CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Mechanisms of humic substances (HS) on enzymatic degradation rate of PCP and phenanthrene was first elucidated in this study. Even though degradation ability of enzyme to degrade the model aromatic pollutants (Ullah et al., 2000; Chupungars et al., 2008) as well as inhibitory effects of HS (Gianfreda and Bollag, 1994; Zavarzina et al., 2004) and sorption efficiency of HS to the pollutants (Paolis and Kukkonen, 1997; Schellenberg, 1984; Peuravuori et al., 2001; Salloum et al., 2002; Vacca, 2005) have been reported by many researches, no one has ever experimented on enzyme kinetics using HS as inhibitors for PCP and phenanthrene degradation by ligninolytic enzyme. Moreover, the mechanism by which HS influence enzymatic degradation rate has never been elucidated.

In part 1 experiment, PCP was used as a model substrate for enzymatic degradation using purified laccase from *Trametes versicolor*. Aldrich humic acid (AHA), Leonardite humic acid (LHA), Suwannee River fulvic acid (SRFA), and Waskish peat fulvic acid (WFA) were used as HS representatives. We found that HS showed inhibitory effect for laccase enzyme on PCP degradation rate. As HS concentrations were increased, PCP degradation rates were decreased. Followed Michaelis-Menten equation, the inhibition of enzyme fit to a competitive type model. However, HS could not inactivate the enzyme. Binding experiment showed PCP could be sorbed into HS. The calculated degradation rate, computed from freely dissolved PCP available after bound to HS, was less than the experimental degradation rate of PCP. This could imply that enzyme has the ability to degrade not only PCP but also HS, which existed in the system. We also found that HS reacted

with laccase and 465 – nm absorption of the mixture of HS – laccase was decreased during the reaction time. As a result, HS could compete with PCP for enzyme degradation. It confirmed the results of which HS exhibited competitive inhibition to enzyme and HS could be substrates for the enzyme. PCP degradation rate was dependent on nature and characteristics of HS. The higher aromatic groups and molecular weight of HS showed more inhibitory and binding efficiencies.

Later studies of phenanthrene enzymatic degradation rate using crude ligninolytic enzymes from *Agrocybe* sp. CU 43 and the same HS along with a dissolved organic matter (DOM) from paddy field soil was investigated. In this part, we studied phenanthrene rather than PCP because our preliminary study found that PCP could not be a substrate for the crude fungal enzyme while phenanthrene showed an obvious degradable result. HS and DOM showed the inhibitory effect either by competitive, uncompetitive, or linear mix inhibition types, for phenanthrene degradation. Once we imitated a complicated nature by using crude fungal enzyme and various types of HS and DOM from rice paddy field soil, we found several types of inhibition effects for phenanthrene degradation. The different inhibition types for the complex samples was expected because crude fungal enzymes comprised of various types of ligninolytic enzymes such as laccase, lignin peroxidase, and manganese peroxidase (Chupungars et al., 2008). As the concentration of HS and DOM were increased, enzymatic degradation rates of phenanthrene were decreased. HS and DOM could not inactivate the enzyme. Moreover, phenanthrene showed that it could be sorbed into HS and DOM. The ratio less than 1 was found for calculated degradation rate using unbound phenanthrene concentration and the experimental rate of enzymatic degradation. Therefore, the enzyme was not only able to degrade freely unbound phenanthrene but it also had to degrade analogous substrate, which was HS

or DOM, resulting lower experimental phenanthrene degradation rate than the calculated rate. HS and DOM could cause the inhibitory effect because they could bind to phenanthrene and protect the contaminant from enzymatic degradation as well as HS and DOM could act as substrates for ligninolytic enzymes. Physicochemical properties of HS, especially their aromatic functional groups and molecular weight, could influence the inhibitory effects and binding capacities.

From our results, we proposed the model that once aromatic pollutants enter the environment including HS, the pollutants would be firstly sorbed by HS and the bound pollutants would not readily released from HS. Then, the free pollutants were bioavailable and degraded by the enzymes, while HS could compete for pollutant enzymatic degradation. As a consequence, enzymatic degradation rate of pollutants were slower due to the presence of HS. Nature and extent of HS such as types and % aromaticity of HS could significantly affect on binding and inhibitory effect. Both PCP and phenanthrene showed HS' percent aromaticity was vulnerable to sorption capacities of the pollutants.

Our findings suggested that transformation of aromatic pollutants in nature including soil and water ecosystems were affected by the presence of HS. Sorption of aromatic pollutants to humic and fulvic acids may alter their reactivity in the environment. The reactivity such as bioavailability and biotransformation may be decreased. Because HS are ubiquitous in nature, accurate determinations of the pollutants – HS substance in term of binding coefficient and enzymatic degradation rate are of essential importance in assessing the fate and transport of aromatic pollutants in terrestrial and aquatic systems. The actual bioremediation needed to consider amount and characteristics of HS exist in the site because HS could be a remediation hindrance and extend the remediation time. For bioremediation

achievement, HS characteristics such as % aromaticity and molecular weight should be essentially studied.

Moreover, our findings also suggested that binding efficiency was related to HS' aromaticity and molecular weight in some extent. In particular, those HS' characteristics could control the rate of enzymatic pollutant degradation. Therefore, this information benefits the knowledge that binding capability of HS to pollutants could imply the slower enzymatic degradation rate. Types and characteristics of pollutants to be remediated are in addition important. Our results showed that degradation rates of PCP were higher than those of phenanthrene at the same HS concentration addition. The higher degradation rate of PCP was mainly due to the less ability of HS to sorb PCP. We found the lower K_{dom} of PCP than phenanthrene for all types of HS. In this case, we calculated the K_{dom} values of phenanthrene by using phenanthrene concentration in water phase equal to the chemical solubility (1 mg/L) so that sorption efficiency of two model compounds could be compared. Significantly higher phenanthrene binding (49.15%) than PCP binding (22.53%) to soil has been reported by Saleem, et al. (1998) in aquifer material, consisting of geological material and water in Libby, Montana, Superfund site. Degradation rate constants and log K_{dom} of PCP and phenanthrene were shown in Table 5.1. Therefore, for actual bioremediation we might experiment on binding isotherms to predict comparable degradation among the remediation sites. Dissolved organic matter which is a component of HS is also important in bioremediation. For example, in application of wastewater treatment by enzymes, environmental conditions and species or strains of microbes should be well studied to specific problems. Wastewater DOM is highly heterogeneous in size and chemical composition as reported by Imai et al. (2000). Determination of DOM chemical structure is difficult due to of this complex

composition. Preliminary study of sorption efficiency might be introduced to be better understanding of the treatment strategies.

For further study, the characteristics of DOM such as % aromaticity and molecular weight are needed to be analyzed. Their uniqueness is important to predict the binding efficiency and inhibitory effect in enzymatic degradation of the pollutants. Moreover, HS physicochemical properties can be used to compare the potential of remediation approach among different bioremediation sites. For example, the next research may conduct experiment on PAHs with different K_{ow}, and HS with different % aromaticity and find the relationship of enzymatic degradation rate and these particular factors.

Table 5.1 Degradation rate constants and sorption of PCP and phenanthrene to 10 mg/L of HS

HS	Degradation rate constant (hr^{-1})		Log K_{dom}	
	PCP	Phenanthrene	PCP	Phenanthrene
Without HS	2.23×10^{-1}	2.23×10^{-3}	-	-
AHA	1.62×10^{-1}	1.05×10^{-3}	4.65	5.05
LHA	4.77×10^{-2}	9.63×10^{-4}	4.81	5.08
SRFA	1.35×10^{-1}	1.27×10^{-3}	4.09	4.91
WFA	1.09×10^{-1}	1.13×10^{-3}	4.56	4.93