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Research review paper

Pre-treatments to enhance the biodegradability of waste activated sludge: Elucidating the rate limiting step

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A B S T R A C T

Pre-treatments for waste activated sludge (WAS) are, in most cases, an attempt to increase the biodegradation and/or improve hydrolysis rate of WAS after anaerobic digestion. This review presents an extensive analysis of WAS pre-treatments effectiveness focusing on increasing the biodegradability. In the first part of the review, WAS is considered as a cluster of organic components: proteins, carbohydrates, humic substances and cells. Based on this breakdown into components, the effect of different pre-treatments on each component (and in combination) is described. Also, possible reasons for the contradictory results frequently found among different studies dealing with the same pre-treatment are included. In the second part, the review describes the effects on volatile solids removal by digestion after pre-treatment and on the dewaterability of the final digestate. The energy balance and potential limiting factors for each pre-treatment are also taken into account. From the published works it is concluded that some pre-treatment techniques, such as thermal hydrolysis, thermal phased anaerobic digestion and low-temperature pre-treatment are effective ways to increase energy production and to improve other sludge properties, such as dewatering. However, these techniques are very energy intensive and require a large capital outlay, so research on milder pre-treatment techniques is valuable.

1. Introduction

Waste activated sludge (WAS) and primary sludge are the main by-products from conventional activated sludge treatment and their disposal is relevant because it causes around 50% of the total operational costs of a sewage treatment plant (Kroiss, 2004; Pilli et al., 2011). Methods like anaerobic digestion (AD) are used to reduce the costs associated with sludge disposal. AD is a widely used sludge treatment method. Its advantages include low energy input, methane production and reduction of sludge quantity. However, its application is often limited by low biodegradability and high retention times, because the complexity of WAS limits the efficacy of the biological process (Ruffino et al., 2016). For instance, in WAS originating from biological nutrient removal wastewater treatment plants (WWTP), only about 30–35% of the organic solids are anaerobically biodegraded in a mesophilic reactor at 20–25 days solids retention time (SRT) (Hiraoka et al., 1985; Ruffino et al., 2015; Valo et al., 2004). To overcome these drawbacks, a substantial amount of research has been done with the aim of increasing both the rate and extent of WAS bioconversion. As a result, several conditioning techniques or *pre-treatments* of sludge prior to AD have been tested in bench, pilot, and full scale. These methods have been applied mostly to WAS, as primary sludge is easier to biodegrade (Kuglarz et al., 2013; Müller, 2001).

The effects of each pre-treatment method depend on its inherent mechanisms. For instance, Salsabil et al. (2010) postulated that the degree of sludge solubilization depends upon the kind of treatment (mechanical, oxidative, thermal) rather than upon the specific energy input. Similarly, Cella et al. (2015) concluded that the innate characteristics of the pre-treatment method are likely more important than energy input. The objectives of this review paper are a) to identify the effects of different pre-treatment methods on the proteins, carbohydrates, humic substances and cells contained in the WAS; b) to assess and compare the biological degradability (hydrolysis rate, biogas production and removal of organic matter), dewaterability of the digestate

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Abbreviation: AD, anaerobic digestion; AOP, advanced oxidation process; BMP, biochemical methane potential; CER, cation exchange resin; CHP, combined heat power; COD, chemical oxygen demand; DNA, deoxyribonucleic acid; EPS, extracellular polymeric substances; E_s, specific energy; LCA, life cycle assessment; n/a, not available; n/d, not determined; n/f, not found; PS, primary sludge; SRT, solids retention time; TPAD, thermal phased anaerobic digestion; TS, total solids; US, ultrasonic; VFA, volatile fatty acids; VS, volatile solids; VSS, volatile suspended solids; WAS, waste activated sludge; WWTP, wastewater treatment plant

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Table 1
Literature review of WAS composition. Values as %VS (unless otherwise stated).

Bacterial Biomass	Protein	Carbohydrate	Humic substances	DNA	Uronic acids	Wastewater source	Type of treatment	Determination method for bacterial biomass; exopolymeric substances (EPS) and humic substances	Reference
15%*	25*	7*	16*	< 1*		Municipal	n/a This sample was a biofilm	Polysaccharide: anthrone method (glucose as standard) Uronic acids: m-hydroxydiphenil sulfuric acid method (D-glucuronic as standard) DNA: 4′,6′-diamidino-2-phenylindole Protein: Lowry method (corrected for humic substances) Humic substances: modified Lowry methods Cell number: epifluorescence microscopy	(Jahn and Nielsen, 1998)
11–24	76–89				n/d	n/a	n/a	Bacterial biomass: acridine orange staining and confocal laser scanning microscopy Other components: subtraction of cell volume from total volume	(Zhang and Fang, 2001)
n/d	"Most abundant fraction"	"Similar as humic substances"	15–20	n/d	n/d	n/a	n/a	cation exchange resin (CER) (Dowex®)	(Frølund et al., 1995)
10–15	46–52	17	18–23	n/d	n/d	n/a	Biological and chemical nutrient removal	CER (Dowex®); 4′,6′-diamidino-2-phenylindole; staining and acridine orange	(Frølund et al., 1996)
9–14*	n/d	n/d	n/d	n/d	n/d	n/a	Biological nutrient removal	Acridine orange staining	(Münch and Pollard, 1997)
n/d	n/d	n/d	17 (of TOC)	n/d	n/d	Domestic	n/a	Sodium hydroxide and sulphuric acid	(Riffaldi, 1982)
n/d	46.1	13.5	7.7	n/d	n/d	10 sources	n/a	CER (Dowex®)	(Mikkelsen and Keiding, 2002)
n/d	50	16–19	16–19	n/d	1	Domestic	Biological and chemical nutrient removal	CER (Dowex®)	(Nielsen et al., 1996)
n/d	62.4	10.8	26.4	0.4	n/d	n/a	n/a	CER (Dowex®)	(Schmid et al., 2003)
n/d	59	15.9	24.9	0.2	n/d				
n/d	53.4	17.8	28.6	0.2	n/d				
14.5*	n/d	n/d	n/d	n/d	n/d	Municipal	–	SYBR® green staining	(Foladori et al., 2010)
n/d	27.1	10.4	24.4	2	n/d	Domestic and industrial	Nutrient removal	CER (Dowex®)	(Wilén et al., 2003)
n/d	48.4	8.8	26.2	1.6	n/d	Domestic	Secondary		
n/d	32.2	11.3	16.1	1.9	n/d	Domestic	Nutrient removal		
n/d	37.8	11.6	16.4	3.5		Domestic	Nutrient removal		
n/d	31.5	8.6	15.6	2.1		Domestic	Nutrient removal		
n/d	27.1 (as VSS)	10.4 (as VSS)	24.4 (as VSS)	n/d		50% domestic, 50% industrial	C, N and P removal	CER (Dowex®)	(Jin et al., 2004)
n/d	48.4 (as VSS)	8.8 (as VSS)	26.2 (as VSS)	n/d		Mainly domestic	Carbon removal		
n/d	31.8 (as VSS)	11.1 (as VSS)	15.9 (as VSS)	n/d		Mainly domestic	Carbon and nitrogen removal		
n/d	38.2 (as VSS)	11.8 (as VSS)	16.6 (as VSS)	n/d		Mainly domestic	C, N and P removal		
n/d	31.5 (as VSS)	8.6 (as VSS)	15.6 (as VSS)	n/d		Mainly domestic	C, N and P removal		
n/d	40.0 (as VSS)	7.6 (as VSS)	8.7 (as VSS)	n/d		Oil refinery	Carbon removal		
n/d	32.4 (as VSS)	9.3 (as VSS)	31.0 (as VSS)	n/d		Leachate	Carbon and nitrogen removal		

n/a: not available.
n/d: not determined.
* Based on COD.

and energy consumption between the pre-treatments. The paper considers only the effects of individual pre-treatments (combinations of pre-treatments are excluded). It starts with an introduction of how anaerobic biodegradability and biodegradation should be defined and gives an overview of the composition of WAS. Then, several pre-treatment methods are discussed based on their underlying mechanisms; the effects on the main WAS components and their strengths and limiting factors. Finally, a discussion and conclusion with an overview of present knowledge gaps is presented.

1.1. *Anaerobic biodegradability and biodegradation*

Chemical oxygen demand (COD) solubilization is commonly used as a parameter to evaluate the effectiveness of pre-treatment (Neumann et al., 2016). Indeed, applying certain pre-treatment conditions, some studies have documented that there is a relationship between COD solubilization and biodegradation (Bougrier et al., 2008; Uma Rani et al., 2012). However, in other cases, higher COD solubilization did not result in a higher biogas production, but sometimes in the same or even lower biogas production (Dhar et al., 2012; Haug et al., 1978; D.-H. Kim et al., 2013; J. Kim et al., 2013b; Nazari et al., 2016; Sapkaite et al., 2017). Since COD solubilization is apparently not an accurate parameter to predict the effectiveness of subsequent biogas production (Sapkaite et al., 2017), until now, batch or (semi)continuous, anaerobic digestion methods are the only useful and accurate tools to assess the effect of pre-treatments on the anaerobic biodegradability of WAS. In this sense, biodegradability is a characteristic of sludge, and in anaerobic digestion processes it is composed of hydrolysis rate and biodegradation extent. Biodegradation is determined via biomethane potential (BMP) test, which in turn is determined by cumulative methane production. This methane production depends, amongst other variables, on activity of the inoculum, temperature, sludge retention time, inoculum-substrate ratio, BMP and hydrolysis rate (Angelidaki et al., 2009; Chen and Hashimoto, 1980; Holliger et al., 2016; Wang et al., 2013). Many papers, however, use different digestion times, which make comparison of data difficult; others report biogas or methane production without disclosing COD or volatile solids (VS) of WAS, making the calculation of biodegradability impossible. As a consequence, in this paper, we mostly use biodegradation to assess the effectiveness of pre-treatments.

A standard method for BMP determination is published in Holliger et al. (2016): research would benefit from adapting to this methodology in order to be able to better compare data. In addition, by only giving information regarding biodegradation, it is not clear if a pre-treatment only has an effect on the BMP, or also on the hydrolysis rate constant, or on both. To be able to accurately perform inter-study comparison, BMP and hydrolysis rate should be both reported (instead of only showing the multi-interpretable change in biogas production).

As mentioned, because many papers do not mention BMP nor the hydrolysis rate, the biodegradation (B_0), shown in Eq. (1), will be used to assess the effectiveness of pre-treatments in this review. This value is presented as "absolute biodegradation" and it is calculated based on the given data from the different studies reviewed. B_0 is the actual methane production of a sludge sample divided by the maximum theoretical conversion of COD to methane (0.35 L CH_4/gCOD, at standard temperature and pressure) (D.-H. Kim et al., 2013; Mottet et al., 2009). This parameter, however, does not consider what is needed for bacterial cell growth, their maintenance, nor the COD reduction due to the presence of other electron acceptors. The contribution of growth and maintenance to COD removal has been reported to be 5–10% of organic material degraded (Angelidaki and Sanders, 2004). This means that an underestimation of the biodegraded part occurs because not all biodegraded COD is transformed into methane. Whenever possible, in this manuscript the apparent first order hydrolysis rate (k_{hyd}) and volatile solids (VS) reduction are also used along with B_0 to assess the effects of pre-treatments on anaerobic biodegradability of WAS.

$$B_0(\%) = 100 \times \frac{Measured\ methane\ production\ \left(NmL\frac{CH_4}{gCOD_{added}}\right)}{350\ mL\frac{CH_4}{gCOD_{added}}} \tag{1}$$

1.2. *Composition of waste activated sludge*

To comprehend the effects of the different pre-treatments on the anaerobic biodegradability of WAS, its composition should be known. Table 1 shows the results of a collection of studies that focused on the composition of WAS in terms of viable and nonviable cells, proteins, carbohydrates, humic acids and DNA. Despite a myriad of studies that provide WAS characterization in terms of COD, proteins and carbohydrates, only a few show the concentration of cells, active biomass and humic substances. From Table 1, a remarkable variation of the WAS composition is observed. For instance, in terms of %VS, it is composed of 10–24% bacterial biomass; 7–19% carbohydrates; 25–62.4% proteins; 7.7–28.6% humic substances; and $<$ 3.5% DNA. It is important to note that the method used to determine each component influences the results (Bourven et al., 2012; Comte et al., 2006; Wilén et al., 2003). Nonetheless, from Table 1, it is concluded that WAS is composed mostly of proteins and humic substances with some bacterial biomass and carbohydrates (listed in decreasing order of %VS).

Proteins, DNA and carbohydrates are anaerobically biodegradable compounds. However, when they are combined into an organized structure like WAS, their biodegradability apparently decreases (Stuckey and McCarty, 1984). Cells are difficult to break down under anaerobic digestion, showing their recalcitrant nature (Foladori et al., 2015; Wett et al., 2010). Similarly, the presence of humic substances is challenging for anaerobic digestion as they affect enzymatic activity by immobilizing enzymes, which, consequently lowers biodegradability (Azman et al., 2015a, 2015b; Fernandes et al., 2015; Frølund et al., 1995; Wetzel, 1991). In addition, humic substances themselves are difficult or impossible to degrade anaerobically (Nielsen et al., 1997; Pinnekamp, 1989; Tian et al., 2015a; Zahmatkesh et al., 2016). Since humic substances account for 10–20% of the COD of WAS, the maximum attainable anaerobic biodegradation of WAS cannot exceed 80–90%. Therefore, an assessment of a pre-treatment method must consider that obtaining a 100% degradation is difficult unless oxidative methods are incorporated.

2. Pre-treatment methods

In the following sections, several sludge pre-treatment methods will be discussed. First a process description of the pre-treatment will be given, whereupon the effect of the pre-treatment on the different individual WAS components will be discussed.

2.1. *Thermal pre-treatment < 100 °C*

2.1.1. *Process description and mode of action*

Mild-thermal or low-temperature thermal pre-treatment consists of the application of a temperature in the range of 55-100 °C from minutes to several hours. It is differenciated from thermal phased anaerobic digestion (TPAD) in which longer times (in the range of 1–3 days are applied). TPAD is analized in the following section. Low-quality heat (e.g. waste heat) can be applied through heat exchangers. The operational parameters are temperature and application time. Its main effect is the disintegration of cell membranes and a concomitant solubilization of organic compounds (Nazari et al., 2016).

2.1.2. *Effects on WAS*

2.1.2.1. Floc and cell disruption. Reduction in particle size (i.e. deflocculation) has been observed in the range of 50-95 °C (Laurent et al., 2009a; Prorot et al., 2011), which, in turn, reduces sludge dewaterability (Pérez-Elvira et al., 2010), but could increase hydrolysis rate because of the increased surface area (Vavilin et al., 2008). Regarding cell disruption, according to Forster et al. (2002) > 95% of the bacterial biomass in WAS consists of Gram-negative bacteria. Gram-negative bacteria are more sensitive to heat than Gram-positive (Salton and Horne, 1951). Prorot et al. (2011) observed that lysis of a portion of the cells already occurred at 50 °C and treatment times of 20 min, and also that cell lysis increased proportionally to temperature up to 95 °C. Salton and Horne (1951) also visually determined that cell disruption occurred from 70 to 100 °C and 5 min application times, and that different bacterial strains were disrupted at different temperatures. Thus, during low-temperature thermal pre-treatment, the cell wall of at least a fraction of the bacterial biomass is ruptured and the internals of the bacteria should become available for digestion. As a consequence, a (relatively small) increase in WAS biodegradability is expected.

2.1.2.2. Carbohydrates, proteins and humic substances. The effect of low-temperature thermal pre-treatment on carbohydrates is an increase in solubilization, as unpuobserved by Dong et al. (2015) at 60 °C, and by Appels et al. (2010) at 80 °C. The solubilization of carbohydrates could increase the biodegradation rate during anaerobic digestion, but probably not the biodegradability of WAS, because carbohydrates are usually biodegraded to a large extent by anaerobic microorganisms. An exception are extracellular polymeric substances (EPS) of aerobic granular sludge (Guo et al., 2018, unpublished results), which are also present in WAS.

Protein denaturation typically occurs above temperatures of 75 °C (De Graaf, 2000). Denaturation makes proteins more prone to biodegradation (Anson, 1938), so an increase in biodegradation rate would be expected at pre-treatment temperatures above 75 °C. The solubilization of proteins is higher than that of carbohydrates (Appels et al., 2010; Dong et al., 2015), which results in the availability of more easily degradable organic matter. Nevertheless, Appels et al. (2010) and Dong et al. (2015) observed that the solubilization of both components did not exceed 20% of their total fraction. This suggests that a significant amount of carbohydrates and proteins remain bound in the WAS matrix, forming a heat stable fraction.

Humic acids only become more flexible above their glass transition temperature at about 70-80 °C (Kolokassidou et al., 2007). However, the fraction of solubilized humic substances remained constant from 75 to 120 °C (Laurent et al., 2009b) and from 25 to 80 °C (Zhen et al., 2012), suggesting that humic acids are not affected during low-temperature thermal pre-treatment. Nonetheless, the presence of humic-like substances could affect WAS biodegradability. For instance, Wang et al. (2015) stated that protein-like components could be trapped by humic-like components thus forming molecular assemblies and making proteins less susceptible to microbial degradation (Azman et al., 2015b; Tan et al., 2008; Wang et al., 2015). A humic acid-protein assembly would mean that proteins are protected from denaturation and degradation thus limiting WAS biodegradability and explaining why proteins are not completely degraded, even at applied pre-treatment temperatures of around 90 °C, as observed by Appels et al. (2010).

2.1.2.3. Hydrolysis rate and biodegradation. Liao et al. (2016) and Prorot et al. (2011) have reported that low-temperature thermal pre-treatment increases hydrolysis rates at temperatures ranging from 60 to 70 °C. Sanders et al. (2000) and Vavilin et al. (2008) stated that when dealing with particulate organic material (such as WAS), the decrease in particle size results in higher hydrolysis rates during anaerobic digestion. Complementarily, the solubilization of organic material could also speed up the hydrolysis rate.

Regarding sludge biodegradation, despite most studies claim an increase, Prorot et al. (2011) found no significant impact in methane yield, even though organic matter solubilization and cell lysis occurred. The operational conditions were a temperature of 95 °C and an application time of 20 min. For the studies that report an increase in biodegradation, the varying results are shown in Table 2. An explanation for the different outcomes after pre-treatment may be the relation between temperature and treatment time, as has been observed by Hiraoka et al. (1985). This hypothetical relation may be observed (for instance) in the study of Appels et al. (2010) (Table 2, entries g and h), which shows similar methane production at 80 °C coupled with an application time of 60 min and at 90 °C with an application time of 15 min. Probably because of the temperature-time relation, an analysis of the biodegradation of WAS must consider the effect of both parameters.

Pre-treatments with a temperature range of 50–95 °C and an application time of 60 min or less (entries from a to j) generally result in increased methane production, even though the results are very different among the studies (Table 2). A word of caution must be said about the paper of Appels et al. (2010) (entries e, g and h) in which the control sample had a very low biodegradation, probably because of a low inoculum-substrate ratio of 0.10 during the digestion and a very limited solubilization for the sample that was pre-treated at 70 °C. The changes in the different elements of WAS, as described above, are due to the following factors: disruption of cell membranes; maximized solubilization of carbohydrate and protein; and protein denaturation at temperatures above 75 °C. These factors could influence the observed increase in biodegradation at 50-95 °C and application times of < 60 min.

Increased application times (6–15 h) at a moderate temperature increase (60-70 °C), seems to result not only in an increased biodegradation, but also in steadier results (Table 2, entries k to o). The observed increase in methane production cannot be explained solely by the COD solubilization, as its maximum release is reached at times lower than one hour. It is hypothesized that the pre-treatment may have had an effect on the particulate fraction when longer application times are applied, thus increasing its biodegradation. A further increase in application times (up to 1–7 days) at a temperature between 55 and 70 °C, seems to result in a similar or even lower biodegradation compared to the untreated samples (Table 2, entries p to s). A possible explanation is the occurrence of the amino-carbonyl (Maillard) reaction (Appels et al., 2010; Liao et al., 2016), which occurs at a low rate at temperatures of 60 °C (Chiu et al., 2009). The products of the Maillard reaction are refractory; and decrease digestibility; and inhibit proteolytic and glycolytic enzymes activity (Friedman, 1996); and are generally detrimental to cells and organisms (Szwergold, 2013).

Finally, as observed in entries t to w, a simultaneous increase in temperature (between 80 and 90 °C) and application time (3 to 10 h) results in conflictive and spread results. The results in this range are comparable to the ones obtained at 60-75 °C and application times between 3 and 15 h.

2.1.3. Limiting factors, advantages and perspectives

Low-temperature thermal pre-treatment is a potential inexpensive way to increase the sludge biodegradability. Because the influence of both time and temperature, a proper selection of both parameters may be fundamental. The mechanism of the temperature-time dependence remains unclear and should be further studied. Nonetheless, steady and more reliable increases in methane production have been found at 60-75 °C and application times between 3 and 15 h. At temperatures of 80-90 °C conflictive results have been found, probably due to the increased rate of the Maillard reaction. Finally, the option of using low quality heat has a positive effect on the energy balance of the entire process (Table A.1). An unfortunate side-effect of this technique is the worsening of the dewaterability of the digestate (Table 14).

2.2. Temperature phased anaerobic digestion (TPAD)

2.2.1. Process description and mode of action

TPAD separates the digestion process into at least two stages, each one providing optimum conditions for hydrolytic/acidogenic and acetogenic/methanogenic microorganisms. The selection mechanisms

Table 2
Effects on the biodegradation of WAS after thermal pre-treatment < 100 °C.

Entry	Temperature, °C	Application time	Change in biodegradation (vs. control)[a]	Change in CH_4 production (vs. control)	Change in biogas production (vs. control)	Reference
Temperature 50-95 °C Application times ≤ 60 min						
a	50	30 min		↑ 13.8%		Dhar et al. (2012)
b	60	60 min			↑ 30%	Hiraoka et al. (1985)
c	60	60 min			↑ 12%	Ennouri et al. (2016)
d	70	30 min		↑ 18.8%		Dhar et al. (2012)
e	70	60 min		↑ 1.6%		Appels et al. (2010)
f	72	7.5 min	↑11% (from 29 to 40)			Vergine et al. (2014)
g	80	60 min		↑ 124%		Appels et al. (2010)
h	90	15 min		↑ 123%		Appels et al. (2010)
i	90	30 min		↑ 13.2%		Dhar et al. (2012)
j	95	20 min	=			Prorot et al. (2011)
Temperature 60-75 °C Application times between 3 and 15 h						
k	60	6 h	↑ 8% (from 21 to 29)			J. Kim et al. (2013b)
l	70	3 h	↑ 7% (from 32 to 39)			Ruffino et al. (2015)
m	70	9 h			↑ 50%	Climent et al. (2007)
n	70	15 h	↑ 6% (from 32 to 38)			Ruffino et al. (2015)
o	75	6 h	↑ 14% (from 21 to 35)			J. Kim et al. (2013b)
Temperature 55-70 °C Application times between 1 and 7 days						
p	55	24 h	↑ 1% (from 38 to 39)			Wang et al. (2014)
q	70	24 h	↑ 3% (from 38 to 41)			Wang et al. (2014)
r	70	1–7 days		↑ 5%		Gavala et al. (2003)
s	70	3 days			↓ 10%	Climent et al. (2007)
Temperature 80-90 °C Application times between 3 and 10 h						
t	80	3 h	↑ 9% (from 28 to 37)			Ruffino et al. (2015)
u	80	10 h		↓ from 291 to 281 mL/gVS		Nielsen et al. (2011)
v	90	3 h	↑ 9% (from 28 to 37)			Ruffino et al. (2015)
w	90	6 h	↑ 5% (from 21 to 26)			Kim, Yu, and Lee (2013b)

(and operational parameters) are temperature, application time and pH. Temperature has more relevance to the outcomes of the process compared to application time (Ge et al., 2011a). For the acidogenic phase, temperatures between 45 °C and 70 °C and retention times of 2–6 days are used, while for the methanogenic phase mesophilic or thermophilic conditions with solids retention time of 14–30 days are used.

2.2.2. Effects on WAS

2.2.2.1. Floc and cell disruption / carbohydrates, proteins and humic substances. Ghasimi et al. (2016b) observed that thermophilic hydrolysis might be increased due to a high sludge loading leading to accumulation of protein-like substances in the reactor broth, which might be related to hydrolytic enzymes. However, to our best knowledge no studies have been conducted dealing with the fate of cells, proteins, carbohydrates and humic substances in the first stage of TPAD. Nonetheless, the effects are expected to be similar to those described for thermal pre-treatment below 100 °C, since the same range of temperatures are used, while only application times are lengthened. As TPAD involves a biological stage coupled with a temperature increase, the mechanism of action and the contribution of both chemical and biological conversions require further research.

2.2.2.2. Hydrolysis rate and biodegradation. From Table 3, it is clear that TPAD increases the WAS biodegradation. J. Yu et al. (2013b) observed an increase of 84.8% in methane production in an acidogenic stage at 45 °C with a retention time of 4 days, while Ge et al. (2011a) found no increase in methane production at 50 °C and 60 °C, but an increased hydrolysis rate in the subsequent methanogenic stage. The increase in biodegradability observed in the same study at 70 °C with 4 days of retention time, suggests that the effects of Maillard reaction, usually occurring during thermal pre-treatment < 100 °C, were absent in TPAD. A reason could be that the increased enzymatic activity in the first stage of TPAD (Ghasimi et al., 2016b) can alleviate the effects of the formation of Maillard products due to the increased presence of hydrolytic enzymes or to the fact that some organisms have enzymatic ways to protect themselves against the Maillard reaction products (Szwergold, 2013). During the acidogenic stage, Ge et al. (2011b) reported the production of methane even at residence times shorter than four days, and at temperatures between 50 °C and 60 °C.

Thermophilic conditions, applying 2–3 days retention time, have been reported to provide better results in terms of increased biodegradation (Bolzonella et al., 2007; Ge et al., 2011a; Riau et al., 2012; Q. Wang et al., 2016). The pH seems to act as a selection mechanism, which impacts biodegradability; with pH values close to neutrality giving increased biodegradability (Ge et al., 2011a) or volatile fatty acids (VFA) yields (Q. Wang et al., 2016), as compared to pH values between 4 and 5. According to the reported studies, the best results during the acidification step occur at temperatures between 65 and 70 °C; retention times ranging from 2 to 3 days; and a pH of 6–7.

2.2.3. Limiting factors, advantages and perspectives

Riau et al. (2012) observed that even though the acidogenic effluent has poor dewaterability, a subsequent mesophilic digestion was able to reduce the ‘time-to-filter’ by > 50% compared to a single-stage anaerobic digestion with the same process duration. This suggests that with this technique the addition of an acidification step may reduce the required amounts of polyelectrolyte for dewatering the digestate. A comparison of the biodegradation changes for low-temperature thermal pre-treatment and TPAD in Table A.1 shows more prominent increases for TPAD, even though similar temperatures are applied. A possible explanation could be the higher production of protein-like substances at 55 °C compared to 35 °C conditions (Ghasimi et al., 2016b) caused by the presence of biological activity during the pre-treatment.

Table 3
Effects on the biodegradation of WAS after TPAD.

Entry	Acidogenic stage			Methanogenic stage			Change in biodegradation (compared to single stage)	Change in CH_4 production (compared to single stage)	Reference
	Temperature, °C	Retention time, day	pH	Temperature, °C	Retention time, day	pH			
a	45	4	6.05	35	16	–		↑85%	(J. Yu et al., 2013b)
b	50	2	7	37	14	–	34%[a]		(Ge et al., 2011a)
c	55	6	7.54	35	24	7.53	↑ 18% (from 39 to 57)		(Wu et al., 2016)
d	55	2	6.8	55	18	8.2	↑ 11% (from 33 to 44)		(Leite et al., 2016)
e	60	2	7	37	14	–	41%[a]		(Ge et al., 2011a)
f	65	2	7	37	14	–	43%[a]		(Ge et al., 2011a)
g	65	2	6.3	55	18	7.9	↑ 5% (from 51 to 56)		(Bolzonella et al., 2012)
h	70	2	7	37	14		48%[a]		(Ge et al., 2011a)
i	70	2	–	35	14	–	↑ 15% (from 16 to 31)		(Ge et al., 2011b)

2.3. Thermal pre-treatment ≥100 °C

2.3.1. Process description and mode of action

High-temperature thermal pre-treatment or thermal hydrolysis relies on the application of temperatures above 100 °C and was originally developed to sterilize the sludge and produce class A biosolids. To provide heat, steam is commonly used through heat exchangers or more commonly by direct injection (Pilli et al., 2014). Both the steam injection and the temperature increase, rise the pressure, which is suddenly released once the target values of temperature, pressure and application time are reached. In such cases, not only the effect of temperature is present but also that of the abrupt pressure drop, which increases both solubilization (Donoso-Bravo et al., 2011) and methane production (Sapkaite et al., 2017). The relevant parameters are temperature, application time, pressure, and the presence of a sudden pressure drop. Compared with application time, temperature has a higher influence on solubilization (Li and Noike, 1992; Valo et al., 2004; Xiao and Liu, 2006). Cambi™ and Exelys™ are industrial-scale technologies consisting of one or more pressurized tanks; reaching up to 160 °C for 30 min and 6 bar for Cambi™; and up to 180 °C for 60 min and 10 bar for Exelys™. Cambi™ works in batch mode; whilst Exelys™ operates in plug flow mode. "Steam explosion" in the Cambi process is achieved by suddenly reducing the pressure to two bar. Heat is then recovered and returned to the preheating stage. A detailed description of these processes is provided by Pilli et al. (2014).

The mechanism of thermal hydrolysis is best understood by highlighting two observations from Bougrier et al. (2008). Firstly, they observed that from 95 to 170 °C, the extent of biogas production from the soluble fraction was higher than that from the particulate fraction. Secondly, the observed increase in biodegradation was caused by the transfer of organic matter from the particulate fraction to the soluble one, but did not lead to an increase in the biodegradation of each fraction (Bougrier et al., 2008), while Pérez-Elvira et al. (2016) found that the biodegradation of the solid fraction remained constant after pre-treatment at 170 °C for 50 min. At temperatures above 190 °C, biodegradation decreases compared to lower temperatures and can even be lower than the control (Mottet et al., 2009; Stuckey and McCarty, 1984).

2.3.2. Effects on WAS

2.3.2.1. Floc and cell disruption. Temperatures from 100 up to 120 °C were found to reduce particle size (Gao et al., 2013; Laurent et al., 2009b); which follows the trend observed in low-temperature pre-treatment. In contrast, at 170 and 190 °C, the particle size increased compared to the untreated sample, which was explained by the creation of chemical bonds (Bougrier et al., 2006). In a follow-up study, Bougrier et al. (2008) observed a temperature threshold of 150 °C, below which temperature worsened dewaterability and above which temperature improved it. The decrease and increase in particle size is also reflected in the dewatering properties of the digestate.

Already at 70–95 °C permeabilization and cell destruction was observed (Prorot et al., 2011; Salton and Horne, 1951); and therefore it is expected that temperatures above 100 °C will provoke extensive cell disruption and release of the intracellular content. Extensive solubilization seems to influence the observed increase in biodegradability for most of the studies reported (Table 4). However, as described below, care should be taken to avoid the negative effects of excessive temperatures. Moreover, considering the relatively low amount of bacterial mass in WAS (Table 1, 10–24%), the impact of temperature on other organic compounds needs to be considered.

2.3.2.2. Carbohydrates, proteins and humic substances. The melting point of sugars (at 170 °C) causes caramelization, and thus the formation of organic acids, aldehydes, and ketones. Caramelization does not involve proteins and should not be confused with the Maillard reaction (Vaclavik and Christian, 2008). Above 170 °C, degradation of sugars is expected to occur. However, Wilson and Novak (2009) observed that below 220 °C, the effect of thermal hydrolysis on carbohydrates was solubilization, not degradation. This fact agreed with their observation that pure carbohydrate solutions were not largely converted to mono or dimeric sugar units at 220 °C (Wilson and Novak, 2009). Regarding proteins, extensive denaturation is expected at such high temperatures as well as degradation at temperatures around 190 °C and 220 °C, since ammonia concentration had been observed to increase up to nine times compared to the control (Wilson and Novak, 2009). This matched with the fact that protein degradation of pure bovine serum albumin was observed for the same temperature range (Wilson and Novak, 2009). Below 170 °C, evidence of extensive protein degradation has not been observed (Bougrier et al., 2008; Morgan-Sagastume et al., 2011). An increase in enzymatic activity is not expected to play a significant role during high-temperature pre-treatment of WAS. In fact, enzyme degradation is a pivotal factor in the loss of enzymatic activity at temperatures in excess of 80 °C (Daniel et al., 1996). Additionally, even though enzyme immobilization (e.g. with humic substances) is known to increase the half-live of enzymes, it only increases the denaturation temperature by 25 °C (Unsworth et al., 2007)."

Finally, Wilson and Novak (2009) reported that from 130 to 220 °C, the relative solubilization of polysaccharides was higher than that of proteins, which is the opposite to what has been observed for low-temperature pre-treatment. It is hypothesized that for a temperature > 100 °C most of the proteins are already solubilized, whereas the bound carbohydrates only start to be solubilized at 130 °C. Also, the Maillard reaction could take place, likely to a higher extent as compared to temperatures below 100 °C, since reaction rates increase with temperature (Dwyer et al., 2008). Finally, at temperatures above 110 °C humic acids start to decompose (Kolokassidou et al., 2007), while at

Table 4
Effects on the biodegradation of WAS after thermal pre-treatment ≥100 °C.

Entry	Temperature, °C	Application time, minutes	Pressure, MPa	Soluble COD	Change in biodegradation (vs. control)	Change in biogas production (vs. control)	Reference
a	100	10	0.1	↑ from 4.1 to 24.8%	↑ 11% (from 12 to 23)		(Gao et al., 2013)
b	110	30	n/d	↑ from 8.7 to 12.2%	↑ 6% (from 47 to 53)		(Mottet et al., 2009)
c	120	10	0.1	↑ from 4.1 to 75.9%	↑ 19% (from 12 to 31)		(Gao et al., 2013)
d	121	30	0.152	↑ from 8.1 to 17.7%	↑ 8% (from 26 to 34)		(Kim et al., 2003)
e	130	15	n/d			↑ 14% (as CH_4)	(Nielsen et al., 2011)
f	130	45	n/d			↑ ≈ 68%	(Pinnekamp, 1989)
g	134	20	0.312	↑ from 0.0 to 14.1%	↑ 15% (from 31 to 46)		(Gianico et al., 2013)
h	135	30	n/d		↑ 6% (from 49 to 55)		(Bougrier et al., 2007b)
i	165	30	n/d	↑ from 8.7 to 25.2%	↑ 9% (from 47 to 56)		(Mottet et al., 2009)
j	170	0*	n/d	40.5% (initial value not disclosed)		↑ 1.59 times	(Bougrier et al., 2006)
k	170	15	n/d			↑ 9.2% (as CH_4)	(Nielsen et al., 2011)
l	170	45	n/d			↑ 74%	(Pinnekamp, 1989)
m	170	60	n/d		↑ 20% (from 25 to 45)		(Valo et al., 2004)
n	175	60	n/d		↑ 13% (from 48 to 61)		(Stuckey and McCarty, 1984)
o	190	0*	n/d	49.0% (initial value not disclosed)		↑ 1.59 times	(Bougrier et al., 2006)
p	190	15	n/d		↑ 13% (from 49 to 62)		(Bougrier et al., 2007b)
q	190	30	n/d	≈63% (initial value not disclosed)		↑ ≈ 2.6 times	(Bougrier et al., 2008)
r	200	60	n/d		↑ 9% (from 48 to 57)		(Stuckey and McCarty, 1984)
s	210	30	n/d	≈67% (initial value not disclosed)		↑ ≈ 2.1 times	(Bougrier et al., 2008)
t	220	30	n/d	↑ from 8.7 to 33.4%	↓ 6% (from 47 to 41)		(Mottet et al., 2009)
u	220	45	n/d			↑ ≈ 11%	(Pinnekamp, 1989)
v	225	60	n/d		↑ 4% (from 48 to 52)		(Stuckey and McCarty, 1984)
w	250	60	n/d		=		(Stuckey and McCarty, 1984)
x	275	60	n/d		↓ 6% (from 48 to 42)		(Stuckey and McCarty, 1984)

n/d: not determined.

* No holding time.

150-180 °C dissociation occurs (Bobleter, 1994; Garrote et al., 1999).

2.3.2.3. Hydrolysis rate and biodegradation. The hydrolysis rate increases after application of thermal hydrolysis (Bougrier et al., 2006; Gao et al., 2013) and so does the biodegradation, as observed in Fig. 1 and Table 4. A parabolic behavior for WAS biodegradation as a function of pre-treatment temperature is observed in Fig. 1, with the highest values in the range of 170–190 °C. Indeed, it has been widely documented that the maximum methane production is found at temperatures around 170–190 °C (Bougrier et al., 2008; Bougrier et al., 2007b; Pinnekamp, 1989; Stuckey and McCarty, 1984) despite the fact that COD solubilization continues to increase in proportion to temperatures up to 220 °C (Mottet et al., 2009) (Table 4, entry t). Similarly, Bougrier et al. (2007b) and Pinnekamp (1989) tested pre-treatments at 190 and 220 °C respectively, and the maximum biodegradation was found below those temperatures, in contrast to VS destruction, which increased in proportion to temperature. Such observations suggest that the pre-treated sludge is mineralized under harsh pre-treatment conditions (Zhen et al., 2014).

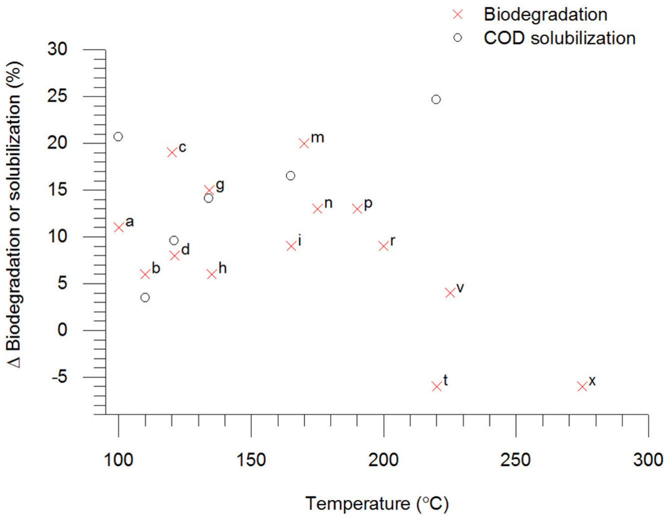


Fig. 1. Change in biodegradation and COD solubilization due to thermal pre-treatment above 100 °C. Letters refer to entries in Table 4.

According to Stuckey and McCarty (1984) the observed peak in methane production could be caused by two competing mechanisms: the conversion of particulate organics into biodegradable dissolved matter, which increases methane yield; and the formation of soluble but refractory compounds that do not contribute to biogas production. The decline in methane production has been widely ascribed to Maillard reactions. Maillard reactions result in the formation of melanoidins (Neumann et al., 2016). Actually, due to their recalcitrant nature, melanoidins are also known as synthetic humic acids (Blondeau, 1989). The occurrence of the Maillard reaction produces color changes (Vaclavik and Christian, 2008), which correlate with the brownish supernatant of digested sludge observed by Bougrier et al. (2007b) at a temperature of 190 °C. At 135 °C or less (well below the methane production peak at 160–190 °C), the formation of refractory products also already takes place as discussed for low-temperature pre-treatment, which could reduce WAS biodegradability. In contrast, it is likely that conditions up to 190 °C promote a higher organic matter solubilization that outcompetes the formation of refractory compounds (Table 4, entry h). In addition, at 170 °C, the caramelization of sugars starts, potentially producing the aldehyde furfural ($C_5H_4O_2$), which has been shown to inhibit anaerobic biological processes at a concentration of around 2 g/L (Ghasimi et al., 2016a). Thus, a temperature of around 190 °C is postulated as the temperature above which the formation of refractory components outcompetes the formation of biodegradable soluble components, thereby reducing biodegradation. The exact temperature, however, depends on sludge composition, and probably other factors.

2.3.3. Limiting factors, advantages and perspectives.

Attention should be given to the effect of the refractory solubilized fraction resulting from thermal hydrolysis, as it may pose negative effects when returned back to the wastewater headworks. A way to minimize the effects of refractory compounds is to increase digestion times (whenever possible) (Stuckey and McCarty, 1984), provided the methanogenic consortia are able to adapt to these compounds. Adaptation may occur by allowing sufficient time for developing the required hydrolytic enzymes (Ghasimi et al., 2016b).

Thermal hydrolysis has been observed to increase biogas production, with the largest impact on low-loaded activated sludge processes, for sludge samples with low initial biodegradation (Bougrier et al., 2008; Pinnekamp, 1989) and for digested sludges that are post-treated and then re-digested (Pinnekamp, 1989). In addition to its original objective of sludge sterilization (implying suitability for land disposal if regulations allow), thermal hydrolysis also increases the dewaterability of the digestate (at above 150 °C) and results into high VS removal. Compared to low-temperature pre-treatment, thermal hydrolysis cannot operate using only low quality waste heat ($\leq$ 80 °C), high quality heat ($>$ 100 °C) is also required, which can negatively impact the energy balance as observed in Table A.1 and Table B.1. To get a closed heat balance (or as closed as possible) dewatered WAS with a high VS concentration ($>$ 16%) should be fed to the pre-treatment, instead of thickened or dewatered sludge with a VS concentration of around 3–6%, as compared to low-temperature pre-treatment Table B.1. Moreover, in many cases the required minimum VS concentration had to be 20–25%, or some primary sludge had to be co-digested to get a closed heat balance during thermal hydrolysis.

2.4. Microwave pre-treatment

2.4.1. Process description and mode of action

Microwaves are high-frequency (around 2.45 GHz) electromagnetic waves, which create a changing electromagnetic field. This causes rapid alignment and realignment of dipoles in polar molecules (such as water and some EPS molecules), and thus generates friction that liberates heat (Vaclavik and Christian, 2008). In addition to the thermal effect, an "athermic" effect related to the breaking of the polymeric network due to the rotation of molecules has been hypothesized (Eskicioglu et al., 2006). Nonetheless, it has been demonstrated that under identical experimental conditions (heating/cooling rates and net absorbed energy), athermal effects do not exist, or at least, are insignificant compared to the thermal effects (Hosseini Koupaie and Eskicioglu, 2016; Mehdizadeh et al., 2013; Sólyom et al., 2011; Vergine et al., 2014).

Similar to conventional thermal pre-treatment (heat transfer by convection), temperature increase is the most relevant parameter during microwave pre-treatment (Hosseini Koupaie and Eskicioglu, 2016; Mehdizadeh et al., 2013). Increase in biodegradation is in proportion to applied temperature, for the low-temperature range (65-85 °C) (Kennedy et al., 2007) as well as for the high temperature range 110-175 °C (Toreci et al., 2011; Toreci et al., 2010). Other important factors are the temperature increase rate, or ramp rate (Hosseini Koupaie and Eskicioglu, 2016) and the sludge solids concentration, as it affects the absorption of microwaves (Eskicioglu et al., 2007b) and thus the energy actually delivered to the sample.

2.4.2. Effects on WAS

2.4.2.1. Floc and cell disruption. Similarly to low-temperature pre-treatment, the mean particle size of the pre-treated sludge seems to decrease under microwave application (Kennedy et al., 2007; Yi et al., 2014). Unfortunately, no studies were found regarding the relationship between particle size distribution and temperature increase, at temperatures higher than 100 °C, although a further decrease in particle size is expected at temperatures around 150 °C, followed by an increase after this temperature, as has been reported for thermal hydrolysis.

Cella et al. (2015) found that the highest microbial destruction occurred at 2.62 kJ/g TS at a temperature of 80 °C and 9 min of application time, which lowered the live/dead ratio from around 3 for the control to around 0.25 for the pre-treated sample. An additional input of energy did not cause significantly higher microbial death. Eskicioglu et al. (2007a) postulated that cell disruption is due to the absorbance of microwaves in the lipid bilayer of cell membranes. Several studies have reported an increase in cell disruption by using microwaves as compared to conventional heating at the same final temperature, the difference potentially being explained by the aforementioned athermic effect (Eskicioglu et al., 2007c; Hong et al., 2006; Hong et al., 2004; Kakita et al., 1995). However, in those studies, either the temperature increase rate or the energy actually absorbed by the sample was not strictly controlled. As a consequence, a higher energy input could have been actually delivered resulting in a higher cell death. Therefore, it is concluded that cell disruption exists during microwave pre-treatment, but the treatment is dominated by thermal effects, just as for conventional thermal pre-treatment.

2.4.2.2. Carbohydrates, proteins and humic substances. The observation that merely thermal effects are determining during microwave pre-treatment is also confirmed by trends observed for both thermal and microwave pre-treatments:

- Proportional increase in soluble COD at temperatures below 100 °C (Eskicioglu et al., 2007c; Kennedy et al., 2007) as well as above 120 °C (Eskicioglu et al., 2009; Toreci et al., 2009). Specifically, a proportional increase in the concentration of soluble sugars, protein and humic acids at 50-160 °C (Eskicioglu et al., 2007a; Mehdizadeh et al., 2013).
- Higher relative solubilization of proteins compared to carbohydrates (Eskicioglu et al., 2007c; Uma Rani et al., 2013). No degradation of proteins and sugars up to 80 °C (8.23 kJ/gTS) (Appels et al., 2013).
- Initial increase of reducing sugars in the supernatant at 50 and 75 °C and subsequent decrease at 96 °C, probably explained by the occurrence of the Maillard reaction (Eskicioglu et al., 2007a).

2.4.2.3. Hydrolysis rate and biodegradation. In contrast to conventional thermal pre-treatment, conflicting results have been observed regarding the hydrolysis rate. Park et al. (2004) and Hosseini Koupaie et al. (2017) observed an increase; Eskicioglu et al. (2007b) found no change; and Toreci et al. (2011) found a decrease. The latter could be explained by inhibition caused by toxic by-products formed during microwave pre-treatment, because temperatures up to 175 °C had been used. Another possible explanation is the use of non-acclimated inoculum during batch digestion tests. Regarding sludge biodegradation, results match with observations for low and high temperature pre-treatment. Most of the studies observed an increase in biodegradation (Table 5), although some papers reported no increase (Cella et al., 2015; Eskicioglu et al., 2008).

The effect of the temperature increase rate during pre-treatment on sludge biodegradation should not be neglected. It has been observed that a slower temperature increase, enhances methane production for both low and high temperature scenarios (Eskicioglu et al., 2009; Hosseini Koupaie and Eskicioglu, 2016; Park and Ahn, 2011; Toreci et al., 2011). This could be related to the application time-temperature

Table 5
Effects on the biodegradation of WAS after microwave pre-treatment.

Entry	Temperature, °C	Application time, minute	Irradiation energy, kJ/L	Specific energy (E_s), kJ/gTS	Change in biodegradation (vs. control)	Change in CH_4 production (vs. control)	Change in biogas production (vs. control)	Reference
a[*]	60	7	1029	16.07 (ca.)	↓ 3% (from 53 to 50)			(Eskicioglu et al., 2008)
b[*]	80	8	1175	18.37 (ca.)	↑ 1% (from 53 to 54)			(Eskicioglu et al., 2008)
c[*]	80	3.5	336	8.23				(Appels et al., 2013)
d[*]	85	4.5	493	16.42 (ca.)		↑ 16%		(Kennedy et al., 2007)
e	91.2	7	588	19.55		↑ 30%		(Park et al., 2004)
f[*]	96	3	450	21.65 (ca.)			↑17%	(Eskicioglu et al., 2007b)
g[*]	96	6	900	17.51 (ca.) as kJ/gSS	↑ 10% (from 33 to 43)			(Coelho et al., 2011)
h[*]	100	9	1322	20.66 (ca.)	↑ 15% (from 53 to 68)			(Eskicioglu et al., 2008)
i	120	10	1470	22.96 (ca.)	↑ 1% (from 53 to 54)			(Eskicioglu et al., 2008)
j	130	46	3335	81.54 (ca.)	↑ 9% (from 46 to 55)			(Chi et al., 2011)
k	160	16	2880	501.8 (ca.) as kJ/gSS		↑ 11%		(Doğan and Sanin, 2009)
l	190	39	2830	69.13 (ca.)	↑ 11% (from 46 to 57)			(Chi et al., 2011)
m	n/d	n/d	975	38.08		↑ 46%		(Martínez et al., 2016)
n	n/d	n/d		20		↑ 6%		(Serrano et al., 2016)

n/d = not disclosed.
ca. = calculated value based on the parameters of the study.
[*] Performed with mixed sludge (WAS = 58%; primary sludge =42%, by volume).

dependence, that was observed by Hosseini Koupaie et al. (2017) for microwave pre-treatment under 90-120 °C and with application times of 1 to 2 h. However, insufficient studies, on the time-temperature dependency with microwave pre-treatment, were found in literature to draw general conclusions regarding this mechanism.

2.4.3. *Limiting factors, advantages and perspectives*

Despite the inherent differences between microwave and ultrasonic (US) pre-treatments, comparisons have been carried out under similar specific energy consumption. For microwaves, applying 2.62 kJ/g TS at a temperature of 80 °C and 10 min of application time, and US pre-treatment, applying 2.37 kJ/g TS at a temperature < 35 °C, it was found that microwaves caused a fourfold to fivefold greater cell death, but this did not result in significantly different biodegradation (Cella et al., 2015). Westerholm et al. (2016) made a similar observation. This suggests that both microwave and ultrasonic pre-treatment result in comparable outcomes regarding biodegradation. Applying an alternative electromagnetic frequency (13.56 MHz versus the conventional 2.45 GHz) did not change the biogas production rate and biodegradation significantly (Hosseini Koupaie et al., 2017), although it was two times more energy efficient compared to the conventional frequency. A potential drawback of microwave pre-treatment is the observed short term inhibition of digestion after pre-treatment (Eskicioglu et al., 2007b; Toreci et al., 2011), however no studies have been performed to further understand its cause. Interestingly, microwave pre-treatment has been reported to improve the dewaterability of the digestate at temperatures below 96 °C (Coelho et al., 2011; Eskicioglu et al., 2007b; Wang and Li, 2016), in contrast to the results of low-temperature thermal pre-treatment. Based on the discussions in previous sections, similar improvements to anaerobic digestion can be achieved with low-temperature or thermal hydrolysis pre-treatments. Furthermore, microwave pre-treatment requires a significantly higher energy expense (Table A.1), due to the fact that electricity is required, in contrast to the waste heat required for low-temperature pre-treatment.

2.5. *Freezing and thawing*

2.5.1. *Process description and mode of action*

Freeze and thaw consists of the freezing of sludge, usually at temperatures around -20 °C for several hours and a subsequent thawing process at room temperature. The formation of ice crystals causes physical damage to the cells (Vaclavik and Christian, 2008). The sequence of mechanisms is: a) at 0 °C, ice crystals are formed in the extracellular solution. Intracellular content remains liquid as it contains fluids with lower freezing points compared to extracellular content (Thomashow, 1998). The ice front pushes particulate matter together, forming clusters; b) solute concentrations just outside the cell increase due to the freezing of the extracellular solution. This causes osmotic pressure that leads to cell dehydration and shrinking (Wang et al., 2001); c) as freezing time increases or temperature is further decreased, to between −2 and -10 °C, the intracellular content freezes and expands due to internal ice formation that then leads to cell lysis (Thomashow, 1998).

Very limited research focused on increasing methane production with this method has been reported. Most of the relevant studies focused on dewatering effects. Temperature, freezing rate and pre-treatment time (curing time) are relevant operational parameters that affected dewaterability (Hu et al., 2011; Vesilind and Martel, 1990; Wang et al., 2001). Lower freezing rates and colder temperatures result in better dewatering (Hu et al., 2011; Vesilind and Martel, 1990). Refreezing, in multiple freeze and thaw cycles, enlarges ice crystals, contributing to further cell disintegration (Vaclavik and Christian, 2008).

2.5.2. Effects on WAS

2.5.2.1. Floc and cell disruption. It has been reported that the ice front formed during freezing of WAS squeezes the particulate fraction together into larger particles that tend to keep their new size after thawing (Gao, 2011; Wang et al., 2001). Other studies showed that freeze and thaw pre-treatment reduced the particle size of the sludge flocs and increased the settleability. Similarly, when subjected to cooling the proteins precipitate or aggregate (Chang et al., 1996; Heller et al., 1999; Yu et al., 2006). This suggests compaction of the sludge flocs due to the freezing process (Hu et al., 2011). As almost all studies report an increase in dewaterability, but with different trends in the modification of particle sizes, both increase in particle size and increase in density could be the mechanism behind dewaterability increase.

The freeze and thaw pre-treatment causes a decrease in the fraction of viable cells (Diak and Örmeci, 2016; Wang et al., 2001). Slow freezing is more preferable than rapid freezing or excessively lower temperatures (Wang et al., 2001). Authors concluded that at -80 °C, the freezing speed was so rapid that some bacteria survived due to the prevention of the elution of proteins and carbohydrates. Cell survival at freezing temperatures could be due to the presence of cryoprotectant components, such as proteins and fats and glycerol (Montusiewicz et al., 2010).

2.5.2.2. Carbohydrates, proteins and humic substances. COD solubilization increases linearly with freezing time, suggesting that long retention times could be required to have significant solubilization (Hu et al., 2011). Wang et al. (2001) observed a 25-fold increase in soluble proteins and an approximately 4-fold increase in soluble carbohydrates after 24 h of freezing at -10 °C. They also observed a higher degree of solubilization in the case of freezing at -10 °C as compared to freezing at -80 °C. This was related to higher cell disruption at higher temperatures. Even though protein denaturation occurs as a result of freezing, freezing does not completely stop enzyme activity (Vaclavik and Christian, 2008). Regarding carbohydrates, the expelling of water from starch (an effect of the retrogradation reaction) is more likely to occur in WAS that is exposed to the effects of freeze–thaw cycles (Vaclavik and Christian, 2008).

2.5.2.3. Hydrolysis rate and biodegradation. No information was found concerning the effect of freeze and thaw pre-treatments on the hydrolysis rate. However, an increase is expected since cell disruption and change in particle size occurs. In the few studies regarding biogas potential, increased biogas production has been reported (Jan et al., 2008; Montusiewicz et al., 2010; Pabón Pereira et al., 2012).

2.5.3. Limiting factors, advantages and perspectives

Similar COD solubilization was observed for freeze and thaw and high-temperature thermal (103 °C) pre-treatment (Gao, 2011). Unfortunately, the anaerobic biodegradability and biogas production was not studied. The only successful freeze and thaw systems are found in natural freezing and thawing systems in open beds (Hellström, 1997; Kinnunen et al., 2014; Vesilind and Martel, 1990). In these sludge beds, the sludge is spread into thin layers during the winter months and allowed to freeze, while in warmer weather the sludge thaws and the water drains out, leaving a dry WAS (Wang et al., 2001). Although this mechanism is beneficial for improving dewaterability, it could cause a decrease in the biodegradation of sludge, because soluble COD also leaks out if the sludge is not in an enclosed volume. Since no energy input is required and an increase in methane production is expected, freeze and thaw using naturally occurring conditions has a positive energy balance (Table A.1). However, this technique is restricted to cold regions during the coldest seasons and is dependent on the actual climatic conditions (not all winters are similarly cold). Also, in order to reduce the size of the bed, research on applicable exposure times need to be performed. Artificial freezing does not seem to be a practical option, because of the amount of electricity required, and the hypothetical large freezers for storing the sludge. For instance, active freezing of sludge from 10 to -25 °C assuming a total solids concentration of 40 g/L and a coefficient of performance of 2.75 (Taib et al., 2010), would demand an expense of 4.25 kJ/gTS in electric energy. Nonetheless, freeze and thaw increases the biomethane potential of WAS, while also probably improving the dewaterability of the digestate. Moreover, in contrast to other pre-treatment techniques, the formation of refractory compounds or undesirable by-products is probably absent at freezing temperatures.

2.6. Ultrasonic pre-treatment

2.6.1. Process description and mode of action

Ultrasonic pre-treatment results in cavitation, a disturbance in the liquid resulting in the formation, growth, and implosion of bubbles (Chatel, 2016). In order for cavitation to occur, weak points in the liquid must exist, such as suspended particulate matter (Chatel, 2016). The efficacy of ultrasonic pre-treatment of WAS depends on ambient conditions and operational parameters (Delmas et al., 2014). Depending on the selection of ultrasonic parameters, the sudden collapse of bubbles creates shear forces and/or formation of hydroxyl radicals.

Parameters such as frequency, ultrasonic density (Eq. 2), temperature and VS concentration should be considered for a complete picture of the performance of ultrasonic pre-treatment. One of the most widespread operational parameters for ultrasonic pre-treatment of WAS is the specific energy requirement, E_s (Eq. 3), an umbrella term encompassing power, volume of the sample, application time and the concentration of solids. Although useful for assessing the energy consumption, it should be employed carefully as it does not replace the detailed selection of the main parameters of ultrasonic pre-treatment. Table 6 lists the relevant parameters and their effects during sonication of WAS.

$$D\left[\frac{kW}{L}\right] = \frac{Power\ (kW)}{Volume\ (L)} \tag{2}$$

$$E_s\left[\frac{kJ}{gTS}\right] = \frac{D\left(\frac{kW}{L}\right) \times time\ (s)}{Solids\ concentration\ \left(\frac{gTS}{L}\right)} \tag{3}$$

2.6.2. Effects on WAS

2.6.2.1. Floc and cell disruption. Ultrasonic pre-treatment has been used as a dispersion technique for aggregates (Foladori et al., 2007; Jorand et al., 1995) such as WAS flocs. A reduction in particle size diameter is expected, which would make the organic matter more accessible for enzymatic attack (Jorand et al., 1995; Vavilin et al., 2008).

For temperature controlled sonication, particle size decreased as the dosage of specific energy increased (Feng et al., 2009). This in turn results in an increase in hydrolysis rate, as will be described below.

An effect of ultrasonic pre-treatment is the break-up or damage of the bacterial cell wall (Jorand et al., 1995). Indeed, cell disruption has been documented by flow cytometry of WAS as presented in Table 7.

Table 6
Parameters with influence in the sonication of WAS.

Parameter, (unit)	Remarks
Frequency, (kHz)	Frequency determines whether cavitation or formation of hydroxyl radicals dominates: low frequencies (20–80 kHz) lead to physical effects (shockwaves, microjets, microconvection) (Chatel, 2016; Pilli et al., 2011); while high frequencies (150–2000 kHz) favour the production of hydroxyl radicals (Chatel, 2016; Tiehm et al., 2001). For WAS ultrasonic pre-treatment, the range of 20 to 41 kHz has been commonly used. Between 41 and 3217 kHz, higher particle size reduction and supernatant turbidity was observed at 41 kHz (Tiehm et al., 2001). Recently, the use of audible frequencies (< 20 kHz) has been assessed with promising results. At lower frequencies, like 12 kHz, higher COD solubilization has been observed than at 20 kHz (Delmas et al., 2014; Tuan et al., 2016; R. Wang et al., 2016a). Jiang et al. (2009) however had the highest COD solubilization at 25 kHz of the tested 19, 25, 40 and 80 kHz.
Ultrasonic density, acoustic power or power density, D, (kW/L)	Power density is part of the parameters included in the calculation of the specific energy (Eq. 3). It has been observed that under the same value of E_s, the higher the power density, the higher the solubilization (Pérez-Elvira et al., 2009; Show et al., 2007).
Temperature	Cavitation increases the temperature of the medium as a function of the sonication time (Chatel, 2016; Nguyen et al., 2016) and power density (Rombaut et al., 2014). Temperature uncontrolled sonication led to higher COD solubilization compared to temperature controlled sonication (Tuan et al., 2016). However, the effect of shear forces by cavitation decreases at increasing temperatures, so it should be kept below 80 °C (Delmas et al., 2014; Huan et al., 2009; Tuan et al., 2016).
Solids concentration, (gTS/L)	There is an optimum in the range of 1–3% TS for solubilization (Sahinkaya, 2015; Show et al., 2007) and biodegradation (Pilli et al., 2016). According to Show et al. (2007), an excess of solids in sludge results in a high energy loss during sonication, thus reducing the effectiveness of pre-treatment.
Application time, (s)	It has been observed for temperature controlled ultrasonic pre-treatment that increasing pre-treatment time over 60 min had essentially no effect on the sludge characteristics (Chu et al., 2001). However, for temperature uncontrolled ultrasonic pre-treatment, the effects of increasing pre-treatment time are increases in temperature and higher cellular death (Zielewicz, 2016).

Entries a and b, were performed with very low TS concentrations that are not representative of thickened WAS that is used for AD, yielding excessive specific energy values. However, Cella et al. (2015), employed sewage sludge at 42 gTS/L and found that even though the percentage of damaged bacterial cell walls increased from around 33 to 50% (Table 7, entry c), the cell death did not increase overall WAS biodegradation; which might be attributed to the relatively low amount of cells in WAS (Cella et al., 2015). Nonetheless, more detailed research is necessary to determine the specific energy required to damage or rupture cell walls in thickened WAS.

2.6.2.2. Carbohydrates, proteins and humic substances. During ultrasonic pre-treatment, for both temperature-controlled and uncontrolled conditions, the solubilization of proteins is higher compared to carbohydrates (Table 8), which matches with some findings from thermal pre-treatment below 100 °C. During temperature-controlled ultrasonic pre-treatment, only a marginal increase in VFA concentration was observed (Cella et al., 2015). It remains unclear whether sonication is able to break down carbohydrates and proteins, as well as its effect on humic substances, which leaves ample room for further research.

2.6.2.3. Hydrolysis rate and biodegradation. Hydrolysis rate increases with ultrasonic pre-treatment (Braguglia et al., 2012; Kianmehr et al., 2013; Zorba and Sanin, 2013). This can be ascribed to the inherent floc dispersion (de-agglomeration) which leads to an increased surface area; and also because of better mixing and diffusion of the components (Bougrier et al., 2006; D.-H. Kim et al., 2013; Rombaut et al., 2014; Sotodate et al., 2009).

Regarding sludge biodegradation, due to the associated increase in temperature (which could reach up to 70 °C) under uncontrolled conditions, an analysis must consider the effect of temperature in order to differentiate between thermal and ultrasonic effects on the biodegradation. As shown in Table 9, when temperature was kept below 45 °C, the increase in biodegradation was in the range of 1–5.5% (entries a to d), even though extensive COD solubilization occurred (Kianmehr et al., 2013). An explanation could be that most of the cell walls are only partially damaged or ruptured during ultrasonic pre-treatment and because biopolymers are only solubilized but not degraded. However, the study from Braguglia et al. (2015) (entry e) is an exception to this trend, as the increase in biodegradation was 14%. In addition, a full-scale study in Nieuwgraaf WWTP, The Netherlands, where about 35% of the total WAS stream was exposed to sonication at temperatures below 30 °C (the temperature increase was 7 °C) and $D = 0.096$ kW/L, resulted in a E_s of about 8 kJ/gTS. No difference between the sonication process and the untreated process was observed, neither for soluble COD increase nor for VS reduction (STOWA, 2013). On the other hand, when control of temperature is not carried out (entries f-g), biodegradation increased in the order of 7–18%, as well as

Table 7
Effect of ultrasounds application in cell disruption.

Entry	Solids concentration	Ultrasonic density, (kW/L)	Specific energy (E_s)	Damaged cells (%)	Comment	Reference
a	0.00253 g TSS/L*	0	0	13	Control	(Guo et al., 2014)
		2.0	95,000 kJ/gTSS	20	Sonicated sample	
b	0.006–0.0088 gTSS/L*	0	0	18	Control	(Foladori et al., 2007)
		0.3–1.5	20,000 kJ/gTSS	25	Sonicated sample	
c	42 gTS/L	0	0	≈33	Control	(Cella et al., 2015)
		1	2.37 kJ/gTS	≈50	Sonicated sample	

* Low solids concentrations are caused by dilution.

Table 8
Release of biopolymers after ultrasonic pre-treatment.

Entry	Temperature, °C	Ultrasonic density, (kW/L)	E_S, kJ/gTS	Change in soluble carbohydrate (vs. control)	Change in soluble protein (vs. control)	Change in soluble humic substances (vs. control)	Reference
a	30	0.65	2.5–21	↑ ≈ 9 times	↑ ≈ 23 times		(Tian et al., 2015b)
b	"un-controlled"	0.1	100	↑11.4 times	↑ 13.3 times	↑ 3.9 times	(Jaziri et al., 2012)
c	"no increase detected"	0.5	2.209	↑ 8.0 times	↑ 10.9 times		(Braguglia et al., 2015)
d	"no increase detected"	0.5	8.838	↑25 times	↑25 times		(Braguglia et al., 2015)
e	Not disclosed	0.18	13.14 (as kJ/gSS)	↑0.5 times			(Brown and Lester, 1980)

methane production (entry h). It could therefore be inferred that thermal effects most likely causes the difference in sludge biodegradation. According to Salsabil et al. (2009), the production of biogas was proportional to E_s in the range 3.6–108 KJ/gTS, thus a higher energy input may result in remarkable increases in biodegradation. Nonetheless, E_s values of 14.3 and 25.9 kJ/gTS required hypothetical sludge biodegradation of 160 and 261%, respectively, implying that energy should be supplied in order to reach a neutral energy balance (Table B.2).

The previous results suggest that temperature-controlled ultrasonic pre-treatment has lower methane yield compared to temperature-uncontrolled conditions. Tuan et al. (2016), already suggested that a better performance is obtained from the coupled effect between sonic waves and temperature. In other words, the effect of ultrasonic pre-treatment alone (i.e. excluding thermal effects) barely increases the WAS biodegradation, while an increase in temperature results in higher biodegradation, probably described by the mechanisms of thermal pre-treatment below 100 °C. Finally, application of ultrasonic pre-treatment at 80 °C resulted in similar COD solubilization to the sole application of temperature at 80 °C (Delmas et al., 2014).

2.6.3. Limiting factors, advantages and perspectives

The energetic efficiency of ultrasonic pre-treatment is a burden because two energy conversions (with their respective energy loses) are carried out: conversion of electrical energy to mechanical vibration, and the conversion of mechanical vibration into cavitation (Pérez-Elvira et al., 2010). For instance, Chatel (2016) remarked that the conversion of electrical energy provided by the generator into acoustic energy transmitted to the medium is currently about 30–40% efficient for low-frequency ultrasound. This could be a reason why ultrasonic pre-treatment generally leads to negative electric energy balances, as observed in Table A.1. The main drawbacks of ultrasonic pre-treatment are thus a) electricity is required instead of heat and; b) a WAS solids concentration below 4% is required, as a higher solids concentration decreased the efficiency of the ultrasonic pre-treatment (Pilli et al., 2016; Sahinkaya, 2015). Even though temperature-controlled ultrasonic pre-treatment results in a marginal rise in biodegradation, better outcomes are observed for temperature-uncontrolled ultrasonic pre-treatment. However, the observed increases in biodegradation are still less than those achieved by low-temperature pre-treatment.

2.7. Hydrodynamic cavitation, milling and homogenization

2.7.1. Process description and mode of action

In addition to ultrasonic pre-treatment, hydrodynamic cavitation, milling and homogenization are also examples of mechanical pre-treatments. Cavitation can be produced by ultrasonic pre-treatment, but also by the flow of liquid under controlled conditions through venturi tubes or orifice plates (Kumar and Pandit, 1999; Lee and Han, 2013). The resulting cavitation bubbles result in disintegration of the sludge (Hirooka et al., 2009). According to Kim et al. (2008), the inclination angle and the number of venturi constrictions have been shown to be factors that affect the amount of COD solubilization.

Sludge milling consists of a grinding mechanism in which moving beads impact the sludge particles by provoking their breakage. Jung et al. (2001) observed that the collision frequency between the moving beads correlated with sludge solubilization rates.

Finally, homogenization is a method for cell disruption, originally developed for the stabilization of food and dairy emulsions (Zhang et al., 2012). During homogenization, sludge is pressurized to between 30 and 150 MPa for 3–30 min, after which the sample is accelerated through a convergent section and collides on an impact ring. As a result, the effects of pressure gradient, cavitation and shear are present (Zhang et al., 2012). During homogenization, pressure is the most significant factor for increasing the solubilization of COD (Li et al., 2014; Wahidunnabi and Eskicioglu, 2014; Zhang et al., 2012), while the number of homogenization cycles is less relevant (Zhang et al., 2012).

Milling and homogenization can result in temperature increases up to 43 °C (Jung et al., 2001; Zhang et al., 2012). This is lower than the threshold temperature required to cause significant changes in the physicochemical characteristics of WAS (see section 2.1). Furthermore, mechanical pre-treatments use electricity as energy input.

2.7.2. Effects on WAS

2.7.2.1. Floc and cell disruption. Homogenization (temperature not mentioned) reduced the floc size: the median size of WAS was reported to become 3 to 4 times smaller (Fang et al., 2015). Similarly, a reduction in particle size was observed for milling (Lajapathi Rai et al., 2008). Cell disruption has also been reported; ball milling with temperature control, at an energy input of around 5 kJ/gTS, achieved a cell inactivation of 30%, while it increased to 80% at 35 kJ/gTS (Lajapathi Rai et al., 2008). This shows that for extensive cell disruption to occur, energy inputs must be substantially high.

2.7.2.2. Carbohydrates, proteins and humic substances. During ball milling, COD solubilization increased in proportion to energy input, similar to cell disruption (Lajapathi Rai et al., 2008). The same trend was observed for homogenization at pressures up to 60 MPa (Fang et al., 2015). Nonetheless, the solubilization extent was limited to about 17% for a homogenization pressure of 80 MPa and E_s = 5.351 kJ/gTS, while both protein and polysaccharide concentrations in sludge supernatant increased by a factor 1.4 (Zhang et al., 2012). For a depressurization at 0.52 MPa (75 psi), the soluble COD concentration in pre-treated sludge increased by 20 ± 2%, while soluble protein

Table 9
Effects of ultrasonication on WAS biodegradation.

Entry	Temperature	Ultrasonic density, (kW/L)	E_s, kJ/gTS	Change in biodegradation (vs. control)	Change in CH_4 production (vs. control)	Notes	Reference
Temperature control							
a	Started at 25 °C and “not increased significantly”	1.9	3.8	↑ 3% (from 20 to 23)			(Seng et al., 2010)
b	$T < 45$ °C	3.7		↑1–3%			(Kianmehr et al., 2013)
c	$T < 35$ °C	0.165	2.37	↑2% (from 41 to 43)		mix of primary sludge and WAS (33:67)	(Cella et al., 2015)
d	$T < 30$ °C	0.65	9	↑ 6% (from 56 to 62)		mix of primary sludge and WAS (1:1)	(Tian et al., 2015b)
e	“no temperature increase detected”	0.6	4.42	↑14% (from 34 to 48)		mix of primary sludge and WAS (33:67)	(Braguglia et al., 2015)
No temperature control							
f	No-controlled	7.5		↑ 7% (from 36 to 43)			(D.-H. Kim et al., 2013)
g	No-controlled	≈ 13.3	2.57	↑ 18% (from 42 to 60)			(Pérez-Elvira et al., 2010)
h	up to 70 °C	0.51			↑ 74%		(Apul and Sanin, 2010)

increased by 72% relative to the control (Dhar et al., 2011). The solubilization values of some mechanical pre-treatments are considerably lower compared to other techniques with comparable energy inputs (Braguglia et al., 2015; Tian et al., 2015b). No information could be found regarding a possible change in the structure of carbohydrates, proteins and humic acids during the mechanical pre-treatments.

2.7.2.3. Hydrolysis rate and biodegradation. Limited increases in hydrolysis rate, e.g. from 0.14 to 0.16 d^{-1}, have been observed for homogenization and hydrodynamic cavitation (Dhar et al., 2011; Elliott and Mahmood, 2012; Lee and Han, 2013). Recorded increases in biodegradation were from 38 to 43% (Dhar et al., 2011) and from 13 to 15% (Lee and Han, 2013) for the same pre-treatments.

2.7.3. Limiting factors, advantages and perspectives

For ball milling and homogenization, it has been observed that a higher concentration of solids in the sample resulted in lower solubilization (Baier and Schmidheiny, 1997; Zhang et al., 2012). For hydrodynamic cavitation with temperature control, Kim et al. (2008) observed that a higher concentration of solids (in the range 0.5 to 4%TS) resulted in higher solubilization. The latter behavior is contradictory to what has been found for ultrasonic pre-treatment (Section 2.7), on which an optimum value for solubilization has been found to be around 3% TS. It could be hypothesized that ultrasonic pre-treatment is limited in its ability to generate cavitation in concentrated sludge, whereas hydrodynamic cavitation may be less dependent of the solids concentration of WAS. In fact, the few comparisons between hydrodynamic and ultrasonic cavitation are contradictory: Lee and Han (2013) compared ultrasonic pre-treatment (apparently with no temperature control) and hydrodynamic cavitation using WAS with about 1% TS at comparable specific energy values, and found that COD solubilization was similar in both cases. In contrast, Kim et al. (2008) found that ultrasonic pre-treatment reached three times higher COD solubilization as compared to a venturi using WAS at 4% TS.

During ball milling the solubilization of organic material was not in-line with the enhanced biodegradation (Baier and Schmidheiny, 1997; Elliott and Mahmood, 2012; Wett et al., 2010). In all cases, the biodegradation remained low. This observation is puzzling and deserves further research, because of its significant implications for volume reduction in full scale-facilities. Finally, despite more information being required regarding the energy consumption of hydrodynamic cavitation, milling and homogenization, these techniques seem to result in a WAS biodegradation and energy requirements that are fairly comparable to other techniques, such as low-temperature pre-treatment. However, relevant differences are that energy must be provided in the form of electricity and there is the requirement of pressurized vessels for homogenization and hydrodynamic cavitation.

2.8. Chemical pre-treatments

This category is sub-divided into alkaline pre-treatment, acid pre-treatment and advanced oxidation processes that use radicals to decompose organic matter.

2.8.1. Alkaline pre-treatment

2.8.1.1. Process description and mode of action. Alkaline pre-treatment consists of the increase in pH of the WAS matrix by means of an alkali, such as NaOH or $Ca(OH)_2$, amongst others. The structure, surface properties and electrostatic charge of extracellular polymeric substances (EPS) are modified due to pH changes (Wang et al., 2012).

The physicochemical properties of EPS depend on whether its functional groups, such as carboxylic or sulfate groups ($pK_a \approx 4$, Wang et al., 2012) and amino groups, (pK_a 7.0–9.0, Wang et al., 2012) are deprotonated or protonated. A pH increase caused by alkaline pre-treatment could therefore have a pivotal effect on the structure of the WAS matrix.

One effect of alkaline pre-treatment is the dissociation of acidic groups in EPS, which causes electrostatic repulsion between the negatively charged EPS (Wingender et al., 1999) and increases its solubilization. The repulsion is mostly due to the ionization of carboxyl and amino groups from proteins (Liao et al., 2002). Saponification of the lipid by-layer of the cell membrane (and a subsequent release of intracellular contents) and protein denaturation due to extreme pH values is another effect (Vaclavik and Christian, 2008). Relevant control parameters are the target pH and the (amount of) reagent used. Application times in the order of two hours are common; it has been found that around 60–82.9% of the total COD is released after 10–24 h (Chang et al., 2002; Li et al., 2008).

The type of reagents used, especially the metal salts, also affect dewaterability of digestate and VS reduction. For instance higher COD solubilization has been observed for sodium hydroxide compared to calcium hydroxide when using the same dose (in charge equivalents) (Li et al., 2008; Rajan et al., 1988). Similarly, at pH 12 with NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$ the COD solubilization values were 39.8%, 36.6%, 10.8% and 15.3%, respectively (Kim et al., 2003). Also, a higher VS removal has been observed for NaOH (34%) compared to $Ca(OH)_2$ (30%) (Ray et al., 1990). The same effect has been measured for the WAS biodegradation: Ray et al. (1990) observed that under the same alkali dose the use of NaOH caused a significantly higher biogas production compared to $Ca(OH)_2$. In contrast, the dewatering performance of WAS, could be improved by adding calcium hydroxide instead of sodium hydroxide (Su et al., 2013). The lower COD solubilization and a better dewaterability obtained with calcium hydroxide could be caused by the calcium bridging (Wang et al., 2012; R. Wang et al., 2016b). Guan et al. (2012) observed that Ca^{2+} interacted with proteins, phenols and carboxylic and hydroxyl functional groups in the sludge flocs. This leads to re-flocculation of dissolved organic polymers, and thus to a decrease in soluble COD (Jin et al., 2009).

2.8.1.2. *Effects on WAS*

2.8.1.2.1. Floc and cell disruption. It has been observed that the average particle size of the sludge during alkaline pre-treatment decreases (Doğan and Sanin, 2009; Kim et al., 2003; Xiao et al., 2015). The high pH values cause floc break-up, which relates to an increase in hydrolysis rate and worsened dewaterability of the digestate. With regard to cell disruption, in the pH range of 8.0–12.5, Xiao et al. (2015) observed that most of the damage to the cell wall and cell membrane occurred at pH 10.00–12.50 and pH 9.00–12.50, respectively. Similarly, in a pure culture of *Flovabacterium aquatile* (a representative bacteria of WAS), disruption was observed to occur up to a pH of 12.30 (Erdincler and Vesilind, 2000), while Doğan and Sanin (2009) determined that pH 11 did not significantly cause cell damage compared to pH 12–12.5. Literature data suggest that extensive cell disruption is achieved at pH around 10–12.

2.8.1.2.2. Carbohydrates, proteins and humic substances. The solubilization of organic matter has been reported to be low at pH < 10 and was found to increase with increasing pH (Chang et al., 2011; Valo et al., 2004; Xiao et al., 2015). However, a pH value of 13, only lead to a marginal increase in solubilization of organic matter as compared to pH 12 (Chang et al., 2011). During alkaline pre-treatment, proteins are solubilized (Brown and Lester, 1980; Doğan and Sanin,

Table 10
Effects on the biodegradation of WAS after alkaline pre-treatment.

Entry	Temperature, °C	Reagent	pH	Required reagent to reach pH, mg reagent/gTS_{sludge}	Change in biodegradation (vs. control)	Change in biogas production (vs. control)	pH neutralization	Reference
pH from 8 to 11.6								
a	25	NaOH	8.07	76	↑ 13% (from 28 to 41)		No neutralization	(Lin et al., 1997)
b	“Ambient”	NaOH	10	Not disclosed		↑15.4%	Down to pH 6.8	(Shao et al., 2012)
c	20	NaOH	10.10	40	↑ 4% (from 30 to 34)		Down to pH 8.5	(Ruffino et al., 2016)
d	20	NaOH	11.60	80	↑ 5% (from 26 to 31)		Down to pH 7.5	(Ruffino et al., 2016)
e	20	NaOH	11.60	80	↑ 4% (from 26 to 30)		Down to pH 8.5	(Ruffino et al., 2016)
pH ≥ 12.00								
f	“Ambient”	NaOH	≈ 12.0	150.3	↑ 35% (from ≈17 to ≈52)		Not disclosed	(Penaud et al., 1999)
g	“Ambient”	NaOH	12	Not disclosed		↓18.1%	Down to pH 6.8	(Shao et al., 2012)
h	“Room”	KOH	12	213		=	Not disclosed	(Valo et al., 2004)
i	“Ambient”	NaOH	12	184	↑ 3% (from 26 to 29)		Not disclosed	(Kim et al., 2003)
j	“Ambient”	NaOH	≈ 12.2	852.9	↓12% (from ≈17 to ≈5)		Not disclosed	(Penaud et al., 1999)
k	25	NaOH	12.26	128	↑ 13% (from 28 to 41)		No neutralization	(Lin et al., 1997)

2009; Shao et al., 2012), and partly degraded (Wang et al., 2012). In contrast to proteins, at pH 10 and with application of $Ca(OH)_2$, the hydrolysis of carbohydrates was not significantly affected (Su et al., 2013). Despite humic acids being stable compounds, considerable changes are observed in high pH solutions. For instance, their solubility increases in direct proportion to pH (Tipton et al., 1992).

2.8.1.2.3. Hydrolysis and biodegradation. After neutralization of the pre-treated sample, Shao et al. (2012) observed that the hydrolysis rate increased applying pH 10, but it was reduced using pH values of 11 and 12. The increase in hydrolysis rate could be explained by the solubilization of organic matter and the observed particle size reduction of WAS. The reduction in particle size can be ascribed to the formation of refractory products under highly alkaline conditions. Table 10 lists results from alkaline pre-treatments at room temperature. The majority of the reported studies had been performed with NaOH dosing. From intra-study comparison, it was concluded that different pH values result in different biogas yields. For example, after pre-treatment and neutralization, Shao et al. (2012) observed that biogas production was the highest when applying pH 10, and decreased in the following order: pH 9, pH 8, pH 11. In that study, the biogas production at pH 12 was 18.1% lower than the control. Similarly, Valo et al. (2004) found that the biogas production using pH 12 (without post-neutralization but using a substrate:inoculum ratio of 1:10 *v*/v) was the same as the control. In general, pH values lower than 12 increased the biodegradation only marginally, i.e. by about 5% in absolute terms (Table 10, entries a to e). In contrast, above pH 12, the effects on biodegradation or biogas production become variable and contradictory (entries f to k), which could be caused by:

1) the formation of refractory compounds. Despite the observation that biodegradation remains the same or is even reduced at pH 12 (Penaud et al., 1999; Valo et al., 2004), an observed continuous increase in COD solubilization (Chang et al., 2011; Uma Rani et al., 2012; Valo et al., 2004), suggests formation of soluble but refractory compounds. A similar trend is observed for thermal hydrolysis.

2) inhibition during anaerobic digestion caused by chemical reagents. Sodium concentrations of 3 g Na^+/L or higher can inhibit digestion (Feijoo et al., 1995; D.-H. Kim et al., 2013). On the other hand, Penaud et al. (1999), found that just the presence of hydroxyl ions decreased biodegradation at a dosage of 327 mg NaOH/gTS or 5.7 g Na^+/L (the presence of a buffer during the digestion was not clear). Moreover, after adaptation to high salinity conditions, sludge methanogenesis may proceed well, even under marine conditions (Zhang et al., 2013).

A final consideration regarding biodegradation is the adjustment of pH before anaerobic digestion. Pre-treatment of sludge at pH 12 in a semi-continuous digestion, without pH adjustment after pre-treatment, increased biodegradation by 13% (Table 10, entry k), resulting in a pH of 7.84 in the digester (Lin et al., 1997). Provided that ammonium toxicity is more likely to occur at higher pH (Chen et al., 2008; Hansen et al., 1998), the previous result suggests that anaerobic digestion can cope with high pH values, thus avoiding or at least reducing the need of a neutralization step. Acclimation is also a possibility, as reviewed by Chen et al. (2008).

2.8.1.3. Limiting factors, advantages and perspectives. A clear disadvantage of alkaline pre-treatment is the cost of chemical reagents and the addition of inorganics to the WAS. The production of chemicals requires energy (Folke et al., 1996; Kent, 2013), and therefore the energy feasibility of the process should be considered for alkali addition. Application of alkaline pre-treatment increases biodegradation in a similar degree as low-temperature thermal pre-treatment (see Table 2 and Table 10), although the energy input for alkaline pre-treatments seems lower according to Table A.1, even when the required energy for the manufacture of the alkali is considered.

2.8.2. Acid pre-treatment

2.8.2.1. Process description and mode of action. During acid pre-treatment, pH ranges between 1 and 5.5 and chemical reagents such as HCl, H_2SO_4, H_3PO_4 and HNO_2 are commonly used. In order to form HNO_2 (the protonated form of nitrite), a nitrite solution is added to reach a concentration of around 1 g NO_2^-/L, while the pH is lowered to around 5.5. The resulting concentration of HNO_2 can be calculated via methods shown by prior research (Wang et al., 2013; T. Zhang et al., 2015a). In contrast to other acids, HNO_2 does not seem to completely act under a pH-related mechanism (Zahedi et al., 2016). Lipid peroxidation (Horton and Philips, 1973) and the disruption of the cell envelope (Pijuan et al., 2012; Wu et al., 2018), are also effects of HNO_2. For acid pre-treatment, the protonated states of the functional groups in EPS result in more dense and compact structures at a lower pH because of hydrophobicity and intermolecular hydrogen bonds (Wang et al., 2012). At low pH, hydrogen ions react with the ionized carboxyl groups to form undissociated acid groups and therefore carboxylic acid groups are present in their un-ionized form. Thus, ionization of the acid groups is suppressed, causing aggregation. The highest flocculation efficiency is achieved near the isoelectric point (Wang et al., 2012). Regarding proteins, their isoelectric point differs for each one of them, and depends upon the ratio of free ionized carboxyl groups to free ionized amino groups (Vaclavik and Christian, 2008).

2.8.2.2. Effects on WAS

2.8.2.2.1. Floc and cell disruption. Below the pH value of the isoelectric point of the sludge, a positive charge is present within the EPS, which prevents aggregation (Liao et al., 2002; Wang et al., 2012) and causes a reduction in particle size. For instance, at a pH 0.98 with application of H_2SO_4, Guo et al. (2014) observed that the average particle size was reduced from 159.62 to 97.18 µm. Similarly to alkaline pre-treatment, saponification of the cell wall could also occur under acidic pH values (Charton, 1975). Under acidic conditions, about 15% of the bacterial cells suffered from damaged cell membranes, compared to about 10% for the control (Guo et al., 2014). In contrast, when HNO_2 was used, even at pH 6, viable cells accounted for 20% in contrast to 80% for the control (Pijuan et al., 2012). A significant reduction in

Table 11
Effects on the biodegradation of WAS after acid pre-treatment.

Entry	Temperature, °C	Reagent	pH	Required reagent to reach pH, mg reagent/gTS_{sludge}	Change in biodegradation (vs. control)	pH neutralization	Reference
a	Not disclosed	HCl	1.00	369	↑12% from 41 to 53%	Up tp pH 6.8	(Devlin et al., 2011)
b	Not disclosed	HCl	2.00	184	↑6% from 41 to 47%	Up tp pH 6.8	(Devlin et al., 2011)
c	25	NO_2^- (then formed HNO_3)	5.5 (controlled)	19.3	↑9% from 36 to 45%	Not disclosed	(Wang et al., 2013)
d	25	NO_2^- (then formed HNO_3)	5.5 (controlled)	18.2	↑20% from 33 to 53%	Not disclosed	(T. Zhang et al., 2015a)

viable cells was also found by Wu et al. (2018), suggesting the biocidal effect of HNO_2.

2.8.2.2.2. Carbohydrates, proteins and humic substances. Even though mild acid pre-treatment at pH 4 and 5 is able to solubilise COD, alkaline pre-treatment is reported to be more effective for releasing COD (Chen et al., 2007; Guo et al., 2014). Acid pre-treatment with HCl or H_3PO_4 is effective for COD solubilization but, only at pH values of 1–2 (Devlin et al., 2011; Sahinkaya, 2015), with a 4-fold and a 6-fold increase in solubilization of carbohydrates and proteins, respectively (Devlin et al., 2011). On the other hand, application of HNO_2 at pH 5.5 even led to deamination (T. Zhang et al., 2015a). Humic acids are expected to precipitate under acidic conditions ($pH < 2$), while fulvic acids remain in solution (Ghabbour and Davies, 2001). Humic and fulvic acids can form complexes with proteins, which are difficult to degrade, but lowering the pH can cause the release of proteins from these complexes, increasing bio-availability (Zahedifar et al., 2002). However, if the pH is adjusted before digestion, humic acid-protein complexes will be re-established. HNO_2 was also able to solubilize carbohydrates and proteins (Li et al., 2016).

2.8.2.2.3. Hydrolysis and biodegradation. Application of acid can lead to an increase in bioconversion rate (Devlin et al., 2011; Wang et al., 2014) as well as biodegradation (Devlin et al., 2011). Biodegradation increased substantially when the applied pH values reached 1 and 2 (Table 11, entries a and b), but not at pH values of 3 to 6 (Devlin et al., 2011), when using different doses of HCl. The application of HNO_2 (Table 11, entries c and d), resulted in similar increases in biodegradation compared to hydrochloric acid (HCl), although it may inhibit methanogens (Li et al., 2016).

2.8.2.3. Limiting factors, advantages and perspectives. Acid pre-treatment requires pH levels of $pH \leq 2$ to deliver positive results (Devlin et al., 2011; Sahinkaya, 2015). Such a low pH value requires considerable amounts of both acid and alkaline solutions to adjust the pH. Also, the reactors should be made of a material able to withstand these low pH values, thus increasing capital investment. To our knowledge no studies have been conducted on the effect of pH readjustment after acid pre-treatment. However, it is widely known that methanogenic organisms are easily inhibited at low pH, inferring that neutralization is mandatory for acid pre-treatment. Regarding the energy balance, added chemicals have an energy value and therefore acid pre-treatment with HCl results in a similar energy balance and sludge biodegradation compared to alkaline pre-treatment (Table A.1), although a lower solubilization during pre-treatment and a lower VS reduction during digestion is reached. The use of HNO_2 for pre-treatment seems to provide comparable biodegradation increases as with the use of HCl, but at milder pH values (e.g. 5.5). Furthermore, rejected water produced after digestate dewatering could be used as a precursor for HNO_2 (Wang et al., 2013). As a consequence, HNO_2 could be a sustainable, yet effective solution for WAS pre-treatment. However, additional research is required to unveil the underlying mechanisms of pre-treatment with HNO_2 and to confirm the observed results.

2.8.3. Advanced oxidation processes (AOPs)

2.8.3.1. Process description and mode of action. Advanced oxidation relies on the oxidation abilities of hydroxyl radicals, a highly reactive and short-living species. Its reactivity is due to its standard electrode potential of +2.3 V (Farr and Kogoma, 1991). As a consequence, hydroxyl radicals react with (almost) every organic substance present in WAS without any selective mechanism. Advanced oxidation can even lead to the complete mineralization of WAS (Xu et al., 2010). The formation of radicals originates from processes such as ozonation and Fenton chemistry and from compounds such as hydroperoxides (i.e. hydrogen peroxide). These three oxidative agents will be discussed below.

Ozone (O_3) is an unstable molecule produced by the electrical stimulation of oxygen. Once produced, it is transferred from the gaseous into the liquid phase, where it decomposes into radicals and reacts with WAS (Bougrier et al., 2006). Ozone breaks high molecular weight organic compounds into lower weight products, such as carboxylic acids, hydrophilic acids, carbohydrates, amino acids and VFA (Bougrier et al., 2007a; Bougrier et al., 2006; Salsabil et al., 2010). The formation of acid compounds correlates with a typical decrease in the pH of about 1 or even 2 units after ozonation (Bougrier et al., 2006; Chu et al., 2008; Tian et al., 2015b). Yang et al. (2013) observed that ozone first reacted with the soluble fraction of the sludge and then oxidized the particulate fraction. However as more intracellular substances are released, the soluble fraction can have a scavenging effect on the ozone (Cesbron et al., 2003). In line with this finding, Bougrier et al. (2006) observed that the biodegradation of the particulate fraction was barely modified.

The Fenton reaction leads to the formation of hydroxyl radicals through the reduction of hydrogen peroxide by reduced transition metals (typically iron), working as catalysts (Kohanski et al., 2007). The rate and extent of the Fenton reactions are dependent on iron and hydrogen peroxide concentration, as well as on the pH of the solution (Erden and Filibeli, 2011; Sahinkaya, 2015). At pH values of 4 and higher, ferric ion precipitates and loses its catalytic activity (W. Zhang et al., 2015b). For WAS pre-treatment mainly pH values of 2 or 3 have been applied (Bao et al., 2015; Gong et al., 2015). Three features make this technique appealing: a) hydroxyl radical formation; b) low pH similar to acid pre-treatment; and c) improved dewaterability of the digestate due to coagulation by iron addition (Neyens and Baeyens, 2003). However, the addition of iron salts and other chemical reagents to adjust the pH leads to increased costs and energy usage.

With the aim of reducing resource consumption, milder pre-treatments have been studied. The standalone application of H_2O_2 oxidizes organic compounds to CO_2 and water (Eskicioglu et al., 2008) without leaving detrimental by-products nor disturbing sludge pH (Jung et al., 2014), in contrast to ozonation.

2.8.3.2. Effects on WAS

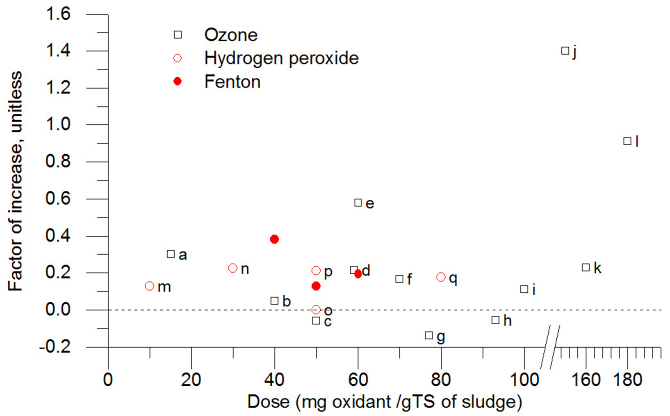
2.8.3.2.1. Floc and cell disruption. Oxidizing agents reduce the particle size of WAS (Demir and Filibeli, 2012). However, the reduction ceases at 100 to 160 mg O_3/g TS (Bougrier et al., 2006). Oxidizing agents also disrupt cell walls, as up to 50% of microorganisms were observed to be deactivated at an ozone dose of 20 mg O_3/g TSS (Chu et al., 2008).

2.8.3.2.2. Carbohydrates, proteins and humic substances. An important effect during AOPs is the mineralization process. It has the advantage of reducing the volume of solids, but the drawback of converting organic carbon to CO_2 (Déléris et al., 2000), resulting in less organic matter available for biogas production. Mineralization (measured by the reduction in COD) seems to increase with increased doses of oxidizing agent. A low dose of oxidizing agent (70 mg O_3/g TS) was reported to result in minimal mineralization (Braguglia et al., 2012), while high doses > 200 mg O_3/g TS resulted in extensive mineralization (Ahn et al., 2002; Déléris et al., 2000). Sugars and humic acids showed the highest reduction in concentration during oxidative pre-treatment (Eskicioglu et al., 2008).

The reaction between radicals and proteins could lead to

Table 12
Effects on the biodegradation of WAS after AOPs pre-treatments.

Entry	Type of AOP	Oxidant dose, mg reagent/ gTS_{sludge}	Oxidant dose, mmol reagent/gTS_{sludge}	Change in biodegradation (vs. control)	Change in biogas production (vs. control)	Reference
a	Ozone	15	0.31		↑ 30%	(Bougrier et al., 2007a)
b	Ozone	40	0.83		↑ 4.7%	(Silvestre et al., 2015)
c	Ozone	50	1.04		↓ 5.8%	(Braguglia et al., 2012)
d	Ozone	59	1.23		↑ 21.6%	(Silvestre et al., 2015)
e	Ozone	60	1.25		↑ 58%	(Bougrier et al., 2007a)
f	Ozone	70	1.46		↑ 16.7%	(Braguglia et al., 2012)
g	Ozone	77	1.60		↓ 13.8%	(Silvestre et al., 2015)
h	Ozone	93	1.94		↓ 5.6%	(Silvestre et al., 2015)
i	Ozone	100	2.08		↑ 11%	(Bougrier et al., 2006)
j	Ozone	150	3.13		↑ 140%	(Bougrier et al., 2007a)
k	Ozone	160	3.33		↑ 23%	(Bougrier et al., 2006)
l	Ozone	180	3.75		↑ 91%	(Bougrier et al., 2007a)
m	H_2O_2	10	0.29	↑ 4% (from 33 to 37)		(T. Zhang et al., 2015a)
n	H_2O_2	30	0.88	↑ 8% (from 33 to 41)		(T. Zhang et al., 2015a)
o	H_2O_2	50	1.47		= (as CH_4)	(Zhou et al., 2015)
p	H_2O_2	50	1.47	↑ 7% (from 33 to 40)		(T. Zhang et al., 2015a)
q	H_2O_2	80	2.35	↑ 6% (from 33 to 39)		(T. Zhang et al., 2015a)
r	Fenton	pH 3 Fe^{2+} = 4 mg/gTS H_2O_2 = 40 mg/gTS	1.18	↑ 12% (from 20 to 32)		(Sahinkaya, 2015)
s	Fenton	pH 2 Fe^{2+} = 7 mg/gTS H_2O_2 = 50 mg/gTS	1.47	↑ 6% (from 48 to 54)		(Zhou et al., 2015)
t	Fenton	pH 3 Fe^{2+} = 4 mg/gTS H_2O_2 = 60 mg/gTS	1.76		↑ 19.3% (as CH_4)	(Erden and Filibeli, 2011)



Note: for Fenton reaction, H_2O_2 is shown

Fig. 2. Effect of oxidant doses in the biogas production. Letters refer to entries in Table 11. This graph is shown in Appendix C, using mmol of oxidant/gTS in the x-axis.

deactivation and degradation of enzymes (Farr and Kogoma, 1991). Silvestre et al. (2015) hypothesized that the change in the secondary and tertiary structure of the amino-acids could explain the observed higher biogas potential. On the other hand, the reaction between hydroxyl radicals and humic substances could increase the soluble COD concentration without the formation of biodegradable organic substrates (Goldstone et al., 2002).

2.8.3.2.3. Hydrolysis rate and biodegradation. As indicated above, pre-treatments with AOPs reduce the mean particle size of WAS at low doses of oxidizing agents, which results in an increased hydrolysis rate. However, high doses of oxidizing agents were observed to lead to a decrease in hydrolysis rate (Appels et al., 2011; Silvestre et al., 2015; Tian et al., 2015b; T. Zhang et al., 2015a; Zhou et al., 2015). Liu et al. (2015) showed an inhibitory effect of AOPs on anaerobic digestion.

Increasing the doses of an oxidizing agent, leads to an increase in sludge biodegradation, however, exceeding certain doses could lead to a decrease in biodegradation, sometimes even lower than the control (Braguglia et al., 2012; Silvestre et al., 2015; T. Zhang et al., 2015a). The dose at which the maximum biodegradation could be achieved, differs among the various studies (Table 12), and is probably because of the variability in the composition of WAS that was used. The presented data, suggests that ozonation performs best around 150 mg O_3/gTS, although lower doses could be sufficient for improvement over control (Table 12 entries d to f). The lower biogas production at higher doses could be due to a) inhibitory conditions; b) mineralization of organic biodegradable compounds; and c) formation of refractory compounds (Bougrier et al., 2006; Liu et al., 2015; Silvestre et al., 2015).

At similar hydrogen peroxide concentrations, the Fenton pre-treatment outcomes are comparable to addition of H_2O_2 (Table 12, entries r to t and entries m to q respectively, Fig. 2). This is unexpected because the low pH of the Fenton reaction should favor the formation of more radicals, thus increasing organic matter degradation. The acidic environment should also increase the WAS biodegradation.

2.8.3.3. Limiting factors, advantages and perspectives. From an energy consumption viewpoint, 50% of the energy demand during ozonation is used for the production of pure oxygen as an input element (Müller et al., 2004). As a consequence, ozonation is scarcely applied to sludge because of its high electrical energy consumption (Xu et al., 2010). The Fenton reaction requires not only H_2O_2 but also other reagents to acidify and neutralize the pH of the WAS. Fenton-treated sludge contains iron-hydroxide complexes, which could limit the usage alternatives of the digested sludge in the final disposal stage (Sahinkaya, 2015). On the other hand, the sole addition of hydrogen peroxide involves only the energy required for producing the reagents. Fig. 2 shows that the three AOPs techniques presented seem to deliver similar biogas or methane increases up to an oxidizing dose of 100 mg/

g TS. Then, if the improvements in biogas production are comparable between AOPs, the best alternative seems to be H_2O_2 addition, according to the energy balance (Table A.1). H_2O_2 addition can be considered the technique with the lowest energy demand. A niche application of AOPs could be the oxidation of refractory substances (e.g. mineralization of humic substances), or for the treatment of digested sludge, as it contains a higher fraction of refractory substances compared to WAS (Kobayashi et al., 2009).

2.9. Biological pre-treatment

2.9.1. Process description and mode of action

Bacteria and archaea break down organic matter with the help of enzymes. Due to their catalytic nature, enzymes hydrolyze complex molecules at milder pH values, and temperatures, and without the production of hazardous waste, compared to chemical or physical pre-treatment methods (Parawira, 2012). The products of hydrolysis are available for further conversion. Enzymes are located both inside and outside the cells. The latter are further divided into cell surface bound (ectoenzymes) and free form (exoenzymes). Exoenzymes are located in water and/or adsorbed within the extracellular polymeric substances of the sludge matrix (Burgess and Pletschke, 2008). However, in contrast to ectoenzymes, exoenzymes do not show relevant lytic activity (Burgess and Pletschke, 2008). In order to increase the lytic potential, intracellular enzymes should be released by disrupting the cell membrane (Kavitha et al., 2014). However, free enzymes are prone to self-degradation before sludge hydrolysis starts (Müller, 2001). In order to overcome self-degradation, the enzymes must be immobilized onto solid media such as substrate, extracellular polymeric substances and flocs, which make them more stable (Burgess and Pletschke, 2008; Matsumoto and Ohashi, 2003) and usable as pre-treatment. On the other hand, immobilization has been postulated to decrease the efficiency of the pre-treatment (Kavitha et al., 2014; Parawira, 2012).

There are two ways to do enzymatic pre-treatment: active addition of enzymatic solutions, which according to Parmar et al. (2001) makes the pre-treatment economically infeasible; or by bioaugmentation. The latter method fosters the endogenous enzyme (or enzyme-producing microorganisms) (S. Yu et al., 2013). Recently, surfactants that can be biologically produced by many different microorganisms, such as rhamnolipids, have been used for pre-treatment (He et al., 2016; Kavitha et al., 2016, Kavitha et al., 2014; Zhou et al., 2013). According to Zhou et al. (2013), rhamnolipids increase the solubility of compounds and provoke the EPS to detach from the attached cell surface. Also, they observed an increase in hydrolytic enzyme activity, and postulated that rhamnolipids hindered the immobilization of naturally present enzymes in the sludge or liberated the trapped enzymes within the floc matrix.

2.9.2. Effects on WAS

2.9.2.1. Floc and cell disruption. By testing an amylase-producing-stain, a protease-producing stain and a blend of both, S. Yu et al. (2013) found that the mean particle size decreased from 87.4 μm (control) to 74.1, 85.4, and 65.8 μm for amylase, protease and blended pre-treated samples, respectively. The effect of protease was negligible in particle size reduction. They hypothesized that the reduction in particle size was not caused by the direct conversion from solid to soluble particles, but because enzymes acted against EPS that have a role in sludge aggregation. In contrast, with a protease treatment at a dosage of 100 mg/L the average particle size reduced from 128 to 81 μm (Yi et al., 2014). Operation at sub-optimal levels of enzyme may explain the conflicting results.

Endogenous enzymes did not cause significant lysis, indicating that the observed effects related to increasing soluble organic matter mainly derived from EPS, whereas enzymatic treatment on sludge integrity was limited (S. Yu et al., 2013). Sesay et al. (2006) measured viable cell counts after application of α-amylase, cellulase and proteinase, and observed an insignificant amount of cell lysis. Finally, based on scanning electron microscopic imaging and the increase in DNA, Yi et al. (2014) inferred that protease treatment did not result in bacterial cells being destroyed.

2.9.2.2. Carbohydrates, proteins and humic substances. After protease pre-treatment, only a low amount (4%) of proteins were solubilized, while the amount of soluble humic substances increased from 361 to 1108 mg/L (Yi et al., 2014) probably because of the destruction of protein-humic acid assemblies.

2.9.2.3. Hydrolysis rate and biodegradation. In some cases, the hydrolysis rate of enzymatically pre-treated WAS increased with the sole addition of a protease (Ushani et al., 2016), while in other cases a blend of amylase and protease was required to increase the hydrolysis rate (S. Yu et al., 2013). The results concerning WAS biodegradation are conflicting. In some cases, enzymatic pre-treatment (with amylase only, and amylase with protease) led to an enhancement of the specific biogas production (S. Yu et al., 2013). While in other cases the biodegradation did not increase using these enzymes (Bayr et al., 2013) nor using solely proteases (S. Yu et al., 2013). The added enzymes may increase the amount of biogas produced, due to their own digestion, and this could be a cause for the contradictory results observed. Unfortunately, no mass balances were found to corroborate this hypothesis. Finally, rhamnolipids does not seem to be a suitable pre-treatment for increasing methane production, as the activity of methanogens is inhibited (Zhou et al., 2013). Nonetheless, acidifiers are not impaired during this process, making it a suitable method for VFA production from WAS.

2.9.3. Limiting factors, advantages and perspectives

It seems that the sole addition of proteases does not result in significant solubilization (Yi et al., 2014) nor biodegradation increase (S. Yu et al., 2013), while amylase seems to increase both parameters. As different enzymes have varying pH and temperature optima and tolerance ranges, it is possible that the enzyme had to work at suboptimal levels (Burgess and Pletschke, 2008), resulting in the spread of results observed during enzymatic pre-treatment. Even though information is missing about the optimum conditions required for the use of an enzyme blend on a complex substrate such as WAS, the costs of even low-purity enzymes is still prohibitive (Parawira, 2012). Even though, bioaugmentation could be a strategy for producing the required enzymes, this also has its drawbacks. For instance, culturing the proper microorganisms demands pH control and substrate addition.

3. Overall discussion

A brief summary of the effects of different pre-treatments on the main components of WAS is presented in Table 13. In Table 14 a qualitative assessment of the effects of different pre-treatments on the sludge characteristics, regarding degradability and economic feasibility, is shown.

From Table 14 it is clear that most of the pre-treatments increase the hydrolysis rate. The improvement is particularly high for thermal and ultrasonic pre-treatments. Since hydrolysis is regarded as the rate-limiting step in WAS treatment, an increased hydrolysis rate is indicative

Table 13
Effects of pre-treatments on some WAS components.

Pre-treatment technique	Bacterial biomass	Protein	Carbohydrate	Humic substances	Effects on other components or in combinations
Thermal < 100 °C	Cell wall starts rupturing at ≥ 50 °C	Denaturation occurs at around 75 °C	Only solubilization	Unaffected. Solubilization up to around 75 °C and they become flexible at 70-80 °C (T_{glass})	Proteins and humic acids form assemblies, protecting the former from biological degradation Proteins and carbohydrates: amino carbonyl reaction occurs even at 60 °C, and rate increasing in proportion to application time
Temperature phased anaerobic digestion (TPAD)	Same as thermal < 100 °C pre-treatment	Same as thermal < 100 °C pre-treatment	Same as thermal < 100 °C pre-treatment	Same as thermal < 100 °C pre-treatment	Same as thermal < 100 °C pre-treatment
Thermal > 100 °C	Extensive cell wall and membrane disruption	Extensive denaturation Protein degradation starting at > 170-190 °C	Theory: caramelization at 170 °C (formation of aldehydes and ketones) Practice: solubilization and low degree of degradation into mono or dimeric sugars at 220 °C	≥ 110 °C gradual decomposition of humic acids, resulting in the production of CO_2 and CO Dissociation at 150-180 °C	Maillard reaction proceeds faster as compared to thermal pre-treatment below 100 °C
Microwave	Cell lysis due to thermal effects	Same as thermal pre-treatment < 100 °C and thermal hydrolysis, depending on temperature			
Freeze and thaw	Limited cell lysis probably because of cryoprotectant components (e.g. glycerol)	Enzymes still working Protein denaturation and formation of aggregates			
Ultrasonic (temperature < 40 °C)	Extensive deflocculation Limited cell disruption	Solubilization, probably caused by deflocculation			
Hydrodynamic cavitation, milling and homogenization	Limited cell lysis due to grinding or cavitation	Solubilization			
Alkaline	Extensive cell disruption at pH 12	Destruction of amide groups and unordered random coil conformation at pH 5.7–10.5	At pH 10 and application of $Ca(OH)_2$, hydrolysis of carbohydrate was not significantly affected	Solubilization and formation of negative charges	Saponification of lipids
Acidic	Limited cell membrane disruption	Denaturation and hydrolysis	Solubilization (but in a lower extent as alkaline)	Precipitation	Hydrolysis of esters
AOPs	Potential damage to cells by lipid peroxidation	Inactivation of proteins by modification of amino acid. In the absence of oxygen, cross-linking was observed		Production of inorganic carbon	
Biological	No cell lysis	With protease, very limited solubilization and degradation into amino acids	With amylase, limited solubilization, but relatively higher than proteins	Limited solubilization probably because of destruction of humic acid and protein complexes	

Table 14

Qualitative assessment of the effect of different pre-treatments on the sludge characteristics regarding degradability and economic feasibility.

Pre-treatment technique	Hydrolysis rate (k_{hyd})	Biodegradation (B_0)	VS removal	Dewaterability of digestate	Economical potential for increased methane production	Economical potential for increased VFA production	Investment costs	Operational costs
Thermal < 100 °C	↑↑	↑/↓	↑	↓	↑	↑/↓	Low	Low
TPAD	↑↑	↑↑	↑↑	↑	↑↑	↑/↓	Low	Low
Thermal > 100 °C	↑↑	↑↑	↑↑	↑	↑↑	↓	High	High
Microwave	↑/↓	↑↑	↑↑	↑	↑	↑/↓	High	High
Freeze and thaw	n/f (increase is expected)	↑	↑	n/f (improvement is expected)	↑/↓	↑/↓	Low/High	Low/High
Ultrasonic	↑↑	↑	↑	↓	↓	↓	High	High
Hydrodynamic cavitation, milling and homogenization	↑	↑	↑↑	↑	↑	↑/↓	Low	Low/High
Alkaline	↑/↓	↑	↑↑	↓	↑/↓	↑↑	Low	High
Acidic	↑/↓	↑	=	↑	↓	↓	High	High
AOPs	↑/↓	↑/↓	↑↑	↑	↑/↓	↑/↓	High	High
Biological	↑	↑/↓	n/f (increase is expected)	n/f (improvement is expected)	↑/↓	↑/↓	Low	High

Notes:↑ increase, ↓ decrease, = no change, n/f: not found in literature.

for a potential increased solids loading rate to existing sludge digesters without retrofitting the sewage treatment plant. However, in some cases chemical and microwave pre-treatments have shown conflicting results regarding the resulting hydrolysis rate. Likely, this can be ascribed to the excessive utilization of chemicals or energy inputs, which can potentially create refractory, inhibitory or even toxic products that cannot be degraded during batch tests. Thermal hydrolysis, TPAD and microwave pre-treatments all substantially increase the sludge biodegradation. In this respect, the effect of low-temperature pre-treatment on biodegradation remains somewhat uncertain. The latter could be related to the applied temperature increase rate, which has also been considered relevant for other thermal pre-treatments such as microwave and freeze and thaw (Hosseini Koupaie et al., 2017; Montusiewicz et al., 2010). Montusiewicz et al. (2010) considered the similarity of the freezing and thermal treatment effects as well, in the sense that the temperature-change rate could have an impact on the outcome of the process. Nonetheless, further research regarding the specific mechanisms of low-temperature pre-treatment are still missing.

As shown in Appendix 0 (Table B.1), the minimum amount of VS required to achieve a neutral energy balance is around 30 gVS/L for low-temperature thermal pre-treatment and about 160 gVS/L for thermal pre-treatment above 100 °C (thermal hydrolysis). In addition to the higher temperature, other characteristics of thermal hydrolysis that explain the higher minimum solids concentration are: i) the low potentials for the recovery of high-quality heat from the combined heat power plant (CHP) to be used to produce steam, and ii) a lower efficiency of heat exchange to pre-heat the incoming sludge. In order to reduce the required solids concentration, in practice, non-treated primary sludge is blended with treated WAS before digestion. This step increases methane production, and allows a lower solids concentration to be used without affecting the overall energy balance. Another option is the direct generation of steam by the biogas, instead of burning the biogas in a CHP for electricity production.

Thermal hydrolysis, TPAD, alkaline, microwave, and mechanical pre-treatments are all methods to substantially increase VS removal and thus reduce the volume of the digestate to be disposed. Microwave, ultrasonic and mechanical pre-treatment techniques all have a high electricity requirement. The pre-treatment itself could lead to an increase in biodegradation, but the energy balance regarding electricity production of these pre-treatments becomes less positive or even negative. For instance, according to Appendix 0 (Table B.2), absolute biodegradation, even exceeding the theoretical limit of 100%, is required to achieve a neutral electricity-balance in some cases, such as for ultrasonic pre-treatment. In the latter case, increasing the solids concentration is not an option as it may reduce the efficiency of the ultrasonic pre-treatment. For microwave pre-treatment, the energy consumption is even higher: a hypothetical biodegradation of 100% after pre-treatment would only cover 50% of the energy requirement (Appendix 0, Table B.2). For alkaline pre-treatments, the costs of chemical reagents remain as a principal drawback. The freeze and thaw pre-treatment requires a large surface area and naturally occurring freezing temperatures. This pre-treatment is only applicable in a few regions because of these naturally limiting factors. If freezing temperatures are to be achieved artificially, the electrical consumption would make the energy balance negative.

Regarding dewaterability, low-temperature, ultrasonic and alkaline pre-treatments, seem to reduce the dewatering ability of the digestate. In contrast, TPAD, thermal hydrolysis, acid, AOPs and microwave pre-treatments seem to improve this parameter. Floc size is known to have substantial impact on sludge dewaterability (Gao, 2011). However, the reduction in particle size after the pre-treatment does not necessarily reduce the dewaterability of the digestate, as observed for acid, AOPs and mechanical techniques. Finally, with increasing attention on VFA production instead of methane, alkaline pre-treatment looks particularly attractive, since alkali dosing is often required to promote VFA formation.

The selection of an optimal pre-treatment method depends on (natural) local conditions, but also on economics and regulations. Firstly, full-scale disintegration technologies are feasible only if the sludge disposal costs are high (Müller et al., 2004). Secondly, investment and operational costs must be considered (Table 14). In general, chemical and enzymatic pre-treatments are not favored because of the dosage of reagents, whereas thermal hydrolysis seems ideal when pasteurization or sterilization of sludge is required by regulations prior to land disposal or agricultural reuse. Thermal hydrolysis also results in a high level of solids reduction, although investment and operational costs are high as well. It should be realized that due to fluctuating energy prices, the focus on increasing methane production is not always economically appealing. In fact, solids reduction is more relevant than methane production for countries such as the Netherlands, where costs for disposal are high, since incineration is the sole outlet. VS reduction as well as increased dewaterability is not only relevant for disposal costs, but also lower required polyelectrolyte dosing could increase the economic feasibility of the pre-treatment method.

Life cycle assessment (LCA) could shed light on the overall cost effectiveness of each technique; however, studies performing LCA on sludge pre-treatment are missing in literature. Pre-treatments based on milder temperature and pH conditions, seem to represent a good opportunity to develop less resource-demanding pre-treatments. Low-temperature, TPAD and hydrodynamic cavitation are techniques that have shown interesting results without the use of harsh conditions and can be considered cost effective pre-treatment techniques.

Despite several studies comparing different pre-treatment techniques, direct comparisons are often unfair. The operational parameters of some techniques are occasionally assayed far from their optimum values, with disproportionate energy inputs or are set at values that could produce recalcitrant or even toxic by-products, resulting in reduced or low performance. It is very important to consider these factors when doing comparisons between the different pre-treatments. On the other hand, considerable research has also been done to assess the synergetic effects of the combination of several different pre-treatments. For instance, Doğan and Sanin (2009) observed that the deteriorated dewaterability caused by alkaline pre-treatment can be improved by the incorporation of microwave irradiation and the protein release can be higher compared to the summation of the release by each method individually. Synergies have also been observed by Kim et al. (2010) and Joo et al. (2015). However, the combination of two pre-treatment techniques does not always result in a direct additive effect (Eskicioglu et al., 2008; Sahinkaya, 2015; Yeneneh et al., 2013), but may lead to an increase in the consumption of energy and/or chemical reagents.

Regarding research gaps, it is clear that pre-treatments such as low-temperature or TPAD result in low electrical energy consumption compared to the more sophisticated methods, such as ultrasonic, microwave and freeze and thaw (if artificial freezing would be used). The exact working mechanisms of these pre-treatments are missing. Finally, information about the production of recalcitrant or inhibitory by-products, not only during thermal hydrolysis, but also during chemical pre-treatments, is still missing. These by-products could become problematic during anaerobic digestion of the pre-treated sludge and/or the subsequent side-stream treatment of the concentrated sludge reject water, or even for the treatment of the recycled reject water in the mainstream water line of the wastewater treatment plant.

Finally, the use of COD solubilization for judging the efficiency of pre-treatments must be discouraged. Until now, the sludge biodegradation assays or BMP tests remain as the only method to accurately assess the performance of WAS pre-treatments to maximize methane production. Proper inter-study comparisons between pre-treatments are only possible if hydrolysis rate, biodegradation and/or BMP values, and COD balances of the samples are reported. Also, additional efforts should be taken to characterize the sludge into its specific components and not merely into VS and/or COD. The use of thickened sludge in performing research on pre-treatment methods must also be promoted to account for real-world conditions. For calculations regarding the economic viability of a pre-treatment the amount of energy consumed (heat and/or electrical) by the equipment, energy losses, energy actually delivered to the sample, and in the case of chemical pre-treatments, the actual amount of reagent used, should be mentioned. Valuable add-ons are the change in dewaterability and VS reduction. All these features would increase the reproducibility and comparability of results, despite the varying composition of WAS.

4. Conclusion

Based on the current knowledge, the best alternative for a pre-treatment method is defined based on the objectives of the treatment (the listed pre-treatment techniques are not specifically ordered):

- Increasing methane production: thermal pre-treatment $> 100\,^{\circ}C$, TPAD and microwave
- Increasing volatile solids removal: thermal pre-treatment $> 100\,^{\circ}C$, TPAD and alkaline
- Improving the dewaterability of digestate: thermal pre-treatment $> 100\,^{\circ}C$, TPAD and microwave
- Lowering energy consumption: TPAD and thermal pre-treatment $< 100\,^{\circ}C$
- Lowering operating and capital costs: TPAD and thermal pre-treatment $< 100\,^{\circ}C$

Microwave, however, uses electrical energy and is thus economically not advantageous. Therefore, overall, low- and high-temperature pre-treatment and TPAD are the most promising pre-treatment methods.

Finally, to thoroughly understand the different mechanisms of different pre-treatment methods, more attention should be paid to the conversion of, and structural change in, the different (complex) components of waste activated sludge during their application.

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Appendix A

Table A.1
Energy balance.

Pre-treatment conditions	Methane generation, L_{CH4}/ $kgVS_{fed}$	Biodegradation, %	TS, g/L	VS/TS	Heat consumed, kJ/ gTS*	Electricity consumed, kJ/ gTS	Heat produced, kJ/gTS	Electricity produced, kJ/ gTS	Heat balance, kJ/ gTS	Electricity balance, kJ/ gTS	VS reduction, %	Reference
Thermal pre-treatment < 100 °C												
Control	138	28	47.40	0.73	2.52	0.00	−1.84	−1.36	0.68	−1.36	27.0	(Ruffino et al., 2015)
Pre-treated: Temperature: 80 °C Treatment time: 3 h	179	37	47.40	0.73	6.49	0.00	−2.39	−1.77	4.10	−1.77	33.5	
Pre-treated: Temperature: 90 °C Treatment time: 3 h	182	37	47.40	0.73	7.37	0.00	−2.43	−1.80	4.94	−1.80	37.5	
Control	167	32	38.20	0.70	3.13	0.00	−2.13	−1.58	1.00	−1.58	34.5	
Pre-treated: Temperature: 70 °C Treatment time: 3 h	202	39	38.20	0.70	6.96	0.00	−2.58	−1.91	4.38	−1.91	36.0	
Pre-treated: Temperature: 70 °C Treatment time: 15 h	199	38	38.20	0.70	6.96	0.00	−2.54	−1.88	4.42	−1.88	37.0	
Control	126	21	12.35	0.80	9.75	0.00	−1.84	−1.36	7.91	−1.36	35.0	(Kim et al., 2013b)
Pre-treated: Temperature: 60 °C Treatment time: 6 h	171	29	12.35	0.80	18.21	0.00	−2.49	−1.85	15.71	−1.85	45.0	
Pre-treated: Temperature: 75 °C Treatment time: 6 h	208	35	12.35	0.80	23.29	0.00	−3.04	−2.25	20.24	−2.25	45.0	
Pre-treated: Temperature: 90 °C Treatment time: 6 h	155	26	12.35	0.80	28.36	0.00	−2.27	−1.68	26.10	−1.68	45.0	
Control	203	38	48.70	0.81	3.06	0.00	−2.99	−2.21	0.07	−2.21	n/a	(Wang et al., 2014)
Pre-treated: Temperature: 35 °C Treatment time: 24 h	203	38	48.70	0.81	3.06	0.00	−2.99	−2.21	0.07	−2.21	n/a	
Pre-treated: Temperature: 55 °C Treatment time: 24 h	206	39	48.70	0.81	4.60	0.00	−3.03	−2.25	1.57	−2.25	n/a	
Pre-treated: Temperature: 70 °C Treatment time: 24 h	216	41	48.70	0.81	5.89	0.00	−3.18	−2.35	2.71	−2.35	n/a	
Thermal phased anaerobic digestion (TPAD)												
Control (single-stage): Temperature: 35 °C SRT: 14 days	90	16	25.40	0.69	4.53	0.00	−1.13	−0.84	3.40	−0.84	48	(Ge et al., 2011b)
	170	31	25.40	0.69	10.29	0.00	−2.13	−1.58	8.16	−1.58	48.	

Pre-treated: Acidogenic stage: Temperature: 70 °C SRT: 2 days; Methanogenic stage: Temperature: 35 °C SRT: 14 days												
Control (single-stage): Temperature: 55 °C SRT: 20 days	288	51	58.00	0.78	3.71	0.00	− 4.08	− 3.02	− 0.36	− 3.02	48	(Bolzonella et al., 2012)
Pre-treated: Acidogenic stage: Temperature: 65 °C SRT: 2 days; Methanogenic stage: Temperature: 55 °C SRT: 18 days	313	56	58.00	0.78	4.39	0.00	− 4.43	− 3.28	− 0.04	− 3.28	55	
Control (single-stage): Temperature: 35 °C SRT: 30 days	220	39	46.20	0.78	2.75	0.00	− 3.14	− 2.32	− 0.39	− 2.32	40.9	(Wu et al., 2016)
Pre-treated: Acidogenic stage: Temperature: 55 °C SRT: 6 days; Methanogenic stage: Temperature: 35 °C SRT: 24 days	320	57	46.20	0.78	4.46	0.00	− 4.56	− 3.38	− 0.10	− 3.38	49.1	
Control (single-stage): Temperature: 55 °C SRT: 20 days	130	33	62.40	0.69	3.45	0.00	− 1.64	− 1.21	1.81	− 1.21	34	(Leite et al., 2016)
Pre-treated: Acidogenic stage: Temperature: 55 °C SRT: 2 days; Methanogenic stage: Temperature: 55 °C SRT: 18 days	173	44	62.40	0.69	3.41	0.00	− 2.18	− 1.61	1.23	− 1.61	38	
Thermal pre-treatment > 100 °C												
Control	261	49	14.50	0.81	8.25	0.00	− 3.84	− 2.84	4.41	− 2.84	39	(Bougrier et al., 2007b)
Pre-treated: Temperature: 135 °C Treatment time: 30 min	292	55	14.50	0.81	37.08	0.00	− 4.31	− 3.19	32.77	− 3.19	41	
Pre-treated: Temperature: 190 °C Treatment time: 15 min	327	62	14.50	0.81	52.93	0.00	− 4.82	− 3.57	48.11	− 3.57	57	
Control	154	31	20.80	0.69	10.02	0.00	− 1.94	− 1.43	8.09	− 1.43	43	(Gianico et al., 2013)
Pre-treated: Temperature: 134 °C Treatment time: 20 min Pressure: 312 kPa	223	46	20.50	0.70	26.28	0.00	− 2.86	− 2.11	23.42	− 2.11	46	
Control	128	25	17.10	0.70	7.00	0.00	− 1.64	− 1.21	5.36	− 1.21	34.8	(Valo et al., 2004)
	228	45	17.10	0.70	40.00	0.00	− 2.91	− 2.16	37.08	− 2.16	66.9	

Pre-treated: Temperature: 170 °C Treatment time: 60 min Pressure: not disclosed												
Microwave pre-treatment												
Control: Mixed sludge, primary and WAS 42:58 v/v	308	53	64.00	0.73	0.35	0.00	−4.12	−3.05	−3.77	−3.05	n/a	(Eskicioglu et al., 2008)
Pre-treated: Temperature: 100 °C Mixed sludge, primary and WAS 42:58 v/v	397	68	64.00	0.73	0.35	20.66	−5.30	−3.93	−4.96	16.73	n/a	
Control: Mixed sludge, primary and WAS 42:58 v/v	192	33	51.40	0.70	0.29	0.00	−2.45	−1.82	−2.16	−1.82	37.4	(Coelho et al., 2011)
Pre-treated: Temperature: 96 °C Mixed sludge, primary and WAS 42:58 v/v	254	43	51.40	0.70	0.29	17.51	−3.25	−2.40	−2.95	15.11	44.1	
Control:	245	46	40.90	0.77	1.00	0.00	−3.41	−2.53	−2.41	−2.53	n/a	(Chi et al., 2011)
Pre-treated: Temperature: 190 °C	304	57	40.90	0.77	1.00	69.13	−4.24	−3.13	−3.24	66.00	n/a	
Freezing and thawing												
Control Mixed sludge, primary and WAS 60:40 v/v	277	57	41.00	0.77	0.37	0.00	−3.86	−2.86	−3.49	−2.86	51.9	(Montusiewicz et al., 2010)
Pre-treated: Freezing stage: Temperature: -25 °C SRT: 1 day; Thawing stage: Temperature: 20 °C SRT: 0.5 day; Mixed sludge, primary and WAS 60:40 v/v	309	63	41.00	0.77	0.37	0.00	−4.30***	−3.18	−3.93	−3.18***	53.8	
Ultrasonic pre-treatment												
Control	221	41	42.00	0.83	0.36	0.00	−3.35	−2.48	−2.99	−2.48	50.0	(Cella et al., 2015)
Pre-treated: US density: 1 kW/L Specific energy: 2.37 kJ/gTS Temperature: < 35 °C Mixed sludge, primary and WAS 33:67 v/v	231	44	42.00	0.83	0.36	14.28	−3.50	−2.59	−3.14	11.69	51.5	
Control	170	34	27.70	0.68	0.59	0.00	−2.10	−1.56	−1.52	−1.56	37	(Braguglia et al., 2015)
Pre-treated: US density: 0.5 kW/L Specific energy: 2.209 kJ/gTS Temperature: "no increase detected"	240	48	27.30	0.68	0.60	4.42	−2.99	−2.21	−2.39	2.20	40	
Control	248	42	33.30	0.83	0.49	0.00	−3.73	−2.76	−3.24	−2.76	n/a	

Pre-treated: US density: ≈13.3 kW/L Specific energy: 2.57 kJ/gTS Temperature: un-controlled	349	60	33.30	0.83	0.49	2.57	−5.25	−3.88	−4.76	−1.31	n/a	(Pérez-Elvira et al., 2010)
Control	114	20	30.00	0.87	0.68	0.00	−1.81	−1.34	−1.13	−1.34	19.6	(Seng et al., 2010)
Pre-treated: US density: 1.9 kW/L Specific energy:3.8 kJ/gTS Temperature: “not increased significantly”	129	23	30.00	0.87	0.68	3.80	−2.05	−1.52	−1.37	2.28	22.2	
Hydrodynamic cavitation, milling and homogenization												
Control	159	35	52.50	0.67	0.31	0.00	−1.95	−1.44	−1.64	−1.44	n/a	(Wett et al., 2010)
Pre-treated: Ball milling E_s = 1.76 kJ/gTS (assumed that 60% of biogas was methane) (assumed that VSS = VS)	249	54	52.50	0.67	0.31	1.76	−3.05	−2.26	−2.74	−0.50	n/a	
Control	72	13	9.85	0.65	1.07	0.00	−0.85	−0.63	0.22	−0.63	n/a	(Lee and Han, 2013)
Pre-treated: Hydrodynamic cavitation E_s = 1.20 kJ/gTS	81	15	9.85	0.65	1.07	1.20	−0.96	−0.71	0.12	0.49	n/a	
Alkaline pre-treatment												
Control: pH: 6.70 Mix sludge, primary and WAS 50:50 v/v	96	26	38.00	0.68	0.15	0.00	−1.20	−0.89	−1.05	−0.89	20.5	(Kim et al., 2003)
Pre-treated: pH: 12.00 Dose: 184 mg NaOH/gTS Neutralization: not disclosed Mix sludge, primary and WAS 50:50 v/v	109	29	38.00	0.68	4.07	1.18	−1.35	−1.00	2.72	0.18	29.8	
Control: pH: 6.86	154	28	10.15	0.75	1.49	0.00	−2.12	−1.57	−0.63	−1.57	35	(Lin et al., 1997)
Pre-treated: pH: 8.07 Dose: 76 mg NaOH/gTS Neutralization: not performed	239	41	10.53	0.69	3.05	0.49	−2.98	−2.21	0.07	−1.72	36	
Pre-treated: pH: 12.26 Dose: 128 mg NaOH/gTS Neutralization: not performed	210	41	12.48	0.69	3.94	0.82	−2.63	−1.95	1.31	−1.13	42	
Control: pH: 6.5–7.5	122	26	8.00	0.70	1.89	0.00	−1.56	−1.15	0.33	−1.15	n/a	(Ruffino et al., 2016)
	145	31	8.00	0.70	3.71	0.52	−1.85	−1.37	1.86	−0.85	n/a	

Pre-treated: pH: 11.60 Dose: 80 mg NaOH/gTS Neutralization: yes, down to pH 7.5												
Pre-treated: pH: 11.60 Dose: 80 mg NaOH/gTS Neutralization: yes, down to pH 8.5	143	30	8.00	0.70	3.69	0.52	−1.82	−1.35	1.87	−0.83	n/a	
Control: pH: 6.5–7.5	142	30	8.00	0.70	1.89	0.00	−1.34	−1.80	0.09	−1.34	n/a	
Pre-treated: pH: 10.10 Dose: 40 mg NaOH/gTS Neutralization: yes, down to pH 8.5	160	34	8.00	0.70	2.78	0.26	−1.51	−2.04	0.74	−1.25	n/a	
Acid pre-treatment												
Control: pH: 6.84 (assumed that 60% of biogas was methane)	141	31	50.00	0.74	0.18	0.00	−1.90	−1.41	−1.72	−1.41	32.7	(Devlin et al., 2011)
Pre-treated: pH: 2.13 Dose: 184 mg HCl/gTS Neutralization: yes, up to pH 6.8 (assumed that 60% of biogas was methane)	156	36	50.00	0.78	5.19	1.37	−2.22	−1.64	2.97	−0.27	34.4	
Control: pH: 6.4	201	36	42.60	0.79	0.84	0.00	−2.89	−2.14	−2.05	−2.14	n/a	(Wang et al., 2013)
Pre-treated: pH: 5.5 Dose: 19 mg NO_2^-/gTS Neutralization: not disclosed	255	45	42.60	0.79	0.84	0.00**	−3.67	−2.72	−2.83**	−2.72	n/a	
Control: pH: 6.5–6.9	164	33	36.10	0.83	1.00	0.00	−2.47	−1.83	−1.48	−1.83	n/a	(T. Zhang et al., 2015a)
Pre-treated: pH: 5.5 Dose: 18.2 mg NO_2^-/gTS Neutralization: not disclosed	260	53	36.10	0.83	1.00	0.00**	−3.92	−2.90	−2.93**	−2.90	n/a	
AOPs: Ozonation												
Control: Mix sludge, primary and WAS 1:1 (TS based)	314	56	16.70	0.78	0.68	0.00	−4.44	−3.29	−3.76	−3.29	n/a	Tian et al., 2015b
Pre-treated: Dose: 36 mg O_3/gTS Mixed sludge, primary and WAS 1:1 (TS based)	362	64	16.70	0.78	0.68	3.14	−5.13	−3.79	−4.45	−0.65	n/a	
	114	26	16.10	0.86	0.91	0.00	−1.78	−1.32	−0.87	−1.32	n/a	

Control: (assumed that 60% of biogas was methane)												(Bougrier et al., 2007a)
Pre-treated: Dose: 150 mg O_3/gTS (assumed that 60% of biogas was methane)	278	63	16.10	0.86	0.91	13.10	−4.33	−3.21	−3.42	9.89	n/a	
AOPs: Hydrogen peroxide												
Control	164	33	36.10	0.83	1.00	0.00	−2.47	−1.83	−1.48	−1.83	n/a	(T. Zhang et al., 2015a)
Pre-treated: Dose: 30 mg H_2O_2/gTS	201	41	36.10	0.83	1.30	0.00	−3.03	−2.24	−1.73	−2.24	n/a	
Control	242	48	13.90	0.73	1.35	0.00	−3.20	−2.37	−1.85	−2.37	n/a	(Zhou et al., 2015)
Dose: 50 mg H_2O_2/gTS	245	48	13.90	0.73	1.86	0.00	−3.24	−2.40	−1.38	−2.40	n/a	
Control	308	53	64.00	0.73	0.35	0.00	−4.12	−3.05	−3.77	−3.05	n/a	(Eskicioglu et al., 2008)
Pre-treated: Dose: 1000 mg H_2O_2/gTS Mixed sludge, primary and WAS 42:58 v/v	304	52	52.00	0.73	10.56	0.00	−4.04	−2.99	6.51	−2.99	n/a	
AOPs: Fenton												
Control	242	48	13.90	0.73	1.35	0.00	−3.20	−2.37	−1.85	−2.37	n/a	(Zhou et al., 2015)
Pre-treated: pH 2.0 $H_2O_2 = 50$ mg/gTS $Fe^{2+} = 7$ mg/gTS	273	54	13.90	0.73	5.46**	0.00	−3.61	−2.67	1.85**	−2.67	n/a	

Notes:The signs for energy computations are negative for energy production and positive for energy consumption.
n/a: not available.
SRT: solids retention time.

[*] Includes the heat and electricity required for the production of the chemical reagents used for chemical pre-treatments.

[**] Excludes the energy required for the chemical reagents.

[***] Assumes natural freezing is performed.

Assumptions regarding energy calculations:

Only the energy required for the pre-treatment itself and for anaerobic digestion was considered.

Specific heat of sludge	4.18	MJ/°C m^3
Specific latent heat of fusion	333	MJ/m^3
Density of WAS	1000	kg/m^3
Ambient temperature	10	°C
Energy content of CH_4	36	MJ/m^3 CH_4
Density of methane	0.656	kg/m^3
Heat transfer coefficient	0.7	W/m^2°C
Digester specific surface	0.5	m^2 of digester walls and roof/ m^3 sludge
Theoretical methane production	0.35	LCH_4/gCOD
Efficiency CHP (electricity)	0.37	-
Efficiency CHP (heat)	0.5	-

Appendix B

Table B.1
Energy balance for thermal pre-treatment < 100 °C and thermal pre-treatment > 100 °C.

Sample type	Temperature, °C	Application time, h	Methane production, LCH$_4$/kgVS$_{added}$	VS g/L	COD/VS	VS/TS	VS reduction, %	Recuperation efficiency of heat exchanger	Heat recuperation efficiency of CHP
Thermal pre-treatment < 100 °C									
Control	10	0	138	34.8	1.39	0.73	27.00%	0.7	0.5
Pre-treated	80	3	179	34.8	1.39	0.73	33.50%	0.7	0.5
Pre-treated	90	3	182	34.8	1.39	0.73	37.50%	0.7	0.5
Control	10	0	167	26.77	1.49	0.70	34.50%	0.7	0.5
Pre-treated	70	3	202	26.77	1.49	0.70	36.00%	0.7	0.5
Pre-treated	70	15	199	26.77	1.49	0.70	37.00%	0.7	0.5
Control	10	0	126	7.0	1.68	0.80	35.00%	0.7	0.5
Pre-treated	60	6	171	9.9	1.68	0.80	45.00%	0.7	0.5
Pre-treated	75	6	208	9.9	1.68	0.80	45.00%	0.7	0.5
Pre-treated	90	6	155	9.9	1.68	0.80	45.00%	0.7	0.5
Control	10	24	203	39.4	1.52	0.81	35.00%	0.7	0.5
Pre-treated	35	24	203	39.4	1.52	0.81	35.00%	0.7	0.5
Pre-treated	55	24	206	39.4	1.52	0.81	35.00%	0.7	0.5
Pre-treated	70	24	216	39.4	1.52	0.81	35.00%	0.7	0.5
Thermal pre-treatment > 100 °C									
Control	10	0	359	11.8	1.51	0.81	39.00%	0.5	0.2 (as steam)
Pre-treated	135	0.5	383	11.8	1.51	0.81	41.00%	0.5	0.2 (as steam)
Pre-treated	190	0.5	308	11.8	1.51	0.81	57.00%	0.5	0.2 (as steam)
Control	10	0	154	14.4	1.40	0.69	43.00%	0.5	0.2 (as steam)
Pre-treated	134	0.33	223	14.4	1.40	0.70	46.00%	0.5	0.2 (as steam)
Control	10	0	128	12.0	1.45	0.70	34.80%	0.5	0.2 (as steam)
Pre-treated	170	1	228	12.0	1.45	0.70	66.90%	0.5	0.2 (as steam)

Sample type	Required energy for heating, MJ/m^3	Energy released as heat, MJ/m^3	Digester temperature, °C	Sludge temperature after pre-treatment, °C	Required energy for heating sludge (for digester), MJ/m^3	Digestion time, d	Heat loss digester, MJ/m^3	Overall energy balance, MJ/m^3	Minimum VS concentration required to close balance, gVS/L	Reference
Thermal pre-treatment < 100 °C										
Control	0	−86	35	10	105	20	15.1	−71	6	(Ruffino et al., 2015)
Pre-treated	88	−112	35	31	17	20	15.1	−9	32	
Pre-treated	100	−114	35	34	4	20	15.1	1	35	
Control	0	−80	35	10	105	20	15.1	−65	5	
Pre-treated	75	−97	35	28	29	20	15.1	−7	25	
Pre-treated	75	−96	35	28	29	20	15.1	−6	25	
Control	0	−16	35	10	105	21	15.9	0	7	(Kim et al., 2013b)
Pre-treated	63	−30	35	25	42	21	15.9	48	26	
Pre-treated	82	−37	35	29.5	23	21	15.9	60	26	
Pre-treated	100	−28	35	34	4	21	15.9	89	42	
Control	0	−144	37	10	113	44	35.9	−108	10	(Wang et al., 2014)
Pre-treated	31	−144	37	17.5	82	44	35.9	−77	18	
Pre-treated	56	−146	37	23.5	56	44	35.9	−54	25	

Pre-treated	75	−153	37	28	38	44	35.9	−42	29	
Thermal pre-treatment > 100 °C										
Control	0	−31	35	10	105	20	15.1	−15	6	(Bougrier et al., 2007b)
Pre-treated	261	−33	35	72.5	0	20	15.1	244	100	
Pre-treated	334	−26	35	90	0	20	15.1	323	157	
Control	0	−16	55	10	188	15	20.4	4	18	(Gianico et al., 2013)
Pre-treated	259	−23	55	72	0	15	20.4	256	174	
Control	0	−11	35	10	105	20	15.1	4	16	(Valo et al., 2004)
Pre-treated	334	−20	35	90	0	20	15.1	330	213	

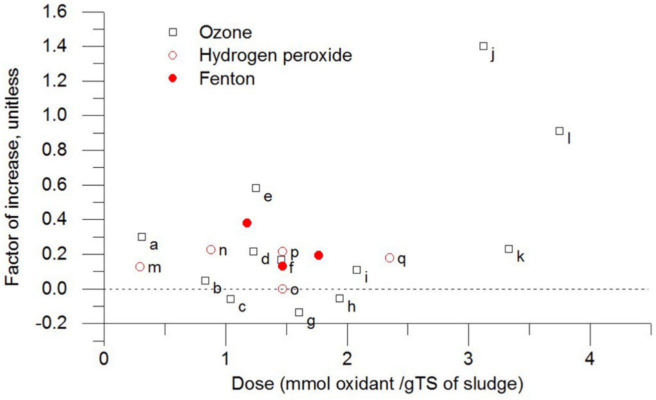
Table B.2
Energy balance for ultrasonic and microwave pre-treatments.

Sample	Notes	CH_4 production, L CH_4/kgVS	COD/VS	Biodegradation, %	VS, g/L	VS/TS	Electricity consumption, kJ/gTS	Pre-treatment specific energy (as electricity), MJ/m^3	Electricity released, MJ/m^3	Required heat to warm the sludge, MJ/m^3	Heat losses in digester, MJ/m^3	Heat released, MJ/m^3	Heat balance, MJ/m^3	Electricity balance, MJ/m^3	Biodegradation required for neutral electricity balance, %	Reference
Ultrasonic pre-treatment																
Untreated		114	1.61	20	26.2	0.87	0	0.00	−60.6	112.86	20.4	−53.8	79.5	−60.65		(Seng et al., 2010)
Treated		129	1.61	23	26.2	0.87	3.79	114.00	−68.6	112.86	20.4	−60.8	72.4	45.37	38	
Untreated		248	1.67	42	27.5	0.83	0.00	0.00	−138.5	104.5	15.1	−122.8	−3.1	−138.48		(Pérez-Elvira et al., 2010)
Treated		349	1.67	60	27.5	0.83	25.90	858.00	−194.9	104.5	15.1	−172.8	−53.1	663.12	261	
Untreated	Mixed sludge PS:WAS = 33:67	170	1.42	34	18.8	0.68	0.00	0.00	−65.0	112.86	16.3	−57.7	71.5	−65.03		(Braguglia et al., 2015)
Treated	Mixed sludge PS:WAS = 33:67	240	1.42	48	18.7	0.68	4.40	120.00	−91.1	112.86	16.3	−80.8	48.4	28.87	64	
Untreated	Mixed sludge PS:WAS = 33:67	221	1.53	41	35.0	0.83	0.00	0.00	−156.8	104.5	15.1	−139.0	−19.4	−156.85		(Cella et al., 2015)
Treated	Mixed sludge PS:WAS = 33:67	230	1.51	44	35.0	0.83	14.29	600.00	−163.7	104.5	15.1	−145.2	−25.5	436.26	160	
Microwave pre-treatment																
Untreated	Mixed sludge PS:WAS = 42:58	308	1.66	53	47.0	0.73	0.00	0.00	−293.9	96.14	22.3	−260.6	−142.2	−293.93		(Eskicioglu et al., 2008)
Treated	Mixed sludge PS:WAS = 42:58	290	1.66	50	47.0	0.73	16.07	1034.64	−276.4	96.14	22.3	−245.0	−126.6	758.27	187	
Untreated	Mixed sludge PS:WAS = 42:58	192	1.67	33	36.1	0.70	0.00	0.00	−140.7	104.5	15.1	−124.8	−5.1	−140.74		(Coelho et al., 2011)
Treated	Mixed sludge PS:WAS = 42:58	254	1.67	43	36.1	0.70	17.51	900.00	−186.2	104.5	15.1	−165.0	−45.4	713.82	210	
Untreated		245	1.52	46	31.3	0.77	0.00	0.00	−155.7	188.1	40.8	−138.0	90.9	−155.71		(Chi et al., 2011)
Treated		304	1.52	57	31.3	0.77	69.13	2827.42	−193.2	188.1	40.8	−171.3	57.7	2634.21	838	

Assumptions regarding energy calculations:

Specific heat of sludge	4.18	MJ/°C m^3
Density of WAS	1000	kg/m^3
Ambient temperature	10	°C
Energy content of CH_4	36	MJ/m^3 CH_4
Density of methane	0.656	kg/m^3
Heat transfer coefficient	0.7	W/m^2°C
Digester specific surface	0.5	m^2 of digester walls and roof/ m^3 sludge
Theoretical methane production	0.35	LCH_4/gCOD
Efficiency CHP (electricity)	0.37	-
Efficiency CHP (heat)	0.5	-
Efficiency CHP (steam)	0.2	-

Appendix C



Note: for Fenton reaction, H_2O_2 is shown

Fig. C.1. Effect of oxidant doses in the biogas production. Letters refer to entries in Table 12.

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