

Article

Vetiver Phytoremediation of Nitrate-Rich Munition Wastewater: Biomass Valorization Within a Circular Economy Framework

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Abstract

Industrial munition facilities generate nitrate-rich wastewater that requires effective and sustainable treatment before discharge. While vetiver grass (*Chrysopogon zizanioides*)-based phytoremediation has shown promise in nitrogen removal, most studies have been limited to small-scale experiments under low nitrogen concentrations, providing limited guidance for system-level design under elevated loading. This study evaluates a greenhouse-scale phytoremediation system using vetiver grass to treat wastewater containing 1000 mg N/L nitrate and 50 mg/L COD. Plant coverage densities of 2%, 4%, and 6% (*w/v*) were assessed to quantify optimum density as a design parameter for optimizing nitrate removal. A 6% coverage achieved the highest efficiency, reducing nitrate by 75% to 253 mg N/L over four months. Plant growth, chlorophyll, protein content, and antioxidant enzyme activity were analyzed to elucidate physiological adaptation under sustained nitrate stress. Harvested biomass was subsequently valorized into biochar and bioethanol, with TCLP tests confirming minimal leaching risk. By integrating performance optimization, stress-response analysis, and biomass conversion, this work advances vetiver phytotechnology toward scalable, design-oriented nitrogen removal within a circular treatment framework.

Keywords: nitrate; vetiver; antioxidant enzymes; biochar; bioethanol; circular economy

1. Introduction

Nitrogen-containing compounds, particularly nitrate, nitrite, and ammonium, are among the most prevalent contaminants detected in both drinking water sources and industrial wastewaters. Wastewater effluents from munition manufacturing facilities containing nitrogen compounds require pretreatment in accordance with regulatory guidelines prior to discharge. Conventional wastewater treatment processes primarily rely on chemical and biochemical methods, which break down energetic compounds (ECs) to inorganic nitrogen species, including nitrate, nitrite, and ammonium [1,2]. These effluents are a significant contributor of nitrogen compounds in aquatic systems if released without adequate treatment. These nitrogen species can contribute to eutrophication, oxygen depletion, and degradation of downstream aquatic ecosystems. As regulatory requirements change, there is an increasing need for sustainable, low-energy treatment technologies capable of treating wastewater with high concentrations of nitrogen compounds.



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Constructed wetland systems, designed for wastewater treatment, provide a practical, economical, and environmentally friendly substitute for traditional treatment approaches [3]. Nitrogen species removal in these engineered and managed systems depends on the synergy of naturally occurring biological, chemical, and physical processes found in wetlands. The primary constituents of these processes are sediments, aquatic plants (macrophytes), and bacteria that interact within the plant rhizosphere [4]. However, most wetland-based systems have been designed for dilute or moderately contaminated waters. Hence, their performance under high nitrogen concentrations is not well understood. Specifically, there is a need to evaluate and optimize design parameters related to treatment efficiency, such as plant density, plant stress responses, and hydraulic parameters.

Various wetland macrophytes, including *Typha* spp., *Phragmites australis*, and *Scirpus* spp., have been widely investigated for nitrogen removal in constructed wetlands. However, their applications have largely focused on municipal or agricultural wastewater with relatively low to moderate nitrogen concentrations, whereas information on plant performance under high-strength industrial nitrate conditions remains limited [5]. Among the candidate macrophytes suitable for use in high nitrogen systems, vetiver grass (*Chrysopogon zizanioides*) has several advantages, such as exhibiting rapid growth capabilities, an extensive root system, and the ability to tolerate a wide range of environmental stresses. The Sunshine variety of vetiver is designated as a noninvasive plant by the United States Department of Agriculture (USDA), lacking the ability to produce seeds or propagate via rhizomes [2]. Vetiver grass has become a popular choice for the effective phytoremediation of industrial wastewaters [6]. Recent studies have further demonstrated the potential of vetiver-based constructed wetlands for improving wastewater quality by optimizing operational parameters such as plant density and hydraulic retention time, highlighting the importance of system-level design in nature-based treatment technologies [7]. Furthermore, in floating island systems treating municipal wastewater, vetiver achieved nitrate removal efficiencies of up to 39% after a 30-day hydraulic retention time, confirming its potential for nitrogen removal under engineered wetland conditions [8]. Previous research using vetiver grass in wastewater systems has primarily focused on lower nitrogen concentrations [9–11]. Panja et al. (2018) first used vetiver under very high nitrate concentrations (as extreme as 352,734 mg N/L) [2] in a bench-scale study using wastewater from a munition plant. Vetiver displayed toxicity symptoms and reduced biomass at that concentration. A subsequent bench-scale investigation demonstrated that vetiver could tolerate nitrate concentrations up to 1850 mg N/L [12]. However, these studies were confined to short-term, bench-scale experiments, providing limited insight into plant–water interactions. Larger-scale evaluations under more realistic conditions remain unexplored. The influence of plant density on nitrate removal kinetics and associated physiological trade-offs under sustained high nitrogen exposure remains unknown. Moreover, the role of oxidative stress responses, such as antioxidant enzyme activity, as indicators of plant performance under nitrogen stress has not been systematically investigated.

Beyond contaminant removal, there is also growing interest in aligning wastewater treatment with circular economy principles. Using high-biomass plants such as vetiver is conducive to waste reduction and generating value-added products [13,14]. Vetiver biomass can be repurposed into value-added products such as biochar and bioethanol [15]. However, the impact of high nitrogen exposure on biomass quality and suitability for downstream recovery pathways has not been comprehensively evaluated, limiting the development of integrated treatment systems that couple pollutant removal with resource recovery [16].

This study addresses these knowledge gaps by evaluating the performance of a vetiver-based system under elevated nitrogen loading conditions in wastewater. Specifically, we

investigated the role of plant coverage density as a key design parameter influencing treatment efficiency. The objectives of this study were to (i) evaluate nitrate removal efficiency under different plant density coverage conditions, (ii) examine associated changes in physicochemical parameters and antioxidant enzyme responses under nitrogen stress, and (iii) explore the feasibility of valorizing harvested biomass into biochar and bioethanol within a circular management framework. By integrating greenhouse-scale performance evaluation, plant density optimization, physiological stress analysis, and biomass valorization within a single framework, this work advances vetiver-based phytoremediation beyond proof-of-concept studies toward a scalable, design-oriented application for high-strength industrial nitrogen removal. Overall, this study provides an integrated evaluation of vetiver grass for high-strength nitrate removal and resource recovery, offering insights into its potential role in sustainable wastewater treatment systems.

2. Materials and Methods

2.1. Synthetic Wastewater Media

Synthetic wastewater was formulated to simulate munition effluent using KHCO_3 (1250 mg/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (7.5 mg/L), $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (26.25 mg/L), KH_2PO_4 (18.75 mg/L), $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ (225 mg/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (150 mg/L), and 1.05 mL/L of trace element solution. Sodium nitrate (NaNO_3) was used as the nitrate source, and the nitrate concentration was adjusted to 1000 mg N/L based on earlier findings that vetiver tolerates ≤ 1850 mg N/L in batch studies [12] but showed decline at 1500 mg N/L in preliminary greenhouse tests. COD was set to 50 mg/L using glucose.

2.2. Experimental Design

Vetiver slips, approximately 8 to 10 inches in length, were planted in a controlled greenhouse environment at a temperature of 25 °C and subjected to a 14 h photoperiod for a duration of two months. Following this period, the plants were carefully removed from their pots, and the roots underwent a thorough cleansing process to eliminate any soil particles. Both the roots and shoots received precise trimming and they were acclimatized in the hydroponic system before it is transferred to the 50-gallon water tanks (containing 30-gallon synthetic wastewater). The vetiver grass was secured within the water tank using nets and zip ties, ensuring its stability.

Wastewater samples (50 mL) were initially collected in triplicate at time points of 0, 3, and 7 days, followed by weekly sampling thereafter until day 120. Periodic modifications to the volume were implemented to compensate for the reduction in water due to evapotranspiration.

2.3. Mesocosm Setup

Five 50-gallon tanks were used for setting up a mesocosm scale constructed wetland. Three plant coverage rates were tested, which were 2, 4 and 6% (*w/v*) vetiver in the 50-gallon water tanks (containing 30-gallon synthetic wastewater). In addition, two control experiments were conducted. One control experiment was using 0% (*w/v*) plant coverage rate (Control #1). The other control experiments included a 2% (*w/v*) vetiver coverage rate, but there was no nitrate in the synthetic wastewater (Control #2).

These two control experiments were conducted in the study to establish precise baseline conditions for comparison. The first control experiment involved a tank with a 0% (*w/v*) plant coverage rate. This control was used to provide a clear reference point, allowing us to observe the natural processes within the system when no vetiver plants were present, serving as a control for the absence of plant-mediated effects. The second control experiment maintained a 2% (*w/v*) vetiver coverage rate, similar to the experimental tanks,

but with a unique distinction: the synthetic wastewater in this control did not contain any nitrate. This specific control condition was designed to isolate and assess the impact of nitrate levels in the absence of other potential factors. By creating this control, we gain a clearer understanding of the role that nitrate plays in the observed effects of the vetiver-covered tanks, highlighting its specific contribution to the overall outcome.

2.4. Chemical Analyses

Nitrate concentrations were measured in triplicate using an Ion Chromatograph (IC, Dionex ICS-5000+ AS-AP, Thermo Scientific, Waltham, MA, USA). COD was quantified using HACH TNT 821/822 kits and a DR6000™ UV-VIS spectrophotometer (Hach Company, Loveland, CO, USA). pH and electrical conductivity (EC) were monitored using calibrated Accumet™ pH meters (Thermo Fisher Scientific, Waltham, MA, USA) and Oakton ECTestr® conductivity meters (Cole-Parmer, Vernon Hills, IL, USA).

2.5. Vetiver Growth

Initial and final whole-plant fresh weight and root/shoot length were recorded to assess biomass response under high nitrate loading.

2.6. Measurement of Chlorophyll

Chlorophyll a, chlorophyll b, and total chlorophyll were measured on days 0 and 120. Shoots (100 mg) were extracted with 80% acetone, incubated at 4 °C for 24 h, and analyzed spectrophotometrically [17,18].

2.7. Protein and Antioxidant Enzymes Activity

Protein was extracted using the Pierce Plant Total Protein Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) and quantified with the BCA assay. Activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase were analyzed using Cayman Chemical enzyme assay kits (Cayman Chemical, Ann Arbor, MI, USA), following Liu et al. (2013) for methodological consistency [18].

2.8. Production and Characterization of Biochar and Bioethanol

Spent roots were pyrolyzed at 500 °C for 60 min under N₂ (0.1 mL/min) to produce biochar. Characterization included BET surface area, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), elemental analysis, cation exchange capacity, and Toxicity Characteristic Leaching Procedure (TCLP leaching) [19]. Shoots were processed for bioethanol via washing, drying, grinding, acid pre-treatment, and simultaneous saccharification and fermentation (SSF) with *Saccharomyces cerevisiae*, following methods adapted from [20]. Ethanol yield was evaluated using HPLC and distillation.

2.9. Statistical Analysis

Data was collected in triplicate and analyzed using two-way ANOVA at a 95% confidence level.

3. Results and Discussion

3.1. pH and EC

During the 4-month experiment, pH (7.7–8.3) and EC (8.5–9.5 µS/cm) remained stable with minimal fluctuations (Table S1), indicating consistent conditions, similar to findings by Stefanakis et al. (2011) [21]. This indicates that the treatment effects were likely to be plant-driven, and not due to physico-chemical fluctuations.

3.2. Growth of Vetiver

Plant height and weight were recorded at day 0 and day 120, with results summarized in Table S2. Root and shoot heights increased across all treatments, with slight shoot gains in the 4% and 6% coverages. Biomass increased only in the nitrate-free control, while all nitrate-treated conditions showed weight loss. These trends indicate nitrogen-induced stress consistent with previous reports [22]. The reduction in biomass despite substantial nitrate removal suggests that prolonged exposure to elevated nitrate concentrations imposed metabolic stress, creating a physiological trade-off between nutrient assimilation and growth maintenance under high-strength loading conditions.

3.3. Chlorophyll Measurement

Chlorophyll a, chlorophyll b, and total chlorophyll were measured at day 120 to assess plant health (Figure S1) and compared with initial values. Control #2 retained only 40% of its original total chlorophyll, while all nitrate-treated conditions showed an almost complete and significant decline ($p < 0.05$). These reductions align with previous reports of chlorophyll loss under nitrogen-induced phytotoxicity [6].

3.4. Nitrate Concentration

Nitrate removal under different vetiver coverage rates is shown in Figure 1. The 6% (w/v) vetiver treatment achieved the highest removal, reducing nitrate by 75% from 1000 mg N/L to 253 mg N/L ($p < 0.05$). The 4% coverage removed over 60%, while the 2% coverage lowered nitrate by 400 mg N/L to 603 mg N/L. In Control #1 (no vetiver), nitrate declined only to 725 mg N/L, and Control #2 (no nitrate) showed no change. Overall, removal efficiencies reached 75%, consistent with earlier findings using floating treatment wetlands [23] and studies showing 75–85% nitrogen reduction with vetiver-based floating systems [9].

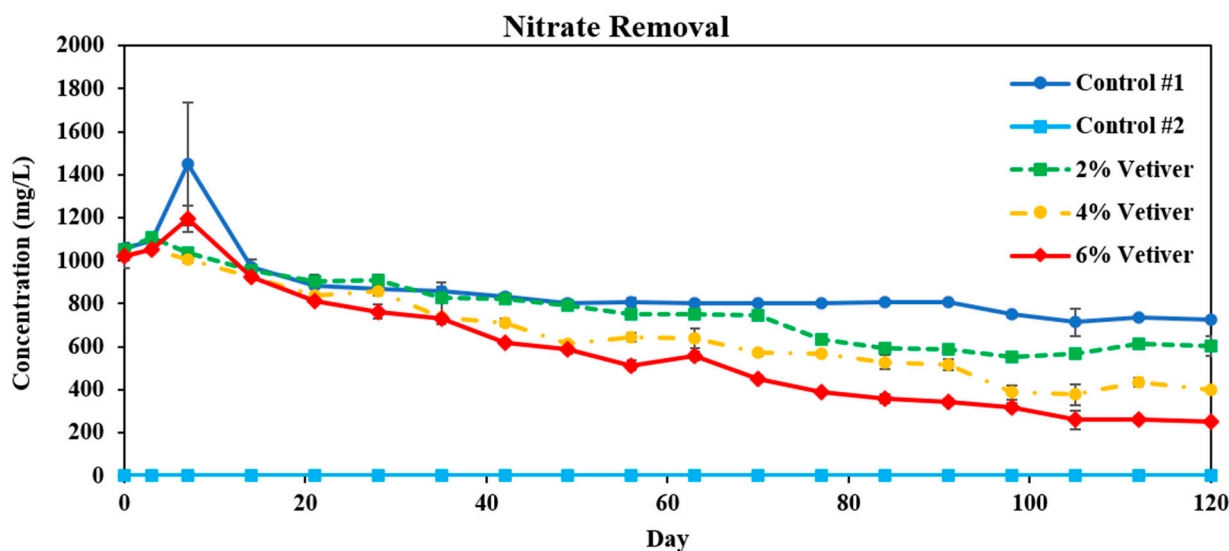


Figure 1. Effects of different vetiver grass coverage rates (including Control #2: 2% vetiver, no nitrate) on nitrate removal under an initial 1000 mg N/L nitrate concentration over 120 days.

The progressive increase in removal efficiency with plant coverage demonstrates that plant density is a primary factor driving treatment performance. To further evaluate nitrate uptake dynamics, first and second-order kinetic models were examined. The first-order model provided a better fit and was used for comparison. Apparent first-order rate constants increased from 0.003 day^{-1} in the control to 0.005, 0.0087, and 0.0132 day^{-1} for the 2%, 4%, and 6% (w/v) vetiver treatments, respectively, indicating faster nitrate

removal with increasing plant density. This density-dependent increase in first-order rate constants establishes quantitative thresholds for optimizing phytoremediation performance at the greenhouse scale. The ability to quantify density-dependent rate enhancement represents a key advance, which will help in designing remediation systems for wastewater. Mechanistically, higher coverage likely increased root surface area, rhizosphere microbial activity, and coupled plant-microbe nitrogen transformations, thereby enhancing nitrate assimilation capacity. Notably, the controls exhibited a partial reduction in nitrate levels, which indicates some background microbial activity; however, a significantly higher rate of removal from planted systems confirms the dominant role of vetiver plants in the removal process.

3.5. COD Concentrations

COD levels varied significantly across treatments (Figure S2). In Control #1, COD rose sharply to 250 mg/L after four months ($p < 0.05$), while Control #2 reached 140 mg/L. In the 2% and 4% vetiver coverages, COD increased to 230 mg/L, and at 6% coverage it reached 207 mg/L. The comparatively lower COD at higher plant coverage suggests improved stabilization through enhanced plant-microbial interactions.

Under sustained high nitrate loading, partial plant senescence likely induced the release of root-derived dissolved organic carbon, altering COD dynamics. COD increased by 50 mg/L in the first two months, 100 mg/L in the third month, and 80 mg/L in the final month. Initial reductions likely reflected organic oxidation and microalgal uptake, whereas later increases were associated with root exudates and microbial byproducts [24,25]. Overall, results highlight vetiver's key role in stabilizing COD in wastewater.

3.6. Protein Concentration of Plant Samples

Figure S3 shows changes in protein concentrations in vetiver roots and shoots over four months. In Control #2, root protein decreased from 3.82 to 1.9 mg/mL ($p > 0.05$), while all vetiver coverage treatments showed small but consistent protein increases. Notably, the 6% (w/v) coverage exhibited the largest rise, from 4.57 to 5.43 mg/mL. These trends align with studies demonstrating that nitrogen availability enhances protein synthesis in plants, such as increased protein in poplar under elevated nitrate [26] and protein gains in *Salvinia molesta* under ammonium exposure [27]. The observed increase in protein concentration under nitrate treatments likely reflects enhanced nitrogen assimilation and incorporation into amino acids and stress-related proteins. In particular, elevated protein levels may be associated with upregulation of antioxidant enzymes and other defense mechanisms activated under high nitrate loading, consistent with the enzyme activity patterns observed in this study.

3.7. Antioxidant Enzymes of Vetiver Plants

This study evaluated nitrogen-induced oxidative stress in vetiver by monitoring antioxidant enzyme activity over four months. Nitrogen uptake generates reactive oxygen species (ROS), activating the plant's antioxidant defense system [28]. The initial antioxidant reaction to nitrogen stress was evaluated through the assessment of Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-s-transferase (GST), and Superoxide Dismutase (SOD) activities. The findings are presented in Figure 2.

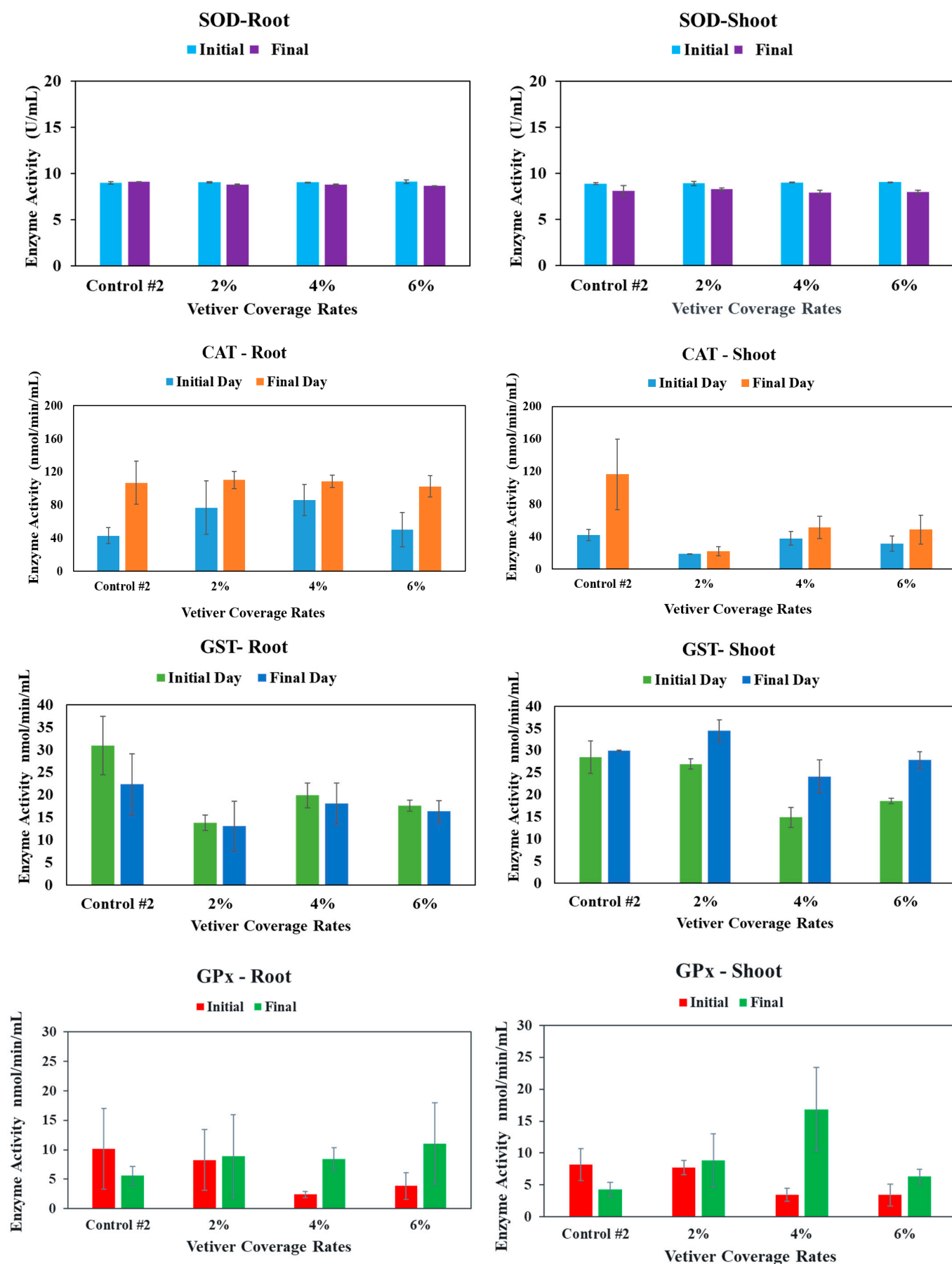


Figure 2. Temporal changes in root and shoot antioxidant enzyme activities of vetiver under different coverage rates, including the Control #2 treatment, during the 120 day experimental period.

Superoxide dismutase, usually the first line of defense against superoxide radicals [28], showed no significant changes in either roots or shoots across all treatments ($p > 0.05$). CAT, which converts H_2O_2 to water [29], increased in all treatments, with greater rises in roots than shoots. CAT levels in controls tripled, while 2%, 4%, and 6% vetiver coverages rose by 30–50%. The pronounced CAT response suggests that hydrogen peroxide detoxification played a dominant role in mitigating nitrate-induced oxidative stress. Compared with the other antioxidant enzymes, CAT exhibited the strongest response because hydrogen peroxide appears to have been the predominant reactive oxygen species generated under prolonged high-nitrate exposure. Catalase has a high catalytic capacity for rapidly scavenging H_2O_2 without requiring additional reducing substrates, making it particularly effective under sustained oxidative stress [30]. In contrast, SOD remained unchanged, suggesting that superoxide radical formation was not the limiting factor in this system.

GPx activity, though much lower than CAT, increased under vetiver treatments but decreased in controls. The strongest increases occurred in 4% and 6% vetiver, whereas Control #2 showed a 50% decline. Despite these trends, GPx changes were not statistically significant ($p > 0.05$).

GST, which detoxifies ROS by binding them to glutathione [31], decreased in roots under all treatments but increased markedly in shoots. Significant GST rises in shoots ($p < 0.05$), were observed, indicating higher stress response in aerial tissues.

These greenhouse study results are consistent with our earlier batch bottle experiments. Although no previous studies have examined such high nitrogen concentrations, our findings align with research showing increased antioxidant activity under nitrogen stress, including nitrate-induced responses in forage crops [32] and ammonium-induced oxidative stress in *Myriophyllum*, tobacco, *Arabidopsis*, and wheat [33–35]. Overall, vetiver activated multiple antioxidant pathways to mitigate nitrogen-driven ROS formation.

3.8. Impact of Varying Coverage Rates on the Production of Biochar

Table 1 shows biochar yields from vetiver exposed to nitrate at different coverage rates. The highest yield (34%) occurred in Control #2, while 6% vetiver produced 30%. Although previous studies link higher carbon content to increased char yield [36,37], no direct correlation was observed in this study. These differences suggest that nitrate exposure during plant growth may have influenced biomass composition prior to pyrolysis, thereby affecting carbonization behavior. Table 1 also indicates that lower vetiver coverage produced biochar with higher carbon and lower oxygen content, showing a strong negative correlation (90.7, $p > 0.05$). Although not statistically significant, this trend suggests enhanced carbon condensation under lower coverage conditions. Decreases in H/C and O/C ratios also reflected enhanced aromaticity and reduced hydrophilicity [38,39], indicating improved structural stability and adsorption potential.

Table 1. Biochar yield and elemental analysis of spent vetiver roots.

Samples	Biochar Yield%	%C	%N	%H	%O	H/C	O/C	%P	%Al	%Si
Control #2	33.7	65.0	0.5	2.2	20.9	0.034	0.322	0.028	0.001	0.001
2% Vetiver	32.75	72.7	0.8	2.1	15.7	0.029	0.216	0.025	0.009	0.002
4% Vetiver	32.28	66.6	0.9	2.0	20.2	0.029	0.304	0.043	0.007	0.002
6% Vetiver	30.36	66.5	0.4	2.1	20.2	0.031	0.304	0.053	0.005	0.002

Note: Control #2: 2% vetiver, no nitrate.

Figure S4 shows that cation exchange capacity (CEC) declined with nitrate exposure; 2% vetiver yielded significantly lower CEC ($p < 0.05$), likely due to loss of oxygen-containing functional groups and increased aromaticity [40,41]. The BET surface areas of

the biochars produced from the control, 2%, 4%, and 6% vetiver coverage treatments were 298.2, 312.3, 336.7, and 349.8 m²/g, respectively, which are comparable to values reported for root-derived biochar produced at 500 °C [42]. TCLP testing confirmed that RCRA metals in all biochar samples remained below regulatory thresholds, matching findings by Nagara et al. (2024) [14]. Importantly, high surface areas were maintained in nitrate treatments, which shows that high-strength nitrate exposure does not compromise biochar quality; although it may influence functional group chemistry and adsorption behavior.

SEM imaging (Figure 3) showed highly porous, fibrous structures across all nitrate-treated biochars, with well-developed cavities enhancing adsorption potential. Control biochar displayed lower pore volume. FTIR spectra (Figure S5) showed a broad O-H stretching band near 3400 cm⁻¹ and more pronounced peaks around 1400 cm⁻¹ in the nitrate-treated samples. The band near 1400 cm⁻¹ may be attributed to phenolic O-H bending and other oxygen-containing functional groups, indicating changes in the surface chemistry of the biochar following nitrate exposure [43]. Peaks near 900 cm⁻¹ were assigned to C-H bending vibrations commonly associated with alkane structures [44,45].

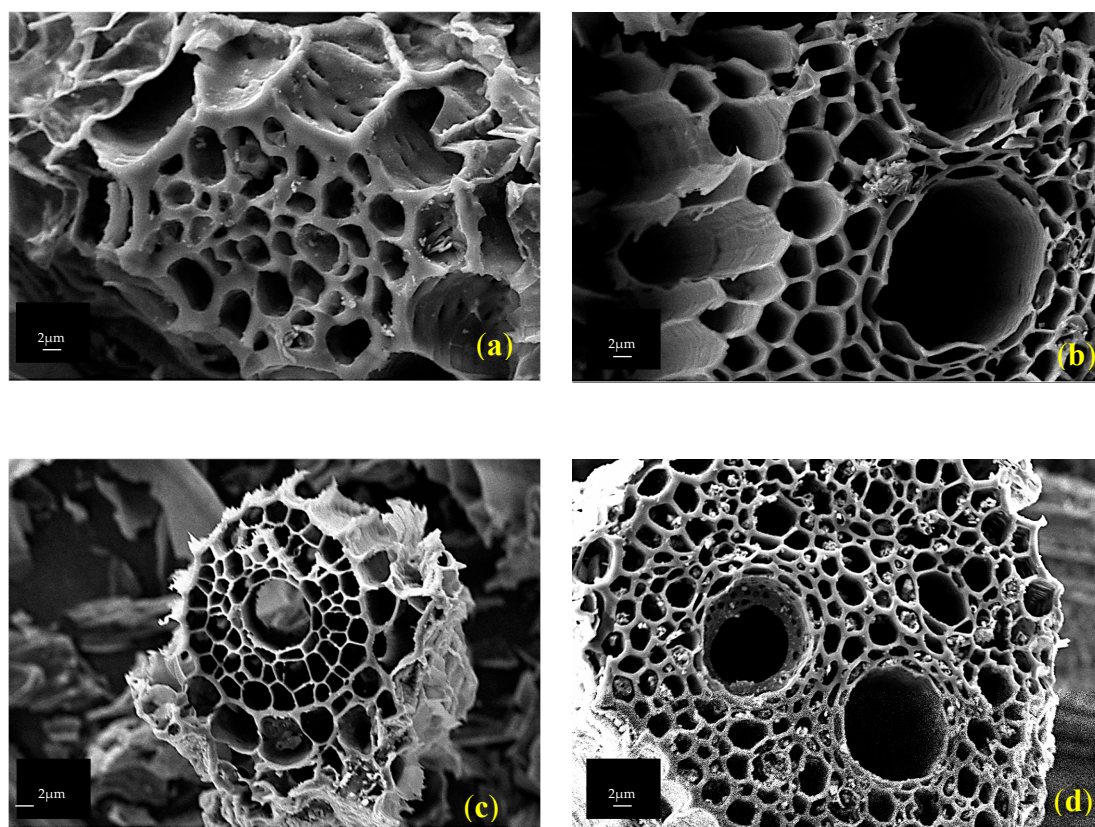


Figure 3. SEM analysis of roots of vetiver: (a) Control #2: 2% vetiver, no nitrate, (b) 2% vetiver (*w/v*), (c) 4% vetiver (*w/v*), (d) 6% vetiver (*w/v*).

XRD analysis (Figure S6) identified the mineral components of the biochar, revealing an amorphous and disordered carbon structure observed around 24°, particularly in the biochar derived from the 2% nitrate solution, which had the highest peak intensity. This indicated a higher level of structural disorder, likely due to the absorption of nitrate ions by the vetiver plant [46]. A peak around 40° indicated alterations in oxygen-containing compounds, with slight variations in phase suggesting nutrient absorption into the biochar structure, although the fundamental structure remained unchanged [47,48].

3.9. Impact of Varying Coverage Rates on the Production of Bioethanol

Figure 4 shows the lignocellulosic composition of spent vetiver, with cellulose, hemicellulose, lignin, and extractives making up roughly 33%, 33%, 14%, and 10% of total biomass. No significant differences ($p > 0.05$) were observed across nitrate treatments, indicating that nitrate uptake did not alter vetiver's structural composition—consistent with findings by Neve et al. (2022) [20]. Cellulose content was comparable to that of other C4 grasses [49,50]. The stability of lignocellulosic fractions under elevated nitrate suggests that prolonged exposure did not disrupt cell wall architecture or carbon partitioning within plant tissues.

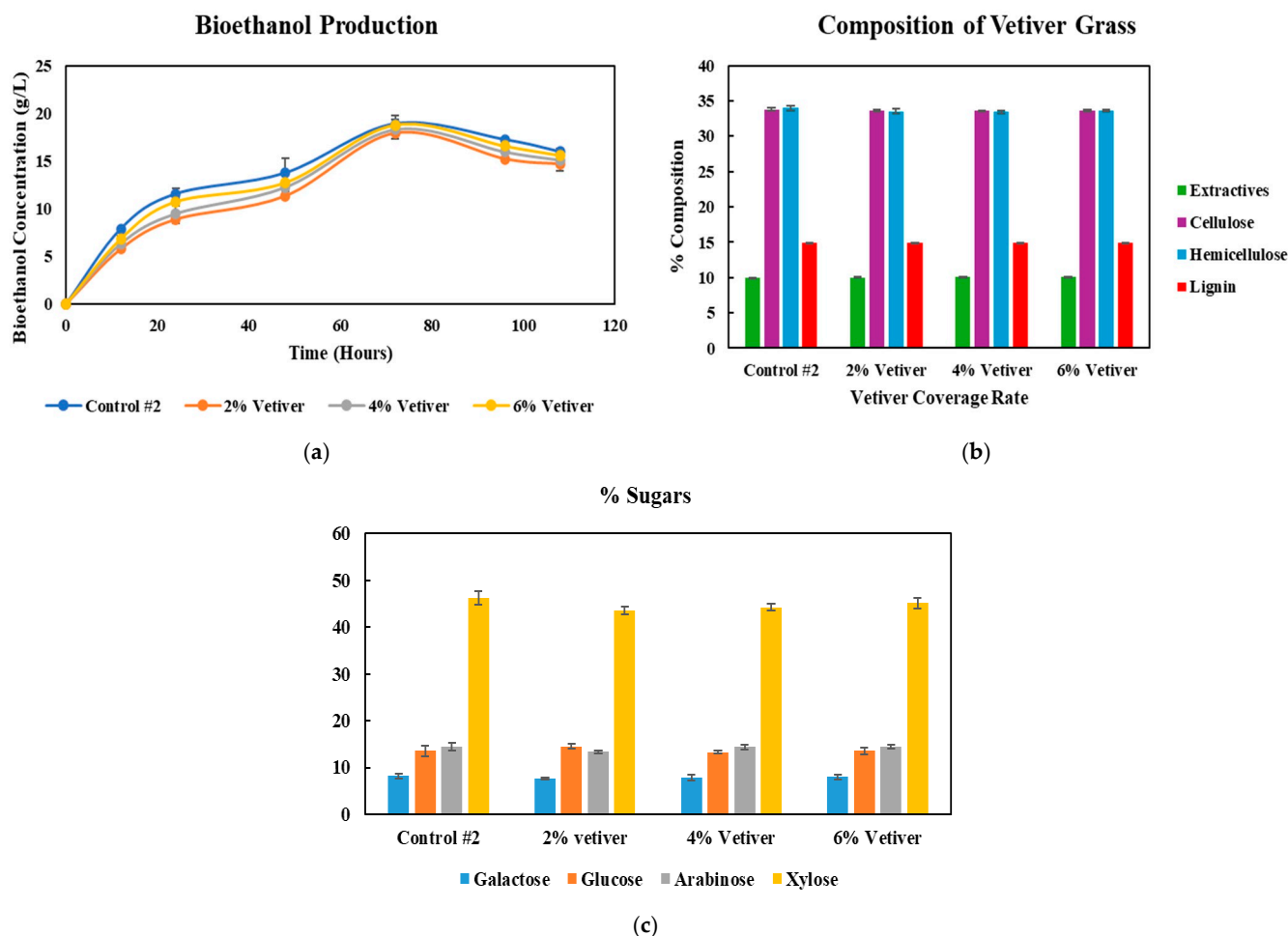


Figure 4. Bioethanol production, composition, and carbohydrate content of spent vetiver shoots under different treatments, including the Control #2 treatment (2% vetiver without nitrate addition). (a) Bioethanol production during simultaneous saccharification and fermentation over 120 h. (b) Biomass composition, including extractives, cellulose, hemicellulose, and lignin. (c) Monomeric sugar composition, including galactose, glucose, arabinose, and xylose.

Bioethanol production from fermented shoots (Figure 4) increased slightly with nitrate exposure but was not significantly different from the control. Fermentation stalled after 72 h, likely due to alcohol inhibition and accumulated reducing sugars [51]. The highest ethanol concentration (18.89 g/L) was obtained from Control #2, following patterns in holocellulose content [20]. Importantly, nitrate exposure did not negatively impact fermentable sugar availability or ethanol productivity, indicating preservation of biomass conversion potential. The recovered ethanol exhibited a purity of 98.30%, density of 0.79 g/mL, and water content of 1.70%, with a clear appearance, consistent with previous vetiver-derived bioethanol [14].

Sugar profiles from two-stage acid–alkali pretreatment (Figure 4) showed higher yields than single-stage methods [52], with xylose dominant (43.5–46.25%). Collectively, these results demonstrate that biomass generated under high-nitrate phytoremediation retains structural integrity and fermentability, supporting its integration into a circular resource recovery framework.

3.10. Economic and Practical Benefits of Biomass Valorization

The production of biochar and bioethanol from harvested vetiver biomass provides both practical and economic advantages by converting a waste by-product of phytoremediation into value-added products. Biochar can be utilized as a low-cost adsorbent, soil amendment, and carbon sequestration material, thereby reducing biomass disposal costs while providing additional environmental benefits. Likewise, bioethanol production offers a renewable energy source that can partially offset the operational costs associated with phytoremediation systems. Integrating contaminant removal with biomass valorization therefore improves resource utilization, supports circular economy principles, and enhances the overall sustainability and economic feasibility of wastewater treatment applications [53].

4. Conclusions

This study demonstrates that plant coverage density is a critical design parameter governing nitrate removal under high-strength loading, with 6% (w/v) vetiver coverage achieving the highest nitrate removal, efficiency, and the fastest kinetics. While effective nitrate uptake was observed, prolonged exposure induced significant physiological stress, evidenced by the loss of biomass, decline in chlorophyll, and activation of the antioxidant defense systems. Among the antioxidant enzymes, CAT-mediated detoxification emerged as the dominant mechanism enabling tolerance to nitrate-induced oxidative stress.

Beyond nitrate removal, this study shows that biomass generated under elevated nitrate treatment retains its structural integrity and can be converted into value-added products, including biochar with high surface area and bioethanol with consistent yield and purity. Overall, this study advanced phytoremediation from a removal-focused approach to a design-oriented, mechanistically informed circular framework. Although the results demonstrate the strong potential of vetiver-based phytoremediation for treating high-strength nitrate-containing wastewater, several limitations should be acknowledged. This study was conducted under controlled greenhouse conditions using a single wastewater composition and treatment duration. Future research could evaluate long-term field-scale performance under varying environmental conditions, investigate the influence of seasonal variability and hydraulic loading, and assess the long-term stability and economic feasibility of large-scale implementation. Nevertheless, the findings indicate that optimizing plant coverage density while integrating biomass valorization through biochar and bioethanol production can enhance both treatment efficiency and resource recovery. These characteristics support the practical application of vetiver-based systems as a sustainable, circular economy approach for large-scale treatment of nitrogen-rich industrial wastewaters.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18151889/s1>, Table S1. Variations in pH and EC levels over 120 days under different coverage rates. Table S2. Vetiver growth under various plant coverage rates. Figure S1. Chlorophyll contents in vetiver leaves at time 0 (Initial Day) and after 120 days (Final Day). Figure S2. Effects of different vetiver grass coverage rates on COD over 120 days. Figure S3. Effects of different vetiver grass coverage rates on protein concentration over 120 days. Figure S4: Cation exchange capacity from roots of vetiver under different coverage conditions. Figure S5: FTIR spectra of the vetiver root biochar. Figure S6: XRD diffractogram of the vetiver root biochar.

Author Contributions: Conceptualization, D.S.; methodology, A.A. and R.D.; validation, A.A., Z.Z., C.C. and R.D.; formal analysis, A.A.; investigation, A.A.; data curation, A.A., Z.Z. and R.D.; writing—original draft preparation, A.A.; writing—review and editing, R.D., Z.Z., C.C. and D.S.; visualization, A.A.; resources, C.C. and D.S., supervision, D.S.; project administration, D.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ECs	energetic compounds
Chl	chlorophyll
ROS	reactive oxygen species
CAT	catalase
GST	glutathione S-transferase
SOD	superoxide dismutase
GPX	glutathione peroxidase
COD	Chemical Oxygen Demands

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