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Study on Extreme Microbial Degradation of Petroleum Hydrocarbons

Hong Li, Xiaokang Li, Tao Yu, Feifei Wang 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. Some oil-contaminated areas are often accompanied by extreme environments including high salinity, low temperature, and high temperature. The petroleum-degrading microorganisms isolated from these extreme environments can quickly adapt to extreme environments, grow and metabolize in extreme environments, and show great potential in the field of oil pollution control. This article reviews species, adaptation mechanism and research progress of oil-degrading bacteria in three extreme environments of hypersaline, low temperature and high temperature, so as to provide theoretical basis for further research and application.

1. Introduction

Oil as one of the most important energy in the industry, the product has been extensively used in life. The huge demand for oil has led to a growing range of energy exploitation and oil spills occur frequently simultaneously. Large quantities of petroleum pollutants enter the environment causing serious degradation of land and water ecosystems. Especially in recent years, the Polar Regions, the salt lakes and the marine areas are often contaminated with high levels of petroleum hydrocarbons. These systems have considerable economic, ecological and scientific value.

Bioremediation technology utilizes microorganisms to degrade toxic pollutants to harmless products such as CO_2, H2O and other inorganic compounds [1]. Microbial degradation of petroleum hydrocarbon pollutants has long been considered as an environmentally friendly, cost-effective and efficient biological treatment. There are different kinds of microorganisms to degrade petroleum hydrocarbon, and their physiological characteristics such as salt resistance, temperature resistance and saline-alkali resistance are also different. The conventional microorganisms have significantly less metabolic capacity and less degradation, and are unable to function in extreme environments. Extremophiles are adapted to grow and thrive under these adverse conditions. Hydrocarbon degrading extremophiles are thus ideal candidates for the biological treatment of polluted extreme habitats and scientific value. It plays an important role in the control of oil pollution in extreme environments.

2. Microbial Degradation of Petroleum Hydrocarbons in Hypersaline Environments

High salinity concentration has a great impact on the physiological characteristics and life activities of microorganisms, including disruption cell membrane, denaturation of enzyme, and reduction of solubility of oxygen and hydrocarbons. Therefore, bioremediation of saline environments without costly dilution of salt-laden soil and water requires halophilic or halotolerant organisms that tolerate high salt concentrations.

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2.1. Species of Halophilic Microorganism
Microorganisms that need salt to grow are called halophiles. Halophiles are classified into three groups according to their optimal salt concentration for growth: slightly halophilic, moderately halophilic, and extremely halophilic. The slightly halophiles are mostly marine microorganisms, and the optimum salt concentration is 1% to 3%(w/v); The moderately halophiles are mainly fungi, and the optimum salt concentration is 3% to 15%(w/v); The extremely halophiles are basically archaea and the optimum salt concentration is 15% to 32%(w/v) [2].

2.2. Adaptations of Halophilic Microorganism to Their Environments [3, 4]

2.2.1. Accumulation of inorganic salt ions. Halophile accumulates $K^+$and Cl^- in its cells to maintain its osmotic balance with the environment. Extreme halophiles of the archaeal Halobacteriaceae family such as H. salinarum, Haloarcula marismortui, Halococcus morrhuae maintain their osmotic balance by concentrating K^+inside cells.This is achieved by the concerted action of the membrane-bound proton-pump bacteriorhodopsin, ATP synthase, and the Na^+/H^+ antiporter and results in an electrical potential that drives the uptake of K^+ into cells via a K^+-uniport mechanism.

2.2.2. Accumulation of compatible organic solutes. The water potential inside the cell is higher than outside in hypersaline environment. In order to avoid cell dehydration due to water outflow from the cell, low compatible molecular organics are synthesized by the halophile, e.g., glutamine, glycerol, betaines, proline, and glutamate. In addition, the sugars sucrose and trehalose are osmolytes vital to stabilizing microbial membranes.They protect microbial proteins from denaturation in saline environments and maintain cell structure stability.

2.2.3. Changes in protein structure. The halophile protein tissue has a unique adaptability to the high salt environment and promotes folding of the riparophilic peptide chain to form a functional protein, ensuring proper functioning of the enzyme activity.

2.3. Microbial Degradation of Petroleum Hydrocarbons in Hypersaline Environments
In the last two decades there has been impressive progress in the area of hydrocarbon degradation in hypersaline environments. Li et al.[5] have isolated a *Planococcus* capable of degrading BTEX in the presence of 0.5%~25% NaCl from oil-contaminated soil. Wang et al.[6] confirmed that the halophile group had no significant effect on the removal of phenanthrene in the presence of 0.1% NaCl, while the phenanthrene could be completely degraded within 5 days in the presence of salt concentrations 5%. Khemili-Talbi et al.[7] have isolated an extremely halophilic archaea from highly mineralized wastewater. It was found that the halophilic archaea had the potential of degrading phenol, pyrene and naphthalene at a concentration of 25% of NaCl and produced biosurfactant.

3. Microbial Degradation of Petroleum Hydrocarbons in Low Temperature Environments
In the past, most studies on oil-degrading bacteria have focused on normal temperature environment. In recent years, it has been reported that petroleum hydrocarbon components can also be degraded by indigenous microorganisms in various land and marine ecosystems, including arctic, alpine, Antarctic soil and sediment. Such microorganisms, which are normally living in low temperature environments, are known as cold-adapted microorganisms. They are able to rapidly adapt to the pollution environment and therefore, cold-adapted microorganisms are becoming increasingly concerned.

3.1. Species of Cold-adapted Microorganism
Cold-adapted microorganism is classified into two groups according to the growth temperature: psychrophilies and psychrotrophs. Both of these microorganisms are able to grow at temperatures around 0°C. Psychrophiles have an optimum growth temperature below 15°C and do not grow above 20°C, whereas psychrotrophs (cold-tolerant) have optimum and maximum growth temperatures above 15°Cand 20°C. At present, there are known cold-adapted microorganisms including bacteria, cyanobacteria, fungi and psychrophilic archaea[8].

3.2. Adaptations of Cold-adapted Microorganisms to Their Environments

At extreme conditions, low-temperature microorganisms develop a series of unique vital signs adapted to the environment. The unsaturated fatty acids and short-chain fatty acids increased and the cell membrane fluidity was enhanced under low temperature. The cold-adapted microorganism is capable of producing degradation enzymes with specific environmental adaptation mechanisms at low temperatures. These enzyme molecules contain enzyme genes capable of being expressed at low temperatures, so that the cryogenic enzyme molecule is more flexible, reduces the activation energy of the petroleum hydrocarbon degradation reaction, and improves the catalytic activity of the enzyme.

Some cold-adapted microorganisms have antifreeze proteins and cold protective substances, among which antifreeze proteins creat a thermal lag by binding with ice crystals on the surface of the proteins, reducing the freezing point of body fluids or cellular water, so that the cold-adapted microorganisms can survive at low temperatures; Cold protective substances can prevent protein denaturation and polymerization, improve the physicochemical reaction environment in the cells of microorganisms, make nutrients stay and concentrate in the cells, and prevent extracellular enzymes from cold denaturation and inactivation.

The cold adaptation capacity of psychrophilic microorganisms is also related to their genetic material. On the one hand, substances such as ribosome extract and RNA polymerase in the psychrophilic microorganisms have good activity at low temperature due to their unique physical and chemical properties, which can maintain protein folding rate.Various enzymes can be normally expressed at low temperature. On the other hand, the cold-adapted microorganism will produce a large amount of cryoprotein when it is impacted by low temperature. In cell responses such as transcription, translation and protein folding of genetic material, low temperature makes cryoprotein express rapidly. These unique physical and chemical properties enable the microorganism to decompose normally at low temperature [9, 10]

3.3. Microbial Degradation of Petroleum Hydrocarbons in Low Temperature Environments

The results have shown that the oil degradation rate can be improved by optimizing the environmental conditions, such as temperature and PH. In addition, biostimulation and bioaugmentation of contaminated cold sites has been tested as a bioremediation strategy. Biostimulation means to stimulate the activity of enzymes in microorganisms by adding different levels of nutrient elements and electronic receptors, such as O_2 and H_2O_2, and the degradation rate of microorganisms to petroleum hydrocarbon pollutants is improved. Romina et al.[11] have studied chronically contaminated Antarctic soil at Carlini Station. Total hydrocarbon removal (71 %) was detected after 30 days when adding fish meal. Paudyn et al.[12] increased oxygen content through soil tillage in the Arctic diesel contaminated soil , and it was found the removal rate of petroleum hydrocarbon pollutants reached above 80% after three years. Bioaugmentation refers to the enhancement of microbial degradation of petroleum contaminants by inoculating low temperature petroleum hydrocarbon degrading bacteria and changing the original quantity and composition of the bacteria. Mohn et al.[13] confirmed over 80 percent of petroleum hydrocarbons were degraded by the addition of cold-adapted microorganisms in soil of polar cold regions remediation tests.

4. Microbial Degradation of Petroleum Hydrocarbons in High Temperature Environments

In 1965, Thomas D. Brock has isolated an extremely thermophilic bacterium in hot spring water at 80°Cfor the first time. Since then, it has been found that some microorganisms can often survive in hot environments, such as deserts and volcanoes. Such microorganisms are called thermophilic microorganisms.

4.1. Species of Thermophilic Microorganism

The optimal growth temperature of thermophilic microorganisms can reach above 40°C, and the maximum growth temperature of most thermophiles ranges between 50°Cand 70°C. Optimal growth of extreme thermophiles and hyperthermophiles is 70–80°C and above 80°C, respectively. At present, the known thermophiles are basically eubacteria and archaea [14].

4.2. Adaptations of Thermophilic Microorganism to Their Environments

The adaptation mechanism of thermophilic bacteria in high-temperature environment is related to various factors, including special cell membrane structure, thermophilic protein, thermophilic enzyme and its genetic material. Firstly, there's a lot of structurally specific complex fats in the membrane, which can make the cell membrane more stable at high temperature. Secondly, thermophilic microorganisms can produce some thermophilic proteins with stable structure in high temperature environment. Thirdly, the chemical modification of certain metal ions in the thermophiles may also enhance the thermal stability of the protein. In addition, thermophilic bacteria have unique thermophilic enzyme, which contains special amino acid, hydrogen bond, ion pair or disulfide bond, which improves thermophilic enzyme thermal stability. Finally, most thermophilic microorganisms have significantly higher G and C bases contents in DNA double helix structure or tRNA than conventional microorganisms. More hydrogen bonds are produced to maintain the stability of the structure. It is the combination of these factors that makes the thermophilic microorganisms have high thermal stability [15, 16].

4.3. Microbial Degradation of Petroleum Hydrocarbons in High Temperature Environments

With the increase in demand for petroleum energy, the problem of oil pollution comes as the scope of oil development expands to high-temperature areas. The most known oil-degrading bacteria are medium temperature microorganisms, which have poor degradation effect on petroleum hydrocarbon under the high temperature environment. Therefore, thermophilic microorganisms are of great importance to the treatment of oil pollution in high temperature environments. Jiang et al.[17] have isolated six thermophilic oil-degrading strains from the oil-contaminated soil at Daqing Oilfield, China, including the Bacillus licheniformis WY2. The study found that the strain WY2 optimal growth in the presence of 0.5% v/v crude oil and pH 7 was observed at 52-80°Cand the content of total petroleum hydrocarbons was reduced by 52%. Wang et al.[18] have reported the isolation thermophilic oil-degrading bacteria group capable of degrading saturated hydrocarbon, aromatic hydrocarbon components, asphaltene and asphaltene as the source of carbon at temperature 45°Cfrom the oil-contaminated soil at Daqing Oilfield . Audrius et al.[19] have isolated a number of thermophilic bacteria capable of utilizing naphthalene as a sole source of carbon from a high-temperature oilfield in Lithuania. These isolates were able to utilize several other aromatic compounds, such as anthracene, benzene, phenol, benzene-1, 3-diol, protocatechuic acid as well.

5. Conclusions

So far, extremophiles capable of degrading petroleum hydrocarbons have been found in oil-contaminated areas under high pressure, high temperature, low temperature or high salt. The use of extremophiles to treat petroleum hydrocarbon pollution has been taken seriously by researchers in the world. However, the degradation of petroleum hydrocarbon by these extremophiles is still limited to its degradation characteristics and related degradation enzymes. The mechanism of these microorganisms to adapt to the extreme environment and the specific ways to degrade petroleum hydrocarbons still need to be further researched. The great potential to exploit microbes in the field of oil pollution and how to apply them efficiently to the site is still a key issue that needs to be studied further in the future.

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