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Review

Recent Advance on the Effects of Biochar on Constructed Wetlands: Treatment Performance and Microorganisms

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Abstract: Constructed wetlands (CWs) is a kind of green environmental protection technology, which is widely used in sewage treatment. The traditional CWs is faced with the problem of low treatment effect of high concentration sewage. In recent years, biochar, as a new type of adsorption material, has been used in CWs because of its advantages of large specific surface area, strong adsorption capacity and wide material sources. This paper systematically summarized the characteristics of biochar and the preparation of biochar by studying the changes of microorganisms added to CWs, and compared the effects of different treatment methods coupled with biochar on the treatment performance of CWs, the effects of biochar coupled with CWs on enzyme activity, functional genes, metabolites and microbial communities were investigated. This review summarizes how different preparation methods affect the properties of biochar and how these biochar cause changes in the microorganisms added to CWs.

Keywords: constructed wetlands; biochar; enzyme activities; microbial communities

1. Introduction

Most developing countries face serious water pollution problems, especially in underdeveloped rural areas[1]. A large amount of nitrogen and phosphorus sewage is put into the natural water body, which has a serious impact on people's life. Constructed wetland (CW), as a low-cost ecosystem, has the advantages of small area, simple operation, green, easy maintenance, and so on, and is widely used in sewage treatment[2], and has a high removal rate of chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total nitrogen (TN), total phosphorous (TP), and so on[3]. However, CWs often faces the problems of insufficient carbon source, insufficient dissolved oxygen and low treatment effect at low temperature[1]. Therefore, some improvements are often carried out in CWs at present, such as the addition of carbon source, electrochemical system coupling, intermittent aeration, biochar enhancement and so on.

Biochar has been widely used in pollutant treatment in CWs due to its large specific surface area, high porosity and strong adsorption performance, and its porous property has a high ability to intercept ammonia nitrogen[4]. It also has good removal effect on heavy metal ions Cd^{2+} and Pb^{2+}[5]. Due to its high specific surface area and high microporous structure, it has been found that granular biochar has high adsorption capacity for micropollutants compared with gravel and other substrates[6]. The traditional CW nitrogen removal is generally through nitrification and denitrification. Denitrification generally faces the problem of insufficient carbon source. Biochar can provide carbon source to promote its reaction, and biochar has strong adsorption capacity to absorb NH_4^+-N[7]. Biological nitrification and denitrification are the main ways of nitrogen removal and are largely dependent on environmental factors. Nitrification is the conversion of NH_4^+-N to NO_3^--N, which is strictly dependent on the dissolved oxygen (DO) concentration. Denitrification is a

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heterotrophic process in which microorganisms convert NO_3^--N into nitrogen or nitrous oxide, which is considered to be an important process for nitrogen removal in CWs and is dependent on carbon sources[8]. Biochar can also increase the denitrification capacity of microorganisms by providing electrons and facilitating electron transfer[9]. The presence of biochar in a CW coupled electrochemical system can reduce the consumption of external organic compounds and enhance the denitrification process. In addition, biochar can be modified by Fe^{3+} produced by the anode. The oxygen-containing functional groups of biochar can interact with Fe^{2+}/Fe^{3+} to form Fe-O-C complexes[10]. Biochar can also promote the growth of plants in CWs[11], For example, biochar provides a large area for microbes to attach and form biofilms, which enhances the removal of biological contaminants. Biochar can absorb pollutants (nitrogen and phosphorus) and then slowly release them and other chemicals for plant growth[12].

This paper first reviews the coupling of biochar and four ways to enhance the removal of nitrogen and heavy metals. While biochar enhances denitrification, it produces some greenhouse gases, and some functional microorganisms, such as *Bacillus* and *Lactococcus*, also change. The mechanism of microbial community change is still unclear and needs further study, and then provide a scientific basis to improve the performance.

2. Improving the performance of constructed wetlands using biochar

Biochar is a carbon-rich material. The raw materials of biochar production are waste biological materials, compost, manure, sludge and so on. The raw material for biochar can also come from plants grown in CWs. These waste and reusable raw materials are of great significance for the mitigation of environmental pollution[13]. Biochar is a kind of solid material rich in carbon obtained by pyrolysis of biomass under the condition of high temperature and oxygen deficiency[14].

The adsorption capacity of biochar made of different materials is also different[15]. The hydraulic retention time affects the adsorption capacity of biochar[16]. Under different hydraulic conditions, coir shell and shell with the highest biochar content in the matrix also have better removal effect on pollutants and stronger wastewater purification capacity[17]. Biochar produced by different methods also has different ability to fix metals[18]. The physical and chemical properties of biochar are mainly determined by the temperature of biomass materials and pyrolysis. Temperature determines the aromatization of biochar, the number of surface functional groups and the size of surface area. With the increase of temperature, the number of surface functional groups of biochar decreases[14].

Biochar in CW has different removal processes for different pollutants. The adsorption of heavy metal ions by biochar is a physical process, and the adsorption capacity depends on the pore structure and surface chemical properties of biochar. It includes specific surface area, pore size distribution, and the types and quantities of surface functional groups. The highly developed pore structure enhances the adsorption capacity by increasing the reaction contact area, and the functional groups absorb metal ions through chemical interactions[19]. The adsorption of ammonium ions by biochar is mainly controlled by cation exchange, surface complexation with oxygen-containing functional groups and the formation of ammonium magnesium phosphate compounds[20]. Under the conditions of intermolecular hydrogen bond and direct electrostatic attraction, COD in CWs added with biochar has a good removal effect[21], At the same time, due to the action of π-π, both greenhouse gases and total nitrogen have a good removal effect[22]. Strong intermolecular π bond, hydrogen bond and electrostatic attraction have been proved to be the main reasons for the high adsorption of pollutants by biochar[12], Some studies have also found that the higher the concentration of biochar, the better the absorption effect of NH_4^+-N[23]. CWs produce greenhouse gases during operation[24], The addition of biochar can reduce greenhouse gas emissions due to its adsorbability[22], It also has a good removal effect on phosphorus[25]. Dissolved organic matter (DOM) will be produced in the process of biochar preparation at high temperature, and the ability of biochar to release DOM is closely related to the efficiency of nitrogen removal[26].

2.1. Performance enhancement through biochar and immobilizated microorganisms

Microbial immobilization is a process that restricts the movement of microbial cells within a certain range, and the enzyme activity and stability of microorganisms can be improved through immobilization[27].

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Studies have shown that the combination of immobilized enzymes and biochar can greatly improve the efficiency of micropollutant removal in CWs. The porous structure of biochar can provide carriers for these immobilized enzymes, and the immobilized enzymes combined with biochar can greatly improve the removal efficiency of micropollutants[28]. The fixation of arbuscular mycorrhizal fungi with biochar promoted the removal of ibuprofen and diclofenac in CWs[29].

Combining the immobilized denitrifying bacteria with the carbon source while maintaining the activity of the immobilized denitrifying bacteria can enhance the denitrification process. The use of external carbon sources alone or immobilization of denitrifying bacteria can improve the removal of organic matter and nutrients in the CW, but there are few studies on the combination of carbon sources and denitrifying fixation. Yu's study showed that the denitrifying bacteria can maintain their biological activity and stability when the particles made from the joint immobilization of rice husk and Pseudomonas fluorescence are put into the horizontal subsurface flow constructed wetland (HSFCW). The stable proliferation improves the ratio of carbon to nitrogen in CW wastewater and alleviates the problem of insufficient carbon source in CW. Meanwhile, COD, NH_4^+-N and TN all have high removal rates[30].

When biochar is combined with bacteria, biochar can enhance the microbial interpretation of pollutants, and fixing denitrifying bacteria on biochar can significantly improve the removal efficiency of nitrate, showing a synergistic effect in the binding process. However, the properties of biochar, such as porosity, surface properties and microbial stability, all have certain effects on the binding of biochar and bacteria. The release of nutrients, organic carbon and organic nitrogen compounds from biochar can also accelerate the growth of bacteria on biochar[9]. The adsorption and immobilization effects of sludge biochar on low-temperature mixed bacteria were studied. It is found that the addition of sludge biochar increased the specific surface area of immobilize particles and protect microorganisms from the interference of hydraulic erosion[14]. The combination of biochar and microbial compound agent has a better treatment effect on heavy metals such as arsenic in CW[23]. The combination of anaerobic ammox bacteria and biochar also has a good treatment effect on landfill leachate[31].

2.2. Combined enhancement through biochar and oxygen supply

Nitrification and denitrification is an important method for nitrogen removal in CWs. In order to better remove TN, alternate aerobic and anoxic environments need to be provided for denitrifying bacteria. Low concentration of DO will inhibit the conversion of ammonia nitrogen by nitrifying bacteria into nitric nitrogen[4]. Traditional CW nitrogen removal generally removes nitrogen in water by nitrification and denitrification under the action of nitrifying bacteria and denitrifying bacteria. Nitrifying bacteria are aerobic bacteria, and denitrifying bacteria are anaerobic bacteria. Nitrification is essential for the removal of TN, and efficient nitrification usually requires sufficient dissolved oxygen[32]. Therefore, the control of oxygen concentration is a crucial factor for efficiency nitrogen removal in CWs. Two common ways to control oxygen concentration are intermittent aeration and tidal flow. Combining biochar with intermittent aeration and tidal flow, it is found that both ways can promote the removal of nutrients. But the latter is more practical and effective, and can reduce greenhouse gas emissions[1].

Intermittent aeration can provide sufficient DO to promote nitrification and denitrification, and also provide an anaerobic environment in the interval of aeration, with lower energy consumption and cost. Biochar can provide a carbon source for the reaction while also reducing greenhouse gas production [33]. Biochar combined with intermittent aeration made of different materials also has different efficiency against pollutants. The biochar made from cow dung with taro as the matrix has a high treatment effect on COD, NO_3^-- N, NH_4^+-N, SO_4^{2-} and PO_4^{3-}, when it is put into the CW with intermittent aeration. In particular, the average removal rate of total coliform reached 97%[34]. Aeration can significantly reduce DOM (humus, protein, tryptophan) in CW[35]. High DO concentrations promote the growth of heterotrophic bacteria and increase the thickness of the substrate surface biofilm, thereby increasing the risk of substrate clogging[36].

Different C/N ratios also have different impacts on nitrogen removal in CW[37]. A higher C/N ratio will have a negative impact on NH_4^+-N removal in CWs and a higher C/N ratio will produce higher oxygen consumption, inhibit the growth of autotrophic bacteria, and lead to a decrease in nitrification efficiency[38]. Intermittent aeration technology can better provide alternating aerobic

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environment and anaerobic environment for nitrification and denitrification reaction, and biochar combined intermittent aeration has a good effect on the treatment of low carbon nitrogen ratio wastewater. The combined operation of biochar and intermittent aeration was applied to subsurface flow constructed wetlands (SFCWs) with different nitrogen ratios. It was found that when C/N was less than 7, there was a significant difference in TN removal effect between adding biochar and not adding biochar, because porous biochar could provide an anaerobic environment, which was conducive to the biofilm formation of denitrifying bacteria. As the C/N ratio increases from 10, there is no significant difference in the removal of TN with or without biochar, because the carbon source is sufficient. In these CWs with biochar, organic matter is rapidly reduced by hydrogen bonding, hydrophobic attraction and electrostatic attraction[7]. The CW with biochar combined intermittent aeration under low influent intensity has no significant improvement in COD removal rate compared with the CW with simple intermittent aeration. Under the condition of sufficient oxygen, although biochar has adsorbability, the adsorption rate of biochar for COD is much lower than the degradation rate of aerobic microorganisms[33]. Table 1 is about the effects of different biochar combined intermittent aeration on COD and nitrogen. However, at high influent intensity, the removal efficiency of the former is more obvious, possibly because the existence of π-π skeleton on the surface of biochar leads to high COD removal rate, and organic molecules can be easily adsorbed by electrostatic attraction and intermolecular hydrogen bonds[39]. At the same time, the nitrogen removal efficiency of both intermittent aeration and tidal flow decreased with the increase of influent intensity, indicating that at low influent intensity, weak denitrification would limit nitrogen removal, while high influent intensity would lead to low nitrification[39].

Table 1. Effect of different biochar combined intermittent aeration on COD and nitrogen.

CW techniques	oxygen supply	Biochar source	COD/N	Wastewater type	Removal efficiency (%)				N_2O emission flux ($\mu g \cdot m^{-2} \cdot h^{-1}$)	References
					COD	NH_4^+-N	$N0_3^-$-N	TN		
SSFCWs	Intermittent aeration	Bamboo	<7	Synthetic wastewaters	89%-99%	97%-99%	-	46%-98%		[7]
VFCWs	Intermittent aeration	Oenanthe javanica	low C/N	Synthetic wastewaters	95%-97%	63%-98%		63%-82%	271–884 μg m^{-2} h^{-1}	[40]
VFCWs	Intermittent aeration	Oenanthe javanica	low C/N	Synthetic wastewaters	94.90%	99.10%		52.70%	60.54 μg m^{-2} h^{-1}	[33]
VFCWs	Intermittent aeration		low C/N	Synthetic wastewaters	>90%	>99%		>67%		[2]
VFCWs	Intermittent aeration		0.5	Synthetic wastewaters	97%	99%		96%		[32]

2.3. Performance enhancement through biochar coupling electrochemical

Electrochemical coupled biochar CW is a feasible and ecologically sustainable technology for treating low carbon to nitrogen ratio tail water of wastewater treatment plants. The electrochemical and biochar coupling system has a significant nitrogen removal effect, mainly because, in the autotrophic denitrification process, H_2 and Fe^{2+} provided by the cathode and anode are used as electron donors, and the addition of biochar as substrate can improve the activity, diversity and abundance of microorganisms. The electrochemical system can reduce the redox potential and DO in the CW, and the ferrous ions produced can promote the circulation of iron and nitrogen. At the same time, the iron ions in the system can also combine with biochar.

The CW microbial electrochemical system includes CW microbial fuel cell and CW microbial electrolytic battery. The CW and microbial electrolytic cell mainly rely on applied voltage to promote the transfer and utilization of electrons to achieve efficient redox process[41]. The application of electrochemical system in CW can reduce oxidation reduction potential(ORP) value and DO value, provide reduction environment, and improve nitrogen removal efficiency[42].

Microbial electrochemical technology in wastewater treatment requires a large number of conductive materials to promote its extracellular electron transfer and biodegradation. Biochar surface has a large number of electroactive surface oxygen-containing functional groups, which can reversibly exchange electrons[43]. As a conductive substrate, biochar can provide enough specific surface area for the growth of electroactive microorganisms, promote electron transfer. The efficiency of denitrification can be improved by promoting the activity of denitrification enzyme[10].

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In addition to being an electron donor, iron can also maintain reducing hypoxia conditions for denitrification, and dissolved iron ions can promote bacterial growth and precipitation of phosphate groups. Based on the redox theory, negative electrode materials or microbial fuel cells are introduced into the CW system to improve the electron transfer efficiency[36].

Biochar and electrochemical coupling, while supplementing organic matter as an external carbon source, can comprehensively improve nitrogen removal efficiency through autotrophic and heterogeneous nitrogen removal. Functional groups as electron donors provide organic carbon sources for anaerobic denitrification. Fe^{2+} and H_2 produced by anode and cathode can be used as electron donors to enhance the removal of nitrite. At the same time, iron can be combined with biochar to improve the adsorption capacity of biochar. Therefore, the absorption capacity of NO_3^--N is improved[10]. A microbial fuel cell consists of an anode, a cathode, and an external resistor through which electrons are transferred to the anode. The anodes usually undergo oxidation by microorganisms, converting organic pollutants into free electrons, protons, and carbon dioxide[44]. Modification of the anode surface can enhance the enrichment of electroactivated bacteria. Zeng Li et al pointed out that biosynthesized FeS/BC hybrid particles can enhance the activity of electroactivated bacteria[45]. Dremoval effect of ammonia nitrogen was better by adding biochar to CWs and microbial batteries at low temperatures. The study showed that electricity itself contributes to the oxidation of NH_4^+, and NH_4^+ can be removed directly and indirectly through non-biological electrochemical oxidation. Through the fuel cell, supplementary electrons can be generated in the degradation kinetics, and the study found that the combination of tidal flow and microbial fuel cell has a significantly better removal effect on ammonia nitrogen than a single tidal flow constructed wetland, up to 83%[46]. Figure 1 shows the mechanism of electrochemically coupled biochar.

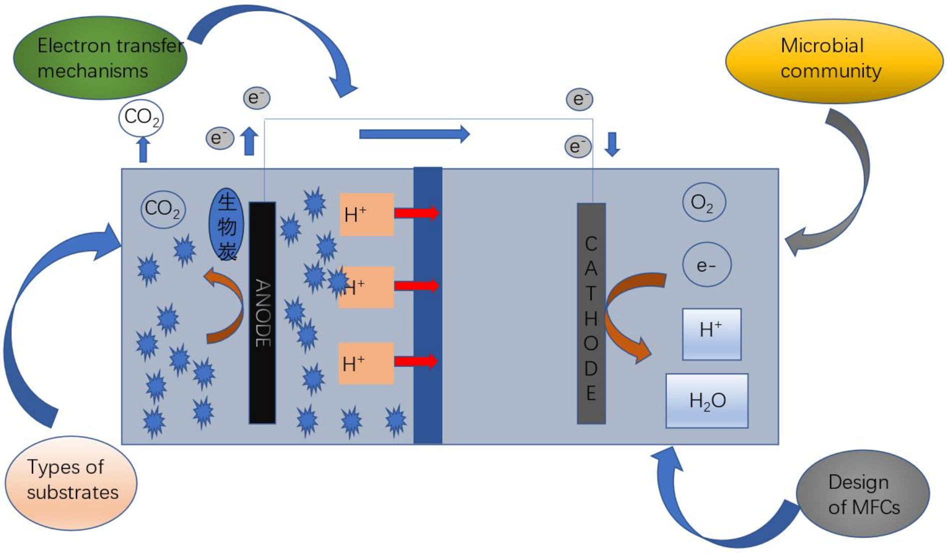

Figure 1. Mechanism diagram of electrochemically coupled biochar.

2.4. Performance enhancement through biochar modification

The sorption ability of biochar could be further raised through diversemodification methods. Among them, the microbial N removal process(nitrification, denitrification and anammox) could be facilitated by Fe in different chemical forms and valence (e.g. Fe^{2+}, Fe^{3+}, Fe_2O_3, Fe_3O_4, zero valent iron). Fe-supported biochar could effectively promote the microbial denitrifi-cation in various systems to improve the NO_3^--N removal[47]. Demonstrated that Fe modified biochar could enhance the microbial quantity and activity then influ-enced N removal process. Thus, biochar modified by Fe was consideredto be the most promising amendment in CWs to enhance the N removal. Under low temperature conditions, the performance of CWs to treat pollutants will be greatly reduced, and the addition of biochar alone cannot achieve a good effect. A large number of studies have found that modified biochar has a good effect on the treatment of pollutants in CWs at low temperature[48]. The adsorption capacity of biochar for nitrate is limited, and usually modified methods are used to improve the adsorption capacity of biochar in CWs[49]. Biochar can be physically, chemically or biologically modified to functionalize or activate it, mainly in terms of surface area, hydrophobicity, pore volume, pore number and number of functional groups[13]. The modified biochar can increase the adsorption capacity of nitrogen, phosphorus and other micro-pollutants in CWs. The commonly used modification methods are metal modification, hydrochloric acid modification and so on. Metal modification methods generally include magnesium biochar adsorption of phosphate through functional group action and complexation, and biochar can also be modified by Fe^{2+}, Fe^{3+}, Fe_2O_3, zero-valent iron, etc. Iron-modified biochar can increase the positive charge on its surface and reduce the negative charge[50]. The surface of biochar modified with iron contains a large number of active functional groups of iron oxide, which enhances the adsorption capacity of pollutants[51].

The adsorption capacity of biochar was greatly improved by heating activation of biochar with concentrated hydrochloric acid. There are hydroxyl, phenolic hydroxyl, cyclolide peroxide and other oxygen-containing groups in the modified biochar. The existence of these groups leads to different surface hydrophilicity and surface acidity of biochar, resulting in different surface charges. The surface negative charge of biochar modified by concentrated hydrochloric acid is greatly reduced, and the positive charge is increased, which enhances the adsorption of negatively charged nitrate ions. At the same time, in the process of heating activation, a large amount of cellulose decomposes, the surface sediment is reduced, and micro-pores are formed on the surface of biochar. In the process of high temperature activation, the surface area of biochar increased, the surface adsorption point of biochar increased, and the adsorption of nitrate by biochar increased[52]. Substrate modification and bacterial modification were applied to the vertical flow constructed wetland (VFCW) to treat NH_4^+-N, NO_3^--N, TN, TP and COD. At the same time, the adsorption capacity of the matrix was greatly improved after the improved VFCW[53]. The humic acid (HA) doped into activated carbon of biomass waste and modified biochar by phosphoric acid activation also had a good removal effect on heavy metals in water[54]. The surface function and adsorption capacity of activated carbon can be improved by using phosphoric acid to activate biochar and in-situ modification[19]. The coupled CW by chemical reduction and denitrification of biochar and iron microorganisms has a good effect on nitrate removal. When biochar was added to CW, the quantity and activity of microorganisms were further increased[21]. During the modification process, iron will react with the surface of biochar to form a large number of particles, resulting in a rough surface of biochar and improved exchange capacity. At the same time, iron may block the pore structure of biochar and affect the adhesion of microorganisms, but large particles will form a thin layer of iron oxide on the surface of biochar, strengthening the adsorption and fixation of anions[55]. Hydrochloric acid can reduce the negative charge on the surface of biochar, increase the positive charge, and improve the adsorption of anionic nitrate by enhancing the electrostatic attraction[50]. The results show that when the ferric chloride modified biochar (Fe-B) is added to CW, when the hydraulic retention time (HRT) is 96h, the treatment effect of the wastewater with C/N less than 3 is particularly obvious. Biochar has a high adsorption capacity for NH_4^+-N, and the iron-modified biochar also has a strong capacity for nitrification of NH_4^+-N and microorganisms. At the same time, due to its physical and chemical properties, Fe-B can trap nitrite nitrogen well and improve its processing capacity[21]. However, the iron ions produced by the modified biochar prepared by pyrolysis of ferric chloride will affect the water environment, and the excessive iron content will lead to the proliferation of iron bacteria, resulting in pipeline blockage[52]. Modified biochar can also improve the quality of soil in CWs, promote the absorption of pollutants by CWs plants, and promote the growth of wetland organisms[56]. The DOM released from porous biochar provides a good living environment and carbon source for microorganisms[57]. Substrate adsorption and microbial degradation are the main mechanisms for pollutant removal in CWs, and the removal effect of TN in CWs is better through matrix modification by using biochar in aerobic zone and microbial modification by using denitrifying bacteria in saturated zone[58]. The addition of amorphous $Fe(OH)_3$ to biochar modified system can provide stronger performance than wood chip system, reduce SO_4^{2-} production and increase the removal rate of nitrate nitrogen[59]. Figure 2 shows the correlation mechanism of iron-modified biochar on CWs.

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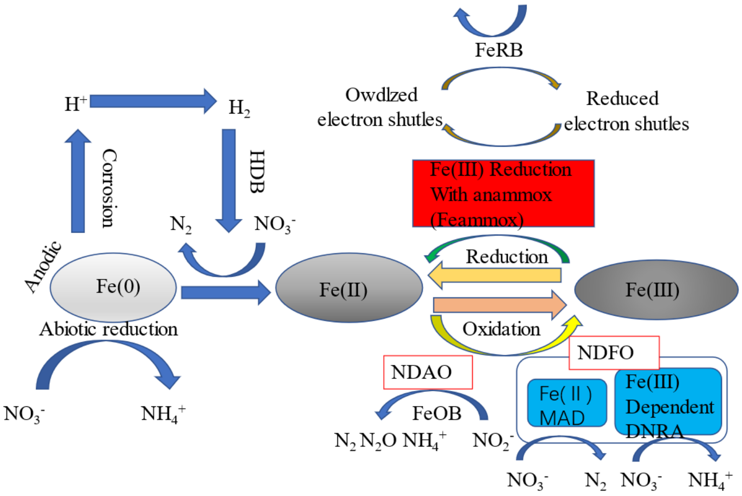

Figure 2. Correlation mechanism diagram of iron modified biochar on CWs.

3. Effects of biochar on microbial communities in constructed wetlands

3.1. Effects of biochar on the composition and structure of EPS

Extracellular polymers (EPS) are macromolecular polymers secreted or released by microbial cells (bacteria, archaea and micro-eukaryotes), and the secretion of EPS is considered to be a basic microbial adaptation[8]. The substances released by biochar are mainly tryptophan-like substances, and the DOM released by biochar immobilizes heavy metals in water[60].

The two types of EPS exist at outer surface of microbial cells, which can be classified as soluble EPS (S-EPS) and bound EPS (B-EPS). Furthermore, according to difficulty level of extraction, B-EPS can be categorized as loosely B-EPS (LB-EPS) and tightly B-EPS (TB-EPS). The main substances released by biochar are tryptophan-like substances. The content of DOM released by biochar to immobilized heavy metals in water is S-EPS, followed by bound TB-EPS and LB-EPS. S-EPS is a macromolecular substance produced by microorganisms through metabolism and self-dissolution. When the dissolved oxygen in the CW is insufficient, S-EPS will accumulate, because the production of microbial metabolism is greater than its degradation. When biochar is put into the CW, the content of S-EPS will be reduced TB-EPS exists on the surface of cells and is tightly bound to the cell wall, which is one of the reasons for the high content of TB-EPS[37].

Extracellular polymers can also be produced by cellular decomposition, and these substances are mainly polysaccharides, proteins, nucleic acids, and lipids. There are two kinds of polysaccharides on the surface of microbial cells, S-EPS and B-EPS, among which B-EPS can be divided into LB-EPS and compact TB-EPS according to the difficulty of extraction. LB-EPS was dispersed in the outer layer of cells, and TB-EPS was deposited in the inner layer[61]. The addition of biochar significantly reduced the total content of EPS, resulting in a change in EPS composition. Biochar leads to an increase in the proportion of protein in EPS, a decrease in the proportion of humus, and a decrease in the number of highly polar functional groups, thereby increasing the hydrophobicity of EPS and promoting cell aggregation[62].

The composition and physicochemical properties of extracellular polymers contribute to the maintenance of biological structure and the treatment of wastewater. The high adsorbability of EPS can increase the removal rate of ammonia nitrogen, nitrite and nitrate. However, if the EPS production is too high, it will form a dense biofilm, resulting in wetland blockage. The aeration rate, substrate type and oxygen concentration in wetland all have influence on the yield of EPS. The content of EPS in different matrix layers is different. Due to the high concentration of DO in the upper matrix and the low concentration of DO in the lower matrix, the microbial content in the upper matrix increases and more extracellular polymers are produced. At the same time, the smaller the matrix

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particle size, the more EPS production, because the smaller the matrix particle size, the larger the specific surface area, the more adsorption sites provided[37].

Biochar addition can significantly change the composition, functional group, and molecular weight distribution of EPS, which may indirectly affect the treatment effect of CWs[8], Intermittent aeration results in EPS reduction[63].

3.2. Effect of biochar on enzyme activity

Substrate enzymes in CWs are catalysts with biological functions, which play an important role in the conversion of nitrogen and organic matter in wastewater. Common substrate enzymes include *ammonia nitrogen oxygenase* (*AMO*), *nitrate reductase* (*NAR*), *dehydrogenase* (*DHA*) and *phosphatase* (*PST*), among which nitrate reductase, as a key enzyme in the first step of denitrification, shows different activities in different artificial CWs. With the increase of biochar content, the activity of nitrate reductase continues to increase. But when the biochar content is too high, it drops slightly[2].

Ferric is a necessary factor in the synthesis of dehydrogenated coenzyme, nitrite reductase and nitrate reductase. Fe-modified biochar is beneficial to the enrichment of reducing agent and the enhancement of N-cycle enzyme activity, thus improving the removal of nitrate nitrogen in CWs. Humic acid enhances the denitrification rate by promoting the electron transfer activity of nicotinamide adenine dinucleotide to denitrifying enzyme. In the process of bacterial binding with biochar, the electrons generated by metabolic process are directly transferred to nitric reductase through biochar[9]. The use of modified biochar to treat domestic sewage with different carbon to nitrogen ratio can reduce N_2, mainly because the modified biochar can promote the nitrification and denitrification process, support more denitrifying bacteria containing *nosZ* and promote the activity of N_2O reductase in denitrification[13]. Denitrifying bacteria containing *nosZ* enzyme will increase after the addition of biochar in CWs, N_2O production will decrease[59].

3.3. Effects of biochar on functional genes

Denitrifying bacteria are a large group, and nitrite reductase genes (*nirS* and *nirk*) and nitrous oxide reductase genes (*norZ*) are commonly used as functional genes to study denitrification process[64]. Figure 3 is a genetic map of nitrogen removal. The combination of biochar and bacteria showed that the expression of *narG* gene was significantly increased. Biochar is an adsorbent of nitrate, and its high porosity and large specific surface area provide conditions for the formation of denitrifying bacteria microbial biofilms, which are mainly reflected in the increase of *nirS* gene *nirK* gene abundance. During the operation of CWs, greenhouse gas CH_4 is produced, and the production of CH_4 is usually related to the abundance of *mcrA* genes. The abundance of *nirK*, *nirS* and *nosZ* genes in the modified biochar was significantly higher than that in the unmodified biochar. At the same time, the low emission of N_2O also confirmed that biochar can induce *norZ* gene transcription[22]. At high C/N ratio, the abundance of denitrification functional genes increased significantly[21]. The acidic gene group content of biochar modified by humic acid is higher than that of modified biochar, because the acidic group binds to the carbon surface and improves its tensile strength in the π-π interaction during the preparation of active biochar[54].

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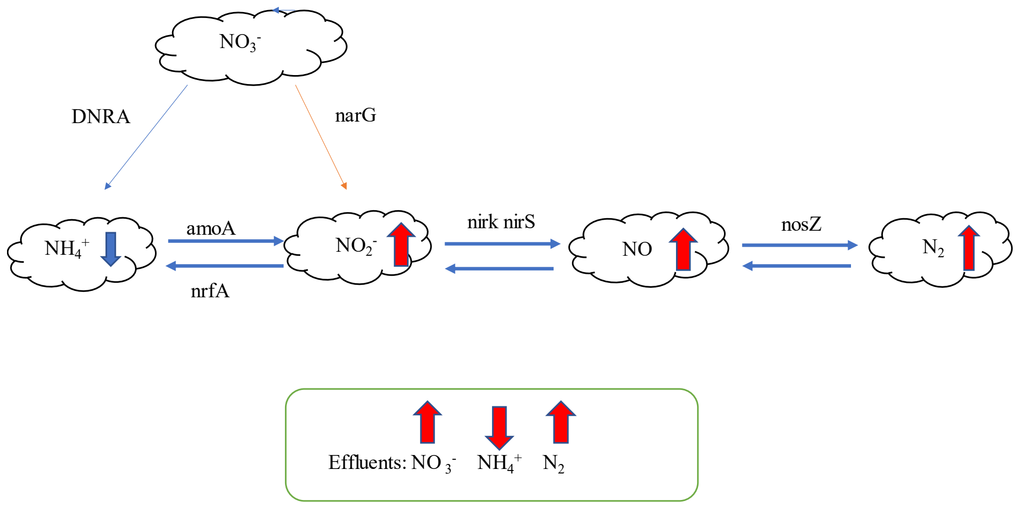

Figure 3. Gene map of nitrogen removal.

The denitrification process is carried out mainly by heterotrophic denitrifi-cation bacteria (HDB) and autotrophic denitrification bacteria (ADB) by which nitrite or nitrate could be transformed into nitrogen gases ultimately. The abundance of relevant functional genes can be determined by quantitative polymerase chain reaction, such as nitrite reductase genes (*nirS* and *nirK*) and nitrous oxide reductase genes (*norZ*) are used to study denitrification processes[47], *czcA* gene associated with cadmium removal. Zhang et al. found that the combined use of zeolite and iron carbon-based had a high removal rate of Cd, and *czcA* gene was significantly increased after the addition of zeolite and iron carbon-based in CWs[65]. Bimetallic Fe-Cu/polyvinylpyrrolidone modified biochar can increase the abundance of denitrification functional genes in wetlands, so as to improve the denitrification capacity of wetlands and convert more nitrate into N_2[66]. The addition of FeO can reduce NO_3^--N by providing electrons. The increase of denitrification function gene *narG*, *nirK* and *nirS* confirmed that the addition of iron and copper can increase the electron for denitrification and the removal rate of nitrate nitrogen[21].

3.4. Biochar on microbial Alpha diversity

When biochar was added, the diversity and abundance of microbial communities decreased, but functional bacteria associated with nitrogen and phosphorus removal increased[67]. In CWs, the dominant bacteria are divided into three phyla, namely *Proteobacteria*, *Saccharomycetes* and *Gonorrhoea*. *Proteobacteria* are the dominant bacteria species in all CW systems. The abundant *Geobacter* genus on biochar in *Proteobacteria* can degrade aromatics released by biochar and reduce the production of CH_4[1]. The enrichment degree of functional bacteria will be improved after the addition of composite biochar made from sewage sludge, food waste and straw, such as, Bacillus and *Lactococcus* genera[68]. The phyla related to nitrogen removal changed significantly after the addition of biochar, such as *Proteobacteria*, *Actinobacteria*, and *Chloroplecta*[69].

After the addition of biochar, the abundance of denitrifying bacteria increased significantly. Microbial diversity and abundance in CWs with biochar added have no effect on various C/N[21]. Different CWs showed that the evolution of microbial communities showed that *Actinomycetes*, *Proteobacteria* and *Bacteroidetes* played an important role in the elimination of antibiotic resistance[35]. The addition of biochar usually can increase the abundance of functional microorganisms related to organic matter degradation and nitrogen removal in the treatment of CWs, such as *Dow*, *Dechloromonas*, *Thiobacillus*, *Hydrophilia*, *Pseudomonas*, *Nitrospira* and *Nitrosomonas*, etc, and enhance the organic matter removal and nitrogen removal capacity of CWs by increasing the abundance of these bacteria[13], After biochar was added to SFCWs, the microbial community showed significant changes, and the relative abundance of *Dow*, *Candida Albicans*, *Dechloromonas*, *Bacillus Desulphuricum*, *Chlorococcus* and *Thiobacillus* all increased[8].

The diversity index of microbial community was also affected by different biochar supplemental levels. The relative abundance of nitrosomonas and nitrospirochetes increased after the addition biochar in the CW, and the diversity index of microbial community increased from 7.54 to 7.81 when the biochar supplemental level was 20.0%[12]. At the same time, the Shannon index and Simpson index of CWs with added biochar are higher than those without added biochar, and the chao and Ace indexes are almost higher than those without added biochar[38].

When coupled electrochemically to biochar, biochar can increase microbial diversity, while cellulose has the opposite effect. In the electrochemically coupled biochar CW, the number of *Proteobacteria* and *Firmicutes* will increase, and the abundance of *Spirillum* nitrate will also increase[10]. In the CW and microbial electrolytic cell, electricity enhanced the denitrification of the cathode, and the abundance of *Bacillus*, *Methylperoxide* and *Aeromonas Hydrophila* was higher, while the abundance of *Thiobacillus* and *Pseudomonas* were also higher[41]. The number of *Proteobacteria* and *Firmicutes* was increased and the number of bacteroidetes and acidobacteria was decreased. Meanwhile, the abundance of *Spirillum* nitrate was also increased after the addition of biochar. Denitrifying bacteria are the main heterogeneous denitrifying bacteria in CW system[10].

DO is a key determinant of microbial community composition, as it can influence the relative abundance of aerobic and anaerobic groups as well as the overall diversity of microorganisms. In the biochar CW with aeration, the proportion of nitrifying bacteria and denitrifying bacteria increased significantly, tidal flow promoted the growth of bacteria, and intermittent aeration reduced the number of bacteria[1]. The combined use of intermittent aeration and biochar increased the microbial abundance of CWs, but also decreased the microbial diversity. If the number of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria increased significantly under intermittent aeration, the abundance of actinomyces in CWs would increase[37]. The relative abundance of CD tolerant bacteria (*Bacteroidetes*) and denitrifying bacteria (*Proteobacteria* and *Bacteroidetes*) increased significantly after the addition of iron carbon-based groups to CWs based on zeolite[65]. Zhang et al. explored the effects of the amount of biochar added on the treatment performance, enzyme activity and microbial community of domestic sewage with low carbon to nitrogen ratio treated by aerated CWs, and found that in their system, the phylum-level dominant microorganisms were *Proteobacteria*, *Discobacteria* and *Phytoplankton*[2]. The pathway of CH_4 production was changed by increasing the abundance of geobacterium in CWs[38].

The addition of modified biochar supported Fe-Cu/polyvinylpyrrolidone to CWs improve the richness and diversity of CW microorganisms. Some reports have shown that zero-value iron nanoparticles can damage cells, but modified biochar supported Fe-Cu/polyvinylpyrrolidone did not reduce the richness and diversity of CW microbial communities. This may be because the presence of bimetals prevents the contact between the zero-valent iron nanoparticles and the cells. The addition of bimetallic modified biochar also increased the abundance of proteus associated with nitrogen removal. The abundance of iron-oxidizing bacteria and iron-reducing bacteria was increased when the modified biochar was added to the CW[70]. After iron modification, the abundance of *Proteobacteria* and *Methanobacteria* was increased under alternating aerobic and anoxic conditions[51].

3.5. Effects of biochar on microbial community structure

The type of substrate in CW can affect the establishment and growth of microbial community[29]. The mutual transformation of microorganisms in CWs is crucial for the operation of CWs and the removal of pollutants, because microbial community composition is closely related to wastewater treatment performance, functional stability and greenhouse gas emissions. Therefore, the analysis of microbial community structure is conducive to improving the design and operation of CWs[1]. Biochar made of different materials has different effects on the changes of microbial communities[71]. The coupling between nitrogen converting microbes usually results in higher energy and material conversion efficiency[62].

When biochar was added to CW, different bacterial community structures were generated in phyla, class, family and genus[37]. The addition of biochar will change the microbial community structure of CW, and the dissolved organic matter released by biochar can enrich denitrifying bacteria[57]. The researchers explored the microbial communities of CWs by studying phyla, class and genus, and found that phyla were mainly composed of *Proteobacteria*, *Firmicutes* and *Bacteroides*. *Proteobacteria* is an electrochemically active phylum in CWs, which plays a certain role in the removal of nitrogen, phosphorus and organic matter in CWs. At the group level, the dominant groups included *α-Proteobacteria*, *γ-Proteobacteria*, *Clostridium* and *Bacteroides*, among which bacteroides were more abundant in iron-rich regions. At the same time, biochar, as a conductive material, can enhance the electron transfer between methanogens and geobacteriaceae and promote the production of CH_4[38]. Complex biochar will also affect the CWs community. Studies have found that functional bacteria such as *Bacillus* and *Lactococcus* will be enriched when biochar made from sewage sludge, food waste and straw co-fermentation products is added to CWs[68]. The metabolic functions of wetland substrate bacteria mainly focus on chemical heterotrophic, nitrate reduction, nitrite reduction and aromatic hydrocarbon degradation[72]. The surface charge of biochar can promote the binding of microbial cells and wastewater compounds, and promote the growth of microorganisms in the void[73].

Limited oxygen in CWs can inhibit the activity of heterotrophic microbial communities, which are essential for COD removal in CWs[34]. Therefore, the concentration of DO is one of the key factors affecting the microbial community structure, and the combination of the two oxygen supply modes and biochar can affect the microbial community structure and enhance the important microbial metabolism. Tidal flow creates alternating wet and dry conditions during operation and has a greater impact on microbial communities than intermittent aeration[1]. The combined use of intermittent aeration and microorganisms can affect the community structure of microorganisms[63].

The voids on the surface of biochar can provide a suitable environment for the propagation of microorganisms, increase the removal rate of ammonia nitrogen, and increase the redox potential to enhance nitrification[37]. The mutual transformation of microorganisms plays an important role in the removal of pollutants in CWs, and the composition of microbial community plays an important role in the wastewater treatment performance and functional stability of CWs. The porous structure of biochar promotes the growth of plants and the attachment and reproduction of microorganisms in wetlands by fixing nutrients and trace elements[1]. Denitrification intensity is closely related to microbial activity. At the same time, when the modified biochar was added to the CW, the microbial community would change, and iron-related bacteria would replace the denitrifying bacteria[70].

4. Conclusions

This paper reviews the research progress of microbial changes in CWs after biochar addition. The source and preparation method of biochar will affect the treatment performance of biochar. When biochar is combined with different treatment technologies, the removal efficiency of ammonia nitrogen, total nitrogen and COD will increase, the production of greenhouse gases in CWs will decrease, and the change of microbial community structure will also have a significant impact. The combined use of biochar and intermittent aeration has the highest pollutant removal efficiency, and the control of oxygen concentration is the key factor to achieve efficient nitrogen removal in CWs. The combination of biochar and these treatment techniques increases the diversity of nitrogen-removing microorganisms in the microbial community. The combined use of each treatment technology and biochar needs further study on the removal of pollutants and the quantitative changes of community microorganisms in CWs.

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References

1. Ji B, Chen J, Mei J, et al. Roles of biochar media and oxygen supply strategies in treatment performance, greenhouse gas emissions, and bacterial community features of subsurface-flow constructed wetlands [J]. Bioresource Technology, 2020, 302: 122890. https://doi.org/https://doi.org/10.1016/j.biortech.2020.122890
2. Zhang Y, Li M, Dong L, et al. Effects of biochar dosage on treatment performance, enzyme activity and microbial community in aerated constructed wetlands for treating low C/N domestic sewage [J]. Environmental Technology & Innovation, 2021, 24: 101919. https://doi.org/https://doi.org/10.1016/j.eti.2021.101919
3. Parde D, Patwa A, Shukla A, et al. A review of constructed wetland on type, treatment and technology of wastewater [J]. Environmental Technology & Innovation, 2021, 21: 101261. https://doi.org/https://doi.org/10.1016/j.eti.2020.101261
4. Feng L K, Wang R G, Jia L X, et al. Can biochar application improve nitrogen removal in constructed wetlands for treating anaerobically-digested swine wastewater [J]. Chmical Engineering Journal, 2020, 379. https://doi.org/10.1016/j.cej.2019.122273
5. Zhang J, Cheng S, He F, et al. Effects of Cd^{2+} and Pb^{2+} on the substrate bioflms in the integrated vertical-flow constructed wetland [J]. Journal of Environmental Sciences, 2008, 20(8): 900-6. https://doi.org/https://doi.org/10.1016/S1001-0742(08)62184-1
6. Lei Y, Langenhoff A, Bruning H, et al. Sorption of micropollutants on selected constructed wetland support matrices [J]. Chemosphere, 2021, 275. https://doi.org/10.1016/j.chemosphere.2021.130050
7. Zhou X, Wang R, Liu H, et al. Nitrogen removal responses to biochar addition in intermittent-aerated subsurface flow constructed wetland microcosms: Enhancing role and mechanism [J]. Ecological Engineering, 2019, 128: 57-65. https://doi.org/https://doi.org/10.1016/j.ecoleng.2018.12.028
8. Deng C, Huang L, Liang Y, et al. Response of microbes to biochar strengthen nitrogen removal in subsurface flow constructed wetlands: Microbial community structure and metabolite characteristics [J]. Science of The Total Environment, 2019, 694: 133687. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.133687
9. Liu Y, Liu S, Yang Z, et al. Synergetic effects of biochars and denitrifier on nitrate removal [J]. Bioresource Technology, 2021, 335: 125245. https://doi.org/https://doi.org/10.1016/j.biortech.2021.125245
10. Zhong L, Yang S-S, Ding J, et al. Enhanced nitrogen removal in an electrochemically coupled biochar-amended constructed wetland microcosms: The interactive effects of biochar and electrochemistry [J]. Science of The Total Environment, 2021, 789: 147761. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.147761
11. Li J, Fan J, Zhang J, et al. Preparation and evaluation of wetland plant-based biochar for nitrogen removal enhancement in surface flow constructed wetlands [J]. Environmental Science And Pollution Researchr, 2018, 25(14): 13929-37. https://doi.org/10.1007/s11356-018-1597-y
12. Zhuang L-L, Li M, Li Y, et al. The performance and mechanism of biochar-enhanced constructed wetland for wastewater treatment [J]. Journal of Water Process Engineering, 2022, 45: 102522. https://doi.org/https://doi.org/10.1016/j.jwpe.2021.102522
13. Deng S, Chen J, Chang J. Application of biochar as an innovative substrate in constructed wetlands/biofilters for wastewater treatment: Performance and ecological benefits [J]. Journal of Cleaner Production, 2021, 293: 126156. https://doi.org/https://doi.org/10.1016/j.jclepro.2021.126156
14. Xing C, Xu X, Xu Z, et al. Study on the Decontamination Effect of Biochar-Constructed Wetland under Different Hydraulic Conditions [J]. Water, 2021, 13(7). https://doi.org/10.3390/w13070893
15. Feng L, Liu Y, Zhang J, et al. Dynamic variation in nitrogen removal of constructed wetlands modified by biochar for treating secondary livestock effluent under varying oxygen supplying conditions [J]. Journal of Environmental Management, 2020, 260: 110152. https://doi.org/https://doi.org/10.1016/j.jenvman.2020.110152
16. Hamada M S, Ibaid Z Z, Shatat M. Performance of citrus charcoal and olivepomace charcoal as natural substrates in the treatment of municipal wastewater by vertical flow subsurface constructed wetlands [J]. Bioresource Technology Reports, 2021, 15: 100801. https://doi.org/https://doi.org/10.1016/j.biteb.2021.100801
17. Xing C J, Xu X X, Xu Z H, et al. Study on the Decontamination Effect of Biochar-Constructed Wetland under Different Hydraulic Conditions [J]. Water, 2021, 13(7). https://doi.org/10.3390/w13070893
18. Zhang Z, Solaiman Z M, Meney K, et al. Biochars immobilize soil cadmium, but do not improve growth of emergent wetland species Juncus subsecundus in cadmium-contaminated soil [J]. Journal of Soils And Sediments, 2013, 13(1): 140-51. https://doi.org/10.1007/s11368-012-0571-4
19. Liu H, Cheng C, Wu H. Sustainable utilization of wetland biomass for activated carbon production: A review on recent advances in modification and activation methods [J]. Science of The Total Environment, 2021, 790: 148214. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.148214
20. Cui X, Hao H, Zhang C, et al. Capacity and mechanisms of ammonium and cadmium sorption on different wetland-plant derived biochars [J]. Science of The Total Environment, 2016, 539: 566-75. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.09.022

21. Hou W, Wang S, Li Y, et al. Influence of modified biochar supported Fe-Cu/polyvinylpyrrolidone on nitrate removal and high selectivity towards nitrogen in constructed wetlands [J]. Environmental Pollution, 2021, 289. https://doi.org/10.1016/j.envpol.2021.117812
22. Chen X, Zhu H, Bañuelos G, et al. Biochar reduces nitrous oxide but increases methane emissions in batch wetland mesocosms [J]. Chemical Engineering Journal, 2020, 392: 124842. https://doi.org/https://doi.org/10.1016/j.cej.2020.124842
23. Guo X F, Cui X Y, Li H S. Effects of fillers combined with biosorbents on nutrient and heavy metal removal from biogas slurry in constructed wetlands [J]. Science of The Total Environment, 2020, 703. https://doi.org/10.1016/j.scitotenv.2019.134788
24. Bonetti G, Trevathan-Tackett S M, Hebert N, et al. Microbial community dynamics behind major release of methane in constructed wetlands [J]. Applied Soil Ecology, 2021, 167: 104163. https://doi.org/https://doi.org/10.1016/j.apsoil.2021.104163
25. Bolton L, Joseph S, Greenway M, et al. Phosphorus adsorption onto an enriched biochar substrate in constructed wetlands treating wastewater [J]. Ecological Engineering, 2019, 142: 100005. https://doi.org/https://doi.org/10.1016/j.ecoena.2019.100005
26. Li J, Fan J L, Zhang J, et al. Preparation and evaluation of wetland plant-based biochar for nitrogen removal enhancement in surface flow constructed wetlands [J]. Environmental Science And Pollution Research, 2018, 25(14): 13929-37. https://doi.org/10.1007/s11356-018-1597-y
27. Kizito S, Wu S, Kirui W K, et al. Evaluation of slow pyrolyzed wood and rice husks biochar for adsorption of ammonium nitrogen from piggery manure anaerobic digestate slurry [J]. Science of The Total Environmente, 2015, 505: 102-12. https://doi.org/10.1016/j.scitotenv.2014.09.096
28. Madadi R, Bester K. Fungi and biochar applications in bioremediation of organic micropollutants from aquatic media [J]. Marine Pollution Bulletin, 2021, 166: 112247. https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112247
29. Hu B, Hu S S, Vymazal J, et al. Arbuscular mycorrhizal symbiosis in constructed wetlands with different substrates: Effects on the phytoremediation of ibuprofen and diclofenac [J]. Journal of Environmental Management, 2021, 296. https://doi.org/10.1016/j.jenvman.2021.113217
30. Yu G, Peng H, Fu Y, et al. Enhanced nitrogen removal of low C/N wastewater in constructed wetlands with co-immobilizing solid carbon source and denitrifying bacteria [J]. Bioresource Technology, 2019, 280: 337-44. https://doi.org/https://doi.org/10.1016/j.biortech.2019.02.043
31. Jia W, Yang Y C, Yang L Y, et al. High-efficient nitrogen removal and its microbiological mechanism of a novel carbon self-sufficient constructed wetland [J]. Science of The Total Environment, 2021, 775. https://doi.org/10.1016/j.scitotenv.2021.145901
32. Jia L, Wang R, Feng L, et al. Intensified nitrogen removal in intermittently-aerated vertical flow constructed wetlands with agricultural biomass: Effect of influent C/N ratios [J]. Chemical Engineering Journal, 2018, 345: 22-30. https://doi.org/https://doi.org/10.1016/j.cej.2018.03.087
33. Zhou X, Wang X, Zhang H, et al. Enhanced nitrogen removal of low C/N domestic wastewater using a biochar-amended aerated vertical flow constructed wetland [J]. Bioresource Technology, 2017, 241: 269-75. https://doi.org/https://doi.org/10.1016/j.biortech.2017.05.072
34. Chand N, Suthar S, Kumar K, et al. Enhanced removal of nutrients and coliforms from domestic wastewater in cattle dung biochar-packed Colocasia esculenta-based vertical subsurface flow constructed wetland [J]. Journal of Water Process Engineering, 2021, 41: 101994. https://doi.org/https://doi.org/10.1016/j.jwpe.2021.101994
35. Feng L K, Wu H M, Zhang J, et al. Simultaneous elimination of antibiotics resistance genes and dissolved organic matter in treatment wetlands: Characteristics and associated relationship [J]. Chemical Engineering Journal, 2021, 415. https://doi.org/10.1016/j.cej.2021.128966
36. Zhuang L-L, Yang T, Zhang J, et al. The configuration, purification effect and mechanism of intensified constructed wetland for wastewater treatment from the aspect of nitrogen removal: A review [J]. Bioresource Technology, 2019, 293: 122086. https://doi.org/https://doi.org/10.1016/j.biortech.2019.122086
37. Zhou X, Chen Z, Li Z, et al. Impacts of aeration and biochar addition on extracellular polymeric substances and microbial communities in constructed wetlands for low C/N wastewater treatment: Implications for clogging [J]. Chemical Engineering Journal, 2020, 396: 125349. https://doi.org/https://doi.org/10.1016/j.cej.2020.125349
38. Guo Z Z, Kang Y, Hu Z, et al. Removal pathways of benzofluoranthene in a constructed wetland amended with metallic ions embedded carbon [J]. Bioresource Technology, 2020, 311. https://doi.org/10.1016/j.biortech.2020.123481
39. Zhou X, Jia L, Liang C, et al. Simultaneous enhancement of nitrogen removal and nitrous oxide reduction by a saturated biochar-based intermittent aeration vertical flow constructed wetland: Effects of influent strength [J]. Chemical Engineering Journal, 2018, 334: 1842-50. https://doi.org/https://doi.org/10.1016/j.cej.2017.11.066

40. Zhou X, Gao L, Zhang H, et al. Determination of the optimal aeration for nitrogen removal in biochar-amended aerated vertical flow constructed wetlands [J]. Bioresource Technology, 2018, 261: 461-4. https://doi.org/https://doi.org/10.1016/j.biortech.2018.04.028
41. Yu B, Liu C, Wang S, et al. Applying constructed wetland-microbial electrochemical system to enhance NH4+ removal at low temperature [J]. Science of The Total Environment, 2020, 724: 138017. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.138017
42. Zhong L, Yang S S, Ding J, et al. Enhanced nitrogen removal in an electrochemically coupled biochar-amended constructed wetland microcosms: The interactive effects of biochar and electrochemistry [J]. Science of The Total Environment, 2021, 789. https://doi.org/10.1016/j.scitotenv.2021.147761
43. Prado A, Berenguer R, Esteve-Núñez A. Electroactive biochar outperforms highly conductive carbon materials for biodegrading pollutants by enhancing microbial extracellular electron transfer [J]. Carbon, 2019, 146: 597-609. https://doi.org/https://doi.org/10.1016/j.carbon.2019.02.038
44. Prathiba S, Kumar P S, Vo D-V N. Recent advancements in microbial fuel cells: A review on its electron transfer mechanisms, microbial community, types of substrates and design for bio-electrochemical treatment [J]. Chemosphere, 2022, 286: 131856. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.131856
45. Li Z, Zhang P, Qiu Y, et al. Biosynthetic FeS/BC hybrid particles enhanced the electroactive bacteria enrichment in microbial electrochemical systems [J]. Science of The Total Environment, 2021, 762: 143142. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.143142
46. Saeed T, Miah M J, Khan T. Intensified constructed wetlands for the treatment of municipal wastewater: experimental investigation and kinetic modelling [J]. Environmental Science And Pollution Research, 2021, 28(24): 30908-28. https://doi.org/10.1007/s11356-021-12700-8
47. Jia W, Sun X, Gao Y, et al. Fe-modified biochar enhances microbial nitrogen removal capability of constructed wetland [J]. Science of The Total Environment, 2020, 740: 139534. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.139534
48. Wu H, Ma W, Kong Q, et al. Spatial-temporal dynamics of organics and nitrogen removal in surface flow constructed wetlands for secondary effluent treatment under cold temperature [J]. Chemical Engineering Journal, 2018, 350: 445-52. https://doi.org/https://doi.org/10.1016/j.cej.2018.06.004
49. Wang B, Liu S Y, Li F Y, et al. Removal of nitrate from constructed wetland in winter in high-latitude areas with modified hydrophyte biochars [J]. Korean Journal of Chemical Engineering, 2017, 34(3): 717-22. https://doi.org/10.1007/s11814-016-0308-y
50. Wang H, Xu J, Sheng L. Preparation of straw biochar and application of constructed wetland in China: A review [J]. Journal of Cleaner Production, 2020, 273: 123131. https://doi.org/https://doi.org/10.1016/j.jclepro.2020.123131
51. Sha N Q, Wang G H, Li Y H, et al. Removal of abamectin and conventional pollutants in vertical flow constructed wetlands with Fe-modified biochar [J]. Rsc Advances, 2020, 10(72): 44171-82. https://doi.org/10.1039/d0ra08265a
52. Wang B, Liu S-y, Li F-y, et al. Removal of nitrate from constructed wetland in winter in high-latitude areas with modified hydrophyte biochars [J]. Korean Journal of Chemical Engineering, 2017, 34(3): 717-22.
53. Sun Y, Zhou P, Zhang N, et al. Effects of matrix modification and bacteria amendment on the treatment efficiency of municipal tailwater pollutants by modified vertical flow constructed wetland [J]. Journal of Environmental Management, 2021, 281: 111920. https://doi.org/https://doi.org/10.1016/j.jenvman.2020.111920
54. Guo Z, Zhang J, Kang Y, et al. Rapid and efficient removal of Pb(II) from aqueous solutions using biomass-derived activated carbon with humic acid in-situ modification [J]. Ecotoxicology and Environmental Safety, 2017, 145: 442-8. https://doi.org/https://doi.org/10.1016/j.ecoenv.2017.07.061
55. Min L, Zhongsheng Z, Zhe L, et al. Removal of nitrogen and phosphorus pollutants from water by $FeCl_3$-impregnated biochar [J]. Ecological Engineering, 2020, 149: 105792. https://doi.org/https://doi.org/10.1016/j.ecoleng.2020.105792
56. Kasak K, Truu J, Ostonen I, et al. Biochar enhances plant growth and nutrient removal in horizontal subsurface flow constructed wetlands [J]. Science Of The Total Environment, 2018, 639: 67-74. https://doi.org/10.1016/j.scitotenv.2018.05.146
57. Xu J, Liu X, Huang J, et al. The contributions and mechanisms of iron-microbes-biochar in constructed wetlands for nitrate removal from low carbon/nitrogen ratio wastewater [J]. Rsc Advances, 2020, 10(39): 23212-20. https://doi.org/10.1039/d0ra03609a
58. Sun Y P, Zhou P C, Zhang N, et al. Effects of matrix modification and bacteria amendment on the treatment efficiency of municipal tailwater pollutants by modified vertical flow constructed wetland [J]. Journal of Environmental Management, 2021, 281. https://doi.org/10.1016/j.jenvman.2020.111920
59. Easton Z M, Rogers M, Davis M, et al. Mitigation of sulfate reduction and nitrous oxide emission in denitrifying environments with amorphous iron oxide and biochar [J]. Ecological Engineering, 2015, 82: 605-13. https://doi.org/10.1016/j.ecoleng.2015.05.008

60. Jia L, Wu W, Zhang J, et al. Insight into heavy metals (Cr and Pb) complexation by dissolved organic matters from biochar: Impact of zero-valent iron [J]. Science of The Total Environment, 2021, 793: 148469. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.148469
61. Zhou X, Chen Z H, Li Z R, et al. Impacts of aeration and biochar addition on extracellular polymeric substances and microbial communities in constructed wetlands for low C/N wastewater treatment: Implications for clogging [J]. Chemical Engineering Journal, 2020, 396. https://doi.org/10.1016/j.cej.2020.125349
62. Tang S, Liao Y, Xu Y, et al. Microbial coupling mechanisms of nitrogen removal in constructed wetlands: A review [J]. Bioresource Technology, 2020, 314: 123759. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123759
63. Feng L K, Liu Y, Zhang J Y, et al. Dynamic variation in nitrogen removal of constructed wetlands modified by biochar for treating secondary livestock effluent under varying oxygen supplying conditions [J]. Journal Of Environmental Management, 2020, 260. https://doi.org/10.1016/j.jenvman.2020.110152
64. Jia W, Sun X, Gao Y, et al. Fe-modified biochar enhances microbial nitrogen removal capability of constructed wetland [J]. Science of The Total Environment, 2020, 740. https://doi.org/10.1016/j.scitotenv.2020.139534
65. Zhang Y, Li Y, Wang J, et al. Interactions of chlorpyrifos degradation and Cd removal in iron-carbon-based constructed wetlands for treating synthetic farmland wastewater [J]. Journal of Environmental Management, 2021, 299: 113559. https://doi.org/https://doi.org/10.1016/j.jenvman.2021.113559
66. Hou W, Wang S, Li Y, et al. Influence of modified biochar supported Fe–Cu/polyvinylpyrrolidone on nitrate removal and high selectivity towards nitrogen in constructed wetlands [J]. Environmental Pollution, 2021, 289: 117812. https://doi.org/https://doi.org/10.1016/j.envpol.2021.117812
67. Zheng C, Zhang X, Gan L, et al. Effects of biochar on the growth of Vallisneria natans in surface flow constructed wetland [J]. Environment Science And Pollution Research. https://doi.org/10.1007/s11356-021-15399-9
68. Meng F C, Feng L J, Yin H J, et al. Assessment of nutrient removal and microbial population dynamics in a non-aerated vertical baffled flow constructed wetland for contaminated water treatment with composite biochar addition [J]. Journal of Environment Management, 2019, 246: 355-61. https://doi.org/10.1016/j.jenvman.2019.06.011
69. Liang Y, Wang Q, Huang L, et al. Insight into the mechanisms of biochar addition on pollutant removal enhancement and nitrous oxide emission reduction in subsurface flow constructed wetlands: Microbial community structure, functional genes and enzyme activity [J]. Bioresource Technology, 2020, 307: 123249. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123249
70. Peng Y, He S, Wu F. Biochemical processes mediated by iron-based materials in water treatement: Enhancing nitrogen and phosphorus removal in low C/N ratio wastewater [J]. Science of The Total Environment, 2021, 775: 145137. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.145137
71. Jia L, Li C, Zhang Y, et al. Microbial community responses to agricultural biomass addition in aerated constructed wetlands treating low carbon wastewater [J]. Journal of Environmental Management, 2020, 270: 110912. https://doi.org/https://doi.org/10.1016/j.jenvman.2020.110912
72. Shen X T, Zhang J, Xie H J, et al. Electron shuttles enhance phenanthrene removal in constructed wetlands filled with manganese oxides-coated sands [J]. Chemical Engineering Journal, 2021, 426. https://doi.org/10.1016/j.cej.2021.131755
73. Saeed T, Haque I, Khan T. Organic matter and nutrients removal in hybrid constructed wetlands: Influence of saturation [J]. Chemical Engineering Journal, 2019, 371: 154-65. https://doi.org/10.1016/j.cej.2019.04.030