



Rapid Optimization of CHO Cell Productivity Using AI-Powered Metabolite Profiling

Determine analyte identities and accurate concentrations from raw mass spectral data in minutes

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<15 min cloud-based data processing replaces weeks-long conventional standard calibration



72 (supernatant) & **115** (cell extract) metabolites named and quantified



5-200 nM Limit of quantitation achieves excellent sensitivity

Highlights

Challenge: Metabolite profiling provides a real-time snapshot of cellular health, making it an ideal technology for monitoring bioprocesses and identifying cell culture optimization strategies. However, these profiling methods are slow, cumbersome, and labor-intensive, often requiring 6–9 weeks of scientific staff effort to deliver a biologically actionable result.

Pyxis Deployment: ~~The AI-powered platform~~ The AI-powered platform Pyxis™ enables rapid metabolite identification and accurate concentration determination from raw liquid chromatography mass spectrometry (LC-MS) data, simplifying cellular nutritional and metabolism assessments for productivity optimization. Here, we demonstrated Pyxis' performance in monitoring a two-week bioprocess that produced the NIST monoclonal antibody (mAb) reference standard utilizing Chinese Hamster Ovary (CHO) cells. CHO cells are widely used in biomanufacturing applications, including the production of mAbs and other biologics.

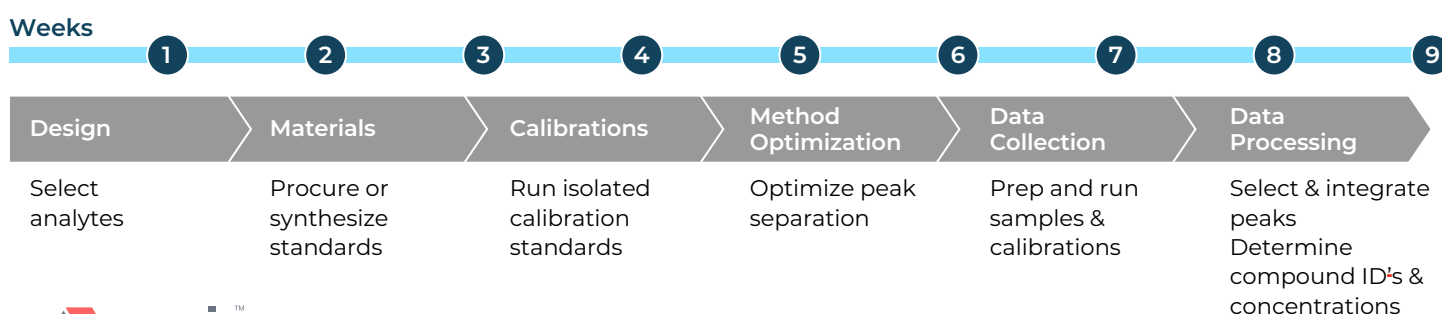
Proof-of-Concept: Within 15 minutes, raw LC-MS data were processed, identifying and determining the concentration of 72 metabolites in cell culture media and 115 metabolites in cell extracts. Examples of metabolic characteristics that contribute to cell viability are presented in this paper, including the availability of choline and other nutrients in cell culture media, as well as central carbon intermediates and redox-responsive analytes in cell extracts. These data exemplify how Pyxis can streamline bioprocess monitoring and identify metabolic strategies for improving product yield.

Pyxis: A revolution in bioprocess monitoring

The rise of biomanufacturing, including the generation of proteins, gene therapy components, and living cell therapies, necessitates continuous improvements in manufacturing efficiency. Streamlining workflows and implementing real-time monitoring enhance bioprocessing quality, reduce

costs, and promote sustainability.¹ Increasingly, process engineers are improving product yield by turning to comprehensive MS-based metabolite profiling methodologies to survey active cell health, ensure scalability, and identify metabolic bottlenecks.^{2,3} Metabolite profiling enables actionable insights into process improvement by covering both routinely measured and mechanistic biochemicals that constitute cellular nutrition and metabolism.

A. Conventional LC-MS Workflow



B. pyxis™ Workflow

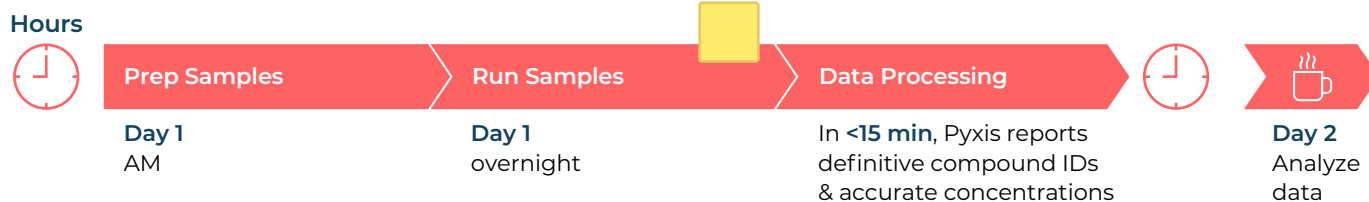


Figure 1. Workflow details for (A) Conventional and (B) Pyxis-based methodology. Both methods comprise running samples on LC-MS but differ in sample preparation and data processing. Pyxis converts traditional weeks-long method development, running of standards, and manual processing into an AI-powered metabolite data deliverable within 15 minutes. Annotated metabolites with accurate concentrations can be obtained from a 96-well plate within 48 hours.

Accurate assessment of cellular metabolism and media component concentrations is essential for precisely adjusting their levels and achieving optimal cell product yields. Conventional LC-MS workflows, however, demand extensive planning and significant investment in quantitative analytical method development using traditional calibration curves and costly labeled internal standards. Metabolite annotation is the most time-consuming part of the workflow, whereby users employ a variety of software tools, libraries, and reference databases to confidently identify features of importance. Such methods often require 6–9 weeks to execute and are restricted to compounds for

which internal standards can be procured or synthesized (Figure 1A).

Pyxis leverages a proprietary Large Spectral Model (LSM) to query the raw LC-MS spectra, thereby rapidly determining confident metabolite identifications and concentrations without the need for traditional calibration curves, internal standards, and reference databases or libraries.^{4,5} Users simply run their analytical samples, apply the Pyxis software, and obtain a data table of the annotated metabolites within 15 minutes (Figure 1B). Thus, metabolite ID and accurate concentration data can be obtained from a 96-well plate containing samples and blanks within 48 hours.

The experiment: Pyxis identifies over 100 CHO cell metabolites in under 15 minutes

To validate Pyxis in a bioprocessing setting, Matterworks partnered with Johnson & Johnson Innovative Medicine to profile CHO cell spent media and cell pellets collected daily over two 13-day, 10-L bioreactor runs. CHO cells produced the NIST mAb reference material as proof of concept. Proteins were precipitated with a mixture of 50:30:20 methanol:acetonitrile:water, and samples were centrifuged. Spent media supernatants were diluted 150-fold. LC-MS acquisition was conducted using HILIC chromatography, optimized for the detection of polar metabolites, and implemented on a ThermoFisher Scientific Vanquish Horizon UHPLC system coupled to an Orbitrap Exploris 120. Raw

LC-MS data (224 files) were processed in the cloud using Pyxis in less than 15 minutes.

Cell viability (Figure 2A) and viable cell density (Figure 2B) served as key performance indicators for the overall cell culture. Cell viability was well-maintained for the first week and subsequently declined from days 7–13. Viable cell density increased to a peak at day 8 and then steadily declined.

To ensure confidence in data quality, Pyxis reports metabolite concentrations only above the pre-validated limit of quantitation (LoQ) for each analyte. Overall, Pyxis identified 72 biochemicals in spent media (i.e., cell culture supernatants) and 115 compounds in cell pellet extracts. Metabolite LoQs ranged from 5 nM (carnitine and pyridoxine) to 100 nM (valine).

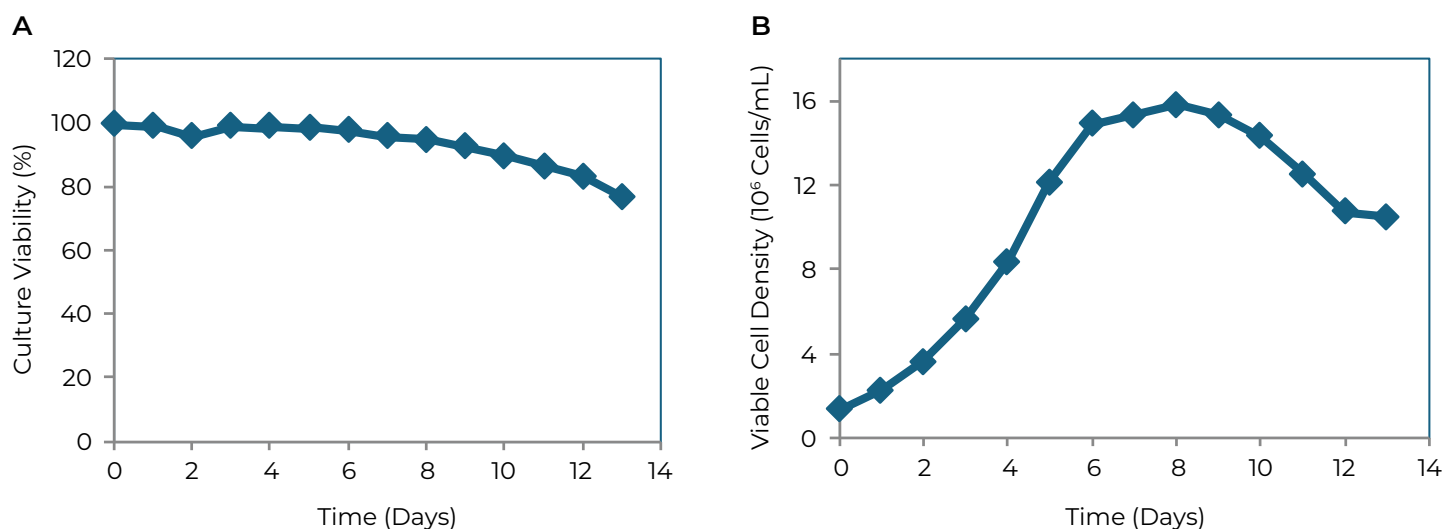


Figure 2. Key performance indicators for CHO cell bioprocessing, including (A) cell viability and (B) cell culture viability density.

Key Results

1. Choline depletion may explain the progressive decline in cell viability

We used differential metabolite analysis to identify CHO cell metabolites that may have contributed to the observed decline in cell viability beginning after Day 6. The volcano plot in Figure 3A identifies CHO cell pellet extract metabolites with significantly different concentrations on Day 12, when cell viability was decreasing, compared to Day 6, when it was still near 100%. Choline levels were particularly low during the viability decline phase, and inspection of its concentration over the bioreactor runs revealed its levels were essentially at the LoQ by Day 9 (Figure 3B). Coincidentally, choline concentration in CHO

cells declined precipitously between Days 9 and 10 and continued to fall in subsequent days. These findings strongly indicate that the cells had depleted all available choline in the media, and intracellular concentrations fell below the optimal level.

Choline is an integral component of membrane phospholipids, with phosphatidylcholine serving as the primary lipid in CHO cell membranes.⁶ Maintaining adequate choline in CHO cell cultures supports robust cell growth and high product titers. In a related metabolomics study, choline depletion in CHO cells and spent media was linked to reduced yield, and optimizing media choline levels subsequently improved titers.⁷ Deploying Pyxis in such workflows can markedly accelerate media optimization and cell nutrient availability in biomanufacturing settings.

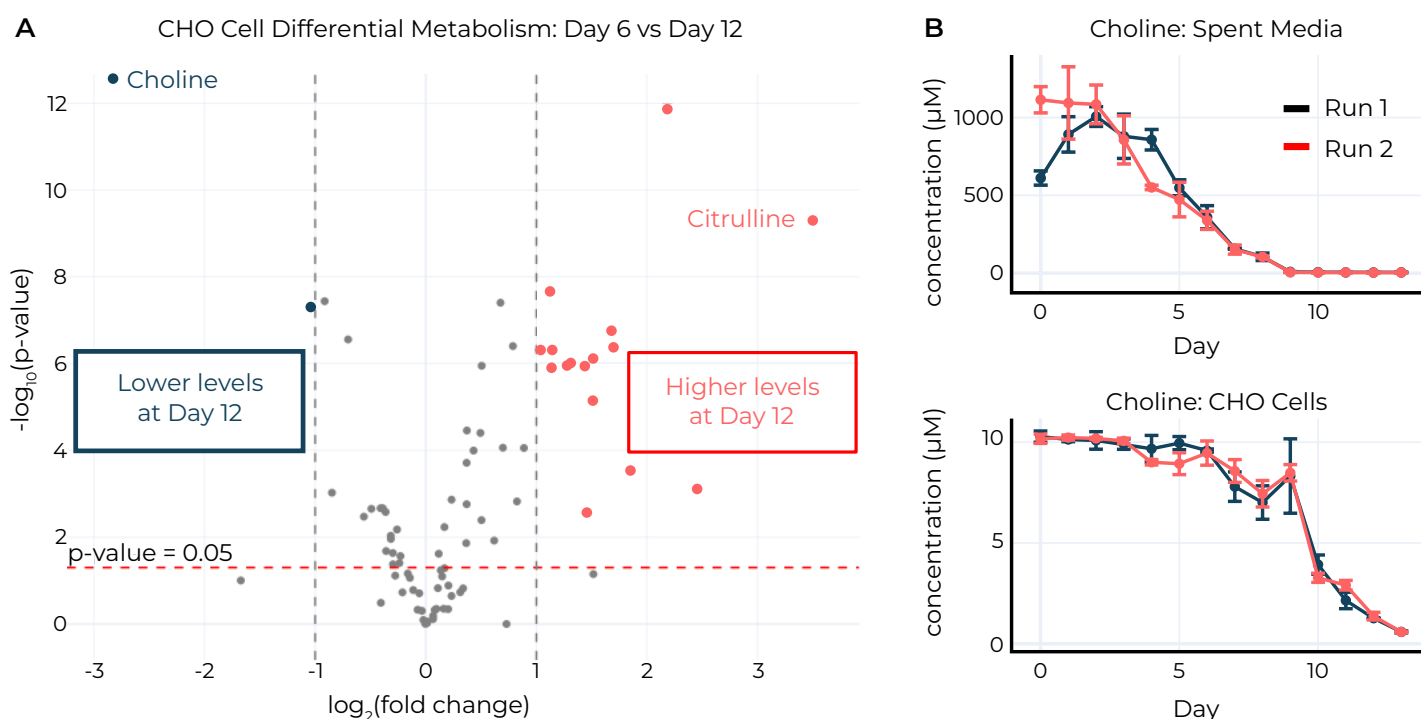


Figure 3. Choline depletion may have contributed to a decline in cell viability starting at Day 6. (A) Volcano plot comparing CHO cell pellet metabolite levels at Day 6 to Day 12. Metabolites are colored blue or red, depending on whether their levels were significantly ($p < 0.05$ and $|\log_2(\text{fold change})| > 1$) lower or higher at Day 12 compared to Day 6, respectively. (B) Accurate concentration (in μM) of choline in spent media (above) and cell pellets (below) over the bioreactor course. Black and red lines correspond to different bioreactor runs, and the error bars represent ± 1 standard deviations of three technical replicates. For supernatants, the concentrations are multiplied by the 150X dilution factor. For cell pellets, the concentration reflects the extraction of 5×10^6 cells into 1 mL of extraction solution.

2. Monitoring of central carbon metabolism and lactate production

Metabolites of central carbon metabolism, including tricarboxylic acid (TCA) cycle intermediates and lactate, are critical surrogates for cellular energy production (Figure 4A). Cells undergoing aerobic respiration produce lower levels of lactate, and analysis of TCA cycle intermediates provides a window into lipid and amino acid synthesis, as well as as mitochondrial energetics.⁸

Here, Pyxis determined the concentrations of lactate,

citrate, succinate, fumarate, pyruvate, and other metabolites in spent media and CHO cell pellets, providing rapid, daily checks of cellular energetics (Figure 4B). Controlling the rate of lactate production is often a powerful strategy for improving product titer. Optimizing TCA cycle intermediates supplied in the media can more effectively modulate lactate levels compared to pyruvate or lactate.⁸ Measuring the absolute concentration of all these compounds with Pyxis provides an almost real-time ledger of central carbon contributors for rapidly achieving cell productivity.

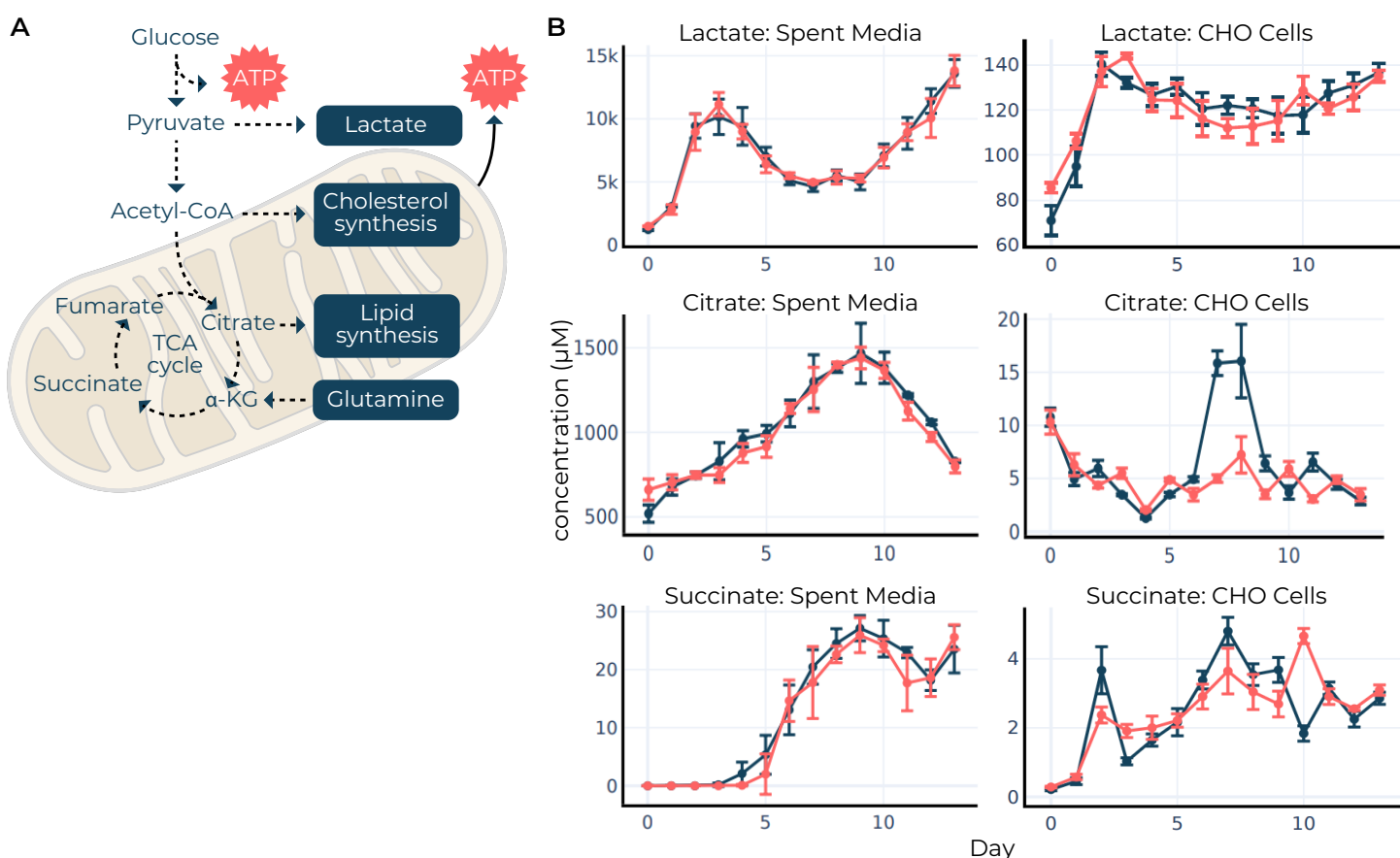


Figure 4. Spent media and CHO cell metabolomic monitoring of central carbon metabolism. (A) Overview of mitochondrial energetic metabolism. (B) Determined concentration (in μM) of example energetic metabolites in spent media (left) and cell pellets (right) over the bioreactor course. Black and red lines correspond to different bioreactor runs, and the error bars represent ± 1 standard deviations of three technical replicates. Concentrations reflect the 150X dilution factor (spent media) or the extraction of 5×10^6 cells into 1 mL of extraction solution.

3. Glutathione ratio (GSH/GSSG) and related amino acids inform on cellular redox state

Protein production often increases oxidative stress in CHO cells through the generation of reactive oxygen species (ROS), and the balance of these molecules with free thiols, such as cysteine and glutathione (GSH), influences the cellular redox state.⁹ Failing to control these parameters adequately can lead to product aggregation, endoplasmic reticulum (ER) stress, and reduced cell productivity.¹⁰ Most process development analytics only measure extracellular variables, ignoring the intracellular environment.¹⁰ While enzymatic kits measuring intracellular GSH and glutathione disulfide (GSSG) are commonly employed in cell line development workflows, comprehensive LC-MS-based methods enhance redox analysis to include the amino acid components of GSH (cysteine, glycine, and glutamate), the GSH/GSSG ratio, and other markers and mitigators of ROS, both inside and outside the

cell. This comprehensive set of measurements offers greater insight into the redox state of the antibody-producing CHO cells, suggesting approaches to improve product yields and quality.

In this study, Pyxis provided daily intracellular accounts of GSH and GSSG, yielding a GSH/GSSG ratio (Figure 5A). Additionally, the micromolar levels of cystine (the oxidized form of cysteine), were measured in both spent media and cell pellets (Figure 5B). Collectively, these measurements reflect a controlled intracellular redox environment. The ability to track intracellular and extracellular redox mediators and subsequently optimize relevant media components has been shown to increase cell viability and productivity, improve product titer, and reduce antibody aggregation.^{9,11} Such process improvements are especially relevant for disulfide bond-containing therapeutics, such as bispecific antibodies.¹⁰

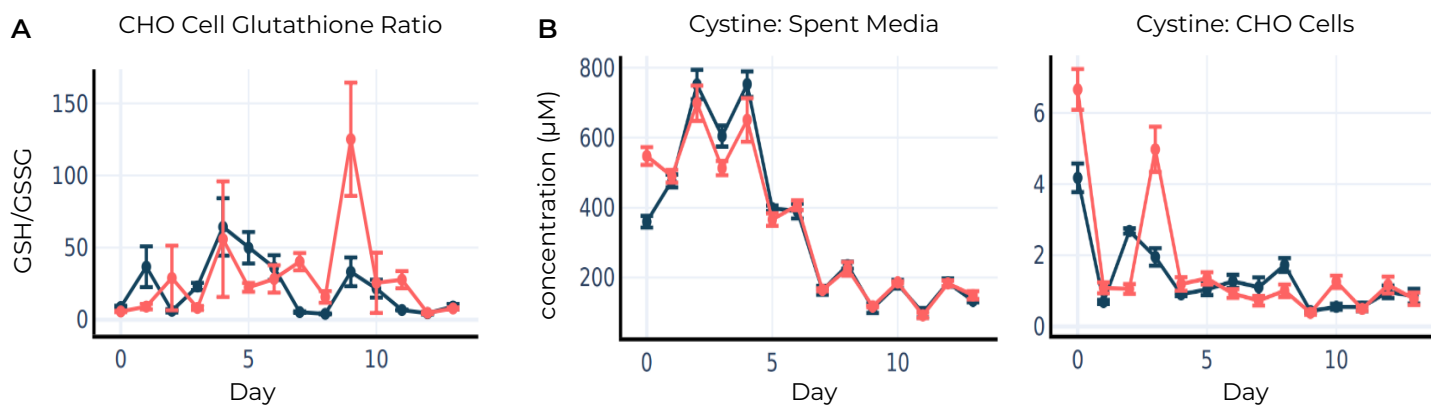


Figure 5. Cellular redox state monitoring through accurate concentration determination of glutathione and related compounds. (A) Calculated intracellular reduced-to-oxidized glutathione ratio (GSH/GSSG) over the bioreactor time courses. (B) Accurate concentration (in μM) of oxidized cystine in spent media (left) and cell pellets (right) over the bioreactor course. Black and red lines correspond to different bioreactor runs, and the error bars represent ± 1 standard deviations of three technical replicates. Concentrations reflect the 150X dilution factor (spent media) or the extraction of 5×10^6 cells into 1 mL of extraction solution.

Summary

Comprehensive and quantitative metabolic analysis can guide actionable strategies for optimizing bioprocesses, provided the data are promptly delivered. Traditional methods for identifying compounds and measuring concentrations require significant investment in time, reagents, and trained personnel. Pyxis streamlines these resource-heavy steps, providing accurate metabolite annotations and concentrations within minutes. Pyxis-mediated accounting of diverse biochemicals supports cell productivity efforts throughout the bioprocessing pipeline, including culture media development, metabolic and genetic engineering, and bioreactor scale-up, ultimately leading to process standardization.

This study highlights near real-time biochemical insights during bioreactor runs enabled by Pyxis, including:

- Opportunities to quickly optimize cell viability and product titer through dynamic assessment of both cellular metabolism (cell extracts) and media components (cell supernatants).
- Insights into mitochondrial function, glucose consumption, and lactate production through the monitoring of energetic compounds.
- More efficient cell line development through a comprehensive view of cellular health, redox states, and metabolism.

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