Enhanced production of periplasmic interferon alpha-2b by *Escherichia coli* using ion-exchange resin for *in situ* removal of acetate in the culture

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**ABSTRACT**

The possibility of using *in situ* addition of anion-exchange resin for the removal of acetate in the culture aimed at improving growth of *E. coli* and expression of periplasmic human interferon-α2b (PrIFN-α2b) was studied in shake flask culture and stirred tank bioreactor. Different types of anion-exchange resin were evaluated and the concentration of anion-exchange resin was optimized using response surface methodology. The addition of anion-exchange resins reduced acetate accumulation in the culture, which in turn, improved growth of *E. coli* and enhanced PrIFN-α2b expression. The presence of anion-exchange resins did not influence the physiology of the cells. The weak base anion-exchange resins, which have higher affinity towards acetate, yielded higher PrIFN-α2b expression as compared to strong anion-exchange resins. High concentrations of anion-exchange resin showed inhibitory effect towards growth of *E. coli* as well as the expression of PrIFN-α2b. The maximum yield of PrIFN-α2b in shake flask culture (501.8 μg/L) and stirred tank bioreactor (578.8 μg/L) was obtained at ion exchange resin (WA 30) concentration of 12.2 g/L. The production of PrIFN-α2b in stirred tank bioreactor with the addition of ion exchange resin was about 1.8-fold higher than that obtained in fermentation without ion exchange resin (318.4 μg/L).

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1. Introduction

Commercial production of recombinant proteins for industrial and pharmaceutical applications has increased significantly in recent years. In addition, advance in the field of biotechnology has led to an enormous development of recombinant bacterium especially *Escherichia coli*. Production of proteins through *E. coli* gains more advantages than the other hosts due to its high growth rate and better understanding of the cultivation process. Interferon, glycoprotein which comes under cytokines family, can be classified into three types (I, II, and III). Interferon-alpha2b is under type I, which is used for the treatment of hairy cell leukemia, chronic hepatitis B and C [1-3].

During aerobic fermentation of *E. coli*, mixed organic acids such as lactic acid, succinic acid, acetic acid and carbonic acid were produced as co-products which caused reduction in the culture pH [4]. The accumulation of these organic acids, especially acetic acid, inhibited growth of the recombinant protein-producing cells [5,6]. In addition, the presence of these organic acids in the culture disrupted the intracellular oxidative metabolism of *E. coli* when they diffused into the cell in unionized form and dissociated within the cells into ionized form. Consequently, growth of *E. coli* is inhibited by the acidic pH of external environment due to the loss of pH homeostasis [7]. *E. coli* is generally capable of maintaining protein stability by regulating cytoplasm pH, ranging from 7.2 to 7.8 [8]. *E. coli* cells responded immediately when the pH of extracellular environment decreased due to the accumulation of mixed organic acids. Thus, intracellular pH of *E. coli* need to be recovered by the regulation mechanism [9]. However, recovery from the acidification was reduced or halted due to the presence of organic acids such as acetate or benzoate [10].

Growth characteristics and acetate production of several *E. coli* strains have been compared in different fermentation methods.