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Response Characteristics of the Community Structure and Metabolic Genes of Oil-Recovery Bacteria after Targeted Activation of Petroleum Hydrocarbon-Degrading Bacteria in Low-Permeability Oil Reservoirs

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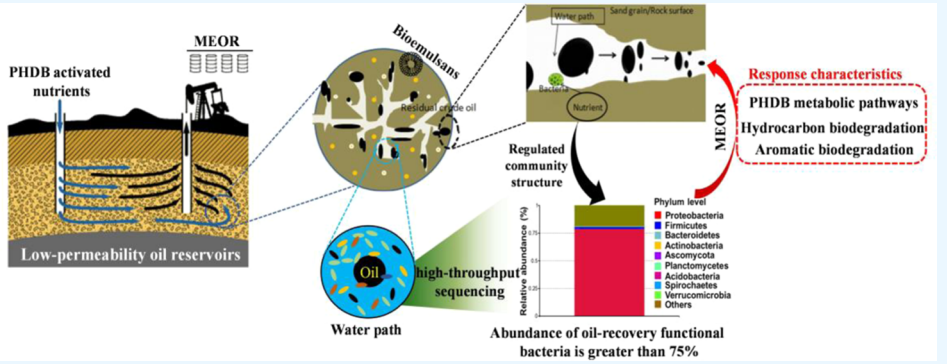

ABSTRACT: The microbial enhanced oil recovery (MEOR) process has been identified as a promising alternative to conventional enhanced oil recovery methods because it is eco-friendly and economically advantageous. However, the knowledge about the composition and diversity of microbial communities in artificially regulated reservoirs, especially after activating petroleum hydrocarbon-degrading bacteria (PHDB) by injecting exogenous nutrients, is still insufficient. This study utilized a combination of high-throughput sequencing and metagenomics technology to reveal the structural evolution characteristics of the indigenous microbial community in the reservoir during the PHDB activated for enhanced oil recovery, as well as the response relationship between the expression of its oil production functional genes and crude oil biodegradation. Results showed that *Pseudomonas* (>75%) gradually evolves into a stable dominant microbial community in the reservoir during the activation of PHDB. Besides, the gene expression and KEGG pathways after crude oil undergoes biodegradation by PHDB show that the number of genes related to petroleum hydrocarbon metabolism dominates the metabolism (21.98%). Meanwhile, a preliminary schematic diagram was drawn to illustrate the evolution mechanism of the EOR metabolic pathway after the targeted activation of PHDB. Additionally, it was found that the abundance of hydrocarbon-degrading enzymes increased significantly, and the activity of alcohol dehydrogenase was higher than that of aldehyde dehydrogenase and monooxygenase after PHDB activation. These research results not only filled in and expanded the theoretical knowledge of MEOR based on artificial interference or regulation of reservoir oil-recovery functional microbial community structure but also provided guidance for the future application of MEOR technology in oil field operations.

1. INTRODUCTION

Microbial enhanced oil recovery (MEOR) technology is a major topic in the areas of oil field exploration, characterized by its strong adaptability to reservoir environments, environmentally friendly nature, and cost-effectiveness, as a green and low-carbon technique for enhancing oil recovery.[1,2] Compared with traditional tertiary oil recovery technologies (such as conventional water flooding, air injection flooding, and chemical agent flooding, etc.),[3−5] MEOR activates functional bacteria for oil recovery by injecting specific nutrients into the oil reservoir or selects oil-producing functional microorganisms from the *in situ* environment of the reservoir to produce specific metabolites through ground fermentation and then injects them into the oil reservoir, ultimately achieving the purpose of improving oil recovery.[6−8] However, although MEOR has the potential to be an economically attractive application, the processes involved in MEOR are complicated and the understanding of all of the oil-displacement

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mechanisms is still vague. The knowledge about the composition and diversity of microbial communities in artificially regulated reservoirs, especially after activating oil-producing bacteria by injecting exogenous nutrients, is still insufficient. This knowledge is crucial for understanding the ecological evolution characteristics and metabolic processes of reservoir microbes during microbial flooding, as well as for selecting strategies for MEOR applications in reservoirs.

During the process of MEOR, indigenous activation microbial enhanced oil recovery technology has been recognized to have the advantages of strong adaptability, good compatibility with the reservoir, and low cost.[9,10] In this process, crude oil is degraded by synergistic effects of microbes and metabolites, leading to improved swept volume and enhanced oil recovery.[11−14] Various mechanisms for enhancing oil recovery, such as viscosity reduction, improved fluidity, and increased oil displacement, have been summarized in numerous previous research reports on MEOR processes.[15−18] However, a slight drawback is that these mechanisms only broadly elucidate the enhanced oil recovery process of oil-producing functional microorganisms in reservoir environments from a macroscopic perspective. They did not reveal from a microscopic perspective how the enhanced oil-recovery genes of these functional microorganisms are expressed due to changes in microbial community structure and how the corresponding metabolic pathways are affected.

Noteworthily, biological viscosity reduction of crude oil is one of the most attractive mechanisms in MEOR. In this process, heavy components are converted into light components, which fundamentally change the properties of crude oil, reduce the viscosity of crude oil, improve the fluidity of crude oil, and thus improve oil recovery.[19] For example, some microorganisms in reservoirs can grow and reproduce directly with petroleum hydrocarbon as a carbon source, which is called petroleum hydrocarbon-degrading bacteria (PHDB). The oil recovery mechanisms of PHDB in reservoirs have been summarized and described in our previous reports.[16,19] Besides, the diversity and evolutionary characteristics of reservoir microorganisms exhibit a myriad of changes with different oil recovery methods and production cycles in the reservoir.[20,21] Alkan et al. reported that the diversity of bacteria in reservoir brine could affect the MEOR flooding performance since each microbe has different metabolic pathways and produces different end-products.[22] However, there are still few research reports on microbial metabolic pathways that activate oil-enhancing microbes by injecting nutrients. Thus, studying the response characteristics of the structure of other oil-producing bacterial communities and their metabolic gene expression after targeted activation of PHDB in reservoirs is of great significance. It can provide further theoretical guidance for the future on-site application of MEOR technology.

Compared to 16S rRNA gene profiling, metagenomic analysis provides information about the potential metabolism of microorganisms.[23] Metagenomic approaches are to conduct statistical analysis on the gene information on microorganisms, predict the function of microorganisms by using their gene composition, and fundamentally analyze the mechanism of microbial action.[24,25] Sierra-Garcia et al. revealed through metagenomics-based approaches that high proportions of hydrocarbon-degrading genes are found in several hydrocarbon environments.[26] It can help provide evidence for MEOR while also providing insight into rich genetic reserves. To evaluate the application potential of microorganisms at the molecular level, there are mainly three functional databases in common use at present,[27−29] Kyoto Encyclopedia of Genes and Genomes (KEGG) database, evolutionary genealogy of genes: nonsupervised orthologous groups (eggNOG) database, and carbohydrate-active enzymes database. Therefore, it is crucial to comprehensively elucidate the genomic spectrum of hydrocarbon biodegradation in crude oil under the symbiotic or mutually beneficial interactions of in situ activated oil-producing functional microorganisms and other microbes in the reservoir environment.

Previous studies have shown that the activation time scale and metabolic characteristics of oilfield oil-recovery functional bacteria in response to different nutrient activation agent components and concentrations also vary.[16,19] Therefore, a combination of the high-throughput sequencing technology and metagenomics methods in this study is needed to carry out sequencing on the genes of targeted activated oil production functional microbial flora in produced fluids from oil wells. To analyze the intermediate products and transformation pathways of petroleum hydrocarbon biodegradation after PHDB activation, bioinformatics analyses are used to specifically investigate the association of community gene functions and their relationship to petroleum hydrocarbon degradation mechanisms during the transformation of petroleum hydrocarbons into metabolic products and/or during energy transfer. Here, we describe evidence for a new relationship between crude oil biodegradation and oil-enhancing gene characteristics of PHDB activation based on the response characteristics of the community structure and metabolic genes of oil-producing bacteria that are unnaturally regulated in low-permeability oil reservoirs. The research results further expand the potential of activating indigenous PHDB in reservoirs to improve oil recovery in low-permeability reservoirs.

2. MATERIALS AND METHODS

2.1. Enrichment Culture for PHDB. The production water samples used for PHDB activation were collected from the Yanchang oilfield located in Northwest China. Characteristics of production water samples are shown in Table S1. PHDB enrichment medium was made based on the modification of the medium in the literature[19] as follows: crude oil 2.0 (wt %), KH_2PO_4 2.0 g/L, Na_2HPO_4 1.5 g/L, NH_4Cl 2.0 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, $NaNO_3$ 4.0 g/L, and 1.0 mL trace element solution. The temperature and pH of the reservoir were 30 ± 2 °C and 7.5−8.5, respectively. In this study, 300 mL of production water sample was injected into facultative anaerobic bottles of 500 mL in total volume with an enrichment medium to activate the PHDB. The bottles were incubated at 30 °C while shaking at 150 rpm for 5−7 days. To obtain reliable data, all treatments were performed in duplicate.

2.2. Metagenomic DNA Extraction. One hundred and fifty milliliters of PHDB enrichment experiment water sample was filtered through a 2.5 μm glass filter with a 50 mm glass chimney filter unit to remove impurities. Then, the filtrate was filtered through a 0.22 μm cellulose acetate filter to collect the microbes. The total DNA of the sample was extracted using the power water DNA Isolation Kit (supplied by Beijing Tiangen Biochemical Technology Co., Ltd.) according to the manufacturer's instructions, and DNA contents above 1 μg were used to construct the library.

2.3. Metagenomic Sequencing and Polymerase Chain Reaction (PCR) Amplification. First, a total amount of 1 μg of DNA sample was used as an input material for the

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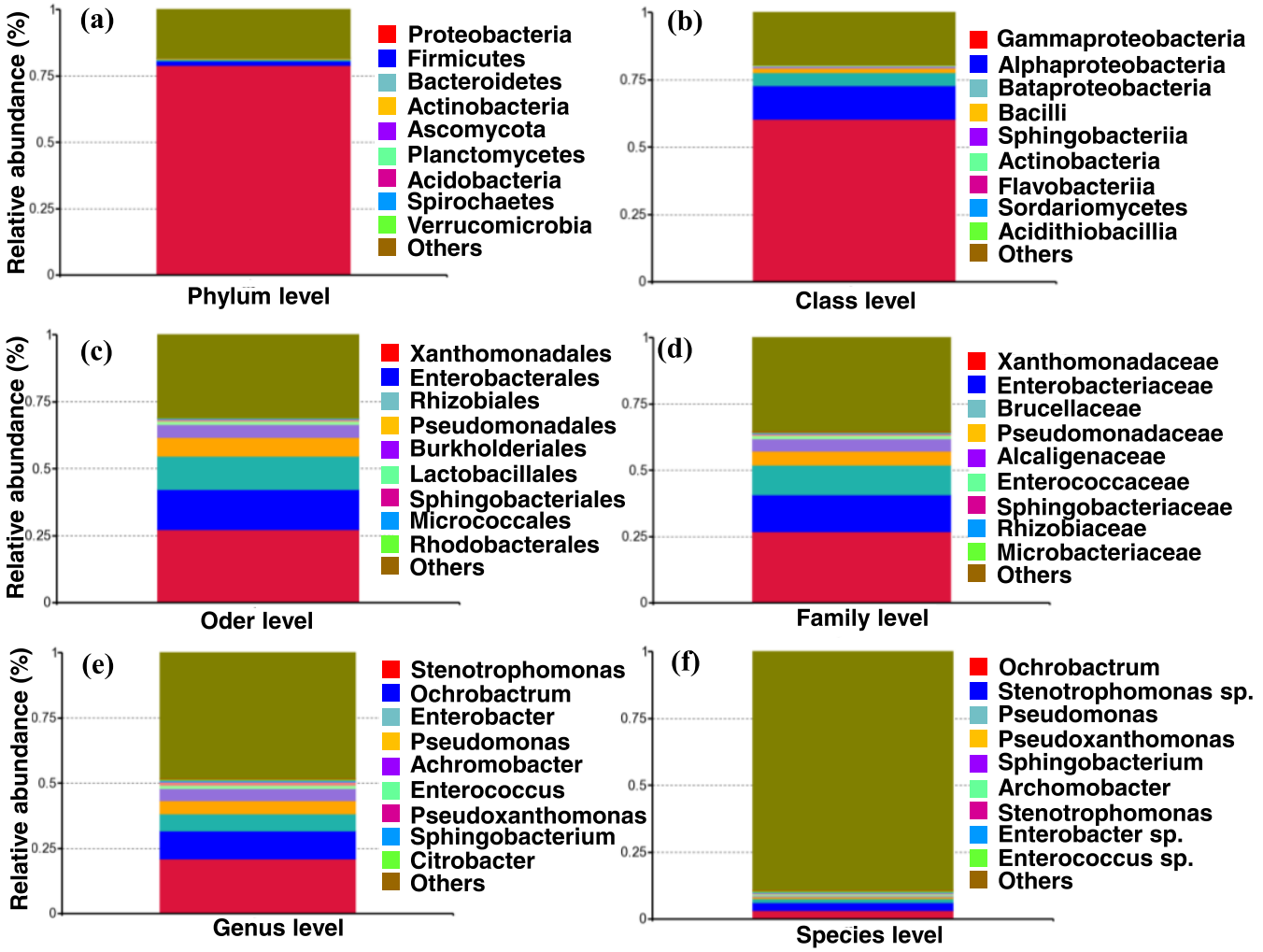

Figure 1. Statistical results of bacterial community composition at phylum (a), class (b), order (c), family (d), genus (e), and species (f) levels.

DNA. Purified DNA was subjected to metagenomic sequencing on the Illumina Miseq platform. Second, the DNA sample was fragmented by sonication to a size of 350 bp, and then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. The PCR program is described in our previous study.[16,19] The mixed PCR products were then sent to the Beijing Institute of Genomics, Chinese Academy of Sciences, for sequencing conducted on a Miseq platform.

2.4. Date Analysis of Metagenomic Sequencing. The DNA sample was lyophilized and shipped to the Beijing Institute of Genomics, Chinese Academy of Sciences, for metagenomic analysis. The testing methods and primer usage for metagenomic techniques can be found in our previous reports.[19] First, species composition and function could be annotated according to the gene prediction results, and a series of data mining analyses could be conducted based on the annotation results.[30] Second, according to the results of gene comparison, the metabolic pathway of the mixed bacteria degrading petroleum hydrocarbons was constructed. The degradation mechanism of petroleum hydrocarbon crude oil was analyzed fundamentally. Finally, the cluster profiler[31] and path-view[28] functions (the R computing package) were used to analyze the distribution of the KEGG-Orthology functions within pathways that may be related to the degradation of petroleum hydrocarbons.[32] Details of additional operational methods can be found in a previous publication.[19]

3. RESULTS AND DISCUSSION

3.1. PHDB Community Evolution after Activation. Based on our previous research,[16,19] the primary factor hindering the abundant proliferation of indigenous oil-recovery functional microbes in reservoirs is a lack of sufficient nutrients. Sequencing of 16S rRNA gene amplicons revealed that with the activation of the oil-producing functional bacterial community in the oil reservoir, *Proteobacteria* (>75%) gradually evolved into the dominant microbial community (Figure 1). The facultative anaerobic metabolism of *Proteobacteria* phylum reportedly leads to the degradation of alkanes and aromatic hydrocarbons and the synchronous production of metabolites to facilitate crude oil biodegradation.[33,34] Besides, *Gammaproteobacteria* (58.5%), *Xanthomonadales* (25.4%), *Xanthomonadaceae* (26.6%), *Stenotrophomonas* sp. (20.5%), and *Ochrobactrum* (5.1%) were the main dominant bacteria at class, order, family, genus, and species level under nutrient stimulation conditions, respectively. Many references demonstrated that the phylum *Proteobacteria* (accounted for more than 60% of the total clones), mainly *Pseudomonas* and *Acinetobacter*, performs the bulk of the activity of hydrocarbon degradation, including alkanes, aerobic

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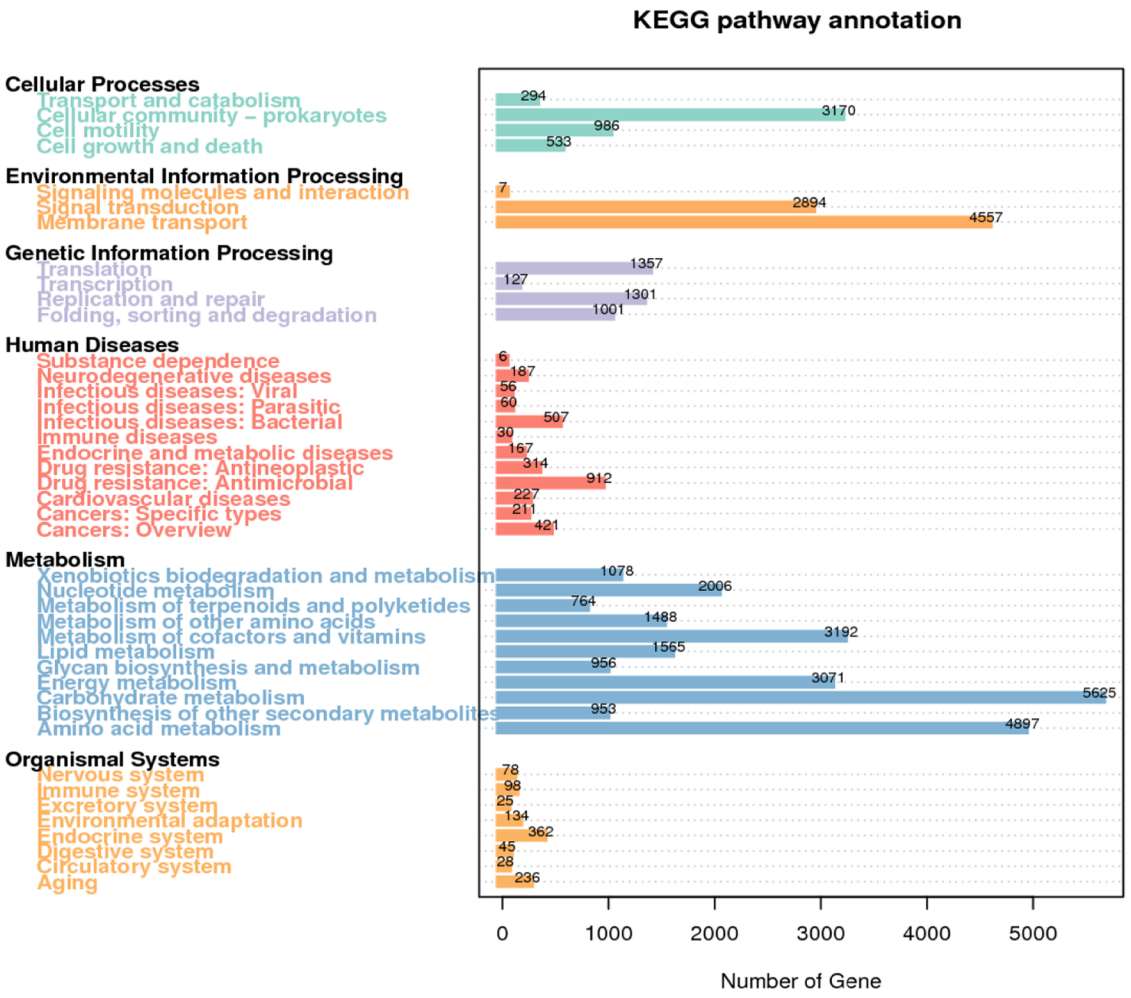

Figure 2. Number of KEGG annotated genes in the PHDB metabolic pathway and their corresponding functional expression characteristics for EOR.

hydrocarbons, asphaltenes, and resins.[35−37] The results showed that the nutrient injection had a profound effect on the diversity, composition, and relative number of the indigenous oil-recovery functional microorganisms.

It is worth mentioning that the genus *Stenotrophomonas* sp. (20.5%) gradually evolved into a dominant oil-recovery bacterium after the nutrient injection in this study, with its relative abundance increasing by approximately 10%. On the contrary, other conventional oil-recovery functional bacterial species in oil production ecosystems, such as the genera *Marinobacter*, *Acinetobacter*, and *Halomonas*, decreased gradually or were not detected. Overall, these results demonstrated that injecting specifically tailored nutrients into the reservoir can selectively activate beneficial oil-recovery functional bacteria (such as PHDB), enhancing or inducing their gradual evolution into a microbial community with oil-recovery capabilities adapted to low-permeability reservoir environments, thus promoting MEOR.

3.2. Activation Characteristics of Oil-Recovery Functional Genes after PHDB Stimulation. To further evaluate the gene expression and KEGG metabolic pathways specifically involved in PHDB crude oil degradation, we conducted separate statistical analyses for the single assembly gene catalogue length (Figure S1), mixed assembly gene catalogue length (Figure S2), and the number of CAZy unigenes genes (Figure S3), respectively. The specific data for the single assembly gene catalogue length, mixed assembly gene catalogue length, and the number of cAZy unigenes genes can be found in the Supporting Information. Based on this, using the comparative data on the number of functional gene and combining it with the corresponding expression levels of oil-recovery functional genes, the metabolic pathways of crude oil biodegradation after activating PHDB were constructed.

In Figure 2, the KEGG pathway was annotated to obtain information on various biological pathways, such as various metabolic pathways, synthetic pathways, signaling pathways, etc. The research results found that the number of genes dominated metabolism, followed by environmental information processing and then cellular processes. Among them, the number of metabolic genes of hydrocarbons in metabolism was as high as 5625, accounting for 21.98% of metabolism. The number of amino acid metabolism genes was 4897, accounting for 19.13% of the total metabolism. The proportion and order of the above gene expression indicate that the stimulating effect of nutrients during the activation of PHDB is greater than environmental factors, thereby demonstrating the close relationship between nutrients and community development.

According to the eggNOG cluster diagram (Figure 3), the protein of the PHDB was mainly concentrated in energy production and conversion of the C (energy production and conversion) taxon, which accounted for 5.03%. The existence of this taxon could convert nutrients into the energy required

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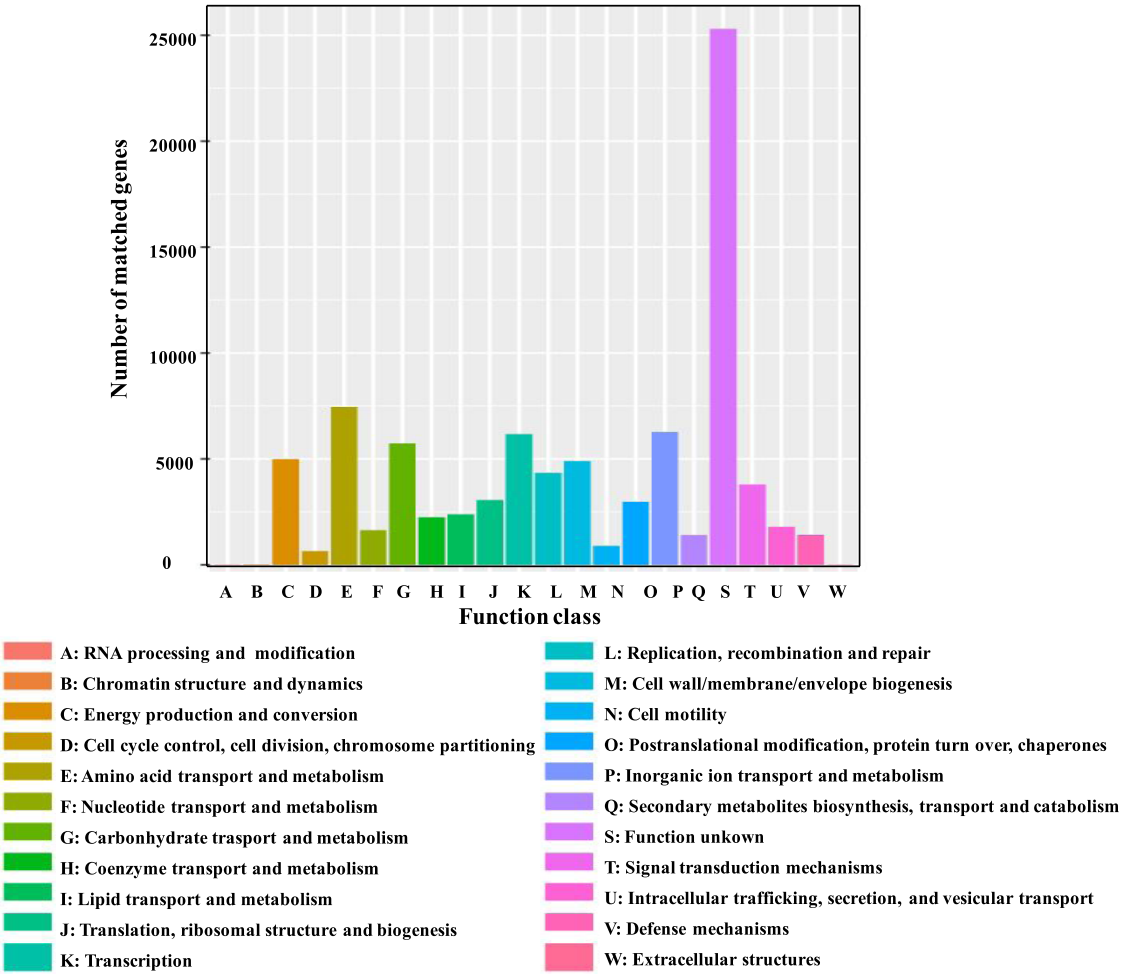

Figure 3. Number of eggNOG annotated genes in the PHDB metabolic pathway and their corresponding functional expression characteristics for EOR.

by the growth, reproduction, and metabolism of PHDB. There was 7.52% of the amino acid transport and metabolism of taxon E (amino acid transport and metabolism). The presence of this gene contributed to the synthesis of surface-activated substances produced by PHDB and the emulsification and degradation of crude oil. There was 5.78% of G (carbon hydrate transport and metabolism) taxon transport and metabolism of carbohydrates; the presence of this gene was conducive to the degradation of hydrocarbons in crude oil by PHDB. And it provided carbon sources and energy for the growth of microorganisms. The inorganic ion transport and metabolism of taxon P (inorganic ion transport and metabolism) accounted for 6.33%. The presence of this gene was helpful for PHDB in absorbing inorganic salt ions in nutrients and optimizing the activation effect of endogenous microorganisms. They also included the transcription of 6.23% of the K (transcription) taxon and the functional location of 25.5% of the S (function unknown) taxon. Furthermore, based on the expression characteristics of functional genes, we speculate that metabolites produced by the PHDB also promoted the degradation of other members in the consortium. The above phenomenon further confirmed that PHDB showed the ability to degrade metabolites accumulated by other members, alleviating the inhibitory effect of metabolites on the degradation ability of such bacteria during the biodegradation of petroleum hydrocarbons, which consequently improved the degradation efficiency.

3.3. Evolutionary of Hydrocarbon Biodegradation Pathways after PHDB Activation. According to the results of gene comparison, we found that the enzyme-encoding genes involved in hydrocarbon metabolism and its related metabolic pathways are key to the microbial degradation of petroleum. Understanding the biodegradation pathway of alkane hydrocarbons by PHDB was important from the standpoint of biotransformation that the existence of an abundance of hydrocarbon-degrading enzymes was proved after PHDB activation. These petroleum microbes contained oxygenase enzymes that could introduce oxygen into the terminal, nonactivated position of different alkane hydrocarbons in crude oil.[38,39] Medium and long-chain alkanes were oxidized by integral-membrane nonheme diiron monooxygenases (alkB) or alternatively by heme iron-containing cytochrome P450 monooxygenases, and P450 of terminal monooxygenase was mainly responsible for the degradation of medium alkanes. alkB and P450 could complement each other.

Figure 4a shows that the first enzyme of the alkane-biodegradation pathway was an integral-membrane alkane 1-monooxygenase (alkB, EC: 1.14.15.3) that hydroxylates alkanes at the terminal position. The terminal oxidation of alkanes by alkB generated primary fatty alcohols, which were further oxidized to aldehydes by alcohol dehydrogenase (ADH,

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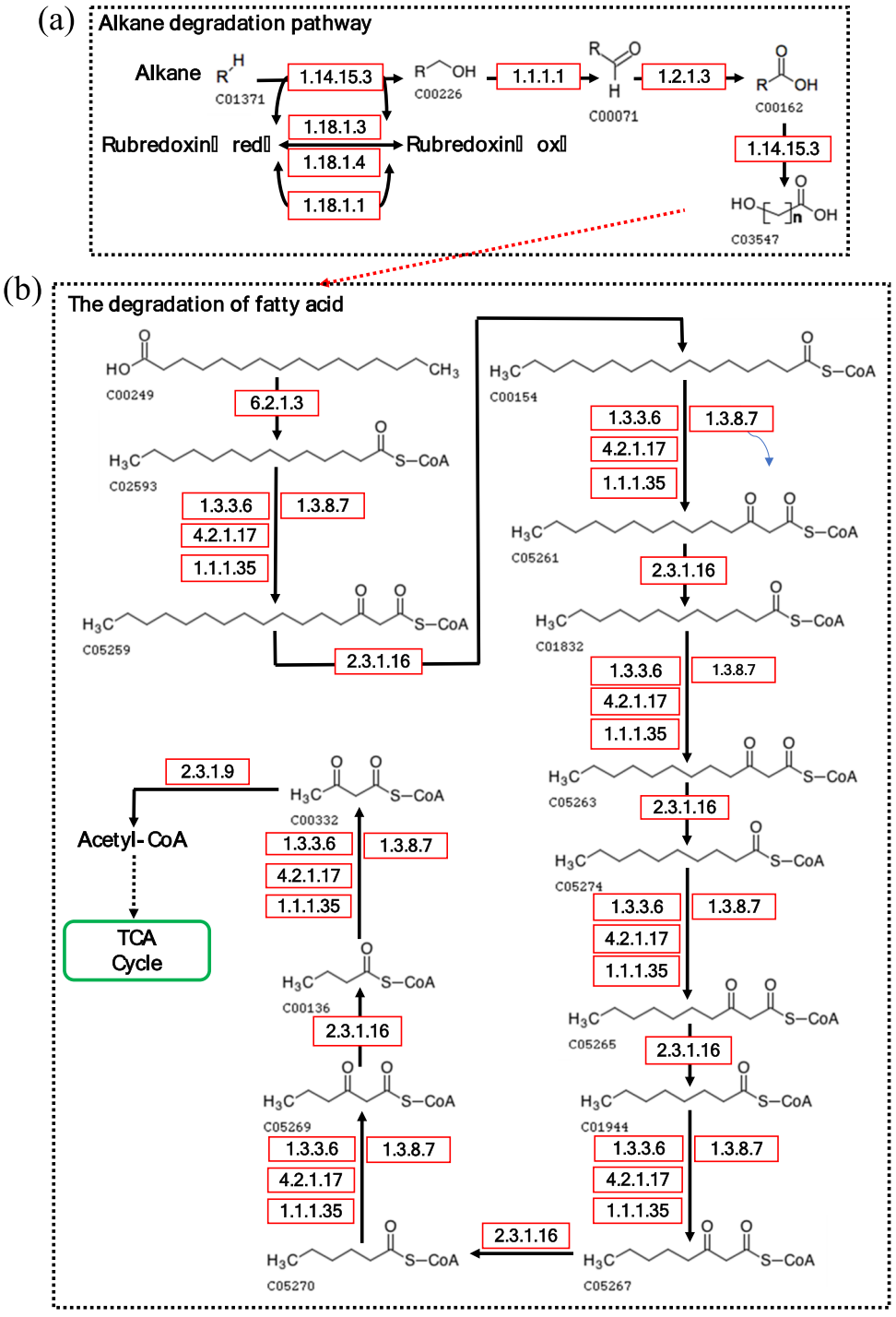

Figure 4. Evolutionary characteristics of biodegradation metabolic pathways of alkanes (a) and fatty acids (b) in crude oil after activating PHDB.

EC: 1.1.1.1). Then, the aldehydes were further oxidized to fatty acids by aldehyde dehydrogenase (ALDH, EC: 1.2.1.3), and the fatty acids continued to generate ω-hydroxyl fatty acids under the action of integral-membrane alkane 1-monooxygenase (alkB, EC: 1.14.15.3). According to this metabolic pathway, it could be prematurely judged that the degradation of alkanes by PHDB belonged to the double-terminal monooxygenation pathway. AlkB required two soluble electron transfer proteins named rubredoxin (alkG) and rubredoxin reductase (alkT). Rubredoxin reductase could transfer electrons from NADH to Rubredoxin via its cofactor FAD, which transfers electrons from NADH to the rubredoxin, which was then transferred to alkane hydroxylase to further promote oxidative degradation of alkanes. In the aerobic metabolism of alkanes, monooxygenase, alcohol dehydrogenase, and aldehyde dehydrogenase were very important, and the corresponding gene codes in the PHDB were 3, 56, and 35, respectively. Studies have shown that there was a positive correlation between the number of coding genes and the enzyme activity corresponding to the gene. Therefore, it could be inferred that the intracellular alcohol dehydrogenase activity after PHDB activation was significantly higher than that of aldehyde dehydrogenase and monooxygenase.

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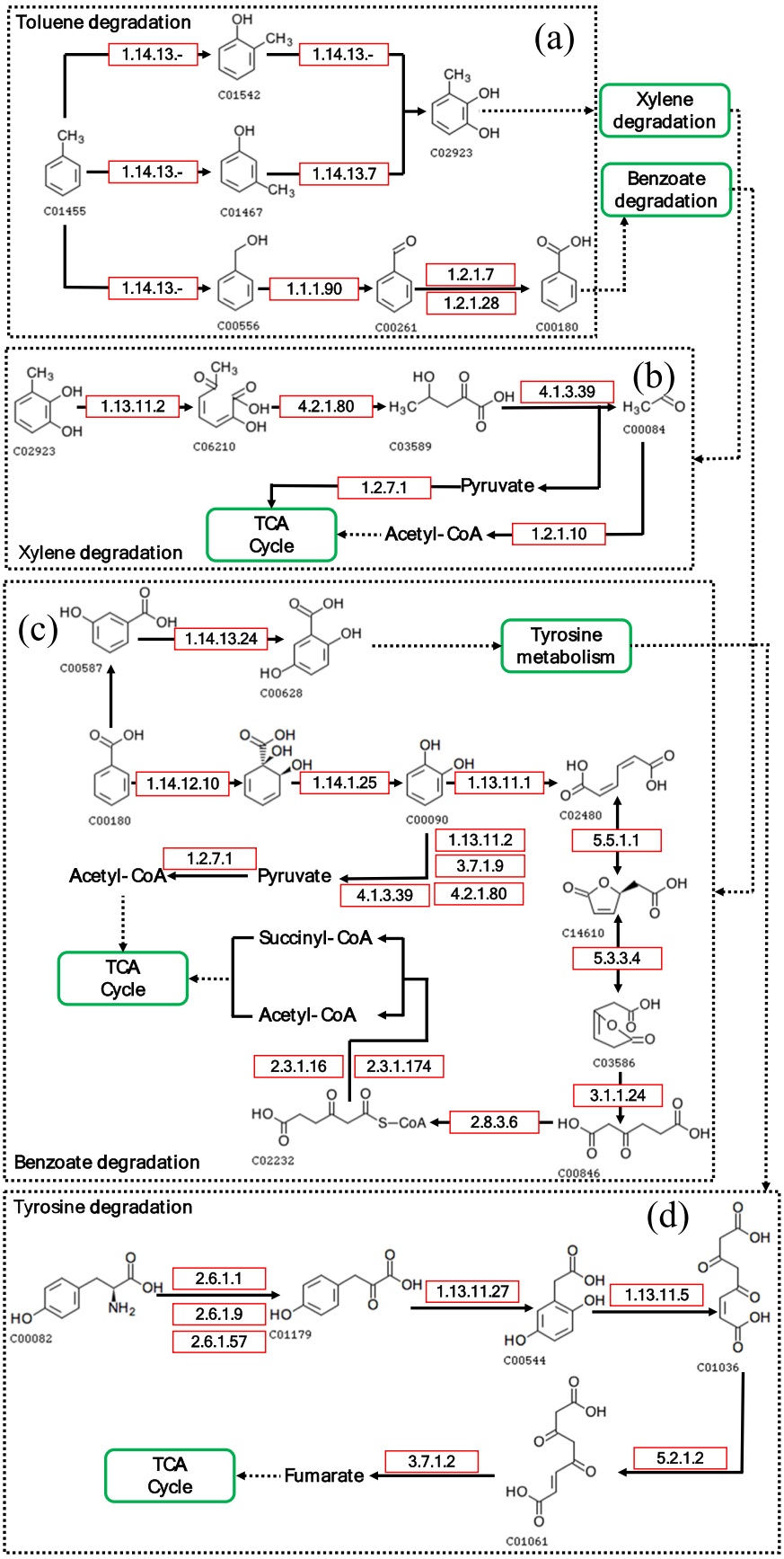

Figure 5. Evolutionary of biodegradation metabolic pathways of iconic aromatic hydrocarbon components in crude oil after activating PHDB. (a) Toluene biodegradation pathways, (b) xylene biodegradation pathways, (c) benzoate biodegradation pathways, and (d) tyrosine biodegradation pathways.

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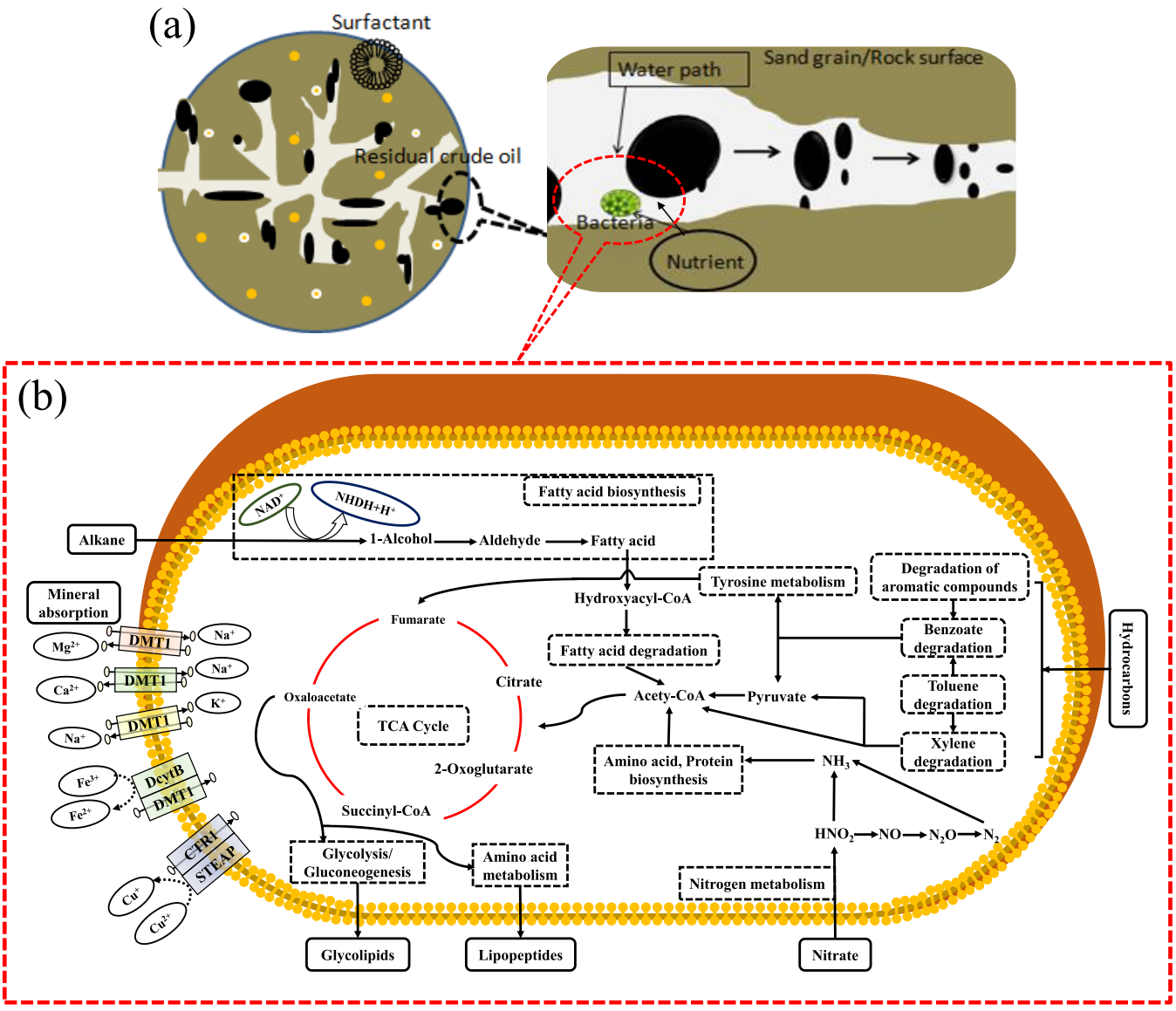

Figure 6. Evolutionary of the metabolic pathway mechanism for EOR after targeted activation of PHDB. (a) The EOR pathway for driving residual crude oil after activating PHDB, (b) Gene expression pathways for EOR in PHDB cells.

In Figure 4b, fatty acids (the main components of the bioacids produced by PHDB) were eventually produced by either single-terminal or double-terminal biodegradation pathways of alkanes. Thus, the biodegradation pathway of fatty acids and related genes was further analyzed. Long-chain acyl-CoA synthetase (fadD, EC 6.2.1.3) was the key enzyme in the fatty acid degradation pathway, and the number of its genes in the PHDB was 35. There were 3 acyl-CoA oxidase (EC: 1.3.3.6) and 55 acyl-CoA dehydrogenase (EC: 1.3.3.6, 1.3.99) genes, respectively. The gene quantity of enoyl-CoA hydratase (echA, EC 4.2.1.17), 3-hydroxyacyl-CoA dehydrogenase (HADH, EC: 1.1.1.35), acetyl-CoA acyltransferase (fadA, EC 2.3.1.16), and acetyl-CoA C-acetyltransferase (atoB, EC:2.3.1.9) were 68, 46, 25, and 66, respectively. Under the catalysis of these enzymes, fatty acids generated acetyl-CoA, entered the tricarboxylic acid cycle (TCA cycle), and finally oxidized to CO_2 and water. By clarifying the expression characteristics of the genes and enzymes involved in crude oil biodegradation after activation of PHDB, we can better explain why there is a linear relationship between the high biomass of oil-recovery functional bacteria and the increased oil recovery.

3.4. Evolutionary of Aromatic Biodegradation Pathways after PHDB Activation. The evolution of the biodegradation metabolic pathways of the iconic aromatic hydrocarbon components in crude oil after activation of PHDB was further analyzed (Figure 5). In Figure 5a, the degradation of toluene was initiated with ring hydroxylation by monooxygenase (EC 1.14.13) via adding one oxygen to the carbon on aromatic rings or of methyl substituent groups; the hydroxylation reaction generates several intermediates (such as catechol and benzoate, etc.), which were further fed into the central pathways of benzoate degradation or xylene degradation and the intermediate catechol was subsequently oxidized by catechol 2,3-dioxygenase (dmpB, xylE, EC 1.13.11.2); the cleavage products were further transformed into pyruvate or acetyl-CoA, which entered the TCA cycle for energy metabolism (Figure 5b). The intermediate benzoate was subsequently oxidized by 3-hydroxybenzoate 6-monooxygenase (nagX, EC:1.14.13.24) or benzoate/toluate 1,2-dioxyge-

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nase subunit α (benA-xylX, EC: 1.14.12.10) (Figure 5c). The cleavage products were further fed into the central pathways of tyrosine degradation or transformed into pyruvate, acetyl-CoA, or succinyl-CoA, which later entered the TCA cycle for energy metabolism.

In Figure 5d, the former was further transformed into pyruvate by tyrosine degradation and entered the TCA cycle for energy metabolism. The evolutionary characteristics of aromatic hydrocarbon biodegradation pathways after the activation of PHDB show that in the mechanism of PHDB utilizing the degradation of crude oil components (i.e., viscosity reduction) to enhance oil recovery, the degradation of aromatic hydrocarbons by PHDB exhibits a preference order, namely, toluene > xylene > benzoate > tyrosine. This order also indirectly reflects the close correlation between the microbial degradation sequence of crude oil components and the expression of their functional genes, which is consistent with the strength of oil-recovery functional gene expression after PHDB activation.

3.5. Metabolic Pathways for EOR after Targeted Activation of PHDB. Based on the above research results, we have preliminarily depicted a schematic diagram illustrating the evolution of EOR metabolic pathways following targeted activation of PHDB (Figure 6). Figure 6a depicts from a macro level how PHDB degraded and dispersed the residual crude oil in the reservoir after nutrient activation, further promoting the formation of small oil droplets in the crude oil, resulting in increased recovery. As can be seen from Figure 6b, the alkanes in crude oil enter the cell through the microbial double membrane, generate alcohol and aldehyde through the action of enzymes, and finally oxidize to fatty acid, which enters the tricarboxylic acid cycle through acetic acid-CoA. Phosphate is partially converted into H_2PO_4 through microbial metabolism, which is used in the synthesis of lecithin, nucleic acid, and ATP, and promotes the degradation of fatty acids.[40] It can also degrade other hydrocarbons such as aromatic hydrocarbons, naphthalene, polycyclic aromatic hydrocarbons, etc.[41] Aromatic hydrocarbons are finally degraded to generate ketone propionate and then enter the tricarboxylic acid cycle through acetic acid-CoA; part of naphthalene is degraded into tyrosine and directly enters the tricarboxylic acid cycle, a part of it generates phenylpropanoid, and polycyclic aromatic hydrocarbons also generate phenylpropanoid through degradation metabolism and then generate propionate ketone, which enters the tricarboxylic acid cycle through acetic acid-CoA.

The same nitrogen-containing substance enters the cell through the microbial double membrane, and through a series of redox reactions, part of it is converted into N_2, and the other part is used for the biosynthesis of amino acids, proteins, etc., and then enters the tricarboxylic acid cycle through acetate-CoA. The utilization of all substances by microorganisms will eventually enter the tricarboxylic acid cycle, in which part of oxalate acetate is transferred to sugar degradation, gluconeogenesis, and synthesis of glycolipid surfactants, and part of it is transferred to amino acid metabolism to synthesize lipopeptide surfactants.

Based on the above-mentioned metabolic pathway of PHDB, a complete and systematic closed-circuit circulation system for biodegrading crude oil is formed, especially biosurfactants, which help to reduce the interfacial tension of oil and water and accelerate the stripping of crude oil from rock surfaces.[42] Simultaneously, crude oil would be further split into smaller oil droplets under the action of microbial cells (Figure 6a), which was more conducive to the utilization of microorganisms. In conclusion, the activation of PHDB can effectively drive the synergistic combination of other reservoir microbial communities, leveraging the characteristics of each bacterial member to increase oil recovery. This growth metabolic feature and its cooperative win mode give petroleum hydrocarbon-degrading microbial communities an advantage over local competitors in the field of water-flooded reservoirs. This research provides insight into the mechanisms of MEOR after artificial interference or regulation of MEOR mechanisms that will contribute to future site operations.

4. CONCLUSIONS

Microbial enhanced oil recovery (MEOR) is considered an economical and environmentally friendly tertiary oil recovery technology and has become a hot topic in the development of green and low-carbon oil recovery technologies. Investigating the response characteristics of PHDB to the community structure and metabolic genes of oil-producing bacteria after targeted activation in low-permeability reservoirs is of great significance and can provide a further theoretical basis and framework for MEOR. Results showed that petroleum hydrocarbon biodegradation occurred gradually through the action of various metabolic enzymes in a specific metabolic sequence, and there was a positive correlation between the number of coding genes and the enzyme activity corresponding to the gene. In the future, a more comprehensive study should be carried out to verify that the targeted activation of oil-enhancing functional microorganisms that produce different metabolites will be more conducive to enhanced oil recovery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10334.

Distribution statistics of the single assembly gene catalogue length (Figure S1); distribution statistics of the mixed assembly gene catalogue length (Figure S2); statistical analysis of the number of CAZy unigenes genes (Figure S3); characteristics of the production water (Table S1) (PDF)

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Notes

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■ REFERENCES

(1) Saravanan, A.; Ponnusamy, S. K.; Kilaru, H. A.; et al. A review on systematic approach for microbial enhanced oil recovery technologies: Opportunities and challenges. *J. Cleaner Prod.* **2022**, *258*, No. 120777.
(2) Wu, B.; Xiu, J. L.; Yu, L.; Huang, L. X.; et al. Research advances of microbial enhanced oil recovery. *Heliyon* **2022**, *8* (11), e11424.
(3) Yuan, S. Y.; Wang, Q. New progress and prospect of oilfields development technologies in China. *Pet. Explor. Dev.* **2018**, *45* (45), 698−711.
(4) Guo, H.; Li, Y.; Kong, D.; et al. Lessons learned from alkali/surfactant/polymer-flooding field tests in China. *SPE Reservoir Eval. Eng.* **2019**, *22*, 78−99.
(5) Radnia, H.; Nazar, A. R. S.; Rashidi, A. Application of nanoparticles for chemical enhanced oil recovery. *Iran. J. Oil Gas Sci. Technol.* **2018**, *7*, 1−19.
(6) Patel, J.; Borgohain, B.; Kumar, M.; Sen, R.; et al. Recent developments in microbial enhanced oil recovery. *Renewable Sustainable Energy Rev.* **2015**, *52*, 1539−1558.
(7) Niu, J. J.; Liu, Q.; Lv, j.; Peng, B. Review on microbial enhanced oil recovery: Mechanisms, modeling and field trials. *J. Pet. Sci. Eng.* **2020**, *192*, No. 107350.
(8) Gao, C. Experiences of microbial enhanced oil recovery in Chinese oil fields. *J. Pet. Sci. Eng.* **2018**, *166*, 55−62.
(9) Ke, C.; Sun, W.; Li, Y.; Lu, G.; Zhang, Q.; Zhang, X. Microbial enhanced oil recovery in Baolige Oilfield using an indigenous facultative anaerobic strain Luteimonas *huabeiensis sp*. *J. Pet. Sci. Eng.* **2018**, *167*, 160−167.
(10) Gaol, C. L.; Ganzer, L.; Mukherjee, S.; Hakan, A. Parameters govern microbial enhanced oil recovery (MEOR) performance in real-structure micromodels. *J. Pet. Sci. Eng.* **2012**, *205*, No. 108814.
(11) Gudiña, E. J.; Pereira, J. F. B.; Costa, R.; Coutinho, J. A. P.; Teixeira, J. A.; Rodrigues, L. R. Biosurfactant-producing and oil-degrading Bacillus subtilis strains enhance oil recovery in laboratory sand-pack columns. *J. Hazard. Mater.* **2013**, *261*, 106−113.
(12) Patel, J.; Borgohain, S.; Kumar, M.; Rangarajan, V.; Somasundaran, P.; Sen, R. Recent developments in microbial enhanced oil recovery. *Renewable Sustainable Energy Rev.* **2015**, *52*, 1539−1558.
(13) She, H. C.; Kong, D. B.; Li, Y. Q.; Hu, Z. Q.; Guo, H. Recent advance of microbial enhanced oil recovery (MEOR) in China. *Geofluids* **2019**, *2019*, 1871392.
(14) Polson, E. J.; Buckman, J. O.; Bowen, D. G.; Todd, A. C.; Gow, M. M.; Cuthbert, S. J. An environmental-scanninge Electron-microscope investigation into the effect of biofilm on the wettability of quartz. *SPE J.* **2010**, *15*, 223−227.
(15) Wang, D.; Sun, S. S.; Cui, K.; Li, H. L.; Gong, Y. J.; Hou, J. R.; Zhang, Z. Y. Wettability alteration in low-permeability sandstone reservoirs by "SiO_2-Rhamnolipid" nanofluid. *Energy Fules* **2019**, *33*, 12170−12181.
(16) Cui, K.; Zhang, Z. Y.; Zhang, Z. Z.; et al. Stimulation of indigenous microbes by optimizing the water cut in low permeability reservoirs for green and enhanced oil recovery. *Sci. Rep.* **2019**, *9*, No. 15772.
(17) Cui, K.; Wang, C. J.; Li, L.; Guo, K.; et al. Controlling the hydro-swelling of smectite clay minerals by Fe(III) reducing bacteria for enhanced oil recovery from low-permeability reservoirs. *Energies* **2022**, *15*, 4393.
(18) Brown, L. R. Microbial enhanced oil recovery (MEOR). *Curr. Opin. Microbiol.* **2010**, *13*, 316−320.
(19) Li, H. L.; Lai, R. Q.; Jin, Y. L.; et al. Directional culture of petroleum hydrocarbon degrading bacteria for enhancing crude oil recovery. *J. Hazard. Mater.* **2020**, *390*, 122160−122171.
(20) Phulpoto, I. A.; Hu, B.; Wang, Y.; Ndayisenga, F.; Li, J.; Yu, Z. Effect of natural microbiome and culturable biosurfactants-producing bacterial consortia of freshwater lake on petroleum-hydrocarbon degradation. *Sci. Total Environ.* **2012**, *751*, No. 141720.
(21) Elumalai, P.; Parthipan, P.; AlSalhi, M. S.; Huang, M.; Devanesan, S.; Karthikeyan, O. P.; et al. Characterization of crude oil degrading bacterial communities and their impact on biofilm formation. *Environ. Pollut.* **2021**, *286*, No. 117556.
(22) Alkan, H.; Mukherjee, S.; Koegler, F.; et al. Reservoir engineering of in-situ meor; impact of microbial community. *J. Pet. Sci. Eng.* **2022**, *195*, No. 107928.
(23) Muangchinda, C.; Rungsihiranrut, A.; Prombutara, P.; Soonglerdsongpha, S.; Pinyakong, O. 16S metagenomic analysis reveals adaptability of a mixed-PAH-degrading consortium isolated from crude oil-contaminated seawater to changing environmental conditions. *J. Hazard. Mater.* **2018**, *357*, 119−127.
(24) Delmont, T. O.; Robe, P.; Cecillon, S.; Clark, I. M.; Constancias, F.; Simonet, P.; et al. Accessing the soil metagenome for studies of microbial diversity. *Appl. Environ. Microbiol.* **2011**, *77*, 1315−1324.
(25) Mirete, S.; Morgante, V.; González-Pastor, J. Functional metagenomics of extreme environments. *Curr. Opin. Biotechnol.* **2016**, *38*, 143−149.
(26) Sierra-Garcia, I. N.; Belgini, D. R. B.; Torres-Ballesteros, A.; et al. In depth metagenomic analysis in contrasting oil wells reveals syntrophic bacterial and archaeal associations for oil biodegradation in petroleum reservoirs. *Sci. Total Environ.* **2020**, *715*, 10708−10717.
(27) Napp, A. P.; Pereira, J. E. S.; Oliveira, J. S.; Silva-Portela, R. C. B.; Agnez-Lima, L. F.; Peralba, M. C. R.; et al. Comparative metagenomics reveals different hydrocarbon degradative abilities from enriched oil-drilling waste. *Chemosphere* **2018**, *209*, 7−16.
(28) Kanehisa, M.; Sato, Y.; Furumichi, M.; Morishima, K.; Tanabe, M. New approach for understanding genome variations in KEGG. *Nucleic Acids Res.* **2019**, *47*, D590−D595.
(29) Song, B.; Li, Z.; Li, S.; Zhang, Z.; Fu, Q.; Wang, S.; et al. Functional metagenomic and enrichment metatranscriptomic analysis of marine microbial activities within a marine oil spill area. *Environ. Pollut.* **2021**, *274*, No. 116555.
(30) Fernandes, C.; Kankonkar, H.; Meena, R. M.; Menezes, G.; Shenoy, B. D.; Khandeparker, R. Metagenomic analysis of tarball-associated bacteria from Goa, India. *Mar. Pollut. Bull.* **2019**, *141*, 398−403.
(31) Xiang, Y. P.; Xiong, W. P.; Xu, R.; Yang, Z. H.; et al. Metagenomic analysis reveals microbial metabolic potentials alterations under antibiotic stress during sludge anaerobic digestion. *J. Environ. Chem. Eng.* **2023**, *11*, No. 110746.
(32) Tully, B. J.; Nelson, W. C.; Heidelberg, J. F. Metagenomic analysis of a complex marine planktonic thaumarchaeal community from the gulf of maine. *Environ. Microbiol.* **2012**, *14*, 254−267.

(33) Vasileva-Tonkova, E.; Gesheva, V. Biosurfactant production by antarctic facultative anaerobe *Pantoea* sp. during growth on hydrocarbons. *Curr. Microbiol.* **2007**, *54*, 136−141.
(34) Xia, W. J.; Shen, W. J.; Yu, L.; Zheng, C. G.; Yu, W. C.; Tang, Y. C. Conversion of petroleum to methane by the indigenous methanogenic consortia for oil recovery in heavy oil reservoir. *Appl. Energy* **2016**, *171*, 646−655.
(35) Rahman, K. S. M.; Thahira-Rahman, J.; Lakshmanaperumalsamyb, P.; Banata, I. M.; et al. Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresour. Technol.* **2002**, *85*, 257−261.
(36) Gargouri, B.; Contreras, M. M.; Ammar, S.; Segura-Carretero, A.; Bouaziz, M. Biosurfactant production by the crude oil degrading *Stenotrophomonas* sp. B-2: chemical characterization, biological activities and environmental applications. *Environ. Sci. Pollut. Res.* **2017**, *24*, 3769−3779.
(37) Yun, Y.; Gui, Z. Y.; Su, T. Q.; et al. Deep mining decreases the microbial taxonomic and functional diversity of subsurface oil reservoirs. *Sci. Total Environ.* **2022**, *821*, No. 153564.
(38) Sharma, S.; Pandey, L. M.; et al. Production of biosurfactant by *Bacillus subtilis* RSL-2 isolated from sludge and biosurfactant mediated degradation of oil. *Bioresour. Technol.* **2020**, *307*, No. 123261.
(39) Das, N.; Bhrigu, D.; Piyush, P. Correlation of soil microbiome with crude oil contamination drives detection of hydrocarbon degrading genes which are independent to quantity and type of contaminants. *Environ. Res.* **2022**, *215*, No. 114185.
(40) Saravanan, A.; Kuma, P. S.; et al. A review on systematic approach for microbial enhanced oil recovery technologies: Opportunities and challenges. *J. Cleaner Prod.* **2020**, *258*, No. 120777.
(41) Zhou, H. H.; Liu, Q.; Jiang, L. J.; Shen, Q.; et al. Enhanced remediation of oil-contaminated intertidal sediment by bacterial consortium of petroleum degraders and biosurfactant producers. *Chemosphere* **2023**, *330*, No. 138763.
(42) Das, S.; Das, N.; Choure, K.; Pandey, P. Biodegradation of asphaltene by lipopeptide-biosurfactant producing hydrocarbonoclastic, crude oil degrading *Bacillus spp*. *Bioresour. Technol.* **2023**, *382*, No. 129198.

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