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DEVELOPING A NEW ELECTROCHEMICAL ENZYME BIOSENSOR FOR HIGHLY SENSITIVE DETERMINATION OF HEAVY METAL IONS

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Aim. This work was purposed to develop a new electrochemical enzyme biosensor for the highly sensitive determination of heavy metal ions.

Methods. The electrochemical transducers consisted of two pairs of gold-interdigitated electrodes that connected to the impedimetric measuring device. Urease was immobilized with glutaraldehyde on one electrode pair, while a reference membrane with BSA was prepared similarly. Measurements were conducted in 5 mM PBS (pH 7.35) using differential mode to compensate for non-specific signal variations.

Results. The biosensor detected multiple HMIs with high sensitivity and showed high work reproducibility (RSD = 2.28%). EDTA treatment successfully restored enzyme activity, allowing various reuses. The sensor retained 85% activity after 18 months at 4 °C, confirming its long-term stability.

Conclusions. This biosensor provides a cost-effective, sensitive, and reusable platform for sensitive HMIs detection in wastewater, offering an alternative to conventional analytical techniques.

Keywords: biosensor, enzyme inhibition, urease, heavy metal ions.

Heavy metal ion (HMIs) contamination is a serious environmental issue, especially in industrial wastewater. Textile production is one of the significant sources of HMIs such as silver (Ag), cobalt (Co), cadmium (Cd), nickel (Ni), zinc (Zn), copper (Cu), and lead (Pb). These metals accumulate in the environment and pose significant risks to human health [1]. Traditional detection methods, such as inductively coupled plasma optical emission spectrometry and atomic absorption spectroscopy, are highly sensitive but require expensive equipment and trained personnel and are not suitable for real-time monitoring [2, 3]. Therefore, there is a need for cost-effective, rapid, and portable methods for HMIs detection.

Biosensors based on enzyme inhibition offer a promising alternative due to their high specificity, sensitivity, and ability to provide continuous monitoring. In this study, an impedimetric biosensor based on urease inhibition was developed and tested for detecting HMIs in textile wastewater before and after treatment.

Aim. This work was purposed to develop a new electrochemical enzyme biosensor for the highly sensitive determination of HMIs.

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Methods. The electrochemical transducers consist of two identical pairs of gold interdigitated electrodes obtained by gold sputtering onto a ceramic plate. The sensitive surface of each electrode pair was approximately 1.0–1.5 mm. The transducers were connected to the portable measuring device. The immobilization procedure was performed as follows. The enzyme solution was prepared by dissolving 5% urease (EC 3.5.1.5, E. coli (115 U/mg)) in 20 mM phosphate buffer solution (PBS), pH 6.5, containing 10% bovine serum albumin (BSA) and 10% glycerol. The mixture for the reference membrane was prepared by the same procedure using BSA instead of enzymes. The solutions were mixed with 1% aqueous solution of glutaraldehyde (GA) in a 1:1 ratio and were deposited on the electrodes. Then, the membranes were dried in open air at room temperature. Before starting the experiments, the electrodes with membranes were washed out in excess of unbound components using PBS. Measurements were carried out at room temperature in an open cell filled with five mM PBS, pH 7.35. Non-specific changes in the output signal associated with fluctuations in temperature, medium pH, and electrical noise were compensated by using the differential mode of measurement. At least three series of experiments were performed.

The HMIs measurement procedure (Fig. 1) involves recording the initial biosensor response (A_0) to 2 mM urea, followed by 30 min incubation in the sample with HMIs. The response (A_i) is then measured again, with inhibition reducing the signal. Residual activity (R%) and inhibition level (L%) are calculated as $R\% = (A_i/A_0)\times100$ and $L\% = [(A_0 - A_i)/A_0]\times100$. For reuse, the biosensor is reactivated by 30 min incubation in 5 mM EDTA, and the response (A_r) is measured to assess reactivation efficiency.

Results and Discussion. The biosensor showed high sensitivity to HMIs, detecting Ag, Co, Cd, Ni, Zn, Cu, and Pb (Fig. 2). Based on the obtained results, the sensitivity of the biosensor enables the detection of HMIs concentrations exceeding the maximum permissible limits in water. The response was stable, with a relative standard deviation (RSD) of 2.28%, indicating high reproducibility. The optimal incubation time in the solution containing HMIs was determined to be 20–30 minutes. After inhibition, the biosensor was successfully reactivated using EDTA, allowing multiple uses. The reactivation efficiency depended on the reactivation time (the optimal regeneration time using EDTA was established at 30 minutes) and the concentration of chelating agents. The biosensor retained 85% of its initial activity after 18 months of storage at 4 °C, demonstrating excellent long-term stability.

Conclusions. A new electrochemical enzyme biosensor for highly sensitive determination of HMIs was developed. The biosensor exhibited high sensitivity, excellent reproducibility, and long-term stability. The possibility of multiple reuses after EDTA reactivation was demonstrated.

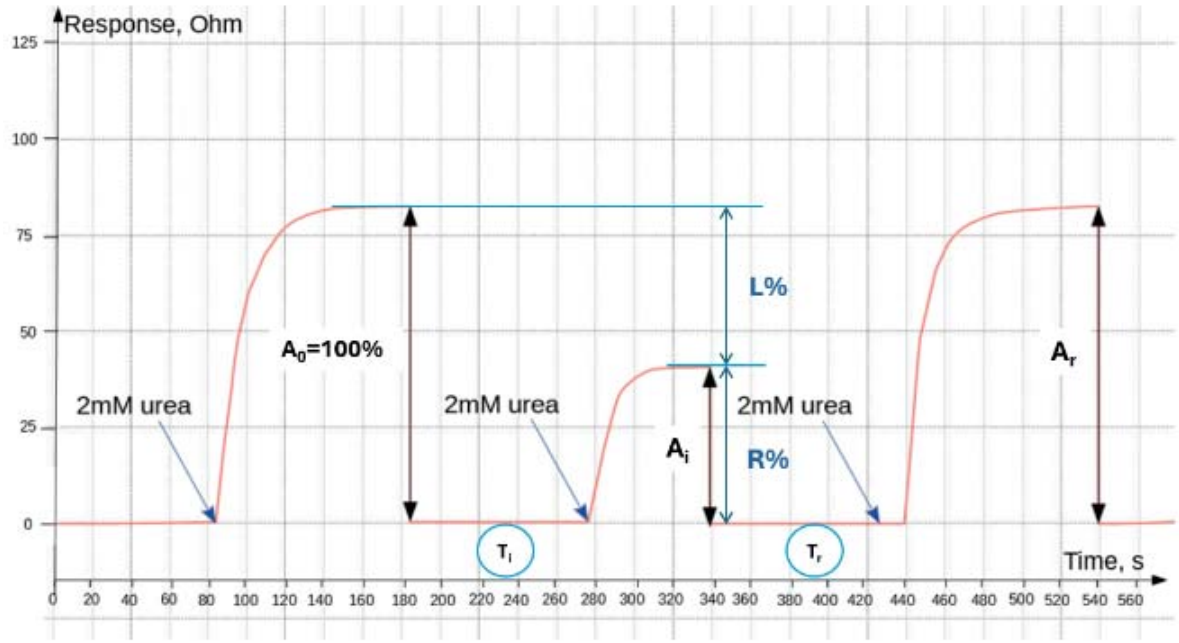

Fig. 1. **Inhibitory determination of heavy metal ions by biosensor:**
A_0 — response before inhibition; A_i — response after inhibition; A_r — response after reactivation; T_i — inhibition time (30 min); T_r — reactivation time (30 min)

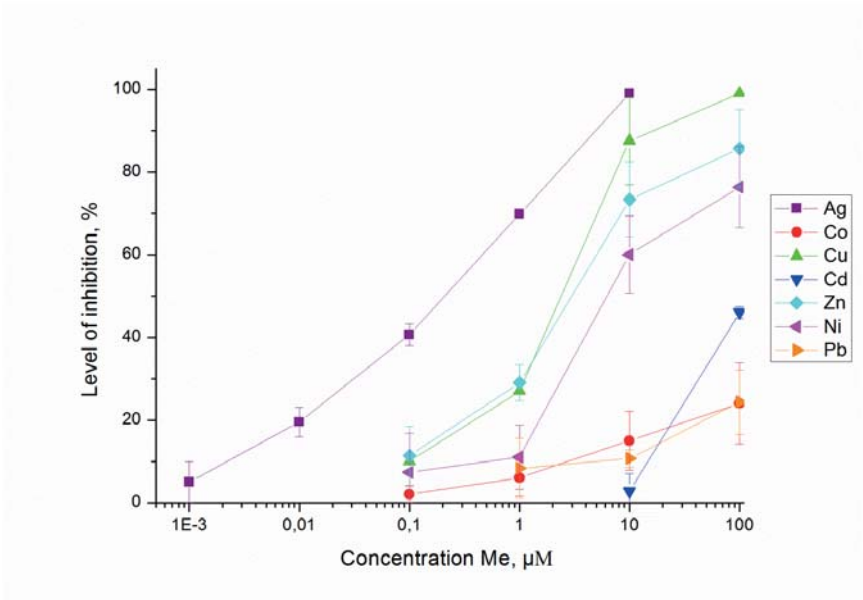

Fig. 2. **Dependence of inhibition level of urease-based biosensors on different concentrations of HMIs Measurements were carried out in 5 mM phosphate buffer solution, pH 7.35; concentration of urea 2.0 mM.** All measurements were performed with a minimum of three replicates

This biosensor provides a practical and efficient solution for environmental monitoring, offering a valuable alternative to conventional methods.

Authors' contribution

VAB performed experiments, data analysis, and presentation; OOS aimed for data analysis; SVD aimed for data analysis; and VMP managed the project and developed a plan of experiments.

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