



Digestate Post-Treatment Strategies for Additional Biogas Recovery: A Review

Cristiane Romio *[®], Michael Vedel Wegener Kofoed and Henrik Bjarne Møller

Department of Biological and Chemical Engineering, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark; mvk@bce.au.dk (M.V.W.K.); henrikb.moller@bce.au.dk (H.B.M.)

* Correspondence: ane.romio@bce.au.dk; Tel.: +45-528-198-77

Abstract: Anaerobic digestion (AD) is a process in which microorganisms, under oxygen-free conditions, convert organic matter into biogas and digestate. Normally, only 40-70% of biomass is converted into biogas; therefore, digestate still contains significant amounts of degradable organic matter and biogas potential. The recovery of this residual biogas potential could optimize substrate utilization and lower methane emissions during digestate storage and handling. Post-treatment methods have been studied with the aim of enhancing the recovery of biogas from digestate. This review summarizes the studies in which these methods have been applied to agricultural and wastewater digestate and gives a detailed overview of the existing scientific knowledge in the field. The current studies have shown large variation in outcomes, which reflects differences in treatment conditions and digestate compositions. While studies involving biological post-treatment of digestate are still limited, mechanical methods have been relatively more explored. In some cases, they could increase methane yields of digestate; however, the extra gain in methane has often not covered treatment energy inputs. Thermal and chemical methods have been studied the most and have yielded some promising results. Despite all the research conducted in the area, several knowledge gaps still should be addressed. For a more thorough insight of the pros and cons within post-treatment, more research where the effects of the treatments are tested in continuous AD systems, along with detailed economic analysis, should be performed.

Keywords: anaerobic digestion; digestate; lignocellulosic biomass; sludge; post-treatment

1. Introduction

The need to develop and improve sustainable energy resources is evident due to the finite nature of fossil fuels and the greenhouse gases (GHG) emissions associated to their use, and the transition to a recycling-based society should be encouraged [1]. Anaerobic digestion (AD) is highly interesting in this context, as it can function in waste treatment by the production of a renewable energy carrier (biogas, mainly composed of CH_4 and CO_2) and the production of an organic fertilizer [2].

Biogas can be produced from many types of organic waste materials, including sludge from wastewater treatment plants (WWTP) and livestock and agricultural wastes, such as crop residues and manure [3]. These materials are interesting due to their abundance and availability worldwide. For these wastes, the first step in AD, hydrolysis, has been considered as rate limiting [4]. Microorganisms involved in hydrolysis synthesize and secrete specific enzymes to hydrolyze polymers of carbohydrates, proteins, lipids, and other minor components, which are used by other groups of microorganisms to further produce CH_4 and CO_2 [5].

The hydrolysis rate is normally limited by the complex structure of these materials. Agricultural biomasses have high contents of lignocellulose, which is the complex and rigid matrix of plant cells and is mainly composed of cellulose, hemicellulose, and lignin strongly linked to each other [6]. Cellulose consists of an unbranched polymer of D-glucose



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). units organized in crystalline or amorphous structures, and the crystalline region is more hardly accessible for hydrolytic enzymes. Hemicellulose is a branched amorphous polymer composed mainly of xylose units. Lignin presents an amorphous structure composed of units of p-coumaryl, coniferyl, and sinapyl [7]. Lignin is hardly degraded anaerobically [6], and it represents a barrier to the enzymatic attack of cellulose and hemicellulose. Regarding sludge, secondary sludge is usually considered more difficult to degrade than primary sludge [8], due to its complex floc structures composed of extracellular polymer substances (EPS) and microbial cell walls [9]. Lignocellulose can also be present in sludge, primarily within the primary sludge, which also contains fats and solids retained in the primary clarifier.

It is estimated that only around 50 to 60% of sludge [10] and 50 to 70% of agricultural biomass are converted into biogas during AD [11]. Besides the recalcitrance of some materials, the semi-continuous operation of digesters, non-optimized process conditions and economic reasons also make the complete biomass degradation difficult to achieve. This means that the material leaving the digester, called digestate, still contains some biogas potential, which could be further exploited. Common methane yields for the whole digestate (WD), which include both the solid and liquid fractions, reported in this review vary between approximately 35 and 170 mL/g VS. When only the solid fraction of digestate (SFD) is considered, these yields can be as high as 300 mL/g VS.

During AD, the most easily degradable fractions of biomass are degraded first, resulting in the accumulation of more recalcitrant fractions on digestate. For instance, during AD of agricultural residues, hemicellulose is degraded faster than cellulose, resulting in accumulation of cellulose and lignin [12]. Post-treatment aims at enhancing the hydrolysis of the recalcitrant fractions and hereby exploit the residual biogas potential of the digestate. Two system configurations involving post-treatment of digestate and its re-digestion have been proposed: (1) treatment of part of digestate and its recirculation back to the digester, and (2) inter-stage treatment of digestate prior to feeding it to a second digester (Figure 1).



Figure 1. System configurations integrating biomass pre-treatment or digestate post-treatment with AD: (a) undigested biomass pre-treatment; (b) digestate post-treatment with recirculation; (c) digestate post-treatment in an inter-stage configuration.

The most commonly studied approach in AD is pre-treatment (Figure 1), where raw undigested substrate is treated by thermal, mechanical, chemical or biological methods prior to AD, in order to open up its structure and increase its degradability. However, a post-treatment of digestate might be advantageous over pre-treatment of certain biomasses. Pretreatment can act on both the fractions, which are easy and hard to degrade during the following AD process, whereas a post-treatment only targets fractions that were not degraded during AD. The value proposition of the concept of post-treatment is that energy, chemicals, and money are only spent on biomass fractions recalcitrant to biological degradation, to increase treatment efficiency while reducing treatment expenditures. Evidently, a substitution of pre-treatment of raw biomass by a post-treatment of digestate would not always be possible to stand alone, since some biomasses, especially the ones containing larger particles, would often require an initial pre-treatment. In these cases, post-treatment could be a complementary approach.

The biogas recovery from digestate could render the whole AD process as more environmentally and economically sustainable. By producing more biogas from the same substrate, the demand for biomass feedstock, sometimes expensive or limited, could be reduced and there could be a more efficient management of the biogas plant resources [13]. Furthermore, reduced residual methane potential from the final digestate could lower the risks of methane emissions during its handling, storage, and subsequent application. Agricultural digestates are frequently employed as fertilizers, while sludge-based digestates normally require safe disposal, such as incineration. Increasing biomass degradation reduces the volume of final digestate, hereby decreasing transportations costs to farmlands/incineration sites and their associated GHG emissions.

This review summarizes studies in which thermal, mechanical, chemical, and biological treatment technologies have been applied to agricultural and sludge-based digestate aiming to recover additional biogas. The studies included were identified in Google Scholar through the search of the following keywords combined: *anaerobic digestion, digestate, sludge, lignocellulose, manure fibers, treatment, post-treatment, inter-stage treatment,* and *digestate recirculation*. Each of the overall treatment technologies are discussed, covering (1) their basic mechanism; (2) a comprehensive review of different results; and (3) a discussion of the perspectives of the methods. Unless otherwise stated, the results reported refer to the increase in methane yields obtained from single batch AD tests of treated WD in comparison to an untreated control.

2. Thermal Post-Treatment

In lignocellulosic biomass, hydrothermal treatments at the temperature range of 160–220 °C are suitable to reach hemicellulose solubilization. Hemicelluloses are broken down into high molecular weight soluble fragments and acetyl groups are split off, resulting in the formation of acetic acid. In a process called auto-hydrolysis, hydronium ions, formed by water auto-ionization and from the generated acetic acids, further catalyze the breakdown of hemicellulose polysaccharides into oligomers and monomers, which can be utilized by AD microorganisms. Cellulose and lignin suffer limited alteration, but, by hemicellulose solubilization, cellulose becomes more exposed to hydrolytic enzymes [14]. In sludge, thermal treatment at temperatures up to 150 °C causes mainly the solubilization of carbohydrates due to EPS disruption. At higher temperatures, proteins are also solubilized and it is thought that they are released from microbial cell lysis [15]. In both lignocellulosic material and sludge, the efficiency of thermal treatments are dependent on treatment conditions, which should be severe enough to promote organic matter solubilization, but not excessively intense. Overly severe treatments at high temperatures or lengthy treatments can lead to the formation of complex, recalcitrant, and inhibitory compounds [16].

A variant of thermal treatment is wet or steam explosion, where biomass is directly heated or exposed to steam at temperatures up to 240 °C and high pressure for few minutes, and then a sudden depressurization is followed, leading to an explosion inside the biomass. In addition to the effects of temperature, the following explosion works as a mechanical treatment, leading to structural breakdown of the biomass [16].

Digestate thermal treatments have been tested at both moderate (<100 $^{\circ}$ C) and high temperatures (>100 $^{\circ}$ C) sometimes followed by an explosion step. A summary of conducted studies are presented in Table 1. More extensive research has been conducted with sludge-based digestate than with agricultural digestate. Some authors have compared the

efficiencies of inter-stage treatment configuration with pre-treatment and have shown that the first strategy could be advantageous in terms of overall methane production. Thermal treatments that employed acids or bases as catalysts will be covered in Section 4. Thermal treatments combined with high pressures aiming the formation of hydrochar and the reutilization of the process water for biogas production are not covered in this review, but have been discussed by Wang and Lee (2020) [17].

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Moderate Temperature	WD, agricultural	80 °C, 1 h	Batch, 35 °C, 65 days	70 ± 2	-19%	[12]
Moderate Temperature	SFD, agricultural	80 °C, 3 h	Batch, 35 °C, 30 and 340 days	Day 30: 61 ± 4.7 Day 340: 179 ± 12.2	-21% (day 30) -4% (day 340)	[18]
Moderate Temperature	SFD, agricultural	80 °C, 3 h	Batch, 55 °C, 30 and 340 days	Day 30: 82 ± 1.8 Day 340: 215 ± 7.3	-40% (day 30) -24% (day 340)	[18]
Moderate Temperature	SFD, agricultural	80 °C, 1 h	Batch 35 °C, 65 days	90 ± 1	-12%	[12]
Moderate Temperature	WAS and WD ^a	80 °C, 10–48 h at pH 7.1 or pH 9.3	Batch, 37 °C Pre: 40 days Inter-stage: 21 + 19 days	291	-3% (pre, pH 7.1, 10 h) 5% (pre, pH 9.3, 10 h) Between +11% and +20% (inter-stage, pH 7.1, 10-48 h) ^b +31% (inter-stage, pH 9.3, 10 h) ^b	[19]
High Temperature	WD, agricultural, HRT of 40, 100 and 150 days	120 °C, 30 min	Batch, 40 °C, 56 days	$71.4 \pm 5.3 (40 \text{ days HRT}) 116.9 \pm 11.3 (100 \text{ days} HRT) 156.9 \pm 7.4 (150 \text{ days HRT})$	+115% (40 days HRT) -16% (100 days HRT) +12% (150 days HRT)	[20]
High temperature	SFD, agricultural	100 °C, 6 h + 135 °C, 1 h	Batch, 37 °C, 50 days	62	+48%	[21]
High temperature	SFD, agricultural	100 °C, 6 h + 135 °C, 1 h	Batch, 53 °C, 50 days	71	+54%	[21]
High Temperature	SFD, agricultural	230 °C, 15 min	Batch, 52 °C ^g	Not reported	+29%	[22]
High temperature	SFD, agricultural	180 °C, 30 min	Continuous, 53 °C, 20 days HRT	≈80	+48%	[23]
High Temperature	WAS and WD ^a	130 °C, 15 min	Batch, 37 °C Pre: 40 days Inter-stage: 21 + 19 days	291	+13% (pre) +9 (inter-stage) ^b	[19]
High Temperature	WAS and WD ^a	170 °C, 15 min	Batch, 37 °C Pre: 40 days Inter-stage: 21 + 19 days	291	+9% (pre) +29% (inter-stage) ^b	[19]
High Temperature	WD, sludge-based	120–190 °C, 60 min	Batch, 35 °C, 60 days	52	+246%, +327% and +304% (120, 170 and 190 °C)	[24]
High Temperature	WD, mixed (agricultural and sludge-based)	120–190 °C, 30 and 60 min	Batch, 35 °C, 60 days	147	Between 0% and +52%	[24]
High Temperature	WD, sludge-based	130–210 °C, 30 min	Batch, 35 °C, 30 days	46.47	Between +178% and 255%	[25]
High Temperature	Primary sludge and WD ^a	130–210 °C, 30 min	Batch, 35 °C Pre: 30 days Inter-stage: 30 + 30 days	203.68 (30 days) 222.79 (30 + 30 days)	Between -8% and +31% (pre) Between +13% and 23% (inter-stage) ^b	[25]
High Temperature + explosion	SFD, agricultural	145–180 °C, 10–20 min, with/without O ₂	Batch, 38 °C, 48 days	≈89	+79%, +108% and +136% (10 min at 145, 165 and 180 °C) +82% (20 min, 165 °C) +106% (10 min, 165 °C, with O ₂)	[26]

 Table 1. Summary of studies about thermal post-treatment of digestate for additional biogas recovery.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
High Temperature + explosion	SFD, agricultural	180 °C, 10 min	Continuous, 38 °C, 20 days HRT, 2.5-3.5 g VS/I/day, co-digestion of manure and treated/untreated digestate (1:1 VS basis)	180 (raw manure) 111 (raw manure + untreated digestate)	+75% ^e	[26]
High Temperature + explosion	WD, sludge-based	165–200 °C, 0–50 min	Batch, 35 °C, 20 days	≈76	Between +26% and 125%	[27]
High Temperature + explosion	Mixed sludge and SFD ^a	170 °C, 30 min	Semi-batch, 38 °C, 12.5–18 days HRT, 2–4 g VS/1/day Configurations: single AD, pre-treatment + AD, AD + AD, AD + inter-stage treatment + AD	330.5 \pm 11.2 ^f (single AD) 365.5 \pm 4.3 ^f (AD + AD)	+4% (pre-treatment + AD) ^{c,f} +31% (AD + inter-stage treatment + AD) ^{d,f}	[28]
High Temperature + explosion	Mixed sludge and SFD ^a	165 °C, 30 min	Continuous, 38–39 °C, 3–5 g VS/1/day, inter-stage	$505\pm81~{ m f}$	n.d	[29]

Table 1. Cont.

n.d.: not determined. ^a Pre-treatment (of raw sludge) and inter-stage treatment (of digested sludge) strategies were compared. ^b Considering the overall methane yields (accounting all AD steps). ^c Compared to single-AD. ^d Compared to double AD. ^e Compared to the reactor being fed with manure and untreated digestate. ^f In mL bioga/g TS. ^g Unknown duration.

2.1. Thermal Post-Treatment at Moderate Temperature

Although there have only been a few studies, thermal treatments at moderate temperature have been in general not harsh enough to enhance agricultural digestate biodegradability. Kaparaju and Rintala (2005) [18] and Sambusiti et al. (2015) [12] obtained lower methane yields compared to control when treating WD and SFD at 80 °C for 1–3 h. When processing waste activated sludge (WAS), however, an inter-stage treatment at the same temperature applied between two batch AD stages, conducted by Nielsen et al. (2011) [19], improved the overall methane yield in comparison to a single-step AD. Overall methane yields increased by 11–20% with treatment durations of 10–48 h, with the highest increase at 10 h treatment. The authors furthermore demonstrated a 31% increase in methane yield by combining the inter-stage thermal treatment (80 °C, 10 h) with a pH increase (from 7.1 to 9.3), which was obtained via removal of CO₂ from the slurry by purging it with N₂.

2.2. Thermal Post-Treatment at High Temperature

Thermal treatments at high temperatures (120–230 °C, 15–60 min) have been demonstrated to increase methane yields of both agricultural and sludge-based digestates in batch and continuous AD tests. The digestate origin seems to be an important factor in the degree of effectiveness of the treatment, with the general best results obtained with sludge-based digestate. Studies investigating the optimal treatment temperature have been conducted solely with sludge-based digestates, showing that, in this case, optimal temperature is around 150–170 °C. In general, higher temperatures have induced higher degrees of organic matter solubilization; however, this has not always been reflected in methane yields. The influence of treatment duration has not been investigated at a great extent with any digestate types; therefore, drawing conclusions is difficult.

Menardo et al. (2011) [20] post-treated agricultural digestate samples at 120 °C for 30 min and showed that the treatment effectiveness varied accordingly to the HRT of the main digester from which samples were collected. Thermal treatment reduced the methane yield by 16% for digestate sampled at a digester operating at 100 days HRT and increased it by 12% when a digestate from a 150 days HRT AD process was employed. For digestate coming from a biogas plant with shorter HRT (40 days), however, an increase of 115%

on the methane yield was observed. By post-treating the solid fraction of manure-based digestate (6 h at 100 $^{\circ}$ C followed by 1 h at 135 $^{\circ}$ C), Khan and Ahring (2020) [21] observed a 54% increment in methane yield in a batch test. At higher temperature (180 $^{\circ}$ C for 15 min), Bruni et al. (2010) [22] obtained a 29% increase in methane yield of SFD. In continuous reactors, Khan and Ahring (2021) [23] observed a 48% increase in methane yield after a treatment of SFD at 180 $^{\circ}$ for 30 min.

Nielsen et al. (2011) [19] treated digested WAS at 130 and 170 °C for 15 min in an interstage treatment configuration and obtained overall methane yields increments of 9 and 29%, respectively, in comparison to a single step AD. Bjerg-Nielsen (2018) [24] post-treated a WAS-based digestate at 120, 170 and 190 °C for 60 min and obtained methane yields increases of 246, 327, and 304%, respectively. When the same treatment was applied to a digestate originated from the co-digestion of the same WAS and wheat straw, the increase in methane yield was limited to up to 52%, with the highest yield also achieved at 170 °C. Thermal post-treatment of primary sludge-based digestate also proved efficient. Yuan et al. (2019) [25] performed treatments at 130–210 °C for 30 min and obtained methane yields increments between 178 and 255%, with the highest increases at 150 and 170 °C and the lowest at 210 °C.

2.3. Thermal Post-Treatment at High Temperature Followed by Explosion

Treatments by steam/wet explosion (110–200 °C, 0–50 min) have been efficient at improving methane yields of both agricultural and sludge-based digestates in batch and continuous AD. Optimal treatment temperature seems to lie around 180 °C and treatment durations between 10 and 30 min. Studies aiming to improve the degradability of agricultural digestate are scarce while, for sludge-based digestate, there are commercially available technologies, such as the CAMBI[®] (Asker, Norway) process.

Biswas et al. (2012) [26] applied steam explosion (145, 165, and 180 °C, 10 min) to the solid fraction of manure-based digestate and observed methane yields increments between 79 and 136%, increasing accordingly to temperature. At 165 °C, the authors investigated the effects of prolonging the treatment to 20 min and of adding O_2 during treatment, but none of the alternatives could further increase the methane yield. In continuous digesters, SFD treated at 180 °C for 10 min was co-digested with raw manure and the methane yield obtained was 75% higher than the yield of a reactor that was fed with raw manure and untreated digested fibers.

Ortega-Martinez et al. (2016) [27] applied steam explosion (110–200 °C, 0–50 min) to sludge-based digestate and observed methane yields increments between 26 and 125%, with the highest yield obtained at 180 °C and 30 min. A higher temperature rendered lower methane yield increments and some indication of inhibition exhibited as longer lag-phase durations. Shana (2016) [28] investigated the effects of treating the solid fraction of sludge-based digestate at 170 °C for 30 min and feeding it to semi-continuous digesters. An inter-stage treatment configuration with thermal explosion between two anaerobic digesters (the first being fed with untreated sludge) increased the overall biogas yield by 44, 31 and 38% when compared to a single step AD, double step AD and single AD preceded by steam explosion pre-treatment of raw sludge, respectively. Rus et al. (2017) [29] reproduced the inter-stage treatment configuration of the experiment of Shana (2016) [28] in pilot-scale and obtained a similar overall biogas production (505 ± 81 versus 478.6 ± 21.9 mL/g TS obtained by Shana (2016) [28]). The system operated for 15 months, and a stable process was demonstrated as possible, with volatile fatty acids (VFA), NH₃, and pH levels within appropriate ranges.

2.4. Thermal Post-Treatment—Discussion and Perspectives

In summary, thermal treatment efficiency has been shown to depend on digestate characteristics and operational conditions (temperature and duration). For agricultural digestates, thermal treatments at moderate temperatures have not been efficient while treatments at high temperatures followed, in some studies, by an explosion step, could enhance methane yields at varying levels. Treatments at all temperature ranges applied to sludge-based digestates have been in general more effective, yielding higher methane yield increments, however, also at varying degrees. Due to the scarcity of studies available and differences on digestate characteristics, it seems difficult to assess whether an explosion step after heat application is justifiable.

Normally, organic matter solubilization increases with treatment temperature and duration [19,27]; however, the same trend is not always reflected on the methane yield, which could be an indication of formation of inhibitory compounds. Inhibitors might include heterocyclic compounds, such as furfural and hydroxymethylfurfural, and phenols. It is not clear, whether these products are toxic to AD microorganisms or if they are simply very difficult to degrade, hampering the further increase of biogas yields [30]. Therefore, careful optimization of process parameters would be required prior to its implementation at larger scales. Additionally, experiments involving continuous AD operation would also be important, as they would allow investigating the microbial community capability to tolerate or adapt to the presence of inhibitors.

Some authors have shown that applying a thermal treatment to sludge-based digestate rather than to the raw substrate could be advantageous regarding the overall methane yield. Normally, higher methane yields are obtained for raw substrates, however, the relative increase in methane yields caused by the thermal treatment have been in many cases higher for the digestate. Nielsen et al. (2011) [19] and Shana (2016) [28] compared the overall methane yields of two system configurations: a single-stage AD preceded by the pre-treatment of raw sludge and a double-stage AD intermediated by the treatment of the digestate originated in the first digester. Nielsen et al. (2011) [19] observed that AD with an inter-stage treatment at 80 $^{\circ}$ C for 10 h yielded 24% more methane than the pre-treatment approach at same conditions. At 170 °C and 15 min, the methane yield in the inter-stage treatment configuration was 18% higher. Shana et al. (2016) [28] demonstrated that an inter-stage treatment configuration (170 $^{\circ}$ C, 30 min with explosion) provided a 38% higher methane production than the pre-treatment strategy. Yuan et al. (2019) [25] performed a similar experiment and observed a slightly increased overall methane yield with the inter-stage treatment strategy. However, in their study, the HRT of the AD in inter-stage treatment configuration was twice as long as the AD following a pre-treatment, and therefore, a direct comparison is not applicable. Nevertheless, they demonstrated that the methane yield increments obtained by the treatment of digestate (178–255%) were indeed higher than the ones observed when treating raw sludge (18–31%). The same trend was confirmed by Ortega-Martinez et al. (2016) [27], who obtained 22 and 125% increases on methane yields of raw and digested sludge, respectively.

Regarding agricultural digestate, the opposite tendency was observed by Menardo et al. (2011) [20], when comparing treatment (120 °C, 30 min) efficiencies in digestate and raw slurry in batch tests: a 175% increase in methane yield was observed when pre-treating slurry while methane yields of digestate samples changed between a 16% reduction and a 115% increase. In a continuous AD test, Biswas et al. (2012) [26] showed that the co-digestion of treated (180 °C, 10 min with explosion) digested fibers and raw manure could increase the methane production by only 8% when compared to the digestion of untreated manure alone. Additional studies comparing pre-treatment and post-treatment of digestate, both agricultural and sludge-based, would be beneficial as they could strengthen the evidences of the trends observed.

Despite some indications of the higher efficiency of thermal treatments of digestate (namely of sludge-based digestate) rather than of raw substrate, it would be important to evaluate how the energy balance and the economy would be affected. With an inter-stage treatment configuration, there should be extra costs and energy demand for operating a second digester. Additionally, a positive energy balance would be dependent on the TS/VS contents of the sample. Despite an increase in methane yield due to the thermal treatment, Bjerg-Nielsen (2019) [24] obtained a lower energy gain as extra methane than the energy used for treatment due to the low TS/VS contents of the WD employed. A

post-treatment of the SFD rather than of the WD should therefore be more advantageous. Moreover, if the thermal treatment is applied to SFD rather than to the solid fraction of undigested biomass, treatment costs and energy consumption should be lowered since solids volume should be reduced during AD. By post-treating SFD, Rus et al. (2017) [29] showed that the inter-stage treatment configuration was superior to the pre-treatment approach in regards to the energy balance. Furthermore, in an economic analysis, they showed that although the inter-stage treatment yielded the highest capital expenditure costs, it also led to the highest internal rate of return and the lowest payback in years. Thermal post-treatment of SFD could therefore be a viable option, especially in biogas plants equipped with combined heat and power units, since low-cost waste heat could be applied as energy input for the treatment. Nevertheless, the number of studies in which energy balances and economic analysis have been conducted are still limited and are therefore recommended for future works.

3. Mechanical Post-Treatment

3.1. Comminution

Comminution, such as chipping, grinding and milling, are purely mechanical techniques employed to directly reduce the particle size of biomass and cellulose crystallinity, increasing the surface area and decreasing the degree of polymerization. With increased surface area, the biomass can be more exposed to hydrolytic enzyme attack, and heat and mass transfer can be improved, hereby enhancing the biogas production [31].

Milling has been applied to agricultural SFD with varying outcomes (Table 2). Kaparaju and Rintala (2005) [18] obtained reduced methane yields in comparison to the untreated control, while Bruni et al. (2010) [32] and Khan and Ahring (2021) [23] observed methane yields increments of up to 10%. In a study conducted by Monlau et al. (2019) [33], the achieved methane yield increase was of 31%. Lindner et al. (2015) [34] demonstrated that the varying degree of effectiveness of the mechanical treatment could be linked to the digester HRT from which the digestate was sampled. By applying milling (2–10 min) to the SFD, the highest methane yield increments (65–170%) were obtained for the treated samples originated from a digester operating with a long HRT (160 days). When the SFD originated from digesters operating at HRT of 24.5 days, the methane yield increases were restricted to 3–16%. Tsapekos et al. (2016) [35] could increase the methane yield by 15–45% through mechanical treatment using batch AD. In continuous AD, however, this increment was restricted to 7%, which underlines the need for studies on post-treatment in continuous systems to evaluate its real potential.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH ₄ /g VS)	Methane Yield Increase Untreated/Treated	Reference
Comminution	SFD, agricultural	Size reduction to <2 mm	Batch, 52 °C, 80 days	Not reported.	+10%	[32]
Comminution	SFD, agricultural	Size reduction to <1 mm	Batch, 35 °C, 30 and 340 days	$61 \pm 4.7~(30~{ m days})$ $179 \pm 12.2~(340~{ m days})$	—16% (30 days) —33% (340 days)	[18]
Comminution	SFD, agricultural	Size reduction to <1 mm	Batch, 55 °C, 30 and 340 days	$82 \pm 1.8~(30~{ m days})$ $215 \pm 7.3~(340~{ m days})$	−32% (30 days) −42% (340 days)	[18]
Comminution	SFD, agricultural, HRT of 14.5 and 160 days	Rotational speed of 500 min ⁻¹ , 2–10 min	Batch, 37 °C, 35 days	$\begin{array}{c} 23 \pm 3.3 \ ^{a} \ (\text{HRT of} \\ 160 \ \text{days}) \\ 229 \pm 18.8 \ ^{a} \ (\text{HRT of} \\ 24.5 \ \text{days}, \text{hay/straw} \\ \text{feeding}) \\ 291 \pm 28 \ ^{a} \ (\text{HRT of} \\ 24.5 \ \text{days}, \text{maize silage} \\ \text{feeding}) \end{array}$	Between +65% and +170% (HRT of 160 days) Between +11% and +16% (HRT of 24.5 days, hay/straw feeding) Between +3% and +9% (HRT of 24.5 days, maize silage feeding)	[34]
Comminution	Dried SFD, agricultural	Frequency of 20 s ⁻¹ , 30 min	Batch, 35 °C, 25 days	101.5	+31%	[33]
Comminution	SFD, agricultural	Size reduction to <3 mm	Continuous, 53 °C, 20 days HRT	$\approx \! 80$	+9%	[23]
Comminution	SFD, agricultural	Manual shearing	Batch, 53 °C ^d	42	Between +15% and +45%	[35]

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Comminution	SFD, agricultural	Manual shearing	Continuous, 53 °C, 15 days HRT, 2.0–2.5 g VS/l reactor/day, co-digestion of manure and SFD (0.7:0.3 ratio VS basis)	211 (manure) 143 (manure + untreated SFD)	+7% ^e	[35]
Ultrasonication	WD, agricultural	3000–15,000 kJ/kg TS	Batch, 37 °C, 13 days, treated/untreated digestate ratio of 1	$241.9 \pm 4.10 \ ^{\rm b}$	Between -10% and 8%	[36]
Ultrasonication	WD, agricultural	3000–50,000 kJ/kg TS	Batch, 37 °C, 56 days, treated/untreated digestate ratio of 3	Not reported	Between -4% and $+21\%$	[37]
Ultrasonication	WD, agricultural	1500–3000 kJ/kg TS	Continuous, 37 °C, 20–30 days HRT, 2.42–3.60 g VS/1/day, recycling ratio (digestate/raw manure) of 1	022 ± 010-0.23 ± 0.09 ^{c,d} (20 days HRT); ≈0.14 ^{c,d} (30 days HRT)	-18% (20 days HRT, 3000 kJ/kg TS) -17% (20 days HRT, 1500 kJ/kg TS) +18% (30 days HRT, 1500 kJ/kg TS)	[38]
Ultrasonication	WD, sludge-based	600–50,000 kJ/kg TS	Batch, 37 °C, 28 days, treated/untreated digestate ratio of 3	169.4	Between +7% and +25%	[39]
Ultrasonication	WD, sludge-based	14,868–59,472 kJ/kg TS	Batch, 37 °C, 24 days	38	Between +60% and 133%	[40]

Table 2. Cont.

^a: mL methane/g digestate. ^b: mL biogas/g VS. ^c: l biogas/g VS/day. ^d Unknown duration. ^e In comparison to the reactor fed with manure and untreated SFD.

3.2. Ultrasonication

A few studies have shown that ultrasonication of agricultural and sludge-based digestate could improve digestate methane yields at varying degrees (Table 2), depending on the specific energy applied and on the digestate recalcitrance. The application of ultrasound to liquids results in the growth and collapse of pre-existing microbubbles by a process called cavitation [41]. When the breakdown of these bubbles is violent enough, it leads to the formation of high mechanical stress capable of disrupting particulate matter [39].

In a study with agricultural digestate, Somers et al. (2018) [36] showed that ultrasonication at 15,000 kJ/kg TS increased the biogas yield by 8%. Boni et al. (2021) [37] could increase the digestate methane yield by 19 and 21% when applying ultrasonication at 20,000 and 50,000 kJ/kg TS, respectively. At lower energy intensities, minimal impacts on digestibility were observed. In a continuous digester operating with digestate recirculation, however, at an applied energy intensity as low as 1500 kJ/kg TS, Azman et al. (2020) [38] demonstrated that the methane production rate could be increased by 18% after an adaptation period and adjustment of HRT and organic loading rate (OLR).

When treating sludge-based digestate with ultrasonication, Garoma and Pappaterra (2018) [40] obtained methane yields increments between 60 and 133%, increasing with the specific energy applied, between 14,868 and 59,472 kJ/kg TS. The same tendency was reported by Boni et al. (2016) [39], with methane yields increasing between 7 and 25% with applied energy of 6000–50,000 kJ/kg TS.

Normally, within a same sample, the higher the ultrasound energy intensity applied, the higher the methane yields, however, significant increases occur only up until a threshold is achieved, as observed by Boni et al. (2016 and 2021) [37,39]. After this point, further improvements in methane yields are limited. Additionally, since ultrasonication is a non-selective treatment, depending on its intensity, it might cause microbial cell lysis. The loss

of microbes within digestate could negatively affect the methane yields, requiring longer adaption times and compromise the digester stability. For instance, Boni et al. (2016) [39] observed that the lag-phase time during AD increased with the intensity of the treatment.

3.3. Mechanical Post-Treatment—Discussion and Perspectives

Mechanical treatments, such as comminution and ultrasonication, have achieved varying outcomes when used as post-treatment of digestate, with efficiencies dependent on the energy applied and on digestate characteristics. Although some positive results have been obtained, both treatments are highly energy demanding and the energy input might be higher than the energy gain as extra methane. For instance, in the case of the study conducted by Monlau et al. (2019) [33], the energy consumption of milling was estimated to be as high as 57.4 kWh/kg TS (or 206,640 kJ/kg TS). When applying ultrasonication, Garoma and Pappaterra (2018) [40] observed that a maximum of only 10.8% of the energy input could be recovered as methane when digestate was ultrasonicated at 14,868 kJ/kg TS. At 59,472 kJ/kg TS, the energy recovered was even lower, solely 3.8%. Even when applying a much lower energy intensity, 1500 kJ/kg TS, Azman et al. (2020) [38] showed that ultrasonication led to a negative energy balance. Part of the energy input can be dissipated as heat. Garoma and Pappaterra (2018) [40], for instance, observed a temperature increase on digestate of up to 17.3 K. Therefore, unless the generated heat is recovered to substitute process energy, the post-treatments discussed in this section are likely energetically unviable for additional recovery of methane from digestate.

4. Chemical Post-Treatment

4.1. Oxidative Post-Treatment

Oxidative treatments include the application of oxidants, such as ozone or hydrogen peroxide. These treatments aim to partially degrade the organic matter through the action of oxygen radical species. In the case of sludge-based digestate, the most desired action of the oxidant is normally the disintegration of microorganism cells. Ozone, for instance, can penetrate the cell wall, damage the cell membrane, and destroy the structures formed by the linkages among microorganisms and the surrounding EPS. As a result, soluble substances are released, which can be further degraded during AD [42]. In lignocellulosic biomass, the purpose of an oxidative treatment is mainly to cause delignification by converting lignin to lower molecular weight compounds by reactions such as electrophilic substitution, side chain displacements, and oxidative cleavage of aromatic ring ether linkages. Normally, these compounds are not degraded anaerobically, but the partial solubilization of lignin makes cellulose and hemicellulose more available for enzymatic attack during AD [43].

Since oxidants cannot act selectively on the biomass fractions desired, intense oxidative treatments might lead to the complete mineralization into CO_2 of some portions of organic matter, which are, therefore, no longer available for AD. Additionally, the efficiency of oxidative treatments on digestate might be impaired by its usual high carbonate content. Carbonate ions can act as hydroxyl radical scavengers, limiting the radicals' action [36].

A summary of studies applying oxidative treatment is presented on Table 3. When treating agricultural digestate with ozone and hydrogen peroxide (5–30 g oxidant/kg TS, 2 h), Somers et al. (2018) [36] observed limited increments on methane yields, not exceeding 13%. On sludge-based digestate, positive effects of ozone treatment (0.16 g O_3/g SS, 10–15 min) were observed by Battimelli et al. (2003) [44]. The recirculation of treated digestate to a continuous system digesting WAS lead to an increase of chemical oxygen demand (COD) removal rate of 43–66%, depending on the recirculation rate applied (0–100%), with the methane yield in removed COD basis approximately constant throughout the experiment. The maximum COD removal was obtained at recirculation rate of 25%. The authors mentioned that the ozonation of digestate presented additional advantages, such as deodorization and decreased viscosity; however, foaming was observed.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Oxidative (H ₂ O ₂)	WD, agricultural	5, 10, and 30 g H ₂ O ₂ /kg TS, 2 h	Batch, 37 °C, 13 days, treated diges- tate/untreated digestate (w/w) of 1	$427.2 \pm 19.33~^{a}$	Between +8% and +12%	[36]
Oxidative (O ₃)	WD, agricultural	5, 10, and 30 g O ₃ /kg TS ¹	Batch, 37 °C, 13 days, treated diges- tate/untreated digestate (w/w) of 1	356.5 ± 43.52 ^a	Between —9% and +13%	[36]
Oxidative (O ₃)	WD, sludge-based	0.16 g O ₃ /g SS, 10–15 min	Continuous, 35 °C, 28 days HRT, 1.1 g COD/1/day, recycling ratio (WD/raw WAS) of 0–1	348 ± 9 ^b (no digestate recirculation)	Between +43% and +66%	[44]
Thermo-acidic (H ₃ PO4)	SFD, agricultural	0.04 g H ₃ PO ₄ /g TS, 160 °C, 15 min	Batch, 52 °C, 80 days	Not reported	+8%	[32]
Thermo-acidic (H ₂ SO ₄)	SFD, agricultural	0.021–0.07 g H ₂ SO ₄ /g TS, 230 °C, 15 min	Batch, 52 °C ¹	Not reported	+67% (0.021 g H ₂ SO ₄ /g TS, 155 °C) +43% (0.023 g H ₂ SO ₄ /g TS, 160 °C) +6% (0.023 g H ₂ SO ₄ /g TS, 180 °C)	[22]
Acidic (HNO ₂)	WD, sludge-based	0, 0.77, 1.54, 2.31, 3.08 and 3.85 mg N/L; pH of 5.5, 22 °C and 24 h	Batch, 37 °C, 4 days	37 (22 °C, 24 h, no HNO ₂)	+38%, +22%, +3%, -3% and -19% (at 0.77, 1.54, 2.31, 3.08 and 3.85 mg N/L)	[45]
Acidic (HCl)	WD, sludge-based	pH 2–6 (corrected with HCl), 25 °C, 1 h	Batch, 35 °C, 20 days	0.11 ^c	+54%, +63% and +9% (25 °C at pH 2, 4 and 6)	[8]
Thermo-acidic (HCl)	WD, sludge-based	pH 2–6 (corrected with HCl), 100 and 180 °C, 1 h	Batch, 35 °C, 20 days	0.11 ^c	Between +63% and 190%	[8]
Thermo-acidic (HCl and H ₂ SO ₄)	SFD, sludge-based	pH 5–6 (corrected with HCl and H ₂ SO ₄), 170 °C, 1 h	Continuous, 15.4–20 days HRT, recycling rate (SFD/raw sludge) of 30%	0.92–1.15 ^d (no digestate recirculation)	Between +14% and 21%	[8]
Alkaline (ammonia)	SFD, agricultural	3.2 g ammonia/g TS; 1–5 days, 22 and 55 °C	Batch, 37 °C, 35 days	≈77–110 ^e	Between -5% and $+80\%$	[46]
Alkaline (ammonia)	SFD, agricultural	0.5–3.2 g ammonia/g TS; 1–5 days, 22 °C	Batch, 37 °C, 35 days	64-81	Between +76% and 205%	[47]
Alkaline (CaO)	SFD, agricultural	6%, 8% and 10% CaO (w/w); 15 °C; 5–25 days	Batch, 52 °C, 80 days	Not reported	Between +15% and +66% (6% CaO) Between +19% and +57% (8% CaO) Between +18% and +50% (10% CaO)	[32]
Alkaline (NaOH)	WD, agricultural	10 g NaOH/kg TS, 40 °C, 24 h	Batch, 35 °C, 65 days	70 ± 2	-40%	[12]
Alkaline (NaOH)	SFD, agricultural	10 g NaOH/kg TS, 40 °C, 24 h	Batch, 35 °C, 65 days.	90 ± 1	-10%	[12]
Alkaline (NaOH)	WD, agricultural	20 to 60 g NaOH/kg VS, 35 °C, 65 h	Batch, 35 °C, 118 days	100 ± 6 (35 °C, 65 h, no NaOH)	Between -7% and -1%	[48]
Alkaline (NaOH)	SFD, agricultural	20 to 60 g NaOH/kg VS, 35 °C, 65 h	Batch, 35 °C, 118 days	301 ± 43 (35 °C, 65 h, no NaOH)	Between -10% and +13%	[48]

Table 3. Summary of studies about chemical post-treatment of digestate for additional biogas recovery.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Alkaline (NaOH)	SFD, agricultural	20 g NaOH/kg VS, 35 °C, 65 h	Continuous, $35 ^{\circ}$ C, $\approx 20 \text{days}$ HRT, 2.0–2.5 kg VS/m ³ /day, recycling ratio (SFD/raw manure and grass silage) of 10%	182 ± 20 (no SFD recirculation); 143 ± 30 (untreated SFD recirculation)	−11% ^f +13% g	[48]
Alkaline (NaOH)	SFD, agricultural	20 g NaOH/kg VS, 25 °C, 30 min	Continuous, 53 °C, 20 days HRT	≈ 80	+11%	[23]
Alkaline (NaOH)	SFD, agricultural	40 g NaOH /kg VS, 20 °C, 48 h	Batch, 35 °C, 30 and 340 days	$61 \pm 4.7~(30~{ m days})$ $179 \pm 12.2~(340~{ m days})$	+0% (30 days) —13% (340 days)	[18]
Alkaline (NaOH)	SFD, agricultural	40 g NaOH /kg VS, 20 °C, 48 h	Batch, 55 °C, 30 and 340 days	$82 \pm 1.8~(30~{ m days})$ $215 \pm 7.3~(340~{ m days})$	—5% (30 days) —20% (340 days)	[18]
Thermo-alkaline (NaOH)	SFD, agricultural	40 g NaOH /kg VS, 80 °C, 3 h	Batch, 35 °C, 30 and 340 days	$61 \pm 4.7~(30~{ m days})$ $179 \pm 12.2~(340~{ m days})$	+6% (30 days) —14% (340 days)	[18]
Thermo-alkaline (NaOH)	SFD, agricultural	40 g NaOH /kg VS, 80 °C, 3 h	Batch, 55 °C, 30 and 340 days	$82 \pm 1.8~(30~{ m days})$ $215 \pm 7.3~(340~{ m days})$	+7% (30 days) —16% (340 days)	[18]
Thermo-alkaline (NaOH)	SFD, agricultural	20 g NaOH/kg TS, 55 °C, 3 days	Batch, 35 °C ¹	129–150	Between 30% and +46%	[49]
Thermo-alkaline (NaOH)	SFD, agricultural	40 g NaOH/kg TS, 160 °C, 15 min	Batch, 52 °C, 80 days	Not reported	+26%	[32]
Thermo-alkaline (NaOH)	SFD, agricultural	20–60 g NaOH/kg TS, 55 °C, 24 h	Batch, 53 °C ¹	42	Between +48% and +300%	[35]
Thermo-alkaline (NaOH)	SFD, agricultural	20–60 g NaOH/kg TS, 90 and 121 °C, 20 min	Batch, 53 °C ¹	42	Between $\approx +114\%$ and $+320\%$	[35]
Thermo-alkaline (NaOH)	SFD, agricultural	40 g NaOH/kg TS, 121 °C and 60 g NaOH/kg TS, 55 °C	Continuous, 53 °C, 15 days HRT, 2.0–2.5 g VS/I/day, co-digestion of manure and SFD (0.7:0.3 ratio VS basis)	211 (manure) 143 (manure + untreated SFD)	+25% ^g (40 g NaOH/kg TS, 121 °C) +26% ^g (60 g NaOH/kg TS, 55 °C)	[35]
Thermo-alkaline (NaOH)	SFD, agricultural	10–30 g NaOH/kg TS, 100 °C, 6 h + 135 °C, 1 h	Batch, 37 °C, 50 days	62	Between +65% and 144%	[21]
Thermo-alkaline (NaOH)	SFD, agricultural	10–30 g NaOH/kg TS, 100 °C, 6 h + 135 °C, 1 h	Batch, 53 °C, 50 days	71	Between 89% and 180%	[21]
Thermo-alkaline (NaOH)	SFD, agricultural	10–30 g NaOH/kg VS, 180 °C, 30 min	Continuous, 53 °C, 20 days HRT	≈ 80	Between +86% and +127%	[23]
Alkaline (NaOH)	WD, sludge-based	pH correction with NaOH 1 mol/L to 8, 9 and 10, 25 °C, 24 h	Batch, 37 °C, 8 days	55 ± 0.3	Between +5% and +25%	[50]
Alkaline (NaOH)	WD, sludge-based	0.1 M NaOH, 30 min	Continuous, 35 °C, 20 days HRT, feeding with raw sludge and recycling ratio (volume of recycled digestate/volume of digester) of 5–15%	Not reported	+33% (recycling ratio of 5%) Lower than +33% for higher recycling ratios	[51]

Table 3. Cont.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Thermo-alkaline (NaOH)	WAS and WD (7 and 15 HRT) ^h	40 g NaOH/kg TS, 70 and 90 °C, 90 min	Batch, 35–38 °C, 21 days	166 ± 15 (WAS) 143 ± 3 (WD, 7 days HRT) 47 ± 1 (WD, 15 days HRT)	+40% and +66% (WAS, at 70 and 90 °C) +29% and +56% (WD, 7 days HRT, at 70 and 90 °C) +131% and +184% (WD, 15 days HRT, at 70 and 90 °C) +23% and +16% (inter-stage, 7 + 13 days with treatment at 70 and 90 °C) ^{i,k} \approx -26% and -38% (inter-stage, 15 + 5 days with treatment at 70 and 90 °C) ^{i,k}	[52]
Thermo-alkaline (NaOH)	WD, sludge-based	0.1 M NaOH, 175 °C, 1 h	Continuous, 35 °C, 30 days HRT, recycling rate (WD/raw WAS) of 30%	57% j	+24%	[53]
Thermo-alkaline (KOH)	WAS and WD ^h	170 °C, pH 10 (adjusted with KOH), 15 min	Batch, 37 °C, 40 days Pre: 40 days Inter-stage: 19 + 21 days	291	+2% (pre) +29% (inter-stage) ^k	[19]

Table 3. Cont.

^a In mL biogas/g VS. ^b In mL CH₄/g TCOD removed. ^c In g COD-CH₄/COD-substrate. ^d In g COD-CH₄/day. ^e In mL CH₄/g TS. ^f In comparison to no SFD recirculation. ^g In comparison to recirculation of untreated SFD. ^h Pre-treatment (of raw sludge) and inter-stage treatment (of digested sludge) strategies were compared. ⁱ Based on modeling, not determined experimentally. ^j Based on influent COD. ^k Considering the overall methane yields (accounting all AD steps). ¹ Unknown duration.

4.2. Acidic Post-Treatment

Acidic treatments usually include the use of HCl, H₂SO₄, or H₃PO₄ alone or in combination with thermal treatment. In lignocellulose, acidic treatment targets the hemicellulose fraction, decomposing it mainly into pentose sugars, which can be readily hydrolyzed during AD. Removal of hemicellulose also makes the cellulose more available for enzymatic attack. At certain conditions, small portions of cellulose can be removed as well as acid-soluble fractions of lignin [31]. Few studies have tested the application of acids as a post-treatment of digestate (Table 3) and they have shown that the degree of effectiveness seems to be highly dependent on treatment parameters (temperature, duration, acid species and concentration), with inhibitors often formed under inappropriate conditions.

Thermal–acidic treatments of digested manure fibers were conducted with H_3PO_4 (4% *w/w* TS, 160 °C, 15 min) [32] and H_2SO_4 (2.1–7.0% *w/w* TS, 155–180 °C, 15 min) [22]. H_3PO_4 increased the methane yield by 8% while H_2SO_4 increased it by 6–67%. With H_2SO_4 , the highest methane yield was obtained at the mildest conditions while inhibition was observed at the most severe ones.

In sludge, the mechanism behind organic matter disintegration through application of acids seems to be linked to the solubilization of organic-binding metals (OBM) from carbohydrates and proteins by destroying their bridging and hydrogen-bonding interactions. Metals influence the stability of sludge flocs as they bridge EPS in the floc matrix. Besides increasing the energy barrier for sludge solubilization, OBM can also take up the binding sites of sludge particulates for enzymes, making the hydrolysis even more difficult. Usually, the higher the OBM content, the lower the methane yield [54]. Therefore, the solubilization of OBM by acids can support sludge flocs destabilization and enhance methane production.

On sludge-based digestate, studies were conducted with the application of HCl, H_2SO_4 , and HNO_2 . Takashima and Tanaka (2014) [8] examined the effects of acidic and thermo-acidic treatment with HCl (25–180 °C, pH 2–6, 1 h). The pure acidic treatment (at 25 °C) led to an increase in methane yield of 9–63%, with the highest yield obtained at pH 4. The combination of thermal and acidic treatment increased the methane yield by 63–190%, with the optimal conditions found at 180 °C and pH adjusted to 6. At similar

treatment conditions (170 °C, pH 5–6, 1 h), the recirculation of SFD treated with HCl or H_2SO_4 to a continuous digester being fed with untreated sludge could increase the methane production by a lower degree (14–20%). The authors hypothesized that the accumulation over time of non-biodegradable compounds could explain the difference of results obtained in batch and continuous AD tests. The presence of non-biodegradable compounds was assessed by the increase in color intensity of digestate, likely linked to the occurrence of Maillard reactions, common at high temperatures.

The treatment of sludge-based digestate with HNO₂ relies on a different mechanism than solubilization of OBM. HNO₂ and its derivatives (NO, N₂O₃, and NO₂) are biocidal agents hypothesized to damage lipids, proteins carbohydrates and DNA in cells and extracellular polymeric substances by reacting with them [55] and releasing organic matter that can be hydrolyzed during AD. Zhang et al. (2016) [45] conducted a 4-day AD batch test, where it was observed that methane production could increase by 38 and 22% when digestate was treated with HNO₂ at concentrations of 0.77 and 1.54 mg N/l, respectively. Higher HNO₂ concentrations, however, led to reduced methane yields.

4.3. Alkaline Post-Treatment

Alkaline treatment has been shown to increase methane yields (Table 3); however, treatment efficacy depends on alkali species, operational parameters, and digestate characteristics. Studies involving treatment of lignocellulosic digestate have included the application of ammonia, CaO, and NaOH while NaOH and KOH have been tested in alkaline post-treatments of sludge-based digestate. In both types of digestate, NaOH has been the most explored agent and has proven to be efficient, in most cases, only when combined with a thermal treatment.

In lignocellulosic biomass, the intermolecular ester bonds, which cross-link xylan hemicelluloses with lignin, are damaged during alkaline treatment resulting in delignification, which can make cellulose and hemicellulose more easily accessible for microbial hydrolysis [31]. Furthermore, cellulose can be swollen, leading to a decrease on crystallinity [3], which can enhance its digestion.

By applying ammonia soaking (3.2 g/g TS, 1–5 days, 22 and 55 °C), Jurado et al. (2013) [46] observed between a 5% reduction and an 80% increase in methane yields when treating digested manure fibers. The optimal treatment conditions were 22 °C and 3 days. The same optimal duration was confirmed by Mirtsou-Xanthopoulou et al. (2014) [47], who obtained increases in methane yields of up to 205% when treating digested manure fibers at 22 °C. The authors also observed that an ammonia concentration as low as 0.5 g/g TS was sufficient for the treatment and that higher concentrations did not lead to significant improvements on methane yields.

The treatment of digested manure fiber with CaO (6–10% w/w TS, 15 °C, 5–25 days) was shown to increase methane yields by 15–66% [32]. When using 6% and 8% CaO, 10 days treatment were found to increase the methane yield by approximately 60%. Longer treatment had limited additional effect while a 10% CaO concentration lead to lower improvements on digestate methane yield.

Reduced yields as a consequence of treatment with NaOH have also been shown in other studies. Treatments with NaOH (12–60 g/kg VS, 24–65 h) resulted in reduced methane yields for both WD [12,50] and SFD [12,18]. Jagadabhi et al. (2008) [48] reported an increase in methane yield of 13%, when treating SFD with 20 g NaOH/kg VS for 65 h, while higher alkali doses affected the methane yields negatively. The authors furthermore extended the experiment to a continuous AD system, with recirculation of untreated and alkaline treated SFD. In both cases, methane yields decreased in comparison to the system without recirculation. However, when compared to the system recirculating untreated SFD, the recirculation of alkaline treated SFD resulted in 13% higher methane production, in agreement with the batch test result. In continuous AD, Khan and Ahring (2021) [23] obtained a similar methane yield increase (11%) when comparing the digestion of treated

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and untreated SFD. The treatment was conducted with the same alkali concentration (20 g NaOH/kg VS) but for a shorter period (30 min).

The combination of NaOH and thermal treatment has been investigated at several conditions, with varying degrees of success. Treatment at moderate temperature (40 g NaOH/kg VS, 80 °C, 3 h) had limited effect on the methane yield of SFD, resulting in a 7% increase in a study conducted by Kaparaju and Rintala (2005) [18]. Conversely, Brémond et al. (2021) [49], obtained 30-46% increase in methane yields of SFD samples when treating them with 20 g NaOH/kg TS at 55 °C for 3 days. Tsapekos et al. (2016) [35] observed a 48–300% increase in methane yield in response to increased concentrations of NaOH when treating SFD with 20–60 g NaOH/kg TS (55 $^{\circ}$ C, 24 h). At higher temperatures (90 and 121 °C, 30 min), the authors reported that the methane yields increased by 114–320%. Interestingly, the treatments with the lowest concentrations of NaOH were those most affected by increasing the temperature from 90 to 121 °C. In a continuous digester fed with manure and treated SFD, however, methane yields were limited to a 26% increase when compared to the yields when co-digesting manure and untreated SFD. When compared to the digestion of manure alone, methane yields were lower [35]. At alkali concentrations of 10–30 g NaOH/kg TS (6 h at 100 °C followed by 1 h at 135 °C), Khan and Ahring (2020) [21] observed methane yields increments between 65 and 188% in batch tests. In continuous AD, treatments with the same alkali concentration, but at higher temperature (180 $^{\circ}$ C, 30 min), lead to methane yields increase of 86–127% [23]. With 40 g NaOH/kg TS (160 $^{\circ}$ C, 15 min), Bruni et al. (2010) [32] obtained an increase of 26% in methane yield in batch AD.

Alkali treatment on sludge causes the dissolution or destruction of floc structures and cell walls, making organic matter more available for enzymatic hydrolysis. In flocs, EPS and gels can be destructed because high pH causes proteins to lose their natural shapes, causes saponification of lipids, and hydrolysis of RNA. After the disruption of flocs, microbial cells are exposed to an environment with high pH, and cannot keep the appropriate turgor pressure, resulting in cell disruption and release of their intracellular material to the bulk liquid [56].

The treatment of sludge-based digestate with KOH (170 °C, 15 min, pH 10 adjusted with KOH) was evaluated by Nielsen et al. (2011) [19]. An inter-stage configuration with thermo-alkaline treatment between two digesters improved the overall methane yield of WAS by 28% while a pre-treatment strategy followed by AD had a minimal impact. Results indicated that the inter-stage strategy was advantageous over a pre-treatment configuration; however, the addition of KOH seemed unnecessary as a similar increase in methane yield was obtained with the thermal treatment alone (Table 1).

The effects of NaOH post-treatment of sludge-based digestate have been in general positive. Song et al. (2019) [50] obtained a 25% increase in methane yield of digestate when adjusting its pH to 10 with NaOH and treating it for 24 h. The combination of alkaline and thermal treatment (40 g NaOH/kg TS, 70 and 90 °C, 90 min) resulted in methane yield increments of 29–56% and 131–184% for digestate samples originated from digesters operating at 7 and 15 days HRT, respectively, with the highest yields obtained at 90 °C treatment [52]. The same treatment conditions applied to undigested sludge improved its methane yield by 40-66%. Based on these results, the authors estimated and compared the overall methane yields that would have been obtained in a system with thermo-alkaline pre-treatment of WAS (followed by 20 days AD) and in a system with inter-stage treatment (7 + 13 days AD or 15 + 5 days AD). At both treatment temperatures, the inter-stage treatment after a first digestion period of 7 days was estimated to yield 16–23% more methane than the pre-treatment strategy. When the inter-stage treatment would be applied after 15 days digestion, however, the overall methane yields at both treatment temperatures were estimated to be lower, underlying the fact that careful testing of biomasses and conditions are necessary to evaluate whether an inter-stage treatment can be advantageous over a pre-treatment.

Alkaline treatment of sludge-based digestate with NaOH was also evaluated in continuous AD systems receiving raw WAS and recirculated digestate. Li et al. (2013) [51] observed that alkaline treatment (0.1 mol/L NaOH, 30 min) could increase the methane production by 33% at a recycling ratio of treated digestate of 5%. Recycling ratios of 10% and 15% also increased the biogas production but at a lower extent. Takashima et al. (1996) [53] obtained 24% higher methane production when recirculating thermal-treated digestate (0.1 mol/L NaOH, 175 °C, 1 h) at a recycling ratio of 30%. Despite the positive result, the methane recovery was lower than the one expected based on the increase of the measured particulate material decomposition. Both studies indicate that some refractory compounds or inhibitors might have been formed during the post-treatment.

4.4. Chemical Post-Treatment—Discussion and Perspectives

Very few studies have involved the treatment of digestate with oxidants and acids, making it difficult to draw solid conclusions about their efficiencies. Based on the reported studies, oxidative treatments have had significant positive impacts on sludge-based digestate only. Acidic treatments have proven to be able to improve degradability of both agricultural and sludge-based digestates under certain conditions of acid concentration and temperature. Some acidic treatments have been applied in combination with thermal treatment and it is difficult to determine whether the addition of an acid has had a positive impact beyond that which would have been obtained with a thermal treatment alone. Alkaline treatments, especially with NaOH, have been more widely explored. In general, when applied alone, NaOH has had a negative or very limited positive impact on improving methane yields while they have been efficient when combined with a thermal treatment. Alkaline treatments with CaO and ammonia have been shown to enhance digestibility of agricultural digestate while KOH has not affected sludge-based digestate, however, the limited number of studies does not allow solid conclusions.

As mentioned, chemical treatment efficiency depends on treatment conditions (chemical species, concentration, temperature, and duration). In some studies [8,22,32,45,48], it was observed that the methane yield of treated material could increase with chemical concentration until a certain point, but decreased at higher chemical concentration, being sometimes even lower than the untreated control. This trend might be linked to the addition or formation of inhibitors to the AD system.

The chemical species applied on the treatment might be direct inhibitors of the AD, when in high concentrations. This is the case of ammonia, sulfate (SO_4^{2-}) , and certain ions, as reviewed by Chen et al. (2008) [57]. Ammonia, in its free form, can diffuse microbial cell membranes, alter the intracellular pH, and cause proton imbalance. Sulfate can primarily inhibit AD by stimulating the activity of sulfate-reducing bacteria, which compete with methanogens for organic substrates. Secondary inhibition results from the formation of sulfide, which is toxic to several bacterial groups [57]. The addition of ions, especially cations, can cause bacterial cells to dehydrate due to osmotic pressure. Among cations, sodium (Na⁺) has particularly important inhibitory potential [57]. Some of the reported studies has showed that alkaline treatment with NaOH failed completely or caused a certain degree of inhibition. The reasons behind this are not yet understood, since authors claimed that Na^+ concentration in the AD system should have been below inhibitory limits (3.5 g/L for moderate inhibition and 8 g/L for strong inhibition, accordingly to Chen et al. (2008) [57]). Acidic treatment with HNO₂ at high concentrations also caused some inhibition; and the reasons are not clear either. HNO₂ could have a biocidal effect on the microbes inside the anaerobic digester; however, the authors [45] claimed that, at the tested concentrations, it would be significantly diluted and rapidly removed by denitrification when entering the digester. Another possibility could be that denitrifiers in the digester compete with methanogens for carbon sources [58]. Inhibitors might also be formed during the chemical treatment. Special attention has been given to inhibitors formed during acidic treatments or at high temperatures, such as furfural and hydroxymethylfurfural [7].

Due to the possible presence of inhibitors, chemical treatments must be carefully examined to operate at appropriate conditions. Concentration of inhibitors in the digester can be reduced by mixing the treated material with other streams, implementing a washing step (when the treated material is mostly solid), adding an absorbent, among others [57]. In the case of high concentrations of ammonia, a stripping step could be employed. Alkaline treatments at high pH can alter the NH_4^+/NH_3 equilibrium, favoring the free ammonia form, which might be stripped out of the system. This might be seen as an advantage when treating digestates with high ammonia concentration, minimizing inhibition risks during AD. However, it is important that a system to recover ammonia exists to avoid emissions to the atmosphere.

In most studies in which chemical treatments have been applied, only batch studies have been performed. Although some positive results have been obtained, it would be important to extended tests to continuous AD systems for a clearer evaluation of the impact of the applied treatments. Since many chemical treatments include inhibition risks, longterm operation of continuous digesters could show how microbial communities would deal with possible accumulation of inhibitors and increasingly ionic load and if they would have the capability to adapt to them.

Economic analysis would also be important. Pure chemical treatments have the advantage of being less energy demanding than thermal and mechanical treatments. However, chemicals must be purchased, representing additional costs. In the case of treatments with ammonia, it could be obtained on site; however, processes to generate/capture it are not cost-free. Harsh treatment conditions, such as elevated or low pH, might require suitable reactors to conduct the treatment and pH neutralization of the biomass prior to AD might be necessary, increasing the treatment capital and running costs [43]. It would also be important to evaluate how the addition of chemicals would affect the application of the final digestate and if the economy would be impacted. For instance, when digestates are employed as fertilizers, certain chemicals could impair fertilizer quality, while others, such as KOH, could have a positive value as fertilizer for farmers, who could be willing to pay more for the enriched digestate and thereby help to cover some of the costs of the treatment.

Studies evaluating whether the chemical treatment of digestate (with further recirculation or the inter-stage approach) could be advantageous over a pre-treatment strategy have not been much explored, and are evidently recommended. The reduction of solids during AD could imply that lower doses of chemicals would be required to treat digestate rather than raw substrate. However, the usual higher buffer capacity of digestate makes this reasoning less simple. The buffer capacity could affect the treatment efficacy [59] and higher buffer capacities would demand higher doses of acids or alkalis to achieve a certain pH.

5. Biological Post-Treatment

5.1. Enzymatic Post-Treatment

During enzymatic post-treatment, different enzymes are added to accelerate the process of hydrolysis and expose different parts of the complex polymers to microbial degradation. For depolymerization of lignocellulose, several enzymes are required. Cellulases include endoglucanases, which cleave internal bonds of cellulose, exoglucanases, which act on external regions of cellulose, and β -glucosidases, which hydrolyze soluble oligosaccharides to glucose [60]. Hemicellulases include different enzymes, such as endo-1,4-β-xylanases, which cleave the xylan backbone, and β-D-xylosidases, which cleave xylose monomers from the non-reducing ends of xylooligosaccharides and xylobiose. As hemicellulose is a branched polymer, various other enzymes are required to remove the side groups from the xylan backbone, such as α -L-arabinofuranosidases, α -D-glucuronidases, acetylxylan esterases, ferulic acid esterases, and p-coumaric acid esterases [61]. Ligninolytic enzymes are oxidative enzymes mainly composed of peroxidases and laccases, which require H_2O_2 and O_2 to act, respectively. Lignin peroxidases oxidizes non-phenolic aromatic compounds while Manganese peroxidases and laccases target the oxidation of phenolic compounds [62,63]. On sludge, the main enzymes required to disintegrate its structure are proteases, lipases, and glycosidic enzymes. Proteases break peptide bonds of

proteins, lipases catalyze the breakdown of fats into fatty acids, and glycosidic enzymes are used for the degradation of different carbohydrates present in the sludge [64].

Studies involving enzymatic post-treatments are scarce and have been applied solely to agricultural digestate (Table 4). Sambusiti et al. (2015) [12] treated WD and SFD with an enzyme cocktail containing cellulases and hemicellulases. The methane yield of the WD increased by 51% with the addition of enzymes, while this increase was only 13% when SFD was treated, although the contents of hemicellulose and cellulose of the SFD were higher. The authors suggested that the results might be related to higher concentrations of certain soluble compounds in WD, which could shield protein-compatible sites of lignin and, in this way, prevent that cellulases and hemicellulases unproductively bind to them. To date, there appears to be no consensus on whether cellulases, for instance, binding to lignin, is a reversible or irreversible process [65,66]. However, even in the case of a reversible binding to lignin, irreversible structural changes on the enzyme could occur, affecting the subsequent adsorption to the active site to which it is designed to attach [66]. Minimizing irreversible binding or deactivation of enzymes by lignin requires a better understanding of the underlying mechanisms and it might be particularly important on the post-treatment of digestate, since its lignin content should be higher than in undigested biomass. Currently, investigations aiming to reduce enzymes binding to lignin include the addition of external agents, such as non-catalytic proteins like bovine serum albumin to shield unspecific binding [67], or the implementation of treatments to reduce lignin content, or to modify its surface area [65,68,69].

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH ₄ /g VS)	Methane Yield Increase Untreated/Treated	Reference
Enzymatic (xylanases and glucanases)	WD, agricultural	0.15 mL/g TS, pH 5, 40 °C, 24 h	Batch, 35 °C, 65 days	70 ± 2	+51%	[12]
Enzymatic (xylanases and glucanases)	SFD, agricultural	0.15 mL/g TS, pH 5, 40 °C, 24 h	Batch, 35 °C, 65 days	90 ± 1	+13%	[12]
Enzymatic (laccases and cellulases)	SFD, agricultural	0.5–2 U/g TS (cellulases), 0.5–84 U/g TS (laccases), pH 4–7, 37 °C, 20 h, with O_2 supply	Batch, 52 °C ^h	Not reported	No effect	[32]
Thermo-acidic (H ₃ PO ₄) + enzymatic (laccases)	SFD, agricultural	H ₃ PO ₄ at 4% (<i>w/w</i> TS), 160 °C, 15 min, 48–59 U/g TS, pH 5.5, 37 °C, 20 h	Batch, 52 °C ^h	Not reported	+18%	[32]
Thermo-alkaline (NaOH) + enzymatic (laccases)	SFD, agricultural	NaOH at 4% (<i>w/w</i> TS), 160 °C, 15 min, 48–59 U/g TS, pH 5.5, 37 °C, 20 h	Batch, 52 °C ^h	Not reported	+34%	[32]
Aeration	SFD, agricultural	1.5–33 l air/h/kg TS, 1.75–6 days	Batch, 35 °C ^h	153–171	Between -21% and $+0\%$	[49]
Fungal SSF (Phanerochaete flavido-alba)	SFD, agricultural	Sterilization by autoclaving, 30 °C, 60% moisture, 21 days	Batch, 52 °C, 20 days	≈76 ^{c,d} (digested grass) ≈166 ^{c,d} (digested corn stover) ≈129 ^{c,d} (digested wheat straw)	-10% (digested grass) -2% (digested corn stover) +8% (digested wheat straw)	[70]
Fungal SSF (Pleurotus ostreatus)	SFD, agricultural	Sterilization by autoclaving with 2% CaO (<i>w/w</i>), 20–25 °C, 75% moisture, 5.5–21 days	Batch, 35 °C ^h	232 ± 12 e	Between15% and 51%	[13]
Fungal SSF (Stropharia rugosoannulata)	SFD, agricultural	Sterilization by autoclaving with 2% CaO (<i>w/w</i>), 20–25 °C, 75% moisture, 5.5–21 days	Batch, 35 °C ^h	167 ± 3 °	Between -17% and +1%	[13]

Table 4. Summary of studies about biological post-treatment of digestate for additional biogas recovery.

Treatment	Digestate Characteristics	Treatment Conditions	Post-AD Conditions	Methane Yield Untreated (mL CH4/g VS)	Methane Yield Increase Untreated/Treated	Reference
Fungal SSF (Cephalotrichum stemonitis)	SFD, agricultural	No sterilization, 25 °C, 67% moisture, 10 days	Batch, 40 °C, 50 days	$22\pm3~^{\mathrm{f,g}}$	+72%	[71]
Fungal SSF (Coprinopsis cinerea)	SFD, agricultural	No sterilization, 25 °C, 70–75% moisture, 10–20 days	Batch, 40 °C, 75 days	44 ^f g	+120% and 143% (10 and 20 days)	[72]
Fungal SSF (Cyclocybe aegerita)	SFD, agricultural	No sterilization, 25 °C, 70–75% moisture, 10–20 days	Batch, 40 °C, 75 days	44 ^f g	+136% and +102% (10 and 20 days)	[72]
Fungal SSF (Cephalotrichum stemonitis)	SFD, agricultural	No sterilization, 25 °C, 70–75% moisture, 10–20 days	Batch, 40 °C, 75 days	44 ^f g	+166% and +214% (10 and 20 days)	[72]

Table 4. Cont.

^c In mL biogas /g TS. ^d Yield for the autoclaved digestate and culture media (no fungal biomass). ^e Yield for the autoclaved digestate and fungal media. ^f In mL CH₄/g, ^g Yield not accounting fungal biomass and culture media. ^h Unknown duration.

One approach with the objective of modifying lignin is the application of laccases. The mere application of laccases is not supposed to cause significant depolymerization of lignin but it could enhance the action of other enzymes by minimizing their unproductive binding to lignin. Laccases can modify the surface area of lignin: it can increase the amount of carboxylic groups on surface, lowering hydrophobicity and increasing the negative surface charge, which repulses cellulases [73]. In a study conducted by Bruni et al. (2010) [32], laccases were applied to digested manure fibers, alone and together with cellulases. However, it is not clear whether all the enzymes were applied concomitantly or if laccases were applied before the addition of cellulases, as would be recommended. Nevertheless, no effects were observed on methane yields due to the enzymatic treatment alone. The treatment with laccases was furthermore combined with steam treatment and H₃PO₄ or NaOH. Steam treatment alone with H₃PO₄ and NaOH resulted in 8% and 26% increase in methane yield, respectively. When laccases were added, the increases in methane yield were higher, 18% and 34%. The results show that the enzymatic treatment alone had no effect; however, enzymes improved the methane yield of steam treated fibers. Steam treatment may have contributed to ease the tight association of lignocellulose, making it more accessible for enzymatic attack. As previously seen, steam treatment can increase biomass biodegradability mainly by hemicellulose solubilization. However, it can also promote unproductive binding of enzymes to lignin, hindering hydrolysis in the digester [73]. Laccase addition to steam treated digestate might have alleviated unproductive binding, thus, enhancing methane production. The combination of enzymes with other treatment methods could in this way have complementary effects, leading to treatments that are more efficient and to the enhanced fulfillment of the potential of enzymes.

5.2. Microbial Aerobic Post-Treatment

In aerobic treatments, facultative anaerobic and aerobic microorganisms degrade organic matter in the presence of oxygen, which is used as a final electron acceptor. Aerobic treatments can include simple aeration or aeration in the presence of an added specific microbial culture or consortia [74]. Simple aeration makes use of hydrolytic activities of endogenous aerobic or facultative anaerobic communities in digestate. In the case of agricultural digestate, the main purpose of the treatment is that the presence of oxygen can favor the ligninolytic activities of endogenous fungi and bacteria [74]. The decomposition of lignin could make cellulose and hemicellulose more accessible to hydrolytic enzymes during subsequent AD, possibly increasing methane yields. However, during aerobic treatment, other fractions of digestible organic matter might be mineralized into CO_2 by microbial metabolism [74]. This was observed by Brémond et al. (2021) [49] when applying

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short aeration treatment (1.75–6 days) to agricultural SFD, which caused a reduction in methane yields.

In order to increase the treatment specificity towards a certain fraction of biomass, microbial cultures can be applied. For this purpose, the use of white-rot fungi (WRF) has been tested in the treatment of lignocellulosic biomass. WRF are known for their ability to selectively decompose lignin. The hyphae of WRF secrete ligninolytic enzymes, which catalyze oxidative reactions during lignin depolymerization [75]. During hyphal expansion and penetration, the lignocellulose structure can furthermore be physically disrupted and pores formed, which increase the surface area of the structures and hereby facilitates enzymatic attack [75]. Digestate potentially has a higher concentration of lignin compared to that of most biomasses since the lignin fraction remains mostly unaltered while other fractions of biomass are degraded during AD. Therefore, the employment of fungi in post-treatment of digestate could be an alternative to improve its degradability. Few studies have applied fungal post-treatment to digestate and they are summarized on Table 4.

López et al. (2013) [70] applied fungal treatment for 21 days to agricultural SFD with Phanerochaete flavido-alba. The authors observed that fungal growth was much slower on digestate than on undigested substrates. Degradation of cellulose and lignin during treatment was low while between 30 and 60% of hemicellulose was degraded. Methane yields during AD did not show any improvement. Brémond et al. (2020) [13] showed that treatments for 5.5–21 days with Pleurotus ostreatus and Stropharia rugosoannulata were not successful either on increasing methane yields of SFD. Apparently, microbial activity of endogenous microorganisms initially present on digestate could not be completely suppressed, even after a sterilization step prior to fungal inoculation. This was indicated by reduced VS content in a digestate sample not inoculated with fungi, which was likely caused by organic matter loss as CO_2 due to microbial respiration. In addition to causing organic matter loss, the presence of endogenous microorganisms can affect the WRF growth, as they compete with them for nutrients [75]. The use of fungi also cause organic matter loss through fungal respiration. For positive effects on methane yields to occur, lignin should be the main fraction consumed and the possible enhancement on biodegradability caused by delignification should offset any VS losses caused by the consumption of digestible fractions, such as cellulose and hemicellulose. Treatments with both *P. ostreatus* and *S.* rugosoannulata in the study of Brémond et al. (2020) [13] lead to further VS losses and reduction of methane yields.

Interestingly, Zanellati et al. (2020 and 2021) [71,72] reported successful fungal treatments over 10–20 days even when employing non-sterilized SFD. Methane yields increments ranged between 72% and 214% when treating SFD with *Cephalotrichum stemonitis*, *Coprinopsis cinerea*, and *Cyclocybe aegerita*. However, these increments were over-estimated since the methane yields from the fungal biomass and culture media were not discounted, and changes on VS content and lignocellulosic composition of SFD were limited. In all of the above studies, it would have been interesting to analyze whether ligninolytic enzymes were indeed produced, and not only analyze the methane yield, to estimate the actual effect of the applied fungi.

The reason behind the varying outcomes from the mentioned studies could rely on digestate composition, mainly low C/N ratios and high pH. Low C/N ratios negatively affect ligninolytic enzymes excretion, which occurs during the secondary metabolism of WRF under nutrients starvation, usually at nitrogen limitation [75]. High pH could be detrimental for fungal growth and enzymatic activities [72]. In the study conducted by Brémond et al. (2020) [13], for instance, pH and C/N ratio were not optimal and might have negatively influenced the fungal growth and secondary metabolism. The pH of the digestate after sterilization was 11.4, while the optimal reported pH for *P. ostreatus* and *S. rugosoannulata* are 6.5–8.7 and 6.5–7.5, respectively [13]. In the studies by Zanellati et al. (2020 and 2021) [71,72], however, the pH of SFD was lower, between 8.4 and 9.4 and a slight acidification was observed after treatment. The C/N of the SFD

studied by Brémond et al. (2020) [13] was 23.6, which might not have been high enough to promote enzymes excretion. For instance, the optimal C/N ratio for *S. rugosoannulata* is 50–100 [13]. The C/N ratios from the studies by Zanellati et al. (2020 and 2021) [71,72] were not reported, but an ammonia stripping step prior to fungal inoculation was performed, which should have lowered N levels, allowing the C/N ratios to approach optimal ranges. The potential of fungi as a post-treatment method is therefore still not clear, and will require future tests at conditions optimal to fungal growth.

5.3. Biological Post-Treatment—Discussion and Perspectives

Biological treatments have the advantage of requiring low investment costs and are considered environmental-friendly due to the low/none demand for chemicals and low energy inputs [76]. In comparison with treatments applying microorganisms, enzymatic treatments have the advantage of taking action immediately, while microbial processes might require long acclimation times [77]. However, enzymatic treatments are known for being cost-intensive. In this sense, the employment of microorganisms could be a cheaper option, also allowing desired enzymes to be continuously produced by them. However, microorganisms have more specific environmental requirements, tolerating a narrower range of conditions than enzymes.

Studies involving biological treatments on digestate are scarce. Enzymatic treatments with cellulases, hemicelluloses and laccases have been proved efficient at enhancing agricultural SFD degradability, however, the limited number of studies do not allow solid conclusions. Fungal treatments have failed in some cases and succeeded in others, indicating that their efficacies might be linked to digestate characteristics. Barriers to the full-scale implementation of biological treatments, however, exist, even when applied to raw substrates. Therefore, many research opportunities remain. Regarding enzymatic treatments, possible investigations could include the application of various enzymes cocktails and operational conditions (dosing, temperature, pH), and the determination of the optimal enzymes addition configuration (as a separated treatment step or added directly into the digester). As discussed, unproductive binding of enzymes to lignin might be an important process on reducing the efficacy of the enzymes in the digestate. Better understanding of such binding and methods to minimize it could contribute to the optimization of enzymatic treatment methods.

With respect to fungal treatment, various fungal strains and operational conditions (temperature, humidity, and duration) at digestate samples from different origins could be tested. As mentioned, a sterilization step prior to fungal inoculation, where native microbes are inactivated, is normally required. Decontamination would make full-scale processes challenging and research on low-cost and high-efficient approaches to sterilize biomass is still needed [75]. Another approach would be screening fungal strains that can thrive in unsterilized biomass. The usual high pH and low C/N ratios of digestate can also represent an obstacle for selective delignification to occur. Alternatives to overcome this problem could include a prior ammonia-stripping step, or mixing digestate with other substrates to increase C/N ratios and decrease pH. Santi et al. (2015) [78] observed faster growth rates of some fungal strains when using a mixture of corn silage digestate and wheat straw in comparison to the utilization of corn silage digestate or wheat straw alone. Isikhuemhen et al. (2009) [79] obtained enhanced productivity of *Agrocybe aegerita* mushroom when supplementing wheat straw with up to 50% digestate. These studies aimed for the production of edible fungi; therefore, no AD tests were conducted.

6. General Discussion and Perspectives

Post-treatment of digestate is an emerging strategy to maximize biomass utilization and biogas generation, enabling a more sustainable operation of biogas plants and potentially also benefiting their economy. The objective of post-treatment is to improve the degradation of recalcitrant biomasses not readily degraded during AD, which could be advantageous over the traditional pre-treatments, in which chemicals and energy are applied to both easy and hardly degradable biomasses. Biological post-treatments on digestate, such as simple aeration, fungal solid-state fermentation (SSF), and addition of enzymes have not been studied to a great extent, and are still not used commercially. Mechanical treatments, such as comminution and ultrasonication, have been explored more widely, and are, in some cases, efficient at enhancing degradability of digestate. However, energy recovered as methane is often low compared to the energy input. Thermal and chemical post-treatments have been the most widely studied technologies and both methods have proven to be, in some cases, efficient at enhancing degradability of agricultural and sludge-based digestates. The success of these treatments, however, depends on treatment conditions. High temperatures, lengthy treatments, or high doses of chemicals are frequently linked to some degree of inhibition of AD. Digestate characteristics also influence the treatment efficacy. Digestate composition depends on the biomasses fed to the digester, inoculum source, AD operational conditions, and configuration [80]. Unfortunately, in most studies, digestates have not had their composition determined and have been characterized only in terms of basic parameters, such as TS/VS contents, pH, ammonia, and VFA concentrations. Assessing digestates composition in more detail like the content of their polymers (cellulose, hemicellulose, lignin, proteins, and lipids) could identify the sources of variation in the results obtained, and guide the choice of the most appropriate treatment methods to support a more stringent comparison of methods and conditions.

As reported, AD have been conducted only in batch tests in most studies on posttreatment. Operating digesters in continuous mode could allow for a clearer evaluation of the post-treatment methods. It would be important to verify if the digester being fed with treated digestate would be able to operate under stable conditions and deliver a satisfactory methane production in long-term, especially if inhibitors potentially form during treatment. Some authors have shown that an inter-stage treatment configuration (between two digesters) could be advantageous over a pre-treatment strategy in terms of overall methane yields obtained [19,28]. More studies alike are recommended to evaluate whether this approach could be generally applied or are restricted to some digestate types and treatment methods.

Energy balances and economic analyses are also limited in the literature, making it difficult to evaluate the real impact that digestate post-treatment could have on biogas plants. A thorough economic analysis was conducted by Brémond et al. [49] in a theoretical study which could serve as a basis for future works. The authors considered different strategies within digestate recirculation: (1) simple addition of SFD to the existing feedstock ration and (2) partial replacement of feedstock by SFD. Different ratios of the produced SFD to be recirculated at different biogas plants were considered. The impacts of direct SFD recirculation were estimated to increase the production of biogas between 0.6% and 5.7%. The partial replacement of feedstock by SFD was estimated to allow for a saving of 64 to 1431 tons/year of feedstock, while maintaining the biogas production constant. None of the strategies combined with an alkaline post-treatment with NaOH prior to SFD recirculation proved economic viable, based on the results obtained experimentally (lab batch AD) by the same authors. However, these outcomes cannot be extrapolated to all cases. As observed in this review, the effects of post-treatments on methane yields varied widely, depending on treatment methods, conditions, and digestate characteristics. Additionally, treatment expenditures and savings with feedstock costs can vary from country to country, therefore, case-to-case analysis are recommended.

There is also a knowledge gap on the effects of digestate post-treatment on the final digestate characteristics and how digestate handling and utilization would be impacted. For digestates normally employed as fertilizers, a re-digestion might lead to further degradation of proteins, increasing ammonium concentration and, therefore, improving its fertilizer quality. While a reduced residual methane yield in digestate could reduce methane emissions during digestate storage and land application, a higher ammonium content could result in increased ammonia and N_2O emissions. In the case of chemical treatments, the impacts the added chemical species on fertilizer quality and value should also be taken into consideration. It is also not clear how re-digestion of digestate affects the final digestate dewaterability. An improved dewaterability could reduce the transport costs of SFD and, in the case of sludge-based digestate, the costs associated with incineration. Takashima and Tanaka [8] showed that thermal–acidic treatment improved dewaterability of sludge-based digestate, while Battimelli et al. (2003) [44] demonstrated that ozone post-treatment lead to poorer filterability at high ozone concentrations. Thermal treatment did not alter digestate dewaterability of sludge-based digestate in the study conducted by Yuan et al. (2019) [25], while it required a higher polymer dose in the study authored by Shana (2016) [28].

7. Conclusions

Post-treatment of digestate could be a complementary approach or an alternative to traditional pre-treatment to enhance biogas plant methane outputs. Additional methane recovery from the same substrate could benefit biogas plant operating economies, and reduce methane emissions during digestate storage and handling, favoring a more sustainable operation. This review covered the post-treatment technologies applied to agricultural and sludge-based digestate. Studies involving biological treatments of digestate, such as short aeration, fungal treatment, and application of enzymes, are still scarce in the literature. Mechanical treatments, such as comminution and ultrasonication, even though effective in some cases at increasing methane yields of digestate, seem to be too energy intensive, so that the additional methane produced is not high enough to cover the treatment energy inputs. Thermal, chemical (oxidative, alkaline, and acidic), and thermo-chemical treatments proved to be efficient at increasing methane yields of digestate; however, their efficacy depends on treatment parameters and digestate characteristics, and have shown, at certain conditions, to cause downstream inhibition in the following AD step. The majority of studies have been applied in AD batch tests only, so that extending tests to continuous systems is recommended, to evaluate the effects in real systems as well as more comparisons between post-treatment of digestate and pre-treatment of undigested substrates. Despite the fact that some very promising results have been presented, in terms of effects on methane yields, the economic feasibility of the process should be examined carefully.

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Abbreviations

- AD anaerobic digestion
- BMP biochemical methane potential
- COD chemical oxygen demand
- C/N carbon to nitrogen ratio
- CSTR continuous stirred tank reactor
- EPS extracellular polymeric substances
- GHG greenhouse gases
- HRT hydraulic retention time
- OBM organic-binding metals

OLR	organic loading rate
SFD	solid fraction of digestate
SS	suspended solids
SSF	solid state fermentation
TS	total solids
VFA	volatile fatty acid
VS	volatile solids
WAS	waste activated sludge
WD	whole digestate
WRF	white-rot fungi
WWTP	wastewater treatment plant

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