Medium Optimization for Hen Egg White Lysozyme Production by Recombinant Aspergillus niger Using Statistical Methods

R. Gheshlaghi, J.M. Scharer, M. Moo-Young, P.L. Douglas

Department of Chemical Engineering, University of Waterloo, 200 University Avenue West, N2L 3G1 Waterloo, Ontario, Canada;
telephone: 519-888-4567, ext. 6981; fax: 519-746-4979;
e-mail: rgheshla@engmail.uwaterloo.ca

Received 3 August 2004; accepted 18 January 2005

Published online 1 April 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20474

Abstract: Statistics-based experimental design was used to investigate the effect of medium components (starch, peptone, ammonium sulfate, yeast extract, and CaCl2·2H2O) on hen’s egg white lysozyme production by Aspergillus niger HEWL WT-13-16. A 2²−1 fractional factorial design augmented with center points revealed that peptone, starch, and ammonium sulfate were the most significant factors, whereas the other factors were not important within the levels tested. The method of steepest ascent was used to approach the proximity of optimum. This task was followed by a central composite design to develop a response surface for medium optimization. The optimum medium composition for lysozyme production was found to be: starch 34 g L⁻¹, peptone 34 g L⁻¹, ammonium sulfate 11.9 g L⁻¹, yeast extract 0.5 g L⁻¹, and CaCl2·2H2O 0.5 g L⁻¹. This medium was projected to produce, theoretically, 212 mg L⁻¹ lysozyme. Using this medium, an experimental maximum lysozyme concentration of 209 ± 18 mg L⁻¹ verified the applied methodology. © 2005 Wiley Periodicals, Inc.

Keywords: Aspergillus niger; hen egg white lysozyme; experimental designs; response surface methodology; medium optimization

INTRODUCTION

Traditionally, recombinant proteins have been produced by bacteria (e.g., Escherichia coli) or mammalian cells such as Chinese hamster ovary (CHO) cells. These hosts, however, have several disadvantages as recombinant protein producers. Bacteria typically lack the mechanism for performing satisfactory post-translational modifications. Mammalian cells may be used to overcome this, however, they are relatively fragile, require expensive media, and often have bioprocess problems in conventional bioreactors.

Filamentous fungi have been used for the industrial production of a wide variety of native products such as antibiotics (e.g., penicillin and cephalosporine), organic acids (citric, acetic, and formic acids), and commercial enzymes (e.g., protease, catalase, amylase) (Rawool et al., 2001). Their ability to secrete large quantities of enzymes (e.g., >20 g L⁻¹ of glucoamylase; Wongwicharn et al., 1999) has encouraged their use for producing recombinant proteins. Furthermore, heterologous protein synthesis in Aspergillus hosts has been proven effective, because the protein products usually fold correctly with efficient formation of disulfide bridges, so a high proportion of the product is in an active conformation (Martinelli and Kinghorn, 1994). A number of heterologous proteins have already been expressed in Aspergillus species such as calf chymosin (Van Hartingsveldt et al., 1990), phytase (Van Gorcom et al., 1990), hen’s egg white lysozyme HEWL (Archer et al., 1990), and tissue plasminogen activator t-PA (Wiebe et al., 2001).

The over-expression of a recombinant cell product is often the primary goal in a biotechnological process. In this regard, several strategies such as strain development, medium optimization, bioprocess optimization, and mathematical modeling have been widely used. When developing an industrial fermentation, medium development is of utmost importance. Metabolite concentrations and growth of cells are strongly influenced by medium composition such as the carbon source, nitrogen source, and inorganic salts (Haq et al., 2003; Swift et al., 2000).

It is not an easy task to explore all the main nutritional factors and try to obtain their optimum levels in a process. Furthermore, published experimental results concerning medium composition are often conflicting. For example, the addition of 10 mM of Ca²⁺ has been reported to have both positive effect (Gyamerah et al., 2002), as well as negative effect (Spencer et al., 1999) on HEWL production. The traditional method of optimization involves varying one factor at a time, while keeping the others constant. This strategy is simple and easy to implement with no need for statistical analysis; although, it may require a relatively large number of experiments and frequently fails to anticipate the optimal condition (Kalil et al., 2000). This important shortcoming is due to the inability of the approach to consider the effect of possible interactions between factors. The