

Article

Process Optimization for the Adsorption of Inhibitors in Corn Stover Prehydrolysate by Cow Manure Biochar for Lactic Acid Fermentation

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Abstract

The pretreatment of lignocellulosic biomass generates inhibitory compounds that severely limit the efficiency of subsequent enzymatic biocatalytic conversions during fermentation. Biochar can be used for inhibitor removal by adsorption, but its efficiency depends on tailored process conditions. In this study, the cow manure biochar (CMB) was applied in the detoxification of prehydrolysate generated from dilute acid pretreatment of corn stover, and the detoxification process was optimized by the response surface method (RSM). At the optimal detoxification condition (53 °C, 118 min, and the biochar loading of 4.5% *w/v*), the detoxified prehydrolysate achieved a lactic acid (LA) production of 42.89 g/L with an 85.67% yield, while a removal efficiency of 46.47% was obtained for the major inhibitors in the prehydrolysate. The reusability of CMB was investigated by water-washing, thermal, and NaOH regenerations. All methods obtained over 80% regeneration performance, and the lactic acid yield remained above 35 g/L after two regeneration cycles. CMB regenerated by water washing maintained 81.86% of its initial adsorption capacity after two cycles, achieving a lactic acid concentration of 36.83 g/L. These results suggested that water washing could serve as a simple and potentially sustainable regeneration approach for maintaining biochar performance in biocatalytic systems.

Keywords: biochar; prehydrolysate; adsorption; detoxification; fermentation; regeneration

1. Introduction

The recycling of agricultural waste has become an essential strategy for promoting sustainable development globally. Lignocellulosic agricultural wastes, such as corn stover and wheat straw, are abundant in cellulose and hemicellulose, along with other components [1]. The hydrolysis of cellulose yields glucose, while that of hemicellulose produces xylose. Consequently, they may serve as promising candidates to replace the current starch platform for conversion into valuable bioproducts [2,3]. However, the complex structure of lignocellulose and the resistance of cellulose are strengthened by its high degree of polymerization and crystallinity, making it challenging to obtain monosaccharides [4]. Thus, lignocellulose must first be pretreated to break its recalcitrance. However, the degradation products generated from carbohydrates and lignin during this process can be detrimental to subsequent enzymatic hydrolysis and enzymatic biocatalytic conversion, significantly limiting the efficient utilization of lignocellulose [5,6]. For instance, Zdarta et al. prepared



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prehydrolysate from birch via nanofiltration of sulphuric acid-hydrothermally pretreatment, in which the concentrations of formic acid, acetic acid, and furfural reached 0.92, 12.05, and 2.70 g/L, respectively [7]. Tan et al. prepared poplar powder prehydrolysate using 3% *w/w* H₂SO₄ at 170 °C, which contained 15.11 g/L acetic acid and up to 7.5 g/L total carbonyl inhibitors. Subsequent ethanol fermentation of the prehydrolysate showed that almost no ethanol was produced [8]. Therefore, detoxification of the prehydrolysate is necessary to enhance fermentation efficiency and product yield.

Because of special properties such as porous structure, numerous functional groups, and ample nutrients [9], biochar has recently emerged as a versatile material with wide-ranging applications, such as soil amelioration [10], enhancement of crop production [11], organic compounds adsorption [12], and heavy metal adsorption [13]. In the last few years, biochar has also been identified as a simple and effective method for detoxifying the inhibitors present in prehydrolysate. For instance, Do Nascimento et al. successfully removed 52% of furfural, 100% of hydroxymethyl furfural (HMF), and 40.4% of acetic acid from the acid prehydrolysate of sisal bagasse using biochar derived from the residues of açai endocarp gasification [14]. Monlau et al. reported that biochar prepared from digestates of a mixture of energy crops, crop residues, and manure was able to completely remove furfural from the dilute acid prehydrolysate of corn stover [15]. Shen et al. prepared corn stover biochar (CSB) for the removal of inhibitors from dilute acid prehydrolysate. The results showed that CSB detoxification reduced the number of compounds in the prehydrolysate by eight, with an overall inhibitor removal efficiency of 30.0% [16]. The efficiency of adsorption removal of inhibitors depends on the detoxification conditions, including temperature, time, pH, biochar loading, etc. Detoxification performed under optimized conditions could significantly improve the fermentability of prehydrolysate. The response surface methodology (RSM) is a commonly employed experimental and statistical approach used to determine the optimal conditions of a given process [17,18]. Deng et al. used acetic acid removal efficiency and sugar loss in dilute ammonia prehydrolysate of energy cane bagasse as detoxification indicators, and optimized the activated carbon dosage, pH, and contact time using RSM, aiming to maximize the removal of non-sugar compounds while minimizing the loss of fermentable sugars [19]. Sarawan et al. employed RSM to optimize the detoxification of acid-pretreated sorghum leaf prehydrolysate using activated charcoal, with optimal conditions of 134.73 min contact time, 25 °C, 5% *w/v* activated charcoal loading, and pH 3.76. Under the optimal conditions, activated charcoal detoxification significantly enhanced subsequent bioethanol production [18].

Given the promising potential of biochar for detoxification and other applications, its recycling and regeneration performance is a crucial indicator for assessing its effectiveness in practical scenarios. Biochar with strong reusability can maintain effective adsorption capacity after simple regeneration treatments. The reuse of biochar not only reduces the demand for new biochar but also contributes to environmental protection and decreases energy consumption [20]. Earlier studies have investigated the regeneration performance of biochar, with recyclability typically ranging from 50% to 90%, depending on the feedstock and regeneration conditions [21,22]. The technologies for reusing biochar primarily include thermal regeneration, chemical regeneration, and biological regeneration [23]. Among these, thermal and chemical regeneration are the more critical types of biochar regeneration. Ledesma et al. investigated the thermal regeneration of activated carbon saturated with p-nitrophenol and revealed that the thermal treatment proceeds through several complex stages [24]. Moreover, Jiang et al. demonstrated that nitrogen-doped biochar regenerated with NaOH exhibited superior recycling capability, with the Pb(II) removal efficiency remaining at 84% after five regeneration cycles [25]. However, these studies were typically conducted using single-component model pollutants and evaluated

regeneration performance primarily based on adsorption capacity. Research on the regeneration performance of biochar following prehydrolysate detoxification remains scarce. More importantly, recyclability has seldom been assessed based on lactic acid production from real corn stover prehydrolysate.

This study employed sustainable and eco-friendly cow manure biochar (CMB) to detoxify corn stover prehydrolysate and aimed to optimize the detoxification conditions using RSM to enhance its biocatalytic performance for lactic acid production. The CMB was regenerated through three methods (water-washing, thermal, and NaOH regeneration), and its recyclability was evaluated based on the fermentability of the detoxified prehydrolysate. By integrating the RSM-optimized detoxification process with multiple biochar regeneration strategies, this study linked biochar regeneration performance with lactic acid fermentation, using fermentation productivity rather than inhibitor concentration as the ultimate indicator to assess regeneration feasibility. Overall, this work provides a feasible approach for the utilization of lignocellulosic agricultural wastes.

2. Results and Discussion

2.1. Adsorption Kinetics

To investigate the adsorption behavior of inhibitors in the prehydrolysate onto CMB, adsorption kinetic experiments were conducted, and the experimental data were fitted using the pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models (Figure 1 and Table S1). The results indicated that the adsorption of inhibitors onto CMB was better described by the PSO model, suggesting that chemisorption dominated the adsorption process. Among the investigated inhibitors, furfural exhibited the highest adsorption capacity. According to the PSO fitting results, the equilibrium adsorption capacity of CMB for furfural reached 7.06 mg/g. The kinetic results indicated that the adsorption rate was highest within the initial 30 min, during which approximately 70% of the equilibrium adsorption capacity was achieved. Five inhibitors reached adsorption equilibrium at around 75 min, while all seven compounds achieved equilibrium within 120 min. These observations provided an appropriate time range for the subsequent optimization experiments.

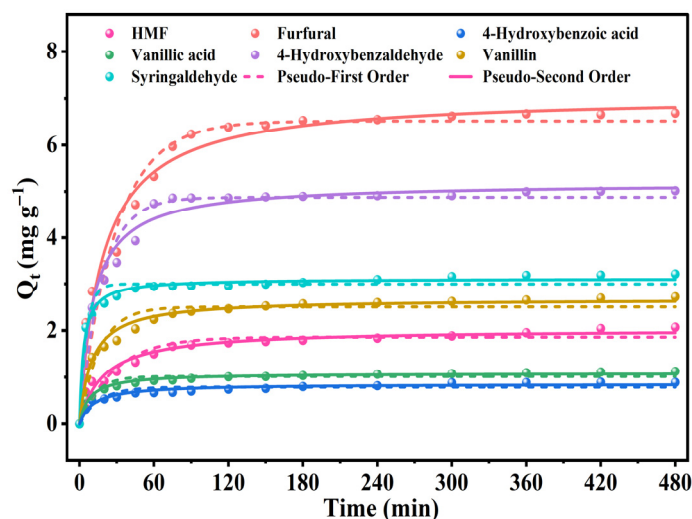


Figure 1. Adsorption kinetics of inhibitors by CMB.

2.2. Optimization by RSM

To maximize detoxification performance, three variables (temperature, detoxification time, and biochar loading) were systematically investigated using RSM. The experimental approach designed by BBD, including temperature (25, 40, 55 °C), detoxification time

(30, 60, 120 min), and biochar loading (1, 3, 5% *w/v*), generated 17 experimental sets. The analysis of the detoxification conditions for the prehydrolysate and LA fermentation results is shown in Table 1.

Table 1. Response surface test design and results.

Run	Temperature (°C)	Detoxification Time (min)	Biochar Loading (% <i>w/v</i>)	72 h LA Concentration (g/L)	Predicted LA Concentration (g/L)
1	55	60	5	42.25	42.75
2	25	60	5	33.57	32.08
3	55	30	3	22.16	20.57
4	40	60	3	18.71	18.81
5	40	120	5	41.32	41.39
6	40	30	1	7.78	7.39
7	40	60	3	19.35	18.81
8	25	120	3	16.64	17.57
9	40	60	3	19.18	18.81
10	25	60	1	8.47	7.97
11	55	120	3	28.16	27.76
12	55	60	1	12.45	13.94
13	40	30	5	29.43	30.35
14	40	60	3	19.25	18.81
15	25	30	3	12.12	13.18
16	40	120	1	8.51	7.92
17	40	60	3	17.55	18.81

The quadratic polynomial regression equation describing the relationship between LA concentration, temperature, detoxification time, and biochar loading was obtained from the response surface methodology data as follows:

$$Y = 19.98 + 4.39X_1 + 2.89X_2 + 14.11X_3 + 0.7018X_1X_2 + 1.18X_1X_3 + 2.63X_2X_3 + 1.69X_1^2 - 1.91X_2^2 + 3.68X_3^2 \quad (1)$$

where Y represents the response of LA concentration, X_1 , X_2 , and X_3 refer to the level of temperature, detoxification time, and biochar loading, respectively. The analysis reveals that the magnitude of coefficients associated with linear terms directly correlates with variable influence on LA concentration. According to this relationship, the relative effects of the independent variables on LA concentration were determined as follows: biochar loading shows the most significant influence ($\beta_{X_3} = 14.11$), followed by temperature ($\beta_{X_1} = 4.39$), while detoxification time exerts the weakest direct effect ($\beta_{X_2} = 2.89$). All interactive terms display positive coefficients, indicating synergistic effects between paired variables in enhancing LA concentration.

The analysis of variance (ANOVA) results for fitting the response surface models are shown in Table S2. The regression model F value is 107.20, and the *p* value is found to be below 0.0001, indicating that the regression model is highly significant. The lack-of-fit term is insignificant, which suggests that the model is statistically meaningful and could be used to predict LA production [26]. The coefficient of determination (R^2) is 99.28%, indicating that the quadratic regression equation fits the data very well and there is a significant linear relationship between the response value and the variables [27]. Variables with *p*-values below 0.05 within the 95% confidence level are considered statistically significant. Thus, X_1 , X_2 , X_3 , X_2X_3 , X_1^2 , X_2^2 , and X_3^2 in LA concentration are significant, as their *p*-values are below 0.05. The influence of different variables on LA fermentation is ranked as follows: biochar loading (X_3) has the most significant impact, followed by temperature (X_1), while detoxification time (X_2) has the least effect. The main influence chart for the variables affecting the LA concentration is shown in Figure S1. An increase in detoxification temperature

resulted in a significant improvement in LA concentration, while higher biochar loading also correlated with elevated LA levels. In contrast, longer detoxification time led to a slow increase in the production of LA. This conclusion is consistent with the quadratic polynomial regression equation and ANOVA results.

Figure S2 shows the actual and predicted values and the residuals versus the predicted plot for the LA concentration. Figure S2a visually represents the relationship between predicted and actual values. The adjusted coefficient of determination ($\text{adj. } R^2$) is 0.9835, and the predicted coefficient of determination ($\text{pre. } R^2$) is 0.9037 (Table S2). This suggests that the predicted LA concentrations are in good agreement with the experimental results, indicating a strong correlation between the predicted and actual values [28]. Figure S2b displays a residual vs. predicted plot showing a normal distribution with all data points falling within the expected range [29].

3D and 2D contour surface graphics are used to thoroughly evaluate the interaction between the studied variables (Figure 2). Figure 2a illustrates the interaction between the two variables, temperature and detoxification time, during detoxification. The lactic acid concentration demonstrated a pronounced positive correlation with elevated temperature. Moreover, LA concentration increased with time at lower temperatures of detoxification of prehydrolysate. In contrast, at higher temperatures of detoxification, LA concentration remained relatively stable over time. This suggested that biochar adsorption has reached saturation at a relatively higher temperature [30,31]. As shown in Figure 2b, increasing the temperature and the biochar loading created a greater possibility for the inhibitors to reach the surface of the biochar. Therefore, by increasing both the temperature and the biochar loading, the adsorption effect can be improved, thus resulting in higher concentrations of LA [29]. According to Figure 2c, Equation (1), and the ANOVA results, a clearer interaction was observed between the detoxification time and biochar loading variables, which significantly affected the LA concentration. In general, increasing the biochar loading led to a higher adsorption effect under the condition of fixed time; similarly, as the time increased under the condition of fixed biochar loading, the resulting LA concentrations were increased.

The Design Expert 11.1.2.0 statistical analysis software generated 100 potential solutions, from which the optimal conditions were selected based on a composite desirability value of 1.00. According to the aforementioned findings from the optimization model by RSM, 53 °C, 118 min, and 4.5% *w/v* biochar loading were identified as the optimum detoxification conditions (ODCs). Detoxification of the prehydrolysate was performed using CMB under these conditions, and the major inhibitors in the prehydrolysate were determined prior to and following detoxification (Figure 3a). After detoxification with CMB, the concentrations of main inhibitors in the prehydrolysate decreased, with furfural achieving the highest removal efficiency of 98.2% and an adsorption capacity of 3.49 mg/g; in contrast, acetic acid exhibited a much lower removal efficiency. Lee et al. further demonstrated that the adsorption performance of activated charcoal for furfural was significantly superior to that for aliphatic carboxylic acids, likely because activated charcoal preferentially adsorbs compounds with higher hydrophobicity [32]. The lactic acid fermentation performance before and after detoxification is shown in Figure 3b. The lactic acid concentration was only 7.63 g/L after 72 h of fermentation in the untreated (UT) group. With the detoxification temperature and biochar loading increased, the movement of inhibitors was enhanced, and biochar-inhibitor contact probability improved, thereby promoting adsorption [33]. The experiment achieved the highest LA concentration of 42.89 ± 0.18 g/L, giving a yield of 85.67% under the predicted optimum conditions (Figure 3b), closely aligning with the expected value of 42.35 g/L. This indicates that the optimization model is quite effective.

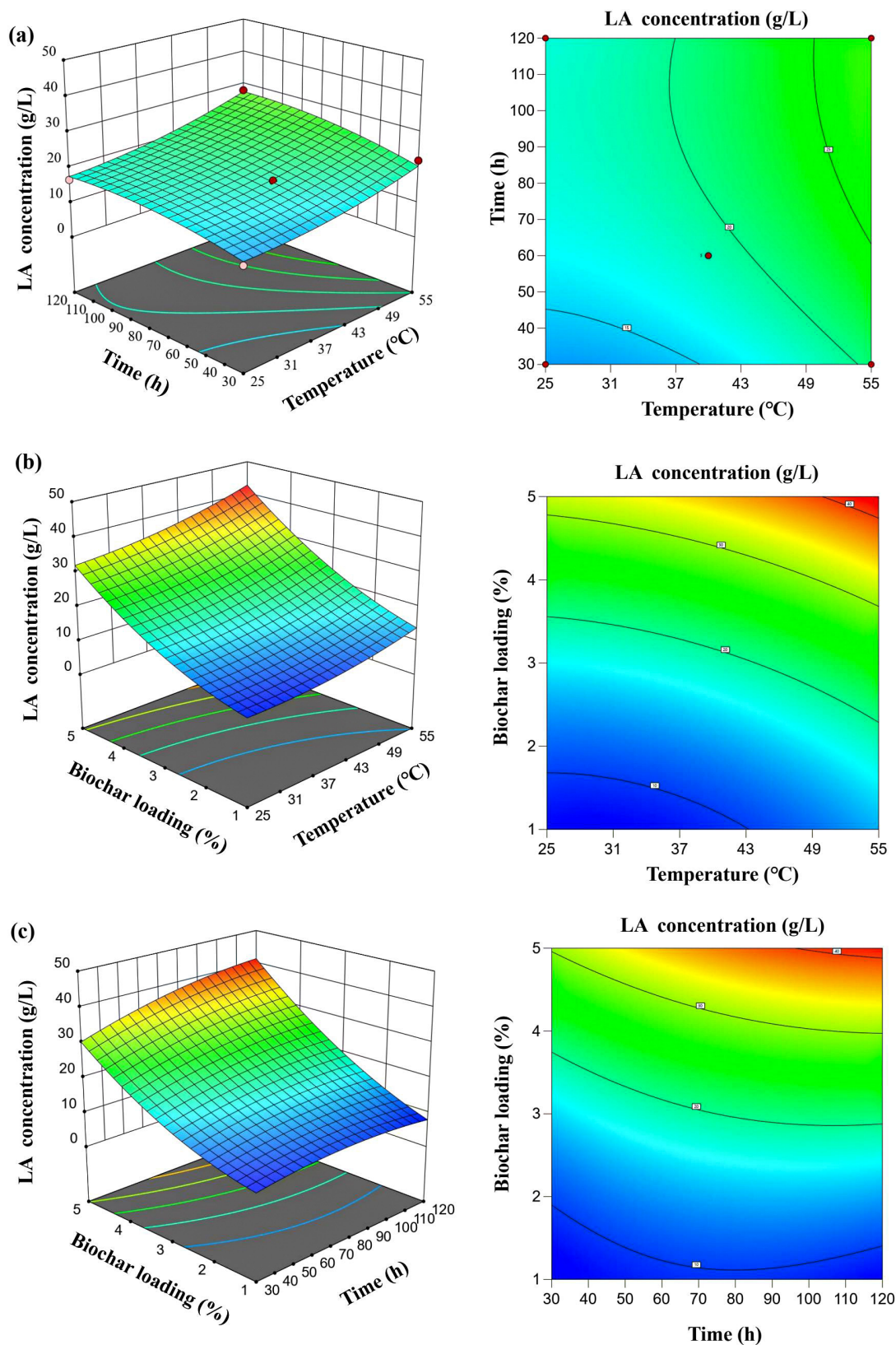


Figure 2. Interaction of three factors on the LA concentration: (a) temperature and detoxification time, (b) temperature and biochar loading, and (c) detoxification time and biochar loading.

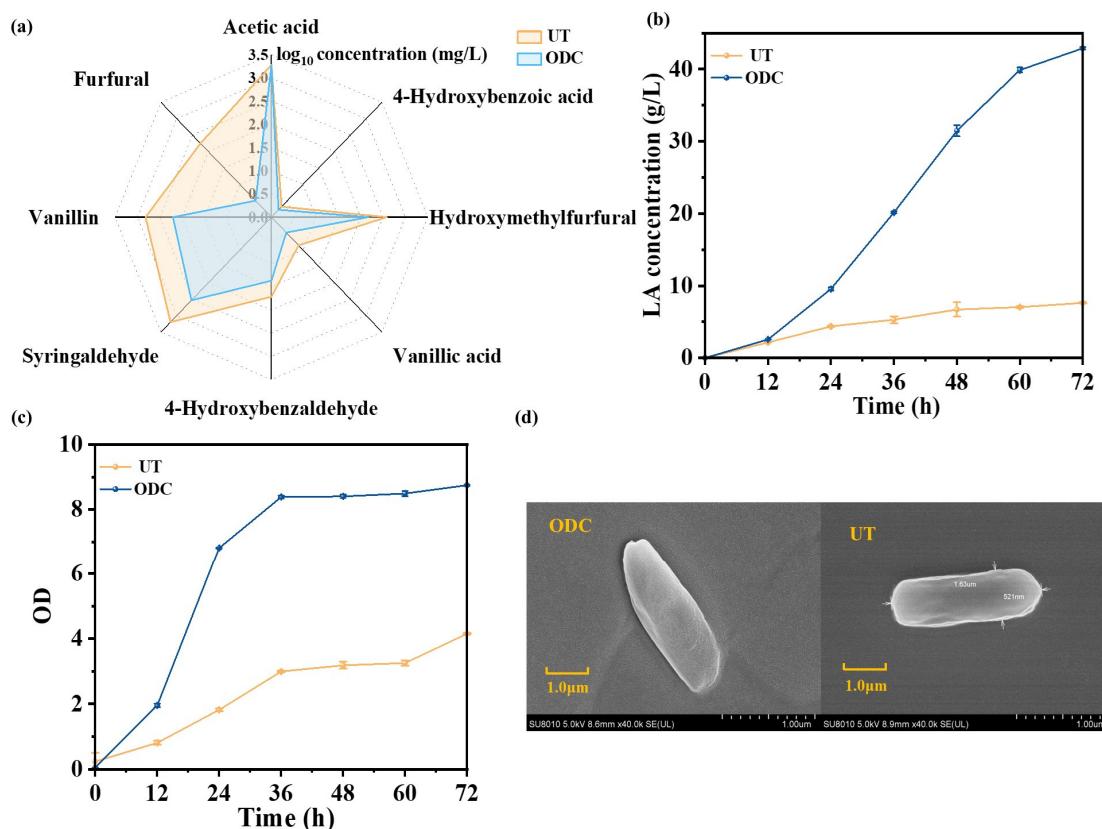


Figure 3. The detoxification and fermentation efficacy under the predicted optimum conditions. (a) The concentrations of inhibitors in the prehydrolysate. (b) Lactic acid production profiles during fermentation. (c) OD values during fermentation. (d) Scanning electron micrographs of cells.

Additionally, the OD values and microbial morphology during the fermentation process are presented in Figures 3c,d, respectively. The OD was 8.75 ± 0.02 at 72 h, performed at the optimal condition predicted by the model. The OD trend aligned with the lactic acid production profiles (Figure 3b). In the UT group, the lactic acid bacteria did not exhibit the typical straight-rod morphology; instead, their cell surfaces displayed fine corrugations, and the cells appeared slightly widened in an adaptive response to environmental stress. In contrast, the cells in the ODC group retained the standard rod shape, with smooth cell surfaces and a plump, intact morphology (Figure 3d). Glucose metabolism proceeded via the glycolytic (EMP) pathway. The effects of inhibitors on microbial metabolism were presented in Figure 4. Pretreatment-derived inhibitors infiltrated the intracellular environment by compromising the structural integrity of the biocatalyst membrane. These inhibitors triggered severe oxidative stress, leading to a substantial accumulation of reactive oxygen species (ROS) in the cytoplasm [34]. From a mechanistic perspective, excessive ROS accumulation not only caused oxidative damage to genomic DNA but also directly attacked the catalytically active sites of key enzymes involved in the glycolytic pathway [35], inducing conformational distortion of enzyme proteins and reducing their substrate affinity. Ultimately, this redox imbalance interrupted the electron transport chain, resulting in the complete inactivation of the biocatalyst [36]. Our previous studies further confirmed that inhibitors markedly elevated intracellular ROS levels, accompanied by a significant decrease in the activities of core biocatalytic enzymes, such as lactate dehydrogenase (LDH) [16].

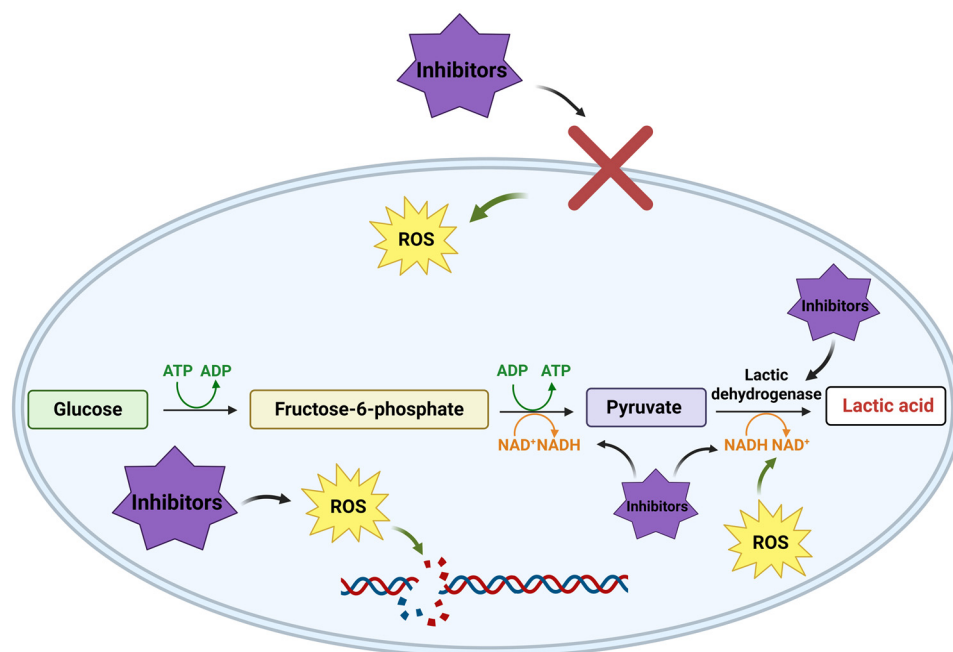


Figure 4. The effects of inhibitors on microbial metabolism.

2.3. Biochar Regeneration

Over the past few years, biochar has been extensively applied in prehydrolysate detoxification. However, once biochar reaches its adsorption capacity or its utilization is completed, it turns into solid waste and poses a risk of leaching organic contaminants, which may lead to secondary pollution. Therefore, the recycling performance of biochar is an important indicator for evaluating its practical application [37].

The elemental compositions of CMB and the regenerated biochars are shown in Table 2. After CMB adsorbed the inhibitors of prehydrolysate, its C and O content increased significantly. The inhibitors that were adsorbed on the surface of biochar altered its initial elemental composition. CMB-H₂O-I showed a significant recovery in C and O content, indicating that water-washing can remove some inhibitors from the biochar surface. However, there was no significant difference in the C content between CMB-H₂O-II and CMB-after, suggesting that CMB-H₂O-II was ineffective at removing inhibitors on the biochar surface. Additionally, repeated water washing leached substantial amounts of K, Ca, Mg, and Si from the biochar ash, resulting in an absolute decrease in ash mass, which in turn caused the fixed-carbon mass fraction to increase [38]. The increase in C content during thermal regeneration was attributed to the increase in the degree of aromatization and graphitization due to the thermal treatment. The increase in C content during thermal regeneration occurred because the thermal regeneration treatment enhanced the degree of aromatization and graphitization [39]. For both CMB-NaOH-I and CMB-NaOH-II, the C content decreased significantly yet remained higher than that of CMB-before, indicating that a portion of the inhibitors had been desorbed from the biochar surface. Similar phenomena were reported by Liu et al., who found that the carbon content of biochar regenerated with NaOH after ciprofloxacin adsorption was higher than that of the raw biochar. This observation suggested that a portion of the adsorbed compounds could be removed during NaOH regeneration, while some residues likely remained on the biochar surface due to relatively strong chemisorption and pore-filling effects [40]. After two rounds of recycling, the elemental composition of the biochar showed little change following either NaOH or thermal regeneration, indicating that both methods exhibited stable regeneration performance.

Table 2. The elemental components of regenerated CMB.

Sample	C %	H %	N %	S %	O %
CMB-before	38.89 ± 0.20	1.76 ± 0.12	1.82 ± 0.13	0.58 ± 0.002	10.49 ± 0.10
CMB-after	55.21 ± 0.24	2.87 ± 0.20	2.88 ± 0.22	0.56 ± 0.012	12.54 ± 0.22
CMB-H ₂ O-I	47.94 ± 0.17	2.25 ± 0.24	2.32 ± 0.10	0.48 ± 0.008	10.49 ± 0.06
CMB-H ₂ O-II	53.10 ± 0.21	2.61 ± 0.09	2.69 ± 0.19	0.56 ± 0.010	11.37 ± 0.20
CMB-500-I	46.67 ± 0.10	1.92 ± 0.13	2.30 ± 0.18	0.49 ± 0.012	9.68 ± 0.11
CMB-500-II	47.19 ± 0.12	1.83 ± 0.18	2.28 ± 0.26	0.50 ± 0.006	8.09 ± 0.14
CMB-NaOH-I	43.39 ± 0.09	1.20 ± 0.06	1.33 ± 0.26	0.44 ± 0.011	8.61 ± 0.17
CMB-NaOH-II	45.49 ± 0.11	2.25 ± 0.26	2.47 ± 0.07	0.47 ± 0.013	7.92 ± 0.23

After regeneration, the specific surface area as well as the pore volume of the CMB increased, as listed in Table 3. It can be observed that the original pore structure of CMB could be effectively regenerated through two rounds of simple water washing. This indicated that water-washing regeneration cleaned the clogged pores, enlarged the pore sizes, and removed water-soluble salts [41]. Thermal regeneration (CMB-500-I) exhibited a tenfold enhancement in specific surface area (50.63 m²/g), accompanied by reduced pore diameter and expanded pore volume. CMB-500-II had a specific surface area of 35.52 m²/g, showing a slight reduction in pore diameter and volume compared to CMB-500-I. During the thermal regeneration, the adsorption sites of biochar were increased under high temperatures, while surface carbonization formed new pores, increasing biochar's surface area. In the process of thermal regeneration, the inhibitors were decomposed, the number of adsorption sites on the biochar increased at high temperatures, and surface carbonization created new pores, thereby enlarging the specific surface area. This indicated that thermal regeneration had an improved effect on the biochar's pore structure. The specific surface area of CMB-500-I and CMB-500-II was significantly higher than that of CMB-H₂O-I and CMB-H₂O-II. However, the average pore diameter had decreased compared to the CMB-H₂O-I. This indicated that thermal regeneration could further optimize the biochar's pore structure, with a noticeable increase in the number of micropores and mesopores. Chemical regeneration was also one of the most commonly used methods to regenerate adsorbent materials. CMB-NaOH-I and CMB-NaOH-II achieved 12.12 times and 5.84 times the surface area increments relative to the original CMB, respectively. This enhancement occurred because the NaOH solution could solubilize some inhibitors onto the biochar, alter its surface structure, and increase the specific surface area [33,42]. The specific surface area of CMB-NaOH-I was similar to that of 500-I but was significantly higher than that of CMB-H₂O-I. However, the specific surface area of CMB-NaOH-II was slightly lower than that of CMB-500-II. This suggested that as the number of regeneration cycles increases, the performance of the NaOH regeneration might not be as stable as that of thermal regeneration.

Table 3. Structural properties of regenerated CMB.

Sample	S _{BET} (m ² /g)	Average Pore Diameter (nm)	Average Pore Volume (cm ³ /g)
CMB-before	4.11 ± 0.21	3.212 ± 0.15	0.016 ± 0.001
CMB-after	1.82 ± 0.11	1.190 ± 0.02	0.011 ± 0.002
CMB-H ₂ O-I	6.50 ± 0.19	3.442 ± 0.11	0.025 ± 0.003
CMB-H ₂ O-II	4.28 ± 0.17	1.171 ± 0.09	0.019 ± 0.001
CMB-500-I	50.63 ± 0.41	1.193 ± 0.07	0.047 ± 0.006
CMB-500-II	35.52 ± 0.24	1.157 ± 0.07	0.046 ± 0.009
CMB-NaOH-I	54.30 ± 0.44	1.200 ± 0.03	0.074 ± 0.008
CMB-NaOH-II	28.12 ± 0.29	1.166 ± 0.06	0.049 ± 0.001

The pore size distribution can further explain the regeneration effect of CMB. Macropores are generally responsible for substance diffusion, mesopores serve as mass transfer channels, and micropores provide adsorption and trapping sites [43]. Thus, the pore size distribution among the three types of pores in regenerated biochar was analyzed, and the methods suitable for CMB regeneration were researched. The pore size distribution of the regenerated biochars is presented in Figure 5; the results of the pore size distribution correspond to Table 3. The results demonstrated that CMB-H₂O-I effectively removed adsorbed inhibitors and restored the pore size distribution of CMB, while CMB-H₂O-II exhibited lower restoration efficacy than CMB-H₂O-I under identical conditions.

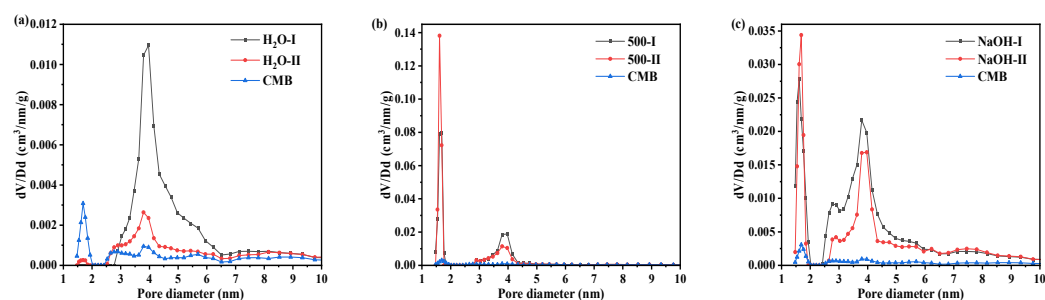


Figure 5. Pore size distribution of regenerated CMB (a) Water-washing regeneration. (b) Thermal regeneration. (c) NaOH regeneration.

The pore distribution of CMB-500-I and CMB-500-II was highest at 1–2 nm, indicating that after regeneration at 500 °C, the biochar had developed a richer micropore structure, and the pore distribution at 3–5 nm had also increased. For the NaOH regeneration method, both the micropore and mesopore quantities increased by CMB-NaOH-II and CMB-NaOH-I. The common inhibitors in the prehydrolysate have molecular sizes of approximately 1 nm; therefore, the increased abundance of micropores (1–2 nm) and mesopores (3–5 nm) provides more accessible adsorption sites and diffusion pathways for these molecules. Compared to the water-washing regeneration, the biochar obtained from thermal regeneration and NaOH regeneration exhibited a better pore size distribution as shown in Figure 5, indicating their enhanced potential for the adsorption of inhibitors [44,45].

As illustrated in Figure S3, the nitrogen adsorption–desorption isotherms of the six regenerated biochars all conformed to type IV isotherms, indicating that mesoporous structures predominated as their primary pore configuration [44]. The maximum nitrogen adsorption capacities were 15.74 cm³/g, 45.04 cm³/g, and 30.82 cm³/g for CMB-H₂O-I, CMB-NaOH-I, and CMB-500-I, respectively. In the low relative pressure (P/P_0) range, the curve displayed upward convex curvature, suggesting the formation of a monolayer of nitrogen molecules on the surface of biochar [46]. A pronounced hysteresis phenomenon emerged in the higher relative pressure region, attributed to the capillary condensation of nitrogen [47]. As the relative pressure approached 1, nitrogen gas was adsorbed onto the larger pores, causing the curve to rise. Notably, the adsorption–desorption curve of biochars obtained by NaOH regeneration and thermal regeneration failed to close in the low-pressure region. This could be attributed to either irreversible adsorption in mesoporous materials or the presence of abundant micropores from which nitrogen could not escape completely during desorption [48].

Based on the SEM macroscopic observations of the biochar surface morphology (Figure 6). After two regeneration cycles using three different regeneration methods, no obvious fragmentation or disintegration of biochar particles was observed in the SEM images. After water-washing regeneration, the pore structure of the biochar was partially restored. However, residual inhibitors were still clearly visible on the biochar surface, and partial pore collapse was observed, indicating that water-washing incompletely removed

adsorbed inhibitors. In contrast, thermally regenerated biochar exhibited a significant increase in micropore and mesopore development, exhibiting a more uniform and ordered pore structure. This suggested that thermal regeneration not only decomposed inhibitors but also promoted further pyrolysis, generating additional porous structures. After NaOH regeneration, the micropores and mesopores were effectively restored, demonstrating that NaOH efficiently desorbed the inhibitors from the biochar surface. These results indicated that, in terms of physical structure, biochars regenerated using all three methods exhibited good stability. Among them, the NaOH-regenerated and thermally regenerated biochars showed better structural stability than the water-washing regenerated biochar. These observations are consistent with the data shown in Table 3 and Figure 5.

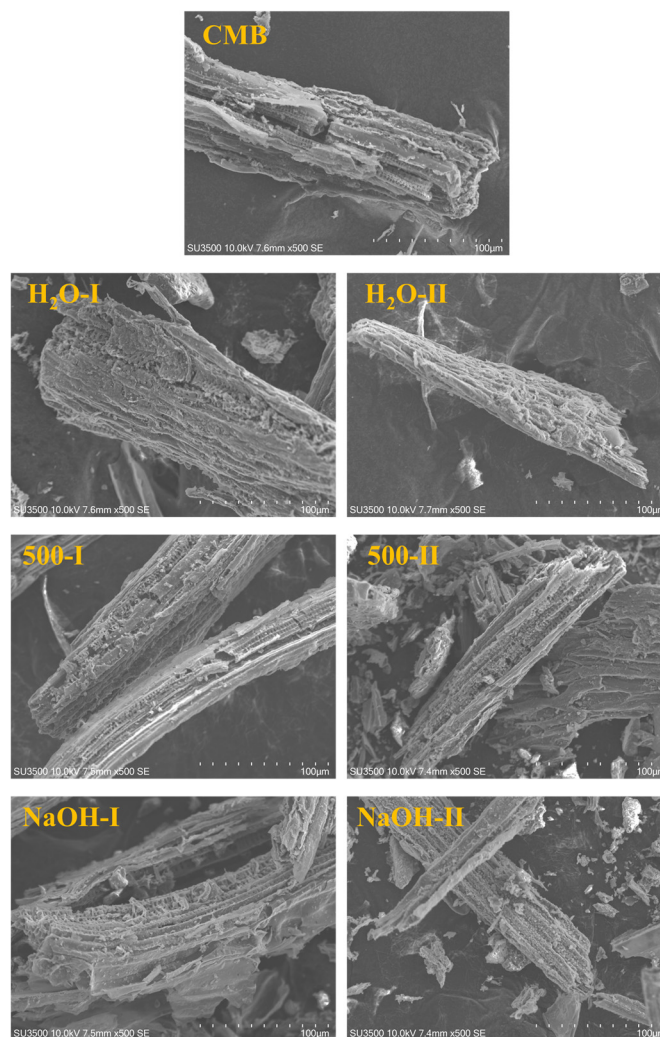


Figure 6. The microstructure of regenerated CMB.

Their ATR-FTIR spectra were recorded to describe the changes in surface chemical functionalities of regenerated CMBs (Figure 7). After water-washing regeneration, an increased intensity of -OH functional groups ($3700\text{--}3200\text{ cm}^{-1}$) was observed on the biochar surface. This enhancement was attributed to the removal of soluble ash components and inorganic salts during water washing, which exposed previously shielded surface -OH functional groups [49,50]. The absorption peak at 2330 cm^{-1} corresponds to atmospheric CO_2 . The absorption band around 1600 cm^{-1} is assigned to the C=O stretching vibration. In CMB- H_2O -I and CMB- NaOH -I, this peak intensity was largely restored. In contrast, CMB- H_2O -II exhibited a peak shift and intensity change, which may be attributed to hydrogen bonding between inhibitors and the C=O groups on the surface of biochar [51].

The decrease in the number of C=O functional groups in CMB-500-II may be attributed to decarboxylation and decarbonylation reactions occurring on the biochar surface as thermal regeneration cycles increased, which consequently reduced the adsorption performance of CMB-500-II [52]. The ATR-FTIR spectra are consistent with the elemental analysis results in Table 2.

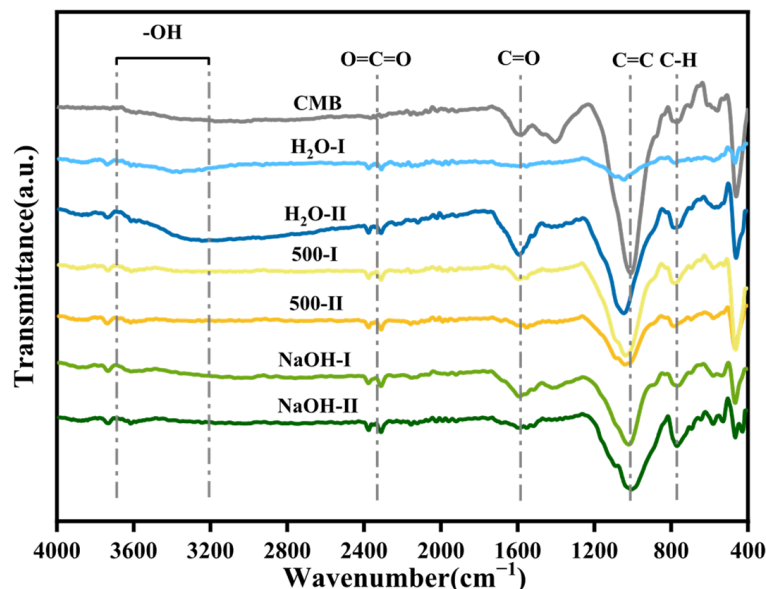


Figure 7. ATR-FTIR spectra of regenerated CMB.

The LA fermentation results of the detoxified prehydrolysate by different regenerated CMBs are shown in Figure 8. The regenerated CMBs with three different methods all maintained a certain detoxification capacity on the prehydrolysate, and the lactic acid fermentability after detoxification was improved compared with the UT group. Following the first regeneration, CMB-H₂O-I exhibited significantly lower LA volumetric productivity compared to CMB, whereas CMB-NaOH-I showed a gradual increase within 72 h, and CMB-500-I maintained a similar productivity level to CMB. At 72 h, the concentrations of LA in CMB-H₂O-I, CMB-500-I, and CMB-NaOH-I were measured at 10.97 ± 0.33 g/L, 31.27 ± 0.17 g/L, and 38.31 ± 0.01 g/L, respectively. At 72 h, the concentrations of LA in CMB-H₂O-II, CMB-500-II, and CMB-NaOH-II were measured at 14.45 ± 0.22 g/L, 25.22 ± 1.34 g/L, and 33.89 ± 0.05 g/L, respectively. However, after the first regeneration, biochar regenerated via three methods (CMB-H₂O-I, CMB-500-I, and CMB-NaOH-I) exhibited the recyclability of $85.34 \pm 1.8\%$, $98.89 \pm 1.1\%$, and $92.42 \pm 1.9\%$, respectively. After the second regeneration, the recyclability of CMB-H₂O-II, CMB-500-II, and CMB-NaOH-II was $81.86 \pm 0.8\%$, $83.51 \pm 0.7\%$, and $81.50 \pm 1.6\%$, respectively. As the regeneration round increases, the adsorption efficiency of biochar decreases, likely due to increased aggregation on the biochar surface [42,53]. Moreover, after two regeneration cycles using three different regeneration methods, the biochars maintained recyclability values above 80%, indicating their good stability in the prehydrolysate. The adsorption performance of biochar is closely related to its physicochemical stability during the detoxification process. Although minor changes in surface properties were observed after detoxification, the overall adsorption capacity toward inhibitory compounds remained sufficient to maintain effective detoxification. This removal of inhibitors alleviated microbial inhibition during fermentation, which directly contributed to improved lactic acid recovery. These results demonstrate the interdependence of biochar degradation, adsorption performance, and lactic acid recovery.

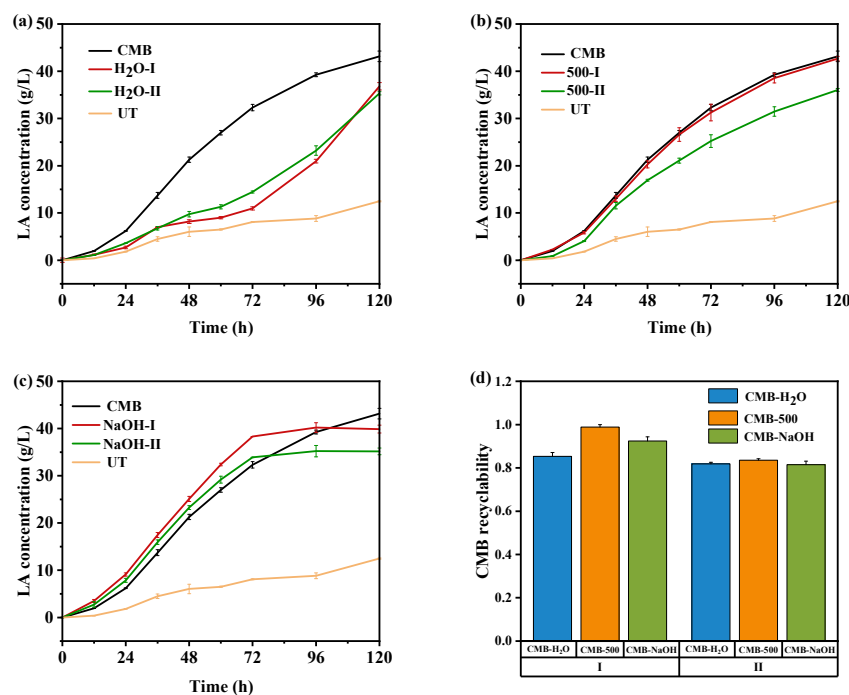


Figure 8. The impact of different regeneration methods on the detoxification fermentation of CMB. (a) LA fermentation after water-washing regeneration, (b) LA fermentation after thermal regeneration, (c) LA fermentation after NaOH solvent regeneration, and (d) CMB recyclability after three regeneration methods.

Compared with thermal and NaOH regeneration methods, water-washing regeneration exhibited lower fermentation productivity. This was attributed to the fact that, during NaOH regeneration, NaOH effectively reacted with and removed the adsorbed inhibitors, thereby exposing more accessible adsorption sites and enhancing the removal of inhibitors by CMB through the pore filling mechanism. Thermal regeneration increased the degree of aromatization and graphitization of CMB. The enhanced aromaticity may have facilitated π - π interactions between the aromatic domains of biochar and inhibitors (Figure 9). Similar interaction mechanisms have been reported in previous studies. For example, Chen et al. investigated plant residue materials as biosorbents for the removal of aromatic compounds and found that adsorption capacity increased with increasing aromaticity of the biosorbent, supporting the potential role of π - π interactions in adsorption processes [54]. Furthermore, Liu et al. have indicated that water washing may induce pore collapse, which could also contribute to the diminished adsorption performance of water-washing regenerated biochar [41]. This finding is consistent with the observations in Figure 6. However, the LA recyclability of both CMB-H₂O-I and CMB-H₂O-II consistently reached around 80%, demonstrating that water washing remains a practical and effective regeneration method for biochar reuse in fermentation systems when operational conditions are optimized. Furthermore, the regenerated biochar obtained through thermal regeneration is slightly more effective for detoxifying the prehydrolysate than NaOH regeneration and water-washing regeneration. Zeng et al. investigated the thermal regeneration and solvent regeneration of bermudagrass biochar. The biochar regenerated by thermal treatment at 300 °C for 3 h achieved a recyclability of 99–100% over four adsorption-regeneration cycles. In contrast, when 1 M NaOH was used for regeneration, the recyclability of the biochar was 86.69%, which was lower than that of thermal regeneration [22]. This is consistent with the results of our study.

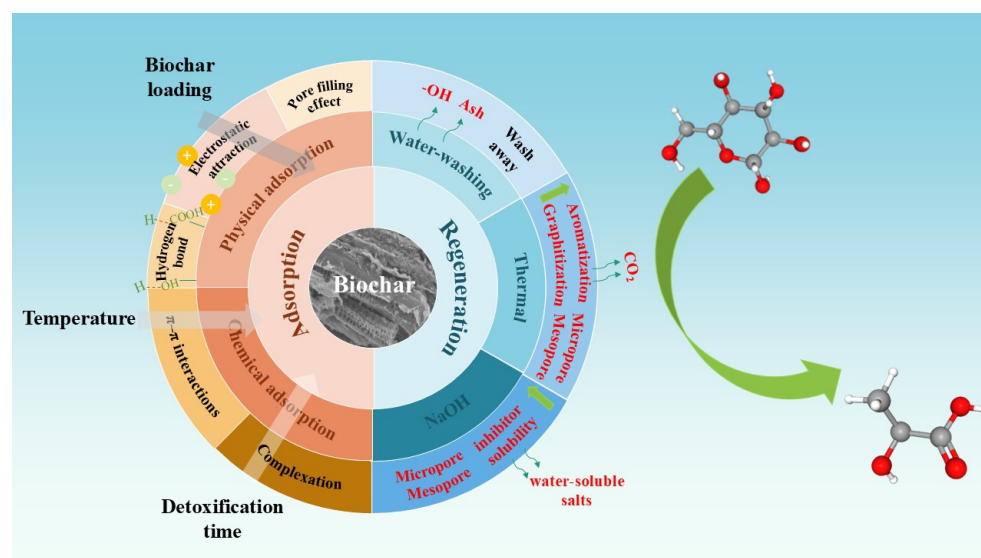


Figure 9. Adsorption and regeneration mechanisms of CMB.

Table 4 summarizes the regeneration performance of biochars after the adsorption of organic compounds reported in previous studies. Thermal, alkali, and water-washing treatments are commonly applied regeneration methods. Regeneration performance strongly depends on feedstock properties, regeneration strategy, and the nature of the adsorbed compounds. For example, wood biochar regenerated by water washing retained only 58.6% recyclability for sulfonamides after two cycles [54], whereas durian peel biochar exhibited even lower recyclability (13–33%) after three cycles under different regeneration treatments [40]. In contrast, certain biochars, such as giant mud crab shell biochar, maintained >90% dye removal for up to six cycles under hot water washing [55]. In the present study, after two regeneration cycles using three different methods, the recyclability remained around 80% (Figure 8). This relatively stable regeneration performance may be attributed to the intrinsic structural characteristics of cow manure biochar. During regeneration, thermal treatment enhanced aromaticity and micropore development, while NaOH treatment facilitated desorption through the disruption of surface interactions. Although water washing resulted in comparatively lower structural restoration, it effectively removed inhibitors and sustained fermentation performance. Compared with previous studies that primarily focused on adsorption performance, the present work further evaluated regeneration effectiveness by considering its impact on lactic acid production. By incorporating fermentation results, this assessment provides additional insight into the practical reuse of biochar in biorefinery systems.

Table 4. Studies on biochar regeneration methods for the removal of organic compounds.

Adsorbent	Preparation Conditions	Regeneration Methods	Regeneration Rounds	Regeneration Performance	Reference
Wood biochar	The wood chips were heated to 650 °C within 60 min and pyrolyzed for 100 min	Water-washing regeneration	2 cycles	The recyclability for sulfonamide removal after the second cycles is 58.6%	[21]
Durian peel biochar	Durian peel powder and KOH pellets were mixed at a 1:1 <i>w/w</i> ratio and pyrolyzed at 700 °C for 2 h under a nitrogen atmosphere at a heating rate of 10 °C/min	Thermal, alkali, hydrothermal regeneration	3 cycles	The recyclability for ciprofloxacin removal after the third cycles are 33%, 23%, and 13%, respectively	[40]

Table 4. Cont.

Adsorbent	Preparation Conditions	Regeneration Methods	Regeneration Rounds	Regeneration Performance	Reference
Giant mud crab shell biochar	Crab shells were heated to 500 °C and pyrolyzed for 2 h	Hot water-washing regeneration	9 cycles	>90% methyl violet removal for 6th regeneration cycles	[55]
Pinus patula biochar	The biochar was prepared via gasification in a reverse downdraft reactor at atmospheric pressure with an air flow rate of 146 ± 4.35 L/min ($0.12 \pm 3.58 \times 10^{-3}$ kg/m ² /s)	Thermal solvent regeneration with 75% C ₂ H ₆ O	7 cycles	The recyclability of Indigo Carmine after seven cycles are 55%	[56]
Cow manure biochar	Cow manure was pyrolyzed at 500 °C for 1 h under a nitrogen atmosphere at a heating rate of 10 °C/min	Water-washing, thermal, NaOH solvent regeneration	2 cycles	The recyclability for inhibitor removal after second cycles are 81.86%, 81.50%, and 83.51%, respectively	this study

3. Materials and Methods

3.1. Chemicals and Microorganisms

MRS cultural medium, glucose, yeast extracts, glucose and lactic acid standard solutions (1 g/L) were obtained from Sigma Aldrich (Wuxi, China); sulfuric acid, sodium hydroxide, and calcium carbonate were supplied by Sinopharm Chemical Reagent Company (Shanghai, China); acetic acid, furfural, hydroxymethylfurfural (HMF), 4-hydroxybenzoic acid, vanillic acid, 4-hydroxybenzaldehyde, vanillin, and syringaldehyde were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). The strain employed for lactic acid fermentation was *Lactobacillus pentosus* (ATCC 8041, Manassas, VA, USA).

3.2. Preparation of Biochar from Cow Manure

The cow manure used in this study was collected from Jinyindao Ranch (Beijing, China). It was dried in an oven at 105 °C until a constant weight was achieved and then stored in a sealed bag for further use. The dried cow manure was ground with a high-speed grinder (RT-34, Taiwan RongCong Precision Technology Co., Taichung City, Taiwan, China) and sieved through a 20-mesh screen. 5 g of cow manure were placed in a quartz boat (100 mm × 40 mm × 20 mm) and thermally treated at 500 °C for 1 h under a nitrogen atmosphere in a tube furnace (GSL-1100X, Hefei Kejing Materials Technology Co., Hefei, China) at a heating rate of 10 °C per minute. The obtained biochar, referred to as cow manure biochar (CMB), was thoroughly mixed before use.

3.3. Preparation of Prehydrolysate from Dilute Acid Pretreatment of Corn Stover

Corn stover used in this study was obtained from the Shangzhuang Experimental Station of the China Agricultural University in Beijing, China. The material was air-dried until the moisture content was less than 10%, and subsequently ground to pass through a 20–40 mesh sieve. The corn stover powder was blended with 1.0% *w/w* sulfuric acid solution at a solid-to-liquid ratio of 1:2.5% *w/v*. The dilute acid pretreatment was then carried out at 121 °C for one hour using an MLS-350 autoclave (Sanyo Electric Co., Osaka, Japan). After pretreatment, the prehydrolysate was separated from pretreated corn stover solids by vacuum filtration.

3.4. Adsorption Kinetic Study of Inhibitors onto CMB

The adsorption kinetic experiments were conducted using seven common inhibitors present in dilute acid prehydrolysate as target adsorbates, and the adsorption data were

fitted using the pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models. The detailed experimental methods are described in Supplementary Material S1.

3.5. The Detoxification of Prehydrolysate by Biochar

To optimize the conditions for biochar detoxification, RSM was applied in this study. Three variables, i.e., temperature, detoxification time, and biochar loading, were investigated, and three levels were selected for each variable, as shown in Table S2. Using the Design Expert 11.1.2.0 statistical analysis software, the Box–Behnken design (BBD) was employed to design the detoxification experiments. Seventeen runs of detoxification experiments in total were performed based on the three factors: temperature, X_1 (°C); detoxification time, X_2 (min); biochar loading, X_3 (% w/v).

Detoxification experiments were conducted in flasks in a shaker incubator at ambient temperature. The shaking speed was kept at 200 rpm. After detoxification, CMB was collected via vacuum filtration and subsequently dried at 60 °C in an oven. The used CMB was then subjected to a characterization and regeneration study. The detoxified prehydrolysate was collected for LA fermentation. The final LA concentration was chosen as the response for RSM analysis. The experimental results were analyzed using variance analysis (ANOVA). Moreover, a second-order polynomial equation (presented below) was used as the regression model to fit the experimental data:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum \sum_{j=1}^k \beta_{ij} X_i X_j \quad (2)$$

where Y represents the response (LA concentration); β_0 is the intercept; β_i is the coefficient of the linear item; β_{ii} is the coefficient of the quadratic item; β_{ij} is the interactive coefficient; X_i and X_j represent the independent variables; and k represents the number of independent variables.

3.6. Lactic Acid Fermentation

Before LA fermentation, the detoxified prehydrolysate was neutralized to pH 6.0 using NaOH. Glucose and yeast extract were incorporated into the prehydrolysate to obtain final concentrations of 50 g/L and 15 g/L, respectively. CaCO_3 (0.56 g/g-glucose) was added aseptically to maintain the pH throughout the fermentation process. The prehydrolysate without biochar detoxification was prepared similarly and referred to as the untreated (UT) group. Both the prehydrolysate and the pure glucose solution were sterilized using 0.22 μm sterile filter membranes. The seed culture of *Lactobacillus pentosus* (ATCC 8041) was prepared in an MRS medium at 37 °C until its optical density (OD_{600}) reached approximately 1.5. Afterward, fermentation was initiated by adding 10% v/v of seed culture. LA fermentation was performed in 50 mL serum bottles in an incubator shaker at 37 °C and 150 rpm for 120 h. Samples were taken every 12 h using a syringe for carbohydrate, LA, and OD analysis. The values of OD_{600} during the fermentation process were tested to determine cell biomass using a microplate reader (Multiskan FC, Thermo Scientific, Waltham, MA, USA). The fermentation experiments were carried out in duplicate.

3.7. Biochar Regeneration

To assess the reusability of the CMB prepared in this study, two rounds of regeneration were performed, and three regeneration methods were investigated: simple washing regeneration, thermal regeneration, and solvent regeneration. In water-washing regeneration, the detoxified CMB was repeatedly washed with deionized water until a neutral pH was achieved. It was then oven-dried at 105 °C to obtain the regenerated biochar, which was designated as CMB- H_2O . In thermal regeneration, CMB after detoxification was loaded into a tubular furnace and treated at 500 °C for one hour in a nitrogen atmosphere to

obtain the regenerated biochar, referred to as CMB-500. In solvent regeneration, NaOH solution was selected for the desorption of inhibitors adsorbed onto biochar in acidic prehydrolysate. The CMB after detoxification was dried and mixed with a 1 mol/L NaOH solution. This mixture was loaded into a shaker for one hour under room temperature conditions, and then the regenerated biochar solid was collected, washed with deionized water until neutrality was achieved, and then dried at 105 °C in an oven until constant weight was achieved. The regenerated biochar was designated as CMB-NaOH. The regenerated biochars were evaluated for their adsorption capacity in prehydrolysate detoxification, following the previously described procedure.

In the second round of regeneration, the same regeneration and adsorption test procedure was carried out. The biochar obtained from the first regeneration was labeled as I, while the biochar obtained from the second regeneration was labeled II, e.g., CMB after the first thermal regeneration was named CMB-500-I, and CMB after the second thermal regeneration was named CMB-500-II. The specific surface area and pore size of the regenerated biochar were determined as described in Section 3.8. Moreover, the final lactic acid concentration of 120 h was utilized as an indicator to evaluate the regeneration performance of CMB. The regeneration experiments were carried out in duplicate.

3.8. Characterization of Biochars

The elemental composition (C, H, N, S and O) of the biochar was determined using an elemental analyzer (UNICUBE, Elementar, Langenselbold, Germany) operated in CHNS mode. All measurements were conducted in triplicate. Approximately 1.5–2.5 mg of each dried sample was accurately weighed into a tin capsule prior to analysis. Combustion was performed at 1000 °C under a pure oxygen atmosphere, with high-purity helium used as the carrier gas at a flow rate of 100 mL/min. The oxygen content was determined by a subtractive (difference) method. The specific surface area (S_{BET}) and pore size of biochar were analyzed by the N_2 adsorption–desorption method, measured with a surface area and pore size analyzer (Nova Station A, Quantachrome, Boynton Beach, FL, USA). Each sample was degassed at 200 °C under vacuum for 12 h. The specific surface area and total pore volume were evaluated by the multi-point BET method, with S_{BET} calculated over a relative pressure (P/P_0) range of 0.02–0.20, while the pore size distribution was derived from the DFT method [57]. The surface functional group information of biochars was characterized by the attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy using an MIR spectrometer (ALPHA-II, Bruker, Karlsruhe, Germany), with spectra recorded over a wavenumber range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} and 64 scans. The surface morphology of the biochars was observed using scanning electron microscopy (SEM, SU3500, Hitachi, Tokyo, Japan). Cell morphology was observed using scanning electron microscopy (SEM, SU8010, Hitachi, Japan). The detailed sample preparation and experimental conditions are described in the Supplementary Material S2.

3.9. HPLC Analysis

Quantification of key inhibitors in the prehydrolysate before and after biochar detoxification was determined by HPLC (e2695, Waters, Milford, MA, USA) with a C18 chromatographic column (5 μm , 4.6 mm $\times$ 250 mm) and a UV/Vis detector (2489, Waters, USA) operating at a detection wavelength of 254 nm. The concentrations of sugars and LA during the fermentation process were determined by high-performance liquid chromatography (HPLC) (e2695, Waters, USA) equipped with a refractive index (RI) detector (2414, Waters, USA) and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The column temperature was kept at 55 °C, with 5 mM sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min. Calibration curves for the inhibitors were established based

on the linear relationships between compound concentrations and peak heights. Samples collected during the detoxification and fermentation processes were centrifuged, and the supernatants were diluted tenfold, filtered through a 0.22 µm sterile membrane filter, and transferred into HPLC vials for subsequent analysis.

Lactic acid yield is calculated according to the following equation:

$$\text{LA yield} = \frac{\text{Final LA concentration} - \text{Initial LA concentration}}{\text{Initial sugar concentration}} \times 100\% \quad (3)$$

The recyclability of regeneration biochar is calculated as follows:

$$\text{Biochar recyclability} = \frac{\text{LA concentration after regenerated biochar detoxification}}{\text{LA concentration after CMB detoxification}} \times 100\% \quad (4)$$

4. Conclusions

In this study, the detoxification conditions for biochar produced from cow manure (CMB) were successfully optimized using RSM. The results indicated that biochar loading is the most dominant factor affecting the performance of the subsequent LA fermentation. The optimal detoxification condition obtained is as follows: a temperature of 53 °C, detoxification time of 118 min, and biochar loading of 4.5% *w/v* with a theoretical lactic acid concentration of 42.35 g/L. After optimization, the fermentability of prehydrolysate was significantly enhanced, achieving a final LA concentration of 42.89 g/L with an 85.67% yield. The adsorption performance of biochar could be recovered by regeneration. Three ways of regeneration all showed satisfactory results, among which water-washing regeneration would be a preferred choice due to its simple operation. After two rounds of water-washing regeneration, the adsorption ability of CMB was maintained at 81.86%, highlighting its potential for industrial applications. Future research could further investigate the regeneration mechanism of biochar and the stability of its long-term cyclic use, as well as promote its large-scale application in biorefining through economic analysis.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/catal16030242/s1>, Text S1: Adsorption kinetic study of inhibitors onto CMB; Text S2: Scanning electron microscopy (SEM) analysis; Figure S1: Main influence chart for LA concentration; Figure S2: The statistical plots for comparing predicted and experimental values for the LA concentration: (a) predicted vs. experimental response and (b) residuals vs. predicted response; Figure S3: Nitrogen adsorption-desorption curve of regenerated CMB; Table S1: Analysis of kinetic parameters for the adsorption of seven inhibitors by CMB; Table S2: ANOVA results for LA concentration; Table S3: Factors and levels in the experimental design of biochar detoxification.

Author Contributions: Y.S. performed experiments, analyzed samples, and wrote the draft; H.Z. and X.H. took part in the performance of experiments; C.W. took part in the performance of experiments and analyzed samples; S.S. designed the experiments and edited the manuscript; D.B. and L.H. reviewed and edited the manuscript. The manuscript was written through the contributions of all authors. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare that they have no competing interests.

References

- Xu, L.; Liaqat, F.; Sun, J.; Khazi, M.I.; Xie, R.; Zhu, D. Advances in the vanillin synthesis and biotransformation: A review. *Renew. Sustain. Energy Rev.* **2024**, *189*, 113905. [\[CrossRef\]](#)
- Wang, Y.Z.; Yang, J.; Wei, H.; Hou, R.; Shi, J.; Jin, Z.; Yang, F.; Hu, J.; Gao, M.-T. Reduction of fermentation-associated stresses by straw-based soluble saccharides for enhancing ethanol production. *J. Agric. Food Chem.* **2020**, *68*, 5863–5872. [\[CrossRef\]](#) [\[PubMed\]](#)
- Bustamante, D.; Tortajada, M.; Ramón, D.; Rojas, A. Production of D-lactic acid by the fermentation of orange peel waste hydrolysate by lactic acid bacteria. *Fermentation* **2019**, *6*, 1. [\[CrossRef\]](#)
- Wu, S.; Shi, S.; Liu, R.; Wang, C.; Li, J.; Han, L. The transformations of cellulose after concentrated sulfuric acid treatment and its impact on the enzymatic saccharification. *Biotechnol. Biofuels Bioprod.* **2023**, *16*, 36. [\[CrossRef\]](#)
- Han, X.; Li, L.; Wei, C.; Zhang, J.; Bao, J. Facilitation of L-lactic acid fermentation by lignocellulose biomass rich in vitamin B compounds. *J. Agric. Food Chem.* **2019**, *67*, 7082–7086. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ingram, L.O.; Aldrich, H.C.; Borges, A.C.; Causey, T.B.; Martinez, A.; Morales, F.; Saleh, A.; Underwood, S.A.; Yomano, L.P.; York, S.W.; et al. Enteric bacterial catalysts for fuel ethanol production. *Biotechnol. Prog.* **1999**, *15*, 855–866. [\[CrossRef\]](#)
- Zdarta, J.; Thygesen, A.; Holm, M.S.; Meyer, A.S.; Pinelo, M. Direct separation of acetate and furfural from xylose by nanofiltration of birch pretreated liquor: Effect of process conditions and separation mechanism. *Sep. Purif. Technol.* **2020**, *239*, 116546. [\[CrossRef\]](#)
- Tan, X.; Wu, X.; Wang, X.; Tao, Y.; Li, Y.; Tang, S.; Zhang, Q.; Sheng, Y. Chitosan-assisted dilute acid pretreatment with enhanced prehydrolysate fermentability and enzyme digestibility of pretreated substrates. *Int. J. Biol. Macromol.* **2025**, *310*, 143231. [\[CrossRef\]](#)
- Alam, M.; Dhillon, S.K.; Ismail, S.; Dhar, B.R. Biochar and granular activated carbon mitigate polystyrene nanoplastics inhibition in dark biohydrogen fermentation of sludge. *ACS EST Eng.* **2024**, *5*, 487–499. [\[CrossRef\]](#)
- Godlewska, P.; Schmidt, H.P.; Ok, Y.S.; Oleszczuk, P. Biochar for composting improvement and contaminants reduction. A review. *Bioresour. Technol.* **2017**, *246*, 193–202. [\[CrossRef\]](#) [\[PubMed\]](#)
- Biederman, L.A.; Harpole, W.S. Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *GCB Bioenergy* **2012**, *5*, 202–214. [\[CrossRef\]](#)
- Zhang, P.; Li, Y.; Cao, Y.; Han, L. Characteristics of tetracycline adsorption by cow manure biochar prepared at different pyrolysis temperatures. *Bioresour. Technol.* **2019**, *285*, 121348. [\[CrossRef\]](#)
- Chen, G.; Wang, C.; Tian, J.; Liu, J.; Ma, Q.; Liu, B.; Li, X. Investigation on cadmium ions removal from water by different raw materials-derived biochars. *J. Water Process Eng.* **2020**, *35*, 101223. [\[CrossRef\]](#)
- Do Nascimento, B.F.; De Araujo, C.M.B.; Do Nascimento, A.C.; Da Silva, F.L.H.; De Melo, D.J.N.; Jaguaribe, E.F.; Lima Cavalcanti, J.V.F.; Da Motta Sobrinho, M.A. Detoxification of sisal bagasse hydrolysate using activated carbon produced from the gasification of açai waste. *J. Hazard. Mater.* **2021**, *409*, 124494. [\[CrossRef\]](#) [\[PubMed\]](#)
- Monlau, F.; Sambusiti, C.; Antoniou, N.; Zabaniotou, A.; Solhy, A.; Barakat, A. Pyrochars from bioenergy residue as novel bio-adsorbents for lignocellulosic hydrolysate detoxification. *Bioresour. Technol.* **2015**, *187*, 379–386. [\[CrossRef\]](#) [\[PubMed\]](#)
- Shen, Y.; Luo, L.; Chong, K.; Yang, Y.; Blersch, D.; Balasundram, V.; Shi, S.; Han, L. The targeted removal of aromatic inhibitors in dilute acid prehydrolysate of corn stover by KOH-modified biochar for the efficient production of lactic acid. *J. Environ. Manag.* **2025**, *395*, 127902. [\[CrossRef\]](#)
- Arminda, M.; Josué, C.; Cristina, D.; Fabiana, S.; Yolanda, M. Use of activated carbons for detoxification of a lignocellulosic hydrolysate: Statistical optimisation. *J. Environ. Manag.* **2021**, *296*, 113320. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sarawan, C.; Suinyuy, T.N.; Sewsynker-Sukai, Y.; Gueguim Kana, E.B. Optimized activated charcoal detoxification of acid-pretreated lignocellulosic substrate and assessment for bioethanol production. *Bioresour. Technol.* **2019**, *286*, 121403. [\[CrossRef\]](#)
- Deng, F.; Cheong, D.-Y.; Aita, G.M. Optimization of activated carbon detoxification of dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate by response surface methodology. *Ind. Crops Prod.* **2018**, *115*, 166–173. [\[CrossRef\]](#)
- Li, H.; Dong, X.; da Silva, E.B.; de Oliveira, L.M.; Chen, Y.; Ma, L.Q. Mechanisms of metal sorption by biochars: Biochar characteristics and modifications. *Chemosphere* **2017**, *178*, 466–478. [\[CrossRef\]](#)
- Guan, Y.; Ma, C.; Zhou, J.; Wang, H.; Yang, H. Enhancing sulfonamide removal from water using wood biochar: Adsorption mechanisms and regeneration potential. *J. Taiwan Inst. Chem. Eng.* **2025**, *167*, 105910. [\[CrossRef\]](#)
- Zeng, S.; Kan, E. Adsorption and regeneration on iron-activated biochar for removal of microcystin-LR. *Chemosphere* **2021**, *273*, 129649. [\[CrossRef\]](#)
- Salvador, F.; Martin-Sanchez, N.; Sanchez-Hernandez, R.; Sanchez-Montero, M.J.; Izquierdo, C. Regeneration of carbonaceous adsorbents. Part II: Chemical, Microbiological and Vacuum Regeneration. *Microporous Mesoporous Mater.* **2015**, *202*, 277–296. [\[CrossRef\]](#)
- Ledesma, B.; Román, S.; Álvarez-Murillo, A.; Sabio, E.; González-García, C.M. Fundamental study on the thermal regeneration stages of exhausted activated carbons: Kinetics. *J. Therm. Anal. Calorim.* **2013**, *115*, 537–543. [\[CrossRef\]](#)
- Jiang, S.; Yan, L.; Wang, R.; Li, G.; Rao, P.; Ju, M.; Jian, L.; Guo, X.; Che, L. Recyclable nitrogen-doped biochar via low-temperature pyrolysis for enhanced lead(II) removal. *Chemosphere* **2022**, *286*, 131666. [\[CrossRef\]](#)

26. Yin, P.; Wang, Z.; Qu, R.; Liu, X.; Zhang, J.; Xu, Q. Biosorption of heavy metal ions onto agricultural residues buckwheat hulls functionalized with 1-hydroxyethylidenediphosphonic acid. *J. Agric. Food Chem.* **2012**, *60*, 11664–11674. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Simões dos Reis, G.; Bergna, D.; Tuomikoski, S.; Grimm, A.; Lima, E.C.; Thyrel, M.; Skoglund, N.; Lassi, U.; Larsson, S.H. Preparation and characterization of pulp and paper mill sludge-activated biochars using alkaline activation: A Box-Behnken design approach. *ACS Omega* **2022**, *7*, 32620–32630. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Xu, S.; Lan, K.; Li, J.; He, T.; Hu, C. Separation of lactic acid from synthetic solutions and the mixture directly derived from corn stover by aqueous two phase extraction. *Sep. Purif. Technol.* **2018**, *204*, 281–289. [\[CrossRef\]](#)
29. Rahimi, M.S.; Esfahanian, M. Modeling and optimization of adsorption behavior of lactic acid onto zirconium-based metal-organic framework: Response surface methodology (RSM). *Sep. Sci. Technol.* **2024**, *60*, 49–65. [\[CrossRef\]](#)
30. Coleman, B.S.L.; Easton, Z.M.; Bock, E.M. Biochar fails to enhance nutrient removal in woodchip bioreactor columns following saturation. *J. Environ. Manag.* **2019**, *232*, 490–498. [\[CrossRef\]](#)
31. Hylton, J.; Hugen, A.; Rowland, S.M.; Griffin, M.; Tunstall, L.E. Relevant biochar characteristics influencing compressive strength of biochar-cement mortars. *Biochar* **2024**, *6*, 87. [\[CrossRef\]](#)
32. Lee, S.C.; Park, S. Removal of furan and phenolic compounds from simulated biomass hydrolysates by batch adsorption and continuous fixed-bed column adsorption methods. *Bioresour. Technol.* **2016**, *216*, 661–668. [\[CrossRef\]](#)
33. Fan, S.; Wang, Y.; Wang, Z.; Tang, J.; Tang, J.; Li, X. Removal of methylene blue from aqueous solution by sewage sludge-derived biochar: Adsorption kinetics, equilibrium, thermodynamics and mechanism. *J. Environ. Chem. Eng.* **2017**, *5*, 601–611. [\[CrossRef\]](#)
34. Xia, W.; Zhu, N.; Mei, J.; Peng, Y.; Song, F.; Ding, S.; Li, F.; Zhou, X. Biocatalytic detoxification of ochratoxins A/B by a fungal dye-decolorizing peroxidase: Mechanistic insights and toxicity assessment. *Toxins* **2025**, *17*, 438. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Günther, A.; Kulawik, M.; Sip, S.; Zalewski, P.; Jarmołowska-Jurczyszyn, D.; Stawicki, P.; Bednarczyk-Cwynar, B. Targeting oxidative stress in carcinogenesis: Oleanolic acid and its molecular pathways. *Antioxidants* **2026**, *15*, 67. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Jilani, S.B.; Olson, D.G. Mechanism of furfural toxicity and metabolic strategies to engineer tolerance in microbial strains. *Microb. Cell Factories* **2023**, *22*, 221. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Verma, N.; Devi, N.L. Removal of atmospheric pollutants using biochar: Preparation, application, regeneration and its future research. *Air Qual. Atmos. Health* **2025**, *18*, 1205–1224. [\[CrossRef\]](#)
38. Niu, Y.; Tan, H.; Hui, S.e. Ash-related issues during biomass combustion: Alkali-induced slagging, silicate melt-induced slagging (ash fusion), agglomeration, corrosion, ash utilization, and related countermeasures. *Prog. Energy Combust.* **2016**, *52*, 1–61. [\[CrossRef\]](#)
39. Zhang, M.; Shu, L.; Shen, X.; Guo, X.; Tao, S.; Xing, B.; Wang, X. Characterization of nitrogen-rich biomaterial-derived biochars and their sorption for aromatic compounds. *Environ. Pollut.* **2014**, *195*, 84–90. [\[CrossRef\]](#)
40. Liu, X.; Huang, P.; Ma, W.; Jin, F.; Tai, L. Unveiling adsorption mechanisms and regeneration challenges of durian peel biochar for ciprofloxacin removal: Batch experiments and DFT study. *J. Water Process Eng.* **2025**, *75*, 107991. [\[CrossRef\]](#)
41. Liu, C.; Qiu, T.; Cao, W.; Liu, H.; Zhao, W.; Mostafa, E.; Zhang, Y. Water-washing treatment can change biochar microstructure: Microwave pyrolysis of biomass. *Energy Sources Part A* **2024**, *46*, 2132–2146. [\[CrossRef\]](#)
42. Alsawy, T.; Rashad, E.; El-Qelish, M.; Mohammed, R.H. A comprehensive review on the chemical regeneration of biochar adsorbent for sustainable wastewater treatment. *npj Clean Water* **2022**, *5*, 29. [\[CrossRef\]](#)
43. Muzyka, R.; Misztal, E.; Hrabak, J.; Banks, S.W.; Sajdak, M. Various biomass pyrolysis conditions influence the porosity and pore size distribution of biochar. *Energy* **2023**, *263*, 126128. [\[CrossRef\]](#)
44. Da Silva, M.C.F.; Schnorr, C.; Lütke, S.F.; Knani, S.; Nascimento, V.X.; Lima, É.C.; Thue, P.S.; Vieillard, J.; Silva, L.F.O.; Dotto, G.L. KOH activated carbons from Brazil nut shell: Preparation, characterization, and their application in phenol adsorption. *Chem. Eng. Res. Des.* **2022**, *187*, 387–396. [\[CrossRef\]](#)
45. Chen, Y.; Zhang, X.; Chen, W.; Yang, H.; Chen, H. The structure evolution of biochar from biomass pyrolysis and its correlation with gas pollutant adsorption performance. *Bioresour. Technol.* **2017**, *246*, 101–109. [\[CrossRef\]](#)
46. Brunauer, S.; Emmett, P.H.; Teller, E. Adsorption of Gases in Multimolecular Layers. *J. Am. Chem. Soc.* **1938**, *60*, 309–319. [\[CrossRef\]](#)
47. Thommes, M.; Kaneko, K.; Neimark, A.V.; Olivier, J.P.; Rodriguez-Reinoso, F.; Rouquerol, J.; Sing, K.S.W. Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report). *Pure Appl. Chem.* **2015**, *87*, 1051–1069. [\[CrossRef\]](#)
48. Li, Y.; Shao, J.; Wang, X.; Deng, Y.; Yang, H.; Chen, H. Characterization of modified biochars derived from bamboo pyrolysis and their utilization for target component (furfural) adsorption. *Energy Fuels* **2014**, *28*, 5119–5127. [\[CrossRef\]](#)
49. Mukhopadhyay, S.; Masto, R.E.; Sarkar, P.; Bari, S. Biochar washing to improve the fuel quality of agro-industrial waste biomass. *J. Energy Inst.* **2022**, *102*, 60–69. [\[CrossRef\]](#)
50. Liu, Z.; Hoekman, S.K.; Balasubramanian, R.; Zhang, F.-S. Improvement of fuel qualities of solid fuel biochars by washing treatment. *Fuel Process. Technol.* **2015**, *134*, 130–135. [\[CrossRef\]](#)

51. Chen, X.; Guo, Y.; Zhang, H.; Cheng, F.; Jiao, Z. Coke powder improving the performance of desulfurized activated carbon from the cyclic thermal regeneration. *Chem. Eng. J.* **2022**, *448*, 137459. [[CrossRef](#)]
52. Li, Y.; Jin, H.; Liu, W.; Su, H.; Lu, Y.; Li, J. Study on regeneration of waste powder activated carbon through pyrolysis and its adsorption capacity of phosphorus. *Sci. Rep.* **2018**, *8*, 778. [[CrossRef](#)]
53. Singh, M.; Ahsan, M.; Pandey, V.; Singh, A.; Mishra, D.; Tiwari, N.; Singh, P.; Karak, T.; Khare, P. Comparative assessment for removal of anionic dye from water by different waste-derived biochar vis a vis reusability of generated sludge. *Biochar* **2022**, *4*, 13. [[CrossRef](#)]
54. Chen, B.; Yuan, M.; Liu, H. Removal of polycyclic aromatic hydrocarbons from aqueous solution using plant residue materials as a biosorbent. *J. Hazard. Mater.* **2011**, *188*, 436–442. [[CrossRef](#)] [[PubMed](#)]
55. Mohd Faizal, A.N.; Putra, N.R.; Abdul Aziz, A.H.; Agi, A.; Ahmad Zaini, M.A. Giant mud crab shell biochar: A promising adsorbent for methyl violet removal in wastewater treatment. *J. Clean. Prod.* **2024**, *447*, 141637. [[CrossRef](#)]
56. Gallego-Ramírez, C.; Chica, E.; Rubio-Clemente, A. A study of the feasibility of pinus patula biochar: The regeneration of the indigo carmine-loaded biochar and its efficiency for real textile wastewater treatment. *Processes* **2024**, *12*, 939. [[CrossRef](#)]
57. Bardestani, R.; Patience, G.S.; Kaliaguine, S. Experimental methods in chemical engineering: Specific surface area and pore size distribution measurements—BET, BJH, and DFT. *Can. J. Chem. Eng.* **2019**, *97*, 2781–2791. [[CrossRef](#)]

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