Thesis Title	Production of Heterologous Protein by Recombinant *Saccharomyces cerevisiae*
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ABSTRACT

The growth of a protease-deficient recombinant yeast strain, *Saccharomyces cerevisiae* harboring a pEB4 plasmid which contained a yeast *GAL10* promoter and the *E. coli* β-galactosidase gene was characterized in non-selective and selective media. When the yeast cells were grow in non-selective medium buffered with 0.15 M citrate-phosphate in shake-flasks, the highest cell concentration and lower ethanol production were obtained at 30 °C and pH 4.5. Statistic factorial design experiments indicated that C/N and C/P ratios of 18.9 and 266, respectively, were needed for high biomass production in selective medium. Requirements for vitamins, amino acids, and growth factors were investigated by Plackett-Burman design. Expression of heterologous β-galactosidase gene induced by 15-40 g/l galactose resulted in maximum enzyme activity after 30 h of flask cultivation. Furthermore, maximum specific enzyme activity obtained when the induction time of β-galactosidase enzyme was employed at the initial stage of cell growth in either flask or bioreactor.

Growth kinetics and levels of β-galactosidase expression were also investigated in a bioreactor. The maximum specific growth rate of 0.19 h^{-1} in batch cultivation was obtained when glucose was employed as the sole carbon source in synthetic medium. Moreover, higher specific growth rate (0.36 h^{-1}) was achieved in complex media. The expression level of β-galactosidase in batch cultivation either in synthetic or complex medium were 1.112 and 1.655 U/mg protein, respectively. The optimal fed-batch cultivation condition was achieved for the high production of β-galactosidase (6.5 U/mg protein) corresponding to 9.6-9.86 % of total yeast soluble protein, with high productivity (0.111 U/mg protein-h). The final biomass concentrations achieved were 7.4-11.9 g DCW/l in the fed-batch cultivations which were 3-4.8 times higher than biomass concentration obtained from batch cultivation in synthetic medium (2.5 g DCW/l)