Journal of Environmental Chemical Engineering 13 (2025) 118879

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Sustainable valorization of slaughterhouse solid waste: A review of characterization, conditioning, hydrolysis and biotransformation strategies

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ARTICLE INFO

Keywords:
Slaughterhouse waste
Waste characterization
Hydrolysis
Anaerobic digestion
Sustainable waste management
Animal by-products (ABPs)

ABSTRACT

The meat processing industry generates significant quantities of waste, including solid animal by-products (ABPs) and slaughterhouse wastewater (SWW). For instance, based on global meat production in 2020 (approximately 250 million tons), slaughterhouse activities can generate up to 2250 million m^3 of SWW and around 375 million tons of ABPs annually. While SWW is typically treated on-site using conventional technologies, ABPs are managed in external facilities employing traditional methods such as incineration, landfilling or rendering. These practices not only intensify environmental and health issues but also hinder the potential of valorization of ABPs into high-value products. Recent advancements in biological processes have explored the transformation of ABPs into valuable products, such as biogas through anaerobic digestion (AD), predominantly at lab-scale. However, the hydrolysis of ABPs remains a critical bottleneck due to their complex composition and high organic content, requiring extended processing times and large reactor volumes, which limit industrial-scale applications. This review evaluates current and emerging practices for the treatment and valorization of slaughterhouse waste, with a focus on transforming these residues into high-value products, promoting sustainability in the meat industry. This study identifies and characterizes ABP fractions suitable for biological processes and emphasizes the role of waste conditioning and hydrolytic strategies aimed at enhancing the efficiency of biological based processes. Key factors influencing hydrolysis performance are discussed alongside the integration of pretreated waste in industrial AD process. The review provides a critical analysis of existing methods, highlighting their gaps, and identifies opportunities to optimize biological waste conversion for sustainable and high value bioproduct generation.

1. Introduction

In the past years, a significant increase in the meat production and consumption has been reported in the literature, with a global meat production of 252 million tons in 2020 [39,49]. This trend is expected to continue due to the increasing global population together with the rising of income levels. The expansion of the meat processing sector is accompanied by high levels of waste generation [38]. Waste generated by the meat slaughtering and processing industry, includes solid animal by-products (ABPs) and slaughterhouse wastewater (SWW). For instance, 1.6–9.0 m^3 of water is used per ton of cattle carcass and 1.6–8.3 m^3 of water per ton of pig carcass, according to the Food and Agriculture Organization (FAO) [11].

The characterisation of SWW stream has been extensively reported, depending on the processed animal [37,5]. On the one hand, SWW are normally treated in-situ using wastewater treatment facilities. These facilities generally combine primary treatment, to separate large particles or solids, followed by a physicochemical or biological treatment, that focuses on the reduction of organic matter concentration by using either anaerobic or aerobic strategies. A key step in SWW treatment

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https://doi.org/10.1016/j.jece.2025.118879
Received 30 May 2025; Received in revised form 1 August 2025; Accepted 22 August 2025
Available online 29 August 2025

involves the use of a flotation unit prior to the biological step, to efficiently separate fats, resulting in a solid side-waste that requires further management and disposal [4].

On the other hand, ABPs, catalogued as solid residue and considered on average up to 60 % of live weight of the processed animal, are regarded unsuitable for human consumption [21]. These residual materials present significant challenges due to their pathogenic nature and high organic load composed of proteins, lipids and inorganic fractions, with concentration varying across geographic regions, animal type and processing practices [36].

Typically, ABPs are composed of different fractions obtained in the slaughtering process that are usually combined to create mixed streams commonly managed by external companies following current regulations. The treatment and disposal of ABPs is regulated worldwide. In the EU, for example, Directive EC No 1069/2009 defines ABPs as materials of animal origin that are not intended for human consumption and may have a potential risk to public and animal health [10]. Also, this EU Directive regulates the management, disposal and use of ABPs by conventional means due to the potential risk to public and animal health.

Three different categories are defined in the Directive EC No 1069/2009: category I, considered high risk material, including parts of animals suspected to be infected or containing environmental contaminants, disposed of by incineration; category II, or intermediate risk, including manure, digestive tract content and animals that die other than by slaughter or not suitable for human consumption, that is safe to be used as animal feed or other purposes when pressured or sterilized; and category III, which includes low risk materials that are safe for human consumption but not intended for it, which may be used under strict conditions for pet food, animal feed, composting or biogas production. While category I ABPs must be disposed of by specialized biohazard waste management companies, ABPs that fall in categories II and III are potential fractions that can be used for resources recovery when appropriate treatment is applied [10].

The use of traditional approaches for managing ABPs, such as landfilling or incineration as a final disposal may raise environmental concerns and health hazards when not properly executed, and they fail to add value to the ABPs materials [31]. This is also the case for the rendering industry, which processes mixed ABPs from slaughterhouses through grinding and cooking, to produce materials, such as animal feed or fats generally destined for low-value markets.

In response to these limitations, alternative strategies aligned with circular economy principles have gained interest recently among researchers and meat processing companies, aiming to valorise slaughterhouse waste into higher-value products such as biomaterials or biofuels [21,36,17]. In this context, anaerobic digestion (AD) is particularly relevant for resource recovery, enabling the production of biogas (with yields of 0.3–0.8 m^3 biogas kg^{-1} depending on the ABP source) and digestate, which can be used as solid amendment [21].

The typical steps for stabilization of both slaughterhouse waste streams are shown in Fig. 1. Solid wastes are classified according to ABPs categories, of which only category II and III materials can be treated via AD after undergoing conditioning and hygienization steps. A hydrolytic step prior to AD is often recommended to enhance the overall process efficiency. In the case of SWW fat is commonly and primarily separated using a flotation unit. The recovered fat is thus treated as a solid waste, while the remaining wastewater typically undergoes aerobic treatment. Alternatively, it may be used as a dilution media for solid substrates intended for AD.

However, the complex composition of ABPs poses significant challenges for large-scale implementation, requiring extensive reactor volumes and prolonged operating times to achieve effective hydrolysis and transformation. At present, most studies have been conducted at laboratory scale, using synthetic or isolated ABP fractions, leaving the feasibility of treating mixed ABPs streams under industrial conditions largely unexplored [13,42]. The efficiency of AD is highly dependent on the selection of appropriate hydrolytic strategies, tailored to specific

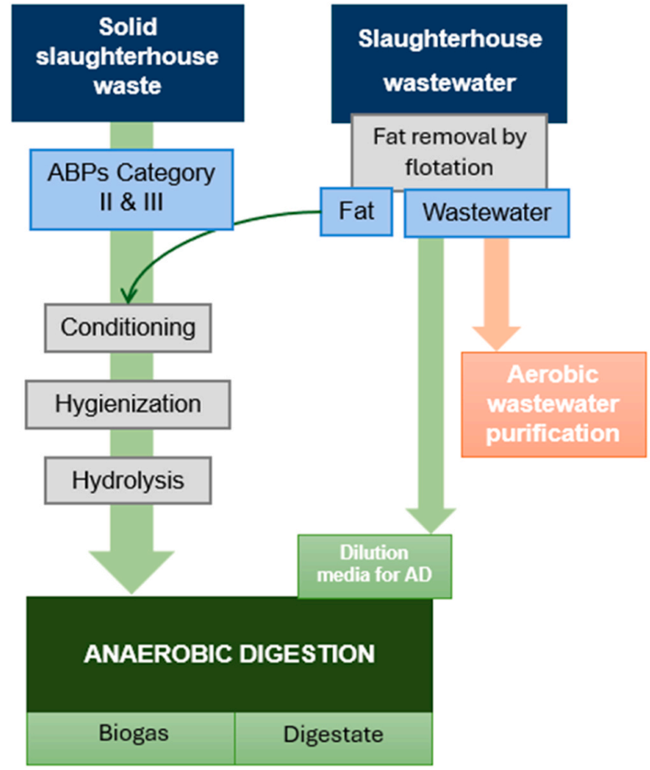

Fig. 1. Typical steps for the stabilization of slaughterhouse solid waste and wastewater through anaerobic digestion.

waste composition. Additionally, waste conditioning, including particle size reduction or pH adjustment, is crucial for optimizing pretreatment efficiency and also, to efficiently conduct the AD process. Without a well-defined hydrolytic strategy, the need for larger reactor volumes and extended retention times would increase operational costs and limit process scalability [27].

The increasing interest in slaughterhouse waste management and the valorisation of ABPs is reflected in the growing number of publications addressing these topics. Fig. 2 shows the bibliometric evolution of slaughterhouse waste research from 2010 to 2024, based on annual publication counts retrieved from Science Direct. Three keyword queries were used: “slaughterhouse waste” (including both solid and liquid waste), “slaughterhouse waste anaerobic digestion” and “hydrolysis slaughterhouse”. An upward trend is observed, with general publications on slaughterhouse waste increasing from 134 articles in 2010 to 785 in 2024. Moreover, specific studies on anaerobic digestion and hydrolysis reached 313 and 340 articles, respectively, in 2024.

In this sense, this study aims to explore the overall slaughterhouse waste treatment and management practices. It highlights the potential of bio-based processes to transform ABPs into high-value products, thereby enhancing sustainability in the abattoir industry. Different waste streams generated in slaughterhouses were identified, in terms of their origin and their characterization, to envisage its possible standardization as feedstock for further biotransformation. In addition, a comprehensive review was conducted to identify demonstrative case-studies dealing with conditioning and hydrolysis of several types of ABPs and of lipid-rich waste streams from wastewater treatment plants (WWTPs) treating SWW. Each case was deeply analysed to obtain comparable and useful information from an engineering perspective. The analysis exemplifies the most common types of conditioning and hydrolytic strategies implemented to enhance the biotransformation and resource recovery from ABPs, through AD.

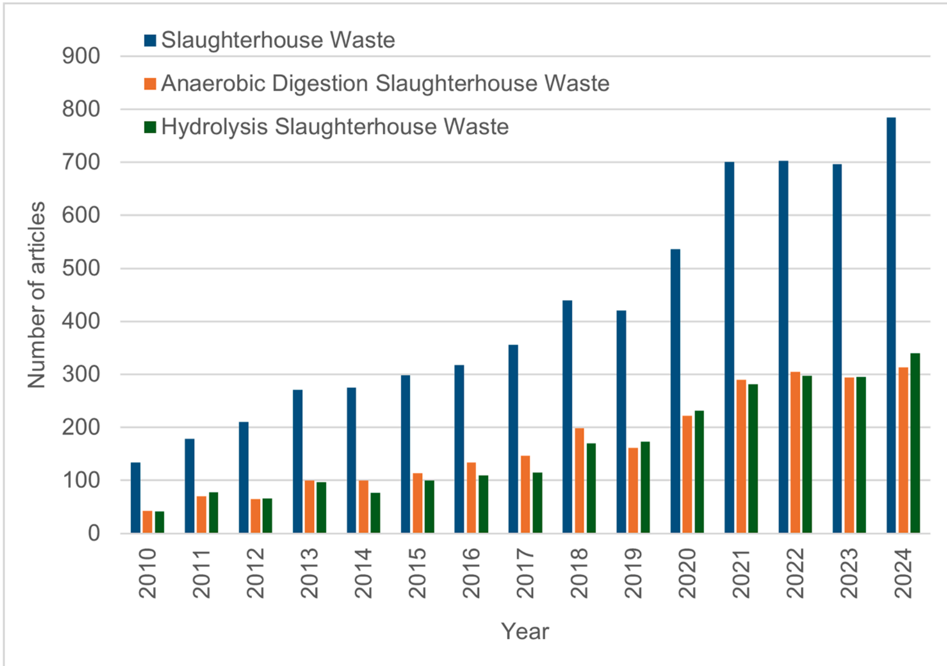

Fig. 2. Bibliometric evolution of "Slaughterhouse waste"; "Anaerobic Digestion Slaughterhouse Waste"; "Hydrolysis Slaughterhouse Waste", in ScienceDirect, from 2010 to 2024.

2. Slaughterhouse waste origin and characteristics

In 2022, meat production in the European Union was 22.1 tons (t) of pig meat, 13.0 t of poultry, 6.6 t of bovine meat and 0.5 t of sheep and goat meat, according to European Commission figures [9]. The main livestock producer in the EU is Spain, with almost 60 million heads of farm animals in 2022, followed by France and Germany, with around 40 million heads of livestock in each [9].

The production of edible meat involves the slaughtering process of animals resulting in ABPs, which entails specific legislation for their management in the European Union. The common steps including bleeding, evisceration, carcass washing and splitting take place as

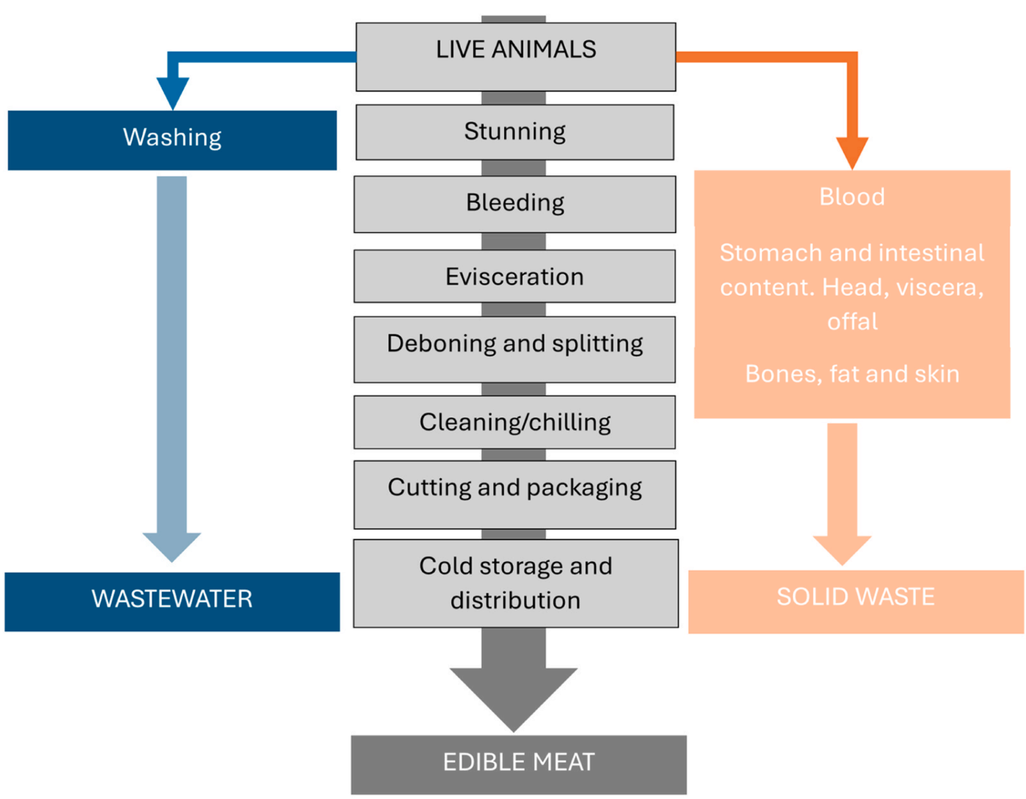

Fig. 3. General slaughtering process, products and by-products. Adapted from [31].

depicted in Fig. 3 [31]. In this stepwise process, the edible part of the animal is separated and further processed to obtain meat for human consumption, and the inedible part or the material not placed on the market are generated as solid by-products, comprising bones, blood, skin, trimmings, organs, viscera, horns, hoofs, feet, skull, etc. [47]. ABPs may also include bodies of dead animals on farms along with other materials produced such as manure, guano, eggshells, feathers, wool and beeswax, and former foodstuffs such as milk, eggs or meat that is not suitable for human consumption [6].

In the meat processing industry, the by-products can reach 66 % of the live load of cattle, 80 % of the live load of pigs and 62 % of the live load of broilers, depending on the different animal characteristics [21]. In general terms, around 60 % of the processed animal is inedible for human consumption and needs to be managed and disposed of.

Generally, the carcass accounts for most of the ABPs, representing ca. 60 %, while the fat content represents around 10–15 % of the total animal waste weight. Organs and offal collectively account for 10–15 %, hide and skin around 10 % and blood approximately 4–5 % of the total body weight. The other ABPs, such as feet, horns or hair, constitute a small percentage of the animal's weight. These percentages may differ depending on the animal, as highlighted by Jayathilakan et al. [18].

Identification and determination of the main compositional fractions of slaughterhouse waste appears to be a fundamental step to assess the most suitable pretreatment and hydrolytic strategy to enable resource recovery via biobased processes. However, intended analytical physicochemical characterization of the different waste and effluents obtained in the slaughtering process is rarely performed. When the residue is treated in a rendering process or by AD, only a basic characterization is conducted, limiting a comprehensive waste comparison throughout the available studies [22]. Moreover, the characterization of main ABPs streams is not mandatory for their treatment or disposal [10].

The available studies show a large variation of the organic matter present in ABPs. These differences are mainly explained by the type of processed animal, geographical location, and slaughtering process applied [18].

In general terms, the solid waste obtained from the abattoir is basically organic matter, containing mainly proteins and fats. In Table 1, the characterization found in the literature of poultry, cattle, and pork ABPs is presented. Large values of volatile solids (VS) confirm the high content of organic matter in this type of waste.

In the case of pork slaughterhouse by-products, they can be differentiated into two main groups, protein-rich, containing more than 70 % protein, such as blood, bone flour and hair, or lipid rich, such as fat. Regarding poultry by-products, such as feathers, which are considered protein-rich, can be up to 8 % of the waste generated from the slaughtering of these animals. Soft waste can make up to 23 %, containing a wide range of protein and lipid content. Cattle by products, are typically characterized as combined streams, rather than individually ABPs, and are categorized as soft, red or white offal depending on the blood content [21]. Soft offal is mainly composed of lipids (58 %) and proteins (27 %). On the contrary, white and red offal are primarily protein-rich, containing 18–26 % of total solids (TS) as protein.

In most cases, the most abundant fraction corresponds to protein, except for the fat by-product obtained from pork and soft offal obtained from cattle, in which the main fraction corresponds to lipids.

Table 1
General characterization of ABPs as reported in the literature. VS: volatile solids; TS: total solids.

Animal	Type of Waste	VS (%)	Proteins (% TS)	Lipids (% TS)	Reference
Pork	Blood	16.8	83.1	0.28	[16]
	Fat	99.2	0	100	
	Hair	35.8	74.4	10.0	
	Bones	41.6	43.8	12.2	
Poultry	Feathers	80	80	3	[45]
	Blood	-	95	-	[31]
	Soft waste	78.4–90	43.7–62.7	18.0–28.2	
Cattle	Soft offal	92.7	26.5	58.4	[51]
	White offal	TS: 29.4	25.9	2.5	[26]
	Red offal	TS: 24.8	18.5	4.9	

3. Traditional management and disposal of slaughterhouse waste and current alternatives

The traditional disposal techniques for the ABPs include landfilling, incineration, composting and rendering. Landfilling is the most common method in the developing countries, as it is a simple and cost-effective method. However, the use of this technique is currently diminishing in all countries due to the associated contamination problems such as groundwater pollution, soil and odor contamination [3]. The other most common alternative in developed countries is incineration, which decomposes ABPs by reaching extreme temperatures of up to 850ºC. This alternative strategy can reduce land utilization, and it is a mandatory technology to apply to category I ABPs [21]. ABPs composting might be mainly applied to waste comprised into category II or category III which have been previously sterilized [50]. Composting process is essentially occurring in two phases, a thermophilic and a mesophilic one for several weeks and months, respectively [32]. This method is considered an inexpensive alternative and environmentally acceptable, but it requires high microbial diversity to degrade the organic compounds and ensure sufficient temperature and time to degrade the pathogenic bacteria present in the waste [12]. In addition, low value materials are obtained throughout composting, in comparison with other biological-based processes.

Rendering is a thermal process that converts ABPs into marketable materials, providing a cost-effective alternative for slaughterhouse waste management. This process treats inedible ABPs from category II or III through heat and pressure to separate proteins from fats. The protein fraction is processed into protein meals for animal feed, pet foods or fertilizers [18], while fats are extracted and used as feedstock in oleochemical industry to produce soaps, detergents, pharmaceuticals and biodiesel [47]. Nevertheless, rendering faces several challenges, such as high energy consumption, odor emissions and pathogen contamination [35]. Furthermore, rendering primarily aims to waste utilization rather than maximizing the economic value of ABPs, resulting in a relatively low-value by-products.

While the mentioned traditional methods provide a means of waste management, they often fail to maximize the potential value of ABPs. As environmental concerns and circular economy principles gain importance, a shift toward more sustainable and resource-efficient strategies has emerged. Recent advancements are focused on transforming ABPs into high value bioproducts and bioenergy, reducing environmental impact while creating marketable alternatives for industries [21].

In this context, strategies focused on protein or fat recovery have been explored. For instance, protein extraction to obtain keratin from nails, feathers or hair is used in the pharmaceutical, biomedical and cosmetic industries [35]. Moreover, animal blood serves as a source of functional proteins, including plasma proteins and globin for food applications [47]. Beyond protein recovery, thermochemical processes such as pyrolysis are also gaining attention. This process involves the thermo-chemical decomposition of organic matter in absense of oxygen , yielding three main products: bio-oil, syngas and biochar. Bio-oil serves as a renewable energy source and syngas can be utilized for heat and power generation and offers potential as biofertilizer. Nevertheless, pyrolysis comes with high initial costs and technical complexity, requiring precise control of temperature and conditions to optimize product yield and quality [28]. Besides, biodiesel production from the transesterification of lipid-rich slaughterhouse waste has also gained interest. The efficiency of this conversion depends on several factors, including reaction time, temperature, and the oil-to-alcohol ratio [39].

Although this strategy is a low-cost alternative for biodiesel production, the high presence of free fatty acids (FFA) in the feedstock can lead to a low conversion rate. Besides, a significant drawback of this process is that the resultant biodiesel may not meet international biodiesel standards, posing challenges for its widespread adoption [43].

Efforts within the EU, such as the ELLIPSE and Water2REturn projects [52,8] aim to valorize slaughterhouse waste and integrate it into the circular economy, transforming it into bioplastics, bio-based fertilizers and other bioproducts. These projects help close waste loops and reduce landfill reliance, turning ABPs into valuable resources that support sustainable sectors across Europe. Yet, the mentioned projects do not make their way to a commercial scale due to the high initial investment, lack of demand and market maturity.

Among the various recent valorization strategies discussed, AD of slaughterhouse waste emerges as the most suitable biological process aimed to revalorize these waste into biogas at large scale [16]. It is reported that large amount of biomethane can be recovered from ABPs, considering that biogas can be used to generate electricity, potentially producing 1.98 m^3 of CH_4 from processing a pig, equivalent to 22.4 kWh/pig, or, for cattle, producing 4.70 m^3 of CH_4 equivalent to 53.1 kWh/cattle. These productions represent 66 % and 32 % of electricity consumed in the slaughtering process of pig and cattle, respectively ([50]). Furthermore, the biogas production capacity is expected to be enlarged by 2026, with the construction and implementation of large-scale slaughterhouse waste digesters.

A comprehensive overview of traditional and current strategies for slaughterhouse waste management is presented in Fig. 4 highlighting their advantages, disadvantages and the final products obtained from each approach.

However, AD drawbacks are also known, related to waste seasonality and lipid and protein presence. Lipids can cause foam formation due to their hydrophobic capacity and improper mixing can lead to microorganisms adhering to fat particles, causing them to float [14]. Additionally, the hydrolysis of lipids can generate long chain fatty acids (LCFAs), that might negatively affect by inhibiting microbial activity through adsorption to cell membranes and surfactant properties [40]. Moreover, the presence of proteins requires hydrolysis to improve its solubilization but can generate ammonia, causing ammonia inhibition. For these reasons, efforts to improve AD, such as the implementation of co-digestion strategies, pretreatment of waste, or microbial adaptation to the residue are being explored nowadays at lab- and pilot-scale [21]. Lab-scale studies focusing on optimization of AD lack of standardized methods for the waste characterization, conditioning, and hydrolysis, and for the comparison of the AD process yields. For this reason, an in-depth analysis of case-studies focused on the valorization of slaughterhouse waste was conducted.

Table 2 summarizes selected studies demonstrating that different

Table 2
Summary of AD studies on slaughterhouse by-products: substrate, operational parameters and performance (based on [50]).

Feedstock & (Co-substrate)	Conditioning	Operation Conditions	CH_4 yield	Reference
Pork liver (Pork fat)	Grinding < 6 mm	16 L. CSTR OLR 2 g VS L^{-1} d^{-1}	1.2 L CH_4 L^{-1} d^{-1} (0.75 L CH_4 g^{-1} VS)	[7]
Poultry SBP (Sewage sludge)	Grinding < 2 mm	20 L. Batch 42 d	0.40 L CH_4 g^{-1} VS	[19]
Swine SBP (-)	-	0.5 L Batch 60–70 d	0.99 L CH_4 g^{-1} VS	[30]
Poultry (Fruit and vegetable wastes)	Shredded into 1–2 cm pieces	50 L Batch 35 d	121 L CH_4	[41]

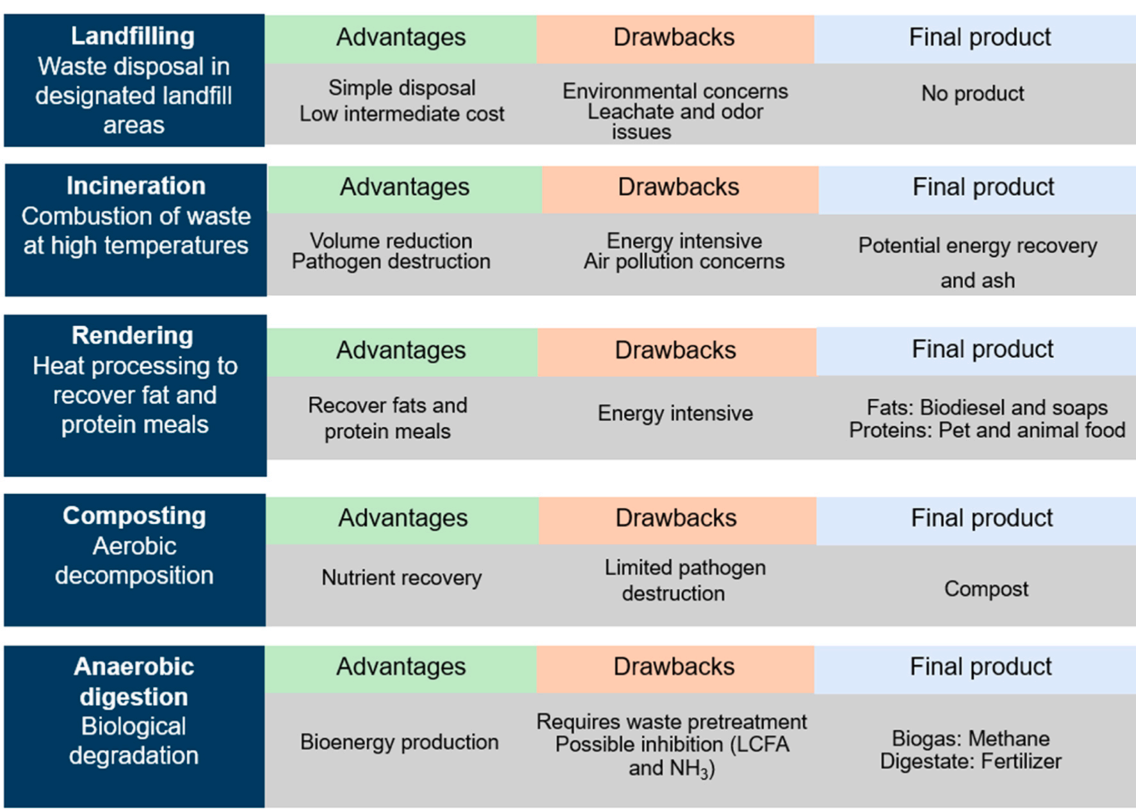

Fig. 4. Overview of slaughterhouse waste management techniques: main advantages, drawbacks and types of end product.

operational conditions have been explored to prove that AD of slaughterhouse waste can be achieved. Different slaughterhouse by-products can be used as substrates, either alone or in co-digestion. Conditioning by grinding to reduce particle size has been commonly used to increase substrate accessibility. Most experiments were conducted in batch mode, with the exception of Dalantai et al. [7] whose applied a continuous stirred tank reactor (CSTR) at an organic loading rate (OLR) of 2 g VS L^{-1} d^{-1} , achieving a methane production rate of 1.2 L CH_4 L^{-1} d. Reported methane yields varied widely among studies, rangning from 0.40 to 0.99 L CH_4 g^{-1} VS [19,30]

4. Towards high added-value products: slaughterhouse waste valorization

Proper identification and determination of the main characteristic parameters of slaughterhouse waste, the conditioning step and the hydrolytic conditions appear to be fundamental towards valorization of ABPs using biological based processes. In this sense, a comprehensive review of the available literature on the hydrolysis, conditioning, and biological transformation of ABPs was conducted, aiming to consolidate and analyze relevant studies. A literature review covering publications since the year 2000 was performed using keywords such as "animal by-products" or "slaughterhouse waste", along with "hydrolysis" or "pretreatment". The selected literature covers different geographical areas and was systematically analyzed by categorizing the information into different sections: waste characterization, conditioning, hydrolytic strategies, and biotransformation of slaughterhouse waste. Each section was further divided into different subsections to facilitate a structured comparison, focusing on the most applied hydrolytic strategies, their feasibility for industrial scale implementation, and their potential to obtain comparable and useful information from an engineering perspective. The ultimate aim was to exemplify the most common employed types of pretreatments or hydrolytic strategies for its commercial scale-up and for its optimization for obtaining high-value products. The resulting information analysis is depicted in Table 3.

The case-studies found can be differentiated in two major areas: solid ABPs and fats (lipids). Specifically, nine of the thirteen case-studies focus on fat waste, while four deal with ABPs. For fat waste, the sources include flotation units installed at in-situ WWTP [15,2,23,29,34,48] (case-studies 1,3, 5, 6, 9 and 11) as well as direct collection from animals [2,24,25] (case-studies 2, 4 and 7). In the case of ABPs, poultry waste is reported in Strong and Gapes, [44] and by Teshnizi et al. [46], (case--studies 8 and 12, respectively), while mixed pork waste is described in case-study 13 [16]. Case-studies 9 and 10 [23] report digestive tract and drumsieve waste, but do not specify where the origin is pork or beef.

In terms of conditioning, the most common strategy for fat waste was dilution to adjust fat concentration and solubilize this waste to be treated as a liquid effluent, while grinding was the primary conditioning method for ABPs. The hydrolytic strategies used across case-studies can be classified into enzymatic or thermochemical methods, which include thermal, alkaline, ultrasound, acid, the use of bacterial products, or sterilization. A clear tendency to apply enzymatic hydrolysis in fat-related case-studies was identified to perform a further comparison to thermochemical strategies. Regarding biotransformation, AD is performed in all case-studies, with exceptions in Masse et al. [24] and in Teshnizi et al. [46].

4.1. Characterization of the slaughterhouse waste in selected case-studies

The characterization of slaughterhouse waste is challenging due to

Table 3
Selected case-studies classified according to waste type.

Case-study	Waste	Conditioning	Hydrolytic strategy	Applied biotransformation	Reference
1	Fat from swine slaughterhouse waste	Dilution using a phosphate buffered saline (PBS) solution	Enzymatic	Anaerobic digestion	[29]
2	Fat particles from pork and beef	Dilution 50:50 using slaughterhouse wastewater from beef and hog abattoir	Enzymatic Alkaline	None	[24]
3	Fat from flotation unit	Addition of fat to wastewater from flotation unit of a poultry slaughterhouse, to obtained and concentration of 800 mg L^{-1}.	Enzymatic	Anaerobic digestion	[48]
4	Commercial pork fat	Filtered wastewater from hog slaughterhouse supplemented with pork fat particles	Enzymatic	Anaerobic digestion	[25]
5	Floatable fat from poultry processing industry	None	Enzymatic	Anaerobic digestion	[34]
6	Floatable fat	Mixing	Thermal and alkaline	Anaerobic digestion	[2]
7	Flesh fat from cattle carcass	Sieving	Thermal and alkaline	Anaerobic digestion	[2]
8	Poultry slaughterhouse waste	Obtention of dried powder and dilution 1:4 waste-to-distilled water. Homogenization and centrifugation, use of supernatant.	Enzymatic	None	[46]
9	Digestive tract	Grinding	Thermal Ultrasound Alkaline Acid Bacterial product	Anaerobic digestion	[23]
10	Drum sieve waste	Non	Thermal Ultrasound Alkaline Acid Bacterial product	Anaerobic digestion	[23]
11	Floatable fat from cattle slaughterhouse wastewater treatment plant	Stirring	Thermobaric Chemical Thermochemical	Anaerobic digestion	[15]
12	Chicken feathers	Cutting	Thermal under oxidative atmosphere Thermal under inert atmosphere	Anaerobic digestion	[44]
13	Mixed pork waste	Homogenization	Thermal Sterilized Alkaline	Anaerobic digestion	[16]

its composition, primarily consisting of fats and proteins but fluctuating based on the type of animal, the slaughtering methods, and geographical location. In the case-studies analyzed, waste characterization was recovered and from this data, characteristic ratios were calculated, as observed in Table 4. Hence, a high variability in the reported residues considered as slaughterhouse waste is observed.

Lipids constitute a great fraction of slaughterhouse waste, but the analytical protocols to quantify them differ markedly among studies, making cross-case comparison difficult. In Masse et al. [25] lipids and long chain fatty acids (LCFA) were characterized according to the Roese-Gottlieb method, a gravimetric procedure originally designed for dairy fat. In contrast, Valladão et al. [48] determined the oil and grease (O&G) content using the hexane extraction method. In fat used in Ning et al. [29], the ratio VS/TS is 89 %, reassuring the high organic content of the fat obtained, also a 91 % of the TS obtained corresponds to fat concentration. The floatable fat obtained from the poultry industry [34] contains 16 % fat concentration based on TS, meaning that it is more diluted than the fat previously mentioned. The fat obtained from the flotation unit [2] contains 38 % of TS/COD, and 89 % VS/TS, also involving a high organic content. When COD and lipids are characterized, similar ratios of lipid COD are obtained (43 and 37 % in [25] and [30]). In short, the variability in fat content is influenced by the different analytical methods and the lack of common characteristic ratios to compare the fat content of the studied wastes. For solid animal by-products, the reference procedure for quantifying total fat is ISO 1444–1996 (AOAC 954.02), which determines the lipid content through acid hydrolysis and solvent extraction.

Table 4

Characterization of ABPs classified according to case-studies. TS: total solids; VS: volatile solids; COD: chemical oxygen demand; tCOD: total COD; sCOD: soluble COD; O&G: oil and grease; C: carbon; N: nitrogen; TKN: total Kjeldahl nitrogen; VFA: volatile fatty acids; NH_4^+: ammonium; FOG: fats, oil and grease.

Case-study[(1)]	Waste	Parameter	Ratios
1	Fat from swine slaughterhouse waste	Fat: 836 g kg^{-1} TS: 917 g kg^{-1} VS: 815 g kg^{-1}	VS/TS: 89 % Fat/TS: 91 % C/N: 0.63
4	Commercial pork fat	Lipid: 519 mg L^{-1} tCOD: 1213 mg L^{-1}	Lipid/tCOD: 43 %
5	Floatable fat from poultry processing industry	TS: 9.3 g L^{-1} pH: 5.4 Proteins: 13.2 g L^{-1} COD: 41 g L^{-1} O&G: 7.1 % Lipids: 15.1 g L^{-1}	Lipid/COD: 37 % Lipid/TS: 16 % Protein/COD: 32 % TS/COD: 23 %
6	Floatable fat	COD: 0.41 g kg^{-1} TS: 155 g kg^{-1} VS: 135 g kg^{-1}	TS/COD: 38 % VS/TS: 89 %
7	Flesh fat from cattle carcass	COD: 1.68 g g^{-1} TS: 935 g kg^{-1} VS: 934 g kg^{-1}	TS/COD: 56 % VS/TS: 99 %
8	Poultry slaughterhouse waste	Protein TKN: 450 mg g^{-1}	-
9	Digestive tract	pH: 7.2 TS: 120 g kg^{-1} VS: 11 g kg^{-1} sCOD: 4 g L^{-1} Total VFA: 2.6 g L^{-1} NH_4^+: 0.13 g L^{-1}	VS/TS: 92 % TS/sCOD: 3.6 %
10	Drum sieve waste	pH: 6.6 TS: 140 g kg^{-1} VS: 140 g kg^{-1} sCOD: 0.85 g L^{-1} Total VFA: 0.2 g L^{-1} NH_4^+: 0.19 g L^{-1}	VS/TS: 100 % TS/sCOD: 6 %
11	Floatable fat from cattle slaughterhouse wastewater treatment plant	pH: 4.2 TS: 145 g kg^{-1} VS: 14.21 g kg^{-1} tCOD: 205 g L^{-1} sCOD: 33.5 g L^{-1} FOG: 90 g L^{-1} VFA: 6.4 g L^{-1}	VS/TS: 98 % TS/tCOD: 141 % FOG/COD: 44 % FOD/TS: 62 %
12	Chicken feathers	Moisture: 11 % Ash: 12.8 % VS: 87.1 % C: 46.8 % N: 13 %	VS/TS: 98 %
13	Mixed pork waste	TS: 269 g kg^{-1} VS: 23.2 g kg^{-1} N: 27.4 g kg^{-1} Lipids: 40 g L^{-1} Proteins: 136 g L^{-1}	VS/TS: 86 % Lipids/TS: 21 % Protein/TS: 75 %

[(1)] References for each case-study can be found in Table 3

Regarding the characterization of ABPs different from fat, mostly found in Luste et al. [23] and in Strong and Gapes [44] the most analyzed parameter is the solids concentration. A high content of VS in most of the reported waste by them exhibits a characteristic ratio VS/TS of almost 100 %. Another parameter often reported is the nitrogen content, related to proteins. Its characterization methods also vary, as Teshnizi et al. [46] analyzed the total Kjeldahl nitrogen (TKN), and Luste et al. [23] analyzed the ammonium concentration. This highlights the need for a standardization of the nitrogen measurement to determine the protein fraction. A reliable analysis to determine the protein content is to determine TKN and convert the value to protein using the 6.38 factor, recommended by the FAO for meat and dairy-based substrates.

Characterization of poultry feathers showed a moisture content from 11 %, and C and N content being 46.8 % and 13 %, respectively [44]. The characteristic ratio C/N showed a higher value in the chicken feathers in comparison to the processing waste, shown in the study, explained by the presence of keratin in feathers.

Luste et al. [23] characterized different waste obtained from slaughtering industries handling cows and pigs. Wastes of interest were drum sieve waste and digestive tract (case-studies 9 and 10). The TS obtained range was about 12–14 % and the soluble COD from 0.85 to 4.0 g L^{-1}. These parameters result in the lowest ratio TS/COD of 3.6 % in digestive tract residue and 6 % in drum sieve. The obtained total volatile fatty acids (VFA) concentration ranged between 0.2 and 2.6 g L^{-1} and the ammonia concentration was around 0.1 g L^{-1}.

Mixed pork waste, reported in case-study 13 by Hejnfelt and Angelidaki, [16] shows a high organic content with a VS/TS ratio of 86 %, considering a protein-rich residue, as 75 % of TS are reported to be proteins, in comparison to lipids, which are only 21 % of TS.

Despite abundant studies, slaughterhouse waste characterization remains fragmented and often incomplete, as regulations only require hygienic safety criteria, not complete physicochemical waste characterization before disposal or valorization. From the case-studies shown in Table 4, TS are reported in 77 % of the cases and COD is reported in 71 %. Far fewer studies contain key fractions such as protein or lipids (35–40 % of the case-studies), resulting in datasets that cannot be rigorously compared. In general terms, the characterization varies depending on the objective of the article, giving information about organic matter if it is crucial to assess the performance of the study.

Our review table 4 highlights extreme variability, underscoring urgent need for unified protocols. We therefore recommend a systematic workflow combining representative sampling, a core suite of analysis (TS/VS, tCOD/sCOD) and the determination of fat, measuring the total lipid content via acid-hydrolysis and Soxhlet extraction, and of protein contents by measuring the TKN. From these parameters, normalized ratios could be assessed and compared among different wastes. Moreover, knowing the complete waste composition enables assessing the most suitable hydrolytic strategy and the determination of the most favorable fermentation parameters.

4.2. Conditioning of the slaughterhouse waste in selected case-studies

Slaughterhouse waste conditioning is usually required to ensure physical conditions to satisfactorily perform hydrolytic and biological fermentation steps, so it is considered a pre-hydrolytic process. These techniques modify the initial characteristics of the residue: particle size reduction, dilution or fat concentration adjustments are performed. Dilution consists in modifying the composition to guarantee a certain concentration which is normally applied to fat-rich streams. Generally, SWW obtained from the meat processing industry, after being treated in the flotation unit, is used as diluting agent for these fat streams, and its characterization is shown in Table 5. The values of COD range between 1.0 and 3.2 g L^{-1}, while TS varies in a wider range. From the analyzed parameters, different characteristic ratios can be gathered. However, no comparison can be easily made as different physicochemical parameters are reported depending on the article.

The use of SWW to dilute fat generates a rich-lipid liquid effluent as reported in Table 6. The final fat concentration is typically adjusted based on the objective of the study, with different fat concentrations explored in each analyzed case-study or compared in the same case-study, as in Masse et al. [24] and in Masse et al. [25].

Although SWW is employed for dilution, one exception is found in Ning et al. [29], where phosphate buffered saline (PBS) was used instead, adjusting the fat concentration to 5 % (w v^{-1}). Dilution of fat is employed to convert the solid waste into a homogenous liquid waste, facilitating the subsequent hydrolysis and fermentation. This step also fixes the target fat concentration, ensuring hydrolysis is evaluated under well-defined and reproducible conditions. The presence of diluting media enhances hydrolysis, as enzymes have a more homogenous effect over waste. For example, in Masse et al. [25], dilution of fat to 5 % w v^{-1} resulted in higher COD release when studying AD, when compared to 20 % w v^{-1} fat.

Another widely used strategy for waste conditioning is particle size reduction, achieved through grinding or milling. This procedure increases the surface area available for hydrolysis, enhancing organic matter degradation and accelerating its conversion into simpler compounds [23]. Additionally, particle size reduction contributes to substrate homogenization, ensuring more uniform processing conditions and it is required in the ABPs regulation. In Strong and Gapes [44] chicken feathers were cut into pieces, of less than 2 mm long and in Hejnfelt and Angelidaki [16] where the mixed pork waste was homogenized in a blender resulting also in a particle size of less than 2 mm. In the case of Luste et al. [23] digestive tract was grinded using a kitchen blender to ensure particle size of less than 12 mm, as ABP regulation imposes. Moreover, in Battimelli et al. [2] sieving or mixing the waste was performed before pretreatment to further standardize the composition of ABPs. In one case, dried powder was obtained and further diluted 1:4 using distilled water [46]. The effects of grinding may translate into faster solubilization rates and in consequence, facilitate access to the organic matter of the waste. Results of hydrolysis efficiency are further discussed in the next section.

Table 5
Characterization of slaughterhouse wastewater (SWW) from diverse sources, classified according to case-studies. TS: total solids; VS: volatile solids; COD: chemical oxygen demand; tCOD: total COD; sCOD: soluble COD; O&G: oil and grease; P: phosphorus; TKN: total Kjeldahl nitrogen.

Case-study(1)	Type of SWW	Characteristics	Ratios
2	Slaughterhouse wastewater from beef and hog abattoir	TS: 5.6 g L^{-1} VS 1.5 g L^{-1} pH 5.4	VS/TS: 27 %
3	Wastewater from flotation unit of a poultry slaughterhouse	COD: 1.01 g L^{-1} O&G: < 10 mg L^{-1} TKN: 216 mg L^{-1} Total P: 14 mg L^{-1}	TKN/COD: 21 % P/COD: 1 %
4	Filtered wastewater from hog slaughterhouse	Lipids: 157 mg L^{-1} tCOD: 3192 mg L^{-1} sCOD: 2295 mg L^{-1}	Lipids/tCOD: 7 % sCOD/tCOD: 72 %

(1) References for each case-study can be found in Table 3

Table 6
Main characteristics of the conditioning process applied to solid slaughterhouse waste, according to case-studies. PBS: phosphate buffered saline.

Case-study(1)	Waste	Conditioning procedure	Characteristics of conditioned waste
1	Fat from swine slaughterhouse waste	Dilution with PBS 0.01 M	5 % w v^{-1} fat
2	Fat particles from pork and beef	Dilution 50:50 SWW and distilled water	200–1000 mg fat L^{-1}
3	Fat from flotation unit	Dilution with SWW	800 mg fat L^{-1}
4	Commercial pork fat	Cutting and mixing	Particle size 2–10 mm
6	Floatable fat	Mixing	-
7	Flesh fat from cattle carcass	Sieving	Screen size 5 mm
8	Poultry slaughterhouse waste	Heating at 110°C and drying at 45–55°C for 24 h and dilution with distilled water (1:4)	Final TS 12 %
9	Digestive tract	Grinding	< 12 mm
11	Drum sieve waste	Stirring	-
12	Floatable fat from cattle slaughterhouse wastewater treatment plant	Cutting	< 2 mm
13	Chicken feathers	Blender Homogenization	< 2 mm

(1) References for each case-study can be found in Table 3

4.3. Hydrolysis of slaughterhouse waste

Slaughterhouse waste is rich in organic fractions, particularly fats and proteins, which can be efficiently used as feedstock for AD [44]. Typically, the hydrolytic step, which involves breaking down complex organic matter into simpler molecules such as fatty acids, alcohols, monosaccharides and amino acids, is recognized as the rate-limiting step in the anerobic digestion of this kind of waste. However, the use of monomers as substrates in the biotransformation process enhances the efficiency of the AD process by reducing hydraulic retention times and allowing the reduction of reactor volumes [1].

In the reported case-studies, the hydrolytic strategies applied are primarily focused on lipids and proteins, as they are the main fractions present in ABPs (Table 4).

On the one hand, enzymatic hydrolysis utilizes specific enzymes to catalyze the breakdown of complex molecules under mild pH and temperature conditions. These enzymes, typically obtained from biological processes, are biodegradable and suitable for scalable processes involving the treatment of large substrate volumes. However, the excessive cost of enzyme production and their substrate specificity can limit their applicability [20]. On the other hand, chemical hydrolysis is an efficient method that rapidly transforms complex substrates into simpler ones. Its versatility allows it to be used with a wide range of feedstocks. However, the use of chemical agents can involve some environmental concerns due to potential toxicity and the generation of inhibitory compounds, such as salts, which can hinder subsequent biological processes [33]. Physical hydrolysis involves mechanical strategies, such as milling or grinding. These methods require less energy in comparison to thermal processes and do not involve the use of chemical additives, reducing the cost of the processes and the hazards related to chemical agents. The primary advantage of mechanical pretreatment is waste homogenization, and reduction of the particle size [14]. A general overview of the main advantages, limitations, and some key features of enzymatic, chemical, and thermal hydrolysis applied to slaughterhouse waste is presented in Fig. 5.

In general terms, enzymatic and physic-chemical strategies for ABPs have been studied at lab-scale. Specifically, in the analyzed case-studies the enzymatic strategies are focused on lipid hydrolysis to avoid its accumulation and its negative effect on the subsequent anaerobic digestion. Only Teshnizi et al. [46] (case-study 8) applied enzymes to obtain protein hydrolysates. Conversely, chemical and physical strategies, or their combination, have been reported by other authors, dealing with fat-added waste or ABPs mixtures. Lipid hydrolysis or COD solubilization are the directly reported strategies to evaluate the efficiency of the hydrolysis step, but AD yield is used as an indirect way to evaluate the hydrolytic effect on waste. This is extensively explained in the following paragraphs.

4.3.1. *Enzymatic hydrolysis*

Enzymatic strategies were the most common reported pretreatment strategies for fat hydrolysis. Fats are conditioned by dissolving them into SWW, which were then considered lipid-rich streams, requiring pretreatment prior to AD to enhance digestion (Section 4.2). The enzymatic process is commonly performed using a specific commercial enzyme that allows proper hydrolysis of the lipid content, such as lipases. In Table 7, enzyme characteristics, dosage and conditions are reported, as well as with the treatment yield and performance.

Commercial lipases were commonly employed in most studied cases, except for case-study 3 where the enzyme was produced by solid state fermentation (SSF) using an available secondary waste stream such as seed oil extraction supplemented with molasses [48]. These authors also reported the characterization of enzymatic activity levels by the formation of free acids by automatic titration. For the other case-studies, no activity characterization of the enzyme is reported.

Applied strategies consisted in the application of preselected doses under standard conditions of pH (around 7), and temperature (30–37ºC), at lab-scale, from 90 mL to 5 L of working volume (wv). The duration of the hydrolysis process was set from 4 to 24 h, depending on the study. To the best of authors knowledge, the analyzed case-studies did not investigate the best conditions to perform the enzymatic treatment, only a comparison between strategies or a comparison to control samples was performed. However, in case-study 5 different enzymatic conditions of pH (7 or non-adjusted), and process time (12 h and 24 h) were evaluated, to determine the optimal one [34]).

The effectiveness of lipid hydrolysis was generally evaluated through the measure of LCFA or Free Fatty Acids (FFA) since these compounds are released during fat hydrolysis. Lipid hydrolysis can also be measured as lipid removal or a decrease in tryacylglicerides (TAG) concentration. In only one case (case-study 2; [24]) particle size reduction is used to assess the hydrolysis yield. Ning et al. [29] in case-study 1 does not directly evaluate the enzymatic effect over hydrolysis.

Enzyme dosages were reported in terms of weight – volume (w v^{-1}) concentration or weight- dry weight (w dw^{-1}) concentration, resulting in a wide range of values which was generally difficult to compare. Therefore, for allowing a proper comparison, enzyme dosage was standardized in terms of waste TS content, since this parameter was the most widely reported in the ABP waste characterization. Accordingly, the obtained enzymatic dose ranged from 1 % to 11 % enzyme weight per dry weight (w dw^{-1}) of waste as shown in Table 7, resulting in a wide reported dosage for the enzymatic as it is conditioned to the enzyme activity, origin and production.

The lowest reported dosage was found in Pascale et al. [34] (case-study 5), which performed the hydrolysis of floatable fat obtained from the poultry industry using a solid commercial lipase under various pH and time conditions at laboratory scale. In that study, a lipase activator, specifically calcium ions at a concentration of 0.15 % (w w^{-1}), was introduced. The enzymatic dosage was 1 % (w dw^{-1}), and the conditions were optimized. The results demonstrated a 2.35-fold increase in acidity index and an eightfold increase in LCFA when the pH was adjusted to 7 over a 24-hour period.

Besides, the same enzymatic dosage was used by Valladão et al. [48] (case-study 3), using fat-supplemented SWW and pretreating it at laboratory scale using lipase sourced from SSF. Operating conditions were temperature at 30ºC and reaction time between 4 and 24 h, using an

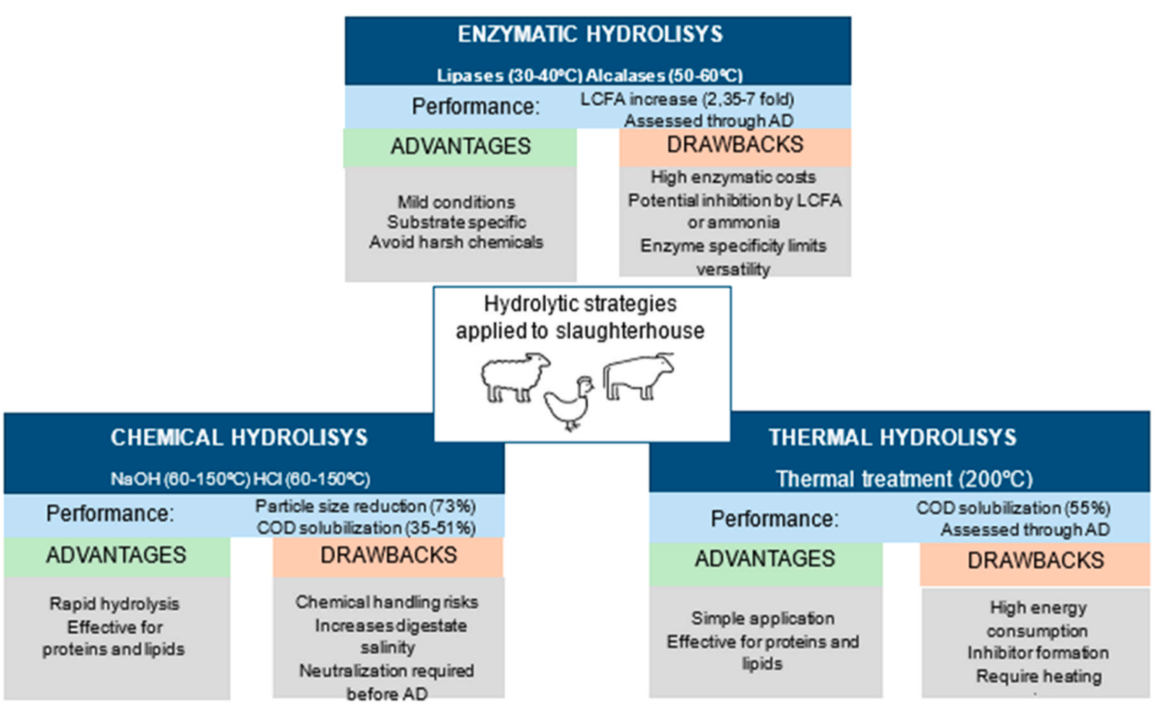

Fig. 5. Overview of hydrolytic strategies for slaughterhouse waste: condition, performance and practical considerations.

Table 7
Enzymatic hydrolytic strategies classified according to case-studies. TAG: tryacylglicerides; FFA: free fatty acids; SSF: solid state fermentation; EA: enzymatic activity; UA: units of activity.

Case-study(1)	Waste	Enzyme characteristics	Enzyme's dosage	Conditions	Treatment yield and performance
1	Fat from swine slaughterhouse waste	Commercial lipase from pig pancreas	1.1–10.9 % (w dw^{-1})	Optimum pre-fixed: 37 °C, pH 7, 24 h	Not assessed directly
2	Fat particles from pork and beef	Three commercial lipases: Pancreatic, plant and bacterial lipase	Wide range, depending on the enzyme.	Different fat concentrations	LCFA increase and particle size reduction
3	Fat from flotation unit	SSF lipase. EA: 37.3 UA g^{-1}	1 % (w v^{-1}) from SSF	30 °C, 4–24 h	Reduction of TAG molecules and increase of FFA 7 %
4	Commercial pork fat	Commercial pancreatic Lipase	11.5 % (w dw^{-1})	Not optimized: 5.5 h, 35 °C	LCFA 4x increment and neutral fat hydrolysis 35 %
5	Floatable fat from poultry processing industry	Solid commercial Lipase. EA: 11500 U g^{-1}	1.0 % (w dw^{-1})	pH 7 or non-adjusted. 12–24 h. 30 °C	2.35-fold acidity index increases. 8 times LCFA increase
8	Poultry slaughterhouse waste	Commercial Alcalase. EA: 2.4 UA g^{-1}	1.5–5.5 % (w dw^{-1})	50–70 °C, 6.5–8.5 pH	12.7 % hydrolysis degree and 87.6 % protein recovery

(1) References for each case-study can be found in Table 3

enzymatic dosage of 1 % (w v^{-1}). The study reported a notable reduction in TAG achieving an 83 % hydrolysis efficiency. Additionally, an increase of FFA was observed within the first 4 h of reaction time, but a final decrease of FFA levels was observed, attributed to endogenous microorganism consumption.

Moreover, a range of enzymatic dosages were studied to hydrolyze diluted fat (case-study 1 [29]), at laboratory scale, in 125 mL reaction volume. Enzymatic dosage ranged from 1.1 % to 10.9 % (w dw^{-1}) and prefixed enzymatic conditions of temperature 37ºC, pH 7 and a reaction duration of 24 h were explored. The hydrolytic yield was not assessed directly; instead, it was inferred through subsequent AD.

Experiments at high enzymatic dosage (11.5 % (w dw^{-1}) were conducted by Masse et al. [25] (case-study 4) using commercial pancreatic lipase under non-optimized laboratory conditions. Two control experiments were conducted: one omitting the addition of the enzyme, and another without both, added fat and enzyme. Results indicated a four-fold increase in LCFA and a 33 % hydrolysis of fats.

Comparison of different commercial lipases has been only conducted in case-study 2. Efficacy of three commercial lipases, sourced from the pancreas, plant or bacteria at different concentrations, was performed to hydrolyze pork or beef fat at different concentrations [24]. Enzymatic hydrolysis was different depending on the enzyme used, and a wide range of dosage was studied. Particle size reduction, assessed with mesh filters, was used to determine hydrolytic efficiency. Additionally, the impact of lipases on LCFA release was studied. When pancreatic lipase was added, a significant reduction of 60 % of particle size of the added fat was observed at 4 h, being more effective in beef than in pork fat. COD measurements did not reveal any significant increase using lipases, indicating that COD was not a good indicator of enzymatic lipolysis. Regarding the LCFA increase, it achieved a 15.5 mg L^{-1} concentration in pork fat using lipases from the pancreas. Bacterial lipase was efficient in particle size reduction but at higher doses and prolonged duration (24 h), while the plant-derived lipase had no effect on particle size reduction. This study concluded that pancreatic lipase emerged as the most effective pretreatment strategy although no investigation of fermentation of the hydrolysate was conducted.

Regarding protein hydrolysis, it was explored by Teshnizi et al. [46] (case-study 8) using alcalase, to produce protein hydrolysates. This study employed a central composite design of five levels investigating different temperatures (ranging from 50 to 70ºC), different pH conditions (ranging from 6.5 to 8.5) and different enzyme to substrate ratio, from 1.5 to 5.5 v v^{-1}. The pH was controlled during the experiment by adding 2 N sodium hydroxide and the enzyme was inactivated by heating at 85ºC for 25 min. This pretreatment resulted in a protein hydrolysis degree of 12.7 %, measured using pH-stat method, and 87.6 % of protein recovery in the supernatant, in the conditions of 60ºC, pH 7.5 and enzyme to substrate dosage of 3.5 % (v v^{-1}).

Although enzymatic pretreatment has been widely applied for the hydrolysis of fats in ABPs, issues related to reproducibility and optimization of operational conditions remain largely unaddressed. Most of the reviewed studies applied fixed enzyme dosages and standard conditions (e.g., pH ~7, temperature 30–37°C), without conducting a systematic evaluation of optimal parameters for each specific ABP matrix. Only a few cases, such as Pascale et al. [34] and Teshnizi et al. [46], explored different pH, temperature, and enzyme-to-substrate ratios to identify optimal conditions, highlighting the influence of these variables on hydrolysis efficiency. Moreover, enzyme activity was rarely characterized by using a standardized unit, limiting the comparability of results across studies. While some efforts have been made to standardize enzymatic dosage, the lack of complete waste and enzyme characterization continues to hinder reproducibility. The heterogeneity of ABPs—such as poultry, pork, or beef fat—further complicates standardization, as shown in Masse et al. [24], where enzyme performance varied significantly depending on the fat type and enzyme origin. These findings emphasize the need for complete substrate characterization, more comprehensive optimization studies and standardized protocols to improve reproducibility and ensure effective enzymatic pretreatment across diverse ABP streams

4.3.2. Chemical and physical hydrolysis

Chemical strategies, using alkaline or acidic agents, and physical ones, such as thermal or ultrasound, have also been employed to hydrolyze the slaughterhouse waste organic matter in the identified cases-studies. Following the enzymatic strategy, alkaline hydrolysis, particularly using sodium hydroxide, is the second most reported method as it effectively breaks down complex organic matter by inducing lipid saponification and peptide bond cleavage [2]. Results from literature studies are collected in Table 8, presenting the strategy, dosage, conditions, treatment yield and performance.

Alkaline strategies have been applied to diluted fats as well as to ABPs obtained directly from the slaughterhouse process. In the case-studies reviewed, the duration of the experiments was approximately 4 h. Various temperatures, ranging from 60 to 150ºC, were explored in alkaline-thermal treatments. The dosage of NaOH varied across the different studies. Although several efforts have been made in order to standardize this parameter, it was not possible since the parameters measured to characterize the residue differed considerably. To facilitate comparison of alkaline strategies, dosages were expressed as grams of NaOH per gram of lipid or per gram of TS, in the case-studies in which the calculation was possible. A concentration of 0.5–1.2 g NaOH g TS^{-1}

Table 8
Physical and chemical hydrolysis classified according to case-studies. VSS: volatile suspended solids; DOC: dissolved organic carbon.

Case-study(1)	Waste	Strategy	Dosage	Conditions	Treatment yield and performance
2	Fat particles from pork and beef	Alkaline	0.67–5.3 g NaOH g^{-1} Fat	23 ºC, 4 h	Particle size reduction 73 % when using 2 g NaOH g^{-1} Fat and sCOD, no significant increase
6	Floatable fat	Thermal and alkaline	0.14 g NaOH g^{-1} lipid	60–120 and 150 ºC, 3 h	LCFA distribution. Anaerobic digestion
7	Flesh fat from cattle carcass	Thermal and alkaline	1.5 g NaOH g^{-1} lipid	60–120 and 150 ºC, 3 h	LCFA distribution. Anaerobic digestion
9	Digestive tract	Thermal	-	120 ºC	COD solubilization: not possible to measure
		Ultrasound	-	24 kHz, 5600 kJ kg^{-1} TS	COD solubilization: 27 %
		Alkaline	0.5–1.2 g NaOH 2 M g^{-1} TS	pH 12–12.2, 4 h	COD solubilization: 51 %
		Acid	0.2–1.2 g HCl 6 M g^{-1} TS	pH 2–2.5, 4 h	COD solubilization: 14 %
		Bacterial product (protease, amylase and lipase)	Liquid Certizyme 5. 60 mg L^{-1}	24 h	COD solubilization: 62 %
10	Drum sieve waste	Thermal	-	120ºC	COD solubilization: 55 %
		Ultrasound	-	24 kHz, 5600 kJ kg TS^{-1}	COD solubilization: 88 %
		Alkaline	0.5–1.2 g NaOH 2 M g^{-1} TS	pH 12, 4 h	COD solubilization: 35 %
		Acid	0.2–0.7–1.2 g HCl 6 M g^{-1} TS	pH 2–2.5, 4 h	COD solubilization: 0.6 %
		Bacterial product (protease, amylase and lipase)	Liquid Certizyme 5. 60 mg L^{-1}	24 h	COD solubilization: 54 %
11	Floatable fat from cattle slaughterhouse wastewater treatment plant	Thermobaric	Autoclaved	121 ºC, 30 min	COD solubilization: 20.8 %
		Chemical	7 g NaOH L^{-1}	24 h	COD solubilization: 48.2 %
		Thermochemical	Combination: 7 g NaOH L^{-1} and autoclaved	121 ºC, 30 min	COD solubilization: 50.7 %
12	Chicken feathers	Thermal under oxidative atmosphere	20 bar pure air (20–22 % oxygen)	50–100–150–200 ºC	DOC increase from 540 mg L^{-1} to 6000 mg L^{-1} at 200ºC. 55 % conversion of VSS to DOC
		Thermal under inert atmosphere	20 bar pure nitrogen	50–100–150–200 ºC	DOC yield achieved at 200ºC of 270 mg C g VSS C^{-1}
13	Mixed pork waste	Thermal	-	70ºC, 1 h. Waste concentration: 5, 20, 50 and 80 %	Methane yield
		Sterilized	-	133ºC, 300kPa, 20 min. Waste concentration: 5, 20, 50 and 80 %	Methane yield
		Alkaline	50 and 100 g NaOH kg^{-1} VS	Waste concentration: 5 %	Methane yield

(1) References for each case-study can be found in Table 3

is reported in case-studies 9 and 10 [23] and 0.2–1.5 g NaOH g $^{-1}$ lipid were also reported in case-studies 6 and 7 [2].

Few studies included direct evaluation of the pretreatment strategy, and no common methodology was used to report the degree of hydrolysis. In case-studies 2, 9 and 10 [23,24] the solubilization of the COD is reported to assess different percentages depending on the hydrolytic strategy applied over diluted fat, digestive tract content and drum sieve waste. Additionally, changes in LCFA distribution were evaluated in case-studies 6 and 7 [2]. In case-studies whose evaluation is inconclusive, the hydrolysate is subsequently valorized through AD to determine the pretreatment strategy's efficiency. The articles mentioned are reported in Table 9.

Masse et al. [24] compared enzymatic and alkaline pretreatment by adding NaOH to pork and beef fat particles conditioned by dilution. Lab-scale tests were conducted at 23ºC, for 4 h. Particle size and sCOD were evaluated and no significant increase in sCOD was observed in pork fat hydrolysis. However, 73 % particle size reduction of fat was observed at the lowest NaOH dosage.

Fat from flotation unit from cattle slaughterhouse and flesh fat from cattle carcass was used as substrate in thermo-alkaline hydrolysis, reported in case-studies 6 and 7 [2]. Different temperatures (60, 120 and 150ºC) and a NaOH dosage of 0.156 gNaOH gTS^{-1} were applied to the lipid-rich ABPs for 3 h, with proper mixing. LCFA were measured using gas chromatography, revealing no changes in LCFA distribution, in percentage, across all temperatures when comparing raw waste to pretreated samples. The resulting LCFA concentration was not shown, and hydrolysate underwent AD.

Different hydrolytic strategies were applied to various ABPs, in case-studies 9 and 10 [23], to compare COD solubilization. Digestive tract content and drum sieve waste were studied under alkaline conditions using a range of NaOH from 0.5 to 1.2 gNaOH 2 M g^{-1} TS, reaching a pH of 12 over 4 h. Soluble COD evolution and particle size reduction were examined, achieving a solubilization of 51 % in the digestive tract content and 35 % in the drum sieve waste. In comparison to the mentioned alkaline strategy, thermal, ultrasound, acid and bacterial products were studied. The highest COD solubilization for digestive tract was achieved when using bacterial products, resulting in 62 % COD solubilization, while the ultrasound strategy applied to the drum sieve waste achieved 88 % COD solubilization. Regarding particle size, a 56 % decrease was observed for digestive tract content using bacteria product, and 11 % reduction for drum sieve waste compared to untreated materials. In the thermal strategy, soluble COD measurement was hindered by water evaporation. Overall, pretreated materials exhibited higher sCOD and sCOD/VS ratios than non-pretreated waste.

The application of thermobaric, chemical and the combination of both strategies, known as thermochemical pre-treatment to dissolved air

Table 9
Anaerobic digestion of hydrolysates classified according to case-studies. WTP: waste treatment plant.

Case-study[(1)]	Waste	AD operation mode	AD Conditions	Inoculum	AD performance
1	Fat from swine slaughterhouse waste	Lab-scale wv 250 mL	Lipase hydrolysate. Organic Loading: 20 g VS L^{-1} SSW, 37 °C, 50 days	Biogas plant sludge. 2.75 % VS	800 mL biogas g VS^{-1} added. Lipase added vs. control: increase biodegradability 72 % (control 61.4 %)
3	Fat from flotation unit	Lab scale, wv 90 mL	Lipase pretreated effluent and medium. pH 7, 30 °C.	Slaughterhouse. COD:VSS 1:1. 11.5 g VSS L^{-1}	4 times improvement CH_4 concentration pretreated residue vs control
4	Commercial pork fat	Pilot scale, 42 L.	Diluted enzymatic hydrolysate (lipid concentration 550 mg L^{-1}). 25 °C	Adapted from hog slaughterhouse. 14.5 g VSS L^{-1}	82 % transformation tCOD to CH_4 in enzymatic hydrolyzed vs 78 % without hydrolysis.
5	Floatable fat from poultry processing industry	Lab-scale, wv 60 mL	Lipase hydrolysate. Batch, 35 °C, 5 days, no agitation	From industrial digester treating poultry processing wastewater. 1,4:1 w w^{-1} Sludge:waste.	Specific CH_4 production: 2.46 mL g^{-1} waste in hydrolysate vs 1.3 mL g^{-1} waste control
6	Floatable fat	Lab scale. 5 L reactor	Thermal-alkaline hydrolyzed residue. 35 and 60 °C	From pilot reactors treating winery effluent	Best biogas production pretreated residue 120ºC, 617 mL biogas g^{-1} VS
7	Flesh fat from cattle carcass	Lab scale. 5 L reactor	Thermal-alkaline hydrolyzed residue. 35 and 60 °C	From pilot reactors treating winery effluent	Best biogas production pretreated residue 120ºC, 1168 mL biogas g^{-1} VS
9	Digestive tract	Lab scale. 2 L	35 °C, pH 7. Pretreated residue (all conditions tested)	Digested sewage sludge from municipal WTP. VS 1.8 %	CH_4 production: 320 mL CH_4 g^{-1} VS added (alkaline and bacterial treated) vs 400 mL CH_4 g^{-1} VS added untreated
10	Drum sieve waste	Lab scale. 2 L	35 °C, pH 7. Pretreated residue (all conditions tested)	Digested sewage sludge from municipal WTP.	CH_4 production: 340 mL CH_4 g^{-1} VS added (thermal) vs 230 mL CH_4 g^{-1} VS added untreated.
11	Floatable fat from cattle slaughterhouse wastewater treatment plant	Lab scale. 400 mL	37 °C, pH 7. Pretreated residue (all conditions tested). Triplicate and control.	Anaerobic sludge from slaughterhouse. Ratio 3:1 VS	Methane yield obtained: 8.5 % thermobaric, 8.5 % thermochemical and 3.3 % chemical greater, in comparison to control strategy.
12	Chicken feathers	Lab scale. 100 mL	36 °C. Low shaking speed. Thermal hydrolyzed residue (all temperatures)	Pond sediment.	140ºC pretreatment: 3–4-fold CH_4 yield increase compared to control. From 40–125 mL CH_4 g^{-1} VSS.
13	Mixed pork waste	Lab scale. 0.5–2 L	37 °C and 55 °C. Pretreated residue (all conditions tested)	Mesophilic or thermophilic inoculum from biogas plant.	600 mL biogas g^{-1} VS in untreated condition. No significant effect was observed regarding the biodegradability of treated residue.

[(1)] References for each case-study can be found in Table 3

flotation (DAF) sludge waste was studied by Harris et al. [15], defined as case-study 11. The use of an autoclave to perform the thermobaric strategy resulted in no increase in soluble COD, but the application of an alkaline agent (NaOH) enhanced the solubilization, resulting in 48.2 % soluble COD. The combination of both strategies, by adding the alkaline agent prior to autoclave the waste resulted in a greater enhance in COD, achieving 50.7 %. However, this method is restricted to laboratory scale experiments as it performance involves high costs at industrial scale.

Additionally, an alkaline strategy was applied to mixed pork waste in case-study 13 [16]. Two NaOH concentrations (50 and 100 g NaOH $kgVS^{-1}$) were tested with 5 % mixed pork waste. This strategy was compared to heating (70ºC for 1 h) and sterilization (133ºC), which were tested in different waste concentrations (5–80 %). Direct evaluation of the hydrolysis was not conducted; instead, the hydrolyzed residue served as the substrate for AD, allowing for a comparison of AD yields among different hydrolysates. Results are explained in the next section (Table 9).

Thermal strategies have also been explored to enhance the solubilization of protein-rich ABPs. In case-study 12, the thermal hydrolysis of feathers was investigated under both oxidative and inert atmospheres. Conducted by Strong and Gapes, [44] at laboratory scale, the study involved subjecting the ABPs to different temperatures (70, 140 and 200ºC). Depending on the experimental condition, either an excess of oxygen or the addition of nitrogen was employed. The concentration of dissolved organic carbon (DOC) was studied, revealing an increase in DOC concentration as the temperature of the pretreatment increased, under both oxidative and inert atmosphere. Specifically, under oxidative conditions at 200ºC, the DOC concentration increased from 540 to 6000 mg L^{-1}, resulting in a conversion of 55 % of volatile suspended solids (VSS) to DOC.

Although enzymatic and physical-chemical pretreatments have been widely studied independently, their combined application remains largely unexplored in the context of ABPs hydrolysis. The reviewed case-studies primarily applied these strategies separately, with enzymatic hydrolysis focusing on lipid breakdown under mild conditions, and physical-chemical methods, including alkaline, thermal, ultrasound, or thermobaric treatments, targeting solubilization and particle size reduction. However, the integration of these approaches could provide synergistic effects by improving substrate accessibility and enhancing overall hydrolysis efficiency. For instance, alkaline-thermal treatment [2,23] led to significant COD solubilization and particle size reduction, potentially enhancing subsequent enzymatic activity. Similarly, Harris et al. [15] reported improved solubilization when combining NaOH addition with thermobaric treatment, suggesting that chemical and thermal pretreatment may enhance the effectiveness of subsequent enzymatic hydrolysis. Despite these promising indications, none of the case-studies in the reviewed literature systematically assessed the sequential or simultaneous application of enzymatic and physical-chemical methods. Further research is needed to evaluate the operational feasibility and potential synergistic effects of integrated pretreatment strategies.

4.4. Biotransformation of slaughterhouse waste

The biotransformation strategy used to valorize the hydrolyzed slaughterhouse waste was AD in 11 of the 13 case-studies analyzed. In two case-studies, 2 and 8, the hydrolytic efficiency was not evaluated through biotransformation. Table 9 summarizes the main parameters of

AD for the hydrolyzed ABPs, including AD conditions, inoculum characteristics and AD yield. Two different strategies can be observed regarding the study of the hydrolysate performance in AD. When only one pretreatment strategy was reported, the AD of the hydrolysate was compared to a control. Conversely, when multiple strategies or conditions were evaluated, a comparative analysis of AD efficiency of each studied condition was conducted. The substrate for the AD was typically the hydrolysate obtained from the pretreatment strategies, although modifications may be made to adjust the characterization of the AD substrate, as seen in Valladão et al. [48] (case-study 3), where the waste was diluted to achieve a lipid concentration of 550 mg L^{-1}. The objective of all the studies was the evaluation of the pretreatment strategies, not the optimization of the AD conditions.

Anaerobic digestion of ABPs was primarily conducted at laboratory scale, with reactor volumes ranging from small experimental setups (60–400 mL) to bench-scale systems of up to 5 L. Only one case-study reported a pilot-scale configuration of 42 L. Throughout the reviewed case-studies, the systems employed were conventional in design, typically equipped to ensure adequate mixing and temperature control. The common approach was batch operation mode in mesophilic conditions, using a previously adapted inoculum. However, two studies explored the comparison between thermophilic (55–60ºC) and mesophilic (30–37ºC) conditions, assessing methane production under both conditions. Inoculum characterization was detailed in only two articles, with a VSS around 11.5 g L^{-1}. When reported, the most common sludge-to-waste inoculation ratio was 1:1 and 1.4:1.

The performance of AD was evaluated primarily by measuring methane or biogas production, along with assessing COD and VS removal from the ABP. In Pascale et al. [34] (case-study 5), methane content was analyzed using gas chromatography. Similar analytical methods were employed in other case-studies (3, 4, and 13). However, Battimelli et al. [2], in case-studies 6 and 7, employed a mass flowmeter for continuous measurement of biogas and Luste et al. [23], in case-studies 9 and 10, utilized the water displacement method to measure biogas volume and gas chromatography to analyze methane content.

When comparing the efficiency of enzymatic pretreatment applied to fat-rich ABPs, a minimal increase in biogas production was observed for pretreated residue compared to controls without pretreatment (case-studies 1, 4, and 5), caused by the presence of LCFA that could inhibit the methane production. Conversely, Valladão et al. [48] (case-study 3), observed a four-fold improvement in methane concentration when using the hydrolyzed residue compared to control, contrary to what was expected, as LCFA inhibition was not shown. This phenomenon was attributed to the use of real effluents that contain other constituents that modify the effect of the toxic compounds on the anaerobic biomass.

Besides, AD of hydrolyzed aerofloation fats (case-study 6) and flesh fat from cattle carcass (case-study 7) was studied at 5 L working volume under mesophilic and thermophilic conditions [2]. Theoretical biogas potentials were calculated based on the composition of slaughterhouse waste, estimated to be approximately 1300 mL gVS^{-1} for both by-products studied. However, the degraded load was the parameter of interest as it provides information by considering the time needed for degradation and the amount of biomass. For instance, pretreatment of carcass waste at 120ºC resulted in a degraded load of 2 g VS $L^{-1}d^{-1}$ in comparison to 0.2 g VS L^{-1} d^{-1} achieved by raw waste, yielding 1168 mL biogas g^{-1} VS fed, in mesophilic conditions. Pretreated aeroflotation fat waste was digested in mesophilic conditions and showed no increase in methane production in comparison to raw waste production, reaching a biogas concentration of 617 mL biogas g^{-1} VS when treated at 120ºC.

The efficiency of chemical treatment based on NaOH did not show improvement in biogas production, in comparison to untreated ABPs. For instance, Luste et al. [23] reported that AD of treated digestive tract content showed no increase of methane production in comparison to untreated waste. This lack of improvement was attributed to factors such as the reduction of VS during pretreatment, inhibitory concentrations of VFA or re-crystallization of cellulose. The increase of particle size of the residue by 22 ± 9.8 % across various hydrolytic strategies (excluding bacterial product treatment) supported this phenomenon. Furthermore, drum sieve waste produced 48 % more methane after thermal treatment, resulting in 340 mL CH_4 g^{-1} VS added. This was attributed to concentration of the waste due to evaporation of water during thermal hydrolysis.

Methane production was assessed for feather residue pretreated thermically under inert and oxidative atmospheres in the study by Strong and Gapes [44]. The pretreatment at 140ºC exhibited the highest methane yield, resulting in 125 mL g^{-1} VSS, with no significant difference observed under inert or oxidative atmospheres. However, methane production differed when using pretreated waste at 200ºC, with a noted decline in oxidative pretreated residue due to a reduced carbon availability for methane generation and the generation of refractory compounds.

In Harris et al. [15], reported as case-study 11, the great increase in COD solubility was observed in chemical and thermochemical strategies. However, the results obtained from the biochemical methane potential (BMP) assay contradicted what was expected, by showing an increase in specific methane production of the thermobaric strategy even though a decrease in the VFA obtained was observed in this case, as only 2.3 g L^{-1} of VFA were obtained in comparison to 14.5 g L^{-1} and 21.0 g L^{-1} obtained in the chemical and thermochemical strategies. In the thermobaric case, no inhibitory effect regarding LCFA concentration was observed, and the lag period of 5 days was not observed, showing an improvement of digestion time by 3 days. The chemical and thermochemical pretreatments achieved a reduction of 2 days and 1 days, in comparison to the control, regarding the methane production.

Hejnfelt and Angelidaki [16], in case-study 13, evaluated the pretreatment yield through batch AD experiments under thermophilic and mesophilic conditions, using untreated, heated or sterilized residue. No increase in the biodegradability or methane yield was observed compared to untreated mixed waste, reaching a theoretical yield of 600 mL biogas g^{-1} VS. Sterilization had no effect on methane yield, but increased the methane production per kg of residue, as water evaporated. Regarding the comparison between thermophilic or mesophilic conditions, no difference was observed when using 5 % dilution of the residue. When using 50 % dilution of the mixed pork waste, no methane was obtained under thermophilic conditions due to ammonia inhibition.

A key limitation observed across the analyzed case-studies is the lack of standardized methodologies for evaluating AD performance. Variations in inoculum origin and operational modes hinder the comparability of results in the reported studies. Standardization of AD setup, including inoculum-to-substrate ratios, hydraulic reaction times and pH, as well as of performance indicators, such as methane yield, COD and VS removal, along with standardization of pretreatment strategies, would enhance reproducibility and enable more meaningful comparisons between studies. Establishing such methodological standards is particularly important for industrial-scale applications, where reproducible and predictable performance is essential for integrating ABPs valorization into existing waste management systems.

5. Conclusions

Slaughterhouse waste presents significant potential for valorization through biological processes despite considerable variation related to animal origin and processing methods. Traditionally, ABPs have been managed through landfilling, composting and rendering, resulting in low-value outputs such as animal feed and fertilizers.

The valorization of slaughterhouse waste through biological processes, particularly AD, presents a promising pathway for resource recovery. However, the heterogeneity of ABPs and the lack of standardized methodologies for their characterization, conditioning, and hydrolysis remain major barriers to reproducibility and scalability.

This review highlights the need to establish standardized protocols for waste characterization, including determination of VS and COD and quantification of protein and lipid content. These parameters are essential to calculate characteristic ratios that can be used to classify the waste and to guide subsequent pretreatment selection.

Conditioning strategies such as adjusting fat concentration or reducing particle size have been widely applied across cases-studies. However, further investigation of the specific effect of conditioning is needed along with the development of standardization procedures for this process.

Hydrolytic strategies can be broadly categorized into enzymatic and physicochemical strategies, both showing potential to enhance waste hydrolysis. Enzymatic hydrolysis, although particularly effective for lipid-rich streams, suffer from uneven dosages and poorly characterized enzyme activities. Physicochemical methods, such as alkaline or thermal treatments, are more versatile but may require high energy inputs or result in the formation of inhibitory compounds. The integration of both strategies remains underexplored and could offer synergistic benefits.

Among biological technologies, AD is considered the primary process for assessing waste conversion efficiency and evaluating the impact of different hydrolytic strategies. However, performance comparisons across studies are hindered by variability in operating conditions and evaluation of performance parameters. While enzymatic pretreatment effectively enhances fat hydrolysis, its impact on biogas production remains limited when compared to untreated controls. Chemical pretreatments improve particle size reduction and waste solubilization by increasing sCOD but they do not consistently improve AD yields due to potential inhibitory effects. Factors such as water evaporation during thermal hydrolysis or the release of inhibitory compounds can negatively affect digestion performance. In this context, co-digestion strategies appear as a promising approach to mitigate inhibition and dilute inhibitory compounds, potentially improving overall process stability.

To advance the field, future research should focus on developing standard protocols for slaughterhouse waste characterization and pretreatment evaluation, optimizing pretreatment conditions, tailored to waste characterization and exploring the integration of enzymatic and physicochemical hydrolytic methods. Additionally, efforts must be made to investigate the scale-up potential and assess the economic feasibility of the most promising alternatives.

CRediT authorship contribution statement

Montserrat Jiménez-Urpi: Writing – original draft, Methodology, Investigation. **Sergi Carbonell-Chacón:** Writing – review & editing, Validation, Supervision. **Irene Jubany:** Writing – review & editing, Supervision. **Guillermo Baquerizo:** Writing – review & editing, Validation, Supervision, Conceptualization. **María Eugenia Suárez-Ojeda:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Author Montserrat Jiménez-Urpi received support from Pla de Doctorats Industrials de la Secretaria d'Universitats i Recerca del Departament d'Empresa i coneixement de la Generalitat de Catalunya (AGAUR) through an industrial PhD grant (2021 DI 00116). M.E. Suárez-Ojeda thanks to the grant ORIGEN PID2021–126102OB- I00 funded by CIN/AEI/10.13039/501100011033 and by ERDF: A way of making Europe from the European Union for funding her participation in this work. M.E. Suárez-Ojeda is member of the GENOCOV research group, Grup de Recerca Consolidat de la Generalitat de Catalunya (2021 SGR 515) and coordinator of the CYTED BioFuturo network (424RT0157).

Data availability

Data will be made available on request.

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