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Purification, Characterization and Kinetic Study of Alpha Naphthyl Acetate Esterase From Atta Flour
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Abstract

As the most promising alternative to detect pesticides, enzyme-based electrochemical biosensors have gained considerable attention due to its operational simplicity and reliability. Alpha naphthyl acetate esterase (ANAE) enzyme, found in atta flour, can be used to detect pesticides. The crude ANAE enzyme was extracted from atta flour, filtered through a PTFE membrane and then purified using an aqueous two-phase separation system (ATPS). Polyethylene glycol (PEG) was used as it could enhance the catalytic activity and stability of enzyme in the ATPS. The ATPS, composed of PEG 1000/NaH₂PO₄ and PEG 1000, NaH₂PO₄/(NH₄)₂SO₄, was performed at 4 °C and pH 5.0. The molecular weight of ANAE was assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of the target esterase was found to be around 65 kilodaltons (kDa). The optimal condition of ANAE to catalyze alpha naphthyl acetate as substrate was at 40 °C with pH 8 of phosphate buffer and 15 min incubation time. The Michaelis-Menten parameters of the purified enzyme were 9.765 mM and 0.084 mM/min, respectively for Km and Vmax. This purified ANAE can be applied to fabricate of screen-printed electrode as biosensor for the detection of pesticides. © The Author(s).

Author Keywords

alpha naphthyl acetate esterase (ANAE); aqueous two-phase separation system (ATPS); biosensor; enzyme; Michaelis-Menten; pesticide

Index Keywords

Biosensors, Catalyst activity, Electrophoresis, Esters, Molecular weight, Naphthalene, Nitrogen compounds, Phase separation, Purification, Sodium dodecyl sulfate, Sulfur compounds; Alpha naphthyl acetate esterase, Aqueous two phase, Aqueous two-phase separation system, Characterization studies, Electrochemical biosensor, Esterase enzymes, Kinetic study, Michaelis-Menten, Separation systems, Two-phase separation; Pesticides

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