Optimizing CHO Cell Culture Conditions

Temperature, Impeller Speed, pH, and DO Parameters that Maximize Productivity

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Production of recombinant monoclonal antibodies (mAbs) for therapeutic use is a multibillion dollar industry. At present, mammalian cell lines are the workhorses of the biotechnology industry, due to the requirement for mammalian-type, post-translational modifications. A large proportion of commercially valuable mAbs are produced in Chinese hamster ovary (CHO) cells.

Development of antibody-producing mammalian cell lines is very costly, therefore, the use of scale-down bioreactors that are representative of large-scale reactors (>5,000 L) are essential for cost-effective development of mammalian cell lines. In this article, we describe a set of experiments aimed at defining a standard set of conditions for the growth of CHO cells with maximum productivity.

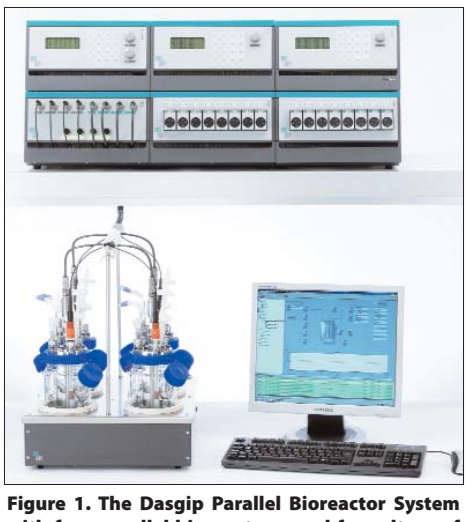

Figure 1. The Dasgip Parallel Bioreactor System with four parallel bioreactors used for culture of CHO cells

These experiments were undertaken to identify standard bioreactor culture conditions that provide the starting point for optimization of conditions for any CHO cell line grown in **Dasgip's** (www.dasgip.com) Parallel Bioreactor System (*Figure 1*). The Dasgip system comprises a temperature-controlled stirring block housing four parallel 700 mL vessels equipped with pH and pO_2 electrodes, dosing lines, gas supply lines and exhaust gas vents fitted with condensers. pH control was achieved through automated addition of acid (1M HCl) and base (1M NaOH). Dissolved oxygen set points were maintained by regulation of oxygen in the mass flow controlled gas mixture.

A model GS-CHO cell line secreting a recombinant IgG4 mAb was used to define the parameters. The cells were cultured in CD-CHO medium (Invitrogen, **Life Technologies**) in batch culture. Four sequential experiments were performed to test the effects of impeller speed, pH, temperature, and dissolved oxygen on growth and antibody production of the cells (*Figure 2*).

Both growth and antibody-production characteristics were used to define the best conditions from each experiment. They were then used as the starting conditions for the next variable to be tested. Initial culture conditions were: temperature 37°C, pH 7.0, impeller speed 80 rpm, minimum dissolved oxygen not controlled.

Initially the effect of impeller speed (80, 110, 140, and 170 rpm) on the cells was tested (*Figures 2A and 2B*). Increased impeller speeds improve the oxygen transfer rate in the growth medium, but also increase the shear stress on cells, which may have been responsible for the longer lag phase as the impeller speed increased. Although the growth rate was similar at 80 and 110 rpm, the decline phase was more rapid at 110 rpm.

At an impeller speed of 140 rpm, the

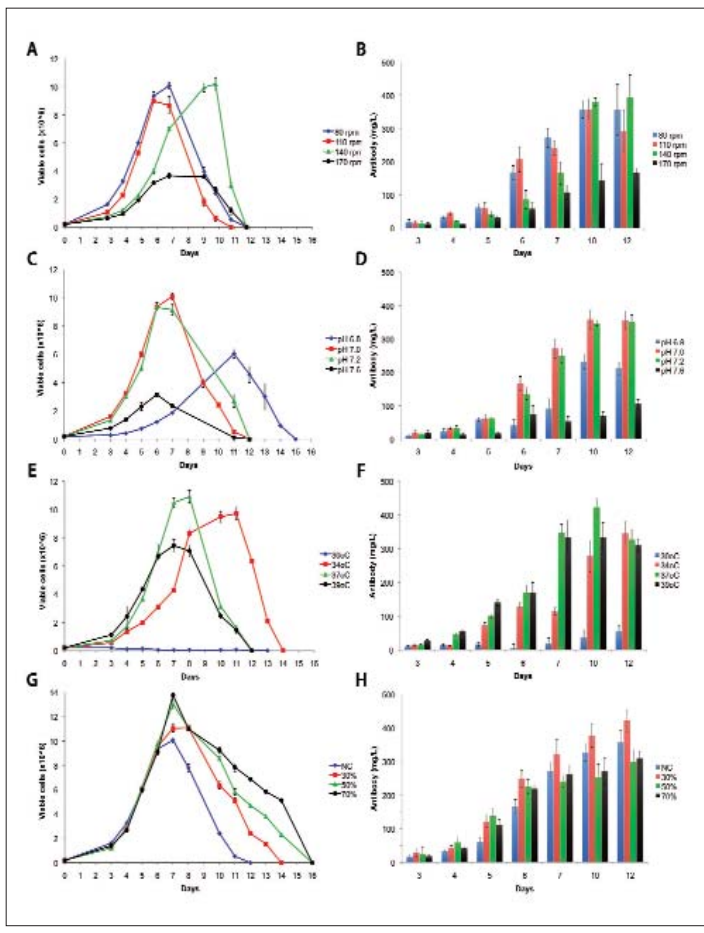

Figure 2. Effects of culture conditions on the growth (left panels) and antibody production (right panels) of CHO cells: Variables tested were impeller speed (A and B), pH (C and D), temperature (E and F), and minimum dissolved oxygen (DO) set point (G and H). No increases in antibody titer were observed beyond day 12 in any of the experiments. pH control was achieved through acid/base addition. The DO concentration was prevented from falling below the minimum DO set point (e.g., 30%) by automatically increasing the oxygen ratio in the mass flow controlled gas mixture. NC indicates the DO concentration is not controlled. Viable cell counts (A, C, E, and G) represent the average of three replicates. Antibody titers (B, D, F, and H) represent three replicates with two technical replicate readings of each. Standard deviation error bars are shown for each value.

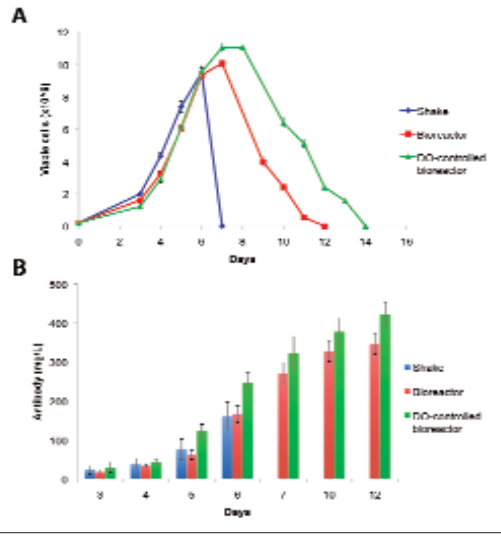

Figure 3. Comparison of batch cultures of CHO cells in shake flasks (shake) and the Dasgip Parallel Bioreactor System without (bioreactor) and with (DO-controlled bioreactor) a 30% minimum DO set point: The growth (A) and antibody production (B) of the cells were monitored. The bioreactor data corresponds to the noncontrolled (NC) data and the DO-controlled bioreactor data corresponds to the 30% minimum DO set point data (30%) from Figures 2G & H. Viable cell counts (A) represent the average of three replicates. Antibody titers (B) represent three replicates with two technical replicate readings of each. Standard deviation error bars are shown for each value.

pattern of growth was shifted by three to four days, but cells still obtained a similar maximum cell density to lower impeller speeds. There was a significantly lower growth rate and maximum viable cell number when the speed was increased to 170 rpm. The maximum antibody titer was similar at 80, 110, and 140 rpm, but decreased at 170 rpm. Therefore, 80 rpm was chosen as the optimal speed because it combined the best growth rates with optimum antibody production.

Once the optimal impeller speed was established, a series of experiments examined the effect of pH (pH 6.8, 7.0, 7.2, and 7.6) on growth and antibody production (*Figures 2C* and *2D*). The optimum pH was in the region of 7.0 to 7.2 where similar growth patterns and antibody titers were achieved. The higher (pH 7.6) and lower (pH 6.8) values resulted in decreased growth rates and viable cell numbers as well as lower antibody titres. Therefore, the pH used for subsequent experiments was pH 7.0.

The penultimate set of experimental conditions examined the effects of temperature (30, 34, 37, and 39ºC) (*Figures 2E* and *2F*). At 34ºC there was a long lag phase and a decreased maximum viable cell number compared to cells grown at 37ºC. However, at 34ºC the cells exhibited an increased stationary phase (from one to three days), extending the duration of the culture by two days.

This data was also examined in relation to antibody production, which exhibited an initial lag period before reaching values similar to that observed with cells grown at 37ºC by day 12. By contrast, cells grown at 39ºC initially grew more quickly than cells grown at 37ºC; however, cells at 39ºC had a decreased maximum viable cell number and decreased antibody production. At 30ºC the cells did not grow, and they simply entered a slow decline phase with a small amount of antibody production. The temperature used for the last experiment was 37ºC.

The final set of experiments tested the effect of maintaining a minimum dissolved oxygen (DO) level (no control or minimum set points of 30%, 50%, 70%) in the bioreactors (*Figures 2G* and *2H*). Following overnight equilibration by sparging of air, initial DO levels in the medium were set at 100%. As the cells grew, the DO concentration in the medium decreased.

The DO concentration was prevented from falling below the minimum DO set point (e.g., 30%) by automatically increasing the oxygen ratio in the gas mixture using the mass flow controllers. In the conditions identified as "no control", the cells were grown without DO control and, consequently, under these conditions the DO level was allowed to fall without any intervention at a minimum set point.

Increasing the minimum DO concentration (from NC to 70% DO) resulted in increased maximum viable cell numbers (from 1 x 10^7 to 1.4 x 10^7) and overall length of culture (from 12 to 16 days). The increased growth and viability with increased DO concentration was not mirrored for antibody production. While the 30% set point improved both growth and productivity, the two highest DO levels (50% and 70%) resulted in decreased antibody titers.

The findings from these experiments led us to define our standard conditions for growth of CHO cell lines as: temperature 37ºC, pH 7.0, impeller speed 80 rpm, minimum dissolved oxygen 30%. (These conditions correspond to the 30% minimum DO set point data [30%] from *Figures 2G* and *2H*).

The data presented in this article defines a standard set of conditions that can be used for the growth of CHO cell lines using the Dasgip Parallel Bioreactor System. These conditions were determined in nine weeks and provide a starting point for the further optimization of culture conditions for specific cell lines expressing different recombinant proteins (e.g., mAb variants).

Comparison with Shake Flasks

Shake flasks are routinely used for growth of cell lines and are generally believed to provide a reasonable estimate of cell-line behavior in the first days of growth in a bioreactor. Comparison of CHO cells grown in the Dasgip Parallel Bioreactor System to cells grown in shake flasks demonstrated that the growth and antibody-production characteristics over the first five days of culture were similar for shake flasks and the non-DO controlled bioreactor (*Figures 3A* and *3B*). However, the increased DO concentration in the medium in the DO-controlled bioreactor resulted in increased antibody production.

Shake flasks fail to maintain the appropriate aeration to support batch culture viability beyond day seven, and therefore it is likely that the growth of cells in the Dasgip Parallel Bioreactor System will provide a better scale-down model for comparison to large-scale fermentation over the duration of a complete batch culture (batch or fed-batch). The design features of the Dasgip Parallel Bioreactor System combines precise control of the culture conditions with ease of use and gives data that offers predictive information which is directly relevant to designing large-scale culture processes. GEN

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