A Review of the Mechanism of Microbial Degradation of Petroleum Pollution

Xiaokang Li, Hong Li and Chengtun Qu*

Shaanxi Oil and Gas Pollution Control and Reservoir Protection Key Laboratory, Xi’an Shiyou University, 710065, Xi’an
E-mail: xianquct@163.com

Abstract. A large amount of petroleum hydrocarbon pollutants will be produced during the oilfield exploitation process. If traditional treatment methods of physical or chemical methods are used, problems such as incomplete treatment and secondary pollution will occur. In order to achieve better treatment effects, the microbial degradation method is currently used to treat petroleum hydrocarbon pollutants. Studying the mechanism of microbial degradation of petroleum hydrocarbon pollutants is conducive to exploring the path of petroleum pollution remediation technology and improving the efficiency of pollution control. In this review, the degradation mechanism of alkanes, alkenes and aromatics is discussed in detail, and the research directions of microbial pollution control are analyzed and discussed.

1. Introduction
Petroleum exploitation, transportation and refining process may pollute the environment, and the leakage of crude oil during mining, transportation and refining is the main cause of environmental pollution. The leakage of crude oil is scattered in soil and water environment. If the scattered crude oil was not treated in time, it will seriously affect the normal life of people in the surrounding area, and also will have a toxic effect on the soil and water environment [1]. The serious harm caused by oil leakage has been widely reported at home and abroad [2-3]. For the treatment of petroleum hydrocarbon pollutants, physical methods, chemical methods and the combination of the two methods have been used widely. However, these methods often cannot completely remove the emulsified oil and dissolved oil in water and soil, and the treatment cost is higher. At the same time, it will cause repeated pollution to the surrounding environment, so physical or chemical methods can only be used in emergency handling [4]. Biological methods were used to degrade petroleum hydrocarbon pollutants by some oil-loving microorganisms in the environment. This method does not cause repeated pollution and has low processing cost. There are many kinds of microorganism that can degrade petroleum hydrocarbon pollutants in nature. By using suitable petroleum degrading microorganisms, some toxic and harmful petroleum hydrocarbon pollutants can be degraded into harmless substances, and even some hydrocarbon substances can be thoroughly degraded [5]. However, the existing micro-biotreatment technology is not efficient in the treatment and remediation of crude oil leakage. In order to improve the degradation efficiency of petroleum degrading bacteria to petroleum hydrocarbon pollutants, we must have an in-depth understanding of the mechanism of petroleum degradation bacteria to degrade petroleum hydrocarbon pollutants, and lay a good foundation for subsequent research and application.

2. Mechanism of Microbial Degradation of Petroleum Pollutants
The degradation of petroleum hydrocarbons by microorganisms is mainly caused by the catalysis of
intracellular enzymes. The process of microbial degradation of petroleum hydrocarbons has four main steps: First, petroleum pollutants are emulsified by surfactants secreted by microorganisms; Second, the emulsified petroleum hydrocarbon is adsorbed by the surface of the microorganism; Then, the petroleum hydrocarbon adsorbed on the surface of the cell membrane enters the cell membrane through active transport or passive transport, endocytosis. Finally, the petroleum hydrocarbon entering the cell undergoes an enzymatic reaction with the corresponding enzyme to achieve the purpose of degrading the pollutant.

The degradation ability of petroleum hydrocarbons in microorganisms is a critical factor in pollution remediation. The degradation pathway of alkanes is divided into single-end oxidation, double-end oxidation and secondary-end oxidation. These three oxidation modes are implemented in different positions on the carbon chain to form alcohol, aldehyde, acid, acid and CoA. And then the β oxidation is completed to provide energy for microbial life activities [6-7].

The degradation mode of cycloalkane is slightly different with paraffin. First, the cycloalkane is oxidized by the hydroxylase to form a cycloalkanol according to fig 1. The cycloalkanol takes off the hydrogen to produce naphthenic ketone. Under the action of the ketoxygenase, the naphthenic ketone is converted into caprolactone. And then a H₂O molecule was inserted into the ring of caprolactone to break the ring. Whose progress produces the 6-hydroxycaproic acid that is further oxidized to 6-oxohexanoic acid by 6-hydroxycaproate dehydrogenase. The dicarboxylic acid is formed through the oxidation by aldehyde dehydrogenase readily. Finally, the dicarboxylic acid is further oxidized through the tricarboxylic acid cycle to maintain the life activity of the microorganism [8-9]. However, it is also said that the degradation of naphthenes also has the degradation caused by co-metabolism and symbiosis, but this is generally only for the degradation of terpenes, which is more difficult to degrade than alicyclic hydrocarbons.

Figure 1. Process of degradation of naphthenes by petroleum-degrading bacteria

Figure 2. Degradation of olefins by petroleum-degrading bacteria

There is more than one degradation method for olefin degradation. The double bond is reduced to a secondary alcohol, and the secondary alcohol is oxidized to a ketone. The ketone is then esterified by the bacteria to form an ester. The ester bond is then cleaved to form two parts, namely primary alcohol
and fatty acid [10]. According to the Figure 2, microorganisms directly oxidize epoxides formed by the addition of olefins to aldehydes or ketones, and then gradually oxidize them to be used by microorganisms without generating intermediate alcohols [11]. There are also some linear paraffins directly dehydrogenated in the microorganism to form olefins, and then further oxidized into alcohols, aldehydes, and acids by the catalytic action of the enzyme to generate energy by β oxidative decomposition.

The degradation of aromatic hydrocarbons is more complicated than other hydrocarbons. The same aromatic hydrocarbons will have different degradation sites due to their different sites of degradation. For example, Arthrobacter and Mycobacterium have more than one degradation pathway for phenanthrene. They are opened at different positions from the phenanthrene ring, and may form products such as 2-hydroxy-1-naphthoic acid or 2, 2-biphenyldioic acid. [12]. The degradation of different polycyclic aromatic hydrocarbons is basically the same. Polycyclic aromatic hydrocarbons are degraded. First, a ring of polycyclic aromatic hydrocarbons is oxidized to form a trans-dihydro-dihydroxy compound or catalyzed by a microenzyme in a microorganism. a benzene ring initial oxide of a cis-dihydro-dihydroxy compound, which is dehydrogenated to form a diol epoxide. The epoxidative cleavage of the diol forms an alcohol with less than one ring and produces salicylic acid, catechol and The product such as gentisic acid then continues to oxidize, and the falling chain hydrocarbon undergoes an alkane oxidation pathway to form a carboxylic acid, which reacts with CoA to form acetyl-CoA, which provides energy for self-metabolism through the TAC cycle [13-14]. For polycyclic aromatic hydrocarbons with more than four rings, as the number of benzene rings increases, it is difficult to be degraded by microorganisms, and can only be oxidatively degraded by co-metabolism. Complex polycyclic aromatic hydrocarbons are also opened and produced in various ways. Intermediate products such as trans-diols, phenols, naphthalenes, and epoxides are all toxic to microorganisms, so existing biotechnology can only be applied to the degradation of low-concentration polycyclic aromatic hydrocarbons.

![Figure 3. Process for degradation of monocyclic aromatic hydrocarbons by petroleum-degrading bacteria](image)

3. Summary
In recent years, the state has strongly advocated the use of clean energy, but the use of diesel, gasoline and petroleum-based products has not decreased, so the amount of oil pollutants is also high, which has a negative impact on people's production, life and environmental protection. As the country pays more and more attention to pollution control, the use of high-efficiency, no secondary pollution, economic microbial combined treatment technology has become the main means of petroleum degradation, and it is constantly developing, but due to the limitations of microbial characteristics, biotechnology There are also many drawbacks. For example, the characteristics of refractory degradation of high-concentration petroleum pollutants cannot be repaired by microorganisms, and the microorganisms themselves are susceptible to environmental influences, and the degradation effect will be greatly reduced with slight changes.

(1) Construct genetically engineered bacteria with high efficiency and environmental adaptability.
Bioengineering methods, such as genetic recombination, protein modification, and molecular integration techniques, are used to completely renovate petroleum-degrading bacteria to construct ideal strains for the specialization of microbial pollution control.

(2) Bio-enhancement and biostimulation of complex petroleum-degrading bacteria. Further degradation of petroleum contaminants is achieved by adding appropriate nutrients and changing F/M values or by adding exotic microorganisms to stimulate the oil-degrading flora.

(3) Optimization and combination of processes. Design a more complete and sophisticated treatment process and combine with the original treatment process to reduce the degradation cycle of petroleum degrading bacteria and increase the degradation efficiency. Strengthening the optimization and innovation of the petroleum refining process, such as bio-electrochemical treatment combined with biological and electrochemical methods to treat petroleum pollution, can not only improve the degradation efficiency, but also convert petroleum pollutants into electricity and hydrogen for people to use.

(4) Improve the environmental risk level and evaluation system of bioremediation technology, reduce the unnecessary risks arising in the process of governance, and formulate the treatment process in the appropriate environment.

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