



Dewaterability of thermophilic digestate: Characteristics and underlying processes

A heuristic comparison of mesophilic digestion, thermophilic digestion and digestate ammonia stripping on the site of Bath WWTP, Netherlands

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By

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Abstract

In a WWTP operating with full scale mesophilic digestion, a thermophilic digestion pilot and a digestate ammonia stripping pilot were installed. Benefits of the aforementioned sludge treatment processes, such as energy and nutrient recovery, are extensively recognized. However, drawbacks, such as deficient sludge dewatering, are amply disregarded despite their substantial influence on the treatment scheme and treatment cost. Consequently, the installations research resolved to determining the causality of the unsatisfactory dewaterability. The digester feed, mesophilic digestate, thermophilic digestate and ammonia stripped thermophilic digestate were compared. Harsher sludge treatments led to a severe deterioration in dewaterability. An increase in presence of colloids resulted in a worsening of dewaterability, to an extent originating from size and surface charge of colloids; unlike variations in presence and size of supracolloids, which were inconsequential. Moreover, inferior dewaterability was strongly associated with the increased concentrations of carbohydrates, proteins and humics in the bulk and loosely bound phases, and with the decreased presence of proteins in the tightly bound phase. Furthermore, the decreased calcium and increased iron presences in the bulk and tightly bound phases were correlated to a degradation in dewaterability; whilst sodium, potassium, ammonium and magnesium in all phases were found of no or insignificant influence. Recognizing the dewatering determinant factors enables the search for digestate specific conditioning and dewatering methods.

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Chapter 1 Introduction

1.1. Research incentives

Excess sludge poses a serious threat to both human health and environmental protection (Wei et al., 2009) due to the hazardous nature of part of the constituents: pathogenic microorganisms, dangerous organic and inorganic compounds, heavy metals and micropollutants (Serrano et al., 2016). Moreover, surplus sludge exhibits an excessive cost of handling and disposal (Wei, 2009; Serrano, 2016). As a result, the digestion process aims at diminishing organic, pathogenic and solids content (Park et al., 2006). To further decrease the volume of waste to be disposed (Suhartini, 2014; Houghton, 2000), the resulting digestate slurry is routinely physically or chemically conditioned (Qi et al., 2011), and dewatered through centrifuges, belt presses or filter presses (Abu-Orf et al., 2004).

Anaerobic digestion of primary and secondary sludges converts organic matter to CO₂ and CH₄ through bacterial hydrolysis, acidogenesis, acetogenesis and methanogenesis processes (Houghton, 2000; Tchobanoglous, 2014). Anaerobic digesters are typically operated, as a function of the digestion temperature and the ensuing bacterial colonies and biochemical processes, with one of the two approaches: mesophilic (30- 38°C) or thermophilic (50- 60°C) digestion (Buhr & Andrews, 1977). Whilst mesophilic fermentation is a common, efficient and reliable process; thermophilic stabilization gives compelling assertions of an enhanced digestion: boosted hydrolysis rate, improved volatile solids and pathogens removal, elevated organic loading rate, diminished solids retention time, lowered reactor volume, enhanced biogas production and reduced digestate production (Coelho, 2011; Suhartini, 2014; Buhr, 1977).

Furthermore, thermophilic digestion results in a higher ammonium concentration in the digestate (Coelho, 2011; Suhartini, 2014), improving the prospect for an effective ammonia recovery air stripping process (Gustin, 2011; Bonmati, 2003; Walker, 2011; Laurenzi, 2013). By ensuring a 9-11 pH and a 30-80°C temperature in the stripping tower (Gustin, 2011; Bonmati, 2003), the equilibrium shifts from ammonium to ammonia (Gustin, 2011; Walker, 2011). An air flow both: removes CO₂ from the digestate, thus increasing the pH and decreasing the base addition requirement (Liu et al., 2015); and increases the interaction surface and vapor pressure, thus removing the ammonia gas from the column (Walker et al., 2011). The resulting gaseous mix passes an acid trap, where ammonia is retained (Swartz et al., 1998). Depending on the positioning of the ammonia stripper unit, the process can either: help prevent NH₃ toxicity in the anaerobic digester which encumbers hydrolysis and methanogenesis, for pre- digester strippers (De la Rubia et al., 2010); or lower NH₄⁺ digestate content in order to attain disposal regulatory requirements, for post- digester strippers (Liao et al., 1995). Moreover, for both types, the resulting ammonium salt is a marketable and valuable resource in agriculture and industry (Laurenzi, 2013; Lei, 2007; Matassa, 2015).

Bath WWTP, under the patronage of Waterschap Brabant Delta RWZI, currently stabilizes primary and secondary sludges through full scale mesophilic digestion. However, a focus on energy and nutrient recovery, as well as the other benefits of thermophilic digestion and thermophilic digestate ammonia recovery, prompted a research initiative on the practicality of the two processes. For this purpose, a pilot scale thermophilic digester and a pilot scale thermophilic digestate ammonia stripper were installed on site. The scheme was part of the research program “Programma Demonstratie Energie-Innovatie” (“DEI”), in collaboration with Colsen BV and STOWA. Various entities and individuals contributed to the project through diverse investigations.

Based on previous experiences, Waterschap Brabant Delta RWZI anticipated a deficient dewatering behavior of the thermophilic digestate and the ammonia stripped thermophilic digestate, by comparison with the mesophilic digestate. Reducing the costs of treatment and disposal of the slurries, by establishing efficacious methods of conditioning and dewatering, was deemed essential in determining the practicality of the processes. A knowledge deficit on the causes of inferior dewaterability of digestates hindered the search for solutions, although it originated the present research.

The focus of this study was centred on understanding the sludge characteristics and underlying processes that result in the contrasting dewaterability behaviours of mesophilic and thermophilic digestate, in order for Waterschap Brabant Delta RWZI to translate the findings into efficient practical conditioning and dewatering methods, through their own research. A peripheral scope was to include the ammonia stripped thermophilic digestate into the investigation.

1.2. Research questions

A series of factors were considered to be potentially detrimental to the dewaterability of mesophilic, thermophilic and ammonia stripped thermophilic digestates. The possibly problematic parameters stemmed both the research questions and the research approach, in the search for the causes of inferior dewatering characteristics of the aforementioned sludges.

This work attempted to answer the following enquiries:

- What is the influence of mesophilic digestion, thermophilic digestion and thermophilic digestate ammonia stripping on the dewaterability?
- Can dewaterability be associated to variations in particle size or charge?
- Can a relation be found between dewaterability and changes in the presence of main extracellular polymeric substances (carbohydrates, proteins and humics) in the different sludge phases (bulk, loosely bound and tightly bound sludge)?
 - Is there a correlation between dewaterability and differences in the concentrations of common cations (ammonium, potassium, sodium, calcium, magnesium, iron and aluminium) in the separate sludge phases (bulk, loosely bound and tightly bound sludge)?

1.3. Research background

1.3.1. Introduction

Sludge suspensions encompass substantial amounts of free and bound water (Coelho et al., 2011), with the latter occurring as interstitial water, vicinal water and hydration water, within the floc, particle or cell (Vesilind, 1994). Whilst free and interstitial water can be removed gravitationally or mechanically, respectively; vicinal and hydration water can be eliminated solely through drying at median or high temperatures, respectively (Vesilind, 1994).

Within the sludge flocs, a preponderant proportion of water is linked with the extracellular polymeric substances network (“EPS”) through hydrogen bonds (water molecules to EPS hydroxyl groups) and electrostatic interactions (water permanent dipole to EPS functional groups) (Coelho, 2011; Neyens, 2004). Furthermore, EPS creates physical entanglement ties and chemical electrostatic bonds with the microbial cells, and absorbs wastewater inorganic and organic compounds, forming a highly hydrated heterogenous matrix, the sludge floc (Morgan, 1990; Wingender, 1999; Wilen, 2003). Moreover, EPS protects cells from harmful compounds or conditions, maintains cells hydrated and represents for cells an alternative source of energy in nutrient shortages (Wingender, 1999; Sutherland, 2001).

EPS can be divided into solution soluble and floc bound EPS (Nielsen & Jahn, 1999), with the latter being subdivided into a dispersible outer layer called loosely bound EPS (“LB-EPS”), a robust inner layer called tightly bound EPS (“TB-EPS”) and a pellet EPS (Li & Yang, 2007). The LB-EPS is present in a lower amount compared to the TB- EPS, and is considered to be a diffusion of the TB- EPS (Li & Yang, 2007).

The EPS is the result of cell lysis, metabolic excretion and absorption of wastewater constituents (Urbain et al., 1993), and is composed of various high molecular weight polymers (Morgan, 1990; Wingender, 1999). In sludges, proteins are typically predominant, succeeded by humics, and followed in descending order of concentrations by polysaccharides, lipids, nucleic acids and others (Frolund 1996; Dignac, 1998; Mikkelsen, 2002). The ratio between different biopolymers, in sludge floc and solution, is relatively constant (Urbain et al., 1993).

In addition, EPS presents numerous charged groups such as: carboxyl, phosphoric, sulfhydryl, phenolic, and hydroxyl (Leis & Flemming, 2002). The negatively charged EPS fractions are predominant (Urbain et al., 1993) and are attached through cation induced ion bridging or double layer compression (Sheng et al., 2010). The most commonly present cations in sludge floc are Ca^{2+} , Fe^{3+} and Al^{3+} equally, and are followed by K^+ , Na^+ and Mg^{2+} (Wilén et al., 2003). However, the concurrent occurrences of other interactions, as polymer bridging, steric interactions and hydrophobic interactions, are plausible (Wilén, 2003; Li, 2012).

1.3.2. Influence of extracellular polymeric substances on sludge dewaterability

Extracellular polymeric substances and dewaterability

Due to their major contribution to the composition of the sludge floc with approximately 20-25% of TS and 33-42% of VS (Frolund, 1996; Wilén, 2003), as well as due to their outer positioning in the sludge floc, floc EPS and its components exert direct influence on the sludge floc surface and interior properties (Morgan, 1990; Sheng, 2010).

As it participates in the floc formation process, the presence of EPS in a certain amount is favourable to dewatering (Li, 2007; Houghton, 2002). However, surpassing that quantity results in unfavourable dewaterability, increased negative surface charge and lower hydrophobicity (Houghton, 2002; Wilén, 2003; Mikkelsen, 2002).

A particular phase of EPS, namely the LB-EPS, appears to significantly negatively impact floc formation and dewatering behaviour (Li & Yang, 2007), as it is the main interaction surface of the sludge floc, due to its outer position, and contains a substantial amount of bound water, due to its low density structure (Li, 2007; Yang, 2009).

Proteins and dewaterability

Proteins are the primary biopolymer in soluble and floc EPS phases (Wilén et al., 2003). Overall, proteins are negatively charged due to their negative carboxyl groups content (Sobeck & Higgins, 2002) and despite their positive amino groups composition (Shin et al., 2001). Consequently, their presence in floc results in a negative surface charge of sludge flocs (Jin, 2004; Dignac, 1998).

Additionally, floc proteins are reportedly capable of storing high quantities of bound water and, as a result, sludges display inferior dewaterability behaviour in their presence (Jin, 2004; Cetin, 2004; Yu, 2008). Noticeably, the supernatant and LB- EPS proteins contribute the most to the poor liquid solid separation in spite of their low concentrations, compared to the TB- EPS and pellet proteins (Yu et al., 2008).

Humics and dewaterability

Humics contribute strongly to the EPS composition as the secondary biopolymer in soluble and floc EPS phases (Wilén et al., 2003). Humics are not formed or degraded in the sludge in typical conditions (Nielsen et al., 1996), and enter the floc matrix by absorption from the wastewater due to an elevated binding capability with the floc (Urbain, 1993; Esparza-Soto, 2003). For a particular sludge, varying concentrations of humics indicate a release due to loss of Ca^{2+} or Fe^{3+} as binders (Gjessing, 1976).

No correlations are found between floc humics content and floc surface charge, bound water or dewaterability (Jin et al., 2004).

Carbohydrates and dewaterability

Carbohydrates are the tertiary biopolymer in soluble and floc EPS phases (Wilén et al., 2003) and appear preponderantly in the TB-EPS and pellet, compared to the supernatant and LB- EPS (Yu et al., 2008).

Due to the presence of carboxylic and phosphate groups in carbohydrates (Sobeck, 2002; Shin, 2001), an increased concentration of floc carbohydrates leads to a more negative surface charge of the floc (Liao, 2001; Jin, 2004). However, reports conflict over the role of carbohydrates in dewaterability. According to various papers, carbohydrates improve (Cetin & Erdinçler, 2004), deteriorate (Jin et al., 2004) or do not affect dewaterability (Yu et al., 2008).

1.3.3. Influence of cations on sludge dewaterability

Cations and dewaterability

Due to their key role in bridging the EPS fractions and, thus, in forming the floc matrix with the entrapped cells, particles, and organic and inorganic substances (Urbain et al., 1993), the impact of the presence of cations on the sludge floc characteristics is significant.

Cationic bonds favour negative and hydrophilic biopolymer fractions (Wilén, 2003; Urbain, 1993), with a major affinity for proteins and a minor affinity for carbohydrates (Dignac et al., 1998), and, thus, strengthen the floc matrix and improve dewaterability. Cation binding in the LB-EPS phase results in the most significant dewaterability enhancement (Li, 2005).

Notable is the capacity of cations to perform ion exchange processes or to fill unsatisfied valences in the floc network: Na^+ can replace Ca^{2+} and Mg^{2+} (Higgins & Novak, 1997), Fe^{3+} can replace Mg^{2+} , Ca^{2+} , K^+ and Na^+ (Li, 2005), and Ca^{2+} can fill unsatisfied negative charges resulting from Fe^{3+} reduction and removal from floc (Nielsen & Keiding, 1998).

Monovalent cations and dewaterability

The presence of monovalent cations in sludge exhibits an insignificant or detrimental impact. Addition of Na^+ or K^+ leads to floc breakage and to dewaterability deterioration, evaluated through: solubilization of biopolymers, and increase in sludge volume index, capillary suction time and sludge resistance to filtration (Higgins, 1997; Sobek, 2002).

Divalent cations and dewaterability

Ca^{2+} and Mg^{2+} display strong associations with proteins and no correlations with carbohydrates (Urbain, 1993; Higgins, 1997). At addition, Ca^{2+} distributes preponderantly to the bulk sludge, with much smaller quantities reaching the other floc phases (Li et al., 2012). This reflects as an insignificant decrease in bulk EPS, LB-EPS and TB-EPS fractions and a negligible increase of pellet EPS (Li et al., 2012).

Accordingly, the presence of Ca^{2+} and Mg^{2+} in the sludge, leads to an amelioration in the sludge dewaterability indicators, measured as improved: sludge volume index, cake solids, optimum polymer dose, capillary suction time and sludge resistance to filtration (Jin, 2004; Higgins, 1997; Sobek, 2002; Li, 2012).

The divalent cation addition decreases floc surface charge, but solely in the outer floc layer (Wilén et al., 2003).

Trivalent cations and dewaterability

Fe^{3+} displays a powerful binding capability to proteins and, to a lesser extent, to carbohydrates (Li, 2005). Furthermore, Fe^{3+} and Al^{3+} substantially shift biopolymers from bulk EPS, LB-EPS and, to a lower degree, TB-EPS to pellet EPS (Li, 2005).

As a result, increased concentrations of Fe^{3+} and Al^{3+} in sludge lead to an important enhancement in dewaterability behaviour (Jin, 2004; Li, 2012; Li, 2005). Oppositely, Fe^{3+} removal prompts deflocculation and release of cells, particles and biopolymers (Nielsen & Keiding, 1998).

Opinions are divided regarding the influence of Fe^{3+} presence on floc surface charge, with some researchers claiming no impact (Li, 2005; Nielsen, 1998) and other scientists declaring a reduction (Wilén et al., 2003) or neutralization of surface charge (Li et al., 2012).

1.3.4. Influence of other slurry characteristics on sludge dewaterability

Aside the biopolymeric and cationic network, other elements influence the dewaterability behaviour of sludges.

Sludge suspensions are colloidal and supracolloidal systems, which through the overall negative charge of the flocs and particles, the low settling velocity of flocs and particles, the broad particle size range of the particles and the high compressibility of biosolids, are rendered difficult to separate from the liquid phase (Sobeck, 2002; Qi, 2011; Karr, 1978; Wilen, 2003). The two latter characteristics are of direct impact on mechanical dewatering: a broad particle size range results in migration of fines in the sludge skeleton and obstruction of pores and a high compressibility of sludges under pressure leads to floc deformation and pore closure (Qi et al., 2011).

1.3.5. Dewaterability of anaerobic digestate

Properties of anaerobic digestate

Changes in sludge characteristics ensuing fermentation in batch and continuous systems and at distinct temperatures and retention times, are presented in Table 1 and are discussed further, in order to obtain an overview of the effects of the anaerobic digestion process.

The anaerobic sludge displays similarities to the primary or secondary excess sludge: a TB-EPS phase quantitatively majoritarian compared to the LB-EPS phase (Ye et al., 2014); a protein fraction predominant in both the LB-EPS and the TB-EPS compared to carbohydrates (Fang, 1996; Sponza, 2002); and a Ca^{2+} cation preponderant compared to Na^+ , K^+ , Mg^{2+} , Fe^{3+} and Al^{3+} (Fang, 1996; Morgan, 1990).

However, during anaerobic digestion, sludge dewaterability deteriorates compared to the excess sludge feed, as denoted by the increased: capillary suction time, sludge resistance to filtration, optimum polymer dose and turbidity. Thus, it is of interest to find the dissimilarities between the inflow and outflow of an anaerobic digester, as they could be the causes of the inferior dewaterability of anaerobic digestate.

Firstly, proteins and humics are strongly solubilized during fermentation, whilst carbohydrates are not or in moderate amounts released to solution. Concomitantly, a decrease in floc or a shift from TB-EPS to LB-EPS of proteins and carbohydrates is noticeable. The variations in floc proteins are best correlated to poor dewaterability.

Secondly, protein digestion and cell lysis are signalled by increased concentration of soluble NH_4^+ and K^+ , respectively, whilst Na^+ displays no meaningful variation. Meanwhile, divalent (Ca^{2+} and Mg^{2+}) and trivalent (Fe^{3+}) cations are heavily released into solution.

Lastly, the quantitative increase of particles in the 0.45- 10 μm size range is powerfully associated to a worsening in anaerobic digestate dewaterability.

Properties of anaerobic mesophilic digestate versus anaerobic thermophilic digestate

Dewaterability of anaerobic thermophilic digestate is poorer than that of anaerobic mesophilic digestate, as measured by capillary suction time and turbidity (Coelho, 2011; Mikkelsen, 2002).

However, a singular study (Mikkelsen & Keiding, 2002) investigates the differences between the two sludges, although the research does not correlate the discrepancies in digestate properties to the variations in dewaterability behaviours. Thermophilic anaerobic digestion, by comparison to mesophilic anaerobic digestion, presents lower concentrations of floc biopolymers (carbohydrates, proteins and humics), and displays lower soluble proteins, higher soluble humics and similar soluble carbohydrates presence (Mikkelsen & Keiding, 2002). Moreover, the calcium and iron ions are lower in the floc of the thermophilic anaerobic digestate (Mikkelsen & Keiding, 2002). Furthermore, a lower floc surface charge, a similar average floc size and a wider floc size distribution were observed for the thermophilic anaerobic digestate (Mikkelsen & Keiding, 2002).

Reference	System type	Chemical addition	Particle surface charge "SC"	Particle size "ps"	Sludge resistance to filtration "SfF"	Capillary suction time "CST"	Optimum polymer dosage "OPP"	Turbidity "TU"	Effects						Observations
									Extracellular polymeric substances "EPS"			Cations			
								Proteins "PR"	Carbohydrates "CA"	Humics "HU"	Monovalent	Divalent	Trivalent		
Novak et al., 2003	AN batch 20°C 20d	-	-	-	++ correl. sol. EPS & PR no correl. sol. CA	-	++ correl. sol. EPS & PR + correl. sol. CA	-	↗ sol. PR	↗ sol. CA	-	↗ sol. NH4+ & K+ ≈ sol. Na+	≈ sol. Ca2+ & Mg2+	≈ sol. Fe3+, possibly pp. as FeS	≈ Pepsinase & glucoisidase
Novak, 2010	AN batch 20°C 30d	-	-	-	++ correl. 0.45µm-1.5µm PS	++ correl. 0.45µm-1.5µm PS	++ correl. 0.45µm-1.5µm PS	-	-	-	-	-	-	-	-
Wilen et al., 2000	AN batch 20°C 4h	-	-	-	-	-	-	↗	↗ sol. PR	↗ sol. CA	↗ sol. HU	-	-	-	Release of bacterial cells
Park et al., 2006	AN batch 25°C 30d	-	-	-	-	-	-	-	↗ sol. PR	≈ sol. CA	-	↗ sol. Ca2+ & Mg2+	↗ sol. Fe3+	-	-
Foladori et al., 2015	AN reactor	-	-	-	-	-	-	-	-	-	-	-	-	-	Cell death but not cell lysis
Park & Novak, 2007	AN batch 35°C 30d	-	-	-	-	-	-	-	↗ sol. PR	≈ sol. CA	-	≈ sol. Ca2+ & Mg2+	-	-	-
Bruus et al., 1993	AN storage 14d	-	-	-	-	-	-	↗	-	-	-	-	-	↗ sol. COD	-
Luo et al., 2015	AN batch 50°C 24h	-	↗ Zeta potential	-	-	↗	-	↗	-	-	-	-	-	-	-
Luo et al., 2015	AN batch 50°C 24h	Ca2+	↘ Zeta potential	↗	-	↘	-	↗	↗ sol. PR	↗ sol. CA	-	↗ Ca2+ requirement	-	↗ sol. COD	-
Nielsen et al., 1996	AN storage 20°C 12d	-	-	-	-	-	-	↗	↘ sol. PR ↘ flocc PR	↘ sol. CA ↘ flocc CA	≈ sol. HU ↗ flocc HU	-	-	-	-
Rasmussen et al., 1994	AN storage 20°C 14d	-	-	↗ 0.45-10µm fraction ≈ > 10µm fraction	↗	-	-	↗	-	-	-	↗ sol. K+ & NH4+	↗ sol. Ca2+ & Mg2+	↗ sol. Fe2+	SO42- and Fe3+ reduction & FeS precipitation
Sponza, 2002	AN reactor	-	↘ + correl. EPS PR	↘ + correl. EPS PR	-	-	-	-	↘ flocc PR	↘ flocc CA	-	↗ sol. Mg2+	-	-	-
Yang et al., 2010	AN batch 40-50°C 4h	Protease and danyliase	-	-	-	-	-	-	-	-	-	-	-	↗ sol. COD	Release of colloids
Houghton et al., 2000	AN reactor 35°C	-	-	-	-	↗	-	-	↗ flocc PR	↘ flocc CA	-	-	-	-	-
Ye et al., 2014	AN batch 20d	-	-	-	-	-	-	-	↗ LB-EPS PR ↘ TB-EPS PR	↗ LB-EPS CA ↘ TB-EPS CA	-	-	-	-	-

Table 1 Influence on anaerobic digestion on sludge characteristics

1.3.6. Extracellular polymeric substances extraction and characterization difficulties

EPS extraction difficulties

EPS extraction and characterisation exhibits severe impediments due to the lack of a standardized method and due to the drawbacks of each available approach.

An ideal EPS extraction method should fulfil several criteria: maximum extraction efficiency, minimum cell lysis, no distortion or disruption of EPS components and no interference with subsequent measurements (Frolund et al., 1996). Sheng (2010) and Park (2007) present comprehensive overviews of the typical drawbacks of the regularly employed extraction methods:

- Cation exchange resin extraction (Frolund et al., 1996) displays an extraction bias towards Ca^{2+} and Mg^{2+} bound biopolymers, predominantly lectin like proteins and carbohydrates.
- Sulphide extraction (Park & Novak, 2007) displays a disproportionate preference for Fe^{3+} and Al^{3+} bound proteins, disregarding other EPS components.
- Alkaline treatment (Brown & Lester, 1980) causes severe cell lysis.
- EDTA extraction (Fang & Jia, 1996) interferes with the ensuing proteins measurements.
- HCHO/NaOH method (Liu & Fang, 2002) interferes with the following carbohydrates analyses.

Due to the non-specific, non-interfering and non-cell disruptive approach, and despite the inferior extraction efficiency (Sheng et al., 2010), the heat extraction method of (Li & Yang, 2007) appears to be more appropriate for the characterization of sludges on which little preexisting knowledge is available. Additionally, unlike the other approaches, the method allows for a differentiation between the LB-EPS and TB-EPS (Sheng, 2010; Li, 2007).

EPS characterisation difficulties- Proteins and humics characterisation

Proteins and humics do not possess a standardized characterisation method for compound identification and quantification, causing difficulties in comparing or replicating research results.

Three methods of protein characterisation are often encountered in existing research: Kjeldahl-N method (AOAC, 1995), Bradford method (Bradford, 1976) and Lowry method (Lowry et al., 1951) according to Avella (2010). With the Kjeldahl-N method being based on an assumed nitrogen content of all proteins, and the Bradford method measuring solely macromolecular proteins (Raunkjær et al., 1994); the Lowry method appears to be an obvious choice, despite the non-linearity of the standard curve at high concentrations, the variation in chromogenicity and the interference of substances (Peterson, 1979; Box, 1983; Lowry, 1951). This is supported by studies showing that only part of solution proteins are detected by the Bradford method, unlike the Lowry method (Davis, 1988; Raunkjær, 1994).

The Lowry method, later incorporated a modification which accounts for the interference of humic substances in the protein measurement (Frolund et al., 1995), allowing the method to be more precise than all aforementioned methods (Frolund, 1995; Frolund, 1996). As an advantage, the use of the corrected Lowry method in protein determination allows for the concomitant quantification of humics concentrations. As a disadvantage, high concentration of both humics and proteins in solution give non-linear a response, whilst low concentrations of humics in solution give an overestimation of humics concentration and an underestimation of proteins concentration (Avella et al., 2010), making the choice of a measuring range difficult.

EPS characterisation difficulties- Carbohydrates characterisation

The drawbacks of the carbohydrates characterisation methods, in combination with the absence of a standardized testing, can lead to incomparable or opposite research outcomes.

The anthrone method (Dreywood, 1946) and phenol method (DuBois et al., 1956) are commonly used approaches for the determination of carbohydrates content (Raunkjær et al., 1994). However, the phenol method shows lower (Handa, 1966; Koehler, 1952) or equal variation compared to the anthrone method (Frolund et al., 1996), as anthrone does not develop the same colorimetric response to different carbohydrate subtypes (Koehler, 1952). Furthermore, the phenol method measures all oligosaccharides and polysaccharides, whilst the anthrone method quantifies methylated sugars and pentoses alone (DuBois et al., 1956).

Considering the above, Dubois's approach appears superior. However, it is important to account for the non-linearity of the calibration curves at high concentration of sugars, and for the differences in extinction coefficients of distinct sugars (DuBois et al., 1956).

1.4. Conclusion on research background

Examining the existing research, it becomes apparent that the majority of studies concerning dewaterability behaviour are focused on aerobic sludges. Surprisingly, this thoroughly explored section contains numerous contradictions, which could have stemmed from the applied methods of EPS extraction and characterisation or from the discrepancies in sludges. Notably, omissions of components (humic acids) or of sludge characteristics (the differentiation between LB-EPS and TB-EPS) are common.

Furthermore, considering the limited number of investigations on anaerobic digestate and the scarce number of studies on the differences between mesophilic and thermophilic anaerobic digestates, and considering these researches are not oriented on explaining the variations in dewaterability, the need to understand the dewaterability behaviour becomes apparent. Moreover, being a fairly new method, references to the dewaterability characteristics of ammonium stripped digestate are not found.

The reasons above further incentivised the search for a comprehensive overview on the relations between anaerobic digestate characteristics and dewaterability capabilities. Although not an ideal quantitative determination, this research aimed to qualitatively determine the components responsible for the deficient anaerobic digestate dewaterability and open the path to a more directional research in the future.

Chapter 2 Materials and methods

2.1. Samples and analyses

For the ensuing analyses the sludge specimens were collected from 4 locations within Bath WWTP, Netherlands, specifically: the pilot scale thermophilic digester influent, the full scale mesophilic digester effluent, the pilot scale thermophilic digester effluent and pilot scale thermophilic digester ammonium stripper effluent. Individual sludge types were sampled on 3 occasions at various dates (Table 2), namely:

Dates	Digester feed ("DF")	Mesophilic digestate ("MD")	Thermophilic digestate ("TD")	NH ₃ Stripped thermophilic digestate ("ASTD")
Tue 6/9/2016	✓	✓	✓	✓
Thu 15/9/2016	✓	✓	✓	✓
Wed 21/9/2016	✗	✗	✗	✓
Mon 28/11/2016	✓	✓	✓	✗

Table 2 Sampling dates

Measurements were effected in biological triplicate on single samples and for discrete parameters (Table 3) The sludge specimens were analysed for volatile solids, colloid and suspension particle size, colloid particle charge, sludge resistance to filtration, capillary suction time and extracellular polymeric substances composition. The bulk, loosely bound and tightly bound extracellular polymeric substances phases were employed for the quantification of chemical oxygen demand, biopolymers presence (proteins, carbohydrates and humics) and cations content (NH₄⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Fe³⁺, Al³⁺). Dewaterability indicators were normalized for total solids content, whilst extracellular polymeric substances components were normalized for volatile solids concentration.

Analyses on specimens	Volatile and total solids		
	Particle size		
	Particle charge		
	Sludge resistance to filtration		
	Capillary suction time		
	Bulk, loosely bound and tightly bound extracellular polymeric substances	Chemical oxygen demand	
		Biopolymers	Carbohydrates
	Cations	Proteins	
		Humics	
		NH ₄ ⁺	
		Na ⁺	
K ⁺			
Mg ²⁺			
Ca ²⁺			
Fe ³⁺			
Al ³⁺			

Table 3 Analyses

2.2. Extracellular polymeric substances extraction

2.2.1. EPS extraction method

Extracellular polymeric substances (“EPS”) were extracted using the heat extraction method of Morgan (1990) and modified by Li (2007) in order to accommodate a triple EPS extraction from the sludge mixture: a gentle extraction resulting in the bulk EPS (“Bulk- EPS”), a mild extraction resulting in the release of loosely bound EPS (“LB- EPS”), and a harsh extraction resulting in the release of tightly bound EPS (“TB- EPS”).

A quantity of 40ml of sludge suspension was phase separated through centrifugation at 4000g for 5min. The resulting centrate was collected for further analysis as Bulk- EPS. The resulting pellet was resuspended employing 12mL of 0.05% NaCl solution at room temperature, and, subsequently, rediluted to the original volume using 0.05% NaCl solution preheated at 70°C. The newly attained suspension presented an instantaneous final temperature of approximately 50°C, and was promptly vortexed at 4000g for 10 min. The resulting liquid was collected for further analysis as LB- EPS. The resulting pellet was resuspended to the original volume using 0.05% NaCl solution preheated at 60°C. The mix was maintained in a 60°C water bath for 30 min, and was, afterwards, centrifuged at 4000g for 15 min. The resulting supernatant was collected for further analysis as TB- EPS. All resulting EPS extracts were filtered over 0.45 µm polyether sulfone filter (VWR International- Radnor, USA), and refrigerated at 4°C up to the time of analysis.

2.2.2. Solids loss during EPS extraction

During the EPS extraction procedure, due to the poor and disparate settling characteristics of sludge specimens and the weak centrifugation, a variable loss of solids from the pellet appears with each sludge centrifugation and centrate collection. As multiple parameters were normalized for the volatile solids content from which the EPS was extracted, the values had to be corrected for the pellet loss. As a result, a prior measurement of percental solids loss for individual sludge types and for discrete centrifugation steps was undertaken. The obtained values (Annex 2- Table A1) were deducted from the measured solids content when reporting EPS components.

2.3. Extracellular polymeric substances characterization

2.3.1. Carbohydrates measurement and calibration

The carbohydrates (“CA”) measurement was performed according to the laboratory’s specific method of te Poele (2006) adjusted from Rosenberger (2003) and, in its turn, adapted from DuBois (1956). To a volume of 2ml of sample, 1ml of 5% phenol solution was added. The mixture was mixed immediately and allowed to react for 10 min. Following, 5ml of 97% sulphuric acid were spurted into the specimen tube and the sample was allowed to react for 10 min before being heavily mixed and allowed another 30 min of idleness at room temperature in order to develop the specific yellow- orange colour. Lastly, the mix was moved in a 4cm cuvette and absorbance at 487nm wavelength against a demiwater blank was measured employing a Genesys 10S UV-VIS Thermo Scientific spectrophotometer (Thermo Fisher-Bleiswijk, Netherlands).

The calibration curve between absorbance and carbohydrates concentration was build using glucose as standard and the correlation equation and curve (Annex 2- Table A2 and Figure A1) were used to determine the actual carbohydrates concentrations in the EPS extracts.

2.3.2. Proteins measurement and calibration

The proteins (“PR”) measurement was conducted according to the laboratory’s specific method of te Poele (2006) adjusted from Rosenberger (2003) and, in its turn, adapted from the correction of the Lowry (1951) method by Frolund (1996).

A volume of 3.5ml of a previously prepared mix of 100:1:1 of 143 mM NaOH and 270 mM Na₂CO₃, 57 mM CuSO₄ and 124 mM C₄H₄Na₂O₆ was added to a 2.5ml of sample. The mix was blended immediately and allowed to react for 10 min. Following, 0.5ml of a previously prepared mix of 2:1 Folin-Ciocalteu phenol: demiwater were added to the test tube. The blend was vortexed and allowed to develop the typical blue color for 45 min at room temperature. The sample was transferred to a 4cm cuvette and the absorbance value was measured at a 750nm wavelength against a demiwater blank utilizing a Genesys 10S UV-VIS Thermo Scientific spectrophotometer (Thermo Fisher-Bleiswijk, Netherlands).

The calibration curve between absorbance and proteins presence was build using glucose as standard and the correlation equation and curve (Annex 2- Table A3 and Figure A2) were used to determine the actual proteins concentrations in the EPS extracts.

2.3.3. Humics measurement and calibration

The humics (“HU”) measurement was conducted according to the laboratory’s specific method of te Poele (2006) adjusted from Rosenberger (2003) and, in its turn, adapted from the correction of the Lowry (1951) method by Frolund (1996).

The humics measurement methodology is analogous to the proteins measurement procedure, with a singular distinction. In the humics measurement, the demiwater substitutes the 270 mM Na₂CO₃ solution from the proteins analysis.

The calibration curve between absorbance and humics concentration was build using humic acid as standard and the correlation equation and curve (Annex 2- Table A4 and Figure A3) were used to determine the actual humics concentrations in the EPS extracts.

2.3.4. Proteins- humics reciprocal interference

Proteins measurements were corrected in order to prevent the interference with the humics measurements, according to the equation (Eq.1) derived by (Frolund et al., 1996). Similarly, humics measurements were corrected in order to prevent the interference with the proteins measurements, according to the equation (Eq.2) derived by (Frolund et al., 1996).

$$Real\ Abs_{.Proteins} = 1.25 \cdot (Measured\ Abs_{.Proteins} - Measured\ Abs_{.Humics}) \quad (Eq. 1)$$

$$Real\ Abs_{.Humics} = 1.25 \cdot Measured\ Abs_{.Humics} - 0.25 \cdot Measured\ Abs_{.Proteins} \quad (Eq. 2)$$

where: Abs= absorbance.

2.4. Other measurements

2.4.1. Volatile and total solids

Volatile solids (“VS”) and total solids (“TS”) were measured according to Standard Methods (Eaton et al., 2005).

2.4.2. Particle size

Particle size average (“PS”) and distribution (“PSD”) of sludge suspension were determined on the specimen unfiltered, utilizing a Malvern Mastersizer 2000 machine (Malvern- Malvern, UK) based on the laser diffraction method.

Particle size average (“PS_{coll}”) of the sludge colloid was determined on the specimen filtered over a 1µm cellulose filter paper (Whatman- Maidstone, UK), employing a Malvern Zetasizer Nano series Nano-ZS machine (Malvern- Malvern, UK) based on the dynamic light scattering technique.

2.4.3. Surface charge

Firstly, the zeta potential average of the sludge colloid (“SC_{coll}”) was evaluated through the electrophoretic light scattering technique, using a Malvern Zetasizer Nano series Nano-ZS machine (Malvern- Malvern, UK).

Secondly, the zeta potential average (“SC_{coll}”) and distribution (“SCD_{coll}”) of the sludge colloid were sized through the microscopic electrophoresis method, utilizing a Zetamètre ZetaCompact machine (CAD Instruments- Les Essarts le Roi, France).

The surface charge was calculated from the zeta potential according to the equation of (Loeb et al., 1961) (Eq.3). The Debye length was evaluated through the formula of (Chassagne et al., 2016) (Eq.4).

$$\sigma_s = \frac{\varepsilon_0 \cdot \varepsilon_r \cdot k_B \cdot T}{e \cdot z} \cdot k \cdot \left[2 \cdot \sinh\left(\frac{e \cdot z}{k_B \cdot T} \cdot \frac{\zeta}{2}\right) + \frac{4}{k \cdot a} \cdot \tanh\left(\frac{e \cdot z}{k_B \cdot T} \cdot \frac{\zeta}{4}\right) \right] \text{ (Eq. 3)}$$

where: σ_s =surface charge; k_B = Boltzmann constant; T= temperature; e= elementary charge; z= valence; ζ = zeta potential; a=average particle diameter.

$$k^2 = \frac{k_E}{\varepsilon_0 \cdot \varepsilon_r \cdot D} \text{ (Eq. 4)}$$

where: k=Debye length; k_E =conductivity; ε_0 = absolute permittivity of vacuum; ε_r = relative permittivity of water; D= ionic diffusion coefficient.

2.4.4. Capillary suction time

Capillary suction time (“CST”) measurement on sludge suspension was executed utilizing a 304M Triton Electronics apparatus (Triton Electronics- Dunmow, United Kingdom) and a Whatman #17 chromatography paper (Whatman- Maidstone, UK), according to Standard methods (Eaton et al., 2005) and derived from (Baskerville, 1968; Gale, 1967). The measured value was normalized for the total solids content (Eq.5). CST measures the time required for the water released from sludge, through capillary suction generated by the absorbent filter, to extend on the filter paper between 2 predetermined diameters.

$$CST = \frac{t}{V \cdot w} \text{ (Eq. 5)}$$

where: CST=capillary suction time; t= suction time; V= sample volume; w=solids concentration.

2.4.5. Sludge resistance to filtration

Sludge resistance to filtration (“SRF”) test were performed on the sludge suspension according to the method of (Christensen & Dick, 1985) employing a Whatman #1 flat sheet cellulose membrane filter (Whatman- Maidstone, UK). The final value was calculated according to the formula of (Novak et al., 1988) (Eq.6 and Eq.7). SRF measures the rate at which water is released from sludge when filtered over a membrane and subjected to additional pressure.

$$\frac{t}{V} = \frac{\mu \cdot w \cdot SRF}{2 \cdot P \cdot A^2} \cdot V + \frac{\mu \cdot R_m}{P \cdot A} \quad (\text{Eq. 6})$$

where: t= filtration time; V= filtered volume; μ = liquid viscosity; w= solids concentration; SRF= sludge resistance to filtration; P= applied pressure; A=filter area; R_m =filter medium resistance.

$$SRF = \frac{2 \cdot P \cdot A^2 \cdot a}{\mu \cdot w} \quad (\text{Eq. 7})$$

where: a= slope of linear plot dV/dt vs V

2.4.6. Cations

Na^+ , NH_4^+ , K^+ , Mg^{2+} and Ca^{2+}

Monovalent and divalent cations (Na^+ , NH_4^+ , K^+ , Mg^{2+} and Ca^{2+}) were measured from EPS extracts, using the ion chromatography procedure through an 883 Basic IC Plus machine (Metrohm- Herisau, Switzerland) operating on a 3M NaOH eluent at a flux of 0,7 ml/min.

Fe^{3+} and Al^{3+}

Trivalent cations (Fe^{3+} and Al^{3+}) were measured from EPS extracts using photometric cell tests (Merck-Fontenay sous Bois, France) and a Spectroquant NOVA60 machine (Merck Millipore- Darmstadt, Germany). Iron measurements include Fe(III) and Fe(II).

2.4.7. Chemical oxygen demands

Chemical oxygen demand (“COD”) was evaluated on the EPS extracts using photometric cell tests (Hach- Düsseldorf, Germany) on a Hach DR 3900 machine (Hach- Düsseldorf, Germany).

Chapter 3 Site and installations

3.1. Site and installations

3.1.1. Bath WWTP

Bath WWTP, Netherlands under the administration of Waterschap Brabantse Delta RWZI, handles a mix of residual waters: municipal wastewater from the residents of NW North Brabant and industrial wastewater from the manufacturing facilities of Westerschelde.

3.1.2. Mesophilic anaerobic digester- full scale

The current treatment scheme includes the treatment of a blend of primary and secondary waste sludges with the aid of two full scale anaerobic mesophilic digesters (Figure 1) operated at 33- 35°C. With a 20days retention time and a 5430m³ volume, both digesters can sustain a loading rate of 1.48kg organic dry matter per m³ per day or approximately 15.4tons of dry solids per day. The sludge line is continued with the dewatering of the digestate with belt band presses, and with the transportation of the sludge cake to an offsite location.

The feeding ratio of primary to secondary sludge to the mesophilic digester depends on the wastewater inflow and, thus, presents high seasonal and daily variabilities and influences the content of the digester and of the samples. Figure 2 and Table 4 present the daily mesophilic digester feed over the observation period and the 20 days moving average mesophilic digester feed on the sampling dates. Primary sludge was both majoritarian and majoritarian, representing 40-60 % of the mesophilic anaerobic digester feed.



Figure 1 Mesophilic digester

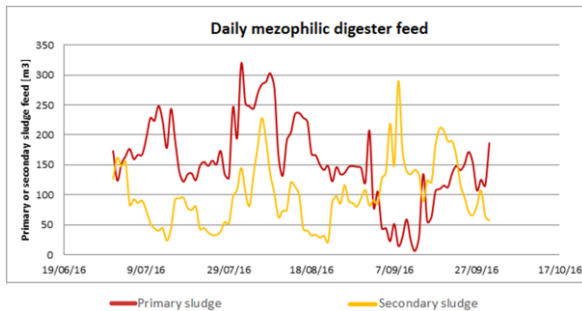


Figure 2 Daily mesophilic digester feed

Mesophilic digester feed				
	Primary sludge		Secondary sludge	
	[m3]	[%]	[m3]	[%]
20 days Moving average mesophilic digester feed				
Tue 6/9/2016	125.87	59	86.91	41
Thu 15/9/2016	80.46	38	128.86	62
Mon 28/11/2016	N.A.	N.A.	N.A.	N.A.
Overall mesophilic digester feed 01/07/16- 30/09/16				
Avg.	154.81	60	101.08	40
Stdev.	67.61	26	53.23	21

Table 4 Mesophilic digester feed

3.1.3. Thermophilic anaerobic digester- pilot scale

The primary and secondary sludge were mixed in a 3.072m³ buffer tank at 15°C and atmospheric pressure before being fed to the thermophilic anaerobic digester (Figure 3). The thermophilic fermenter pilot had an effective volume of 18m³, and operated with a 20days retention time at a temperature of 52-55°C. The resulting digestate was partially wasted in the sewer and partially fed to the NH₃ stripper.

The thermophilic fermenter daily feeding and 20 days moving average feed on the sampling dates are presented in Table 5 and 6. Unlike the mesophilic digester, the thermophilic digester feeding ratio of primary to secondary sludge was relatively constant and did not depend on the wastewater inflow. Primary sludge was majoritarian, representing 70-80 % of the thermophilic anaerobic digester feed.



Figure 3 Thermophilic digester

Thermophilic digester feed					
		Primary sludge		Secondary sludge	
		[m3]	[%]	[m3]	[%]
Daily thermophilic digester feed					
Jul- Sept	Mon-Thu	0.73	74	0.26	26
	Fri	1.55	67	0.76	33
Oct- Dec	Mon-Thu	0.82	82	0.18	18
	Fri	1.78	77	0.53	23

Table 5 Daily thermophilic digester feed

Thermophilic digester feed					
		Primary sludge		Secondary sludge	
		[m3]	[%]	[m3]	[%]
20 days Moving average thermophilic digester feed					
Tue 6/9/2016		12.67	71	5.17	29
Thu 15/9/2016		13.40	71	5.43	29
Mon 28/11/2016		14.34	80	3.50	20

Table 6 Thermophilic digester feed

3.1.4. Ammonia stripper- pilot scale

The thermophilic digestate was used as the sludge feed source for the operation of the 330L NH₃ recovery pilot (Figure 4). A temperature of 60°C and an increased pH through both aeration and addition of either Na(OH) or Mg(OH)₂ ensured the correct conditions for the 6- 24h batch NH₃ stripping process. The produced NH₃ gas was transported to a HNO₃ acid wash and where the fertilizer NH₄(NO₃) was formed.

3.1.5. Primary and secondary sludge digester feed

During the observation period (July- September), the solids content of the individual primary and secondary sludge sources were regularly assessed. Primary sludge showed an average solids concentration of 4.75g/L of which 67% was organic; whilst secondary sludge displayed an average solids content of 6.49g/L of which 67% was organic. Despite the similar organic content of both sludge sources, the types of organic fraction were indubitably disparate and presented different states or potentials of biodegradation. The differentiated feeding ratios of primary to secondary sludge for the mesophilic and thermophilic digesters might have affected the comparison of the two processes.



Figure 4 Ammonia stripper

3.2. Operation limitations

Aspects of the operation of the mesophilic digester, thermophilic digester and ammonia stripper were disclosed subsequent to the research planning, and the sludge sampling and analysis. The following limitations quickly ensued and negatively impacted the research objectives:

- The mesophilic and thermophilic digesters were fed different ratios of primary to secondary sludge.
- The thermophilic digester was fed a different ratio of primary to secondary sludge in the last sampling by comparison to the first and second samplings.
- The pH in the ammonia stripper was increased with either NaOH or $Mg(OH)_2$ and with varying base amounts, with no disclosure on the characteristics of each batch.
- Due to a differentiated and precipitated shut down of the ammonia stripper installation, the last sampling of this source was collected on an earlier day than the other locations.

Chapter 4 Associated research

The observed installations were the object of study of diverse external entities. Relevant results stemming from these researches are presented below, under the observation that the results are attributed to the company which produced them and that this study has not contributed.

Royal Haskoning DHV and Aiforo

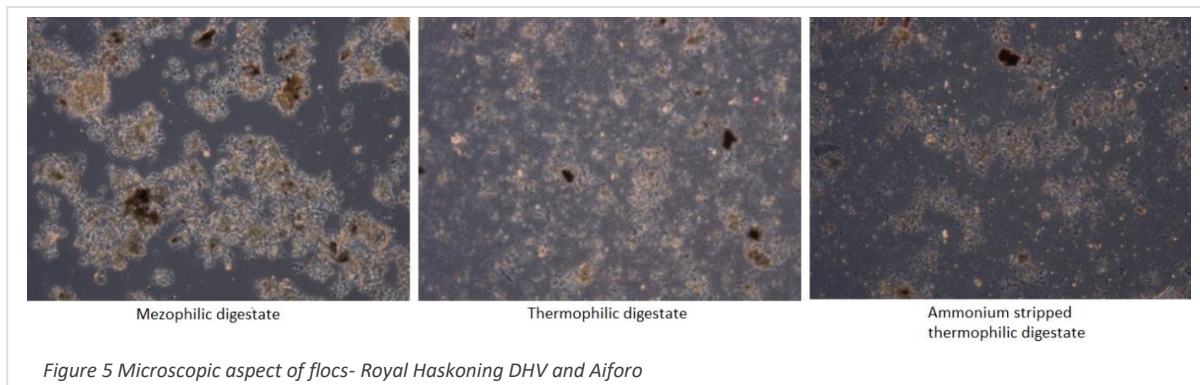
Fe^{3+} , Ca^{2+} and Mg^{2+} were added in high concentrations in order to compare their effect on the sludge dewaterability, quantified as capillary suction time (“CST”), and on the floc surface charge, quantified as polyelectrolyte required to reach the isoelectric point (“PE”) (Table 7). Ammonia stripped sludge followed by thermophilic digestate presented higher negative surface charges than mesophilic digestate, possibly impeding flocculation. Although all cations improved the dewaterability and reduced the surface charge, by comparison to Ca^{2+} and Mg^{2+} , Fe^{3+} presented the most poignant positive effect. However, the dosages of cations were undisclosed, and it could not be evaluated if they accounted for the differences in valence and binding capacity of individual cations.

	Blank	Fe^{3+}	Mg^{2+}	Ca^{2+}
	CST [sec]			
Mesophilic digestate	420	185	355	300
Thermophilic digestate	1260	820	1050	950
NH_3 Stripped thermophilic digestate	1665	895	1245	1070
	PE added to isoelectric point [ml]			
Mesophilic digestate	2.8	2.3	2.12	-
Thermophilic digestate	4.12	3.04	3.48	-
NH_3 Stripped thermophilic digestate	8.5	3.7	6.2	5.5

Table 7 Effect of cation addition on CST and PE required

The research included microscopic observations of the different sludge typologies (Figure 5). The floc disruption and release of particles were noticeable in the thermophilic digestate and even more so in the ammonia stripped thermophilic digestate, and correlated to the poor dewaterability characteristics of the sludges.

The research included microscopic observations of the different sludge typologies (Figure 5). The floc disruption and release of particles were noticeable in the thermophilic digestate and even more so in the ammonia stripped thermophilic digestate, and correlated to the poor dewaterability characteristics of the sludges.



A range of tested polymers revealed only cationic polymers exhibited a significant positive effect on dewaterability. However, anionic polymers or mixes of anionic and cationic polymers presented no or, respectively, negligible improvements, going to show the preponderance of negatively charged particles.

For the chosen cationic biopolymer BC 470 L, optimum dosages were determined to be 12g PE/ kg TS for mesophilic digestate, 21g PE/ kg TS for thermophilic digestate and 25g PE/ kg TS for ammonia stripped digestate, clearly indicating the poor flocculation and dewaterability behaviors. Visual observation during the tests were also noteworthy. The mesophilic sludge formed firm definite flocs, easily drainable and with few flocs becoming attached or lost through the band press. The thermophilic sludge presented a weak floc, with material being attached and passing the band press. The ammonia stripped sludge formed limited flocs, and substantial amounts of particles were pushed through the filter material.

Particle size distribution was determined utilizing a laser diffraction based particle size LS 12 320 Beckman Coulter analyzer (Beckman Coulter-Brea, USA). The d50 was evaluated at 70.59µm for mesophilic digestate, 34.17µm for thermophilic digestate and 32.08µm for ammonia stripped sludge (Figure 6). Although a decrease in average particle diameter is apparent, the distribution becomes narrower and no differentiation occurs between the thermophilic and ammonia stripped sludge.

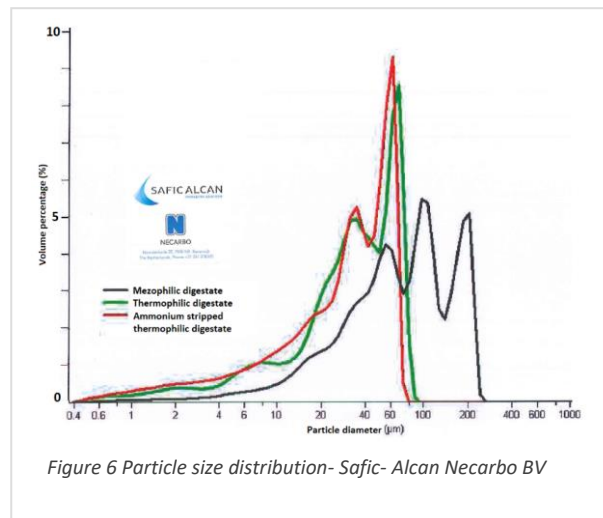


Figure 6 Particle size distribution- Safic- Alcan Necarbo BV

Waterschap Brabantse Delta RWZI

The minimum polymer dosage for attaining an acceptable dewaterability was visually determined using a high molecular weight biopolymer Praestol K232L 0.2% active (Necarbo- Beverwijk, Netherlands) and a room filter press on the disparate sludge specimens. The mesophilic digestate could reach dry cake solids of 18% using 11 g polymer / kg dry solids, the thermophilic digestate required 24 g polymer/ kg dry solids to attain hardly 13% dry cake solids, whilst the ammonia stripped sludge did not reach a satisfactory dewaterability even with 39 g PE / kg dry cake solids.

The addition of 1eq Fe³⁺/ kg dry solids decreased the polymer requirement and increased the attained dry solids cake. The thermophilic digestate necessitated only 12 g polymer/ kg dry solids to reach 16% dry solids cake. Moreover, the ammonia stripped sludge could be dewatered to 13% dry solids cake using 18.1 g polymer / kg dry solids. The positive effect of Fe³⁺ on dewaterability was reiterated with an ensuing test by varying the quantity of the trivalent cation (Table 8).

Other test divulged additional characteristics: the direct correlation pH and CST for both thermophilic and ammonia stripped sludge (Table 9) and the direct relation between increased Mg(OH)₂ addition in the stripping process and deterioration of dewaterability (Table 10).

	Fe ³⁺ [eq/kg TS]	CST [sec]
Thermophilic digestate	0	885.1
	0.61	743.5
	1.21	481.9
	1.82	281.5
NH ₃ Stripped thermophilic digestate	0	1688.7
	1.04	610.4
	1.56	230.5
	2.08	100.4

Table 8 Fe³⁺ and CST dependence

	pH	CST [sec]
Thermophilic digestate	7.88	986.5
	5.95	792.4
	4.1	716.9
NH ₃ Stripped thermophilic digestate	9.24	1640.2
	6.94	1175.3
	4.01	767.1

Table 9 pH and CST dependence

	Mg(OH) ₂ base [L]	CST [sec]
NH ₃ Stripped thermophilic digestate	0	1492.7
	9.6	1686.7
	11.6	2459.9
	13	2517.7

Table 10 Mg(OH)₂ and CST dependence

Chapter 5 Results and discussion

5.1. Capillary suction time

Capillary suction time was used as means to determine the dewaterability behaviour of the sludges. Complete overviews of the results are presented in Annex Table A5.

The CST values (Figure 7), normalized for TS content, increased with the increased harshness of the treatment to which the sludges were subjected to. The lowest CST was obtained for the DF (620 sec/g TS), followed in ascending order of obtained values by MD (1569 sec/g TS), TD (4133 sec/g TS) and ASTD (5835 sec/g TS).

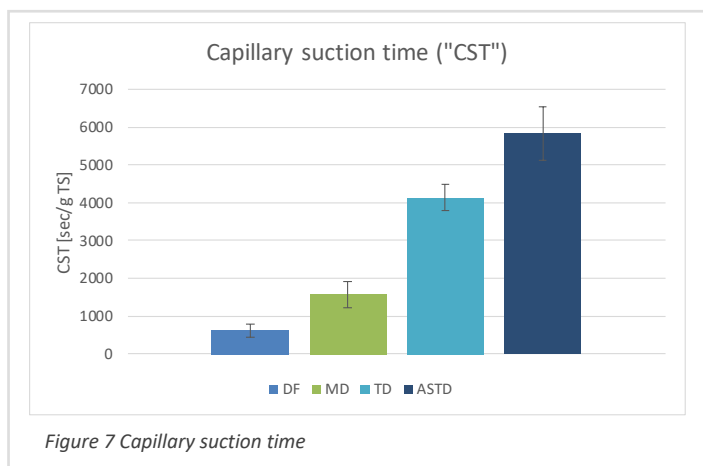


Figure 7 Capillary suction time

The negative impact of anaerobic digestion on dewaterability was exhibited, in addition to which thermophilic digestate presented inferior liquid solids separation compared to mesophilic digestate. The ammonia stripping procedure, when applied to thermophilic digestate, further aggravated the dewaterability of the sludge, attaining exacerbated values.

This is in line with other researches where it is found that fermentation has a negative impact on CST (Novak, 2010; Houghton, 2000; Luo, 2015), particularly anaerobic digestion at 55°C when compared to one at 35°C (Coelho, 2011; Mikkelsen, 2002). This is also in line with the findings of the Bath WWTP water board.

5.2. Sludge resistance to filtration

Sludge resistance to filtration was used as means to determine the dewaterability behaviour of the sludges. Complete overviews of the results are presented in Annex Table A6.

The SRF values (Figure 8), normalized for TS content, raised with the increased abrasiveness of the treatment to which the sludges were subjected to. The DF displayed a minor resistance to filtration ($2.23 \cdot 10^{10}$ cm/g TS) compared to the other sludges. Anaerobic digestion had a negative impact on dewaterability, as expected (Novak, 2010; Novak, 2003; Rasmussen, 1994), with MD ($9.48 \cdot 10^{10}$ cm/g TS) continuing to display superior liquid solid separation characteristics when compared to TD ($15.99 \cdot 10^{10}$ cm/g TS). The ASTD ($22.98 \cdot 10^{10}$ cm/g TS) exhibited a major resistance to filtration by analogy to the aforementioned sludges.

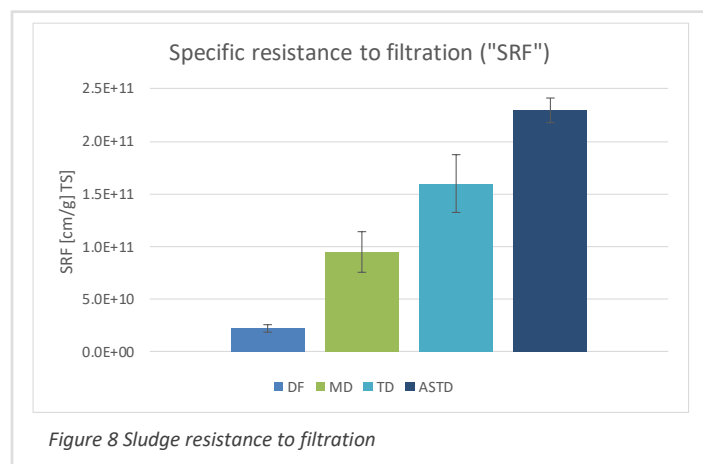


Figure 8 Sludge resistance to filtration

Both dewaterability indicators showed identical dewaterability behaviours for all sludges. A strong positive correlation (+0.98) was obtained between CST and SRF.

5.1. Volatile and total solids

Volatile solids and total solids assessments were primarily employed for the normalization of additional variables in order to account for variations of solids content in between sludge types and in between samplings within sludge types. Complete overviews are presented in Annex Table A5 and A6.

Notable in these measurements were 2 elements.

Firstly, the contrast between the quasi-constant suspension VS/TS ratios of the MD, TD and ASTD and the increasing colloidal VS/TS ratios, compared to the DF (Figure 9). The increase in the ratio between the colloidal organic and total colloidal fraction is, reported to the DF content, 28% higher for MD, 83% higher for TD and 102% higher for the ASTD. This could point a preferential digestion of supracolloidal fractions, accompanied by deflocculation.

Secondly, the increase in both organic and inorganic colloidal (and implicitly sub-colloidal) fraction, as the processes through which the sludges were submitted became harsher (with the sole exception of TS_{coll} in MD). Colloidal volatile solids concentrations elevated, compared to DF, with 14% for MD, 100% for TD and 173% for ASTD. ASTD VS_{coll} increased with 39% having TD as reference. Colloidal total solids concentrations presented a drop of 11% for MD, and a rise of 8 % for TD and of 35% for ASTD, compared to DF. Strong positive correlations were found between colloidal VS, TS and VS/TS, and CST (+0.99, +0.88 and +0.99, respectively), as well as SRF (+0.96, +0.81 and +0.98, respectively). This indicates that an enlarged presence of colloids, organic or inorganic, resulted in a poor dewaterability behaviour of sludges. Moreover, the more consistent increase of VS_{coll} than TS_{coll} , possibly indicated a higher release from floc of cells, biopolymers and other organics than of inert colloids.

The general quantitative increase in the colloidal fraction under anaerobic conditions is noticed in other anaerobic systems (Yang, 2010; Novak, 2010), and is strongly associated with the deterioration of dewaterability indicators (Novak, 2010). Such a release is often accompanied by a release of bacterial cells (Wilen et al., 2000), and of soluble COD as a measurement for soluble biopolymer (Yang et al., 2010).

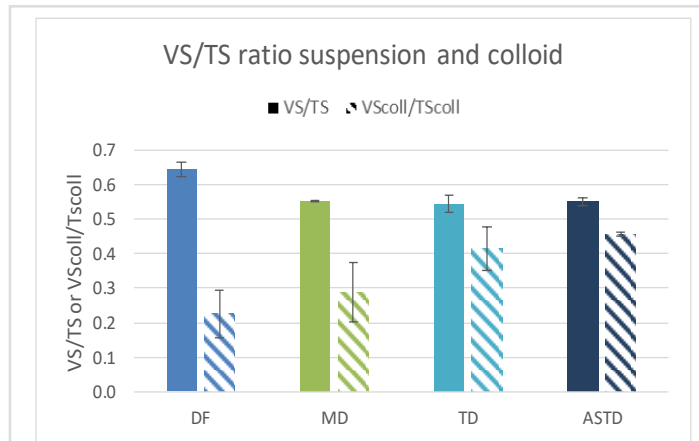


Figure 9 Volatile solids to total solids ratio suspension and colloid

Source	VS _{coll}	TS _{coll}	VS _{coll} /TS _{coll}
	Avg. per sludge type [g/L]	Avg. per sludge type [g/L]	Avg. per sludge type [-]
DF	0.84	3.74	0.23
MD	0.96	3.33	0.29
TD	1.68	4.06	0.41
ASTD	2.31	5.05	0.46

Table 11 Volatile solids, total solids and ratio colloid

5.4. Particle size

5.4.1. Suspension- Particle size

Suspension average particle size summary is depicted in Annex Table A9.

Suspension average particle size (Figure 10), reported as D_{50} , was measured and correlations to dewaterability of sludges were evaluated. The average particle size of the digesters feed was estimated to 80.0 μm , which decreased to values of 55.1 μm for MD and 53.0 μm for TD, as a result of fermentation. Thermophilic digestate, further subjected to ammonia stripping, presented a growth in particle flocs dimension, reaching a particle diameter of 63.8 μm .

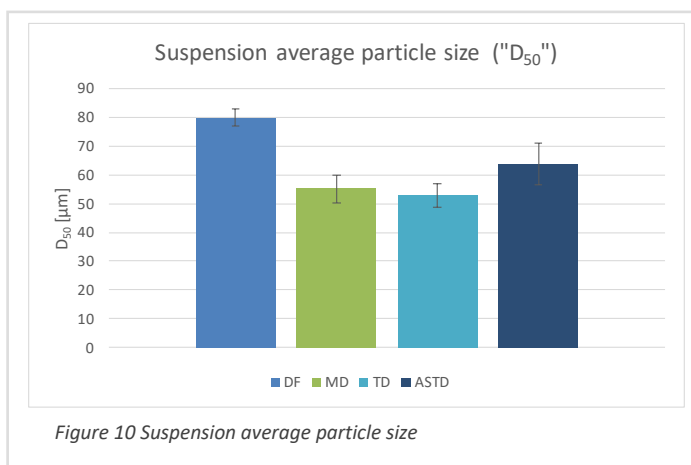


Figure 10 Suspension average particle size

Whilst for the anaerobic digestion process an overall decrease in the average particle diameter was evident; for the thermophilic vs mesophilic digestion no trend could be found, as the D_{50} variation fell well within the standard deviations limits. For ASTD an apparent reflocculation, due to magnesium addition in the form of $\text{Mg}(\text{OH})_2$ base or due to reoxidation of iron, was unlikely, as a cation bridging mechanism would have also flocculated colloids and would have resulted in: a reduced colloidal solids concentration and an inflated colloidal average diameter. Considering the opposite occurred, it appears more likely that supra-colloids from the lower size range of the ASTD were deflocculated due to the treatment harshness, whilst higher size range flocs maintained their structure. No correlations were found between the suspension D_{50} and dewaterability indicators CST (-0.44) and SRF (-0.54).

Decrease in suspension particle size during anaerobic digestion (Sponza, 2002), as well as the lack of difference between digestion at dissimilar temperature were reported before (Mikkelsen & Keiding, 2002). Additionally, other studies could not detect a relation between the supracolloidal sludge fraction and the sludge dewaterability (Rasmussen, 1994; Mikkelsen, 2002). The reduction of particle size with the increase harshness of the treatment, including the ASTD, was also measured by Waterschap Brabant Delta RWZI collaborators.

5.4.2. Suspension- Particle size distribution

Suspension particle size distributions are depicted in detail in Annex Table A9 and Figures A4- A6.

Aside from the average particle size, the distribution of particle size (Figure 10, 11 and 12) possesses the potential to influence liquid solid separation.

The method displayed several shortcomings. In the lower detection range, particles were not distinguished and included in the distribution, probably due to overshadowing from the larger flocs. In the higher detection range, a high standard deviation was found between triplicates, possibly due to scarce sizeable flocs which randomly appeared in the measured specimens.

Moreover, an increased variability in the data might have resulted from difficulties in correctly estimating the particles size in the cases of large flocs, which typically present non-defined and non-spherical shapes.

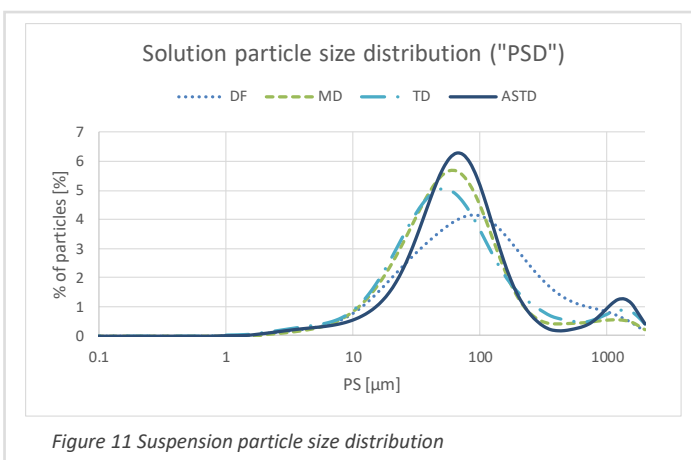
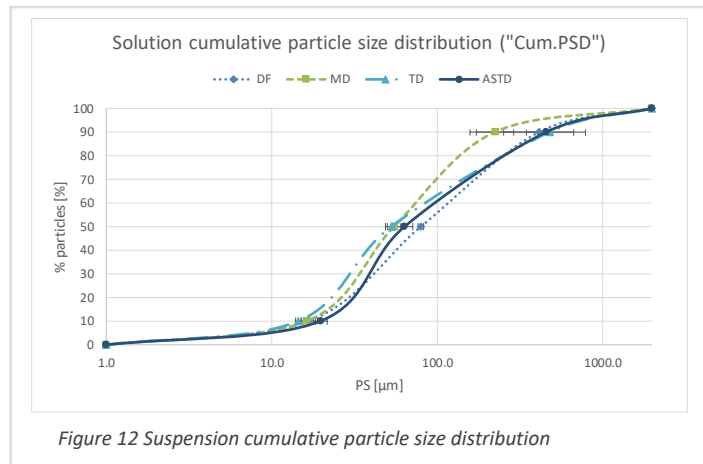


Figure 11 Suspension particle size distribution

The digester feed presented the broadest distribution with the largest particles and flocs. By comparison, the mesophilic and thermophilic digestion decreased the spread of the particle size distributions and the diameters of the sludge fragments, to approximately similar values. The ammonia stripped thermophilic digestate exhibited the narrowest particle size distribution, and, as seen earlier, the average size of the flocs moderately raised compared to the anaerobic digestates. A broader distribution causes a migration of fines, generating blinding of filtration cake and filtration media, and leading to elevated resistances to filtration (Qi et al., 2011). Considering the later, as well the facts that particle size distribution became narrower with the increased roughness of the sludge treatment and with the worsening of dewatering behaviour, it was considered implausible that, in this study, PSD was the cause of unsatisfactory dewaterability.

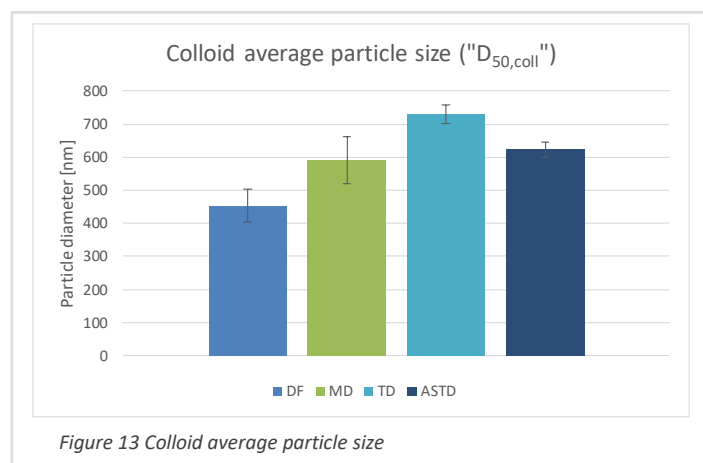


The results were in contradiction with the research of Mikkelsen (2002), where thermophilic digestion resulted in a wider particle size distribution compared to mesophilic digestion, and the parameter was, in effect, strongly associated to dewaterability. However, the results are partially confirmed by the Safic-Alcan Ncarbo BV study, where equally narrower distributions were observed for TD and ASTD, but with a broader particle size spread for the MD.

5.4.3. Colloid- Particle size

Colloid average particle size complete results are presented in Annex Table A10.

The average particle size of the sludge colloidal (and implicitly sub-colloidal) fraction (Figure 13), reported as $D_{50, coll}$, is presented hereunder. The average colloid size of the digesters feed was estimated to 452.4 nm, which enlarged to values of 592.4 nm for MD and 730.3 nm for TD, as a result of fermentation. Thermophilic digestate, further subjected to ammonia stripping, presented a decrease in colloid dimension, reaching a diameter of 623.5 nm.



Digestates compared to feed, and thermophilic digestate compared to mesophilic digestate, displayed a higher average colloid size. It is assumed that the increased in $D_{50, coll}$, resulted not from the flocculation of colloidal particles, but from the supra-colloidal fraction destruction accompanied by the release of colloidal particles with sizes in the upper measuring range of the machine. Whilst release of lower sized colloids might have occurred for MD and TD, it was surpassed by the inflow of high diameter colloids, so it did not reflect on the average colloid size. The ammonia stripped sludge possibility went through a similar process of receiving new high diameter colloids as a result of floc destruction, but due to the severity of the treatment, flocs were simultaneously severely fragmented and released lower sized colloids, which reflected in a decrease of the $D_{50, coll}$ value. This notion was supported by the mild inverse relation (-0.89) found between colloidal and suspension D_{50} .

From the $V_{S, coll}$ it was already known of the negative impact of the colloidal organics on dewaterability. It was possible that an inflated average colloid diameter $D_{50, coll}$ had a minor influence in the deterioration of dewaterability, as measured by the weak correlations to both CST (+0.70) and SRF (+0.73).

Unfortunately, no information could be derived on the colloid size distribution, as a broader colloidal size range could have been the underlying cause for the $D_{50, \text{coll}}$ to dewaterability correlation.

An enlarged quantity of particles in the 0.45- 1.5 μm or 0.45- 10 μm ranges, during anaerobic digestion, is also observed and correlated to a deterioration in CST, SRF and OPD by Rasmussen (1994) and Novak (2010).

5.5. Particle surface charge

5.5.1. Colloid- Surface charge

Colloids surface charge measurements are presented in complete overview in Annex Table A11, for Electrophoretic light scattering measurement technique, and in Annex Table A12, for Microscopic electrophoresis measurement technique.

Average colloid surface charge and zeta potential values from Electrophoretic light scattering measurement technique (Figure 14 and Table 12) are discussed hereunder. Average colloid surface charge and zeta potential values from Microscopic electrophoresis measurement technique are not discussed due to the large standard deviations between replicates, which could have been the result of a too high particle concentration in the analysed specimens.

The calculation of the electrical potential difference at the particle surface (surface charge), revealed that colloids from all sludge surfaces were negatively charged. The lowest surface charge was found in the digesters feed, whereas any applied process, be it mesophilic digestion, thermophilic digestion or ammonia stripping, resulted in an increase in the negativity of the surface charge to similar levels.

A higher negative surface charge could have prevented particles from aggregating into flocs and negatively impacted dewaterability, but did not explain the differences in dewaterability between MD, TD and ASTD. Low to average correlation were found between SC and CST (-0.77) and SRF (-0.86), where the more negative the charge on the particle surface the higher the CST and SRF values were determined.

Existing reports are contradictory to the results of this study, as anaerobic digestate compared to feed (Sponza, 2002), and thermophilic digestion compared to mesophilic digestate (Mikkelsen & Keiding, 2002), are considered to lower SC, and SC is considered not to be engaged into the increased difficulty of removing water from sludge.

However, literature often refers to the zeta potential parameter (apparent electrical potential differential at the slipping plane) as the major interaction plane in electrostatic repulsion, despite it not accounting for the conductivity of the solution.

In the current case, zeta potential became more negative with the increased roughness of the treatment to which the sludge was subjected to, making flocculation and dewaterability increasingly difficult. ZP was found to negatively impact dewaterability and was strongly negatively associated with CST (-0.90) and SRF (-0.96) both.

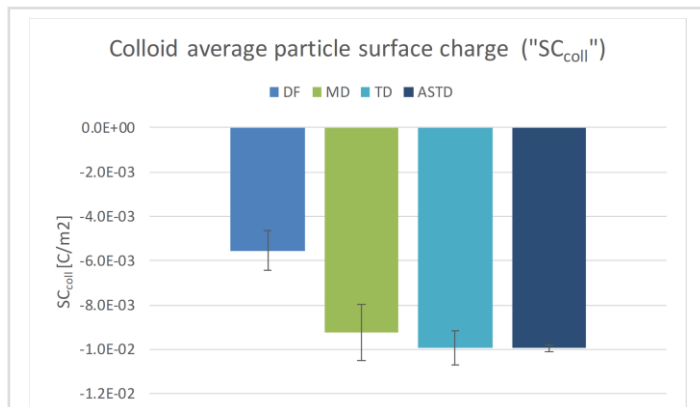


Figure 14 Colloids average particle surface charge- Electrophoretic light scattering technique

Particle surface charge colloids		
Source	Zeta potential	Surface charge
	Avg. per sludge type [mV]	Avg. per sludge type [C/m ²]
DF	-12.44	-0.0055
MD	-15.73	-0.0093
TD	-16.75	-0.0099
ASTD	-17.86	-0.0100

Table 12 Colloids average particle surface charge and ZP- Electrophoretic light scattering technique

The findings are supported in this case by researchers claiming an increase in the negativity of the ZP for anaerobic digestion (Luo et al., 2015). However, contradictions exist, with other studies claiming a decrease of ZP with thermophilic digestion compared to mesophilic digestion (Mikkelsen & Keiding, 2002). The current findings are partially supported by the findings of the other collaborators in the present project. Whilst a more negative surface charge was determined for all digestates compared to the feed, they further observed that ASTD presented the highest negative charge, followed by TD and by MD.

5.5.2. Colloid- Surface charge distribution

Colloidal surface charge distributions determined through microscopic electrophoresis measurement technique are presented in Annex Table A13 and Figures A9- A11. Results are not discussed due to the large standard deviations between replicates, which could have been the result of a too high particle concentration in the analysed specimens. Not accounting for the size distribution of colloids by using an average particle diameter might have furthered inflated variation.

5.6. Chemical Oxygen Demand

Chemical oxygen demand measurements were undertaken in order to observe shifts, degradations or variations in extractability of organic matter. A complete overview of the obtained COD concentrations is presented in Annex Table A14.

The COD concentrations (Figure 7 and Table 13), normalized for VS content, displayed an increase in Total, Bulk and LB EPS, accompanied by a decrease in TB EPS, going from DF to MD, TD and ASTD, with few exceptions.

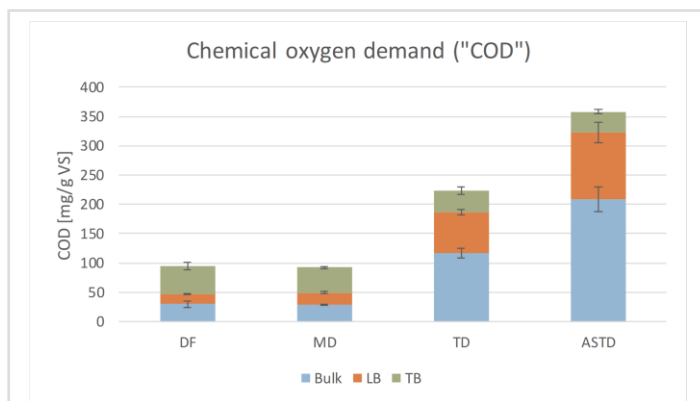


Figure 15 Chemical oxygen demand

Source	Chemical oxygen demand ("COD")			
	Avg. per sludge type [mg/g VS]			
	Bulk	LB	TB	Total
DF	30.17	17.15	48.26	95.58
MD	28.50	20.81	43.60	92.91
TD	116.61	70.33	36.58	223.52
ASTD	209.36	113.86	34.77	357.99

Table 13 Chemical oxygen demand

By comparison to the feed, soluble COD raised with 286% during thermophilic digestion and decreased with 6% during mesophilic digestion. The solution COD variation in MD is insignificant, as it lies within the standard deviations of the measurements. As in the case of anaerobic digestion, solubilization of COD is expected (Foladori, 2015; Bruus, 1993; Rasmussen, 1994; Yang, 2010), it was suggested that at 35°C the rate of hydrolysis of organic matter (or reduction of nitrate and phosphate) exceeded that of COD solubilization, whilst the opposite occurred at 55°C. This allowed for soluble COD to accumulate in the TD. The further soluble COD supplementation in ammonia stripped thermophilic digestate with 593% compared to DF, accompanied by the fact that 6h stripping batches could not have resulted in significant, if any, organic matter removal, suggests a vigorous COD solubilization. Soluble COD appeared to generally present a positive relation with CST (+0.97) and SRF (+0.93), even with the MD outlier included.

The COD content in the LB phase raised with the increased harshness of the sludge treatment. Anaerobic digestion resulted in an increase of 21% for MD and 300% for TD, suggesting higher temperature fermentation resulted in higher transfer of organic content to the outer layer of the EPS ad floc. A higher shift of COD to the LB region was noted also in ASTD, which was with 563% higher than in DF. A raised LB COD content appears to denote poor dewaterability, with correlations of +0.99 to CST and +0.95 to SRF.

The COD content in the TB phase decreased with the increased intensity of the sludge treatment. Anaerobic digestion resulted in a decrease of 18% for MD and 24% for TD, suggesting higher temperature fermentation resulted in a release of organics from the firm inner EPS network to the diffuse outer EPS

network or solution. A higher shift of COD from the TB region was noted also in ASTD, which was with 28% lower than in DF. A decreased TB COD concentration appear to denote worsened dewaterability, with correlations of -0.97 to CST and -0.98 to SRF.

Overall, a release of organic matter, measured as COD, was observed in the Bulk and LB phases, which could have originated from the TB or, to a lesser extent, pellet phases. This corresponded with the decrease in organics in the TB phase. It is important to note that, quantitatively the amount of COD lost from TB does not balance with the amount of COD gained in Bulk and LB, which probably reflects differences in extraction efficiencies between the 3 EPS phases. Furthermore, variable extraction efficiencies appear to transpire between different sludge types. Moreover, degradation of organic matter could not be assessed, but it was assumed it would occur preponderantly in the soluble phase and not in the LB or TB phases, as the 2 latter are used as energy sources only in cases of substrate shortages.

As COD reflects, among other components, EPS biopolymeric content, and soluble or loose EPS biopolymeric content denotes weak flocs and poor dewatering, it was comprehensible that poor dewaterability was positively correlated to Bulk and LB COD and negatively correlated to TB COD.

5.6. Extracellular polymeric network

5.6.1. Biopolymers

Overview of full results on the presence of biopolymers components, normalized for VS content, in the EPS phases can be found in Annex Table A15- A16. Firstly, results occasionally presented important standard deviations between the samples of the same sludge from the three different sampling dates, but not between the triplicates of the same sludge from the same sampling date. As a result, the presumption that the deviations originated from variabilities in samples characteristics was made. Secondly, variations in extraction efficiencies for different sludge types and EPS phases were observed. As a consequence, the heat method for EPS extraction was deemed unreliable. Thirdly, correlations between parameters are indicators of a possible relation, not a confirmation of an association, and were considered as such. Finally, the differentiation between degradation, shift or increased extractability of EPS biopolymeric components could not be assessed.

The BP concentrations in the soluble and LB phase (Figure 19 and Table 14) elevated with the increasing abrasiveness of the process to which the sludge was subjected. Concurrently, the TB phase did not show meaningful BP content variations, except for the BP content raise in the ASTD sludge.

Compared to the feed, Bulk BP content increased with 14% when anaerobically digested at 35°C, with 339% when fermented at 55°C and with 758% when NH₃ stripped. As BP behaves as an adhesive to other floc components, the increased presence of BP in Bulk suggested floc disintegration and release of floc components, and had a negative effect on dewatering behaviour. Bulk BP, as expected, correlated strongly with CST (+0.97) and SRF (+0.94).

LB BP showed a similar behaviour to Bulk BP, and increased with 28% in MD, 255% in TD and 493% in ASTD, compared to DF. An enlarged LB fraction suggested floc weakening and an increased amount of entrapped water in the outer floc layer, which was supported by the association to CST (+0.98) and to SRF

Source	Bulk	LB	TB	Total
Avg. per sludge type [mg/g VS]				
Carbohydrates ("CA")				
DF	1.50	1.42	0.10	3.02
MD	2.43	2.30	5.05	9.77
TD	11.52	6.89	3.78	22.19
ASTD	26.49	15.43	4.67	46.58
Proteins ("PR")				
DF	5.65	4.49	11.29	21.43
MD	5.15	4.55	8.77	18.47
TD	9.00	6.16	4.67	19.83
ASTD	14.49	9.33	5.63	29.45
Humics ("HU")				
DF	7.90	5.64	16.03	29.58
MD	9.69	7.94	13.45	31.09
TD	45.57	27.97	15.58	89.11
ASTD	88.23	43.81	33.59	165.63
Biopolymers ("BP")				
DF	15.06	11.55	27.42	54.03
MD	17.27	14.79	27.27	59.33
TD	66.08	41.03	24.03	131.14
ASTD	129.21	68.57	43.88	241.66

Table 14 Biopolymers

(+0.95), and denoted poor dewaterability. The negative impact of LB BP on dewatering was emphasized before, although on activated sludge by Li (2007).

TB BP concentrations remained quasicontant between DF, MD and TD, and only surged in ASTD. The stripped sludge exhibited 60% more content than DF. No relation was found with TB BP, and CST and SRF (+0.66 and +0.67, respectively). This may have originated from the impossibility of differentiating shifts, degradation or improved extractions of BP components.

Whilst for DF and MD, TB BP was the predominant component, suggesting a strong dense floc; for the TD and ASTD, TB BP was the minority constituent and was surpassed by LB and, even more so, bulk BP. This consolidates the above notion, that Bulk and LB BP in excess deteriorate liquid solid separation. In confirmation, following low temperature fermentation the TB phase was measured by other researchers to be predominant compared to LB (Ye et al., 2014).

Unexpectedly, humics were the predominant fraction in all sludges and in all EPS groups, despite typically being the 2nd or 3rd reported dominant BP component, if reported at all (Dignac, 1998; Frolund, 1996; Mikkelsen, 2002). Proteins, compared to carbohydrates, dominated all EPS groups of DF and MD; whilst for the TD and ASTD, the roles reversed, with carbohydrates outweighing proteins. In accordance, on low fermentation temperature AN sludge, proteins were discovered in higher presence than carbohydrates both in LB and TB phases (Fang, 1996; Sponza, 2002).

Bulk and LB BP perfectly correlated with Bulk and LB COD (+1.00 and +1.00, respectively), whilst no association was found between TB BP and TB COD (-0.51). This may be the outcome of COD in Bulk and LB phases having BP as a majority fraction; whilst COD in TB phase having bacterial cells or other absorbed organics as predominant components.

However, BP is simply a term describing all extracellular biopolymers and its influence on dewaterability is only a reflection of the major BP components dictating the liquid solid separation behaviour.

5.6.2. Carbohydrates

The carbohydrates concentrations (Figure 16 and Table 14), normalized for VS content, displayed an increase in Bulk, LB EPS and TB EPS going from DF to MD, TD and ASTD, with few exceptions.

Bulk CA increased with 61% in MD and with 666% in TD compared to the feed, suggesting a higher rate of solubilization than rate of glucosidase for TD than MD. Furthering thermophilic digestate through the ammonia stripping process resulted in a further CA solubilization with 1663% higher than the DF. Bulk CA correlated strongly with CST (+0.96) and SRF (+0.93), thus having an influence on dewaterability. Partial support of these results can be found in literature concerning anaerobic digestion. Study outcomes vary from researches in which soluble carbohydrates content increased (Park, 2007; Park, 2006), to studies in which it was constant (Novak, 2003; Wilen, 2000; Luo, 2015). Contradictory, was also the analysis of Mikkelsen (2002), where thermophilic digestate presented constant soluble CA when compared to mesophilic digestate.

LB carbohydrates increased with mesophilic digestion (+62%) and, even more so, with thermophilic digestion (+385%), indicating either an inflated shift from the TB fraction or an increased extractability for TD compared to MD. ASTD presented a further solubilization of carbohydrates compared to DF (+956%). LB carbohydrates also correlated strongly with CST (+0.96) and SRF (+0.94).

Determinable carbohydrates content in the tightly bound EPS was low at the moment sludge was fed to digesters, but resulted in a soaring of the concentrations after the fermentation and ammonia stripping (+37.8- 50.5%), finally reaching contiguous values. The increased presence of CA in the TB floc phase demonstrated a disconnection of the EPS network binds, which allowed for a more facile extraction and a release to Bulk and LB phase. The relation was inexistent to weak between TB CA, and CST and SRF (+0.59 and +0.72, respectively). Literature study gives opposite outcomes, with fermentation decreasing EPS CA concentrations through digestion (Sponza, 2002; Nielsen, et al., 1996) or shift to looser EPS (Ye, 2014), especially for thermophilic digestion versus mesophilic digestion (Mikkelsen & Keiding, 2002).

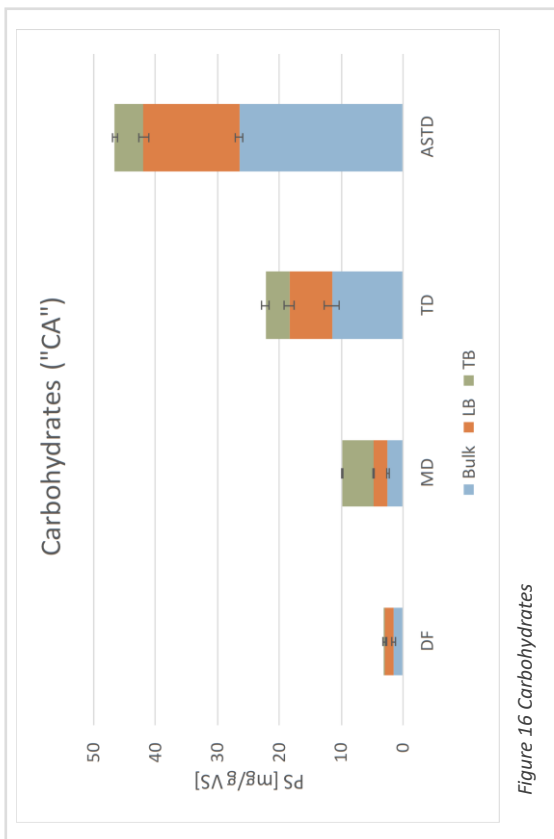


Figure 16 Carbohydrates

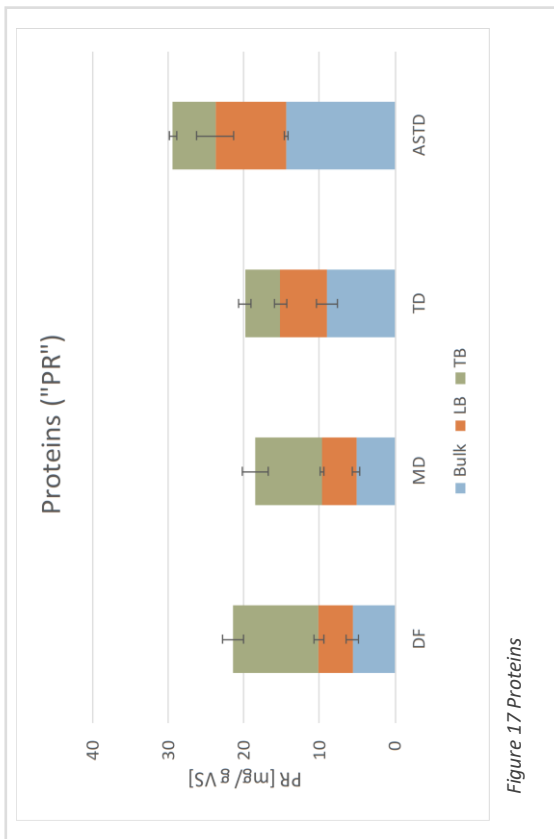


Figure 17 Proteins

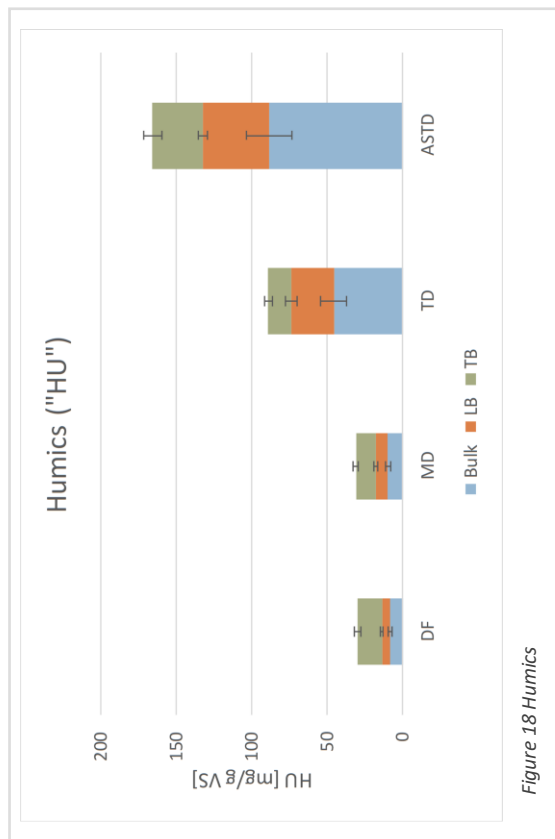


Figure 18 Humics

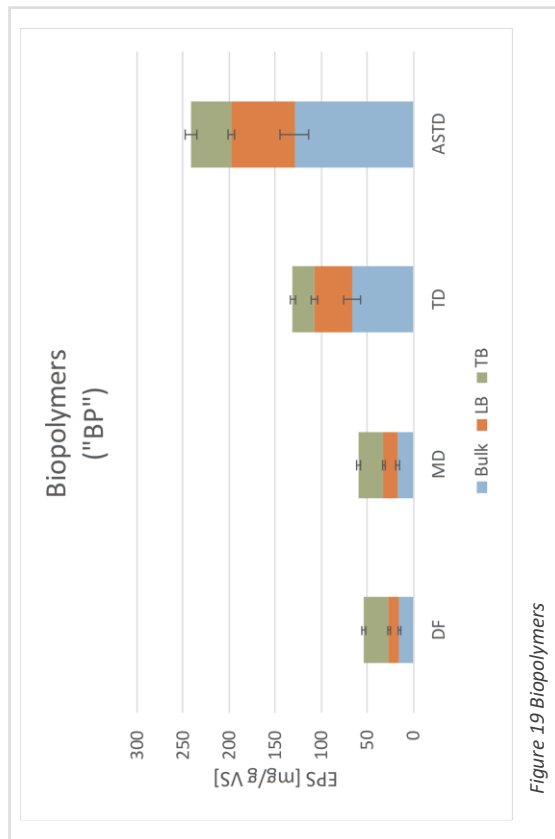


Figure 19 Biopolymers

In general, most CA were found in Bulk EPS, followed by LB EPS, and least CA were determined in TB EPS.

5.6.3. Proteins

The proteins concentrations (Figure 17 and Table 14), normalized for VS content, displayed no exact overall trend.

DF and MD displayed similar protein content in the Bulk and LB phases, with the only difference being the decreased in TB protein (-22%) for the later compared to the former. A possible digestion and/or shift of proteins from the TB to LB and Bulk phases, followed by their prompt and equal digestion, would have left the impression of no protein content variation in the 2 later phases.

TD exhibited an increase in Bulk (+59%) and LB (+37%) protein, and a decrease in TB (-59%) protein compared to DF. Similarly to the MD, TD resulted in a digestion and transfer of proteins from TB to Bulk and LB phases. However, in this case it was possible that the rate of protein release had overridden the rate of protein consumption, resulting in a protein accumulation in the later 2 phases. Mikkelsen (2002) discovered that thermophilic digestion reduces soluble and EPS PR more than mesophilic digestion, with the prior contradicting this study and the later supporting this research.

ASTD presented higher concentrations of protein in Bulk (+156%) and LB Bulk (+108%), when compared to DF. However, TB PR (-50%) in ASTD remained lower than DF. As TB PR digestion in a 6h ammonia stripping batch appeared unlikely, a shift between phases likely occurred.

In general, proteins changed from being predominant in TB phase for DF and MD, to being majoritarian in the Bulk and LB phase for TD and ASTD. Increased solution proteins in anaerobic digestion are heavily reported (Novak, 2003; Wilen, 2000; Luo, 2015; Park, 2007; Park, 2006). However, conflicting studies report both a decrease in EPS PR (Nielsen, 1996; Sponza, 2002), an increase in EPS PR (Houghton et al., 2000) and a constant EPS PR accompanied by a shift from TB to LB phases (Ye et al., 2014).

Since proteins are notoriously high in water content (Cetin, 2004; Jin, 2004; Yu, 2008), their presence in loosely bound EPS is an indicator of high amounts of bound water in floc. Concomitantly, the decreased of proteins presence in tightly bound EPS and release to Bulk and LB EPS is an index of loss of floc network strength and integrity. All 3 signals indicate a worsening in dewaterability behaviour, and were found to be correlated to with the water removal capability from floc. Bulk, LB and TB PR correlated strongly with CST (+0.95, +0.94 and -0.90, respectively) and SRF (+0.91, +0.92 and -0.90, respectively). Supernatant and LB proteins having large contributions to a deterioration in dewaterability is confirmed by previous reports (Yu et al., 2008), but not references are found regarding TB proteins.

5.6.4. Humics

The humics concentrations (Figure 18 and Table 14), normalized for VS content, displayed an increase in Bulk and LB EPS going from DF to MD, TD and ASTD, with few exceptions. TB EPS showed a stable content, with the exception of the rise in ASTD sludge.

Humics, not being produced or digested in significant amounts, are a better indicator of extraction efficiency variations (Gjessing, 1976) and EPS phase changes than the other 2 EPS components: carbohydrates and proteins.

Soluble HU increased with 22% in MD and with 477% TD compared to the feed, suggesting a higher rate of solubilization for TD than MD. The effect of the ammonia stripping process was a further solubilization of humics: with 1016% higher than the DF. Bulk HU correlated strongly with CST (+0.97) and SRF (+0.94), thus having an influence on dewaterability. During fermentation, Wilen (2000) also reports an increase in soluble HU, whilst Nielsen (1996) observes steady soluble HU concentrations. Mikkelsen (2002) similarly discovered that thermophilic digestion increases in soluble HU content when compared to mesophilic digestion.

LB carbohydrates increased with mesophilic digestion (+41%) and, even more so, with thermophilic digestion (+396%), indicating an increased extractability for TD compared to MD. ASTD presented a further

solubilization of humics compared to DF (+676%). LB humics were also strongly associated with CST (+0.99) and SRF (+0.96).

Humics in tightly bound EPS were relatively stable and within standard deviation in DF, MD and TD. Only ammonia stripping displayed a doubling of extractible humics, showing for this particular case a severe and deep disruption of the EPS and floc matrix. The relation between TB HU, and CST and SRF was weak (+0.78 and +0.75, respectively). In accord to this study, Nielsen (1996) report increasing EPS HU concentrations during anaerobic digestions. Mikkelsen (2002) noticed a different behaviour, with thermophilic digestion reducing EPS HU compared to mesophilic digestion.

Considering the binding power of humics approaches that of proteins, and considering humics are not notably produced or digested (Nielsen et al., 1996), the accumulation of HU in Bulk and LB EPS phases may have indicated a weakening of floc and a release of cells and organic and inorganic colloids, typically associated to poor dewaterability. This fact was confirmed by the discovered correlations. In contradiction, no correlations are found between humics content and dewaterability in the study of Jin (2004).

For DF and MD humics were preponderant in TB phase, whilst for TD and ASTD humics became prevalent in the Bulk and LB phases, reiterating the notion of a weakened floc due to the severity of treatments it was subjected to.

5.7. Cations

5.7.1. Cations

Overview of full results on the presence of cations, normalized for VS content, in the EPS phases can be found in Annex Tables A17- A19 and Figures A12- A15. All difficulties determined in EPS biopolymers characterisation applied for EPS cation characterisation.

Total cations (Table 15 and Figure 26) presented a steep rise in concentrations during anaerobic digestion and ammonia stripping, but reached similar levels for all processes in all EPS phases without displaying a particular trend.

Bulk cations rose with 96%, 147% and 98% respectively, for MD, TD and ASTD respectively, compared to the feed. LB cations increased in concentrations with 102%, 110% and 142% respectively, for MD, TS and ASTD respectively, compared to DF. Compared to DF, cations in the TB phase surged with 71% in mesophilic digestate and only with 33% in thermophilic digestate, whilst ammonia stripping resulted in 84% higher concentrations.

Total cations in Bulk and TB were found to not or hardly correlate to the dewaterability indicators CST (+0.67 and +0.61, respectively) and SRF (+0.73 and +0.73, respectively), whilst total cations in LB were mildly to strongly associated to CST (+0.84) and SRF (+0.92), reinforcing the role of loosely bound sludge in the floc structure and dewaterability behaviour.

The preponderate quantity of cations was determined to be in suspension, with LB and TB phases following in descending order. This may have been due to cations being more bound to the EPS and floc towards the centre of the floc, resulting in a decreased extractability of these fractions or due to cations remaining unutilized in the EPS bridging and floc forming process.

In all sludge types and EPS phases, monovalent cations were in majority ($\text{NH}_4^+ > \text{Na}^+ > \text{K}^+$), followed by divalent cations ($\text{Ca}^{2+} > \text{Mg}^{2+}$), and trivalent cations were in minority ($\text{Fe}^{3+} > \text{Al}^{3+}$).

However, total cation is only the sum off all cation and its influence on dewaterability is only a reflection of the individual cations dictating the liquid solid separation behaviour.

5.7.2. Sodium

The sodium concentrations (Figure 20 and Table 15), normalized for VS content, displayed no meaningful variation in any of the EPS phases and in any of the sludges.

Soluble Na^+ decrease with 16%, 11% and 7% respectively, for MD, TD and ASTD respectively, compared to the DF. LB sodium concentrations diminished with 11%, 30% and 12% respectively, for MD, TS and ASTD

respectively, compared to DF. Compared to DF, TB Na⁺ content decreased with 7% in mesophilic digestate and with 44% in thermophilic digestate, whilst ammonia stripping resulted in 19% higher concentrations. Similarly to this study, during anaerobic digestion, meaningful variations in Na⁺ content are not noted by Novak (2003).

Bulk, LB and TB Na⁺ concentrations could not be correlated to the dewaterability indicators CST (+0.24, -0.59 and +0.09, respectively) and SRF (+0.06, -0.60 and +0.11, respectively), thus presenting no influence on the liquid solid separation process in this research. However, the presence of Na⁺ has the potential to deteriorate sludge dewaterability as it enters an ion exchange process with Ca²⁺ and Mg²⁺ (Higgins & Novak, 1997; Sobek & Higgins, 2002) and weakens floc bonds, but this was not applicable in this case.

5.7.3. Potassium

The potassium concentrations (Figure 21 and Table 15), normalized for VS content, displayed no trendline variations in any of the EPS phases and in any of the sludge types.

K⁺ increased in soluble fraction through MD (+63%) and even more so through TD (+100%) when compared to the feed, suggesting increased cell lysis (Novak et al., 2003) during fermentation, especially at elevated temperatures. Increased solubilization of K⁺ during anaerobic digestion is frequently remarked (Novak, 2003; Park, 2006; Rasmussen, 1994; Sponza, 2002). A decrease in Bulk K⁺ in ASTD (-19%), when compared to TD, could be explained by: reabsorption in the pellet of K⁺ due to a positive valence void following ammonia stripping, or by precipitation with another compound. The LB and TB K⁺ concentrations raised for MD (+77% and +37%, respectively) and for TD (+57% and +1%, respectively), and varied for ASTD (+52% and -8%, respectively), when compared to the same fractions in the feed. Whilst for TD a shift of K⁺ from LB and TB to solution was possible, for ASTD reabsorption to pellet or precipitation seemed the 2 possible choices.

Bulk, LB and TB K⁺ concentrations could not be correlated to the dewaterability indicators CST (+0.65, +0.41 and -0.53, respectively) and SRF (+0.70, +0.55 and -0.37, respectively), thus presenting no influence on the liquid solid separation process. Contrary to other researchers, in this case, the presence of K⁺ did not deteriorate sludge dewaterability (Higgins, 1997; Sobek, 2002).

Source	Bulk	LB	TB	Total
	Avg. per sludge type [mmol/g VS]			
Sodium ("Na+")				
DF	1.6678	0.9436	0.6418	3.2532
MD	1.3944	0.8411	0.5952	2.8307
TD	1.7003	0.6797	0.3615	2.7415
ASTD	1.5955	0.8292	0.7656	3.1903
Potassium ("K+")				
DF	0.1626	0.0787	0.0512	0.2925
MD	0.2643	0.1396	0.0702	0.4742
TD	0.3250	0.1238	0.0516	0.5005
ASTD	0.2638	0.1200	0.0471	0.4309
Ammonium ("NH4+")				
DF	0.2785	0.1385	0.1809	0.5979
MD	2.7061	1.4668	0.8776	5.0505
TD	3.5084	1.7506	0.7845	6.0435
ASTD	2.5477	1.9644	0.8465	5.3585
Calcium ("Ca2+")				
DF	0.0719	0.0325	0.0221	0.1265
MD	0.0460	0.0184	0.0087	0.0731
TD	0.0241	0.0120	0.0057	0.0419
ASTD	0.0179	0.0164	0.0006	0.0349
Magnesium ("Mg2+")				
DF	0.0665	0.0273	0.0148	0.1087
MD	0.0022	0.0045	0.0111	0.0177
TD	0.0074	0.0059	0.0074	0.0207
ASTD	0.0244	0.0189	0.0112	0.0545
Iron ("Fe2++Fe3+")				
DF	0.0008	0.0010	0.0018	0.0035
MD	0.0007	0.0012	0.0025	0.0044
TD	0.0022	0.0048	0.0039	0.0108
ASTD	0.0032	0.0083	0.0033	0.0148
Total cations ("TotC")				
DF	2.2483	1.2220	0.9131	4.3834
MD	4.4137	2.4716	1.5656	8.4509
TD	5.5679	2.5776	1.2152	9.3606
ASTD	4.4531	2.9584	1.6763	9.0877

Table 15 Cations

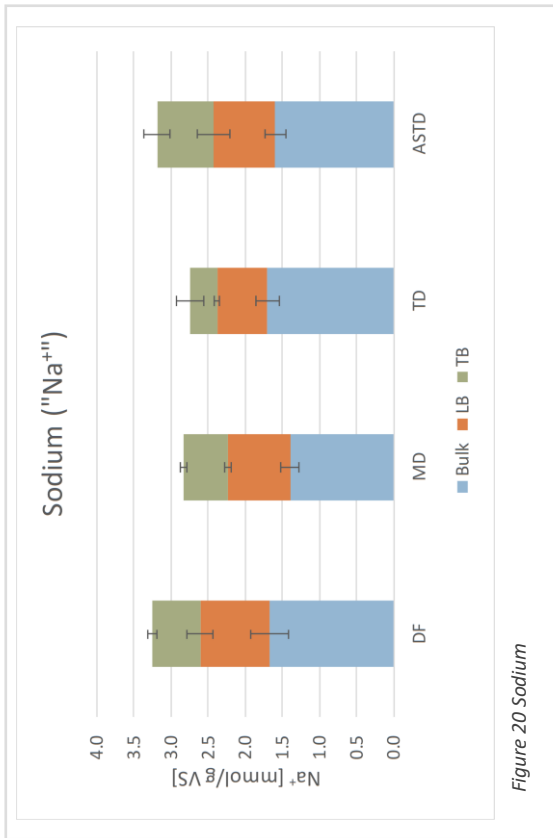


Figure 20 Sodium

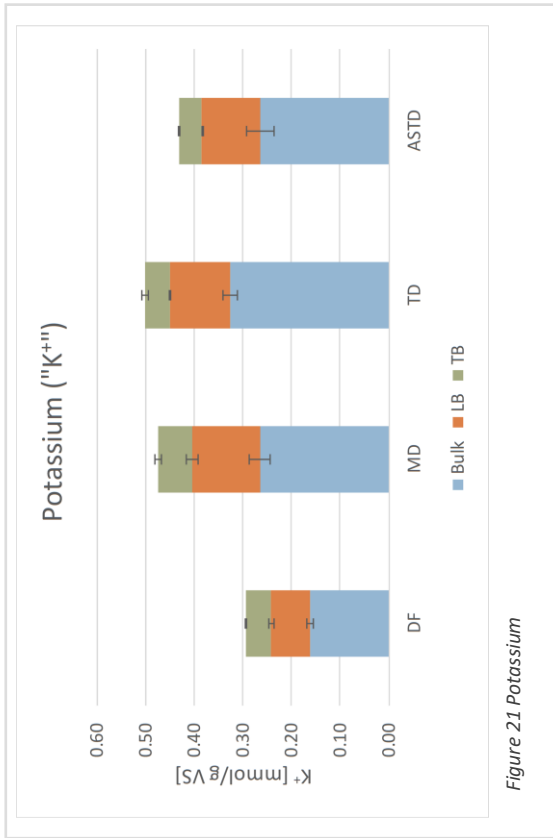


Figure 21 Potassium

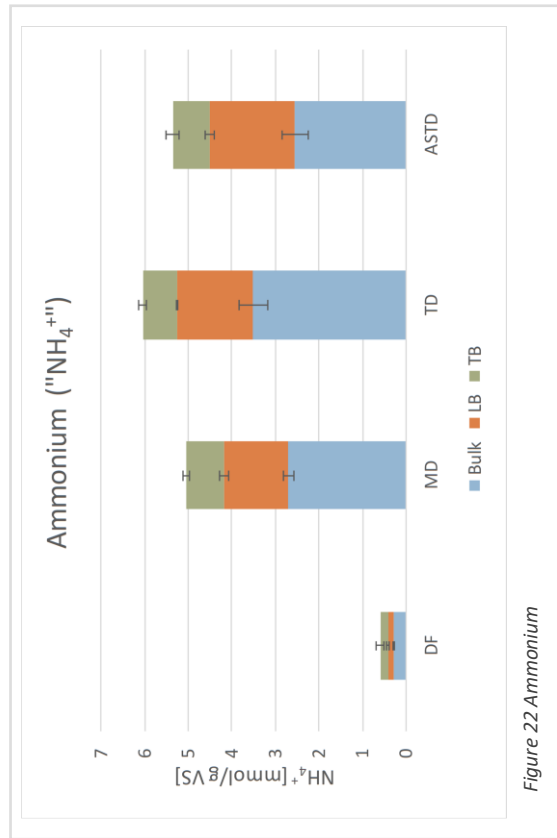


Figure 22 Ammonium

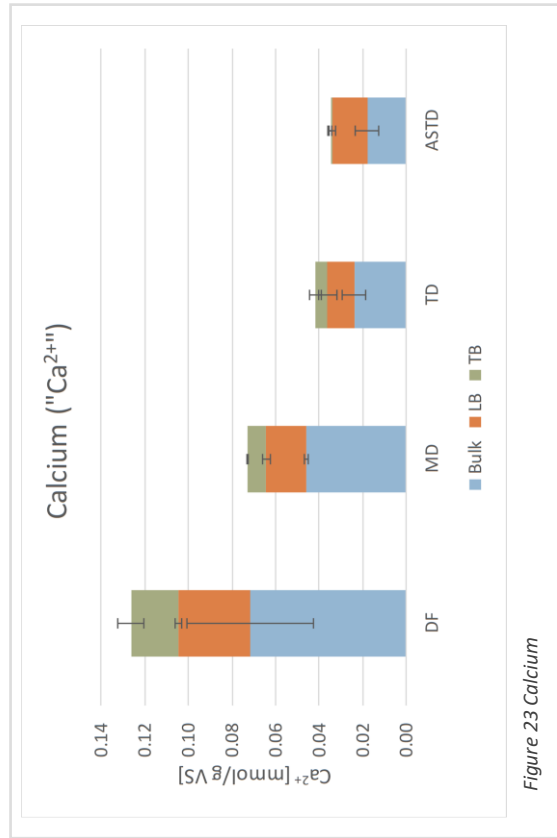


Figure 23 Calcium

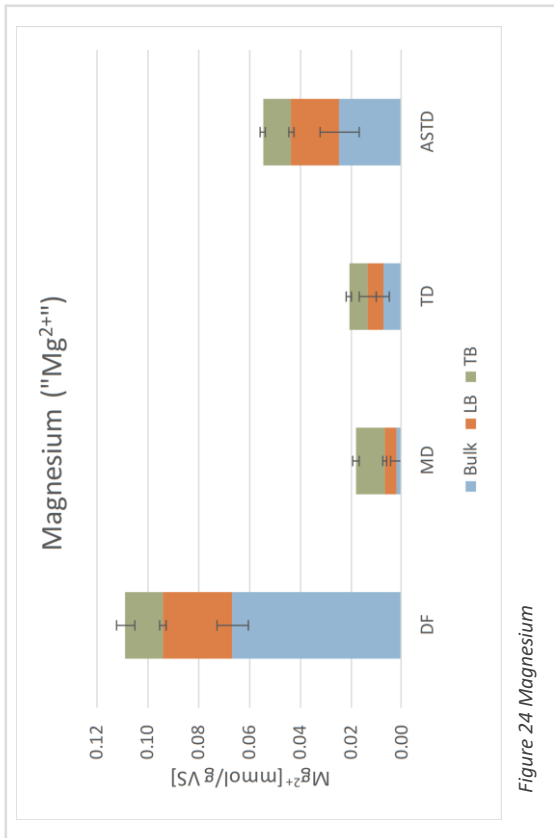


Figure 24 Magnesium

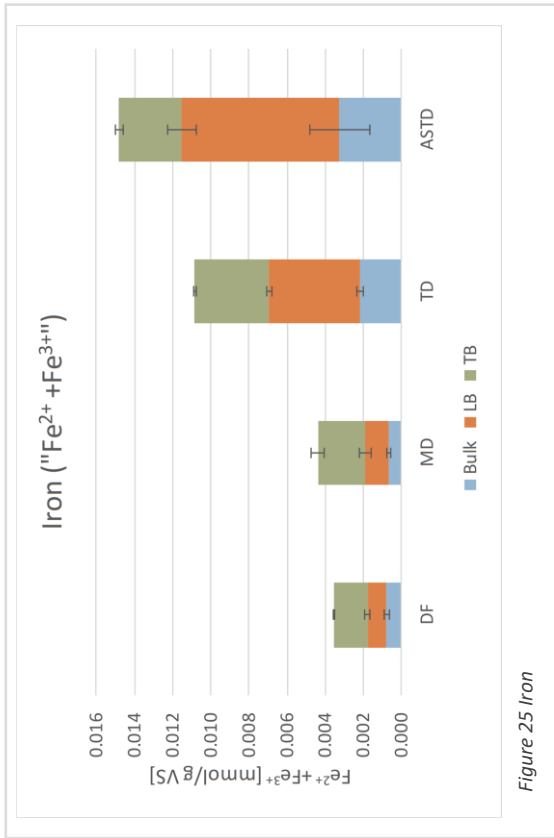


Figure 25 Iron



Figure 26 Cations

5.7.4. Ammonium

Ammonium concentrations (Figure 22 and Table 15), normalized for VS content, increased in all EPS phases with the increased harshness of treatment, except the soluble NH_4^+ in ASTD which was stripped.

Compared to the feed, the ammonium concentrations in the Bulk phases increased with MD (+871%) and even more so with TD (+1159%), which could be the effect of an increased protease at 55°C compared to 35°C. Increased solubilization of ammonium during anaerobic digestion was previously observed in various researches (Novak, 2003; Park, 2006; Foladori, 2015; Park, 2007; Rasmussen, 1994). A drop in Bulk NH_4^+ was noted in the ASTD (-27%), when compared to the TD, which corresponded to the ammonia stripping process. However, the stripping process appeared limited and inefficient compared to the performance of other ammonia strippers (Bonmati, 2003; Walker, 2011; Gustin, 2011; Lauren, 2013). LB ammonium concentrations enlarged with 959%, 1163% and 1318% respectively, for MD, TS and ASTD respectively, compared to DF. TB ammonium concentrations enlarged with 385%, 334% and 369% respectively, for MD, TS and ASTD respectively, compared to DF. According to the ammonium presence in the EPS phases, thermophilic digestion protease appeared to be more active in the solution and in the loosely bound floc fraction; whilst for the tightly bound floc fraction mesophilic digestion degraded more proteins. The increase in LB and TB NH_4^+ of ASTD compared to TD was assumed not to have originated from protease, but from the stripping process making the component more available for extraction without actually stripping it.

Bulk and TB NH_4^+ concentrations presented none to extremely low associations with the dewaterability indicators CST (+0.64 and +0.63, respectively) and SRF (+0.71 and +0.76, respectively), whilst LB NH_4^+ presented a median to strong relationship with CST (+0.84) and SRF (+0.91). Correlations between ammonia and dewaterability may have come as a secondary effect of the strong correlations between proteins, and CST and SRF, without NH_4^+ having an actual influence.

5.7.5. Calcium

The calcium concentrations (Figure 23 and Table 15), normalized for VS content, decreased in all phases with the increased harshness of the treatment to which the sludge was subjected to, with few exceptions. Some high standard deviations were found and can be seen in the graphical representation.

Soluble Ca^{2+} decrease with 36%, 66% and 75% respectively, for MD, TD and ASTD respectively, compared to the DF. Contrary to this report, soluble calcium during anaerobic digestion is frequently reported to increase (Rasmussen, 1994; Bruus, et al., 1993; Park, 2006) or stagnate (Park, 2007; Novak, 2003). LB calcium concentrations diminished with 43%, 62% and 49% respectively, for MD, TS and ASTD respectively, compared to DF. Compared to DF, TB Ca^{2+} content decreased with 60% in mesophilic digestate and with 74% in thermophilic digestate, whilst ammonia stripping resulted in 97% lower concentrations. The decrease of calcium in all EPS phases could have been the result of: solubilization followed by, more likely, immediate precipitation with a common anion or by, less likely, filling of the void of required positive valences created by the reduction of Fe^{3+} in anaerobic conditions through cation exchange of Ca^{2+} with Fe^{2+} (Nielsen & Keiding, 1998). Floc calcium decrease in floc has been also reported by Mikkelsen (2002) for MD and, even more so, for TD.

Bulk and TB Ca^{2+} concentrations presented strong associations with the dewaterability indicators CST (-0.94 and -0.89, respectively) and SRF (-0.97 and -0.96, respectively), whilst LB Ca^{2+} presented a low correlation with CST (-0.74) and SRF (-0.80).

5.7.6. Magnesium

The magnesium concentrations (Figure 24 and Table 15), normalized for VS content, diminished severely in all phases of the MD. In the TD and the ASTD, the Mg^{2+} presence in all phases increased compared to MD, but remained lower than the DF. Some high standard deviations were found and can be seen in the graphical representation.

Soluble Mg^{2+} decrease with 97%, 89% and 63% respectively, for MD, TD and ASTD respectively, compared to the DF. Opposite to this research, soluble magnesium during anaerobic digestion is frequently

reported to increase (Sponza, 2002; Bruus, et al., 1993; Park, et al., 2006) or stagnate (Park & Novak, 2007) (Novak et al., 2003). LB magnesium concentrations diminished with 84%, 78% and 31% respectively, for MD, TS and ASTD respectively, compared to DF. Compared to DF, TB Mg^{2+} content decreased with 25% in mesophilic digestate and with 50% in thermophilic digestate, whilst ammonia stripping resulted in 24% lower concentrations. The variations in magnesium in all EPS phases could have been the result of: solubilization followed by immediate precipitation with a common anion or, to a lower degree, decreased extractability due to floc binding. However, the reason for which the decrease in magnesium was most accentuated with the least harsh treatment, could not be found. Increased magnesium concentrations in all EPS phases of ASTD, compared to TD, were owed to the $Mg(OH)_2$ base addition in the ammonia stripping process.

Bulk, LB and TB Mg^{2+} concentrations could not be correlated to the dewaterability indicators CST (-0.42, -0.17 and -0.59, respectively) and SRF (-0.55, -0.29 and -0.62, respectively), thus presenting no influence on the liquid solid separation process.

5.7.7. Iron

The iron concentrations (Figure 25 and Table 15), normalized for VS content, increased in all phases with the increased harshness of the treatment to which the sludge was subjected to. It is important to be kept in mind that the iron measurements include both Fe(II) and Fe(III).

Soluble Fe concentrations surged with 11%, 178% and 315% respectively, for MD, TD and ASTD respectively, compared to the DF. The solubilization of iron (di- or trivalent) in anaerobic digestion is commonly remarked in literature (Bruus, 1993; Park, 2006; Rasmussen, 1994). However, the iron release can appear as a constant concentration, due to precipitation of the released cation as FeS (Novak et al., 2003). LB iron presence enlarged with 20%, 375% and 723% respectively, for MD, TS and ASTD respectively, compared to DF. Compared to DF, TB Fe content increased with 42% in mesophilic digestate and with 122% in thermophilic digestate, whilst ammonia stripping resulted in 88% higher concentrations. Contrary to this study, Mikkelsen (2002) observed a decrease in floc iron in MD and, even more so, in TD which could be true if the measurement included only the trivalent iron cation. The escalating Fe concentration in all phases of the anaerobic digestion were the effect of iron reduction, resulting possibly in: loss of binding strength with other EPS components, cation exchange with calcium, release from floc and increased extractability. The same does not apply for ammonia stripped thermophilic digestate, as the stripper operates aerobically. It was considered reoxidation of iron would lead to rebinding in floc with all EPS components (CA, PR and HU) which were available in high concentrations, but this process was not observed. An explanation for the increased iron concentrations in ASTD further release of iron during floc disintegration. If a precipitation as iron hydroxides due to increased pH, the $Fe(OH)_3$ should have presented a particle size lower than $0.45\mu m$ in order to pass the sample filtration process and be measured at a later time.

Bulk and LB Fe^{3+} concentrations presented extremely strong correlations with the dewaterability indicators CST (+0.98 and +0.98, respectively) and SRF (+0.93 and +0.95, respectively), whilst LB Fe^{3+} presented a median relationship with CST (+0.83) and SRF (+0.83). Existing research similarly strongly correlates the presence of bound iron with improved dewaterability (Jin, 2004; Li, 2012; Li, 2005; Nielsen, 1998). The stronger influence of iron on dewaterability was also remarked by the researches associated to the Bath WWTP project. Similar to this study, they remarked iron presented the highest binding power, followed in decreasing order of bridging strength by calcium and magnesium.

5.7.8. Aluminium

Bulk, LB and TB concentrations of aluminium were determined to be close or below detection limit, and thus were not presented or discussed.

5.8. Other observation

Due to the high correlations found between Ca^{2+} and Fe^{3+} , and dewaterability indicators, it was of interest to determine if the two cations were associated in any manner to the biopolymers components (Table 16).

Ca^{2+} could be mildly associated with the Bulk phase of CA, PR and HU and with the TB phase of CA and PR, without presenting any other associations. Existing literature shows strong correlation between calcium, and protein and humics, but no association between calcium and carbohydrates (Gjessing, 1976; Higgins, 1997; Urbain, 1993). Ca^{2+} displayed, as expected due to its lower valence, a diminished binding power compared to Fe^{3+} .

Fe^{3+} or Fe^{2+} strongly correlated with the Bulk and LB phases of carbohydrates and humics, but not with their TB phase. The presence of Fe was accurately associated with the presence of proteins in all EPS phases, confirming the Fe preference for proteins along with its capacity to bind alternative compounds: carbohydrates and humics (Li, 2005; Gjessing, 1976).

	Bulk CA	LB CA	TB CA	Bulk PR	LB PR	TB PR	Bulk HU	LB HU	TB HU
Bulk Ca^{2+}	-0.83	-	-	-0.80	-	-	-0.85	-	-
LB Ca^{2+}	-	-0.54	-	-	-0.50	-	-	-0.64	-
TB Ca^{2+}	-	-	-0.89	-	-	0.89	-	-	-0.58
Bulk Fe^{3+}	0.98	-	-	0.98	-	-	0.99	-	-
LB Fe^{3+}	-	0.99	-	-	0.98	-	-	1.00	-
TB Fe^{3+}	-	-	0.63	-	-	-0.99	-	-	0.31

Table 16 Correlations calcium and iron to carbohydrates, proteins and humics

Chapter 6 Conclusions and recommendations

6.1. Conclusions

In a WWTP operating with full scale mesophilic digestion, a thermophilic digestion pilot and a digestate ammonia stripping pilot were installed with the purpose of determining the origin of the inferior dewaterability of the sludges resulting from the aforementioned processes. The grounds for the initiative were economical and practical in nature, as the disadvantage of high cost of treatment and disposal of sludges could outweigh the advantage of energy or nutrient recovery, and as the search for an efficient counteractive applied treatment would be a black box approach without understanding the causes of the poor dewaterability. Existing research is primarily focused on the association between characteristics of primary and secondary sludges and filtration potential, and presents serious limitations and contradictions. An analysis on the characteristics of anaerobic digestates and the relation to dewaterability behaviour was not found.

Consequently, the current research attempted to find the potential causes of the unsatisfactory drainage by comparing four sludges: digester feed, mesophilic digestate, thermophilic digestate and ammonia stripped thermophilic digestate. Numerous parameters were analysed and verified for a correlation to dewaterability. Table 19 presents the influence of each parameter on the dewaterability and its variation in each digestate type.

Firstly, anaerobic digestion and, even more so, anaerobic digestate ammonia stripping resulted in a worsening in dewaterability, as signalled by the capillary suction time and the sludge resistance to filtration. Furthermore, anaerobic thermophilic digestion displayed a poorer dewaterability compared to anaerobic mesophilic digestion.

The inferior dewaterability was the consequence of a severe floc destruction accompanied by a substantial release of colloidal and subcolloidal fractions, whilst the supracolloidal fraction remained unaltered and inconsequential. Moreover, as dewaterability deteriorated, the released components were more organic than inorganic in nature, indicating a release of bacterial cells, biopolymers and other organics. Furthermore, the high negative influence of an increased colloidal fraction on dewaterability appears to be related in majority to the quantity and solely in minority to the size or charge of colloids.

The idea of release of organic material, from the tightly bound floc matrix to the solution and to the loosely bound floc network, resulting in poor drainage was reinforced by the chemical oxygen demand measurement. Further, the Bulk and LB biopolymers presented a similar behaviour and correlation to dewaterability as the COD, unlike the TB biopolymers, suggesting a loosening and breakage of the floc network and a strong negative impact of the soluble and diffuse floc phases on dewaterability. Moreover, TB biopolymers were the predominant component in digester feed and anaerobic mesophilic digestate; whilst Bulk and LB biopolymers dominated the anaerobic thermophilic digestate and ammonia stripped thermophilic digestate, suggesting a diffuse floc capable of holding large amounts of water whilst incapable or retaining wastewater components for the two latter sludge types. The influence of individual biopolymeric compounds was also investigated. Humics were the predominant EPS fraction in all sludges. Proteins were the 2nd most common component in DF and MD; whilst carbohydrates were the 2nd most common component in TD and ASTD. Similar to the overall biopolymer, the increased presence of carbohydrates, proteins and humics in both Bulk and LB phases was strongly correlated to inferior filtration of digestates, whereas in the TB phase only the decreased presence of proteins was associated to substandard drainage of slurries.

As biopolymers and cations create the floc network in which the other floc constituents are immobilized, the cationic presence and distribution were analysed. Total cations mildly to strongly a positively correlated to the unsatisfactory dewaterability in the LB phase, whereas the total cations

concentrations in Bulk and TB phases were found of no or weak indication. For all sludges the preponderant quantity of cations was determined to be in suspension, with LB and TB phases following in descending order, showing the full bridging capacity of cations was unutilized. The influence of individual cationic components was also examined. In all sludge types and EPS phases, monovalent cations were in majority ($\text{NH}_3 > \text{Na}^+ > \text{K}^+$), followed by divalent cations ($\text{Ca}^{2+} > \text{Mg}^{2+}$), whilst trivalent cations were in minority ($\text{Fe}^{3+} > \text{Al}^{3+}$). Monovalent cations presented no significant correlations to dewaterability indicators. Similarly, the magnesium divalent cation and the aluminium trivalent cation were found of insignificant correlation to sludge drainage capacity. Oppositely, the calcium divalent cation and the iron trivalent cation were found responsible for the dewaterability behaviour. Whilst a decrease in Ca^{2+} was associated to poor dewaterability in all EPS phases: strongly correlated in Bulk and TB phases and weakly related in LB phase; increase in Bulk and TB EPS Fe concentrations very strongly negatively impacted sludge drainage, and increase in LB EPS Fe presence negatively averagely related with dewaterability ability.

Lastly, an association between biopolymers and cations was examined. Ca^{2+} could be mildly negatively associated with the Bulk phase of carbohydrates, proteins and humics, and with the TB phase of carbohydrates and proteins, without presenting any other associations. Fe^{3+} or Fe^{2+} strongly positively correlated with the Bulk and LB phases of carbohydrates and humics, but not with their TB phase. The presence of Fe was accurately positively associated with the presence of proteins in all EPS phases.

In conclusion, the release of colloids, independent of their nature, was the origin of the poor dewaterability behaviour. The phenomenon was accompanied by an increase in carbohydrates, proteins and humics in solution and loosely bound floc matrix, and by a decrease of calcium and an increase of iron presences in the bulk and tightly bound floc network. From the extracellular polymeric substances components, proteins and iron display the strongest impact on digestate dewaterability. Recognizing the dewatering determinant factors enables the search for digestate specific conditioning and dewatering methods

6.2. Recommendations

Throughout the current study a number of general research impediments and limitations became apparent, and a requirement for their remediation or a need for their investigation became unequivocal.

First and foremost, there is need for the unification and standardization of extracellular polymeric substances extraction and characterisation methods, in order to avoid contradictory outcomes of studies and in order to allow for the research to produce accurate and coherent theories. The utilized of extracellular polymeric substances extraction and characterisation methods should display constant efficiencies independent of the sludge type, extracellular polymeric substances phase or biopolymer fraction, in order to allow for a proper sludge characterisation and for a distinction between degradation and shift of components. To this aim, innovative methods for extracellular polymeric substances extraction and characterisation should also be attempted (ex: gas chromatography, scanning electron microscopy, environmental scanning electron microscopy, atomic force microscopy, confocal laser scanning, quartz crystal microbalance, X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, Raman spectroscopy, 3-Dimensional excitation–emission matrix fluorescence spectroscopy, nuclear magnetic resonance, high-performance size exclusion chromatography, time of flight secondary ion mass spectrometry as reviewed by Sheng (2010)).

Second, other techniques for dewaterability determination and sludge characterisation could be essayed, in order to improve determination precision or to bring additional information (ex: titration for surface charge characterisation; sieving and filtering for particle size fractionation characterisation; hydrophobicity, optimum polymer dose, viscosity, cake compressibility and cake permeability for dewaterability determination).

Last but not least, although the current study indicated the factors which impacted dewaterability in the comparison between anaerobic mesophilic, thermophilic and ammonia stripped thermophilic digestates, a following research should investigate the manner and the proportion in which these factors influenced dewaterability. A different ensuing investigation should identify practical measures to counter dewaterability deficiencies.

Parameter	Influence on dewaterability	Variation in sludge compared to DF		
		MD	TD	ASTD
Capillary suction time	-	↗	↗↗	↗↗↗
Sludge resistance to filtration	-	↗	↗↗	↗↗↗
Volatile solids colloid	High	↗	↗↗	↗↗↗
Total solids colloid	Mediu	↘	↗↗	↗↗↗
Volatile solids/Total solids colloid	High	↗	↗↗	↗↗↗
Particle size suspension	None	↘↘	↘↘↘	↘
Particle size colloid	Low	↗	↗↗↗	↗↗
Surface charge colloid	Low to medium	↗↗	↗↗	↗↗
Bulk Chemical oxygen demand	High	=↘	↗↗	↗↗↗
LB Chemical oxygen demand	High	↗	↗↗	↗↗↗
TB Chemical oxygen demand	High	↘	↘↘	↘↘↘
Bulk Biopolymers	High	↗	↗↗	↗↗↗
LB Biopolymers	High	↗	↗↗	↗↗↗
TB Biopolymers	None	=↘	=↘	↗
Bulk Carbohydrates	High	↗	↗↗	↗↗↗
LB Carbohydrates	High	↗	↗↗	↗↗↗
TB Carbohydrates	None to low	↗↗↗	↗	↗↗
Bulk Proteins	High	=↘	↗↗	↗↗↗
LB Proteins	High	=↗	↗↗	↗↗↗
TB Proteins	High	↘	↘↘↘	↘↘
Bulk Humics	High	↗	↗↗	↗↗↗
LB Humics	High	↗	↗↗	↗↗↗
TB Humics	Low	↘↘	↘	↗↗
Bulk Cations	None	↗	↗↗↗	↗↗
LB Cations	Medium to high	↗	↗↗	↗↗↗
TB Cations	Low	↗↗	↗	↗↗↗
Bulk Sodium	None	↘↘	=↗	=↘
LB Sodium	None	↘	↘↘↘	↘↘
TB Sodium	None	=↘	↘↘	↗↗
Bulk Potassium	None	↗↗	↗↗↗	↗↗
LB Potassium	None	↗↗↗	↗↗	↗↗
TB Potassium	None	↗↗	=↗	=↘
Bulk Ammonium	None to low	↗↗	↗↗↗	↗
LB Ammonium	Medium to high	↗	↗↗	↗↗↗
TB Ammonium	None to low	↘↘	↘↘	↘↘
Bulk Calcium	High	↘	↘↘	↘↘↘
LB Calcium	Low to medium	↘	↘↘↘	↘↘
TB Calcium	Medium to high	↘	↘↘	↘↘↘
Bulk Magnesium	None	↘↘↘	↘↘	↘
LB Magnesium	None	↘↘↘	↘↘	↘
TB Magnesium	None	↘↘	↘↘↘	↘↘
Bulk Iron	High	=↘	↗↗	↗↗↗
LB Iron	Medium	=↗	↗↗	↗↗↗
TB Iron	High	↗	↗↗↗	↗↗

Table 17 Results overview

Bibliography

- Abu-Orf, M., Muller, C., Park, C., & Novak, J. (2004). Innovative technologies to reduce water content of dewatered municipal residuals. *Journal of residuals science & technology*, 1(2), 83-91.
- AOAC. (1995). *Official Methods of Analysis 16th Edition*. Gaithersburg: AOAC International.
- Avella, A., Görner, T., & de Donato, P. (2010). The pitfalls of protein quantification in wastewater treatment studies. *Science of the total environment* 408(20), 4906-4909.
- Baskerville, R., & Gale, R. (1968). A simple automatic instrument for determining the filterability of sewage sludge. *J. Instit. Water Pollut. Control* 67, 233–241.
- Bonmati, A., & Flotats, X. (2003). Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre-or post-treatment to mesophilic anaerobic digestion. *Waste management* 23(3), 261-272.
- Box, J. (1983). Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water research*, 17(5), 511-525.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72(1-2), 248-254.
- Brown, M., & Lester, J. (1980). Comparison of bacterial extracellular polymer extraction methods. *Applied and Environmental Microbiology* 40(2), 179-185.
- Bruus, J., Christensen, J., & Rasmussen, H. (1993). Anaerobic storage of activated sludge: effects on conditioning and dewatering performance. *Water Science and Technology* 28(1), 109-116.
- Buhr, H., & Andrews, J. (1977). The thermophilic anaerobic digestion process. *Water Research* 11(2), 129-143.
- Cetin, S., & Erdinçler, A. (2004). The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. *Water Science and Technology* 50(9), 49-56.
- Chassagne, C., Dubois, E., Jiménez, M., Van Der Ploeg, J., & Van Turnhout, J. (2016). Compensating for electrode polarization in dielectric spectroscopy studies of colloidal suspensions: theoretical assessment of existing methods. *Frontiers in chemistry* 4.
- Christensen, G., & Dick, R. (1985). Specific Resistance Measurements: Methods and Procedures. *J. Environ. Eng.* 111, 258.
- Coelho, N., Droste, R., & Kennedy, K. (2011). Evaluation of continuous mesophilic, thermophilic and temperature phased anaerobic digestion of microwaved activated sludge. *Water research* 45(9), 2822-2834.
- Davis, E. M. (1988). Protein assays: review of common techniques. *Am. Biotechnol. Lab.* 6, 28-37.
- De la Rubia, M., Walker, M., Heaven, S., Banks, C., & Borja, R. (2010). Preliminary trials of in situ ammonia stripping from source segregated domestic food waste digestate using biogas: Effect of temperature and flow rate. *Bioresource technology* 101(24), 9486-9492.
- Dignac, M., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., & Scribe, P. (1998). Chemical description of extracellular polymers: implication on activated sludge floc structure. *Water Science and Technology* 38(8-9), 45-53.
- Dreywood, R. (1946). Qualitative test for carbohydrate material. *Industrial & Engineering Chemistry Analytical Edition* 18(8), 499-499.
- DuBois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28, 350-356.
- Eaton, A., Clesceri, L., Rice, E., Greenberg, A., & Franson, M. (2005). APHA: standard methods for the examination of water and wastewater. *Centennial Edition., APHA, AWWA, WEF, Washington, DC.*

- Esparza-Soto, M., & Westerhoff, P. (2003). Biosorption of humic and fulvic acids to live activated sludge biomass. *Water research* 37(10), 2301-2310.
- Fang, H., & Jia, X. (1996). Extraction of extracellular polymer from anaerobic sludges. *Biotechnology techniques* 10(11), 803-808.
- Foladori, P., Velho, V., Costa, R., Bruni, L., Quaranta, A., & Andreottola, G. (2015). Concerning the role of cell lysis-cryptic growth in anaerobic side-stream reactors: the single-cell analysis of viable, dead and lysed bacteria. *Water research* 74, 132-142.
- Frolund, B., Griebe, T., & Nielsen, P. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology* 43(4), 755-761.
- Frolund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research*, 30(8), 1749-1758.
- Gale, R., & Baskerville, R. (1967). Capillary suction method for determination of the filtration properties of a solid/liquid suspension. *Chem. Ind.* 9, 336-355.
- Gjessing, E. T. (1976). *Physical and chemical characteristics of aquatic humus*. Ann Arbor, Michigan: Ann Arbor Science.
- Gustin, S., & Marinsek-Logar, R. (2011). Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. *Process Safety and Environmental Protection* 89(1), 61-66.
- Handa, N. (1966). Examination on the applicability of the phenol sulfuric acid method to the determination of dissolved carbohydrate in sea water. *J. Oceanogr. Soc. Japan* 22(3), 79-86.
- Higgins, M. J., & Novak, J. T. (1997). Dewatering and settling of activated sludges: The case for using cation analysis. *Water Environment Research*, 69(2), 225-232.
- Higgins, M., & Novak, J. (1997). The effect of cations on the settling and dewatering of activated sludges: laboratory results. *Water Environment Research*, 69(2), 215-224.
- Houghton, J., & Stephenson, T. (2002). Effect of influent organic content on digested sludge extracellular polymer content and dewaterability. *Water Research* 36(14), 3620-3628.
- Houghton, J., Quarmby, J., & Stephenson, T. (2000). The impact of digestion on sludge dewaterability. *Process Safety and Environmental Protection* 78(2), 153-159.
- Jin, B., Wilén, B., & Lant, P. (2004). Impacts of morphological, physical and chemical properties of sludge flocs on dewaterability of activated sludge. *Chemical Engineering Journal* 98(1), 115-126.
- Karr, P., & Keinath, T. (1978). Influence of particle size on sludge dewaterability. *Water Pollution Control Federation*, 1911-1930.
- Koehler, L. (1952). Differentiation of carbohydrates by anthrone reaction rate and color intensity. *Analytical Chemistry* 24(10), 1576-1579.
- Laureni, M., Palatsi, J., Llovera, M., & Bonmati, A. (2013). Influence of pig slurry characteristics on ammonia stripping efficiencies and quality of the recovered ammonium-sulfate solution. *Journal of Chemical Technology and Biotechnology* 88(9), 1654-1662.
- Lei, X., Sugiura, N., Feng, C., & Maekawa, T. (2007). Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas purification. *Journal of hazardous materials* 145(3), 391-397.
- Leis, A., & Flemming, H. (2002). Carbon transformations and activity in biofilms. *Encyclopedia of Environmental Microbiology*.
- Li, H., Wen, Y., Cao, A., Huang, J., Zhou, Q., & Somasundaran, P. (2012). The influence of additives (Ca²⁺, Al³⁺, and Fe³⁺) on the interaction energy and loosely bound extracellular polymeric substances (EPS) of activated sludge and their flocculation. *Bioresource technology*, 114, 188-194.
- Li, J. (2005). Effects of Fe (III) on floc characteristics of activated sludge. *Journal of Chemical Technology and Biotechnology* 80(3), 313-319.

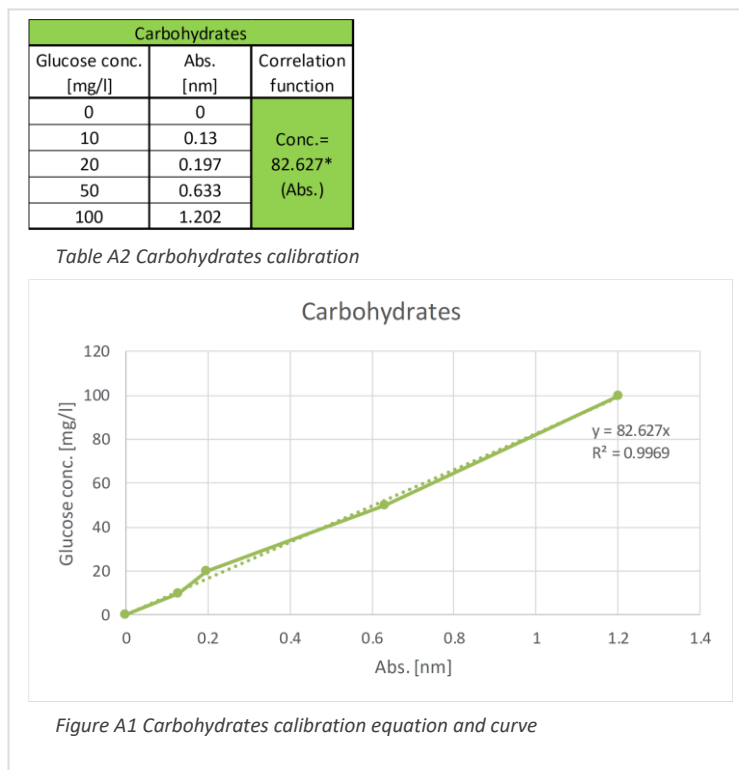
- Li, X., & Yang, S. (2007). Influence of loosely bound extracellular polymeric (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research* 41, 1022- 1030.
- Liao, B., Allen, D., Droppo, I., Leppard, G., & Liss, S. (2001). Surface properties of sludge and their role in bioflocculation and settleability. *Water research* 35(2), 339-350.
- Liao, P., Chen, A., & Lo, K. (1995). Removal of nitrogen from swine manure wastewaters by ammonia stripping. *Bioresource Technology* 54(1), 17-20.
- Liu, H., & Fang, H. (2002). Extraction of extracellular polymeric substances (EPS) of sludges. *Journal of biotechnology* 95(3), 249-256.
- Liu, L., Pang, C., Wu, S., & Dong, R. (2015). Optimization and evaluation of an air-recirculated stripping for ammonia removal from the anaerobic digestate of pig manure. *Process Safety and Environmental Protection* 94, 350-357.
- Loeb, A., Overbeek, J., & Wiersema, P. (1961). The electrical double layer around a spherical colloid particle. *Journal of The Electrochemical Society*, 108(12), 269C-269C.
- Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193(1), 265-275.
- Luo, K., Yang, Q., Li, X., Zhang, S., Pang, Y., Li, X., & Liao, X. (2015). Effect of Calcium Ions on Dewaterability of Enzymatic-Enhanced Anaerobic Digestion Sludge. *Applied biochemistry and biotechnology* 176(8), 2346-2357.
- Matassa, S., Batstone, D., Hulsen, T., Schnoor, J., & Verstraete, W. (2015). Can direct conversion of used nitrogen to new feed and protein help feed the world? *Environ. Sci. Technol.* 49 (9), 5247–5254.
- Mikkelsen, L., & Keiding, K. (2002). Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water research* 36(10), 2451-2462.
- Morgan, J., Forster, C., & Evinson, L. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research* 24(6), 743- 750.
- Neyens, E., Baeyens, J., & Dewil, R. (2004). Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *Journal of hazardous materials* 106(2), 83-92.
- Nielsen, P., & Jahn, A. (1999). Extraction of EPS. *Microbial extracellular polymeric substances*, 49-72.
- Nielsen, P., & Keiding, K. (1998). Disintegration of activated sludge flocs in presence of sulfide. *Water Research*, 32(2), 313-320.
- Nielsen, P., Frølund, B., & Keiding, K. (1996). Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage. *Applied microbiology and biotechnology* 44(6), 823-830.
- Novak, J., Goodman, G., Pariroo, A., & Huang, J. (1988). The blinding of sludges during filtration. *J. Water Pollut. Control Fed.* 60, 206–214.
- Novak, J. T. (2010). The role of organic colloids in dewatering. *Drying Technology*, 28(7), 871-876.
- Novak, J., Sadler, M., & Murthy, S. (2003). Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Research*, 37(13), 3136-3144.
- Park, C., & Novak, J. T. (2007). Characterization of activated sludge exocellular polymers using several cation-associated extraction methods. *Water research* 41(8), 1679-1688.
- Park, C., Abu-Orf, M., & Novak, J. (2006). The digestibility of waste activated sludges. *Water environment research* 78(1), 59-68.
- Peterson, G. (1979). Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. *Analytical biochemistry* 100(2), 201-220.
- Qi, Y., Thapa, K., & Hoadley, F. (2011). Application of filtration aids for improving sludge dewatering properties – A review. *Chemical Engineering Journal* 171, 373– 384.

- Rasmussen, H., Bruus, J., Keiding, K., & Nielsen, P. (1994). Observations on dewaterability and physical, chemical and microbiological changes in anaerobically stored activated sludge from a nutrient removal plant. *Water research* 28(2), 417-425.
- Raunkjær, K., Hvitved-Jacobsen, T., & Nielsen, P. (1994). Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. *Water research*, 28(2), 251-262.
- Rosenberger, S. (2003). Charakterisierung van belebtem Schlamm in Membranbelebungsreaktoren zur Abwasserreinigung PhD thesis. *University of Berlin*.
- Serrano, A., Hendrickx, T., Elissen, H., Laarhoven, B., Buisman, C., & Temmink, H. (2016). Can aquatic worms enhance methane production from waste activated sludge? *Bioresource technology* 211, 51-57.
- Sheng, G., Yu, H., & Li, X. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnology advances* 28(6), 882-894.
- Shin, H., Kang, S., & Nam, S. (2001). Effect of carbohydrate and protein in the EPS on sludge settling characteristics. *Water Science and Technology* 43(6), 193-196.
- Sobeck, D., & Higgins, M. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water research*, 36(3), 527-538.
- Sponza, D. T. (2002). Extracellular polymer substances and physicochemical properties of flocs in steady and unsteady-state activated sludge systems. *Process Biochemistry* 37(9), 983-998.
- Suhartini, S., Heaven, S., & Banks, C. (2014). Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: performance, dewaterability and foam control. *Bioresource technology* 152, 202-211.
- Sutherland, I. (2001). Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147(1), 3-9.
- Swartz, E., Shi, Q., Davidovits, P., Jayne, J., Worsnop, D., & Kolb, C. (1998). Uptake of gas-phase ammonia by aqueous/aqueous acid solutions. *Journal of Aerosol Science* 29, S985-S986.
- Tchobanoglous, G., Burton, F., Stensel, H., & Tsuchihashi, R. (2014). *Wastewater engineering: treatment and reuse*. New York: McGraw-Hill.
- te Poele, S. (2006). Fouling in Ultrafiltration of WWTP Effluent PhD Thesis. *Delft University of Technology*.
- Urbain, V., Block, J., & Manem, J. (1993). Bioflocculation in activated sludge: an analytic approach. *Water Research* 27(5), 829-838.
- Vesilind, P. (1994). The role of water in sludge dewatering. *Water Environment Research* 66(1), 4-11.
- Walker, M., Iyer, K., Heaven, S., & Banks, C. (2011). Ammonia removal in anaerobic digestion by biogas stripping: An evaluation of process alternatives using a first order rate model based on experimental findings. *Chemical Engineering Journal* 178, 138-145.
- Wei, Y., Zhu, H., Wang, Y., Li, J., Zhang, P., Hu, J., & Liu, J. (2009). Nutrients release and phosphorus distribution during oligochaetes predation on activated sludge. *Biochemical Engineering Journal* 43(3), 239-245.
- Wilen, B. M., Jin, B., & Lant, P. (2003). The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water research*, 37(9), 2127-2139.
- Wilen, B., Keiding, K., & Nielsen, P. (2000). Anaerobic deflocculation and aerobic reflocculation of activated sludge. *Water Research*, 34(16), 3933-3942.
- Wingender, J., Neu, T., & Flemming, H. (1999). What are bacterial extracellular polymeric substances? *Microbial extracellular polymeric substances: characterization, structures and function*. Berlin Heidelberg: Springer-Verlag, 1-18.
- Yang, Q., Luo, K., Li, X., Wang, D., Zheng, W., Zeng, G., & Liu, J. (2010). Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. *Bioresource Technology* 101(9), 2924-2930.
- Yang, S., & Li, X. (2009). Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions. *Process Biochemistry* 44(1), 91-96.

- Ye, F., Liu, X., & Li, Y. (2014). Extracellular polymeric substances and dewaterability of waste activated sludge during anaerobic digestion. *Water Science and Technology* 70(9), 1555-1560.
- Yu, G., He, P., Shao, L., & He, P. (2008). Stratification structure of sludge flocs with implications to dewaterability. *Environmental science & technology* 42(21), 7944-7949.

Source	Stage	Volatile solids loss from initial content		Total solids loss from initial content	
		Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]
DF	1st centrifugation	1.55	0.07	1.32	0.06
	2nd centrifugation	1.09	0.17	1.03	0.17
	Total	2.65	0.18	2.35	0.18
MD	1st centrifugation	2.44	0.02	1.87	0.04
	2nd centrifugation	1.31	0.14	1.13	0.13
	Total	3.75	0.14	3.00	0.13
TD	1st centrifugation	7.53	0.02	5.03	0.07
	2nd centrifugation	2.87	0.04	2.33	0.05
	Total	10.40	0.05	7.36	0.09
ASTD	1st centrifugation	11.50	0.02	9.76	0.02
	2nd centrifugation	2.35	0.02	2.10	0.03
	Total	13.84	0.03	11.85	0.03

Table A1 Solids loss during the centrifugation steps of EPS extraction



Proteins		
BSA conc. [mg/l]	Abs. [nm]	Correlation function
0	0	Conc. = 191.41* (Abs.)
10	0.068	
20	0.134	
50	0.2815	
100	0.501	

Table A3 Proteins calibration

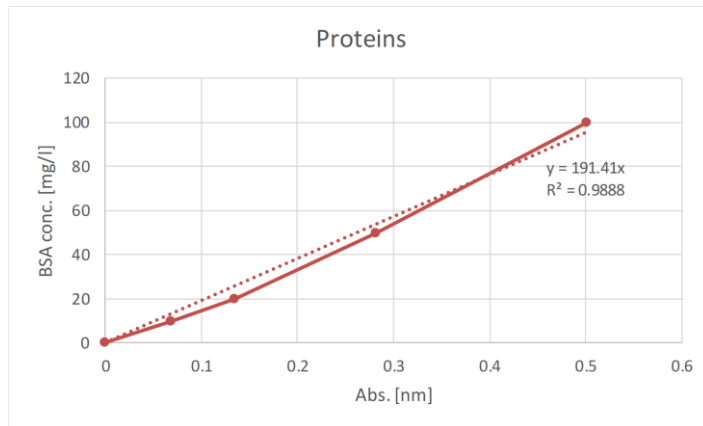


Figure A2 Proteins calibration equation and curve

Humics		
HA conc. [mg/l]	Abs. [nm]	Correlation function
0	0	Conc. = 471.9* (Abs.)
14.28	0.04	
28.56	0.072	
71.4	0.166	
142.8	0.29	

Table A4 Humics calibration

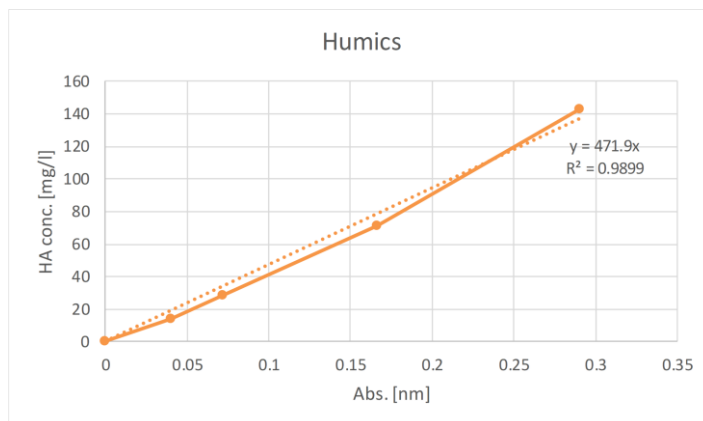


Figure A3 Humics calibration equation and curve

Source	Sampling	Capillary suction time ("CST")							
		Suction time				Capillary suction time			
		Avg. per sampling [sec/6.4ml]	Stdev. per sampling [s/6.4 ml]	Avg. per sludge type [s/6.4 ml]	Stdev. per sludge type [s/6.4 ml]	Avg. per sampling [sec/g TS]	Stdev. per sampling [sec/g TS]	Avg. per sludge type [sec/g TS]	Stdev. per sludge type [sec/g TS]
DF	1	112.6	12.7	178.2	59.8	470.1	17.6	620.1	170.4
	2	192.5	12.8			805.3	10.8		
	3	229.5	22.2			584.9	15.1		
MD	1	308.0	10.9	432.8	112.4	1178.5	5.6	1569.3	341.6
	2	464.2	11.0			1810.7	3.9		
	3	526.2	17.2			1718.8	5.1		
TD	1	819.8	32.1	998.1	196.7	3788.4	6.1	4133.9	351.3
	2	965.6	46.3			4490.6	7.6		
	3	1209.1	67.1			4122.7	8.7		
ASTD	1	1175.1	15.4	1301.5	150.0	5230.4	2.2	5835.5	696.3
	2	1262.2	28.0			5679.7	3.7		
	3	1467.2	63.7			6596.5	6.8		

Table A5 Capillary suction time

Source	Sampling	Specific resistance to filtration ("SRF")							
		Slope V/t - V ("a")				Specific resistance to filtration			
		Avg. per sampling [sec/cm ⁶]	Stdev. per sampling [sec/cm ⁶]	Avg. per sludge type [sec/cm ⁶]	Stdev. per sludge type [sec/cm ⁶]	Avg. per sampling [cm/g TS]	Stdev. per sampling [cm/g TS]	Avg. per sludge type [cm/g TS]	Stdev. per sludge type [cm/g TS]
DF	1	2.3497	0.0314	2.7673	0.4535	2.23E+10	4.94E+09	2.23E+10	3.43E+09
	2	2.7027	0.0180			2.57E+10	7.41E+09		
	3	3.2497	0.2760			1.88E+10	3.02E+10		
MD	1	8.3504	0.2132	11.5191	3.0272	7.26E+10	9.12E+09	9.48E+10	1.92E+10
	2	11.8257	0.7927			1.05E+11	2.40E+10		
	3	14.3814	0.3552			1.07E+11	8.88E+09		
TD	1	18.1515	1.0030	16.7596	2.2762	1.91E+11	1.97E+10	1.60E+11	2.71E+10
	2	14.1329	1.9845			1.49E+11	4.99E+10		
	3	17.9944	1.2738			1.39E+11	2.53E+10		
ASTD	1	23.0797	2.6807	22.5520	1.1878	2.34E+11	4.13E+10	2.30E+11	1.16E+10
	2	21.1918	0.8027			2.17E+11	1.38E+10		
	3	23.3844	2.9829			2.39E+11	4.53E+10		

Table A6 Sludge resistance to filtration

Source	Sampling	Volatile solids ("VS")				Total solids ("TS")				VS/TS ratio ("VS/TS")			
		Avg. per sampling [g/L]	Stdev. per sampling [g/L]	Avg. per sludge type [g/L]	Stdev. per sludge type [g/L]	Avg. per sampling [g/L]	Stdev. per sampling [g/L]	Avg. per sludge type [g/L]	Stdev. per sludge type [g/L]	Avg. per sampling [-]	Stdev. per sampling [-]	Avg. per sludge type [-]	Stdev. per sludge type [-]
DF	1	24.6060	0.0287	29.3555	9.4570	37.4162	0.1450	45.3558	13.8050	0.6576	0.0040	0.6452	0.0206
	2	23.2146	0.4991			33.5948	0.7389			0.6215	0.0292		
	3	40.2459	0.1618			61.2964	0.1123			0.6566	0.0044		
MD	1	22.6178	0.1610	23.7311	2.3490	40.8351	0.1106	42.9104	4.2828	0.5539	0.0076	0.5531	0.0007
	2	22.1458	0.2692			40.0605	0.3113			0.5528	0.0144		
	3	26.4298	0.0759			47.8355	0.1818			0.5525	0.0048		
TD	1	19.0638	0.2595	20.4564	2.8712	33.8125	0.0949	37.7441	6.9976	0.5638	0.0139	0.5448	0.0235
	2	18.5471	0.1173			33.5965	0.1973			0.5521	0.0086		
	3	23.7584	0.1230			45.8233	0.3070			0.5185	0.0085		
ASTD	1	18.9177	0.1421	19.2016	0.2787	35.1033	0.1832	34.8602	0.2110	0.5389	0.0091	0.5509	0.0109
	2	19.2124	0.1525			34.7234	0.2789			0.5533	0.0113		
	3	19.4748	0.0241			34.7540	0.1190			0.5604	0.0036		

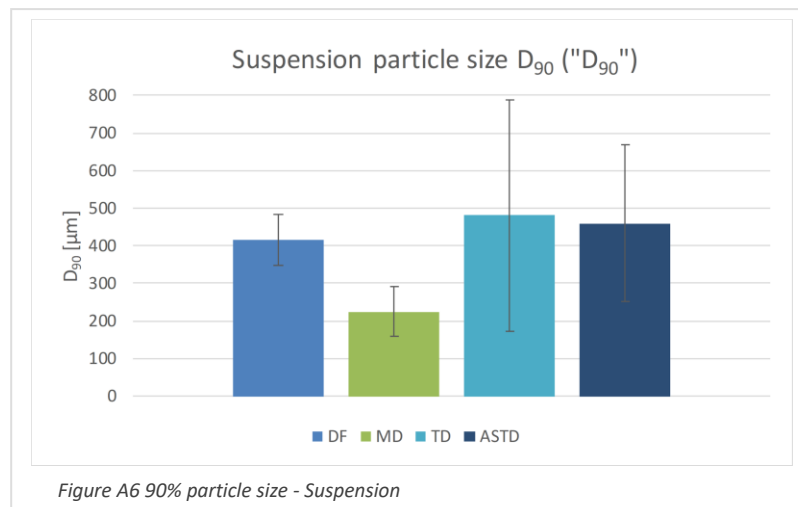
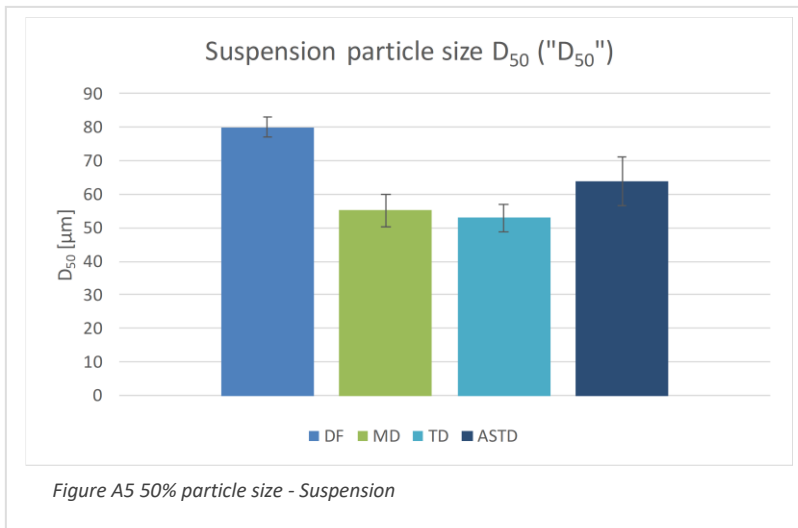
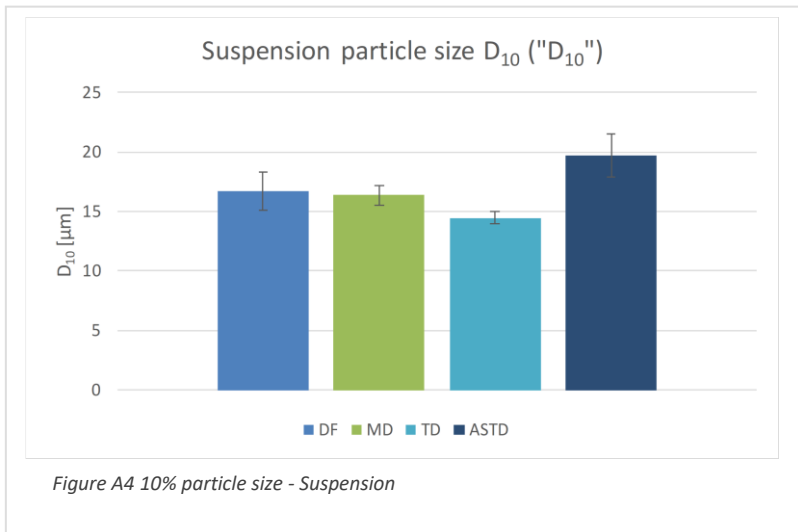
Table A7 Volatile and Total Solids - Suspension

Source	Sampling	Volatile solids colloids ("VS _{coll} ")				Total solids colloids ("TS _{coll} ")				VS/TS colloids ratio ("VS _{coll} /TS _{coll} ")			
		Avg. per sampling [g/L]	Stdev. per sampling [g/L]	Avg. per sludge type [g/L]	Stdev. per sludge type [g/L]	Avg. per sampling [g/L]	Stdev. per sampling [g/L]	Avg. per sludge type [g/L]	Stdev. per sludge type [g/L]	Avg. per sampling [-]	Stdev. per sampling [-]	Avg. per sludge type [-]	Stdev. per sludge type [-]
DF	2	0.6707	0.0310	0.8430	0.2438	3.7725	0.2080	3.7412	0.0442	0.1778	0.0719	0.2257	0.0678
	3	1.0154	0.0182			3.7100	0.0229			0.2737	0.0190		
MD	2	0.7533	0.0365	0.9618	0.2948	3.3132	0.0366	3.3291	0.0226	0.2274	0.0497	0.2886	0.0866
	3	1.1703	0.0390			3.3451	0.0211			0.3498	0.0339		
TD	2	1.4415	0.0163	1.6835	0.3422	3.9107	0.0706	4.0580	0.2083	0.3686	0.0213	0.4132	0.0631
	3	1.9255	0.0626			4.2053	0.1527			0.4579	0.0488		
ASTD	2	2.0931	0.0158	2.3053	0.3001	4.6301	0.0361	5.0479	0.5908	0.4521	0.0108	0.4563	0.0060
	3	2.5176	0.0614			5.4656	0.0766			0.4606	0.0281		

Table A8 Volatile and Total Solids - Colloid

Source	Replicate	Particle size and cumulative particle size distribution ("Cum.PSD")											
		D ₁₀				D ₅₀				D ₉₀			
		Avg. per sampling [µm]	Stdev. per sampling [µm]	Avg. per sludge type [µm]	Stdev. per sludge type [µm]	Avg. per sampling [µm]	Stdev. per sampling [µm]	Avg. per sludge type [µm]	Stdev. per sludge type [µm]	Avg. per sampling [µm]	Stdev. per sampling [µm]	Avg. per sludge type [µm]	Stdev. per sludge type [µm]
DF	1	17.3	0.2	16.7	1.6	77.2	1.0	80.0	3.1	337.5	37.5	415.8	67.8
	2	17.8	0.7			79.6	6.8			456.7	118.3		
	3	14.9	1.3			83.3	9.4			453.1	103.1		
MD	1	15.8	0.1	16.3	0.8	52.1	0.6	55.1	4.9	160.6	10.2	224.2	65.9
	2	16.0	0.6			52.4	3.3			219.7	145.0		
	3	17.3	0.4			60.9	2.5			292.3	80.6		
TD	1	14.3	0.1	14.5	0.5	49.1	0.5	53.0	4.2	836.0	97.2	480.9	307.6
	2	15.1	1.1			52.4	5.0			295.0	132.5		
	3	14.1	0.2			57.4	1.4			311.7	87.3		
ASTD	1	17.7	0.9	19.7	1.8	58.1	4.1	63.8	7.3	675.3	486.0	460.0	208.5
	2	20.8	1.2			71.9	6.1			445.7	408.0		
	3	20.7	0.8			61.2	3.6			259.0	82.5		

Table A9 Particle size - Suspension



Source	Replicate	Particle size colloids ("PS _{coll} ")			
		Average particle size			
		Avg. per sampling [nm]	Stdev. per sampling [nm]	Avg. per sludge type [nm]	Stdev. per sludge type [nm]
DF	2	417.3	2.7	452.4	49.6
	3	487.5	2.6		
MD	2	542.0	4.0	592.4	71.2
	3	642.7	58.0		
TD	2	750.7	13.8	730.3	28.8
	3	709.9	16.6		
ASTD	2	607.2	11.6	623.5	23.1
	3	639.8	17.8		

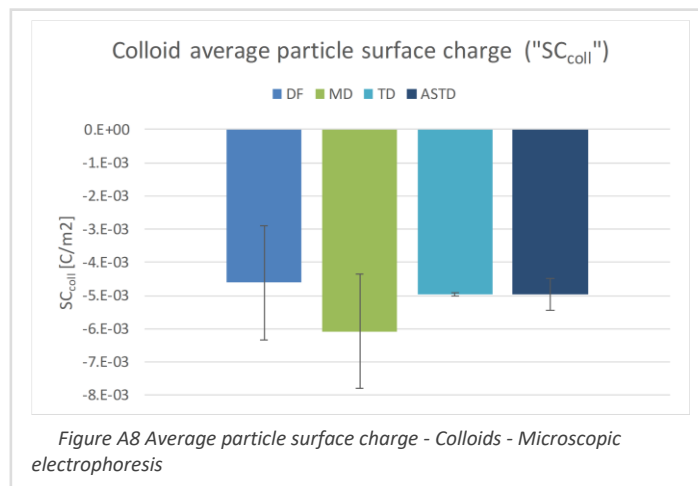
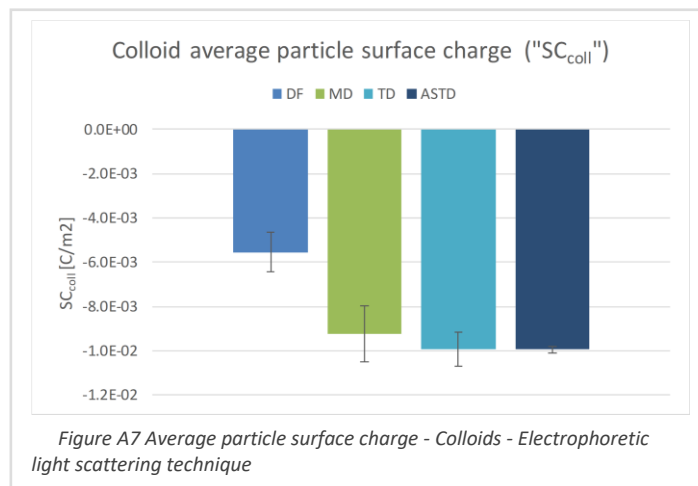
Table A10 Particle size - Colloid

Source	Sampling	Particle surface charge colloids ("SC _{coll} ")									
		Conductivity		Zeta potential				Surface charge			
		Avg. per sampling [mS/cm]	Stdev. per sampling [mS/cm]	Avg. per sampling [mV]	Stdev. per sampling [mV]	Avg. per sludge type [mV]	Stdev. per sludge type [mV]	Avg. per sampling [C/m ²]	Stdev. per sampling [C/m ²]	Avg. per sludge type [C/m ²]	Stdev. per sludge type [C/m ²]
DF	2	5.92	0.05	-13.57	0.15	-12.44	1.60	-0.0062	7.48E-14	-0.0055	9.10E-04
	3	5.39	0.17	-11.31	0.92			-0.0049	2.75E-13		
MD	2	10.67	0.17	-16.54	1.97	-15.73	1.15	-0.0101	1.45E-13	-0.0093	1.27E-03
	3	8.95	0.23	-14.92	0.59			-0.0084	2.29E-13		
TD	2	10.11	0.40	-15.79	2.49	-16.75	1.36	-0.0094	3.51E-13	-0.0099	7.52E-04
	3	9.86	0.47	-17.71	2.35			-0.0105	4.27E-13		
ASTD	2	7.28	0.05	-19.31	0.86	-17.86	2.06	-0.0098		-0.0100	1.46E-04
	3	10.68	0.29	-16.40	0.20			-0.0101	2.41E-13		

Table A11 Conductivity, zeta potential and surface charge - Colloid - Electrophoretic light scattering technique

Source	Sampling	Particle surface charge colloids ("SC _{coll} ")									
		Conductivity		Zeta potential				Surface charge			
		Avg. per sampling [mS/cm]	Stdev. per sampling [mS/cm]	Avg. per sampling [mV]	Stdev. per sampling [mV]	Avg. per sampling [mV]	Stdev. per sampling [mV]	Avg. per sampling [C/m ²]	Stdev. per sampling [C/m ²]	Avg. per sludge type [C/m ²]	Stdev. per sludge type [C/m ²]
DF	2	5.06	0.08	-13.84	0.18	-18.54	6.66	-0.0058	1.34E-13	-0.0046	1.73E-03
	3	0.57	0.03	-23.25	0.48			-0.0034	4.81E-13		
MD	2	4.76	0.06	-17.73	0.22	-24.85	10.07	-0.0073	1.10E-13	-0.0061	1.72E-03
	3	0.58	0.00	-31.97	0.27			-0.0049	2.34E-14		
TD	2	1.85	0.00	-19.15	0.10	-25.96	9.63	-0.0049	7.37E-15	-0.0050	3.84E-05
	3	0.58	0.00	-32.77	0.24			-0.0050	3.54E-14		
ASTD	2	1.40	0.00	-20.49	0.12	-27.40	9.77	-0.0046	1.61E-14	-0.0050	4.79E-04
	3	0.59	0.00	-34.30	0.11			-0.0053	8.76E-15		

Table A12 Conductivity, zeta potential and surface charge - Colloid - Microscopic electrophoresis

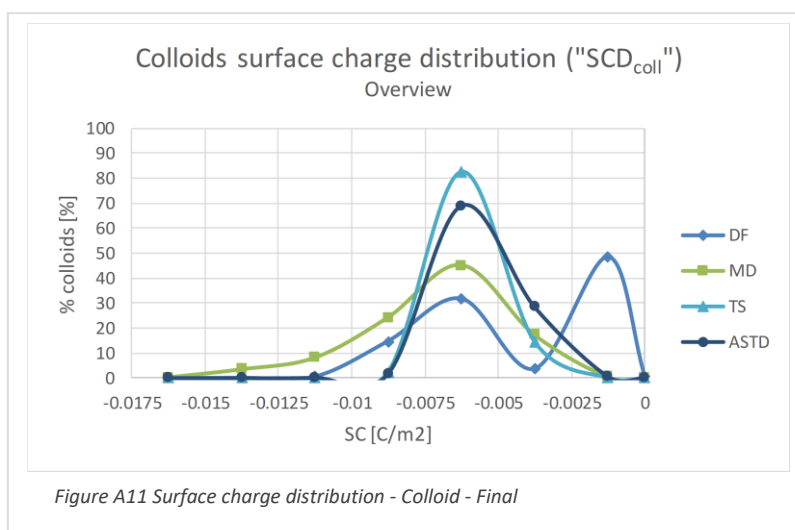
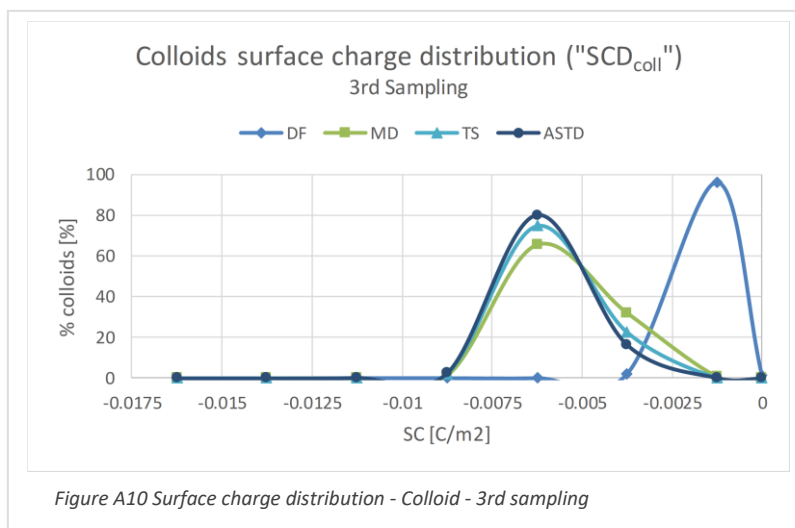
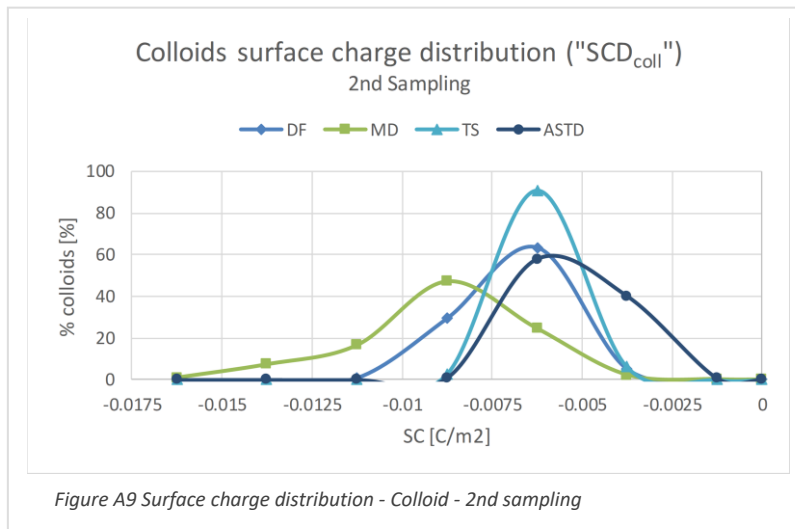


2nd Sampling								
SC range	Surface charge distribution ("SCD") % colloidal particles							
	DF		MD		TS		ASTD	
Avg. [C/m2]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]
0	0.12	0.04	0.00	0.00	0.00	0.00	0.00	0.00
-0.0013	0.51	0.33	0.20	0.13	0.08	0.08	0.89	0.26
-0.00375	5.44	1.39	2.35	0.37	6.41	0.99	40.26	1.26
-0.0063	63.36	4.09	24.78	1.62	90.64	1.11	57.90	0.90
-0.00875	29.42	3.44	47.50	1.46	2.85	0.32	0.85	0.55
-0.0113	1.04	0.07	16.66	0.61	0.00	0.00	0.00	0.00
-0.01375	0.10	0.04	7.40	1.26	0.00	0.00	0.00	0.00
-0.0163	0.00	0.00	0.97	0.37	0.00	0.00	0.00	0.00

3rd Sampling								
SC range	Surface charge distribution ("SCD") % colloidal particles							
	DF		MD		TS		ASTD	
Avg. [C/m2]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]
0	1.83	0.23	0.00	0.00	0.00	0.00	0.00	0.00
-0.0013	96.25	7.28	0.90	0.25	0.19	0.08	0.29	0.06
-0.00375	1.91	0.37	32.24	1.52	22.75	2.48	16.64	0.57
-0.0063	0.00	0.00	65.57	2.26	74.78	2.51	80.23	1.34
-0.00875	0.00	0.00	1.27	0.39	2.21	0.32	2.56	0.45
-0.0113	0.00	0.00	0.02	0.03	0.06	0.07	0.28	0.10
-0.01375	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-0.0163	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Overall								
SC range	Surface charge distribution ("SCD") % colloidal particles							
	DF		MD		TS		ASTD	
Avg. [C/m2]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]	Avg. [%]	Stdev. [%]
0	0.98	1.21	0.00	0.00	0.00	0.00	0.00	0.00
-0.0013	48.38	67.70	0.55	0.50	0.13	0.08	0.59	0.42
-0.00375	3.68	2.50	17.30	21.14	14.58	11.56	28.45	16.70
-0.0063	31.68	44.80	45.18	28.84	82.71	11.21	69.06	15.79
-0.00875	14.71	20.81	24.39	32.69	2.53	0.45	1.71	1.20
-0.0113	0.52	0.73	8.34	11.77	0.03	0.05	0.14	0.20
-0.01375	0.05	0.07	3.70	5.23	0.00	0.00	0.00	0.00
-0.0163	0.00	0.00	0.48	0.68	0.00	0.00	0.00	0.00

Table A13 Colloid- Surface charge distribution- Microscopic electrophoresis



Source	Phase	Sampling	Chemical oxygen demand ("COD")			
			Avg. per sampling [mg/g VS]	Stdev. per sampling [mg/g VS]	Avg. per sludge type [mg/g VS]	Stdev. per sludge type [mg/g VS]
DF	Bulk	1	34.73	0.13	30.17	5.48
		2	24.09	0.16		
		3	31.70	0.05		
	LB	1	17.64	0.17	17.15	0.63
		2	16.44	0.10		
		3	17.36	0.05		
	TB	1	52.85	0.04	48.26	6.49
		2	71.44	0.02		
		3	43.67	0.04		
MD	Bulk	1	27.26	0.02	28.50	1.60
		2	30.31	0.01		
		3	27.92	0.01		
	LB	1	18.49	0.07	20.81	2.01
		2	21.82	0.07		
		3	22.12	0.08		
	TB	1	25.68	0.07	43.60	1.80
		2	42.33	0.06		
		3	44.88	0.10		
TD	Bulk	1	118.69	0.18	116.61	7.70
		2	108.08	0.07		
		3	123.06	0.09		
	LB	1	65.18	0.05	70.33	4.88
		2	74.89	0.03		
		3	70.92	0.04		
	TB	1	30.23	0.04	36.58	6.26
		2	36.77	0.01		
		3	42.75	0.08		
ASTD	Bulk	1	222.16	0.13	209.36	20.87
		2	185.28	0.09		
		3	220.64	0.03		
	LB	1	112.17	0.07	113.86	17.29
		2	131.93	0.01		
		3	97.47	0.05		
	TB	1	37.06	0.05	34.77	3.78
		2	30.41	0.04		
		3	36.85	0.10		

Table A14 Chemical oxygen demand*

*Values marked with a strikethrough were considered outliers and were not included in the average or standard deviation, as their inclusion led to a standard deviation above 20%

Source	Phase	Sampling	Carbohydrates ("CA")				Proteins ("PR")				Humics ("HU")			
			Avg. per samplin g [mg/g VS]	Stdev. per samplin g [mg/g VS]	Avg. per sludge type [mg/g VS]	Stdev. per sludge type [mg/g VS]	Avg. per samplin g [mg/g VS]	Stdev. per samplin g [mg/g VS]	Avg. per sludge type [mg/g VS]	Stdev. per sludge type [mg/g VS]	Avg. per samplin g [mg/g VS]	Stdev. per samplin g [mg/g VS]	Avg. per sludge type [mg/g VS]	Stdev. per sludge type [mg/g VS]
DF	Bulk	1	2.07	0.16	1.50	0.25	8.85	0.18	5.65	0.80	6.64	0.22	7.90	1.21
		2	1.33	0.20			5.08	0.25			8.02	0.23		
		3	1.68	0.13			6.22	0.03			9.05	0.03		
	LB	1	1.72	0.21	1.42	0.26	4.02	0.42	4.49	0.66	4.81	0.11	5.64	0.93
		2	1.34	0.05			4.96	0.05			5.48	0.24		
		3	1.21	0.03			2.23	0.07			6.65	0.02		
	TB	1	4.70	0.11	0.10	0.11	12.29	0.17	11.29	1.40	14.64	0.24	16.03	1.96
		2	6.43	0.05			10.30	0.08			28.45	0.07		
		3	4.55	0.15			6.25	0.07			17.42	0.03		
MD	Bulk	1	2.31	0.06	2.43	0.13	5.29	0.10	5.15	0.48	7.87	0.09	9.69	1.68
		2	2.40	0.02			5.55	0.06			10.03	0.04		
		3	2.56	0.08			4.62	0.17			11.18	0.02		
	LB	1	2.20	0.04	2.30	0.15	4.59	0.08	4.55	0.23	5.20	0.05	7.94	1.28
		2	2.47	0.11			4.76	0.07			7.03	0.01		
		3	2.22	0.09			4.30	0.12			8.85	0.05		
	TB	1	3.35	0.02	5.05	0.12	7.58	0.05	8.77	1.72	8.40	0.19	13.45	1.45
		2	4.96	0.14			10.74	0.08			14.48	0.24		
		3	5.13	0.05			7.99	0.10			12.43	0.27		
TD	Bulk	1	12.80	0.19	11.52	1.29	10.50	0.07	9.00	1.40	45.83	0.32	45.57	8.67
		2	11.54	0.11			8.77	0.03			36.77	0.12		
		3	10.21	0.11			7.72	0.20			54.11	0.02		
	LB	1	6.37	0.05	6.89	0.83	6.02	0.03	6.16	0.84	23.90	0.06	27.97	3.76
		2	7.85	0.08			7.07	0.08			28.69	0.11		
		3	6.46	0.14			5.41	0.05			31.32	0.02		
	TB	1	3.11	0.16	3.78	0.58	5.51	0.13	4.67	0.81	13.81	0.10	15.58	2.49
		2	4.19	0.10			4.61	0.20			17.34	0.14		
		3	4.04	0.06			3.90	0.25			21.69	0.05		
ASTD	Bulk	1	27.12	0.09	26.49	0.67	21.63	0.03	14.49	0.24	70.69	0.10	88.23	15.27
		2	25.79	0.11			14.32	0.15			95.35	0.10		
		3	26.55	0.06			14.66	0.23			98.64	0.06		
	LB	1	14.81	0.10	15.43	0.88	15.15	0.16	9.33	2.46	45.89	0.44	43.81	2.93
		2	16.05	0.14			11.07	0.11			41.74	0.05		
		3	10.71	0.07			7.59	0.07			60.60	0.06		
	TB	1	4.43	0.08	4.67	0.34	9.10	0.02	5.63	0.49	37.10	0.05	33.59	6.01
		2	7.55	0.10			5.97	0.11			37.02	0.37		
		3	4.91	0.08			5.28	0.15			26.64	0.04		

Table A16 Biopolymers- Carbohydrates, Proteins and Humics*

*Values marked with a strikethrough were considered outliers and were not included in the average or standard deviation, as their inclusion led to a standard deviation above 20%

Source	Phase	Biopolymers ("BP")	
		Avg. per sludge type [-]	Stdev. per sludge type [-]
DF	Bulk	15.06	1.48
	LB	11.55	1.17
	TB	27.42	2.41
MD	Bulk	17.27	1.75
	LB	14.79	1.31
	TB	27.27	2.25
TD	Bulk	66.08	8.88
	LB	41.03	3.94
	TB	24.03	2.69
ASTD	Bulk	129.21	15.29
	LB	68.57	3.93
	TB	43.88	6.04

Table A15 Biopolymer- Total

Phase	Sampling	Sodium ("Na ⁺ ")				Ammonium ("NH ₄ ⁺ ")				Potassium ("K ⁺ ")			
		Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]
Bulk	1	1.4862	0.0011	1.6678	0.2568	0.2933	0.0079	0.2785	0.0146	0.1581	0.0004	0.1626	0.0064
	2	1.8494	0.0015			0.2781	0.0031			0.1671	0.0009		
	3	0.6754	0.0021			0.2640	0.0028			0.1005	0.0013		
LB	1	0.8165	0.0007	0.9436	0.1797	0.1354	0.0037	0.1385	0.0234	0.0745	0.0003	0.0787	0.0060
	2	1.0707	0.0007			0.1634	0.0034			0.0830	0.0003		
	3	0.4366	0.0019			0.1168	0.0044			0.0512	0.0004		
TB	1	0.6009	0.0005	0.6418	0.0578	0.1499	0.0014	0.1809	0.0941	0.0502	0.0012	0.0512	0.0015
	2	0.6827	0.0011			0.2865	0.0017			0.0523	0.0014		
	3	0.3071	0.0014			0.1062	0.0086			0.0254	0.0033		
Bulk	1	1.3077	0.0006	1.3944	0.1225	2.7900	0.0006	2.7061	0.1187	0.2489	0.0003	0.2643	0.0219
	2	1.4811	0.0016			3.3529	0.0019			0.3256	0.0009		
	3	0.9786	0.0007			2.6221	0.0006			0.2798	0.0004		
LB	1	0.8065	0.0007	0.8411	0.0490	1.5308	0.0007	1.4668	0.1088	0.1287	0.0004	0.1396	0.0110
	2	0.8758	0.0013			1.5285	0.0016			0.1507	0.0005		
	3	0.6327	0.0010			1.3412	0.0018			0.1396	0.0011		
TB	1	0.5668	0.0040	0.5952	0.0401	0.8048	0.0019	0.8776	0.0723	0.0639	0.0010	0.0702	0.0063
	2	0.6236	0.0006			0.9494	0.0020			0.0766	0.0008		
	3	0.4515	0.0006			0.8784	0.0007			0.0702	0.0003		
Bulk	1	1.5869	0.0061	1.7003	0.1603	3.4592	0.0041	3.5084	0.3248	0.3355	0.0111	0.3250	0.0149
	2	1.8137	0.0005			3.8549	0.0005			0.3145	0.0004		
	3	1.0134	0.0017			3.2110	0.0024			0.2351	0.0011		
LB	1	0.6555	0.0005	0.6797	0.0343	1.7693	0.0006	1.7506	0.0264	0.1232	0.0003	0.1238	0.0009
	2	1.3091	0.0007			2.3651	0.0016			0.1899	0.0004		
	3	0.7040	0.0014			1.7320	0.0016			0.1245	0.0008		
TB	1	0.2303	0.0005	0.3615	0.1855	0.7164	0.0008	0.7845	0.0963	0.0470	0.0005	0.0516	0.0065
	2	0.8646	0.0008			1.1128	0.0027			0.0878	0.0009		
	3	0.4927	0.0008			0.8525	0.0014			0.0563	0.0008		
Bulk	1	1.4415	0.0031	1.5955	0.1335	2.3272	0.0050	2.5477	0.3118	0.2307	0.0016	0.2638	0.0291
	2	1.6655	0.0030			2.7682	0.0036			0.2853	0.0019		
	3	1.6795	0.0017			4.2078	0.0024			0.2754	0.0010		
LB	1	0.6761	0.0019	0.8292	0.2165	1.2930	0.0017	1.9644	0.1141	0.1194	0.0012	0.1200	0.0009
	2	1.3939	0.0002			2.0451	0.0006			0.2059	0.0004		
	3	0.9823	0.0014			1.8837	0.0020			0.1206	0.0009		
TB	1	0.2506	0.0009	0.7656	0.1793	0.5023	0.0026	0.8465	0.1505	0.0466	0.0007	0.0471	0.0006
	2	0.8923	0.0002			0.9529	0.0015			0.0954	0.0004		
	3	0.6388	0.0018			0.7400	0.0022			0.0475	0.0007		

Table A17 Cations - Sodium, Ammonium and Potassium*

*Values marked with a strikethrough were considered outliers and were not included in the average or standard deviation, as their inclusion led to a standard deviation above 20%

Source	Phase	Sampling	Calcium ("Ca ²⁺ ")				Magnesium ("Mg ²⁺ ")				Iron ("Fe ²⁺ +Fe ³⁺ ")			
			Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per samplin g [mmol/g VS]	Stdev. per samplin g [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]
DF	Bulk	1	0.0924	0.0016	0.0719	0.0289	0.0735	0.0007	0.0665	0.0063	0.0020	0.0022	0.0008	0.0001
		2	0.0514	0.0230			0.0612	0.0114			0.0009	0.0066		
		3	0.1819	0.0008			0.0648	0.0023			0.0007	0.0046		
	LB	1	0.0336	0.0007	0.0325	0.0015	0.0259	0.0004	0.0273	0.0014	0.0019	0.0048	0.0010	0.0001
		2	0.0314	0.0007			0.0286	0.0044			0.0009	0.0005		
		3	0.0801	0.0024			0.0274	0.0035			0.0011	0.0004		
	TB	1	0.0264	0.0003	0.0221	0.0062	0.0211	0.0017	0.0148	0.0035	0.0031	0.0023	0.0018	0.0001
		2	0.0177	0.0009			0.0173	0.0006			0.0017	0.0056		
		3	0.0386	0.0014			0.0123	0.0051			0.0018	0.0056		
MD	Bulk	1	0.0576	0.0025	0.0460	0.0009	0.0179	0.0088	0.0022	0.0020	0.0018	0.0005	0.0007	0.0001
		2	0.0466	0.0091			0.0036	0.0188			0.0006	0.0014		
		3	0.0454	0.0051			0.0008	0.0264			0.0008	0.0009		
	LB	1	0.0195	0.0029	0.0184	0.0017	0.0176	0.0023	0.0045	0.0008	0.0021	0.0023	0.0012	0.0003
		2	0.0126	0.0068			0.0039	0.0128			0.0010	0.0026		
		3	0.0172	0.0057			0.0051	0.0075			0.0014	0.0075		
	TB	1	0.0087	0.0048	0.0087	0.0001	0.0120	0.0003	0.0111	0.0013	0.0027	0.0014	0.0025	0.0004
		2	0.0034	0.0086			0.0079	0.0059			0.0021	0.0018		
		3	0.0088	0.0057			0.0101	0.0022			0.0027	0.0024		
TD	Bulk	1	0.0280	0.0041	0.0241	0.0055	0.0211	0.0038	0.0074	0.0024	0.0051	0.0026	0.0022	0.0002
		2	0.0755	0.0033			0.0091	0.0059			0.0021	0.0038		
		3	0.0202	0.0032			0.0057	0.0048			0.0023	0.0014		
	LB	1	0.0090	0.0018	0.0120	0.0044	0.0130	0.0017	0.0059	0.0035	0.0047	0.0004	0.0048	0.0001
		2	0.0309	0.0027			0.0084	0.0035			0.0022	0.0026		
		3	0.0151	0.0016			0.0035	0.0009			0.0049	0.0028		
	TB	1	0.0028	0.0124	0.0057	0.0027	0.0080	0.0022	0.0074	0.0008	0.0039	0.0007	0.0039	0.0001
		2	0.0048	0.0055			0.0068	0.0043			0.0016	0.0035		
		3	0.0067	0.0017			0.0052	0.0062			0.0038	0.0026		
ASTD	Bulk	1	0.0141	0.0004	0.0179	0.0053	0.0298	0.0045	0.0244	0.0077	0.0080	0.0014	0.0032	0.0016
		2	0.0326	0.0041			0.0190	0.0118			0.0021	0.0043		
		3	0.0217	0.0054			0.0040	0.0028			0.0044	0.0010		
	LB	1	0.0049	0.0037	0.0164	0.0016	0.0198	0.0011	0.0189	0.0012	0.0077	0.0016	0.0083	0.0007
		2	0.0175	0.0025			0.0181	0.0007			0.0039	0.0007		
		3	0.0152	0.0022			0.0018	0.0244			0.0088	0.0023		
	TB	1	0.0001	0.0432	0.0006	0.0007	0.0119	0.0060	0.0112	0.0011	0.0036	0.0012	0.0033	0.0002
		2	0.0011	0.0127			0.0104	0.0037			0.0032	0.0031		
		3	0.0071	0.0076			0.0012	0.0275			0.0032	0.0051		

Table A18 Cations - Calcium, Magnesium and Iron*

*Values marked with a strikethrough were considered outliers and were not included in the average or standard deviation, as their inclusion led to a standard deviation above 20%

Source	Phase	Monovalent cations		Divalent cations		Trivalent cations		Total cations	
		Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]	Avg. per sludge type [mmol/g VS]	Stdev. per sludge type [mmol/g VS]
DF	Bulk	2.1088	0.2573	0.1384	0.0296	0.0011	0.0001	2.2483	0.2590
	LB	1.1609	0.1814	0.0598	0.0020	0.0013	0.0001	1.2220	0.1814
	TB	0.8739	0.1104	0.0369	0.0071	0.0022	0.0001	0.9131	0.1106
MD	Bulk	4.3648	0.1720	0.0482	0.0022	0.0007	0.0001	4.4137	0.1720
	LB	2.4476	0.1198	0.0228	0.0019	0.0012	0.0003	2.4716	0.1198
	TB	1.5430	0.0830	0.0198	0.0013	0.0028	0.0004	1.5656	0.0830
TD	Bulk	5.5337	0.3625	0.0315	0.0060	0.0027	0.0002	5.5679	0.3625
	LB	2.5542	0.0433	0.0180	0.0056	0.0054	0.0001	2.5776	0.0437
	TB	1.1976	0.2091	0.0131	0.0028	0.0045	0.0001	1.2152	0.2091
ASTD	Bulk	4.4069	0.3405	0.0423	0.0093	0.0038	0.0016	4.4531	0.3406
	LB	2.9136	0.2447	0.0353	0.0020	0.0094	0.0007	2.9584	0.2448
	TB	1.6591	0.2341	0.0118	0.0013	0.0054	0.0002	1.6763	0.2341

Table A19 Cations - Monovalent, Divalent, Trivalent and Total

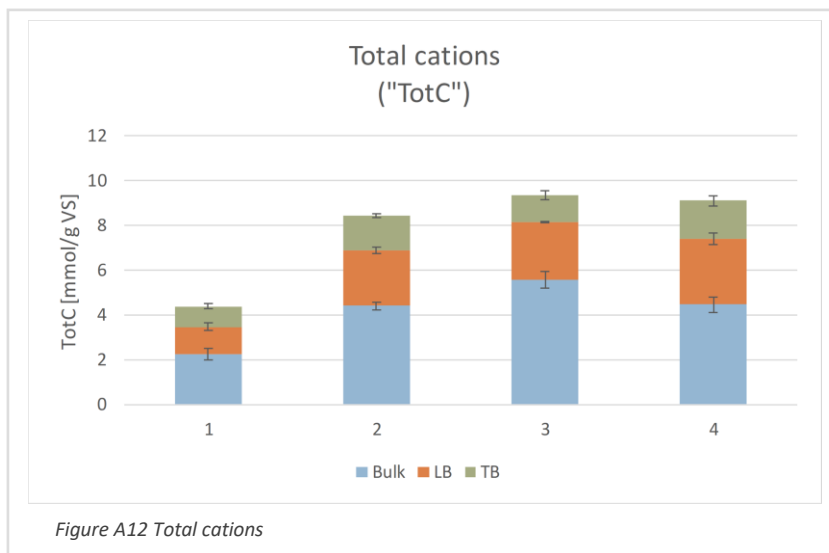
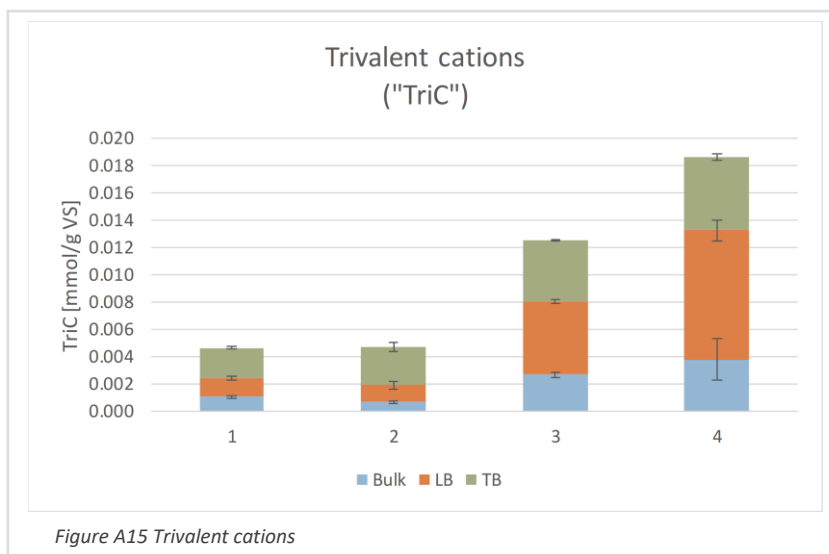
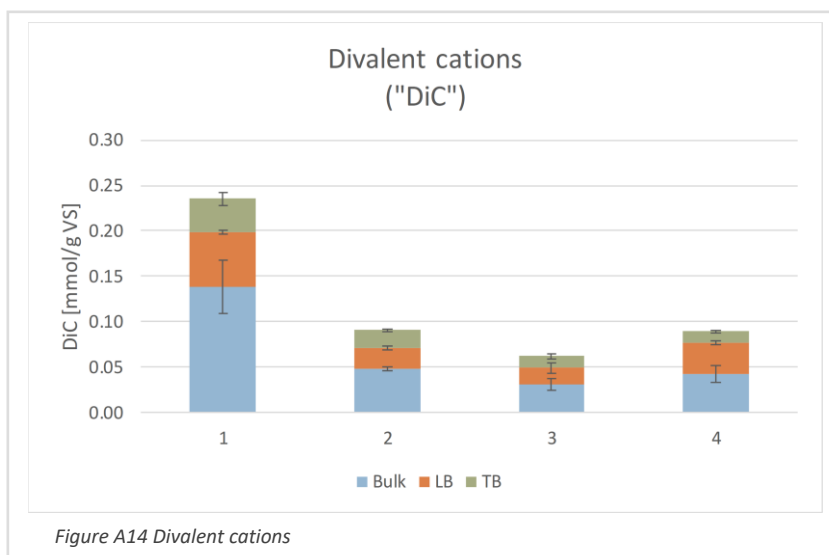
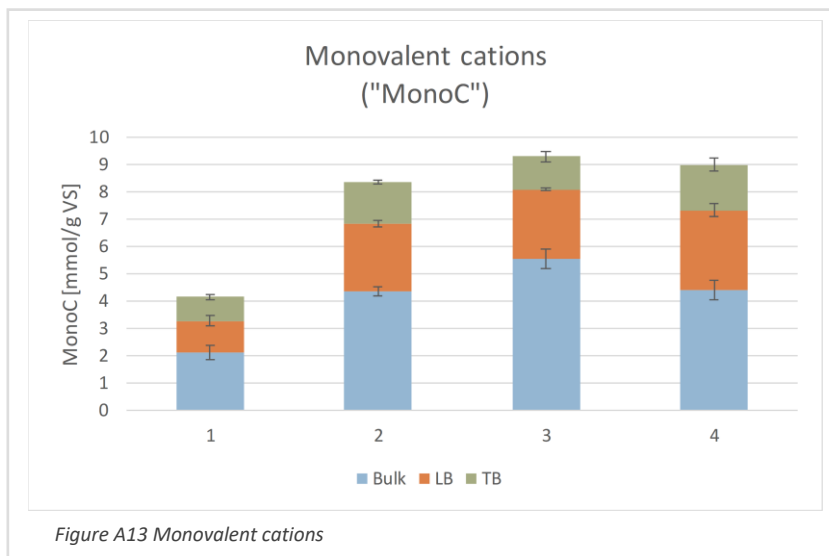


Figure A12 Total cations



Abbreviations

Abbreviations

- ASTD- Ammonia stripped thermophilic digestate
- BP- Biopolymers
- CA- Carbohydrates
- COD- Chemical oxygen demand
- CST- Capillary suction time
- DF- Digester feed
- EPS- Extracellular polymeric substances
- HU- Humics
- MD- Mesophilic digestate
- PR- Proteins
- PS- Particle size
- PSD- Particle size distribution
- SC- Surface charge
- SCD- Surface charge distribution
- SRF- Sludge resistance to filtration
- TS- Total solids
- TD- Thermophilic digestate
- VS- Volatile solids
- ZP- Zeta potential

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