CHAPTER I

INTRODUCTION

1.1 Statement of problem

Polyethers have a long history as specialty polymers used as raw materials for synthesizing detergents or polyurethanes and in their original forms in lubricants, antifreezing agents, inks, cosmetics, etc (Huang *et al.*, 2005). They are either water-soluble or oily liquids, which eventually find their way into either environment or wastewater systems. The polyethers include polyethylene glycol (PEG), polypropylene glycol (PPG), polytetramethylene glycol (PTMG), polybutylene glycol (PBG), polyglycerin, polyglycidol, polyisobutylene oxide, polyglyoxylate and others, including various copolymers synthesized from ethylene oxide and propylene oxide.

Among these compounds, polyethylene glycol (PEG) is an important group of non-ionic synthetic water-soluble polymers of ethylene oxide (Huang *et al.*, 2005). Because of their low toxicity and lack of skin irritation, PEGs are widely used in the pharmaceutical industry in the preparation of ointments, suppositories, tablets, and solvents for injection, as well as in cosmetics such as creams, lotions, powders, cakes, and lipsticks. PEGs are also used as intermediates in the preparation of resins such as alkyd and polyurethane resins, and as components in the manufacture of lubricants, antifreeze agents, wetting agents, printing inks, adhesives, shoe polish, softening agents, sizing agents, and plasticizers. In addition, PEG has been used as resin gels that are used to immobilize enzymes or microbial cells, and for the chemical modification of enzymes (Kawai, 2002).

Chemically unsubstituted polypropylene glycol (PPG) is a synthetic polymer consisting of 1,2-propane oxide, which includes structural isomers derived from primary and secondary alcohols, and has various optical isomers due to asymmetric carbons. PPG has straight or branched chain structures (diol and triol types, respectively) (Tachibana *et al.*, 2002). PPG (approximately 1,000 daltons) is usually a water-insoluble and oily liquid, unlike PEG which is water-soluble up to few million daltons. PPG is used in solvents for drugs and as the ingredients of paints, lubricants, inks and cosmetics, though most are transformed to polyurethanes or surface-active agents. PPG and PEG are synthesized into block copolymers and used as amphoteric surfactants, where PEG is a hydrophilic and PPG is a hydrophobic constituent (Kawai, 2002).

The annual worldwide production of PEG and PPG were in the range of one million metric tons (Peretti *et al.*, 2005). At this time, the production level has been increased due to an expansion in the need for nonionic surfactants and other products including PEGs and PPGs, though no exact data are available on the total amounts of PEG and PPG produced and used. This is partly due to the fact that nonionic surfactants and other derivatives are synthesized from ethylene oxide or propylene oxide, and not from PEG or PPG. The production level of PPG is greater than that of PEG, but most PPG is transformed into polyurethanes. However, the amount of PEG entering water streams is greater than that of PPG, based on these applications (Kawai, 2002).

These chemically stable compounds so far do not form much of an environmental problem because they can be assimilated by biological degradation (Tachibana *et al.*, 2002). However, due to the extensive use of PEG and PPG, they can enter and pollute the industrial sites and the environment and thus become environmental problem since they can neither be recycled nor incinerated.

Therefore, understanding the biodegradation pathway as well as microorganisms and enzymes involved is necessary.

In line with this, there have been extensive screening and isolation of bacteria capable of using PEGs and PPGs as carbon and energy source. It has been reported so far that PEG-utilizing bacteria do not grow on PPG and *vice versa*, suggesting that the biological degradation pathways of PEG and PPG could be dissimilar (Tachibana *et al.*, 2003). On the contrary, we found that the bacterium *Pseudomonas* sp. PE-2 can be grown on both PEG and PPG. PEG and PPG dehydrogenase activities were induced during growth on either PEG or PPG and might be responsible for the assimilation of PEGs/PPGs. Therefore, partial purification and characterization of polyethylene glycol dehydrogenase (PEG-DH) and polypropylene glycol dehydrogenase (PPG-DH) will be needed for understanding of PEG and PPG degradation processes.

1.2 Objectives

Since PEG-DH and PPG-DH have been reported to be involved in the assimilation of PEG and PPG, in this research, partial purification and characterization of the PEG-DH and PPG-DH from *Pseudomonas* sp. PE-2 capable of growing on either PEG or PPG were investigated.

1.3 Scope of study

1.3.1 To determine optimum concentration of PEG or PPG for cell growth and induction of PEG/PPG-degrading enzymes

1.3.2 To examine localization of PEG/PPG-degrading enzymes

1.3.3 To prepare partially purified PEG/PPG-degrading enzymes

1.3.4 To characterize the partially purified PEG/PPG-degrading enzymes

1.4 Expected benefits

The PEG-DH and PPG-DH partially purified from *Pseudomonas* sp. PE-2 will be unique enzymes and efficiently degrade "high molecular weight" PEG and PPG. The characteristic of enzymes will be useful information for the remediation application on site contaminated with PEGs and PPGs.

1.5 Thesis organization

This thesis is comprised of seven different chapters. First, chapter I provide the introduction part of this research. Second, the theoretical background and literature review are described. Third, the research methodology is explained. After that, the results and discussions are demonstrated and then these results are concluded. Finally, the suggestion and future works are orderly described.