



ASSESSMENT OF DIFFERENT APPROACHES FOR THE ADAPTATION OF CHO CELLS TO SERUM-FREE MEDIUM

A. Rita Costa, M. Elisa Rodrigues, Mariana Henriques, Joana Azeredo, Rosário Oliveira

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho,

Campus de Gualtar 4710-057, Braga, Portugal

E-mail: anaritamc@deb.uminho.pt

KEYWORDS

CHO cells; Cell adaptation approaches; Serum-free medium; suspension culture

INTRODUCTION

The most common processes for the large-scale production of biopharmaceuticals require the use of suspended cells and serum-free (SF) medium, in order to achieve higher productivities more safely and less costly. Indeed, suspension cultures allow a surface-to-volume ratio much higher than adherent cultures, allowing increased cell densities and consequently higher productivities. For its turn, the interest in adapting cells to grow in SF medium is related to the many disadvantages that serum presents from the manufacturing and safety point of view. This disadvantages include the batch-variable concentrations of components which jeopardize the process consistency; the burden put on downstream processing; the high costs of serum; and the risk of transmission of animal diseases to humans.

However, this process of adaptation of cells to both SF medium and suspension can take a long time to be achieved.

Objective

To test different methodologies and conditions for the adaptation of CHO cells to grow in suspension and SF medium.

MATERIALS AND METHODS

Cells were adapted to suspension culture using shake flasks, under 100 rpm agitation, with DMEM medium supplemented with 10 % serum, 1 % L-glutamine, 1 % selective pressure and 0.1 % Pluronic F68. Cell density and viability were monitored daily during culture. Both suspended and adherent cells were adapted to serum-

free medium using methodologies of direct and a gradual adaptation. The direct approach consisted at the complete serum removal in one passage. For its turn, the gradual adaptation was based on a sequential reduction of serum percentage. Furthermore, this work also evaluated different media, both for adherent and suspended cell lines. In the first case, the media tested was (i) DMEM without serum and (ii) CHO-III(A). For the second case, the media evaluated was (i) DMEM without serum, (ii) CD-CHO and (iii) CHO-S-SFM-II. In other attempts to improve serum-free adaptation, L-Glutamine concentration in the medium was increased, the selective pressure was reduced, and non-essential amino acids were used.

RESULTS

It was possible to observe that cells were easily adapted to suspension, but not to serum-free medium. The direct methodology failed completely, resulting in total cell death. Using a gradual methodology, adherent cells showed better adaptation to CHO-III(A), although it was not possible to lower serum percentage more than 0.1 %. Suspended cells adapted better with CHO-S-SFM-II, but the lowest serum percentage achieved was 2.5 %. The other attempts to improve serum-free adaptation have failed.

CONCLUSIONS

The present work suggests that a gradual methodology is the best approach to serum-free adaptation. Furthermore, the choice of serum-free medium proves to be of utmost important for the success of cell adaptation. Indeed, CHO-III(A) and CHO-S-SFM-II had the most positive outcome for adherent and suspended cells, respectively. The results also demonstrate that serum-free adaptation is an extremely time-consuming process that needs to be simplified and



accelerated. This will only be possible after achieving a better understanding of the process.

AUTOBIOGRAPHIES

A. RITA COSTA went to University of Minho, where she studied Biomedical Engineering completing the Master Integrated cycle in Clinical Engineering in 2007. She spent three months training at Hospital de São Marcos de Braga. In 2008 she began working on a project at University of Minho, in collaboration with the biopharmaceutical company Biotecnol and the Institute of Molecular Pathology and Immunology of the University of Porto. Currently she is working at University of Minho, on Professor Joana Azeredo's group, performing her PhD on Enhancement of N-glycosylation of Monoclonal Antibodies, working in mammalian cell culture. Her e-mail address is anaritamc@deb.uminho.pt and her web page is <http://www.ceb.uminho.pt/pessoas/pid.aspx?id=295>.

M. ELISA RODRIGUES went to the University of Minho, where she studied Biomedical Engineering, completing the Master Integrated cycle in Clinical Engineering in 2007. She trained for three months at Hospital de São Marcos de Braga, before starting working on a collaboration project between University of Minho, the biopharmaceutical company Biotecnol and the Institute of Molecular Pathology and Immunology of the University of Porto, in 2008. Now at University of Minho she is working on Professor Rosário Oliveira's group, performing her PhD on Optimization of Monoclonal Antibodies Production in Wave and Stirred Tank Reactors. Her e-mail address is elisarodrigues@deb.uminho.pt and her web page is <http://www.ceb.uminho.pt/pessoas/pid.aspx?id=292>.

MARIANA HENRIQUES graduated in Biological Engineering in 1998 at University of Minho. She then

post-graduated in Industrial Engineering in 2000 and obtained her PhD degree in Chemical and Biological Engineering in 2005 at the University of Minho. She is currently an invited assistant at the Department of Biological Engineering at University of Minho, and her main research interests are *Candida* species virulence factors, monoclonal antibodies production processes, and characterization of biomaterials. Her e-mail address is mcrh@deb.uminho.pt and her web page can be found at <http://www.deb.uminho.pt/pessoas/docente.aspx?id=28>.

ROSÁRIO OLIVEIRA graduated in Chemical Engineering at University of Luanda, in 1975. She obtained her PhD degree in Engineering Science in 1991 at the University of Minho. She is currently a full professor at the Department of Biological Engineering of the University of Minho. She is the head of the master degree in Environmental Management and head of the Biofilm research group. Her main research interests are in microbiology and environmental sciences, and animal cell culture technology. Her e-mail is roliveira@deb.uminho.pt and her web page is <http://www.deb.uminho.pt/pessoas/en/docente.aspx?id=36>.

JOANA AZEREDO graduated in Biological Engineering at University of Minho in 1994, where she further obtained her PhD degree in Chemical and Biological Engineering in 1998. She is currently an associate professor at the Department of Biological Engineering at University of Minho, where she leads the bacteriophage research group. She is also a member of the Biofilm research group, with further research interest in animal cell culture technology. Her e-mail address is jazeredo@deb.uminho.pt and her web page can be found at <http://www.deb.uminho.pt/pessoas/docente.aspx?id=17>.