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Biochemical Methane Potential Tests to Evaluate Anaerobic Digestion Enhancement by Thermal Hydrolysis Pretreatment

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Abstract

This study evaluates the effects of thermal hydrolysis (TH) pretreatment on anaerobic digestion (AD), through results obtained by biochemical methane potential (BMP) tests under mesophilic conditions (35 °C). Thickened sludge from a wastewater treatment plant (WWTP) was thermally treated under two different temperatures (150 and 170 °C) and reaction times (30 and 60 min). Results show a significant increase in soluble COD, compared with the untreated sludge, when sludge was treated at 170 °C for 60 min. Moreover, the following BMP tests point out that TH pretreatment of sludge accelerated the AD rate and increased the biogas yield contributing to an increase in methane production, ranging between 17 and 24% compared with the raw sludge. Furthermore, the hydrolysis constant was estimated and methane production and degree of disintegration of the TH pretreated sludge were correlated, in order to deep the knowledge on the hydrolysis as the AD rate-limiting step. Further, the combined effects of TH pretreatment and AD on sludge show a reduction of total and volatile solids up to 19% and 24%, respectively.

Keywords Thermal hydrolysis pretreatment · Anaerobic digestion · Organic solubilization · BMP · Sludge hydrolysis

Introduction

Sewage sludge is the main waste produced by wastewater treatment plants (WWTPs) [1]. In the last century, the amount of this residue has increased due to the high water demand of an increasing population, to industrialization and urbanization, and to the higher level of wastewater treatment [2]. Sludge management, including treatment and disposal, could account up to 65% of the total operational costs of a WWTP [3]. The main sludge disposal options, used over the years, are incineration, landfilling, and land application. Today, all these strategies are not anymore economically and environmentally sustainable, due to the need for expensive machinery, the use of non-renewable resources, the high space requirement, the stringent regulatory limits, and the high generation of greenhouse gases (GHGs) emissions [2, 4]. Therefore, the development of new sludge management strategies, both cost-effective and environmentally sustainable, is a challenging issue, which still requires considerable efforts from the scientific and industrial communities. Among all sludge management technologies, AD has been applied as an efficient way to reduce the final amount of solids for disposal [5]. The AD process is considered one of the most cost-effective, technologies because it allows obtaining both high energy and resource recovery from sewage sludge [6]. AD of sludge is a microbiological process that transforms biodegradable organic matter into biogas (60 –70 vol% of methane, CH_4) in anaerobic conditions (i.e., in the absence of oxygen, O_2), reducing the mass of residual solids, contributing to reduce the pathogens present in the sludge and removing odors [1, 7]. AD is a slow process that mainly involves the following steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. It is well known that the rate-limiting step of the AD process is hydrolysis [8, 9], which implies the solubilization of intracellular biopolymers of degradable organics and their conversion to lower molecular weight compounds. Therefore, in order to

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speed up the AD process of sludge, and in particular of waste activated sludge (WAS), a pretreatment of the sludge would be beneficial, in order to contribute to disrupt the flocks and the cell walls of the WAS structure, by damaging the physical structure of organic solids and enhancing the release of intracellular matter. Thus, there is a high interest to develop pretreatments of sludge capable to enhance the hydrolysis step of the AD process reducing the digestion rates and the retention time and increasing the biogas production [10, 11]. To improve the sludge anaerobic digestion efficiency, various pretreatment technologies have been developed, such as thermal hydrolysis (TH), ultrasound, and alkali treatment [12, 13]. Among these pretreatments, the TH and related heat treatment processes have long been used in sludge processing for different purposes, such as increasing organic loading rate, improving biodegradability, and enhancing dewaterability [14]. Originally, TH process was used for conditioning the sludge and improving its dewaterability. Further researches were performed, with the aim of improving the settleability and filterability of sludge by altering the sludge's physical characteristics [15]. Performing these research studies, the TH process was found to destroy the structural integrity of microbes and cause the lysis of cell walls, releasing cell contents in the aqueous phase, which became more available for biological degradation [1]. Today, TH process has been recognized as a well-established and commercially implemented technology to improve AD efficiency, especially for the digestion of WAS [16, 17]. The effectiveness of the TH process applied to sludge was found to be enhanced at high temperature (T) and reaction time (RT). Essentially, under T ranging between 130 and 200 °C, with RT between 15 and 60 min, a hydrolysis reaction occurs to break down complex molecules in sludge into simpler compounds, improving the bioavailability of sludge contents for AD [18]. Actually, the disintegration of sludge and the solubilization of biodegradable organic matter could occur also at T lower than 100 °C, but with much higher RT, ranging from 5 to 24 h [19, 20], thus making the process less sustainable. The TH pretreatment of sludge is an advantageous process, because it does not require chemicals, thus reducing costs and being an environmental friendly technology [21].

The effectiveness of the TH process, prior to the AD process, is correlated to the increase in the amount of biogas produced, in comparison to the untreated sludge, given a certain T and RT, which are the main operative parameters of the TH pretreatment process.

In this scenario, the objective of this study is to apply the TH process prior to AD of a thickened sludge under four different operative conditions, obtained by varying T and RT, to potentially improve the biogas production and digestion efficiency. The enhancement in biogas production is determined by biochemical methane production (BMP) tests, which allowed to evaluate the hydrolysis kinetics constant. The BMP assay has proved to be a relatively simple and reliable method that gives valuable information for optimizing the design and functioning of an anaerobic digester. However, there are many factors that may influence the BMP assay, such as the inoculum and substrate characteristics and the experimental conditions. Despite the literature related to BMP assays is extensive, several different batch methods have been utilized for measuring methane potentials, which are difficult to compare making it difficult to draw precise and generalized conclusions [22].

Therefore, the novelty of this research is to collect and compile results obtained in the BMP assay using thickened sludge from a municipal WWTP as substrate, which has a particular composition and characteristic, with the aim of providing an accurate literature database in relation to the T and RT selected and in comparison with the untreated raw sludge. In addition, results reported in the present study contribute to increase the knowledge about the enhancement in biogas production due to TH pretreatment prior the AD process, thus providing to public and private WWTP owners an accurate reference on the possibility to use the BMP assay as a valuable tool to set the optimal TH pretreatment operative conditions.

Material and Methods

Sludge Sampling and Characterization

The sludge used for TH tests was sampled from the sludge dynamic thickener of Trento Nord WWTP, Italy. The sludge was stored at 4 °C and used for all the TH tests. The characteristics of the raw sludge are as follows: total solid (TS) 4.98 ± 0.6%, volatile solids (VS) 3.68 ± 0.6%, total chemical oxygen demand (TCOD) 51.6 ± 0.7 g L^{-1}, soluble chemical oxygen demand (sCOD) 6.7 ± 0.3 g L^{-1}, total Kjeldahl nitrogen (TKN) 4.2 ± 0.2 g L^{-1}, soluble phosphorus (PO_4^{3-}-P) 1.8 ± 0.1 mg L^{-1}, and pH 6.7 ± 0.1.

Thermal Hydrolysis Reactor

A batch-type reactor was used for the TH process. The whole system consists of a high pressure stainless steel reactor, equipped with pressure transmitter, pressure gauge, double thermocouple, electrical band heater, inlet and outlet valves; a temperature control panel with temperature and pressure recorders; and finally, a plastic graduated cylinder, with the lower part submerged into water, to allow the measure of the amount of gas produced during the thermal process (data not of interest for the present investigation and thus not reported in the following). The stainless steel reactor (AISI 316), having a total internal volume of about 50 mL, was designed for a maximum temperature and pressure, respectively, of 300 °C and 140 bar. More details about the reactor and the whole system assembly, including P&I diagrams and a picture, are reported in Fiori et al. [23] and Basso et al. [24].

In this study, the thermal pretreatment of sludge was conducted at four operating conditions, different in T and RT, namely at 150 °C and 30 min (test #1), 150 °C and 60 min (test #2), 170 °C and 30 min (test #3), and 170 °C and 60 min (test #4). These operative parameters were chosen on the basis of previous literature studies. In particular, Carrère et al. [13] reported that several studies revealed an improvement of sludge characteristics at 150 °C, with an optimal temperature in the range of 160–180 °C and treatment times from 30 to 60 min.

The maximum pressure reached in the various TH tests ranged from 6 to 10 bar with the sludge sample volume held at a maximum value of 35 mL.

Before the beginning of each test, the headspace of the reactor was flushed using N_2, in order to create an inert atmosphere and avoid oxidation of the sample during TH. The temperature was then increased to the TH temperature set point and maintained for 30–60 min, depending on the residence time chosen for the test. At the end of each test, the reactor was cooled down to 30 °C by means of a stainless steel disk kept at − 24 °C and placed below the reactor. After cooling, the gas produced in the reactor was expanded by opening the reactor outlet valve and let passing through a plastic graduated cylinder previously filled with water. After that, the reactor was opened and the sludge collected and used for further tests and analysis.

Biochemical Methane Potential Tests

Each sludge sample, after the TH test, was assessed in the biochemical methane potential (BMP) assay, in order to determine the specific biogas and methane yields. The BMP assays were conducted over 28 days using serum bottles (volume 135 mL, sealed with polypropylene red rubber stopper) inside a thermostatic bath set at 35.0 ± 0.1 °C. The bottles were inoculated with anaerobically digested sludge from an anaerobic digester located in Reggio Emilia, Italy, which treats zootechnical waste from local companies. The characteristics of the inoculum were TS 5.63%, VS 3.87%, TCOD 52.8 g L^{-1}, sCOD 1.0 g L^{-1}, organic nitrogen (N_{org}-N) 1.8 g L^{-1}, ammonium nitrogen (NH_4^+-N) 2.0 g L^{-1}, PO_4^{3-}-P 22.7 mg L^{-1}, alkalinity 5000 mg $CaCO_3$ L^{-1}, and pH 7.5. Notably, the inoculum was pre-incubated for 14 days (35.0 ± 0.1 °C) in order to minimize its residual biodegradable organic matter content.

A feeding/inoculum ratio (F/I) equal to 0.5 g VS g^{-1} VS was used, as suggested by Angelidaki et al. [25] and Neves et al. [26]; thus, the liquid volume in the serum bottle was 100 mL: 30 mL of sludge (before or after TH pretreatment) plus 70 mL of inoculum (headspace volume 35 mL). Before the beginning of the BMP tests, the headspace of each bottle was purged with N_2 for 5 min, in order to ensure not oxidizing conditions.

All the tests involving the sludge from Trento Nord WWTP were carried out in triplicate; more in detail, 12 serum bottles were used for BMP tests with TH pretreated sludge plus inoculum, and 3 serum bottles were used for BMP tests with raw thickened sludge plus inoculum. In addition, two serum bottles were used containing only the inoculum (blank BMP) in order to have a BMP assay to be used as a baseline.

Gas pressure was measured periodically (every day in the first 12 days, when biogas production was high, every 1–3 days later) in the headspace of each serum bottle by using a manometer which allowed the amount of biogas produced to be measured. After that, the headspace was depressurized to atmospheric pressure, allowing the gas to flow in a plastic graduated cylinder with the lower part submerged in a NaOH (2M) solution. In this way, the high pH of the solution caused the dissolution of CO_2 in water and thus the gas measured was only composed of CH_4. The amount of CH_4 produced was evaluated considering the change in the liquid level in the graduated cylinder. The amounts of biogas and CH_4 measured at laboratory conditions were converted to volumes of biogas and CH_4 at standard conditions, using the ideal gas law ($p_0 = 1.01325\ bar$ and $T_0 = 273.15\ K$) in order to make comparable all the tests performed.

Analytical Method and Calculations

Several chemical analyses were performed in order to characterize the raw thickened sludge, the inoculum, and the TH pretreated sludge. TS, VS, TCOD, and sCOD were measured in accordance with standard methods [27]. NH_4^+-N, N_{org}-N, TKN, and PO_4^{3-}-P concentrations were quantified, according to APAT-CNR-IRSA [28]. Alkalinity was measured only for the BMP inoculum sludge using a prepared kit for determination (Hach Lange) to assess the buffering capacity of the AD system in neutralizing the drop of pH due to the production of volatile fatty acids (VFAs). The pH was measured with a VWR™ sympHony™ meter.

Data collected during 28 days of BMP assay allowed the determination of specific biogas production (SBP), expressed as mL_{biogas} g^{-1} VS, and the specific methane production (SMP), expressed as mL CH_4 g^{-1} VS, as for, respectively, Eqs. (1) and (2):

$$SBP = \frac{V_{biogas\ TH} - V_{biogas\ blank}}{VS_{TH}} \qquad (1)$$

$$SMP = \frac{V_{CH_4\ TH} - V_{CH_4\ blank}}{VS_{TH}} \qquad (2)$$

where $V_{biogas\ TH}$ and $V_{CH4\ TH}$ are, respectively, the volume of biogas and the volume of methane produced by the TH pretreated sludge plus the inoculum (mL); $V_{biogas\ blank}$ and

$V_{CH4\ blank}$ are, respectively, the volume of biogas and the volume of methane produced by the inoculum (blank) (mL); VS_{TH} is the mass of volatile solids of the TH pretreated sludge inside the BMP bottle (g VS). Similarly, SBP and SMP were calculated also for the raw thickened sludge.

The sewage sludge disintegration degree (DD) was calculated as for Eq. (3), which allowed the assessment of the fraction of particulate substances solubilized by the TH process, with respect to the initial insoluble fraction [1, 29].

$$DD(\%) = \frac{sCOD - sCOD_0}{TCOD_0 - sCOD_0} \times 100 \quad (3)$$

where sCOD is the soluble COD of the TH-treated sludge, and $sCOD_0$ and $TCOD_0$ are, respectively, the soluble COD and the total COD of the raw thickened sludge.

The BMP tests allowed determining the hydrolysis constant (k_h), assuming hydrolysis could be described by a first-order kinetics equation. k_h could be evaluated as for Eq. (4):

$$ln\frac{B_\infty - B}{B_\infty} = -k_h \cdot t \quad (4)$$

where B_∞ is the final (total, cumulative) methane production and B is the methane production at time t. The value of k_h is represented by the slope (in absolute value) of the trend line obtained by plotting Eq. (4), considering 5 consecutive days of BMP assay.

To evaluate and compare the combined effect of RT and T of the different TH pretreatment conditions, the severity factor (log R_0) was calculated following Eq. (5) [30, 31]:

$$\log R_0 = \log\left(t \cdot exp\left(\frac{T - 100}{14.75}\right)\right) \quad (5)$$

where t refers to reaction time (min), T is the temperature (°C), 100 is the base temperature (100 °C), and 14.75 is the activation energy based on the assumption that the reaction is hydrolytic and the overall conversion is first order [31]. This expression only takes into account time and temperature and does not consider other process variables.

Statistical Analysis

One-way ANOVA and Tukey's HSD multiple comparison tests were performed to verify statistical significance in the datasets at a 95% confidence interval. Statistical analysis was performed using XLSTAT. The results were expressed as means ± standard deviation of three parallel measurements.

Results and Discussion

Effects of TH Pretreatment on Sludge Characteristics

TH pretreatment of sludge was performed in the range of 150–170 °C and 30–60 min of residence time, using the same sludge sample for all the experimental tests. Figure 1 shows the changes in terms of DD, TCOD, and sCOD concentration of the sludge due to the TH pretreatment.

According to Table 1, in tests #1 (T = 150 °C; RT = 30 min) and #2 (T = 150 °C; RT = 60 min), the TCOD concentration accounts for, respectively, 51.9 ± 0.8 g TCOD L^{-1} and 52.6 ± 0.3 g TCOD L^{-1} while in tests #3 (T = 170 °C; RT = 30 min) and #4 (T = 170 °C; RT = 60 min) is equal to 55.4 ± 1.8 g TCOD L^{-1} and 53.9 ± 1.4 g TCOD L^{-1}, respectively. The small variation of the TCOD concentration before and after the thermal pretreatment falls within the 10% analytical error. Results of the ANOVA analysis show that there are no significant differences between the TCOD concentration of the raw sludge and tests #1, #2, and #4, while only test #3 revealed statistical differences.

From Fig. 1, a larger increase of sCOD, compared with the raw sludge, can be observed in all the tests performed, meaning that particulate polymeric compounds and microbial cells were disrupted by the thermal treatment releasing organics from the cells [32].

The sCOD concentration, reported in Table 1, in tests #1, #2, #3, and #4, was equal to, respectively, 14.8 ± 0.7, 15.8 ± 0.8, 17.3 ± 0.9, and 18.9 ± 0.4 g sCOD L^{-1} (sCOD of the raw sludge, 6.7 ± 0.3 g sCOD L^{-1}). While the increment between the TCOD concentration of the raw sludge and the TH-treated sludge was minimal in all the tests performed, varying in the range 1–7%, on the contrary, a significant increase in sCOD concentration was observed accounting for 118, 134, 156, and 180%, respectively, in tests #1, #2, #3 and #4, compared with the raw sludge. Results confirm that TH process resulted in significant COD solubilization. The most notable increase in sCOD (180%) was observed with test #4, where the treatment temperature and residence time were the highest of all the experiments carried out. In addition, there was a notable increase in the sCOD fraction of the TCOD for the sample of test #3 (156%), where the treatment temperature was the same of test #4, while the residence time was equal to half. Thus, results highlight that doubling the residence time of the raw sludge inside the TH reactor gave rise to an increase of about 24% sCOD concentration. This result was also observed for tests #1 and #2, where the increase was even lower. Moreover, comparing tests performed at 150 and 170 °C, results reveal that a high COD solubilization corresponded to tests carried out at 170 °C, confirming that higher temperatures promote a better COD solubilization.

ANOVA analysis revealed that sCOD concentrations of all the tests performed are significantly different from the raw

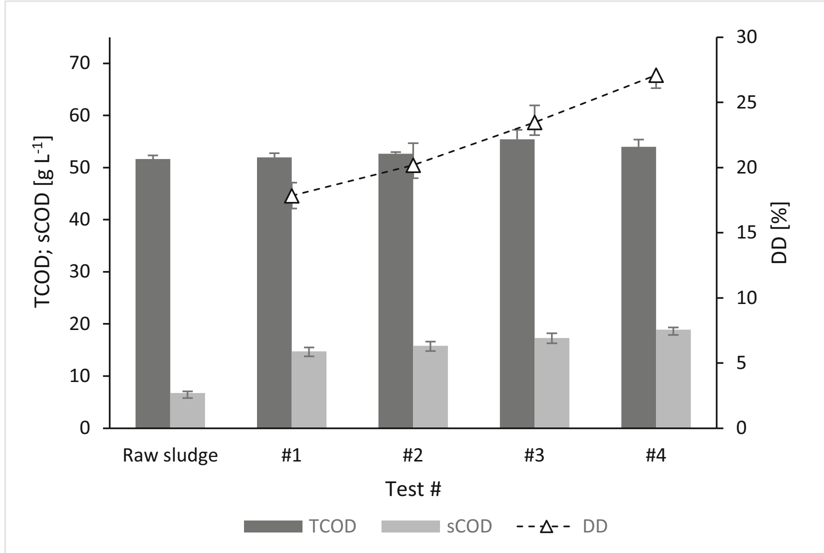

Fig. 1 TCOD, sCOD, and DD trends of untreated (raw sludge) and TH pretreated sludges: #1 T = 150 °C—RT = 30 min; #2 T = 150 °C—RT = 60 min; #3 T = 170 °C—RT = 30 min; #4 T = 170 °C—RT 60 min

sludge; on the contrary, each test is comparable with the others.

The DD, evaluated according to Eq. (3) and reported in Fig. 1 and Table 1, confirms the COD solubilization of sludge due to TH pretreatment. The DD was equal to 17.9 ± 0.9%, 20.2 ± 1.0%, 23.5 ± 1.7%, and 27.1 ± 1.3%, respectively, in tests #1, #2, #3, and #4, confirming an increase as pretreatment temperature and residence time increased. Organic matter, which could be transformed into biogas in the subsequent AD process, was transferred to a large extent to the hydrolysate due to TH. The major organic components of hydrolysate were proteins and carbohydrates, which were solubilized due to the TH process [33]. Thus, results of the DD confirm that TH pretreatment was effective and promoted hydrolysis and solubilization. Results of the ANOVA analysis performed on the DD percentage show that there are significant differences between tests #1, #3, and #4, while test #2 is comparable with DD percentage obtained from tests #1 and #3. Furthermore, referring to Table 1, it is evident that the DD percentage increased linearly with the severity factor as a direct consequence of the cell disruption that takes place during the thermal pretreatment [34], obtaining a solubilization of 27.1 ± 1.3% of the particulate matter at the highest severity factor evaluated (log R_0 = 3.8 in test #4).

Results observed are consistent with prior findings that showed a linear increase in COD solubilization between 150 and 180 °C and reaction time between 0 and 90 min [35, 36].

As it could be seen from Table 1, the TKN concentrations were 4.2 ± 0.2, 4.2 ± 0.2, 4.5 ± 0.2, and 4.9 ± 0.2 g TKN L^{-1}, respectively, in tests #1, #2, #3, and #4, while that of the raw sludge was equal to 4.2 ± 0.2 g TKN L^{-1}.

Thus, results indicate that the TKN concentrations of tests #1 and #2, when the treatment temperature was equal to 150 °C, were equal to that of the raw sludge. On the contrary, the TKN concentration increased much more during tests #3 and #4, performed at a higher temperature (170 °C), with both a RT of 30 and 60 min. As expected, the ANOVA analysis revealed that there are no significant differences between the TKN concentration of the raw sludge and those of tests #1, #2, and #3; on the contrary, TKN concentration measured in test #4 is statistically different from all the others except from test #3.

Table 1 Sludge characteristics before and after the TH pretreatment

Parameter	Before TH test	After TH tests			
	Raw sludge	Test #1	Test #2	Test #3	Test #4
Total COD (g TCOD L^{-1})	51.6 ± 0.7	51.9 ± 0.8	52.6 ± 0.3	55.4 ± 1.8	53.9 ± 1.4
Soluble COD (g sCOD L^{-1})	6.7 ± 0.3	14.8 ± 0.7	15.8 ± 0.8	17.3 ± 0.9	18.9 ± 0.4
DD (%)	–	17.9 ± 0.9	20.2 ± 1.0	23.5 ± 1.7	27.1 ± 1.3
log R_0	–	2.9	3.3	3.5	3.8
Total Kjeldahl nitrogen (g TKN L^{-1})	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.5 ± 0.2	4.9 ± 0.2
Soluble phosphorous (mg PO_4^{3-}-P L^{-1})	1.8 ± 0.1	1.9 ± 0.1	2.9 ± 0.1	13.7 ± 0.7	9.2 ± 0.5
pH (–)	6.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1

Considering the PO_4^{3-}-P concentration, results reported a significant increase, especially when the treatment temperature was 170 °C. Data reported in Table 1 shows that the PO_4^{3-}-P concentration varied from 1.8 ± 0.1 mg PO_4^{3-}-P L^{-1} of the raw sludge to 1.9 ± 0.1, 2.9 ± 0.1, 13.7 ± 0.7, and 9.2 ± 0.5 mg PO_4^{3-}-P L^{-1}, respectively, for tests #1, #2, #3, and #4. This result confirms the previous findings about the role of the RT: increasing the RT of the TH pretreatment did not cause a significant variation in the solubilization of the thickened sludge. The system revealed to be much more sensitive to T variation. Results of the ANOVA analysis performed on the PO_4^{3-}-P concentration show that there are significant differences between the raw sludge and tests #2, #3, and #4, while test #1 is comparable with PO_4^{3-}-P concentration of the raw sludge and test #2.

As expected, TH pretreatment of sludge increased the TKN and soluble phosphorous concentrations due to the mechanism of cell lysis promoted by high temperature. Indeed, the TH pretreatment of sludge destroys the structural integrity of microbes and causes the lysis of cell walls releasing cell contents that are composed also by nutrients.

BMP Tests

The BMP tests were operated under mesophilic conditions for 28 days until biogas production had almost ceased. As reported in the "Biochemical Methane Potential Tests" section, the TH-treated sludge was added to the inoculum sludge in order to perform the BMP assays. The characteristics of the sludge samples, after adding the TH-treated sludge to the inoculum sludge, are reported in Table 2. The average SBP and SMP trends, evaluated according to Eqs. (1) and (2), are reported in Fig. 2a and b, respectively. SBP and SMP trends with standard deviation are reported in Supplementary Materials. BMP tests show that TH pretreatment enhanced the anaerobic biodegradability, accelerating the biogas production rate of sludge, compared with the untreated thickened sludge. According to Fig. 2a, the highest cumulative biogas production was achieved at the end of test #2, where the thickened sludge was pretreated at T = 150 °C and RT = 60 min, accounting for 359 ± 14 mL biogas g^{-1} VS. This result is quite similar to that obtained performing the test with the TH pretreated sludge at 170 °C for 30 min (test #3), where the SBP, after 28 days, reached the value of 343 ± 13 mL biogas g^{-1} VS. Thus, there is a very small difference between tests #2 and #3, despite that they differed in both TH treatment temperature and residence time: indeed, the severity factor of test #2 resembles that of #3. Moreover, test #4 (T = 170 °C and RT = 60 min) shows a SBP equal to 329 ± 47 mL biogas g^{-1} VS, a little bit lower than test#3, highlighting once more that increasing the RT of the sludge inside the TH reactor did not have significant effects on sludge characteristics. Finally, the lowest SBP value was observed for test #1 (T = 150 °C and RT = 30 min) accounting for 305 ± 88 mL biogas g^{-1} VS. However, as shown from Fig. 2a, all the SBP values ascribed to the TH pretreatment of sludge were notably higher than that of the raw thickened sludge (226 ± 39 mL biogas g^{-1} VS). Thus, the BMP tests testify that biogas production was significantly increased due to the enhancement of the anaerobic biodegradability resulting from the TH pretreatment. Correctly, Eqs. (1) and (2) take into account also the biogas and methane production due to the inoculum sludge: as shown in Fig. 2a and b, such productions were very limited due to inoculum pre-incubation performed (see "Biochemical Methane Potential Tests"). ANOVA analysis revealed that there are only significant differences between the SBP of the raw sludge and test #2.

Concerning the SMP (Fig. 2b), results are mostly similar to those of the SBP. Test #2 reported the highest methane production achieving, after 28 days of BMP assay, a value of 237 ± 14 mL CH_4 g^{-1} VS. Further, the SMP values of tests #3, #4, and #1 are quite close to each other accounting for, respectively, 213 ± 14, 205 ± 11, and 204 ± 61 mL CH_4 g^{-1} VS. It is important to highlight that all the conditions studied yielded a considerable increase in the total methane production, compared with the raw thickened sludge (160 ± 24 mL CH_4 g^{-1} VS).

However, at 170 °C, the biogas and the methane yields were lower than, respectively, the biogas and the methane yields obtained at 150 °C. This finding is consistent with previous studies, where it is reported that the anaerobic biodegradability of sludge is species-specific and depends mainly on the organic solubilization and structure destruction [37, 38].

Figure 2 shows that in the first 2 weeks, the cumulated biogas volumes significantly increased (both in general terms and in comparison with the raw thickened sludge), testifying a significant effect of the TH pretreatment on the anaerobic digestion. From day 15 to day 24, there was a slight increase in the SBP. After 24-day digestion, there were no obvious changes in the entire gas production. This finding is shown more clearly in Fig. 3, where the daily biogas production (DBP) is reported. The DBP trend with standard deviation is reported in Supplementary Materials. The maximum DBP was observed between day 5 and day 6; after that, the daily biogas production decreased till day 11, and further, on only small biogas production peaks were observed between days 13 and 24. The first peak shows that the amount of organic substrate that can be easily digested anaerobically increased after the TH treatment [39] and the transformation of macro-molecule components into micro-molecule components, such as volatile fatty acids (VFAs).

Data reported in Fig. 3 indicates that all the performed TH pretreatments of sludge improved the early degradation steps of anaerobic digestion, i.e., the hydrolysis process, which occurred in the first period, but slightly affected the latter steps,

Table 2 Sludge characteristics before and after the BMP tests

Parameter	Before BMP tests					After BMP tests				
	Raw sludge	Test #1	Test #2	Test #3	Test #4	Raw sludge	Test #1	Test #2	Test #3	Test #4
Total COD (g TCOD L^{-1})	52.5 ± 4.9	52.6 ± 5.0	52.8 ± 4.5	53.6 ± 6.0	53.2 ± 5.6	55.9 ± 11	50.7 ± 4.5	43.9 ± 2.5	48.7 ± 1.8	42.7 ± 10.6
Soluble COD (g sCOD L^{-1})	2.7 ± 0.1	5.1 ± 0.2	5.4 ± 0.2	5.9 ± 0.3	6.4 ± 0.3	4.4 ± 0.2	2.6 ± 0.1	2.9 ± 0.1	4.0 ± 0.1	2.9 ± 0.1
Ammonium nitrogen (g NH_4^+-N L^{-1})	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	3.1 ± 0.2	2.1 ± 0.1	2.0 ± 0.1
Organic nitrogen (g N_{org}-N L^{-1})	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	1.7 ± 0.1	3.2 ± 0.2	2.9 ± 0.2	1.4 ± 0.1	2.0 ± 0.1
Soluble phosphorous (mg PO_4^{3-}-P L^{-1})	16.4 ± 1.2	16.4 ± 1.1	16.7 ± 1.2	20.0 ± 1.0	18.6 ± 1.0	23.1 ± 1.2	25.5 ± 1.3	25.3 ± 1.3	27.6 ± 1.4	23.0 ± 1.1
Total solids (TS) (%)	5.44 ± 0.8	5.34 ± 0.7	5.32 ± 0.7	5.38 ± 0.7	5.44 ± 0.8	4.55 ± 0.1	4.56 ± 0.1	4.68 ± 0.1	4.59 ± 0.3	4.41 ± 0.7
Volatile solids (VS) (%)	3.82 ± 0.8	3.71 ± 0.8	3.72 ± 0.8	3.78 ± 0.8	3.78 ± 0.8	3.01 ± 0.1	2.98 ± 0.2	3.05 ± 0.1	3.02 ± 0.1	2.89 ± 0.5
pH (−)	6.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1

the acetogenesis, and methanogenesis processes [32, 33]. Actually, this is what should be expected as the TH pretreatment of sludge enhances the hydrolysis, which is the limiting stage of the entire AD process.

Results of the SBP and SMP, diminished of the values of the blank sample, allowed an assessment of the percentage of CO_2 and CH_4 produced by each TH pretreated sludge. The percentages of CO_2 and CH_4 were in the range 35–39% and 61–65%, respectively. The highest CH_4 percentage (65%) corresponded to test#1 performed at a temperature of 150 °C and a reaction time of 30 min. Test #2 reported the same percentage of CH_4 (64%), while the lowest value (61%) was obtained with both tests #3 and #4. Since data revealed that there is no strong difference between the CH_4 percentage obtained from all the experimental tests, it is not possible to find a correlation between the operative conditions of thermal pretreatment and the methane concentration in the produced biogas. However, all the tests performed led to a percentage of CH_4 produced greater than 60%, in accordance with many literature studies [21, 40, 41]. Moreover, considering the SBP and SMP of the raw sludge and comparing those productions with those obtained by the tests with TH pretreated sludge, the increase of the CH_4 percentage due to the TH could be obtained. In accordance with data reported in Fig. 2a and b, test #2 showed the highest increase in SBP and SMP, compared with the raw sludge, leading to 24% of CH_4 overproduction followed by tests #3, #4, and #1, where the overproduction accounted for, respectively, 19%, 18%, and 17%. These results confirm that the TH pretreatment enhanced the methane production in respect to the raw thickened sludge.

Results of the present study were compared with those reported in the literature in order to assess the efficiency of the TH performed. The comparison with the literature reveals that the SBP and SMP here obtained are quite in line with those of previous studies [21, 40], while the percentage of CH_4 produced, comparing data referred to the same TH temperature and reaction time, was, in some cases, lower [41, 42].

Hydrolysis Rate Constant Evaluation

The determination of k_h constant was performed using data obtained from the BMP tests and by using Eq. (4). k_h expresses the time required by a substrate to accomplish the hydrolysis step into the anaerobic digester. The lower the $1/k_h$ value, expressed in days, the less the time required to hydrolyze the sludge. The hydrolysis rate constants for tests #1, #2, #3, and #4 resulted, respectively, 0.14, 0.17, 0.16, and 0.17 day^{-1}, while that of the raw thickened sludge was equal to 0.15 day^{-1}. This value is consistent with Shimizu et al. [43], who reported that the first-order digestion rate constant for sludge is 0.15 day^{-1}. Considering $1/k_h$, results reveal that, except for test #1 where $1/k_h$ was equal to 6.9 days (T = 150 °C and RT = 30 min), all remaining $1/k_h$ values were lower than that of the raw sludge ($1/k_h$ = 6.7 days), accounting for 6.1, 6.0, and 5.9 days, respectively, for tests #2, #3, and #4. This data confirms that TH enhanced the hydrolysis of sludge, which is the limiting factor of the anaerobic digestion process, making it fast and more effective. In particular, the best result was obtained with the test performed at 170 °C for 60 min, accounting for a reduction in the time needed for hydrolysis of 12%, followed by tests #3 and #2 with a reduction of, respectively, 9% and 10%.

Correlation Between Total Methane Production and Disintegration Degree of TH Pretreated Sludge

In this section, the sludge solubilization performance, in terms of DD of the TH pretreated sludge, is correlated with the total methane production in the subsequent mesophilic tests (Fig. 4), in order to identify the limiting factor for methane

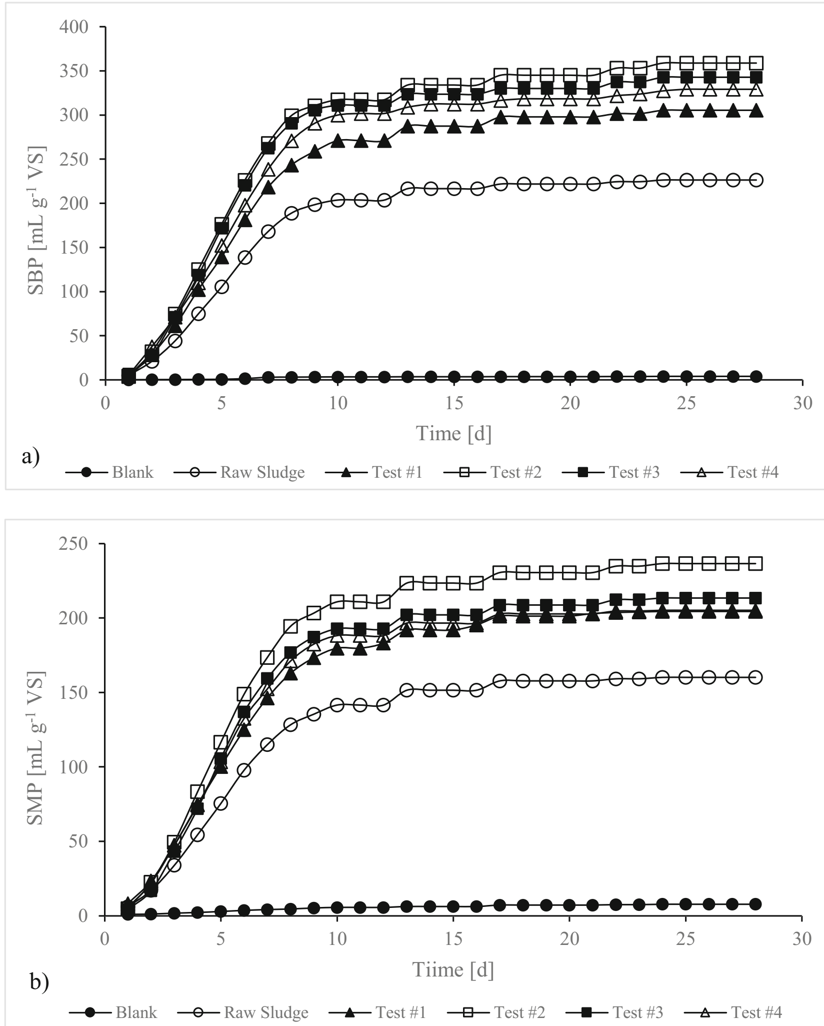

Fig. 2 **a** SBP and **b** SMP of the TH, blank (inoculum) and raw sludge

production in AD. As reported by Abelleira-Pereira et al. [44], many authors agree that the hydrolytic microorganisms' low performance is the kinetics bottleneck for methane production in AD process, while other authors reported that the soluble organics do not influence the BMP [45], but the methane production is influenced by the slow increase in methanogens [18].

Figure 4 testifies the higher solubilization of thickened sludge after TH process not always corresponded to an improvement on total methane production. The higher methane production corresponded to a 20.2% of DD, while at higher values of DD, 23.5% and 27.1%, a lower methane production was observed. Moreover, lower methane productions were associated to higher TH treatment temperature, meaning that

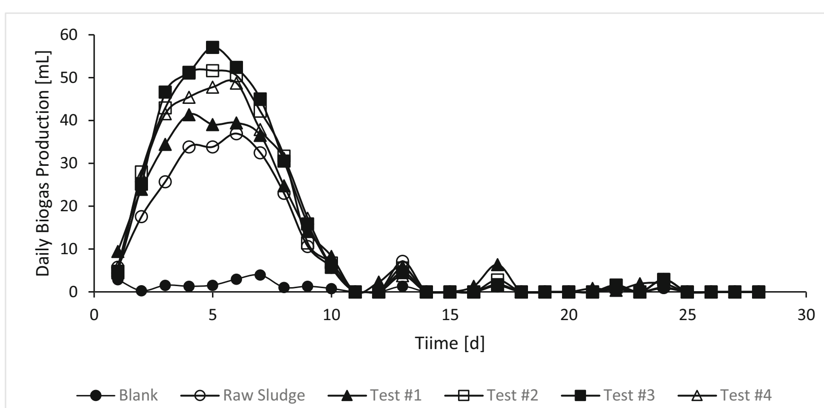

Fig. 3 Daily biogas production

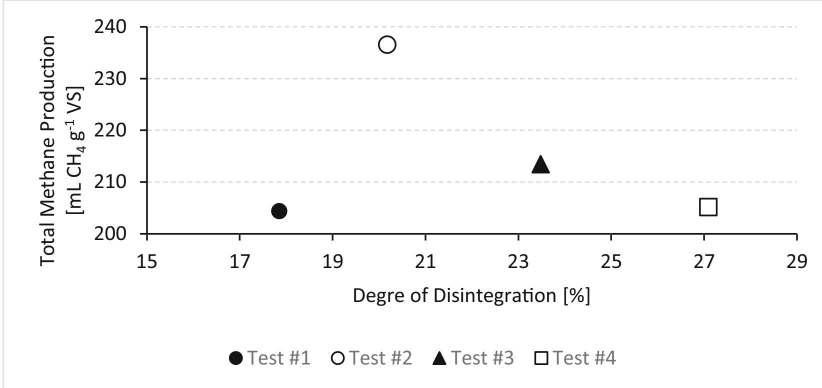

Fig. 4 Correlation between DD and total methane production

higher pretreatment severity does not always result in a higher biogas production yield, although the DD (%) increases considerably [46]. Results of the present study confirm the explanation given by Dwyer et al. [34], who reported that increasing the pretreatment intensity, a worsening of the biodegradability of the solubilized organic matter, could occur. Thus, results suggest that solubilization of organic matter is not the only responsible for the increase in methane production during AD.

Combined Effects of TH and AD on Sludge Characteristics

At the end of the BMP tests, the sludge was subjected to chemical analyses to evaluate the combined effects of TH and AD and for comparison with the raw thickened sludge, only subjected to AD. Data before and after the BMP tests are reported in Table 2.

Results revealed a TS and VS reduction for all the samples after the BMP tests. The highest TS reduction was achieved at 170 °C and reaction time of 60 min (19%) corresponding to the highest VS reduction (24%), too. The results also show that TH pretreatment of sludge at 150 °C and 60 min (test #2) contributed a little to the TS and VS reduction in the sludge, even though methane production from BMP test was the highest in this study. In test #2, the BMP tests implied a reduction of 12% in TS and 18% in VS. Moreover, results showed that after 28 days of AD, around 50% of the soluble COD was still detected in the TH-treated sludge, meaning that not all the solubilized organics were used for biogas production. The insoluble COD, on the other hand, further reduced by 6–15% after AD in tests #2, #3, and #4, in which the largest reduction was noticed for the pretreated sludge at 170 °C for 60 min.

Changes in N fraction were also measured in this study, so as to clarify the effect of TH and AD on nitrogen conversion after different TH conditions.

The organic nitrogen was mainly degraded and converted to ammonium nitrogen. As shown from data reported in Table 2, this finding is evident for tests #3 and #4, where the highest temperature was applied. On the contrary, for tests #1 and #2, the organic nitrogen increased, meaning that solubilization of organics occurred but the organic nitrogen converted to ammonium nitrogen to a limited extent. Nitrogen compounds had very high concentrations at the end of BMP tests, around 2–3 gNH_4^+-N L^{-1} and 1–3 gN_{org}-N L^{-1}: considering that pH was 8.0 after each test, performing TH at higher TS concentrations and, consequently, higher N concentrations could lead to inhibition of the anaerobic bacteria for both alkaline environment and high ammonia concentration.

Moreover, in all the tests performed, an increase in the soluble phosphorous concentration was detected, which was higher in the sludge pretreated samples, meaning that the combination of TH and AD process contributed to soluble phosphorous release.

Conclusions

The TH pretreatment of sludge positively affects the solubilization of organic matter in the sludge. For all the tests performed, TH of sludge increased the soluble COD compared with the untreated sludge. Results of the BMP tests highlight that TH also contributed to enhance the biogas and methane production. The highest cumulative biogas and methane productions were achieved when the thickened sludge was pretreated at 150 °C for 60 min, accounting for 359 ± 14 mL biogas g^{-1} VS and 237 ± 14 mL CH_4 g^{-1} VS. All the tests performed showed a CH_4 percentage ranging between 61 and 65%. Further, the evaluation of the hydrolysis rate constant confirmed that TH contributed to decreasing the time required by the sludge to accomplish the hydrolysis step into the anaerobic digester. However, the correlation between total methane production and degree of disintegration of the TH pretreated sludge highlighted that solubilization of organic matter was not the only responsible for the increase of methane production during AD. Finally, the combined effects of TH and AD gave rise to a considerable reduction in TS and VS concentrations.

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References

1. Abelleira J, Pérez-elvira SI, Sánchez-oneto J et al (2012) Advanced thermal hydrolysis of secondary sewage sludge a novel process combining thermal hydrolysis and hydrogen peroxide addition. Resour Conserv Recycl 59:52–57
2. Pilli S, Yan S, Tyagi RD, Surampalli RY (2015) Thermal pretreatment of sewage sludge to enhance anaerobic digestion: a review. Crit Rev Environ Sci Technol 45:669–702
3. Ferrentino R, Langone M, Andreottola G, Rada EC (2014) An anaerobic side-stream reactor in wastewater treatment: a review. WIT Trans Ecol Environ 191:1435–1446
4. Ferrentino R, Merzari F, Andreottola G (2019) Optimization of Fe^{2+}/H_2O_2 ratio in Fenton process to increase dewaterability and solubilisation of sludge. Environ Technol in press
5. Oliveira JV, Duarte T, Costa JC, Cavaleiro AJ, Pereira MA, Alves MM (2018) Improvement of biomethane production from sewage sludge in co-digestion with glycerol and waste frying oil, using a design of experiments. Bioenergy Res 11:763–771
6. Ding HH, Chang S, Liu Y (2017) Biological hydrolysis pretreatment on secondary sludge: enhancement of anaerobic digestion and mechanism study. Bioresour Technol 244:989–995
7. Appels L, Degrève J, Van Der Bruggen B et al (2010) Influence of low temperature thermal pre-treatment on sludge solubilisation, heavy metal release and anaerobic digestion. Bioresour Technol 101:5743–5748
8. Bouallagui H, Marouani L, Hamdi M (2010) Performances comparison between laboratory and full-scale anaerobic digesters treating a mixture of primary and waste activated sludge. Resour Conserv Recycl 55:29–33
9. Merzari F, Langone M, Andreottola G, Fiori L (2019) Methane production from process water of sewage sludge hydrothermal carbonization. A review. Valorising sludge through hydrothermal carbonization. Crit Rev Environ Sci Technol 49:947–988
10. Khanal SK, Grewell D, Sung S, van Leeuwen J(H) (2007) Ultrasound applications in wastewater sludge pretreatment: a review. Crit Rev Environ Sci Technol 37:277–313
11. Pilli S, Bhunia P, Yan S, LeBlanc RJ, Tyagi RD, Surampalli RY (2011) Ultrasonic pretreatment of sludge: a review. Ultrason Sonochem 18:1–18
12. Neumann P, Pesante S, Venegas M, Vidal G (2016) Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. Rev Environ Sci Biotechnol 15:173–211
13. Carrère H, Dumas C, Battimelli A et al (2010) Pretreatment methods to improve sludge anaerobic degradability: a review. J Hazard Mater 183:1–15
14. Barber WPF (2016) Thermal hydrolysis for sewage treatment: a critical review. Water Res 104:53–71
15. Hii K, Baroutian S, Parthasarathy R, Gapes DJ, Eshtiaghi N (2014) A review of wet air oxidation and thermal hydrolysis technologies in sludge treatment. Bioresour Technol 155:289–299
16. Odeby T, Netteland T, Solheim OE (1996) Thermal hydrolysis as a profitable way of handling sludge. Chemical Water and Wastewater Treatment IV. Springer, Berlin
17. Kepp U, Machenbach I, Weisz N, Solheim OE (2000) Enhanced stabilisation of sewage sludge through thermal hydrolysis - three years of experience with full scale plant. Water Sci Technol 42:89–96
18. Strong PJ, Mcdonald B, Gapes DJ (2011) Combined thermochemical and fermentative destruction of municipal biosolids : a comparison between thermal hydrolysis and wet oxidative pretreatment. Bioresour Technol 102:5520–5527
19. Nazari L, Yuan Z, Santoro D, Sarathy S, Ho D, Batstone D, Xu C(C), Ray MB (2017) Low-temperature thermal pre-treatment of municipal wastewater sludge: process optimization and effects on solubilization and anaerobic degradation. Water Res 113:111–123
20. Liu X, Xu Q, Wang D, Zhao J, Wu Y, Liu Y, Ni BJ, Wang Q, Zeng G, Li X, Yang Q (2018) Improved methane production from waste activated sludge by combining free ammonia with heat pretreatment: performance, mechanisms and applications. Bioresour Technol 268:230–236
21. Kim D, Lee K, Park KY (2015) Enhancement of biogas production from anaerobic digestion of waste activated sludge by hydrothermal pre-treatment. Int Biodeterior Biodegradation 101:42–46
22. Müller WR, Frommert I, Jörg R (2004) Standardized methods for anaerobic biodegradability testing. Rev Environ Sci Biotechnol 3: 141–158
23. Fiori L, Basso D, Castello D, Baratieri M (2014) Hydrothermal carbonization of biomass: design of a batch reactor and preliminary experimental results. Chem Eng Trans 37:55–60
24. Basso D, Weiss-Hortala E, Patuzzi F, Castello D, Baratieri M, Fiori L (2015) Hydrothermal carbonization of off-specification compost: a byproduct of the organic municipal solid waste treatment. Bioresour Technol 182:217–224
25. Angelidaki I, Alves M, Bolzonella D, Borzacconi L, Campos JL, Guwy AJ, Kalyuzhnyi S, Jenicek P, van Lier JB (2009) Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci Technol 59:927–934
26. Neves L, Oliveira R, Alves MM (2004) Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. Process Biochem 39:2019–2024
27. APHA, AWWA, WEF (2012) Standard methods for examination of water and waste water. 22nd Edition, American Public Health Association, Washington DC
28. APAT-CNR-IRSA (2003) Metodi analitici per le acque. Volume Primo, Roma
29. Kim DH, Jeong E, Oh SE, Shin HS (2010) Combined (alkaline+ ultrasonic) pretreatment effect on sewage sludge disintegration. Water Res 44:3093–3100
30. Overend RP, Chornet E (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos Trans R Soc Lond A 321:523–536
31. Ferreira LC, Souza TSO, Fdz-Polanco F, Pérez-Elvira SI (2014) Thermal steam explosion pretreatment to enhance anaerobic biodegradability of the solid fraction of pig manure. Bioresour Technol 152:393–398
32. Xue Y, Liu H, Chen S, Dichtl N, Dai X, Li N (2015) Effects of thermal hydrolysis on organic matter solubilization and anaerobic digestion of high solid sludge. Chem Eng J 264:174–180
33. Han Y, Zhuo Y, Peng D, Yao Q, Li H, Qu Q (2017) Influence of thermal hydrolysis pretreatment on organic transformation characteristics of high solid anaerobic digestion. Bioresour Technol 244: 836–843
34. Dwyer J, Starrenburg D, Tait S, Barr K, Batstone DJ, Lant P (2008) Decreasing activated sludge thermal hydrolysis temperature reduces product colour, without decreasing degradability. Water Res 42:4699–4709
35. Everett JG (1972) Dewatering of wastewater sludge by heat treatment. Water Pollut Control Fed 44:92–100
36. Hung-Wei L, Xiao S, Le T, Al-Omari A, Higgins M, Boardman G, Novak J, Murthy S (2014) Evaluation of solubilization characteristics of thermal hydrolysis process. Conference Proceedings in WEFTEC

37. Vlyssides AG, Karlis PK (2004) Thermal-alkaline solubilization of waste activated sludge as a pre-treatment stage for anaerobic digestion. Bioresour Technol 91:201–206
38. Li Y, Park SY, Zhu J (2011) Solid-state anaerobic digestion for methane production from organic waste. Renew Sust Energ Rev 15:821–826
39. Zhong W, Zhang Z, Luo Y, Sun S, Qiao W, Xiao M (2011) Effect of biological pretreatments in enhancing corn straw biogas production. Bioresour Technol 102:11177–11182
40. Donoso-bravo A, Pérez-elvira S, Aymerich E, Fdz-polanco F (2011) Assessment of the influence of thermal pre-treatment time on the macromolecular composition and anaerobic biodegradability of sewage sludge. Bioresour Technol 102:660–666
41. Qiao W, Yan X, Ye J, Sun Y, Wang W, Zhang Z (2011) Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. Renew Energy 36:3313–3318
42. Choi JM, Han SK, Lee CY (2018) Enhancement of methane production in anaerobic digestion of sewage sludge by thermal hydrolysis pretreatment. Bioresour Technol 259:207–213
43. Shimizu T, Kudo K, Nasu Y (1993) Anaerobic waste-activated sludge digestion- a bioconversion mechanism and kinetic model. Biotechnol Bioeng 41:1082–1091
44. Abelleira-Pereira JM, Pérez-Elvira SI, Sánchez-Oneto J, de la Cruz R, Portela JR, Nebot E (2015) Enhancement of methane production in mesophilic anaerobic digestion of secondary sewage sludge by advanced thermal hydrolysis pretreatment. Water Res 71:330–340
45. Appels L, Lauwers J, Gins G et al (2011) Parameter identification and modeling of the biochemical methane potential of waste activated sludge. Environ Sci Technol 45(9):4173–4178
46. Xu G, Chen S, Shi J, Wang S, Zhu G (2010) Combination treatment of ultrasound and ozone for improving solubilization and anaerobic biodegradability of waste activated sludge. J Hazard Mater 180: 340–346