FROM SHAKE FLASKS TO BIOREACTOR USING VOLUMETRIC POWER INPUT AS SCALE-UP CRITERIA IN A FILAMENTOUS BACTERIAL CULTURE

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Introduction. One of the essential parameters to specify cultivation conditions of microorganisms in shake flasks is the agitation speed. Nevertheless, filling volume, size and geometry of the flasks affect the total amount of energy delivered to the fluid, measured as volumetric power input (P/V) [1]. It is one of the crucial values for characterizing and scaling-up cultures [2]. In this work, the P/V measured in Coiled Flask (CF) and Conventional Normal Flasks (NF) was used to scale-up to a 1 L bioreactor and to determinate if this scale-up strategy can be used in the production of recombinant proteins by *S. lividans.*

Methods. The strategy was based on the information generated by CF and NF in terms of the P/V during the growth *S. lividans* cultures. The evolution of the P/V was characterized on-line, using the method described by Büchs et al. [3]. Biomass was evaluated by dry weight, morphology was analyzed by Image Analysis in order to determine the average diameter aggregate was determined [4]. Rheological parameters (flow consistency index, K and flow behavior index, n) of the culture broth were measured using a 50 mm plate/plate geometry in a controlled stress rheometer (Physica MCR 101 Modular Compact Rheometer).

Results. The P/V for the filamentous bacteria *S lividans* 66 strain 1326 cultures, measured in CF and NF was 0.20 and 0.44 kW/m^3 respectively. Based on these data, as well as the dimensions of the bioreactor and its geometry correction, agitation speed was calculated by equation 1. The P/Vs of flasks were tested in the bioreactor by shaking up to 260 rpm (B260) and 340 rpm (B340).

$$P = N_p D^5 n^3 \rho \qquad \textbf{Equation 1}$$

In the same power input of the stirred bioreactor, it was similar the behavior observed in conventional and coiled shake flasks, in terms of the specific growth rate (μ). However, in terms of morphology there are vast differences so the P/V is not the only responsible for the morphological changes on productive cultures of *S. lividans*, also local energy dissipation and flow patterns may be playing an important role. A maximum of 4.8 ± 0.15 and 2.1 ± 0.05 g/L of biomass was obtained in CF and NF respectively, while in 1.0 L bioreactor the biomass was of 3.7 ± 0.12 and 4.0 ± 0.89 g/L for 340 and 260 rpm.

Table 1. Summary of experiments in shake flasks and stirred bioreactor, conducted under similar power input

			Rheological parameters	
	μ (h^{-1})	Diameter (μm)	K (Pa.s)	n
NF	0.073±0.004	362.6±63.2	0.035	0.49
B260	0.077±0.007	146.3±42.6	0.057±0.012	0.61±0.04
CF	0.11±0.009	163.7±43.0	0.024	0.58
B340	0.083±0.004	134.3±4.80	0.440±0.006	0.59±0.08

The P/V is not only responsible for the morphological changes and productive cultures of *S. lividans*. Local energy dissipation and flow patterns may be playing an important role.

Conclusions. The power input, as a scale-up criterion (from flasks to 1L bioreactor), reproduce well the specific growth rate but, It did not reproduce culture behavior (particularly biomass concentration and morphology at 0.20 kW/m^3).

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