OTR12 - Effects of L-alanyl-L-glutamine media supplementation on batch hybridoma growth and monoclonal antibody production

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Introduction:

As the demand for monoclonal antibodies (mAb) is increasing, there is a significant interest in developing optimized cell culture processes for hybridoma. Optimization process comprises a number of variables, including the selection of better producer hybridomas, culture media and bioreactor culture conditions. L-glutamine is an unstable essential amino acid involved in hybridoma energy production, cell growth and antibody synthesis. However, L-glutamine breaks down to ammonium that can, at least, lower hybridoma growth and mAb production. Dipeptides of L-glutamine with L-alanine or L-glycine are stable forms, which can be used in cell culture media to avoid its negative effects.

Objective:

Evaluate the effects of L-alanyl-L-glutamine on hybridoma growth kinetics and mAb productivity.

Methodology:

The murine hybridoma cell line 90DA5/CB5/AA3, which produces mouse immunoglobulin (Ig) G1κ against PBP2a protein, was used in a series of batch experiments performed in roller bottles during 7 days. The medium utilized was DMEM high glucose (4.5g/L; LONZA) supplemented with glutamine or L-alanyl-L-glutamine (6.4mM; Gibco) and 10% v/v fetal calf serum (FCS). Cell counts were performed in Neubauer chamber, under optical microscope, after dilution in Trypan Blue 0,4%. After cell counting, each sample was centrifuged (200g, 10min) and the supernatant frozen for further analysis. Murine IgG (Mouse-IgG ELISA, Roche), L-glutamine (YSI2700 analyzer) concentrations were determined. Specific cell growth rate (μ) and doubling time (dt) were calculated using the differential method, during the exponential growth phase. Specific Lglutamine consumption rate (qSglu) and IgG production rate [qP(I-

gG)] were estimated by plotting total cell concentration, cumulative substrate consumption or production, versus the integral of viable cells (IVC) and fitting the plots with a regression coefficient of close to one.

Results:

L-alanyl-L-glutamine compared to L-glutamin- -supplemented media increased hybridoma cell growth, after 7 days, as measured by IVC (212390000 and 169267500 cell.h/mL, respectively) and extended the stationary phase (2.05+0.13 and 0.87+0.08 x106 cell/mL at 96h, respectively). Interestingly, it did not affect the maximum viable cell concentration (2.70+0.17 and 2.60+0.06 x106 cells/mL at 72h, respectively), μ (0.023 and 0.023h-1, respectively) and dt (30 and 30h, respectively). In addition, free glutamine concentration during hybridoma cultivation with L-alanylL-glutamine-supplemented medium differed from glutaminesupplemented medium since it started with low levels (0.035 versus 1.180g/L), peaked at 24h (0.844 versus 0.853g/L) remained above control (0.523 versus 0.320g/L) and both returned to basal levels at 72h. Of note, spontaneous release of glutamine from L-alanyl-L-glutamine was observed in cellfree medium supplemented with FBS at 4 and 37oC. After 7 days of hybridoma cultivation in batch mode, L-alanylL-glutamine-supplemented medium presented an increase of 55% in antibody volumetric productivity when compared to glutamine-supplemented medium (70.7 and 45.4μg/mL, respectively) and an increased qP(IgG) at exponential phase (6.0 versus 4.5 x10-7μg/cell.h).

Conclusion:

L-alanyl-L-glutamine supplementation increased the hybridoma cell growth and significantly increased antibody volumetric productivity.

Keywords: Hybridoma, IgG Production, L-alanyl-L-glutamine supplementation