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Summary of PhD Thesis

Title: Microbial Degradation of PAHs and Environmental Genomics in Bioaugmentation Systems Using Isolated Yeasts.

Polycyclic aromatic hydrocarbons (PAHs) which contain two or more fused aromatic hydrocarbon rings, are hazardous environmental pollutants, and are widespread in nature because of anthropogenic activities. While it has been demonstrated that PAHs could be degraded by many bacterial isolates, little is known about PAH degradation by yeast, especially for high molecular weight (HMW) PAHs. In this thesis, the performance of isolated yeast strains to degrade HMW-PAHs was investigated in liquid culture, slurry reactor and different biological wastewater treatment systems. The molecular techniques such as fluorescent *in situ* hybridization (FISH) with oligonucleotide probes (EUB338 for most bacteria, SRB385 for sulfate reducing bacteria & PD1 for yeasts), 16S r RNA gene clone libraries and denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S and 26S rRNA genes were used to study microbial structure dynamics of the above biological systems. Variations of some functional genes (*nahAc & C230*) responsible for the degradation of aromatic compounds were also followed.

The main results are given as below:

1) Five yeast strains, belonging to 4 different genera and growing fast on agar plates coated with benzo(a) pyrene as the sole carbon source, were acquired from oil contaminated soil, and were identified based on sequencing of D1/D2 domain of 26S rRNA encoding genes. PCR-DGGE was successfully used to differentiate and authenticate the selected isolates. One of the strains identified as *Pichia anomala* was capable of degrading naphthalene, phenanthrene and chrysene, singly, and benzo(a)pyrene in combination. All of the 3 other PAHs could be utilized as the carbon source for the cometabolic degradation of

benzo(a)pyrene with naphthalene as the best one.

2) Slurry reactor systems inoculated with the above five yeasts removed all of the 16 PAHs contained in the weathered crude oil with a high efficiency. Five- and six-ring PAHs could be effectively removed through co-metabolism. DGGE was successfully used for the tracing of yeast population changes during biodegradation. Three of the five yeast strains remained in the system over the whole 6 week treatment with *Candida maltosa-like* and *Pichia nguilliermondii* as the predominant ones. This is the first report on the PCR-DGGE analysis of yeast populations in a slurry reactor.

3) Three biological treatment systems, inoculated respectively with activated sludge (AS), activated sludge plus mixed yeast culture (SY), and mixed yeast culture (MY), were constructed for treating the biologically treated produced water supplemented with chrysene and benzo(a)pyrene as HMW-PAHs. All of the three systems demonstrated high efficiency in removing low molecular weight (LMW) PAHs. However, only the MY and SY systems demonstrated significant removal of HMW-PAHs.

4) PCR-DGGE analysis indicated that all of the five yeast strains inoculated remained in the SY and MY systems as the dominating populations, and FISH results showed that the relative abundance of yeast population was over 10% in the two systems, suggesting that the five effective yeast strains played a key role in the removal of chrysene and benzo(a) pyrene. FISH results also indicated that bacteria and yeast coexisted in all of the three systems, with SRBs as the dominating population in eubacteria. The existence of catabolic genes (*nahAc & C230*), which were responsible for LMW-PAHs degradation, were confirmed in all of the three systems. These results suggested that bioaugentation for the removal of HMW-PAHs from wastewater was possible by inoculating effective yeast strains.