

# Pretreatment Strategies for Sewage Sludge to Improve High Solid Anaerobic Digestion

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**Abstract** - High solid anaerobic digestion (HSAD) of sewage sludge is one of the most efficient, effective, and environmentally sustainable remediation techniques; however, the presence of complex floc structures, hard cell walls, and large amounts of molecular organic matter in the sludge hinder HSAD hydrolysis. As a result, pre-treatment of sewage sludge is required to speed up hydrolysis and improve HSAD efficiency. Pretreatment approaches for enhancing sewage sludge HSAD are included in this review, which include mechanical, chemical, thermal, and biological procedures. The merits and disadvantages of various pretreatment processes, as well as their effectiveness, are explored, and recent advances for increased biogas production are reviewed.

**Key Words:** Anaerobic digestion, Sewage Sludge, digestion, High Solid, Pre-treatment.

## 1. INTRODUCTION

SS is mostly made up of dewatered microbial biomass and is created in WWTPs during biological, chemical, and physical treatments. Pathogens, heavy metals, and other toxic elements are also present in SS [1]. Around 2.1 million tons of dry solids are produced annually in Egypt due to the technologies utilized for wastewater treatment, which are mostly activated sludge based. The majority of WWTPs in Egypt lack proper sludge stabilization facilities, except for the WWTPs in high living standards governorates (e.g., Cairo, Alexandria, and Giza). Therefore, about 85% of the non-stabilized sludge is improperly disposed and directly used for agricultural purposes.

Application to agricultural soil (after AD, composting, or chemical treatment), incineration, landfilling, and recycling as building materials are among of the SS treatment and disposal options used [3]. The advantage of AD is that it produces methane (CH<sub>4</sub>), which is the major ingredient of biogas (55–65%), resulting in a renewable energy source. In conventional digesters processing SS, specific CH<sub>4</sub> output typically varies between 0.19 and 0.24 Nm<sup>3</sup> kg VS<sub>i</sub>—n<sup>-1</sup> [4] and is dependent on the SRT used in the wastewater treatment line [5].

AD also minimizes the amount of sludge solids that must be disposed of, stabilizes the sludge, eliminates microorganisms, and reduces odour emissions [7,8]. The following are the main disadvantages of AD of SS: (1) low reaction rates (due to slow hydrolysis of bacterial aggregates), resulting in large reaction volumes and high digester investment costs; (2) process vulnerability and low resilience to inhibitor (e.g. ammonia) accumulation; (3) production of hydrogen sulphide (H<sub>2</sub>S) and volatile silicon compounds, which impede biogas production and utilisation. Other drawbacks include a high buffer required for pH regulation, ineffective treatment of diluted waste, and elevated heavy metal concentrations in the ADS [8].

The amount of TS in a food has a big impact on how well it digests. Pumping and mixing of the sludge become difficult at greater TS content, hence conventional SS AD is done with a TS percentage of between 2 and 6 percent [8]. However, TS content can be increased by up to 25% to (1) reduce storage space within the WWTP and (2) lower transportation costs. Increased TS levels increase digester capacity while reducing water addition to the feed substrate [9]. HSAD, on the other hand, causes the buildup of inhibitory metabolites such H<sub>2</sub>S, FAN, and LCFA, all of which can disrupt or slow down methanogenic activity [10,11]. DSS also has a high viscosity, which can make mixing and pumping activities difficult.

Chemical, thermal, biological, or a combination of these pretreatment procedures degrade the complex sludge structure [26]. These pre-treatment procedures have been shown to lower solid bulk, rupture complex EPSs, and boost methane generation during HSAD in a pilot-scale laboratory experiment. Pretreatment is essential to manage substrates in order to maximize their use in HSAD, and it also enhances substrate biodegradability, increases soluble substrate amount, reduces SS viscosity, promotes microbial degradation accessibility, and lowers total sludge management cost [32]. Because of these advantages, SS pretreatment is considered to be critical for effective HSAD and has gained worldwide attention. This study examines different pretreatment approaches that are required for SS management, with the primary goal of gaining a better understanding of currently used pretreatment procedures.

## 2. HSAD OF SS OVERVIEW

In most WWTPs, sludge treatment includes thickening and dewatering, which separates the solid and liquid components of the sludge so that it can be readily handled for eventual disposal. Polyelectrolytes are frequently used to improve floc adhesion and dewatering effectiveness. Due to the high-water content of treated sludge, anaerobic digesters typically require large operating volumes, resulting in insufficient biogas output to meet the WWTP's energy demands [11,12]. As a result, in small WWTPs and highly urbanized locations with limited land, typical AD is not always anticipated [13]. Small towns and cities have been merging increasingly frequently in recent decades to make infrastructure, such as sewage and waste treatment facilities, more effective and convenient to use [14]. Centralized AD plants that accept DSS from many WWTPs can act as hubs for AD, lowering sludge treatment costs both operationally and financially. Digesting sludge with a high TS concentration allows for lower treatment volumes while keeping the same VS loading rate, resulting in lower energy consumption and transportation costs. As a result, centralizing SS valorisation may result in energy-neutral or even positive AD plants. However, the high TS content of DSS raises several challenges for the digestion process:

- The hydrolytic stage of AD of SS is often the rate-limiting phase, as secondary sludge is rich in difficult-to-digest bacterial cells [15]. The polyelectrolytes used as flocculants slow down the hydrolysis process even more, and have recently been demonstrated to reduce methane generation [16]. To promote SS biodegradability and hydrolysis, thermal and ultrasonic pretreatments, chemical conditioning, and cavitation are commonly used [7]. However, due to rheological problems, applying these procedures to DSS is not always possible.
- The anaerobic breakdown of proteinaceous material by SS HSAD results in substantial ammonia buildup. SS has a high protein level, with protein content ranging from 12 to 29 percent of TS for main sludge and 25 to 50 percent of TS for secondary sludge [17]. Under thermophilic circumstances, the likelihood of ammonia inhibition rises dramatically, as the fraction of FAN (the most toxic form of ammonia) rises with temperature [18]. As a result, ammonia removal is frequently required to prevent the anaerobic process from being disrupted.
- When concentrated or dewatered, SS is a pseudoplastic fluid that exhibits yield stress and viscoelastic behavior [19]. For adequate pumping and mixing at high TS concentrations, rheological and CFD analyses, as well as the installation of specialized devices, may be necessary, resulting in significantly greater investment and operational costs than standard AD systems.
- Biogas from Sewage Sludge by Anaerobic Digestion is usually utilized in (1) boilers for heat generation, (2) combustion engines for combined heat and electricity production, and (3) biomethane upgrading plants. Prior to using biogas, these technologies normally necessitate

purification pretreatments [20]. HSAD of SS produces biogas that is high in H<sub>2</sub>S, VOC, and siloxanes, necessitating biogas treatment.

Aside from these drawbacks, ADS has a lot of promise as a soil amendment and/or fertilizer [21]. ADS typically contains 30–55 percent stabilized OM, up to 3 percent total nitrogen, 0.7–1.5 percent total phosphorus, 0.7 percent total potassium, and varying quantities of magnesium, sulphur, and heavy metal ions. Dry SS has a heat value of between 12 and 15 MJ kg<sup>-1</sup> [22].

## 3. PRETREATMENTS TO ENHANCE THE ANAEROBIC BIODEGRADABILITY OF DSS

Sewage sludge complex organic matter (OM) composition provides barriers to effective AD. The presence of a complex floc structure (extracellular polymeric substances, EPSs), resistant cell walls, and other high molecular weight OM in sludge has been reported as a barrier to AD hydrolysis in several investigations [6]. Because of the hydrolysis issue, the retention period is longer, the bioreactor is larger, and the biogas yield is lower. Several studies have recommended sludge preparation prior to AD to promote hydrolysis and accelerate methane production [13]. Chemical, thermal, biological, or a combination of these pretreatment procedures degrade the complex sludge structure [5]. These pre-treatment procedures have been shown to lower solid bulk, rupture complex EPSs, and boost methane generation during AD in a pilot-scale laboratory experiment. Pretreatment is essential to manage substrates in order to maximize their use in AD, and it also enhances substrate biodegradability, increases soluble substrate amount, reduces SS viscosity, promotes microbial degradation accessibility, and lowers total sludge management cost [19]. Despite the previously noted advantages of anaerobic SS treatment, AD is characterized by lengthy retention durations (20 days) and low VS degradation (30–50 percent) [9]. The sluggish hydrolysis of the cell aggregates that make up secondary sludge has been recognized as the source of these restrictions by scientists [23].

Indeed, SS mostly contains microbial aggregates (flocs), which are microorganisms bound together by EPS, which are often composed of proteins, polysaccharides, and humic-like substances, in addition to a considerable amount of water (>75 percent). EPS forms a three-dimensional matrix bonded to the surface of the cells, which shields the microorganisms included in the aggregate. It is generated through microbial metabolism and lysis or adsorbed from the bulk solution. EPS, in particular, prevents cell rupture and lysis, increases floc strength, and reduces floc dewaterability and biodegradability [24]. Furthermore, each microorganism is protected by a cell membrane that is made up of a phospholipid bilayer with embedded proteins and serves as a physiochemical barrier to direct AD [8].

Furthermore, during the thickening and dehydration processes, cPAM is commonly added in the range of 2.5–10 g

kg TS—1 to limit the hydrolysis rate and reduce methane production [25]. cPAM is utilized to agglomerate the flocs and other particles present in the sludge by charge neutralization and interparticle bridging, resulting in increased dewaterability and lower DSS transport costs.

Many investigations in the previous decade have concentrated on mechanical, thermal, and chemical pretreatments with the goal of eliminating microbial clumps and cells before AD of SS. The above-mentioned slowly biodegradable biomasses are changed to lower molecular weight and quicker biodegradable compounds as a result of these pre-treatments, boosting the hydrolysis rate, VS conversion efficiency, and subsequent bio-methane generation of HSAD.

### 3.1 Thermal Pretreatments

The use of thermal pretreatment to improve HSAD hydrolysis is a well-known commercially established technology [27,29]. SS and other wastes are heated to high temperatures in the thermal pretreatment process, which causes hydrolysis and increases the digestibility of SS and other wastes [30]. This pretreatment method breaks down cell membranes, resulting in soluble organic substrates that are easily hydrolyzed during digestion [29]. Pathogen sterilization, sludge volume reduction, odour elimination, and improved sludge dewaterability are all advantages of thermal pretreatments [27]. The thermal processing of SS has been done at various temperatures (50–250 °C) [29]. Low-temperature (less than 100 °C), high-temperature (more than 100 °C), [26].

#### 3.1.1 Low-Temperature Pretreatment

Low-temperature thermal pretreatment has been shown to be an efficient approach for improving CAD when treating sludge with a TS of 2-5. Thermal pre-treatment can increase the hydrolysis of sludge particles and macromolecular organic compounds while also lowering the viscosity of high-solids sludge, according to theory. In fact, as the temperature of the sludge rises, it becomes more fluid [87], [88]. The optimal parameters for low-temperature thermal pretreatment of municipal wastewater sludge were established by Nazari et al. [89]. According to their findings, the best temperature, time, and pH for pre-treatment were 80°C, 5 hours, and pH 10 correspondingly. sCOD increased to 18.3 7.5 percent under these conditions, but VS declined to 27.7 12.3% [89].

According to Xiaocong Liao, after 30 minutes of treatment at 60, 70, and 80 degrees Celsius, 9.1, 13.0, and 16.6% of sludge solids were dissolved, and sludge flow indices increased from 0 to 0.098, 0.195, and 0.249, respectively. As a result, thermal pretreatment at 60, 70, and 80 degrees Celsius increased the quantity of accessible substrates and decreased sludge viscosity, resulting in biogas production in batch anaerobic digestion experiments increasing by 7.3, 15.6, and 24.4 percent, respectively, and apparent kinetic constants increasing from 0.19 d<sup>-1</sup> to 0.29, 0.39, and 0.39 d<sup>-1</sup>,

respectively, compared to the control. The improved digestion following low-temperature thermal pretreatment confirmed the efficacy of this technique to accelerate high-solids anaerobic digestion, as well as increase biogas yield.

#### 3.1.2 High-Temperature Pretreatment

Treatment duration and applied temperature are important factors in sludge solubilization [6]. The COD and VFA ratios will increase as the temperature rises. In a mixture of primary sludge and WAS, Aboufoth et al. found that the best temperature range for OM solubilization was 175–200 °C [70]. During the 60–120 and 60–240-minute treatment periods, the COD solubilization ratio increased from 11.25 percent to 15.1 percent and 25.1 percent, respectively, at 175 °C. Due to the impact of high-temperature pretreatment, there has been some fluctuation in biogas production results. Climent et al. observed that high-temperature pretreatment had no effect on methane production, but Carrère et al. discovered that it increased biogas output by up to 150 percent [71].

When low-temperature thermal treatments are compared with high-temperature thermal treatments by using the same sludge and the same reactors, laboratory study highlighted that the best results are obtained with operational temperatures in the range of 140–160 °C [36]. Specifically, Xue et al. [36] reported that, with respect to raw or pretreated DSS (16.7 %TS) at temperatures < 120 °C, the biogas production increased by 6–16 % and the SRT could be reduced from 18 to 20 d to 12–14 d after a sludge pretreatment carried out at 140–160 °C. However, another study found that even when the pretreatment temperature was raised to 140–165°C, the organic content became more solubilized, with soluble COD increasing from 32 to 45 g L<sup>-1</sup>, the additional solubilized material was not degradable due to the formation of melanoidins, which are not biodegradable [38]. Lower temperatures would also minimize the ammonification of proteins in the sludge, lowering the quantity of ammonia in the pretreated sludge fed into the HSAD digestion [39].

### 3.2 Physical Pretreatments

Pretreatment with physical and mechanical agents disintegrates solid particles, lowering their size and hence increasing particle surface area, which aids the AD process [11]. According to much research, bigger particles have a lower chemical oxygen demand (COD) and produce less biogas [39].

#### 3.2.1 Ultrasonication

One of the most well-studied and successful mechanical pretreatment procedures for improving sludge biodegradability is ultrasonication. In cavitation, ultrasonication produces hydro-mechanical shear forces that damage the sludge structure [31]. During sludge pretreatment, many physical parameters such as ultrasonic frequency, temperature, and density have been shown to influence the cavitation process [40]. On solubilization,

biological activity stimulation, and enzyme release, ultrasonication has physical, chemical, and biological impacts [26]. The effect of ultrasonication on COD particle size may cause the peak in the particulate fraction (more than 1600 nm) to move to the smallest size range (less than 2 nm) [42]. The frequency and duration of sonification play a crucial impact in boosting the AD process. Li et al. achieved a 53.8 percent increase in methane generation with a quick drop in Methanocorpusculum abundance and dewaterability deterioration in waste activated sludge (WAS) at a density of 0.5 W/mL, frequency of 20 kHz, and sonification for 80 minutes in waste activated sludge (WAS) [40].

Ultrasonication increased biogas production by more than 40% at a low specific energy input and around 15% at a moderate specific energy input, according to Appels et al. [41]. Furthermore, ultrasonic WAS pretreatment lowered WAS levels, improved dewatering ability, and induced COD release from biosolids [43]. According to earlier research, ultrasonication is the most extensively employed pretreatment procedure for increased biogas generation and sludge dewaterability during WAS AD [44]. The fundamental disadvantage of ultrasonication pretreatment, however, is the high energy cost [45]. Furthermore, not all research found that ultrasonication pretreatment improved biogas generation and the elimination of volatile solids (VS). After WAS ultrasonication, Sandino et al. found only a minor increase in mesophilic methane production and VS destruction [46].

### 3.2.2 High-Pressure Homogenization

Pretreatment with high-pressure homogenization (HPH) involves an abrupt pressure gradient (up to 900 bar), cavitation, strong shearing forces, significant turbulence, and subsequent depressurization, which results in a high soluble COD (SCOD) concentration and macromolecule hydrolysis [27,47]. Zhang et al. discovered that the best energy-efficient HPH treatment for SS with a total solid (TS) percentage of 2.48 percent was at a homogenization pressure of 30 MPa with a single homogenization cycle [48]. Furthermore, for a 9.58-g/L TS sludge, a maximal sludge disintegration degree (COD) of 43.94 percent was reached at 80 MPa with four homogenization cycles [49]. HPH boosted biogas production while lowering odor-causing volatile Sulphur compounds in the digester headspace from municipal waste sludge [50]. HPH, on the other hand, has been shown to have a minor impact on pathogen elimination throughout the AD process [29].

### 3.2.3 Microwave Irradiation

Microwave irradiation pretreatment is another option for WAS AD pretreatment. Microwave irradiation pretreatment uses wavelengths between 1 mm and 1 m, with frequencies between 300 MHz and 300 GHz. Microwave pretreatment has been shown to boost biogas generation by 50%, resulting in effective organic compound solubilization [52]. Additionally, in a semi-continuous manner, microwave pretreatment of SS AD boosted methane output and biodegradability by 20% and 70%, respectively [53]. Park

and Ahn looked at the effect of microwave pretreatment on the mixture of primary and secondary sludge during AD and found a 3.2-fold increase in the sCOD to total COD (tCOD) ratio and a 41 percent reduction in VS [54]. This was complemented by a 53% increase in daily biogas output with a shorter hydraulic retention period. Microwave irradiation helps kill harmful bacteria during AD, in addition to increasing biogas output. After irradiation at 70 °C (900 W; hydraulic retention time = 15–25 days) before AD, Kuglarz et al. found that microwave pretreatment reduced *Clostridium perfringens* by 50%, total bacteria by 77 percent, and *Salmonella* spp. and *Escherichia coli* by 100 percent. In addition, compared to untreated sludge, 35 percent more methane was created [55].

## 3.3 Chemical Pretreatments

Chemical pretreatment, which uses chemical reagents such as acids, alkalis, and oxidants to hydrolyze the sludge and boost biogas production by enhancing cellulose biodegradability, is the most promising strategy for complex organic waste annihilation [26,29,56]. Various chemical procedures for AD have been investigated, including alkali and acid pretreatment, as well as ozonation [29]; however, chemical pretreatment is not suited for quickly biodegradable compounds [57]. The chemical pretreatment outcome is mostly determined by the organic compound properties, the method utilized, and the chemical variety used.

### 3.3.1 Alkali Pretreatment

Alkali pretreatment is a widely used approach for disrupting sludge cells and EPSs, allowing OM to be dissolved without leaving toxic residues in the downstream processes. The reaction can be carried out at room temperature and pressure, with little energy requirements [26,30]. The solubilization effectiveness of COD during alkali pretreatment relies on the type of chemicals used for pretreatment, which are enumerated in descending order of efficiency: NaOH > KOH > Mg(OH)<sub>2</sub> > Ca(OH)<sub>2</sub>, and their concentrations [26]. The amount of solubilization is related to the chemical dosage. Large doses are related with increased solubilization in general, while exceptionally high doses are associated with reduced HSAD activity [58]. Wei et al. investigated the effect of alkali pretreatment on hydrogen production from SS and found that at an initial pH of 11.0, maximal hydrogen production was 10.32 mL/g COD [59]. Another study found that after alkali pretreatment of WAS with a pH of 12.0, sCOD increased from 200 to 8000 mg/L [60]. Li et al. achieved a rate of 38.3 percent SS organic decomposition and a biogas output of 0.65 L/g volatile suspended solids (VSSs) in comparison to 30.3 percent and 0.64 L/g in the control [61].

### 3.3.2 Acid Pretreatment

Acid pretreatment for SS AD has gotten far less attention than alkali pretreatment, yet it is more successful at treating lignocellulosic compounds contained in SS because it promotes hydrolytic microbe concentration and lignin breakdown under acidic conditions [26]. COD and other

macromolecules solubilization is affected by pH, and following acid pretreatment of SS at pH 3.3, 58 percent and 52 percent decreases in tCOD and VSS solubilization were achieved, respectively. Biogas production is also boosted as a result of the increased solubilization. After acid pretreatment of WAS at pH 2.0, Devlin et al. saw a 14.3% increase in methane output [61]. Acid pretreatment of SS boosted methane production while also increasing hydrogen-producing bacteria [62]. Strong acidic pretreatment, on the other hand, may produce inhibitory by-products such as furfural and hydroxymethylfurfural [29]. Concentrated acid, on the other hand, is not recommended for acid pretreatment due to its corrosive nature and the possibility of increased costs in the neutralization process, which could jeopardise downstream processing [63].

### 3.3.3 Ozonation

Ozone (O<sub>3</sub>) is a powerful oxidant that has sparked a lot of interest in WAS pretreatment. In comparison to other chemical pretreatments, this approach does not raise the salt concentration and leaves no chemical residues [64]. Both directly and indirectly, ozonation reacts with organic substances. The hydroxyl radicals are used in the indirect ozone reaction, whereas the direct reaction includes fast ozone breakdown into radicals. This mechanism is dependent on the reactant structure, which aids in the biodegradation of recalcitrant chemicals [29]. Biogas output increased by 200 percent following sludge ozonation before AD, according to Ak et al. [66], and biogas production doubled when combined with a light ozone treatment (at 1.33 mg O<sub>3</sub>/g VSS). Furthermore, with WAS treatment, an ozone dose of 0.15 g O<sub>3</sub>/g TSs resulted in an increase in SCOD from 4% to 37% and a 2.4-fold increase in biogas production [67]. Ozonation increases biogas generation and sludge solubilization while also removing pathogenic bacteria from the wastewater treatment system [68]. Although ozone has a substantial influence on SS AD, the key downsides of ozonation are ozone instability and the high energy requirement for ozone generation [26].

## 3.4 Biological Pretreatments

Biological pretreatments are environmentally benign strategies that predigest and increase the AD hydrolysis stages using aerobic, anaerobic, and enzymatic processes [29]. These stages can be made better by using a complex matrix of bacteria that work together to disintegrate the floc structure of sludge and other organic substances [46]. Although eco-friendly and cost-effective, this pretreatment method is time-consuming and requires adequate microbial proliferation parameters [30].

### 3.4.1 Aerobic Pretreatments

Before HSAD, sludge can be treated with air and aerobic or facultative anaerobic microorganisms as an aerobic pretreatment [31]. The micro-aeration technique involves injecting oxygen into the treatment system, which aids in the faster hydrolysis of complex organic compounds by

increasing the hydrolytic activities of the endogenous microbial population [72]. Both aerobic and facultative anaerobic microorganisms benefit from the oxygenic environment in terms of hydrolytic activity. These bacteria are valuable biological resources that can be exploited to cure SS before AD [32]. Exoenzymes that slowly biodegrade substrates that would otherwise remain refractory in anaerobic settings are stimulated by micro-aeration pretreatment [72]. High temperatures (> 70 °C) combined with oxygen enhance the production of hydrolytic enzymes by the hydrolytic microbial population (e.g., proteases). This pretreatment is also known as the auto hydrolytic procedure because these hydrolytic enzymes promote sludge solubilization and organic compound breakdown during AD [26]. Micro-aeration treatment before AD has been proven in several studies to improve not just the AD hydrolysis step but also the overall methane production [72–74]. With infected and non-inoculated substrates, Lim and Wang observed that micro-aeration pretreatment improved methane output by 21% and 10%, respectively [72]. Another study revealed a 20% increase in methane yield after micro-aeration pretreatment, indicating that short-term oxygen pretreatment does not reduce anaerobic methanogens' methanogenic activity [73]. Montalvo et al. optimized the airflow rate, pretreatment time, and temperature for microaerobic pretreatment of SS [74] and found that 0.3 vvm, 48 h, and 35 °C were the best conditions for greater hydrolytic activity. Micro-aeration pretreatment of SS boosted methane generation by 211 percent in this situation [74] compared to the procedure without pretreatment. *Trichoderma viride* pretreatment of organic waste improves AD hydrolysis and, as a result, increases methane generation [75]. *Bacillus licheniformis*, a thermophilic proteolytic bacterium, was found to be effective for sludge pretreatment, resulting in improved OM stabilization and gas generation [32]. Bioaugmentation for sludge pretreatment with *Geobacillus thermodenitrificans*, an aerobic thermophilic bacterium, resulted in a 21% reduction in VS and a 2.2-fold increase in methane generation [76]. Another study used a temperature of 55 °C to improve the AD of mixed sludge, resulting in a 12 percent increase in biogas output and a 27–64 percent reduction in VS [77]. Overall, these investigations suggest that an aerobic pretreatment with oxygen or a thermophilic hydrolytic microbial population overcomes the hydrolysis process issues and boosts biogas generation during AD.

### 3.4.2 Anaerobic Pretreatments

Anaerobic pretreatments can be conducted by predigesting the substrates in mesophilic or thermophilic environments [26]. Temperature phased anaerobic digestion is a typical method for anaerobic pretreatment of SS (TPAD). A main or hyper thermophilic digester is followed by a secondary mesophilic digestion in the TPAD system for sludge pretreatment [29]. Under mesophilic circumstances, it ensures improved acetogenesis and methanogenesis. Two-stage AD [27,31] is another term for this pretreatment technique. Higher biogas production, enhanced floc and solid structure disintegration, reduced quality thermal energy

consumption, and pathogen elimination during thermophilic digestion are just a few of the advantages of TPAD [27].

Using a TPAD to predigest SS has been the subject of several investigations. At 45 °C, a recent study using TPAD on wastewater sludge digestion discovered a 77 percent reduction in VS and a methane releasing rate of 3.55 0.47 L CH<sub>4</sub> /L day [78]. Another study found that TPAD increased biogas yield by enhancing sludge AD, resulting in a 37–43% increase in methane generation [79]. Bolznella et al. investigated the effect of extreme thermophilic prefermentation and found that it increased methane yield by 30–50% when compared to single-stage mesophilic and thermophilic testing [80]. For better degradability and higher methane generation, Ge et al. suggested a retention time of 1–2 days, a pH range of 6–7, and a temperature of 65 °C [81]. All of these investigations found that using a thermophilic-mesophilic TPAD improves hydrolysis, reduces VS, and boosts biogas generation.

### 3.4.3 Enzyme-Assisted Pretreatments

Enzyme-assisted SS pretreatments have sparked a lot of interest as a way to improve AD hydrolysis [82]. Hydrolytic enzymes added to a pre-treatment system improve sludge solubilization, EPS degradation, and biogas production [26]. The following four enzymatic addition techniques have been documented by Brémond et al. : 1) addition in a specialised pretreatment vessel, 2) direct addition in a single-stage digester, 3) direct addition in a two-stage process digester's hydrolysis and acidification vessel, and 4) addition in recirculated AD leachate [31]. Several parameters such as activity, specificity, amount, enzyme stability, temperature, and pH should be examined and tuned for efficient enzymatic pretreatment [82]. Carbohydrates, proteins, and limited lipids make up the majority of sludge in wastewater treatment plants. Because WAS contains EPS-rich flocs that are less biodegradable [31], carbohydrases, proteases, and lipases are the most commonly utilized enzymes for enzymatic sludge pretreatment [30]. Enzymes such as protease, amylase, glycosidase, and glucosidase have been reported to improve anaerobic digestibility and increase biogas production, and enzymes such as protease, amylase, glycosidase, and glucosidase have been reported to improve anaerobic digestibility and increase biogas production. A protease pretreatment produced by utilizing *Bacillus licheniformis* resulted in a 26 percent increase in biogas generation [82]. Chen et al. investigated the effects of lysozyme, protease, and -amylase pretreatments on WAS hydrolysis and degradability and discovered that lysozyme was the most effective of the enzymes tested [83]. When compared to protease and amylase, lysozymes increased sCOD concentration in the sludge by 2.23 and 2.15 times, respectively, and improved sludge flocculation disintegration [86]. Enzymatic processing of activated sludge, food waste, and their mixtures has been done with fungal mash (*Aspergillus awamori*). Pretreatment of these hydrolytic enzyme-rich substrates with fungal mash resulted in a 54.3 percent reduction in VS and a 1.6–2.5-fold increase in methane output [84]. For large-scale pretreatment, Odnell et

al. recommended that specialized enzymes that are more adapted to sludge conditions are necessary [85]. Several enzymes were tested for enzymatic activity, lifetime, and biogas production (cellulose, -amylase, protease, lysozyme, subtilisin, and trypsin). The study found that all of the enzymes tested had a short activity lifetime (less than 24 hours) in WAS and anaerobic digester sludge. Among the investigated enzymes, only subtilisin exhibited a considerable increase in biogas production (37 percent) [85]. For sludge pretreatment, the effects of endogenous enzymes such as amylase, protease, and a mixture of amylase/protease were studied. It was established that a combined enzymatic treatment proved to be better for biogas production; however, for sludge solubilization and acidification, amylase was better than protease or mixed enzymes [86]. All of these studies imply that enzymatic pretreatment can improve sludge AD and biogas production; however, more research is needed to identify specific enzymes for various substrates so that more effective SS AD can be developed.

## 4. CONCLUSIONS

Finally, various disintegration techniques have been investigated to pretreat SS prior to AD in order to speed up the hydrolysis process and increase biogas output. Pretreatment procedures such as physical, chemical, thermal, and biological expedite SS solubilization, which improves solid organic waste biodegradation and increases substrate solubility. Because of its potential to promote OM solubilization and pathogen suppression, thermal pretreatment has been widely used in industry. The addition of acid or base to thermal pretreatments boosts biogas output. Ultrasonication is a common pretreatment method for improving biogas production and dewaterability. HPH produces a high sCOD and decreases Sulphur compounds that cause odours. During Alzheimer's disease, electrokinetic disintegration greatly boosts microbial diversity. Another promising option for biogas production and sludge biodegradability is chemical pretreatment. Anaerobic pretreatment improves AD, boosts methane output, and requires little energy. All of these pretreatments have the potential to boost biogas yield; nevertheless, the sludge solubilization and biogas generation rates have varied depending on the substrates and facilities used. To address the economic and energy challenges, more study is needed on the present pretreatment methods. Finally, in terms of energy balance and environmental sustainability, it is critical to establish standardized methodologies for each pretreatment approach.

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