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The role of biochar on alleviating ammonia toxicity in anaerobic digestion of nitrogen-rich wastes: A review

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Abstract: This paper reviewed the mechanisms of biochar in relieving ammonia inhibition. Biochar affects nitrogen-rich waste's anaerobic digestion (AD) performance through four ways: promotion of direct interspecies electron transfer (DIET) and microbial growth, adsorption, pH buffering, and provision of nutrients. Biochar enhances the DIET pathway by acting as an electron carrier. The role of DIET in relieving ammonia nitrogen may be exaggerated because many related studies don't provide definite evidence. Therefore, some bioinformatics technology should be used to assist in investigating DIET. Biochar absorbs ammonia nitrogen by chemical adsorption (electrostatic attraction, ion exchange, and complexation) and physical adsorption. The absorption efficiency, mainly affected by the properties of biochar, pH and temperature of AD, can reach 50 mg g^{-1} on average. The biochar addition can buffer pH by reducing the concentrations of VFAs, alleviating ammonia inhibition. In addition, biochar can release trace elements and increase the bioavailability of trace elements.

Keywords: Ammonia stress; Adsorption; Microbial immobilization; Direct interspecies electron transfer; Biochar

1. Introduction

Anaerobic digestion (AD) is an effective technology for treating organic wastes and producing biogas and energy (Yang et al., 2021a). However, in processing nitrogen-rich wastes, such as livestock manure (Zhou et al., 2021), food waste (Chuenchart et al., 2020), and sludge (Yuan et al., 2016), ammonia is produced at high concentrations, leading to instability, low efficiency and even failure of the AD system, known as ammonia inhibition (Zheng et al., 2021). Recently, the issue of ammonia inhibition has gradually received attention. Many strategies have been proposed to alleviate ammonia inhibition, including 1) adjustment of temperature or pH turn NH_3 into NH_4^+, thereby reducing the toxicity of ammonia nitrogen (Karlsson and Ejlertsson, 2012); 2) supplement of methanogenic archaea with high resistance to ammonia nitrogen or acclimation under high ammonia nitrogen concentration (Yan et al., 2021); 3) removal of ammonia nitrogen by converting ammonia nitrogen to N_2 by nitrification or denitrification (Kwon et al., 2019); 4) addition of nutrients such as trace elements to enhance the ability of microorganisms to resist high ammonia nitrogen concentration. Although these methods can effectively alleviate ammonia inhibition, their application in the large-scale biogas project of nitrogen-rich substrates is still far from commercialization because they have apparent disadvantages such as low operability or the potential risk of environmental pollution.

Biochar is a carbon-rich material produced from biomass pyrolysis (Chiappero et al., 2020). Due to the high specific surface area, porosity, conductivity, redox-property, and functional groups (Huang et al., 2021), biochar is an effective additive in alleviating ammonia inhibition (Wei et al., 2020; Indren et al., 2020a; Xu et al., 2018). Biochar has a potential application value as an additive to alleviate ammonia inhibition considering the high operability and environmental friendliness. In general, the efficiency of biochar in relieving ammonia inhibition mainly benefits from its capacity of adsorption (Shi et al., 2013), immobilizing microorganisms (Wei et al., 2020; Pandey et al., 2020), releasing trace elements (Indren et al., 2020a) and enhancing the direct interspecies electron transfer (DIET) (Ma et al., 2019). The performance of biochar depends on the properties of biochar, biochar addition ratio, operating conditions (e.g., temperature and pH) of AD, and the total ammonia nitrogen (TAN) concentration. Biochar provides an ideal microenvironment for the growth and survival of microorganisms due to its porous structure (Yan et al., 2021). The addition of biochar can also increase the concentration of microorganisms, thereby improving the resistance of the microbial consortium to the high TAN concentration (Wei et al., 2020). Wang et al. (2017) reported that biochar could act as an electron carrier to promote DIET between bacteria and archaea to enhance methane production. The ability of biochar is beneficial for biogas production because high TAN concentration will inhibit the DIET pathway. Biochar is rich in trace elements (e.g. Fe, Co, Ni, Mo, Zn, Se, Cu, and Mn), which may be conducive to the growth of microorganisms and thus increase their resistance to ammonia nitrogen (Banks et al., 2012; Indren et al., 2020a).

Over the past ten years, the biochar application in AD has been reviewed in several papers (Chiappero et al., 2020; Qiu et al., 2019; Fagbohungbe et al., 2017; Kumar et al., 2021). These reviews provided some valuable information. However, none of them focused on the AD system with high ammonia nitrogen. For the common digestion systems, the main role of biochar may be to counteract the weakened buffer capacity caused by the accumulation of VFAs. Interestingly, for the AD of nitrogen-rich substrates, the mechanism of biochar to alleviate ammonia inhibition is more complicated. In the current review, we provide a comprehensive analysis of the mechanism of biochar addition on mitigating ammonia inhibition based on biochar properties, including conductivity, redox-property, adsorption, rich in trace elements, and porous. Particular attention is paid to the interactions between biochar and microorganisms, including interspecies electron transfer and immobilization. According to this interaction, some potential application modes of biochar in AD systems with high ammonia nitrogen concentration are discussed in detail.

2. ***Ammonia toxicity in anaerobic digestion***

The digestion system often faces the risk of ammonia inhibition when the C/N ratio of the substrate is lower than 15, such as livestock manure, food waste, sludge, and microalgae (Cai et al., 2021). NH_3 can penetrate cell structure and cause the imbalance of protons, and high NH_4^+ concentration can damage the enzymes' structure. Ammonia inhibition will lead to low digestion efficiency due to the low conversion efficiency of VFAs. Under ammonia inhibition, the range of ammonia nitrogen concentrations may be very wide, ranging from 2 to 25 g L^{-1} (Poirier et al., 2016). Several review papers relate to ammonia inhibition (Capson-Tojo et al., 2020). Therefore, this point is only briefly mentioned in the current review. Ammonia inhibition includes two states, namely complete ammonia inhibition and "inhibited steady-state." For the former, the microorganisms completely lose their metabolic activity. Regarding the latter, methane production remains stable even though the digestion system is inhibited by ammonia nitrogen. NH_4Cl, $(NH_4)_2CO_3$, NH_4HCO_3, urea, and $(NH_4)_2SO_4$ were often used to simulate the different TAN concentrations in previous studies (Yan et al., 2019; Yang et al., 2018; Bonk et al., 2018). Complete ammonia inhibition will generally happen when TAN concentration is above 14 g L^{-1} (see e-supplementary material). However, the TAN concentration is rarely greater than 15 g L^{-1} in the actual AD system without extra supplement of nitrogen source. Therefore, "inhibited steady-state" is the most common ammonia inhibition type. Under the "inhibited steady-state" condition, the VFAs concentration and pH (> 7) are high (Zheng et al., 2021).

Ammonia inhibition will lead to low digestion performance, caused by the imbalance of microorganisms (mainly bacteria and archaea) (Cho et al., 2017). Compared with methanogenic archaea, bacteria have higher resistance to ammonia toxicity (Capson-Tojo et al., 2020). Acetoclastic methanogen dominates in the unstressed AD system, and the acetoclastic pathway is the main methane-producing pathway. *Methanosaeta* and *Methanothrix* are common strict acetoclastic methanogens (Su et al., 2015). Ammonia nitrogen is the main factor driving the succession of methanogenic archaea's community (Bonk et al., 2018). Some methanogenic archaea have high ammonia nitrogen resistance because of their particular morphology and structure. For instance, Capson-Tojo et al. (2020) reported that the AD system had a high digestion efficiency when the NH_3 concentration was above 1000 mg L^{-1} due to the succession of methanogenic archaea from *Methanosaeta* to *Methanoculleus*. Interestingly, the succession of methanogenic archaea has a certain sequence along with the increase of TAN concentration. The strict acetoclastic methanogen (*Methanosaeta*) will be replaced by versatile methanogenic archaea (*Methanosarcina*) and then the hydrogenotrophic methanogens (*Methanothermobacter*, *Methanoculleus*, *Methanobrevibacter*, *Methanospirillum*, *Methanolinea*, *Methanomassiliicoccus,* and *Methanosphaera*) (Cai et al., 2021). Therefore, the hydrogenotrophic pathway will dominate in the AD system with a high TAN concentration. The syntrophic acetate oxidation bacteria (SAOB) are an essential partner of hydrophilic methanogens. Cai et al. (2021) showed that SAOB was sensitive to H_2 partial pressure. The community structure of bacteria will also change along with the succession of methanogenic archaea's community.

3. *Biochar properties associated with mitigation of ammonia inhibition*

The properties of biochar determine its effect on alleviating and eliminating ammonia inhibition when used as an additive in the AD with high ammonia nitrogen. A detailed review of this topic can be found in the literature (Weber et al., 2018). This section only provides a brief overview of biochar characteristics related to reliving ammonia inhibition. The physicochemical properties of biochar play a significant role in alleviating ammonia inhibition. They include elemental composition, ash content, porosity (the percentage of pore volume to the total volume of biochar), specific surface area, graphite structure, redox properties, and pH. For instance, graphite structure and redox properties correlate with electrical conductivity (see in Section 5.1). Ash content, pH, and hydroxyl groups affect the surface charge of biochar (Tan et al., 2020) (Details are discussed in Section 5.4.1). Porosity and specific surface area affect the growth of microorganisms (see in Section 5.2). Key physicochemical properties of biochar are affected by pyrolysis temperature (see e-supplementary material). The ratio of H/C, N/C, and O/C can represent the number of hydroxyls, amino and carboxylic groups, respectively, which can affect the adsorption capacity of ammonia nitrogen on the biochar (Li et al., 2017) (Details are discussed in Section 5.4.1). Wood vinegar ($pH < 7$) is gradually produced, and carbon is slowly mineralized as temperature increases. Therefore, biochar's pH and ash content increase progressively along with the rise of pyrolysis temperature (see e-supplementary material). The pH and ash content levels affect the ability of biochar to mitigate ammonia inhibition, mainly through chemisorption and trace element concentrations, respectively (see in Sections 5.4.1 and 5.3).

4. *Effect of biochar addition on anaerobic digestion with high ammonia nitrogen*

concentration

As shown in Table 1, many studies proved the positive effect of biochar in AD systems with high TAN concentrations. Biochar addition can increase the methane yield (Sugiarto et al., 2021; Mumme et al., 2014), enhance digestion efficiency, such as shortening the digestion time (Indren et al., 2020), and improve biogas quality (mainly CH_4 content) (Shen et al., 2015; Wei et al., 2019). Some biochars still contain a large proportion of decomposable carbon, especially those produced at low pyrolysis temperatures. The remained decomposable carbon contributes to methane yield (Munne et al., 2014). The accumulation of VFAs quickly occurs in the high ammonia nitrogen system, making the digestion system fragile (Zheng et al., 2021). Biochar can provide high buffering capacity, leading to strong resistance to VFAs (Wei et al., 2020; Indren et al., 2020b). Xu et al. (2018) reported that biochar could also adsorb VFAs due to its porosity. Biochar can adsorb ammonia nitrogen to relieve ammonia inhibition because of polar functional groups and porous structures (Masebinu et al., 2019). The porous structure of biochar is beneficial to the growth of microorganisms, contributing to the enhancement of digestion efficiency (Indren et al., 2020a). The porosity and conductivity of biochar will improve DIET and help electron transfer (Wei et al., 2020). Biochar addition can increase the CH_4 content to more than 90% (Wei et al., 2019). This can be explained from two aspects. On the one hand, the direct adsorption of CO_2 by hydrophobic sites on biochar increases CH_4 content (Wei et al., 2020). On the other hand, biochar can promote the interaction between hydrogenotrophic methanogens and SAOB, which accelerates the conversion of CO_2 to methane (Shen et al., 2015). Conductivity, redox property, functional groups, high pH and alkalinity, rich in trace elements, high specific surface, and porous are the critical properties of biochar, which can affect digestion performance (see e-supplementary material). The average adsorption capacity of biochar without modification for ammonia nitrogen is about 50 mg g^{-1}. As shown in Table 3, the addition of biochar is positive for the digestion system with high ammonia nitrogen concentration when the amount of biochar is in the range of 2-40 g L^{-1}. Besides the positive effect, some researchers also reported that biochar could affect digestion performance negatively (Wei et al., 2020; Mumme et al., 2014; Rasapoor et al., 2020). Chen et al. (2021) found that when the biochar addition exceeded a certain amount, the volumetric biogas production rate decreased (Indren et al., 2020b). Excessive biochar addition may damage the digestion system in two ways. First, adding a large amount of biochar will lead to the deficiency of nitrogen source when the ammonia nitrogen concentration is low. In this case, microbial growth and metabolism will be inhibited because ammonia nitrogen is a nitrogen source for microorganisms. Second, biochar has high pH (6.8-11.3) (Table 3). According to the chemical equilibrium of ammonia nitrogen, the high pH of biochar can promote the conversion of NH_4^+ to NH_3, which is unfavourable to the AD system because NH_3 is more toxic than NH_4^+ (Wei et al., 2020). Excessive biochar leads to an increase in the pH of the digestion system, which sometimes is extremely detrimental to microorganisms. Therefore, the effect of biochar on alleviating ammonia inhibition is related to the TAN concentration and the amount of biochar supplementation. The biochar addition ratio needs to be optimized based on different TAN concentrations when biochar is used as an additive in the digestion system to enhance biogas production and alleviate ammonia nitrogen.

5. Mechanisms of biochar in relieving ammonia inhibition

Based on the previous reports, there are mainly four mechanisms of biochar in reducing ammonia inhibition, namely 1) acceleration of the DIET pathway, 2) assistance on growth and attachment of microorganisms, 3) provision of nutrients and 4) adsorption of ammonia nitrogen and pH buffering. These were discussed in detail as follows.

5.1 Direct interspecies electron transfer

5.1.1 Electron transfer of biochar

The conductivity and redox properties are the main factors affecting electron transfer efficiency (Feng et al., 2021). They are influenced by the biochar's graphitic structure and functional groups, respectively. Oxygen-containing functional groups (OCFGs) are biochar's most important functional groups. Among these OCFGs, the phenolic hydroxyl group is the main electron-donating group, and the quinone, carbonyl, and carboxyl groups are the main electron-accepting groups. These characteristics will affect electron transfer mechanisms (Figure 1). When the pyrolysis temperature is lower than 500 °C, the electrical conductivity of biochar is low, and there are prominent redox peaks on the cyclic voltammetry curve, which indicates the electron transfer mode of biochar is determined by the functional groups (Feng et al., 2021). Interestingly, the number of OCFGs decreases and the conductivity of biochar increases along with an increase of pyrolysis temperature. At the same time, the peak current of the redox peak decreases, and the cyclic voltammetry curve gradually changes into "shuttle shape", which indicates that the electron transfer mode of biochar gradually changes to another type, which mainly depends on the conductivity of biochar. Similarly, Sun et al. (2017) reported that directly transfer electrons through conductivity was more than three times faster than through OCFGs when biochar's H/C and O/C ratios were less than 0.35 and

0.09, respectively. Therefore, when some strategies are used to enhance the electron transfer rate of biochar, the properties of biochar (OCFGs and redox properties) should be considered first.

5.1.2 Effect of direct interspecies electron transfer on reliving ammonia inhibition

In the AD system, the interspecies electron transfer (IHT) pathway (Martins et al., 2018) and direct interspecies electron transfer (DIET) are two-electron transfer pathways related to methane production. IHT includes acetoclastic, hydrogenotrophic, and methylotrophic pathways. DIET is more efficient and stable than IHT (Yang et al., 2017) since the velocity of electron transfer of DIET is several times higher than that of IHT (Viggi et al., 2014). Generally, the connection of DIET among different microorganisms are biological electrical connections such as conductive pili and e-transport protein (Park et al., 2018). Under ammonia-inhibited conditions, the expression of the biological electrical connections is repressed, which leads to the inhibition of the DIET pathway. Interestingly, the DIET pathway can be enhanced by supplementing conductive materials such as biochar. Biochar possesses conductivity and redox properties because of graphite structure and OCFGs (Martins et al., 2018). Therefore, it can be an electron carrier to enhance DIET, thereby accelerating methane production (Wang et al., 2017; Yan et al., 2021). For instance, Chen et al. (2014) found that the DIET between *Geobacter metallireducens, Geobacter sulfurreducens* and *Methanosarcina barkeri* was through biochar instead of biological electrical connections. Pan et al. (2019) also reported that the DIET had high energy efficiency and the interaction of microorganisms could be enhanced by adding biochar. *Geobacter* species is a type of bacteria that can cooperate with archaea through DIET and involve the degradation of ethanol, propionate, and butyrate. For example, Zhao et al. (2016) found that *Geobacter* and *Methanosaeta* or *Methanosarcina* could degrade propionic acid and butyric acid into methane through the DIET pathway. Wang et al. 2021 reported that rice straw biochar could improve the DIET pathway between *Geobacter, Methanosaeta,* and *Methanosarcina*. Besides *Geobacter* species, Syntrophic acetic acid oxidizing bacteria (SAOB) are also the most commonly reported bacteria potentially involved in DIET. Westerholm et al. (2012) claimed that supplementation of biochar in the high ammonia nitrogen system resulted in a 9-fold increase in the abundance of *Syntrophaceticus schinkii*, and an increase in the proportion of the *Methanothermobacter thermautotrophicus*. However, if only supplemented *Methanothermobacter thermautotrophicus*, *Syntrophaceticus schinkii* did not increase. This suggests that biochar provides a connection for the DIET between *Syntrophaceticus schinkii* and *Methanothermobacter thermautotrophicus*, resulting in the rapid recovery of the digestion performance of ammonia inhibited system. Biochar also provides a suitable living environment for these microorganisms participating in the DIET pathway to ensure their function. For example, Wang et al. (2018) found that biochar could simultaneously increase the abundance of Anaerolineaceae and *Methanosaeta*. Under different ammonia nitrogen conditions, the microorganisms participating in DIET may be different. As shown in Table 2, many methanogenic archaea can participate in the DIET pathway mediated by biochar, such as *Methanosarcina*, *Methanosaeta,* and *Methanospirillum* (Pan et al., 2019; Indren et al., 2020b). This allows biochar to be used at different TAN concentrations to mediate the DIET pathway. Figure 2 clearly shows the succession mechanism of the DIET pathway mediated by biochar under different TAN concentrations. As discussed in section 2, ammonia nitrogen is a critical parameter in determining the community composition of methanogenic archaea in the anaerobic digestion of nitrogen-rich wastes. Biochar mainly mediates the DIET pathway between bacteria and *Methanosaeta* at relatively low ammonia nitrogen concentrations (FAN < 100 mg L^{-1}). *Methanosarcina* is the main archaea participating DIET-mediated by biochar when the FAN concentration is in the range of 100-500 mg L^{-1}. Hydrogenotrophic methanogens become dominant archaea when the FAN concentration is higher than 500 mg L^{-1}. In this case, biochar promotes the DIET pathway as an electron carrier for hydrogenophilic methanogens and bacteria. Therefore, biochar enhances the DIET pathway regardless of the ammonia-inhibited state, benefiting from the non-specificity of biochar's ability to immobilize microorganisms.

5.1.3 The role of direct interspecies electron transfer may be exaggerated

A previous study showed that high ammonia nitrogen concentration would weaken the DIET pathway (Yan et al., 2020). The improvement of digestion performance is often attributed to DIET enhancement after supplementing biochar (Ma et al., 2019). However, some studies did not give enough evidence about DIET enhancement. For example, microorganisms with electrical activity (such as *Geobacter* species and *Syntrophus aciditrophicus*) were not enriched by the supplement of biochar in some reports. Although there may still be many electroactive microorganisms that have not yet been discovered, it is often unreasonable to rely solely on the succession of the microbial community (based on 16S rDNA data) to claim the presence or enhancement of DIET. From this perspective, the effect of biochar on enhancing the DIET pathway and then alleviating ammonia inhibition may be exaggerated (Yan et al., 2020). The developments of molecular biology and bioinformatics technology make it possible to research the DIET pathway in the complex digestion system. First, it is necessary to use high-throughput sequencing technology and q-PCR technology to explore the existence of some known electroactive microorganisms such as *Geobacter* species. In this case, combining fluorescence in situ hybridization (Fish) and electron microscope technology is an efficient way to observe agglomerates' generation and the spatial structure of electroactive microorganisms. Second, combining metagenomics and metaproteomics and annotation databases (KEGG and GO) can effectively detect the related gene and gene expression participating in electronic delivery such as PilA, OmcS, and OmcE when the known electroactive microorganisms cannot be found. At the same time, testing microbial DIET ability after the isolation of microorganisms is an effective method to find the electroactive microorganisms.

The contribution of the DIET pathway to relieving ammonia inhibition is still not clear after the biochar supplement. Some experiments should be carried out to determine this. For example, zeolite or other materials without conductivity can be used as a control group to explore the contribution of the DIET pathway to alleviating ammonia inhibition.

5.2 Effect of biochar on promoting microbial growth in the ammonia-stressed system

Biochar can immobilize microorganisms and then enhances the ability to resist ammonia nitrogen toxicity. The enrichment depends on the parent material of biochar, the number, and types of pores. The size of bacteria and archaea is in the range of 400-2000 and 100-15000 nm, respectively. The mesopores (2-50 nm) and micropores (0.1-50 nm) are not large enough for microorganisms. Therefore, macropores are the most critical parameter for microbial colonization. The number of macropores determines the colonization ability (Indren et al., 2020a). Biochar provides a suitable micro-environment for microorganisms to survive in stressed environments. Microorganisms and their DNA were found in the pores of biochar (Mumme et al., 2014; Indren et al., 2020a; Fagbohungbe et al., 2016). The interaction between biochar and microorganisms has been studied. Yan et al. (2021) and Indren et al. (2021) reported that biochar could promote the colonization of hydrogenophilic methanogens and versatile methanogen, leading to an increase in AD's resistance to ammonia nitrogen. While, Shen et al. (2020) and Zhang et al. (2019b) revealed that biochar could only promote the growth of acetoclastic methanogens. These results illustrate that the immobilized effect of biochar on microorganisms is not specific (Wei et al., 2020). Interestingly, the immobilization effect of biochar may be related to the cell morphology of the archaea when multiple archaea with the same methanogenesis pathway exist. It still requires further research to explore these unknown. Besides archaea, the effect of biochar's adsorption and immobilization on bacteria is also often reported (Indren et al., 2020b). For example, Wei et al. (2020) reported that the *Rhodobacter sp*., which was responsible for hydrolysis, dominated the biochar-added reactor, and the abundances of *Paludibacter sp*. (producing VFAs) and *Proteinclasticum sp*. (producing H_2) increased by 39.4% and 46.25, respectively (Wang et al., 2017).

In addition to immobilizing microorganisms, biochar can also affect the growth of suspended microorganisms. The methanogenic archaea enriched by biochar is closely related to the methanogenic archaea growing in suspension, which are affected by ammonia nitrogen concentrations of ammonia-stressed AD systems. The species of methanogenic archaea in suspension are mainly affected by ammonia nitrogen concentration. Interestingly, biochar itself can promote the succession of microorganisms. For instance, Giwa et al. (2019) reported that *Methanothrix* and *Methanosarcina* dominated in biochar-added and control reactors, respectively, when the TAN concentration was greater than 2.45 g L^{-1}. Similarly, Ma et al. (2019) reported that biochar could promote the succession of *Methanosaeta* to *Methanosarcina*, which led to the increase of capacity of resisting ammonia nitrogen inhibition. The adsorption capacity, pH, and buffering capacity of biochar may be the key factors affecting the microbial species in the suspension. These characteristics of biochar obviously affect the growth environment of microorganisms. This may be an indirect mechanism that biochar affects the structure of the archaeal community. The direct mechanism of biochar to promote the succession of microorganisms in suspension still needs to be further studied.

5.3 ***Provision of nutrients***

Although there is no direct relationship between trace elements and the adsorption ability, many articles have proved that they could alleviate ammonia inhibition by improving microbial activity, especially methanogenic archaea (Molaey et al., 2018b; Molaey et al., 2018c). Biochar contains abundant macro-elements (C, H, O, K, Na, and Ca) and trace elements (Fe, Co, Ni, Mn, Mo, Se, and Zn) (Wambugu et al., 2019; Indren et al., 2020a; Sanchez et al., 2021). The elemental composition of biochar is directly related to the parent material. Indren et al. (2020a) compared the composition of trace elements in wood pellet, wheat straw, and sheep manure biochar and found that Fe, Mn, and Zn content in sheep manure biochar was the most abundant. Wambugu et al. (2019)

tested the elemental composition in the leachate of the biochar and found that the potassium content was higher than 1 g kg^{-1}, while the Fe, Co, Ni, and Mn content were less than 10 mg kg^{-1}. Compared with wood and straw biochar, sludge and livestock manure biochar contain higher ash content. For instance, the ash content of sludge biochar is 40-70%, while the ash content of coconut shell biochar is only 2-4%, which means that there are more mineral elements in the sludge (Zhang et al., 2019). Sugiarto et al. (2021) extracted the biochar's leachate using citric acid and found that the leachate was rich in Fe and could enhance digestion performance due to the increase of *Clostridia* and *Methanosaeta*.

In general, macronutrients are sufficient in digestion systems, while the deficiency of trace elements is often reported (Molaey et al., 2018a; Bhatnagar et al., 2020). The deficiency of trace elements will lead to volatile fatty acids accumulation. Supplementation of trace elements is often used as a vital strategy to alleviate ammonia inhibition (Cai et al., 2021b). Biochar can promote digestion performance due to the abundance of trace elements. For example, Sanchez et al. (2021) reported that the content of acetyl-CoA synthase and methyl coenzyme M in the biochar-added treatment was higher, which might be related to the presence of Fe, Co, Ni, and Mn. Similarly, Yue et al. (2019) also found that biochar could promote the growth of microorganisms due to the existence of trace elements. Interestingly, Cai et al. (2018) claimed that the deficiency of trace elements in the digestion system was also related to the low bioavailability of trace elements. In the AD systems with high ammonia nitrogen, the pH is often higher than 8, leading to low bioavailability of trace elements. The bioavailability of trace elements (Fe, Co, Ni) can be enhanced by biochar, thereby increasing the abundance of related enzyme genes and enzyme activity (Qi et al., 2021). Generally, chemical ligands such as EDTA and EDDS are commonly used to regulate the bioavailability of trace elements (Cai et al., 2019). Some bio-ligands such as SMPs and EPS can also regulate the bioavailability of trace elements. Biochar can increase the concentration of SMP and EPS by improving the activity of microorganisms, which leads to the high bioavailability of trace elements (Qi et al., 2021).

5.4 Adsorption of ammonia nitrogen by biochar

5.4.1 Adsorption mechanism and affecting factors

As shown in Figure 4, there are four mechanisms for ammonia nitrogen adsorption, including chemical adsorption (electrostatic attraction, ion exchange, and complexation) and physical adsorption (Masebinu et al., 2019; Fagbohungbe et al., 2016). Biochar surfaces usually are negatively charged because of the presence and dissociation of OCFGs such as carboxyl, lactone, phenol, carbonyl, lactol, anhydride, pyrone, ether, chromene, and quinone (Tomczyk et al., 2020; Yan et al., 2021; Qiu et al., 2019). The negative surface charge (NSC) is positively correlated with the surface polarity of biochar, which determines the ability of chemical adsorption (Tian et al., 2020; Rasapoo et al., 2020). Electrostatic attraction, ion exchange, and complexation are related to the number of OCFGs (Masebinu et al., 2019; Qambrani et al., 2017; Ahmad et al., 2014). Compared with complexation and electrostatic attraction, the ion exchange plays a more significant role in chemical adsorption (Vu et al., 2018). All the four adsorption pathways are affected by biochar characteristics (functional groups type and number, porosity, and specific surface area) and digestion conditions such as pH and cation concentration.

The specific surface area (2.32 to 766.00 $m^2 g^{-1}$) and porosity are positively correlated with physical adsorption (mainly intraparticle diffusion and surface site absorption). The specific surface area mainly affects the capacity of surface site absorption. The porosity affects the physical adsorption of ammonia nitrogen by influencing intraparticle diffusion. Functional groups mainly affect chemical adsorption. Figure 5a showed the typical functional groups on the surface of biochar (Tang et al., 2019; Zhang et al., 2020; Xue et al., 2019). Hydroxyl, carboxylic, and amino are the main functional groups contributing to ammonia nitrogen absorption. The cationic exchange capacity (CEC) is positively related to the number of OCFGs of biochar. Ion exchange happens because of the possible metal exchange between NH_4^+ and alkali metals (K^+, Mg^{2+}, $Ca^{2+,}$ and Na^+) available on the biochar surface (Masebinu et al., 2019). The OCFGs gradually disappear with a further rise in the pyrolysis temperature (Figure 5b). Among these functional groups, the order of stability is amine and amides < polysaccharides or phosphodiesters < $-CH_3$, -OH, and -NH groups < carbonyl, carboxyl, and aromatic ring (Figure 4b). The decrease of functional groups reduces polarity, which is detrimental for chemisorption (Ahmad et al., 2014). Therefore, chemical attraction capacity is sensitive to the change of pyrolytic temperature of producing biochar (Ahmed et al., 2016a; Yin et al., 2017).

pH has a close relationship with NSC (Tan et al., 2020). The pH of the digestion system affects the adsorption of biochar by affecting the form of ammonia nitrogen and the surface charge of biochar. First, pH influences the charge on the surface of biochar by affecting OCFGs' protonation and deprotonation. Among these OCFGs, hydroxyl and carboxyl have a decisive effect on the SNC, and the number of the hydroxyl group is higher than that of carboxyl. Second, NH_4^+ can be transformed to NH_3 as the pH increases (Zheng et al., 2021). Biochar adsorbs NH_3 only by physical adsorption, which is less efficient than the adsorption of NH_4^+. As a result, the ability of biochar to adsorb ammonia nitrogen decreases as the pH increases (Du et al., 2005; Yang et al., 2018). Adding biochar into AD often leads to a higher system's pH. Therefore, excessive biochar loading can negatively affect AD performance (Zhang et al., 2019)

As shown in Figure 6, the point of zero charges (pH_{pzc}) of the biochar is also a critical parameter affecting the adsorption ability of the biochar to ammonia nitrogen. The pH_{pzc} is the pH of the digestion system under which there is no charge on the surface of biochar. In general, the pH_{pzc} is in the range of 2-8 (Song et al., 2018). At $pH_{pzc,}$ the adsorption ability of biochar to NH_4^+ through the electrostatic attraction pathway is weak (Shi et al., 2013). When the pH is lower than pH_{pzc}, the positively charged NH_4^+ is repelled by the biochar with a positive charge. Simultaneously, the positive charge and the amount of H^+ on the surface of biochar decrease as the pH of the digestion system increases ($pH < pH_{pzc}$) so that the adsorption capacity of the biochar for ammonia nitrogen increases. The H^+ will compete with NH_4^+ for the functional groups on the biochar surface, reducing adsorption efficiency. Biochar is mainly negatively charged when the pH of the digestion system is higher than pH_{pzc}. The lower the pH_{pzc} is, the more negative charges on the surface. In this case, the adsorption capacity of biochar to adsorb ammonia nitrogen through electrostatic attraction, ion exchange, and complexation is enhanced.

5.4.2 Adsorption enhancement by modification

The average absorption capacity of unmodified biochar is about 50 mg g^{-1}, which is insufficient to relieve ammonia inhibition, especially for the digestion system with high ammonia concentrations (> 6 g L^{-1}). Therefore, the main mechanism of reducing ammonia inhibition for biochar does not originate from the adsorption capacity if the biochar is not modified (Lu et al., 2016). Modification is an effective way to enhance the absorption capacity by increasing functional groups and specific surface area and increasing the adsorption site, mainly including acid, alkali, and magnetic modification (Chen et al., 2021; Wang et al., 2019; Li et al., 2020; Qin et al., 2017). Other modified methods such as carbonaceous materials and organic solvents have obvious disadvantages (mainly potential pollution and high cost). Therefore, these methods are not discussed in this review. The effect of the modified biochar on the performance of anaerobic digestion with high TAN concentration was summarized and listed in Table 3.

It is evident from Table 3 that acid (hydrochloric acid, nitric acid, sulfuric acid, and phosphoric acid) modification can increase the number of acidic functional groups (3.5-7.4 times) (Qiu et al., 2019). In addition, acid washing removes some of the minerals, opening up pores and thus leading to high specific surface area (Wang et al., 2019). These changes will improve the adsorption efficiency of biochar to ammonia nitrogen. In addition, acid modification can help to form new functional groups with high adsorption capacity. For instance, after biochar modification using 0.2 mol L^{-1} H_2SO_4, the amount of carboxyl group decreased while the amount of the lactone group increased due to the dehydration effect of carboxyl and hydroxyl (Chen et al., 2021; Wang et al., 2014; Ahmed et al., 2016b). The ability of biochar to adsorb ammonia nitrogen through chemical adsorption (mainly electrostatic adsorption and complexation) increased by 1.6

times because of the newly formed lactone groups. In addition, the increase of acidic oxidizing groups can increase the hydrophilicity and cation exchange capacity of biochar (Wahab et al., 2012). Interestingly, acid modification also can increase the aromaticity of biochar (Shi et al., 2013), which is beneficial for ammonia nitrogen adsorption. The acid-modified biochar can also reduce the pH_{pzc} of the biochar, making it have higher adsorption capacity in the digestion system with neutral pH.

Alkali, such as KOH and NaOH, is also often used to modify biochar to increase specific surface area and functional groups (Wang et al., 2019). The increase in the specific surface area might be due to the corrosiveness of strong alkalis, which can make the surface of biochar rough. However, there is no related report on the mechanism of alkali affecting the number of functional groups. KOH has stronger alkaline than NaOH. To improve the adsorption capacity, the concentration of NaOH and KOH should be higher than 3 M and 2 M, respectively. The effect of alkali on modification also depends on the type of biochar. For example, the specific surface area and total pore number of corncob waste biochar increase after NaOH modification, leading to a 42% increase in biochar's absorption capacity (Vu et al., 2018). However, Fan et al. (2010) found that NaOH modification only had a slight effect on the surface area of bamboo biochar. It is worth noting that these cations in these alkalis will compete for adsorption sites with NH_4^+, which will lead to a decrease in absorption for NH_4^+ (Yang et al., 2018; Zheng et al., 2020). Therefore, the alkali concentration used for modification should not be too high. The alkalinity of biochar is enhanced after alkali modification. Therefore, special attention needs to be paid to the potential negative impact of pH on the digestion system when strong alkali is applied to modify biochar.

Another modification method of biochar is magnetization using iron salt. Biochar is easier to be recycled after magnetization (Hu et al., 2019; Qin et al., 2017). The iron oxide attached to the surface of the biochar can increase the specific surface area, and the Fe-O functional group provides new active sites, thereby increasing the adsorption capacity (Wang et al., 2021a). For instance, Qin et al. (2017) reported that the surface area of biochar increased by 20 times after modification using $FeCl_3$ solution, which led to a 25% increase of methanogenic archaea. The magnetized biochar may have a strong electrical conductivity, which benefits the DIET pathway (Qin et al., 2017). The performance of the magnetized biochar is stable. Hu et al. (2019) reported that the magnetic biochar still had strong adsorption capacity after being recycled four times.

Although biochar modification can improve biochar quality, it may negatively affect the environment (potential secondary pollution), especially chemical-modified methods. Some researchers developed some new modification methods. Recently, Huang et al. (2021) reported that gas-modified biochar could significantly reduce the pore size of biochar and increase the specific surface area. Gas modification may mainly change the physical adsorption capacity of biochar for ammonia nitrogen. However, the adsorption performance of modified biochar using this way still needs to be determined in actual high ammonia nitrogen digestion system.

6. *The potential application mode of biochar in relieving ammonia inhibition*

The essence of ammonia inhibition is the imbalance of microbial consortium in the AD system. Under ammonia inhibition, the methanogenic activity of methanogenic archaea is suppressed. Bioaugmentation can effectively relieve ammonia inhibition by adding one or several pure strains, especially methanogenic archaea with high ammonia nitrogen resistance (Fotidis et al., 2014). However, pure strains are difficult to colonize. They have no advantage in competing with indigenous microorganisms. Some researchers reported that exogenous supplementation of ammonia-tolerant microbiology consortium, consisting of several kinds of microorganisms with a mutual relationship, made the microbiota more resistant and easier to colonize (Wang et al., 2015). For example, It was reported that the ammonia nitrogen resistance of *Methanosarcina* was greatly improved in the presence of SAOB (Wang et al., 2015). Therefore, future research should focus on supplementing microbiology consortium with mutual relationships. Heitkamp et al. (2021) found that the concentration of microorganisms attached to biochar was higher than the average concentration of suspended and attached growth microorganisms. This means that biochar can be used as a carrier for microorganisms to alleviate ammonia inhibition and is an ideal partner for bioaugmentation. In addition, biochar is not specific for microbial enrichment, which is beneficial for enriching microorganisms with different functions simultaneously.

The inhibition of bacteria and archaea by high ammonia nitrogen leads to the accumulation of VFAs such as propionic and butyric acids (Zheng et al., 2021). The DIET pathway also be suppressed in high ammonia concentrations (Yan et al., 2020). As shown in Figure 3, there are two promising application modes in reliving ammonia inhibition considering the combination of biochar and bioaugmentation. For the first one, the microbial consortium, containing propionate and butyrate degrading bacteria, SAOB, and methanogenic archaea with high ammonia resistance, can be enriched on biochar by restrictive culture. For example, the microbial consortium with high ammonia nitrogen resistance can be obtained by only using propionic and butyric acids as carbon sources under the conditions of gradient ammonia nitrogen acclimation. The second mode is the enrichment of electroactive microorganisms on biochar such as *Geobacter* species (Xu et al., 2022). For instance, Zhang et al. (2016) reported that electroactive bacteria could be enriched when ethanol was used as the sole carbon source (Zhao et al., 2016). Biochar magnetization (discussed in section 5.4.4) can be introduced into both modes considering the reuse of biochar (Qin et al., 2017; Hu et al., 2019). The microbiology consortiums loaded on the magnetic biochar can also be recovered. Therefore, magnetic biochar together with bioaugmentation is a promising combination way to relieve ammonia inhibition.

7. The prospectives for future research

1. The adsorption capacity of ammonia nitrogen by biochar is affected by the pH of the AD system. The AD system with high ammonia nitrogen usually has a pH value ranging from 7 to 8.5. The addition of alkaline biochar would change the system's pH and thus affect the subsequent adsorption capacity. Therefore, it is essential to study biochar's adsorption capacity under different pH.

2. The effect of biochar on the alleviating ammonia nitrogen depends on several factors such as the properties of biochar, biochar addition ratio, the digestion conditions (temperature and pH), and TAN concentration. Therefore, for a given substrate, the use of biochar as an additive to remove ammonia nitrogen needs to be examined and optimized individually. Biochar modification can also be deployed to improve the adsorption efficiency. However, the existing modifications such as acid, alkali, and oxidant modification still face many challenges such as high cost and potential environmental risks. Safe, efficient, and

practical modification methods should be developed in future.

3. The average adsorption capacity of unmodified biochar is less than 50 mg g^{-1}, and sometimes the adsorption capacity may not be the primary mechanism for biochar to relieve ammonia inhibition. Therefore, a deep understanding of the working mechanisms of biochar in reducing ammonia inhibition is still needed, particularly in the high ammonia nitrogen concentration.

4. The main archaea are versatile methanogen (*Methanosarcina*) and hydrogenotrophic methanogen (mainly *Methanobacterium*, *Methanoculleus, Methanothermobacter*) in the digestion system with high ammonia nitrogen. It has been reported that the beneficial effect of biochar on the AD system with high ammonia nitrogen is caused by promoting the DIET pathway. The hydrogenotrophic pathway is the main methane-producing way under high ammonia nitrogen conditions. Therefore, it is essential to reveal how biochar promotes the growth of versatile methanogen, hydrogenotrophic methanogen, and SAOBs. In addition, biochar is not specific for microbial enrichment, which is beneficial for enriching microorganisms with different functions simultaneously. Magnetic biochar can be well combined with bioaugmentation to enhance its effectiveness in mitigating ammonia inhibition.

5. The contribution of DIET to the mitigation of ammonia inhibition by biochar is still unclear because many studies do not provide sufficient evidence to prove the existence of DIET. The developments of molecular biology and bioinformatics technology (16S rDNA high-throughput sequencing, q-PCR, Fish, electron microscope technology, metagenomics and metaproteomics, and KEGG or GO)

make it possible to research the DIET pathway in the complex digestion system. Therefore, these techniques can be used in the future to explore the mechanisms of ammonia inhibition alleviation by biochar.

8. *Conclusions*

The mechanisms of biochar to alleviate ammonia inhibition are complicated. The adsorption and buffer capacity are not the main mechanisms, especially under high ammonia nitrogen conditions. The actual contribution of DIET to the mitigation of ammonia inhibition by biochar may be exaggerated because many related studies don't provide definite evidence. Magnetic biochar combined with bioaugmentation is a promising mode in mitigating ammonia inhibition based on biochar's capacity of immobilizing microorganisms. Current bioinformatics and molecular biology techniques should be used in conjunction to investigate the mechanism of alleviating ammonia inhibition by biochar.

E-supplementary data for this work can be found in e-version of this paper online.

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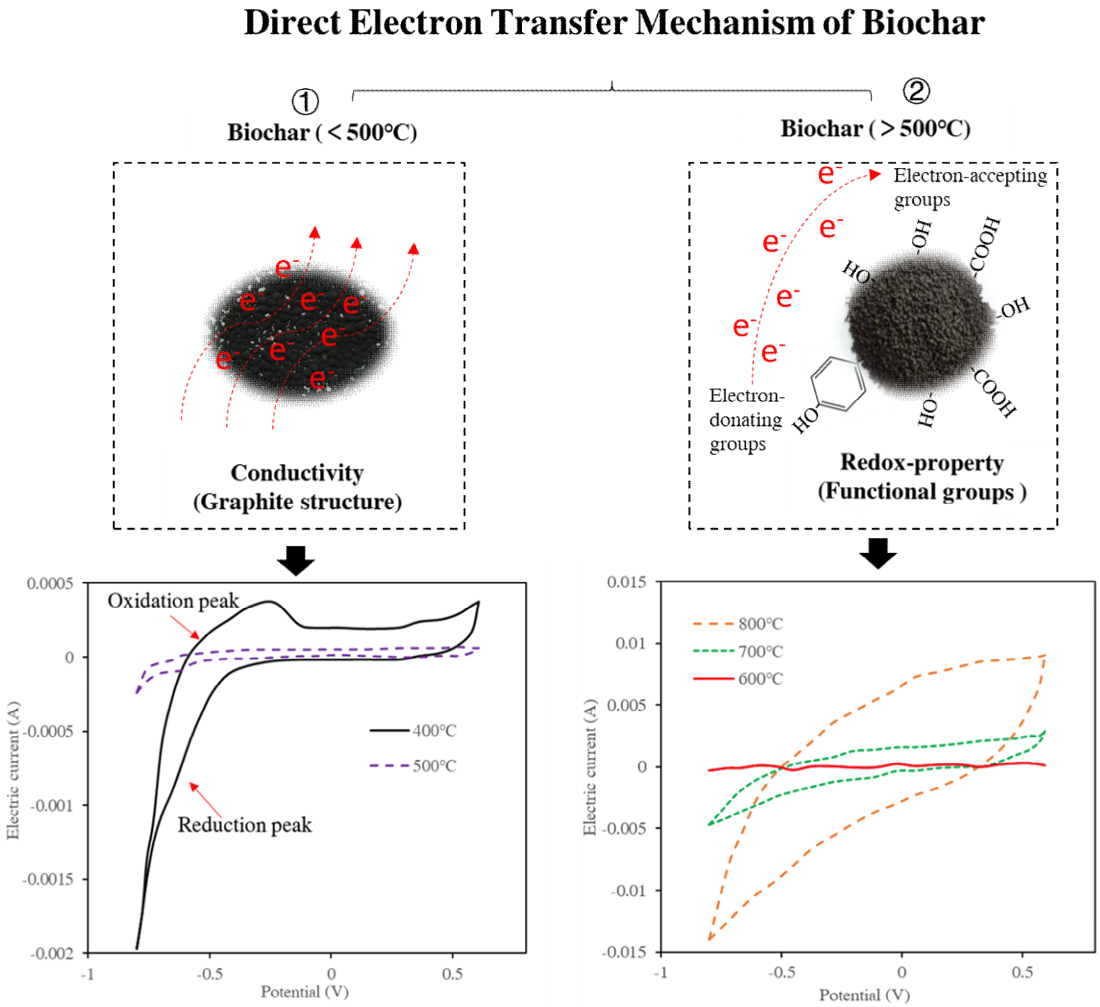

Figure 1. The two mechanisms of electron transfer in biochar considering different pyrolysis temperatures (>500℃ and <500℃). Some information originated from Sun et al. (2017) and Feng et al. (2021).

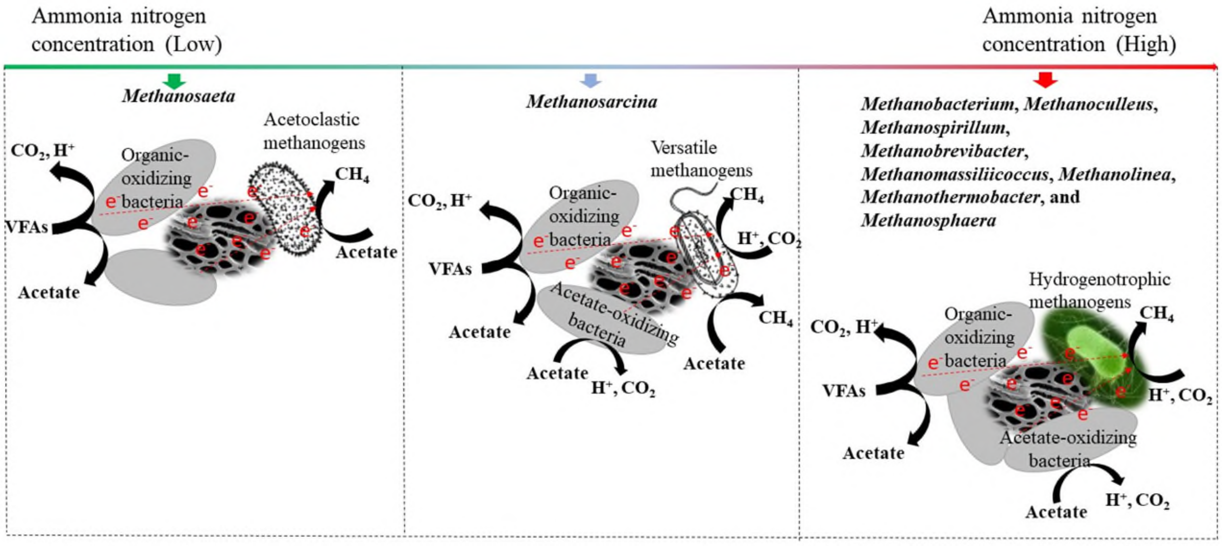

Figure 2. The succession mechanism of DIET pathway mediated by biochar under different ammonia nitrogen concentrations (Baek et al., 2018; Wang et al., 2021c).

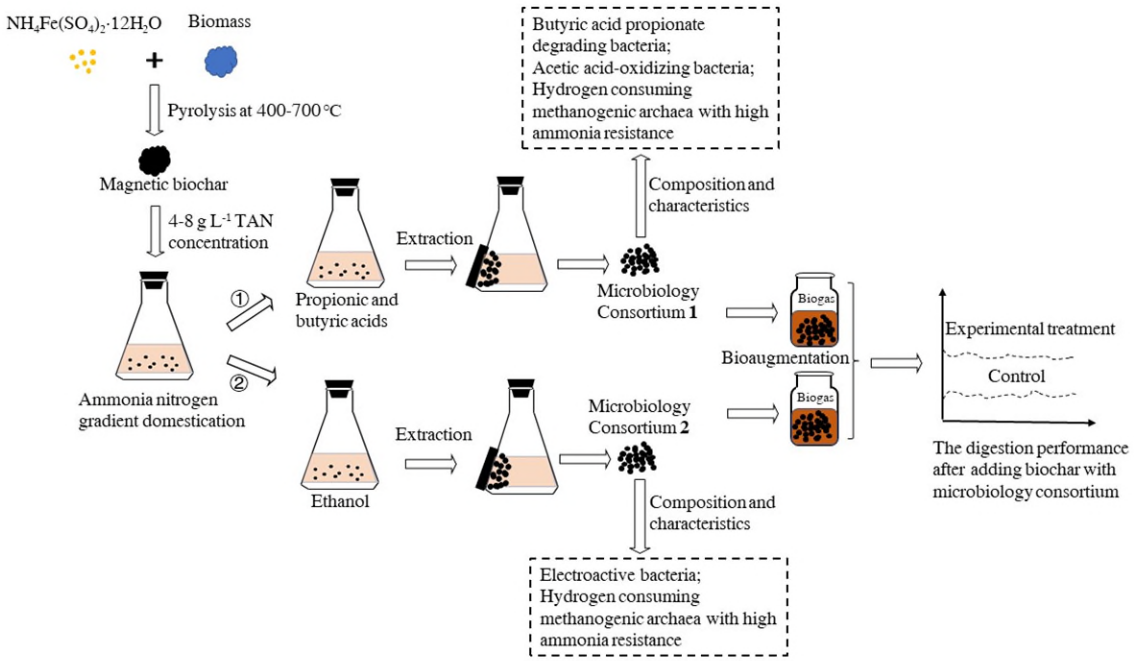

Figure 3. Magnetic biochar combined with two typical microbiology consortiums as a promising way to relieve ammonia inhibition.

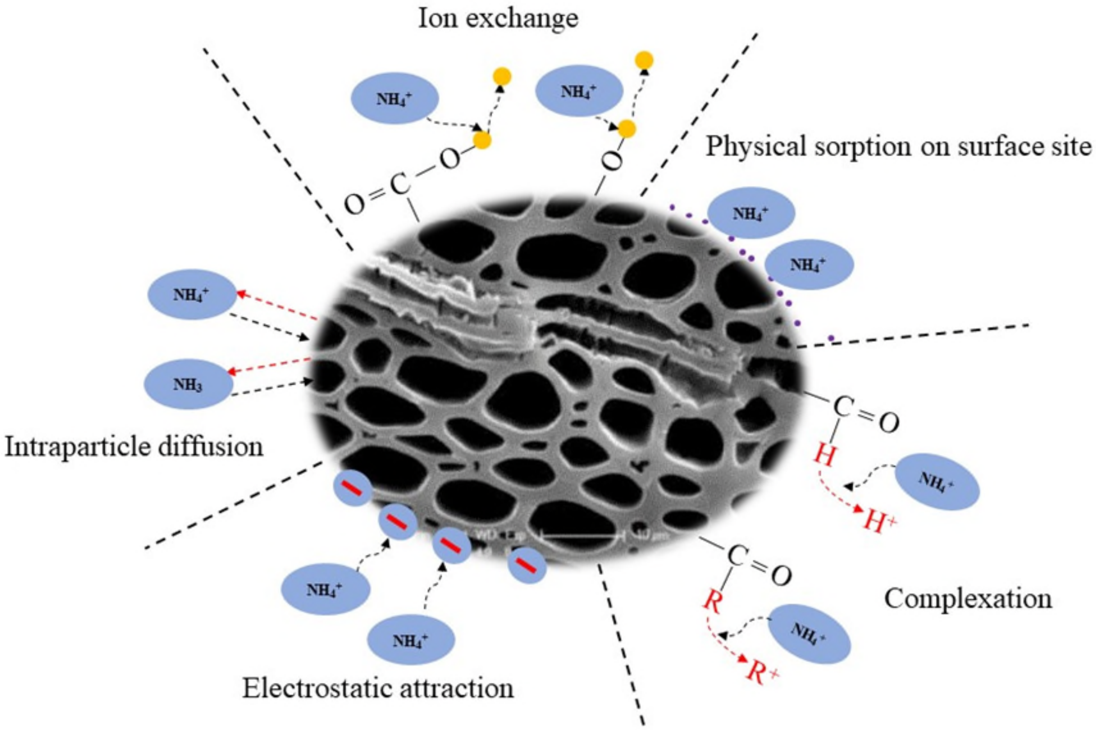

Figure 4. Mechanism of ammonia nitrogen adsorption on biochar (Chen et al., 2021; Wang et al., 2021b; Li et al., 2017).

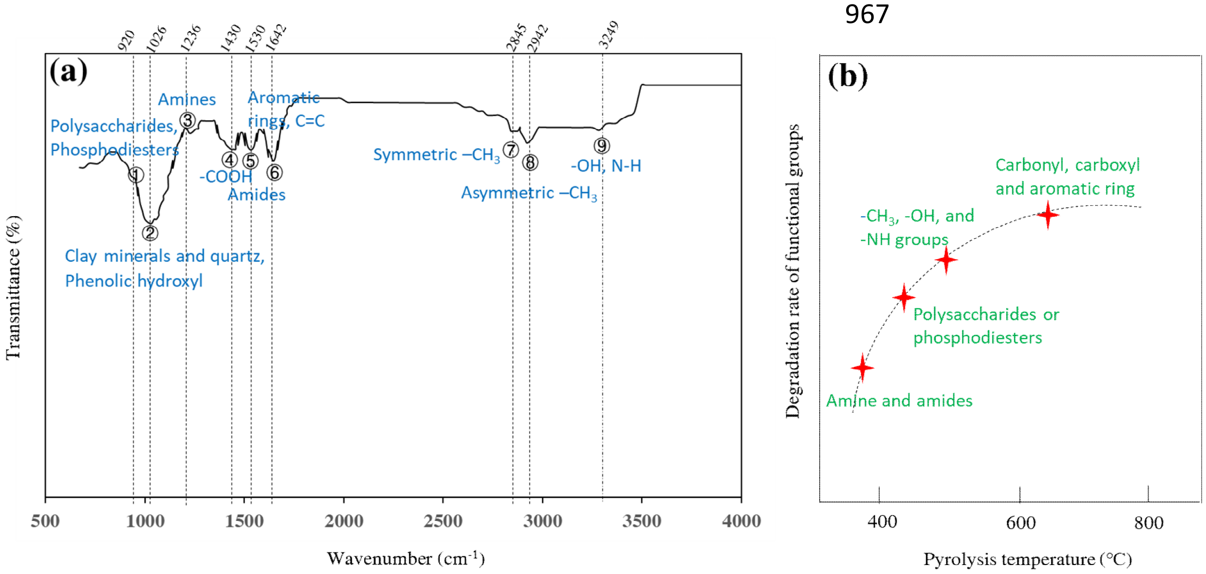

Figure 5. Typical functional groups on the surface of biochar that determine ammonia absorption (a); degradation rate of functional groups (b). Some information originates from Tang et al. (2019), Zhang et al. (2020) and (Xue et al., 2019)

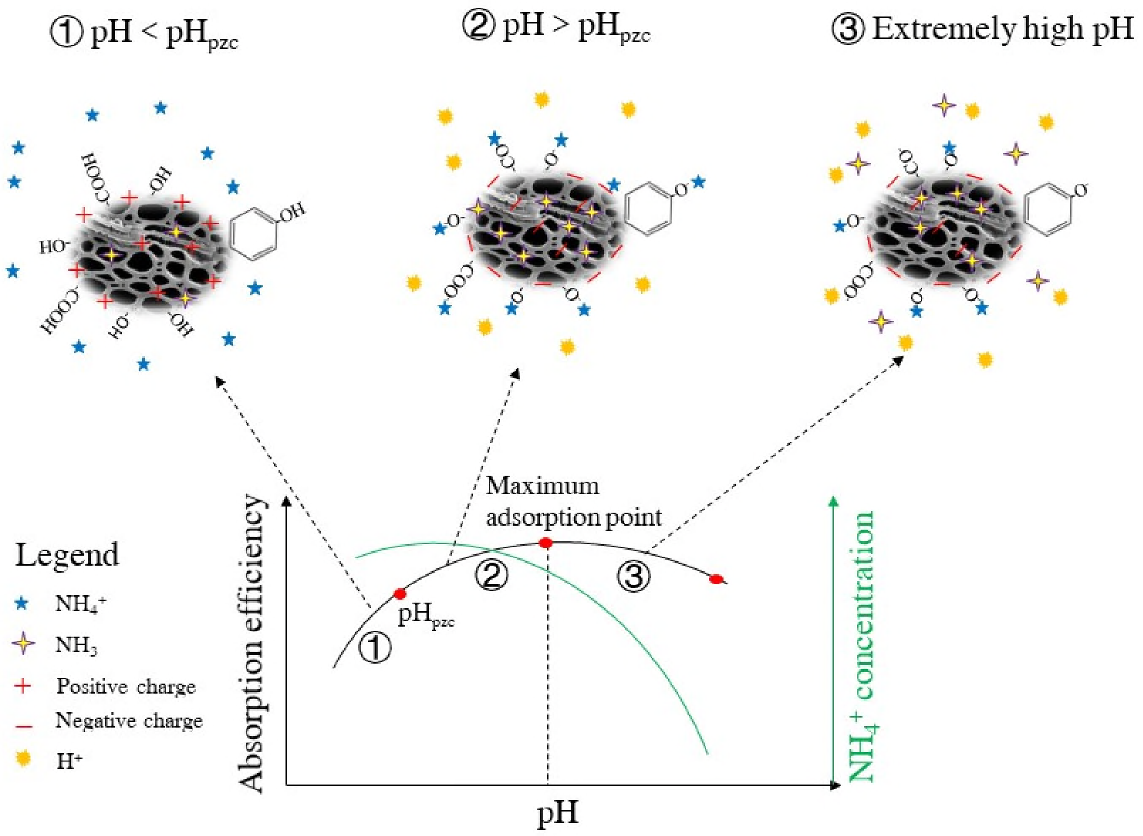

Figure 6. The relationship between the pH in the digestion system and the adsorption efficiency of ammonia nitrogen by biochar.

Table 1. Effect of biochar on the performance of AD with high ammonia nitrogen

Biochar type	pyrolysis conditions	Average pore size (nm)	Ash content (wt%)	pH	Specific surface area of biochar (m^2 g^{-1})	TAN concentration of digestion system (g L^{-1})	Addition ratio of biochar (g L^{-1})	TAN removal (mg g^{-1})	Performance	Reference
Straw biochar	750 °C	-	55.20	11.24	12.00	4	4	6.65	Methane yield was improved 35%. The number of *Methanosarcina thermophila* increase.	Yan et al., 2021
Corn stover biochar	600 °C	5.9	-	10.1	302.6	0.62	-	-	Methane content and yield increased by 28.9% and 17.8%, respectively.	Wei et al., 2020
Woody biochar	1100 °C	-	9.40	10.08	766.00	4	4	41.94	Methane yield was improved 24%. DIET was enhanced.	Yan et al., 2021
Woody biochar	500-600 °C	0.3-0.45	16.92	9.26	209	0.99-16	-	-	Biochar reduced the TAN concentration and increased the methane yield (32-36%) and enhanced tolerance of *MST* to ammonia nitrogen. It also enhanced DIET between bacteria and methanogens. .	Pan et al., 2019
Paper sludge and wheat husks (1:2 (v/v))	500 °C	-	-	-	-	2.1-6.6	20	2.4-6.8	Methane yield was improved 32%. When the TAN is below 2.1g/L, biochar can alleviate ammonia inhibition.	Mumme et al., 2014
Wheat straw digestate	230 °C	-	-	-	-	2.1-6.6	20	3.0-8.2	No noticeable effect was observed	Mumme et al., 2014
Coconut shell biochar	-	< 2	-	-	774.48	-	5-40[a]	-	The methane yield was improved 13%. Biochar	Shen et al., 2020

									enhanced the growth of acetoclastic methanogens.	
Rice straw biochar	600 °*C*	21.5	5.19	6.83	65.18	0.9-3.5	2-15	150-650	Biogas production was increased by 78.3% when the dose of biochar was 15g/L.	Cheng et al., 2020
Corn straw biochar	400-600 °*C*	-	17.1-20.8	8.2-8.3	29.8-56.6	4.28	8	55.0-98.8	Methane yield increased by 57.5-87.1%. The stability and buffering capacity increased.	Zhang et al., 2019
Coconut shell biochar	400-600 °*C*	-	2.5-3.6	9.3-9.7	16.1-26.3	4.28	8	76.3-98.8	Methane yield increased by 32.8-42.2%. The stability and buffering capacity increased.	Zhang et al., 2019
Sewage sludge biochar	400-600 °*C*	-	42.2-67.3	8.7-11.1	2.32-12.7	4.28	8	91.3-110	Methane yield increased by 22.4-49.1%. The stability and buffering capacity increased.	Zhang et al., 2019
Rice straw biochar	160-260 °*C*	13.0	-	7.3	19.4	2.08	2-10	25	Biochar enhances the intensity of functional groups and the immobilization of microorganism, which strengthens the conversion of organic acids to biogas.	Xu et al., 2018

Note: a, the dose of biochar was calculated based on 1 kg=1 L

Table 2: Potentially related microorganisms (mainly bacteria and archaea) participated DIET with biochar in AD systems.

Substrate	Biochar type	TAN concentration and pH	Related electron donating bacteria	Related electrotrophic methanogen	Reference
Pig manure	Rice husk	TAN: 4.14-12.24; pH: 6.77-8.02	*Defluviitoga*, *Thermovirga* and *Cloacibacillus*	*Methanothrix*	Yang et al., 2021b
Glucose; H_2/CO_2	Straw and spruce Woodchip	pH: 6.25-7.25	*S. schinkii sp.28*	*Methanothermobacter thermautotrophicus sp.3*	Yan et al., 2021
Food waste	Waste wood	-	*Syntrophomonas*	*Methanosarcina*	Cui et al., 2021
Ethanol	Wood chips	-	*Pseudomonas*	*Methanosaeta*	Qi et al., 2021
Dairy manure	Dairy manure	-	*Clostridium, Syntrophomonas and Syntrophus*	*Methanobacterium, Methanolinea and Methanomassiliicoccus*	Jang et al., 2021
Synthetic wastewater,	Rice straw	-	*Bacteroidetes*, *Smithella*, *Desulfovibrio* and *Geobacter*	*Methanosaeta, Methanosarcina*	Wang et al., 2021
Waste activated sludge	Sewage sludge	pH=7.1	*Syntrophomonas*, and *Peptococcaceae*	*Methanosaeta, Methanobacterium*	Wu et al., 2019

Table 3. The effect of biochar modification on its capacity for removing ammonia nitrogen in AD systems.

Biochar type	Biochar production condition	Modification method	Effect of modification on biochar characteristics	Effect of modified biochar on ammonia removal	Reference
Corn stalk and rice hull	Pyrolysis at 450-550 °C	H_2SO_4 (0.2 M): Biochar = 1:50. The mixture shaken at 200 r min^{-1} at 60 °C for 24 h	Decreasing carboxyl group and increasing the lactone group, which means that the number of acidic OCFGs rise. At the same times, new substituents form.	Chemical adsorption (mainly electrostatic adsorption and complexation) of modified biochar to NH_4^+-N was enhanced.	Chen et al., 2021
-	-	HNO_3 (5.0 M): Biochar =1:50. HCl: Biochar =1:50.	Enhancing the available functional groups and improving the quality of biochar's structure (homogenous).	Capacity of adsorbing ammonia nitrogen was enhanced.	Shi et al., 2013
Corncob wastes	Pyrolysis at 400 °C, 90 min	NaOH (0.3 M): Biochar =1:20 (v/w)	Total pore and specific surface area of modified biochar increase.	Adsorption capacity of biochar for ammonia nitrogen increased by 42% compare with raw biochar.	Vu et al., 2018
Rice straw	Pyrolysis at 500 °C for 2 h in N_2 environment	Rice straw: $FeCl_3$ solution = 1:8 for 2 h.	Specific surface area and active sites increase after magnetization.	Total amount of methane archaea increased by 25% and methane production greatly increased.	Qin et al., 2017
Sludge	Pyrolysis at 500 °C for 1 h in N_2 environment	1 g biochar mixed with 0.90 g $NH_4Fe(SO_4)_2\cdot 12H_2O$ and 0.39 g $(NH_4)_2Fe(SO4)_2\cdot 6H_2O$	The active sites increase after magnetization.	Adsorption capacity of modified biochar for ammonia nitrogen reached 17. 52 $mg\cdot g^{-1}$.	Wang et al., 2021a

The role of biochar on alleviating ammonia toxicity in anaerobic digestion of nitrogen-rich wastes: a review

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