Effects of chemically assisted ultrasonication treatments on anaerobic digestion of sewage sludge

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2016

Tian, X. (2016). Effects of chemically assisted ultrasonication treatments on anaerobic digestion of sewage sludge. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/144043>

<https://doi.org/10.32657/10356/144043>

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**EFFECTS OF CHEMICALLY ASSISTED
ULTRASONICATION TREATMENTS ON
ANAEROBIC DIGESTION OF SEWAGE
SLUDGE**

TIAN XINBO

SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING

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SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING

A THESIS SUBMITTED TO

NANYANG TECHNOLOGICAL UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF

DOCTOR OF PHILOSOPHY

2016

Acknowledgements

First of all, I would like to express my sincere gratitude to my supervisor Prof. Ng Wun Jern for his kind guidance and encouragement. Prof. Ng Wun Jern is always supportive to my work, shows me the right direction for my research and encourages me when I am down. With his help, I realize how to become a creative and qualified engineer.

Many thanks go to Prof. Lee Changsoo. Although Prof. Lee Changsoo no longer works at NTU, he brought me into NTU and showed me the necessary fundamentals to be a good researcher which are essential for my research career.

I would also like to thank Dr. Antoine Trzcinski, who always provides me constructive suggestions and shares his research experience with me.

Special thanks to the Public Utilities Board, Singapore for sponsoring the project and providing sludge samples for analysis and Xylem Water Solutions Herford GmbH for providing the ozone generator.

I would also like to acknowledge center director Prof. Liu Yu, lab manager Ricky Lim and all AEBC members. They are all very friendly and helpful.

Last but not least, I would like to thank my parents Mr. Tian Hongwei and Mdm. Li Xiaoying. They always support me with love and share every happiness and sorrow with me.

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Abstract

Sewage sludge is a problematic by-product of wastewater treatment. Due to the high organic and pathogenic content, it needs to be stabilized before safe disposal. Anaerobic digestion is generally applied to stabilize sewage sludge, because it not only reduces the organic content in sludge but also generates biogas which can be used for energy supply. However, anaerobic sludge digestion is a time-consuming process because hydrolysis of particulate organics in sludge limits the overall anaerobic digestion rate. Sludge treatment is often integrated with an anaerobic digester to solubilize the solids as well as to enhance the overall anaerobic digestion performance. Sludge treatment can be integrated in two different configurations. It can be applied to feed sludge before anaerobic digestion as pre-treatment. Alternatively, it can be achieved by treating the digested sludge after anaerobic digestion and recycling the treated digested sludge to the original reactor, which is known as post-treatment. In this study, the effects of individual ultrasonication (ULS), sequentially combined ULS and ozonation (ULS-Ozone) and simultaneously combined ULS and alkaline (ULS+ALK) treatments on feed sewage sludge (i.e. pre-treatment) and digested sludge (i.e. post-treatment) were investigated.

ULS pre-treatment showed limited performance in solubilizing feed sewage sludge and increasing the sludge biodegradability because it is essentially “single” effect - mechanical disintegration. Synergistic VSS solubilization was observed in feed sludge after the ULS-Ozone pre-treatment. In addition, some of the organics solubilized by ultrasound can be further broken down by ozone, resulting in solubilization of low molecular weight (MW) components ($MW < 27$ kDa). The ULS+ALK pre-treatment not only induced synergistic COD solubilization but also generated smaller organics with MW around 5.6 kDa in feed sludge. The biogas production increase following anaerobic digestion of ULS, ULS-Ozone, ULS+ALK treated feed sludge with semi-continuous anaerobic reactors were 20.7%, 35.5% and 24.6%, respectively at SRT of 10

days.

ULS-Ozone and ULS+ALK post-treatments were found to result in synergistic COD solubilization in digested sludge. Despite the similar extent of COD solubilization (from 200 mg/L to around 2,600 mg/L), the solubilization products were different after the ULS-Ozone and ULS+ALK treatments of digested sludge. The ULS+ALK treatment of digested sludge primarily solubilized macromolecules with MW over 500 kDa. However, the ULS-Ozone treatment of digested sludge not only solubilized macromolecules with MW higher than 500 kDa but also released organics with MW around 103 kDa. The biogas production from the semi-continuous anaerobic digesters increased by 9.8%, 10.7%, 17.8% after the ULS, ULS-Ozone and ULS+ALK treated digested sludge were recycled to the anaerobic digester, respectively at SRT of 20 days and recycle ratio of 5%.

List of publications

Technology Disclosure:

1. **Tian, X.**, Trzcinski, A.P., and Ng, W.J. (2015). Treatment for enhancing biodegradability of digested sludge. Submitted to NTUitive on 8th December, NTU reference: TD/341/14.

Journal publications:

1. Trzcinski, A.P., **Tian, X.**, Wang, C., Lin, L.L., and Ng, W.J. (2015). “Combined ultrasonication and thermal pre-treatment of sewage sludge for increasing methane production”; Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 50(2), 213-223.
2. **Tian, X.**, Trzcinski, A.P., Wang, C., Lin, L., and Ng, W.J. (2014). “Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment”; chemosphere, in press, DOI: 10.1016/j.chemosphere.2014.09.007.
3. **Tian, X.**, Trzcinski, A.P., Wang, C., Lin, L., and Ng, W.J. (2014). “Insights on the solubilization products after combined alkaline and ultrasonic pre-treatment of sewage sludge”; Journal of Environmental Sciences, in press, DOI: 10.1016/j.jes.2014.07.024.
4. **Tian, X.**, Trzcinski, A.P., Lin, L.L., and Ng, W.J. (2015). “Impact of ozone assisted ultrasonication pre-treatment on anaerobic digestibility of sewage sludge”; Journal of Environmental Sciences, accepted paper.

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List of Symbols

ALK	Alkaline
BMP	Biochemical methane potential
BI	Biodegradability increase
COD	Chemical oxygen demand
CST	Capillary suction time
DD	Disintegration degree
EEM	Excitation emission matrix
EPS	Extracellular polymeric substances
FA	Fulvic acid
FI	Fluorescence intensity
HA	Humic acid
MW	Molecular weight
PS	Primary sludge
RR	Recycle ratio
RI	Refractive index
SCOD	Soluble chemical oxygen demand
SEC	Size exclusion chromatography

SRT	Solids retention time
SMP	Soluble microbial products
TOC	Total organic carbon
TS	Total solids
TSS	Total suspended solids
TWAS	Thickened waste activated sludge
UF	Ultrafiltration
ULS	Ultrasonication
ULS+ALK	Ultrasonication+alkaline
ULS-Ozone	Ultrasonication-ozonation
VFAs	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge

Chapter 1 Introduction

1.1 Background

Anaerobic digestion is a commonly used technology for sewage sludge stabilization in a wastewater treatment plant. Aside from the solids stabilization, biogas is produced during the anaerobic digestion process. However, anaerobic sludge digestion is a slow process with solids retention time (SRT) around 20 to 30 days (Appels et al., 2008). Hydrolysis of particulate organics in sludge is the rate-limiting step in sludge digestion (Eastman and Ferguson, 1981, Pavlostathis and Giraldo-Gomez, 1991). Sludge is often treated before it is fed into an anaerobic digester, which is known as pre-treatment, to solubilize the solids and hence accelerate the subsequent anaerobic digestion.

Ultrasonication (ULS) has been reported as an effective sludge pre-treatment process (Tiehm et al., 1997, Tiehm et al., 2001). The collapse of cavitation bubbles during ULS imposes substantial hydro-mechanical shear force on the particulate matters in sludge. This mechanical force breaks up biological flocs, ruptures microbial cells and results in solubilization of intracellular and extracellular polymers. In spite of its advantages, ULS pre-treatment has limitation because it is essentially “single” effect - mechanical disintegration (Lehne et al., 2001, Khanal et al., 2007). Chemical methods have been attempted to enhance the effectiveness of ULS pre-treatment by introducing another disintegration mechanism (Gronroos et al., 2005, Liu et al., 2008, Kim et al., 2010, Xu et al., 2010, Gong et al., 2015). Among the reported chemical methods, ozone and alkaline (ALK) treatments were found to be relatively effective and can induce synergistic effects when combined with ULS pre-treatment.

In comparison with individual ULS pre-treatment, greater sludge solubilization and methane yields from anaerobic sludge digestion can be achieved after ozone assisted ULS pre-treatment (Xu et al., 2010, Yang et al., 2012, Yang et al., 2013) and ALK assisted ULS pre-treatment (Chiu et al., 1997, Wang et al., 2005, Jin et al.,

2009, Kim et al., 2010). However, there is lack of fundamental knowledge on the characteristics of the solubilized compounds after the aforementioned pre-treatments. In addition, the synergistic mechanisms between the chemical methods (i.e. ozone and ALK) and ULS pre-treatment need to be further investigated.

Alternatively, anaerobic sludge digestion can be enhanced with post-treatment where digested sludge is treated and recycled to the original anaerobic digester. Takashima et al. (1996) indicated pre-treatment targets not only slowly biodegradable but also easily biodegradable solids in feed sludge. As a result, some of the energy and chemical input during pre-treatment step is wasted on solubilizing the easily biodegradable organic particulates in feed sludge without increasing the overall sludge biodegradability. In contrast, post-treatment more specifically targets the slowly biodegradable solids and recalcitrant compounds in digested sludge. In view of such potential advantages, treatment of digested sludge and thereafter digesting the treated digested sludge again can be an alternative for more economical improvement of anaerobic digestion performance (Nielsen et al., 2010, Takashima et al., 1996). However, ULS, ozone assisted ULS and ALK assisted ULS treatments have not been applied as post-treatments in previous literature. Consequently, information on their corresponding impact on digested sludge solubilization as well as the overall anaerobic digestion performance is not readily available.

1.2 Objectives and scope

The objective of this project is to provide a comprehensive evaluation on the effects of ULS, ozone assisted ULS and ALK assisted ULS treatments in two treatment configurations (i.e. pre-treatment and post-treatment). The limitation of individual ULS pre-treatment is first discussed in Chapter 4 based on its impact on sludge disintegration and biodegradability improvement. The ozone and ALK treatments are then combined with ULS to improve the effectiveness of ULS pre-treatment in Chapters 5 and 6, respectively. The possible synergistic effects and the

corresponding synergisms in the combined pre-treatments shall also be presented in these chapters. Afterwards, the impact of ULS, ozone assisted ULS and ALK assisted ULS pre-treatments on anaerobic digestion performance are investigated with semi-continuous anaerobic digesters in Chapter 7. Lastly, the effects of ULS, ozone assisted ULS and ALK assisted ULS post-treatments on sludge properties and anaerobic digestion performance of semi-continuous reactors are elaborated in Chapter 8 and 9, respectively. In addition, a detailed comparison between the impact of the aforementioned pre-treatments and post-treatments on anaerobic digestion performance is presented in Chapter 9.

Chapter 2 Literature Review

2.1 Composition of sewage sludge

Sewage sludge consists of two sludge streams which are primary sludge (PS) and waste activated sludge (WAS). Information on these two types of sludge is listed in Table 2-1. PS is collected from the primary sedimentation tank which contains 2-8% readily settleable solids with 60-80% organic content (Davis, 2011). WAS is collected from the secondary clarifier after the activated sludge process. Total solids (TS) content of WAS is around 0.5-2% with 60-85% organic content (Davis, 2011). PS is superior to WAS in terms of anaerobic digestibility (Barber, 2014). PS consists mainly of more easily degradable organics ; whereas, WAS consists mainly of intact biological flocs which is hard to degrade (Davis, 2011, Barber, 2014). The organics in WAS exist in the form of extra- and intra- cellular substances which are well protected by the sludge matrix from being hydrolyzed.

Table 2-1 Sewage sludge composition (Davis, 2011)

Sludge category	Production sites	Sludge composition and characteristics
Primary sludge	Sedimentation tank	Readily degraded organics TS: 2-8% VS: 60-80% of TS
Waste activated sludge	Secondary clarifier after activated sludge process	Microorganism flocs TS: 0.5-2% VS:60-85% of TS

2.2 Anaerobic digestion of sewage sludge

PS and WAS are problematic due to their high organic contents and noxious odor. Sludge stabilization is needed to reduce the organic fractions, odors as well as amounts (Bitton, 2005). Anaerobic digestion is a widely accepted process to treat the sludge for safe disposal. It has the advantage of high stabilization degree, low residual sludge production, no aeration requirements and generation of methane which can be used as an energy source (McCarty, 1964). Anaerobic digestion is a biological process which converts organics into methane and carbon dioxide with different bacteria populations in a certain sequence. The fundamentals, drawbacks

and possible solutions to the drawbacks of anaerobic sludge digestion will be introduced in the following paragraphs.

2.2.1 Fundamental biochemistry of anaerobic digestion

Anaerobic digestion is generally divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis steps (Rittman and McCarty, 2001, Bitton, 2005). Each step is dominated by different microorganisms and these are hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria and methanogens, respectively.

Hydrolytic bacteria can be either facultative or obligate anaerobic bacteria (Zehnder, 1988). They either secrete exoenzymes or have hydrolase attached to their cell walls (Kaseng et al., 1992). With the aid of hydrolase, biopolymers such as carbohydrates, proteins and lipids can be hydrolyzed into smaller organics. The products of hydrolysis are further used by acidogenic bacteria to produce short-chain organic acids as intermediates or direct to acetate for methanogens (Deublein and Steinhauser, 2008). This step is known as acidogenesis. Acetogenesis is the continuation of acidogenesis where acetogenic bacteria convert the intermediates (e.g. butyrate and alcohols) into acetate, hydrogen and carbon dioxide. The products from acetogenesis are converted to methane by methanogens according to two mechanisms: cleavage of acetate and oxidation of hydrogen by carbon dioxide. Jeris and McCarty (1965) have shown that around 70% of methane production originates from acetate cleavage and the rest is formed via hydrogen oxidation.

2.2.2 Bottleneck in anaerobic sludge digestion and possible solutions

Despite the advantages of anaerobic sludge digestion, it is a slow process with SRT around 20 to 30 days (Appels et al., 2008). As anaerobic digestion consists of four sequential steps, it is rate limited by the slowest step among all the steps (Hill, 1977). It has been widely recognized that hydrolysis of particulate organics limits the anaerobic sludge digestion rate (Eastman and Ferguson, 1981, Pavlostathis and Giraldo-Gomez, 1991).

In order to overcome this bottleneck, sludge treatment is often applied to solubilize the solids in sludge to accelerate the rate of hydrolysis and so enhance the overall anaerobic digestion performance. The treatment configurations are categorized into pre-treatment and post-treatment according to the treatment location.

Figure 2-1 Process configuration of sludge pre-treatment

Pre-treatment is the most commonly used treatment method and is applied to the feed sludge before it is fed into the anaerobic reactor as shown in Figure 2-1. Sludge pre-treatment has been first reported by Stuckey and McCarty (1978). Thereafter, different pre-treatment methods have been vastly investigated such as mechanical (Lehne et al., 2001, Baier and Schmidheiny, 1997), alkaline (ALK) (Rajan et al., 1989, Ray et al., 1990, Mendez et al., 2002, Kim et al., 2003), and thermal (Stuckey and McCarty, 1984, Li and Noike, 1992). In addition, pre-treatments with different disintegration mechanisms have also been combined for a higher effectiveness, such as ALK assisted thermal (Valo et al., 2004, Vlyssides and Karlis, 2004) and ALK assisted mechanical (Jin et al., 2009, Cho et al., 2014) pre-treatments.

Figure 2-2 Process configuration of sludge post-treatment

Alternatively, post-treatment can also be integrated to anaerobic sludge digestion to improve its performance. Post-treatment is realized by treating the digested sludge and recycling the treated digested sludge back to the original anaerobic reactor as shown in Figure 2-2. The concept was first proposed by Gossett et al. (1982). They found the performance of thermal treatment was more efficient when the substrate (i.e. municipal refuse) was prior biodegraded than the situation where the thermal treatment was directly applied to the substrate (Gossett et al., 1982). The post-treatment was first integrated to anaerobic sludge digestion as an alternative to pre-treatment by Takashima et al. (1996). Thereafter, other post-treatments, such as ALK (Li et al., 2013) and ozone (Battimelli et al., 2003) post-treatments, have also been reported. There are also papers showing post-treatment as superior to pre-treatment in terms of improving the anaerobic digestion effectiveness (Rivero et al., 2006, Takashima, 2008). Despite the potential advantages, the number of reports in the literature is relatively limited.

2.2.3 Treatment methods selected in this study

Ultrasonication (ULS) is one of the most commonly applied treatment techniques. It is a fast, effective and easily retrofittable process (Foladori et al., 2010). In addition, many successful full-scale applications have been reported in the literature (Hogan et al., 2004, Xie et al., 2007). Researches have pointed out that effectiveness of ULS treatment could be further improved when it was assisted with chemical methods (Kim et al., 2010, Xu et al., 2010). Ozone and ALK treatments are two commonly used chemical methods to enhance the ULS treatment. Synergistic effects had also been observed due to the positive interactions between different disintegration mechanisms (Kim et al., 2010, Xu et al., 2010). Therefore, ULS, ozone assisted ULS and ALK assisted ULS treatments will be investigated in this study.

2.3 Introduction to individual treatments

2.3.1 Ultrasonication treatment

2.3.1.1 Disintegration mechanisms

Ultrasound imposes sono-chemical, thermal, and mechanical effects on the sludge (Doulah, 1977, Riesz et al., 1985, Leighton, 1994). Impact of these three mechanisms varies from the ultrasonic frequency applied. ULS treatment is generally conducted at low ultrasonic frequency (around 20 kHz) because higher chemical oxygen demand (COD) was reported to be solubilized under lower ultrasonic frequencies at the same ULS duration (Tiehm et al., 2001). Mechanical effect is the predominant disintegration mechanism for low frequency ULS sludge treatment because easily collapsed transient cavitation bubbles are preferentially formed during low frequency ULS process (Tronson et al., 2002). Wang et al. (2005) further confirmed the predominance of the mechanical effects by showing the insignificance of the sono-chemical and thermal effects at 20 kHz ULS. However, it should be noted researchers have reported the thermal effect (i.e. rise

in temperature) may increase the soluble chemical oxygen demand (SCOD), although the increase was significantly lower than the SCOD increase induced by the mechanical disruption (Chu et al., 2001, Liu et al., 2009).

Gallipoli and Braguglia (2012), however, found high frequency ULS treatment (200 kHz) was more effective than low frequency ULS treatment (20 kHz) in increasing the sludge SCOD when the specific energy was over 30,000 kJ/kg TS. This is because the sono-chemical effect induced by high frequency ULS process could chemically solubilize organic solids which could not be mechanically disintegrated under low frequency ULS treatment (Gallipoli and Braguglia, 2012). However, in many cases, the applied specific energy input was lower than 30,000 kJ/g TS (Bougrier et al., 2005, Bougrier et al., 2006, Erden and Filibeli, 2010) where the low frequency ULS was more effective in terms of COD solubilization. Therefore, the ULS treatment in this study will also be conducted under low frequency of 20 kHz.

2.3.1.2 Quantification units

Different quantifications of ULS energy input have been reported in the literature. Power input (W) and ULS time (s) are direct expressions (Tiehm et al., 1997, Chu et al., 2001). However, these quantification units have obvious limitations, as volume and concentration of the sludge to be treated could heavily influence the ultrasonication performance (Wang et al., 2005, Akin, 2008). The most commonly reported quantification unit is specific energy input (J/g TS) which takes the sludge volume, sludge concentration and ultrasonic energy input into consideration. Therefore, the specific energy input will be used in this study to quantify the ULS energy input.

2.3.2 Ozonation treatment

2.3.2.1 Disintegration mechanisms

Ozonation is a widely reported chemical sludge treatment technology. Ozone chemically reacts with the sludge and destroys microorganism cell components (Chu et al., 2009, Yan et al., 2009). It is also capable of solubilizing the extracellular polymeric substances and breaking down the solubilized complex macromolecules (Yan et al., 2009). In addition, ozone is able to convert refractory organic matters into biodegradable forms (Volk et al., 1993, Nishijima et al., 2003). Ozonation is a fast process without resulting in extra sludge production compared to other chemical pre-treatments. However, ozonation may induce mineralization which converts organic carbon into inorganic carbon dioxide (Ahn et al., 2002, Lee et al., 2005). Therefore, a proper ozone dosage needs to be selected.

2.3.2.2 Quantification units

Many papers quantified the ozonation treatment in terms of ozonation time (s) (Zhang et al., 2009, Xu et al., 2010). Use of ozonation time allows calculation of energy input when power of the generator was given. However, the ozone production efficiency varied among different devices and the applied ozone dosage was not available. In addition, sludge concentration also has impact on ozonation performance. Therefore, the more reasonable ozone quantification unit would be the ozone dosage which is the ozone mass received by each gram of total solids ($\text{g O}_3/\text{g TS}$) (Goel et al., 2003a, Erden and Filibeli, 2011). A number of papers also quantified the ozone as ozone received per gram TSS ($\text{g O}_3/\text{g TSS}$) (Yan et al., 2009). However, since ozone reacts with both suspended and soluble solids, use of ozone received per gram TS is more reasonable. Therefore, the ozone dosage will be used in this study to quantify ozonation.

2.3.3 Alkaline treatment

2.3.3.1 Disintegration mechanisms

ALK treatment, a chemical treatment process, has been reported for sludge treatment since late 1970s (Stuckey and McCarty, 1978, Ray et al., 1990). Addition of alkali provides large quantity of hydroxyl ions and increases sludge pH. This creates a hypotonic environment under which microorganism cells cannot withhold a proper turgor pressure and lyzed (Neyens et al., 2003). Hydroxyl ions react with the lipids and proteins components in the cell wall and membrane, inducing cell lysis (Neyens et al., 2003). In addition, organic solids in sludge become increasingly negatively charged with increase in pH (Katsiris and Kouzeli-Katsiri, 1987). De-agglomeration of biological flocs happens due to the electrostatic repulsion leading to solubilization of some extracellular polymers (Neyens et al., 2003). Anaerobic sludge digestion can be enhanced due to the aforementioned disintegration mechanisms. However, addition of alkali was accompanied by the increase of dissolved solids concentration (e.g. sodium ions) which may have negative impact on the subsequent anaerobic digestion (Feijoo et al., 1995).

2.3.3.2 Quantification units

The most commonly used ALK treatment quantification unit is alkali concentration (mol/L) (Ray et al., 1990, Chiu et al., 1997, Lin et al., 1997, Li et al., 2012, Şahinkaya and Sevimli, 2013). In some studies, the alkali dosage (g alkali/g TS) was also provided by converting the alkali concentration according to the sludge concentration (Park et al., 2012, Seng et al., 2010). However, this term is not used as commonly as the alkali concentration in literature. pH is another generally used quantification unit (Liu et al., 2008, Kim et al., 2010, Hu et al., 2009). However, even at the same applied alkali concentration, pH may vary depending on the sludge alkalinity and original sludge pH. Furthermore, it has been shown that sludge pH changed after alkali addition due to the reaction between hydroxyl ions

and sludge components (Kim et al., 2010). Therefore, the applied alkali concentration will be used in this study to quantify the ALK treatment and the applied alkali dosage will be provided as well.

2.4 Pre-treatment

2.4.1 Individual ultrasonication pre-treatment

2.4.1.1 Sludge solubilization

The hydro-mechanical force caused by ULS solubilizes the particulate organics in sludge. SCOD, the most commonly used chemical indicator, is often used to quantify the sludge solubilization. SCOD increased after ULS pre-treatment as a result of the solubilization of organic solids. Apart from SCOD, disintegration degree (DD) is also commonly used to quantify the disintegration extent (Muller et al., 1998). This term allows comparison of different sludges. As increase in DD and SCOD is due to COD solubilization, the discussion based on these two terms will not be differentiated. It was calculated as follows (Muller et al., 1998):

$$DD = (SCOD_T - SCOD_0) / (SCOD_{NaOH} - SCOD_0) \times 100\%$$

Where, $SCOD_T$ is the soluble chemical oxygen demand (SCOD) of treated sample, $SCOD_{NaOH}$ is the SCOD of sample immersed in 1 mol/L NaOH (1:1, V/V) at 90 °C for 10 min and $SCOD_0$ is the SCOD of the untreated sample.

Lehne et al. (2001) suggested that the maximum DD caused by ULS treatment should be around 50%. This is because ULS, a mechanical process, could maximally solubilize half of the organics as ALK treatment does (Lehne et al., 2001). This is in good agreement with the latter studies where the maximum DD after the ULS pre-treatment was around 50% (Bougrier et al., 2005, Erden and Filibeli, 2010). This is a potential drawback of the ULS pre-treatment as some of the organics cannot be mechanically solubilized. In addition, SCOD would initially increase fast with increasing specific energy input but the COD solubilization rate

would become lower after a certain energy input (Bougrier et al., 2005, Naddeo et al., 2009, Erden and Filibeli, 2010). This is due to the exhaustion of easily disruptable solids or lack of gas bubbles for cavitation bubble formation (Khanal et al., 2007). These aforementioned observations showed the limitations of ULS pre-treatment in sludge solubilization due to its individual disintegration mechanism.

2.4.1.2 Influence on anaerobic digestion

A summary of the effects of ULS pre-treatment on anaerobic digestion is as shown in Table 2-3. Tiehm et al. (1997) reported that ULS treatment of feed sludge improved anaerobic sludge digestion performance. In a semi-continuous reactor at SRT of 22 days, VS reduction increased from 45.8% to 50.3% after the ULS pre-treatment. Moreover, the ULS pre-treatment could shorten the SRT without decreasing the solids removal efficiency of the anaerobic reactor. For example, 47.3% VS removal rate was achieved following anaerobic digestion of ULS treated feed sludge at SRT of 12 days which was higher than the VS removal rate following anaerobic digestion of untreated feed sludge at a longer SRT of 22 days (45.8% VS removal). Wang et al. (1999) further investigated the effects of ULS pre-treatment with batch anaerobic digestion tests. Soluble proteins and carbohydrates first increased during anaerobic digestion of untreated feed sludge as a result of the enzymatic hydrolysis of the particulate organics. However, the biopolymers had already been solubilized in ultrasonicated feed sludge and were directly utilized since the beginning of the anaerobic digestion (Wang et al., 1999). In addition, VS destruction increased from 27% to 30%, 32%, 37%, and 39% and methane production increased from 200 mL CH₄/g VS_{added} to 220 mL CH₄/g VS_{added}, 250 mL CH₄/g VS_{added}, 320 mL CH₄/g VS_{added}, and 330 mL CH₄/g VS_{added} after 10 minutes, 20 minutes, 30 minutes and 40 minutes ULS pre-treatments, respectively (Wang et al., 1999). Nickel and Neis (2007) found that the improvement in biogas production and VS removal induced by ULS treatment of feed sludge was higher when the reactor was operated at a shorter SRT. For example, biogas production increased by 16% following anaerobic digestion of

ULS treated feed sludge at SRT of 8 days; while, the same ULS treatment condition only resulted in 11% increase in biogas production when the SRT was 16 days. Braguglia et al. (2012) suggested that high frequency (200 kHz) to be an alternative to conventional low frequency (~ 20kHz) sludge disintegration because high frequency ultrasonication is able to sonochemically disintegrate the solids that cannot be mechanically disintegrated. VS reduction and biogas production increased by 35% and 40% respectively following anaerobic digestion of the high frequency ultrasonicated feed sludge at specific energy input of 25,000 kJ/kg TS. However, no comparison had been provided between the low frequency and high frequency ULS at the same specific energy input.

Bougrier et al. (2005) demonstrated the importance of sludge solubilization on the subsequent biogas recovery by anaerobically digesting the supernatant and pellet of the untreated and ultrasonicated feed sludge. Biogas production was higher from the supernatant of the ultrasonicated feed sludge than from the supernatant of the untreated feed sludge (Bougrier et al., 2005). However, no obvious difference in biogas production was observed between the pellet of the ultrasonicated and untreated feed sludge (Bougrier et al., 2005). Bougrier et al. (2006) observed a linear relationship between SCOD and methane production for ultrasonicated feed sludge. This observation allows prediction of sludge anaerobic biodegradability with SCOD data. However, it should be pointed out that some solids in sludge cannot be mechanically solubilized according to Lehne et al. (2001). Therefore, the methane production improvement caused by the ULS treatment was limited by its capability of sludge solubilization. In order to overcome this limitation, chemical methods can be used to assist ULS sludge treatment. Among the tested chemical methods in literature, ozone and ALK treatments are relatively effective and can induce synergistic effects when combined with ULS pre-treatment (Kim et al., 2010, Xu et al., 2010). Therefore, the effects of ozone assisted ULS pre-treatment and ALK assisted ULS pre-treatment will be introduced in the following sections.

Table 2-2 Summary of influence of ultrasonication pre-treatment on anaerobic sludge digestion

Reference	Sludge characteristics	Ultrasonication conditions	Anaerobic digestion conditions	Results
Tiehm et al. (1997)	PS and WAS	31 kHz Time: 64 s 3.6 kW	Semi-continuous Control (SRT: 22 d) Test (SRT: 22, 16, 12 and 8 d)	VS reduction: Control: 45.8% Test: 50.3% (SRT: 22 d) Test: 49.3% (SRT: 16 d) Test: 47.3% (SRT: 12 d) Test: 44.3% (SRT: 8 d)
Wang et al. (1999)	TWAS (TS: 3.3-4%)	9 kHz 2 W/mL Time: 10, 20, 30, 40 min	Batch (12 d, 36 ± 1 °C)	10 min: CH ₄ (+14%), VS reduction (+10%) 20 min: CH ₄ (+45%), VS reduction (+18%) 30 min: CH ₄ (+60%), VS reduction (+37%) 40 min: CH ₄ (+65%), VS reduction (+44%)
Tiehm et al. (2001)	WAS (TS: 2.59%)	41 kHz Time: 7.5, 30, 60, 150 min	Semi-continuous (SRT: 8 d, 37 °C)	7.5 min: CH ₄ (+3.7%), VS reduction (+5.6%) 30 min: CH ₄ (+21.4%), VS reduction (+27.0%) 60 min: CH ₄ (+40.1%), VS reduction (+46.0%) 150 min: CH ₄ (+55.4%), VS reduction (+56.7%)
Bougrier et al. (2006)	TWAS (TS: 20 g/L)	20 kHz, 0.45 W/mL 6,250, 9,350 kJ/kg TS	Batch (24 d, 37 °C)	6,250 kJ/kg TS: CH ₄ (+47%) 9,350 kJ/kg TS: CH ₄ (+51%),
Nickel and Neis (2007)	WAS (TS: 0.5-4%)	31 kHz, 3600 W Time: 90 s	Semi-continuous (SRT: 16 and 8 d, 37 °C)	SRT 16 d: Biogas (+11%), VS reduction (+37%) SRT 8 d: Biogas (+16%), VS reduction (+41%)
Xie et al. (2007)	PS and TWAS (TS: 1-2%)	20 kHz, 3000 W Time: 1.5 s	Full-scale (SRT: 30 d)	Biogas (+45%)
Neis et al. (2008)	TWAS (25% treated)	31kHz	Full-scale	Biogas (+30%) VS reduction (+28.6%)
Pérez-Elvira et al. (2009)	WAS (TS<0.5%)	20kHz, 100W Time: 16s	Continuous (SRT: 20 d)	Biogas (+36.4%) VS reduction (+25%)

Apul and Sanin (2010)	WAS	24kHz 0.51W/mL Time: 10,15, 20 min	Batch (48 d, 36 ± 1 °C)	10 min: CH ₄ (+6.4%) 15 min: CH ₄ (+17.9%) 20 min: CH ₄ (+1.3%)
Erden and Filibel (2010)	BS (TS: 2.14%)	20 kHz 0.0864 W/mL 9,690 kJ/kg TS	Batch (35 d, 36 ± 1 °C)	Biogas (+ 44%)
Braguglia et al. (2012)		200kHz, 0.3-0.33 W/mL 25,000 kJ/kg TS	Batch (20 d, 37 °C) F/I ratio:0.5 and 0.1	F/I=0.5: Biogas (+40%), VS reduction (+9%) F/I=1: Biogas (+3.7%), VS reduction (+34.8%)

F/I: feed to inoculum ratio

BS: Biological sludge

PS: primary sludge

WAS: waste activated sludge

2.4.2 Ozone assisted ultrasonication pre-treatment

2.4.2.1 Sludge solubilization

Xu et al. (2010) first combined ULS and ozonation to treat feed sludge. They found the combined ULS and ozone treatment induced higher SCOD solubilization than individual ULS and ozone treatments did. The SCOD in the ULS, ozone and combined treated feed sludge were 797, 2,347 and 3,341 mg/L, respectively (Xu et al., 2010). Xu et al. (2010) investigated the influence of the combination sequence and found simultaneous combination of ULS and ozone induced slightly higher COD solubilization than ozone followed by ULS. However, the sequence where ULS was conducted prior to ozonation has not been investigated in that work. Yang et al. (2012) used response surface methodology to predict the optimum combination condition for WAS solubilization. However, influence of the selected ozone dosage range on the response (i.e. SCOD) was not significant. It is possible that the ozone dosage had little impact on sludge solubilization during the combined treatment. However, it is also possible that the selected range in that work was either too high or too low such that its corresponding impact on the response was not obvious. Yang et al. (2013) provided insights into the solubilized organics using excitation emission matrix (EEM) fluorescent spectroscopy after ULS, ozone and ozone assisted ULS treatments of feed sludge. They found the fluorescence intensities (FI) of proteins, simple amino acids and humic acid (HA)-like substances in the combined treated feed sludge were higher than those in the ULS and ozone treated feed sludge (Yang et al., 2013).

Although synergistic COD solubilization had not been observed in the study of Xu et al. (2010) or Yang et al. (2012), synergistic VSS solubilization was observed in Yang et al. (2013). This suggested combination of ULS and ozone for treatment of feed sludge resulted in extra solids solubilization than the sum of the solids

solubilization induced by the individual ULS and individual ozone treatments at the same conditions. Xu et al. (2010) suggested that ULS treatment enhanced the ozone mass transfer during the combined treatment. Yang et al. (2012) speculated that ULS enhanced the decomposition of ozone into hydroxyl ions and the ozone bubbles on the other hand enhances the cavitation effects caused by the ultrasound. However, these possible synergisms are based on scientific postulations, but are not supported with experimental data in the respective studies. Therefore, these information gaps need to be addressed in this study.

2.4.2.2 Influence on anaerobic digestion

In addition to sludge solubilization, combined ULS and ozone pre-treatment was found to improve anaerobic biodegradability of the feed sludge. Anaerobic biodegradability of feed sludge increased by 93% and 106% after 30 minutes and 45 minutes of combined pre-treatment (Xu et al., 2010). However, long treatment time might induce negative effect on sludge biodegradability. For example, 120 minutes of the combined pre-treatment only induced 75% increase in the sludge anaerobic biodegradability. The possible reasons were formation of refractory compounds and mineralization of organics due to the ozone overdose.

Aside from the aforementioned report, information on the anaerobic digestion of the combined treated feed sludge was limited. In addition, no previous studies had investigated the anaerobic digestion of combined ULS and ozone treated feed sludge with continuous anaerobic digestion reactors. Consequently, information on the influence of SRT and organic loading rate on biogas production and solids removal following anaerobic digestion of the combined treated feed sludge was not available. In addition, the combined treatment would also have impact on the residual soluble organics in the anaerobic digested sludge, especially the formation of refractory compounds due to ozonation. Insights into the residual organics in the digested sludge would be useful in understanding the influence of the combined pre-treatment on the anaerobic system. However, the relevant information is not

available in literature and needs to be investigated.

2.4.3 Alkaline assisted ultrasonication pre-treatment

2.4.3.1 Sludge solubilization

Use of alkali to improve the performance of ULS pre-treatment has been introduced by Chiu et al. (1997). They demonstrated that combined ULS and ALK pre-treatment induced higher COD solubilization than individual ULS and ALK pre-treatments. Wang et al. (2005) showed that higher SCOD solubilization was obtained at higher pH at the same ULS time. Similar observations were reported by Li et al. (2010).

Jin et al. (2009) compared the combination sequence in terms of COD solubilization. They found the simultaneous combination of ULS and ALK pre-treatment resulted in higher COD solubilization than sequential combination of ULS and ALK treatment (Jin et al., 2009). Kim et al. (2010) found that the combined ULS and ALK pre-treatment resulted in synergistic sludge disintegration. This indicated the combined pre-treatment induced higher COD solubilization than the sum of individual pre-treatments at the same conditions. It was hypothesized that addition of alkali made the microbial cells more vulnerable to ULS pre-treatment and thus increased COD solubilization (Kim et al., 2010, Jin et al., 2009). Şahinkaya and Sevimli (2013) also observed synergistic effects after the combined pre-treatment. They suggested that ULS pre-treatment could release more biopolymers which became more effectively degraded by hydroxyl ions (Şahinkaya and Sevimli, 2013). Although many synergistic mechanisms have been suggested, no concrete evidence has been provided in literature. In addition, fewer insights have been provided into the characteristics of the solubilized compounds after the combined pre-treatment. Therefore, more research is needed to shed light on the solubilization products as well as the synergistic mechanisms.

2.4.3.2 Influence on anaerobic digestion

Combined ULS and ALK treatment of feed sludge enhanced the subsequent anaerobic digestion performance. Kim et al. (2010) found that the methane production improved by around 55% along with 17% increase in VS reduction after combined ULS and ALK pre-treatment (pH 9 + 7000 kJ/kg TS). Seng et al. (2010) investigated the influence of SRT on the anaerobic digestion of ULS and ALK assisted ULS treated feed sludge. For all the tested SRTs (10, 15 and 25 days), anaerobic digestion of the combined treated feed sludge resulted in greater methane production and higher VS removals than anaerobic digestion of the ULS treated and untreated feed sludge (Seng et al., 2010). The VS reduction (24.8%) during anaerobic digestion of the combined treated feed sludge at SRT of 15 days was higher than the VS reduction during anaerobic digestion of the control sludge (19.6%) and ultrasonicated sludge (22.2%) at SRT of 25 days (Seng et al., 2010). This indicated that the ALK assisted ULS treatment of feed sludge could shorten the SRT from 25 days to 15 days without decreasing the VS removal rates during anaerobic digestion.

In addition to the improvement in anaerobic digestion effectiveness, ALK assisted ULS treatment of feed sludge also solubilized more compounds which might have negative impact on the anaerobic digestion system. For example, Kim et al. (2010) suggested soluble nitrogenous species increased by 20% due to the enhanced anaerobic digestion (from degradation of proteins solubilized by ALK assisted ULS pre-treatment) and this may impose burden for the subsequent nitrogen removal step. In addition, most of the soluble nitrogen was ammonia which could be inhibitory to anaerobic digestion process (Kim et al., 2010). Apart from the potential of ammonia-nitrogen formation, refractory organics may also accumulate in the digested sludge as a result of the enhanced solubilization. However, relevant studies are not available in the past literature and this information gap needs to be closed.

2.4.4 Soluble compounds characterization

It has been mentioned in Section 2.4.1.2 that the compounds in feed sludge solubilized after pre-treatment are directly related to the subsequent anaerobic digestion effectiveness. Therefore, an understanding of the nature of the solubilized compounds would be essential. Proteins and carbohydrates are two most commonly reported solubilization products which contributed to the COD solubilization (Wang et al., 1999, Wang et al., 2006b, Kim et al., 2010). These biopolymers are released from the solubilization of extracellular and intracellular polymeric substances in biological flocs during the pre-treatment (Wang et al., 2006b). However, the proteins and carbohydrates results were measured chemically and there may be interference by other compounds. Furthermore, these measurements only provided general characteristics without providing other detailed information, such as molecular weights (MW) of the solubilized compounds. Eskicioglu et al. (2006) indicated the importance of the MW and found the anaerobic biodegradability and biodegradation rate of the soluble organics highly related to their MWs.

In addition, Yang et al. (2013) and Luo et al. (2013) demonstrated the feasibility of using EEM fluorescent spectroscopy to characterize the solubilization products after pre-treatment. The EEM fluorescent spectroscopy has the advantage of a fast and accurate process and it is not influenced by other interferences (Luo et al., 2013). Fluorescent characteristics of the solubilized compounds could be a good supplement to the proteins and carbohydrates measurements. In addition, Luo et al. (2013) also indicated the EEM fluorescent spectroscopy was able to characterize change in soluble organics in the digested sludge after pre-treatment. Despite the advantages of the aforementioned techniques, these have not been widely applied to characterize the solubilization products in the ULS, ozone assisted ULS and ALK assisted ULS treated feed sludge and the residual soluble organics in the digested sludge following anaerobic digestion of the aforementioned treated feed sludge.

2.5 Post-treatment

As introduced in Section 2.2.2, post-treatment is treatment of the digested sludge and recycling the treated digested sludge back to the original anaerobic reactor. The rationale for post-treatment and pre-treatment is similar, wherein both aim to rupture the microbial cells and release the extra- and intra- cellular substances in sludge. However, studies on post-treatment are relatively limited in numbers compared with those on pre-treatment. Takashima et al. (1996) first used post-treatment process to enhance the anaerobic digestion of WAS. They observed the TSS removal rate of an anaerobic digestion system increased from 61% to 100% after 0.1 mol/L ALK post-treatment at 175 °C (Takashima et al., 1996). Ozonation has also been investigated to enhance the anaerobic sludge digestion as post-treatment (Battimelli et al., 2003, Goel et al., 2003b, Yasui et al., 2005). Afterwards, thermal and thermochemical post-treatments have also been tested to improve the gas as well as the solids removal rate during the anaerobic digestion process (Rivero et al., 2006, Takashima, 2008, Nielsen et al., 2010, Takashima and Tanaka, 2010). Li et al. (2013) improved the biogas recovery from anaerobic digestion of sewage sludge with ALK post-treatment. However, to the best of our knowledge, no previous work studied the influence of ULS, ozone assisted ULS and ALK assisted ULS post-treatments on anaerobic sludge digestion. Therefore, the literature review was conducted based on other reported post-treatment methods. The works conducted previously and the questions raised during application of other post-treatment methods will be introduced in the following sections. This would be helpful in designing the experiments of the ULS, ozone assisted ULS and ALK assisted ULS post-treatments which are to be investigated in this study. Furthermore, reports comparing pre-treatment and post-treatment in previous literature will also be summarized.

2.5.1 Sludge solubilization

Post-treatment solubilizes microbial cells and other organic solids in digested

sludge, resulting in COD solubilization and VSS reduction. Takashima and Tanaka (2008) found thermal treatment at 170 °C for one hour increased the SCOD in digested sludge from 0.59 to 8.64 g/L and reduced the VSS concentration in digested sludge from 14.4 to 9.2 g/L. Combination of chemical treatment methods (ozone, oxygen, Na₂CO₃, HCl and H₂O₂) with thermal treatment further improved the solubilization of digested sludge (Takashima and Tanaka, 2008). However, the SCOD increase was not related to the VSS reduction after the treatments of the digested sludge. For example, combining thermal treatment with 0.01 and 0.05 g O₃/g VS treatment decreased the VSS concentrations in digested sludge from 14.4 to 8.6 and 6.2 g/L, respectively; while, the corresponding SCOD concentrations in digested sludge increased from 0.59 to 8.83 and 8.81 g/L which were similar after the two treatment conditions (Takashima and Tanaka, 2008). The most effective thermochemical post-treatment in terms of COD solubilization was when 6.0 g/L Na₂CO₃ was added during the thermal treatment (170 °C, one hour) which increased the SCOD in digested sludge from 0.59 to 12.7 g/L (Takashima and Tanaka, 2008). However, the most effective post-treatment in terms of VSS solubilization was when 34 g H₂O₂/L treatment was applied together with the thermal treatment which decreased the VSS concentration in digested sludge from 14.4 to 5.2 g/L (Takashima and Tanaka, 2008).

Takashima (2008) compared the effects of thermal pre-treatment and thermal post-treatment (120 °C, one hour) in terms of COD solubilization. The thermal post-treatment increased the SCOD in digested sludge from 0.71 to 3.92 g/L; while the net SCOD increase in feed sludge was higher (from 4.26 to 8.56 g/L) after the thermal pre-treatment (Takashima, 2008). This is because the feed sludge (for pre-treatment) had higher TCOD at 46.7 g/L; while the TCOD of the digested sludge (for post-treatment) was only 28.9 g/L which left fewer organic particulates to be solubilized. Nielsen et al. (2010) compared moderate temperature thermal treatment (80 °C, 10 hours), high temperature thermal treatment (130 °C and 170 °C, 15 minutes) and ALK assisted high temperature thermal treatment (170 °C + pH 10) on solubilization of feed sludge and digested sludge. The decrease in VSS/VS was

more obvious for the digested sludge than that for the feed sludge at all the tested treatment methods (Nielsen et al., 2010). This indicated the thermal and ALK assisted high temperature thermal treatments were more effective when used as post-treatment than as pre-treatment in terms of solubilizing the organic solids. Furthermore, they found the high temperature thermal post-treatment was more effective than the moderate temperature thermal post-treatment (Nielsen et al., 2010). The maximum VSS/VS ratio in digested sludge decreased from 94% to 69% after the moderate thermal post-treatment for 10 hours (Nielsen et al., 2010). However, the VSS/VS decreased significantly from 95% to 71% and 57% after 130 °C, 170 °C thermal post-treatments for only 15 minutes, respectively (Nielsen et al., 2010). In addition, they found the ALK treatment could further enhance the thermal treatment in terms of sludge solubilization. The VSS/VS ratio decreased to from 95% to 29% when the 170 °C thermal treatment was conducted at pH 10 (Nielsen et al., 2010).

Li et al. (2013) tested the influence of ALK treatment on digested sludge. SCOD increased from 490 to 1,630 mg/L after the digested sludge had been treated at 0.1 mol/L NaOH concentration for 30 minutes (Li et al., 2013). In addition to COD solubilization, soluble polysaccharides in digested sludge increased from 72 to 136 mg/L due to the cell lysis (Li et al., 2013). However, they found the soluble proteins in digested sludge only increased from 2.2 to 4.4 mg/L after the ALK post-treatment (Li et al., 2013). As the main cell component, the increase in soluble proteins due to the ALK post-treatment was relatively low in comparison to the increase in soluble polysaccharides. This is because during ALK treatment proteins might be broken down to amino acids and small peptides that could not be detected by the coomassie brilliant blue method used in that study (Li et al., 2013).

Apart from biopolymers solubilization, formic, acetic and propionic acids were also detected after thermal treatment of digested sludge although they only contributed to a minor part of the SCOD increase (Takashima and Tanaka, 2008). Li et al. (2013) also observed increase in VFAs concentration after ALK treatment

of digested sludge and suggested the VFAs contained in acidogenic bacteria were released after the post-treatment process. In addition, Li et al. (2013) also pointed out that the VFAs could also be formed due to the alkalisation of the glycerol and fatty acids during the ALK treatment. Similar to treatment of feed sludge, insights into the solubilization products after treatment of digested sludge remained at the biochemical level, and information on the MW distribution and fluorescent characteristics would be helpful in understanding the nature of the solubilized compounds.

2.5.2 Influence on anaerobic digestion

2.5.2.1 Batch anaerobic digestion test

Takashima and Tanaka (2008) investigated the anaerobic biodegradability of thermal and chemically assisted thermal treated digested sludge with batch anaerobic digestion test. The thermal post-treatment (170 °C) increased the anaerobic biodegradability of digested sludge by around 60%; while, using chemical methods (Na_2CO_3 , HCl, H_2O_2 and O_3) to assist the thermal post-treatment did not improve the methane recovery from the digested sludge compared to the individual thermal post-treatment (Takashima and Tanaka, 2008). Besides, combination of 34 g H_2O_2 /L or 0.05 g O_3 /g VS chemical treatment with the thermal treatment decreased the methane production from the digested sludge (Takashima and Tanaka, 2008). Similarly, Nielsen and co-workers (2010) found thermal (170 °C) and ALK assisted thermal (170 °C + pH 10) treated digested sludge had similar anaerobic biodegradability, although the ALK assisted thermal treated digested sludge had higher VS solubilization than the thermally treated digested sludge. This is normally related to the nature of the solubilized compounds. For example, solubilization of refractory or toxic compounds may not necessarily improve the sludge digestibility. Takashima and Tanaka (2014) suggested soluble color compounds formed due to acid-thermal treatment of digested sludge may not be biodegradable which resulted in a low methane recovery from the anaerobic

digestion. This emphasized the importance of understanding the nature of solubilized compounds as well as their effects on the subsequent anaerobic digestion.

2.5.2.2 Continuous anaerobic digestion test

Post-treatments improved biogas production and solids removal efficiency from anaerobic sludge digestion as summarized in Table 2-3. Post-treatment performance varied from studies due to the difference in sludge, treatment methods and operating conditions. For example, the ALK assisted thermal and individual ozonation post-treatments only enhanced the solids removal rate during anaerobic digestion but did not have impact on the methane production from the anaerobic reactor (Takashima et al., 1996, Battimelli et al., 2003); while the combined thermal and oxidative post-treatment did increase the methane production by around 40% (Rivero et al., 2006).

Table 2-3 Summary of various post-treatments on anaerobic sludge digestion

References	Post-treatment condition	Anaerobic digestion condition	Improvement in anaerobic digestion ^c
(Takashima et al., 1996) ^b	0.1 mol/L NaOH, 175 °C, 1h	RR: 1%	+63.9% VSS removal
(Battimelli et al., 2003)	0.16 g O ₃ /g TS	α : 15%	+41.7% COD
			+11.1% SS removal
		α : 25%	+83.3% COD
			+50% SS removal
		α : 50%	+19.4% COD
(Yasui et al., 2005) ^a	24 kg O ₃ /day		-36.1% SS removal
		α : 100%	+75% COD
			-5.5% SS removal
			+30% CH ₄
		SRT: 45 days, RR: 2-4%	
(Rivero et al., 2006)	2 g H ₂ O ₂ /g VSS 2 g H ₂ O ₂ /g VSS at 90 °C	SRT: 30 days, RR: 20%	+10% VSS removal
			+40.6% CH ₄
(Takashima, 2008)	120 °C, 1h		+60.4% VSS removal
		SRT: 20 days, RR: 5%	+21.4% CH ₄
(Li et al., 2013) ^b	0.1 mol/L NaOH, 30 min		+18.7% VS removal
		SRT: 20 days, RR: 5%, 10% and 15%	+33% biogas (RR: 5%)
(Takashima and Tanaka, 2014) ^a	170 °C, 1h, pH 5-6	SRT: 20 days, α : 30%	+14-21% CH ₄
			+200-250% VSS removal

^a: Digested sludge was separated with a liquid/solid separation device and recycled the solid-rich fraction to extend the SRT

^b: Post-treatment was conducted after 12 hours of feed (24 hours as one cycle)

^c: the increase in gas production (methane or biogas) was not reported when the increase was not obvious

α : ratio between recycled sludge to inflow sludge

RR: ratio between recycled sludge to total working volume

Recycle rate has also been recognized as an important factor which has impact on the anaerobic digestion process. Higher recycle rate during post-treatment is known to reduce the amount of active anaerobic microorganisms in anaerobic digester (Battimelli et al., 2003, Li et al., 2013). However, the investigation on the effects of recycle rate on the overall anaerobic digestion process was limited. Battimelli et al. (2003) have shown COD and SS removal rates started to decrease when the ratio between recycled sludge rate to the inflow sludge rate (α) exceeded 25% with integration of ozonation (0.16 g O₃/g TS) post-treatment. They suggested that lower recycle rate ($\alpha < 25\%$) did not provide enough easily biodegradable substrate and did not make a difference to the anaerobic reactor; while, higher recycle rate reduced the activity of anaerobic bacteria and decreased the anaerobic digestion effectiveness (Battimelli et al., 2003). Li et al. (2013) also investigated the

influence of recycle rate for post-treatment. However, in their study, the recycle rate was expressed as the ratio of recycled sludge to the total working volume (RR: recycle ratio) which was different from Battimelli et al. (2003). As these two terms were inter-changeable, the corresponding α value is also calculated for comparison. Li et al. (2013) observed 33% increase in biogas production when ALK treated (0.1 mol/L NaOH, 30 minutes) digested sludge was recycled at RR of 5% (α : 100%) at SRT of 20 days. However, they also observed biogas production started to decrease when the RR increased to 10% (α : 200%) and 15% (α : 300%) (Li et al., 2013). According to Li et al. (2013), higher RR resulted in more inactivation of the anaerobic microorganisms which had negative impacts on the anaerobic digestion or might even fail the anaerobic digestion. The two aforementioned studies suggest the importance of an appropriate recycle rate and indicated the potential negative effects of higher recycle rate. However, it should be noted that the influence of RR on the anaerobic digestion performance is treatment method and operating conditions dependent. For example, Rivero et al. (2006) observed increase in biogas production with integration of combined thermal and oxidative post-treatment when the anaerobic reactor was operated at RR of 20%. In addition to RR, SRT of the anaerobic digester should have impact on the performance of post-treatment. However, previous research only tested the post-treatment at a fixed SRT but rarely investigated the influence of SRT on the performance of anaerobic reactors with post-treatment integrated. Therefore, the influence of RR and SRT on the reactors with integration of ULS, ozone assisted ULS and ALK assisted ULS post-treatments will be investigated in this study.

In a number of studies, digested sludge was thickened prior to post-treatment (Goel et al., 2003b, Takashima and Tanaka, 2014). The advantage of this configuration is the energy can concentrate on the solids in the digested sludge. Takashima (2008) also suggested recycling of concentrated digested sludge increased the biomass amount which compensated for the inactivation of anaerobic microorganisms due to the treatment of digested sludge. However, this also increased the amount of recalcitrant organics in the anaerobic digestion system. Takashima and Tanaka

(2014) pointed out the persistent solids would accumulate in the anaerobic reactor if the digested sludge was prior thickened, because these persistent solids could not be removed via sludge wasting. In addition, integration of the centrifugation step also increased the complexity, capital and operational cost of the overall process. Therefore, this will not be investigated in this work.

2.5.2.3 Comparison between post-treatment and pre-treatment

Rivero et al. (2006) tested the oxidative (2 g H₂O₂/g VSS) and the thermal assisted oxidative (2 g H₂O₂/g VSS at 90 °C) treatments when they were applied to feed sludge as pre-treatment and applied to digested sludge as post-treatment. No obvious improvement in daily methane production was observed due to the integration of oxidative pre-treatment (+2%) and oxidative post-treatment (no obvious change) (Rivero et al., 2006). Nevertheless, the VSS removal rate increased by 31.7% and 10% due to the integration of oxidative pre-treatment and post-treatment, respectively (Rivero et al., 2006). The thermal assisted oxidative post-treatment induced, however, better anaerobic digestion performance than the thermal assisted oxidative pre-treatment did. Daily methane production decreased by 41.1% due to the integration of the thermal assisted oxidative pre-treatment; while, 40.6% increase in daily methane production was observed when the thermal assisted oxidative treatment was integrated in the recycle line (Rivero et al., 2006). The improvements in VSS removal rate after anaerobic digestion were similar when the thermal assisted oxidative post-treatment (+60.4%) and pre-treatment (+58.1%) were applied (Rivero et al., 2006). Takashima (2008) compared the effectiveness of thermal pre-treatment (SRT: 20 days, 120 °C) and thermal post-treatment (SRT: 20 days, 120 °C, RR: 5%). The methane production increase due to post-treatment (+21.4%) was higher than that due to pre-treatment (no obvious change) (Takashima, 2008). In addition, the improvement in VS destruction after anaerobic digestion due to the post-treatment (+18.7%) was superior to that due to the pre-treatment (+12.7%) (Takashima, 2008). However, these two studies are the only available studies comparing pre-treatment and post-treatment. Therefore,

more studies are needed to compare other treatment methods and in different treatment configurations.

2.6 Objectives

The aim of this thesis is to provide a comprehensive evaluation on the effects of ULS, ozone assisted ULS and ALK assisted ULS treatments in different treatment configurations (i.e. pre-treatment and post-treatment). Performance of each treatment will be first evaluated based on their effects on sludge solubilization and sludge anaerobic biodegradability. Their impacts on the anaerobic digestion of sewage sludge will also be determined with semi-continuous anaerobic digesters in terms of biogas production, solids removal rate and properties of the digested sludge. More detailed objectives are shown as follows:

- To understand the limitations of individual ULS pre-treatment
- To determine the synergistic mechanisms in ozone assisted ULS and ALK assisted ULS pre-treatments
- To provide insights into the solubilization products after ozone assisted ULS and ALK assisted ULS pre-treatments and to evaluate their impact on the digestibility of the pre-treated sewage sludge
- To investigate the impact of the aforementioned pre-treatments on anaerobic digestion of sewage sludge with semi-continuous reactors at different SRTs
- To provide information on the solubilization products after ULS, ozone assisted ULS and ALK assisted ULS treatments of digested sludge as well as their influences on the biodegradability of the digested sludge
- To evaluate the impact of the aforementioned post-treatments on anaerobic digestion of sewage sludge with semi-continuous reactors at different SRTs and RRs
- To understand the impact of treatment configurations on the effectiveness of the aforementioned treatments

Chapter 3 Methodology

3.1 Sludge samples for treatment

Mixtures of primary sludge and thickened waste activated sludge (ratio around 1:1 based on dry solids) were collected from a local municipal used water treatment plant for pre-treatment purpose. The primary source of the wastewater is municipal wastewater and the solid residence time of the activated sludge tank is 2.5 days. The waste activated sludge is first thickened by centrifuge with the aid of polymer before mixing with the primary sludge.

The mixture sludge was collected every three weeks and stored in a cold room with a constant temperature of 4 °C for experiments. The sludge was sampled at similar total solids (TS) concentration in range of 14.5-17.2 g/L to minimize variations among experiments. A table of the feed sludge characteristics is shown in Table 3-1. Anaerobically digested sludge was taken from 1 L laboratory scale semi-continuous anaerobic reactors for post-treatment purpose. The anaerobic reactors were fed with the aforementioned sludge mixture and operated at SRT of 10 and 20 days.

Table 3-1 Main characteristics of the tested sewage sludge sample

Parameter	Value range
Total solids (g L ⁻¹)	14.5-17.2
Volatile solids (g L ⁻¹)	11.8-13.4
Total suspended solids (g L ⁻¹)	13.2-15.9
Volatile suspended solids (g L ⁻¹)	11.2-13.3
Total COD (mg L ⁻¹)	16,800-25,000
Soluble COD (mg L ⁻¹)	400-1,200
Soluble proteins (mg L ⁻¹)	< 100
Soluble carbohydrates (mg L ⁻¹)	< 50
pH	6.0-6.5

3.2 Physical-chemical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured in accordance with standard methods (APHA, 1998). Soluble and total COD were measured based on the closed reflux

colorimetric method described in standard methods (APHA, 1998). Protein concentration was determined with the Lowry's method (1951) using bovine serum albumin (Sigma, US) as standard and a UV spectrophotometer (Shimadzu, UV-1800) against a blank at wavelength of 750 nm. Carbohydrate concentration was determined with the sulfuric-phenol method (DuBois et al., 1956) using D-Glucose (Merck, Germany) as standard with a UV spectrophotometer (Shimadzu, UV-1800) against a blank at wavelength of 485 nm. The soluble samples were obtained by first centrifuging the sludge at 10,000 rpm for 10 minutes. The supernatant was then filtrated through 0.45 µm syringe filters for SCOD, proteins and carbohydrates analysis. Dewaterability was measured in accordance with the standard methods in terms of capillary suction time (CST) (APHA, 1998).

3.3 Sludge solubilization quantification

3.3.1 SCOD increase

Sludge solubilization was quantified in terms of SCOD increase ($SCOD_+$) which was the SCOD difference before and after the treatment as shown in:

$$SCOD_+ = SCOD_{after} - SCOD_{before}$$

3.3.2 Disintegration degree

Sludge disintegration degree was also used to express the extent of sludge solubilization. It was calculated as follows (Muller et al., 1998):

$$DD = (SCOD_T - SCOD_0) / (SCOD_{NaOH} - SCOD_0) \times 100\%$$

Where, $SCOD_T$ is the soluble chemical oxygen demand (SCOD) of treated sample, $SCOD_{NaOH}$ is the SCOD of sample immersed in 1 mol/L NaOH (1:1, V/V) at 90 °C for 10 min and $SCOD_0$ is the SCOD of the untreated sample.

3.3.3 Volatile suspended solids solubilization

VSS solubilization was also employed to quantify the solubilization of organic

solids as follows:

$$\text{VSS solubilization} = \frac{VSS_b - VSS_a}{VSS_b} \times 100\%$$

Where VSS_b stands for the VSS concentration before each pre-treatment; VSS_a stands for the VSS concentration after each pre-treatment.

3.4 Analytical methods

3.4.1 pH

Sludge pH was measured with a pH meter (Agilent, model 3200P) with an accuracy of 0.001. Buffer solutions of pH 4, pH 7 and pH 10 (Fisher, UK) were used to calibrate the pH meter before measurement.

3.4.2 Particle size distribution

Particle size distribution was measured with a particle size analyzer (Shimadzu, model SALD-3101). One mL sludge samples were used for the measurement. A laser light source was emitted from a semiconductor light source, diffracted by the particles in the sample and then collected on a photo sensor. Sizes and numbers of the particles were measured according to the diffracted laser signal. Median and mean diameters were used to quantify the particle size distribution.

3.4.3 Total organic carbon analysis

Total organic carbon (TOC) was analyzed with a Multi N/C 2100S (Jena, Germany). A sample was prepared by first centrifuging sludge at 10,000 rpm for 10 minutes. The supernatant was then filtrated through a 0.45 μm membrane syringe filter to remove the small particulates before injection. One mol/l hydrochloric acid was used to acidify the sample to remove the inorganic carbon fraction. The sample was then combusted at 950 $^{\circ}\text{C}$ and the produced carbon dioxide was detected with a focus radiation non-dispersive infrared sensor.

3.4.4 VFAs analysis

A gas chromatograph (Agilent Technologies 7890A GC system) with a 30m×320µm×0.5µm column and flame ionization detector was used for VFAs analysis. The sample was prepared by first centrifuging sludge at 10,000 rpm for 10 minutes. Supernatant was filtrated through a 0.2 µm membrane syringe filter to remove the small particulates. 0.1 mL diluted formic acid (10%) was added into 0.9 mL sample to acidify the sample before measurement.

3.4.5 Biogas composition

Gas composition was analyzed with a gas chromatograph (Agilent Technologies 7890A GC system) with two thermal conductivity detectors. Five columns were installed for determination of CH₄, CO₂ and H₂. They are: (1) an Agilent HayeSep R 0.9 m X 1/8" X 2.0 mm packed column, (2) an Agilent HayeSep C 3.0 m X 1/8" X 2.0 mm packed column, (3) an Agilent MolSieve 5A 3.0 m X 1/8" X 2.0 mm packed column, (4) an Agilent HayeSep Q 0.9 m X 1/8" X 2.0 mm packed column, (5) an Agilent MolSieve 13X 3.0 m X 1/8" X 2.0 mm packed column.

3.4.6 Size exclusion chromatography

A high performance liquid chromatograph (Agilent Technologies, 1260 LC system) was used for the MW distribution analysis using the PL aquagel-OH 8µm MIXED-M column. Milli-Q water was used as mobile phase with a flow rate of 1 mL/min. Calibration was done using polyethylene glycol and polyethylene oxide standards with molecular weight of 500 kDa, 70 kDa, 4 kDa, 600 Da and 106 Da with the refractive index (RI) detector. Both RI and UV (254 nm) detector were used for the detection of the eluted substances. The sample was first centrifuged at 10,000 rpm for 10 minutes. The supernatant was then filtered through a 0.2 µm membrane filter for injection.

3.4.7 Apparent molecular weight distribution

Apparent MW distribution of soluble organics was obtained via ultrafiltration (UF) fractionation as described by Eskicioglu et al. (2006). The UF process was conducted in a manufactured dead-end stirred cell (370mm × 120mm). Supernatant was fractionated into different MW ranges with polyethersulfone UF membranes (Sartorius, Germany) with molecular weight cut-offs of 300 kDa, 30 kDa and 5 kDa, respectively.

3.4.8 Fluorescent spectroscopy analysis

A fluorescence spectrometer (LS 55, Perkin Elmer, USA) was used to measure the fluorescence intensity (FI) of the soluble fluorescent products. The measurement procedure was previously described by Wu et al. (2011). Excitation was from 230 to 520 nm with 5-nm intervals. Emission was collected from 230 to 550 nm with 5-nm increments. Samples were pre-diluted with DI water to avoid the measured FI exceeding the maximum level. The compounds were identified based on their Ex and Em wavelengths as reported in the literature. Peaks of simple protein-like substances such as tyrosine proteins appeared in the Ex/Em range of Ex < 250 nm, Em < 350 nm (Determann et al., 1994, Chen et al., 2003). Fulvic acid (FA)-like substances were detected in Ex/Em range of Ex < 250 nm, Em > 380 nm (Determann et al., 1994, Chen et al., 2003, Her et al., 2003). Peaks of SMP-like substances fell within the Ex/Em range of Ex: 250-280 nm, Em < 380 nm (Determann et al., 1994, Coble, 1996, Baker, 2001, Chen et al., 2003). HA-like substances were detected within Ex/Em range of Ex > 250 nm, Em > 380 nm (Determann et al., 1994, Coble, 1996, Mobed et al., 1996, Her et al., 2003).

3.5 Treatment methods

Ultrasonication (ULS) was performed with an ultrasonicator (Misonix, Q700) as shown in Figure 3-1. The ultrasound frequency was 20 kHz with maximum power input at 700 Watts. ULS energy was quantified in terms of specific energy input

and the calculation is as follows (Lehne et al., 2001):

$$\text{Specific energy input} = (P \times t) / (V_{\text{sludge}} \times \text{TS})$$

where, P (W) is power input of the ultrasonicator, t (sec) is the time of ULS, V_{sludge} (L) is volume of treated sludge. During ULS, temperature of the sample was monitored and maintained at about 30 °C with an ice-water bath.

Figure 3-1 Picture of ultrasonicator

Ozonation was performed with an ozone generator (Wedeco, GSO 30) as shown in Figure 3-2. Pure oxygen was used as feed gas and converted to ozone with a high voltage converter. The power input of the ozone generator was 180 Watts. The applied ozone dosage was quantified according to the potassium iodide method (Konsowa, 2003).

Figure 3-2 Picture of ozone generator

The flow of ozone generation process is as shown in Figure 3-3. The ozonation was conducted in a one litre glass bottle ozone reactor and a stone diffuser was installed to produce fine ozone bubbles and enhance mass transfer. A glass bottle was placed before the reactor to avoid backflow of sludge into ozone generator. Two glass bottles filled with potassium iodide (KI) were placed after the ozone reactor to remove the residue ozone. Optimum combination sequence of ULS and ozonation was determined before commencing the main experiment. The Ultrasonication-Ozonation (ULS-Ozone) sequence was performed by applying ozonation after ULS and the Ozonation-Ultrasonication (Ozone-ULS) sequence was conducted by dosing ozone prior to ULS.

Figure 3-3 Demonstration of sludge ozonation process

NaOH was selected for ALK pre-treatment due to its reported higher impact on sludge (Kim et al., 2003). Sodium hydroxide pellets were dissolved to make a 3 mol/L stock solution. Designated NaOH concentrations were achieved by adding different volumes of stock solution to the sludge sample. The sludge samples were then mixed at 200 rpm for 10 minutes at room temperature (25 °C). The ULS and ALK treatments were combined simultaneously (ULS+ALK) because the simultaneously combined treatment is reported to induce higher COD solubilization than the sequentially combined treatments (i.e. ULS-ALK and ALK-ULS) (Jin et al., 2009). The ALK and ULS+ALK treated samples were neutralized with hydrochloric acid before they were used for anaerobic digestion.

3.6 Anaerobic digestion

3.6.1 Batch anaerobic digestion test

Sludge anaerobic biodegradability was quantified with biochemical methane potential (BMP) assays in accordance with the modified method described by Owen et al. (1979). Hereby, the biodegradability is defined as the methane production from each gram of COD added into the reactor. Anaerobic digested sludge taken from a full-scale anaerobic reactor at a local wastewater treatment plant was degassed at 35 °C for one week before it was used as inoculum. Ten mL of substrate sludge (untreated and treated feed sludge) and 30 mL of degassed inoculum were used to assess the change in sludge anaerobic biodegradability after pre-treatment; while, 50 mL of substrate sludge (untreated and treated digested sludge) and 50 mL of degassed inoculum were used to determine the change in sludge anaerobic biodegradability due to post-treatment. After the sludge addition, a mixture of 20% CO₂ and 80% N₂ was used to purge each serum bottle for three minutes to create an anaerobic environment. The bottles were then capped with septum and aluminum rings and incubated in an incubator shaker at 35 °C. The biogas volumes were regularly measured using a wetted glass syringe. The biogas composition was determined as described in Section 3.4.5.

3.6.2 Semi-continuous anaerobic digestion test

Semi-continuous anaerobic digestion tests were conducted in 1.2 L glass bottles with 1 L working volume at 35 °C. An example of the anaerobic digestion is as shown in Figure 3-4. Biogas was collected with a Tedlar gas bag. The biogas volume was measured daily with a gas meter (Ritter, Germany). Sludge in the reactor was replaced (i.e. addition and withdrawn) daily. However, sludge transfer schemes were different between pre-treatment and post-treatment. The methods would be described in the following sections.

Figure 3-4 Example of a semi-continuous anaerobic digester

3.6.2.1 Pre-treatment

One litre of seed sludge, which was taken from a continuous anaerobic digester with SRT of 28 days from a local used water treatment plant, was fed into each reactor before starting the experiment. 100 and 50 mL sludge aliquots were removed daily from the reactors and replaced with the same amount of feed sludge

to obtain SRT of 10 and 20 days, respectively. The reactors operating at 10 days SRT were referred to as Control10, ULS10, ULS-Ozone10 and ULS+ALK10 as these received the untreated, ULS treated, ULS-Ozone treated and ULS+ALK treated sewage sludge as feed, respectively. Similarly, the reactors with 20 days SRT were referred to as Control20, ULS20, ULS-Ozone20 and ULS+ALK20. The feed sludge had been purged with nitrogen gas for one minute before feeding into the anaerobic reactors. Each reactor was run for three SRTs so that process stability may be assumed. Feed sludge in storage was changed every three weeks. Each batch of feed sludge was manually adjusted to keep a consistent TS concentration of around 15 g/L, although COD of the batches did vary slightly. Daily biogas production was normalised by dividing daily gas production by amount of COD fed into the reactor.

3.6.2.2 Post-treatment

One litre of seed sludge, which was taken from a continuous anaerobic digester with SRT of 28 days from a local reclamation plant, was fed into each reactor before starting the experiment. Three experiment reactors testing the ULS post-treatment (ULS reactor), ULS-Ozone post-treatment (ULS-Ozone reactor), and ULS+ALK post-treatment (ULS+ALK reactor) were run together with a control reactor (i.e. without integration of post-treatment). Similar to pre-treatment, 100 and 50 mL sludge aliquots were daily discarded from the reactors and replaced with the same amount of feed sludge to obtain SRT of 10 and 20 days, respectively. However, for the experimental reactor (i.e. with integration of post-treatment), more sludge was withdrawn and recycled for post-treatment as shown in Table 3-1. The treated digested sludge had been purged with nitrogen gas for one minute before recycling to the anaerobic reactors. The RR was calculated as the ratio of the volume of recycled sludge to the total working volume of the anaerobic reactor as defined by Li et al. (2013). Before starting the post-treatment tests, all the reactors were operated at SRT of 10 days without conducting post-treatment for 20 days to stabilize the reactor and to obtain similar reactor performance. Afterwards, the

anaerobic reactors were tested at three different conditions sequentially as shown in Table 3-2.

Table 3-2 Operating conditions of each reactor with post-treatments

	Condition I	Condition II	Condition III
SRT (days)	10	10	20
Volume of daily discarded sludge (mL)	100	100	50
Volume of daily recycled sludge (mL)	50	100	50
RR (%)	5	10	5

Chapter 4 Effects of ultrasonication pre-treatment on sewage sludge

4.1 Introduction

An understanding of the advantages and limitations of individual ultrasonication (ULS) pre-treatment (i.e. treatment of feed sludge) is important if chemical methods are to be used to further improve its performance. In this chapter, change in feed sewage sludge properties, solubilization of COD and biopolymers were used to evaluate the impact of ULS treatment on feed sewage sludge. Molecular weight (MW) distribution analysis and ultrafiltration (UF) fractionation of the supernatant were conducted to provide insights into the MW distribution of the solubilization products. In addition, batch anaerobic digestion assays were conducted to assess the improvement of feed sludge biodegradability after pre-treatment. A first-order model was then used to fit the biodegradability and digestion time to assess the change in the anaerobic digestion rate after the ULS pre-treatment. Part of the results in this chapter has been published in Journal of Environmental Science and Health, Part A.

4.2 Sludge characteristics change

As shown in Figure 4-1 (a), soluble COD, proteins and carbohydrates in feed sludge increased linearly with ULS specific energy input up to 21 kJ/g TS. Solubilization of these biopolymers was good indication of release of extra-cellular intra-cellular polymeric substances. When the specific energy input exceeded 21 kJ/g TS, the solubilization of COD, proteins and carbohydrates became slow with the increasing specific energy input. This showed the limitation of high energy input ULS treatment in sludge solubilization. Khanal et al. (2007) suggested that this limitation is due to the exhaustion of easily disintegrated organics particles and exhaustion of dissolved gas which suppress cavitation bubbles formation. Chu et al. (2001) have shown during ULS the easily breakable materials (e.g. porous biological flocs and bacteria) were first quickly disintegrated and after a certain

ULS specific energy input the effects on sludge disintegration became relatively insignificant. This explained the fast COD solubilization before 21 kJ/g TS. However, the left hardly disintegrated particles would consume more ULS energy to be solubilized afterwards. This resulted in the slow COD solubilization rate when ULS specific energy input exceeded 21 kJ/g TS. In addition, as introduced in Eller (1969) and Zhao et al. (2015), ultrasound wave needs gas cavity to induce cavitation effect when propagating through sludge. With the exhaustion of dissolved gas in sludge, the cavitation effect became relatively inefficient despite the increase in specific energy input.

ULS pre-treatment resulted in significant particle size reduction in feed sludge. Mean and median diameter decreased significantly from 45 μm to 15 μm after 9 kJ/g TS ULS pre-treatment as shown in Figure 4-1 (b). Further increase in ULS specific energy input was not efficient in reducing particle size in feed sludge. Besides, both mean and median diameter increased slightly when the specific energy input was over 21 kJ/g TS. Similar observations have been reported previously as re-flocculation effect due to the cell lysate solubilization (Bougrier et al., 2005, Erden and Filibeli, 2010). Sludge dewaterability is often used as an indicator of the sludge structure integrity because small particle size and high concentration of soluble biopolymers resulted in poor sludge dewaterability (Wang et al., 2006a). As shown in Figure 4-1 (b), CST in feed sludge increased significantly from 125 s to 430 s after 9 kJ/g TS ULS pre-treatment, indicating deterioration of feed sludge dewaterability. Afterwards, the increase in CST with increasing ULS specific energy input became negligible, and the CST reached around 490 s after 63 kJ/g TS ULS pre-treatment. These observations suggested the effects of ULS pre-treatment on disintegrating the feed sludge structure became inefficient after 9 kJ/g TS.

Figure 4-1(a) Change in SCOD, soluble proteins and carbohydrates concentrations in feed sludge with specific energy input after ultrasonication pre-treatment (b) Change in CST, median diameter and mean diameter in feed sludge with specific energy input after ultrasonication pre-treatment (error bars indicate standard deviations)

4.3 Molecular weight distribution

Calibration was conducted with standard polymers as described in Section 3.4.6. A linear relationship was derived between the log value of MW (Da) and retention time (Rt: min) with a correlation coefficient of 99.2% as shown follows:

$$\text{Log}(MW) = 9.8223 - 0.6748(Rt)$$

MW distribution of organics in the supernatant of the control and ultrasonicated feed sludge is as shown in Figure 4-2 (a). MWs of the standard polymers are shown

as dash lines. High MW compounds eluted earlier and had a shorter retention time because they did not go as deep into the gel pores as low MW ones (Trzcinski et al., 2011, Aquino et al., 2006). According to the change in the UV signal response, ULS pre-treatment was able to solubilize compounds with MW over 500 kDa as well as compounds with MW lower than 106 Da. However, it should be noted that the SEC was calibrated with polyethylene glycol and polyethylene oxide which are linear polymers; while, the organics in the sludge supernatant were mainly three-dimensional complex organics such as proteins and carbohydrates. These compounds might have different retention time in the SEC column than the polyethylene glycol and polyethylene oxide which had the same MW as them. Therefore, the SEC results could provide the MW range rather than exact MW of the solubilized compounds. Supernatant of the control and ultrasonicated feed sludge was further fractionated with UF fractionation process to obtain the COD fraction in each MW range. As shown in Figure 4-2 (b), COD fraction of the organic compounds with MW over 300 kDa had the most obvious increase after the ULS treatment of feed sludge. It increased from 15.8% to 21.7%, 40.8% and 43.9% after 3, 9 and 21 kJ/g TS ULS pre-treatment had been applied, respectively. This is because the sludge matrix which consists of high MW extracellular polymeric substances and intra-cellular biopolymers was solubilized due to the ultrasonic disruption (Eskicioglu et al., 2006); while, the solubilization of low MW compounds was relatively insignificant due to lack of chemical effect during ULS. This finding is in accordance with previous results that ULS treatment mainly mechanically breaks the microbial cells in sludge which releases the high MW extra- and intra- cellular substances (Wang et al., 2006b). Therefore, it was reasonable to suggest that increasing ULS specific energy input over 21 kJ/g TS would further increase the fraction of high MW organics (MW > 300 kDa). This would also have influenced the subsequent anaerobic digestion performance.

Figure 4-2 (a) Molecular weight distribution chromatograms of organics in the supernatant of control and ultrasonicated feed sludge (b) COD fraction in different MW ranges in control and ultrasonicated feed sludge

4.4 Anaerobic biodegradability

Feed sludge anaerobic biodegradability (methane production from each gram of COD added into the reactor) was improved after ULS pre-treatment as shown in Figure 4-3 (a). Sludge biodegradability increased with increasing specific energy input. However, ULS treatment of feed sludge at high specific energy input may not be economical. It was found that 9 kJ/g TS ULS pre-treatment improved the feed sludge biodegradability by 14.8%; whereas, when the specific energy input

was 7 times higher (i.e. 63 kJ/g TS), the biodegradability increased by 31.8% which was only 2.2 times higher compared to the improvement induced by 9 kJ/g TS ULS pre-treatment. This could be related to the limitation of high energy input ULS treatment in sludge solubilization as stated in Section 4.2

It has been indicated in previous studies that the sludge biodegradability was related to SCOD in the sludge (Gronroos et al., 2005, Bougrier et al., 2005). Therefore, to evaluate the possibility of using SCOD data to predict change in biodegradability after ULS treatment of feed sludge, the sludge ultimate biodegradability and SCOD concentration were plotted as shown in Figure 4-3 (b). The coefficient of determination (R^2) of the fit is 94.83%, indicating a strong correlation. Based on the fitting equation shown in Figure 4-3 (b), every 1,000 mg/L increase in SCOD concentration could result in 13 mL $\text{CH}_4/\text{g COD}_{\text{added}}$ increase in biodegradability which is consistent with Bougrier et al. (2005) who observed that biogas increase in ultrasonicated sludge originated mainly from the soluble fraction. This meant that increasing SCOD concentration would be an option to further improve the sludge ultimate anaerobic biodegradability.

Figure 4-3 (a) Biochemical methane potential results of control and ultrasonicated feed sludge (Biodegradability: methane production from each gram of COD added into the reactor) (b) Linear fitting between sludge biodegradability and SCOD concentration for control and ultrasonicated feed sludge (error bars indicate standard deviations)

4.5 Anaerobic process kinetic analysis

The first-order model was used to determine the rate-constant of anaerobic digestion with the data obtained (Trzcinski and Stuckey, 2012).

$$Y=Y_{\max}(1 - \exp(k_d t))$$

Where, Y is the biodegradability at time t (mL CH₄/g COD_{added}), k_d is the rate-constant of the anaerobic digestion (day⁻¹) and Y_{max} is the ultimate biodegradability (mL CH₄/g COD_{added}).

The values of the fitted variables and the measured data are compared in Table 4-1. The predicted ultimate biodegradability was consistent with the measured biodegradability and all the values of the coefficient of determination (R²) are higher than 98%. These indicated the obtained data fitted well in the model and the model can be used for predicting process performance. As shown in Table 4-1, the rate-constant “k_d” increased from 0.171 to 0.201 after 9 kJ/g TS ULS pre-treatment, indicating the methane production rate of feed sludge was accelerated after ULS treatment. This indicated easily degradable organics were first solubilized after 9 kJ/g TS ULS specific energy input. Solubilization of these organics not only enhanced the biogas production but accelerated the biogas production rate. However, further increase of specific energy input over 9 kJ/g TS did not further increase the methane production rate. This meant some of the organics solubilized by ULS were slowly biodegradable when the ULS specific energy input was over 9 kJ/g TS. Although these organics could be ultimately digested to methane, longer digestion time was still needed for such conversion. This is possibly related to the increase in COD fraction due to macromolecules with MW over 300 kDa with increasing specific energy input as reported in Section 4.3. It has been shown in the literature that these macromolecules are relatively slowly biodegradable compared to the smaller ones (Eskicioglu et al., 2006). Therefore, solubilization of these high MW compounds after ULS treatment of feed sludge could contribute to a higher methane recovery eventually but did not necessarily further enhance the anaerobic digestion rate.

Table 4-1 Summary of the first-order fitting and measured results for control and ultrasonicated feed sludge

Sludge	First-Order fitting			Measured results	
	$Y_{\max}$ (mL CH ₄ /g COD _{added})	k_d (Day ⁻¹)	R^2 (%)	Biodegradability (mL CH ₄ /g COD _{added})	Increase (%)
Control	191.5 (4.2)*	0.171 (0.012)	99.33	196.9 (1.2)	N/A
9 kJ/g TS	215.6 (6.2)	0.201 (0.020)	98.64	226.1 (1.7)	14.8
15 kJ/g TS	219.5 (5.6)	0.194 (0.017)	98.97	229.1 (4.8)	16.4
21 kJ/g TS	231.2 (5.3)	0.187 (0.014)	99.18	239.0 (0.4)	21.4
42 kJ/g TS	248.0 (5.7)	0.192 (0.015)	99.19	254.5 (2.1)	29.3
63 kJ/g TS	254.2 (5.5)	0.205 (0.016)	99.22	259.5 (1.0)	31.8

*Numbers in bracket stand for standard deviation

4.6 Summary

ULS pre-treatment was able to reduce the particle size and increase the SCOD in feed sewage sludge. However, the effects of ULS pre-treatment on COD solubilization became less obvious when the specific energy exceeded 21 kJ/g TS because of the exhaustion of easily disintegrable organic particles and exhaustion of dissolved gas which then suppressed cavitation bubbles formation. Therefore, to further enhance the sludge solubilization, a chemical method should be used to assist the ULS treatment rather than further enhance the ULS specific energy input. And in the subsequent experiments, the maximum ULS specific energy input would be limited to 21 kJ/g TS beyond which the individual effect of ULS treatment on sludge solubilization became relatively inefficient.

Solubilization products after ULS treatment of feed sludge were found to be high MW extracellular and intracellular polymers substances. The COD fraction of organics with MW over 300 kDa increased with increasing ULS specific energy input.

Feed sludge biodegradability was linearly related to the SCOD concentration after ULS pre-treatment, indicating biodegradability could be improved by further enhancing COD solubilization. Although increase of ULS specific energy input over 9 kJ/g TS further enhanced the sludge anaerobic biodegradability, the anaerobic digestion rate-constant, which is obtained by fitting the biodegradability data with the digestion time, was not further improved with increasing ULS specific energy input.

Chapter 5 Effects of ozone assisted ultrasonication pre-treatment on sewage sludge

5.1 Introduction

The effects of individual ultrasonication (ULS) pre-treatment on sewage sludge have been discussed in Chapter 4. In this chapter, ozonation, a chemical treatment method, was combined with the ULS pre-treatment to further enhance sludge solubilization as well as sludge anaerobic digestibility. Due to the supplementary effects of these two treatments, combination of ULS and ozonation had been suggested to have larger impact on feed sludge disintegration (Xu et al., 2010, Yang et al., 2012, Yang et al., 2013). However, information in the literature on such combination is scarce and the synergistic mechanisms between the two pre-treatments are still ambiguous. This chapter aims to investigate the positive interactions between the two pre-treatments and seek explanations for the synergistic effects. The optimum combination sequence was determined and changes in the sludge characteristics after individual and combined pre-treatments measured. In addition to the conventional parameters such as SCOD for measurement of sludge solubilization, size exclusion chromatography (SEC) measurement was conducted to determine the change in molecular weight (MW) distribution of the soluble organics after each pre-treatment. Batch anaerobic digestion tests were then conducted to evaluate the influence of the pre-treatments on feed sludge anaerobic biodegradability. The results discussed in this chapter have been published in Chemosphere.

5.2 Comparison of ultrasonication and ozonation

For ultrasonicated feed sludge, SCOD₊ increased linearly with specific energy input as shown in Figure 5-1 (a). The SCOD₊ was 3,450 mg/L after 21 kJ /g TS ULS pre-treatment and values did not plateau. However, for ozonated feed sludge, SCOD₊ increased markedly to around 2,400 mg/L at an ozone dosage of 0.02 g O₃

/g TS and then plateaued at around 3,700 mg/L at higher ozone dosages as shown in Figure 5-1 (b). Proteins and carbohydrates concentrations were converted to equivalent COD concentration with factors of 1.5 and 1.07, respectively as described previously (Rittman and McCarty, 2001). Proteins and carbohydrates were responsible for around 80% of the COD solubilization measured after both pre-treatments. The equivalent COD of the solubilized proteins and carbohydrates in feed sludge after 21 kJ /g TS ULS pre-treatment were 2,370 mg/L and 450 mg/L, respectively. The equivalent COD of the solubilized proteins and carbohydrates in feed sludge were 2,500 mg/L and 560 mg/L after 0.08 g O₃ /g TS ozonation, respectively. These are consistent with reported results, showing that ultrasound and ozone solubilized the extra-cellular and intra-cellular biopolymers in sludge (Wang et al., 2006b, Zhang et al., 2009).

Figure 5-1 (a) Change in soluble COD, equivalent COD of proteins and carbohydrates in feed sludge with specific energy input after ultrasonication pre-treatment (b) Change in soluble COD, equivalent COD of proteins and carbohydrates in feed sludge with ozone dosage after ozonation pre-treatment (c) Change in feed sludge pH with specific energy input and ozone dosage (SCOD₊: the SCOD increase induced by pre-treatment) (error bars indicate standard deviations)

In addition, ultrasound and ozone resulted in different changes in pH value. The feed sludge pH remained relatively constant at around 5.9 with increasing specific energy input as shown in Figure 5-1 (c). However, the feed sludge pH decreased

obviously with increasing ozone dosage. The sludge pH dropped from 5.9 to 5.2 after 0.04 g O₃ /g TS ozonation. These results were consistent with past reports and indicated that ultrasound and ozone did not disrupt the sludge in the same way (Bougrier et al., 2006). The pH drop during ozonation treatment was due to the formation of acidic compounds. Yasui and Miyaji (1992) observed the formation of carboxylic acids during human waste ozonation. Bougrier et al. (2006) suggested that the pH decrease was due to the formation of VFAs from the degradation of lipid compounds in sludge. Yan et al. (2009) found that the lactic acid concentration increased after ozonation due to the oxidation of the soluble macromolecules. This difference in the solubilization products after the two pre-treatment processes used would have impact on subsequent anaerobic degradation.

5.3 Combination sequence determination

In order to investigate the interaction between ultrasound and ozone, various combination sequences were tested. The SCOD₊ changes in each sequence are as shown in Figure 5-2 (a) and 5-2 (b). The maximum SCOD₊ values obtained in the ULS-Ozone and Ozone-ULS sequences were around 4,000 and 3,800 mg/L, respectively showing an improved COD solubilization compared to each individual treatment. Such SCOD concentration increase was slightly higher than the maximum SCOD increase (3,300 mg/L) reported in a previous study treating WAS with combined ultrasonication and ozonation process (Xu et al., 2010). Although WAS has been shown to be more susceptible to ULS treatment than mixtures of PS and WAS, a higher solubilization was observed in this study in comparison to Xu et al. (2010). This is because the mixed sludge used in this study had a higher TS concentration (around 17 g/L) than the WAS (around 10 g/L) used in the previous work (Xu et al., 2010). Therefore, more solids were available for solubilization.

Figure 5-2 (a) Change in SCOD₊ with ozone dosage in control and ultrasonicated feed sludge (at various specific ultrasonic energy inputs) in sequence of ULS-ozone (b) Change in SCOD₊ with specific energy input in control and ozonated feed sludge (at various ozone dosages) in sequence of Ozone-ULS (SCOD₊: the SCOD increase induced by pre-treatment) (error bars indicate standard deviations)

Although the SCOD₊ values were similar between the two selected sequences in most of the combination conditions, the Ozone-ULS sequence did not appear to be substantially advantageous because the SCOD₊ increase was marginal during the subsequent ULS step, especially at high prior ozone dosage (e.g. 0.12 g O₃ /g TS) as shown in Figure 5-2 (b). This is because ozone significantly solubilized the solids in sludge and fewer solids were available for the ultrasound attack. This

indicated the ozonation step should be conducted subsequent to the ULS step. Therefore, the ULS-Ozone sequence was chosen for subsequent experiments.

5.4 Sludge solubilization

Prior ULS step did not enhance the COD solubilization induced by ozone. The COD solubilized by ozone decreased with increase in the prior ULS specific energy input as shown in Figure 5-3 (a). For example, the SCOD₊ induced by 0.048 g O₃ /g TS ozonation was 2,600 mg/L for untreated feed sludge but was only 1,500 mg/L and 400 mg/L for feed sludge which had been pre-ultrasonicated at 9 kJ /g TS and 21 kJ /g TS, respectively. This is because changes in SCOD can be due to not only organic solubilization but also degradation of organics to oxidized species such as CO₂. In this work, the total COD of the 9 kJ/g TS ultrasonicated sludge decreased from 24.6 to 23.4, 20.7 and 20.5 g/L after being ozonated by 0.012, 0.024 and 0.036 gO₃/g TS, respectively. Previous works have shown that mineralization and degradation of the soluble organics due to ozone oxidation resulted in SCOD decrease (Ahn et al., 2002, Erden and Filibeli, 2011). In addition, this could also be related to the solubilization of flocculants due to the prior ULS step. It has been indicated in previous studies that cell lysate solubilized by ultrasound could act as flocculants (Ruiz-Hernando et al., 2015, Erden and Filibeli, 2010, Bougrier et al., 2005). Higher prior ULS specific energy input would result in a higher concentration of such flocculants. Colloidal organics solubilized by the ozonation may not be removed by the 0.45 µm filter and contributed to the SCOD concentration when concentration of such flocculants was low. However, when concentration of such flocculants was high, the organics solubilized by the subsequent ozonation may be substantially re-flocculated and removed by the 0.45 µm filter. This would contribute to the lower SCOD solubilization at the same applied ozone dosage when the prior ULS specific energy input was higher.

Figure 5-3 (a) SCOD₊ induced by different ozone dosages in control sludge and ultrasonicated feed sludge (at various specific energy inputs) (b) VSS solubilization induced by different ozone dosages in control sludge and ultrasonicated feed sludge (at various specific energy inputs) (error bars indicate standard deviations)

Foladori et al. (2010) suggested that the VSS could be used as an alternative to represent the particulate organics in sludge sample. The VSS solubilization induced by ozone for both control and pre-ultrasonicated sludge is as shown in Figure 5-3 (b). In contrast with the SCOD results, a greater VSS solubilization due to ozonation was obtained when the prior ULS specific energy input was lower than 12 kJ /g TS. The highest VSS solubilization induced by ozone was 41.3% when

0.048 g O₃ /g TS ozonation was applied after 9 kJ /g TS ULS. The same ozone dosage applied without prior ULS only induced 21.1% VSS solubilization. This implied that ultrasound made the organic solids easily attacked by ozone. Agglomerations of particulates would have become smaller in size after ultrasonic dispersion resulting in a higher specific surface area, and therefore affording a greater probability of contact with ozone. This is important because the half-life of ozone is only 30 minutes (Tian et al., 2014), and is likely much lower in practice as it may react with none target inorganic materials before it has the chance to do so with the target organic solids. Besides, smaller particles are more readily ozonated than bigger ones because of a lower mass transfer resistance (Cesbron et al., 2003). Similar synergistic solids solubilization was reported in a previous study. Yang et al. (2013) reported that combined ULS and ozonation treatment of feed sludge solubilized 6.1% more solids than the sum of solids solubilized by individual ULS and ozonation treatments.

VSS solubilization induced by ozone started to decrease when the prior ULS specific energy was higher than 12 kJ /g TS. This was possibly due to the reaction between ozone and solubilized organics released by the prior ultrasound. Cesbron et al. (2003) showed that the soluble and particulate organics competed for ozone in a sludge ozonation system. During sludge ozonation, soluble organics fixed the thickness of an effective mass transfer film and the thickness of this film is dependent on the concentration of the soluble organics. When diameter of particles are smaller than this film, ozone could react simultaneously with the particles and soluble organics; while when diameter of particles are larger than the film, ozone reacts first with the soluble organics until the thickness of the aforementioned mass transfer film is thin enough (i.e. concentration of soluble organics is low enough) for the ozone to react with the particles and soluble fractions simultaneously. Concentration of soluble organics increased with ULS specific energy input but size of solid organics in sludge did not change much with the ULS specific energy input after 9 kJ/g TS energy ULS pre-treatment as stated in Section 4.2 . The high organics concentration would form a screening effect on the solid organics which

protected the solid organics against the attack by ozone. As a result, ozone would first react with soluble organics before it could react with the solid organics. Therefore, at the same ozone dosage, the corresponding VSS reduction induced by the ozone decreased with the increase in prior ULS specific energy input.

5.5 Molecular weight distribution

MW distributions of organics in the control and treated feed sludge were measured with a calibrated SEC. The calibration equation is shown as described in Section 4.3:

$$\text{Log}(MW) = 9.8223 - 0.6748(Rt)$$

As shown in Figure 5-4, no peak with the same retention time was detected in ultrasonicated and ozonated feed sludge, indicating solubilization of different compounds after both pre-treatments. High MW compounds ($Rt < 6$ min) were found to be the main solubilized compounds after both pre-treatments as shown in both the UV and RI signals. The corresponding MWs of these compounds were over 500 kDa according to the retention time of the largest standard polymer (500 kDa) shown as dash line. Such high MW compounds were reported as cell fragments and extracellular polymers in WAS with MW as high as 10^5 kDa (Pavoni et al., 1972, Namkung and Rittmann, 1986, Schiener et al., 1998, Aquino et al., 2006). It should be noted that RI is a universal detector which detects most of the eluted substances if these are present in sufficient concentrations, while the UV 254 nm detector provides good sensitivity towards aromatic compounds (Trzcinski et al., 2011). For both ultrasonicated and ozonated feed sludge, the detected peaks had similar retention times in both the UV and RI detector signals as shown in Figure 5-4 (a) and 5-4 (b), suggesting most of the solubilized high MW compounds were possibly aromatic compounds. Yang et al. (2013) reported that tryptophan proteins were the main solubilization products after ULS pre-treatment while humic acids (HAs) and fulvic acids (FAs) were the primary solubilization products after ozonation pre-treatment.

In addition, low MW compounds ($8 \text{ min} < Rt < 13.5 \text{ min}$) were also detected in

both treated feed sludge but not as obvious as the high MW ones. MWs of these polymers were lower than 27 kDa according to the aforementioned calibration equation. Peaks with retention time longer than 13.5 min were also detected. These peaks should stand for compounds which are smaller than 106 Da (retention time of the standard polymer is shown as the dash line). However, these compounds were not necessarily organic compounds with MW smaller than 106 Da but small organics around this MW due to the characteristics difference between the detected compounds and standard polymers (i.e. polyethylene glycol and polyethylene oxide) as discussed in Section 4.3. UV response of such compounds was lower in the ozonated sludge than that in the control sludge as shown in Figure 5-4 (a). This decrease in UV response of the simple organic compounds was because of the mineralization effect induced by ozone (Weemaes et al., 2000, Ahn et al., 2002). Simple organic compounds were oxidized into carbon dioxide.

Figure 5-4 MW distribution chromatograms of the soluble substances in control, ultrasonicated (15 kJ /g TS) and ozonated feed sludge (0.06 g O₃ /g TS) in (a) UV signal (254 nm) (b) RI signal

The MW distribution of the soluble organics in the ultrasonicated and the ULS-Ozone treated feed sludge are shown in Figure 5-5 (a) and (b). Organic solids were mainly solubilized in form of low MW components (8 min < Rt < 13.5 min, MW < 27 kDa) as shown by the arrows in Figure 5-5 (b). In addition, the response increase in high MW compounds was not as marked as was observed when sludge was only ozonated as shown in Figure 5-4 (b).

Some of the high MW components released by ULS were found to be very sensitive to ozone attack. The peak which represented high MW components (Rt: 4 min) disappeared after ozonation was applied as shown by an arrow in Figure 5-5 (a). This suggested prior ULS released more organics which could be ozone scavengers. Consequently, the reaction between these soluble organics and ozone

became more pronounced compared to the situation when ozone was directly applied to the untreated feed sludge. It should be borne in mind that organic solids solubilization by ozone was also enhanced in ultrasonicated sludge (≤ 12 kJ /g TS) as shown in Figure 5-3 (b). This meant that the prior ULS step enabled better utilization of ozone by the soluble and particulate organics in feed sludge. This is important because utilization of the applied ozone had significant influence on performance of a sludge ozonation system (Chu et al., 2008, Manterola et al., 2008). Despite the synergistic effects caused by the combined treatment, the potential of refractory compounds formation should not be neglected. Yang et al. (2013) had observed solubilization of HA-like substances when ozone was used to treat a WAS sample. Such solubilization was enhanced when the ULS and ozonation pre-treatment were combined simultaneously (Yang et al., 2013). Therefore, it is reasonable to hypothesize such formation of HA-like substances may also happen when ULS and ozonation were applied sequentially. Macromolecules containing HAs (such as extracellular polymeric substances) were first mechanically released due to the ULS and then chemically degraded to HA-like substances and other compounds due to the ozone oxidation. This might also contribute to the degradation of high MW components (Rt: 4 min) as shown in Figure 5-5 (b). In addition, solubilization of the primary sludge (PS) fraction may also contribute to such formation of refractory products. For example, residual toilet paper fibers or lint commonly found in PS contains lignin which could be degraded into HAs due to the ULS-Ozone pre-treatment.

Figure 5-5 MW distribution chromatograms of the soluble substances in ultrasonicated sludge (9 kJ /g TS) and ultrasonicated feed sludge (9 kJ /g TS) with various subsequent ozone dosages in (a) UV signal (254 nm) (b) RI signal

5.6 Anaerobic digestion

Change in anaerobic biodegradability (methane production from each gram of COD added into the reactor) of the control, ozonated, ultrasonicated and ULS-Ozonated feed sludge during the biochemical methane potential (BMP) assay is shown in Figure 5-6. Biodegradability of the ultrasonicated feed sludge increased in the first 4 days of anaerobic digestion. However, a lag phase was observed for both the ozonated and ULS-Ozonated feed sludge in the first 4 day of the anaerobic

digestion. Their corresponding biodegradabilities in the first 4 day were lower than the untreated feed sludge (i.e. control). This was because the oxidized species generated by ozone had inhibited the methanogens as these are known to thrive at very low redox potentials (Weemaes et al., 2000). After day 7, most of the oxidized species were reduced via acidification and no further inhibition effect was observed. The biodegradability then increased much faster in the ozonated and ULS-Ozonated feed sludge compared to the untreated and ultrasonicated sludge. For all the tested sludge samples, the increase in biodegradability became insignificant after 15 days of anaerobic digestion and reached plateau values after 22 days.

The ULS-ozone treated feed sludge had ultimate biodegradability of 265 mL CH₄ /g COD_{added} which was 34.7% more compared to the untreated sludge (196 mL CH₄ /g COD_{added}). However, the sum of ultimate biodegradability increase (BI) was only 27.7% when ULS (9.9%) and ozonation (17.8%) had been applied at the same conditions. One possibility for this synergistic increase is the synergistic VSS solubilization as mentioned in Section 5.4. Besides, the MW reduction in the sequential combined treatment may also contribute to the synergistic biodegradability increase. Eskicioglu et al. (2006) indicated that the macromolecules with MW higher than 300 kDa were mostly complex cell fragments and HAs which were harder to be anaerobically biodegraded. In this work, the soluble high MW components (MW > 500 kDa) released by ultrasound were effectively degraded by ozone and most of the organic solids were solubilized in form of low MW components (MW < 27 kDa) which benefited the subsequent anaerobic digestion.

Figure 5-6 Biochemical methane potential results for control, ozonated, ultrasonicated and ULS-Ozonated feed sludge (Biodegradability: methane production from each gram of COD added into the reactor) (error bars indicate standard deviations)

However, it should be noted that the increases in methane production were only 10.9%, 6.6% and 15.4% after the ULS, ozonation ULS-Ozone pre-treatments, respectively. These figures were lower than the increases in anaerobic biodegradability for the ozonated and ULS-Ozonated feed sludge. Such difference was because the ozonated and ULS-Ozonated sludge had lower total COD amount than the control and ULS treated feed sludge as a result of the mineralization effect as mentioned in Section 5.4. However, in practical condition, the lower total COD concentration in the feed sludge also indicated sludge could be fed into the anaerobic reactor at a higher flow rate to achieve the same COD loading rate. Nevertheless, it should also be noted that such COD loss also implied some ozone dosage was wasted in converting organics to carbon dioxide rather than to readily biodegradable components. It was also reported in a previous study that high ozone dosage would create negative effect on biogas production (Silvestre et al., 2015). Therefore, a lower ozone dosage was recommended for the subsequent experiment. Reduction of ozone dosage would not only save some energy but also reduce the

loss of biodegradable organics during ozonation. Therefore, a lower subsequent ozone dosage (0.012 g O₃/g TS), at which obvious sludge solubilization was observed, will be tested in the subsequent semi-continuous anaerobic digestion tests.

5.7 Summary

Ultrasound and ozone were found to disrupt the feed sewage sludge differently and induce synergistic effects when sequentially combined. The ULS-Ozone sequence was shown to be advantageous compared to the Ozone-ULS sequence. The ULS-Ozone pre-treatment worked as follows:

- Prior ultrasonication helped disperse the biological flocs in feed sludge and enhanced the reaction between ozone and the organic solids.
- The resulting organic solids were then solubilized by ozone in the form of low MW organics (MW < 27 kDa).
- The concentration increase of high MW compounds (MW > 500 kDa) during the ozonation was relatively insignificant.
- Ozone utilization by the ultrasonicated feed sludge was better compared to non-ultrasonicated sludge. Some of the high MW compounds solubilized by ultrasound were likely ozone scavengers and hence effectively degraded by ozone.
- Synergistic sludge biodegradability increase was observed due to the aforementioned synergistic actions.

Chapter 6 Effects of alkaline assisted ultrasonication pre-treatment on sewage sludge

6.1 Introduction

The effects of ultrasonication (ULS) and ultrasonication-ozonation (ULS-Ozone) pre-treatments on sewage sludge have been introduced in Chapters 4 and 5, respectively. In this chapter, alkaline (ALK) treatment was used to assist the ULS pre-treatment to further improve feed sludge solubilization and sludge anaerobic biodegradability. The reported literature showed COD solubilization during ULS process could be significantly enhanced at higher pH (Chiu et al., 1997, Wang et al., 2005). Synergistic increase in sludge disintegration degree (DD) was observed after the simultaneous combination of ULS and ALK (ULS+ALK) pre-treatment (Kim et al., 2010). Notwithstanding these reports, there is relative lack of fundamental knowledge on the characteristics of the solubilized compounds following such combined pre-treatment process. This chapter aims to shed more light on the solubilization products with size exclusion chromatography and excitation emission matrix fluorescent spectroscopy. The possible synergistic mechanisms for the positive interactions between the ULS and ALK pre-treatments were also discussed. In addition, batch anaerobic digestion tests were then conducted to investigate the influence of the solubilized substances on feed sludge biodegradability. Results reported in this chapter have been published in Journal of Environmental Sciences.

6.2 Comparison of alkaline and ultrasonication treatment

The sludge pH increased from 6.5 to 7.8, 9.7, 12.2 and 12.8 after NaOH concentrations of 0.01, 0.02, 0.05 and 0.1 mol/L had been applied to feed sludge respectively. The corresponding NaOH dosages were 0.025, 0.05, 0.125 and 0.25 g NaOH/g TS, respectively. As shown in Figure 6-1 (a), the SCOD concentrations were 1,300, 1,700, 3,400 and 4,800 mg/L which corresponded to DD of 0.8%,

4.0%, 19.6% and 32.8% at NaOH concentrations of 0.01, 0.02, 0.05 and 0.1 mol/L, respectively. The obvious sludge disintegration at higher NaOH concentration (NaOH > 0.05 mol/L, pH >12.2) was the consequence of chemical induced cell lysis. Alkali addition increased the sludge pH value and created a hypertonic environment in microbial cells. Cell membranes could not withstand the resulting turgor pressure and so lost integrity (Neyens et al., 2003). However, sludge disintegration was not significant (DD < 4%) when the applied NaOH concentration was lower than 0.02 mol/L (pH < 9.7). The SCOD increase was likely supplemented by the de-agglomeration of biological flocs with the increase of pH (Katsiris and Kouzeli-Katsiri, 1987, Hu et al., 2009).

The ULS pre-treatment caused considerable disintegration in feed sludge even at low specific energy input. As shown in Figure 6-1 (b), 6 kJ/g TS ULS treatment was able to increase the SCOD in feed sludge to 2,400 mg/L which corresponded to a DD of 11.7%. The SCOD increased linearly with the specific energy and reached approximately 4,700 mg/L (the corresponding DD: 32.8%) after 21 kJ/g TS ULS pre-treatment.

Figure 6-1(a) Change in SCOD and DD in feed sludge with applied NaOH concentrations after alkaline pre-treatment (b) Change in SCOD and DD in feed sludge with specific energy inputs after ultrasonication pre-treatment (error bars indicate standard deviations)

6.3 Sludge solubilization

Change in feed sludge SCOD concentration after the ULS+ALK pre-treatment is as shown in Figure 6-2 (a). The SCOD increased with increase in both the specific energy input and the applied NaOH concentration. These results were in good agreement with a previous study where the ULS+ALK pre-treatment was conducted in the NaOH concentration range of 0-0.04 mol/L and the ULS specific energy input range of 3.75-15 kJ/g TS (Jin et al., 2009). Wang et al. (2005) also reported the COD solubilization induced by 30 minutes of ULS treatment of feed sludge increased from around 400 to 2,000 mg/L when the sludge pH was increased from 6.8 to 12. The maximum SCOD concentration in feed sludge observed in this study was around 11,000 mg/L when 21 kJ/g TS ULS pre-treatment was combined with 0.1 mol/L ALK pre-treatment. Such SCOD increase

was the result of intracellular and extracellular organics solubilization. The concentrations of soluble proteins and carbohydrates in feed sludge increased significantly after the ULS+ALK pre-treatment as shown in Figure 6-2 (b) and (c). Proteins, the principle components of cells, were solubilized as a result of cell lysis (Wang et al., 2006b, Kim et al., 2010). The solubilization of polysaccharides in cell walls and extracellular polymeric substances (EPS) contributed to the increase in soluble carbohydrates concentration (Wang et al., 2006b). Significant solubilization of proteins due to ULS+ALK pre-treatment was observed by Liu et al. (2008). In their study, around 67% of proteins in a WAS sample was solubilized when ULS pre-treatment was conducted at pH 12 for 60 minutes. The DD increased to 50%, 67%, 80% and 91% when 21 kJ/g TS ULS was conducted at NaOH concentrations of 0.01, 0.02, 0.05 and 0.1 mol/L, respectively as shown in Figure 6-2 (d). 50% sludge disintegration was achieved when 21 kJ/g TS ULS pre-treatment was combined with 0.01 mol/L ALK pre-treatment. This was significantly higher than the DD induced by individual 21 kJ/g TS ULS pre-treatment (DD: 32.8%). Considering the DD caused by 0.01 mol/L ALK pre-treatment was only 0.8%, the combination of ALK treatment with the ULS process obviously induced synergistic sludge disintegration (i.e. synergistic COD solubilization). Similar observation was reported in a previous study (Kim et al., 2010). 58.9% DD was obtained when ULS treatment (7.5 kJ/g TS) was combined with ALK treatment (pH 12); whereas, the DD induced by the ULS and the ALK treatment alone were only 19.5% and 21.4%, respectively. A possible reason for such synergistic disintegration was the NaOH made the sludge structure more vulnerable to the mechanical disruption caused by ultrasound (Jin et al., 2009, Kim et al., 2010, Tyagi et al., 2014). In order to provide more information on the synergisms, the solubilized organics were then subsequently characterized with size exclusion chromatography (SEC) and excitation emission matrix (EEM) fluorescence spectroscopy.

Figure 6-2 Change in (a) SCOD concentration (b) equivalent COD concentration of soluble proteins (c) equivalent COD concentration of soluble carbohydrates (d) disintegration degree in feed sludge after ULS+ALK pre-treatment with specific energy input at different applied NaOH concentrations (error bars indicate standard deviations)

6.4 Molecular weight distribution

Molecular weight (MW) distributions of organics in the control and treated feed sludge were measured with a calibrated SEC. The calibration equation is shown as described in Section 4.3:

$$\text{Log}(MW) = 9.8223 - 0.6748(Rt)$$

MW distribution chromatograms of control, ALK treated (0.05 mol/L), ULS treated (21 kJ/g TS) and ULS+ALK treated feed sludge are as shown in Figure 6-3 (a). The retention time of the standard polymers are shown for comparison. As shown in Figure 6-3 (a), compounds with retention time shorter than 6 minutes were

solubilized in all the treated feed sludge. These compounds had MW higher than 500 kDa as they eluted earlier than the largest standard polymer (MW: 500 kDa). Compounds with retention time longer than 8 minutes were also solubilized (the earliest peak was from the ULS treated feed sludge, but it was not obviously noted due to the large scale difference compared to the ULS+ALK treated feed sludge). The corresponding MW of these compounds should be less than 27 kDa according to the calibration equation as introduced above. A large UV response at retention time around 9 minutes (MW: 5.6 kDa) was observed in the ULS+ALK treated feed sludge. However, such peak was not observed in either of the individual ALK or ULS treated feed sludge. Therefore, solubilization of these substances was likely due to the synergistic actions between the ULS and ALK pre-treatments. Formation of such small macromolecules was possible because the macromolecules solubilized by ULS treatment were further hydrolyzed by NaOH (Şahinkaya and Sevimli, 2013). Examples of such degradation would be the saponification reactions of lipids and the ALK hydrolysis of proteins. Using SEC to characterize the solubilization products after sludge pre-treatment has not been reported previously and can provide insights into the MW distribution change of the soluble organics due to the pre-treatment processes.

The supernatant was fractionated with UF membranes based on their MWs range as determined by the SEC. Apparent MW distribution in terms of TOC (% W/W) for different MW ranges was then measured to obtain quantitative results as shown in Figure 6-3 (b). The TOC results showed good agreement with the SEC results as presented in Figure 6-3 (a). The TOC fraction of components with MW larger than 300 kDa in the supernatant of feed sludge increased after the pre-treatment processes, indicating solubilization of macromolecules. This TOC fraction was only 7.8% in the control sludge and increased to 60%, 16% and 42.3% after ALK, ULS and ULS+ALK pre-treatments, respectively. A review of the literature suggested UF fractionation on the supernatant of ULS+ALK treated feed sludge had not been reported previously. However, Eskicioglu et al. (2006) did report the COD fraction with MW over 300 kDa increased from 16.9% to 29.5% and 24.7%

after conventional thermal and microwave thermal pre-treatment, respectively. In addition, it was observed that the effects of ALK treatment were different when it was applied on its own and applied together with the ULS treatment. Individual ALK treatment increased the TOC fraction of organics with MW larger than 300 kDa from 7.8% (control) to 16% (ALK); while ALK treatment decreased the corresponding fraction from 60% (ULS) to 42.3% (ULS+ALK) when it was combined with the ULS treatment. This was likely because the alkaline hydrolysis of macromolecules solubilized by the ULS pre-treatment as introduced previously in this Section. As a result, COD fraction of high MW macromolecules decreased while simpler organics were formed as degradation products. These smaller compounds in the supernatant would benefit the subsequent anaerobic digestion.

Figure 6-3 (a) Molecular weight distribution chromatograms of the soluble substances in the control, ALK, ULS and ULS+ALK treated feed sludge in UV 254 nm signal (b) TOC mass fraction of each molecular weight range in the supernatant of control, ALK, ULS and ULS+ALK treated feed sludge

6.5 Fluorescent products characterization

Fluorescent products were characterized with EEM fluorescence spectroscopy to provide more insights into the solubilized compounds in feed sludge. As shown in

Figure 6-4, each spectrum was divided into I, II, III, IV and V as summarized by Chen et al. (2003). According to the Ex/EM range described in Section 3.4.8, peaks in Region I and II represent simple protein like substances and peaks in Region III represent FA-like substances. However, no obvious peaks were observed in Region I, II and III in both the control and treated feed sludge samples as shown in Figure 6-4. It was possible that some of the protein-like and FA-like peaks were “over-shadowed” by other peaks or some simple proteins were aggregated with polysaccharides and detected in Region IV as SMP-like products (Ng and Ng, 2010).

Peaks of SMP-like matters (in Region IV) were observed in all the spectra as shown in Figure 6-4. FI of the SMP-like peak was found to increase after various pre-treatment processes. The FI values of the observed SMP-like peaks in both control and treated feed sludge are as shown in Table 6-1. FI of the SMP-like peak only increased from 463 to 531 after 0.05 mol/L ALK pre-treatment. Solubilization of the SMP-like substances was more significant after 21 kJ/g TS ULS pre-treatment and FI of the corresponding peak increased from 463 to 768. Addition of NaOH to the ULS process further enhanced the SMP-like substances solubilization. FI of the observed SMP-like peak in the ULS+ALK (21 kJ/g TS + 0.05 mol/L) treated feed sludge was 839 which was 9.2% higher than that in the ULS treated feed sludge (FI: 768). These results showed that the ULS+ALK treatment of feed sludge resulted in synergistic solubilization of SMP-like substances. These SMP-like substances were mainly extracellular and intracellular substances solubilized as a result of sludge structure disintegration (i.e. biological flocs disintegration and cell lysis). This correlated well with the high concentration of soluble proteins and carbohydrates as shown in Figure 6-2 (b) and (c) and also confirmed the synergistic effect that addition of NaOH made the sludge structure more readily disintegrated by ULS.

Table 6-1 Fluorescence intensity of the observed peaks in control and treated feed sludge

Sample	SMP-Like (Ex/Em)	FI	HA-like (Ex/Em)	FI
Control	280/375	463	350/400	43
ALK	280/370	531	350/450	91
ULS	280/370	768	370/425	80
ULS+ALK	290/360	839	350/440	201

SMP: Soluble microbial products; HA: Humic acid; FI: Fluorescence intensity

Peaks of HA-like substances in Region V were also observed in all the spectra. FI of the HA-like peak in feed sludge was found to increase after various pre-treatments and the FIs of the observed peaks are listed in Table 6-1. Individual ALK or ULS pre-treatment only slightly increased the FI of the HA-like peaks. The FI increased from 43 to 91 and 80 after 0.05 mol/L ALK pre-treatment and 21 kJ/g TS ULS pre-treatment, respectively. Interestingly, FI of the HA-like peak was significantly high in the ULS+ALK treated feed sludge as highlighted by a red ellipse in Figure 6-4 (d). FI of the highlighted peak was 201 as shown in Table 6-1. This was higher than that in the ALK treated (FI: 91), the ULS treated feed sludge (FI: 80) and even their sum (i.e.: 171). This indicated ALK and ULS pre-treatments had synergistic effects on the solubilization of HA-like substances which has not been reported previously. The synergistic mechanism was proposed to be as follows. HA in sludge is normally adsorbed onto the activated biomass (Esparza-Soto and Westerhoff, 2003). HA is known to be soluble in basic condition but is relatively insoluble in neutral and acidic conditions (Stevenson, 1994). When only ALK pre-treatment was applied, most of the HAs remained attached on the biological flocs due to lack of mechanical disruption. Ultrasound disrupted the sludge matrix and mechanically set free the HAs trapped in biological flocs. However, these HAs remained insoluble due to a neutral pH during individual ULS pre-treatment. Thus, the corresponding FI increases of HA-like substances in both individual ALK and individual ULS pre-treated sludge were not obvious. When ULS was performed under alkaline condition, HAs were mechanically set free by ultrasound and were then solubilized in the ALK solution (pH: 12.2). The solubilized HAs then contributed to the synergistic COD solubilization as mentioned in Section 6.3. This synergistic solubilization of HA-like substances may also have influenced the subsequent anaerobic digestion.

Figure 6-4 Excitation emission matrix fluorescent spectra of the supernatant in (a) Control (b) ALK treated (0.05 mol/L) (c) ULS treated (21 kJ/g TS) (d) ULS+ALK (21 kJ/g TS+ 0.05 mol/L) treated feed sludge

6.6 Anaerobic biodegradability

Biochemical methane potential (BMP) assay was used to test the change in feed sludge anaerobic biodegradability after various pre-treatment processes. As shown in Figure 6-5, the anaerobic biodegradability (methane production from each gram of COD added into the reactor) of all the treated feed sludge was higher than the control during the first four days of the BMP assay, because the pre-treatment step solubilized particulate organics and so accelerated the anaerobic digestion. The feed sludge anaerobic biodegradability only increased by 17.8% and 5.7% after 0.02 and 0.05 mol/L ALK pre-treatments had been applied, respectively. This indicated a NaOH dosage of 0.02 mol/L was more suitable for enhancing the biodegradability of the sewage sludge and it was selected for the subsequent tests. The DD obtained by 0.02 and 0.05 mol/L ALK pre-treatment were 4.0% and 19.6% as shown in Section 6.2. This meant that the biodegradability increase (BI) after individual ALK pre-treatment was not necessarily related to the applied NaOH

concentration or DD. Therefore, further increase of sludge anaerobic biodegradability via higher NaOH dosage was not an option. Anaerobic biodegradability of feed sludge was also improved after ULS pre-treatment. The anaerobic biodegradability increased from 175.8 to 202.7 mL CH₄/g COD_{added} (+15.3%) after 11.5 kJ/g TS ULS pre-treatment. However, the anaerobic biodegradability only increased to 212.2 mL CH₄/g COD_{added} (+20.7%) when the specific energy input was doubled to 21 kJ/g TS. This suggested increase in specific energy input could further improve the sludge anaerobic biodegradability but was relatively inefficient after a certain energy threshold.

The ULS+ALK treated feed sludge had higher biodegradability in comparison with the ULS treated and the ALK treated sludge. The ultimate sludge anaerobic biodegradability increased from 175.8 to 229.4 (+30.5%) and 242.3 (+37.8%) mLCH₄/g COD_{added} by combining 11.5 and 21 kJ/g TS ULS pre-treatment with 0.05 mol/L ALK pre-treatment, respectively. This showed that using NaOH to enhance ULS pre-treatment was an alternative for further improving sludge biodegradability instead of increasing NaOH dosage or ULS energy individually. The BI in this work was slightly lower compared to the previous results from Kim et al. (2010). One possible reason is the NaOH concentration in this work was higher compared to theirs, the inhibition effect of sodium ions might have decreased methane production (Feijoo et al., 1995). It is also possible that the longer digestion time in this work allowed slowly degradable compounds in untreated feed sludge to be digested; the relative increase in the treated feed sludge was therefore less. The results of this study were from batch reactors with digestion time of 30 days; whereas, the results of Kim et al. (2010) were based on a continuous reactor with solids retention time of 20 days. This was confirmed by the results of Seng et al. (2010) where the methane production after anaerobic digestion of ULS+ALK treated waste activated sludge (WAS) were 17.3%, 31.1% and 42.1% higher than the methane production after anaerobic digestion of untreated WAS at solids retention time (SRT) of 25, 15 and 10 days, respectively.

Figure 6-5 Biochemical methane potential results of control, ALK, ULS and ULS+ALK treated feed sludge (Biodegradability: methane production from each gram of COD added into the reactor) (error bars indicate standard deviations)

Nevertheless, contribution of the synergistic effects to the anaerobic biodegradability increment was also observed. For example, BI after ULS+ALK (21 kJ/g TS + 0.05 mol/L) pre-treatment was 37.8%; whereas, the BI were only 5.7% and 20.7% after ALK and ULS pre-treatments had been applied individually under the same conditions, respectively. Obviously, the BI induced by the ULS+ALK pre-treatment (i.e. 37.8%) was significantly higher than the BI induced by individual ALK pre-treatment (i.e. 5.7%), individual ULS pre-treatment (20.7%) or the numerical summation of both terms (i.e. 26.4%). Such biodegradability improvement due to the ULS+ALK pre-treatment had not been emphasized in previous works and could be related to the synergistic sludge disintegration as

mentioned in Section 6.3. As mentioned in Section 6.3 and 6.5, solubilization of SMP-like products such as proteins and carbohydrates were significantly enhanced due to the ULS+ALK treatment of feed sludge. Solubilization of those compounds effectively accelerated the hydrolysis step and thus enhanced the methane production (Wang et al., 1999). In addition, the combined pre-treatment generated smaller organics due to the interactions between ALK and ULS treatments as mentioned in Section 6.4. This was also beneficial for the subsequent anaerobic digestion. Besides, synergistic solubilization of HA-like compounds might also contribute to the synergistic biodegradability improvement. HAs are generally considered as persistent in anaerobic digestion. However, Ho and Ho (2012) found low concentrations of HAs (less than 5 g/L) could enhance methane production by serving as electron acceptors of the fatty acids degradation. Therefore, further investigation of the synergistically solubilized HAs may be helpful to the mechanism investigation.

6.7 Summary

SCOD in feed sewage sludge increased maximally from 1,200 to 11,000 mg/L after the ULS+ALK pre-treatment. The hydroxyl ions were found to further degrade the macromolecules solubilized by ULS treatment. This synergistic action generated smaller organics with MW around 5.6 kDa. Combination of ALK treatment with ULS treatment could enhance solubilization of the SMP-like substances by making the cell walls more vulnerable to ultrasound disruption. HA was also found to be released due to the synergistic actions between alkali and ultrasound.

Individual ALK and ULS pre-treatments have their respective limitations in improving feed sludge anaerobic biodegradability. Whereas, the ULS+ALK pre-treatment could further enhance the sludge biodegradability due to the aforementioned synergistic effects between ULS and ALK treatments. The sludge anaerobic biodegradability increased by 30.5% and 37.8% when 0.05 mol/L ALK pre-treatment was combined with 11.5 kJ/g TS and 21 kJ/g TS ULS pre-treatment, respectively.

Chapter 7 Effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline pre-treatments on anaerobic digestion of sewage sludge in semi-continuous reactors

7.1 Introduction

The effects of ultrasonication (ULS), ultrasonication-ozonation (ULS-Ozone) and ultrasonication+alkaline (ULS+ALK) treatments on sewage sludge have been introduced in previous chapters. In this chapter, the ULS, ULS-Ozone and ULS+ALK treated feed sludge were anaerobically digested with semi-continuous reactors at SRT of 10 and 20 days. The biogas productions and solids removal efficiencies were measured to assess the possible enhancement with such pre-treatments. Furthermore, dewaterability of the digested sludge and soluble residual organics in the anaerobic digested sludge were also characterized to evaluate the impact of treatment of feed sludge on the characteristics digested sludge. At the end of this chapter, economic analysis was conducted to compare the tested pre-treatment methods.

The ULS, ULS-Ozone and ULS+ALK conditions tested in this chapter were selected following consideration of the results reported in previous chapters. The ULS energy input was selected at 9 kJ/g TS because further increase in ULS energy input over 9 kJ/g S is not economical as discussed in Section 4.4. The ozone dosage for ULS-Ozone treatment was 0.012 g O₃/g TS. As described in Section 5.6, 0.036 g O₃/g TS ozonation treatment of feed sludge induced mineralization of organics. Reduction of ozone dosage would not only save energy but also reduce the loss of biodegradable organics during ozonation. Therefore, a lower ozone dosage of 0.012 g O₃/g TS, at which synergistic sludge solubilization was observed when combined with ULS treatment of feed sludge, was selected for ULS-Ozone pre-treatment in this chapter. NaOH concentration of 0.02 mol/L (NaOH dosage: 0.05 g NaOH/g TS) was selected for the ULS+ALK treatment of feed sludge because higher NaOH concentration introduced more sodium ions which induced negative effects on

sludge anaerobic biodegradability as described in Section 6.6.

7.2 Biogas production and solids removal

Biogas production from the anaerobic reactors is as shown in Figure 7-1 (a) and (b). Anaerobic biodegradability of feed sewage sludge was higher at SRT of 20 days with its longer substrate-microbe contact time. At both SRTs, the daily biogas production was higher from the reactors fed with treated feed sludge than from the control reactor. Average daily biogas production from each reactor is as shown in Table 7-1. These values were calculated by averaging the daily biogas production in the third SRT. At SRT of 10 days, daily biogas production increased from 256 to 309 (+ 20.7%), 348 (+ 35.9%) and 319 (+24.6%) mL biogas/g COD_{fed} as a result of the ULS, ULS-Ozone and ULS+ALK treatments of feed sludge, respectively. At SRT of 20 days, daily biogas production increased from 313 to 337 (+ 7.7%), 393 (+25.5%) and 365 (+ 16.6%) mL biogas/g COD_{fed} due to the ULS, ULS-Ozone and ULS+ALK treatments of feed sludge, respectively. Such increase in biogas production was because the pre-treatments solubilized particulate macromolecules in sludge which accelerated the rate-limiting hydrolysis step. The ULS-Ozone and ULS+ALK treatments of feed sludge solubilized more organics than the ULS treatment, thus more biogas production was observed. For all the reactors, the methane content was relatively stable at around 65%. In addition, no VFAs (including acetate) accumulation in the digested sludge was observed and pH value remained near neutral throughout the anaerobic digestion test (around 7.0 to 7.2) in all the reactors. This suggested the ULS, ULS-Ozone and ULS+ALK treatments of feed sludge did not cause stress on the acetogenesis and methanogenesis step in the reactors. Methane produced from each gram of COD removed was also improved after the tested pre-treatments. Similar observations were reported by Apul and Sanin (2010) after ULS pre-treatment and by Lin et al. (1997) after ALK pre-treatment. Theoretically, each gram of COD removed could maximally produce 350 mL CH₄ at standard condition (or 395 mL at 35 °C) (Lin et al., 1997). In this study, the highest methane production from each gram COD removed was 347 mL

from the ULS-Ozone10 reactor. This observed value was close to the maximum value, indicating the removed organics were almost completely utilized for methane production. However, in practice, this value was calculated according to biogas production, total COD concentration in feed sludge and total COD concentration in post-digestion sludge. The measurement errors of these parameters might have influenced the measured methane production based on COD removal.

Figure 7-1 Daily biogas production from the reactors at (a) SRT of 10 days (Control10, ULS10, ULS-Ozone10 and ULS+ALK10) (b) SRT of 20 days (Control20, ULS20, ULS-Ozone20 and ULS+ALK20) (error bars indicate standard deviations)

The average VS and VSS concentrations in the digested sludge are as shown in Table 7-1 and the corresponding VS removal efficiencies after anaerobic digestion against the untreated feed sludge were 35.6%, 38.3%, 42.1% and 41.2% for control10, ULS10, ULS-Ozone10 and ULS+ALK10 reactors, respectively. The corresponding increase in VS removal rates after anaerobic digestion due to the

ULS, ULS-Ozone and ULS+ALK treatments of feed sludge were 7.6%, 18.3% and 15.7%, respectively. Solids removal efficiency was higher at the longer SRT. The VS removal rates after anaerobic digestion of the untreated, ULS, ULS-Ozone and ULS+ALK treated feed sludge were 37.3%, 40.9%, 45.3% and 44.1% at SRT of 20 days, respectively. The corresponding improvements in VS destruction after anaerobic digestion were 9.7%, 21.4% and 18.2% due to the ULS, ULS-Ozone and ULS+ALK treatments of feed sludge. Higher VS removal efficiency indicated more organic matters were digested and converted into biogas. Similarly, integration of pre-treatment step also improved the VSS removal efficiency as shown in Table 7-1. The increase in VSS removal efficiency indicated particulate organics in the pre-treated sludge could be better hydrolysed for the subsequent anaerobic digestion process.

The daily biogas production and solids removal efficiency were in the order of ULS-Ozone > ULS+ALK > ULS > control for the tested reactors. In addition, it was noted the daily biogas production and solids removal efficiencies of the ULS-Ozone10 and ULS+ALK10 reactors were higher than those of the Control20 reactor. However, daily biogas production and solids removal rates of the ULS10 reactor were lower than those of the Control20 reactor. This suggested the ULS-Ozone and ULS+ALK treatments of feed sludge could halve the SRT without affecting digestion performance; whereas, the individual ULS treatment of feed sludge was not able to provide such advantage.

Table 7-1 Performance of the semi-continuous anaerobic digesters at the assumed steady state

Reactor	SRT 10 days				SRT 20 days			
	Control10	ULS10	ULS-Ozone10	ULS+ALK10	Control20	ULS20	ULS-Ozone20	ULS+ALK20
Biogas (mL/day g COD _{fed}) (n=10 and 20) ^a	256 ± 5 ^c	309 ± 6	348 ± 9	319 ± 7	313 ± 7	337 ± 10	393 ± 12	365 ± 8
Methane percentage (%) (n=5)	64.2 ± 0.9	65.2 ± 0.8	65.6 ± 0.6	65.1 ± 0.8	64.9 ± 0.5	64.9 ± 1.3	65.4 ± 1.2	65.0 ± 1.3
Post-digestion TS (mg/L) (n=5 and 8) ^b	10650 ± 602	10410 ± 401	9720 ± 370	10580 ± 527	10306 ± 180	10001 ± 764	9375 ± 236	10231 ± 233
Post-digestion VS (mg/L) (n=5 and 8) ^b	8340 ± 383	7990 ± 344	7500 ± 245	7620 ± 202	8125 ± 252	7644 ± 253	7081 ± 217	7243 ± 437
Post-digestion TSS (mg/L) (n=5 and 8) ^b	9885 ± 468	9340 ± 171	8330 ± 220	8970 ± 501	9481 ± 164	8769 ± 153	8189 ± 371	8613 ± 415
Post-digestion VSS (mg/L) (n=5 and 8) ^b	8005 ± 469	7640 ± 155	6760 ± 248	7038 ± 165	7619 ± 258	7056 ± 213	6606 ± 206	6706 ± 246
Post-digestion TCOD (mg/L) (n=4)	12,321 ± 667	11733 ± 482	10470 ± 831	11476 ± 769	11215 ± 1059	10850 ± 794	10191 ± 737	10253 ± 921
Post-digestion SCOD (mg/L) (n=4)	193.6 ± 28.1	256.8 ± 34	588.7 ± 53.3	296.3 ± 30.3	181.6 ± 24.3	227 ± 15.1	440 ± 33.5	245.7 ± 19.5
Post-digestion proteins (mg/L) (n=3)	73.3 ± 1.5	81.7 ± 1.6	166.6 ± 3.3	88.3 ± 1.8	74.7 ± 1.1	89.7 ± 0.8	158.6 ± 1.9	93.6 ± 1.6
Post-digestion carbohydrates (mg/L) (n=3)	19.7 ± 0.6	28.8 ± 4.3	61.7 ± 0.8	33.8 ± 0.6	13.7 ± 0.7	22.9 ± 0.4	34.6 ± 2.4	24.5 ± 2.1
Post-digestion total VFAs (mg/L) (n=3)	Nd*	Nd	Nd	Nd	Nd	Nd	Nd	Nd
CST in digested sludge (s) (n=3)	56.9 ± 2.8	145.6 ± 6.2	179.1 ± 10	173.9 ± 7	50.3 ± 3.5	101.4 ± 5.1	120 ± 6.1	120.2 ± 8.8
Methane (mL/day g COD _{removed})	299 ± 6	334 ± 6	347 ± 9	332 ± 7	295 ± 7	306 ± 9	342 ± 10	312 ± 7

* not detectable (< 10 mg/L)

^a n = 10 at SRT of 10 days and n = 20 at SRT of 20 days^b n = 5 at SRT of 10 days and n = 8 at SRT of 20 days^c numbers after the “±” stand for the standard deviations

7.3 SCOD and dewaterability in digested sludge

The average SCOD concentrations in the digested sludge during anaerobic digestion are as shown in Table 7-1. The treatments of feed sludge increased the post-digestion SCOD concentration at both SRTs. The post-digestion SCOD of the ULS-Ozone reactor was much higher than that of the ULS and ULS+ALK reactors. The SCOD in the digested sludge from the ULS-Ozone reactors were around 300 and 200 mg/L higher than that from the ULS reactors operating at SRT of 10 and 20 days, respectively; while, the SCOD in the ULS-Ozone treated feed sludge was 1,200 mg/L higher than the SCOD in the ULS treated feed sludge. These increases in post-digestion SCOD were much lower compared to the increase in SCOD in feed sludge due to the subsequent ozonation (around 1,200 mg/L). This indicated much of the COD solubilized by the subsequent ozonation treatment of feed sludge was biodegraded and only a relatively small fraction accumulated in the anaerobic reactors. However, the post-digestion SCOD concentration of the ULS+ALK reactors did not increase obviously compared to the post-digestion SCOD of the ULS reactors at SRT of 10 and 20 days. This indicated combination of ALK treatment with ULS treatment did not increase the amount of soluble recalcitrant organics in the anaerobic digested sludge.

It is known that biopolymers are a major component of sludge (Rittman and McCarty, 2001). Averaged values of soluble carbohydrates and proteins concentration in the digested sludge during the last three sampling days are compared in Table 7-1. Soluble carbohydrates and proteins concentrations in the digested sludge from the ULS-Ozone reactor were much higher than their corresponding concentrations in the digested sludge from the ULS, ULS+ALK and control reactors at both SRTs. This suggested undigested biopolymers contributed to the higher SCOD in the digested sludge from the ULS-Ozone reactors.

The influence of SRT on the residual carbohydrates and proteins concentrations were different. As shown in Table 7-1, soluble carbohydrates concentrations

decreased obviously when all the reactors had longer residence time. For example, the residual soluble carbohydrates concentration decreased from 62 to 35 mg/L when SRT of the ULS-Ozone reactor increased from 10 to 20 days. This is because the solubilized carbohydrates after the treatments of feed sludge were mainly complex polysaccharides from extra- and intra- cellular structures (Tian et al., 2014, Wang et al., 2006b). Longer residence time was needed for sufficient degradation. However, the soluble proteins concentrations in the digested sludge did not show obvious difference between SRT of 10 and 20 days for all the reactors. The residual proteins were likely to be functional proteins or enzymes which could not be degraded via microbial utilization (Park et al., 2008). In addition, HAs which were generated during the anaerobic digestion could be mistakenly detected as proteins with the Lowry's method used.

The dewaterability of the digested sludge also decreased due to the treatments of feed sludge. At SRT of 10 days, CST of the digested sludge from the control, ULS, ULS-Ozone and ULS+ALK reactor were 56.9, 145.6, 179.1 and 173.9 s, respectively. Dewaterability of the digested sludge further improved at a longer residence time. At SRT of 20 days, CST of the digested sludge from the control, ULS, ULS-Ozone and UA reactor were 50.3, 101.4, 120 and 120 s, respectively. This was because treatment of feed sludge solubilized biopolymers which could bind with free water and worsen the sludge dewaterability (Wang et al., 2006a). Some of these biopolymers were persistent after anaerobic digestion and deteriorated the dewaterability of the digested sludge.

7.4 Molecular weight distribution

Molecular weight (MW) distribution of organics in the digested sludge was measured with a calibrated size exclusion chromatography (SEC). The calibration equation is shown as follows as described in Section 4.3:

$$\text{Log}(MW) = 9.8223 - 0.6748(Rt)$$

MW chromatograms of the soluble substances in the digested sludge from reactors operating at 10 days SRT and 20 days SRT are as shown in Figure 7-2 (a) and (b),

respectively. Detected peaks were divided into five groups (A to F) in ascending order of retention time. MWs of the components in these peaks are in the descending order from A to F because larger compounds were retained for a shorter time in the column and eluted earlier.

7.4.1 High MW compounds

At SRT of 10 days, responses of peak A (Rt: 4.0 min) and B (Rt: 6.2 min) in the digested sludge from the ULS-Ozone reactor were significantly higher than those from the control, ULS and ULS+ALK reactors as shown in Figure 7-2 (a). Compounds detected in these peaks were macromolecules with MW higher than 500 kDa because retention time of these peaks was shorter than the retention time of the largest tested standard polymer (MW: 500 kDa, Rt: 6.2 min). This indicated anaerobic digestion of ULS-Ozone treated feed sludge resulted in accumulation of soluble polymeric substances in the digested sludge. This was due to the subsequent ozonation process because these compounds were not observed when feed sludge was only treated with ULS.

Similarly, the responses of peak C (Rt: 8.2 min) and peak D (Rt: 8.8 min) with MW around 19.4 kDa and 7.7 kDa, respectively were also obviously higher in the digested sludge from the ULS-Ozone reactors than those in the digested sludge from the control, ULS and ULS+ALK reactors. However, it should be noted that peak E (Rt: 9.4 min) instead of peak D was detected in the digested sludge of the ULS+ALK reactors. This was likely related to the ALK hydrolysis of soluble macromolecules during the ULS+ALK treatment of feed sludge as mentioned in Section 6.4. This generated new substrates compared to the ULS and ULS-Ozone pre-treatments and anaerobic digestion of these compounds might result in formation of different residual organics in the anaerobic digested sludge.

Responses of peak A, C, D and E were lower at SRT of 20 days than at SRT of 10 days as compared in Figure 7-2 (a) and (b). This indicated some of the compounds detected in these peaks were slowly biodegradable compounds. They were not biodegraded at SRT of 10 days but could be degraded at SRT of 20 days. Some of

these compounds were likely to be carbohydrates because the results reported in Section 7.3 showed some carbohydrates were not biodegradable at SRT of 10 days but became biodegradable at SRT of 20 days. At SRT of 20 days, hardly any difference was observed between the MW chromatograms of the digested sludge from the control, ULS and ULS+ALK reactors as shown in Figure 7-2 (b). However, responses in peak A, B, C and D were still significantly higher in the digested sludge from the ULS-Ozone reactor. This indicated considerable amounts of polymeric substances remained undigested in the ULS-Ozone reactor even at SRT of 20 days. These residual soluble polymeric compounds in the anaerobic digested sludge were possibly related to solubilization of persistent compounds after the ULS-Ozone treatment of feed sludge (Yang et al., 2013, Tian et al., 2014). These results correlated very well with the increase in biopolymers in the digested sludge as observed in Section 7.3. This indicated hydrolysis of these soluble polymeric substances limited the overall performance of the anaerobic digestion for the ULS-Ozone treated feed sludge. Biogas production from the ULS-Ozone reactors could be further increased if these organics were utilized.

Figure 7-2 Molecular weight distribution chromatograms of (a) supernatant in the digested sludge from reactors operating at SRT of 10 days (b) supernatant in the digested sludge from reactors operating at SRT of 20 days

7.4.2 Low MW compounds

Peak F (Rt: 13.3 min) was detected in the digested sludge from the ULS-Ozone reactors as shown in Figure 7-2 (a) and (b). Although these compounds were organics with low MW, they were not monomers or other easily biodegradable components because they remained undigested at SRT of 20 days. Therefore, they were possibly short chain alkenes or aromatics which are by-products from degradation of larger molecules and could be detected by the UV 254 nm detector. Formation of these by-products is related to the subsequent ozonation process because the response was only obviously higher in the digested sludge from the ULS-Ozone reactor. It is possible that the complex polymers in the untreated and ULS treated feed sludge could only be broken down via biodegradation; while, dosing ozone could provide different degradation pathways by chemically breaking down the high MW biopolymers into smaller fragments (Tian et al., 2014). Besides, ozone can convert some refractory compounds into biodegradable ones (Nishijima et al., 2003). These aforementioned factors could generate different substrates and anaerobic digestion of these new substrates could contribute to the accumulation of the detected by-products.

7.5 Fluorescent products characterization

Excitation emission matrix (EEM) fluorescence spectroscopy analysis was conducted to measure the fluorescence intensity (FI) of fluorescent compounds in the supernatant of digested sludge from the reactors. The EEM spectra of all the samples are as shown in Figure 7-3 (a)-(h). FI of the detected peaks was shown in numbered and colour contour lines for reference. According to the Ex/Em range introduced in Section 3.4.8, the main peaks were HA-like (as highlighted with a white arrow in Figure 7-3 (c)) and FA-like substances (as highlighted with a white arrow in Figure 7-3 (e)). These substances were released from the biodegradation of the extracellular polymeric substances, sludge pellets and refractory components (e.g. lignin) in sewage sludge (Luo et al., 2013). Aside from these two groups,

SMP-like substances and simple protein-like matters were also detected in each spectrum. However, their FIs were relatively low and over-dominated by peaks of HA-like and FA-like substances.

Figure 7-3 Excitation emission matrix fluorescent spectra of the supernatant in the digested sludge from (a) control10 (b) ULS10 (c) ULS-Ozone10 (d) ULS+ALK10 (e) control20 (f) ULS20 (g) ULS-Ozone20 (h) ULS+ALK20 reactors

7.5.1 Humic acid-like substances

At SRT of 10 days, the FI of HA-like substances were similar in the digested sludge from the control and ULS reactors as shown in Figure 7-3 (a) and (b). In contrast, the FI of the HA-like substances were significantly higher in the digested sludge from the ULS-Ozone reactor as shown in Figure 7-3 (c). This confirmed some proteins detected with Lowry's method in Section 7.3 were attributed to humic substances. This is because the ULS-Ozone treatment of feed sludge disintegrated the sludge better and solubilized more HA-containing substances in comparison with the ULS treatment. Biodegradation of these solubilized HA-containing substances resulted in a higher concentration of HAs as by-products. These HAs should contribute to the residual polymeric substances in the digested sludge from the ULS-Ozone reactors (e.g Peak C, in Figure 7-2 (b)) as discussed in Section 7.4.1, because HAs are known to be persistent and have high MW (Li et al., 2009, Stevenson, 1994). Similarly, FI of the HA-like substances was slightly higher in the digested sludge from the ULS+ALK reactor than in the digested sludge from the ULS reactor. Such increase in HAs during anaerobic digestion of treated feed sludge was in good agreement with results obtained by Luo et al. (2013). They observed anaerobic digestion of enzymatically treated waste activated sludge (WAS) resulted in higher FI of HA-like substances compared to anaerobic digestion of untreated WAS in the digested sludge (Luo et al., 2013).

By comparing the EEM spectra in Figure 7-3 (a), (b), (d) to Figure 7-3 (e), (f), and (h), FI of the HA-like matters in the digested sludge from the control, ULS and ULS+ALK reactors were both found to increase at the longer retention time. This was likely because sludge were better digested at the longer retention time which released more HAs as anaerobic digestion by-products. In contrast, FI of the HA-like substances were similar in the digested sludge from the ULS-Ozone10 and the ULS-Ozone20 reactors as shown in Figure 7-3 (c) and (g). This indicated the HA-containing substances were mostly biodegraded and the HAs were released into the supernatant within 10 days of anaerobic digestion for the ULS-Ozone treated feed

sludge. A longer digestion time did not further increase the FI of the HA-like substances in the anaerobic digested sludge.

7.5.2 Fulvic acid-like substances

FI of the FA-like substances were similar in the digested sludge from the control, ULS and ULS-Ozone reactors at SRT of 10 days as shown in Figure 7-3 (a), (b), (c) and (d). By comparing Figure 7-3 (a), (b) with Figure 7-3 (e), (f), FI of the FA-like substances in the digested sludge from the control and the ULS reactors were both found to increase when the SRT increased to 20 days which was similar to the observations on the HA-like matters. However, the FI of the FA-like substances in the digested sludge from the ULS-Ozone and ULS+ALK reactors decreased when the SRT increased from 10 days to 20 days, indicating the FA-like compounds became biodegradable at a longer digestion time. Such biodegradability increase of the FA-like substances is related to the use of chemical treatment (e.g. ozone or alkali) to enhance ULS treatment of feed sludge. For example, ozone was reported to increase of biodegradability of FAs in previous studies (Volk et al., 1997, Kozyatnyk et al., 2013). Such increase in biodegradability of FAs is a potential advantage of ULS+ALK and ULS-Ozone pre-treatments and had not been emphasized in previous studies.

7.6 Economic analysis

An economic analysis was conducted to evaluate the ULS, ULS-Ozone and ULS+ALK pre-treatment of feed sewage sludge. The values used for this analysis were taken from Cho et al. (2014) and Chu et al. (2009) as shown in Table 7-2.

Table 7-2 Practical values used for economic analysis

Parameter	Values
Daily sludge influent	200 m ³ /d
CHP efficient for electricity generation	30%
CHP efficient for waste heat generation	50%
Sludge cake disposal cost	53 \$/ton
Electricity price	0.106 \$/kwh
Waste heat price	0.07 \$ /Mcal
Ozone electricity price	15.23 kwh/kg O ₃
Alkali cost	0.5\$/kg NaOH

The cost and gain from the pre-treatments were listed in Table 7-3 on an annual basis. It was noted using pre-treatment induced negative economic effects on the anaerobic digestion process. This was mainly due to the high cost of ULS treatment. The performance of ULS process was highly dependent on the efficiency of the disintegrator (Zielewicz, 2016). It has been indicated in previous research that the laboratory scale ULS could not been operated at the equally high intensity and efficiency as full-scale application (Pérez-Elvira et al., 2009). Therefore, lower ULS specific energy input should be needed to achieve the similar performance in practical application. For example, Xie et al. (2007) obtained around 45% increase in biogas production when only 1.44 kwh/m³ ULS pre-treatment was applied. Such ULS energy input was equivalent to only around 0.34 kJ/g TS ULS specific energy input in this work by assuming the TS concentration was 15 g/L. In addition, the obtained results showed that using ozone to assist ULS increased the cost balance; while, the ULS+ALK treatment did not show obvious advantage compared to ULS pre-treatment in terms of cost balance. Therefore, ozone should be used to assist ULS for treatment of feed sewage sludge in view of economics. However, it should also be pointed out that the values used in Table 7-2 were taken from literatures and these values may vary from places. For example, for ULS-Ozone pre-treatment, which resulted in lower TSS concentration in the digested sludge, might have larger positive cost recovery for places where solids disposal was expensive. In addition, it was also found that higher SRT resulted in higher cost balance for all

the reactors. This was because longer digestion time was given and thus sludge could be better degraded for biogas production during anaerobic digestion. However, it should also be borne in mind that shorter SRT could reduce the size of the reactor which decreased the capital cost.

Table 7-3 Economic analysis results for the semi-continuous anaerobic digestion tests with integration of ULS, ULS-Ozone and ULS+ALK pre-treatments on annual basis

Reactor	ULS cost(\$)	Ozone cost(\$)	ALK cost(\$)	Sludge disposal(\$)	Recovered electricity(\$)	Recovered heat(\$)	Cost balance(\$)	Compared to control(\$)
SRT 10 days								
Control10	-	-	-	47,806	65,552	259,734	277,479	-
ULS10	-290,175	-	-	45,171	79,123	313,507	57,284	-220,195
ULS-Ozone10	-290,175	21,413	-	40,286	89,110	353,076	90,311	-187,168
ULS+ALK10	-290,175	-	29,200	43,381	81,684	323,653	42,580	-234,899
SRT 20 days								
Control20	-	-	-	45,852	80,147	317,565	351,860	-
ULS20	-290,175	-	-	42,409	86,293	341,915	95,624	-256,236
ULS-Ozone20	-290,175	21,413	-	39,604	100,632	398,732	148,172	-203,688
ULS+ALK20	-290,175	-	29,200	41,655	93,463	370,324	102,757	-249,103

7.7 Summary

This chapter compared the impact of ULS, ULS-Ozone and ULS+ALK pre-treatments on sewage anaerobic sludge digestion. The biogas production and solids removal efficiencies from the tested anaerobic reactors were in the order of ULS-Ozone > ULS+ALK > ULS > control reactors at both SRT of 10 and 20 days. ULS-Ozone and ULS+ALK treatments of feed sludge could shorten the anaerobic SRT from 20 days to 10 days without adverse impact on anaerobic digestion performance. Soluble polymeric substances were found to accumulate in the anaerobic digested sludge following anaerobic digestion of ULS-Ozone treated feed sludge. Such digested sludge had deteriorated dewaterability. Although some of these polymers were anaerobically degradable at 20 days SRT, most were HA-like substances and persistent. In addition, biodegradability of FA-like substances was improved due to the ULS-Ozone and ULS+ALK treatments of feed sludge. In addition, the economic analysis showed using ozone to assist ULS pre-treatment of feed sludge increased the cost balance; while, the ULS+ALK treatment did not show obvious advantage compared to ULS pre-treatment in terms of cost balance.

Chapter 8 Effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline post-treatments on digested sludge

8.1 Introduction

The effects of ULS, ULS-Ozone and ULS+ALK pre-treatments (i.e. treatment of feed sludge) on anaerobic sludge digestion have been introduced in previous chapters. As introduced in Figure 2-2 in Section 2.2.2, treatment can not only be applied to feed sludge as pre-treatment but also be applied to digested sludge (the treated digested sludge is recycled to the anaerobic reactor) as post-treatment. Although other treatment methods (e.g. ozone, thermal, alkaline) have been reported for treatment of digested sludge in previous studies (Battimelli et al., 2003, Takashima, 2008, Li et al., 2013), the literature has not reported ULS, ULS-Ozone and ULS+ALK treatments for digested sludge.

Therefore, this chapter aims to investigate the effects of the ULS, ULS-Ozone and ULS+ALK treatments on digested sludge (i.e. post-treatment). In order to facilitate comparison with the pre-treatment results, the same treatment conditions as in Chapter 7 were selected for ULS, ULS-Ozone, ULS+ALK treatments tested in this chapter. Changes in digested sludge properties, characteristics of the solubilization compounds and digested sludge anaerobic biodegradability were investigated in this chapter.

8.2 Particle size reduction

Change in median particle diameter results is as shown in Figure 8-1. Chemical post-treatments did not have obvious influence on the digested sludge particle size. The median diameter in digested sludge had only changed from 45.3 μm to 43.1 and 48.3 μm after the ozone and ALK post-treatments, respectively. The slight increase in median diameter was possibly because some flocs were re-flocculated with the aid of electropositive organic polymers solubilized after the ALK treatment (Li et al., 2008). In contrast, the ULS post-treatment showed better

capability of reducing particles sizes than the chemical methods and had reduced the median diameter from 45.3 to 15.9 μm . ULS treatment had mechanically reduced the floc whereas, chemical methods could only chemically lyse the cells or extra-polymeric substances without causing significant change in particle sizes (Bougrier et al., 2006). When the chemical methods were used to enhance the ULS post-treatment, no obvious change in median diameter was observed compared to the case of individual ULS post-treatment. This indicated combining either ozone or ALK treatment with ULS treatment did not enhance floc size reduction in the digested sludge.

Figure 8-1 Effects of various post-treatments on the mean diameter of the digested sludge (error bars indicate standard deviations)

8.3 COD and biopolymers solubilization

SCOD in the digested sludge increased after the post-treatments as shown in Figure 8-2(a). ULS post-treatment was obviously more effective than the ozone and ALK

post-treatments in terms of COD solubilization. The ozone and ALK post-treatments only increased the SCOD in digested sludge from 200 mg/L to 650 and 500 mg/L, respectively; whereas, the ULS post-treatment was able to increase the digested sludge SCOD to 1,500 mg/L. The SCOD was further increased to 2,600 and 2,650 mg/L when the ULS post-treatment was assisted with ozone and ALK post-treatments, respectively. COD solubilization contributed by the ozone and ALK post-treatments was significantly improved when these chemical post-treatments were combined with ULS post-treatment as shown in Figure 8-2 (a). This suggested the two chemical post-treatments induced synergistic COD solubilization when combined with ULS post-treatment. It has been introduced in Section 6.3 that the synergistic COD solubilization observed after ULS+ALK treatment of feed sludge is because the alkali addition made the cells in feed sludge more vulnerable to ultrasound attack. Therefore, it is reasonable to assume that the alkali addition also weakened the cell walls in the digested sludge which resulted in synergistic COD solubilization after ULS+ALK treatment of digested sludge. However, it should be noted that digested sludge contained less viable biomass than the sewage sludge as most of these biomass were utilized during anaerobic digestion. Apart from undigested cells, the residual organics in digested sludge might also be attributed to organics which were protected by cells and zoogaea (Li et al., 2013). Therefore, addition of alkali might also de-agglomerate the zoogaea in the digested sludge which made the organics more available to the ultrasound attack. Although ULS-Ozone treatment of digested sludge induced synergistic COD solubilization, synergistic SCOD solubilization had not been observed when ULS-Ozone was applied for treatment of feed sludge as reported in Section 5.4. It had been suggested by Cesbron et al. (2003) that soluble organics in sludge created screening effect on the solid organics if the soluble organics reacted actively with ozone, but if the soluble organics could not react with ozone, such screening effect was not obvious. Therefore, it is possible ozone did not readily react with the organics solubilized by ultrasound and the screening effect was not observed, but instead specifically solubilized the particulate organics in digested sludge during

the combined ULS-Ozone treatment of digested sludge. In addition, smaller particles created by the ULS treatment enhanced the mass transfer of the subsequent ozonation process which resulted in the synergistic COD solubilization.

Figure 8-2 Change in (a) SCOD (b) soluble proteins (c) soluble carbohydrates in digested sludge due to various post-treatments (error bars indicate standard deviations)

Concentration of soluble biopolymers in digested sludge also increased after various post-treatments. As shown in Figure 8-2 (b) and (c), the concentration increases of soluble proteins and carbohydrates after the ozone and ALK post-treatments were relatively insignificant in comparison with the increases after the ULS post-treatment. In addition, the solubilization of proteins and carbohydrates due to the ozone and ALK post-treatments became more obvious when they were combined with ULS post-treatment. These results indicated the synergistic interactions between the ULS and the chemical treatments helped disintegrate the biological flocs, resulting in the release of biopolymers from the digested sludge.

8.4 Molecular weight distribution

MW distribution of the soluble substances in the control and treated digested sludge were also measured to provide more information on the solubilization products after the tested post-treatments. MW distribution chromatogram of the standard polymer mixture solution is as shown in Figure 8-3 (a). A linear relationship was derived between the log value of MW (Da) and retention time (Rt: min) with a correlation coefficient of 99.12%:

$$\text{Log (MW)} = 14.666 - 1.4855(\text{Rt})$$

The MW distributions of the soluble organics in the digested sludge without the ULS post-treatment (i.e. control, ozone and ALK) and with the ULS post-treatment (i.e. ULS, ULS-Ozone and ULS+ALK) were compared in Figure 8-3 (b) and (c). The MW distribution results showed good agreement with the SCOD results as stated in Section 8.3. The ozone and ALK post-treatments were relatively incapable of solubilizing the digested sludge and thus the corresponding peak response increases were low as shown in Figure 8-3 (b). However, the UV responses of the soluble organics were obviously higher after the ULS, ULS-Ozone and ULS+ALK treatments as shown in Figure 8-3 (b) and (c).

The soluble organics in the control and treated digested sludge were detected in peak A, B, C and D. The retention times of peak A (Rt: 4.7 min) and B (Rt: 5.8 min) were shorter than the retention time of the largest standard polymer (Rt: 6.2 min). Therefore, MWs of the compounds detected in peak A and B were larger than 500 kDa. The compounds detected in peak C (Rt: 7.2 min) had MW around 103 kDa according to the calibration equation. Due to the high MWs, these compounds (i.e. in peak A, B and C) were possibly from solubilization of cellular polymeric substances and humic substances from the biological flocs in the digested sludge. ULS-Ozone and ULS+ALK post-treatment obviously increased the UV responses of macromolecules in the supernatant of the digested sludge as shown in Figure 8-3 (c). However, such increases were relatively negligible when the ozone and ALK post-treatments were individually applied as shown in Figure 8-3 (b). This indicated that the synergistically solubilized organics to be macromolecules.

In addition to the aforementioned macromolecules, peak D with retention time around 8.9 minutes (MW: 7.6 kDa) was also detected in the supernatant of all the tested digested sludge. The UV response of peak D decreased after the ozone and ALK post-treatments. It is possible that the corresponding compounds were broken down due to the chemical attack induced by the ozone and ALK post-treatments. However, the UV responses of peak D in the supernatant of the ULS-Ozone and ULS+ALK treated digested sludge were higher than that in the supernatant of the

ULS treated digested sludge. This indicated the synergistic effects in the ULS-Ozone and ULS+ALK treatments also resulted in solubilization of compounds detected in peak D.

Furthermore, the MW distributions of the solubilized compounds were different after the ULS-Ozone and ULS+ALK treatments of digested sludge as compared in Figure 8-3 (c). For example, the ULS-Ozone treatment increased the UV responses of peak A, B and C in the supernatant of digested sludge; while, the ULS+ALK treatment only significantly increased the UV responses of peak A but had relatively low impact on the UV responses of peak B and C in the supernatant of digested sludge compared to the ULS-Ozone post-treatment. This indicated the solubilized compounds in digested sludge due to the ULS-Ozone and ULS+ALK treatments were different.

Figure 8-3 Molecular weight distribution chromatograms of (a) mixture of standard polymers solution (b) supernatant of control, ozone treated and ALK treated digested sludge (c) ULS treated, ULS-Ozone treated and ULS+ALK treated digested sludge

8.5 Fluorescent products characterization

The excitation emission matrix (EEM) spectra of the supernatant of the control and treated digested sludge are compared in Figure 8-4 (a)-(f). The most obvious change in fluorescence intensity (FI) increase was observed in the SMP-like and HA-like substances according to the Ex/Em range as introduced in Section 3.4.8. The maximum FI of the SMP-like and HA-like peaks were summarized in Table 8-

1.

The FI increase of the SMP-like substances in digested sludge increased after the tested post-treatments due to solubilization of extra- and intra- cellular polymers. The FI increase of the SMP-like substances in digested sludge was relatively insignificant after the ozone and ALK post-treatments (from 443 to 453 and 450); while, the ULS post-treatment showed obvious increase in SMP-like matters (from 443 to 620) in digested sludge which was in accordance with the biochemical results.

FI of the SMP-like substances was the highest (FI: 820) after the ULS+ALK treatment of digested sludge as shown in Table 8-1. This is in good agreement with the biopolymers results as observed in Section 8.3 and confirmed that alkali addition made the cells in digested sludge more vulnerable which resulted in more cell lysis during the ULS+ALK treatment. However, FI of the SMP-like peak in the ULS-Ozone treated digested sludge (FI: 589) was slightly lower than the ULS treated digested sludge (FI: 620), despite the higher soluble biopolymers concentration as observed in Section 8.3. This indicated the soluble biopolymers in the ULS-Ozone treated digested sludge could not be fluorescently detected. It is possible that the solubilized compounds did not have fluorescent characteristics or the fluorescence intensity was weakened at a higher redox potential due to ozonation.

Table 8-1 Maximum fluorescence intensity (FI) of the observed peaks in the supernatant of the control and treated digested sludge

Sample	SMP-Like (Ex/Em)	FI	HA-like (Ex/Em)	FI
Control	260/375	443	330/425	71
Ozone	260/380	453	330/425	73
ALK	260/380	450	330/340	91
ULS	270/375	620	330/420	122
ULS-Ozone	270/380	589	340/425	193
ULS+ALK	280/370	820	350/445	225

SMP: Soluble microbial products; HA: Humic acid; FI: Fluorescence intensity

FI of the HA-like substances were also found to increase after the treatments of digested sludge. The FI increase in the HA-like substances was relatively insignificant from 71 to 73 and 91 after the ozone and ALK treatments of digested sludge, respectively; while, the FI of the HA-like substances was increased to 122

after the ULS treatment of digested sludge. In addition, FI of the HA-like substances in digested sludge became even higher when ULS treatment was combined with the chemical methods. FI of the HA-like peaks increased to 193 and 225 after the ULS-Ozone and ULS+ALK treatments of digested sludge, respectively. Such increase in the HA-like substances is related to better disintegration of the biological flocs and as a result the HAs contained in EPS, sludge pellets and some refractory compounds were released (Yang et al., 2013, Luo et al., 2013). However, it should not be neglected that ULS+ALK post-treatment increased the pH of the digested sludge which favoured the solubilization of HAs as observed previously in Section 6.5. Although HAs are known to be persistent, such solubilization of HA-like substances is often related to better disintegration of the sludge structure.

Figure 8-4 EEM spectra of the supernatant of (a) control (b) Ozone treated (c) ALK treated sludge (d) ULS treated (e) ULS-Ozone treated (f) ULS+ALK treated digested sludge

8.6 Batch anaerobic digestion test

8.6.1 Gas production

The control, ULS, ULS-Ozone and ULS+ALK treated digested sludge were anaerobically digested to determine the improvement in sludge biodegradability due to the post-treatments. As shown in Figure 8-5 (a), the methane production from the treated digested sludge was significantly higher than the untreated digested sludge. Biodegradability (methane production from each gram of COD fed into the reactor) of the digested sludge was also improved after the post-treatments. The biodegradability of the digested sludge increased from 68.6 to 89.5, 110.4 and 97.3 mL CH₄/g COD_{added} after ULS, ULS-Ozone and ULS+ALK post-treatments, respectively. Methane production from the digested sludge increased from 54.7 to 70.2 mL CH₄ (+28.3%) after the ULS post-treatment. Such increase was much higher than that observed in Section 5.6 (around 10%) when the same ULS energy was applied to feed sewage sludge as pre-treatment. The feed sludge used for pre-treatment contained both PS and WAS in 1:1 TS ratio. These sludge, especially PS, contains more easily biodegradable solids than digested sludge. Treatment energy could be wasted on solubilizing these biodegradable components without increasing the sludge biodegradability. However, easily biodegradable solids in feed sludge were biologically degraded by anaerobic digestion. Treatment of digested sludge could focus on solubilization of the solids that are less biodegradable.

Methane production from the digested sludge improved from 54.7 to 81.1 (+48.3%) and 76.3 mL CH₄ (+39.5%) after the ULS-Ozone and ULS+ALK post-treatments, respectively. These increases in methane production were higher than that induced by the ULS post-treatment because the ULS-Ozone and ULS+ALK treatments of digested sludge released more organics for anaerobic digestion than the ULS treatment did. The batch tests results showed the ULS, ULS-Ozone and ULS+ALK post-treatments were able to improve methane production of digested sludge. As a result, the overall methane recovery from an anaerobic digestion system could

potentially be increased with greater solids destruction when treated digested sludge is re-digested (either by recycling to the original digester or to a downstream digester).

Figure 8-5 (a) Change in methane production from the control and treated digested sludge (b) Change in SCOD during the batch anaerobic digestion of the control and treated digested sludge (error bars indicate standard deviations)

8.6.2 Change in SCOD during anaerobic digestion

The change in SCOD in the serum bottles during the anaerobic digestion is as shown in Figure 8-5 (b). The SCOD in the untreated digested sludge only fluctuated slightly during the anaerobic digestion tests. This indicated the COD solubilized by the hydrolytic bacteria were immediately used for biogas production. However, SCOD dropped significantly during anaerobic digestion of the treated digested sludge. This indicated that the hydrolysis bottleneck was better overcome. However, it should be noted that anaerobic digestion of treated digested sludge also resulted in higher post-digestion SCOD than control at the end of the batch anaerobic digestion test. This is because such treatment of digested sludge could have resulted in solubilization of refractory compounds. For example, HA-like matters were solubilized after the ULS, ULS-Ozone and ULS+ALK post-

treatments as discussed in Section 8.5.

8.7 Summary

This chapter demonstrated the possibility of using ULS, ULS-Ozone and ULS+ALK treatments to disintegrate the anaerobically digested sludge to further enhance methane production. Although the ozone and ALK treatments were ineffective in disintegrating the digested sludge when applied on their own, their performance in disintegrating digested sludge was significantly improved when combined with ULS post-treatment. Synergistic COD and biopolymers solubilization was observed after the ULS-Ozone and ULS+ALK post-treatments of digested sludge. However, the synergistically solubilized substances were different in terms of molecular weights and fluorescent characteristics. Anaerobic biodegradability of the digested sludge was significantly improved after the post-treatments. Methane production from re-digesting the digested sludge was increased by 28.3%, 48.3% and 39.5% after the ULS, ULS-Ozone and ULS+ALK post-treatments, respectively in batch BMP tests after 30 days of anaerobic digestion.

Chapter 9 Effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline post-treatments on anaerobic digestion of sewage sludge in semi-continuous reactors

9.1 Introduction

The effects of ultrasonication (ULS), ultrasonication-ozonation (ULS-Ozone) and ultrasonication+alkaline (ULS+ALK) treatments on digested sludge characteristics have been introduced in Chapter 8. In this chapter, the ULS, ULS-Ozone and ULS+ALK post-treatments were integrated to semi-continuous anaerobic reactors (i.e. the post-treated digested sludge was recycled back to the reactor) at SRT of 10 and 20 days. The change in biogas production, solids removal efficiency during anaerobic digestion and properties of the post-digestion sludge were measured to assess the possible enhancement with the tested post-treatments. In addition, the economic analysis was also conducted after each tested post-treatments. The obtained results were then compared with the results collected in Chapter 7 (integration of pre-treatment) to evaluate the influence of treatment configuration on the overall anaerobic digestion performance as well as economic feasibility.

9.2 Biogas production

Daily biogas production from each reactor is as shown in Figure 9-1. The daily gas productions from the four reactors were similar in the first 20 days stabilization period (no post-treatment integrated), indicating the performance of each reactor was similar before the post-treatments were integrated. Integration of the post-treatments improved the daily biogas production from 20 days onwards as shown in Figure 9-1. The methane composition of the biogas did not show obvious difference (at around 65%) for all the reactors during application of the three conditions. This meant the post-treatment step did not impact the biogas composition. To quantitatively compare the change in biogas production increase, the daily biogas production from each reactor was averaged when the gas

production became stable as shown in Table 9-1.

Table 9-1 Summary of the reactor performance after the ULS, ULS-Ozone and ULS+ALK post-treatments

	Control	ULS	ULS-Ozone	ULS+ALK
Condition I (SRT 10 days, RR 5%)				
Daily biogas production (mL/d) (n=9)	500 ± 12*	526 ± 9	525 ± 12	541 ± 6
Post-digestion TS (mg/L) (n=5)	11460 ± 481	10720 ± 309	10830 ± 292	11390 ± 392
Post-digestion TSS (mg/L) (n=5)	9980 ± 220	9710 ± 606	9240 ± 487	9840 ± 198
Post-digestion VS (mg/L) (n=5)	8470 ± 333	7940 ± 420	8160 ± 429	8140 ± 397
Post-digestion VSS (mg/L) (n=5)	7840 ± 219	7530 ± 202	7280 ± 394	7630 ± 211
Post-digestion SCOD (mg/L) (n=5)	182 ± 6	224 ± 7	237 ± 8	220 ± 7
CST in post-digestion sludge (s) (n=3)	64.7 ± 4.3	113.8 ± 8.3	148.7 ± 7.4	128.1 ± 6.8
Condition II (SRT 10 days, RR 10%)				
Daily biogas production (mL/d) (n=9)	474 ± 8	512 ± 9	498 ± 8	526 ± 7
Post-digestion TS (mg/L) (n=5)	10960 ± 378	10780 ± 275	10500 ± 252	11300 ± 362
Post-digestion TSS (mg/L) (n=5)	9690 ± 368	9570 ± 529	9140 ± 608	9490 ± 595
Post-digestion VS (mg/L) (n=5)	8150 ± 406	7920 ± 431	7710 ± 222	7760 ± 347
Post-digestion VSS (mg/L) (n=5)	7740 ± 111	7700 ± 82	7310 ± 342	7390 ± 403
Post-digestion SCOD (mg/L) (n=5)	184 ± 11	228 ± 3	242 ± 3	234 ± 4
CST in post-digestion sludge (s) (n=3)	63.1 ± 3.3	127.4 ± 5.4	143.9 ± 6.3	131.2 ± 5.1
Condition III (SRT 20 days, RR 5%)				
Daily biogas production (mL/d) (n=11)	279 ± 5	306 ± 5	309 ± 5	329 ± 7
Post-digestion TS (mg/L) (n=5)	11820 ± 480	11320 ± 649	11470 ± 160	12230 ± 850
Post-digestion TSS (mg/L) (n=5)	10560 ± 227	9850 ± 173	9800 ± 509	9860 ± 403
Post-digestion VS (mg/L) (n=5)	8710 ± 399	8160 ± 282	8080 ± 354	8210 ± 530
Post-digestion VSS (mg/L) (n=5)	8460 ± 393	7750 ± 364	7540 ± 531	7700 ± 285
Post-digestion SCOD (mg/L) (n=5)	225 ± 6	245 ± 4	270 ± 11	246 ± 4
CST in post-digestion sludge (s) (n=3)	74.6 ± 4.4	134.8 ± 5.7	156 ± 5	143.8 ± 5.4

*numbers after the “±” stand for standard deviation

Under condition I, the increases in biogas production due to the ULS, ULS-Ozone and ULS+ALK post-treatments were 5.2%, 7.1% and 8.2%, respectively. In addition, the biogas increases due to the ULS-Ozone and ULS+ALK post-treatments were slightly higher than that induced by the ULS post-treatment. The ULS, ULS-Ozone and ULS+ALK post-treatments increased the daily biogas production by 8%, 4.9% and 11.1%, respectively under condition II. The biogas increases due to the ULS (8%) and ULS+ALK (11.1%) post-treatments were higher than the corresponding biogas production increases under condition I (5.2% and 8.2%, respectively), because more digested sludge was treated and recycled as substrate at the higher RR. However, the biogas production increase for the ULS-Ozone reactor was lower under condition II than that under condition I. Li et al. (2013) observed a decrease in biogas production when the RR (treated at: 0.1 mol/L NaOH, 30 minutes) exceeded 5%. According to Li et al. (2013), the decrease

in biogas production was related to the inactivation of anaerobic microorganisms at the higher RR. However, in this study, increase of RR from 5% to 10% only decreased the biogas production from the ULS-Ozone reactor but increased the biogas production from the ULS and ULS+ALK reactors. Therefore, the influence of RR on the biogas production was highly dependent on the treatment methods selected. In addition, no VFAs concentration was detected in the digested sludge of all the reactors under condition I and II, suggesting the post-treatments did not inhibit the methanogenesis step even when the reactors were operated at SRT of 10 days. However, it should be noted that the cost for the treatment was doubled when the amount of recycled sludge was doubled; while, the corresponding biogas production did not increase proportionally for all the tested post-treatment methods. This indicated post-treatment at RR of 10% was not economical and RR of 5% was therefore recommended. The optimum operating condition for post-treatments in terms of enhancing biogas production was condition III where the ULS, ULS-Ozone and ULS+ALK post-treatments enhanced the biogas production by 9.8%, 10.7% and 17.8%, respectively. This is likely related to the longer SRT under condition III. On one hand, the longer SRT provided sufficient time for the biodegradation of the recycled treated digested sludge. On the other hand, the digested sludge contained fewer biodegradable solids at the longer SRT. Consequently, the post-treatment energy could concentrate on slowly biodegradable solids in the digested sludge which also enhanced the overall anaerobic digestion.

Figure 9-1 Daily biogas production from the control and experiment reactors (with post-treatments)

The biogas production increase in the semi-continuous anaerobic digestion reactors due to the post-treatments and pre-treatments are compared in Table 9-2. The ULS-Ozone pre-treatment resulted in higher biogas production increase than the ULS-Ozone post-treatment at both tested SRTs. This indicated the ULS-Ozone was more suitable for treatment of feed sewage sludge than for treatment of digested sludge in enhancing biogas production. This was possibly related to the effects of the treated sludge on the anaerobic digestion process. The feed sludge acted as substrate for anaerobic digestion; whereas, the digested sludge not only acted as substrate for the anaerobic digestion but also contained active anaerobic microorganisms which were essential for the anaerobic digestion (Battimelli et al., 2003, Li et al., 2013). Consequently, selection of pre-treatment method should primarily rely on its impact on feed sludge biodegradability; while, selection of post-treatment method should not only focus on its impact on digested sludge biodegradability, but also take its negative effects on anaerobic bacteria inactivation in digested sludge into consideration. Therefore, the lower biogas

production increase observed in the ULS-Ozone post-treatment configuration was due to the inactivation or lysis of essential anaerobic microorganisms (e.g. hydrogentrophic methanogens) in the digested sludge. This could have negated its positive effects on the biodegradability improvement of the digested sludge. Similarly, the pre-treatment configuration was more advantageous than the post-treatment configuration in terms of enhancing biogas production at SRT of 10 days for the ULS and ULS+ALK treatments. In contrast, the post-treatment configuration performed slightly better at SRT of 20 days for these treatments.

Table 9-2 Performance comparison when the ULS, ULS-Ozone and ULS+ALK treatments were integrated as pre-treatments and post-treatments under different operating conditions

Parameter	Treatment	ULS	ULS-Ozone	ULS+ALK
Biogas increase (%)	Pre-treatment	20.7	35.9	24.6
	SRT: 10 days			
	Pre-treatment	7.7	25.5	16.6
	SRT: 20 days			
	Post-treatment	5.2	7.1	8.2
	SRT: 10 days, RR: 5%			
	Post-treatment	8	4.9	11
	SRT: 10 days, RR: 10%			
	Post-treatment	9.8	10.7	17.8
Solids removal efficiency increase (%)	SRT: 20 days, RR: 5%			
	Pre-treatment	7.6	18.3	15.7
	SRT: 10 days			
	Pre-treatment	9.7	21.4	18.2
	SRT: 20 days			
	Post-treatment	11.7	6.8	7.3
	SRT: 10 days, RR: 5%			
	Post-treatment	4.7	9.1	8
	SRT: 10 days, RR: 10%			
Post-digestion SCOD concentration (mg/L)	Post-treatment	9.5	10.9	8.6
	SRT: 20 days, RR: 5%			
	Pre-treatment	194 to 257	194 to 589	194 to 296
	SRT: 10 days			
	Pre-treatment	182 to 227	182 to 440	182 to 246
	SRT: 20 days			
	Post-treatment	182 to 224	182 to 236	182 to 220
	SRT: 10 days, RR: 5%			
	Post-treatment	184 to 228	184 to 242	184 to 234
	SRT: 10 days, RR: 10%			
	Post-treatment	225 to 245	225 to 270	225 to 246
	SRT: 20 days, RR: 5%			

9.3 Solids removal after anaerobic digestion

Recycling treated digested sludge to the original reactor reduced the post-digestion solids concentration as shown in Table 9-1. The average post-digestion VS concentrations of the ULS, ULS-Ozone and ULS+ALK reactors were lower than that of the control reactor; while, the post-digestion VS did not show obvious difference among the experiment reactors under the same operating condition. This indicated the ULS-Ozone and ULS+ALK post-treatments were not able to further reduce the post-digestion VS compared to the ULS post-treatment. The tested post-treatments resulted in the highest VS removals after anaerobic digestion under condition III where the increases in VS removal rates (against the feed sludge) were 9.5%, 10.9% and 8.6% due to the integration of ULS, ULS-Ozone and ULS+ALK post-treatments, respectively. This was possibly because relatively longer residence time was given for digesting the treated digested sludge under condition III (SRT 20 days) than under condition I and II (SRT 10 days).

The improvement in VS removal efficiency after anaerobic digestion due to integration of the post-treatments and pre-treatments are compared in Table 9-2. At the same SRT, the improvement of VS removal rates due to the integration of pre-treatment was higher than that due to the integration of post-treatment for most of the tested conditions. This indicated the pre-treatment configuration was relatively effective in terms of enhancing the VS destruction during anaerobic digestion compared to the post-treatment configuration. This was because the post-treatments induced cell lysis in the recycled anaerobic digested sludge. Although the treated digested sludge was recycled to sustain the same SRT, fewer microorganisms were actually available for the biodegradation of organic solids. This negated the biodegradability improvement of the digested sludge caused by the post-treatments, resulting in limited improvements in VS removals after anaerobic digestion. Therefore, greater VS reduction would be expected when the treated digested sludge is re-digested in a second digester placed in series. In addition, it should also be pointed out that the ULS and ULS-Ozone treatment cost was actually reduced

when they were used as post-treatments to treat digested sludge. The ULS energy and ozone dosage was applied on TS basis. The TS was reduced after anaerobic digestion, resulting in relatively lower energy input and chemical (ULS and ozone) consumption. These could compensate some of the solids disposal costs.

9.4 SCOD and dewaterability in digested sludge

Recycling treated digested sludge to the original reactor increased the SCOD concentration in digested sludge as shown in Table 9-1. The post-digestion SCOD concentration from the tested reactors was in the order of ULS-Ozone > ULS+ALK > ULS > control reactors which was similar to what was observed in Section 8.6.2 after the batch assay. The increase in post-digestion SCOD was because the soluble organics in the recycled treated digested could not be anaerobically degraded and accumulated in the reactor. Dewaterability of the digested sludge also deteriorated with integration of post-treatment as shown in Table 9-1. Such deterioration of the digested sludge dewaterability is because the soluble residual polymers in the post-digestion sludge kept the water from being dewatered as discussed in Section 7.3.

The impact of post-treatments and pre-treatments on post-digestion SCOD concentration is compared in Table 9-2. The ULS pre-treatments and post-treatments resulted in similar increase in the post-digestion SCOD. Likewise, the increases in post-digestion SCOD were similar due to the ULS+ALK pre-treatments and post-treatments. However, the increase in post-digestion SCOD due to the ULS-Ozone post-treatment was significantly lower than that induced by the ULS-Ozone pre-treatment. It is possible that the recalcitrant organics in the digested sludge were converted to biodegradable ones during the ULS-Ozone post-treatment and were biodegraded during the re-digestion.

9.5 Economic analysis

An economic analysis was conducted for the tested post-treatments as shown in

Table 9-3. Similar to pre-treatment, no increase in cost balance was observed for the tested post-treatments due to the high ULS cost. Furthermore, using ozone and ALK to assist the ULS post-treatment did not increase the cost balance. This indicated that the beneficial effects (i.e. biogas production increase and reduction in solids disposal cost) caused by the chemical treatments could not compensate the cost of these chemical treatments during post-treatment. Therefore, chemical treatments should not be used to assist ULS post-treatment in view of economics.

Although the highest biogas production increase was observed at SRT of 20 days and RR of 5% as mentioned in Section 8.7.1, the highest cost balance was obtained for all the reactors at SRT of 10 days and RR of 5%. This was because the volume of the reactor at SRT of 10 days was only half of the size than the reactor at SRT of 20 days. Therefore, when the same portion (5% of the reactor volume) of the sludge was treated, the energy used for treatment was also halved.

The economic analysis results of the tested post-treatments were also compared with the economic analysis results of the tested pre-treatments in Table 7-3. The increase in the recovered electricity and heats (due to biogas production increase) were higher in pre-treatment configuration than in post-treatment configuration; while, the treatment cost of in the post-treatment configuration was much lower than the cost of in the post-treatment configuration when the same sludge amount was treated. This was because the digested sludge contained lower TS. The ULS and ozone treatments were applied on TS basis. Lower energy was therefore needed when applied to the digested sludge. In addition, it was found that the reduction in sludge disposal cost was not obvious after post-treatments because of the poor performance in increasing solids removal efficiency, especially at SRT of 10 days. The values used for this economic analysis were collected from literatures and these values may vary from places. Based on the aforementioned discussions, the post-treatment configuration is recommended when the cost for ULS and ozone are high or when the reactor has a high TS removal rate and the post-digestion TS concentration is low; while, the pre-treatment configuration is recommended when the solids disposal cost is high or when the values of the electricity and heat

generated from the biogas are high.

Table 9-3 Economic analysis results for the semi-continuous anaerobic digestion tests with integration of ULS, ULS-Ozone and ULS+ALK post-treatments on annual basis

Reactor	ULS cost(\$)	Ozone cost(\$)	ALK cost(\$)	Sludge disposal(\$)	Recovered electricity(\$)	Recovered heat(\$)	Cost balance(\$)	Compared to control(\$)
SRT 10 days, RR 5%								
Control	-	-	-	48,266	71,128	281,829	304,692	-
ULS	103,689	-	-	46,960	74,827	296,484	220,622	-84,030
ULS-Ozone	104,753	15,460	-	44,687	74,685	295,921	205,705	-98,987
ULS+ALK	110,170		29,200	47,589	76,961	304,939	194,942	-109,750
SRT 10 days, RR 10%								
Control	-	-	-	46,863	67,430	267,174	287,741	-
ULS	208,539	-	-	46,283	72,835	288,593	106,607	-181,134
ULS-Ozone	203,123	14,989	-	44,203	70,844	280,702	89,231	-198,510
ULS+ALK	218,599	-	29,200	45,896	74,827	296,484	77,617	-210,124
SRT 20 days RR 5%								
Control20	-	-	-	51,071	79,379	314,521	342,830	-
ULS20	218,985	-	-	47,637	87,061	344,959	165,398	-177,432
ULS-Ozone20	221,887	16,374	-	47,395	87,915	348,341	150,599	-192,231
ULS+ALK20	236,589	-	29,200	47,685	93,605	370,887	151,017	-191,812

9.6 Summary

Biogas production and solids destruction rates during anaerobic digestion with semi-continuous reactors were enhanced when the treated digested sludge was recycled to the anaerobic reactor. The ULS+ALK had the best effectiveness among the tested treatments in terms of enhancing biogas production; while, the tested post-treatments showed similar effectiveness in decreasing the post-digestion VS. The impact of the post-treatments on the anaerobic digester performance was related to the SRT and RR of the reactors. Longer SRT and RR of 5% were recommended for the post-treatment configuration in view of process performance. The maximum daily biogas production increase observed in this work was 17.8% when the semi-continuous anaerobic reactor was integrated with the ULS+ALK post-treatment and operated at SRT of 20 days and RR of 5%.

In view of cost, neither ozone nor alkali treatment could increase the cost balance when combined with ULS post-treatments. Therefore, chemical methods should not be used to assist the ULS post-treatments. The treatment cost for the post-treatment configuration was lower in comparison to the pre-treatment configuration; while, the biogas recovery and solids reduction were more obvious for the pre-treatment configuration than the post-treatment configuration.

Chapter 10 Conclusions and recommendations for future work

10.1 Conclusions

10.1.1 Limitations of individual ultrasonication pre-treatment

- Ultrasonication (ULS) pre-treatment was able to reduce the particle size and increase the SCOD concentration in feed sewage sludge. However, its effects on COD solubilization became less obvious when the specific energy exceeded 21 kJ/g TS.
- COD fraction of soluble organics with MW over 300 kDa in feed sludge increased with increasing specific energy input. As most of these high MW macromolecules were slowly biodegradable, the first-order anaerobic digestion rate constant obtained from anaerobic digestion of the ultrasonicated feed sludge did not increase further after the specific energy input exceeded 9 kJ/g TS.
- ULS pre-treatment has limitation in improving feed sludge biodegradability, because increase in ultrasonic energy input was not proportionally related to the increase in sludge biodegradability. When the specific energy input was 7 times higher (from 9 to 63 kJ/g TS), the increase in biodegradability was only 2.2 times (from 14.8% to 31.8%) higher.

10.1.2 The synergistic mechanisms in ultrasonication-ozonation and ultrasonication+alkaline pre-treatments

- Application of ozonation after the ULS pre-treatment could further increase COD solubilization and induce synergistic VSS solubilization in feed sewage sludge. ULS helped to disperse the biological flocs and enhanced the reaction between ozone and the organic solids in sludge. Some of the high MW (MW > 500 kDa) compounds solubilized by ultrasound were ozone scavengers and effectively degraded by ozone into smaller organics.

These aforementioned synergistic effects resulted in synergistic sludge biodegradability increase.

- COD solubilization in feed sewage sludge was significantly enhanced when ALK treatment was combined with the ULS treatment. Addition of NaOH to the ULS process could enhance solubilization of the SMP-like substances by making the cell walls more vulnerable to ultrasound disruption. In addition, the hydroxyl ions were found to further degrade the macromolecules solubilized by the ULS treatment. This synergistic action generated smaller organics with MW around 5.6 kDa. HAs were also found to be released as a result of the synergistic actions between alkali and ultrasound. Due to the aforementioned positive interactions, synergistic biodegradability increase of feed sludge was observed.

10.1.3 The effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline pre-treatments on anaerobic sludge digestion in semi-continuous reactors

- The ULS-Ozone and ULS+ALK pre-treatments had higher effectiveness than the ULS pre-treatment in terms of enhancing biogas production and solids removal efficiency during anaerobic digestion of sewage sludge with semi-continuous reactors. The biogas production and solids removal efficiencies for the tested reactors were in the order of ULS-Ozone > ULS+ALK > ULS > control reactors at both SRT of 10 and 20 days.
- ULS-Ozone and ULS+ALK treatments of feed sludge could shorten the anaerobic SRT from 20 days to 10 days without adverse impact on the anaerobic digestion performance; while, the ULS pre-treatment was not able to provide such advantage.
- Soluble polymeric substances were found to accumulate in the anaerobic digested sludge following anaerobic digestion of ULS-Ozone treated feed sludge. Such digested sludge had deteriorated dewaterability. Although

some of these polymers were biodegradable at 20 days SRT, most of them were HA-like substances and persistent. In addition, biodegradability of FA-like substances was improved due to the ULS-Ozone and ULS+ALK treatments of feed sludge.

- ULS-Ozone pre-treatment resulted in higher cost balance compared to individual ULS pre-treatment; while, ULS+ALK pre-treatment did not show obvious advantages compared to individual ULS pre-treatment in terms of economics.

10.1.4 The effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline post-treatments on digested sludge characteristics

- The ozone and ALK post-treatments were poor in disintegrating the digested sludge when applied individually, but their performance in disintegrating the sludge was significantly improved when they were combined with ULS post-treatment. Synergistic COD and biopolymers solubilization was observed during ULS-Ozone and ULS+ALK treatments of the digested sludge.
- The synergistically solubilized substances were different after the ULS-Ozone and ULS+ALK treatments of digested sludge according to the MWs and the fluorescent characteristics analysis.
- Methane production from the digested sludge increased by 28.3%, 48.3% and 39.5% after the ULS, ULS-Ozone and ULS+ALK post-treatments, respectively in batch BMP tests after 30 days anaerobic digestion time.

10.1.5 The effects of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline post-treatments on anaerobic sludge digestion in semi-continuous reactors

- Recycling the ULS, ULS-Ozone and ULS+ALK treated digested sludge to

the anaerobic reactor enhanced the biogas production and solids destruction rate of the anaerobic reactors. The ULS+ALK post-treatment was the most effective treatments among the tested treatments in terms of enhancing biogas production; while, the tested post-treatments had similar performance in reducing the post-digestion VS.

- The impact of the post-treatments on the anaerobic digester performance was related to the SRT and RR of the reactors. Long SRT and RR of 5% were recommended for the post-treatment configuration. The maximum daily biogas increase obtained in this study was 17.8% when the semi-continuous anaerobic reactor was integrated with the ULS+ALK post-treatment and operated at SRT of 20 days and RR of 5%.
- In view of cost, neither ozone nor alkali treatment could increase the cost balance when combined with ULS post-treatments. Chemical treatments should not be used to assist the ULS treatment when post-treatment configuration was integrated.

10.1.6 Impact of treatment configurations on the effectiveness of ultrasonication, ultrasonication-ozonation and ultrasonication+alkaline treatments

- The ULS-Ozone pre-treatment had greater effectiveness than the ULS-Ozone post-treatment in terms of enhancing biogas production at SRT of 10 and 20 days. Similarly, the pre-treatment configuration was more advantageous than the post-treatment configuration in terms of enhancing biogas production at SRT of 10 days for the ULS and ULS+ALK treatments; while, the post-treatment configuration performed slightly better than the pre-treatment configuration at SRT of 20 days for these treatments.
- The pre-treatment configuration was more effective than the post-treatment configuration in enhancing the solids destruction during the anaerobic digestion for the tested treatments. However, the advantage of post-

treatment configuration lies on the lower treatment cost because the ULS specific energy input and ozone dosage were TS based. The digested sludge contained lower solids content compared to the feed sludge, thus a lower treatment cost was needed.

- Although treatment configurations did not have obvious impact on the post-digestion SCOD for the ULS and ULS+ALK treatments, the ULS-Ozone post-treatment did result in a lower post-digestion SCOD than the ULS-Ozone pre-treatment. The refractory compounds in the recycled digested sludge might be converted to biodegradable ones after the ULS-Ozone post-treatment. They were then biodegraded after the treated digested sludge was recycled and re-digested.

10.2 Recommendations for future work

- This study investigated the effects of ULS, ULS-Ozone and ULS+ALK treatments on the enhancement of anaerobic sludge digestion performance. Microbial biology studies, such as quantitative real-time polymerase chain reaction (Q-PCR) and pyrosequencing can be conducted in the future to understand the change in microbial communities in anaerobic reactors with the integration of the treatments.
- ULS-Ozone treatment of feed sludge was found to significantly increase the post-digestion SCOD concentration and amount of HAs in the supernatant of the anaerobic digested sludge. In some wastewater treatment plant, the supernatant of the digested sludge is recycled to the activated sludge tank. Therefore, future studies can be conducted to evaluate the impact of recycling such supernatant on the activated sludge process.
- Long SRT was found to enhance the anaerobic digestion performance with post-treatments integrated and the longest SRT tested in this thesis was 20 days. In order to further improve the anaerobic digestion performance, effects of post-treatment on anaerobic reactors with longer SRT of 25 and

30 days are recommended to be tested in future work.

- It has been indicated in this thesis that during the post-treatment configuration the inactivation of the anaerobic microorganisms in treated digested sludge had negative effects on anaerobic digestion. However, information on the sensitivity of the anaerobic microorganism in the digested sludge to each treatment method was not available. Therefore, future studies with fluorescence in situ hybridization (FISH) tests are recommended to investigate the disruption of different microbial consortia in digested sludge due to post-treatment.
- This thesis investigated the effects of ULS, ULS-Ozone and ULS+ALK post-treatments on a single-stage reactor. In a two-stage anaerobic digestion system, the treated digested sludge can alternatively be digested in a downstream anaerobic reactor (also known as inter-stage treatment) instead of being recycled to the original digester. This would overcome the negative effects (i.e. anaerobic microorganisms inactivation) caused by the post-treatment configuration. Therefore, future studies are suggested to investigate the effects of ULS, ULS-Ozone and ULS+ALK treatment when they are applied as inter-stage treatment in a two-stage anaerobic digestion system.
- This study provided a detailed comparison of the impact of the ULS, ULS-Ozone and ULS+ALK treatments on anaerobic sludge digestion. However, one of the main restrictions in this study is the efficiencies of the treatment devices. In this work, all the treatments were conducted with laboratory devices which generally had lower intensities than full-scales ones. Therefore, pilot scale or even full scale tests are needed in the future to understand the effects of the ULS, ULS-Ozone and ULS+ALK in real application and to obtain economic analysis results.

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