**Addition of biochar significantly promoted the biodegradation of phenol by phenol-degrading bacteria and its metabolomics analysis**

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**Abstract:** The use of biochar to assist in microbial degradation is seen as a key strategy for eliminating organic pollutants. However, the specific ways in which biochar aids this process are not fully understood. This study examined how biochar affects the degradation of phenol by phenol-degrading bacteria (PDB). The findings indicate that when PDB was combined with biochar, phenol removal reached 82.4% at a concentration of 1300 mg/L, surpassing 41.6% phenol removal ratio from single microorgranism treatment. Microbial community analysis indicated that there was a notable increase in *Gammaproteobacteria, Serratia*, *Raitonia* and *Enterobacter* in the biochar-assisted sample compared to those in the sample without biochar. The pathway of phenol degradation was confirmed via GC-MS analysis and metabolomic analysis, the findings revealed that the phenol degradation pathway used by PDB was an ortho-cleavage pathway. PICRUSt2 analysis suggested that the addition of biochar might enhance PDB metabolism by modulatiing the degradation of inprotocatechuate, toluene and phenylacetate. Metabolomic analysis indicated that biochar demonstrated potential to augment PDB biodegradation by promoting Oxygen-driven metabolism and fueling bacterial cell energy needs. This study sheds light on how biochar affects PDB biochemical breakdown and offers useful information for the potential use of biochar-supported microbial technology in phenol degradation.

**Keywords**: phenol; biochar; biodegradation; microbial community; metabolomics

1. **Introduction**

Phenol is a significant organic pollutant commonly present in industrial wastewater. It emerges from multiple industrial activities,examples like fabric manufacturing, coal transformation, oil refining, coking operations, production of leather, resin production, coconut crafting, perfume production, and drug manufacturing (Arya et al., 2011) (Jiang et al., 2015) (Dong et al., 2024) (Liu et al., 2024). Owing to its harmful properties and long-lasting nature, the phenol removal has garnered global attention (Jiang et al., 2015). Existing approaches for treating phenol wastewater, including both physical and chemical approaches, are costly and result in the production of additional pollutants.(Dong et al., 2024). Compared with these methods, biological processes are a promising avenue for treating phenol-containing wastewater due to their high efficiency, energy savings, low cost, and minimal environmental impact. Nonetheless, the high toxicity of phenol inhibits the growth of microorganisms, posing challenges in employing separate microbiological methods for its treatment (Dong et al., 2024).

Many previous studies have shown that microorganisms have potential to degrade phenol, including for example *Pseudomonas.*sp.*.*(Filipowicz et al., 2020; Li et al., 2021; Tyagi et al., 2024; Zhao et al., 2020), *Bacillus* sp.(Arutchelvan et al., 2005), *Yeast trichosporon* (Alexieva et al., 2008; Liu et al., 2011; Park et al., 2009), filamentous fungi(Santos and Linardi, 2004), *Micromycetes*, denitrifying bacteria, [*Scenedesmus*](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/scenedesmus), and [*Chlorella*](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/chlorella)(Kong et al., 2019; Xiao et al., 2019), among others. However, the biodegradation efficiency of phenol-degrading bacteria is lower than expected in practical applications, possibly due to inadequate viability and bioactivity when faced with adverse environmental conditions. To address this issue, researchers have proposed the combination of biochar with bioremediation methodologies (Liu et al., 2024; Liu et al., 2021; Nie et al., 2021) Biochar, known for its porous sarrangement and strong environmental resilience, can serve as the microbial habitat and provide nutrient elements to microorganism (Qi et al., 2021). In the study conducted by Lin et al (Lin and Cheng, 2020) in batch and continuous flow bioreactors, the removal rates of phenol degradation ranged from 59.3% to 92% for free cells and from 87.5% to 92% for immobilized cells. Sahand110 which is a novel strain of phenol-degrading bacteria, isolated from coking effluent demonstrated the capability to withstand and fully breakdown elevated phenol concentrations of as high as 600 mg/L within 72 hours under optimal thermal conditions and acidity level (Shahabivand et al., 2022). Biochar has been reported to promote the production of extracellular polymers (EPSs), which could affect the biodegration of bacteria(Shi et al., 2019). Furthermore, microorganisms usually degrade phenol through their metabolism, so the changes in microbial metabolomics induced by biochar play a crucial role within this procedure. PICRUSt2 analysis suggested that the addition of biochar might enhance phenol-degrading bacteria (PDB) metabolism by modulatiing the degradation of inprotocatechuate,toluene and phenylacetate. Metabolomic analysis indicated that biochar demonstrated potential to augment PDB biodegradation by promoting Oxygen-driven metabolism and fueling bacterial cell energy needs.

The objective of this study was to investigate the degradation pathways of phenol by PDB and the biodegradation mechanisms influenced by biochar. Microbiological and metabolomic analyses were subsequently conducted to evaluate the effects of PDB biodegradation.

1. **Materials and methods**
   1. Biochar adsorption experiment

The adsorption performance of biochar prepared using different methods was tested in conical flasks for phenol adsorption. A phenol solution was mixed with biochar at a concentration of 0.6% (w/v) in an oscillating incubator set to a temperature of 30 ◦C and a shaking speed of 120 rpm. Samples were collected every 8 h, and the mixture was shaken at room temperature for 72 h to achieve adsorption equilibrium.

* 1. Phenol biodegradation process by PDB

PDB was isolated from the the aerobic sludge of a wastewater treatment plant (Shanxi, China) in our previous work (Dong et al., 2024). The strain was cultured in basic medium (BM) ((NH4)2SO4 0.472 g/L, MgSO4⋅7 H2O 0.05 g/L, K2HPO4 0.2 g/L, MnSO4⋅4 H2O 0.01 g/L, FeSO4 0.01 g/L, and NaCl 0.12 g/L). Firstly, PDB was activated in BM for 24 h at 30°C with shaking at 120 r/min. Then 0.5 % volume of the activated medium was transferred into 100 mL BM and cultured to the logarithmic growth phase. The removal of phenol was studied across different initial levels, spanning concentrations from 300 to 1700 mg/L, with the inclusion of 5% (w/v) the above bacterial solution inoculation, with or without the presence of biochar (6% w/v). The solutions were cultured at 30 ℃ and 160 rpm for 72 hours, after which the samples were collected at 8-hour intervals. All experiments were performed three times. Subsequently, the phenol levels of the samples were determined via high-performance liquid chromatography (HPLC) with Waters Corpration (USA) (Dong et al., 2024).

* 1. Examining the structure of the microbial community

To investigate the composition of the microbial community, the bacterial suspension collected were subjected to high throughput sequencing analyses, after the reaction (72 h), the bacterial suspension spun at 10000 rpm for 5 minutes to obtain the target bacteria in triplicate, and Beijing Novozymes Technology Co., Ltd, was used to analyze the microbial diversity through the Illumina Miseq sequencing platform.

* 1. GC-MS analysis

The intermediate products of phenol degradation were identified through GC-MS analysis using GC 6890N and MSD 5975C (Agilent Technologies, USA). After 48 hours, the sample was removed from the minimal Salt medium (MSM) and centrifuged at 10000 revolutions per minute at 4 ℃ for 15 minutes. The supernatant was extracted three times with an equal volume of ethyl acetate (analytical grade), dried over Na2SO4 (>99.5% purity), and concentrated to 0.5 mL using a rotary evaporator. Pretreated samples (1 μL) were analyzed by GC-MS with pure helium (99.999%) used as the carrier gas at a constant flow rate of 1.0 mL/min. The samples were separated on a DB-5MS column (30 m\*0.25 mm, 0.25 μm，Agilent Technologies) using programmedprogrammed to increase from 60 °C (1 min) to 200 °C (5 min) at a rate of 5 °C/min, and then increase to 280 ℃ (5 min) at a rate of 5 °C/min and the electron energy were set to 70 eV.

* 1. Metabolomics

Samples (1 mL) were extracted from the MSM for metabolic analysis at 72 h. Beijing Novozymes Technology Co., Ltd. conducted the metabolite analysis. Metabolites were extracted from bacterial cells using a solution containing methanol and chloroform. The analyzed sample extracts were scrutinized using a Vanquish UHPLC system (Thermo Fisher, Germany) connected to either an Orbitrap Q ExactiveTM HF or Orbitrap Q ExactiveTM HF-X mass spectrometer (Thermo Fisher, Germany) at Novogene Co., Ltd. (Beijing, China). The metabolites were annotated using the NIST database available at <https://webbook.nist.gov/chemistry/.> Partial least squares discriminatant analysis (PLS-DA) was conducted using MetaboAnalyst 4.0 to discern the overall metabolic profile distinctions among groups. The VIP value and P value (T test) from the PLS-DA model were used as criteria for determining the metabolites exhibiting differential expression (VIP value > 1 and P value < 0.05). The metabolites showing differential expression were next compared to the Kyoto Encyclopedia of Genes and Genomes (KEGG) to detabase to determine their associated metabolic pathways.

1. **Results and discussion**
   1. Removal rate of phenol by biochar

Based on the outcomes of the adsorption experiments, a phenol background solution with an initial concentration of 1300 mg/L was selected to explore the adsorption process of phenol onto biochar prepared at various pyrolysis temperatures. The results are depicted in Fig S1. After a 32-hour adsorption period, the phenol concentration in each experimental group progressively stabilized, signifying that the adsorption process in the biochar had reached an equilibrium state. Analyzing the data from the figure reveals that the removal efficiencies for phenol by biochars pyrolyzed at 600°C, 800°C, and 1000°C (D-BC600, D-BC800, and D-BC1000, respectively) were 12.4%, 15.9%, and 18.5%, respectively, under the same high phenol concentration conditions. The phenol adsorption by biochar exhibited a biphasic pattern, characterized by an initial rapid adsorption phase followed by a gradual transition to a slower adsorption rate, ultimately leading to equilibrium.

* 1. Enhancement of Phenol Biodegradation by Biochar

Fig S2 shows how well PDB removes phenol at levels ranging from 100 mg/L to 1200 mg/L. Efficient removal was attained solely at lower phenol concentrations, specifically 100 and 200 mg/L, resulting in removal efficiencies of 100% and 80%, respectively. When the concentration of phenol was relatively high (400 and 800 mg/L), the efficiency decreased to 20-40%. When the concentration of phenol reached 1700 and 1900 mg / L, the bacteria could not survive. At high concentrations (as depicted in Fig S2), the initiation of phenol breakdown was significantly delayed. The growth of the strain under different initial phenol concentrations was investigated. As described in Fig S3, the strains exhibited the fastest growth at a low phenol level of 300 mg/L. However, the eventual bacterial density (OD600 value of 0.307) was notably less than that observed in the system with a phenol concentration of 700 mg/L, yielding an OD600 value of 0.491. This observation suggested that a low levels of phenol did not supply a sufficient carbon source, which the strains quickly utilized.

The effect of phenol level on microorganisms has been extensively documented. Ling Zhao et al. (Zhao et al., 2020) discovered that successful elimination of *Pseudomonas citronellolis* occured solely at phenol levels of 100 and 200 mg/L, resulting in removal rates of 100% and 80%, respectively. Nevertheless, at higher phenol levels (400 and 800 mg/L), the removal rate decreased to only 20-40%, and the microorganisms did not survive at levels of 1000 and 1200 mg/L. The recently identified strain Rhodococcus pyridinivorans PDB9T N-1, as reported by Manas Barik et al. (Barik et al., 2021), could fully degrade phenol at concentrations exceeding 1600 mg/L in a brief period. Aneela Iqbal et al. (Iqbal et al., 2017) reported that *Achromobacter* sp. (AIEB-7), *Pseudomonas* sp. (AIEB-4), and *Alcaligenes* sp. (AIEB-6) could effectively biodegrade phenol at concentrations of 250, 500, and 750 mg/L, respectively. Nevertheless, the degradation rates decreased to only 81%, 72%, and 69%, respectively, upon exposure to 1000 mg/L. Additionally, *Bacillus* sp. (AIEB-1), *Enterobacter sp.* (AIEB-3), and *Acinetobacter sp.* (AIEB-2) showed significant degradation of benzene at levels of 250, 500, and 750 mg/L, respectively.

The addition of biochar to the bacterium significantly enhanced the removal of phenol (Fig 1). At a phenol level of 1300 mg/L (Fig 1), the promotion effect of D-BC600 was significantly less pronounced than that of to D-BC800 and D-BC1000. Specifically, at this concentration, the phenol removal rates were 82.4% for D-BC800 and 75.9% for D-BC1000.

* 1. Bacterial community structure

We conducted a 16S rRNA metagenomics analysis to explore how the addition of biochar affects the bacterial community. The operational taxonomic unit (OTU) was employed to categorize variety within microbial communities using 16S rRNA sequence similarity. We identified 11 OTUs common to both groups (PDB and DBC). Alpha diversity analysis assessed the variety of species complexity in each sample using multiple indices, including the observed species and Chao1, Shannon and Simpson indices. The leveling off of the rarefaction curves suggested adequate sequencing data coverage for all bacterial species within the community (Fig. 2a). A notable disparity in species richness was observed between microbial communities with and without biochar addition (Fig. 2b). Comparative analysis of microbial communities with and without biochar addition revealed a notable increase in the class *Gammaproteobacteria* added biochar (Fig. 2c). The rise in *Gammaproteobacteria* communities was mainly attributed to an increase in the communities belonging to the phylum Proteobacteria (Fig. 2d). *Proteobacteria* is the dominant phylum in pharmaceuticals, oil refineries, coking wastewater treatment facilities (WWTPs) and sewers (Bai et al., 2011; Zhu et al., 2012). *Gammaproteobacteria* show great adaptability in degrading pollutants and are found in different biotreatment systems, including wastewater containing phenol, household wastewater and wastewater generated from coking processes (Dosta et al., 2011; Jiang et al., 2016; Tian et al., 2020). Liu et al. (Liu et al., 2016) effectively isolated a strain (PA) with the ability to degrade pheonl from industrial wastewater originating from petrochemical processes. Analysis of the 16S rDNA sequence indicated that this strain belongs to *Acinetobacter calcoaceticus* within the *Gammaproteobacteria* group. The strain efficiently removed 91.6% of the initial 800 mg/L phenol within 48 hours and demonstrated resilience to phenol levels as high as 1700 mg/L. These results suggest that *Gammaproteobacteria* have promising potential for treating phenolic wastewater.

At the genus level, a significant enrichment of *Serratia*, *Raitonia* and *Enterobacter* was observed in the biochar-amended sample compared to the sample without biochar (Fig. 2e). *Ralstonia* and *Enterobacter* took over as the predominant genera, replacing *Bacillus*. These findings suggest that the addition of biochar caused a change within the microbial communities, especially the primary ones, which could impact the functions of the system. Prior research has spotlighted *Bacillus* as possessing robust phenol tolerance and exclusive carbon usage. Radulovic (Radulovic et al., 2018) found *Serratia* and *Bacillus* to be strains that are tolerant to phenol in the area around the roots of floating duckweed and capable of withstanding phenol levels of at a minimum concentration of 500 mg/L. Notably, *Serratia* was identified as the predominant strain associated with phenolremoval. In addition, Ke (Ke et al., 2018) demonstrated the effective breakdown of phenol and its ability to be reused by fixing *Bacillus* sp. SAS19 on porous carbon gels. Ralstonia (Jamshidian et al., 2013) showed the ability to break down phenol and p-nitrophenol resulting in phenol breakdown up to 1000 mg/L. From a polluted soil, Nilotpala Pradhan (Pradhan and Ingle, 2007) decomposed phenol as the exclusive origin of carbon and energy, enduring and breaking down phenol at levels as high as 1050 mg/L. Furthermore, *Serratia* sp. LJ-1 screened by Lu et al. (Lu et al., 2014) degraded both phenol and ammonium. Additionally, *Enterobacter* was observed to have a notable association with phenol breakdown (Huang et al., 2016). These findings imply that several functional microorganisms might work together to achieve efficient phenol elimination.

The phylogenetic study of communities was performed through the estimation of unobserved states (PICRUSt2) analysis to predict how the observed changes in the microbiome following biochar addition might affect microbial functions. The analysis revealed significant functional alterations in the pathways related to protocatechuate degradation II (ortho-cleavage pathway) (P = 0.007), phenylacetate degradation I (aerobic) (P = 0.003), and toluene degradation III (aerobic) (via p-cresol) (P = 0.038) (Fig S5). The degradation of protocatechuate II through the ortho-cleavage pathway is an essential biological process in phenol degradation. Numerous studies have demonstrated that microorganisms utilize orthogonal cleavage for phenol degradation. Nalia Filowicz (Filipowicz et al., 2020) determined the metabolic pathway for phenol degradation by *Candida subhashii* (strain A01), *Candida oregonenis* (strain B02), and *Schizoblastosporion starkeyi-henricii* (strain L01) to involve the orthogonal cleavage, which was confirmed through an enzymatic assay. Enzyme activities and the genes encoding enzymes involved in phenol breakdown were examined through whole-genome sequencing (WGS) to determine the phenol degradation of *Arthrobacter* sp. strains AQ5-05 and AQ5-06, which were first found in Antarctica. Both strains were found to possess all the necessary genes necessary for phenol degradation, specifically those involved in ortho-cleavage. Validation was carried out through enzymatic assays for catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, revealing the presence of only catechol 1,2-dioxygenase in both strains, matching the WGS findings (Lee et al., 2022). Zhang et al. (Zhang et al., 2023) analyzed the enzyme activities and catA gene expression and suggested that SAS26 first metabolized phenol into catechol using phenol hydroxylase, followed by its oxidation through the ortho-cleavage pathway catalyzed with the help of catechol 1,2-dioxygenase. Overll, microbial community succession caused by modified biochar addition may contribute to changes in phenol metabolism.

* 1. Identification of the phenol breakdown intermediate

To identify the intermediates generated by the degradation of phenol in the pure PDB system and the biochar-immobilized PDB system, qualitative analysis was conducted using GC-MS. By analyzing the mass spectrometry standard libraries were compared, a preliminary structural analysis of the unknown compounds was performed.

The metabolites identified through GC-MS measurements are illustrated in Fig. 3a and 3b. The components of the pure PDB-degraded phenol system included acetaldehyde, ethyl acetate, ethyl propionate, 4-methyl-2-pentanone, phenol, 1,2-cyclohexanedio, n-hexadecane, 2,4-dimethyl-heptane, 7-tetradecene, and 9-hexadecenoic acid. In the biochar-immobilized PDB-degraded phenol system, the detected compounds included acetaldehyde, ethyl acetate, ethyl propionate, isopropyl acetate, 4-methyl-2-pentanone, phenol, n-hexadecane, and 2,4-dimethyl-heptane. An MS diagram of the intermediates is shown in Fig. 4. The results demonstrated that catechol was fully degraded into acetaldehyde, 2,4-dimethylheptane, 4-methyl-2-pentanone and other chain-like small molecules through ring-opening degradation catalyzed by enzymes such as orthocyclic lyase.

Previous studiess have demonstrated that the microbial decomposition of phenol occurs in three stages. Initially, phenol is transformed into catechol through the catalysis by the phenol hydroxylase gene. Afterward, catechol undergoes ring-opening cleavage to form chain-like small molecules. The third stage results in the production of water and carbon dioxide. Although, the products of the first stage were not detected in this study, but the chain-like small molecules from the second stage were identified.

* 1. Metabolomic analysis of phenol degradation by PDB with biochar

According to Section 3.4, the addition of biochar affects phenol degradation by PDB, with changes in the microbial community structure. To investigate the underlying mechanism, nontargeted metabolic techniques were employed to compare the pathways for phenol degradation in samples with and without biochar. A total of 162 distinct annotated metabolites were identified in the two sample categories. These included 45 lipids and lipid-like molecules, 25 organic acids and derivatives, 17 phenylpropanoids and polyketides, 16 organoheterocyclic compounds, 15 benzenoids, 9 organic oxygen compounds, 9 nucleosides, nucleotides, and analogues, 1 organic nitrogen compound, and 1 organosulfur compound (Fig. 5a).

Multivariate statistical analyses were initially applied to metabolites data. In the present study, the PCA results (Fig. 5b) showed clear separation indicating, which indicated that the metabolites with addition of biochar to the metabolites significantly altered the metabolites in the samples, consistent with the GC-MS results. PC1 explained 55.91% of the variance in the original dataset. PC2 accounts for 9.25% of the variance in the original datase. PLS-DA (Fig. 5c and 5d) is a multivariate statistical method with supervised pattern recognition that is effective in removing study-irrelevant effects and thereby identifying differentially abundant metabolites. In this study, Q2 was 0.95, suggesting an appropriate model fit. The PLS-DA score plot revealed important differences between the two sets.

PLS-DA and PCA were carried out on the metabolites to entry the metabolic differences caused by adding biochar during the breakdown process of PDB. Further screening for differentially abundant metabolites was conducted using FC and VIP, with the main metabolites selected based on VIP > 1.0, FC > 1.5 or FC < 0.667 and a P value < 0.05 selected for further screening (Haspel et al., 2014; Heischmann et al., 2016; Sreekumar et al., 2013). The findings, as shown in Fig. 5b and 5c, showed a distinct division of the samples into two clusters, indicating that the addition of biochar changed the bacterial metabolite profile. Subsequently, 81 compounds with noticeable statistical variation were also identified. These metabolites with altered expression were standardized, hierarchically grouped, and depicted in a heatmap (Fig. 6), with 60 metabolites showing decreased expression and 21 metabolites showing increased expression (Fig S4). The prevailing upregulation implies that the addition of biochar stimulated bacterial. metabolism.

KEGG pathway analysis indicated that the upregulated compounds were primarily associated with phenylalanine metabolism, biosynthesis of antibiotics, purine metabolism, glutathione metabolism, biosynthesis of amino acids, and arachidonic acid metabolism (Fig. 7). These pathways are mainly linked to amino acid metabolism, which is crucial for numerous cellular physiological processes. Amino acid metabolism regulates the movement of substances across cellular membranes and aids in synthesizing and activating crucial cellular enzymes, thereby influencing the breakdown of organic contaminants (Qi et al., 2022). Several amino acids were upregulated, implicating diverse metabolic pathways, such as aminobenzoate degradation, glycine, serine and threonine metabolism, tryptophan metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis. Importantly, a notable rise in L-tryptophan was detected in the BC treatment group, indicating a potential increase in cell replication and the activity of PDB.

As can be seen in Section 3.4, GC-MC did not detect the products of the first stage, but the first products catechol and cis-muconic acid, were detected in the metabolomics analysis, which clarified the phenol degradation pathway as follows: phenol was transformed into catechol by 2-monooxygenase (NADH) P0/A0, and then ortho-cleavage of catechol to cis-muconic acid in the presence of catechol 1,2-dioxygenase and cis-muconic acid was further broken down to succinyl 3-oxoadipyl-CoA and succinyl-CoA, which finally entered the citric acid cycle. In 3.3, PICRUSt2 analysis predicted significant functional alterations in the pathways related to pro-catecholate degradation II (orthogonal cleavage pathway) (P = 0.007) following biochar addition (Fig S5). It was further demonstrated that PDB degraded catechols via the ortho-cleavage pathway and that the addition of biochar affected the breakdown of catechols, KEGG analysis revealed that the expression of cis-muconic acid, a differentially abundant metabolite, was downregulated, suggesting that the addition of biochar facilitated the phenol decomposition, promoting the breakdown of cis-succinic acid into the subsequent product.

1. **Conclusion**

The findings demonstrate that biochar significantly enhanced the phenol biodegradation of PDB, especially at phenol levels exceeding 1300 mg/L. Microbial community analysis suggested that the addition of biochar caused a change within the microbial communities. The phenol degradation pathway confirmed that phenol was first hydroxylated to a catechol. Then catechol split forming cis, cis-muconate, and then metabolized into succinyl-CoA and acetyl-CoA. Finally, it enteres the tricarboxylic acid cycle. PICRUSt2 analysis revealed that biochar addition may lead to promote the degradation of PDB metabolism by the pathways related to pro-catecholate degradation II. KEGG analysis reveals that biochar has the potential to promote amino acid metabolism, which is crucial for amino acid metabolism in cellular physiological processes. It may assist in the synthesis and activation of key cellular enzymes by regulating the transport of substances across cell membranes, thereby promoting the degradation of PDB. Notably, the level of L-tryptophan significantly increased in the biochar-treated group, a finding that suggests an enhancement in cellular replication and PDB activity.

**CRediT authorship contribution statement**

**Jing Dong:** Conceptualization, Methodology, Writing - review & editing. **Li Ren:** Data curation, Validation, Writing - original draft. **LingLi Xu:** Validation. **YuXiang Liu:** Writing - review & editing. **Ke Yuan:** Formal analysis.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Data availability**

Data will be made available on request.

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Fig. 1. Effect of biochar on phenol removal.



Fig. 2. Species dilution curve (a) Good’s coverage; (b) Chao1 and relative abundance of species in the top10 histogram; (c) class; (d) phylum; (e) genus.



Fig. 3. GC plots of the pure PDB system and biochar immobilized PDB system at 72 h; (a) GC diagram of the pure PDB product; (b)GC diagram of the biochar-immobilized PDB product.



Fig. 4. MS diagram and structure of the outputs of (a) ethyl propionate; (b) acetaldehyde; (c) 4-methyl-2-pentanone; (d) 1,2-cyclohexanedio, and (e) n-hexadecane; (f) 2,4-dimethyl-heptane; (g) 7-tetradecen; (h) 9-hexadecenoic acid; (i) Isopropyl acetat.



Fig. 5. Metabolite classification pie chart (a), PCA (b), and score plots of PLS-DA (c) for the different metabolites and (d) permutation test.



Fig. 6. Heatmap analysis of differentially expressed metabolites.



Fig. 7. KEGG pathway analysis of upregulated differentially expressed meetabolites.