

Preferential Degradation of Aromatic Hydrocarbons over Aliphatic Hydrocarbons in Mineral Oil by a Microbial Consortium

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| 著者 | BACOSA HERNANDO PACTAO |
| 号 | 8 |
| 学位授与機関 | Tohoku University |
| 学位授与番号 | 環博第48号 |
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| 氏名 | バコサ ヘルナンド パクタオ BACOSA HERNANDO PACTAO |
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| 指導教員 | 東北大学教授 井上 千弘 |
| 論文審査委員 | 主査 東北大学教授 井上 千弘 東北大学教授 細矢 憲 東北大学教授 西村 修 東北大学准教授 須藤 孝一 (工学研究科) |

論文内容要旨

Bioremediation of mineral oil hydrocarbon components is an attractive approach of cleaning up oil contaminated sites. It is environmentally friendly, cost-effective, applicable over large areas, and capable of complete destruction of the contaminant. Microbial populations which are the major players in bioremediation have critical roles with regards to their composition, metabolic capabilities, and growth. Previous studies reported that the aliphatic components of mineral oil were preferably degraded than the more toxic aromatic hydrocarbons. In a series of experiments, this research work investigated several aspects of the microbial consortium that preferentially mineralized aromatic hydrocarbons in mineral oil. It aimed to evaluate its ability to degrade the equivalent carbon number (EC) hydrocarbon fractions in mineral oil, and binary mixture of aliphatic-aromatic hydrocarbons. It also aimed to elucidate its microbial composition and community dynamics during the degradation process.

The consortium was initially investigated in aerobic batch cultures containing kerosene as the sole carbon and energy source. After three days of incubation, the consortium degraded 20% of kerosene. The aromatic fractions EC >7-8 and EC >8-10 were completely degraded after three days while the aliphatic fractions EC >6-8 and EC >8-10 were only partially degraded. The aromatic EC >10-12 fraction was the third most degraded, and the aliphatic EC >10-12 and EC >12-16 fractions were the least degraded fractions. The first order rate constants for the aromatic fractions ranged from 0.12/d to 0.51/d and from 0.06/d to

0.32/d for the aliphatic fractions. It should be noted also that fractions containing compounds of lower molecular weights were degraded more quickly than fractions with higher molecular weight components. When incubated in the soil, the consortium's patterns of degradation of kerosene hydrocarbon fractions were very similar to that of the liquid culture. Aromatic hydrocarbon fractions were more readily degraded than the corresponding aliphatic fractions. Based on the cloning and sequencing of the 16S rRNA gene, the microbial community was predominantly identified as Betaproteobacteria of the genera *Achromobacter*, *Alcaligenes*, *Cupriavidus*, and *Burkholderia*; and Gammaproteobacteria of the genus *Rhodanobacter*. The microbial consortium preferentially utilized aromatic fractions, which are more toxic over aliphatic fractions. This finding is useful when considering risk-based bioremediation and the microbial community could potentially degrade the more toxic aromatic hydrocarbons components in petroleum-contaminated environments.

The consortium was further investigated in the degradation of binary mixtures of aliphatic and aromatic hydrocarbon compounds. Degradation experiments were performed under aerobic conditions in sealed bottles containing liquid medium and n-octane, n-decane, or n-dodecane as representative aliphatic hydrocarbons or toluene, ethylbenzene or *p*-xylene as representative aromatic hydrocarbons. The results demonstrated that the consortium degraded *p*-xylene more rapidly than n-octane. It degraded toluene, ethylbenzene and *p*-xylene more rapidly than n-decane. The same results were also obtained in n-dodecane-aromatic tandem where toluene, ethylbenzene, and *p*-xylene were preferably degraded. The substrate preferences of the consortium showed that the aliphatic-aromatic hydrocarbon binary mixtures reflected its degradation ability of complex hydrocarbon compounds such as kerosene. These results suggest that aliphatic-aromatic binary systems could be used as a tool to rapidly determine the degradation preferences of a microbial consortium.

To determine the dynamics of the major bacterial genera in the consortium, real-time PCR targeting the 16S rRNA gene was performed. Bacterial cells were gathered periodically from batch cultures containing n-octane, *p*-xylene, n-octane-*p*-xylene, or kerosene. The dynamics of the major bacterial genera in the consortium was investigated and the putative role of each major bacterial group was determined. *Achromobacter/Alcaligenes* did not degrade hydrocarbons, but utilized the metabolic intermediates. They were initially inhibited by aromatic hydrocarbons and started to increase when certain metabolites were

formed. *Burkholderia* was the major aromatic hydrocarbon degrader which substantially increased at the earlier stage of the experiment. It could be inhibited by competition with other bacteria or by metabolites as evident by its decrease during the later part of the experiment. *Cupriavidus* was the major aliphatic degrader. It also utilized intermediate metabolites resulting in sustained increase throughout the experimental period. *Rhodanobacter* was inhibited by aromatics, utilized intermediate metabolites, but did not degrade hydrocarbons.

The findings of this study are indispensable contribution detailing the role of the microbial consortium in the degradation of aromatic hydrocarbons. This consortium is a potential source of isolates to construct a defined synthetically mixed culture that can efficiently degrade toxic aromatic hydrocarbons in petroleum-contaminated environments. Bacterial populations that prefer to degrade aromatic compounds are essential in the bioremediation of complex petroleum hydrocarbon products and the reduction of associated health risk.

論文審査結果の要旨

石油系炭化水素で汚染された海洋や土壌のバイオレメディエーションにおいては、油分解微生物が脂肪族炭化水素を優先的に分解し、毒性の強い芳香族炭化水素はあまり分解しないことが知られている。リスクベースのバイオレメディエーションを行う上で、優れた芳香族炭化水素分解能力を保有する微生物コンソーシアムを獲得することは重要な課題となっている。本論文は自然界から得られた芳香族炭化水素を優先的に分解する微生物コンソーシアムについて取りまとめたもので、以下の5章で構成されている。

第1章では、石油系炭化水素の微生物分解に関して概観するとともに、特に芳香族炭化水素類の微生物分解についての現状と課題がまとめられている。

第2章では、油田周辺の石油汚染土壌から新たに見出された高い油分解活性を有する微生物コンソーシアムが、石油中の単環芳香族を脂肪族炭化水素の約10倍の速度で分解するユニークな特徴を有していることが示されている。このコンソーシアムを用いて石油汚染土壌から毒性の高い単環芳香族類を優先的に分解させることにより、当該土壌のもたらす健康リスクをすみやかに低減できることが議論されている。

第3章では、前章で得られたコンソーシアムならびに一般に得られる脂肪族を優先的に分解するコンソーシアムを用いて、芳香族-脂肪族炭化水素2成分系での分解実験を行い、微生物コンソーシアムによる鉱物油中炭化水素の優先利用は芳香族-脂肪族炭化水素2成分系における分解パターンが反映されることが示されている。

第4章では、芳香族炭化水素を優先的に分解する微生物コンソーシアムを用い、芳香族-脂肪族炭化水素2成分系および石油の分解過程における微生物の動態を解析した結果が述べられ、*Burkholderia* 属細菌が芳香族炭化水素を直接的に分解し、その分解生成物を *Cupriavidus* 属細菌がさらに利用することなどが示されている。

第5章では、本研究で得られた結果が総括されている。

以上のように、本論文では従来報告されていなかった芳香族炭化水素を優先的に分解する微生物コンソーシアムを見出し、その保有する特徴を炭化水素の成分分析、微生物群集解析の両面から明らかにしており、学術的な意義は高いものがある。また油汚染土壌の浄化にこのコンソーシアムを使用することにより、効果的なバイオレメディエーションの実施が期待できるので実用上の価値も高い。

よって、本論文は博士(環境科学)の学位論文として合格と認める。