（学位第３号様式）

学　位　論　文　要　旨

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題　目	Bioremediation of oil-contaminated seawater and sediment by an oil-degrading bacterial consortium （石油分解細菌コンソーシアムを用いた石油汚染海洋環境の微生物修復）

This study describes a continuum of research aimed at developing a bioremediation technology for oil-contaminated marine environment. First, isolation and identification of bacterial strains with oil-degrading potential from previously contaminated tropical soil (Guimaras Island, Philippines) was done. Eighteen bacterial strains were isolated and identified by 16S rRNA gene sequence analysis. The results indicated that the isolates belonged mostly to Gammaproteobacteria and Alphaproteobacteria with most strains able to utilize *n*-alkanes and heavy oil as carbon source but only few were able to grow in phenanthrene. Strains closely related to *Pseudomonas aeruginosa, Marinobacter mobilis*, *Halomonas* sp. and *Gaetbulibacter* sp. were selected for further *in vitro* oil-degrading analysis and were immobilized individually and as a consortium by attaching to cocopeat biocarrier and by encapsulating into sodium alginate. Results of *in vitro* oil degradation studies revealed higher oil reduction in treatments receiving the consortium than individual strains in both free-living and immobilized forms.

Once the effectiveness of the consortium to degrade heavy oil was established, its bioremediation potential in seawater was examined. Oil-degrading abilities of the immobilized bacterial consortium in cocopeat, rice hull powder and sodium alginate capsules were compared with that of the free-living form and natural microflora of the seawater. Scanning electron microscopy revealed colonization and strong attachment of bacterial cells on the surface of the cocopeat and rice hull powder yielding significantly higher ($p<0.05$) oil reduction compared to treatments supplemented with the same consortium in free living and encapsulated forms in a 60-day *in vitro* seawater bioremediation trial. Higher cultivable bacterial counts and significantly higher degradations ($p>0.05$) in both aliphatic and aromatic fractions were obtained in treatments augmented with carrier-immobilized consortia. The developed immobilized cells showed sustained activities and viabilities during storage in low temperature for six months.

In the next study, the efficiency of cocopeat-immobilized bacterial consortium in remediating sediment artificially-contaminated with heavy oil was determined. A 3^2 factorial microcosm experiment was conducted to investigate the effects of the different combination of the bacterial cells and inorganic nutrients to degradation of hydrocarbons and bacterial community shifts in the contaminated sediment. Temporal changes in residual heavy oil components, total cultivable and oil-degrading bacterial counts and bacterial community dynamics in the different treatments within 60 day-period were determined. Results showed positive influences of nutrient and bacterial cell addition to the heavy oil degradation. Addition of immobilized cells combined with supplementation with inorganic nutrients resulted to significantly highest reduction ($p<0.05$) in total petroleum hydrocarbons (68.9%) and sustained high numbers of oil-degrading and heterotrophic bacteria throughout the experimental period.

The effects of the different remediation agents to the bacterial community shifts were determined by PCR-DGGE. Statistical analysis of the DGGE profile revealed differences in responses and adaptation mechanisms by indigenous microflora to oil contamination and addition of different remediation agents. Immobilization of the bacterial consortium improved survival of the augmented strains. Addition of inorganic nutrients promoted growth of bacterial groups closely related to those with hydrocarbon-degrading capacities. Persistence of augmented cells and growth of oil-degrading bacterial groups may have contributed to enhanced oil degradation.