Biomethane production improvement by hydrothermal pretreatment of thickened waste activated sludge

Ahmad Shabir Razavi, Farokhlaqa Kakar, Ehssan Hosseini Koupaie, Hisham Hafez and Elsayed Elbeshbishy

ABSTRACT

This study evaluated the impact of hydrothermal pretreatment on thickened waste activated sludge (TWAS) for solubilization enhancement and biomethane production improvement through the mesophilic anaerobic digestion process. In order to assess the effect of temperature, retention time and severity index (SI) of the hydrothermal pretreatment, TWAS was exposed to fifteen different pretreatment conditions within a combination of 10 different pretreatment temperature range (150–240 °C), five different retention times (5–30 min) and five different severity indexes (SI = 3, 3.5, 4, 4.5 and 5). The solubilization enhancement was observed in all hydrothermally pretreated samples with the highest solubilization efficiency of 49% in pretreatment conditions of 200 °C and 10 min retention time within the corresponding SI = 4. Biomethane production was not improved in all fifteen pretreatment conditions, pretreatment with SI beyond 4 decreased the biodegradability of TWAS. The highest biomethane production was observed in the pretreatment condition of 170 °C and 10 min with a 40% increase compared to non-pretreated TWAS.

Key words | anaerobic digestion, biomethane recovery, hydrothermal pretreatment, solubilization, thickened waste activated sludge

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HIGHLIGHTS

- Hydrothermal pretreatment of thickened activated sludge for enhanced anaerobic digestion.
- Maximum improvement of solubilization and methane recovery of 49 and 40%.
- Maximum solubilization was achieved under the '200 °C, 1,551 kPa, 10 min' condition.
- Highest methane yield was obtained under the '170 °C, 786 kPa, 10 min' condition.

INTRODUCTION

Population growth along with high living standards have brought a noticeable challenge for management of sludge produced during wastewater treatment. It is estimated that produced wastewater sludge globally exceeds ten thousand tons per day. On average, a typical wastewater treatment plant produces one hundred thousand dry tonnes of sludge per year (Liu *et al.* 2012b). Disposing of this amount of sludge in a more sustainable way with a small environmental footprint has brought many efforts and ended up with different methods for sludge management. Among all treatments, anaerobic digestion (AD) is known as a sustainable approach for sludge treatment. It is a vital process prior to disposal for stabilization of sludge, and it has been used worldwide in modern wastewater treatment plants and is considered to be an environmentally friendly and economical approach for sludge management (Vlyssides & Karlis 2004; Ashley *et al.* 2008).

In order to improve the decomposition and solubilization of TWAS during the hydrolysis stage, many pretreatment methods have been explored including thermal,

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mechanical, and chemical (Valo *et al.* 2004; Climent *et al.* 2007; Brodeur *et al.* 2011). Of all the pre-treatment techniques, hydrothermal pre-treatment revealed the highest improvement in the solubilization of TWAS by disrupting cell walls and releasing the intracellular matter into the liquid phase, which makes it available for microorganisms during AD (Wilson & Novak 2009).

Many full-scale wastewater treatment facilities are currently utilizing hydrothermal pre-treatment to accelerate biogas production through AD. In addition to enhanced solubilization and biogas production, improved dewaterability of wastewater sludge has also been reported via hydrothermal pretreatment (Donoso-Bravo *et al.* 2011). Hydrothermal pretreatment is an effective method to achieve organic matter degradation by combining high temperature and pressure in order to achieve solubilization and cell destruction of sludge, and it does not need a chemical catalyst, which makes it cost effective and environmentally friendly (Kim *et al.* 2015).

In hydrothermal pretreatment, the three primary variables that manipulate the process are temperature, pressure, and retention time. Many studies have investigated the effect of pre-treatment temperature on sludge reduction and biomethane production and most of the studies reported that thermal pre-treatment increases the hydrolysis of sludge up to a certain range of temperature; however, if the temperature exceeded the optimum conditions (160–210 °C), the biodegradability of sludge decreases (Eskicioglu *et al.* 2006; Kim *et al.* 2015; Han *et al.* 2017). Some researchers reported that if the pre-treatment temperature exceeds 200 °C, it negatively affects the AD process due to the polymerization of toxic and refractory compounds (Stuckey & McCarty 1984; Dhar *et al.* 2012). Donoso-Bravo *et al.* (2011) investigated the effect of different temperatures ranging from (120–220 °C) using one hour retention time for primary and secondary sludge; they found that the optimum pretreatment temperature for biogas production was 180 °C and increasing the temperature beyond this point resulted in a decrease in the biogas production. However, other findings show that a pretreatment temperature of 210 °C is the optimal condition for biomethane production from TWAS (Kim *et al.* 2015). Kim *et al.* (2015) studied the effect of thermal hydrolysis on TWAS with temperatures ranged from 150 °C to 270 °C, the results of this study demonstrated that the optimal pretreatment temperature for biogas production was 210 °C. Similar findings have been found in another study that reported the optimum pretreatment temperature for biomethane production from high solids content to be 120 °C–160 °C (Li *et al.* 2014).

Compared to the effect of temperature, the retention time (holding time) during the pretreatment has shown insignificant effects on the hydrolysis of sludge under high pre-treatment temperatures (100 °C), but under a low range of temperatures, the holding time could considerably affect the solubilization and degradability of wastewater sludge (Wilson & Novak 2009b; Han *et al.* 2017).

Hydrothermal pretreatment, beside sterilizing the sludge, can also enhance the dewaterability of waste by dissolving the extracellular polymeric substances of the flocs and decreasing the sludge disposal and shipment cost (Di Capua *et al.* 2020). The extracellular polymers in secondary treatment are an important factor for biofloculation and settling of sludge but a major obstacle for dewatering the sludge. The concentration of extracellular polymers can vary in WAS and it has a diverse linear relation with the dewaterability of sludge: higher extracellular polymers means lower dewaterability. In thermal pretreatment, cell destruction also occurs due to the pressure difference and further contributes to the hydrolysis rate of the waste (Di Capua *et al.* 2020). In a study that investigated the availability of extracellular polymers and the dewaterability of WAS, it was shown that up to a level extracellular polymer can help the dewaterability of WAS, and when the extracellular polymers reach a higher level and decrease the number of small particles, it highly influences the dewaterability of WAS (Neyens & Baeyens 2003). The effect of hydrothermal pretreatment on dewaterability of municipal biomass waste consisting of WAS, kitchen waste and fruit waste also shows that it can be enhanced. The dewatered cakes from WAS, kitchen waste and fruit waste show a dewaterability enhancement of 60.4%, 55.1% and 46.7% respectively (Liu *et al.* 2012a).

One of the objectives of the pretreatment is to reduce the particle size of substrates with high fiber content and to improve gas production and digestion rate. The active surface area is related to the particle size, and by reducing the size more surface of the organics become available for the microorganisms and results in a biodegradability increase. In a study by Zhang & Banks (2013) on organics of municipal solid waste, it was shown that the particle size does not have any impact on the biodegradability of the feedstock, but when considering the particle size with the digestion system, it can help the biomethane production. Although, in another study, the results conclude that a smaller mean particle size can increase the biogas production by 28% and excessive size reduction causes reduced biomethane production by aggregation of volatile fatty acids (Izumi *et al.* 2010).

Although the hydrothermal pretreatment is now industrialized and practiced in more than 40 full-scale wastewater treatment plants using different thermal pretreatment technologies (Cambi®, Biothelys®, Exelys®, TPH®, Lysotherm® and Turbotec®), yet there is still no general agreement on the optimum condition. According to previous researches, the accepted operational condition has a wide range of heating temperatures ranging from 120 °C to 230 °C and retention time varying from 20 to 60 min (Sapkaite *et al.* 2017). Although some of the previous research has investigated the effect of hydrothermal pretreatment on enhanced wastewater sludge disintegration and AD performance, to the best of the authors' knowledge no comprehensive study considering the effect of Severity Index (SI) under a wide range of temperatures, pressures, and holding times has been performed. SI is a parameter used to combine the effect of pretreatment temperature, pressure and retention time in a single factor. It is an important parameter for hydrothermal pre-treatment system design adopted in industrial application as it helps to simultaneously evaluate the effect of three affective variables. Therefore, the aim of this research is to fill the gap and find the optimum hydrothermal pretreatment condition for TWAS disintegration among five different SIs and also within each SI. Also, the objective of this study was to investigate the effect of temperature, pressssure, and holding time on the solubilization of TWAS and the subsequent mesophilic AD process within five different SI values.

MATERIAL AND METHODS

Inoculums and substrate characteristics

The inoculum and substrate (TWAS) were collected from Ashbridge Bay wastewater treatment plant located in Toronto (Ontario, Canada). The inoculum was taken from mesophilic anaerobic digesters performing at 34–38 °C and fed with a mixure of primary sludge (PS) and TWAS at a flowrate of 6,420 m^3/day and 1,600 m^3/day, respectively. TWAS is the sludge produced in the secondary treatment (conventional activated sludge). The characteristics of the inoculum and TWAS used in this study are summarized in Table 1.

Thermal pretreatment

TWAS was pretreated by Parr 4,848 high pressure/temperature reactor equipped with a proportional integral derivative (PID) programming unit and automated tuning ability, which makes it possible to control the thermal profile precisely. The thermal reactor used was equipped with a mechanical mixer with a speed controller connected to a controller software (SpecView) that allows control of parameters within different loops while providing real-time plotting. The Parr 4,848 reactor is capable of working with a maximum temperature of 275 °C and a maximum pressure of 1,900 psi and has a capacity of 2 liters. One liter of TWAS was added to the hydrothermal reactor and then the reactor valves were sealed during the thermal pretreatment to keep the pressure and vapors inside. The mechanical mixer was configured to rotate at 150 RPM. The temperature was increased throughout three loops to control the process and keep the temperature at the target. For this purpose, the first loop raised the temperature to a certain degree, which was 30 degrees less than the target, with a heating ramp rate of 3 °C/min. During the second loop, the ramp rate was decreased to 1 °C/min to prevent exceeding the temperature beyond the target temperature. During the third loop, the temperature was kept constant, reaching the desired retention time. By the end of the retention time, the heater was turned off while the mechanical mixer was kept running, and the cooling system that circulates water by pumping was kept turned on until the temperature was below 50 °C so that the reactor could be opened.

Table 1 | The characteristics of the inoculum and TWAS used in this study[a]

Parameters	Inoculum	TWAS
Total COD (mg/L)	18,700 ± 220	49,600 ± 1,500
Soluble COD (mg/L)	900 ± 60	2,580 ± 480
TSS (mg/L)	15,300 ± 430	34,000 ± 3,000
VSS (mg/L)	11,600 ± 280	25,400 ± 2,000
Total protein (mg/L)	ND[b]	1,000 ± 150
Soluble protein (mg/L)	ND[b]	260 ± 30
Total carbs (mg/L)	ND[b]	3,560 ± 430
Soluble carbs (mg/L)	ND[b]	110 ± 20
Ammonia (mg/L)	600 ± 60	251 ± 7
Alkalinity (mg/L)	3,600 ± 230	1,063 ± 126
pH	7.8 ± 0.1	6.3 ± 0.2

[a]TWAS, Thickened Waste Activated Sludge; COD, chemical oxygen demand; TSS and VSS, total and volatile suspended solids.
[b]ND, Not determined.

Experimental design and procedure

Solubilization study

TWAS has been hydrothermally pretreated within five different SI values to compare the effect of temperature, pressure, and holding time on the degree of solubilization among pre-treated samples and also between the non-pretreated and pre-treated samples. Under each SI value, three different combinations of temperature, retention time, and pressure were considered, resulting in a total number of 15 pre-treatment scenarios. The SI values used in this study were 3, 3.5, 4, 4.5, and 5. The range of temperatures was from 150 °C to 240 °C, and the retention time was varied from 5 to 30 minutes. The experimental design of this study is shown in Table 2.

SI is a parameter used to combine the effect of pretreatment temperature and retention time in a single factor (Aita & Kim 2010; Razavi *et al.* 2019). The SI was calculated using Equation (1).

$$\text{Severity Index (SI)} = \log \exp[(T - 100)/14.75 \times t] \quad (1)$$

where T and t are the pre-treatment temperature (°C) and retention time (min), respectively.

The percentage improvement (P) in soluble chemical oxygen demand (SCOD) in this study was calculated using Equation (2).

$$P(\%) = (SCOD_P - SCOD_R)/(TCOD_R - SCOD_R) * 100 \quad (2)$$

where $SCOD_P$ is the SCOD concentration of pretreated samples, and $SCOD_R$ and $TCOD_R$ are the SCOD concentration and TCOD concentration of raw (non-pretreated) TWAS, respectively.

The suspended solids reduction efficiency (R) was calculated using Equation (3).

$$R\ (\%) = (SS_R - SS_P)/SS_R \times 100 \quad (3)$$

where SS_R and SS_P are the suspended solids (TSS or VSS) concentrations before and after the hydrothermal pretreatment.

Batch anaerobic digestion

Following the solubilization study, the effects of hydrothermal pre-treatment on the anaerobic digestibility of TWAS was evaluated. Biochemical methane potential (BMP) assays were conducted in triplicates under mesophilic conditions (38 °C). The BMP experiment included fifteen pretreated and one non-pretreated sample, resulting in 48 assays in total. The BMP was conducted in 500 mL glass bottles. In order to correct the biogas produced from the inoculum, three bottles were used as a blank containing only inoculum (without substrates). The volume of inoculum was fixed in each bottle at 250 mL, the volume of substrate was calculated based on a food to microorganism (F/M) ratio of 1 g $TCOD_{substrate}$/g VSS_{seed}. Additional alkalinity ($NaHCO_3$) was added as a buffering agent with a concentration of 4 g/L to avoid a pH drop during the AD process. Before sealing the bottles, the inoculum was purged with nitrogen gas to remove the available oxygen. After adding the substrate and inoculum, the glass bottles were sealed by rubber septa and plastic caps to ensure an anaerobic condition. The sealed glass bottles were placed in a shaker rotating at 100 rpm and set at temperature of 38 °C.

Analytical methods

All the water and gas quality analysis such as TSS, VSS, TCOD, SCOD, carbohydrates, proteins, VFAs, particle size distribution (PSD), biogas production, and biogas compositions were analyzed as described in our previous paper (Kakar *et al.* 2019).

Statistical analysis

Multifactor analysis of variance (ANOVA) with 95% confidence level ($\alpha = 0.05$) evaluated the statistically significant effect of the experimental factors (i.e. temperature, retention time, pressure and SI) in Minitab 19. The analysis of ANOVA in a two-level interaction was performed to evaluate the

Table 2 | The experimental design used in this study

Severity Index (SI)	3.0 ± 0.05	3.5 ± 0.05	4.0 ± 0.05	4.5 ± 0.05	5.0 ± 0.05
Pretreatment pressure (kPa), temperature (°C), retention time (min)					
Pretreatment Scenarios #1	475, 150, 30	786, 170, 30	1247, 190, 20	1565, 210, 20	2323, 220, 30
Pretreatment Scenarios #2	613, 160, 20	999, 180, 15	1551, 200, 10	2323, 220, 10	2806, 230, 15
Pretreatment Scenarios #3	786, 170, 10	1247, 190, 10	1909, 210, 10	2806, 230, 5	3364, 240, 20

effects of experimental factors. Fisher's least significant difference was also calculated for all pairs of means.

RESULT AND DISCUSSION

Effects of hydrothermal pretreatment on COD solubilization prior to AD

TWAS samples were hydrothermally pretreated at fifteen different temperatures and retention times within five different SIs, as presented in Table 2. Figure 1(a) shows the SCOD concentration of the pretreated samples in a comparison to non-pretreated. Figure 1(b) demonstrates the percentage improvement in SCOD. The results of the experiment reveal that solubilization enhancement of TWAS was achieved in all hydrothermal pretreated conditions. As shown in Figure 1(a), with increasing SI, the SCOD increased until the SI reached 4, after which it stabilized. The highest SCOD concentration of 25,400 ± 729 mg/L was achieved at a pretreatment condition of 200 °C and 10 min within SI = 4 compared to about 2,580 ± 1,351 mg/L SCOD for non-pretreated samples. The lowest value of the SCOD concentration for the pretreated samples was 15,100 mg/L, which was observed at pretreatment condition of 150 °C and 30 min within SI = 3. Those data revealed that increasing the SI beyond 4 did not enhance the solubilization. The SCOD enhancement is caused by transformation of particulate organic polymers such as proteins, carbohydrates, and lipids to simpler monomers such as amino acids, sugars, and long chain fatty acids (Bougrier *et al.* 2008; Xue *et al.* 2015).

As illustrated in Figure 1(b), within all fifteen pretreatment conditions, the degree of solubilization improvement ranged between 27% and 49%. Organics fractions that represent COD in TWAS are mostly protein (40%), lipids (25%) and polysaccharides (15%) (Wilson & Novak 2009). The rate limiting step for fats disintegration is fatty acid oxidation and not hydrolysis, so the improvement in solubilization is most likely due to the hydrolysis of protein and polysaccharide (Liu *et al.* 2012b). During the hydrolysis stage of AD, proteins are converted to peptides and amino acids, which later are transformed to volatile fatty acids (VFAs). The same reactions happen during thermal pretreatment, through which macromolecular proteins are converted first to small molecules of peptides, and are further degraded to amino acids (Gagliano *et al.* 2015). Thermal hydrolysis also degrades polysaccharides like cellulose and starch to smaller molecules of glucose, and at higher temperature, smaller carbohydrate molecules can interact with amino acids and form the Maillard reaction or cause non-enzymatic browning of sugar by caramelization (pyrolysis) (Gerrard 2005). As was also hypothesised by other researches, carbohydrate is a more easily degradable compound during the

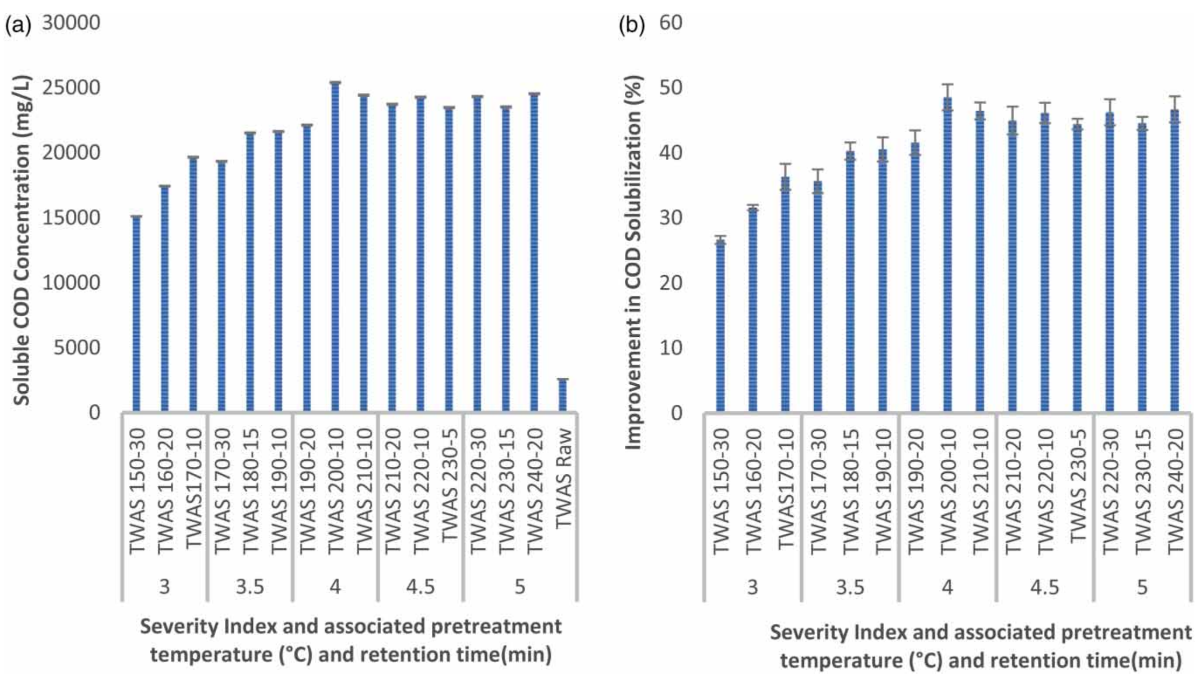

Figure 1 | Effect of hydrothermal pretreatment on solubilization; (a) soluble COD concentration, (b) soluble COD improvement comparing to non-pretreated.

hydrothermal pretreatment compared to protein. Therefore, in hydrothermal pretreatment with lower SI, the SCOD improvement is referred more to carbohydrate degradation but higher intensity of pretreatment disintegrates protein to smaller organic compounds like amino acids (Bougrier *et al.* 2008). The availability of degraded carbohydrate like glucose and amino acids with suitable temperature (higher pretreatment intensity) can form the Maillard reaction, which causes inhibitory effects for COD solubilization and the subsequent AD process. It can also be observed in Figure 1(b) that increasing the severity of the pre-treatment condition improves the soluble COD up to a certain level (in this case, SCOD was 25,400 mg/L at SI = 4) and increasing pretreatment intensity beyond this range adversely affects the process of solubilization. This trend was also observed in other studies evaluating the hydrothermal pretreatment of different wastes. In previous studies that investigated the effect of thermal pretreatment on physical and chemical properties of three different municipal biomass wastes (kitchen waste, fruit/vegetable waste and wastes activated sludge), its result revealed that a higher intensity of hydrothermal pretreatment condition can negatively affect the solubilization of COD (Liu *et al.* 2012b; Li *et al.* 2016). According to the literature, higher pretreatment temperature of WAS increases the ammonia concentration as a consequence of protein solubilization and decreases the concentration of carbohydrate due to Maillard reactions (Cesaro & Belgiorno 2014).

During hydrothermal pretreatment, the three main parameters affecting the solubilization are temperature, pressure, and retention time. As illustrated in Figure 1, the retention time has minimal effect on the solubilization of COD and the pre-treatment temperature is the dominant parameter in the hydrothermal pretreatment. Considering the overall results of the solubilization study, lower pretreatment temperature cannot increase the solubilization of TWAS effectively and higher pretreatment temperature with SI beyond 4 adversely affect the overall process of solubilization.

Effect of hydrothermal pretreatment on suspended solid reduction

The change in the concentration of TSS and VSS is illustrated in Figure 2(a) and 2(b), which demonstrates the reduction efficiency in TSS and VSS after hydrothermal pretreatment.

As shown in Figure 2(a), the hydrothermal pretreatment has considerably reduced the TSS and VSS concentration compared to the non-pretreated samples. The highest TSS and VSS reduction was achieved at pretreatment conditions of 220 °C and 10 min. The TSS concentration decreased from 34,100 ± 3,400 mg/L for the raw sample to a lowest value of 11,400 ± 5,400 mg/L and the VSS concentration decreased from 25,400 ± 2,500 mg/L for the raw sample to a lowest value of 6,800 ± 2,700 mg/L at pretreatment conditions of 220 °C and 10 min. As shown in Figure 2(b), the reduction efficiency in TSS and VSS ranged from 25% to 67% and from 31% to 73% for TSS and VSS, respectively. On the other hand, the TSS and VSS reduction efficiency showed an increasing trend until an SI of 4.5; however, increasing the intensity of pretreatment beyond the SI of 4.5 decreased the efficiency of pretreatment in terms of suspended solids reduction. The decrease in TSS and VSS reduction efficiency in more intense pretreatment conditions (higher than SI = 4.5) can be explained as a result of polymerization of inorganic compounds with organics and formation of insoluble macromolecular polymers that reduce the efficiency of hydrothermal pretreatment. The polymerization can be because of the Maillard reaction, which happens between degraded carbohydrates such as glucose and fructose with degraded protein like amino acids or formation of hybrid polymers by reaction of organic and inorganic compounds (Liu *et al.* 2012b).

The particle size of the feedstock can affect the anaerobic biodegradability rate as it increases the substrate utilization coefficient (Halalsheh *et al.* 2011). One of the objectives in the pretreatment process is to reduce the particle size of the substrate to improve the biogas production and digestion rate. There is a correlation between active surface area with the particle size and by reducing the particle size, more surface of organics become available for the microorganisms, which results in an increase in biodegradability. The results of the particle size distribution in this study support the suspended solids reduction by showing a reduction in particle size of the pretreated TWAS samples compared to non-pretreated samples. The reduction of particle size of the pretreated samples and the control sample were evaluated by D10, D50, D90. The results revealed that the D10, D50 and D90 reduced from 27, 76, and 152 μm to lowest values of 12, 39, and 75 μm, respectively, at pretreatment conditions of 220 °C and 10 min, see Figure 3. In a study conducted by Izumi *et al.* (2010) on food waste, they evaluated the effect of particle size reduction and solubilisation on biomethane recovery using bead milling to grind and batch anaerobic digestion. It was found that smaller mean particle size can increase the biogas production by 28% and excessive size reduction causes reduced biomethane production by aggregation of volatile fatty acids (Izumi *et al.* 2010).

According to the results illustrated in Figure 2, the hydrothermal pretreatment had a higher effect on VSS

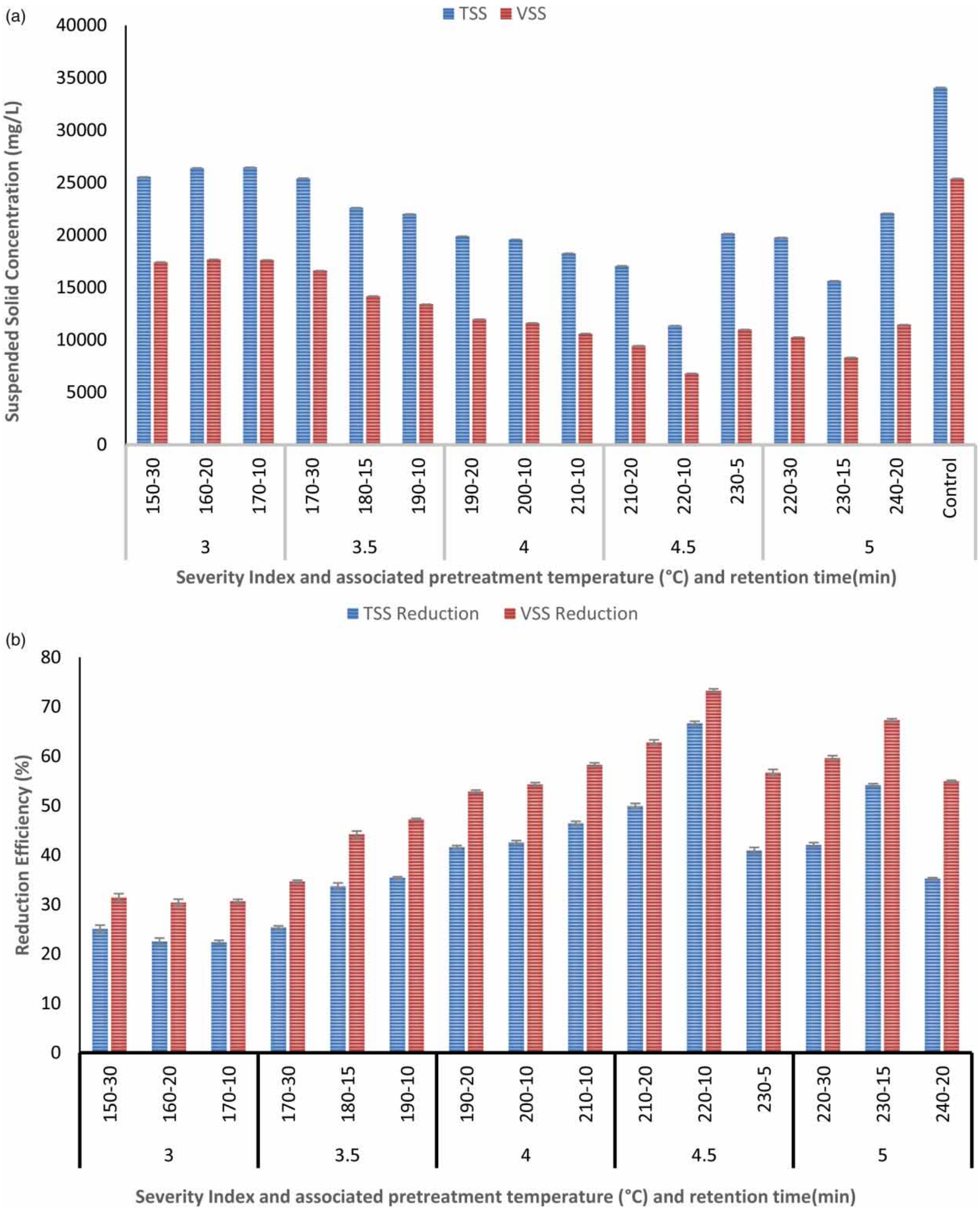

Figure 2 | The effect of hydrothermal pretreatment on suspended solids; (a) suspended solid concentration (mg/L), (b) suspended solid reduction efficiency (%).

reduction compared to TSS. This can be explained by the fact that thermal hydrolysis mainly caused the destruction of organic matter rather than the inorganic compounds. The same findings have been reported by other researches (Jin *et al.* 2016; Razavi *et al.* 2019).

Comparing the effect of retention time and the temperature, it was found that the retention time had less effect on TSS and VSS reduction compared to the pretreatment temperature. This finding is in an agreement with the findings of another study investigating the effect of time in a thermal pretreatment at a lab and pilot scale study on waste activated sludge. In this study, six experimental conditions were assessed with retention times ranging from 0 to 30 minutes and temperature of 170 °C. The result of the study

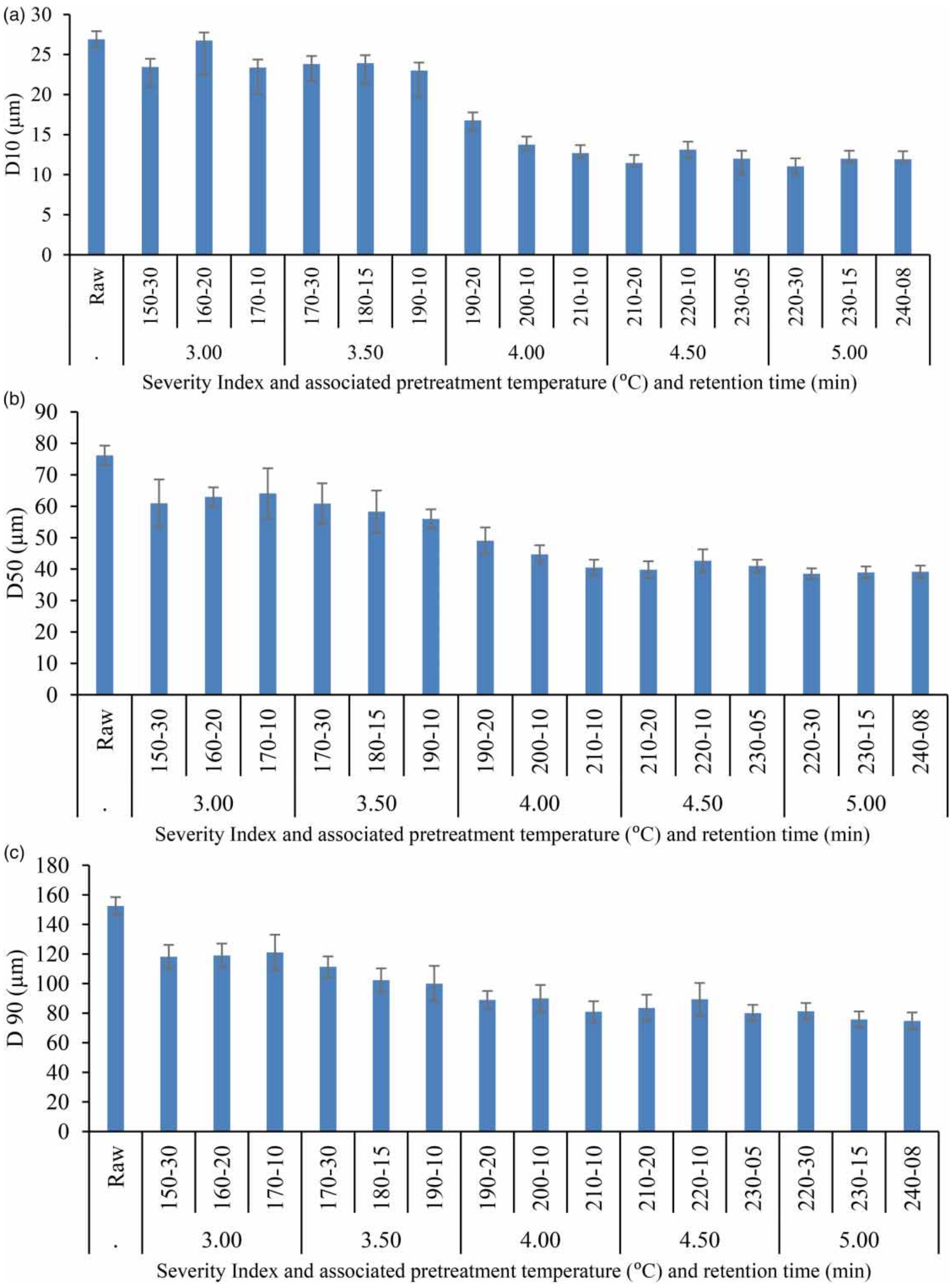

Figure 3 | The effect of hydrothermal pretreatment on particle sizes; (a) D10, (b) D50, (c) D90.

evaluating the effect of retention time in thermal pretreatment revealed that a reaction time of up to 15 min can influence the solubilization and beyond that the effect of retention time is negligible (Donoso-Bravo *et al.* 2011).

With respect to the results of the TSS and VSS reduction efficiency, it can be concluded that the lower temperature and SI is not a suitable condition for hydrothermal pretreatment to disintegrate the TWAS. Also a pretreated condition with SI above 4.5 results in polymerization and reduces the TSS and VSS reduction efficiency.

Impact of hydrothermal pretreatment on anaerobic digestion of TWAS

Following the solubilization study, the BMP test of TWAS was performed in an anaerobic condition within batch reactors for all 15 pretreated (pretreated conditions in Table 2) and one non-pretreated samples. The BMP assay continued for 51 days. The results of the cumulative methane yield for all pretreated and non-pretreated samples are shown in Figure 4(a) and 4(b).

According to Figure 4(a), the methane production followed the same trend for all pretreated and non-pretreated samples. No significant lag phase was observed as a result of delay in the adaptation of microbial populations to the new environment. This observation can be explained as both substrate (TWAS) and inoculum being from the same wastewater treatment plant, equipped with mesophilic anaerobic digesters. Figure 4(b) illustrates the biodegradability of TWAS after hydrothermal pretreatment, as shown in the figure, was not improved under all the pretreated conditions since the methane production improvement demonstrates a different trend compared to that of the solubilization. The highest solubilization was observed in pretreated conditions within SI = 4 and all pretreated condition improved soluble COD, while TWAS exposed to the most intensive hydrothermal pretreatment condition with SI higher than 4.5 showed lower methane yield compared

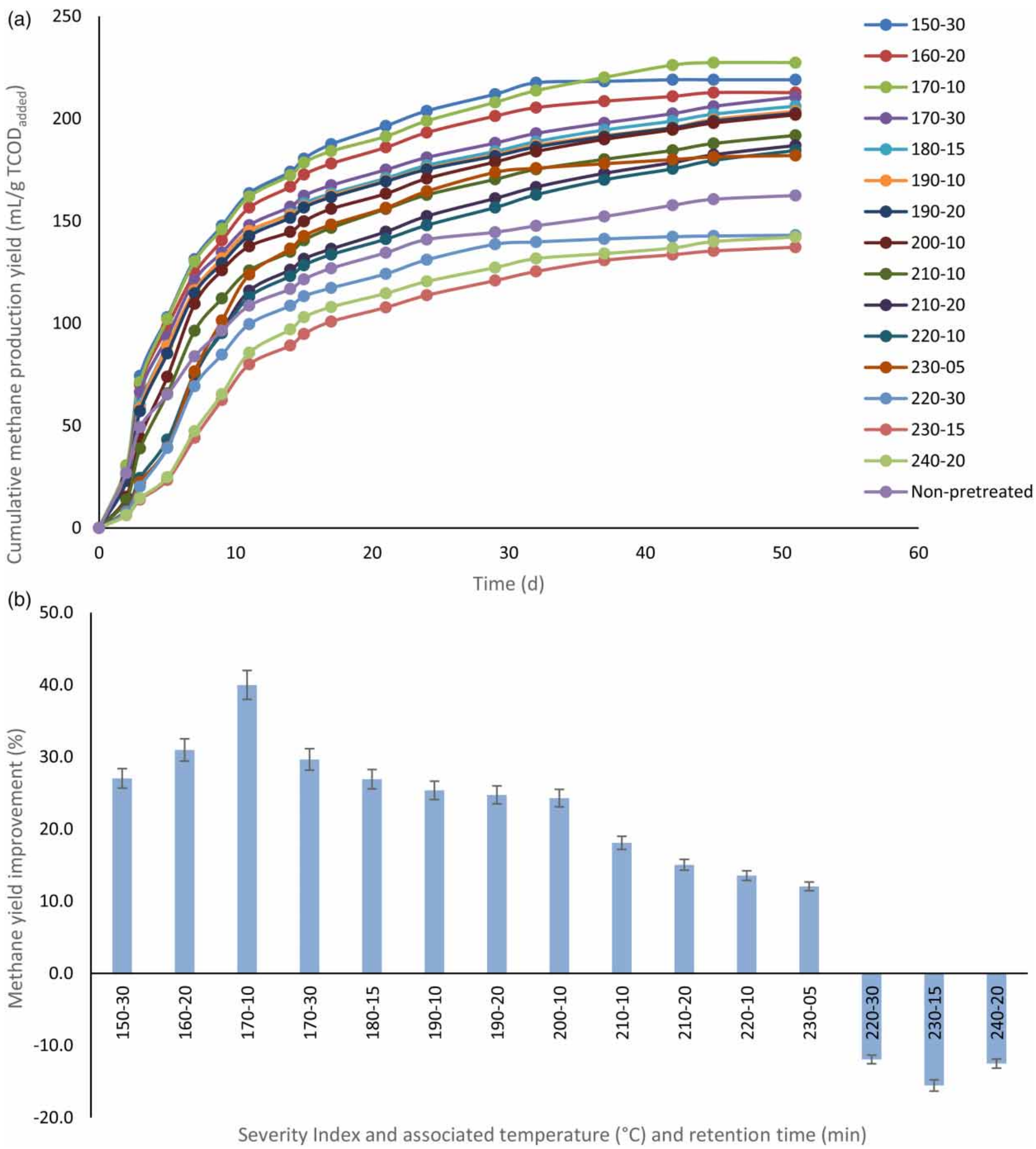

Figure 4 | (a) Cumulative methane production yields of non-treated and pre-treated TWAS; (b) the improvement in cumulative methane yield of the BMP digesters (@ the STP of 0 °C and 1 atm) (the error bars in the chart are the standard deviations of three samples).

to non-pretreated samples. This explains that TWAS biodegradability decreased after pretreatment under severe conditions and that adversely affects the overall AD performance in terms of biogas production. This result is supported by a previous study in which Xue *et al.* (2015) studied the thermal hydrolysis of organic matter solubilisation and anaerobic digestion of sludge. In this study, the range of thermal pretreatment temperatures was from 60 to 180 °C, and it was reported that biogas yield increased by 16.5% in a pretreatment condition of 160 °C while a higher pretreatment temperature of 180 °C decreased the biogas yield.

The methane production improved by 12–40% within pretreatment conditions between SI 3 and 4.5 with the highest methane yield produced in the least pretreated intensity SI = 3, with pretreated temperature of 170 °C with 10 min retention times. The increased bio-methane production by hydrothermal pretreatment was also reported by other researches but with a higher efficiency. In this regard, Valo *et al.* (2004) reported a 45% improvement after WAS pretreated at 170 °C. The pretreatment condition with SI beyond 3 and pretreatment temperature higher than 170 °C shows a mild reduction in bio-methane yield with the intensity of pretreatment; when the pretreatment condition reaches SI = 5 and pretreated temperature of 220 °C with 30 min retention time, the methane production yield gets lower than the non-pretreated TWAS. The AD is a complex and delicate process involving different clusters of bacteria with sensitivity to several processing parameters like pH, ammonia, VFAs, and hydrogen (Appels *et al.* 2008). The decrease in bio-methane yield for pretreated TWAS with more intense pretreatment condition might be as a result of free ammonia enhancement after pretreatment. This result can be caused by the formation of toxic refractory compounds such as Amadori and melanoidin caused by high pretreatment temperature, which has been also reported by previous studies (Appels *et al.* 2008; Pilli *et al.* 2015).

According to the result, during the first four days, the highest biomethane production rate happened in non-pretreated and low-intensity pretreated digesters. This observation can be related to the high initial concentration of carbohydrate. According to the results, the highest total carbohydrate concentration was observed at 160 °C and 170 °C. Pretreatment temperatures higher than 170 °C exhibit a decreasing trend in carbohydrate concentration and also biomethane production yield during the first four days of the operation period. After the first four days, the biomethane production seems comparatively similar for all samples. As some other researchers reported, carbohydrate is the first component of organic matter that is consumed in AD to produce biogas and protein and lipid degradation starts when carbohydrate depletes (Yang *et al.* 2015). Therefore, it is hypothesized that the improved biogas production in lower pretreatment temperature is due to the high availability of carbohydrate. Protein hydrolysis starts by increase in pretreatment temperature, and it degrades to its smaller molecules; high temperature with availability of small carbohydrate molecules make a suitable condition for formation of hydrochar polymers, which are not degradable biologically (Ding *et al.* 2017).

According to the results illustrated in Figures 1 and 4, there is no correlation between the concentration of SCOD and methane yield during the pretreatment, as SCOD shows an increasing trend reaching the maximum value at SI to 4 while the maximum methane yield has been observed for samples pretreated with SI = 3. In addition to pretreatment temperature, retention time is also a determining factor in hydrothermal pretreatment. The illustrated results from the main effect plot and cumulative methane yield demonstrate that the effect of retention time is not statistically significant considering the same pretreatment temperature with different retention times (see Figure 5(a)). As an example, under two different SIs with the same pretreatment temperature of 170 °C and two different retention times of 10 and 30 min, the cumulative methane yield decreased from 135 to 133 mL/gTCOD$_{Added}$, this can be also observed in pretreatment conditions of 190 °C with 10 and 20 min retention times. These observations revealed that the dominant parameter in hydrothermal pretreatment is the temperature and the retention time's influence on methane yield is not significant. Therefore, enhancement of the methane production by hydrothermal pretreatment is more affected by pretreatment temperature than retention time. As in the main effect plot of methane yield in Figure 5, the effect of pretreatment temperature and SI shows the same trend, while retention time shows the highest efficiency in 10 min and higher retention time can negatively affect the methane production.

In conclusion, the biodegradability of TWAS was improved under the pretreatment with lower severity (160–170 °C). Increasing the intensity of pretreatment temperature beyond 170 °C most likely forms non-biodegradable matters that reduce the efficiency of AD. It is also concluded that retention time beyond 10 min will not have any significant effect on methane production.

Methane production rate

During the AD experiment, TWAS reached its maximum biomethane production rate after 3 days for all pretreated and non-pretreated samples and after that, it showed a

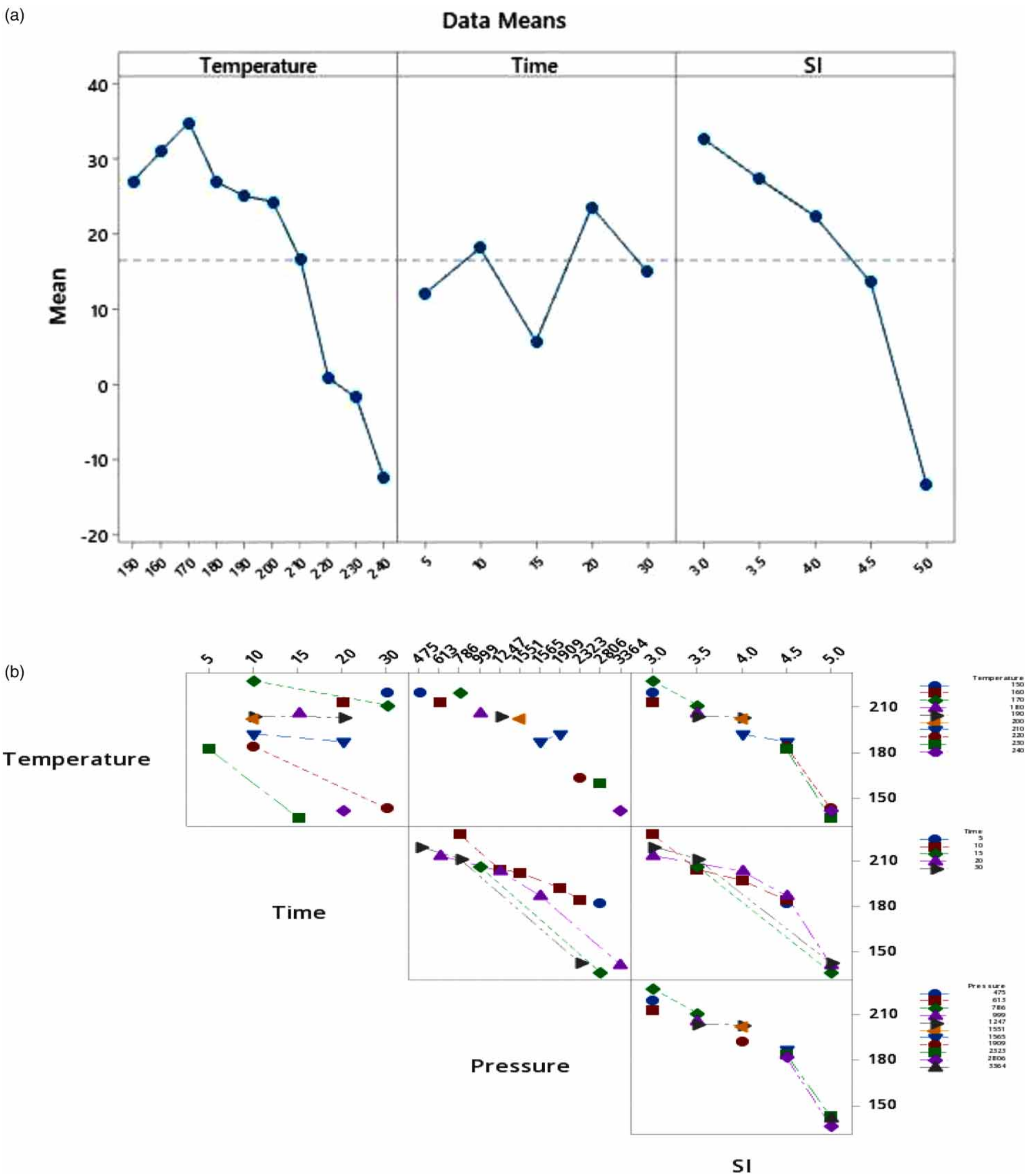

Figure 5 | (a) The main-effect plot of methane yield improvement; (b) interaction plot of pretreatment temperature (°C), retention time (min), pressure (kPa) and severity index.

reduction in daily production rate. Figure 6(a) compares the biomethane production rate among pretreated and non-pretreated digesters. Figure 6(b) shows the highest methane production rate.

The highest methane production rate was observed in the least intensive pretreatment condition of 160 °C-20 min with 22 mL/g COD_{added}/d and the lowest methane production of 7 mL/g COD_{added}/d was obtained in the most

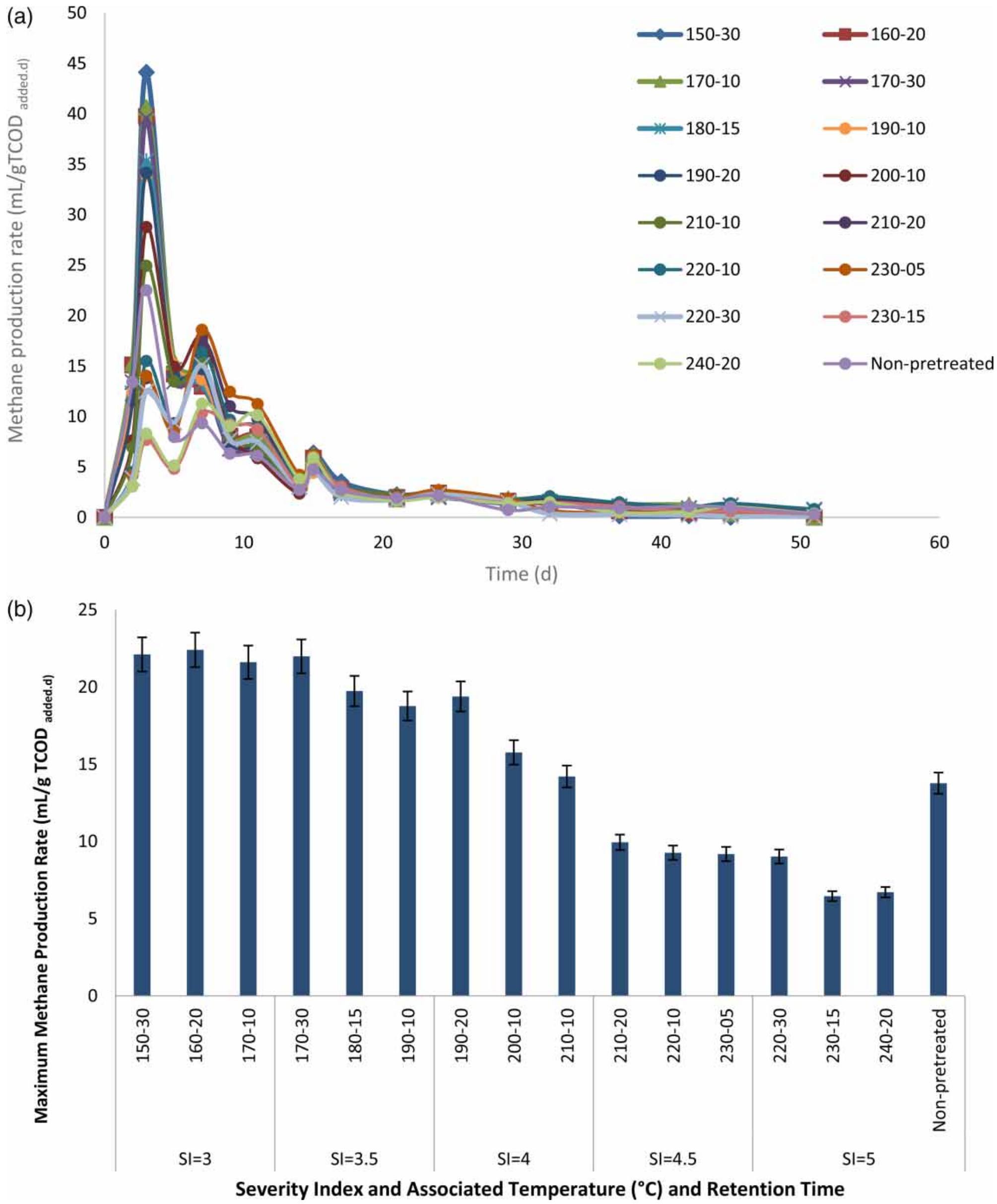

Figure 6 | The effect of hydrothermal pretreatment on (a) daily methane production rate (mL/g $TCOD_{added.d}$; (b) Maximum methane production rate (mL/g $TCOD_{added.d}$).

severe condition of 240 °C–20 min. The biomethane production rate showed a decreasing trend with the increase in pretreatment SI. In pretreatment conditions with SI > 4, the highest methane production rate was lower than that of the control. This finding is in an agreement with the result of methane production yield in which the biomethane improvement showed that pretreatment condition with severity higher than 210 °C–10 min has a negative impact on the bioconversion of pretreated TWAS to biomethane. One of the components of TWAS is the carbohydrates, which are the fastest degradable matter during AD. The highest concentration of soluble carbohydrate was obtained in the same condition that has the highest methane production rate and degradability illustrated in Figure 5(b). This can be explained as the produced biomethane in AD process of TWAS mostly coming from digestion of carbohydrates and protein. A large portion of biomethane (60–70%) was produced during the first 11 days of the experiment. Observing this, it can be concluded that most of the biogas was produced by carbohydrate being the fastest biodegradable component of organic waste.

For pretreatment conditions that have lower production rate compared to the non-pretreated sample, it can also explain the Maillard reaction happening between soluble protein and carbohydrate, which form non-biodegradable matter. This results in lower carbohydrate concentration compared to non-pretreated TWAS, which shows a lower production rate. Carbohydrate and amino acids are the main components of melanoidins and the formation of this refractory matter in AD is reported to have a relation with the concentration of carbohydrate and amino acid (Liu *et al.* 2012a).

CONCLUSION

The results of this study revealed that despite solubilization enhancement for all hydrothermal pretreatment conditions, the biomethane production does not follow the same trend as solubilization. Also, the optimum pretreatment condition for sludge disintegration and methane production were different. The optimum hydrothermal pretreatment condition for highest COD solubilisation happened at 200 °C and 10 min retention time with a concentration of 25,400 ± 729 mg/L and 49% improvement within the SI of 4.0 ± 0.05 compared to the control, while the ideal condition for maximum methane production yield was achieved at 170 °C and 10 min retention time within lowest severity index (SI = 3.0 ± 0.05) demonstrating 40% improvement comparing to the control (259 mL/g $TCOD_{added}$). Beyond this optimal condition for methane production, the biomethane production decreased with increasing SI. The results also revealed that the highest SI shows a lower biomethane production compared to the control, which adversely affected the overall process.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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