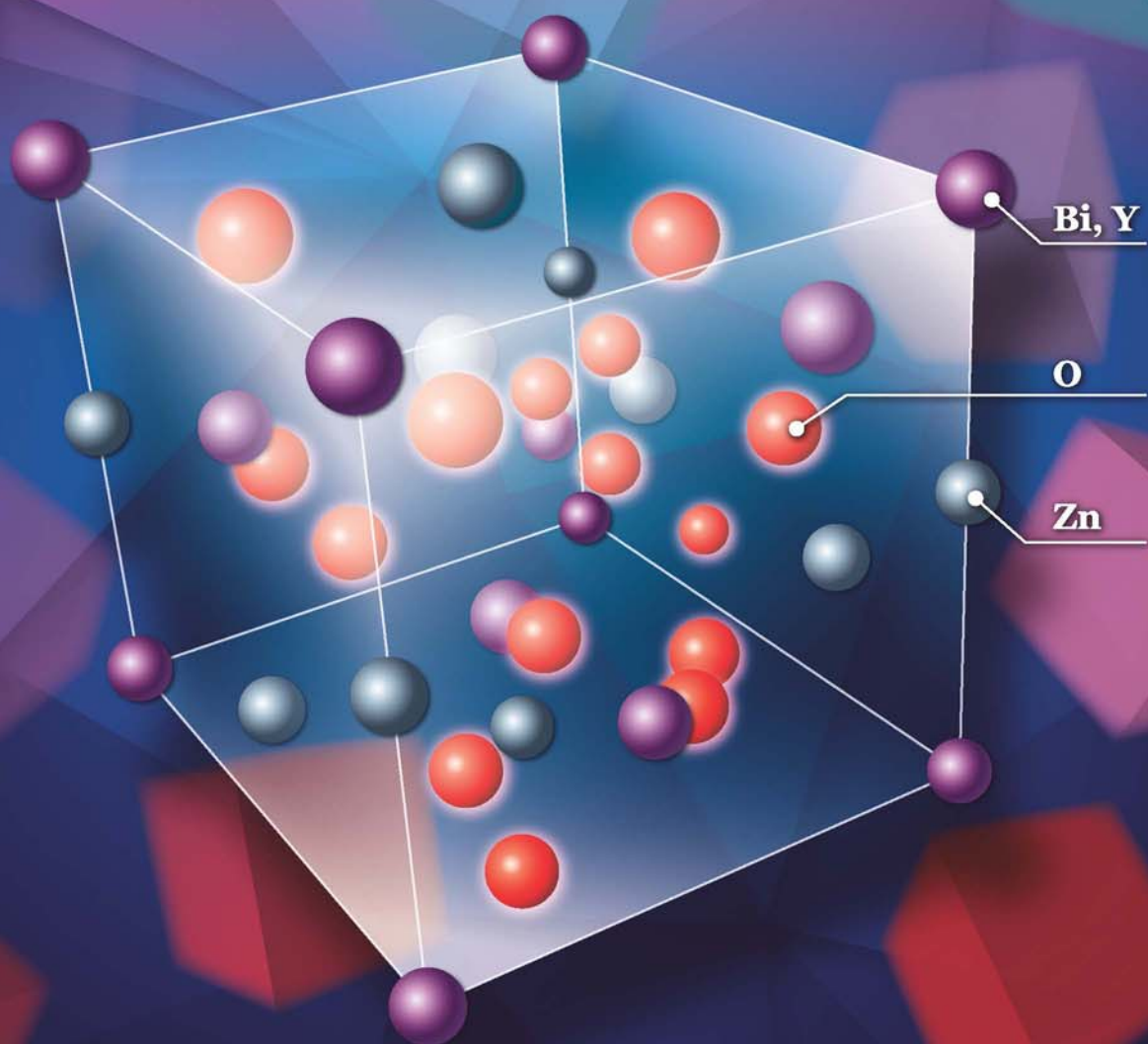


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Insights on the solubilization products after combined alkaline and ultrasonic pre-treatment of sewage sludge

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ABSTRACT

This work provides insights on the solubilization products after a simultaneous combination of alkaline and ultrasonic (ALK + ULS) pre-treatment of sewage sludge. Soluble chemical oxygen demand (SCOD) increased from 1200 to 11,000 mg/L after such treatment. Organics with molecular weight around 5.6 kDa were solubilized because of the synergistic effect of ultrasound and alkali. Organics with molecular weight larger than 300 kDa increased from 7.8% to 60%, 16% and 42.3% after ULS, ALK and ALK + ULS treatment, respectively. Excitation emission matrix fluorescence spectroscopy analysis identified soluble microbial product-like and humic acid-like matters as the main solubilization products. Sludge anaerobic biodegradability was significantly enhanced with the simultaneous application of ALK + ULS pre-treatment. ALK + ULS pre-treatment resulted in 37.8% biodegradability increase compared to the untreated sludge. This value was higher compared to the biodegradability increase induced by individual ALK pre-treatment (5.7%) or individual ULS pre-treatment (20.7%) under the same conditions applied.

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Introduction

Sludge is a by-product of wastewater treatment which requires further treatment and disposal. Anaerobic digestion is often used given its advantages of high degree of organic stabilization and methane production (Gronroos et al., 2005). Anaerobic digestion is, however, a relatively slow process. Much of the sludge organics are particulate macromolecules and can only pass through cell membranes and be utilized by microorganisms when hydrolyzed into soluble simple organics (Pavlostathis and Giraldo-Gomez, 1991). Hydrolysis of particulate macromolecules is the rate-limiting step in

sludge anaerobic digestion (Eastman and Ferguson, 1981; Pavlostathis and Giraldo-Gomez, 1991). The hydrolysis of waste activated sludge (WAS) is especially slow because of its composition. WAS comprises intact microbial cells which are enclosed by extracellular polymeric substances (EPS) and other organic fibers. This complex structure protects microorganisms from being lysed and thus slows hydrolysis. In order to overcome this rate-limiting step, pre-treatment processes are often applied to solubilize the sludge for anaerobic digestion.

Ultrasonic (ULS) pre-treatment, a mechanical process, has been reported to be an effective sludge pre-treatment method (Tiehm et al., 1997). The collapse of cavitation bubbles during

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ultrasonication imposes substantial hydro-mechanical shear force on the particulate matters in sludge. This mechanical force breaks up biological flocs and ruptures microbial cells, resulting in solubilization of intracellular and extracellular polymers.

Chemical oxygen demand (COD) solubilization during ultrasonication process could be significantly enhanced with the aid of NaOH addition (Chiu et al., 1997). Wang et al. (2005) confirmed that higher ultrasonic sludge disintegration was obtained at a higher pH with the multi-variable linear regression method. Synergistic increase in sludge disintegration degree (DD) was observed when alkaline (ALK) and ULS pre-treatments were applied simultaneously (Kim et al., 2010). Notwithstanding these reports, there is a relative lack of fundamental knowledge on the characteristics of the solubilized compounds following such combined pre-treatment process. This work aims to shed more light on the solubilization products in terms of molecular weights (MWs) and fluorescence intensities.

MWs of the solubilized organics are important as these may impact on the downstream biological process (Eskicioglu et al., 2006). Size exclusion chromatography (SEC) has been used to measure MW of organic substances in water samples (Aquino et al., 2006; Her et al., 2003; Trzcinski et al., 2011). However, such approach has not yet been reported for the assessment of the change in soluble organics due to a pre-treatment step. Therefore, SEC measurement was conducted in this work to characterize the MW of solubilized substances after pre-treatment. Afterwards, the soluble organics were fractionated into different MW ranges with ultrafiltration (UF) membranes. COD of each MW fraction was determined to complement the SEC results. Excitation emission matrix (EEM) was an emerging technique used to characterize the solubilization products after the sludge pre-treatment process (Luo et al., 2013; Yang et al., 2013). However, relevant research on its application to characterize the solubilization products following ALK + ULS pre-treatment has not been reported. Therefore, EEM fluorescence spectroscopy analysis was conducted in this study to fill the information gap. Fluorescence intensities of soluble microbial product (SMP) and humic acid (HA) substances were measured to provide novel insights on the solubilization products after ALK + ULS pre-treatment. Anaerobic digestion tests were also conducted to investigate the influence of the solubilized substances on subsequent anaerobic digestion.

1. Materials and methods

1.1. Sludge samples

Samples of a mixture of primary sludge and thickened WAS (ratio around 1:1 based on dry solids, total solids (TS): 15–17 g/L) were collected from a local municipal used water treatment plant. Sludge pH was relatively constant, varying from 6.3 to 6.5.

1.2. Analytical methods

COD was measured in accordance with Standard Methods (APHA, 1998). Protein concentration was determined with Lowry et al. (1951) using a UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) against a blank at wavelength 750 nm. Bovine serum albumin was used as the standard. Carbohydrate concentration was determined with the sulfuric-phenol method (DuBois et al., 1956) against a blank at wavelength 495 nm. D-Glucose was used as the standard. Protein and carbohydrate concentrations were converted to equivalent COD concentration with factors of 1.5 and 1.07, respectively as described previously (Rittman and McCarty,

2001). TOC was analyzed with a Multi N/C 2100S TOC analyzer (Analytik-Jena, Jena, Germany). Sludge pH was measured with a pH meter (3200P, Agilent, Santa Clara, CA, USA). Sludge DD was expressed as the extent of sludge solubilization. It was calculated as follows (Muller et al., 1998):

$$DD = (\text{SCOD}_T - \text{SCOD}_0) / (\text{SCOD}_{\text{NaOH}} - \text{SCOD}_0) \times 100\% \quad (1)$$

where SCOD_T is the soluble chemical oxygen demand (SCOD) of treated sample, $\text{SCOD}_{\text{NaOH}}$ 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.

1.3. Sludge pre-treatments

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. Various NaOH concentrations were achieved by adding different volumes of stock solution to the sludge sample. Applied NaOH concentrations were 0.01, 0.02, 0.05 and 0.1 mol/L which corresponded with NaOH dosages of 0.025, 0.05, 0.125 and 0.25 g NaOH/g TS, respectively. The sludge samples were then mixed at 200 r/min for 10 min at room temperature (25°C).

ULS pre-treatment was performed with an ultrasonicator (Q700, Misonix Qsonica, Newton, CT, USA). The ultrasound frequency was 20 kHz with a maximum power input at 700 W. The power input was around 130 W when applied to a treated sludge volume of 200 mL. Ultrasonication energy was quantified in terms of specific energy input and the calculation is as follows (Lehne et al., 2001):

$$\text{Specific energy input} = (P \times t) / (V_{\text{sludge}} \times \text{TS}) \quad (2)$$

where, P (W) is the power input of the ultrasonicator, t (sec) is the time of ultrasonication, and V_{sludge} (L) is the volume of treated sludge. During ultrasonication, the temperature of the sample was monitored and maintained at about 30°C with an ice-water bath. The maximum specific energy input was 21 kJ/g TS.

ALK and ULS pre-treatments were conducted simultaneously (ALK + ULS), as ALK + ULS treatment had been reported to have higher impact on sludge compared to sequential combinations (Chiu et al., 1997; Jin et al., 2009). ALK + ULS pre-treatment was performed by sonicating the sludge while it was being mixed at a designated NaOH concentration.

1.4. Size exclusion chromatography

A high performance liquid chromatography (HPLC) (1260 LC system, Agilent, Santa Clara, CA, USA) was used for the SEC analysis using the PL aquagel-OH 8 μm MIXED-M column. Milli-Q water was used as mobile phase with a flow rate of 1 mL/min. A PL aquagel-OH 8 μm guard column was installed in front of the main column. The sample was first centrifuged at 10,000 r/min for 10 min. The supernatant was then filtered through a 0.2 μm membrane filter before injection. UV (254 nm) detector was used for the 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. A linear

relationship was derived between the log value of MW (Da) and retention time (Rt, min) with a correlation coefficient of 99.2%:

$$\log(\text{MW}) = 9.8223 - 0.6748 (\text{Rt}) \quad (3)$$

1.5. Apparent molecular weight distribution

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

1.6. EEM fluorescence spectroscopy analysis

EEM fluorescence spectroscopy is not only a sensitive but also a selective technique which is ideal for complex environmental samples (Luo et al., 2013). It detects target substances based on their Excitation (Ex) and Emission (Em) wavelengths without destroying samples (Luo et al., 2013).

A fluorescence spectrometer (LS 55, Perkin Elmer, Waltham, MA, 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). Ex was from 230 to 520 nm with 5-nm intervals. Em was collected from 230 to 550 nm with 5-nm increments. Samples were pre-diluted 100 times 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 literatures. Peaks of simple protein-like substances such as tyrosine proteins appeared in Ex/Em range of Ex < 250 nm, Em < 350 nm (Chen et al., 2003; Determann et al., 1994). Fulvic acid (FA)-like substances were detected in Ex/Em range of Ex < 250 nm, Em > 380 nm (Chen et al., 2003; Determann et al., 1994; Her et al., 2003). Peaks of SMP-like substances fell in Ex/Em range of Ex: 250–280 nm, Em < 380 nm (Baker, 2001; Chen et al., 2003; Coble, 1996; Determann et al., 1994). HA-like substances were detected in Ex/Em range of Ex > 250 nm, Em > 380 nm (Coble, 1996; Determann et al., 1994; Her et al., 2003; Mobed et al., 1996).

1.7. Anaerobic biodegradability determination

Biochemical methane potential (BMP) assays were conducted in serum bottles to quantify sludge anaerobic biodegradability (Owens et al., 1979). For ALK and ALK + ULS pre-treated samples, the pH was pre-adjusted with 0.1 mol/L HCl for the BMP assays. 10 mL substrate sludge and 30 mL seed sludge (degassed at 35°C for one week) were added into the serum bottles. A mixture of 20% CO₂ and 80% N₂ was used to purge each bottle for 3 min to create an anaerobic environment. All bottles were incubated in an incubation shaker at 35°C. The biogas volumes were regularly measured using a wetted glass syringe. The biogas composition was determined with gas chromatography (7890A GC system, Agilent, Santa Clara, CA, USA).

2. Results and discussion

2.1. Sludge solubilization in individual pre-treatments

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 respectively. As shown in Fig. 1a, the SCOD concentrations were 1300, 1700, 3400 and 4800 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 (Hu et al., 2009; Katsiris and Kouzeli-Katsiri, 1987).

The ULS pre-treatment caused considerable sludge disintegration even at low specific energy input. As shown in Fig. 1b, 6 kJ/g TS ultrasonication was able to increase the SCOD concentration to 2400 mg/L which corresponded to a DD of 11.7%. The SCOD concentration increased linearly with the specific energy input. SCOD concentration reached approximately 4700 mg/L and the corresponding DD was 32.8% after 21 kJ/g TS ultrasonication.

2.2. Sludge solubilization in combined pre-treatment

Change of SCOD concentration during the combined treatment is shown in Fig. 2a. The SCOD concentration increased with the increase in both the specific energy input and the applied NaOH concentration. These results were in good accordance with a previous study when the ALK + ULS treatment was conducted in

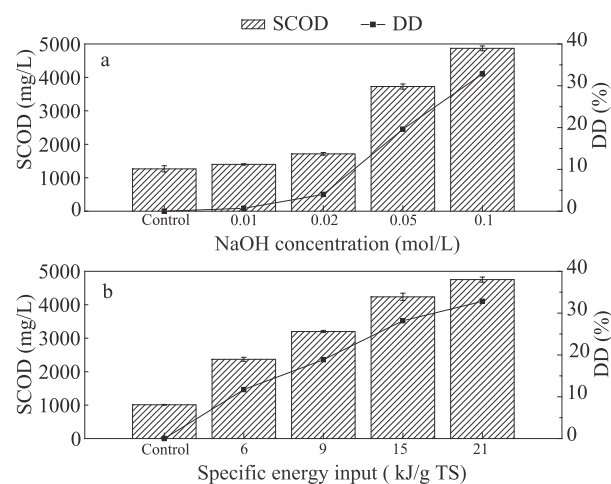


Fig. 1 – Change in soluble chemical oxygen demand (SCOD) and disintegration degree (DD) with applied NaOH concentrations (a), and change in SCOD and DD with specific energy inputs (b).

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 min ULS pre-treatment increased from around 400 to 2000 mg/L when the sludge pH was increased from 6.8 to 12. The maximum SCOD concentration 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 organic solubilization. The concentrations of soluble proteins and carbohydrates increased significantly in the combined treatment as shown in Fig. 2b and c. Proteins, the principle components of cells, were solubilized as a result of cell lysis (Kim et al., 2010; Wang et al., 2006). The solubilization of polysaccharides in cell walls and EPS contributed to the increase in soluble carbohydrates concentration (Wang et al., 2006). Significant solubilization of proteins due to ALK + ULS pre-treatment was observed by Liu et al. (2008). In their study, around 67% of proteins in WAS sample was solubilized when ULS treatment was conducted at pH 12 for 60 min. The DD increased to 50%, 67%, 80% and 91% when 21 kJ/g TS ultrasonication was conducted at NaOH concentrations of 0.01, 0.02, 0.05 and 0.1 mol/L, respectively as shown in Fig. 2d. 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 addition of NaOH to the ultrasonication process obviously induced synergistic 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 DDs induced by the ULS and the ALK treatment alone were only 19.5% and 21.4%, respectively. A possible reason of 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). However, under the aforementioned combination condition, the damage to sludge caused by the alkaline pre-treatment was limited (DD: 0.8%). Therefore, apart from the enhancement of the mechanical disintegration due to ultrasound, it is possible that alkali addition also enhanced the sonochemical effects of cavitation effect through the formation of radicals. The solubilized organics were then subsequently further characterized to seek for the possible synergistic mechanisms.

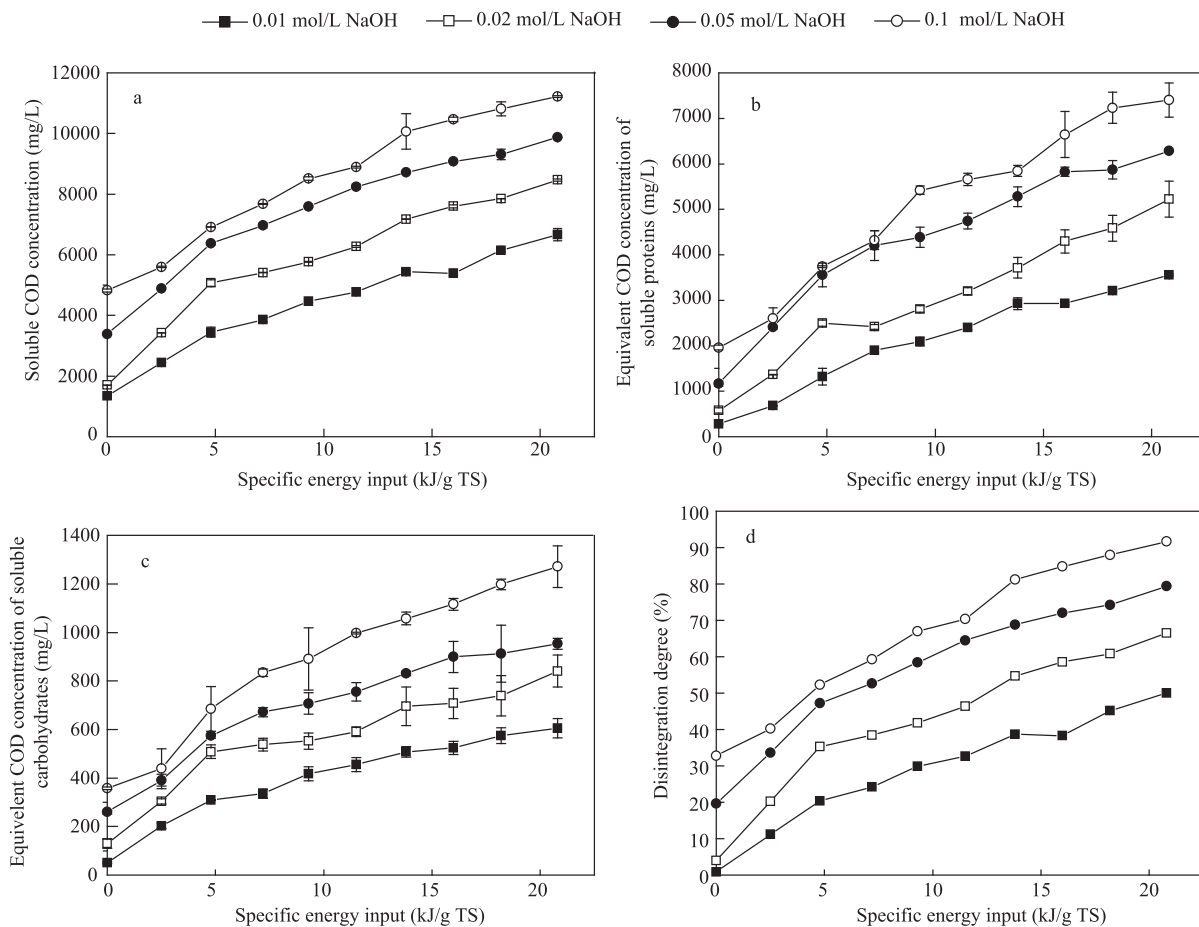


Fig. 2 – Change in soluble chemical oxygen demand (SCOD) concentration (a) equivalent chemical oxygen demand (COD) concentration of soluble proteins (b) equivalent COD concentration of soluble carbohydrates (c) disintegration degree (d) with specific energy input at different applied NaOH concentrations.

2.3. Molecular weight distribution

MW distribution chromatograms of control, ALK pre-treated (0.05 mol/L), ULS pre-treated (21 kJ/g TS) and ALK + ULS pre-treated sludge are shown in Fig. 3a. The retention time of the standard polymers are shown for comparison. 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 (Aquino et al., 2006; Trzcinski et al., 2011). As shown in Fig. 3a, compounds with retention time shorter than 6 min were solubilized in all the pre-treated samples. 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 min were also solubilized (the earliest peak was from the ULS pre-treated sludge, but it was not obviously noted due to the large scale difference compared to the ALK + ULS pre-treated sludge). The corresponding MW of these compounds was less than 27 kDa according to the calibration equation as introduced in Section 1.4. A large UV response at retention time around 9 min (MW: 5.6 kDa) was observed in the ALK + ULS pre-treated sample. However, such peak was not observed in either of the individual applied ALK or ULS pre-treated sludge. Therefore, solubilization of these substances was likely due to the synergistic effects between ULS and ALK pre-treatments. Using SEC to characterize the solubilization products after sludge pre-treatment has not been reported previously and could provide insights on the MW distribution change 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 Fig. 3b. The TOC results showed good agreement with the SEC results as presented in Fig. 3a. The TOC fraction of components with MW larger than 300 kDa increased after the pre-treatment process, indicating

solubilization of macromolecules. This TOC fraction was only 7.8% in the control sludge and had increased to 60%, 16% and 42.3% after ALK, ULS and ALK + ULS pre-treatments, respectively. A review of the literature would suggest that UF fractionation on the supernatant of ALK + ULS treated 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 treatment, respectively. It should be noted that the COD is a general indicator which is influenced not only by organics but also by inorganic interferences such as ammonium. Therefore, the results of this work confirmed that the increase had been due to organics instead of inorganics.

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% (ALK + ULS) when it was combined with ULS treatment. This was likely because the macromolecules solubilized by the ULS pre-treatment could be chemically degraded by the hydroxyl ions during the ALK + ULS treatment (Şahinkaya and Sevimli, 2013). Examples of such degradation would be the saponification reactions of lipids and the alkaline hydrolysis of proteins. Meanwhile, simpler organics were formed as degradation products. This accounted for the compounds observed at retention time of 9 min (MW: 5.6 kDa) in the supernatant of the ALK + ULS treated sample as shown in Fig. 3a.

2.4. Fluorescent products characterization

Fluorescent products were characterized with EEM fluorescence spectroscopy to provide more insights on the solubilized compounds. As shown in Fig. 4, each spectrum was

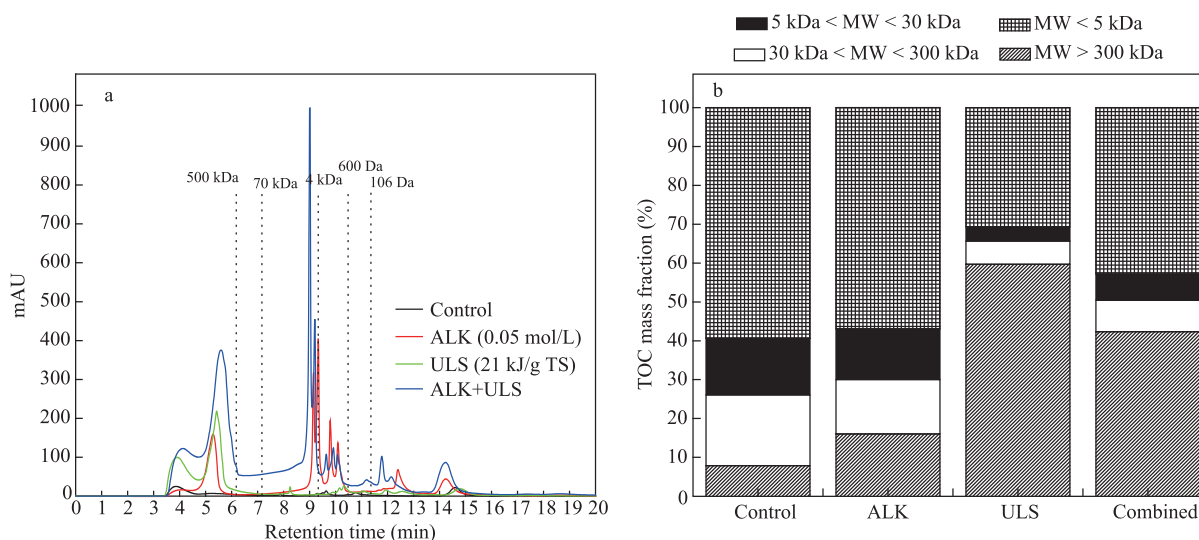


Fig. 3 – (a) Molecular weight distribution chromatograms of control, alkaline (ALK) and ultrasonic (ULS) and ALK + ULS pre-treated sludge in UV 254 nm signal, (b) TOC (total organic carbon) mass fraction of each molecular weight range in control and pre-treated sludge.

divided into I, II, III, IV and V as summarized by Chen et al. (2003). According to the Ex/Em range described in Section 1.6, peaks in Regions I and II represent simple protein like substances and peaks in Region III represent FA-like substances. However, no obvious peaks were observed in Regions I, II and III in both control and pre-treated sludge samples as shown in Fig. 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 Fig. 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 the control and the pre-treated sludge samples are shown in Table 1. FI of the SMP-like peak only slightly 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 ultrasonication process further enhanced the SMP-like substance solubilization. FI of the observed SMP-like peak in ALK + ULS (0.05 mol/L + 21 kJ/g TS) pre-treated sludge was 839 which was 9.2% higher than that in the ULS pre-treated sludge (FI: 768). These results confirmed the positive interactions between the ALK and ULS pre-treatments in terms of solubilizing microbial products and this correlated well with the high concentration of soluble proteins and carbohydrates as shown in Fig. 2b and c.

Peaks of HA-like substances in Region V were also observed in all the spectra. FI of the HA-like peak was found to increase after various pre-treatments and the FIs of the observed peaks are listed in Table 1. Individual ALK or ULS

Table 1 – Fluorescence intensity of the observed peaks in control and pre-treated sample.

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
Combined	290/360	839	350/440	201

SMP: Soluble microbial products; HA: humic acid; FI: fluorescence intensity.

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 ALK + ULS pre-treated sludge as highlighted by a red ellipse in Fig. 4d. FI of the highlighted peak was 201 as shown in Table 1. This was higher than that in the ALK pre-treated (FI: 91), the ULS pre-treated sludge (FI: 80) and even their sum (i.e.: 171). This indicated that 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 activate biomass (Esparza-Soto and Westerhoff, 2003). HA was known to be soluble in basic condition but 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 the 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.

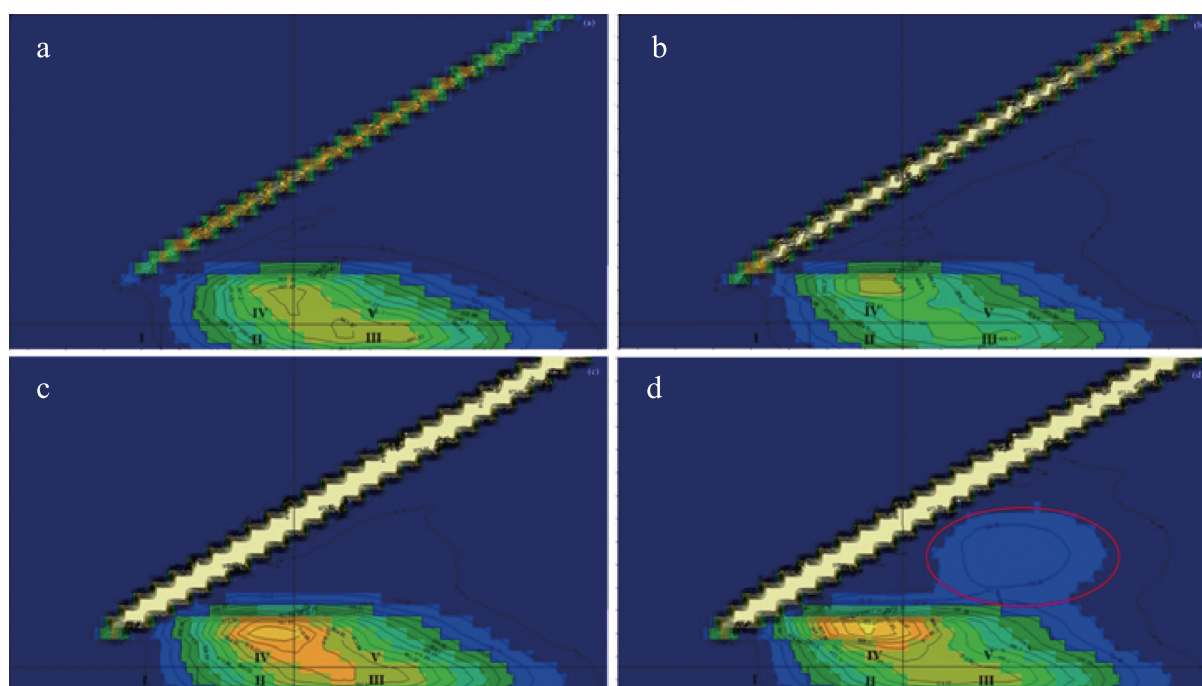


Fig. 4 – EEM spectra of control sludge (a) Alkaline (ALK) pre-treated sludge (0.05 mol/L) (b) Ultrasonic (ULS) pre-treated sludge (21 kJ/g TS) (c) and ALK + ULS pre-treated sludge (0.05 mol/L + 21 kJ/g TS) (d).

Thus, the corresponding FI increases in both individual ALK and individual ULS pre-treatment were not obvious. When ultrasonication was performed under a basic condition, HA was mechanically set free by ultrasound and could also be solubilized in the alkaline solution (pH: 12.2). The solubilized HAs then contributed to the synergistic COD solubilization as mentioned in Section 2.2. This synergistic solubilization of HA-like substances may also have influenced the subsequent anaerobic digestion.

2.5. Anaerobic biodegradability

BMP assay was used to test the change in sludge anaerobic biodegradability change after various pre-treatment processes. As shown in Fig. 5, the anaerobic biodegradability of all the pre-treated samples 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 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. However, the DDs obtained by 0.02 and 0.05 mol/L ALK pre-treatment were 4.0% and 19.6% as shown in Section 2.1. 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 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 ultrasonication. However, the anaerobic biodegradability only increased to 212.2 mL CH₄/g COD_{added} (+20.7%) when the specific energy input was double 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 aforementioned limitations in individual pre-treatment were not observed after the combined pre-treatment. The

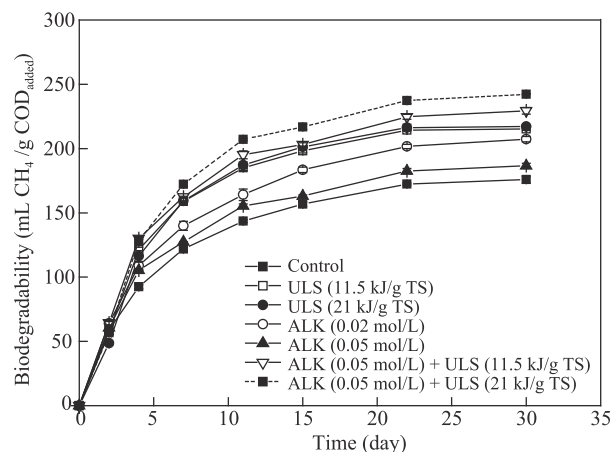


Fig. 5 – Biochemical methane potential (BMP) results of control, alkaline (ALK), ultrasonic (ULS) and ALK + ULS pre-treated sludge.

ultimate sludge anaerobic biodegradability increased from 175.8 to 229.4 (+30.5%) and 242.3 (+37.8%) mL CH₄/g COD_{added} by combining 11.5 and 21 kJ/g TS ULS pre-treatment to 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 rather than keeping increasing NaOH dosage or ULS energy. The BI in this work was slightly lower compared to the previous results from Kim et al. (2010). One possible reason is that 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 sludge to be digested; the relative increase in the pre-treated 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 solid retention time of 20 days. This was confirmed by the results of Seng et al. (2010) where the methane productions of ALK + ULS pre-treated sludge were 17.3%, 31.1% and 42.1% higher than the untreated sludge at solid retention time of 25, 15 and 10 days, respectively.

Nevertheless, contribution of the synergistic effects to the anaerobic biodegradability increment was also observed. For example, BI after ALK + ULS (0.05 mol/L + 21 kJ/g TS) pre-treatment was 37.8%; whereas, the BIs 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 ALK + ULS 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 ALK + ULS pre-treatment has not been emphasized in previous works and could be related to the synergistic sludge disintegration as mentioned in Section 2.2. As mentioned in Sections 2.2 and 2.4, solubilization of SMP-like products such as proteins and carbohydrates was significantly enhanced due to the combined pre-treatment. 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 treatment as mentioned in Section 2.3. 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 recalcitrant in anaerobic digestion. However, Ho and Ho (2012) found that low concentrations of HAs (less than 5 g/L) could enhance methane production by serving as electron acceptors of the fatty acid degradation. Therefore, further investigation of the synergistically solubilized HAs may be helpful to the mechanism investigation.

3. Conclusions

The SCOD concentration increased maximally from 1200 to 11,000 mg/L due to the ALK + ULS treatment. During the ALK + ULS pre-treatment, 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. Addition of NaOH to the ultrasonication process could enhance solubilization of the SMP-like substances. HA was also found to be released due to the synergistic actions between alkali and ultrasound.

Individual ALK and individual ULS pre-treatments have their respective limitations in improving sludge anaerobic degradability. However, such limitation was reduced in ALK + ULS pre-treated sludge. 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. Therefore, ULS and ALK pre-treatment should be combined rather than individually applied for the benefit from the synergistic effects.

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