ABSTRACT

In the presented doctoral thesis, the development of bio-sensing receptor for detection/detoxification of organophosphate compounds is described.

In this work, we report on the development of bio-sensor receptors for successful detection and detoxification of organophosphorus compounds using sol-gel technology. A novel sol-gel immobilization method was developed and used to immobilize the hexahistidine-tagged organophosphorus hydrolase enzyme (His_6-OPH) on various SiO_2 and TiO_2 materials. The influence of the used immobilization technique (entrapment, covalent attachment and adsorption), and nature of the supporting material on retained catalytic properties of the enzyme was studied.

We describe the optimising of the sol-gel process parameter, including aging time of the sol, water/silane ratio, type of the precursor (TMOS and TEOS), and the precursors ratio (TEOS/GPTMS and TMOS/MTMOS) for the successful entrapment of His_6-OPH within SiO_2 sol-gel layers. Furthermore, we developed bio-sensor receptors based on mesoporous TiO_2 films and SiO_2 particles. His_6-OPH was immobilized on mesoporous TiO_2 thin films with uniform (9 nm) and bimodal (13-38 nm) pore size distributions, and SiO_2 particles with pore size distributions of 20 and 6 nm, through covalent attachment and physical adsorption.

Enzyme immobilized on all supports follows the Michaelis-Menten kinetics and retains its catalytic activity. The prepared bio-sensing SiO_2 sol-gel layers retained enzyme activity up to 90 % with expected differences between the starting sols' compositions. Enzyme activity of mesoporous TiO_2 and SiO_2 materials depends strongly on the used immobilization technique (covalent attachment vs. physical adsorption) and porosity of the material, where covalent attachment on more porous material seem as the better choice. The bio-sensor receptors show good activity, and enhanced stability with respect to the free enzyme at extreme conditions of pH and temperature. In addition, the bio-sensor receptors can be easily separated from the reaction media and reused multiple times without significant loss of activity.

Keywords: bio-sensor receptor, sol-gel, organophosphates, His_6-OPH, SiO_2 and TiO_2, mesoporous material, immobilization