103b Role of Protein Binding in the Remediation of Pentachlorophenol from Aqueous Streams by a Two-Stage Bioreactor System

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Pentachlorophenol is a toxic chemical that is found in paper mill bleach plant effluent, treated outdoor wood products, soil, ground water and in human urine and serum. Its ubiquitous presence in the environment is due to its prolific use as a biocide, herbicide, pesticide and wood preservative prior to 1984 when it was banned by the EPA in all consumer products and listed as a priority pollutant. Since 1984, use of PCP had been restricted to the pressure and thermal treatment of wood used in commercial or industrial applications. Toxicity of PCP in mammals is attributed to its ability to uncouple mitochondrial oxidative phosphorylation, and to lead to liver disease and DNA strand breakage. The LD50 (lethal dose, 50% kill) of PCP to rats is 27mg/kg. The oxidative products of PCP are tetrachlorohydroquinone (TCHQ) and tetrachlorobenzoquinone (TCBQ), highly reactive compounds but much less toxic than PCP when taken orally. The LD50 of TCHQ is 3319mg/kg and the LD50 of TCBQ is 4000mg/kg. When PCP is metabolized by oxidative pathways, for example in mammals, it produces TCHQ and TCBQ which have been shown to directly bind to liver and blood proteins as well as directly cause DNA strand breakage. However, when TCHQ and TCBQ are metabolized, they do not cause the same type of damage. The white-rot fungus Trametes versicolor has been shown to be highly effective at bioremediation of PCP, producing TCHQ as one of its primary metabolites. In a two-stage bioreactor system T. versicolor was immobilized in polyurethane cubes and utilized to remediate PCP from aqueous solution as well as produce active extracellular fluid. This two-stage bioreactor system was evaluated for its effect on PCP remediation, the presence of the reactive intermediate TCHQ and the role that apparent protein binding had on PCP remediation. Binding of PCP and TCHQ is thought to occur at the sulfhydryl groups on protein. A model protein compound, bovine serum albumin, was reacted with the solutions from the bioreactor system to determine its effect on free compounds and apparent protein bound compounds in solution. Model compounds that contain sulfhydryl groups, such as dithiothreitol, glutathione, and cysteine, were reacted with reactor solutions and a non-reactor solution to determine the effect of sulfhydryl groups on free and apparent protein bound compounds. Treatment of PCP aqueous solutions by the two-stage bioreactor system, utilizing T. versicolor, results in over 99% remediation of free PCP. The reactive intermediate TCHQ was present in each stage of the reactor scheme in concentrations about one-tenth of the concentrations of free PCP in solution. Reactor solutions were also found to contain protein bound PCP and TCHQ. The amount of protein bound PCP was about 10% of the concentration that was free in solution. Protein binding of PCP exhibited a strong dependence on the concentration of reactor protein. Solutions that were reacted with model protein and model sulfhydryl containing compounds exhibited a number of interactions with PCP and the model compounds. When bovine serum albumin was reacted with solutions from the reactor where the immobilized fungus was not present the concentration of free PCP in solution was reduced by about 22%. The model sulfhydryl containing compounds were shown to have a dramatic effect on reducing the amount of free PCP in non-reactor solutions and solutions from the reactor where immobilized cells were not present. However dithiothreitol had to be present for significant effect to occur. Glutathione and cysteine alone did not reduce free PCP significantly, however in the presence of dithiothreitol these compounds reduced free PCP by 49% and 38% respectfully. Dithiothreitol alone was able to reduce the concentration of free PCP by 28%. Sulfhydryl interactions of PCP play on important role in removing this toxic compound from aqueous solutions. With the knowledge that protein binding and more specifically sulfhydryl binding occurs with toxins in solutions, new remediation techniques can be developed that remediate certain compounds from the environment.