Eco-energetic management of activated sludge derived from slaughterhouse wastewater treatment: pre-treatments for enhancing biogas production in anaerobic conditions.

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In this paper different pre-treatments (involving temperature, pressure and enzymatic processes) were applied to activated sludge from slaughterhouse wastewater treatment with the aim of improving biogas production in anaerobic processes. In order to quantify the efficiency of the above mentioned pre-treatments, the degree of hydrolysis was evaluated by removal of biodegradability parameters. In addition, the biomethane production and productivity were evaluated by Biomethane potential tests. Results showed that all the pre-treated samples obtained higher removal percentages for Total Chemical Oxygen Demant, Total Solids and Volatile Solids obtaining between 2 and 3.2 times higher values than those without pre-treatments. However, regarding removal of Total Suspended Solds (TSS) and biomethane yield (Y_{CH4}), the best results were obtained for two different pre-treatments: (i) Enzymatic pre-treatment in combination with thermal pre-treatment at 120 °C (THE) obtaining %TSSremoval = 13.5 ± 0.8 % and Y_{CH4} = 425 mL CH4/ g VS and (ii) Thermal pre-treatment by steam injection at 160 °C followed by a sudden decompression (THSD_160) with values of %TSSremoval = 9.56 ± 1.36% and Y_{CH4} = 425 mL CH4/ g VS. Both pre-treatments were considered as optimal pre-treatments of activated sludge from slaughterhouse wastewater treatment, showing twice the productivity of non-pretreated sludge sample (WT).

Introduction

In recent years, there has been an increase in industrial sewage sludge produced by wastewater treatment plants. By 2020, 13 million tonnes of dry solids of waste activated sludge (WAS) are expected to be produced in the European Union[1]. This waste shows a high organic load, including microbial aggregates with filamentous bacterial strains, organic and inorganic particles, extracellular polymeric substances (EPS) and a large amount of water. Depending on the producing industry, it can also contain toxic substances. Disposing of this sludge represents around 50% of total costs in a wastewater treatment plant. Moreover, it can have severe environmental impacts and can cause a public health hazard[2,3]. For these reasons, over the last decade different environmentally and economically sustainable WAS management technologies have been developed[4], including aerobic and anaerobic stabilisation processes[5]. In large facilities anaerobic digestion (AD) is usually preferred over its aerobic counterpart because, as well as having a low environmental impact, it supplies part of the plant's energy requirements and is a viable alternative to conventional energy production[6].

In anaerobic processes, microorganisms transform the organic compounds into carbon dioxide and methane (a valuable biofuel). This involves several different, interconnected, stages: hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis. It is known that the hydrolysis stage is the rate-limiting phase in AD because microorganisms must transform complex organic matter into a lower molecular weight bioavailable structures easy to be digested by the microbial population. However, when the degradation is insufficient, biogas production in the AD process decreases substantially .[7]

In order to improve the biogas produced by mesophilic and thermophilic AD processes of industrial WAS, different technologies such as pre-treatments have been proposed in the literature[8]. The aim of these pre-treatments is to improve sludge solubility and biodegradability, releasing the intracellular substances by rupturing the cell wall and making them more accessible to subsequent microbial actions and hence increasing biogas production through the AD process[9]. These pre-treatments include physical treatments (thermal hydrolysis, microwave, ultrasonic and/or electrokinetic disintegration, high-pressure homogenisation); chemical (acid, alkali and advanced oxidation processes) and biological (temperature phase anaerobic digestion, microbial electrolysis cell and enzymatic treatments)[3].

Thermal hydrolysis is a well-known and efficient technology used for improving the solubilisation of industrial and municipal sludge at laboratory and industrial scale[10]. This technology has been tested over a wide range of temperatures (60-180°C) and operation times (5min to several hours). Presenting several advantages[11–14], it:

(i) To significantly improve the biodegradability and solubilisation of activated and primary sludge by disintegrating biomass cells walls and membranes;
(ii) To allow significant higher loading rates resulting in smaller digestion plants;
(iii) To improve the rate of biogas production;
(iv) To disinfect sludge, providing pathogen-free biosolids and reducing odour and pathogen regrowth;
(v) To reduce sludge viscosity, thus improving its dehydration capacity by altering its structure and releasing bound water;
(vi) To eliminate scum and foaming and to produce conditions which do not encourage foaming, enhancing the sludge's sedimentation capacity;
(vii) To minimize inhibition by hydrogen sulphide
(viii) To significantly reduce the additional requirements for heating the sludge previously to other downstream processes.

If combined with other effective pre-treatments, the final effect of this kind of technology could be improved. In this sense, some industrial TH technologies such as Cambi®, Biothelys®, Exelys® and Turbotec® are implemented in combination with sudden decompression after high pressure thermal hydrolysis. This type of system involves temperature and pressure, injecting steam directly into the substrate and, after a variable residence time, the pressure is suddenly released. This thermal hydrolysis and sudden decompression (THSD) technology (also known as thermal steam explosion) generates a final significant increase in the amount of methane produced after AD. Several works in the literature reveal the sturdiness of this technology. Ferreyra et al.[15] increased the methane production up 200 % comparing to untreated samples when applying the THSD pre-treatment (170 °C, 30 min) before the anaerobic digestion of pig manure; Sapkaite et al.[16] obtained 30-41% solubilization increase and 25-72% increase in methane production testing different times (5-35 min) and heating temperature (140-170 °C) when applying THSD pre-treatment before AD of sewage sludge.

The solubilisation of organic substrates by combining some physical pre-treatments with enzymatic pre-treatments has been widely demonstrated[17,18]. However, there are no studies in the literature about the effect of the combination of TH and enzymatic treatment on slaughterhouse wastes. Enzymatic pre-treatments are also efficient pre-treatments and are mainly applied to ligno-cellulosic wastes to make their content more accessible and biodegradable[19–21]. However there are only a few studies in the bibliography concerning the use of enzymes to hydrolyse slaughterhouse wastes as a pre-treatment or directly in the AD digester as an inductor, either alone or in co-digestion [22–25]. In this sense, Müller et al. [26] and Agabo et al. [23] conclude that, in order to avoid the negative effect of endogenous hydrolytic enzymes on extracellular enzymes, a previous physical contact between enzymes and slaughterhouse wastes is needed in a pre-treatment step prior to AD.

For this reason, this work has proposed combinations of different pre-treatments: (1) TH only; (2) TH with enzymatic treatment (THE) and (3) TH with sudden decompression at different temperatures (120°C: THSD_120, 140°C: THSD_140 and 160°C: THSD_160) as options for improving AD biomethane production.

Since the popular methodology of Owen et al. [27] was published, Biomethane Potential tests (BMP test) have been used to characterise a wide variety of substrates and have become important tools for investigating possible pre- and post- digestion treatment options [27]. The main objective of this work, therefore, was to study the influence of these pre-treatments on the anaerobic digestion of slaughterhouse activated sludge (ASS) coming from a slaughterhouse wastewater treatment facility by determining the biodegradability and biomethane production using the BMP tests.

Experimental

Feedstock used

The sludge used in this work is an activated sludge resulting from the aerobic treatment of slaughterhouse wastewater in the Matadero del Sur S.A. facilities. The aerobic treatment was operated in a sequential batch reactor (SBR) with an HRT (hydraulic retention time) of 24h and a purification efficiency of 80%. Depending on the specific tasks being performed in the slaughterhouse, the wastewater influent can vary greatly over the week. The average of total chemical oxygen demand (CODt) content over a week is around 5,000 mg O_2/L, although some peaks of 10,000 mg O_2/L were detected during the sampling.

Sludge, whose composition is shown in Table 1, was concentrated by soft centrifugation (1000 x g, 5 min, 4 °C) up to 13 ± 0.5 % w/v of dry weight (DW). The total sludge dry matter was mostly insoluble (98.67 ± 0.25 % w/w), and it showed pH values of 6.5 ± 0.3.

Table 1. Sludge composition

Parameters	Composition (% w/w)
Total volatile solids	85.24 ± 0.16
Proteins	15.38 ± 1.31
Lipids	29.80 ± 0.71
Carbohydrates	40.06 ± 1.59
Total Carbon (TC)	56.95 ± 0.73
Total Nitrogen (TN)	2.46 ± 0.21

Inoculum

The mesophilic inoculum came from a laboratory digester treating sewage sludge and operating at stable conditions: 35°C and HRT = 20 days. The percentage of inoculum used in BMP tests was 40% which is considered optimum for biogas production in this kind of substrates[23]. Table 2 shows the main characteristics of the inoculum used in this study in terms of Total and Soluble Chemical Oxygen Demand (CODt, CODs,) and Total and Volatile Solids (TS and VS).

Table 2. Characterisation of the inoculum

Parameters	CODt	CODs	TS	VS
Values (g/L)	7.8	1.5	7.4	2.8

Pre-treatments essays

Table 3 shows the nomenclature given to the pre-treated and non-pre-treated samples.

Table 3. Samples nomenclature according to the pre-treatment applied to ASS.

Sample name	Description of pre-treatment
WT	**Without pre-treatment**

TH	**Autoclaved (121 °C)**
THE	**Autoclaved (121°C) + enzymatic**
THE_dil	**THE sample + distilled water (1:2)**
THSD_120	**Steam injection up to 120 °C + sudden decompression**
THSD_140	**Steam injection up to 140 °C + sudden decompression**
THSD_160	**Steam injection up to 160 °C + sudden decompression**

WT sample corresponds to the raw sample without any pre-treatment. Thermal hydrolysis (TH) was performed by placing 1 L of sludge samples in 5-L flasks in an autoclave (Raypa steam steriliser) at 121 °C, 30 min respectively. THE samples combined both autoclaving at 121 °C (TH samples) and enzymatic hydrolysis pre-treatments. Enzymatic hydrolysis was performed using 0.1% (v/w) protease (bioprotease LA-450 from Biocon Española S.A.) as the hydrolytic agent. The process took place in batch, in a 2-L Bio-bundle bioreactor operating under controlled conditions of stirring (150-rpm) and temperature (55 °C) over 180 min. The pH was also controlled (8.5) using KOH as neutralising agent. In addition, THE samples were diluted with distilled water at a ratio of 1:2 in order to obtain THE_dil samples.

Finally, thermal hydrolysis with sudden decompression (THSD) was performed in the pilot thermal hydrolysis unit described by Ferreira et al. [15] with slight operational modifications. Briefly, in a thermal hydrolysis unit consists of a 2-L hydrolysis reactor connected via a decompression valve to a 5-L vessel. Operating discontinuously, 1 L of sludge was heated in the hydrolysis reactor by injecting live steam (12 bars) from a boiler up to reaching 120, 140 and 160 °C (THSD_120, THSD_140 and THSD_160, respectively). After 30 minutes at the desired temperature, the sudden decompression valve is opened in a sudden decompression, releasing the hydrolysed sludge to the atmospheric 5-L flash tank.

Solubilisation and molecular weight of the soluble organic component of sludge were analysed both at the beginning and at the end of each pre-treatment.

The solubility reached in each treatment was determined by the difference in percentage of Total Suspended Solid between the sample without and with pre—treatment (%$TSS_{removal}$)[28]

Anaerobic digestion tests (BMP)

BMP tests were performed according to literature[27,29]. It was used 250-mL serum bottles with a working volume of 150 mL using an orbital shaker at 85 rpm under mesophilic conditions (35 ± 1 °C). The digesters were loaded with a mixture of inoculum and substrates at a final ratio of 3:2 (v:v). The substrates were the activated waste sludge, which had been pre-treated as explained in Table 3.

The control reactor, containing only anaerobic inoculum and distilled water, was also incubated in order to determine background inoculum gas production. All of the reactors were run in triplicate. Data presented in this work correspond to average values.

Prior to incubation, the pH was adjusted to 7.0 employing 2 M sodium hydroxide solution. All of the reactors were subsequently purged with 100% N_2 for 3-4 min to maintain anaerobic conditions and then sealed with rubber septum and metal caps.

Biogas production and biogas composition were determined daily during the digestion period. At the end of the said period, data on total solid (TS) volatile solid (VS), volatile fatty acids (VFA) and both total and soluble chemical oxygen demand (CODt, CODs, respectively) were determined for all of the reactors in order to calculate the efficiency of the biological treatment. Biogas production was determined indirectly by measuring the daily cumulative pressure inside the bottles using pressure transducers. Pressure data were used to determine the volume of biogas at standard temperature and pressure, according to the ideal law of gases .[29] Cumulative methane volume was calculated as the sum of the daily methane volume. Methane yield (Y_{CH4}) corresponded to the normalised net volume of methane per kg of initial VS ($mL_{CH4}/kgVS_{initial}$) was calculated as indicated in Eq. (1).

$$Y_{CH_4}\left(\frac{NmL}{kgVS_{initial}}\right)=\frac{Volume_{CH4}}{initial\ Kg\ VS}$$

Experimental biomethane potential (BMPexp) was calculated as the asymptote of the methane yield curve. Substrate biodegradability was also calculated in terms of VS removal and CODt removal after BMP.

Physicochemical characterization

The pH values from ASS samples were measured using a pH meter (Hanna HI2202-02, Hanna instruments). Carbohydrates were determined according to standard AOAC methods.[30] Lipid content was determined gravimetrically with hexane for 12 h in a Soxhlet extractor.[31] The total carbon and nitrogen contents of ASS were analysed with a Leco Elemental Analyser, model CHNS-932. The concentrations of total solids (TS), total dissolved solids (TDS), total suspended solids (TSS) and total volatile solids (TVS) were measured according to standard methods[30]. Total COD (CODt) was determined photometrically at 585 nm according to German standard methods DIN 38 409-H41-1, 1980. Soluble COD (CODs) was also determined by filtering with 0.7-μm Millipore AP-4004705. The thermoreactor used was manufactured by MERCK and the Heλios α type spectrometer was manufactured by Electron Corporation.

Chromatographic techniques

Molecular-mass distribution of the soluble organic component of sludge were determined by size-exclusion chromatography using a JASCO LC-4000 system, using a Superdex PeptideTM 10/300 GL column (optimum separation range 0.1–7 kDa). Samples were previously centrifuged at 12,000 × g for 30 min at 4 °C to remove insoluble debris and the supernatant was passed through a 0.2-μm filter and loaded into a 0.02-mL loop connected to the JASCO system. The column was equilibrated and then eluted with 0.25 M Tris-HCl buffer (pH 7.00) in isocratic mode at a flow-rate of 0.5 mL min−1. Proteins/peptides were detected at 215 nm with a JASCO UV-4075 UV/Vis detector module coupled to the column.

Volatile fatty acids (VFA) (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and heptanoic acid) were determined by gas chromatography (GC-2010 Plus, Shimadzu). For VFAs analysis, the sample was filtered through Millipore GVWP025000 0.22 μm glass fibber filters. Total acidity was calculated by adding the individual fatty acids together.[29]

Gas composition was determined employing a gas chromatography technique (GC-2010 Shimadzu). The gases analysed (H_2, CH_4, CO_2, O_2 and N_2) were measured by means of a thermal conductivity detector (TCD) at 250 °C using a Supelco Carboxen 1010 Plot column. The oven temperature was programmed between 35 and 200 °C. The carrier gas was nitrogen at a pressure of 35 kPa[29].

Results and discussion

Effect of pre-treatments

The effect of the applied pre-treatments on ASS was evaluated based on both the total sludge dry matter solubilisation and the molecular weight profile of the sludge soluble organic component. Both parameters are directly linked to the product organic matter availability for methanogenic bacteria and the molecular weight profile shows also the hydrolysis degree of the product. Figure 1 shows the effect of the different pre-treatments on sludge solubilisation as $\%TSS_{removal}$ in each pre-treatment studied.

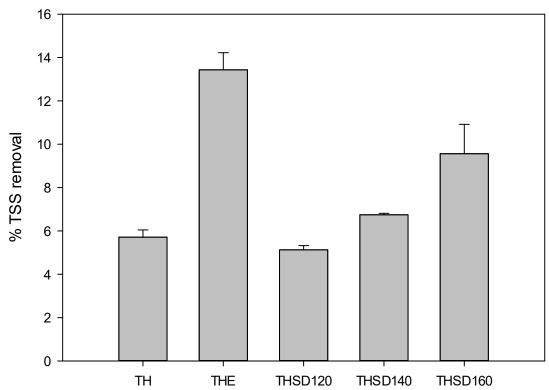
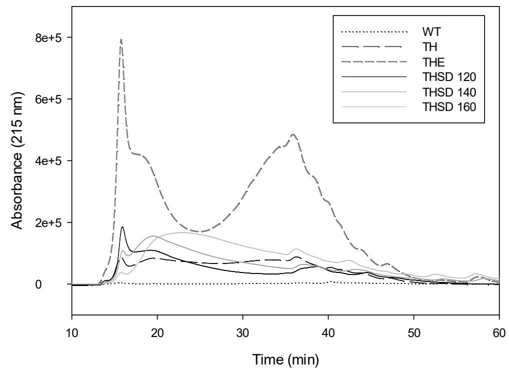


Figure 1. Removal of TSS (as %) at each experimental condition.

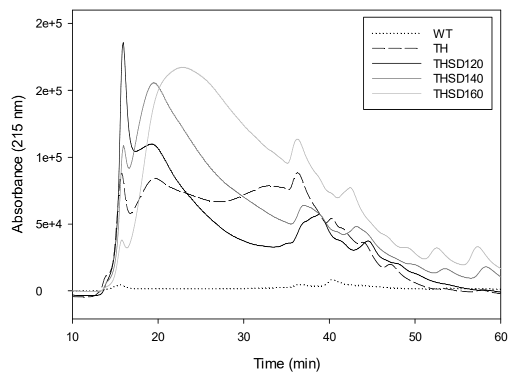
As can be seen in Figure 1, TH pre-treatment obtained $\%TSS_{removal}$ = 5.96 ± 0.01%. The combination of thermal and biological pre-treatment (THE) led to the highest $\%TSS_{removal}$ values (13.5 ± 0.8 %). THSD pre-treatments obtained 5.13 ± 0.20 %, 6.75 ± 0.07% and 9.56 ± 1.36 %, for 120°C, 140°C and 160°C tests, respectively. These results were lower than %TSS removal obtained by using other reported pre-treatments in sewage sludge such as: hydro-thermal at ≥ 200°C [32] and chemical pre-treatments ($\%TSS_{removal}$ = 30-55%)[33]. Therefore, the $\%TSS_{removal}$ results in each pre-treatment depend highly upon different parameters such as the operation time, operation temperature and the origin of the sample. [32]

The effect of the different pre-treatments on the dry matter solubility is also reflected in the molecular exclusion HPLC analysis. The molecular weight profiles of the samples soluble organic components at each condition (Figure 2) are shown together with the molecular weights distributions (Table 4). Due to the low soluble organic content of non-pre-treated ASS, the molecular weight profile of the WT sample was practically flat (Figure 2, Table 4). In terms of both solubility and bioavailability, pre-treated samples, however, showed important changes in the chromatographic profile. According to solubility results, THE showed the best molecular weight profile with the highest conversion of high molecular weight molecules into low ones (48.4 % for <1 KDa sizes molecules, Table 4). This implies a high bioavailability degree of the product for methanogenic bacteria.

On the other hand, although the two pre-treatments performed at 120°C temperature condition, reached similar solubility rates (as it can be seen in Figure 1), the TH product had a more interesting profile in terms of bioavailability, since it achieved 45.7 % conversion of soluble content into <1 KDa-size molecules, compared to the 37.8 % achieved by the THSD_120 treatment, (Table 4).



(a)



(b)

Figure 2. Molecular exclusion HPLC chromatogram (215 nm) representing the molecular weight profile of the soluble organic components of ASS after each treatment. (a) all pre-treatments; (b) enlarged image of non-enzymatic treatments.

Regarding THSD pre-treatments, the operating temperatures had effects on both the solubility and the molecular profile (Figures 1 and 2 and Table 4). Increasing temperatures led to a greater conversion of high molecular weight molecules (> 5 KDa) into low molecular weight ones. Compared with THSD_120, THSD_140 slightly reduces the fraction above 5 KDa (2.83 %), increasing the fraction between 1 and 5 KDa (3.56 %), and maintaining an almost similar fraction under 1 KDa. However, THSD_160 considerably decreased the fraction above 5 KDa (14.3 %), increasing the fraction between 1 and 5 KDa by 7.73 %, and also increasing the fraction under 1 KDa by 6.55 %, showing a molecular weight distribution pattern similar to the TH treatment (Table 4).

In conclusion, THE was the best pre-treatment applied for hydrolysing the samples, leading to a highly soluble and bioavailable product, while THSD_120 was the pre-treatment that had the least effect on the product. Among the non-enzymatic pre-treatments, THSD_160 achieved the highest solubilisation rates, with an interesting molecular size profile due to the high content in <1 KDa size molecules, very similar to that shown by the TH treatment. So finally, therefore, although higher-temperature pre-treatments achieved higher solubilisation rates, TH was the treatment that achieved best results at lower temperatures (120°C).

Table 4. Molecular weight distribution of the soluble organic fraction of ASS after applying the different treatments (ND: Not detected).

Molecular weight (KDa)	**WT (%)**	**TH (%)**	**THE (%)**	**THSD_120 (%)**	**THSD_140 (%)**	**THSD_160 (%)**
> 5	**ND**	**37.8**	**38.4**	**50.8**	**48.0**	**36.5**
3 - 5	**ND**	**5.09**	**3.28**	**4.19**	**5.33**	**6.44**
1 - 3	**ND**	**11.6**	**9.93**	**7.25**	**9.67**	**12.7**
< 1	**ND**	**45.7**	**48.4**	**37.8**	**37.0**	**44.33**
0.3 - 1	**ND**	**13.9**	**17.0**	**6.94**	**8.32**	**11.4**
< 0.3	**ND**	**31.7**	**31.5**	**30.8**	**28.7**	**33.0**

BMP tests results

The biodegradability parameters of pre-treated samples were determined according to the BMP tests procedure[27,29]. In these essays, the daily generation of biomethane was registered throughout the whole experiment, as well as during the final removal of organic matter expressed as COD and TS. Table 5 collects the data of the removal percentage in each sample in terms of COD, CODs, TS and VS after anaerobic digestion treatment. All the deviations of the parameters were between 1 and 5%, corresponding to experimental errors.

Table 5. Removal of total and soluble COD (CODt, CODs) and total and volatile solids (TS, VS).

Sample	**REMOVAL (%)**			
	CODt	**CODs**	**TS**	**VS**
WT	**20.4**	**30.1**	**21.7**	**31.0**
TH	**60.9**	**77.9**	**45.1**	**59.0**
THE	**65.4**	**79.2**	**50.5**	**62.3**
THE_dil	**63.8**	**77.5**	**47.0**	**57.4**
THSD_120	**63.2**	**71.0**	**38.6**	**53.3**
THSD_140	**67.0**	**78.5**	**41.5**	**57.1**
THSD_160	**69.4**	**77.9**	**46.5**	**61.4**

As it can be observed, there was a slight degradation of the main parameters in the case of non-samples ($\%CODt_{removal}$ = 20.4; $\%CODs_{removal}$ = 30.1%; $\%TS_{removal}$ = 21.7 and $\%VS_{removal}$ = 31%). However, all of the pre-treatments applied increase the removal of total and soluble organic matter in comparison with the WT sample.

Figure 3 shows the biomethane yield in terms of the volatile solids in all BMP tests, enlarging the data results for the first 14 days. As it can be observed in the enlarged image, the maximum productivity of non-pre-treated samples reached 215 ml CH4/ gVS in 6 days. However, the productivity values of the pre-treated samples were, in all cases, higher than in the untreated one. The behaviour of biomethane yield will be compared by groups and in general in the following discussion.

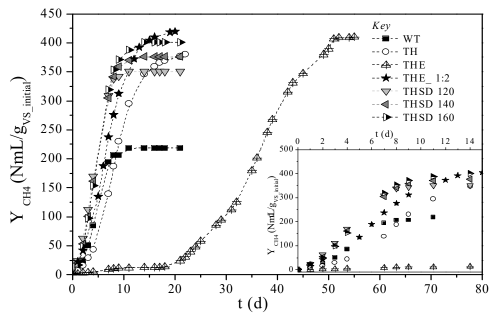


Figure 3. Biomethane yield (as mlCH4/gVSinitial) during experimental time in each test.

BMP tests of TH and THE

TH obtained $\%CODt_{removal}$ = 60.9% and $\%CODs_{removal}$ = 77.9%, corresponding to 2.98 and 2.58 times higher than WT. On the other hand, when TH was used, $\%TS_{removal}$ and $\%VS_{removal}$ only increased by a factor of 2.07 and 1.90, respectively. THE was the best pre-treatment for solids removal with 50.5% and 62.3% for $\%TS_{removal}$ and $\%VS_{removal}$ respectively, being 2.32 and 2.01 times higher than in WT. $\%CODt_{removal}$ and $\%CODs_{removal}$ were 65.4% and 79.2% respectively, being 3.20 and 2.63 times higher than WT. Comparing both pre-treatments, the THE treatment increase slightly the solubility in terms of these studied parameters in 1-5%.

With regard to biomethane yield, 375 ml $CH4/gSV_{initial}$ in TH was obtained against 425 ml CH4/g $SV_{initial}$ when THE was the pre-treatment applied. Both results imply an increment in biomethane yields of 1.74 and 1.97 times for TH and THE, respectively, in comparison with the non-pre-treated sample. These results are in the same range as other data reported in the literature for AD of thermal hydrolysis and enzymatic pre-treated sludges (Table 6).

TH pre-treatment has been previously tested in other kind of sludges in the literature. Choi et al.,[34] used TH for treating sewage sludge obtaining an increase of 1.3 in Y_{CH4} at temperatures 200 °C and reaction time of 30 min. Mirmasoumi et al.[35] obtained Y_{CH4} increase of 1.5 by TH at 90 °C during 30 min. Zhang et al.[36] studied the AD of dewatered sludge after pre-treatment for 30 min in a hyperbaric environment (160 °C, 0.55 MPa). Results obtained only 33% of CODt removal and an improve of 1.21 of Y_{CH4}. These reduced results can be due to the low content of VS (50% w/w) in the sludge which is low compared to European sludges[36]. Similar reduced results were obtained for saline sludge TH pre-treatment at 120° during 3h.[37]

THE pre-treatments can be also compared with other enzymatic pre-treatments reported in the literature (Table 6). Yu et al. [38] used and endogenous protease isolated from *Aeromonas hydrophila* and used during 7h in Sewage sludge obtaining reduced values of removal parameters and biomethane yield in comparison with this work. However, Agabo et al.[23] added the same protease in 0.3% (v/v) directly in the digester as enhancers of AD of SS, obtaining only an increase of 1.71 in Y_{CH4}. But when the protease was used as endogenous protease during a previous step of fermentation, the increase in Y_{CH4} was 3.89 times, but the removal of VS was only 25%.

Table 6. BMP results after thermal hydrolysis and enzymatic treatments of different sludges in the bibliography

Sample	Pre-treatment	Removal(%) CODt; VS	Y_{CH4}* increase (times higher)	Reference
ASS	TH	70.6; 59.0	1.74	This work
SS	TH	61.2; NM	1.3	34
SS	TH	NM; 38.1	1.5	35
DMS	TH	33; NM	1.21	36
Saline WAS	TH	NM; 32.7	1.29	37
ASS	THE	65.4; 62.3	1.97	This work
WAS	Enzymatic	11.1; NM	1.18	38
SS	Enzymatic	NM; 29.0	1.71	23
SS	Enzymatic	NM; 25.0	3.89	23

ASS: activated slaughterhouse sludge;SS: sewage sludge; DMS: dewatered municipal sludge; WAS: waste activated sludge; TH: Thermal Hydrolysis; THE: thermal and enzymatic hydrolysis;NM: Not measured Y_{CH4}: Biomethane productivity in base of TVS*

BMP tests of THE and THE_dil

Overloading a digester may disturb the digestion of waste if such waste is quickly hydrolysed and acidified, creating an over-accumulation of VFAs. During AD an excess of VFAs usually inhibits the methanogenesis phase[39]. In addition, ASS is commonly lipid-rich, so it is probably the long-chain fatty acids hydrolysis that limits the overall process[11] leading to the appearing of stages of latency observed in Figure 3. This can be explained by the high content of organic matter dissolved in the medium after THE pre-treatment, can cause shifts in microbial populations. Hence, methane yields returning to normal levels only occurs when the populations develop a tolerance to higher loading rates[40].

It is well-known that when there is an excess of organic matter, the population involved in anaerobic digestion cannot address the overall degradation. In THE experiment it was observed a long lag phase in biogas generation (20 days), which was probably due to an excess of highly bioavailable soluble organic matter (including VFAs). In order to avoid this problem and study how affect the initial organic matter concentration on the AD process, a new experiment with a 1:2 dilution of THE sample was developed.

THE_dil results show that, in spite of diluting the total organic matter from 22.4 g/L to 11.1g/L, the removal percentage of the main biodegradability parameters did not reduce correspondingly. Indeed, similar removal parameters for COD and solids: $\%CODt_{removal}$ = 63.8%; $\%CODs_{removal}$ = 77.5%; $\%TS_{removal}$ = 47.0% and $\%VS_{removal}$ = 57.4% were obtained. Results show that despite similar results in the removal of biodegradability parameters (Table 5), there was a great difference in BMP time when the pre-treated substrate was diluted (THE_dil). As it can be observed in Tables 5 and 6, THE treatment is the most efficient for increasing soluble organic matter content, but the initial CODt was very high (32.1 gCODt/L). THE and THE_dil samples reached the maximum biomethane yield (415 ml CH4/ g VS) after 50 days whereas the sample THE_dil required only 20 days. These results are in concordance with the hypothesis of some authors stating that after an initial instance of overloading, the digestion performance is improved due to an increased diversification of methanogenic microorganisms that create resistance to high organic load. [41]

BMP tests of THDS_120, THSD_140 and THSD_160

In the case of THSD treatments, the organic matter removals parameters increased with the temperature. In this sense, the maximum removal percentages were obtained when the THSD pre-treatment was operated at 160°C. At this condition, the removal percentages were: $\%CODt_{removal}$ = 69.4%; $\%CODs_{removal}$ = 77.9%; $\%TS_{removal}$ = 46.5%; $\%VS_{removal}$ = 61.4%. These results imply removal percentages that are 3.40, 2.58, 2.14, and 1.98 times respectively higher than the WT.

The biomethane productivity values obtained were 350 ml CH4/gVS for THSD_120; 375 ml for THSD_140 and 400 ml CH4/gVS for THSD_160. These imply an improvement factor in biomethane yield of 1.62, 1.74 and 1.86, respectively, which are in the range (1.4-2.6 times) found in the literature[11,13,16,17]. In general, therefore, a slight increase of biomethane yield was observed when the temperature was increased, the best result was obtained when the sample was pre-treated at 160°C. This is in agreement with the literature, where the optimal operating conditions for improving AD by TH were at the range of 160-180 °C[39]. In fact, temperatures above 170-190 °C can provoke an opposite effect, mainly due to Maillard reactions that result in the formation of barely biodegradable compounds called melanoidins[42]. In addition, Ortega-Martínez et al. reported that not only the temperature was important, but also the time. In this sense, the recommended pre-treatment times were 30-60 minutes[43].

In addition, it is important to remark that all of the above needed 10-15 days to reach maximum biomethane yield levels.

BMP test general results

All of the proposed pre-treatments achieved high solubilisation and hydrolysis rates, which resulted in higher biogas production in comparison with untreated slaughterhouse sludge (WT). In order of decreasing solubilisation rates: THE (13.2%) > THSD_160 (9.87%) > THSD_140 (8.07%) > TH (6.6%) > THSD_120 (6.25%). On average, removal values of characterisation parameters were: $\%CODt_{removal}$ = 65.0% ± 2.74; $\%TS_{removal}$ = 44.9 % ± 3.86 and $\%TVS_{removal}$ = 58.4 % ± 2.98 for all the pre-treatments in comparison with $\%CODt_{removal}$ = 20.4 %; $\%TS_{removal}$ = 21.7 % and $\%TVS_{removal}$ = 31.0 % for un-treated sample. In this sense, pre-treatments achieved 2-3.5 times greater removal percentages of main biodegradability parameters after BMP tests.

In general, when using THSD as pre-treatments, there was a slight increase in the biomethane yield when the temperature increased. THE and THSD_160 were the two pre-treatments that achieved the highest solubilisation rates (13.4 ± 0.79 % and 9.56 ± 1.36 % $TSS_{removal}$ respectively), and together with TH they achieved the highest hydrolysis rates, resulting in highly bioavailable products constituted by 48.4 %, 45.7 % and 44.3 % of molecules with a size of <1 KDa, respectively. These two pre-treatments (THE and THSD_160) obtained the highest biogas productivity with final values of around 415 mL CH4/ gVS – twice the methane production of WT sample. Organic overloading in THE sample (32.1 gCODt/L) caused a 30-day lag phase, this being the time needed by microorganisms in order to adapt after the hydrolysis stage. In order to reduce this time, THE was diluted by 50% (THE_dil).

It is important to remark that both treatments THSD_140 and TH reached similar methane production rates (350 - 355 ml CH4/gVS), but the former requires less time – around 7 days – whereas the latter requires 15 days, more than double the time.

Conclusions

In summary, Slaughterhouse factories can have an additional added-value as bio-refineries if the slaughterhouse residues (such as sludge) are used as feedstock in anaerobic digestion generating high volumes of biomethane. For this purpose, it is normally proposed a TH pre-treatment not only due to that this method increases the solubility of the sludge and hence generates higher biomethane production but also this pre-treatment can serve as a method of sanitation previously to treatment of slaughterhouse by-product treatment according to European Comision.[44] However, in this study, for increasing biogas generation in AD of ASS, it is proposed a combination of thermal with other methods such as sudden decompression at 160°C or enzymatic pre-treatments. By these both methods, the biomethane production is increased in 9-12% in comparison with only TH method as a consequence of a slightly higher solubilisation of feedstock (1-10%). These results could result in significant economic saving and environmental emission reductions at industrial scale. In this sense, new studies using these pre-treatments at industrial scale must be developed in order to compare not only the industrial efficiency of biomethane production but also the economic and environmental aspects of each applied proposed pre-treatment.

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