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Green Remediation Technology for Total Petroleum Hydrocarbon-Contaminated Soil

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Abstract: In order to improve the bioremediation efficiency of petroleum-contaminated soil, five test groups were selected in this study, including native bacteria, *Acinetobacter venetianus*, *Vetiveria zizanioides* L., and *Vetiveria zizanioides* L. combined with *Acinetobacter venetianus* and biochar to compare the degradation efficiency of petroleum-contaminated soil. The results of the study showed that after a six-month bioremediation, there was no significant difference between the native bacteria and the A.V. bacteria group in the removal efficiency of TPH, and the proportion of degradable TPH accounts for about 50~70%. The removal efficiency of TPH could be increased by 18.1–29% by increasing the phytoremediation of *Vetiveria zizanioides* L. The cultivation of *Vetiveria zizanioides* L. could not only stabilize the soil's pH and conductivity but could also increase the soil's bacterial abundance. It was suggested that bioremediation could be carried out through the combination of native bacteria and the planting of *Vetiveria zizanioides* L. Although the addition of biochar to the soil was able to improve the remediation effect of *Vetiveria zizanioides* L., it would increase the soil conductivity and reduce the abundance of soil bacteria. Therefore, it was recommended to reduce the conductivity of biochar before adding it, which may improve processing efficiency.

Keywords: bioremediation; phytoremediation; total petroleum hydrocarbon; biochar

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1. Introduction

Petroleum has always been a very important energy source for humans. However, during the process of extraction, storage, transportation, and use of petroleum, accidental leakage often causes soil pollution [1–3]. Aliphatic, cycloaliphatic, and aromatic hydrocarbons are mainly included in petroleum components, which were collectively referred to as total petroleum hydrocarbons (TPH). Soil TPH contamination could be sorted into high volatility, low carbon number TPHg contamination, such as gasoline, and low volatility, high carbon number TPHd contamination, such as diesel, heavy oil, lubricating oil, etc. Soil pollution caused by high volatile TPHg was generally treated by physical methods, such as soil vapor extraction (SVE) and air sparging (AS). For soils with low volatile TPHd contamination, chemical, biological, or thermal treatment techniques were more commonly used [3]. With the advantages of safety, economy, low energy consumption and having little impact on soil properties, bioremediation was considered to be an environmentally friendly green remediation technology [1–5].

The application of microbial degradation and phytoremediation was included in bioremediation. The common methods for treatment with microorganisms included

landfarming, bio-piling, etc. [6]. Many studies have screened strains with high TPH-degradation efficiency from oil-contaminated sites to accelerate the biodegradation efficiency [3,7–11]. *Pseudomonas* sp., *Acinetobacter* sp., and *Rhodococcus* sp. had been proven to have a high degradation ability of petroleum [3,7]. Research indicated that there were various types of genes in the *Acinetobacter* strain, i.e., *alkB*, *alma*, and *cyp153*, that could degrade alkanes of varying chain lengths. *Acinetobacter venetianus*, a species of bacteria that was notable for degrading alkanes, had been a hot research topic regarding TPH-contaminated soil bioremediation [12–15]. Phytoremediation is a technology that uses plants and associated microorganisms for the remediation of contaminates [16,17]. Phytoremediation results in a green landscape and pollution remediation effects, therefore, was a more popular remediation technology compared to physical and chemical remediation techniques [18–20]. For petroleum-contaminated soil, the main mechanisms of phytoremediation were as follows: (1) Metabolism in plants, which converted, decomposed, or synthesized TPH into plant cells in a process referred to as phytotransformation or phytodegradation; (2) Plant roots release low-molecular-weight organic acids, providing microorganisms in the rhizosphere soil with a carbon source or nutrient source, which promotes the decomposition of pollutants by rhizosphere microorganisms in a process called rhizoremediation [21,22]; (3) The evapotranspiration of plant leaves causes pollutants to evaporate into the atmosphere in a process called phytovolatilization [18–20]. Phytoremediation is highly dependent on the selection of plants capable of tolerating the polluted environment and maintaining decent plant vigor [23]. *Vetiveria zizanioides* L. is a perennial herb that is able to grow in a variety of harsh environments [18,19]. Studies pointed out that *Vetiveria zizanioides* L. had the potential to remediate oil-contaminated soil. Studies had also shown that *Vetiveria zizanioides* L. was capable of decomposing fresh and weathered oil-contaminated soils. The cultivation of *Vetiveria zizanioides* L. mainly increased the bioavailability of TPH in the soil and therefore improved the degree of biodegradation of hydrocarbons in the soil [18].

Biochar has the characteristics of porosity and high specific surface area, and when it was applied to soil, soil aeration could be increased and soil fertility could also be improved [4,24–30]. Wang et al. (2017) [31] studied the decomposition of petroleum in soil; the degradation efficiency of the added bulrush straw biochar was 46.9%, and the degradation efficiency without the addition was 28.2%. Kong et al. (2018) [32] studied the remediation efficiency of PAH-contaminated soil. The removal rate of PAH without biochar was about 27.7%; with the addition of 5% wheat straw or sawdust biochar, the PAH removal rate could be increased to about 47.5–55.7%. Aziz et al. (2020) [33] studied diesel-contaminated soil and found that the degradation efficiency of soil with waste biochar was at least two times higher than that without biochar. This was mainly due to the synergistic effect of biostimulation and bioaugmentation by biochar [25–28]. The application of biochar was able to achieve the effect of agricultural waste recycling and soil carbon sequestration, which was a good material for soil petroleum pollution remediation.

Historically, TPH-contaminated soil from a petroleum-polluted site in central Taiwan was used to conduct experiments with different bioremediation combination methods in this study, which included the use of native bacteria, *Acinetobacter venetianus*, and *Vetiveria zizanioides* L., as well as the combination of *A. venetianus* and biochar. The main objective was to improve the TPH's bioremediation efficiency by using various bioremediation methods on TPH-contaminated soil to identify the most economical and effective bioremediation technology among the applied methods.

2. Materials and Methods

The soil tested in this study was collected from a polluted site in a petroleum depot in central Taiwan. The coordinates of the site are 120/32/36, 24/17/57 and the target pollutants were petroleum hydrocarbons (TPH) with high carbon numbers (C_{10}~C_{40}). Contaminated soils with different concentrations were sieved and uniformly mixed into high and low concentrations of soil. The high TPH soil concentration was about 8000 mg/kg;

the low TPH soil concentration was about 3000 mg/kg. The experiments were conducted in pots; each pot contained about 30 kg of soil. They were divided into five experimental groups (A–E), and three replicate experiments were performed for each experimental group. Group A, the control group, used the native bacteria that originally existed in the soil to degrade TPH. Group B was the petroleum-decomposing bacteria test group, for which the confirmed *A. venetianus* was selected for the test. *A. venetianus* bacteria were cultivated and provided by Chinese Petroleum Corporation (CPC), Taiwan. For the preparation of *A. venetianus* solution, we took 600 mL of nutrient medium (Nutrient Broth) and placed it in a 2-L conical flask, followed by 1% of the *A. venetianus* bacterial solution cultured from diesel-contaminated soil, and incubated it in a rotary shaker for 17–19 h at 150 rpm at 30 °C. After incubation, the cultures were centrifuged, and the suspensions were collected for experiments; the concentrations of *A. venetianus* were about 4×10^8~8×10^8 CFU per milliliter. The bacterial liquid was divided into two additions. The first time was one week before the test, and one liter of stock solution was added to each pot, diluting 1:1 with tap water and mixing it into the test soil. The second time was three weeks later when 1 L of stock solution was also added to each pot, diluted 1:1 with tap water, and watered directly on the soil. Group C was the experimental group that involved planting *Vetiveria zizanioides* L. Group D was the experimental group that involved adding *A. venetianus* bacteria and planting *Vetiveria zizanioides* L. The E group was the experimental group in which 2.5% biochar was added to the soil and was cultivated with *Vetiveria zizanioides* L. Biochar, made from the plant *Vetiveria zizanioides* L., was cultivated at the petroleum-contaminated site of CPC. It was prepared by taking the upper part of *Vetiveria zizanioides* L., air-drying the plant, then carbonizing it at 550 °C for an hour. About 750 g of biochar was added to each pot and was evenly mixed before planting *Vetiveria zizanioides* L. *Vetiveria zizanioides* L. was purchased from a horticultural company that is situated in Tianwei Township, Changhua County. The experimental group of *Vetiveria zizanioides* L. was cultivated with two clumps for each pot.

All experimental groups were given the same growth conditions of water and natural sunshine. At sunset time on sunny days, they were watered with tap water to make the soil saturated with water. The soil was collected every two months, and the rhizosphere soil was taken from the experimental group of planting *Vetiveria zizanioides* L. The basic characteristics of the soil were also analyzed, including pH, conductivity, organic matter content, and TPH concentration. For soil pH measurement, 20 g of air-dried soil was taken, and 20 mL of reagent water was added and stirred. Then, the supernatant was taken and measured with a pH electrode. As for soil electrical conductivity, 10 g of air-dried soil was taken, and 50 mL of deionized water was added in. After being shaken at 140 rpm for an hour, it was filtered with Whatman No.5 filter paper, which was directly measured with a conductivity meter. The soil organic matter content was measured using the dry combustion method at 540 °C [34]. For soil TPH concentration analysis, we followed the method NIEA S703.62B, with 2 g of soil taken and 10 mL of n-hexane added. Then, it was extracted via ultrasonic, concentrated under reduced pressure, and then TPH quantified by GC-FID. For GC-FID, an Agilent DB-1 column (30 m × 0.32 mm) was used, and the initial temperature of oven was maintained at 40 °C for 3 min, then heated to 320 °C at a rate of 6 °C/min and held at 320 °C for 10 min. The Next Generation Sequencing (NGS) was used for soil microbiological analysis before the test and five months after the test. The DNA of the samples was extracted using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany). We took 2 ng of DNA; the primers 341F and 805R were used for PCR amplification of the 16S rRNA genes. The PCR amplifications were performed using KAPA HiFi DNA Polymerase (Kapa Biosystems, Wilmington, MA, USA). The amplified PCR products were subjected to 2 × 301 bp Paired-end Sequencing using the MiSeq System (Illumina, San Diego, CA, USA).

3. Results

3.1. Changes in Basic Properties of Test Soil

The soil of the pot experiment was collected from the contaminated site of the petroleum depot, which was contaminated by high carbon fraction of oil. Soils were contaminated with different concentrations and were uniformly mixed into high and low concentrations. The average concentration of TPH was 3029 ± 908 mg/kg in the low-concentration soil collected from three replicate samples and was 7865 ± 1436 mg/kg for the high-concentration soil. The particle size analysis of the soils with the two concentrations showed that the proportion of sand particles accounted for about 98.0%, and silt accounted for about 2.0%. Before the test, the low concentration soil's (3000 mg/kg) pH, conductivity, and organic matter content were 6.97, 171.2 μS/cm, and 7.43%, respectively. As for the high-concentration soil (8000 mg/kg), the pH, conductivity, and organic matter content were 7.60, 167.7 μS/cm, and 8.67%, respectively.

The soil TPH biodegradation test was divided into five test groups from A to E. The changes in soil properties of each experimental group were shown in Figure 1, which had undergone bioremediation experiments for 6 months. Figure 1a showed the change in soil pH. After six months of testing, the pH of the soil in each test group increased. The pH values of low and high concentration were 8.18 and 8.75, respectively, in the soil of the native bacteria group (Group A), and those in the *A. venetianus* bacteria (Group B) were 8.13 and 9.09, respectively. The pH values of low and high concentrations of *Vetiveria zizanioides* L. (Group C), were 7.86 and 8.17, respectively. In Group D, *Vetiveria zizanioides* L. was cultivated and *A. venetianus* bacteria was added, the pH values of the low and high concentrations of those were 7.74 and 8.29, respectively. As for Group E, *Vetiveria zizanioides* L. was cultivated and biochar was added, and its pH values for low and high concentrations were 7.57 and 8.08, respectively. It could be clearly seen from Figure 1a that the pH increases of the experimental groups C, D, and E cultivated with *Vetiveria zizanioides* L. are less than those of experimental groups A and B without planting *Vetiveria zizanioides* L.

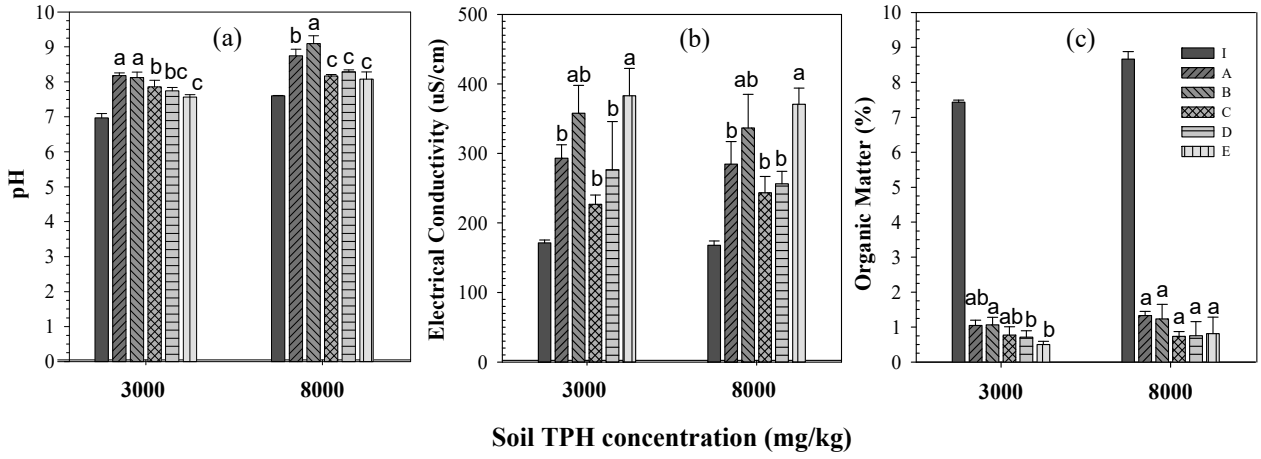

Figure 1. Changes in soil properties before and after bioremediation experiments. (**a**) for pH; (**b**) for electrical conductivity; (**c**) for organic matter. "I" stands for the initial condition before testing. "A": used the native bacteria; "B": added the petroleum-decomposing bacteria; "C": planted *V. zizanioides*; "D": added *A. venetianus* bacteria and planted *V. zizanioides*; "E": 2.5% biochar was added to the soil and planted *V. zizanioides*. Identical lowercase letters on bar indicate statistically insignificant differences; differences indicate significant differences.

Figure 1b showed the change in soil conductivity. The initial conductivities before the high and low concentration soil tests were 167.7 μS/cm and 171.2 μS/cm, respectively. After six months of testing, the soil conductivity for both high and low concentration soil has increased, and the variation trend of electrical conductivity among different

test groups of soil with two concentrations is consistent. The conductivity of Group E (*Vetiveria zizanioides* L. + biochar 2.5%) increased the most. The conductivities of the high and low concentration soils were 370 μS/cm and 383 μS/cm, respectively. The group that increased the second most was Group B. With the addition of *A. venetianus* bacteria, the high and low concentrations of soil conductivity were 357 μS/cm and 336 μS/cm, respectively. Followed by Group A, the native bacteria group, the high and low concentrations of soil conductivity were 293 μS/cm and 284 μS/cm, respectively. The conductivities of high and low concentrations of soil in Group D (*Vetiveria zizanioides* L. + *A. venetianus* bacteria) were 276 μS/cm and 256 μS/cm, respectively. The conductivity of the experimental group C, with only *Vetiveria zizanioides* L. cultivated had the smallest increase, and the conductivities of the soil with high and low concentrations was 227 μS/cm and 243 μS/cm, respectively. In addition to the experimental group E, a test group cultivated with *Vetiveria zizanioides* L. increased little in its conductivity. However, the conductivity of the experimental group E increased more, which was presumed to be mainly due to the higher conductivity of biochar.

Figure 1c showed the change in soil organic matter content. The initial soil organic matter content before the test was 7.43% and 8.67% in the low and high concentration soils respectively. After six months of testing, the organic matter content of all test groups was significantly reduced. The organic matter contents of the low concentration soil in the A and B test groups were 1.04% and 1.06%, respectively. The organic matter content of the three experiment groups cultivated with *Vetiveria zizanioides* L. could be lower than 1%, and the average was between 0.5% and 0.77%. The organic matter content of the high concentration soil in the A and B test groups were 1.33% and 1.24%, respectively. The organic matter content of the three experimental groups cultivated with *Vetiveria zizanioides* L. could also be lower than 1%, with an average of 0.73 to 0.81%.

3.2. Changes in Soil TPH Concentration

The soil TPH concentration in this study was divided into two types: high concentration and low concentration. The initial concentration of TPH in the high and low concentration soil was about 8000 mg/kg and 3000 mg/kg, respectively. The soil TPH biodegradation test was divided into five test groups from A to E. During the experiment, the rhizosphere soil was collected every 2 months to analyze the TPH concentration, and the analysis results were shown in Figure 2.

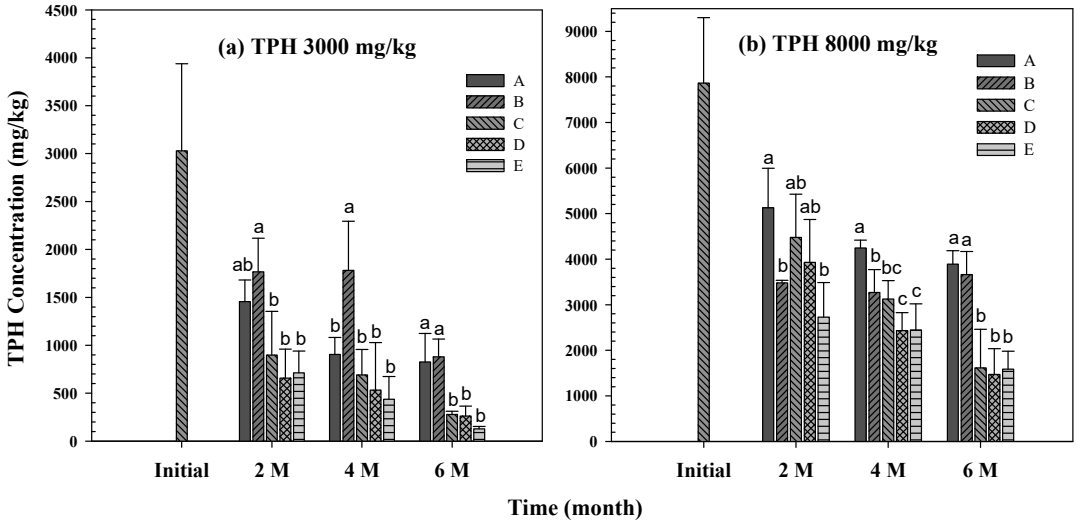

Figure 2. Changes of TPH concentration in different bioremediation test groups. (**a**) for initial soil TPH concentration was 3000 mg/kg; (**b**) for initial soil TPH concentration was 8000 mg/kg. "M" stands for test time (month). Identical lowercase letters on bar indicate statistically insignificant differences; differences indicate significant differences.

Figure 2a showed the changing TPH concentration in each experimental group of low-concentration (3000 mg/kg) TPH-contaminated soil. Group A was a native bacteria control group, the average concentration of soil TPH was 1455.3 ± 226.4 mg/kg 2 months later, and the degradation rate was 52.0%. At four months, the average concentration of soil TPH was 904.7 ± 178.0 mg/kg, and the average degradation rate was 70.1%. After testing for 6 months, the average concentration of soil TPH was 826.3 ± 296.5 mg/kg, and the degradation rate was 72.7%. In group B, the *A. venetianus* bacteria testing group was added. After two months, the average soil TPH concentration was 1765.3 ± 352.6 mg/kg, and the degradation rate was 41.7%. The average concentration of soil TPH at four months was 1781.3 ± 512.0 mg/kg, and the degradation rate was 41.2%. After six months of testing, the average soil TPH concentration was 878.3 ± 186.0 mg/kg, and the degradation rate was 71.0%. There was no significant difference in the degradation efficiency of *A. venetianus* bacteria in the soil and the control group. Group C, the experimental group of planting *Vetiveria zizanioides* L., the average concentration of TPH in the rhizosphere soil of which was 897.2 ± 456.8 mg/kg two months after planting, which was lower than the regulatory standard of 1000 mg/kg, and the degradation rate was 70.4%. At four months, the average concentration was 691.3 ± 265.2 mg/kg, and the degradation rate was 77.2%. After planting for six months, the average soil TPH concentration was merely 278 ± 33.8 mg/kg, and the degradation rate could run up to 90.8%. Group D was the experimental group with *A. venetianus* bacteria added and *Vetiveria zizanioides* L. cultivated simultaneously. After two months, the average concentration of TPH in the rhizosphere soil was 656.9 ± 303.2 mg/kg, and the degradation rate was 78.3%. At about four months, the average concentration was 531.7 ± 496.4 mg/kg, and the degradation rate was 82.5%. At six months, the average soil TPH concentration was 263.0 ± 102.8 mg/kg, and the degradation rate was 91.3%. The cultivation of *Vetiveria zizanioides* L. and the addition of *A. venetianus* bacteria could improve the efficiency of TPH degradation. Two months after testing, the removal efficiency increased by 7.9%. There was also a 5.3% and 0.5% increase after testing for four months and six months. The longer the test time was, the less efficiency the *A. venetianus* strains improved. Group E was the experimental group in which *Vetiveria zizanioides* L. was cultivated after 2.5% biochar was added to the soil. After two months, the average concentration of TPH in the rhizosphere soil was 532.0 ± 227.9 mg/kg, and the degradation rate was 82.4%. At four months, the average concentration of soil TPH was 436.4 mg/kg, and the degradation rate was 85.6%. After testing for six months, the average soil TPH concentration was 128.7 ± 23.3 mg/kg, and the degradation rate was 95.8%. Compared with experimental group C, by adding biochar to the soil while *Vetiveria zizanioides* L. is cultivated, the TPH removal rate could be increased by about 5–8.5%.

Figure 2b showed the changing of TPH concentration in each experimental group of high-concentration polluted soil (TPH concentration about 8000 mg/kg). The average concentration of soil TPH in control group A was 5125.7 ± 870.3 mg/kg at two months, and the degradation rate was 34.8%. At four months, the average concentration of soil TPH was 4243.3 ± 176.0 mg/kg, and the degradation rate was 46.1%. After six-month testing, the average soil TPH concentration was 3893.0 ± 292.3 mg/kg, and the degradation rate was 50.5%. As for the test group B with *A. venetianus* bacteria added, the average concentration of soil TPH, two months after testing was 3665.0 ± 504.1 mg/kg, and the degradation rate was 53.4%. At 4 months, the average concentration of soil TPH was 3479.7 ± 58.2 mg/kg, and the degradation rate was 55.8%. The average soil TPH concentration was 3269.0 ± 502.5 mg/kg, and the degradation rate was 58.4% after six months of testing. Compared with the control group, there was an extra 8% increase in the removal rate by adding *A. venetianus* bacteria. In Group C, with *Vetiveria zizanioides* L. cultivated, the average concentration of soil TPH at two months was 4478.3 ± 947.5 mg/kg, and the degradation rate was 43.1%. At 4 months, the average concentration was 3125.0 ± 402.7 mg/kg, and the degradation rate was 60.3%. After testing for 6 months, the average soil TPH concentration was 1615.7 ± 844.9 mg/kg, and the degradation rate was nearly 80%. Group D, with *A. venetianus* bacteria added and meanwhile planting *Vetiveria zizanioides* L., the average

concentration of soil TPH at two months was 3929.7 ± 941.4 mg/kg, and the degradation rate was 50.0%. At four months, the average concentration was 2432.0 ± 396.0 mg/kg, and the degradation rate was 69.1%. 6 months after testing, the average soil TPH concentration was 1468.7 ± 567.1 mg/kg, and the degradation rate was 81.3%. Compared with Group C, with only *Vetiveria zizanioides* L. cultivated, the removal efficiency of soil TPH in testing Group D increased by 6.98–8.81% at 2–4 months, and only increased by 1.87% at 6 months. In Group E, with biochar added and *Vetiveria zizanioides* L. cultivated, the average concentration of soil TPH at two months was 2728.7 ± 757.1 mg/kg, and the degradation rate was 65.3%. At four months, the average concentration of soil TPH was 2442.3 ± 577.9 mg/kg, and the degradation rate was 69.0%. After six months of testing, the average soil TPH concentration was 1588.3 ± 389.3 mg/kg, and the degradation rate was nearly 80%.

3.3. The Changing of Soil Bacteria

For the two TPH concentration test soils in this study, before the bioremediation test and after the five-month test, the bacterial phase analysis results of soil samples in each test group were shown in Figure 3 and Table 1. Figure 3a for the soil concentration of TPH was 3000 mg/kg. Before the test, the bacteria in the soil included 715 genera and 1092 species. After testing for 5 months, there were 604 genera and 772 species remaining in test group A, and 663 genera and 873 species in test group B; Groups A and B both decreased. The experimental Groups C, D, and E, which were cultivated with *Vetiveria zizanioides* L., had a more abundant bacterial phase. The experimental group C, with only *Vetiveria zizanioides* L. cultivated, contained 865 genera and 1123 species, which was the most abundant. The second most group was group D., with *A. venetianus* bacteria added and *Vetiveria zizanioides* L. cultivated, there were 829 genera and 1119 species contained. Figure 3b for the TPH concentration was 8000 mg/kg soil, and 695 genera and 1078 species were included in the bacteria in the soil before the test. After a five-month experiment, the bacterial phase of the test group A also decreased, with only 627 genera and 827 species remaining. The experimental group D with *Vetiveria zizanioides* L. cultivated and *A. venetianus* bacteria added had the most abundant bacterial phase, which included 790 genera and 1082 species. Unclassified bacteria accounted for a relatively high proportion in the bacterial phase of the test soil, which accounted for about 42% in soil with a TPH concentration of 3000 mg/kg and about 62% in soil at 8000 mg/kg, indicating that there were still quite a few microorganisms in the soil that had not been fully studied.

This study aimed at the petroleum-degrading bacteria *Acinetobacter venetianus*, and the content ratio changes in the soil before and after the test were shown in Figure 4. Before the test, the contents of *A. venetianus* bacteria in the two soils with TPH concentrations of 3000 mg/kg and 8000 mg/kg accounted for about 0.24% and 0.28%, respectively. After a five-month experiment, all test groups, including the test groups B and D with added *A. venetianus* bacteria, showed that the *A. venetianus* bacteria content in the soil was significantly lower than that before the test. The proportion of *A. venetianus* bacteria remaining in the test group B with a TPH concentration of 3000 mg/kg soil was the highest about 0.18%. The group with the second highest concentration was test group D for about 0.034%, in which *Vetiveria zizanioides* L. was cultivated and *A. venetianus* bacteria was added. For TPH concentration 8000 mg/kg soil, although there were relatively abundant *A. venetianus* bacteria at the beginning, the content of which in all test groups had a significant reduction. The content in the test group B was the highest about 0.014%, showing that the soil in this experiment had no assistance in the sustained growth of *A. venetianus* bacteria. The higher the TPH concentration was, the higher the reduction rate of *A. venetianus* bacteria would be. *A. venetianus* bacteria had been confirmed to be capable of degrading TPH. However, in this study, the growth of *A. venetianus* was not as expected. It was speculated that *A. venetianus* bacteria should be the bacteria that was able to degrade fresh oil. The soil in this experiment was weathered petroleum, and therefore could not be used by *A. venetianus*.

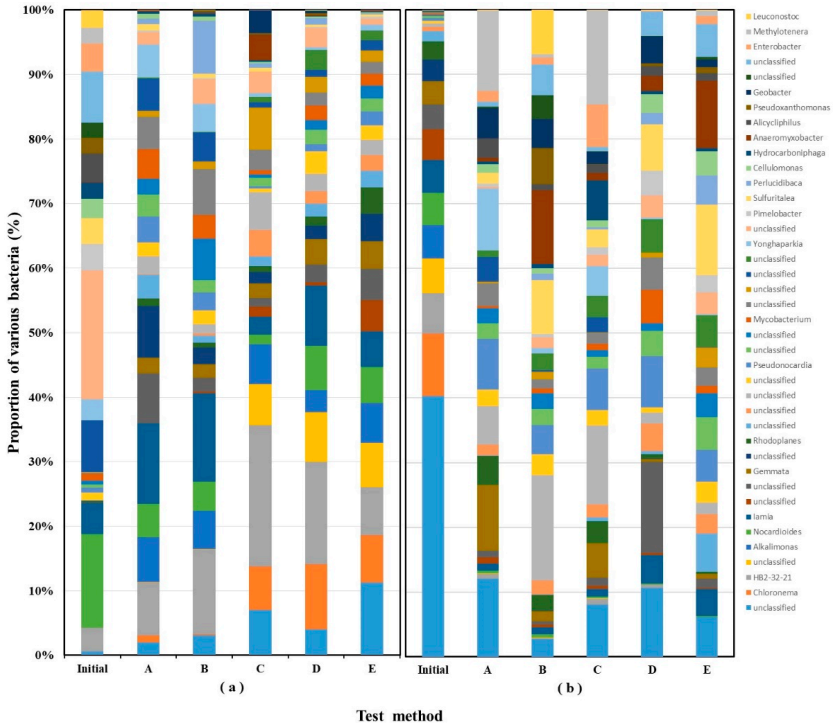

Figure 3. Changes of bacterial phases in different bioremediation test groups. (**a**) for TPH conc. 3000 mg/kg; (**b**) for TPH conc. 8000 mg/kg.

Table 1. Changes in soil bacterial phase abundance before and after the experiment.

Test Method	TPH Concentration 3000 mg/kg		TPH Concentration 8000 mg/kg	
	Genus	Species	Genus	Species
Before Test	715	1092	695	1078
A	604	772	627	827
B	663	873	725	966
C	865	1123	721	947
D	829	1119	790	1082
E	791	1028	706	940

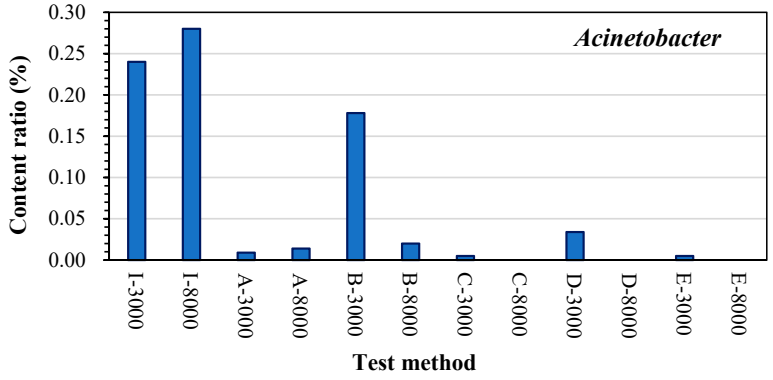

Figure 4. Changes of soil *A. venetianus* bacteria content in different experimental groups before and after the experiment. "3000" and "8000" stand for the soil TPH concentration were 3000 mg/kg and 8000 mg/kg respectively. "I" stands for the sample before testing; "A": used the native bacteria; "B": added the petroleum-decomposing bacteria; "C": planted *V. zizanioides*; "D": added *A. venetianus* bacteria and planted *V. zizanioides*; "E": 2.5% biochar was added to the soil and planted *V. zizanioides*.

4. Discussion

It was found that the soil pH and conductivity increased during the bioremediation process from the comparison of soil characteristics before and after the experiment. Compared with the experimental groups A and B, there was less of an increase in pH and conductivity that in test group C and D, which were cultivated with *Vetiveria zizanioides* L., indicating that the microbial remediation process increased the phytoremediation of *Vetiveria zizanioides* L., which had assistance to stabilize soil properties, including pH and electrical conductivity. In addition, this study found that soil conductivity increased with the addition of biochar. Although the TPH removal efficiency of test group E (with *Vetiveria zizanioides* L. cultivated and biochar added) was slightly better than that of experimental group C, it was found that there was a slightly decrease in the abundance of soil with the addition of biochar from the results of bacterial phase analysis. This indicated that biochar could inhibit the growth of microorganisms, which could probably be related to the increase of electrical conductivity. If the conductivity of biochar can be reduced before being added, washing it with water, for instance, perhaps the degradation efficiency of TPH in test group E can be further improved.

The results of the TPH degradation test showed that the removal efficiency of the in-situ bacteria group reached 70% at four months for low-concentration soil but only increased slightly by 2.6% in the following two months, indicating that the proportion of TPH could be easily degraded by native bacteria in low-concentration soil for about 70%. The degradation efficiency of TPH by *A. venetianus* bacteria is slower than that of the native bacteria in the first four months, indicating that the degradation of the in-situ bacteria could be inhibited with the addition of *A. venetianus* bacteria. Besides, *A. venetianus* bacteria did not play a role in the initial stage. The TPH decomposition rate of the *A. venetianus* bacteria test group also reached about 70% by six months, which was close to that of in situ bacteria. In high-concentration soil, the removal efficiency of Group A was about 46% at four months and 50.5% at six months, with an increase merely about 4.5%, which was similar to the changes in low-concentration soils, indicating that almost all TPH could easily be decomposed by native bacteria at four months. In the first two months, the decomposition efficiency of TPH by adding *A. venetianus* bacteria to high-concentration soil was obviously better, and the removal rate could reach up to 50% or more. However, TPH did not increase significantly in the next 4 months. Comparing the change of TPH removal rate with Figure 4, there were still more *A. venetianus* bacteria remaining in test group B after 5 months in low-concentration soil. Therefore, *A. venetianus* bacteria may play a role in the decomposition efficiency of TPH in the next two months. As for high-concentration soil, the concentration of *A. venetianus* bacteria in test group B was much similar to that in test group A at five months, indicating that *A. venetianus* bacteria did not play a role in the later period and also there was no significant increase in TPH removal rate. The test results of group A and B showed that the proportions of TPH that were easily degraded by native bacteria were similar to *A. venetianus* bacteria in the soil. Low-concentration soil accounted for about 70% and high-concentration soil for about 50%. For high-concentration soil, adding *A. venetianus* bacteria was more effective, but the removal efficiency of adding *A. venetianus* bacteria and native bacteria was not much different under sufficient decomposition time.

Comparing the TPH degradation efficiency of test groups C and D with those of test groups A and B, respectively, we could understand the efficacy of *Vetiveria zizanioides* L. cogongrass phytoremediation. In low concentration soil, the TPH degradation efficiency of group C, which was cultivated with *Vetiveria zizanioides* L., had increased more by an average of 18.1% compared to test group A. Also, the average increase of experimental group D was 20.3% compared with that of experimental group B. For high-concentration soil, the TPH degradation efficiency increased by an average of 29.0% in test group C, which *Vetiveria zizanioides* L. was cultivated. Compared with the test group B, the test group D had an average increase of 23.0%. The research results showed that the remediation effect of TPH-contaminated soil could be effectively improved by phytoremediation. For TPH, being difficult to be degraded by microorganisms, the degradation efficiency

could be further improved by 18.1~29% through phytoremediation. Indicated by the soil characteristics and soil bacteria phase, cultivating *Vetiveria zizanioides* L. not only had the effect of stabilizing the soil characteristics but is also able to reduce the changes in soil pH and conductivity. It was speculated that a suitable environment was provided for the growth of microorganisms, leading to an increase of the abundance of bacterial phases, which contributes more decomposition of TPH. In addition, the metabolism of *Vetiveria zizanioides* L. was helpful for the absorption, degradation, and evapotranspiration of TPH decomposition products, which accelerated the degradation of TPH in the soil of the test group of cultivating *Vetiveria zizanioides* L.

The number of bacterial species in test groups A and B decreased. It was speculated that with the progress of bioremediation, TPH and various nutrients in the soil decreased gradually. Due to the reduction of nutrients, the number of bacteria was reduced. However, in the groups growing plants, because the roots of the plants excreted various organic matter could provide a source of nutrients; therefore, the microorganisms could grow sustainably.

A. venetianus bacteria had been confirmed to be capable of degrading TPH. However, in this study, the growth was not as it was expected. On the contrary, a downward trend in growth was shown. The soil in this experiment was regarded as weathered petroleum, and it was speculated that *A. venetianus* bacteria should be the bacteria that was able to degrade fresh oil. As a result, in this experiment, with the addition of the *A. venetianus* bacteria test group, the degradation efficiency of TPH was not improved.

5. Conclusions

In the high and low concentrations of TPH-contaminated soil tested in this study, the proportion of TPH easily degraded by native bacteria and *A. venetianus* bacteria accounted for about 50% and 70% respectively. The removal efficiency of TPH could be increased by 18.1–29% through the phytoremediation of *Vetiveria zizanioides* L. The cultivating of *Vetiveria zizanioides* L. not only had the effect of stabilizing soil pH and conductivity but could also increase the abundance of soil bacteria. Besides, the landscape greening of the site could also be increased. The removal efficiency of adding *A. venetianus* bacteria was not much different from that of native bacteria. It was suggested that for the bioremediation of petroleum-contaminated soil, the native bacteria could be a combination of cultivating *Vetiveria zizanioides* L. The addition of biochar to soil could improve the remediation effect of *Vetiveria zizanioides* L., but the addition of this had led to the increase of soil conductivity and may therefore reduce the abundance of soil bacteria. It was suggested that the conductivity of biochar could be reduced before testing, making it possible to improve the treatment efficiency more.

The planting of *Vetiveria zizanioides* L. can significantly promote the TPH degradation efficiency in the soil and can be used as a phytoremediation technology for TPH contamination.

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