CURRENT RESEARCH AND DEVELOPMENT IN BIOTECHNOLOGY ENGINEERING AT IIUM

VOLUME III

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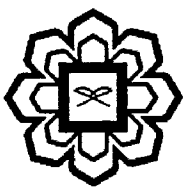

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CHAPTER 5

DETERMINATION OF OPTIMAL RANGE OF POST-INDUCTION TEMPERATURE FOR PRODUCTION OF SOLUBLE RECOMBINANT BROMELAIN IN *ESCHERICHIA COLI* USING ONE-FACTOR-AT-A-TIME (OFAT) APPROACH.

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ABSTRACT

In this work, a one-factor-at-a-time (OFAT) experiment for determining the optimal preliminary range of post-induction temperature (°C), before subsequent optimization of recombinant bromelain production using Response Surface Methodology (RSM), are discussed. Bromelain from *Ananas comosus*, in nature, being a eukaryotic plant-origin proteolytic enzyme, has been subjected to post-translational modification such as glycosylation and presence of native molecular chaperones for aiding the protein folding. However, attempts to express this enzyme in *Escherichia coli* might incur undesirable potential drawback, which is, high tendency of the recombinant bromelain to express in the form of inactive insoluble wasted fractions known as inclusion bodies. Induction temperature has been known to be one of the controllable factors which could minimize the formation of inclusion bodies. Here, post-induction temperatures between 21 – 41°C were studied and their effects on the recombinant bromelain specific activity (U/mg) were evaluated. We found that post-induction temperature around 26°C marked highest value of specific activity (~14 U/mg) and therefore, shall be used as a center-point for the next RSM analysis.

Keywords: recombinant bromelain, *Escherichia coli*, post-induction temperature, OFAT.

INTRODUCTION

For decades there has been huge interest of research studies involving a group of catalytic protein known as hydrolases. This group of protein, or enzyme, autonomously catalyzes the cleavage, or hydrolysis, of chemical bonds within the nascent structure of a biological molecule. In nature, they predominantly and diversely exist in the physiological process for all major species of organisms in our biosphere – in mammalian, bacteria, fungus and plants. In this work, interest will be on a "species" of