Norwegian University of Life Sciences
Faculty of Chemistry, Biotechnology and Food Science

Philosophiae Doctor (PhD)
Thesis 2019:36

Dewatering of digested biomass

Avvanning av utråtnet biomasse

Oda Kjørlaug Svennevik

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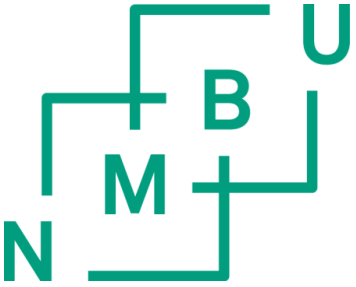
Ås (2019)



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Oda Kjørlaug Svennevik

Ås, 2019

Summary

The world's population is increasing and as cities grow in population and area, efficient and safe handling of sewage is becoming increasingly important. Wastewater treatment generates two products; purified water and sewage sludge. More sustainable options than landfilling of untreated sewage sludge and other organic wastes are desired. Technologies such as the anaerobic digestion (AD) process have been employed to convert a portion of the organic material to renewable energy in the form of biogas. The remaining solid residue after AD (digestate) has been reduced in volume and is more attractive for other disposal options such as land application in the agricultural sector. To minimize the amount of water in the digestate before transportation, solids are separated from the water in a dewatering process. Technologies such as the thermal hydrolysis process (THP) have been developed to increase biogas production and improve dewatering. The amount of water removed from the digestate has a substantial impact on the volume of dewatered cake and thus the transportation costs. It is therefore important to understand the mechanisms underlying the dewatering process. This thesis aimed at improving the understanding of why digestates dewater differently, how this can be predicted and what the effect of the THP is, either in front of AD (Pre-AD THP) or after AD (Post-AD THP). A wide range of digestates from 35 commercial full-scale plants were collected as a part of this study.

This study is based on five research papers, which gave the following main results:

Overall, the AD substrate composition was shown to highly influence the dewatered cake solids from digestates with conventional AD, Pre-AD THP and Post-AD THP. Thermogravimetric analysis (TGA) was used to predict dewatered cake solids by measuring the free water content. Different dewatering devices produced different cake solids despite having similar free water content, however, the impact was minimal compared to the impact of differences in digestate physicochemical parameters. A universal factor describing the water retention capacity of the digestate was identified and termed C/N•ash. This factor was found to correlate linearly with the predicted cake solids by TGA when plants with conventional AD were separated

from Pre-AD THP plants. Moreover, when applied to full-scale data, the C/N•ash was found to correlate linearly with both dewatered cake solids and the polymer dose used in the dewatering process.

Both Pre-AD THP and Post-AD THP were found to improve predicted cake solids of dewatered digestate by increasing the amount of free water. Post-AD THP gave bigger improvement in cake solids than Pre-AD THP. Centrifuging the Post-AD THP digestate at 80 °C increased the dewatered cake solids compared to predictions by TGA at 35 °C.

The substrate composition also influenced the amount of melanoidin-associated compounds in Pre-AD THP digestate quantified as the concentrations of soluble colloidal chemical oxygen demand, color and dissolved organic nitrogen.

Post-AD THP results in a sterilized biosolid fraction where pathogens can grow without microbial competition if recontaminated during storage and handling. This scenario was efficiently mitigated by the addition of a mixed microbial community from compost to the dewatered biosolids. The compost added a robust and diverse microbial community capable of outcompeting the added *Escherichia coli* and suppressing its' growth in Post-AD THP biosolids. The results provide a simple solution for control of pathogen recontamination.

Overall, this study has identified a parameter that can easily be measured in a commercial laboratory providing information on the water holding capacity of digestates. Predictive tools have been developed and the effect of sludge composition on both dewaterability and the concentration of melanoidin-associated compounds have been quantified. A solution to control pathogen growth in Post-AD THP biosolids has been thoroughly verified showing increased robustness of compost-inoculated biosolids.

Sammendrag

Med en økende verdensbefolkning og større byer er det viktig med et effektivt og trygt system for å håndtere avløp og kloakk. Rensing av kloakk gir to produkter; renset vann og slam. Andre alternativer enn å deponere ubehandlet slam og andre organiske avfallsstrømmer er ønskelig og teknologier som anaerob utråtning har blitt brukt til å konvertere deler av det organiske materialet til fornybar energi i form av biogass. Den faste fraksjonen som er igjen etter anaerob utråtning (råtnerest) har da blitt redusert i volum, stabilisert og er et mer attraktivt produkt for annet bruk, som for eksempel gjødsel i landbruket. For å redusere vannmengden i råtneresten før transport blir den faste fraksjonen separert fra vann fraksjonen i avvanningsprosessen. Teknologier som den termisk hydrolyse prosessen (THP) har blitt utviklet for å øke biogass produksjonen og forbedre avvanningen. Mengden av vann som kan bli separert fra råtneresten har stor betydning for volumet av avvannet kake som må transporteres og kostnadene forbundet med dette. Derfor er det viktig å forstå de styrende mekanismene bak denne prosessen. Denne avhandlingen ønsket å forbedre forståelsen av hvorfor råtnerester avvanner ulikt, hvordan dette kan måles og effekten av THP enten før eller etter anaerob utråtning. Et bredt spekter av råtnerester fra 35 kommersielle full-skala anlegg ble samlet inn i løpet av dette prosjektet for å studere forskjeller mellom ulike anlegg.

Denne avhandlingen er basert på fem forskningsartikler som ga følgende resultater:

Substratet som går til utråtning har stor innflytelse på tørrstoff konsentrasjonen av avvannet kake. Dette gjaldt for råtnerester fra vanlig utråtning og råtnerester med THP før eller etter råtnetanken. Termogravimetrisk analyse (TGA) ble brukt til å predikere tørrstoffkonsentrasjonen av avvannet kake ved å måle mengden fritt vann. Ulike avvanningsmaskiner ga ulike tørrstoffkonsentrasjoner i avvannet kake selv med lignende innhold fritt vann i råtneresten, men denne effekten var liten sammenlignet med forskjellene i fysiske og kjemiske parametere. En universell faktor som reflekterte vannbindingsevnen til råtneresten ble funnet og kalt C/N•ash. En lineær korrelasjon ble funnet mellom denne faktoren og predikert tørrstoffkonsentrasjon av avvannet kake når konvensjonelle utråtningsprosesser ble separert fra de med THP før råtnetanken. Det ble

også funnet lineære sammenhenger mellom C/N•ash og avvannet kake og polymerforbruk fra full-skala avvanningsprosesser med THP før råtnetanken.

THP både før og etter råtnetanken økte predikert tørrstoffkonsentrasjon i avvannet kake ved å øke andelen fritt vann i råtneresten. THP etter råtnetanken ga den største forbedringen i avvannet kake sammenlignet med THP før råtnetanken. Direkte avvanning etter THP av utråtnet slam viste at tørrstoffkonsentrasjonen av avvannet kake kunne ytterligere forbedres ved å sentrifugere på 80 °C sammenlignet med TGA prediksjoner ved 35 °C.

Substratsammensetningen påvirket konsentrasjoner av Maillard produkter målt som løst kolloidalt kjemisk oksygenforbruk, farge og løst organisk nitrogen i råtnerester med THP før råtnetanken.

Inokulering av sterilisert råtnerest med aktiv mikroflora fra kompost i to dager eliminerte vekst av *Escherichia coli* (*E. coli*) etter rekontaminering. Dette kan være en effektiv strategi for å beskytte mot rekontaminering.

Oppsummert har denne studien kommet frem til en parameter som kan måles i kommersielle laboratorier og brukes til å beskrive vannbindingsevnen til ulike råtnerester. Modeller for å predikere tørrstoff av avvannet kake har blitt utviklet og substratblandingens innvirkning på avvanning og produkter fra Maillard reaksjonen har blitt studert. En løsning for å eliminere vekst av *E. coli* i steril råtnerest har blitt verifisert og viste økt motstandsdyktighet mot rekontaminering ved inokulering med kompost.

List of papers

Paper I

Svensson, K., Kjørlaug, O., Higgins, M. J., Linjordet, R., & Horn, S. J. (2018). Post-anaerobic digestion thermal hydrolysis of sewage sludge and food waste: Effect on methane yields, dewaterability and solids reduction. Water Research, 132, 158-166.

Paper II

Oda K. Svennevik, Odd Egil Solheim, Greeley Beck, Geir H. Sørland, Kjell R. Jonassena, Ester Rus, Bjørge Westereng, Svein J. Horn, Matthew J. Higgins, Pål J. Nilsen (2019). Post anaerobic digestion thermal hydrolysis increases the concentration of dry solids in dewatered cake

Manuscript

Paper III

Oda K. Svennevik, Greeley Beck, Ester Rus, Bjørge Westereng, Matthew Higgins, Odd Egil Solheim, Pål J. Nilsen, Svein J. Horn (2019). CNAsh - a novel parameter predicting cake solids of dewatered digestates.

Water Research, revised manuscript

Paper IV

Oda K. Svennevik, Pål J. Nilsen, Odd Egil Solheim, Bjørge Westereng, Svein J. Horn (2019). Quantification of soluble melanoidin-associated compounds in commercial thermal hydrolysis digestates.

Manuscript

Paper V

Oda K. Svennevik, Kjell R. Jonassen, Kine Svensson, Live H. Hagen, Bjørge Westereng, Pål Nilsen, Svein Horn, Lars Bakken (2019). Pathogen growth in sterile digestates can be eliminated by inoculation with a complex microbial community.

Manuscript

Abbreviations

AD	Anaerobic digestion
AOB	Ammonium oxidizing bacteria
AS	Activated Sludge
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
C/N	Carbon to nitrogen ratio
CST	Capillary suction time
CSTR	Continuous stirred-tank reactors
ddPCR	digital droplet polymerase chain reaction
DON	Dissolved organic nitrogen
E. coli	*Escherichia coli*
EPA	Environmental protection agency
EPS	Extracellular polymeric substances
F/M	Food to Microorganism
LFNMR	Low field nuclear magnetic resonance
MAD	Mesophilic anaerobic digestion
M/D	Monovalent to divalent
MBR	Membrane biofilm reactor
MBBR	Moving bed biofilm reactor
MPN	Most probable number
NOB	Nitrite oxidizing bacteria
PAO	Phosphate accumulating organisms
PCA	Principal component analysis
Pre-AD THP	Thermal hydrolysis before AD
Post-AD THP/PAD-THP	Thermal hydrolysis after AD
PS	Primary Sludge
SRF	Specific resistance to filtration

SRT	Sludge retention time
SSFW	Source separated food waste
SSM	Safe sludge matrix
TAD	Thermophilic anaerobic digestion
TCB	Thermotolerant coliform bacteria
TGA	Thermogravimetric analysis
THP	Thermal hydrolysis process
UV	Ultraviolet absorbance
VAR	Vector attraction reduction
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

1 Introduction

1.1 Wastewater treatment – purification of sewage

1.1.1 Wastewater treatment

Modern wastewater treatment as we know it today has been evolving since the 18th century, accelerated by events such as "The Great Stink" of London in 1858 as reviewed by Neal (2006). Sewage and industrial waste were directly discharged into the river Thames polluting the river as well as the river banks. Because of these unsanitary conditions, diseases spread quickly and the construction of pipelines and pumping stations began to transfer the sewage away from the city. Following the pipelines, dedicated sewage treatment systems were designed and were at the time considered the most significant improvement for human health in the last 100 years (Jenkins and Wanner, 2014).

Wastewater treatment plants (WWTPs) today are designed to receive and treat sewage to reduce health risks and pollution associated with this waste-stream. However, as cities are growing with increasing population density, stricter legislations have been enforced to protect the water recipients downstream of the WWTPs. Nutrients such as nitrogen, phosphorus and organic material can cause eutrophication, leading to excessive algae production and oxygen depletion in receiving water bodies (Metcalf and Eddy, 2014). Wastewater treatment reduces the amount of pathogens, and the concentrations of nutrients and toxic compounds that may negatively affect water ecosystems. Lately, increased attention has been given to the limited reserves of some nutrients, such as phosphorus (Cordell and Neset, 2014). This has given the WWTPs an opportunity to move from disease and pollution control to becoming an important part of the circular economy as nutrients can be recovered from the wastewater. In some areas of the world shortage in portable water is an increasing concern and the WWTPs can also serve as water reclamation plants, recycling the water instead of discharging it to rivers and oceans (Tram Vo et al., 2014).

The wastewater entering treatment plants is usually a mix of domestic wastewater, industrial wastewater, infiltration from pipe leakage and stormwater from heavy rainfall or snowmelt (Metcalf and Eddy, 2014). The main goal of a WWTP is to meet

regulatory discharge requirements set by local authorities and the process configuration of a WWTP will vary depending on these requirements. However, a combination of physical, mechanical, chemical and biological treatment is often used as illustrated in Figure 1.

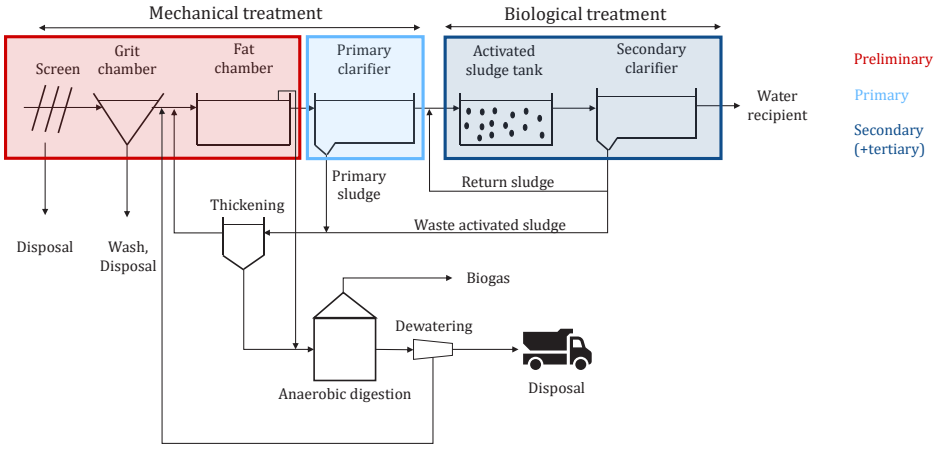

Figure 1: Typical configuration of a WWTP divided into mechanical and biological treatment. Preliminary treatment with screening, grit and fat removal is followed by primary sedimentation, activated sludge treatment and secondary clarification before the water is discharged. The sludge fractions produced are treated by anaerobic digestion before being dewatered and disposed. Adapted from (Metcalf and Eddy, 2014).

A preliminary treatment is typically the first step in a WWTP and includes the mechanical and/or physical removal of debris, grit and fat to reduce maintenance cost and potential operational problems downstream (Metcalf and Eddy, 2014).

Primary treatment removes suspended solids and organic material either by settling, chemically assisted settling or filtration (Metcalf and Eddy, 2014). The remaining supernatant then enters the biological treatment process while the settled solids, often termed primary sludge, goes to sludge treatment.

The configuration of the biological treatment depends on discharge limits set by local regulations. The main objective of the biological treatment is to remove nitrogen, phosphorus and organic material. The most common biological treatment process in the world is the Activated Sludge (AS) process which is based on aerobic suspended growth of bacteria to purify the wastewater (Barnard, 1975, Jenkins and Wanner, 2014). The AS process has been used since the early 1900s originally for the removal of organic

material by aeration of the activated sludge (Jenkins and Wanner, 2014). In the AS process, heterotrophic bacteria oxidize organic material under aerobic conditions, lowering the concentration of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) in the wastewater producing new microbial biomass and CO_2 (Metcalf and Eddy, 2014). Later, the AS process was optimized to also biologically remove nitrogen and phosphorus (Barnard, 1975). Separate chambers were suggested to manipulate the process parameters such as aeration for the removal of nitrogen and phosphate in addition to COD.

Nitrogen removal is usually divided into several process steps, performed by different bacteria in separate chambers in the AS process. Firstly, Nitrification is performed in an aerobic two-step process where ammonium oxidizing bacteria (AOB) such as *Nitrosomonas* convert ammonium to nitrite, followed by conversion of nitrite to nitrate by nitrite oxidizing bacteria (NOB) such as *Nitrospira* (Jenkins and Wanner, 2014). Secondly, the wastewater enters the anoxic Denitrification process where nitrate is converted to N_2 by oxidizing organic material. Denitrification is performed by a large diversity of heterotrophic bacteria (Jenkins and Wanner, 2014).

Phosphate can be chemically precipitated from the wastewater as crystals such as struvite or removed biologically (Mehta et al., 2015). Enriching the activated sludge with phosphate accumulating organisms (PAOs) such as *Accumulibacter* is achieved by alternating between aerobic and anaerobic conditions (Nielsen et al., 2012). The PAOs can accumulate a high fraction of phosphate compared to non-PAOs, thus effectively removing the phosphate from solution (Nielsen et al., 2012). This is commonly called “luxury uptake” (Barber, 2014) and the phosphate is removed from the wastewater treatment line through removal of the microbial biomass.

Other configurations for the purification of wastewater have also been developed such as the moving bed biofilm reactor (MBBR) and membrane biofilm reactor (MBR) (Ivanovic and Leiknes, 2012).

1.1.2 Sludge production

Primary sedimentation or filtration will generate primary sludge (PS) that behaves like a colloidal suspension where particles are linked by van der Waals forces (Markis et al., 2014). PS is readily biodegradable in anaerobic digestion (AD) and dewaters well (Kopp and Dichtl, 2001a, Barber, 2014)

In the AS process, new biomass is constantly generated, and a fraction is continuously wasted to control the "Food to Microorganism" (F/M) ratio. The F/M ratio is defined as the total applied substrate rate (g BOD/d) divided by total microbial biomass (g volatile suspended solids (VSS)) with typical values of 0.10-0.5 g BOD/g VSS•d for activated sludge systems (Metcalf and Eddy, 2014). To ensure a stable F/M ratio, accumulated activated sludge must be continuously removed from the system. The wasted microbial biomass is commonly called waste activated sludge (WAS). Variations in plant operation and microbial growth rates due to temperature, or weather conditions such as snow melting and rainfall, will affect the F/M ratio of the biological treatment and the sludge retention time (SRT) (Metcalf and Eddy, 2014). The amount of WAS produced and its' SRT will therefore depend on seasonal weather conditions. The SRT of WAS has also been linked to dewaterability, with longer SRTs having a negative impact on the dewatering process (Barber, 2014). WAS, which may contain up to 80 % extracellular polymeric substances (EPS), has strong water holding capacities (Neyens et al., 2004) and has been described as a viscous gel-like material linked by hydrogen bonds and electrostatic forces (Markis et al., 2014). WAS dewaters poorly compared to PS and has consequently been the focus of several dewatering studies (Kopp and Dichtl, 2001a, Barber, 2014, Christensen et al., 2015). WAS is usually blended with PS prior to further treatment.

Tertiary treatment of the wastewater after biological treatment is practiced at some plants. This process chemically precipitates the remaining phosphate or organic matter, mainly serving as a final polishing step before the water is discharged to the recipient (Metcalf and Eddy, 2014). The sludge produced is commonly blended with WAS before further processing.

1.2 Anaerobic digestion – biological treatment of solid waste

Increased attention to global warming which is associated with increased greenhouse gas emissions due to fossil fuel consumption has accelerated research and implementation of renewable energy production. To reduce these emissions the EU implemented in 2015 the Circular Economy Action Plan (European Commission, 2015) where reduction of landfill waste and an increase of waste re-use and recycling have been identified as important parameters. Biological (anaerobic digestion, esterification, fermentation and electro fuel cells) and physicochemical (pyrolysis, gasification and incineration) methods are currently practised to convert waste to energy (Bhatia et al., 2018). Energy recovery from sewage sludge has traditionally been achieved by anaerobic digestion (AD) (Mills et al., 2014), and the most common feedstocks are currently manure, sewage sludge, municipal solids waste and food waste (Achinas et al., 2017). The feedstock is typically anaerobically digested at mesophilic or thermophilic conditions by a microbial community for 15-20 days (Metcalf and Eddy, 2014).

Digester microbiology

Both bacteria and archaea contribute to the AD process and are responsible for the four main steps of anaerobic digestion: disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 2). The hydrolysis is commonly much faster than the disintegration, making the disintegration the rate limited step of AD (Batstone et al., 2002a). Pre-treatments such as the thermal hydrolysis process performs the disintegration step prior to AD, thus improving the kinetics of the AD process (Perez-Elvira et al., 2010).

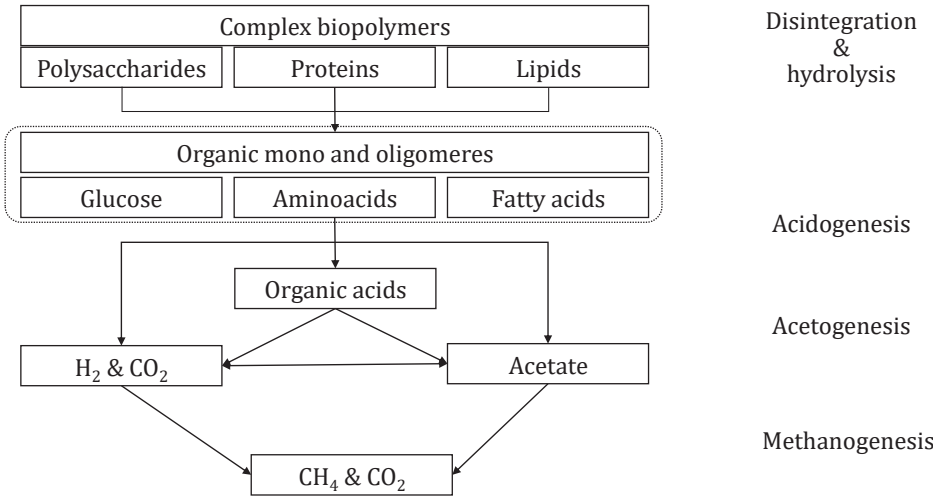

Figure 2: Microbial process of anaerobic digestion divided into disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis. Adopted from Batstone et al. (2002b).

A large and diverse group of bacteria is responsible for hydrolysis and fermentation. They have been identified as facultative and obligate anaerobic bacteria including organisms such as *Clostridium* ssp., *Peptococcus anaerobus*, *Bifidobacterium* spp., *Desulphovibrio* spp., *Corynebacterium* spp., *Lactobacillus*, *Actinomyces, Staphylococcus* and *Escherichia coli* (Metcalf and Eddy, 2014).

The final step of methanogenesis is performed by strict obligate anaerobic archaea such as *Methanobacteriales, Methanococcales, Methanomicrobiales,* and *Methanopyrales* (Metcalf and Eddy, 2014). Hydrogenotrophic methanogens oxidize hydrogen and use CO_2 as a carbon source to produce methane:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \text{Equation 1}$$

Aceticlastic methanogens in the order *Methanosarcinales* such as *Methanosarcina* and *Methanosaeta* are able to cleave acetate to form methane (Metcalf and Eddy, 2014):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Equation 2}$$

A stable AD process will depend on a balance between the different microbial processes to prevent accumulation of intermediates, which may cause inhibition and even process failure.

Different operational strategies such as mesophilic AD (MAD) or thermophilic AD (TAD) with or without pre-treatment can lead to differences in the microbial community and pathways for methanogenesis (Kirkegaard et al., 2017, Chen et al., 2018). Thermophilic and THP digestion have shown to favour the combination of acetoclastic and hydrogenotrophic methanogen, while conventional mesophilic digestion is dominated by acetoclastic methanogens (Kirkegaard et al., 2017, Chen et al., 2018)

Co-digestion

Co-digestion of organic waste is becoming increasingly popular (Khalid et al., 2011, Braguglia et al., 2018) due to several reasons. The European Commission action plan on circular economy aims to reduce waste generation and increase recycling of materials and resources, and food waste is defined as one of the targets (European Commission, 2015). AD is already implemented in many WWTP and pre-treatment technology is available and can easily be implemented to sanitize and increase the capacity of existing plants. Several studies have indicated beneficial effects such as balancing of nutrients and increased load of biodegradable matter leading to increased biogas yield (Khalid et al., 2011). Addition of food waste has also been reported to improve dewaterability of the digestate (Higgins et al., 2017a).

Biogas utilization

The degradation of organic matter in anaerobic digesters results in a biogas rich in methane. This biogas is commonly used in combined heat and power systems (CHP) (Mills et al., 2014) producing heat and electricity that can be used within the plant. The biogas can also be cleaned and upgraded to bio-methane that can be injected to natural gas grids or used as vehicle fuel (Mills et al., 2014).

The solid residue remaining after AD (digestate) is typically dewatered, separating solids from liquid to reduce transportation costs and ease the handling of the digestate for its final end-use.

1.3 Separation of solids from liquid: Dewatering

The process of separation of solids from liquid is called dewatering. The process produces a cake often termed biosolids and a dewatering liquor commonly returned to the inlet of the WWTP. Although not important for the main purpose of WWTPs, purification of wastewater, the operational cost of dewatering and cake disposal can amount to 30-50 % of a WWTPs' annual budget (Mikkelsen and Keiding, 2002). Consequently, the optimization of this process is crucial for the plants' economy. The dewatered solids are usually transported from the WWTPs by trucks to its end-use. The dewatering efficiency will thus impact the quantity of solids and hence the number of trucks needed for transport. This will influence the carbon foot-print of the plant as well as the number of heavy transport vehicles passing through nearby neighbourhoods. Current research on the dewatering process can be divided into the following main topics discussed in the next sections:

- Physicochemical properties of the sludge
- Effects of pre- or post-treatments
- Chemical conditioning and types of dewatering devices

1.3.1 Physicochemical properties of the sludge

Activated sludge floc composition

Sludge flocs have been defined as fractal-like structures kept together by electrostatic and hydrophobic forces in addition to physical entanglement (Christensen et al., 2015). Organic debris, inorganic particles, microorganisms and extracellular polymeric substances (EPS) (Figure 3) make up most of the sludge flocs (Nielsen et al., 2012).

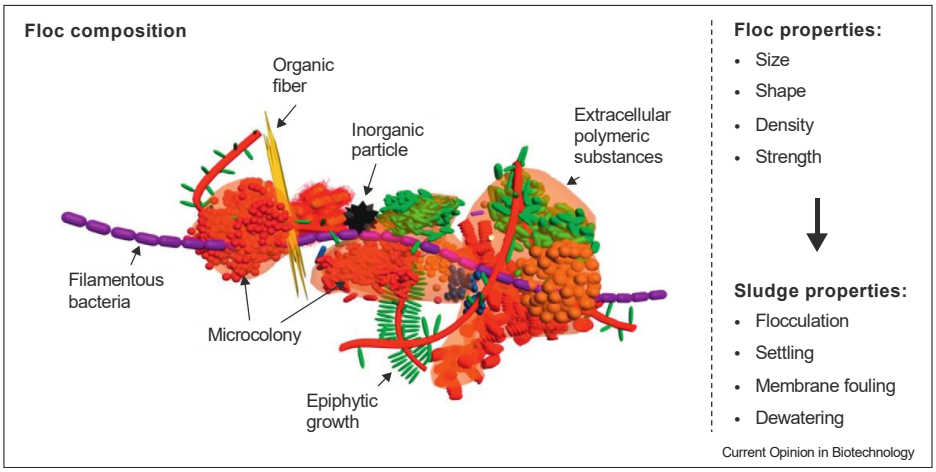

Figure 3: Floc composition, properties and resulting sludge properties in activated sludge affecting dewatering (Nielsen et al., 2012).

The physicochemical properties of activated sludge flocs reflect both wastewater composition and process configuration (Christensen et al., 2015). The ratio of monovalent to divalent (M/D) ions has been shown to affect floc composition, settling and dewatering properties of activated sludge (Higgins and Novak, 1997a, Higgins and Novak, 1997b). High M/D ratio had a negative effect on dewatering due to the decreased binding between biopolymers and the floc, and an optimum M/D ratio of 1 has been suggested (Higgins and Novak, 1997a). Finally, both M/D ratio and calcium to magnesium ratio have been found to be important when evaluating the cation balance (Higgins and Novak, 1997a). In addition, the presence of divalent cations under acidic conditions has been shown to improve the biosorption of humic and fulvic acids to activated sludge, thus improving the removal of these compounds from the wastewater (Esparza-Soto and Westerhoff, 2003).

Several aspects of the floc structure of biological sludge have been studied in relation to dewatering. However, EPS is probably the most studied floc component in dewatering research (Liu and Fang, 2003).

Extracellular polymeric substances

The important role of EPS, which may constitute up to 80 % of the activated sludge biomass (Neyens et al., 2004), is due to its strong water binding capacity (Christensen et al., 2015). Microorganisms are embedded in the gel-like and highly hydrated EPS

biofilm matrix (Neyens et al., 2004), which is responsible for the structural and functional integrity of the flocs.

Varying amounts of EPS is generated due to fluctuations in wastewater composition, environmental stress forming a protective layer around the bacteria (Shi et al., 2017, Ye et al., 2011). The most important factors have been summarized by Shi et al. (2017) and are shown in Figure 4. In addition, the choice of biological wastewater treatment configuration such as fixed-film, activated sludge or MBR will also affect the EPS production (Shi et al., 2017).

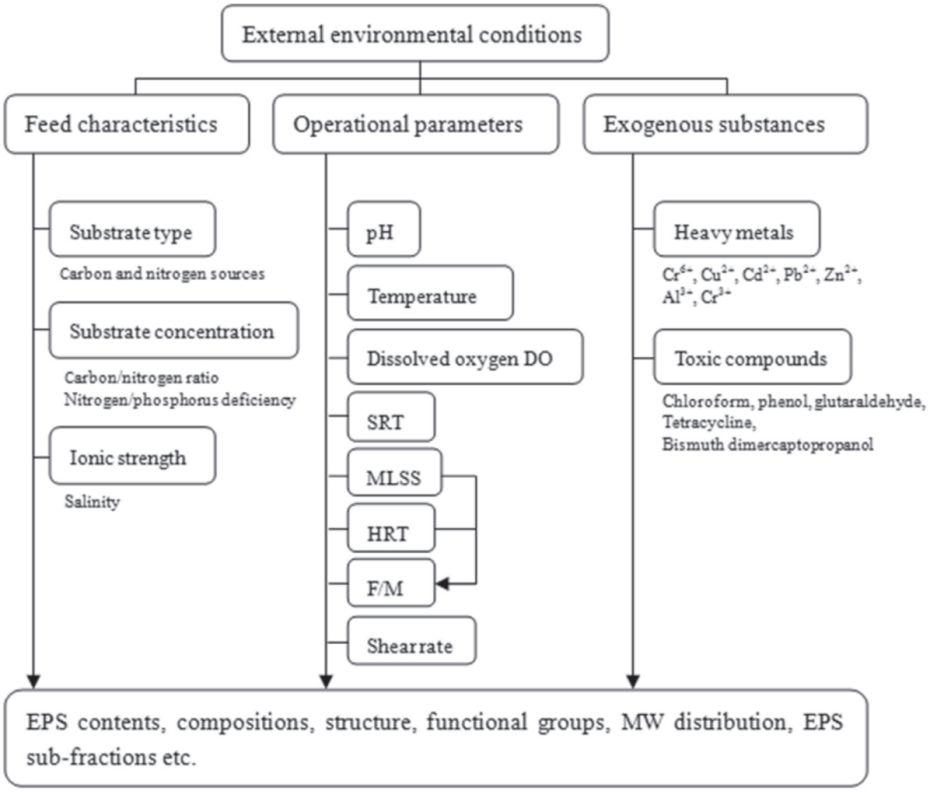

Figure 4: Schematic presentation of important factors that influence extracellular polymeric substances (EPS) characteristics in activated sludge during wastewater treatment (Shi et al., 2017).

Comparing literature regarding EPS can be challenging due to the lack of standardized methods (Christensen et al., 2015). However, many authors classify EPS into tightly bound, loosely bound and suspended EPS. Substitute factors such as organic matter

content has also been suggested to relate EPS to dewatering (Skinner et al., 2015). Soluble EPS has also been related to sludge rheology (Hong et al., 2018).

Sludge rheology

Several factors influencing sludge rheology have been summarized by Hong et al. (2018), including type of sludge, total solids concentration, temperature, pH, dose of polymer and chemical composition, especially concentration of biopolymers and organics. The rheological behaviour of primary sludge has not been thoroughly studied, and the main body of literature on sludge rheology is focusing on biological sludge (WAS) (Hong et al., 2018). However, primary sludge has lower viscosity at similar total solids concentration compared to WAS (Hong et al., 2018). Thus, the ratio of primary and biological sludge (PS/WAS) is an important process parameter for both flow hydrodynamic, digestion and dewatering (Hong et al., 2018). Researchers also have found a correlation between EPS content and viscosity (Hong et al., 2018), and moisture distribution and viscosity (Dai et al., 2014). In addition, sludge temperature has also been shown to be a key parameter, reducing viscosity with increasing temperature (Klinksieg et al., 2007).

The amount of EPS and rheological properties seem to be related to the water holding capacities of the sludge flocs and hence its' dewaterability.

Moisture distribution

The physical confinement of water in sludge has been studied for decades, and several classifications and definitions have been applied (Kopp and Dichtl, 2001b, Vaxelaire and Cezac, 2004, Vesilind, 1994). In this study, the following definitions were used (Figure 5):

- Free water: water not bound to particles
- Interstitial water: water bound by capillary forces
- Surface (vicinal) water: water bound by adsorption and adhesion forces
- Bound (intercellular) water: water inside microbial cells and in hydrate minerals (water of hydration).

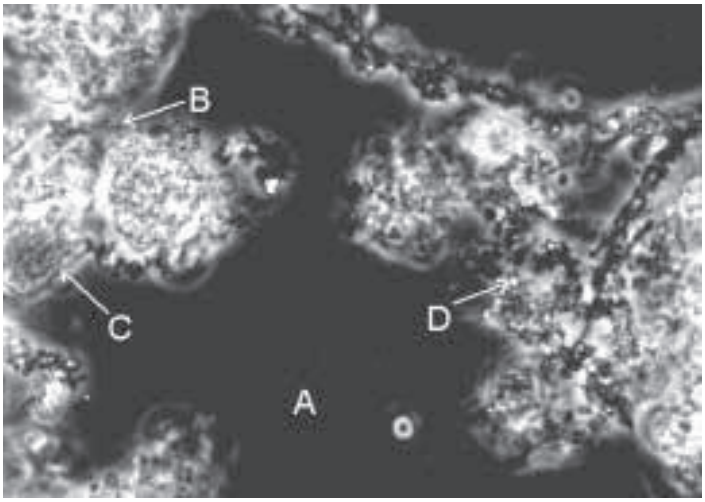

Figure 5: Moisture distribution in a sewage sludge floc (Kopp and Dichtl, 2001b)

Several methods to determine the moisture distribution exist and have been summarized by Vaxelaire and Cezac (2004). The moisture distribution in relation to dewatering has been studied in the laboratory using techniques such as the capillary suction time (CST) test, specific resistance to filtration (SRF) or the sludge volume index (SVI) (Vaxelaire and Cezac, 2004). However, only a few studies have related their findings to full-scale dewatering. Kopp and Dichtl (2001b) on the contrary did relate their drying test (thermogravimetric analysis (TGA)) results to full-scale performance. Their results showed a linear relationship between the water fraction they termed free water and the fraction removed by full-scale centrifuge dewatering (Kopp and Dichtl, 2001b).

To improve sludge dewatering the structural integrity of the sludge needs to be changed (Neyens and Baeyens, 2003). Heat pre-treatment has been shown to reduce the water retention capacity of EPS (Neyens et al., 2004). Thus, the effect of pre- or post-treatments could be linked to changes in the moisture distribution.

1.3.2 Pre- or post-treatment

The anaerobic digestion process has been employed as a mean of stabilizing sludge and producing energy since the early 1900s (Cameron et al., 1900). Several technologies have been used to improve the AD process, ensuring pathogen destruction, better process performance and more efficient dewatering. Different types of pre- or post-treatments may disintegrate the floc structure and improve dewaterability, and are summarized by Neyens and Baeyens (2003) as:

- Heat treatment in the range 40 – 180 °C
- Chemical treatment using ozone, acids or alkali
- Mechanical disintegration
- Freezing and thawing
- Biological hydrolysis with or without enzyme addition

Thermal hydrolysis (steam explosion) has been applied world-wide to improve the AD processes since the first full-scale installation in 1995 at Hamar, Norway (Ødeby et al., 1996). Hydrothermal treatment, typically carried out at 165 °C for 30 minutes, has been shown to give several benefits including increased substrate organic loading rates to the digester and improved dewatering of the digestate (Barber, 2016). Today, THP is used as a pre-treatment of substrates going to anaerobic digestion (Pre-AD THP, Figure 6). Pre-AD THP sterilize the feedstock and reduces the viscosity allowing a more concentrated feed to the AD process (Barber, 2016). Moreover, THP disintegrates and hydrolyses the sludge, and since disintegration is the rate limiting step in AD (section 1.2), this allows for the retention time in digesters receiving THP treated sludge to be lower compared to conventional plants, still achieving the same stabilization. Thus, the throughput of the existing AD plant can be increased or the number of digesters needed when building new AD plants can be reduced. This again can save valuable space at WWTPs located in densely populated areas.

There is a general agreement in literature that Pre-AD THP improves dewaterability of digestates, but the mechanisms are not well understood. However, dewatering efficiency seems to depend on both sludge characteristics and dewatering device (Barber, 2016). Neyens et al. (2004) suggested that THP improves dewaterability in two steps: Firstly, by reducing the water retention properties of EPS and secondly by reducing the amount of fine flocs by promoting flocculation. Reduced viscosity due to

increased THP treatment temperature has also been linked to improved dewatering (Higgins et al., 2017b).

Recently a new plant configuration has been explored to optimize energy efficiency and the dewatering process: the so-called Post-AD THP (Figure 6). In Post-AD THP the THP unit is placed after the AD process as a dewatering aid. Dewatered digestate is treated in the THP and then directly, without cooling, flocculated and dewatered in a centrifuge (Kjorlaug, 2015, Kolovos et al., 2016). Full-scale tests have shown promising results in terms of dewatering, with a wet mass reduction of more than 60 % compared to conventional AD dewatering (Kolovos et al., 2016). Another factor making Post-AD THP different from Pre-AD THP is the recirculation of final dewatering liquor back to the digester instead of the WWTP (Figure 6). THP solubilize organic material (Suarez-Iglesias et al., 2017) and the Post-AD THP liquor is returned to the digester to improve biogas production. The dewatering liquor from dewatering before the THP unit is thus the only water outlet after digestion, being returned to the WWTP (Figure 6).

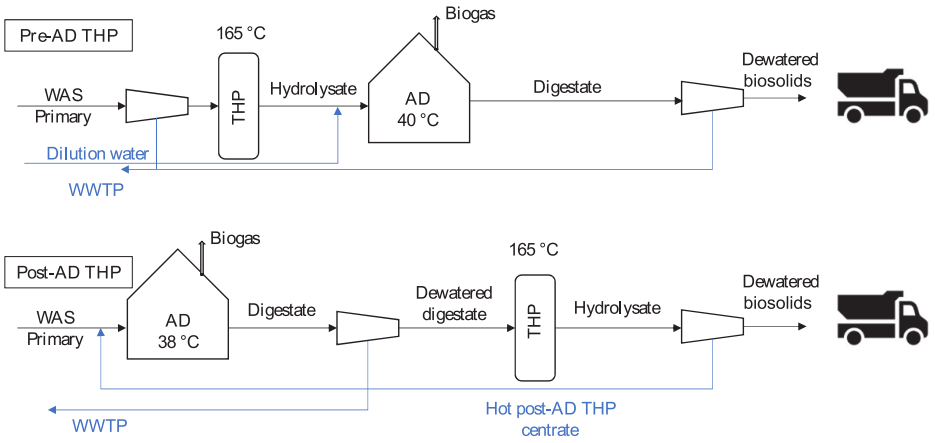

Figure 6: Process drawing of the Pre-AD THP and Post-AD THP configuration in a wastewater treatment plant with anaerobic digestion

1.3.3 Chemical conditioning and dewatering device

Chemical conditioning

Originally, lime was commonly used in dewatering of sludge. However, in modern sludge dewatering organic polymers usually made from polyacrylamide (condition agents) and metal salts are typically added to the digestate prior to the dewatering device to induce particle aggregation and improve the water release rate and quality of the dewatering liquor (Novak, 2006). The building blocks of organic polymers are individual monomer units linked together in linear, branched or structured configurations (Metcalf and Eddy, 2014). The charge and molecular weight of the polymers are determined by functional groups attached to the polymer chains (Metcalf and Eddy, 2014). A wide range of polymers exists, and tests are commonly applied on-site to choose the best agent.

Cationic polymers are the most commonly used polymers in digestate dewatering due to the negative charge of sludge particles (Novak, 2006). The polymer dose required for efficient separation of solids from liquid depends on sludge type, mixing, and dewatering device (Novak, 2006, Metcalf and Eddy, 2014). Linear relationships have been found between the concentration of soluble protein and polysaccharides and required polymer dose for optimal dewatering (To et al., 2018). Dual conditioning with inorganic metal salts was found to reduce the needed dose of organic polymer for digestates containing high levels of proteins, polysaccharides and COD (Murthy et al., 2000). Another factor that can reduce polymer dose, is the choice of dewatering device since centrifuges have been shown to typically require more polymer than belt presses (Novak, 2006).

Dewatering device

Several different dewatering devices can be used including centrifuges, belt presses, rotary presses, screw presses and recessed-plate filter press (Metcalf and Eddy, 2014). The most commonly used dewatering devices are centrifuges and belt presses. Although requiring higher polymer doses, centrifuges typically produce higher cake solids than belt presses (Novak, 2006). Recently hydraulic filter presses have been introduced in sludge dewatering showing promising results in terms of the dry solids of the dewatered

biosolids (cake solids) compared to centrifuges and belt presses (Thunberg, 2010). The choice of dewatering device will depend on several factors such as sludge type, desired cake solids and liquor quality, in addition to process configurations (Metcalf and Eddy, 2014).

1.3.4 Summarizing remarks

Most of the literature on dewatering has focused on biological sludge as it is the most difficult sludge type to both digest and dewater. However, digestates will typically be based on a combination of PS and WAS and thus embody the physicochemical parameters of both, which has been shown to be fundamentally different (Barber, 2014). Pre-AD THP has been found to change the floc structure, degrade the water holding capacities of EPS and reduce viscosity, all factors which are linked to the moisture distribution. However, little literature is available on the effect of THP on the moisture distribution, and in particular the free water content, which could link these observations to full-scale dewatering expectations.

Irrespectively of conventional AD or the Pre-AD THP configuration, flock structure, EPS, viscosity and the free water content seem to be linked to the AD substrate blend, more specifically to the ratio of PS, WAS and other co-substrates. However, measuring these ratios and estimating the subsequent effect after digestion can be challenging due several aspects. This could be due to inadequate mixing in the digester and the often-limited accuracy of combining manual sampling and flow meters. To date no general parameter exists that sufficiently fingerprints digestates linking it to dewatering performance. Such a parameter could help linking fundamental research and experience from full-scale operations before conducting detailed trials.

The few studies that compare their results to full-scale performance typically use data from one or a few plants which limit the universality of the observations. Therefore, this thesis had an emphasis on using a large range of digestates to investigate the variations and trends seen between different plants and substrate blends.

Regardless of the configuration, the sludge treatment will create three main products: biogas, dewatered cake and liquor. The main body of sludge digestion research focus on the generation of biogas. However, the choices among technologies and process configurations will also affect the quality and amount of dewatered cake and liquor which can be equally or more important in an economical perspective.

1.4 Dewatering liquor

The physicochemical properties of the dewatering liquor can be related to several aspects of the sludge treatment such as the substrate blend, the digestion process, application of pre-or post-treatment and the choice of chemical conditioning agent and dewatering device. The dewatering liquor is usually sent back to the wastewater treatment process (Oleszkiewicz and Barnard, 2006). The sludge dewatering liquor is often more concentrated than the plant influent, which could make it more cost efficient to treat the liquor separately in a dedicated treatment process (Oleszkiewicz and Barnard, 2006).

Application of Pre-AD THP will increase the soluble organic material in the digester feed and more proteins are converted to ammonium (Barber, 2016, Suarez-Iglesias et al., 2017), both of which will increase the soluble nutrient concentration in the dewatering liquor. In addition, the organic loading rate is typically increased leading to more concentrated system in terms of solids and nutrients in the digester (Barber, 2016). Consequently, the nutrient concentration in the dewatering liquor will be higher in THP plants than in conventional AD plants.

Due to the high temperature and treatment time in thermal hydrolysis, production of soluble refractory compounds has been reported (Dwyer et al., 2008b, Bougrier et al., 2007, Barber, 2017). It is hypothesized to be by-products from Maillard reactions, where carbohydrates and amino acids polymerize and form colloidal compounds called melanoidins (Dwyer et al., 2008b). Melanoidins give the dewatering liquor a deep brown color and decrease the ultraviolet transmission (UVT), causing potential impacts on downstream processes such as UV disinfection (Dwyer et al., 2008b). Using molecular weight fractionation Dwyer et al. (2008b) analyzed the soluble fraction after Pre-AD THP treatment. The largest fraction of melanoidins was found in the > 10kDa range. This fraction had the highest concentration of color, dissolved organic carbon and UV absorbance.

The digestion process will reduce the concentration of dissolved organic nitrogen (DON) (Higgins et al., 2017b). In addition, the dewatering process has been shown to reduce DON due to polymer addition which sequester some of it in the cake (Higgins et al.,

2017b) (Figure 7A). In addition, UV absorbing compounds can also be significantly reduced due to polymer conditioning and dewatering (Higgins et al., 2017b) (Figure 7B). Melanoidin associated compounds can be reduced by advanced oxidation processes (Dwyer et al., 2008a) or by coagulation with inorganic coagulants (Dwyer et al., 2009).

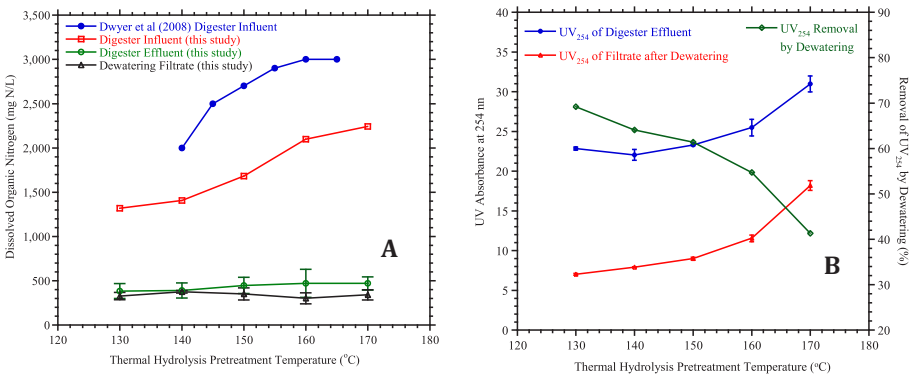

Figure 7: Effect of Thermal Hydrolysis Pretreatment temperature and dewatering on DON (Figure 7A) and UV absorbance (Figure 7B). (Higgins et al., 2017b)

Increased concentration of soluble recalcitrant organics in the dewatering liquor can increase the concentration of these compounds in the WWTP effluent. In areas with strict effluent discharge limits, this can be a disadvantage of installing THP. Combining published research (Dwyer et al., 2008b) and full-scale reports (Barber, personal communication) there are indications that the digestion substrate blend also can have an impact on the formation of melanoidin-associated DON encouraging further investigation into this topic by comparing several digestates.

In addition to the quality of the dewatering liquor, the quality of the dewatered biosolids is important. The level of pathogen destruction and cake dryness will largely impact the disposal options available and the cost of this disposal.

1.5 Dewatered biosolids

After dewatering, several options for biosolids cake handling are available including agricultural applications, landfill, incineration, pyrolysis and gasification (Mills et al., 2014). The choice of disposal option will vary depending on local legal regulations and geographical location. Regardless of disposal option, transportation by trucks, storage and human exposure are inevitable. Hence, the cake dryness and hygienic quality are important.

Thermotolerant coliform bacteria (TCB) and *Escherichia coli* are two common indicators used to measure the extent of fecal contamination in sewage sludge (Paruch and Mæhlum, 2012). TCB is a general indicator group, embodying fecal pathogens from humans, other mammals and birds in addition to non-fecal bacteria naturally found in the environment (Paruch and Mæhlum, 2012). *E. coli* is a more accurate indicator since it is found exclusively, and in large numbers, in feces from humans and warm blooded animals and is not found in the environment (Paruch and Mæhlum, 2012).

Growth of pathogenic bacteria in the dewatered biosolids can be due to two main mechanisms: regrowth/reactivation or recontamination. Regrowth/reactivation implies that the sludge treatment process did not sufficiently destroy pathogens. Recontamination implies the biosolids have been exposed to an external contamination either in the sludge treatment line or during storage.

To ensure safe handling, regulations such as the U.S. EPA 40 CFR Part 503 (Iranpour et al., 2004) and the U.K. Safe Sludge Matrix (SSM) (Gale, 2005) have been enforced. Enhanced treatment such as the THP will ensure destruction of pathogens and meet the highest standards of the SSM and Class A in U.S. EPA 40 CFR Part 503 requiring low levels of pathogens. However, EPA has also included extra requirements for so-called Vector Attraction Reduction (VAR). While the time and temperature requirements will reduce the chance for regrowth of pathogens, the VAR regulations are added to prevent extensive growth of pathogens due to recontamination. Recontamination with subsequent growth of pathogens was observed in post-AD pasteurized sludge in the 1980s (Ward et al., 1999), leading to the implementation of regulations similar to VAR. The biosolids are commonly stored for a period, especially if they are applied in

agriculture, and recontamination followed by rapid pathogen growth can present a risk during storage and handling. There are several options to comply with VAR including drying, 38 % volatile solids reduction by anaerobic or aerobic digestion (Iranpour et al., 2004). To achieve Class A requirements enhanced treatment such as THP and thermophilic digestion must be applied before AD.

Over the last couple of decades observations of sudden increase in TCB and *E.* coli after centrifuge dewatering have been reported, although low levels complying with regulations were measured before centrifugation (Cheung et al., 2003, Higgins et al., 2007). By combining results from classical enumeration methods and qPCR of *E. coli* Higgins et al. (2007) found that during digestion *E. coli* enters a viable, but non-culturable state. Consequently, they are not detected by classic enumeration methods and this can lead to underestimation of fecal pollution. However, after centrifuge dewatering, these bacteria are reactivated, show rapid growth and can again be detected by enumeration. Three MAD plants and one temperature phased AD (TPAD) plant (TAD followed by MAD in series) were investigated by Higgins et al. (2007). Only digestates from the TPAD plant did not show any regrowth. Later, several process configurations have been studied showing different results depending on AD configuration or pre-treatment (Figure 8) (Murthy et al., 2010).

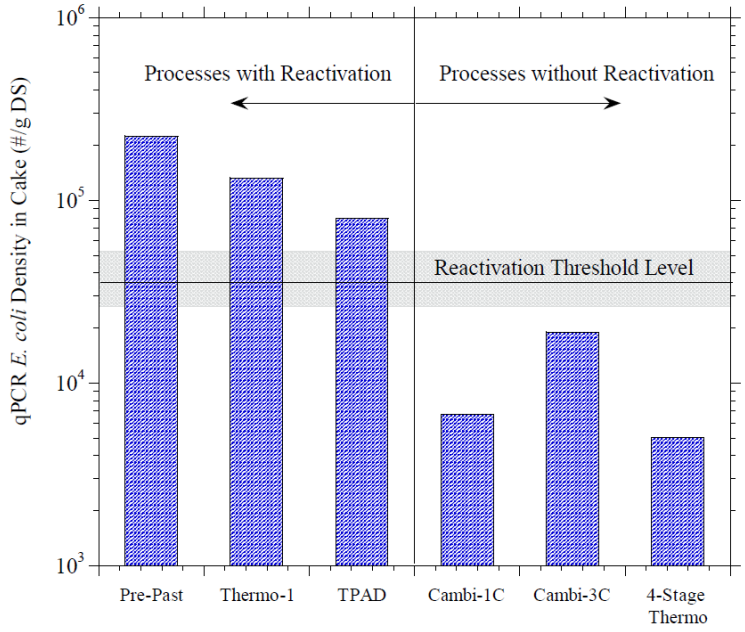

Figure 8: "Comparison of E. coli densities for different digestion/pre-treatment processes and centrifuge dewatering, with and without reactivation" (Murthy et al., 2010).

Digestates treated with Pre-AD THP (Cambi, Figure 8) and 4 stage TAD did not show reactivation of *E. coli*. Contradictory to findings by Higgins et al. (2007), TPAD showed regrowth.

Sterilization by THP followed by organic matter reduction and microbial inoculation during the AD process successfully eliminates reactivation and meet the VAR requirements (USEPA, 2003). However, when applying the THP unit after digestion the microbial inoculation is before sterilization which can make the biosolids less resilient towards recontamination due to the lack of microbial competition.

The Post-AD THP configuration shows promise in increased dewaterability (Kolovos et al., 2016), which can give large savings in operational cost. However, the dewatered biosolids will no longer comply with the VAR requirements since the thermal treatment is after the AD process. Inoculation of pasteurized digestate in a mesophilic digester for 6 days can prevent pathogen growth and ensure Class A (Ward et al., 1999). However, with the Post-AD THP process this would require cooling before digestion and

dewatering. This again will lead to higher investment costs and a potential loss of dewatering efficiency due to the digestion process itself and lower temperature in the centrifuge (Kopp and Dichtl, 2001a, Klinksieg et al., 2007). Biological inoculation with a pathogen-free microbial community after dewatering could therefore be an interesting option to stabilize the sterilized and dewatered biosolids and prevent pathogen recontamination. A similar approach has been explored in the aquaculture sector to increase larval viability (Vadstein et al., 2018).

2 Thesis aims and outline of work

The overall aim of this thesis was to get a better understanding of important factors influencing dewatering by studying a wide range of digestates.

To achieve the overall aims, the research was divided into the following sub-objectives: firstly, improve the understanding of factors affecting separation efficiency related to the thermal hydrolysis process (**Paper I, II and III**), digestate composition (**Paper III**), and dewatering device (**Paper III**). Secondly, study the soluble fraction of THP digestates with the aim of quantifying melanoidin-associated compounds by chemical/physical methods and understanding the effect of different sludge compositions on the formation of these compounds (**Paper IV**). Finally, study the quality of the Post-AD THP dewatered cake in terms of resilience against pathogenic growth from recontamination *by Escherichia coli* (**Paper V**).

This thesis is based on these five papers which have the following outline:

In **Paper I** conventional pre-treatment at 70 °C was compared with Post-AD THP. The COD solubilization and dewaterability at different reaction times and temperatures in the Post-AD THP were evaluated by using batch-tests and thermogravimetric analysis (TGA). Continuous stirred-tank reactors (CSTR) were used to compare the two digestion configurations in terms of biogas production.

In **Paper II** samples from 32 full-scale plants were used to study the effect of Post-AD THP on predicted cake solids by TGA. The effect of Post-AD THP on the moisture distribution was quantified by using low field nuclear magnetic resonance (LFNMR) and TGA. A full-scale trial was conducted to compare predictions by TGA at 35 °C to full-scale dewatering at 80 °C. Water diffusion rates of Post-AD THP digestates were determined at 35 °C and 80 °C to study the effect of temperature on water mobility.

In **Paper III** different full-scale dewatering devices and digestate physicochemical properties were investigated in relation to predicted cake solids by TGA. Principal component analysis (PCA) was used to identify physicochemical parameters affecting

the free water content related to digestion substrate. A combined physicochemical parameter was identified to predicted cake solids by TGA and full-scale dewatering including both polymer and dewatered cake solids.

In **Paper IV** the effect of different sludge blends and source separated food waste on the concentration of soluble colloidal COD, colour, UV and DON was quantified using seven full-scale Pre-AD THP digestates.

In **Paper V** the resilience of the sterilized Post-AD THP biosolids against *Escherichia coli* (*E. coli*) recontamination was investigated. Sterilized Post-AD THP biosolids were inoculated with compost for 48 hours. Sterilized and inoculated Post-AD THP was then compared to two other dewatered biosolids with approved hygienization methods: TAD and Pre-AD THP. The samples were recontaminated with wastewater containing *E. coli*. Growth of *E. coli* was monitored through standard enumeration methods (viable counts) and digital droplet PCR. Microbial respiration was measured with a robotized incubation system. The microbial diversity of the biosolids was assessed using 16S rRNA gene sequencing.

3 Main results and discussions

3.1 Paper I

In this paper two treatment technologies for sewage sludge and source separated food waste (SSFW) were tested in relation to methane production and predicted dewatered cake solids, namely pasteurization (conventional AD) and post anaerobic digestion thermal hydrolysis (PAD-THP). Digested cakes from sewage sludge and SSFW were investigated separately to assess the effect of different PAD-THP treatment temperatures and reaction times on COD solubilization, bio-methane potential and predicted cake solids concentration after dewatering. Two anaerobic semi-continuous laboratory digesters were used to investigate the digester performance of both pasteurization and PAD-THP when co-digesting sewage sludge and SSFW. Two digester feedstocks were made: one where the feed was heated at 70 °C for 1 hour, and another where raw, un-treated, sludge was blended together with the dewatering liquor from after PAD-THP.

The sewage sludge digestate cake showed better performance after PAD-THP in terms of biogas yield of centrate and predicted cake solids after dewatering compared to the digestate cake from SSFW. The SSFW digestate is rich in lignocellulosic material and thus has a higher content of fiber than the sewage sludge digestate. This could explain the differences seen, as lignocellulosic material has been shown to have a higher optimum THP treatment temperature compared to sewage sludge.

Results from semi-continuous digesters co-digesting food waste and sewage sludge showed an improved volumetric methane yield (7 %) and increased COD reduction (68 % to 74 %) in the PAD-THP digestion compared to the digester with pasteurized feed.

Following PAD-THP the increase in predicted cake solids concentration after dewatering was greater for the sewage sludge digestate than the SSFW. The sewage sludge digestate original cake solids was 17 % DS and after PAD-THP the predicted cake solids concentration had increased to 43 % DS. The SSFW digestate improved the predicted cake solids from 34 % DS to 46 % DS. The practical implication of these findings is a 60 % reduction in mass of cake that needs disposal in the case of sewage

sludge (Figure 9). Further improvments in cake reduction can also be expected due to the increased volatile solids reduction from recirculation of the centrate to the AD process.

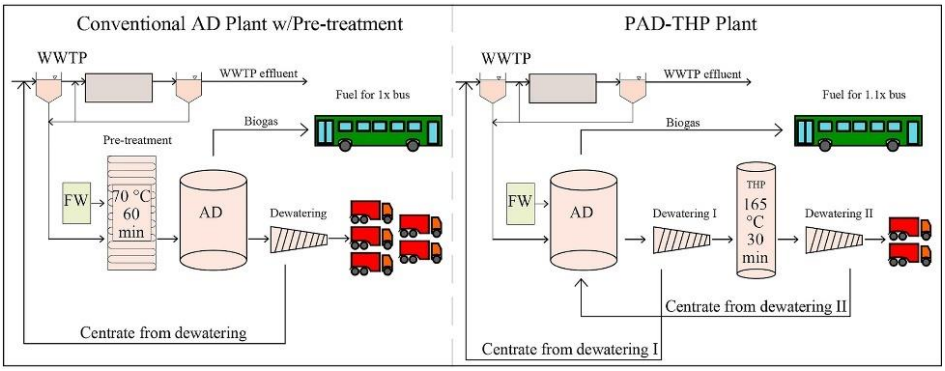

Figure 9: Illustration of the two different scenarios tested with semi-continuous laboratory digesters comparing pasteurization (Conventional AD Plant) and PAD-THP.

3.2 Paper II

In Paper I, a different effect of THP on dewatered cake solids of sewage sludge digestate and SSFW digestate was demonstrated. Paper II therefore investigated the effect of Post-AD THP on a broad range of digestates from 32 commercial full-scale plants. This study investigated the effect of Post-AD THP on moisture distribution, and predicted cake solids after dewatering, and the effect of dewatering at higher temperatures. Low field nuclear magnetic resonance and TGA were combined to determine the moisture distribution of digestates and Post-AD THP digestates. TGA was further used to predict the dewatered cake solids after Post-AD THP treatment in the laboratory. Dewatering at 80 °C in the Post-AD THP configuration compared to conventional dewatering temperatures (20-35 °C) was investigated by laboratory centrifugation and measurement of water diffusion rates. A full-scale trial was conducted to investigate if higher cake solids can be achieved when dewatering at 80 °C, compared to predictions by TGA at 35 °C.

The free water fraction was measured for 32 Post-AD THP digestates and compared to the original cake solids (Figure 10). The results showed a consistent increase in dry solids concentration in dewatered cake across the sample set compared to original cake dry solids concentration. This data set included three plants with fiber rich substrates such as source separated food waste and manure. In contrast to findings in Paper I, these plants followed the same trend as the sludge samples.

Determination of the moisture distribution in eight digestates and Post-AD THP digestates showed a significant change in the interstitial and free water fraction. The free water fraction increased, while the interstitial water fraction decreased.

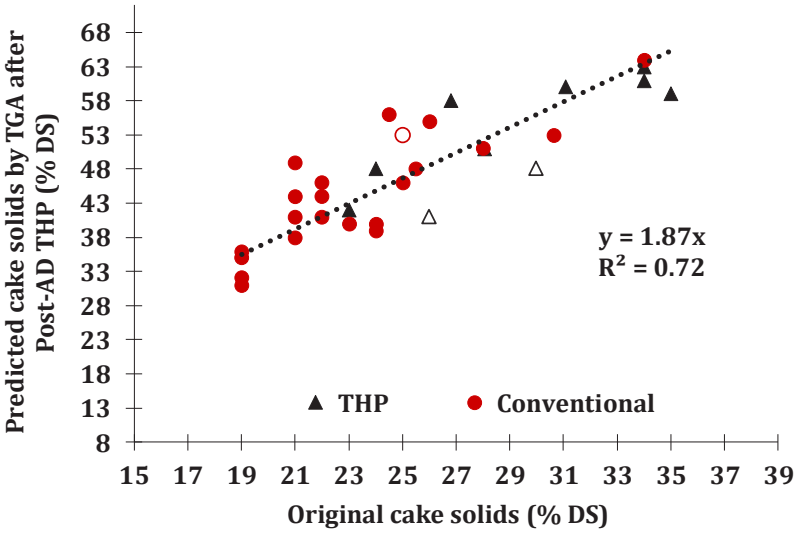

Figure 10: Effect of Post-AD THP on predicted dry solids concentration of dewatered cake compared to dry solids concentration of original dewatered cake. Open symbols represent digestates from source separated food waste.

Furthermore, water extraction from centrifuged pellets was 21 % higher at 80 °C compared to 20 °C. Higher soluble COD concentrations were found in the supernatants generated by centrifugation at 80 °C compared to 20 °C, indicating that organic compounds such as lipids and collagenous compounds were solubilized and thus probably releasing trapped water. The water diffusion rates of Post-AD THP digestates were higher at 80 °C compared to 35 °C. This could contribute to improved full-scale dewatering efficiency as the water release rate is important due to the limited time in the centrifuge. Full-scale trial confirmed that more water could be extracted at 80 °C than what was predicted by TGA at 35 °C, yielding up to 21 % higher cake solids concentrations when the applied polymer dose was increased.

3.3 Paper III

In Paper II, considerable variation in dewaterability of different digestates was observed. In Paper III, factors that could explain this variation in dewaterability was investigated. The influence of different dewatering devices and digestate physicochemical parameters were studied by combining laboratory analysis and full-scale data.

Dewatered cake solids were predicted by measuring the free water content with TGA and compared to full-scale dewatered cake solids for 15 commercial full-scale plants. The free water fraction measured by TGA corresponded to the fraction removed by centrifuges in full-scale (R^2 = 0.98). Dewatered cakes made by hydraulic filter presses were 9 % drier than those from centrifuges, while 7 % higher cake solids were achieved with centrifuges compared to belt presses. The largest possible difference in dewatered cake solids due to dewatering device in this data-set was 5 % DS and cannot explain the large variance in Figure 11. Thus, other factors are influencing dewatered cake solids more than the dewatering device.

A novel digestate physicochemical parameter was defined by multiplying the carbon to nitrogen ratio with the ash content of the dried solids (C/N•ash). C/N•ash was compared to the predicted cake solids by TGA for 22 commercial full-scale plants. A strong linear relationship was found when Pre-AD THP digestates were separated from conventional digestates (Figure 11). The C/N•ash factor was accurately reflecting the physicochemical properties influencing the dewatered cake solids. C/N•ash was compared to reported polymer dose from eight full-scale Pre-AD THP plants, showing a linear relationship also between these two parameters. In conclusion, C/N•ash reflects the water retention properties of different digestates irrespectively of dewatering device and sludge origin and can be used as a general predictor of dewatered cake solids concentration.

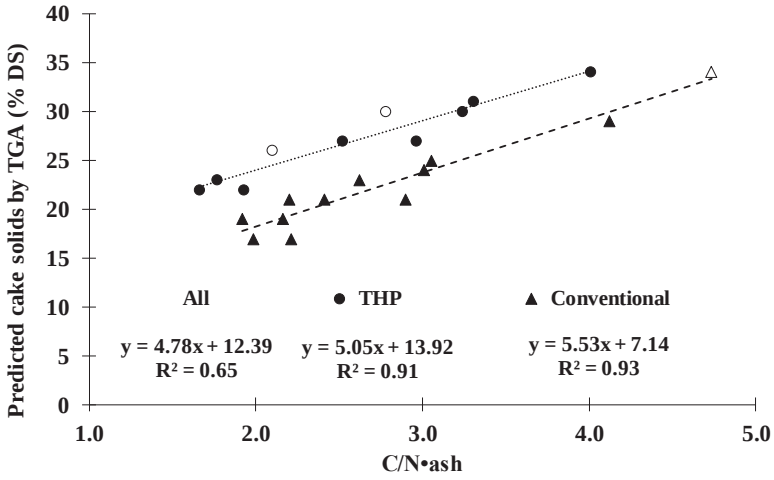

Figure 11: The mass C/N ratio was multiplied with the relative content of ash (mass fraction), both measured on dry solids basis. The C/N•ash was compared to predicted cake solids by TGA for sewage sludge, food waste and co-waste AD digestates from conventional and Pre-AD THP plants. Results from linear regression analysis of all, conventional and Pre-AD THP plants are displayed. Pure food waste AD plants are indicated with open symbols.

3.4 Paper IV

The effect of different THP substrates on soluble melanoidin-associated compounds in digestates were investigated. Digestates from seven full-scale Pre-AD THP plants were divided into three groups and compared. The three groups were digestates with high amounts of WAS (High WAS), more PS than WAS (High PS) and source separated food waste (FW). High WAS and High PS had been treated at 165 °C in the THP, while FW had been treated at 145 °C. Soluble colloidal COD (CODsc), colour, UVA and DON were measured in the digestates and compared by ANOVA and Tukey's pair-wise comparison to identify differences between the three groups.

High amount of WAS in the THP substrate gave higher concentrations of CODsc, colour and DON in the digestate compared to the other groups (Figure 12). UVA varied considerably between the plants tested (Figure 12) but showed 88 % increase from the lowest concentration to the highest concentration in the data-set.

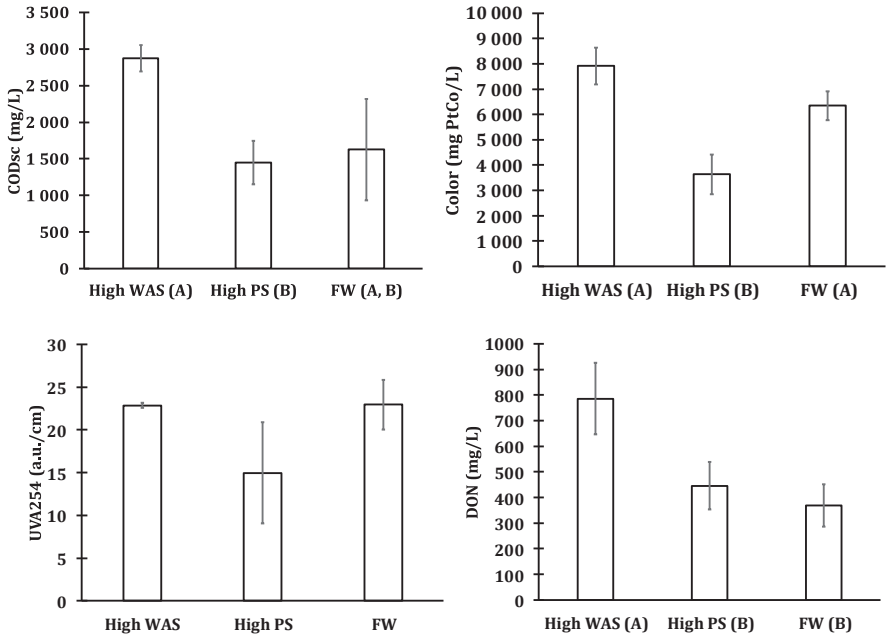

Figure 12: Soluble melanoidin-associated compounds in digestates fed with more waste activated sludge (WAS) than primary sludge (PS) (High WAS), more PS than WAS (High PS) and food waste (FW). Concentrations of soluble colloidal COD (CODsc), colour, ultraviolet absorbance at 254 nm (UVA254) and dissolved organic nitrogen (DON) with the standard deviation in each group are presented. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter are significantly different.

In this study, equal or lower concentrations of soluble melanoidin-associated compounds were found in the FW compared to High WAS and High PS. This indicates that treating FW at 145 °C can limit the formation of these compounds to a level not significantly different from sludge treated at 165 °C.

The type of substrate was found to influence the soluble melanoidin-associated compounds in the digestates tested.

3.5 Paper V

In this study, the efficiency of inoculating the Post-AD THP biosolids with a microbial community from compost to suppress the growth of *E. coli* after recontamination was investigated.

The addition of compost bacteria effectively suppressed the growth of both viable *E. coli* (Figure 13A) and *E. coli* gene (*uidA*) abundance (Figure 13B) after recontamination of Post-AD THP biosolids with wastewater. The sterile Post-AD THP biosolids reached low levels of *E. coli* in line with legislations in Norway and the USA within 13 days. This period could be substantially reduced by inoculation with compost. The effective colonization of compost bacteria in the Post-AD THP biosolids was also supported by high respiration and higher microbial diversity compared to the sterile Post-AD THP biosolids.

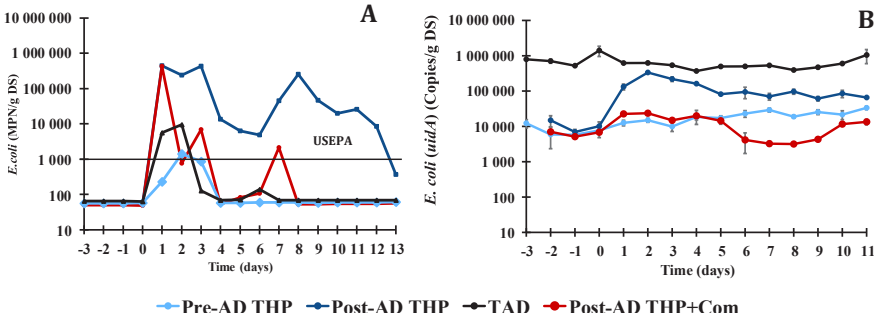

Figure 13: Abundance of *E. coli* throughout the experimental period. Panel A shows the viable counts (MPN), Panel B shows the abundance of *uidA* gene quantified by ddPCR, both plotted against time (time = 0 is the time of recontamination, sampling at day 0 was done before recontamination). USEPA limit of 1000 MPN/gDS is indicated by the black line. The Norwegian limit of 2500 MPN/gDS is not shown. It should be noted that the very high MPN values for Post-AD THP at day 1 and 3 are minimum estimates (all MPN tubes were positive).

The results show that by inoculating the Post-AD THP biosolids for two days the growth of *E. coli* after recontamination can be reduced. Addition of a second digester after sterilization as suggested by Ward et al. (1999) would require higher investment costs. Hence, the approach proposed in this study with inoculation after dewatering could be an attractive alternative strategy.

4 Concluding remarks and future perspectives

The main objective of this thesis was to improve the understanding of mechanisms impacting dewatering of digested biomass with focus on the effect of the thermal hydrolysis process (THP) and substrate composition. Based on the results from this study the following conclusions can be made:

Thermal hydrolysis improves the dewaterability of digested biomass by increasing the amount of free water leading to higher predicted dry solids concentration of dewatered cake. Both THP configurations studied (Pre-AD THP and Post-AD THP) resulted in higher dewatered cake solids compared to dewatering of conventional digestates. However, Post-AD THP resulted in the largest improvement.

In this thesis, a wide range of digestates were used to identify mechanisms explaining the variations seen between different digestates. Overall, the results from this study show that digestate dewaterability highly depends on the digestate physicochemical properties. A physicochemical parameter was found that can be easily measured and used to fingerprint the water holding capacity of a wide range of different digestates irrespectively of biomass origin and dewatering device. This parameter, termed C/N•ash, reflects both the organic and inorganic fraction of the digestate by combining the carbon to nitrogen ratio and ash content of the dry solids.

In this study we investigated the differences between digestate from different full-scale plants. However, seasonal variations within the same plant may have an impact on the dewatering. Thus, sampling one plant over one year could be done to investigate if the C/N•ash would describe these changes.

C/N•ash can also be included in the planning of new plants or optimization of existing plants. C/N•ash can help identify suitable digestates for testing of dewatering devices, as in greenfield projects testing of such equipment is done on other existing plants. Process guarantees are commonly set years before they must be demonstrated in the full-scale set-up. In the case of discrepancies between guarantees and actual

performance, the C/N•ash could help identify if the sludge blend has changed or if the dewatering process is not performing according to expectations.

The composition of the feedstock treated by the THP was shown to influence the production of some melanoidin-associated compounds. This could have an impact on the plant effluent but not necessarily all months of the year. Variations in rain- and snowfall and the potential loss of efficiency of the wastewater biology in colder winter months has been shown to give fluctuations in the concentration of these compounds in the effluent at some plants. Hence, further research is needed to identify potential mitigation techniques that could easily be switched on and be operated only when needed.

Growth of *E. coli* in the Post-AD THP biosolids after recontamination was successfully suppressed by inoculation of the sterile Post-AD THP biosolids with compost. Two days inoculation in room-temperature was used for this study to simulate the typical storage capacity in receiving containers at WWTPs. However, other conditions could potentially improve the effectiveness of this step. Factors such as temperature, time, air control and biomass mixing are among the parameters that should be further investigated to improve this step.

Overall, this thesis has shown the importance of substrate composition when assessing dewatering performance in addition to the influence of the two THP configurations Pre-AD THP and Post-AD THP. Dewatering performance is crucial for WWTPs economy and hence an important factor to consider when planning new facilities. This work has provided fingerprinting parameters and predictive tools that can help plan new or optimize existing facilities thereby saving valuable time and money. Moreover, this work has provided a practical solution to the problem of recontamination of sterile Post-AD THP biosolids.

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PAPER I

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Post-anaerobic digestion thermal hydrolysis of sewage sludge and food waste: Effect on methane yields, dewaterability and solids reduction

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ABSTRACT

Post-anaerobic digestion (PAD) treatment technologies have been suggested for anaerobic digestion (AD) to improve process efficiency and assure hygenization of organic waste. Because AD reduces the amount of organic waste, PAD can be applied to a much smaller volume of waste compared to pre-digestion treatment, thereby improving efficiency. In this study, dewatered digestate cakes from two different AD plants were thermally hydrolyzed and dewatered, and the liquid fraction was recirculated to a semi-continuous AD reactor. The thermal hydrolysis was more efficient in relation to methane yields and extent of dewaterability for the cake from a plant treating waste activated sludge, than the cake from a plant treating source separated food waste (SSFW). Temperatures above 165 °C yielded the best results. Post-treatment improved volumetric methane yields by 7% and the COD-reduction increased from 68% to 74% in a mesophilic (37 °C) semi-continuous system despite lowering the solid retention time (from 17 to 14 days) compared to a conventional system with pre-treatment of feed substrates at 70 °C. Results from thermogravimetric analysis showed an expected increase in maximum TS content of dewatered digestate cake from 34% up to 46% for the SSFW digestate cake, and from 17% up to 43% in the sludge digestate cake, after the PAD thermal hydrolysis process (PAD-THP). The increased dewatering alone accounts for a reduction in wet mass of cake leaving the plant of 60% in the case of sludge digestate cake. Additionaly, the increased VS-reduction will contribute to further reduce the mass of wet cake.

1. Introduction

Anaerobic digestion (AD) is commonly used in waste management for treatment of organic wastes such as sewage sludge and food waste, with the aim of waste stabilization, methane generation and production of a digestate that can be used as a fertilizer. AD processes typically have long retention times, meaning large digesters and thus large plant footprints. The waste stabilization efficiency and digestate quality depends on the characteristics of the waste and the AD technology. Technologies assuring a high hygienic quality of the digestate as well as high waste stabilization rates are key for a successful AD plant, and pre-treatment technologies such as the thermal hydrolysis process (THP) has been extensively used to improve process performance (Barber, 2016; Carrere et al., 2016; Neyens and Baeyens, 2003).

THP has increased degradation rates and biogas yields for a wide range of wastes, including sewage sludge and lignocellulosic biomasses (Bauer et al., 2014; Dereix et al., 2006; Estevez et al., 2012; Horn et al., 2011b; Lizasoain et al., 2016; Vivekanand et al., 2013; Wilson and Novak, 2009). The optimum temperature and time combination during THP pretreatment depends on the type of substrate. THP treatment has resulted in reduced capillary suction time (CST) and filtration time of sludge, both parameters important for the rate of the dewatering process (Dereix et al., 2006; Everett, 1972; Haug et al., 1978). However, CST and filtration methods are not necessarily correlated with maximum cake solids (Kopp and Dichtl, 2001). Technologies that increases the total solids (TS) in dewatered digestate have a large potential for reducing the storage silo footprint as well as transportation costs for disposal of the

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digestate cake. Although improved dewaterability is well documented in sludge after THP (Everett, 1972; Haug et al., 1978; Neyens and Baeyens, 2003; Skinner et al., 2015), the mechanism is not well understood, and the optimum THP treatment conditions for different wastes are largely unknown.

THP-based technologies result in solubilization of organic material and thus release of readily degradable organic matter to the liquid fraction (Dereix et al., 2006). In Norway, pre-treatment of waste is mainly applied to meet health regulations where the minimum requirement is heating for 1 h at 70 °C for sludge and waste of animal origin (Nærings- og fiskeridepartementet, 2007). Although pre-treatment results in a reduction of pathogenic bacteria in the digestate and improved process performance (Bagge et al., 2005; Lang and Smith, 2008; Wang et al., 1997), large fractions of the waste are readily bio-degradable and do not benefit from such treatment. Post-anaerobic digestion treatment (PAD) has recently been suggested as an alternative (Sambusiti et al., 2015; Thygesen et al., 2014). This means that only a fraction of the original waste needs to be treated, while still ensuring a hygienic end-product for land application. In a typical PAD-THP setup, the digestate would be dewatered, treated with THP and then after THP undergo a subsequent dewatering, where the liquid fraction is recirculated to the anaerobic digester. The patented Cambi Solid-Stream™ (Kjorlaug et al., 2015; Kolovos et al., 2016; Solheim and Nilsen, 2014) is based on this idea, and involves post-treatment of digestate cake using THP.

So far, only one full-scale plant has installed a PAD-THP process, which is the Cambi SolidStream™ (Amperverband in Olching, Germany). No laboratory scale studies have been published on this topic. Thus, many of the mechanisms of the technology are not well documented and understood. For example, recirculation of the centrate from the post-treated digestate can result in a reduction of sludge retention time (SRT), which could reduce the efficiency of the AD process (Jang et al., 2014), possibly counteracting the beneficial effect of post-treatment. In addition, optimal THP conditions found for other substrates will not necessarily apply to biogas digestates, and studies of how digestate cakes of different origin respond to THP are lacking. A third unknown factor of THP is the effect on digestate dewaterability. Up to now, the effect of THP treatment on different digestate cakes is not described in the literature.

The objectives of this study were to evaluate the effect of:

1) thermal hydrolysis conditions (time and temperature) on the solubilization of COD and resulting biogas production from digestate cakes using biochemical methane potential (BMP) tests; and
2) PAD-THP on digester performance and overall solids reduction using semi-continuous anaerobic digesters.

2. Materials and methods

The experimental work was in part performed at the Biogas Laboratory at the Norwegian University of Life Sciences (Ås, Norway) and at the Environmental Engineering Laboratory at Bucknell University (Lewisburg, PA, USA). Due to differences in the laboratory equipment at the two locations, it was not possible to use the same methods for all analyses; however, we consider the methods used compatible.

2.1. Experimental design

This study is based on two experiments. The first experiment was designed to find the optimal THP conditions for two digestate cakes using the biochemical methane potential (BMP) test. The second experiment was designed to investigate how the Solid Stream approach affects the performance of semi-continuous anaerobic digesters operated until steady state conditions were achieved.

2.2. Materials for THP conditions experiment

We obtained centrifuged digestate cake from two different full-scale AD plants. One cake was from a food waste anaerobic digester operating in the thermophilic range (52–53 °C; Hadeland and Ringerike waste company (HRA), Ringerike, Norway), and source separated food waste (SSFW) was its sole substrate. HRA pretreats the SSFW according to Norwegian regulations at 70 °C for 1 h. The second cake was from an anaerobic digester operating in the mesophilic range (35 °C) treating sludge and collected at Hampton Roads Sanitation District's (HRSD) Nansemond Treatment Plant (Suffolk, Virginia, USA). HRSD's plant treats a mix of primary and waste activated sludge (WAS) from a Bio-P process. Both plants use high solids centrifuges for dewatering.

2.3. THP conditions experiment

The digestate cakes (HRA and HRSD) were used for testing different post-treatment conditions. The post treatment of HRA digestate cake was performed in Norway, using a small Cambi mini test steam explosion unit with a reactor volume of 1 L (CAMBI GROUP AS, Asker, Norway), while the HRSD digestate cake was post-treated in a larger Cambi mini test steam explosion unit at Bucknell University with a reactor volume of 5 L (CAMBI GROUP AS, Asker, Norway). The characteristics of the two cakes prior to post-treatment are presented in Table 1.

To examine the effect of different THP conditions on BMP and dewatering properties of digestate cakes, a set of seven different pre-incubation times and temperatures, spanning from 134 °C to 175 °C and from 20 min to 30 min, was applied. The lowest temperature was not tested with the 20 min treatment because this combination of time and temperature does not fulfill the current regulations for sanitation. Pre-incubation time was measured from the time the desired temperature in the reactor was reached. The post-treated digestate cakes were separated in a centrifuge at 2000 relative centrifugal force (RCF) for 30 min and the liquid and solid fractions were used in BMP and dewatering tests. The BMP results for the liquid fraction is presented on the basis of COD and the solid fraction on the basis of TS, because much of the liquid COD was volatiles that would result in falsely low TS measurements, and the solid fraction contained particulates making COD-measurements

Table 1
Characteristics of digestate cakes from HRA and HRSD. Standard deviations are listed in parenthesis. All percentages are on the basis of TS with the exception of Ash which is on the basis of wet weight.

	Unit	HRA	HRSD
TS	%	18.5 (0.3)	21.8 (0.1)
VS	%	73.1 (0.4)	68.4 (0.4)
Ash	%	5.0 (0.7)	6.9 (0.1)
COD	g/L	226 (2.6)	183 (17)
COD:VS		1.7	1.2
C	%	42.7 (0.3)	32.7 (0.2)
H	%	5.45 (0.09)	5.47 (0.08)
N	%	2.43 (0.05)	4.70 (0.1)
S	%	0.49 (0.02)	1.95 (0.01)
C:N		17.6 (0.5)	7.0 (0.2)
ADF[a]	%	54.6 (0.3)	31.4 (1.3)

[a] ADF = Acid detergent fiber.

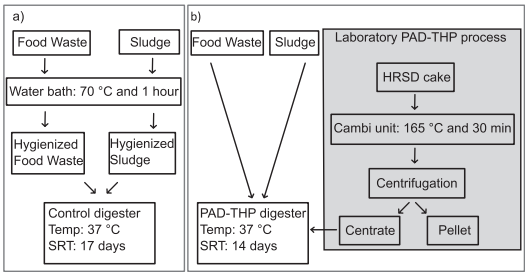

Fig. 1. Experimental setup for CSTR experiment. a) In the control digester, substrates were heated at 70 °C for 1 h in a water bath prior to feeding. b) In the PAD-THP digester, untreated food waste and sludge was used as substrate. Centrate was produced by thermal hydrolysis of HRSD cake and separated using a laboratory centrifuge. The centrate was fed to the digester together with food waste and sludge.

less reliable. BMP results of the solid fraction is given on the basis of TS instead of VS to directly relate the methane yield of the cake to predicted cake solids.

2.4. Semi-continuous stirred tank reactors experiment – PAD-THP

For the semi-continuous stirred tank reactor (CSTR) experiments, feed sludge (primary sludge and waste activated sludge (WAS)) was shipped overnight from HRSD weekly and stored at 5 °C until use. Waste used for the digester fed with conventional pre-treated waste was pretreated upon arrival by heating in closed containers in a water bath for one hour at 70 °C. The initial heating of the waste to reach 70 °C took approximately 1 h. A preprocessed food waste called an Engineered BioSlurry (EBS) was supplied by Waste Management, Inc. The food waste was a homogenized product of different commercial food wastes, including pre-consumer and post-consumer organic waste streams such as waste from restaurants, grocery stores, expired packaged goods, food processors and residential organics. The food waste samples were stored in a freezer until use. In order to get enough centrate for the PAD-THP digester, it was decided to only use centrate from post-treatment of HRSD cake from the full-scale digester and not from the laboratory digesters. This decision was based on the results from the THP-conditions experiment on dewatering properties which revealed that we were not able to dewater the post-treated cake to the final cake solids achieved by a full scale centrifuge with a laboratory centrifuge, hence, much of the centrate would be left in the pellet and not recirculated to the laboratory digester. Another reason for the decision was that digestate is lost both in the initial dewatering before THP to reach a TS of 16%, but even more in the Cambi unit because some of the digestate is deposited on the inside walls of the THP reactor. The treated digestate cake was separated by centrifugation while still hot at 3000 RCF for 30 min in the laboratory centrifuge, and this centrate was used in the PAD-THP digester (Fig. 1).

Two, 10 L CSTRs were used in the experiment, one control-digester using conventional technology fed with hygenized sludge and food waste (70 °C for 1 h) and one PAD-THP digester fed with untreated sludge, food waste, and centrate from THP-treated HRSD cake (treated for 30 min at 165 °C). The feed characteristics are presented in Table 2. The ratio of food waste, sludge and centrate was 15:100:10 on a wet weight basis and approximately 10:20:1 on the basis of COD (Table 3). The digesters were fed once a day, immediately after digester wasting. Biogas was transported through a tube in the headspace of the digester to a PF-8000 respirometer from Respirometer Systems and Applications (Springdale, Arkansas, USA) which recorded total gas volume and gas production rates. In addition, a second tube was connected to a gas bag that collects or supplies gas during the brief periods of wasting and feeding in order to maintain a stable concentration of gases in the headspace and not create a vacuum or pressure in the digester. The bag has a valve that is opened only during the feeding and wasting operation. A sealed septum port in the top of each reactor allows sampling of the headspace for gas composition. Both reactors were inoculated with digestate from two lab-scale digesters digesting sewage sludge from HRSD at day 0 of the experiment.

A co-digestion scenario with mixing of food waste and sewage sludge for digester feed was chosen in order to test the effect of PAD-THP on a digester already operating under high organic loading rates, and low SRT. Concentrated food waste have 2–3 times the COD concentration of sewage sludge (Table 2), and can be used to improve volumetric biogas yields of sewage sludge digesters. Organic loading rate (OLR) and sludge retention time (SRT) is presented in Table 3. The SRT is lowered as a consequence of the recirculation-stream. A low SRT (17 and 14 d), compared to conventional plants, was used in the experiment in order to be able to observe a possible reduced efficiency due to the lowering of SRT.

2.5. Analyses

Total solids (TS) and volatile solids (VS) were determined gravimetrically by drying at 105 °C and subsequent burning at 550 °C. Volatile fatty acids (VFAs) in the CSTR effluent were measured using an Agilent 5890 (Agilent Technologies, Santa Clara, California, USA) gas chromatograph (GC) with a flame ionizing detector (FID) equipped with a 30 m × 0.53 mm × 1 μm film thickness Supelco Nukol Fused Silica capillary column (Catalog # 25357). Samples were first centrifuged at 3000 × *g* for 15 min, and then

Table 2
Feedstock characteristics used for CSTR experiment. Standard deviations are listed in parenthesis.

	Unit	Untreated sludge	Hygenized sludge	Untreated food waste	Hygenized food waste	PAD-THP centrate
COD	g/L	92.2 (6.9)	102 (8.3)	270 (29.7)	261 (13.2)	38.7 (4.9)
S-COD	g/L	3.0 (0.7) *	12.6 (1.6) *	96.8 (11.4)	96.5 (7.5)	35.7 (3.3)
TS	%	6.3 (0.4)	6.9 (0.8)	13.4 (0.2)	13.4 (0.5)	2.8 (0.2)
VS	% of TS	79 (0.7)	79 (1.0)	91 (1.0)	90 (1.4)	88 (1.9)
TAN[a]	mg/L	221 (45) *	363 (117) *	396 (66)	388 (82)	1248 (318)
PO4-P	mg/L	541 (394)	781 (474)	493 (202)	477 (226)	712 (505)
Acetic acid	mg/L	578 (258)	592 (133)	3383 (1833)	3157 (1950)	934 (202)
Propionic acid	mg/L	408 (164)	277 (148)	132 (23)	127 (20)	666 (113)
tVFAs	mg/L	1211 (567)	1022 (333)	3677 (1873)	3540 (1837)	2202 (411)
pH		5.7 (0.2) *	6.0 (0.3) *	3.9 (0.1)	3.9 (0.1)	6.9 (0.2)

* values of untreated and hygenized substrates are significantly different (P-value < .05).
[a] TAN = total ammonia nitrogen.

Table 3
Experimental design of CSTR experiment. The organic content of the centrate is not included in the organic loading rate (OLR) for the PAD-THP digester, because this is a recirculation-stream and not fresh substrate added to the digester feed. The difference in OLR is caused by the difference between the pasteurized and non-pasteurized feed. Standard deviations are listed in parenthesis.

	Unit	Control	PAD-THP
FW	g/day	75	75
Sludge	g/day	500	500
Centrate	g/day	0	150
Digester volume	L	10	10
Feed mass	g/day	575	725
SRT	day	17	14
OLR on COD basis	g/L*d	7.1 (0.48)	6.8 (0.52)
OLR on VS basis	g/L*d	3.6 (0.27)	3.4 (0.14)

supernatant was filtered through a 0.45-μm filter. Next, 0.5 mL of sample was placed in a gas chromatography vial and diluted with 0.5 mL of deionized water. Then 50 μL of methanesulfonic acid was added to the vial, and the vial was capped. Samples were auto-injected into the gas chromatograph at a volume of 1 μL. The injector temperature was 238 °C, and the oven was first held at 105 °C for 4.00 min, followed by a 5 °C/min ramp to 145 °C, followed by a 10 °C/min ramp to 190 °C and a hold of 5.50 min. The detector temperature was 200 °C.

COD was determined using commercial kits (Merck in Norway and CHEMetrics in the USA). For determination of soluble fraction of COD, the samples were prepared by centrifugation (RCF of 23,907 for 10 min) prior to filtration. A 0.2 μm syringe filter was used for THP conditions experiments, as this excludes more particulates than the 0.45 μm filter and will give a more correct measure of true solubles. For the CSTR experiment 0.45 μm filters were used, as this filter pore size is more common when evaluating effluent quality from anaerobic digesters.

CHNS analysis was performed on a Vario El Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) by combusting the dried samples at 1150 °C under a constant flow of oxygen gas.

Acid Detergent Fiber (ADF) was analyzed with an Ankom^{200} Fiber Analyzer (ANKOM Technology, Macedon, New York, USA) according to manufacturer's description using F58 filter bags to retain more of the fiber fraction.

Biochemical methane potential tests were performed as previously described by Horn et al. (2011a,b). In brief: inoculum and substrate was mixed in 500 mL bottles with rubber septa sealed with aluminum screw caps. Pressure and biogas composition was measured using an electronic manometer (GMH 3161 Greisinger Electronic, Regenstauf, Germany) and an Agilent 3000A GC (Agilent Technologies, Santa Clara, California, USA) for 30 days. All BMP tests were performed in triplicate, including blanks with only inoculum and positive controls containing cellulose. Substrate to inoculum ratio was 1:3 on the basis of VS.

Prediction of cake solids were measured with a thermogravimetric method using the conditions described by Kopp and Dichtl (2001). In brief: A Netzsch Simultaneous TG-DTA/DSC Apparatus STA 449 F1 Jupiter® (NETZSCH-Geräutebau GmbH, Selb, Germany) was used with drying at 35 °C and a constant flow of nitrogen (20 mL/h). The drying curve was analyzed to find the amount of free water. This amount of water was assumed to correspond to the maximum water mass possible to remove from the digestate by high solids centrifuges, and hence a theoretical maximum TS of the sludge cake was estimated. The setup was calibrated using monodisperse silica particles of diameters 1.86 μm, 4.08 μm, 7.75 μm (Cospheric LCC, Santa Barbara, California, USA).

2.6. Calculations

COD and VS reduction, cake reduction and the volume of centrate recirculated to the PAD-THP CSTR was calculated based on mass balance. Equations are formulated in the supplemental material.

Statistical analysis were performed with the software R. For the THP conditions experiment a 2- way analysis of variance (ANOVA) was used with the parameters time, temperature and cake origin. A paired *t*-test was conducted to test if the daily methane production of the two CSTRs were significantly different, and if the pre-treatment of the substrates in the CSTR experiment influenced the measured parameters. All statistical tests were performed at the significance level of 95%.

3. Results and discussion

3.1. Testing of different THP conditions for post-treatment

Digestate cakes from two AD plants were thermally hydrolyzed with different combinations of time and temperature, ranging from 20 to 30 min and 134–175 °C, respectively. The aim was to investigate how different post-treatment conditions affected the two cakes with regards to solubilization, specific methane yield and dewaterability. The results from these experiments are presented and discussed in this section.

3.1.1. Solubilization and methane potential in centrate

The amount of soluble COD relative to the total COD can be used as a measure of solubilization during thermal hydrolysis. The effect of THP treatment time and temperature on digestate cakes from HRA and HRSD are presented in Fig. 2a. ANOVA showed that THP temperature and cake origin had a significant effect on the solubilization of the digested cake (p-value < .001 and < .01, respectively). The ANOVA also showed that the response to THP temperature was significantly different for the two cakes (p-value < .01), where soluble COD increased more in HRSD cake compared to HRA cake.

The highest solubilization of the HRSD cake was 187% higher than the lowest; for HRA cake the highest solubilization was 50% higher than the lowest. Additionally, the highest solubilization of HRSD cake was 113% higher than the highest solubilization of the HRA cake. For the HRSD cake (digested sludge) the highest solubilization was achieved for the 30 min treatment at 175 °C (32%), while the lowest solubilization for the 30 min treatment at 134 °C (10%). The increase in solubilization is greater between 152 °C and 165 °C compared to 165 °C and 175 °C for both the 20 min and the 30 min treatment time. For the HRA cake (digested food waste), the increase in solubilization over the temperature range (134 °C–175 °C) is small. Here the 30 min treatment at 175 °C gave the highest solubilization (15%), while 30 min at 134 °C gave the lowest solubilization (9%).

Several authors have observed that solubilization increased with increasing temperature and time of the THP (Haug et al., 1978; Li and Noike, 1992; Wilson and Novak, 2009). Haug et al. (1984) found that solubilization of WAS was highest for 30 min at 175–200 °C. Li and Noike (1992) tested WAS at temperatures up to 175 °C for 30 min and found that, generally, the solubilization increased with increasing temperature. Wilson and Novak (2009) tested WAS and primary sludge with pretreatment temperatures up to 220 °C for 2 h, and also found that the solubilization increased with increasing temperature. The results presented here agree with the previous findings in published literature on WAS and primary sludge. However, the degree of solubilization seems to be feedstock dependent.

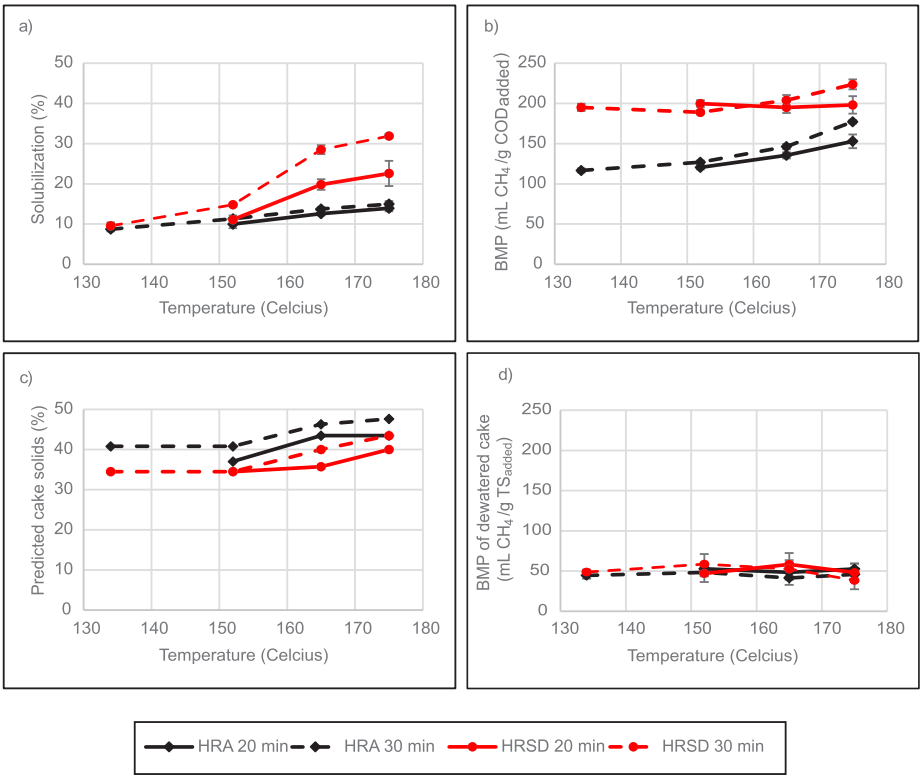

Fig. 2. a) Degree of solubilization achieved by thermal hydrolysis of cake from HRA and HRSD as soluble COD per total COD. Standard deviation of the COD measurements are presented as error bars. b) BMP of centrate from thermally hydrolyzed cake on the basis of COD added. Standard deviations of the COD measurements and methane measurements are presented as error bars. c) Predicted maximum TS in dewatered sludge cake. No replicates was made for this analysis, with the exception of the least uniform sample: untreated HRA, which had a RSD of 4% between triplicates. d) BMP in dewatered cake. Standard deviations of the TS measurements and methane measurements are presented as error bars.

In a PAD-THP, the centrate after post-treatment will be recirculated to the AD for additional biogas production. Therefore, in this study, the BMP of the centrate produced from the different post-treatment conditions was determined. ANOVA showed that the thermal treatment resulted in significantly different specific methane yields of the centrate from the two post-treated cakes (p-value < .001). The different centrates originating from HRSD cake in all cases yielded more methane compared to the centrates from the HRA cake (Fig. 2b). Temperature of the THP treatment also had a significant impact on methane yields of the centrates (p-value < .01), and the response to the temperature of the THP treatment was significantly different for the two cakes (p-value < .05). Increasing temperatures made the centrate from PAD-THP of HRA cake more available for conversion to methane. For centrates from PAD-THP of HRSD cake the response to higher temperature was smaller than for HRA cake, but the overall conversion to methane was significantly higher for centrates from HRSD cake.

Several authors have observed that methane yields increase with increasing temperature and time up to a certain level as a result of the treatment, before the effect levels off or decreases (Li and Noike, 1992; Stuckey and McCarty, 1984). Stuckey and McCarty (1984) found the optimum temperature for WAS to be 175 °C. Li and Noike (1992) found that the methane yield of WAS leveled off at a temperature of around 150 °C. Our results show increase in methane yields for the centrate from both cakes for temperatures up to 175 °C and treatment time 30 min.

The differences in solubilization and specific methane yield between the HRSD and HRA cakes can have several explanations; first, the pretreatment was performed with two different THP pilots and with different water-content in the incoming sludge cake (14.2% TS in HRSD cake and 18.5% TS in HRA cake; data not shown). This could have had an effect on the mixing of steam and digestate cake in the mini Cambi SE test unit, giving a lower treatment efficiency for the HRA compared to HRSD.

Second, it has been shown that the effect of THP depends on waste characteristics (Bougrier et al., 2008; Wilson and Novak, 2009), and the characteristics of the two cakes used in this experiment were different (Table 1). The HRSD cake, coming from an anaerobic digester treating sludge from a Bio-P plant, had a higher ash content compared to the HRA cake from the food waste plant and a lower COD:VS ratio. The HRSD cake also had a lower carbon content and higher nitrogen and sulfur contents. Plant material and lipids generally have a high C:N ratio, while protein have lower C:N ratios. A high COD:VS ratio is an indication of a more energy dense material, containing for instance more lipids. Further, analysis of acid detergent fiber (ADF), confirmed a larger fiber fraction (74% higher ADF) in the digestate cake from HRA compared to the

digestate cake from HRSD. The larger fiber fraction in HRA digestate cake indicates that this waste contains more plant material compared to the HRSD digestate cake.

Primary sludge and WAS has previously been reported to be efficiently solubilized at 165 °C (Wilson and Novak, 2009), and the maximum increase in methane yield have also been found around 30 min treatment time at 165 °C (Haug et al., 1978; Li and Noike, 1992; Stuckey and McCarty, 1984). Bougrier et al. (2008) found that at temperatures lower than 150 °C, carbohydrate solubilization was more important than protein solubilization in activated sludge. Several authors have found that lignocellulosic biomass solubilizes more at treatment temperatures higher than 175 °C (Horn et al., 2011a, 2011b; Vivekanand et al., 2013). Bauer et al. (2014) found only small increases in methane yields when treating late harvested straw at temperatures between 160 °C and 220 °C and treatment times of 15 min, while Lizasoain et al. (2016) tested treatment temperatures between 160 °C and 220 °C and treatment times of 5–20 min, and found that the methane yield of reed was highest for the treatment at 200 °C for 15 min, while only a small increase in methane yield was observed at 160 °C. Hence, a possible explanation of the lower solubilization and lower increase in methane yield of digestate from HRA compared to HRSD could be that the HRA digestate cake has more resemblance to lignocellulosic wastes (i.e. high fiber content) while the HRSD digestate cake may have more resemblance to primary sludge and WAS (i.e. higher protein content). The larger increase in solubilization observed for HRSD cake between temperatures 152 °C and 165 °C compared to the increase in solubilization of HRA cake, could also be explained by a lower protein content in the HRA cake (Bougrier et al., 2008).

3.1.2. Dewatering properties

The costs associated with the transport and disposal of the digestate cake are significant for many AD plants, and with a typical water content of around 80% in the cake, improved dewatering would be beneficial. We determined the dewatering properties as predicted maximum solids for the digestate cakes after post-treatment (Fig. 2c) according to the thermogravimetric method described by Kopp and Dichtl (2001). ANOVA showed that both temperature and cake origin had a significant effect on the maximum cake solids (p-value < .001 and < .01, respectively), where higher temperature resulted in better dewaterability. Above a temperature of 152 °C, the cake solids increased with increasing temperature and treatment time for both digestate cakes. Maximum predicted TS for the treated cakes were 43 and 46% for the HRSD and HRA cakes, respectively.

The dewatering properties of the original untreated cakes were very different: the HRA cake showed the ability to be dewatered to the predicted TS of 34.0% (data not shown), while the HRSD cake only achieved the predicted TS of 17.0% (data not shown) Thus, the post-treatment was clearly most efficient for the HRSD cake.

The observed difference in efficiency of the thermal hydrolysis could again be explained by the origin of the two digestate cakes. In sludge, water is bound inside viable cells (Vesilind, 1994) and between microbial cells in flocs (Higgins and Novak, 1997; Li and Yang, 2007). The amount of bound water is effected by high concentrations of phosphate, disturbing the cation-bridging of the sludge-flocs, as well as the concentration of extracellular polymeric substances (EPS). If the amount of intracellular and floc-bound water is less in food waste cakes (such as the HRA cake), the potential for improving the dewaterability of these types of digestate cakes will also be lower.

The results presented here demonstrate that post-treatment could increase the maximum cake solids in digestate cakes, and thereby cut the transportation costs of AD plants significantly. The predicted increase in maximum cake solids was from 34% up to 46% for the HRA digestate cake, and from 17% up to 43% for the HRSD digestate cake (Fig. 2c). Alone, the increase in maximum cake solids contributes to the reduction of final wet cake mass after post treatment by 26% of the original untreated cake for the HRA digestate cake and 60% of the original untreated cake for the HRSD digestate cake. Hence, the practical implication for plants similar to HRSD of implementation of PAD-THP technology will be that for every ten trucks that is needed for digestate cake transportation today, only four will be needed if thermal hydrolysis post-treatment is implemented. In addition, the relative reduction in wet cake mass will depend on the digestate cake treated. A digestate cake that is already dewatered to 30% before PAD-THP will have less potential for reduced wet cake mass, compared to a digestate cake that only dewaters to 15%. It will therefore be of paramount importance to take several considerations into account when making decisions on post-treatment.

3.1.3. Residual methane potential in cake

The residual methane potential of the cake fraction is of interest when evaluating to what extent the remaining methane potential in the digestate cake has been exploited by PAD-THP. Because of the small volume of post-treated digestate available, and the limitations of the laboratory equipment, it was not possible to produce a cake with equal properties as full-scale cake. The separation in the laboratory centrifuge resulted in a pellet with higher water content compared to the predictions of water content after full scale dewatering presented in section 3.1.2. To overcome this challenge, BMP was measured on the pellet and the results were then corrected by subtracting the BMP coming from the centrate which would be removed during dewatering in a full scale plant. The BMP based on these calculations are presented in Fig. 2d.

ANOVA showed no significant difference in BMP of the two cakes at different THP treatment times or temperatures. BMP of the untreated HRA digestate cake and the HRSD digestate cake treated for 60 min at 70 °C and centrifuged was found to be 75 and 63 mL CH_4/g TS_{added}, respectively (data not shown). The BMP of the treated HRA cakes was in all cases lower compared to the untreated cake, and ranged from 41 to 53 mL/g TS_{added}. The BMP of the treated HRSD cakes was in all cases lower than the cake treated at 70 °C for 60 min, and ranged from 38 to 59 mL/g TS_{added}. The BMP of the centrates was observed to be much higher (Fig. 2b) compared to the BMP of the cake presented here, and improved separation of the liquid fraction will lower the methane yield from the cake. Although there still is some methane potential left in the digestate cakes, it is not evident that this will be emitted as methane during storage or after land application. BMP-tests are designed to give the maximum methane yield from a test material, and will therefore give a worst-case scenario; other parameter such as oxygen and moisture access will influence the methane production from the cake during storage and after land application.

3.2. CSTR experiment

Based on the batch testing, the treatment of digestate cake for 30 min at 165–175 °C gave the best results in regard to solubilization, specific methane yields of the centrate, and dewaterability. In a full scale system, the centrate after post-treatment will be recirculated to a continuous digester. Hence, results from batch experiments do not give the full picture of the impact of solid stream on an AD system. In order to evaluate the impact of the recirculation on continuous processes, two semi-continuous CSTRs were run in parallel, co-digesting food waste and sewage sludge, with a low SRT and high OLR (Table 2). The control digester received hygenized feed which was a mix of sewage sludge and food waste, to meet the health regulations (heat treated at 70 °C for 60 min), while the PAD-

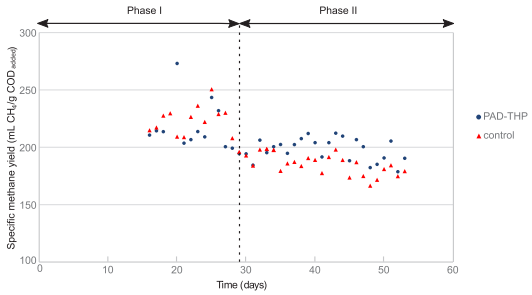

Fig. 3. Volumetric methane yield during the time of the experiment. The first 28 days (Phase I), the PAD-THP reactor did not receive any centrate.

THP digester received unhygenized feed and centrate from the PAD-THP (30 min at 165 °C) HRSD-cake.

During the first phase of the experiment (the first two SRTs), the PAD-THP digester was operated without adding centrate. In this period, the control digester had a higher volumetric methane yield compared to the PAD-THP digester (Fig. 3). From day 28, the second phase of the experiment, centrate was added with the feed to the PAD-THP digester every day. During the second phase, both reactors performed well, achieving COD-reduction of around 70% with low residual VFA-concentrations in the effluent and a close to neutral pH (Table 4). The PAD-THP digester performed better than the control in regard to volumetric methane yield and COD-reduction, with an average increase in volumetric methane yield of 7%. It also had marginally higher concentration of acetic-, propionic- and total volatile fatty acids as well as higher TAN, PO_4^--P and S-COD in the effluent. After the PAD-THP centrate was added, the volumetric yield of the PAD-THP digester was higher compared to the control digester for all days. Both the daily volumetric methane yield and specific methane yield was significantly higher in the PAD-THP digester (p-value < .001 and < .01, respectively) compared to the control digester.

The relative moderate increase in volumetric methane yield (7%) compared to the observed 25% (Kjorlaug et al., 2015) and 50% (Kolovos et al., 2016) increase in volumetric biogas yield in the full scale plant in Germany could be a result of a higher methane yield in the control digester in this study due to the pretreatment of the feed substrates. The hygenization of the feed for the control digester resulted in a different feed sludge composition for the two digesters. Based on the paired *t*-test, three of the measured parameters were significantly different for untreated and hygenized sludge; S-COD (p-value < .001), TAN (p-value < .01) and pH (p-value < .05), while none of the parameters were significantly different for untreated and hygenized food waste. This difference indicates that the pre-treatment solubilized some of the feed sludge and degraded some of the proteins in the sludge (Table 2).

The feed for the PAD-THP digester included centrate. In order to get enough and consistent centrate, it was decided to only use centrate from post-treatment of HRSD cake and not from the laboratory digesters. The COD content of the centrate was to a large degree soluble, with a ratio of S-COD:COD of 92%, had a strength of 39 g COD/L and the TS was 88% organic (Table 2). In comparison, analyses of samples from the full scale Cambi plant in Germany showed a centrate strength of 40 g COD/L and a VS of 85–90% (Kjorlaug et al., 2015). A high conversion rate of food waste to methane could explain why a smaller difference in methane yields was observed in this study compared to the results from the full scale plant (Kjorlaug et al., 2015; Kolovos et al., 2016). The full scale plant does not receive food waste, and the higher the conversion rate in the control digester, the less will the potential of improvement of methane yields be.

In addition to feed composition, the SRT of the two digesters were different. The SRT was set to be 17 days for the control digester, resulting in a SRT of 14 days for the PAD-THP digester. Both SRTs are low compared to the SRTs of conventional full-scale plants, which commonly operates with average SRTs of 20–25 days in the US. We chose a low SRT in order to evaluate the suitability of PAD-THP for digesters that is already operating close to their limit. As many sewage plants implement co-digestion of sludge and food waste, SRTs are also lowered, as long as the digester volume remains unchanged. However, the low SRT in this study could have contributed to the smaller difference between methane yields of the control digester and the PAD-THP digester, compared to what has been observed in the full-scale plant in Germany (Kjorlaug et al., 2015; Kolovos et al., 2016).

Differences in laboratory scale and full-scale configuration may also have influenced the results presented in this section. In a full scale PAD-THP system the solids content of the influent can be higher than in a pre-hygienization system and because of this the SRT can be higher. The centrate will be continuously produced from the effluent and will respond to changes in solids reduction and effluent characteristics. Another difference in full-scale digesters is that they receive feed continuously contrary to the laboratory reactors that was fed once per day. The effects of feeding frequency on anaerobic digesters is not well documented. We think that by using the adaptations described in section 2.4, the results presented in section 3.2 is a conservative estimate of what can be achieved in full-scale.

3.3. Major differences between pre-treatment and post-treatment

Researchers have shown that pre-pasteurization processes can

Table 4
Results from CSTR experiment. Standard deviations are listed in parenthesis.

	Unit	Control Digester	PAD-THP Digester
COD reduction	%	68 (2)	74 (1)
VS reduction	%	63 (1)	72 (1)
TAN[a]	mg/L	2110 (253)	2161 (466)
PO_4-P	mg/L	499 (38)	542 (64)
Acetic acid	mg/L	73 (14)	84 (19)
Propionic acid	mg/L	16 (7)	23 (9)
tVFAs	mg/L	98 (15)	120 (31)
S-COD	g/L	5 (1.6)	8 (0.9)
pH		7.3 (0.1)	7.3 (0.1)
Specific methane yield on COD basis	mL/g COD_{added}	186 (9.9)	197 (9.4)
Specific methane yield on VS basis	mL/g VS_{added}	365 (17)	415 (20)
Volumetric methane yield	L/L*d	1.32 (0.060)	1.41 (0.067)

[a] TAN = total ammonia nitrogen.

experience reactivation and regrowth of indicator organisms such as fecal coliforms and *E. coli* (Chen et al., 2011; Higgins et al., 2007). This is thought to be due to inadequate time-temperature treatment associated with pre-pasteurization which does not completely inactivate the organisms. With PAD-THP, all digestate cake is treated at 165 °C for 30 min, which effectively inactivates pathogens, minimizing the risk of reactivation and regrowth. Pre-THP would also achieve the same effect and minimize the risk of reactivation and regrowth.

Post-digestion treatment will improve the overall energy balance. The amount of solids that are heat treated is less with PAD-THP compared to pre-AD hygienization or pre-THP because of the solids reduction that occurs during digestion. For example, in comparing pre-THP to PAD-THP, the heat treatment is applied at the same solids concentration, around 16%, but with PAD-THP, the total solids to be treated could be reduced by 50% or more due to biodegradation that occurs in the digester. Increased solids reduction and conversion to biogas influences the energy balance both directly through methane production and indirectly through lowering the heat loss of the PAD-THP system. With PAD-THP most of the energy used for sterilization is returned to the digester for heating by returning the centrate.

Another advantage of PAD-THP compared to pre-THP and pre-hygenization is the improvement in cake solids during final dewatering which can have a large beneficial impact on economics. Pre-THP has been shown to improve final dewaterability after digestion, however, digestion reduces the extent of dewaterability compared to the solids immediately after pre-THP. Hasan et al. (2017) reported that the cake solids after dewatering of a mixed primary and secondary sludge that had undergone pre-THP was around 43%, but after subsequent anaerobic digestion the cake solids decreased to around 31%. In the PAD-THP scheme, the solids are thermally hydrolyzed and dewatered immediately, which improves cake solids. In addition, the solids are dewatered at higher temperatures immediately following PAD-THP which further improves cake solids.

4. Conclusion

This study obtained novel insights into the differences in the effect of PAD-THP on digestate cakes from a food waste plant and a wastewater treatment plant.

Post-treatment improved methane yields both in batch and in a semi-continuous system and improved the extent of dewaterability of the digestate cakes. The effect of the post-treatment was influenced by digestate cake characteristics, and the treatment was more efficient for the cake from a plant treating sewage sludge compared to the cake from a plant treating source separated food waste. Improved VS reduction in the anaerobic digester and improved dewatering of the treated digestate cake assured that the final cake product was stable, with low residual methane potential. Our estimates suggests a reduction in final wet cake mass due to improved dewatering from PAD-THP of 60% of the original wet cake mass for digestate cake from an anaerobic digester treating sewage sludge from a Bio-P plant.

Our results indicate that thermal hydrolysis of digestate cake is an efficient technology for improving methane production and dewaterability in conventional anaerobic digesters, and performs better compared to the conventional pre-treatment technology at 70 °C. Practically, the technology will improve methane yields and the extent of digestate dewaterability, increase the AD plants income through higher methane production and reduce the AD plants transportation and disposal costs through reduction of final wet cake mass.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.01.008.

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PAPER II

Post anaerobic digestion thermal hydrolysis increases the concentration of dry solids in dewatered cake

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Abstract

Organic waste fractions such as sewage sludge, food waste and manure can be stabilized by anaerobic digestion (AD) to reduce the density of pathogenic bacteria and produce renewable energy in the form of biogas. Following AD the digested solid fraction (digestate) is usually dewatered to reduce the volume before transportation. Post-AD treatments such as the post-AD thermal hydrolysis process (Post-AD THP) have been developed to improve the dewatering, but the mode of action is not well understood. In this study, low field nuclear magnetic resonance and thermogravimetric analysis (TGA) were used to study the moisture distribution before and after Post-AD THP. Samples from 32 commercial full-scale plants were used to assess the impact of Post-AD THP on a broad range of raw materials. Maximum dry solids concentration of dewatered cake after Post-AD THP was predicted by TGA. Centrifugation and water diffusion rates were used to study dewatering mechanisms at 80 °C compared to 20-35 °C. Finally, a full-scale Post-AD THP centrifugal trial was conducted and compared to predictions by TGA. Post-AD THP changed the moisture distribution of the samples by increasing the free water fraction. A consistent improvement in predicted dry solids concentration of dewatered cake was achieved across the 32 samples tested, on average increasing the dry solids concentration by 87 %. Dewatering Post-AD THP digestates at 80 °C improved dry solids concentration of dewatered cake above the predictions by TGA at 35 °C.

Keywords: Anaerobic digestion, biogas, dewatering, moisture distribution, thermal hydrolysis

1 Introduction

Anaerobic digestion (AD) is a common method to reduce and stabilize sewage sludge and organic waste (Aguilar et al., 2017). The process results in two end-products: biogas and digestate. The biogas can be directly used at the plant as an energy source in combined heat and power (CHP) engines. At some plants the biogas is upgraded to vehicle fuel or injected to the gas grid (Mills et al., 2014). The digestate is usually dewatered to separate the solids from water, producing a cake and a liquid fraction. Minimizing the water content in the dewatered cake reduces the total cost of dewatering and cake handling that can represent 30-50 % of a plants operational budget (Mikkelsen and Keiding, 2002). Efficient dewatering will depend on sludge physicochemical properties, polymer type and dose for particle aggregation and type of dewatering device (Novak, 2006). However, pre-treatment methods altering the sludge structure are necessary to significantly improve dewaterability (Neyens and Baeyens, 2003). Recent full-scale and laboratory studies on the application of the post-AD thermal hydrolysis process (Post-AD THP) have shown encouraging results with large improvements in dewatering (Kolovos et al., 2016, Svensson et al., 2018).

Pre- or post-treatment processes such as heat treatment, chemical treatment, mechanical disintegration, freezing and thawing and biological hydrolysis with or without enzyme addition have been explored to intensify the digestion process and improve dewatering efficiency (Neyens and Baeyens, 2003). The THP is an established hydrothermal treatment process traditionally applied prior to the AD process (Pre-AD THP). Pre-AD THP typically operates at 165°C sterilizing the biomass and enabling increased digester loading rates through reduced sludge viscosity (Barber, 2016). In addition, improved biogas production and digestate dewaterability have been reported (Barber, 2016). However, the THP was originally developed as a dewatering aid directly in front of the dewatering device (Barber, 2016). More than 60 % reduction in dewatered cake for disposal has been reported for a full-scale Post-AD THP plant (Kolovos et al., 2016) and similar results were found in the laboratory for sewage sludge digestate (Svensson et al., 2018). However, Post-AD THP on fiber-rich source separated food waste (SSFW) digestate resulted in only 26% wet cake reduction (Svensson et al., 2018). To date, only one full-scale plant is using Post-AD THP.

To better understand the mechanisms influencing the dewaterability after Post-AD THP a wide range of raw materials need to be tested.

The positive influence on dewatering by lowering viscosity has been studied by several authors, and could explain the positive influence of THP on dewatering (Klinksieg et al., 2007, Miryahyaei et al., 2019). Poor dewaterability of biological sludge has been attributed to the strong water binding capacities of microbial extracellular polymeric substances (EPS) which could represent up to 80 % of the biomass (Christensen et al., 2015, Neyens et al., 2004). Application of sludge treatment such as thermal hydrolysis has been shown to degrade the EPS, hence reducing the water retention properties of sludge (Neyens et al., 2004). Therefore, the reduction of viscosity caused by THP could be associated with changes in the moisture distribution. Pre-AD THP increase the dewaterability of sludge (Barber, 2016). However, reported improvements in dry solids concentration of dewatered cake due to industrial application of Post-AD THP surpass the effect of Pre-AD THP (Gerstner, 2017), making it a promising technology for plants with high cake disposal costs. The effect of Post-AD THP on the moisture distribution has to our knowledge so far not been studied but may explain the superior dewatering performance of this technology.

Additionally, increasing the digestate temperature has been reported to have a positive effect on both sludge viscosity and dewatering (Klinksieg et al., 2007). However, the highest temperature studied by Klinksieg et al. (2007) was 55 °C while the Post-AD THP dewaters at 80 °C. Hence, more research is needed to understand the effect of temperature on dewatering.

To better understand the mechanisms and universality of the Post-AD THP technology a wide range of biomass samples were analyzed in this study. The aims were to: 1) study the effect of Post-AD THP on the free water and predicted dry solids concentration of dewatered cake and 2) study the effect of high temperature dewatering on the dry solids concentration of dewatered cake

2 Materials and methods

2.1 Samples

A wide range of digestates and dewatered digestates were collected from a total of 32 plants in Europe, Asia and Oceania to study the effect of Post-AD THP on predicted dry solids concentration of dewatered cake (Table 1). Dry solids concentration of dewatered digestate cake or predicted dry solids concentration of dewatered cake by thermogravimetric analysis from Plants A-H, J and L-U have earlier been published by Svennevik et al. (2019).

All samples were shipped to the Norwegian University of Life Sciences in Norway and stored at 4 °C until analyzed.

TABLE 1

2.2 Thermal hydrolysis treatment

To assess the impact of Post-AD THP two THP pilots were used. Dewatered digestate from Plants A-H (Table 1) was treated in a THP pilot at Reading Sewage Treatment Works, UK, previously described by Shana (2015). Dewatered raw sludge or digestates from Plant I- AF were treated with the Cambi Thermal Hydrolysis pilot (Cambi Group AS, Norway) located at the Norwegian University of Life Sciences (Horn et al., 2011). Both units were operated similarly and digestates were hydrothermally treated by steam injection to 6 barg for 45 minutes followed by direct steam explosion. The samples were stored at 4 °C until analyzed.

2.3 Thermogravimetric analysis

Thermogravimetric analysis (TGA) was used to determine the free water content in accordance to Kopp and Dichtl (2001b) with minor modifications described by Svensson et al. (2018) and Svennevik et al. (2019). In brief; 100 mg samples were dried at 35 °C in a Netzch Simultaneous Thermogravimetry-Differential Thermal Analysis/ Differential scanning calorimetry (TG-DTA/DSC) Apparatus STA 449 F1 Jupiter® with a constant nitrogen flow of 20 mL/min. The drying curve was analyzed as described by Svennevik et al. (2019) to identify the transition point between free and interstitial water. Calibration was done with monodisperse silica particles of diameters 1.86 μm, 4.08 μm and 7.75 μm

(Cospheric LCC, USA). Predicted dewaterability measured five times on the same sample was 40.6 ± 0.7 % DS.

The maximum dry solids concentration of dewatered cake can be predicted based on the assumption that the free water measured by the TGA can be removed in full-scale centrifugation (Kopp and Dichtl, 2001b). The prediction method has shown good results when compared to full-scale dewatered cakes (Kopp and Dichtl, 2001b, Svennevik et al., 2019).

2.4 Low-field nuclear magnetic resonance

Low-field nuclear magnetic resonance (LFNMR) was used to determine bound water and water diffusion rates.

2.4.1 Bound water

Bound water in digestates and Post-AD THP digestates from Plants A-H were determined as described by Beck et al. (2018) and Svennevik et al. (2019). In brief: five mL of sample were analyzed by a Bruker mq20 minispec with a 0.47 T permanent magnet (Bruker, Billerica, MA, USA). The temperature was controlled at 22 °C with a BVT 3000 nitrogen temperature control unit (Bruker, Billerica, MA, USA). Spin-spin relaxation time (T_2 relaxation time) was measured by using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Carr and Purcell, 1954, Meiboom and Gill, 1958). The bound water was defined by the peak with the shortest relaxation time and calculated in relation to the total peak areas.

2.4.2 Water diffusion rate

Water diffusion rates (WDR) were measured on a 21 MHz (0.5 T) LFNMR spectrometer supplied by Anvendt Teknologi AS. WDR was used to investigate the effect of temperature (80 °C, 35 °C) on Post-AD THP digestates from Plants A, B, E-G and K. The instrumentation produces pulsed magnetic field gradients up to 400 G/cm for diffusion and one-dimensional image experiments. Three mL of sample were filled in LFNMR sample tubes of 18 mm diameter and adjusted to either 35 °C or 80 °C by heated air for 10 minutes. CPMG was used to measure T_2 relaxation time (Carr and Purcell, 1954, Meiboom and Gill, 1958) and a set of diffusion measurements at different observation times (Sørland, 2014). To

investigate the repeatability of the water diffusion rate measurement, one sample was split into three and measured in triplicate yielding an average water diffusion rate of 2.7 ± 0.1 e^{-9} m^2/s.

2.5 Moisture distribution

Three main water fractions were used in this study; free water, interstitial water and bound water (Kopp and Dichtl, 2001b). The moisture distribution in digestates and Post-AD THP digestates from Plant A-H were determined as described by Svennevik et al. (2019). In brief: Free water was determined by TGA as described in section 2.3 and bound water by LFNMR as described in section 2.4.1. Interstitial water was quantified by subtracting the amount of free water from bound water. All samples were normalized to 3 % DS by mathematically adjusting the free water content, to allow comparison between different samples.

2.6 Centrifugation

2.6.1 Laboratory centrifugation

To study the effect of temperature (20 °C and 80 °C) on dewatering of Post-AD THP digestate a Beckman Model J2-MC Centrifuge with a JS-7.5 rotor was used for centrifugation. The samples were centrifuged at 1889 G for 30 minutes. Samples were stabilized at room temperature or heated in a heating cabinet 80 °C prior to centrifugation. After centrifugation the supernatant was decanted for soluble chemical oxygen demand (COD) analysis and the remaining pellets were analyzed for dry solids.

2.6.2 Full-scale centrifugation

The full-scale trial was done at Plant K with a mobile centrifuge from GEA Westfalia. Digestate at 80 °C was provided from the full-scale installation. The polymer Zetag 9118 (BASF, Germany) at a concentration of 0.2 % active substance (AS) was used. Polymer dose is reported as kg AS/ton DS.

2.7 Characterization Analysis

The dry solids (DS) concentration was measured gravimetrically by drying a sample at 105 °C to constant weight. Standard deviation represents three measurements on the same sample.

Soluble chemical oxygen demand (COD) was measured after filtration at 0.45µm with Merck Spectroquant® COD Cell Test. Standard deviation represents three measurements on the same sample.

ADF was analyzed according to manufacturer's recommendations using an Ankom^{200} Fiber Analyzer (ANKOM Technology, Macedon, New York, USA) with F58 filter bags.

2.8 Statistical analysis

Single factor analysis of variance (ANOVA) was performed in Microsoft Excel to assess if two data sets were significantly different at a significance level of 0.05.

3 Results and Discussion

3.1 The effect of Post-AD THP on free water and predicted dry solids concentration of dewatered cake

A wide range of digestates from 32 commercial full-scale plants with dry solids concentration of dewatered cake ranging from 19-35 % DS were used to test the universality of Post-AD THP. Plants C, D, F and G were sampled for Post-AD THP testing twice, one year apart. There was no significant difference in predicted concentration of dry solids in dewatered cake by TGA for the 2015 (data not shown) and 2016 (used in Figure 1) samples demonstrating good reproducibility of sampling procedure, treatment and analysis. Dry solids concentration of original dewatered cakes and predictions by TGA for Plants A-H, J and L-U have earlier been published by Svennevik et al. (2019). Predicted dry solids concentration of dewatered cake by TGA of Post-AD THP digestates were compared to the dry solids concentration of original dewatered cake (Figure 1).

FIGURE 1

After Post-AD THP a significant increase ($p < 0.001$) in predicted dry solids concentration of dewatered cake was found compared to the dry solids concentration of the original

dewatered cake (Figure 1). Regardless of the original cake dryness or any use of pre-AD treatment the predicted dry solids concentration of dewatered cake were on average increased by 87 % by application of Post-AD THP at 165 °C for 45 minutes. The consistent increase indicates that both sludge type and thermal pre-treatment history impact the results of Post-AD THP.

Samples from Plant P, T and U contained source separated food waste (SSFW), showing an average improvement of 77 ± 31 %. This is substantially higher than the result from Svensson et al. (2018) where a 35 % improvement was found after Post-AD THP of SSFW digestate. This could be due to the fiber content in the digestates, as Plant P, T and U had a lower fiber content than the digestate tested by Svensson et al. (2018) (data not shown).

To better understand the reason for the observed change in free water (Figure 1), eight digestates (Plants A-H) were selected for moisture distribution analysis before and after Post-AD THP treatment. The digestates had a large span in primary sludge to waste activated sludge ratios, and different thermal pre-treatments or no pre-treatment. The moisture distribution was determined as described in section 2.5. A significant increase in free water ($p < 0.001$) and reduction in interstitial water ($p < 0.001$) were observed after Post-AD THP (Figure 2). However, no significant difference in bound water was measured (Figure 2).

FIGURE 2

The increase in free water was almost equal to the reduction in interstitial water, jointly accounting for 99% of the change in moisture distribution (Figure 2). Kopp and Dichtl (2001b) suggested that capillary forces between the sludge flocs bind the interstitial water to the sludge. We hypothesize that the change in interstitial and free water can be attributed to the reduction of these capillary forces restricting the interstitial water fraction from behaving like free water. This can potentially be due to the degradation of EPS as THP has been shown to break down the water holding capacity of EPS (Neyens et al., 2004). Since the free water has been linearly correlated to the dry solids concentration of dewatered cake in full-scale (Kopp and Dichtl, 2001b, Svennevik et al., 2019), the change in moisture

distribution is probably the main reason for the improved dewaterability after Post-AD THP reported by Kolovos et al. (2016) and Svensson et al. (2018).

In addition to differences in types of raw materials and any use of pre-treatment, the AD process is typically operated at either mesophilic (MAD) or thermophilic (TAD) temperatures. The influence of these two configurations on predicted dry solids concentration of dewatered cake after Post-AD THP was investigated using samples from Plant X, which ran these processes in parallel. The same predicted dry solids concentration in dewatered cake by TGA was found for both digestates (38 % DS, data not shown).

The results provide novel information about the effect of Post-AD THP on an extensive data set, showing the universality of the technology to improve the concentration of solids in the dewatered cake. In addition, the effect of high temperature dewatering could promote further water extraction.

3.2 High temperature dewatering

The effect of dewatering at 80 °C compared to 20 or 35 °C was investigated in the laboratory to see if more water could be removed.

3.2.1 Laboratory trials

10 Post-AD THP digestates were centrifuged in the laboratory at 20 °C and 80 °C to study the effect of increased temperature on water extraction (Figure 3).

FIGURE 3

The experiment demonstrated a significant average increase of 22 ± 3 % ($p < 0.01$) in water extraction from the pellet when increasing the digestate temperature from 20 °C to 80 °C. Using samples between 5 and 55 °C Klinksieg et al. (2007) developed a rheological model to predict dewaterability at elevated temperatures. Assuming their equation is valid up to 80 °C, an increase in cake solids of 23 % could be expected. Hence, our result (22%) agrees well with the model developed by Klinksieg et al. (2007). Several factors may explain this increase.

Increasing the temperature up to 70 °C has been linked to the destruction of vicinal (surface) water (Vesilind, 1994). If surface water decreases at 70 °C interstitial water that has a lower binding energy than surface water (Kopp and Dichtl, 2001a) can to some extent be converted to free water. Based on this, our hypothesis is that when dewatering at 80 °C the binding forces previously restricting interstitial and surface water from behaving like free water are weakened and the dry solids concentration of the pellet was therefore increased.

Moreover, the increase in free water may be linked to the structural integrity of lipids or collagenous compounds at different temperatures. Lipids in aqueous media have higher water diffusion coefficients and solubility at higher temperatures (Chipasa and Mędrzycka, 2006). Thus, when solubilized at 80 °C this could potentially release some of the interstitial water to the free water fraction. The soluble chemical oxygen demand (COD) of the supernatants obtained from centrifugation at 20 °C and 80 °C was measured, showing

significantly higher concentrations of soluble COD in the 80 °C supernatant ($p = 0.04$) (Figure 4) supporting this hypothesis.

FIGURE 4

In addition to the effect on structural integrity of lipids or collagenous compounds trapping water in the sludge matrix, water will also have a lower viscosity at 80 °C than 20 °C (Korson et al., 1969), which may also explain the positive influence of temperature on dewatering reported by Klinksieg et al. (2007).

LFNMR can provide information on the mobility of water at different temperatures by measuring the water diffusion rate (WDR) in the digestate. The effect of temperature on the water diffusion rate in six Post-AD THP digestates was therefore measured by LFNMR (Figure 5). The lowest stable temperature with the current set-up was obtained at 35 °C and the WDRs at this temperature were compared to WDRs at 80 °C (Figure 5).

FIGURE 5

The WDRs at 35 °C and 80 °C were significantly different ($p < 0.001$) with 98 ± 14 % higher WDRs at 80 °C compared to 35 °C. Korson et al. (1969) found that water had a 103 % higher viscosity at 35 °C compared to 80 °C. The digestates tested (Figure 5) consist of a large fraction of water (93 ± 3 %, data not shown), hence the reduced viscosity of this water could potentially improve the water release rate during dewatering. This can positively influence full-scale dewatering since the time in the centrifuge is limited, making the rate of water release important (Kopp and Dichtl, 2001b).

Full-scale centrifuge dewatering at 20-35 °C has been correlated to the fraction of free water measured by TGA at 35 °C (Kopp and Dichtl, 2001b). This study indicates that this can be further improved by dewatering at 80 °C. A full-scale trial was therefore initiated to investigate if higher dry solids concentration in dewatered cake could be achieved than predicted by TGA and if so, at what polymer dose.

3.2.2 Full-scale dewatering

The total cost of the full-scale dewatering process will depend on two factors: the polymer dose, as this product can be expensive and achieved concentration of dry solids in the dewatered cake, as this is directly related to the mass of cake for disposal. Full-scale Post-AD THP digestate (Plant K) with various amounts of polymer was used to study full-scale dewaterability at 80 °C (Figure 6).

FIGURE 6

The concentration of dry solids in dewatered cake was predicted by TGA at 35 °C to be 39 % DS, assuming optimal polymer conditioning before centrifuge dewatering. The prediction by TGA was achieved after centrifugation with a polymer dose of 12 kg active substance (AS)/ton DS when operating at 80 °C (Figure 6). This was the same polymer dose previously used with a Pre-AD THP configuration at the same plant.

The prediction of TGA would normally be the upper limit when dewatering at 35 °C with a centrifuge, and further polymer dosage would not increase the concentration of dry solids in the dewatered cake as reported by Kopp and Dichtl (2001b). However, in this study, increasing the polymer dose further increased the dry solids concentration of dewatered cake up to 47.5 % DS using 24 kg AS/tDS (Figure 6). This corresponds to a 21 % increase in cake dryness compared to TGA predictions, supporting the positive effect of higher temperatures on the dry solids concentration in dewatered cake.

3.3.3 Research implications

This study has shown that predicted dry solids concentration in dewatered cake after Post-AD THP from 32 commercial full-scale plants depended on the original dewaterability, but a consistent increase was found. The cost of dewatering and cake handling can have a large influence on the operational budget, hence the ability to predict the expected dry solids concentration of dewatered cake is important. Consequently, the improvement of 87 % across the broad sample range studied will provide important information to support cost/benefit analysis prior to investments in plant upgrades or planning of new plants.

Dewatering at 80 °C showed further potential for improvement in dry solids concentration in dewatered cake when increasing the polymer dose compared to the predictions by TGA for conventional dewatering at 35 °C. The proposed mechanisms are the solubilization of lipids and proteins and the increased diffusion rates of water. The practical implication of this is a balance between the increased cost of polymer at higher dose compared to the reduced cost of transport and processing of the dewatered Post-AD THP digestate. These are important factors to include in the operational budget, and the cost of polymer must be balanced with the cost of cake handling.

4 Conclusions

In this study, novel insights across a broad range of raw materials were found on the impact of Post-AD THP on dewatering. The following conclusion were made:

- Post-AD THP changed the moisture distribution of digestates by releasing the interstitial water into the free water fraction.
- Post-AD THP gave a consistent improvement in the dry solids concentration in dewatered cakes predicted by TGA, with an average increase of 87 %.
- Dewatering at 80 °C increased the amount of water extractable by a centrifuge above the predictions by TGA at 35 °C.

Acknowledgement

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Figure captions

Figure 1: Effect of Post-AD THP on predicted dry solids concentration of dewatered cake compared to dry solids concentration of original dewatered cake. Hollow markers represent digestates from source separated food waste.

Figure 2: Average change in moisture distribution due to Post-AD THP for Plants A-H. Data in Supplementary Material A.

Figure 3: Effect of temperature on the dry solids concentration in centrifuged pellet.

Figure 4: Soluble COD of supernatant from centrifugation at 20 °C and 80 °C

Figure 5: Water diffusion rates of post-AD digestates for Plant A, B, E, F, G and K at 35 °C and 80 °C. Test of standard deviation of selected sample showed very low standard deviation (less than 0.1 10^{-9} m^2/s)

Figure 6: Full-scale dewatering of Post-AD THP digestate at 80 °C. Dewatered cake solids (% DS) as function of polymer dose (kg active substance (AS)/ ton DS).

Table 1 Technical details of full-scale plants sampled in this study, with or without thermal treatment. Digestates from either mesophilic digestion (MAD), thermophilic digestion (TAD) or not digested (No AD). SSFW = source separated food waste.

Plant ID	Thermal treatment	Digestion process and raw material	Continent
Plant A	Pre-AD THP	MAD, sewage sludge	Europe
Plant B	Pre-AD THP	MAD, sewage sludge	Europe
Plant C	Pre-AD THP	MAD, sewage sludge	Europe
Plant D	Pre-AD THP	MAD, sewage sludge	Europe
Plant E	Pre-AD THP (WAS Only)	MAD, sewage sludge	Europe
Plant F	Pasteurization	MAD, sewage sludge	Europe
Plant G	None	MAD, sewage sludge	Europe
Plant H	None	MAD, sewage sludge	Europe
Plant I	Pre-AD THP	MAD, sewage sludge	Europe
Plant J	None	MAD, sewage sludge	Europe
Plant K	Post-AD THP	MAD, sewage sludge	Europe
Plant L	None	MAD, sewage sludge	Europe
Plant M	None	MAD, sewage sludge	Europe
Plant N	None	MAD, sewage sludge	Europe
Plant O	None	TAD, sewage sludge	Europe
Plant P	Pasteurization	MAD, SSFW and manure	Europe

Plant ID	Thermal treatment	Digestion process and raw material	Continent
Plant Q	Pasteurization	MAD, pulp and paper sludge and fish waste	Europe
Plant R	Pre-AD THP	MAD, sewage sludge	Europe
Plant S	Pre-AD THP	MAD, sewage sludge	Europe
Plant T	Pre-AD THP	MAD, SSFW	Europe
Plant U	Pre-AD THP	MAD, SSFW	Europe
Plant V	Pre-AD THP	MAD, sewage sludge, extended aeration	Europe
Plant W	None	MAD, sewage sludge	Oceania
Plant X	None	MAD and TAD sewage sludge	Europe
Plant Y	None	MAD, sewage sludge	Asia
Plant Z	None	MAD, sewage sludge	Europe
Plant AA	None	MAD, sewage sludge	Europe
Plant AB	None	MAD, sewage sludge	Europe
Plant AC	None	MAD, sewage sludge	Europe
Plant AD	None	MAD, sewage sludge	Europe
Plant AE	Pasteurization	TAD, sewage sludge and food waste	Europe
Plant AF	None	MAD, sludge, wine industry	Europe

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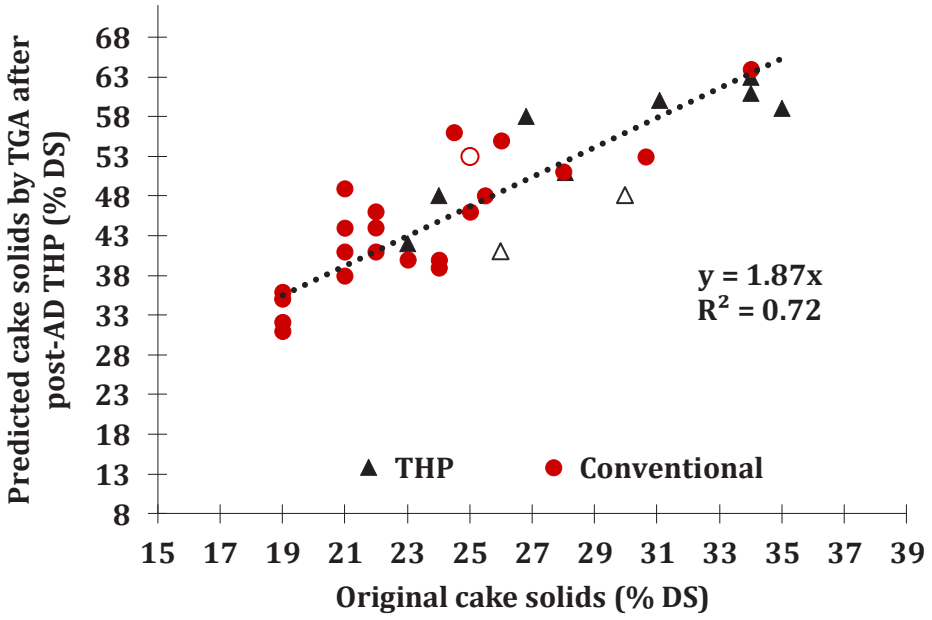

447

448 **Figure 1: Effect of Post-AD THP on predicted dry solids concentration of dewatered cake compared to**
449 **dry solids concentration of original dewatered cake. Hollow markers represent digestates from**
450 **source separated food waste.**

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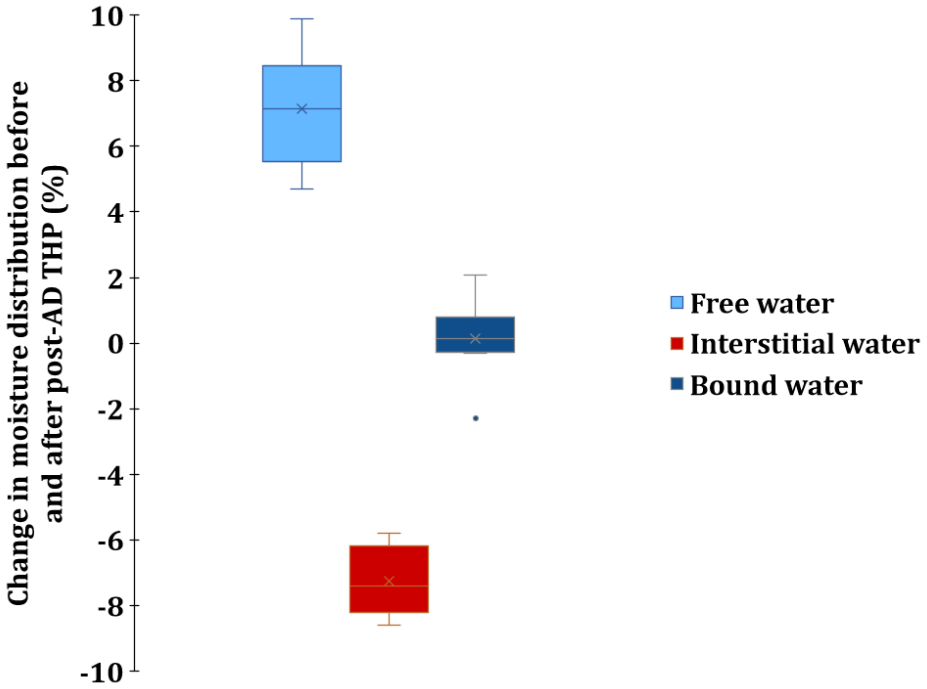

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453 **Figure 2: Average change in moisture distribution due to Post-AD THP for Plants A-H. Data in**
454 **Supplementary Material A.**

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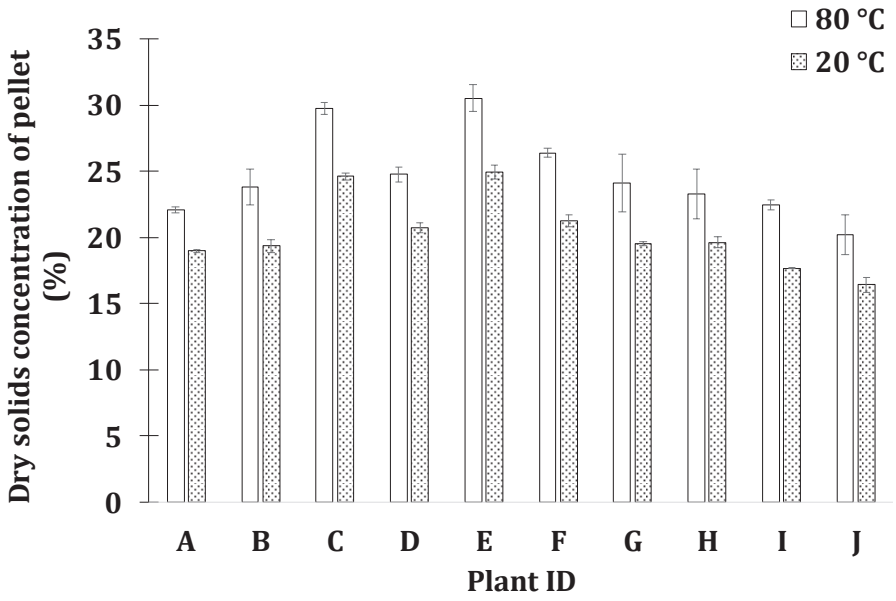

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458 **Figure 3: Effect of temperature on the dry solids concentration in centrifuged pellet.**

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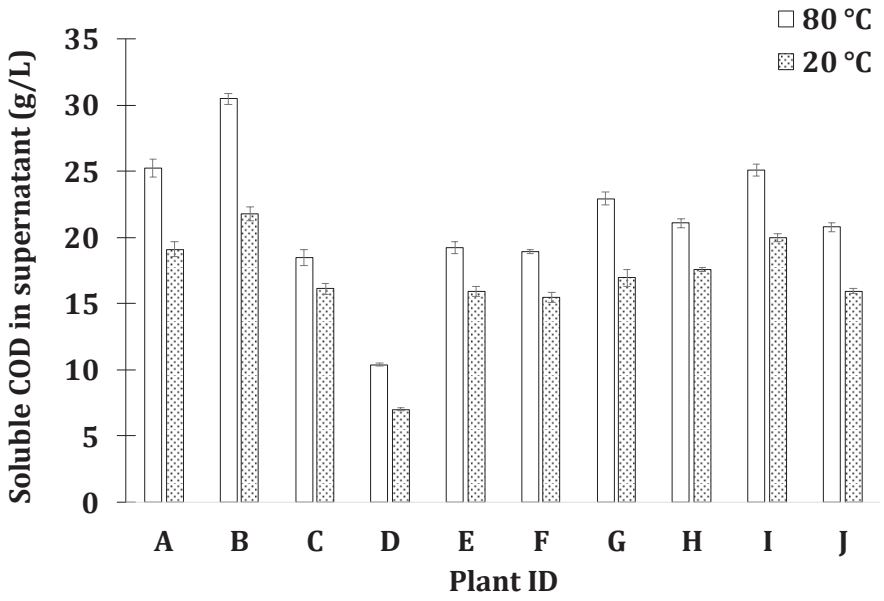

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462 **Figure 4: Soluble COD of supernatant from centrifugation at 20 °C and 80 °C**

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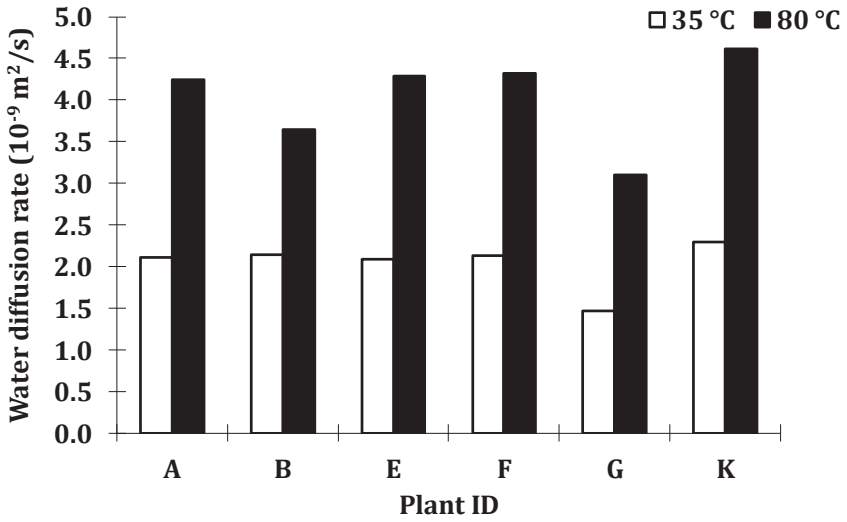

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466 **Figure 5: Water diffusion rates of post-AD digestates for Plant A, B, E, F, G and K at 35 °C and 80 °C. Test**
467 **of standard deviation of selected sample showed very low standard deviation (less than 0.1 10^{-9} m^2/s)**

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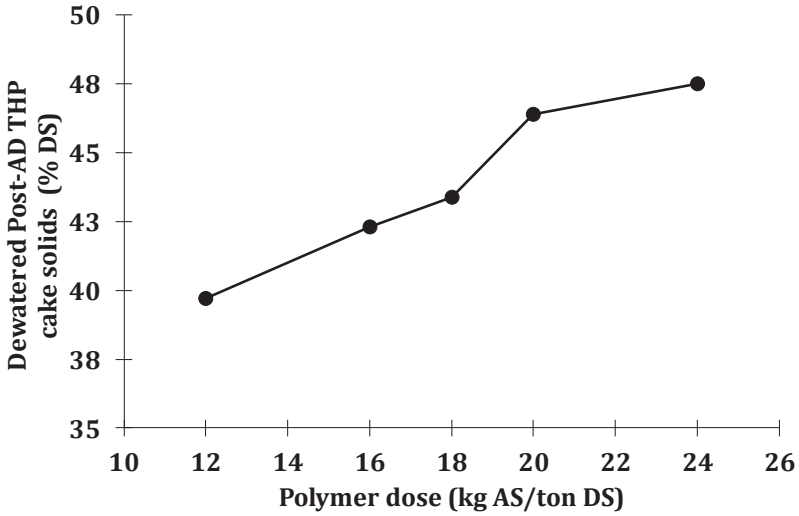

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471 **Figure 6: Full-scale dewatering of Post-AD THP digestate at 80 °C. Dewatered cake solids (% DS) as**
472 **function of polymer dose (kg active substance (AS)/ ton DS).**

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Post anaerobic digestion thermal hydrolysis increases the concentration of dry solids in dewatered cake

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Supplementary Material

Table S1 Moisture distribution in digestates and Post-AD THP digestate from Plants A-E (Pre-AD THP) and Plants F-H (None-THP)

Plant ID	Free water (%*)		Interstitial water (%*)		Bound water (%*)	
	Digestate	Post-AD THP	Digestate	post-AD THP	Digestate	post-AD THP
A	89.7	95.9	9.8	3.8	0.5	0.3
B	89.8	96.8	10.0	2.7	0.2	0.5
C	93.1	97.8	6.8	0.1	0.02	2.1
D	92.0	97.3	6.3	0.5	1.7	2.2
E	89.2	97.5	9.2	1.2	1.6	1.3
F	89.5	96.8	9.9	1.6	0.7	1.6
G	88.7	97.2	11.1	2.5	0.2	0.2
H	84.5	94.4	12.0	4.5	3.4	1.1
Average all plants	89.6 ± 2.5	96.7 ± 1.1	9.4 ± 2.0	2.1 ± 1.5	1.0 ± 1.2	1.2 ± 0.8

* % of total water

PAPER III

CNAsh - a novel parameter predicting cake solids of dewatered digestates

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Abstract

Efficient digestate dewatering is crucial to reduce the volume and transportation cost of solid residues from anaerobic digestion (AD) plants. Large variations in dewatered cake solids have been reported and predictive models are therefore important in design and operation of such plants. However, current predictive models lack validation across several digestion substrates, pre-treatments and full-scale plants. In this study, we showed that thermogravimetric analysis is a reliable prediction model for dewatered cake solids using digestates from 15 commercial full-scale plants. The tested digestates originated from different substrates, with and without the pre-AD thermal hydrolysis process (THP). Moreover, a novel combined physicochemical parameter (C/N•ash) characterizing different digestate blends was identified by multiplying the C/N ratio with ash content of the dried solids. Using samples from 22 full-scale wastewater, food waste and co-waste plants, a linear relationship was found between C/N•ash and predicted cake solids for digestates with and without pre-AD THP. Pre-AD THP improved predicted cake solids by increasing the amount of free water. However, solids characteristics like C/N ratio and ash content had a more profound influence on the predicted cake solids than pre-AD THP and type of dewatering device. Finally, C/N•ash was shown to have a linear relationship to cake solids and reported polymer dose from eight full-scale pre-AD THP plants. In conclusion, the novel parameter C/N•ash can be used to predict dewatered cake solids regardless of dewatering device and sludge origin.

Keywords: Anaerobic digestion, ash, biogas, C/N, dewatering, thermal hydrolysis

1 Introduction

As the world's population is growing the demand for dedicated sewage and organic waste treatment increases. Wastewater treatment generates two main streams of organic residues: primary sludge (PS) from initial sewage sedimentation and waste activated sludge (WAS) from aerobic biological treatment of the sewage liquid phase. PS and WAS can be used as substrate for anaerobic digestion (AD), a biological process converting organic matter to renewable energy in the form of biogas. Organic waste from households or industry can be treated by co-digestion with PS and WAS or digested separately. Regardless of substrate, the digested residue (digestate) typically contains 95-98% water. To reduce transportation costs, efficient separation of water from solids (dewatering) is crucial. Thus, polyelectrolytes (polymers) are added to the digestate to bind particles into larger aggregates resulting in increased water release rate (Kopp and Dichtl, 2001b). However, large variations in dewatered cake solids are reported in literature despite similar AD configurations (Barber, 2016) implying that digestate physicochemical properties could be important in explaining these variations. Digestate dewatering and disposal can represent 30-50 % of a full-scale plants' annual operating cost (Mikkelsen and Keiding, 2002). Consequently, predicting the expected cake solids is important for the design and optimization of full-scale AD plants.

Predictive models on dewatering have been developed or investigated by several authors (Nellenschulte and Kayser, 1997, Kopp and Dichtl, 2001b, Klinksieg et al., 2007, Skinner et al., 2015, To et al., 2018). Small particles have been negatively correlated to dewatered cake solids (Nellenschulte and Kayser, 1997). However, this does not include the effect of pre-treatments such as the thermal hydrolysis process (THP) that reduces particle size and still improves dewaterability (Neyens et al., 2004). Several methods of quantifying dewaterability have been

studied including the use of filtration models (Skinner et al., 2015), replication of the full-scale process in the laboratory (To et al., 2018), rheological analysis (Klinksieg et al., 2007) and thermogravimetric analysis (TGA) (Kopp and Dichtl, 2001b). While most studies correlate their predictions to a small number of full-scale results, Kopp and Dichtl (2001b) found a linear relationship between their predicted cake solids and results from 33 full-scale plants digesting sewage sludge. The model assumes that polymer product and dosage in addition to selected dewatering device will be optimized by respective vendors in full-scale to reach the predicted maximum cake solids by TGA. However, it has not been validated for pre-treated digestates or other substrates than sewage sludge. Summarized, the literature on predictive models lacks comparison to a wide range of full-scale data or does not include the effect of different substrates and pre-treatments. All models are limited in the way that no single physicochemical parameter has been found linking dewaterability to a range of digestion substrates like PS, WAS or other organic wastes.

WAS containing up to 80 % extracellular polymeric substances (EPS) has strong water holding capacities (Neyens et al., 2004, Skinner et al., 2015, Christensen et al., 2015). WAS has been described as a viscous gel-like material linked by hydrogen bonds and electrostatic forces (Markis et al., 2014). In contrast, PS behaves like a colloidal suspension where particles are linked by the much weaker van der Waals forces (Markis et al., 2014). Thus, WAS has higher viscosity (Hong et al., 2018) and less free water than PS, leading to poor dewaterability (Kopp and Dichtl, 2001a, Neyens et al., 2004, Christensen et al., 2015). The PS to WAS ratio is thus an important factor in digestate dewaterability. Despite the negative effect of WAS and EPS on dewaterability, no standard method for measuring EPS has been developed (Christensen et al., 2015). Surrogates such as the volatile solids (VS) concentration have been suggested (Skinner

et al., 2015), but are not valid when digesting co-wastes such as food waste (Higgins and Rajagopalan, 2017). Additionally, the VS content in PS and WAS is similar (Suarez-Iglesias et al., 2017) and can therefore not explain the differences between these two substrates. However, a decreasing trend in dewaterability of digestates when the VS content increased was observed by Kopp and Dichtl (2001b) and they suggested this could be due to different behavior of organic and inorganic particles. Nicholson et al. (2018) investigated the effect of the carbon to nitrogen (C/N) ratio on dewaterability and found that an increase in C/N ratio coincided with increased dewaterability. However, both Kopp and Dichtl (2001b) and Nicholson et al. (2018) suggested that used alone it could not accurately predict dewaterability. Identifying a parameter that can describe these differences will help predict dewatered cake solids for conventional AD plants. However, the digestion substrate mix is often fixed, and to improve dewaterability pre-treatment such as the THP is needed (Neyens and Baeyens, 2003).

The THP as AD pre-treatment (pre-AD THP) improves dewaterability, but dewatering efficiencies depend on digestate characteristics and dewatering device (Barber, 2016). Improved dewaterability has been linked to reduced viscosity (Higgins et al., 2017). This could be due to the solubilization of EPS, weakening the water holding capacities of the flocs in WAS (Neyens et al., 2004). However, the mechanisms explaining the effect of pre-AD THP on dewatering and linking it to digestion substrates are not well documented.

In conclusion, a universal physicochemical parameter is needed to describe digestate dewaterability and the effect of pre-AD THP. In this study, we have developed a standardized and universal predictive model for dewatered cake solids by using a diverse sample set from full-scale plants. Based on digestates from a range of wastewater, food-waste and co-waste plants, the objectives of this study were 1) to validate TGA as method to predict digestate cake

solids after full-scale dewatering for several digestion substrates with and without pre-AD THP, 2) to identify a universal digestate physicochemical parameter that can be used to predict cake solids, and 3) to investigate the effect of pre-AD THP on predicted cake solids

2 Materials and methods

2.1 Samples

Digestates were collected from a total of 22 plants in Europe and USA, with and without pre-AD THP, to study the effect of physicochemical properties and pre-AD THP on dewatered cake solids (Table 1). Additionally, full-scale dewatered digestates were collected at the outlet of the dewatering device at 15 plants to compare cake solids predictions by TGA to full-scale dewatering results (Plants A-O). Plants P-V did not run a dewatering process or used additives complicating direct comparison to the other dewatered digestates. Thus, comparison of TGA predictions and full-scale results for these plants were not valid or possible.

Digestates collected from Plants A-H in the United Kingdom (UK) were studied in most detail to identify physicochemical parameters affecting predicted cake solids. These plants used mesophilic anaerobic digestion (MAD) of sewage sludge with various pre-treatment methods, allowing comparison of conventional and pre-AD THP digestates. Two configurations of THP before AD (pre-AD THP) were sampled: THP treating both PS and WAS and only the WAS. In these plants, all THPs were operated at 165 °C for 30 minutes while pasteurization involved pre-treatment of PS and WAS at 70 °C for one hour.

Data from Plant I and J has earlier been published by our research group (Svensson et al., 2018).

All samples were shipped to the Norwegian University of Life Sciences in Norway and stored in dark, airtight containers at 4 °C until analyzed.

TABLE 1

2.2 Thermogravimetric analysis

Thermogravimetric analysis (TGA) was used to determine the free water and predict dewatered cake solids in accordance to Kopp and Dichtl (2001b) with minor modifications described by Svensson et al. (2018). In brief; 100 mg samples were dried at 35 °C in a Netzsch Simultaneous TG-DTA/DSC Apparatus STA 449 F1 Jupiter® with a constant nitrogen flow of 20 mL/min. The drying curve was analyzed and the linear region prior to the change in drying rate due to the transition between free and interstitial water was identified. Linear regression analysis of this region identified the line defining the free water evaporation immediately before the transition. The deviation between the drying curve and free water defining line was calculated to identify the point between free and interstitial water. The drying curves are enclosed in Supplementary Material A. Calibration was done with mono-disperse silica particles of diameters 1.86 µm, 4.08 µm and 7.75 µm (Cospheric LCC, USA). Repeatability was investigated using five replicates on the same sludge sample. Predicted cake solids was found to be 40.6 ± 0.7 %DS.

2.3 Low-field nuclear magnetic resonance

Low-field nuclear magnetic resonance (LFNMR) was used to determine bound water in digestates from Plants A-H. LFNMR allowed a non-invasive measurement of the bound water in the digestate and, as opposed to dilatometric measurements, does not require any sample alteration such as freezing.

A Bruker mq20 minispec with a 0.47 T permanent magnet (Bruker, Billerica, MA, USA) was used to perform the LFNMR measurements previously described by Beck et al. (2018) to define bound water for Plants A-H. In brief; five mL of each sample was pipetted into a pre-weighed glass LFNMR tube and the total weight was recorded. The probe region of the LFNMR was stabilized at room temperature (22 °C) using a BVT 3000 nitrogen temperature control unit (Bruker, Billerica, MA, USA). Two minutes were allowed for the sample to equilibrate in the instrument before data acquisition. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to measure the spin-spin relaxation time (T_2 relaxation time) of the samples with 32,000 echoes, gain 66 dB, 8 scans and a recycle delay of 5 seconds. The pulse separation (τ) for the measurements was optimized for the different sets of samples to allow full T_2 relaxation while minimizing τ. The CPMG decay curves were analyzed by continuous non-negative least squares (NNLS) fitting (Lawson and Hanson, 1974, Whittall et al., 1991) using PROSPA 3.2 (Magritek, Aachen, Germany). The NNLS fitting in PROSPA provides a continuous distribution of T_2 values. 512 data points were determined for each fit and a smoothing parameter of 0.5 was selected. For each peak, both T_2 values corresponding to maximum peak intensity and peak area were determined.

2.4 **Moisture distribution**

In this study the classification from Kopp and Dichtl (2001a) and Vesilind (1994) with three main water fractions was used; free water not bound by particles, interstitial water bound by capillary forces, surface water bound by adhesive forces and intracellular water including the water of hydration. The sum of surface and intracellular water was termed bound water. The amount of free, interstitial and bound water was determined by combining data from LFNMR and TGA analyses.

The free water was determined by TGA as described in section 2.2, and total water was determined by drying a sample at 105 °C until constant weight. Bound water was determined by LFNMR, where the area of the peak with shortest relaxation time was calculated in relation to the total area of all peaks. Interstitial water was calculated by subtracting the free and bound water from the total amount of water. Different pre-treatments and operational strategies of the AD process in Plants A-H lead to different digestate dry solids (DS) concentrations. To calculate and compare the moisture distribution between digestates all results were normalized theoretically to 3 % DS to evaluate the percentage of free, interstitial and bound water. Normalization also allowed the comparison to previous results on moisture distribution applying TGA and dilatometric measurements (Kopp and Dichtl, 2001a).

2.5 **Composition Analysis**

Digestates were analyzed for DS, VS, ash, carbon, nitrogen, iron and aluminum (Plants A-H) and acid detergent fiber (ADF) (Plants I, P and V) in the search for a physiochemical factor reflecting the digestion substrate that could predict cake solids after dewatering.

The DS, VS and ash concentration were measured gravimetrically in triplicates by drying a sample at 105 °C to constant weight followed by combustion at 550 °C.

Carbon and nitrogen were measured by combusting a dried sample (105 °C overnight) at 1150 °C with a constant flow of oxygen gas in a Vario El Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

PS/WAS ratios on dry solids basis were communicated by plant owner.

Iron and aluminum were analyzed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma – Optical emission Spectroscopy (ICP-OS). Samples were acidified and digested at 85 °C overnight before being analyzed by ICP-MS or ICP-OS.

ADF was analyzed according to manufacturer's recommendations using an Ankom^{200} Fiber Analyzer (ANKOM Technology, Macedon, New York, USA) with F58 filter bags.

2.6 **Statistical analysis**

Principal Components Analysis (PCA) was performed on physicochemical parameters from Plants A-H (Supplementary Material B, Table SB1) using the software Past (Hammer et al., 2001). The correlation matrix was used since the variables were measured in different units.

3 Results and Discussion

3.1 Predicted cake solids by TGA compared to full-scale results

Full-scale digestates and dewatered digestates originating from several different substrates, with and without pre-AD THP were used to validate the TGA as a good method for prediction of cake solids.

The cake solids from 15 full-scale dewatered digestates were successfully predicted by TGA and ranged from 17-34 % DS (R^2 = 0.90, Figure 1A), which is in line with previous findings from centrifuge dewatering by Kopp and Dichtl (2001b) (R^2 = 0.92), despite three different dewatering devices being used in the full-scale plants (centrifuge, belt press and hydraulic filter press). Some authors challenge the validity of drying tests, such as TGA, because the results depend on several factors such as material, sample size and drying conditions (Vaxelaire and Cezac, 2004). However, when calibrating with mono-disperse silica and using the same drying conditions and sample size, these issues were overcome for our samples. The samples included digestates from digesters treating pure food-waste, sewage sludge and a blend of sewage sludge and food-waste (Figure 1A). Additionally, eight plants had pre-AD THP installed. Hence, we conclude that TGA is a reliable method for predicting dewatered cake solids on digestates originating from different substrates and with pre-AD THP.

FIGURE 1

Because dewatering devices function differently, one explanation for the difference in cake solids is the use of different dewatering devices. To investigate the dewatering devices' impact on final cake solids, we therefore grouped centrifuges, belt presses and hydraulic filter presses to determine if dewatering device would result in different correlations to the free water

fraction measured by TGA (Figure 1B). Grouping the results and applying linear regression analysis revealed that 7 % higher cake solids were achieved with centrifuges compared to belt presses, although having similar free water content (Figure 1B). This observation corresponds well with literature where centrifuges were reported to achieve 2-7 % higher cake solids compared to belt presses (Novak, 2006). Samples from hydraulic filter presses were 9 % dryer than those from centrifuges, although having similar free water content. According to Kopp and Dichtl (2001b) all the free water measured by TGA are removed in full-scale centrifuges. Hence, this implies that some of the interstitial water is accessed with a hydraulic filter press. Other authors have argued that some interstitial water can also be removed in dewatering of sludge by centrifugation or filtration (Vesilind, 1994, Novak, 2006). However, published full-scale data on hydraulic filter presses compared to centrifuges and belt presses in sludge dewatering were not found. Nevertheless, industrial testing supports that hydraulic filter presses access more water than centrifuges and belt presses (Thunberg, 2010).

The largest influence of dewatering device in the cake solids range tested would be 5 % DS, comparing a belt press and a hydraulic filter press. Although dewatering device influenced the achieved cake solids, the variation across the data set (17-34 % DS) cannot be explained by dewatering device. Hence, the difference observed in cake solids (17 % DS) must be determined by digester substrate or operation. In the following Section we therefore focused on identifying a universal physicochemical parameter, with the aim of relating this parameter to the water holding properties of digestates.

3.2 Digestate physicochemical properties

The current body of literature on sewage sludge dewatering suggest that PS/WAS ratio, the amount and composition of the organic matter (VS, C/N), the inorganic matter and the moisture distribution are important parameters. This study aimed at identifying a parameter that could describe digestate dewaterability. Eight plants (Plants A-H) were selected for detailed analysis of different physicochemical parameters. These plants had a wide range of PS/WAS ratios and different pre-treatment methods.

The physicochemical characteristics of digestates from Plants A-H (Supplementary Material B, Table SB1) were combined in a principal component analysis (PCA) to identify the influence of various parameters on the free water content (Figure 2). Two principal components (PC) described 87 % of the variance in the dataset. For PC 2 (15 %) the variance was mostly related to the moisture distribution (free, interstitial and bound water). Large amounts of free water correlated negatively with large amounts of interstitial and bound water similar to findings by Kopp and Dichtl (2001a).

FIGURE 2

PC 1 described 72% of the variation and was related to physicochemical parameters typically measured at WWTPs or in commercial laboratories and the ratio of PS/WAS.

Plant A, B and E (Figure 2, QA) and Plant H (Figure 2, QC) had the highest amount of VS, carbon and nitrogen in the digestate. In addition, these plants had high amounts of WAS compared to PS. High amounts of VS and WAS has been shown to correlate negatively with dewatering performance (Kopp and Dichtl, 2001b, Kopp and Dichtl, 2001a, Skinner et al., 2015). Although similar in VS, carbon and nitrogen content, Plant A, B and E groups in QA while Plant H is found

in QC. The reason for this could be the application of THP in Plants A, B and E giving higher amount of free water compared to Plant H.

High concentrations of iron and aluminum, ash, high PS/WAS ratio and high carbon/nitrogen (C/N) ratio was found for plants in QB and QD. These parameters had a positive impact on free water and hence the predicted cake solids (Figure 2, PC1). Plant C, having the highest C/N ratio and concentration of iron and aluminum was also the plant with the highest amount of free water in the digestate (Figure 2, QB).

The moisture distribution was mainly described by PC2 which contributed only to 15 % of the variance in the data-set. Additionally, measuring the moisture distribution require instruments not normally present at WWTPs or in commercial laboratories. The physicochemical parameters primarily described by PC1 were therefore considered more useful.

The negative axis of PC1 is described by VS, carbon and nitrogen all representing the organic fraction of the sludge. The positive axis of PC1 is described by a combination of organic (C/N) and inorganic fractions (ash, iron and aluminum). Literature suggests that both organic and inorganic sludge content is important for the dewatering performance (Kopp and Dichtl, 2001b, Skinner et al., 2015, Miryahyaei et al., 2019) Thus the parameters described by the positive PC1 axis are potential predictors of dewatering and therefore discussed in more detail.

The role of cations in dewatering has been studied by several authors (Higgins and Novak, 1997, Park et al., 2006). The divalent bridging theory (Higgins and Novak, 1997) linked the charge of divalent cations to the stabilization of sludge flocs and improved dewaterability by binding to the negatively charged EPS and particles. The ratio between monovalent and divalent cations has been shown to correlate with polymer dosage and dewaterability across multiple substrates

(Higgins and Rajagopalan, 2017). The relatively high concentrations of iron and aluminum (Supplementary Material B, Table SB1) probably arise from the addition of inorganic coagulants in the wastewater treatment plant (WWTP). As pointed out by Park et al. (2006) inorganic cations might be better expressed as a fraction of the ash.

The positive impact of low volatile solids (VS) content and hence high ash content on predicted cake solids (Figure 2) has also been reported by others (Skinner et al., 2015, Kopp and Dichtl, 2001b). Kopp and Dichtl (2001b) explained the positive influence of ash by the density difference between organic and inorganic particles and the binding of more water by capillary forces to organic particles. Miryahyaei et al. (2019) showed that the addition of up to 0.07 g inert material/g WAS reduced viscosity of the digestate and improved dewaterability. However, addition of food waste low in ash content has also been shown to improve dewaterability of sewage sludge (Higgins and Rajagopalan, 2017). Although there seems to be an agreement in literature that the ash content plays a role in dewatering, an additional factor is needed to correct for the differences seen between PS and WAS and when adding a co-substrate.

This factor could be the C/N ratio. Several authors have observed that WAS contains more nitrogen and protein than PS and that WAS dewaters less than PS (Kopp and Dichtl, 2001a, Suarez-Iglesias et al., 2017, Higgins and Rajagopalan, 2017). The C/N ratio of PS was found to be substantially higher than WAS (Higgins and Rajagopalan, 2017, Nicholson et al., 2018) and was suggested to be an indicator of EPS (Nicholson et al., 2018). In addition to the difference observed in C/N of PS and WAS, Svensson et al. (2018) found a clear difference between C/N ratios of source separated food waste (SSFW) digestate and WAS digestate. The SSFW digestate dewatered to 34 % DS, outperforming the WAS digestate which dewatered to 17 % DS. Hence, the C/N ratio reflects the superior dewatering performance of PS and food waste compared to

WAS. The amount of PS and WAS going to digestion can be challenging to accurately predict in full-scale as this factor depends on flow meters and manual sampling conducted at sometimes irregular intervals. Therefore, we propose to use C/N as a substitute for the PS/WAS ratio as this is a more general factor that also can be used for other substrates such as food waste.

Summarized, we identified four factors influencing dewatering positively; C/N, PS/WAS, ash and iron and aluminum. However, since iron, aluminum and other cations are a part of the ash, and C/N can be used as substitute for the PS/WAS ratio, we suggest only using two factors when relating digestate physiochemical properties to the free water and predicted cake solids: C/N ratio and ash.

These two factors have been studied separately in relation to dewatering, but alone any of these two parameters cannot accurately describe the variance in dewatering . In this study we therefore tried combining these parameters to investigate if the effect of both inorganic and organic material could explain predicted cake solids. Compared to organic material such as EPS, ash has a low water holding capacity which makes it an important parameter to include when assessing dewaterability. Additionally, inorganic compounds have also been shown to act as a skeleton adding a more rigid and incompressible structure to the sludge rendering it easier to mechanically dewater (Qi et al., 2011). This effect, including improved drainage and passage of water, is probably more important for dewaterability than just the low water holding capacity of ash (Qi et al., 2011). Therefore, to combine the effect of the organic and inorganic composition of the digestate we multiplied the two factors together to create one combined factor, denoted C/N•ash. Both factors point in the same direction, i.e. both high C/N and high ash indicate low water binding and thus good dewaterability. We then investigated its correlation with predicted cake solids.

3.3 Predicting cake solids from physicochemical parameters

The sample set (Plants A-V) was used to study the correlation between C/N•ash and predicted cake solids by TGA on a broad range of digestion substrates (Figure 3). The test matrix included digestion substrates such as sewage sludge, pulp and paper sludge, fish waste, food waste and manure.

FIGURE 3

Generally, the data showed a linear relationship between C/N•ash and predicted cake solids for all plants ($R^2 = 0.65$). However, when separating the pre-AD THP plants and conventional AD plants a much stronger linear relationship was obtained ($R^2 = 0.91$ and $R^2 = 0.93$). Two equations were identified by linear regression to predict dewatered cake solids of conventional and pre-AD THP digestates by using C/N•ash (Figure 4). The results show that the variation in cake solids can be described by digestate physicochemical properties defined by C/N•ash.

The difference in predicted cake solids between the food waste plants (Figure 3) cannot be explained by different PS/WAS ratios. Svensson et al. (2018) found a higher concentration of acid detergent fibers (ADF) in SSFW digestate compared to WAS digestate. Increasing ADF concentrations in the food waste digestates (Plant I, P and V) corresponded with increasing cake solids and C/N ratios in this study (data not shown). Thus, the C/N•ash also reflects physicochemical properties in food waste digestates that are related to predicted cake solids.

C/N described the water binding capacity of the organic fraction. The amount of ash corrects for the inorganic material with low water binding capacity and skeleton building mechanisms. The combined C/N•ash was found to successfully describe the water binding capacity of

digestates. The results suggest that the negative effect of high amounts of WAS can be counteracted if high amounts of ash is present in the digestate.

Overall, these results provide a novel correlation that can be used to predict the dewatered cake solids of conventional and pre-AD THP digestate from the physicochemical parameter C/N•ash. The two equations (Figure 3) also make it possible to study how the installation of a pre-AD THP will influence the predicted cake solids.

3.4 Effect of pre-AD THP on predicted cake solids

The linear regression analysis of conventional and pre-AD THP plants (Figure 3) was further used to study the effect of pre-AD THP on predicted cake solids.

The pre-AD THP changes the moisture distribution and increases the amount of free water for a given sludge blend, thereby increasing the dewatered cake solids (Figure 3). In the C/N•ash range studied (1.7-4.7) the pre-AD THP improved dewaterability by 5-7 % DS depending on the digestate. However, the difference in cake solids described by C/N•ash was 17 % DS. Hence, digestate physiochemical properties have a bigger effect on the predicted cake solids than the application of pre-AD THP.

3.5 Relating physicochemical parameters to full-scale dewatered cake solids and polymer dose

The main goal of the dewatering process is to reduce the wet mass of cake by achieving the highest possible cake solids concentration. On the other hand, the amount of polymer used to achieve the desired cake solids is also of economic importance. The polymer dose (kg active substance (AS)/ton DS) and measured cake solids for eight full-scale pre-AD THP dewatering processes (Plants A-E and L-N) were compared to C/N•ash to relate C/N•ash to full-scale dewaterability (Figure 4).

FIGURE 4

Linear relationships were found for both cake solids ($R^2 = 0.79$) and polymer dose ($R^2 = 0.90$) as a function of C/N•ash (Figure 4). The highest reported polymer dose was at pre-AD THP plants digesting more WAS than PS (C/N•ash ≈ 1.7-1.8), and the lowest reported polymer dose from a plant digesting only PS (C/N•ash ≈ 4) (Figure 4). This is in agreement with literature where WAS was readily solubilized in the THP compared to PS (Suarez-Iglesias et al., 2017), and increasing concentrations of soluble biopolymers have been linked to increased polymer dosage in sludge dewatering (To et al., 2018). The results show that, in addition to being correlated to the free water fraction measured by TGA, the C/N•ash is highly relevant to predict the full-scale dewatering process, including both cake solids and polymer dose for pre-AD THP plants.

3.6 Practical applications

Although the importance of the PS/WAS ratio is known, in full-scale operation the calculation of this ratio can be challenging due to dependence on flow meters as well as manual sampling by plant operators. Change in plant operation due to seasonal weather conditions, variation in co-waste quality and the digestion process itself also make it difficult to predict the effect of the substrate on digestate dewatering. More controlled conditions can be applied in laboratory studies, but comparison to full-scale and other laboratory studies can be difficult due to the lack of standardized analytical methods (To et al., 2016). In addition, large variations in sludge characteristics can also make it challenging to compare sludge blends used in different studies (To et al., 2016). For instance, it is known that sludge age of WAS will change the dewatering performance (Barber, 2014), and such difference in dewaterability will not be adequate described by using the PS/WAS ratio. This study has identified a physicochemical parameter (C/N•ash) that overcomes these challenges. This will make it easier to compare the sludge blends used in different dewatering studies.

In the industry the C/N•ash will give realistic expectations for the dewatering process. This can support cost/benefit analysis prior to investments in several ways. As an example, for a pre-AD THP plant, the dewatering process can result in:

1) 23 % DS in cake solids using 16 kg AS polymer/ton DS (Figure 4, Plant A, C/N•ash = 1.7), or

2) 34 %DS in cake solids using 4 kg AS polymer/ton DS (Figure 4, Plant M, C/N•ash = 4).

The different digestates described by C/N•ash will thus predict very different operational budgets for a plant, including both expenses for polymer and disposal. Consequently, including

C/N•ash in economic analysis can identify if optimization of the wastewater treatment process is necessary to maximize the amount of PS in the AD substrate. The C/N•ash correlation is thus a powerful tool in the planning and budgeting of full-scale plants.

In greenfield projects testing, demonstration and comparison of relevant dewatering devices are done on existing plants. C/N•ash can therefore help identify suitable digestates for testing. It can also eliminate the need for full-scale testing completely, saving valuable time and money in an investment process.

Process guarantees, including dewatering expectations, are commonly set years before they must be demonstrated in the actual set-up. If discrepancies are observed between expected and actual values after project completion, C/N•ash can be used to identify if the dewatering process is not performing according to expectations or if the sludge blend has changed.

4 Conclusions

This study presents novel insight regarding the impact of digestate physicochemical properties and pre-AD THP on dewatered cake solids. The following conclusions were made:

1. TGA is a good method to predict full-scale dewatered cake solids. The method was valid for digestates from sewage sludge, food waste and co-digestion of these substrates, as well as pre-AD THP digestates.
2. A universal factor describing digestion substrates was found by multiplying the C/N ratio by the ash content of digestate dry solids (C/N•ash).
3. Strong linear relationships were found between C/N•ash and predicted cake solids by TGA for 22 full-scale plants when separating conventional and pre-AD THP digestates. Dewatered cake solids of both conventional and pre-AD THP digestates can thus be predicted from C/N•ash.
4. Pre-AD THP improved predicted cake solids of digestates by increasing the amount of free water. Pre-AD THP had the largest effect on digestates with more WAS than PS.
5. C/N•ash showed linear relationships with both dewatered cake solids and polymer dose of full-scale pre-AD THP plants.

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Figure legends

Figure 1: Cake solids predictions by TGA compared to cake solids after full-scale dewatering for all dewatering devices tested (A). Data points in white triangles were earlier published by Svensson et al. (2018). Δ = conventional food waste digestion, ▲ = conventional digestion of sewage sludge, ● = pre-AD THP of sewage sludge, ○ = pre-AD THP and co-digestion of food waste and sewage sludge. Cake solids predictions by TGA and full-scale results from Figure 1A were grouped into different dewatering devices with their respective linear regression lines fit to intercept at 0 (Figure 1B): belt presses ($y = 0.95x$, $R^2 = 0.97$), centrifuges ($y = 1.02x$, $R^2 = 0.98$), hydraulic filter presses ($y = 1.11x$, $R^2 = 0.90$) and all dewatering devices ($y = 1.02$, $R^2 = 0.90$).

Figure 2: PCA of physicochemical parameters and moisture distribution for Plant A-D (THP), Plant E (WAS-THP) and Plant F-H (None-THP). The four quadrants (Q) have been divided into A, B, C and D to ease the discussion.

Figure 3: The C/N ratio was multiplied with ash, both measured on dry solids basis. The C/N•ash was compared to predicted cake solids by TGA for sewage sludge, food waste and co-waste AD digestates from conventional and pre-AD THP plants. Results from linear regression analysis of all, conventional and pre-AD THP plants are displayed. Pure food waste AD plants are indicated with hollow markers. Data for Plant I (C/N•ash = 4.7) and J (C/N•ash = 2.2) have earlier been published by Svensson et al. (2018).

554 Figure 4: The relationship between reported polymer dose (kg Active Substance (AS)/ ton DS),
555 full-scale dewatered cake solids (%DS) and digestate physicochemical parameters described by
556 C/N•ash for eight full-scale pre-AD THP plants.

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560 **TABLES**

561 Table 1: Technical details of the plants sampled

Plant ID	Thermal treatment	Digestion process Raw material	Continent	Dewatering device
Plant A	Pre-AD THP	MAD, sewage sludge	Europe	Hydraulic filter press
Plant B	Pre-AD THP	MAD, sewage sludge	Europe	Hydraulic filter press
Plant C	Pre-AD THP	MAD, sewage sludge	Europe	Belt press
Plant D	Pre-AD THP	MAD, sewage sludge	Europe	Belt press
Plant E	Pre-AD THP (WAS-THP)	MAD, sewage sludge	Europe	Hydraulic filter press
Plant F	Pasteurization	MAD, sewage sludge	Europe	Centrifuge
Plant G	None	MAD, sewage sludge	Europe	Centrifuge
Plant H	None	MAD, sewage sludge	Europe	Centrifuge

Plant I*	Pasteurization	MAD, food waste	Europe	Centrifuge
Plant J*	None	MAD, sewage sludge	USA	Centrifuge
Plant K	None	TAD, sewage sludge	Europe	Centrifuge
Plant L	Pre-AD THP	MAD, sewage sludge	Europe	Hydraulic filter press
Plant M	Pre-AD THP	MAD, sewage sludge	Europe	Centrifuge
Plant N	Pre-AD THP	MAD, sewage sludge and food waste	Europe	Centrifuge
Plant O	None	MAD, sewage sludge	Europe	Belt press
Plant P	Pre-AD THP	MAD, food waste	Europe	N/A
Plant Q	Pasteurization	MAD, pulp and paper sludge and fish waste	Europe	N/A
Plant R	None	MAD, sewage sludge	Europe	N/A

Plant S	None	MAD, sewage sludge	Europe	N/A
Plant T	None	MAD, sewage sludge	Europe	N/A
Plant U	Pasteurization	MAD, food waste and manure	Europe	N/A
Plant V	Pre-AD THP	MAD, food waste	Europe	N/A

562 *Data from Svensson et al. (2018)

563 N/A = not available

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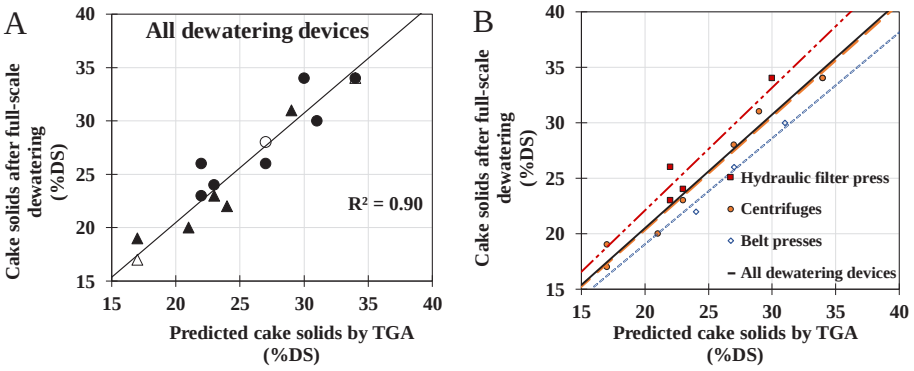

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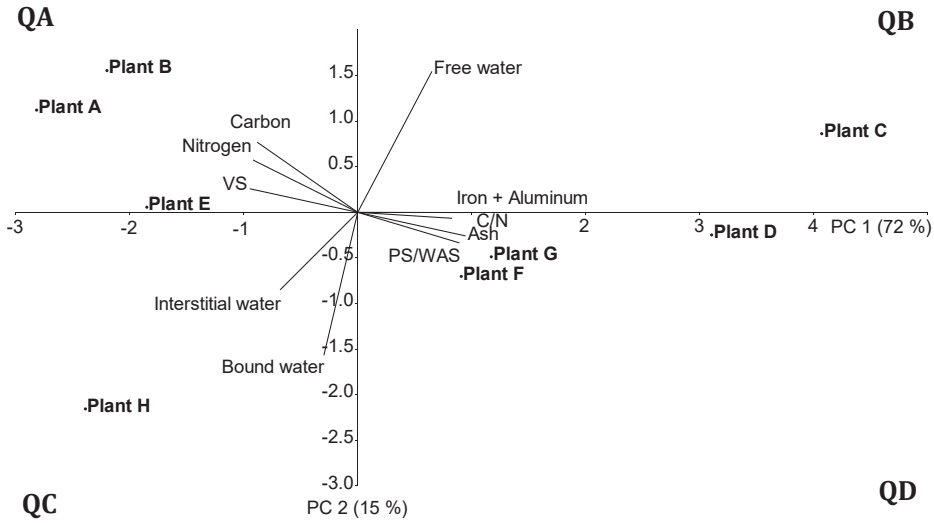

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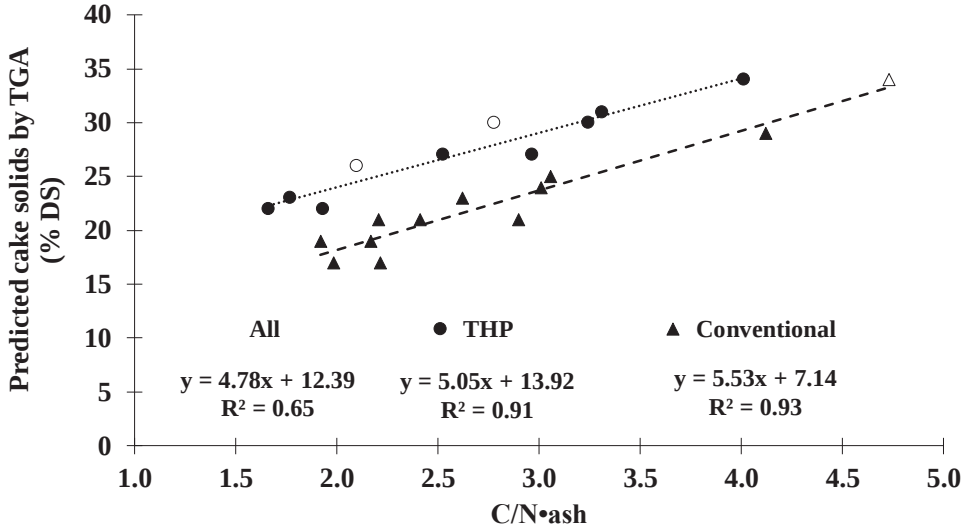

Figure 3: The C/N ratio was multiplied with ash, both measured on dry solids basis. The C/N•ash was compared to predicted cake solids by TGA for sewage sludge, food waste and co-waste AD digestates from conventional and pre-AD THP plants. Results from linear regression analysis of all, conventional and pre-AD THP plants are displayed. Pure food waste AD plants are indicated with hollow markers. Data for Plant I (C/N•ash = 4.7) and J (C/N•ash = 2.2) have earlier been published by Svensson et al. (2018).

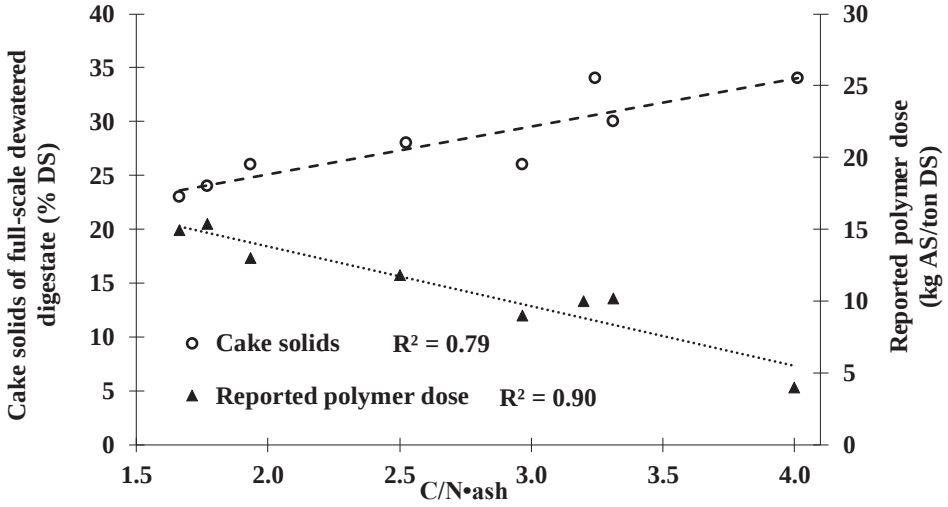

Figure 4: The relationship between reported polymer dose (kg Active Substance (AS)/ ton DS), full-scale dewatered cake solids (%DS) and digestate physicochemical parameters described by C/N•ash for eight full-scale pre-AD THP plants.

CNAsh a novel parameter predicting cake solids of dewatered digestates

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Supplementary Material

Supplementary Material A: TGA drying curves

Drying curves for 20 digestates are presented in Figure SMA-1 to Figure SMA-20. Data for Plant I and J was published by Svensson et al. (2018) and is therefore not included.

All figures show the TGA drying curve and free water defining line twice. The full drying curve (left) and a focused drying curve around the point of transition between free and interstitial water with deviation analysis in frame (right). Both the drying curve and the free water defining line is plotted as a function of mass water/mass solids, and the deviation analysis is displayed in frame (right).

The free water defining line was calculated from the equation enclosed on the left figure, resulting from linear regression of the region before the transition from free to interstitial water. The deviation between the drying curve and the free water line was calculated and used to identify the point defined by Kopp and Dichtl (2001) as "A" (right, in frame).

The predicted cake solids after centrifugation was calculated according to Kopp and Dichtl (2001): Predicted cake solids = $\frac{1}{1+\frac{mass\ water}{mass\ solids}}$

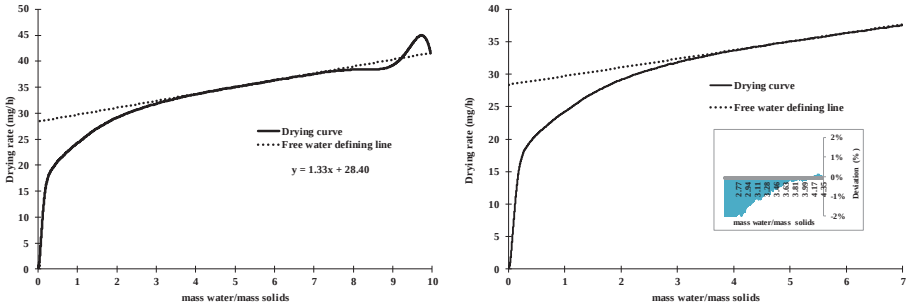

Figure SMA-1: Plant A

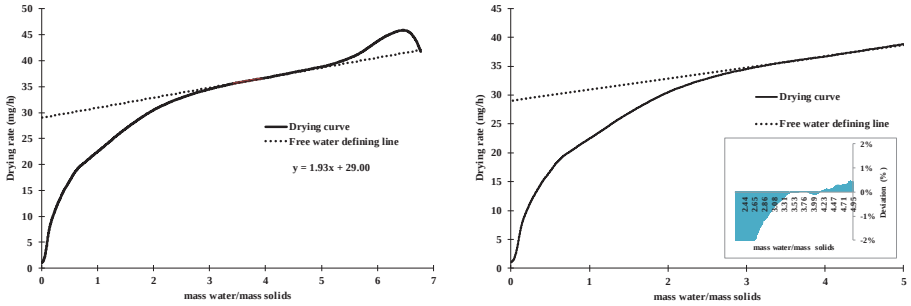

Figure SMA-2: Plant B

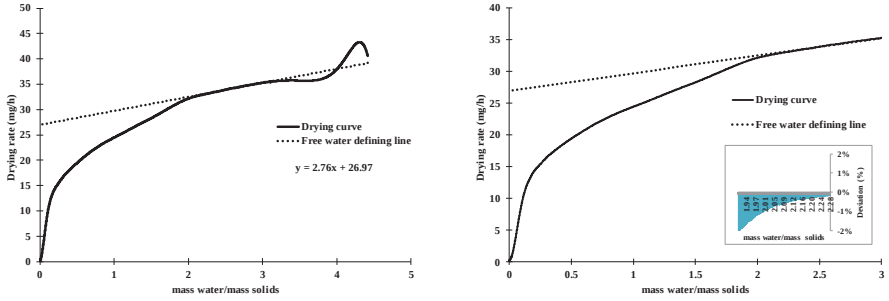

Figure SMA-3: Plant C

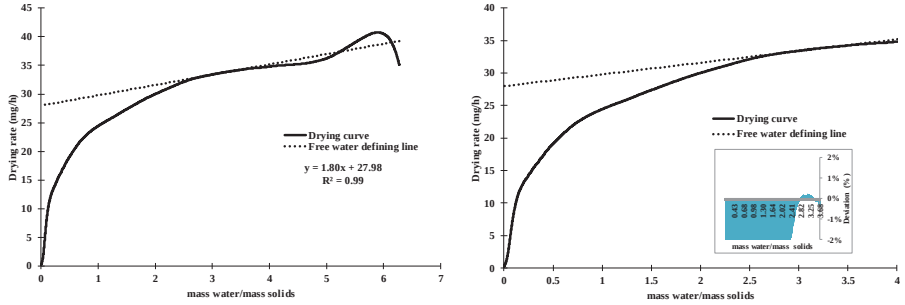

Figure SMA-4: Plant D

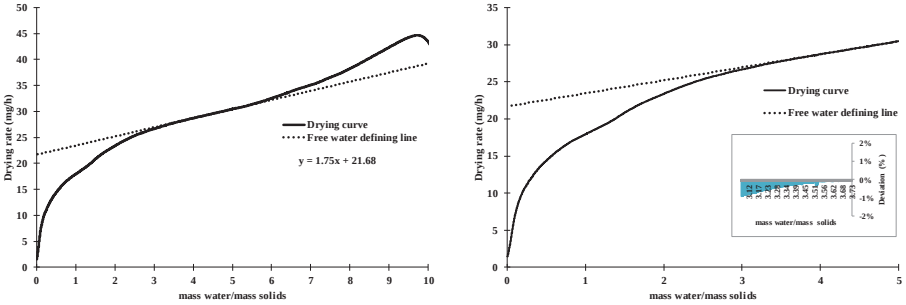

Figure SMA-5: Plant E

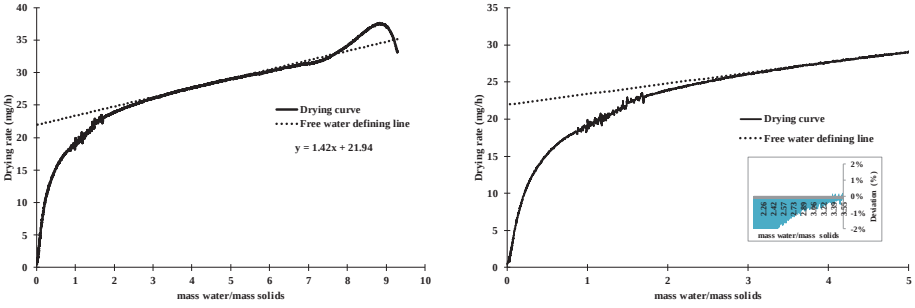

Figure SMA-6: Plant F

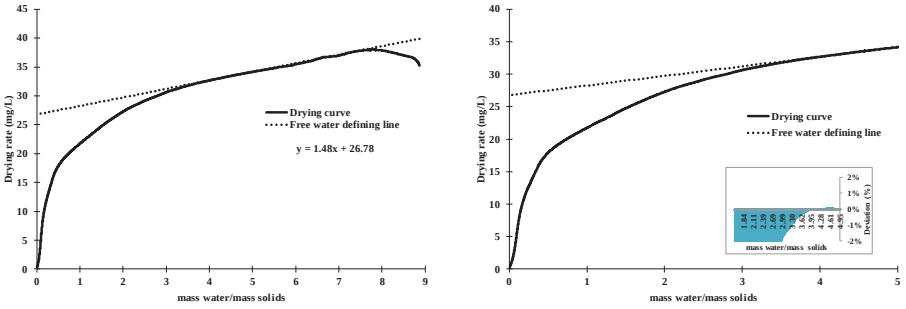

Figure SMA-7: Plant G

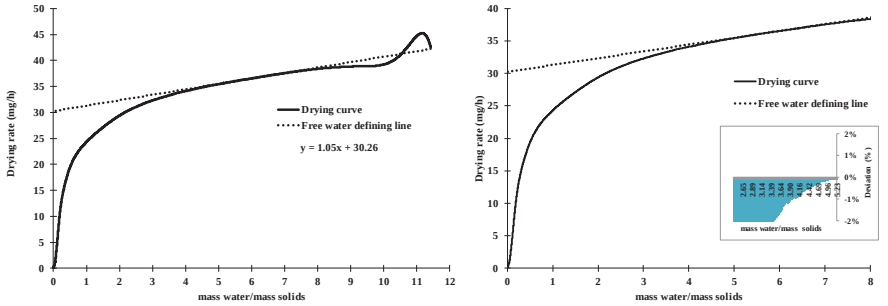

Figure SMA-8: Plant H

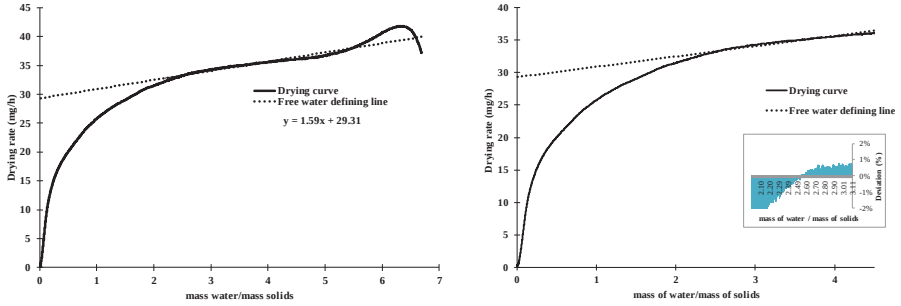

Figure SMA-9: Plant K

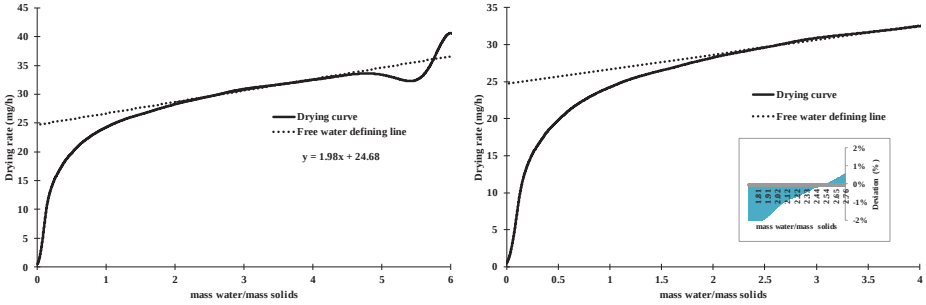

Figure SMA-10: Plant L

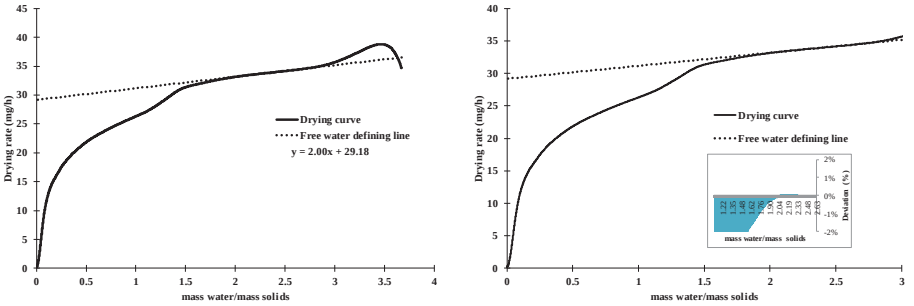

Figure SMA-11: Plant M

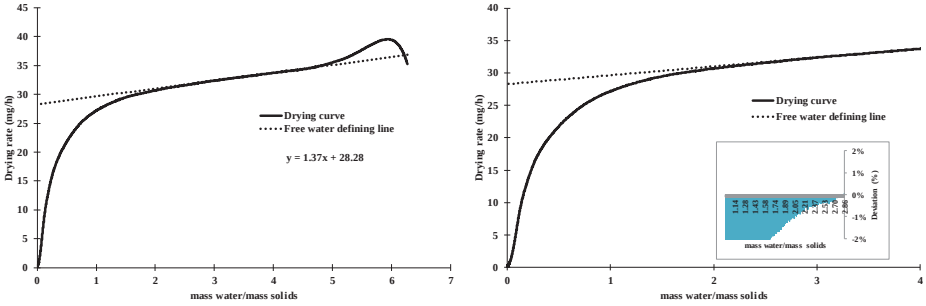

Figure SMA-12: Plant N

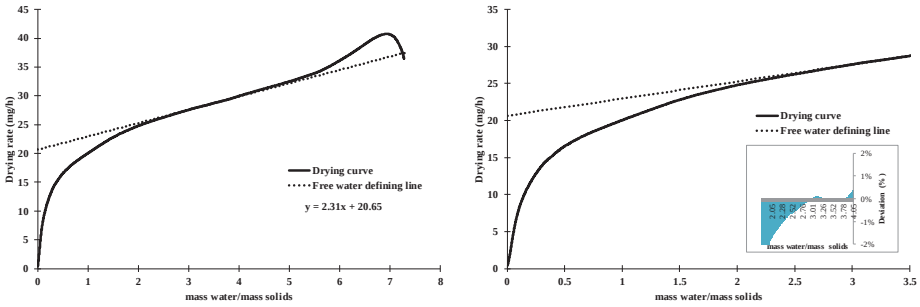

Figure SMA-13: Plant O

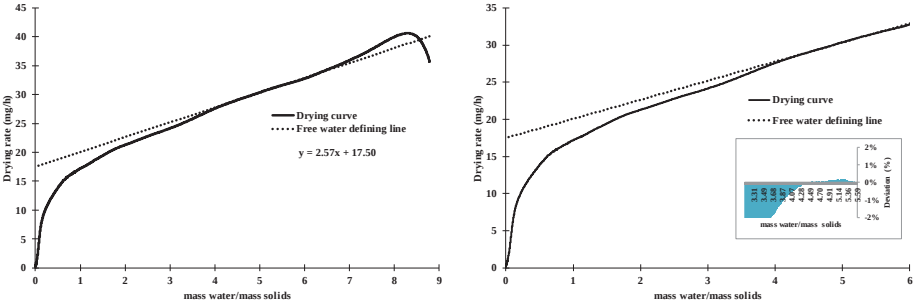

Figure SMA-14: Plant P

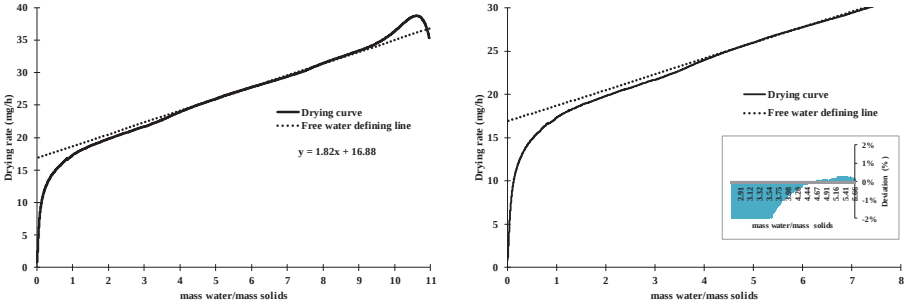

Figure SMA-15: Plant Q

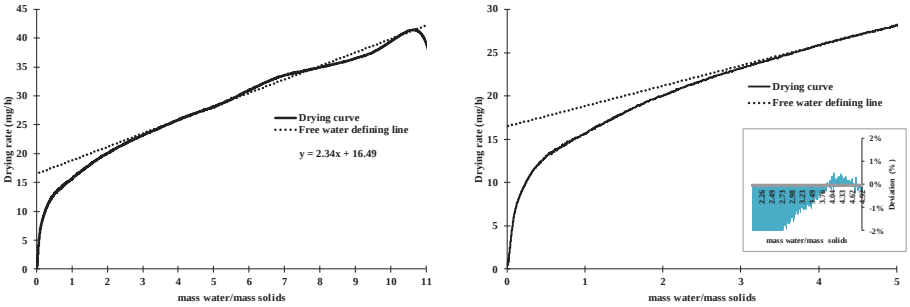

Figure SMA-16: Plant R

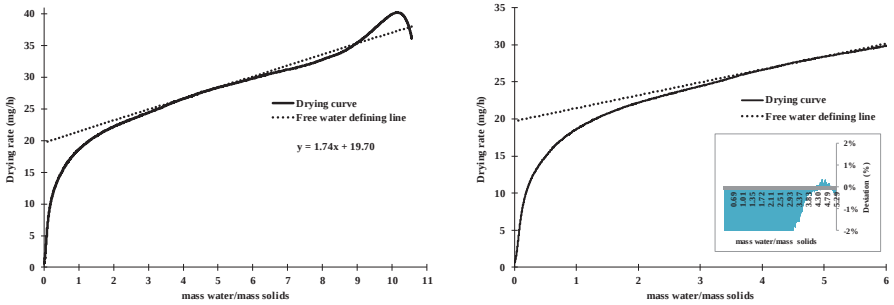

Figure SMA-17: Plant S

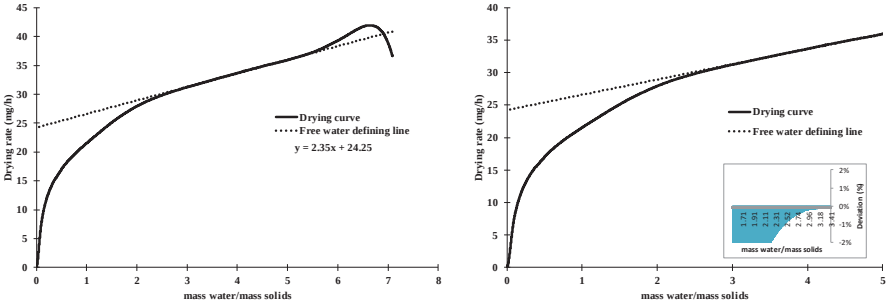

Figure SMA-18: Plant T

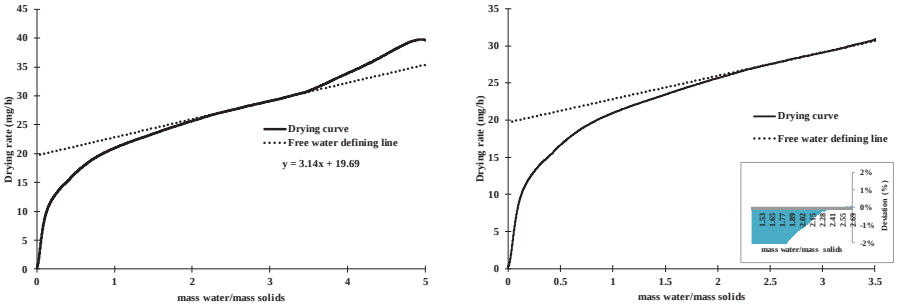

Figure SMA-19: Plant U

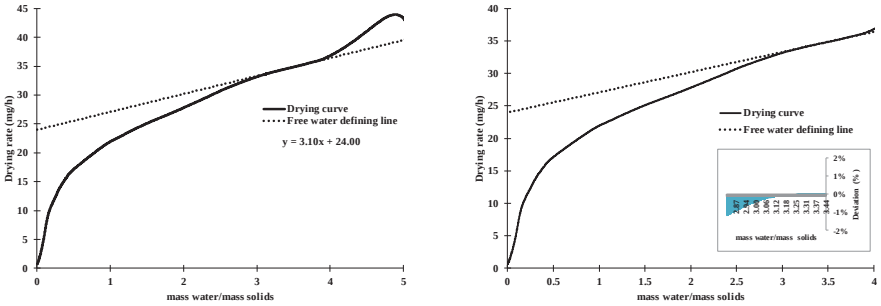

Figure SMA-20: Plant V

KOPP, J. & DICHTL, N. 2001. Prediction of full-scale dewatering results of sewage sludges by the physical water distribution. *Water science and technology,* 43**,** 135-143.

SVENSSON, K., KJØRLAUG, O., HIGGINS, M. J., LINJORDET, R. & HORN, S. J. 2018. Post-anaerobic digestion thermal hydrolysis of sewage sludge and food waste: Effect on methane yields, dewaterability and solids reduction. *Water research*.

Supplementary Material B: Physicochemical parameters for Plants A-H

Table S1: Physicochemical parameters for Plants A-H. Plants A-F (pre-AD THP AD), Plants F-G (conventional AD)

Parameter	Unit	Plant A	Plant B	Plant C	Plant D	Plant E	Plant F	Plant G	Plant H
VS	% of DS	71.8 ± 0.1	71.0 ± 0.1	58.6 ± 0.2	57 ± 1	70.70 ± 0.03	62.0 ± 0.2	62.9 ± 0.1	69 ± 1
Ash	% of DS	28.2 ± 0.1	29.0 ± 0.	41.4 ± 0.2	43 ± 1	29.3 ± 0.03	38.0 ± 0.2	37.1 ± 0.1	31 ± 1
PS/WAS	-	0.3	0.4	1.5	1.5	0.7	1.5	1.5	0.4
Carbon	% of DS	37.9 ± 0.1	38.0 ± 0.1	30.9 ± 0.2	30.0 ± 0.6	36.8 ± 0.2	31.5 ± 0.2	30.8 ± 0.4	35.1 ± 0.2
Nitrogen	% of DS	6.46 ± 0.02	6.19 ± 0.05	3.87 ± 0.03	4.32 ± 0.08	5.56 ± 0.03	4.6 ± 0.1	4.8 ± 0.1	5.51 ± 0.02
C/N	-	5.9	6.1	8.0	6.9	6.6	6.9	6.5	6.4
Iron + Aluminum	% of DS	3.0	3.9	9.0	7.7	3.2	5.1	6.9	3.5
Free water	% of total water	89.7	89.8	93.1	92	89.2	89.5	88.7	84.5
Interstitial water	% of total water	9.8	10.0	6.8	6.3	9.2	9.9	11.1	12.0
Bound water	% of total water	0.5	0.2	0.02	1.7	1.6	0.7	0.2	3.4

Supplementary Material C: C/N•ash related to predicted cake solids by TGA and full-scale dewatered cake solids

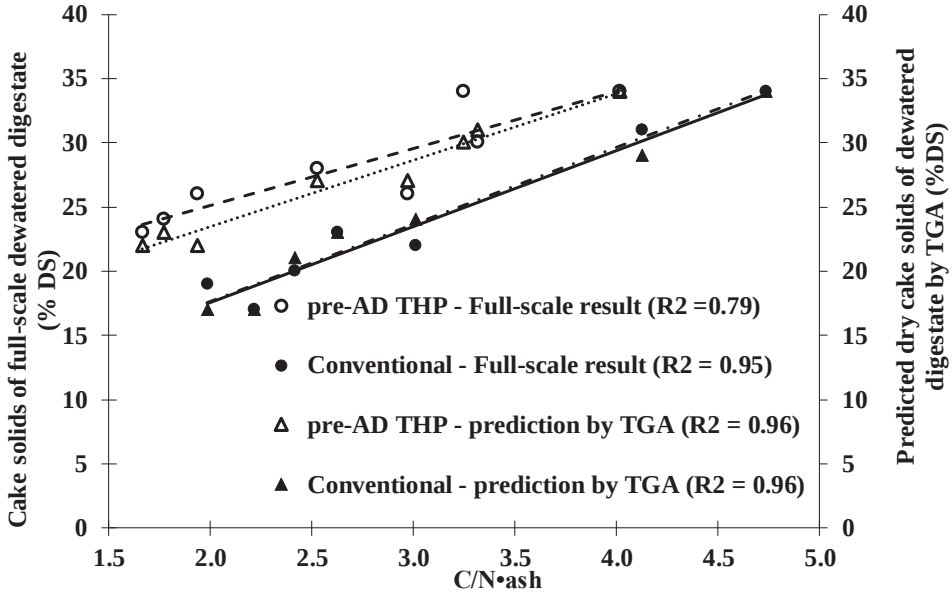

Figure SMC: Predicted digestate cake solids by TGA and full-scale results compared to C/N•ash for pre-AD THP and conventional digestion plants with their respective linear regression lines.

PAPER IV

Quantification of soluble melanoidin-associated compounds in commercial thermal hydrolysis digestates

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Abstract

Solid residues termed primary sludge (PS) and waste activated sludge (WAS) are generated from wastewater treatment can be stabilized through anaerobic digestion (AD). With the increasing focus on circular economy and waste management, other organic wastes such as food waste (FW) are also digested together with sewage sludge or separately. Application of the thermal hydrolysis process (THP) prior to AD results in several benefits; such as higher loading rates, improved substrate biodegradation rate, improved dewatering and destruction of pathogens. However, higher loading rates, improved digestion and solubilization of the substrate due to THP increase the concentration of nutrients in the return liquor from post AD dewatering entering the wastewater treatment plant. In addition, melanoidins, soluble recalcitrant compounds associated with Maillard reactions, have been identified after THP. Melanoidins could impact downstream processes and water effluent discharge concentrations. In this study, the soluble fraction of digestates from seven commercial full-scale THP plants were analyzed to investigate whether similar melanoidin-associated compounds could be detected across several THP substrates. Three main digestate groups were studied; digestate from AD treating a higher proportion of WAS than PS (High WAS), digestates from AD treating a higher proportion of PS than WAS (High PS) and digestate from AD treating source separated food waste (FW). The soluble colloidal chemical oxygen demand (CODsc), color, ultraviolet absorbance at 254 nm (UVA254) and dissolved organic nitrogen (DON) were quantified and compared. High WAS resulted in higher concentrations of CODsc, color and DON compared to High PS. FW had higher concentration of color than High PS and lower concentrations of DON compared to High WAS. Large variations of UVA254 were found between the different digestates studied. In conclusion, the THP substrate influences the concentration of soluble melanoidin-associated compounds in digestates. The results provide novel information on the effect of different THP substrates and can be used in the planning new WWTPs and optimization of existing facilities.

Keywords: biogas, digestate, Maillard, thermal hydrolysis

1 Introduction

Wastewater treatment plants (WWTPs) combine physical, chemical and biological processes mainly to reduce the concentration of nutrients discharged to the water recipient (Metcalf and Eddy, 2014). The wastewater treatment process will in addition to purified water create two main solid residues: primary sludge (PS) and waste activated sludge (WAS). Both sludge types require stabilization and sanitation before further handling and transportation to its end-use. Anaerobic digestion is commonly used for this purpose, also allowing energy recovery in the form of biogas from the degradation of organic material (Mills et al., 2014). In addition to sewage sludge other organic wastes such as food waste and manure can also be treated by AD (Achinas et al., 2017). During the AD process organic material is degraded which sometimes result in higher concentrations of soluble nutrients after digestion compared to the plant influent (Metcalf and Eddy, 2014).

Application of pre-treatment methods such as the thermal hydrolysis process prior to AD (Pre-AD THP) will further increase the soluble nutrient concentration by several mechanisms. Firstly, the Pre-AD THP will reduce the viscosity of the substrate, allowing higher digester loading rates and hence higher concentrations of solids and nutrients in the digester (Barber, 2016). Secondly, the THP solubilize the substrates, giving higher concentrations of soluble nutrients in the digester feed (Bougrier et al., 2007, Suarez-Iglesias et al., 2017). Due to the high operating temperature of the THP, typically 165 °C, the formation of recalcitrant melanoidins have been reported by several authors, arising from the Maillard reactions between sugars and proteins (Dwyer et al., 2008b, Tampio et al., 2014, Gupta et al., 2015, Higgins et al., 2017). Thirdly, improved degradation of organic material will also further increase the concentration of ammonium in the digestate compared to conventional AD (Barber, 2016). Finally, increased ammonium concentration typically shifts the methanogenic community from *Methanosaeta* to *Methanosarcina* (Calli et al., 2005). Further, *Methanosarcina* has lower affinity for acetate resulting in higher residual acetate concentrations in the digestate (De Vrieze et al., 2012).

Regardless of Pre-AD THP or conventional AD the produced digestate is typically dewatered and the liquid fraction returned to the wastewater treatment plant. The return liquor can be 5 % of the total flow into the WWTP when applying Pre-AD THP

(Dwyer et al., 2008b). Although small in volume, the relatively high concentration of nutrients will increase the load on the biological nutrient removal process, and recalcitrant components may accumulate and increase the concentration of organic compounds in the plant effluent. Consequently, research into the formation of melanoidins in THP is important for the planning and optimization of the whole WWTP.

Melanoidins consists of a large range of different molecules that are formed via condensation during Maillard reactions (Maillard, 1912) at high temperatures (140-165 °C), which is a typical temperature range in THP processes. Melanoidins related to THP treatment have been defined as recalcitrant compounds having a higher molecular weight than 10kDa with strong color and high ultraviolet absorbance (UVA254) capacity (Dwyer et al., 2008b, Tampio et al., 2014, Gupta et al., 2015, Higgins et al., 2017). Maillard reactions may cause production of thousands of distinct chemical compounds from only a few initial precursors (Hemmler et al., 2018), and thus melanoidins are a tremendously complex pool of compounds. Formation of melanoidins also frequently occurs in food stuffs (Morales et al., 2012). Melanoidin-associated compounds have strong chelating properties and can complex with minerals and polymers and thus be significantly reduced by e.g. coagulation with aluminum-sulphate, preferentially removing the melanoidin-associated fractions in the > 10kDa range (Dwyer et al., 2009). The organic matter related melanoidin compounds can thus be attributed to the colloidal material passing a 0.45 µm filter, the soluble colloidal fraction. This fraction may influence the plant effluent chemical oxygen demand (COD) and can thus be expressed as soluble colloidal COD (CODsc). One typical characteristic of the melanoidin-associated compounds is the strong brown color, which has been shown to increase with increasing THP temperature (Dwyer et al., 2008b). The increase in THP temperature has also been linked to the increase in UV absorbing compounds (Dwyer et al., 2008b, Higgins et al., 2017) which can have a negative impact on downstream processes as reported by Dwyer et al. (2008b). Compounds identified as the dissolved organic nitrogen (DON) after THP are also melanoidin associated (Dwyer et al., 2008b, Gupta et al., 2015, Higgins et al., 2017). The contribution of total nitrogen to eutrophication processes in sensitive water recipients has led to strict legislations concerning the concentration of nitrogen in the WWTPs effluent in some areas (Oleszkiewicz and Barnard, 2006). Consequently, several studies have been initiated to

investigate melanoidin-associated DON (Dwyer et al., 2008b, Dwyer et al., 2008a, Dwyer et al., 2009, Higgins et al., 2017).

In general, THP has been shown to improve anaerobic digestibility of sewage sludge within the optimum temperature operating range of 160 °C to 180 °C (Barber, 2016). However, the formation of components affecting anaerobic degradability negatively has been reported from thermal treatment of food waste at 160 °C (Liu et al., 2012). Consequently, commercial full-scale Pre-AD THP on food waste is normally operated at lower temperatures than with sewage sludge. However, operating above 133 °C for 20 minutes enables the plants to receive other organic wastes such as animal by-products and still comply with current legislations (European Parliament, 2002, Sargalski et al., 2007). These lower temperatures reduce the melanoidin formation in these protein and carbohydrate rich streams that are particularly prone to melanoidin formation.

Although several studies on single substrates have been conducted in the laboratory, there is a lack of comparison across different commercial full-scale plants operating at relevant conditions according to their substrate. The formation of melanoidin-associated compounds can make an impact on the WWTP configuration and operation. In areas with low discharge limits this is an important parameter to evaluate prior to choice of sludge treatment technology and more research is needed to understand the impact of different THP substrates.

The objective of this study was to investigate if THP substrate influences the concentration of the following soluble melanoidin-associated compounds in the digester effluent: 1) CODsc 2) Color, 3) UVA254 and 4) DON.

The soluble fraction of digestates from seven commercial full-scale Pre-AD THP plants was analyzed to quantify the concentration of soluble melanoidin-associated compounds depending on different substrates at relevant THP conditions. The digestates contained either predominantly WAS, more PS than WAS or FW, and were grouped accordingly to perform statistical analysis across the different substrate blends.

2 Materials and Methods

2.1 Plant samples

Digestates from seven commercial full-scale Pre-AD THP plants were sampled to investigate the effect of different substrates on the soluble fraction (Table 1).

All plants treating sewage sludge had the same THP operating conditions (165 °C in 30 minutes). The plants treating source separated food waste were operated at lower temperature and shorter time (145 °C in 20 minutes).

TABLE 1

2.2 Analyses

Chemical Oxygen Demand (COD) was measured in closed glass vials with Merck Spectroquant® COD Cell Test. Soluble COD was defined by filtration at 0.45µm (Whatman, GE Healthcare).

Coagulation was performed on samples filtered at 1.2µm (Whatman, GE Healthcare) and 60 mL filtrate was coagulated with 1.5 mL aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$, 54 g/L) at pH 6 for 30 minutes and let to settle for 30 minutes. The supernatant was then filtered at 0.45µm to determine the truly soluble COD (CODts). Soluble colloidal COD (CODsc) was calculated by subtracting CODts from CODs.

Color was measured by a Palintest Photometer 7100 and automatically converted to the unit mg platinum-cobalt (PtCo)/L by installed calibration curve from manufacturer. According to manufacturer each unit is equivalent to the color produced by 1 mg/L platinum in the form of chloroplatinic acid in the presence of 2 mg/L cobaltous chloride hexahydrate. UVA was measured at 254 nm in a 1 cm quartz cell with a spectrophotometer (Synergy H4Microplate reader, Biotek). The samples were diluted and filtered at 0.45µm before spectrophotometric analysis.

Total Nitrogen (TN) and ammonium-nitrogen were measured using Merck Spectroquant® Cell Tests. DON was calculated by subtracting TN from ammonium-nitrogen.

The dry solids (DS) concentration was determined gravimetrically by drying the sample at 105 °C until constant weight.

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167 2.3 Statistical analysis

168 Minitab was used for ANOVA and Tukey pair-wise comparison at a significance level of
169 0.05.

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3 Results and Discussion

3.1 Summary of laboratory analysis of digestates

The soluble fraction of digestates from Plants A-G was analyzed to identify differences between digestates originating from different substrates (Table 2). The digestates were divided into three groups: High WAS: plants with more WAS than PS, High PS: plants with higher amounts of PS than WAS and FW: plants with food waste (FW) (Table 2).

TABLE 2

The results from the High WAS in this study showed an average color of 8784 ± 1857 mg PtCo/L (Table 2). This is in line with findings from Dwyer et al. (2008b) that studied pure WAS in batch tests and found the concentration of residual color to be 8686 ± 500 mg PtCo/L. A significant reduction in color ($p < 0.01$) was measured due to removal of the CODsc fraction, averaging at 70 ± 10 % (Color-$Color_{ts}$, Table 2). This supports that the CODsc fraction is associated the color formation as suggested by Dwyer et al. (2009).

The average UVA254 of High PS, 21 ± 8 a.u. (Table 2) is slightly lower than found by Higgins et al. (2017) for similar digestates from CSTR digestion in the laboratory. However, the discrepancy between our results and Higgins et al. (2017) is smaller than the variance within the High PS group in this study.

The High WAS group in this study show considerably lower UVA254, 25 ± 3 a.u. (Table 2) than what reported by Dwyer et al. (2008b) (45.5 ± 2 a.u.) after batch-test of pure WAS sludge. Plant A and B in the High WAS group have 70-80% WAS compared to 100 % WAS in the study of Dwyer et al. (2008b) which could explain these differences.

The average DON concentration of the High PS group in this study was 632 ± 120 mg/L (Table 2). Dwyer et al. (2008b) did not measure DON after their batch-test but Higgins et al. (2017) reported a DON concentration of 470 mg/L in the digestate. This is lower than what was measured for the High PS group, but larger variations were observed within the group than the variation between this study and Higgins et al. (2017).

To correct for different DS concentrations in the digestates, the soluble components were normalized to 4 % DS by using the correction factor in Table 2 before statistical analysis was performed between the three groups.

3.1.1 CODsc

The average CODsc concentrations in each group were compared (Figure 1).

FIGURE 1

The results show a large span in the measured CODsc concentrations (Figure 1) with an increase of 185 % comparing the lowest and highest concentration in the data-set. ANOVA analysis showed that there is a significant difference between the groups ($p = 0.04$) and Tukey pair-wise comparison showed that High WAS had significantly higher concentrations of CODsc than High PS (Figure 1). WAS generally has a higher protein content and solubilize more easily in THP compared to PS (Suarez-Iglesias et al., 2017), and this can explain the higher potential for melanoidin formation in High WAS compared to High PS.

The High WAS group had a relatively low deviation within the group (6 %). This is in line with findings from Bougrier et al. (2008) where little difference on melanoidin formation was found after THP treatment of five different full-scale samples of WAS.

The FW group had a large internal variation with 86 % higher CODsc concentration in Plant G compared to Plant F. Food waste as AD substrate can vary considerably, and differences in anaerobic biodegradability between different food waste fractions have been observed and linked to melanoidin-associated COD (Liu et al., 2012). Although not statistically significant the FW group showed on average 43 % less CODsc than the High WAS group. This is in contrast to the findings of Liu et al. (2012) where WAS showed an improved biodegradation as opposed to the food waste samples, and the difference was linked to melanoidin formation. We hypothesize that the difference between our result and Liu et al. (2012) can potentially be attributed to the different THP reaction temperatures. Food waste is rich in compounds that could lead to formation of melanoidins hence selecting parameters that reduce melanoidin synthesis is important. Choosing a temperature of 145 °C, which is the lower temperature range of Maillard reactions, melanoid formation is reduced. The results (Figure 1) show that by treating food waste at 145 °C the formation of melanoidin-associated COD can be controlled and are not significantly different than when operating sludge at 165 °C.

3.1.2 Color

The most reported characteristic of the melanoidin-associated compounds is the strong brown color of the liquor arising from the Maillard reaction. The average concentrations of color (mg PtCo/L) of each group were compared (Figure 2).

FIGURE 2

The results show large variation between the lowest and highest color concentration. An increase of 189 % was found from the lowest concentration in the High PS group to the highest concentration in the High WAS group. Significant differences between the groups were found by ANOVA analysis ($p < 0.01$), and Tukey pair-wise comparison showed that High WAS and High PS were significantly different as well as High PS and FW (Figure 2).

Less variation in the FW group was found for color compared to CODsc. This could be due to other compounds in food waste contributing to color formation than melanoidin-associated compounds.

3.1.3 Ultraviolet absorbance

Ultraviolet absorbance at 254 nm (UVA254) has been studied by several researchers (Dwyer et al., 2008b, Dwyer et al., 2009, Wilson and Novak, 2009, Higgins et al., 2017) since it can have a direct impact on downstream UV disinfection processes. An increase of UV absorbing compounds will increase the energy demand of the process. UVA254 measured as absorbance units per centimeter and compared (Figure 3).

FIGURE 3

Variations in UVA254 were observed between groups, but also within the groups especially for High PS. Comparing the lowest UVA254 from the High PS group to the highest UVA254 from the FW group, a 142 % increase was found. However, the differences between the groups were not significant. This could be due to the high UVA254 of Plant E, giving rise to the high standard deviation of the High PS group. Plant E also had the highest concentration of CODts (Table 2) which indicates that other UV absorbing organic compounds could also be present in this digestate. This could potentially originate from various industries contributing to the inlet flow of the WWTP.

However, findings by Wilson and Novak (2009) also show similar UVA254 for PS and WAS after THP, but before digestion.

3.1.4 Dissolved organic nitrogen

Concentrations of dissolved organic nitrogen (DON) were compared between the different groups (Figure 4).

FIGURE 4

The High WAS group had a substantially higher DON concentration than the other two groups. An increase of 185 % was found from the lowest DON in FW and the highest DON in High WAS. ANOVA showed that there was a significant difference between the groups ($p = 0.03$). A significant difference between High WAS and High PS was identified by the Tukey pair-wise comparison (Figure 4). Although the same trend was observed by Dwyer et al. (2008b) and Higgins et al. (2017) showing increased concentrations of DON at higher THP temperatures, Dwyer et al. (2008b) generally reported higher DON concentrations. The results from this study is in line with the hypothesis from Higgins et al. (2017) that higher amounts of WAS in the substrate result in higher concentrations of DON after THP.

A significant difference was also found between High WAS and FW. The FW group in this study had the lowest average concentration of DON. This could be due to relatively lower amounts of nitrogen and protein in the food waste compared to WAS. Although food waste fractions can vary between plants and regions Liu et al. (2012) reported lower concentrations of protein in the food waste fractions studied compared to WAS.

3.2 Practical implications

The practical implications of these findings are that the THP substrate should be a part of the cost/benefit analysis when planning the installation of THP. The results from this study can help evaluate if the impact on the plant effluent will be acceptable or other measures must be implemented. This could be optimization of the dewatering process which has shown to reduce the concentration of both DON and UVA254 in the dewatering liquor compared to the digestate (Higgins et al., 2017).

4 Conclusions

Based on the soluble fraction of seven commercial full-scale Pre-AD THP digestates we observed that:

- High amounts of waste activated sludge in the substrate resulted in higher concentrations of CODsc, color and dissolved organic nitrogen in the digestate compared to digestates with high amounts of primary sludge and source separated food waste.
- Source separated food waste treated at 145 °C showed equal or lower concentrations of all components compared to sludge operated at 165 °C.
- Large variations in UVA254 were observed between the plants, from 15 -30 a.u./cm.

In conclusion, the type of substrate affects the concentration of melanoidin-associated compounds in THP digestates

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Figure legends

Figure 1: Average concentrations of CODsc in groups High WAS, High PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter are significantly different.

Figure 2: Average concentration of color in groups High WAS, High PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter are significantly different.

Figure 3: Concentration (absorption units) of Ultraviolet absorbing compounds in groups WAS, PS and FW.

Figure 4: The concentration of dissolved organic nitrogen (DON) divided into the three groups WAS, PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter (A or B) are significantly different.

394 Table 1 Technical details of plants sampled: digestion substrate, fraction of primary
395 sludge (PS) and waste activated sludge (WAS), THP operating conditions and location.

Plant ID	Substrate	THP operating conditions	Location
Plant A	Sewage sludge PS/WAS = 0.3	165 °C – 30 minutes	England
Plant B	Sewage sludge PS/WAS = 0.4	165 °C – 30 minutes	England
Plant C	Sewage sludge PS/WAS = 1.5	165 °C – 30 minutes	England
Plant D	Sewage sludge PS/WAS = 1.5	165 °C – 30 minutes	England
Plant E	Sewage sludge PS/WAS = 1.5	165 °C – 30 minutes	Norway
Plant F	Food waste	145 °C – 20 minutes	Norway
Plant G	Food waste	145 °C – 20 minutes	Norway

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Table 2 Analysis of the soluble fraction of digestate from Plants A-G. Dry solids (DS) concentrations were used to calculate the correction factor relating samples to 4 % DS.

Parameter	Unit	Plant A High WAS	Plant B	Plant C High PS	Plant D	Plant E	Plant F FW	Plant G
CODts	mg/L	3 868 ± 126	2 250 ± 96	2 924 ± 224	2 343 ± 110	5 577 ± 175	3 337 ± 270	5 085 ± 47
CODsc	mg/L	3 287 ± 157	3 034 ± 115	2 255 ± 228	1 589 ± 128	2 325 ± 180	1 012 ± 288	2 605 ± 632
DON	mg/L	823 ± 63	893 ± 69	555 ± 134	570 ± 110	770 ± 93	380 ± 78	381 ± 63
$Color_s$	mg PtCo/L	10 097	7 470	5 061	4 172	6 245	6 007	7 311
$Color_{ts}$	mg PtCo/L	1 885	1 426	1 420	1 185	2 862	2 336	2 043
UVA254	a.u./cm	28	23	15	30	19	25	21
DS	%	4.79 ± 0.01	4.04 ± 0.00	5.77 ± 0.04	5.82 ± 0.08	5.58 ± 0.04	3.57 ± 0.01	4.95 ± 0.30
Correction factor	-	0.84	0.99	0.70	0.72	0.69	1.12	0.81

a.u./cm = absorption unit per centimeter

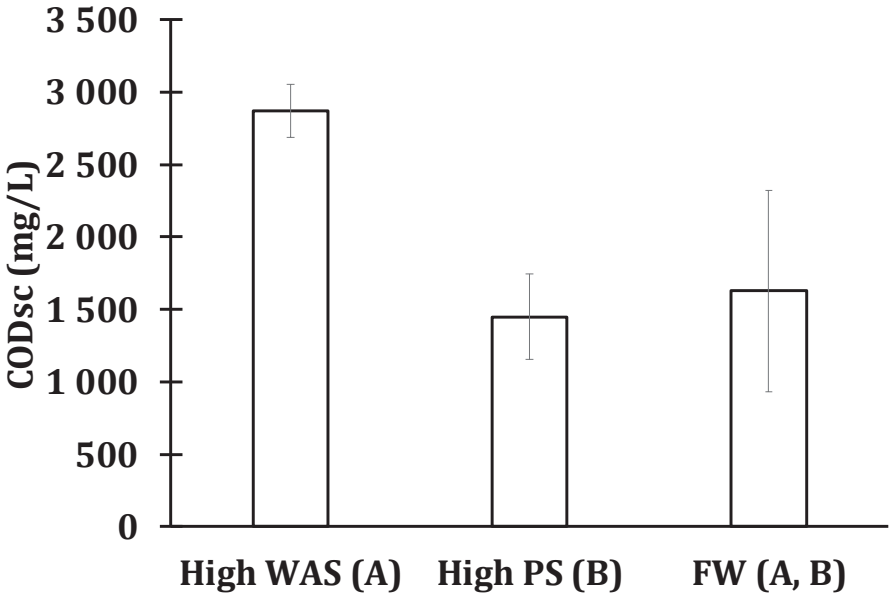


Figure 1: Average concentrations of CODsc in groups High WAS, High PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter are significantly different.

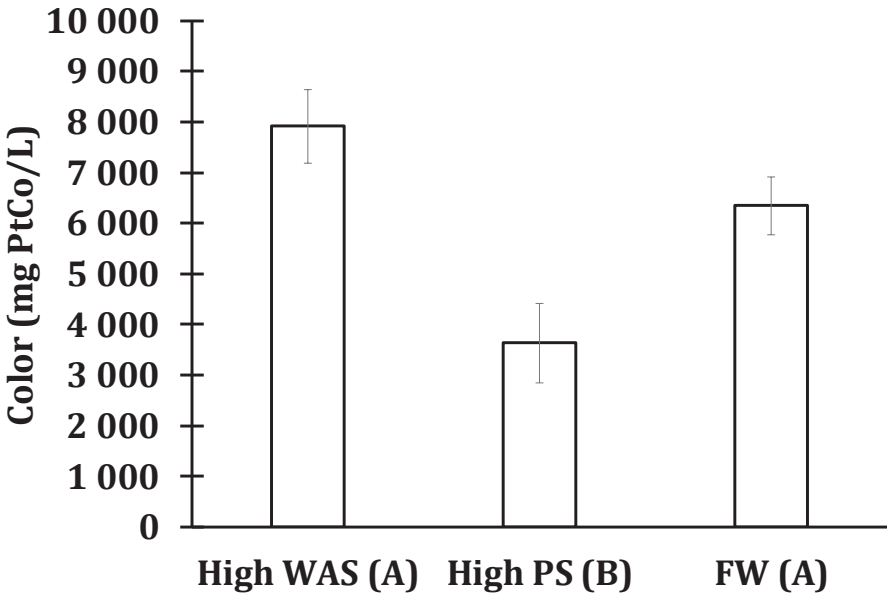
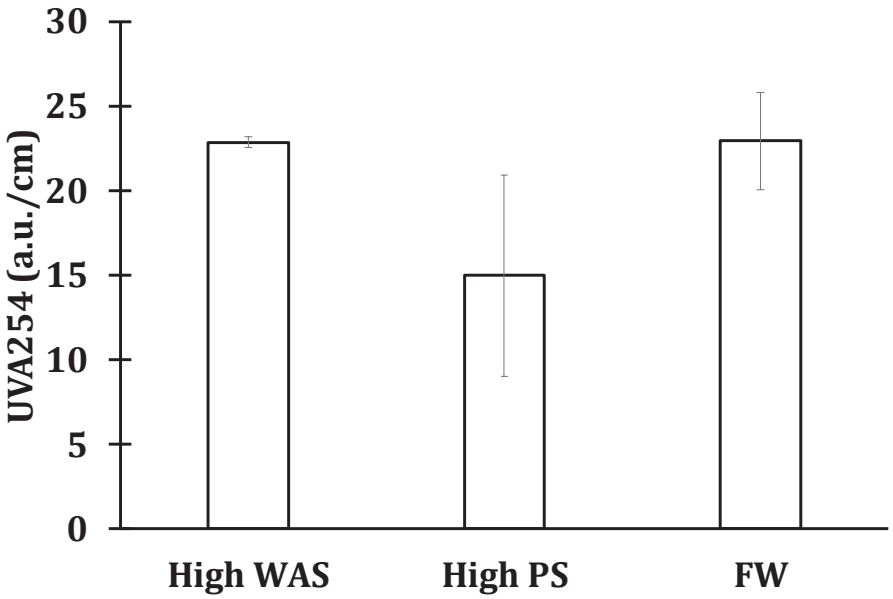


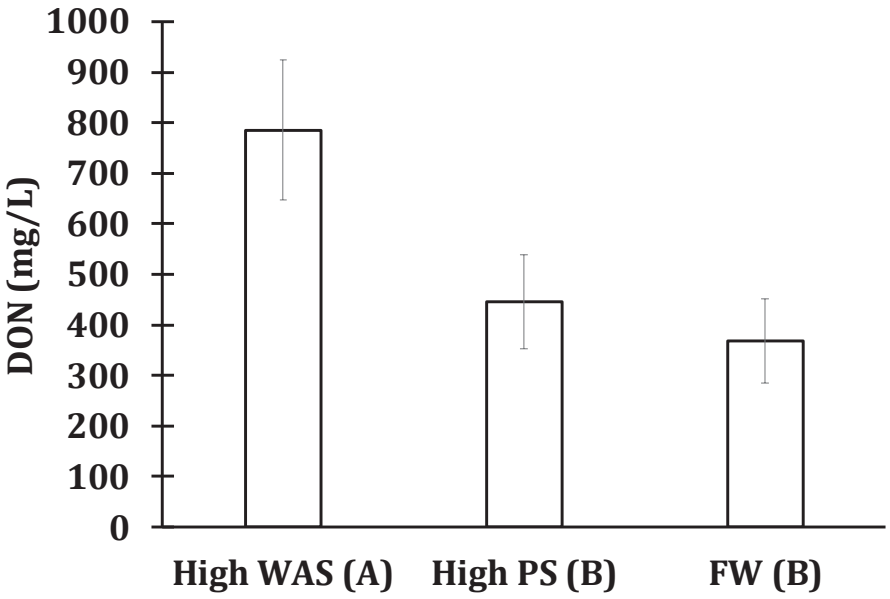
Figure 2: Average concentration of color in groups High WAS, High PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis behind each group. Groups that do not share a letter are significantly different.



415

416 **Figure 3: Concentration (absorption units) of Ultraviolet absorbing compounds in groups WAS,**
417 **PS and FW.**

418



419

420 **Figure 4: The concentration of dissolved organic nitrogen (DON) divided into the three groups**
421 **WAS, PS and FW. The results of Tukey pair-wise comparison are indicated in parenthesis**
422 **behind each group. Groups that do not share a letter (A or B) are significantly different.**

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424

PAPER V

Pathogen growth in sterile digestates can be eliminated by inoculation with a complex microbial community

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Abstract

Sludge from wastewater treatment systems is normally stabilized by anaerobic digestion (AD), and the digestates can be used to fertilize crops. This represents a health risk, however, unless pathogens are effectively eliminated. Such sanitation has traditionally been achieved by thermophilic digestion, or partial sterilization before or after AD, and more recently by Thermal Hydrolysis Processing (THP), which effectively sterilizes the material. THP prior to AD (Pre-AD THP) sterilizes the sludge before microbial inoculation in the digester. However, THP after AD (Post-AD THP) results in a sterile digestate. Although both may seem attractive from a health perspective, there is a risk for growth of pathogens in the Post-AD THP biosolids due to lack of competitors: it is practically impossible to avoid microbial recontamination (including pathogens) during handling and storage, and these organisms will rapidly colonize the sterile material. In theory, the growth of pathogens could be suppressed by establishing a complex community of harmless bacteria, due to competition for substrates and/or antagonism. We tested this in a laboratory incubation of THP treated digestates, using the growth/survival of *E. coli* as a pathogen model, introduced by contaminating the material with wastewater. Compost bacteria were introduced 2 days prior to the wastewater contamination in the Post-AD THP biosolids. During the aerobic incubation, we monitored respiration, total bacterial population (ddPCR of 16S rRNA), bacterial diversity (16S rRNA amplicon sequencing) and abundance of *E. coli* as viable counts (most probable number) and gene abundance (ddPCR, primers targeting the *uidA* gene in *E. coli*). The results demonstrate convincingly that the compost bacteria effectively colonized the material (high respiration), and suppressed *E. coli*: viable counts decreased rapidly (albeit fluctuating) while its gene abundance remained nearly constant. In contrast, the *E. coli* gene abundance increased by 1-2 orders of magnitude, and viable counts were sustained high in the material without compost bacteria until day 13. In conclusion, pathogen growth in sterile digestates can be eliminated by inoculation with a complex microbial community.

Keywords: anaerobic digestion, biogas, *E. coli*, sanitation, thermal hydrolysis, vector attraction reduction

1 Introduction

Wastewater treatment creates two main products: water and sludge. While the water is discharged to recipients like rivers or oceans, the sludge needs further treatment and stabilization before land application. This can be done through anaerobic digestion (AD) at mesophilic or thermophilic temperatures where organic material is converted to biogas, reducing the mass of solids and the number of fecal pathogens. Implementation of treatment processes such as the thermal hydrolysis process (THP) can enhance the AD process and improve sanitation through sterilization at 165 °C (Barber, 2016). The solid residue remaining after AD (digestate) is commonly dewatered before transportation to its final end-use. The treated and dewatered solid residue is often termed biosolids (USEPA, 2003). Which treatment processes that are required for the biosolids to be used on agricultural land are dictated by local authorities.

The use of biosolids as fertilizers is regulated by local legislation, requiring treatment that minimize the risk for transmitting infectious diseases. Regulations usually set limits for fecal coliforms, *Salmonella*, *Helminth ova* and enteric viruses (Iranpour et al., 2004). Quality control focuses on reducing these indicators in the sludge, and acceptable levels are normally obtained by thermal drying, thermophilic anaerobic digestion (TAD) in batch or multi-stage, pasteurization, conditioning with lime and the pre-AD thermal hydrolysis process (Pre-AD THP) (Iranpour et al., 2004, Murthy et al., 2011). In anaerobic digested sludge *Salmonella* is present in low numbers compared to fecal coliforms (Sidhu and Toze, 2009). Therefore, the indicator group most commonly used for biosolids is thermotolerant coliform bacteria (TCB) and the more specific bacterium *Escherichia coli* (*E. coli*) (Higgins et al., 2007, Fane, 2016, Chen et al., 2011). However, there is an ongoing debate on whether the absence of culturable indicator organisms is an adequate safety criterium (Sidhu and Toze, 2009). For instance, it has been shown that fecal coliforms and *E. coli* may enter a "viable but non-culturable state" during digestion (Higgins et al., 2007). Consequently, the commonly used multiple-tube fermentation most probable number (MPN) technique may grossly underestimate the number of metabolically intact (but non-culturable) cells. Therefore, researchers have used both the MPN technique and the polymerase chain reaction (PCR) technique to identify fecal regrowth in centrifuged biosolids (Chen et al., 2006, Higgins et al., 2007, Chen et al., 2011). While biosolids are produced continuously throughout the year, the

period for land application is limited and season dependent. Consequently, the biosolids must be stored until use. Ensuring safe handling therefore requires knowledge on re-growth of pathogens in biosolids with different sanitation methods, but also the biosolids' resistance to external recontamination during storage.

In addition to pathogen reduction, it is important to reduce the attraction of vectors such as flies and insects during storage and handling. Specific legislations such as the Vector Attraction Reduction (VAR) requirements (USEPA, 2003) have been introduced as a consequence of reports on recontamination and growth of pathogens in sterilized biomass (Ward et al., 1999).

The THP has traditionally been applied before the AD process (Pre-AD THP, Figure 1) to ensure a more efficient anaerobic digestion, hence reducing the available organic material in the digestate. Sterilization at 165 °C, reduction in available organics and microbial inoculation are all factors than can be expected to avoid regrowth of *E. coli* and make the biosolids less attractive to vectors. Consequently, this process will meet the highest standard for biosolids hygienization (Class A) and the VAR requirements. Thermophilic anaerobic digestion (TAD, Figure 1) operating at 55 °C in 2 hours batch-mode is meeting the hygienization requirements in Norway (Landbruks- og matdepartementet, 2003) and would also meet VAR requirements due to reduction of organic matter and microbial inoculation. However, the THP process has recently been applied after AD (Post-AD THP, Figure 1) to substantially enhance the dewatering process and reduce the wet mass of biosolids for disposal (Svensson et al., 2018). While this ensures absence of all pathogens, it also eliminates all other microbes, and this could increase the risk for recolonization by pathogens during storage unless dried to 75% dry weight (USEPA, 2003). An alternative to drying could be to inoculate the material with harmless microbes immediately after THP treatment and dewatering as a part of the cake handling and storage facility.

FIGURE 1

The overall objective of this study was to investigate if inoculating the dewatered Post-AD THP biosolids with a mixture of harmless bacteria hinder extensive growth of *E. coli* after recontamination.

To achieve this, we tested the effect of inoculating post-AD THP treated biosolids with pathogen-free compost on subsequent growth of *E. coli*. The source of *E. coli* was wastewater, added two days after the compost inoculation. For comparison, the study included two biosolids approved by Norwegian legislation (TAD and Pre-AD THP) (Landbruks- og matdepartementet, 2003). The MPN method and digital droplet PCR were used to monitor the growth of *E. coli*. A robotized incubation system was used to monitor the microbial respiration. 16S rRNA sequencing was used to investigate the microbial diversity of the biosolids before and after recontamination, in addition to the effect of compost addition to the Post-AD THP biosolids.

2 Material and methods

2.1 Samples

2.1.1 Plant sampling and compost

Three plants with different hygienization methods were selected for analysis (Table 1). Dewatered biosolids were collected at the outlet of the dewatering equipment in airtight containers at Plant A and B at the same day and stored overnight at 4 °C before the experiments were started. Dewatered Post-AD THP biosolids were collected at the centrifuge outlet at Plant C in airtight containers, shipped to the Norwegian University of Life Sciences and stored at 4 °C until the experiment was started. To ensure a sterile biosolids after sampling and transportation from Plant C, this biosolids was autoclaved at 121 °C for 15 minutes prior to the experiment.

Dewatering liquor was collected at all three plants to measure the soluble chemical oxygen demand of the liquid phase of the biosolids.

TABLE 1

The autoclaved biosolids sample from Plant C was split in two. One part was blended with 10 volume % compost and the other was used directly. Compost was provided by the Norwegian company Høst. According to manufacturer the compost was based on yard waste, processed at 60-70 °C in 2-3 months with frequently turning and air supply, and subsequent vermicomposting. The compost was documented free of thermotolerant coliforms (TCB) and *E. coli* by enumeration according to NS-EN ISO 9308-2:2014. The compost was stored at 4 °C and left at room temperature the last 24 hours before start-up.

All samples were passed through a sieve with a mesh size of 4 mm to separate out clumps and particles making it easier to get uniform and representative samples. The sieve was washed and sanitized by a 70 volume % ethanol solution between different samples. In total, four sample sets were prepared for analysis (Table 2).

2.1.2 Source of recontamination

Wastewater collected from the primary sedimentation tank at Plant B was used as the source of *E. coli.* The sample was transferred to the Norwegian University of Life

Sciences in an airtight container and stored at 20 °C until use. Presence of *E. coli* in the sample was confirmed by enumeration by a commercial laboratory prior to recontamination of samples sets.

2.2 Robotized incubation system for respiration kinetics

To investigate the respiration rates of the microbial community with and without recontamination with wastewater, a robotized incubation system connected to a gas chromatograph (7890A, Agilent Technologies) and a NO analyzer (Model 200E Chemilumenescence NO analyzer, Teledyne) previously described by Molstad et al. (2016) was used. In brief: The system allows for automated time incremental sampling and monitoring of O_2, N_2, NO, N_2O, CO_2 and CH_4. The sample volume used for analysis is replaced by helium. Six sterilized serum vials (120mL) were used per sample set (recontaminated + control). 2 g of biosolids were added to each vial, capped with an airtight rubber septum, and incubated aerobically at 20 °C. Mass loss of gasses due to sampling and dilution with helium was corrected for.

After 48 hours three flasks were recontaminated by spiking with 50 µL wastewater, while the other three replicate flasks received equal amounts of sterile distilled water by a syringe. To secure aerobic conditions, O_2 was injected when needed to keep the O_2 concentration in the headspace within the interval 10-20 vol%. The molar ratio of O_2 consumption to CO_2 production was close to 1 (Supplementary Material D), and CO_2 was chosen to display the respiratory data as it was less affected by injection of O_2 (the rate of O_2 consumption could not be calculated for those time intervals when O_2 was injected).

2.3 Preparation of samples

In parallel with the incubation experiment, a large set of samples with identical treatments were placed in 5o mL Falcon tubes, designed for destructive sampling to determine the viable counts and gene abundances throughout the experiment. For the samples that were to be recontaminated with wastewater, triplicates for each sampling day were prepared in 50 mL sample tubes by adding 23 g sample to each tube at the

start of the experiment. This resulted in 39 tubes per sample set. For the control samples, that were not spiked with wastewater, duplicates of 700 mg sample for each sampling day were prepared in 2 mL cryogenic tubes at the start of the experiment. The samples were all placed in closed plastic boxes at room temperature (20°C) with the cap loose to allow air diffusion into the tube. Each sample set was placed in separate containers and the bottom was covered with tissues wetted with distilled water to avoid desiccation of the samples. The plastic containers were opened for spiking and sampling, otherwise left closed. At each sampling point two tubes were frozen for ddPCR and 16S rRNA gene sequencing at -80°C at each sampling time point. One tube from the 50 mL set was shipped to a commercial laboratory in a cooler containing one cooler brick at each sampling point for enumeration of *E. coli*.

An incubation period of 48 hours was chosen to allow the microbial community of the compost to blend into the biosolids from Plant C and mature before recontamination. 48 hours typically represent the storage capacity of receiving containers at wastewater treatment plants to reduce work hours in weekends, hence simulating the maturing time available in full-scale systems. After 48 hours recontamination was induced by distributing 0.5 mL of wastewater across the top layer of the samples. The volume of wastewater contamination was based on initial investigations with the incubation robot (data not shown). The tubes were capped, shaken and left with the cap loosely placed on top of the tube.

2.4 Microbial analysis

2.4.1 Enumeration of *E. coli* by standard cultivating methods

Enumeration of *E. coli* was done by an accredited commercial laboratory according to NS-EN ISO 9308-2:2014 to assess the viable count of *E. coli*.

2.4.2 DNA extraction and purification

Genomic DNA from duplicate samples from each sample time point was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany). The entire process was carried out according to the manufacturer's protocol. The bead beating was performed with a FastPrep 24 at 4m/s and 45 seconds.

2.4.3 Digital PCR

ddPCR was performed on both technical replicate DNA extracts for each sampling point. The ddPCR reaction mix contained 10 µL QX200 ddPCR EvaGreen Supermix (Bio-Rad), 2 µL of DNA template/extract, and 100 nM final concentration of primer pairs PRK341F/PRK806R (5'-CCTACGGGRBGCASCAG-3', 5'-GGACTACYVGGGTATCT-3') (Eurofins Genomic) targeting the V3-V4 hypervariable region of prokaryotic 16S rDNA (Yu et al. 2005), *or* primer pairs ECF_uidA/ECR_uidA (5'-CGGAAGCAACGCGTAAACTC-3', 5'-TGAGCGTCGCAGAACATTACA-3') (Eurofins) targeting a region of the beta-glucuronidase reporter gene (*uidA)* specific for *E.coli* (Silkie et al., 2008) (Table 2). 20 µL reaction mix and 70 µL Droplet generation oil for EvaGreen (Bio-Rad) was used to generate droplets in a QX200 droplet generator (Bio-rad). 40 µL of oil droplet suspension was transferred to 96 well twin.tec PCR plates (Eppendorf) and heat sealed with aluminum foil (PX1™ PCR plate sealer (Bio-Rad)).

TABLE 2

PCR cycling conditions (Table 3) when amplifying *uidA* primer products (90 bp) were in accordance with the QX200™ ddPCR™ EvaGreen Supermix PCR protocol with an annealing/extension temperature of 63 °C. The cycling conditions when amplifying 16S rDNA (465 bp) were 95 °C for 5 minutes (enzyme activation/denaturation), 40 cycles at 95 °C for 30 seconds (denaturation) and 55 °C for 30 seconds (annealing/extension) and 45 seconds at 72 °C (extension), followed by signal stabilization for 5 minutes at 4 °C and 5 minutes at 90 ^{0}C. All PCR reactions were conducted in a 2720 Thermal Cycler (Applied Biosystems) with 2 °C s^{-1} ramp rate, a lid temperature of 105 °C and a final 4 °C hold step. PCR products were analyzed in a QX200 droplet reader (Bio-Rad), and the data was analyzed in the Quantasoft™ Analysis Pro 1.0.596 software (Bio-Rad).

TABLE 3

2.4.4 16S rRNA gene sequencing and bioinformatics analysis

The extracted DNA was subjected for 16S rRNA gene sequencing in order to assess taxonomic composition and dynamics of the prokaryotic community. For this, a two-step nested PCR was carried out, firstly amplifying the V3-V4 region using the primer pair PRK341F(5'-CCTACGGGRBGCASCAG-3')/PRK806R(5'-GGACTACYVGGGTATCTAAT-3') (Yu et al., 2005), followed by a second PCR for

attachment of dual indices and Illumina sequencing adaptors. Both PCRs were performed using 1× HotFirePol blend master mix (Solis BioDyne), and amplicons were purified with AMPure XP beads (Beckman-Coulter, USA) using the manufactures protocol. Equimolar concentrations of the amplicon libraries were pooled and the final library was quantified with the QX200™ Droplet Digital™ PCR System (Bio-Rad, USA) using primers targeting the Illumina adapters. Finally, the library was sequenced on an Illumina MiSeq system (Illumina, USA), using the MiSeq v3 reagent kit with 300-bp paired-end reads.

The resulting sequences were processed using USEARCH associated algorithms. Initially, paired end reads were merged, filtered (maxee = 1, minimum length = 350 bp), dereplicated and singletons discarded. Sequences were then clustered to operational taxonomic units OTUs at 97% identity threshold and checked for chimeric sequences. Finally, taxonomy was assigned through the uclust method implemented in QIIME (Caporaso et al., 2010), using the SILVIA database (Quast et al., 2012). UniFrac distances (weighted and unweighted) were generated through the beta_diversity.py script, also in QIIME. Samples with low sequencing depth, i.e. below 10,000 sequencing reads per sample (in total 7 samples, see Supplementary Material H), were excluded from the downstream analysis. The generated OTU data was mined and statistically analyzed and visualized using Calypso (Zakrzewski et al., 2016). Taxa less than 0.01 % relative abundance across all samples have been filtered out from the final diversity plots, and all analysis are based on total sum normalization (TSS) + square root transformed data, unless stated otherwise.

3 Results and discussion

3.1 Growth of *E. coli*

Growth of *E. coli* in the four sample-sets were monitored by both MPN enumeration (viable count of *E. coli*) (Figure 2A) and ddPCR (*uidA* gene) (Figure 2B). The regulatory requirements for Class A biosolids in Norway are 2500 MPN/gDS (Landbruks- og matdepartementet, 2003) and in the USA the limit is 1000 MPN/gDS (USEPA, 2003).

FIGURE 2

In the Post-AD THP material, i.e. material that was practically sterile before recontamination high viable counts were recorded initially, and fluctuated throughout the incubation, declining to low levels after 13 days (Figure 2A), while the abundance of *uidA* (Figure 2B) peaked 2 days after recontamination and remained high throughout. Adding compost to this material (Post-AD THP+Com) evidently suppressed *E. coli*: the viable counts declined rapidly to very low values after the first spike, and the *uidA* gene abundance remained low compared to that without compost.

In the material that was sterilized before anaerobic digestion (Pre-AD THP), *E. coli* remained low throughout (both viable counts and *uidA* abundance). In the unsterilized digestate from Plant A (TAD), the viable counts of *E. coli* were low throughout, while *uidA* abundance remained high, much higher than in any of the others.

The addition of compost to the Post-AD THP biosolids was effective in reducing the time to reach levels of *E. coli* below the Norwegian and USEPA regulatory requirements for Class A in the Post-AD THP+Com biosolids (Figure 2A). The results show that 13 days after recontamination the levels of *E. coli* in the Post-AD THP biosolids will be within regulatory requirements, and that the addition of compost can reduce this period to 4-8 days (Norwegian or USEPA regulations, respectivly), 2-6 days longer than for the currently accepted method TAD. Pre-AD THP did not exceed the Norwegian regulations after recontamination and had only one day above the USEPA regulations. This is in line with work by other researchers showing that substrate and oxygen availability stimulate growth of fecal coliforms, but that low levels are reached within two weeks (Chen et al., 2011).

The soluble organic material in the biosolids was measured as chemical oxygen demand (COD) in the dewatering liquor of TAD, Pre-AD THP and Post-AD THP. As expected the Post-AD THP had the highest soluble COD concentration (32.3 ± 0.6 mg COD/L, data not shown) due to the thermal hydrolysis treatment immediately before dewatering. TAD had the second largest soluble COD concentration (10.7 ± 0.2 mg COD/L, data not shown) followed by the Pre-AD THP (3.5 ± 0.1 mg COD/L, data not shown). The availability of substrate and oxygen has been identified as important factors stimulating microbial growth in dewatered digestates (Chen et al., 2011). The extent of *E. coli* growth in the biosolids tested in this study can be linked to the concentration of soluble COD in the dewatering liquor. Hence our results are in line with findings from Higgins et al. (2007) and Chen et al. (2011). The faster decline of culturable *E. coli* in Post-AD THP+Com compared to Post-AD THP could therefore be due to the competition for substrate with the compost microbial community and subsequent substrate depletion. The fluctuating levels of culturable *E. coli* for Post-AD THP during the 13-day period (Figure 2A) could be due to the alternation between different carbon feed sources, and the adaptation of the bacterial community to these substrates.

The TAD biosolids had a higher number of *uidA* copies than any of the other biosolids before and after recontamination. This could be due to differences in the amount of extracted DNA between samples but more likely related to the lower treatment temperature this biosolids has experienced (55 °C) compared to the others (165 °C). The latter can be supported by the findings of Kirkegaard et al. (2017) where no overlap between the bacterial community of the digester feed sludge and digestates in plants with THP was detected, in contrast to both mesophilic and thermophilic digestates without THP.

Increased microbial activity due to recontamination can be measured as increased respiration rates and this was investigated using a robotized incubation system.

3.2 Respiration

The rates of CO_2 production throughout the incubation period are shown in Figure 3. The O_2 consumption rates were more or less a replica of these figures because the ratio between O_2 consumption and CO_2 production rates (O_2/CO_2 molar ratio) was ~1

328 throughout the entire incubation of all materials, except for an initially higher O_2/CO_2
329 ratio in Pre-AD THP (first 10-20 hours), as shown in Supplementary Material D.

330 **FIGURE 3**

331 The Post-AD THP control (Figure 3A) should in principle be sterile, hence without any
332 respiration. Nevertheless, we could detect low but significant rates of CO_2 production
333 and equivalent rates of O_2 consumption (Supplementary Material D). This could be due
334 to abiotic reactions, or a combination of abiotic reactions and low biological activity by
335 organisms introduced with the distilled water added at time 0. When this material was
336 contaminated with sewage water (Post AD THP, Figure 3A), a conspicuous series of
337 respiratory spikes were detected at day 1, 3, 5 and 7. In principle, this could be artifacts
338 created by spikes of acidity (releasing CO_2) to the headspace, but the rates of O_2
339 consumption spiked in exactly the same way (Supplementary Material D, Figure SD1B).
340 Thus, there were indeed spikes of respiration throughout in this material. The most
341 plausible explanation is that the bacteria introduced with the sewage water exploited
342 different fractions of the available organic material at different times, depending on the
343 initial number of organisms able to exploit each fraction.

344 Adding compost to the Post-AD THP material (Figure 3B) had a striking effect on the
345 respiration: it was initially low, but increased gradually to peak after 3 days, but
346 remained high throughout compared to that with sewage water only (Figure 3B versus
347 3A). Adding sewage to this material had negligible effects on the respiration in this
348 material, except for a stronger spiking of the respiration after 3 days.

349 The material that had been THP treated prior to AD (Pre-AD THP, Figure 3C) had low
350 respiration rates throughout, peaking at day 2. Adding sewage water to this material
351 (Pre-AD THP versus Pre-AD THP control) enhanced the respiration spike but had
352 otherwise no significant effect on the respiration.

353 The material that had not been THP treated at all (TAD, Figure 3D) had a similar
354 respiration kinetics as Pre-AD THP (Figure 3C), but higher rates throughout, and a
355 stronger peak after 2 days. As for the others, the addition of sewage water had marginal
356 effects apart from a slightly stronger respiration peak after 2 days.

357 The incubation was continued for 25 days, and the cumulated CO_2 at this time point was
358 used to compare the fraction of total organic carbon mineralized in the various

materials, depending on the organisms present. The results (Supplementary Material D, Table SD1) show that after 25 days, the organisms in the compost mineralized 8-9 % of the total C in Post-AD THP, while the organisms in the sewage water mineralized only 2-3 %. The indigenous organisms in the Pre-AD THP mineralized 6-7 % of total C (adding *E. coli* had a marginal effect on this), while the indigenous organisms in TAD mineralized 9-10 %.

Taken together, these results demonstrate that the three materials contain a significant fraction of degradable organic material that can sustain respiratory metabolism and growth, and that organisms in the compost evidently have a capacity to utilize more of these compounds than the organisms in sewage water (including *E. coli*). It appears likely, therefore, that inoculation with compost would minimize the potential of growth of pathogens by scavenging the available carbon substrates. Any antagonistic effects would add to the suppression of pathogens.

3.3 Growth of prokaryotic population (16*S* rRNA)

Total 16S rRNA genes in DNA extracts were quantified (Figure 4) with ddPCR to assess growth and to adjust for bias in DNA extraction when interpreting *uidA* gene copies (see below). As expected, the 16Sr RNA gene abundance was low initially in Post-AD THP but increasing (albeit fluctuating) after recontamination (at day 0), presumably by growth. The first sharp increase in 16S rRNA coincided with the first spike of respiration (Figure 3A) while the subsequent spikes of respiration were hardly reflected in the 16S rRNA abundance. Adding compost to this material (Post-AD THP+Com) resulted in high 16S rRNA from the very beginning (probably compost 16*S* rRNA), a transient decline after 4 days, i.e. just after the transient peak in respiration (Figure 3B), followed by an increase to a relatively stable level throughout the rest of the incubation.

In theory, the spikes of respiration (Figure 3) should reflect high growth rates, hence increase in the 16Sr RNA gene abundance. However, it seems likely that the respiration spikes are due to high respiration (hence net growth) of fractions of the community, and that their net growth is balanced by death of others. The only apparent exception the early net increase in 16S rRNA genes after recontamination of Post-AD THP material without compost, which coincided with the first respiration spike (Figure 3A). It is

interesting to inspect the apparent growth yield per mol O_2 for this period: the net increase in 16S rRNA abundance between day 1 and 2 was $1.2*10^{10}$ copies g^{-1} DS. The oxygen consumption during the same period was 100 µmol g^{-1} DS. If we assume 1 cell per 16S rRNA gene copy, this implies a growth yield of $1.2*10^{14}$ cells mol^{-1} O_2. This is just slightly lower than the growth yield for oxic growth of the model bacterium *Paracoccus denitrificans*, determined to be $1.5*10^{14}$ cells mol^{-1} O_2 (Bergaust et al., 2010).

FIGURE 4

3.4 *E. coli* (*uidA*) compared to prokaryotic population (16S rRNA)

Biases due to inadequate DNA extraction with commercial kits makes it challenging to compare different types of samples. The ratio of *uidA* copies to the prokaryotic population (16S rRNA) was therefore calculated to obtain less biased estimates of the relative abundance of *E. coli* in the different biosolids throughout the incubation. This ratio (*uidA*/16S rRNA) is plotted against time in Figure 5.

FIGURE 5

The *uidA*/16S rRNA abundance ratio was <12 $*10^{-6}$ throughout the entire incubation, hence, *E. coli* constituted a marginal fraction of the total microbial population in all materials.

In the Post-AD THP material without compost, the *uidA/16s* ratio increased sharply during the first two days after contamination with sewage water (Figure 5), coinciding with the first respiration peak in this material (Figure 3A). This suggests that when *E. coli* has no other competitors than those present in the sewage water, it grows faster than the average of the entire population. However, the *uidA/16s* declined gradually throughout the subsequent 5 days (2-7), coinciding with the next two spikes of respiration (Figure 3 panel A). As noted previously, we interpret the spiking of respiration in this material as a succession of different groups of organisms. It is tempting to speculate that the second and third peak of respiration is driven by organisms that suppress *E coli,* either by antagonistic effects or by competing for carbon substrates.

The effect of adding compost to the Post-AD THP is striking, but must be interpreted with care: prior to sewage water treatment (day -2, -1, and 0), the *uidA/16s* ratio was ~10 times lower than that without compost. This is attributable to dilution with compost bacteria (total 16S rRNA was approximately 10 times higher with than without compost, Figure 4). Spiking with sewage water increased the *uidA/16s*, but it remained low except for a spike at day 4. This spike could suggest a late proliferation of *E coli*, but the absolute abundance of *uidA* (Figure 2B) lends no support to this. A more likely explanation is that a fraction of the bacteria introduced with the compost died out at this point in time, seen as a decline in 16S rRNA abundance (Figure 4).

In summary, the gene abundance and the respiration kinetics suggested successive growth/death by different fractions of the organisms introduced with the compost and the sewage water. In order to inspect this, we sequenced 16S rRNA amplicons and analyzed them with bioinformatic tools.

3.5 Diversity

Bioinformatic analysis of 16S rRNA gene amplicons showed differences in both alpha and beta diversity between different biosolids (Figure 6).

FIGURE 6

All tested alpha-diversity matrixes (Figure 6A and Supplementary I) indicate a higher microbial diversity in Post-AD THP when compost was added. Accordingly, compost possessed the highest microbial diversity amongst all samples in the current experiment. It is therefore reasonable to suggest that the addition of compost overall enriches the community in Post-AD THP, thus providing an environment where *E. coli* is rapidly outcompeted. This complies with the lower *E. coli* density observed by the viable count and ddPCR results (Figure 2). Furthermore, the multivariate analysis (Figure 6B and Supplementary Material J) used to assess the community composition within each sample demonstrated that all Post-AD THP+Com samples had a highly similar prokaryotic community throughout the incubation. Overall, spiking with wastewater did not have any noticeable impact on the community composition for the TAD and Pre-AD THP biosolids, as the control samples cluster closely together with its re-contaminated counterparts (Figure 6B). Post-AD THP+Com with and without wastewater also cluster together, and as expected, in close proximity to the compost. Despite originating from the same plant (Plant C), Figure 6B strongly indicates that the microbial composition in Post-AD THP differs severely depending on whether compost was added or not. Moreover, Post-AD THP without compost is the only sample that undergoes a notable temporal change in community composition (Figure 6B; dark blue, filled symbols) after recontamination with wastewater. This supports our interpretation of the conspicuous respiration kinetics in this material: the bursts (spikes) of respiration reflects activity (hence growth) of different fractions of the

459 bacterial community of the sewage water, thus causing major shifts in community
460 composition.

461 Overall, these results suggest that the biosolids from Post-AD THP treatments benefit
462 from addition of compost, providing a more robust and competitive microbial
463 community that effectively minimize the risk for pathogen growth. It may also stabilize
464 the material by mineralizing a larger fraction of available organic material than could be
465 achieved by random contamination of sewage material.

466 The suppression of pathogens by establishing other bacteria has an interesting parallel
467 in aquaculture, where management that sustains a stable microbial community secures
468 better survival of larvae than management that involves frequent disinfection (Vadstein
469 et al., 2018).

470

4 Conclusion

This study presents novel insight into *Escherichia coli* (*E. coli*) recontamination of various biosolids and the addition of a harmless bacteria to suppress growth of *E. coli* in sterilized biosolids. The following observations were made:

1. Addition of compost to sterilized post-anaerobic digestion thermal hydrolysis (Post-AD THP) biosolids reduced the growth of *E. coli* after recontamination with wastewater compared to the sterilized biosolids.
2. The Norwegian and USEPA regulatory requirements were met for the sterilized Post-AD THP biosolids after 13 days. However, the addition of compost reduced this period substantially.
3. Addition of compost to Post-AD THP biosolids provided a more robust and diverse microbial community able to outcompete the *E. coli* for substrate.

In conclusion, pathogen growth in a sterile digestate can be eliminated by inoculation with a complex microbial community.

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Figure legends

Figure 1: Process configurations studied. Thermophilic anaerobic digestion (TAD), pre-anaerobic digestion thermal hydrolysis (Pre-AD THP) and post-anaerobic digestion thermal hydrolysis (Post-AD THP).

Figure 2: Density of *E. coli* throughout the incubation showing the viable counts (MPN) (A) and the abundance of *uidA* gene quantified by ddPCR (B), both plotted against time (time = 0 is the time of recontamination, sampling at day 0 was done before recontamination). USEPA limit of 1000 MPN/gDS is indicated by the black line. It should be noted that the very high MPN values for Post-AD THP at day 1 and 3 are minimum estimates (all MPN tubes were positive).

Figure 3: Respiration rates in robotized incubation system. Respiration of Post-AD THP (A), Post-AD THP+Com (B), Pre-AD THP (C) and TAD (D) recontaminated with wastewater and their respective controls. Samples were recontaminated with wastewater on day 0. One TAD replicate showed large deviation from the two others and was therefore excluded.

Figure 4: ddPCR results of the prokaryotic population targeting the 16S rRNA gene. Recontamination with wastewater was done at day 0. Each recontaminated sample set and controls are enclosed in Supplementary Material F.

Figure 5: ddPCR results of *E. coli* in relation to the prokaryotic population (16S rRNA) in different biosolids.

Figure 6: Microbial diversity (at OTU level) in the different biosolids as expressed through the Shannon index and Community richness (A) and PCoA using the unweighted UniFrac matrix (B). The data is not TSS normalized for the alpha diversity matrices (A) but rarefied to the sample with lowest sequencing depth (55 335 reads). Additional diversity plots are provided in the Supplementary Material I and J.

580 Table 1 Plant specifications. Geographical location, type of anaerobic digestion process
581 and thermal treatment.

Plant ID	**Plant location**	**Anaerobic digestion process**	**Thermal treatment**
Plant A	Norway	Thermophilic – 55 °C	None
Plant B	Norway	Mesophilic – 40 °C	165 °C in 30 minutes (Pre-AD THP)
Plant C	Germany	Mesophilic – 38 °C	165 °C in 40 minutes (Post-AD THP)

582
583

584 Table 2 Primers used for digital droplet polymerase chain reaction

Primer name:	Target:	Sequence:	Amplicon length (bp):	Reference:
PRK341F	*16S*	5'-CCTACGGGRBGCASCAG-3'	465	Yu et al. (2005)
PRK806R	*16S*	5'-GGACTACYVGGGTATCT-3'		
ECF_uidA	*uidA*	5'-CGGAAGCAACGCGTAAACTC-3'	90	Silkie et al. (2008)
ECR_uidA	*uidA*	5'-TGAGCGTCGCAGAACATTACA-3'		

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587

588 Table 3 Digital droplet polymerase chain reaction cycling conditions

	PRK806R/PRK341F			ECG_uidA/ECR_uidA		
	T (°C):	Duration:	Cycles:	T (°C):	Duration:	Cycles:
Enzyme	95	5 min		95	5 min.	
activation/DNA	95	30 sec.		95	30 sec.	
denaturation						
			40			40
Anealing/Extension	55	30 sec.	cycles.	63	1 min	cycles
Extension?	72	45 sec.				
Signal stabilization	4	5 min.		4	5 min.	
	90	5 min		90	5 min.	
Hold	4	Indef.		4	Indef.	

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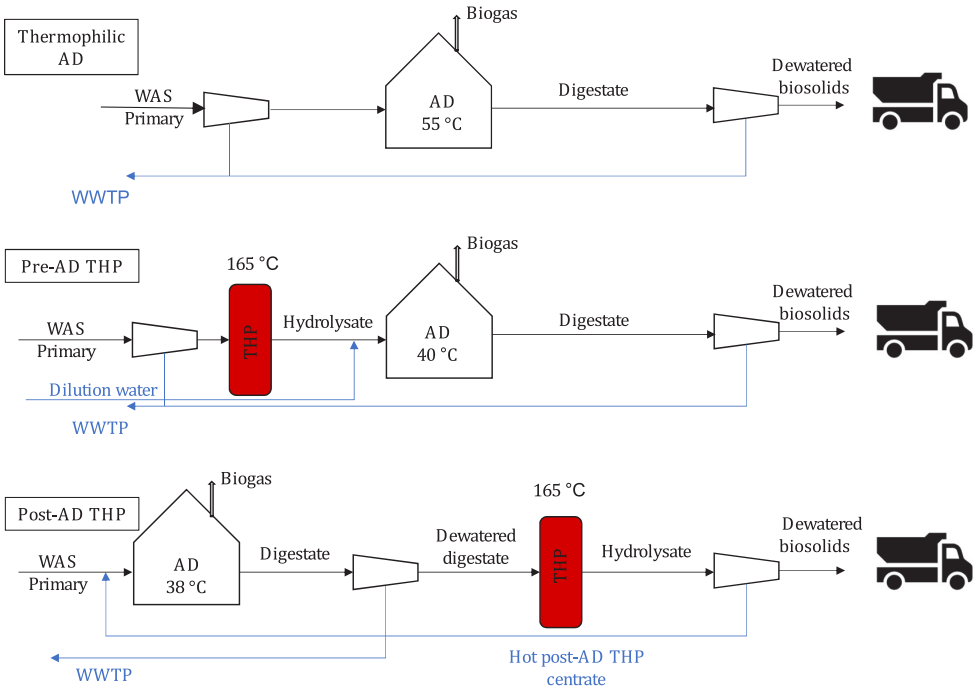

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594 **Figure 1: Process configurations studied. Thermophilic anaerobic digestion (TAD), pre-anaerobic**
595 **digestion thermal hydrolysis (Pre-AD THP) and post-anaerobic digestion thermal hydrolysis**
596 **(Post-AD THP).**

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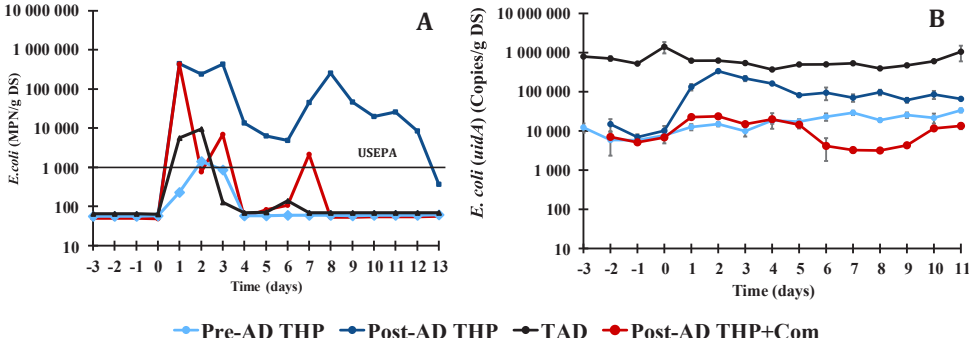

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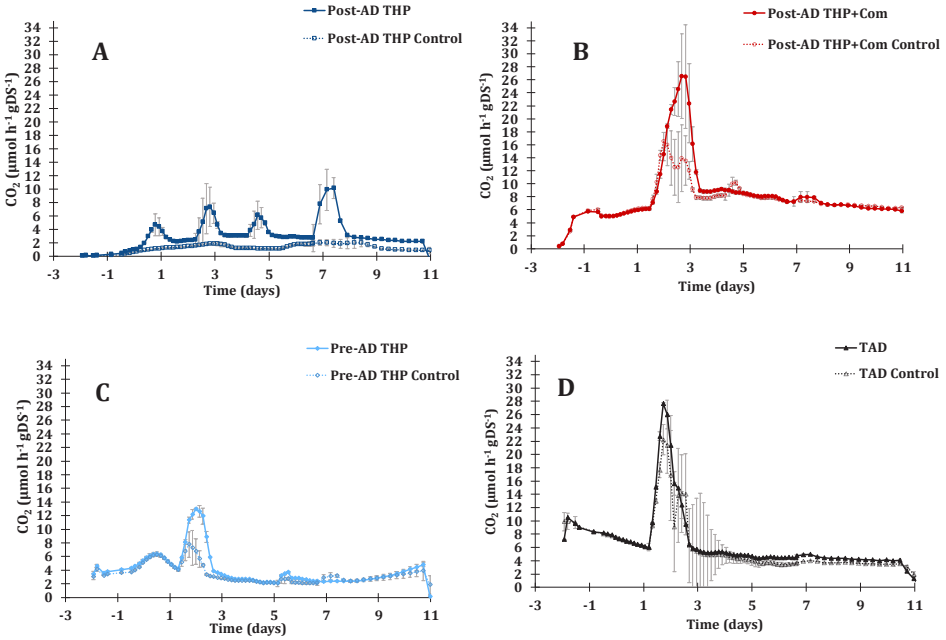

610

611 **Figure 3: Respiration rates in robotized incubation system. Respiration of Post-AD THP (A), Post-**
612 **AD THP+Com (B), Pre-AD THP (C) and TAD (D) recontaminated with wastewater and their**
613 **respective controls. Samples were recontaminated with wastewater on day 0. One TAD replicate**
614 **showed large deviation from the two others and was therefore excluded.**

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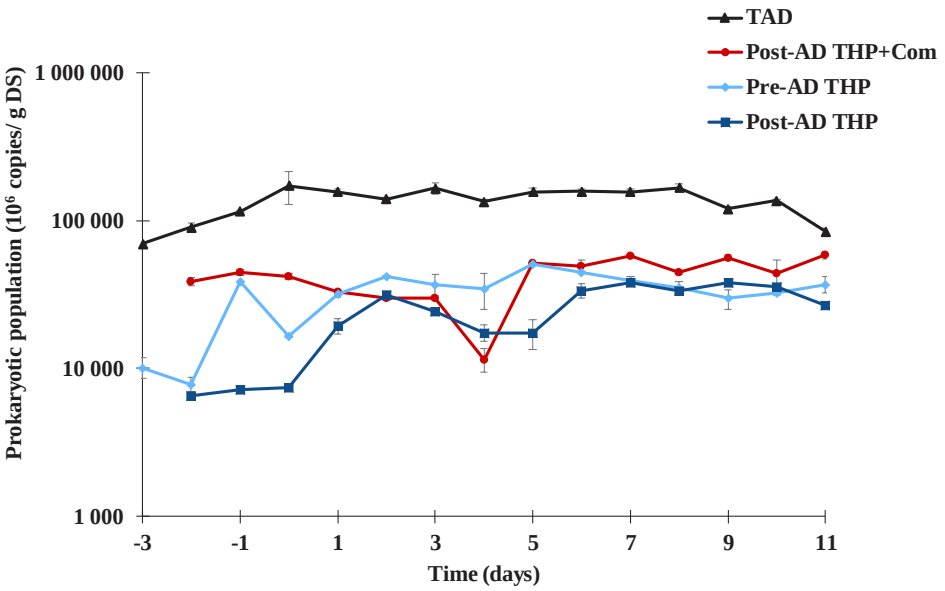

616

617 **Figure 4: ddPCR results of the prokaryotic population targeting the 16S rRNA gene.**
618 **Recontamination with wastewater was done at day 0. Each recontaminated sample set and**
619 **controls are enclosed in Supplementary Material F.**

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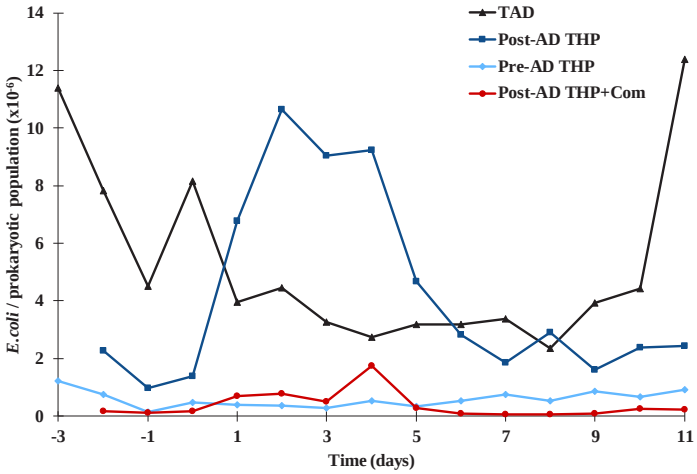

622

623 **Figure 5: ddPCR results of *E. coli* in relation to the prokaryotic population (16S rRNA) in different**
624 **biosolids.**

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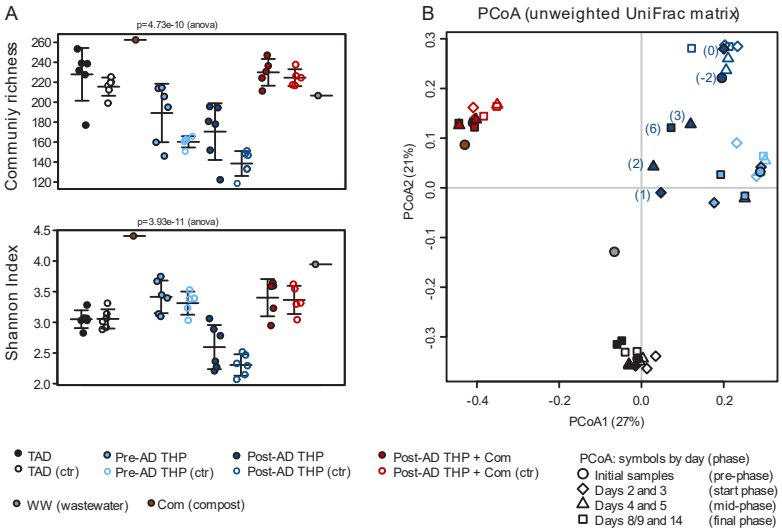

Figure 6: Microbial diversity (at OTU level) in the different biosolids as expressed through the Shannon index and Community richness (A) and PCoA using the unweighted UniFrac matrix (B). The data is not TSS normalized for the alpha diversity matrices (A) but rarefied to the sample with lowest sequencing depth (55 335 reads). Additional diversity plots are provided in the Supplementary Material I and J.

Pathogen growth in sterile digestates can be eliminated by inoculation with a complex microbial community: Supplementary Materials

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[c] Vestfjorden Avløpsselskap, Slemmestad, Norway

[*]Corresponding author: svein.horn@nmbu.no

Supplementary Material A

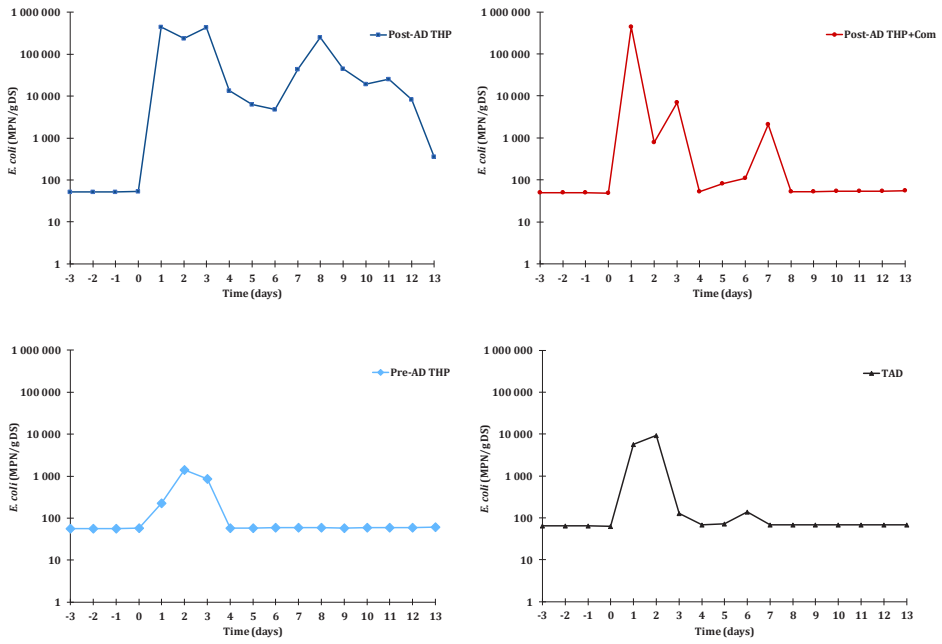

Figure SA1: viable counts of *E. coli* by enumeration method

Supplementary Material B:

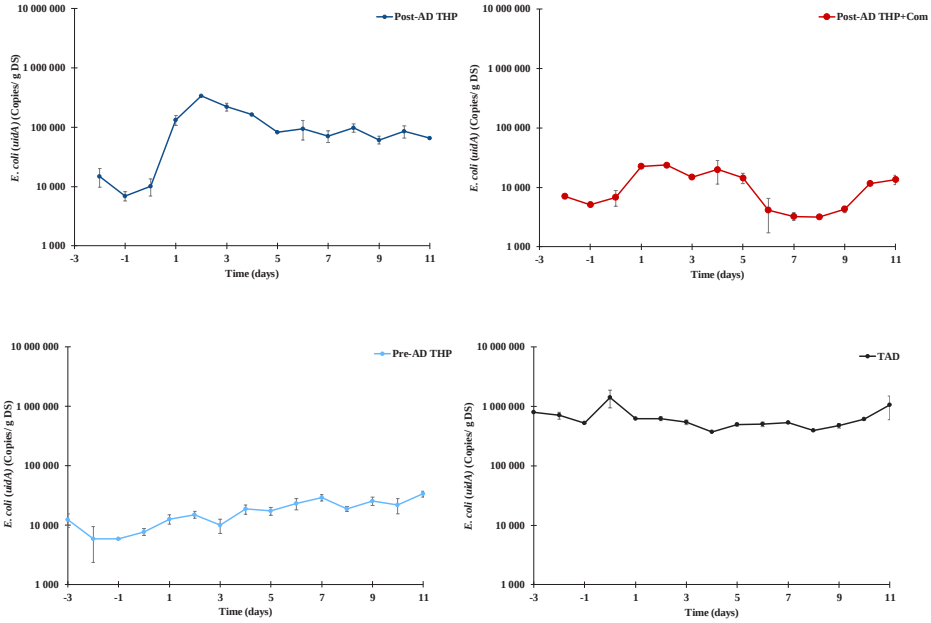

Figure SB1: ddPCR of *E. coli* (*uidA*) of recontaminated samples

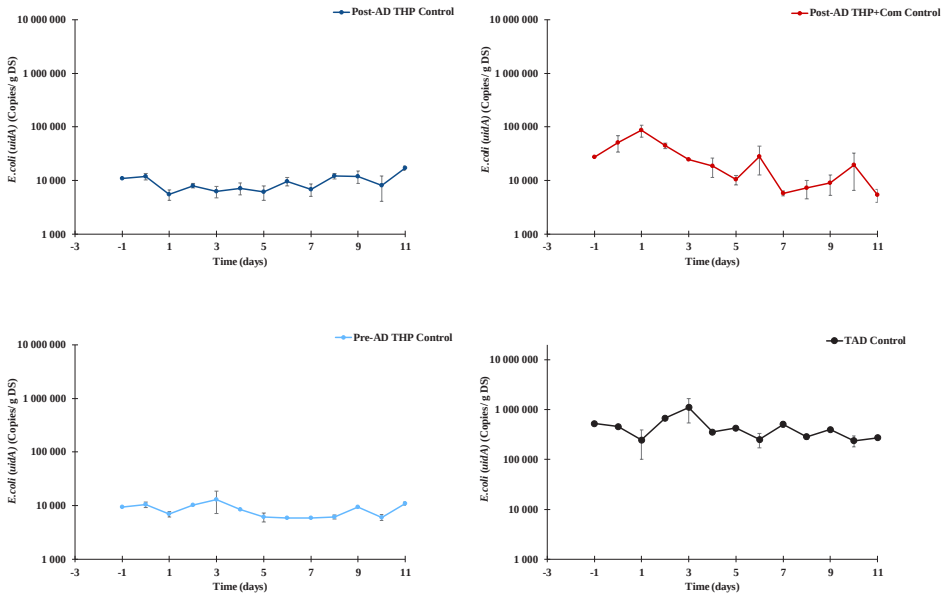

Figure SB2: ddPCR of *E. coli* (*uidA*) of control samples

Supplementary Material C

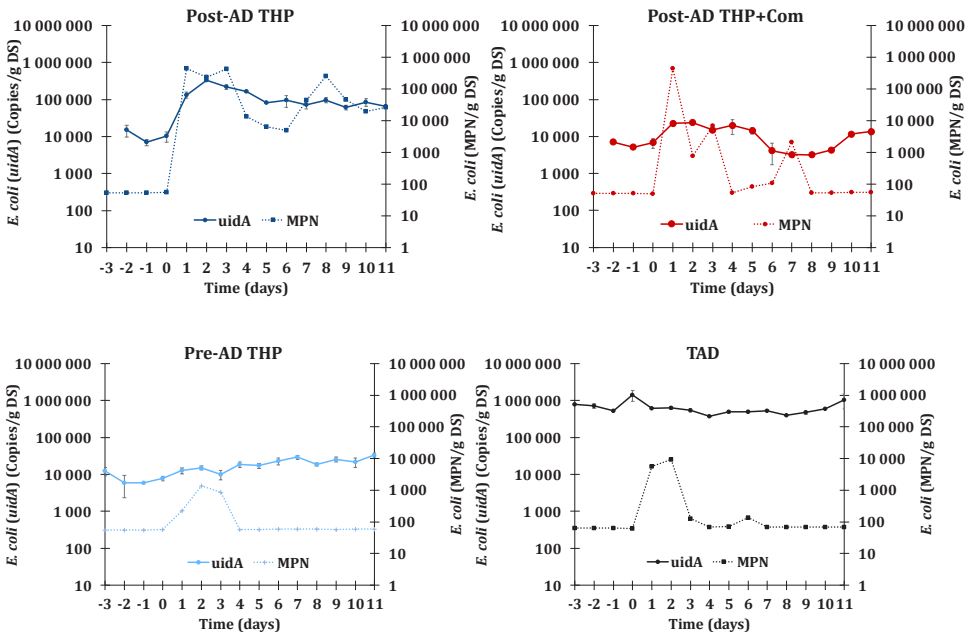

Figure SC1: Viable counts of *E. coli* and *uidA* gene abundance for same sample

Supplementary Material D

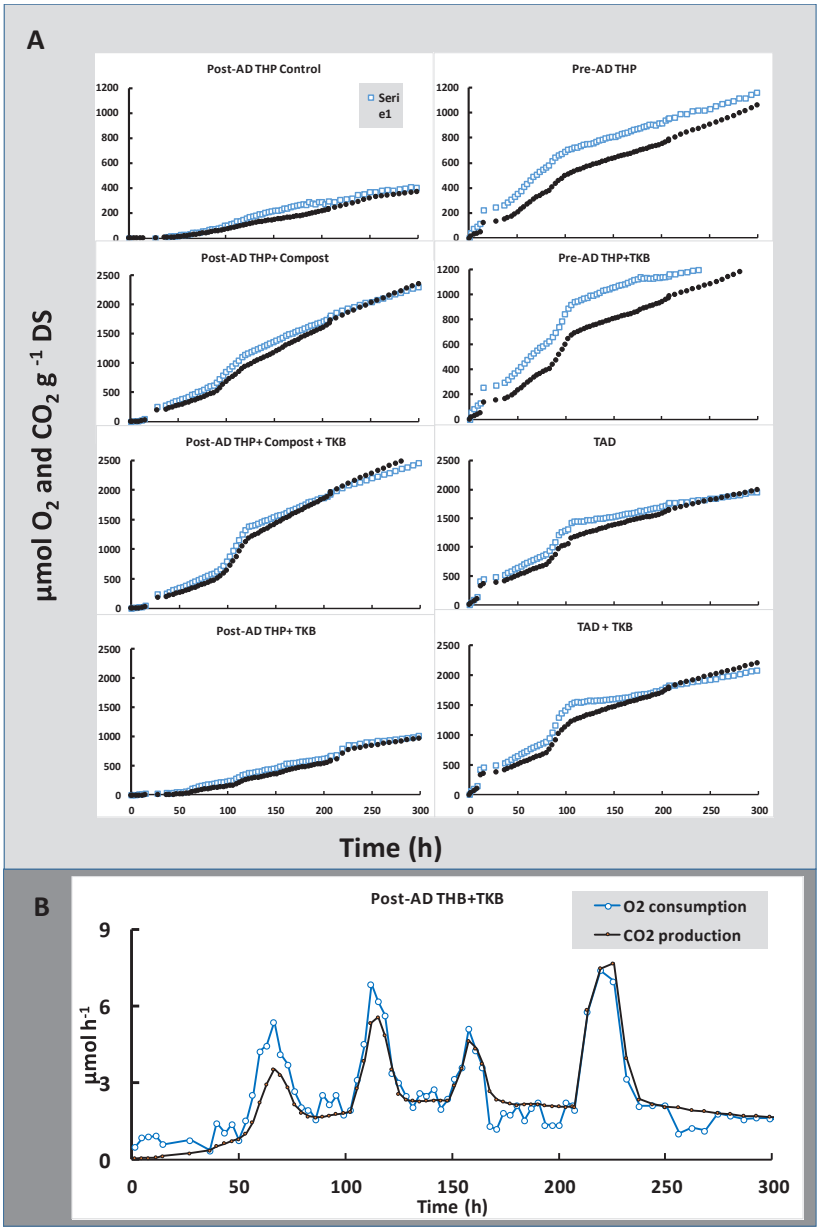

Figure SD1: Ratio between O_2 consumption and CO_2 production for all samples (A) and Post-AD THP (B).

Ratio between O_2 consumption and CO_2 production.

Figure SD1A shows the cumulated O_2 consumption and CO_2 production (μmol $vial^{-1}$) throughout the first 300 hours of incubation. The two curves are practically identical for all materials; hence the molar O_2/CO_2 ratio was ~1 throughout. The only exception was the early phase (0-15 h) for the material that had been THP treated prior to anaerobic digestion (Pre AD THP): here we find that the oxygen consumption rate exceeded the CO_2 production. We speculated that this could be due to high concentration of fatty acids in this material, but the analysis of these acids did not suggest higher concentrations of fatty acids in Pre-AD THP than in the others. A more plausible explanation would be that the early high oxygen consumption was due to abiotic oxidiation of Fe^{2+}. Figure SD1B shows the rates of O_2-consumption and CO_2 production (μmol $vial^{-1}$ h^{-1}) in the material that was THP treated after AD and inoculated with sewage water (after ~48 hours). This is just to demonstrate that the O_2 consumption rate spiked at intervals throughout, exactly as the CO_2 production.

Summary Material D (continued)

To judge the availability of organic C for microbial mineralization, depending on the organisms present, we present the cumulated amount of CO_2 produced after 600 hours of incubation (Table SD1). The unit is mg CO_2-C per g total C in the material (assuming 50% C in dry material). The results for Post-AD THP suggest that the organisms in the sewage water were able to mineralize only 2-3 % of total C in the material within 600 h of incubation, while the compost organisms mineralized 8-9%.

Table SD1: cumulated amount of CO_2 produced after 600 hours of incubation

Material	µmol CO_2 $vial^{-1}$	mg CO_2-C g^{-1} total C
Post AD THP	429	13.7
Post-AD THP+*E. coli*	1126	35.7
Post AD+compost+*E. coli*	3096	92.9
PostAD+Compost+*E. coli*	3169	99.1
Pre-AD THP	1975	66.2
Pre-AD+THP+*E. coli*	2197	74.7
TAD	2447	94.0

Supplementary Material E

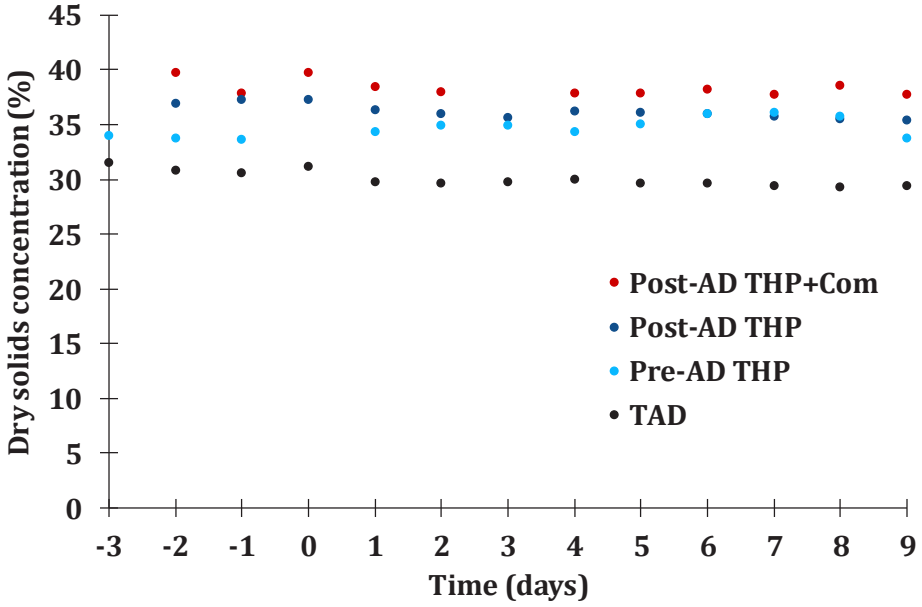

Figure SE1: Dry solids concentration of samples from day -3 to 9.

Supplementary Material F

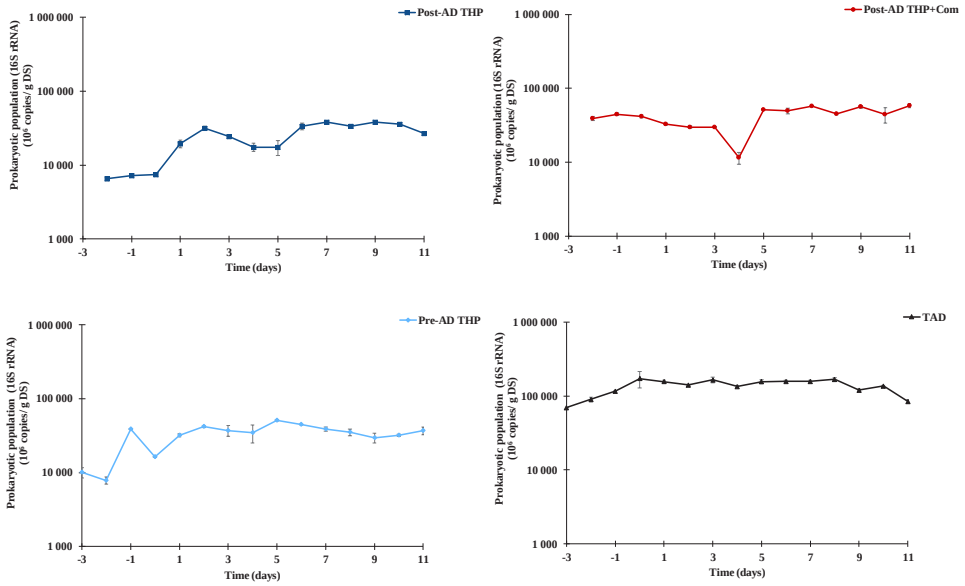

Figure SF1: ddPCR of 16S rRNA recontaminated samples

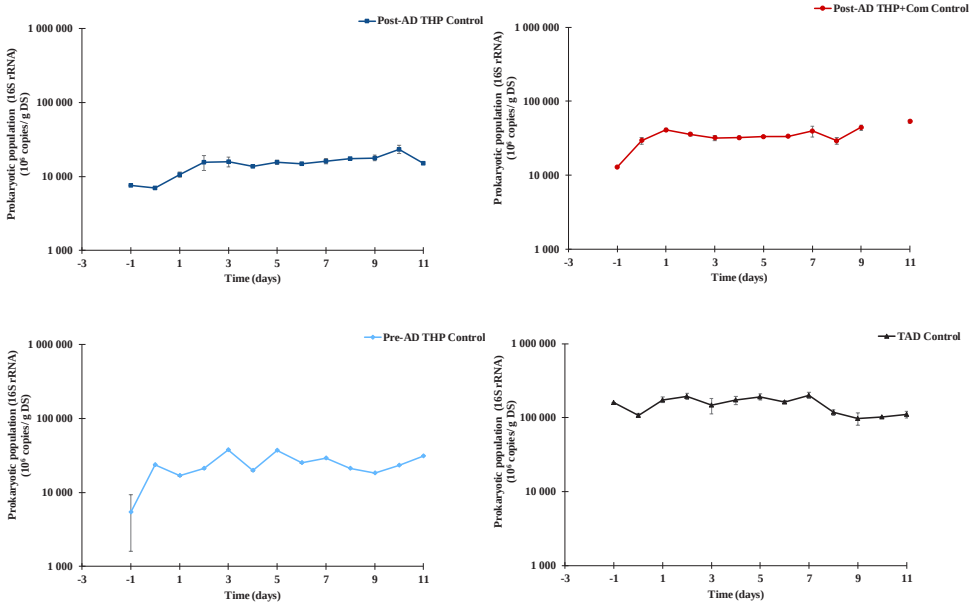

Figure SF2: ddPCR of 16S rRNA control samples

Supplementary Material G

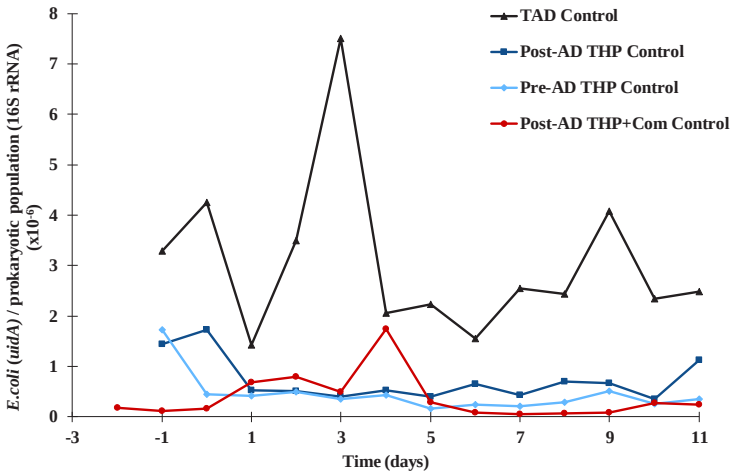

Figure SG1: ***E. coli(uidA) /*** **prokaryotic population (16S rRNA) of control samples**

Supplementary Material H

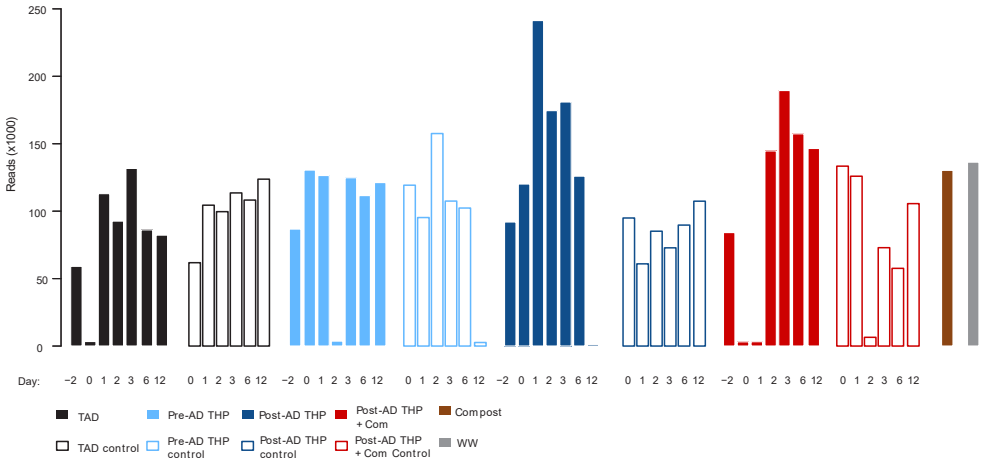

Figure SH1: Sequencing reads per sample

Supplementary Material I

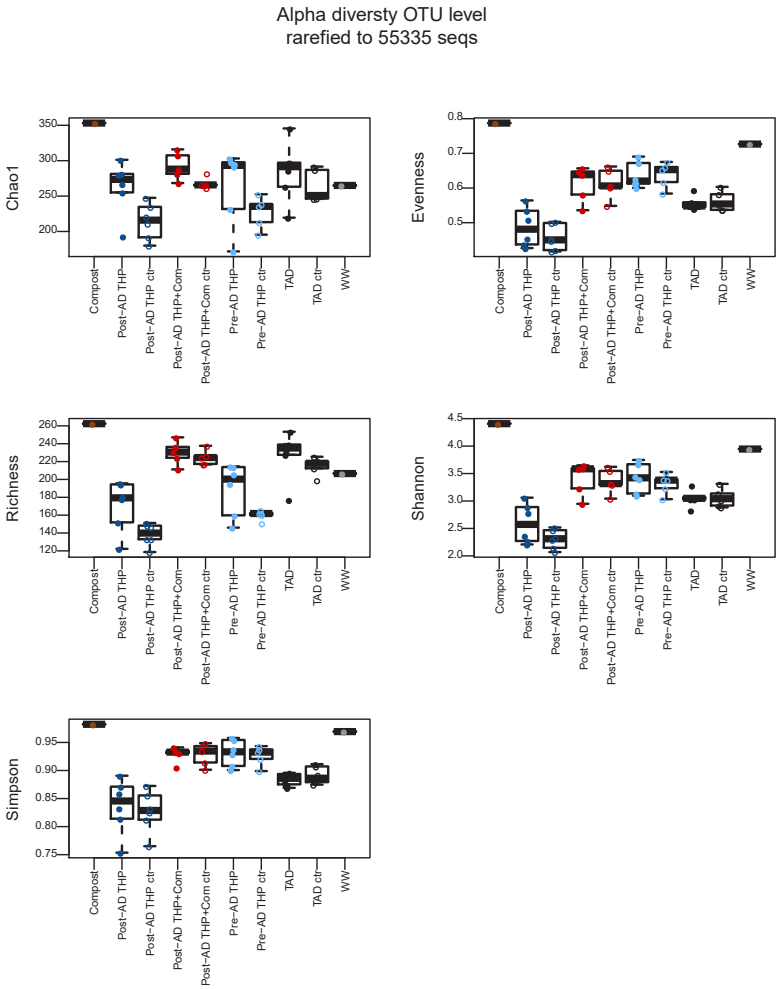

Figure SI1: Alpha diversity metrices at OTU level. Chao1, Evenness, Richness, Shannon and Simpson.

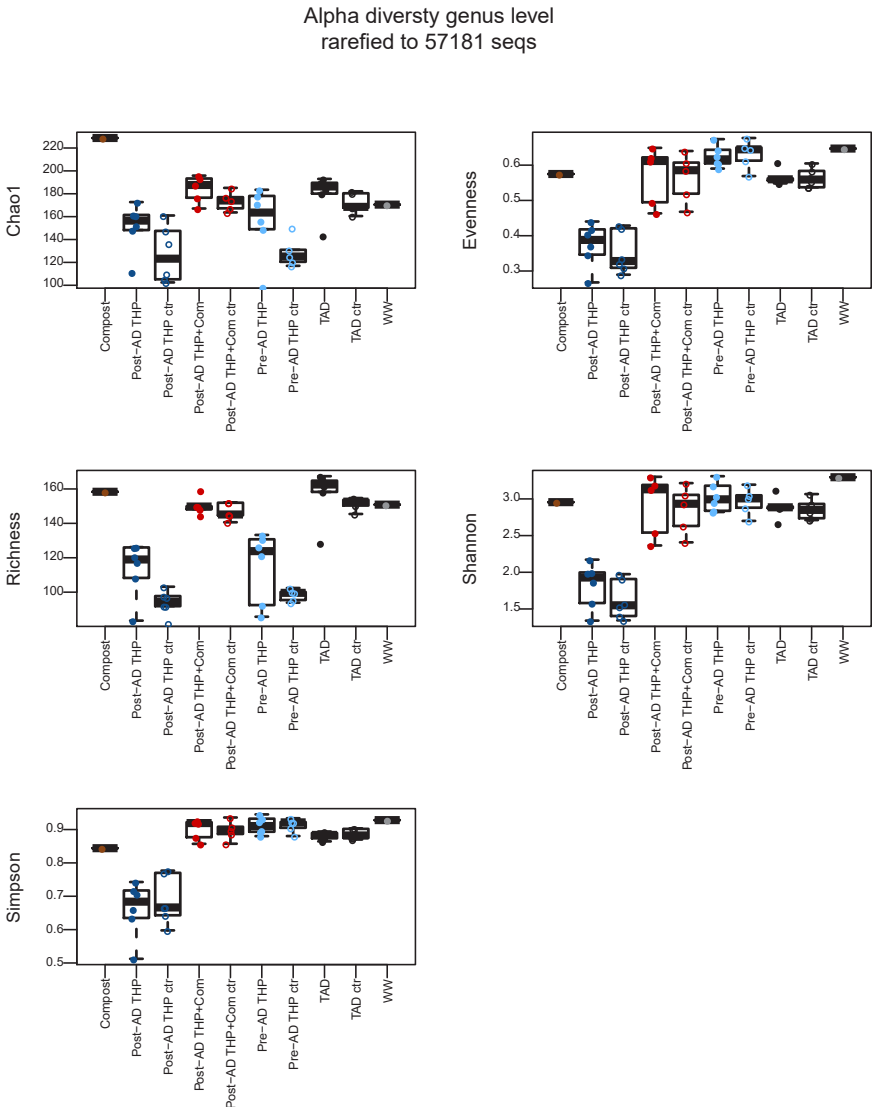

Figure SI2: Alpha diversity on genus level. Chao1, Evenness, Richness, Shannon, Simpson.

Diversity vs. Time

All diversity matrices are based on OTUs

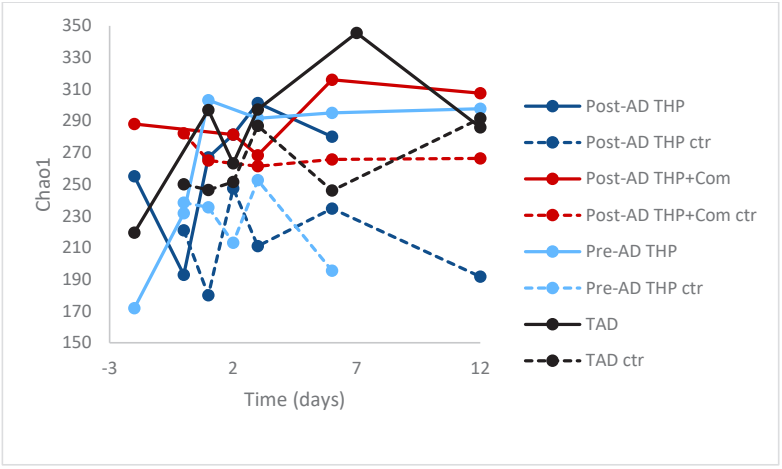

Figure SI3: Alpha diversity calculated with Chao1 for all samples vs time

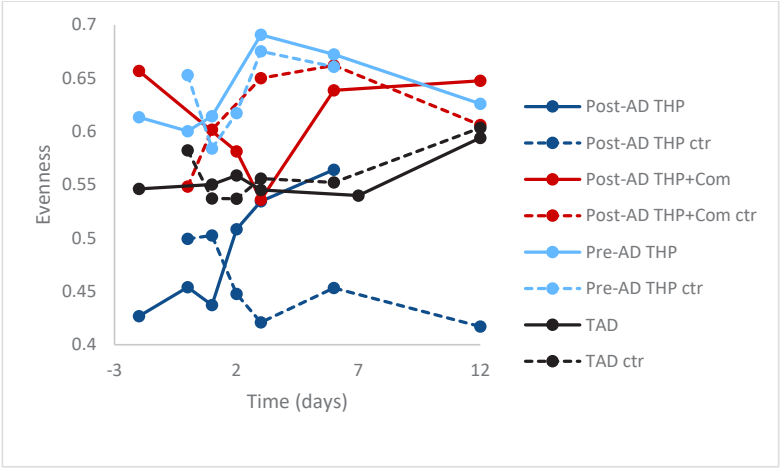

Figure SI4: Alpha diversity calculated with Evenness for all samples vs time

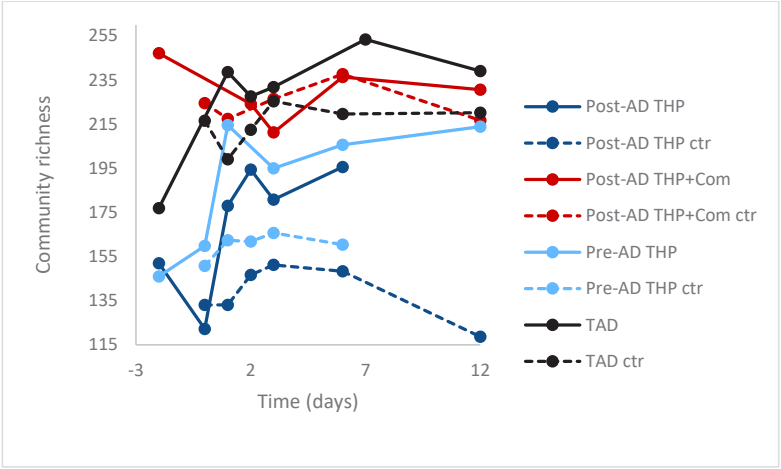

Figure SI5: Alpha diversity calculated with Community richness for all samples vs time

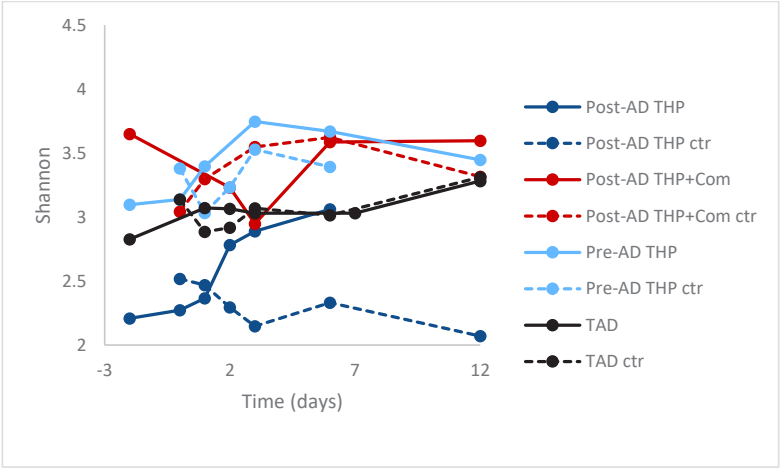

Figure SI6: Alpha diversity calculated with Shannon index for all samples vs time

Supplementary Material J

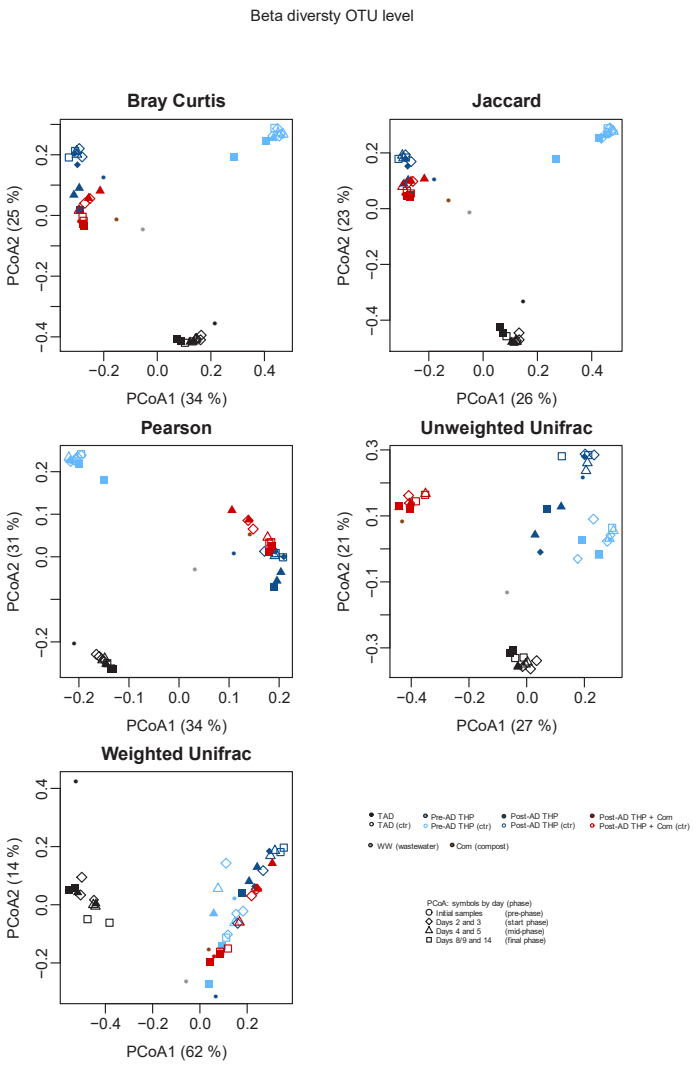

Figure SJ1: Beta diversity metrices at OTU level. Bray Curtis, Jaccard. Pearson, Unweighted Unifrac and Weighted Unifrac.

Errata list

Page/Paper	Line	Changed from	Changed to
Xi/xii		WWTP moved from page xi	WWTP moved to page xii
Paper II	103	digestates,	digestates and
Paper II	203	Figure 2	Figure 1
Paper II	205	L-V	L-U
Paper II	207	Figure 2	Figure 1
Paper II	211	Figure 2	Figure 1
Paper II	216	Plant P, U and V	Plant P, T, and U
Paper II	219	Plant P, U and V	Plant P, T, and U
Paper II	245	Plant Y	Plant X
Paper III	267	Plant A, B and C	Plant A, B and E
Paper III	268	Plant A, B and C	Plant A, B and E
Paper III	281	suggest	suggests
Paper III	328	an	and
Paper IV	296	digestated	digestate
Paper IV	404	Figure 5	Figure 1
Paper IV	411	Figure 6	Figure 2
Paper IV	416	Figure 7	Figure 3
Paper IV	420	Figure 8	Figure 4
Paper V	311	(Figure 1A)	(Figure 2A)
Paper V	569	Supplementary Material G	Supplementary Material F
Paper V	619	Supplementary Material G	Supplementary Material F

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