ABSTRACT
OXIDATIVE ENZYME-BASED OPTICAL BIOSENSORS FOR DETECTION OF SUBSTITUTED PHENOLS IN ENVIRONMENTAL SAMPLES

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Tyrosinase-based fiber optic biosensor and bilirubin oxidase- based flow injection biosensors were developed for quantification of phenols and pentachlorophenol, respectively. The application of these biosensor systems in environmental monitoring was also studied. Tyrosinase was immobilized in a polyethersulfone membrane mounted in a reaction cell coupled with fiber optics connected to a miniaturized spectrophotometer. The miniaturized spectrophotometer operates in visible range using a diode optimized at 565 nm, with the enhancement of absorbance of the chromophoric product by barbituric acid. Under optimum conditions, this biosensor system gave linear responses to phenol, catechol and p-cresol up to 0.3 mM (phenol and catechol) and 0.2 mM (p-cresol). The detection limit (S/N = 3) was determined to be 0.01 mM for catechol and phenol and 0.1 mM for p-crresol. Bilirubin oxidase (BOX) was immobilized in aminopropyl glass beads and the flow injection (FI) biosensor system for pentachlorophenol (PCP) was designed using a substrate recycling scheme. PCP was efficiently converted to 1,4-tetrachloro-p-benzoquinone (1,4-TCBQ) and then to 1,4-tetrachloro-p-hydroquinone (1,4-TCHQ) by bis(trifluoroacetoxy)iodobenzene (BTFAIB) and zinc powder, respectively. BOX rapidly oxidized 1,4-TCHQ to 1,4-TCBQ, which in turn was readily reduced back to 1,4-TCHQ in the presence of excess 2-Nicotinamide Adenine Dinucleotide (NADH). Under optimized conditions the rate of NADH consumption, measured as the absorbance decrease at 340 nm, yielded a 1,4-TCHQ detection limit of 250 nM. The detection limit was improved to 25 nM by using a fluorescence detector with excitation and emission wavelengths of 345 and 450 nm, respectively. Water samples spiked with phenol were analyzed using the tyrosinase-based fiber optic biosensor while PCP contaminated soil samples were analyzed using the bilirubin oxidase-based flow injection biosensor. The results compared well with the reference methods. The tyrosinase-based biosensor system is nonspecific to phenol; but it can be useful for fast contaminant monitoring and screening. The bilirubin oxidase-based biosensor system is substrate specific to 1,4-TCHQ and, or 1,4-TCBQ.