Effects of Lowering Culture Temperature in a Continuous Perfusion Platform When Optimizing Upstream Bioprocessing

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Abstract

Upstream bioprocess development generally involves the study and characterization of a number of parameters as they relate to their impact on cell growth, and volumetric productivity among others. These process variables are many, and their corresponding effects and ideal parameters will vary based on the nature of the cell line and molecule under study, as well as the cell culture platform utilized. While fed-batch culture has become quite popular, continuous perfusion systems continue to offer strategically advantageous benefits for the production of unstable or complex recombinant proteins. It is, however, necessary to recognize the fundamentally different effects that varying process parameters can have based on the cell culture platform used. Reduced temperature, for example, has been shown to stimulate increased productivity in batch and fed-batch culture and, while this phenomenon is frequently exploited in current bioprocesses, the implementation of a temperature reduction strategy has the potential to upset the somewhat delicate process of continuous perfusion.

Here, we will examine the complex role of temperature during the process optimization of a disposable CHO cell perfusion based platform utilizing an Alternating Tangential Flow (ATF) filtration system. The effects of lower culture temperatures as they relate to cellular growth rate, purge rate, and filter retention will be explored as well as strategies for identifying optimal temperatures to maximize the volumetric productivity and overall value of an upstream bioprocess.

Materials and Methods

Cell Culture

CHO cell preculture was thawed and propagated in shake flasks for fewer than 30 generations prior to inoculation of bioreactors. A ramp-up phase at a higher temperature was carried out early in the culture to allow for rapid cellular proliferation and minimization of overall culture time. Perfusion was initiated during the early stages of the culture and was brought to maximum rate prior to the first temperature shift. All temperature shifts were executed on a linear gradient over 48 hours.

ATF Perfusion

Perfusion was performed using a Refine® ATF perfusion system. Retention rates were calculated daily based on product concentration differences between bioreactor and harvest streams (pre and post ATF filtration respectively).

Biomass Removal

Biomass removal was initiated based on cellular growth rates and adjusted as needed to maintain steady state viable cell densities within the reactors.

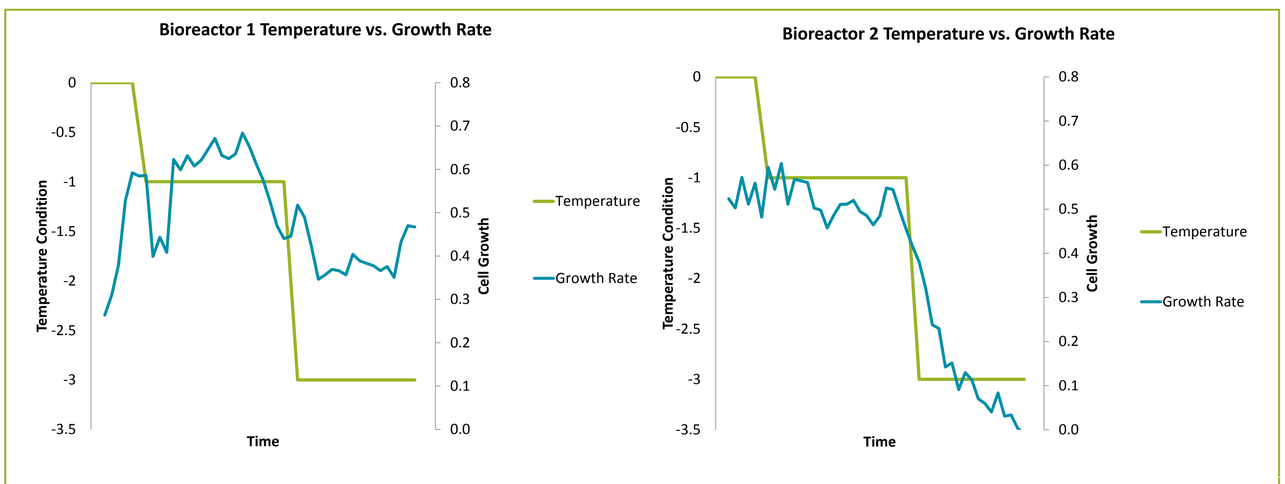

Figure 1: Decreased temperature led to decreased cellular growth rates

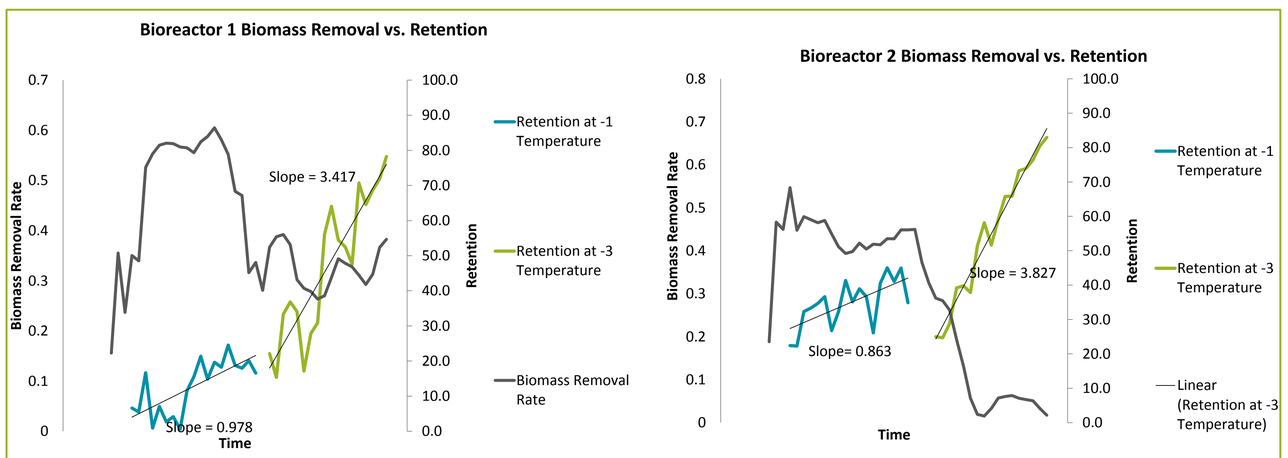

Figure 2: ATF retention rates increase as biomass removal rates decrease

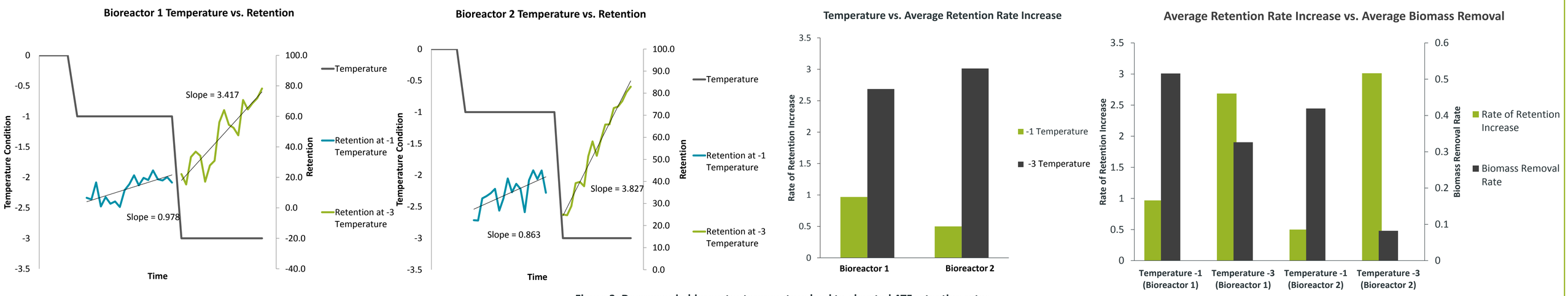

Figure 3: Decreases in bioreactor temperature lead to elevated ATF retention rates

Results

Bioreactors were run at each of two temperature conditions (-1 and -3). ATF retention as well as daily biomass removal and cellular growth rates were monitored over the course of the bioreactor runs. **Figure 1** depicts the temperature profiles for each bioreactor run as well as cellular growth rates. Both reactors exhibited a decrease in growth rate corresponding to the -3 temperature relative to the -1 temperature condition. In order to maintain steady cellular densities within the bioreactors, the decreased growth rates associated with lowering the vessel temperatures necessitated a decrease in biomass removal rate of approximately 40% for Reactor 1 and 80% for Reactor 2.

As a means of measuring the effects of temperature and subsequent biomass removal rates on ATF retention, the rate of retention increase was calculated based on the slope of the line of daily retention. **Figure 2**, highlights the inverse relationship between daily biomass removal rates and the slope of ATF retention increase. Both reactors exhibited a sharp increase in retention rate, greater than 3-fold, following decreases in biomass removal. Finally, **Figure 3** shows ATF retention data relative to the temperature profiles of each reactor as well as average retention rate increases and biomass removal decreases. Both bioreactors displayed a strong correlation between decreasing temperature and biomass removal rates and increasing rates of ATF retention.

Conclusion

The data presented here show that lower growth rates associated with a decrease in temperature necessitate a concomitant decrease in biomass removal, leading to an increased rate of ATF retention. This retention is likely the result of accumulation of cell matter within the reactor which is incapable of passing through the ATF filter membrane and thus can only be removed by means of removing biomass. Therefore, it is critical to assess both the potential positive as well as negative effects that a temperature reduction strategy might have on an ATF perfusion platform.

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