

Know Your Process: On-line and At-line Analytics for Upstream Bioprocess Key Nutrients

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Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Overview

In bioprocess development, point-of-need analytics are required to monitor critical process parameters and achieve desired product quality attributes. Repligen enables data-driven, accelerated process optimization with the following analytical bioprocess devices: On-line PATsmartTM MAVEN[ ] for glucose and lactate and PATsmartTM REBEL[ ] Analyzer for spent media analysis.

Automated monitoring of glucose and lactate plus low-level glucose control with the MAVEN System together with understanding the amino acid consumption profiles with the REBEL System, can lead to higher growth/viability, lower lactate, and decreased COGS. Defined control strategies enable more intensified and stable cell culture processes, such as perfusion, which may see dramatically improved productivity. This results in a more efficient and profitable process for biopharmaceuticals manufacturing.

Real-time Monitoring of Glucose and Lactate and Glucose Control

Automated feeding enabled by frequent on-line measurement of glucose can be used to create consistent and stable glucose levels in cell culture. This reduces the stress of variable (alternating high and low levels of nutrient) conditions that often occur in bolus feeding strategies. The outcome of the optimized glucose feeding strategy is improved cell growth and viability (cell culture longevity and reproducibility) as well as reduced metabolite profiles.

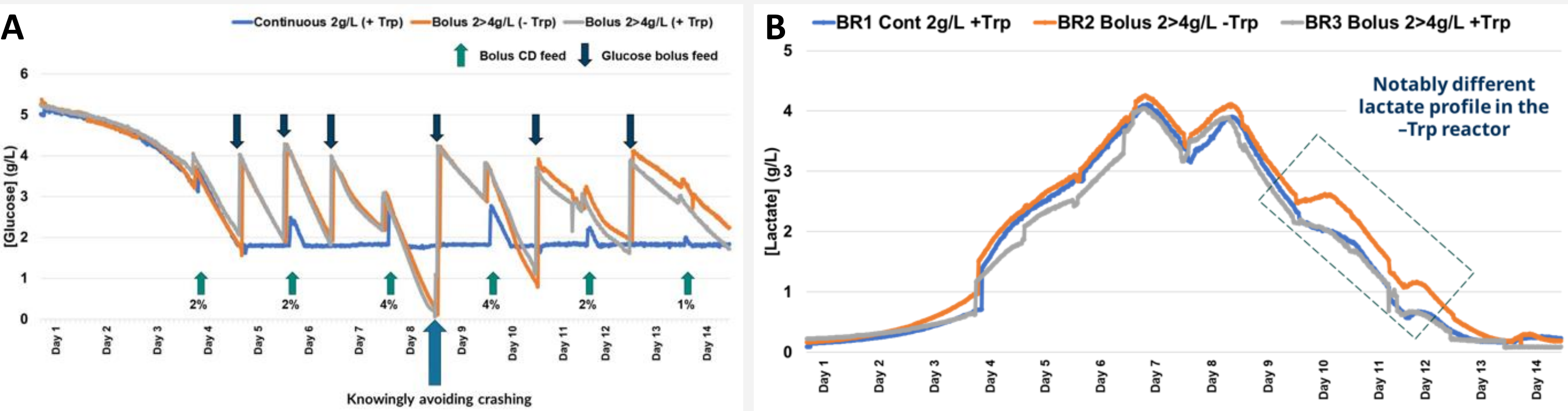


Figure 1. (A) Glucose measurements using the MAVEN in a CHO 10 L bioreactor run. Bioreactor 1: Continuous 2 g/L glucose, controlled by MAVEN; Bioreactor 2: Bolus 2–4 g/L glucose (–Trp); Bioreactor 3: Bolus 2–4 g/L glucose (+Trp). On day 8, the glucose levels dropped close to zero in bioreactors 2 and 3. A potential cell culture crash was avoided by monitoring the glucose levels closely with the MAVEN real-time measurements and adjustment of glucose feeding time. **(B)** Lactate profiles in run 1 for all three bioreactors. The real-time measurements showed a shift in the days 9–13 for the bioreactor that was not fed extra tryptophan in the amino acid mix.

MAVEN: On-line Automated Analysis and Data Processing with In Situ Probe Sampling

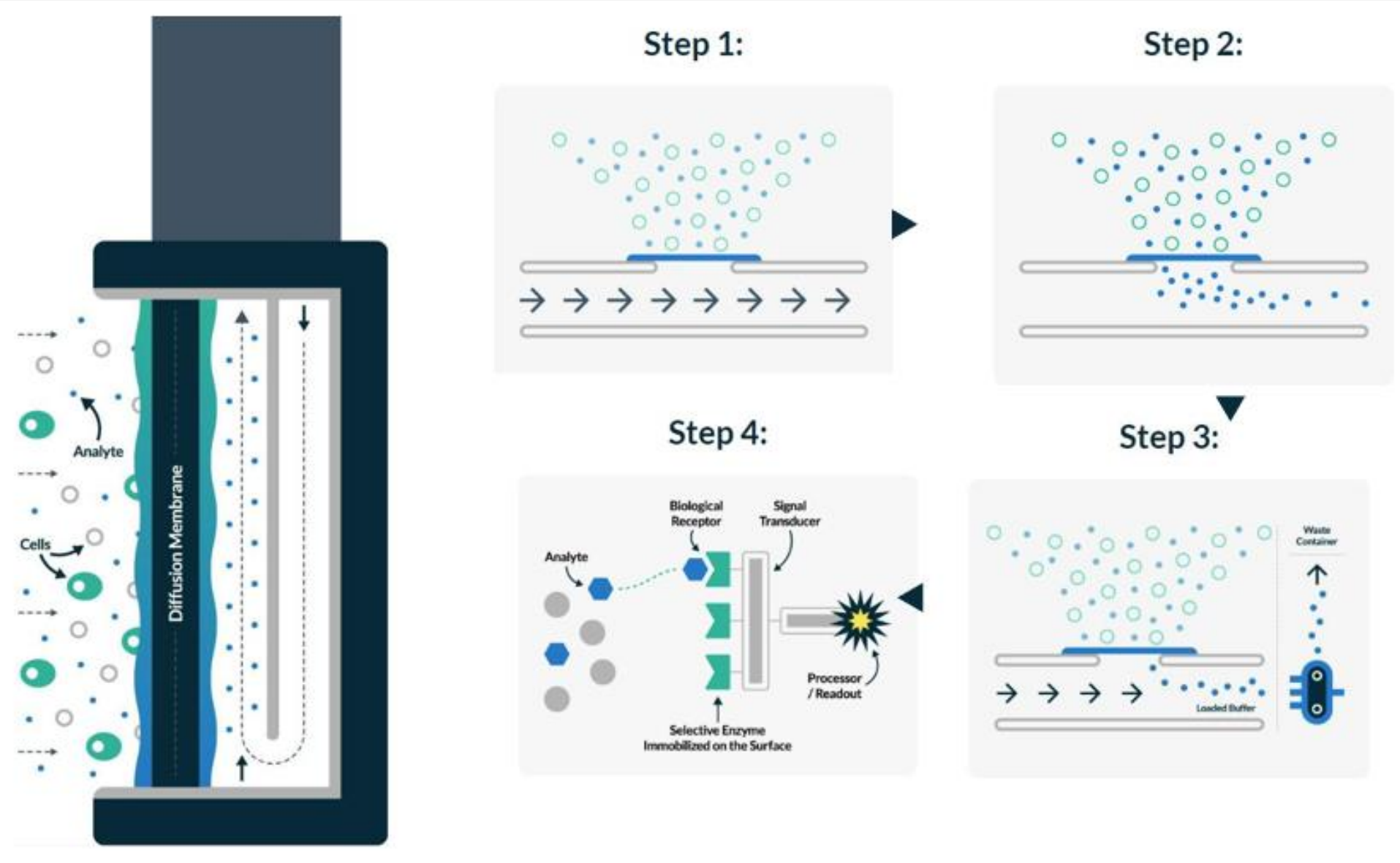


Figure 2. Automatic, on-line glucose and lactate monitoring

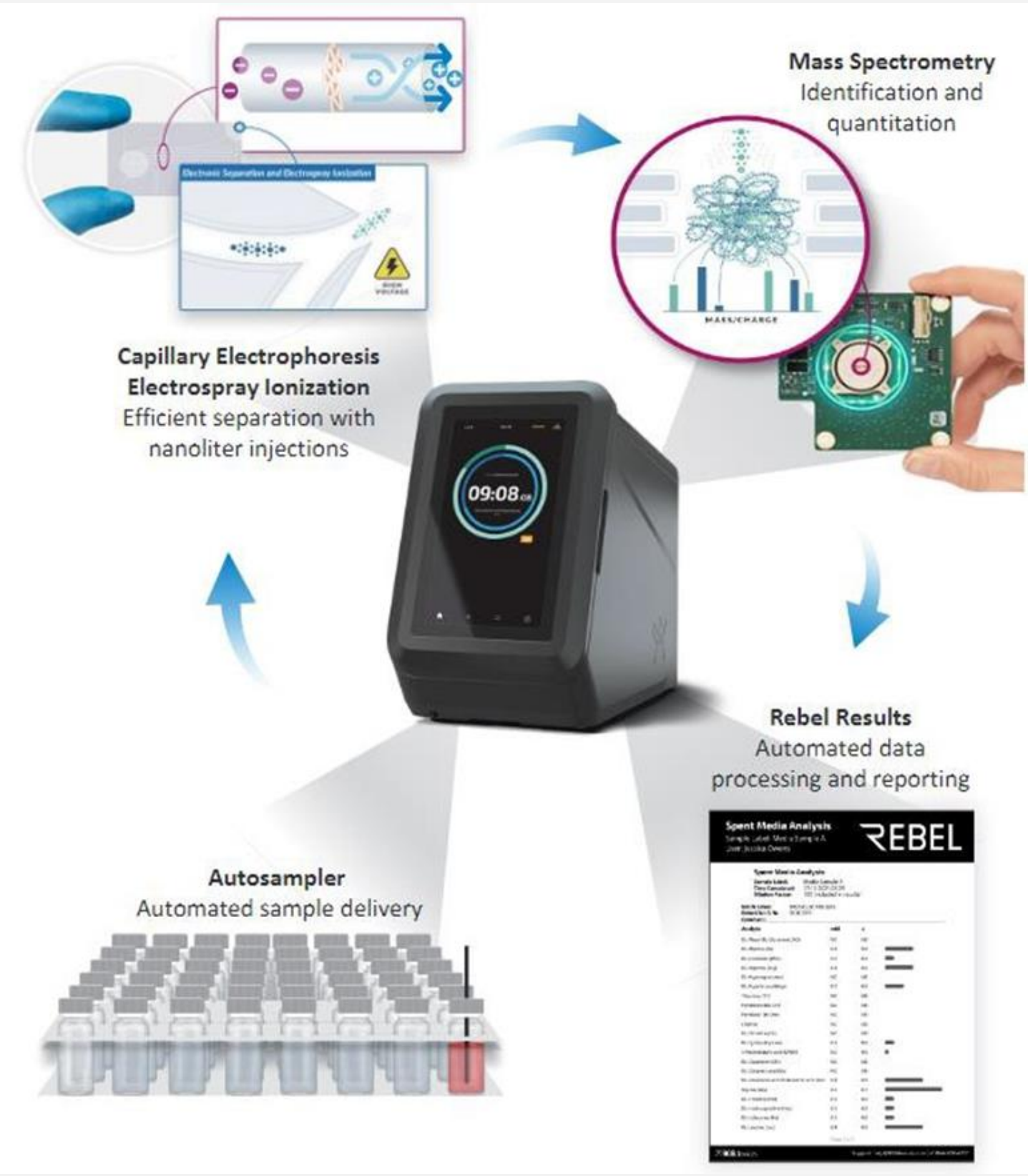
- Diffusion probe installed in bioreactor connects to biosensor
- Enzyme-based biosensor detects glucose to 0.01 g/L and lactate to 0.05 g/L
- Small molecules diffuse through the semipermeable membrane into the buffer solution
- Biosensors are replaced after 5,000 measurements; therefore, the lifetime of a sensor is dependent on the measurement frequency.
- No loss of bioreactor volume
- Significantly reduced risk of contamination as compared to sample pulls
- Enables on-line analytics and process control

MAVEN Bioreactor In Situ Probes and Perfusion Flow Cells



Figure 3. Diffusion probes have been developed for stainless steel bioreactors and for single-use bag bioprocesses. The probes are autoclaved with other bioprocess materials. Using the probe for frequent measurements reduces the risk of contamination from sample pulls and the manual work involved.

Actionable Information on Your Bioprocess at the Point of Need



- Minimal sample requirement as low as 10 µL
- Simple sample prep: spin and filter, then dilute
- Integrated analyzer includes autosampler, separation, detection, analysis, and reporting
- Analysis run-time ~10 min per sample
- Consumable kit optimized for 200 replicate analyses

Figure 4. The REBEL at-line cell culture media analyzer

Spent media analysis: Process and media optimization

Fresh media analysis: Media fingerprinting, screening, and stability

Process Modeling: Mechanistic data-driven modeling



Figure 5. PATsmartTM REBEL[ ] Spent Media Analysis Kit

REBEL Analyte Panel

Amino Acids	Alanine	Glutamic Acid	Leucine	Serine
	Alanyl-glutamine	Glutamine	Lysine	Threonine
	Arginine	Glycine	Methionine	Tryptophan
	Asparagine	Histidine	Phenylalanine	Tyrosine
	Aspartic Acid	Isoleucine	Proline	Valine
Vitamins, etc.	Choline	Pyridoxal	Thiamine	Betaine
	Nicotinamide	Pyridoxine		
Amines	�-alanine	GABA	Methylhistidine	Sarcosine
	Citrulline	Hydroxyproline		

REBEL Provides Critical Bioprocess Data

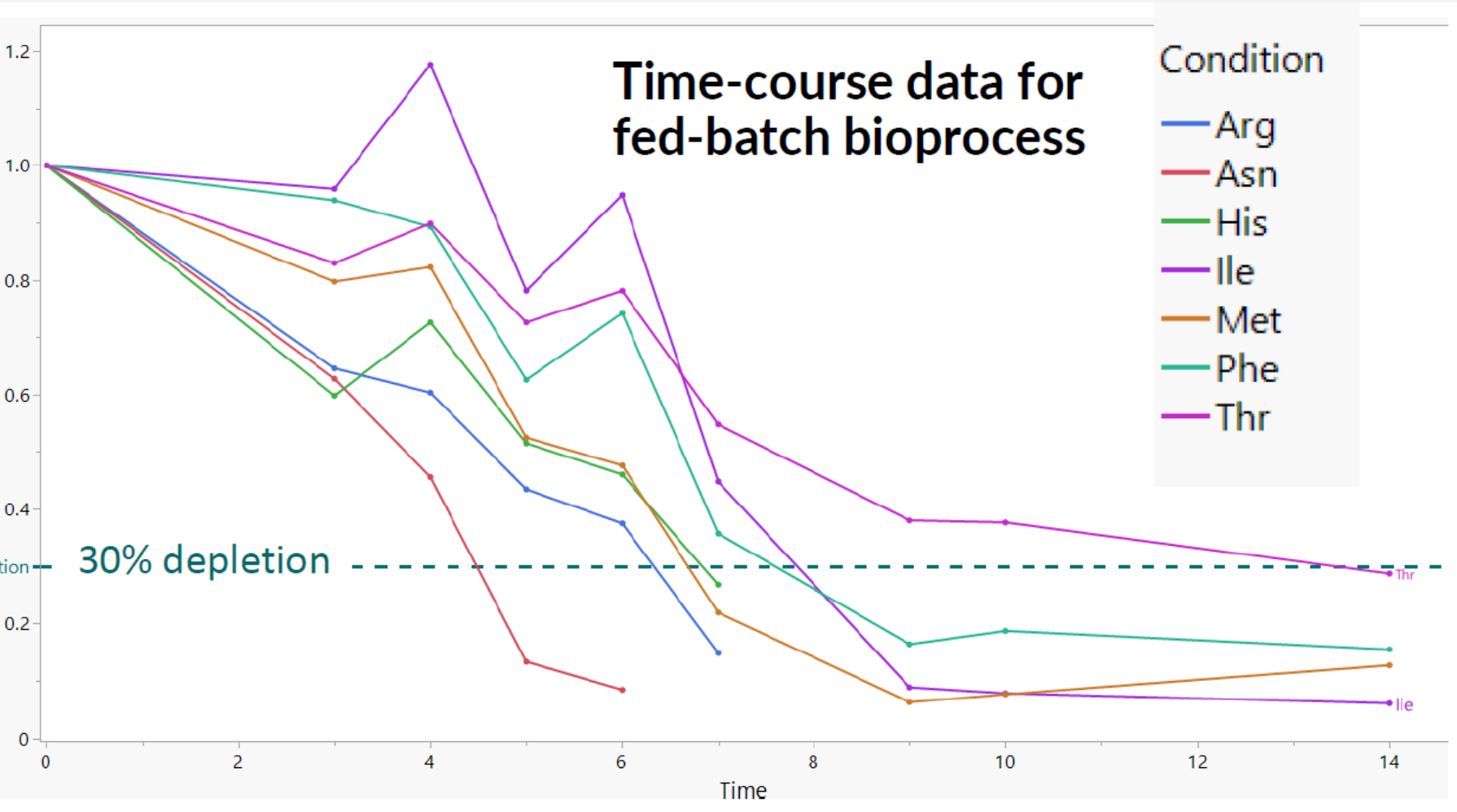


Figure 6. REBEL is used for frequent measurements of the cell culture media to analyze several components during the timecourse. In this graph, the data for the different analytes are normalized. Some amino acids were depleted below 30% of the starting condition before the mid-point of the process. Depletion may hinder growth or cause misincorporations in the final product.

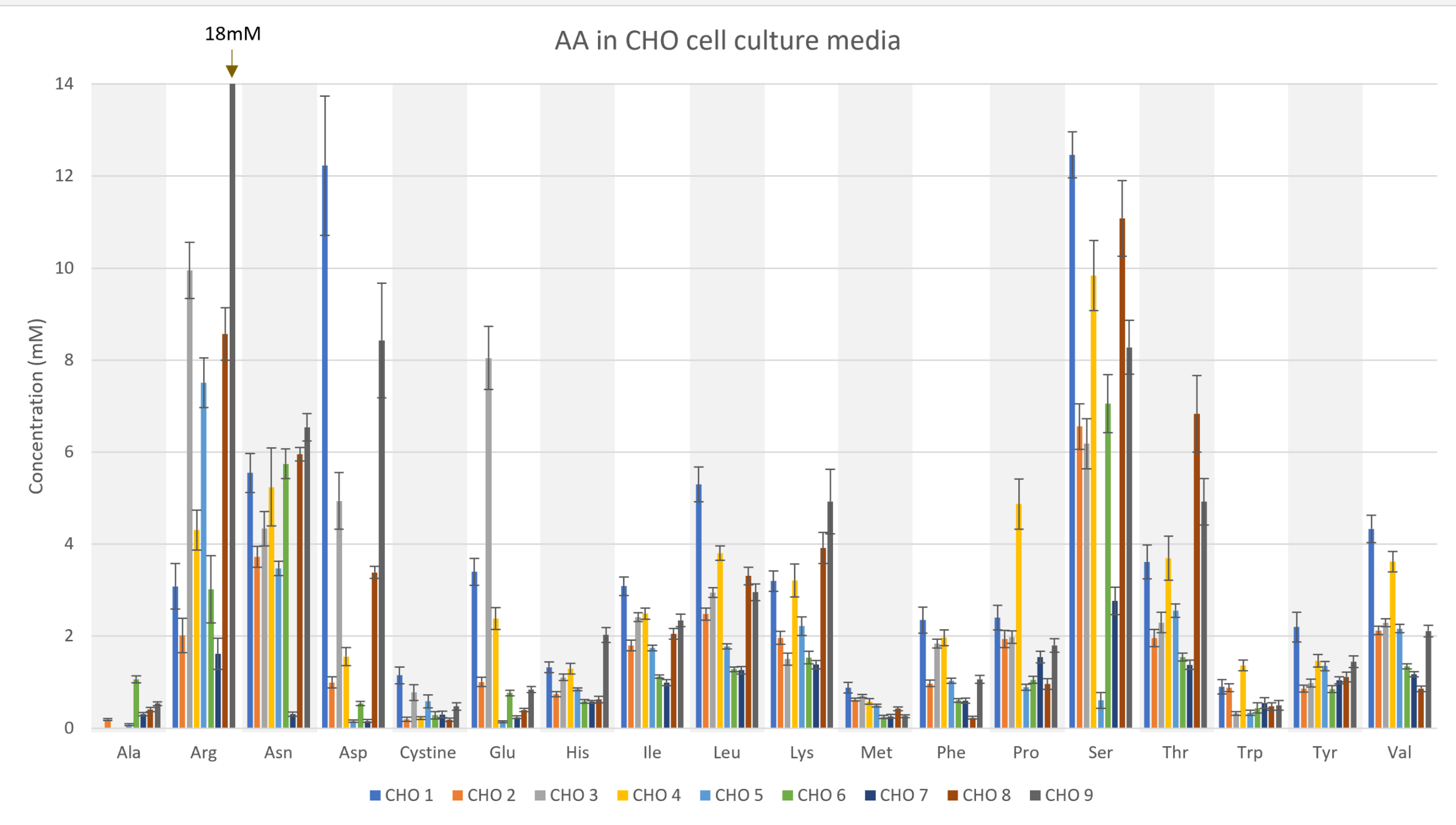


Figure 7. Check your media analyte panel and know what you’re dealing with!

- Analysis of common commercial media reveals analyte diversity.
- Some CHO cell line strains and populations have different basal and feed media needs. What is right for your bioprocess?
- Test diverse media with a variety of component levels and concentrations.



Learn more at repligen.com