ABSTRACT

Title of Thesis: ENHANCED PRODUCTION OF BIOSOLIDS BY IMPROVED ACTIVATED SLUDGE CLARIFICATION AND STRUCTURED WATER ANALYSIS

Xiaocen Liu, Master of Science, 2017

Thesis Directed By: Dr. Birthe V. Kjellerup
Department of Civil and Environmental Engineering, University of Maryland

Biosolids contain high contents of soil-required nutrients, so that have been widely applied in land application. The production of the biosolids depends on the clarification performance and dewaterability of the sludge, which are influenced by bioflocculation and structured water content, respectively. Therefore, research on sludge bioflocculation improvement and structured water content determination were proposed in this study. The result indicated that different activated sludge exhibited various bioflocculation limitations. Influence of the sludge characteristics such as the extracellular polymeric substances (EPS) composition, viscosity and floc size on the structured water content were also investigated. The results indicated that no significant correlation was observed between the EPS composition and the structured water content, however, the sludge floc size was positively correlated with it. The bioflocculation limitations
were pinpointed, and how floc size influenced the structured water content needed for further studies to improve sludge dewaterability, therefore, enhance the biosolids production quantitatively and qualitatively.
ENHANCED PRODUCTION OF BIOSOLIDS BY IMPROVED
ACTIVATED SLUDGE CLARIFICATION AND STRUCTURED WATER
ANALYSIS

by

Xiaocen Liu

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
[Master of Science]
[2017]

Advisory Committee:
Professor Birthe Kjellerup, Chair
Dr. Alba Torrents
Dr. Haydée De Clippleir
Acknowledgements

This project carried out during May, 2016 to August, 2017 in the research laboratorium of the Blue Plains Advanced Wastewater Treatment Plant (DC Water). I want to express my gratitude to the Blue Plains Advanced Wastewater Treatment Plant for funding the entire project. Without this support, the research would not have been performed.

I also want to thank The Department of Civil and Environmental Engineering at the University of Maryland at College Park for the support that made this project possible.

I own my deepest gratitude to my advisor at the University of Maryland Dr. Birthe Veno Kjellerup for her guidance and support on my study and research, and I learnt a lot from her. She gave me valuable suggestions on how to lead a graduate project and encouraged me to be curious about all the observations about my project. She made significant contribution on the completion of this thesis.

I am deeply grateful to my supervisor at DC Water Dr. Haydée De Clippeleir. Her suggestions and advice were important and helped me carry out the project. Without her help, I would not have been able to complete this project.
Table of Contents

Acknowledgements........................................................................................................... ii
Table of Contents............................................................................................................... iii
List of Tables ....................................................................................................................... v
List of Figures ..................................................................................................................... vi
List of Abbreviations .......................................................................................................... viii
Chapter 1: Introduction..................................................................................................... 1
  1.1 Problem Statement ...................................................................................................... 1
  1.2 Objectives .................................................................................................................. 2
Chapter 2: Literature Review.......................................................................................... 4
  2.1 Biosolids..................................................................................................................... 4
  2.2 Sludge Clarification and Bioflocculation................................................................. 5
  2.3 Anaerobic Digestion................................................................................................... 7
  2.4 Dewatering Process.................................................................................................. 7
  2.5 Structured Water ....................................................................................................... 8
  2.6 Extracellular Polymeric Substances (EPS).............................................................. 12
Chapter 3: Manuscript 1: Identification of coagulation, flocculation and floc strength limitations in activated sludge using modified jar tests ........................................ 14
  Abstract .......................................................................................................................... 14
  3.1 Introduction............................................................................................................... 15
  3.2 Materials and Methods............................................................................................ 19
    3.2.1 Sample location and acquirement ...................................................................... 19
    3.2.2 Polymer types and preparation ....................................................................... 19
    3.2.3 Modified Jar test .............................................................................................. 20
    3.2.4 Classic and novel settling metrics ................................................................... 22
    3.2.5 Extracellular polymeric substances ................................................................. 22
  3.3 Results....................................................................................................................... 23
    3.3.1 High-rate activated sludge .............................................................................. 23
    3.3.2 Bioaugmented high-rate activated sludge ...................................................... 26
    3.3.3 Biological nutrient removal sludge ................................................................. 28
  3.4 Discussion.................................................................................................................. 34
    3.4.1 Evaluation of modified jar tests ...................................................................... 34
    3.4.2 Limitations in HRAS sludge ........................................................................ 35
    3.4.3 Impact of bioaugmentation of HRAS on floc formation limitations ............. 39
    3.4.4 Impact of SRT and organic loading rate on floc formation limitations ... 41
  3.5 Conclusion ................................................................................................................. 42
  3.6 References ................................................................................................................ 42
Chapter 4: Manuscript 2: Influence of extracellular polymeric substance (EPS) on the structured water content in the sludge and objective measurements of the structured water content .......................................................... 45
  Abstract .......................................................................................................................... 45
  4.1 Introduction............................................................................................................... 46
  4.2 Materials and Methodology ..................................................................................... 53
    4.2.1 Sample locations and acquisition ................................................................... 53
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.2 Determination of the structured water content</td>
<td>54</td>
</tr>
<tr>
<td>4.2.3 Extracellular polymeric substances (EPS) extraction</td>
<td>56</td>
</tr>
<tr>
<td>4.2.4 Extracellular polymeric substances (EPS) Composition</td>
<td>57</td>
</tr>
<tr>
<td>4.2.5 Floc Size quantification</td>
<td>60</td>
</tr>
<tr>
<td>4.2.6 Approaches for Determination of the Structured Water Content</td>
<td>60</td>
</tr>
<tr>
<td>4.3 Results and Discussion</td>
<td>61</td>
</tr>
<tr>
<td>4.3.1 Determination of the Structured Water Content</td>
<td>61</td>
</tr>
<tr>
<td>4.3.2 Structured Water Content Values</td>
<td>71</td>
</tr>
<tr>
<td>4.3.3 Impact of EPS on the Structured Water Content</td>
<td>77</td>
</tr>
<tr>
<td>4.3.4 Impact of Dynamic Viscosity on the Structured Water Content</td>
<td>94</td>
</tr>
<tr>
<td>4.3.5 Impact of Floc Size on the Structured Water Content</td>
<td>96</td>
</tr>
<tr>
<td>4.3.6 Assessment of the approaches for determination of the Structured Water Content</td>
<td>97</td>
</tr>
<tr>
<td>4.4 Conclusion</td>
<td>101</td>
</tr>
<tr>
<td>4.5 Acknowledgements</td>
<td>103</td>
</tr>
<tr>
<td>4.6 References</td>
<td>103</td>
</tr>
<tr>
<td>Chapter 5: Conclusion</td>
<td>107</td>
</tr>
<tr>
<td>References (Chapter 1, 2 and 5, excluding manuscript references)</td>
<td>114</td>
</tr>
</tbody>
</table>
List of Tables

Table 3.1. Intrinsic characteristics of the tested sludge types 30
Table 4.1. EPS analysis for five sludge types 78
Table 4.2. Floc sizes for four sludge types 97
List of Figures

Figure 2.1. Wastewater treatment process diagram---------------------------------4
Figure 2.2. Moisture distribution in a sewage sludge floc---------------------9
Figure 2.3. Drying curve-----------------------------------------------------11
Figure 3.1. Orthokinetic and gravitational flocculation curves for activated sludge
----------------------------------------------------------------------------------------31
Figure 3.2. Settling velocity distribution (line) -----------------------------31
Figure 3.3. Polymer response curves ------------------------------------------32
Figure 3.4. Orthokinetic flocculation curve -----------------------------------33
Figure 3.5. Settling velocity distribution (scatter) --------------------------34
Figure 4.1. Moisture distribution in a sewage sludge floc---------------------49
Figure 4.2. Drying curve-----------------------------------------------------50
Figure 4.3. Drying test set up -----------------------------------------------56
Figure 4.4. Drying rate curve for the THP sludge (forward rolling average) ----64
Figure 4.5. Weight curve for the THP sludge (forward rolling average) --------69
Figure 4.6. Structured water content from the drying rate curve and Monod model
---------------------------------------------------------------------------------------------73
Figure 4.7. Structured water content from the weight curve ---------------------75
Figure 4.8. Cake TS of five sludge types---------------------------------------77
Figure 4.9. Relationship between the COD and the structured water content ----80
Figure 4.10. The structured water content and EPS composition (baseline A)84-85
Figure 4.11. The structured water content and EPS composition (baseline B)86-87
Figure 4.12. The structured water content and EPS composition (baseline C)88-89
Figure 4.13. The structured water content and EPS composition (baseline D) 90-91
Figure 4.14. The structured water content and EPS composition (Monod) 92-93
Figure 4.15. Dynamic viscosity of the primary sludge 95
Figure 4.16. Flocs of five sludge types under microscope view 96
List of Abbreviations

AWWTP - advanced wastewater treatment plant
Bio-HRAS - Bioaugmented high-rate activated sludge
BNR - Biological nutrient removal sludge
BP – Branched polymer
C - PolyDADMAC
COD – Chemical oxygen demand
CSV - Critical settling velocity
DLVO - Derjaguin-Landau-Verwey-Overbeek
EPS - Extracellular polymeric substances
HRAS – High-rate activated sludge
ISV - Initial settling velocity
LB-EPS – Loosely bound extracellular polymeric substances
LOSS - Limit of Stoksian settling
LP – Linear polymer
M/D ratio - monovalent to divalent ratio
PN – Protein
PRC – Polymer response curve
PS – Polysaccharides
OFC – Orthokinetic flocculation curve
SRT - Solis retention time
SWC – Structured water content
SVD - Settling velocity distribution
SVI - Sludge volume index
TB-EPS – Tightly bound extracellular polymeric substances
TOF - Threshold of flocculation
TS – Total solids
TSS – Total suspended solids
VSS – Volatile suspended solids
WWTP – Wastewater treatment plant
Chapter 1: Introduction

1.1 Problem Statement

In wastewater treatment plants, sludge from different treatment stages confluences in the anaerobic digestion tank, where the final treatment process of the sludge produces biogas (methane) for energy recovery. Thereafter, the treated sludge is dewatered, and the solids collected after the dewatering process is referred to as biosolids. Biosolids contain high concentrations of nitrogen and phosphorus, and the material is subsequently used for land applications such as farming, silviculture, soil reclamation and others (Esteller et al., 2009; Wang, Shammas and Hung, 2007).

The total amount of the sludge that is separated in various treatment processes is the source for the biosolids. Therefore, the sludge clarification is an important process that influences the biosolids production. However, activated sludge (secondary sludge) is considered as clarification limited (Li et al., 2016), where metal salts and high molecular weight polymers are added to improve the separation. Floc formation is an important process that determines the activated sludge clarification. Therefore, a study that investigates the limitations that prevent the floc formation in the activated sludge is proposed (Chapter 3).

Reduction of the moisture content from the biosolids can reduce the volume and total weight, which reduces the need for biosolids storage and transportation requirements thus reducing the costs for biosolids handling and therefore also environmental foot print including a reduction of greenhouse gases from handling and transportation. The dewatering process aims to remove as
much water as possible to achieve a small biosolids volume. However, the
dewatering process also has limitations (Vaxelaire, 2001). A proportion of the
water in the biosolids cannot be removed by traditional dewatering treatments,
and this part of water is referred to as structured water. Measurements of the
structured water content in various sludge types served as a good estimate of the
dewatering ability and biosolids quality (Neyens, 2004; Lee, Lai and Mujumdar,
2006). However, current measurements of the structured water content are either
dependent on subjective evaluations or require complicated approaches (Smith
and Vesilind, 1995). Therefore, objective measurements of the structured water
content using the drying test are proposed (Chapter 4).

1.2 Objectives

The objective of this research was to study the biosolids production
considering both sludge sources and dewatering ability. Pinpointing the
clarification limitations of the activated sludge paves for further studies aiming at
improving the clarification capacity, so that increased volumes of sludge can be
treated in the anaerobic digestion process. Sludge dewaterability determines the
moisture content in the biosolids (Murthy, Novak and Holbrook, 2000). Objective
measurements of the structured water content in the sources of sludge can provide
information about the dewaterability of these sludge types.

The objectives of the study were:

(1) Pinpoint bioflocculation limitations for the activated sludge;
(2) Determine objective measurements of the structured water content for different sludge types and study the sludge characteristics influences on the structured water content;

(3) Provide suggestions for further study in this field that aiming to increase the biosolids production and quality.

In this study, a manuscript titled: “Identification of coagulation, flocculation and floc strength limitations in activated sludge using modified jar tests” evaluating bioflocculation limitations in high–rate activated sludge is shown in Chapter 3 for objective (1).

A second manuscript titled: “Influence of extracellular polymeric substance (EPS) on the structured water content in the sludge and objective measurements of the structured water content” evaluated objective measurements of structured water content and the influence of sludge characteristics on the structured water was present in Chapter 4 for objective (2). Suggestions on further studies about biosolids production was shown in Chapter 5 for objective (3).
Chapter 2: Literature Review

2.1 Biosolids

Municipal sewage is treated through various types of wastewater treatment plants, where a series of biological processes remove or transform the organic matter, nutrients, and pathogenic bacteria to meet the regulatory requirements for the effluent enabling discharge to receiving water systems (Wang et al., 2008) (Figure 2.1).

Figure 2.1. Wastewater treatment process diagram.

The wastewater is constituted of two main parts: 1) the liquid part, which contains water and soluble organic or inorganic matters, while 2) makes up the solid part, which contains insoluble particles including organic matter and bacteria (Painter and Viney, 1959). In the wastewater, these two parts are mixed together, and one objective in wastewater treatment is to separate them to obtain clean effluent. During gravitational separation, the solids settle to the bottom of
the sedimentation tank as sludge, and the sludge from each treatment stage is collected for further treatment referred to as the solids treatment process. First, the thermal hydrolysis process makes the sludge more biodegradable under high temperature and pressure, the sludge will then be stabilized through the anaerobic digestion process, where anaerobic bacteria and archaea decompose the organic compounds in the sludge to produce biogas for energy recovery and thereby reduce the sludge volume (Wang, Shammas and Hung, 2007). The sludge from the anaerobic digestion tank will then be dewatered to reduce the water content, whereby the volume will be further reduced in addition to limiting potential issues with odor during storage and transportation for land application purposes (Parkin and Owen, 1986). The biosolids contains a high concentration of nutrients such as carbon, nitrogen, phosphorous as well as micro nutrients such as zinc, copper and nickel (Esteller et al., 2009). This content makes biosolids applicable as fertilizer and for soil amendment in land application such as farming, silviculture, soil reclamation and others (Wang, Shammas and Hung, 2007; EPA, 2002).

As the increase in the population takes place, more municipal sewage will be produced (Vorosmarty, 2000). Therefore, evaluation of the solids treatment process and biosolids production will become increasingly important and invite more public attention.

2.2 Sludge Clarification and Bioflocculation

Sludge clarification refers to the solid separation in gravitational sedimentation tanks in each treatment process (Chao and Keinath, 1979). Floc
formation and settlement are the most important processes that determine the sludge clarification efficiency. Bioflocculation is a complicated process that refers to the floc formation that takes place in the activated sludge. The two processes coagulation and flocculation are important for bioflocculation (Amuda and Amoo, 2007). In coagulation, discreet particles in the wastewater combine into aggregates, whereas these aggregates attach to each other and form microflocs during the flocculation process. These interactions can be described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, considering both molecular interactions, attractive Van De Waals forces, repulsive electrostatic forces, and solid particle electrification (Derjaguin & Landau, 1993; Verwey et al., 1999). In wastewater treatment, charge neutralization and polymer addition are commonly applied for improving the bioflocculation process. Solids particles of the sludge are negatively charged (Jin, Wilén and Lant, 2003) thus they can combine with positively charged cations to form electroneutral flocs. Therefore, decreasing the monovalent/divalent cation ratio (M/D ratio) by dosing divalent cations such as calcium and magnesium ions can increase the efficiency of the electroneutral floc formation thus improving bioflocculation (Higgins, Tom and Sobeck, 2004). Polymers, on other hand, can have linear or branched structures to provide junctions for solid particles to attach thus increasing the floc size and strength (Poduska and Hicks, 1979; Singh et al., 2003). Activated sludge from the secondary treatment process is an important source of biosolids. However, it is considered as bioflocculation limited (Li et al., 2016), and the limitation would originate from both coagulation and flocculation steps (Busch and Stumm, 1968;
Therefore, pinpointing the limitation can inform potential solutions that can improve the sludge clarification process.

2.3 Anaerobic Digestion

Anaerobic digestion is a biological process, where anaerobic bacteria and archaea transform organic material in the sludge and form biogas that is mainly composed of methane, carbon dioxide, water vapor and trace hydrogen sulfide (Lastella et al., 2002). Removal of carbon dioxide, water vapor and other produced gases from the raw biogas results in improved quality of biogas thus mainly methane. Biogas production is applied as a means of energy recovery from the wastewater treatment process and can be used by the facilities for producing mechanical energy, heating the anaerobic digester, supplying electricity (Deng et al., 2014). The volume of the sludge is reduced after anaerobic digestion due to the production of methane and the remaining biomass (called digestate) will be dewatered to produce biosolids.

2.4 Dewatering Process

Dewatering involves the solid-liquid separation process that removes moisture from the sludge (Stasta et al., 2006). Digestate from the anaerobic digestion process is dewatered, and the water content often decreases from 95% in anaerobic digestion to 70-85% in the dewatered solids (Wang et al., 2008). Water is commonly removed from the sludge by filtration and centrifugation (Wakeman, 2007). The water that is removed through this process is called filtrate and is
commonly returned to secondary treatment processes. Dewatered solids are referred to as “cake solids”. The moisture content in the cake solids varies and is influenced by both sludge characteristics and the applied dewatering technology (Novak, 2006). Various dewatering technologies result in different total solids (TS) content of the cakes. The cake TS can range from 24-42% for pressure and vacuum filtered sludge, while the TS content ranges from 20-40% for belt and centrifuge filtered solids (Werther and Ogada, 1999). The cake solids obtained could be applied for land application as biosolids. As the population continues to increase, increased cake solids production is expected due to increased volumes of municipal wastewater. Therefore, issues with storage, transportation and management of these cake solids will require solutions. As a result, it is important to study the mechanisms of different dewatering technologies to achieve higher dewaterability and less moisture content in the cake solids.

2.5 Structured Water

As mentioned above, dewatered biosolids have high moisture contents, since not all water can be removed with current mechanical dewatering technologies (Werther and Ogada, 1999; Tsang and Vesilind, 1990). Four types of water have been identified in sludge (Vesilind, 1994): (1) Free water: water that is unaffected by the capillary force and moves freely in the sludge; (2) Interstitial water: water that is trapped into interstitial places between the sludge flocs due to capillary forces; (3) Surface water: water that is attached to the surface of the sludge flocs due to adhesive forces; (4) Chemically bound water: water that is
tightly bound to the sludge flocs due to chemical interactions such as hydration water (Figure 2.2) (Kopp and Dichtl, 2000). There is a continuous debate about the determination of the structured water content, and different approaches for measuring the structured water content have been applied (Vaxelaire and Cézac, 2004).

Figure 2.2. Moisture distribution in a sewage sludge floc: A: free water; B: interstitial water; C: surface water; D: chemically bound water (Kopp and Dichtl, 2000).

One operational determination of the structured water content is the moisture that does not freeze under the temperature that would freeze free water (Colin and Gazbar, 1995). Based on this definition, Heukelekian and Weisberg
(1956) proposed the dilatometric measurement of the structured water content. This approach assumed that when the sludge was frozen to a temperature below -20°C, the volume change of the sludge would only be caused by freezing of the free water. Therefore, the free water content was determined by recording the volume change of the sludge, and the structured water content was calculated by subtracting the free water content from the total water content in the sludge (Wu, Huang and Lee, 1998).

Another operational determination of the structured water content was proposed by Lee and Hsu (1995), where, the structured water content was defined as the moisture that was excluding the free water. The structured water content can be obtained via a drying test (Lee and Hsu, 1995), which successfully provided a full drying curve of the sludge sample instead of a number such as the dilatometric measurement provided. A constant amount of sludge was dried under controlled temperature and relative humidity resulting in a drying curve that showed the relationship between the drying rate and the remaining moisture content in the sludge sample, and the drying rates varied for different moisture types (Figure 2.3) (Lee and Hsu, 1995). The structured water content was obtained according to the change of the drying rates. In Figure 2.3, the moisture content in the sludge at note B indicates the structured water content of the sludge. However, the determination of note B is visual and subjective, which means that interpretation of note B can lead to different structured water contents (Lee and Hsu, 1995; J. Kopp and N. Dichtl, 2000). Thus, an objective
measurement without subjective interpretation is necessary for determination of the structured water content in different sludge types.

**Figure 2.3.** Drying Curve. Moisture evaporation rate vs. moisture content remains in the sludge. A-B: free water evaporation period; B-C: interstitial water evaporation period; C-D: surface water evaporation period; D-E: chemically bound water evaporation period (Lee and Hsu, 1995).

In this thesis, structured water is defined as the moisture content in the sludge that excludes the free water similar to Lee and Hsu (1995), and the content will be determined based on the drying test results (described above). Five
objective approaches based on the drying test will be provided and evaluated
according to their theoretical and practical interpretations.

2.6 Extracellular Polymeric Substances (EPS)

EPS are natural polymers secreted by the microorganisms in the sludge
that provides important functional and structural properties for the biofilm/sludge
flocs (Liu and Fang, 2002). EPS is mainly composed of protein (PN),
polysaccharides (PS), humic substances, lipids, DNA and inorganic compounds
(Sheng, Yu and Li, 2010). Bioflocculation efficiency and dewaterability of the
sludge are influenced by the EPS content and composition (More et al., 2014;
Neyens, 2004). The PN/PS ratio is an important parameter of the EPS, and studies
indicate that a PN/PS ratio smaller than 1 might lead to poor bioflocculation and
lower dewaterability (Morgan, Forster and Evison, 1990; Basuvaraj, Fein and
Liss, 2015). EPS can be bound to the bacteria attached to the sludge structure thus
forming a net-like structure to hold the biofilm together. EPS is often classified
into two parts: 1) loosely bound EPS (LB-EPS) and 2) tightly bound EPS (TB-
EPS) according to their connection with the bacteria cells and EPS extraction
methodologies (More et al., 2014). The content of the LB-EPS and TB-EPS in the
sludge is also an important parameter that influences sludge bioflocculation and
dewaterability. Basuvaraj (2015) indicated that sludge with more TB-EPS content
(TB-EPS/LB-EPS ratio >2.5) had improved settleability and dewaterability
compared to sludge with less TB-EPS (TB-EPS/LB-EPS ratio<1.5). Considering
the influence of EPS on the sludge dewaterability, studies on the influence of EPS
on the structured water content are proposed. A previous study indicated that sludge with total EPS content more than 20 mg/g VSS exhibited a higher structured water content (7-24 g H₂O/ g MLSS) than sludge with less EPS content (structured water content: 6-15 g H₂O/ g MLSS) (Liao et al., 2000). Therefore, in this study, it is essential to investigate the relationship between the structured water content and the sludge EPS characteristics such as PN/PS ratio, LB-EPS, TB-EPS and total EPS content.
Chapter 3: Manuscript 1: Identification of coagulation, flocculation and floc strength limitations in activated sludge using modified jar tests

Keywords: solid separation, high-rate activated sludge, branched polymer, bioaugmentation, jar test

Abstract
Floc formation is a complicated and vital process in the activated sludge system. However, elevated effluent suspended solids in activated sludge systems are common and frequently the exact cause is unknown. This study utilized multiple modified jar tests with FeCl3, polyDADMAC, linear polymer (LP) and branched polymer (BP) to assess the presence coagulation, flocculation and floc strength limitation present in high-rate activated sludge (HRAS), bioaugmented HRAS (bioHRAS) and biological nutrient removal sludge (BNR). HRAS was found to be flocculation and coagulation limited, whereas these limitations where mitigated by bioaugmentation. BioHRAS was found to be floc strength limited. BNR had no apparent limitation but benefitted from both coagulant and polymer addition. Polymers in combination with orthokinetic test are useful diagnostic tool to identify floc limitations where after appropriate measures can be taken to mitigate these limitations.
3.1 Introduction

Solid separation is imperative towards the success of a wastewater treatment plant (WWTP). Without it, effluent quality limits would not be obtainable. Activated sludge consists of bacteria whose density approximates water (Andreadakis, 1993). As such, activated sludge has to coagulate and flocculate in order to achieve solid separation. Solids separation is a complex process involving (1) coagulation of primary colloids into aggregates and (2) subsequent flocculation into microflocs (<50 µm). When exposed to tranquil conditions commonly found in clarifiers, microflocs will further flocculate into macroflocs (>50 µm) and settle out. Both coagulation and flocculation of activated sludge have similar mechanisms and have been used interchangeably in the past. For clarity sake, ‘floc formation’ shall henceforth be used to denote the complete process (coagulation followed by flocculation) as described above, while ‘coagulation’ and ‘flocculation’ will be used for the appropriate sub-processes (1) and (2) respectively.

Floc formation is most commonly described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Derjaguin & Landau, 1993; Verwey et al., 1999) which describes two distinct processes: (1) double layer compression and (2) bridging (Hermasson, 1999; Langelier & Ludwig, 1949; Mer & Healy, 1963). The DVLO theory combines the attractive van der Waals interactions with the repulsive double layer interactions originating from Coulomb forces between charged particles. Activated sludge cells are negatively charged (Liao et al., 2001; Wilen et al., 2003), thus these negative repulsive forces must be overcome before
the attractive van der Waals forces allow for the formation of bacterial cells into aggregates. This energy barrier is function of the total surface charge of the bacterial cell. Bridging involves divalent cations (Ca$^{2+}$, Fe$^{2+}$, Mg$^{2+}$) or high molecular weight molecules like polymers to electrostatically clump cells together. The effectiveness of this process is mediated by the surface charge and the amount of cations present and is sensitive towards changes in the monovalent to divalent (M/D) ratio. While both processes occur for both coagulation and flocculation, Mer and Healy (1963) established that coagulation is more influenced by the amount of charge to be charge neutralized while flocculation depends on successful collisions of aggregates which are subsequently held together by cationic bridges. Thus, coagulation happens perikinetically, while flocculation is orthokinetically driven. Not all collisions are successful however, with orthokinetic collision efficiency, i.e. the percentage of total collisions that is successful, being the major macroscopic metric to assess flocculation potential. Hence, mixing energy is commonly applied to promote orthokinetic over perikinetic floc formation.

Floc formation can be artificially induced or improved by the addition of chemicals like metal salts and synthetic polymers. Ferric chloride is commonly used in primary treatments to coagulate inorganic suspended solids into primary sludge and the removal of phosphate or as conditioning agent in dewatering. Synthetic cationic polymers are mainly used as conditioning agents in dewatering to improve total cake solids and aid solid separation in secondary clarifiers. Many types exist, depending on the application. Charge neutralization and particle
destabilization in the coagulation step can be achieved by adding a polyDADMAC-type polymer which typically has a very high charge density but relatively low molecular weight. Collision efficiency in the flocculation step can be improved with high molecular weight polyamide-type polymer. These polymers can be linear in structure, maximizing the molecular weight and thus minimizing optimal dosage, or branched, which improves floc strength. Given these different effects, these different types of polymer could potentially be used to pinpoint coagulation, flocculation or floc strength limitations in sludge.

Activated sludge floc formation is mediated through extracellular polymeric substances (EPS), which act as a biopolymer where double layer compression and bridging can take place. EPS is predominantly made out of protein, polysaccharides and to a lesser extent humic acids and DNA (Frolund et al., 1996). Multiple studies have suggested that the structure and composition of EPS is one of the main factors affecting bioflocculation, citing total amount and the protein (PN) over polysaccharide (PS) ratio being crucial to aggregation proficiency.

Clearly, activated sludge floc formation is a complex process involving physical (DVLO/bridging) and biological (EPS production) processes. Many limitations in the floc formation process and it is not always clear where in the floc formation process the limitation persists. As such, evaluating strategies to mitigate elevated suspended solids in secondary clarifiers due to poor floc formation is not always straightforward. Blue Plains advanced wastewater treatment plant (AWWTP) in Washington, DC, USA currently has two secondary
treatment trains (East and West, Solids retention time (SRT) = 1-2 days) and one biological nutrient removal (BNR, SRT = ~ 20 days) system. The East secondary system is at the time of writing being bio-augmented with BNR sludge to achieve some nitrogen removal in the secondary reactors. The West secondary reactors historically have poor effluent suspended solids (ESS) (33 ± 16 mg total suspended solids (TSS) L$^{-1}$), while the East reactors perform marginally better (25 ± 22 mg TSS L$^{-1}$), although unexplained spikes in ESS persist. The BNR reactor performs excellent (6 ± 2 mg TSS L$^{-1}$). Mancell-Egala et al. (2017) did a comprehensive study on the settling properties of these three systems and determined that flocs formed in these three reactors were significantly different. West produced small flocs with slow settling velocity distribution, while East produced bigger flocs with faster settling velocities, most likely due to the bio-augmentation. A good correlation with between the threshold of flocculation (TOF), a metric for collision efficiency, and ESS was found for West secondary. This gave first indications that West might be limited in floc formation, although it is unclear whether this is limited in the flocculation or coagulation step. This correlation did not hold for East, indicating another limitation is at play. Due to the size of the flocs produced, Mancell-Egala et al. (2017) suggested that floc strength might be the limiting factor, although no conclusive evidence was given.

In this research, the possible floc forming limitations will be further explored. Orthokinetic flocculation tests will be used to assess the sludge’s floc formation. Synthetic polymers (polyDADMAC and linear and branched polyamide type) as well as FeCl$_3$ will be used to artificially improve a part of the
floc formation process to pinpoint coagulation, floculation or floc strength limitations present in East, West and BNR.

3.2 Materials and Methods

3.2.1 Sample location and acquirement

Blue Plains Advanced Wastewater Treatment Plant is the largest advanced wastewater treatment plant of this kind in the world, treating over 1.1 million cubic meters of sewage per day and serving District of Columbia and part of Maryland and Virginia. Samples for this study were obtained from two secondary systems (West and East; SRT = 1-2 days) and one BNR reactor (SRT = ~ 20 days). A full description of aforementioned reactors can be found in Mancell-Egala et al. (2017). Samples were collected using buckets or submersible centrifugal pumps when significant amount of sludge was needed and were acquired from between June to August 2016. All experiments were performed within a few hours of sampling.

3.2.2 Polymer types and preparation

Ferric Chloride (Fisher Scientific, USA) and PolyDADMAC (SNF Polydyne FL-4520, USA) were used as coagulant. The polyDADMAC was a very high charge density, low molecular weight polymer. PolyDADMAC was freshly diluted to 0.2% w/w using the company provided stock media at the same day of the experiment. Linear polyamide polymer with high-molecular weight and 10% charge density (SNF Polydyne, Clarifloc SE-1163, USA) and a medium-
molecular weight branched polyamide polymer with 10% charge density (SNF Polydyne, Clarifloc C-3220, USA) were used as flocculent polymers. Linear and branched polymer solutions (0.2% w/w) were prepared and activated on the same day as the experiment by slowly adding the polymer granules in deionized water and stirring the solution at 300 RPM for 30 minutes.

3.2.3 Modified Jar test

The standardized jar test (ASTM, 1995), was modified to better represent the real conditions seen in a clarifier, while still having enough resolution to determine differences in floc formation behavior. Diluted Sludge was poured into a modified Nalgene® 4L graduated cylinder (ø = 10 cm) and mechanically mixed at 245 s⁻¹ (500 RPM) for 10 seconds with an IKA Eurostar 60 (IKA, USA) mixer equipped with two 4-bladed axial flow impellers when liquid prepared polymer was added. Subsequently, the sludge was further agitated at 112 s⁻¹ (300 RPM) for 30 seconds to enmesh the polymer within the flocs. When two polymers were added, these two steps were repeated for every polymer. Mixing was throttled down to 22 s⁻¹ (100 RPM) for 10 minutes to allow for flocculation. Ten minutes was chosen as this was deemed sufficient for steady state floc formation to happen (Biggs & Lant, 2000; Wahlberg et al., 1994). The graduated cylinder was instantly baffled to dissipate kinetic energy and sludge was allowed to settle. After 1 minute, clamps 5 cm below the liquid level were opened and sludge was allowed to rapidly gravity drain into a sample cup. The TSS collected in this cup represent the fraction of total TSS that settled slower than 3 m h⁻¹. This test was
used as the basic procedure for creating the orthokinetic flocculation curve (OFC) (section 3.2.3, (1)), polymer response curve (PRC) (section 3.2.3, (2)), settling velocity distribution test (SVD) (Section 3.2.3, (3)).

(1) **Orthokinetic test**

Orthokinetic tests were used to assess the floc formation at different concentrations under non-rate-limiting conditions. The modified jar test was used at different sludge concentrations ranging from 100 mg TSS L\(^{-1}\) to 1500 mg TSS L\(^{-1}\), and thus chosen in flocculant settling range (below LOSS). Optimal polymer doses were spiked in these test after determination using the polymer response curve (Section 3.2.3, (2)). The control curve was subjected to the same protocol without the addition of polymer to include the effect of rapid mix on floc formation in the results.

(2) **Polymer response curve**

A polymer response curve (PRC) assessed the influence of different polymer concentrations on the floc formation. An orthokinetic curve without the addition of polymer was created prior to the test and a sludge concentration where 20% of the sludge was removed was chosen. At this concentration, floc formation was limited enough to have enough resolution for the effect of polymer dosage to be observed.

(3) **Settling velocity distribution test**

A discreet settling velocity distribution (SVD) of the sludge was obtained by subjecting it to different settling times: 5 min (CSV = 0.6 m/h), 2 min (CSV = 1.5 m/h), 1 min (CVS = 3 m/h) and 20 s (CSV = 9 m/h). SVDs were obtained at
the same sludge concentration as the polymer response curves. To assess the impact of shear on the SVD, both 22 s\(^{-1}\) or 91 s\(^{-1}\) (260 RPM) was applied for 10 minutes as flocculation step.

### 3.2.4 Classic and novel settling metrics

Sludge volume index (SVI) and initial settling velocity (ISV) were determined at 3.5 g TSS L\(^{-1}\) in a Nalgene® 2L settleometer according to the standard methods (APHA, 2005). Limit of Stoksian settling (LOSS) determines the sludge concentration where flocculent settling transitions into hindered settling and was measured according to Mancell-Egala et al. (2016) Threshold of flocculation (TOF) measures the minimal sludge concentration required for settleable flocs to form when subjected to a 2 min flocculation and settling time, which corresponds to a critical settling velocity (CSV) of 1.5 m/h. Six gradient concentrations from 100 mg/L to 1000 mg/L were prepared. Detailed modus operandi can be found in (Mancell – Egala et al., 2016).

### 3.2.5 Extracellular polymeric substances

Extracellular polymeric substances were extracted using a modified heat extraction method based on Li and Yang (2007). 2.5 mg TSS of freshly sampled sludge was centrifuged at 4000 s\(^{-1}\) for 5 minutes. The pellet was resuspended in 10 ml, pH adjusted (pH = 7.2), phosphate saline buffer (PBS) containing 2 mM Na\(_3\)PO\(_4\), 4 mM KH\(_2\)PO\(_4\), 9 mM NaCl and 1 mM KCl at 60 °C and immediately vortexed for 1 minute to shear of the loosely bound (LB) EPS. The sludge was
subsequently centrifuged at 4000 s\(^{-1}\) for 10 minutes and the supernatant collected as LB-EPS. Next, the pellet was resuspended in 10 ml PBS and incubated at 60 °C for 30 minutes. Lastly, the sludge was centrifuged for 15 minutes at 4000 s\(^{-1}\) and the tightly bound (TB) EPS fraction was recovered in the supernatant. Both LB and TB EPS were filtered through a 1.5 µm glass microfiber filter (Whatman, USA) and stored at -20 °C for subsequent analysis.

LB and TB EPS samples were analyzed for chemical oxygen demand (COD), total soluble protein (TP) and total polysaccharide (TS). COD was determined using Hach® kits. TP were determined using the The Lowry method (Lowry et al., 1951) was used using a modified Lowry Protein Assay kit (Thermo Fisher, USA) and bovine serum albumin (BSA) as standard. TS were determined using the DuBios method (DuBois et al., 1956) where glucose was used as a standard.

3.3 Results

3.3.1 High-rate activated sludge

The West reactor at Blue Plains AWTP has been operating as a high-rate activated sludge (HRAS) reactor at short SRT (1 – 2 days) and showcased the poorest performances in terms of effluent quality and collision efficiency (as depicted by TOF) of the three reactors assessed at Blue Plains (Table 3.1). Gravitational flocculation did not create any flocs faster than 1.5 m h\(^{-1}\) until the threshold of flocculation was reached, while applying orthokinetic shear at 20 s\(^{-1}\) induced the formation of flocs faster than 3 m h\(^{-1}\) even at the lowest concentration.
tested (Figure 3.1A). No significant difference (p-value = 0.5) was observed
between the gravitational and orthokinetic slope (-81 ± 28 %TSS g TSS\(^{-1}\) and -95
± 13 %TSS g TSS\(^{-1}\) respectively). Steady state orthokinetic floc formation was
achieved at 558 mg TSS L\(^{-1}\) (as indicated by the slope flattening out), where 48 ±
1 % of the sludge was unable to form flocs faster than 3 m h\(^{-1}\). The settling
velocity distribution showed that flocs predominantly (40%) settle between 1.5 –
3 m h\(^{-1}\). When harsher orthokinetic shear (90 s\(^{-1}\)) was applied, the distribution
shifted to the left, with 0.6 – 1.5 m h\(^{-1}\) being the predominant settling class.

HRAS sludge did not respond well to increasing dosages of coagulants
PolyDADMAC (C) and FeCl\(_3\) as indicated by the low final improvement at high
dosage (C= 16.9 ± 2.3 %; FeCl\(_3\) = 13.8 ± 3.4 %) as shown in Figure 3.3A. FeCl\(_3\)
yielded an immediate improvement at low concentrations, but did not respond to
an increase in dosage. C did respond to increasing dosages but failed to
outcompete FeCl\(_3\). Both linear polymer (LP) and branched polymer (BP) showed
a clear increase in the formation of flocs that settle faster than 3 m h\(^{-1}\) (Figure
3.3B). However, no significant difference in flocculation response was observed
between both polymers’ slopes (493 ± 8 % improvement (g polymer kg TSS\(^{-1}\))^\(-1\)
and 605 ± 158 % improvement (g polymer kg TSS\(^{-1}\))^\(-1\) for LP and BP
respectively; p-value = 0.343). Addition of 0.5 mg polyDADMAC g TSS\(^{-1}\) did
not improve the polymer response at low concentrations as indicated by the
similar slope to LP and BP (442 ± 119 % improvement (g polymer kg TSS\(^{-1}\))^\(-1\)),
however steady state was reached at a smaller dosage (Figure 3.3C).
Independent of the treatment, the percentage of sludge that formed flocs slower than 3 m h\(^{-1}\) decreased when sludge with increasing concentrations was subjected to orthokinetic shear. This decrease flattened out when steady state floc formation was achieved. C did not have observable effect on WEST sludge, and was not significantly different from the control (Figure 3.4A). LP and BP had a similar but profound effect on HRAS sludge as their slopes were nonsignificant from each other (LP = -14.0 ± 0.6 % improvement (g polymer kg TSS\(^{-1}\))\(^{-1}\); BP = -13.5 ± 1.3 % improvement (g polymer kg TSS\(^{-1}\))\(^{-1}\); p-value = 0.587) (Figure 3.4B). However, at 1555 mg TSS L\(^{-1}\), BP significantly (p-value = 0.02) outperformed LP in removal percentage of flocs (13.0 ± 2.7 % versus 1.7 ± 0.3 % for LP and BP respectively). Combination of polymer and PolyDADMAC did not yield significant improvements over using polymer alone (Figure 3.4C).

Figure 3.2A shows the settling speed distribution and the effect of increased shear this distribution. Intrinsically, West flocs predominantly settled slower than 3 m h\(^{-1}\) and are resistant to shear as indicated by the small increase (12.5±2.51%) in flocs settling slower than 3 m h\(^{-1}\). LP and BP performed similarly, creating flocs with settling speeds predominantly in the 3-9 m h\(^{-1}\) range and no significant change in floc strength was observed (Figure 3.5A). When polyDADMAC was combined with LP, fast settling flocs (> 9 m h\(^{-1}\)) were created, but were very prone to breakup when shear was increased. A Combination of polyDADMAC and BP performed worse than BP alone.
3.3.2 Bioaugmented high-rate activated sludge

Similar to the West reactor, East is operated as a high-rate activated sludge reactor with an average SRT of 1 – 2 days. East however is bioaugmented with about 0.2 kg TSS_{BNR} kg TSS_{East}^{-1} d^{-1}, mainly to allow for some nitrification in the secondary reactor. Effluent quality of the bioaugmented high-rate activated sludge (BioHRAS) reactor was better than the non-bioaugmented, but big fluctuations were present as indicated by the high standard deviation (Table 3.1). Collision efficiency was better than the conventional HRAS as indicated by TOF value. The slope (-99 ± 17 %TSS g TSS^{-1}) was, while (borderline) statistically nonsignificant from HRAS (p-value = 0.09) or BNR (p-value = 0.07), higher than both (Figure 3.1B). With addition of orthokinetic shear, a consistent downwards slope was observed (-51 ± 4 %TSS g TSS^{-1}) with increasing sludge concentration and flattened out at 1087 mg TSS L^{-1} at 46% solids slower than 3 m h^{-1} remaining (Figure 3.1B). The drop of orthokinetic flocculation occurs at lower concentration around 500 mg/L. Gravitational flocculation still has its limiting percentage around 45% while orthokinetic flocculation continuously goes downwards. The settling velocity distribution is similar to the non-bioaugmented sludge with a peak showing at the 1.5 – 3 m h^{-1} class (Figure 3.2B). However, the overall distribution is more right skewed whereas the non-bioaugmented HRAS sludge is more left skewed. Applying harsher shear (90 s^{-1}) deteriorated the 3 – 9 m h^{-1} class (from 21 ± 4 % to 3 ± 3 % in that respective class), while the 0.6 – 1.5 m h^{-1} class became more prominent (13 ± 3 % to 38 ± 8 % for 20 s^{-1} and 90 s^{-1} respectively).
Figure 3.3D shows that neither polyDADMAC nor FeCl₃ had any visible improvement on bioHRAS sludge. Only at very high polyDADMAC dosage (1 g polymer kg sludge⁻¹) a minor improvement of 18 ± 3 % over the control could be detected. LP and BP have induced a similar response on EAST sludge with similar slope (LP = 229.3 ± 31.7 % improvement (g polymer kg TSS⁻¹)⁻¹; BP = 219.8 ± 6.6 % improvement (g polymer kg TSS⁻¹)⁻¹; p-value = 0.660) and maximum obtainable improvement (LP = 82.2 ± 2.8 %; BP = 84.5 ± 2.5 %; p-value = 0.353). Combining 0.5 g polyDADMAC kg TSS⁻¹ with increasing concentrations of LP showed a significant improvement in response (639.8±75.7 % improvement (g polymer kg TSS⁻¹)⁻¹) compared to LP alone (p-value = 0.001), but plateaued at a similar maximum improvement point.

PolyDADMAC does not have observables effect on the orthokinetic profile for bioHRAS sludge, as the slope did not differ significantly (p-value=0.077) from the control, where no polymer is dosed (Figure 3.4D). LP showed a big improvement over the control treatment, while combining both polyDADMAC with LP did not yield any additional improvement over only dosing LP (LP = 122 ± 1 %TSS g TSS⁻¹; C+LP = -119 ± 4 %TSS g TSS⁻¹; p-value=0.347) (Figure 3.4D/E). BP had a significantly bigger effect on the orthokinetic profile than LP as it obtained a steeper slope (200 ± 2 %TSS g TSS⁻¹; p-value = 0.0001). However, this advantage disappeared when the sludge concentration reached 1000 mg TSS L⁻¹, resulting in the similar maximum removal potential (LP = 6.0 ± 0.8 %, BP = 6.3 ± 0.5 %). Combining polyDADMAC with BP counteracted the observed improvements over the other polymer treatments.
BioHRAS showed the highest intrinsic shear sensitivity of all sludges tested (Figure 3.5B). PolyDADMAC had no effect on this. All flocculent polymer showed a clear impact on the settling distribution with an increase in the 3 – 9 m h\(^{-1}\) and > 9 m h\(^{-1}\) class. In the case of LP, the formed flocs were weak and easily broken up as indicated by the sharp increase in particles settling slower than 3 m h\(^{-1}\). BP however was able to create strong flocs that were indifferent to the increased shear. This advantage disappeared when polyDADMAC was used in combination with BP.

3.3.3 Biological nutrient removal sludge

Within the treatment line of Blue Plains AWTP, the BNR reactor receives the wastewater from the East and West reactors. Designed for nitrification/denitrification, the average SRT of the reactor is 20 days and receives a low loading compared to the HRAS reactors. As such, the effluent suspended solids were the lowest of the tested reactors and collision efficiency was high (Table 3.1). Gravitational settling formed flocs that settled faster than 1.5 m h\(^{-1}\) at the lowest concentration tested (100 mg TSS L\(^{-1}\)), but the slope (-70 ± 23 %TSS g TSS’1) was similar to HRAS sludge (Figure 3.1C). When orthokinetic shear was applied, the sludge behaved similarly to the high-rate sludge, however produced more fast settling flocs at high concentration with the slope flattening out at 31 ± 4 %. Finally, BNR sludge had the highest observed EPS content of the tested sludges (Table 3.1).
FeCl$_3$ did not show a clear response on BNR sludge except when very high dosages were applied (Figure 3.3G). PolyDADMAC (C) did show an obvious trend even at low concentrations, in contrast with both HRAS sludges. Its slope and thus response is significantly larger than HRAS’ and bioHRAS’ slope (p-value = 0.006 and 0.0001 respectively). LP and BP performed indistinct from each other (311.9 ± 34.2 % improvement (g polymer kg TSS$^{-1}$)$^{-1}$ and 318.2 ± 78.4 % improvement (g polymer kg TSS$^{-1}$)$^{-1}$ respectively (Figure 3.3H). LP achieved a higher but insignificant maximum improvement compared to control (90.3 ± 3.0 %) than BP (87.1 ± 3.6 %). When 0.5 g polyDADMAC was combined increasing concentrations of LP, a sharp increase in the lower dosages was observed as indicated by the high slope (495.2 ± 136.8 % improvement (g polymer kg TSS$^{-1}$)$^{-1}$) (Figure 3.3I), however, the slope quickly flattened out at 54 ± 6 % at 0.1 g LP kg TSS$^{-1}$ and remained constant.

Unlike the HRAS sludges, 0.5 g PolyDADMAC kg TSS$^{-1}$ had a profound effect when floc formation at BNR was assessed at increasing sludge concentrations (-123 ± 7 %TSS g TSS$^{-1}$) (Figure 3.4G). LP has the steepest slope (-312 ± 21 %TSS g TSS$^{-1}$) (Figure 3.4H) and did significantly differ from the BP slope (-247 ± 30 %TSS g TSS$^{-1}$, p-value = 0.04) and the combined polyDADMAC and LP slope (-117 ± 12 %TSS g TSS$^{-1}$, p-value = 0.001) (Figure 3.4I). All treatments were able to reach the same steady state flocs formation (3.8 ± 0.7 %, 5.5 ± 1.8 % and 5.4 ± 0.3 % for LP, BP and C + LP respectively).

BNR produced flocs that are strong as indicated by the indifference of the sludge’s settling velocity distribution to low or high shear forces (Figure 3.2C).
Adding LP or BP helped achieving faster settling flocs that were more prone to break up (Figure 3.5C). Combining polyDADMAC with LP produced slower settling flocs than dosing polymer alone, however the flocs were completely indifferent to higher shear forces.

Table 3.1. Intrinsic characteristics of the tested sludge types.

<table>
<thead>
<tr>
<th></th>
<th>Bio-augmented HRAS</th>
<th>Bio-augmented HRAS</th>
<th>Bio-augmented BNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor performance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Process name</td>
<td>West</td>
<td>East</td>
<td>BNR</td>
</tr>
<tr>
<td>Effluent TSS</td>
<td>33.1 ± 12.4</td>
<td>23.8 ± 28.9</td>
<td>6.96 ± 4.49 mg TSS L⁻¹</td>
</tr>
<tr>
<td>Settleability parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOF</td>
<td>535 ± 139</td>
<td>369 ± 60</td>
<td>295 ± 12 mg TSS L⁻¹</td>
</tr>
<tr>
<td>LOSS</td>
<td>1706 ± 539</td>
<td>801 ± 259</td>
<td>1287 ± 307 mg TSS L⁻¹</td>
</tr>
<tr>
<td>ISV</td>
<td>3.37 ± 1.24</td>
<td>1.36 ± 0.95</td>
<td>2.29 ± 1.05 m h⁻¹</td>
</tr>
<tr>
<td>SVI30</td>
<td>88 ± 81</td>
<td>154 ± 60</td>
<td>122 ± 46 ml g⁻¹</td>
</tr>
<tr>
<td>Specific EPS content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total EPS</td>
<td>110 ± 37</td>
<td>93 ± 6</td>
<td>135 ± 10 mg COD g VSS⁻¹</td>
</tr>
<tr>
<td>PN/PS ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB-EPS</td>
<td>0.76 ± 0.85</td>
<td>1.85 ± 1.47</td>
<td>2.03 ± 0.76 mg BSA mg glucose⁻¹</td>
</tr>
<tr>
<td>TB-EPS</td>
<td>1.98 ± 0.57</td>
<td>2.23 ± 0.74</td>
<td>2.01 ± 0.35 mg BSA mg glucose⁻¹</td>
</tr>
<tr>
<td>Total EPS</td>
<td>1.63 ± 0.38</td>
<td>2.19 ± 0.96</td>
<td>2.00 ± 0.13 mg BSA mg glucose⁻¹</td>
</tr>
</tbody>
</table>
Figure 3.1. Orthokinetic and gravitational flocculation curves for HRAS (A) bioaugmented HRAS (B) and BNR sludge (C).

Figure 3.2. Settling velocity distribution at 20 s\(^{-1}\) (solid rectangles) and 90 s\(^{-1}\) (hollow rectangle) for HRAS (A) bioaugmented HRAS (B) and BNR sludge (C).
Figure 3.3. Polymer response curves for HRAS (A-C) bioaugmented HRAS (D-F) and BNR sludge (G-I).
Figure 3.4. Orthokinetic flocculation curves for HRAS (A-C) bioaugmented HRAS (D-F) and BNR sludge (G-I).
Figure 3.5. Settling velocity distributions for HRAS (A), bioaugmented HRAS (B) and BNR (C) sludge at 20 s⁻¹ (solid circle) and 90 s⁻¹ (hollow circle).

3.4 Discussion

3.4.1 Evaluation of modified jar tests

Orthokinetic flocculation curves, where different concentrations of sludge are subjected to a velocity gradient to promote (or obstruct at high gradients) floc formation, are designed to assess steady state floc formation at different amounts of collisions. As these concentrations increase, the steady state floc morphology...
and size will change, which is represented by a change in the amount the solids travel faster than 3 m h⁻¹. At low concentrations, less collisions happen, which resulted in smaller flocs assuming that the density of the flocs does not change. All sludges behaved similar each other below 500 mg l⁻¹ as indicated by the nonsignificant difference between the sludge’s OFC slopes. As such, PRC curves obtained at these concentrations only depict the influence of the polymer on collision efficiency without interference of the intrinsic stokssian characteristics of the sludge. At high concentrations, collisions are more prevalent and thus flocs were allowed to reach their maximum size as indicated by the curves in Figure 3.4 to flatten out.

3.4.2 Limitations in HRAS sludge

Effluent suspended solids measured in the effluent is the result of the coagulation and flocculation capabilities of the sludge. Consistent high effluent suspended solids observed in the HRAS reactor (Table 3.1) gives an indication that coagulation, flocculation, or both is limited. The control OFC curves relieved that HRAS and bioHRAS were significantly less able to form flocs faster than 3 m h⁻¹ compared to BNR at high concentrations, suggesting lower collision efficiency. Furthermore, the conventional HRAS sludge has the worst TOF value of all reactors tested, indicating an intrinsic floc formation deficiency.

PRC curves (Figure 3.3) give valuable information on how floc formation intrinsically, i.e. independent of the amount of collisions, responds to the dosed polymer. As such, it is a direct measurement of collision efficiency of the primary
particles and sludge. HRAS sludge showcased the biggest response in floc formation of all sludges when LP or BP were dosed as the 50% more flocs faster than 3 m h⁻¹ were formed. This strong initial response was a first indication that HRAS sludge might be limited in the flocculation step. Similarly, when 0.5 g polymer kg TSS⁻¹ was dosed, the OFC curves of the HRAS sludge improved, indicating that big or dense flocs were formed at lower concentration indicating improved collision efficiency. BP and LP followed the similar trend at increasing concentrations steady state flocs began to form. At this point BP created more flocs faster than 3 m h⁻¹, which became significant (p-value = 0.02) at the final concentration tested.

HRAS sludge has been known to form pinpoint flocs, which are a strong indicator of a floc formation deficiency. Pinpoint flocs have been known to be denser than conventional flocs and BP with its branched structure might take advantage of that by creating compact dense flocs rather than big ones. Low SVI and high LOSS (Table 3.1) further indicated small flocs formed as the former indicates good compressibility and the latter high tolerance to hindered settling. In both cases the sludge flocs are fairly non-interactive with each other and have been described as being coagulation limited as well as adding coagulant has been proposed as a solution to pinpoint flocs.

If the sludge is flocculation limited, only adding coagulant might does not yield any effect as only the final floc is measured in the test. As such, 0.5 g polyDADMAC kg TSS⁻¹ to HRAS sludge had no effect on the floc morphology as indicated by Figure 3.4A. Assessing if a sludge is coagulation limited can
hence only be assessed due to indirect effect and synergies with polymer addition when flocculation is limited as well. However, the PCR curve revealed a near instantaneous improvement of 10 – 15% when FeCl$_3$ was dosed, indicating that iron did aid the coagulation process. PolyDADMAC, being a low molecular weight polymer, will act as a flocculent at higher dosage as contrary to FeCl$_3$, it can act as a nucleation site for bridging, hence the shallow slope observed when only coagulant was dosed in Figure 3.3A. Figure 3.3C where both polyDADMAC and increasing amounts of LP were dosed did attain steady state faster than dosing polymer alone. Moreover, at 0.02 g LP g TSS$^{-1}$, C + LP significantly (p-value = 0.01) increased the collision efficiency compared to only LP, indicating that a coagulation was contributing to a lower overall collision efficiency. However, as 0.5 g polyDADMAC per g TSS$^{-1}$ was dosed on top of the LP, the additional gain in collision efficiency might just be a result from the nucleation site capabilities of polyDADMAC as described above. The addition of polyDADMAC + LP did produce significantly faster flocs (> 9 m h$^{-1}$) than dosing BP or LP alone, indicating that by improving coagulation and flocculation, bigger or denser flocs were created than when only polymer was dosed. These flocs were brittle, as they deteriorated once more shear was applied, which indicates that they are most likely bigger than denser, as strength of the flocs generally goes down when its size increases. The OFC curves for (bio)HRAS did not contain any information regarding coagulation limitations as coagulation is relatively independent of concentration as it more influenced by total charge than the amount collisions in contrary to flocculation. Therefore, flocculation is relatively slow compared to
coagulation. Hence, Figure 3.4C where both polyDADMAC and LP were used did not show any additional improvement over using polymer alone. However, at very low concentrations (< 150 mg TSS L\(^{-1}\)), LP nor BP had a significant improvement compared to the control (p-value = 0.42 and 0.26 for LP and BP respectively), indicating that the polymer has no effect on the sludge. High molecular weight polymers are designed to act on bigger particles and hence require aggregates before they have any effect. As such, this is a final indication that HRAS sludge might be coagulation limited as well as flocculation limited as no aggregates were able to reach the required size for the polymer to have any effect on. In the case of bioHRAS, and BNR which show no other indication of being coagulation limited, both LP and BP did have a significant effect on the amount of the flocs formed that settled faster than 3 m h\(^{-1}\).

EPS is the driver behind floc formation in activated sludge systems. The EPS of HRAS sludge did not differ significantly from the bioaugmented variant or BNR. Most studies show a negative correlation between settleability and EPS amount, however these studies assess settleability in terms of SVI, a parameter which has recently been scrutinized to not reflect normal clarifier behavior (Mancell-Egala et al., 2016). HRAS sludge had a very low protein over polysaccharide ratio (PN/PS ratio) in the loosely bound fraction of the EPS (Table 3.1). The repulsive forces between activated sludge cells have been contributed to LB-EPS (Li & Yang, 2007), while another study found that LB-EPS was responsible for attractive forces (Liu et al., 2010). However, a low PN/PS ratio has been more univocally accepted as an indicator for poor floc formation (Liao et
al., 2001; Morgan et al., 1990), and thus might explain the floc formation characteristics.

### 3.4.3 Impact of bioaugmentation of HRAS on floc formation limitations

Bioaugmentation of the high-rate activated sludge reactor with BNR sludge improved the overall proficiency of the solid separation as indicated by the lower effluent suspended solids (Table 3.1). However, effluent quality was quite eccentric in time as denoted by the high standard deviation, indicating a more situational limitation that might not always have an impact on effluent suspended solids. Bioaugmentation also improved the intrinsic collision efficiency of the sludge as noted by the lower obtained TOF value compared to the HRAS reactor. The shallower PCR slope for LP and BP (Figure 3.3E) indicate that bioaugmentation helped mitigating the flocculation limitation, but as no impact of polyDADMAC can be seen on the final floc formation (Section 3.4.4), flocculation assumed to be limiting. Dosing polyDADMAC and linear polymer increased the response of the latter on the sludge (figure 3.3F), however no effect of polyDADMAC nor FeCl3 was observed. Hence bioaugmentation of HRAS sludge mitigated a possible coagulation limitation, and changed the floc morphology of the sludge as indicated by the higher SVI and lower LOSS and ISV. This was also observed for this particular reactor in Mancell-Egala et al. (2017), where images revealed bigger flocs than the conventional HRAS reactor tested. Next, the OFC strengthen this hypothesis as a significant different between the slope of LP and BP was observed. As final floc size increases with
concentration until a maximum has been achieved at high concentration, the significant slope difference between LP and BP can be explained by BP creating stronger flocs that are less prone to breakup. BP, with its branched nature, creates more compact flocs with lower fractal dimension (Hjorth et al., 2008). This advantage of BP over LP diminished when the sludge concentration was increased, which can be explained by the larger impact of macro floc formation in the (tranquil) sedimentation step. When the concentration was high, too many collisions happen to observe the effect of floc strength as everything will be allowed to reflocculate. As such, no difference between LP and BP can be observed at higher concentrations.

Figure 3.2B also suggests a floc strength limitation, where almost all sludge in the 3-9 m h\(^{-1}\) class deteriorated into lower class when the sludge was subjected to 90 s\(^{-1}\) instead of 20 s\(^{-1}\) compared to the conventional HRAS. This was further explored in Figure 3.5B where dosing BP made the sludge very resilient to shear stress compared to LP. The linear structure of LP and high molecular weight promotes floc size which makes the flocs even more prone to breakup. Interestingly, when polyDADMAC was added in combination with BP, the benefit of the latter disappeared. One explanation is that the neutralization of charge caused an excess of positive charge, with less bridging as effect and a more brittle floc that is more sensitive to shear as result.
3.4.4 Impact of SRT and organic loading rate on floc formation limitations

The biological nutrient reactor had the lowest effluent suspended solids of the tested reactor (Table 3.1), as well as the lowest observable TOF and formation of gravitational induced flocs at the lowest concentration tested (Figure 3.1C). Therefore, BNR sludge can be considered not limited in floc formation for all practical reasons. BNR was also very resilient to shear stress as no significant change in settling velocity was found when the sludge was subjected to a higher velocity gradient. Both the PCR and OFC curve showed a greater affinity for LP than BP, which was especially visible at higher polymer dosages (Figure 3.3H) and very low sludge concentrations (Figure 3.4H). Given the very good intrinsic floc forming properties of BNR sludge, BNR aggregates are most likely larger than HRAS’ which explains LP’s bigger affinity. Moreover, BNR is a high SRT, low organic loading rate sludge, which is for creating denser flocs as the ash levels in the sludge increases with SRT.

There was a clear impact of polyDADMAC in both the PRC (figure 3.3G) and OFC (Figure 3.4G) tests, which might indicate a severe coagulation limitation. However, given that coagulation is a precursor of flocculation and BNR showed very good effluent suspended solids achieved and intrinsic floc formation properties, this apparent limitation might be a side effect of the test setup. Only the final floc is measured, as such, it is hard to distinguish between a limitation in the coagulation or flocculation. BNR’s flocculation might be non-limited enough for any improvement in coagulation to be seen in the final floc
morphology. If the sludge is (severely) flocculation limited as is the case for (bio)HRAS sludge, this is not the case as discussed above.

3.5 Conclusion

Three different sludge types where assessed for their possible floc formation limitations using modified jar tests. Three main conclusions can be summarized:

Conventional HRAS shows great affinity polymer but is non-discriminatory towards linear or branched polymer. This sludge can be considered predominantly flocculation limited with coagulation limitation becomes apparent at low concentrations. To mitigate limitations, dosing linear polymer is advised.

Bioaugmented HRAS shows greater affinity for branched than linear polymer. While being flocculation limited, floc strength is the main issue. As such, addition of branched polymer or careful design of clarifier headworks might be appropriate to mitigate ESS spikes.

BNR sludge shows no indication of a coagulation, flocculation or strength limitation. Since flocculation was not limited, low molecular weight coagulants did show an improvement in overall floc formation. Hence, cheap mannich-type coagulants might be appropriate if effluent suspended solids limits are especially stringent.

3.6 References


Chapter 4: Manuscript 2: Influence of extracellular polymeric substance (EPS) on the structured water content in the sludge and objective measurements of the structured water content.

Keywords: Activated sludge, Biosolids, Structured water content, Extracellular polymeric substances (EPS), Dewatering properties

Abstract

Structured water is the moisture content in the sludge that cannot be removed by conventional dewatering processes and it remains in the dewatered biosolids thus causing extra cost for the storage, transportation and disposal of the biosolids. In this study, the drying test that was applied for determination of the structured water content in five sludge types (primary sludge, secondary sludge, sludge before thermal hydrolysis process (THP), sludge after THP and sludge after anaerobic digestion (AD)) were proposed and evaluated. Two baselines simulating free water evaporation (baseline A and baseline B) combined with the drying rate curve, two baselines simulating weight changed caused by free water evaporation (baseline C and baseline D) combined with the weight curve and Monod model were applied as five determinations of the structured water content in the sludge. Results indicated that baseline A and baseline D considered all experimental impactors such as temperature, relative humidity and wind speed; baseline B could be applied when experiment condition was strictly controlled. Weight curve with baseline C was limited by environmental impactors. Monod
model provided mathematical expression of drying rate, however it was sensitive to the experimental environment. Sludge extracellular polymeric substances (EPS) composition, floc size and dynamic viscosity were also analyzed for their impact on the structured water content. The results indicated that there was no significant correlation between the tightly bound EPS, organic matter content, protein (PN) content, polysaccharide (PS) content and PN/PS ratio of the sludge EPS and the structured water content. Structured water content was negatively correlated with loosely bound (LB)-EPS polysaccharides ($R^2=0.57$ and 0.64 for baseline A and baseline B, respectively). Dynamic viscosity of the sludge did not influence the moisture evaporation, while the floc size of the sludge was positively correlated with the structured water content ($R^2=0.77$ and 0.60 for baseline A and baseline B, respectively). The biosolids quality could be enhanced by reducing the moisture content in the sludge, and possible treatments involved increasing floc size and decreasing LB-EPS polysaccharide contents.

4.1 Introduction

In wastewater treatment facilities, municipal wastewater is treated to remove the organic matters, pathogens and nutrients, and large amount of sludge is produced throughout the treatment processes. This sludge is collected and further treated to remove pathogens and moisture content. Dewatered sludge is referred to as biosolids, and it contains high level of nutrients such as organic matter, nitrogen and phosphorous, thus it has significant potential for land application (Lu, He and Stoffella, 2012). Class A biosolids that meets the US
Environmental Protection Agency’s (USEPA) requirements have been applied in agriculture and public green places as fertilizer (Roy et al., 2011). Resource recovery facilities take charge of the waste disposal and recycling. Biosolids are produced by dewatering the sludge from the wastewater processes at multiple stages such as primary and secondary clarification. Maximum separation between the solids part and the water content is desired to achieve effluent clarity, to reduce energy consumption for biosolids handling processes at the resource recovery facility, to limit the need for storage space after the final biosolids treatment and to reduce the requirements for transportation to the land application sites (Wakeman, 2007). Sources of the biosolids are made up of primary sludge from the primary clarification as well as the biomass produced during the treatment processes, where organic carbon, nitrogen and phosphorus are removed by biological processes and precipitation. Industrial or domestic sludge can contain up to 95% water on a mass basis (Colin and Gazbar, 1995). Thus, dewatering of the sludge is applied as a process that separates water from solids. Often, more than 40% of the total cost of wastewater treatment is spent on biosolids treatment, separation and disposal (Ruiz-Hernando, Labanda and Llorens, 2010). However, current dewatering treatment approaches are still leaving the biosolids with a high moisture content ranging from 88% to 65% (Vaxelaire, 2001), and improved dewaterability is continuous challenge. Dewaterability of biosolids correlates with the structured water content and the water distribution within the individual sludge flocs (Kopp and Dichtl, 2000). In the sludge flocs, water can be present in different compartments.
depending on the binding characteristics such as adsorption, capillarity forces and chemical bonds. Vesilind (1994) developed a classification of the types of water in sludge flocs, which had been applied in previous studies (Vaxelaire and Cézac, 2004; Tsang and Vesilind, 1990): (1) Free water: water that is unaffected by capillary force; (2) Interstitial water: water that is trapped into interstitial places between the sludge flocs due to capillary forces; (3) Surface water: water that is attached to the surface of the sludge flocs due to adhesive forces; (4) Chemically bound water: water that is tightly bound to the sludge flocs due to chemical interactions such as hydration water (Figure 4.1). Tsang and Vesilind (1990) showed that only the free water (1) and a small proportion of the interstitial water (2) can be removed by mechanical dewatering processes, thus the combination of interstitial water, surface water and hydration water was left within the biosolids (Wu, Huang and Lee, 1998). In this study, the structured water is defined as the moisture content in the sludge excluding free water. An approach for objective measurement of the structured water content in various sludge types is required to better understand the current limitations present in sludge dewatering. Furthermore, identification of the mechanism by which water binds to the sludge flocs will provide information regarding solutions to improve dewaterability and to achieve a reduced moisture content in biosolids.
**Figure 4.1.** Moisture distribution in a sewage sludge floc (freely after Kopp and Dichtl, 2000). Free water moved between flocs; Interstitial water was trapped in the interstitial places in the flocs; Surface water was adsorbed onto the surface of the floc particles; Chemically bound water bound to the floc particles through chemical bonds.

One approach that has been used by several research groups (Robinson and Knocke, 1992; Lee and Hsu, 1995; Chu and Lee, 1999) to measure the water distribution of biosolids is the drying test. One of the advantages of this approach is that the drying test provides a full curve of the drying period instead of just providing a number of the structured water content. Another advantage is that the drying test is easy to perform because it does not require the use of specialized equipment, thus it can be setup in any laboratory (Lee and Hsu, 1995; Matsuda, Kawasaki and Mizukawa, 1992). In this approach, a sludge sample is dried in a closed system at a constant temperature and relative humidity. The moisture loss is caused by evaporation of free water followed by structured water. The approach
was based on a hypothesis stating that different moisture types within the sludge have various evaporation rates (Vaxelaire and Cézac, 2004). Therefore, a change in the evaporation rate indicates a transition between two types of water associated with the sludge flocs. A drying curve were applied to describe the relationship between the water content and the evaporation rate (Figure 4.2).

**Figure 4.2.** Drying curve for the drying test (Lee and Hsu, 1995). The drying rate for different water types were different: A-B indicated free water evaporation rate; B-C indicated interstitial water evaporation rate; C-D indicated surface water evaporation rate; D-E indicated chemically bound water evaporation rate.
There has been a controversy regarding the determination of the structured water, and the content of structured water in the sludge floc depends on various measurement technologies (Vaxelaire, 2004). One applied determination of structured water defines it as water that does not freeze at -20 °C, at which free water would freeze (Colin and Gazbar, 1995). A dilatometric test that is based on this hypothesis can be applied to quantify the structured water content (Lee, 1996). In the method, a certain amount of sludge sample was subjected to freezing temperatures until -20 °C was reached. The samples were then reheated to the room temperature. It was hypothesized that only free water would freeze below -20 °C, so that the volume change of the sludge sample only would be caused by the free water freeze (Smith and Vesilind, 1995). The content of the free water in the sludge was determined by the volume change and then subtracted from the total water content of the biosolids to obtain the structured water content (Wu, Huang and Lee, 1998). The dilatometric test has been applied for determination of the structured water content because it provides contents of the structured water content directly (Vaxelaire, 2004, Smith and Vesilind, 1995). However, a value of the structured water content alone is not as convincing as a full curve provided by the drying test. Furthermore, the requirements for the precision of the equipment for the dilatometric test combines to make this method less practical.

Extracellular Polymeric Substances (EPS) play an important role in the biosolids dewatering processes (Neyens, 2004), and previous studies indicate that the EPS content can influence the moisture distribution and dewaterability (Houghton, 2001; Mikkelsen, 2002). Bound EPS was defined as insoluble EPS
that binds with bacterial cells to form a net-like structure that is involved in holding the sludge flocs (biofilms) together. It has further been classified as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS): TB-EPS is tightly attached on the bacterial cell surface, while LB-EPS pervades into the surrounding environment at the outer layer of the biofilm, forming a loose coverage of the TB-EPS (Wingender, Neu and Flemming, 1999). Proteins (PN) and polysaccharide (PS) are two major components of EPS (Sheng, Yu and Li, 2010). TB-EPS generally contains more protein, with a PN to PS ratio of 1.6 to 2.0, whereas LB-EPS contained a higher proportion of polysaccharide, with PN to PS ratio ranging from 0.7 to 0.9 (Basuvaraj, Fein and Liss, 2015). The later study also showed that TB-EPS leaded to the formation of granular flocs, which correlated with improved biosolids settleability and dewaterability. Meanwhile, a high LB-EPS content caused formation of pinpoint flocs with small floc size, which exhibited poor settleability and dewaterability (Basuvaraj, Fein and Liss, 2015). In another study, it was shown that a high LB-EPS content prevented efficient flocculation, settleability and dewaterability of a sludge (Li and Yang, 2007). This study also found that the interstitial water content present in the LB-EPS fraction (4.3% of total water content) was increased compared to the water content in the TB-EPS fraction (0.2% of total water content). Therefore, LB-EPS might result in a higher structured water content in the sludge.

The establishment of an objective method for determination of the structured water content in biosolids from DC Water was applied towards several sample types. The main focus was put on establishing an approach based on the...
previously described drying test that would be capable of providing a full drying curve to measure the different types of water present in the biosolids. Establishment of an objective approach would represent an improvement compared to currently applied approaches, where the determination of the structured water content is partially subjective (Chen et al., 1997; Lee, Lai and Mujumdar, 2006; Vaxelaire and Cézac, 2004). In this current study, objective approaches for quantifying the structured water content were applied for sludge with different characteristics obtained from different process steps at DC Water. The relationships between the structured water content and sludge characteristics like the EPS composition, floc size and the dynamic viscosity were investigated at the same time.

The samples were collected throughout the solid treatment processes. Based on the data obtained from the drying test, five objective approaches were developed and applied for evaluating the water content in the collected biosolids samples. The methodologies were all assessed and evaluated according to their theoretical and practical meanings. Analysis of the sludge viscosity and EPS content were performed for all samples to study the relationship between them and the structured water content.

4.2 Materials and Methodology

4.2.1 Sample locations and acquisition

The Blue Plains Advanced Wastewater Treatment Plant is the largest advanced wastewater treatment plant in the world, treating over 1.1 million cubic
meters (300 million gallons) of sewage per day and serving the District of Columbia and parts of Maryland and Virginia (DC Water, 2017). Five sludge types were studied in this project, following the solids treatment processes of the Blue Plains from November 2016 to June 2017. The sampling locations were: primary sludge collected at the primary gravity thickener (TS=5%); the biological activated sludge (the secondary sludge) (TS=5%) collected at the secondary thickening tank; the mix of primary and secondary sludge (40% of primary sludge and 60% of secondary sludge based on mass) (TS=5%) collected at a blended temporary storage tank (blending tank No. 2 in DC water) prior to the thermal hydrolysis process (THP); the thermal-hydrolyzed mixed sludge (Cambi sludge, the THP sludge) (TS=10%) collected after the THP process; the anaerobically digested sludge (the AD sludge) (TS=5%) collected at a blended temporary storage tank (blending tank No. 3 in DC water) before dewatering. The samples were collected using 500 ml plastic cups, and they were subjected to the drying test immediately after collection.

4.2.2 Determination of the structured water content

The drying test was operated to determine the structured water content in the sludge samples. In Figure 4.3, a closed chamber (54.5*54.5*38 cm) made out of Plexi glass was set up in the laboratory at Blue Plains Advanced WWTP. The temperature and relative humidity (RH) inside the closed chamber were controlled by an air condition unit and magnesium nitrate (Mg(NO₃)₂), respectively, and a detector (OM-92 temperature/humidity Data Logger, OMEGA) was placed in the
chamber to record real-time temperature and RH every 30 seconds during the
drying period of each sample. An air flow system, fixed in two opposite walls of
the chamber, provided recycled air flow (speed= 0.52 m/s), accelerating the
drying process. An electronic scale recorded the weight changes due to water loss
from three grams of sludge sample every 30 seconds, and the data was logged
through RS232 Data Logger software (Eltima Software 2.7). Aluminum pans
(diagram=5.4 cm, height=1.5 cm) were pretreated at 550°C for at least 20 minutes
to remove potential moisture before placed on the scale. The sludge samples
covered the surface of the aluminum pan to achieve an even drying area. Air
condition and magnesium nitrate were setup at least 40 minutes before the drying
test was started to reach steady state experimental conditions: temperature of
22°C and RH of 60%. Total solids (TS) and volatile solids (VS) were measured in
duplicate at the same day for each sample by heating five grams of sludge at
105°C and 550°C for at least 24 hours and 20 minutes, respectively (Dinuccio et
al., 2010).
Figure 4.3. Drying test set up. Electronical scale measured the weight change of the drying sample, and weight data was logged through the computer software (RS232); Magnesium nitrate controlled the relative humidity; air flow accelerated the evaporation of the sludge; temperature/relative humidity probe recorded the experimental impactors.

4.2.3 Extracellular polymeric substances (EPS) extraction

EPS were extracted in triplicate by using a heat extraction method developed by Li and Yang (2007) where all compounds of the EPS were extracted simultaneously. A phosphate saline buffer solution (PBS) was applied for extraction of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). PBS was prepared by mixing 2 mM Na₃PO₄, 4 mM KH₂PO₄, 9 mM NaCl and 1
mM KCl, with pH adjusted to 7.2. The sludge samples were diluted until total suspended solids (TSS) equaled to 1000 mg/L, after which 25 mg of the diluted samples were used for EPS extraction. The samples were centrifuged at 4000 rpm for five minutes. Then the supernatant was discarded and the concentrated solid pellet was resuspended in 10 ml heated (60°C) PBS followed by vortexing for one minute. The samples were then centrifuged for 10 minutes at 4000 rpm and the liquid supernatant was collected as LB-EPS. For the TB-EPS extraction, 10 ml of heated (60°C) PBS was added to the solid pellet and immediately vortexed for one minute. The mixture was subsequently transported to a tube with cap and heated at 60°C for 30 minutes. After heating, the sample was centrifuged at 4000 rpm for 15 minutes, and the supernatant was collected as TB-EPS (Li and Yang, 2007). All extracted EPS samples were labeled by the sludge type and date and kept at -20°C until further analysis. TSS and VSS of the diluted samples were measured in duplicate by heating at 105°C and 550°C for at least one hour and 20 minutes, respectively (El-Kamah et al., 2010).

4.2.4 Extracellular polymeric substances (EPS) Composition

(1) Soluble Protein Analysis:

The total protein content in the extracted EPS fractions was measured using an improved method developed from the Lowry method (Lowry et al., 1951) because it was widely applied in previous studies (Dulley and Grieve, 1975; Markwell et al., 1978; Peterson, 1979). This method was based on the reaction between the copper ions and peptides bound in the protein using Folin
reagent (Folin and Ciocalteu, 1927). A commercial modified Lowry Protein Assay kit (Thermo Fisher Scientific, USA) was applied for the total protein measurement. In triplicate, EPS samples were defrosted at 25°C and 400 µL of each EPS sample was transferred into a labeled glass tube. 400 µL of deionized water was prepared as a control. Two ml of the Modified Lowry Protein Assay solution (Thermo Fisher Scientific, USA) was added to each sample tube, the tubes were then sealed and mixed completely by shaking 10 times and incubated at 25°C for 10 minutes. At this point, 200 µL of Folin’s reagent (Thermo Fisher Scientific, USA) was added, and the tubes were immediately vortexed for one minute and incubated at 25°C for 30 minutes. Thereafter, the total protein concentration in each sample was determined by light transmission using a HACH DR2800 Spectrophotometer (HACH, Loveland, Colorado) at 750 nm.

(2) Soluble Polysaccharide Analysis:

The total polysaccharide concentration in the EPS was measured according to an improved measured developed from the DuBios method (DuBois et al., 1956) because it was widely applied in previous studies (Cuesta et al., 2003; Escot et al., 2001; Siddhanta et al., 1999). This approach was based on a colorimetric method to determine polysaccharide concentration. In triplicate, EPS samples were thawed, and two mL of each EPS sample was transferred into a labeled glass tube. 200 mL of deionized water was prepared as a control. 0.125 mL of 80% (by weight) phenol solution was added to each test tube, after which five mL of 98% (by weight) sulfuric acid was added immediately. Tubes were
then sealed and incubated for 10 minutes without mixing at 25°C. The tubes were then vortexed and incubated at 25°C in a water bath for 10 minutes. Thereafter the total polysaccharide concentration was determined by light transmission determined by using a HACH DR2800 Spectrophotometer (HACH, Loveland, Colorado) at 485nm.

(3) Chemical Oxygen Demand (COD) Analysis:
The organic matter concentration of the extracted EPS samples was determined as the Chemical Oxygen Demand (COD). The COD concentration was determined to study the influence of the organic matter content in the sludge on the structured water content. For this method, HACH® COD kits (HACH, Loveland, Colorado) were applied according to the manufacturer instructions (HACH, COD manual, Loveland, Colorado). In this approach, the strong oxidant potassium dichromate (K₂Cr₂O₇) was used to oxidize organic compounds present in the sample. During the reaction, the orange-red dichromate ion (Cr₂O₇²⁻) was reduced to the green chromic ion (Cr³⁺). The total concentration of Cr⁶⁺ and Cr³⁺ were measured colorimetrically after a two-hour reaction at 150 °C. After this, the concentration of Cr⁶⁺ and produced Cr³⁺ were measured by using the HACH DR2800 Spectrophotometer (HACH, Loveland, Colorado) stored program for low range (3–150 mg/L) and high range COD (20-1500 mg/L) measurement, respectively.
4.2.5 Floc Size quantification

The floc size distribution in the five types of sludge was determined to evaluate the influence on the structured water content. Fresh sludge samples were collected from the Blue Plains Advanced WWTP and transported, using ice bags to prevent bacterial growth, to the research laboratory at University of Maryland at College Park for quantification of the sludge floc size distribution using microscopic techniques. A capillary tube was used to take sludge samples by slightly touching the microscope slide, and the sample was then covered by a coverslip. The floc sizes were measured using a biological trinocular microscope (Motic AE31 series, HongKong, China), with a 10X objective and a ruler placed in one eye piece, where the length of each unit indicated 100 µm. The floc size was determined by counting 500 flocs for each sample (five randomly selected spots on the slide, 20 flocs for each spot, five replicates). The sizes of the 500 flocs were measured and recorded in order according to their sizes. Three characteristics of flocs were then defined using these floc size data: (1) average floc size, which was determined as the average value of 500 floc sizes; (2) maximum floc size, which was determined as average of the largest 10 flocs; and (3) minimum floc size, which was determined as average of the smallest 10 flocs.

4.2.6 Approaches for Determination of the Structured Water Content

From the drying test, water evaporated during the whole drying period, and the weight of the drying sample was recorded every 30 seconds. The drying rate was defined as the mass of water that could evaporate per hour, and it was
calculated by dividing the weight difference between two recordings with the time interval (30 s). Two types of curves were applied showing the results of the drying test. One was the drying rate (g/hr) versus moisture content (g \( \text{H}_2\text{O}/\text{g TS} \)) curve, and the other was the weight (g) versus time (hr) curve. To eliminate the influence caused by random fluctuations from the data obtained from the drying test and to obtain a flat curve, a rolling average was applied for the drying data analysis (Martin, 2001). The raw data of the weight change (K_i) was recorded chronologically (K_1 indicated the beginning of the drying test), and the average value of proximal 20 records was calculated as a new weight change (S_i), and the new weight changes was applied to make curves. If S_i was calculated as the average of date from K_{i-20} to K_i, then it was defined as the “forward rolling average”. If S_i was calculated as the average of data from K_i to K_{i+20}, then it was defined as the “backward rolling average”. Even though the shapes of the curves were similar independent of applying a forward or a backward rolling average, the results of the structured water content were different, and the difference of the results could exceed 0.1 g.

### 4.3 Results and Discussion

#### 4.3.1 Determination of the Structured Water Content

The structured water contents of the five sludge types were determined using five objective approaches. As mentioned in section 4.2.6, drying rate curve and weight curve were applied to represent the results of drying test.
### 4.3.1.1 Drying Rate Curve: drying rate (g/hr) versus moisture content (g H2O/g TS):

The drying rate curve (Figure 4.4) started from the right end (time =0) with a higher moisture content and moved leftward as the evaporation happened. It showed that the drying rate kept constant at the first half period of the drying test (higher moisture content), followed by a decrease when the moisture content reached around 3 g H2O/ g TS. The constant drying rate indicated free water evaporation, and when the drying rate started to decrease, another moisture type, which was structured water, began to evaporate (J. Kopp and N. Dichtl, 2000). The fluctuation of the drying rate curve made it difficult to determine the time, when the drying rate started to decrease, which was directly correlated to the value of structured water content. Therefore, the theoretical free water evaporation rate was considered as a proper baseline for the real drying rate, and the determination of drying rate drop was obtained by analysis of the difference between the theoretical free water evaporation rate and the real drying rate.

1. **Penman’s Equation for the Theoretical Evaporation Rate (baseline A)**

   Penman’s equation (Eqn 1) (Penman, 1948) has been widely applied to calculate the evaporation rate for the free water present at big surface areas like lakes and rivers. The original Penman’s equation considered solar radiation, vapor pressure, latent heat, psychrometric coefficient and wind speed as input data (Penman, 1948). However, not all data were available and necessary for the test of waste water samples. A simplified Penman’s equation (Eqn 2) was applied in this
study for the calculated drying rate, considering only temperature, relatively humidity (RH) and wind speed, and detailed assumption and approximation referred to Valiantzas (2006). Free water density and pan surface area were required for unit transition from (mm/d) to (g/hr). The calculated evaporation rate was plotted as the baseline A for the theoretical free water evaporation rate, and the absolute difference between real drying rate and the baseline A was defined as the absolute error. In Figure 4.4, the orange line indicated the baseline A, and it was a linear line that paralleled to the X-axis. During the drying period, temperature, RH and wind speed kept constant at 22°C, 60% and 0.52 m/s respectively, so that calculated evaporation rate from Penman’s equation did not change. The gray line (Figure 4.4) indicates the absolute error between the baseline A and the real drying rate, and a drop was observed when the real drying rate started to decrease. To determine when the drying rate started to decrease, a threshold for the relatively error was set. The absolute error curve kept steady when the data was within the moisture content ranging from 15 to 5 g H₂O/g TS. Assuming the absolute error of this range matched normal distribution, the average and standard deviation (STDV) were calculated using this part of data, and the threshold was set as “average - 3*STDV”. This threshold defined that 99.7% of the absolute errors closed to the average were considered as acceptable values, and the other 0.3% absolute errors were farther away from the average so that be considered as having big difference from the free water evaporation (baseline A) (Benhidour and Onisawa, 2010). This threshold selected the data that were away from the free water evaporation, and the structured water content was

63
obtained as the average of the five smallest moisture contents, when the absolute error was larger than the threshold, instead of the smallest moisture content, to eliminate the influence of random errors.

\[
E_{\text{pen}}(\text{mm/d}) = \frac{\Delta \cdot (Rn)}{\Delta + \gamma} \cdot \frac{y}{\gamma + \Delta} \cdot \frac{6.43 \cdot (f_u)\lambda}{\lambda} \quad \text{(Eqn 1)}
\]

\[
E_{\text{pen}}(\text{mm/d}) = 0.052(T + 20)(1-RH/100)(1-0.38 + 0.54u) \quad \text{(Eqn 2)}
\]

**Figure 4.4.** Drying rate curve for the THP sludge when applying forward rolling average. Baseline A represented the free water evaporation rate from Penman’s equation, and it showed the influence of the environmental impactors; Baseline B represented the free water evaporation rate from the weight curve, and it showed a constant drying rate; The first drop on the right side (A) was caused by the experimental humidity stabilization; The second drop on the left side (B) indicated the structured water evaporation.
(2) Weight Data for the Theoretical Drying Rate (baseline B)

The weight of the drying sample decreased as the drying process was taking place, thus a weight (g) versus time (hr) curve could be applied to show the drying test result (Figure 4.5). A proportional relationship between the weight and the drying time was observed until the weight curve started to flatten out, and the slope of the “weight versus time curve” indicated the drying rate. Therefore, the proportional relationship in the weight curve and the slope of this part could be considered as another theoretical free water evaporation rate baseline. The proportional part, which occurred at the first half of the weight curve, indicated the free water evaporation (J. Kopp and N. Dichtl, 2000), while the smooth part, which occurred at the last half of the weight curve, indicated the completion of the evaporation process. Therefore, the beginning of the structured water evaporation should occur between the proportional part and the smooth part.

Based on the principle of making as much data as possible and at the same time, avoiding the influence of the structured water evaporation, weight data from 3 - 20 hours of the drying period was considered as the free water evaporation and applied for the free water evaporation rate calculation (detailed discussion could be found in Section 4.3.1.2). Therefore, a constant evaporation rate, which was equal to the slope of the proportional part was plotted in the “drying rate versus moisture content curve” as baseline B (yellow line in Figure 4.4). The absolute difference between the real drying rate and the baseline B was defined as the absolute error (light blue line in Figure 4.4). The absolute error curve for baseline
B and the real drying rate kept steady when the data was within the moisture content ranged from 15 to 5 g H₂O/g TS. Assuming the absolute data of this part matched the normal distribution, the average and standard deviation were calculated using this part of data, and the threshold was set as “average-3*STDV”. Same as baseline A, this threshold could select the real drying rate data that were away from the free water evaporation (baseline B) (Benhidour and Onisawa, 2010). The structured water content was obtained as the average of the five smallest moisture contents when the absolute error was smaller than the threshold.

4.3.1.2 Weight Curve: weight (g) versus time (hr)

As mentioned above (Section 4.3.1.1), the weight of the drying sample and the drying time could be plotted and showed the result of the drying test. In Figure 4.5, the proportional part at the beginning period of the drying test indicated the weight change caused by free water evaporation, and the smooth part at the terminal period of the drying test indicated the completeness of water evaporation. The beginning of the structured water evaporation occurred between these two parts. Similar to the drying rate curve, a theoretical weight change caused by free water evaporation was applied as a comparison, and the structured water content was determined by analyzing the error between the theoretical and real weight curve.

(1) Proportional Part for the Theoretical Weight Change (baseline C)
The free water evaporation process was stable with a constant drying rate (J. Kopp and N. Dichtl, 2000). Therefore, theoretically, a fixed weight difference between adjacent recordings should be observed in the weight curve during this process, where a proportional relationship between the weight (g) and the drying time (hr) was shown. The drying test required three hours for experimental environment stabilization, and during this time, the weight change was not stable influenced by the relatively lower relative humidity (<60%), so that the weight data within this time was not considered for data analysis. To simulate the evaporation of free water, the weight data from 3 - 20 hours of the drying period was considered as this proportional part because a linear relationship between the weight and the drying time was shown during this period for all the samples. The slope and the interception were calculated indicating the free water evaporation rate and the moisture content in the sludge before the drying test, respectively. In Figure 4.5, the orange line showed the modeled weight curve for the free water evaporation as baseline C. An absolute error shown as the grey line indicated the absolute difference between the real weight change and the baseline C. The “absolute error” (grey line) kept stable and small for around 20 hours, then it increased as an exponential function until reached its maximum value. During the free water evaporation process, the real weight was similar like the baseline C, and the “absolute error” was stable and small. When the structured water started to evaporate, the drying rate decreased because the molecular acting force and capillary forces between the structured water and the particles prevented the structured water from evaporating as easily as the free water. Therefore, the real
weight lost became smaller than the modeled weight loss (baseline C), which only considered the free water evaporation, causing the “absolute error” increased. The “absolute error” increased even further when the moisture that had stronger acting force with solid particles started to evaporate. The baseline C was set to zero when the modeled weight was negative because the weight of sludge sample could not be smaller than zero g. An exponential function was found to represent the “absolute error” using weight data from 20 hours to the maximum value (R^2>0.96), and the slope was calculated using data every five minutes to show how the “absolute error” changed. The structured water content was obtained as the water content when the slope reached 50% of the maximum slope.

(2) Calculated Drying Rate for the Theoretical Weight Change (baseline D)

Penman’s equation provided the calculated evaporation rate for the free water, and it could be transited into weight change as a predicted weight change (baseline D) for comparison with the real weight change. In Figure 4.5, the yellow line indicated that the weight change came from the Penman’s calculated evaporation rate, and the light blue line indicated the absolute difference between the real weight and baseline D. For all samples, the “absolute error” was approximately zero with the average and the standard deviation (STDV) of the entire data both smaller than 0.005 g. The baseline D represented the weight change caused by free water evaporation using the evaporation rate calculated by Penman’s equation. When the structured water started to evaporate, the real drying rate decreased, and the real weight loose would also be smaller than the
baseline D. As mentioned above (Section 4.3.1.2, (1)), during the first three to 20 hours, it was the free water that evaporated, and data of this period was applied for the threshold determination. Assuming the absolute error data matched the normal distribution, a threshold was set up as the value of the “average + 3*STDV”, and it selected the data that had big difference from the baseline D. The structured water content was determined as the moisture content when the “absolute error” was bigger than threshold.

Figure 4.5. Weight curve for the THP sludge when the forward rolling average was applied. Baseline C represented the expected drying sample weight change for the free water evaporation; Baseline D represented the weight change from the Penman’s calculated evaporation rate; The error for baseline C fit an exponential function.
4.3.1.3 Monod Model to Determine the Structured Water Content

The drying rate curve had the similar shape like the Monod curve (Eqn 3), which described the mathematical relationship between the growth rate of microorganisms ($\mu$) and the concentration of limiting substrate ($S$) (Strigul, Dette and Melas, 2009). Two coefficients in the equation, $\mu_{\text{max}}$ and $K_s$, indicated the maximum growth rate of the microorganisms and the value of substrate when the growth rate reached a half of the maximum rate, respectively. In this study, the drying rate represented the growth rate of the microorganisms, and the moisture content in the sludge represented the limiting substrate. The drying rate was higher when the moisture was higher (similar to “sufficient substrate”) and then decreased when less moisture was left in the sludge (similar to “limited substrate”). A non-linear regression model was used to fit the Monod equation on the data using “Rstudio” software (RStudio®, Boston, MA). The structured water content was determined as the water content in the sludge when the slope of the matched Monod curve equaled to 0.008. Several other thresholds were attempted, and resulted indicated that a small change of the threshold ($\pm$ 0.001) could lead to a big difference of the structured water content ($\geq$ 0.1 g). The 0.008 was determined as the threshold because it leaded to realistic structured water content for all sludge types.

$$\mu = \mu_{\text{max}} \cdot \frac{S}{K_s+S} \quad (\text{Eqn 3})$$
4.3.2 Structured Water Content Values

Figure 4.6 and Figure 4.7 showed the structured water content for the five sludge types using the drying rate curve and weight curve, respectively.

4.3.2.1 Structured Water Content Values from the Drying Rate Curve

When applying the drying rate curve, the results of the structured water content for a particular sludge type were similar, independent of the baselines A and B. This was because baseline A indicated the evaporation rate of the free water under a certain temperature and relatively humidity, which was supposed to be constant according to Kopp and Dichtl (2000). On the other hand, the baseline B indicated the average evaporation rate of the first 20 hours, which was within the period of the free water evaporation. Therefore, the baseline A and the baseline B both represented the evaporation rate of the free water from two aspects, and they had similar values (Figure 4.4, orange line for the baseline A and yellow line for the baseline B). When the structured water started to evaporate, the real drying rate decreased and diverged from the free water evaporation rate. The difference between the structured water evaporation rate and the expected free water evaporation rate became even larger when more bound water started to evaporate, until the difference reached the threshold set above. Since the baseline A and the baseline B had similar values and were both paralleled to the X-axis, the time when a difference was observed between the real
drying rate and the two baselines were similar, causing similar results of the structured water content.

When applying the same baseline, no matter the Penman’s calculated drying rate (baseline A) or the constant drying rate (baseline B), the results of the structured water content were higher when use the backward rolling average than use the forward rolling average. That was because that when applying the rolling average, it moved the original “drying rate versus moisture content” curve slightly rightward and leftward for the forward rolling average and backward rolling average, respectively. Therefore, considering the shape of the drying rate curve (Figure 4.4), when the difference between the real drying rate and free water evaporation rate reached the threshold, which also indicated the obvious decrease in real drying rate caused by the transition from the free water evaporation to the structured water evaporation, the X-axis value for backward rolling average was slight higher than the forward rolling average.

The Monod model caused the structured water content smaller than the other measurements for all sludge types except for the Cambi sludge (after THP), and that was caused by the choice of the threshold for the Monod model measurement. “Rstudio” software provided a mathematical expression of the drying rate. The curve plotted by the mathematical expression was named as the modeled curve, and it started to indicate the drying rate instead of the real drying rate curve. The slope of the modeled curve increased as the moisture content left in the sludge decreased, so that a smaller threshold could lead to a higher
structured water content. In this study, the threshold was determined as 0.008 because it provided realistic structured water contents for all sludge types.

Figure 4.6 indicated that anaerobic sludge (AD) had highest structured water content. Wang, Shammas and Hung (2007) proved that during the anaerobic digestion process, while the anaerobic microorganisms break down the organic matters, hydrous particles were produced, and that might be the reason why the AD sludge contained higher structured water than other sludge types. Previous studies measured even higher structured water contents for the anaerobic digestion sludge, which were larger than five g H₂O/ g TS (Tsang et al., 1990).

**Figure 4.6.** Structured water content from the drying rate curve and Monod model. Baseline A and B measured similar structured water content for each sludge type, while Monod model measured smaller contents; Anaerobic digestion sludge had highest structured water content among the five sludge types.
4.3.2.2 Structured Water Content Values from the Weight Curve

Figure 4.7 showed discrepant values of the structured water content of the sludge. When applying the weight curve, two measurements that considered the baseline C and the baseline D caused different structured water contents, respectively. Baseline C failed to contain the influence of environmental conditions such as temperature, relative humidity and wind speed into calculation, and at the same time, it assumed a constant temperature (T) and relative humidity (RH) during the whole drying test. The temperature and relative humidity within the Plexi chamber fluctuated slightly according to probe records. Baseline C had large standard deviation because it was influenced by the experimental conditions (T and RH). On the other hand, baseline D considered the environmental impactors (T, RH and wind speed) into its calculation because its weight change was calculated from Penman’s equation. However, the standard deviation of baseline D was still large, indicating weight curve was not as good as the drying rate curve considering the stability of the data.
Figure 4.7. Structured water content based on results from the weight curve.

Baseline C had large standard deviation because it was sensitive to the experimental environment changes; Baseline D indicated that secondary and AD sludge had higher structured water content than other sludge types.

4.2.3.3 Evaluation of the Structured Water Content with Cake TS

The biosolids product obtained after the dewatering process is commonly referred to as cake solids, which would be applied in land application as biosolids (Werther and Ogada, 1999). Structured water, which could not be removed by the physical dewatering process remained in the cake solids. Therefore, the TS content of the cake solids should be correlated with the structured water content, which then could be applied to predict the cake solids based on the calculated structured water content. The TS of the cake solids for each sludge types was measured by Zhang et al. (2017), and was applied to assess the structured water contents from this project. Zhang et al. (2017) measured the cake TS by dosing
coagulant polymer, followed by centrifuge filtering and simulating the dewatering process of DC Water. The structured water content was shown as \((g \text{ H}_2\text{O}/g \text{ TS of the sludge})\) thus the cake TS could be calculated as \(1/ (1+\text{structured water content})\). Figure 4.8 showed the predicted cake TS based on the five approaches for determining the structured water in this project.

The results showed that the structured water content failed to accurately predict the cake TS of the sludge for all five approaches (Figure 4.8). In this project, the structured water was defined as the moisture in the sludge that excluded free water, and the values of the structured water content were determined based on the drying performance (drying rate or weight loss), which was different from the free water evaporation. To compare the drying performance with the free water evaporation performance, five thresholds were determined to assess the difference between them. When the difference was larger than the threshold, the moisture content at that point was determined as the structured water content (see Section 4.3.1). This indicated that the predicted cake TS based on the structured water content contained chemically bound water, surface water and all the interstitial water. On the other hand, a previous study indicated that the dewatering process could remove free water and a proportion of the interstitial water from the sludge (Tsang and Vesilind, 1990). This indicated that the “true” cake TS was determined, when the cake solids contained chemically bound water, surface water and only part of the interstitial water. The proportion of the interstitial water that was removed through dewatering process was still counted as the structured according to this project, thus, the predictions
overestimated the water content in the cake solids, so that they underestimated the
cake TS. However, the cake TS of the secondary sludge and the sludge before
THP was overestimated, indicating underestimate of the structured water content.
These two sludge types contained more EPS than the other sludge types (Table
4.1). The high EPS content could reduce the sludge dewaterability (More et al.,
2014) and cause high moisture content in the cake TS.

**Figure 4.8.** Cake TS calculated by structured water contents determined by five
objective measurements as well as true cake TS. The structured water content of
this project failed to predict cake TS because of the influence of interstitial water
and EPS content in the sludge.

**4.3.3 Impact of EPS on the Structured Water Content**

Tightly bounded and loosely bounded EPS were extracted for the five
sludge types, and COD, PN and PS were measured for further analysis of the
relationship between the structured water content and EPS composition. The
concentrations of each compounds were listed in Table 4.1. The importance of these results will be discussed in the subsequent sections.

**Table 4.1.** EPS analysis for five sludge types, based on COD, PN, PS, PN/PS ratio and TB-EPS/LB-EPS ratio. Average and standard deviation are shown.
4.3.3.1 Impact of COD on the Structured Water Content

COD values showed the content of organic matter in the sludge, and the COD values for each sludge type could be explained by the mechanisms of different treatment processes. Municipal sewage contained high level of organic matters from residential drains. Therefore, the primary sludge, which captured the suspended solids in the municipal sewage by gravitation, had higher organic matter shown as high COD values (Table 5.1). Secondary sludge was compositio

by the microorganisms that consumed the organic carbon in the primary sludge for growth. The sludge before THP (BT2) was the mixture of the secondary and primary sludge (primary sludge and secondary sludge count for 40% and 60% of the total sludge by weight, respectively) by weight, so that it had the COD value between the two sludge types. THP process made the organic compound of the sludge more biodegradable by compressing the sludge under high temperature. In DC Water, the THP process was achieved using Cambi technology, and the sludge after THP was be referred as Cambi sludge to highlight this technology. In the THP process, organic matter was degraded into smaller molecules and inorganic matter, so that the COD value of Cambi sludge was relatively small. In the AD process, smaller molecules of organic matter from the THP are degraded into inorganic matters, so that smallest COD value was observed. However, the COD value was still high in the AD sludge (Table 5.1) because the AD process could not achieve 100% biodegradation efficiency, and the remaining organic matters that failed to be degraded cause the COD content in the AD sludge.
No significant correlation between the COD content and the structured water content was observed for what? \((R^2<0.3)\) (Figure 4.9). Therefore, the COD value of a sludge type, which represented the influence of each treatment process on organic matters, failed to correlate with the structured water content of a sludge.

**Figure 4.9.** The relationship between the COD and the structured water content. \(R^2\) for each fitted line were all smaller than 0.3, indicating no linear relationship between the structured water content and the COD content of the sludge, no matter which approach was applied for structured water content measurement.
4.3.3.2 Impact of PN and PS on the Structured Water Content

PN has in other studies been shown to have a high water-holding capacity (More et al., 2014; Sponza, 2002). Therefore, a sludge type that contained a high PN content was supposed to have a higher structured water content. Figure 4.10-4.14 (A) showed the impact of PN on the structured water content for the five sludge types, but no correlation was observed between these two parameters (R^2<0.2). Figure 4.10-4.14 (B) showed the impact of PS on the structured water content for the same five sludge types. Similar to PN content, there was no correlation between the structured water content and the PS content (R^2<0.1).

However, when the PS data were compared to the drying rate curve and baseline A, a stronger correlation between the structured and the LB-EPS-PS was observed (R^2=0.57). A similar correlation was also observed, when a comparison between the drying rate curve and baseline B took place (R^2=0.64). Even though the R^2 was smaller than 0.9 and could not indicate any significant correlation, it was still larger than the other R^2 values. It indicated that compared with any types of PN and TB-EPS-PS, the structured water content was negatively correlated with the LB-EPS-PS.

Although neither Figures 4.10-4.114 (A) nor Figures 4.10-4.14 (B) showed strong correlations between the structured water content of a sludge and its PN or PS alone, there was still a possibility that the PN and PS worked together to influence the structured water content of a sludge. Since the PN/PS ratio was an important parameter for the EPS (Ren et al., 2016), the influence of
its PN/PS proportion on the structured water content was studied for LB-EPS, TB-EPS and total EPS (Figure 4.10-4.114 (C)). However, even though a trend that a sludge that had a larger PN/PS ratio had a higher structured water content was observed for all figures, it still lacked a significant correlation with a large R². No matter which approach was applied to determine the structured water content, the R² for the fitted lines were all smaller than 0.4, indicating no significant correlation was observed between the PN/PS ratio and the structured water content.

4.3.3.3 Impact of TB-EPS/LB-EPS Ratio on the Structured Water Content

TB-EPS had higher PN/PS ratio than the LB-EPS (Table 5.1), and a sludge that had larger PN/PS ratio contained a higher structured water content (Figure 4.10-4.14 (C)). Therefore, the content of TB-EPS, LB-EPS and TB-EPS/LB-EPS ratio were plotted to study how they would influence the structured water content of the sludge. Figure 4.10-4.14 (D) showed that there was no correlation between the TB-EPS (R²<0.1) or the TB-EPS/LB-EPS ratio (R²<0.1) and the structured water content. When apply the drying rate curve and baseline A, larger R² value was observed between the structured water content and the content of the LB-EPS (R²=0.29), however, it still failed to indicate any obvious correlation.

There was another fact that made it even more complicated to study the influence of TB-EPS and LB-EPS content on the structured water content. TB-EPS was considered to have higher water-holding capacity because it contained
more PN than PS (Basuvaraj, Fein and Liss, 2015). However, for some sludge type like the primary sludge, its LB-EPS had higher PN/PS ratio than that of the TB-EPS of other sludge types (Table 4.1). Therefore, even the primary sludge had smaller TB-EPS/LB-EPS ratio (1.9) (Table 4.1), its LB-EPS could be considered as a material that had high capacity to hold moisture, making the overall structured water content higher than other sludge types.
Figure 4.10. The relationship between structured water content measured by drying rate curve (baseline A) and (A) PN; (B) PS; (C) PN/PS ratio; and (D) EPS.
Figure 4.11. The relationship between structured water content measured by drying rate curve (baseline B) and (A) PN; (B) PS; (C) PN/PS ratio; and (D) EPS.
Figure 4.12. The relationship between structured water content measured by weight curve (baseline C) and (A) PN; (B) PS; (C) PN/PS ratio; and (D) EPS.
Figure 4.13. The relationship between structured water content measured by weight curve (baseline D) and (A) PN; (B) PS; (C) PN/PS ratio; and (D) EPS.
PN-Structured water content - Monod

(A)

PN-Structured water content - Monod

(B)

1684
Figure 4.14. The relationship between structured water content measured by Monod model and (A) PN; (B) PS; (C) PN/PS ratio; and (D) EPS.
4.3.4 Impact of Dynamic Viscosity on the Structured Water Content

Dynamic viscosity was an important characteristic of the sludge, and a higher dynamic viscosity indicted that the sludge was more resistant to shearing flow. The acting force between the different levels of the sludge might also have influence on the evaporation of free water. This study hypothesized that a sludge type that had a higher viscosity had a higher structured water content. Blending two letters of the primary sludge at 18,000 rpms for 1 minutes (Waring Commercial, #WSB) successfully increased the viscosity of the sludge by 60%, as shown in Figure 4.15. The TB-EPS was the main fraction of the net-like EPS (Yuan, Wang and Feng, 2014) so that determined the structure of the sludge flocs (Ding et al., 2015). The fast blending destructed the sludge flocs into smaller pieces and released the TB-EPS so that increased the viscosity of the sludge. The structured water content of the blended sample was determined using the measurement that applying the drying rate curve and baseline A. The structured water content of the primary sludge decreased from 2.12 g H$_2$O/g TS to 1.50 g H$_2$O/g TS after blending test, which indicated that increasing the viscosity could decrease the structured water content of a sludge type. The result failed to confirm the hypothesis. Two possible explanations were raised to explain this phenomenon. First, dynamic viscosity described the sludge resistance to the shearing flow, however, during the whole drying test, the sample was placed in the aluminum tank steadily, with no shearing flow occurred, so that the vertical evaporation was not affected by the dynamic viscosity. Second, blending the sludge sample at a high mixing speed could cause shear force and centrifugal
These two effects could break the flocs into smaller pieces, so that release part of the interstitial water, which was supposed to be trapped in the cracks of the flocs, into free water. Therefore, blending the primary sludge finally decreased the structured water content in the sludge.

Dynamic viscosity of other sludge types should be measured and analyzed considering their TS and structured water content, so that more data would be available to make a full curve about the relationship between the viscosity and the structured water content.

**Figure 4.15.** Dynamic viscosity for the primary sludge before blending (orange) and after blending (blue).
4.3.5 Impact of Floc Size on the Structured Water Content

Flocs of the five sludge types under the microscope were shown in Figure 4.16, and the floc sizes based on 500 flocs of each sample was listed in Table 4.2. The secondary sludge had the biggest flocs followed by the sludge before THP and primary sludge, and the Cambi sludge had the smallest floc size. AD sludge flocs did not show clear floc shapes under the microscope, and it was more like a mash with pieces of particles. In the AD sludge, large flocs that connected to each other were observed, as well as some tiny flocs that distributed in the extra places, making it hard to determine the floc size. Considering AD and secondary sludge, which both had big floc size also had more structure water content, floc size could be considered as an important characteristic of a sludge that was correlated with SWC ($R^2=0.77$ and 0.60 for baseline A and baseline B, respectively).

Figure 4.16. Flocs of five sludge types under microscope view. Secondary and anaerobic digestion (AD) sludge had larger floc sizes than other sludge types.
Table 4.2. Floc sizes for the primary sludge, secondary sludge, sludge before THP and sludge after THP.

<table>
<thead>
<tr>
<th>unit: μm</th>
<th>average</th>
<th>max</th>
<th>min</th>
</tr>
</thead>
<tbody>
<tr>
<td>primary</td>
<td>26.8</td>
<td>109</td>
<td>5</td>
</tr>
<tr>
<td>secondary</td>
<td>68.3</td>
<td>243</td>
<td>10</td>
</tr>
<tr>
<td>before THP (BT2)</td>
<td>41.9</td>
<td>108</td>
<td>10</td>
</tr>
<tr>
<td>Cambi (THP)</td>
<td>21.9</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

4.3.6 Assessment of the approaches for determination of the Structured Water Content

The five objective measurements of the structured water content all aimed at eliminating the subjective judgement caused by visual observations or contrived choices when determine the structured water content. The structured water content determined when applying the drying rate curve had similar values for each sludge type. However, applying the Monod model and weight curve usually caused different results. Therefore, a comprehensive discussion about the five measurements was necessary, considering both theoretical meaning and practical meaning.

4.3.6.1 Penman’s Equation for the free water evaporation rate (baseline A)

Penman’s equation was a widely applied estimate for the free water evaporation, however, it fitted the big surface water systems like lakes.
(Valiantzas, 2006). In this study, an aluminum tank (diameter=54.17 mm) was applied as the container of the drying sample, which was apparently not a big surface area. The fringe effect would not be neglected in this study. Simplified Penman’s equation was applied in this study because only temperature, relative humidity and wind speed were required, which were accessible from the drying test. This measurement was the only one that applied all the environmental impacts into the structured water content determination among the five measurements. The limited researches on the free water evaporation with small surface area and their complicated model was another reason of why other model was not applied (Koerselman and Beltman, 1988). The free water evaporation in the environmental water system was not same as that in the sludge. The interaction between the water molecules and the sludge flocs would influence the free water evaporation. For all drying samples, the absolute error between the drying rate data and the baseline A kept small and steady from 5-15 g H₂O/ g TS, indicating that it belonged to the free water evaporation period. Therefore, this part of data was applied to set the threshold.

4.3.6.2 Slope of the weight curve for the free water evaporation (baseline B)

The real drying rate was the result of the weight lost with 30s divided by the time interval, therefore, the slope of the weight vs. time curve should represent the drying rate. During the first half of the drying test, it was free water that evaporated, and it should have a constant drying rate theoretically, even though in the experimental condition, the drying rate fluctuated slightly affected by the
slight change of the experimental environment. The slope of the weight curve using data from 3-20 hours could be considered as the average evaporation rate of the free water, therefore was applied as the baseline B for the free water evaporation. This was also a good way to combine the information of the drying rate curve and weight curve together. The threshold of the structured water content was set considering the data from 5-15 g H₂O / g TS to keep coincidence with baseline A. This measurement was more influenced by the experimental factors than the last one (baseline A). A saltation in the environmental conditions, no matter temperature, relative humidity or wind speed changed, could cause a variation in the drying rate. However, such variation was neutralized and could not be shown in the baseline B when calculating the average drying rate as the free water evaporation rate. Therefore, this measurement could only be considered when the experimental environment was controlled scrupulously.

4.3.6.3 Monod model to simulate the drying test

Monod model was applied to simulate the drying rate curve, aiming to find the mathematical expression of the complicated curve for further data analysis. The drying rate curve had the similar figure shape of the Monod model curve, and “Rstudio” software successfully provided the coefficients for the function expression. This was the only measurement that provided a mathematical expression of the drying rate. However, the Monod model did not fit all the samples well, especially when the free water drying rate fluctuated obviously. The threshold of the structured water content was determined as the slope of the
Monod model started to be bigger than 0.08 considering the reasonable structured water content values. Other threshold could be chosen and other mathematical analysis of the Monod model could be considered based on particular requirement.

4.3.6.4 Proportional part of weight curve for the free water evaporation

(baseline C)

As discussed above, the first half of the drying test was the free water evaporation, and since it had constant drying rate, a proportional linear part of the weight curve could be observed and served as the free water evaporation baseline. The error between the baseline C and the real weight change was plotted then, and an exponential function was determined to represent the error plot. These steps did help to provide an objective measurement of the structured water content, however, too many calculations on the raw data introduced multistep error between the final data and the raw data. Also, the accomplishment of this measurement based on the determination of the exponential function of the error between the baseline C and the real weight change. The coefficients of the exponential function had significant influence on the structure water content.

4.3.6.5 Weight change from the calculated drying rate for the free water evaporation (baseline D)

Applying the calculated free water evaporation rate to the weight curve was another attempt to combine the information of the two curves together. The
weight change from the calculated free water evaporation rate fitted to the real
weight change best (Figure 4.5), and for most (>80%) drying tests, it was hard to
tell the difference of the baseline D and the real weight change visually. This
approach considered the experimental environmental impactors such as
temperature, relative humidity and wind speed into calculation when applying the
Penman’s equation.

4.4 Conclusion

The five approaches for determination of the structured water content in
the sludge meet the requirements as an objective measurement with both
advantages and shortcomings. Applying the drying rate curve and baseline A
approach considered the impact of environmental conditions such temperature
and relative humidity into the calculation, so this approach could be applied when
these experimental conditions were unstable. Penman’s equation estimated the
free water evaporation rate under big surface area, however, the drying test
applied aluminum pan (diameter =5.4 cm) as the sludge container, and the
influence of the fringe effect such as the adsorption interaction between the sludge
and aluminum pan could not be ignored in this case. Baseline B provided a
constant drying rate simulating the free water evaporation under fixed temperature
and humidity. However, it failed to represent the influence of the experimental
condition changes. The weight curve provided a flatter curve than the drying rate
curve, but neither baseline C nor baseline D could be considered as a good
measurement. The problems that occurred in the baseline B approach also
occurred for the baseline C approach. Baseline D combined the information of the drying rate curve and weight curve together, and it also took experimental impactors into consideration. The Monod model was the only one that provided a mathematical expression of the drying rate. However, results indicated that it was influenced by the experimental conditions such as temperature and relative humidity and could not be applied when the experimental conditions fluctuated.

Structured water referred to the moisture content that could not be removed by physical dewatering process, so that could be applied to predict cake TS for a sludge (Werther and Ogada, 1999). However, the structured water content determined based on the five approaches in this project could not predict the cake TS of each sludge type accurately. The dewatering process removed the free water as well as a proportion of the interstitial water from the sludge, which resulted in a lower moisture content in the cake solids and a higher cake TS compared with the results of this project. However, for the sludge types that contained more EPS reduced dewaterability of the sludge was observed leading to increased moisture content in the cake solids. This caused a reduced cake TS compared with the results of this project.

There was no strong correlation between the EPS sludge composition or dynamic viscosity and the structured water content ($R^2 < 0.5$) was observed. Among the EPS compounds, a larger $R^2$ was observed between the structured water content and the LB-EPS-PS ($R^2 = 0.64$), indicating a negative correlation, however, it still failed to indicated a significant correlation. The structured water
content was positively correlated with the floc size of the sludge ($R^2=0.77$ and 0.60 for baseline A and baseline B, respectively).

4.5 Acknowledgements

The authors appreciated the support from the Blue Plains Advance Wastewater Treatment Plant (DC Water), who funded this study and made it possible. The Civil and Environmental Department of the University of Maryland also provided valuable support on this project.

4.6 References


Chapter 5: Conclusion

Biosolids production is important to the solid treatment process in the wastewater treatment facilities. It contains high content of nutrients beneficial for soil reclamation thus biosolids have been widely applied in land applications as fertilizer. This study investigated the enhancement of the biosolids production from two aspects: 1) sludge clarification, which increased the source of the biosolids and enhanced the biosolids production quantitatively, and 2) sludge dewaterability, which determined the moisture content in the biosolids and enhanced the biosolids production qualitatively. Removing moisture content from the biosolids reduced its volume, so that saved cost and energy consumption of the biosolids storage, transportation and disposal.

Bioflocculation was an important step for the activated sludge clarification. The high-rate activated sludge bioflocculation limitations were studied by modified jar tests and polymer dosing. Polymer dosing involving coagulant, linear polymer and branched polymer enhanced the coagulation, flocculation and floc strength, respectively. The results indicated that the three activated sludge types that were tested at DC Water exhibited different limitations (Chapter 3). High-rate activated sludge (HRAS) was flocculation limited as well as coagulation limited, and dosing experiments with coagulant and polymers both caused improvements of the sludge bioflocculation. Bioaugmentation with sludge from biological nutrient removal (BNR) reactor mitigated the flocculation limitation in the HRAS. However, bioaugumented high-rate activated sludge (BioHRAS) was floc strength limited, and branched polymer enhanced its
bioflocculation by strengthening the sludge flocs (Chapter 3, Figure 3.5). BNR sludge showed the best bioflocculation behavior among the three activated sludge types, with least effluent total suspended solids (TSS) (6.96 ± 4.49 mg/L), indicating no flocculation and floc strength limitations.

The structured water content is important for the dewaterability of the sludge, since it determines the moisture content in the biosolids. To test this parameter, a drying test was evaluated using five sludge types (primary sludge, secondary sludge, sludge before thermal hydrolysis process (THP), sludge after THP and sludge after anaerobic digestion (AD)) in the Blue Plains WWTP. Based on these experiments five approaches were assessed in this study. The results showed that the five approaches could be used under different circumstances:

- The drying rate curve and baseline A could be applied when the influence of the experiment temperature (T), relative humidity (RH) or wind speed was significant because the equation of baseline A took these environmental impactors into consideration.
- Baseline B could be applied only when the experimental environment (T, RH and wind speed) was constant.
- The weight curve and baseline C also depended on the experimental conditions such as T, RH and wind speed. The data analysis of this approach involved multistep calculation such as rolling average, curve fitting and slope calculation, where multiple errors caused by each step could not be avoided for this measurement.
Baseline D fitted the real weight change best with small absolute difference between the real weight change and the baseline D (range from 0.0001g to 0.001g). It also took environmental impactors (T, RH and wind speed) into consideration because of the calculated evaporation using Penman’s equation.

The Monod model provided a mathematical expression of the drying rate. However, it could only be applied when experimental conditions such as T, RH and wind speed were constant, under which the real drying rate curve had an approximate shape of the Monod model.

The structured water content determined by these five objective determinations did not match the cake TS of the five sludge types because different from this project, which considered all the interstitial water as the structured water, the dewatering process removed a proportion of interstitial water from the anaerobic sludge, so that reduced the moisture content in the cake solids, causing a higher cake TS than the calculated cake TS using the structured water content from this project. For the sludge that contains high content of EPS such as the secondary sludge, which reduced the dewaterability of the sludge, more moisture content was left in the cake solids, resulting a smaller cake TS compared with the calculated cake TS using the structured water content from this project. The relationship between the structured water content and the sludge EPS composition, dynamic viscosity and floc size were also studied, and no strong correlation was observed between either of them (Chapter 4). However, when
apply the drying rate curve, an obvious stronger correlation between the soluble polysaccharide content in the loosely bound extracellular polymeric substances (LB-EPS-PS) and the structured water content ($R^2=0.57$ and 0.64 for baseline A and B, respectively) was observed, and increased LB-EPS-PS content caused a decrease in the structured water content. The size of the sludge flocs was also influencing the structured water content ($R^2=0.77$ and 0.60 for baseline A and baseline B, respectively). Larger flocs provided more space within the flocs, so they could trap more interstitial water than smaller flocs. From another aspect, small flocs could be considered as the result of breaking up of the larger flocs. During the break up, the floc structure was destroyed, and interstitial water that was used to be trapped inside the flocs was released into free water. Therefore, increased sludge floc size caused increase in the structured water content.

In the Blue Plains Advanced Wastewater Treatment Plant (DC Water), 500 tons of wet biosolids are produced every day, with total solid content around 30% (DC Water, 2017). The facility spends money, manpower and energy on the management of these biosolids. 350 tons of water were disposed along with 150 tons of dry biosolids. The cost caused by storage and transportation of the wet biosolids could be reduced by reducing the moisture content in the biosolids and enhancing the biosolids production quality. Conventional dewatering processes successfully remove free water from the sludge, so that it is the structured water that remains in the biosolids (Tsang and Vesilind, 1990). Finding out the sludge characteristics that determine its structured water content could provide solutions that reduce the structured water content of the sludge, so that achieve less
Future Research Perspectives

The five approaches of the structured water content measurement all achieve the objective of the thesis (objective (2)). However, the data analysis of these measurement could be further improved: 1) The Penman’s equation used for calculating the free water evaporation rate was estimated based on a big surface area. Instead, water evaporation on small surface area models (Birdi, Vu and Winter, 1989; Erbil, McHale and Newton, 2002) could be applied to match the real drying test condition (diameter of drying sample=5.4 cm); 2) The determination of the thresholds applied for the five approaches all use the combination the “average” and the “standard deviation”, assuming the data matched normal distribution. Instead, particular distribution of the data should be determined for all approaches, such as Chi-square distribution and multimodal distribution, and the threshold determination for each approach should consider its data distribution characteristics. Therefore, improvement on the data analysis mathematically and statistically should be proposed to improve the objective measurements of the structured water content.

In this study, a negative correlation between the LB-EPS-PS and structured water content was observed. However, the mechanism of how the polysaccharide content influenced the structured water content is still unclear. A possible hypothesis is that some functional groups or special structure in the LB-
EPS-PS might impact the water-holding capacity of the sludge (Reeves et al., 1996). Fluorescent dyes can stain various structures in the EPS according to the target of the dye (Madea and Ishida, 1967; Wood and Fulcher, 1983). For example, Fluorescein Labeled Concanavalin A (Con A) stains the “\(\beta\)-D-PS” structure in the polysaccharide (Lawrence, Neu and Swerhone, 1998). Therefore, qualitative and quantitative staining experiments could be applied in a further study to detect whether particular structures in the polysaccharide cause the influence on the structured water content. A study investigating the LB-EPS-PS composition, structure and its influence on the sludge structured water content should be proposed for this aspect.

This study also indicated that the structured water content in the sludge was positively correlated with its floc size. More detailed research that evaluates whether it is the interstitial space within the flocs or other conditions such as the floc structure or the composition of the microorganisms in the flocs that cause the influence on the structured water content should also be proposed (Basuvaraj, Fein and Liss, 2015).

This study could lead to another project that focus on how LB-EPS-PS and floc size influence the structured water content in the sludge and what sludge characteristics determine the floc size. Therefore, the objectives of the project could be determined as: 1) Investigate which structure of the polysaccharide in the loosely bound extracellular polymeric substances that influences the sludge structured water content; 2) Investigate how sludge floc size influences the sludge structured water content; 3) Study which characteristics of the sludge that
determines the floc size. Staining test using fluorescent dyes could detect various structures in the polysaccharide, so that investigate whether particular structures in the LB-EPS-PS determined the structured water content in the sludge. Biological trinocular microscope measures the floc size of various sludge types, and floc morphology and smaller structure within the flocs such as its interstitial space could be determined using even more precise microscopes. The relationship between the structured water content and these floc characteristics could be studied to study how floc size influences the structured water content. Thereafter, how the floc morphology and size be determined could be investigated. EPS is an important compound of the sludge, and its composition such as protein (PN) content, polysaccharide (PS) content and PN/PS ratio should be analyzed to study its influence on the floc size.
References (Chapter 1, 2 and 5, excluding manuscript references)


Lastella, G., Testa, C., Cornacchia, G., Notornicola, M., Voltasio, F. and Sharma,


