CHAPTER 1

INTRODUCTION

1.1 General ideas

Alkaline proteases are very important industrial enzymes. They are used widely in detergent, cleaning stains and soils containing proteins (blood, grass, milk, gravy, tomato sauce, etc.), food, pharmaceutical, leather and film industries, as well as in waste processing industries (Hayashi et al, 1967). Alkaline protease acts as a biocatalyst cutting proteins into peptides chains under the presence of water, according to the following reaction:

$$\text{Protein} + H_2O \xrightarrow{\text{Alkaline protease}} \text{Peptides}$$

One of the conventional methods for alkaline protease production using aqueous two-phase system comprises the following three processing steps:

Bio-reaction ---→ Extraction -----→ Back extraction

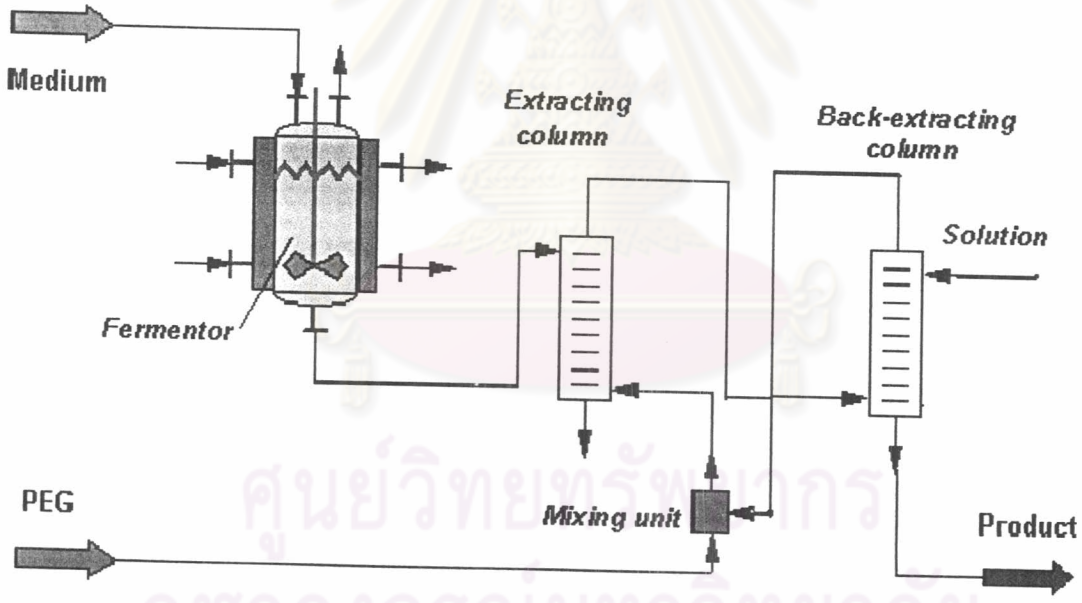

Figure 1.1: Traditional fermentation process using aqueous two-phase system

In Figure 1.1, a stream of medium goes into a main fermentor for enzyme production after which the fermented broth is fed to an extracting column which is also fed with an aqueous solution of Polyethylene Glycol (PEG) for enzyme extraction. The top phase from the extracting column is subsequently pumped into a back-extracting column for further enzyme purification and PEG recovery. A mixing unit is used for mixing reused and fresh PEG before being fed back to the extracting column.

To produce alkaline protease, the fermentation process is carried out using some microorganisms such as alkalophilic *Bacillus pumilus*, *Spilosoma oblique, Bacillus subtilis*, *Bacillus thuringiensis* etc. (Hotha et al, 1997. Lee et al, 1990. Fikret et al, 2003). However, low production yields, product inhibition, and complexity of recovery system result in high production costs remain problem areas.

Therefore, in order to overcome the problem due to product inhibition to microorganisms, extractive fermentation -a combined fermentation, and product extraction in a single unit- is considered in this research work for alkaline protease production. Extractive fermentation using aqueous two-phase system is a promising alternative to the conventional process, since it provides a non-denaturing natural environment for biomolecules, and stabilizes cells (Lee, et al, 1990; Hotha et al, 1997; J. Planas et al, 1996).

Aqueous two-phase system (ATPs) was first developed in Sweden during the mid-1950s for separation of macromolecules, cells, and organelles. As hinted by its name, aqueous two-phase is an aqueous, liquid–liquid, biphasic system that could be obtained either by mixing aqueous solution of two polymers, or aqueous solution of polymer and salt (Albertsson, 1958). This polymer-salt system results in higher selectivity in protein partitioning, leading to an enriched product with high yields in an extraction step. In addition, aqueous two-phase systems offer an effective extraction process for various biomolecules. Their advantages are short process time, and possible attainment of high product yield, and purity. Moreover, extraction using aqueous two-phase system is an economical technology with low energy consumption, low labor cost requirement, and has great potential for further process development (R. Gupta et al 2001). Scale-up processes based on aqueous two-phase systems are, furthermore, simple, and a continuous steady state is possible. Therefore, the aqueous two-phase system was chosen in this research work as an appropriate system for extractive fermentation of alkaline protease production of which knowledge in this field is still very limited.

Aqueous two-phase systems of interest in this research work comprise polyethylene glycol (PEG) and potassium phosphate. Although previous works of other groups concentrated on extractive cultivation of alkaline protease in ATPs containing PEG and dextran T500 (Lee et al, 1990), but the exorbitant price of dextran T500 limits its use (Hotha et al, 1997). PEG is a macromolecule with chemical formula of $H(OCH_2CH_2)_nOH$. Due to its nontoxic character, this chemical is commonly used in cosmetics, food, and pharmaceutical products. The biocompatible character of PEG explains success of this polymer in biotechnology applications. In addition, it is also commonly used for liquid-liquid partitioning and precipitation of biomacromolecules (Albertsson, 1986). More importantly, research work in our group previously revealed that partition coefficient of alkaline protease determined at the value of 49.1 at pH 7.5 in PEG

1000/ PO^{3-}_4 system is relatively high (Chuayyok, 2001). Therefore, it should be a suitable for extractive fermentation in this research work.

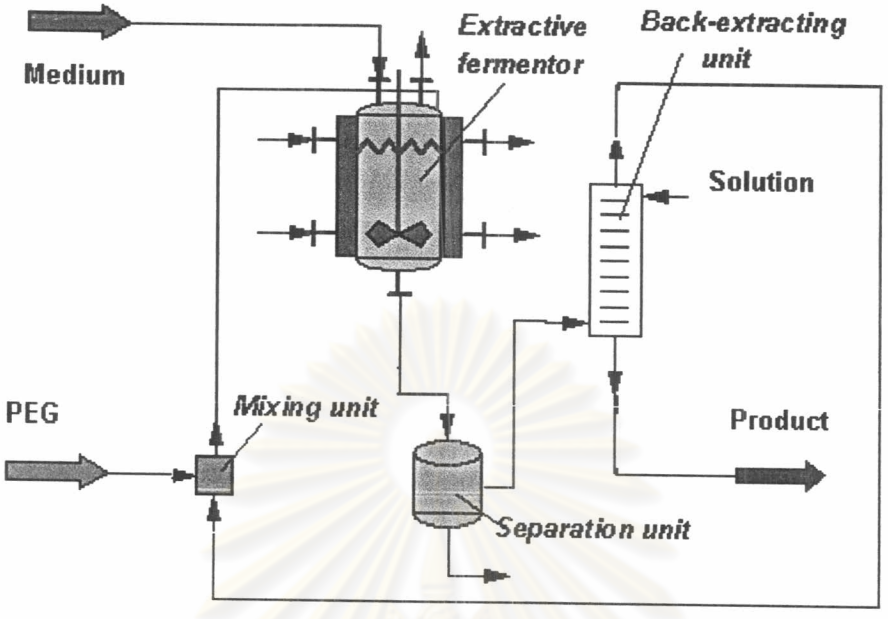

Figure 1.2: Extractive fermentation process

In Figure 1.2, the cells are cultivated in an extractive fermentor with APTs of PEG and phosphate. After fermentation, both phases will be pumped to separating unit for phase separation. The top phase will then be moved to back-extracting column for enzyme purification and PEG recovery. The reused PEG is refreshed with fresh PEG in mixing unit before taken back to extractive fermentor. In this process, an extracting column and a fermentor are combined to a single unit of extractive fermentor (Figure 3). The process is considered simpler than the process shown in Figure 1.

1.2 Objective

To select a suitable aqueous two-phase systems for batch extractive alkaline protease fermentation.

1.3 Scope of work

The scope of the research work will cover:

1.3.1 Investigation of extractive fermentation for alkaline protease production using PEG 1000/ potassium phosphate aqueous two-phase systems.

1.3.2 Exploration and selection of other ATPs if PEG 1000/ potassium phosphate system is not suitable for extractive fermentation.

1.3.3 Study effects of system volume ratio on:

- Partition coefficient of alkaline protease, and glucose
- Enzyme productivity and purity

1.4 Expected benefits from the research

1.4.1 Deeper understanding of the extractive fermentation process for alkaline protease production.

1.4.2 Results from this study may benefits process development of alkaline protease production.

1.5 Research procedures

1.5.1 Literature survey

1.5.2 Perform experimental studies on extractive fermentation for alkaline protease production

1.5.3 Data analysis and conclusion

1.5.4 Write the completed report