TEMPERATURE SHIFT AND SUPPLEMENTATION REDUCTION IN FED BATCH CULTURES: IMPACT ON MONOCLONAL ANTIBODY`S QUALITY

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Introduction. A systematic strategy of supplementation adapted to kinetic cell growth in fed-batch cultures provides a suitable administration of nutrients. Supplementation function prevents overfeeding which may cause inhibitory concentration of some nutrients components in the bioreactor[(1)]. Subphysiological temperature (<37 ºC) provides a decresed in cell death which also benefits protein production rates. Product quality has been observed to be temperature dependent as well[(2)]. Higher product quality at lower culture temperatures has been attributed to lower protease activity and lower activity of other deleterious temperature-sensitive enzymes.[(3)]
The aim of this work was to modulate the profile of supplementation at low temperature to assess the impact on the quality of a IgG monoclonal antibody.

Methods. Two cultures in Single Use Bioreactor (Hyclone, MA) were run with the following conditions: T 36.3°C, pH 7.0+/-0.5, DOT 35% of air saturation. Supplementation with concentrate media started when the cell density was half of the exponential growth phase and decreased 50% of the inital flow rate at the end of that fase, in this moment the temperature also was reduced. Quality of monoclonal antibody was evaluated determining its isoform profile by HPLC-CEX. The cultures were compared with a control reactor (no temperature shift and no supplementation reduction). IgG was quantified by affinity protein A HPLC, dialy sampling was made for kinetic parameters.

Results. Table 1 present results for the three cultures. It was demonstrated that subphysiological temperature contributes lowering the percentage of acidic isoforms and therefore provides a better quality of product. Establishing a proper feed flow rate avoided nutrients accumulation in the bioreactor, so lower osmolality profile was obtenined, this could decrease stress on cells (fig 2B)

We indentified positive effects on the integral of viable cells and specific productivity (fig 3A) and this strategy was able to extend the cultures 48 hours more with a viability greater than 80% in both bioreactors (fig 1B). The results shown in the graphs 1A and 2A indicate an increase both in cell concentration and specific cell growth rate, therefore the increase in the concentracion of Mab in correlated with these parameters.

Table 1. Effect of reduction of feed flow rate and temperature in CHO cells cultures

ID culture	Final viable cell denstity (10^6)	Final viability (%)	Final Osmalarity (mosm/Kg)	Normalized Mab titer	Acids isoform(%)	Purificable Mab (%)
Control	6.24	46.94	690	1	21	36
SUB 1	20.25	86.41	394	1.1	12.36	34.16
SUB 2	17.43	80.25	498	1.5	14	26.2

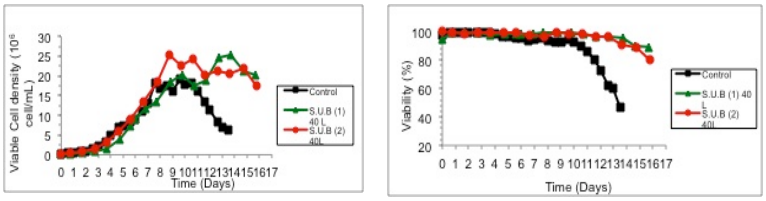

Fig.1 (A) Viable cell density, (B) Viability

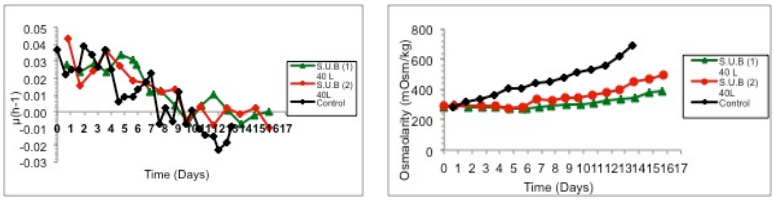

Fig.2 (A) Specific cell grow rate, (B) Osmalarity

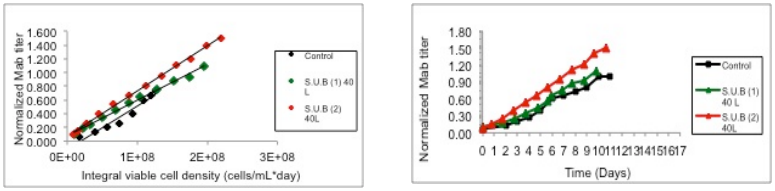

Fig.3 (A) Specific productivity slope, (B) (A) Normalized Mab titer

Conclusions. A proper profile of supplementation allows significant improvement and overall yield of process. A temperaturte shift from 36.3 to 32°C resulted in a better quality of IgG.

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