Effect of anaerobic digestion temperature on sludge quality

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Assignment of Diploma Thesis

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Study Programme: Environmental Technology and Engineering
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Specialisation:

Subject of Diploma Thesis:
Effect of anaerobic digestion temperature on sludge quality

Directions for Elaboration:
Make the literature review on the topic of the main characteristics and differences between mesophilic and thermophilic anaerobic digestion of sludge. Find the details concerning sludge quality characterization and select the methods which can be used for sludge quality comparison.

In the practical part of your thesis operate two laboratory-scale digesters for the study of the mesophilic and thermophilic sludge anaerobic digestion process products. Compare the quality of digested sludge, reject water and biogas with special attention to the sludge VSS/TSS ratio, dewaterability and foaming properties.
Recommended Literature:


and other according to the instructions of supervisor.

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Head of Department

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Dean

Prague 16.02.2015
DECLARATION

This thesis/dissertation was written at the Department of Water Technology and Environmental Engineering of the University of Chemistry and Technology in Prague, The Czech Republic, 2015

I hereby declare that this thesis is my own work. Where other sources of information have been used, they have been acknowledged and referenced in the list of used literature and other sources.

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In Prague,
on 21, August 2015
Acknowledgment

First and for most I am grateful to God and mother of Jesus who gave me the survival and ability to go through not only the thesis but through the entire steps since I left home. God gives me the caliber when times brings convergent stresses.

My heartfelt thank goes to my supervisor, Professor Ing. Pavel Jenicek. I am not exaggerating when I say he is one of the very few persons who influenced my life. With him I learned a lot, his wisdom, politeness, pragmatic and curious supervisory roles will remain in my heart.

I want to thank all members of the anaerobic digestion research team in the department of water and environmental engineering, University of Chemistry and Technology, Prague, Czech for their contributions to this thesis one way or another and for their team spirit.

I would also like to thank Mariajesus Matamala Venegas-Puga of Chile especially for coming to the laboratory with me during the week ends.
Abstract

Sludge management takes great share of the cost spent in the wastewater treatment plants (WWTPs) where sludge anaerobic digestion (AD) is one such major unit. Sludge AD is practiced mainly because of its environmental and energy advantages. Sludge quality after AD in terms of dewaterability, reject water characteristics as well as foaming phenomena is a concern either economically or environmentally. In that respect this thesis compared the difference in sludge quality between mesophilic and thermophilic waste activated sludge (WAS) after AD using completely stirred tank reactors (CSTRs).

Based on the VFAs, pH, and gas composition as well as volatile solid (VS) degradation follow ups there were no major process stability problems encountered by both temperature CSTRs. However following the increase in the OLR, the thermophilic CSTR is often found to be the slower to adapt based on the biogas yield. Hence the biogas yield and percentage methane of the thermophilic CSTR is behind the mesophilic despite its fast hydrolysis and relatively better VS destruction.

Regarding the results for the mesophilic sludge, the mean CST (seconds), extent of dewaterability (% water removed), ammonia nitrogen (Nammon in mg/l) and the soluble COD (CODsol in mg/l) are 852±180, 62.9±1.7, 1484±153.5, and 2315.7±407.6 respectively. Meanwhile the foaming potential (FP), foam stability (IS) and the specific biochemical methane yield in ml-CH₄/g-VSS are 4.4±1.7, 0.7±0.12 and 193 respectively. Whereas for the thermophilic sludge, the mean CST, extent of dewaterability, Nammon and CODsol are 1109±211, 65±1.8, 1581±120.5, and 4740.6±1122.8 respectively. Meanwhile the FP, IS and specific biochemical methane yield are 8.9±1.6, 0.4±0.09 and 258.5 respectively.

Based on our study with a maximum organic loading rate (OLR) achieved at 2.82 g-VS/l/d, the CST (p-value≈0), Nammon (p-value≈0.01) and CODsol concentration (p-value≈0) as well as the FP (p-value≈0) are significantly better as quality for the mesophilic sludge. However, the CST studied on batch test AD did not show a significant difference (p-value≈0.2) between the two temperature conditions. Conversely, the centrifugal dewaterability (p-value≈0.2) between the two temperature conditions. Conversely, the centrifugal dewaterability (p-value≈0.2) and the IS (p-value≈0) are significantly better as quality for the thermophilic sludge. Further the biochemical methane yield from the thermophilic sludge batch AD is higher than the mesophilic counterpart along with better volatile suspended solid (VSS) removal which is 81% compared to the 76% VSS removal by the mesophilic WAS batch AD.
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AD/AD:</td>
<td>Anaerobic digestion / Anaerobic digester</td>
</tr>
<tr>
<td>ANNAMOX:</td>
<td>Anaerobic ammonium oxidation</td>
</tr>
<tr>
<td>APHA:</td>
<td>American Public Health Association</td>
</tr>
<tr>
<td>AWWA:</td>
<td>American Water Works Association</td>
</tr>
<tr>
<td>BMP:</td>
<td>Biochemical methane potential</td>
</tr>
<tr>
<td>Centri_dewater:</td>
<td>Centrifugal dewaterability</td>
</tr>
<tr>
<td>COD:</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>CODt:</td>
<td>Total chemical oxygen demand</td>
</tr>
<tr>
<td>CODsol:</td>
<td>Soluble chemical oxygen demand</td>
</tr>
<tr>
<td>CST:</td>
<td>Capillary suction time</td>
</tr>
<tr>
<td>CSTR:</td>
<td>Completely stirred tank reactor</td>
</tr>
<tr>
<td>CWWTP:</td>
<td>Central wastewater treatment plant</td>
</tr>
<tr>
<td>DM:</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DS:</td>
<td>Dissolved solid</td>
</tr>
<tr>
<td>EC:</td>
<td>European Commission</td>
</tr>
<tr>
<td>EEC:</td>
<td>European Economic Commission</td>
</tr>
<tr>
<td>EPS:</td>
<td>Extracellular polymeric substance</td>
</tr>
<tr>
<td>EU:</td>
<td>European Union</td>
</tr>
<tr>
<td>FP:</td>
<td>Foaming potential</td>
</tr>
<tr>
<td>FS:</td>
<td>Fixed solid</td>
</tr>
<tr>
<td>GC:</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HRT:</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>Nammon:</td>
<td>Ammonia nitrogen</td>
</tr>
<tr>
<td>OLR:</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>PC-WWTP:</td>
<td>Prague central wastewater treatment plant</td>
</tr>
<tr>
<td>Rpm:</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>RTF:</td>
<td>Resistance to filtration</td>
</tr>
<tr>
<td>SRT:</td>
<td>Solid retention time</td>
</tr>
<tr>
<td>tDM:</td>
<td>Tone of dry matter</td>
</tr>
<tr>
<td>TS:</td>
<td>Total solid</td>
</tr>
<tr>
<td>TDS:</td>
<td>Total dissolved solid</td>
</tr>
<tr>
<td>TSS:</td>
<td>Total suspended solid</td>
</tr>
<tr>
<td>UK:</td>
<td>United kingdom</td>
</tr>
<tr>
<td>USEPA:</td>
<td>United states environmental protection agency</td>
</tr>
<tr>
<td>VFA:</td>
<td>Volatile fatty acid</td>
</tr>
<tr>
<td>VM:</td>
<td>Volatile matter</td>
</tr>
<tr>
<td>VS:</td>
<td>Volatile solid</td>
</tr>
<tr>
<td>VDS:</td>
<td>Total dissolved volatile solid</td>
</tr>
<tr>
<td>VSS:</td>
<td>Volatile suspended solid</td>
</tr>
</tbody>
</table>
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1. Introduction

Wastewater treatments plants (WWTPs) are huge management facilities to existing and emerging cities. As a by-product of the WWTP operations, immense primary and secondary sludge is produced and its management is becoming a challenge either economically or environmentally. For instance, in the European Union (EU) alone about 30,000 tons of sludge dry mass (DM) are generated every day with projection of 10% increase by 2020 (Jiang et al., 2014). In terms of expenditure, 30-40% of the capital cost and 50% of the operational cost of these plants is taken up by the sludge management (Appels et al., 2008, Lau et al., 2013).

The economic inefficiencies of WWTPs and the huge volume of sludge with regard to storage, transport and stabilization are issues of concern that have to be dealt by professionals in the field in order to solve such challenge and optimize cost of those WWTPs. Though there are alternatives to sludge management, recently the down side with dumping, land filling and incineration triggered the need for other better alternatives. Thus the reuse of sludge for land application and recovery of energy are getting much attention along with other valorization efforts. In this regard, the anaerobic digestion (AD) of sewage sludge is favored for its advantages over others. Among its advantages, the process simplicity, the recovery of clean energy (biogas), a reduction of 30 to 50% of the sludge volume, the destruction of pathogens, removal of bad odor, land application of the stabilized sludge are the main ones (Nges and Liu, 2010).

On the other hand, the volume of sludge AD reactors, transport and storage of sludge, the reject water characteristics as well as the recovery of those plant nutrients within the sludge (Verstraete and Vlaeminck, 2011) are among the main drivers to still deal with sludge AD. In relation to those issues described, the temperature of sludge AD has to be considered for its effect on sludge dewaterability, reject water ammonia and the CODsol content, foaming phenomena as well as the biogas yield aspects of the sludge handling.

In the past most reports regarding comparison of mesophilic and thermophilic AD are focused on biogas yield and biogas quality. Regarding the energy advantages, some reports show thermophilic sludge AD is preferred to mesophilic one (Zabraska et al., 2002), (Ge et al., 2011) and (Gavala et al., 2003). Though the heat demand by the mesophilic reactors is lower than the demand by the thermophilic. Issues of process stability with regard to operational conditions are also compared between these two thermal systems (Smith & McCarthy, 1986) & (De la Rubia, 2002) in favor of mesophilic systems for better stability. However, most reports are still conflicting on the biogas yield and quality, the reject water as
well as the digestate quality issues (CHI et al., 2010), (T. AMANI, 2011) and (Cavinato et al., 2013) where the latter issues are even hardly explored. Thus, in this thesis it is hypothesized that operating temperature of the sludge AD would have a consequence on the digestate and reject water quality, foaming potential and stability (FP & IS) between mesophilic and thermophilic sludge AD in a continuously stirred tank reactor (CSTR).

Further, the methanogenic activity test or the biochemical methane potential (BMP) assay on waste activated sludge (WAS) is run in batch bottles along with pure cellulose and acetic acid substrates. All the tests are performed using standard methods. As a result, some study parameters like foaming phenomena and soluble COD (CODsol) concentration in the reject water showed a statistically significant disparity in favor of the mesophilic sludge. Conversely, other parameters like the foam stability and centrifugal dewaterability as well as the BMP level show promising results in favor of the thermophilic sludge as detailed in the result section of this document.

2. Rationale of the study

The general motivation for this research is economic and environmental concern with regard to sludge management, particularly WAS AD. Despite the fact that the WWTPs have the potential to be energy self sufficient and be source to recyclable nutrients as well as water, it is known that WWTPs consumes huge energy for aeration, heating and other related processes units that make this sector costly or economically demanding and it is still environmentally a concern too. To approach these problems, the energy recovery via AD is given attention and sludge AD is an existing practice in the WWTPs. At the same time, sludge AD is a concern due to the quality of the reject water and the digestate with respect to environmental pollution and sludge storage as well as transport. These concerns are possible affected by the process parameters specially temperature of sludge AD. Therefore the rationale for this study is to contribute to the optimization of sludge AD from comparison of mesophilic and thermophilic sludge AD with respect to sludge dewaterability and reject water characteristics as well as foaming phenomena. It also conducted a BMP assay on WAS. As a result, this study provides scientific evidences to an informed decision in the sector. It also provides such facts and figures to related studies and also identifies gaps to be filled in upcoming studies.
3. Literature review

3.1. Typical composition of sewage sludge and the discharge rules

The composition of sludge is a result of the source and treatment process applied to it. The major components of typical sludge are water and organic matter. The organic fraction in sludge is expressed in terms of the volatile solid (VS) or COD, suspended (SS) or dissolved solids (DS), as well as nitrogen and phosphorus contents. Those solids expressions are also important from the technological point of view like the loading rate determination to digesters.

In regard to the degree of demand for treatment, various national and international rules are set for different type of sludge use and disposal. For example, the EU has regulation under the title of Urban Wastewater Treatment Directive 91/271/EEC which sets the minimum levels to be met prior to discharge to surface water based on the population size. The nitrogen and phosphorus limits apply to sensitive areas mainly to freshwater lakes. Similarly there are rules with regard to pathogen, odour and stability of sludge. The sludge composition is classified in to the following categories according to the report of the European Commission (EC), 2001.

A : primary sludge, primary sludge with physical/chemical treatment or high pollution load
B1 : biological sludge (low load)
B2 : biological sludge from clarified water (low and middle load)
C : mixed sludge (mix of A and B2 types)
D : digested sludge. The respective sludge composition is thus summarized in table1.

Table 1. The composition of wastewater sludge with respect to the treatment applied

<table>
<thead>
<tr>
<th>Content</th>
<th>Unit</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Dry matter</td>
<td>g/L</td>
<td>12</td>
</tr>
<tr>
<td>Volatile matter</td>
<td>%DM</td>
<td>65</td>
</tr>
<tr>
<td>C</td>
<td>%VM</td>
<td>51.5</td>
</tr>
<tr>
<td>H</td>
<td>%VM</td>
<td>7</td>
</tr>
<tr>
<td>O</td>
<td>%VM</td>
<td>35.5</td>
</tr>
<tr>
<td>N</td>
<td>%VM</td>
<td>4.5</td>
</tr>
<tr>
<td>S</td>
<td>%VM</td>
<td>1.5</td>
</tr>
<tr>
<td>C/N</td>
<td>Unit less</td>
<td>11.4</td>
</tr>
<tr>
<td>P</td>
<td>%DM</td>
<td>2</td>
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<tr>
<td>Cl</td>
<td>%DM</td>
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</tr>
<tr>
<td>K</td>
<td>%DM</td>
<td>0.3</td>
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<tr>
<td></td>
<td>%DM</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Al</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ca</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Fe</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mg</td>
<td>0.6</td>
<td>0.6</td>
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<td>Fat</td>
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<td>8</td>
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<tr>
<td>Protein</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>Fibers</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Calorific value</td>
<td>kWh/DM</td>
<td>4200</td>
</tr>
</tbody>
</table>

(Comission, 2001b, Comission, 2001a)

### 3.2. Anaerobic digestion and process stability

As a system, AD consists of a series of microbiological processes that convert organic compounds to methane and carbon dioxide, hydrogen sulfide, water and other products in the absence of oxygen. It reduces the VS in the fed sludge by 35%-60%, depending on the operating conditions and sludge quality. As a complex and synthrophic microbiological process, AD mainly involves bacteria and archaea. The major synthrophic processes can be classified into four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis and these processes happen at the same time with different degree of conversions (Figure 2). In most cases the 70% methane is produced from the acetate and the rest from the hydrogen. 

The hydrolysis stage is the rate limiting stage that splits polymers mainly into monomers and oligomers. The acidogenesis stage is the transformation of monomers into short and medium chain acids and alcohols which is a concern due to the acidification of digesters that marks the need to monitor digesters to avoid process failures. The acetogenesis stage is the conversion of short chain acids to hydrogen, carbon dioxide and acetate. In the methanogenesis stage the carbon dioxide, hydrogen and acetate are converted mainly to methane and water.

The conversion of acetate to methane is the major pathway in AD. All the processes are taken place under very low oxidation reduction potential with reference to hydrogen and hence for the methanogenesis it goes down to -300mV or has to be less than -200mV (Appels et al., 2008). The methanogenesis stage is performed by the most sensitive microbes of the anaerobic process that belong to the third domain of life-archaea. The two major species within archaea are the hydrogenotrophic ones which feed on hydrogen and the acetoclastic ones which feed on the acetate. The species involved are those *Methanoseta concilii*, *Methanobacterium beijingense* and *Methanosarcina formcoccus* and others (Bitton, 2005), (Ho et al., 2013).

With respect to sludge AD, biogas can be recovered from the raw sewage sludge, which is mostly difficult to handle, or from use of the waste activated sludge (WAS) or a mixture of both (Figure 1).
The challenges with AD including the sludge AD are process instability, long startup and digestion time, mal-odorous nuisance mainly due to H$_2$S, and vulnerability to xenobiotic compounds. Process stability in AD in fact depends on the type of substrate used; the higher the degradability of the substrate the more the process instability. Process stability in AD is also dependent on the stages of degradation by the different organisms doing the job in the system (Figure 2). In this aspect of AD, process stability may impact the thermophilic AD differently from that of the mesophilic.

Source: (Weedermann et al., 2013)
Although the overall process in AD is exergonic (the change in Gibb's free energy is negative), some steps such as propionate oxidation are sensitive to build up of H₂ which is an issue of process stability. This process has to be in syntrophy with methanogens or sulfur reducers to proceed under standard conditions. Kaspar & Wuhrmann clearly stated that hydrogen and acetate are inhibitors of microbial degradation of propionate and ethanol hence the partial pressure of hydrogen and alkalinity have to be used as a monitoring parameters to sludge AD (Lee and Zinder, 1988), (Kaspar and Wuhrmann, 1978).

In complex waste mixtures like sludge 30% of the electron flow occurs through oxidation of fatty acids. Thus there is high production of the diatomic hydrogen which has to be reduced by the organisms responsible in order for the process to proceed safely. Propionate is the most produced intermediate in AD of organics. Propionate oxidation produces hydrogen molecule whose partial pressure has to be between 10^-4 and 10^-6 atmosphere for the system to operate efficiently and for the oxidation of ethanol its value ranges 10^-1 and 10^-6 (McCarty and Smith, 1986).

Appels and colleagues (2008) noted that process stability and control is more an issue to thermophilic anaerobic reactors compared to mesophilic. The reason to that is due to the increase of free ammonia in the system which inhibits the microorganisms therein. The amount of free ammonia depends on the total ammonia, temperature and pH in the system. Free ammonia increase with pH and temperature. The resulting instability leads to an increase of volatile fatty acids (VFAs) that causes a decrease in pH and hence a decrease in free ammonia that enables the process to remains stable. However, the amount of methane production is reduced due to ammonia. A concentration of free ammonia ranging 560–568mg NH₃-N/l leads to inhibition of 50% methanogenesis at pH 7.6 under thermophilic conditions. Total inhibition can occur at a concentration of 10 g-N/l. A VFA concentration of 2g/l is also reported to inhibit cellulolytic hydrolysis dictating that the concentration as acetic acid has to be kept lower than the inhibitory concentration (Apples et al., 2008), (Sung and Liu, 2003), (Rajagopal et al., 2013) (Table 2). Generally ammonia concentration of less than 200mg/l is desired to ADs since nitrogen is also necessary for microbes.

Regarding the VFAs, the acids are various in ADs and have cooperative effect on the bacteria and archaea working there. Thus the individual concentration of those short as well as medium molecular sized acids do matter in the process stability with varying magnitude of the impact. For example, an acetic acid concentration of 2400mg/l and butyric concentration of 1800mg/l do not have inhibitive effect on methanogens. However, a propionic acid concentration of 900mg/l resulted in a significant inhibition of the methanogens (Franke-Whittle et al., 2014). This emphasizes the prior need to monitor propionate acids in ADs.

In a rather different view point, Rubia and co-workers stated in their review that another issue of process stability of concern with the thermophilic digestion of sludge is related to the procedure in which the
thermophilic inoculum is taken. Hence the reviewers recommended that fast increase of temperature to target point with the gradual raising of organic loading rate (OLR) can be an approach to this problem. The choice of AD technology is linked to process stability. As a result some authors recommend two-staged configuration of CSTRs (Kim et al., 2002) (De la Rubia et al., 2013).

Table 2. Typical process stability parameters and control values

<table>
<thead>
<tr>
<th>Condition</th>
<th>Parameter</th>
<th>Value</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionate oxidation</td>
<td>Hydrogen partial pressure</td>
<td>$10^{-4}$ to $10^{-6}$ atmosphere</td>
<td>McCarthy &amp; Smith, 1986</td>
</tr>
<tr>
<td>Ethanol oxidation</td>
<td>Hydrogen partial pressure</td>
<td>$10^{-1}$ to $10^{-6}$ atmosphere</td>
<td>McCarthy &amp; Smith, 1986</td>
</tr>
<tr>
<td>Total process inhibition</td>
<td>Free ammonia</td>
<td>10g-N/l</td>
<td>Apples et al., 2008</td>
</tr>
<tr>
<td>Inhibition of 50% methanogens</td>
<td>Free ammonia</td>
<td>560–568mg NH$_3$-N/l</td>
<td>Apples et al., 2008</td>
</tr>
<tr>
<td>Significant inhibition to methanogens</td>
<td>Propionic acid concentration</td>
<td>900mg/l</td>
<td>Whittle et al., 2014</td>
</tr>
</tbody>
</table>

3.3. Thermophilic versus mesophilic sludge AD: state of the art

3.3.1. Comparison of mesophilic and thermophilic AD of sludge

In most studies comparison of mesophilic and thermophilic AD are tied to process speed and biogas yield. The kinetics of a whole sludge AD is faster in thermophilic AD than mesophilic (Chi et al., 2010), (Ge et al., 2011) in general. Further, the relative digestion kinetics of pure substances is investigated targeting the particular step which is affected by temperature variation during the AD process. In this regard Ge et al., found out in 2011 that hydrolysis is the most temperature affected stage which strongly followed the Arrhenius relationship. With activation energy of $31 \pm 4$ kJ mol$^{-1}$, an increase of 1.5x for each 10 °C of temperature increase is reported. Further Li and co-workers found out that the rate limiting stages of anaerobic process affected by temperature includes the acidogenesis in the mesophilic case in addition to the hydrolysis phase (Li et al., 2015).

Mesophilic AD has been practiced most because of its lower energy demand for heating and better stabilization whereas thermophilic AD is preferred for its high biochemical reaction and low retention time in addition to stabilization. The later is more accepted for its conformity to discharge limits imposed by environmental regulations. For example, a thermophilic sludge meets the "class A" bio-solid product by United States Environmental Protection Agency (USEPA).
In a study by Gavala et al., in 2003, thermophilic sludge AD was found to be effective on organic matter removal and methane production with some pretreatment and given shorter time for digestion. According to them, the pre-treatment of sludge showed positive effect on the methane production rate when followed by both mesophilic and thermophilic AD. Whereas the methane potential was positive only when followed by mesophilic digestion. The authors, however, reported that pretreatment of sludge at 70°C showed more positive effect for the secondary sludge digested at mesophilic temperature which would be the improved solubility of COD and the relative process stability of mesophilic systems. The later is affirmed by other researchers where thermal pre treatment is known to improve biogas yield by 21 and 31% (Ruffino et al., 2015).

The desirable effect of thermal pretreatment on methane production rate can be linked to the microbial diversity and abundance (Khemkhao et al., 2012) As expected, the full scale study by Cavinato et al., (Cavinato et al., 2013) showed that the biogas yield and hence VS removal for the thermophilic digester was found to be far better than the mesophilic ADs, however the methane content expressed as percentage of the gas showed no difference between the two temperatures. Interestingly, this study showed that there was no significant difference in process stability between the two thermal digesters from the determination of pH and alkalinity which is not the case in most related studies.

The experimental study by Amani and co-workers in 2011 compared the quality and quantity of biogas between mesophilic and thermophilic batch AD of WAS which also compared its dewaterability. According to this group, thermophilic AD improved the dewaterability of sludge. The seemingly preferable advantage in case of mesophilic AD is that the slightly higher amount of volumetric methane production, because of difference in biogas yield, over thermophilic though the biogas quality did not show a difference. The later is, however, a direct opposite of what is reported within a year by Chi and team mates.

In an advancement to AD, new concepts called co-phasing which exchange sludge between spatially separated thermophilic and mesophilic digesters showed improved process stability as well as better effluent quality compared to the single stage mesophilic digesters (Song et al., 2004). The data on effluent quality showed increased \( \text{NH}_4^+ \)-N (mg/L) for thermophilic digesters as it was the case in the earlier studies (Nges & Liu, 2010).

In a study by Suhartini and co-workers, thermophilic and mesophilic AD of sugar beet pulp was compared by varying the OLR over a long duration of reaction time. They found out that digestate dewaterability was better for the thermophilic AD. The researchers also noted however that the increase in OLR from 4-g VS/l-day to 5 g VS/l-day deteriorated the dewaterability of both digestate (Suhartini et al., 2014). Thus, here it is worth to mention that the dewaterability issue of sludge AD is not clearly explained in those previous studies.
An 'attractive' earlier study by Moen et al., 2003, showed that the significant difference between the mesophilic and thermophilic AD of sludge with regard to biogas yield lay in the solid retention time (SRT). The difference between the two temperature digesters is feasible between the 6th and 20th days of the SRT. A statistically significant difference in VS destruction, with a 4% solid content on average, was observed at the 15th days of the SRT (Moen et al., 2003). Since similar studies have been made reporting that over 90% of the biogas is produced with in the first 14 days, this would be an ideal SRT for a batch AD setting (Nges & Liu, 2010). However, the study also noted that the shorter the SRT the more the undigested will the VS be.

Regarding effluent quality, Nges and Liu found out that thermophilic digesters discharge higher VFAs compared to the mesophilic ones where valeric, propionic and acetic acids were dominants which is mainly due to the faster hydrolysis followed by unbalanced consumption of the acids including by the methanogens (Nges and Liu, 2010).

3.3.2. Sludge dewaterability and the temperature of sludge digestion

Typical concentration of dewatered sludge increases from activated sludge through raw to stabilized sludge as 15 – 25, 20 – 30 and 25 – 40 in %TS correspondingly (Lecture from Professor Pavel Jenicek of the University of Chemistry and Technology, Prague, Czech). The later is ranging highest and stabilization through sludge AD is an existing and energy giving, among its benefits, alternative that again highly depends on the temperature of digestion.

As noted earlier, dewatering the sludge is useful for many reasons. These include minimization of volume, cheaper and easier storage and transport of the sludge, save energy for its drying and incineration, improve handling and stabilization etcetera. The extreme low dewaterability of sludge incurs a cost which takes share of 25-50% the overall cost of wastewater treatment (Zhou et al., 2014). Thus an efficient treatment method of sludge is a clear demand. In this regard, this research compares dewaterability difference between mesophilic and thermophilic sludge.

In a study by Zhou et al., 2014, dewaterability of sludge was significantly correlated to sludge EPS content. In thermophilic ADs increase of fatty acids occur because of the increased protein degradation. This increase of fatty acids leads to increased ammonia and COD release which brings increase of colloidal charges which consequently impairs sludge filterability. Colloidal charge is strictly related to CST. As a result increase of colloidal charge in thermophilic ADs worsens sludge dewaterability (Braguglia et al., 2015).

In an advanced anaerobic process sludge often undergoes chain of pre-treatment mainly thermal to enhance stabilization. In a study by Braguglia et al., 2014, thermal pretreatment before AD of sludge thus worsened the dewaterability of the sludge though it gave out improved solids destruction and biogas
yield. In a related comparative study, using the semi-continuous flow completely mixed reactors, the sludge dewaterability was worse for thermophilic case despite its superiority in sludge reduction efficiency (Braguglia et al., 2015), (Chi et al., 2010).

Sludge dewatering is not only limited to issues of volume minimization and subsequent liquor treatments. It is also important as sludge conditioning which is a desired precondition for the solid management including odor removal and reducing putrescibility. In an associated effort, various physicochemical approaches have been practiced to dewater sludge according to the USEPA.

3.3.3. Sludge pretreatment and its impact on dewaterability

Most dewaterability related past studies were not intended for comparison of mesophilic and thermophilic sludge. Rather, they are oriented to study the effect of physicochemical and mechanical pretreatments on biodegradability and dewaterability as well as on sludge rheology. Among such studies, Liu et al., (2014) investigated the effect of sludge pretreatment with microwave-acid process and they found improved sludge dewaterability compared to that of the untreated sludge (Liu et al., 2014).

In another study, sludge was digested using semi-continuous mesophilic AD where sludge floc disintegration using ultrasound showed a decrease in dewaterability due to the increase of fine particles and bound water. As a result the CST increased from 1 to 15 seconds of L/g-TS. Whereas in this same study the use of ozone for pretreatment impacted only slight increase in CST. Thus the authors suggest that optimization of both degree of disintegration and HRT are necessary to improve the dewaterability after sonication (Braguglia et al., 2009). In addition, sludge was digested using semi-continuous mesophilic AD and it is also reported that low energy ultrasound treatment is generally used to improve sludge dewaterability (Braguglia et al., 2012).

Contrary to Zhou and colleagues (Zhou et al., 2002) the addition of glucose was found to increase the amount of EPSs produced and it lower the CST of sludge as reported by Houghton & Stephenson (2002). However, Houghton and Stephenson suggested an optimum level of EPS sought for better sludge dewaterability with a value of 17.2 mg g\(^{-1}\) of the suspended solid (SS) (Houghton and Stephenson, 2002). Anaerobic sludge sampled from a large scale reactor in an Australian WWTP was tested for dewaterability using a CST apparatus from Triton Electronics Ltd., UK. The use of metal cations and chitosan to flocculate sludge after AD was found to improve the dewaterability of sludge which could be part of a physicochemical post-treatment of sludge (Lau et al., 2013).

3.4. Sludge management and rheology

Those other important sludge characteristics that needs to be dealt with in order to manage sludge also include its physicochemical, sanitary and rheological properties. AD sludge is found to behave as a non-Newtonian fluid (Baudez et al., 2011). Therefore manipulating the rheological property of sludge would...
one way or another affect the dewaterability of the sludge. The rheological property and quality of sludge liquor is again affected by temperature (Farno et al., 2014).

The mixing, transport or pumping and heat exchange systems design during sludge management depends on the rheological property of the sludge. Filtration and "expression" which is acted up on the sludge with hydraulic pressure to squeeze out water from filter cake are among those methods applied to test sludge dewaterability (Feng et al., 2014).

A more rigorous study by Wang et al., in 2014 showed that hydrothermal treatment at an elevated temperature, with a threshold temperature of 120-150°C, gave a further reduction of 19 to 47% of the moisture content compared to that made at room temperature with a residence time of 30 minutes (Wang et al., 2014). However, other factors also matter in the fluidity of sludge like the amount of solid is worth to mention here (Jiang et al., 2014). According to Urrea et al., 2014, if a temperature of up to 200 °C treatment is applied to sludge as thermal treatment, the sludge turns to behave like a Newtonian fluid (Urrea et al., 2015).

### 3.5. Foaming in AD

Foaming in ADs is related to increase demand in digester working volume, impairing mass transfer and increase of energy input for mixing as well as structural damage (Junker, 2007) (Shimp et al., 2010). In a fermentation process where AD is its extreme case, there are two types of foams mainly based on stability: the stable and unstable foams. For instance, stable foams are formed when the yeast *Moniliella* became nitrogen-limited and are stabilized by secreted polysaccharides. The drivers of foam formation, as in the case of fermentation, are extra cellular proteins, excreted products and cell lysis products. Differing from fermentation, AD process foams are usually in a complex three phase forms caused by surfactants and the biogas production in there. Generally, the level of foaming in fermentation increase in height with gas flow rate.

Foaming in ADs is seasonal. The sudden increase of biogas yield and VFA production are related to foam formation (Subramanian and Pagilla, 2014). The SRT showed increase in foaming with lower retention and the correlation of foaming with higher polymeric substance concentration and lower calcium concentration was determined by (Carroll et al., 2013). Practically surface sludge spray and reduced mixing are used as a remedy to foaming issue.

### 3.6. The biochemical methane potential testing

The BMP assay is an essential approach to determine amount of organics in a given substrate that can be anaerobically converted to methane under optimum conditions for which several batch assays are developed (Hansen et al., 2004), (Shanmugam and Horan, 2009), (Labatut et al., 2011) (Anglidaki et al., 2009). In our batch experiment serum bottles are used to determine the BMP of WAS which is also used
as feed for the CSTRs. An inoculum blank and pure substrate controls are also studied according to Anglidaki et al., 2009. The specific methane yield is used to evaluate the potential efficiency of thermophilic and mesophilic ADs.

4. Goal of the study

4.1. The general goal
The goal of this study is to compare thermophilic and mesophilic sludge AD in terms of digested sludge quality, reject water characteristics and FP as well as IS with keeping track of the biogas production and its composition as well as follow up of the process stability.

4.2. Specific objectives
- To characterize the composition of the WAS, mesophilic inoculum and the thermophilic inoculum in terms of their DS, TS, VS, pH, COD, NH₃-N and FS
- To monitor biogas production and biogas quality during AD of WAS in CSTRs
- To monitor the AD process stability by solid analysis, VFAs & pH measurements
- To determine and compare the CODsol & the Nammon contents of the reject water during ADs at the mesophilic and thermophilic temperatures
- To determine and compare the FP and IS of AD sludge between mesophilic and thermophilic systems
- To determine and compare the dewaterability of the sludge digested at mesophilic and thermophilic temperatures using CST and centrifugation followed by drying
- To compare the BMP of WAS along with pure cellulose and acetic acid as standard substrates

5. Methods and materials

5.1. Experimental design
The temperature of sludge AD is the factor in this study which is set at two levels; 35±1 for mesophilic and 55±1 for thermophilic systems operated continuously for nearly 100 days. The response variables are the dewaterability measured either as CST in seconds or as amount of water removed (extent of dewaterability), the CODsol in mg/l, the NH₃-N in mg/l, and FP as well as IS expressed as coefficients. To guarantee reproducibility, the analysis is performed in several runs. Regarding the BMP batch test the specific biochemical methane yield is taken as a response variable with temperature and substrate as
factors that are at two and three levels respectively. The tests are made in triplicate in most cases if not several.

5.2. Experimental setup
The sludge is digested in a CSTR. The decant from those CSTRs is analyzed for those study variables; dewaterability, reject water characteristics and the foaming. The biogas is measured using gas meter and analyzed using GC. Therefore the setup includes all those activities centered by the CSTRs according to Figure 3.

![Figure 3. Schematic view of the whole set of the experiment](image)

5.3. The CSTRs construction
Two 10L volume CSTRs are used for the sludge mesophilic and thermophilic AD. Over all, the construction, inoculation, operation, and monitoring of the CSTR is performed according to Usack and co-workers (Usack et al., 2012). Figure 4 shows the actual setup of WAS AD experiment. An electro-mechanical mixer is applied at a rate of 55/56 rpm to both digesters which are wrapped by a resistant heater controlled with temperature sensors which are set at the respective temperatures; 35±1 and 55±1°C.
Connected tubular and located on top, a gas meter is used to determine volume of biogas produced that passes through the foam trap.

Figure 4. Snap shot of the CSTRs AD setup before commencement

5.4. Analytical methods

5.4.1. The solids analysis

All the solids in the sludge samples are determined according to the standard methods outlined in APHA (The American Public Health Association, 1999). The TS is determined by drying the sludge sample in an oven at 105°C. This temperature is maintained for 3 hours which is assumed to evaporate all the water in the sample and the sample is weighed to constant weight. The VS is determined by combusting the solid sample after the oven drying in a furnace at a temperature of 550°C for an hour. Because of complete mineralization the organic matter in the sample with insignificant inorganic matter volatilizes at this ignition temperature. The TDS and VDS are determined from the centrifuged sample after separation of the suspended fraction. The SSs are then obtained from calculation of the difference between the total
and the dissolved part. The residue after the ignition of sample at 550 is the inorganic fraction called fixed solid (FS).

5.4.2. The COD analysis
Use of the COD instead of the VS as a parameter of organic strength is also possible since both tells the amount of organic matter in a sample directly or indirectly where the former is faster to determine though it is hardly representative. The COD test is an indirect measure of the degradable portion of an environmental sample. To do the CODt as well as the soluble fraction the chemical digestion method is applied (AGENCY, 1993). The sludge sample is mineralized chemically in a strong oxidizing agent in an incubator set at a temperature of 150 for 2 hours. Afterwards the absorbance of the sample is measured at the 600 nanometer wavelength which is based on change of color of the potassium dichromate.

5.4.3. The N ammon analysis
The NH$_3$-$N$ is determined using Kjeldahl Method or Kjeldahl digestion. With this method the sludge sample is first heated by sulfuric acid to decompose the organic matter and release reduced nitrogen as ammonium sulfate. Decomposition is complete when dark color of the sample is changed to clear colorless product. The solution is then distilled with small amount of caustic soda that converts the ammonium ion into ammonia. The amount of ammonia and hence the amount of nitrogen is determined by back titration. Since the ammonia reacts with the known amount of boric acid in a flask which is connected to the end of the condenser, the excess acid is then titrated with sodium carbonate using pH indicator.

5.4.4. The VFA analysis
VFA can be analyzed using different methods. GC, spectrophotometer, and titration can be applied to measure VFA. However, use of GC is preferred for its accuracy, precision and detection of individual acids (Victor et al., 2014). Capillary column GC with polar stationary phase and flame ionization detection is used for the separation and determination of individual VFAs in GC-2010. The GC is equipped with flame ionization detector and capillary column CP- Vax 58 (25 meters length and 0.25millimeters internal diameter). The oven temperature is set at 70$^\circ$C and it is increased to 134$^\circ$C with a rate of 15 $^\circ$C/min and with an isotherm for 1 minute. Injection temperature is 270$^\circ$C and a split mode (all sample goes to the column) is applied.

5.4.5. The dewaterability test
Two standard methods are in place to study the dewaterability; the CST and resistance to filtration (RTF). The CST method and the extent of dewaterability which is based on amount of water removed by
centrifugation are applied in this work. Usually the test is made in a room temperature to control effect of temperature on dewaterability, and the CST is recorded according to the standard method by APHA (Association, 1999). The extent of dewaterability is almost based on the combined principle of centrifugal separation and the TS determination followed by simple calculations.

5.4.6. The analysis of foaming

Regarding foaming study the bubble test is applied (Jenicek et al., 2011). This method is based on bubbling of nitrogen gas in to the sludge sample in a graduated glass cylinder at a predetermined gas velocity for 5 minutes that induces foam in a gradual increase in volume. The foam is then left for another 5 minutes after stoppage of bubbling to determine the stability of the foam as it normally get sunk in volume in few minutes.

5.5. Equipment and reagents used

5.5.1. The solids analysis

The solid samples are analyzed using aluminum cups folded around a mold and are weighted first using analytical balance. A scissor and hard plastic cup is used to tear and mould the aluminum sheet in to cups. Pipettes of 5ml volume with pipetting aids are used to measure subsamples. Beakers of larger volume and magnetic stirrers are used to keep the sample homogenized. After sub-sampling the oven, furnace, paper cups, balance and centrifuge as well as measuring cylinders and containers are used. For entry and calculation of results spread sheet is used on a computer networked with the balance.

5.5.2. The COD analysis

To do the COD analysis sulfuric acid and potassium dichromate as strong oxidizers, vials, automatic sampler, oven, and volumetric cylinders are used. A spectrophotometer, DR3900 (HACH LANGE), is used to determine COD after incubation of vials containing the sludge sample and oxidizers in DRB 200 digester. Centrifuge is used to separate the solid from the supernatant to study the CODsol.

5.5.3. The Nammon analysis

The NH$_3$-N is determined using Distillation Unit K-350. Accessories like glass tubes, distilled water, indicator solution, titrant, etcetera are applied, all are arranged in a set.

5.5.4. The VFA analysis

Regarding the VFA analysis, the centrifuge to separate the expressed water, syringe to pump sample, 0.2μm pore size syringe filter to filter sample before injection and micro pipette to measure sample as well as vials to hold sample are used before the GC analysis. The VFA determination is made using GC-2010.
5.5.5. The dewaterability test
The models used in this CST study are two types which are produced in different times with different limit on CST reading capacity (CST3 v01. 2001, FIMA BRUNO, Czech) (Figure 5). To do the extent of dewaterability, a centrifuge, reservoir tubes, aluminum cups, analytical balance, oven, and some other equipment are used.

![Figure 5. The CST testing equipment during testing](image)

5.5.6. The pH measurement
Digital pH probe (SENTRON, SI4007400-010) is used to measure the pH and the temperature of sludge sample almost every day with frequent calibration.

5.5.7. The foaming analysis
To do the foaming analysis nitrogen gas is used to bubble the sludge sample which is placed in a graduated cylinder of 3.8 litters volume. A glass fitted by ceramic sieve is used to uniformly distribute the gas in to the sludge sample in the cylinder. The velocity of gas is set to 1l/m using gas meter and a timer (Figure 6).
5.6. Procedures followed

The biogas production, pH and operation parameters like OLR, mixing and temperature are recorded almost daily. The various solids and CODsol are monitored 2-3 times a week, the dewaterability and ammonium nitrogen tests were made as frequent as needed so does the foaming phenomena both done right after the startup. The methanogenic activity test is performed once. Thus all activities are performed using standard procedures as detailed later.

5.6.1. Substrate and inoculum Sampling

The thermophilic inoculum and the WAS is collected from PC-WWTP and the mesophilic inoculum is brought from Česká Lípa, located in north Bohemia. Composite samples of thickened WAS as substrate is grabbed in intervals using dipper fit with an extendable handle. Samples are collected using cleaned and labeled plastic jars, transported on a pushcart and some time in a car. Personnel safety is considered during such time. The samples collected are put in to a refrigerator set at a temperature of 4°C. In the later times thickened WAS sample is collected from an outlet in storage system at site.
5.6.2. Sludge solids determination

Sludge sample in the jar is first manually mixed to take homogenous sub-sample. After homogenizing the sludge a sub-sample of 50-100ml volume is taken in beakers. The sub-sample measured is continuously stirred using a magnetic stirrer till the analytic samples are all taken.

In the meantime an aluminum foil is tear in to rectangular pieces using a scissor. The foil cut in to further pieces is mold in to cups. The cups are labeled referring to which sample it holds and whose sample is there. At the same time part of the sludge sample is centrifuged at 1300rpm for 10minutes to take the expressed water sample for the DS analysis.

10ml samples each are taken using pipettes and are poured in to the aluminum cups after weighting the cups themselves. The sample with the cup is dried at a temperature of 105±1°C for 3 hours in an oven for TS content analysis. Later after cooling the samples in desiccators they are weighed using analytical balance. After weighting the dried sample is moved in to combustion in a furnace for an hour at a temperature of 550°C after firmly folding each cup to avoid sample loss. Then the samples after ignition are cooled in a desiccators again before weighting. All the measurement values are entered in to excel sheet on a computer connected with the analytical balance.

5.6.3. The COD, Nammon and VFAs,

To quantify the CODsol, samples of 5ml sludge supernatant from the centrifuged sample are measured using pipette. For determining the CODt homogenized sludge sample is sub-sampled without centrifugation. The sub-samples are poured to their respective 50ml volume flask for dilution in which the rest of volume is filled with distilled water. In case of the CODt further dilution in to a 100ml volumetric flask is applied because of the detection limit of the spectrophotometer. After those dilutions the samples are transferred to beakers for convenience of taking the subsamples after shaking to uniform concentration.

From the beakers of the final dilution the 2.5ml subsample is transferred to the digesting glass vials in to which 1.5ml of potassium dichromate and 3.5ml of sulfuric acid are added from the respective dispensers. The culture tubes are caped tightly. The mix are then homogenized there after they are boiled for two hours in an oven at 150°C. After digestion the samples are cooled to room temperature and the COD is measured using absorbance in a spectrophotometer. Always before the actual a blank with distilled water (zero COD) is read using the spectrophotometer.

In order to determine the Nammon, 10ml samples of sludge are taken using measuring pipette which is poured to the glass tube in to which rest 40ml distilled water is added. In another glass 25ml of bottle boric acid is added. Afterwards, they are fit to the Kjeldahl digester in their respective place. The
digestion and distillation time takes 6 minutes. After that the flask with the boric acid is titrated with sodium carbonate using drops of the pH indicator.

Regarding the VFAs, random volume of sludge sample is centrifuges first triple times using labeled plastic tubes. After centrifugation the supernatant is pipetted, transferred and filtered through a 0.2µm diameter syringe filter to ease injection in to the GC. Afterwards, 1ml of sub-sample is taken. The analyte is poured in to the glass vials that are labeled accordingly and stored in freezer until analysis using the GC-2010.

5.6.4. The FP and IS

To do the foaming phenomena, the velocity of the nitrogen gas is first adjusted using gas meter and timer to a rate of 1l/minute. After that the sludge sample is measured using 300ml cylinder following mixing on a magnetic stirrer. The sludge is then poured to a graduated 3.8l volume cylinder in to which the gas is bubbled via tube inserted with the diffuser. The bubbling is done for 5 minutes to determine the FP. After that sludge volume reached following bubbling is recorded, the gas is then disconnected and is allowed another 5minute before recording the next reduced sludge volume. After all the values are calculated according to the method mentioned earlier. The FP and IS are calculated according to equation below (Jenicek et al., 2011).

\[
FP = \frac{V_5}{V_0} \quad IS = \frac{(V_{ST} - V_0)}{(V_5 - V_0)}
\]

Where:

\(V_0\) = volume of sludge at the beginning of measuring
\(V_5\) = volume of foamy sludge after 5 min of bubbling
\(V_{ST}\) = volume of foamy sludge 5 min after the nitrogen gas flow stop

5.6.5. The CST and extent of dewaterability tests

To do the dewaterability, the CST unit is first connected to power output. The WATMAN filter paper is packed in between the two rectangular blocks. The top block is fixed to the bottom by side clamps and the upper block is also connected to the timer unit via cable that is connected to two electrodes spaced by a distance of 1cm. In the centre of the upper block is a circular hole in which a stainless steel cylinder called the reservoir is loosely fit, resting on the blotter paper and it is rotated partly. The 5ml sludge sample is put in to the reservoir after homogenization. After the water from the sludge passes down the filter paper it moves radially first touching the front electrode by then the timer starts counting. By the time the water from the sludge sample touches the posterior electrode the timer stops counting that time is
recorded for the particular sample and the test continues for the subsequent sample after changing the filter paper and cleaning the reservoir to refill.

Concerning the procedure for determination of extent of dewaterability, first plastic tubes are numbered serially and are weighed. The 10ml homogenized sludge samples are pipetted in to those tubes and are weighed again. The tubes are then centrifuged at 1300rpm for 10minutes. The expressed water after centrifugation are transferred to a weighed and correspondingly labeled paper cups prepared similar to the TS analysis procedure. The cups with the expressed water as well as the consolidated sludge with the tubes after centrifugation are weighed again and every time the measurements are entered to an excel sheet. The aluminum cups with their sample are oven dried for 3hours at 105°C and the cups are weighed again. After all the water removed by the centrifugation is divided to the actual water content of the sludge determined in separate procedure using equivalent samples. The fractions obtained are then expressed as percentages defining the extent of dewaterability.

5.6.6. The BMP test

To study the methanogenic activity (BMP) the protocol by Angelidaki et al., 2009 is applied. Thus glass bottles of 120ml volume with 80ml slurry and 40 ml gas space are used with rubber and aluminum ring stopper on top. Inoculating thermophilic and mesophilic inocula in to the glass bottles at 50ml volume is done first to each bottle. The substrates are then poured at an initial loading of 0.5g-CODt per g-VSS of inoculum. After filling the slurry the bottles were sealed with rubber stopper and aluminum lid. The inside of each bottle is then flashed with nitrogen gas for about two minutes before they are placed in to their respective thermal chamber. The biogas yield from those bottles is measured using the liquid displacement system while the biogas quality is determined using the GC-2014 (Angelidaki et al., 2009). A blank or endogenous sample and control cellulose and acetic acid substrates were all also run side by side in triplicates (Figure 7).
5.8. Data analysis and interpretation

5.8.1. Hypotheses (research) questions

There are 4 null and their corresponding alternate hypotheses in this study:

1) The first one is that there is no difference in sludge quality (dewaterability) between thermophilic and mesophilic sludge AD.
   - The alternative to this hypothesis consequently is that there is difference in sludge quality (dewaterability measured in seconds as CST as well as centrifugal extent of dewaterability) between thermophilic and mesophilic sludge AD.

2) The second one is that there is no difference in reject water quality (NH$_3$-N & CODsol in mg/L) between thermophilic and mesophilic sludge AD.
   - The alternative to this hypothesis is therefore there is difference in reject water quality between thermophilic and mesophilic sludge AD.

3) The third hypothesis is that there is no difference in foaming phenomena (FP & IS) between thermophilic and mesophilic sludge AD.
   - So the alternative to this hypothesis is that there is difference in foaming phenomena (FP & IS) between thermophilic and mesophilic sludge AD.

4) The fourth one is if the BMP of WAS differ between mesophilic and thermophilic batch AD or not

5.8.2. Analysis and interpretation of the results

The data collected from every measurement is recorded in an excel sheet as per the standard protocol in the school and summary statistics are calculated from there with construction of preliminary graphs. Later
the data are converted to an R readable format and are statistically analyzed using the freeware R-3.1.2 for Windows. Since the study is on the effect of temperature of digestion on anaerobic sludge quality a t-test is performed. The statistical tests thus are run on ammonia concentration versus temperature, CST versus temperature, CODsol concentration versus temperature and foaming phenomena versus temperature. Further, the outputs from the analysis are interpreted and are also discussed against related findings from scientific journals and other relevant publications.

5.9. Quality assurance and quality control
To ensure accuracy and precision, standard procedures are followed and analyses are made in several runs. Blank assessments and references are also considered. Calibration of instruments is performed whenever deemed important to minimize systemic error. The use of in-process monitoring and regulators helped monitor the operation of the test runs. Whenever there is suspect with the expected operation of the CSTRs a batch bottle test is run side by side.

6. The result and discussion
Based on the design of the experiments outlined in the methodology section of this paper this study compared the quality of a WAS anaerobically digested at mesophilic (35±1°C) and thermophilic (55±1°C) temperatures using CSTRs. The CSTRs are mixed at 55±1rpm. This study also determined the foaming phenomena of the digestate and the BMP of WAS substrate along with inoculum blank and other pure substrates. Begun with the characterization and seeding of adapted inocula to both CSTRs as well as the WAS feed, the AD process variables are monitored regularly, both the reject water and the digestate are analyzed physicochemically and compared between the two systems. Thus the outcome of the thesis is presented accordingly in this section.

6.1. Characterization of inputs
The characterization of solids in sludge is an important ground to know the amount of organic and inorganic contents so as to predict the degree of stabilization needed and to monitor the progress during digestion as well as to evaluate the efficiency of degradation. Later after digestion, use of the ratios of solids is also useful to monitor and evaluate extent of stabilization of the feed based on the relative existence of components (Table 3 and 4). Further to know the organic strength of the sludge and other constituents determining the COD and Nammon is also necessary. Therefore, both inocula and the WAS that is not pretreated are characterized first for their solids, the COD and Nammon content.
After bringing the samples the analyses of those samples are performed within 24 hours time. Till analyses are made samples are kept in their respective temperature by using labeled & resistant plastic bottles.

Each similar test is performed using consistent sample volume. For the solids and Nammon analysis, the sludge sample volume of 10ml is used while proper sample volume of centrifuged and diluted sludge are used to both the CODsol and the CODt. Measurement of CODt is made by diluting the samples by a factor of 50. The DS and the CODsol samples are centrifuged first at an angular speed of 1300rpm for 10minutes. After centrifugation, 10ml of the expressed water is pipetted in to the drying cups to do the DS. For the CODsol, 2.5ml of sample is pipetted after dilution in to the digestion glass vials. To do the COD different dilution rates; 5 times for the mesophilic and 10 times for the thermophilic sludge samples are applied because of their variations in VS concentration with respect to the detection limit of the spectrophotometer. Whereas the WAS is analyzed without dilution. All the three samples are analyzed at around 19°C (room temperature).

Table 3. Result from the physicochemical characterization of the WAS, the mesophilic inoculum & the thermophilic inoculum in mean ± (standard deviation)

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>TS (mg/l)</th>
<th>TDS (mg/l)</th>
<th>VS (mg/l)</th>
<th>VDS (mg/l)</th>
<th>TSS (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAS</td>
<td>7.0</td>
<td>7723.3 (15.3)</td>
<td>710.0(17.3)</td>
<td>5203.3 (35.1)</td>
<td>103.3 (15.3)</td>
<td>7013.3</td>
</tr>
<tr>
<td>Mesophilic inoculum</td>
<td>7.7</td>
<td>22753.3 (86.2)</td>
<td>1606.7(15.3)</td>
<td>13143.3 (155.7)</td>
<td>566.7 (20.8)</td>
<td>21146.7</td>
</tr>
<tr>
<td>Thermophilic inoculum</td>
<td>8.0</td>
<td>27166.7 (257.7)</td>
<td>2835.7(5.8)</td>
<td>15440.0 (628.6)</td>
<td>1863.3 (25.2)</td>
<td>24330</td>
</tr>
</tbody>
</table>

The various solids determined in these analyses are thus in increasing concentration in order from the WAS to mesophilic inoculum and to thermophilic inoculum (Table 3 and 4) this would be due to the location of sampling for both inocula and also the amount of biomass present in those inocula as well as degree of treatment applied at the WWTP. Further the sludge is dewatered before it is fed to both ADs at those WWTP sites thus the WAS is more dilute than AD sludge or inoculum.

The solids expressed as TS and VS as well as the ratio between these two slightly varies from what is reported by the European Commission (E.C) (Commission, 2001) in this case. The amount of TS for WAS is slightly higher in our case while it is lower for the inocula so their ratio varies accordingly. However, given the fact that the stabilization in these inocula may not be complete and the EC report is years older and the EU is representation of sum of countries the deviation is tolerable.
Table 4. Result from the physicochemical characterization of the WAS, the mesophilic inoculum & the thermophilic inoculum in mean ± (standard deviation)

<table>
<thead>
<tr>
<th>Sample</th>
<th>VSS (mg/l)</th>
<th>VSS/TSS</th>
<th>CODs (mg/l)</th>
<th>CODt (mg/l)</th>
<th>CODt/VS</th>
<th>N-NH+_N-NH (mg/l)</th>
<th>FS (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAS</td>
<td>5100</td>
<td>0.73</td>
<td>46 (8.7)</td>
<td>7033.3 (763.8)</td>
<td>1.4</td>
<td>45.9 (2)</td>
<td>2520 (26.4)</td>
</tr>
<tr>
<td>Mesophilic inoculum</td>
<td>12576.7</td>
<td>0.59</td>
<td>918.3 (45.4)</td>
<td>21633.3 (1069.3)</td>
<td>1.6</td>
<td>1024.2 (3.9)</td>
<td>9606.7 (65.1)</td>
</tr>
<tr>
<td>Thermophilic inoculum</td>
<td>13576.7</td>
<td>0.56</td>
<td>3083.3 (106.9)</td>
<td>24100.0 (983.6)</td>
<td>1.5</td>
<td>1523.8 (19.7)</td>
<td>11726.7 (391.4)</td>
</tr>
</tbody>
</table>

According to the result on VSS/TSS ratio, it is expected that a WAS before the AD has a ratio which ranges between 0.7-0.8 (70-80%) due to more degradable organic matter expressed as VS. Accordingly the stabilized WAS and hence the inocula are expected to reach down to 0.5 of that same ratio (50%) which is based on a relative measure of stabilization. Thus the value in the table lies within this assumption though the stabilization requires longer time for both inocula.

The CODsol of the WAS sample falls around the lower value of the range published by Zanetti and co-worker which is 51-135 mg/l. The Nammon is lower by comparison to a case study in Germany which is 650mg/l for an activated sludge that was actually from a waste stream high in nitrogen content. Conversely the CODt of the sludge sample in our case falls between the CODt of raw sewage sludge and WAS as reported by Zanetti and colleagues and Meyer and Wilderer (Zanetti et al., 2012, Meyer and Wilderer, 2004).

As to the CODt/VS ratio, it is generally suggested that this ratio lies within standard deviation around the mean value of 1.5 which actually varies among different reports. The finding here for those inocula as well as the WAS (Table 4) are not far deviated according to the report by Parker and colleagues (Parker et al., 2008). However, according to Kabouris and co-workers (Kabouris et al., 2008) the CODt/VS ratio is lower compared to what they found out on a thickened WAS, 1.94. Conversely, it is a little higher than what is reported by El-Haji et al., in 2007. Therefore, it is evident that various sludge sources have different outcomes as a matter of the nature of the waste itself, the treatment applied at the WWTPs and, if any, pretreatment applied to the sludge samples.

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Compared to the WAS, the mean ammonia concentration of both inocula is far higher than what is desired for methanogenesis process which is below 200mg/l (Table 4) however the inhibitory level is much higher than that. The thermophilic sludge is even 1.5 times higher than the mesophilic by comparison. Free ammonia concentration of 560–568mg NH₃-N/l is claimed to reduce methane
production by 50% (Apples et al., 2008). Total inhibition can occur at a concentration of 10 g-N/l (Sung and Liu, 2003). Further the pH of the thermophilic inoculum is relatively higher. In any case, the variability of these 'indicator' ratios between the two inocula has to be minimized before seeding to our CSTRs in this study. By doing so the later comparison on sludge quality can be performed on common ground and also it speeds up the time needed for the establishment of the pseudo-steady state condition.

6.2. The AD startup

6.2.1. Feed characterization and digester inoculation

The inoculation of both digesters is performed by filling both CSTRs with adapted and 'equilibrated' inocula to 10L volume on day one. The next day, the first feeding of digesters with WAS is done after feed characterization and following decantation of an equivalent volume of mixed liquor in the system using the mid-port of both digesters. Feeding with a gradually increasing OLR with digestion time continues with the corresponding increase of the decant.

Even though the WAS of PC-WWTP is characterized initially, the feed to our CSTRs is determined for its solids and COD contents because of the thickening of the sludge at site. Thus to exactly determine the organic content of the substrate, for our desired feed to fit to the conservative OLR (45g-TS/L), the solid content is determined every time new substrate is brought. Thus, up on TS determination if the TS of the WAS is above the desired loading concentration it is diluted or if it is below it is concentrated. The result of most of the characterization of feed are summarized by date in table 5. According to the solid analysis of feed it looks interesting that almost all of the WAS samples are very close 0.7 in VS:TS.

<table>
<thead>
<tr>
<th>Date</th>
<th>TS</th>
<th>TSS</th>
<th>VS</th>
<th>VSS</th>
<th>CODt</th>
<th>VS/Ts</th>
<th>CODt/VS</th>
<th>VSS/TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-4-15</td>
<td>60</td>
<td>39.4</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-5-15</td>
<td>40</td>
<td>35.2</td>
<td>26</td>
<td>24</td>
<td>51.7</td>
<td>0.7</td>
<td>1.99</td>
<td>0.7</td>
</tr>
<tr>
<td>13-5-15</td>
<td>57</td>
<td>37.6</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-6-15</td>
<td>72</td>
<td>71.8</td>
<td>49.1</td>
<td>49.0</td>
<td>56.8</td>
<td>0.7</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>07-7-15</td>
<td>66</td>
<td>49.6</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-7-15</td>
<td>61.6</td>
<td>45.4</td>
<td>70.9</td>
<td>0.74</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The OLR is gradually increased with varied concentration over three weeks of the startup times and even beyond. It was increased from 120ml of the 45g/l-TS concentration to 300ml within the first 4 days and was kept so until 8th day of operation. From the 9th day on the loading increased to 400ml. Sometimes the feeding is interrupted during the weekends. The maximum feed volume reached in our experiment is
however 750ml that begun from day 71 of the digester running and in effect the HRT is lowered from 20
days to 13 in the last two weeks.

6.2.2. AD process monitoring and stability
The stability of ADs and establishment of the pseudo-steady state is verified based on the biogas yield
and biogas composition, pH, and VFAs released during digestion as well as VS destruction and the ratio
of solids which have to be followed strictly during the startup period and beyond.

6.2.2.1. Biogas yield, pH and OLR
The startup period was more dedicated to the attainment of a pseudo-steady state in both temperature
systems. As a result it was necessary to quantify the input and output condition of both digesters and
monitor the pH, VFAs, and the solids in the mean time. The biogas yield, pH and temperature of both
digesters are followed daily except some of the weekends.

During the startup time the mesophilic digester was relatively stable. Conversely, little biogas generation
is observed by the thermophilic digester, during the first three days it was even none due to gas meter
problem. The peaks in biogas volume recorded are usually following the weekends due to longer or
extended temporal readings and hence longer digestion time. The biogas yield trend line is broken at
some point due to gas meter problem and hence missing records of the biogas yield (Figure 8).
During those initial times it was necessary to trace back the causes of the missing biogas production by
the thermophilic CSTR. Assumptions were tested regarding leakage and as a result some tubing were
replaced, change of gasbag and other leak sealing efforts are made as appropriate. After a week the gas
production of both digesters showed narrowed variation as shown by the first point on figure 8 though the
thermophilic system is slower to start gas production following new feed. It was almost always improving
behind the mesophilic system both in biogas yield and methane content mainly following an increase in
OLR.

Further, to address the problem related to the biogas yield by the thermophilic CSTR, batch AD of the
decants from both CSTRs and analysis of the solids is performed side by side which showed rather higher
biogas yield and relatively better VS removal by the thermophilic sludge. Thus change and exchange of
the gas meters is made a number of times which brought somehow improvements though it is also good
to consider the biochemical process inside the thermophilic CSTR in more detail. The lower gas yield is
likely to be due to lower acidogenesis and hence lower methane production by the thermophilic digester
since the VS destruction is not in agreement with biogas production in comparison to the mesophilic
condition.

Yet again, the result in lower biogas yield to thermophilic digester is not unique to this current work that
an earlier study by Chi et al (2010) also showed same result. The higher biogas yield by the mesophilic
CSTR also agrees with Ruffino et al., 2015 and Chi et al., 2010. Chi et al., have found a statistically significant difference in biogas quality in favor of the mesophilic system. In our case the methane content on average were 61.3% and 64.3% for the thermophilic and mesophilic WAS ADs respectively. On the other hand, some authors reported better biogas yield and even better methane content by the thermophilic system (Li et al., 2015; Gavala et al., in 2003). Thus these contradicting results suggests the need to resolve the gap with an appropriate method.

![Figure 8. The biogas yield by both CSTRs during startup and beyond](image)

A point of interest with this event of the biogas yield by the thermophilic digester is that it disagree with the result of VS/TS ratio, where the lower is the better (Figure 9) even though the VS/TS is an estimator and not a direct measure for the biogas yield. Since AD is a complex and four stage process, higher or faster hydrolysis may not guarantee faster acetogenesis or methanogenesis. Rather, in ADs the efficiency of biogas yield is determined by the acetogenesis stage which results in 70% of the methanation from acetate reduction (Ali Shah et al., 2014). Additionally for the better VS/TS ratio, a reason could be the solubility of organic matter that is improved in the thermophilic digester as it can also be witnessed from its lower VSS/TSS ratio as compared to the mesophilic reactor (Figure 9). In a related fact, it is proved
that the rate limiting step in AD is hydrolysis which is most affected by temperature that behave according to the Arrhenius's theory. The hydrolysis rate in thermophilic AD is twice as compared to the mesophilic part (Ge et al., 2011).

The lower VS/TS ratio does not mean complete degradation in to CH₄ and CO₂. In this regard, acidogenesis stage is known to bring effect on digester pH. The pH of our thermophilic digester is not disturbed significantly. It was around 8.2 and 7.8 for thermophilic and mesophilic sludge respectively (Annex-I). That implies the rate of hydrolysis in the thermophilic CSTR is not accompanied by the rate of acidogenesis. Thus the problem signifies the relevance of visualizing the biochemical process in more details to understand the relation among time, temperature, substrate concentration and biogas yield by the thermophilic anaerobic CSTRs across the stages of AD. To suggest in this respect, the secondary fermentation bacteria have to be active enough to take advantage of the fast hydrolysis in such synthrophic metabolic pathway. In that case supplementing such group of bacteria could help.

In a related affair, some authors reported about optimum HRT for a maximum biogas yield (Kim et al., 2006). Others found out even a statistically significant difference in biogas yield between thermophilic and mesophilic digestion between 6th and 20th days of the SRT. Thus such issues suggest that there are more factors to consider in order to optimize biogas yield in a thermophilic CSTR. However, it is difficult to draw strong conclusion on difference in the biogas production between the mesophilic and the thermophilic CSTRs. Based on the gained results, the measured data are in contradiction with some process parameters and guarantee that the biogas data are correct and complete to make a strong conclusion.

Regarding pH as stability indicator, no significant problem of stability has occurred. Though relatively the pH for the mesophilic CSTR was low it was almost stable throughout (Annex I). Despite the favorable and stable pH & gradual improvements in biogas quality by the thermophilic system, an increase in the OLR (Annex II) often slows down the biogas yield following feeding. This suggests that the process stability in terms of performance as compared to the mesophilic digester is not good. This idea can support the early study by Cavinto et al., in 2013. In other words, process sensitivity to an increased OLR is more prominent to the thermophilic system that still agrees with the idea by Appels and colleagues (2008). Further, it signifies on the comfort with operation of a mesophilic digester compared to the thermophilic one.
6.2.2.2. The VFAs

Except the acetic acid concentration during the earlier days, the total as well as the component VFAs are higher for the thermophilic CSTR (Figure 10a-10d) which agrees with the review by Appels and colleagues (2008). This is also associated with an increase of free ammonia and is known to negatively impact the methanogenic process in ADs. The increase in ammonia concentration follows from the increase efficiency of protein degradation.

Franke-Whittle et al., 2014 mentioned the reference quantities regarding process inhibition levels of VFA components. Those reference figures states that an acetic acid concentration of up to 2400mg/l and butyric acid concentration of to 1800mg/l do not have inhibitive effect on methanogens. However, a propionic acid concentration of 900mg/l resulted in a significant inhibition of the methanogens. Thus none of the acid concentration dictated process instability in this study (Figure 10a-d). The relatively higher propionic and butyric acid concentration by the thermophilic digester is still associated with the faster hydrolysis and acidogenesis (Nges and Liu, 2010) process.
Figure 10. The acetic acid (a), the propionic acid (b), the butyric acid (c) & the total VFAs (d) between the two CSTRs during startup.
6.3. The dewaterability issue

6.3.1. The dewaterability test
Despite some limitations with the CST method, it is proved useful long ago and is a standard method still (Vesilind, 1988). It is easy, fast and cost effective, and moreover the test unit is portable (Scholz, 2005). It is widely accepted that a short CST is associated with good sludge dewaterability (Peeters, 2011). In our experiment, 2-3 times a week the dewaterability of the sludge samples from both CSTRs are tested using capillary suction units and later together with centrifugation where significant amount of the tests are performed after the startup of the AD. Before testing for dewaterability, the temperature of both sludge samples is lowered to room temperature, even though temperature alone does not affect dewaterability unless it is moderated by other physicochemical properties (Sawalha and Scholz, 2012).

![Figure 11. The dewaterability as CST for both sludge during the startup period](image)

The mesophilic sludge's dewaterability property looks to be more variable compared to the Thermophilic during the startup. However, during this time the CST for both digesters' sludge increased with digestion time (Figure 11). As the AD time advances after the startup period, the CST for the thermophilic CSTR digestate becomes higher than the mesophilic (Figure 12).
Regarding statistical analysis, all the data are checked for normality of distribution before conducting the two sample t-tests. The result on distribution test as verified by the box plots and the Q-Q plots there is no systemic deviation (Figure 24). Given that the sample is biological, the slight deviations are tolerable and there were negligible outliers that are just errors.

The mean CST for the mesophilic sludge is 852 with a standard deviation of 180 while for the thermophilic it is 1109 with a standard deviation of 211, all measured in seconds. In terms of the specific CST, the mean value in seconds related to TSS concentration in g/l for the mesophilic sludge is 35 with a standard deviation of 7.7 whereas it is 52 s.l/g for the thermophilic sludge with a standard deviation of 11.5.

The Welch two sample t-test is used to evaluate if the mean CST of the two sludge is different (the test is carried out two-sided, at the 5% significance level). On a 95% confidence [-357, -158], the true mean difference in CST in seconds is significantly lower for the mesophilic sludge (p-value≈0).

There could be several reasons for why the mean CST is significantly higher by the thermophilic sludge. As one possible cause, the filterability of the thermophilic digestate could be impaired due to fine...
particles that are in suspension. Studies reported that sludge dewaterability is strongly related to sludge particle size distribution and those small particles contained in the sludge (Fitria et al., 2014). For the water contained in the sludge to reach the electrodes of the CST unit, it has to filter through the WHATMAN filter paper first. Thus the relatively higher fine suspensions in the thermophilic sludge could have blocked the fine pores of the filter paper causing resistance for the water to filter through. Supportive to our argument, the increase of fine particles and bound water is related to worse dewaterability in earlier studies (Braguglia et al., 2009; Zhou et al., 2014; Scholz, 2005). Further, this current result agrees with the works by Braguglia and Chi and their colleagues (Braguglia et al., 2015), (Chi et al., 2010). The bad dewaterability of the thermophilic sludge measured in CST by this study also supports earlier associative findings by Braguglia and co-workers (Braguglia et al., 2015) and Chi et al., 2010. The higher CST for the thermophilic digester in this current study however contradicts the report by T. Amani et al., in 2011 which is actually done on a batch AD system.

Though the two most common standard methods to test dewaterability are the CST and RTF, neither the RTF nor the CST are incomprehensive to describe and model the dewatering property of sludge (Smollen, 1986) and are also difficult to model (Scholz, 2005). Supporting the two old but standard methods, centrifugal dewatering is a fast process that separates wastewater solids from liquids (USEPA, 1987). Centrifugal dewatering is an existing practice in WWTPs. Thus a side by side examination on centrifugal dewaterability is done in this study. To do that, 10ml of digestate was centrifuged at 1300rpm for 10minutes after the sludge sample is homogenized using magnetic stirrer before sampling. The result from centrifugal dewatering and subsequent solid analysis is expressed as extent of dewaterability (% of water removed). As a result, contrary to the CST outcome, better dewaterability for the thermophilic digestate is found with this method (Figure 13).
Statistically, the mean extent of dewaterability for the mesophilic sludge is 62.9% with a standard deviation of 1.7% while for thermophilic sludge it is 65% with a standard deviation of 1.8%. Thus, on a 95% confidence [-3.3, -1.4], the true mean difference in the extent of dewaterability as percentage water removed is significantly lower for the mesophilic sludge (p-value≈0 ) in favor of the thermophilic sludge. Since centrifugal dewatering in the WWTPs is an existing practice despite its noise and high power consumption, this finding is promising to keep doing with it.

6.3.2. Dewaterability tests on a batch mesophilic and thermophilic AD of WAS

For further curiosity and comparison, measurement of the CST for the batch AD is done earlier (Figure 14). In the batch AD case, the thermophilic peak in CST is attained before the mesophilic (Figure 14) that could be related to the faster hydrolysis and relatively higher fine particle suspension which hinders filterability similar to the sludge from CSTRs. Overall the CST is improved along the digestion days except the deviation by the thermophilic digester in the end. Both lowest and highest CST records are made for mesophilic sludge signifying its variability in comparison to the thermophilic sludge though it needs statistical verification. The trend in CST along digestion time resembles with the pattern in the CSTR sludge. The mean CST in seconds for the mesophilic sludge is 466 while it is 537 for the thermophilic sludge.

The two bench scale batch bottle AD digested sludge are statistically analyzed if there is a true mean difference in CST between the mesophilic and the thermophilic sludge. Thus on a 95% confidence the true difference in mean CST in seconds between the mesophilic sludge and the thermophilic sludge from
the batch AD is not statistically significant (p-value≈0.2). However the difference between the two sludge samples could still have technical implications. The variability in CST is so higher for the mesophilic with a standard deviation of 159 seconds sludge compared to the thermophilic sludge with 114 seconds.

6.4. The reject water characteristics

Another focus area of our study was evaluation of the difference in reject water quality between thermophilic and mesophilic sludge. The reject water quality from both CSTRs was analyzed during and after the startup period with respect to CODsol and Nammon concentrations.

With regard to Nammon, it is clear from figure 15 & 16 that ammonia concentration is higher for the thermophilic CSTR digestate compared to the mesophilic. Both sludge ammonia values are higher than the ammonia which was present in the feed before digestion. The thermophilic liquor is maintained at relatively higher ammonia concentration at least during the startup period. The slopes of that Nammon trend do not have such big difference but the intercept which marks the inherent properties of both systems in the beginning (Figure 15).

From statistical analysis, the mean reject water Nammon concentration in mg/l by the mesophilic sludge liquor is 1484 while it is 1581 for the thermophilic. These mean values of the reject water Nammon concentration from both reactors is lower than reported (2000-4000mg/l) value by Zanetti et al., in 2012 which could be due the lower initial concentration in the feed. The variability in ammonia concentration is relatively higher for mesophilic than that of the thermophilic which is 153.5 and 120.5 mg/l respectively.

On a 95% confidence interval [-169.4, -24.3], the mean Nammon concentration by mesophilic sludge is significantly lower (p-value≈0.01) than that of the thermophilic. Within each system, however, there is very little variability compared to the difference between the two systems. The higher ammonia by the thermophilic digester could be associated with the faster breakdown of protein or other nitrogen compounds due to the faster hydrolysis of compounds in the WAS.
Figure 14. The CST against digestion time for batch AD of WAS

Figure 15. The Nammon concentration in the sludge by both CSTRs during the startup period
The ammonia concentration trend by the two CSTRs showed little similarity with time (Figure 16) that agree with an earlier related study (Cavinato et al., 2013). As a matter of fact, given the shorter the HRT the ammonia of the thermophilic digester is higher than the mesophilic which can be attributed to the relatively faster hydrolysis in the former. However since both substrates are the same with longer HRT the gap in ammonia concentration between the two sludge could be narrowed.

According to some literature the process inhibitive concentration of ammonia is not that specific depending on the type of substrate and temperature of digestion, however it is most cited that the threshold concentrations for initial inhibition by ammonia falls in the range of 1500–1900 mg total Nammon/l (Gebauer, 2004). Thus accordingly even the peak ammonia concentration in our thermophilic digester is within the threshold limit related to process inhibition. Moreover there was no process inhibition due to this as it can be referred on the consistent pH and other process parameters.
Figure 17. The CODsol trend during
Still, this large content of Nammon
surpass discharge limit which are subject to local restrictions. Environmentally the impact of such

Figure 18. The CODsol trend after the
startup period in both sludge AD reject water
in the reject water could have environmental implications and could
surpass discharge limit which are
ammonia concentration on those sensitive ecosystems is already justified. In this respect, aquatic suffocation because of the subsequent nitrification reaction and toxicity due to free ammonia can be mentioned, the later species depends on temperature and pH of water body. Thus rules like "The fishing act" of the EU is sort of legal aspects towards limit of discharge of ammonia in effluents from WWTPs. In another dimension, the Nammon from AD of WAS can be recovered or recycled to the biological wastewater treatment units since biological nitrogen removal is still economical (Zanetti et al., 2012). Another approach to minimize such ammonia concentration in the effluent from AD could be feeding it to an ANAMOX unit (Zhang et al., 2008).

Over the significant difference in Nammon trend in the reject water, the CODsol trend shows bigger difference (Figure 17 and 18) between the mesophilic and thermophilic sludge. Additionally, variability exists within each WAS AD system along the digestion period. In this attribute to reject water the higher concentration is also recorded for the thermophilic sludge. Though the feed to both digesters is identical in the CODt, due to temperature effect the CODsol in the thermophilic digestate is far higher than the mesophilic. By the same fact, the hydrolysis stage in this regard also increased the solubility of the organic matters in the thermophilic sludge.

In another scenario the higher amount of suspended particles could also contribute to high CODsol as described by a related study (Abbasi et al., 2015). The higher COD in the thermophilic digester agrees with the result from Baudez et al., 2011 that expressed as increased solubilization of sludge at higher temperature, though it is contrary to Ge et al., 2011.

The mean CODsol concentration in mg/l is 2316 for the mesophilic reject water whereas it is 4741 for the thermophilic which agrees to the range of value (2700-5000mg/l) reported by Zanetti et al., 2012. Since the CODt is high in the initial substrate, the higher CODsol in the thermophilic sludge is brought by the fast hydrolysis in the thermophilic system. The variability of the COD concentration is higher by the thermophilic sludge compared to its mesophilic counterpart: 1123 and 408 g/l respectively. The result on the CODsol in the sludge from the mesophilic and the thermophilic digesters was statistically analyzed if the true mean difference between them is not zero. As a result, the mean CODsol concentration of the mesophilic sludge is significantly lower (p-value≈0) than the thermophilic liquor on a 95% confidence [-2838.5, -2011.4].

As a matter of consequence, the COD of the reject water particularly for the thermophilic AD could be a concern environmentally therefore the reject water must be treated (Comission, 1991). As an option, if the Nammon in the reject water itself is not high enough for the COD consumption by the ammonia oxidizing and reducing bacteria, it can be suggested that the COD of the reject water especially from the thermophilic reactor can be fed to nitrification/denitrification units in WWTPs.
6.5. The foaming phenomena

Another sludge quality parameter investigated in this research is the foaming phenomena. A foam is generally defined as a gas-liquid dispersion with over 95% gas and rest is the surround liquid. Even though the foam from sludge AD can also contain fine particles. Regarding foaming in AD there is little scientific report available either on the exact implication of foaming or on the root cause of the problem. Of those very few works, a survey of foaming on full scale biogas plants by Kougias and colleagues could be mentioned. They generally expressed the problem with foaming in ADs as it brings economical and environmental impact (Kougias et al., 2014). Most impacts of foaming studied on water treatment plants and/or on WWTPs are explained by operational inefficiency and demand of extra energy of mixing. However, it is clear that foam takes up volume in digesters and hence it demands an extended volume of digesters in AD than without foam. Further mixing in ADs is an existing practice to keep microbes and substrate in uniform contact. Thus the energy of mixing could be higher if foaming can also be a concern there. Further foaming in ADs limits gas recovery and it is responsible for 20–50% biogas production loss according to the Danish survey by Kougias and co-workers. In addition, the cleaning cost is also another concern of foaming. Thus it can be said that the cost and operational inefficiency implication holds to sludge ADs too as indicated in some reports (Ganidi et al., 2009) (Junker, 2007; Shimp et al., 2010). Moreover, it is possible that the foam could bring damage to mechanical systems due to low density liquid transport with the gas in the system.

With that regard, in our experiment foaming phenomena of both mesophilic and thermophilic sludge were followed by conducting the bubbling test and by simple observation. From our observation during sludge sampling and while using the level probe to check change in digester volume, the mesophilic digester forms foaming little higher than in the thermophilic CSTR that supports the related report by Ganidi et al., 2009.

Mainly the bubbling test is conducted to determine foaming phenomena in this study. Though there is an alternative to measure foaming, the bubbling test is favored. The FP is also correlated with temperature especially between 4 and 20°C thus foam sludge samples are brought to room temperature before testing by bubbling to fairly compare the two sludge (Fryer et al., 2011). From use of the two accepted indices for foaming, IS and FP, several tests are run in our study to evaluate the difference between the foam condition in the two temperature sludge. Thus, it is clear from figures 19 and 20 that the FP is consistently higher for the thermophilic CSTR while the foam stability is consistently higher for the mesophilic digester except an outlandish case in the former which could be due to non-systematic error. The finding of higher potential by the thermophilic sludge disagrees with the reports by Suhartini and colleagues (Suhartini et al., 2014) and Zabranska et al., 2002.
The variability in IS by the two sludge most behave in a similar fashion (Figure 19). However, the two sludge started with opposite scores near the 30th day and also near the last digestion times. The mean IS for the mesophilic sludge is 0.7 whereas it is 0.4 for the thermophilic. The variability of stability is relatively higher for the mesophilic sludge (0.12) compared to its mesophilic counterpart (0.09). The IS by both CSTRs was statistically analyzed if the true mean difference between them is different from zero. As a result, the mean IS of the mesophilic sludge is significantly higher (p-value ≈ 0) than the thermophilic sludge on a 95% confidence level [0.24, 0.4].

In the case of the FP the trend with digestion time between the sludge got wider with increase in time (Figure 20). The thermophilic sludge showed consistently higher FP over the mesophilic sludge which is rather more stable. During the bubbling experiments the highest foam volume is usually reached before the 5th minute of bubbling, often during the first three minutes especially for the mesophilic sludge. Aside from that, the foam structure is also different where uniform structure with lower sized foams is typical of the thermophilic sludge.
Accordingly, the mean FP by the mesophilic sludge is 4.4±1.7 and it is 8.9±1.6 by the thermophilic sludge. In this regard both sludge showed nearly similar variability. However, based on the statistical analysis, we found that the mean FP between the mesophilic and thermophilic sludge differs (95% CI [3.5, 5.5]) significantly (p-value≈0) and is higher for the thermophilic sludge.

In order to bring solution to foaming problem an identification of its causes is a step forward. The cause for foaming include intense agitation, aeration or gasification and presence of surfactants as it is mentioned in some reports. In addition, organic overloading, the filamentous bacteria, EPS, other hydrophobic substances are also associated with foaming in ADs. Moreover, some compounds present in AD substrates are surface active agents which are most related and studied as contributors to foaming in ADs (Carroll et al., 2013; Ganidi et al., 2009). Nowadays antifoaming agents are abundant commercially (Kougias et al., 2014) though practically surface sludge spray and reduced mixing are used as a remedy.
6.6. The BMP assay

The BMP assay is used to get information for a proposed biogas supply and hence helps in the design of AD, to select potential substrates or their mix among waste streams, to see the kinetic behavior of the digestion and to optimize substrate mix. The limit with such test is however that it does not give information on the continuous process and the biochemical methane yield from such bench scale studies is sometimes higher compared to actual operations (Browne et al., 2013).

Along with the evaluation of sludge quality from mesophilic and thermophilic digesters it was necessary to determine the BMP of the WAS used as fed. Thus, in order to determine the BMP of the WAS a batch BMP assay is conducted according to the protocol by Anglidaki et al., 2009 on the 46th day of CSTRs' digestion time. The test on the WAS is conducted together with cellulose and acetic acid for standard substrates and an endogenous blank for correction of the biochemical methane yield from the inocula. All the tests are run in triplicates to generate reproducible result. The dosing of test components is calculated on the basis of the VSS of sludge and the COD of the substrate. Accordingly the three media are fed in the ratio of 0.5g-COD per g-VSS. The BMP test is run for 44 days. As noted on the protocol, 50ml of the two inocula are fed to each 120ml serum bottle. Thus the formulation made is summarized in table 6. The remaining space of each bottle is filled with tap water which is made to 80ml total slurry volume. The gas space left in each bottle was 40ml.

Table 6. Substrate portion of the BMP assay fed to both temperature bottles

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mesophilic</th>
<th></th>
<th>Thermophilic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>COD\textsubscript{t} (g/l)</td>
<td>9.5</td>
<td>28.4</td>
<td>1190</td>
<td>23.1</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>50</td>
<td>11.52</td>
<td>0.28</td>
<td>14</td>
</tr>
<tr>
<td>VSS (g/l)</td>
<td>13.06</td>
<td>49.03</td>
<td></td>
<td>12.29</td>
</tr>
</tbody>
</table>

After the substrates are made to slurry in the glass bottles with the inoculum each bottle is rubber caped and flushed with nitrogen gas. After headspace flushing to remove rest of oxygen the thermophilic and the mesophilic groups of bottles are placed in their respective thermal chamber. The mesophilic temperature is set at 35±1\textdegree C and the thermophilic at 55±1\textdegree C. The content of each bottle is manually mixed by shaking after gas measurement every day.

Accordingly the methane yield in the mesophilic batch systems is over 86\% of the expected theoretical yield for cellulose and the acetic acid. For sludge, it is around 57\% of the expected theoretical yield from
a pure substrate that is obviously because of the nature of medium and it is not equally degradable like those pure media. Compared to those pure media, it is nearly 30% less in methane yield but it should be borne in mind that it is a secondary medium brought after an activated sludge process (Figure 21). The BMP test yield for the WAS (>200ml-CH$_4$/g-COD) that is digested at the mesophilic temperature is in agreement with the report by Carrère et al., 2010, but it is lower than what is reported by Pontoni et al., (Pontoni et al., 2015).

![Figure 21. Specific cumulative biochemical methane yield from the mesophilic BMP assay](image)

The biochemical methane yield in the thermophilic batch AD assay is greater than the mesophilic systems. It was about 95% of the theoretical yield per unit gram of COD for the pure substrates; cellulose and the acetic acid (Figure 22). Still better yield in the WAS thermophilic batch AD is recorded at little over 74% of the theoretical yield (Figure 22) for pure substrate. However, similar to the mesophilic sludge digestion, the biochemical methane yield by the WAS thermophilic batch assay is lower than those pure media. That happened due to the less degradability of WAS. The BMP of WAS is even proved to be significantly lower than that of the mixture of primary and secondary sludge (Girault et al., 2012).
In both temperature of digestion the acetic acid and cellulose showed slight difference in degradation rates. For the acetic acid reaching early peak which is due to the solubility of the substrate compared to both the sludge and cellulose. Conversely, the production of methane becomes slightly higher for the cellulose after the 15th day of digestion which is still due to the difference in the degradability of the substrate since cellulose takes longer time to degrade relative to acetic acid and hence there is availability of the substrate is prolonged in the system. In the case of cellulose and sludge BMP assay the rate of methane production is faster by the thermophilic bottles which is due to the faster hydrolysis as a result of the higher temperature of digestion (Ge et al., 2011).
Figure 23. The specific cumulative biochemical methane yield during the BMP assay as compared between mesophilic and thermophilic WAS AD

The rate of biochemical methane yield from the WAS AD in the thermophilic case is much faster than the mesophilic with the disparity widening with increase in time (Figure 23). The reason for that to happen is due to the fast hydrolysis in the thermophilic case.

It was described earlier in this document that the biogas yield by the mesophilic CSTR is higher than the yield by the thermophilic CSTR. The HRT and SRT were mentioned as possible causes for the lower yield by the thermophilic CSTR even though that could need further study. Apparently supportive, the methane yield by the thermophilic batch bottles is found to be higher than the mesophilic bottles due to longer sludge retention time. In addition the reactor volume and the amount of reactants are basically different in these two different technologies.

Overall, thanks to long term adaptation of anaerobic bacteria consortia to WAS as substrate, the specific methane yields achieved are very high (especially thermophilic) in comparison with literature data (Jenicek et al., 2013).
After digestion is ceased the sludge is analyzed for its pH, Nammon, CODsol, and solids (Table 7). Overall, the pH values of all digesters are well in the optimum range. Relatively the pH for the thermophilic bottles is little higher compared to the mesophilic pH readings. The highest Nammon by the WAS fed digesters is due to the nature of the substrate that obviously contains protein compounds. The CODsol compared between the mesophilic and the thermophilic sludge is higher for thermophilic in all kinds of substrates and the control. This over two fold concentration of CODsol by the thermophilic sludge is attained due to high background of thermophilic inocula and the effect of temperature on hydrolysis and the subsequent dissolution of compounds. The effect of dissolution is also evident from the TDS and VDS determined after digestion. Conversely, the TSS and VSS are higher for the mesophilic sludge. The VSS removal by the thermophilic digester is higher than by the mesophilic WAS batch AD which are 81% and 76% respectively.

Since the extent of mineralization and hence the biochemical methane yield in all the substrates is better by the thermophilic batch systems so does is the solid removal which can be understood from the TS and VS determined after digestion for both temperature sludge. The TS and VS concentrations are low after digestion in the thermophilic sludge in all the substrate as well as the control cases.
Figure 24. The summary of box plots of the study variables between the two categories of AD
7. Conclusions and recommendation

The physicochemical characteristics of the mesophilic and the thermophilic sludge are different in some aspects of quality. The mesophilic sludge is higher in the TSS content and is also higher in the VS/TS ratio. Conversely the thermophilic sludge is higher in TDS and lower in VS/TS ratio. Since the VS/TS ratio is low for the thermophilic sludge it indicates better degradation efficiency by the system. In a related fact the differences in these parameters of sludge influenced its dewaterability. Based on the specific CST result the dewaterability of the thermophilic sludge is lower than the mesophilic sludge (35 and 52s.l/g respectively) on average. However, the extent of dewaterability of the thermophilic sludge by centrifugation is relatively higher than the mesophilic sludge (65 and 62.9% respectively), desirable for the former.

Regarding the reject water characteristics, the CODsol of the thermophilic sludge is far higher than the mesophilic sludge which is on average 4741 mg/l and 2316 mg/l respectively. Still the thermophilic sludge is higher in Nammon concentration compared to the mesophilic sludge that is 1581mg/l and 1484 mg/l on average. These higher CODsol and Nammon concentrations thus need further treatment of the reject water.

Regarding foam phenomena, the thermophilic sludge foam is less stable than the mesophilic sludge foam (0.4 and 0.7 respectively) while the potential for foaming is higher by the thermophilic sludge.

Further, WAS as a secondary waste is still a useful substrate for AD in order to produce clean energy based on the BMP assay result.

Therefore before choosing the temperature of digestion between mesophilic and thermophilic the situation in our study dictated that considering the biogas yield from a specific technological or substrate point of view alone is not enough. Thus multi parametric optimization is a necessary approach to deal with the matter which considers various factors like economic, technologic and environmental dimensions.

As recommendation, it is necessary to study microbial diversity and abundance in the WAS AD using CSTRs as it is linked to methane production rate (Khemkhao et al., 2012). In other words, the microbial diversity and abundance could differ among inocula thus it is good to study such differences among inocula. Additionally, the result on dewaterability of sludge depends on the method applied to testing, therefore it is necessary to review existing methods for testing AD sludge dewaterability. The selection and design of technology for full scale sludge dewatering should be based on the results of different types of dewaterability tests.
8. References


Annex

I. The pH trend in both CSTRs

II. OLR in ml at a concentration of 45g/l-TS in to those 10l volume reactors