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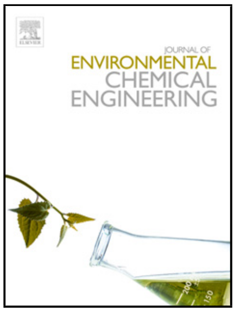

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Author: Xinbo Tian Antoine Prandota Trzcinski Leonard Lin Wun Jern Ng

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Enhancing sewage sludge anaerobic “re-digestion” with combinations of ultrasonic, ozone and alkaline treatments

Xinbo Tian[a,c], Antoine Prandota Trzcinski[b*], Leonard Lin[d], Wun Jern Ng[c]**

[a] Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore 637141, Singapore

[b] School of Civil Engineering & Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, 4350 Australia

[c] School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

[d] Public Utilities Board, Water Reclamation (Plants) Department, 20 Pioneer Road, Singapore 628507, Singapore

**Corresponding author*

Email address: antoine.trzcinski@usq.edu.au

Tel: +61 4631 1617

** WJNg@ntu.edu.sg

Abbreviations: ***AD:*** Anaerobic Digestion; ***ALK*:** Alkaline; ***COD:*** Chemical Oxygen Demand; ***CST*:** Capillary suction time (second); ***DI:*** Deionized water; ***Em***: emission wavelength (nm); ***Ex***: excitation wavelength (nm); ***FA***: fulvic acid; ***FI***: fluorescence intensity; ***HA***: humic acid; ***MW***: molecular weight (Da); ***PS:*** Primary sludge; ***Rt*:** retention time (min); ***SCOD:*** Soluble Chemical Oxygen Demand; ***SEC:*** Size Exclusion Chromatography; ***SMP***: soluble microbial products; ***SRT:*** solids retention time; ***TS:*** Total Solids; ***ULS***: ultrasonic; ***ULS-Ozone***: combined ultrasonic and ozone; ***ULS+ALK***: combined ultrasonic and alkaline; ***WAS***: waste activated sludge;

Highlights

- Median diameter of bioflocs dropped from 45.3 to 15.9 μm after ULS treatment
- SCOD increased from 200 to 2,648 mg/L after ULS+ALK post-treatment
- SMP and HA-like substances were produced as a result of combined post-treatments
- Methane production increased by 48.3% after ULS-Ozone post-treatment.
- Ozone and ALK induced synergistic effects when combined with ULS treatment.

Abstract

This study investigated the feasibility of using ultrasonic (ULS), ozone assisted ultrasonic (ULS-Ozone) and alkaline assisted ultrasonic (ULS+ALK) post-treatment to target the persistent organic fraction in anaerobically digested sludge in order to increase methane recovery. Synergistic COD solubilization in digested sludge was observed when ozone (0.012 g O_3 g^{-1} TS) and alkaline (0.02M for 10 min.) treatment was combined with ULS treatment. The digested sludge Soluble Chemical Oxygen Demand (SCOD) increased from 200 mg/L to 1,500, 2,600 and 2,650 mg/L after the ULS, ULS-Ozone and ULS+ALK treatments, respectively. Different compounds were, however, solubilized after the ULS-Ozone and ULS+ALK treatments as indicated by size exclusion chromatography (SEC). ULS+ALK treatment primarily solubilized macromolecules with molecular weight (MW) over 500 kDa; while, the ULS-Ozone treatment solubilized macromolecules with MW higher than 500 kDa and also organics with MW around 103 kDa. The methane production from "re-digestion" of the treated digested sludge increased by 28.3%, 48.3% and 39.5% after the ULS, ULS-Ozone and ULS+ALK treatments, respectively.

Keywords: Sewage sludge; ultrasound; ozone; alkaline; anaerobic digestion; post-treatment.

1. Introduction

The waste activated sludge process produces between 180 and 270 kg of sludge per megalitre of wastewater treated (Metcalf and Eddy, 2014), and the biosolids management system is considered cost-intensive as it typically accounts for 25-60% of the total operational costs of conventional activated sludge-based wastewater treatment plants (Canales et al., 1994; Verstraete & Vlaeminck, 2011). Anaerobic digestion (AD) is a commonly used technology for sewage sludge stabilization in wastewater treatment plant. Aside from the solids stabilization, biogas is produced. However, AD 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). Pre-treatment of sludge before AD is often applied to solubilize these solids to accelerate subsequent digestion. However, Takashima et al. (1996) indicated pre-treatment not only targets the slowly biodegradable solids but also the easily biodegradable solids in waste activated sludge (WAS). As a result, part of the energy and chemical input during pre-treatment would then be wasted on solubilizing the easily biodegradable organic particulates without increasing overall sludge biodegradability. Such wastage of pre-treatment energy and chemicals would be present when pre-treatment is applied to sludge containing primary sludge (PS) (e.g sewage sludge) which would contain more biodegradable solids. In view of such potential inefficiency, post-treatment of the digested sludge and thereafter digesting the treated digested sludge again could be an alternative for more economical improvement of anaerobic digestion performance (Nielsen et al., 2010, Li et al., 2013). Compared to pre-treatment, post-treatment would more specifically target the solids which are more difficult to be biodegraded in digested sludge. However, reports on post-treatment are relatively limited in numbers.

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. Therefore, pre-treatment methods are also potentially suitable for post-treatment. For example, the reported pre-treatment methods such as alkaline (ALK), ozone and thermal pre-treatment (Ray et al., 1990, Goel et al., 2003, Bougrier et al., 2006) have all been reported to be feasible for post-treatment as well (Battimelli et al., 2003, Takashima, 2008, Li et al., 2013).

Ultrasonication (ULS), a proven pre-treatment method, has, however, not been reported for post-treatment. Ultrasonic pre-treatment can be enhanced when assisted with chemical methods (Kim et al., 2010, Xu et al., 2010). This work aims to investigate the feasibility of applying ultrasonication and chemically (with alkali or ozone) assisted ultrasonication for treatment of digested sludge (i.e. post-treatment). Change in digested sludge physical properties was assessed with particle size distribution. Sludge solubilization was measured chemically in terms of Chemical Oxygen Demand (COD), proteins and carbohydrates and fluorescently in terms of soluble microbial products (SMP) and humic acid (HA). Furthermore, molecular weight (MW) distribution of the soluble substances was also investigated to shed light on the solubilization products. Batch AD tests on the treated digested sludge were then conducted to evaluate the influence of post-treatment on improvement of biodegradability if the digested sludge was digested again.

2. *Materials and Methods*

2.1. Sludge samples

Digested sludge (Total solids content or TS: around 15 g/L) was taken from a laboratory-scale semi-continuous anaerobic reactor fed with a mixture PS and WAS (at solids ratio = 1:1) with SRT of 10 days.

2.2. Analytical methods

Chemical Oxygen Demand (COD), solids concentrations and dewaterability were measured in accordance with standard methods (APHA, 1998). The particle size distribution was measured in triplicate with a particle size analyzer (Shimadzu, model SALD-3101). Sludge pH was measured with a pH meter with an accuracy of 0.01 (Agilent, model 3200P). Protein concentration was determined with Lowry's method (1951) using bovine serum albumin as standard and a UV spectrophotometer (Shimadzu, UV-1800) against a blank at 750 nm. Carbohydrate concentration was determined with the sulfuric-phenol method against a blank at 495 nm (DuBois et al., 1956). D-Glucose (Merck, Germany) was used as standard. The soluble fraction was obtained by first centrifuging sludge at 10,000 rpm for 10 minutes. The supernatant was then filtered through a 0.45 μm membrane filter before testing SCOD (Soluble Chemical Oxygen Demand).

2.3. Digested Sludge Post-treatment conditions

2.3.1 Ultrasonication treatment

ULS treatment was performed with an ultrasonicator (Misonix, Q700) at 20 kHz. During ultrasonication the temperature was monitored and maintained at below 30 °C with an ice-water bath to avoid thermal hydrolysis of sludge at higher temperature. Based on prior tests, the specific energy input was set at 9 kJ/g TS (Tian et al., 2014b). A control sludge sample without any form of post-treatment was also analysed along the tested post-treatments.

2.3.2 Ozone treatment

Ozonation was performed with an ozone generator (Wedeco, GSO 30). A stone diffuser was installed to produce fine ozone bubbles and to enhance ozone mass transfer. The applied ozone dosage of 0.012 g O_3 g^{-1} TS was also set based on prior tests (Tian et al., 2014b).

2.3.3 Alkaline treatment

Alkaline (ALK) treatment was carried out using sodium hydroxide (Sigma-aldrich). A 3M NaOH stock solution was added to the digested sludge sample to reach a concentration of 0.02M. The digested sludge samples were then mixed at 200 rpm for ten minutes at room temperature (25°C).

2.3.4 Combined treatment

For chemically assisted ULS treatment, the combination sequence was determined based on earlier tests (Tian et al., 2014a, Tian et al., 2014b). Combined ULS and ozone (ULS-Ozone) treatment was conducted by applying ozonation after ULS treatment. Combined ultrasound and alkaline (ULS+ALK) treatment was applied by ultrasonicating the sludge after NaOH addition.

2.4. Size Exclusion Chromatography (SEC)

A HPLC (Agilent Technologies 1260 LC system) was used for SEC 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. A PL aquagel-OH 8μm guard column was installed in front of the main column. The sample was first centrifuged at 10,000 rpm for 10 minutes. The supernatant was then filtered through a 0.2 μm membrane filter before injection. UV (254 nm) detector was used for detection of the eluted substances. Calibration was done using polyethylene glycol and polyethylene oxide standards with MW of 500 kDa, 70 kDa, 4 kDa, 600 Da and 106 Da.

2.5. Excitation-emission matrix (EEM) fluorescence 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 wavelength (Ex) was from 230 to 520 nm with 5-nm intervals. Emission wavelength (Em) was collected from 230 to 550 nm with 5-nm increments. Samples were pre-diluted 50 times with DI water to prevent the measured peak intensity from exceeding the maximum level.

Fluorescent compounds were detected according to their Ex and Em wavelengths as summarized by Chen et al. (2003). Peaks of simple protein-like substances were measured in the Ex/Em range of Ex < 250 nm, Em < 350 nm. Peaks of soluble microbial product (SMP)-like substances were fluorescently detected in the Ex/Em range of Ex: 250-280 nm, Em < 380 nm. Fulvic acid (FA)-like substances appeared in the Ex/Em range of Ex < 250 nm, Em > 380 nm and Humic acid (HA)-like substances were observed in the Ex/Em range of Ex > 250 nm, Em > 380 nm.

2.6. Anaerobic digestion tests

AD tests were conducted in triplicate as described by Owens et al. (1979). The ULS+ALK treated digested sludge was neutralized with 6M HCl before anaerobic digestion. 50 mL digested sludge was supplemented with 50 mL degassed inoculum and placed in a 150 mL serum bottle. Each serum bottle was flushed with nitrogen for three minutes to create anaerobic conditions. The bottles were incubated on an incubator shaker at 35°C. Biogas volume was measured with a wetted glass syringe. The biogas composition was determined with gas chromatography (Agilent Technologies 7890A GC system). Change in SCOD was monitored during the anaerobic digestion period.

2.7. Statistical analysis

The results are presented as mean ± standard deviation (SD). T-tests to determine statistical differences between treatments were carried out by comparing the critical value through ANOVA one-way analysis of variance (SPSS Statistics V17.0). Comparisons were considered significantly different at $p < 0.05$.

3. Results and Discussion

3.1. Particle size reduction

Median particle diameter results are shown in Figure 1. Chemical post-treatments without ULS post-treatment did not have a great impact on the digested sludge particle size. The median diameter slightly changed from 45.3 μm to 43.1 and 48.3 μm (statistically different at 95% confidence) after the ozone and ALK post-treatments, respectively. The slight increase in median diameter can be explained by flocs re-flocculating 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 reduced the median diameter significantly from 45.3 to 15.9 μm. ULS treatment mechanically reduced the floc whereas, chemical methods could only chemically lyze the cells or extra-polymeric substances without causing significant change in particle sizes (Bougrier et al., 2006). When the chemical methods were used in combination with ULS, the treatment resulted in statistically greater median diameter compared to the individual ULS post-treatment. This indicated combining either ozone or ALK to ULS post-treatment would not enhance floc size reduction in digested sludge.

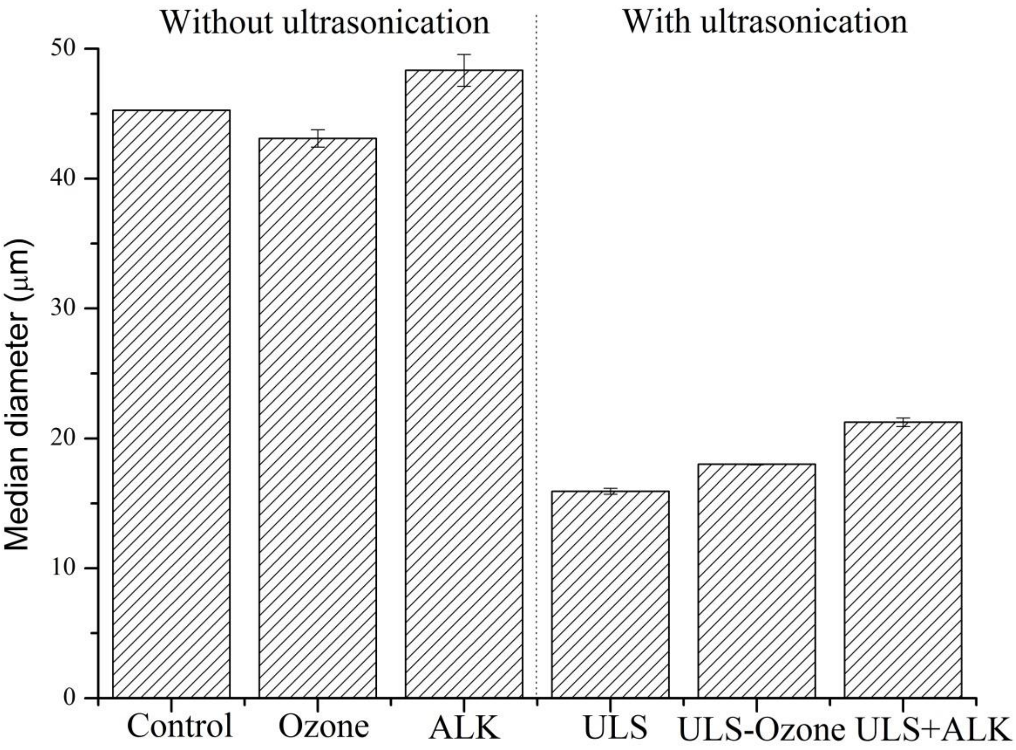

Figure 1. Effect of investigated post-treatments on the mean diameter of biological flocks in digested sludge. Error bars indicate the standard deviation of triplicate measurements.

3.2. COD and biopolymers solubilization

SCOD concentrations of the sludge increased after the post-treatment steps, as shown in Figure 2a. 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 concentrations from 200 ± 1.1 mg/L to 647.5 ± 9.7 and 501 ± 23.8 mg/L, respectively; whereas, the ULS post-treatment was able to increase the SCOD to 1,502 ± 6.5 mg/L. SCOD further increased to 2,611± 14.2 and 2,648 ± 66.1 mg/L when the ULS post-treatment was assisted with ozone and ALK post-treatment, respectively. COD solubilization by the ozone and ALK post-treatments was significantly improved when these post-treatments were combined with ULS post-treatment, as shown in Figure 2a. This suggested the chemical post-treatments induced synergistic COD solubilization when combined with ULS post-treatment. Although such synergistic SCOD increase has not been reported before for post-treatment of digested sludge synergistic SCOD solubilization had been reported for combined ultrasonication and alkaline pre-treatment of WAS (Kim et al., 2010). Kim et al. (2010) had suggested the alkali addition made the cells in WAS more vulnerable to ultrasound attack and induced synergistic COD solubilization. However, synergistic SCOD solubilization had not been observed when ULS and ozone were combined for pre-treatment, though the smaller particles created by the ULS step benefited the mass transfer of the subsequent ozonation process. Tian et al. (2014b) suggested the reaction between ozone and some of the organics solubilized by ultrasound decreased the net SCOD during combined ultrasound and ozone pre-treatment. Therefore, it is possible ozone did not readily react with the organics solubilized by ultrasound, but instead specifically solubilized the particulate organics in digested sludge during the combined ultrasound and ozone post-treatment which resulted in the synergistic COD solubilization.

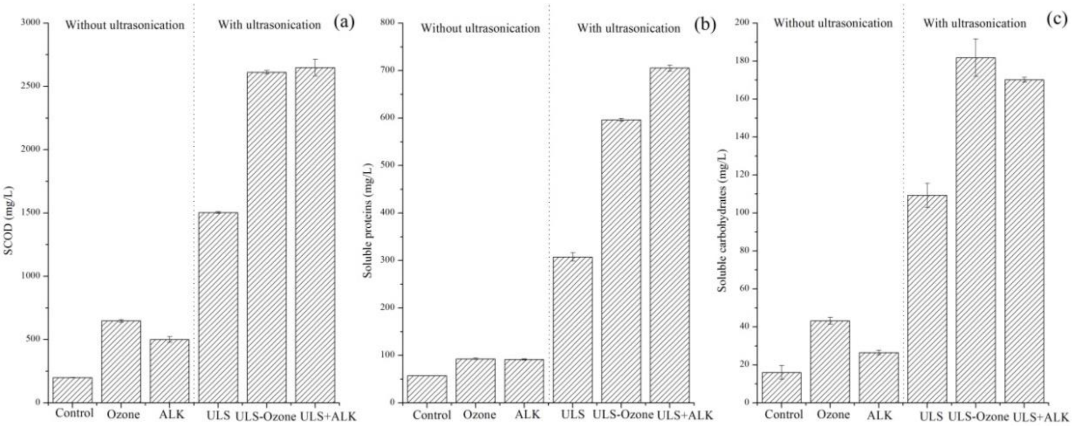

Figure 2. Change in (a) Soluble Chemical Oxygen Demand (SCOD) (b) soluble proteins (c) soluble carbohydrates due to chemical, ultrasound and chemically-assisted ultrasound post-treatments. Error bars indicate the standard deviation of triplicate measurements.

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

3.3. Molecular weight distribution

MW distribution of the standard polymer mixture solution is shown in Figure 3a. 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 samples without and with the ULS post-treatment were compared separately in Figures 3b and 3c. The MW distribution results showed good agreement with the SCOD results as stated in Section 3.2. The ozone and ALK post-treatments were relatively incapable of solubilizing the sludge and thus the corresponding peak response increases were low as shown in Figure 3b. However, the UV responses of the soluble organics were obviously higher after the ULS or chemically assisted ULS treatments, as shown in Figures 3b and 3c.

The soluble organics in the control and treated digested sludge were detected as 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. Combination of the ozone or ALK post-treatment to the ULS post-treatment obviously increased the UV responses of macromolecules, as shown in Figure 3c. However, such increases were relatively negligible when the ozone and the ALK post-treatments were individually applied, as shown in Figure 3b. This correlated well with the synergistic COD solubilization as discussed in Section 3.2 and indicated that the synergistically solubilized organics have MW higher than 500 kDa. Although similar SCOD concentrations were obtained, the MW distributions of the solubilized compounds were different after the ULS-Ozone and ULS+ALK post-treatments. For example, the ULS-Ozone post-treatment increased the UV responses of peak A, B and C; while, the ULS+ALK post-treatment only significantly increased the UV responses of peak A and had relatively little impact on the UV responses of peak B and C. This indicated the solubilized compounds due to the ULS-Ozone and ULS+ALK treatments were different.

In addition to the aforementioned macromolecules, peak D with a 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 ozone and alkaline post-treatments increased the UV responses of peak D when they were combined with ULS post-treatment. This indicated the synergistic effects in the ULS-Ozone and ULS+ALK treatments also resulted in solubilization of compounds detected in peak D. However, such increase was relatively less obvious in comparison with the increase in peak A, B and C.

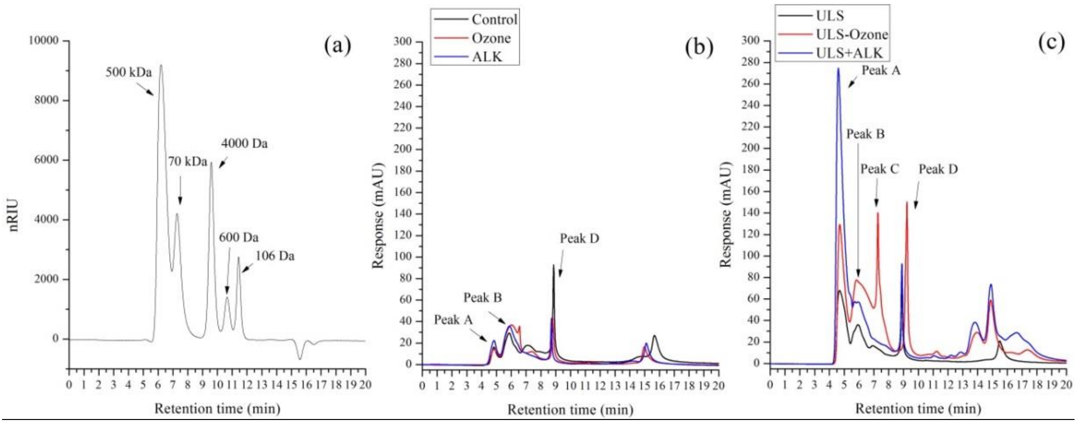

Figure 3. Molecular weight distribution chromatograms of (a) Mixture of standard polymers solution (b) control, ozone treated and ALK treated digested sludge (c) ULS treated, ULS-Ozone treated and ULS+ALK treated digested sludge

3.4. Fluorescent products characterization

The EEM spectra of the supernatant of the control and treated digested sludge are compared in Figures 4 a-f. The most obvious change of fluorescence intensity (FI) increase was observed in the SMP-like and HA-like substances according to the Ex/Em range as introduced in Section 2.5. The maximum FI of the SMP-like and HA-like peaks were summarized in Table 1.

The FI increase of the SMP-like substances increased after post-treatment due to the solubilization of extra- and intra- cellular polymers. The FI increase of the SMP-like substances was relatively insignificant after the ozone and the 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) which was in accordance with the biochemical results.

FI of the SMP-like substances was the highest (FI: 820) when ULS post-treatment was combined with ALK post-treatment, as shown in Table 1. This is in good agreement with the biopolymers results as observed in Section 3.2 and supported the conclusion that the ALK post-treatment made the cells more vulnerable which resulted in more cell lysis during ultrasonication. However, FI of the SMP-like peak in the ULS-Ozone treated sample (FI: 589) was slightly lower than the ULS treated sample (FI: 620), despite the higher soluble biopolymers concentration as observed in Section 3.2. This indicated the soluble biopolymers in the ULS-Ozone post-treated sludge could not be fluorescently detected. It is also 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 1. Maximum fluorescence intensity (FI) of the observed peaks in the supernatant of the control and post-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 post-treatments. The FI increase in the HA-like substances was relatively insignificant from 71 to 73 and 91 after the ozone and ALK post-treatments, respectively; while, the FI of the HA-like substances was increased to 122 after ULS post-treatment. In addition, FI became even higher when ULS treatment was combined with the chemical methods. FI of HA-like peaks increased to 193 and 225 after ULS-Ozone and ULS+ALK post-treatments, 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 sample which favoured the solubilization of HAs as observed previously (Tian et al., 2014a). Although HAs are known to be non-biodegradable, such solubilization of HA-like substances is often related to better disintegration of the sludge structure.

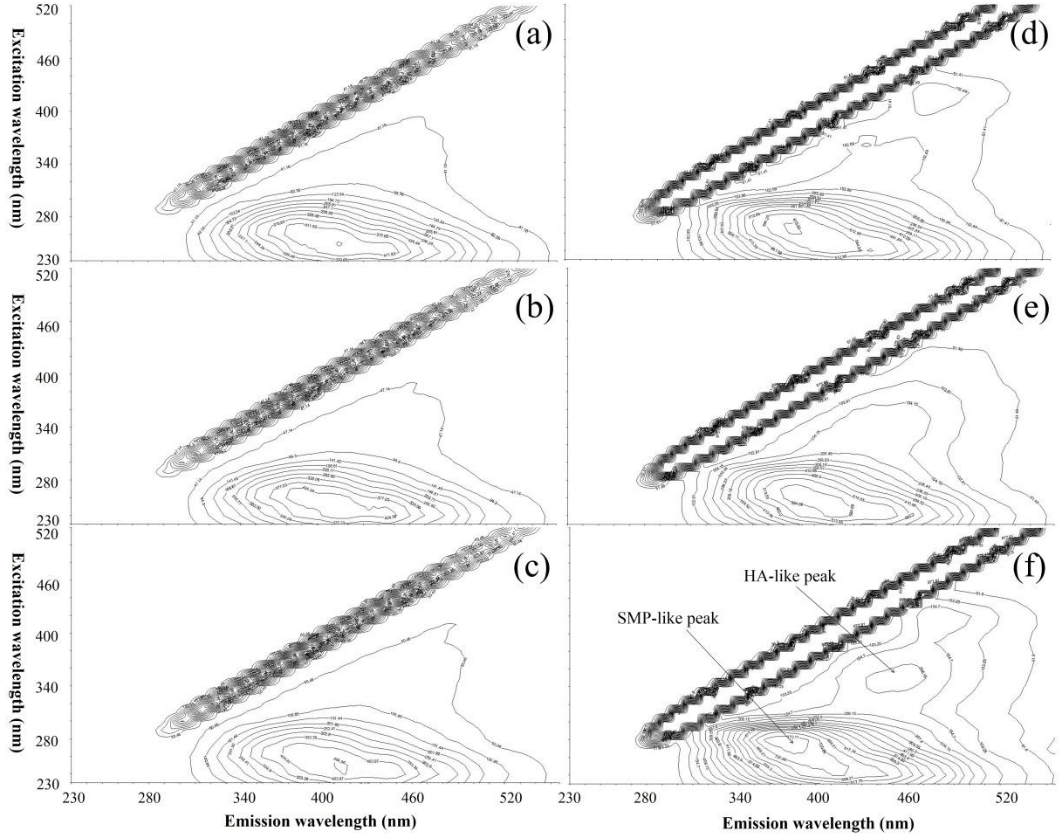

Figure 4. EEM spectra of the supernatant of (a) control (b) Ozone treated digested sludge (c) ALK treated digested sludge (d) ULS treated digested sludge (e) ULS-Ozone treated digested sludge (f) ULS+ALK treated digested sludge.

3.5. Anaerobic digestion

3.5.1. Gas production

The control, ULS, ULS-Ozone and ULS+ALK post-treated digested sludge were anaerobically digested to determine improvement in methane production due to the post-treatments. As shown in Figure 5a, methane production was significantly improved with the post-treatments. Methane produced increased by 28.3 % from 54.7 ± 0.1 to 70.2 ± 0.1 mL CH_4 after the ULS post-treatment. Such increase was much higher than that observed in our previous study (around 10%) when the same energy was applied to sewage sludge as pre-treatment (Tian et al., 2014b). The sludge used for pre-treatment contained both PS and WAS in 1:1 ratio. Energy would have been wasted on solubilizing biodegradable solids in sludge during pre-treatment without necessarily increasing the sludge biodegradability. However, easily biodegradable components in feed sludge could be biologically degraded by anaerobic digestion. Post-treatment of the digested sludge could then focus on solubilisation of the less biodegradable solids. Methane production improved from 54.7 ± 0.1 to 81.1 ± 0.5 and 76.3 ± 1.1 mL CH_4 after the ULS-Ozone and ULS+ALK post-treatments, respectively. This increase in methane production was because the ULS-Ozone and ULS+ALK post-treatments released more organics for anaerobic digestion in comparison to the ULS post-treatment. The methane production from the ULS-Ozone treated sludge was higher than that from the ULS+ALK treated sludge despite of the similar SCOD concentration as mentioned in Section 3.2. This was possibly because ozone was not only able to solubilize organics but also able to convert non-biodegradable solids into biodegradable ones; whereas, ALK treatment was unable to do so. The batch tests results showed the ULS and chemically assisted ULS post-treatments were able to improve biodegradability of digested sludge. As a result, the overall methane recovery from an AD system could potentially be increased with greater solids destruction when post-treated digested sludge is re-digested – either by recycle to the original digester or to a downstream digester.

As the ULS and chemically aided ULS post-treatment had not been reported before, the results from this work were compared with previous studies using different post-treatment methods. Takashima (2008) obtained 130% to 200% improvement in CH_4 production when 120°C thermal post-treatment was applied; while, Takashima and

Tanaka (2014) observed 172.7% - 190.9% increase in sludge biodegradability when the post-treatment was conducted in pH range of 2-6. However, the methane production increase after the ULS, ULS-Ozone and ULS+ALK treatments in this work were in the range 28.3% to 48.3%. In some studies, thermal treatment at medium (60-80°C) and high temperature (130-170°C) and pressure (up to 21 bar) resulted in biogas production increase in the range 30-80% (Barber, 2016), but the efficiency depends on the type of sludge, sludge rheology, inoculum used in the AD test and scale of the process. Compared to high temperature and pressure treatments, the methods described in this study do not require a boiler and cooling step prior to the digester and require less investment.

Aside from the different efficiencies of the compared treatment methods, such performance difference could also be attributed to the sludge age (i.e. SRT) difference of the tested digested sludge. In this work, the digested sludge was taken from a semi-continuous anaerobic reactor with SRT of 10 days in this study, while, the digested sludge used in previous studies was taken from an anaerobic digester with SRT of 20 days. As a result, the digested sludge in this work should contain more readily biodegradable solids which may absorb the treatment energy without increasing the biodegradability and weaken the treatment efficiency.

In addition, it was reported chemical methods had not enhanced the thermal post-treatment in terms of methane production. Takashima and Tanaka (2008) found addition of Na_2CO_3, H_2O_2 and HCl did not further improve the methane production following thermal post-treatment at 170°C; while, ozone only slightly improved methane production from 148 to 150 mL $CH_{4/g}$ VS_{added} (+1.3%). Similarly, Nielsen et al. (2010)

observed similar ultimate methane production when 170°C thermal treatment was applied individually (377 mL $CH_4/_g$ VS_{added}) and when it was assisted with ALK treatment at pH 10 (374 mL $CH_4/_g$ VS_{added}). However, the results of this work showed ozone and ALK treatments were good supplements to the ULS treatment by further increasing the methane production significantly. The corresponding improvements were 15.5 % (ozone: from 70.2 to 81.1 mL CH_4) and 8.7% (ALK: from 70.2 to 76.3 mL CH_4). This is possibly because combined thermal and chemical treatments would create extreme conditions which may result in formation of recalcitrant compounds; while, combined ULS and chemical treatments may not have such negative effects.

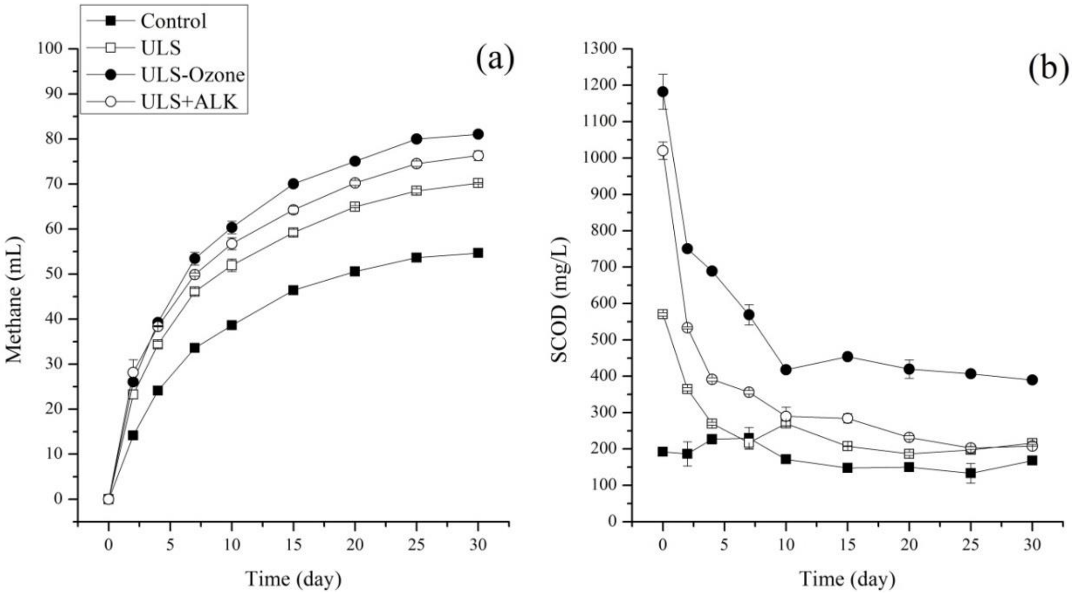

Figure 5. (a) Change of methane production from the control and post-treated digested sludge (b) Change of SCOD during the batch anaerobic digestion of the control and post-treated digested sludge. Error bars indicate the standard deviation of triplicate tests. The error bars were omitted when smaller than the marker.

3.5.2. Change in SCOD during anaerobic digestion

The change of SCOD in the serum bottles during anaerobic digestion is shown in Figure 5b. The SCOD in the control 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 post-treated digested sludge. This indicated that the hydrolysis bottleneck was better overcome after post-treatment. However, it should be noted post-treatment also resulted in higher residual SCOD at the end of the anaerobic digestion test ($p<0.05$). Such increase in residual SCOD was also observed in ULS and ULS+ALK pre-treatments (Tiehm et al., 2001, Kim et al., 2010) and thermal post-treatment (Takashima, 2008). Such treatment of the digested sludge could have resulted in solubilization of refractory compounds. For example, the solubilization of HA-like matters increased after ULS, ULS-Ozone and ULS+ALK post-treatments as discussed in Section 3.4. Luo et al. (2013) also pointed out that pre-treatment step could solubilize compounds which were further biodegraded to HAs and these HAs remained in the digested sludge due to poor biodegradability.

4. Conclusions

This work demonstrated possibility of using ULS treatment and chemically assisted ULS treatment to disintegrate anaerobically digested sludge to enhance methane production. Although the ozone and the ALK post-treatments were poor in disintegrating the digested sludge when applied on their own, their performance was significantly improved when combined with ULS post-treatment. Synergistic COD and biopolymers solubilization was observed during ULS-Ozone and ULS+ALK post-treatments of the digested sludge. However, the synergistically solubilized substances were different according to the MWs and the fluorescent characteristics analysis. Methane production from re-digesting the treated digested sludge was increased by 28.3%, 48.3% and 39.5% after the ULS, ULS-Ozone and ULS+ALK post-treatments, respectively.

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References

APHA 1998. *Standard Methods for the Examination of Water and Wastewater, 20th edition,* Washington, D.C.

APPELS, L., BAEYENS, J., DEGREVE, J. & DEWIL, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science,* 34**,** 755-781.

Barber, W. P. F. 2016**.** Thermal hydrolysis for sewage treatment: A critical review. *Water Research*, 104, 53-71.

BATTIMELLI, A., MILLET, C., DELGENES, J. P. & MOLETTA, R. 2003. Anaerobic digestion of waste activated sludge combined with ozone post-treatment and recycling. *Water Science and technology,* 48**,** 61-68.

BOUGRIER, C., ALBASI, C., DELGENES, J. & CARRERE, H. 2006. Effect of ultrasonic, thermal and ozone pre-treatments on waste activated sludge solubilisation and anaerobic biodegradability. *Chemical Engineering and Processing,* 45**,** 711-718.

Canales, A., Pareilleux, R.J.L., Goma, G., Huyard, A. 1994. Decreased sludge production strategy for domestic wastewater treatment. *Water Science and Technology*, **30**, 97-106.

CHEN, W., WESTERHOFF, P., LEENHEER, J. A. & BOOKSH, K. 2003. Fluorescence Exication-Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. *Environmental Science & Technology,* 37**,** 5701-5710.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry,* 28**,** 350-356.

EASTMAN, J. A. & FERGUSON, J. F. 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal of the Water Pollution Control Federation,* 53**,** 352-366.

GOEL, R., TOKUTOMI, T. & YASUI, H. 2003. Anaerobic digestion of excess activated sludge with ozone pretreatment. *Water Science and technology,* 47**,** 207-214.

KIM, D.-H., JEONG, E., OH, S.-E. & SHIN, H.-S. 2010. Combined (alkaline+ultrasonic) pretreatment effect on sewage sludge disintegration. *Water Research,* 44**,** 3093-3100.

LI, H., JIN, Y., MAHAR, R., WANG, Z. & NIE, Y. 2008. Effects and model of alkaline waste activated sludge treatment. *Bioresource Technology,* 99**,** 5140-5144.

LI, H., ZOU, S., LI, C. & JIN, Y. 2013. Alkaline post-treatment for improved sludge anaerobic digestion. *Bioresource Technology,* 140**,** 187-191.

LOWRY, O., ROSEBROUGH, N., FARR, A. & RANDALL, R. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* , 193**,** 265-275.

LUO, K., QI YANG, XIAO-MING LI, HONG-BO CHEN, XIAN LIU, GUO-JING YANG & GUANG-MING ZENG 2013. Novel insights into enzymatic-enhanced anaerobic digestion of waste activated sludge by three-dimensional excitation and emission matrix fluorescence spectroscopy. *Chemosphere,* 91**,** 579-585.

Metcalf & Eddy, AECOM 2014. *Wastewater engineering: treatment and resource recovery,* page 392. Fifth Edition. McGraw-Hill. New York.

NIELSEN, H. B., ANDERS THYGESEN, THOMSEN, A. B. & SCHMIDT, J. E. 2010. Anaerobic digestion of waste activated sludge - comparison of thermal pretreatments with thermal inter-stage treatments. *Journal of Chemical Technology and Bioechnology,* 86**,** 238-245.

OWENS, W. F., STUCKEY, D. C., HEALY, J. B., YOUNG, L., Y & MCCARTY, P. L. 1979. Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity. *Water Research,* 13**,** 485-492.

PAVLOSTATHIS, S. G. & GIRALDO-GOMEZ, E. 1991. Kinetics of Anaerobic Treatment. *Water Science and Technology,* 25**,** 35-59.

RAY, B. T., LIN, J.-G. & RAJAN, R. V. 1990. Low-Level Alkaline Solubilization for Enhanced Anaerobic Digestion. *Research Journal of the Water Pollution Control Federation,* 62**,** 81-87.

TAKASHIMA, M. 2008. Examination on Process Configurations Incorporating Thermal Treatment for Anaerobic Digestion of Sewage Sludge. *Journal of Environmental Engineering,* 134**,** 543-549.

TAKASHIMA, M., KUDOH, Y. & TABATA, N. 1996. Complete anaerobic digestion of activated sludge by combining membrane separation and alkaline heat post-treatment. *Water Science and Technology,* 34**,** 477-481.

TAKASHIMA, M. & TANAKA, Y. 2008. Comparison of thermo-oxidative treatments for the anaerobic digestion of sewage sludge. *Journal of Chemical Technology & Biotechnology,* 83**,** 637-642.

TAKASHIMA, M. & TANAKA, Y. 2014. Acidic thermal post-treatment for enhancing anaerobic digestionof sewage sludge. *Journal of Environmental and Chemical Engineering,* 2**,** 773-779.

TIAN, X., CHONG WANG, ANTOINE P. TRZCINSKI, LEONARD LIN & WUN JERN NG 2014a. Insights on the solubilization products after combined alkaline and ultrasonic pre-treatment of sewage sludge. *Journal of environmental Sciences,* In press.

TIAN, X., CHONG WANG, ANTOINE P. TRZCINSKI, LEONARD LIN & WUN JERN NG 2014b. Interpreting the synergistic effect in combined ultrasonication-ozonation sewage sludge pre-treatment. *Chemosphere,* In press 10.1016/j.chemosphere.2014.09.007.

TIEHM, A., NICKEL, K., ZELLHORN, M. & NEIS, U. 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Research,* 35**,** 2003-2009.

Verstraete, W., Vlaeminck, S.E. 2011. ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. *International Journal of Sustainable Development & World Ecology*, **18**(3), 253-264.

WU, B., KITADE, T., CHONG, T. H., UEMURA, T. & ANTHONY, G. F. 2011. Impact of membrane bioreactor operating conditions on fouling behavior of reverse osmosis membranes in MBR-RO processes. *Desalination,* 311**,** 37-45.

XU, G., CHEN, S., SHI, J., WANG, S. & ZHU, G. 2010. Combination treatment of ultrasound and ozone for improving solubilization and anaerobic biodegradability of waste activated sludge. *Journal of hazardous materials,* 180**,** 340-6.

YANG, S.-S., GUO, W.-Q., MENG, Z.-H., ZHOU, X.-J., FENG, X.-C., ZHENG, H.-S., LIU, B., REN, N.-Q. & CUI, Y.-S. 2013. Characterizing the fluorescent products of waste activated sludge in dissolved organic matter following ultrasound assisted ozone pretreatments. *Bioresource Technology*, 131**,** 560-563.