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Research on the Functional Microbe Activation System in a Post-Polymer Flooded Reservoir

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Abstract: Further exploitation of the residual oil underground in post-polymer flooded reservoirs is attractive and challenging. Microbial-enhanced oil recovery (MEOR) is a promising strategy to enhance the recovery of residual oil in post-polymer flooded reservoirs. Identifying and selectively activating indigenous microorganisms with oil displacement capabilities is an urgent requirement in the current design of efficient microbial-enhanced oil recovery technologies. This study combines high-throughput sequencing with functional network analysis to identify the core functional microbes within the reservoirs. Concurrently, it devises targeted activation strategies tailored to oligotrophic conditions through an analysis of environmental factor influences. The feasibility of these strategies is then validated through physical simulation experiments. With nutrient stimulation, the overall diversity of microorganisms decreases while the abundance of functional microorganisms increases. The core displacement results showed that the oil recovery factor increased by 3.82% on the basis of polymer flooding. In summary, this research has established a system for the efficient activation of functional microorganisms under oligotrophic conditions by utilizing bioinformatics, network analysis, and indoor simulation systems. This achievement will undoubtedly lay a solid foundation for the practical implementation of microbial enhancement techniques in the field.

Keywords: MEOR; indigenous microorganisms; environmental factor; functional microbe

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1. Introduction

The globally increasing demand for crude oil has prompted the oil industry to enhance oil production from primary, secondary, and tertiary recovery methods to fulfill the global requirement. Among the most mature, effective, and successful chemical-enhanced oil recovery (CEOR) methods is polymer flooding [1–3], which involves the injection of a polymer solution into the reservoir with a suitable concentration to diminish the mobility ratio of the water–oil system (M) as much as possible, and hence increase the sweep efficiency [4–7]. However, frequent injections of polymers create more extensive pore paths, resulting in the quick movement of injection fluid out of the oil-bearing strata. This ultimately lowers the oil recovery potential with a significant percentage (~50%) of the original oil remaining in place, unextracted [8]. The efficient extraction of this part of the crude oil and tackling the problem of boosting oil recovery even further post-polymer flooding is a serious subject confronting various oilfields. Regrettably, there has not yet been an efficacious chemical approach discovered that can succeed in further enhancing oil recovery rates in reservoirs subsequent to chemical flooding treatments [9]. Recently, researchers have predicted the potential role of indigenous microbial populations

in improving recovery in post-polymer flooded reservoirs, viewing it as a promising alternative strategy [10].

Microbial-enhanced oil recovery (MEOR) utilizes microorganisms and their metabolites such as biopolymers, biosurfactants, enzymes, solvents, and bioacids to modify the flow characteristics of residual oil, thereby enhancing oil recovery [11–13]. According to the different sources of microorganisms, this technology mainly includes indigenous microbial-enhanced oil recovery (IMEOR) and exogenous microbial-enhanced oil recovery [14]. IMEOR is widely used due to its advantages such as high metabolic activity, strong adaptability of strains, simple process, and low cost [15]. The mechanism by which indigenous microorganisms enhance oil recovery involves the injection of activators to stimulate metabolic activity and the production of metabolites within the reservoir's functional microorganisms. These microorganisms interact with crude oil, rocks, and water, thereby enhancing the efficiency of water flooding and ultimately enhancing oil recovery rates. The primary functional microorganisms in reservoirs include surfactant-producing microbes and hydrocarbon-degrading microbes [16,17]. Surfactant-producing microbes produce biosurfactants that improve the interface properties of oil, water, and rocks, which makes crude oil flow more easily in subsurface oil reservoirs. In addition, hydrocarbon-degrading microbes degrade the heavy hydrocarbons and heterocyclic hydrocarbons in the crude oil [18,19]. An increase in oil production is associated with an increase in functional microorganisms, which is currently the primary theoretical basis of MEOR technology [20,21]. The core of IMEOR lies in the targeted activation of functional microorganisms within the reservoir. Currently, scholars, both domestically and internationally, have obtained substantial information regarding the composition of microbial communities in various oil reservoirs through numerous studies. However, they have yet to establish a consistent pattern for the activation of these functional microorganisms [22].

The indigenous microorganisms in oil reservoirs have been in an oligotrophic state for a long time, and introducing a substantial amount of nutrients from outside may paradoxically suppress their growth [23]. Studies show that under limited nutrient conditions, microbial metabolic activities can be enhanced instead. This is because when cells are in a starvation state they exhibit hydrophobicity and substances in the microbial cell walls can act as surfactants to improve crude oil recovery rates [24]. In recent years, high-throughput sequencing and its associated analyses have played a crucial role in studying bacterial community distributions in ecological contexts. Gao et al. [25] employed high-throughput sequencing to examine the bacterial community distributions in oil–water samples from various geographical and environmental settings, uncovering that the environment indeed exerts an influence on these distributions. Under oligotrophic conditions, microorganisms rely on petroleum hydrocarbons as their carbon source to sustain their life activities [26]. In this case, inorganic salts such as phosphorus and nitrogen sources are the main factors limiting the functional activities of microorganisms. Therefore, targeted adjustment in the composition and concentration of inorganic salts through the relationship between functional microorganisms and environmental factors is essential for achieving targeted regulation of functional microorganisms and enhancing microbial recovery rates.

IMEOR benefits from the increasing understanding of the assemblages of microbial communities underground. The analysis and understanding of a microbial community structure before and after activation by activators serves as one of the primary bases for evaluating the activation effect of the activators. In this paper, we use high-throughput sequencing to analyze the composition and function of microbial communities in reservoirs after polymer flooding, discuss the response relationship between environmental factors and functional microbial communities, and quickly establish a set of oligotrophic and efficient activation systems in combination with dynamic and static experiments, which will certainly lay the foundation for on-site implementation of microbial-enhanced oil recovery.

2. Materials and Methods

2.1. Samples Collection

According to the conditions for indigenous microbial oil recovery, produced fluids after polymer flooding were collected to analyze the diversity of microbial communities and used for activation experiments [13,27]. The water samples collected from the production well were immediately stored in a 10 L plastic container. The containers were filled to maintain an anaerobic condition by avoiding oxygen intrusion. Then, the containers were transported in a cooler filled with ice blocks within 48 h and stored at −20 °C until DNA extraction. Before collecting samples, all containers were subjected to high-pressure sterilization and water sample rinsing for analysis to ensure the accuracy of the microbial investigation data.

2.2. PCR Amplification and 16S rRNA Gene Sequencing

The extracted total DNA was sent to the Majorbio Company (Shanghai, China) for 16S rRNA gene high-throughput sequencing. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) through a thermocycler PCR instrument [28]. PCR reactions were performed in triplicate using a 20 μL mixture containing 12.5 μL of dNTP and Taq enzyme premix, 0.8 μL of each primer (5 μM), and 10 ng of template DNA. The PCR reactions were conducted by referring to the previous citation [29]. The PCR products were detected on a 2% agarose gel and subsequently purified using the DNA Gel Extraction Kit (BoRi, Jinhua, China).

QIIME 2 was used to process the amplicon sequencing data, and the divisive amplicon denoising algorithm (DADA2) [30] was used to perform quality control, denoising, splicing, and dechimerization of the original sequencing sequence to obtain the amplified sequence variant, amplicon sequence variant (ASV).

2.3. Sequencing Data Analysis

Redundancy analysis (RDA) is an ordination method that evolved from correspondence analysis and combines it with multiple regression analysis, where each step of the calculation involves regression against environmental factors. This analysis is primarily used to reveal relationships between microbial communities and environmental factors. Through this analysis, one can examine the relationships among environmental factors, samples, and microbial communities, as well as the associations between any two of these entities. RDAs were performed using the 'vegan' and 'ggplot2' packages of the R 4.3.1 software. Functional Annotation of Prokaryotic Taxa (FAPROTAX) software utilizes the current literature on cultured strains to map prokaryotic branches (such as genera or species) to established metabolic or other ecological-related functions [31]. Commonly used in ecological interpretation of 16S marker gene data, it can quickly identify sample taxa with specific metabolic phenotypes. Species functional studies were conducted using FAPROTAX software version 1.2.5 and the results were imported into Gephi software (https://gephi.org) for visualization using the Fruchterman–Reingold layout.

2.4. Activation of Indigenous Functional Microbes Test

Fow_ JO was the produced fluid from the reservoir after polymer flooding, rev_ FA1_ JO was cultured in screening Scheme 1, and rev_WJY_JO was a basic inorganic salt culture. The experiment utilized a method of directly adding a nutrient activator into the water samples to activate the indigenous microbial community. A basic inorganic salt nutrient activator (K_2HPO_4, 1.0 g/L; KH_2PO_4, 1.0 g/L; $MgSO_4$, 0.5 g/L; NH_4Cl, 1.0 g/L; $CaCl_2$, 0.02 g/L; $FeCl_3$, trace) and 5 mL of crude oil were placed in a 150 mL Erlenmeyer flask, and then sterilized under high pressure at 121 °C for 15 min. Then, under sterile conditions, 10 mL of the experimental water sample was injected into each triangular flask, separately. After shaking, the triangular flask was incubated at 45 °C and 150 rpm for 8 days. All

treatments were conducted in duplicate to ensure the reliability of the data. All solutions and cultures were transferred using sterile needles and syringes.

To maximize the activation of oil recovery functional bacteria, we adjust the composition and concentration of inorganic salts in a targeted manner based on the relationship between functional microbes in the reservoir and environmental factors. At the same time, we analyzed the changes in microbial composition in production fluid samples resulting from variations in inorganic nutrients.

2.5. Bacteria Growth Curve

The culture medium was collected at different time points (1, 2, 4, 6, and 8 days) under sterile conditions. Subsequently, the collected medium was centrifuged at 1000 rpm and 4 °C for 10 min. After centrifugation, the pellet was washed three times with sterile water. The resulting suspension was then diluted with sterile water to its original volume. The cell density was measured by detecting the optical density at a wavelength of 600 nm using ultraviolet light. The bacterial growth curve can be plotted with the cultivation time on the x-axis and the absorbance value on the y-axis [32].

2.6. Determination of Crude Oil Biodegradation Rate

Following the method proposed by Gao et al. [25] the weight method was employed to determine the degradation rate of petroleum by mixed bacterial strains. Each experiment was conducted in triplicate, and the average value was calculated for each group [33]. The degradation rate of crude oil was quantified according to the following equation:

$$H = \frac{m_1 - m_2}{m_1} \times 100\%$$

where m_1 is the mass of residual oil in the control medium (g), and m_2 is the mass of residual oil in the culture medium inoculated with bacteria (g).

2.7. Core Oil Displacement Experiment

Initially, the weighed core was vacuumed at −0.1 MPa for 5 h and then the core was saturated with formation water. The weight of the core after water saturation was measured and the pore volume and porosity of the core were calculated. The core after saturated water was placed in a constant temperature box at 45 °C for aging and wetting for 12 h, then the saturated oil experiment was carried out, and finally it was placed in a constant temperature box for aging for 24 h. The primary water flooding stage was carried out until the rate of water content reached more than 98%, and then the water flooding was stopped. After injecting 0.5 PV polymer segment plugs for polymer flooding, the subsequent water flooding was carried out to follow up, and when the rate of water content was 98% or more, the flooding experiment was stopped to evaluate the effect of the polymer flooding. Finally, after injecting the 0.3 PV activator, the model was closed and placed in 45 °C constant temperature chambers to incubate for 7 days. The water flooding process concluded when the water content rate reached over 98%, and the impact of microbial-enhanced oil recovery (MEOR) was assessed.

3. Results

3.1. Characteristics of Microbial Community in Oil Reservoirs after Polymer Flooding

The analysis of microbial community structure was primarily conducted on the produced fluid of polymer-flooding block oil wells in Daqing Oilfield, resulting in a total of 726 bacterial ASVs. The bacterial species composition included a total of 32 phyla, 61 classes, 106 orders, 159 families, 218 genera, and 245 species. At the family level, the relative abundance of species indicated that (Figure 1A) the main functional bacteria in the samples were *Synergistaceae*, accounting for 25.19%, *Coprothermobacteriaceae*, accounting for 10.45%, *Microcystaceae*, accounting for 9.49%, *Arcobacteriaceae*, accounting for 8.54%,

Clostridiaceae, accounting for 8.01%, Pseudomonadaceae, accounting for 4.99%, *Tepidanaracter*, accounting for 2.60%, and *Moraxellaceae*, accounting for 2.17%.

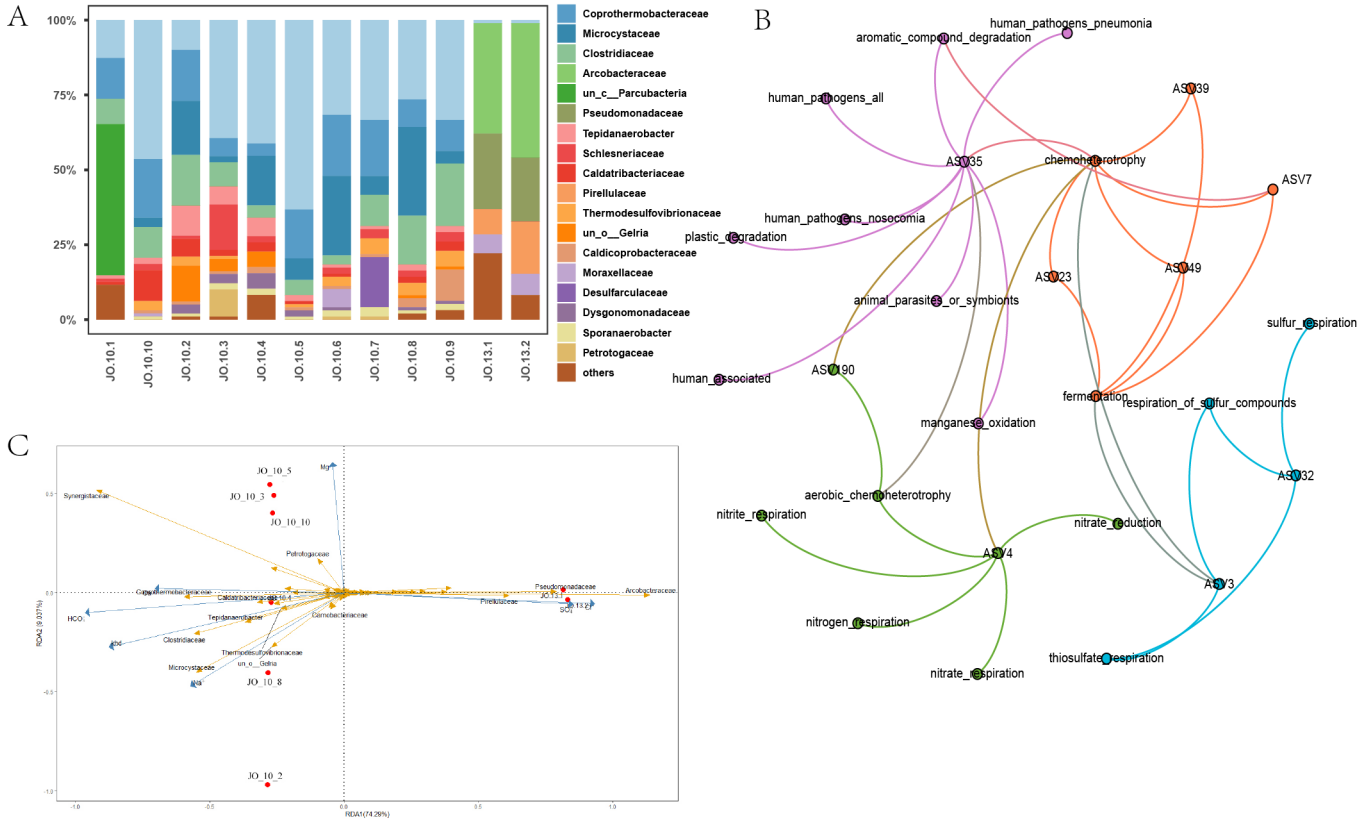

Figure 1. (**A**) Dominant microbial composition of bacteria at the family level. (**B**) Analysis of functional networks based on the family level. (**C**) Spatially constrained, distance-based redundancy analysis of plot-based quantitative bacterial community composition. The polymer-flooded samples are shown. Arrows indicate the direction of the maximum change in variables. Blue arrows indicate environmental factors; Yellow arrows represent bacteria from different families; The angle between species and environmental factors represents the positive and negative correlation between species and environmental factors. khd, degree of mineralization (total salinity of water).

Identifying indigenous microbial communities with oil recovery capabilities is an urgent requirement for designing efficient microbial-enhanced oil recovery (MEOR) technologies. The FAPROTAX software was used to predict the function of the species. The results showed that the produced fluid after polymer flooding mainly had aromatic compound degradation, chemoheterotrophic, aerobic chemoheterotrophy, respiration of sulfur compounds, fermentation, nitrate reduction, and other functions (Figure 1B). Among them, chemoheterotrophic processes were mainly executed by *Clostridiaceae*, *Sporanaerobacter*, *Coprothermobacteriaceae*, *Moraxellaceae*, *Rhodocyclac*, and *Pseudomonadaceae*. Aromatic compound degradation was mainly performed by *Clostridiaceae*, *Moraxellaceae*, and *Pseudomonadaceae*. Aerobic chemoheterotrophy was shown primarily by *Moraxellaceae* and *Rhodocyclaceae*. The fermentation was typically associated with *Clostridiaceae*, *Sporanaerobacter*, and *Coprothermobacteriaceae*.

The interaction between functional microorganisms and environmental factors in the reservoir was analyzed using redundancy analysis. RDA was performed using the samples, with the functional bacteria in the samples as response variables, and the reservoir physicochemical properties as explanatory variables. The results showed that the total explanatory rate of differences in bacterial community structure by physicochemical factors was 83.33% (RDA1, 74.29% + RDA2, 9.037%) (Figure 1C). In the correlation analysis between bacterial communities and environmental factors, the environmental factors that had the greatest

impact on the distribution of bacterial communities were SO_4^{2-}, Cl^-, HCO_3^-, and mineralization, followed by Ca^{2+}, Na^+, and Mg^{2+}. *Synergistaseae*, *Coprothermobacteriaceae*, and *Clostridiaceae* were negatively correlated with Cl^- and SO_4^{2-}, while these bacteria were positively correlated with Mg^{2+}, HCO_3^-, Na^+, mineralization, and Ca^{2+}. Pseudomonadaceae was positively correlated with Cl^- and SO_4^{2-}, while being negatively correlated with Ca^{2+}, Na^+, and Mg^{2+}.

3.2. Research on Activation of Functional Microorganisms

Through functional network analysis, it has been identified that microorganisms able to perform functions such as aromatic compound degradation, chemoheterotrophic metabolism, aerobic chemoheterotrophy, fermentation, and hydrocarbon degradation mainly belong to *Pseudomonadaceae* and *Moraxellaceae*. After obtaining the composition of functional microbial communities, we aim to selectively activate these functions. This process involves identifying the environmental factors that influence the structure of functional microbial communities. To achieve this, we conducted a distance-based redundancy analysis to identify the main factors influencing the growth activities of functional microbes in oil reservoirs. The nutritional components in MEOR stimulation were adjusted according to the growth requirements of functional microorganisms to optimize their growth needs and maximize their functional potential. The redundancy analysis revealed that *Pseudomonadaceae* was positively correlated with Cl^- and SO_4^{2-}, while being negatively correlated with Ca^{2+}, Na^+, and Mg^{2+}. In experiments, a basic inorganic salt medium was used to stimulate produced water samples after polymer flooding. The mixed bacteria were enriched and exhibited crude oil degradation effects on this basal medium, with an oil degradation rate of 14.38%. Based on these studies and the results of the previous experiments, combined with the analysis of environmental factors, $CaCl_2$, NH_4Cl, and $MgSO_4$ were selected as the modifying factors. Building on this, we have designed nine experimental schemes to explore the variations in microbial growth in produced water samples after polymer flooding. This was achieved by adjusting the concentrations of these three salts (refer to Table 1). After the adjustment, the crude oil degradation rate increased to 20.95%, representing a 45.68% improvement over the degradation rate under the basal inorganic salt medium conditions (Table 2).

Table 1. Screening schemes for inorganic salts with different concentrations.

Title 1	K_2HPO_4 g/L	KH_2PO_4 g/L	$MgSO_4$ g/L	NH_4Cl g/L	$CaCl_2$ g/L	$FeCl_3$
Scheme 1	1.00	1.00	0.25	0.75	0.03	trace
Scheme 2	1.00	1.00	0.50	0.75	0.01	trace
Scheme 3	1.00	1.00	0.75	0.75	0.02	trace
Scheme 4	1.00	1.00	0.25	1.50	0.02	trace
Scheme 5	1.00	1.00	0.50	1.50	0.03	trace
Scheme 6	1.00	1.00	0.75	1.50	0.01	trace
Scheme 7	1.00	1.00	0.25	2.25	0.01	trace
Scheme 8	1.00	1.00	0.50	2.25	0.02	trace
Scheme 9	1.00	1.00	0.75	2.25	0.03	trace

Table 2. Comparison of degradation rate between rev_WJY_JO and rev_FA1_JO medium.

Index	Medium		Increase (%)
	rev_WJY_JO Medium	rev_FA1_JO Medium	
degradation rate (%)	14.38	20.95	45.68

An 8-day activation experiment was conducted under aerobic conditions, measuring the absorbance of the bacterial community at 600 nm. The results showed that on the first day, only the OD600 value of Scheme 1 exceeded 0.8. In the subsequent experiments, bacterial colonies under Schemes 2–4 all achieved rapid growth, while the OD600 value

of the other schemes was still less than 0.8. On the eighth day, the growth trends of Schemes 1, 2, and 4 all tended to stabilize. Scheme 3 demonstrated a downward trend (refer to Figure 2A). When analyzing the optical density (OD) value results, it is important to consider that bacterial growth may be influenced by other factors. Emulsification was also used as an evaluation index. The emulsification results showed that Scheme 1, Scheme 2, Scheme 3, and Scheme 4 had a consistent and effective emulsification effect throughout, as evidenced by the OD600 value change curve. Among them, the emulsification effect of Scheme 1 changed the fastest and was the most noticeable. On the second day, a significant amount of crude oil dissolved in the fermentation liquid without adhering to the wall. The particles were fine and uniform, creating a homogeneous suspension that resembled ink. Therefore, Scheme 1 is determined to be the best activation scheme. Compared with the basic inorganic salt medium, the OD600 value of Scheme 1 gradually increased during the 8-day activation experiment and was higher than that of the inorganic salt medium. The bacterial activation efficiency was higher, and the growth rate was faster (Figure 2B).

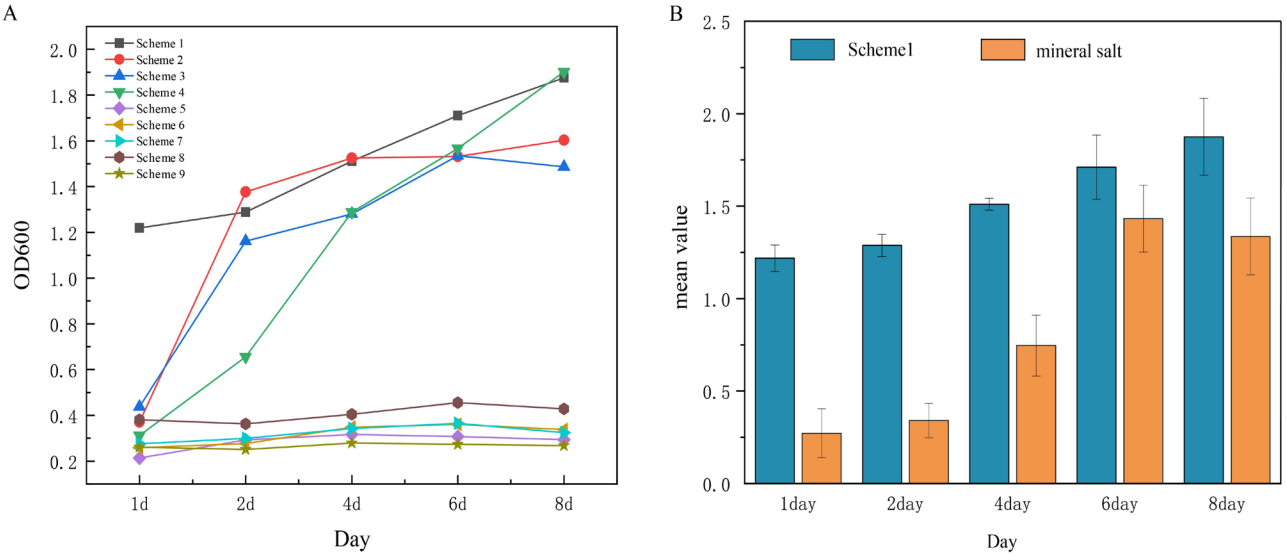

Figure 2. (**A**) Changes in cell growth after 8 days of culture of mixed strains under different protocols. (**B**) Changes in cell growth of mixed strains after 8 days of incubation in basal and optimized media.

3.3. Core Displacement Experiment

Based on the evaluation results of the aforementioned activation systems, Scheme 1, which exhibited the highest activation, was chosen as the optimal activator system. A 600 MD core was used to evaluate the oil displacement efficiency of the optimal formula system. The core oil displacement experiment process involved water flooding, polymer flooding, water flooding, microbial flooding, and water flooding.

The recovery rate of the core samples during the water flooding process was 34.44%. As water injection continued, the pressure gradually decreased and remained at a relatively low level. This may be because the saturated oil in the core was replaced by water. During the polymer flooding stage, the recovery rate increased rapidly, while the water content rate decreased consistently. After reaching the maximum water content saturation, the crude oil recovery rate increased by 18.5% compared to water flooding alone. Afterward, the 0.3 PV activation Scheme 1 was injected into the core. The injection pressure increased, leading to the formation of new driving energy, and the flooding pressure eventually stabilized at 0.298 MPa. After shutting down the well for 7 days, subsequent water flooding was carried out. After injecting 1.13 PV of formation water, the water content of the produced fluid increased to its maximum and remained stable at 98%. The displacement pressure ultimately stabilized at 0.187 MPa. On the basis of polymer flooding, the crude oil recovery increased by 3.82%, and the total recovery reached 56.94% (Figure 3).

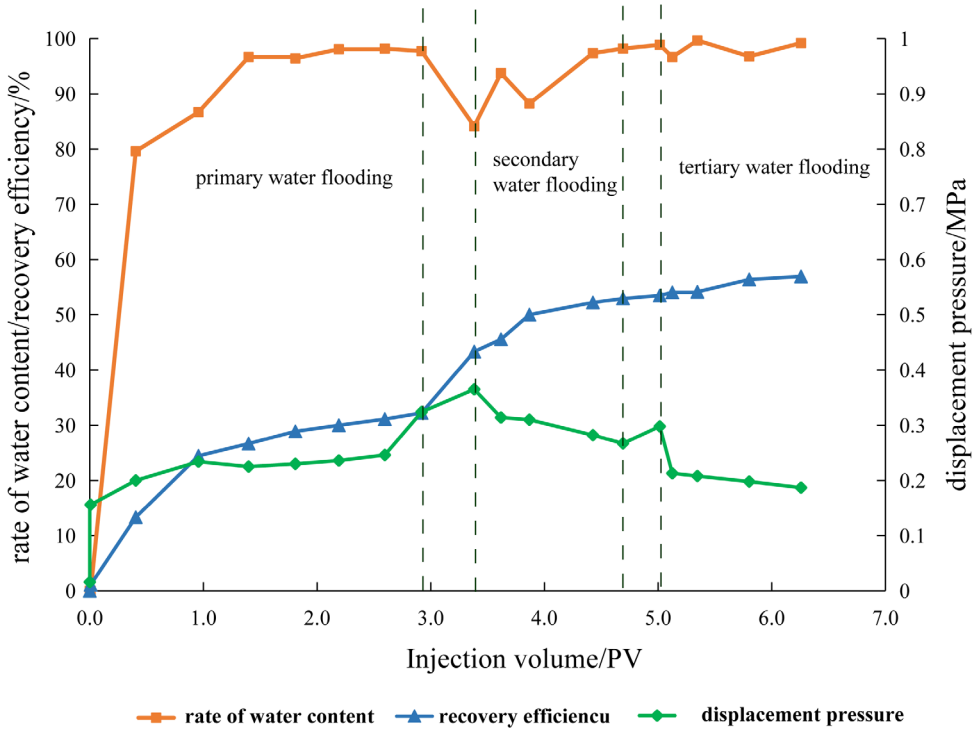

Figure 3. Injection pressure, rate of water content, and recovery efficiency were observed with injection volume at various stages of injection.

3.4. Changes in Community Structure during Activation

The changes in microbial community structure were highly influenced by environmental factors. Adding certain nutrients can alter the original microbial community structure and potentially improve the degradation efficiency of organic matter.

In the pre-culture sample, the fow_JO sample contained 53 species at the family level. The main functional bacterial groups were *Pseudomonadaceae* and *Moraxellaceae*, accounting for 65% and 6% abundance, respectively. In the cultured sample, the rev_FA1_JO and rev_WJY_JO samples each contained 14 family-level species, and the number of species significantly decreased. The main functional bacterial groups in the rev_FA1_JO sample were *Comamonadaceae*, *Hydrogenophilaceae*, and *Enterobacteriaceae*, with abundances of 67%, 12%, and 5%, respectively. In the rev_WJY_JO sample, the main functional bacterial groups were *Hydrogenophilaceae* and *Comamonadaceae*, accounting for 76% and 10% of the total abundance, respectively. Compared the species composition structure of the samples before and after cultivation, the main groups changed from the initial dominant group Pseudomonadaceae to *Comamonadaceae* in sample rev_FA1_JO and *Hydrogenophilaceae* in sample rev_WJY_JO, respectively (Figure 4A).

Figure 4B showed that the primary functional groups in the core bacterial communities in the reservoir after polymer flooding were chemoheterotrophy, aerobic chemoheterotrophy, hydrocarbon degradation, thiosulfate respiration, and fermentation. Among these, the hydrocarbon degradation functional groups were predominant. This indicates the presence of many hydrocarbon-degrading microorganisms in the reservoir that utilize organic matter to obtain a supply of nutrients. After cultivation, the functions in the reservoir were significantly activated. The main functional groups in the core bacterial groups in rev_FA1_JO included aromatic compound degradation, dark hydrogen oxidation, respiration of sulfur compounds, methanogenesis, etc. The main functional groups in the core bacterial groups in rev_WJY_JO included chemoheterotrophy, aerobic chemoheterotrophy, anaerobic chemoheterotrophy, nitrite respiration, fermentation, etc.

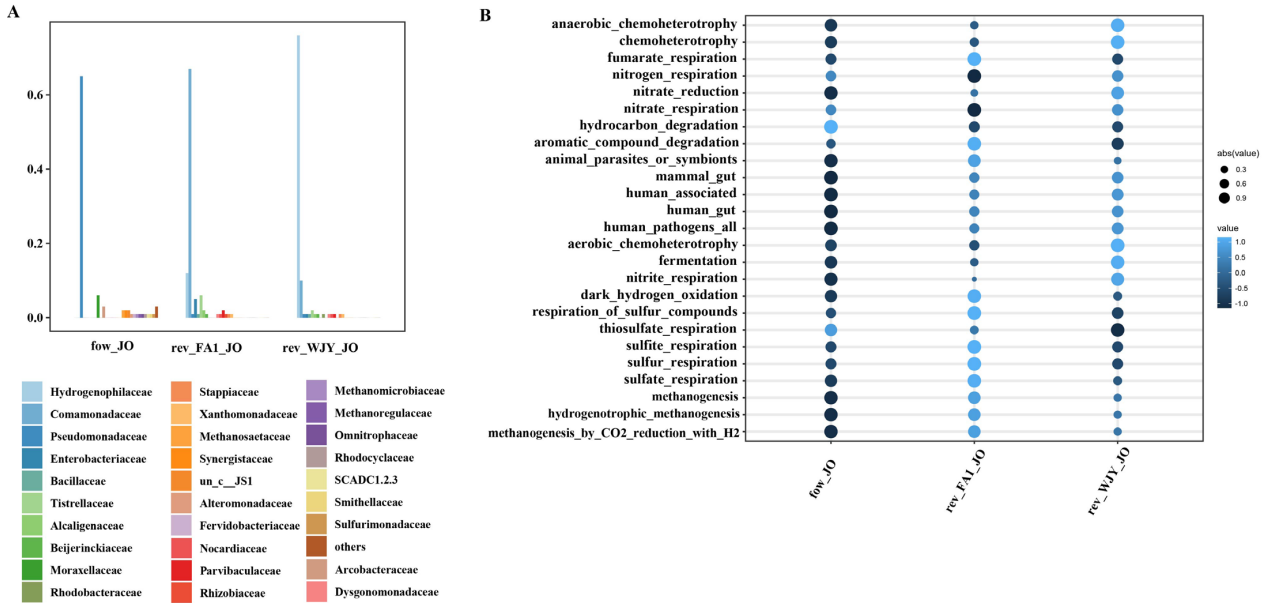

Figure 4. (**A**) Composition of bacterial community structure in the produced liquid after polymer flooding before and after the experiment. (**B**) Changes of the functional groups in the core bacterial microbiota at different stages.

4. Discussion

After polymer flooding, a significant amount of residual oil, approximately 50%, is retained in the reservoir, along with the residual polymers that have adsorbed onto the rock formations [34]. Microbial-enhanced oil recovery (MEOR) is a promising strategy to enhance the recovery of residual oil in post-polymer flooded reservoirs. Shi et al. [35] screened five facultative anaerobic bacteria from the Daqing Oilfield after polymer flooding. These strains exhibited the capability to utilize both oil and polymers as carbon sources for growth and reproduction. The results revealed that the screened bacteria could produce organic acids and active substances, degrade polymers and crude oil, and enhance oil recovery by 5%. Patel et al. [36] discovered that microorganisms involved in microbial-enhanced oil recovery utilize residual hydrolyzed polyacrylamide (HPAM) as a nitrogen source for growth, reproduction, and crude oil metabolism. Furthermore, they produce surfactants within micropores, which facilitate polymer degradation. This mechanism provides a viable solution to address reservoir plugging issues, thereby enhancing the efficiency of oil recovery. However, the polymers and crosslinkers commonly used in polymer flooding may exhibit biotoxicity, exerting toxic effects on indigenous reservoir microorganisms. Therefore, understanding the microbial community structure after polymer flooding is a prerequisite for implementing IMEOR.

Different types of oil reservoirs, due to variations in environmental conditions and dissimilar development approaches, can lead to significant differences in microbial community structures. These disparities in microbial communities inevitably affect the screening of subsequent activators and the evaluation of their effectiveness. Consequently, conducting analyses of indigenous microbial communities in various types of oil reservoirs, clarifying the characteristics of these reservoir microbial communities, identifying their predominant oil displacement functional bacteria, and subsequently formulating targeted activation strategies for these functional microorganisms is imperative for designing efficient technologies for MEOR [37,38]. Studies have shown that nutrient-rich environments favor fast-growing bacteria, whereas nutrient scarcity selects bacteria with efficient nutrient utilization. In oligotrophic environments, microorganisms are more likely to alleviate resource constraints and sustain life processes through "group cooperation". This was the so-called "hunger game" hypothesis, which focused on environmental nutrient supply, individual growth strategies, and interspecies interactions. IMEOR requires synergistic interactions

among microbial communities. Therefore, it is necessary to ensure that various oil displacement functional bacteria work together synergistically while also enhancing their activation rates. In this paper, a rapid establishment of an oligotrophic activation system is achieved by exploring the relationship between functional flora and environmental factors. This is achieved through a combination of dynamic and static experiments, providing reference methods and pathways for improving recovery after polymer flooding (Figure 5A,B). This activation system addresses the challenges of lengthy screening cycles and low activation efficiency, significantly impacting the implementation of microbial-enhanced oil recovery technology in various types of oil reservoirs.

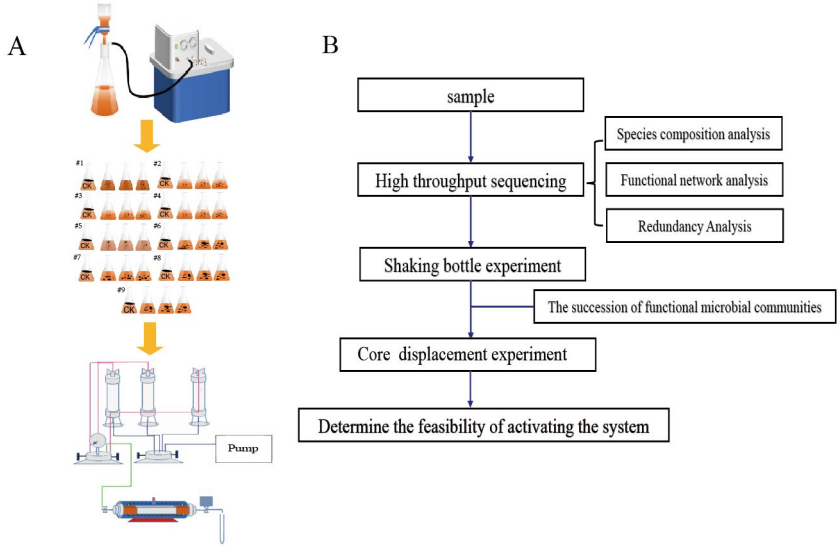

Figure 5. (**A**) Schematic diagram of oligotrophic activation system screening. (**B**) Rapid screening process in an oligotrophic activation system.

The results show that the degradation rate of the highest culture medium increased from 14.38% to 20.95%, representing a total increase of 45.68%. Due to varying nutrient levels leading to different activation functions, the FAPROTAX functional analysis (Figure 4B) revealed that the primary functions of the microbial communities in the fow_JO included hydrocarbon degradation, thiosulphate respiration, chemoheterotrophy, fermentation, etc. Biodegradation is of paramount importance in MEOR. From one perspective, bacteria can degrade hydrocarbons in crude oil [39] to obtain carbon for energy, growth, and reproduction. From another perspective, the transformation of heavy components into lighter ones leads to a fundamental alteration of crude oil properties, resulting in reduced viscosity and enhanced flowability. Consequently, this facilitates increased oil recovery. In the fow_JO, the microorganisms mainly responsible for chemoheterotrophy and hydrocarbon degradation functions were *Synergistaceae*, *Pseudomonadaceae*, and *Moraxellaceae*. *Synergistaceae* belongs to the phylum of autotrophic bacteria and plays a crucial role in the biological conversion of organic matter into methane and carbon dioxide [40]. *Pseudomonas* is a widely reported functional bacterium that can be used for microbial oil recovery. Its primary function in oil recovery is to degrade crude oil and generate biosurfactants [41]. *Moraxellaceae* also belongs to the Proteobacteria phylum, which is involved in the degradation and fermentation of organic components, such as hydrocarbon degradation. To activate these oil displacement functions, $MgSO_4$, NH_4Cl, and $CaCl_2$ were selected as regulatory factors based on RDA, and different schemes of indoor shaking bottle experiments were conducted. The addition of activators effectively stimulates various microbial functions in the reservoir. In the rev_FA1_JO, the main transformations occurred in aromatic compound degradation, dark hydrogen oxidation, and methanogenesis functional groups. In rev_WJY_JO, the focus was on fermentation, chemoheterotrophy, nitrite respiration, etc. The activation effects in two different schemes indicate that rev_FA1_JO activates the

methane production function. In the middle of the oil reservoir, facultative microorganisms and anaerobic microorganisms coexist, producing H_2, CO_2, small molecular acids, and alcohols through anaerobic fermentation. In the anoxic conditions of the deep reservoir environment, methanogenic microorganisms enhance the fluidity of crude oil by producing methane through methanogenesis [42]. Meanwhile, the degradation function of aromatic compounds was activated, leading to the production of small molecule substances, acids, etc., which are beneficial for enhancing crude oil recovery. Compared with rev_WJY_JO, rev_FA1_JO demonstrates a quicker activation rate of functional microorganisms, which is more conducive to activating the oil displacement function.

Environmental variables such as temperature, chemical composition of the formation brine, and stochastic processes [43,44] have all been found to be important drivers of distinct microbial assemblages in oil reservoirs. The structure of the original microbial community changes with different reservoir environments. When activators are injected, different types of activators will have different selective activation effects on the microorganisms, resulting in a decrease in microbial species and an increase in abundance [45]. The species abundance of *Pseudomonadaceae* gradually decreased from 65% before activation and finally reached its lowest value at 1%. However, the abundance of *Comamonadaceae*, *Hydrogenophilaceae*, and *Enterobacteriaceae*, which are also part of the Proteobacteria phylum, increased to 67%, 12%, and 5%, respectively (Figure 4A). Some members of the *Comamonadaceae* possess the ability to degrade hydrocarbon compounds in crude oil. They break down these compounds into simpler molecules through metabolic activities, thereby facilitating the release and flow of hydrocarbons within the reservoir, which in turn enhances recovery rates. *Hydrogenophaga* exhibits certain activity in the anaerobic cultivation of methanogens that use coal as a substrate [46] and can degrade and utilize biphenyl-based organics [47]. Therefore, *Hydrogenophaga* may participate in methanogenesis as a fermentative bacterium. Under aerobic and anaerobic conditions, certain bacterial species within the genera *Enterobacter* sp. have exhibited the ability to degrade PAM/HPAM, achieving degradation rates ranging from 16% to 91% [48]. The decline of *Pseudomonadaceae* may be due to a synergistic effect with the families of *Comamonadaceae* and *Hydrogenophilaceae* bacteria. More importantly, these synergistic microbial populations with a greater abundance contribute to a more robust function in the reservoir microbial ecosystem. In this study, in order to further validate the activation system, core oil displacement experiments were conducted based on the optimal scheme. The results show that on the basis of polymer flooding, the oil recovery is increased by 3.82%. Therefore, exploring the relationship between functional microorganisms and environmental factors can provide an important reference value for the rapid activation and targeted mediation of functional bacteria.

5. Conclusions

In order to rapidly activate the oil displacement function microorganisms and enhance the recovery rate of post-polymer flooded reservoirs, we deliberately adjusted the composition and concentration of inorganic salts in the oligotrophic activation system, based on the relationship between functional microorganisms and environmental factors. Meanwhile, changes in the microbial function before and after activation were analyzed. A targeted activation strategy was established under an oligotrophic stimulation system through the integration of static and dynamic experiments.

The functional groups in the core bacterial groups in the post-polymer flooded reservoir were mainly dominated by hydrocarbon degradation, chemoheterotrophic, aromatic compound degradation, and fermentation functional groups. Through indoor shaking bottle experiments, it has been proven that the aromatic compound degradation and methanogenesis function microbial communities were significantly activated. The degradation rate of the optimized culture medium increased from 14.38% to 20.95%, a total increase of 45.68%. Based on microbial community functional analysis and RDA analysis, the regulatory factors were determined to be $MgSO_4$, NH_4Cl, and $CaCl_2$, respectively. The indigenous functional microbes showed remarkable abundance, and the beneficial function-

ing bacteria were stimulated in the IMEOR process. *Comamonadaceae*, *Hydrogenophilaceae*, and *Enterobacteriaceae* dominated the activation process. The core flood results showed that the oil recovery factor increased by 3.82% on the basis of polymer flooding, with good oil displacement efficiency.

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References

1. El-hoshoudy, A.N. Experimental and theoretical investigation for synthetic polymers, biopolymers and polymeric nanocomposites application in enhanced oil recovery operations. *Arab. J. Sci. Eng.* **2022**, *47*, 10887–10915. [CrossRef]
2. Ruiz-Cañas, M.C.; Quintero-Perez, H.I.; Castro-Garcia, R.H.; Romero-Bohorquez, A.R. Use of nanoparticles to improve thermochemical resistance of synthetic polymer to enhanced oil recovery applications: A review. *CTF-Cienc. Tecnol. Y Futuro* **2020**, *10*, 85–97.
3. Tavakkoli, O.; Kamyab, H.; Shariati, M.; Mohamed, A.M.; Junin, R. Effect of nanoparticles on the performance of polymer/surfactant flooding for enhanced oil recovery: A review. *Fuel* **2022**, *312*, 122867. [CrossRef]
4. Firozjaii, A.M.; Saghafi, H.R. Review on chemical enhanced oil recovery using polymer flooding: Fundamentals, experimental and numerical simulation. *Petroleum* **2020**, *6*, 115–122. [CrossRef]
5. Gbadamosi, A.O.; Junin, R.; Manan, M.A.; Agi, A.; Yusuff, A.S. An overview of chemical enhanced oil recovery: Recent advances and prospects. *Int. Nano Lett.* **2019**, *9*, 171–202. [CrossRef]
6. Salem, K.G.; Tantawy, M.A.; Gawish, A.A.; Gomaa, S.; El-hoshoudy, A.N. Nanoparticles assisted polymer flooding: Comprehensive assessment and empirical correlation. *Geoenergy Sci. Eng.* **2023**, *226*, 211753. [CrossRef]
7. Standnes, D.C.; Skjevrak, I. Literature review of implemented polymer field projects. *J. Pet. Sci. Eng.* **2014**, *122*, 761–775. [CrossRef]
8. She, Y.-H.; Zhang, F.; Xia, J.-J.; Kong, S.-Q.; Wang, Z.-L.; Shu, F.-C.; Hu, J.-M. Investigation of biosurfactant-producing indigenous microorganisms that enhance residue oil recovery in an oil reservoir after polymer flooding. *Appl. Biochem. Biotechnol.* **2011**, *163*, 223–234. [CrossRef]
9. Hou, J.; Song, K.; Wen, Y. Development Direction of Enhanced Oil Recovery Techniques in Polymer Flooded Mature Oilfields. *Futur. Technol.* **2023**, *2*, 47–61.
10. Ren, G.; Wang, J.; Qu, L.; Li, W.; Hu, M.; Bian, L.; Zhang, Y.; Le, J.; Dou, X.; Chen, X.; et al. Compositionsand co-occurrence patterns of bacterial communities associated with polymer-and ASP-flooded petroleum reservoir blocks. *Front. Microbiol.* **2020**, *11*, 580363. [CrossRef]
11. Cui, K.; Sun, S.; Xiao, M.; Liu, T.; Xu, Q.; Dong, H.; Wang, D.; Gong, Y.; Sha, T.; Hou, J.; et al. Microbial mineralization of montmorillonite in low-permeability oil reservoirs for microbial enhanced oil recovery. *Appl. Environ. Microbiol.* **2018**, *84*, e00176-18. [CrossRef] [PubMed]
12. Geetha, S.J.; Banat, I.M.; Joshi, S.J. Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). *Biocatal. Agric. Biotechnol.* **2018**, *14*, 23–32.
13. She, H.; Kong, D.; Li, Y.; Hu, Z.; Guo, H. Recent advance of microbial enhanced oil recovery (MEOR) in China. *Geofuids* **2019**, *2019*, 1871392. [CrossRef]
14. Gong, X.C.; Liu, Z.S.; Guo, P.; Chi, C.Q.; Chen, J.; Wang, X.B.; Tang, Y.Q.; Wu, X.L.; Liu, C.Z. Bacteria in crude oil survived autoclaving and stimulated differentially by exogenous bacteria. *PLoS ONE* **2012**, *7*, e40842. [CrossRef] [PubMed]
15. Wu, B.; Xiu, J.; Yu, L.; Huang, L.; Yi, L.; Ma, Y. Biosurfactant production by Bacillus subtilis SL and its potential for enhanced oil recovery in low permeability reservoirs. *Sci. Rep.* **2022**, *12*, 7785. [CrossRef] [PubMed]
16. Markande, A.R.; Patel, D.; Varjani, S. A review on biosurfactants: Properties, applications and current developments. *Bioresour. Technol.* **2021**, *330*, 124963. [CrossRef] [PubMed]

17. Xia, W.; Du, Z.; Cui, Q.; Dong, H.; Wang, F.; He, P.; Tang, Y. Biosurfactant produced by novel Pseudomonas sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons. *J. Hazard. Mater.* **2014**, *276*, 489–498. [CrossRef]
18. Suri, N.; Gassara, F.; Stanislav, P.; Voordouw, G. Microbially enhanced oil recovery by alkylbenzene-oxidizing nitrate-reducing bacteria. *Front. Microbiol.* **2019**, *10*, 453830. [CrossRef] [PubMed]
19. Liu, Y.-F.; Chen, J.; Liu, Z.-L.; Shou, L.-B.; Lin, D.-D.; Zhou, L.; Yang, S.-Z.; Liu, J.-F.; Li, W.; Gu, J.-D.; et al. Anaerobic degradation of paraffins by thermophilic Actinobacteria under methanogenic conditions. *Environ. Sci. Technol.* **2020**, *54*, 10610–10620. [CrossRef]
20. Vigneron, A.; Alsop, E.B.; Lomans, B.P.; Kyrpides, N.C.; Head, I.M.; Tsesmetzis, N. Succession in the petroleum reservoir microbiome through an oil field production lifecycle. *ISME J.* **2017**, *11*, 2141–2154. [CrossRef]
21. Lin, J.H.; Zhang, K.C.; Tao, W.Y.; Wang, D.; Li, S. Geobacillus strains that have potential value in microbial enhanced oil recovery. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 8339–8350. [CrossRef] [PubMed]
22. Gao, P.; Li, G.; Le, J.; Liu, X.; Liu, F.; Ma, T. Succession of microbial communities and changes of incremental oil in a post-polymer flooded reservoir with nutrient stimulation. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 2007–2017. [CrossRef] [PubMed]
23. Harvey, R.W.; Harms, H.; Landkamer, L. Transport of microorganisms in the terrestrial subsurface: In situ and laboratory methods. *Man. Environ. Microbiol.* **2007**, *70*, 872–897.
24. Sheehy, A. Oil Recovery Using Microorganisms. WO1992001780A1, 6 February 1992.
25. Gao, P.; Tian, H.; Li, G.; Zhao, F.; Xia, W.; Gu, J.D.; Le, J.; Ma, T. Environmental conditions and diffusion-limited microbial transfer drive specific microbial communities detected at different sections in oil-production reservoir with water-flooding. *bioRxiv* **2020**, 2020-12.
26. Sun, G.Z.; Qian, Q.; Hu, J.; Wang, Z.L.; Li, X.M.; Wang, W.D. Study on the Oil Recovery Mechanism of Endogenous Microorganisms in the Z3 Oil Reservoir under Different Types of Activation Agents. *J. Xi'an Shiyou Univ. Nat. Sci. Ed.* **2019**, *2*, 8.
27. Yuan, S.Y.; Wang, Q. New progress and prospect of oilfields development technologies in China. *Petrol. Explor. Dev.* **2018**, *4*, 698–711. [CrossRef]
28. Barberán, A.; Bates, S.T.; Casamayor, E.O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2012**, *6*, 343–351. [CrossRef]
29. Gloor, G.B.; Hummelen, R.; Macklaim, J.M.; Dickson, R.J.; Fernandes, A.D.; MacPhee, R.; Reid, G. Microbiome profiling by illumina sequencing of combinatorial sequence-tagged PCR products. *PLoS ONE* **2010**, *5*, e15406. [CrossRef] [PubMed]
30. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
31. Sansupa, C.; Wahdan, S.F.M.; Hossen, S.; Disayathanoowat, T.; Wubet, T.; Purahong, W. Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria? *Appl. Sci.* **2021**, *11*, 688. [CrossRef]
32. Zhao, F.; Li, P.; Guo, C.; Shi, R.J.; Zhang, Y. Bioaugmentation of oil reservoir indigenous Pseudomonas aeruginosa to enhance oil recovery through in-situ biosurfactant production without air injection. *Bioresour. Technol.* **2018**, *251*, 295–302. [CrossRef]
33. Gao, W.; Gao, X.; Mi, T.; Han, B.; Zhang, Y.; Li, X.; Yin, X.; Sun, C.; Li, Q.; Cui, Z.; et al. Degradation potential and diversity of oil-degrading bacteria isolated from the sediments of the Jiaozhou Bay, China. *Acta Oceanol. Sin.* **2019**, *38*, 54–64. [CrossRef]
34. Seright, R.S.; Wang, D. Polymer flooding: Current status and future directions. *Pet. Sci.* **2023**, *20*, 910–921. [CrossRef]
35. Shi, M.; Wei, L.; Liao, G.; Han, P.; Hou, Z.; Chen, X.; Wang, Y. Laboratory study on MEOR after polymer flooding. In *SPE International Improved Oil Recovery Conference in Asia Pacific*; SPE: Kuala Lumpur, Malaysia, 2003; p. SPE-84865.
36. Patel, J.; Borgohain, S.; Kumar, M.; Rangarajan, V.; Somasundaran, P.; Sen, R. Recent developments in microbial enhanced oil recovery. *Renew. Sustain. Energy Rev.* **2015**, *52*, 1539–1558. [CrossRef]
37. Mokhtari, M.; Ozbayoglu, E.M. Laboratory investigation on gelation behavior of xanthan crosslinked with borate intended to combat lost circulation. In *SPE International Production and Operations Conference and Exhibition*; SPE: Tunis, Tunisia, 2010; p. SPE-136094.
38. Sun, G.Z.; Song, X.; Wu, X.L.; Wang, W.D.; Li, X.M.; Wang, Z.L. Study on Activation of Endogenous Functional Microorganisms in Block 3 of Oil-Contaminated Areas by Different Components of Long-Acting Activators. *J. Xi'an Shiyou Univ. Nat. Sci. Ed.* **2017**, *32*, 112–118.
39. Xu, X.; Liu, W.; Tian, S.; Wang, W.; Qi, Q.; Jiang, P.; Gao, X.; Li, F.; Li, H.; Yu, H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. *Front. Microbiol.* **2018**, *9*, 2885. [CrossRef] [PubMed]
40. Yahai, Z.J.L. Research progress on interspecies electron transfer of methane-producing microorganisms through mutual oxidation. *Microbiol. Bull.* **2015**, *42*, 920–927. [CrossRef]
41. Youssef, N.; Elshahed, M.S.; McInerney, M.J. Microbial processes in oil fields: Culprits, problems, and opportunities. *Adv. Appl. Microbiol.* **2009**, *66*, 141–251. [PubMed]
42. Varjani, S.J.; Upasani, V.N. Core flood study for enhanced oil recovery through ex-situ bioaugmentation with thermo-and halo-tolerant rhamnolipid produced by Pseudomonas aeruginosa NCIM 5514. *Bioresour. Technol.* **2016**, *220*, 175–182. [CrossRef]
43. Stegen, J.C.; Lin, X.; Konopka, A.E.; Fredrickson, J.K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **2012**, *6*, 1653–1664. [CrossRef]
44. Ren, H.; Xiong, S.; Gao, G.; Song, Y.; Cao, G.; Zhao, L.; Zhang, X. Bacteria in the injection water differently impacts the bacterial communities of production wells in high-temperature petroleum reservoirs. *Front. Microbiol.* **2015**, *6*, 505. [CrossRef] [PubMed]

45. Lin, J.; Hao, B.; Cao, G.; Wang, J.; Feng, Y.; Tan, X.; Wang, W. A study on the microbial community structure in oil reservoirs developed by water flooding. *J. Pet. Sci. Eng.* **2014**, *122*, 354–359. [CrossRef]
46. Beckmann, S.; Lueders, T.; Krüger, M.; von Netzer, F.; Engelen, B.; Cypionka, H. Acetogens and acetoclastic Methanosarcinales govern methane formation in abandoned coal mines. *Appl. Environ. Microbiol.* **2011**, *77*, 3749–3756. [CrossRef] [PubMed]
47. Lambo, A.J.; Patel, T.R. Cometabolic degradation of polychlorinated biphenyls at low temperature by psychrotolerant bacterium Hydrogenophaga sp. IA3-A. *Curr. Microbiol.* **2006**, *53*, 48–52. [CrossRef]
48. Joshi, S.J.; Abed, R.M. Biodegradation of polyacrylamide and its derivatives. *Environ. Process.* **2017**, *4*, 463–476. [CrossRef]