Effects of Pretreatments on the Solubilization and Theoretical Methane Production of Waste Activated Sludge from a Brazilian *Eucalyptus* Kraft Pulp Mill

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Waste activated sludge (WAS) originating from kraft pulp mill effluent treatment plants represents an important environmental challenge for this industry. Anaerobic digestion is a promising option for WAS treatment, with the added benefit of biogas production. This paper presents the application of thermal, thermal-alkaline, and mechanical pretreatment methods in order to promote the solubilization of organic matter to enhance the anaerobic digestion of the WAS from a Brazilian *Eucalyptus* bleached kraft pulp mill. A total of 16 pretreatment operating conditions were compared. Chemical analyses showed an improvement in organic matter solubilization, with an increase greater than 7-fold for soluble chemical oxygen demand and 4-fold for biochemical oxygen demand. Nutrient solubilization showed an increase greater than 10-fold for total Kjeldahl nitrogen and 3-fold for total phosphorus. Theoretical biochemical methane potential was improved from 211 mLCH$_4$/gVS for raw sludge to 333-343 mLCH$_4$/gVS after mechanical pretreatment, 314-360 mLCH$_4$/gVS after thermal pretreatment, and 373-378 mLCH$_4$/gVS after thermal-alkaline pretreatment. In general, thermal-alkaline pretreatment showed the best results for all the evaluated parameters, with the advantage of requiring lower temperature and retention time when compared to thermal conditions.

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INTRODUCTION

In addition to consuming significant quantities of fresh water, the pulp and paper (P&P) industry is one of the world's largest generators of effluents. Despite the continuous reduction of water use, from about 200 m^3/ADT in the 1960's to the current 25 m^3/ADT in modern kraft pulp mills, an average of approximately 60 m^3/ADT of effluent is generated (Reeve and Silva 2000; Karlsson 2010).

Effluent treatment plants (ETP) generate considerable amounts of waste activated sludge (WAS), which is usually incinerated or disposed of in landfills. Sludge generation in Canadian mills was estimated at 50 kg/ADT, and WAS corresponds to approximately 30% of this total (Elliott and Mahmood 2005). Sludge management represents a large financial burden on P&P mills. Approximately 60% of the total effluent treatment costs are spent on sludge management and disposal (Kamali *et al*. 2016). Therefore, there is a growing demand for methods that minimize the costs by reducing the volume of WAS or reducing the cost of the treatment and final disposal.

Anaerobic digestion is a promising treatment for WAS, with the additional benefit of producing biogas. However, WAS from P&P mills contains complex lignocellulosic compounds, which inhibit the digestion of the substrate. Considering the slow rates of substrate removal (0.3 to 11 gCOD/gVSS.d) (Rajagopal *et al*. 2013) and slow bacterial growth rates (0.02 to 0.04 gVSS/gCOD removed) (Mermillod *et al*. 1992) inherent in the anaerobic processes, it requires high-volume reactors operating with a high retention time, turning this option economically unfavorable (Meyer and Edwards 2014).

Pretreatment technologies maximize the soluble fraction of the sludge organic matter prior to applying anaerobic digestion. Different methods have been investigated based on biological, chemical, thermal and mechanical processes (Elliott and Mahmood 2007; Meyer and Edwards 2014; Li *et al*. 2019). Defined as “methods of sludge disintegration”, pretreatment leads to a desired soluble chemical oxygen demand and/or protein increase in the liquid phase, resulting in an easier conversion of organic matter to methane. Promising results related to the increase of biogas production, organic matter removal, and reduction of the retention time required for the anaerobic digestion have been reported (Elliott and Mahmood 2007; Meyer and Edwards 2014).

Thermal, thermal-alkaline, and mechanical sludge pretreatments for anaerobic digestion are the most studied methods (Elliott and Mahmood 2007; Meyer and Edwards 2014; Li *et al*. 2019). The results of research are promising, and a considerable increase in methane production has been observed using WAS from P&P mills, *e.g.*, 110 mL/mgCOD for thermal-alkaline and 115 mL/mgCOD for thermal pretreatments, compared with 30 mL/mgCOD for raw sludge (Wood *et al*. 2009, 2010), and more than 100% increase after mechanical pretreatment (Elliott and Mahmood 2012).

For P&P mill sludge, thermal pretreatment conditions include temperatures between 70 and 190 °C (Bayr *et al*. 2013; Huang 2015) and retention time from 10 to 60 min (Wood *et al*. 2009; Wood *et al*. 2010; Bayr *et al*. 2013). Usually, longer retention times are related to lower temperatures. Thermal-alkaline pretreatment was tested at pH 12 and 140 °C for 60 min (Wood *et al*. 2009, 2010). Mechanical methods included mechanical shearing (1500 rpm in alkaline conditions for 30 min), sonication (20 kHz), and high-pressure homogenization of 83 MPa in alkaline conditions (Elliott and Mahmood 2012). Commercial pretreatment plants are available, based on thermal hydrolysis process, *e.g.*, Cambi® (Norway) and Lystek® (Canada), both suitable for anaerobic digestion optimization by disintegration of microbial cell walls and hydrolyses of complex macromolecules into simpler compounds from several biosolids. Nevertheless, there are still gaps in the knowledge, such as the effects of pH on the solubilization of organic matter at different temperatures and the effects of the retention time during the mechanical disintegration.

The effects of pretreatment are still unclear, considering the large discrepancy between the results reported in the literature, even when the same pretreatment types were compared, *e.g.*, 60 mLCH$_4$/gVS (Kinnunen *et al*. 2015) to 182 mLCH$_4$/gVS (Zhang *et al*. 2016) for thermal pretreatment. Most studies have been limited to investigating the increase in biogas production through biochemical methane potential (BMP) tests, without verifying the real effects of pretreatment on sludge solubilization (for example, COD and nutrient solubilization before and after the pretreatment tests). Considering that the increase in biogas yield occurs due to the previous solubilization of the organic matter caused by the pretreatment, as evidenced in several studies (Wood *et al*. 2009, 2010; Bayr *et al*. 2013; Karlsson *et al*. 2011; Kinnunen *et al*. 2015; Veluchamy *et al*. 2017, 2018), a deeper understanding of the effects of pretreatment is needed for the optimization of the anaerobic process.

Although experimental BMP tests are widely applied for determining methane yield and anaerobic digestion configurations, there are some methodologies used to save costs and time from this process by using the theoretical biochemical methane potential (BMP_{th}) of a substrate considering its organic composition. Methodologies based on the elemental composition for the determination of theoretical production fit better with the experimental results and behavior (Nielfa *et al*. 2015). BMP_{th} is widely recognized in order to give an indication of the maximum methane production expected from a specific waste. A review on the methods for determination of biomethane potential mentioned that theoretical studies are significant and constitute an interesting option, especially in cases where access to laboratory facilities is limited (Jingura and Kamuso 2017). For P&P sludge, the methane yield ranges from 40 to 60% of the theoretical potential (Rodriguez-Chiang and Dahl 2015).

The objective of this research was to present and evaluate different thermal, thermal-alkaline, and mechanical pretreatments, in order to find the best condition for organic matter and nutrient solubilization and theoretical methane production for WAS generated in a Brazilian *Eucalyptus* bleached kraft pulp mill.

EXPERIMENTAL

Sampling

The WAS samples were collected from an ETP of a bleached kraft pulp mill located in Southeast Brazil. The mill uses *Eucalyptus* sp. as raw material for pulp production (approximately 1 Mt of ADT/year). The effluent treatment process consists of a primary clarifier followed by a conventional activated sludge plant. Approximately 15 kg of WAS/ADT of pulp (dry basis) is generated. Approximately 300 L of WAS were collected from the return line of the secondary clarifier and stored according the sampling and samples preservation methods (USEPA 2013): 50-L plastic flasks, at temperatures below 4 °C, with additional samples placed into acidified glass flasks (H_2SO_4 to $pH < 2$) for chemical oxygen demand, nitrogen, and phosphorus analyses.

Pretreatment Tests

Figure 1 summarizes the experimental design of the pretreatment tests.

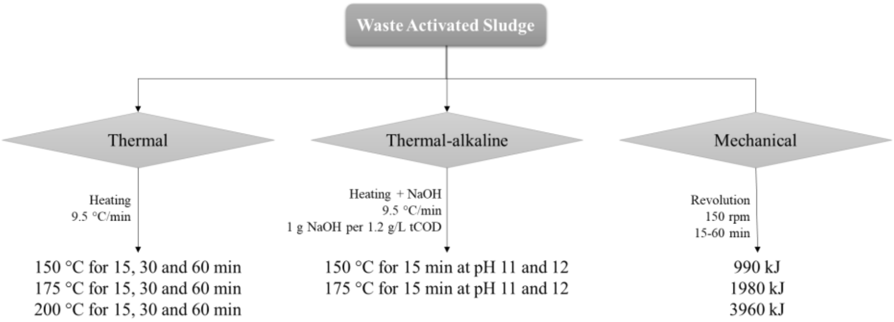


Fig. 1. Experimental setup for pulp mill waste activated sludge pretreatment tests

In all, 16 different thermal, thermal-alkaline, and mechanical pretreatment operating conditions were tested in duplicate, resulting in 32 tests. The tested conditions were chosen based on published results for pretreatment prior to anaerobic digestion tests using WAS from a municipal wastewater treatment plant (Valo *et al*. 2004) and WAS from pulp and paper ETPs (Wood *et al*. 2009; Elliott and Mahmood 2012; Bayr *et al*. 2013).

Thermal (T) and thermal-alkaline (TA) pretreatment tests were carried out in a 20-L pressurized Parr 4848 M Reactor (Parr Instrument Company, Moline, USA), with an internal mixer (200±10 rpm) and thermometer. The reactor is made of stainless steel and designed to withstand high temperatures and pressures. For each pretreatment test, 4 L of WAS were used. After loading the sludge sample, the headspace of the reactor was flushed with nitrogen gas to exclude oxygen and prevent any oxidation of the organic compounds. Heating rate was measured in 9.5 °C per minute. Retention time was measured from the moment that the desired temperature was reached.

For thermal-alkaline pretreatment, pH adjustment was carried out by using sodium hydroxide (NaOH), a pH probe, and a shaker table. The experiment required 5.5 g to achieve pH 11 and 6.2 g to achieve pH 12, resulting in a relation of approximately 1 g NaOH per 1.2 g/L tCOD. The pH adjustment was performed immediately before the pretreatment tests started, in order to verify if the pH increase contributes to a reduction of the required retention time.

Mechanical (M) pretreatment was carried out using a centrifugal mill (REGMED Jokro MJK-6, São Paulo, Brazil), according to NBR 14.346 (NBR 14.346 1999), using 16 g (dry basis) of WAS previously disintegrated. It was tested under three conditions in terms of energy input: 990 kJ (150 rpm for 15 min), 1980 kJ (150 rpm for 30 min) and 3960 kJ (150 rpm for 60 min). Due to the equipment characteristics, rotation could not be changed and the influence of stirring speed was not evaluated.

Analytical Methods

Chemical characterization of WAS, performed before and after the pretreatment tests, included the following parameters: total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) – method 5220-D (APHA/AWWA 2012); biochemical oxygen demand (BOD_5) – method 5210 (APHA/AWWA 2012); total phosphorus (P) – method 4500-P (APHA/AWWA 2012); total Kjeldahl nitrogen (TKN) – method 1687 (EPA 2001a); ammonia-nitrogen (NH_3-N) – method 1689 (EPA 2001b); volatile solids (VS) – method 2540 (APHA/AWWA 2012); and elemental composition (C, H, N, O) – according to the analyst's manual of TruSpec Micro CHN and TruSpec O (LECO, St. Joseph, MI , USA). For sCOD it was used filters with 0.45 µm pore size. All the analysis was performed in triplicate and the results are expressed as the mean ± standard deviation.

WAS Disintegration and Theoretical BMP

Sludge floc structure was observed before and after pretreatment tests using a digital microscope (RoHS®, Nanjing, China).

The empirical biomass formula ($C_aH_bO_cN_d$) was determined according to Rittmann and McCarty (2012). BMP_{th} was calculated using the stoichiometric equation (Eq. 1) based on the atomic composition of the WAS using the Buswell equation (Eq. 2).

$$C_aH_bO_cN_d + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4}\right) H_2O \rightarrow$$
$$\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8}\right) CO_2 + dNH_3 \text{ (Eq. 1)}$$

$$BMP_{th} = 22.4 * \left[\frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right)}{12.017a + 1.0079b + 15.999c + 14.0067d}\right] * 1000 \text{ (Eq. 2)}$$

Statistical Analysis

Data analysis was performed using the Ryan-Joiner test for verifying whether the results were normally distributed. Statistical significance was examined by analysis of variance (ANOVA) and *post hoc* Tukey test to evaluate the difference of the averages for data with normal distribution. The Kruskal-Wallis method was used when the results did not follow a normal distribution, and the *post hoc* Dunn test was employed to evaluate the difference of the averages. The probability level of $p < 0.05$ was considered significant. Minitab 17.1.0 (MINITAB®, 2016, State College, PA, USA) and XLSTAT 2020.4.1 (Addinsoft, 2020 – New York, USA) software were used in order to perform the statistical tests, and SigmaPlot 11.0 (Systat Software, 2008, Chicago, IL, USA) was used to produce the graphs.

RESULTS AND DISCUSSION

Organic Matter Solubilization and Floc Disintegration

Total COD raised from 7.55 g/L (raw sludge) to 8.43 to 9.12 g/L (M pretreatment), to 8.81 to 11.04 g/L (T pretreatment), and to 10.85 to 12.07 g/L (TA pretreatment), an increase of 12 to 60% (Fig. 2). The increase in sCOD was even greater, from 1.11 g/L (raw sludge) to 7.30 to 7.50 g/L (M pretreatment), to 7.33 to 10.73 g/L (T pretreatment) and to 9.21 to 11.46 g/L (TA pretreatment), an increase of 656 to 1,030%. The maximum tCOD (12.07 g/L) and sCOD (11.46 g/L) values from these tests were obtained with the TA conditions of 175 °C for 15 min at pH 12.

Statistically, the best results for tCOD were achieved with the TA conditions of 175 °C for 15 min at pH 12 (12.07 g/L), 150 °C for 15 min at pH 12 (11.35 g/L), and 175 °C for 15 min at pH 11 (11.09 g/L) (see the Appendix, for Supplementary Information, Tables S1 and S2). For sCOD, the best results were with the TA conditions of 175 °C for 15 min at pH 12 (11.46 g/L) and with the T conditions of 175 °C for 30 min (10.73 g/L). For both tCOD and sCOD, the lowest solubilization was shown with the M tests, significantly lower than the TA results.

BOD_5 also sharply rose from 0.57 g/L (raw sludge) to 2.43-2.60 g/L (M pretreatment), to 2.34-2.77 g/L (T pretreatment) and to 2.77-2.94 g/L (TA pretreatment), an increase of 427-516%. The maximum BOD_5 (2.94 g/L) was obtained from the TA conditions of 175 °C for 15 min at pH 11. Mechanical pretreatment conditions showed a significantly lower BDO_5 solubilization when compared with the TA tests (see Supplementary Information, Tables S1 and S2).

Pretreatment increased the organic matter content and significantly increased sCOD/tCOD and BOD_5/tCOD ratios. An initial sCOD/tCOD ratio of 0.15 was achieved for the raw sludge, with a final ratio above 0.80 after all tested pretreatment approaches. For BOD_5/tCOD, the initial ratio of 0.08 was raised to 0.24-0.30 after the sludge pretreatments. These results indicate that there was an extensive hydrolysis of the particulate organic matter in addition to an increase in the biodegradable content of the organic matter as a result of the sludge pretreatment application (see Supplementary Information, Fig. S1).

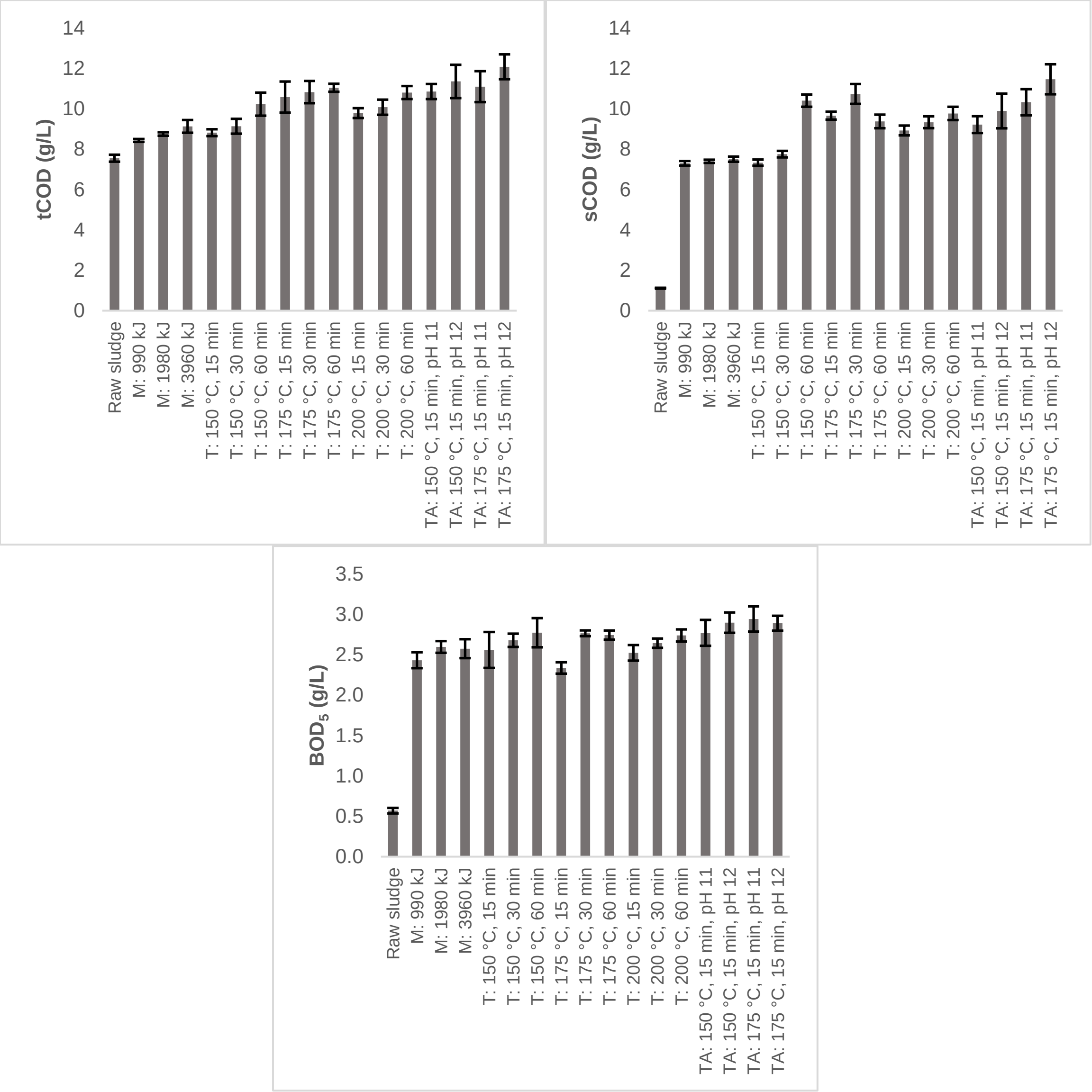


Fig. 2. Total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD) and biochemical oxygen demand (BOD_5) solubilization after mechanical (M), thermal (T) and thermal-alkaline (TA) pretreatment tests for kraft pulp mill waste activated sludge

Mechanical sludge pretreatment techniques help to remove small fibers that are potentially difficult to degrade in a subsequent anaerobic digester (Elliott and Mahmood 2012), but the results are always inferior to those achieved when higher temperatures or alkalinity are applied. A study where three different mechanical methods for pretreatment of P&P mill WAS were compared showed the best results for solubilization and for biogas production when mechanical homogenization was combined with WAS alkalization (Elliott and Mahmood 2012). This indicates that the use of high temperatures and alkalization are more effective to rupture the cell membranes than mechanical disintegration alone.

The present results were similar to those reported for thermal and thermal-alkaline pretreatments of pulp mill WAS (Table 1). Wood *et al*. (2009, 2010) found a 6-fold increase in sCOD concentration after T pretreatment of 170 °C for 60 min and a 7-fold increase after TA pretreatment of 140 °C for 60 min at pH 12, using WAS from a sulfite pulp mill. Bayr *et al*. (2013) achieved a 4-fold increase after 70 °C for 40 min and a 9-fold increase after 150 °C for 10 min, also using WAS from a sulfite mill. The present results are also similar to those reported for municipal sewage WAS, where the fraction of sCOD increased more than 10 times using the T pretreatment (Valo *et al*. 2004). Donoso-Bravo *et al*. (2011) and Zhang *et al*. (2016) demonstrated that temperature affects sCOD, due to the effect on the bacterial cell, leading to a sub sequential cell breakage and macromolecular destruction.

Table 1. Soluble Chemical Oxygen Demand (sCOD) before and after Pretreatment Tests in Pulp Mill Waste Activated Sludge

Pretreatment	**sCOD (g/L)**		**Increase**	**Reference**
	Before	After		
Mechanical conditions	1.1	7.3-7.5	7-fold	This study
Thermal conditions	1.1	7.3-10.7	7-10-fold	This study
Thermal-alkaline conditions	1.1	9.2-11.5	9-11-fold	This study
Thermal: 70 °C, 40 min	1.0	4.0	4-fold	Bayr *et al*. (2013)
Thermal: 150 °C, 10 min	1.0	9.0	9-fold	Bayr *et al*. (2013)
Thermal: 170 °C, 60 min	1.4	8.5	6-fold	Wood *et al*. (2009)
Thermal-alkaline: 140 °C, 60 min, pH 12	1.4	9.7	7-fold	Wood *et al*. (2009)
Mechanical shear: 1500 rpm, pH 12	1.4	2.9	2-fold	Elliott and Mahmood (2012)

The destruction of the sludge floc was observed after the pretreatment tests by microphotography (Fig. 3).

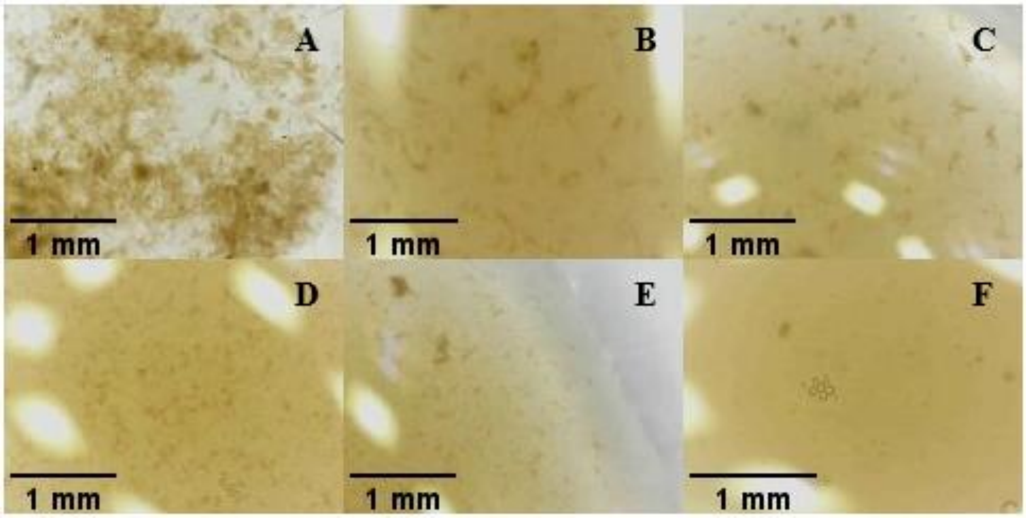


Fig. 3. Microphotograph of waste activated sludge: raw sludge (A); after mechanical pretreatment at 3960 kJ (B); after thermal pretreatment at 150 °C for 15 min (C); after thermal pretreatment at 200 °C for 15 min (D); after thermal-alkaline pretreatment at 150 °C for 15 min at pH 11 (E); after thermal-alkaline pretreatment at 175 °C for 15 min at pH 12 (F)

The results showed that at either temperature, alkali addition and mechanical rotation disrupted the sludge floc structure when compared with the raw sludge sample. Most of the sludge macromolecules were degraded into small units after pretreatment, which is desirable, since these are easier for the microorganisms to decompose in order to increase the methane yield in the anaerobic digestion stage. Similar results were shown by Lin *et al*. (2009; 2010), with sludge disruption seen after the biological and alkaline tests. In both studies, the authors showed that the increase in sCOD was accompanied by the disruption of the sludge floc caused by the pretreatment applied.

A possible mechanism for improvement of biogas production in anaerobic digestion is additional COD and BOD_5 solubilization as a result of pretreatment, making the organic matter more available for digestion. Previous studies showed COD solubilization to be the principal mechanism for enhancing anaerobic digestibility (Wood *et al*. 2009, 2010; Bayr *et al*. 2013). A rise of 24% in methane production was observed when sCOD increased 9 times after thermal pretreatment (Bayr *et al*. 2013). It is the first step in improving biogas production and making the anaerobic technology of WAS viable for full-scale implementation in P&P mills.

Solubilization of Nutrients

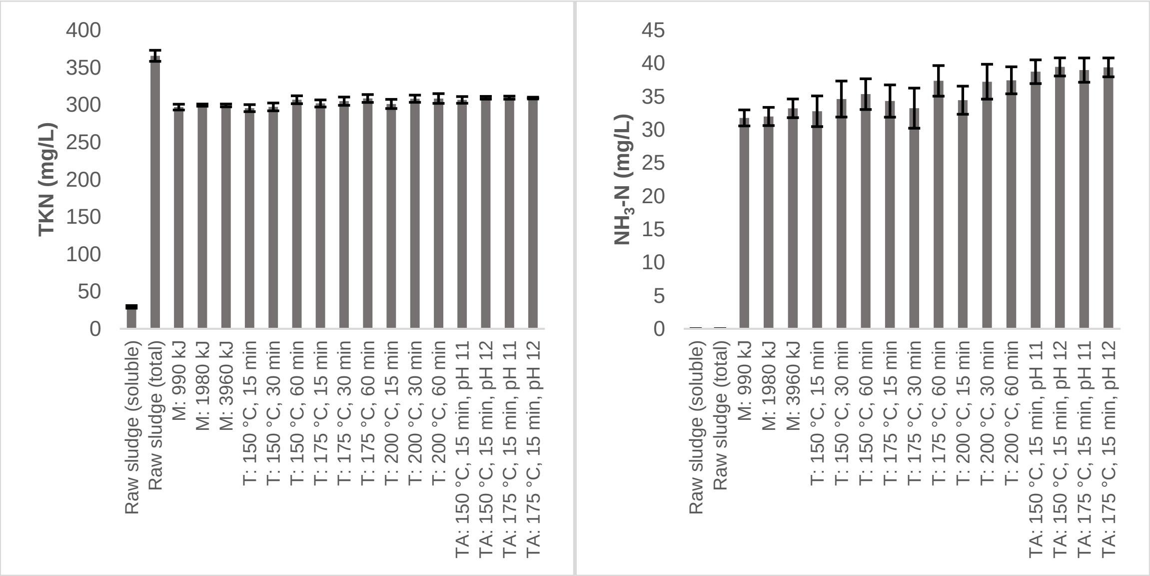
Chemical tests showed that the amount of TKN in the raw sludge was 366 mg/L, but only 29 mg/L (8%) of this was in the soluble form. All pretreatment conditions improved the solubilization. The soluble form of TKN rose to 297-300 mg/L (M pretreatment), to 296-309 mg/L (T pretreatment) and to 307-310 mg/L (TA pretreatment) (Fig. 4). The maximum TKN (310 mg/L) from these tests was obtained with TA conditions of 150 °C for 15 min at pH 12 and 175 °C for 15 min at pH 11.

Mehrdadi *et al*. (2012) also showed TKN solubilization after WAS pretreatment. The authors reported increased values from 5.6% to 170.8% of TKN using ultrasonic wave irradiation, explaining that solubilizing the WAS and consequently supplying nitrogen components to the biological treatment units would accelerate the treatment process.

NH_3-N content was undetected in the raw sludge but showed a concentration of 32-33 mg/L after the M pretreatment, 33-37 mg/L after the T pretreatment and 39 mg/L after the TA pretreatment. The increase in NH_3-N can be explained by the protein decomposition, as already demonstrated by Lin *et al*. (2009, 2010) after alkaline and biological pretreatments using P&P mill WAS.

The reported results demonstrated an NH_3-N increase after pretreatment tests for pulp mill WAS ranging from 2-fold (Lin *et al*. 2009) to 11-fold (Lin *et al*. 2010) (Table 2). Zhang *et al*. (2016) also highlighted the quick release of ammonia with temperatures above 100 °C, which can be explained by the protein conversion to peptides and volatile fatty acids of smaller molecular weight, and also by the ammonia production through the cleavage of amine functional groups of ammonia acids (-NH_2), as well as from the destruction of the peptide bonds (-NH-CO-).

The decomposition of organic matter releases nitrogen compounds, mainly those containing proteins, amino acids and urea (Suschka and Grübel 2014). Nitrogen is essential for the growth and maintenance of microorganisms, but its inhibitive effect on methane production can be observed at high concentrations. Despite the increase in the NH_3-N concentration, it is still below the inhibitive range. The inhibitory effects of ammonia have been related to the methanogenesis phase in anaerobic digestion (Calli *et al*. 2005; Yan *et al*. 2021). Yan *et al*. (2021) cite some problems related to the application of thermal strategies for sludge hydrolysis, including high residual ammonia. Despite this, a critical threshold concentration was reported to range from 1,500 to 7,000 mg/L (Rajagopal *et al.* 2013).



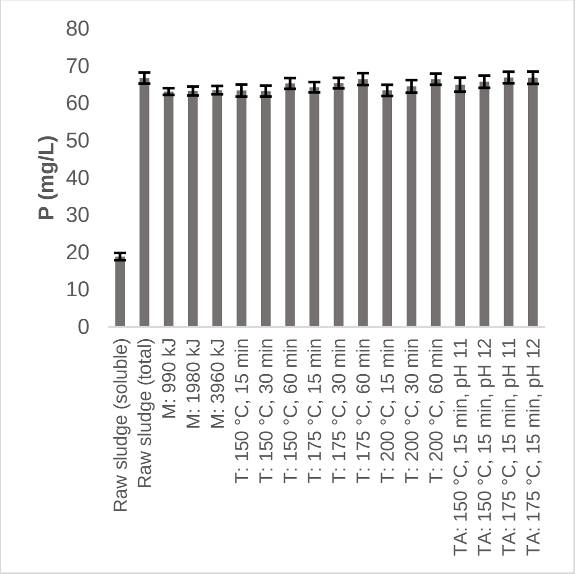


Fig. 4. Total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N) and total phosphorus (P) solubilization after mechanical (M), thermal (T) and thermal-alkaline (TA) pretreatment tests for kraft pulp mill waste activated sludge

The concentration of total P was 67 mg/L in the raw sludge, but only 19 mg/L (28%) was in the soluble form. Pretreatment raised the soluble concentrations of P to 63 to 64 mg/L (M pretreatment), to 63 to 66 mg/L (T pretreatment), and to 65 to 67 mg/L (TA pretreatment). Statistically, the results showed that all the P content was solubilized after TA pretreatments (see Supplementary Information, Tables S1 and S3).

An increase in P-solubilization in WAS was observed after irradiation with ultrasonic waves from 2 to 116%. This is important because P is one of the initial substrates for the initiation of microbial activity in biological treatments (Mehrdadi *et al*. 2012). While orthophosphate (PO_4^{-3}, HPO_4^{-3}, H_2PO_4) is already available for biological metabolism, the polyphosphate needs a hydrolysis step to convert it to the orthophosphate form, which is, in natural processes, a slow reaction.

Table 2. Ammonia-nitrogen (NH_3-N) before and after Pretreatment Tests in Pulp Mill Waste Activated Sludge

Pretreatment	**NH_3-N (mg/L)**		**Increase**	**Reference**
	Before	After		
Mechanical conditions	un$^{(1)}$	32-33	-	This study
Thermal conditions	un$^{(1)}$	33-37	-	This study
Thermal-alkaline conditions	un$^{(1)}$	39	-	This study
Alkaline: 4 gNaOH/100 g TS sludge	0.85$^{(2)}$	1.24$^{(2)}$	2-fold	Lin *et al*. (2009)
Alkaline: 8 gNaOH/100 g TS sludge	0.85$^{(2)}$	1.38$^{(2)}$	2-fold	Lin *et al*. (2009)
Alkaline: 16 gNaOH/100 g TS sludge	0.85$^{(2)}$	1.24$^{(2)}$	2-fold	Lin *et al*. (2009)
Mechanical shear: 1500 rpm, pH 12	47	93	2-fold	Elliott and Mahmood (2012)
Sonication: 20 kHz	47	205	4-fold	Elliott and Mahmood (2012)
High-pressure Homogenization: 83,000 kPa in alkaline conditions	47	538	11-fold	Elliott and Mahmood (2012)
$^{(1)}$undetected, $^{(2)}$g/L				

Nutrient solubilization and nutrient recycling capacity were demonstrated by Sun *et al*. (2013) after thermal treatment in sewage sludge (180 to 240 °C for 30 to 90 min). The authors showed that 40 to 70% of the nitrogen and 10 to 15% of the phosphorus in sewage sludge could be dissolved into the liquid phase, suggesting that temperature is effective for solubilizing the sludge. The results of the present research suggest that pH also has an important role in the solubilization, along with temperature.

WAS nutrient solubilization is important since anaerobic digestion consists of biochemical processes carried out by essential microorganisms. Nutrients are essential for the success of microorganism growth and maintenance. Despite the large amount of nitrogen and phosphorus compounds in WAS, the major parts are in insoluble forms, forcing the hydrolyzation by the microorganisms and limiting the biological processes. Through the previous solubilization of nutrients caused by pretreatment, there was an improvement in the anaerobic digestion stage due to the acceleration of the hydrolyze phase.

Theoretical Biochemical Methane Potential

Table 3 presents the results obtained for the C/N ratio, VS content, empirical formula, and theoretical biochemical methane potential. Improvements in the C/N ratio, VS, and BMP_{th} were shown after all pretreatments tests as a result of sludge solubilization.

Table 3. Carbon/Nitrogen (C/N) Ratio, Volatile Solids (VS), Empirical Formula, and Theoretical Biochemical Methane Potential (BMP_{th}) for Raw and Pretreated Kraft Pulp Mill Waste Activated Sludge

Pretreatment	C/N	VS (g/g)	Empirical Formula	BMP_{th} ($mLCH_4/gVS$)
Raw sludge	6	0.35	$C_7H_{13}O_9N$	211
M: 990 kJ	8	0.37	$C_9H_{14}O_8N$	333
M: 1980 kJ	8	0.38	$C_9H_{14}O_8N$	333
M: 3960 kJ	8	0.37	$C_9H_{14}O_8N$	343
T: 150 °C, 15 min	8	0.38	$C_9H_{14}O_8N$	314
T: 150 °C, 30 min	8	0.39	$C_9H_{14}O_8N$	345
T: 150 °C, 60 min	8	0.40	$C_9H_{14}O_8N$	355
T: 175 °C, 15 min	8	0.38	$C_9H_{13}O_7N$	346
T: 175 °C, 30 min	8	0.41	$C_9H_{14}O_7N$	359
T: 175 °C, 60 min	8	0.41	$C_9H_{14}O_7N$	360
T: 200 °C, 15 min	7	0.38	$C_8H_{12}O_7N$	344
T: 200 °C, 30 min	8	0.40	$C_9H_{14}O_8N$	354
T: 200 °C, 60 min	8	0.39	$C_9H_{14}O_7N$	360
TA: 150 °C, 15 min, pH 11	9	0.41	$C_{10}H_{16}O_8N$	373
TA: 150 °C, 15 min, pH 12	8	0.42	$C_{10}H_{14}O_8N$	373
TA: 175 °C, 15 min, pH 11	8	0.42	$C_9H_{12}O_6N$	378
TA: 175 °C, 15 min, pH 12	8	0.43	$C_9H_{14}O_7N$	376

The C/N ratio improved from 6 in the raw sludge to 7-9 after pretreatment tests, with better results under the TA conditions. Despite improvements, the C/N ratio remained below the optimal value indicated for anaerobic digestion, which is between 20 and 30 (Banks and Heaven 2013). A better C/N ratio may be achieved with co-digestion. Nevertheless, there are no reports of inhibition related to the unbalanced C/N ratio. Park *et al*. (2012) did not achieve an increase in the C/N ratio after different alkaline and ultrasonic pretreatments using pulp mill WAS; however, this did not affect the increase in methane production, even with an initial C/N ratio around 10.

Elemental composition was changed as the VS contend changed. The values raised from 0.35 gVS/g (raw sludge) to 0.37-0.43 gVS/g after pretreatment tests. An increase on the VS values was accompanied by an increase in C in the empirical formula as a result of cells walls rupture and organic matter released. Liew *et al*. (2022) presented some examples about the importance of VS content for biogas production; according to their review, higher contents of VS result in more produced energy in the form of biogas.

BMP_{th} increased in all the pretreatments tested by at least 100 $mLCH_4/gVS$. The results ranged from 211 $mLCH_4/gVS$ (raw sludge) to 333-343 $mLCH_4/gVS$ (M pretreatment), to 314-360 $mLCH_4/gVS$ (T pretreatment, and to 373-378 $mLCH_4/gVS$ (TA pretreatment), an increase of 149-179%. The maximum BMP_{th} was obtained with the TA conditions, all exceeding 373 $mLCH_4/gVS$. In contrast, the minimum values were obtained with the T condition of 150 °C for 15 min (314 $mLCH_4/gVS$) and with all the M tests (333-343 $mLCH_4/gVS$).

In practice, no reaction goes to full completion, and it is impossible to have total breakdown of cellulosic materials. Although the results only give a maximum biogas production potential and overestimate the real biogas yields, the results seem to be very promising when comparing the BMP_{th} values with literature (Table 4). The application of a limiting factor (*f*) was proposed to give more reliable results and eliminate the discrepancy between hypothetical and real biogas amount when Buswell equation was applied (Achinas and Euverink 2016). Applying the *f* factor (adjusted BMP_{th}), the results of this research were still higher than the experimental values determined by other authors (Bayr *et al*. 2013; Kinnunen *et al*. 2015).

Table 4. Biochemical Methane Potential (BMP) for Pulp Mill Waste Activated Sludge

Pretreatment	Theoretical BMP_{th}	Adjusted BMP_{th}	Experimental BMP	Reference
Raw sludge	211 $mLCH_4/gVS$	169 $mLCH_4/gVS$	-	This study
Mechanical conditions	333-343 $mLCH_4/gVS$	266-274 $mLCH_4/gVS$	-	This study
Thermal conditions	314-360 $mLCH_4/gVS$	251-288 $mLCH_4/gVS$	-	This study
Thermal-alkaline conditions	373-378 $mLCH_4/gVS$	298-303 $mLCH_4/gVS$	-	This study
Thermal: 150 °C, 10 min	-	-	134 $mLCH_4/gVS$	Bayr *et al*. (2013)
Thermal: 70 °C, 40 min	-	-	112 $mLCH_4/gVS$	Bayr *et al*. (2013)
Thermal: 80-134 °C, 20-120 min	-	-	60-124 $mLCH_4/gVS$	Kinnunen *et al*. (2015)

The BMP results provide an indication of the biodegradability of a substrate and its potential for producing methane via anaerobic digestion. Such information allows a direct assessment of the biogas yields achieved by the anaerobic process. From an economic perspective for P&P mills, these results show that methane can be obtained from WAS and that the investment in anaerobic digestion technology may provide energy (biogas) and also fertilizer (digestate) that can be applied in the forestry field, reinforcing the production of raw material for the industry. In comparison, Meyer *et al*. (2020) explained that anaerobic digestion system requires a lower capital cost than other techniques, citing that it could cost at least 3 times less than incineration.

One possibility to be considered is the use of heat energy, commonly in the form of high-pressure steam generated in the boilers, as the heat source for the pretreatment and anaerobic reactors, thus reducing the operating costs. The biogas produced can be burned in a combined heat and power (CHP) plant to simultaneously generate electricity and heat. Considering the possible process routes, a CHP system can also produce heat for the pretreatment and anaerobic reactors and electrical power for the ETP. This can reduce the operational costs for the pretreatment while providing power for pumps and aerators, making the ETP a self-sufficient sector in terms of energy. Another way for reducing the operating costs was presented by Jian *et al*. (2021), using black liquor (pH 12.8, 4.1 g/L of residual alkali) to replace NaOH for the pretreatment using sludge from a soda pulping mill. The authors showed that adding black liquor increased sCOD concentration and increased the amount of methane produced, demonstrating the economic feasibility of this strategy.

CONCLUSIONS

1. Pulp mill waste activated sludge (WAS) solubilization was achieved with thermal, thermal-alkaline, and mechanical pretreatments. For organic matter solubilization, the bests results were achieved with the thermal-alkaline (TA) operating conditions of 175 °C for 15 min at pH 12 for tCOD and sCOD and 175 °C for 15 min at pH 11 for BOD_5.
2. The TA pretreatment also showed the maximum solubilization values for nutrients, in special the operation conditions of 150 °C for 15 min at pH 12, 175 °C for 15 min at pH 11 and 175 °C for 15 min at pH 12. This showed the powerful influence of pH on the solubilization, despite the low retention time (only 15 min).
3. All pretreatments improved the potential methane production by at least 100 $mLCH_4/gVS$. The bests results were achieved with the TA operating conditions, in the range of 373-378 $mLCH_4/gVS$.
4. Considering the variables studied, pH seems to be the most important parameter in the solubilization process. In general, mechanical conditions resulted in the worst results. Thermal tests, despite the higher temperature (up to 200 °C) and greater retention time (up to 60 min), resulted in lower solubilization when compared with the thermal-alkaline processes, although there was a statistical similarity for sCOD, BDO_5, TKN, NH_3-N and P. Thermal-alkaline pretreatment showed the best values for all the evaluated parameters, with the additional advantage of requiring lower temperature (150 to 175 °C) and retention time (15 min).
5. Future research should include a pilot scale tests for economic evaluation of both pretreatment and anaerobic digestion processes; also, for study other variables, such as the use of other sources of alkalinity, metals solubilization and the influence of pretreatment on the volatile organic acid concentrations.

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APPENDIX

Supplemental Information

Table S1. Results for Ryan-Joiner Normality Test for Total (tCOD) and Soluble (sCOD) Chemical Oxygen Demand, Biochemical Oxygen Demand (BOD_5), Total Phosphorus (P), Total Kjeldahl Nitrogen (TKN), and Ammonia-Nitrogen (NH_3-N)

Source of Variation	**Mean**	**St Dev**	**N**	**RJ**	***p*-value**
tCOD	10,103	1,205	99	0.991	> 0.100
sCOD	8,902	1,918	99	0.906	< 0.010
BOD_5	2,615	412	99	0.777	< 0.010
P	63.56	8.058	102	0.597	< 0.010
TKN	297.7	48.51	102	0.565	< 0.010
NH_3-N	33.56	9.005	102	0.755	< 0.010

Table S2. Statistical Results for Total (tCOD) and Soluble (sCOD) Chemical Oxygen Demand and Biochemical Oxygen Demand (BOD_5)

Pretreatment	**tCOD (g/L)** $^{(1)}$	**sCOD (g/L)** $^{(2)}$	**BOD_5 (g/L)** $^{(2)}$
Raw sludge	7.55±0.17 $^{2.29}{}_{i}$	1.11±0.02 $^{1.95}{}_{e}$	0.57±0.03 $^{6.02}{}_{d}$
M: 990 kJ	8.43±0.07 $^{0.86}{}_{hi}$	7.30±0.11 $^{1.55}{}_{e}$	2.43±0.10 $^{4.05}{}_{cd}$
M: 1980 kJ	8,747±85 $^{0.97}{}_{h}$	7.39±0.08 $^{1.06}{}_{e}$	2.60±0.07 $^{2.80}{}_{bcd}$
M: 3960 kJ	9.12±0.32 $^{3.46}{}_{fgh}$	7.50±0.13 $^{1.72}{}_{de}$	2.57±0.12 $^{4.55}{}_{bcd}$
T: 150 °C, 15 min	8.81±0.17 $^{1.92}{}_{gh}$	7.331±0.15 $^{2.07}{}_{e}$	2.56±0.22 $^{8.69}{}_{bcd}$
T: 150 °C, 30 min	9.13±0.37 $^{4.04}{}_{fgh}$	7.75±0.16 $^{2.08}{}_{de}$	2.68±0.08 $^{3.07}{}_{bc}$
T: 150 °C, 60 min	10.22±0.57 $^{5.59}{}_{cde}$	10.40±0.30 $^{2.93}{}_{ab}$	2.77±0.18 $^{6.52}{}_{ab}$
T: 175 °C, 15 min	10.57±0.77 $^{7.27}{}_{bcde}$	9.66±0.20 $^{2.04}{}_{abc}$	2.34±0.07 $^{3.01}{}_{cd}$
T: 175 °C, 30 min	10.82±0.55 $^{5.10}{}_{bcd}$	10.73±0.49 $^{4.58}{}_{a}$	2.76±0.04 $^{1.28}{}_{ab}$
T: 175 °C, 60 min	11.04±0.20 $^{1.79}{}_{bcd}$	9.37±0.34 $^{3.58}{}_{bcd}$	2.74±0.06 $^{2.07}{}_{ab}$
T: 200 °C, 15 min	9.78±0.24 $^{2.50}{}_{efg}$	8.92±0.24 $^{2.72}{}_{cde}$	2.52±0.01 $^{3.84}{}_{cd}$
T: 200 °C, 30 min	10.07±0.38 $^{3.73}{}_{def}$	9.33±0.29 $^{3.14}{}_{bcd}$	2.64±0.06 $^{2.17}{}_{bc}$
T: 200 °C, 60 min	10.80±0.32 $^{2.98}{}_{bcd}$	9.76±0.33 $^{3.37}{}_{abc}$	2.74±0.07 $^{2.73}{}_{ab}$
TA: 150 °C, 15 min, pH 11	10.85±0.37 $^{3.43}{}_{bcd}$	9.21±0.42 $^{4.52}{}_{bcd}$	2.77±0.16 $^{5.78}{}_{ab}$
TA: 150 °C, 15 min, pH 12	11.35±0.82 $^{7.26}{}_{ab}$	9.89±0.86 $^{8.69}{}_{abc}$	2.90±0.13 $^{4.35}{}_{a}$
TA: 175 °C, 15 min, pH 11	11.09±0.77 $^{6.92}{}_{bc}$	10.32±0.65 $^{6.27}{}_{ab}$	2.94±0.16 $^{5.31}{}_{a}$
TA: 175 °C, 15 min, pH 12	12.07±0.62 $^{5.09}{}_{a}$	11.46±0.74 $^{6.48}{}_{a}$	2.89±0.09 $^{3.19}{}_{a}$

$^{(1)}$ compared with Tukey test; $^{(2)}$ compared with Dunn test

Mean ± standard deviation; Coefficient of variation statistical test

Means that do not share a letter are significantly different considering 95% confidence

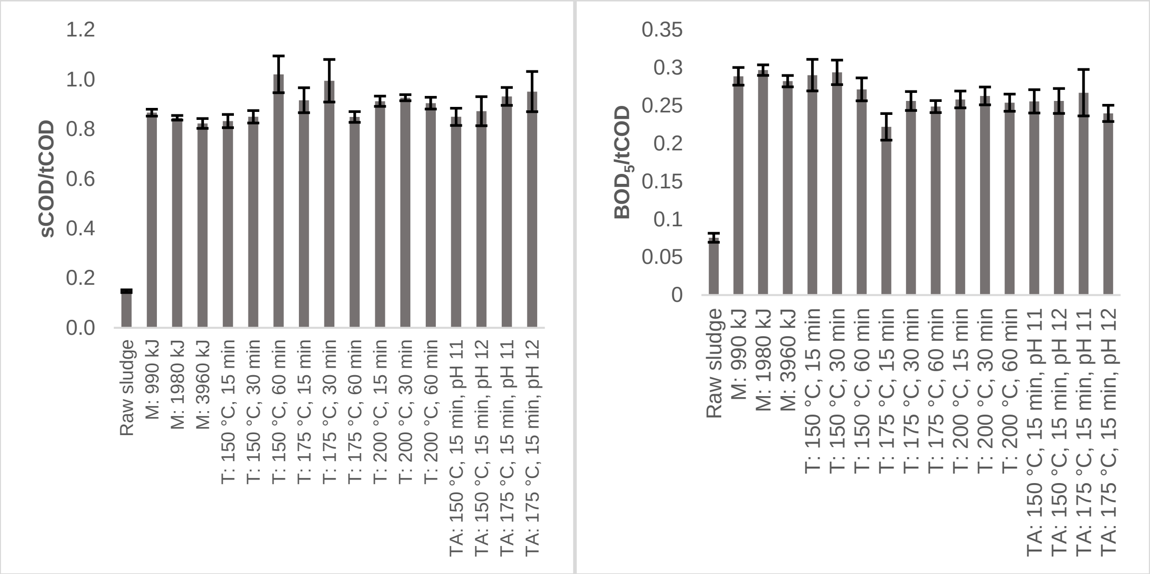

Fig. S1. Soluble chemical oxygen demand versus total chemical oxygen demand (sCOD/tCOD) and biochemical oxygen demand versus total chemical oxygen demand (BOD_5/tCOD) before and after pretreatment tests

Table S3. Statistical Results for Total Kjeldahl Nitrogen (TKN), Ammonia-Nitrogen (NH_3-N), and Total Phosphorus (P)

Pretreatment	**TKN** $^{(1)}$	**NH_3-N** $^{(1)}$	**P** $^{(1)}$
Raw sludge (soluble)	29±2 $^{5.97}{}_{f}$	un$^{(2)}$	19±1 $^{5.11}{}_{f}$
Raw sludge (total)	366±7 $^{2.02}{}_{a}$	un$^{(2)}$	67±1 $^{2.23}{}_{a}$
M: 990 kJ	297±4 $^{1.30}{}_{ef}$	32±1 $^{3.80}{}_{ef}$	63±1 $^{1.44}{}_{ef}$
M: 1980 kJ	300±1 $^{0.48}{}_{def}$	32±1 $^{4.29}{}_{ef}$	63±1 $^{1.90}{}_{def}$
M: 3960 kJ	299±2 $^{0.64}{}_{ef}$	33±1 $^{4.23}{}_{ef}$	64±1 $^{1.76}{}_{cdef}$
T: 150 °C, 15 min	296±5 $^{1.59}{}_{ef}$	33±2 $^{7.05}{}_{ef}$	63±2 $^{2.56}{}_{cdef}$
T: 150 °C, 30 min	297±5 $^{1.77}{}_{ef}$	35±3 $^{7.85}{}_{cde}$	63±1 $^{2.31}{}_{cdef}$
T: 150 °C, 60 min	307±5 $^{1.72}{}_{abcd}$	35±2 $^{6.54}{}_{bcde}$	65±1 $^{2.22}{}_{abcd}$
T: 175 °C, 15 min	302±5 $^{1.58}{}_{cdef}$	34±2 $^{7.08}{}_{cdef}$	64±1 $^{2.14}{}_{bcde}$
T: 175 °C, 30 min	305±6 $^{1.82}{}_{bcde}$	33±3 $^{9.09}{}_{def}$	65±1 $^{2.14}{}_{abc}$
T: 175 °C, 60 min	309±5 $^{1.70}{}_{abc}$	37±2 $^{6.17}{}_{abc}$	66±2 $^{2.41}{}_{ab}$
T: 200 °C, 15 min	301±6 $^{2.04}{}_{cdef}$	34±2 $^{6.15}{}_{cde}$	63±2 $^{2.37}{}_{cdef}$
T: 200 °C, 30 min	308±5 $^{1.56}{}_{abc}$	37±3 $^{7.05}{}_{abcd}$	65±2 $^{2.62}{}_{bcde}$
T: 200 °C, 60 min	309±7 $^{2.11}{}_{abc}$	37±2 $^{5.43}{}_{abc}$	66±2 $^{2.26}{}_{ab}$
TA: 150 °C, 15 min, pH 11	307±4 $^{1.47}{}_{abc}$	39±2 $^{4.61}{}_{ab}$	65±2 $^{2.90}{}_{abcde}$
TA: 150 °C, 15 min, pH 12	310±2 $^{0.59}{}_{ab}$	39±1 $^{3.46}{}_{a}$	66±2 $^{2.51}{}_{ab}$
TA: 175 °C, 15 min, pH 11	310±2 $^{0.66}{}_{ab}$	39±2 $^{4.69}{}_{ab}$	67±2 $^{2.31}{}_{a}$
TA: 175 °C, 15 min, pH 12	309±1 $^{0.38}{}_{ab}$	39±1 $^{3.61}{}_{ab}$	67±2 $^{2.48}{}_{a}$

$^{(1)}$ compared with Dunn test; $^{(2)}$ undetected
Mean ± standard deviation; Coefficient of variation statistical test
Means that do not share a letter are significantly different considering 95% confidence