Effect of humic substances on the anaerobic digestion of secondary sludge in wastewater treatment plants: a review

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Abstract

Anaerobic digestion is a promising technology for energy recovery from secondary sludge, yet the presence of humic substances in wastewater limits anaerobic digestion. In particular, humic substances make secondary sludge denser and more compact, reducing the availability of organic matter for biodegradation. Here we review the impact of humic substances on the anaerobic process, with emphasis on humic substances properties, effect on sludge structure and composition, effect on hydrolysis, acidolysis and methanogenesis, evolution of humic substrances, and strategies to counteract negative impact. Strategies include removing humic substances, pretreatment of secondary sludge prior anaerobic digestion, and addition of metal salts, enzymes and organisms. We observed that humic substances with a high E4/E6 ratio, representing the absorbance determined at 465 nm and 665 nm, a low carbon/nitrogen ratio, and a low aromaticity are easier to digest anaerobically. The liquid-solid phases distribution of humic substances influences anaerobic digestion efficiency, and the repolymerisation of humic substances during anaerobic digestion reduces sludge degradability.

Keywords Humic substances · Structure and composition of sludge Dynamic evolution · Mitigate inhibition strategies Metal salts

1. Introduction

Energy recovery from secondary sludge in anaerobic digestion processes is crucial for wastewater treatment plants (Li et al. 2017a). However, the presence of humic substances in secondary sludge poses challenges to achieving high anaerobic digestion efficiency (Li et al. 2019b; Liu et al. 2015). Understanding the impact of humic substances on anaerobic digestion performance and developing effective mitigation strategies are of paramount importance, yet a comprehensive summary of their effects remains lacking.

The activated sludge process has been widely adopted by wastewater treatment plants to treat wastewater (He et al. 2021; Liu et al. 2023). Organic and inorganic substances in wastewater are absorbed into activated sludge and eventually end up in secondary sludge, which becomes an ideal resource for renewable energy production (Pan et al. 2022). Anaerobic digestion has been regarded as an effective approach to recovering chemical energy from secondary sludge (Li et al. 2017a; Liu et al. 2021b; Wang et al., 2017; Wang et al. 2019a; Zhang et al. 2022b), its conversion efficiency of organic matter to energy is usually less than 50% (McCarty et al. 2011; Raposo et al. 2012). The presence of humic substances naturally occurring in secondary sludge and their release into the liquid phase of anaerobic digestion has been identified as a significant factor hindering anaerobic digestion's efficiency (Li et al. 2019b; Liu et al. 2015). Moreover, the presence of stubborn bacterial cell walls and refractory organic matter in secondary sludge also greatly impacts the anaerobic digestion efficiency (Li et al.

2019a). Diverse methods have been adopted to enhance methane accumulation in anaerobic digestion, such as optimization of operational conditions (Wu et al. 2019), the addition of additives (Feng et al. 2014), and pre-treatment before anaerobic digestion (Li et al. 2016). While these techniques facilitated the destruction and the subsequent energy recovery from secondary sludge, they also released more humic substances from the solid to the liquid phase, becoming an inhibitor to energy recovery from secondary sludge in anaerobic digestion.

Humic substances are typical compounds in secondary sludge, accounting for 20–30% of volatile solids (Xu et al. 2020). They usually possess the characteristics of a large molecular size, aromatic structure, and oxygen-containing functional groups, which complicate decomposition and further utilisation by anaerobes (Li et al. 2017b). Humic substances in secondary sludge are sourced from dead microorganisms, animal residues, and plant degradation and polymerisation (Liang et al. 2021; Xu et al. 2020), while some have been found to generate *in situ* during anaerobic digestion processes due to the transformation of protein-like substances (Dai et al. 2013). The inhibition effects of humic substances on anaerobic digestion have been observed to reduce methane production (Li et al. 2021; Liu et al. 2019a), likely due to the inhibition by humic substances of functional enzyme activities associated with hydrolysis and acidification (Huang et al. 2021), as well as competition between humic substances and methanogens for electrons (Li et al. 2019b). However, inconsistent observations have also been reported in which humic substances acted as promoters that facilitated methane yield

(Huang et al. 2021; Zhou et al. 2014). The effect of humic substances on anaerobic digestion processes and the associated mechanisms thus remains poorly summarised. Furthermore, various technologies have been proposed to alleviate the inhibition of anaerobic digestion processes by humic substances, such as the removal of humic substances from wastewater, the pretreatment of secondary sludge before anaerobic digestion, the addition of metal salts, and the addition of enzymes or the introduction of endogenous or exogenous organisms (Azman et al. 2015; Hu et al. 2022; Li et al. 2014; Li et al. 2021; Millati et al. 2020). However, the performance of these methods has greatly varied (Fernandes et al. 2015; Li et al. 2021). To date, a comprehensive summary of the mechanisms and mitigation strategies related to humic substances in anaerobic digestion processes remains lacking, which hinders the development of energy recovery from secondary sludge using these processes.

This review aims to introduce novel insights into the impact of humic substances on anaerobic digestion processes, addressing key aspects that have not been comprehensively summarized before. Specifically, this review focuses on Ⅰ) unveiling the characteristics of humic substances and their impact on the structure and composition of secondary sludge; Ⅱ) investigating the intricate influence of humic substances on crucial steps of anaerobic digestion processes, i.e., hydrolysis, acidification, and methanogenesis, shedding new light on their complex interactions; Ⅲ) exploring the dynamic evolution of humic substances in anaerobic digestion processes, providing novel insights into their transformation and behavior; and Ⅳ) examining the effects and mechanisms of methods that mitigate inhibition of anaerobic digestion by humic substances, presenting promising approaches for improving energy recovery efficiency. This review deepens our understanding of the effects of humic substances on anaerobic digestion processes and provides challenges and research recommendations for future research development.

2. Influence of humic substances on secondary sludge structure

2.1 Characteristics of humic substances

Humic substances are the second-most abundant organic substance in secondary sludge, accounting for 20–30% of the volatile solids (Wang et al. 2023; Xu et al. 2020). They originate from wastewater and are enriched in secondary sludge due to biosorption (Kulandaivelu et al. 2020; Ryu et al. 2021). Nonetheless, the definitive structure of humic substances remains undefined, owing to their diverse chemical composition (Baveye and Wander 2019). Noteworthy, the average elemental composition of humic substances is relatively consistent, mainly composed of carbon, oxygen, nitrogen, hydrogen, and sulfur (Nardi et al. 2021). Previous studies have reported that humic substances originated from numerous materials such as leonhardite, soil, and compost manure, which typically exhibit similar levels of carbon content, but differ in terms of oxygen, hydrogen, and nitrogen content. In contrast, humic substances obtained from secondary sludge are characterized by a high concentration of nitrogen and hydrogen and a low concentration of oxygen in their composition (Yang and Li 2016). Humic substances also contain diverse functional groups, including carboxyl, alcoholic hydroxyl, quinone, ketone, and phenolic groups (Lipczynska-Kochany 2018; Wang et al. 2022c). This structure allows humic substances to combine with diverse organic matter, such as organic acids, fatty acids, lipids, amino acids, and others (Nardi et al. 2021).

Based on water solubility, humic substances can be further divided into three categories: humic acid (soluble at pH > 2), fulvic acid (soluble at all pH values), and humin (insoluble at all pH values) (Ghaneian et al. 2014; Liu et al. 2019a). These fractions can vary remarkably in their chemical composition, molecular size, and properties, such as hydrophobicity, and aromaticity (Rigobello et al. 2017). Moreover, the structure of humic substances is dynamic and can be influenced by various environmental factors such as pH, and ionic strength, which dominates the size and shape of the specific fraction of humic substances (Klučáková 2018).

2.2 Effect of humic substances on secondary sludge structure and composition

Typically, sludge flocs (Fig. 1) mainly contains three layers, namely soluble, lightly bound, tightly bound extracellular polymeric substances (Liu et al. 2020), and consisted of humic substances, protein, carbohydrate, bacteria cell, metal ions, and other organic matters, and so on (Kulandaivelu et al. 2020; Wang et al. 2023). Humic substances develop strong links (dotted line in Fig. 1) with inorganic, such as metal ions and organic matter, such as proteins and polysaccharides, which lowers the solubility of the latter in secondary sludge. Furthermore, the functional groups of humic substances react with biodegradable organic matter through absorption, sedimentation, and aggregation (He et al. 2016; Siddiqui et al. 2020; Xu et al. 2020; Zhou et al. 2023), and with inorganic matter through the bridge, electrostatic, and steric interactions (Calabi-Floody et al. 2011; Zhang et al. 2021b), which result in a more compact, denser, and stable secondary sludge structure (Fig. 1). For example, the addition of humic substances enhanced the fractal dimension of sludge by 8.7% and decreased the total surface energy, soluble total organic carbon, and transverse relaxation times by 5.8%, 36.1%, and 24.4%, respectively, indicating that humic substances can combine with organic matter in secondary sludge and improve the sludge floc structural stability (Xu et al. 2020). Additionally, the enhancement of extracellular polymeric substances' spatial configuration, along with the decrease in its random-coil degree due to humic substances, reduces the active sites in extracellular polymeric substances in combination with extracellular enzymes (Xu et al. 2020), which inhibits the hydrolysis and acidification potential of secondary sludge in anaerobic digestion processes.

Apart from influencing the structures of organic substances in secondary sludge, humic substances also affect their organic substance composition (Yang et al. 2019) due to their nature as recalcitrant substances and important components of the organic matter (Wang et al. 2019b). Studies have confirmed that pretreatment efficiently promotes sludge anaerobic digestion efficiency, as shown by significant changes in recalcitrant substance content, such as humic substances during pretreatment conditions (Liu et al.

2020; Wang et al. 2022b). However, in most cases, the hydrolysis degree of secondary sludge is represented by protein and polysaccharide variations, while the recalcitrant organic substance variation is not included (Lu et al. 2020). A suitable index is thus needed to describe the variation of recalcitrant organic substances. Three-dimensional fluorescence spectroscopy can provide a pathway to analyze the amount of recalcitrant organic substances and a reference for future studies (Geng and Zhou 2022). This technology has been successfully used to quantify variations of humic substances content, such as changes from 33.26% to 23.05%, 36.2% to 15.9%, and 29.4% to 10.5% after pretreatment with sodium percarbonate (Wang et al. 2022b), heat-CaO_2 (Liu et al. 2019b), and sulfite (Liu et al. 2020), respectively. Overall, the impact of humic substances on the structure and composition of secondary sludge might affect the efficiency of anaerobic digestion, as discussed in the following.

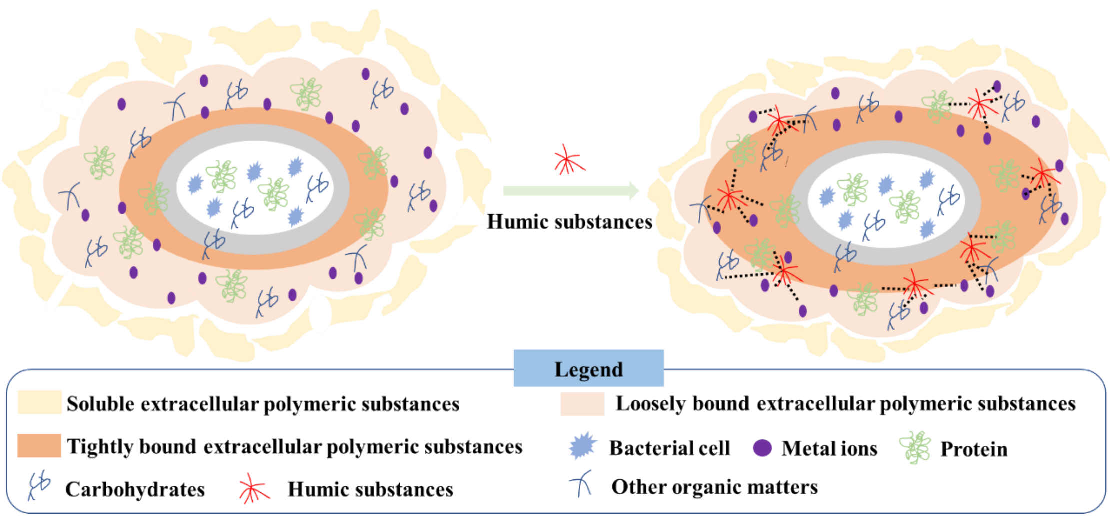

Figure 1. Proposed humic substances' interactions with organic and inorganic substances in sludge. The presence of humic substances can make secondary sludge denser and more compact, reducing the availability of organic matter.

3. Humic substances on the influence of each stage in anaerobic digestion

Anaerobic digestion of secondary sludge typically involves three interlinked stages: hydrolysis, acidification, and methanogenesis (Guo et al. 2022). In the hydrolysis stage, polysaccharides and proteins are transformed into monosaccharides and amino acids. Hydrolysis products are further transformed into short-chain fatty acids in the acidification stage. These acidification products are ultimately transformed into methane as the end-product of anaerobic digestion. The impact of humic substances on anaerobic digestion efficiency in each stage and the associated mechanisms are discussed in the following sections (and summarised in Table 1 and Fig. 2).

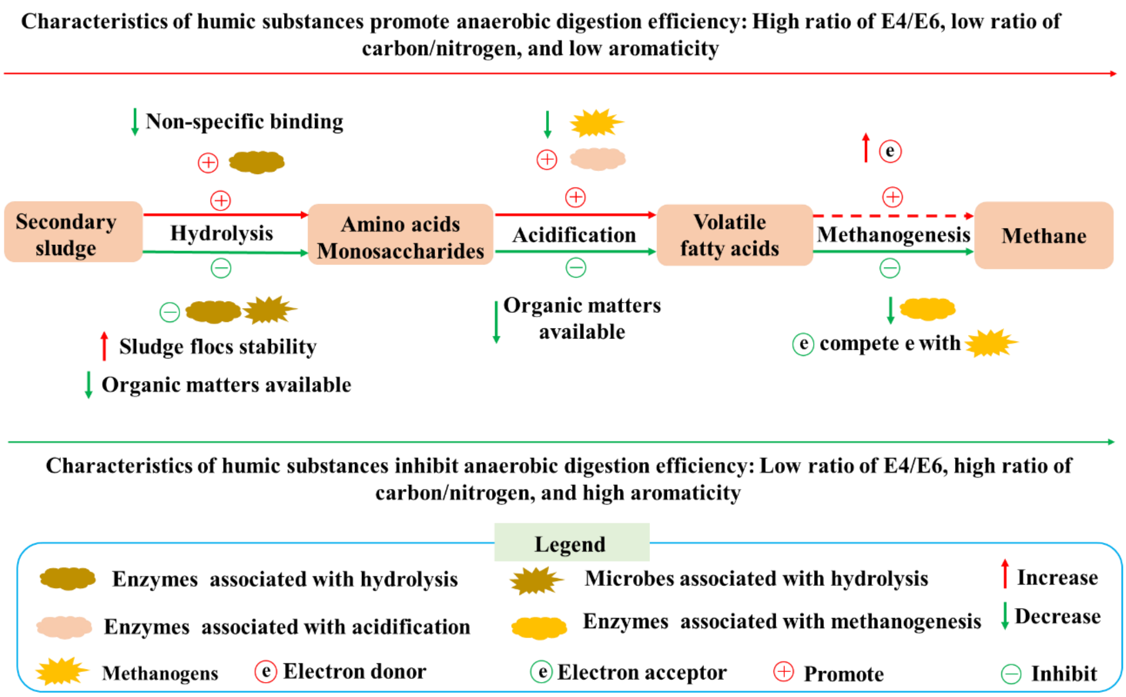

Figure 2. Influence of humic substances on each stage of anaerobic digestion. Note: E4/E6 refers to the ratio of absorbance determined at 465 nm and 665 nm (E6) of humic

substances.

Table 1 Influences of humic substances on each step in the anaerobic digestion of secondary sludge

Hydrolysis	Acidification	Methanogenesis	Note	References
Positive: reduces non-specific binding of enzymes, enhances enzymatic hydrolysis efficiency	**Not available**	**Not available**	Commercial humic substances	Tang et al. (2020a)
Positive: improves hydrolytic enzyme activity due to its strong hydrophobicity and protection of enzyme activity	**Positive**: quinone structure of humic substances serves as an electron acceptor	**Negative**: inhibits acetoclastic methanogen activity and blocks the conversion of acetyl-CoA to 5-methyl-tetrahydromethanopterin	Commercial humic substances (SHHA) Higher hydrophobicity and aromaticity	Liu et al. (2015)
Positive: electron transfer capacity of humic substances promotes hydrolase activity, i.e., protease and amylase	**Positive**: electron transfer capacity of humic substances promotes enzyme activities related to acidification, such as acetate kinase, butyric kinase, and phosphotransacetylase	**Negative**: electron transfer capacity of humic substances inhibits F420-reducing hydrogenase activity	Humic substances extracted from sludge	Wang et al. (2022a)
Positive: increases microorganism activity and metal regulation by chelation	**Not available**	**Not available**	Humic substances extracted from peat	Hartung (1992)
Negative: inhibits hydrolytic	**Negative**: low ratios of	**Positive**: increases the activity of	Humic substances	Huang et

protease activity through humic substances accumulation outside of anaerobe cells	E4/E6 and carbon/nitrogen make humic substances more difficult to decompose in anaerobic digestion	enzymes associated with methanogenesis due to small molecular humic substances passing through the cell membrane and accelerating electron transfer	extracted from sludge	al. (2021)
Negative: inhibits key enzyme activity, such as α-amylase and protease due to the high mobility of humic substances and easy access to enzymes	**Positive**: quinone functional groups of humic substances possess good electron transfer chains and behave as catalysts	**Negative**: lowers enzyme F420 activity, humic acid competes for electrons with acetate and prevents its conversion to methane, and humic acid might compete for metabolites, which blocks the methanogenesis pathway	Commercial humic substances	Li et al. (2019b)
Negative: inhibits hydrolytic bacteria activity, such as *Clostridiales*, *Bacteroidales*, and *Anaerolineales*)	**Not available**	**Negative:** inhibits the activity of hydrogenotrophic methanogens *Methanobacteriaceae*, *Methanomicrobiales*-WCHA208, and Unassigned *Thermoplasmata* WCHA1-57	Commercial humic substances	Azman et al. (2017)
Negative: increases sludge floc structural stability, reducing organic content availability	**Negative**: less amount of low molecular quality organic substances available	**Negative**: changes the accessibility of hydrolysis products, leading to poor anaerobic bioconversion	Commercial humic substances	Xu et al. (2020)

Note: E4/E6 refers to the ratio of absorbance determined at 465 nm and 665 nm of humic substances. SHHA means commercial humic substances from Shanghai Reagent Company.

3.1 Hydrolysis stage

Conflicting observations have been reported regarding the influence of humic substances on the hydrolysis stage of anaerobic digestion (Table 1). Tang et al. (2020a) demonstrated that the efficiency of enzymatic hydrolysis increased from 64.9% to 81.8% in the presence of humic substances (obtained from waste wheat straw), which reduced the non-specific binding of hydrolase. Liu et al. (2015) revealed that the strong hydrophobicity of commercial humic substances benefitted the formation of protective micelles, which enhanced hydrolytic enzyme activity, including protease and α-glucosidase, thus accelerating hydrolysis. Similar observations were reported by Wang et al. (2022a) using humic substances derived from sludge and by Hartung (1992) using humic substances obtained from peat, which respectively increased the electron transfer capacity or the activity of microorganisms responsible for hydrolytic enzyme secretion and hydrolysis promotion.

In contrast, Huang et al. (2021) found that proteinase activity decreased by 20% under a 7.5 mg humic substances/g volatile solids stress condition, which was attributed to hydrolytic protease inhibition due to the interaction between humic substances and functional enzymes or substrates and the accumulation of humic substances (from sludge) outside anaerobe cells. This research also found that a low E4/E6 ratio (refers to the ratio of the absorbance determined at 465 nm and 665 nm) and high carbon/nitrogen ratio under humic substances stress conditions make humic substances more difficult to decompose in the hydrolysis stage of anaerobic digestion and increase the possibility of the combination between enzymes and humic substances (Huang et al. 2021). The presence of humic substances resulted in up to 38.2% inhibition of hydrolysis due to their superior mobility and easy contact with enzymes, i.e., proteinase and α-amylase, and such inhibition effects were dependent on both the concentration and structure of humic substances (Li et al. 2019b). Azman et al. (2017) observed up to 40% inhibition of hydrolysis efficiency by humic substances, which was attributed to the reduced hydrolytic bacterial activity, such as *Clostridiales*, *Bacteroidales*, and *Anaerolineales*. Another study additionally revealed that humic substances enhanced sludge floc structural stability, which reduced the organic content bioavailable to anaerobes in the liquid phase, thus inhibiting hydrolysis efficiency (Xu et al. 2020).

Inconsistent observations of the impact of humic substances on hydrolysis are likely caused by the sources, structures, molecular weights, functional groups, and concentrations of humic substances, as well as the microbial community in the secondary sludge. Specifically, the presence of humic substances has promoted hydrolysis as in Tang et al. (2020a), Liu et al. (2015), and Huang et al. (2021), but the magnitudes of these changes have varied greatly. This may be caused by the different sources of humic substances (waste wheat straw versus commercial humic substances versus peat). Nonetheless, although both Wang et al. (2022a) and Huang et al. (2021) utilized humic substances from sludge, hydrolysis was promoted in the former but inhibited in the latter study. This discrepancy might be due to differences in the humic substances' structure and functional groups. Those in Wang et al. (2022a) possessed higher nitrogen-content and oxygen-containing groups than those in Huang et al. (2021), which potentially increased the electron transfer capacity. This higher capacity subsequently may have made the interactions between negatively charged humic substances and enzymes stronger than those between hydrophobic interactions and enzymes, resulting in the release of hydrolytic enzymes and facilitating their activity (Li et al. 2019a; Liu et al. 2015). Moreover, greater inhibition of hydrolysis efficiency by humic substances was observed in (Azman et al. 2017), although the same commercial humic substances were used. Such differences might be related to variations in the humic substances concentrations and corresponding differences in the microbial community adaptation capacity.

3.2 Acidification stage

Most studies have revealed the positive effects of humic substances on the acidification process during anaerobic digestion (Li et al. 2019b; Liu et al. 2015; Wang et al. 2022a). For example, the short-chain fatty acid yield increased from 1379 mg chemical oxygen demand (used as an indicator that measures the amount of oxygen consumed by inorganic and organic compounds)/L to 2741 mg chemical oxygen demand/L when the dosage of SHHA (commercial humic substances from Shanghai Reagent Company) increased from 420 mg-SHHA/g volatile solids to 1120 mg-SHHA/g volatile solids, possibly because the presence of quinone structures in the humic substances accelerated the bioreactions of pyruvate to acetyl-CoA and glyceraldehyde-3p to D-glycerate 1,3-

diphosphate (Liu et al. 2015). Li et al. (2019b) also observed a 101.5% increase in acidogenic efficiency at 150 mg humic substances/g volatile solids. Additionally, a recent study revealed that humic substances contributed to electron transfer chains and acted as catalysts, promoting the activities of enzymes responsible for the acidification stage, such as acetate kinase, phosphotransacetylase, and butyric kinase (Wang et al. 2022a). Notably, the electron transfer chain contains two parts (electron acceptor capacity and electron donor capacity); the former was determined by nitrogen content, humification index, oxygen-containing groups, and aromatic groups (Tan et al. 2017), while the latter was only determined by nitrogen content and oxygen-containing groups (Wang et al. 2022a).

In contrast, some studies showed an inhibitory effect of humic substances on the acidification stage of anaerobic digestion (Huang et al. 2021; Xu et al. 2020). The concentration of short-chain fatty acids decreased from 9800 to 4800 mg/L, and the acidification rate was inhibited by 50% when the humic substances concentration increased from 1.0 to 2.5 mg humic substances/g volatile solids (Huang et al. 2021). This phenomenon might be attributed to the low E4/E6 and high carbon/nitrogen ratios of humic substances, making them more difficult to decompose and reducing the number of organic substances available for subsequent short-chain fatty acid production (Xu et al. 2020). The inconsistent observations regarding the impact of humic substances on the acidification stage of anaerobic digestion likely arose from differences between the structure, molecular weight, and functional group content, such as phenolic group and carboxyl group of the humic substances. The commercial humic substances separately adopted by Liu et al. (2015) and Li et al. (2019b) enhanced short-chain fatty acids to varying degrees, which was ascribed to differences in the molecular weight and chemical structure of the humic substances. In addition, the maximum short-chain fatty acids were obtained with different concentrations of humic substances (1120 versus 150 mg humic substances/g volatile solids) and functional group contents, i.e., phenolic group and carboxyl group, leading to differences in both the quinone structure number in humic substances and the bioconversion of organic substances to short-chain fatty acids (Li et al. 2019b; Liu et al. 2015).

3.3 Methanogenesis stage

Many studies have found that the addition of humic substances inhibits methane production in anaerobic digestion (Azman et al. 2017; Li et al. 2019b; Liu et al. 2015; Wang et al. 2022a; Xu et al. 2020). For instance, methane production was severely inhibited by 14% to 97% when the SHHA levels were increased from 430 mg humic substances/g volatile solids to 1430 mg humic substances/g volatile solids (Liu et al. 2015). This inhibition was mainly caused by the competition of humic substances with acetoclastic methanogens for electrons, severely inhibiting acetoclastic methanogen activity and the bioreaction of acetyl-CoA to yield 5-methyl-tetrahydromethanopterin (Liu et al. 2015). Azman et al. (2017) observed a similar inhibition effect but attributed the lower methane yield to lower activity of hydrogenotrophic methanogens, such as *Methanobacteriaceae*, *Methanomicrobiales*-WCHA208, and Unassigned

Thermoplamata WCHA1-57 rather than that of acetoclastic methanogens, due to the lower acclimatization capacity of the hydrogenotrophic methanogen microbial community with the addition of humic substances. Li et al. (2019b) found that the methanogenic efficiency decreased by 52.2% after adding 150 mg humic substances/g volatile solids, which may have been due to lower F420 activity (a key enzyme in methane biosynthesis), competition between humic substances and acetate for electrons, and blocking between humic substances and methanogens for metabolites. In addition, Xu et al. (2020) found that a reduced methane yield with humic substances addition was due to humic substances changing the bioaccessibility of hydrolysis products, which resulted in poor bioconversion from glucose to biogas.

Nonetheless, controversial outcomes have been reported in some studies. Huang et al. (2021) found that methanogenesis was enhanced approximately two-fold, and coenzyme F420 activity increased by 19% under 10 mg humic substances/g volatile solids conditions. The enhanced enzyme activity might have been caused by small molecular weight humic substances passing through the cell membranes of anaerobes and enhancing electron transfer capacity. This result was further confirmed by Zhao et al. (2023a), in that study, small molecular humic substances acted as electron donors, accelerating the electron transport capacity of methanogens and resulting in a 15.8–80.8% enhancement of methane production. Noteworthy, the promotion effect of humic substances on methane production was observed during the thermal hydrolysis pretreatment of sludge which caused the variation of the humic substances molecular structure, including carbon/nitrogen ratio, functional group, elemental composition, and E4/E6 ratio of humic substances (Huang et al. 2021; Zhao et al. 2023a). Additionally, the low concentration of humic substances adopted in those studies has also been found to play a key role in facilitating methane production. However, in traditional wastewater treatment plants, secondary sludge does not undergo thermal hydrolysis pretreatment before entering anaerobic digestion reactors due to high energy demand, which increased the operating costs of the wastewater treatment plants, and the concentration of humic substances in the secondary sludge is typically much higher than the concentration required to promote methane production (0.2-0.3 g/g volatile solids (humic substances content in secondary sludge) versus 0.05 g/g volatile solids (the adopted humic substances concentration that observed to enhance the methane production after the pretreatment of secondary sludge)).

Taken together, it can be concluded that the high concentration of humic substances in the secondary sludge (far higher than the concentration of humic substances required for promoting methane production) has a negative impact on the performance of anaerobic digestion in the traditional wastewater treatment plants. Based on this, measures need to be taken to alleviate the inhibitory effects of humic substances on anaerobic digestion and improve its overall efficiency. A deeper understanding of the dynamic evolution of humic substances during anaerobic digestion is crucial in formulating strategies to mitigate their inhibitory effects on the process. In the following sections, we will first discuss the dynamic evolution of humic substances and then explore strategies to mitigate their inhibitory effects.

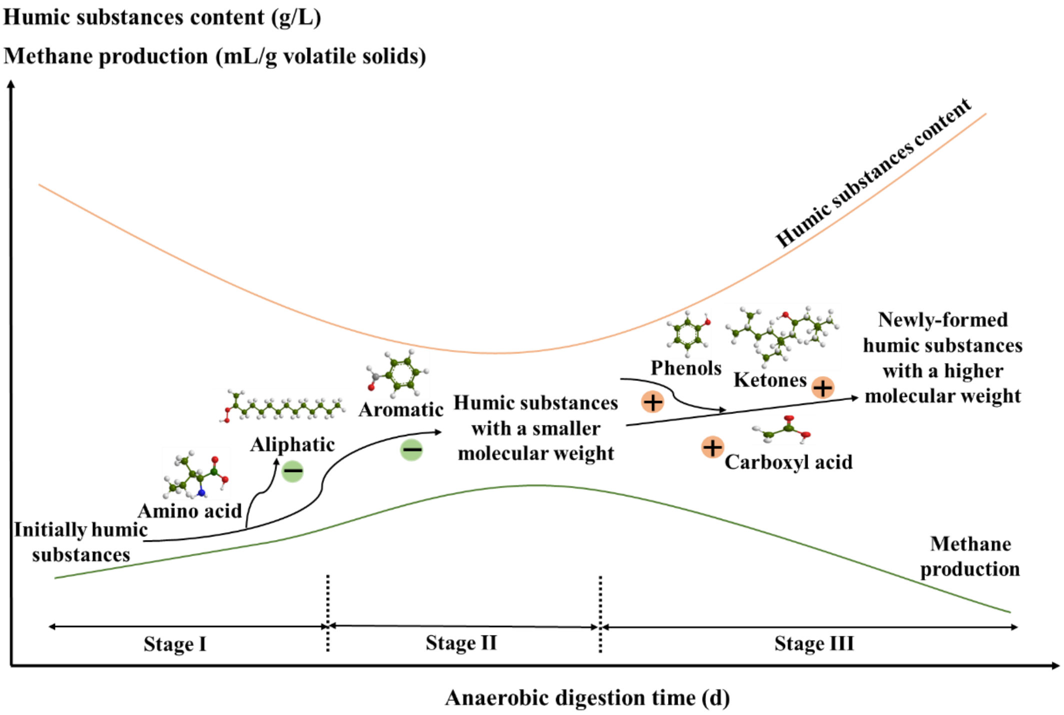

Figure 3. Dynamic evolution of humic substances in anaerobic digestion. Note: Stage I, stage II, and stage III represent the consumption of labile aliphatic and exposure of aromatic in humic substances, biodegradation of labile aromatic structure in humic substances, and repolymerization of humic substances, respectively. The minus sign in the green circle and the plus sign in the orange circle mean depolymerization and repolymerization, respectively. The distribution of humic substances in liquid and solid phase affect anaerobic digestion efficiency, and the repolymerisation of humic substances in anaerobic digestion reduces sludge degradability.

4. Evolution of humic substances during anaerobic digestion

Studying the dynamic evolution of humic substances in anaerobic digestion processes helps to provide an in-depth understanding of their behavior and influencing factors in anaerobic digestion reactors, thereby benefiting to development and implement strategies that improve the overall performance of anaerobic digestion. Variations of the humic substances' content and structure have been commonly reported during the anaerobic digestion process. The dynamic evolution of humic substances during anaerobic digestion typically passes through three phases (Tang et al. 2018) (Fig. 3): 1) superficial labile aliphatics are extensively consumed by microorganisms as energy sources, causing aromatic exposure that enhances the aromaticity of humic substances; 2) some labile aromatic structures in humic substances are further biodegraded by anaerobes; and 3) more stable and unsaturated humic substances structures form due to the repolymerisation of humic substances with new aromatics. The variations of the specific content and structure of humic substances in anaerobic digestion are discussed in detail in the following sections.

4.1 Evolution of humic substances content

Several studies have demonstrated *in situ* biosynthesis, degradation, and repolymerisation of humic substances during anaerobic digestion (Dai et al. 2013; Li et al. 2013; Wang et al. 2021b). For example, Li et al. (2013) not only revealed that humic substances were the key components of extracellular polymeric substances but also found that they emerged in microbe cells. Dai et al. (2013) observed that the humic substances content in digested sludge (30.1%) was higher than that in raw secondary sludge (20.0%), indicating the generation of humic substances during anaerobic digestion, where humic substances content augmentation was attributed to the transformation of protein-like substances. Wang et al. (2021b) confirmed the simultaneous degradation and repolymerisation of humic substances in anaerobic digestion, as the humic substances content first decreased from 8.4 g/L to 4.6 g/L but then increased to 7.7 g/L during mesophilic anaerobic digestion. Liu et al. (2019a) controversially observed that humic substances were not formed but rather degraded in anaerobic digestion. Specifically, the humic substances content in the anaerobic digestion system with humic substances-free sludge remained almost unchanged but decreased in the anaerobic digestion system with sludge from wastewater treatment plants. These conflicting observations might be attributed to two aspects: 1) the complex composition of humic substances in real sludge had a high percentage of small molecular weight moieties and aliphatic moieties, which were oxidized in anaerobic digestion, leading to reduced humic substances content (Li et al. 2013); and 2) the organic loading rate of sludge from wastewater treatment plants was higher than that of humic substances-free sludge (0.077 mg/L versus 0.06 mg/L). This indicated that more aliphatic moieties of humic substances could be oxidized, which may explain the inconsistency of humic substances variation (Liu et al. 2019a). Additionally, the distribution of humic substances in the solid and liquid phases during anaerobic digestion processes has been shown to vary significantly; the humic substances content in the solid phase gradually decreased by 47.6%, while that in the liquid phase increased by 92.2% (Liu et al. 2019a). Tang et al. (2020b) similarly reported that 49% of humic substances were released from the solid to the liquid phase during anaerobic digestion.

The distribution of humic substances in solid and liquid phases, rather than the overall humic substances content, are more likely to influence anaerobic digestion performance.

4.2 Evolution of the humic substances structure

Changes in the humic substances structure due to anaerobic digestion can be mostly observed in three aspects: 1) increased humification degree of humic acid and fulvic acid, as evidenced by augmented total acidity (phenolic and carboxyl moieties) (Liu et al. 2019a); 2) degradation of aliphatic moieties by microorganisms due to their simple, linear, and vulnerable structure, while aromatic moieties are subsequently more exposed to the environment (El Fels et al. 2014; Wang et al. 2021b); and 3) the repolymerisation of aromatic moieties in humic substances. Through repolymerisation, organic compounds combine with aromatic moieties in humic substances through the actions of enzymes and microorganisms to form more complex structures, which are more difficult for bacteria to decompose and utilize. The repolymerisation of aromatic moieties generally follows three main routes: 1) consumption of aliphatic moieties conducive to transforming polysaccharides into other oxidized compounds (Liu et al. 2019a); 2) polymerization of non-humic substances or biodegradable fulvic acid with humic substances aromatic structures (Awasthi et al. 2017); and 3) use of C=C in aromatics from lignin to compose the basic structures of newly formed humic substances (Zheng et al. 2014).

The anaerobic digestion process changes humic substances structures by increasing the molecular size and the amounts of aromatic structures and oxygen functional groups. In an anaerobic digestion reactor, the ratio of oxygen/carbon was raised from 0.32 to 0.39, while the ratio of carbon/nitrogen was reduced from 9.06 to 6.41, accompanied by an increase in humic substances total acidity from 2.32 mmol/g to 3.29 mmol/g (Li et al. 2017b). The proportion of humic substances with average molecular weights over 50 kDa and 100 kDa also rose from 14.7% to 18.4% and from 65.3% to 85.5%, respectively (Li et al. 2017b). Wang et al. (2021a) revealed that the contents of oxygen-containing and aromatic moieties after anaerobic digestion increased from 37% to 45% and from 41% to 66%, respectively, indicating augmented degrees of condensation and humification. Liu et al. (2019a) also observed that the variation in the humic substances structure was caused by the consumption of aliphatic moieties and the enrichment of aromatic moieties. Notably, the release of humic substances from the solid to the liquid phase during anaerobic digestion shows that it promotes humic substances solubilization. This phenomenon might be explained by the augmentation of oxygen-containing functional groups after anaerobic digestion, which improves the hydrophilicity of humic substances and thus enhances their solubility (He et al. 2023; Liu et al. 2019a).

Table 2 Methods to mitigate the inhibition of anaerobic digestion by humic substances

Technologies	Conditions	Enhancement of biogas production	References
Pretreatment of secondary sludge before anaerobic digestion	140 mg calcium peroxide/g volatile solids	47.6%	Wang et al. (2019)
	78.91 mg calcium hypochlorite/g volatile solids	33.8%	Hu et al. (2022)
Addition of metal salts	5 mM calcium ion	128%	Azman et al. (2015)
	5 mM magnesium ion	146%	
	5 mM iron ion	145 %	
	100 mg/L calcium ion, 70 mg/L aluminium ion	Not available	Li et al. (2021)
Addition of enzymes	Hydrolytic enzymes	Not available	Fernandes et al. (2015)
	Amylase	19%	Yu et al. (2013)
	Protease	16%	
	Amylase and protease	20%	
Introduction of endogenous or exogenous organisms	*Ochrobactrum* sp. POC9	20%	Poszytek et al. (2018)

5. Strategies to mitigate the inhibition of anaerobic digestion by humic substances

To mitigate the inhibition effect of humic substances on anaerobic digestion performance, several methods have been adopted, including the removal of humic substances from wastewater, the pretreatment of secondary sludge before anaerobic digestion, the addition of metal salts to reduce humic acid binding ability, the addition of enzymes to bind with humic substances or promote humic substances decomposition, and the introduction of endogenous or exogenous organisms to facilitate humic substances degradation (Table 2). The performance of each strategy is discussed in the following sections.

5.1 Removal of humic substances from wastewater

Researchers have developed numerous methods to remove humic substances from wastewater, including physicochemical treatments such as adsorption, filtration, advanced oxidation processes, electrochemical, and so on. (Algamdi et al. 2019; Sinha and Ghosal 2023; Yin et al. 2020; Zhang et al. 2021a). Humic substances removal can achieve high efficiencies, reaching up to 99% through adsorption using magnetic biochar at a lower pH of 3-4 (Zhang et al. 2021a), 94.5% via ultrafiltration membranes (graphene oxide-polyethersulfone) (Algamdi et al. 2019), 96% with the addition of TiO_2/coconut shell powder (Sinha and Ghosal 2023), and 90% through electrochemical treatment (Yin et al. 2020). Additionally, biological treatments involving the use of enzymes responsible for humic substances degradation have also been investigated

(Zahmatkesh et al. 2017; Zhu et al. 2022). Notably, the incorporation of purified peroxidase has shown great potential in achieving nearly complete elimination of humic substances from wastewater (Zhu et al. 2022). These methods have effectively removed humic substances from wastewater and reduced their accumulation in secondary sludge, thereby enhancing the subsequent anaerobic digestion efficiency of secondary sludge. However, it is crucial to acknowledge the potential challenges associated with these approaches. Implementing physicochemical treatments usually demands substantial energy and chemical inputs, resulting in increased operational costs for wastewater treatment plants, posing economic challenges for large-scale applications (Rashtbari et al. 2020). Moreover, the stability of biological treatments employing enzymes for humic substances degradation can be affected by environmental factors, leading to fluctuations in microbial activity (Zhu et al. 2022). Although these extraction approaches can be successfully applied at the laboratory scale, their practical and economic feasibility in pilot or full-scale applications remains questionable due to extremely high operational costs.

5.2 Pretreatment of secondary sludge before anaerobic digestion

Pretreatment of secondary sludge means the addition of compounds with high oxidation capacities, such as calcium peroxide, calcium hypochlorite, sodium perborate in terms of enhancing the biodegradation of humic substances (Hu et al. 2022; Millati et al. 2020). These compounds can destroy the structures of humic substances, leading to a reduction of polymerization and generating biodegradable organic matter with small molecular weights. For example, the pretreatment of secondary sludge with calcium peroxide (0.14 g/g volatile solids) enhanced methane production by 47.6%, which was mainly attributed to the degradation of humic substances and the generation of biodegradable organic substances (Wang et al. 2019b). Calcium hypochlorite (78.91 mg/g volatile solids) pretreatment of secondary sludge enhanced methane production by 33.8%, partly due to enhanced humic substances biodegradability, and reduced the humic substances concentration (Hu et al. 2022). Studies have also shown that sodium perborate, peracetic acid, and ozone improved humic substances degradation (Ai et al. 2019; Ding et al. 2019; Yin et al. 2011). However, compounds with high oxidation capacities not only degrade humic substances in sludge but also other soluble organic substances, resulting in the oxidation of many soluble organic substances to carbon dioxide and ultimately providing fewer organic substances to anaerobes for subsequent biogas production. Also, the high prices of these compounds have limited their application.

5.3 Addition of metal salts

A variety of metal salts, such as calcium, magnesium, iron, and aluminum, have been added to anaerobic digestion to alleviate the inhibition caused by humic substances, with potential mechanisms shown in Fig. 4. The individual addition of calcium, magnesium, or iron salts greatly reduced the inhibition of humic substances on hydrolysis, with hydrolysis efficiencies increasing by 65%, 75%, and 72%, respectively (Fernandes et al. 2015). The corresponding methane production was also enhanced by

128%, 146%, and 145% compared with the respective group without added metal salts (Fernandes et al. 2015). This might be attributed to the presence of diverse functional groups, i.e., carboxylic, ketones, phenols in humic substances in sludge, which provide superior metal-binding abilities through complexation and adsorption reactions (He et al. 2016). Interestingly, the addition of sodium and potassium salts did not significantly mitigate the inhibition of humic substances on hydrolysis efficiency, which might due to the lower complexation ability of sodium and potassium salts with humic substances (Azman et al. 2015). The addition of 100 mg/L calcium or 70 mg/L aluminum salts greatly reduced the inhibition of humic substances on hydrolysis efficiency by up to 65%, which was likely caused by the binding of metal salts with humic substances through sweep flocculation, cation exchange, and electrostatic forces (Li et al. 2021). The binding of metal salts to humic substances reactivated hydrolytic enzymes and eventually increased the degradation of organic substances in anaerobic digestion (Li et al. 2021). Furthermore, synergistic effects in alleviating inhibition by humic substances can be achieved by the joint addition of two metal ions at low concentrations. Specifically, the addition of calcium at 50 mg/L and aluminum salt at 10 mg/L restored 80% of hydrolysis efficiency in anaerobic digestion in the presence of humic substances (Li et al. 2021). These studies have shown that metal salt addition can mitigate inhibition by humic substances in the hydrolysis stage, and the enhancement of hydrolysis efficiency increases the availability of soluble organic substances to anaerobes for subsequent methane bioproduction.

Notably, humic substances bind more effectively to heavy metal ions, such as mercury, cadmium, copper than to the other metal ions mentioned above, i.e., aluminum, calcium, magnesium, and iron (Zhao et al. 2023b). However, the addition of heavy metal salts to the anaerobic digestion system would pose an extreme risk to the ecosystem, such that aluminum, calcium, magnesium, and iron are better options when using metal salts to mitigate inhibition by humic substances on anaerobic digestion performance.

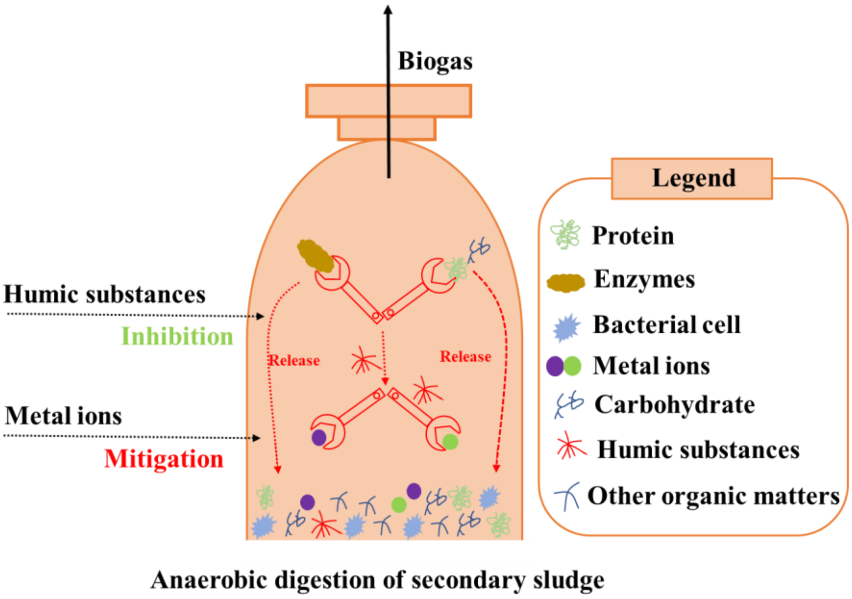

Figure 4. Metal salts mitigate the inhibitory effect of humic substances on anaerobic digestion performance. Metal salts can effectively reduce the inhibitory effect caused by humic substances during anaerobic digestion.

5.4 Addition of enzymes or the introduction of endogenous or exogenous organisms

The addition of hydrolytic enzymes, such as protease, α-amylase, was shown to reduce the negative influence of humic substances on anaerobic digestion performance (Fig. 5) (Fernandes et al. 2015). This is to likely occur because additional hydrolytic enzymes attach to humic substances, which prevents the interaction of humic substances with intrinsic hydrolytic enzymes produced by anaerobes within the anaerobic digestion process (Fernandes et al. 2015). A similar conclusion was also reached by Yu et al. (2013), who observed that the addition of protease, amylase, and their combination enhanced the yield of biogas, with values of 16%. 19% and 20%, respectively.

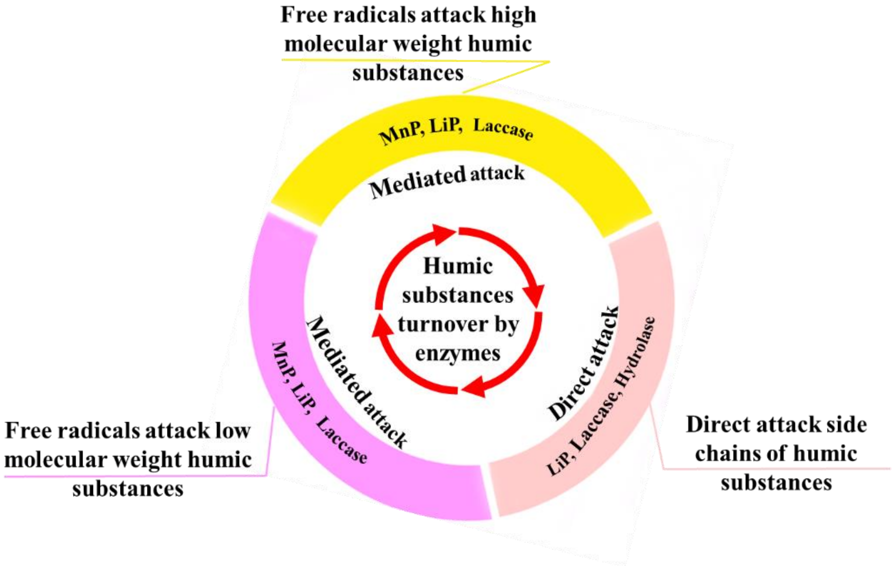

Figure 5. Proposed metabolic pathway of humic substances degradation and transformation by enzymes. Note: MnP and LiP represent manganese peroxidase and lignin peroxidase, respectively. Adding enzymes can enhance the degradation of humic substances, benefiting anaerobic digestion efficiency.

Laccase, manganese peroxidase, and lignin peroxidase facilitated the degradation and transformation of humic substances (proposed model shown in Fig. 5) (Negi and Das 2023). These enzymes mainly attack humic substances along three pathways: 1) direct attack on the side chains by laccases and lignin peroxidase; 2) mediated attack on high molecular weight humic substances by free radicals, resulting in the generation of low molecular weight humic substances; and 3) mediated attack on the low molecular weight humic substances by free radicals, resulting in their degradation, incorporation, mineralization, and polymerization. For example, the addition of laccase transformed humic substances into several small molecular substances, such as fulvic acid, and humic acid (Lisov et al. 2021; Zahmatkesh et al. 2016). Such humic substances with small molecular weights can pass through the cell membranes of anaerobes, enhancing the efficiency of electron transfer capacity and methane yield and eventually relieving the inhibition of anaerobic digestion processes by humic substances (Huang et al. 2021). In addition, manganese peroxidase and lignin peroxide can also catalyze the depolymerization of humic substances into substances with small molecular weights (Zhang et al. 2022a). However, enzyme activity is influenced by many factors, i.e., temperature, pH, humic substances concentration, and enzymes are generally expensive, which limits their application in the real world.

Organisms associated with hydrolysis and methanogenesis may also overcome the inhibition of humic substances, owing to the production of enzymes or repair of their membranes. As a result, these microorganisms can tolerate high concentrations of humic substances and avoid their negative impact on the performance of anaerobic digestion (Yap et al. 2018). The presence of humic substances in anaerobic digesters has been found to be positively correlated with organisms belonging to *Actinomycetales*, *Syntrophobacterales*, *Pedosphaerales*, and *Methanosaeta*. These organisms may be considered a potential candidate for the bioaugmentation of the sludge exposes to humic substances (Yap et al. 2018). Poszytek et al. (2018) reported that the bioaugmentation of *Ochrobactrum* sp. POC9 in secondary sludge, remarkably enhanced the performance of anaerobic digestion, resulting in a 20% enhancement of methane production. The effectiveness of the bioaugmentation strategy exposed to high concentrations of humic substances in anaerobic digestion has not been extensively studied. However, introducing exogenous anaerobic organisms that are tolerant to humic substances or have the capability of degrading them, maybe a promising alternative to overcome the inhibition of anaerobic digestion caused by humic substances, especially when endogenous organisms cannot adapt to the presence of humic substances. For instance, Li et al. (2019a) reported a phenomenon where long-term acclimatization of secondary sludge to the high concentration of humic substances (the ratio of humic substances and volatile solids was 20%) did not result in the adaptation of the microbial community to humic substances and therefore did not enhance the performance of anaerobic digestion. On the contrary, anaerobic digestion efficiency decreased by 74%.

6. Perspective

The presence of humic substances makes the sludge structure denser and more compact, resulting in fewer organic substances bioavailable for subsequent anaerobic digestion (He et al. 2016; Xu et al. 2020). Unfortunately, few studies have focused on mitigating the impact of humic substances on the sludge structure. While extensive studies have investigated humic substances removal from wastewater, including adsorption (Sounthararajah et al. 2015), membrane filtration (Ma et al. 2010), electrochemical treatment (Shan et al. 2016), and enzymatic treatment (Zahmatkesh et al. 2017), such methods have limited application in the real world due to their high costs (Zhu et al. 2022). Therefore, more attention should be given to exploring cost-effective methods of removing humic substances from wastewater before it enters biological treatment processes in wastewater treatment plants. Additionally, organic substances in secondary sludge contain biodegradable organic substances and recalcitrant organic matter, but the degree of hydrolysis is typically evaluated only by the variation of biodegradable organic substances, while the recalcitrant organic matter is not included (Lu et al. 2020). Pretreatment has been extensively and effectively adopted to promote anaerobic digestion efficiency (Li et al. 2016). For example, the humic substances content changed from 33.3% to 23.1%, from 36.2% to 15.9%, and from 29.4% to 10.5% with the pretreatment of sodium percarbonate (Wang et al. 2022b), heat-CaO_2 (Liu et al. 2019b), and sulfite (Liu et al. 2020), respectively. Therefore, recalcitrant organic matter variation is also needed as an index to describe the changes in organic substances in sludge and thus comprehensively and accurately represent the degree of hydrolysis.

The repolymerisation of aromatic moieties in humic substances tends to generate humic substances with a larger molecular size, more aromatic structures, and oxygen functional groups, and less biodegradability to anaerobes (Awasthi et al. 2017; Zheng et al. 2014). Although phenol oxidases play an important role in the repolymerisation of humic substances (Nozhevnikova et al. 2019), no relevant research has explored the reduction of phenol oxidase activity in the anaerobic digestion of sludge to inhibit the repolymerisation of humic substances. Therefore, more research should investigate the inhibition of phenol oxidase activity and prevention of humic substances repolymerisation.

Inconsistent observations regarding the impact of humic substances on each stage of anaerobic digestion, such as hydrolysis, acidification, and methanogenesis, may be attributable to the different sources, structures, molecular weights, functional groups, and concentrations of humic substances across studies (Huang et al. 2021; Liu et al. 2015). Some have reported that humic substances can act as electron acceptors, which can compete for electrons with methanogens and thus lead to the inhibition of methane production (Tan et al. 2017). However, humic substances can also act as electron donors that accelerate electron transfer capacity and enhance methane production (He et al. 2019; Xiao et al. 2019). The role of humic substances as electron donors or acceptors has been found to depend on the oxygen-containing groups and nitrogen content in humic substances (Wang et al. 2022a). For example, thermal hydrolysis pretreatment significantly changed the humic substances structure, i.e., E4/E6 ratio and carbon/nitrogen ratio in sludge, which augmented methane production by 200% (Huang et al. 2021). Noteworthy, the promotion effect of humic substances on anaerobic digestion occurred in the pretreatment of secondary sludge, i.e., thermal hydrolysis, and with low concentration, i.e., 0.05 g/g volatile solids of humic substances added to anaerobic digestion reactors. However, this phenomenon is rare in actual wastewater treatment plants due to the complex structure of humic substances, i.e., low E4/E6 ratio, high carbon/nitrogen ratio, and the high concentration (0.2-0.3 g/g volatile solids) of humic substances in the secondary sludge. Therefore, humic substances present an inhibitory effect on the efficiency of secondary sludge anaerobic digestion in traditional wastewater treatment plants. Thus, future studies should aim to enhance the electron-donating capacity of humic substances by altering the ratio of E4/E6 and carbon/nitrogen. This can help to reduce the binding of humic substances with enzymes and provide more electrons for methanogens, ultimately leading to improved performance of anaerobic digestion. Additionally, most previous studies have been performed in batch experiments (Li et al. 2017b; Li et al. 2019b). Future investigations are required to better understand the long-term effects of humic substances on the anaerobic digestion of secondary sludge in larger-scale applications and provide more crucial information for engineering applications.

Studies have reported several strategies to mitigate the inhibition of anaerobic digestion by humic substances, such as the removal of humic substances from wastewater, the pretreatment of secondary sludge before anaerobic digestion, the addition of metal salts, the addition of enzymes, or the introduction of endogenous and exogenous organisms (Li et al. 2014). The pretreatment of secondary sludge before anaerobic digestion has been limited by the high demand for energy, and chemicals. Alternatively, the addition of metal salts, such as calcium, magnesium, iron, and aluminum, seems to be a plausible method to relieve the inhibition of anaerobic digestion by humic substances (Azman et al. 2015; Li et al. 2021). However, the metal ion addition approach has been mainly applied at the laboratory scale (Azman et al. 2015). Pilot-scale and full-scale research, as well as investigations of the ecological impacts of metal ion addition, remain necessary. Moreover, adding enzymes requires the maintenance of optimal conditions for enzyme activity. However, in practical application, enzyme activity is often inhibited due to suboptimal conditions. Furthermore, the high cost of enzymes also limits their application. On the other hand, the introduction of exogenous anaerobes with the ability to degrade humic substances could be a promising method to overcome the inhibition of anaerobic digestion caused by humic substances. This can provide more substrates for subsequent anaerobic digestion processes, thereby facilitating anaerobic digestion efficiency. Further research is needed to explore the potential of this approach and determine the optimal conditions for introducing exogenous anaerobes into the anaerobic digestion system.

In addition, various metabolic pathways of methane production in anaerobic digestion have been proposed to involve a range of enzymes and metabolites (Fig. 6) (Liu et al. 2015; Zhang et al. 2020). Enzymes at various concentrations play a crucial role in methane production but are highly dependent on the microbial community. Microbes in sludge determine the secretion of relevant enzymes responsible for hydrolysis, acidification, and methanogenesis (Du et al. 2021; Liu et al. 2022; Liu et al. 2021). Although several studies have explored the responses of microbial communities to diverse stress conditions, such as pH, temperature, salinity, the enrichment of microorganisms communities varies significantly under different stress conditions. Few studies have focused on the influence of humic substances on microbial community evolution and the mechanism of regulation between enzymes and microbes (Tang et al. 2020b). Future studies are thus required to elucidate these phenomena, using molecular biology approaches combined with omics technology, such as genomics, proteomics, and transcriptomes to provide new insights regarding the mitigation of the inhibition effects of humic substances on anaerobic digestion performance.

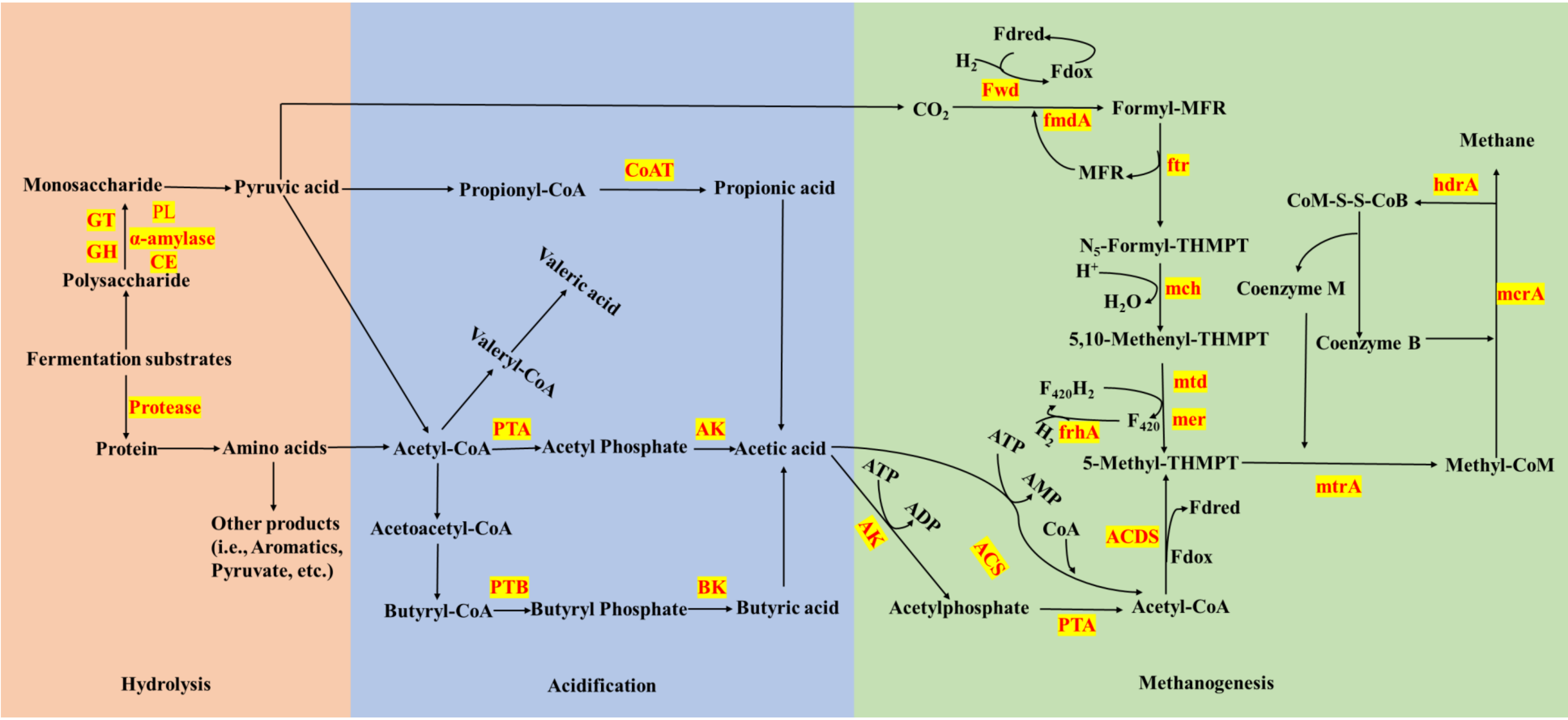

Figure 6. Metabolic pathway of methane production in anaerobic digestion of secondary sludge. Note: GH, Glycoside hydrolase; GT, Glycosyltransferases; PL, Polysaccharide lyase; CE, Carbohydrate esterase; CoAT, CoA transferase; PTA, phosphotransacetylase; AK, acetate kinase; PTB, phosphotransacetylase; BK, butyric kinase; ACS, acetyl-coenzyme A synthase; frhA, coenzyme F420 hydrogenase; THMPT,

tetrahydromethanopterin; fmdA, formyl-MFR dehydrogenase; ftr, formyl-MFR-THMPT formyltransferase; mch, methenyl-THMPT cyclohydrolase; mtd, 5,10-methylene-THMPY dehydrogenase; mer, methylene-THMPT reductase; ACDS, Acetyl-CoA decarbonylase; hdrA, heterodisulfide reductase; mcrA, methyl coenzyme reductase; and mtrA, THMPT methyltransferase. Clarifying the relationship between microorganisms and enzymes involved in the degradation of humic substances during anaerobic digestion processes is beneficial for promoting the degradation of humic substances.

7. Conclusion

This review comprehensively summarises and presents insights into the effect of humic substances on the performance of anaerobic digestion on secondary sludge. The key findings of this review suggest that the presence of humic substances makes secondary sludge denser and more compact, reducing the number of organic substances bioavailable to anaerobes for subsequent anaerobic digestion. Moreover, the effect of humic substances on each step of anaerobic digestion is case-specific and is determined by several factors, including source, structure, molecular weight, and concentrations of humic substances. However, in traditional wastewater treatment plants, the presence of humic substances typically inhibits anaerobic digestion efficiency in traditional wastewater treatment plants, due to the complex structure and high concentration in the secondary sludge. Subsequently, the distribution of humic substances in the solid and liquid phases affects anaerobic digestion efficiency, and the repolymerization of humic substances during the anaerobic digestion process reduces the bioavailability of organic substances, resulting in a stronger inhibitory effect on the performance of anaerobic digestion. Increasing the E4/E6 ratio and decreasing the carbon/nitrogen ratio in the molecule structure of humic substances can enhance their electron donor capacity and lead to an improvement in anaerobic digestion efficiency by enhancing the electron transfer capacity and methane production. Finally, the addition of metal salts and the introduction of exogenous anaerobes are potentially effective in mitigating the inhibition of anaerobic digestion caused by humic substances, with the former reducing humic substances' binding ability and the latter improving their degradation.

Declarations

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Consent to participate Not applicable

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Availability of data and material Not applicable

Code availability Not applicable

Authors' contributions Zhenyao Wang: Conceptualization, Writing Original Draft, Critical Thinking. Xuan Li: Critical Thinking, Validation, Review & Editing. Muhammad Ahmar Siddiqui: Review & Editing. Huan Liu: Review & Editing. Ting Zhou: Review & Editing. Lei Zheng: Review & Editing. Siyu Huang: Review & Editing. Li Gao: Review & Editing. Carol Sze Ki Lin: Review & Editing. Qilin Wang:

Supervision, Review & Editing.

References

Ai, P., Zhang, X., Dinamarca, C., Elsayed, M., Yu, L., Xi, J., Mei, Z. (2019). Different effects of ozone and aqueous ammonia in a combined pretreatment method on rice straw and dairy manure fiber for enhancing biomethane production. Bioresour Technol, 282, 275-284. http://doi.org/10.1016/j.biortech.2019.03.021

Algamdi, M.S., Alsohaimi, I.H., Lawler, J., Ali, H.M., Aldawsari, A.M., Hassan, H.M. (2019). Fabrication of graphene oxide incorporated polyethersulfone hybrid ultrafiltration membranes for humic acid removal. Sep Purif Technol, 223, 17-23. http://doi.org/10.1016/j.seppur.2019.04.057

Awasthi, M.K., Wang, M., Chen, H., Wang, Q., Zhao, J., Ren, X., Li, D.-s., Awasthi, S.K., Shen, F., Li, R. (2017). Heterogeneity of biochar amendment to improve the carbon and nitrogen sequestration through reduce the greenhouse gases emissions during sewage sludge composting. Bioresour Technol, 224, 428-438. http://doi.org/10.1016/j.biortech.2016.11.014

Azman, S., Khadem, A.F., Plugge, C.M., Stams, A.J., Bec, S., Zeeman, G. (2017). Effect of humic acid on anaerobic digestion of cellulose and xylan in completely stirred tank reactors: inhibitory effect, mitigation of the inhibition and the dynamics of the microbial communities. Appl Microbiol Biotechnol, 101(2), 889-901. http://doi.org/10.1007/s00253-016-8010-x

Azman, S., Khadem, A.F., Zeeman, G., van Lier, J.B., Plugge, C.M. (2015). Mitigation of Humic Acid Inhibition in Anaerobic Digestion of Cellulose by Addition of Various Salts. Bioengineering (Basel), 2(2), 54-65. http://doi.org/10.3390/bioengineering2020054

Baveye, P.C., Wander, M. (2019). The (bio) chemistry of soil humus and humic substances: Why is the “new view” still considered novel after more than 80 years? Front Environ Sci, 7, 27. http://doi.org/10.3389/fenvs.2019.00027

Calabi-Floody, M., Bendall, J.S., Jara, A.A., Welland, M.E., Theng, B.K.G., Rumpel, C., Mora, M.d.l.L. (2011). Nanoclays from an Andisol: Extraction, properties and carbon stabilization. Geoderma, 161(3-4), 159-167. http://doi.org/10.1016/j.geoderma.2010.12.013

Dai, X., Luo, F., Dai, L., Dong, B. (2013). Degradation of extracellular polymeric substances (EPS) in anaerobic digestion of dewatered sludge. Procedia Environ Sci, 18, 515-521. http://doi.org/10.1016/j.proenv.2013.04.069

Ding, W., Cao, S., Jin, W., Zhou, X., Wang, C., Jiang, Q., Huang, H., Tu, R., Han, S., Wang, Q. (2019). Ozone disinfection of chlorine-resistant bacteria in drinking water. Water Res, 160, 339-349. https://doi.org/10.1016/j.watres.2019.05.014

Du, W., Huang, X., Zhang, J., Wang, D., Yang, Q., Li, X. (2021). Enhancing methane production from anaerobic digestion of waste activated sludge with addition of sodium lauroyl sarcosinate. Bioresour Technol, 336, 125321. http://doi.org/10.1016/j.biortech.2021.125321

El Fels, L., Zamama, M., El Asli, A., Hafidi, M. (2014). Assessment of biotransformation of organic matter during co-composting of sewage sludge-lignocelullosic waste by chemical, FTIR analyses, and phytotoxicity tests. Int Biodeterior Biodegrad, 87, 128-137. http://doi.org/10.1016/j.ibiod.2013.09.024

Feng, Y., Zhang, Y., Quan, X., Chen, S. (2014). Enhanced anaerobic digestion of waste activated sludge digestion by the addition of zero valent iron. Water Res, 52, 242-50. http://doi.org/10.1016/j.watres.2013.10.072

Fernandes, T.V., van Lier, J.B., Zeeman, G. (2015). Humic acid-like and fulvic acid-like inhibition on the hydrolysis of cellulose and tributyrin. Bioenergy Res, 8(2), 821-831. http://doi.org/10.1007/s12155-014-9564-z

Geng, Y.K., Zhou, Y. (2022). Reduction of refractory Maillard reaction products by Fe(3+) during thermal hydrolysis pretreatment and enhanced sludge biodegradability. J Hazard Mater, 430, 128400. http://doi.org/10.1016/j.jhazmat.2022.128400

Ghaneian, M.T., Morovati, P., Ehrampoush, M.H., Tabatabaee, M. (2014). Humic acid degradation by the synthesized flower-like Ag/ZnO nanostructure as an efficient photocatalyst. J Environ Health Sci Eng, 12(1), 1-7. http://doi.org/10.1186/s40201-014-0138-y

Guo, H., Tian, L., Wang, Y., Zhu, T., Tong, Y., Liu, Y. (2022). Improved methane production from the two-phase anaerobic digestion and dewaterability of anaerobically digested sludge by β-cyclodextrin pretreatment. J Clean Prod, 363, 132484. http://doi.org/10.1016/j.jclepro.2022.132484

Hartung, H. (1992). Stimulation of anaerobic digestion with peat humic substance. Sci Total Environ, 113(1-2), 17-33. http://doi.org/10.1016/0048-9697(92)90014-J

He, D., Xiao, J., Wang, D., Liu, X., Fu, Q., Li, Y., Du, M., Yang, Q., Liu, Y., Wang, Q., Ni, B.J., Song, K., Cai, Z., Ye, J., Yu, H. (2021). Digestion liquid based alkaline pretreatment of waste activated sludge promotes methane production from anaerobic digestion. Water Res, 199, 117198. http://doi.org/10.1016/j.watres.2021.117198

He, E., Lu, C., He, J., Zhao, B., Wang, J., Zhang, R., Ding, T. (2016). Binding characteristics of Cu(2+) to natural humic acid fractions sequentially extracted from the lake sediments. Environ Sci Pollut Res Int, 23(22), 22667-22677. http://doi.org/10.1007/s11356-016-7487-2

He, X.S., Yang, C., You, S.H., Zhang, H., Xi, B.D., Yu, M.D., Liu, S.J. (2019). Redox properties of compost-derived organic matter and their association with polarity and molecular weight. Sci Total Environ, 665, 920-928. http://doi.org/10.1016/j.scitotenv.2019.02.164

He, Z., Wang, F., Zou, Z., Tang, C., Zhou, A., Liu, W., Ren, Y., Li, Z., Wang, A. (2023). Recent advances and perspectives in roles of humic acid in anaerobic digestion of waste activated sludge. Chem Eng J, 466, 143081. http://doi.org/10.1016/j.cej.2023.143081

Hu, J., Li, Z., Tao, W. (2022). How dose calcium hypochlorite promote the methane production from sludge anaerobic digestion: A mechanism study from enhanced biodegradability of recalcitrant substances. J Water Process Eng, 50, 103268. http://doi.org/10.1016/j.jwpe.2022.103268

Huang, F., Liu, H., Wen, J., Zhao, C., Dong, L., Liu, H. (2021). Underestimated humic acids release and influence on anaerobic digestion during sludge thermal hydrolysis. Water Res., 201, 117310. http://doi.org/10.1016/j.watres.2021.117310

Klučáková, M. (2018). Size and charge evaluation of standard humic and fulvic acids as crucial factors to determine their environmental behavior and impact. Front Chem, 6, 235. http://doi.org/10.3389/fchem.2018.00235

Kulandaivelu, J., Choi, P.M., Shrestha, S., Li, X., Song, Y., Li, J., Sharma, K., Yuan, Z., Mueller, J.F., Wang, C. (2020). Assessing the removal of organic micropollutants from wastewater by discharging drinking water sludge to sewers. Water Res, 181, 115945. http://doi.org/10.1016/j.watres.2020.115945

Li, D., Zhou, Y., Tan, Y., Pathak, S., Majid, M.B., Ng, W.J. (2016). Alkali-solubilized organic matter from sludge and its degradability in the anaerobic process. Bioresour Technol, 200, 579-86. http://doi.org/10.1016/j.biortech.2015.10.083

Li, H., Jin, C., Zhang, Z., O'Hara, I., Mundree, S. (2017a). Environmental and economic life cycle assessment of energy recovery from sewage sludge through different anaerobic digestion pathways. Energy, 126, 649-657. http://doi.org/10.1016/j.energy.2017.03.068

Li, H., Li, Y., Jin, Y., Zou, S., Li, C. (2014). Recovery of sludge humic acids with alkaline pretreatment and its impact on subsequent anaerobic digestion. J Chem Technol Biotechnol, 89(5), 707-713. http://doi.org/10.1002/jctb.4173

Li, H., Li, Y., Li, C. (2013). Characterization of humic acids and fulvic acids derived from sewage sludge. Asian J Chem 25(18), 10087. http://doi.org/10.14233/ajchem.2013.15162

Li, H., Li, Y., Li, C. (2017b). Evolution of humic substances during anaerobic sludge digestion. Environ Eng Manage J, 16(7). http://doi.org/10.30638/eemj.2017.171

Li, J., Hao, X., van Loosdrecht, M.C., Yu, J., Liu, R. (2019a). Adaptation of semi-continuous anaerobic sludge digestion to humic acids. Water Res, 161, 329-334. http://doi.org/10.1016/j.watres.2019.06.016

Li, J., Hao, X., van Loosdrecht, M.C.M., Liu, R. (2021). Relieving the inhibition of humic acid on anaerobic digestion of excess sludge by metal ions. Water Res, 188, 116541. http://doi.org/10.1016/j.watres.2020.116541

Li, J., Hao, X., van Loosdrecht, M.C.M., Luo, Y., Cao, D. (2019b). Effect of humic acids on batch anaerobic digestion of excess sludge. Water Res, 155, 431-443. http://doi.org/10.1016/j.watres.2018.12.009

Liang, T., Elmaadawy, K., Liu, B., Hu, J., Hou, H., Yang, J. (2021). Anaerobic fermentation of waste activated sludge for volatile fatty acid production: recent updates of pretreatment methods and the potential effect of humic and nutrients substances. Process Saf Environ Prot, 145, 321-339. http://doi.org/10.1016/j.psep.2020.08.010

Lipczynska-Kochany, E. (2018). Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: A review. Chemosphere, 202, 420-437. http://doi.org/10.1016/j.chemosphere.2018.03.104

Lisov, A., Belova, O., Zavarzina, A., Konstantinov, A., Leontievsky, A. (2021). The Role of Laccase from Zygomycetous Fungus Mortierella elasson in Humic Acids Degradation. Agronomy, 11(11). http://doi.org/10.3390/agronomy11112169

Liu, H., Li, X., Zhang, Z., Nghiem, L., Gao, L., Wang, Q. (2021a). Semi-continuous anaerobic digestion of secondary sludge with free ammonia pretreatment: Focusing on volatile solids destruction, dewaterability, pathogen removal and its implications. Water Res, 202, 117481. https://doi.org/10.1016/j.watres.2021.117481

Liu, H., Xu, Y., Geng, H., Chen, Y., Dai, X. (2022). Contributions of MOF-808 to methane production from anaerobic digestion of waste activated sludge. Water Res, 220, 118653. http://doi.org/10.1016/j.watres.2022.118653

Liu, H., Li, X., Zhang, Z., Nghiem, L.D., Gao, L., Batstone, D.J., Wang, Q. (2023). Achieving expanded sludge treatment capacity with additional benefits for an anaerobic digester using free ammonia pretreatment. Chem Eng J, 465, 142846. https://doi.org/10.1016/j.cej.2023.142846

Liu, K., Chen, Y., Xiao, N., Zheng, X., Li, M. (2015). Effect of humic acids with different characteristics on fermentative short-chain fatty acids production from waste activated sludge. Environ Sci Technol, 49(8), 4929-36. http://doi.org/10.1021/acs.est.5b00200

Liu, Q., Li, Y., Yang, F., Liu, X., Wang, D., Xu, Q., Zhang, Y., Yang, Q. (2021b). Understanding the mechanism of how anaerobic fermentation deteriorates sludge dewaterability. Chem Eng J, 404, 127026. http://doi.org/10.1016/j.cej.2020.127026

Liu, R., Hao, X., van Loosdrecht, M.C., Zhou, P., Li, J. (2019a). Dynamics of humic substance composition during anaerobic digestion of excess activated sludge. Int Biodeterior Biodegrad, 145, 104771. http://doi.org/10.1016/j.ibiod.2019.104771

Liu, X., Du, M., Yang, J., Wu, Y., Xu, Q., Wang, D., Yang, Q., Yang, G., Li, X. (2020). Sulfite serving as a pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste activated sludge. Chem Eng J, 385, 123991. http://doi.org/10.1016/j.cej.2019.123991

Liu, X., Xu, Q., Wang, D., Yang, Q., Wu, Y., Yang, J., Liu, Y., Wang, Q., Ni, B.-J., Li, X. (2019b). Enhanced short-chain fatty acids from waste activated sludge by heat–CaO2 advanced thermal hydrolysis pretreatment: parameter optimization, mechanisms, and implications. ACS Sustainable Chem Eng, 7(3), 3544-3555. http://doi.org/10.1021/acssuschemeng.8b05799

Lu, Q., Yu, Z., Wang, L., Liang, Z., Li, H., Sun, L., Shim, H., Qiu, R., Wang, S. (2020). Sludge pre-treatments change performance and microbiome in methanogenic sludge digesters by releasing different sludge organic matter. Bioresour Technol, 316, 123909. http://doi.org/10.1016/j.biortech.2020.123909

Ma, N., Zhang, Y., Quan, X., Fan, X., Zhao, H. (2010). Performing a microfiltration integrated with photocatalysis using an Ag-TiO2/HAP/Al2O3 composite membrane for water treatment: Evaluating effectiveness for humic acid removal and anti-fouling properties. Water Res, 44(20), 6104-6114. http://doi.org/10.1016/j.watres.2010.06.068

McCarty, P.L., Bae, J., Kim, J. (2011). Domestic wastewater treatment as a net energy producer--can this be achieved? Environ Sci Technol, 45(17), 7100-6. http://doi.org/10.1021/es2014264

Millati, R., Wikandari, R., Ariyanto, T., Putri, R.U., Taherzadeh, M.J. (2020). Pretreatment technologies for anaerobic digestion of lignocelluloses and toxic feedstocks. Bioresour Technol, 304, 122998. http://doi.org/10.1016/j.biortech.2020.122998

Nardi, S., Schiavon, M., Francioso, O. (2021). Chemical structure and biological activity of humic substances define their role as plant growth promoters. Molecules, 26(8), 2256. http://doi.org/10.3390/molecules26082256

Negi, B.B., Das, C. (2023). Mycoremediation of wastewater, challenges, and current status: A review. Bioresour Technol Rep, 101409. http://doi.org/10.1016/j.biteb.2023.101409

Nozhevnikova, A., Mironov, V., Botchkova, E., Litti, Y.V., Russkova, Y.I. (2019). Composition of a microbial community at different stages of composting and the prospects for compost production from municipal organic waste. Appl Biochem Microbiol, 55(3), 199-208. http://doi.org/10.1134/s0003683819030104

Pan, X., He, J., Pang, H., Zhang, P., Zou, X., Zhong, Y., Ding, J. (2022). New insight into enhanced short-chain fatty acids production from waste activated sludge through pretreatment of cation exchange resin coupled NaCl addition. J. Environ. Manage., 302(Pt B), 114074. http://doi.org/10.1016/j.jenvman.2021.114074

Poszytek, K., Karczewska-Golec, J., Ciok, A., Decewicz, P., Dziurzynski, M., Gorecki, A., Jakusz, G., Krucon, T., Lomza, P., Romaniuk, K. (2018). Genome-guided characterization of Ochrobactrum sp. POC9 enhancing sewage sludge utilization—Biotechnological potential and biosafety considerations. Int J Environ Res Public Health, 15(7), 1501.

http://doi.org/10.3390/ijerph15071501

Raposo, F., De la Rubia, M., Fernández-Cegrí, V., Borja, R. (2012). Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renewable Sustainable Energy Rev, 16(1), 861-877. http://doi.org/10.1016/j.rser.2011.09.008

Rashtbari, Y., Américo-Pinheiro, J.H.P., Bahrami, S., Fazlzadeh, M., Arfaeinia, H., Poureshgh, Y. (2020). Efficiency of zeolite coated with zero-valent iron nanoparticles for removal of humic acid from aqueous solutions. Water Air Soil Pollu, 231(10), 514. http://doi.org/10.1007/s11270-020-04872-9

Rigobello, E.S., Campos, S.X., Azevedo, E.R.d., Dantas, A.D.B., Vieira, E.M. (2017). Comparative characterization of humic substances extracted from freshwater and peat of different apparent molecular sizes. Rev Ambient Água, 12, 774-785. http://doi.org/10.4136/ambi-agua.2022

Ryu, J., Jung, J., Park, K., Song, W., Choi, B., Kweon, J. (2021). Humic acid removal and microbial community function in membrane bioreactor. J Hazard Mater, 417, 126088. http://doi.org/10.1016/j.jhazmat.2021.12608

Shan, L., Liu, J., Yu, Y., Ambuchi, J.J., Feng, Y. (2016). Characterization of persistent colors and decolorization of effluent from biologically treated cellulosic ethanol production wastewater. Environ Sci Pollut Res 23(10), 10215-10222. http://doi.org/10.1007/s11356-016-6220-5

Siddiqui, M.A., Dai, J., Luo, Y., Chen, G. (2020). Investigation of the short-term effects of extracellular polymeric substance accumulation with different backwashing strategies in an anaerobic self-forming dynamic membrane bioreactor. Water Res, 185, 116283. http://doi.org/10.1016/j.watres.2020.116283

Sinha, R., Ghosal, P.S. (2023). A comprehensive appraisal on status and management of remediation of DBPs by TiO2 based-photocatalysts: Insights of technology, performance and energy efficiency. J Environ Manage, 328, 117011. http://doi.org/10.1016/j.jenvman.2022.117011

Sounthararajah, D.P., Loganathan, P., Kandasamy, J., Vigneswaran, S. (2015). Effects of humic acid and suspended solids on the removal of heavy metals from water by adsorption onto granular activated carbon. Int J Environ Res Public Health, 12(9), 10475-10489. http://doi.org/10.3390/ijerph120910475

Tan, W., Xi, B., Wang, G., Jiang, J., He, X., Mao, X., Gao, R., Huang, C., Zhang, H., Li, D. (2017). Increased electron-accepting and decreased electron-donating capacities of soil humic substances in response to increasing temperature. Environ Sci Technol, 51(6), 3176-3186. http://doi.org/10.1021/acs.est.6b04131

Tang, W., Wu, X., Huang, C., Huang, C., Lai, C., Yong, Q. (2020a). Humic acid-assisted autohydrolysis of waste wheat straw to sustainably improve enzymatic hydrolysis. Bioresour Technol, 306, 123103. http://doi.org/10.1016/j.biortech.2020.123103

Tang, Y., Dai, X., Dong, B., Guo, Y., Dai, L. (2020b). Humification in extracellular polymeric substances (EPS) dominates methane release and EPS reconstruction during the sludge stabilization of high-solid anaerobic digestion. Water Res, 175, 115686. http://doi.org/10.1016/j.watres.2020.115686

Tang, Y., Li, X., Dong, B., Huang, J., Wei, Y., Dai, X., Dai, L. (2018). Effect of aromatic repolymerization of humic acid-like fraction on digestate phytotoxicity reduction during high-solid anaerobic digestion for stabilization treatment of sewage sludge. Water Res., 143, 436-444. http://doi.org/10.1016/j.watres.2018.07.003

Wang, D., He, D., Liu, X., Xu, Q., Yang, Q., Li, X., Liu, Y., Wang, Q., Ni, B.J., Li, H. (2019b). The underlying mechanism of calcium peroxide pretreatment enhancing methane production from anaerobic digestion of waste activated sludge. Water Res, 164, 114934. http://doi.org/10.1016/j.watres.2019.114934

Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J., Yuan, Z., (2017). Technologies for reducing sludge production in wastewater treatment plants: state of the art. Sci. Total Environ. 587, 510-521. https://doi.org/10.1016/j.scitotenv.2017.02.203

Wang, Q., Sun, J., Liu, S., Gao, L., Zhou, X., Wang, D., Song, K., Nghiem, L. (2019a). Free ammonia pretreatment improves anaerobic methane generation from algae. Water Research, 162, 269-275. https://doi.org/10.1016/j.watres.2019.06.065

Wang, X., Lyu, T., Dong, R., Liu, H., Wu, S. (2021a). Dynamic evolution of humic acids during anaerobic digestion: Exploring an effective auxiliary agent for heavy metal remediation. Bioresour Technol, 320(Pt A), 124331. http://doi.org/10.1016/j.biortech.2020.124331

Wang, X., Lyu, T., Dong, R., Wu, S. (2022a). Revealing the link between evolution of electron transfer capacity of humic acid and key enzyme activities during anaerobic digestion. J Environ Manage, 301, 113914. http://doi.org/10.1016/j.jenvman.2021.113914

Wang, X., Muhmood, A., Lyu, T., Dong, R., Liu, H., Wu, S. (2021b). Mechanisms of genuine humic acid evolution and its dynamic interaction with methane production in anaerobic digestion processes. Chem Eng J, 408, 127322. http://doi.org/10.1016/j.cej.2020.127322

Wang, Y., Sun, P., Guo, H., Wang, D., Zhu, T., Liu, Y. (2022b). Enhancing Methane Production from Anaerobic Digestion of Waste Activated Sludge through a Novel Sodium Percarbonate (SPC) Pretreatment: Reaction Kinetics and Mechanisms. ACS ES&T Engineering, 2(7), 1326-1340. http://doi.org/10.1021/acsestengg.1c00468

Wang, Z., Li, X., Liu, H., Zhou, T., Qin, Z., Mou, J., Sun, J., Huang, S., Chaves, A.V., Gao, L., Wang, Q. (2023). Bioproduction and applications of short-chain fatty acids from secondary sludge anaerobic fermentation: A critical review. Renew Sust Energ Rev, 183, 113502. http://doi.org/10.1016/j.rser.2023.113502

Wang, Z., Mou, J., Qin, Z., He, Y., Sun, Z., Wang, X., Lin, C.S.K. (2023c). An auxin-like supermolecule to simultaneously enhance growth and cumulative eicosapentaenoic acid production in Phaeodactylum tricornutum. Biresour Technol, 345, 126564. http://doi.org/10.1016/j.biortech.2021.126564

Wu, D., Li, L., Zhao, X., Peng, Y., Yang, P., Peng, X. (2019). Anaerobic digestion: A review on process monitoring. Renewable Sustainable Energy Rev, 103, 1-12. http://doi.org/10.1016/j.rser.2018.12.039

Xiao, X., Xi, B.D., He, X.S., Zhang, H., Li, D., Zhao, X.Y., Zhang, X.H. (2019). Hydrophobicity-dependent electron transfer capacities of dissolved organic matter derived from chicken manure compost. Chemosphere, 222, 757-765. http://doi.org/10.1016/j.chemosphere.2019.01.173

Xu, Y., Lu, Y., Zheng, L., Wang, Z., Dai, X. (2020). Effects of humic matter on the anaerobic digestion of sewage sludge: New insights from sludge structure. Chemosphere, 243, 125421. http://doi.org/10.1016/j.chemosphere.2019.125421

Yang, D., Dai, X., Song, L., Dai, L., Dong, B. (2019). Effects of stepwise thermal hydrolysis and solid-liquid separation on three different sludge organic matter solubilization and biodegradability. Bioresour Technol, 290, 121753. http://doi.org/10.1016/j.biortech.2019.121753

Yang, Y., Li, H. (2016). Recovering humic substances from the dewatering effluent of thermally treated sludge and its performance as an organic fertilizer. Front Environ Sci Eng, 10, 578-584.

http://doi.org/10.1007/s11783-015-0827-5

Yap, S.D., Astals, S., Lu, Y., Peces, M., Jensen, P.D., Batstone, D.J., Tait, S. (2018). Humic acid inhibition of hydrolysis and methanogenesis with different anaerobic inocula. Waste Manage, 80, 130-136. http://doi.org/10.1016/j.wasman.2018.09.001

Yin, D.T., Jing, Q., AlDajani, W.W., Duncan, S., Tschirner, U., Schilling, J., Kazlauskas, R.J. (2011). Improved pretreatment of lignocellulosic biomass using enzymatically-generated peracetic acid. Bioresour Technol, 102(8), 5183-92. http://doi.org/10.1016/j.biortech.2011.01.079

Yin, H., Guo, Q., Lei, C., Chen, W., Huang, B. (2020). Electrochemical-driven carbocatalysis as highly efficient advanced oxidation processes for simultaneous removal of humic acid and Cr (VI). Chem Eng J, 396, 125156. http://doi.org/10.1016/j.cej.2020.12515

Yu, S., Zhang, G., Li, J., Zhao, Z., Kang, X. (2013). Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. Bioresour Technol, 146, 758-761

Zahmatkesh, M., Spanjers, H., Toran, M.J., Blanquez, P., van Lier, J.B. (2016). Bioremoval of humic acid from water by white rot fungi: exploring the removal mechanisms. AMB Express, 6(1), 118. http://doi.org/10.1186/s13568-016-0293-x

Zahmatkesh, M., Spanjers, H., van Lier, J.B. (2017). Fungal treatment of humic-rich industrial wastewater: application of white rot fungi in remediation of food-processing wastewater. Environ Technol, 38(21), 2752-2762. http://doi.org/10.1080/09593330.2016.1276969

Zhang, D., He, H., Ren, Y., Haider, R., Urynowicz, M., Fallgren, P.H., Jin, S., Ali, M.I., Jamal, A., Sabar, M.A. (2022). A mini review on biotransformation of coal to methane by enhancement of chemical pretreatment. Fuel, 308, 121961. http://doi.org/10.1016/j.fuel.2021.121961

Zhang, J., Lu, W., Zhan, S., Qiu, J., Wang, X., Wu, Z., Li, H., Qiu, Z., Peng, H. (2021a). Adsorption and mechanistic study for humic acid removal by magnetic biochar derived from forestry wastes functionalized with Mg/Al-LDH. Sep Purif Technol, 276, 119296. http://doi.org/10.1016/j.seppur.2021.119296

Zhang, Q., Zhao, X., Li, W., Chen, H., Zhu, X., Zhu, H., Zhang, P. (2020). Responses of short-chain fatty acids production to the addition of various biocarriers to sludge anaerobic fermentation. Bioresour Technol, 304, 122989. http://doi.org/10.1016/j.biortech.2020.122989

Zhang, W., Tang, M., Li, D., Yang, P., Xu, S., Wang, D. (2021b). Effects of alkalinity on interaction between EPS and hydroxy-aluminum with different speciation in wastewater sludge conditioning with aluminum based inorganic polymer flocculant. J Environ Sci, 100, 257-268. http://doi.org/10.1016/j.jes.2020.05.016

Zhang, Z., Li, X., Liu, H., Zamyai, A., Guo, W., Wen, H., Gao, l., Nghiem, L., Wang, Q. (2022b). Advancements in detection and removal of antibiotic resistance genes in sludge digestion: a state-of-art review. Biresour Technol, 344, 126197. https://doi.org/10.1016/j.biortech.2021.126197

Zhao, C., Liu, H., Liu, H., Zhan, X., Zhang, X., Fu, B., Shi, M., Lu, S., Huang, F., Cui, M. (2023a). Influence of thermal hydrolysis on sludge anaerobic digestion: Release of humic acid promotes electron transport of methanogen. Chem Eng J, 451, 138455. http://doi.org/10.1016/j.cej.2022.138455

Zhao, P., Wang, A., Wang, P., Huang, Z., Fu, Z., Huang, Z. (2023b). Two recyclable and complementary adsorbents of coal-based and bio-based humic acids: High efficient adsorption and immobilization remediation for Pb (II) contaminated water and soil. Chemosphere, 318, 137963. http://doi.org/10.1016/j.chemosphere.2023.137963

Zheng, W., Lü, F., Phoungthong, K., He, P. (2014). Relationship between anaerobic digestion of biodegradable solid waste and spectral characteristics of the derived liquid digestate. Bioresour Technol, 161, 69-77. http://doi.org/10.1016/j.biortech.2014.03.016

Zhou, S., Xu, J., Yang, G., Zhuang, L. (2014). Methanogenesis affected by the co-occurrence of iron(III) oxides and humic substances. FEMS Microbiol Ecol, 88(1), 107-20. http://doi.org/10.1111/1574-6941.12274

Zhou, T., Zhang, Z., Liu, H., Dong, S., Nghiem, L., Gao, L., Chaves, A., Zamyadi, A., Li, X., Wang, Q. (2023). A review on microalgae-mediated biotechnology for removing pharmaceutical contaminants in aqueous environments: Occurrence, fate, and removal mechanism. J Hazard Mater, 443, 130213. http://doi.org/10.1016/j.jhazmat.2022.130213.

Zhu, X., Liu, J., Li, L., Zhen, G., Lu, X., Zhang, J., Liu, H., Zhou, Z., Wu, Z., Zhang, X. (2022). Prospects for humic acids treatment and recovery in wastewater: A review. Chemosphere, 137193. http://doi.org/10.1016/j.chemosphere.2022.137193