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The effects of specific culture parameters are cell line-dependent and need to be investigated to allow optimization of productivity and product quality. In this study, a biphasic cultivation strategy for a Chinese hamster ovary (CHO) cell line expressing an erythropoietin fusion protein (Epo-Fc) was developed. In biphasic cultivation, culture conditions are changed after accumulating biomass during an initial growth phase to allow maximum recombinant protein expression in the production phase. Cultures were run in batch mode, and after an initial growth phase, cultivation temperature and pH were shifted to allow optimized protein production and quality. Using a design of experiments (DoE) approach, the individual and synergistic effects of cultivation temperature and pH on the responses cell growth, recombinant protein production, and protein aggregation could be systematically evaluated. The results show that all three responses were influenced by the cultivation temperature. Additionally, an interaction effect between pH and temperature was found to be associated with protein aggregation.

By lowering culture temperature and pH from initial 37°C and pH 7.05, a 2.5-fold increase in final product concentration and a reduction in aggregates from 75% to less than 1% could be achieved. Furthermore, lowering temperature and pH substantially reduced cell-specific glucose and glutamine consumption as well as lactate and ammonium production. Increasing cell-specific protein production by optimizing culture conditions significantly contributes to a more economic bioprocess.