

Study on the Effect of Exogenous Stimulation on Microbial Remediation of Petroleum-contaminated Soil

Lirong He^{1,2,3,4 a}, Yingying Sun^{1,2,3,4 b}

¹Shaanxi Provincial Land Engineering Construction Group Co., Ltd, Xi'an, Shaanxi,710075, China;

²Institute of Land Engineering Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an, Shaanxi,710075, China;

³Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Natural Resources, Xian, Shaanxi, 710075, China;

⁴Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an, Shaanxi, 710075, China.

^a156144982@qq.com, ^bsunyy526@163.com

Abstract

With the increasing severity of the oil pollution problem, microbial remediation techniques have been developed for use in oil-contaminated soils and are considered to be one of the most promising remediation techniques due to their low investment, low environmental risk and high adaptability. However, due to their small size, low resistance and unstable activity, microorganisms are easily inactivated in nutrient-poor oil-contaminated soils and often have poor degradation effects on soil contaminants. To address this problem, studies have now shown that the addition of exogenous stimulants can improve the degradation activity of microorganisms and increase the population size to varying degrees, shorten the enzymatic degradation cycle and significantly improve the degradation effect of petroleum pollutants. However, the current research mainly focuses on a single type of exogenous treatment to stimulate the activity of petroleum degrading microorganisms to improve the degradation efficiency, while a single exogenous stimulus cannot completely improve the degradation activity of microorganisms. In the future, we can use typical organic and inorganic stimuli to carry out targeted combinations to maximize microbial degradation efficiency, analyse the response mechanism of petroleum-contaminated soil-microbial ecosystem to exogenous stimuli, explore the best combination of exogenous stimuli, apply microbial stimuli to petroleum-contaminated soil in situ remediation, and provide scientific basis for petroleum-contaminated soil bioremediation and treatment.

Keywords

Oil pollution; Soil microorganisms; Exogenous stimulation.

1. Introduction

Leaking oil wells, crude oil pipelines and leaking storage tanks during the development of oil and gas fields, and marine oil spills cause petroleum-based substances to fall on the ground soil, resulting in soil contamination problems. Petroleum is a complex mixture of thousands of compounds, including saturated alkanes, aromatic hydrocarbons, asphaltenes and non-hydrocarbon compounds [1-3]. Petroleum hydrocarbons (APH) are common organic pollutants with high hydrophobicity and persistence, and their massive enrichment in soil can have a sustained negative impact on soil function and cause varying degrees of harm to microorganisms, plants and animals throughout the ecosystem [4-5]. Therefore, there is an urgent need for research into methods that facilitate environmental remediation to remove or reduce petroleum contaminants from soils. So far, remediation techniques have been developed for petroleum-contaminated soils, among which microbial remediation is considered one of the most promising remediation techniques due to its low investment, low

environmental risk and high adaptability. However, due to their small size, low resistance and unstable activity, microorganisms are easily deactivated in nutrient-poor petroleum-contaminated soils and often have poor degradation effects on the pollutants in the soil [6].

2. The Reinforcing Effect of Exogenous Stimuli on Microbial Remediation of Petroleum-contaminated Soils

Existing studies have shown[7] that the addition of exogenous stimulants can improve the degradation activity and increase the population size of microorganisms to varying degrees, shorten the enzymatic degradation cycle and significantly improve the degradation efficiency of petroleum pollutants, therefore, exogenous stimulation is necessary to improve the remediation of oil-contaminated soil. Some studies have shown[8] that adding nutrients to the soil will activate the activity of enzymes in microorganisms, enhance the distribution and transfer rate of nutrients by the microbial metabolic system, and ensure the efficient and rapid decomposition of crude oil in microorganisms. Witzig et al[9] found that the synthesis efficiency of enzymes in microorganisms will also be reduced when the content of nutrients in the soil is too low, and the function of the metabolic system will be weakened. Wu et al[10] showed that there was a linear correlation between the degradation rate of TPH and the population size and activity of microorganisms.

There are many ways to improve microbial degradation of petroleum contaminants through exogenous stimulation, divided into physical and chemical stimulation. Physical stimulation can change the nature of soil microbial cell membranes and their living environment through physical effects (e.g. aeration, electrical stimulation, etc.), and the contact between microbes and crude oil is enhanced, which in turn improves microbial degradation efficiency. However, physical stimulation is difficult and costly to implement in situ, so most in situ remediation techniques use chemical stimulation to improve microbial degradation of contaminants. Chemical stimulation consists of organic and inorganic stimulation. Under the effect of chemical stimulation, microorganisms accelerate the catabolism of petroleum hydrocarbons, increase the rate of anabolism, reach high quantity and activity in a short time, and enhance the remediation ability of crude oil-contaminated soil.

2.1 The Role of Chemical Stimulation on Microbial Remediation of Petroleum-contaminated Soils

2.1.1 Inorganic Stimulation

Inorganic stimulants are mainly N and P compounds. The appropriate nutrient ratio and addition not only promote the uptake of nutrients by microorganisms and reduce the pollution of the environment by excessive nutrients, but also accelerate the degradation of petroleum pollutants by microorganisms. Within a certain range of pollution concentrations and inorganic fertiliser concentrations, the effect of pollution treatment increased with increasing concentration of exogenous stimulants [11], i.e. increasing microbial activity while enhancing microbial degradation of pollutants. Wu et al [12] found that the addition of N and P inorganic salts stimulated microbial remediation of oil-contaminated soil, and the efficiency of degradation of petroleum hydrocarbons (60%) was higher than that of the control group without the addition of inorganic salts (34%), with higher microbial activity and numbers than the control group. A further study showed that the use of NH_4NO_3 and K_2HPO_4 to stimulate microbial remediation of petroleum contamination revealed the highest biomass of soil microorganisms at a soil C:N:P of 100:10:1, which was about 40% higher than that of the control group, and the degradation of aromatic hydrocarbons reached 43.89% [13]. Kalantary et al [14] analysed the effect of the addition of compounds containing different elements on However, inorganic stimulants, due to their short presence in the soil, tend to change the soil pH and carbon to nitrogen ratio, causing adverse effects on microbial metabolism.

2.1.2 Organic Stimulation

Organic stimulants mainly include amino acid complex fertilizers, humus, plant root secretions, surfactants, organic wastes, etc. Among them, surfactants can well promote microbial growth and

metabolism, but there are differences in the effectiveness of promoting pollutant degradation. Patowary et al [15] applied rhamnolipid (RL) 1.5 g/L to contaminated soil and measured microbial degradation of petroleum hydrocarbons up to 86.1%, while the application of sodium dodecyl sulfate (SDS) achieved only 68.1% biodegradation because rhamnolipid eliminated three PAHs (fluoranthene, benzo[a]fluorene and benzo[a]anthracene) from the soil, while the addition of SDS only served to emulsify the crude oil. when Li et al. used different surfactants to promote microbial remediation of petroleum-contaminated soil, they found that lecithin increased the degradation of hydrocarbons by petroleum-degrading bacteria. The effect of monostearic acid glycerides and cetyl trimethyl ammonium bromide was not significant in promoting the degradation of petroleum hydrocarbons [16]. Hua Li et al [17] concluded that the efficiency of using microorganisms alone to degrade oil-bearing soil was low at 22.68%, and the remediation of diesel-contaminated soil reached 46.11% and 45.32% with the addition of Tween80 and RL, respectively. It can be seen that lecithin, RL and Tween80 are the better microbial remediation auxiliaries at present, while the other surfactants mentioned above have a lower promotion effect. In addition, the effectiveness of surfactants in promoting microbial remediation also depends on the uptake of nutrients by microorganisms and the balance between nutrient levels, as a lack of or an increase in the content of certain elements will reduce the activity of microorganisms.

2.2 The Role of Physical Stimulation on Microbial Remediation of Oil-contaminated Soils.

The addition of exogenous stimulants can significantly promote the degradation effect of microorganisms, and auxiliary some physical stimulation methods will further enhance the remediation efficiency. Some studies have shown that the effect of combined microbial-electric field remediation on petroleum-contaminated soil is significantly improved compared with microbial remediation alone, and the remediation effect increases with increasing electric field strength when the electric field strength is below 0.02 V/cm [18]. Luo et al [19] found that a DC current of 20 mA can increase the hydrophobicity of the cell surface of degrading bacteria and flatten the cell shape, which is conducive to the contact with organic matter and improve the. Therefore, the selection of the appropriate current can effectively improve the degradation effect. Therefore, choosing the right current can effectively promote the electrochemical process in microorganisms, enhance the uptake and decomposition of substances, and play a good role in assisting microorganisms to degrade oil-containing pollutants.

3. Summary

The current research mainly focuses on a single type of exogenous treatment to stimulate the activity of petroleum-degrading microorganisms to improve the degradation efficiency, while a single exogenous stimulation cannot completely improve the degradation activity of microorganisms. In the future, we can use typical organic and inorganic stimuli to carry out targeted combinations to maximise microbial degradation efficiency, analyse the response mechanism of petroleum-contaminated soil-microbial ecosystem to exogenous stimuli, explore the best combination of exogenous stimuli, apply microbial stimuli to petroleum-contaminated soil in situ remediation, and provide scientific basis for petroleum-contaminated soil bioremediation and treatment.

Acknowledgements

The project was supported by Natural Science Basic Research Plan in Shaanxi Province of China (2021JQ-960) and the projects of Land Engineering Construction Group of Shaanxi Provincial (DJNY2020-19 and DJNY2021-27).

References

- [1] V.N. Kavamura, E. Esposito. Biotechnological strategies applied to the decontamination of soils polluted with heavy metals[J]. Biotechnology Advances, 2010, (28):61-69.

- [2] P. Xu, G.M. Zeng, D.L. Huang, et al. Use of iron oxide nanomaterials in wastewater treatment: a review[J]. *Science of the Total Environment*, 2012, 424:1-10.
- [3] G.M. Zeng, M. Chen, Z.T. Zeng. Risks of neonicotinoid pesticides[J]. *Science*, 2013, 340:1403.
- [4] J. Zhang, J. Dai, H. Chen, et al. Petroleum contamination in groundwater/air and its effects on farmland soil in the outskirts of an industrial city in China[J]. *Journal of Geochemical Exploration*. 2012, 118:19-29.
- [5] H. Van De Weghe, G. Vanermen, J. Gemoets, et al. Application of comprehensive two-dimensional gas chromatography for the assessment of oil contaminated soils[J]. *Journal of Chromatography A*, 2006, 1137:91-100.
- [6] A. Borowik, J. Wyszowska¹, M. Wyszowski. Resistance of aerobic microorganisms and soil enzyme response to soil contamination with Ekodiesel Ultra fuel[J]. *Environmental Science & Pollution Research International*, 2017, 24(12): 24346-24363.
- [7] K.S.H. Yu, A.H.Y. Wong, K.W.Y. Yau, et al. Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments[J]. *Marine Pollution Bulletin*, 2005, 51(8-12):1071-1077.
- [8] Z. Du, Y.G. Chen, X. Li. Quantitative proteomic analyses of the microbial degradation of estrone under various background nitrogen and carbon conditions[J]. *Water Research*, 2017, 123(15):361-368.
- [9] M. Witzig, B. Lengowski Melanie, H.R. Zuber Karin, et al. Effects of supplementing corn silage with different nitrogen sources on ruminal fermentation and microbial populations in vitro[J]. *Anaerobe*, 2018, 51:99-109.
- [10] M.L. Wu, W.A. Dick, L. Wei, et al. Bioaugmentation and bio-stimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil[J]. *International Biodeterioration & Biodegradation*, 2016, 107:158-164.
- [11] K. Khan, R. Joergensen. Compost and phosphorus amendments for stimulating microorganisms and growth of ryegrass in a Ferralsol and a Luvisol[J]. *Journal of Plant Nutrition and Soil Science*, 2012, 175(1):108-114.
- [12] Kost, David, Dick, et al. Bioremediation of hydrocarbon degradation in a petroleum contaminated soil and microbial population and activity determination[J]. *Chemosphere Environmental Toxicology & Risk Assessment*, 2017, 169:124-130.
- [13] Wang Yanjie, Li Fayun, Rong Xiangmin et al. Remediation of petroleum-contaminated soil with biomass and nutrient application[J]. *Journal of Agricultural Environmental Science*, 2018, 037(002):232-238.
- [14] R. Kalantary, A. Mohseni-Bandpi, A. Esrafil, et al. Effectiveness of biostimulation through nutrient content on the bioremediation of phenanthrene contaminated soil[J]. *Journal of Environmental Health Science and Engineering*, 2014, 12(1):143.
- [15] P. Bruheim, H. Bredholt, K. Eimhjellen. Bacterial degradation of emulsified crude oil and the effect of various surfactants[J]. *Canadian Journal of Microbiology*, 1997, 43(1):17-22.
- [16] X. Li, Q. Zhao, X. Wang, et al. Surfactants selectively reallocated the bacterial distribution in soil bioelectrochemical remediation of petroleum hydrocarbons[J]. *Journal of Hazardous Materials*, 2017, 344(15):23-32.
- [17] Hua L, Peng XY, Fan Y et al. Analysis of the degradation products of single strains and mixed bacteria for petroleum degradation[J]. *Journal of Shaanxi University of Science and Technology (Natural Science Edition)*, 2014, 000(005):27-31,41.
- [18] Guo S , Fan R , Li T , et al. Synergistic effects of bioremediation and electrokinetics in the remediation of petroleum-contaminated soil[J]. *Chemosphere*, 2014, 109(AUG.):226-233.

- [19]Luo, QS, Wang, H, Zhang, XH, Qian, Y. Effect of direct electric current on the cell surface properties of phenol-degrading bacteria[J].Applied and Environmental Microbiology,2005,71(1):423-427.