Comparison of rBCG-pertussis cultivation in flasks and bioreactor

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1. INTRODUCTION

Tuberculosis is one of the infectious diseases that causes high number of deaths in the world and the BCG vaccine, produced from the bacillus Calmette-Guérin (BCG), is used to prevent it (CERNUSCHI et al., 2018). Another disease of great global concern is whooping cough, caused by *Bordetella pertussis* and the vaccine for its prevention confers complete immunity only after 6 months of life, with the first months being the most vulnerable age group. Knowing that *Mycobacterium bovis* BCG has great potential as a live attenuated vaccine for heterologous antigen presentation, a recombinant BCG strain carrying the subunit 1 of the genetically detoxified pertussis toxin (rBCG-pertussis) was constructed, which could confer immunity in the first days of life against tuberculosis and whooping cough (NASCIMENTO et al., 2000). The cultivation of BCG, however, still has limitations, as it is carried out in static cultures, where BCG cells form a pellicle on medium surface due to the tendency of aggregation and clumping of this microorganism. Thus, the traditional BCG vaccine production in static cultures have many manual operations, low reproducibility and great losses during the production process. Since the rBCG-pertussis vaccine is composed of live microorganisms, the evaluation of viable cell concentration is required to determine the vaccine dose, and the conventional method is by counting colony forming units (CFU) (LEAL et al., 2004). This work aims to evaluate the submerged culture of rBCG-pertussis and the concentration of viable cells in shaken flasks and a 1 L bioreactor.

2. METHODOLOGY

Inoculum cultures were grown in 250 mL Erlenmeyer flasks containing 50 mL of 7H9 modified medium (7H9mfg). The flasks were incubated at 37 ºC, 125 rpm and 7.5% CO_2 until reaching optical density (OD) of 2.0, and then transferred to new flasks to obtain initial OD of 0.2. The cultivation was carried out in 1 L Erlenmeyer flasks containing 200 mL of 7H9mfg, incubated under the same conditions used for inoculum preparation. Once the bacterial growth curve and specific growth rate were known, the process was scaled up to a 1L bioreactor (Bioengineering Ralf 1L) with 600 mL of 7H9mfg. The cultivation was carried out at 37 ºC, initial pH at 6.7, and dissolved oxygen controlled at 20% saturation with air (through a cascade of 150 - 1000 rpm of agitation and 0.1 to 0.5 L/min of airflow). Antifoam 204 was added when necessary. Samples were taken periodically for analysis of OD, dry weight (DW) and CFU/mL.

3. RESULTS AND DISCUSSIONS

The growth curves and concentration of viable cells are shown in Figure 1.

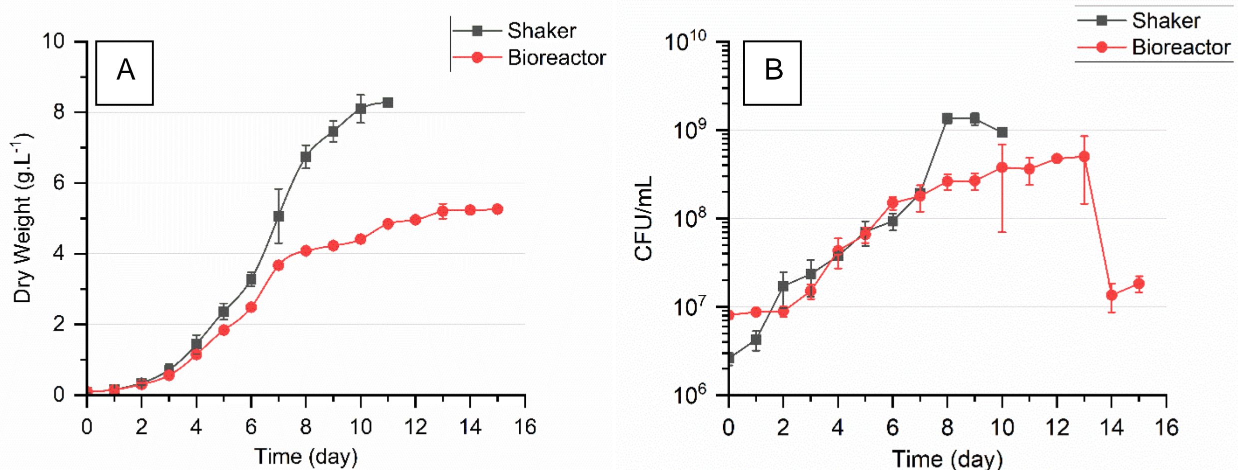

Figure 1. *(A) Growth curve and (B) viable cell concentration (CFU/mL) of rBCG-pertussis grown in 7H9mfg medium in shake flasks and bioreactor. Average and deviation of 2 independent experiments in shaker and a representative experiment in bioreactor (average and deviation os analytical duplicates are shown)*

In flasks, the maximum DW was 8.10 g/L, maximum specific growth rate ($\mu_{máx}$) was 0.68 day^{-1}, and maximum viable cell concentration was 1.36x10^9 CFU/mL. 7H9mfg contains tyloxapol as surfactant to avoid clumping of BCG cells. This detergent makes it difficult to control foam, but even in presence of this surfactant, BCG sticks to reactor wall and sampling system. Therefore, four cultures were performed in bioreactors to fix operational problems related to foam and clumping, and to define the cascade for dissolved oxygen control. In bioreactor, DW reached 5.26 g/L, $\mu_{máx}$ was 0.64 day^{-1}, and viable cell concentration reached 3.63x10^8 CFU/mL. The shaker flask culture yielded higher DW and viable cell concentration than submerged culture, mainly due to the formation of foam and clumping of cells. In conclusion, it is possible to cultivate rBCG-pertussis in submerged cultures, but further investigation is necessary to set a system to deal with foam and clumps and to increase cell concentration. DW reached in bioreactor was 1.4 times higher than previously published data (PASCOE et al., 2020).

5. REFERENCES

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6. ACKNOWLEDGMENTS

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