Steady state and dynamic control performance of the ambr™ automated micro bioreactor system in a CHO cell batch culture

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Introduction
Implementation of high throughput automated systems is recognised as a viable approach in the field of bioprocess development(1). An efficient multi-parallel microscale bioreactor system can unlock development bottlenecks in a variety of bioprocess applications, such as cell line screening, media development, feed and process optimisation, QbD and DoE studies.

Aims
• Illustrate the capability of the ambr system with CHO cell cultures
• Assess the degree of reproducibility for replicate ambr cultures
• Investigate the resolution in CHO cell culture responses for a variety of experimental conditions (signal to noise ratio)
• Demonstrate Process Control Performance in the ambr system
• Steady State Control for standard culture operation
• Dynamic Control for parameter shift applications

CHO Cell Culture
• 24 parallel bioreactors in parallel, as a single experiment
• Standard Conditions: pH 7.1 ± 0.1, DO 40%, 3x10⁵ cells/mL
• 6 (or 3) replicate cultures for standard (or test) conditions
• CHO-K1S cells, Sigma ExCell ACF Medium, max volume 15mL
• baseline Ambr® Alarms, 24h added on days 0, 4, 7
• 37°C, 850 RPM (impeller tip speed 0.5 m/s)

Aim: Test a variety of pH values and ranges at steady state

pH Control Test Setup

- pH 7.1 ± 0.1 (x3 bioreactors)
- pH 7.0 ± 0.2 (x3 bioreactors)
- pH 6.9 ± 0.2 (x3 bioreactors)

pH Control Conclusions
• Good steady state pH control, as ‘wide range’ or ‘specific setpoint’
• Individual vessel control of pH, CO₂, gas and liquid base additions
• Automatic liquid base addition as needed, e.g. through the night
• Base settings are flexible e.g. gain, target pH, base concentration

pH control test results: 7.1 ± 0.1 (x6 bioreactors)

- (1a) pH controlled down to upper limit by CO₂, pH without
- (1b) an example CO₂ gas flow trace
- (2a) pH at lower limit by liquid base additions
- (2b) an example pH trace, saw-tooth line due to base additions

pH control test results: 7.0 ± 0.2 (x3 bioreactors)

- (1a) pH controlled down to upper limit by CO₂ gas input
- (1b) an example CO₂ gas flow trace
- (2a) pH generally fluctuates within the specified range
- Only one base addition was required

pH control test results: 6.9 ± 0.05 (x3 bioreactors)

- pH controlled to a constant level by CO₂ gas addition throughout
- Trace overlaps for clarity
- Small pH fluctuations due to minor pH offset adjustments

Cell Culture Results

• Clear resolution between 3 and 8x10⁴ cells/mL conditions
• Slight scatter is observed in seeded cell density, as a result of the inherent variability in cell seeding. This is due to cumulative small errors in source cell count, sampling & pipetting, and final cell count

Aim: Test a variety of DO values at steady state

DO Control Test Setup

- DO 20% (x3 bioreactors)
- DO 60% (x3 bioreactors)
- DO 40% (x3 bioreactors)

DO Control Conclusions
• Good steady state DO control
• Good resolution between setpoints
• Minor fluctuations occur due to bioreactor cap removal

DO control test results (x12 bioreactors)

- (1a) Excellent steady state DO control without cap removal
- (2a) DO shift test (x3 bioreactors)
- pH shift 7.2 → 7.0
- Thermal shift 37°C → 33°C
- Temp. shift time ~ 1 h
- Time is independent of culture properties

Cell Culture Conclusions
• The ambr system performs very well with CHO cell cultures
• Very good resolution in CHO cell culture responses for parameter shifts (curves for all conditions)
• High reproducibility between ambr CHO culture replicates demonstrates good process control performance within the ambr system

Aim: Test dynamic control, for parameter shifts (x3 bioreactors)

DO shift test (x3 bioreactors)

- VERY high viability with low scatter between replicates
- Viability begins to decline after glucose is depleted, leading to lower viability for high seeding density, and pH 7.1 conditions

Dynamic Control Test Setup

Aim: Test dynamic control, for parameter shifts (x3 bioreactors)

Temperature shift test

- pH shift 7.2 → 7.0
- DO shift 60% → 40 %
- Shifts occur smoothly and under control without overshoot
- Control dynamics rapid compared to CHO cell culture
- Enables common industry process ‘shift’ applications


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