Proceedings of ICEGEE 2026 Symposuim: AI-Based Medicine and Biological Data Analysis
DOI: 10.54254/2753-8818/2026.AU33550

Mechanism Analysis of a Three-way Synergistic Remediation System Comprising Biochar, Petroleum-degrading Bacteria, and Degradation Agents

Wenyao Li

Department of Environmental Engineering, College of Chemical Engineering and Environment, China University of Petroleum, Beijing, China
2023010637@student.cup.edu.cn

Abstract. Oil leakage pollution is a major problem in the global ecological environment. It is high-cost and poor environmentally friendly to repair only with traditional degradation agents. Biochar remediation technology can only adsorb and enrich pollutants without degradation function. Although microbial remediation has environmental advantages, it has problems such as slow remediation rate, insufficient stability and easy loss of bacteria. Therefore, it is particularly important to develop a combination technology of microorganisms and traditional remediation methods. Biochar materials are low cost, wide source and friendly to environment, and can be used as ideal carrier for microbial immobilization. This paper systematically reviews the theoretical basis and synergistic mechanism of biochar, petroleum degrading bacteria and degradation agent ternary synergistic remediation system, expounds the advantages of biochar carrier, petroleum degrading bacteria degradation characteristics and degradation agent action mechanism, deeply analyzes the physical, chemical and biological coupling mechanism and synergistic path of the three, and integrates multi-bacteria synergy and micro-environment regulation to perfect the construction of ternary remediation system. The study also identifies technical bottlenecks across system design and safety, offering theoretical and technical frameworks for sustainable oil spill remediation.

Keywords: Biochar, petroleum degrading bacteria, degradant, synergistic remediation, oil spill pollution

1. Introduction

polycyclic aromatic hydrocarbons, pose severe threats to ecosystems and human health due to their hydrophobicity, persistence, and toxicity. While biochar effectively adsorbs pollutants and immobilizes microorganisms, the overall degradation efficiency remains dependent on microbial metabolism. Consequently, a ternary synergistic system—integrating biochar, microorganisms, and pollutants—can leverage complementary advantages to significantly enhance bioremediation performance.

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.AU33550

Currently, research has primarily focused on the properties of individual components or binary synergistic systems. However, the coupling mechanisms, synergistic modes, and regulation strategies for ternary systems in complex environments remain poorly understood. To address these gaps, this paper systematically reviews the current state of ternary synergistic systems, providing critical theoretical and technical frameworks for the efficient remediation of oil-contaminated sites.

2. Biochar: multifunctional immobilization carrier and pollutant adsorbent

2.1. Preparation and characteristics of biochar

Biochar is a carbon-rich solid material prepared by pyrolysis of biomass under oxygen-limited or oxygen-free conditions. Raw materials include agricultural straw, forestry by-products, urban organic wastes, etc. High specific surface area and pore structure are the basis for loading microorganisms and adsorbing pollutants. With the increase of pyrolysis temperature, the specific surface area and total pore volume of biochar increase. The pore structure provides attachment space for microorganisms and enhances physical adsorption of petroleum hydrocarbons [1,2]. Moreover, the increase of pyrolysis temperature makes the aromaticity of biochar increase and polarity decrease, the high aromatization structure enhances the adsorption of aromatic hydrocarbons through π-π interaction, and the strong hydrophobicity promotes the enrichment of non-polar petroleum hydrocarbons and the attachment of hydrophobic microorganisms [1,2]. Zeta potential regulates biochar's hydrocarbon adsorption and microbial attachment via electrostatic action. Specifically, heterogeneous charges enhance pollutant capture, while a lower absolute Zeta potential promotes the immobilization of negatively charged microbes. Thus, regulating this parameter enables the dual optimization of adsorption and immobilization [1-3].

2.2. Adsorption mechanism of biochar

The adsorption of petroleum pollutants by biochar is the synergistic result of physical adsorption, chemical adsorption and partition. The low temperature carbon prepared at pyrolysis temperature lower than 350 ℃ is dominated by partition, while the high temperature carbon prepared at 500℃~600℃ is dominated by physical adsorption and chemical adsorption [1]. Physical adsorption relies on pore filling and van der Waals forces. Meanwhile, chemical adsorption binds aromatic hydrocarbons through π-π interactions and enhances stability via hydrogen bonding. Additionally, hydrophobic interactions promote the enrichment of non-polar components, leading to high adsorption capacity. Finally, partition mainly occurs in the non-carbonized organic phase of low-temperature biochar [1,3,4].

2.3. Advantages of biochar as microbial carrier

The pore size distribution of biochar provides different scales of attachment space for microorganisms, large specific surface area can form high-density biological matrix, porosity can improve nutrient retention capacity and microbial survival rate, and stable carbon structure can ensure the sustainability of microbial activities [1-3]. Surface oxygen-containing functional groups enhance the adsorption of microbial cells through electrostatic interaction and chemical bonding, and can also improve the bioavailability of nutrients. Biochar contains trace nutrients such as Fe_2O_3 and NaCl, which provide mineral nutrients for microbial metabolism., its Zeta potential adjustability can also optimize its adsorption capacity to microbial cells [1,2]. Through physical shelter, nutrient

DOI: 10.54254/2753-8818/2026.AU33550

supply and microenvironment regulation, the stress resistance and environmental adaptability of microorganisms are significantly improved [1,2,5].

3. Petroleum-degrading bacteria: core biochemical transformation functional unit

3.1. Metabolic pathways of petroleum-degrading bacteria

Petroleum-degrading bacteria degrade hydrocarbons through terminal oxidation, subterminal oxidation and co-metabolism. Key enzymes catalyze the molecular structure change of hydrocarbons, gradually decompose long-chain alkanes into alcohols, aldehydes and other compounds, and finally mineralize them into CO_2 and H_2O [2]. Different hydrocarbon degradation difficulties vary, saturated hydrocarbons are the easiest to degrade, aromatic hydrocarbons are the second, polar substances are the most difficult to degrade [1,2]. The construction of complex bacteria can improve the degradation efficiency of complex petroleum hydrocarbons [6]. Aerobic microorganisms are the core of petroleum hydrocarbon degradation. Bacillus subtilis, Pseudomonas aeruginosa and other strains decompose petroleum components through monooxygenase and dioxygenase ; anaerobic microorganisms such as Desulfovibrio and Thiobacterium denitrans can degrade complex components difficult to be decomposed by aerobic bacteria through dehydroxylation in anoxic environment [7-9]. Complementarity of aerobic and anaerobic groups can broaden hydrocarbon degradation spectrum.

3.2. Protection and enhancement of degradation bacteria by immobilization

Immobilization of microorganisms can significantly improve the survival rate and tolerance of bacteria in a polluted environment. The physical shelter provided by biochar for microorganisms can alleviate the toxicity of heavy metals and organic pollutants, so that immobilized microorganisms can still maintain high degradation activity in a high-concentration polluted environment, while the degradation ability of free bacteria is severely inhibited [1]. Under low pollution concentration, the degradation performance of immobilized microorganisms and free bacteria is stable; In high pollution concentration and heavy metal and organic stress environment, the degradation performance of immobilized microorganisms is significantly better than that of free bacteria [2].

4. Degradation agents: key regulators of synergy

4.1. Biosurfactant degradation agent

Biosurfactants change the existing state of petroleum hydrocarbons through emulsification, construct mass transfer bridges among pollutants, microorganisms and interfaces, and improve the bioavailability of petroleum hydrocarbons [10-12]. When the concentration of rhamnolipid is lower than the critical micelle concentration (CMC), the adhesion between bacteria and hydrocarbons is enhanced by reducing the interfacial tension, increasing the solubility of hydrocarbons and improving the hydrophobicity of bacteria; When the concentration is higher than the CMC, the solubility of hydrocarbons is further increased through micellar solubilization, and it can also be used as a co-metabolic substrate to promote the degradation of strains, and it has no obvious inhibition effect on the growth of bacteria, and the concentration of rhamnolipid can be kept stable during the repair process [10-12]. Biosurfactants can strip pollutants adsorbed on biochar surfaces, expose oxygen-containing functional groups and adsorption sites covered by biosurfactants, avoid

DOI: 10.54254/2753-8818/2026.AU33550

destroying biochar functional groups, and adjust its surface charge properties through intermolecular interactions [13,14].

4.2. Nutrient degradation agent

Water-soluble organic nitrogen source and phosphorus source can significantly promote the growth of oil-degrading bacteria. Organic nutrients not only provide N and P, but also contain trace elements and growth promotion factors, which can stimulate microbial growth more effectively [10]. Biochar has adsorption and slow release effects on nutrients, which can prolong the effective time of nutrients, avoid rapid loss caused by seawater dilution or rain washing, and provide continuous nutritional support for microorganisms [1,2,8].

4.3. Chemical synthetic degradation agent

Chemical synthetic degradation agents mainly include chemical dispersants, oil condensers and oil collectors [15]. They can disperse, condense or enrich oil spills by reducing oil-water interfacial tension and rapidly increasing the contact area between petroleum hydrocarbons and microorganisms. They are important reagents for oil spill emergency treatment [15]. Such reagents can make up for the shortcomings of slow effect of bioremediation and cooperate with biochar adsorption to strengthen petroleum hydrocarbon enrichment, creating conditions for degradation bacteria metabolism.

5. Mechanism analysis of ternary cooperative repair system

5.1. Coupling mechanism of physics, chemistry and biology

The systematic integration of physical, chemical and biological processes is the core of efficient collaborative remediation of petroleum pollution by the ternary system of biochar, degradation agent and petroleum degradation bacteria. Aiming at bottlenecks such as strong hydrophobicity of petroleum hydrocarbons, low bioavailability and difficult colonization of functional bacteria, the ternary system forms a full-chain remediation path through functional complementarity and process linkage.

As a physical core and functional carrier, biochar rapidly adsorbs and enriches petroleum hydrocarbons and blocks migration by virtue of developed pore structure and high specific surface area, and provides shelter space and colonization base for microorganisms [3]. Chemical units play a bridge role, biosurfactants reduce oil-water interfacial tension and solubilize petroleum hydrocarbons, converting refractory components into small molecules and easily usable substrates [13]. The biological unit is the mineralization core. Indigenous degradation bacteria or synthetic microbial communities secrete functional enzymes to completely mineralize petroleum hydrocarbons into harmless substances, and at the same time realize in-situ regeneration of biochar. Metabolite products can also enhance the effect of chemical agents [16]. For heavy metal contamination, microorganisms induce calcium carbonate precipitation Microbially Induced Carbonate Precipitation (MICP) functional bacteria can further convert heavy metal ions into stable carbonate precipitates through metabolic activities, greatly reducing the toxic inhibition of heavy metals on petroleum degradation bacteria [17]. Deep coupling of the three forms a closed loop of enrichment, transformation, mineralization and ecological restoration, breaking through the limitations of a single technology and constructing an efficient green restoration system suitable for oil-contaminated sites [3,13,16].

DOI: 10.54254/2753-8818/2026.AU33550

5.2. Two-way promotion mechanism

Biochar and degrading bacteria have a two-way promoting effect. Biochar improves microbial immobilization efficiency and pollutant tolerance by providing attachment sites, micronutrients and pH buffering; extracellular polymers secreted by degrading bacteria enhance the structural stability of biochar. The organic acids produced by metabolism expose their new adsorption sites, can also decompose unstable carbon elements in biochar, increase pore volume and specific surface area, and improve pollutant fixation capabilities [1,2,5,18]. The bi-directional promotion relationship between biochar and degradation agent is also significant. The addition of a degradation agent can promote the regeneration of biochar surface active sites, among which surfactant degradation agent can reduce the occupation of petroleum hydrocarbon on biochar active sites, and disperse biochar particles and maintain their specific surface area [4,13]. Correspondingly, biochar can enrich surfactants through adsorption, forming local high concentration areas at the solid-liquid interface, and further strengthening the capture and conversion ability of active sites for pollutants [9].

5.3. Three-way cooperative path

Based on the binary synergy mechanism, the ternary synergy system forms the core mechanism of adsorption, fixation, activation and degradation. Through the cascade coupling of biochar physical adsorption, degradation agent interface regulation and biodegradation of degrading bacteria, a complete closed loop from capture to complete mineralization of pollutants is constructed, and key factors such as multi-bacterial synergy and aerobic and anaerobic function complementarity are integrated to form a perfect collaborative remediation path. Biochar rapidly adsorbs and enriches petroleum hydrocarbons through hydrophobic interaction and π-π interaction by virtue of its high specific surface area and developed pore structure, forming a local high concentration area at the solid-liquid interface, which not only reduces the mobility of pollutants, but also strengthens the contact probability between degradation units and substrates [3,4]. Its porous structure can also increase the ventilation of the system and provide oxygen contact sites for aerobic microorganisms [7]. The rough surface and oxygen-containing functional groups of biochar provide multi-dimensional attachment sites for degrading bacteria. Modified biochar can achieve efficient immobilization of microorganisms and induce stable biofilm formation through electrostatic attraction and hydrogen bonding [1,5,19]. Appropriate environmental parameters and inoculation amount can further improve the immobilization efficiency. Physical barriers can also buffer environmental toxicity and reduce the competitive pressure of indigenous microorganisms [2,5,6]. Nutrient elements such as phosphorus can also support microbial growth [8]. In the microenvironment constructed by biochar, degradation agents are locally enriched and activated. Biosurfactants exhibit concentration-dependent regulation in stages, and their parental molecular structures can strip petroleum hydrocarbons from the surface of biochar, release active sites, disperse biochar particles and maintain the accessibility of active sites [13]. Rhamnolipid can also regulate the surface charge properties of biochar, enhance the affinity of active sites with degrading bacteria and pollutants, and do not destroy the functional group structure [14]. Traditional chemical surfactants need to be strictly controlled in concentration to avoid occupying active sites of biochar or destroying oxygen-containing functional groups [13]. Nitrogen and phosphorus nutrients loaded on biochar realize slow-release supply and activate key metabolic enzymes of degrading bacteria [1,2,10]. Surfactants can also destroy oil film continuity, promote oxygen dissolution, alleviate hypoxia problem, reduce biotoxicity of petroleum pollutants and reduce damage to microbial cell membrane [7]. Under the above synergistic effect, the degrading bacteria in the protective

DOI: 10.54254/2753-8818/2026.AU33550

microenvironment obtain sufficient metabolic activation and substrate supply, and realize efficient degradation of petroleum hydrocarbons through the complementary functions of aerobic and anaerobic microorganisms. Aerobic bacteria decompose paraffin, aromatic hydrocarbons and other conventional components, while anaerobic bacteria degrade refractory complex components [7,8]. Multi-bacteria synergy mode is introduced to combine Pseudomonas, Bacillus and other strains with different degradation advantages, which can cover a wider range of pollutant types [8,9]. The combination of surfactant-producing bacteria and degrading bacteria can realize efficient connection of dispersion and degradation, and indigenous microorganisms and exogenous degrading bacteria form a synergistic metabolic network [8,13]. Degradation bacteria efficiently mineralize adsorbed and emulsified petroleum hydrocarbons into CO_2 and H_2O, and the extracellular polymers produced by their metabolism and surfactants jointly modify the active sites of biochar to optimize the adsorption and catalytic microenvironment [1,5,9,10]. They can also decompose unstable carbon components in biochar to realize its modification and regeneration, but it is necessary to avoid excessive microbial propagation blocking the pores of biochar and affecting the adsorption efficiency [18,20]. The repair cycle of ternary synergistic system is several weeks to several months. Although the emergency treatment speed is slower than that of physical and chemical methods, it can degrade thin oil layers and dispersed oil more thoroughly and is more environmentally friendly [15]. The modification of microorganisms and the protection of pores by surfactants can prolong the functional life of biochar [13,18]. Although improper use of surfactants and release of harmful substances of biochar may accelerate its aging failure, reasonable regulation of the distribution ratio and concentration of each group can improve the repair efficiency and maintain the long-term stability of the system [15,20]. Therefore, the carrier and adsorption function of biochar, the mass transfer and activation function of degradation agent, and the metabolism and mineralization function of bacteria form a complete coordinated closed loop, realizing the integrated repair path of concentration, release, degradation and isolation [4,10].

5.4. Shortcomings and technical difficulties of ternary synergy system

Although the advantages of ternary synergy system are significant, there are few studies, mainly due to the following reasons: ternary system research started late, and it needs to be established on the basis of mature single and binary systems. Secondly, the system is not a single addition of three elements, involving nonlinear coupling of multiple processes. The existing detection technology has limited ability to capture microscopic dynamic processes and is difficult to analyze. At the same time, multi-dimensional parameter combination leads to complex experimental design and regulation, high cost and long cycle. In addition, the ternary system lacks multidisciplinary technology platform and interdisciplinary collaboration team, and lacks financial and technical support [16,21,22]. There are still many deficiencies in research and application at this stage. Synthetic microbiome is vulnerable to environmental heterogeneity and the coexistence of multiple pollutants, and its function is attenuated. The carrier stability defect of genetic modification technology further reduces the environmental adaptation ability; The release of exogenous microorganisms may cause horizontal gene transfer, niche competition and other problems, threatening the stability of native ecosystems; the degradation process lacks effective real-time monitoring means, and the accumulation risk of toxic metabolic intermediates is difficult to control; the treatment capacity of some refractory pollutants is insufficient, and it is difficult to adapt to complex pollution scenarios [16,21]. At the technical level, the rational design and construction of synthetic microbiome lacks systematic theoretical support, relies on empirical trial and error, and the automation and high-throughput of degradation element creation have not been realized; the existing

DOI: 10.54254/2753-8818/2026.AU33550

optimization strategies focus on single feature improvement, and it is difficult to realize the balance between system stability and functionality and multi-scenario adaptation; the system research and development has a high demand for multidisciplinary cross integration, and the problem of stable function of artificial life in extreme environment has not been solved [21].

6. Conclusion

Biochar has dual functions of pollutant adsorption and microbial carrier, and can also regulate the microenvironment of the system and provide nutrient support; Biosurfactant in degradation agent improves bioavailability of petroleum hydrocarbon and activates active sites of biochar, nutrient provides continuous support for microbial metabolism, and the three jointly enhance remediation efficiency. The core of ternary system is the synergistic mechanism of adsorption, fixation, activation and degradation. The three are coupled to realize synergistic regulation of remediation cycle and biochar functional life, significantly improving remediation efficiency and system stability. However, at present, the system still has some deficiencies such as limited environmental adaptability, urgent control of biosafety risks, lack of real-time monitoring means, etc. Meanwhile, it faces technical difficulties such as rational design of synthetic microbiome, balance of system stability and functionality, and interdisciplinary integration. Follow-up research should focus on the above-mentioned deficiencies and difficulties, deeply explore the interface behavior and dynamic response mechanism of each component in complex environment, strengthen the rational design research of synthetic microbiome, construct efficient monitoring and risk control system, improve the environmental adaptability and long-term stability of the system, and promote its engineering practical application.

Future ternary collaborative systems will integrate genetic engineering and artificial intelligence to enhance functional strengthening and intelligent regulation. By optimizing microbial strains and leveraging machine learning for real-time monitoring, this integration will drive oil spill remediation toward large-scale, precise, and engineered applications.

References

[1] Jiang, M. H. (2025). Preparation and performance study of immobilization carriers for petroleum-degrading bacteria (Master's thesis, Chongqing University of Technology).
[2] Bootorabi, F., Haapasalo, J., Smith, E., Haapasalo, H. and Parkkila, S. (2011) Carbonic Anhydrase VII—A Potential Prognostic Marker in Gliomas. Health, 3, 6-12.
[3] Ma, L. L. (2017). Study on biochar-based immobilized microorganisms and remediation of petroleum contaminated soil, Southwest Petroleum University.
[4] Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., ... & Ok, Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere, 99, 19-33.
[5] Dou, J. B. (2024). New mechanism and directional regulation of organic pollutants degradation in biochar activated persulfate system, Zhejiang University.
[6] Chen, H. Y. (2025). Study on the remediation of petroleum hydrocarbon pollution by iron-activated biochar immobilized petroleum hydrocarbon degrading bacteria, Chongqing University of Technology.
[7] Ma, X. W., Li, H. H., & Wei, Z. H. (2025). Construction of petroleum-degrading complex flora and optimization of biochar immobilization conditions. Acta Petrolei Sinica (Petroleum Processing Section), 41(5), 1430-1441.
[8] Chi, J. G., Lv, J. L., Gao, Y. C., Wang, J. N., Zhang, W., Zhang, Q., & Kong, X. (2015). Removal of oil from oily seawater by biochar immobilized microbial agents. Industrial Safety and Environmental Protection, 41(6), 13-16+29.
[9] Li, B. H., Huang, J. H., & Ma, Q. (2025). Research status of microbial flora degrading wastewater containing petroleum hydrocarbon pollution. Acta Microbiologica Sinica, 65(6), 2353-2364.
[10] Hua, T., Li, S. N., Di, Z. H., Zhou, B., Zeng, W. L., Zhou, Q. X., & Li, F. X. (2018). Research progress on the mechanism of microbial degradation of petroleum pollutants. Biotechnology Bulletin, 34(10), 26-34.

DOI: 10.54254/2753-8818/2026.AU33550

[11] Xu, H. (2013). Preparation of comprehensive bioremediation agent for sea surface oil spill and evaluation of its strengthening effect, Ocean University of China.
[12] Mao, M. (2022). Study on the effect of surfactant enhanced remediation of petroleum hydrocarbon contaminated aquifer, Chang'an University.
[13] Xiao, P. (2023). Study on surfactant enhanced microbial remediation of petroleum contaminated soil, Southwest Petroleum University.
[14] Ye, Z. C., Su, Y. F., & Yang, Y. F. (2024). Application of microbial remediation technology based on molecular biology in petroleum contaminated environment. Chinese Journal of Biotechnology, 40(3), 739-757.
[15] Zhang, J., Wang, J., & Yu, Y. (2022). Research progress of biochar in remediation of soil contaminated by petroleum hydrocarbons and polycyclic aromatic hydrocarbons. Journal of Guilin University of Technology, 42(1), 183-191.
[16] Pu, W. H., Zhou, L. X., Yang, F., & Yang, C. Z. (2005). Research progress of offshore oil spill prevention and control technology. Marine Sciences, (6), 73-76.
[17] Lan, M. C., Yang, M. Q., Hu, H. Y., Gao, H. J., & Tang, H. Z. (2025). Application of synthetic biology in pollution remediation of petrochemical industry. Chinese Journal of Biotechnology, 41(11), 4321-4336.
[18] Zhang, D. Y., Liu, Z. Q., Yao, L. Y., Fan, X. L., & Gao, J. (2025). Key mechanism and application prospect of microbial induced calcium carbonate precipitation biochar combined technology for remediation of farmland contaminated soil. Journal of Jilin University (Earth Science Edition), 55(6), 1958-1980.
[19] Jiang, F., Lin, Z. Y., Luo, Y. Y., Zhou, M. T., Xiao, R. B., Wang, P., & Huang, F. (2025). Research progress on the influence mechanism of biochar and microorganisms on the morphological transformation of heavy metals in farmland soil. Guangdong Agricultural Sciences, 52(2), 44-57.
[20] Shen, L. L. (2025). Preparation of porous biochar immobilized biological agents and its degradation mechanism of benzopyrene in soil (Master's thesis, Guangdong University of Technology).
[21] Tan, C. L., Liu, Y., Huang, X. G., Zhang, J. Y., & Luo, W. H. (2022). Effects of biochar on soil microbial metabolic activities. Chinese Journal of Eco-Agriculture (Middle and English), 30(3), 333-342.
[22] Lin, X. Y., Li, C. R., Xu, X. H., & Jiang, J. D. (2025). Synthetic microbiome design: From theoretical construction to applications and challenges in environmental bioremediation. Chinese Journal of Biotechnology, 41(11), 4298-4320.