

Biochar–Microbial Synergistic Systems for Nitrogen and Phosphorus Removal in Polluted Waters: Mechanisms, Studies, and Insights

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Abstract

Eutrophication caused by increasing non-point source pollution has resulted in the accumulation of nitrogen and phosphorus in water bodies, leading to severe algal blooms, oxygen depletion, and ecosystem degradation. However, conventional remediation technologies often face limitations in efficiency and sustainability. Biochar, derived from organic waste, has gained attention for its strong adsorption capacity. Recent advances show that combining biochar with functional microorganisms—forming biochar-microbial synergistic systems—can significantly enhance the removal of nitrogen and phosphorus from polluted water. For example, laboratory studies have reported that microbial-immobilised biochar can achieve over 90% removal of ammonium and phosphate, far exceeding the performance of single biochar or free bacteria. Such ecological and environmental benefits are due to the physical and chemical properties of biochar and its ability as a multifunctional carrier for microorganisms, which therefore form a comprehensive purification mechanism of physical adsorption, biological transformation and chemical precipitation. This review systematically summarises the mechanisms, advantages, and recent progress of biochar-microbial synergistic systems, including key factors influencing removal efficiency and the prospects for in-situ application. Additionally, comparative analyses between biochar-microbial synergistic technology and traditional technologies are presented, discussing economic feasibility and current challenges. This review highlights the unique potential of biochar-microbial composites

to achieve high efficiency, environmental friendliness, and practical scalability in water pollution remediation, providing a reference for future research and large-scale applications.

Keywords: biochar-microbial synergistic systems, microbial immobilisation, aquatic nutrient pollution, water environmental management, nitrogen and phosphorus removal, sustainable waste management

1. Introduction

1.1 Current Environmental Situation of Non-point Source Pollution

With the intensification of agricultural runoff, urban rainfall discharge, and the livestock industry, non-point source pollution has become a critical issue worldwide ¹⁻³. Non-point source pollution occurs when pollutants enter water bodies from diffuse and scattered areas without a clear discharge point ⁴. It is more complex to monitor than other forms of water pollution. Non-point source pollution poses a significant threat to biodiversity, water quality, and human health ^{5,6}, as these activities introduce excessive nitrogen and phosphorus into aquatic systems, leading to severe eutrophication, oxygen depletion, and ecosystem degradation ⁷. For example, agricultural non-point source pollution accounts for 57.2% and 67.4% of the nitrogen and phosphorus pollution released into the environment in China, the highest among all pollution pathways ⁸. One study analysed water quality of the main streams of the Yangtze River and the Yellow River using panel data from 46 prefecture-level cities and 18 state-controlled water quality monitoring points from 2004 to 2019, revealing that a unit increase in fertiliser application results in a 0.129-0.196 unit increase in $\text{NH}_3\text{-N}$ concentration ⁹. Another vital pathway of non-point source pollution comes from livestock and poultry. In some typical agricultural provinces, such as Hubei, China, the load source of livestock and poultry non-point source pollution exceeds farming, accounting for 52% and 69% of the total nitrogen and non-point source pollution production in rural areas ¹⁰. In contrast, atmospheric deposition contributes the least of non-point source pollution, only 3.9% of the total in the arid and rain-scarce North China Plain ¹¹.

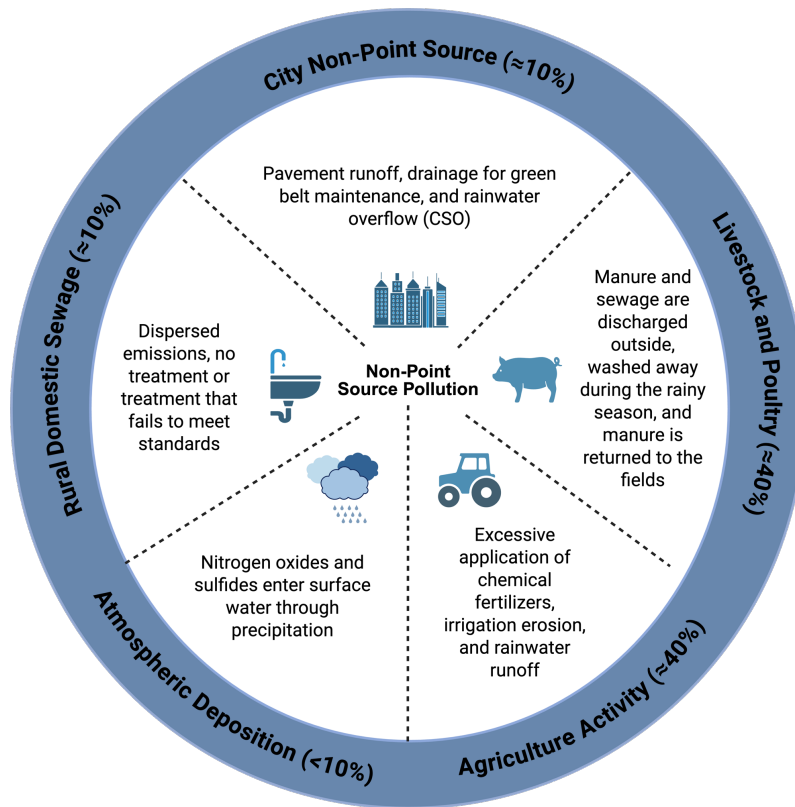


Figure 1. Source, pathway, and its percentage of the total non-point source pollution.

1.2 Application of Biochar in Pollution Remediation

In the context of non-point source pollution, biochar is considered a highly promising material for the remediation of pollution in aquatic habitats. It is a charcoal-like substance produced through pyrolysis, a process that involves high temperatures and low oxygen conditions¹². Recently, numerous studies have focused on the removal of aquatic pollution using biochar^{13,14}. The advantages of biochar include being produced from various raw materials, having low production costs, and being environmentally friendly, as it allows for the recycling of natural products^{15,16}. Some common raw materials are plant residues, agricultural waste, livestock and poultry manure¹⁷.

Additionally, due to its porous structure and large specific surface area, biochar can effectively adsorb pollutants, including nutrients, organic contaminants, and heavy metals^{18,19}.

Furthermore, the oxygen-containing functional groups on the surface of biochar can capture ionic pollutants through ion exchange processes^{20,21}. With these effective characteristics in aquatic pollutant removal, biochar has garnered increasing attention in recent years.

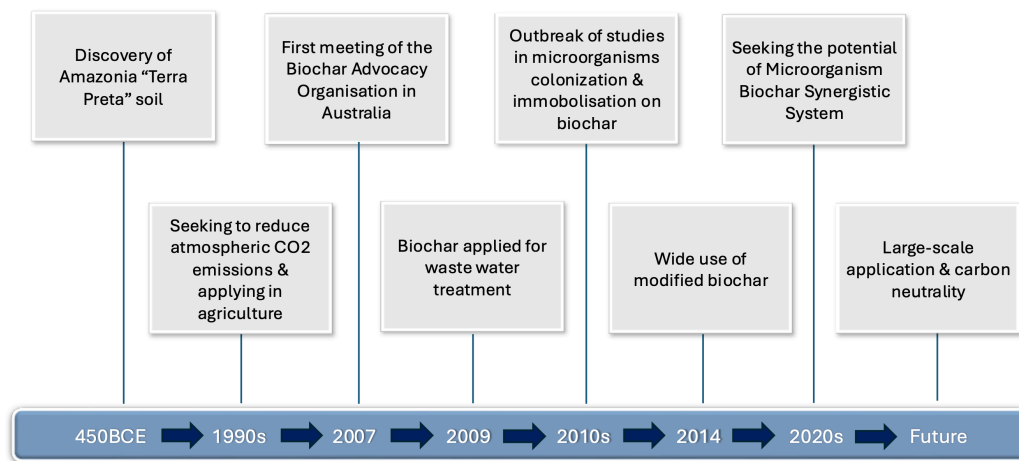


Figure 2. The development of the application of biochar.

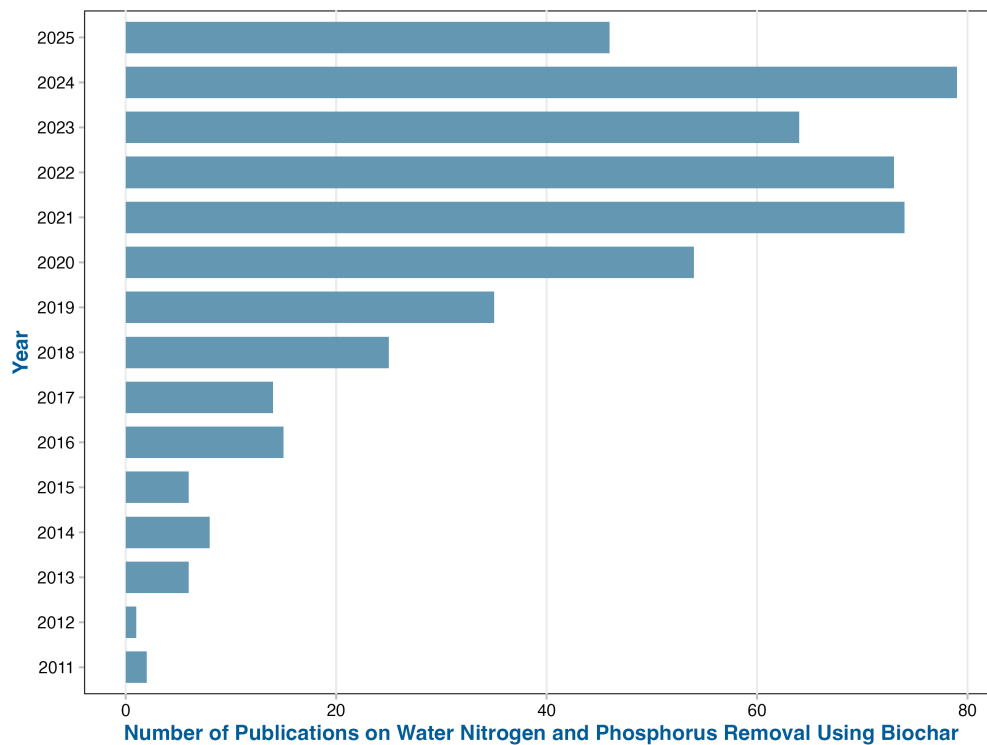


Figure 3. Annual publication on aquatic nitrogen and phosphorus pollutants removal using biochar from 2011 to 2025. Figure generated using data gained from Web of Science (keywords: biochar, aquatic pollution, Nitrogen and Phosphorus pollution, etc.). The number of studies has rapidly increased since 2018, peaking in 2024.

1.3 The Role of Microorganisms in the Removal of Pollutants

Microorganisms play a crucial role in the biochemical cycling of nitrogen and phosphorus in aquatic ecosystems, supporting their self-purification^{22,23}. The main processes of nitrogen

removal include nitrification, denitrification, and ammonification (mineralisation). Ammonifying bacteria convert organic nitrogen into NH_4^+ or NH_3 through ammonification²⁴. Subsequently, nitrifying bacteria in water bodies oxidise NH_4^+ to NO_3^- via nitrification, and denitrifying bacteria reduce NO_3^- to nitrogen gas, thereby achieving effective nitrogen removal through volatilisation^{25,26}.

Polyphosphate accumulation is a biological phosphorus removal method commonly used in wastewater treatment and is naturally found in aquatic ecosystems. Polyphosphate-accumulating organisms (PAOs) absorb excess phosphorus and store it internally as polyphosphate under aerobic conditions²⁷.

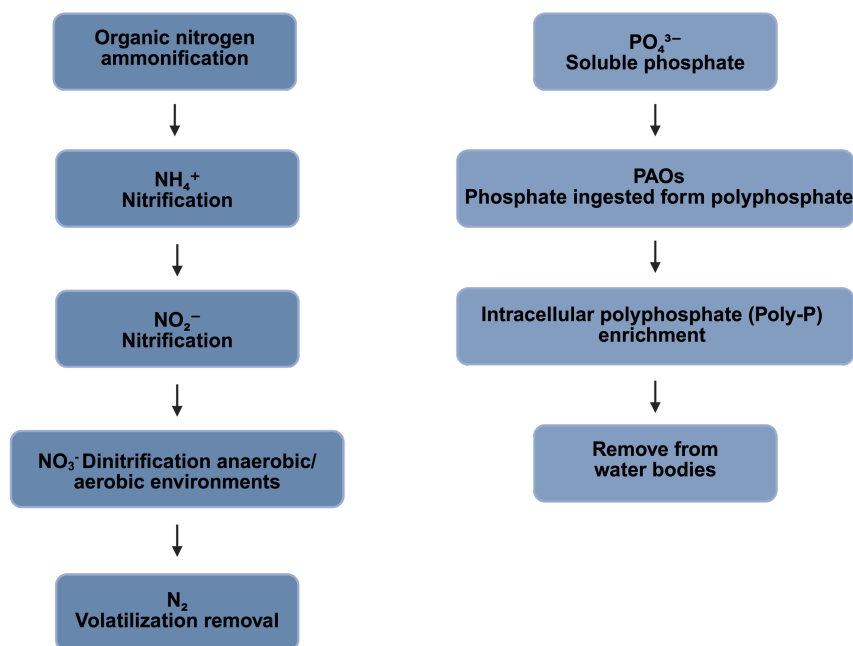


Figure 4. Microbial removal paths of Nitrogen and Phosphorus

1.4 Research Potential and Significance of Biochar - Microbial Synergistic Systems

As non-point source pollution becomes an increasingly critical issue, remediation methods relying solely on biochar or microorganisms face significant limitations^{28–30}. Therefore, researchers are encouraged to explore more effective strategies and technologies for water pollution remediation.

Regarding the role of biochar as a microbial carrier^{31,32}, the synergy of the biochar-microbial system is evident not only spatially but also through its functional complementarity.

Consequently, it offers a new approach for the remediation of nitrogen and phosphorus in aquatic systems. This strategy aligns with future needs for green, efficient, and multi-functional technologies in water environment management.

number of available sites, resulting in a larger surface area on the biochar³⁸. Biochar with a wide range of SSA has been effectively used in aquatic nitrogen and phosphorus removal. For instance, studies indicated that a coffee grounds biochar with SSA (BET) 46.318 m² g⁻¹ effectively removed aquatic ammonium, while a walnut shell biochar with SSA (BET) 967.1084 m² g⁻¹ was used in removing both ammonium and phosphate^{34,39}.

2.1.3 Surface Chemistry

Regarding its chemical properties, biochar features a variety of oxygen-containing functional groups (OFGs), including carboxyl (–COOH), hydroxyl (–OH), and phenolic (–ArOH) groups. These OFGs can interact with nitrogen and phosphorus pollutants, heavy metals, and organic contaminants through mechanisms such as electrostatic attraction, complexation, and hydrogen bonding^{40,41}. Furthermore, OFGs can promote microbial adhesion and immobilisation on biochar, enhancing the rate of biofilm formation⁴². Consequently, the physical and chemical properties of biochar work together to provide a foundation for highly efficient and environmentally friendly water remediation systems.

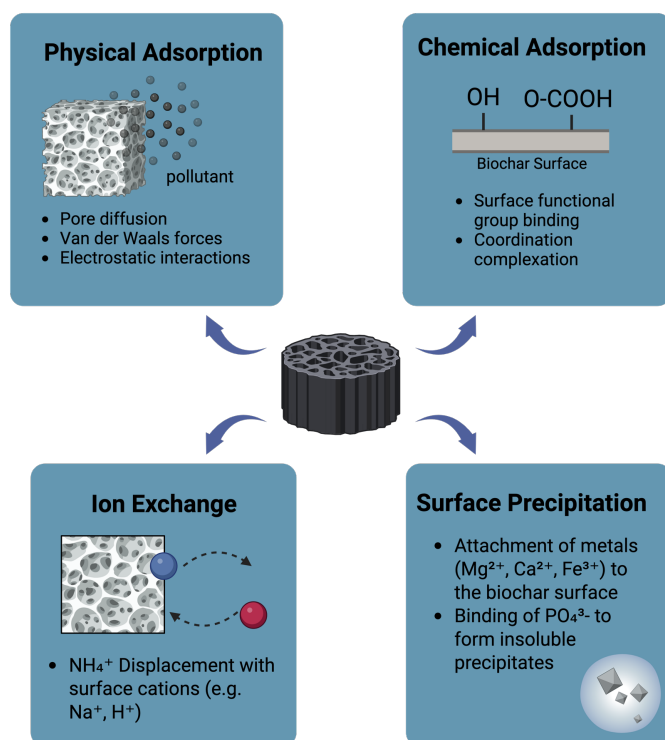


Figure 6. The four mechanisms by which biochar adsorbs pollutants (aquatic nitrogen and phosphorus).

2.2 Nitrogen and Phosphorus Pollutants Adsorption by Biochar

Nitrogen pollutants in water mainly exist as ammonium (NH_4^+) and nitrate (NO_3^-). For ammonium, one of the main adsorption mechanisms of biochar is ion exchange and electrostatic adsorption. The surface of biochar usually carries a negative charge formed by oxygen-, nitrogen-, and sulfur-containing functional groups, which can directly attract and adsorb positively charged ammonium ions^{43–45}. However, since nitrate is negatively charged, common biochar is theoretically unable to attract it. Many studies indicate that untreated biochar cannot adsorb or can only adsorb low levels of nitrate, less than 0.8 mg N g^{-1} ^{46,47}. One approach to address such an issue is to modify biochar. Numerous studies have demonstrated that modified biochar, featuring introduced positive charge sites, can address this issue. It has been shown that La-modified oak sawdust biochar increased nitrate adsorption capacity from 2.02 mg N g^{-1} (unmodified) to $22.58 \text{ mg N g}^{-1}$ ⁴⁸. Some other common modifying elements include Fe, Al, and Mg^{49–51}.

Phosphorus in water typically exists as phosphate. When biochar is used to adsorb such a pollutant, it encounters the same issue. Studies have shown that Al-modified biochar increased the adsorption of both phosphate and nitrate by 20% and 15%, respectively⁵². Modified biochar not only improves electrostatic attraction, but the metal ions loaded on its surface can also react with phosphate to form precipitates⁵³.

The adsorption rate and capacity of pollutants by biochar depend on its feedstock, production temperature, and modification. Different feedstocks result in varying initial chemical properties, including elemental composition and surface functional groups⁵⁴. Furthermore, pyrolysis temperature affects the surface morphology and pore structure of biochar. Higher pyrolysis temperatures result in a rougher surface and larger pore structures⁵⁵. As for SSA, it initially increased and then decreased with the rise in pyrolysis temperature⁵⁵. Moreover, it has been shown that as the pyrolysis temperature increased, the adsorption capacities for NO_3^- -N and NH_4^+ -N significantly increased^{18,56}. Therefore, feedstock and production temperature together influence the initial properties and adsorption capacity of biochar. The ultimate enhancement of anion pollutant adsorption depends on the modification, which introduces positive charge sites⁴⁶. Table 1 summarises the adsorption ability of nitrogen and phosphorus pollutants utilising different types of biochar.

Table 1. Research on Nitrogen and Phosphorus removal using biochar.

| Feedstock | Pyrolysis Temperature | Modification | Pollutant | Adsorption Ability | Reference |
|-----------------------------|-----------------------|-------------------|--|--|-----------|
| Pine sawdust; | 300 °C; | No | NH ₄ ⁺ | 5.38 mg g ⁻¹ | 57 |
| Wheat straw | 550 °C | Modification | | | |
| Poplar chips | 550 °C | Al | NO ₃ ⁻ and PO ₄ ³⁻ | 25.24mg g ⁻¹ and 43.98 mg g ⁻¹ | 52 |
| Biochar | | | | | |
| Bamboo | 370 °C | No | NH ₄ ⁺ | 6.38 mM-g ⁻¹ | 58 |
| Biochar | | | | | |
| <i>Phragmites australis</i> | 600 °C | MgCl ₂ | NH ₄ -N and PO ₄ -P | 30 mg g ⁻¹ and 100 mg g ⁻¹ | 59 |
| Biochar | | | | | |
| Corn Stalk | 550 °C | FeCl ₃ | TN and TP | 60% and 85% | 60 |
| Biochar | | | | | |
| Coffee ground | 500 °C | No | NH ₄ ⁺ | 51.52 mg g ⁻¹ . | 39 |
| Biochar | | | | | |
| Walnut shell | 600 °C | Fe | NH ₄ ⁺ and PO ₄ ³⁻ | 111.8mg g ⁻¹ and 165.0 mg g ⁻¹ | 34 |
| Biochar | | | | | |

2.3 Limitations of Traditional Biochar and Microorganisms Technology

There are, however, limitations of biochar. The adsorption capacity of biochar is finite, and the rate of adsorption greatly depends on particle size, pH, and temperature^{61,62}. Moreover, biochar ageing presents a significant challenge for pollutant adsorption. Various biotic and abiotic factors, including temperature, precipitation, and vegetation cover, influence the physical and chemical properties of biochar⁶³. This can cause the remobilisation of heavy metals and the formation of new pollutants, including polycyclic aromatic hydrocarbons, environmentally persistent free radicals, and colloidal or nano-biochar particles⁶⁴. It has been demonstrated that the adsorption rate of phosphate decreases with the degree of biochar ageing, and the ageing of biochar may impair its ability to adsorb cationic and anionic substances⁶⁵. More importantly, the pollutant-removing ability of biochar is primarily attributed to adsorption, rather than degradation.

As for traditional microorganism technology, significant limitations also exist. Some studies have shown that autotrophic nitrifying bacteria are dependent on the C/N ratio and temperature

in aquatic systems ⁶⁶. Moreover, high nitrogen concentration has a severe inhibiting effect on autotrophic nitrifying bacteria ^{67,68}. Thus, microorganisms in heavily polluted or harsh water bodies are vulnerable, as they often struggle to survive or encounter challenges like low water temperatures and slow reaction rates. Therefore, maintaining microbial community stability and improving the efficiency of nitrogen and phosphorus removal has become increasingly crucial.

Considering these limitations, a comprehensive approach is urgently needed that can efficiently tackle aquatic pollution.

3. The Biochar-Microbial Synergistic System

3.1 Mechanisms of Biochar-Microbial Synergistic System

Remediation approaches that rely only on biochar face low efficiency and poor sustainability issues. To eliminate aquatic nitrogen and phosphorus pollution, functional microbial strains are required that can further convert them. Numerous studies have demonstrated that the biochar-microbial synergistic system is a promising technology that enhances the overall removal process (Table 3). Thus, microbial immobilisation technology has recently attracted widespread attention for addressing water pollution ⁶⁹.

3.1.1 Biochar as a Microbial Carrier

Due to its structure and chemical properties, biochar acts as a vital carrier material for aquatic microorganisms. Microorganisms can attach to biochar through physical adhesion and charge attraction, which is facilitated by its hydrophobic, nonpolar surface, and then form a biofilm ⁷⁰. Additionally, studies show that microorganisms can secrete sticky substances to attach to the surface of biochar ⁷¹. Furthermore, biochar offers abundant habitat for microorganisms because of its high porosity and SSA, enhancing the interaction between organic pollutants and microorganisms ⁷².

Many microorganisms, such as microalgae, have negatively charged surfaces ⁷³. Modified biochar infused with positively charged metals enhances attraction to microorganisms through electrostatic forces, enabling effective microbial colonisation. The surface functional groups of biochar also significantly influence colonisation. A variety of active functional groups, including hydroxyl, sulfonic acid, carboxyl, amino, acylamino, imine, and other hydroxyl and carboxyl groups, can all promote microbial adhesion and growth ⁴². Table 2 summarises the studies on the ability of various biochar as microbial carriers.

Table 2. Research on biochar as a microbial carrier.

| Feedstock | Pyrolysis Temperature | Pyrolysis Duration | Porosity and Pore Size | Microorganisms | Microbial Carrier Effectiveness | Reference |
|------------|-----------------------|--------------------|--|---|---|-----------|
| Maize | 600 °C | 30 min | - | <i>Lupinus angustifolius L.</i> | Improve N and P uptake of plants | 74 |
| Wood | 850 °C | 30 min | - | <i>Lupinus angustifolius L.</i> | Improve N and P uptake of plants | 74 |
| Hard Wood | >700 °C | Few Minutes | 78.5% | <i>Bradyrhizobium japonicum</i> | Population abundance 4.8 log ₁₀ CFU mL ⁻¹ | 75 |
| Soft Wood | >700 °C | Few Minutes | 78.2% | <i>Bradyrhizobium japonicum</i> | Population abundance 6.3 log ₁₀ CFU mL ⁻¹ | 75 |
| Corn Stalk | 250 °C | 1 Hour | <5 um | <i>Stenotrophomonas maltophilia</i> | Effectively remove copper II | 76 |
| Pinewood | 600°C | 1.5 Hours | - | <i>Pseudomonas putida</i> | Support microorganism for 5 months | 77 |
| Pinewood | 600°C | 1-1.5 Hours | - | <i>Enterobacter cloacae UW5</i> | Support high microorganisms' population density | 77 |
| Rice husk | 500°C | 4 Hours | 0.0737 cm ³ g ⁻¹ | <ol style="list-style-type: none"> 1. <i>Rigidoporus vinctus</i> 2. <i>Aspergillus versicolor</i> 3. <i>Purpureocillium lilacinum</i> 4. <i>Aspergillus japonicus</i> | Efficiently remove 64.10% diesel | 79 |

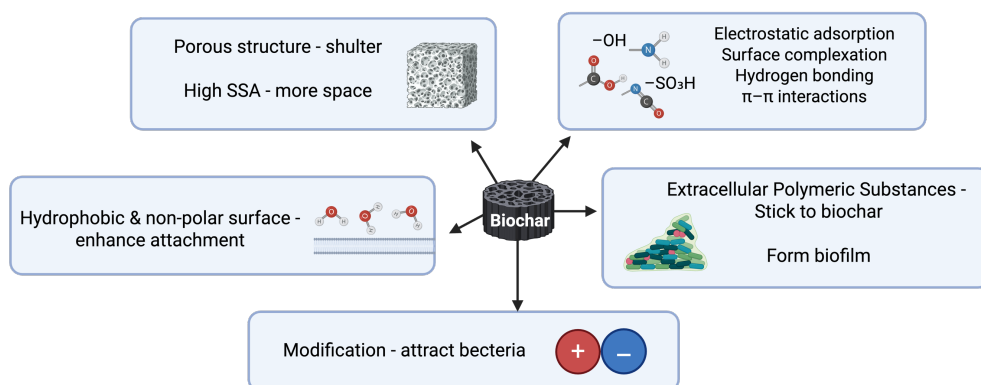


Figure 7. Mechanisms of biochar as a bacterial carrier.

This biochar-microbial synergistic approach has been explored for application in various scenarios, demonstrating effective removal of diverse pollutants, including organic compounds and heavy metals (see Table 4). Moreover, numerous studies have shown that microbial-immobilised biochar effectively reduces nitrogen and phosphorus pollution through adsorption, microbial assimilation, and biochemical transformation⁸⁰. Through the biochar-microbial synergistic system, we can achieve a combined effect where $1+1 > 2$ in treating aquatic nitrogen and phosphorus pollutants.

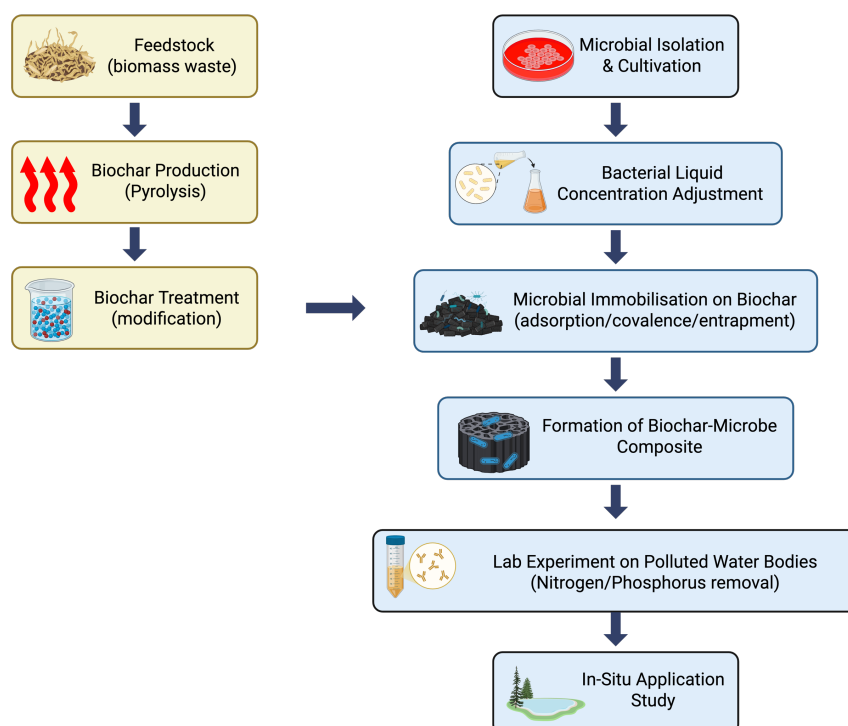


Figure 8. Flow chart of the production and application of microbial-immobilised biochar.

3.2.2 Biochar Facilitates Microorganisms

In the biochar-microbial synergistic system, biochar serves not only as a habitat for microorganisms but also supports their growth and stabilises their community. As a nutrient-rich material, biochar also promotes the development of microbial colonies ^{81,82}. In nitrogen and phosphorus removal processes, bacteria require carbon sources as their primary source of energy. Studies have shown that dissolved organic matter supplied by biochar can accelerate denitrification and microbial activity ⁸³. These easily decomposable carbon sources can also support both heterotrophic denitrification and microbial assimilation ⁸⁴. Moreover, microorganisms can hide within the porous biochar or cover themselves with carbon powder in heavy metal-contaminated environments to reduce exposure ⁸⁵. Therefore, biochar can mitigate the threat of harsh environments to these microorganisms and facilitate pollutant removal. Under such dual effects, the rate at which microorganisms remove nitrogen from water bodies accelerated. Table 3 summarises the aquatic pollutants removal ability of biochar-microbial synergistic technology under different scenarios.

Table 3. Research on the use of biochar-microbial synergistic technology to remove aquatic pollutants and its application scenarios.

| Feedstock | Microorganisms | Target Pollutant | Application Scenarios | Removal Mechanism | Reference |
|------------------------------|--|-------------------|----------------------------|---|---------------|
| Bamboo, Wood | <i>Pseudomonas</i> , <i>Achromobacter</i> , <i>Ochrobactrum</i> , <i>Stenotrophomonas</i> | Nonylphenol | Wastewater treatment | Synergistic adsorption and biodegradation | ⁸⁶ |
| Rice husk | <i>Pseudomonas aeruginosa</i> | Phenanthrene | Water and soil remediation | Synergistic adsorption and biodegradation | ⁸⁷ |
| Eucalyptus wood branch waste | <i>Pseudomonas fluorescens</i> MC46 | Triclocarban | Wastewater treatment | Synergistic adsorption and biodegradation | ⁸⁸ |
| Honeysuckle residue | <i>Bacillus subtilis</i> | Chlortetracycline | Wastewater treatment | Synergistic adsorption | ⁸⁹ |

| | | | | | |
|-----------------------------------|--|-------------------------------|--|--|---------------|
| | | | | and biodegradation | |
| Eichhornia crassipes | <i>Bacillus cereus</i> L5-1 | Phenol | Waste water treatment | Synergistic adsorption and biodegradation | ⁹⁰ |
| Sugarcane bagasse | <i>Bacillus cereus</i> LY-1 | Phenol and Cd ²⁺ | Industrial wastewater treatment | Synergistic adsorption and biodegradation | ⁹¹ |
| Chinese herb residual | <i>Bacillus cereus</i> LZ01 | Chlortetracycline | Agricultural wastewater, aquaculture wastewater | Synergistic adsorption and biodegradation | ⁹² |
| Wheat Straw | <i>Pseudomonas</i> spp. | Petroleum Hydrocarbons | Groundwater Remediation | Synergistic adsorption and biodegradation | ⁹³ |
| Agro- industrial waste | <i>Pseudomonas</i> <i>fluorescens</i> strain MC46 | Triclocarban | Groundwater contamination model | Synergistic adsorption and biodegradation | ⁹⁴ |
| Dolichospe rmum flos- aquae | <i>Proteus mirabilis</i> YC801 | Hexavalent Chromium Cr(VI) | Simulated groundwater remediation | Synergistic adsorption and biodegradation | ⁹⁵ |

3.1.3 Biochar Enhance Microbial Pollutants Degradation

Studies have demonstrated that biochar can improve interspecies electron transfer, thereby enhancing nitrogen cycling ⁹⁶. The redox-active functional groups on the surface of biochar facilitate electron transfer, which can also boost the metabolic activity of microorganisms and improve the efficiency of nitrification, denitrification, and phosphorus removal ⁹⁷. The

synergistic effect of biochar and microorganisms is also evident in their coordinated mechanism for pollutant removal. Microorganisms at the biofilm interface can effectively convert aggregated nitrogen pollutants, speeding up biochemical processes such as nitrification, denitrification, and ammonification^{32,98}. Table 4 summarises the removal ability of aquatic nitrogen and phosphorus utilising biochar-microbial synergistic technology.

Table 4. Research on the use of biochar-microbial synergistic technology for the removal of aquatic nitrogen and phosphorus.

| Feedstock | Microorganisms | N and P Removal | Colonisation Approach | Colonisation Ability | Reference |
|------------------------------|-------------------------------------|--|-----------------------|----------------------|-----------|
| Reed straw | <i>Ochrobactrum sp.</i> | NH ₄ ⁺ -N: 79.39% | Entrapment | High | 99 |
| Bamboo biocahr | <i>Pseudomonas mendocina GL6</i> | NO ₃ ⁻ -N: 8.34 mg (L·h) ⁻¹ | Adsorption | High | 100 |
| Coconut shell | <i>Pseudomonas putida</i> | NH ₄ ⁺ -N: 96.3%, PO ₄ ³⁻ -P: 91.5% | Entrapment | High | 7 |
| Bamboo | <i>Paracoccus sp. YF1</i> | NO ₃ ⁻ -N: 100% within 15 hours | Adsorption | Medium | 101 |
| Walnut shell | <i>Pseudomonas stutzeri XL-2</i> | NH ₄ ⁺ -N: 96.34% - 98.73% | Adsorption | High | 80 |
| Longan seeds | <i>Cupriavidus sp. H29</i> | NO ₃ ⁻ -N: 98.1% | Adsorption | High | 102 |
| <i>Cyperus alternifolius</i> | <i>Zobellella denitrificans A63</i> | NO ₃ ⁻ -N: 87.2%, TN: 93% | Adsorption | High | 103 |

Compared to the traditional "adsorption + dispersion of microorganisms" approach, this composite "adsorption → enrichment → biochemical transformation" improves the exposure of pollutants to microorganisms, significantly boosting the removal of nitrogen and phosphorus in aquatic ecosystems.

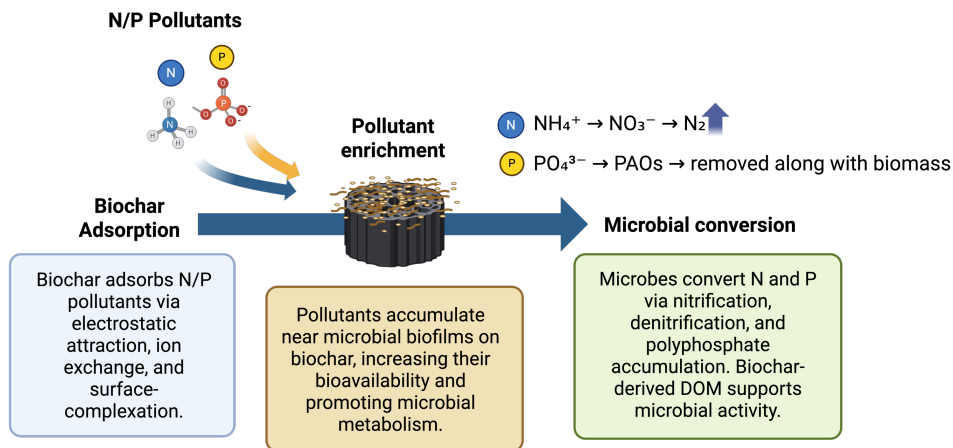


Figure 9. Process of biochar-microbial synergistic system in Nitrogen and Phosphorus removal

3.2 Key Factors Influencing Synergistic Removal of Nitrogen and Phosphorus

In the biochar-microbial synergistic system, the removal of nitrogen and phosphorus pollutants is affected by several factors, including pore size, FOGs, biochar dosage, and surrounding environmental conditions. Table 5 summarises the key factors affecting the biochar-microbial synergistic technology's ability to remove aquatic nitrogen and phosphorus.

Table 5. Research on the key factors affecting biochar-microbial synergistic technology for the removal of aquatic nitrogen and phosphorus.

| Removal Technology | Porosity | Functional Group | Dosage | Environmental pH | Removal Mechanism | Reference |
|--|---|--|------------------------------|------------------|---|-----------|
| Ammonia oxidizing bacteria immobilised biochar | - | Si-O-Si, Si-O, Si-O-Al, Si-O-Mg, -OH, CO_3^{2-} | 1.0 g 50 mL ⁻¹ | - | Adsorption and biodegradation synergistic removal | 99 |
| Aerobic denitrify bacteria immobilised biochar | Pore volume: 0.134 cm ³ g ⁻¹ ; average pore | -COOH, -OH, >C=O, -C=O | 5 g L ⁻¹ | pH = 5.0–9.0 | Adsorption and biodegradation synergistic removal | 92 |

| | | | | | | |
|---|---|---|-----------------------|---|---|----------------|
| | size: 1.75 nm | | | | | |
| Organic-degrading bacteria immobilised biochar | 2–20 nm | –COOH, –OH, C=O, –PO ₄ ^{3–} | 10g L ^{–1} | pH = 5.0–9.0, Optimal pH: 7.0 (N) / 6.0 (P) | Adsorption and biodegradation synergistic removal + metal-assisted precipitation | ⁷ |
| Active slug originated bacteria immobilised biochar | Pore volume: 0.06 cm ³ g ^{–1} Micropore diameter: 1.79 nm | - | 33.3g L ^{–1} | pH = 6.0–9.0, optimal at pH 8.0 | Biological denitrification | ¹⁰¹ |
| Denitrifying bacteria immobilised modified biochar | Pore volume: 2.32 m ³ g ^{–1} Avg. pore diameter: 52.31 nm | C–O, –COO [–] , =C=O, Ar–H | 36 g L ^{–1} | pH = 5, 7, 9, optimum performance at pH 7 | Adsorption and biodegradation + Synergistic effect enhanced by Mg ²⁺ release | ⁸⁰ |

3.2.1 Pore Size and Functional Groups

Micropores (<2 nm) can provide a large SSA, enhancing the adsorption of small particles such as NH₄⁺ and PO₄^{3–} ³⁵. Importantly, porosity and SSA also depend on the raw materials and temperature used during biochar production ³⁷. Studies have shown that biochar produced

from materials with higher lignin content and at moderate temperatures (400-700 °C) exhibits a higher SSA ^{37,104}. However, mesopores (2-50 nm) and macropores (>50 nm) are more suitable for microbial colonization ³⁶. This creates a trade-off when selecting biochar types for addressing aquatic pollution. The FOGs covering the surface of biochar are another key factor. The presence of FOGs simultaneously enhances pollutant adsorption and microorganism adhesion ⁴⁰⁻⁴².

3.2.2 Biochar Dosage

Additionally, the amount of biochar added can have non-linear and counterintuitive effects on the rates of nitrogen and phosphorus removal. One study has shown that as the biochar dosage increases, the adsorption capacity for ammonia nitrogen decreases ¹⁰⁵. However, this trend has not been observed in phosphate adsorption ¹⁰⁶. In contrast, the phosphate adsorption capacity first increases and then decreases ¹⁰⁵. Therefore, identifying the optimum dosage for removing nitrogen and phosphorus pollutants is essential. It has been demonstrated that the phosphate adsorption capacity peaks when the biochar (Ca-pmsBC750N) dosage reaches 1.5 g L⁻¹ ¹⁰⁷. Other studies suggest that the optimal dosage considering both economy and effectiveness should be around 1.0 g L⁻¹ ¹⁰⁸. When the biochar dosage exceeded 1.0 g L⁻¹, the adsorption capacity ceased to increase ¹⁰⁹. However, in high flow rate water bodies (rivers, constructed wetlands), low doses may be quickly diluted or washed away, resulting in an incomplete adsorption-biotransformation chain. The suggested dosage (1.0 g L⁻¹) is more suitable in small-volume closed water bodies (such as aquaculture ponds and landscape lakes).

3.2.3 Environment pH

Changes in the surrounding environment can also impact the removal of nitrogen and phosphorus. FOGs on the surface of biochar are also affected by the pH of the environment, thereby indirectly influencing the removal of nitrogen and phosphorus ¹¹⁰. Studies have shown that as the pH value rises, phosphorus adsorption efficiency decreases ¹¹¹. Furthermore, the survival of microorganisms is also affected by pH, with a range of 6-8 being most suitable for their survival and reproduction ¹¹². Nguyen et al. ¹¹³ indicated that the phosphorus uptake rate of *tetrasphaera* PAOs increased as pH increased from 6 to 8. Additionally, many studies have suggested that the adsorption capacity of biochar increases with increasing environmental pH ^{114,115}. Li et al. ¹¹⁶ showed the same trend, indicating that the adsorption capacity of phosphate peaks at pH 9.

3.3 Lab Research Cases

Based on numerous lab studies, the capacity of nitrogen and phosphorus removal by microbial-immobilised biochar far surpasses that of single biochar and free bacteria. Wang et al.¹¹⁷ systematically examined the removal rate of ammonia nitrogen using modified/unmodified biochar, as well as biochar with and without bacterial immobilisation. The chosen microorganism was heterotrophic nitrifying bacteria, *Pseudomonas sp. Strain-II*, which was isolated from the activated sludge of the sewage treatment plant. The result indicated that the removal rate of modified biochar was 88.6% to 90.93% (48 hours), significantly higher than that of normal biochar (42.2% in 48 hours). Additionally, bacteria-immobilised biochar removed 58.35% of ammonia nitrogen for 5 hours, while bacteria without immobilisation could only remove 20.43% of ammonia nitrogen within the same time. Wang et al.¹¹⁷ indicated that alkali and metal ion modification significantly increased the SSA of biochar, 2.99–3.56 times higher than the original biochar. Furthermore, the modification made the surface of the biochar positively charged under near-neutral conditions, which better attracted negatively charged bacteria and enhanced the adhesion amount. Mg^{2+} complexes with the functional groups on the surface of biochar increased roughness and active sites, which is beneficial to bacterial colonisation. Moreover, high surface alkalinity can promote the deprotonation of NH_4^+ to form NH_3 , thereby accelerating the nitrification process.

Table 6. Laboratory cases of using biochar-microbial synergistic technology for the removal of nitrogen and phosphorus from water bodies.

| Removal Technology | Biochar Type | Pollutant | Removal Rate | Effect and Comparison | Reference |
|---|---|-------------|--------------------------|---|----------------|
| <i>Pseudomonas sp. Strain-II</i> Immobilisation | NaOH+ Mg^{2+} -modified rice husk-derived biochar etc | NH_4^+ -N | 58% (5 Hours) | Unloaded bacteria biochar 20.43% | ¹¹⁷ |
| <i>Paracoccus denitrificans(T)</i> , <i>Pseudomonas(J)</i> , and <i>Raoultella(L)</i> Immobilisation | Peanut shell biochar | NH_4^+ -N | 97.9%~99.1% (30 days) | Microorganisms adsorbed 1.16- 3.44 times better than embedded | ¹¹⁸ |

| | | | | | |
|--|-------------------------------------|--------------------------------|---|---|----------------|
| <i>Bacillus sp. AK3</i> and <i>Alcaligenes</i> <i>sp.</i> | Bamboo biochar | NO_3^- -N | 40% within 6 days | Not effective in P removal | ¹¹⁹ |
| <i>Pseudomonas</i> <i>hibiscicola strain</i> L1 Immobilisation | Peanut shell biochar | NO_3^- -N | from 100.00 to 74.17 $\text{mg}\cdot\text{L}^{-1}$ (120 hours) | As Nitrogen source enhance heavy metal removal | ¹²⁰ |
| <i>Zobellella sp. A63</i> immobilisation | Cyperus alternifolius biochar | NO_3^- -N, TN | 68.2%, 78.6% | Significantly higher than biochar dosage system | ¹⁰³ |
| Biofilm | - | NH_4^+ -N, TP | 91.2%, 99.9% | - | ¹²¹ |
| <i>Chlorella</i> <i>pyrenoidosa</i> embedded | Pine timber biochar | Ammonia Nitrogen, TN, TP | 69.2 %, 43.0 %, 73.8 % | High potential in estuarine environments and saline groundwater | ¹²² |

In the process of the synergistic system removing nitrogen and phosphorus, the primary contributor is the immobilised microorganism. Biochar contributes less than 10% to the removal rate, mainly serving as a support for microbes ¹²³. Other studies listed also highlighted the mechanisms and effectiveness of the microbial-biochar synergistic technology (See Table 6). An et al. ¹²⁰ found that different types of modified biochar influence the microbial immobilisation rate. The adsorption rate of microorganisms on NaOH^- and $\text{NaOH}^+\text{Mg}^{2+}$ -modified biochar is much higher than that of HNO_3^- and Mg^{2+} -modified biochar, leading to an increase in nitrogen and phosphorus removal ¹²⁰. Xue et al. ¹²³ observed that the removal rate of NO_3^- -N increases as the immobilisation capacity rises (from 0 to 40%) and peaks at 40%. However, when immobilisation capacity increases from 40% to 60%, the removal rate declines ¹²³. This was mainly due to the competition for the limited nutrients and microbial aggregation, which leads to a reduction in effective surface area and binding sites. Furthermore, the pollutant concentration in the environment significantly influences the pollutant removal rate of both microorganism-immobilised biochar and free bacteria. A study shows that the pollutant removal rate of microorganism-immobilised biochar drops from 99.6% to 66.5% when the nitrate concentration increases from 50 mg L^{-1} to 300 mg L^{-1} ¹⁰⁰. The pollutant removal rate of

free bacteria also declines from 90.4% to 30.3% ¹⁰⁰. Therefore, high nitrate levels in the environment can exert toxic and inhibitory effects on bacteria ¹⁰⁰. Regarding microorganism-immobilised biochar, the decrease in nitrate removal rate is smaller than that of free bacteria, revealing the protective effect of biochar on bacteria.

Laboratory studies have established the basis for the in-situ use of microorganism-immobilised biochar. Although environmental conditions in water bodies may introduce more complex effects on nitrogen and phosphorus removal in real-world situations, these studies have demonstrated the usefulness and mechanism of the microbial biochar synergistic system.

4.2 Pilot Study for In-situ Application

With the increase in laboratory studies on biochar-microbial synergistic systems, the practical engineering applications of this technology have become more evident. Although there are no actual cases of application in water body treatment, some pilot studies have demonstrated its potential.

A study by Guo et al. ¹²² simulates turbulent water flow in natural water bodies by varying shaking rates. This research investigates the ability of *Chlorella pyrenoidosa*-embedded biochar beads to remove nitrogen, phosphorus, and heavy metals under different flow rates (0 rpm, 80 rpm, 160 rpm, and 240 rpm). It shows that the pollutant removal capacity of *Chlorella pyrenoidosa*-embedded biochar beads exceeds that of using either *Chlorella pyrenoidosa* bacteria or pine timber biochar alone. Additionally, these beads demonstrate strong resistance to high turbulence, which does not affect bacterial growth or the rate of pollutant removal. It suggests that such technology is suitable for use in highly fluid water bodies such as estuaries and tidal zones.

Furthermore, *Chlorella pyrenoidosa*-embedded biochar beads performed well in their removal rate when the salinity was below 21‰ ¹²². At 21‰ salinity, Ca^{2+} is replaced by Na^+ , causing an imbalance in the osmotic pressure of algal cells and even negative removal (release) of organic matter. Thus, a higher salinity negatively impacts their effectiveness. Guo et al. ¹²² also indicated that *Chlorella pyrenoidosa*-embedded biochar beads showed the best performance in neutral or weakly alkaline (pH 7-8) environments. Acid environmental conditions significantly affected adsorption and algal activity. Therefore, *Chlorella pyrenoidosa*-embedded biochar beads are suitable for use in estuarine and marine environments, as well as in saltwater groundwater. Such environments include the Bohai Sea in China, the estuary of the Shenzhen

River, and other regions ^{124,125}. However, such technology has poor adaptability to acidic, high-salt (>21‰) or high-pollution load water bodies. The stability and ecological risks under long-term application are under investigation. This research highlights the potential of this synergistic technology under specific environmental conditions, providing a valuable reference for future projects.

The tidal flow constructed wetlands system is a promising technology for removing aquatic nitrogen and phosphorus. This technology treats various types of wastewaters by mimicking natural pollutant processing and speeds up oxygen transfer ^{126,127}. Zhao et al. ¹⁰³ discovered that nitrogen and phosphorus removal rates can be enhanced by applying *Zobellella sp. A63* immobilised biochar. Such a positive relationship exists under both high C/N and low C/N environmental conditions. Additionally, the use of *Zobellella sp. A63* immobilised biochar also promotes the enrichment of N-removed microorganisms and increases bacterial diversity¹⁰³. Although this study used stimulated wastewater, it demonstrated the potential of microbial biochar synergistic technology to improve the efficiency of the tidal flow constructed wetlands system, thereby further boosting pollutant removal rates.

3.4 The Economy and Sustainability of the Biochar-Microbial Synergetic System

The feasibility of applying biochar-microbial synergistic technology depends on its cost, environmental benefits, and sustainability. Currently, there has been no systematic economic analysis of the biochar-microbial synergetic technology. This may stem from limited open data on the unit capital and the replacement cycle of this technology, indicating that biochar-microbial synergistic technology is not yet widely applied. Other research has compared biochar with other activated carbon materials, and some have investigated the economic benefits of removing phenanthrene from sediment using biochar-immobilised cells ^{128,129}.

To evaluate, we can consider the production cost of biochar and the cultivation expenses of several related pollutant removal technologies that utilise biochar. The feedstock for biochar production is inexpensive or nearly free of charge. Utilising waste organic material to address environmental issues makes it a sustainable and environmentally friendly solution. The main costs of biochar production include electricity, gas, and fuel. For instance, in China, the value of each ton of biochar is approximately \$360, excluding transportation costs ¹³⁰. A techno-economic analysis of utilising biochar pellets in solving agricultural non-point source pollution indicated that the production cost of this technology is \$412.6 t⁻¹, while the unit removal cost of soluble reactive phosphorus ranges from \$165 to \$ 326 kg⁻¹ ¹³¹. As for microbial technology, a

full-scale project using wood chip denitrification bioreactors applied in a farmland drainage outlet reported its nitrogen removal capital cost of \$33 kg⁻¹ per year¹³². Generally, the overall cost of microbial technologies is higher than that of using biochar-based technology for aquatic pollutant removal, primarily due to the construction, maintenance, and operational difficulties associated with these technologies. However, the technical costs of microbial cultivation, strain isolation, and colonisation are still relatively low. Consequently, the cost of the biochar-microbial synergistic technology is moderate, yet no large-scale commercial production currently exists. Most biochar-microbial synergetic products are produced in small quantities by research institutes for scientific purposes. Overall, the environmental and industrial benefits should outweigh its technical costs. The production and application of this technology in practice may depend on government and institutional support and encouragement to promote its adoption and wider use.

5. Challenge, Future, and Prospect

5.1. Current Challenge

Whilst biochar technology has potential, there are no doubt some challenges and limitations. One vital issue is the ageing, which can affect its structural stability and lead to the re-release of pollutants after long-term use. This may cause the failure of supporting microorganisms and hinder carbon sequestration. Therefore, using microbial-immobilised biochar is not a 'one-time fix' for removing nitrogen and phosphorus from water bodies. The effects of biochar ageing on pore structure, surface chemistry, adsorption capacity, and microbial activity remain unclear.

Another critical challenge is the recycling of microbial-immobilised biochar, an area that has not been explored in existing studies. Traditional recycling methods include thermal, chemical, and biological regeneration^{133–135}. These approaches can restore biochar's adsorption capacity and structure. Physical and chemical methods often rely on high temperatures and strong acids or alkalis, which are harmful to immobilised microorganisms¹³⁶. In contrast, biological regeneration employs microorganisms to decompose saturated pollutants, similar to the biochar-microbial synergistic technology. However, its effectiveness remains uncertain. Researchers need to develop regeneration technology under mild conditions (low temperature, weak acid and alkali). Additionally, evaluating the feasibility of biological regeneration under different pollution loads and multi-pollutant conditions is necessary.

The limitations of microorganisms in this technology are also evident. Some studies identified clear ranges of different environmental indicators for microorganism survival⁹². Although it can

protect microorganisms, biochar-microbial synergistic technology is not applicable in extreme environments. The current aim should focus on screening strains with stronger tolerance (salt, temperature, acid and alkali) and studying the surface regulation strategies of biochar to enhance the stability of the microbial community. Table 7 summarises the pros and cons of various nitrogen and phosphorus removal technologies.

Table 7. Comparison of different technologies for the removal of nitrogen and phosphorus.

| Technology | Application Scenarios | N and P Removal Rate | Cost | Sustainability | Operational difficulty | Reference |
|--|---------------------------------------|---|--|---|--|----------------|
| Biochar immobilised with <i>Bacillus cereus</i> W2 | Aquaculture water pollutant removal | Remove 100% NO_3^- -N within 24 hours | Low to moderate- uses natural raw material and the process is simple | High- renewable resource biochar | Moderate- Meticulous strain culture and fixation operations are required | ¹²³ |
| Modified biochar | Restoration of eutrophic water bodies | Maximum P adsorption rate 758.96 mg g^{-1} | Low to moderate- uses natural raw material and the process is simple | High- sustainable feedstock | Low- simple modification in process and direct application | ¹³⁷ |
| Free Bacteria <i>Bacillus cereus</i> W2 | Aquaculture water pollutant removal | Remove 93.5% NO_3^- -N within 24 hours | Low: cost of strain culture and expansion is low and no carrier materials are required | Moderate- biodegradable and eco-friendly, but susceptible to environmental stress | Low- directly add the bacterial solution | ¹²³ |
| Oxidation Ponds | Sewage treatment in | Total N: 1.78 mg L^{-1} ; Total | Low: rely on natural light and | Moderate- does not require additional | Low- is simple and easy to maintain, | ¹³⁸ |

| | | | | | | |
|-----------------------|--|---|--|--|--|-----|
| | developing regions | P: 1.94 mg L ⁻¹ | microorganism | energy but can pollute the receiving water body | but its processing effect depends on natural conditions | |
| Land Treatment System | Rural or peri-urban areas for tertiary P removal | 8–99% depending on soil type, age, loading rate | Very Low: soil and natural ecological processes | High- long-term operation, low carbon and low energy consumption | Low to Moderate-maintenance and long-term management | 139 |
| Constructed Wetlands | Municipal, agricultural, decentralized systems for tertiary wastewater treatment | TP removal: 60–99%; TN removal: up to 90% | Low to moderate: Moderate construction costs and low operation and maintenance costs | High-natural driven system, low carbon footprint | Low to moderate-simple structure but requires proper design and long-term monitoring | 116 |

5.2 Future Study

Further research should focus on selecting suitable biochar feedstocks, modification materials, and pollutant-degrading bacteria to determine the optimal combination through laboratory experiments. Several innovative biochar modification materials, such as nanomaterials and magnetic particles, have been reported to be effective in removing heavy metals and chemical pollutants^{140,141}. Whether these modification materials also enhance nitrogen and phosphorus removal is currently under investigation. Additionally, the impact of modified materials on immobilised microorganisms remains unclear. The interaction between these components should also be carefully considered.

In microbiology, efforts should focus on developing efficient screening and culture technologies for functional microorganisms. While enhancing the efficiency of the biochar-microbial synergistic system, researchers should also aim to reduce technical costs. Future research should further investigate the genetic and metabolic mechanisms of functional expression.

The key research direction of biochar-microbial synergistic technology over the next 5 years should focus on addressing urban black and odorous water bodies, industrial mixed pollutants wastewater, farmland drainage, and non-point source pollution. Future studies should investigate the simultaneous removal of nutrients and organic pollutants, as well as enhance the denitrification and phosphorus fixation capacity, using biochar-microbial synergistic technology.

The success of the biochar-microbial synergistic system in removing nitrogen and phosphorus offers valuable insights for eliminating other types of pollutants. It is important to explore its effectiveness in removing heavy metals, organic compounds, pesticide and insecticide residues, and antibiotics. Lastly, since most current research remains at the laboratory stage, further small-scale field trials should be conducted to verify the real-world benefits of this technology.

5.3 Industrial Applications

Applying the biochar-microbial synergistic system to existing pollutant treatment technologies is a crucial pathway for advancing this approach. Notable examples include using biochar-microbial synergistic products in constructed wetlands and membrane bioreactors (MBRs) to improve pollutant removal efficiency^{103,121}. Additionally, promotion should also consider environmental policies and social support. Government funding, standardised management, and incentive policies are vital in successfully implementing new technologies. Going forward, efforts in this field should focus on linking governments, enterprises, research institutions, and social groups to develop a sustainable model of multi-party collaboration.

6. Conclusion and Outlook

This review comprehensively analysed the mechanisms, advantages, and practical developments of biochar-microbial synergistic systems for nitrogen and phosphorus removal in aquatic environments. Compared to traditional technologies, these systems demonstrate

significant synergistic advantages, particularly enhanced removal efficiency, stability, and environmental adaptability, overcoming key limitations of conventional pollutant remediation. From a technical perspective, recent studies indicate that biochar-microbial composites can achieve over 80–90% removal of nitrogen and phosphorus, with low-cost raw materials and enhanced microbial activity ensuring both economic and ecological sustainability. The co-application of engineered biochar and functional microorganisms offers a promising solution for large-scale water pollution control.

To accelerate practical adoption, policymakers need to standardise biochar quality, support pilot demonstrations in typical regions, and promote cross-disciplinary collaboration. Incentives for recycling and utilising biochar derived from agricultural waste should be incorporated into environmental management policies.

Looking ahead, biochar-microbial synergistic systems are expected to become a key technology for ecological restoration and sustainable environmental industries. Future research should focus on optimising system performance under complex field conditions, developing robust technical standards, and exploring intelligent control methods. The widespread promotion of this technology will significantly improve global water quality and promote resource recycling.

Abbreviations:

OFGS: Oxygen-containing functional groups

SSA: Specific surface areas

PAOs: Poly-phosphate accumulating organisms

C/N: Carbon to nitrogen ratio

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