

Reference

260

Influence of pH on cellular growth of *Pichia pastoris* KM71H by fed-batch process

Wanderley (4), Marcela Silvestre Outtes (1,2,3); Oliveira, Carla (1); Mussatto, Solange Inês (1); Brunaska, Danyelly (2,3,5); Lima Filho, José Luiz (2,3,4); Domingues, Lucília (1); Teixeira, José Antonio (1)

- 1: University of Minho, Portugal;
- 2: Grupo de Prospecção Molecular e Bioinformática (ProspecMol), Brazil;
- 3: Lab. de Imunopatologia Keizo Asami (LIKA), Brazil;
- 4: Programa de Pós-graduação em Ciências Biológicas, Brazil;
- 5: Departamento de Bioquímica, Brazil

E-mail: marcelasow@gmail.com

Keywords: *Pichia pastoris*, Fed-batch fermentation, cellular growth, pH, expression system

Abstract

Pichia pastoris is a methylotrophic yeast that can be genetically engineered to express proteins for industrial use. One of the most important advantages of protein expression in *P. pastoris* is its capability of growing on minimal medium and efficiently secreting heterologous proteins with low secretion levels of endogenous proteins. Operational variables such as pH, temperature, stirring rate, among others, usually affect the microorganism's growth during the fermentation processes. Therefore, the present work aimed to evaluate the influence of pH on cellular growth of *P. pastoris* KM71H by fed-batch process. The fermentation run was carried out in a 1.6 L (total volume) bioreactor, being performed in two phases: In the first stage (24 h), the yeast was batch-cultured in BMGH medium; while in the second stage (72 h), it was cultivated by feed-batch operation with a feeding medium containing 50% glycerol and 12ml/l of trace metal solution. During the overall process, which lasted after 96 h, the aeration and temperature conditions were fixed at 10 ml\L.h, 1.5 vvm and 30°C, respectively. Different pH values were evaluated: 5.0, 5.5 and 6.0. Cellular growth was determined by measuring the fermentation broth UV-spectrophotometric absorbance at 600 nm, which was correlated to a calibration curve (dry weight ´ optical density). Glycerol consumption was detected by HPLC analysis. *P. pastoris* KM71H successfully grew in all the evaluated pH values; but the highest biomass production was observed at pH 5.0 (98.79 g/L). Although *P. pastoris* is reported as being a microorganism able to grow over a wide pH range (from 3 to 7); it was not observed high cell density of *P. pastoris* KM71H strain when cultivated at pHs 5.5 and 6.0. High cellular growth is especially important for proteins secretion, as the concentration of product in the medium is roughly proportional to the concentration of cells in culture. Finally, these results reveal the possibility of obtaining high cell density of *P. pastoris* KM71H by fed-bach cultivation at pH 5.0, which can be a suitable condition for the yeast application in heterologous proteins production. Supported by: CNPq, ISAC-ERASMUS