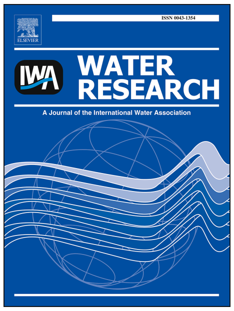

Low-temperature thermal pre-treatment of municipal wastewater sludge: Process optimization and effects on solubilization and anaerobic degradation

Laleh Nazari, Zhongshun Yuan, Domenico Santoro, Siva Sarathy, Dang Ho, Damien Batstone, Chunbao (Charles) Xu, Madhumita B. Ray

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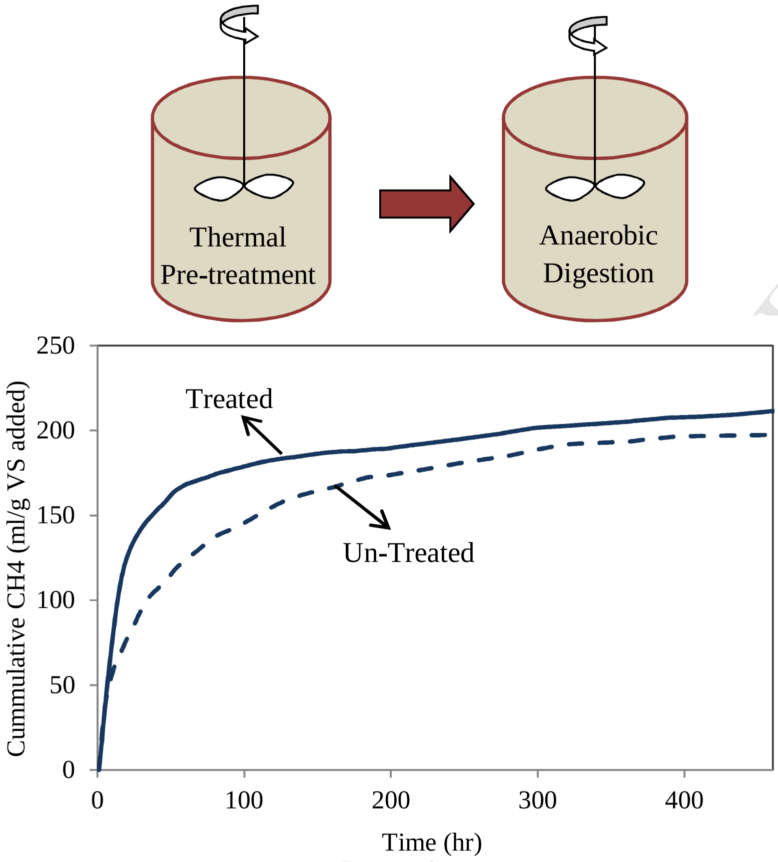
Thermal
Pre-treatment
Anaerobic
Digestion
Treated
Un-Treated
Cummulative CH4 (ml/g VS added)
250
200
150
100
50
0
0
100
200
300
400
Time (hr)

Low-Temperature Thermal Pre-Treatment of Municipal Wastewater Sludge: Process Optimization and Effects on Solubilization and Anaerobic Degradation

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Abstract

The present study examines the relationship between the degree of solubilization and biodegradability of wastewater sludge as a result of low-temperature thermal pre-treatment. The main effect of thermal pre-treatment is the disintegration of cell membranes and thus solubilization of organic compounds. There is an established correlation between chemical oxygen demand (COD) solubilization and temperature of thermal pre-treatment, but results of thermal pre-treatment in terms of biodegradability are not well understood. Aiming to determine the impact of low temperature treatments on biogas production, the thermal pre-treatment process was first optimized based on an experimental design study on waste activated sludge in batch mode. The optimum temperature, reaction time and pH of the process were determined to be 80 ^{o}C, 5 hr and pH 10, respectively. All three factors had a strong individual effect ($p < 0.001$), with a significant interaction effect for temp.pH^2 ($p = 0.002$). Thermal pre-treatments, carried out on seven different municipal wastewater sludges at the above optimum operating conditions, produced increased COD solubilization of 18.3 ± 7.5 % and VSS reduction of 27.7± 12.3 % compared to the untreated sludges. The solubilization of proteins was significantly higher than carbohydrates. Methane produced in biochemical methane potential (BMP) tests, indicated initial higher rates ($p = 0.0013$) for the thermally treated samples (k_{hyd} up to 5 times higher), although the ultimate methane yields were not significantly affected by the treatment.

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Keywords: Thermal treatment, Waste activated sludge, Process optimization, Solubilization, Degradability, Methane production rate

1. Introduction

The conventional activated sludge process is widely used for the removal of organics and nutrients in municipal and industrial wastewater plants due to its high efficiency, cost effectiveness, flexibility, and ease of operation. However, production of large amount of waste activated sludge (WAS) is one of its major drawbacks (Neyens et al., 2004). WAS along with the primary sludge (PS) from primary treatment of wastewater present a significant disposal problem; volume reduction and stabilization are required before disposal (Rajan et al., 1989). Sludge handling and disposal cost could be as high as 50% of the total cost of the wastewater treatment process (Appels et al., 2010; Neyens et al., 2004).

Anaerobic digestion (AD) is the most commonly used method for sludge stabilization to reduce odors, pathogens and volatile solids, where organic materials in sludge are converted to biogas (mainly methane and CO_2). The process consists of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al., 2008; Kim et al., 2015). Anaerobic digestion of WAS is generally limited by the hydrolysis step due to its particulate nature. The hydrolysis step degrades both insoluble organic matters and high molecular weight compounds such as proteins, carbohydrates and lipids into soluble organics (Appels et al., 2008). The major part of the organic compounds in WAS is trapped in a polymeric network formed by extracellular polymeric substances (EPS) (Devlin et al., 2011; Dhar et al., 2012; Nielsen et al., 2011). EPS are highly hydrated structures surrounding the bacterial cell wall. They are of great importance in bioflocculation, settling and dewatering of the sludge. Between 70 and 80% of EPS in WAS can be attributed to proteins and carbohydrates (Neyens et al., 2004). In order to enhance anaerobic digestion, the EPS network should be disintegrated to make the cell contents available to microorganisms (Dhar et al., 2012; Nielsen et al., 2011). Improving solubilization of solids and degradation of hydrolyzed organics have been reported to improve the overall digestion rate and the degree of degradation (Strong and Gapes, 2012).

Different pre-treatment methods such as thermal, chemical, biological and mechanical have been applied prior to AD on both WAS and PS to improve the cell disintegration and hydrolysis steps (Carrère et al., 2010; Devlin et al., 2011; Neyens and Baeyens, 2003). Although, thermal pre-treatments were initially used to improve sludge dewaterability by degradation of gel structure (Kondusamy and Kalamdhad, 2014), they can also destroy the cell walls to release organic compounds for biodegradation, (Neyens and Baeyens, 2003; Nielsen et al., 2011) and decrease the digestate viscosity (Ariunbaatar et al., 2014). Thermal treatments are usually divided into low temperature (< 100 ^oC) and high temperature treatments

(> 100 °C), the latter is also known as thermal hydrolysis. Temperature above 200 °C is not favorable and has been reported to result in degradation of nitrogenous organic material and production of toxic compounds and formation of refractory components due to polymerization reactions (Dhar et al., 2012; Nielsen et al., 2011; Strong and Gapes, 2012; Valo et al., 2004). Combined treatment methods such as thermal and alkali or acid addition have also been investigated (Dhar et al., 2011; Rafique et al., 2010; Tanaka et al., 1997).

Although these methods are reported to enhance organics solubilization, there are different observations on the effect of pre-treatments on biogas production. Many studies have documented that there is a direct relationship between solubilization and biodegradation but with different proportionality. For example, Uma Rani et al. studied the effect of low-temperature thermo-alkali pre-treatment of WAS and found that treatment at 60 °C, pH 12 was optimum for 23% higher COD solubilization and 22% higher suspended solids (SS) reduction with 51% higher biogas production compared to control (Uma Rani et al., 2012). Similarly, 30% improvement in biogas production in a low temperature pre-treatment (70 °C) for 9-72 hrs of a mixture of thickened primary sludge and WAS reported by (Ferrer et al., 2008). Tanka et al. reported an increase in methane production up to 200 % with VSS solubilization of 40-50 % by thermo-alkali pre-treatment of WAS at 130 °C for 5 minutes (Tanaka et al., 1997). Conversely, in a study by Dhar et al. (2012) thermal pre-treatments of municipal WAS at 50, 70 and 90 °C for 30 min caused significant increase in the ratios of SCOD/TCOD compared to the control, with only 13-19% increase in methane production. SCOD increase was due to the disruption of cells in WAS and release of proteins, carbohydrates and lipids, as confirmed by the analysis of SCOD. Nielsen et al. reported the effects of thermal pre-treatment and inter-stage treatment at low (80 °C) and high (130-170 °C) temperatures, and 170 °C/pH 10 for 10-24 hr on WAS (Nielsen et al., 2011). All the treatments, especially those at high temperatures (130 and 170 °C) increased the solubilization of volatile solids and enhanced methane production rate but the treatments at 80 °C and 170 °C/pH 10 did not show any improvement in final methane yield.

The above studies indicated that the effects of thermal/thermo-chemical treatment vary widely exhibiting a complex relationship of temperature, time of treatment, chemical dosage and the type of sludge requiring a comprehensive study comparing the performance of different sludges at comparable conditions. Since low temperature treatments are potentially cost-effective, the objective of the present study is to investigate the effects of low temperature thermal pre-treatment on solubility and digestibility of various types of sludge. Earlier studies involving low temperature pre-treatment were conducted at long treatment times such as 10 h (Nielsen et al., 2011), 72 h (Ferrer et al., 2008), and as high as 7 days (Gavala et al., 2003). Reducing treatment time would improve the cost-effectiveness of the process;

therefore, this study is aimed to investigate the treatment for shorter durations of 1, 3 and 5 hr in batch mode. The pre-treatment conditions such as treatment temperature and time with three different pH conditions (acidic, neutral and basic) were first optimized by an experimental design for the maximum organics solubilization. To the best of our knowledge, no previous study was reported on optimization of the pre-treatment conditions with experimental design. Low temperature thermal pre-treatments of 7 different types of sludge were then carried out at the optimal conditions. The effectiveness of the pre-treatments was investigated by a comprehensive characterization of the treated samples by analyzing changes in proteins and carbohydrates concentrations, elemental and FT-IR analyses. The digestibility of the pre-treated sludge samples was finally evaluated through BMP analysis.

2. Materials and Methods

2.1. Materials

Optimization of the thermal pre-treatment for the maximum solubilization was performed with WAS samples taken from Adelaide Pollution Control Plant (thereafter named as ADE-WAS), London, Ontario. The ADE-WAS samples were taken from rotary drum thickeners every two weeks in order to maintain consistency and sample freshness and stored at 4 ^{o}C prior to the experiments. Six other sludge samples (3 primary, 2 WAS, 1 digestate) from five different wastewater treatment plants were used in this work. Two additional WAS samples were obtained from Oxford Pollution Control Plant (thereafter named as OX-WAS) in London, and St. Mary's Wastewater Treatment Plant (thereafter named as SM-WAS), Ontario. Primary sludge samples were obtained from Adelaide (thereafter named as ADE-PS) and Pottersburg Pollution Control Plant (thereafter named as PO-PS) in London, Ontario. Sieved sludge (thereafter named as S-PO-PS), which is a primary sludge generated by a rotating belt filter as an alternative to primary sedimentation, was collected from Pottersburg Pollution Control Plant. Moreover, a digested sludge sample (thereafter named as G-D), collected from an anaerobic digester at Guelph Wastewater Treatment Plant, Guelph, Ontario, was used as a reference.

The pH of sludge was controlled by adding 1 N sodium hydroxide (NaOH) or 1 N sulfuric acid (H_2SO_4). The acid, base and all other chemicals were obtained from Caledon and Sigma-Aldrich, respectively. All other chemicals used for analysis were purchased from Sigma-Aldrich. The modified Lowry protein assay kit including the reagent (containing cupric sulfate, potassium iodide, and sodium tartrate in an alkaline sodium carbonate buffer), 2N Folin-Ciocalteu reagent and standard solution of bovine serum albumin were purchased from Thermo Scientific (ON, Canada).

2.2. Experimental design

Optimization of the thermal pre-treatment was conducted through a 3^3 full factorial design (three variables at three levels, a total of 27 experiments) to determine the effects of three independent variables (temperature, residence time and initial pH) on COD solubilization of ADE-WAS. Since SCOD is the main parameter for evaluation of sludge solubilization and hydrolysis (Chen et al., 2007; Uma Rani et al., 2012), it was treated as a major output. Optimized conditions were then applied to treat all seven different sludge samples.

The factors and levels used in the experiments are presented in Table 1. For statistical analysis, variable levels were normalised to -1 (low), 0 (central), and 1 (high) according to the following formula.

$$x_i = \frac{Hi+Lo}{2} + X_i \frac{Hi-Lo}{2} \qquad (1)$$

Where Hi is the un-coded high level and Lo is the un-coded low level of the variable.

<INSERT TABLE 1>

Design Expert (version 7.0), Minitab (version 16.0) and Matlab (version 2013b) were used to perform the statistical analysis, the experimental data fitting and response optimization. All three factors and their interactions were analysed by ANOVA, treating factors as continuous (including variables in Matlab using the anovan command) with non-significant interactions discarded to create the final model. The final model was also developed in Microsoft Excel 2010, using the regression tool in the analysis toolbox, simultaneously regressing the multiple factors. A summary of this analysis is provided in supplementary material S1.

2.3. Thermal pre-treatments

Thermal treatments on the sludge sample were performed in a 100 mL stirred batch reactor (Parr 4590 Micro Bench top reactor). In a typical experiment, approximately 70 g of sludge was fed into the reactor. The pH of raw sludge was around 7.6 ± 0.1, and adjusted using approximately 3.5 to 6.5 ml 1N acid or base solution, for acidic and basic conditions, respectively. The reactor was then sealed and the residual air inside the reactor was removed by purging with nitrogen. It was then heated with stirring to the desired temperature. Once, the desired temperature was reached, the reactant content was hold for the stipulated reaction time of 1 hr, 3hr or 5 hr. The reaction was stopped by quenching the reactor in a water/ice bath. Each experiment was run in duplicate or triplicate and the relative errors of the measured variables were mostly within ±4%.

2.4. Biochemical methane production tests

Biochemical methane potential (BMP) tests were measured on an automatic test system AMPTS II (Bioprocess Control, Sweden). Since the optimum pH for methanogenic bacteria is between 6.6 and 7.6, pH of all samples was adjusted before the BMP test by adding appropriate volume of 1 N NaOH or 1 N H_2SO_4. The batch anaerobic reactors were seeded with digestate (VS ~1.1%) collected from Guelph wastewater treatment plant, Ontario, and fed with respective pretreated substrate (e.g. ADE-WAS, OX-WAS, SM-WAS, ADE-PS, PO-PS, S-PS and G-D) at a substrate-to-inoculum ratio of approximately 1:3 on a mass VS basis. Untreated samples were used with seed as the control and seed alone was used in the blank to account for the background methane produced by the seed. All BMP tests were conducted in triplicate at 37 ^oC for approximately 20 days.

BMP data were fitted using Eq (2) to extract the hydrolysis rate coefficient (k) and methane potential B_0 (Jensen et al., 2011):

$$B(t) = B_0(1 - \mathrm{e}^{-\mathrm{k_{hyd}t}}) \qquad (2)$$

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

2.5. Sample analyses

After each experiment, the reactor contents were separated into four fractions for analyses: (i) the particulate (total) fraction of the sludge, (ii) the soluble fraction that was obtained after centrifugation of 10 ml of the pre-treated sludge at 4500 rpm for 10 min followed by filtration through 0.45 µm membrane filters, (iii) the bound or labile fraction that was obtained by centrifuging 5 ml of the pre-treated sludge at 4500 rpm for 10 min. The supernatant was removed and the solids were re-suspended in 50 ml of 50 mM phosphate buffer (pH=8). The solution was then mixed at 1500 rpm for 10 min using a magnetic stirrer. It was then centrifuged at 4500 rpm for 10 min followed by filtration using 1.2 µm filter paper and the filtrate was collected as the bound fraction (Higgins et al., 2008); and (iv) the tightly bound fraction that was obtained by centrifuging 5 ml of the reactor contents at 4500 rpm for 10 min. The supernatant was removed and the solids were re-suspended to a total volume of 50 ml with 1N sodium hydroxide solution. The solution was then mixed at 500 rpm for 2 hrs using a magnetic stirrer. It was centrifuged at 4500 rpm for 10 min following by filtration using 1.2 µm filter paper and the filtrate was collected as the tightly bound fraction (Higgins et al., 2008).

The pH of various aliquots/solvents was measured by the electric probe of SI Analytics potentiometric titrator (TitroLine® 7000). Total solids (TS), volatile solids (VS), total and volatile suspended solids (TSS and VSS), and total chemical oxygen demand (TCOD) were performed on particulates fraction and the soluble chemical oxygen demand (SCOD) was conducted on soluble fraction. All the analyses were performed according to the Standard Methods (American Public Health Association (APHA), 1960).

Protein concentrations of total sludge, soluble, bound and tightly bound fractions were determined using Thermo-Scientific protein kit based on modified Lowry et al. method (Lowry et al., 1951). The color developed in the sample is measured at 750 nm using a Thermo Scientific Evolution 220 UV-Visible spectrophotometer. Soluble and total carbohydrate concentrations were determined using the phenol–sulfuric acid method (Webb, 1985). The absorbance of the digested sample was measured using the spectrophotometer at 490 nm. Total lipids concentrations were measured based on Bligh & Dyer method using methanol-chloroform solution (W.J.Dyer, 1959).

The solids from selected streams were dried in an oven at 105 °C overnight for elemental (CHNS) analysis using a Flash EA 1112 analyzer (Thermo Scientific) employing 2, 5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (BBOT) as the calibration standard. The oxygen concentration was calculated by difference (100% - C% - H% - N% - S% - ash%). The Fourier transform infrared (FT-IR) analyses in 4000-550 cm^{-1} range for soluble fractions were conducted on a PerkinElmer FT-IR spectrometer (Model: LR 64912C)

COD and VSS solubilization after treatments were calculated as follows:

$$COD\ solubilization = \frac{SCOD_t - SCOD_0}{TCOD_0} \times 100 \qquad (3)$$

$$VSS\ solubilization = \frac{VSS_0 - VSS_t}{TSS_0} \times 100 \qquad (4)$$

Where the subscripts refer to the untreated samples (0) and treated samples (t).

3. Results and discussion

3.1. Optimization of thermal pre-treatments

3.1.1 Sludge characterization

The average characteristics of the collected ADE-WAS for experimental design experiments are listed in Table 2. As can be seen, that around 70% of the volatile solid contents are proteins and carbohydrates.

The characteristics of WAS used in this work compares well with literature although some of the parameters such as pH, TS, total and soluble protein are in the slightly higher range.

<INSERT TABLE 2>

The pH of the samples was measured before and after thermal pre-treatments. For alkaline and neutral conditions pH decreased after the pre-treatment, and the drop in pH was greater for alkaline condition (from 10.1 to 8.7) compared to neutral conditions (from 7.6 to 7.1). During alkali treatment the biomass itself consumes some of the alkali (Ariunbaatar et al., 2014) which results in pH reduction. It could also be due to the formation of acidic compounds by degradation of macromolecules (Bougrier et al., 2008). For the acidic pre-treatments, in contrast, pH was slightly increased from 4.1 to 4.3, which was likely attributed to the desorption of proteins or volatilization of acidic compounds (Bougrier et al., 2008).

3.1.2. COD solubilization and solids reduction

Table 3 shows the design of the experiments and the impact of different pre-treatment conditions on SCOD and VSS solubilization of ADE-WAS. After all pre-treatments, the total COD in the pretreated sludge remained almost constant. All pre-treatments resulted in increased COD solubilization (between 2 and 20%) compared to the untreated sludge; similar to the results found in previous pre-treatment studies in this temperature range (Dhar et al., 2012; Rajan et al., 1989; Uma Rani et al., 2012); The increase in SCOD is likely owing to the disruption of WAS microbial cells and release of organic compounds such as proteins, carbohydrates and lipids (Appels et al., 2010; Dhar et al., 2012). VSS solubilization was also in the same range as COD solubilization and changes from 0.45 to 38%. The difference between the VSS and COD solubilization is probably due to the different particle sizes used for VSS and SCOD calculations. VSS represents the particle sizes greater than 1.2 µm, while SCOD represents the particle soluble COD with sizes less than 0.45 µm. The particles in the size range less than 1.2 µm and greater than 0.45 µm are considered as colloidal particles. When VSS solubilization is greater than COD solubilization, suspended solids are transferred into colloidal fractions which are not completely solubilized. This was also confirmed during the filtration of the sludge for separating the soluble phase. After centrifugation of the sludge, it was first filtered by using 1.2 µm filters followed by filtration through 0.45 µm filters. Filtration of this solution was very difficult (even for the thermally treated sample), suggesting the presence of a large volume of colloidal particles (0.45 µm < d < 1.2 µm). On the other hand, greater COD solubilisation over VSS degradation indicates the solubilization of colloidal particles that are not included in VSS measurements. The degree of solubilisation increased with temperature, and at the same treatment temperature and time, solubilization in alkaline condition was higher than that in acidic or neutral conditions.

<INSERT TABLE 3>

3.1.3. Determination of factors affecting COD solubilization

The effects of single variables (temperature, pH and treatment time) on COD and VSS solubilization are shown as main effects plots (Fig. 1a and b), and the results of the ANOVA are shown in Table 4. Fig. 1 depicts the response mean for each variable level connected by a line when other variables are constant (without considering the interaction effects). According to Fig. 1a all three variables show a positive main effect for COD solubilization, implying that increasing each of temperature, time and pH when other parameters are kept constant enhances solubilization of organic matters in the sludge. However, only temperature and pH have a significant effect on VSS solubilisation (p = 0.004, 0.005 respectively). Three hours of treatment and neutral pH caused the lowest mean VSS solubilization, indicating that at these conditions, most of the solubilized organics are in the colloidal fraction, which is not included in VSS determination.

The analysis of variance (ANOVA) presented in Table 4 shows almost all observed variance can be represented by the model (R^2=0.92, p=4.9x10^{12}). All three factors (temperature, time and pH) were found to have significant effects on COD solubilization. Interaction, polynomial, and quadratic effects are not significant, but the interaction of time×pH^2 was found to have a significant effect ($p = 0.0024 < 0.05$), noting that due to normalisation of the coded variables, pH^2 will be either 0 or 1, for coded pH values of 0 or -1,1 respectively. An uncoded model, against raw values could also be fit, (R^2=0.88, p=6x10^{11}, SI S1), in which case the pH quadratic term dropped out.

<INSERT FIG 1>

<INSERT TABLE 4>

The reduced cubic regression model equation (third order polynomial) based on the coded values of the experimental factors as provided in Table 4 is shown below. This equation relates the COD solubilization (%) as a function of temperature (^{o}C), residence time (h), and initial pH of the solution (coded -1, 0, 1) as below:

$$COD\ Solubilization\ \% = 11.61 + 4.28 \times temperature + 1.48 \times time + 5.11 \times pH - 2.66 \times temperature \times pH^2 \qquad (5)$$

3.1.4. Response surface plots and optimization of process conditions

The three dimensional and contour plots for COD solubilization are shown in Fig 2a, b and c. Fig. 2a shows the interaction between temperature and time at constant pH 10. Solubilization shows an increasing trend with temperature and reaction time. The maximum COD solubilization occurs at highest temperature (80 ^{o}C) and reaction time close to 5 hrs.

<INSERT FIG 2>

Fig. 2b represents the interaction between pH and reaction time at constant temperature of 80 ^{o}C. As the pH increases, COD solubilization increases and same trend occurs for reaction time. The maximum solubilization in this case occurs at alkaline pH and at around 5 hrs. In Fig.2c the effect of temperature and pH at constant reaction time of 5 hrs is shown. Increasing both the parameters enhances solubilization of organics.

Based on the results, an optimization was performed by Design Expert (7.0) to maximize the solubilization of the treated sludge, and the recommended optimal conditions are 80 ^{o}C, 5 hrs treatment time, and pH =10 which is the same operating condition as experiment No. 27 in Table 4. The COD solubilization at optimum operating condition predicted to be 19.96 % by the software which is very close to the experimental value of 20.25 % in Table 3. Thus the predicted values and experimental results are in good agreement, and the recommended optimum conditions by Design Expert software are validated.

Similar results for the effect of temperature, residence time and pH have been reported by other researchers. For example, Uma Rani et al. found that temperature (60-80 ^{o}C), plays an important role in enhancing COD solubilization of dairy waste activated sludge (Uma Rani et al., 2012). Bougrier et al. and Valo et al. also reported a constant rise in SCOD of waste activated sludge when the treatment temperature was increased from 170 to 190 ^{o}C and 130 to 170 ^{o}C, respectively (Bougrier et al., 2008; Valo et al., 2004).

The positive effect of increasing the reaction time on COD solubilization was also seen by Uma Rani et al. (2012) where SCOD increased with time up to 24 hours for thermal solubilization of WAS at 6, 9, 12, 24, 36 and 48 h and alkaline conditions (pH=10-12).

The effects of pH on SCOD concentration and hydrolysis of WAS were investigated by Chen et al (Chen et al., 2007). They reported an increase in sludge hydrolysis with pH and found significantly higher SCOD at alkaline pH compared to neutral or acidic pH, which was also confirmed by Uma Rani et al.(2012). At alkaline pH, saponification of lipids in the cell walls may occur, which results in solubilization of membrane and leakage of intracellular material out of the cell (Neyens et al., 2003). Moreover, alkaline pH leads to the dissociation of acidic groups in EPS causing electrostatic repulsion between the negatively charged EPS, which may cause desorption of some of the extracellular polymers and subsequent increase in solubility of organic matters in water (Chen et al., 2007; Neyens et al., 2003). Strong alkali may solubilize EPS not only because of chemical degradation, but also because of the ionization of the hydroxyl groups resulting in extensive swelling and subsequent solubilization (Neyens et al., 2004). On the other hand, the main reaction that occurs when acid is added to the sludge is the hydrolysis of polysaccharides to respective monosaccharides which can solubilise relatively easily. Polysaccharides are generally unstable in strong acids causing hydrolysis of glycosidic linkages; however, they are stable towards degradation in alkaline conditions especially at high temperatures (Neyens et al., 2004). Strong acid conditions may result in production of inhibitory by-products such as furfural and hydroxymethylfurfural (HMF) (Ariunbaatar et al., 2014; Devlin et al., 2011; Rajan et al., 1989).

3.2. Effects of pre-treatment on different sludge types at the optimal operating conditions

Different sludge collected from various wastewater treatment plants were treated at the optimal operating conditions determined above. The characteristics of the untreated and treated sludge are presented in Table 5. The highest VSS solubilization occurred for the primary sludge collected from Pottersburg treatment plant (PO-PS), although the SCOD increase did not correspond with the VSS reduction probably due to more colloidal particles formed after pre-treatment. The high VSS solubilization for the primary sludge is in agreement with an earlier work (Aldin et al., 2010). Primary sludge is easily biodegradable since it consists of more easily digestible carbohydrates and fats, compared to activated sludge which consists of complex carbohydrates, proteins and long chain hydrocarbons. Interestingly, sieved primary sludge (S-PO-PS) did not show similar degree of solubilization as compared to primary clarifier sludge, also treatments seem to be quite effective for COD and VSS solubilization of two WAS samples collected from the City of London. While VSS reduction was low for WAS from St. Mary’s plant, the SCOD was higher indicating the presence of higher amounts of colloidal particles in the sludge. The ratio of % SCOD change to % VSS change after treatment varies from 0.24-2.13, depending on the source of sludge (different plants), rather than the locations within a plant (primary or secondary) indicating uncertain nature of the problem.

<INSERT TABLE 5>

3.3. Proteins and carbohydrates solubilization

Increase in SCOD of the treated WAS originates from the microbial cell lysis resulting in release of various organic compounds. It is well known that proteins and carbohydrates are the main constituents of

EPS of sludge (Chen et al., 2007). In order to investigate the effects of thermal treatments on solubilization of proteins and carbohydrates, some of the primary and WAS samples were selected for proteins and carbohydrates analysis. Adelaide plant's WAS and PS (ADE-WAS and ADE-PS) were selected for this purpose as well as S-PO-PS since it is a primary sludge generated by an alternative method (rotary belt filtration) rather than from primary clarifier.

Fig. 3 shows the total carbohydrates concentration for ADE-WAS, ADE-PS and S-PO-PS before and after thermal treatment at optimum operating conditions. The total carbohydrates concentration has remained almost constant after the treatment with an average experimental error of 10%. This means that carbohydrates did not degrade to volatile fatty acids (VFA) during the low-temperature thermal treatment. There seems to be much larger amount of total carbohydrates in primary sludge compared to WAS, which is in agreement with literature (Ariunbaatar et al., 2014)., while WAS has higher amounts of proteins and lipids However, the concentration of soluble carbohydrates is greater in un-treated WAS compared to primary sludge, as shown in Fig. 4. Thermal treatment does not show a considerable increase in soluble carbohydrates concentration except for S-PO-PS, where the soluble carbohydrates increased from 109 µg/ml in the un-treated sample to around 220 µg/ml in the treated one.

<INSERT FIG 3>

<INSERT FIG 4>

Protein content in the sludge is usually divided into different fractions such as total, soluble, bound and tightly bound fraction. Bound and tightly bound fractions represent the protein loosely attached to the microbial cell wall and the fraction inside the microbial cell, respectively, however the soluble proteins are in the aqueous phase (Dhar et al., 2012; Higgins et al., 2008). The total protein is the combination of these fractions as well as some unknown fractions in the sludge.

<INSERT FIG 5>

According to Fig. 5 the total concentration of protein in sludge is almost constant before and after the thermal treatments, suggesting that total protein remained unchanged at low temperature thermal treatment. As expected, WAS showed greater amount of total proteins compared to PS. It also contained more soluble, bound and tightly bound protein fractions according to Fig. 6. As a result of thermal pre-treatments the concentration of tightly bound fraction considerably decreased for all samples and reached to 43.4 µg/ml, 24.9 µg/ml and 113.17 µg/ml compared to 592.2 µg/ml, 278.1 and 223.7 µg/ml in the untreated samples for ADE-WAS, ADE-PS and S-PO-PS, respectively. This indicates that cell lysis took place during the treatment and the proteins inside the cells were released and transferred from tightly bound fractions to soluble proteins. The treatments were more effective in releasing the tightly bound fraction of WAS compared to primary sludge and this trend was also observed in reduction of bound protein fraction which could explain the higher COD solubilization for WAS compared to PS. The treatments have also resulted in considerable increase in soluble protein fractions. Previous researchers have pointed out the effect of low-temperature treatments on destroying the cell walls and making the proteins accessible for biological degradation (Neyens and Baeyens, 2003). Comparing Fig. 4 and 6, it can be stated that in all cases, increase of soluble protein was much higher than soluble carbohydrates in the same operating condition. Bourgrier et al. (2008) suggested that carbohydrates are mainly located in the exopolymers of sludge structure and proteins are mainly placed inside the cells (Bougrier et al., 2008). It is also well known that both proteins and carbohydrates are the main compositions of EPS (Chen et al., 2007). Considering that exocellular proteins concentration exceed carbohydrates, making them the most abundant component of sludge EPS (Neyens et al., 2004), the higher concentration of soluble proteins compared to carbohydrates suggests that cell lysis occurred during the thermal treatment and the protein concentration is the sum of protein released from EPS as well as the cell lysis.

<INSERT FIG 6>

3.4. Effects of treatments on sludge functional groups

FT-IR analysis of the soluble phase of the selected sludge samples in the range of 4000-550 cm^{-1} was performed to identify the effects of treatments on functional groups. A strong band at 3300 cm^{-1} was observed and attributed to overlapping of O–H stretch of bound water and N-H stretch of protein group. The band located at 1640 cm^{-1} was assigned to the stretching vibration of C=O and C-N (amide 1) peptidic bond of proteins. Since no protein degradation occurred during the treatments, no peaks associated with amino acids or smaller fragments such as NH_3 and carboxylate groups were observed. The same functional groups were observed for all of the selected samples. Thus the thermal treatments at low temperature did not affect the functional group types in sludge samples.

3.5. Elemental analysis of the sludge samples

CHNS analysis was performed on the suspended solids fraction of the same sludge for which proteins and carbohydrates were analyzed. Table 6 shows the results for selected sludge samples.

<INSERT TABLE 6>

A slight decrease of sulfur in treated sludge compared to the untreated sludge indicates the release of sulfur components to the soluble phase. It is also possible that the sulfur has been converted to ferrous sulfide (FeS) or colloidal sulfur during pre-treatment. The sulfur contents in the sludge are not desirable and may contribute to corrosion in combustion engines and lead to unpleasant odor in wastewater treatment plants when converted to hydrogen sulfide (H_2S) and other organosulfur compounds during anaerobic digestion (Dhar et al., 2012, 2011). The nitrogen content of the treated samples also decreased compared to untreated samples. This shows that nitrogen has been transferred to the soluble phase when thermally treated. As the proteins are the primary source of nitrogenous compounds, this suggests that proteins were solubilized during the pre-treatments. The decreased carbon content of the treated samples indicates solubilization of carbohydrates as a result of thermal treatments. Higher reduction of C, H, N and S elements for ADE-WAS treated sample compared to ADE-PS and S-PO-PS confirms the higher VSS solubilization for ADE-WAS (38.78%) compared to ADE-PS (15.17%) and S-PO-PS (18.44%) (Table 4) as these elements represent the volatile matter content of the sludge.

3.6. Impact of low-temperature thermal pre-treatments on methane production potential

The seven sludge samples treated at optimum operating conditions were analyzed for methane production through BMP tests, which represent anaerobic digestibility of sludge. The BMP graphs are provided in the supplementary material S2. The characteristic parameters are summarized in Table 7.

<INSERT TABLE 7>

The degradability of the samples which is translated to final methane production does not show significant improvement in the treated samples compared to the untreated sludge. It actually reduced for the S-PO-PS sludge treated at the earlier optimized conditions. This might be due to the fact that the operating conditions were optimized based on WAS and not on primary sludge, indicating how the nature of sludge determines the outcome. However, the hydrolysis rate coefficient of all treated samples was 1.1 - 2.5 times higher than that of the untreated sludge. Even for the G-D sludge (a digested sludge), k_{hyd} increased more than five times compared to the un-treated G-D. A single tailed *t*-test (for treated > untreated) indicated no significant effect on B_0 ($p = 0.15$) and a weak but significant effect on k_{hyd} with a p value of 0.013. This indicates that thermal pre-treatment enhanced the hydrolysis, which is a rate-limiting step in AD, but did not improve the ultimate digestibility.

Previous studies suggest that solubilization of particulate proteins as a result of pre-treatment will enhance the subsequent digestion of sludge since protein is the least biodegradable component of the sludge compared to carbohydrates and lipids (Neyens and Baeyens, 2003; Uma Rani et al., 2012). In our study, increased protein solubilization did not result in improved methane production from the treated samples. While the COD solubilization was enhanced for the all sludge, it is likely that the thermal pre-treatment was solubilising particulate material which would otherwise been more slowly degradable (hence the increase in hydrolysis coefficient). Another possibility is formation of non-degradable materials such as dioxins, which were reported previously (Ferrer et al., 2008; Mullar, 2001). In our case, it is less likely for dioxins to form as they are associated with the presence of oxygen and high temperature treatments (T > 100 °C) (Appels et al., 2010; Nges and Liu, 2009). However, melanoids can start forming at temperatures lower than 100 °C (even at room temperature) and longer reaction times (from hours to days) and are distinguishable by their brownish color, which was also observed in the soluble phase in our experiments (Ariunbaatar et al., 2014; Nges and Liu, 2009). Thus, it could be concluded that formation of refractory components during the pre-treatments as well as solubilization of non-biodegradable organics or transformation of organics into CO_2 have led to the same or even reduced methane production during the BMP test. For example, Appels et al. obtained a negligible increase of biogas production from sludge pretreated at 70 °C for 60 min (Ariunbaatar et al., 2014). Pre-treatment of household waste and algal biomass at 70 °C for 60 min and 8 hr, did not report any enhancement of biogas production (Ariunbaatar et al., 2014). However, Tanaka et al. observed a 30% increase in methane production when treating WAS in alkaline condition at 60 and 80 °C (Tanaka et al., 1997).

The results from our comprehensive work on 7 different sludges confirm that the high COD and VSS solubilization after the pre-treatments do not necessarily indicate an increase in methane yield. However, the heat treatments improved the hydrolysis rate coefficient during BMP test which could result in increased digester capacity or reduced treatment time. In addition, reduction in bound protein due to thermal pre-treatment also can cause reduction in odor during digestion (Dhar et al., 2011).

4. Conclusions

The effects of low-temperature thermal pre-treatment on sludge solubilization and biodegradability were studied using various types of sludge. The experimental conditions including temperature, reaction time and pH were optimized for maximum COD solubilization using full factorial design and the optimal conditions were determined. The following conclusions can be drawn from this study:

- Higher temperature, longer reaction time and alkaline pH were favorable for increased solubilization of organic matter in WAS. The optimum operating conditions for maximum COD solubilization were determined to be 80 °C, 5 hrs and pH ≈10. COD solubilization at these conditions increased by 20% with a VSS reduction of 44% compared to the untreated sample.
- Pre-treatment resulted in the release of carbohydrates and proteins to the soluble phase. Increase of soluble proteins was much higher than the soluble carbohydrates, as protein released from both EPS and the cell lysis.

- Methane was produced at a higher rate for the thermally pre-treated samples based on the BMP tests results, but the ultimate methane yield was not significantly affected by the pre-treatment.

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Table 1- The factorial design variables and levels

Experimental variables	Symbol	Levels		
		-1	0	1
Temperature (^{o}C)	X_1	40	60	80
Residence time (hr)	X_2	1	3	5
pH	X_3	4	7	10

Table 2- Average of characteristics of collected ADE-WAS sample

Parameter	Value		Ref.
	WAS used in the experiments	WAS used in literature	
pH	7.76 ± 0.1	6.8-7.1	(Pang et al., 2014)
TS (%)	3.91 ± 1.8	1.5-4.4	(Bougrier et al., 2008; Kim et al., 2015)
VS (%)	2.85 ± 1.3	1.1-3.3	(Bougrier et al., 2008; Kim et al., 2015)
TCOD (g/L)	52.4 ± 4.4	21.0-62.0	(Dhar et al., 2011; Kim et al., 2015)
SCOD (g/L)	0.98 ± 0.2	1.4-2.8	(Dhar et al., 2011; Kim et al., 2015)
Total Protein (g/L)	15.2 ± 0.4	2.8-15.7	(Devlin et al., 2011; Pang et al., 2014)
Soluble Protein (g/L)	0.68 ± 0.0	0.05-0.45	(Devlin et al., 2011; Dhar et al., 2011)
Total Carbohydrates (g/L)	4.09 ± 1.2	0.62-6.2	(Devlin et al., 2011; Pang et al., 2014)
Soluble Carbohydrates (g/L)	0.21 ± 0.0	0.1-0.31	(Bougrier et al., 2008; Dhar et al., 2011)
Total Lipids (%)	3.09 ± 0.1	5-12	(Haandel and Lubbe, 2007)

Table 3- The experimental design and the results

Variables in uncoded/original units				Experimental results			
No.	Temp. (^oC)	Residence time (hr)	pH	$SCOD_t$-$SCOD_0$ (mg/l)	VSS_0-VSS_t (mg/l)	COD solubilization (%)	VSS solubilization (%)
1	40	1	4	1200	5900	2.59	13.29
2	40	1	7	1120	200	2.42	0.45
3	40	1	10	6960	6400	15.01	14.41
4	40	3	4	3120	1400	6.73	3.15
5	40	3	7	3720	1334	8.02	3.00
6	40	3	10	6800	7000	14.67	15.77
7	40	5	4	2000	3900	4.31	8.78
8	40	5	7	3560	3600	7.68	8.11
9	40	5	10	8280	8600	17.86	19.37
10	60	1	4	4080	5700	6.98	12.93
11	60	1	7	6040	4200	10.34	9.52
12	60	1	10	7780	9600	13.31	21.77
13	60	3	4	4960	4400	8.49	9.98
14	60	3	7	7320	4000	12.53	9.07
15	60	3	10	10160	7800	17.39	17.69
16	60	5	4	4540	7600	7.77	17.23
17	60	5	7	8980	8000	15.37	18.14
18	60	5	10	10280	9600	17.59	21.77
19	80	1	4	3680	7200	6.82	15.69
20	80	1	7	7000	3400	12.98	7.41
21	80	1	10	9560	15200	17.73	33.12
22	80	3	4	4720	6600	8.75	14.38
23	80	3	7	8120	5000	15.06	10.89
24	80	3	10	10120	17200	18.77	37.47
25	80	5	4	4440	7400	8.23	16.12
26	80	5	7	8480	10000	15.73	21.79
27	80	5	10	10920	17800	20.25	38.78

Table 4. ANOVA results on COD solubilization model considering only significant effects and interactions

Source	Sum of squares	Degrees of freedom	Mean square	F	*p*-Value
Model	649.6	4	162.4	67.55	< 0.0001
Temperature	109.7	1	109.7	45.61	< 0.0001
Time	39.4	1	39.4	16.36	0.0005
pH	469.3	1	469.3	195.21	< 0.0001
$\text{Temperature} \times \text{pH}^2$	28.3	1	28.3	11.77	0.0024
Residual	52.9	22	2.4		
Total	702.5	26			

Table 5. Solubilization of different sludge types treated at the obtained optimum operating conditions

Characteristics	ADE-WAS	OX-WAS	SM-WAS	ADE-PS	PO-PS	S-PO-PS	G-D
Untreated sample							
TS (%)	4.01	3.66	4.78	2.95	3.09	3.82	1.12
VS (%)	2.94	2.55	3.54	2.59	2.62	3.46	0.67
TCOD (g/l)	52.4	44.8	66.1	49.5	47.4	49.9	10.1
SCOD (g/l)	0.98	2.40	1.98	4.90	6.42	4.64	1.24
Treated sample							
VSS_0-VSS_t (mg/l)	17800	18000	5800	4400	13200	6000	2000
$SCOD_t$-$SCOD_0$ (mg/l)	10920	11780	17980	9000	4960	3480	1860
VSS solubilization (%)	38.78	35.86	12.78	15.17	43.42	18.44	29.41
COD solubilization (%)	20.25	26.27	27.18	18.20	10.46	6.97	18.56

Table 6. CHNS results of the suspended solids fractions of selected sludge samples

Samples		C (%)	H (%)	N (%)	S (%)
ADE-WAS	Un-treated	35.84	5.37	6.20	0.91
	Treated	27.58	4.15	3.13	0.57
ADE-PS	Un-treated	47.25	7.07	2.21	0.22
	Treated	45.41	6.68	1.27	0.22
S-PO-PS	Un-treated	46.01	6.86	1.59	0.22
	Treated	44.39	6.68	0.75	0.12

Table 7. Hydrolysis rate coefficients and degradability determined from the BMP tests results using parameter estimation

Parameters		ADE-WAS	OX-WAS	SM-WAS	ADE-PS	PO-PS	S-PO-PS	G-D
B_0 (degradability, ml/g VS added)	Un-Treated	293	190	176.3	489.5	479.6	498.4	159.5
	Treated	305	198	184.5	505.8	437.5	333.2	127.1
k_{hyd} (hydrolysis rate coefficient, hr^{-1})	Un-Treated	0.029	0.017	0.013	0.015	0.019	0.015	0.008
	Treated	0.034	0.039	0.033	0.017	0.023	0.023	0.045

Table 8. Experimental and theoretical values for methane yield

Samples		TMP (ml CH_4/gVS)	BMP (ml CH_4/gVS)	Error %
ADE-WAS	Un-treated	261.18	293.3	10.1
	Treated	126.34	305.3	58.6
ADE-PS	Un-treated	514.96	489.5	5.2
	Treated	438.71	505.8	13.3
S-PS	Un-treated	451.15	498.4	9.5
	Treated	426.77	333.2	28.1

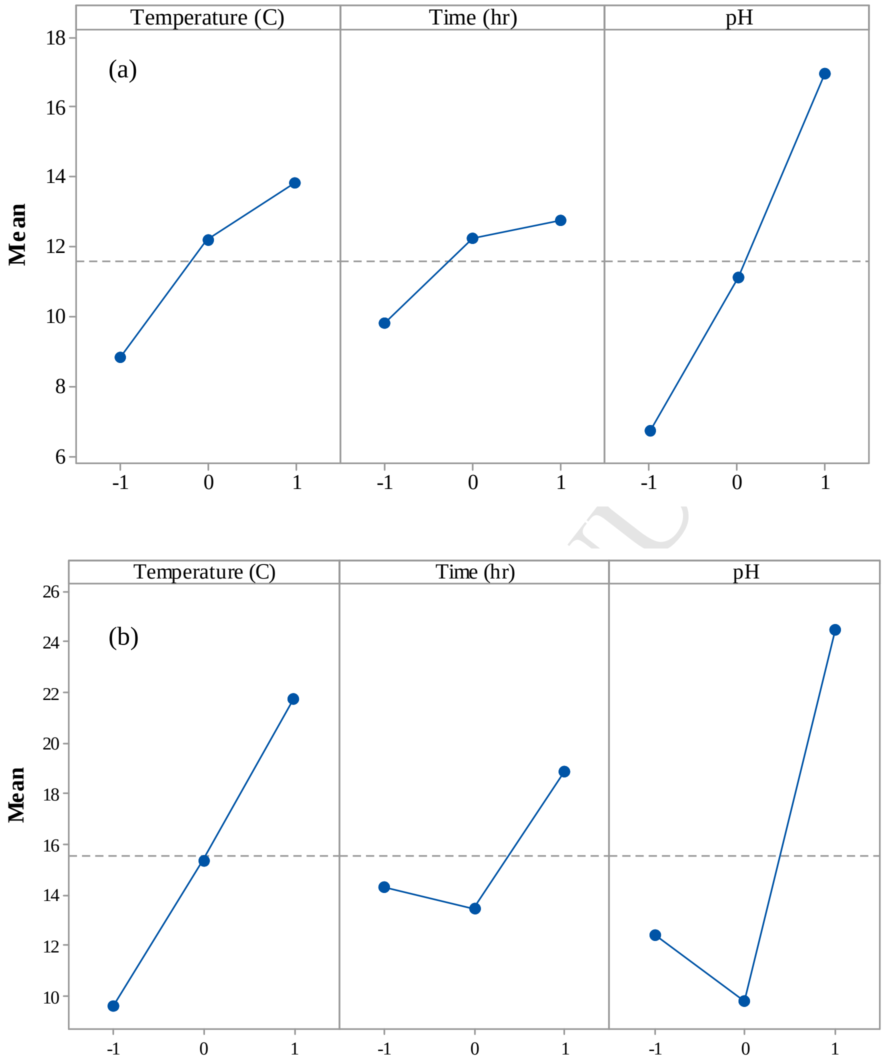

Fig. 1. Main effect plots for SCOD (a) and VSS solubilization (b)

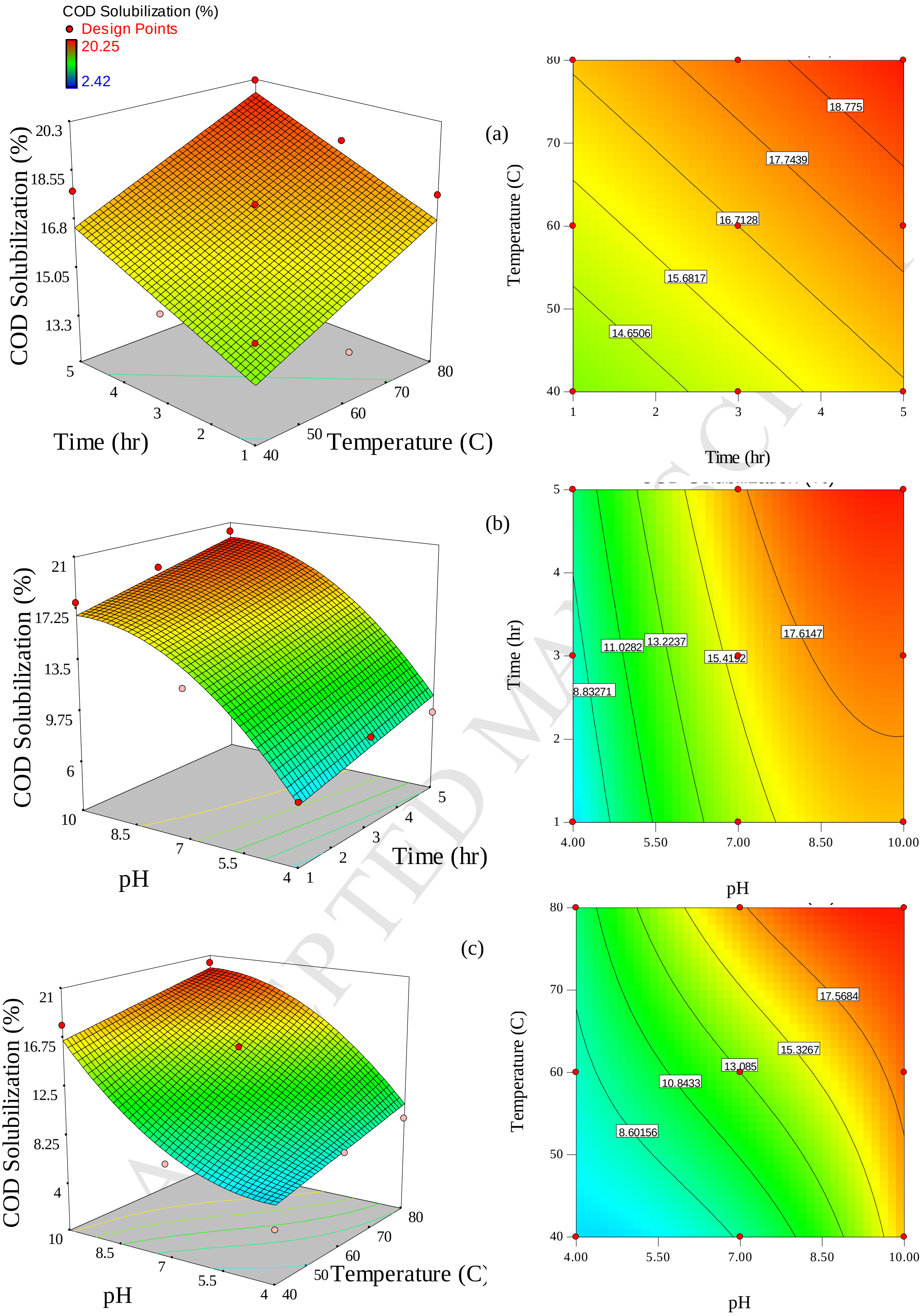

Fig.2. Three dimensional response surface and contour plots for COD solubilization at (a) constant pH (10), (b) constant temperature (80 ^{o}C) and (c) constant time (5 hrs)

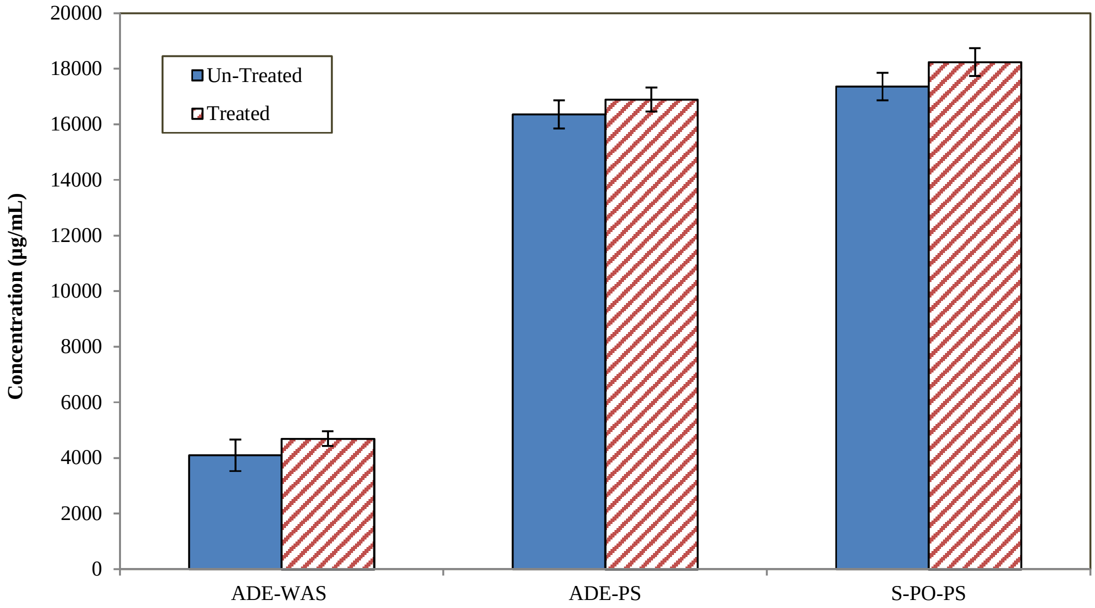

Fig. 3. Total carbohydrates concentration for the different sludge samples treated at optimum operating conditions ($80\ ^{o}C$, 5 hr and pH=10)

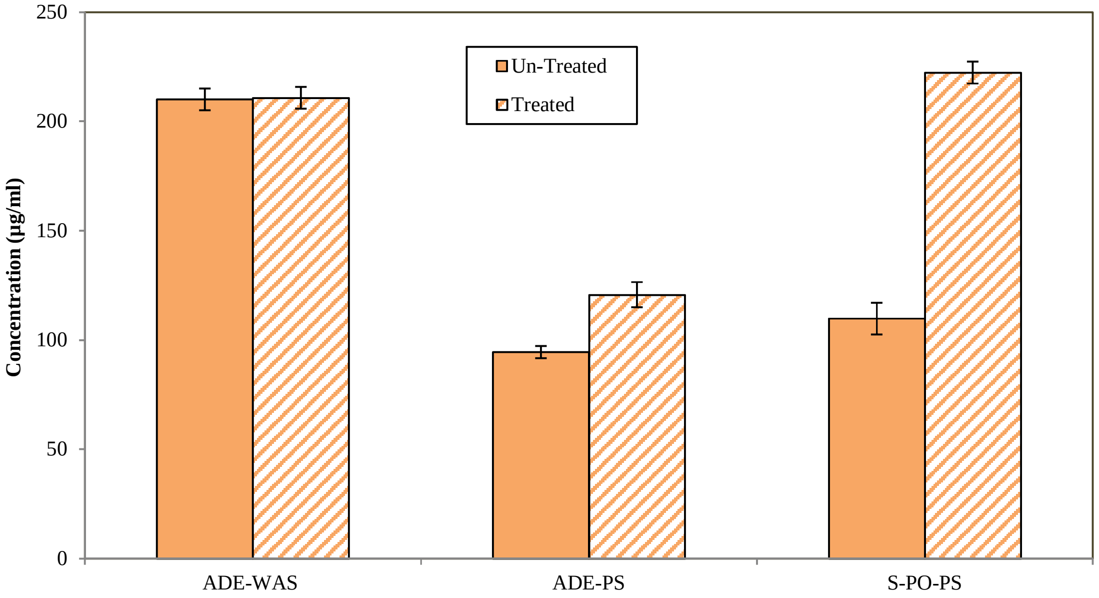

Fig. 4. Soluble carbohydrates concentration for the different sludge samples treated at optimum operating conditions (80 ^{o}C, 5 hr and pH=10)

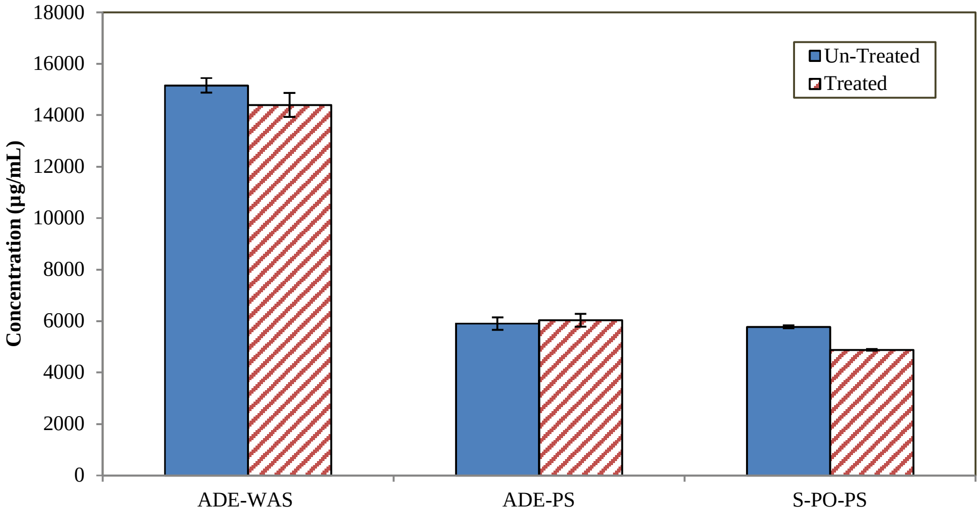

Fig. 5. Total protein concentration for the different sludge samples treated at optimum operating conditions (80 ^{o}C, 5 hr and pH=10)

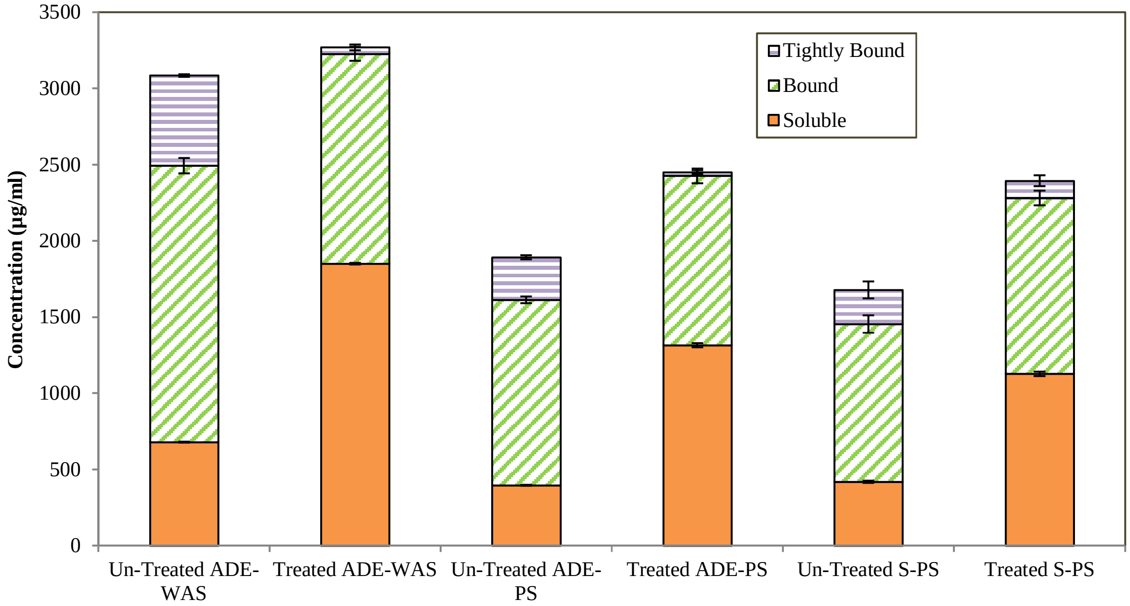

Fig. 6. Different protein fractions concentration for the different sludge samples treated at optimum operating conditions (80 ^{o}C, 5 hr and pH=10)

Highlights:

- Thermal pretreatment was conducted for 7 different sludge from 3 wastewater plants.
- Using CCD, the optimum treatment conditions were determined to be 80 ^{o}C, 5 hr and pH 10.
- COD and VSS solubilization increased as a result of thermal pre-treatments.
- The solubilization of proteins was significantly higher than carbohydrates
- Methane was produced at initial higher rates for the pretreated samples.