CHAPTER VI

CONCLUSIONS

1. The greatest extent of PEG-DH and PPG-DH activities were observed with 3% (v/v) PEG 4000 and 1.5% PPG 1000 (v/v) as sole carbon and energy sources for *Pseudomonas* sp. PE-2, respectively.

2. PEG-DH and PPG-DH of *Pseudomonas* sp. PE-2 are inducible enzymes.

3. Most of the PEG-DH activity (76.6%) was found in the periplasmic fraction, while PPG-DH activity (82%) was found mainly in the cytoplasmic fraction.

4. PEG-DH from *Pseudomonas* sp. PE-2 was partially purified by 30-45% saturated ammonium sulfate precipitation, DEAE-650M Toyopearl and Phenyl-Sepharose CL-4B column chromatography with 14.8% yield, 11.8 purification fold and specific activity of 547.4 nmol/min.mg protein.

5. PPG-DH from *Pseudomonas* sp. PE-2 was partially purified by 60-80% saturated ammonium sulfate precipitation, DEAE-650M Toyopearl and Butyl-Toyopearl column chromatography with 14.1% yield, 51.4 purification fold and specific activity of 480.2 nmol/min.mg protein.

6. PEG-DH from *Pseudomonas* sp. PE-2 was a monomeric protein with the molecular weight of 73.6 kDa, while PPG-DH from *Pseudomonas* sp. PE-2 was a homodimeric protein of two identical subunit size of 36.1 kDa.

7. The optimum pH and temperature of the PEG-DH and PPG-DH were at pH 9.0, 25°C and pH 7.5, 25°C, respectively.

8. The partially purified PEG-DH and PPG-DH were stable in the pH range of 8.0 to 9.5 and 7.0 to 8.0, respectively.

9. Both enzymes were stable below 30°C.

10. Pyrroloquinoline quinone (PQQ) played important role of the cofactor for PEG-DH and PPG-DH.

11. Co^{2+}, Cu^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+} and Ni^{2+} inhibited the PEG-DH activity by 8-100%, while metal ions inhibited the PPG-DH activity approximately 5-30% except Ni^{2+} which could enhance the PPG-DH activity about 38% when compared with Ca^{2+}.

12. The determination of substrate specificity of PEG-DH and PPG-DH towards various hydroxyl compounds showed that among the substrates tested they preferred relatively longer chain primary alcohols than the shorter ones. Contrarily, PEG and PPG at lower molecular weight seemed to be the preferred substrates.

13. The apparent K_m values of PEG-DH for PEG 600, PEG 2000, PEG 4000, PEG 6000 and PEG 8000 were about 0.4, 3.3, 4.6, 30.5 and 28.3 mM and V_{max} were 87.72, 75.19, 76.92, 181.82 and 128.21 nmol min^{-1} mg $protein^{-1}$, respectively.

14. The K_m values of PPG-DH for PPG 725, PPG 1000 and PPG 2000 were about 5.1, 4.6 and 12.6 mM and V_{max} were 38.82, 49.75 and 32.57 nmol min^{-1} mg $protein^{-1}$, respectively.