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MOMENTUM AND MASS TRANSFER PHENOMENA IS INFLUENCED BY THE GEOMETRY AND AGITATION MODE IN SHAKE FLASKS

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Shaking bioreactors are widely applied for screening and bioprocess development projects due to its flexibility and ease of operation, and probably more than 90% of submerged cultures for research purposes are performed in shake flasks [1,2]. Almost all the tasks, such as screening of strains, media optimization, strain development, elucidation of metabolic pathways, investigations of process conditions, and evaluation of fundamental growth kinetics are made in these vessels [3]. In orbitally agitated shake flasks, the momentum and heat transfer is influenced by the geometry of the rotating bulk liquid, that is the contact area between the liquid and the friction area, *i.e.* the flask inner wall (and indentations in baffled flasks, or the coiled stainless steel spring in flasks) [4-6]. On the other hand, the mass transfer (mainly oxygen) is influenced by the wet wall exposed to the surrounding air, this is the mass exchange area [4,7]. Modifications of shake flasks by the introduction of baffles and other enhancements (like stainless steel spring coils) are frequently necessary in order to provide sufficient aeration and shear stress [4]. As an example, we determined how shake flask design determine bacterial morphology, productivity, and the *O*-mannosylation of a recombinant glycoprotein in *S. lividans* cultures [4]. For the scale-up and the understanding of recombinant glycoproteins production in *S. lividans,* we also determined the effect of the volumetric power input (P/V) and oxygen mass transfer in shake flasks, evaluating the oxygen transfer rate (OTR) and carbon dioxide transfer rate (CTR) behavior in three different flask designs using the Respiration Activity Monitoring System (RAMOS) device [6, unpublished data].

On the other hand, A major deficiency in using orbitally shaken flasks as culture bioreactors is that the OTR obtained is comparatively low, and oxygen supply may become limiting if the oxygen demand exceeds the oxygen transfer capacity through the shake flask closure or/and the gas-liquid interface [1-4]. The OTR from headspace air to the liquid phase is described by the gradient in oxygen concentrations multiplied by the volumetric mass-transfer coefficient (k_La). ResonantAcoustic® Mixing (RAM) technology enables noncontact mixing by the application of low frequency acoustic energy applied to a vessel. RAM technology for mixing microbial cultures is purposed like an alternative to solve OTR limitations in shake flasks when combined with the Oxy-Pump® stopper which actively pumps air in and out of the vessel [8]. Since there is no known correlation between the k_La and operating parameters in RAM mixers, we determined an empirical k_La correlation and compare it against the k_La for orbital shake flasks. Further, we followed cellular growth, glucose uptake and dissolved oxygen during an *Escherichia coli* BL21 (DE3 gold) rSMD cultivation in orbital and acoustic shake flasks at equivalent k_La values of 46 and 93 h^{-1} [9].

References:

1. Büchs J (2001) Introduction to advantages and problems of shaken cultures. *Biochem Eng J*, 7:91-98.
2. Zimmermann HF, Anderlei T, Buchs J, Binder M (2006) Oxygen limitation is a pitfall during screening for industrial strains. *Appl Microbiol Biotechnol*, 72:1157-1160.
3. Gupta A, Rao G (2003) A study of oxygen transfer in shake flasks using a non-invasive oxygen sensor. *Biotechnol Bioeng*, 84:351-358.
4. Gamboa-Suasnavart RA, Valdez-Cruz NA, Cordova-Dávalos LE, Martínez-Sotelo JA, Servín-González L, Espitia C, Trujillo-Roldán MA * (2011) The O-mannosylation and production of recombinant APA (45/47 KDa) protein from *Mycobacterium tuberculosis* in *Streptomyces lividans* is affected by culture conditions in shake flasks. *Microb Cell Fact,* 10:110.
5. Mancilla E, Palacios-Morales CA, Cordova-Aguilar MS, Trujillo-Roldán MA, Ascanio G, Zenit R. (2015) A hydrodynamic description of the flow behavior in shaken flasks. *Biochem Eng J,* 99:61-66.
6. Marín-Palacio LD, Gamboa-Suasnavart RA, Valdez-Cruz NA, Servín-González L, Córdova-Aguilar MS, Soto E, Klöckner W, Büchs J, Trujillo-Roldán MA (2014). The role of volumetric power input in the growth, morphology, and production of a recombinant glycoprotein by *Streptomyces lividans* in shake flask cultures. *Biochem Eng J* 90:224-233.
7. Büchs J, Maier U, Lotter S, Peter CP (2007) Calculating liquid distribution in shake flasks on rotary shakers at waterlike viscosities. *Biochem Eng J*, 34:200-208.
8. Applikon Inc. (2003) Application note. http://www.applikon-bio.com/cms3/images/stories/RAM/Application_Note_RAMbio_Ecoli_Ppistoris.pdf
9. Reynoso-Cereceda GI, García-Cabrera RI,Valdez-Cruz NA, Trujillo-Roldán MA (2015) Caracterización del crecimiento bacteriano en matraces agitados por un sistema de resonancia acústica en comparación a la agitación orbital. XVI Congreso Nacional de Biotecnología y Bioingeniería, 21 al 26 de Junio, 2015.