OXYGEN TRANSFER RATE IS AFFECTED BY THE SHAKE FLASK DESIGN IN THE PRODUCTION AND *O*-MANNOSYLATION OF RECOMBINANT APA GLYCOPROTEIN FROM *MYCOBACTERIUM TUBERCULOSIS* IN *STREPTOMYCES LIVIDANS.*

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Introduction. Shake flask are the most frequently used reaction vessels in biotechnology (1). In spite of their large practical importance, very little is known about the characteristic properties of shaken cultures from an engineering point of view. Oxygen transfer rate (OTR) is the most suitable measurable parameter to quantify the physiological state of an aerobic culture since most metabolic activities depend on oxygen consumption (1). The effect of three different shear and oxygenation conditions using conventional (NF), baffled (BF) and coiled (CF) shake flasks on *S. lividans* cultures was evaluated online using the RAMOS device(2). A previous report indicates that different flask designs in *S. lividans* cultures leads to changes in *O*-mannosylation and production of a recombinant protein (3).

Methods. *S.lividans* cultures were performed at 30ºC, 150 rpm, 60 h in 34% sucrose Luria-Bertani medium (3). Additional cultures of *Corynobacterium glutamicum* an d *E. coli* were carried out with the same culture conditions, and in the same *S. lividans* culture medium, in order to challenge the system with a high respiration activity microorganism. OTR during the cultivations was measured using the RAMOS (2). This device analyzes the depletion of oxygen concentration in the gas headspace of the shake flask with an oxygen sensor. The slope of this depletion corresponds to the OTR (2). Three coiled, three baffled and two conventional flasks were used in the same experiment.

Results. The cultures carried out in CF and BF reaches a maximum OTR value of 9.0 mmol/lh after 40 hrs of culture. In contrast, cultures in NF a significantly lower OTR was reached (1.0 mmol/lh). *C. glutamicum* cultures achieved in CF, BF, and NF, 10, 9 and 2 mmol/lh respectively. *E. coli* cultures in CF and BF only achieved 5 mmol/lh and 2 mmol/lh in NF. Low values in *E. coli* cultures are probably due to the high sucrose concentration in culture medium.

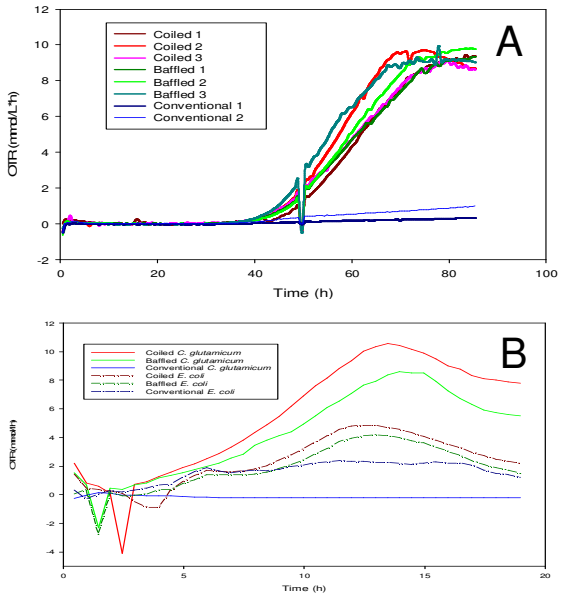

Fig.1 A: OTR during cultivation of *S.lividans* producing rAPA from *M. tuberculosis*. **B:** OTR in *C. glutamicum* and *E. coli* cultures in conventional (blue) baffled (green) and coiled shake flasks (red).

Conclusions. Flask design modifies the OTR in microorganism cultures with low and high respiration activity. These changes could lead to changes in recombinant protein production in *S. lividans* cultures. The knowledge about the oxygen transfer aspects inside flasks can provide new tools for the development and improvement of biotechnology processes, mainly in those producing bacterial recombinant proteins

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