**Physiological Response of *S. clavuligerus* to shear forces in 2-D Rocking Motion and Stirred Tank Bioreactors**

David Gómez-Ríos1\*, Howard Ramírez-Malule2, Peter Neubauer3, Stefan Junne3, Silvia Ochoa 4, Rigoberto Ríos-Estepa1

*1 Grupo de Bioprocesos, Departamento de Ingeniería Química, Universidad de Antioquia (UdeA), Calle 70 No. 52-21, Medellín, Colombia; 2 Escuela de Ingeniería Química, Universidad del Valle, A.A. 25360 Cali, Colombia; 3 Chair of Bioprocess Engineering, Department of Biotechnology, Technische Universität Berlin, Ackerstr. 76, ACK 24, 13355 Berlin, Germany; 4 Grupo de investigación en Simulación, Diseño, Control y Optimización de Procesos (SIDCOP), Departamento de Ingeniería Química, Universidad de Antioquia (UdeA), Calle 70 No. 52-21, Medellín, Colombia.*

*\*Corresponding author: dandres.gomez@udea.edu.co*

**Highlights**

* Low shear forces favor the preservation of macromorphology in *S. clavuligerus.*
* High shear forces promote mycelia thinning and clavulanic acid accumulation.
* Oxygen uptake and clavulanic acid production are affected by mycelia aggregation

**1. Introduction**

*Streptomyces clavuligerus* (*S. clavuligerus*) is a Gram-positive filamentous bacterium notable for producing clavulanic acid (CA), which is a potent inhibitor of β-lactamases enzymes that confer resistance to bacteria against β-lactam antibiotics. CA is traditionally produced in stirred tank reactors (STR); nevertheless, the impact of bioreactor geometry on cell performance and CA production has not been completely understood. The present study aims at performing a comparative analysis of the metabolic response of *S. clavuligerus* to low shear stress in 2-D rocking-motion single-use bioreactor and at high mechanical shear stress in STR.

**2. Methods**

Cultivations of *S. clavuligerus* DSM 41826 in chemically defined media [1] were conducted by duplicate cultivations in a 15 L STR (Techfors S, Infors AG, Bottmingen, Switzerland) and in a 20 L 2-D rocking-motion single-use bioreactor CELL-tainer® (CT) (CELL-tainer Biotech BV, Winterswijk, The Netherlands). Cultivations were operated in batch mode (5 L) during the first 37 h, followed by fed-batch operation (35 mL/h) during 77 h. Cell dry weight (CDW), CA and metabolites quantifications were performed as described by Ramirez-Malule [1]. Mycelia samples were photographed in a Nikon Eclipse Ti2 inverted microscope (Nikon Instruments Inc., Amsterdam, The Netherlands) at 40x and further processed in ImageJ software (U.S. National Institutes of Health, Maryland, USA).

**3. Results and discussion**

In Figure 1, microscopy images (Figures 1a-1c) and biomass concentration developments of *S. clavuligerus* (Figure 1e) for the STR and CT cultivations are presented. In CT cultivations, the calculated specific growth rate (µmax) and maximum biomass concentration were 0.068 h-1 and 10 g/L, respectively. A similar µmax (0.069 h-1), but higher maximum biomass concentration (14.5 g/L) were obtained in STR cultivations. The increase of agitation rate from 300 to 500 rpm in STR caused a decrease in mycelia thickness of 22.5% during the cultivation time (Figure 1e) as consequence of high shear forces. In contrast, the lower shear forces in CT reactor did not cause hyphal fragmentation leading to more aggregated and thicker mycelia. The mycelial thickness in CT increased 30.6% (Figure 1e) during the same time of cultivation and comparable values of dissolved oxygen in the CT reactor. The higher biomass production observed in STR (Figure 1e) was attributed to the generation of a considerable number of mycelial fragments capable to growth and reproduce [2,3].

CA production is promoted by phosphate limiting conditions [1,4]; although the latter condition was reached in STR and CT reactors, CA release was lower in CT cultivations. The maximum specific CA concentrations were 33.44 mg/g CDW and 14.3 mg/g CDW in the STR and CT cultivations, respectively. In this regard, a reduction in oxygen uptake was observed in CT cultivations due to aggregation and clumping of mycelia. Oxygen uptake has a key role in the secondary metabolism of *S.clavuligerus*, since several oxidation steps reactions require molecular oxygen to occur. Thus, CA accumulation was further enhanced by the high shear stress and hyphal fragmentation that prevent the adhesion of filaments and limitations to oxygen diffusion. These results suggest that environmental conditions in CT reactor promoted a different carbon flux distribution as a consequence of less relative stress conditions, leading to a lower activity of secondary metabolism and hence, lower CA accumulation [4].

|  |  |  |
| --- | --- | --- |
| a. | b. |  |

Figure 1. Morphological response of *S. clavuligerus* in STR and CT: a. STR (143 h); b. CT (139 h); e. Time course of biomass in STR (squares) and CT (circles) and mycelia thickness in STR (diamonds) and CT (triangles).

**4. Conclusions**

Low shear forces did not lead to significant hyphal fragmentation or lysis, on the contrary, it promoted mycelial thickening and branching in *S. clavuligerus*. Hence, the 2-D rocking-wave pattern of agitation favored the preservation of macromorphology in this filamentous organism.

Oxygen plays a key role in enhancing the CA productivity, thus the hyphal fragmentation during the exponential phase and moderate cellular stress seem to be critical for attaining high CA titers.

**Acknowledgement:** The authors kindly acknowledge the support of Departamento Administrativo de Ciencia, Tecnología e Innovación– COLCENCIAS grant number 111577657246 CT 432-2017.

**References**

[1] H. Ramirez-malule, S. Junne, M.N. Cruz-bournazou, P. Neubauer, Streptomyces clavuligerus shows a strong association between TCA cycle intermediate accumulation and clavulanic acid biosynthesis, (2018).

[2] E.A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-vaillant, C. Jacquard, H. Klenk, C. Clément, Y. Ouhdouch, P. Van Wezel, Taxonomy , Physiology , and Natural Products of Actinobacteria, Microbiol. Mol. Biol. Rev. 80 (2016) 1–44. doi:10.1128/MMBR.00019-15.Address.

[3] E. Olmos, N. Mehmood, L. Haj Husein, J.L. Goergen, M. Fick, S. Delaunay, Effects of bioreactor hydrodynamics on the physiology of Streptomyces, Bioprocess Biosyst. Eng. 36 (2013) 259–272. doi:10.1007/s00449-012-0794-1.