REFERENCES

Aiken G. R., Mcknight, D.M., Wershaw, R.L., eds. Humic Substances in Soil, Sediment, and Water, Geochemistry, Isolation and Characterization. New York: Wiley, 1985.

Alexander, M. 1995. How toxic are toxic chemicals in soil? Environmental Science and Technology 29: 2713 – 2717.

Ashley, J.T.F. 1996. Adsorption of Cu (II) and Zn (II) by estuarine, riverine and terrestrial humic acids. Chemosphere 33: 2175 – 2187.

Aust, S.D. 1990. Degradation of environmental pollutants by *Phanerochaete chrysosporium*. Microbial Ecology 20: 197 – 209.

Barr, D.P., Aust, S.D. 1994. Pollutant degradation by white rot fungi. Reviews in Environmental Contamination and Toxicology 13: 49 – 72.

Beckett, R., Jue, Z., Giddings, J. 1987. Determination of molecular weight distributions of fulvic and humic acids using flow field-flow fractionation. Environmental Science and Technology 21: 289 – 295.

Bengtsson, G., Zerhouni, P., 2003. Effects of carbon substrate enrichment and DOC concentration on biodegradation of PAHs in soil. Journal of Applied Microbiology 94: 608 – 617.

Berry, D.F., Boyd, S.A., 1985. Decontamination of soil through enhanced formation of bound residue. Environmental Science and Technology 19: 1132 – 1133.

Blumer, M. 1976. Polycyclic aromatic compounds in nature. Scientific American 234: 34 – 45.

Bollag, J. 1992. Decontaminating soil with enzymes: an in situ method using phenolic and anilic compounds. Environmental Science and Technology 26: 1876 – 1881.

Bollag, J., Dec, J., Huang, P.M. 1997. Formation mechanisms of complex organic structures in soil habitats. Advance Agronomy 63: 237 – 266.

Boominathan, K., Reddy, C.A. 1992. Fungal degradation of lignin: biotechnological applications. In D.K. Arora, R.P. Elander, K.G. Muderji (eds.), Handbook of Applied Mycology Vol. 4, pp. 763 – 822. New York: Marcel Dekker.

Bumpus, J.A., Tien, M., Wright, D., Aust, S.D. 1985. Oxidation of persistent environment pollutants by white-rot fungi. Science 228: 1434 – 1436.

Butte, W., Denker, J., Kirsch, M., Hopner, T. 1985. Pentachlorophenol and tetrachlorophenols in wadden sediment and clams Myaarenaria of the Jadebusen after a 14-year period of wastewater discharge containing pentachlorophenol. Environmental Pollution (Series B) 9: 29 – 39.

Calderbank, A. 1989. The Occurrence and Significance of Bound Pesticides Residues in Soil. Environmental Contamination and Toxicology 108: 71 – 103.

Cerniglia, C.E. 1992. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 331 – 338.

Cerniglia, C.E., Heitkamp, M.A. 1989. Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. In U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment, pp. 41 – 68. Boca Raton: CRC Press.

Chefetz, B., Deshmukh, A.P., Hatcher, P.G., Guthrie, E.A. 2000. Pyrene sorption by natural organic matter. Environmental Science and Technology 34: 2925 – 2930.

Chien, Y.Y., Bleam, W.F. 1997. Fluorine-19 nuclear magnetic resonance study of atrazine in humic acid and sodium dodecyl sulfate micelles swollen by polar and nonpolar solvent. Langmuir 13: 5283 – 5288.

Chien, Y.Y., Kim, E.G., Bleam, W.F. 1997. Paramagnetic relaxation of atrazine solubilized by humic micellar solutions. Environmental Science and Technology 31: 3204 – 3208.

Chin, Y.P., Aiken, G., O'Loughlin, E. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environmental Science and Technology 28: 1853 – 1858.

Chin, Y.P., Aiken, G.R., Danielsen, K.M. 1997. Binding of pyrene to aquatic and commercial humic substances: the role of molecular weight and aromaticity. Environmental Science and Technology 31: 1630 – 1635.

Choi, J., Aomine, S. 1974. Adsorption of pentachlorophenol by soils. Soil Science and Plant Nutrition 20: 135 – 144.

Chupungars, K. 2008. Isolation and characterization of polycyclic aromatic hydrocarbon degrading fungi. Doctoral dissertation. Biotechnology Faculty of Science Chulalongkorn University.

Clapp, C.E., Mingelgrin, U., Liu, R., Zang, H., Hayes, M.H.B. 1997. A quantitative estimation of the complexation of small organic molecules with soluble humic acids. Journal of Environmental Quality 26: 1277 – 1281.

Claus, H., Filip, Z. 1988. Behaviour of phenoloxidases in the presence of clays and other soil-related adsorbents. Applied Microbiology and Biotechnology 28: 506 – 511.

Claus, H., Filip, Z. 1990. Effects of clays and or other solids on the activity of phenoloxidases produced by some fungi and actinomycetes. Soil Biology and Biochemistry 22: 483 – 488.

Cozzolino, A., Piccolo, A. 2002. Polymerization of dissolved humic substances catalyzed by peroxidase: effects of pH and humic composition. Organic Geochemistry 33: 281 – 294.

Crosby, D.G. 1981. Environmental chemistry of pentachlorophenol. Pure Applied Chemistry 53: 1051 – 1080.

Cullen, D., Kersten, P. 1992. Fungal enzymes for lignocellulose degradation. In J.R. Kinghorn, G. Turner (eds.), Applied Molecular Genetics of Filamentous Fungi. Glasgow: Blackie Academic and Professional (Chapman and Hall).

Davis, S., Burns, R.G. 1990. Decolorization of phenolic effluents by soluble and immobilized phenol oxidases. Applied Microbiology and Biotechnology 32: 721 – 726.

Dębska, B., Maciejewska, A., Kwiatkowske, J. 2002. The effect of fertilization with brown coal on Haplic Luvisol humic acids. Rostlinná výroba 48: 33 – 39.

Dec, J., Bollag, J. 1997. Determination of covalent and non-covalent binding interactions between xenobiotic chemicals and soil. Soil Science 162: 858 – 874.

Delaune, R.D., Gambell, R.P., Reddy, K.S. 1983. Fate of pentachlorophenol in estuarine sediment. Environmental Pollution (Series B) 6: 297 – 308.

Dercová K., Sejáková, Z., Skokanová, M., Barančíková, G., Makovníková, J. 2007. Bioremediation of soil contaminated with pentachlorophenol (PCP) using humic acids bound on zeolite. Chemosphere 66: 783 – 790.

EPA (US Environmental Protection Agency). 1988. 2,4-D. Reviews in Environmental Contamination and Toxicology 104: 63 – 72.

Essington, M.E. 2004. Organic matter in soil. In Soil and water chemistry: An integratic approach, pp 129 – 181. New York: CRC Press.

Extoxnet (Extension Toxicology Network). 2008. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/metirampropoxur/pentachlorophenol-ext.html

Fakoussa, R.M., Frost, P.J. 1999. In vivo-decolorization of coal-derived humic acids by laccase-excreting fungus *Trametes vesicolor*. Applied Microbiology and Biotechnology 52: 60 – 65.

Fernando, T., Aust, S.D. 1994. Biodegradation of toxic chemicals by white rot fungi. In G.R. Chaudhry (ed.), Biological Degradation and Bioremediation of Toxic Chemicals, pp. 386 – 402. London: Chapman & Hall.

Fersht A. 1985. Enzyme Structure and Mechanism, 2nd edition, W.H. Freeman, New York.

Fitzgibbon, F.J., Nigam, P., Singh, D., Marchant, R. 1995. Biological treatment of distillery waste for pollution remediation. Journal of Basic Microbiology 5: 293 – 301.

Fuzzi, S., Zappoli, S. 1996. The organic component of fog droplets. In: Proceedings of the 12th International Conference on Cloud and Precipitation, pp. 1077 – 1079. Zurich, Switzerland.

Gadad, P., Hongxia, L., Nanny, M.A. 2007. Characterization of noncovalent interactions between 6-propionyl-2-dimethylaminonaphthalene (PRODAN) and dissolved fulvic and humic acids. Water Research. In Press.

Garbarini, D.R., Lion, L.W. 1986. Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environmental Science and Technology 20: 1263 – 1269.

Gauthier, T.D., Seitz, W.R., Grant, C.L. 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene K_{oc} values. Environmental Science and Technology 21: 243 – 248.

Gianfreda, L., Bollag, J.M. 1994. Effect of soils on the behavior of immobilized enzymes. Soil Science Society of America Journal 58: 1672 – 1681.

Grathwohl, P. 1990. Influence of organic matter from soils and sediments from various origins on the sorption of some chlorinated aliphatic hydrocarbons: Implications on K_{oc} correlations. Environmental Science and Technology 24: 1687 – 1693.

Hammel, K.E. 1992. Oxidation of aromatic pollutants by lignin-degrading fungi and their extracellular peroxidases. In H. Siegel, A. Siegel (eds.), Metal Ions in Biological Systems, pp. 41 – 60. New York: Marcel Dekker.

Hammel, K.E., Gai, W.Z., Green, B., Moen, M.A. 1992. Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 58: 1832 – 1838.

Han, M.J., Choi, H-T., Song, H-G. 2004. Degradation of phenanthrene by *Trametes versicolor* and its laccase. The Journal of Microbiology 42: 94 – 98.

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiology Reviews 13: 125 – 135.

Havers, N., Burba, P., Lampert, J., Klowkow, D. 1998. Spectroscopic characterization of humic-like substances in airborne particulate matter. Journal of Atmospheric Chemistry 29: 45 – 54.

Holman, H.Y.N., Nieman, K., Sorensen, D.L., Miller, C.D., Martin, M.C., Borch, T., McKinney, W.R., Sims, R.C. 2002. Catalysis of PAH biodegradation by humic

acid shown in synchrotron infrared studies. Environmental Science and Technology 36: 1276 – 1280.

Huynh, V.B., Chang, H.M., Joyce, T.W., Kirk, K. 1985. Dechlorination of chloroorganics by a white rot fungus. Technical Association of the Pulp Paper Industry 68: 98 – 102.

Imai, A., Fukushima, T., Matsushige, K., Kim, Y.H., Choi, K. 2002. Characterization of dissolved organic matter in effluents from wastewater treatment plants. Water Research 36: 859 – 870.

International Humic Substances Society. 2008. Available from: http://www.ihss.gatech.edu

Itoh, K., Fujita, M., Kumano, K., Suyama, K., Yamamoto, H. 2000. Phenolic acids affect transformations of chlorophenols by *Coriolus versicolor* laccase. Soil Biology and Biochemistry 32: 85 – 91.

Kang, K.H., Dec, J., Park, H.G., Bollag, J.M. 2002. Transformation of the fungicide cydrodinil by a laccase of *Trametes villosa* in the presence of phenolic mediators and humic acids. Water Research 36: 4907 – 4915.

Katase T., Bollag, J.M. 1991. Transformation of trans-4-hydroxycinnamic acid by a laccase of the fungus *Trametes versicolor* – its significance in humification. Soil Science 151: 291 – 296.

Keith, L.H., Telliard, W.A. 1979. Priority pollutants: I. A Prospective View. Environmental Science and Technology 13: 416 – 423.

Kirk, T.K., Farrell, R.L. 1987. Enzymatic 'combustion': the microbial degradation of lignin. Annual Review of Microbiology 41: 465 – 505

Kirk, T.K., Lamar, R.T., Glaser, J.A. 1992. The potential of white rot fungi in bioremediation. In S. Mongkolsuk (ed.), Biotechnology and Environmental Science: Molecular Approaches, pp. 131 – 138. New York: Plenum Press.

Kiss, Gy., Tombácz, E., Varga, B., Alsberg, T., Persson, L. 2003. Estimation of the average molecular weight of humic-like substances isolated from fine atmospheric aerosol. Atmospheric Environment 37: 3783 – 3794.

Kitunen, V.H., Valo, R.J., Salkinoja-Salonen, M.S. 1987. Contamination of soil around wood-preserving facilities by polychlorinated aromatic compounds. Environmental Science and Technology 21: 96 – 101.

Klavins M, and Serzane J. 2000. Use of Humic Substances in Remediation of Contaminated Environments. In L.W. Donale, et al. (eds.), Bioremediation of contaminated soils, pp 217 – 235. New York: Marcel Dekker.

Knapp, J.S., Newby, P.S. 1999. The decolourisation of a chemical industry effluent by white rot fungi. Water Research 33: 575 – 577.

Kotterman, M.J.J., Vis, E.H., Field, J.A. 1998. Successive mineralization and detoxification of benzo(a)pyrene by the white rot fungus *Bjerkandera sp.* strain BOS55 and indigenous microflora. Applied Environmental Microbiology 64: 2853 – 2858.

Kriválcsy, Z., Kiss, Gy., Varga, B., Galambos, I., Sárvári, Zs., Gelencsér, A., Molnár, Á., Fuzzi, S., Facchini, M.C., Zappoli, S., Andraccio, A., Alsberg, T., Hansson, H.C., Persson, L. 2000. Study of humic-like substances in fog and interstitial aerosol by size-exclusion chromatography and capillary electrophoresis. Atmospheric Environment 34: 4273 – 4281.

Kumar, V., Wati, L., Nigam P., Banet, I.M., Yadav, B.S.,Singh, D. Marchant, R. 1998. Decolorization and biodegradation of anaerobically digested sugarcane molasses spent wash effluent from biomethanation plants by white-rot fungi. Process Biochemistry 33: 83 – 88.

Ladd, J.N., Butler, J.H.A. 1971. Inhibition by soil humic acids of native and acetylated proteolytic enzymes. Soil Biology and Biochemistry 3: 157 – 160.

Lamar, R.T., and Dietrich, D.M. 1990. In situ depletion of pentachlorophenol from contaminated soil by *Phanerochaete* spp. Applied and Environmental Microbiology 56: 3093 – 3100.

Lamar, R.T., Glaser J.A., and Kirk, T.K. 1990. Fate of pentachlorophenol in sterile soils inoculated with the white rot basidiomycete *Phanerochaete chrysosporium*: mineralization, volatilization and depletion of PCP. Soil Biology and Biochemistry 22: 433 – 440.

Lamar, R.T.,Glaser, J.A., and Evans, J.W. 1993. Solid-phase treatment of pentachlorophenol-contaminated soil using lignin-degrading fungi. Environmental Science and Technology 27: 2566 – 2571.

Lau, K.L., Tsang, Y.Y., Chui, S.W. 2003. Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52: 1539 – 1546.

MacCarthy, P. 2001. The principles of humic substances. Soil Science 166: 738 – 751.

MacCarthy, P., Rice, J.A. In S.H. Schneider, P.J. Boston (eds.), Proceedings of Chapman Conference on the Gaia Hypothesis, pp. 339 – 345. Cambridge: MIT Press.

Malcolm, R.T., MacCarthy, P. 1986. Limitation in the use of commercial humic acids in water and soil research. Environmental Science and Technology 20: 904 - 911.

Marangoni, A.G. 2003. Reversible enzyme inhibition. In Enzyme kinetics, a modern approach. New Jersey: John Wiley & Sons.

Mazzuoli, S., Loiselle, S., Hull, V., Bracchini, L., Rossi, C. 2003. The analysis of the seasonal, spatial, and compositional distribution of humic substances in a subtropical shallow lake. Acta hydrochimica et Hydrobiologica 31: 461 – 468.

Messerschmidt A. 1997. Multi-copper oxidases. World Scientific. Singapore.

Mileski, G.J., Bumpus, J.A., Jurek, M.A., Aust, S.D. 1988. Biodegradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 54: 2885 – 2889.

Moen, M.A., Hammel, K.E. 1994. Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Applied and Environmental Microbiology 60: 1956 – 1961.

Mougin, C., Jolivalt, C., Briozzo, P., Madzak, C. 2003. Fangal laccases: from structure-activity studies to environment applications. Environmental Chemistry Letters 1: 145 – 148.

Műller-Wegener, U. 1988. Interaction of humic substances with biota. In F.M. Frimmel and R.F. Christman (eds.), Humic substances and their role in the environment, pp. 179 – 192. John Wiley & Sons Limited.

Nam, K., Alexander, M. 1998. Role of nanoporosity and hydrophobicity in sequestration and bioavailability: tests with model solids. Environmental Science and Technology 32: 71 – 74.

Nanny, M.A., Bortiatynski, J.M., Jacqueline, M., Tien, M., Hatcher, P.G. 1996. Investigations of enzymatic alterations of 2,4-dichlorophenol using 13C-nuclear magnetic resonance in combination with site-specific 13C-labeling: understanding the environmental fate of this pollutant. Environmental Toxicology and Chemistry 15: 1857 – 1864.

O'Loughlin, E.J., Traina, S.J., Chin, Y.-P. 2000. Association of organotin compounds with aquatic and terrestrial humic substances. Environmental Toxicology and Chemistry 19: 2015 – 2021.

Olk, D.C. 2006. A chemical fractionation for structure-function relations of soil organic matter in nutrient cycling. Soil Science Society of America Journal 70: 1013 – 1022.

Pan, B., Xing, B., Liu, W., Xing, G., Toa, S. 2007. Investigating interactions of phenanthrene with dissolved organic matter: Limitations of Stern – Volmer plot. Chemosphere 69: 1555 – 1562.

Paolis, F.D., Kukkonen, J. 1997. Binding of organic pollutants to humic and fulvic acids: influence of pH and the structure of humic material. Chemosphere 34: 1693 – 1704.

Paszcynski, A., Crawford, R.L. 1995. Potential for bioremediation of xenobiotic compounds by white-rot fungus *Phanerochaete chrysosporium*. Biotechnology Progress 11: 368 – 379.

Peralta-Zamora, P., de Moraes, S.G., Esposito, E., Antunes, R., Reyes, J., Durán, N. 1998. Decolorization of pulp mill effluents with immobilized lignin and manganese peroxidase from Phanerochaete chrysosporium. Environmental Technology 19: 521 – 528.

Pérez, J., De la Rubia, T., Hamman, O.B., Martínez, J. 1998. *Phanerochaete flavido-alba* laccase induction and modification of manganese peroxidase isoenzyme pattern in decolorized olive oil mill waste waters. Applied and Environmental Microbiology 64: 2726 – 2729.

Perminova, I.V., Frimmel, F.H., Kovalevskii, D.V., Abbt-Braun, G., Kudryavtsev, A.V., Hesse, S. 1998. Development of a predictive model for calculation of molecular weight of humic substances. Water Research 32: 872 – 881.

Perminova, I.V., Frimmel, F.H., Kudryavtsev, A.V., Kulikova, N.A., Abbt-Braun, G., Hesse, S., Petrosyan, V.S. 2003. Molecular weight characteristics of humic substances from different environments as determined by size exclusion chromatography and their statistical evaluation. Environmental Science and Technology 37: 2477 – 2485.

Perminova, I.V., Grechishcheva, N.Y., Petrosyan, V.S. 1999. Relationship between structure and binding affinity of humic substances for polycyclic aromatic hydrocarbons: relevance of molecular descriptors. Environmental Science and Technology 33: 3781 – 3787.

Peuravuori, J., Paaso, N., Pihlaja,K. 2001. Sorption of pentachlorophenol on lake aquatic humic matter. International Journal of Environmental Analytical Chemistry 79: 37 – 51.

Pointing, S.B., Bucher, V.V.D., Vrijmoed, L.L.P. 2000. Dye decolorization by sub-tropical basidiomycetous fungi and the effect of metals on decolorizing ability. World Journal of Microbiology and Biotechnology 16: 199 – 205.

Prasad, G.K., Gupta, R.K. 1997. Decoloruization of pulp and paper mill effluent by two white-rot fungi. Indian Journal of Environmental Health 39: 89 – 96.

RAIS (The Risk Assessment Information System). 2008. Available from: http://rais.ornl.gov/tox/profiles/phenanthrene_c_V1.shtml

Reddy, C.A. 1993. An overview of the recent advances on the physiology and molecular biology of lignin peroxidases of *Phanerochaete chrysosporium*. Journal of Biotechnology 30: 91 – 107.

Reddy, C.A. 1995. The potential for white-rot fungi in the treatment of pollutants. Current Opinion in Biotechnology 6: 320 – 328.

Reddy, C.A., D'Souza, T.M. 1994. Physiology and molecular biology of the lignin peroxidases of *Phanerochaete chrysosporium*. FEMS Microbiological Reviews 13: 137 – 152.

Reddy, C.A., Mathew, Z. 2001. Bioremediation potential of white rot fungi. In G.M. Gadd (ed.), Fungi in bioremediation, pp. 52 – 78. Cambridge: Cambridge University Press.

Reddy, G.V.B., Gold, M.H. 2000. Degradation of pentachlorophenol by *Phanerochaete chrysosporium*: intermediates and reactions involved. Microbioloby 146: 405 – 413.

Reinhammer, B. 1984. In R. Lonthe (ed.), Copper proteins and copper enzymes V.III, pp. 1 – 35. New York: CRC Press.

Roy-Arcand, L. Archibald, F.S. 1991. Direct dechlorination of chlorophenolic compounds by laccases from *Trametes (Coriolus) versicolor*. Enzyme Microbiology and Technology 13: 194 – 203.

Ruggiero, P., Radogna, V.M. 1984. Properties of laccase in humus-enzyme complexes. Soil Science 138: 74 – 87.

Sack, U., Heinze, T.M., Deck, J., Cerniglia, C.E., Rainer, M., Zadrazil, F., Fritsche, W. 1997. Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Applied and Environmental Microbiology 63: 3919 – 3925.

Saleem, A.M., Darwin, L.S., Ronald, C.S., Judith, L.S. 1998. Pentachlorophenol and phenanthrene biodegradation in creosote contaminated aquifer material. Chemosphere 37: 103 – 111.

Salloum, M.J., Chefetz, B., Hatcher, P.G. 2002. Phenanthrene sorption by aliphatic-rich natural organic matter. Environmental Science and Technology 36: 1953 – 1958.

Salloum, M.J., Dudas, M.J., McGill, W.B. 2001a. Variation of 1-naphthol sorption with organic matter fractionation: The role of physical conformation. Organic Geochemistry 32: 709 – 719.

Sayadi, S., Ellouz, R. 1992. Decolourization of olive mill waste-waters by the white-rot fungus *Phanerochaete chrysosporium*: involvement of the lignin-degrading system. Applied Microbial Technology 37: 813 – 817.

Scheel, T., Hofer, M., Ludwig, S., Holker, U. 2000. Differential expression of manganese peroxidase and laccase in white-rot fungi in the presence of manganese or aromatic compounds. Applied Microbiology and Biotechnology 54: 686 – 691.

Schellenberg, K., Leuenberger, C., Schwarzenbach, R.P. 1984. Sorption of chlorinated phenols by natural sediments and aquifer materials. Environmental Science and Technology 18: 652 – 657.

Schliephake, K., Leonergan, G.T., Jones, C.L., Mainwaring, D.E. 1993. Decolourisation of a pigment plant effluent by *Pycnoporus cinnabarinus* in a packed-bed bioreactor. Biotechnology Letters 15: 1185 – 1188.

Schnitzer, M. 1978. Humic substances: Chemistry and Reactions. In M. Schnitzer, S.U. Khan (eds.), Soil Organic Matter. Amsterdam: Elsevier.

Schoemaker, H. E., Harvey, P.J., Bowen, R.M., Palmer, J.M. 1985. On the mechanisms of enzymatic lignin breakdown. FEBS Letters 183: 7 – 12.

Schulten, H.-R. 1996. Three dimensional, molecular structures of humic acids and their interaction with water and dissolved contaminants. International Journal of Environmental Analytical Chemistry 64: 147 – 162.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M. 1993. Environmental organic chemistry. New York: John Wiley & Sons.

Shinozuka, T., Shibata, M., Yamagushi, T. 2004. Molecular weight characterization of humic substances by MALDI-TOF-MS. Journal of the Mass Spectrometry Society of Japan 52(1): 29 – 32.

Solloum, M.J., Dudas, M.J., McGill, W.B. 2001b. Sorption of 1-naphthol to soil and geologic samples with varying diagenetic properties. Chemosphere 44: 779 – 787.

Spadaro, J.T., Renganathan, V. 1994. Peroxidase-catalyzed oxidation of azo dyes: mechanism of disperse yellow 3 degradation. Archives of Biochemistry and Biophysics 312: 301 – 307.

Srinivasan, S.V., Murthy, D.V.S. 1999. Colour removal from bagasse-based pulp mill effluent using a white rot fungus. Bioprocess Engineering 21: 561 – 564.

Stevenson, F.J. 1994. Humus Chemistry: Genesis, Composition, Reaction. New York: John Wiley & Son.

Sutherland, J.B., Selby, A.L., Freeman, J.P., Evans, F.E. 1991. Metabolisms of phenanthrene by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology 57: 3310 – 3316.

Tan, K.H. 2003. Humic matter in soil and the environment, Principles and controversies, pp. 75 – 126. New York: Marcel Dekker.

Thailand Pesticides Database. 2006. Available from www.ipmthailand.org/en/Pesticides/Pesticides.asp

Theophais, G.D., Triantafllos, A.A., Tetrakis, D.E., Pomonis, P.J. 1998. Removal of chlorinated phenols from aqueous solutions by adsorption on alumina pillared clays and mesoporous alumina aluminum phosphates. Water Research 32: 295 – 302.

Thorn, K.A., Folan, D.W., MacCarthy, P. 1989. Characterization of the International Humic Substnaces Society standard and reference fulvic and humic acids by solution state carbon-13 (^{13}C) and hydrogen-1 (^{1}H) nuclear magnetic resonance spectrometry. In Water Resources Investigations Report, pp. 89 – 4196. U.S. Geological Survey, Denver, CO.

Thurston, C.F. 1994. The structure and function of fungal laccase. Microbiology 140: 19 – 26.

Uhle, M.E., Chin, Y-P., Aiken, G.R., McKnight, D.M. 1999. Binding of polychlorinated biphenyls to aquatic humic substances: The role of substrate and sorbate properties on partitioning. Environmental Science and Technology 33: 2715 – 2718.

Ullah, M.A., Bedford, C.T., Evans, C.S. 2000. Reactions of pentachlorophenol with laccase from *Coriolus versicolor*. Applied Microbiology and Biotechnology 53: 230 – 234.

United States Department of Agriculture, Natural Resources Conservation Service. Available from: http://soils.usda.gov/technical/classification/taxonomy/

Vacca, D.J., Bleam, W.F., Hickey, W.J. 2005. Isolation of soil bacteria adapted to degrade humic acid-sorbed phenanthrene. Applied and Environmental Microbiology 71: 3797 – 3805.

Wang, C.J., Thiele, S., Bollag, J.M. 2002. Interaction of 2,4,6-trinitrotoluene (TNT) and 4-amino-2,6-dinitrotoluene with humic monomers in the presence of

oxidative enzymes. Archives of Environmental Contamination and Toxicology 42: 1 – 8.

Wang, Y, Yu, J. 1998. Adsorption and degradation of synthetic dyes on the mycelium of *Trametes versicolor*. Water Science and Technology 38: 233 – 238.

Wikipedia, available on line: http://en.wikipedia.org/wiki/Ultisol

Wild, S.R., Harrad, S.J., Hones, K.C. 1992. Pentachlorophenol in the U.K. environment. I: A Budget and Source Inventory. Chemosphere 24: 833 – 845.

Wilson, S.C., Jones, K.C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environmental Pollution 81: 229 – 249.

Wondrack, L., Szano, M., Wood, W.A. 1989. Depolymerization of water soluble coal polymer from subbituminous coal and lignite by lignin peroxidase. Applied Biochemistry and Biotechnology 20: 765 – 779.

Xing, B., McGill, W.B., Dudas, M.J. 1994. Cross-correlation of polarity curves to predict partition coefficients of nonionic organic contaminants. Environmental Science and Technology 28: 1929 – 1933.

Yaropolov, A.I., Skorobogat'Ko, O.V., Vartanov, S.S., Varfolomeyev, S.D. 1994. Laccase. Properties, catalytic mechanism, and applicability. Applied Biochemistry and Biotechnology 49: 257 – 280.

Yavmetdinov, I.S., Stepanova, E.V., Gavrilova, V. P., Lokshin, B.V., Perminova, I.V., Koroleva, O.V. 2003. Isolation and characterization of humin-like substances produced by wood-degrading white rot fungi. Applied Biochemistry and Microbiology 39: 257 – 264.

Zappoli, S., Andracchio, A., Fuzzi, S., Facchini, M.C., Gelencser, A., Kiss, G., Krivacsy, Z., Molnar, A., Meszaros, E., Hansson, H. C., Rosman, K., Zebuhr, Y.

1999. Inorganic, organic and macromolecular components of fine aerosol in different areas of Europe in relation to their water solubility. Atmospheric Environment 33: 2733 – 2743.

Zavarzina, A.G., Demin, V.V., Nifant'eva, T.I., Shkinev, V.M., Danilova, T.V., Spivakov, B.Ya. 2002. Extraction of humic acids and their fractions in poly(ethylene glycol)-based aqueous biphasic systems. Analytica Chimica Acta 452: 95 – 103.

Zavarzina, A.G., Leontievsky, A.A., Golovleva, L.A., Trofimov, S.Y. 2004. Biotransformation of soil humic acids by blue laccase of *Panus tigrinus* 8/18. Soil Biology and Biochemistry 36: 359 – 369.

Zhang, F., Knapp, J.S., Tapley, K.N. 1998. Decolourisation of cotton bleaching effluent in a continuous fluidized-bed bioreactor using wood rotting fungus. Biotechnology Letters 20: 717 – 723.

Zhang, F., Knapp, J.S., Tapley, K.N. 1999. Decolourisation of cotton bleaching effluent with wood rotting fungus. Water Research 33: 919 – 928.

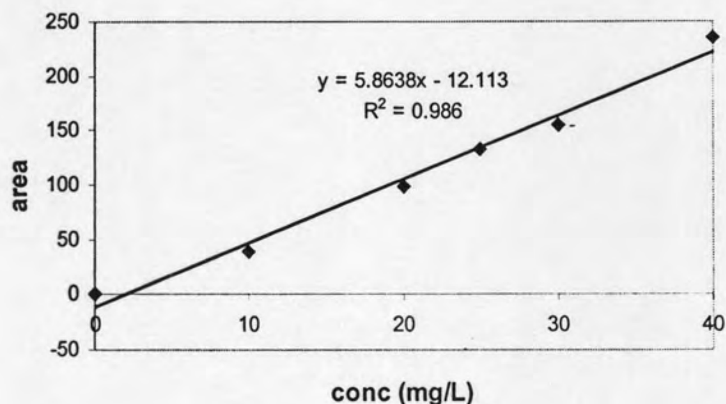
Zhang, L.H., D. Jenssen. 1994. Studies on intrachromosomal recombination in SP5/V79 Chinese hamster cells upon exposure to different agents related to carcinogenesis. Carcinogenesis 15: 2303 – 2310.

APPENDICES

APPENDIX A: Enzyme kinetics for PCP with and without HS as inhibitors

A.1 PCP calibration curve

PCP determination procedure provided calibration curve

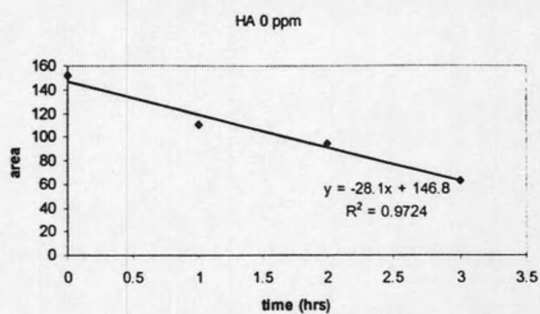


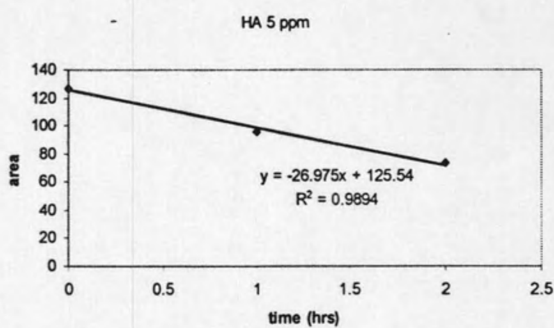
A.2 Determination of inhibitor concentration

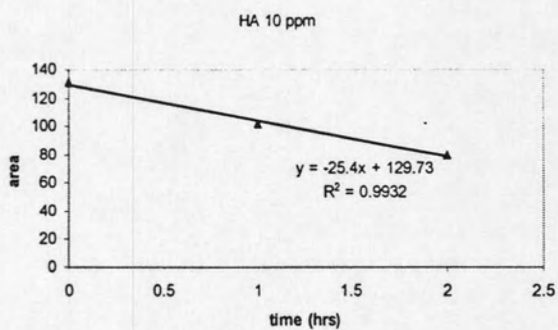
To find the amount of HS that yielded linear kinetics for PCP degradation. It was to preliminary experiment to select the HS concentration. PCP 30 mg/L plus laccase 20 units/mL at 5, 10, 15 mg/L of AHA and SRFA was used.

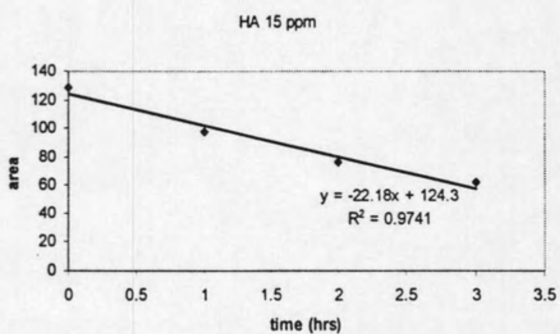
time (hrs)	0	1	2	3
PCP	151.5	109.9	94.1	63.1
PCP + AHA 5 mg/L	127.15	95.35	73.2	69.3
PCP + AHA 10 mg/L	130.95	101.9	80.15	76.5
PCP + AHA 15 mg/L	128.5	97.55	76.45	61.6
PCP + SRFA 5 mg/L	127.55	95	64	40
PCP + SRFA 10 mg/L	144.3	105.85	92.75	81.95
PCP + SRFA 15 mg/L	140.85	116.85	98.2	73.35

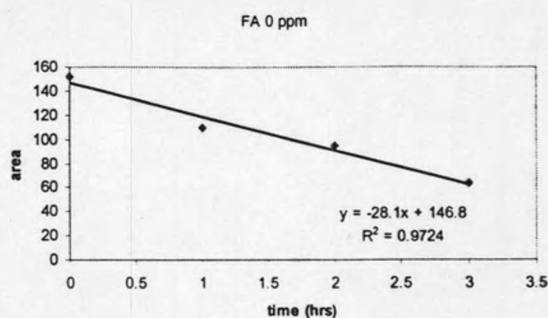
The relationship between area of the peak analyzed by GC-FID and time was plotted as followed.

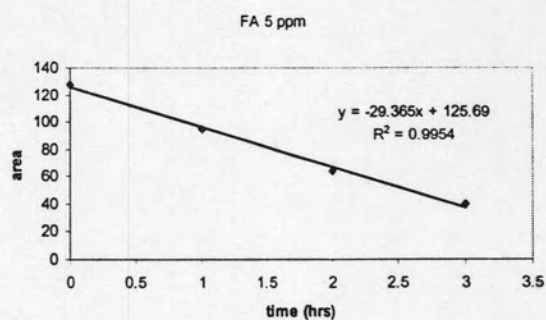

HA 0 ppm
160
140
120
100
80
60
40
20
0
area
y = -28.1x + 146.8
R² = 0.9724
0
0.5
1
1.5
2
2.5
3
3.5
time (hrs)

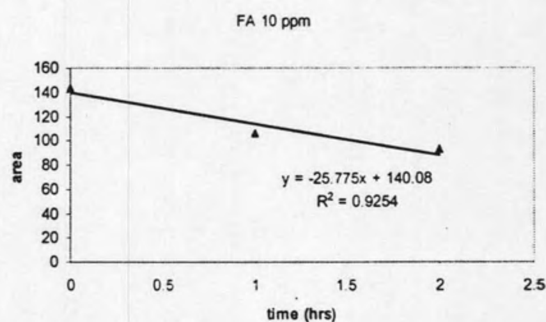

HA 5 ppm
140
120
100
80
60
40
20
0
area
y = -26.975x + 125.54
R² = 0.9894
0
0.5
1
1.5
2
2.5
time (hrs)

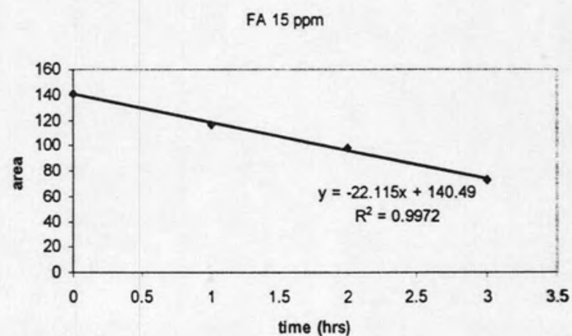

HA 10 ppm
140
120
100
80
60
40
20
0
area
y = -25.4x + 129.73
R² = 0.9932
0
0.5
1
1.5
2
2.5
time (hrs)


HA 15 ppm
140
120
100
80
60
40
20
0
area
y = -22.18x + 124.3
R² = 0.9741
0
0.5
1
1.5
2
2.5
3
3.5
time (hrs)

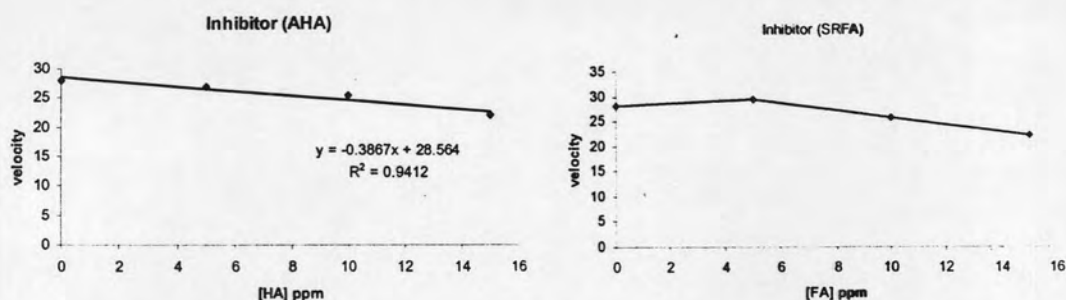








Then, the relationship between the velocity of enzyme for PCP degradation and concentration of HS that act as an inhibitor was plotted.



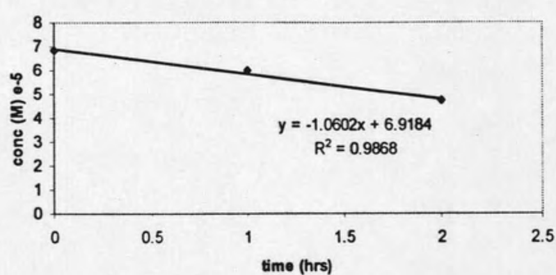
Therefore, 10 and 15 mg/L of HS were chosen for further analysis. Also, the extrapolate concentration of HS 20 mg/L was experimented.

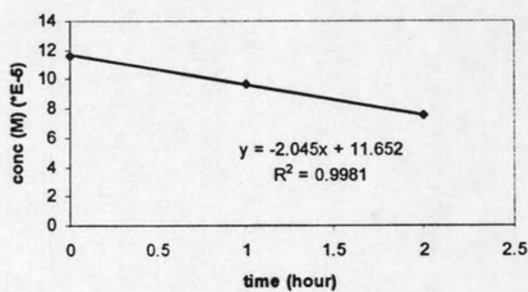
A.3 Enzyme kinetics for PCP

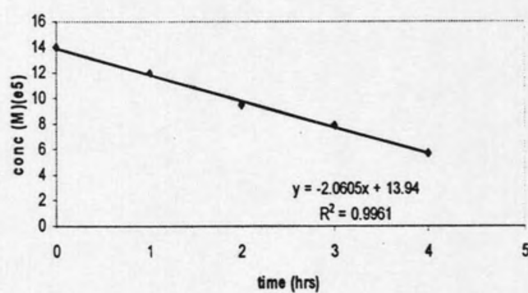
In the experiment, 10, 20, 25, 30, 40 mg/L of PCP were mixed with 20 units/mL of laccase and measured PCP concentration at time 0, 1, 2, 3, 4 hrs by GC-FID. The data and graph between their velocity and concentration are shown below.

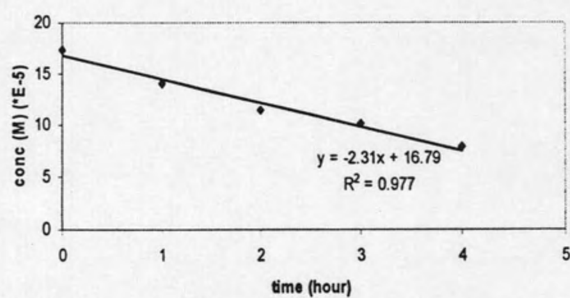
PCP concentration (M) at time 0 – 4 hrs, when 10, 20, 25, 40 mg/L of PCP were added as substrates.

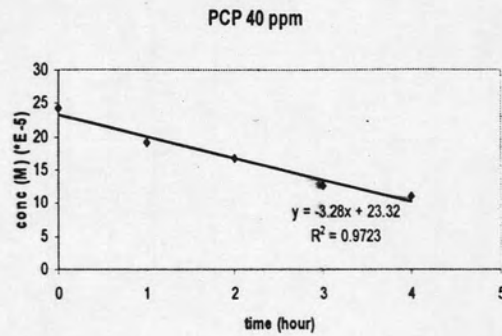
time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	6.8477E-5	0.000116	0.000140209	0.000174	0.000242
1	5.99952E-5	9.71E-05	0.000119489	0.000141	0.000192
2	4.72726E-5	7.51E-05	9.46493E-05	0.000114	0.000167
3	4.72726E-5	6.53E-05	7.93216E-05	0.000101	0.000126
4	5.14529E-5	6.25E-05	5.72689E-05	7.85E-05	0.000111


PCP 10 ppm
conc (M) e-5
time (hrs)
y = -1.0602x + 6.9184
R2 = 0.9868
0
1
2
3
4
5
6
7
8
0.5
1.5
2.5


PCP 20 ppm
conc (M) (*E-5)
time (hour)
y = -2.045x + 11.652
R2 = 0.9981
0
2
4
6
8
10
12
14
0.5
1
1.5
2
2.5

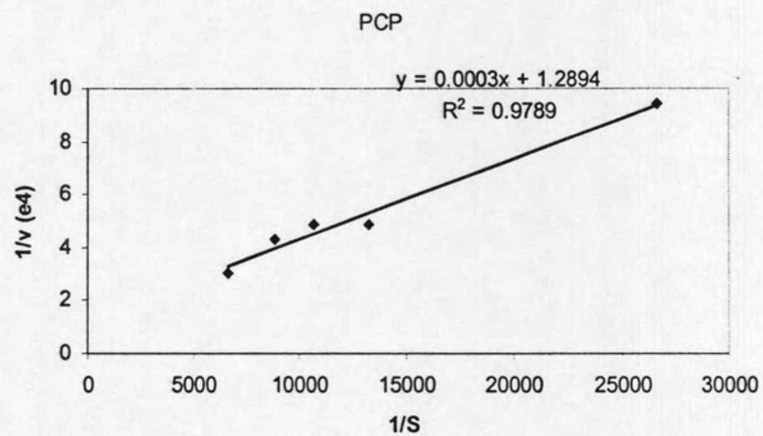

PCP 25 ppm
conc (M)(e5)
time (hrs)
y = -2.0605x + 13.94
R2 = 0.9961
0
2
4
6
8
10
12
14
16
1
3
5


PCP 30 ppm
conc (M) (*E-5)
time (hour)
y = -2.31x + 16.79
R2 = 0.977
0
5
10
15
20
1
2
3
4



Slope of each PCP concentration was its velocity. Then, k_m and V_{max} for PCP was determined by plotting 1/V and 1/S

1/S	1/v (e4)
26635	9.4321
13317.5	4.88998
10654	4.8532
8878.333	4.329
6658.75	3.04878



Calculation for K_m and V_{max}

y-intercept = $1/V_{max}$, y-intercept = 1.2894e4, $V_{max} = 1.16 \pm 0.19$ μM/min

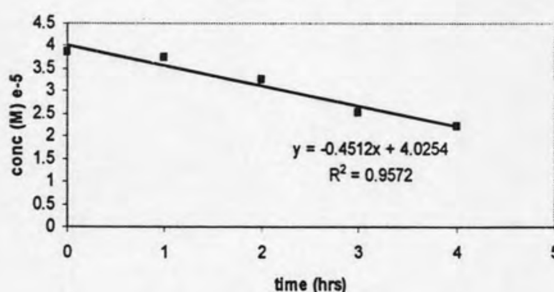
slope = K_m/V_{max}, slope = 0.003e4, $K_m = 0.21 \pm 0.03$ mM

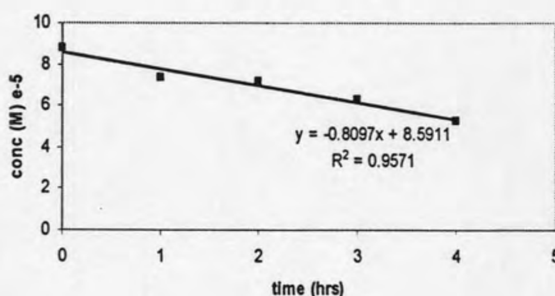
A.4 Enzyme kinetics for PCP when AHA 10 mg/L was added as an inhibitor

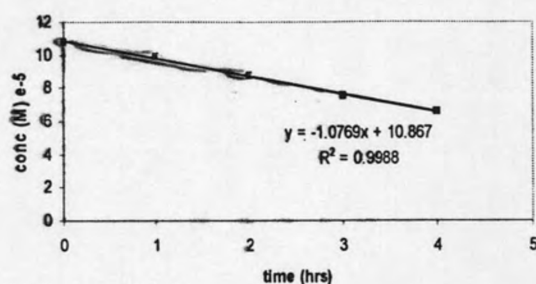
In the experiment, 10, 20, 25, 30, 40 mg/L of PCP and 10 mg/L of AHA were mixed with 20 units/mL of laccase and measured PCP concentration at time 0, 1, 2, 3, 4 hrs by GC-FID. The data and graph between their velocity and concentration are shown below.

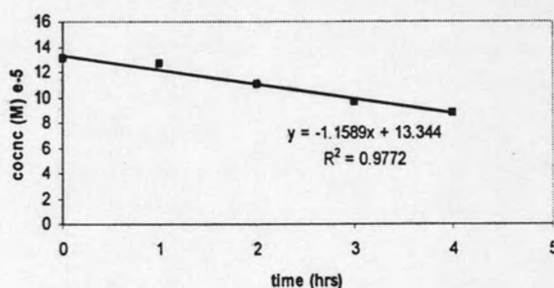
PCP concentration (M) at time 0 – 4 hrs, when 10, 20, 25, 40 mg/L of PCP were added as substrates and 10 mg/L of AHA was added as inhibitor

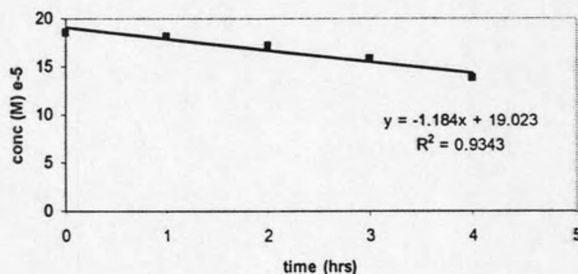
time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.87065E-05	8.8041E-05	0.000108131	0.000130462	0.000185254
1	3.74616E-05	7.33709E-05	9.87084E-05	0.000126133	0.000180505
2	3.24822E-05	7.15515E-05	8.73324E-05	0.000110774	0.000171599
3	2.52429E-05	6.27609E-05	7.57457E-05	9.66975E-05	0.000158174
4	2.22553E-05	5.28595E-05	6.57677E-05	8.72366E-05	0.000137222





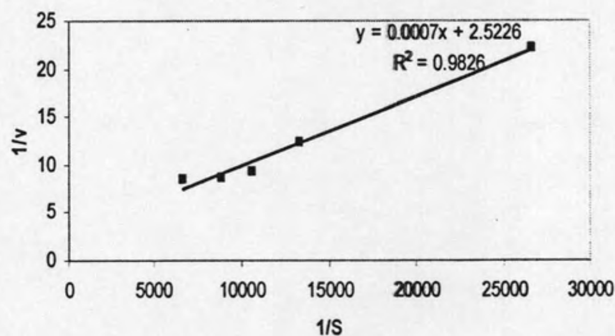






Slope of each PCP concentration was its velocity. Then, K_m and V_{max} for PCP as 10 mg/L AHA added was determined by plotting 1/v and 1/S

1/S	1/v(e4)
26635	22.16312057
13317.5	12.35025318
10654	9.28591327
8878.333	8.628872206
6658.75	8.445945946

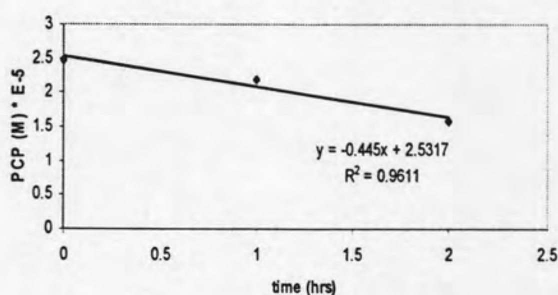


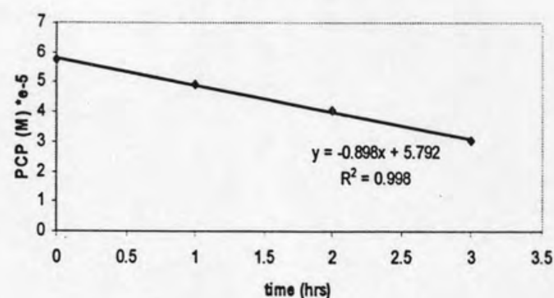
A.5 Enzyme kinetics for PCP when AHA 15 mg/L was added as an inhibitor

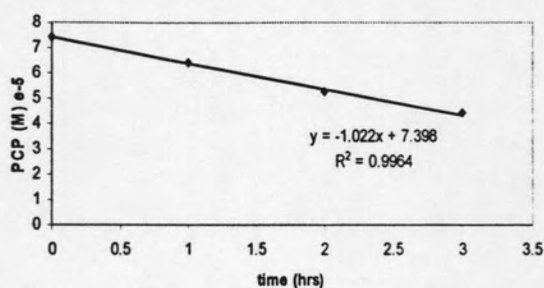
In the experiment, 10, 20, 25, 30, 40 mg/L of PCP and 15 mg/L of AHA were mixed with 20 units/mL of laccase and measured PCP concentration at time 0, 1, 2, 3, 4 hrs by GC-FID. The data and graph between their velocity and concentration are shown below.

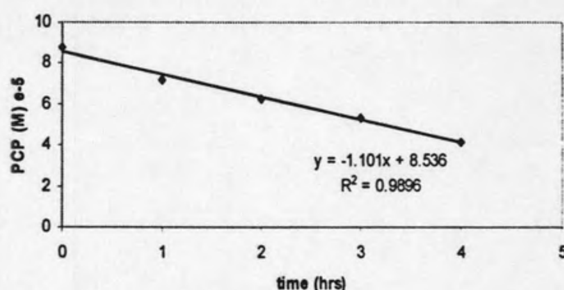
PCP concentration (M) at time 0 – 4 hrs, when 10, 20, 25, 40 mg/L of PCP were added as substrates and 15 mg/L of AHA was added as inhibitor

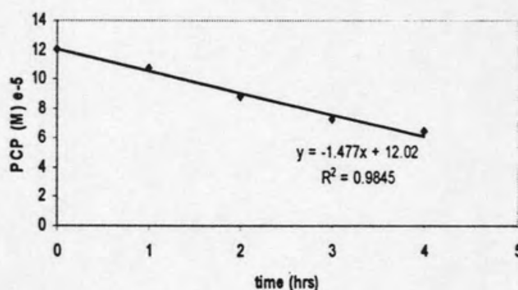
time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	2.48285E-05	5.75035E-05	7.42285E-05	8.74535E-05	0.000120554
1	2.19285E-05	4.93035E-05	6.40535E-05	7.17785E-05	0.000107679
2	1.59785E-05	4.04535E-05	5.24035E-05	6.25535E-05	8.81785E-05
3	1.44785E-05	3.04785E-05	4.40285E-05	5.35785E-05	7.23785E-05
4	1.43035E-05	3.17785E-05	3.77785E-05	4.15285E-05	6.43535E-05





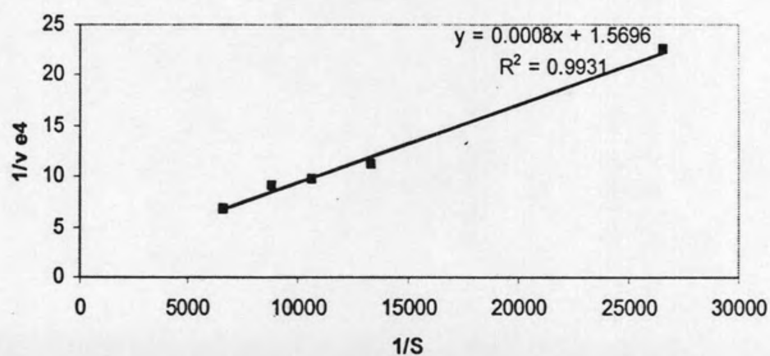






Then, K_m and V_{max} for PCP as 15 ppm AHA added was determined by plotting 1/v and 1/S

1/S	1/v(e4)
26635	22.47191011
13317.5	11.13585746
10654	9.784735812
8878.333333	9.082652134
6658.75	6.770480704



20 mg/L of AHA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	2.39972E-05	7.06372E-05	9.19572E-05	0.000125317	0.000153697
1	2.19972E-05	6.18772E-05	8.90772E-05	0.000110997	0.000155817
2	1.39572E-05	5.62772E-05	7.31372E-05	0.000105657	0.000151457
3	1.39572E-05	5.19772E-05	6.59772E-05	9.72372E-05	0.000135937
4	1.39572E-05	4.74172E-05	5.44172E-05	9.02372E-05	0.000133237

10 mg/L of LHA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.33537E-05	6.96745E-05	9.47009E-05	0.000110749	0.000173924
1	3.18099E-05	6.41086E-05	8.85256E-05	0.000105142	0.000170552
2	2.95347E-05	6.21178E-05	8.64942E-05	9.51478E-05	0.000167342
3	2.85191E-05	5.98833E-05	8.22283E-05	8.90131E-05	0.000157917
4	2.60002E-05	5.82176E-05	7.84094E-05	8.93381E-05	0.000142357

15 mg/L of LHA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	2.78778E-05	6.2196E-05	8.27115E-05	0.000105545	0.000144177
1	2.61255E-05	5.8988E-05	8.20914E-05	0.000108026	0.000141535
2	2.61794E-05	5.69122E-05	7.77781E-05	0.000103335	0.000135928
3	2.55594E-05	5.55642E-05	7.34917E-05	9.19313E-05	0.000131372
4	1.9952E-05	4.84472E-05	6.4838E-05	9.02868E-05	0.000119806

20 mg/L of LHA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.82999E-05	7.60957E-05	0.000100283	0.000128485	0.000183261
1	3.35399E-05	7.24399E-05	9.79853E-05	0.00012747	0.000175755
2	3.34206E-05	6.94557E-05	9.64932E-05	0.000124008	0.000175517
3	3.48381E-05	6.67549E-05	9.32105E-05	0.000120188	0.00017298
4	3.48679E-05	6.65311E-05	8.87042E-05	0.000110594	0.000165669

10 mg/L of SRFA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.3121E-05	8.43049E-05	0.000107414	0.000122799	0.000168017
1	3.1092E-05	6.66479E-05	9.28457E-05	0.000100781	0.000151087
2	2.9244E-05	6.01363E-05	8.03071E-05	8.78182E-05	0.000130613
3	2.5337E-05	5.0172E-05	6.71325E-05	7.42498E-05	0.000110018
4	2.352E-05	3.71488E-05	7.85202E-05	7.41287E-05	7.44921E-05

15 mg/L of SRFA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.54401E-05	6.9017E-05	9.4262E-05	0.00011523	0.00017676
1	3.16989E-05	6.5904E-05	8.5553E-05	0.00010828	0.00016163
2	2.84922E-05	6.4961E-05	7.98E-05	0.00010064	0.00012988
3	2.49082E-05	6.0968E-05	7.1783E-05	9.0395E-05	0.00012495
4	2.35878E-05	5.2574E-05	6.8545E-05	8.2567E-05	0.00010366

20 mg/L of SRFA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.67E-05	4.23E-05	8.28E-05	0.000114	0.000198
1	2.54E-05	4.49E-05	6.11E-05	7.38E-05	0.000177
2	2.08E-05	2.85E-05	3.47E-05	6.7E-05	0.000142
3	2.08E-05	2.08E-05	2.89E-05	6.67E-05	0.000138
4	2.08E-05	2.08E-05	3.45E-05	5.4E-05	8.66E-05

10 mg/L of WFA + PCP

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	3.67E-05	7.9E-05	0.000102	0.000125	0.000174
1	3.43E-05	7.33E-05	9.93E-05	0.000116	0.000166
2	3.37E-05	7.09E-05	9.49E-05	0.000113	0.000162
3	3.19E-05	6.85E-05	9E-05	0.000106	0.000157
4	2.73E-05	6.23E-05	8.09E-05	9.78E-05	0.000139

15 mg/L of WFA + PCP

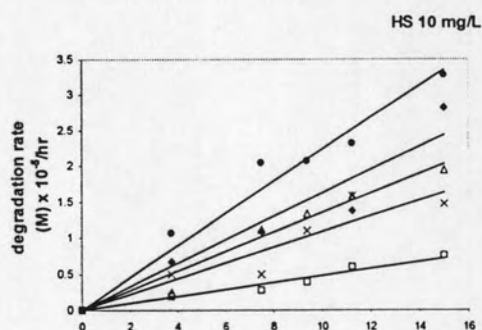
time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	4.26E-05	9.27E-05	0.000118	0.000158	0.000223
1	4.03E-05	8.7E-05	0.000113	0.000146	0.000208
2	3.9E-05	8.37E-05	0.00011	0.000141	0.000198
3	3.62E-05	7.91E-05	0.000105	0.000135	0.000189
4	3.21E-05	7.26E-05	9.38E-05	0.000126	0.000184

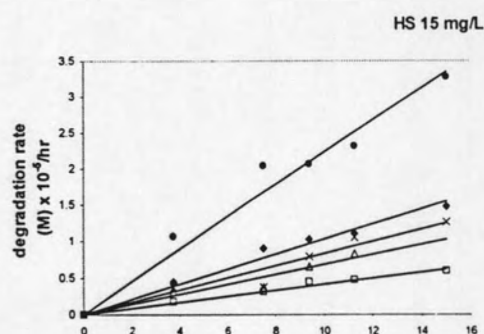
20 mg/L of WFA + PCP

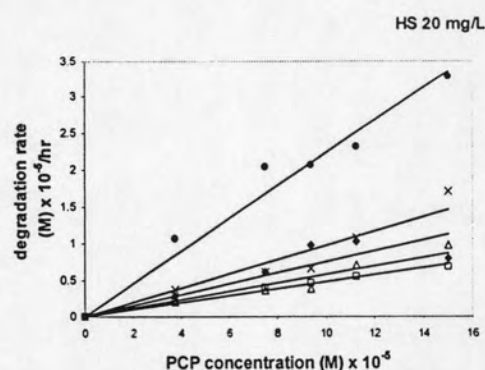
time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	4.3E-05	8.9E-05	0.000101	0.00015	0.000203
1	3.89E-05	8.24E-05	9.21E-05	0.000139	0.000193
2	3.57E-05	7.73E-05	8.05E-05	0.000128	0.000194
3	3.42E-05	7.12E-05	7.85E-05	0.000119	0.000162
4	2.59E-05	7.49E-05	8.13E-05	0.000100	0.000133

APPENDIX B: PCP degradation rate with and without HS

This information is for section A: PCP degradation rate. Figure shows the degradation rate of PCP by laccase enzyme at various concentrations of PCP and different HS types and concentration addition. The slope of each graph represents k' for each condition.



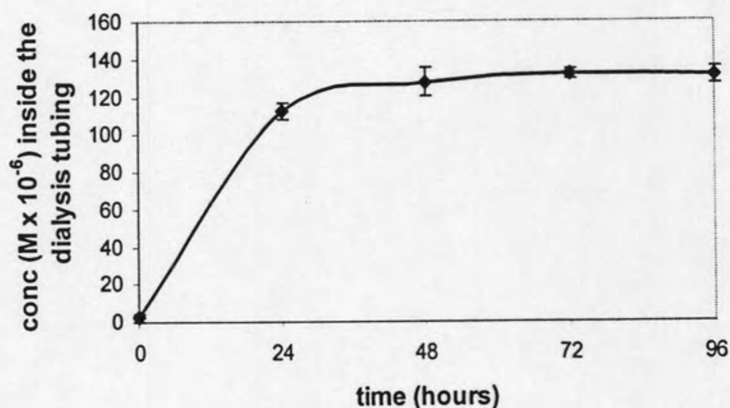




PCP degradation rate when HS 10, 15, 20 mg/L were added. Symbols represent HS, ●: no HS, ♦: AHA, □: LHA, Δ: SRFA, ×: WFA

APPENDIX C: Kinetics of PCP-HS binding

This information is for section G: PCP – HS binding studies, to find the equilibrium time for PCP inside and outside the dialysis tubing. It was necessary to examine the kinetics of PCP-HS association to determine the length of time needed to reach equilibrium in the dialysis-binding experiments. After an initial rapid rise in the PCP concentration within the dialysis bag, equilibrium appeared to be attained within 3 days. Therefore, dialysis-binding experiments were run for 3 days to ensure that system equilibrium was attained.



Kinetics of PCP binding by LHA

APPENDIX D: Calculation for PCP degradation rate in section 3.2.3: PCP – HS desorption studies

Using k′ from Table 3.2 HS concentration equals to 20 mg/L, and PCP concentration is 15 mg/L to calculate PCP degradation rate (dc/dt). The values can be derived from equation $dC/dt = k'[C]$. For example, for AHA, $dC/dt = (7.75 \times 10^{-2}\ hr^{-1}) \times (5.63 \times 10^{-5}\ M) = 4.36 \times 10^{-6}\ M/hr$.

APPENDIX E: Calculation for desorption rate of PCP from HS in section 3.3.8: PCP – HS desorption studies

Using K_{dom} in Table 3.6 and % desorption from Figure 3.8 (The values for % desorption of AHA, LHA, SRFA, and WFA at 24 hrs were 25, 17, 53, and 32 %, respectively and linear isotherm was assumed for desorption at 24 hrs). Default values were 20 mg/L of HS and 15 mg/L of PCP concentration. The example is for PCP desorption rate from AHA.

$$K_{dom}\ \text{L/kg.OC} = C_{dom} / C_w$$

$$C_{dom} = (C_t - C_w) / C_{HS}$$

$$4.49 \times 10^4\ \text{L/kg.OC} = \left[\frac{5.63 \times 10^{-5}\ \text{mol/L} - Cw}{20\ \text{mg.OC/L}}\right] \times 1/Cw$$

$$C_w = 2.97 \times 10^{-5}\ \text{mol/L}$$

$$(C_t - C_w)/C_{HS} = C_{dom}$$

$$C_{dom} = (5.63 \times 10^{-5} - 2.97 \times 10^{-5}\ \text{mol/L})/20\ \text{mg/L}$$

$$C_{dom}\ \text{at 20 mg/L HS} = 2.66 \times 10^{-5}\ \text{mol/L}$$

25 % of bound PCP was released from AHA at 24 hr

$$= 2.66 \times 10^{-5} \times 0.25 / 24$$

$$\text{Desorption rate} = 2.77 \times 10^{-7}\ \text{M/hr}$$

APPENDIX F: Calculation for Table 3.7: Experimental and calculated PCP degradation rate constants in section 3.3.10

Using PCP concentration of 15 mg/L (5.63×10^{-5} M) and k' values from Table 2, we can calculate experimental PCP degradation rate by dC/dt = k'.[C], for example, degradation rate for AHA 10 mg/L addition = $(1.62 \times 10^{-1})(5.63 \times 10^{-5}) = 9.12 \times 10^{-6}$ M/hr

The calculated PCP degradation rate can be found by using Kdom. This example is also for 10 mg/L of AHA.

$$K_{dom} \text{ L/kg.OC} = C_{dom} / C_w$$

$$C_{dom} = (C_t - C_w) / C_{HS}$$

$$4.49 \times 10^4 \text{ L/kg.OC} = \left[\frac{5.63 \times 10^{-5} \text{ mol/L} - Cw}{10 \text{ mg.OC/L}} \right] \times 1/Cw$$

$$C_w = 3.89 \times 10^{-5} \text{ mol/L}$$

C_w is a freely dissolved PCP. Since the PCP enzymatically degradation experiment with and without HS was carried out for 4 hr – initial reaction, we used 4 hrs to find the calculated PCP degradation rate.

$$\text{Calculated PCP degradation rate} = 3.89 \times 10^{-5} \text{ M} / 4 \text{ hr}$$

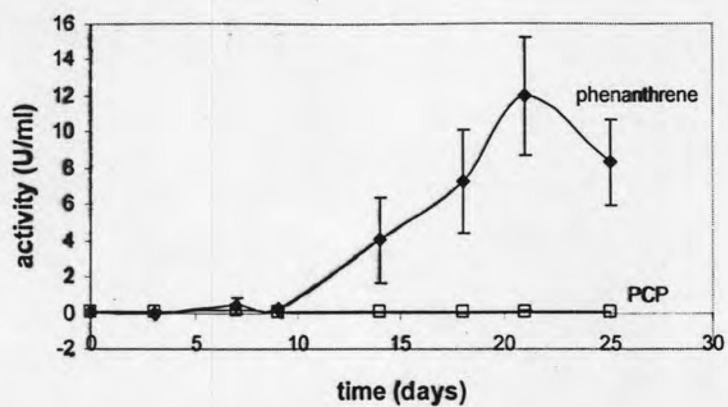
$$= 9.71 \times 10^{-6} \text{ M/hr}$$

APPENDIX G: Testing for aromatic contaminants of the fungus in liquid medium

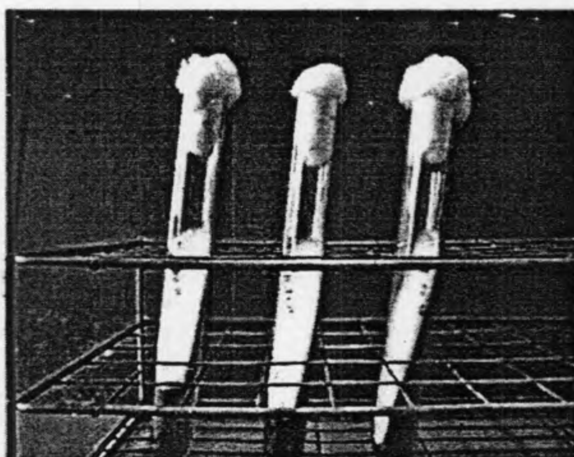
The white rot fungi from yanaghi mushroom are kindly given by Dr. Panan Rerngsamran. Inoculums are prepared from the fungus by growing on malt extract agar for 10 days at room temperature. Then, 5 – 6 pieces of 1 x 1 cm agar containing mycelia are transferred to 100 ml malt extract broth in a 500 ml Erlenmeyer flask and incubated at 28°C on an orbital mixer incubator (Ratek) at 120 rpm for 14 days. The cultured broth is transferred to a sterilized centrifuge tube and centrifuged at 10000g for 10 minute and washed twice with sterilized deionized water. The supernatant is decanted and the fungal pellet is weighed. Ten grams of the pellet is transferred to N-limiting medium 100 ml in 500 ml Erlenmeyer flask and incubated at 28°C on an orbital mixer incubator at 120 rpm for 7 days. Then, 100 ppm of either PCP or phenanthrene is added and incubated at the same condition. At the cultivated time, 10 ml of the broth is centrifuged (Sorvall® Biofuge Stratos) at 10000g for 20 minutes. The supernatant which contains ligninolytic enzymes is tested for their activities.

G.1 Optimum time for enzyme cultivation

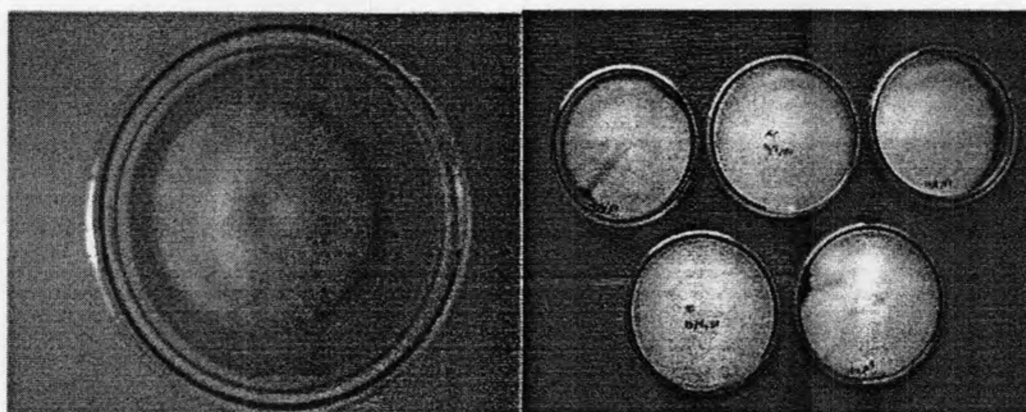
Preliminary study found that the induction of ligninolytic enzyme as measured by catechol assay showed negative result for using PCP as an inducer. In contrast, phenanthrene showed more preferable in enzyme productivity. The result also clarified that day 21 was the optimum time for enzyme cultivation.



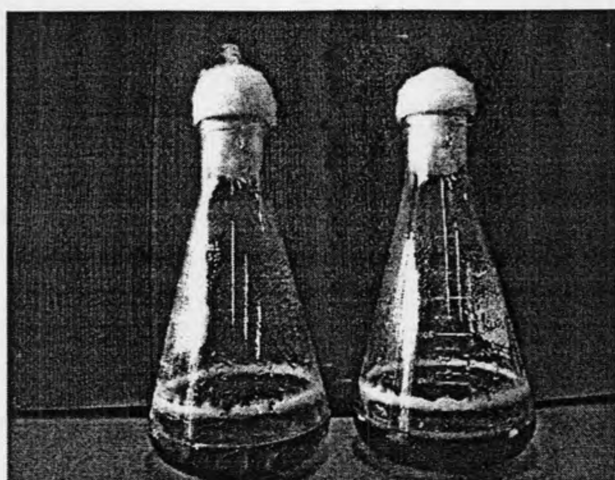
Optimum time for enzyme induction

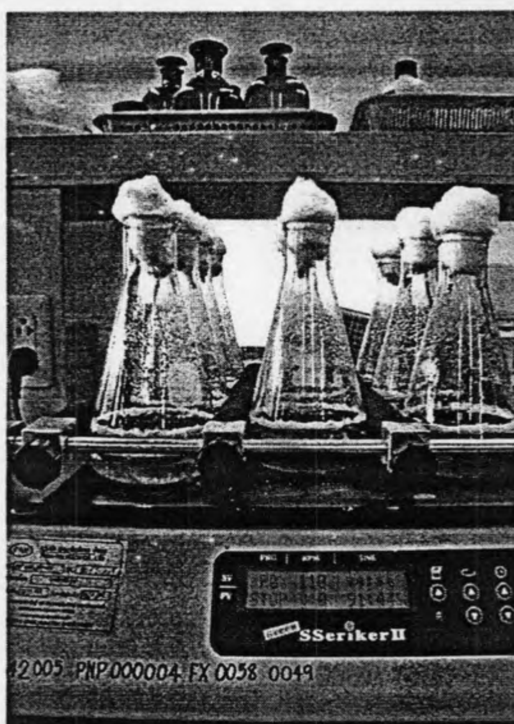


Inoculums on malt extract slant



Inoculums on malt extract agar





***Agrocybe* sp. CU 43 in malt extract broth**

G.2 Media for fungi rising

Malt extract agar per liter

Malt extract	20	grams
Peptone	5	grams
Glucose	20	grams

Agar	15	grams
Deionized water	1,000	ml

Sterilize at 121°C, 15 lbs/in^2 for 15 minutes

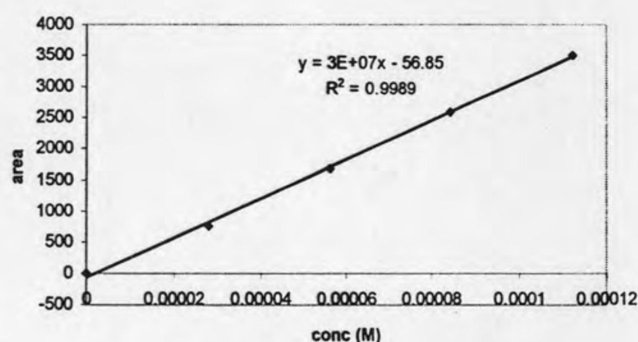
N-limiting medium per liter

Glucose	10	grams
Ammonium nitrate (NH_4NO_3)	0.1	grams
Potassium hydrogen phosphate (KH_2PO_4)	1	grams
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5	grams
Iron II heptahydrate ($FeSO_4.7H_2O$)	0.01	grams
Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)	0.001	grams
Manganese sulfate ($MnSO_4$)	0.001	grams
Cupper sulfate hexahydrate ($CuSO_4.5H_2O$)	0.001	grams

Adjust pH to 4.5 and sterilized at 121°C, 15 lbs/in^2 for 15 minutes

APPENDIX H: Enzyme kinetics for phenanthrene with and without HS and DOM addition

H.1 Phenanthrene calibration curve by GC-FID



Average phenanthrene concentration data for calculate K_m and V_{max} for phenanthrene using ligninolytic enzymes were shown as below

Phenanthrene degradation

time (hours)	0 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	4.61167E-06	3.17E-05	7.07483E-05	0.000110935	0.001388483
1	5.33167E-06	3.09E-05	6.52617E-05	0.000103182	0.001346083
2	4.24833E-06	2.99E-05	6.48017E-05	0.000101902	0.00131435
3	4.12833E-06	2.9E-05	6.22083E-05	0.000103515	0.00128175
4	0.000003875	2.74E-05	0.000059415	9.45983E-05	0.001233517

10 mg/L of AHA + phenanthrene

time (hours)	0 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.81E-06	3.15E-05	6.09E-05	9.52E-05	0.000135
1	3.49E-06	3.1E-05	6.1E-05	9.46E-05	0.000127
2	3.44E-06	3.02E-05	6.03E-05	8.55E-05	0.00012
3	3.24E-06	2.88E-05	5.6E-05	8.09E-05	0.000112
4	3.23E-06	2.74E-05	5.52E-05	7.88E-05	0.000102

15 mg/L of AHA + phenanthrene

time (hours)	0 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	2.8E-06	3.26E-05	5.88E-05	8.77E-05	0.000123
1	2.57E-06	3.12E-05	5.72E-05	8.52E-05	0.000111
2	2.4E-06	3.05E-05	5.36E-05	8.21E-05	0.00011
3	2.51E-06	2.95E-05	5.16E-05	7.94E-05	0.000107
4	2.41E-06	2.79E-05	5.29E-05	7.92E-05	0.0001

10 mg/L of LHA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.85E-05	7.73E-05	0.000111	0.000162
1	3.5E-05	7.24E-05	0.000111	0.000163
2	3.41E-05	6.88E-05	0.000107	0.000158
3	3.07E-05	6.84E-05	0.000104	0.000154
4	2.46E-05	6.67E-05	9.8E-05	0.000144

15 mg/L of LHA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.97E-05	7.76E-05	0.000126	0.000171
1	3.6E-05	7.59E-05	0.000125	0.000171
2	3.33E-05	7.3E-05	0.000125	0.000169
3	3.19E-05	7.16E-05	0.000121	0.000164
4	1.77E-05	7.07E-05	0.000119	0.000161

10 mg/L of SRFA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.31E-05	6.64E-05	9.97E-05	0.000132
1	3.33E-05	6.49E-05	9.52E-05	0.000128
2	3.3E-05	6.33E-05	9.03E-05	0.000125
3	3.07E-05	6.18E-05	8.64E-05	0.000121
4	2.91E-05	6.05E-05	8.4E-05	0.000114

15 mg/L of SRFA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.43E-05	7.14E-05	0.000102	0.000138
1	3.36E-05	6.66E-05	0.0001	0.000135
2	3.21E-05	6.47E-05	9.46E-05	0.000133
3	3.14E-05	6.26E-05	9.2E-05	0.000131
4	3.06E-05	5.91E-05	8.63E-05	0.000115

10 mg/L of WFA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.69E-05	7.83E-05	0.000109	0.000148
1	3.34E-05	6.92E-05	0.000104	0.000139
2	3.3E-05	6.75E-05	0.000101	0.000136
3	3.22E-05	6.69E-05	9.93E-05	0.000135
4	3.18E-05	6.6E-05	9.69E-05	0.000132

15 mg/L of WFA + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.89E-05	7.46E-05	0.000109	0.000152
1	3.61E-05	7.54E-05	0.000108	0.000147
2	3.49E-05	7.1E-05	0.000104	0.000145
3	3.39E-05	6.92E-05	0.000102	0.000136
4	3.28E-05	6.56E-05	1E-04	0.000135

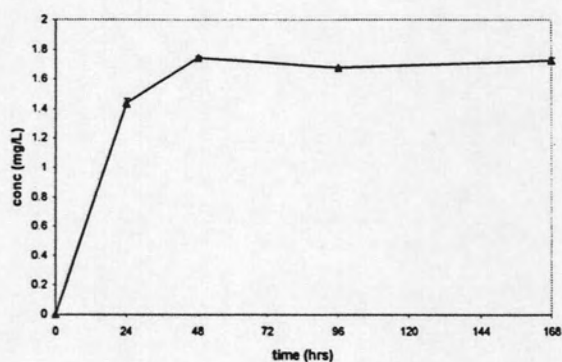
10 mg/L of DOM + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.42E-05	6.69E-05	9.5E-05	0.000128
1	3.41E-05	6.49E-05	9.5E-05	0.000125
2	3.36E-05	6.34E-05	9.42E-05	0.000124
3	3.17E-05	6.42E-05	9.36E-05	0.000121
4	3.1E-05	6.18E-05	9.17E-05	0.000122

15 mg/L of DOM + phenanthrene

time (hours)	5 mg/L	10 mg/L	15 mg/L	20 mg/L
0	3.64E-05	6.91E-05	0.000101	0.000143
1	3.27E-05	6.44E-05	9.88E-05	0.000135
2	3.12E-05	6.09E-05	9.52E-05	0.000132
3	3.25E-05	5.94E-05	9.48E-05	0.000131
4	3.19E-05	6.11E-05	9.43E-05	0.000132

APPENDIX I: Equilibrium time for binding study



Equilibrium dialysis analysis of sorption of 20 mg/L of phenanthrene by a 15 mg/L of LHA solution.

APPENDIX J: Calculation for Table 4.7: Experimental and calculated phenanthrene degradation rate constants in section 4.3.8.

Using phenanthrene concentration of 20 mg/L (1.12×10^{-4} M) and k' values from Table 4.2, we can calculate experimental phenanthrene degradation rate by dC/dt = k'[C], for example, degradation rate for AHA 15 mg/L addition = $(1.09 \times 10^{-3})(1.12 \times 10^{-4}) = 1.22 \times 10^{-7}$ M/hr

For calculated phenanthrene degradation rate, use K_p from table 4.3, which was experimented by using 15 mg/L of HS or DOM and 20 mg/L of phenanthrene, to calculate bound phenanthrene. The following is the example of calculated phenanthrene degradation rate for AHA:

K_p = 0.64 mol/kg

Bound phenanthrene to HS 15 mg/L = 0.64 mol/kg * 15 mg/L * 10^{-6} kg/mg

Bound phenanthrene = 9.60×10^{-6} mol/L

Free phenanthrene = 1.12×10^{-4} M – 9.60×10^{-6} M

= 1.02×10^{-4} M

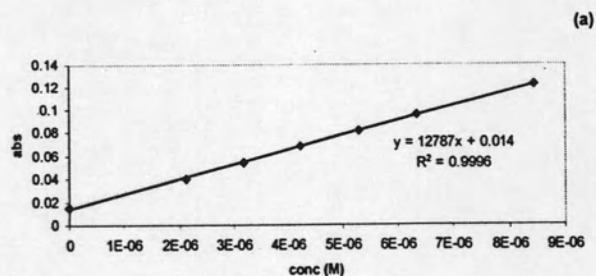
The binding experiment was performed in 96 hr – equilibrium time, which is still in within the duration of phenanthrene enzymatic degradation rate, we compared to.

Calculated phenanthrene degradation rate considering only unbound phenanthrene = 1.02×10^{-4} M / 96 hr = 1.06×10^{-6} M/hr

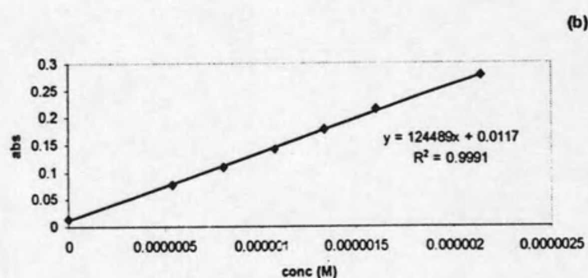
APPENDIX K: Kinetics of HS and laccase

Standard curves of HS calibrated by UV-vis spectrophotometry at 465 nm absorption were shown below.

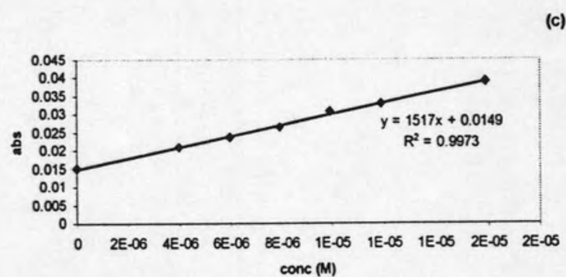
(a)



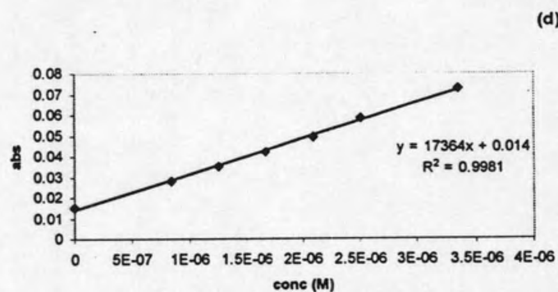
(b)



(c)



(d)



Standard curves by UV-vis spectrophotometer at 465 nm – absorbance for HS; (a): AHA, (b): LHA, (c): SRFA, (d): WFA.

HS were incubated with laccase enzyme and measured their light absorption at 465 nm every 24 hours for 96 hours. Their average absorbance was as shown below. Average 465 nm - absorbance of laccase at 465 nm at 0 – 96 incubation times were 0.3838, 0.3851, 0.3991, 0.3713, 0.3847, respectively.

AHA + laccase

time (hours)	10 mg/L	15 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	0.4554	0.4581	0.4682	0.4804	0.5327	0.5506
24	0.4328	0.4502	0.4478	0.4485	0.5251	0.5499
48	0.4449	0.4657	0.4437	0.4525	0.5167	0.5306
72	0.4238	0.4302	0.4249	0.4166	0.4929	0.5338
96	0.4343	0.4526	0.4042	0.4033	0.4873	0.5155

LHA + laccase

time (hours)	10 mg/L	15 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	0.4767	0.5122	0.5336	0.5877	0.6477	0.7043
24	0.4834	0.5226	0.5233	0.5809	0.6301	0.7147
48	0.4892	0.5359	0.5175	0.5807	0.6209	0.6944
72	0.4575	0.4857	0.5149	0.5613	0.6349	0.6831
96	0.4637	0.4843	0.5122	0.5338	0.6133	0.6642

SRFA + laccase

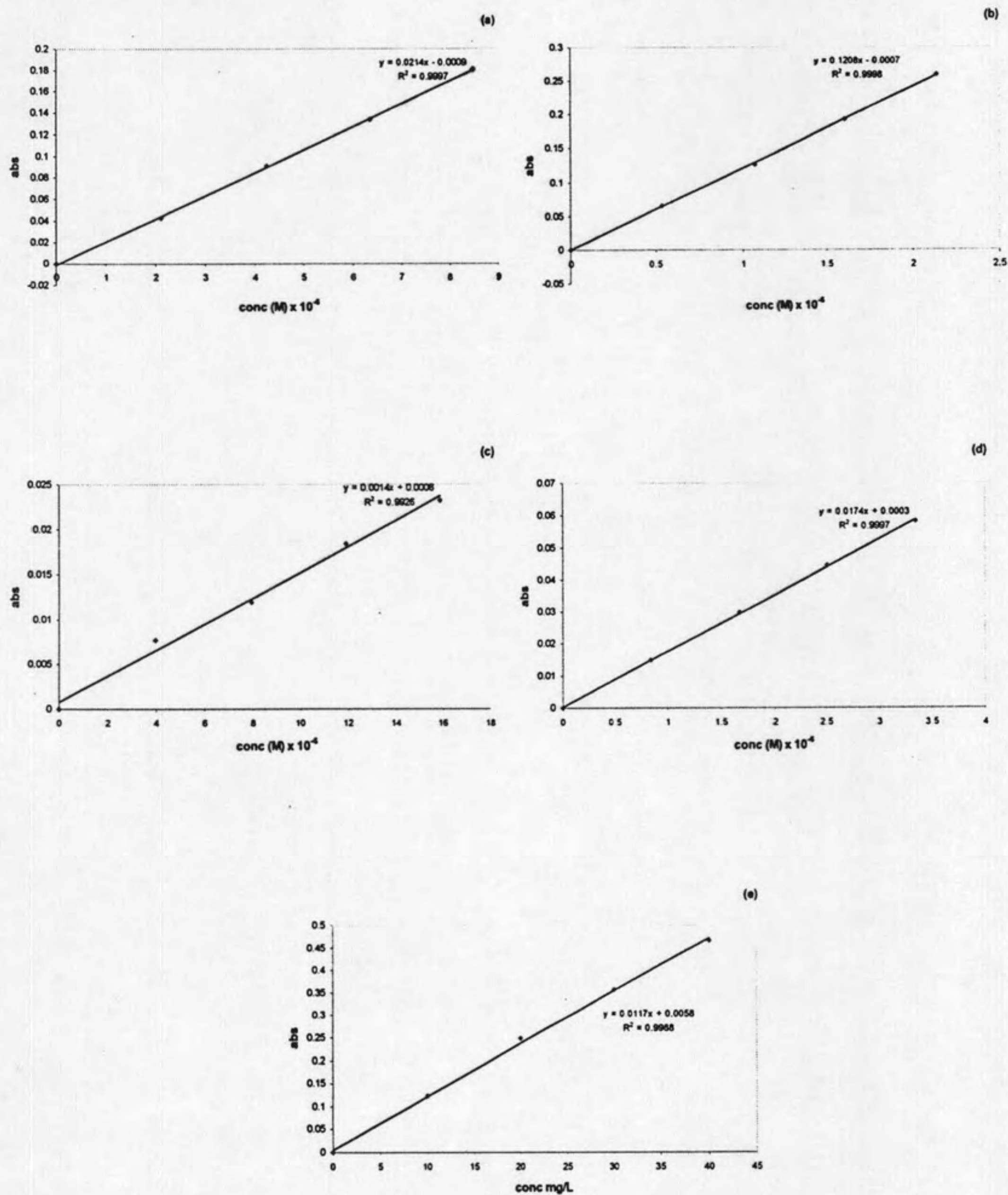
time (hours)	15 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	0.4502	0.4124	0.4283	0.4688	0.4813
24	0.4226	0.4055	0.4243	0.4423	0.4584
48	0.4443	0.4146	0.4482	0.4319	0.4699
72	0.4056	0.3835	0.4085	0.4308	0.4513
96	0.4199	0.3936	0.3907	0.4393	0.4193

WFA + laccase

time (hours)	10 mg/L	20 mg/L	25 mg/L	30 mg/L	40 mg/L
0	0.4188	0.4803	0.4720	0.4886	0.5298
24	0.4245	0.4735	0.4566	0.4754	0.4817
48	0.4137	0.4548	0.4588	0.4626	0.4748
72	0.3982	0.4433	0.4112	0.4548	0.4614
96	0.3897	0.4409	0.4064	0.4501	0.4536

APPENDIX L: Kinetics of HS, DOM and ligninolytic enzymes

Calibration curves of HS and DOM analyzed by UV-vis spectrophotometric method were shown.



Standard curves by UV-vis spectrophotometer at 465 nm – absorbance for HS; (a): AHA, (b): LHA, (c): SRFA, (d): WFA, (e): DOM

HS and DOM were incubated with crude ligninolytic enzyme from *Agrocybe* sp. CU 43 and measured their light absorption at 465 nm every 24 hours for 96 hours by UV-vis spectrophotometer. Their average absorbance was as shown in table below. Average 465 nm - absorbance of the enzyme at 465 nm at 0 – 96 incubation times were 0.0506, 0.0517, 0.0523, 0.0511, 0.052 respectively.

AHA + ligninolytic enzyme

time (hours)	10 mg/L	20 mg/L	30 mg/L	40 mg/L
0	0.0985	0.1467	0.1983	0.2450
24	0.1038	0.1511	0.2099	0.2551
48	0.1031	0.1534	0.2116	0.2626
72	0.1073	0.1577	0.2118	0.2624
96	0.1029	0.1582	0.2108	0.2655

LHA + ligninolytic enzyme

time (hours)	10 mg/L	20 mg/L	30 mg/L	40 mg/L
0	0.1211	0.1867	0.2541	0.3221
24	0.1242	0.1897	0.2590	0.3322
48	0.1244	0.1908	0.2611	0.3358
72	0.1253	0.1926	0.2631	0.3362
96	0.1259	0.1979	0.2641	0.3382

SRFA + ligninolytic enzyme

time (hours)	10 mg/L	20 mg/L	30 mg/L	40 mg/L
0	0.0573	0.0642	0.0706	0.0771
24	0.0577	0.0654	0.0716	0.0787
48	0.0583	0.0661	0.0721	0.0791
72	0.0588	0.0668	0.0724	0.0800
96	0.053	0.0669	0.0731	0.0811

WFA + ligninolytic enzyme

time (hours)	10 mg/L	20 mg/L	30 mg/L	40 mg/L
0	0.0676	0.0828	0.0967	0.1137
24	0.0689	0.0839	0.0988	0.1149
48	0.0704	0.0849	0.1001	0.1159
72	0.0717	0.0862	0.1029	0.1169
96	0.0731	0.0881	0.1030	0.1178

DOM + ligninolytic enzyme

time (hours)	10 mg/L	20 mg/L	30 mg/L	40 mg/L
0	0.2051	0.3395	0.4588	0.5245
24	0.2151	0.3744	0.4994	0.6054
48	0.2156	0.3836	0.5000	1.2742
72	0.2192	0.3880	0.5238	1.4134
96	0.2217	0.3921	0.5309	1.4421

APPENDIX M Proceeding paper

08R2-09

บทบาทของกรดฮิวมิคและฟูลวิคต่อการย่อยสลายสารเพนตะคลอโรฟีนอลที่เร่งปฏิกิริยาโดยแลคเคส

The Effect of Humic and Fulvic Acids on Laccase Catalyzed Degradation of Pentachlorophenol

อาภาภรณ์ ศิริพรประสาร[1*] มาร์ค เอ แนนนี่[2,3] และ เอกวัล ลือพร้อมชัย[4,5]

Apaporn Siripornprasarn[1*] Mark A. Nanny[2,3] and Ekawan Luepromchai[4,5]

Received 15 January 2007 ; received in revised form .... February 2007 ; accepted .... February 2007

บทคัดย่อ

เพื่อเพิ่มประสิทธิภาพวิธีการบำบัดทางชีวภาพ เราจำเป็นต้องเข้าใจถึงบทบาทของสารฮิวมิคต่อการย่อยสลายสารปนเปื้อน บทบาทที่เป็นไปได้ของกรดฮิวมิคและกรดฟูลวิค คือ: 1) สามารถเป็นสารตั้งต้นของเอนไซม์ 2) สามารถทำลายคุณสมบัติการเร่งปฏิกิริยาของเอนไซม์ที่มีหน้าที่ย่อยสลายสารปนเปื้อน หรือ 3) ไม่ทำปฏิกิริยากับเอนไซม์ แต่ปกป้องสารปนเปื้อนจากการย่อยสลายโดยเอนไซม์ การย่อยสลายสารเพนตะคลอโรฟีนอล (50 ppm) โดยแลคเคส (20 ยูนิต) ที่ถูกสกัดจาก *Trametes versicolor* ผสมกับกรดฮิวมิคของอัลดริช (400 ppm) หรือกรดฟูลวิคจากแม่น้ำสุวรรณี (400 ppm) ที่อุณหภูมิ 25°C และ pH 5 ถูกใช้เป็นระบบจำลองเพื่อพิสูจน์สมมติฐานสามข้อนี้ ความเข้มข้นของสารเพนตะคลอโรฟีนอลถูกวัดทุกๆ 12 ชั่วโมง เป็นเวลา 72 ชั่วโมง ด้วย GC ความสามารถในการเร่งปฏิกิริยาของเอนไซม์ถูกวัดด้วยสารแคททีโคล 10 mM และวัดการเปลี่ยนแปลงของการดูดกลืนแสงที่ 440 nm การเปลี่ยนแปลงของโครงสร้างทางเคมีของกรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณีที่มีผลมาจากการย่อยสลายด้วยเอนไซม์ถูกวัดด้วยการตรวจวัดการดูดกลืนแสง UV-VIS ของส่วนผสมของปฏิกิริยาที่ 200 ถึง 800 nm. ผลการทดลองแสดงว่าเมื่อไม่มีกรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณี สารเพนตะคลอโรฟีนอลถูกย่อยสลายด้วยเอนไซม์ 97% ในเวลา 72 ชั่วโมง กรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณีลดการย่อยสลายสารเพนตะคลอโรฟีนอลเป็น 80% และ 86% ตามลำดับในช่วงเวลาเดียวกัน อย่างไรก็ตาม ความสามารถในการเร่งปฏิกิริยาของแลคเคสไม่มีการเปลี่ยนแปลงและมีความสามารถในการเร่งปฏิกิริยาอย่างเต็มที่ตลอด

[1*]Ph. D. Student, International Postgraduate Program in Environmental Management (Hazardous Waste Management), Graduate School, Chulalongkorn University, Bangkok 10330, Thailand;

[2]Associate Professor, School of Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK 73019-1024, USA;

[3]Institute scientist,Institute for Energy and the Environment, Sarkeys Energy Center, University of Oklahoma, Norman, OK 73019-1024, USA;

[4]Lecturer,Department of Microb iology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand;

[5]Lecturer, National Research Center for Environmental and Hazardous Waste Management (NRC-EHWM), Chulalongkorn University, Bangkok 10330, Thailand;

[*]Tel : 0-2218-8132, Fax : 0-2218-8212, e-mail : capaporn@hotmail.com

ช่วงเวลาการทดลอง ทำให้ลบล้างสมมติฐานที่ว่ากรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณีสามารถทำลายความสามารถในการเร่งปฏิกิริยาของเอนไซม์ การเปลี่ยนแปลงทางเคมีของกรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณีโดยแลคเคสตามที่ถูกวัดด้วย UV-VIS มีนัยสำคัญหลังจาก 8 วัน ดังนั้น เราจึงเสนอว่ากรดฮิวมิคของอัลดริชหรือกรดฟูลวิคจากแม่น้ำสุวรรณี สามารถทำหน้าที่เป็นสารตั้งต้นของเอนไซม์แลคเคสและรบกวนอัตราการย่อยสลายของสารเพนตะคลอโรฟีนอล ผลการทดลองชี้แนะว่าการย่อยสลายสารเพนตะคลอโรฟีนอลระหว่างการบำบัดทางชีวภาพในธรรมชาติอาจต่ำกว่าในการทดลองเนื่องจากสารฮิวมิค

คำสำคัญ : เพนตะคลอโรฟีนอล; แลคเคส; กรดฮิวมิค; กรดฟูลวิค; การบำบัดทางชีวภาพ

Abstract

To improve the efficiency of bioremediation strategies, we must understand the effects of humic substances on pollutant biodegradation. The possible roles of HA and FA are that they: 1) can act as competitive enzymatic substrates, 2) can deactivate the enzyme responsible for biodegradation, or 3) are enzymatically inert but sequester the pollutant substrate and protect it from enzymatic degradation. Degradation of pentachlorophenol (PCP) (50 ppm) by purified laccase (20 units activity) isolated from *Trametes versicolor*, in combination with either Aldrich humic acid (AHA) (400 ppm) or Suwannee River fulvic acid (SRFA) (400 ppm), at 25°C and pH 5 was used as a model system to test these three hypotheses. PCP concentrations were monitored every 12 hours for 72 hours by GC. Enzyme activity was measured with 10 mM catechol and monitoring absorbance changes at 440 nm. Changes in the AHA or SRFA chemical structure resulting from enzymatic degradation were detected by monitoring the UV-VIS absorbance of the reaction mixtures over 200 to 800 nm. Results showed that without AHA and SRFA, PCP was enzymatically degraded by 97% within 72 hours. The presence of AHA and SRFA resulted in lower PCP degradation of 80% and 86%, respectively, during the same time period. However, laccase activity was unchanged and fully active throughout the reaction period disproving the hypothesis that AHA and SRFA deactivate laccase. Chemical changes in AHA and SRFA by laccase, as monitored by UV-VIS, were significant after 8 days. Therefore, we proposed that AHA and SRFA act as competitive substrates for laccase and decrease the enzymatic degradation rate of PCP. The results suggested that the extent of PCP degradation during bioremediation in natural environment could be lower than in laboratory due to the presence of humic substances.

Keywords : pentachlorophenol; laccase; humic acid; fulvic acid; bioremediation

Introduction

Aromatic pollutants are ubiquitous environmental contaminants found in air, soil, and aquatic environments. Most of them are toxic to living organisms, and some of them and their metabolites are mutagenic and carcinogenic to humans. Therefore, the knowledge of mechanisms involving the reduction of aromatic pollutants by taking the advantage of microorganisms and their enzymes is critical. Oxidoreductase enzymes produced by fungi such as laccase can convert aromatic molecules of xenobiotics to cation radicals and finally CO_2 [1]. As a result, they are detoxified and

mineralization. However, the degrading activities of these extracellular enzymes in the environment may be altered due to the interactions with dissolved organic matter (DOM). Humic substances (HS) including humic acid (HA) and fulvic acid (FA) are major components of DOM and normally found in the terrestrial and aquatic environment. It has been believed that HS have an ability to shield aromatic pollutants from degradation. In the present of oxidoreductases, the bioavailability of pollutants can be decreased through binding to HS mediated by the enzymes [2-5]. Meanwhile, it was found that the enzymes can break down HS into lower-molecular-mass products. For example, extracellular manganese peroxidase was reported to be a crucial enzyme in the degradation process of coal-derived HS [6, 7]. Consequently, the bound pollutants may be released into the environment after HS degradation. To understand the mechanisms involving soil decontamination, this research would focus on the roles of HS to alter enzymatic degradation of aromatic pollutants.

How HS play a role in the system was the key question in the whole research. Three hypotheses for HS' roles were

1. HS can compete with aromatic pollutants for enzyme. HS is a competing enzymatic substrate because HS contains numerous functional groups that are known to be oxidized by oxidoreductive enzymes. Therefore, some HS may act as analogous substrates and disturb the equilibrium of the enzymatic reaction. If this hypothesis is true, modification of humic and fulvic molecules will be observed. UV-VIS spectrophotometer can be used in the analysis of HA and FA's structure changes. Regarding to enzyme kinetic measurement characterized followed Michaelis-Menten equation (1)

$$v = V_{max} S / (K_m + S) \qquad (1)$$

where v is the velocity or rate of enzyme catalyzed reaction, S is the substrate concentration, K_m is the Michaelis-Menten constant and V_{max} is the maximum reaction rate. V_{max} of the condition without HA and FA will be the same, while K_m is increased.

2. HS disables (denatures/deactivates) the enzyme. HS and enzymes interact directly; forming enzyme-humic substance complex. Moreover, HS are able to modify the active center by changing the quaternary or tertiary structure of the enzyme protein. Therefore, HS disable enzyme activities. Changing in HS structure will not be observed. To test whether HS disables enzyme, catechol, which is an ensure substrate for laccase, will be added. If HS disables enzyme, catechol will not be enzymatically degradable. The decrease in the amount of aromatic pollutant will be due to sorption into HS.

3. HS is not a competitive substrate but protects aromatic pollutants (sequestration). Pollutants are associated with HS in sound manner by adsorption and oxidative coupling (solution-phase interactions). Aromatic pollutants are agglomerated into HS. After incorporation, humic molecules may not react with enzymes; therefore, HS help protect aromatic pollutants. Enzyme activities will be active and HS structure may or may not change while aromatic pollutants decrease. Moreover, we will observe lower V_{max} due to substrate is incorporated into HS.

We expected that this information would enhance the potential for designing and improving aromatic pollutants bioremediation strategies. For example, HS could be an important variation by acting as an obstacle for bioremediation successfulness.

Materials and Methods

Chemicals

Pentachlorophenol (PCP) was purchased from Aldrich Company, USA. Stock solution containing 10^3 ppm PCP was prepared in hexane to obtain the absolute dissolution.

Purified laccase from *Trametes versicolor* (Fluka Company, USA) was used in the experiment. The assay for laccase activities was analyzed by using 10 mM catechol in 100 mM sodium acetate buffer pH 5.0 by an increase in absorbance at 440 nm. One unit of laccase activity is defined as that which caused a change in absorbance of 1.0 $min^{-1}ml^{-1}$ at 25°C [8]. Regarding to gain the highest activity of laccase, the enzyme was diluted in 0.2 M sodium acetate buffer pH 5 [8]. Enzyme was prepared freshly in each analysis.

Aldrich humic acid (AHA) (Fluka Company, USA) was originated from lilgnite. AHA was dissolved in 0.5 M NaOH solution and adjusted its volume by distilled water. Then, it was filtered by Whatman Glass microfibre filters. Suwannee River fulvic acid (SRFA) was purchased from International Humic Substances Society (Brookings, SD, USA; http://www.ihss.gatech.edu/). SRFA was obtained from Suwannee River, which was rises in the Okefenokee Swamp in South Georgia, USA. The swamp contains extensive peat deposits. SRFA was dissolved in distilled water.

PCP determination

The PCP concentrations were determined by gas chromatography (6890N, Agilent Technologies) equipped with flame ionization detector. The following instrument parameters were used: column, HP 5 MS (30 m x 0.25 mm id x 0.25 µm); carrier, helium 33 cm/sec constant flow; oven, 35°C for 5 min 35 - 220°C at 8°C/min; injector, 5 µl splitless 250°C, read time, 13.5 min.

PCP degradation studies

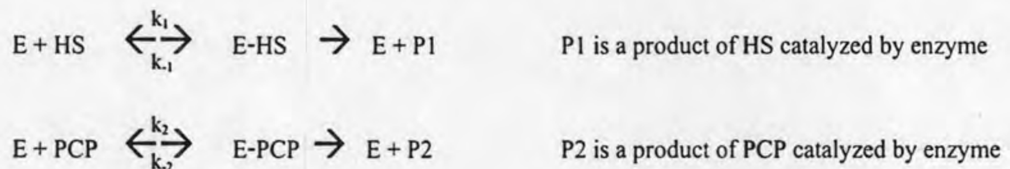
To quantify substrate loss, reactions of 50 ppm of PCP, 20 units of laccase and 400 ppm of HS were investigated at room temperature at pH 5. All reactions were performed statistically and contained in a final volume of 0.4 ml. The reaction mixtures were shaken continuously at 200 rpm, and then they were sacrificed at each analysis. PCP concentrations were monitored at intervals throughout the time for reaction. At each measurement, hexane was added for extraction by shaking at 200 rpm for 1 hour. The supernatant was dehydrated by sodium sulfate and analyzed by GC-FID.

HS structure studies

To examine HA and FA structural changes, UV-VIS spectroscopic method was used because it is the most widely used for preliminary characterization of humic substances [9]. Spectra known to be useful in quantitative characterizing HA and FA are the absorbance at 280, 365, 465, 665 nm and the carbon-normalized UV absorbance at 254 nm [10]. The absorbance was measured with spectrophotometer (Specord 40, Analytik Jena AG).

Enzyme kinetics

To confirm hypothesis 1; HS is a competing enzymatic substrate. K_m and V_{max} of the reaction mixtures with and without HA and FA need to be investigated. Firstly, 30 ppm of PCP was used as a substrate for 20 units/ml of laccase enzyme. 5, 10, 15 ppm of either AHA or SRFA were added as competitive inhibitors. PCP concentrations were analyzed every hour for 4 hours. Then the concentrations of AHA and SRFA providing the linear relationship between PCP concentrations and time were selected. Then, 20 units/ml of enzyme was incubated with the 10, 20, 25, 30, 40 ppm of substrate concentrations for a set period of time over which the reaction is still linear. The amount of substrate changes was plotted (ordinate) versus time (abscissa). The slope was represented as velocity of enzyme (v). To find K_m and V_{max}, plot the data as 1/v versus 1/S. Either HA or FA was added as competitive inhibitors. The same experiment was carried out, then, the result of K_m and V_{max} of the conditions with and without HA and FA was compared. If HS is acting as a competitive substrate, we should observe same V_{max} and higher K_m. The reaction of the mixture with PCP, HS and laccase enzyme (E) can be shown as



Results

Effects of AHA and SRFA on biodegradation of PCP by laccase

After incubation 50 ppm of PCP, with and without 400 ppm of AHA or SRFA and 20 units/ml of laccase, it was shown that PCP was decreased upon the time of reaction. The highest degradation, 97% in 72 hours, was observed in the mixture without HS. AHA and SRFA lowered PCP degradation to 80% and 86%, respectively, in the same period of time (Figure 1). Enzymatic degradation was calculated from the concentration of PCP at intervals (Ct) divided by the concentration of PCP at initial (C0). Laccase was active in all conditions.

HS structure studies

Even though the series of wavelength detected by UV-VIS spectrophotometer that are useful to characterize HS structure was proposed by Macmil et al [10], in this study only absorbance at 365 nm has been used for HS structure change studies because at the other wavelengths, HS's absorbance was disturbed by PCP and laccase. It was found that chemical changes in AHA and SRFA by laccase were significant after 8 days (Figure 2).

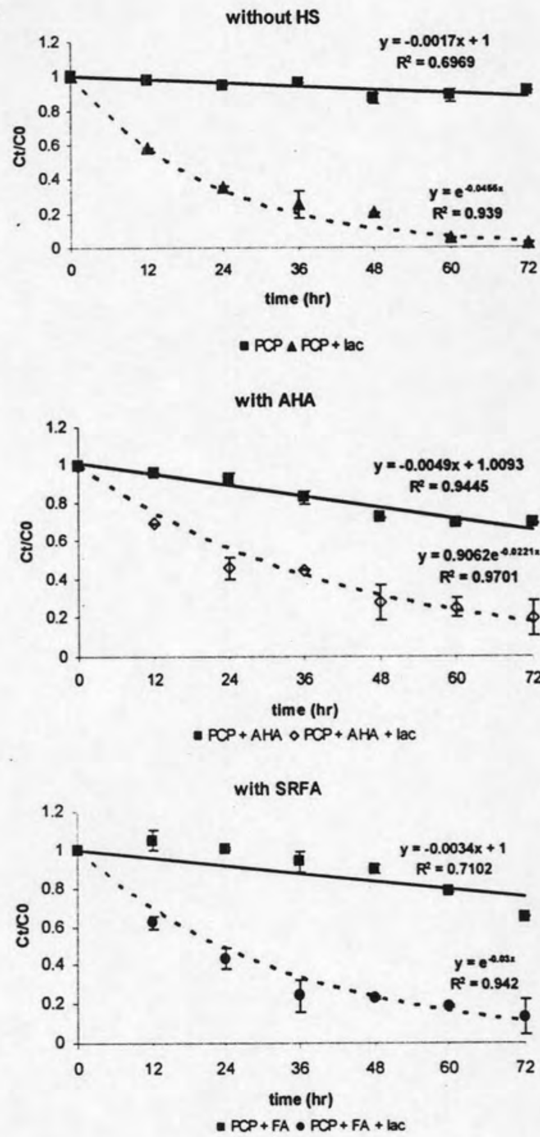


Figure 1 PCP degradation in the absence and presence of HS. Y-axis is [PCP] at cultivated time (Ct) divided by at initial time of reaction (C0). X-axis is time (hours).

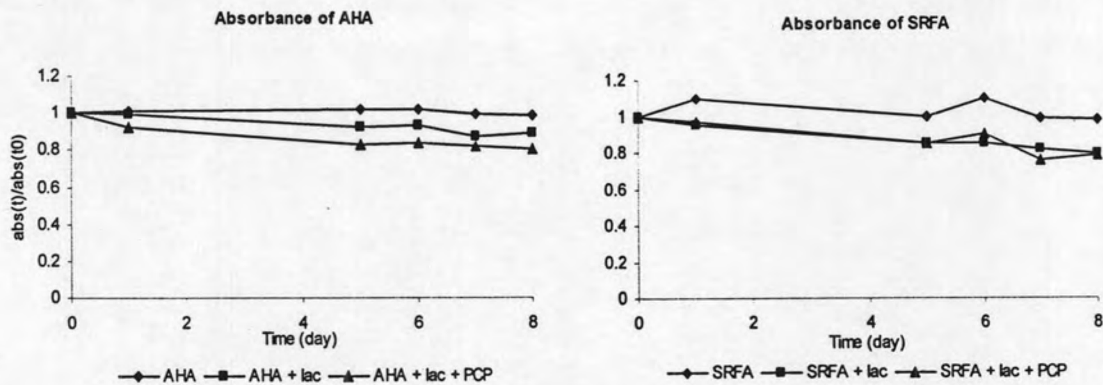


Figure 2 Absorbance of humic substances at 365 nm in the present and absence of laccase.

Inhibitory effects of HS to laccase in PCP biodegradation

The reaction of 30 ppm PCP with 20 units/ml of laccase over 4 hours with 10 and 15 ppm of either AHA or SRFA yielded the linear kinetics. Therefore, 10 and 15 ppm of AHA and SRFA were selected in the K_m and V_{max} measurement experiment. The reaction of PCP with 20 units/ml of laccase showed Michaelis-Menten kinetics in the substrate range of 10 – 40 ppm with a K_m value of 0.18 mM and a V_{max} of 0.001 mM min^{-1}. In the addition of 10 and 15 ppm AHA, K_m values were increasing to 0.32 and 0.51 mM, respectively. With 10 and 15 ppm SRFA, the K_m values were 0.51 and 0.92 mM, respectively. The V_{max} with HS addition resulted 0.001 mM min^{-1}. Figure 3 showed lineweaver-Burke plot, where AHA and SRFA were added as inhibitors. The effect of AHA and SRFA on catalytic activity of laccase for PCP was concluded in table 1.

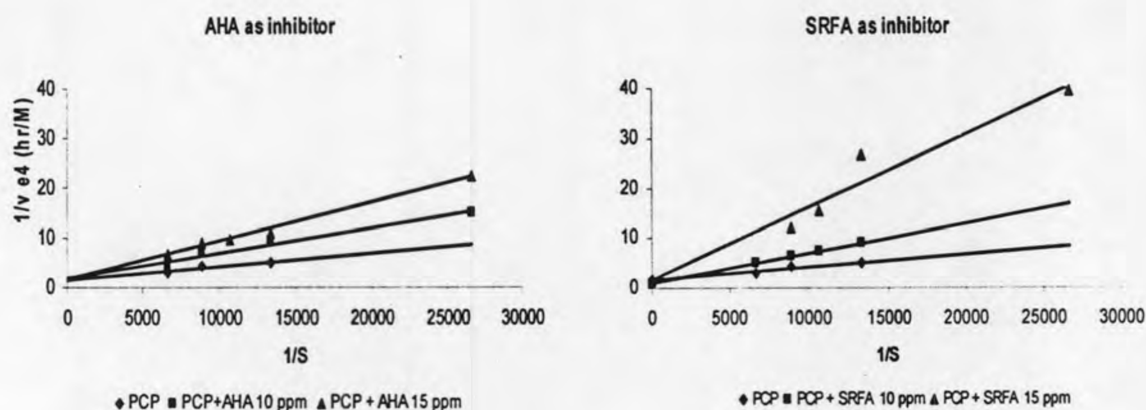


Figure 3 Lineweaver-Burke Plot when AHA and SRFA were added as inhibitors.

Table 1 Effect of AHA and SRFA on K_m and V_{max} values

HS	HS (ppm)	K_m (mM)	V_{max} (mM min^{-1})
Control (no HS)	-	0.18	0.001
AHA	10	0.32	0.001
	15	0.51	0.001
SRFA	10	0.51	0.001
	15	0.92	0.001

Discussion

Laccase from *Trametes versicolor* has previously known to remove PCP from solution at pH 5, depending on initial PCP concentration and amount of laccase [8]. In this research, PCP was enzymatically degraded by laccase both in the presence and absence of HS. With no HS, PCP degradation was higher than with HS existence. Enzyme activity was found to be active throughout the incubation time and, consequently, invalidate hypothesis 2; HS can deactivate enzyme.

HS structure was changed because of the enzyme. Therefore, either hypothesis 1 or 3 was true. Due to increasing K_m values and similar V_{max} followed Michaelis-Menten equation with higher concentration of HS addition, hypothesis 1; HS can act as a competing substrate, was possible.

The K_m value of 0.18 mM for PCP indicated low specificity of the enzyme for the substrate, which confirms by Ullah et al (2000) [8]. Compared K_m of AHA and SRFA addition at the same concentration, K_m of SRFA addition was higher than of AHA. Therefore, SRFA seemed to act as analogous substrate for laccase and compete to PCP degradation more than AHA. The nature and extent of enzymatic degradation should vary depending on characteristics of a given HS. % aromaticity in HS, which is proven to be susceptible to be degraded by laccase, was typically more in humic acids. % aromaticity in AHA and SRFA was 41% and 24%, respectively [11, 12]. However, the result of K_m was not correlated to % aromaticity in HS. This could be explained that those HS were originating different. SRFA was an aquatic fulvic acid, while AHA was a terrestrial humic acid. The oxygen content of the aquatic SRFA (43.9%) [13] was greater than that of the terrestrial AHA (25.1%) [14], which roughly reflects differences in the number of polar functional groups present on the carbon skeleton. Moreover, weight-average molecular weights of AHA and SRFA, which were 4731 and 2519 [15], could affect the PCP enzymatic degradation rate. The smaller molecule may be more readily for enzyme to be catalyzed.

In conclusion, we proposed that HS could be an analogous substrate and it could compete with PCP for oxidoreductive enzymes. Several researchers claimed that decreased amount of pollutant was due to binding and oxidative coupling of the pollutant and HS mediated by enzyme. For example, Park et al (2000) found the same trend for PCP degradation by laccase [16]. They found that with humic acid, chlorophenol transformation by laccase remained the same or reduced compared to without humic acid. They explained that laccase was incapable of generating sufficient oxidation products to enhance binding of chlorinated phenols and humic acid. However, this research initiated the novel idea that at certain concentration of the reaction mixtures, HS could be competitive inhibitor for enzyme and reducing aromatic pollutant degradation rate. HS which is the most widespread and ubiquitous natural nonliving organic materials in terrestrial and aquatic environments and represent a major fraction of soil organic matter should be more concerned in bioremediation technique.

References

[1] Bollag, J., Dec, J., and Huang, P.M. 1997. Formation mechanisms of complex organic structures in soil habitats, Adv Agron, 63, 237 – 266.

[2] Berry, D.F., and Boyd, S.A., 1985. Decontamination of soil through enhanced formation of bound residue, Environ Sci Technol, 19, 1132 – 1133.

[3] Calderbank, A. 1989. The occurrence and significance of bound pesticides residues in soil, Environ Cont Tox, 108, 71 – 103.

[4] Bollag, J. 1992. Decontaminating soil with enzymes: an in situ method using phenolic and anilic compounds, Environ Sci Technol, 26, 1876 – 1881.

[5] Dec, J., and Bollag, J. 1997. Determination of covalent and non-covalent binding interactions between xenobiotic chemicals and soil, Soil Sci, 162, 858 – 874.

[6] Ziegenhagen, D., and Hofrichter, M. 1998. Degradation of humic acids by manganese peroxidase from the white-rot fungus *Clitocybula dusenii*, Basic Microbiol, 38, 289 – 299.

[7] Hofrichter, M., Ziegenhagen, D., Sorge, S., et al. 1999. Degradation of lignite (low-rank coal) by ligninolytic basidimycetes and their manganese peroxidase system, Appl Microbiol Biotechnol, 52, 78 – 84.

[8] Ullah, M.A., Bedford, C.T., and Evans, C.S. 2000. Reactions of pentachlorophenol with laccase from *Coriolus versicolor*, Appl Microbiol Biotechnol, 53, 230 – 234.

[9] Nurmi, J.T., and Tratnyek, P.G. 2002. Electrochemical properties of natural organic matter (NOM), fraction of NOM, and model biogeochemical electron shuttles, Environ Sci Technol, 36, 617 – 624.

[10] Macmil, S.L., AbuBakr, B., Nanny, M.A. et al. Enzymatic transformation of humic substances by naphthalene dioxygenase (NDO). Personal communication.

[11] Ashley, J.T.F. 1996. Adsorption of Cu (II) and Zn (II) by estuarine, riverine and terrestrial humic acids, Chemosphere, 33, 2175 – 2187.

[12] Thorn, K.A., Folan, D.W., and MacCarthy, P. 1989. Characterization of the International Humic Substances Society standard and reference fulvic and humic acids by solution state carbon-13 (^{13}C) and hydrogen-1 (^{1}H) nuclear magnetic resonance spectrometry, Water Resources Investigations Report 89 – 4196. U.S. Geological Survey, Denver, CO.

[13] Available: http://www.ihss.gatech.edu/ [January, 2007].

[14] Malcolm, R.T., and MacCarthy, P. 1986. Limitation in the use of commercial humic acids in water and soil research, Environ Sci Technol, 20, 904 - 911.

[15] O'Loughlin, E.J., Traina, S.J., and Chin, Y.-P. 2000. Association of organotin compounds with aquatic and terrestrial humic substances, Environ Tox Chem, 19, 2015 – 2021.

[16] Park, J.-W., Dec, J., Kim, J.-E. et al. 2000. Dehalogenation of xenobiotics as a consequence of binding to humic materials, Arch Environ Cont Tox, 38, 405 - 410.

08R2-09

ทำเนียบวิทยากร

ชื่อบทความ	บทบาทของกรดฮิวมิคและฟูลวิคต่อการย่อยสลายสารเพนตะคลอโรฟีนอลที่เร่งปฏิกิริยาโดยแลคเคส The Effect of Humic and Fulvic Acids on Laccase Catalyzed Degradation of Pentachlorophenol
ผู้นำเสนอบทความ	นางอาภาภรณ์ ศิริพรประสาร
สถานที่ทำงาน	นิสิตปริญญาเอก หลักสูตรสหสาขาวิชาการจัดการสิ่งแวดล้อม (การจัดการของเสียอันตราย) บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
โทรศัพท์	081-821-2101
ประวัติการศึกษา	- ปริญญาตรี วท.บ. (อุตสาหกรรมเกษตร) สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหาร ลาดกระบัง พ.ศ. 2537 - ปริญญาโท M.Sc., The University of Georgia, USA, พ.ศ. 2541 - กำลังศึกษาต่อปริญญาเอก หลักสูตรสหสาขาวิชาการการจัดการสิ่งแวดล้อม จุฬาลงกรณ์มหาวิทยาลัย พ.ศ. 2547
ตำแหน่งหน้าที่ปัจจุบัน	นักวิชาการสิ่งแวดล้อม 5 สำนักจัดการกากของเสียและสารอันตราย กรมควบคุมมลพิษ
ประสบการณ์	- นักเรียนทุนกองทุนพัฒนาข้าราชการพลเรือน ศึกษาต่อปริญญาโทที่สหรัฐอเมริกา พ.ศ. 2539-2541 - ได้รับทุน JICA อบรมหลักสูตร Risk Assessment and Monitoring for Environmental Chemicals ที่ประเทศญี่ปุ่น พ.ศ. 2546
ผลงาน	-

BIOGRAPHY

Mrs. Apaporn Siripornprasarn, was born on January 6, 1972 in Bangkok. Thailand. She finished her secondary school from Trium Udom Suksa, Bangkok. After that, she joined and graduated in Agricultural Industry, Faculty of Agriculture at King Mongkut Institute of Technology Ladkrabang and graduated in 1994. Afterwards, she won the scholarship from Thai government to study Master's degree in Environmental Health Science at The University of Georgia, USA and graduated in 1998. Then, she worked as an environmental scientist at Pollution Control Department, Thailand since 1999 to 2004. After that she started her Ph.D. degree in International Programs in Environmental Management, Chulalongkorn University and completed the program in 2008.