# Research on the Impact of Alkylbenzene Sulfonate Surfactants in Use in Oilfields on Native Biodiversity of Mining Areas and Response to Environmental Remediation of Native Biodiversity

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Abstract: In order to clarify the effect of alkylbenzene sulfonate surfactants used in oil field on native biodiversity in mining area. In this paper, high-throughput sequencing and biodiversity analysis were performed on the soil continuously polluted by alkylbenzene sulfonate surfactants. Clostridia, BPC102 and Bacteroidia became the dominant bacteria in the soil environment, with strong self-repair response of environmental organisms. Bacteria S035, Bacilli and Dothideomycetes showed negative response, indicating that alkylbenzo sulfonate surfactants inhibited and affected the growth, development and reproduction of these native organisms. The results showed that alkylbenzene sulfonate surfactant had obvious effects on soil biodiversity in mining area, which provided scientific basis for environmental impact assessment and environmental management of surfactants.



## 1 INTRODUCTION

Alkylbenzene sulfonate surfactants are widely used in the field of chemical flooding in low-permeability oilfields for enhanced oil recovery due to their excellent oil displacement performance. As an important oil field EOR chemical agent, the application range of surfactants is still expanding and the consumption is also increasing day by day. In the process of use, a large amount of waste water and waste residues containing surfactants are inevitably discharged and infiltrated into the soil. Large-scale industrial use of alkylbenzene sulfonate surfactants urgently needs to clarify its impact on the native biodiversity of oilfields and mining areas and the response of native biodiversity to its environmental bioremediation.

The environmental behavior of alkylbenzene sulfonate surfactants in soil mainly includes migration, adsorption and degradation. As an important place for energy exchange of various substances recycling machines, soil is usually the destination of migration, retention and deposition of pollutants in the environment. After surfactants enter the mining environment, they will first have a certain impact on the biodiversity of the mining area. The impact degree is positively correlated with the impact of local biodiversity, and negatively correlated with the bioremediation response of local biodiversity. If the native biodiversity has a strong response to the environmental bioremediation of alkylbenzene sulfonate surfactants, it indicates that the oil field has a high tolerance of alkylbenzene sulfonate surfactants and a large marginal safety concentration, that is, the environmental biological reference value is large, and the environmental biological toxicity is small. On the other hand, if the native biodiversity responds weakly to the environmental bioremediation of alkylbenzene sulfonate surfactants. The microbial flora that can degrade the surfactant cannot be enriched in a short period of time, the tolerance of alkylbenzene sulfonate surfactants in the oilfield will be low and the safety marginal concentration will be small. In other words, alkylbenzene sulfonate surfactants have low environmental biological reference value and high environmental biological toxicity in oilfield mining area. Therefore, it is of great significance to study the effects of alkylbenzene sulfonate surfactants on native biodiversity and the response of native biodiversity to environmental bioremediation.



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In this paper, different types of bioreactors with alkylbenzene sulfonate surfactants as the only pollution source have been designed and continuously operated. The second-generation Qualcomm sequencing technology (Illumina MiSeq) was used to conduct high-throughput sequencing of 16S rDNA V3~V4 regions and ITS1 regions on the soil which was continuously polluted by alkylbenzene sulfonate surfactants. The sequencing results were evaluated by OTU cluster analysis, Alpha diversity, species composition and abundance analysis methods, which provided a theoretical basis for the ecological and environmental protection in the oilfields and mining areas.

# 2 METERIALS AND METHODS

## 2.1 Experimental Materials

Experimental target material: alkylbenzene sulfonate surfactant used in an oilfield

Experimental soil: Fresh soil randomly collected from a domestic oilfield chemical flooding enhanced oil recovery test mining area, remove surface rocks and other impurities, mix well and pass through a 2mm sieve.

Experimental equipment: In order to obtain the influence of alkylbenzene sulfonate surfactants on local biodiversity after entering the soil environment and the response of local biodiversity to bioremediation of characteristic pollution sources, a bioreactor was designed as shown in Figure.1. The prepared surfactant solution is continuously introduced into the reactor soil from the top of the bioreactor. The bottom is provided with an outlet from which the solution can seep out. The surrounding sampling holes are used to collect soil samples at different contamination times.

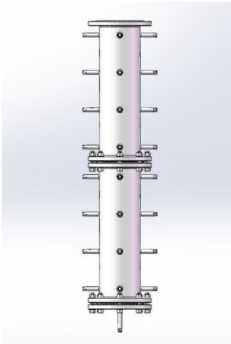


Figure 1: Schematic diagram of bioreactor.

## 2.2 Experimental Methods

A peristaltic pump was used to add the prepared surfactant solution at a certain flow rate (calculated based on the actual leakage) from the top of the reactor, and keep the experimental temperature relatively constant. Samples were taken on day 7 (represented by D), day 30 (represented by E) and day 60 (represented by F), and set a group of blank samples for control (represented by V) at the same time. The obtained soil samples were stored in sterilized sealed bags at −80 °C and microbial sequencing was performed as soon as possible. The whole experiment was carried out under dark conditions.

# 3 RESULTS AND ANALYSIS

## 3.1 OTU Cluster Analysis

In order to facilitate analysis in the study, a single marker is artificially set for a Taxonomic unit, namely OTU (Operational Taxonomic Units). In order to understand the number of species and genera in the sequencing results of a sample, it is necessary to classify the sequence. Through the classification operation, sequences are divided into many groups according to their similarity, and one group is an OTU.

Figure.2 is a Venn diagram of the number of bacterial OTU in soil sample. As shown in the figure, a total of 5276 OTU were obtained in group D, 4951 OTU in group E, 5078 OTU in group F, and 4600 OTU in group V of control group. The sequence of OTU numbers in the four soil samples is D > F > E > V. The richness of bacterial groups was the highest in the day7, and the lowest in the blank group. The number of OTUs in the four groups was compared in pairs: there were 2694 OTUs shared by group V and Group D, 2413 OTUs shared by group V and Group E, 2204 OTUs shared by group V and group F, 2815 OTUs shared by group D and group E and 2573 OTUs shared by group E and group F. It can be seen that the bacterial groups in the soil samples of day7 and day30 had the highest consistency and the smallest difference, while day60 had the lowest consistency and the largest difference. This indicated that as the pollution time of the alkylbenzene sulfonate on the soil is prolonged, the difference of the bacterial groups in the soil is greater.

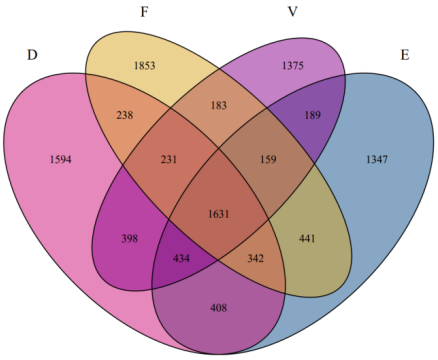



Figure 2: The Venn diagram of the number of bacterial OTUs in soil samples.

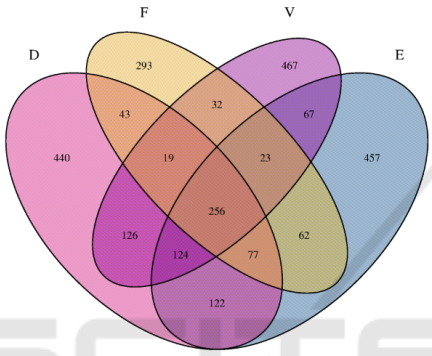



Figure 3: The Venn diagram of the number of fungal OTUs in soil samples.

As shown in the figure, there were 1114 OTUs in the control group, 1207 OTUs in the D group, 1188 OTUs in the E group and 805 OTUs in the F group. The number of OTU in the four groups of soil samples was D > E >V > F. Fungal species richness was highest in soil samples after 7 days of contamination, and lowest in soil samples after 60 days of contamination. The pairwise comparison results show that the number of OTUs shared by group V and D is 525, the number of OTUs shared by group D and E is 579, the number of OTUs shared by group E and F is 418, and the number of OTUs shared by group V and F is 418. The total number of OTUs is 470, the number of OTUs shared by groups V and F is 330, and the number of OTUs shared by the 4 groups of soil samples is 256. After comparison, it was found that the consistency of fine fungal groups was the highest and the difference was small between the soil samples at day7 and day30. While the consistency was the lowest and the difference was the largest between the soil samples at day60 and the control group. It can be seen that with the extension of time, the difference of fungal groups in soil contaminated by alkylbenzene sulfonate surfactant gradually increased.

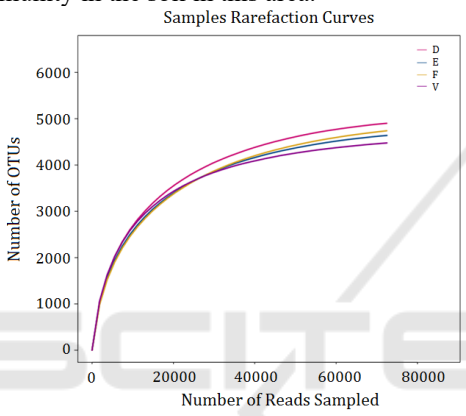
## 3.2 Alpha Diversity Index Analysis

MiSeq platform was used to perform high-throughput sequencing on four groups of soil samples by sequencing while synthesizing. The sequencing results are shown in Table1. Shannon index and Simpson index are usually used to estimate the diversity of OTU species in microbial communities. They are also commonly used to estimate the diversity of microorganisms in sample. The larger the Shannon index value, the higher the community diversity. The smaller the Simpson index value, the higher the community diversity. It can be seen from Table1 that the order of the species diversity of bacteria and fungi in the four groups of soil samples is V> D> E> F. This shows that as time goes by, the microbial diversity of the soil contaminated by surfactants has declined, and the surfactants have an inhibitory effect on the growth of microorganisms. The coverage of each sample was more than 99.90%, indicating that the sequencing effect was ideal, and the diversity analysis results fully reflected the information of microbial species in the area.

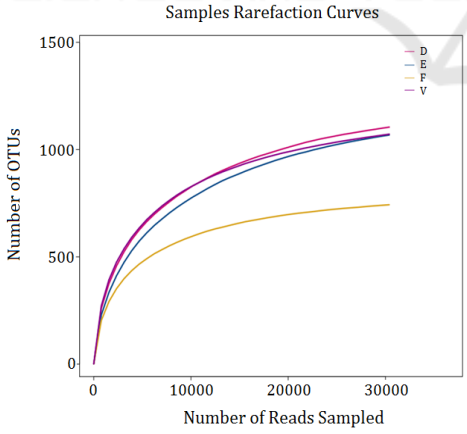
Table 1: Diversity analysis of bacterial microbial index of soil samples.

| Group | Bacteria | | | Fungi | | |
|---|---|---|---|---|---|---|
| | Shannon | Simpson | Coverage, % | Shannon | Simpson | Coverage ,% |
| D | 7.12± 0.115 | 0.0018± 0.00035 | 99.91 | 4.82 ±0.285 | 0.0236 ±0.00890 | 99.95 |
| E | 6.90± 0.393 | 0.0042± 0.00483 | 99.93 | 4.52 ±0.586 | 0.0410 ±0.04184 | 99.94 |
| F | 6.71± 0.904 | 0.0093± 0.01571 | 99.92 | 3.68 ±1.320 | 0.1398 ±0.17308 | 99.96 |
| V | 7.18± 0.085 | 0.0014± 0.00018 | 99.91 | 4.96 ±0.193 | 0.0180 ±0.00412 | 99.94 |

Random sampling of sequencing sequences is used to construct a curve based on the number of extracted sequences and the number of OTU represented by them, that is the dilution curve. Generally, when the curve tends to be flat, it indicates that the number of samples is reasonable. Figure.4(a)and Figure.4(b) are the dilution curves of soil samples. It can be seen that the dilution curves of bacteria and fungi of the samples are basically flat, indicating that the sequencing and sampling are reasonable and can truly reflect the microorganisms in the soil samples. Combined with the coverage of each sample, it shows that most of the microbial groups are included in the sequencing results, which can truly reflect the composition of the microbial community in the soil in this area.




(a) Bacteria




(b) Fungi

Figure 4: Dilution curve of the sample.

## 3.3 Analysis of Community Structure

As shown in Figure.5, more than 70 types of bacteria were detected in the four groups of soil samples. Among them, β-Proteobacteria, α-Proteobacteria, Acidobacteria, Δ-Proteobacteria and γ-Proteobacteria are the absolute dominant species. The proportions of the five dominant bacteria in the blank group were 9.04%, 8.55%, 9.62%, 7.16%, and 4.96%, respectively. By day 7, the proportions of these five bacteria were 11.04%, 8.35%, 9.97%, 7.01% and 6.41%. By day 30, the proportions of the five dominant bacteria were 10.95%, 10.14%, 7.90%, 8.93%, and 7.13%. By day 60, the proportions of the five bacteria were 12.46%, 10.50%, 8.24%, 9.24% and 6.92%, respectively. In general, the relative content of the five dominant bacteria did not change much with the prolongation of pollution time, and their growth and reproduction in the soil were relatively stable, and they were not greatly affected by alkylbenzene sulfonate surfactants.

Figure.6 is a Heatmap based on the genus level. The Heatmap can reflect the similarity and difference in species composition of all samples at a specific taxonomic level. The more similar species or samples are, the closer they are to each other in the cluster tree, which can also indicate that certain bacterial groups may have specific distributions.

The relative abundance of Clostridia in soil samples at day30 and day60 was higher than day7 and black group. Similarly, the relative abundances of bacteria BPC102 and Bacteroidia were higher in the soil samples on day60 than in other soil samples, indicating that under the continuous pollution of alkylbenzene sulfonate, Clostridia, BPC102 and Bacteroidia gradually became the dominant bacteria in the soil. However, the relative abundance of bacteria S035 and Bacilli decreases with the extension of the experiment time, indicating that the growth and propagation of bacteria S035 and Bacilli are inhibited by alkylbenzene sulfonate.

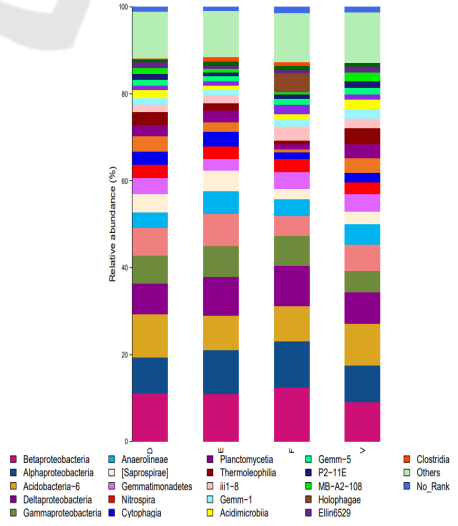



Figure 5: Histogram of bacterial community composition at class classification level.

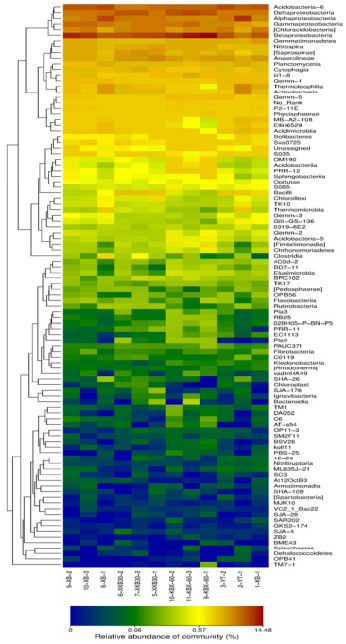


Figure 6: Heatmap at class classification level.

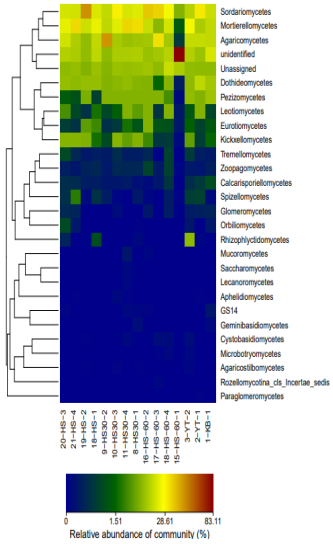


Figure 8: Heatmap at class classification level.

Figure 7 is a histogram of fungal community composition based on class classification level. More than 20 types of fungi were detected in the four groups. Among them, the relative proportions of Sordariomycetes, Mortierellomycetes and Agaricomycetes were 21.49%~28.03%, 10.81%~27.95%, 13.29~19.62%, which were relatively high in soil samples, and were the absolute dominant fungal species. The relative content of Dothideomycetes in the blank group was 10.15%, which decreased to 2.02% on day 60, indicating that alkylbenzene sulfonate would inhibit the growth and reproduction of Dothideomycetes.

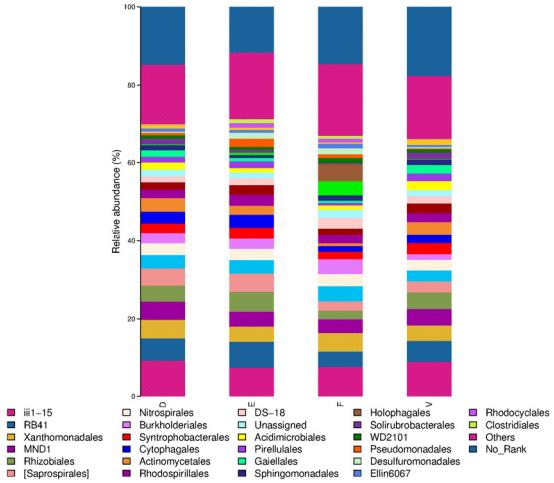


Figure 7: Histogram of bacterial community composition at class classification level.

# 4 CONCLUSION

In this paper, the oil field soil continuously polluted by alkylbenzene sulfonate surfactants was taken as the research object. The results of high-throughput sequencing and biodiversity analysis were as follows:

(1) As the soil is continuously polluted by alkylbenzene sulfonate surfactants for a longer time, the abundance and diversity of bacteria and fungi in the soil are decreasing, and the group differences of microorganisms are gradually increasing. It shows that alkylbenzene sulfonate surfactants have a significant impact on the native biodiversity in the soil environment of mining areas.

(2) Microbial sequencing showed that alkylbenzene sulfonate contaminated soil had dominant bacteria, They were Betaproteobacteria, Alphaproteobacteria, Alphaproteobacteria, Acidobacteria, Deltaproteobacteria, Gammaproteobacteria, Clostridia, BPC102 and Bacteroidia.

(3) Clostridia, BPC102 and Bacteroidia had positive response to alkylbenzene sulfonates, and gradually became the dominant bacteria in the soil environment of the oil field. It showed that the native biodiversity of the oilfield mining area had a strong environmental biological self-repair response to alkylbenzene sulfonate surfactants.

(4) The relative content of bacteria S035, Bacilli, and fungus Dothideomycetes (Polycystomycetes) decreased with the extension of the contamination time, that is, it showed a negative response to alkyl

benzene sulfonates. The results showed that alkylbenzenesulfonate surfactants inhibited and affected the growth, development and reproduction of these native organisms.

It can be seen that alkylbenzene sulfonate surfactant have an obvious impact on the diversity of soil biodiversity in mining areas. Therefore, their use and discharge must consider environmental capacity, fundamentally reduce their direct discharge to the environment, and increase the treatment of wastewater and waste residue containing surfactants.

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