TRANSFORMATION AND UTILIZATION OF SOLUBILIZED SUBSTANCES FROM PRETREATED SLUDGE

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SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING

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TRANSFORMATION AND UTILIZATION OF SOLUBILIZED SUBSTANCES FROM PRETREATED SLUDGE

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A thesis submitted to the Nanyang Technological University in
partial fulfilment of the requirement for the degree of
Doctor of Philosophy

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

Date 12/12/2019. Zhou Yan

Authorship Attribution Statement

This thesis contains material from 3 papers published in the following peer-reviewed journals in which I am listed as an author.

Chapter 4 is published as D. Lu, K.K. Xiao, Y. Chen, Y.N.A. Soh, and Y. Zhou. Transformation of dissolved organic matters produced from alkaline-ultrasonic sludge pretreatment in anaerobic digestion: From macro to micro. *Water Research* **142**, 138-146 (2018). DOI: 10.1016/j.watres.2018.05.044.

The contributions of the co-authors are as follows:

- A/Prof Zhou suggested project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by Dr. Xiao and Dr. Chen.
- I co-designed the study with A/Prof Zhou and Dr. Xiao. I conducted all the lab work, including sludge preparation, sludge treatment, anaerobic digestion and organic compounds characterization. I also analyzed the data.
- Dr. Chen assisted in the collection of sludge.
- Ms. Soh assisted in the test and analysis of organics composition with Gas Chromatography-Mass Spectrometry and Ultra Performance Liquid Chromatography-Mass Spectrometry.

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- A/Prof Zhou suggested the project direction and edited the manuscript drafts.

- I prepared the drafts of the manuscript. The manuscript was revised by Dr. Sun.
- I performed all the sludge preparation, sludge treatment, anaerobic digestion and characterization of organic compounds. I also analyzed the data.
- Dr. Sun assisted in the collection of sludge and provided the guidance for sludge preparation.

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- A/Prof Zhou suggested the project direction and edited the manuscript drafts.
- I prepared the drafts of the manuscript. The manuscript was revised by Dr. Wu and Dr. Jiang.
- I performed all the sludge preparation, sludge treatment, anaerobic digestion and characterization of organic compounds. I also analyzed the data.
- Dr. Qian assisted in the experiment of biochar production and provided the guidance for related characterization.
- Dr. Cao assisted in the collection of sludge and provided the guidance for sludge preparation.

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SUMMARY

Waste activated sludge (WAS) is not only a potential environmental pollution but also an alternative resource. Technologies that could achieve both sludge reduction and resource recovery are studied in this Ph.D project. The primary aim of this research is to investigate and utilize the solubilized substances produced from sludge pretreatment. Pretreatment methods employed in this thesis are alkaline (ALK), ultrasonic (ULS) and thermal hydrolysis pretreatment (THP).

Firstly, the organics released by ALK, ULS and combined ALK-ULS pretreatment as well as their transformation in anaerobic digestion (AD) are investigated. ALK-ULS pretreated sludge has the maximum methane production. However, such pretreatment releases not only easily biodegradable substances but also more recalcitrants. Thus, more residual dissolved organic matters (DOMs) are detected after digestion. The steroid-like matters, alkanes and aromatics are the main components of the residual low molecular weight (LMW) DOMs. With the investigation of higher molecular weight (MW) residuals, recalcitrant compounds like flavonoids and benzene derivatives are detected. The results suggest that further treatment should be considered to reduce these refractory residuals.

Secondly, DOMs in THP sludge liquor and their transformation during AD are characterized. Sludge with 172 °C treatment shows the maximum methane production due to the significant release of LMW DOMs. The effluent from THP sludge digesters contains more DOMs residues. Over half of the residual LMW components are steroid-like compounds and aromatics. Further profiling of higher MW residuals also detects recalcitrant compounds. Therefore, polishing step should be considered to reduce the recalcitrant residuals in THP AD liquor.

When the liquid and solids fractions of THP sludge (THP-L and THP-S) are separately digested, most of organics in THP-L can be removed within 15 days, while THP-S only contributes 31.0% to the total methane production. In order to improve the total resource recovery from THP sludge, an integrated process, namely, methane generation from THP-L coupled with biochar production from THP-S is proposed, which results in an energy surplus. Additionally, THP-L digested sludge has better

dewaterability and reduced viscosity. The proposed new sludge treatment process therefore has lower operating cost and higher value returns.

Finally, the feasibility of using THP-L as fertilizer is evaluated. With 165 ºC treatment, the contents of nitrogen, phosphorus and free amino acids (FAAs) in the THP-L are comparable to the commercial fertilizer. Especially, plant growth-promoting compounds (e.g. FAAs and indole-3-acetic acid) can be generated under this temperature. More importantly, THP-L products (< 180 ºC) do not have inhibition but have promotion effect on the plant and soil bacteria growth. By comparing nutrients content, organics content, the potential phytotoxicity and inhibition effect, it is concluded that THP-L from 165 ºC treatment is expected to be a valuable organic fertilizer. The findings lead to a new resource recovery approach and help to solve sludge treatment problem.

Overall, this thesis provides new and detailed insights into the transformation of DOMs during sludge pretreatment and AD, proposes new approaches to utilize these DOMs, offers new direction for sludge treatment and resource recovery.

LIST OF PUBLICATIONS

Journal Papers

1. **Lu, D.,** Xiao, K., Chen, Y., Soh, Y.N.A. and Zhou, Y. (2018) Transformation of dissolved organic matters produced from alkaline-ultrasonic sludge pretreatment in anaerobic digestion: From macro to micro. *Water Research* 142, 138-146.

2. **Lu, D.,** Sun, F. and Zhou, Y. (2018) Insights into anaerobic transformation of key dissolved organic matters produced by thermal hydrolysis sludge pretreatment. *Bioresource Technology* 266, 60-67.

3. **Lu, D.,** Wu, D., Qian, T., Jiang, J.K., Cao, S.B. and Zhou, Y. (2020) Liquid and solids separation for target resource recovery from thermal hydrolyzed sludge. *Water Research,* 115476.

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Chapter of Text Book

1. **Lu, D.,** Le, C. and Zhou, Y. (2019) Identification of recalcitrant compounds after anaerobic digestion with various sludge pretreatment methods. Post Treatments of Anaerobically Treated Effluents, IWA publishing, 279-293.

Conference Proceedings

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
AMPTS	Automatic Methane Potential Test System
ANOVA	Analysis of Variance
BMP	Biochemical Methane Potential
BSA	Bovine Serum Albumin
CST	Capillary Suction Time
Da	Dalton
DD	Disintegration Degree
DOC	Dissolved Organic Carbon
DOMs	Dissolved Organic Matters
EPS	Extracellular Polymeric Substances
FID	Flame Ionization Detector
g	Gravitational Acceleration
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HA	Humic Acids
HMW	High Molecular Weight
HMs	Heavy Metals
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
HS	Humic Substances
LCFA	Long Chain Fatty Acids
LC-OCD-OND	Liquid Chromatography coupled with Organic Carbon Detector and Organic Nitrogen Detector
LLE	Liquid-Liquid Extraction
LMW	Low Molecular Weight
MW	Molecular Weight
PN	Proteins
PS	Polysaccharides
sCOD	Soluble Chemical Oxygen Demand

SEC	Size-Exclusion Chromatography
SEI	Specific Energy Input
SPE	Solid Phase Extraction
SRT	Sludge (Solid) Retention Time
tCOD	Total Chemical Oxygen Demand
THP	Thermal Hydrolysis Pretreatment
TOC	Total organic carbon
TS	Total Solids
TSS	Total Suspended Solids
UV/Vis	Ultraviolet-Visible
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge

CHAPTER 1 INTRODUCTION

1.1 Background and problem statement

Rapid urbanization and industrialization in the past two centuries have resulted in the growth of domestic and industrial wastewater treatment plants (WWTPs), with which clean water could be regenerated from wastewater. As an inevitable by-product of WWTPs, huge amount of waste activated sludge (WAS) is produced, resulting in environmental and health issues (Li et al. 2016, Raheem et al. 2018, Zhen et al. 2017). Sludge is a secondary environmental pollution source. Sludge disposal is required to meet strict environmental regulations, but sludge treatment technologies are limited (Eshtiaghi et al. 2013). More attention should therefore be paid to dispose, treat and/or reuse sludge.

Several methods have been applied in practice to handle sludge, such as incineration, combustion and landfilling (Agarwal et al. 2015, Hale et al. 2012). However, these processes cause either air pollution and/or energy and resource wastage. It is well known that WAS contains numerous microbes, organic matter and inorganic ingredients, which could be regarded as resources nowadays (Thomsen et al. 2017). Therefore, the development of techniques to achieve both sludge treatment and resource recovery is of great significance.

As one of the oldest and well-studied sustainable biological processes, anaerobic digestion (AD) is widely used to reduce sludge, recover bioenergy and stabilize the biomass (Yuan and Zhu 2016). However, the rate-limiting step of hydrolysis slows down the overall AD process and hampers the application of this technology (Tyagi et al. 2014). Pretreatment can disrupt sludge flocs and bacterial cells, releasing intracellular and extracellular substances. Thus, various pretreatment approaches, such as chemical, thermal, mechanical and biological pretreatment have been developed to accelerate the hydrolysis step and subsequent AD efficiency (Abelleira-Pereira et al. 2015, Wei et al. 2017). Among these methods, alkaline (ALK) and ultrasonic (ULS) pretreatment techniques attract great attention (Ruiz-Hernando et al. 2014, Zhang et al. 2007). The combined pretreatment of these two methods was reported to reduce the energy requirement, improve sludge disintegration as well as enhance the biogas production (Kim et al. 2010a). Besides, thermal hydrolysis pretreatment (THP) has also been successfully installed at the full-scale for more than a decade (Pilli et al. 2014). It is well-documented that THP of sludge could obtain higher volatile solids (VS) reduction (235% > control), enhance biodegradability, improve dewaterability and reduce viscosity (Carrere et al. 2008, Ennouri et al. 2016a).

After pretreatment, a large amount of dissolved organic matters (DOMs) would be released, which are considered as the direct assimilable carbon source to microorganisms compared with particulate organic matter. DOMs generated with different sludge pretreatment methods may have different compositions and fate. However, previous studies mainly focused on either qualitative or semi-quantitative characterization of the overall DOMs, without a comprehensive analysis of the specific key compounds. It is still not clear how these DOMs are produced in the pretreatment step and how the key DOMs are transformed during the subsequent AD process. Besides, the methane production is closely related to sludge solubilization efficiency (Carrere et al. 2008). To date, most of the research studied the correlation between the total removal of organic matters (volatile solids removal in particular) and the methane production with/without sludge pretreatment. The detailed correlation between the specific organics and biogas generation has not been systematically investigated.

With THP, up to 60% of sludge solubilization can be obtained (Bougrier et al. 2008, Lu et al. 2018a), which indicates abundant DOMs would be present in the liquid fraction of THP sludge (THP-L). The solids fraction of THP sludge (THP-S) would have less readily biodegradable organic matters, resulting in the less biogas generation and/or longer digestion period (meaning larger reactors). Investigation on the needs to digest THP-S and biogas production from THP-L and/or THP-S are required. Besides, THP could significantly improve sludge dewaterability and flow behavior. But such good properties could not be maintained when it is mixed with anaerobic sludge for anaerobic digestion (Zhang et al. 2018). Thus, it would make more sense to carry out the dewatering step prior to AD to treat the liquid and solids portions more efficiently. To make the wastewater management more energy-efficient, such separation needs to be proposed and evaluated.

In addition, there are large amount of nutrients, such as nitrogen (N), phosphorus (P) and potassium (K) remaining in sludge after AD. Sludge land application is an efficient way to utilize the nutrients and organics in the sludge. But the presence of hazardous substances, like heavy metals (HMs) and pathogens, limits the direct application of sludge as solid fertilizer (Wang et al. 2019a). THP of sludge could effectively remove pathogens, improve dewaterability, solubilize organic matters and nutrients (Lu et al. 2018a, Zhang et al. 2018), indicating the fertilizer potential of THP-L fraction but such possibility has not been explored. Sludge subject to different temperature TH would result in distinctly different content of soluble organic matters, nutrients and inorganic substances (Sun et al. 2014). It is likely that the soluble products released under different temperature may also vary the fertilizing effects of the sludge on plants. Certainly, the TH temperature should not be too high given a more sustainable approach would be preferred. Last but not least, the soluble organics of sludge are reported to be capable of promoting plant growth (Sun et al. 2014), however, few studied the specific organic compounds that may promote plant growth. These issues need to be studied in order to better understand the fertility of THP-L products and evaluate the feasibility of using THP-L fraction as the organic fertilizer.

1.2 Objectives

The main objectives of this Ph.D study are to:

1) Characterize the key DOMs produced during ALK-ULS pretreatment and their transformation during AD;

2) Investigate the transformation of DOMs generated from THP in sludge anaerobic digestion;

3) Propose an energy-efficient method to separate liquid and solids fraction of THP sludge to recover target resources;

4) Evaluate the fertilizer potential of THP-L fraction.

1.3 Organization of the thesis

This thesis comprises eight chapters. Each chapter elaborates the content as follows:

Chapter 1 - Introduction

This chapter provides a brief introduction of the background, problems statement and main objectives of the thesis.

Chapter 2 - Literature review

This chapter provides information on the sludge treatment status, fundamental knowledge of anaerobic digestion and various pretreatment methods. Especially, the DOMs as well as their characterization methods are emphasized. Also, the development of techniques for sludge land application are presented. Finally, the limitation and knowledge gaps are discussed.

Chapter 3 - Materials and methods

This chapter presents the description of the general materials and common analytical methods used in this thesis.

Chapter 4 - Transformation of dissolved organic matters produced from alkaline-ultrasonic sludge pretreatment in anaerobic digestion

This chapter characterizes the key DOMs formed during ALK and/or ULS pretreatment and studies the impact of such organics on AD process. The recalcitrant compounds that may be potentially formed during pretreatment or AD are identified and discussed.

Chapter - 5 Insights into anaerobic transformation of key dissolved organic matters produced by thermal hydrolysis sludge pretreatment

The DOMs generated under low, medium and high temperature are analyzed and their transformation during the subsequent AD is investigated in this chapter. After AD, the residual DOMs are characterized and discussed.

Chapter - 6 Liquid and solids separation for target resource recovery from thermal hydrolysis pretreated sludge

The AD performance of THP sludge, THP-L and THP-S is investigated respectively and their rheological properties during AD are explored in this chapter. An integrated system to handle both liquid and solids portions of THP sludge for biogas generation and biochar production in an economical way is proposed and evaluated.

Chapter – 7 Thermal hydrolyzed sludge liquor as organic fertilizer

This chapter characterizes the content of THP-L products generated under different temperature, including nutrients and DOMs, especially plant growth-promoting organic compounds. The toxicity to soil microbes and phytotoxicity of THP-L products are evaluated.

Chapter - 8 Conclusions and recommendations

This chapter summarizes the major findings of this thesis. Some recommendations are proposed for future study.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This literature review starts with a brief introduction of sludge treatment status and fundamental anaerobic digestion process. Then the definition of pretreatment methods and their implication are discussed. Additionally, a review of dissolved organic matters characterization methods is presented, with special attention focused on analysis of components groups and individual compound. The sludge physiochemical properties are also reviewed in this chapter. Finally, the potential of using sludge products as fertilizer is reviewed and discussed.

2.2 The status of sludge production and treatment

Wastewater is treated by WWTPs to regenerate the cleaner water. The number of WWTPs is rapidly increasing with the development of industrialization and urbanization in the world. As an inevitable by-product, sludge is produced during wastewater treatment process, which has imposed negative impact on human and environment (Raheem et al. 2018). For example, 220,000 tons of dry sewage sludge is produced annually in Sweden and 6.5 million tons/year is produced in USA (Lundin et al. 2004, Zhen et al. 2017). Globally, about 45 million tons of dry sludge is produced per year, which is equivalent to 2.0 billion population equivalent (PE) (Zhang et al. 2017). What's more, the cost for sludge treatment is as high as 150 – 350 USD/dry ton in Australia and 165 – 550 USD/dry ton in Europe (Zhang et al. 2017). Generally, the operation cost for sludge treatment could reach as high as 50% of the total operation cost for WWTPs (Hii et al. 2014). Notably, the regulations for sludge reuse/disposal become more stringent and the public concerns are increasing. All these of issues emphasize the importance of developing the proper techniques to enhance the efficiency of sludge treatment and final disposal.

Wasted Activated Sludge (WAS) is the residual semi-solid material left over after biological treatment of wastewater, which is mainly composed of functional microbes and secreted extracellular polymeric substances (EPS) (Tyagi and Lo 2013, Wu et al. 1998). Typically, WAS contains 59 - 88% (w/v) decomposable organic substances, including 50 - 55% carbon, 25 - 30% oxygen and 10 - 15% nitrogen (Raheem et al. 2018). Various valuable resources, including water (water content above 80 wt%), organic substances (proteins, polysaccharides, etc.) and nutrients (such as nitrogen, phosphorus and potassium) are present in sludge, which could be recovered with proper treatment techniques. Among them, water is the important asset that can be recycled through treatment processes. Organic substances can be recovered directly or used to generate energy. Nutrients are especially valuable in agricultural application. In conventional WWTPs, about 90% of the incoming phosphorus load is incorporated into the sewage sludge (Desmidt et al. 2015). It should be pointed that the hazardous substances such as pathogenic microbes, heavy metals (HMs) and recalcitrant compounds are also present in sludge, which would be harmful to the environment and cause pollution if not properly handled (Li et al. 2018).

Many sludge treatment technologies are available for the safe disposal, energy generation and resource recovery, such as landfill, incineration, AD and land application. The most common ways to handle sewage sludge in the US are landfill, incineration and land application (Gude 2015). With incineration, up to 90% of sludge volume reduction as well as the destruction of pathogens could be achieved simultaneously. The ashes generated (30 wt%) could be used to produce building materials or disposed into the landfills (Wu et al. 2016). However, incineration has drawbacks. High operation cost and generation of fly ashes and hazardous gases (such as CO, NO_x, SO_x and dioxins) suggest it is not eco-friendly and sustainable (Jin et al. 2015). For land deficient countries, landfill is not a viable option any more (Nizami et al. 2017). The leachate from sludge landfill would be a cause for groundwater contamination and subsequent aquatic pollution (Mitrano et al. 2017). AD process and land application are the commonly used methods to effectively treat sludge and utilize resources. Via AD process, organic matters in sludge can be converted to biogas energy. The produced methane can be used for generating electricity and/or heat, which compensates the total operation cost of WWTPs (Carrere et al. 2010). Land application of sewage sludge used to be a popular way to reutilize the sludge resource in many countries. But the HMs accumulated in soils transfer along the food chain, being the threat to the health of humans and animals (Chang et al. 2018). If the problems of hazardous substances can be solved, land application of sludge will be an ideal method as it can recycle the nutrients and organic matters sustainably.

The urgent sludge problems and current demand for a sustainable society call for the economic and environmentally friendly approaches to treat sludge and make full use of its valuable substances. Thus, the following literature review will focus on the sludge treatment with AD and land application technologies.

2.3 Anaerobic digestion of sludge

2.3.1 Basic principles of anaerobic digestion

AD is commonly used to degrade the organic matters by a diverse and closely dependent group of microbes in the absence of oxygen or nitrates (McCarty and Smith 1986). The whole degradation process consists of different reaction steps, including hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gujer and Zehnder 1983, Jain et al. 2015) as shown in Figure 2.1. During the first step of hydrolysis, complex organics like proteins (PN), polysaccharides (PS), lipids and nucleic acids are solubilized and hydrolyzed into the smaller soluble substances (e.g. monosaccharides, long-chain fatty acids (LCFAs) and amino acids) with the assistance of enzymes. Then acidogenesis process is mediated by acidogenic (organic acid forming) bacteria. In acetogenesis, the intermediate metabolites are converted to acetate (HAc), hydrogen (H_2) and CO_2 by hydrogen-producing acetogenic bacteria. Finally, the methanogens transform the previous products into the biogas (CH_4 and CO_2). In this step, HAc can be formed with H_2 and CO_2 by hydrogen-oxidizing acetogens first and split into CH_4 and CO_2 by aceticlastic methanogens. In general, more than two-third of the methane is produced from HAc fermentation, while the rest is generated from CO_2 reduction (Jain et al. 2015). The generation of methane with low energy input is the key merit of AD process, which promotes the application of AD to treat various organic waste, especially sludge.

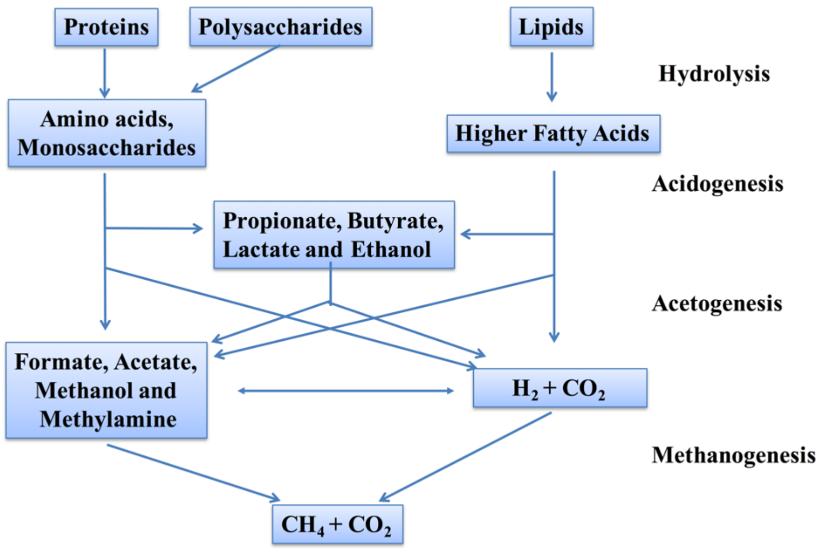

Figure 2.1 Anaerobic process reactions adapted from Gujer and Zehnder (1983).

2.3.2 Anaerobic digestion of WAS

WAS (return sludge) is a microbial matrix composed of microorganisms, EPS and influent solids. These microbial-originated EPS are a complex mixture of biopolymers, which is relatively recalcitrant to AD by nature with only 30 - 50% biodegradability (Li and Noike 1992). These three-dimensional, gel-like EPS also protect cells from rupture and lysis. Except the protection from EPS, bacteria also possess a hard cell envelope, which is composed of glycan strands crosslinked by peptides, presenting physical and chemical barriers to direct AD. Generally speaking, WAS with more EPS content and microbial cells is sturdier and will be more difficult to be hydrolyzed. Besides, the degradability of WAS is also affected by some other parameters, such as aerobic/anoxic fraction, the inerts content from the upstream catchment, and in particular, the aerobic sludge age (Mottet et al. 2010). The biodegradability is lower for activate sludge with long-sludge age and higher for the primary sludge. In the conventional mesophilic AD process, the volatile solids (VS) reduction of WAS was studied to be 30 - 50% and the biogas yield was typically around 270 - 385 CH_4/g VS (Appels et al. 2008, Dai et al. 2016).

Owing to the immobilized organic matters in sludge and high resistance of WAS to biodegradation, the sludge disintegration or organic matters hydrolysis is known to be the rate-limiting step for AD process. Using pretreatment to disrupt EPS matrix and cell walls to release the extracellular and intracellular substances and making them more accessible to microorganisms have been widely studied (Carrere et al. 2010). Bougrier et al. (2008) reported that the higher temperature treatment could lead to higher WAS disintegration, with the best performance of methane production at 170 - 190 °C. Ultrasonic pretreatment was also studied to reduce the sludge particle size, making organic matters more accessible for enzymatic attack (Vavilin et al. 2008). In Singapore, a full-scale sludge digester (4500 m^3) fed with sonicated sludge was studied for 6 months, producing 45% more methane than the control digester (Xie et al. 2007). In a word, the biodegradability of WAS is low, and the hydrolysis rate is slow. Pretreatment prior AD can both accelerate the hydrolysis and improve the inherent biodegradability of sludge, which has been widely studied.

2.4 Pretreatment methods

So far, various sludge pretreatment options, such as thermal, mechanical, chemical, biological or combined treatment, have been performed at laboratory, pilot scale and full scale. Most of them have achieved different levels of success by speeding up the hydrolysis rate and enhancing the biogas generation (Cesaro and Belgiorno 2014). In this section, the treatment approaches including alkaline, ultrasonic and thermal processes are reviewed.

2.4.1 Alkaline pretreatment

Alkaline (ALK) pretreatment is promising in sludge disintegration due to the multiple advantages, such as the simple device, ease of operation, low cost and high methane conversion efficiency (Jin et al. 2009). With the addition of base, it can be carried out at the ambient or moderate temperature (Kim et al. 2010a). By dissociation of acidic groups in EPS, saponification of the lipid by-layer of the cell membrane and denaturation of PN, alkali can help to release the intracellular substances effectively (Liao et al. 2002). In addition, ALK pretreatment has shown high attractiveness in the production of short-chain fatty acids (SCFAs) during sludge fermentation (especially at pH of 10) (Chen et al. 2017).

The reagents and pH values are the main control parameters affecting the pretreatment performance. Several ALK reagents can be used to operate ALK pretreatment, inducing NaOH, $Ca(OH)_2$, KOH, $Mg(OH)_2$ and CaO. The effectiveness of each alkali is closely related to its affinity to the organic components. As a result, a ranking of efficacy (NaOH > KOH > $Mg(OH)_2$ and $Ca(OH)_2$) was found, of which NaOH was the most efficient to accelerate the sludge solubilization and enhance biodegradability (Kim et al. 2003). An increase of 83% compared to control in biogas production was observed when NaOH was applied to treat the pulp and paper sludge (Lin et al. 2009). High pH values can cause the breakup of sludge flocs, resulting in the increased hydrolysis rate and worsened dewaterability. The pH ranges of 10.0 - 12.5 and 9.0 - 12.5 were observed to rupture the most of cell walls and cell membranes (Xiao et al. 2015). However, pretreatment with the overloaded NaOH (pH > 12) is not suitable for AD process. Ammonia inhibition would be enhanced under higher pH and high concentration of sodium (3 g/L or higher) can also inhibit the anaerobic activity and decrease the biogas generation (Dai et al. 2017, Li et al. 2012). Moreover, the increase of recalcitrant compounds (e.g. humic substances) during pH modification would reduce the sludge biodegradation (Tian et al. 2015a). Other disadvantages of ALK pretreatment like the increased mineral content in the digested sludge and re-neutralization of treated sludge should also be taken into account (Carrere et al. 2010).

2.4.2 Ultrasonic pretreatment

Ultrasonic (ULS) pretreatment is a well-established technique for sludge treatment. During ULS pretreatment, the sudden and violent collapse of cavitation bubbles leads to extreme conditions (a pressure above 500 bars) (Chu et al. 2002), creates powerful hydro-mechanical shear forces and/or forms highly reactive radicals (H· and ·OH), which induce the breakup of sludge flocs and release of the extracellular and intercellular substances (Xiao et al. 2015).

When operating ULS pretreatment, factors such as frequency, specific energy input (SEI, kJ/kg TS) and sludge concentration should be considered. The low frequency ultrasound (20 - 40 kHz) has been widely applied for sludge disintegration (Zhang et al. 2013). In addition, applying ULS pretreatment with different SEI can cause different levels of sludge disintegration and cells lysis. The commonly reported SEI range for sludge solubilization is from 1000 to 16,000 kJ/kg TS, which could be varied with different sludge concentrations (Carrere et al. 2016). The optimum sludge concentration was reported to be around 20 - 30 g/L for ULS treatment. It is because more particulates in liquid can produce more cavitation sites and more shear forces while homogenous distribution of acoustic waves may be disrupted when sludge concentration is beyond the optimum value (Pilli et al. 2011). Noted that for a given SEI, pretreatment with the higher power intensity was more effective than that with the lower power intensity (longer treatment time) (Tyagi et al. 2014). Further elevation of the applied SEI to 26,000 kJ/kg TS increased the breakup of sludge flocs and the solubilization of particulates into soluble substances, but it consumed much higher energy (Foladori et al. 2007).

Under a lower power input, the inactivation of microbes is found prior to the cell lysis as the energy requirement for cell lysis is higher. Huan et al. (2009) reported that cells started to lyse only when the disintegration degree was over 40%. Besides, during ULS pretreatment, the component of PN could release more than PS (Tian et al. 2015b). However, whether the ultrasonication is able to break down PN and PS is not known, which needs further investigation.

ULS pretreatment has been widely installed in many full-scale and pilot-scale sludge anaerobic digesters, mostly in Germany. The ULS facilities resulted in 25 - 50% increase of digestion rate and 10 - 45% increase of biogas generation (Barber 2005, Carrere et al. 2010). Nevertheless, there are still some limitations for ULS pretreatment application. For example, high energy input is required to achieve substantial gains in degradability for ultrasonic pretreatment. The conversion efficiency between different types of energy is low. Only 30 - 40% of electrical energy could be converted to acoustic energy transmitted to the sludge (Chatel 2017).

To compensate the high energy consumed in ULS pretreatment and maximize the methane recovery, ALK method was employed together to treat sludge. Kim et al. (2010a) reported that the combined ALK-ULS pretreatment could promote the sludge solubilization to 70% from 50% for the individual pretreatment. The synergistic effect of combined pretreatment was also observed, where the disintegration degree achieved in the combined pretreatment is higher compared to the total of the separate pretreatment (Li et al. 2015). In addition, the sequence of each method would also affect the efficiency of sludge disintegration. The sludge solubilization was found to be higher after ALK-ULS treatment compared to that after ULS-ALK pretreatment (Jin et al. 2009).

2.4.3 Thermal hydrolysis pretreatment

2.4.3.1 Low temperature thermal pretreatment

Low temperature thermal pretreatment (LTHP) applies the temperature from 55 to 100 °C, resulting in the disintegration of cell membrane and solubilization of organic components (Nazari et al. 2017). When operating sludge LTHP, waste heat could be reused because of the low temperature. The main parameters for LTHP are temperature and retention time. The particle size of sludge was found to reduce from 50 to 95 °C, increasing the hydrolysis rate (Prorot et al. 2011). Regarding the biogas generation, the increase trend was found at 60 - 75 °C while conflicting results were reported at 80 - 90 °C, which is likely due to the deactivation of enzymes (Nges and Liu 2009). However, the lower temperature treatment requires more retention time, which could last from hours to several days (Gavala et al. 2003, Sun et al. 2016). Besides, the solubilization of PN and PS didn't reach 20% of their total content (Dong et al. 2016). Only 40 - 50% of soluble chemical oxygen demand (sCOD) from LTHP sludge was found to be biodegradable at a hydraulic retention time (HRT) of 24 hours (Paul et al. 2006). What's more, the dewaterability of digestate is also deteriorated with sludge LTHP.

Low temperature pretreatment is also applied in the process of temperature phased anaerobic digestion (TPAD), which uses a higher temperature at thermophilic stage (around 55 ºC), either under anaerobic or aerobic conditions (Carrere et al. 2010). The VS reduction of 54% was observed by treating primary sludge with the thermophilic (HRT of 2 days) - mesophilic (HRT of 13 - 14 days) TPAD (Ge et al. 2010). The increased hydrolysis coefficient rather than an increase in inherent biodegradability is reported to be the key reason of the improved performance. The biological effect like the increased microbial population or production of extracellular hydrolytic enzymes and non-biological effect such as reduced sludge particle size and increased surface area are the possible mechanisms.

2.4.3.2 High temperature thermal pretreatment

High temperature thermal pretreatment (HTHP) relies on the employment of temperature above 100 °C, where the high temperature will promote the disruption of cells and release of the intracellular substances. HTHP was first applied to improve sludge dewaterability because it could effectively destroy the sludge gel structure, decrease the viscosity of sludge and release the bound water (Barber 2016). The significant improvement of dewaterability was obtained through treatment above 150 °C (Bougrier et al. 2008). In addition to the improved dewaterability, HTHP could lead to severe sludge solubilization, which would accelerate the AD rate and greatly enhance sludge biodegradability (Gavala et al. 2003, Li et al. 2017a). The well-known industrial-scale technologies based on HTHP are Cambi™ (150 - 165 °C for 30 min, 8 - 9 bar) and BIOTHELYS (150 - 180 ºC for 30 - 60 min, 8 - 10 bar) (Pilli et al. 2015). Obviously, the most important parameters affecting treatment efficiency are temperature, pressure and operation time. Most studies reported that the optimal temperature for HTHP was from 160 to 180 °C and the treatment time was from 30 to 60 min (Carrere et al. 2010). The pressure of these conditions may vary from 600 to 2500 kPa (Weemaes and Verstraete 1998). Noted to point out that the treatment duration is likely to have less effect compared with treatment temperature for HTHP (Neyens and Baeyens 2003).

Generally, the increase of methane production is in proportion to sludge COD solubilization (Carrere et al. 2008). However, it was found that the increased solubilization was obtained with increasing temperature up to 220 °C, but no increment of methane generation was observed (Dwyer et al. 2008, Mottet et al. 2009). It implied that treatment of excessively high temperature (higher than 170 - 190 °C) could deteriorate biodegradability in spite of improving sludge solubilization. This may be explained by Maillard reactions, where reducing sugars and amino acids would react to form nonbiodegradable and inhibitory by-products (such as melanoidins) (Bougrier et al. 2008). Melanoidins may remain in the anaerobic digester, increasing the color of final effluent (Dwyer et al. 2008). Besides, at 170 °C, the caramelization of sugars also happens, forming organic acids, ketones and aldehydes. The extensive denaturation of PN and their degradation are also expected at temperature around 190 - 220 °C (Wilson and Novak 2009).

The other merits of HTHP include reduction of sludge viscosity, enhancement of sludge handling, sludge sanitation, and no extra energy requirements (positive energy balance by excess biogas production) (Nazari et al. 2017). There are still some potential disadvantages which need to be noted, including largely increased soluble recalcitrant, the deterioration of AD by ammonia inhibition, and final effluent color (Batstone et al. 2010).

Table 2.1 Summary and assessment of different pretreatment methods (further modified from Gonzalez et al. (2018), Zhen et al. (2017)).

Pretreatment	ALK	ULS	Thermal < 100 ºC	Thermal > 100 ºC
Mechanism	Solvation, saphonication	Hydro-mechanical shear forces, oxidizing effect	Heating	Heating
Controlling parameters	Dose, time	Power input, time	Temperature, time, pressure	Temperature, time, pressure
Hydrolysis rate	↑	↑↑	↑↑	↑↑
VS removal	↑↑	↑	↑	↑↑
Dewaterability of digestate	↓	↓	↓	↑
Methane production	↑	↑	↑	↑↑
Investment costs	Low	High	Low	High
Advantages	Increased methane yield, simple device, easy operation， suitable for lignin breakdown	Increased methane yield, effective in the degradation of cellular wastes, easy maintenance	Increased methane yield, sludge reduction, low cost	Increased methane yield, sanitation, odor removal, sludge reduction, improved dewaterability
Disadvantages	Chemical cost, corrosion, toxicity of Na^+, recalcitrant compounds formation, neutralization	High energy demand, probes replacement every 1.5–2 years	Recalcitrant compounds formation, ammonia release	High energy demand and capital cost, recalcitrant compounds formation, ammonia release

Notes: ↑ means increase, ↓ means decrease

The summary and assessment of different pretreatment methods are tabulated in Table 2.1. The evaluation of one suitable pretreatment technique not only depends on the sludge solubilization and methane production but also is assessed by the environmental and energetic effect. Therefore, the techniques for processing pretreated sludge, such as AD, dewatering, transportation and disposal should be considered. In addition, recalcitrant compounds formed during different pretreatment are still not clear. These refractory components may be problematic during AD process and downstream treatment of the concentrated reject water. The specific compounds of the solubilized organics should also be paid attention to, which could help to obtain a better understand of the pretreatment methods.

2.5 Characterization of the solubilized substances

2.5.1 The investigation of solubilized substances after sludge pretreatment

After sludge pretreatment, some of the particulates could be solubilized in the liquid phase as dissolved organic matters (DOMs). For THP sludge, up to 60% sludge solubilization was achieved as the hydrolysis effect could reach the microbial cell nuclei (Lu et al. 2018a). The majority solubilized substances in pretreated sludge are PN, PS and HS (Li et al. 2016). Among them, PN and PS are the major contributors to the methane production during digestion (Lu et al. 2018b). HS would remain in the effluent due to the refractory properties. The presence of residual soluble organic matters may be problematic when treating the effluent at the downstream processes. For example, the content of HS as well as part of PN and PS (not degraded completely) were reported to be the primary factors for membrane fouling in membrane bioreactors (Stuckey 2012). Some soluble substances in the effluent may become precursors for disinfection by-products (Liu and Li 2010). Gu et al. (2018) reported that the diluted supernatant of THP sludge could be applied to the side-stream anammox process. But some organic matters in THP sludge liquor would cause inhibition to the anammox process. For the investigation of the soluble substances, previous studies mainly focused on the total VS/COD or some common chemical groups like PN and PS, the information of the specific compounds or groups is still lacking. To overcome the limitation of characterization techniques, more effort should be made in this filed.

2.5.2 Characterization of the solubilized substances

Characterization of the soluble specific compounds or chemical groups in pretreated sludge has been a challenge because it is a complex mixture of various compounds. The extraction, separation and identification of the individual component are all complicated. Many attempts to characterize the soluble components have been conducted previously, which are mainly applied to characterize one group of compounds with the same characteristics or the individual compound.

2.5.2.1 Characterization of the groups of compounds

Because of the simplicity, colorimetric methods like UV are often used to analyze groups of organics, including PN, PS and HS (Xiao et al. 2017a). For example, Lowry and Bradford methods are widely applied to analyze PN in the wastewater treatment system (Kunacheva and Stuckey 2014). These colorimetric methods putatively provide the concentration of given types of organic components, but no detailed information about their chemical structures and properties is offered. Moreover, some groups of compounds may be interfered by other existing components, resulting in the misestimated quantities (Le and Stuckey 2016).

To determine the main chemical groups of compounds, the analytical method of Fourier Transform Infrared Spectrometry (FTIR) is commonly used. It produces an infrared spectrum over a wide spectral range, showing vibrational modes of atomic bonds of molecules (Li et al. 2016). By determining the functional groups of organics in wastewater treatment systems, FTIR could be used to predict the major components. The tryptophan-like PN derivate HS was proposed when observing the increase of aromatic amine groups (absorbing at 1300 cm^{-1}) (Amir et al. 2006). Wang and Wu (2009) compared the FTIR results of clean and fouled membranes, concluding the presence of PN and PS by observing a large absorption band for the hydroxyl and amino groups (Mei et al. 2014). However, the technique of FTIR can't accurately quantify the amounts of compounds groups because of the discrepancy in absorption intensity and samples preparation problems. This technology is useful for estimating part of the compounds' structure, like functional groups that have adsorption peaks, but it couldn't provide enough information for the detailed compounds identification.

According to the fluorescence characteristics of compounds, the soluble substances could be divided into five regions through Fluorescence Excitation-Emission Matrix Spectroscopy (EEM) method, including tyrosine-like PN, tryptophan-like PN, fulvic acid-like materials, microbial byproduct-like materials and humic acid-like products (Xiao et al. 2017c). EEM technique is known to be selective and rapid. In past years, it has been widely applied to identify soluble compounds in wastewater system by comparing the spectral fingerprints which were produced at different excitation and emission wavelengths (Ex/Em) (Sun et al. 2016). However, the correction and extraction of EEM data are time-consuming, which is still in development.

In recent years, the technique of Liquid Chromatography – Organic Carbon Detection – Organic Nitrogen Detection (LC-OCD-OND) has been used to evaluate the characteristics of organics in water samples (Huber et al. 2011). It is able to differentiate organics from high molecular weight (HMW) to low molecular weight (LMW) based on their different MW and chemical properties, including biopolymers (> 20 kDa), humic substances (HS), building blocks (300 – 500 Da), LMW neutrals and LMW acids (< 350 Da). This technique permits the fractionation and quantification of different groups of components. The successful application of Size Exclusion Chromatography (SEC) coupled with LC-OCD-OND to determine the soluble components in different pretreated sludge liquor was well-documented previously (Li et al. 2016, Sun et al. 2018a). In addition, this technique could be incorporated with other analytical methods, such as UV technique. The content of biopolymers is regarded as the total of high molecular weight (HMW) PN and HMW PS. Thus, the LMW PN and LMW PS could be determined by subtracting HMW PN and HMW PS from the total PN and PS respectively. Sun et al. (2018a) reported that after sludge freezing process with nitrite, the mainly released compounds were readily biodegradable components, including LMW PN, LMW PS, building blocks, LMW neutrals and LMW acids.

2.5.2.2 Identification of the individual compounds

In order to identify the specific individual compound, the advanced technique like Gas Chromatography Mass Spectrometry (GC-MS) has been applied recently. Rather than only offering the overall picture of some groups of compounds, it could identify the separate compound in wastewater treatment systems. The aromatic carboxylic acids and short chain carboxylic acids were proved to be dominant when LMW components in raw wastewater were characterized with GC-MS (Reemtsma and Jekel 1997). During anaerobic process, the increase of aromatic compounds was observed due to the hydrolysis or breakup of HMW substances (Ahring et al. 2015). In a reactor only fed with mineral salts, vitamins, metals, cysteine and sugars, GC-MS was successfully applied to find out the high concentrations of long chain alkenes, long chain alkanes, aromatics, esters and amides in the reactors' effluent (Aquino and Stuckey 2004). It should be noted that the detection of GC-MS only allows for non-polar, volatile (mainly LMW) compounds. Further analytical methods such as Liquid Chromatography Mass Spectrometry (LC-MS) should be considered to detect more non-volatile and HMW substances.

The untargeted metabolomics methods using LC−MS have been applied to many research fields, such as environmental research. Tipthara et al. (2017) developed this technique to profile and identify the metabolites in the supernatant of an anaerobic bioreactor and found out that the increased organic matters after feed were primarily phospholipids, ceramides and cardiolipins. Although more compounds could be identified and determined with these advanced analytical techniques, these technologies were seldom used to analyze the solubilized substances of pretreated sludge due to the more complex content. The preparation methods to extract or concentrate the target compounds should be taken into account and the analytical methods for such complex systems should be developed.

2.6 Change of sludge properties

2.6.1 Sludge dewaterability

Sludge dewatering is an important step for sludge treatment processes as it aims to reduce sludge volume thus decrease the cost of sludge transport and disposal. The water content of sludge is generally up to 80% even after filter press or centrifuge. Due to the special properties of sludge like high hydrophilicity, it is difficult to dewater. To optimize sludge treatment process, dewaterability is one of the most key parameters to be understood. It could be indicated by the capillary suction time (CST), water content of dewatered sludge cake and specific resistance to filterability (SRF) (Liu et al. 2016a).

The dewaterability of sludge could be improved by different treatment processes such as physical and chemical conditioning (Wang et al. 2017). THP is a well-known technique to enhance sludge dewaterability, where EPS destruction and bound water release play important roles (Liu et al. 2019). Besides, other pretreatment methods, such as acidification/ultrasonic pretreatment, ALK pretreatment and Fenton pretreatment could also improve sludge dewaterability (Liang et al. 2015, Xiao et al. 2016). For the investigation of the digested sludge dewaterability, Song et al. (2016) reported that the CST could be reduced by 90% through persulfate and zero valent iron pretreatment. In addition, the change of sludge dewaterability during AD was investigated by Zhang et al. (2018), who found the dewaterability of digested sludge was improved by 3.5% and 5.1% respectively for the unpretreated and THP sludge. It is known that the solids content, organics composition and processes conditions could affect sludge dewaterability (Yeneneh et al. 2016). However, it is always overlooked when new sludge treatment processes are proposed. This property is of great importance when taking the energy effectiveness into consideration.

2.6.2 Sludge rheological properties

The optimal design and operation of sludge treatment need the accurate prediction of parameters for various facilities, such as pumps, pipes and mixing devices, which in turn depends on the properties of sludge, especially the rheological properties. In general, the apparent viscosity could indicate the rheology of Newtonian fluid, whose shear stress and shear rate have linear relationship (Tang and Zhang 2014). But it is well-known that sludge is a non-Newtonian fluid with shear-thinning properties as the shear stress is not linearly proportional to the shear rate (Eshtiaghi et al. 2013, Lotito et al. 1997).

The rheological properties of sludge largely depend on the temperature, sludge origin and solids concentration (Markis et al. 2014). Solids concentration was reported to exhibit an exponential impact on sludge viscosity (Eshtiaghi et al. 2013). The shear stress could be doubled when the total suspended solids (TSS) of raw sludge was increased from 6 to 8% (Füreder et al. 2018). On the other hand, sludge rheology could be modified by treatment processes, including pretreatment and AD. Liu et al. (2016b) reported that microwave-H_2O_2 pretreatment improved sludge flowability and showed positive effect on the rheology properties of digested sludge after AD. Meantime, the decreased consistency index and increased flow behavior index for digested sludge indicate the decreased non-Newtonian flow characteristics. The dissolution of some of the solids may cause a decrease of the yield stress during sludge thermal hydrolysis (Baudez et al. 2013). In addition, the decrease of yield stress and viscosity was found to be partially irreversible due to the irreversible solubilization of solids, denaturation of proteins and destruction of EPS (Farno et al. 2014).

Barber (2016) investigated THP of sludge from 130 to 180 ºC, finding that the apparent viscosity of sludge decreased with the increasing temperature. ULS, ALK and LTHP methods could all reduce sludge viscosity and the reduction was more with higher treatment intensity (Ruiz-Hernando et al. 2014). In addition, the reduction of apparent viscosity of sludge was found during digestion (Guibaud et al. 2004). And the longer solid retention time (SRT) appeared to produce sludge with lower viscosity and better flowability through the continuous digesters (Dai et al. 2014).

As stated above, the rheological properties of sludge through AD or pretreatment have been investigated. To comprehensively assess a pretreatment method prior AD, it is necessary to study the sludge rheology throughout the whole treatment process. Besides, sludge rheology is regarded to be a bridge between its physical-chemical properties and dewaterability (Wang et al. 2017), which would provide a better understanding of the mechanisms for pretreatment on sludge dewatering.

2.7 Fertilizer potential of sludge

Sludge shows the great potential as fertilizer because it is rich in macro nutrients (such as N, P and K) required for plant growth. The organic substances constitute more than half of sludge content, whose addition could improve soil structure, increase water retention and promote the bioavailability of macro and micronutrients (Guidi 1981, Kominko et al. 2017). The decomposition of substances in soil could also be improved, which provides organic sources for soil microbes. Notably, the organic matters of sludge are able to promote plant growth (Day and Katterman 1992). The application of sludge as fertilizer seems to be ideally favorable as it can recycle nutrients and organic matters sustainably. However, the presence of HMs, pathogenic microorganisms, some toxic compounds and unpleasant odor limit the direct application of WAS to the soil. Especially, the HMs could be accumulated in soil and plant, which would be a potential threat to human and animals.

To solve these problems, various approaches have been developed in order to realize the fertilizer application of sludge. The digested sludge was found more suitable to be used as soil fertilizer due to the lower bioavailability of HMs (Liu et al. 2018). Thermally dried anaerobic sludge was reported to have the higher nitrogen recovery and greater amount of mineralizable nitrogen, resulting in an effective rapid release fertilizer (Rigby et al. 2016). Composting is one of the cost-efficient methods to manage and reuse sludge resource. With the addition of absorbents to capture HMs, composted sludge could be used as the soil amendment to provide nutrients and organic matters (Wang et al. 2019b). But the negative effects of this process, such as, incomplete composting process and long stabilization time would limit its application (Nguyen and Shima 2019). Treated sludge ash is another approach to reuse sludge as fertilizer. Herzel et al. (2016) used sodium and potassium additives to thermochemically treat sewage sludge ash. Meanwhile, the volatile HMs were removed through the off-gas treatment, making the ash product a highly bioavailable P-fertilizer. It is known that protein in sludge could be extracted and hydrolyzed to amino acids. The amino acids can be chelated with trace elements and form fertilizer (AACTE), which is an environmentally friendly fertilizer (Liu et al. 2009).

Recently, the liquid fraction of pretreated sludge was also reused as the soil amendment. During sludge THP, a large proportion of nutrients content, like nitrogen (40 - 70%), phosphorus (10 - 15%) and potassium (50 - 70%) could be solubilized (Sun et al. 2013), indicating the valuable fertilizer potential of the liquor products. Besides, the HMs were proved to be less in the liquor (Munir et al. 2018, Sun et al. 2014), meaning the accumulation of HMs will be minor when the liquor product is used for land application. In addition, the recyclability of P in treated sludge was reported to be better than that in soluble inorganic fertilizer, which could reduce terrestrial and aquatic eutrophication (Kahiluoto et al. 2015). Sun et al. (2014) investigated the liquid product of hydrothermally treated sewage sludge and reported the high nitrogen content and low level of HMs in the liquor. The facilitating effect to the crop yield was also observed when applying this liquid product to farmland.

In liquid products of THP sludge, there are lots of active fractions, which are beneficial to plant growth. However, previous studies mostly focused on the macronutrients such as nitrogen, phosphorus and potassium, the information on the specific functional compounds is very scarce. In order to further assess the fertilizer potential of liquid products, the plant growth-promoting organics, such as amino acids, organic acids, phytohormone and other compounds should be investigated. In addition, some toxic components like aromatic compounds could be formed during sludge THP (Lu et al. 2018a), which may be harmful to the soil microbes. Whether the application of THP sludge liquor has negative effects on bacterial growth is still not known.

2.8 Current limitations and research gaps

The inevitable byproduct of WAS produced from WWTPs has been a big challenge with the increasing population and urbanization. Thus, the efficient and sustainable approaches to treat sludge and recover valuable resource have attracted much attention. It is well-known that AD is the common technique to be used to reduce sludge volume, stabilize organics and generate biogas energy. But the hydrolysis of sludge is very slow, limiting its application. Pretreatment is thus developed to accelerate the hydrolysis step and enhance the subsequent AD performance.

Recently, ULS pretreatment has been widely applied prior to AD in the pilot and full-scale sludge treatment plants. To compensate the high energy requirement for ULS pretreatment, ALK method was applied to lower the energy input. The synergistic effect of ALK-ULS pretreatment was reported to greatly enhance sludge solubilization (Kim et al. 2010a). But whether the synergistic effect still exists on sludge biodegradability is not clear. Besides, DOMs are the readily biodegradable substances compared to particulates. The relationship between the specific organics with the biogas generation has not been reported before. Additionally, refractory compounds may be formed during ALK-ULS pretreatment, which are possibly harmful to the downstream treatment processes. However, the information about the recalcitrant compounds is still lacking. Therefore, it is essential to report the detailed characterization of the DOMs during ALK-ULS pretreatment and AD process to have a better understanding of this technology.

THP is another well-known effective sanitary technique for sludge treatment. It has been widely reported to be able to enhance sludge biodegradability and dewaterability (Li et al. 2017a). Additionally, THP is likely to be more efficient than ALK-ULS pretreatment in solubilizing organic matters (Lu et al. 2018a, Lu et al. 2018b). However, for the investigation of such solubilized substances, previous studies mainly focused on the determination of the total organics and/or COD without the detailed characterization of the specific components. How the DOMs are transformed during THP and AD processes is still not clear. Besides, some recalcitrant or inhibitory compounds would be generated during THP, whose information is still limited. Thus, it is important to study the impact of THP on AD performance and characterize the organics transformation during THP and AD processes.

It is worthy to note that up to 60% sludge solubilization could be obtained with THP (Bougrier et al. 2008), indicating large number of organics were solubilized in the liquid fraction of THP sludge (THP-L). In other words, solids may have the low biodegradability and need longer digestion time. Whether there is a necessity to digest the solids fraction of THP sludge (THP-S) has not been investigated before. Thus, the separated digestion of THP-S and THP-L needs to be conducted in order to know the respective contribution of these two fractions to biogas generation. In addition, sludge dewaterability and rheological properties would also affect the treatment processes. But this information about the separated AD is usually overlooked. Previous studies reported that THP sludge can’t maintain its good flowability and dewaterability during AD process (Zhang et al. 2018), suggesting the separation of THP sludge should be operated prior to AD. The economic assessment should be carried out to evaluate the feasibility of such separation.

THP-L products also show fertilizer potential as large amount of nutrients (like nitrogen, phosphorus and potassium) and organic matters are present (Lu et al. 2018a, Sun et al. 2013). However, whether THP-L is suitable to be used for land application is not reported clearly. For example, the information on the HMs content is not clear. Some toxic or inhibitory compounds could be produced and existed in THP-L products, which would exhibit the negative effect on the growth of soil microbes. But such impact has not been studied before. Most importantly, the specific components in THP-L that would be beneficial to plant growth have not been investigated. Thus, more effort should be paid to better understand the valuable fertilizer resource.

CHAPTER 3 MATERIALS AND METHODS

3.1 Introduction

This chapter presents a description of the general materials and common analytical methods used in Chapter 4 - 7. The experiment processes and developed specific methods will be discussed in their respective chapters.

3.2 Reagents and chemicals

Chloroform, dichloromethane, acetone and n-hexane of GC−MS grade were purchased from Merck KGaA (Germany). Acetonitrile, isopropanol, water and methanol (all LC−MS grade) were purchased from Sigma-Aldrich (United States). Formic acid and ammonium formate were purchased from Sigma-Aldrich. The alkane standard mixture (C10 − C40, 50 mg/L) was purchased from Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q water process (Millipore Advantage A10, Merck, France).

3.3 Analytical methods

3.3.1 Solids tests

The measurements of total solid (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were based on Standard Methods (APHA 2005). For TS and VS tests, 5 mL of sample was added to a pre-weighed foil tray and left in a 105 ºC oven (Memmert, USA) for 2 h until constant weight. After cooling down to the room temperature in the desiccator, dried samples were weighed to give a result of TS. The tray was then placed in a muffle furnace at 550 ºC for 1 h, cooled and weighed again to obtain the value of VS. For TSS and VSS tests, the empty aluminum dish together with filter paper was pre-weighed. 2 mL of sample was vacuum filtered. Similarly, the filtered solids and aluminum dish were dried (at 105 ºC), cooled and weighed to get the result of TSS. The residual sample was further volatilized (at 550 ºC), cooled and weighed to determine VSS.

3.3.2 Chemical oxygen demand (COD) tests

Total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) were determined based on the standard method 5220 D (APHA 2005). For sCOD test, sludge sample was centrifuged (10,000 g for 10 min) and filtered through a 0.45 μm nylon membrane filter.

The digestion solution and sulfuric acid reagent were prepared in advance. Digestion solution was first prepared by adding 20.432 g of dried potassium dichromate, 334 mL of concentrated sulfuric acid and 66.6 g of mercury sulfate to 1000 mL of ultrapure water. The mixture was diluted to 2000 mL using a volumetric flask. The sulfuric acid reagent was prepared by adding silver sulfate (technical grade) to the concentrated sulfuric acid (5.5 g Ag_2SO_4/kg H_2SO_4) and stirred overnight.

The test tube containing sample and premixed reagents was heated in a block heater at 150 ºC for 2h. After cooling down, the samples were determined with an Ultraviolet-Visible (UV/Vis) spectrometer at a wavelength of 600 nm. Distilled water was used to operate the blank test. COD standards were used to obtain the calibration curve between COD values and absorbance.

3.3.3 EPS extraction process

EPS fractions of sludge samples were extracted with the method modified from Xiao et al. (2017b). 15 mL of sludge samples was centrifuged (4000 g for 15 min at 4 ºC), the supernatant was collected as soluble EPS (SB EPS). The remaining pellets were resuspended with equivalent volume of 0.05% NaCl solution and put in 70 ºC water bath for 5 min, followed by centrifugation (4000 g for 10 min at 4 ºC) to collect the supernatant as loosely bound (LB) EPS. Then the residual pellets were resuspended again to the original volume, heated at 60 ºC water bath for 30 min. After centrifuging at 4000 g for 15 min under 4 ºC, the supernatant was collected as tightly bound (TB) EPS.

3.3.4 Proteins (PN) and polysaccharides (PS) determination

Soluble PN was determined using the Bicinchoninic acid (BCA) protein assay (23235, Thermofisher, USA). Soluble PS was measured by the phenol-sulfuric acid method (Dubois et al. 1956). 0.025 mL of 80% phenol and 2.5 mL of concentrated sulfuric acid were added into the test tube that contained 1 mL of sample/standard. After standing for 30 min at room temperature, the mixture was determined with an UV/Vis spectrometer at a wavelength of 490 nm. The calibration curve was made using glucose as standard.

3.3.5 Size exclusion chromatography (SEC) coupled with organic carbon detection and organic nitrogen detection (LC-OCD-OND) analysis

The composition of dissolved organic carbon (DOC) was classified with a SEC-OCD-OND system (DOC-LABOR, Karlsruhe, Germany). The observed DOC was mainly divided into hydrophobic dissolved organic carbon (HO DOC), biopolymers (> 20 kDa, including high molecular weight (HMW) PN and HMW PS), humic substances (HS), building blocks (BB, 300 – 500 Da), low molecular weight (LMW) acids (< 350 Da) and LMW neutrals (< 350 Da) according to the respective properties and molecular weight (MW). HO DOC was calculated by subtracting HI DOC (hydrophilic dissolved organic carbon) from the total DOC concentration (Huber et al. 2011). 1g of PN and PS equal to 0.497 and 0.444 g of equivalent carbon based on the proposed chemical formulae of $C_{52.5}H_{6.65}N_{16}O_{21.5}S_2$ and $(C_6H_{10}O_5)n$. The concentration of LMW PS and LMW PN was obtained by subtracting HMW PS and HMW PN from the total PS and PN respectively.

3.3.6 Gas Chromatography–Mass Spectrometry (GC-MS)

Solid phase extraction (SPE) was carried out as a pretreatment to extract organic compounds with purchased SPE cartridges (Waters Oasis! HLB, Waters Corporation, United States). Collected supernatants were filtered through 0.45 μm glass fibre filters. SPE cartridges were conditioned using 10 mL of methanol followed by 20 mL of ultrapure water. Then 50 mL of liquid sample was loaded onto two connected cartridges. The filtrate from this step was collected for the subsequent liquid-liquid extraction (LLE). Finally, the compounds were eluted with 2 mL of methanol, acetone, dichloromethane and n-hexane successively into individual glass vial. The eluent from each cartridge was analyzed separately. Ultrapure water was used as the control to eliminate any contaminations during SPE.

The filtrate through SPE cartridge continued to be treated by LLE. 20 mL of filtrate was diluted to 100 mL with ultrapure water. Mixture solvent composed of n-hexane, chloroform and dichloromethane (volume ratio = 1: 1: 1) was selected as the extraction solvent. Then LLE was conducted on 100 mL of diluted filtrate using 50 mL of mixture solvent. Shook the extraction funnel for 2 min. Then 2 phases were separated within 5 min. The extraction process was repeated twice. Trace water was removed by anhydrous Na_2SO_4. The solvent was removed completely at 40 ºC under vacuum. The organics were dissolved in 1 mL of dichloromethane and proceeded to analyze. All glassware was washed with acetone prior to use. Ultrapure water was used as the control.

Samples prepared from LLE and SPE were analyzed using GC-MS (GCMS-QP2010ULTRA, Shimadzu) separately. Description and analysis of GC-MS operation process can be found in Kunacheva et al. (2017). Briefly, samples extract was injected into an RTX-5MS (30 m × 0.25 mm ID, Restek) column for identification of compounds. Splitless injection was used at 280 °C with carrier gas (helium) at 1 mL/min. The program was designed as follows: 50 °C for 7 min, increase 7 °C/min to 325 °C, hold for 14 min. The MS was operated with the ion source temperature at 230 °C, and mass spectra was acquired from m/z 30 to 580. The chromatographic peaks were identified by both analysis of the mass spectrum, retention time and comparison with the NIST11 library (National Institute of Standards and Technology, Gaithersburg, MD, USA, http://www.nist.gov/srd/mslist.htm). Each compound was semi-quantified using the alkane with the closest retention time (Table 3.1).

Table 3.1 Alkane standards for DOMs quantification.

Compound Name	Formula	Molecular Weight	RT (min)	R^2	RI
Decane	$C_{10}H_{22}$	142	11.61	0.999	1015

Dodecane	$C_{12}H_{26}$	170	17.20	0.999	1214
Tetradecane	$C_{14}H_{30}$	198	21.54	0.999	1413
Hexadecane	$C_{16}H_{34}$	226	25.28	0.997	1612
Octadecane	$C_{18}H_{38}$	254	28.60	0.997	1810
Eicosane	$C_{20}H_{42}$	282	31.60	0.996	2009
Docosane	$C_{22}H_{46}$	310	34.34	0.994	2208
Tetracosane	$C_{24}H_{50}$	338	36.85	0.994	2407
Hexacosane	$C_{26}H_{54}$	366	39.18	0.995	2606
Octacosane	$C_{28}H_{58}$	394	41.32	0.993	2804
Triacontane	$C_{30}H_{62}$	422	43.33	0.994	3003
Dotriacontane	$C_{32}H_{66}$	450	45.21	0.990	3202
Tetratriacontane	$C_{34}H_{70}$	478	47.00	0.991	3401
Hexatriacontane	$C_{36}H_{74}$	506	49.08	0.988	3600
Octatriacontane	$C_{38}H_{78}$	534	51.76	0.983	3800
Tetracontane	$C_{40}H_{82}$	562	55.38	0.980	3997

RT denotes retention time (min); RI denotes retention index

3.3.7 Metabolites and lipids extraction and analysis

The untargeted analysis of sludge samples using Ultra Performance Liquid Chromatography–Mass Spectrometry (UPLC-MS) approach was conducted to profile and identify more compounds with higher MW (< 2 kDa). First, the filtered samples were lyophilized, from which the fractions of polar metabolites and non-polar lipids were extracted following the steps in Tipthara et al. (2017), and analyzed by ACQUITY UPLC (Waters, USA) coupled with a Xevo G2-XS QToF mass spectrometer (Waters, U.K.) with an electrospray ionization (ESI) source. Detailed description and analysis of UPLC-MS operation process can be found in Tipthara et al. (2017). EZinfo (version 3.0.3.0, Umetrics) was used for multivariate analysis of compounds in raw WAS, pretreated sludge and digested sludge samples respectively. The most significant components were determined by orthogonal projection to latent structures discriminant analysis (OPLS-DA) and then exported from S-plot back to Progenesis QI for further analysis and identification. Polar and lipid biomolecules were identified using (1) MetaScope by searching compounds against structural databases (SDFs) for E. coli (http://ecmdb.ca/), LIPID MAPS (http://www.lipidmaps.org/), HMDB (http://www.hmdb.ca/), and ChEBI (https://www.ebi.ac.uk/chebi/) and (2) Chemspider by searching compounds against the KEGG database (http://www.genome.jp/kegg/).

3.3.8 Other analytical methods

pH was measured with a pH meter (Mettler-Toledo, model S220). Total organic carbon (TOC) and total nitrogen (TN) was determined using a total organic carbon (TOC) analyzer (Shimadzu, Japan). The content of ammonia was tested using the Nessler method kit (TNT832, Hach, USA). The concentration of P was determined using the molybdate-ascorbic acid method.

3.4 Calculation

3.4.1 Disintegration degree (DD)

DD was calculated with Eq. (3.1), which can directly show the degree of particulate substance solubilized by pretreatment (Jin et al. 2009).

$$DD\% = \frac{sCOD_p - sCOD_0}{tCOD_0 - sCOD_0} \times 100 \qquad (3.1)$$

where $sCOD_p$ is the sCOD of pretreated sludge, $sCOD_0$ is the sCOD of untreated sludge, and $tCOD_0$ is the tCOD of untreated sludge.

3.4.2 Kinetics analysis

Methane generation data was fitted to a First-Order Model using Eq (3.2) to estimate the hydrolysis rate coefficient (k) and biochemical methane potential B_0 (Jensen et al. 2011).

$$B(t) = B_0 (1 - e^{-kt}) \qquad (3.2)$$

where B(t) is the biochemical methane yield at time t, B_0 is the biochemical methane potential, and t is time.

The digestion of DOMs produced from pretreatment was fitted to the First-Order kinetics model using Eq (3.3) to evaluate the degradation rate constant (k) (Li et al. 2017b).

$$M = e^{-kt + b} \qquad (3.3)$$

where M is the concentration of soluble organics, b is the constant, k is the first-order degradation constant (day^{-1}), and t is the digestion time.

CHAPTER 4 ANAEROBIC TRANSFORMATION OF DOMS PRODUCED FROM ALK-ULS SLUDGE PRETREATMENT

Soluble organic compounds released by ALK, ULS and combined ALK-ULS pretreatment as well as their transformation in AD systems were investigated in this chapter. The maximum methane production was observed with ALK-ULS pretreated sludge (pH 12 and SEI 24 kJ/g TS). The combined treatment likely enhanced the sludge solubilization and produced more LMW substances. However, such pretreatment released not only easily biodegradable substances but also some recalcitrants, such as HS and complex HMW proteins. Thus, more residual DOMs were detected after digestion, which may pose adverse effects on the downstream water treatment. Refractory HS were the main components of the residual DOMs. At the molecular level, a large amount of residual steroid-like matters, alkanes and aromatics were identified. Specific higher MW residual compounds, e.g. polar metabolites (like dipeptide, benzene and substituted derivatives), and non-polar lipids (like LCFAs, flavonoids, sphingolipids, glycerophospholipids and their derivatives) were also identified. The results indicate that further polishing steps should be considered to remove the remaining soluble recalcitrant compounds.

4.1 Introduction

AD is widely used to recover bioenergy and reduce sludge (Yuan and Zhu 2016). But the rate-limiting step of sludge hydrolysis hinders the application (Carrere et al. 2010, Liu et al. 2016b). To accelerate the hydrolysis step, many sludge pretreatment approaches, such as thermal, chemical and mechanical pretreatment have been developed (Abelleira-Pereira et al. 2015, Neumann et al. 2017, Wei et al. 2017). Among them, ALK pretreatment is the most effective chemical method and can greatly solubilize particulate matters and enhance the sludge digestibility in a simple setup (Ruiz-Hernando et al. 2014, Yang et al. 2013). ULS pretreatment also receives attention and has been implemented in many full-scale plants (Zhang et al. 2007). Nevertheless, high energy consumption limits the broad application of ULS (Zhang et al. 2008). By combining with other pretreatment methods, the energy requirement of ULS can be reduced, and the sludge disintegration as well as biogas production can be improved too (Zawieja et al. 2008).

To date, most of the research mainly focused on the relationship between the total organics removal and the methane production. The detailed correlation between the specific organics and biogas generation has not been systematically investigated. In addition, it is not clear how DOMs are produced in the pretreatment step and how the key DOMs are transformed during the subsequent AD process. Besides, the information on the potential inhibitory compounds produced during pretreatment as well as the recalcitrant compounds remained after AD is still lacking. Such study is particularly important when a plant has a water recycling requirement.

The main objectives of this study are to (1) explore the transformation of DOMs after ALK and/or ULS pretreatment, (2) study the impact of such organics on the subsequent AD process, and (3) identify the recalcitrant DOMs after AD process.

4.2 Material and methods

4.2.1 Sludge source

The feed sludge (WAS, pH 6.7) was collected from a local wastewater treatment plant in Singapore. It was settled at 4 ºC for 24 h before use. The anaerobic sludge collected from a mesophilic anaerobic digester in the same plant was used as the inoculum. The characteristics of WAS and seed sludge are listed in Table 4.1.

Table 4.1 Characteristics of WAS and seed sludge.

Parameters	WAS (g/L)	Seed sludge (g/L)
TS	13.62 ± 0.15	15.44 ± 0.23
VS	10.81 ± 0.20	11.23 ± 0.11
TSS	12.45 ± 0.14	14.27 ± 0.22
VSS	10.36 ± 0.18	10.62 ± 0.28
tCOD	15.01 ± 0.23	15.52 ± 0.31
sCOD	0.25 ± 0.10	0.26 ± 0.13

4.2.2 Sludge pretreatment

The pH value of WAS was adjusted from 6.7 to 9, 10, 11 and 12 respectively using NaOH solution (5 mol/L). After that, the WAS samples were incubated for 30 minutes at room temperature, then neutralized to pH 6.7 using HCl solution (5 mol/L) (Jin et al. 2009). Deionized water was used to top up the final sample to the same volume so that the solids concentration in all the samples can be maintained comparable. Triplicates were prepared for each type of pH sludge.

The ULS treatment was operated using an ultrasonicator (Q700, QSonica, Newtown, CT, USA) with the frequency of 20 kHz. The tip of probe was placed 1 cm into sludge samples. The temperature of sludge during ultrasonic pretreatment was controlled within 30 ± 5 ºC by the ice bath. The SEI for sludge samples was set at 0, 6, 12, 18 and 24 kJ/g VS respectively. Triplicates were prepared for each ULS test.

A full factorial experiment was carried out in this study. Feed sludge samples were firstly pretreated with alkali at the pH values of 6.7, 9, 10, 11 and 12 respectively, and then neutralized to pH 6.7. The ALK treated samples were further treated with SEI of 0, 6, 12, 18 and 24 kJ/g VS. Triplicates were prepared for each type of sludge.

After pretreatment, all the treated sludge samples were split into two groups. One group was used for measurement of TS, VS, TSS, VSS, tCOD, sCOD, disintegration degree, PN, PS analyses and EPS extraction. The other group was used as feed for anaerobic digestion.

4.2.3 Anaerobic digestion process

The anaerobic digestion experiment was carried out using an automatic methane potential test system (AMPTS II, Bioprocess Control Company, Sweden). Each reactor had working volume of 400 mL and headspace of 150 mL. The inoculum to substrate VS ratio was 0.98 g : 1 g. Reactors were purged with N_2 to remove dissolved gas. All experiment was conducted at 35 ºC for 30 days with semi-continuous stirring (60 s on and 10 s off, 80 rpm). Control experiment was operated with raw WAS as feed sludge. Blank tests (inoculum and deionized water) were carried out in duplicates to provide the background biogas production from the inoculum. The blank methane production was subtracted. Four replicates were carried out for each type of pretreated sludge. Among them, two replicates were continuously operated for 30 days and samples were taken on the last day for the analysis of residues. The other two replicates were sampled at 0, 2^{nd}, 5^{th}, 10^{th}, 16^{th} and 30^{th} day during AD to monitor the changes of DOMs.

4.2.4 Statistical analysis

Pearson's correlation was determined by the software SPSS version 19.0 (Niu et al. 2013). The extent of correlation was evaluated by the Pearson's correlation coefficient (R), which can range between -1 and +1, where -1 denotes a perfect negative correlation, +1 indicates a perfect positive correlation, and 0 denotes no relationship. A two-tailed t-test for the null hypothesis that regression slope is zero was carried out to determine p value. The correlation will be considered as statistically significant with probability (p value) less than 0.05.

The significance of parameters' difference was evaluated by analysis of variance (ANOVA) and $p < 0.05$ was considered to be statistically significant. The software of SPSS version 19.0 was used for the statistical analysis.

4.3 Results and discussion

4.3.1 Effects of ALK and/or ULS pretreatment on sludge properties

4.3.1.1 Effects on DOC distribution and compositions

The effects of ALK (pH 6.7 - 12) or ULS (0 - 24 kJ/g TS) as well as ALK-ULS pretreatment on the sludge DOC fractions are present in Figure 4.1. For raw sludge, TB DOC fraction was the most dominant in EPS matrix. After pretreatment, part of EPS and microbial fragments would be solubilized and shifted from the inner layers to outer layers, leading to the increase of SB DOC and LB DOC fractions. It is worthy to note that DOMs were more dominant in SB DOC after pretreatment. This part of DOMs is in fact more accessible to anaerobic microorganisms. This confirms that pretreatment can improve the accessibility of organic matters in sludge fractions.

A regression model (Eq 4.1) was established to indicate the relationship between solubilized DOC and operation parameters, i.e. pH and SEI. This implies that the effects of pH (X_1, X_1^2) and SEI (X_2, X_2^2) are all highly significant. The interaction term (X_1X_2) of the pH and SEI was found to be insignificant ($p > 0.05$). A regression coefficient R^2 of 0.955 was obtained, indicating 95.5% of the variations can be explained by the model, and all the estimated parameters were significant with p values less than 0.05. Therefore, this modified model can be used to predict the solubilized DOC using the values of pH and SEI within the design range. Based on this model, the optimal condition for the highest DOC solubilization would be pH of 12 and SEI of 21.6 kJ/g TS.

$$Y = 181.26 - 35.98 X_1 + 12.10 X_2 + 2.55 X_1^2 - 0.28 X_2^2 \qquad (4.1)$$

where X_1, X_2 and Y are pH, SEI (kJ/g TS) and solubilized DOC.

In this study, the optimal solubilization for individual ALK and ULS pretreatment was observed at pH of 12 and SEI of 24 kJ/g TS respectively. Besides, when ultrasonication was applied to the ALK pretreated sludge, the remarkable increase of solubilization extent was observed. It is likely that cell walls weakened by ALK were easily destructed by ULK, resulting in the direct release of the soluble intracellular organics. Therefore, the combined pretreatment under conditions of pH 12 and SEI 24 kJ/g TS showed the best solubilization performance of 247 mg DOC/g VS. In order to investigate the transformation of DOMs with different pretreatment methods, the following three optimal conditions were chosen, namely, combined pH 12 and SEI 24 kJ/g VS (ALK12-ULS24), individual ALK pretreatment at pH 12 (ALK12), ULS pretreatment at SEI 24 kJ/g VS (ULS24).

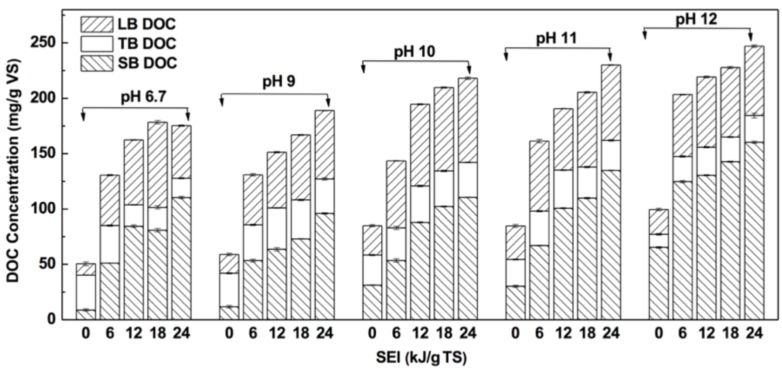

Figure 4.1 Effects of pH and specific energy input (SEI) on DOC fractions.

4.3.1.2 Effects of ALK and/or ULS pretreatment on DOMs compositions

DOMs at the different molecular weight of the pretreated sludge were analyzed by LC-OCD-OND with more specific fractions. The concentration of HI DOC in ALK12-ULS24 pretreated sludge was clearly higher than that in other pretreated sludge as well as in control samples (Figure 4.2). The concentration of HMW PN was higher than HMW PS regardless of pretreatment methods, which is reasonable as 40 - 50% of dry cell weight is PN (Wei et al. 2016). Besides, the proportion of HMW PN in biopolymers increased from 60% in untreated sludge to 73.5%, 82% and 80% in ALK12, ULS24 and ALK12-ULS24 pretreated sludge respectively. It implies that ULS pretreatment may release more HMW PN than ALK pretreatment did. Conversely, ALK pretreatment likely released more HMW PS, as the biopolymers composed of HMW PN and HMW PS.

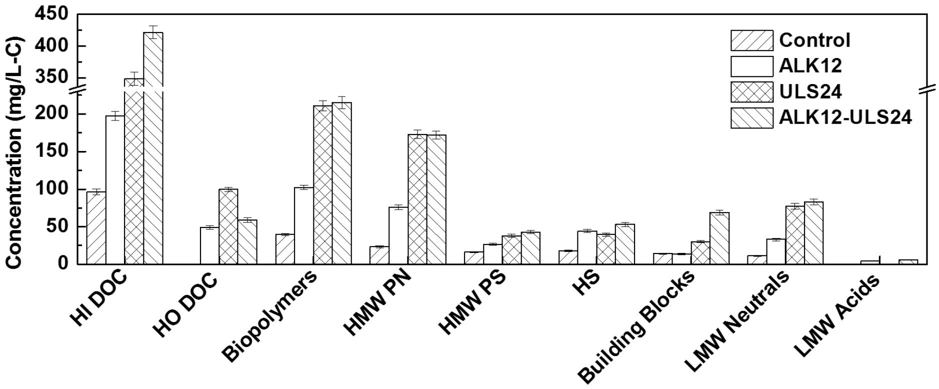

Figure 4.2 Quantification of sub-fractions (mg/L -C) of DOMs after pretreatment.

ALK treatment also released a high concentration of HS (52.87 mg/L -C). The concentration of building blocks (68.89 mg/L -C), which are the breakdown products of HS, was found much higher with combined treatment (Figure 4.2). Thus, ALK12-ULS24 treatment resulted in large amount of HS-like matters, including the complex HS and building blocks. Previous findings reported that part of HS belonged to hydrophobic substances (Penru et al. 2013). In this study, the concentration of HO DOC was lower with ALK treatment, indicating that ALK treatment may be in favour of generating hydrophilic HS rather than hydrophobic HS.

Statistical analysis demonstrates that strong or moderate correlation was found between SEI with HI DOC, HMW PN, HMW PS, LMW neutrals (Table 4.2) and the correlation was significant ($p < 0.01$), which suggests that the ULS treatment was more likely to be a physical way to release these organics that were originally embedded in sludge flocs rather than through chemical reactions. Meanwhile, a moderate correlation ($R = 0.616, p < 0.01$) was observed between pH and LMW acids, indicating the formation of LMW acids may be partly due to the hydrolysis of organic matters by the reaction of alkali. Additionally, sludge DD had a strong positive correlation with SEI ($R = 0.792, p < 0.01$) but a weak correlation with pH ($R = 0.466$, $p < 0.05$), which is in agreement with the reports that ALK treatment at high pH levels played more important roles than that at low pH values in increasing sludge disintegration prior to subsequent ULS pretreatment (Kim et al. 2010a). It further confirms the different roles and mechanisms of ULS and ALK treatment in sludge disintegration. The strong correlation ($R = 0.792$) of statistical significance ($p < 0.01$) between DD and SEI suggests ULS treatment is more controllable in application.

Table 4.2 Pearson's Correlation Coefficients (R) between pretreatment parameters and DOMs compositions.

	DD	HI DOC	HMW PN	HMW PS	Bio polymers	HS	BB	LMW Neutrals	LMW Acids
pH	0.466*	-	-	-	-	-	-	-	0.616**
SEI	0.792**	0.764**	0.917**	0.602**	0.904**	-	-	0.751**	-

**Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level.

- denotes correlation is insignificant ($p > 0.05$).

4.3.2 Effects of pretreatment on methane production

The biodegradability of different pretreated sludge is shown in Figure 4.3. The cumulative methane production (CMP) increased rapidly at the beginning and then gradually reached a steady state during AD process. This confirms that all pretreatment could promote the total biogas production compared to control systems. However, the individual ALK and ULS treatment behaved differently in facilitating the methane production, with the higher initial methane generation rate from ULS treatment. In this study, the methane production rate by ULS pretreatment is higher than other reported studies (Ruiz-Hernando et al. 2014), in which the similar SEI but higher TS content were used. Such performance difference may be related to different sludge characteristics, e.g. sludge concentrations.

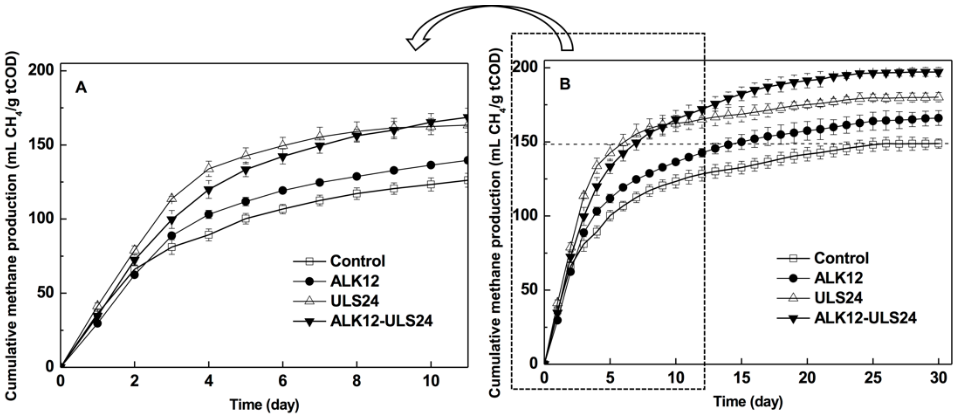

Figure 4.3 Cumulative methane production (CMP) curves of control, ALK12, ULS24 and combined ALK12-ULS24 pretreated sludge. A: Enlargement of the first 10 days. B: Methane production profile in the 30 days operation.

ALK12 pretreatment increased methane production by 11.8% (166.0 ± 5.0 mL CH_4/g tCOD), while the methane production of ULS24 treatment increased by 20.0% (178.2 ± 3.1 mL CH_4/g tCOD) (Figure 4.3). To compare, combined ALK12-ULS24 increased the methane yield by 32.8% (197.1 ± 3.0 mL CH_4/g tCOD). It has been reported that inhibitory effect from Na^+ may affect the methanogens (McCarty and McKinney 1961), hence, the optimum pretreatment conditions for maximum solubilization may not be the best conditions for maximum biogas production. In this study, Na^+ concentration at the highest ALK dosage (0.018 mol/L) is far lower than the inhibition threshold (> 0.2 mol/L) (Hierholtzer and Akunna 2012). It was found out that the optimal pretreatment condition (ALK12-ULS24) for maximum solubilization was also the best condition for maximum biogas production. If the CMP from control reactors is used as the baseline (148.4 ± 2.1 mL CH_4/g tCOD till day 30), ALK12, ULS24 and ALK12-ULS24 groups could achieve the baseline value on day 14, 6 and 7, respectively. These findings are of great significance in reducing the system SRT and reactor size.

To investigate the relationship between methane production and parameters, a regression model (Eq 4.2) was established. This model implies that the effects of pH and SEI are all highly significant. Also, high determination coefficient (R^2 = 0.926) indicates that 92.6% of the variability can be explained by the model. This model indicates that the methane production of pretreated sludge increases with more alkali dosage and energy input within the design range.

$$Z = 201.89 - 15.96\ X_1 + 1.30\ X_2 + 1.09\ X_1^2 \qquad (4.2)$$

where X_1, X_2 and Z are pH, SEI (kJ/g TS) and methane generation (mL CH_4/g tCOD). It should be noted that the hydrolysis rate coefficient of organics in ALK12 treated reactors (k = 0.222 day^{-1}) was lower than that in control reactors (k = 0.232 day^{-1}), whereas the hydrolysis rate coefficient in ULS24 treated reactors (k = 0.277 day^{-1}) was higher than that in control (Table 4.3). The results suggest that ULS24 caused an increase in hydrolysis coefficient, but ALK12 did not. The increase in hydrolysis coefficient for ULS24 is relatively small. The main effect of pretreatment is therefore an increase in bioavailability rather than in the speed of degradation. From Fig. 4.3, ALK treated sludge had a slower initial methane production rate compared to the ULS pretreatment. Detailed explanation will be presented below.

Table 4.3 Estimated k and B_0 after different pretreatment by the First-Order Model.

	k (day^{-1})	B_0 (mL/ g tCOD)	R^2
Control	0.232 ± 0.010	143.2 ± 1.4	0.976
ALK12	0.222 ± 0.008	160.1 ± 1.3	0.985
ULS24	0.277 ± 0.008	176.5 ± 1.1	0.990
ALK12-ULS24	0.235 ± 0.006	193.8 ± 1.2	0.992

4.3.3 Transformation of DOMs during AD

To study the transformation of DOMs, samples were withdrawn from control and ALK12-ULS24 sludge digesters on 0, 2nd, 5th, 10th, 16th, 30th day. The characterization and comparison of DOMs in the two digesters are presented in Figure 4.4. In control digesters, the initial soluble substances concentration was very low, and the concentration was not significantly changed during the following 30 days digestion regardless of the biogas production rate. It seems the availability/accumulation of those compounds was somehow limited by upstream bioreactions. Hydrolysis step is known to limit the overall reaction rate in anaerobic digestion (Carrere et al. 2010), and the subsequent bioreaction kinetics can be much faster. The relatively stable and low concentration of soluble substances observed during AD may confirm hydrolysis was the limiting factor when there was no pretreatment performed. HMW DOMs, including HMW PN and HMW PS, were digested gradually to a lower concentration. On the contrast, some compounds, such as LMW neutrals and building blocks even increased in the first 10 days, which may due to the hydrolysis of biopolymers, HS or some particulate substances.

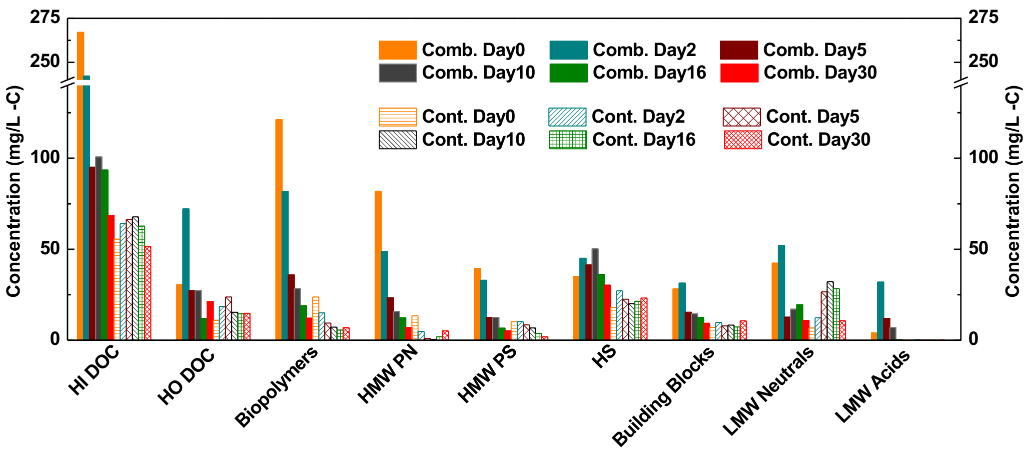

Figure 4.4 Transformation of DOMs during AD for control and ALK12-ULS24 sludge digesters. Comb. denotes ALK12-ULS24 sludge digesters, Cont. denotes control digesters.

Different from the control reactors, a large amount of DOMs were available for AD after pretreatment. The majority of them, such as HO DOC, HMW PN, HMW PS, building blocks and LMW neutrals, were reduced rapidly within the first 5 days. The results showed good agreement with the trend of methane production (Figure 4.3). During AD process, part of biopolymers was hydrolyzed or degraded into LMW neutrals and LMW acids, leading to the elevation of some organics. The amount of HO DOC increased more than 2-fold within the first 2 days and then reduced gradually during digestion. This phenomenon indicates that HO DOC generated by anaerobic bacteria seemed easier to be degraded than that produced by pretreatment step. It is also likely that HO DOC-degrading bacteria needs some acclimation period at the early stage of AD. Generally, LMW DOMs was considered more easily biodegraded than HMW DOMs (Li et al. 2016). These suggest HO DOC generated by bacteria may consist of LMW alkanes and lipids, while those produced by pretreatment were more likely to be hydrophobic HS and HMW hydrocarbons. LMW acids also increased significantly at the beginning, which was mostly associated with the acidification of other organic matters.

PN and PS are the main components in WAS (Wilén et al. 2003). It was found that the consumption of HMW PS was slower than HMW PN (Figure 4.4). This may be due to the lower concentration of HMW PS. Another reason is that the majority of PS exists in the cell walls, which is likely more resistant to the hydrolysis (Li and Noike 1992). From the previous results, ALK12 and ALK12-ULS24 pretreatment likely released a higher percentage of HMW PS in comparison with ULS24 treatment. Thus, this could be one of the reasons for the slower initial methane generation rate in ALK-related pretreated sludge reactors.

In addition, HS was not consumed but accumulated during the first 10 days in both reactors, then declined slightly during the latter period. This proves that HS was less biodegradable due to its polycyclic or heterocyclic aromatic structures (Provenzano et al. 2014). The binding of HS with hydrolytic enzymes may inhibit the hydrolysis efficiency of AD (Azman et al. 2017, Fernandes et al. 2014). From Figure 4.2, the concentration of HS in ALK12-ULS24 and ALK12 pretreated sludge was higher than that in ULS24 treated sludge. Hence, the large amount of refractory HS-like matters produced by ALK treatment may be another reason to slow down the initial biogas generation rate in ALK-related AD groups.

To investigate the correlation between DOMs and CMP, the Pearson's Correlation Coefficients were calculated and described (Table 4.4). CMP was strongly positive ($p < 0.01$) correlated to the concentrations of HMW PN, HMW PS, LMW neutrals and LMW acids. This reveals that the utilization efficiencies of these DOMs were higher in AD. Among them, LMW neutrals had the highest utilization efficiency ($R = 0.932$, $p < 0.01$) followed by HMW PS. In addition, the CMP had a stronger positive correlation with the concentration of HMW PS ($R = 0.859$, $p < 0.01$) than that with HMW PN ($R = 0.707$, $p < 0.01$). This further confirms that HMW PN had the relatively lower utilization efficiency in comparison with HMW PS. There were nearly no correlations between CMP and the concentrations of HS and building blocks, which is reasonable that HS-like substances are generally less biodegradable.

Table 4.4 Pearson's Correlation Coefficients (*R*) between cumulative methane production (CMP) and the available DOMs.

	HI DOC	HMW PN	HMW PS	Bio polymers	HS	BB	LMW Neutrals	LMW Acids
CMP	0.868**	0.707**	0.859**	0.824**	-	-	0.932**	0.785**

**Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level.

- denotes correlation is insignificant ($p > 0.05$).

4.3.4 Identification of the residual DOMs after AD

The higher methane production would consume more organics, leaving less residual VS. But the residual soluble organics are determined by the composition and concentration of DOMs released by various pretreatment. Sludge pretreatment releases both readily usable substance as well as some potential recalcitrant matters which may pass through AD process and be carried over to the subsequent wastewater treatment process. A significant amount of soluble residues were detected in the effluent from AD as shown in Figure 4.4, and the residues were much more in treated sludge AD effluent compared to those in control reactors'.

The distribution of residual DOMs from different digesters was similar (Figure 4.5a). The components of HS, HO DOC and LMW neutrals dominated the residual DOMs, which contributed 35.0%, 22.3%, 16.3% in control reactors and 32.9%, 26.6%, 13.6% in ALK12-ULS24 digesters, respectively. Moreover, the total amount of HS-like substances (HS and building blocks) occupied nearly half of the total residual DOMs. The majority of residual HO DOC, including hydrophobic HS, long chain alkanes and other HMW hydrocarbons, was refractory to hydrolysis or biodegradation. In addition, the amount of residual HMW PS in ALK12 pretreated sludge digesters was about 2 - 3 times higher than other reactors (Figure 4.6). Interestingly, the lower concentration of residual HMW PS in ALK12-ULS24 digesters indicates that the biodegradability of HMW PS produced by ALK pretreatment might be improved with ULS treatment.

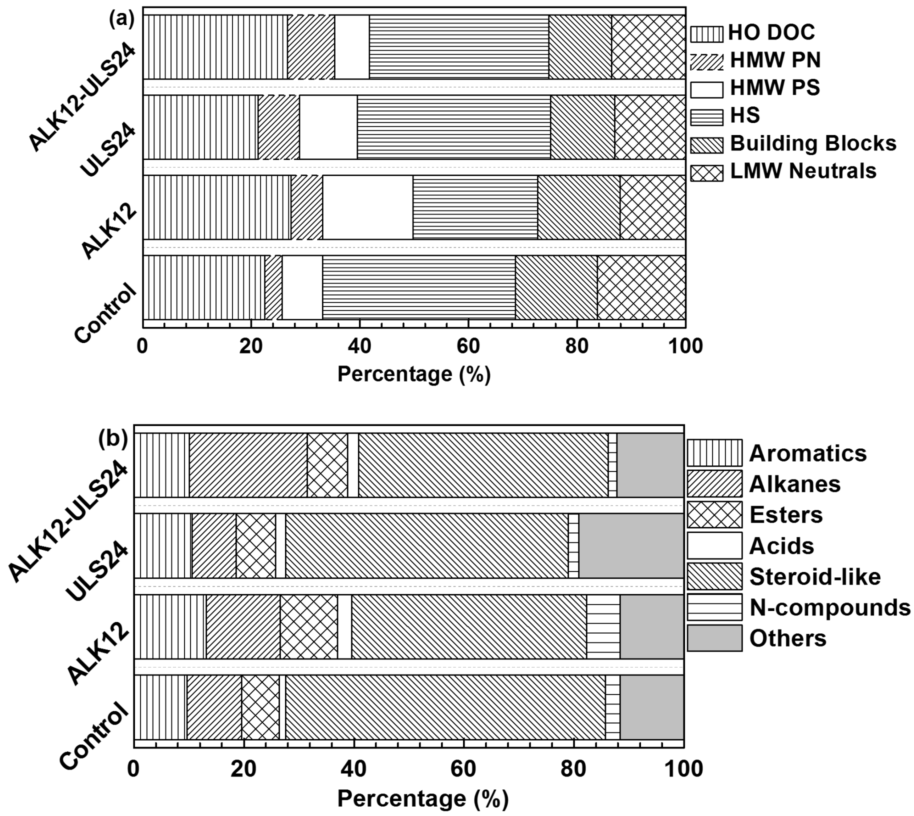

Figure 4.5 (a) The distribution of residual DOMs. (b) The distribution of specific residual LMW DOMs (< 580 Da) after AD.

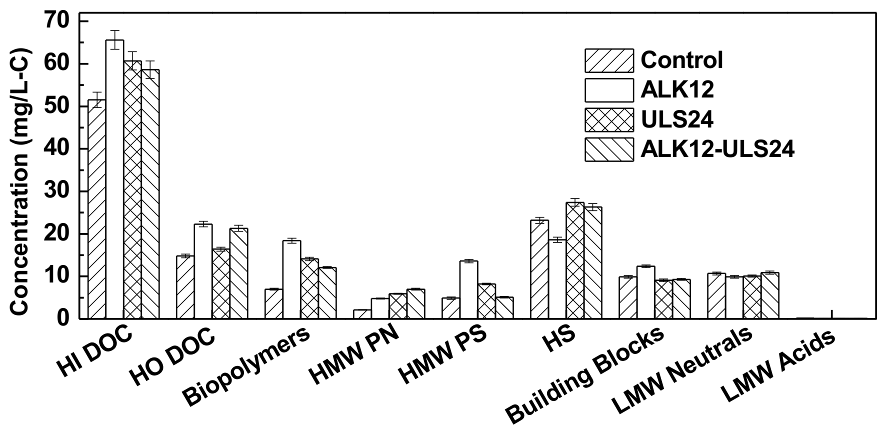

Figure 4.6 Distribution of DOMs after AD.

It was found that LMW substances were the major compounds released by ALK12-ULS24 treatment and the components of LMW neutrals and biopolymers were more related to the biogas production in AD reactors. It would be interesting to investigate the detailed compounds profile and understand how these compounds were transformed in AD. GC-MS identified a total of 53 LMW (<580 Da) compounds with a similarity index above 80% in ALK12-ULS24 AD effluent, while 20 peaks were designated as "unknown". Similar types of compounds were found in digesters effluent with different pretreatment. The identified LMW DOMs were categorized as aromatics (mostly phenolic compounds), alkanes, esters, acids, steroid-like matters, nitrogenated compounds (N-compounds, mostly amide compounds and amino acids) and others (alcohols and aldehydes) depending on the corresponding functional groups and properties.

In control digesters, there were a large amount of polycyclic steroid-like matters (58.1%) left over after AD, followed by alkanes (9.9%) and aromatics (9.7%). In ALK12-ULS24 pretreated sludge digesters, the percentage of steroid-like matters decreased to 45.4% while alkanes and aromatics increased to 21.4% and 10.1% respectively (Figure 4.5b). Among the above compounds, aromatics are generally more refractory to AD because of benzene rings. It seems ALK treatment may release a higher concentration of aromatics that led to higher aromatic residuals as well (Figure 4.7). Similarly, the higher concentration of alkanes produced in ALK12-ULS24 pretreatment may result in higher level of remaining alkanes. Further, N-compounds were also much more in ALK pretreated systems (Figure 4.7), indicating the serious fragmentation of cell membranes and other intracellular organic matters caused by alkali reaction. This may also increase the soluble N in the effluent if nitrogenated compounds were not easily degradable. In addition, the polycyclic steroid-like substances constituted nearly half of the residues in all the AD effluent. Nevertheless, the higher transformation of steroid-like substances in digesters with pretreated sludge implies that the pretreatment could promote the anaerobic utilization of these slowly biodegradable compounds.

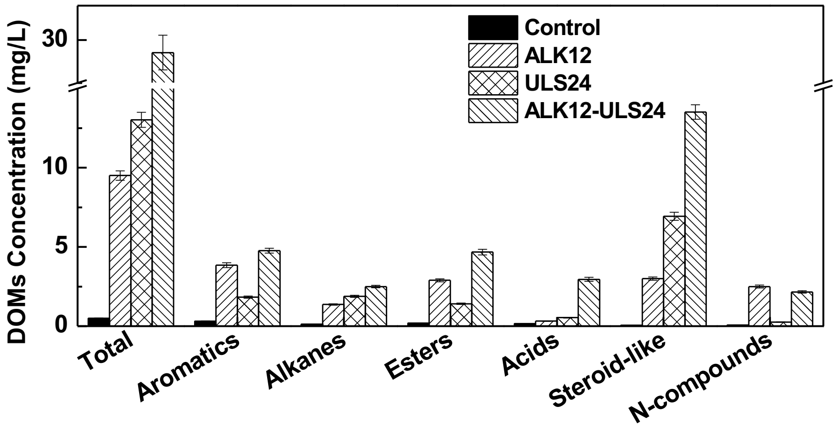

Figure 4.7 Quantification of LMW DOMs after pretreatment.

Many compounds such as alkanes, acetophenone, phthalates, squalene, cholesterol and stigmasterol were identified at significant concentrations after pretreatment and then declined during AD process. Phthalates were found to reduce from 1.28 mg/L to 0.10 mg/L in ALK12-ULS24 treated sludge during AD, indicating that phthalates may be intermediate products generated by pretreatment and could be gradually degraded anaerobically. It is worthy to note that compounds like cis-11-Eicosenamide and glutarimide, N-(3-pentyl)- were observed in digested sludge while they were absent from both raw WAS and pretreated sludge samples. Further, their concentration was higher in the pretreated digested sludge compared to control systems. It is possible that these compounds were microbial by-products in AD.

Since GC-MS can only detect non-polar, relatively volatile and thermo-stable components, non-volatile compounds with higher molecular weight (< 2 kDa) were analyzed using UPLC-MS. The results validated that ALK12-ULS24 pretreatment promoted the release of compounds like amino acids, sugars, fatty acids, alkenes, steroids, sphingolipids, glycerophospholipids and their derivatives. These compounds are mainly fragmentation of cytoplasm, extracellular substances and cell membrane. During AD process, most of them could be digested or transformed to other components as intermediate products. Thus, there were lots of soluble intermediate residues detected in the digested samples, including polar metabolites (like dipeptide, phenylpropanoids, benzenoids and substituted derivatives), and lipids (like diacylglycerols, LCFA, prenol lipids, alkenes, flavonoids, steroids, sphingolipids, glycerolipids, glycerophospholipids and their derivatives).

Additionally, considerable amount of alkenes, LCFA, steroids and flavonoid were also found in pretreated samples, suggesting they were generated during pretreatment and remained slow biodegradability or nonbiodegradability characteristics in AD.

One abundant group of residual compounds had simple chain structures, such as LCFA, alkenes, sphingolipids, diacylglycerols and glycerophospholipids. The number of identified lipids species in residues was more than that of polar metabolites, which may be due to their various homologues and the metabolism of anaerobic bacteria. The LCFA, produced during the hydrolysis or breakup of lipids, may become an inhibitor if the concentration is high. It is reported that LCFA was degradable but the degradation required longer retention time (Salminen and Rintala 2002). The second abundant group of residues contained monocyclic or polycyclic structures. For instance, the residual dipeptides were mainly the breakdown products of aromatic proteins, like tyrosine and tryptophan over other aliphatic proteins. Moreover, it has been well documented that organics like benzenoids and their derivatives were more recalcitrant and even inhibitory to cell growth and methanogenesis (Fernandez et al. 2017, Pham et al. 2013). Besides, phylloflavanine, classified as flavonoid, was proposed to be refractory with multiple complex benzene rings and double bonds. Citreoviridin C (classified as Pyranones and derivatives) was recognized as mycotoxin, which may inhibit the activity of bacterium. These compounds once accumulated in the biological systems can cause serious inhibition. To date, it is still not clear how the recalcitrant compounds would be transformed in the downstream wastewater treatment facilities.

Pretreatment process caused a significant solubilization of sludge, leading to the great increase of soluble HMW PN, HS-like matters, LMW neutrals and HMW PS. During AD, most of LMW neutrals, HMW PS and HMW PN were degraded due to their readily biodegradability. HS-like matters, on the other hand, were nearly nonbiodegradable, and remained in the residues. At molecular level, the compounds containing monocyclic or polycyclic structures (e.g. aromatic dipeptides, benzenoids and their derivatives) are more recalcitrant and even inhibitory to microbes. It should be noted that the solids that were not solubilized in the pre-treatment step may still contribute to the biogas production after long-term digestion, while it is challenging to discuss the correlation between biogas production and solubilization of this particular part of solids.

This study found out that identification and quantification of produced DOMs from pretreatment can be a cost-effective and time-saving method to predict the digestion efficiency of sludge. This study can help to better understand the mechanisms of various pretreatment methods and assist in the design of more effective sludge treatment and post-treatment processes.

4.4 Conclusions

Combined ULS12-ALK24 pretreated sludge displayed the best performance in terms of methane production (197.1 ± 3.0 mL CH_4/g tCOD) due to the enhanced sludge solubilization, especially LMW substances. However, more refractory substances, e.g. HS-like matters also remained after AD process. The pretreatment promoted the release of biodegradable organic matter as well as recalcitrant HS and complex HMW PN, which were hardly biodegradable. LMW residual DOMs mainly composed of steroid-like matters, alkanes and aromatics. HMW residues included polar metabolites and non-polar lipids, most of which were identified as recalcitrant and/or inhibitory compounds. It is recommended that further treatment step for anaerobic digester liquor should be considered to reduce these refractory residuals.

CHAPTER 5 ANAEROBIC TRANSFORMATION OF KEY DOMS PRODUCED BY THERMAL HYDROLYSIS SLUDGE PRETREATMENT

Generally, chemical pretreatment is not favored due to the addition of reagents. Thermal hydrolysis seems ideal especially when sludge sterilization is required. The mechanisms of different pretreatment methods are distinct, resulting in diverse sludge solubilization efficiency and AD performance. THP could achieve higher organic matters solubilization and more decomposition of some macromolecular substances, which would be more beneficial to AD. In this Chapter, the detailed DOMs profile by THP and their transformation during AD were investigated. Among the temperature tested, 172 °C treatment showed the best sludge solubilization and the maximum methane production. The study revealed that high temperature sludge pretreatment mainly improved the release of LMW proteins, LMW neutrals and LMW polysaccharides. Notably, the effluent from thermal treated sludge digesters contained more DOMs residues. The predominant residual DOMs were humic substances, LMW proteins and LMW neutrals. At the molecular level, over 50% of the residual LMW components were slowly biodegradable or nonbiodegradable steroid-like compounds and aromatics. Further profiling of the higher MW compounds detected the recalcitrant or inhibitory compounds, e.g. benzenoids, flavonoids, pyridines and their derivatives.

5.1 Introduction

THP is widely studied to accelerate the sludge hydrolysis efficiency thus improve the subsequent AD performance. It has been successfully installed at the full scale for more than a decade (Pilli et al. 2014). The AD performance and sludge dewaterability were likely to be enhanced with the elevated high temperature from 100 to 180 ºC (Li et al. 2017a). The well-known industrial-scale technologies based on THP are Cambi^{TM} (150 - 165 ºC for 30 min, 8 - 9 bar) and BIOTHELYS (150 - 180 ºC for 30 - 60 min, 8 - 10 bar) (Pilli et al. 2015). It has various inherent merits in sludge treatment, e.g. higher VS reduction, enhanced biodegradability, improved dewaterability and reduced viscosity (Carrere et al. 2008, Ennouri et al. 2016a, Li and

Noike 1992). Temperature above 180 ºC is not favorable which may produce more refractory compounds and reduce the sludge biodegradability (Abe et al. 2013, Bougrier et al. 2008, Stuckey and McCarty 1984). Meanwhile, the low temperature pretreatment (below 100 ºC) has also been developed considering the concerns of huge energy consumption (Appels et al. 2010). Certainly, different temperature will have different treatment mechanisms on sludge. However, the true mechanisms and the profiles of solubilized organic matters under different temperature have not yet been confirmed. This study selected low, medium and high temperature (70, 121 and 172 ºC) thermal treatment methods for comparison.

THP is particularly effective in treating WAS, which consists of considerable not easily biodegradable bacterial cells (Barber 2016). These cells have a sturdy structure of EPS which protects the cells from the hydrolytic enzyme and other attacks from outside (Toreci et al. 2009). The major components of EPS, i.e. PN, PS and HA, amount differently in different sludge flocs layers. Yu et al. (2008) reported that the location of the dominate EPS components in different EPS fractions will affect their accessibility and biodegradability.

It is known that DOMs generated with different sludge pretreatment methods may have different distributions and fate. However, previous studies mainly focused on either qualitative or semi-quantitative characterization of the overall DOMs in EPS matrix, without a comprehensive analysis of the specific key compounds. Further, the transformation of solubilized DOMs during THP and AD process is not clear.

Hence, this study aimed to analyze the generation of DOMs with low, medium and high temperature thermal pretreatment, investigate the transformation of these compounds during AD, and identify the residual compounds after AD process.

5.2 Materials and Methods

5.2.1 Sludge source

The WAS from a local wastewater treatment plant in Singapore was collected as the feed sludge. It was concentrated and stored at 4 ºC before use. Anaerobic sludge collected from a mesophilic anaerobic digester (the same plant) was used as the seed sludge for AD. The properties of sludge samples are presented in Table 5.1.

Table 5.1 Characteristics of raw sludge and seed sludge.

	Units	Seed sludge	Raw sludge
TS	g/L	15.02 ± 0.25	13.13 ± 0.15
VS	g/L	11.24 ± 0.13	10.65 ± 0.21
TSS	g/L	14.12 ± 0.20	12.27 ± 0.12
VSS	g/L	10.60 ± 0.21	10.21 ± 0.16
tCOD	g/L	15.17 ± 0.23	14.37 ± 0.29
sCOD	g/L	0.26 ± 0.11	0.24 ± 0.10
PN	mg/L	96.24 ± 2.47	89.91 ± 3.43
PS	mg/L	54.43 ± 1.12	45.06 ± 1.75
pH	1	7.44 ± 0.02	6.46± 0.02

5.2.2 Sludge pretreatment

The pretreatment at 70 ºC was carried out in a water bath for 30 min. The pretreatment at 121 ºC was carried out in a vertical pressure steam sterilizer (HG-50, Hirayama Manufacturing Corporation, Japan) for 30 min. The pretreatment at 172 ºC was carried out in a pressure reactor (Model 4534, Parr Instrument Company, US), where the temperature was controlled at 172 ± 3 ºC for 30 min. All the pretreated sludge was cooled down to the room temperature before DOMs characterization and use in AD. Triplicates were conducted for each temperature pretreatment and each replicate was characterized separately. The three replicates were then mixed together and used as feed sludge for AD.

5.2.3 Anaerobic digestion process

An AMPTS (Bioprocess Control Company, Sweden) was used to carry out AD experiments. The inoculum : substrate VS ratio was 0.96 g : 1 g. Each reactor had a working volume of 400 mL and a headspace of 150 mL. All reactors with sludge were purged with nitrogen gas for 10 min and sealed immediately to induce the anaerobic environment. The AD reactors were all operated at 35 ºC for about 30 days with the semi-continuous stirring. Control reactors were conducted with raw sludge as the feed sludge. Blank reactors with only seed sludge and deionized water were prepared for determination of the background biogas generation. Other three types of thermally treated sludge were used as pretreated sludge feed. The net gas production was obtained by subtracting the biogas from the blanks. Four reactors were set-up for each type of feed sludge. Two reactors were sampled at 0, 2nd, 5th, 10th, 16th and 30th day respectively to monitor DOMs. The samples from the other two reactors were collected on the 30th day for the residual DOMs analysis.

5.2.4 Energy assessment

The energy input for WAS thermal hydrolysis was calculated according to Eq 5.1 (Lu et al. 2008, Passos and Ferrer 2014). When the pretreated sludge cools down from pretreatment temperature to room temperature, the surplus energy would be recovered by a heat exchanger with an efficiency φ of 85%.

$$E_{THP,\ heat} = \rho Q\gamma\ (Tp\text{-}Tr) - \rho Q\gamma\ (Tp\text{-}Tr)\ \varphi \qquad (5.1)$$

where $E_{THP,\ heat}$: input heat for THP (kJ/d); ρ: density (1000 kg/m^3); Q: sludge flow rate (m^3/d); γ: specific heat (4.18 kJ/kg ºC); Tp: temperature of pretreatment (165 ºC); Tr: room temperature (25 ºC); φ: heat recovery efficiency.

The output energy from anaerobic reactors was calculated according to Eq 5.2.

$$E_{CH_4} = P_{CH_4}\ \xi\eta \qquad (5.2)$$

where E_{CH4}: output energy from methane production (kJ/d); P_{CH4}: methane production (m^3/d); ξ: lower heating value for methane (35800 kJ/m^3); η: Energy conversion efficiency (90%).

The economic values were converted to the energy (0.23 USD/kWh) to evaluate the total process (Liang et al. 2019). The energy analysis was calculated by subtracting the input energy from the total output value.

5.2.5 Statistical analysis

The significance of DOMs composition difference was evaluated by analysis of variance (ANOVA) and $p < 0.05$ was considered to be statistically significant. The software of SPSS version 19.0 was used for the statistical analysis.

5.3 Results and Discussion

5.3.1 Effects of thermal pretreatment on sludge content

5.3.1.1 Effects of thermal pretreatment on DOC distribution and compositions

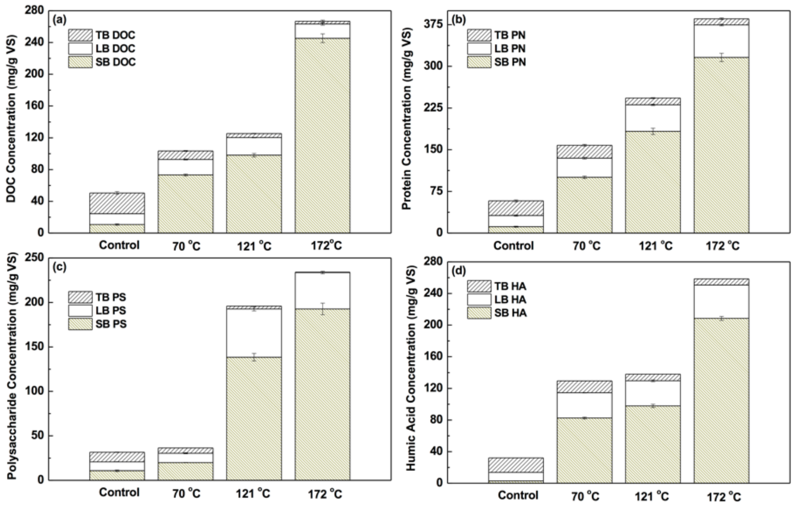

Figure 5.1 Effects of different pretreatment temperature on the concentration of DOC (a), PN (b), PS (c) and HA (d).

The changes of fractionized EPS, PN, PS and HA with different temperature thermal treatments are displayed in Figure 5.1. Clearly, all the thermal pretreatment contributed to the concentration increase of total DOC. The maximum concentration of 266 ± 8 mg DOC/g VS was obtained with the temperature of 172 ºC, which was more than doubled amount compared to the other pretreatment. In addition, the EPS compositions were significant different ($p = 0.019 < 0.05$) in 172 ºC pretreated sludge compared to raw sludge, whereas no significant difference was found among the lower temperature (70, 121 ºC) pretreated sludge and raw sludge (Figure 5.1), indicating the different mechanisms occurred during DOMs solubilization.

From Figure 5.1a, the majority of organics were locked up in TB EPS for raw sludge. After thermal pretreatment, the concentration of organic compounds increased in SB layer and decreased in TB layer, which implies the solubilization of particulates and shift of these substances from inner layers to outside layers. PN, PS and HA are the main components in WAS (Wilén et al. 2003). The changes of these compounds in respective layers show the similar trend with thermal pretreatment. The concentration of PN, PS and HA increased greatly in SB and LB layers, while they decreased in TB fraction. The results confirm that pretreatment can improve the accessibility of these organics to anaerobic microorganisms during AD.

Among the three temperature treatments, the highest concentrations of PN (386 ± 11 mg/g VS), PS (234 ± 6 mg/g VS) and HA (258 ± 4 mg/g VS) were obtained under the pretreatment temperature of 172 ºC (Figure 5.1). The released soluble PN was more than the soluble PS, and this is reasonable that more than half of the organic substances in microorganisms is PN (Li and Noike 1992). Wilson and Novak (2009) reported that below 220 °C, PS were not largely degraded. Regarding PN, evidence of extensive protein degradation has not been observed below 170 °C (Bougrier et al. 2008). In addition, the net production difference of PS between 172 and 121 ºC pretreatment was far less than that between 121 and 70 ºC pretreatment (38 v.s. 159 mg PS/g VS), implying that temperature higher than 121 ºC may not necessarily help on the extracellular PS solubilization. The minor increase of PS content with high temperature may be also related to the Maillard reaction where some released sugars reacted with amino compounds under high temperature (> 150 ºC) (Dwyer et al. 2008). The concentration of HA increased significantly after thermal pretreatment, which may have adverse effects on the downstream process. Azman et al. (2015) also reported that part of cellulose in the cell walls could be converted to humic substances during the thermal treatment process. Overall, PN was remarkably increased with high temperature pretreatment (172 ºC), followed by PS and HA.

5.3.1.2 Effects of thermal pretreatment on released DOMs compositions

More specific fractions of DOMs were investigated and the results are shown in Figure 5.2. Most of the fractions (except HMW PN, LMW neutrals and LMW acids) had a marked increment with the ascending temperature. LMW PN, LMW neutrals and LMW PS composed the majority of the observed DOMs after 172 ºC treatment.

This may be attributed to the disruption of chemical bonds of HMW substances at high temperature. Interestingly, in contrast to the fact that there is higher PN content in biomass (Li and Noike 1992), the concentration of HMW PS was higher than HMW PN after 172 ºC treatment.

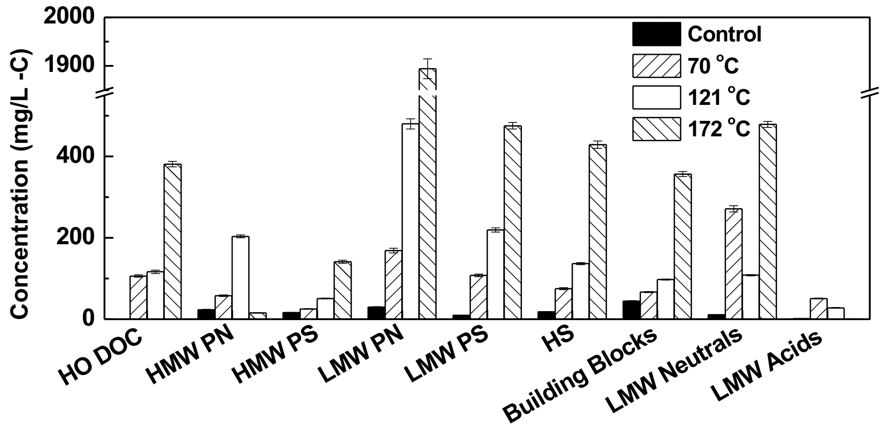

Figure 5.2 Quantification of DOMs (mg/L -C) after different thermal pretreatment.

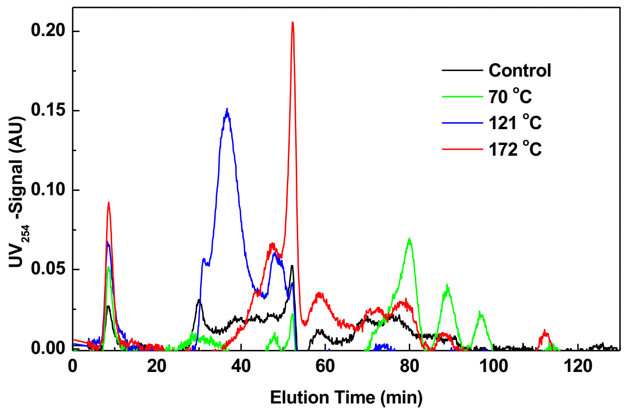

Figure 5.3 LC-OCD chromatogram of control and different thermal pretreated samples in UV_{254}-signal.

The distribution of HMW PN was highly different when the treatment temperature was different. The concentration of HMW PN was the highest with 121 ºC treatment compared to the other temperature pretreatment. Correspondingly, there was a great deal of denatured HMW PN with double binds and benzene ring structures detected in biopolymers (Figure 5.3). It has been reported that protein size would be reduced when the temperature was higher than 150 ºC (Wilson and Novak 2009). Thus, in this study, much more LMW PN other than HMW PN were detected at 172 ºC. Further, at the high temperature, the Maillard reaction of amino acids with reducing sugars could produce HMW heterogeneous polymers (> 10 kDa), which contributed to the significant amount of HS (Bougrier et al. 2008, Dwyer et al. 2008). Therefore, it should be noted that the concentrations of both HS and building blocks increased greatly at the higher temperature. These compounds are able to attach to the active groups of enzymes, leading to the inhibition on AD (Azman et al. 2017, Fernandes et al. 2014).

5.3.2 Effects of thermal pretreatment on methane production in anaerobic digestion

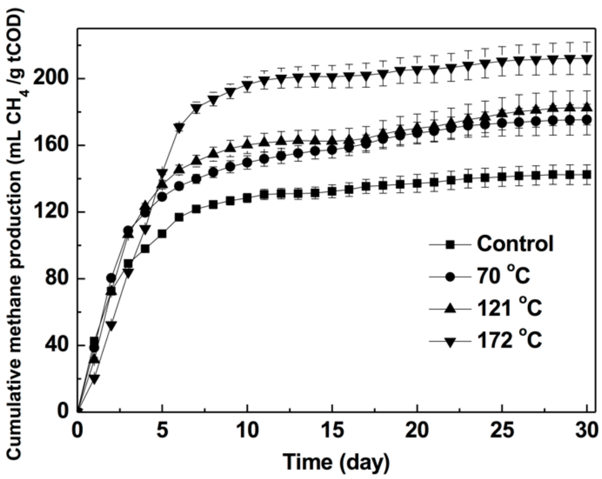

Figure 5.4 Methane production in the different AD reactors fed with control and thermal pretreated sludge.

Figure 5.4 shows the measured methane production from control and THP sludge anaerobic digesters. The biogas production increased rapidly at the beginning and gradually stabilized, which in turn indicated the utilization of available biodegradable organics. It is clear that all the experimental reactors had better methane production performance with thermal treated sludge as feed. Such performance can be explained by the improved sludge disintegration and solubilization. The best performance of methane production was obtained with 172 ºC pretreated sludge (212.1 ± 8.2 mL CH_4/g $tCOD_{feed}$), which is significantly higher compared to other thermal pretreated sludge. In this study, 70 ºC and 121 ºC pretreated sludge digesters (referred as 70 ºC digesters and 121 ºC digesters hereafter) achieved the similar methane production (178.8 ± 7.5 mL CH_4/g $tCOD_{feed}$), with an increase of 25.6% compared to control digesters. Additionally, 172 ºC pretreated sludge reactors (referred as 172 ºC digesters hereafter) produced 142.4 ± 5.3 mL methane per g $tCOD_{feed}$ on 5th day while untreated sludge reactors took 30 days to achieve the same amount, implying thermal pretreatment could remarkably reduce SRT in AD.

5.3.3 DOMs transformation during anaerobic digestion

As 172 ºC treatment provided the best performance in terms of sludge solubilization and biogas production, samples collected from 172 ºC digesters were analyzed to investigate the transformation of key DOMs during AD. In order to compare, samples from control reactors were also analyzed. In the control digesters, the removal rate of organics was limited by the availability of the readily biodegradable organic matters, leading to the minor reduction of the monitored soluble substances (Figure 5.5). The concentration of LMW neutrals even increased at the early stage of digestion due to the degradation of other HMW substances, like HMW PN and HMW PS. The remaining organics may consist of insoluble particulates which are not accessible to bacteria or some soluble nonbiodegradable substances.

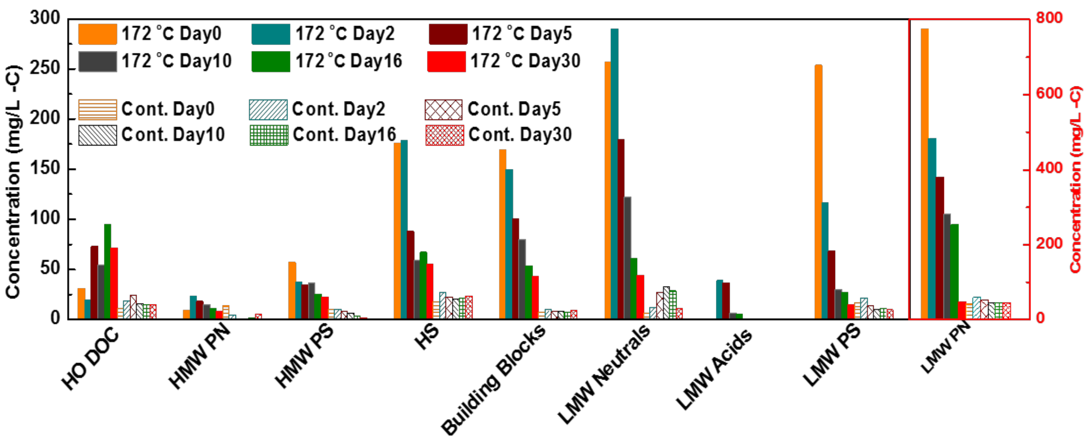

Figure 5.5 Transformation of sub-fractions (mg/L -C) of observed organics in control and 172 ºC digesters during AD.

On the other hand, thermal treatment released abundant readily usable carbon sources which can be digested directly. In the early stage of 172 ºC AD, almost all such components were consumed rapidly, especially the easily biodegradable substances like LMW PN, LMW PS and LMW neutrals (Figure 5.5). Interestingly, LMW PN and LMW PS were the major organic matters transformed during AD (consumed by

727.5 ± 10.6 and 239.4 ± 6.8 mg/L –C respectively), suggesting that they were more associated with the total methane production than other DOMs fractions. According to the concentration profile, the depletion of DOMs was fitted to the first-order kinetics (Li et al. 2017b). Statistical indicators (R^2) were also considered to evaluate the soundness of the model (Table 5.2). The degradation rate constant of LMW PS was higher than that of LMW PN (0.337 v.s. 0.088 day^{-1}), implying the faster utilization of LMW PS.

Table 5.2 Degradation kinetics of DOMs during AD using First-Order kinetics.

	k (day^{-1})	b	R^2
LMW PN	0.088 ± 0.021	6.527 ± 0.097	0.887
LMW PS	0.337 ± 0.060	5.518 ± 0.076	0.956
LMW Neutrals	0.081 ± 0.017	5.653 ± 0.086	0.906

A rapid reduction of LMW neutrals (k = 0.081 day^{-1}) was also observed, since the smaller molecules are thought to be less resistance for contacting bacteria. HS was partially degraded within the first 2 days and decreased slightly onwards. It suggests most of the humic-like substances, mainly associated with the inherent aromaticity and complexity of the polycyclic components, were hardly biodegradable. The HMW DOMs, including HMW PN and HMW PS, only changed slightly during digestion, which suggests that HMW DOMs released by the higher temperature treatment (172 ºC) were more recalcitrant to digestion. Li et al. (2016) concluded that the rate-limiting step for HMW PN degradation was the rupture of aromatic moieties other than the breakup of peptide bonds.

5.3.4 Chemical compositions and characterization of residual DOMs after AD

At this stage, the study found out that the thermal pretreatment released both easily biodegradable DOMs and some relatively recalcitrant substances. Thus, it is reasonable that the concentration of residual DOMs in thermal pretreated AD was higher than that in control anaerobic digesters (Figure 5.6). For instance, the residual DOMs in 172 ºC digesters was 3-fold more than that in control digesters. Besides, the distribution of residual DOMs was distinctly different with different temperature pretreatment.

In control digesters, the residual DOMs were dominated by HS, LMW PN and HO DOC, accounting for 24.4%, 19.2% and 15.6% of total residues, respectively. Part of HO DOC was reported to belong to hydrophobic HS (Penru et al. 2013). Thus, these three types of residuals were refractory to digestion and stayed in the effluent. With the higher temperature (172 ºC) sludge pretreatment, almost all the residual components were more compared to that in control and other temperature digesters. Thus, the discussion on residues will mainly focus on 172 ºC digesters below.

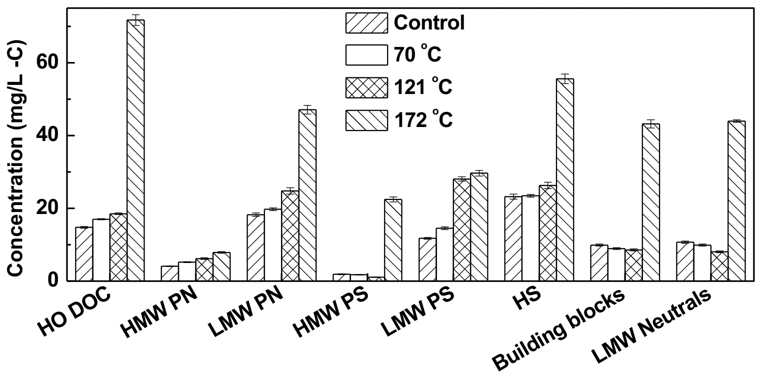

Figure 5.6 Quantification of DOMs (mg/L -C) of control and different thermal pretreated samples after AD.

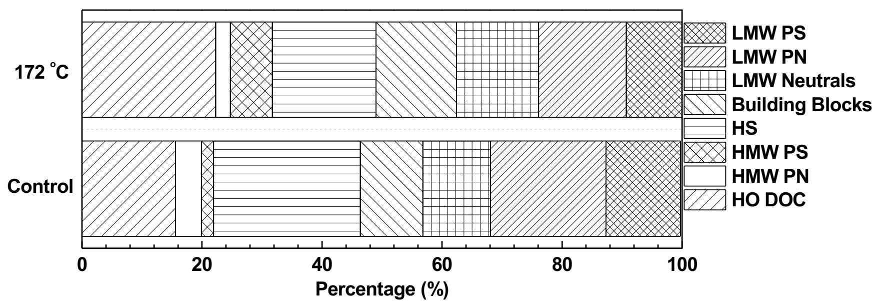

Figure 5.7 Distribution of residual DOMs (mg/L -C) sub-fractions from control and 172 ºC digesters.

It was found that HO DOC, HS, LMW PN and LMW neutrals constituted the majority of the residues in 172 ºC digesters (Figure 5.7). The concentration of residual HO DOC from 172 ºC digesters was 3.9 times higher than other digesters. The remaining HO DOC may contain lots of complex Maillard products, which were nonbiodegradable due to their heterogeneous polycyclic structures (Dwyer et al. 2008). Residual HS-like matters (including HS and building blocks) were almost three folds higher in 172 ºC digesters compared to control, due to the higher production under the higher temperature pretreatment (Figure 5.2) and their nonbiodegradability. The significant amount of LMW PN residues was also observed, which may contain the breakup products of aromatic PN, e.g. tryptophan-like PN.

Generally, LMW substances were more likely to be degraded by microorganisms owing to low mass transfer resistance (Li et al. 2016). However, this study reveals large amounts of LMW PN, LMW PS and LMW neutrals, which should be consumed and transformed to biogas, were still present at the end of AD process. Therefore, it would be interesting to know the compositions of those LMW compounds. In this study, LMW (< 580 Da) specific compounds in digested sludge samples were identified and semi-quantified by GC-MS. The results imply that some released components by thermal hydrolysis treatment were removed in AD and new compounds were also produced during AD with 70 identified compounds reduced to 60 compounds.

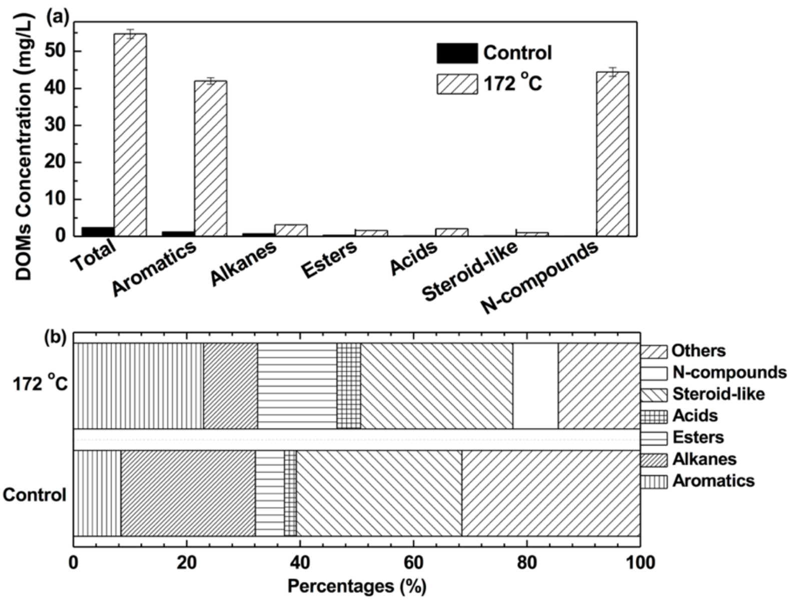

Figure 5.8 (a) Semi-quantification of LMW DOMs (< 580 Da) after 172 ºC temperature pretreatment and control. (b) Distribution of residual LMW DOMs (< 580 Da) from control and 172 ºC digesters.

The identified LMW DOMs were classified into alkanes, esters, acids (compounds with carboxyl groups), aromatics (mostly compounds with benzene rings), steroid-like matters, N-compounds (nitrogenated compounds, mostly amino acids and amide compounds) and others (alcohols, aldehydes and others) (Figure 5.8). It should be noted that alkane with the closest RT was used as standards to quantify the LMW organic matters. As the retention index is different for standards and actual compounds, the concentration derived is only an indication of the abundance of each compound, while the actual concentration of identified compound may be varied.

The concentrations of residual aromatics and N-compounds increased with the elevated treatment temperature and it can be concluded that 172 ºC sludge pretreatment released the highest amount of both compounds (Figure 5.8). The aromatic organics, probably the breakup products of tryptophan-like PN, HS-like matters and other substances, increased by a factor of 7.1 compared to other lower temperature pretreatment. The results suggest that 172 ºC could certainly disrupt the bacterial cells as well as the bio-macromolecules, achieving the best performance of hydrolysis. But the high concentration of soluble N, ammonia in particular, may lead to inhibition on anaerobic processes (Barber 2016).

The LMW residues after digestion in all thermal treated sludge AD reactors were higher than those in control reactors, which may be associated with the higher release of DOMs during pretreatment. The highest amount of steroid-like matters residues was found in the 70 ºC digesters other than 172 ºC digesters. That is reasonable that steroid-like matters are likely to decompose under the higher temperature (evidenced by Figure 5.8a) thus were lower in 172 ºC reactors. With the elevation of pretreatment temperature from 70 ºC to 172 ºC, the percentage of residual steroid-like matters decreased from 54.7% to 26.9%.

For control reactors, the majority of the residual LMW DOMs were steroid-like matters (29.2%) and alkanes (23.7%) (Figure 5.8b). However, in thermal pretreated AD reactors, the compounds of steroid-like matters (26.9%) and aromatics (22.9%) dominated the residues. Minor steroid-like matters were consumed during AD, indicating such polycyclic compounds produced by pretreatment were relatively nonbiodegradable. The steroid-like matters, e.g. cholestenol and coprostanol, are reported to be the degradation products of cholesterol (Jarde et al. 2005, Leeming et al. 1996). Alkanes were considered as slowly biodegradable compounds as they require a longer retention time for digestion under both aerobic and anaerobic conditions (Rojo 2009). Aromatics may also require a longer retention time. For example, the intermediate products phthalates (94% -similarity index) were found to reduce in 172 ºC digesters, indicating that phthalates can be gradually but slowly degraded. In addition, compounds like squalene (87% -similarity index) and erucyl amide (86%) were observed only in digested samples while absent from both raw WAS and treated sludge samples. It is likely that these compounds were produced as by-products of substrate utilization by microorganisms.

UPLC-MS was employed to further investigate the higher MW (< 2 kDa) compounds before and after 172 ºC pretreatment and subsequent AD. By the relative abundance profiles, the significant DOMs before and after 172 ºC sludge pretreatment, as well as the key DOMs from control digesters and 172 ºC digesters were putatively identified.

The results demonstrate that thermal pretreatment mainly assisted in the release of amino acids, dipeptides, fatty acids, purine nucleotides, pyrimidine bases, benzenoids, sugars, glycerophospholipids, steroids and steroid derivatives, which were primarily the breakup products of proteins, polysaccharides, nucleotides and other substances from extracellular, cytoplasm, lysosome, endoplasmic reticulum, nucleus and cell membrane, respectively. Besides, the presence of nucleotides, such as pyrimidine bases, pyrimidine nucleosides and purine nucleosides, confirms that the extent of damage by high temperature pretreatment (172 ºC) reached the microbial cell nuclei.

Lots of residual DOMs were detected in the digested samples, including polar metabolites (benzenoids, peptides, LCFA, pyrrolidines, pyridines and their derivatives) and lipids (flavonoids, steroids and their derivatives). It is known that organics like benzenoids, pyrrolidines, pyridines and their derivatives were more recalcitrant or even inhibitory to cell growth and methanogenesis (Fernandez et al. 2017, Pham et al. 2013). 2-acetylpyrrolidine (classified as pyrrolidines) was observed in both thermal treated and digested sludge. This reveals high temperature pretreatment will produce nonbiodegradable compounds. LCFA (mainly metabolites of lipids) was also considered as an inhibitor by adsorption onto cell walls, of which the digestion is possible but needs longer retention time (Salminen and Rintala 2002).

It is noteworthy that the majority of the residues contained monocyclic or polycyclic structures, implying they were relatively recalcitrant. For instance, most of the residual amino acids and peptides were confirmed to be the breakup products of aromatic PN, like tyrosine and tryptophan over other aliphatic PN. A considerable abundance of thiophene, which is the product of Maillard reaction, was found in 172 ºC digesters. Flavonoids compounds, which are supposed to be refractory with multiple complex benzene rings and double bonds, were also present in the thermal AD effluent.

The organics solubilization after different pretreatment (THP and ALK12-ULS24) and the recalcitrant organic compounds in digesters are compared. After pretreatment, the solubilization of almost all components in THP sludge were higher than that in ALK12-ULS24 pretreated sludge. Particularly, the compounds of HS, building blocks and LMW neutrals in THP sludge were 6 - 8 times higher. However, the content of HMW PN was less in THP sludge. It suggests THP is more efficient to hydrolyze macromolecular compounds to the smaller components. At the molecular level, more LMW (< 580 Da) compounds were released during THP. Aromatic and N-containing compounds are the dominant in THP sludge while steroid-like matters are the major compounds in ALK12-ULS24 pretreated sludge. The compound of thiophene (product of Maillard reaction) was also found in THP sludge. Besides, the compounds of nucleotides, such as pyrimidine bases, pyrimidine nucleosides and purine nucleosides were detected in THP sludge while they were not in ALK12-ULS24 pretreated sludge. These findings further confirm the higher extent of damage by high temperature pretreatment (reaching the microbial cell nuclei).

After digestion, more residual DOMs were in THP sludge digesters compared to that in ALK12-ULS24 pretreated sludge digesters, which is likely due to the higher solubilization of organic matters after thermal hydrolysis. The components of HO DOC, HS and LMW neutrals constituted the majority of the residues in both pretreated sludge digesters. As for the specific residual LMW components, the compounds of steroid-like matters (45.4%), alkanes (21.4%) and aromatics (10.1%) dominated the residues in ALK12-ULS24 pretreated sludge digesters, while steroid-like matters (26.9%) and aromatics (22.9%) were the main LMW residual components in THP sludge digesters. It is consistent with the higher release of aromatic compounds during THP. In addition, the compounds of nucleotides were observed in THP sludge digesters while they were not in ALK12-ULS24 pretreated sludge digesters. Compared with ALK12-ULS24 pretreatment, the higher organics solubilization and higher biodegradability can be obtained with THP sludge. However, more recalcitrant compounds would be released/formed under high temperature. These recalcitrant compounds would be harmful to the downstream treatment, thus, need further polishing before final disposal.

Table 5.3 Energy and cost evaluation of THP for sludge anaerobic digestion (1 m^3 CH_4 produced).

Items	Energy calculated (kWh)	Cost calculated (USD)
Methane production (1 m^3)	9.0	2.1
Energy applied for THP	8.4	1.9
Net cost		0.2

The energy evaluation for 1 m^3 methane produced with sludge THP was tabulated in Table 5.3. The energy applied for thermal hydrolysis was lower than energy output from methane production, indicating THP prior to AD is a cost-efficient technology. It is consistent with Kepp et al. (2000) that energy cost of thermal pretreatment can be covered by biogas production and in addition there would be a significant energy surplus. Besides, the methane yield of THP sludge (212.1 ± 8.2 mL CH_4/g tCOD) is higher than that of ALK12-ULS24 pretreated sludge (197.1 ± 3.0 mL CH_4/g tCOD), which is consistent with the result of higher organics solubilization of THP sludge. Thus, compared with ALK12-ULS24 pretreatment (an energy-negative technique), THP is more preferable.

5.4 Conclusions

This study provided novel insights into the transformation of the key DOMs during THP and AD process. Their impacts on biogas generation were also investigated. Regardless of the improved sludge biodegradability, DOMs (e.g. HO DOC, HS, LMW PN and LMW neutrals) remained more in the effluent of thermal pretreated digesters. At the molecular level, steroid-like matters and aromatics were proved to dominate the LMW DOMs residues. Further study of the higher MW compounds detected the refractory or inhibitory compounds. Overall, THP WAS prior to AD improved the performance with a tradeoff of higher residual DOMs, which requires further consideration.

CHAPTER 6 LIQUID AND SOLIDS SEPARATION FOR TARGET RESOURCE RECOVERY FROM THERMAL HYDROLYZED SLUDGE

Chapter 5 concluded that considerable organic matters could be solubilized in the liquid fraction after sludge THP. It speculates that there is no need to digest the solids part of THP sludge as it contains less readily biodegradable substances. In this chapter, the assumption was verified by digesting the liquid and solids part of THP sludge separately. The THP-L reached the biodegradability (262.6 ± 5.1 mL CH_4/g tCOD) on the 15th day during anaerobic treatment, while THP-S only contributed 30.0% to the total methane production and required more than 30 days digestion time. We investigated the feasibility to convert THP-S into biochar to realize the higher value of the solids fraction. The results prove the produced biochar can be used as slow-release fertilizer. The AD performance and rheological properties of different portions were studied respectively. Finally, a new process to produce biochar from the solids part and generate methane from the liquid fraction was proposed in order to recover resources from THP sludge more effectively. The preliminary energy assessment for this process was performed. The proposed new sludge treatment process has lower operating cost and higher value returns.

6.1 Introduction

THP prior to AD has been successfully implemented for a few years, where sludge is heated up to around 165 ºC under high pressure conditions (Han et al. 2017). The merits of THP, such as improved dewaterability, enhanced biodegradability and reduced sludge viscosity have been well documented recently (Bougrier et al. 2008, Ennouri et al. 2016b). Up to 60% sludge solubilization could achieve with THP (Bougrier et al. 2008, Lu et al. 2018a). This means abundant DOMs would be present in the THP-L. The THP-S would have less readily biodegradable organic matters, resulting in the less biogas generation and/or longer digestion period (meaning larger reactors) (Bougrier et al. 2008). However, there is no literature so far to report the needs to digest the solids fraction of THP sludge and the contribution of biogas production from the solids portion is not clear.

It is well known that sludge displays non-Newtonian flow behavior (Eshtiaghi et al. 2013, Zhang et al. 2016a). Its rheological characteristics are important parameters, which greatly affect sludge treatment processes, including dewatering, drying, mixing, pumping and transport (Chaari et al. 2003, Xia et al. 2009). With better flowability, the energy required to homogenize and transport the feedstock could be reduced due to the lower head loss and pumping power (Zhang et al. 2016a). The different sludge origin and solids content would affect the rheology of sludge (Feng et al. 2014). The dewaterability and viscosity of sludge could be significantly improved by THP (Barber 2016). However, such good properties could not be maintained when THP sludge is mixed with anaerobic sludge for digestion. The dewaterability of mixed sludge (THP sludge mixed with seed sludge) in the digester is generally worse than the original THP sludge (Zhang et al. 2018). Thus, it would make more sense to carry out the dewatering step prior to AD to separate the liquid and solids portions. To date, the information on the feasibility of such separation is lacking.

The main objectives of this study are to (1) investigate the AD performance of THP sludge, THP-L and THP-S, (2) study the rheological properties of different sludge fractions during AD, (3) propose an integrated system to handle both liquid and solids portions of THP sludge in an energy-efficient way. The preliminary energy analysis for the proposed process was performed.

6.2 Material and methods

6.2.1 Sludge source and preparation

WAS was collected from a local municipal water reclamation plant in Singapore. Anaerobic seed sludge was collected from the same plant (a mesophilic anaerobic digester). They were concentrated and stored at 4 ºC before use. The concentrated WAS was thermally hydrolyzed with a pressure reactor (Model 4534, Parr Instrument Company, US) under 165 ± 4 ºC for 30 min. Then the pretreated sludge was cooled down to the room temperature and collected as the original THP sludge. After centrifuged at 6000 g for 10 min, the supernatant and solids part of original THP sludge were collected separately. The original THP sludge, liquid and solids fractions of original THP sludge were diluted with distilled water (named THP sludge, THP-L and THP-S) before used as feed for AD. Three sets of each type of sludge were prepared. Each set was characterized separately. The characteristics of different sludge before anaerobic digestion are shown in Table 6.1.

Table 6.1 Characteristics of different sludge (portion) before anaerobic digestion.

	Seed sludge	Raw WAS	THP sludge	THP-L	THP-S
TS (g/L)	53.9 ± 0.5	49.3 ± 0.3	52.9 ± 0.1	45.7 ± 0.2	57.4 ± 0.3
VS (g/L)	40.2 ± 0.3	40.5 ± 0.1	43.1 ± 0.1	42.4 ± 0.2	42.9 ± 0.2
TSS (g/L)	51.9 ± 1.3	46.5 ± 1.4	35.5 ± 0.4	6.8 ± 0.1	51.3 ± 0.3
VSS (g/L)	38.6 ± 0.7	38.2 ± 1.2	26.6 ± 0.4	6.0 ± 0.1	36.7 ± 0.3
tCOD (g/L)	46.4 ± 0.4	58.3 ± 0.3	82.9 ± 0.7	55.9 ± 0.2	88.7 ± 0.9
sCOD (g/L)	0.79 ± 0.02	0.81 ± 0.02	31.04±0.35	52.68±0.51	14.80 ± 0.28
pH	7.35 ± 0.01	6.43 ± 0.02	5.31 ± 0.02	5.22 ± 0.01	5.48 ± 0.01
CST (s)	1565.0±22.5	1675.1±18.6	201.7 ± 3.3	70.8 ± 3.35	271.9 ±10.1
η (mPa·s)	2833 ±81	3010 ± 112	29 ± 1	7 ± 1	99 ± 6
τ_y (Pa)	36.03 ± 1.14	32.81 ± 1.35	0.55 ± 0.02	0.20 ± 0.01	1.33 ± 0.03

6.2.2 Biochar preparation and determination of elemental content

Biochar was produced through pyrolysis process according to the method described in Qian et al. (2019). Briefly, THP-S was dried (at 70 ºC), ground, and sieved (250 μm). Then it was packed into a crucible and heated in a furnace at 600 ºC for 2 h. After cooling down, biochar was ground and sieved (250 μm) before test. The elements of C, H, N were measured by the elemental analyzer (Elementar, Vario EL Cube). Before the determination of metal elements, biochar was digested following the process reported in Qian et al. (2019). After digestion, the concentrations of Fe, Mg, Na, K, and Ca were tested by ICP-OES (Perkin Elmer Optima 8300), and the concentrations of heavy metals (Cr, Mn, Ni, As, Mo, Cd, Co, Pb, Cu, Al and Zn) were measured using iCAP Q ICP-MS (Thermo Scientific).

6.2.3 Dewaterability tests

Sludge filterability was indicated by capillary suction time (CST). CST was measured using an apparatus (OFITE Capillary Suction Timer, OFI Testing Equipment, Houston, USA) according to Sun et al. (2018a). The improvement of sludge dewaterability was assessed with CST reduction percentage R% according to Eq 6.1.

$$R\ (\%) = \frac{CST_0 - CST_d}{CST_0} \times 100\% \qquad (6.1)$$

where CST_0 represents the CST of the sludge before digestion, CST_d represents the CST of digested sludge.

6.2.4 Rheological tests

Rheological measurements were carried out by a rheometer (Physica MCR102 Modular Compact Rheometer, Anton Paar, Austria) equipped with a concentric cylinder system (cup radius of 28.92 mm, height of 73.10 mm). Prior to each test, the sludge sample was pre-sheared at 300 s^{-1} for 2.5 min, followed by 5 min rest at zero shear rate. The flow tests were performed to investigate the flow behavior of sludge samples. The shear rate ramped up from 9.5 to 1000 s^{-1}. In all the rheological tests, the temperature was maintained at 15 ºC by a chiller. Herschel-Bulkley model (Eq 2) was used to describe the rheological behavior of sludge samples (Miryahyaei et al. 2019).

$$\tau = \tau_y + k\gamma^n \qquad (6.2)$$

where, τ_y is the yield stress (Pa), n is flow behavior index (dimensionless), k is the consistency index ($Pa{\cdot}s^n$).

6.2.5 Energy assessment

The theoretical energy balance was calculated based on the experimental data and previous studies. The input heat for WAS THP and AD processes ($E_{THP,\ heat}$) was calculated according to Eq 6.3 (Lu et al. 2008, Passos and Ferrer 2014). When the pretreated sludge is cooling down from pretreatment temperature to digestion temperature, the surplus energy would be recovered by a heat exchanger with an efficiency φ of 85%. The reactor was assumed to be a cylinder with the diameter to height ratio of 2:1. The reactor wall surface area was calculated to account for heat losses during digestion.

$$E_{THP,\ heat} = \rho Q\gamma\ (Tp\text{-}Tr) - \rho Q\gamma\ (Tp\text{-}Td)\ \varphi + 86.4\ \kappa A\ (Td\text{-}Tr) \qquad (6.3)$$

where $E_{THP,\ heat}$: input heat for THP reactors and digesters (kJ/d); ρ: density (1000 kg/m^3); Q: sludge flow rate (m^3/d); γ: specific heat (4.18 kJ/kg ºC); Tp: temperature of pretreatment (165 ºC); Tr: room temperature (25 ºC); Td: temperature of anaerobic digestion (35 ºC); φ: heat recovery efficiency; κ: heat transfer coefficient (1 W/m^2 ºC) ; A: surface area of reactor wall (m^2).

The input electricity for AD agitation and pumping was estimated according to Eq 6.4 (Lu et al. 2008).

$$E_{elec} = Q\theta + V\omega \qquad (6.4)$$

where E_{elec}: input electricity (kJ/d); Q: sludge flow rate (m^3/d); θ: electricity requirement for pumping (1800 kJ/m^3), V: volume (m^3), ω: electricity requirement for mixing (300 kJ/m^3 d).

The input energy for sludge dewatering was estimated based on the dry solids (DS) according to Eq 6.5 (Xu et al. 2014). Sludge dewatering process contains polyacrylamides (PAM) conditioning and sludge dewatering through belt pressing. Due to the excellent dewaterability of THP sludge, only the belt pressing was considered when separating the liquid and solids fractions (Li et al. 2017a).

$$E_{dewater} = m\chi + m\psi \qquad (6.5)$$

where $E_{dewater}$: input energy for sludge dewatering (kJ/d); m: DS mass flow rate (kg DS/d); χ: electricity requirement for dewatering (369 kJ/kg DS), ψ: Polyacrylamides used for dewatering estimated as energy (550.8 kJ/kg DS).

The energy requirement for sludge pyrolysis process contains drying, heating and pyrolysis steps. The moisture content of THP-S was estimated to be 60 wt% (Li et al. 2017a). The moisture content of dewatered digested WAS and THP sludge (with the addition of PAM) was estimated to be 70 and 65 wt% (Liang et al. 2019, Zhang et al. 2018). The energy input for drying (moisture evaporation) was estimated according to Eq 6.6 (Wang et al. 2012):

$$E_{drying} = [\varepsilon M + \gamma M\ (Tb\text{-}Tr)]\ \frac{m}{(1\text{-}M)} \qquad (6.6)$$

where E_{dry}: input energy for drying (kJ/d); ε: enthalpy of vaporization of water during the drying process (2200 kJ/kg); M: moisture content of wet sludge; γ: specific heat (4.18 kJ/kg ºC); Tb: the boiling point of water (100 ºC); Tr: room temperature (25 ºC); m: DS mass flow rate (kg DS/d).

The energy input for heating up dry sludge was estimated according to Eq 6.7:

$$E_{heat} = \gamma_s m\ (Th\text{-}Tr) \qquad (6.7)$$

where E_{heat}: input energy for heating (kJ/d); γ_s: specific heat capacity of dry sludge (1.95 kJ/kg ºC); Th: the pyrolysis temperature (600 ºC); Tr: room temperature (25 ºC); m: DS mass flow rate (kg DS/d).

The incineration cost was estimated to be 88 USD/tonne DS (Lundin et al. 2004). The energy requirement for 1 kg DS pyrolysis (E_{pyro}) was estimated to be 755 kJ (Wang et al. 2012). The total energy requirement for sludge pyrolysis process was calculated according to Eq 6.8:

$$E_{pyrolysis} = E_{drying} + E_{heat} + E_{pyro} \qquad (6.8)$$

The output energy from digesters was calculated according to Eq 6.9.

$$E_{CH_4} = P_{CH_4}\ \xi\eta \qquad (6.9)$$

where E_{CH4}: output energy from methane production (kJ/d); P_{CH4}: methane production (m^3/d); ξ: lower heating value for methane (35800 kJ/m^3); η: Energy conversion efficiency (90%).

The economic values in this study were converted to the energy (230 USD/MWh) to evaluate the total process (Liang et al. 2019). The energy analysis was calculated by subtracting the input cost from the total output value.

6.3 Results and discussion

6.3.1 Performance of anaerobic digestion with different feed sludge

6.3.1.1 Biodegradability of different feed sludge

The cumulative methane production with different feed sludge is compared in Figure 6.1. The maximum methane production (262.6 ± 5.1 mL CH_4/g $tCOD_{feed}$) was obtained during the digestion of THP-L, which is consistent with the results in Li et al. (2017a), who obtained the cumulative methane yield of 240 - 320 mL/g tCOD by digesting the filtrate from hydrothermally pretreated sewage sludge (180 ˚C, 30 min). This indicates the higher percentage of biodegradable substances was in THP-L and that was lower in THP-S. It should be noted the initial methane production rate in THP sludge reactors was slower than that in WAS reactors, which is consistent with the previous report (Lu et al. 2018a). Besides, the initial methane production rate in THP-S reactors was much slower than that in WAS reactors, while it was much faster in THP-L reactors, suggesting that the rate-limiting factor of THP sludge digestion was more related to the solids substances.

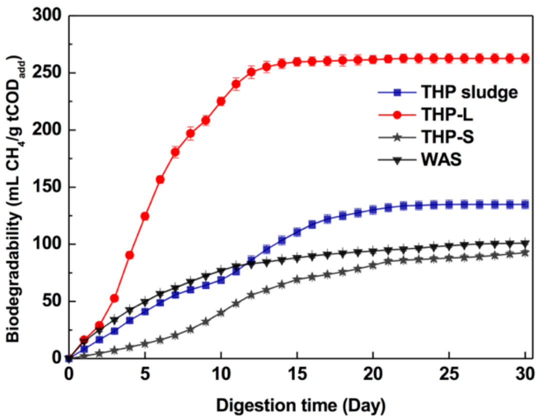

Figure 6.1 Cumulative methane production curves of different sludge (portion) during anaerobic digestion.

The digestion time of THP-L was much shorter than other reactors. The biogas production ceased in THP-L reactors on the around 15th day and it stopped in THP sludge digesters on the 22nd day, while the raw sludge and THP-S digesters continued the biogas generation till 30th day. It confirms the solids fraction is slower to degrade. It should be noted that the digestion time of THP-L and THP sludge in this study is slightly longer than other reported results, which may be due to the much higher sludge concentration and lower inoculum to substrate ratio (Jensen et al. 2011).

6.3.1.2 Contribution of different feed sludge to methane production

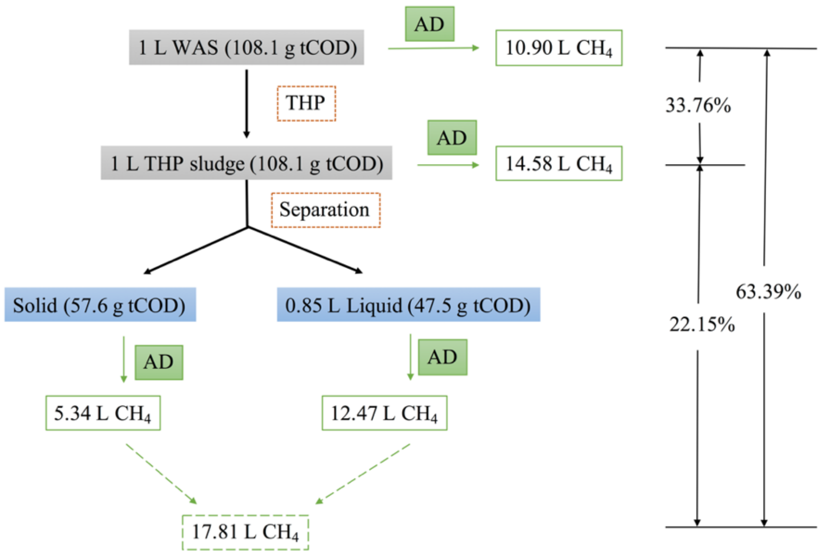

Figure 6.2 Conversion 1 L of WAS to methane through anaerobic digestion process.

Based on 30 days digestion time, the conversion performance of 1 L WAS to methane through the direct digestion of raw WAS, the digestion of THP sludge, or separate digestion of THP-L and THP-S is shown in Figure 6.2. In this study, 1 L of THP sludge was separated to 57.6 g tCOD of solids and 47.5 g tCOD of liquid (0.85 L). The tCOD of solids fraction was estimated by subtracting the tCOD of THP-L from the tCOD of THP sludge. The missing mass of tCOD during operation was estimated to be 5%. The separated THP-AD process markedly improved the methane production by 63.39% compared to the traditional AD process with raw sludge as feed and improved by 22.15% compared to THP sludge AD process without liquid-solid separation. The methane generation of THP-S only accounted for 30.0% of the total methane production and the digestion time was doubled compared to THP-L digestion. If the digestion stops at day 15, the biogas contribution from THP-S would be even less. As the biodegradability of the solids fraction is relatively low, it would make more sense to treat this part of solids by other methods (e.g. incineration or biochar production). The excellent dewaterability of THP sludge also makes the separation of solids feasible (Table 6.1).

6.3.1.3 Soluble substances transformation during anaerobic digestion

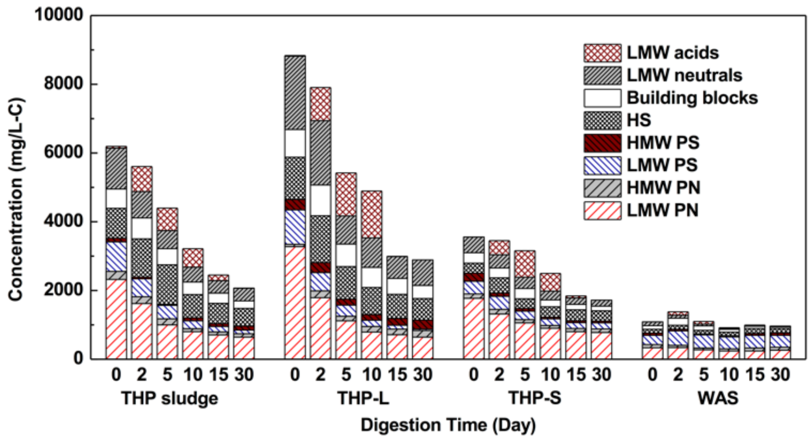

Figure 6.3 Soluble substances transformation of different AD reactors fed with different sludge during digestion.

The specific fractions of soluble organics were determined as shown in Figure 6.3 to study their transformation in anaerobic digesters with different feed sludge. In reactors of THP sludge, THP-L and THP-S, most of the organic matters were consumed including LMW PN, HMW PN, LMW PS, HMW PS, HS, building blocks and LMW neutrals. Among them, LMW PN, LMW neutrals and LMW PS were greatly removed, which accounted for 40.6%, 19.3% , 18.3% of total DOC removal in THP sludge reactors, 42.0%, 22.3%, 15.3% in THP-L reactors and 54.0%, 15.2% and 10.4% in THP-S reactors, respectively. The results imply LMW PN, LMW neutrals and LMW PS are the main contributors to biogas generation. The total initial concentration of LMW PN, LMW neutrals and LMW PS in THP-L reactors was 46.7% more than that in THP sludge digesters and almost 3 times of that in THP-S digesters, which would explain the higher biogas production in THP-L reactors.

It is noteworthy that the concentration of HS-like substances (HS and building blocks) increased within the first 2 days and reduced gradually from the 5th day during digestion in all reactors (Figure 6.3). It is reasonable that the breakdown of other substances (like PN and PS) could form part of HS-like substances which are relatively recalcitrant to anaerobic microbes (Provenzano et al. 2014) and HS-degrading microorganisms likely need some acclimation time at the early stage of digestion. The percentage of HS-like substances reduction was higher in THP-L reactors (20.8%), followed by THP sludge reactors (16.8%) and THP-S reactors (9.5%). In summary, more and relatively easy biodegradable soluble organics existed in THP-L reactors, which facilitates the access of microbes to organics and promotes the sludge biodegradability.

6.3.2 The physicochemical properties of sludge during anaerobic digestion

6.3.2.1 The dewaterability of sludge during anaerobic digestion

The CST values of different digested sludge from THP sludge, THP-L and THP-S reactors were compared as shown in Figure 6.4. A lower CST value of samples indicates better dewaterability. The CST values of digested WAS samples were higher than 2000 s (out of the range of this CST testing equipment), which were not shown in this study. Apparently, the dewaterability of THP-L digested sludge is the best, which may be attributed to the less suspended solids content and the larger surface area of THP-L digested sludge (Miryahyaei et al. 2019). During AD operation, the CST values of all types of digested sludge decreased, indicating that AD would improve the digested sludge dewaterability. This finding could be well supported by Xu et al. (2009) and Zhang et al. (2018). The highest CST reduction of sludge samples was obtained on the 15th day for THP-L reactors (17.0%), 15th day for THP sludge reactors (16.1%) and 30th day for THP-S reactors (3.3%). The improved dewaterability would be of great benefit for sludge volume reduction and help to save huge amounts of operational cost for sludge conditioning and dewatering before further treatment. It should be noted that the worst dewaterability of seed sludge deteriorated the dewaterability of all type of digested sludge once mixed together (Table 6.1). Separating the solids and liquid fractions of THP sludge before AD would make more sense.

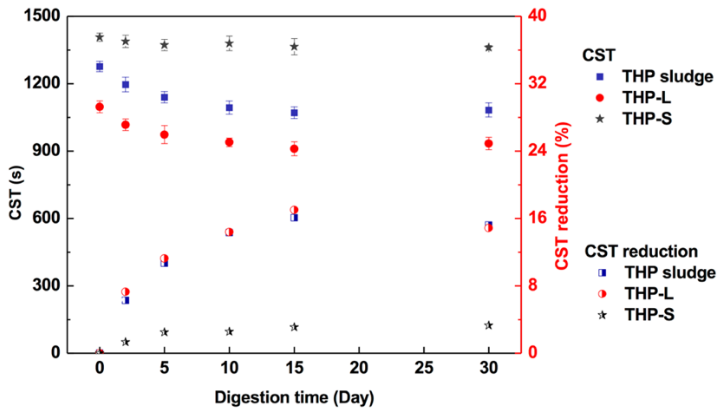

Figure 6.4 The CST values and CST reduction of different sludge during anaerobic digestion.

6.3.2.2 The rheological properties of sludge during anaerobic digestion

The rheological properties of sludge play an important role in heat and mass transfer process during bioreactions, they also affect the design and cost of sludge treatment, such as piping, stirring and transport. Generally, the apparent viscosity (shear rate at 19.4 s^{-1}) of digested sludge decreased with increasing digestion time (Figure 6.5a), suggesting a better flowability of sludge in the digesters. This reduction in apparent viscosity was mainly attributed to the reduction of organic matters in sludge (Mori et al. 2008). Among these four types of digested sludge samples, sludge in WAS reactors showed the most prominent reduction of apparent viscosity, with the reduction of 35.8% during the first five days (Figure 6.5a). The apparent viscosity of sludge in THP related reactors was much lower than that in WAS reactors, suggesting that THP treatment would improve overall sludge properties. In addition, it is reasonable that sludge from THP-L reactors was always less viscous due to the exponential relationship between the sludge viscosity and solids content (Dhar et al. 2012). The rheogram of different AD sludge was well fitted by the Herschel-Bulkley model, where the values of τ_y are shown in Figure 6.5b. Furthermore, the yield stress of digested sludge also decreased with the digestion time, indicating AD weakened the internal structure. In addition, it can be observed that the evolution of yield stress with digestion time was highly consistent with that of apparent viscosity for the same type of feed. The reduction of both parameters for THP related AD sludge is likely induced by the continuous transformation of dissolved materials to biogas during digestion (Zhang et al. 2016a). Comparing the sludge viscosity with CST values, the highly viscous sludge seemed to have poor dewaterability, which is consistent with the finding in Li and Yang (2007).

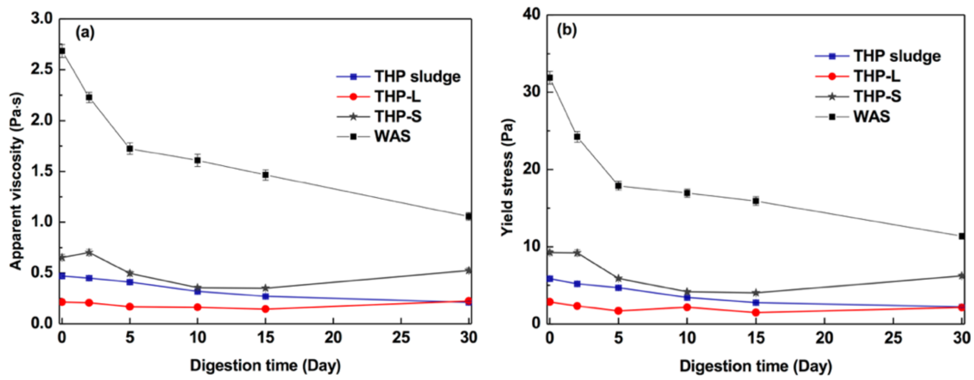

Figure 6.5 Evolution of apparent viscosity (shear rate at 19.4 s^{-1}) and yield stress for sludge samples with digestion time.

The apparent viscosity and yield stress values of the feed sludge are shown in Table 6.1. The original seed sludge and WAS had worse flowability while THP treated sludge (including THP-L, THP sludge and THP-S) behaved better fluidity. After mixing with seed sludge, the viscosity of THP related AD sludge became much higher. It would require more energy to stir or pump this digested sludge effectively. Generally, THP sludge has better dewaterability and flowability. But these good properties would be affected by mixing with seed sludge. Even the digested THP sludge has worse filterability and fluidity compared with the original feed THP sludge. In addition, the liquid fraction of THP sludge could be digested with short retention time and it also has higher biodegradability. By contrast, the solids portion of THP sludge not only produces less methane but also prolongs the digestion period. Thus, it would be promising if the liquid and solids fractions of THP sludge are separated before AD and transferred to different processes.

6.3.3 Biochar production and properties

Apparently, THP-L should be separately treated for energy recovery, while the solids portion of THP-S should be also properly handled with higher recovery value. It was reported that the biochar produced from sewage sludge could be used as the fertilizer (Chen et al. 2015, Qian et al. 2019), and the biochar production from sludge could be energy surplus (Li et al. 2017a). Therefore, the production of valuable biochar would help to solve the problem of sludge reduction and realize the full utilization of sludge.

Table 6.2 Contents of main elements in THP-S biochar.

Elements	mg/g
P	61.82 ± 1.24
Fe	40.07 ± 0.25
Ca	33.15 ± 0.24
Al	22.15 ± 0.34
Mg	9.57 ± 0.13
K	8.12 ± 0.12
Na	2.84 ± 0.01
C	283.30 ± 3.10
H	19.90 ± 0.04
N	40.15 ± 0.15
S	4.89 ± 0.33

Table 6.3 Contents of heavy metals in THP-S biochar.

Heavy metals	mg/g	Regulation limits [a]
Zn	1.615 ± 0.032	2
Cu	0.480 ± 0.021	0.5
Cr	0.095 ± 0.002	0.5
Ni	0.070 ± 0.002	0.1
Mn	0.045 ± 0.001	nr
Pb	0.040 ± 0.001	0.5
Co	0.035 ± 0.001	0.1
Mo	0.025 ± 0.001	nr
As	0.010 ± 0.001	0.02
Cd	0.002 ± 0.001	0.01

Biochar yield from the dry THP-S was 33%. The contents of nutrients are important to evaluate if the biochar is suitable to be applied as a fertilizer. Table 6.2 shows the contents of main elements in biochar, including P, Fe, Ca, Al, Mg, K and Na. The content of P was as high as 61.82 mg/g, which was comparable to P content of 0.2 - 73.0 mg/g in biochar reported in previous literature (Chan and Xu 2009, Sun et al. 2018b). In practice, the addition of biochar to soil was reported to affect the microbial community (Prayogo et al. 2014). Zhang et al. (2016b) proved that applying biochar at a low application rate could improve the grain yields. In addition, the contents of HMs in biochar limit its application as the amounts are strictly regulated (Uysal et al. 2010). The contents of various kinds of HMs in the biochar produced from THP-S are shown in Table 6.3. It appears that the levels of heavy metals in biochar were all below their limits for fertilizer (Nzihou and Stanmore 2013). Moreover, the bioavailability and eco-toxicity of HMs in biochar were significantly reduced after pyrolysis (Devi and Saroha 2014). Thus, biochar produced from the THP-S has great potential to be used as a fertilizer or soil remediation additive.

6.3.4 Energy analysis

The integrated process for target resource recovery from THP sludge is proposed as shown in Figure 6.6. THP-L is separately treated for biogas generation and THP-S is pyrolyzed to produce biochar. For the liquid treatment facility, granular or biofilm based process can be employed to further enhance the conversion efficiency. It is known that expanded granular sludge bed (EGSB) systems have high organic conversion efficiency with organic loading up to 22.5 kg COD/m^3 day (Liu et al. 2012). In this study, the organic loading rate of THP-L reactors was only 1.86 kg COD/m^3 day (0.096 kg COD/kg VSS day). Future study can be focused on employing EGSB reactors to handle the THP-L stream. This would shorten the HRT further. The facilities set-up and operation of the proposed approach are very close to that of the current existing THP - AD process (Figure 6.6). It includes thermal hydrolysis reactor (THP), anaerobic digester (AD), liquid/solids separation with the belt press or centrifuge (sludge dewatering) and sludge dryer (drying) (Barber 2016, Mills et al. 2014). The dried sludge is either sent to incineration plant or pyrolysis plant. The energy consumption of pyrolysis plant or incineration plant is estimated while its capital cost is not considered as incineration plant or pyrolysis plant is normally not part of wastewater treatment facilities in wastewater treatment plant. Furthermore, the sludge transportation cost is not included in this study. The anaerobic reactor that receives THP-L, in fact, should have a much smaller footprint given the shortened digestion time. It suggests the existing sludge treatment facilities and flow in WWTPs can be rearranged to facilitate the process and save the capital cost for the proposed process.

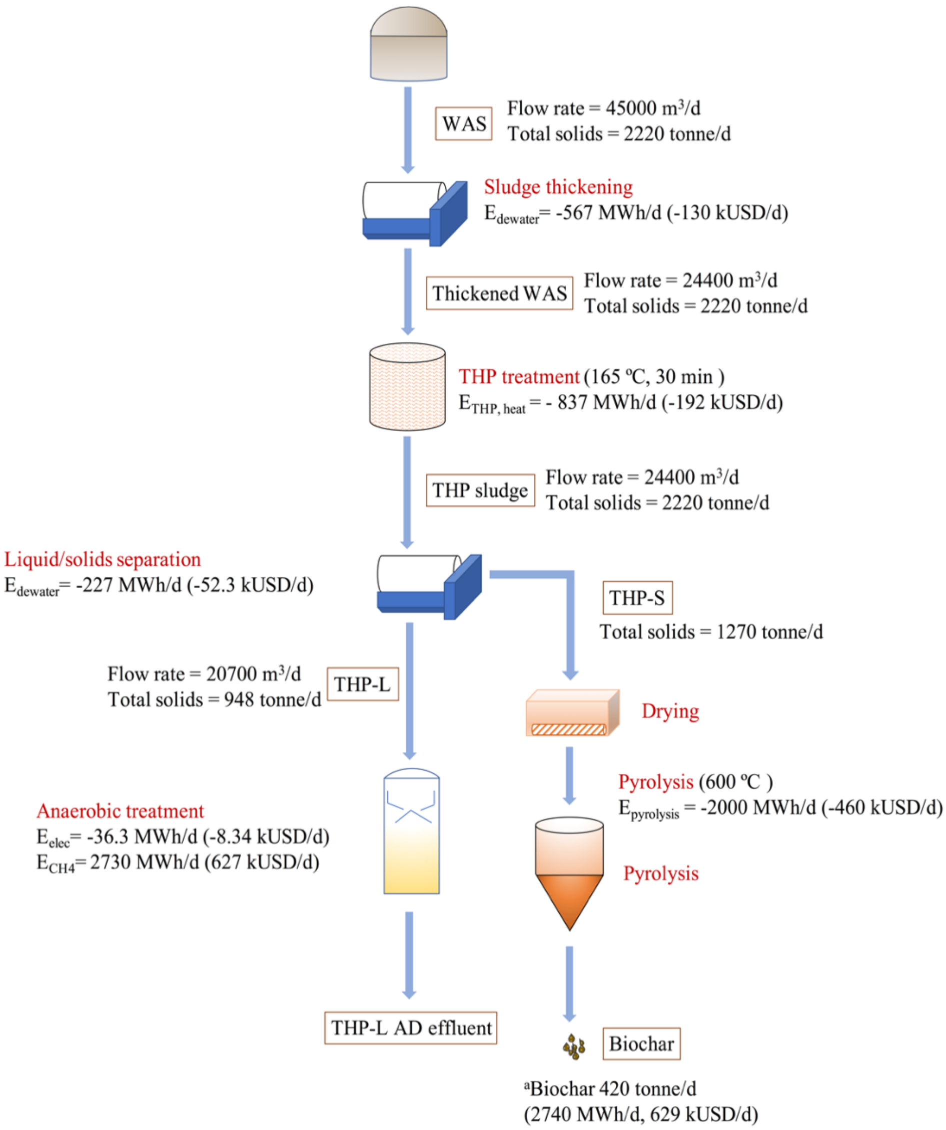

Figure 6.6 Energy analysis of the proposed process.

[a] Biochar price :1.5 USD/kg (Alhashimi and Aktas 2017).

The energy analysis for this sludge management approach was calculated based on a plant with a treatment capacity of 45000 m^3/d WAS. It is obvious this process realizes the energy surplus of 1800 MWh/d (0.81 MWh/tonne DS). For the proposed approach, the main energy cost is for THP-S pyrolysis (2000 MWh/d), and the second major energy input is for sludge thermal hydrolysis (837 MWh/d) followed by the energy requirement for sludge dewatering and liquid/solids separation. Notwithstanding, THP process can be driven by waste heat from various industries. The required energy for thickening and dewatering processes highly depends on sludge properties. The better dewaterability and lower viscosity of THP sludge and THP-L AD sludge would further reduce the total treatment cost. On the other hand, the benefits of produced biochar (2740 MWh/d) accounted for more than half of the total output, followed by the contribution of methane generated by THP-L digestion. Given the excellent dewaterability of THP sludge before AD, the direct separation of THP-S for pyrolysis saves energy requirement for sludge drying. Moreover, the pyrolysis of solids instead of incineration would decrease the release of air pollutants to the atmosphere and also reduce the incineration ashes. As such, the integrated process of methane generation from THP-L treatment coupled with biochar production from THP-S pyrolysis is an environmentally and energy-efficiently sustainable approach.

6.4 Conclusions

This study investigated the feasibility to recover resources by separating the liquid and solids portions of THP sludge. The THP-L and THP-S portions were anaerobically digested separately. Compared to THP sludge digestion, 22.1 % more methane was produced from this separated digestion. However, the solids portion of THP sludge contributed much less to total methane generation (only 30.0%) and prolonged the digestion period (from 15 to 30 days). Thus, in this study, a new approach that converts the solids portion to valuable biochar was proposed. The energy assessment confirms that the integrated approach of biochar production from THP-S pyrolysis and biogas generation from THP-L digestion is energy-efficient. Moreover, the better dewaterability and lower viscosity of THP-L digested sludge make the process more feasible and practicable.

CHAPTER 7 THERMAL HYDROLYZED SLUDGE LIQUOR AS ORGANIC FERTILIZER

In previous chapters, the liquid fraction of THP sludge were anaerobically digested to methane, which is an energy biogas but low value. This chapter proposed a new approach to use it as organic liquid fertilizer given high value chemical compounds that can promote plant growth were identified in THP liquor. Besides high contents of N, P and K, total free amino acids (FAAs) and plant-growth-promoting FAAs also presented in high concentration in the THP liquor. For the first time, phytohormone compound, indole-3-acetic acid, was observed and the content was the highest in THP liquor with 165 ºC treatment (165 ºC THP liquor). Meantime, 165 ºC THP liquor did not have negative impact on soil microbes and seed germination. THP liquor with higher temperature treatment (> 165 ºC) showed toxicity at elevated levels. These results suggest that 165 ºC THP liquor has a great potential to be an organic fertilizer. This chapter provided a new potential application of THP liquor and improved the value of WAS.

7.1 Introduction

WAS contains numerous microbes, organic matters and inorganic ingredients, which could be regarded as a resource (Thomsen et al. 2017). The technologies that can achieve sludge treatment and resource recovery simultaneously carry utmost significance. One option is to convert the organic matters in the sludge to energy products, like methane through AD (Li et al. 2016). However, nutrients, e.g. nitrogen (N), phosphorus (P) and potassium (K), still remain in sludge after AD. Furthermore, long digestion time and low value product - methane, make AD a less attractive technology (Spirito et al. 2014).

WAS for land application has been investigated for many years. This allows the nutrients in the WAS to be recycled and reused. However, the presences of hazardous substances, e.g. heavy metals (HMs), pathogens and unpleasant odor, limit its direct application as solid fertilizer (Wang et al. 2019a). The nutrients and organics are mainly embedded in the sludge particles which would reduce their bioavailability (Warman and Termeer 2005). Thermal hydrolysis pretreatment (THP) of sludge has been studied and applied in various scales due to its high efficiency in sludge reduction, organics solubilization, pathogens removal and improvement in dewaterability (Lu et al. 2018a, Zhang et al. 2018). In the THP process, a large proportion of organic matters, N (40 - 70%) , P (10 - 15%) and K (50 – 70%) could be solubilized into the liquid fraction (Bougrier et al. 2008, Sun et al. 2013). Besides, HMs distribution showed minor part in liquid phase and major part remaining in solids fractions (Munir et al. 2018, Sun et al. 2014). It is expected that the liquid fraction of THP sludge (THP liquor) can be a great fertilizer. However, such feasibility has never been explored.

Sludge subject to different temperature THP would result in distinctly different content of soluble organic matters, nutrients and inorganic substances (Sun et al. 2014). It is likely that the soluble products released under different temperature may also have varied fertilizing effects on plants. Certainly, the THP temperature should not be too high given a more sustainable approach would be preferred. To date, it is not clear if there are any organic matters in the sludge, other than the inorganic nutrients, would be beneficial for plant growth, while only a handful studies mentioned soluble organic compounds of sludge are capable of promoting plant growth (Day and Katterman 1992, Sun et al. 2014). Besides, the potential impact and toxicity of THP liquor on seed germination, soil microorganisms have not been evaluated.

The main objectives of this study are to (1) study the impact of different THP temperature on the release of chemicals of interest in THP liquor, (2) characterize the specific organic compounds which are beneficial to plant growth, (3) investigate the toxicity of THP liquor on plant and soil microbes, (4) evaluate the fertilizing potential of THP liquor products. In this study, the feasibility of using THP liquor for land application is assessed with respective to the nutrients and organics contents, its toxicity to plant and microbes as well as the presence of HMs. The specific plant growth-promoting compounds in THP liquor, including free amino acids (FAAs) and phytohormone compounds were analyzed to provide the insights of the promoting factors.

7.2 Material and methods

7.2.1 Sludge source and treatment

The WAS was collected from a local wastewater treatment plant in Singapore. It was concentrated and stored at 4 ºC before use. The characteristics of sludge sample are listed in Table 7.1. The prepared sludge samples were treated at 121, 140, 165, 180, 200 and 220 ºC respectively in a pressure reactor (Model 4534, Parr Instrument Company, US) for 30 min. All the treated sludge samples were cooled down to the room temperature and centrifuged at 10,000 g for 10 min to collect the supernatant as THP liquor products (named 121 ºC, 140 ºC, 165 ºC, 180 ºC, 200 ºC and 220 ºC THP liquor). Triplicates were conducted for each temperature treatment test.

Table 7.1 Characteristics of WAS sample.

Parameters	WAS (g/L)
Total solids (TS)	15.19 ± 0.08
Volatile solids (VS)	12.28 ± 0.07
Total suspended solids (TSS)	15.08 ± 0.07
Volatile suspended solids (VSS)	12.19 ± 0.06
tCOD	19.23 ± 0.17
sCOD	0.31 ± 0.05

7.2.2 Determination of the specific plant grow-promoting compounds

7.2.2.1 Determination of free amino acids

The cation exchange resin (CER) was used to remove the metal elements from the THP liquor before the determination of FAAs according to the methods reported in Wu et al. (2017). 60 g CER per g VS of THP liquor was mixed at 300 rpm (4 ºC) for 12 h. The liquid phase was harvested by centrifuging the suspension at 10,000 g for 15 min and filtrating through 0.45 μm membrane filters. The obtained liquid samples were then lyophilized for further tests.

The lyophilized samples were dissolved in deionized water to determine FAAs content using the derivatization method with a HPLC as described in Le and Stuckey (2017). Briefly, 21 types of primary and secondary amino acids were calibrated as the internal and external standards. The primary FAAs were derivatized with ortho-phthalaldehyde (OPA) and secondary amino acids were derivatized with 9-fluorenylmethylchloroformate (FMOC). All the derivatives were analyzed using the HPLC with a UV diode array detector (DAD, SPD- M20A) and an Agilent Zorbax Eclipse Plus C18 column.

7.2.2.2 Determination of phytohormone compounds

The THP liquor samples were filtrated (0.45 μm membrane filters) and lyophilized. Then the lyophilized samples were mixed with 80% (v/v) methanol water solution. After sonication for 15 min and centrifugation for 3 min at 14,000 rpm, the supernatant was collected for phytohormone detection. Eight types of phytohormone standards were used for compounds screening, including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), zeatin, dihydrozeatin, kinetin, benzylaminopurine, gibberellic acid A3 and salicylic acid.

A HPLC equipped with a DAD and an Agilent Zorbax C18 column was used to detect these phytohormone compounds. Mobile phase comprised 2.7 g/L formic acid in water (A) and 2.7 g/L formic acid in methanol (B). The proportion of mobile phase B was kept at 10% for the first 3 min, increased from 10% to 48% in following 5 min, further ramped to 90% in subsequent 2 min, maintained at 90% for 2 min and restored to 10% in the last 3 min. The flowrate was set at 0.3 mL/min and the temperature of column chamber was maintained at 30 ± 0.5 ºC. The detector wavelength was set at 254 nm while the UV adsorption data at 190 to 400 nm was simultaneously collected. The data acquisition frequency was set at 20 Hz. The injection volume was 1.0 μL.

7.2.3 Determination of metal elements content

Liquid samples were digested based on the methods in Qian et al. (2019) before the test of metal elements. After digestion, the concentrations of Fe, Mg, Na, K and Ca were determined by ICP-OES (Perkin Elmer Optima 8300), and the concentrations of heavy metals (Cr, Mn, Ni, As, Mo, Cd, Co, Pb, Cu, Al and Zn) were measured using iCAP Q ICP-MS (Thermo Scientific).

7.2.4 Biotoxicity experiment to soil microbes

The pH values of THP liquor products were adjusted to 7.0 ± 0.1. The strain of *P. putida* was precultured in Luria-Bertani (LB) broth (25 g/L) at 30 °C and 150 rpm for 12 h. For the biotoxicity tests, 200 μL of cell suspension culturing in 20 mL of LB broth was carried out as the control groups. Similar cell suspension culturing in the mixed medium was carried out as the experimental groups. The mixed medium contained 10 mL of double strength LB broth and 10 mL of prepared THP liquor (referred as LB-THP medium).

All experiments were performed at 30 °C and 120 rpm in a temperature-controlled incubator. The cell suspension was collected and OD_{600} was determined using a UV/Vis Spectrometer at a wavelength of 600 nm in a 2-hour interval. All inoculation experiments were conducted in triplicates.

7.2.5 Germination tests

Wheat seeds were used for germination tests. Before the experiment started, the wheat seeds were sterilized for 10 min with 10% hydrogen peroxide and then soaked with deionized water for 2 h. The THP liquor samples were diluted to 1000, 500 and 250 mg C/L respectively. Twenty sterilized seeds were placed on a filter paper in a petri dish (90 mm) and moistened with 5 mL of the pre-prepared liquid samples (experimental groups) or deionized water (control groups). All dishes were covered and incubated in the dark at 25 °C for 5 days. The germination percentage, root length and shoot length were recorded (Liao et al. 2014). All germination tests were carried out in triplicates.

7.2.6 Statistical analysis

The significance of germination ratio, root and shoot growth difference was evaluated by analysis of variance (ANOVA) and $p < 0.05$ was considered to be statistically significant. The software of SPSS version 19.0 was used for the statistical analysis.

7.3 Results and discussion

7.3.1 Effects of temperature on sludge solubilization

7.3.1.1 Effects on nutrients release

The impact of THP temperature on the nutrients release was investigated and the concentrations of soluble nutrients are shown in Figure 7.1a. The concentrations of DOC and TN show increase trend with the increasing reaction temperature until 200 °C. When the temperature further raised, the concentrations were found to decrease, which may be due to the carbonization reaction and organic N repolymerization at the higher temperature (> 200 °C) (Zheng et al. 2015). Besides, NH_4^+ also gradually increases with the elevated temperature, implying more than 30% of the nitrogen content is solubilized as ammonium nitrogen which could be easily uptaken by the plant.

As shown in Figure 7.1, the concentrations of P and K increase gradually with the elevated temperature. The solubilization of both components could reach more than 90% of the maximum values at 165 °C. It is noteworthy that the concentration of P in THP liquor is lower at 220 °C than that at 200 °C, which agrees with the findings by Yuan et al. (2018) that a decreased P dissolution was observed when the operation temperature was higher than 200 °C. This may be explained by the enhanced precipitation of P with multivalent metal ions like Mg and Zn at the higher temperature (Wang et al. 2019a).

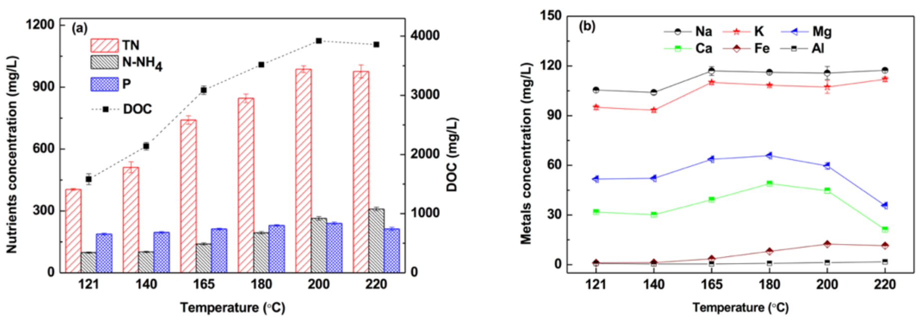

Figure 7.1 Effects of temperature on (a) nutrients, (b) macronutrients release.

The secondary nutrients such as Mg, Ca and trace elements like Fe are essential for plant growth, thus were investigated and present in Figure 7.1b. The concentrations of these components are the highest at 180 or 200 °C. When the reaction temperature is higher, the precipitation, physical adsorption or complexation reaction of those components would be enhanced, resulting in the lower soluble content (Laurent et al. 2011, Wu et al. 2015).

The nutrients content, i.e. N, P and K, in THP liquor products, are compared with the commercial organic liquid fertilizer to evaluate their fertilizer potential. As shown in Table 7.2, the concentrations of both N and P in THP liquor are higher than that in the commercial fertilizer while the content of K is slightly lower. This indicates THP liquor products roughly meet the requirement of nutrients in liquid fertilizer. However, the low temperature THP liquor contains less nutrients, which could be uneconomic.

Table 7.2 Comparison of the content of major nutrients between THP liquor products and the commercial fertilizer (based on dry weight).

THP liquor	N (%)	P (%)	K (%)
121 °C	8.45 ± 0.09	3.93 ± 0.10	1.98 ± 0.06
140 °C	9.34 ± 0.50	3.74 ± 0.08	1.78 ± 0.05
165 °C	9.21 ± 0.45	2.60 ± 0.05	1.35 ± 0.04
180 °C	9.24 ± 0.22	2.51 ± 0.04	1.18 ± 0.03
200 °C	10.14 ± 0.17	2.47 ± 0.06	1.10 ± 0.03
220 °C	11.18 ± 0.35	2.37 ± 0.09	1.24 ± 0.04
Commercial[a]	3.0	1.5	2.4
Commercial[a]	3.7	1.2	3.0

[a] The well-selling organic liquid fertilizer for horticulture in Singapore.

7.3.1.2. Effects on organic matters solubilization

The composition of organic matters in different THP liquor is shown in Figure 7.2. The concentrations of LMW acids and LMW neutrals increase with the elevated temperature, which may be attributed to the breakup of other HMW substances under the high temperature, such as PS and PN. The concentrations of LMW PS and LMW PN reach the respective maximum values at 165 °C and 200 °C. The highest concentration of HS and BB (the breakup product of HS) was found at 180 °C, implying that BB and HS may break up when subject to the extreme environment.

It should be noted that the total organics content of 165 °C THP liquor is greatly higher than that of 140 °C THP liquor, and the organics content of 220 °C THP liquor remarkably reduces, which suggests the temperature between 165 and 200 °C is efficient for more substances solubilization. In these THP liquor products (165 - 200 °C), the organics of LMW PN, LMW neutrals, building blocks and HS make up the majority of the total organic matters. It is well known that compounds with the smaller MW are more easily adsorbed by the plant and utilized by the microbes. In particular, HS and organic acids produced during the hydrothermal process were proved to promote the growth of the plant root (Alvarez and Grigera 2005, Sun et al. 2014). These imply the THP liquor products (165 - 200 °C) would be more suitable for plant growth. In addition, LMW PN (mainly composed of amino acids) accounts for nearly half of the total organics in these THP liquor products. The detailed composition of the FAAs will be discussed below.

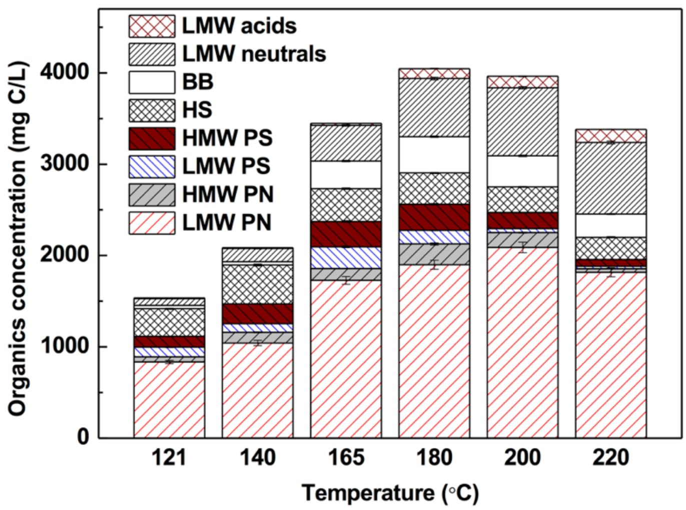

Figure 7.2 The soluble organic matters fractions of different THP liquor products.

7.3.2 Characterization of plant growth-promoting compounds

7.3.2.1 The composition of free amino acids

Table 7.3 The composition of FAAs in different THP liquor products.

FAAs	THP liquor [a]					
	121 °C	140 °C	165 °C	180 °C	200 °C	220 °C
Aspartic acid	0.049 ± 0.001	0.115 ± 0.005	1.565 ± 0.051	1.590 ± 0.060	0.795 ± 0.042	0.538 ± 0.022
Glutamic acid	0.035 ± 0.002	0.158 ± 0.006	0.309 ± 0.010	0.225 ± 0.028	0.313 ± 0.026	0.249 ± 0.013
Asparagine	0.027 ± 0.001	0.107 ± 0.005	- [b]	-	-	-
Serine	0.016 ± 0.002	0.029 ± 0.000	0.278 ± 0.011	0.365 ± 0.020	0.268 ± 0.015	0.123 ± 0.004
Glutamine	-	0.136 ± 0.002	-	-	-	-
Histidine	-	-	0.020 ± 0.000	0.024 ± 0.002	0.072 ± 0.004	0.013 ± 0.001
Glycine	-	0.007 ± 0.000	0.196 ± 0.006	0.190 ± 0.011	0.129 ± 0.004	0.064 ± 0.003
Threonine	0.027 ± 0.002	0.024 ± 0.000	0.045 ± 0.001	-	-	-
Arginine	0.085 ± 0.003	1.386 ± 0.04	0.815 ± 0.039	0.922 ± 0.035	1.444 ± 0.052	1.410 ± 0.077
Alanine	-	-	0.044 ± 0.002	0.095 ± 0.004	0.470 ± 0.026	0.422 ± 0.028
Tyrosine	-	-	-	-	-	-
Cystine	-	-	0.478 ± 0.021	1.011 ± 0.055	0.843 ± 0.039	-
Valine	0.023 ± 0.000	0.168 ± 0.007	0.246 ± 0.030	0.233 ± 0.013	0.238 ± 0.008	0.309 ± 0.022
Norvaline	-	0.027 ± 0.001	0.088 ± 0.000	0.052 ± 0.002	0.056 ± 0.001	0.068 ± 0.005
Tryptophan	-	-	0.019 ± 0.002	0.024 ± 0.002	0.043 ± 0.002	0.037 ± 0.001
Phenylalanine	-	0.056 ± 0.002	0.214 ± 0.015	0.127 ± 0.005	0.143 ± 0.006	0.222 ± 0.007
Isoleucine	0.018 ± 0.001	0.091 ± 0.005	0.229 ± 0.010	0.133 ± 0.005	0.133 ± 0.007	0.188 ± 0.007
Leucine	-	-	0.314 ± 0.012	0.789 ± 0.032	1.417 ± 0.082	1.279 ± 0.046
Lysine	-	0.034 ± 0.002	0.043 ± 0.000	0.036 ± 0.002	-	0.010 ± 0.001
Sarcosine	-	-	0.006 ± 0.000	0.008 ±0.0001	0.007 ± 0.000	0.008 ± 0.000
Proline	0.031 ± 0.001	-	0.060 ± 0.001	0.104 ± 0.007	0.125 ± 0.005	0.163 ± 0.006
Total	0.311 ± 0.008	2.338 ± 0.055	4.969 ± 0.146	5.928 ± 0.289	6.496 ± 0.262	5.103 ± 0.235

[a] The content of FAAs was based on dry weight (g/100 g sample).

[b] - means below detection limit.

As previously discussed, LMW PN occupies a significant portion in the organics from THP liquor, implying the present of large amounts of amino acids. It has been known that amino acids are the important nitrogen source and could be assimilated directly by many plants (Jones and Darrah 1994, Reeve et al. 2008). It is also essential nutrients for many soil microbes. Therefore, the amino acids are important active fraction of organic compounds in an organic fertilizer. The composition of FAAs in different THP liquor products is analyzed and tabulated in Table 7.3.

It is obvious that the total concentration of FAAs increases with the increasing temperature but decreases when the temperature is higher than 200 °C (Table 7.3), which is consistent with the previous results of LMW PN in Figure 7.2. Below 200 °C, the highest FAAs concentration of 6.496 g/100 g sample was obtained, which is comparable with the content (6.81 g/100 g) of reported commercial fertilizer (Kim et al. 2010b). For each specific amino acid, the optimal temperature to achieve the highest value is different from 165 to 200 °C. Among the total amino acids, the concentrations of aspartic acid, glutamic acid, arginine, cystine, leucine and serine are relatively high in THP liquor products (165 - 200 °C). Particularly, leucine that is rich in the products could promote plant growth (Shahollari et al. 2007). Cystine (sulfur-containing amino acid) is the precursor of several metabolites that regulate plant growth (Amir et al. 2002). Additionally, other FAAs that are known to promote plant growth were also observed in THP liquor products. For instance, glutamic acid was known to increase the crop yield and proline could affect the formation of plants cell walls (Ashraf and Foolad 2007). The concentrations of these plant-growth promoting FAAs all show an increasing trend with the increased temperature until 200 °C (Table 7.3). It indicates the condition of higher temperature (within 200 °C) would be likely to generate more plant growth-promoting amino acids, which make THP liquor products more valuable for application as the organic fertilizer.

7.3.2.2 The characterization of phytohormone compounds

Some types of phytohormone compounds are known to benefit to growth. IAA, IBA, zeatin, dihydrozeatin, kinetin, benzylaminopurine, gibberellic acid A3 and salicylic acid have been reported to promote plant growth previously (Fahad et al. 2015). Surprisingly, IAA was observed in THP liquor, while other types of phytohormone compounds were absent. As shown in Table 7.4, IAA is only present when the temperature is lower than 180 °C. This is reasonable that IAA is easily decomposed compound (Nissen and Sutter 1990). The higher concentration of IAA was obtained in THP liquor products generated at 140 to 180 °C. Besides, pyridoxal (one type of Vitamin B6) was observed in the liquid fraction of THP sludge (around 172 °C) (Lu et al. 2018a). These findings confirm that the phytohormone compounds are present in THP liquor products (< 200 °C).

Table 7.4 The content of IAA in different THP liquor products.

THP filtrate	IAA (μg/L)
121 °C	61.3 ± 0.3
140 °C	607.6 ± 3.2
165 °C	662.8 ± 3.6
180 °C	289.1 ± 6.4
200 °C	- [a]
220 °C	-

[a] - means below detection limit.

7.3.3 Evaluation of toxicity of THP liquor products

7.3.3.1 Evaluation of inhibition effect of THP liquor products to microbes

Various compounds that are potentially useful for plant growth (including nutrients and plant growth-promoting compounds) are present in THP liquor products, indicating their feasibility as fertilizer. However, the application could only be realized when there is no toxicity effect. Different LB-THP medium was used to culture *P. putida* cells and the growth curves are compared with control experiments (only LB broth) as shown in Figure 7.3. Obviously, the culture density suggests sigmoidal growth in all medium. The addition of THP liquor appears to stimulate the growth of cells as the values of OD_{600} in experiment groups are higher than that in control groups at the end of incubation. This stimulation phenomenon may be attributed to the addition of nutrients and organics from the THP liquor. According to the growth curves, LB-THP medium (< 180 °C) did not have measureable inhibition while LB-THP medium (> 180 °C) exhibited inhibition effect at the early stage. This could be explained by Wang et al. (2016), which found high temperature would enhance the production of toxic aromatic compounds.

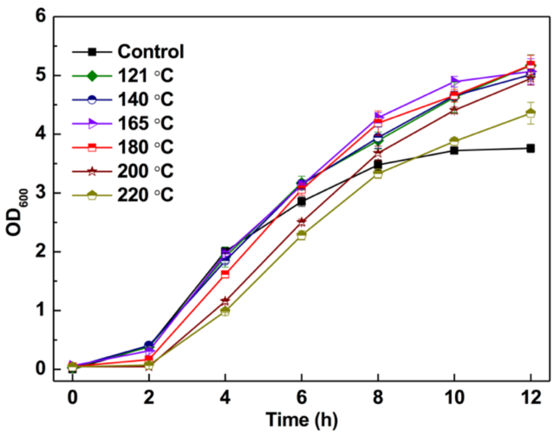

Figure 7.3 Growth curves of *P. putida* cells with different LB-THP medium (optical density measured at 600 nm).

7.3.3.2 Evaluation of the phytotoxicity of THP liquor products to plant

A suitable fertilizer should not have inhibition on both soil microorganisms and plants. The germination tests were performed and the results of germination ratio, root length and shoot length in different THP liquor are recorded in Figure 7.4. Obviously, almost all the original THP liquor products exhibit negative impacts on the germination, root length and shoot length compared with control tests. The inhibition effect is more serious with the elevated THP temperature. The findings are in agreement with Nurdiawati et al. (2015) that found liquid produced from fruit bunch under 220 °C showed more harmful effect on plants than the lower temperature liquid did. The inhibition might be attributed to the toxic compounds, e.g. aromatics formed at high temperature. The high DOC and electric conductivity (EC) values of the original THP liquor would also be harmful to plant. As shown in Table 7.5, the EC values of the original THP liquor are all higher than 1500 µS/cm, which is considered the upper value for growing media (Nurdiawati et al. 2018).

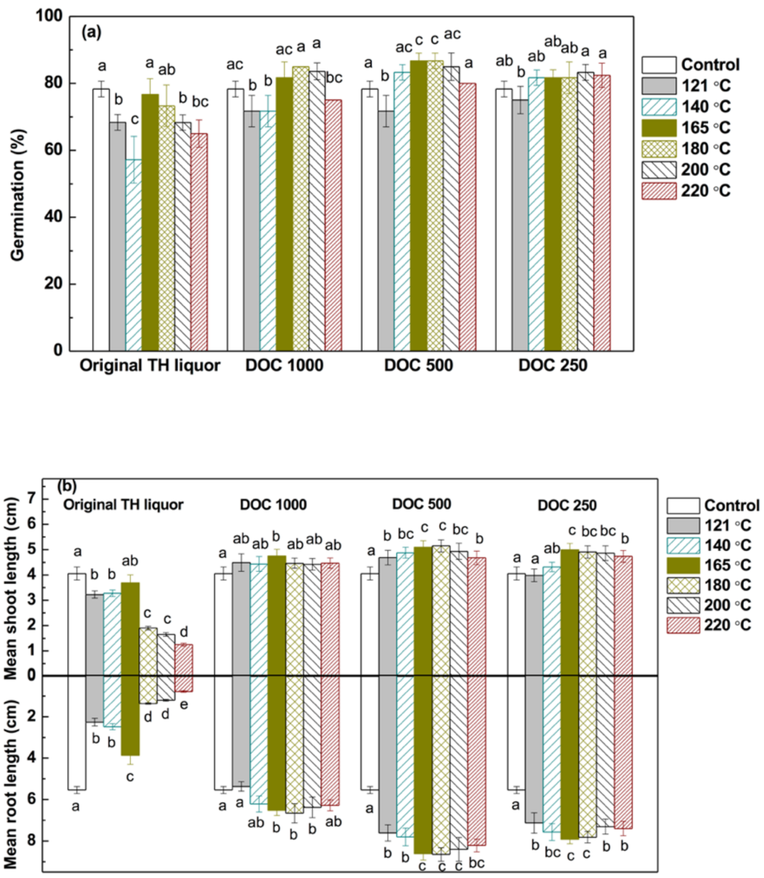

Figure 7.4 (a) Germination ratio, (b) root length and shoot length of wheat seeds in different diluted THP liquor products. Different alphabets above the bars suggest that these values are significantly different at $p < 0.05$ (Liao et al. 2014).

Table 7.5 The electric conductivity (EC) of the THP liquor products.

EC (µS/cm)	121 °C	140 °C	165 °C	180 °C	200 °C	220 °C
Original	2260 ± 23	2200 ± 26	1843 ± 16	2140 ± 17	2620 ± 14	2920 ± 18
DOC (1000mg/L)	1417 ± 34	1105 ± 28	629 ± 12	666 ± 16	687 ± 11	813 ± 28
DOC (500mg/L)	731 ± 22	602 ± 15	320 ± 11	340 ± 10	342 ± 17	411 ± 23
DOC (250mg/L)	387 ± 7	298 ± 16	166 ± 10	181 ± 5	183 ± 13	222 ± 6

Therefore, dilution of the THP liquor prior to germination tests was carried out. From Figure 7.4, diluted THP liquor could relieve the inhibition and even stimulate the root and shoot elongation. When the THP liquor was diluted to 1000 mg C/L, no signs of inhibition were found on seed germination and seedling growth. The further dilution of these liquor products to 500 mg C/L remarkably increase the length of root. This stimulation effect may be explained by the introduction of nutrients and beneficial compounds from THP liquor as well as the reduction of toxic substances in the liquor. Nevertheless, when THP liquor was further diluted to 250 mg C/L, the seed germination, root and shoot elongation remained unchanged or decreased due to the lack of nutrients and organics. As a result, the concentration of 500 mg C/L would be preferable when THP liquor is applied to the plant. With this concentration for application, the root elongation with 165 - 220 °C THP liquor was significant better compared with that of other THP liquor products. The enhanced growth was also observed for the shoot elongation with 140 - 200 °C THP liquor. These suggest 165 - 200 °C THP liquor products with DOC of 500 mg/L are more beneficial to plant growth. It should be noted that the concentration of nutrients in THP liquor products would decrease after dilution. However, the presence of phyto-active compounds in THP liquor shows more value compared with the commercial fertilizer.

7.3.4 The HMs content of different THP liquor products

Table 7.6 Contents of HMs in 165 - 200 °C THP liquor and limits for HMs in soil amendments (mg/kg).

Heavy metals	THP liquor products			Sludge Austria [a]	German fertilizer ordinance (2015) [b]
	165 °C	180 °C	200 °C		
Zn	20.28 ± 0.30	50.00 ± 1.60	81.69 ± 2.90	2000	1000
Cu	1.33 ± 0.03	2.37 ± 0.05	4.89 ± 0.11	500	70
Cr	2.13 ± 0.06	3.55 ± 0.11	7.26 ± 0.19	500	300
Ni	8.23 ± 0.21	10.66 ± 0.20	20.54 ± 0.48	100	80
Mn	12.15 ± 0.29	16.99 ± 0.44	26.08 ± 0.54	nr	nr
Pb	0.37 ± 0.01	0.30 ± 0.01	0.23 ± 0.01	500	150
Co	2.54 ± 0.08	2.58 ± 0.09	3.08 ± 0.07	100	nr
Mo	2.89 ± 0.08	2.65 ± 0.06	2.95 ± 0.06	nr	nr
As	6.68 ± 0.24	29.73 ± 1.51	43.85 ± 2.24	20	40
Cd	0.006 ± 0.0001	0.008 ± 0.0001	0.011 ± 0.0001	10	1.5

[a] According to Nzihou and Stanmore (2013).

[b] According to Wang et al. (2019a).
nr: Not regulated.

Table 7.7 Contents and mass balance of heavy metals in different fractions with 165 °C pretreatment.

	THP sludge	THP-S		THP-L		Mass balance
Heavy metals	mg/g THP sludge	mg/g THP-S	mg/g THP sludge	mg/g THP-L	mg/g THP sludge	%
Zn	0.68	0.972	0.520	0.020	0.009	78
Cu	0.386	0.570	0.305	0.001	0.000	79
Cr	0.031	0.057	0.030	0.002	0.001	101
Ni	0.028	0.035	0.019	0.008	0.004	80
Mn	0.081	0.135	0.072	0.012	0.006	96
Pb	0.010	0.015	0.008	0.001	0.000	85
Mo	0.007	0.010	0.005	0.003	0.001	96

The concentrations of HMs in 165 - 200 °C THP liquor products were determined and compared with the regulations as present in Table 7.6. The content of As in 180 and 200 °C THP liquor products exceeds its limits for soil application. In 165 °C THP liquor, the contents of all HMs are far less than their regulation limits for fertilizer. The dilution prior to application would further lower the HMs concentration. As shown in Table 7.7, HMs would be largely concentrated in solid phase after sludge THP, which further confirmed the low presence of HMs in THP-L. This is consistent with the findings by Wu et al. (2015). What's more, THP could also transform the free HMs into more stable forms (Munir et al. 2018). These findings infer that 165 °C THP liquor is highly possible used for land application because the heavy metals content would not be problematic.

Considering the high contents of nutrients and plant growth-promoting compounds (such as FAAs and IAA), THP liquor products exhibit a great fertilizing potential. Through the soil bacteria incubation and seeds germination tests, 165 °C THP liquor product shows no inhibition effect to bacteria and no phytotoxicity to plant growth. Additionally, the HMs content of this product is within the regulations for fertilizer. Thus, 165 °C THP liquor product could be used as a candidate for organic fertilizer. The utilization of THP liquor as a kind of liquid organic fertilizer could help to solve sludge treatment problem but also produce enormous economic benefits.

7.4 Conclusion

This study investigated the feasibility of thermal hydrolyzed sludge liquor as fertilizer. By comparing the chemical content of the THP liquor produced under different temperature, 165 °C THP liquor shows appropriate N (9.21%) and P (2.60%) that are similar as the commercial fertilizer. The plant growth-promoting organics, including FAAs and IAA, are highly present in this product. In addition, 165 °C THP liquor product was proved to be free of inhibition to soil bacteria. This product has no phytotoxicity and could stimulate plant growth with minor treatment. THP liquor with higher temperature treatment (> 165 ºC) showed toxicity at elevated levels. Therefore, 165 °C THP liquor can be used as an efficient fertilizer for land application.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Overall conclusions

This thesis presents the transformation and utilization of the solubilized substances from pretreated WAS. Through the efficient sludge pretreatment, large amount of particulate substances would be solubilized, which are easily accessible to microbes. Firstly, the characterization of key DOMs during ALK-ULS pretreatment and AD were investigated (Chapter 4). In order to further promote the sludge solubilization, THP was applied. The insights into the anaerobic transformation of DOMs produced by sludge THP were obtained (Chapter 5). THP could not only promote sludge biodegradability but also improve dewaterability, which makes the separation of THP-L and THP-S feasible. The approach for target resource recovery from different portions of THP sludge was proposed and evaluated (Chapter 6). Finally, the high content of solubilized substances in THP liquor make it a potential organic fertilizer, whose investigation and evaluation were conducted (Chapter 7). The major findings and achievements of this thesis are listed as follows:

(1) ALK-ULS pretreatment promoted sludge solubilization and methane production. During pretreatment, both the biodegradable organic matters and the hardly biodegradable substances like recalcitrant HS and complex HMW PN were released. After digestion, polycyclic steroid-like matters, alkanes and aromatics constituted the majority of the residual LMW DOMs. Specific higher MW residual compounds were also investigated, and the recalcitrant compounds like flavonoids and benzene derivatives were detected.

(2) The best sludge solubilization and maximum methane production was obtained at 172 ºC sludge pretreatment. Under this condition, LMW PN, LMW neutrals and LMW PS were the majority of the solubilized compounds and the main contributors for biogas generation. Regardless of the improved sludge biodegradability, DOMs (e.g. HS, LMW PN and LMW neutrals) remained more in the effluent of thermal pretreated digesters. The slowly biodegradable or nonbiodegradable steroid-like compounds and aromatics dominated the LMW DOMs residues. Further study of the higher MW compounds also detected the

recalcitrant or inhibitory compounds, such as benzenoids, flavonoids, pyrrolidines, pyridines and derivatives.

(3) By separated digestion of THP-L and THP-S portions, 22.1 % more methane was obtained compared with THP sludge digestion. THP-S only produced 30.0% of the total methane and prolonged the digestion time. It is recommended to pyrolyze the THP-S to valuable biochar. The new approach of biochar production from THP-S pyrolysis and biogas generation from THP-L digestion was proposed and estimated to be energy-efficient.

(4) The content of nitrogen and phosphorus in 165 °C THP liquor met the requirement for commercial fertilizer. The plant growth-promoting compounds like FAAs (including glutamic acid, tryptophan, leucine and cystine) and IAA were generated under this temperature. Besides, THP liquor (< 180 °C) was free of inhibition to soil microbes. After dilution, THP liquor products showed no phytotoxicity. THP liquor with higher temperature treatment (> 165 ºC) showed toxicity at elevated levels. Therefore, 165 °C THP liquor could be used as a candidate for organic fertilizer.

8.2 Recommendations for future research

In Chapter 4 and Chapter 5, ALK-ULS pretreatment and THP improved the sludge biodegradability while the higher concentration of residual DOMs (refractory compounds included) was present in the effluent after AD. Thus, the polishing step such as post-treatment is suggested to remove the residual substances in digester liquor. However, the related information is lacking. More effort should be paid to develop the post-treatment technologies. On the other hand, if the residual substances in the effluent are not removed, whether they will have the negative effect on the downstream treatment processes (like membrane bioreactor, anammox and disinfection) is not clear. Further research is required to study the impact of the residual recalcitrant components to the downstream treatment processes.

In addition, thermal hydrolysis of sludge at high temperature enhanced the generation of some refractory compounds, especially Maillard byproducts (produced by the reaction of reducing sugars and PN at high temperature) (Bougrier et al. 2008).

Efforts should be made to reduce the generation of byproducts and maintain the good performance of sludge solubilization. The options like combination of different pretreatment methods, addition of additives, stepwise thermal hydrolysis pretreatment could be studied in the future work.

Results in Chapter 6 investigated the feasibility of target resource recovery through the batch study. It could be demonstrated to the continuous reactors to better evaluate its energy consumption. Especially, granular or biofilm-based process could be employed to treat the THP-L efficiently. For example, the EGSB system has been used to treat ALK fermentation liquid of WAS, high-strength organic wastewater and so on (Jing et al. 2016, Zhang et al. 2011). Compared with the high organic loading (up to 22.5 kg COD/m^3 day) of EGSB reactors (Liu et al. 2012), THP-L reactors was only 1.86 kg COD/m^3 day in this chapter. Future study can be focused on employing EGSB reactors to handle the THP-L stream. This would shorten the HRT further.

Finally, according to the preliminary results in Chapter 7, the fertility and safety of the THP liquor products could be obtained. To better assess the proposed technique, the long-term application of THP liquor fertilizer for plant growth in field and pot trials should be studied in future. Additionally, organic fertilizer can increase soil productivity on low-fertility and irrigated soils, providing a means of carbon accumulation by increasing the carbon stock in biomass and soil organic carbon. It is also regarded to lower terrestrial greenhouse gas (GHG) fluxes due to the carbon sequestration (Breunig et al. 2019). It is expected that land application of THP liquor has the potential to lower GHG emissions, but such possibility has not been studied. Life Cycle Assessment (LCA) is commonly used to evaluate the environmental impacts over the whole life cycle of the objectives (Chau et al. 2015). Thus, it would be meaningful to conduct the LCA on the land application of THP liquor.

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