Pretreatment strategies to enhance the biodegradability of waste activated sludge: Focusing on the conversion of refractory substances

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Abstract

Anaerobic digestion (AD) is a low-cost technology widely used to divert waste activated sludge (WAS) to renewable energy production, but is generally restricted by its poor biodegradability which mainly caused by the endogenous and exogenous refractory substances present in WAS. Several conventional methods such as thermal-, chemical-, and mechanical-based pretreatment have been demonstrated to be effective on organics release, but their functions on refractory substances conversion are overlooked. This paper firstly reviewed the presence and role of endogenous and exogenous refractory substances in anaerobic biodegradability of WAS, especially on their inhibition mechanisms. Then, the pretreatment strategies developed for enhancing WAS biodegradability by facilitating refractory substances conversion were comprehensive reviewed, with the conversion pathways and underlying mechanisms being emphasized. Finally, the future research needs were directed to improve the circular bioeconomy of WAS management from the point of removing the hindering barrier of refractory substances on WAS anaerobic biodegradability.

Keywords: Waste activated sludge; Anaerobic digestion; Biodegradability; Refractory substances; Pretreatment.

1. Introduction

Activated sludge process has been successfully applied to treat municipal and industrial wastewater for over a century. During this process, waste activated sludge (WAS) is generated in large quantities as an unavoidable by-product. The annual production of WAS is reported to reach 70 million tons (80% moisture) by 2025, and this value will continue to increase as the population grows, water quality standards improve, and sludge treatment technologies slowly upgrade (Xu et al., 2022). Different from the construction waste, WAS contains huge amounts of organic substances such as protein and carbohydrate. Different from the food waste, WAS also concentrates some hazardous and noxious substances such as antibiotics, heavy metals, pathogenic bacteria, and microplastics. These properties make WAS both resources and pollutants (Xu et al., 2020b; Syafiuddin and Boopathy, 2021). Actually, the organic matter in sludge is not independent and they can interact with each other or with other inorganic compounds, leading to variations in the characteristics of the organic matter and even altering hydrolysis and biogas conversion (Gonzalez et al., 2018; Yan et al., 2022). Considering the goals to recover resources and to reduce carbon footprints during WAS management, cutting-edge research studies on traditional and novel processes driven by circular bioeconomy concept are undergoing (Mannina et al., 2022).

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Anaerobic digestion (AD), one of the such traditional process can simultaneously transfer organic substances into renewable energy production and weaken the concentration and toxicity of hazardous substances, might play a vital role in circular bioeconomy of WAS management (Liu et al., 2019e; Wang et al., 2018a). It was demonstrated that AD process can synchronously achieve the purposes of energy recovery, pathogen inactivation and pollutant degradation (Ariunbaatar et al., 2014; Chen et al., 2022). Biomass plays an important role as a zero-carbon renewable energy source, and its utilization by AD will become one of the key strategies to realize the aim of carbon neutralization.

For all this, AD process is not driven by the mechanical and chemical energy but by the metabolic activity of hydrolytic microbes, acid-producing bacteria, and CO_2-dependent and/or acetate-dependent methanogens, and is therefore susceptible to adverse factors such as control conditions, functional microbial performance, etc. (Liu et al., 2021a; Zhu et al., 2021). In addition, its poor biodegradability is mainly caused by the presence of endogenous and exogenous refractory substances in WAS, which is particularly affected by the content and property of endogenous and exogenous refractory substances (Xu et al., 2022; Fan et al., 2022).

Although several conventional methods such as thermal-, chemical-, and mechanical-based pretreatment have been demonstrated to be effective for organics release, however none of them are targeted (Carrere et al., 2010; Park et al., 2022; Balasundaram et al., 2022). This has led them to become cost-intensive due to high energy and chemical requirements, as well as passive environmental-consequences such as higher-net carbon dioxide emission. For instance, Xue et al. (2015) found that thermal pretreatment promoted sludge solubilization and volatile suspended solids (VSS) reduction greatly, but failed to improve methane production. Wilson and Novak (2009) also observed that thermal treatment increased sludge hydrolysis, but when the temperature exceeds 180 °C, the Maillard effect occurred and refractory organic compounds were further produced, which was detrimental to subsequent sludge treatment. In contrast to proteins and polysaccharides, the endogenous and exogenous refractory substances are not only difficult to degrade but also toxic to anaerobic functional microorganisms (Xu et al., 2022; Wu et al., 2020). It seems more urgent to target these refractory substances, such as changing their structure to improve their bioavailability and reducing their toxicity to functional microorganisms, which may also be more effective in improving the efficiency of AD.

This paper firstly reviewed the presence and role of endogenous and exogenous refractory substances in anaerobic biodegradability of WAS, especially on their inhibition mechanisms. Then, the pretreatment strategies developed for enhancing WAS biodegradability by facilitating refractory substances conversion were comprehensive reviewed, with the conversion pathways and underlying mechanisms being emphasized. Finally, the future research needs were directed to improve the circular bioeconomy of WAS management from the point of removing the hindering barrier of refractory substances on WAS anaerobic biodegradability. The results obtained will provide a deeper understanding of the inhibitory effects and mechanisms derived from the refractory substances in WAS, and are expected to guide the development of effective strategies to mitigate the negative effects of refractory substances in the future.

2. Refractory substances present in WAS

WAS are composed mainly of the cells of various microorganisms, extracellular polymeric substances (EPS), adsorbed inorganic, organic substances and the majority of water (Cao et al., 2021). Due to the complexity of wastewater influent and the diversity of biological treatment processes, WAS generated from wastewater treatment plants (WWTPs) is of complex composition, usually containing refractory residues of some biochemical and chemical-refractory compounds (Fig. 1), which reduce the biodegradability of WAS. Hence, it is vital to understand the refractory substances (including endogenous and exogenous refractory substances) present in WAS, to provide a necessary reference for further research of WAS.

2.1. Main endogenous refractory substances present in WAS

2.1.1. Lignocellulose

Lignocellulose, which originates from the residuals of exogenous materials (including latrine waste, vegetable leftovers, and leaves) in the wastewater influents, accounts for about 40% at WAS of the total organic matter (Li et al., 2018; Wilen et al., 2003). It mainly consists of lignin (0-40%) and holocellulose (hemicellulose (0-85%) and cellulose (15-99%)) (Li et al., 2018). Among them, both cellulose and hemicellulose have anaerobic biodegradability when they exist independently, and hemicellulose is more easily hydrolyzed, while lignin is the main refractory substance and accounts for about 16% of the total organic matter in WAS (He et al., 2022). These components in WAS are usually interconnected by different kinds of bonds, forming a dense structure of cellulose-hemicellulose-lignin complex, which has extreme resistance to the overall biodegradability of lignocellulose and hinders the energy conversion of biosolids (Chandra et al., 2012; Feng and Lin, 2017).

Lignin has a very complex amorphic three-dimensional long-chain aromatic polymer composed of p-coumaryl, coniferyl and sinapyl alcohol (three phenyl propane units), which are linked by various bonds (Frommhagen et al., 2015; Xiao et al., 2020). Furthermore, as an impermeable/resistant layer, lignin forms the structural support which forms a protective barrier against lignocellulosic biomass and prevents the biodegradation of lignocellulose (Chandra et al., 2012). In general, lignin is more difficult to biodegrade in anaerobic environment than in aerobic environment (Feofilova and Mysyakina, 2016; Mishra and Wimmer, 2017). At present, some studies have reported that lignin can affect the anaerobic biochemical process. For example, Mustafa et al. (2016) found that the methane production was negatively affected by lignin from lignocellulosic biomass.

2.1.2. Humic substances

Humic substances are the highly transformed part of non-living natural organic matter (Lipczynska-Kochany, 2018). Actual humic substances contained in the WAS are commonly described as supramolecular associations of several relatively low molecular weight components, which are connected by hydrophobic interactions and hydrogen bonds (H-bonds), and whose content (15-42% of the total organic matter) depends mainly on the WWTPs (Table 1) (Liang et al., 2021; Lipczynska-Kochany, 2018). More specifically, Lurie and Rebhun (1997) indicated that humic substances are complex mixtures of polyaliphatic and polyaromatic compounds, the main components are alkyl or aromatic unit skeletons cross-linked by oxygen and nitrogen atoms of carboxyl, phenol and alcohol hydroxyl, ketone and quinone functional groups (Lipczynska-Kochany, 2018).

Based on water solubility, humic substances are classified into humic acids (HA), insoluble at acidic water ($pH < 2$) but soluble in alkaline conditions; fulvic acids, soluble at any pH levels in water; humin, insoluble at all pH levels in water (Sutton and Sposito, 2005). Humic substances have been identified as inhibitory compounds that critically inhibit the biodegradability or bioavailability of WAS (Azman et al., 2017).

2.1.3. Alginate

Alginate in EPS is a type of uronic acid, which is a major but often overlooked polymer associated with the difficult hydrolysis of WAS, accounting for approximately 7% w/w of the organic matter in WAS (Geng et al., 2021). Alginate is considered as a complete family of linear copolymers with different chemical structures containing blocks of (1,4)-linked β-d-mannuronate (M) and α-l-guluronate (G) residues (Lee and Mooney, 2012). Recent studies have reported that the gel-like materials in WAS which called structural extracellular polymeric substances (St-EPS) was formed by alginate isomers of glucuronic acid and polygalacturonic acid (Zhang et al., 2021). In addition, exopolysaccharides are considered to be key elements in shaping biofilms and providing structural supports to them, and are highly correlated with the structure of the sludge matrix (Felz et al., 2019). While, hydrolysis of EPS in WAS is a rate-limiting step in AD, mainly depending on the resistance of EPS to degradation (Dai et al., 2020). Thus, alginate-like EPS could be involved in the basic structures of sludge, and closely associated wth the biodegradability of sludge.

2.2. Main exogenous refractory substances present in WAS

2.2.1. Polymer flocculants

Polymer flocculants are widely used in WWTPs to significantly improve the performance of wastewater purification and sludge dewatering (Wei et al., 2018). In these processes, large amounts of flocculants are highly enriched by WAS as exogenous pollutants during biological wastewater treatment (Wang et al., 2018b). For example, cationic polyacrylamide (cPAM) concentrations have been reported to be 2.5-10 g/kg of dry sludge in dewatered sludge due to the heavy use of cPAM in the mechanical dewatering process (Liu et al., 2019d).

Among the widely used polymeric flocculants, polymer flocculants (e.g., chitosan and polyacrylamide) can be partially degraded by anaerobic bacteria, but their degradation persistence (the half-life of chitosan was about 61.4 days) is much higher compared to the inherent organic matter (Liu et al., 2021a; Wang et al., 2018b). Consequently, such flocculants concentrated by WAS may affect the series of bio-conversions including lysis, hydrolysis, acidogenesis, and methanogenesis, and thus constitute a potential threat to the AD performance (Appels et al., 2008; Luo et al., 2020). For example, 12 g/kg TSS cationic polyacrylamide reduced the maximum methane production by 37.67% and prolonged the digestion time from 22 to 26 days (Wang et al. (2018b). Poly ferric sulfate showed a similar phenomenon for AD of WAS. As the concentration of poly ferric sulfate increased to 40 g/kg TS, the cumulative methane yield decreased to 56.7% of the control, and the lag time prolonged by 237.0% (Liu et al., 2021c). Furthermore, the inhibition of hydrogenotrophic methanogenesis by ferric polymeric sulfate was found to be much more severe than that of acetotrophic methanogenesis. Liu et al. (2021a) found that chitosan at 8, 16 and 32 g/kg TSS decreased methane production from 166.3 to 154.3, 139.5 and 115.8 mL/g VS.

2.2.2. Pharmaceuticals and personal care products

Pharmaceuticals and personal care products (PPCPs) such as antibiotics and antimicrobials are highly accumulated in WAS due to heavy usage and persistence nature (Huang et al., 2019; Kim et al., 2013). Currently, over half of the world's antibiotics used worldwide are used in livestock, however, these antibiotics inevitably enter into sewage sludge through excretion due to the high dosage concentration and the low absorption and utilization capacity of humans and animals (Le-Minh et al., 2010). For example, Chlortetracycline (CTC), a class of tetracycline antibiotics, was as high as 34.2 mg/kg TSS in WAS (Puicharla et al., 2014). Clarithromycin, a 14-membered ring macrolide antibiotic, accumulated in WAS at concentration levels up to 67 mg/kg dry sludge (Verlicchi and Zambello, 2015). In addition, it has been reported that >90% of triclosan can be removed by adsorption or biodegradation during wastewater treatment, however most of it would enter into the WAS and can be detected at concentrations as high as 0.028-37.189 mg/kg TSS (Butler et al., 2012). Previous studies reported that about 79% of triclocarban (TCC) removed by WWTPs was transferred to solids, and only about 18% of TCC was degraded by nitrification-denitrification processes, leaving a residual of about 4.15 ± 0.77 kg/d in WAS (Lozano et al., 2013). Moreover, due to the low biodegradability, antibiotics and antimicrobials enriched in WAS could affect the reduction and resource recovery of WAS.

2.2.3. Polycyclic aromatic hydrocarbon

Polycyclic aromatic hydrocarbons (PAHs), the representative persistent organic pollutants with two or more aromatic-fused rings, are commonly found in WAS. Typically, PAHs enter into WWTPs with industrial wastewater, and are enriched in WAS after wastewater treatment due to hydrophobicity (Larsen et al., 2009; Siebielska and Sidelko, 2015). Several typical PAHs including naphthalene, acenaphthene, phenanthrene, anthracene, fluorene, and chrysene, were reported to be detected in WAS at concentrations of 22.4, 17.6, 95.3, 6.1, 3.3, and 12.5 mg/kg dry sludge, respectively (Luo et al., 2016).

The persistence of PAHs depends on various factors (e.g., concentration, structure, dispersibility, bioavailability), but generally, the environmental persistence of PAHs is positively correlated with their molecular weight, hydrophobicity, and toxicity (Bamforth and Singleton, 2005). Recent studies have shown the potential for biodegradation of PAHs in sludge under anaerobic conditions. Phenanthrene, a representative PAHs, was partially degraded (47.5%) by anaerobic fermentation at a dose of 100 mg/kg dry sludge (Luo et al., 2016). However, the biodegradation of anaerobic PAHs in sewage sludge is usually limited and its degradation rate depends on the active anaerobic bacterial population (Larsen et al., 2009). Chang et al. (2003) found that compared to municipal sludge samples, PAHs degradation rates in petrochemical sludge samples were usually faster, which may be due to the presence of more PAH-degrading autochthonous microorganisms. Moreover, PAHs degradation is also influenced by the conditions of methanogenic, sulfate-reducing and nitrate-reducing (Chang et al., 2003).

3. Adverse effects of refractory substances on anaerobic biodegradability of WAS

AD has been widely used to recover energy from WAS, but the presence of exogenous and endogenous refractory substances in WAS usually limits its application. Therefore, it is essential to evaluate the impacts of these exogenous and endogenous refractory substances on AD of WAS, especially their inhibition mechanisms (Fig. 2), to provide a theoretical basis for strategies to enhance the biodegradation of WAS.

3.1. Lignocellulose

The components of lignocellulose (cellulose, hemicellulose, and lignin) have different effects on AD. Li et al. (2018) found that cellulose has a higher biomethane potential than hemicellulose and their co-digestion has a synergistic effect to produce more methane. However, lignin is almost undegradable under anaerobic conditions and also reduces the production of methane from holocellulose. The lignin content of lignocellulose is negatively correlated with methane production from AD. It has been indicated that the methane potential of AD of phytoplankton with lower lignin concentration is high, and lignin above 100 g/kg VS causes a significant decrease in biochemical methane potential (Triolo et al., 2012). He et al. (2022) found that in WAS anaerobic fermentation, lignin inhibited methane production which reduced the consumption of short-chain fatty acids (SCFAs), resulting in the accumulated SCFAs.

As mentioned above, although cellulose and hemicellulose are hydrolysable, they tend to be covered by lignin in lignocellulose, forming a stable composite structure that not only prevents from enzymatic attack, but also increases the mass transfer resistance of sludge, which reduces bioconversion efficiency of lignocellulose (Li et al., 2021a). Therefore, understanding the mechanisms of lignin on WAS anaerobic biodegradability becomes essential. Previous studies have found that lignin could inhibit both hydrolysis and methanogenesis processes and that methanogenesis is more vulnerable to the adverse effects of lignin (He et al., 2022). In the anaerobic conditions, lignin inhibits the hydrolysis process by the formation of a protective layer that prevents enzyme entry, and by irreversibly/reversibly binding to the enzyme due to its inherent hydrophobicity, affecting the protein structure of the enzyme (Ju et al., 2013). In addition, since its possesses numerous quinone structures, lignin could receive the remaining electrons in the system, which act as an active terminal electron acceptor, thus preventing the conversion of acetate to methane (Li et al., 2018). In general, as shown in Fig. 2, the inhibition of lignin on WAS anaerobic biodegradability is mainly caused by the pathways of improving mass transfer resistance, ectoenzymes competition, endoenzymes active sites encroachment, and means electrons competition.

3.2. Humic substances

Humic substances such as HA, are refractory macromolecular organic compounds, and their presence limits the conversion of organics to methane. Humic substances represent a high proportion in WAS and have been shown to inhibit the hydrolysis of organics, for instance, Brons et al. (1985) discovered that adding 250 mg per liter of humate inhibited protein hydrolysis. In addition, several studies have reported that HA is an inhibitory compound for hydrolysis and methanogenic processes in AD. For example, Liu et al. (2015) found that SHHA (a commercially available HA) in WAS anaerobic fermentation at 1.0 g/g-sludge inhibited methane up to 97%, thus the accumulation of SCFAs. Li et al. (2019a) observed in batch digestion experiments that biogas production decreased with increasing HA, and an increase in HA:VSS to 15% resulted in a 35.1% inhibition level of biogas production. Semi-continuous experiments of WAS anaerobic digestion showed that HA could reduce methane production rate and prolong lag phase time (Li et al., 2019b).

The combined action of multiple factors led to the influence of HA on WAS anaerobic biodegradation (Fig.2). From the perspective of sludge structure, HA could interact with sludge particles to form a dense structure to enhance the stability of the organic matter structure in sludge, thus increasing the mass transfer resistance of sludge. Moreover, HA could change the spatial configuration of extracellular organic substances, leading to a reduction in the active site of WAS, thus enhancing the ectoenzymes competition (Xu et al., 2020a). From the perspective of enzymes, HA could reduce the activity of key enzymes, such as α-amylase, protease, F_{420} enzyme, thereby promoted competition between other co-cultured bacteria and functional bacteria (including hydrolysis, acidogenesis, methanogenic bacteria) (Li et al., 2019b). In addition, HA has covalent bonds and sweep flocculation interactions with the enzyme, and at high HA levels, the predominant interactions irreversibly turn into covalent bonds, leading to a strong negative effect on hydrolysis (Fernandes et al., 2015). From the perspective of electron transfer system, HA could play as a terminal electron acceptor that receives electrons directly from acetate, thus improving the ectoenzymes competition (Li et al., 2019a). Furthermore, HA could easily cross the outer cell membrane of hydrogenotrophic methanogenic bacteria, destabilizing the cells and leading to cell inactivation (Khadem et al., 2017).

3.3. Alginate

Alginate, a typical St-EPS component, is an important endogenous refractory substance in WAS. Generally, EPS, the main organics (20%-50%) in WAS, contain carboxyl, hydroxyl and other functional groups, which can combine with bivalent cations (Ca^{2+}) commonly found in wastewater, and form stable EPS structure through aggregating and flocculating, resulting in slow hydrolysis of WAS and low AD efficiency (Li et al., 2012; Qian et al., 2021). Notably, alginate has a GG block (i.e., a dimer of G) that can act as an ionic bridge between divalent cations and other glyoxylates (Geng et al., 2021). Although the effects of alginate on WAS anaerobic digestion has not been systematically studied, it is found that anaerobic alginate hydrolytic bacteria (AHB) are rarely enriched in anaerobic reactors, resulting in difficult conversion of alginate. Qian et al. (2021) found that the enrichment of alginate-degrading microbial consortium (ADC) which could excrete alginate lyase by *Algibacter lectus* and *Bacteroides* sp, can be used for highly selective fermentation of WAS. Zhang et al. (2019) showed that extracted EPS could be used by ADC for methane production with acetate as the main intermediate, resulting in a 115%-185% increase in methane production. It is worth noting that these studies on mixed culture promoting the conversion of alginate and isomer in St-EPS contribute to methane and/or SCFAs production from WAS as valuable biochemical products, but it is seldom reported.

3.4 Polymer flocculants

Flocculants, which are abundant in WAS due to their widespread use in wastewater management and sludge dewatering, but can adversely affect the AD efficiency. For example, cPAM has been found that not only decelerate the AD process, but also about 46% of this is degraded, and the major degradation products, including polyacrylic acid, acrylamide and acrylic acid, inhibit all dissolution, hydrolysis, acidogenesis and methanogenesis processes, thereby reduced methane production (Wang et al., 2018b).

Research has reported that polymeric flocculants could alter sludge particle size and specific surface area, creating a "cage" effect to increase the mass transfer resistance between anaerobic bacteria/hydrolytic enzymes and the sludge matrix (Lin et al., 2017; Luo et al., 2020). A study on polymeric flocculants showed that chitosan could inhibit EPS secretion and reduce anaerobic bacterial activity by occupation of indigenous carbohydrate linkages sites (Liu et al., 2021a). The presence of cationic polyacrylamide and poly ferric sulfate significantly reduced the relative activities of protease, acetate kinase and coenzyme F_{420}, resulting in the inhibition of methane production (Fig.2) (Liu et al., 2021c; Wang et al., 2018b). In addition, the presence of poly ferric sulfate also increases the production of H_2S, which is toxic to methanogenic bacteria.

3.5 Pharmaceuticals and personal care products

Antibiotics and antimicrobial agents are known to be detrimental to microbial growth and metabolism, and their presence may therefore adversely affect AD. Several studies have shown that macrolide antibiotics could inhibit WAS anaerobic digestion. For example, it has been observed that rhodopsin at 1000 μg/L reduced the maximum cumulative methane yield from AD by 7.7% as well as the methane production rate by 15.7% (Ni et al. (2020). The maximum methane production rate was reduced by 46.2%with an increase in the clarithromycin concentration from 0 to 2000 mg/kg (Huang et al., 2020). And the effect of other antibiotics such as triclosan on methane production showed a dose-dependent effect. Triclosan at low concentrations had little effect on methane production and as concentrations increased to 1000 and 1500 mg/kg TSS accumulated methane production decreased by 8% and 13.2%, respectively (Wang et al., 2020). Although norfloxacin and sulfamethazine had little effect on methane yield, they depressed the methane production rate (Zhao et al., 2019). Methane production was almost completely inhibited by benzalkonium chloride (a non-oxidizing antimicrobial agent) at a level of 100 mg /L (Flores et al., 2015).

Among all kinds of microorganisms, methanogenic bacteria are more susceptible to these contaminants. CTC could inhibit methanogenesis by increasing acetate kinase activity to stimulate acidification and decreasing F_{420} activity in WAS anaerobic biochemical reactions (Tang et al., 2021b). Roxithromycin only significantly inhibited acidogenesis and methanogenesis processes in AD of WAS, while clarithromycin and triclosan had negative effects on hydrolysis, acidification, acetic acid production and methanogenic processes (Huang et al., 2020; Ni et al., 2020; Wang et al., 2020). Roxithromycin may also bind to peptidyl transferase in 23S rRNA, blocking transpeptide action and mRNA translocation, inhibiting protein synthesis, thus affecting the normal growth of microorganisms (Sibert et al., 1979). Competition between bacteria and methanogenic bacteria is one of the reasons for methane inhibition by clarithromycin. In addition, many antimicrobial agents have hydrophobic/hydrophilic termini similar to those of surfactants (Luo et al., 2019). The extraordinary hydrophobicity of the cytoplasmic membrane of methanogens leads to their vulnerability to these contaminants (Luo et al., 2020). The proton motive force for ATP production in methanogenic archaea is also susceptible to negative effects, leading to significant inhibition of methane production. It is worth noting that PPCPs (such as roxithromycin and clarithromycin), have been shown to promote the proliferation of antibiotic resistance genes (ARGs) in WAS, and environmental risks need to be considered in practice (Zeng et al., 2021).

3.6 Polycyclic aromatic hydrocarbon

Persistent organic pollutants (POPs) enter the WWTP with industrial wastewater, resulting in large quantities of POPs, including PAHs and polychlorinated biphenyls (PCBs) remained in WAS, which may affect anaerobic bacteria in (Feng et al., 2015). PAHs were discovered to inhibit the methanogenic process during the anaerobic fermentation of WAS, inhibited the conversion of SCFAs to methane, and promoted acidification, resulting in increased production of SCFAs (Luo et al. (2016). Yang et al. (2019b) demonstrated that methane production decreased with increasing PCBs, and 100 mg PCB/kg DS significantly decreased methane production by 26.6%. PAHs could enhance the activity of key enzymes involved in acetate production (phosphotransacetylase and acetate kinase) and increase the number of genes encoding them, thereby inhibited the conversion of SCFAs into methane (Luo et al., 2016). Unfortunately, information on the underlying mechanism of PAHs inhibition of methane yield from AD of WAS is still lacking, further research is needed in the future (Yang et al., 2019b).

4. Pretreatment strategies for enhancing WAS biodegradability by facilitating refractory substances conversion

In the field of sludge treatment, AD has the merits of reducing WAS production and recovering methane as bioenergy, but this process is significantly affected by the poor biodegradability of WAS, which pose a challenge for sludge reduction and stabilization. In recent years, to enhance the WAS biodegradability, various pretreatment strategies for facilitating refractory substances conversion have been previously proposed (Fig.3) (Liu et al., 2019a; Akbay et al., 2021; Balasundaram et al., 2022), which provide insights for improving the efficiency of WAS utilization in practical engineering.

4.1. Physical-based pretreatment

4.1.1 Microwave pretreatment

Microwave pretreatment can effectively disintegrate sludge flocs and break cells, and achieve higher degradation efficiency of refractory organic matter and excellent biomethane production. For, example, Liu et al. (2019a) utilized a 12-min microwave to treat PAM reactor and found that after 30 days, the viscosity decreased significantly (from 6.97 ± 0.30 to 5.40 ± 0.21 mPa/s, 22.5%) and the content of PAM's monomeric acrylamide in digester was only 0.37 ± 0.01 mg/L, which did not lead to the accumulation of acrylamide in the digestion residues. This suggests that microwave irradiation destroys the structure of PAM and makes PAM more easily biodegradable. Kor-Bicakci et al. (2020) also found that microwave anaerobic digestion can also be used to reduce TCC levels in biosolids.

4.1.2 Ultrasound pretreatment

Ultrasound generates mechanical shear force through cavitation to disrupt cell membranes and release intercellular material in bulk solution. In addition, hydroxyl radicals based on cavitation can decompose many toxic and refractory substances into simpler forms, such as chlorinated hydrocarbons, aromatic compounds, and textile dyes (Chawla et al., 2014; Tian et al., 2021). However, total mineralization of these refractory contaminants is difficult to obtain by ultrasound alone (Vajnhandl and Le Marechal, 2005). Zhang et al. (2022) used 20 kHz for pretreatment of sewage sludge. However, such sonication conditions for sludge pretreatment did not result in polyfluoroalkyl substances degradation. Conversely, the concentrations of perfluorooctanoic acid, perfluoroheptanoic acid, and perfluorohexanoic acid sonication were increased in the liquid phase after long time treatment (> 15 min). It may be that ultrasound reduced the sludge floc binding capacity and promoted the release of these three perfluoroalkyl acids. Therefore, ultrasonication can only improve the biodegradability of sludge by promoting floc breakage, but it does not work on polyfluoroalkyl substances. This conclusion was also confirmed by Zhang and Li (2018). Ultrasound effectively promotes the dissolution of antibiotics, but does not biodegrade the fluoroquinolones that are refractory by themselves. The same is true for the changes of PAHs. Sonication leached PAHs from sludge solids into liquid phase, but the sonication of PAHs was negligible, just a simple phase transition (Oh et al., 2016).

4.1.3 Thermal hydrolysis pretreatment

Thermal hydrolysis (TH) promoted the biodegradation of WAS and released the refractory organic matter carried by the sludge to the WWTP. Slowly biodegradable or non-biodegradable steroids and aromatics are more than 50% dissolved in the digester of the sludge feed after being treated with TH

(Lu et al., 2018). It is generally believed that low temperature has little influence on the biodegradability of sludge. Prorot et al. (2011) found biodegradability tests (TH below 100 °C) did not show any improvement of the intrinsic biodegradability after the TH, only the increase of the specific digestion rate was observed. Many researchers have reported optimal temperatures for TH in 160 - 180 °C for max methane production (Jeong et al., 2019). Toutian et al. (2020) found the poorly soluble COD increased with increasing TH temperature. Lu et al. (2018) found that with increasing TH temperature (120 to 172 °C), the concentration of HA was observed to increase significantly. It may be caused by a variety of sources, possibly including the release of HA from sludge disintegration, the conversion of cellulose in the cell wall, the Maillard reaction and polysaccharide hydrolysis. However, the possibility of caramelization of reducing sugars from starch cellulose hydrolysis is very low. This suggests that TH treatment can significantly promote the dissolution of soluble refractory organics in AD effluent, but its improvement biodegradability has not been demonstrated. Generally, TH pretreatment can improve the biodegradability of proteins, carbohydrates, lipids and hemicelluloses during AD, but the biodegradability of cellulose and lignin was negligible because TH treatment cannot destroy cellulose crystal structure and heterogeneity. However, thermal hydrolysis effectively reduced the abundance (> 94%) of almost all subtypes of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs). It is a powerful technology for the reduction of tetracyclines, macrolides and lincosamides, which provides the possibility for the transformation of refractory substances and deserves further research (Zhang and Li, 2018).

4.2. Chemical-based pretreatment

4.2.1 Alkaline pretreatment

Most literature related to alkaline treatment enhances other sludge disintegration technologies by adding alkali to make sludge more susceptible to hydrolysis. Liu et al. (2021b) increased the PAM degradation rate to 80.1% by alkaline pre-fermentation. Alkaline pre-fermentation breaks up the firm "PAM sludge" flocs and reduces the molecular weight of PAM, making it more susceptible to microbial degradation (Fig.3). In addition, the lignin and various uronic acid substitutions can remove by alkaline solution on hemicellulose. The remaining biomass pretreated with 4% alkali for 60 minutes showed 46.6% degradation of lignin with 83.2% increase in glucan content (Kaur et al., 2012). However, fluoroquinolones are hardly degraded during AD. The removal rate was lower than 42.02%, and the residual fluoroquinolones were mainly adsorbed on the digested sludge. Because the limiting step in reducing fluoroquinolones is biodegradation, desorption of antibiotics does not improve conversion (Zhang and Li, 2018). But it's worth noting that during the alkaline pretreatment of sludge, microorganisms related to anaerobic digestion will deviate from the optimal growth conditions with the increase of system pH, and the structure of EPS will also become unstable with electrostatic action. Hence, the choice of pH for alkaline pretreatment is also critical.

4.2.2 Ozonation

Ozone, a strong oxidant, can be broken down into free radicals that react directly or indirectly with organics. Refractory substances in sludge can be hydrolyzed by exposing it to highly oxidizing conditions (ozone). As a result, stubborn organic matter will be oxidized to readily biodegradable substances that can be utilized by microorganisms. Li et al. (2021b) observed that ozonation could remove PAHs from sludge. In fact, PAHs adsorbed on the surface of sludge particles were released into the liquid phase during the initial stage of ozonation due to the dissolution of extracellular polymers. Then, the PAHs present in the liquid phase will be degraded again. This suggests that low

O_3 concentrations may cause PAHs to be released into wastewater. Thus, the concentration of O_3 should be strictly controlled (Lin et al., 2016a). Ozonation can also partially degrade some pollutants (Cheng and Hong, 2013). Wang et al. (2018c) found that ozone pretreatment can lead to efficient degradation of antibiotics in sludge (86.4 - 93.6%), mainly through desorption of the binding part of the sludge and subsequent oxidation of the dissolved part. However, this approach has some drawbacks. For instance, some refractory organic pollutants initially adsorbed on the sludge may be released into the wastewater during the sludge ozonation treatment, resulting in a reduce in effluent quality.

4.2.3 Other chemical pretreatment

Chemical pretreatment mainly utilizes chemical agents to interact with microorganisms and EPS by various mechanisms. It can not only improve AD performance, but also promote the transformation of refractory pollutants. For example, the addition of calcium peroxide (CaO_2) could break the hydrolysis limit to recover resources such as SCFAs. Wang et al. (2019) found that CaO_2 pretreatment promoted the degradation of humus, lignocellulose and other typical refractory organisms (eg, triclocarban, norfloxacin, phenanthrene, diclofenac, and loxazone), which could alleviate the inhibitory effect of recalcitrant organics on anaerobic bacteria. Ping et al. (2018) found that a decrease in non-biodegradable humic substances and an increase in readily biodegradable tryptophan-like proteins after adding CaO_2. Humus-like substances may be oxidized by $\cdot OH$ or $\cdot O_2^-$ produced by CaO_2. Another possible explanation is that the presence of CaO_2 can enhance the biosorption potential since multivalent cations (i.e., Ca) can form bridges with humic acid. Similar phenomena have been observed by many researches using chemical agents. Lu et al. (2022) reduced the content of non-biodegradable substances in total organics using ferric chloride-assisted nitrite pretreatment. Yuan et al. (2022) introduced the reducing/chelating agent ascorbic acid (H_2A) into a conventional zero-valent iron (Fe^0)/persulfate (PS) process. The generated $SO_4^{-}\cdot$, $\cdot O_2^{-}$ and $\cdot OH$ were found to degrade refractory organic matter (humus). The formation pathway of $SO_4^{-}\cdot$, O_2^{-} and $\cdot OH$ is as follows:

$$Fe^0 + H_2O \rightarrow Fe^{2+} + H_2\uparrow \tag{1}$$

$$Fe^{2+} + S_2O_8^{2-} \rightarrow Fe^{3+} + SO_4^{-}\cdot + SO_4^{2-} \tag{2}$$

$$Fe^{2+} + H_2A \rightarrow Fe^{2+}H_2A_x \tag{3}$$

$$Fe^{2+}H_2A_x + O_2 \rightarrow Fe^{3+}H_2A_y + \cdot O_2^{-} \tag{4}$$

$$SO_4^{-}\cdot + H_2O \rightarrow \cdot OH + SO_4^{2-} + H^+ \tag{5}$$

Xu et al. (2021) pretreated WAS with $KMnO_4$ at 0.1 g/g TSS, and SCFAs production was observed to increase 7.4 times from WAS. $KMnO_4$ reduced the species and concentrations of pollutants, promoted the degradation of lots refractory substances such as humus and lignocellulose, and provided more substances for the production of SCFAs. GC/MS analysis showed that $KMnO_4$-pretreated humic and lignocellulose produced several small molecular species (such as acids, alkanes, and alcohol organics), some of which were shown to serve as substrates for the production of SCFAs. Lv et al. (2021) found that adding exogenous N-acyl-homoserine lactones (AHLs) can enhance the effectiveness of anaerobic granular sludge by promoting the transformation of refractory organic matter. In addition, emerging organic pollutants (eg, carbamazepine and ibuprofen) often accumulate in sludge. Thermally activated persulfate generated $SO_4^{2-}\cdot$ and $\cdot OH$, macrolide and tetracycline antibiotics were efficiently decomposed by active radicals (> 80%), while quinolone antibiotics were less efficient (Tang et al., 2021a). In addition, Wang et al. (2022) added sodium percarbonate (SPC) to pretreat waste activated sludge. The activated SPC promoted the conversion of organic substrates from non-biodegradable to biodegradable, which were further used by functional microorganisms involved in sludge AD for methane production. The pretreatment of SPC oxidation can be completed within 24 h, means that the SPC-based technology has better sludge treatment effect compared with other strategies like CaO_2, $KMnO_4$, etc. Therefore, economic strategies still need to be considered for practical application, some pretreatment methods can be seen in Table 2.

Yang et al. (2019a) used peroxymonosulfate (PMS) as an additive to pretreat WAS, it was found that PMS pretreatment improved the bioavailability of released organics and degraded refractory organics such as cyclopentasiloxane, heptasiloxane and ethylene glycol. Previous studies reported that sulfite pretreatment may destroy the structure of HA in sludge, which mainly through sulfonation reaction. Liu et al. (2020a) used sulfite pretreat WAS, founding that the abundance of some microorganisms associated with refractory substances also increased during pretreatment, which is further conducive to the biodegradation of WAS. In addition, Liu et al. (2020a) found that the reactive derivatives from acidified-sulfite (e.g., H_2SO_3, HSO_3^-) not only enhanced the abundance of bacteria associated with hydrolysis and acidification (e.g., *Proteiniclasticum* sp., *Alkaliphilus* sp., *Romboutsia* sp., *Tissierella* sp.), but also induce and stimulate the growth of sulfite reducing bacteria (e.g., *Desulfitispora* sp., *Desulfurivibrio* sp.), which increase the conversion of organics (equations (6) and (7)), and further contributed to the resource utilization of WAS (Fig.3).

$$CH_3CH_2COO^- + SO_3^{2-} + 2OH^- \rightarrow S^{2-} + CO_3^{2-} + CH_3COO^- + 2H_2O \quad (6)$$

$$CH_3CH_2CH_2COO^- + 2SO_3^{2-} + 4OH^- \rightarrow S^{2-} + 2CO_3^{2-} + CH_3COO^- + 4H_2O \quad (7)$$

4.3. Biological-based pretreatment

Biological pretreatment is considered as an environmentally friendly technology which is promoted increasingly, providing low-energy input, low-cost disposal and gentler operating conditions. Microbial strains isolated from diverse resources can be applied alone or in combination to break up stubborn polymer structures. For example, the enzymatic treatment of lignocellulosic biomass mainly relies on microbially produced enzymes such as hemicellulase and cellulase. Yuan et al. (2016) pretreated sludge with a microbial consortium (thermophilic microbial consortium MC1), which can effectively improve the biodegradability of cotton stalks. Cellulose, hemicellulose and lignin were significantly degraded by the microbial consortium MC1 after 14 hours. Vitamins and hemicelluloses are converted into sugars. The monomers (sugars) are then converted into volatile organic products. Zhang et al. (2019) enriched the alginate degradation complex (ADC) using alginate as a substrate. Oligomeric alginate lyases were detected in 12 bacterial species by metagenomics and identified to convert alginate to methane. Larsen et al. (2009) applied *Proteiniphilum* acetatigenes for mesophilic anaerobic sludge digestion and observed a positive effect of the introduced strain on the removal of PAHs. Boruszko (2017) supplied the same results in a report. However, the effect of enzymatic reaction alone is limited, so it should be used in combination with other pretreatment methods. The source of the enzyme has a significant impact on the enzyme activity and type, limiting the stability of the enzyme in WAS. An efficient, stable and inexpensive enzyme source needs to be further explored.

4.4. Combined pretreatment

4.4.1 Thermal-chemical combination

The synergistic effect of pyrohydrolysis and alkalinity can achieve better sludge floc disintegration and degradation of complex organic matter. For example, Liu et al. (2019c) pretreated WAS with hot alkali and found that not only the large and hard flocs were broken and the degradation of PAM was improved, but also the accumulation of acrylamide was avoided. Additionally, during TH pretreatment, several reactions between proteins and reducing sugars occur, producing maillard reaction products (MRPs), which resist degradation under both anaerobic and aerobic conditions. Besides, several adverse biological effects are even induced by some compounds of MRPs, including genotoxicity, cytotoxicity, and antimicrobial activity. Geng and Zhou (2022) developed a method to decrease refractory MRP of WAS without affecting solubility. 10 mg/L $FeCl_3$ increased the solubility of WAS after TH pretreatment, and the refractory residues decreased. $FeCl_3$ enhanced the biodegradability and methane production of WAS after TH pretreatment ($10.4 \pm 0.8\%$ increase in yield). The specific analysis is mainly attributed to Fe^{3+} triggering the Fenton-like reaction, leading to the enhanced biodegradability of TH pretreatment solution, and the enrichment of microbial communities related to protein degradation and methane production.

In addition to the high temperature, freezing has also been recently reported to improve sludge flocs biodegradation performance. Liu et al. (2020b) found that the degradation of humic acid-like substrates could be significantly promoted by nitrite-based freezing pretreatment. It supposed that EPS and cells were destroyed though the formation of ice crystals during this nitrite-based freezing process. Simultaneously, the “excess” protons in acidic sludge mixture and extra nitrite would be removed from ice crystals and concentrated into liquid-like ice grain boundaries (i.e., freeze concentration effect), causing a dramatic decrease in pH and increase in nitrite concentration, under which conditions a large number of reactive derivatives of nitrite (e.g., free nitrous acid (FNA), $NO_2^{\cdot}$, NO, N_2O_3) was produced (Equation 8). And those reactive derivatives could as oxidizing species to facilitate the degradation of refractory compounds such as humic substrates, pharmaceuticals, and PPCPs, thus the bio-degradability of solubilized substrates were improved Liu et al. (2020b).

$$2NO_2^- + 2H^+ \leftrightarrow HNO_2 + HNO_2 \leftrightarrow N_2O_3 + H_2O \leftrightarrow NO + NO_2^{\cdot} + H_2O \quad (8)$$

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4.4.2 Ultrasonic-chemical Combination

The ultrasonic-chemical treatment has been shown to be effective in interfering with the chemical structure of organic toxic contaminants and converting them into soluble substrates. For example, Zhao et al. (2020) investigated the influence of ultrasound irradiation combined with ozone (US/O_3) for oxidative pretreatment on the levofloxacin-pressured AD digesters. US/O3 provides ·OH that facilitates oxidative ring opening of levofloxacin and oxidation of other bio-macromolecules in WAS. Sufficient ·OH will promote oxidative ring opening, resulting in the formation of large amounts of short-chain acids, and the conversion of N-containing groups to NH_4^+. This reaction illustrates the enhanced biodegradability after US/O_3 pretreatment. Ultrasonic treatment in combination with FNA has also been found to provide a positive synergistic effect on sludge breakdown and enhancement of the transformation of the released organic matter from non-biodegradable to biodegradable (Niu et al., 2019). Lin et al. (2016b) found that the ultrasonic-Fenton method could not only degrade the PAHs on the sludge surface, but also degrade the cavities and intracellular substances of the sludge. In addition, Shao et al. (2018) used ultrasound combined with linear alkyl benzene sulfonate (LAS) to control the re-flocculation of flocs for the treatment of digested sludge rich in refractory organics. The surfactant linear alkyl benzene sulfonate (LAS) was added immediately after sonication to avoid re-flocculation of dispersed flocs. The SCOD of sonication was found to be higher than that of pristine digested sludge due to the release of a large amount of refractory organics and the degradation of VS. However, this experiment only proves the dissolution of refractory chemical substances, and whether the conversion is not discussed.

5. Challenges and feature perspectives

AD as a method to realize safe disposal and resource recovery of WAS, a by-product of activated sludge processes, contributes significantly to the goal of global carbon neutrality. Compared to other treatment methods (including landfills, composting and incineration), AD is a welcome and stable method, due to its low energy footprint, low cost, and moderate performance (Deena et al., 2022; Kunatsa and Xia, 2022). However, the application of AD is often limited by low biodegradability due to the presence of endogenous and exogenous refractory substances in WAS. It has been reported that these refractory substances at the environmentally relevant concentrations have adversely affected the anaerobic degradability of sludge. The reasons for these influences can be summarized as follows: ① improved mass transfer resistance, ② enhanced ectoenzymes competition, ③ endoenzymes active sites encroachment, ④ electrons competition, ⑤ cytotoxicity. Corresponding, some strategies to improve the performance of AD have been developed. For example, microwave pretreatment could improve the degradability of refractory pollutants, and thus improving the performance of sludge AD. The sulfite pretreatment could enhance the conversion of refractory substances and reduce the toxicity of anaerobic functional microorganisms. These studies investigated the influence behavior and mechanism of these refractory substances on sludge AD and provided an insight into the improvement of sludge degradability.

To improve the biodegradability of WAS which restricted by endogenous and exogenous refractory substances, a significant number of strategies have been developed. However, some improvements and measures are still required to be further explored to pave the way for efficient, economic and environmental large-scale application of AD for WAS treatment:

1). The quantitative methods of typical refractory substances in sludge are relatively mature, but the basic data of their existence forms in sludge, which are closely related to their effect on sludge anaerobic degradability and anaerobic functional microorganisms, is still limited. At present, most of the studies on their anaerobic degradability and the role of anaerobic functional microorganisms are mostly based on model compounds, which may be different from actual situations. Therefore, the following research should pay more attention to their form of existence and structure.

2). Previous studies on the anaerobic degradability of refractory substances and their effects on anaerobic functional microorganisms are mostly based on a single model compound. However, there are many co-existing refractory substances in the actual system, such as the co-existence of HA and organic flocculants, antibiotics and organic flocculants. Thus, the results obtained previously are not representative and cannot reflect the real situation. Therefore, the follow-up work should pay attention to the co-existing environmental pollutants.

3). At present, characterization methods of sludge anaerobic degradability all rely on biochemical methanogenic potential (BMP) test, which has been used for more than 30 years and has fundamental problems such as long experimental period, large experimental error and strict experimental conditions. In recent years, a series of methods, such as UV-vis spectral method and the three-dimensional excitation-emission matrix fluorescence combined with fluorescence regional integration method, have been used to reflect the biodegradability of dissolved substances. However, these studies have some limitations, such as whether the anaerobic degradation can be truly reflected. Therefore, it is urgent to develop more efficient characterization methods of sludge anaerobic degradability. While, the combination of BMP and fluorescence methods may be a research direction.

4). Although some approaches for refractory substances have been developed, most of them are not specific. In addition to promoting the conversion of refractory substances in sludge, these methods also have influence on the original organic or inorganic substances, such as proteins and polysaccharides. As a result, the efficiency of these preprocessing methods has been affected, resulting in most of them are cost-intensive because of the high-energy and chemical-requirements, and passive environmental-consequences such as higher-net carbon dioxide emission. Therefore, it is necessary to develop more targeted pretreatment methods based on in-depth analysis of the action mechanism of these refractory substances, so as to enhance the AD performance of sludge, as well as improve the circular bio-economy of WAS management.

6. Conclusion

This paper reviews several pretreatment methods and process control technologies to enhance the AD of WAS from the perspective of promoting refractory substances or pollutants conversion. The main mechanisms include: 1) Improving the transformation of sludge substrates by physicochemical method, to provide more available substrates for anaerobic microorganisms, and mitigate the toxicity of refractory substances or pollutants on anaerobic microorganisms; 2) Improving the abundance of functional microorganism, to enhance the biotransformation of refractory substances or pollutants. This study provides a new insight into the development of sludge treatment technology for sludge reduction and stabilization.

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