Thesis Title	The Development of Bromoperoxidase Production from Red Algae *Polycarvernosa* sp.
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Abstract

Effect of various cell disintegration techniques on enzyme extraction were studied for the purification. These techniques were ; grinding with detergent (Triton X-100 and sodium deoxycholate), grinding with ultrasonication, freeze and thaw, grinding with freeze and thaw, grinding with agitation. Grinding with agitation (agitation time of 6 hours) provided a good result. Specific activity of bromoperoxidase was increased up to 57.54% when compared with grinding alone. Effects of various precipitants on fractionation by precipitation were also investigated. These precipitants were ; ammonium sulphate, methanol, ethanol and acetone. Fractionation by acetone gave the highest degree of purification (degree of purification is 4.21). At the purification stage, DEAE-Toyopearl was shown to be a greater anion-exchanger than that of DEAE-Sephadex, with 7.24 degree of purification. Comparison of the effect of separation by gel filtration using Sephadex G-75 and Biogel A-0.5 M were also studied. The degree of purification from Sephadex G-75 was 14.42, while from Biogel A-0.5 M was lower. The protease concentration in crude enzyme was 267 μU/ml, but in partial purified enzyme was only 0.002 μU/ml. Molecular weight of the extracted bromoperoxidase was approximately 615,000 dalton. Bromoperoxidase was deactivated by dialysis against EDTA, then reactivated specifically by vanadium. It was found that vanadium 0.6 mM was suitable for partial purified enzyme reactivation. Effect of temperature on stability was studied, partial purified enzyme was more stable than crude enzyme. The partial purified enzyme was stable at acidic pHs down to 4 and at alkaline pHs up to 9, The optimum pH and temperature of the partial

purified enzyme activity were 6 and 55°C respectively. Crude enzyme was stable at acidic pHs down to 4 and at alkaline pHs up to 9. The optimum pH and temperature of crude activity were 6 and 55°C respectively. After maintaining at 4°C for 40 days, crude enzyme activity was decreased to 47.50%, then partial purified enzyme activity was decreased to 26.07 %. Crude enzyme activity and partial purified enzyme activity were decreased to 34.48 % and 30.63 % respectively, when kept at -20°C for 40 days.

Keywords : Bromoperoxidase / *Polycarvernosa* sp. / Red algae