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Preparation of Micron-Scale Activated Carbon-Immobilized Bacteria for the Adsorption–Biodegradation of Diesel Oil

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Abstract: This paper investigated the micron-scale activated carbon (MAC) immobilized diesel-oil-degrading bacteria (bio-MAC) used as remediation materials for the removal of diesel-oil-contaminated water. The high-efficiency indigenous diesel-oil-degrading bacteria were firstly screened and enriched, then the MAC was used as a diesel oil sorbent and biocarrier for the immobilization of degrading bacteria to prepare the bio-MAC material. The removal performance of the bio-MAC was evaluated via a comparison with the freely degrading bacteria and MAC. The SEM results demonstrated that the diesel-oil-degrading bacteria were effectively immobilized and grew well on the surfaces of MAC particles. The concentration of MAC significantly influenced the growth and activity (DHA and LPS) of immobilized bacteria, and the MAC addition of 3.0 g/L was proven to be an optimum amount for the preparation of bio-MAC. The high-throughput sequencing analysis further indicated that the bacteria immobilized on MAC showed higher abundance levels and diversities index values compared to freely suspended bacteria, such as *Pseudomonas*, *Rhodococcus*, *Bacillus* and *Microbacterium*. The FTIR spectroscopy results showed that the bio-MAC could effectively degrade the aliphatic hydrocarbons, alkenes and aromatic compounds of diesel oil to carboxylic acids, esters, alcohols and other metabolites. When the concentration of diesel oil was 1 g/L, the removal efficiency for the diesel oil of bio-MAC reached 86.35% after 15 days, while only 23.82% and 70.97% of the diesel oil was removed using the same amount of free bacteria and MAC, respectively. The prepared bio-MAC showed a synergic effect of adsorption and biodegradation and efficiently removed diesel oil from wastewater.

Keywords: diesel oil; micron-scale activated carbon; immobilized bacteria; adsorption; biodegradation

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1. Introduction

Diesel oil is a light petroleum product mainly consisting of linear, cyclic and branched aliphatic hydrocarbons, alkenes and aromatic compounds [1]. The leakage of diesel oil during the refining, transportation and usage can lead to serious pollution to soil and water. Diesel oil is extremely toxic in nature, as it comprises several mutagenic and carcinogenic compounds [2]. In water, the presence of diesel oil has detrimental effects on aquatic biological resources [3]. Therefore, it is very important to use the necessary repair techniques once the water is contaminated by diesel oil. The conventional techniques for removing diesel oil can be classified as physical, chemical and biological methods [4]. Bioremediation

is a technique utilizing biological organisms to remove the hazardous substances, which has been proven be the most economical and effective method of contaminated water remediation [5]. However, in the actual remediation process, the free bacterial cells usually had poor adaptability to the adverse environmental conditions, and was not suitable for microbial reproduction in the nutrient-deficient water [6]. Moreover, the biodegradation was a slow process and its removal efficiency was very low during the initial stage of the reaction, especially for the treatment of high concentrations of oil-contaminated water, which greatly limited its application [7].

Immobilizing the degrading bacteria on the surface of biocarriers was proven to enhance the activity and stability of the bacteria, and it could also develop the adsorption–biodegradation reaction to decrease the impact of the fluctuations in contaminant concentration [8]. For instance, some carbon-based materials, such as biochar, activated carbon and biofuel ash, were used to immobilize bacteria and adsorb oil pollutants for water–oil separation [9–11]. The immobilization of degrading bacteria on the carbon materials improved the degradation efficiency of the petroleum hydrocarbon in the bioremediation process [12–14]. Among the commonly reported carbon sorbents, activated carbon (AC) is an excellent adsorbent with stable chemical properties [15], a developed microporous structure [16], a large specific surface area and many functional groups [17], which has been widely used as a carrier for microbial cells in the membrane bioreactor process [12]. However, the smaller the particle size of the activated carbon, the higher the adsorption capacity of the contaminants and bacteria. The application of nano-scale activated carbon was usually held back by the particle aggregation, which usually resulted in large-sized particles with limited reactivity and sorption capacity. According to the deep-bed filtration theory, the colloids with a particle size of 1 μm were demonstrated to have strong migration ability in porous media [12]. Thus, the suitable particle size (1 μm) of the powdered activated carbon with good activity and dispersity in water was important for its application. The micron-scale activated carbon (MAC) immobilized degrading bacteria (bio-MAC) was thought to have good remediation ability for diesel-oil-contaminated wastewater [18].

In diesel-oil-contaminated water treatment technologies, adsorption and biodegradation processes were usually involved. However, developing materials to achieve the synergistic remediation of diesel-oil-polluted water via adsorption and degradation was still a challenge. In this study, we present for the first time the results concerning the effects of synergetic adsorption and biodegradation using bio-MAC for the removal of diesel oil from water. The diesel-oil-degrading bacteria were firstly isolated from diesel-oil-contaminated soil. Subsequently, the screened bacteria were immobilized on MAC powder (1 μm) using adsorption method. High-throughput sequencing analysis was further used to evaluate the abundances and diversity changes of degrading bacterial communities after immobilization. Finally, the diesel oil degradation experiments and characterization procedure were carried out to investigate the performance of the bio-MAC compared with the free bacteria and MAC.

2. Materials and Methods

2.1. Chemicals and Biocarrier

The diesel oil (0#) with a density of 0.84 g/mL was obtained from a gas station in Shijiazhuang city of Hebei province in China. The MAC powders with an average diameter of 1 μm were purchased from Shanghai Hainuo charcoal Co., Ltd., Shanghai, China. The obtained MAC powder had a specific surface area of 406.1 m^2/g, a micropore volume of 0.2562 mL/g, an average micropore diameter of 2.524 nm and a zeta potential of 19.99 mV. The contaminated soils were obtained at a diesel station where a leak occurred in the storage tank. The soils were sampled, air-dried and screened through a 2 mm mesh before the experiment. The physical and chemical properties of the soil are listed in Table 1.

Table 1. Total PAH contents and physicochemical properties of the soil.

PAHs Content (μg/kg)	Moisture (%)	pH	Salinity (g/kg)	Organic Matter (g/kg)	Total Nitrogen (g/kg)	Available P (mg/kg)	Available K (mg/kg)	C/N
1065.33	15.90	8.20	22.51	24.41	1.59	29.07	571.88	15

The other chemicals used here included hexane, glutaraldehyde, glycerin, Na_2SO_4, $FeSO_4{\cdot}7H_2O$, $CaCl_2$, KH_2PO_4, K_2HPO_4, NH_4NO_3, $MgSO_4$ and $MgSO_4$, which were of analytical grade and purchased from the Shanghai Sangon Biotech Co., Ltd., Shanghai, China. The Luria–Bertani (LB) broth and agar powder were purchased from Beijing Aoboxing Biotechnology Co., Ltd., Beijing, China.

2.2. Screening and Enrichment of Bacterial Strains

Before the biological culture experiments, the nutrition medium (NM) and mineral medium (MM) were prepared according to the reported method [19]. Firstly, 20 g of LB was added into 1 L distilled water and stirred to a homogeneous mass to obtain the NM, then the NM was heated to 100 °C and cooled to room temperature (25 °C) and the pH value of the NM was adjusted to 7.2–7.4. The MM was made by dissolving 1.0 g K_2HPO_4, 1.0 g KH_2PO_4, 1.0 g NH_4NO_3, 0.5 g $MnSO_4$, 0.01 g $FeSO_4{\cdot}7H_2O$ and 0.02 g $CaCl_2$ into 1 L distilled water, and its pH was adjusted to 7.0. Finally, the NM and MM were sterilized at 121 °C for 30 min and cooled to room temperature (25 °C).

The dominant degrading bacteria were enriched and acclimated using diesel oil as a sole source of carbon. Here, 1 g diesel-oil-contaminated soil was added into 100 mL sterile water and shaken for 4 h, then 10 mL of suspension was taken out and added into 90 mL of fresh MM solution. Finally, 0.01 g 0# diesel oil was added into the MM solution so that the concentration of oil reached 0.1 g/L. The prepared MM solution containing the bacteria was incubated at 30 °C in a shaker (150 rpm) over 7 days. The acclimatization process was achieved by gradually increasing the diesel oil concentration from 0.1 g/L to 1 g/L (concentration increased by 0.2 g/L every 5 days) in the prepared MM solution. During this stage, the cultured bacteria were thought to have acclimatized to the use of diesel oil as a sole carbon source and energy source. Finally, the bacteria with high activity were obtained via domesticating three times in MM solution containing 1.0 g/L diesel oil. The screened bacteria were preserved as a glycerol suspension (20%, w/v) at −80 °C.

Ten of the dominant strains were screened from the cultured bacteria via the plate line separation and purification method, and then the ten strains were further identified using high-throughput sequencing technology. The removal capacity levels of the diesel oil for the ten single strains and mixed strains were also analyzed to evaluate their diesel oil degradation ability in wastewater. The bacteria-free contaminated solution was set as a control group.

2.3. Preparation and Characterization of Bio-MAC

To obtain the optimum quantity of added MAC during the immobilization of degrading bacteria, the MAC suspensions with different concentrations of 1, 2, 3, 4 and 5 g/L were prepared and sterilized at 121 °C for 30 min. After the MAC suspensions were cooled down to room temperature (25 °C), 10 mL of screened diesel-oil-degrading bacteria was added to each MAC suspension liquid. All of the samples were incubated in a shaker (80 rpm) at 30 °C for 30 h to ensure that the diesel-oil-degrading bacteria were adsorbed and immobilized on the MAC powders [20].

The high-resolution scanning electron microscopy (SEM) analysis was used for morphological observations of the prepared materials. In order to conduct the SEM observation without damaging the bio-MAC, the bio-MAC was firstly pretreated via soaking with glutaraldehyde and freeze-drying [21]. In detail, the bio-MAC was soaked for 48 h with glutaraldehyde; dehydrated using ethanol at volume ratios of 30%, 50%, 70%, 90% and 100% for 20 min; then dried naturally and treated using gold sputtering before the SEM

observation. The morphology was observed using scanning electron microscopy (Hitachi, S-4800) with an accelerating voltage of 20 keV.

The functional group changes of the diesel oil in solution before and after the reaction with the degrading bacteria and bio-MAC were analyzed using the Fourier transform infrared (FTIR) spectra using an iS10 FT-IR spectrometer (Nicholas, American) at a wavenumber range of 400–4000 cm^{-1}. The spectrometer's resolution was 4 cm^{-1}, and scanning was performed 32 times. The KBr method was used for the preparation of the samples.

2.4. Influence of MAC Concentration on the Activity of Diesel-Oil-Degrading Strains

In order to identify the influence of the added MAC concentration on the activity of the immobilized bacteria, the activity levels of dehydrogenase and lipase and the concentration of the bacteria in water were also investigated in the diesel oil degradation experiment. Aliquots (10 mL) of bio-MAC (MAC addition was 1, 2, 3, 4 and 5 g/L, respectively) were added to 90 mL of solution at a concentration of 1 g/L diesel oil. The reaction was performed at 30 °C in a shaker (150 rpm) for 15 days. The prepared MM solution without the addition of MAC was used for blank samples. All samples were produced in triplicate.

The concentration of diesel oil in water was measured at 256 nm with a UV–visible spectrophotometer (Shimadzu, UV-2550), which was extracted using normal hexane as the solvent [22]. The dehydrogenase and lipase were measured using the colorimetric method [23]. In detail, the modified tetrazolium salt was used as a hydrogen receptor of dehydrogenase, which was detected at 460 nm. The lipase content was determined using *p*-nitrophenol (PNP) ester as the substrate. The substrate of lipase can hydrolyze to colored *p*-nitrophenol, and the concentration of *p*-nitrophenol could be measured at 405 nm absorbance using a UV–Vis spectrophotometer.

The concentration of degrading bacteria in solution was analyzed by adopting the plate counting method [24]. The original cultured bacterial solution was diluted into different multiples (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}) using the sterilized deionized water. The bacterial solutions were inoculated on the sterilized LB agar and cultured at 30 °C over 30 h in the biochemical incubator (Boxun, BSP-250). Finally, the numbers of bacteria could be calculated in the culture dish.

2.5. Identification of Free and Immobilized Degrading Bacteria

The free and immobilized cells of the degrading bacteria were enriched on the 0.22 μm membrane firstly, then the membrane was cut into pieces for the following total DNA extraction. The DNA extraction, PCR amplification, purification, pooling and sequencing of a region of the 16S rRNA gene were performed using standard procedures by Shanghai Personal Biotechnology Co., Ltd. The 16S rDNA gene of each isolate was PCR-amplified and -sequenced using universal primers 27F (AAGGAGGTGATCCAGCCGCA) and 1492R (GAGAGTTTGATCCTGG CTCAG). Raw data generated from the high-throughput sequencing run were processed and analyzed following the pipelines of Mothur and QIIME.

2.6. Removal of Diesel Oil by Free and MAC Immobilized Bacteria

The adsorption–biodegradation efficiency of diesel oil using bio-MAC was compared with the freely suspended degrading bacteria and MAC. Three different groups were set up: 90 mL MM mixed with 10 mL cultured degrading bacteria, 90 mL MM mixed with 10 mL MAC suspension (3 g/L) and 90 mL MM mixed with 10 mL MAC (3 g/L) immobilized degrading bacteria. Aliquots (0.1 g) of 0# diesel oil were added to the three groups. The 100 mL MM solution containing 0.1 g 0# diesel oil was used as the blank control. The different experimental groups were placed in a shaker (150 rpm) at 30 °C over 15 days. All samples were undertaken in triplicate. The concentrations of residual diesel oil in liquid were extracted using hexane and measured at 256 nm with a UV–visible spectrophotometer, and the minimum detection mass concentration of this method was 0.005 mg/L. The

Equation (1) used for the removal percentages of diesel oil by different materials was as follows:

$$R = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

In the equation, R was the removal percentage of diesel oil (%), C_0 was the concentration of diesel oil in the blank sample (mg/L) and C_t was the concentration of residual diesel oil after reaction with the degrading bacteria or bio-MAC (mg/L).

3. Results and Discussion

3.1. Culture of Diesel-Oil-Degrading Bacteria

As shown in Figure 1, a total of ten bacterial strains with high diesel oil degradation capacity were isolated from the mixed bacteria. According to the results of the 16S rDNA amplification and sequencing step, the ten dominant degrading strains included six kinds of *Brevundimonas*, two kinds of *Staphylococcus* and *Acinetobacter*. The screened bacteria were suggested to have the function of taking up diesel oil as the carbon source to grow and proliferate in solution [25]. Compared to the samples without the addition of degrading strains, the diesel oil was degraded significantly by adding a single strain or mixed strains in water, and the removal efficiency was increased by 17.89–46.05%. The mixed strains had the highest removal efficiency (46.05%) for diesel oil. Moreover, considering that the mixed strains also had the fastest growth rate and maximum population (Figure S1), it was helpful to enhance the adaptability of the bacteria and improve the degradation efficiency of the diesel oil [26]. Therefore, the mixed strains were chosen for immobilization on the MAC in the following experiments.

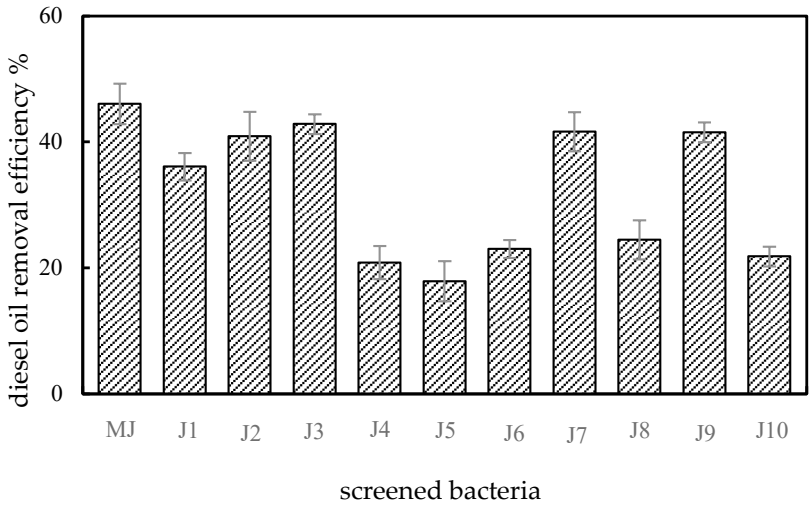

Figure 1. The removal efficiency rates for diesel oil using the screened bacterial strains after incubation: (**CK**) no bacteria; (**MJ**) mixed bacteria; (**J1**) *Brevundimonas diminuta*; (**J2**,**J3**) *Brevundimonas olei*; (**J4**) *Brevundimonas naejangsanensis*; (**J5**) *Brevundimonas nasdae*; (**J6**) *Brevundimonas naejangsanensis*; (**J7**,**J8**) *Staphylococcus saprophyticus*; (**J9**,**J10**) *Acinetobacter johnsonii*.

3.2. SEM Observation of Bacterial Immobilization

As shown in Figure 2, the morphologies of the degradation bacteria, MAC and bio-MAC were observed via high-resolution SEM images. Compared with the images of the separate diesel-degrading bacteria (Figure 2A) and the MAC suspension (Figure 2B), it can be clearly seen that almost all of the mixed bacterial cells were attached to the surfaces of MAC particles, and no free cells were present in solution (Figure 2C). Compared with the traditional activated carbon (AC) materials, the MAC had a larger specific surface area, higher adsorption capacity and greater hydrophobicity [27], indicating a higher adsorption ability of the bacterial colonization [16]. The MAC had a greater van der Waals force and electrostatic action with the negatively charged bacteria according to the DLVO theory [28].

Moreover, it can be seen that the produced extracellular polymeric substances (EPSs) on the surface of MAC had formed a biofilm-like structures (Figure 2C), which could facilitate the immobilization of degrading bacteria [5]. Therefore, there were two possible methods for the attachment of bacterial cells on MAC, including adsorption by MAC and the secretion of EPSs by growing bacteria. The degrading bacteria could be tightly attached to the surface of the MAC, enhancing the adsorption–biodegradation effect of the bio-MAC for the removal of diesel oil in the solution.

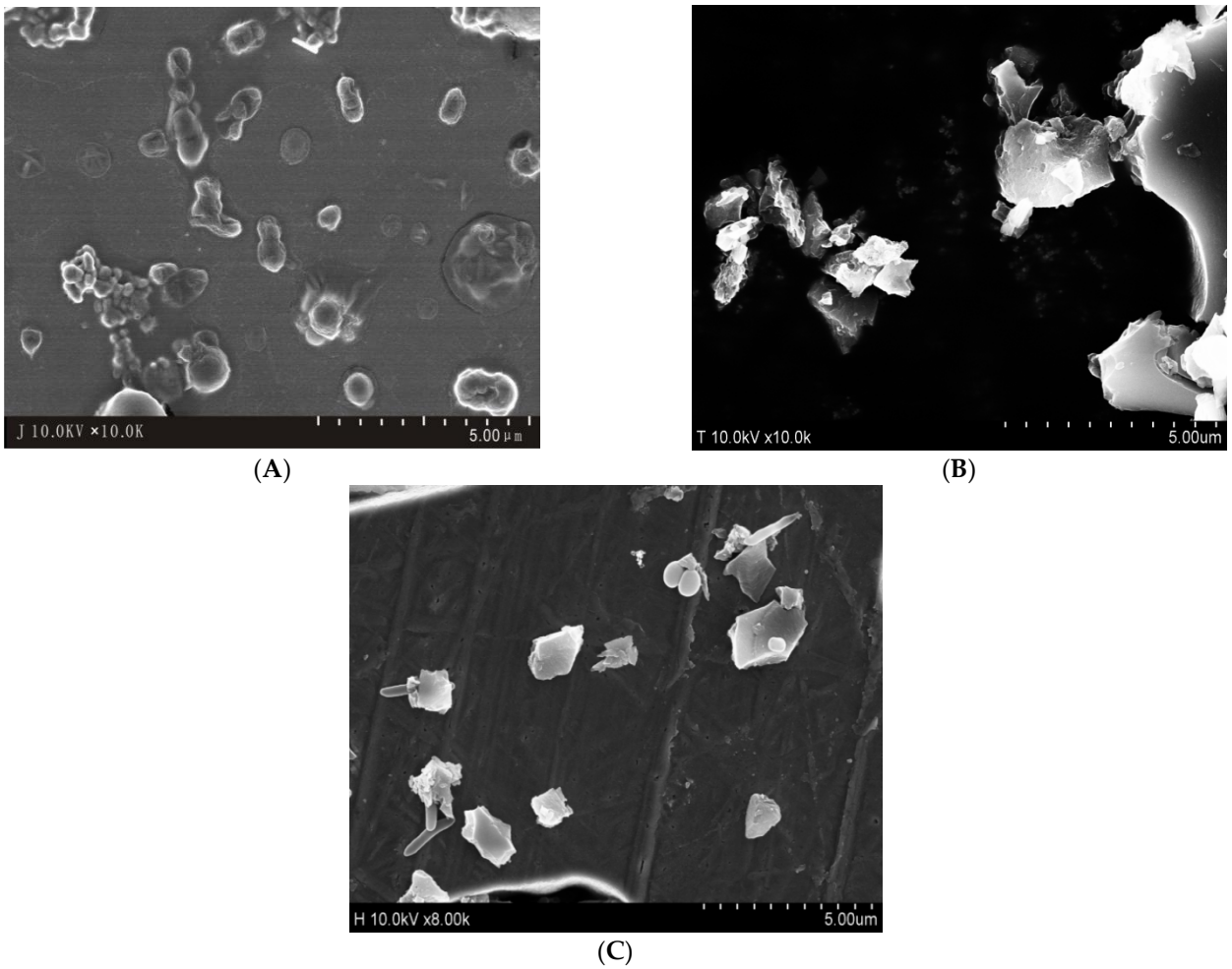

Figure 2. Scanning electronic microscope images: (**A**) degrading bacteria; (**B**) MAC; (**C**) bio-MAC.

3.3. Effect of the MAC Concentration on the Microbial Activity of Bio-MAC

The effects of the added MAC concentration on the diesel oil removal efficiency and bacterial enzymatic activity of bio-MAC are shown in Figure 3. The diesel oil removal percentage and bacterial concentration both increased with the increase in MAC concentration (from 0 to 3.0 g/L) in the bio-MAC, indicating that the increase in the MAC concentration was beneficial to the growth of degrading bacteria. However, when the MAC concentration is higher than 3.0 g/L, the excess presence of MAC may have cause the competitive adsorption of nutrients from the solution and may inhibit the subsequent microbial utilization and reproduction, resulting in a reduction in the diesel oil removal efficiency.

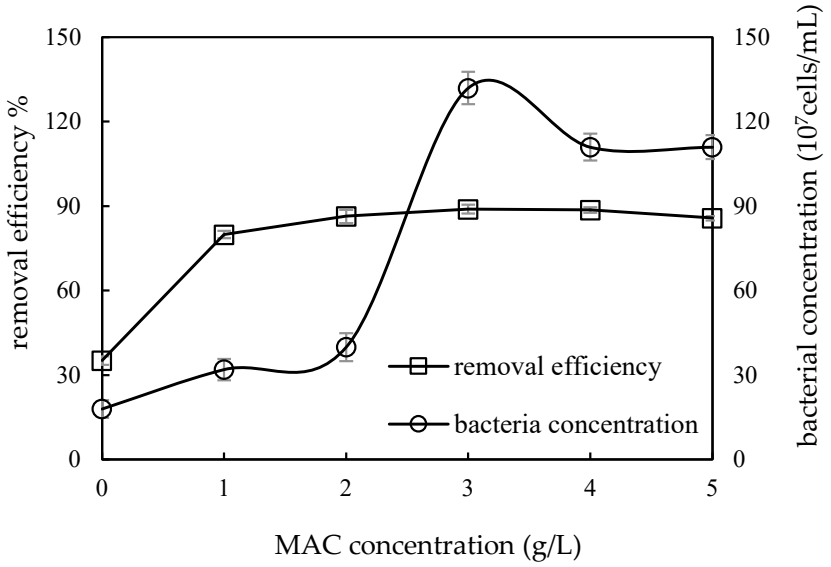

Figure 3. Effects of added MAC concentrations on the removal efficiency of diesel oil and the activity of bacteria (experimental conditions: MAC mass: 0–3 g/L; solution volume: 100 mL; temperature: 30 °C; pH = 7; time: 15 days; rotating speed: 150 r/min).

The effect of the MAC concentration on the enzymatic activity (DHA and LPS) of the bacteria also displayed the same trend (Figure 4). The DHA and LPS values of bacteria increased steadily with the increasing concentration (below 3.0 g/L) of MAC. The strong co-adsorption ability for diesel oil and bacteria by MAC improved the growth of the bacteria and further enhanced the degradation of the diesel oil. The biological load, concentration and activity of immobilized bacteria had a significant relationship with the MAC addition, and the MAC addition of 3.0 g/L was thought to be an optimum amount for the growth of diesel-oil-degrading bacteria.

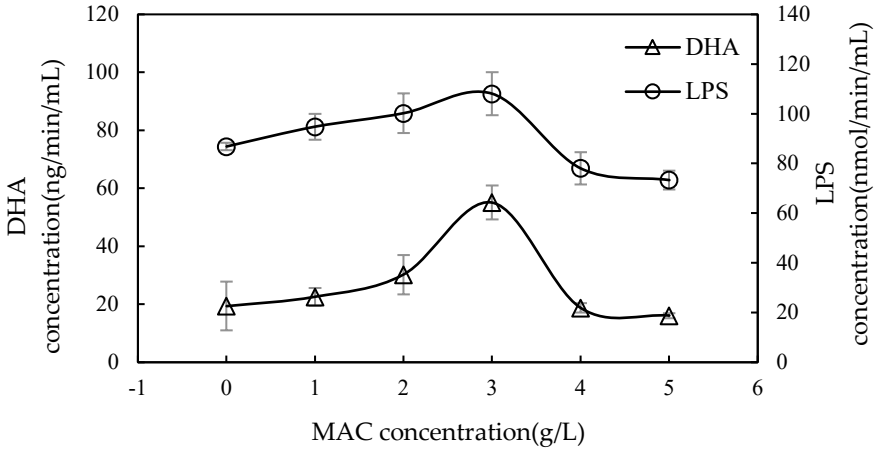

Figure 4. Effects of the added MAC concentration on the DHA and LPS values of bacteria (experimental conditions: MAC mass: 0–3 g/L; solution volume: 100 mL; temperature: 30 °C; pH = 7; time: 15 days; rotating speed: 150 r/min)

3.4. Changes in Community Structures of Degrading Bacteria after Immobilization

The changes in the microbial community compositions and relative abundances after bacterial immobilization on MAC are presented in Figure 5. The abundances of most of the dominant diesel-oil-degrading bacteria increased after the immobilization on MAC, such as *Pseudomonas*, *Bacillus*, *Microbacterium*, *Staphylococcus*, *Luteimonas* and *Caulobacter*. For instance, the *Brevundimonas* was demonstrated to be a main degrading bacteria of

diesel oil [29], and its abundance increased from 53.67 to 58.34% after immobilization. *Pseudomonas*, *Bacillus*, *Rhodococcus* and *Microbacterium* all have petroleum hydrocarbon degradation capability [30], the abundance levels of which increased by 9.07% compared to the free culture condition. In addition, according to the results of the alpha diversity index calculation (Figure S2), the Shannon–Wiener index of the degrading bacteria in bio-MAC was 3.08, which was higher than that of free cells of degrading bacteria (2.91). The increase in the abundance of these functional genera indicated that the growth and activity of degrading bacteria were enhanced through the immobilization by MAC.

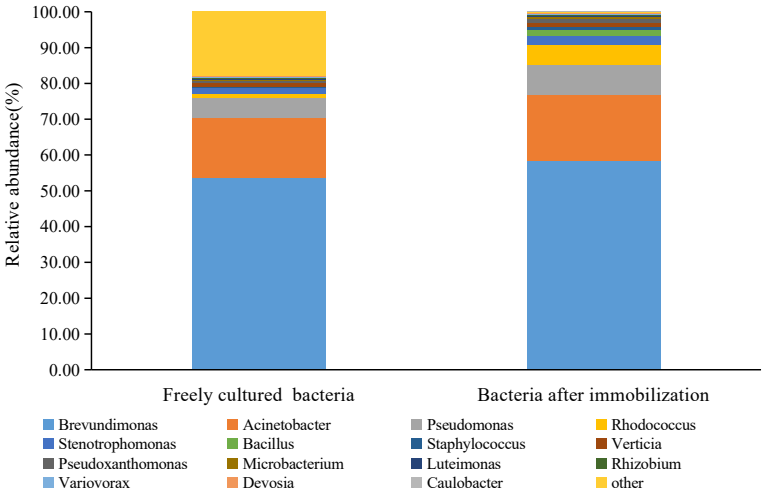

Figure 5. Changes in the bacterial composition and abundance after immobilization.

3.5. FTIR Spectra of Diesel Oil Solution before and after Reaction by Free Bacteria and Bio-MAC

As shown in Figure 6, the bands at 2930.31 cm^{-1} and 2855.58 cm^{-1} of the sample (CK) were the characteristic peaks of saturated hydrocarbons, which were the stretching and bending vibration absorption peaks of methyl, methylene and methine. The peaks in the range of 1470–1430 cm^{-1} were the bending vibration peaks of -C-CH_3 or deformation vibration absorption peaks of O-H, while the band near 1380 cm^{-1} was the vibration peak of the benzene skeleton and the band near 722 cm^{-1} represented the in-plane rocking shock absorption peak of –(CH_2)n-, $n \geq 4$ [31]. The FTIR spectra of the diesel–water solution reacting with free bacteria showed new bands at 1123.81 cm^{-1} and 650 cm^{-1}. According to the literature, the 1123.81 cm^{-1} band was the characteristic absorption peak of the C-O in the ether bond group, while the 650 cm^{-1} band was the stretching vibration peak of acid ions or carboxylic acid molecules. In contrast, more new bands appeared in the FTIR spectra of the diesel–water solution reacting with bio-MAC. For instance, the 2923.07 cm^{-1} band was the stretching vibration peak of carboxylic acid ions or carboxylic acid molecules, the 1731.76 cm^{-1} band was the stretching vibration peak of C=O in the saturated aliphatic chain, the1258.81 cm^{-1} band was the stretching vibration absorption peak of O-CH_3 [32], the bands near 1100 cm^{-1} were the antisymmetric vibration absorption peaks of the carbon-oxygen bond (O-CH_2-R) in the long-chain alkyl and 804.17 cm^{-1} was the out-of-plane bending vibration absorption peak of C-H in the benzene ring.

The bio-MAC could effectively degrade the aliphatic hydrocarbons, alkenes and aromatic compounds in diesel oil, as seen by comparing the FTIR spectra of the diesel–water solution before and after degradation, and the microbial metabolites included carboxylic acids, esters, alcohols and other substances [33]. According to the related research reports, the alkanes could be oxidized to the corresponding aldehydes and fatty acids when single-end oxidation occurred, and they also could be converted into dicarboxylic acids when double-end oxidation occurred [34]. Thus, the pathway of degradation for linear alkanes by bacteria was dominated by single-end oxidation and double-end oxidation to form dicarboxylic acids [35]. The FTIR spectroscopy results indicated that there not only did

single-end oxidation and sub-end oxidation occur, but also double-end oxidation in the degradation of diesel oil by bio-MAC.

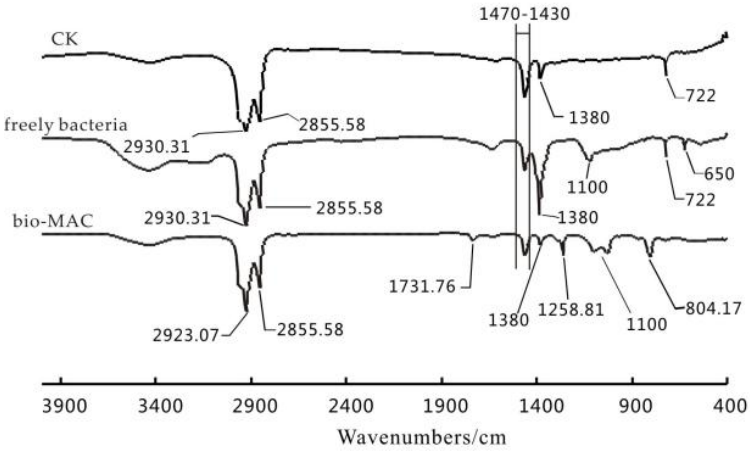

Figure 6. FTIR spectra of the residue diesel oil in wastewater after degradation using bacteria and bio-MAC.

3.6. Adsorption–Biodegradation of Diesel Oil by Bio-MAC

As shown in Figure 7, the removal efficiency rates for diesel oil in contaminated water by freely suspended degrading bacteria, MAC and bio-MAC were 23.82%, 70.97% and 86.35% after 15 days' reaction, respectively. Compared with the same addition amounts of individual degrading bacteria or MAC, the diesel oil removal efficiency by bio-MAC resulted in a significant improvement, which suggested that the bio-MAC had an adsorption–biodegradation effect on the diesel oil removal. The adsorption–biodegradation process could be illustrated with two steps. In the first stage, the adsorption of the diesel oil by MAC played an important role (as a trapper), and virtually no biodegradation occurred during this period. However, the MAC with large specific surface area provided a great survival space for the early growth of degrading bacteria, which significantly increased the abundance of degrading bacteria and enhanced the available contact area between the diesel oil and the immobilized bacterial cells [36]. Once the bacteria had successfully adhered and colonized on MAC, the biodegradation process could start in the subsequent stage, as demonstrated by the FTIR spectroscopy results (Figure 6). The immobilized degrading bacteria could use diesel oil as a carbon source, and then the diesel oil adsorbed on the bio-MAC was degraded. With the continuous degradation of diesel oil by immobilized bacteria, the adsorption capacity of the bio-MAC could be recovered when the adsorbed diesel oil was degraded, ensuring that the bio-MAC had a long-term and efficient removal efficiency for diesel oil. In addition, compared with the other carrier materials of immobilized degrading bacteria (Table 2), bio-MAC was proven to have obvious advantages in the removal of the diesel oil in solution.

Table 2. The summary of the main microorganism immobilization materials used for oil removal in water.

Immobilization Material	Immobilized Microorganisms	Immobilization Method	Contaminant	Removal Rates (%)	References
Wood Chips	*Corynebacterium Variabile* HRJ4	Adsorption	Petroleum Hydrocarbon	78.9	[37]
Expanded Graphite	ODB-1 (*Pseudomonas* sp.)	Adsorption	Diesel Oil	85	[38]
Corncob–biochar	Mixed Strain (Petroleum Hydrocarbon-Degrading Bacteria F-3 and R-7)	Adsorption	Petroleum Hydrocarbon	63.8	[39]

Table 2. *Cont.*

Immobilization Material	Immobilized Microorganisms	Immobilization Method	Contaminant	Removal Rates (%)	References
Spent Mushroom Substrate Biochar	*Ochrobactrum sp. QI*	Adsorption	Saturates	62.04	[40]
Acetic-Acid-modified Ramie Fiber	Mixed Strain (*Acinetobacter Calcoaceticus*, *Acinetobacter Baumannii*, *Acinetobacter venetianus* and *Rhodococcus* sp.)	Adsorption	Crude Oil	85.16	[41]
Sponge	Mixed Strain (Petroleum Hydrocarbon-Degrading Bacteria JZ3 and JZ4)	Adsorption	Oil	82.4	[42]
Alkaline modification firstly and then Acid-modified Attapulgite with Alginate	Mixed Strain (Petroleum Hydrocarbon-Degrading Bacteria)	Adsorption	Petroleum Hydrocarbon	60.54	[43]
MAC	Mixed Strain (*Pseudomonas*, *Rhodococcus*, *Bacillus* and *Rhizobium*, etc)	Adsorption	Diesel oil	86.35	This article

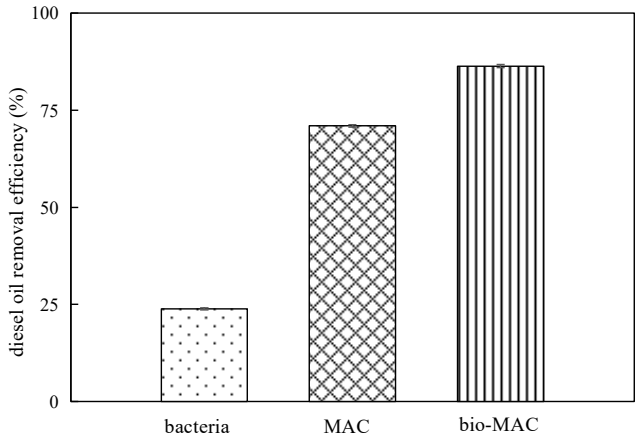

Figure 7. Comparison of the diesel oil removal efficiency rates of degrading bacteria, MAC and bio-MAC.

4. Conclusions

Using an enrichment culture technique, the indigenous diesel-oil-degrading bacteria were successfully isolated and enriched, and the bio-MAC was prepared by immobilizing the degrading bacterial cells on the surface of the MAC. The high-throughput sequencing results demonstrated that the diesel-oil-degrading bacteria showed greater abundance and diversity after immobilizing on MAC. The SEM and FTIR spectroscopy results indicated that the bacteria stabilized on MAC had strong oxidation ability in the degradation of diesel oil. Compared with the freely suspended degrading bacterial cells and MAC, the bio-MAC showed a significantly improved removal efficiency (86.35%) for diesel oil. In the whole adsorption–biodegradation process using bio-MAC, the MAC acts as the trapper of the diesel oil and biocarrier of the degrading bacteria, ensuring the high removal efficiency for the diesel oil. Specially, the adsorption capacity of MAC could recover when the adsorbed diesel oil compounds were biodegraded by the immobilized bacteria. The MAC-immobilized diesel-oil-degrading bacteria were proven to have an adsorption–biodegradation effect on diesel oil, representing a promising way to remove diesel oil from wastewater.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14132061/s1, Figure S1: Growth curve of the screened bacteria; Figure S2: Changes in the Shannon–Wiener index values of bacteria after immobilization on bio-MAC.

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