#

Hydrodynamic behavior of shaking flasks and pilot stirred bioreactors usedforthe production of a recombinant protein by filamentous bacteria

[1]María Soledad Córdova-Aguilar, [1]Ernesto Mancilla, [2]Mauricio A. Trujillo-Roldán, [2]Luz Deisy Marín-Palacio, [1]Gabriel Ascanio, [3]Roberto Zenit, [1]Enrique Soto; [1]Centro de Ciencias Aplicadas y Desarrollo Tecnológico,[2]Instituto de Investigaciones Biomédicas, [3]Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510, México,marisol.cordova@ccadet.unam.mx

Key words: hydrodynamic characterization, particle image velocimetry, turbulence intensity,

Introduction. The hydrodynamic state in shake flasks strongly affects the morphology of the filamentous *Streptomyces lividans*, as well as the productivity and *O*-mannosylation of a recombinant glycoprotein[1].Just a few authors have worked to provide the basis for scaling-up successfully the hydrodynamics between shake-flasks and stirred tanks [2].The aim of this work is to describe experimentally the flow characteristics of agitated flasks and pilot stirred tanks using particle image velocimetry and high speed videos in order to evaluate the main variables involved in the scale up from an engineering point of view

Methods. The configurations of Erlenmeyer flasks tested were: conventional, baffled and coiled as used elsewhere [1] and a 1.0 liters bioreactor (Applikon Biotechnology) equipped with two 3.8 cm diameter Rushton turbines.The velocity fields for horizontal and vertical planes were measured by means of a Particle Image Velocimetry (PIV) technique. High speed videos were recorded to observe the behavior of the interface. The phase locking technique was used to synchronize the image acquisition and position. The fluid was Luria-Bertani's medium modified by the addition of 34% w/v sucrose [1], with the following properties; ρ= 1300 kg m^{-3}, η= 3.5 m Pa s and a surface tension σ= 62 mNm^{-1}. The turbulence analysis was performed from the velocity fields with the equations reported in [3].

Results. The interface shape and the velocity fields were completely different for the flask configurations as shown in figures 1 and 2. Scaling up to the stirred tank by P/V parameter, resulted in morphological and production changes and even so the velocity fields were as the observed in flasks (*data not shown*).

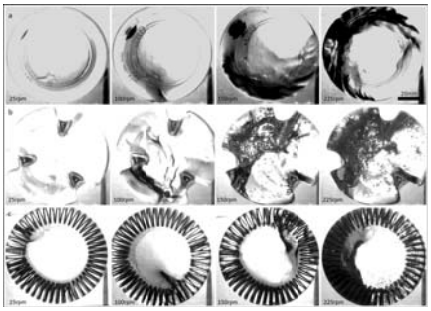

Fig.1 Bottom view of conventional(a) baffled (b) and coiled (c) shake flask configurationsfor different agitation rates.

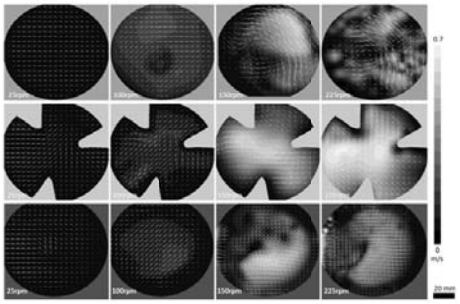

Fig.2 Velocity length scalar maps at 25, 75, 150 and 200 rpm for: a) Conventional, b) Baffled and c) Coiled shake flasks.

Conclusions. The hydrodynamic state in terms of the turbulent variables evaluated in this work, is strongly associated to the changes in morphology and the O-glycosylation profile reported before[1] and both were the basis considered for the scale-up of the process. Not only the power input should be considered, but also, to include the contributions of the hydrodynamic parameters, in order to achieve more accuracy in the scale up.

Acknowledgements.This work was partially financed by PAPIIT-UNAM IN209113, IN108312 and CONACyT grants 178528.

References.

1. Gamboa-Suasnavart, RA, Valdez-Cruz, NA, Córdova-Dávalos, L E, Martínez-Sotelo, JA, Servín-Gonzalez, L, Espitia, C, Trujillo-Roldán, MA. (2011). *Microb.Cell Fact*. 10 (1):110.
2. Suresh, S, Srivastava, VC, Mishra, IM. (2009)..*Crit Rev.Biotechnol.*, 29(4): 255–278.
3. Kundu, PK, Cohen, I M. (2008).Academic Press, pp:537-601.