

Article

Effect of Different Pretreatments on Sludge Solubilization and Estimation of Bioenergy Potential

Reshma Babu *, Gustavo Capannelli and Antonio Comite *

Department of Chemistry and Industrial Chemistry, University of Genoa, 16146 Genoa, Italy; gustavo.capannelli@gmail.com

* Correspondence: reshma.babu@edu.unige.it (R.B.); antonio.comite@unige.it (A.C.)

Abstract: Most of the conventional treatments of waste-activated sludge (WAS) are devoted to their minimization and destruction. On the other hand, the biomass contained in WAS can be utilized as a valuable source of renewable carbon. In this study, the influence of different pretreatments (ultrasonication, chemical, thermal, and combined pretreatments) was explored for sludge solubilization. Effects of the pretreatments were investigated as a function of the solubilization of total solids (TS), volatile solids (VS), and chemical oxygen demand (COD). Concentrations of soluble carbohydrates and total nitrogen were also measured. The most effective pretreatment to hydrolyze sludge was found to be the combined alkali–thermal (pH 12, 75 °C) pretreatment method, leading to TS and vs. solubilization of 9.6% and 17.2%, respectively. Soluble COD, carbohydrates, total nitrogen, and proteins estimated in the liquid phase were 5235 mg/L, 732 mg/L, 430 mg/L, and 2688 mg/L, respectively. Thus, the alkali–thermal method could be used for efficient valorization of WAS. Moreover, the solid fraction from all pretreated samples was further subjected to thermogravimetric analysis to estimate its potential for bioenergy from its higher heating value (HHV), which was found to be in the range of 10–11.82 MJ/kg. This study can provide better insight into the efficient valorization of liquid and solid phases of sludge after pretreatment.

Keywords: waste-activated sludge; pretreatment; ultrasonication; chemical; alkali; thermal; sludge solubilization; higher heating value



Citation: Babu, R.; Capannelli, G.; Comite, A. Effect of Different Pretreatments on Sludge Solubilization and Estimation of Bioenergy Potential. *Processes* **2021**, *9*, 1382. <https://doi.org/10.3390/pr9081382>

Academic Editor: Alberto J. Moya

Received: 2 July 2021

Accepted: 5 August 2021

Published: 9 August 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The European Union with a population of 500 million people is estimated to have a total sludge production of about 13 million tons of dry matter as of 2020 from wastewater treatment plants (WWTPs) [1,2]. In recent times, population and economic factors have led to an increase in the number of WWTPs, and, with stricter effluent discharge laws enforced on them, the rate of biosolid production is expected to increase [3–5]. At present, sludge management involves the disposal of sludge using conventional methods such as incineration, landfilling, and agricultural use [6]. It does not include the valorization of sludge as feedstock for the production of fine chemicals and bioproducts [7]. Within the vision of circular economy, valorization of secondary biomass can offer a sustainable way to obtain useful products such as fuels and chemicals either directly or indirectly [8,9]. To facilitate valorization of sludge, it is important to firstly solubilize the organic content present.

Waste-activated sludge (WAS) mainly comprises microbes. The cell walls and membranes of the microbial cells contain organic carbon that can serve as raw materials for production of value-added materials [10]. However, the cell walls act as physical walls that prevent the easy release of these organics [11]. Hence, it is necessary to pretreat WAS to hydrolyze the cell structure and, therefore, enhance sludge solubilization [12,13].

Several pretreatment techniques have been investigated in the past to improve sludge hydrolysis. These include mechanical [14–16], chemical [17,18], thermal [19,20], and biological [21] methods. Mechanical methods such as ultrasonication improve the solubility

of organic molecules by disrupting sludge flocs through cavitation phenomena [22]. Alkali treatment, as a chemical pretreatment method, involves increasing the sludge pH to 12 with the addition of an alkali and maintaining it for a specified amount of time. This can increase the solubilization of complex organics by destroying cells and flocs [23,24]. In thermal pretreatment, cell hydrolysis of sludge takes place because of a pressure difference. The most utilized temperature range for solubilizing organics varies from 60 °C to 180 °C [25]. Low-temperature pretreatment (<100 °C) requires longer reaction times than high-temperature pretreatment, but they can both be used to efficiently improve the production of biogas from secondary sludge [24].

Additionally, combined pretreatments (e.g., thermal–chemical [26], thermal–chemical–ultrasonic [27], ultrasonic–chemical [28], and thermal–ultrasonic [29]) have been studied previously on WAS. A summary of results from coupled pretreatments is shown in Table 1, suggesting that the combination of different pretreatments can improve sludge hydrolysis and the solubilization of organics.

Table 1. Summary of combined pretreatment methods.

Pretreatments	Conditions	Results	References
Thermal–chemical	130 °C, pH 12, 5 min	Enhanced biodegradability and solid solubilization	[26]
Thermal–chemical	50–90 °C, pH 9–12, 5–180 min	80 °C, pH 11 optimum	[27]
Thermal–chemical–ultrasonic	80 °C, pH 11, 20 kHz, 5–180 min	Improved COD solubilization and suspended solid reduction	
Alkali–ultrasonic	Ambient temperature, 40 meq/L NaOH, 20 kHz, 120 W, 24 h	Enhanced production of volatile acids	[28]
Ultrasonic	20 kHz, 1 h	Efficient hydrolysis on WAS	[29]
Thermal–ultrasonic	Thermal: 30–90 °C, 0–3 h Ultrasonication: 20 kHz, 20 min	Improved hydrolysis for thermal–ultrasonication compared to ultrasonic method	
Thermal–alkali	80 °C, 80 min, 0.1 M NaOH	Improved solubilization of organic matter in thermal–alkali method	[23]
Thermal	80–170 °C, 30 min–12 h		

This study focused on pretreating WAS to disintegrate its cell structure and release organics such as polysaccharides and proteins by using ultrasonication, chemical, thermal, ultrasonication–chemical, ultrasonication–thermal, chemical–thermal, and ultrasonication–chemical–thermal methods. Thus, in addition to single pretreatments, two or three of these individual methods were also combined and experiments carried out. The main objective of this article was to study the effects of different single and coupled pretreatments on the solubilization of secondary sludge obtained from a municipal wastewater treatment plant. A thorough search of the relevant literature did not yield a similar compilation of these seven pretreatments performed on waste-activated sludge from municipal WWTPs all in a single study. Therefore, the aim of this paper was to give better insight into the disintegration efficiency of organics in secondary sludge after the abovementioned pretreatments for their further resource recovery, biotechnological transformation, or energy exploitation. Concentrations of total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), carbohydrates, proteins, and total nitrogen were measured. The solid fraction of sludge after pretreatments was further subjected to thermogravimetric analysis to determine the higher heating value (HHV) for estimating the bioenergy potential.

2. Materials and Methods

2.1. Materials

A municipal WWTP located in Genoa, Italy provided the raw WAS used in this study. Samples obtained were taken after the secondary settler unit of the WWTP. Sludge was stored at 4 °C, and the experiments were carried out with a living sludge. The major composition of raw WAS is given in Table 2. Prior to the pretreatments, sludge was thickened to a TS concentration of 15 g/L with a vs. fraction of 12.2 g/L. Chemicals such as sodium hydroxide (NaOH) used for the tests was purchased from Sigma Aldrich and used as received.

Table 2. Characteristics of raw waste-activated sludge (WAS).

Parameter	Mean Value ± Standard Deviation
pH	~7.0
Total solids (TS)	4.8 ± 0.1 g/L
Volatile solids (VS)	3.7 ± 0.2 g/L
Soluble chemical oxygen demand (SCOD) ¹	500 ± 10 mg/L
Soluble carbohydrates ¹	143 ± 5 mg/L
Total nitrogen ¹	230 ± 35 mg/L

¹ Evaluated on the liquid fraction of sludge.

2.2. Pretreatments

In this study, physical (ultrasonication), chemical (alkali addition), and thermal (heating) methods, as well as their combinations, were applied to WAS. Each pretreatment was done using 80 mL of thickened sludge in 100 mL Pyrex bottles for a reaction time of 1 h.

Ultrasonication (U) was performed using an ultrasonicator bath (Bandelin Sonorex Digitec DT 52 H) operating at a constant and continuous frequency of 35 kHz and heating power of 140 W. The pH of sludge was not controlled, and only a negligible change in pH was observed after ultrasonication. A rise in temperature is expected during sonication due to the cavitation phenomenon [24,30]. However, during pretreatment, the sample was maintained at ambient temperature 25 °C by circulating cold water for a reaction time of 1 h. These ultrasonic parameters were selected following the work by Tiehm et al. [31] and Zhang et al. [32]. It was suggested that a low frequency (below 40 kHz) and longer sonication were optimum for sludge degradation [33,34]. Lowering the frequency produced cavitation bubbles large enough to collapse and permit initiation of strong shear forces in the sludge sample. Furthermore, increasing the sonication time resulted in the disintegration of microbial cell walls and enhanced the solubilization of organic matter [35].

The alkali (chemical) pretreatment (A) was carried out using 5 M NaOH to set the initial pH of the thickened WAS to 12 [36,37]. NaOH was chosen as it was found to better influence sludge solubilization [37]. The sample was stirred continuously at 200 rpm at room temperature for 1 h.

For thermal pretreatment (H), a heating temperature below 100 °C was considered. Microbial cell lysis was effectively studied between 60 °C and 100 °C in the lower temperature range [38–40]. Here, a median temperature of 75 °C was selected and, after heating WAS to the set temperature, the reaction continued for 1 h with constant heating and stirring at 200 rpm. The pH of the sample was not adjusted and remained at 7 before and after pretreatment.

In addition to the abovementioned individual pretreatments, combined pretreatments (ultrasonication–alkali (UA), ultrasonication–heating (UH), alkali–heating (AH), and ultrasonication–alkali–heating (UAH)) were performed to study the effect of the different pretreatments on the solubilization of organic matter in WAS.

The UA method was a combination of ultrasonication and the addition of alkali at a fixed sludge pH of 12. The total time of pretreatment was 1 h. After NaOH addition, WAS was mixed and then sonicated, maintaining the batch experiment at room temperature by circulating cold water.

In the UH method, ultrasonication and thermal pretreatments were combined. The temperature of the sonicator bath was set to 75 °C, and sludge was ultrasonicated for a total pretreatment time of 1 h. The initial pH of the sludge was not modified.

The AH pretreatment combined the addition of NaOH to set the pH of sludge to 12 and subsequent low-temperature thermal pretreatment. WAS was heated at 75 °C with constant stirring at 200 rpm for a reaction time of 1 h.

The UAH method was the combination of ultrasonication, alkali addition, and thermal pretreatments. This pretreatment was performed analogously to the UH method with the difference being that the sludge pH was adjusted to 12 before the start of the experiment using 5 M NaOH. After pH adjustment, sludge was sonicated for 1 h at 75 °C.

2.3. Sample Analyses

After the pretreatments, the solid fraction was separated from the liquid phase by centrifuging (Neya 16, Maharashtra, India) at 7000 rpm for 15 min. The aqueous samples were filtered using 0.2 µm cellulose acetate syringe filters (Merck, Darmstadt, Germany) before the determination of soluble composition. The Merck Spectroquant COD Cell Test (catalog number 114541 from Darmstadt, Germany) and the Merck Spectroquant Nitrogen (total) Cell Test (catalog number 114537 from Darmstadt, Germany) were used to measure SCOD and total nitrogen (TN), respectively. Proteins were estimated by multiplying organic nitrogen concentration by a factor of 6.25 [41]. The soluble carbohydrates were determined using Dubois's colorimetric phenol-sulfuric acid method with D-glucose as a standard solution for calibration [42]. Total solids (TS) and volatile solids (VS) measurements were gravimetrically done on the pretreated WAS samples as per Standard Methods for the Examination of Water and Wastewater 21st edition [43]. For TS calculations, samples were dried to a constant weight at 105 °C and weighed. This sample was then allowed to undergo combustion at 600 °C for measuring VS. All the experimental runs were performed in duplicate, and the results were shown as means ± standard deviation.

The percentage of solubilized TS and vs. were calculated as follows:

$$\% \text{ solubilized TS} = \frac{\text{TS}_i - \text{TS}_f}{\text{TS}_i} \times 100, \quad (1)$$

$$\% \text{ solubilized vs.} = \frac{\text{VS}_i - \text{VS}_f}{\text{VS}_i} \times 100, \quad (2)$$

where TS_i and VS_i refer to TS and vs. before pretreatment, and TS_f and VS_f refer to values after pretreatments. Sludge pH was read using a pH 209 bench top pH meter (Hanna Instruments Italia, Ronchi di Villafranca Padovana, Italy).

2.4. Estimation of HHV by Thermogravimetric Analysis

The energy potential of sludge can be determined from its HHV. Most commonly, a bomb calorimeter is used as a standard to obtain this value; in the absence of this instrument, HHV can be estimated from ultimate and proximate analysis. However, a special instrument is required to carry out ultimate analysis. Although proximate analysis can be done using more commonly used instrumentation, the time needed for the experimental runs is higher and strongly dependent on the operator's skill. Thus, thermogravimetric analysis (TGA) has been successfully used in the past to determine the main analytical parameters such as moisture content (MC), volatile matter (VM), fixed carbon (FC), and ash (A) required for estimating HHV [44,45]. Furthermore, TGA allows faster analysis with smaller quantities of samples. Here, TGA was done using a thermal analyzer (TGA/DSC 1 Mettler Toledo, Milan, Italy) to estimate the HHV of the sludge sample as per ASTM Standard E1131-20 [46]. The experiments were done on the solid part of the sludge obtained after centrifugation before and after all pretreatments. Approximately 7–8 mg of solid samples pre-dried at 60 °C were placed in an alumina crucible for the analysis. In the first segment of the experiment, the sludge sample was heated at 10 °C/min in an inert environment (N_2 gas) from ambient temperature to 110 °C with a hold time of 5 min. Then,

the sample was heated to 950 °C with the same heating rate and an isothermal period of 7 min at this temperature. After this, at 950 °C, carrier gas was switched to O₂ with a dwelling time of 10 min to ensure complete combustion of the sample. N₂ and O₂ flow was maintained at 80 mL/min for the complete run. Prior to the sample run, blank experiments with an empty crucible were performed to obtain baseline correction. For accuracy of results, experiments were done in duplicate and reproducibility of the data was assured. The thermogravimetric (TG) curve was plotted using OriginPro software.

The following proximate parameters were evaluated as per ASTM Standard E1131-20 [46]: MC and VM were calculated in the nitrogen atmosphere; MC was measured as the weight loss between room temperature (starting temperature) and 110 °C; VM was considered to be the difference in mass at 110 °C and 950 °C, assuming that complete devolatilization took place before the combustion stage; FC was evaluated as the mass loss during the isothermal period at 950 °C when the N₂ carrier gas was substituted by O₂; Ash (A) was considered to be the residual mass at 950 °C in the oxidative atmosphere after combustion.

3. Results and Discussion

The pretreated sewage sludge can be used as primary or secondary raw material to produce fuels or biomolecules such as amino acids, proteins, biosurfactants, short-chain fatty acids, and monomers for bioplastics. Here, the focus was to explore the ability of different pretreatments and their combinations to release organic molecules in the liquid phase, thereby enhancing valorization efficiency. Furthermore, the work was specially oriented toward a reduction in total solids, SCOD, carbohydrate solubilization, and protein estimation from nitrogen content.

3.1. Solubilization of TS, VS

Figure 1 shows the percentage of solubilized TS and vs. after the different pretreatments calculated as per Equations (1) and (2) in Section 2.3. The concentrations of TS and vs. in g/L after pretreatments are also represented in Figure 1. In general, for all the pretreatments, the percentage of TS solubilization was lower than vs. solubilization. This is because, in addition to organic matter, TS is also composed of mineral solids which cannot be easily broken down [47]. Among the individual pretreatments, alkali addition with TS 6.2% and vs. 11.2% seemed better than ultrasonication (TS 2.0% and vs. 5.4%) and thermal (TS 2.5% and vs. 7.0%) pretreatments at solubilizing total and volatile solids. Overall, combining at least two or more of the pretreatment methods improved the sludge solubilization compared to the corresponding individual pretreatments. However, combining the ultrasonication method with heating had the least effect on solubilization in terms of both TS (6.5%) and vs. (7.0%) in comparison to other combined pretreatments. WAS solubilization improved when chemical pretreatment was combined with ultrasonication (TS 7.2%, vs. 12.7%) and the values increased greatly for the combined alkali–thermal pretreatment to TS 9.6% and vs. 17.2%. When all three single pretreatments were coupled, the solubilization of TS 9.5% and vs. 17.8% was similar to that of the alkali–thermal method. This suggests that combining ultrasonication with the alkali–thermal pretreatment method did not significantly impact sludge solubilization. Nevertheless, improving WAS solubilization helps to increase the concentration of organic molecules released into the soluble phase.

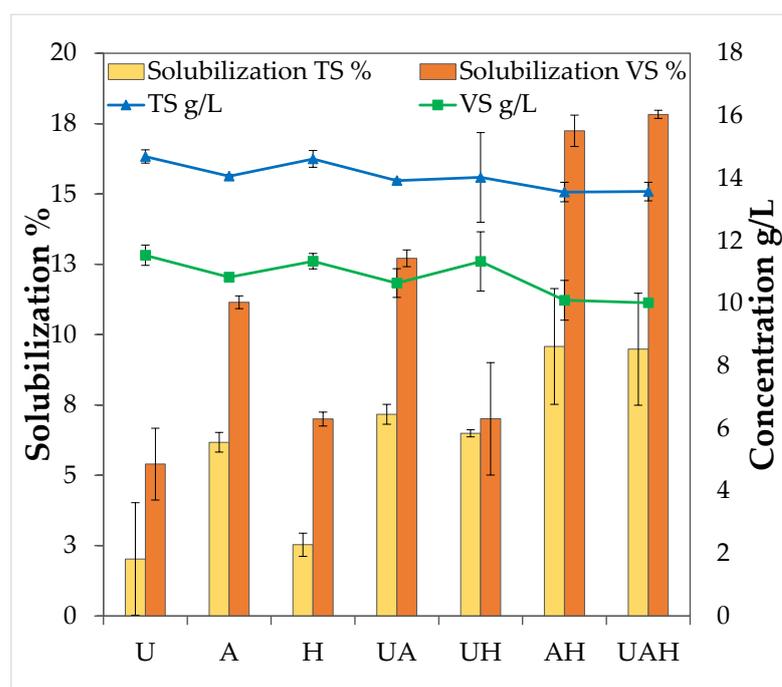


Figure 1. TS and vs. in terms of percentage solubilized and g/L after various pretreatments. U, A, and H represent ultrasonication, alkali, and heating, respectively.

3.2. Solubilization of SCOD and Carbohydrates

The SCOD and soluble carbohydrate concentration are shown in Figure 2, which indicates that all the single and grouped pretreatments had a significant impact on the SCOD measurements as compared to the untreated sludge sample with an SCOD of 500 mg/L. The release of total carbohydrates into the soluble phase of WAS improved with the pretreatments but did not have a strong dependence on the type of pretreatment, varying in the range of 500–800 mg/L. The highest SCOD of 5235 mg/L was achieved by a combination of alkali and heating pretreatments. In general, combined treatments gave better SCOD values of about 3600–5235 mg/L as compared to individual tests with SCOD values in the range 1770–3600 mg/L. This was also confirmed by previous studies [36,48]. Coupling of single pretreatments gave a trend similar to solubilization of TS and VS. The effect of alkali addition proved to be pivotal for effectively solubilizing COD, which is agreement with previous studies [49,50]. Chemical pretreatment alone resulted in an SCOD of 3654 mg/L, which was slightly increased to 4081 mg/L when combined with ultrasonication and improved significantly to 5235 mg/L when combined with thermal pretreatment. Alkali pretreatment was the most efficient among individual pretreatment methods, as well as when combined with ultrasonication, heating, or both, at helping to enhance COD solubilization. Ultrasonication had a lower impact on solubilizing COD in comparison to other single pretreatment methods. This could be the reason that adding ultrasonication to the most impactful alkali–heating pretreatment only slightly changed the SCOD and soluble carbohydrate concentration. Overall, the results suggest that all the different pretreatment methods elevated the release of carbohydrates and COD. However, combined pretreatments with alkali addition solubilized greater quantities of organic molecules in the soluble phase.

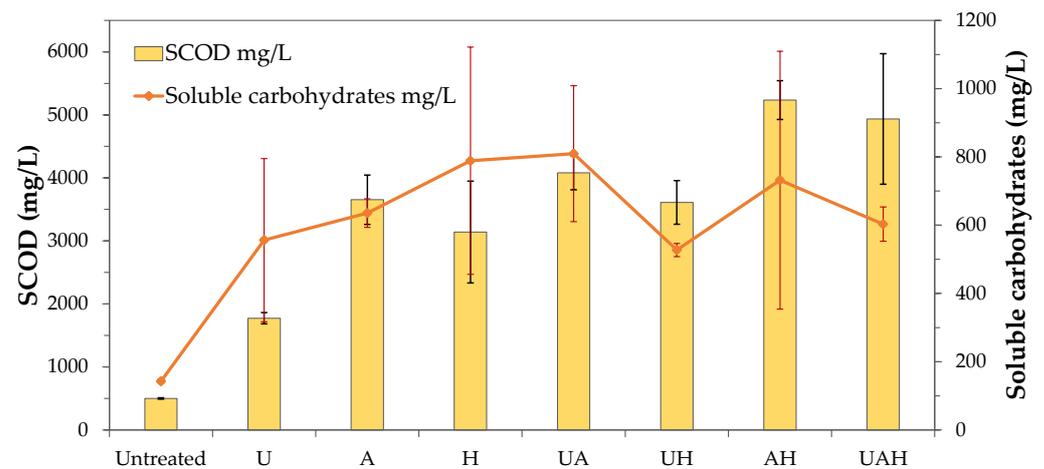


Figure 2. Comparison of different pretreatments in terms of SCOD and carbohydrates.

3.3. Total Nitrogen and Soluble Proteins

One of the significant constituents of the microorganisms found in WAS is represented by proteins, which are comprised of carbon, nitrogen, oxygen, and hydrogen. Disruption of microbial cells releases proteins into the aqueous phase of WAS [16]. The concentration of nitrogen in protein is historically assumed to be 16% [51,52]. Thus, the concentration of proteins in WAS was calculated as 6.25 times the total nitrogen [49]. Here, only TN concentration was measured, and total proteins were estimated by assuming that all the nitrogen released was in an organic form. The concentrations of TN and proteins are shown in Figure 3. The concentration of proteins present in WAS was comparatively similar to the trend observed in SCOD, with a higher quantity of proteins resulting from combined pretreatments than each separate treatment. Ultrasonication alone had a limited effect on protein release; however, when coupled with other pretreatments, it improved the solubilization of proteins. Alkali pretreatment resulted in a protein concentration of 1969 mg/L which gradually rose when combined with ultrasonication, heating, or both, ranging from 2281 mg/L to 3031 mg/L. It was found that increasing the pH of sludge increases protein solubilization [53], whereas the protein release accounts for a higher value than the corresponding carbohydrate solubilized [54], as seen here. Alkali with thermal pretreatment at 75 °C was seen to be the most effective among pretreatments in terms of COD solubilization, as well as carbohydrate and protein transfer, into the soluble phase. Similar results were obtained by Uma Rani et al. [55], who suggested that a low temperature and pH of 12 were important parameters of pretreatment for enhanced solubilization of organic matter. Generally, pretreatments involving the alkali method were found to better solubilize both lower- (below 20 kDa) and higher-molecular-weight (above 20 kDa) proteins compared to other pretreatments due to the enhanced removal of protein nitrogen at pH 12 [56].

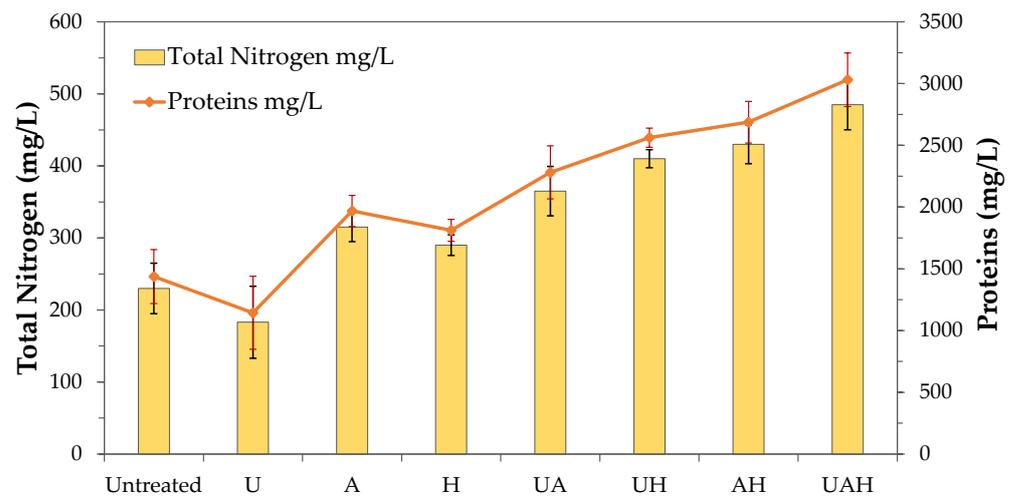


Figure 3. Effect of pretreatments on the concentration of total nitrogen and protein release.

3.4. Effect of Alkali Addition on WAS Solubilization

The effect of adding NaOH on solubilizing the organic fraction of sludge was compared for different pretreatment methods, as shown in Figures 2 and 3. Figure 2 compares the extent of solubilization of COD and carbohydrates, while Figure 3 shows the proteins (estimated from concentration of total nitrogen) for both individual and combined pretreatments. The pH sludge pretreated with alkali was adjusted to 12 using 5 M NaOH solution. Among the individual pretreatment methods, alkali pretreatment had a significant influence on WAS disintegration with respect to ultrasonication, as it resulted in higher concentrations of soluble COD, carbohydrates, and proteins. Similar to these results, Tyagi et al. [57] also found that an alkali pH of 12 was better for the release of organics in the soluble phase. Thermal pretreatment at low temperature with an SCOD of 3141 mg/L was comparable to the soluble COD of 3654 mg/L obtained after alkali addition. However, this value of 3141 mg/L was improved to 5235 mg/L when thermal and alkali pretreatments were combined. The concentration of soluble proteins was also enhanced from 1813 mg/L to 2688 mg/L after AH pretreatment. In general, the type of pretreatment performed had a very limited effect on the solubilization efficiency of carbohydrates. In the combination of ultrasonication and alkali methods, at the end of 1 h, the values of soluble COD, carbohydrates, and proteins was almost doubled with respect to the corresponding initial concentrations after ultrasonication pretreatment. During the UA method, ultrasonication created hydromechanical shear forces that helped to break the WAS flocs. The more opened floc structure resulted in better interaction of the microbial cell, with OH⁻ ions promoting better disintegration. Hence, the sludge solubilization efficiency of UA was higher than individual ultrasonication and alkali methods. Upon combining alkali, ultrasonication, and thermal methods, there was significant WAS disintegration due to the synergistic effects of the three different pretreatments. However, in terms of the release of soluble COD and carbohydrates, the concentrations were quite comparable to the alkali-thermal method, with only a negligible increase in protein concentration with respect to AH. As a result, the effect of alkali addition on sludge solubilization was most prominent when alkali pretreatment was coupled with low-temperature thermal pretreatment.

3.5. Thermogravimetric Analysis

TGA was used for the mass loss experiments to estimate the HHV of the sludge sample before and after pretreatments. TGA analysis was carried out on the solid part of the sludge samples to understand their behavior in terms of energy potential. The results of this analysis can give better insight into the usage of sludge for energy even after pretreatments and the resource recovery phase. MC, VM, FC, and A were calculated from TGA curves as outlined in Section 2.4, and an example of this can be seen in Figure 4a,

showing the TGA curve of the untreated sample. Figure 4b shows the TGA graphs before and after all pretreatments.

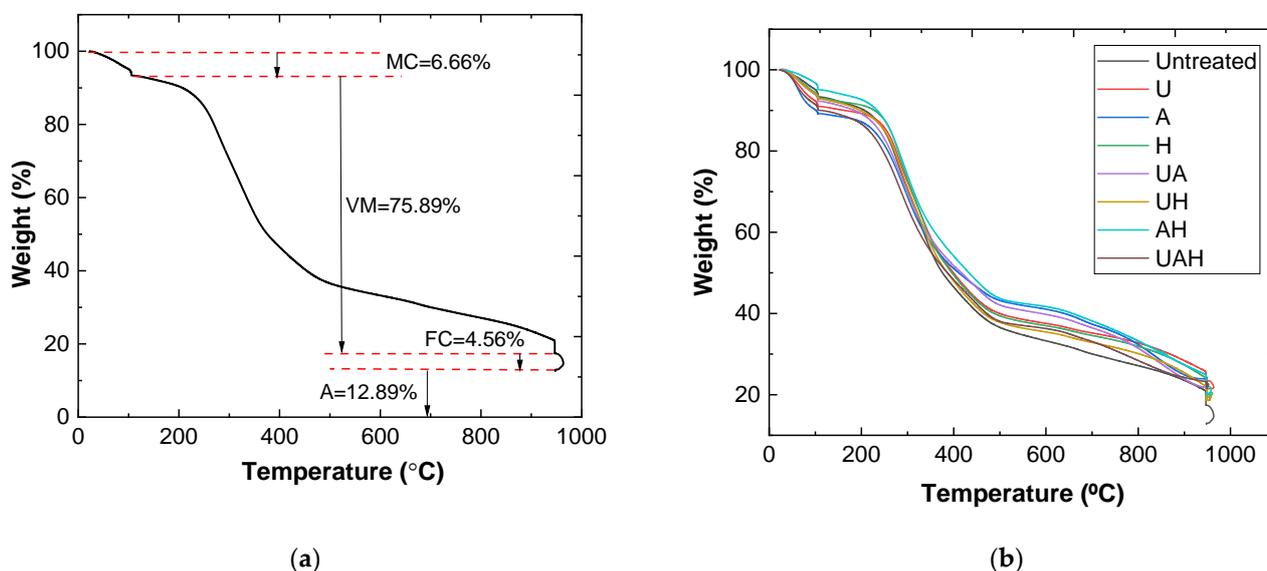


Figure 4. TGA graphs of (a) untreated sludge with proximate parameters shown, and (b) sludge before and after all pretreatments.

For all TG curves, it can be observed that there was an initial mass loss attributed to water volatilization, expressed as MC. In the second stage, there was large weight loss up to 950 °C due to the decomposition of the main volatile constituents of the sludge sample, represented as VM. Significant sludge pyrolysis took place between 200 °C and 550 °C. Biodegradable organic matter was disintegrated in the temperature range of 200–400 °C, whereas most of the nonbiodegradable organics decomposed at 400–550 °C. A comparatively lower loss of weight was noticed from 550 °C to 950 °C, which contributed to the remaining volatilization of organics. At 950 °C, sludge was allowed to completely oxidize in the O₂ environment, and FC and A were measured at this stage. The FC of samples was also measured as the difference in mass percentage between the sample and sum of masses of MC, VM, and A. These results were found to be fully in agreement with those calculated from the TG curves. The TG curves for all alkali-treated samples were observed to be steeper in the 550 °C to 950 °C region. In almost all pretreated sludge samples, especially the alkali ones, FC was found to strongly decrease with respect to the untreated sludge.

HHV was estimated here from thermogravimetric analysis using an empirical equation from the literature [58].

$$\text{HHV} = 0.3536\text{FC} + 0.1559\text{VM} - 0.0078\text{A}, \quad (3)$$

where FC, VM, and A are the weight percentages on a dry basis yielding the HHV in MJ/kg. This correlation was selected as it can be useful for computing HHV from proximate analysis for any kind of biomass material. Moreover, it had an average absolute error and bias error of 3.74% and 0.12%, respectively, highlighting the good relationship between calculated and measured HHV. The HHV results for all the samples are shown in Table 3.

The estimated HHV values according to Parikh et al.'s correlation of the sludge samples after pretreatments using proximate values from TG curves were in the range of 10–12 MJ/kg, while the untreated sludge had an HHV of 13.34 MJ/kg. It is the VM that mainly contributed to the heating value of sludge. From the literature, municipal sludges with VM 23–53% had an HHV of 4.3–13.9 MJ/kg (measured using a bomb calorimeter) [59]. The percentage of VM obtained in this study was around 65–75%, which is comparatively

higher than the above values, with an HHV between 10 and 14 MJ/kg. Due to variability found in FC, a clear comparison was not possible among the different pre-treated samples. However, the proximate analysis using the TG curve gave a reasonable estimation of HHV in case of the absence of expensive equipment, which could be used for quick and preliminary energy studies. Moreover, it can be seen that the HHV value of sludge after pretreatments did not largely differ with respect to the HHV of untreated sludge. Thus, this suggests that, even after pretreatment, there is a possibility to utilize this sludge for further energy recovery.

Table 3. Results of HHV from thermogravimetry.

Sample	HHV MJ/kg
Untreated	13.34 ± 0.65
U	10.90 ± 0.65
A	10.00 ± 0.65
H	11.44 ± 0.65
UA	10.88 ± 0.65
UH	11.47 ± 0.65
AH	11.82 ± 0.65
UAH	10.58 ± 0.65

4. Conclusions

This paper evaluated the effect of different pretreatments on WAS solubilization, which is the first step for a biorefinery exploiting WAS as a source. The pretreatments resulted in an increase in the concentration of dissolved organics. Among individual pretreatments, the alkali addition method was the most significant. The conversion of volatile solids in general was higher for alkali-treated samples, ranging from 11 to 18%. Upon comparing all pretreatments and all tested parameters, the combined alkali–heating pretreatment was found to be the most effective. The highest solubilization of both TS (9.6%) and vs. (17.2%) was obtained for the alkali–heating pretreatment method. Higher SCOD values showed that the pretreatment could be more effective in improving the release of organic soluble molecules in the liquid phase, and that a combination of pretreatments was the most effective. Solubilization of carbohydrates was independent of the pretreatments performed. The amount of nitrogen release was higher for the combined pretreatments involving the alkali step. On the other hand, the higher SCOD compared to the concentration of carbohydrates and estimated proteins revealed the presence of other classes of soluble compounds. Identification of the major contributors to the SCOD is necessary to efficiently valorize WAS. Therefore, alkali-coupled pretreatments could be further explored to produce an aqueous liquor with organic constituents which can undergo subsequent biotechnological steps to produce value-added chemicals/materials. For all the pretreatments, the HHV values were approximately 10–11.82 MJ/kg and did not greatly vary with comparison to the untreated sample. The HHV of the sludge after pretreatments was reduced to a maximum of 10 MJ/kg from 13.34 MJ/kg (untreated sample), which shows that, after resource recovery, the solid part of sludge can be further utilized for its energy properties.

Author Contributions: Conceptualization, A.C.; methodology, A.C.; validation, R.B.; formal analysis, R.B.; investigation, R.B.; resources, G.C.; writing—original draft preparation, R.B.; writing—review and editing, A.C.; visualization, R.B.; supervision, G.C. and A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grant ARGE17-992/5/2 for a PhD funded by the European Social Fund within the Liguria Regional operational program 2014–2020—thematic objective “Education and Training”.

Acknowledgments: The authors acknowledge the financial support received from the European Social Fund—Liguria Regional operational program 2014–2020. They also thank Ticass Scrl, Iren Spa, Micamo, and Active Cells for supporting this project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Collivignarelli, M.C.; Abbà, A.; Miino, M.C.; Torretta, V. What advanced treatments can be used to minimize the production of sewage sludge in WWTPs? *Appl. Sci.* **2019**, *9*, 2650. [\[CrossRef\]](#)
2. Durdevic, D.; Blecich, P.; Juric, Z. Energy recovery from sewage sludge: The case study of Croatia. *Energies* **2019**, *12*, 1927. [\[CrossRef\]](#)
3. Li, W.; Yu, N.; Liu, Q.; Li, Y.; Ren, N.; Xing, D. Enhancement of the sludge disintegration and nutrients release by a treatment with potassium ferrate combined with an ultrasonic process. *Sci. Total Environ.* **2018**, *635*, 699–704. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Oladejo, J.; Shi, K.; Luo, X.; Yang, G.; Wu, T. A Review of sludge-to-energy recovery methods. *Energies* **2019**, *12*, 60. [\[CrossRef\]](#)
5. Suárez-Iglesias, O.; Urrea, J.L.; Oulego, P.; Collado, S.; Díaz, M. Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review. *Sci. Total Environ.* **2017**, *584–585*, 921–934. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Wang, Q.; Wei, W.; Gong, Y.; Yu, Q.; Li, Q.; Sun, J.; Yuan, Z. Technologies for reducing sludge production in wastewater treatment plants: State of the art. *Sci. Total Environ.* **2017**, *587–588*, 510–521. [\[CrossRef\]](#)
7. Samorì, C.; Kiwan, A.; Torri, C.; Conti, R.; Galletti, P.; Tagliavini, E. Polyhydroxyalkanoates and crotonic acid from anaerobically digested sewage sludge. *ACS Sustain. Chem. Eng.* **2019**, *7*, 10266–10273. [\[CrossRef\]](#)
8. Bianchini, A.; Bonfiglioli, L.; Pellegrini, M.; Saccani, C. Sewage sludge drying process integration with a waste-to-energy power plant. *Waste Manag.* **2015**, *42*, 159–165. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Raheem, A.; Sikarwar, V.S.; He, J.; Dastyar, W.; Dionysiou, D.D.; Wang, W.; Zhao, M. Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: A review. *Chem. Eng. J.* **2018**, *337*, 616–641. [\[CrossRef\]](#)
10. Liu, X.; Liu, H.; Chen, J.; Du, G.; Chen, J. Enhancement of solubilization and acidification of waste activated sludge by pretreatment. *Waste Manag.* **2008**, *28*, 2614–2622. [\[CrossRef\]](#)
11. Kim, D.H.; Jeong, E.; Oh, S.E.; Shin, H.S. Combined (Alkaline + ultrasonic) pretreatment effect on sewage sludge disintegration. *Water Res.* **2010**, *44*, 3093–3100. [\[CrossRef\]](#)
12. Gong, C.; Jiang, J.; Li, D. Ultrasound coupled with fenton oxidation pre-treatment of sludge to release organic carbon, nitrogen and phosphorus. *Sci. Total Environ.* **2015**, *532*, 495–500. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Weemaes, M.P.J.; Verstraete, W.H. Evaluation of current wet sludge disintegration techniques. *J. Chem. Technol. Biotechnol.* **1998**, *73*, 83–92. [\[CrossRef\]](#)
14. Kampas, P.; Parsons, S.A.; Pearce, P.; Ledoux, S.; Vale, P.; Churchley, J.; Cartmell, E. Mechanical sludge disintegration for the production of carbon source for biological nutrient removal. *Water Res.* **2007**, *41*, 1734–1742. [\[CrossRef\]](#)
15. Khanal, S.K.; Grewell, D.; Sung, S.; Van Leeuwen, J. Ultrasound applications in wastewater sludge pretreatment: A review. *Crit. Rev. Env. Sci. Technol.* **2007**, *37*, 277–313. [\[CrossRef\]](#)
16. Nah, I.W.; Kang, Y.W.; Hwang, K.Y.; Song, W.K. Mechanical pretreatment of waste activated sludge for anaerobic digestion process. *Water Res.* **2000**, *34*, 2362–2368. [\[CrossRef\]](#)
17. Jih-Gaw, L.; Chang, C.N.; Chang, S.C. Enhancement of anaerobic digestion of waste activated sludge by alkaline solubilization. *Bioresour. Technol.* **1997**, *62*, 85–90. [\[CrossRef\]](#)
18. Lin, Y.; Wang, D.; Wu, S.; Wang, C. Alkali pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. *J. Hazard. Mater.* **2009**, *170*, 366–373. [\[CrossRef\]](#)
19. Barjenbruch, M.; Kopplow, O. Enzymatic, mechanical and thermal pre-treatment of surplus sludge. *Adv. Environ. Res.* **2003**, *7*, 715–720. [\[CrossRef\]](#)
20. Bougrier, C.; Delgenès, J.P.; Carrère, H. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chem. Eng. J.* **2008**, *139*, 236–244. [\[CrossRef\]](#)
21. Ponsá, S.; Ferrer, I.; Vázquez, F.; Font, X. Optimization of the hydrolytic-acidogenic anaerobic digestion stage (55 °C) of sewage sludge: Influence of PH and solid content. *Water Res.* **2008**, *42*, 3972–3980. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Tytla, M. The effects of ultrasonic disintegration as a function of waste activated sludge characteristics and technical conditions of conducting the process—Comprehensive analysis. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2311. [\[CrossRef\]](#)
23. Zhang, Y.; Xu, S.; Cui, M.; Wong, J.W.C. Effects of different thermal pretreatments on the biodegradability and bioaccessibility of sewage sludge. *Waste Manag.* **2019**, *94*, 68–76. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Ruiz-Hernando, M.; Martín-Díaz, J.; Labanda, J.; Mata-Alvarez, J.; Llorens, J.; Lucena, F.; Astals, S. Effect of ultrasound, low-temperature thermal and alkali pre-treatments on waste activated sludge rheology, hygienization and methane potential. *Water Res.* **2014**, *61*, 119–129. [\[CrossRef\]](#)
25. Climent, M.; Ferrer, I.; Baeza, M.d.M.; Artola, A.; Vázquez, F.; Font, X. Effects of thermal and mechanical pretreatments of secondary sludge on biogas production under thermophilic conditions. *Chem. Eng. J.* **2007**, *133*, 335–342. [\[CrossRef\]](#)
26. Tanaka, S.; Kamiyama, K. Thermochemical pretreatment in the anaerobic digestion of waste activated sludge. *Water Sci. Technol.* **2002**, *46*, 173–179. [\[CrossRef\]](#)

27. Kavitha, S.; Kannah, Y.R.; Yeom, I.T.; Do, K.U.; Banu, J.R. Combined thermo-chemo-sonic disintegration of waste activated sludge for biogas production. *Bioresour. Technol.* **2015**, *197*, 383–392. [[CrossRef](#)] [[PubMed](#)]
28. Chiu, Y.C.; Chang, C.N.; Lin, J.G.; Huang, S.J. Alkaline and ultrasonic pretreatment of sludge before anaerobic digestion. *Water Sci. Technol.* **1997**, *36*, 155–162. [[CrossRef](#)]
29. Kim, D.J.; Youn, Y. Characteristics of sludge hydrolysis by ultrasound and thermal pretreatment at low temperature. *Korean J. Chem. Eng.* **2011**, *28*, 1876–1881. [[CrossRef](#)]
30. Chu, C.P.; Chang, B.-V.; Liao, G.S.; Jean, D.S.; Lee, D.J. Observations on changes in ultrasonically treated waste-activated sludge. *Water Res.* **2001**, *35*, 1038–1046. [[CrossRef](#)]
31. Tiehm, A.; Nickel, K.; Zellhorn, M.; Neis, U.; Tiehm, A. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Res.* **2001**, *35*, 2003–2009. [[CrossRef](#)]
32. Zhang, G.; Zhang, P.; Gao, J.; Chen, Y. Using acoustic cavitation to improve the bio-activity of activated sludge. *Bioresour. Technol.* **2008**, *99*, 1497–1502. [[CrossRef](#)] [[PubMed](#)]
33. Xia, Y.; Yang, C.; Zhang, T. Microbial effects of part-stream low-frequency ultrasonic pretreatment on sludge anaerobic digestion as revealed by high-throughput sequencing-based metagenomics and metatranscriptomics. *Biotechnol. Biofuels* **2018**, *11*, 47. [[CrossRef](#)]
34. Pilli, S.; Bhunia, P.; Yan, S.; LeBlanc, R.J.; Tyagi, R.D.; Surampalli, R.Y. Ultrasonic pretreatment of sludge: A review. *Ultrason. Sonochemistry* **2011**, *18*, 1–18. [[CrossRef](#)]
35. Le, N.T.; Julcour-Lebigue, C.; Delmas, H. An executive review of sludge pretreatment by sonication. *J. Environ. Sci.* **2015**, *37*, 139–153. [[CrossRef](#)] [[PubMed](#)]
36. Penaud, V.; Delgenes, J.P.; Moletta, R. Influence of thermochemical pretreatment conditions on solubilization and anaerobic biodegradability of a microbial biomass. *Environ. Technol.* **2000**, *21*, 87–96. [[CrossRef](#)]
37. Kim, J.; Park, C.; Kim, T.H.; Lee, M.; Kim, S.; Kim, S.W.; Lee, J. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. *J. Biosci. Bioeng.* **2003**, *95*, 271–275. [[CrossRef](#)]
38. Prorot, A.; Julien, L.; Christophe, D.; Patrick, L. Sludge disintegration during heat treatment at low temperature: A better understanding of involved mechanisms with a multiparametric approach. *Biochem. Eng. J.* **2011**, *54*, 178–184. [[CrossRef](#)]
39. Rocher, M.; Goma, G.; Pilas Begue, A.; Louvel, L.; Rols, J.L. Towards a reduction in excess sludge production in activated sludge processes: Biomass physicochemical treatment and biodegradation. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 883–890. [[CrossRef](#)]
40. Wang, Q.; Noguchi, C.; Hara, Y.; Sharon, C.; Kakimoto, K.; Kato, Y. Studies on anaerobic digestion mechanism: Influence of pretreatment temperature on biodegradation of waste activated sludge. *Environ. Technol.* **1997**, *18*, 999–1008. [[CrossRef](#)]
41. Sridhar, M.K.C.; Pillai, S.C. Proteins in wastewater and wastewater sludges. *J. Water Pollut. Control Fed.* **1973**, *45*, 1595–1600.
42. Dubois, M.; Gilles, K.; Hamilton, J.K.; Rebers, P.A.; Smith, F. A colorimetric method for the determination of sugars. *Nature* **1951**, *168*, 167–169. [[CrossRef](#)]
43. Eaton, D.A.; Franson, H.M.A. *Standard Methods for the Examination of Water & Wastewater*, 21st ed.; APHA: Washington, DC, USA, 2005; p. 1200.
44. Malucelli, L.C.; Silvestre, G.F.; Carneiro, J.; Vasconcelos, E.C.; Guiotoku, M.; Maia, C.M.B.F.; Filho, M.A.S.C. Biochar higher heating value estimative using thermogravimetric analysis. *J. Therm. Anal. Calorim.* **2020**, *139*, 2215–2220. [[CrossRef](#)]
45. Saffe, A.; Fernandez, A.; Mazza, G.; Rodriguez, R. Prediction of regional agro-industrial wastes characteristics by thermogravimetric analysis to obtain bioenergy using thermal process. *Energy Explor. Exploit.* **2019**, *37*, 544–557. [[CrossRef](#)]
46. ASTM E1131-20. *Standard Test Method for Compositional Analysis by Thermogravimetry*; ASTM International: West Conshohocken, PA, USA, 2020.
47. Qiao, W.; Wang, W.; Xun, R.; Lu, W.; Yin, K. Sewage sludge hydrothermal treatment by microwave irradiation combined with alkali addition. *J. Mater. Sci.* **2008**, *43*, 2431–2436. [[CrossRef](#)]
48. Tanaka, S.; Kobayashi, T.; Kamiyama, K.I.; Signey Bildan, M.L.N. Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. *Water Sci. Technol.* **1997**, *35*, 209–215. [[CrossRef](#)]
49. Niaounakis, M.; Halvadakis, C.P. *Olive Processing Waste Management. Literature Review and Patent Survey*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2006; p. 514. [[CrossRef](#)]
50. Vlyssides, A.G.; Karlis, P.K. Thermal-Alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion. *Bioresour. Technol.* **2004**, *91*, 201–206. [[CrossRef](#)]
51. Suschka, J.; Grübel, K. Nitrogen in the process of waste activated sludge anaerobic digestion. *Arch. Environ. Prot.* **2014**, *40*, 123–136. [[CrossRef](#)]
52. Westgate, P.J.; Park, C. Evaluation of proteins and organic nitrogen in wastewater treatment effluents. *Environ. Sci. Technol.* **2010**, *44*, 5352–5357. [[CrossRef](#)]
53. Chen, Y.; Jiang, S.; Yuan, H.; Zhou, Q.; Gu, G. Hydrolysis and acidification of waste activated sludge at different pHs. *Water Res.* **2007**, *41*, 683–689. [[CrossRef](#)] [[PubMed](#)]
54. Liu, H.; Fang, H.H.P. Extraction of extracellular polymeric substances (EPS) of sludges. *J. Biotechnol.* **2002**, *95*, 249–256. [[CrossRef](#)]
55. Rani, U.R.; Kumar, A.S.; Kaliappan, S.; Yeom, I.T.; Banu, R.J. Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process. *Bioresour. Technol.* **2012**, *103*, 415–424. [[CrossRef](#)] [[PubMed](#)]
56. Xiao, K.; Zhou, Y. Protein recovery from sludge: A review. *J. Clean. Prod.* **2020**, *249*, 119373. [[CrossRef](#)]

-
57. Tyagi, V.K.; Lo, S.L.; Rajpal, A. Chemically coupled microwave and ultrasonic pre-hydrolysis of pulp and paper mill waste-activated sludge: Effect on sludge solubilisation and anaerobic digestion. *Environ. Sci. Pollut. Res.* **2014**, *21*, 6205–6217. [[CrossRef](#)] [[PubMed](#)]
 58. Parikh, J.; Channiwala, S.A.; Ghosal, G.K. A correlation for calculating HHV from proximate analysis of solid fuels. *Fuel* **2005**, *84*, 487–494. [[CrossRef](#)]
 59. Thipkhunthod, P.; Meeyoo, V.; Rangsunvigit, P.; Kitiyanan, B.; Siemanond, K.; Rirksomboon, T. Predicting the heating value of sewage sludges in thailand from proximate and ultimate analyses. *Fuel* **2005**, *84*, 849–857. [[CrossRef](#)]