

## MASTER'S THESIS

### Microemulsions enhanced bioremediation of polycyclic aromatic hydrocarbons (PAHs) contaminated soil using composting technology

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**Microemulsions Enhanced Bioremediation of Polycyclic Aromatic  
Hydrocarbons (PAHs) Contaminated Soil Using Composting Technology**

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Master of Philosophy**

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## Abstract

Soil contamination with polycyclic aromatic hydrocarbons (PAHs), originated from increasing consumptions of coal and petroleum necessitates to the development of innovative technologies to remove PAHs from soil. Thermophilic bioremediation is regarded as one of the most popular strategies to remediate soil contaminated by PAHs. However, the success of bioremediation has been limited by the low aqueous solubility and poor bioavailability of these hydrophobic organic compounds. Microemulsions which have been successfully employed in soil washing to increase the solubility and desorption of hydrophobic pollutants, were evaluated for their feasibility to improve the biodegradation of PAHs compounds in soil using composting approach.

The first phase of the present study aimed at developing microemulsion formulation under thermophilic temperature (55°C) and assessing their effect on solubilizing two representative PAHs, phenanthrene (PHE) and benzo[a]pyrene (B[a]P). Tween 80, linseed oil, with the presence of 1-pentanol to serve as a cosurfactant could form oil-in-water microemulsions under thermophilic condition. Solubilization results showed that the behaviours of microemulsions were similar to surfactants, which showed the equilibrium solubility of PAH compounds increased linearly with increasing concentration of surfactant above critical micelle concentration. In addition, the oil concentration was another important factor in enhancing of solubilization of PAHs. With 250 mg L<sup>-1</sup> linseed oil, there was no difference between the weight solubilization ratio (WSR) values in microemulsions and Tween 80 alone systems; however, when the oil concentration increased to 500 mg L<sup>-1</sup> in microemulsions, the maximum WSR values for PHE and B[a]P were 0.0751 and 0.0133 which were about 1.6 to 2 fold higher than that of their Tween 80 counterparts. Furthermore, the microemulsions containing 1000 mg L<sup>-1</sup> linseed oil did not further increase the solubilization. Due to the structure of microemulsions, the WSR values reached a maximum and were constant even the extra amount of oil was added into the system.

The feasibility of using oil-in-water microemulsions to enhance the bioremediation of PHE and B[a]P in aqueous system was evaluated in the second phase. No additional benefit was observed on the biodegradation of PHE by adding microemulsions consisting 0.2% and 0.4% Tween 80 to aqueous system, which is likely due to the high aqueous solubility of PHE making it readily accessible for microbial degradation. The stimulatory effect of surface active agents on aqueous biodegradation was more pronounced with the higher molecular weight B[a]P. Additionally, the results clearly indicated that the removal rate of B[a]P in microemulsions was higher than that in their respective Tween 80 solution alone systems. And it is also worth to note that the removal rate of B[a]P increased with increasing oil content and shown a significant difference in both 0.2% and 0.4% Tween 80 microemulsion systems. However, a distinct inhibitory effect on PHE and B[a]P degradation was observed in the presence of 0.4% Tween 80 solution, which reduced the removal rate to half as that of control group, only 34.4% and 18.6% removal of PHE and B[a]P, respectively. This might be due to surfactants which might reduce the cell surface hydrophobicity of *Bacillus subtilis* (BUM) and as a result, might inhibit the uptake of hydrophobic pollutants. Microemulsions increased the solubilization of PAHs and might simultaneously suppress the negative effects of Tween 80. Therefore, microemulsions could successfully enhance the solubilization of PAHs and subsequently benefit to the biodegradation.

In the third phase of this study, a series of batch desorption experiments using both microemulsions and Tween 80 solutions was carried out to evaluate their effect on PAHs availability in soil-water system. Various concentrations of surfactant/cosurfactant and oil were used to evaluate their individual influences on the desorption of PHE and B[a]P. Microemulsions did not show an advantage over Tween 80 solutions on the desorption efficacies for PHE. However, a distinct inhibitory effect on B[a]P desorption was observed in the presence of microemulsions. At high concentrations of linseed oil ( $500 \text{ mg L}^{-1}$ ), the desorption rate of B[a]P declined to zero. It might reveal that the high amount of oil in microemulsions sorbed onto soil and provided potential sorptive sites for PAHs especially for high octanol-water partition coefficient ( $K_{ow}$ ) compounds which have high affinity to oil. These results clearly showed that the influences of microemulsions in desorption were completely different from that of solubilization for both PHE and B[a]P. The presence of soil affected the distribution behaviours of both PHE and B[a]P. Therefore, sorption of the individual component in microemulsions should be further investigated to provide more information on the feasibility of using microemulsions as soil washing agents.

The aim of the fourth phase was to develop an innovative bioremediation technique for PAHs contaminated soil by enhancing composting remediation efficiency through addition of microemulsions. In order to gain a better insight into this PAHs remediation strategy, fates and availabilities of PAHs in soil were also investigated. The results demonstrated that pig manure amendment facilitated the removal of both PHE and B[a]P in batch composting study for a period over 56 days. In the soil control without any amendments, the removal rates of PHE and B[a]P were 49.3% and 29.7%, respectively. With the addition of pig manure, PHE could be completely removed within 35 days, whereas 41.5% of B[a]P was removed in 56 days. The combined application of pig manure and surface active agents led to a more extensive removal rate, as 72.0% and 55.5% of B[a]P could be removed in the presence of microemulsion and Tween 80, respectively. However, the addition of microemulsion and Tween 80 did not cause any additional advantage on the biodegradation of PHE because of its comparative higher aqueous solubility. Moreover, a sequential extraction mass balance study on the species of PAHs demonstrated that biodegradation was the predominant means for both PHE and B[a]P removal in soil composting treatment whereas sequestration of PHE and B[a]P to organic matter was insignificant. In the presence of pig manure, the PHE in non-desorbing and bound residual fractions could be released as desorbing fraction for microbial attack. The present results concluded that microemulsion could enhance the biodegradation of PAHs, especially B[a]P in composting process and sorption to the organic fraction played a very minor role. Overall, this work will contribute to significant benefits for risk assessment, and development of remediation strategies for contaminated sites.

It can be concluded that the oil-in-water microemulsions formed by Tween 80, linseed oil and 1-pentanol is a promising alternative to Tween 80 alone system in enhancing the bioremediation of soil contaminated by PAHs under composting condition.

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