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Purification and Properties of 2-hydroxy-1,4-naphthoquinone Reductase

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Quinone reductase is known to be a xenobiotic metabolizing enzyme and is highly inducible in animals following pretreatment with various xenobiotic chemicals including polycyclic aromatic hydrocarbons and other planar aromatic compounds. 2-hydroxy-1,4-naphthoquinone reductase was purified to electrophoretic homogeneity from duck liver, and the subunit molecular mass of the enzyme was estimated to be 40.3 kDa by SDS-polyacrylamide gel electrophoresis. In addition to the 1,4-naphthoquinone, the enzyme also catalyzed the reduction of 4-nitrobenzophenone and 1,4-benzoquinone. The activity of the enzyme was markedly inhibited by Cu^{+2} and Mn^{+2}. The purified enzyme was inactivated by treatment with N-bromosuccinimide, a reagent that modifies tryptophan residue. Kinetic constants for some substrates were determined. And some other molecular and catalytic properties will be presented.

Key words: Purification, property, 2-hydroxy-1,4-naphthoquinone reductase

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Optimal Conditions for Mass Separation of Pullulan from Culture broth of *Aureobasidium pullulans* HP-2001

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Optimal conditions for mass separation and purification of pullulan produced by *Aureobasidium pullulans* HP-2001 in a 100L bioreactor were investigated. *A. pullulans* HP-2001 was cultivated in the medium containing 5.0% (w/v) glucose, 0.25% yeast extract, 0.5% K_2HPO_4, 0.1% NaCl, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.06% $(NH_4)_2SO_4$ at 30°C for 96 hr. The filter press and the tubular type of a continuous centrifuge were used for separation of cells from culture broth. The optimal concentration of the diatomite to mix with culture broth for separation of cells using the filter press was 5.0% (w/v). The optimal feeding rate of culture broth for separation of cells using the tubular continuous centrifuge was 2 L/min. Pullulan in the supernatant after removal of cells was concentrated with 8 times using the ultra filtration system and its recovery yield was 70.3%. Optimal conditions for precipitation of pullulan from the concentrated supernatant after removal of cells were investigated with the orthogonal array method with five distinct levels and three factors. Organic solvents used in this study were ethanol and iso-propanol. Optimal temperature, reaction time and volume of iso-propanol added into the supernatant for isolation of pullulan were 4°C, 24 hr and 3 times, respectively.

Key words: *Aureobasidium pullulans* HP-2001, orthogonal array, Filter press, continuous centrifugation, U/F