Global Biochemical Profiling for Bioprocess Optimization

The Challenge: Optimizing the Production of a Protein Therapeutic

It is widely understood that the production phase of bioprocessing can have a significant impact on the overall profitability of the biopharmaceutical. Organizations must analyze everything from total protein production level to glycosylation patterns as well as overall quality and stability. It is clear that the final product’s profitability can be dramatically changed by the culture process.

Protein production has been greatly improved through process monitoring of important variables such as dissolved O$_2$, pH, lactate and carbon source depletion. Further optimization has been accomplished via formulation development using a cause and effect model as well as spent media analysis looking for a few specific rate limiting media components.

Given the large numbers of variables involved—especially in the composition of media nutrients—it is has traditionally been difficult to achieve globally optimal conditions using these methods.

The Solution: Global Analysis of Biochemicals

Instead of monitoring a handful of biochemicals, comprehensive biochemical profiling analyzes the change in hundreds of biochemicals including metabolites in the cells and components in the media. This “metabolomics” approach allows the analysis across different reactors over time which can help identify and address rate limiting media nutrients and biochemicals related to cell toxicity.

Typical sampling is done every day over a 12 to 14 day run. The samples are spun to separate the spent media from the cells, frozen and then sent to Metabolon for analysis. This type of analysis can be used during any stage of development—from flasks to large reactors.

The result is a comprehensive analysis for optimizing expression, leading to:

- increased product yield and quality
- decreased substrate consumption
- better control of metabolic activity
- lower process costs
- increased process reliability and reproducibility
- maximized operational efficiency

mVision Analysis

Metabolon’s mVision analytical platform is designed and optimized to detect small molecule biomarkers from numerous biochemical classes. Analysis by three unique analytical platforms separates and detects the broadest range of biochemicals as well as provides internal QC for biochemicals common to two or more platforms.
This global, non-targeted approach offers a unique advantage over other metabolomics services that provide only raw data (e.g. mass spec signals) or analyze a limited number of compounds chosen from a restricted panel of detectable chemicals (targeted analysis). With Metabolon’s solution, designing studies can be achieved without the need for a complete understanding of the biology or biochemistry of samples.

**Typical Results from Bioprocessing Analysis**

The top-level result of an example bioreactor analysis is shown in the table below. These results show a large number of biochemicals found in the samples. By measuring biochemicals and metabolites in both cells and media, one can monitor certain species which may be produced in the cell and then transported to the media or species in the media (e.g. nutrients) that are decrease over time but result in the production of carbon-related intermediates within the cell.

The panel below shows several amino acids and how their concentrations change, both in the media and in the cells. Similar panels can be shown for lipids, carbohydrates, energy related compounds, nucleosides and nucleotides.

<table>
<thead>
<tr>
<th>Biochemicals Measured:</th>
<th>Cells</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>388</td>
<td>271</td>
</tr>
<tr>
<td>Common to both</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>Cells Only</td>
<td>190</td>
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<tr>
<td>Media Only</td>
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**Application of Global Biochemical Information**

With this quantity of data, it is most important to put this information into a biochemical context. A complete understanding of global pathways enables researchers to put the data to work.

For example, as illustrated here, unexpectedly high lactate was found in the feed media for this fermentor (lactate was not typically measured in this system). By leveraging the data found in a global biochemical profile, the elevated lactate levels were identified. It was also found that these levels were pushing back on the glucose metabolic pathway giving rise to gluconeogenesis as well as elevated pyruvate and TCA cycle intermediates.

These results correlated with impaired cell growth and production and were easily corrected once the data enabled a clear understanding of the impact on the pathways.

**Identify limiting components**

Utilizing the breadth of information that global biochemical profiling offers, overall cell health and production potential can be readily monitored. This figure shows a prototypical trend of a rate limiting media component. Metabolon’s profiling platform is capable of simultaneously identifying the changes in several media components. As such, rate limiting components can be easily identified, as shown here. Once identified, the limiting component can be supplemented back into the media to significantly improve production.

**Identify Toxic metabolites**

In the analysis depicted below, the identification of toxic metabolite buildup in cells and media were also targeted. Identification and pathway analysis allows for formulation improvements which decreases the accumulation of these metabolites.
Conclusion

Most biochemicals in cell culture applications exhibit a wide range of concentration fluctuations. These examples above highlight the type of relevant information that global biochemical profiling can provide. Through this global analysis, one can not only measure these changes but also put them into biological context and—most importantly—into application.

Typical findings as evidenced across multiple studies in the bioprocessing space have yielded the following results:

- Identification of unexpected rate limiting nutrients
- Pathway modifications for minimization of toxic intermediates
- Formulation dependent catalysis of production intermediates
- Essential metabolite pathway deficiencies
- Sugar pathway modifications for glycosylation optimization

This information has been utilized to drastically impact production process and the value of a global biochemical analysis has been clearly proven to be a valuable tool in bioprocessing.

Looking ahead to global biochemical profiling beyond media formulation, additional applications of the technology are apparent. Uses including clone selection as well as tracking impacts of genetic modifications for novel bioprocessing applications (eg: biofuels and chemical synthesis) are on the rise.