HIGH REPRODUCIBILITY IN THE CULTURE BIOSCIENCES HIGH-THROUGHPUT BIOREACTOR FACILITY.

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ABSTRACT

Parallel experimentation in single-use equipment is an important strategy to reduce bioprocess development time. In this study we demonstrate robust process control of 15 parallel *E. coli* fermentations in the Culture Biosciences high-throughput bioreactor system. Processes run with the same operating parameters showed highly similar growth profiles.

INTRODUCTION

Developing a robust and efficient bioprocess for scale-up is crucial for commercial production. Process optimization requires large numbers of bioreactor experiments for clone screening, media optimization, and operating parameter DOEs. High-throughput screening in bench-scale systems can dramatically accelerate bioprocess development by providing data that is comparable to bench, pilot, and manufacturing scale.

With Culture Bioscience's high throughput bioreactor platform, process optimization can be completed in parallel runs in a fraction of the time ordinarily required. To obtain high quality data, these systems must be able to reliably control industry standard process parameters such as temperature, pH, DO, and feeding strategy. We demonstrate reproducible growth profiles when running fifteen parallel *E. coli* clones: five replicates at three different process temperatures.

MATERIAL AND METHODS

Researchers carried out the process in Culture Bioscience's 250mL single-use bioreactors. Two rushton impellers powered by a magnetic-coupled drive provide high agitation suitable for microbial applications. Bioreactors are equipped with active heating and cooling.

E. coli cultures were grown for 24+ h in media with initial glucose concentration of 30 g/L. The processes were started at 100ml and fed with 500 g/L glucose. Fed batch feeding was initially triggered by DO spike and a complex feed strategy based on DO spike detection was implemented thereafter. The temperature was controlled at three different setpoints: 25°C, 30°C, and 37°C. pH was adjusted to 7 using 7M ammonium hydroxide. Fifteen experiments were run in total with five at each temperature.

Dissolved oxygen was maintained at 20% through cascade control of the stirrer speed and aeration rate. Stirrer speed ranged from 750-3000 rpm corresponding to tips speeds of 0.94-3.77 m/s. Air was sparged through a submerged dip tube at flow rates ranging from 3-16 sL/h (0.5-2.0 vvm).

RESULTS AND DISCUSSION

Process parameters such as temperature, pH, and DO were monitored live. Temperature was adequately controlled for all vessels throughout the run. The pH was also stable throughout the run at pH 7, see **Figure 1**.

As expected the *E. coli* clones held at 37°C had the fastest growth profiles followed by the 30°C and 25°C clones. The feed-start was automatically triggered by a glucose depletion induced DO peak, shown in **Figure 2**. The five vessels at 37°C started feed at 9.0±0.3 h and the five vessels at 30°C started feed at 15.4±0.7 h. The five vessels at 25°C did not reach biomass levels high enough to trigger feeding within the time of the experiment. The range of feed start times for vessels at the same temperature was <5% of the EFT.

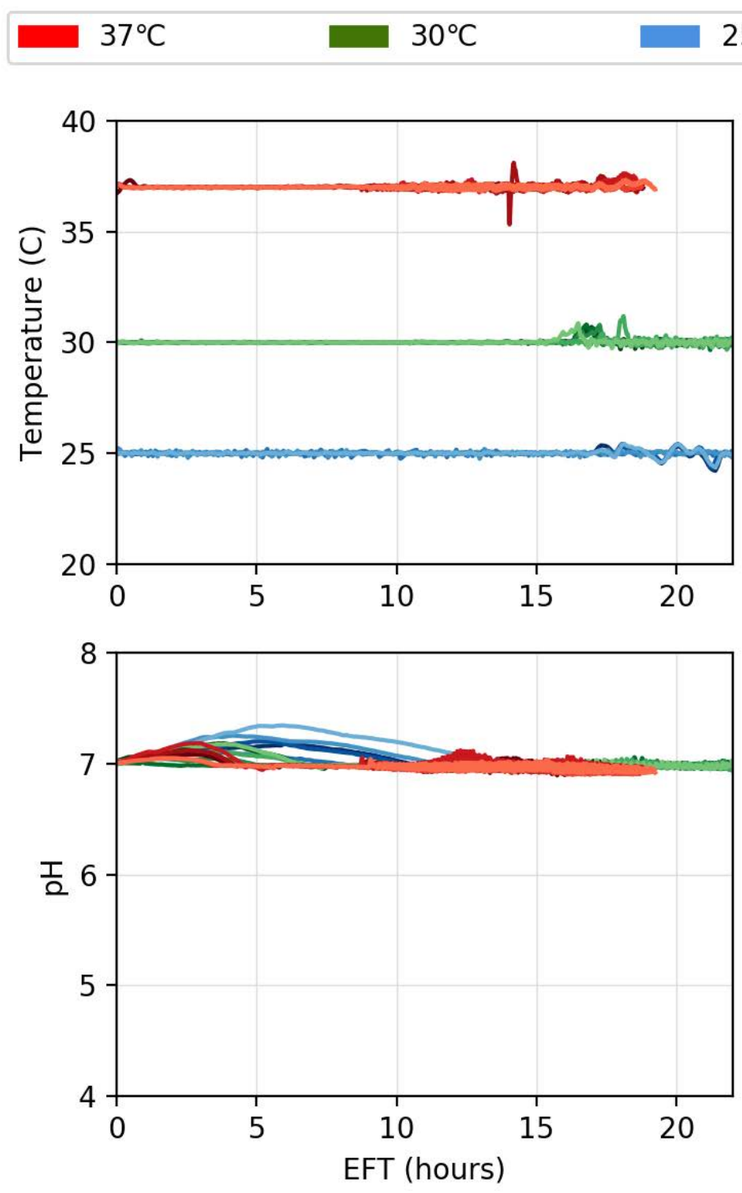

Figure 1. Temperature and pH of parallel fermentation of *E. coli* in 250 mL single-use vessels.

After the initial feed trigger by DO spike, we implemented a complex feeding strategy where a feedback control loop based on DO peak response was used to control feed pump rates.

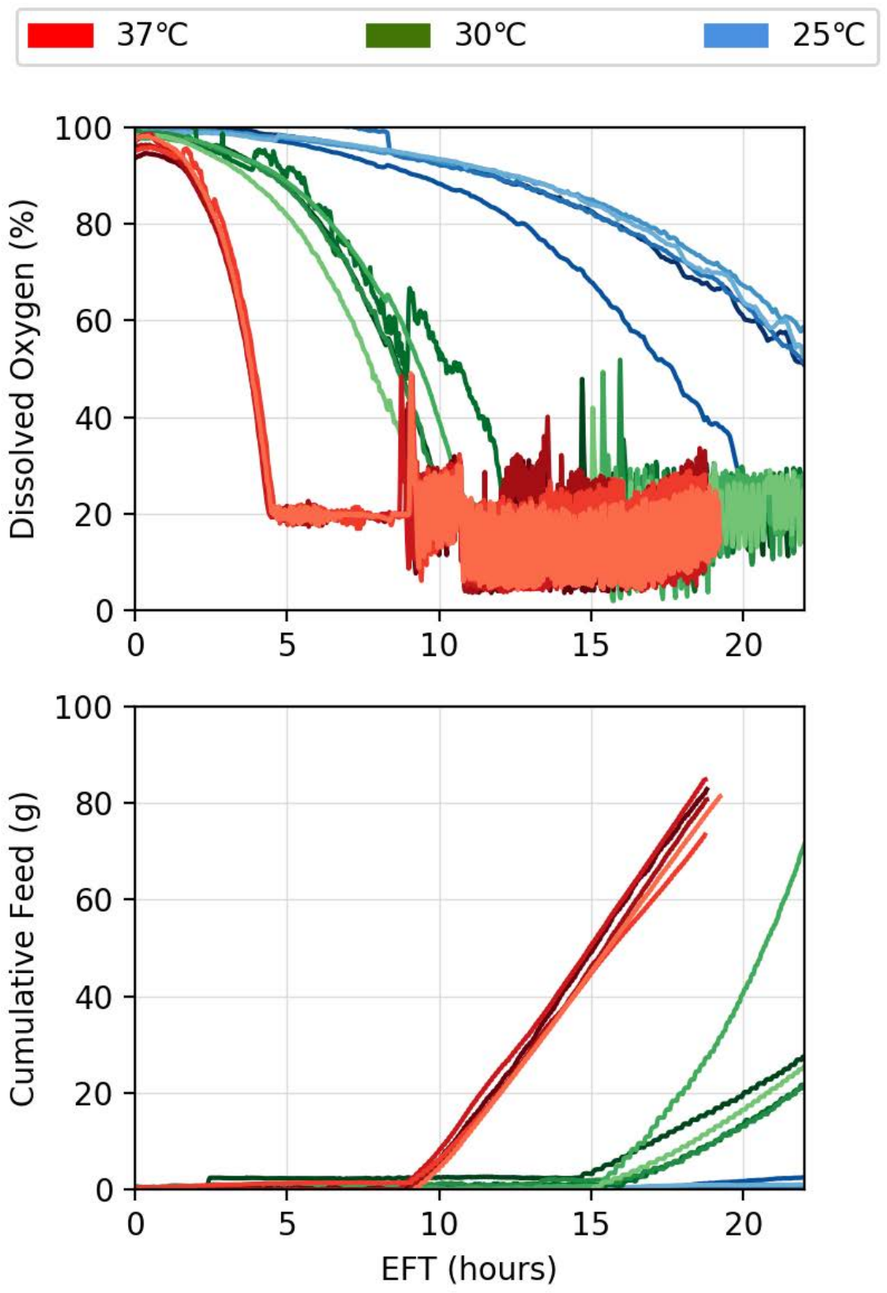

Figure 2. DO and cumulative feed amount of parallel fermentation of *E. coli* in 250 mL single-use vessels.

When the DO level spikes above 30%, the PID controller pumps feed at a fixed mass flow rate until the DO level begins to drop. The mass flow rate is determined from change in weight of the feed bottle, which is mounted on a scale. **Figure 3** shows an example of the dissolved oxygen trace and pump response in a narrow time window during the growth phase.

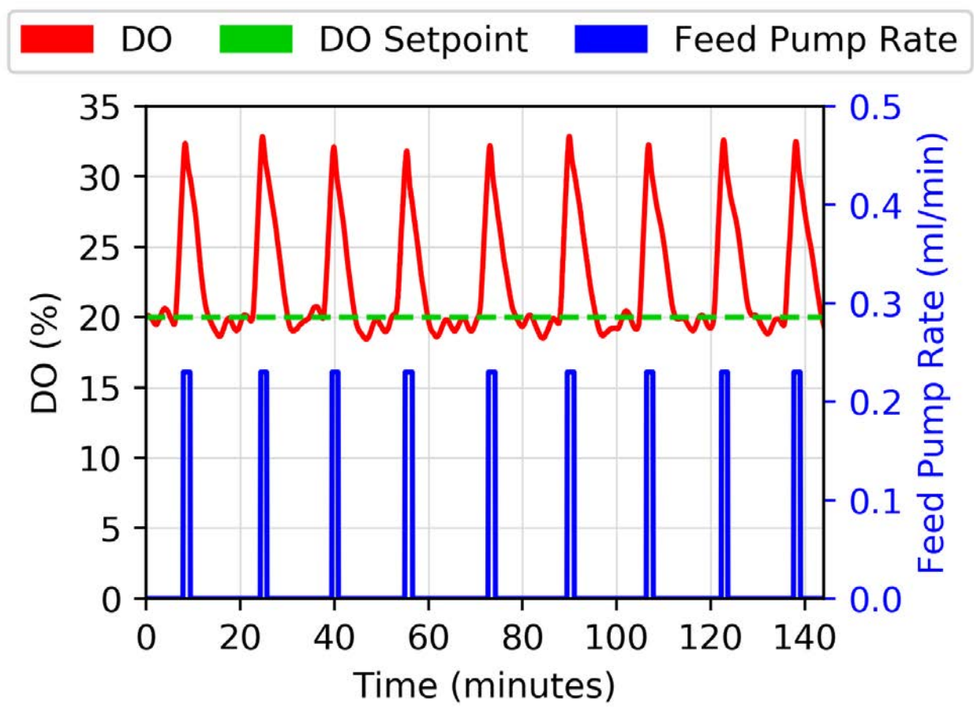

Figure 3. Dissolved oxygen and feed pump rates demonstrating active feedback control by DO peak response for *E. coli* grown at 37°C.

The tight control of fermentation parameters in parallel reactors leads to consistent biomass growth rates among vessels at each temperature (see **Figure 4**). In addition, offgas analysis (**Figure 5**) shows OUR and CER values of up to 500 mmol/L•h for the 37°C clones with stable RQ values in the range of 0.96-1.05 in the fed batch growth phase.

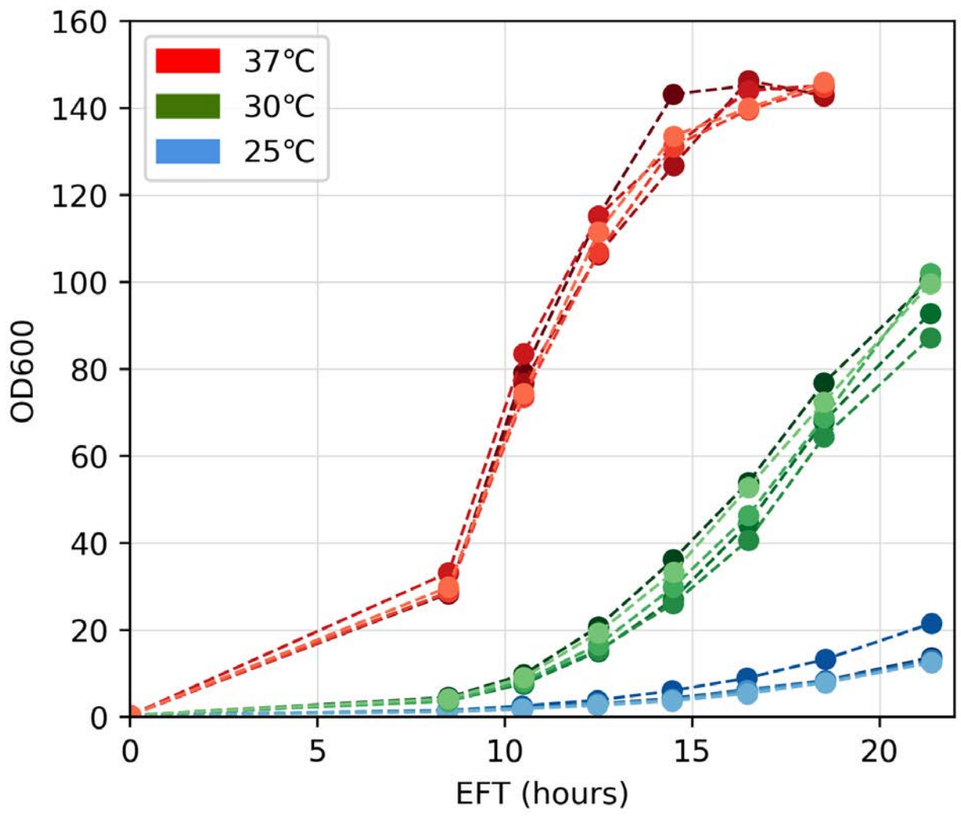

Figure 4. OD600 timepoints tracking *E. coli* growth.

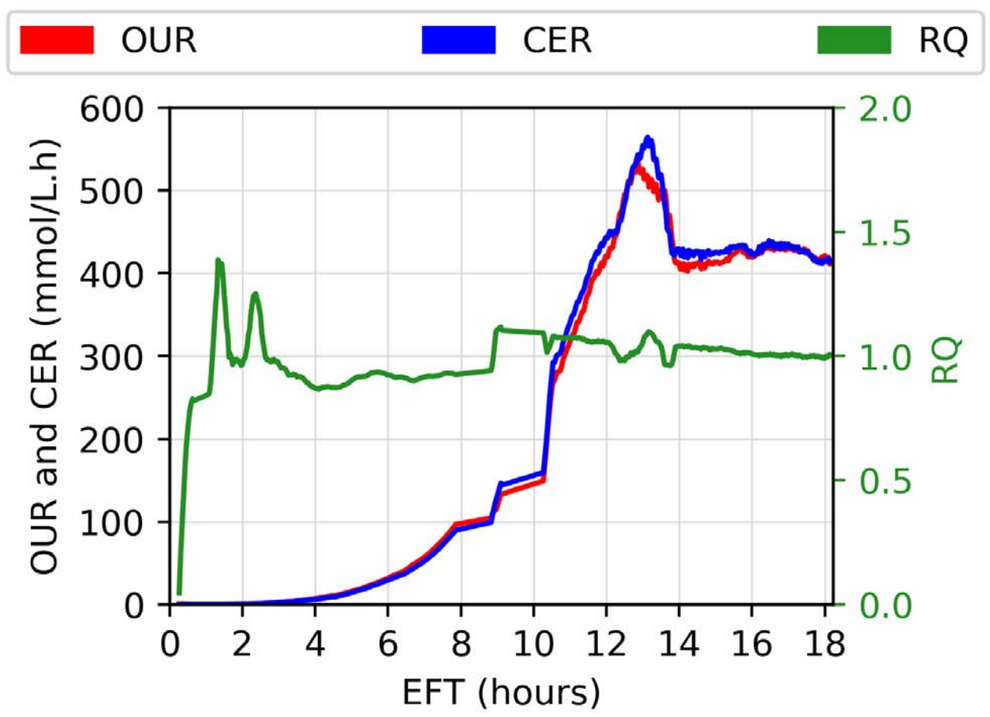

Figure 5. OUR, CER, and RQ analysis from off-gas data collected of *E. coli* grown at 37°C.

The runs at 37°C reached a maximum OD600 of 145 demonstrating the system is suitable for high density microbial fermentation.

CONCLUSION

Culture Biosciences' high-throughput bioreactor system can reliably control critical process parameters leading to high reproducibility of parallel fermentations. The single-use bioreactors are well-designed for microbial fermentation and can achieve high mass and heat transfer. This case study demonstrates our system's ability to generate high quality, reproducible data that can be used to accelerate process development and scale-up.

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