Desulfotomaculia	-0.2468	0.0455	g_Dechloromonas	-0.3918	0.0005
				***		
HN4	c_Syntrophia	-0.4034	0.0001	g_Vitreoscilla	0.4975	0.0000
	c_Leptospirae	-0.3757	0.0004	g_Peptostreptococcus	0.4788	0.0000
	c_Thermacetogenia	0.3469	0.0013	g_Rheinheimera	0.4776	0.0000
	c_Coriobacteriia	0.3326	0.0021	g_Clostridium_sensu_stricto_9	-0.4641	0.0000
	c_KD4-96	0.3124	0.0047	g_midas_g_9199	0.4530	0.0000
CST	c_Syntrophia	-0.5250	0.0001	g_midas_g_134	-0.5039	0.0016
	c_midas_c_796	0.3603	0.0500	g_CAG-352	-0.4765	0.0033
	c_Elusimicrobia	0.3400	0.0665	g_Smithella	-0.4629	0.0044
	c_Desulfomonilia	-0.3029	0.1540	g_Paeniclostridium	-0.4024	0.0474
	c_Hydrogenedentia	0.2893	0.1540	g_Ca_Competibacter	-0.3799	0.0768
CN	c_Syntrophia	-0.2971	0.1883	g_midas_g_8364	-0.4016	0.0159
	c_midas_c_814	-0.2621	0.3219	g_DMER64	-0.3678	0.0434
	c_Desulfitobacteriia	0.2483	0.3345	g_UCG-004	-0.3502	0.0638
	c_Chthonomonadetes	0.2231	0.4337	g_Pseudarcobacter	0.3442	0.0638
	c_D8A-2	0.1940	0.4337	g_midas_g_11550	0.3336	0.0809
TKN	Class	R	P value	Genus	R	P value
	c_midas_c_814	0.3749	0.0062	g_HN-HF0106	0.3993	0.0084
	c_Campylobacteria	-0.2968	0.0906	g_midas_g_22835	0.3988	0.0084
	c_Sumerlaeia	-0.2853	0.0922	g_Moheibacter	-0.3899	0.0090
	c_BRH-c20a	0.2647	0.1421	g_midas_g_2408	0.3830	0.0096
	c_Desulfitobacteriia	-0.2400	0.1568	g_midas_g_4663	-0.3606	0.0227
TOC*	c_Desulfovibrionia	0.3580	0.0139	g_Eggerthella	0.3023	0.0939
	c_midas_c_814	0.3204	0.0358	g_Ruminococcus_torques_group	0.3053	0.0939
	c_Fusobacteriia	-0.2908	0.0566	g_CAG-352	0.3262	0.0939
	c_Sumerlaeia	-0.2948	0.0566	g_midas_g_249	0.2981	0.0939
	c_Aminicenantia	0.2671	0.0888	g_midas_g_35539	0.3368	0.0939
Turb	c_Syntrophia	-0.5818	0.0000	g_Rhodanobacter	-0.5690	0.0000
	c_Polyangia			g_Acetitomaculum	-0.5142	0.0005
	c_midas_c_796	0.3451	0.0542	g_midas_g_1658	-0.4878	0.0014
	c_Negativicutes	0.3395	0.0542	g_Ca_Sarcinithrix	-0.4750	0.0021
	c_Acidimicrobiia	-0.3255	0.0675	g_midas_g_6504	-0.4527	0.0048
VSTS	c_Bacilli	-0.2398	0.4870	g_Desulfovibrio	0.3097	0.2439
	c_Campylobacteria	-0.2720	0.4870	g_Akkermansia	0.3064	0.2439
	c_Coriobacteriia	0.2234	0.4870	g_IMCC26207	-0.3263	0.2439

| | c_Fibrobacteria | 0.2306 | 0.4870 | g_Ruminococcus | 0.3296 | 0.2439 |
| | c_midas_c_18 | -0.2256 | 0.4870 | g_Leuconostoc | 0.3076 | 0.2439 |

Table S4: 7 ANOSIM statistical significance for organisms that differ between fecal sludge parameters

Parameter	R	Sig
Origin of sample (building use)	-0.09	0.8021
Time since last emptied	R: -0.07	0.8776
Type of containment	0.355	1e-04
pH	0.048	0.278
TOC	0.026	0.2110
TKN	0.233	0.1822
NH4_N	0.3304	0.1639

Table S4: 8 P-values of Kruskal Wallis test showing statistical significance differences between fecal sludge characteristics

	TS	TOC	TKN	NH4_N
TS	na	0.48	0.64	0.44
TOC	0.48	na	0.58	0.47
TKN	0.64	0.58	na	0.49
NH4_N	0.44	0.47	0.49	na

Curriculum Vitae

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EDUCATION

May 2018 -Aug 2023	**Ph.D. Civil and Environmental and Geomatic Engineering** ETH Zürich, Zürich, Switzerland
Sept 2012 – Oct 2014	**M.Sc. Environmental Biotechnology** Istanbul Technical University, Istanbul, Turkey
Jan 2004 – Jul 2008	**B.Sc. Biochemistry and Biotechnology** Kwame Nkrumah University of Science and Technology, Kumasi Ghana

EXPERIENCE

2018- Present	**PhD Researcher** studying fecal sludge dewatering and settling Management of Excreta, Wastewater, and Sludge Research Group Department of Sanitation, Water, and Solid Waste for Development (Sandec) Swiss Federal Institute of Aquatic Science and Technology (Eawag) Dübendorf, Switzerland
2014 – 2018	**Project Manager** Quality Environmental Consultancy – Adamus Resources and Asanko Gold mine Ghana
2010 – 2011	**Quality Control Officer** Ayrton Drug manufacturing limited Accra, Ghana
2009 – 2010	**Quality Assurance Officer** Blue skies product Ghana limited Accra, Ghana
2008 – 2009	**Production Supervisor** Blue skies product Ghana limited Accra, Ghana

TEACHING EXPERIENCE

EPFL. Lausanne, Switzerland
Guest lecturer, Sanitary engineering in developing countries EPFL course Env. 402 in SIE department ENAC faculty, October 2019.

Guest lecturer, Sanitary engineering in developing countries EPFL course Env. 402 in SIE department ENAC faculty, October 2020 (Virtual lecture)

Guest lecturer, Sanitary engineering in developing countries EPFL course Env. 402 in SIE department ENAC faculty, October 2021

Guest lecturer, Sanitary engineering in developing countries EPFL course Env. 402 in SIE department ENAC faculty, October 2022

ETH Zurich, Switzerland
Guest lecturer, Sanitation systems and technologies, ETH course 102-0838-00L, Sanitary Engineering in Developing Countries in D-BAUG department, April 2019.

Guest lecturer, Lecturer, Sanitation systems and technologies, ETH course 102-0838-00L, Sanitary Engineering in Developing Countries in D-BAUG department April 2023

Consultant Capacity Development (ConCaD) for Citywide Inclusive Urban Sanitation **(**training)
Guest lecturer, Impact of piped water supply on sanitation, Inclusive urban sanitation- capacity development consultants (YouTube video).

PEER-REVIEWED SCIENTIFIC PUBLICATIONS
BOOK CONTRIBUTIONS
Velkushanova, K., Reddy, M., Zikalala, T., Gumbi, B., Archer, C., Ward, B.J., Andriessen, N., ***Sam, S.B***, Strande, L., Chapter 8. Laboratory procedures and methods for characterization of fecal sludge, Methods for Fecal Sludge Analysis. ISBN: 9781780409122 eBook: 9781780409122 IWA Publishing, London, 2021. (Author)
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PRESENTATIONS AND CONFERENCE PROCEEDINGS

Sam, S.B., Ward, B.J., Andriessen, N., Vogel, M., Strande L., Dewatering of fecal sludge: Missing link for safely managed Sanitation. IWA Congress and Exhibition, Copenhagen, September 2022

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Sam, S.B., Ward, B.J., Morgenroth, E., Strande, L. Water Hub @ NEST: Blackwater. Eawag Symposium Dubendorf, Switzerland, Poster Presentation, 2019. First place, best student presentation.

Ward, B.J., ***Sam S.B.,*** Morgenroth, E., Strande, L. Progress in Fecal Sludge Dewatering: Evaluating a conceptual model and predictors of dewatering performance. Fecal Sludge Management – FSM5, Cape Town, South Africa. Podium Presentation, 2019

Sam, S.B., Dulekgurgen, E., Potential of wastewater treatment systems as Bioresource, biopolymers of floccular and aerobic granular activated sludge. 1st International Conference on Bioresource Technology for Bioenergy, Bioproducts and Environmental Sustainability. Barcelona, Spain October 2016

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Sam, S.B., Dulekgurgen, E., Characterization of Exopolysaccharides from Floccular and Aerobic Granular Activated Sludge as Alginate-Like-ExoPS. International Conference on recycling and reuse, Istanbul, Turkey   June 2014

AWARDS AND RECOGNITIONS

2019	Best Student Presentation 2019 Eawag Symposium
2018	Swiss National Science Foundation doctoral fellowship
2014	Best Graduating international student - Turkish Ministry of Education Awards
2012	3-year full academic scholarship to Istanbul Technical University, Turkey
2008	Dean's Honor Award for First class students (KNUST-Ghana)