

Introduction

As a supporting process in biopharmaceutical workflows, the hydration of media, feeds, and buffers has often been overlooked as an opportunity for process optimization, risk mitigation, and cost reduction. The hydration process requires a high amount of human intervention, which can impact variation in a bioprocess overall. In this study, we evaluated a novel approach to replace manual media preparation methods with automated in order to improve consistency, reduce labor, reduce process time, employ digital controls, and digital electronic batch records that facilitate investigations and data analysis. In the end, we also needed to demonstrate that the resulting culture media perform as well as control batches of the same media prepared by traditional methods. In a single unit operation, the Oceo Rover reliably hydrates and drives sterile filtration of cell culture media resulting in sterile, ready-to-use media that support equivalent cell culture performance.



Methods and Materials

This study was designed to evaluate the performance of a production cell culture medium prepared by various methods:

1. Automated Oceo Rover system
2. From fresh powder by traditional methods
3. Pre-made liquid shipped ready-to-use from supplier
4. From aged powder in Oceo Rover cartridges (aged 3 months)

Cell line: mAb expressing Apollo X model cell line, FUJIFILM Diosynth Biotechnologies (FDB).

Cell culture media: Production medium: Proprietary FBM514, FUJIFILM Irvine Scientific.

Common seed train medium: FDB proprietary medium, FUJIFILM Irvine Scientific.

Common feed media: Cell Boost 7A/7B Feed, Hyclone.

Culture conditions: Fed-batch cultures, duplicate 10 L Bioreactor Runs (A and B) for each condition.

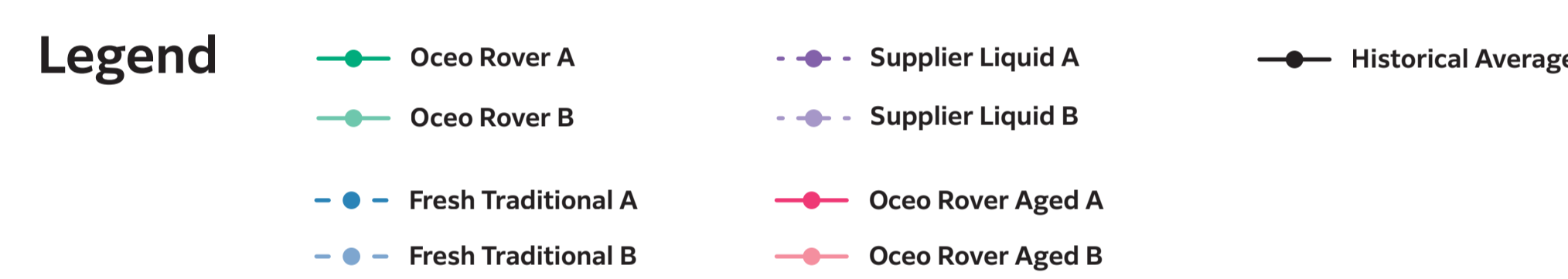
Cell culture performance analysis: Monitor growth and metabolites on NOVA Flex 2: Viable cell density (VCD), Yield, Viability, Cell size, Osmolality, Glucose, Lactate, Glutamine, Glutamate, and Ammonium. Monitor mAb titer on Cedex.

Results

Step	Phase	Parameters
0	Ready	N/A
1	Fill	Phase Volume = 20 L WFI Flow - 10 LPM Conductivity Limit = 3 mS/cm
2	Top w/ Bypass	Cumulative Volume = 25 L PCV-004% Open = 5%
3	Top	Phase Volume = 25 L
4	Bottom	Phase Volume = 30 L
5	Bottom Pulse	Pulse Count = 5 Pulse Duration = 60 seconds Pulse Volume = 5 L
6	Top	Phase Volume = 10 L
7	Bottom	Phase Volume = 55 L
8	Dry Supplement	Phase Volume = 20 L Pressurize Time = 3 seconds
9	Bottom	Cumulative Volume = 200 L
10	Blowout Dry Supplement	Vent Pressure - 0.5 psig Outlet Pressure - 1 psig Max Duration = 20 minutes
11	Finish	N/A

Figure 1. (above) Automation recipe for 200 L FBM514 medium preparation process. Defines a volume-based schedule of phases along with specific parameters for each individual phase.

	Oceo Rover	Traditional Preparation
Time	34 minutes	231 minutes
Volume Prepared	200 L	20 L



Equivalent Cell Culture Growth and Production

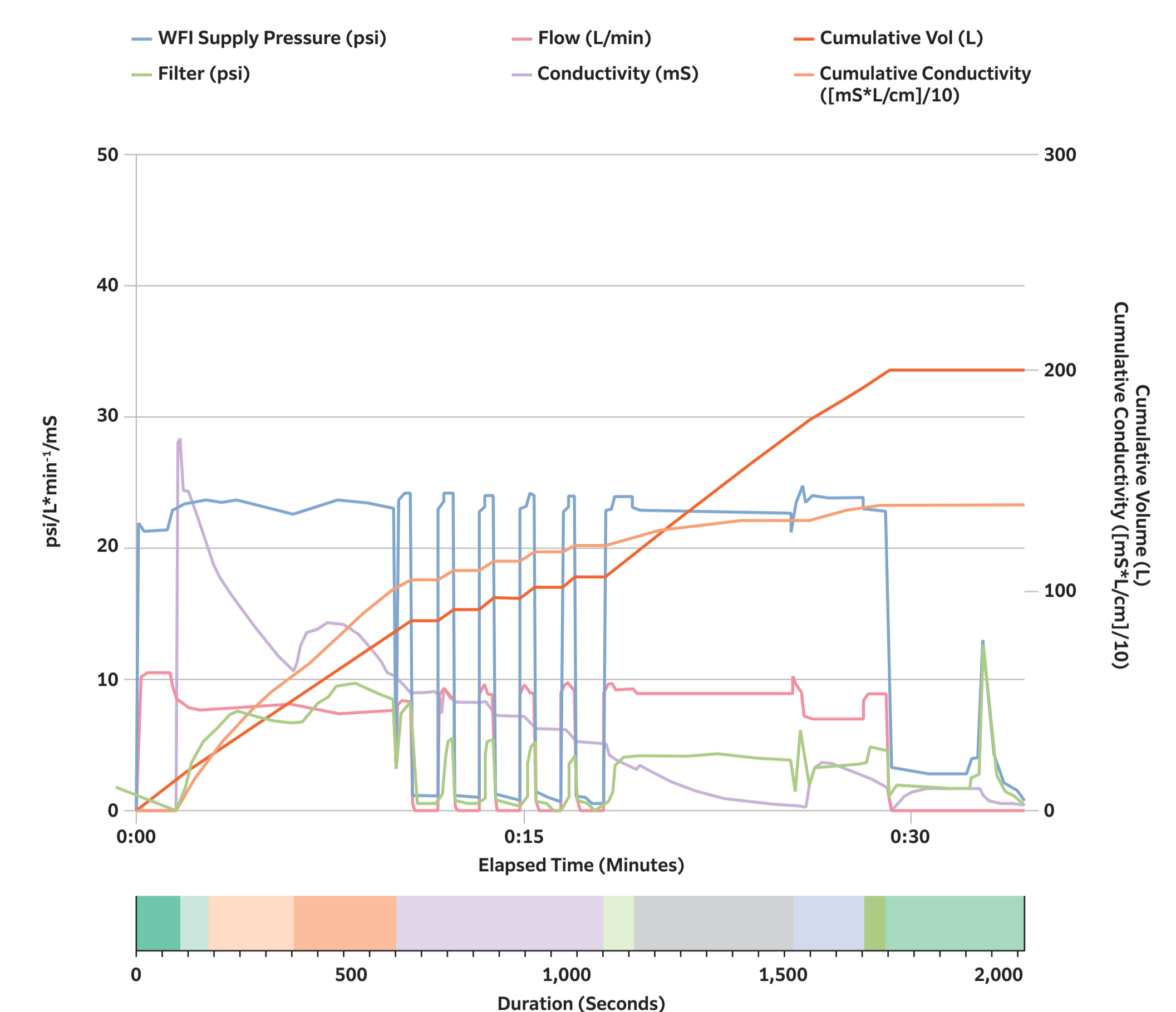
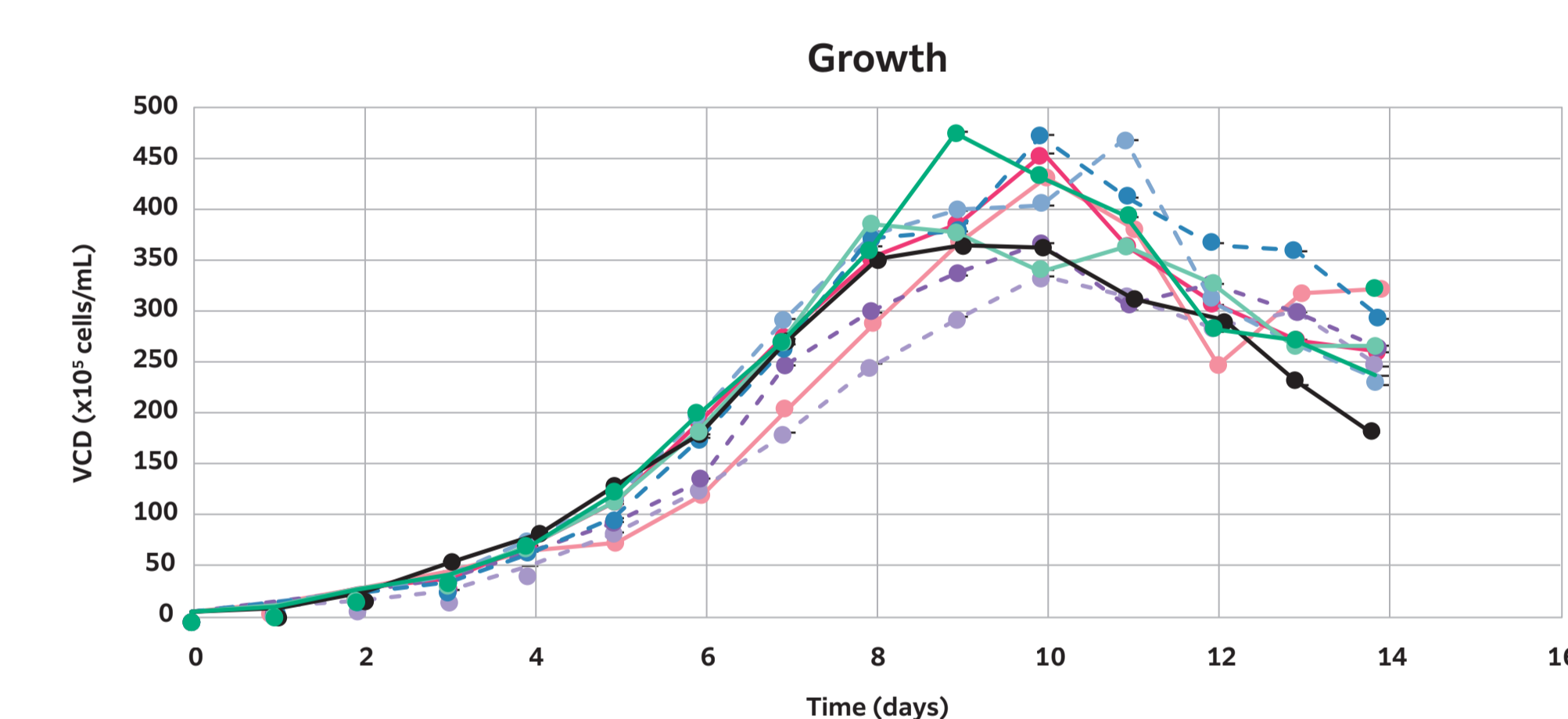
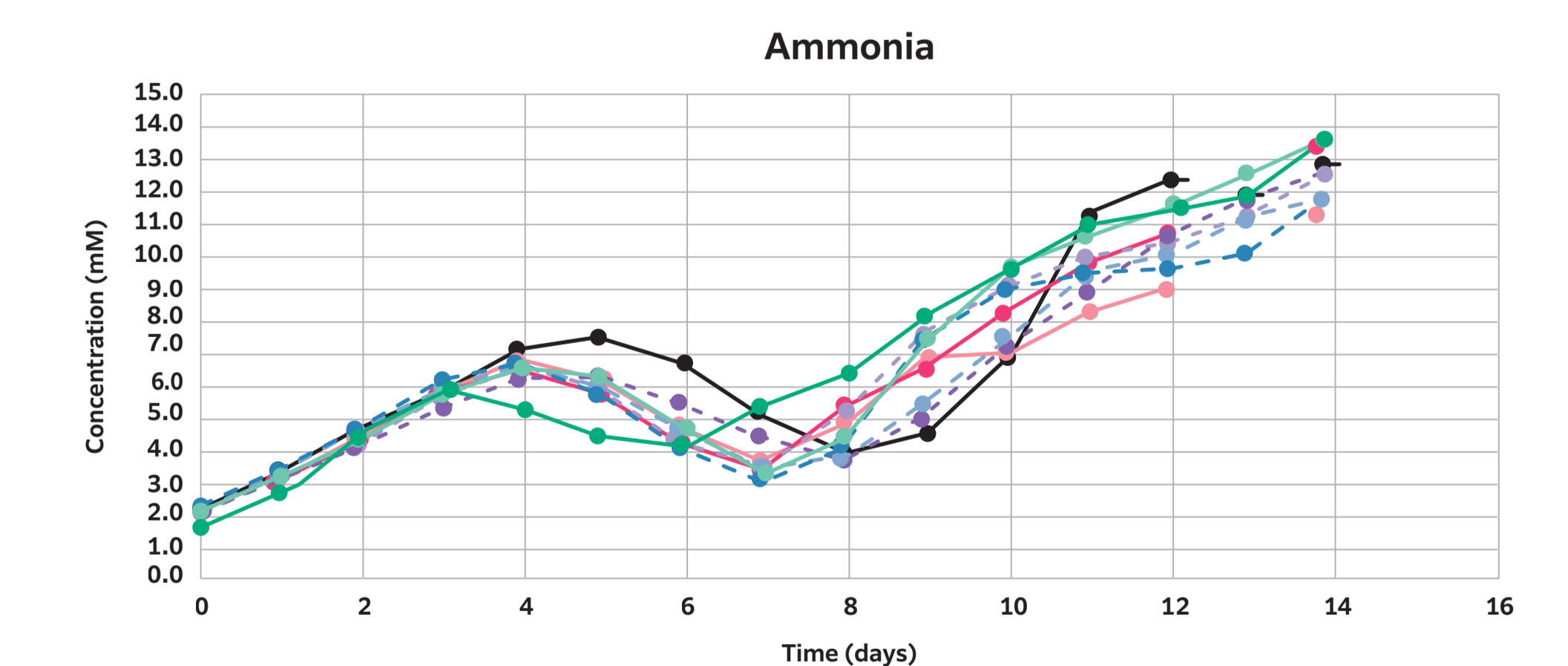
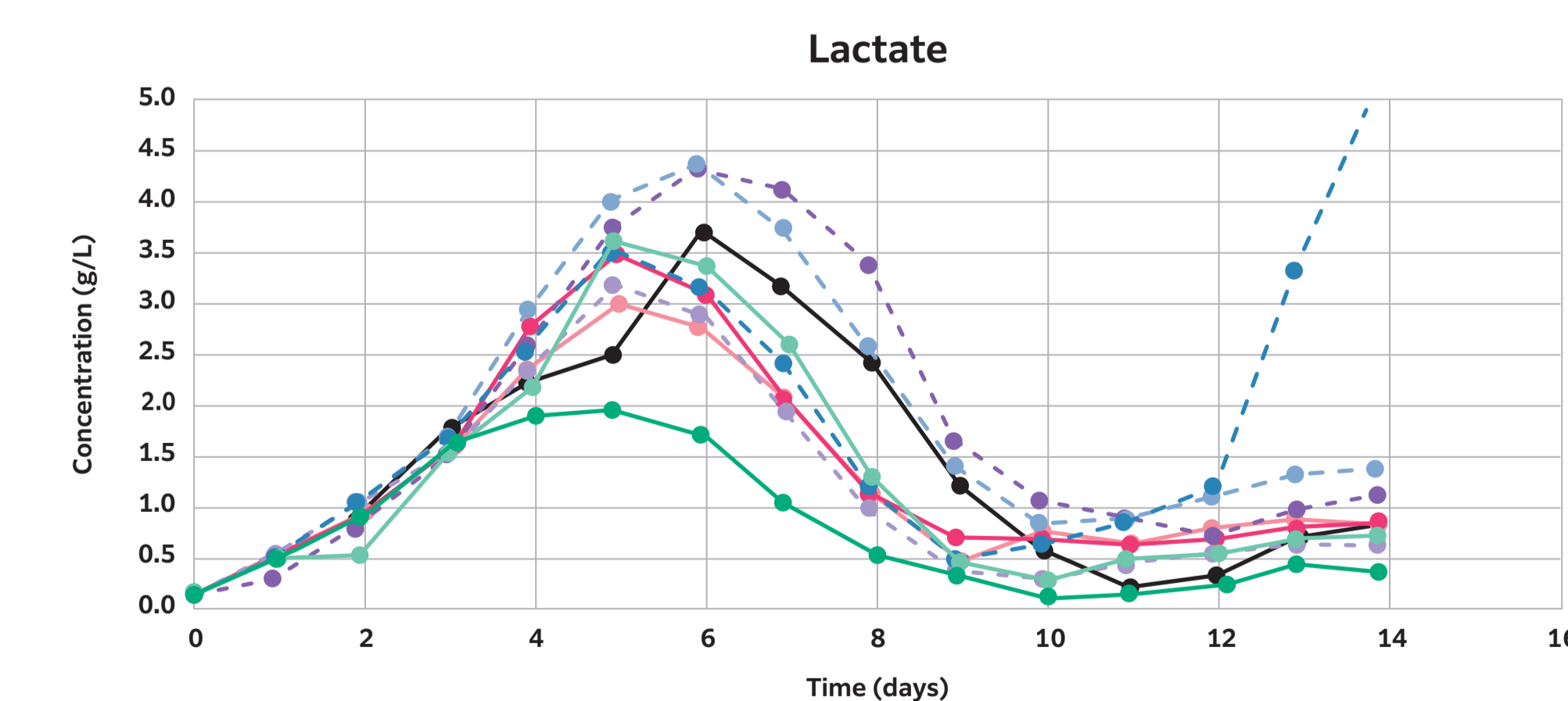
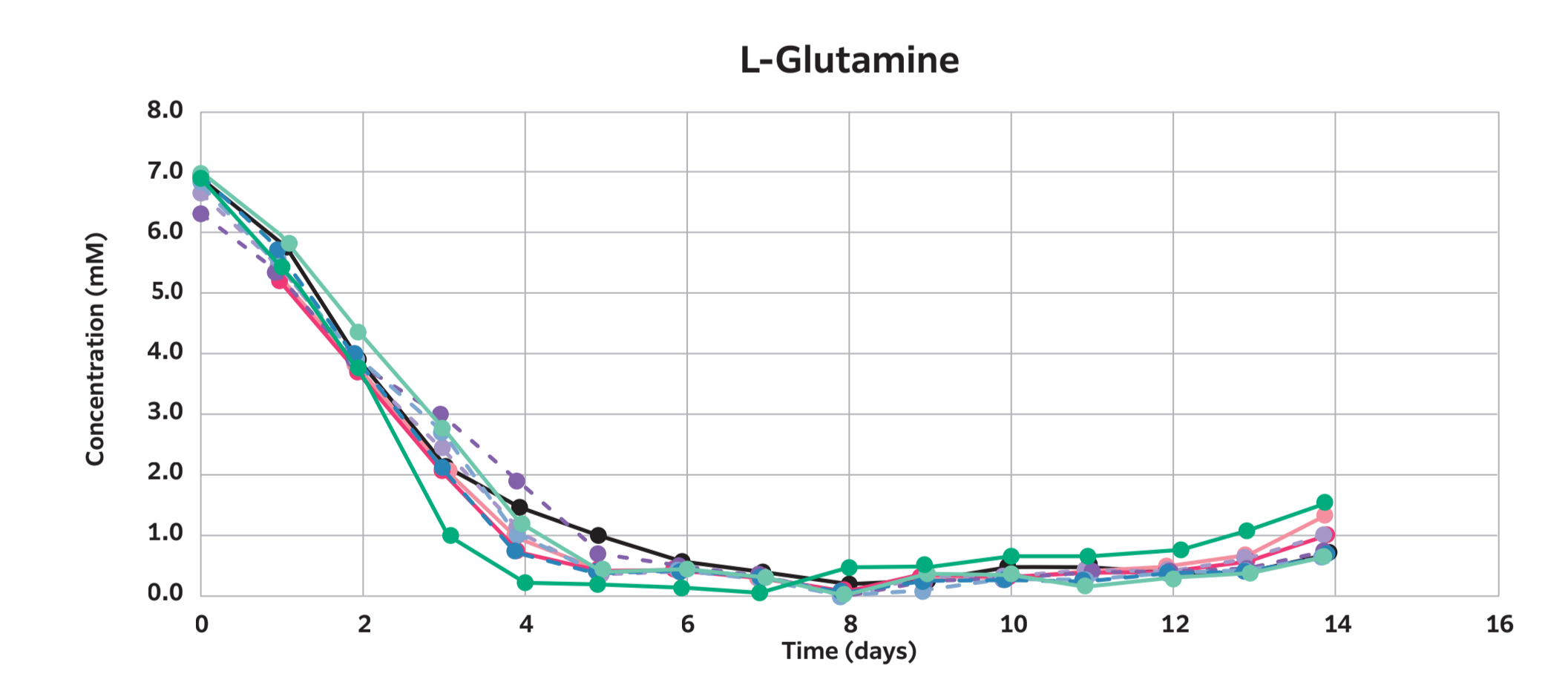
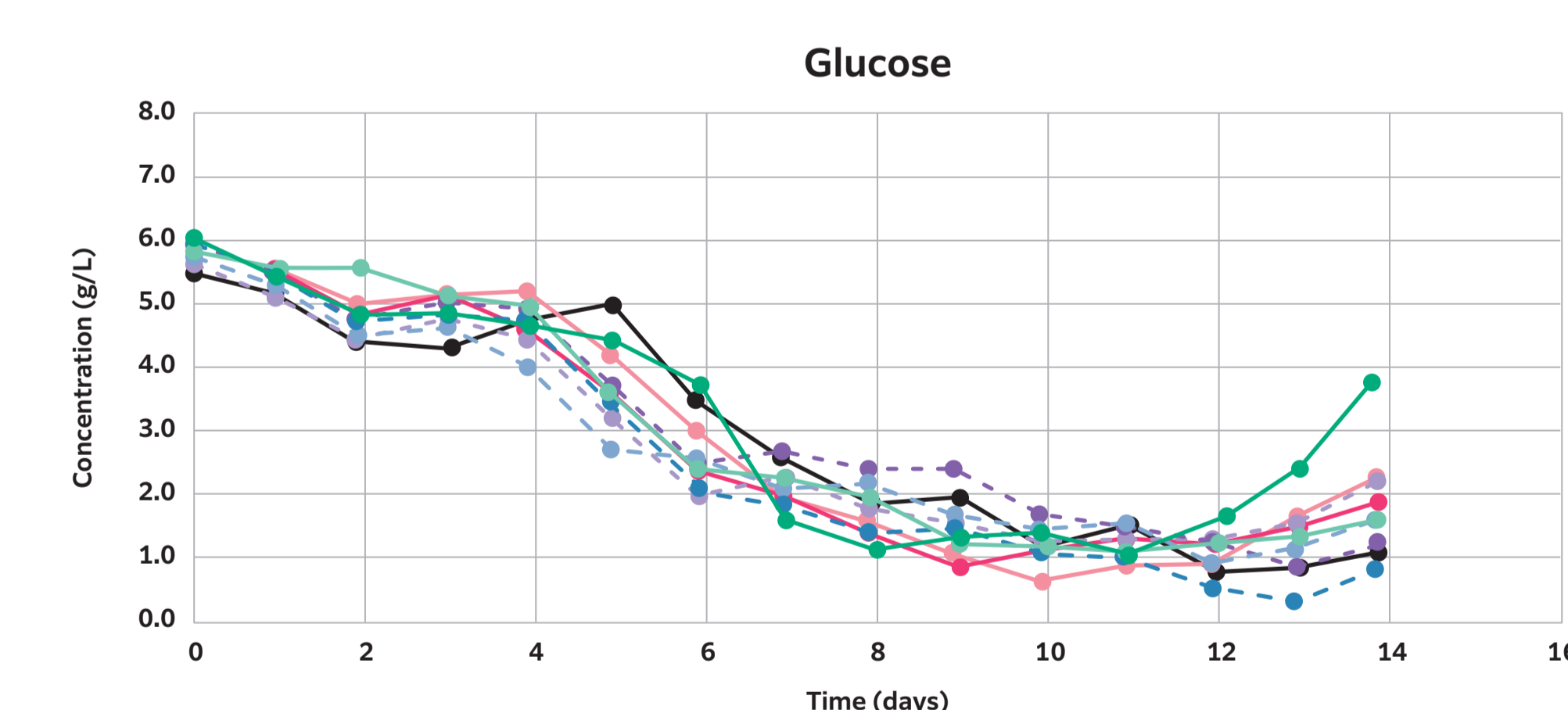


Figure 2. (above) Electronic record of 200 L FBM514 medium preparation process. Provides a complete digital record of the preparation process, including a time course of changing pressures, flow rates, conductivity, cumulative volume, and cumulative conductivity. Color-coded phases correspond to the recipe phases described in Figure 1.

Figure 3. (left) Time and labor reduction. The Oceo Rover method employs pre-measured materials and an automated hydration/filtration unit operation as a way to reduce labor, human error and overall processing time. The overall process time is much shorter even when preparing much larger batches.

Figure 4. (below) Cell culture performance. Performance of 8 media batches were compared to each other and to a historical average in parallel 10 L fed-batch bioreactor cultures.

Equivalent Nutrient and Metabolite Profiles



Summary

The automated Oceo Rover method for media preparation:

- **Delivered equivalent cell culture performance:** In terms of growth, production, nutrient, and metabolite profiles
- **Generated a complete electronic record of the preparation process:** Useful for retrospective analysis and investigations
- **Reduced human intervention:** Addressing a recognized source of variation with traditional preparation methods
- **Saved time:** The automated method was 6.7 times faster than the existing methods even when making 10 times the volume