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Investigations of microcantilever surface functionalization as potential applications to nanobiosensors.

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Cantilever biosensors have attracted considerable interest in several areas due to high specificity of certain biomolecules (antibodies, enzymes, DNA, etc.), detection of biomarkers, simultaneously with high sensitivity and selectivity in very small volumes of sample. Potential applications of these cantilever biosensors include analysis in biomedical, environmental and agricultural [1]. Other applications of interest in agribusiness are the monitoring of pesticides and metals in water. It is reported the utilization of the alkaline phosphatase enzyme in the development of biosensors for detection of metals such as cadmium, cobalt, zinc, nickel and lead in water [2]Alkaline phosphatase enzyme is important for detection of phosphate [3] and heavy metals [2] in surface waters. The purpose of this study is to compare two different types of immobilization of the microcantilever's surface with alkaline phosphatase enzyme: 1) deposition of 20 nm of gold on the microcantilever surface by "sputtering", 2.5 mmol 16-mercaptohexadecanóico acid (thiol) on the gold and 10 µL (5 mg in 1 mL) of alkaline phosphatase enzyme on thiol. 2) 5 µL of (3-Mercaptopropyl)-trimethoxysilane on the microcantilever surface. The experiments were performed with commercially available rectangular silicon tip-less microcantilevers (350 µm length, 30 µm width and 0.5-1.5 µm thickness with a force constant of 0.07 N m^{-1}, NT-MDT Company, Russia). Surface properties of microcantilevers were monitored by contact angle measurements and atomic force microscopy (AFM). The resonance frequencies of microcantilevers were monitored with Dimension V (Veeco) atomic force microscope. A solution of 10 ppm of $PbCl_2$ was utilized. The efficiency for different immobilizations of microcantilevers was measured by exposed them to 10 ppm $PbCl_2$.solution for 10 minutes, then for 10 minutes to a pH 7.4 phosphate buffer solution, after which this cycle was repeated several times. The resonance frequencies of microcantilevers were measured at each cycle. It was observed that the functionalized microcantilever with silane showed a higher efficiency because the alkaline phosphatase enzyme remained linked to the substrate in a larger number of cycles.

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