IMPACT OF INOCULUM PROPERTIES ON BIOREACTOR PERFORMANCE: A CHO CELL CASE OF STUDY

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Key words: CHO cells, CDM, fed-batch bioreactors

Introduction. Mammalian cells are often used for biopharmaceuticals production (e.g. monoclonal antibodies)[1]; inoculum train to reach large-scale bioreactors needs several passages in which cells could lose expression of the recombinant protein, get into a stationary phase and have a direct impact on the production stage[2]. This kind of behavior is directly related to cell's environment (nutrients availability, selection pressure, pH, DO and temperature). In this context, Chemically Defined Media (CDM) is commonly used at commercial scale[3] for cell growth but intrinsic characteristics of media could be use whether proliferation or production.

This work's aim is to define a strategy to generate a robust inoculum for a scale-up process.

Methods. Propagation of the inoculum was carry out in shake flask or wave bags depending the final bioreactor scale. Fed-batch cultures were run in a B. Braun (1 L), Celligen Plus (2 L) or Single-Use Biorreactor (50 L). CDM-A or CDM-B were used for inoculum and/or production. In bioreactor a concentrated feed CB was introduced at day 3 with a 200 mM glutamine solution to restore depleted nutrients; pH was maintained at 6.85 (CO_2-$NaHCO_3$), temperature 36.7 ºC, DO 35% (air saturation). IgG was quantified during the culture by affinity Protein A HPLC. Daily sampling was made to calculate kinetic parameters.

Results. CDM-B was used initially for cells propagation and production in bioreactor, several runs (n>5) were conducted with this strategy but as is shown in Fig. 1 a lag phase (> 3 days) in the culture was presented, this was attributed to a late inoculum state (specific growth rate decays through several passages). In order to get a healthy cell seed we change to CDM-A and others chemically defined medias (data not shown), with this approach inoculum had a higher cell density Fig. 2 and a prolonged exponential phase; bioreactors were run with CDM-A to evaluate IgG production Fig. 3. In another set of experiments different media including CDM-B were evaluated in shake flask to determine the highest specific productivity, resulting this media with this characteristic (data not shown). Changes of media had an impact on cell metabolism, specifically on glutamine and glucose consumption Table 1. Finally, we performed a process with media for different purposes CDM-A for cell proliferation at inoculum stage and CDM-B for production in bioreactor Fig. 3. Kinetic parameters at different stages and mediums are condensed in Table 1.

Table 1. Kinetic parameters

Inoculum	μ, h^{-1} CDM-A	μ, h^{-1} CDM-B	Q_{gluc}, g/L/d CDM-A	Q_{gluc}, g/L/d CDM-B	Q_{gln}, mMol/L/d CDM-A	Q_{gln}, mMol/L/d CDM-B
N+1	0.022 ± 0.0013	0.025 ± 0.0022	0.17	0.05	1.12	0.76
N+2	0.020 ± 0.0016	0.026 ± 0.0019				
N+3	0.018 ± 0.0021	0.024 ± 0.0015				
Bioreactor	0.018 ± 0.0018	0.026 ± 0.0011	0.24	0.07	1.02	0.83

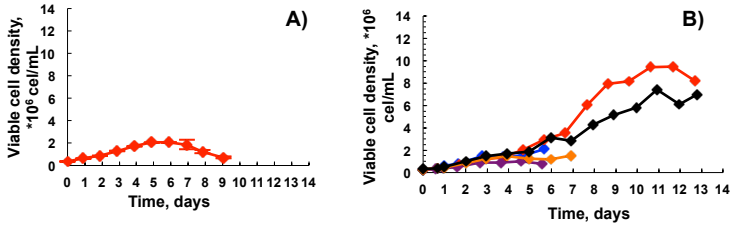

Fig. 1 CDM-B; A) Propagation B) Bioreactor

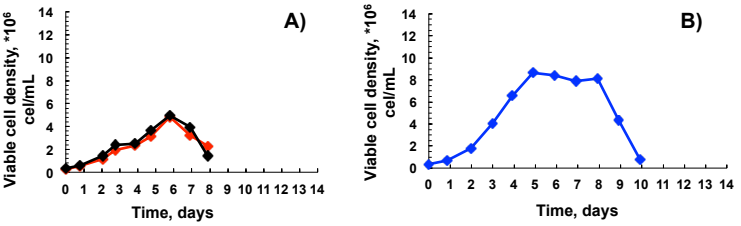

Fig. 2 CDM-A; A) Propagation B) Bioreactor

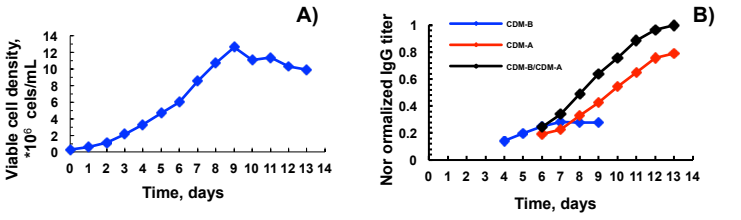

Fig. 3 A) CDM-A Seed/ CDM-B Bioreactor B) Product titer comparison

Conclusions. In this work we provide a solution to eliminate a lag phase and enhance recombinant protein (IgG) titer in bioreactor when mammalian cells had retarded metabolism.

Acknowledgements. The authors would like to thank CONACyT - FINNOVA finantial suport with the project 174106.

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