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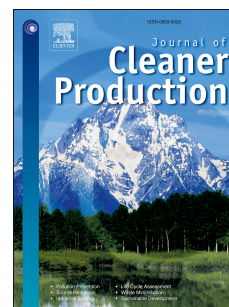
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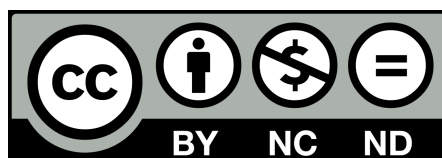
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Abstract

Hygienization reduces the public health risks involved in the application of biowaste to agricultural land. Recent advances in the hygienization of treated biowaste have not been reviewed to date. In many countries, the process involves using low temperature thermal pasteurization. Thermal hygienization accounts for between 6% and 25% of primary energy production in European biogas plants. Hygienization pretreatment can also influence the production of biogas by the treated substrates (from a slight negative effect to a biogas yield surplus of 50% in most cases). Alternative athermal pasteurization technologies (including electro-technology, microwave, pressurization, ultrasound and chemical treatment) have been shown to be capable of considerably reducing the number of bacteria and increasing the methane yield. The performance of these alternatives varies greatly and depends on the type of biowaste, the operational parameters studied, energy input and the method of interpreting the experimental results. Analyses of energy and exergy efficiency, of environmental impacts and of economic feasibility show that thermal hygienization may be the most energy efficient and economical approach when it exploits the wasted heat recovered from other processes. The present study also revealed that the research focus has been confined to the sewage sludge. Studies on the other biowaste, including animal by-products, are needed.

Keywords: Biowaste; Hygienization; Anaerobic digestion; Methane potential enhancement; Thermal pasteurization; Non-thermal pasteurization

1. Introduction

The term biowaste covers a wide range of organic waste produced by human-based biological activities, livestock farming and food-processing industries, including municipal solid waste (MSW), sewage sludge and waste activated sludge (WAS) and animal by-products (ABP) such as animal slurry, animal manure and slaughterhouse waste. Valorization of these kinds of biowaste to recover material and produce energy (Mihai and Ingrao, 2018), accompanied by efficient waste management, is attracting increasing attention (Yong et al., 2016). Anaerobic digestion (AD), one of the promising approaches to the production of biogas, is an efficient source of renewable energy for the co-generation of heat and electricity (Miltner et al., 2017). Bio-methane is also an important raw material for the chemical synthesis industry, and enables a reduction in global greenhouse gas emissions (Xu et al., 2018). Aside from biogas, the solid residue, or digestate, that remains in the anaerobic digester, is rich in nutritional and mineral components. The “return-to-soil” policy for treated waste as fertilizer or soil amendment through application to agricultural land means that waste can be fully exploited (Das et al., 2019).

However, studies in the last two decades revealed the presence of large numbers of various pathogenic microorganisms in biowaste destined for land application. Fecal coliforms, *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter* spp., *Cryptosporidium*, *Giardia*, Hepatitis B/E, Norovirus and Rotavirus were the main microorganisms found in British livestock manures (Hutchison et al., 2004), in swine manure during treatment and storage (Ziemer et al., 2010), animal manure-amended soil (Jaffrezic et al., 2011), land-applied biosolids (Grant et al., 2012), slaughterhouse waste (Franke-Whittle and Insam, 2013), anaerobic digestate (Maynaud

et al., 2016) and swine waste (Sui et al., 2019). These infectious microorganisms can be transmitted from waste to the environment during application on the land and consequently contaminate food and cause outbreaks of human diseases (Sobsey et al., 2006). The spread of antibiotic resistance genes (ARG) from biowaste is also the subject of increasing concern (Chen et al., 2019). Recent results show that the conventional waste treatment is not sufficient to remove ARG from food waste (He et al., 2019). A hygienization step is usually required to control this sanitary risk by inactivating the pathogen, as illustrated in **Fig. 1**. In the present paper, the term “biowaste” is limited to biologically-derived waste that requires hygienization, including animal by-products, sewage sludge and biosolids, as defined by the relevant regulations (details provided in Section 3).

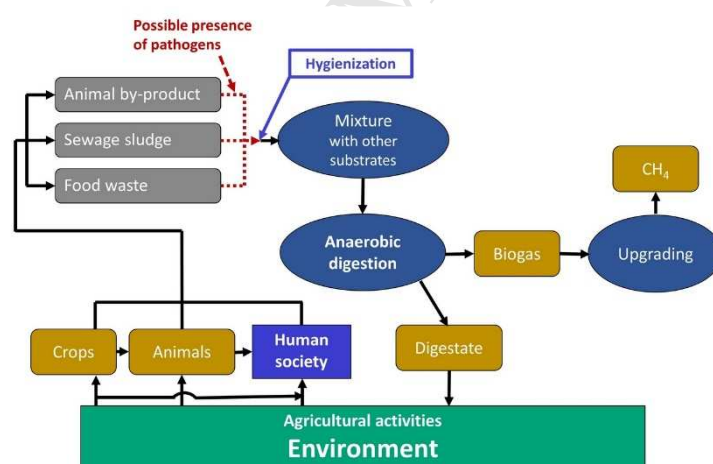


Fig. 1. Pathogen transmission pathway from biowaste to humans and to the natural environment.

For certain categories of biowaste, the hygienization process involves thermal pasteurization before biogas production. This method of pretreatment can also influence the bio-methane potential (BMP) of the substrate. Recent research has focused on

innovative pretreatment methods that help increase the BMP of biowaste in a more energy efficient way (Fan et al., 2017). Electro-technology, microwave (MW), high hydrostatic pressure (HHP), power ultrasound (PUS) and chemical treatment are among the promising more effective alternatives for BMP enhancement (Zhen et al., 2017). Their effect on the hygienization of biowaste has been studied independently, but most of these studies did not link these effects to their influence on BMP. **Fig. 2** is an annual list of papers published on the topic “anaerobic digestion”, “AD with pretreatment” and “AD with hygienization or sanitation” referenced using Web of Science. The proportion of papers dealing with AD and devoted to pretreatment methods increased from 1.67% in 1990 to 24.58% in 2018. On the other hand, only a few articles (< 5 papers per year between 1990 and 2003 and 5 to 20 papers per year since 2003) have concerned hygienization. There was thus a need for a systematic review of the sanitation of biowaste treatment to pave the way for cleaner production of biogas.

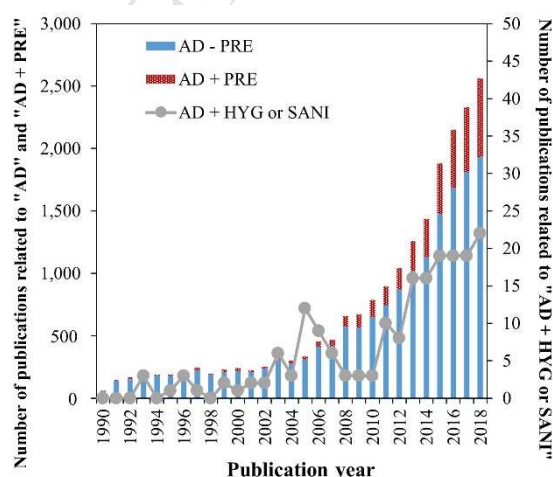


Fig. 2. Bibliometric review of the number of papers published per year on the topic “AD - PRE” (anaerobic digestion without pretreatment), “AD + PRE” (anaerobic digestion with pretreatment) and “AD + (HYG or SANI)” (anaerobic digestion with hygienization or sanitation) in Web of Science Core Collection (Clarivate Analytics) since 1990.

This paper provides a comprehensive review of the hygienization of the biowaste, including 1) the sanitary challenges of biowaste treatment and the corresponding global regulations, 2) conventional thermal hygienization with its energy consumption and its effect on BMP enhancement, 3) a summary of the effects of emerging non-thermal pasteurization technologies on the efficiency of disinfection and the enhancement of BMP of the biowaste and 4) a discussion of cleaner production enabled by hygienization and its future prospects.

2. Literature review

The present paper covers a bibliographical investigation of the literature referenced by Web of Science Core Collection® (Clarivate Analytics, Massachusetts, US), Scopus® (Elsevier, Amsterdam, Netherlands) and Google Scholar® (Alphabet, California, US). The keywords searched involved the combinations of (but not limited to): “anaerobic digestion” with or without “biowaste”, “organic waste”, “biogas (methane) production”, “animal by-product”, “animal slurry (manure)”, “sewage sludge”, “waste activated sludge”, “slaughterhouse waste” and “biosolids” for the general topics; “hygienization”, “sanitation”, “disinfection”, “pathogen removal”, “pathogen inactivation”, “microbial inactivation”, “sanitary risk” and “pathogen transmission” for the topics about hygienization aspect; “energy consumption (efficiency)”, “life cycle assessment”, “heat demand” and “biogas plant” for the topics about the energy efficiency; “pretreatment”, “thermal pretreatment”, “thermal pasteurization”, “pulsed electric field”, “electrical disintegration”, “(power) ultrasound”, “ultrasonication”, “microwave”, “high (hydrostatic) pressure”, “pressurization”, “chemical pretreatment”, “acid pretreatment”, “alkali pretreatment”, “oxidation (ozone) pretreatment” and “BMP enhancement” for the topics about the pretreatment methods.

3. Public health risk and global regulations

3.1 Sanitary challenges of biowaste for public health

Biowaste vehicles a wide range of microorganisms among which almost all species of infectious agents identified as dangerous for humans can be found. **Fig. 3** summarizes the occurrence of six species of pathogens identified in cattle, swine and poultry slurries. The minimum and maximum prevalence of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Cryptosporidium parvum*, *Giardia lamblia* in these animal slurries were 11.9 - 97%, 0 - 78%, 19.8 - 30%, 0 - 71.4%, 53 - 66.7% and 7 - 93.3%, respectively. The figure leads one to conclude that there is a high risk that animal excrement will be contaminated by different pathogenic agents. The origin of contamination is not limited to sick animals. The accumulation of contaminants from the over-spread soil (Martinez et al., 2009) and improper hygiene practices in farm facilities (Gerba and Smith, 2005) are also possible sources of microbiological contamination. Transport of waste is also believed to be another potential source of recontamination (Sahlström, 2003). Bicudo and Goyal (2003) also pointed out that bacteria derived from livestock, whether pathogenic or not, might introduce antibiotic resistance genes into the environment. Public health may therefore be threatened if biowaste is not properly treated and managed (Lowman et al., 2013).

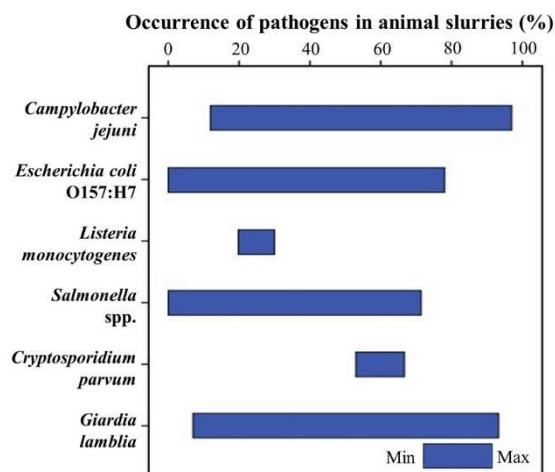


Fig. 3. Occurrence of pathogens in biowastes.

(please refer to the supplementary material for the original data and references of this figure)
 (data of this figure were collected from Quilez et al., 1996; Busato et al., 1999; Watabe et al., 2003; Hutchison et al., 2004; Schouten et al., 2005; Castro-Hermida et al., 2006; Gunn et al., 2007; Esteban et al., 2008; Hölzel and Bauer, 2008; Pourcher et al., 2008; Reinoso and Becares, 2008; Huneau-Salaün et al., 2009; Vilar et al., 2010; Cunault, 2012; Pourcher et al., 2018)

The risk to public health was further reinforced by Hutchison et al. (2004) who compared the concentration of certain pathogens in animal slurries produced by sheep, poultry, pig and cattle in the UK. **Fig. 4** shows the results of their work. The concentrations of the five kinds of pathogens investigated in the animal slurries were generally high, between 10^2 and 10^5 CFU·mL⁻¹. This implies that the safe disposal of this biowaste is a major challenge for public health.

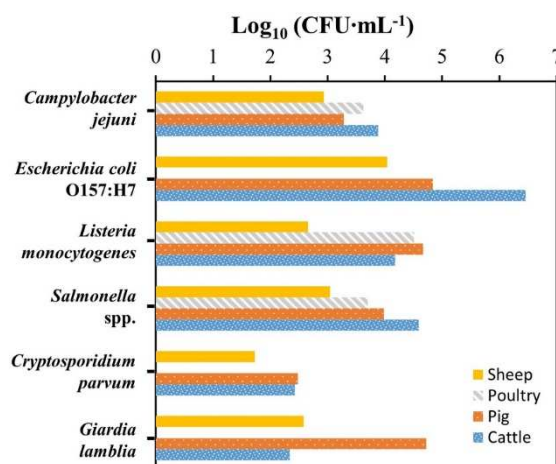


Fig. 4. Example of the abundance of pathogens in animal slurries.
(data of this figure were extracted and converted from Hutchison et al., 2004)

In 2002, the US Environmental Protection Agency (EPA) revealed to the public, for the first time, the interactions between the pathogens in sewage sludge and the illnesses of residents who lived near the land where the sludge was applied. Their findings were delivered in a short communication (Lewis and Gattie, 2002) and in a research article (Lewis et al., 2002), thus drawing medical, political and scientific attention to the issue. One year later, US EPA regulations Part 503 on the control of pathogens in sewage sludge (US EPA, 2003) was introduced. In the meantime, the European Union (EU) issued its first regulation (EC No. 1774/2002) on health rules concerning mandatory hygienization (sterilization) of ABP before anaerobic digestion, which unified various operational parameters proposed by European countries (European Union, 2002). The above-mentioned research and legislation led to an increase in the number of hygienization-related articles published between 2002 and 2005, as shown in **Fig. 2**.

3.2 Global regulations on biowaste hygienization

Biowaste is usually subject to thermal pasteurization for sanitary purposes. Thermal treatment can either be separate from the major transformation processes or be

combined with them. **Table 1** lists the operational hygienization parameters laid down in the regulations of some countries and authorities for different target biowastes. The types of the biowaste that require hygienization and the operational parameters vary with the country. EU commission regulation No. 142/2011 requires that the animal by-products defined as category 2 or 3 (e.g. slaughterhouse and livestock waste) should first be blended and crushed to obtain a particle size of less than 12 mm and then be thermally pasteurized at 70 °C for minimum of 60 min before entering anaerobic digesters (European Union, 2011). The regulation entitles EU member countries to choose alternative processes to thermal treatment for the hygienization of ABP, but only if these processes achieve a 5-log₁₀ reduction of *Enterococcus faecalis* or *Salmonella Senftenberg* (775W, H₂S negative) and a 3-log₁₀ reduction of the thermos-resistant viruses such as parvovirus. For example, the Swedish Board of Agriculture approved an integrated thermophilic sanitation process at 52 °C for 10 h in thermophilic digesters (Grim et al., 2015) as a hygienization treatment. The US EPA regulation states that the sewage sludge must be thermally treated by applying different time-temperature equations to reach the targeted classification of biosolids (for example, 30 min is needed for a treatment at 70 °C for sewage sludge with at least 7% solids). The wide range of operational temperatures and the length of treatment proposed by these countries make it difficult to compare the efficiency of the different pasteurization processes. For this reason, a pasteurization parameter value (F-value) developed by food engineers was introduced. F-value is defined as the time required at the reference temperature to achieve the same pasteurization efficiency (target pathogen reduction ratio) as that obtained by pasteurization at another temperature, assuming a log-linear inactivation

profile of the reference microorganism (Ball, 1923). The F-value is calculated according to **Eq. 1**.

$$F - \text{value} = \int_0^t 10^{\frac{T - T_{\text{ref}}}{z}} dt \quad (1)$$

where the F-value is the pasteurization value at the reference temperature (min), T is the original temperature (°C), T_{ref} is the reference temperature ($T_{\text{ref}} = 70$ °C), t is the original treatment time at T (min), z is the Z value implying the temperature increase required for a 1- log₁₀ reduction of the targeted pathogen's decimal inactivation time (here z is set at 7 °C based on the pasteurization of *Enterococcus faecalis*) (Sörqvist, 2003). To cite one example, in Sweden, the integrated thermophilic sanitation at 52 °C for 10 h has the same pasteurization effect on the *Ent. faecalis* treated at 70 °C (T_{ref}) for 1.6 min (i.e. F-value = 1.6 min). The F-values of the pasteurization efficiency of the corresponding hygienization parameters are in italic in parentheses in **Table 1**. One may judge the EU to be more prudent in establishing the hygienization parameters than its members and other countries across the world. It should be noted that Ireland proposed extremely conservative thermal pasteurization parameters (60 °C for 48 h, 2 times) for the hygienization of ABP, equivalent to an F-value of 214 min at 70 °C (Coultry et al., 2013).

Table 1

Summary of regulatory treatment times and temperatures for the thermal hygienization of biowaste in different countries.

Authorities	Type of waste	Operational parameters of hygienization process			References
		HYG + MAD ^a	HYG + TAD ^b	HYG during AD ^c	
European Union	ABP	70 °C, 1 h (60 min) ^d for all processes			European Union (2011)
Austria and Germany	ABP	70 °C, 1 h (60 min)	70 °C, 0.5 h (30 min)	55 °C, 24 h (10 min)	Amon and Boxberger (1999)
Denmark	ABP	55 °C, 7.5 h (3.2 min)	55 °C, 5.5 h (2.4 min)	55 °C, 6.0 h (2.6 min)	Bendixen (1999)
		60 °C, 3.5 h (7.8 min)	60 °C, 2.5 h (5.6 min)	52 °C, 10 h (1.6 min)	
		65 °C, 1.5 h (17 min)	65 °C, 1.0 h (12 min)	-	
USA	Biosolids	70 °C, 0.5 h (30 min)	70 °C, 0.5 h (30 min)	-	US EPA (2003)
Ireland	ABP	60 °C, 48 h, 2 times (214 min) or 70 °C, 1 h (60 min)			DAFF of Ireland (2008)
China	Biosolids	-	-	55 °C, 5 d (52 min)	Ministry of Health of China (2013)
UK	Catering waste	57 °C, 5.0 h (4.1 min)			UK APHA (2014)
Sweden	ABP	-	-	52 °C, 10 h (1.6 min)	Grim et al. (2015)

^a HYG + MAD: Hygienization and mesophilic anaerobic digestion take place in separate units

^b HYG + TAD: Hygienization and thermophilic anaerobic digestion take place in separate units

^c HYG during AD: Anaerobic digestion is combined with hygienization and takes place in the same unit

^d *F* values are shown in parentheses, based on $T_{ref} = 70$ °C and $z = 7$ °C in **Eq. (1)**, indicating the thermal inactivation of *Ent. faecalis*

In addition to operational parameters, the regulations usually specify acceptable microbial abundance in the final product of the AD process, namely in the digestate, before it is spread on the land. **Table 2** gives several examples of the microbial criteria for the safe disposal of the digestate derived from the AD of ABP, sewage sludge and general agricultural waste in the EU, the US and the US state of California, respectively. *E. coli*, *Enterococcus* spp., *Salmonella* spp. and helminth ova are often selected as indicator bacteria to characterize the quality of the digestate. The criteria have to account for the fact that the AD process also serves as a step in waste sanitation. A number of studies proved that AD, especially thermophilic AD, can significantly reduce the number of infectious agents or traditional indicator microorganisms, such as fecal coliforms, *Salmonella* spp., *E. coli*, *Enterococcus* spp., Helminth ova, phages in sewage sludge (Scaglia et al., 2014), animal slurry (Nolan et al., 2018), swine carcasses and manure (Tápparo et al., 2018) and in cow manure (Qi et al., 2019). A recent review by Zhao and Liu (2019) reported that the reduction in the number of pathogens could be explained by the stressful conditions created by AD for the selection of the methanogenic microorganisms. However, the *Campylobacter* spp. was shown to be able to survive mesophilic anaerobic digestion of cow manure (Qi et al., 2019). Certain spore forming bacteria, such as *Bacillus* spp., are much more resistant to the thermal hygienization pretreatment and to the thermophilic AD process (Bagge et al., 2010). The presence of the infectious viruses in the digestate is still overlooked (Zhao and Liu, 2019). The removal of resistant pathogens and bacterial endospores in biowaste is a recommended research focus in the field of the hygienization of biowaste in the future.

Table 2

Criteria chosen by several authorities for the indicator microorganisms characterizing the target biowaste treated by AD for safe disposal.

Indicators	N	c	m	M	quantity of biowaste considered
EU regulation (EC) 142/2011 – Section III.3.1 concerning animal by-products					
<i>Escherichia Coli</i> or <i>Enterococcaceae</i>	5	1	1,000	5,000	1 g
<i>Salmonella</i> spp.	5	0	0	0	25 g
US EPA under 40 CFR Part 503 – Section 4 concerning sewage sludge					
Fecal coliform	–	–	1,000	–	4 g TS
<i>Salmonella</i> spp.	–	–	3	–	4 g TS
Enteric viruses	–	–	1	–	4 g TS
Viable helminth ova	–	–	1	–	4 g TS
California Title 14 – Section 17896.60 (b) concerning anaerobic digestate					
Fecal coliform	–	–	1,000	–	4 g TS
<i>Salmonella</i> spp.	–	–	3	–	4 g TS

N = number of samples to be tested

m = threshold value for the viable count of bacteria; considered satisfactory if the viable count in all samples does not exceed m

M = maximum value for the viable count of bacteria considered unsatisfactory if the viable count in one or more samples is M or more

c = number of samples the bacterial count of which may be between m and M, the sample still being considered acceptable if the bacterial count of the other samples is m or less

TS: Total solids

4. Thermal hygienization

4.1 Energy consumption

Thermal hygienization of biowaste often takes place in biogas plants, using the heat produced by the co-generation of locally produced biogas. **Table 3** summarizes the heat required by thermal hygienization as a proportion of total primary energy production of several BGP in Europe. Generally, the process consumes 6 - 25% of the local primary energy production except in Ireland which, as mentioned in Section 3.2 above, requires an extremely strict hygienization process (60 °C for 48 h, 2 times) that significantly increases the energy consumed by the hygienization process (Coultry et al., 2013). The authors compared the energy consumption of EU and Irish national hygienization standard in an Irish biogas plant. Their study found that in the case of pasteurization prior to anaerobic digestion, 57% and 4,544% of the biogas output would be used for EU and Irish hygienization while around 30% and 1,893% would be required respectively in the case of pasteurization after digestion. They concluded that the EU parameters were more economical from a financial point of view (Coultry et al., 2013).

The temperature required by the treatment may vary depending on the scale of the BGP and the origins of the biowaste to undergo hygienization. Waste with higher water content (like slurry and municipal solid waste) requires more energy to reach the target sanitation temperature. The difference in the heat demand required by the hygienization process in BGP can also be explained by the different methods of calculation used. Some papers were based on energy auditing within the BGP while others were based on life cycle assessment (LCA), meaning different system boundaries were used for the assessment. For example, some studies included the primary energy efficiency of the heat generation for hygienization whereas others did not. In addition, the functional

parameters of one BGP may differ significantly from another. These may lead to different assumptions and hypotheses concerning the heat recovery ratio, heat exchange efficiency, insulation conditions and energy consumption (Grim et al., 2015).

Table 3

Energy demand of thermal hygienization process in several BGP in Europe (* considering the heat consumption of all the AD units, table adapted from Liu et al., 2018a).

Country	Treatment capacity (kt·y ⁻¹)	Substrates treated	Bio-methane production (10 ⁶ Nm ³ ·y ⁻¹)	Hygienization Operation	AD process	$\frac{Q \text{ required by HYG}}{Q \text{ generated by BGP}}$	References
Sweden*	20-60	several BGPs	-	-	-	6-17%	Berglund and Börjesson (2006)
Germany	10-20	AW, crops, MSW	0.5-0.9	70 °C, 1 h	-	10-15%	Pöschl et al. (2010)
Ireland	10.8	slurry 70%, vegetables 30%	0.5-1.0	70 °C, 1 h	40 °C	30-57%	Coultry et al. (2013)
				60 °C, 48 h, 2 times	40 °C	1,893-4,544%	
UK*	5.1	slurry 50%, AW 50%	0.37	–	40 °C	17%	Whiting and Azapagic (2014)
Sweden	25.2	MSW 82%, ABP 15% Food waste 3%	2.79	52 °C, 10 h	52 °C	9%	Grim et al. (2015)
Sweden	28	food industry waste	1.37	72 °C, 1 h	52 °C	20%	Lindkvist et al. (2017)

HYG: Hygienization; BGP: Biogas plant; AW: Agricultural waste; MSW: Municipal solid waste; ABP: Animal by-products

4.2 Effect on anaerobic digestion

The thermal hygienization of biowaste usually takes place at a temperature below 100 °C for a period of up to several hours. This short-term mild thermal treatment can also serve as a pretreatment step of the substrates before their transformation and hence, affect their behavior in subsequent biogas production. **Fig. 5** summarizes studies reporting short-term thermal pretreatment at < 100 °C for the enhancement of methane yield of four kinds of biowaste: slaughterhouse waste (n = 13), sewage sludge (n = 9), pig slurry (n = 1) and cattle slurry (n = 4). The boxplots represent the medians, the 25th and the 75th percentiles and the estimated intervals at 99% of the reported effect of methane yield enhancement on biowaste. The enhancement of methane yield resulting from mild thermal pretreatment generally ranged between 0 and +50%, with several studies reporting negative effects or an extreme positive effect (between +50 - +500%). The thermal pretreatment enhances the solubilization of COD in the substrates and converts the complex chemical substances into simpler ones (e.g. long-chain fatty acids into volatile fatty acids and proteins into amino acids). The treatment may also cause a morphological modification of the substrate particles, making the hydrolysis process much easier (Luste and Luostarinen, 2010). In addition to intensifying BMP, thermal pasteurization can also increase the maximum methane yield rate of certain biowastes, including cattle, pig and chicken offal (Ware and Power, 2016a), hydrolysis digestate, municipal wastewater sludge, pork liver and slaughterhouse sieving waste (Liu et al., 2018b).

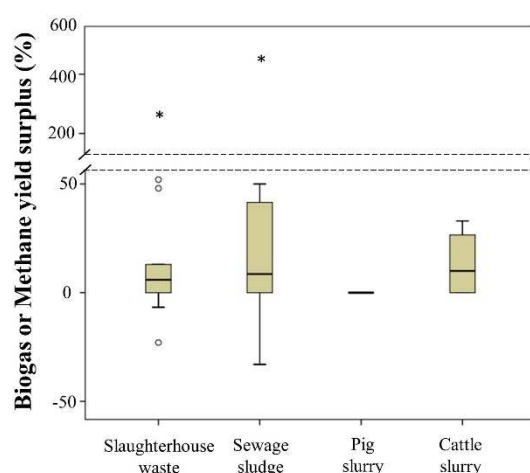


Fig. 5. Summary of short-term mild thermal pretreatment ($< 100\text{ }^{\circ}\text{C}$) related to biogas or methane yield enhancement of biowastes.

(please refer to the supplementary material for the original data and references of this figure)
 (data of this figure were collected from Edström et al., 2003; Climent et al., 2007; Ferrer et al., 2008; Luste et al., 2009; Hejnfelt and Angelidaki, 2009; Luste and Luostarinen, 2010; Rafique et al., 2010; Luste and Luostarinen, 2011; Rodríguez-Abalde et al., 2011; Luste et al., 2012; Yan et al., 2013; Vergine et al., 2014; Grim et al., 2015; Ware and Power, 2016a; Nazari et al., 2017; Liu et al., 2018b)

Thermal pretreatment of most slaughterhouse waste can lead to an increase of +4.3 - +48% of the BMP. However, many researchers found no effect or even a negative effect for certain types of the slaughterhouse waste that are rich in protein and grease, like the content of the digestive tract, grease trap sludge (Luste et al., 2009), pork by-products (Hejnfelt and Angelidaki, 2009), general slaughterhouse waste (Grim et al. 2015), cattle, pig and chicken offal (Ware and Power, 2016a) and blood sludge (Liu et al., 2018b). Thermal treatment of these kinds of waste might produce high concentrations of ammonia, sulfate and acids that are toxic and inhibit the methanogenic process during anaerobic digestion. Edström et al. (2003) studied mesophilic co-digestion during 130 days of pasteurized or non-pasteurized slaughterhouse waste and found a 400% increase in biogas production (from 310 to 1,140 $\text{NmL}\cdot\text{g Volatile Solids}^{-1}$, denoted $\text{NmL}\cdot\text{g VS}^{-1}$ or from 83.7 to 307.8 $\text{NmL}\cdot\text{g Total Weight}^{-1}$, denoted $\text{NmL}\cdot\text{g TW}^{-1}$). This significant enhancement of BMP might be due to the composition of the feedstock, a mixture of

animal by-products including blood, stomach contents, slaughterhouse sludge, food waste and liquid manure. The co-digestion of various feedstock helps balance the C/N ratio, minimize the accumulation of toxic compounds and mitigate the shock of changes in pH during anaerobic digestion.

Sewage sludge is widely studied as a substrate when it comes to the effect of thermal pretreatment on methane production (Carrère et al., 2010) and the intensification of dewaterability (Zhen et al., 2017). BMP enhancement caused by this pretreatment ranged from 0 - +50% in the majority of the studies, whereas Nazari et al. (2017) reported -33% to +4.6% for sewage sludge of eight different origins. These authors observed solubilization of COD in all kinds of sludge, which did not necessarily lead to an increase in BMP. This observation was explained by the formation of inhibitory compounds like ammonia and the high concentration of cationic ions like Na^+ . In the same paper, despite the lack of an increase in biogas yield, the hydrolysis of the sewage sludge was improved by the low-temperature pretreatment, i.e. there was an increase in biogas yield rate during the early stage of AD compared with non-treated sludge. Yan et al. (2013) showed that BMP of the excess sludge treated at 50, 70, 90, 100, 110 and 120 °C was increased by more than 400%. These authors explained the significant difference in the BMP enhancement by the difference in the chemical compositions of the sludges they studied.

In addition to these authors' argument, the marked difference in BMP enhancement of the thermal treatment may also be due to the physical-chemical status of the substrate when it was collected. Quideau et al. (2014) reported that nearly 1% of the BMP could be lost every day during storage of bovine slurry. This loss is due to decomposition of degradable organic matter. Consequently, the organic content that remained in the

substrate was generally hard to break down and hence not easily influenced by mild thermal pretreatment.

Another possible explanation is that the methods used for the determination of BMP by the researchers were not the same. This leads to different interpretations of the term BMP, which may be experimentally observed, graphically determined or mathematically estimated by different models. Experimentally and graphically determined BMP strongly depend on the shape of the methane production curves and the anaerobic digestion time considered, i.e., whether it was long enough for the methane production curves to level out and reach a plateau. Many articles failed to specify how their BMP came out, thus making it difficult to compare their findings with other findings in the literature using the same terminology.

5. Alternative non-thermal pasteurization technologies

5.1 Mechanisms

5.1.1 Electro-technology

The term electro-technology covers a group of electrical applications to the target substrates, including pulsed electric field (PEF), high voltage discharge or other techniques for cell disintegration using electrical power. These technologies have been widely studied in the food industry since the 1960s (Brennan and Grandison, 2012) for the purpose of non-thermal pasteurization and the intensification of extraction and drying processes of food products. Electro-technology mainly aims at rupturing the target bacterial cell membrane by creating permanent and irreversible pores from which the intercellular plasmas of the bacteria leak. Recent studies showed that electro-technology can also lead to the formation of toxic radicals like superoxide radicals (O_2^-)

and hydroxyl radicals ($\cdot\text{OH}$) that have a sublethal effect on the target microorganisms (Wang et al., 2018).

5.1.2 Microwave

Microwave irradiation (MW) involves bulk heating of the target materials through the oscillating realignment of the dipolar molecules (water, in most cases) induced by penetration of the microwave. The frequency of MW is generally 0.9 or 2.45 GHz as water molecules are the main absorbers of the microwave irradiation at these two frequencies (Tyagi and Lo, 2013). In addition to the thermal aspect, athermal effects were also observed during MW pretreatment that denatures macromolecules, like proteins and DNA, by breaking their hydrogen bonds (Toreci et al., 2009). The combined effects help hygienization / pasteurization of the feedstock.

5.1.3 Pressurization

Pressurization refers to a group of non-thermal technologies using extreme high pressure to pasteurize products. High hydrostatic pressure (HHP) is an innovative homogenization technology that provides isostatic pressurization at 100 - 900 MPa to a liquid product for a specified treatment time. This non-thermal treatment pasteurizes the products by causing a phase transition of the lipid bilayers of the microorganisms (Martín et al., 2002). A high level of pressurization can also be achieved by applying pressured neutral gas (e.g. CO_2) to a liquid.

5.1.4 Power Ultrasound

Power ultrasound (PUS) has been widely studied to kill the microbes in food products. This ultra-sonication technology causes cavitation by delivering alternating sonic shock waves (usually at a frequency of 20 - 100 kHz). These waves induce rapid

formation of air bubbles inside the substrates and a rapid increase in local temperature and pressure to inactivate microorganisms (Piyasena et al., 2003).

5.1.5 Chemical treatment

Chemical treatment as an alternative to pasteurization involves adding alkali, acid, ozone and other chemicals to the substrates. It is often combined with other treatments to enhance the efficiency of microbe inactivation. Alkali and acid pretreatments modify the pH of the medium to an extreme level that could stress or inhibit microbial activity. The change in pH can also trigger different physical-chemical reactions, e.g. particle coagulation, breakdown of the cellular membrane and the decomposition of lignocellulose, etc. Ozone treatment creates high concentrations of oxidative free radicals that are toxic to the bacteria.

5.2 Pasteurization efficiency in biowastes

As a substitute for thermal hygienization of the biowaste, any alternative technology needs to show that pasteurization is at least as efficient as that obtained by thermal treatment. In fact, according to studies of food pasteurization, the technologies proposed in Section 5.1 can achieve satisfactory levels of bacterial destruction (Brennan and Grandison, 2012). However, not many research articles deal with the hygienization of biowaste. **Table 4** lists several emerging hygienization technologies cited in the literature (PEF, MW, PUS, pressurization and alkali treatment) for the purpose of pathogen inactivation in different biowastes. The energy input was extracted or calculated from the articles when available.

Fecal coliforms, *Salmonella* spp. and *Escherichia coli* are usually selected as indicator bacteria to characterize the hygienization efficiency of the new technologies. Our review showed that most research focused on hygienization of waste sewage sludge.

Few articles were found on hygienization of other kinds of biowaste (slaughterhouse waste, animal slurries, food waste, etc.). The microwave treatment proved to be the most efficient method of pasteurization, given the significant reduction of indicator bacteria it achieved. This could be due to the coupled thermal and athermal effect induced by microwave irradiation that reinforces bacterial inactivation. Studies of electro-technologies and pressurization for the hygienization of biowaste are scarce and more research is needed.

The electrical energy consumed by these technologies ranged from $3.6 \text{ kJ}_e \cdot \text{g TS}^{-1}$ to $11.4 \text{ kJ}_e \cdot \text{g TS}^{-1}$. In contrast, Coultry et al. (2013) reported a heat input of $5.23 - 7.85 \text{ kJ}_t \cdot \text{g TS}^{-1}$ for thermal hygienization at 70°C for 60 min, given the TS of the substrates ranging between 10% and 15%. Grim et al. (2015) reported consumption of $2.2 \text{ kJ}_t \cdot \text{g TS}^{-1}$ for integrated thermophilic sanitation at 52°C for 10 h.

It should be noted that the simple comparison of the heat input of thermal hygienization and the electricity input of the alternative technology is not appropriate since the efficiency of recovery from primary energy is not the same. In France, nuclear, renewable and fossil energy represent respectively 75%, 15% and 10% of total electricity generation. The energy efficiency of these three energy sources are set at 33%, 100% and 38%, giving an average national energy efficiency of 43.6%. Assuming a 5% loss of primary energy during distribution via the power grid, the reciprocal of 38.6% ($44.6\% \text{ minus } 5\%$) gives 2.58, the regulated conversion factor from electricity to primary energy (the French Republic, 2012). The same factor is set at 3.34 in the United States and 2.60 in Germany, and depends on the energy structure of the country considered (Santos et al., 2013). This means that in terms of the energy consumption, thermal hygienization is more energy efficient than the alternative technologies that

consume electrical power. This advantage will be reduced when more electricity is generated from renewable energy sources: in Sweden, the primary energy factor from electricity was reduced from 1.92 in 2005 (Thollander et al., 2013) to 1.60 in 2018 (International Energy Agency, 2019) thanks to the development of renewable energy sources.

The most widely used substrate in studies of alternative technologies was sewage sludge whose water content usually exceeded 90%, whereas feedstocks subject to hygienization in real biogas plants (like slaughterhouse waste and livestock slurry) have a water content of less than 85%. It is widely accepted that the energy consumed by sludge pretreatment depends to a great extent on the concentration of the sludge (Cano et al. 2015). Conclusions cannot be drawn without quantitative energetic, economic and life cycle analyses. This issue is discussed in Section 6.3.

Table 4

Review of the pasteurization efficiency of several alternative technologies for biowaste hygienization (table adapted from Liu et al., 2018a).

Technology	Type of biowaste	Operational parameters	Target indicators	Microbial reduction	References
PEF	WAS	50 Hz, 0.6 - 1.2 kV·cm ⁻¹	<i>Salmonella</i> spp.	1.4 log10	Keles et al. (2010)
	Animal by-product	0.3 - 3.0 kJ _e ·mL ⁻¹ , 10 - 25 kV·cm ⁻¹	<i>Enterococcus faecalis</i>	0.5 - 3.0 log10	Liu et al. (2019)
			<i>Escherichia coli</i>	0.7 - 3.5 log10	
MW	Primary sludge	7.27 kJ _e ·g TS ⁻¹ , 85 °C	Fecal coliforms	6.8 log10	Hong et al. (2004)
	WAS	11.4 kJ _e ·g TS ⁻¹ , 85 °C	Fecal coliforms	6.5 log10	
	AD sludge	10.1 kJ _e ·g TS ⁻¹ , 65 °C	Fecal coliforms	5.6 log10	
	Sewage sludge	0.4 - 1.2 kJ _e ·mL ⁻¹ , cooled at 45 °C	Coliforms	Complete destruction	Martin et al. (2005)
	Primary sludge	4.86 kJ _e ·g TS ⁻¹ , 65 °C	Fecal coliforms	~5.6 log10	Hong et al. (2006)
	WAS	7.60 kJ _e ·g TS ⁻¹ , 65 °C		~5.4 log10	
	AD sludge	10.1 kJ _e ·g TS ⁻¹ , 65 °C	Fecal coliforms	~3.5 log10	Pino-Jelicic et al. (2006)
	Primary sludge	1 kW _e , 2450 MHz, 110 s	Fecal coliforms	4.2 log10	
	WAS		<i>Salmonella</i> spp.	2.0 log10	
	Thickened WAS	3.49 kJ _e ·g TS ⁻¹ , 80 °C, 9 min	Fecal coliforms	~2 log10	
PUS	WAS	20 kHz, 0.1 - 0.3 W _e ·mL ⁻¹ , 120 min	Total coliforms	3 log10	Chu et al. (2001)
			Heterotrophs	2.4 log10	
	WAS	5 - 27 kJ _e ·g TS ⁻¹	<i>Escherichia coli</i>	4 log10	Ruiz-Hernando et al. (2014)
Pressure	Thickened WAS	3.16 kJ _e ·g TS ⁻¹ , 20 kHz, 10 min	Fecal coliforms	~1 log10	Akgul et al. (2017)
	Sewage Sludge	Pressurised by CO ₂ , 2800 kPa for 23 h	<i>Escherichia coli</i>	No effect	Mushtaq et al. (2018)
	WAS	35 - 157 g NaOH·kg TS ⁻¹ , 24 h	<i>Escherichia coli</i>	4 log10	Ruiz-Hernando et al. (2014)
Alkali	Pre-thickened sludge	pH 10 - 12 for 0 - 4 days	Fecal coliforms	2 - 4.5 log10	Yin et al. (2017)
			<i>Salmonella</i> spp.	2 - 4 log10	
			Faecal streptococcus	2 - 4 log10	

WAS: Waste activated sludge; AD sludge: Anaerobic digested sludge; kJ_e or kJ_t: Kilojoules of electrical or thermal energy; TS: Total solids

5.3 Effect on anaerobic digestion

Several authors undertook comprehensive reviews of pretreatment for the improvement of methane yield (Carrère et al., 2010), energy feasibility (Cano et al., 2015), waste management (Carrere et al., 2016), BMP and enhancement of the dewaterability of the sewage sludge (Zhen et al., 2017) plus details on the mechanisms behind the AD pretreatment process (Li et al., 2019). They also summarized the advantages and the disadvantages of each technology. The present section completes their reviews by considering recent research including that conducted by 2018 and extending the substrates from sewage sludge to all kinds of biowaste for which hygienization is mandatory, e.g. animal slurries, meat-processing sludge, and slaughterhouse waste.

Fig. 6 shows the gain in methane yield of the biowaste obtained using different kinds of pretreatment. Although treatment efficiency depends on the origin of the substrates and on the operational parameters applied, the boxplots enable the comparison of the different technologies and give a general idea of the possible range of methane enhancement that could be obtained with different alternative technologies. The figure shows that the medians of the methane yield enhancement were generally below 50% for the majority of the alternative hygienization processes, including electro-technology ($n = 12$), microwave irradiation ($n = 10$), power ultrasound ($n = 20$), high hydrostatic pressure ($n = 6$) and alkali pretreatment coupled with heating ($n = 7$). Only two studies were available on acidic treatment combined with heating, and they reported an increase in methane production of 14.3% and 17.5%, respectively. A higher median value (100%) was observed with oxidation ($n = 9$).

In addition, several reported values were identified as outliers, indicating they are beyond the 99% possible interval estimated by the boxplot method. The outliers (whose values lie an abnormal distance from other values) are marked by open circles and the extreme outliers (whose values exceed three times the height of boxes) are marked by asterisks. The outliers in the figure are further discussed in the following paragraphs in a review of the possible reasons why they obtained such extremely high results of BMP enhancement. The corresponding absolute BMP values based on volatile solids (VS) and on the total weight (TW) of the substrates are also presented.

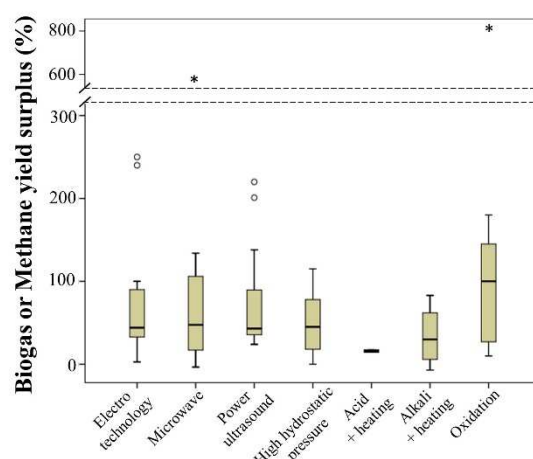


Fig. 6. Summary of alternative technologies for the enhancement of the biogas or methane yield of biowaste.

(please refer to the supplementary material for the original data and references of this figure)

(data of this figure were collected from Tiehm et al., 1997; Engelhart et al., 1999; Wang et al., 1999; Weemaes et al., 2000; Chu et al., 2002; Onyeche et al., 2002; Yeom et al., 2002; Barjenbruch and Kopplow, 2003; Goel et al., 2003; Bien et al., 2004; Valo et al., 2004; Bougrier et al., 2005; Choi et al., 2006; Bougrier et al., 2007; Xie et al., 2007; Braguglia et al., 2008; Carlsson et al., 2008; Rittmann et al., 2008; Lin et al., 2009; Salerno et al., 2009; Salsabil et al., 2009; Jin, 2010; Carrère et al., 2010; Erden and Filibeli, 2010; Yang et al., 2010; Beszédes et al., 2011; Braguglia et al., 2011; Coelho et al., 2011; Devlin et al., 2011; Lee and Rittmann, 2011; Li et al., 2012; Shao et al., 2012; Zhang et al., 2012; Appels et al., 2013; Cheng and Hong, 2013; Uma Rani et al., 2013; Zawieja and Wolny, 2013; Cesaro et al., 2014; Houtmeyers et al., 2014; Ruiz-Hernando et al., 2014; Vergine et al., 2014; Wahidunnabi and Eskicioglu, 2014; Wonglertarak and Wichitsathian, 2014; Ebenezer et al., 2015; Ki et al., 2015; Martín et al., 2015; Riau et al., 2015; Zhou et al., 2015; Pilli et al., 2016; Serrano et al., 2016; Zou et al., 2016; Chamaa, 2017; Safavi and Unnthorsson, 2017; Zhen et al., 2017; Safavi and Unnthorsson, 2018; Mushtaq et al., 2018)

In SCOD/TCOD (soluble/total COD), extracellular polymer content and biogas production, Choi et al. (2006) reported increases of up to 4.5 times (from 0.040 to 0.180), 6.5 times (from 65 to 420 mg·L⁻¹) and 2.5 times (from 52 to 129 NmL·g VS⁻¹ or from 0.972 to 2.37 NmL·g TW⁻¹) respectively, after application of the pulsed electro-power to WAS at 19 kV·cm⁻¹ at a frequency of 110 Hz for 1.5 s. In another study, Ki et al. (2015) treated primary sludge with PEF that increased the concentration of acetate 2.6 times (from 88 to 230 mg·L⁻¹) and current density by 140% (from 1.3 to 3.1 A·m⁻²). This means that the electrical treatment could produce a relatively higher enhancement of methane potential for biowaste. A study by Salerno et al. (2009) concluded that the PEF pretreatment increased the methane potential by 100% in the case of biosolids (WAS) and by 80% in the case of pig manure (absolute BMP values not available). However, the marked increase in methane yield might be due to the use of a non-standard method of interpretation of the data: the biogas yield curves did not level out to enable the precise evaluation of the bio-methane potentials of the substrates. Safavi and Unnthorsson (2018) investigated the effect of PEF on methane production using pig slurry and found that a 58% increase of the methane yield obtained by the electroporation failed to compensate for the energy input of PEF (absolute BMP values not available).

Beszédes et al. (2011), Coelho et al. (2011) and Ebenezer et al. (2015) obtained a maximum 134% increase in biogas yield (from 211 to 495 NmL·g TS⁻¹ or from 57.6 to 135 NmL·g TW⁻¹), 102% (from 199 to 401 NmL·g TS⁻¹ or from 6.25 to 15.7 NmL·g TW⁻¹) and 570% (from 57.5 to 386 NmL·g TS⁻¹ or from 0.05 to 3.36 NmL·g TW⁻¹) respectively for microwaved meat industry sludge and two kinds of activated sludge, compared with the other studies that achieved less than 60% after MW irradiation.

Beszédes et al. (2011) reported that they were incapable of compensating for the energy demand of MW through surplus methane yield whereas Ebenezer et al. (2015) succeeded in saving 50.7% of the operational cost with an optimized energy input of 14 $\text{kJ}_e \cdot \text{g TS}^{-1}$ thanks to the high biogas yield obtained with MW treatment.

The PUS generally enhances the biogas potential of the biowaste by less than 80% whereas Tiehm et al. (1997), Onyeché et al. (2002), Chu et al. (2002) and Zawieja and Wolny (2013) obtained 220% (absolute BMP values not available), 138% (from around 220 to 500 $\text{NmL} \cdot \text{g TS}^{-1}$ or from around 3.50 to 7.95 $\text{NmL} \cdot \text{g TW}^{-1}$), 104% (from 200 to 408 $\text{NmL CH}_4 \cdot \text{g TS}^{-1}$ or from 1.88 to 3.83 $\text{NmL CH}_4 \cdot \text{g TW}^{-1}$) and 200% (from 320 to 963 $\text{NmL} \cdot \text{g VS}^{-1}$ or from 2.06 to 9.10 $\text{NmL} \cdot \text{g TW}^{-1}$) respectively, for various types of sewage sludge. Tiehm et al. (1997) terminated the AD process too early and was consequently unable to obtain the best potential methane values of the PUS-treated and intact sludge. The high values reported in the other three studies could be explained by the variation in the composition of the substrates and the strength of the sonication applied.

The pressurization pretreatment was reported to increase methane yield by between 18% and 78% except for Zhang et al. (2012) who obtained a 110% cumulative rise in methane yield (from 1.6 to 3.4 NL). Mushtaq et al. (2018) found no difference in methane yield using settled sludge (from 304 to 316 $\text{NmL} \cdot \text{g VS}^{-1}$ or from 11.4 to 11.9 $\text{NmL} \cdot \text{g TW}^{-1}$) and using WAS (from 251 to 259 $\text{NmL} \cdot \text{g VS}^{-1}$ or from 5.77 to 5.96 $\text{NmL} \cdot \text{g TW}^{-1}$). The former enhancement could be attributed to the short digestion time practiced by the authors (7 days), which was more representative for the comparison in terms of the methane yield rate rather than the methane potential. The latter study dealt with pressurization with the aid of CO_2 ($p \text{ CO}_2 = 2.8 \text{ MPa}$) for the improvement of

biogas production of co-settled sewage sludge after 70 days of digestion. The authors found that pressurization by CO₂ was not suitable for waste treatment, as it influenced neither the methane yield nor the viable counts of *E. coli* and *S. enterica*.

The BMP could be influenced (in a range of 10% - 83%) by the alkali pretreatment coupled with heating, depending on the alkali concentration and the combined heating operational parameters (time and temperature). It should be noted that Li et al. (2012) found a long lag time (inhibition) for the NaOH-pretreated sludge that produced merely 1.5% more biogas than untreated one (from 517 to 525 NmL·g VS⁻¹ or from 5.58 to 5.67 NmL·g TW⁻¹). This absence of effect may be due to the nature of the substrate, meaning that no further biodegradable COD could be achieved by the NaOH pretreatment. Wonglertarak and Wichitsathian (2014) found that anaerobic degradability could be increased by alkali pretreatment to a degree that depended on the AD incubation temperature: ranging from a 7.6% increase in biogas (from 78.24 to 84.22 NmL·d⁻¹) to 18.6% (from 61.09 to 72.46 NmL·d⁻¹) for the AD in thermophilic and ambient conditions respectively.

The marked incertitude of BMP enhancement is evidenced by oxidation pretreatment, ranging from 16% to 180%, with one extremely high value of 800%. This notable difference could be due to the different oxidation methods studied. Zhou et al. (2015) obtained a 13% increase in methane (from 264 to 298 NmL·g VS⁻¹ or from 2.67 to 3.01 NmL·g TW⁻¹) by using an iron activated peroxidation pre-treatment process (50 mg H₂O₂·g TCOD⁻¹ with the indigenous iron serving as catalyst). Weemaes et al. (2000) used an ozone treatment with 0.05 - 0.2 g O₃·g COD⁻¹, which produced a methane surplus of around 200% (from approximately 110 to 330 NmL·g COD⁻¹ or from 0.83 to 2.21 NmL·g TW⁻¹). This means an improvement in biodegradability ranging from 31.4%

to 93.3%, obtained from the BMP divided by the theoretical maximum BMP of the organic matter ($350 \text{ NmL}\cdot\text{g COD}^{-1}$). This high biodegradability achievement remains a laboratory-scaled research because the substrate was highly diluted. The extremely high methane surplus was obtained by Cheng and Hong (2013), who studied the effect of pressure-assisted ozonation (PAO) and found that with a Feed/Inoculum ratio of 0.8 and after 20 cycles of PAO at 1,040 kPa for 30 s/cycle, the biogas production based on the added COD was improved 8 times (from around 20 to $160 \text{ NmL}\cdot\text{g COD}^{-1}$ or from 0.167 to $1.27 \text{ NmL}\cdot\text{g TW}^{-1}$) compared to the untreated sludge. In addition, the pure ozonation effect without pressurization could also have 2.4 times (from around 20 to $48 \text{ NmL}\cdot\text{g COD}^{-1}$ or from 0.167 to $0.365 \text{ NmL}\cdot\text{g TW}^{-1}$) the biogas potential of the intact samples. Despite the high relative values, the low biodegradability and the low total weight BMP of the substrate may make industrial application less likely.

To conclude, it is clear that, for certain outliers identified in **Fig. 6**, the substrates tested were not easily biodegradable (low BMP values). A slight increase in absolute BMP values induced by the pretreatment could contribute to a high BMP enhancement interpreted by the percentage. It is also worth noting that a number of the papers only discussed the percentage BMP rise but did not report any BMP absolute values. The absence of these basic research data makes it difficult to compare results with those of other papers. It is therefore recommended that the researchers report the absolute values of both the experimental and the modeling data when calculating the BMP enhancement. Less attention has been paid to studies on animal by-products and other regulated biowaste which possess high gross biogas yield (based on total weight). This point is discussed in Section 6.3.

The alternative technologies available for the hygienization of biowaste proved to be efficient in the pasteurization and the enhancement of methane production process. Generally, methane enhancement was increased by around 50%, up to 100% in limited cases with most technologies. Many of the extremely high values reported in the literature might be explained by the different methods used for data interpretation and the over-input of energy. Researchers should be aware of this problem when they compare their research results with those in the literature.

6. Discussion and future outlook

6.1 Sanitary challenges

The sanitary challenges involved in applying biowaste on agricultural land can originate from different sources such as cross transmission due to hygiene protocols being ignored, contamination by the over-fertilized soils and sick animals. However, when applying treated biowaste to the land, many factors determine the survival and decay behaviors of the pathogens in the amended soils, including pH, moisture content, temperature, weather, oxygen accessibility and soil types (Roberts et al., 2016). There is no clear relationship between transmission of zoonotic pathogens in the amended soils and the probabilities of human infection, all depending on the pathogens considered and the methods of evaluation used for quantitative risk assessment (Viau et al., 2011). Another emerging concern is the transmission of antibiotic resistance genes of the bacteria from the biowaste into the environment (Wellington et al., 2013). Since the hygienization regulations do not control the presence of the bacterial endospores and the viruses in the final digestate, one should always be aware that certain non-conventional pathogenic agents might survive all biowaste treatment processes and finally enter the environment (Zhao and Liu, 2019). No further information was available in the

literature about how to cope with this potential sanitary risk during the disposal of the digestate. These subjects are among the main topics that require further research for a better sanitary approach to the disposal of treated biowaste.

6.2 Industrial applications

6.2.1 Hygienization before, during or after anaerobic digestion

The hygienization processes before (pre-hygienization), during (inter-hygienization) or after anaerobic digestion (post-hygienization) play an important role in pathogen reduction efficiency, energy consumption and the quality of the final digestate entering the biogas plant (Haumont et al., 2019).

Most regulations impose pre-hygienization of the biowaste before anaerobic digestion. On one hand, this may require supplementary energy to heat several regulated substrates to a desired temperature (for example, 70 °C in Europe). On the other hand, the pretreated substrate may also preheat the digesters when mixed with other feedstock. No studies are available concerning this energy balance. Pre-hygienization may modify the biogas production kinetics of the substrate. In terms of the pathogen reduction efficiency, it inactivates the pathogens before entering the digesters, permitting further hygienization during the anaerobic digestion that prevents the regrowth of the inactivated microorganisms, as stated in Section 3.2.

Post-hygienization immediately after AD process has been shown to be the most economically effective (Sahlström, 2003) since it requires less energy to heat the digestate which has already been heated to the digestion temperature. However, heating all the digestate rather than just the regulated feedstock would make post-hygienization less efficient. Astals et al. (2012) conducted an energy balance of the post-hygienization process and concluded that a positive energy surplus could only be achieved with a heat

recovery system. Keller (1983) found that the post-hygienized digestate was more vulnerable to regrowth of the indicator pathogens. This author explained the phenomenon by the fact that the post-treatment could induce further degradation of the organic matter thereby favoring bacterial growth. Clements (1983) recommended pre-hygienization of the substrate to minimize the likelihood of recontamination of the sludge, despite the higher energy input. Post-hygienization can cause excessive NH_3 loss that influences the final quality of the digestate as fertilizer (Haumont et al., 2019).

Inter-hygienization, i.e. treating the digestate between the primary and the post digesters, has not been well studied. Like post-hygienization, it has the advantage of lower heat input. However, this intermediary heating could possibly destroy methanogenic microbes present in the digestate and therefore influence post-digestion performance (Haumont et al., 2019).

6.2.2 Commercially available technology

Commercially available technology for higienization is rare. Most biogas plants apply the same operational parameters as for thermal higienization (residence time and temperature) according to the appropriate regulations. The design and the operational mode of the process vary from one biogas plant to another.

When it comes to commercially available alternative technologies, many companies dealing with the sludge pretreatment by electro-technology (e.g. OpenCEL[®], Biocrack[®], PowerMod[®]), pressurization (e.g. MicroSludge[®]), ultrasounds (e.g. Sonix[®], Biosonator[®]), etc. exist or have existed. These commercial technologies are intended to treat the sludge to improve BMP and dewaterability. The alternative solutions for the higienization of biowaste remain to be tested commercially. The transfer of the

commercial technologies from the food processing industry to the waste treatment is also welcomed.

6.3 Cleaner production of hygienization

Carrere et al. (2016) summarized the technical advantages and disadvantages of the pretreatment methods from lab scale to full scale. Zhen et al. (2017) discussed the state of the art of sewage disintegration technologies. Both authors made general comments on the state of the art of the different pretreatment methods and listed several commercially available technologies. The focus was on methane enhancement, improving dewaterability, the cost, the demand for specific energy, operational difficulties and the actual scale of application. The present paper focuses on cleaner production of the thermal and alternative technologies for hygienization.

As discussed in Section 5.2, from the point of view of exergy, the thermal energy is more efficient than electrical power. The general exergy efficiency of the total AD system was estimated at 58.5% in a multi-generation biogas power plant that treated animal manure and crop residues (Ogorure et al., 2018). The authors stated that nearly 80% of the total expenses were related to the exergetic destruction. The authors of another study (Barrera et al., 2016) concluded that an exergy efficiency up to 46% could be achieved in a biogas plant treating vinasse. Most improvement in exergy efficiency could be achieved by recovering the residual heat from the raw vinasse (80 °C) to heat the digesters. Barati et al. (2017) reported an overall exergy efficiency of 72.8% for a biogas cogeneration plant. Almost 70% of the exergy loss was due to the irreversible energy degradation and about 26% of the loss was attributed to the failure to recover the waste heat from the exhaust gas in the combustion chamber. The authors suggested that installing a heat recovery system would also significantly enhance the exergy efficiency.

De Meester et al. (2012) reported that the exergy efficiency of a biogas plant for electricity generation varied from 15.3% to 33.3% and stated that the residual AD digestate had much more exergetic potential than electricity. A life cycle analysis (LCA) proved that the main environmental impact of a biogas plant came from the emission of NO_x in the combined heat and power process (CHP). The overall impacts could be reduced if the production of electricity (CHP) was avoided (Massaro et al., 2015). Pöschl et al. (2010) reported that the energy efficiency of biogas production systems could be improved by up to 65% if the biogas was valorized in the form of the natural gas rather than as electricity. Ware and Power (2016b) conducted a net energy analysis of an Irish biogas plant treating ABP. They found that the biogas plant could be energy self-sufficient since the surplus of the thermal energy could be used for heating in the vicinity of the plant and the electricity generated could subsidize the demand for electricity for biogas production.

These studies confirm that the recovery of the waste thermal energy to heat the reactors (including the hygienizers) and full valorization of the final products (biogas and digestate) would be more exergy-efficient solutions than the generation of the electrical power. The thermal process is thus more advantageous than electricity-consuming technologies when it is performed using local waste heat. After a literature review, Cano et al. (2015) made the same comment, i.e. that thermal pretreatment, rather than other innovative treatments, could enable energy self-sufficiency if waste heat was valorized. These authors also found that the lab-scale pretreatment was not energy feasible for industrial implementation. Innovative technologies require further study to improve energy efficiency and treatment efficiency at larger scales.

If the study boundaries are expanded to pretreatment of the feedstock, the effect of the thermal and the alternative technology on the enhancement of BMP should also be taken into account in energy and exergy balances. Mirmasoumi et al. (2018) conducted an exergo-economic analysis of the anaerobic digestion of sewage sludge assisted by mild thermal pretreatment (70 °C and 90 °C) and found that a pretreatment at 90 °C for 30 min followed by the thermophilic digestion of the sludge reduced the total energy cost by 41.3%. Li et al. (2017) performed a life cycle analysis of the different biogas production pathways of sewage sludge and concluded that thermophilic anaerobic digestion with a high concentration of solids could reduce the environmental impacts by 40% and provide about 30% economic surplus, compared to the other anaerobic digestion methods including thermal hydrolysis.

The moisture content of the substrates plays an important role in reaching an energy balance. Cano et al. (2015) reviewed the dependence of the energy feasibility of a variety of pretreatment methods on the sludge concentration. Comparisons of the biogas yield of different substrates based on total weight is rare, even though this is a key factor considered by biogas plants when they choose their substrates. Monlau et al. (2015) also confirmed that the net energy gain of the chemical pretreatment depended on the TS concentration of the feedstock. Bragulia et al. (2011) considered that thickening the sludge would be a key to achieving positive net energy production. Their study focused on sewage sludge with high water content. Other biowastes, like slaughterhouse waste and animal slurries, generally have higher concentrations of solids and could thus be more appropriate for innovative hygienization / pretreatment technologies. Few analyses of this energy gain and the relevant environmental impacts

were available. Further research on this aspect is of great importance, i.e. examining suitable hygienization / pretreatment methods as a function of the type of biowaste.

7. Conclusions

The high levels of pathogens in biowaste is of growing concern for public health. The relation between application of the anaerobic digestate on the land and human infection remains unclear. Although the hygienization process is believed to remove most of the pathogenic agents, the introduction of the antibiotic resistance genes as well as the survival of the bacterial spores requires more research into their behavior during treatment in the biogas plants and their dispersal into the environment.

The present paper reviewed thermal, electrical, mechanical and chemical pre-hygienization technologies for the safe disposal of anaerobic digested biowaste. Pre-hygienization, either thermal or non-thermal, has an effect on both pathogen reduction (high bacterial reduction) and the biogas yield (between 0 - 50% BMP enhancement in most studies). However, some published results show marked variations (up to 800% increase in BMP) and lack a standard protocol for data interpretation to insure results in the literature are sufficiently comparable.

Analyses of the energy balance and the environmental impacts assess the technical and economic feasibility of available hygienization processes. Thermal hygienization represents 6 - 25% of the total primary energy produced by biogas plants in Europe. The present review shows that, from an exergo-economic view, thermal pre-hygienization valorizing waste heat is generally more efficient than the other alternative approaches that require electrical power.

The concentration of solid in the waste largely affects the net energy yield. Many papers dealt with the sewage sludge with low concentrations of solid. More innovative

hygienization approaches to the biowaste with higher concentrations of solids are thus needed. Slaughterhouse waste and the animal slurry whose hygienization is mandatory in the EU are increasingly interested in biogas plants for energy production because of their high BMP based on wet weight. Future research should also focus on both improving technical efficiency and evaluating feasibility based on the energy, LCA and economic analyses of the implementation of the alternative hygienization technologies.

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Supplementary material

Original literature data of **Fig. 3**, **Fig. 5** and **Fig. 6** are available in **Table S1**, **Table S2** and **Table S3** in the supplementary material.

Nomenclature

ABP	Animal by-products
AD	Anaerobic digestion
ARG	Antibiotic resistance genes
AW	Agricultural waste
BGP	Biogas plant
BMP	Bio-methane potential
CFU	Colony-forming unit
CH ₄	Methane

CHP	combined heat and power process
COD	Chemical oxygen demand
F-value	Pasteurization value
HHP	High hydrostatic pressure
HYG	Hygienization
$\text{kJ}_e \cdot \text{g TS}^{-1}$	Kilojoules of electrical energy per gram of total solids
$\text{kJ}_t \cdot \text{g TS}^{-1}$	Kilojoules of thermal energy per gram of total solids
LCA	Life cycle analysis
MAD	Mesophilic anaerobic digestion
MSW	Municipal solid waste
MW	Microwave irradiation
n	Number of cases considered
PAO	Pressure-assisted ozonation
PEF	Pulsed electric field
PRE	Pretreatment
PUS	Power ultrasound
Q	Energy required or generated
SANI	Sanitation
SCOD	Soluble chemical oxygen demand
T	Operational temperature
T_{ref}	Reference temperature
TAD	Thermophilic anaerobic digestion
TCOD	Total chemical oxygen demand
TS	Total solids

TW	Total weight
VS	Volatile solids
WAS	Waste activated sludge
Z	Z-value

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Table list

Table 1 Summary of regulatory treatment times and temperatures for the thermal hygienization of biowaste in different countries.

Table 2 Criteria chosen by several authorities for the indicator microorganisms characterizing the target biowaste treated by AD for safe disposal.

Table 3 Energy demand of thermal hygienization process in several BGP in Europe (* considering the heat consumption of all the AD units, table adapted from Liu et al., 2018a).

Table 4 Review of the pasteurization efficiency of several alternative technologies for biowaste hygienization (table adapted from Liu et al., 2018a).

Figure captions

Fig. 1. Pathogen transmission pathway from biowaste to humans and to the natural environment.

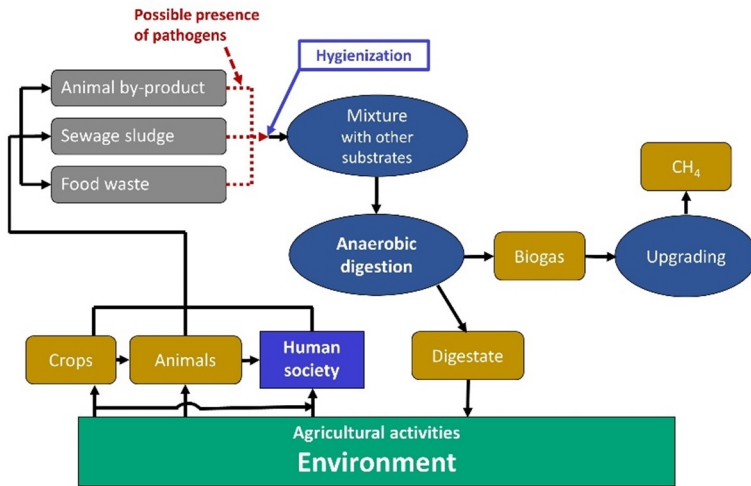
Fig. 2. Bibliometric review of the number of papers published per year on the topic “AD - PRE” (anaerobic digestion without pretreatment), “AD + PRE” (anaerobic digestion with pretreatment) and “AD + (HYG or SANI)” (anaerobic digestion with hygienization or sanitation) in Web of Science Core Collection (Clarivate Analytics) since 1990.

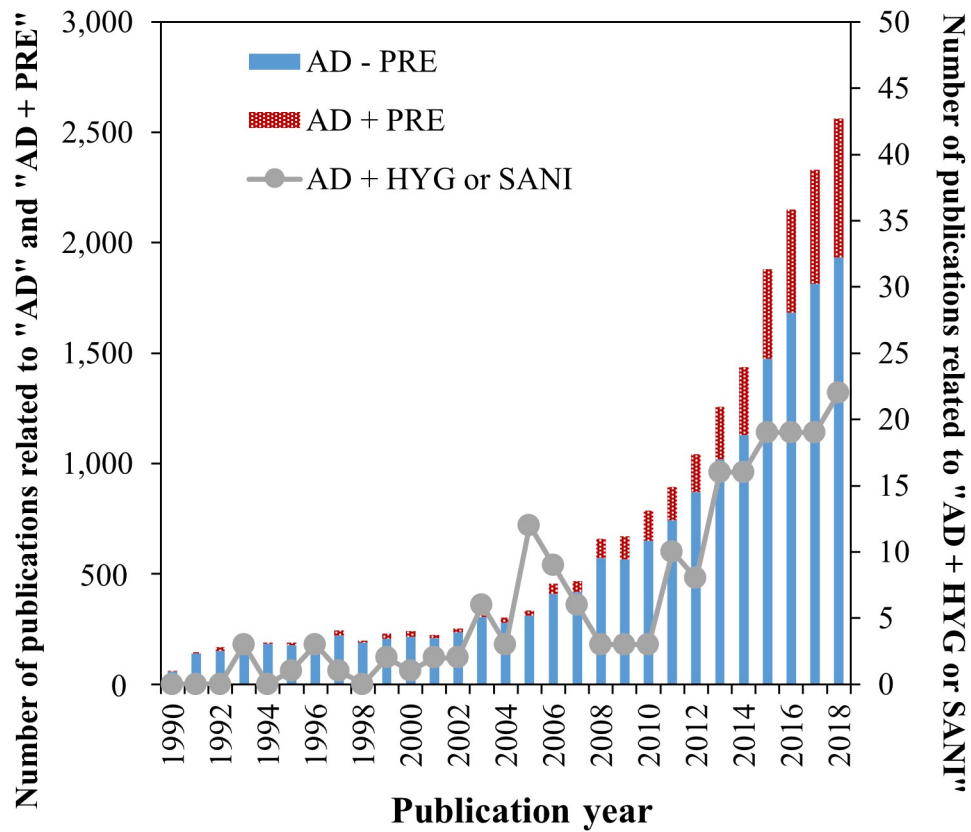
Fig. 3. Occurrence of pathogens in biowastes.

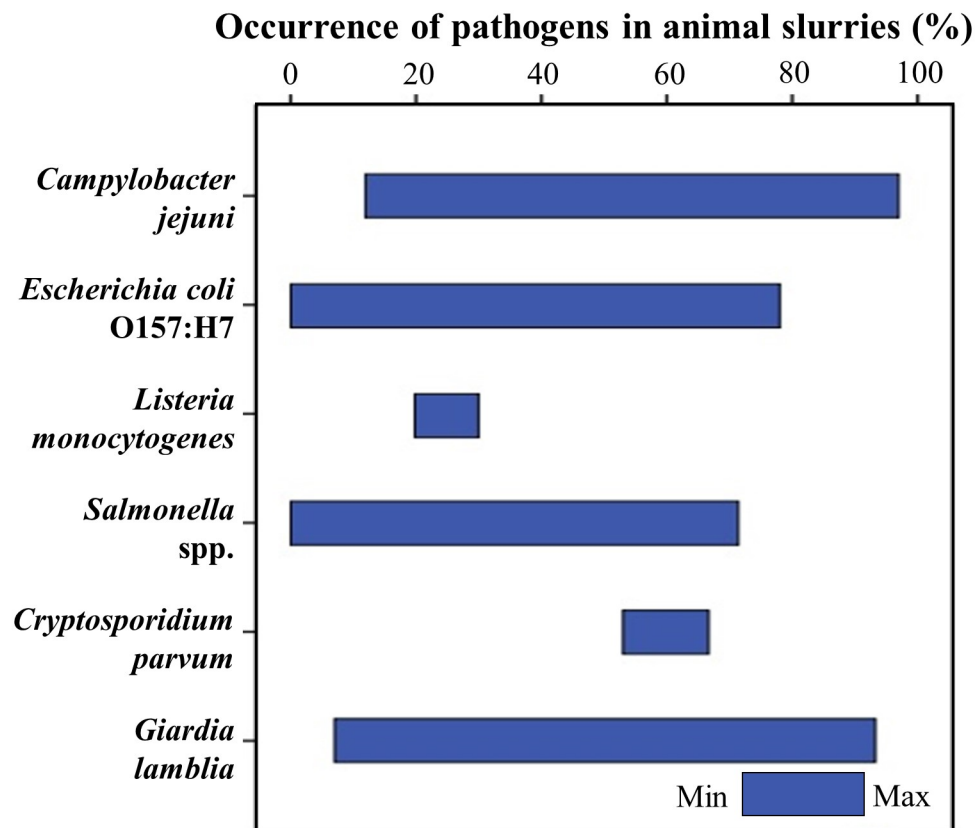
Fig. 4. Example of the abundance of pathogens in animal slurries.

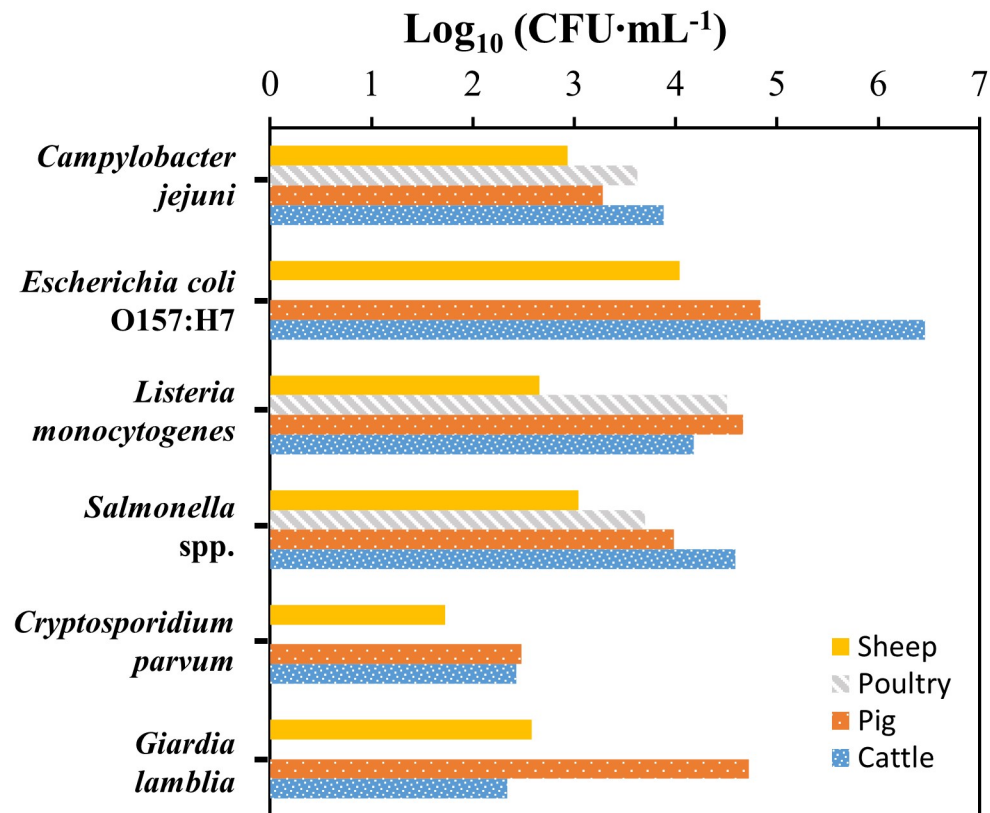
Fig. 5. Summary of short-term mild thermal pretreatment ($< 100\text{ }^{\circ}\text{C}$) related to biogas or methane yield enhancement of biowastes.

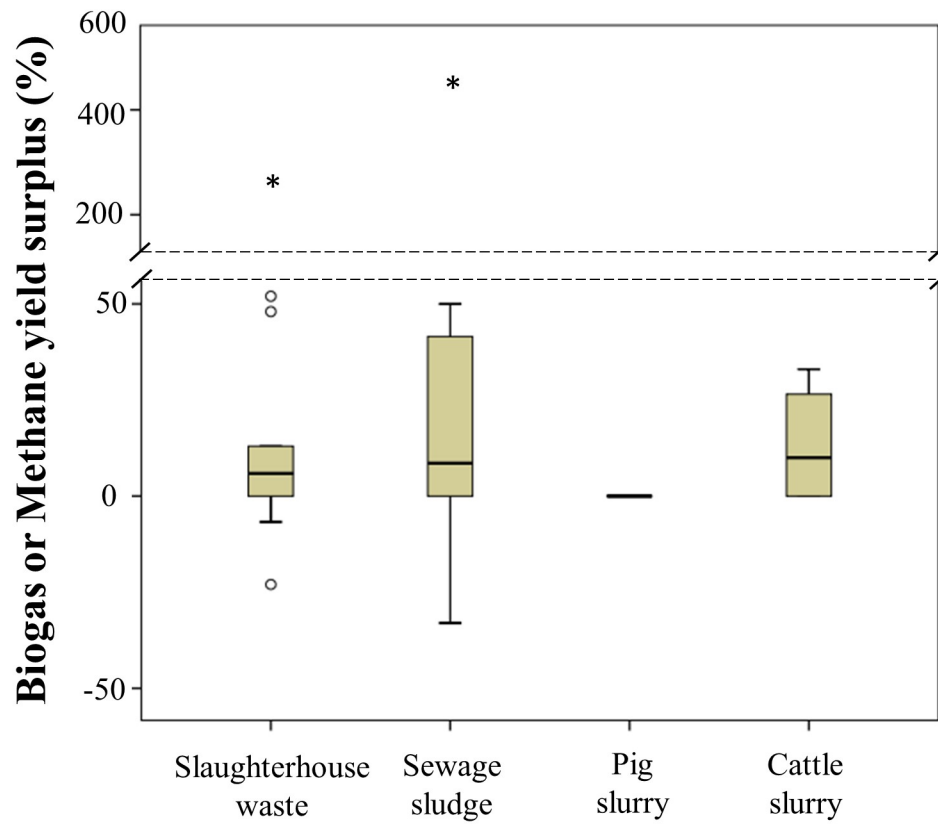
Fig. 6. Summary of alternative technologies for the enhancement of the biogas or methane yield of biowaste.

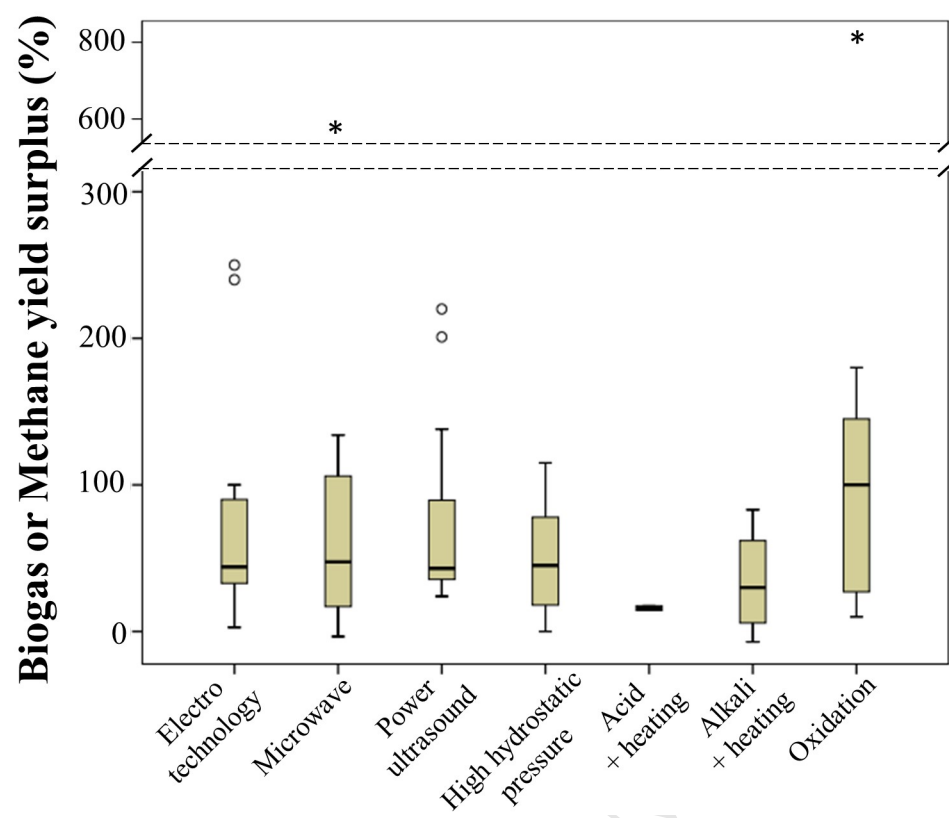












Highlights

- A comprehensive review of the state of the art of the biowaste hygienization
- Global regulations on the hygienization of biowaste were compared
- Thermal hygienization consumes 6 - 25% of the primary energy produced by biogas
- Pre-hygenization influences the bio-methane potential of the treated waste
- The energy efficiency of alternative technologies remains to be improved