Application of microbial remediation in the treatment of offshore oil pollution

Xinru Zhu *
School of Ocean Sciences, China University of Geosciences (Beijing), Beijing, China

*Corresponding author e-mail: 1011181203@cugb.edu.cn

Abstract. With the development of offshore oil industry, the pollution caused by frequent oil spill accidents has brought great pressure to marine ecology. How to repair the oil polluted sea area economically, efficiently and safely has become an urgent problem to be solved in marine ecological environment protection. In this paper, the main factors affecting the bioremediation of marine oil pollution are the polluted sea environment, the physical and chemical properties of oil and the types of microorganisms. The research progress of marine ecological restoration at home and abroad and the main research direction of marine oil pollution ecological restoration technology are summarized. The possible problems in the repair application are analyzed, and the further research direction is proposed.

Key words: Offshore; Oil pollution; Environment; Microbial remediation; Degradation.

1. Introduction
Marine ecosystem is one of the most valuable natural resources, and its ecological value is very important. In the process of oil exploitation, loading and unloading, storage, transportation and use, every link has the risk of oil spill accident. With the rapid development of offshore oil industry and transportation industry, accidents occur frequently, causing varying degrees of damage to marine water quality and ecology, and increasing pressure on marine ecology. High concentration of petroleum pollutants will have a direct toxic effect on animals and plants, while low concentration of petroleum pollutants will aggravate the biological hazards and even threaten human health through bioaccumulation and food chain transmission. When the oil spill spreads to the coastal zone, it will also affect the beach and wetland ecosystem. How to repair the oil polluted sea area economically, efficiently and safely has become an urgent problem for marine ecological environment protection [1].

According to the types of oil input, marine oil pollution can be divided into sudden input and long-term input. Sudden accidents include oil tanker accidents, offshore oil leakage and blowout accidents, and long-term input includes oily sewage, oil leakage from port ships and natural seabed sedimentary rocks, exudates, discharge of civil and industrial wastewater, discharge of oily waste gas, etc. Offshore oil field accidents or oil transportation accidents will seriously pollute the surrounding environment and cause serious damage to the ecosystem. With more and more marine oil spill accidents and ship leakage accidents, the task of marine ecosystem restoration is becoming more and more difficult [2].

After entering the marine environment, oil mainly exists in the form of opaque oil film floating on the sea surface, dissolved emulsion dispersion and condensed matter. The harm of offshore oil pollution
mainly endangers ecology, human health and social industry. So bioremediation of marine oil pollution is very important.

2. Remediation technology of offshore oil pollution
At present, there are many repair methods for marine oil spill accidents, including physical method, chemical method and microbial method. However, the main methods used for oil spill control are physical skimming and absorption. In chemical method, chemical agents (such as oil dispersant, oil coagulant, dispersant, etc.) are put in to remove oil stains. By changing the physical properties of the spilled oil, the thin oil film which is difficult to be recovered by the recovery device for less than 1 minute can be gathered into a thick oil layer or condensed into a viscous semi-solid oil block. Then the spilled oil can be recovered by the recovery device, or directly emulsified and dispersed in sea water. Chemical treatment of oil pollution is fast, but there are some problems such as incomplete oil treatment, secondary pollution and high cost. Microbial method is to transform petroleum pollutants into essential life substances, such as protein, nucleic acid, amino acid, polysaccharide, etc., or decompose them into small molecule organic substances, such as alcohol, phenol, aldehyde, fatty acid, etc., or even directly mineralize them into inorganic substances, such as CO$_2$ and H$_2$O. The degradation of petroleum is characterized by good treatment effect, low cost and friendly environment [3-4].

3. Main factors affecting bioremediation of offshore oil pollution
The environment of the polluted sea area, such as temperature, pH value, nutrients, salinity, oxygen content, will have an impact on the remediation of oil pollution. On the one hand, temperature will affect the physical and chemical properties of petroleum hydrocarbons, on the other hand, it will affect the metabolism of microorganisms. Only in the appropriate temperature range, the microbial enzyme activity can play an effective role. Neutral or partial alkaline marine environment is more suitable for the survival and metabolism of most petroleum degrading microorganisms. Too high or too low pH value will affect their petroleum degradation performance. The metabolic activities of microorganisms are also affected by the high salinity of seawater, such as the absorption of nitrogen by microorganisms. The growth and metabolism of microorganisms need to maintain a certain amount and proportion of various nutrients. Oil leakage will lead to the increase of carbon source in the polluted sea area. Nitrogen, phosphorus and other nutrients become the limiting factors of oil microbial degradation. In the bioremediation process of oil pollution, adding a certain proportion of nitrogen and phosphorus can improve the oil degradation efficiency. The degradation of oil slick on the sea surface is generally carried out by aerobic bacteria. According to the calculation, the amount of oxygen needed to decompose 1g oil is about 3 ~ 4g [5].

The degradation efficiency of petroleum is closely related to its physical and chemical properties, such as the physical state, concentration and composition of petroleum. Due to the variety of petroleum degrading bacteria and the complex chemical structure of petroleum pollutants, the way of microbial degradation of petroleum is very complex. The structure of alkanes is relatively simple, and the main biodegradation pathways are terminal oxidation, alkyl hydroperoxidation and cyclohexane degradation. The degradation of aromatic hydrocarbons by microorganisms is realized by the opening of benzene ring caused by the oxidation of aromatic hydrocarbons by oxidase. Microbial degradation of polycyclic aromatic hydrocarbons (PAHs) is catalyzed by monooxygenase or dioxygenase, which is finally degraded into carbon dioxide and water. In saturated hydrocarbons, straight chain hydrocarbons are the most easily degraded; in aromatic hydrocarbons, bicyclic and tricyclic aromatic hydrocarbons are more easily degraded, and five or more polycyclic aromatic hydrocarbons are difficult to be degraded by microorganisms. On the whole, the order of biodegradation of petroleum hydrocarbons by microorganisms from easy to difficult is straight chain alkanes, branched chain alkanes, cycloalkanes, monocyclic aromatic hydrocarbons, polycyclic aromatic hydrocarbons, heterocyclic aromatic hydrocarbons and asphalt.

Different kinds of oil degrading microorganisms have different remediation effects on oil pollution. Petroleum degrading bacteria can be divided into "specific hydrocarbon type" bacteria and "non-specific
hydrocarbon type" bacteria according to their available carbon sources. "Specific hydrocarbon type" bacteria refer to the bacteria with petroleum hydrocarbon as the sole carbon source. For example, alkaliphilic bacteria can only use saturated alkanes and branched alkanes as carbon sources; ring decomposing bacteria can only use aromatic hydrocarbons (naphthalene, phenanthrene and anthracene) as carbon sources; while oleophilus and Spirillum can only use alkanoates, aliphatic hydrocarbons and alkanols as carbon sources. The available carbon sources of "non-specific hydrocarbon type" bacteria are not limited to petroleum hydrocarbons, such as Vibrio, Pseudoalteromonas, marine Monas and Halomonas, Micrococcus and Staphylococcus, Sphingomonas and Geobacillus.

4. Progress of bioremediation in the treatment of offshore oil pollution
Compared with the physical or chemical degradation of oil polluted sea area, biodegradation is an environmentally friendly way, which is more accepted by the public. In the presence of oxygen molecules, various components of petroleum react with each other under the action of catalytic enzymes, and gradually degrade, and finally transform into carbon dioxide and water. It has many advantages, such as low cost, fast efficiency, no secondary pollution and so on.

Under normal circumstances, petroleum is adsorbed on the surface, transferred to the microbial membrane, and decomposed into three parts: (1) alkanes are converted into fatty acids by some microbial enzymes, and then gradually metabolized into acetyl coenzyme A, which enters the tricarboxylic acid cycle metabolism, and finally converted into carbon dioxide and water; (2) aromatic hydrocarbons are oxidized by oxidase In the process of catechol degradation, ortho and meta ring opening reactions are carried out respectively; (3) polycyclic aromatic hydrocarbons are degraded to ethylene glycol and catechol groups under the catalysis of enzyme, and then further decomposed to acetyl coenzyme A or succinic acid [6].

4.1. Immobilized microorganism technology
Immobilized microorganism technology uses physical or chemical methods to fix free microorganisms in a limited space to improve the microbial density and maintain its biological activity. With the advantages of high reaction efficiency, easy control of reaction and less loss of microorganisms, it has become one of the main research directions of marine remediation. Lu Lei et al. Embedded oil degrading bacteria in PVA and SA, and added activated carbon to optimize the performance of immobilized microspheres in the degradation process, which improved the elasticity and permeability of microspheres, reduced the crushing rate, and significantly improved the microbial degradation efficiency of crude oil. When the initial oil content was 2342 mg / L, the highest degradation rate was 78%, which was about twice as high as that of free bacteria. Jiang Tianxiang et al. Used si-jhs immobilized beads to degrade oily seawater, and the oil degradation rate was as high as 97.8%. Using PVA and SA as carriers, Gao Xiangxing and others immobilized petroleum degrading bacteria group DC10 by embedding method, which improved the degradation efficiency of petroleum degrading bacteria group and enhanced the ability of continuous degradation of petroleum. In order to solve the problems that microorganisms are easy to be diluted and have less effective contact with petroleum hydrocarbons in the practical application of microbial degradation, Wang Xin and others chose light carriers with biological affinity and hydrophobicity to embed and fix different petroleum degrading bacteria, and achieved good degradation effect [7].

4.2. Screening of high efficient petroleum degrading bacteria
Efficient and safe oil degrading bacteria are the key to oil degradation, and the screening of oil degrading bacteria has always been a research hotspot. Li Guoli et al. Screened low temperature oil degrading bacteria producing biosurfactants from oil contaminated soil samples of Qinghai Tibet Plateau. The results show that the strain can utilize many kinds of hydrocarbons in petroleum, but the degradation degree of different hydrocarbons is different. It mainly degrades medium and long-chain alkanes and branched alkanes, but the degradation ability of short-chain alkanes, long-chain alkanes and aromatic hydrocarbons is relatively weak. Tian Yan et al. Studied the enrichment and screening of oil
degrading bacteria in different media, and the results showed that the oil degrading ability of the bacteria enriched in different media was different. Zhang Hui and others screened Biosurfactant Producing Bacteria from long-term oil sludge polluted by petroleum, and used them to degrade petroleum hydrocarbon pollutants in water, with a degradation rate of 99.5%. Li Le et al. Screened and isolated five high efficient oil degrading bacteria from oily sludge, investigated the degradation efficiency of a single strain, combined with the advantages of each strain to construct a composite flora, and its degradation efficiency was significantly higher than that of a single flora [8]. Guo Ping Ping isolated an efficient oil degrading bacterium from the surface seawater of Dalian port to explore the effects of temperature, nutrients and inoculation amount on oil degradation.

4.3. Effect of nutrients on microbial degradation of petroleum

After the "Exxon Valdez" oil spill accident in the United States, in order to speed up the thorough removal of oil, biological nutrients with nitrogen and phosphorus as the main nutrients were added, including IBDU synthesized by isobutyraldehyde and urea, custombien synthesized by urea and formaldehyde, and inipol EAP, an emulsifier with saturated urea composed of water-soluble nitrogen and lipophilic phosphorus ZZ, the results show that the addition of nutrients is helpful to accelerate the complete removal of oil [9-10]. Agota et al. Promoted the degradation of petroleum hydrocarbons by microorganisms in the beach environment of the northern Gulf of Mexico by adding inorganic nutrients. Cui Zhisong et al. Compared the promotion effect of slow-release fertilizer and soluble nutrients on biodegradation, the results showed that the slow-release and attached sediment characteristics of slow-release fertilizer could reduce the loss caused by tidal erosion in practical application, and maintain a relatively stable level of nutrients in the remediation area, which had a better promotion effect on Biodegradation in practical application.

5. Summary

(1) At present, the screening and immobilization of oil degrading bacteria have been widely and deeply studied. However, in the open sea area polluted by oil, the environmental conditions are changeable and difficult to control. Tide, temperature and water temperature, rainfall, light and so on will affect the effect of microbial remediation. The application of oil degrading bacteria in the remediation of marine oil pollution is still faced with the problems of functional stability, genetic stability and environmental risk. Therefore, how to improve the stability and adaptability of petroleum degrading bacteria in natural conditions, and improve the remediation efficiency in practical application need further research.

(2) Although microbial degradation of petroleum is a long-term and complex process, it still attracts more and more attention. It has become one of the best ways to deal with marine oil pollution to seek efficient oil degrading bacteria. First of all, efficient degradation bacteria can be screened and domesticated in nature. Secondly, genetically modified strains can be used to provide stable and efficient strains for pollution control. Finally, in order to better degrade all kinds of components in oil, we can use the way of mixed strains or adding some other surfactants (such as rhamnolipid) which do less harm to the ecological environment, which can more comprehensively solve the pollution problem. Because of its complexity, the research of bioremediation still needs to be further explored, and the research on its degradation mechanism has become a research hotspot of offshore oil remediation.

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