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How Spent Media Analytics Can Support Process Optimization

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Abstract

Cell culture involves growth of cells in an artificial environment. Cell culture medium is a liquid designed to support the growth of cells in this environment. Usually, it contains a mixture of

- an energy source like carbohydrates in the form of sugars;
- amino acids since they are essential building blocks of proteins;
- fatty acids and lipids are particularly important in serum-free media;
- inorganic salts to maintain osmotic balance;
- vitamins and trace elements essential for growth and proliferation of cells;
- buffering system to maintain the pH in the physiological range.

Introduction

Spent media is the media that remains after cell cultivation. Nutrient depletion and metabolite accumulation occur in the medium during cell growth (Figure 1). Additionally, the target product also accumulates which, in certain circumstances, can also become toxic if concentrations become too high.

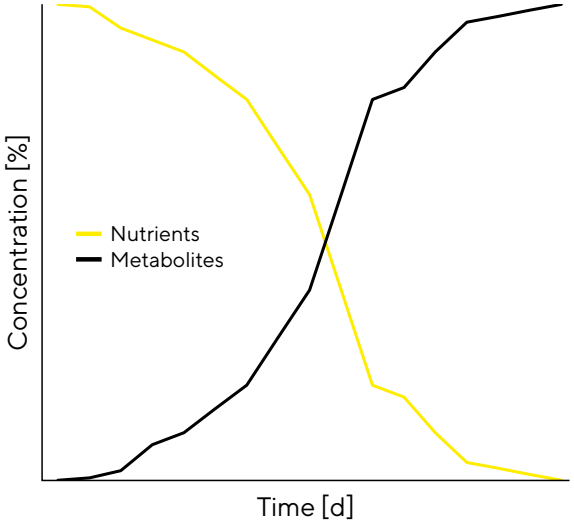

Figure 1: *Exemplary Change in Nutrient and Metabolite Levels During Cell Cultivation.*

Spent media analysis is the study of the used media over the course of the production process. The information gathered facilitates the selection of the right cell culture medium and feed combination, as well as the development of suitable feeding strategies. It is a useful tool that enables us to gain insight into the metabolic processes as well as to track and understand changes in medium composition.

Cell Culture Process Formats

A **batch process** is inoculated with the medium of choice and no further nutrients are added during the cultivation.

In a **fed-batch process,** additional substrates or supplements are added to increase process duration, cell density and productivity. The feed supplement can be added in bolus or continuous mode. The feeding strategy should be adapted to the cell's requirements which can be initially determined using spent media analysis.

Continuous processes can be performed with (perfusion mode) and without cell retention (chemostat mode). Fresh nutrients are continuously added to the culture while spent medium (and cells) are harvested at the same time. With this process strategy, toxic metabolites can be removed and the process is especially suited for unstable products.

At Sartorius Xell, we offer spent media analysis in four service tiers, ranging from basic insights into full process understanding (Table 1):

1. Analysis of only primary parameters like pH value, osmolality, glucose, lactate, glutamine and ammonium.
2. Analysis of essential parameters like amino acids, dipeptides, and vitamins.
3. Measurement and analysis of trace elements and ions for a comprehensive understanding of the bioprocess.
4. Measurement of additional features such as organic acids, polyamines, for in-depth understanding of the bioprocess.

Table 1: *Spent Media Analysis for Various Components.*

Ions and Elements	**Equipment**
Trace Elements	ICP-MS
Phospate	Multimodereader
Ammonium	Multimodereader
Amino Acids	
Free Amino Acids	UHPLC-DAD
Total Amino Acids	UHPLC-DAD
Amines and Peptides	
Dipeptides	UHPLC-MS/MS
Glutathione	UHPLC-MS/MS
Polyamines (Putrescine, Spermidine, Spermine)	UHPLC-MS/MS
Organic Acids	
Tartaric acid, Malate, Malonic acid, Citrate, Pyruvate, Succinate, KetoGlutaric acid, Fumarate, Maleic acid	UHPLC-MS
Vitamins	
Ascorbate	UHPLC-MS/MS
Water-soluble Vitamins	UHPLC-MS/MS
Vitamin E (Tocopherol)	UHPLC-DAD
Pyridoxal Phosphate	UHPLC-DAD
Sugars	
Glucose	Chip-Sensor
Lactate	Chip-Sensor
Sucrose	Chip-Sensor

Since different mammalian cells have different features, the culture media should be designed for optimal growth of each unique cell type or clone. Challenges occur when the composition of the cell culture medium does not match the cells' requirements. An ill-suited medium could underfeed or overfeed the cells. The depletion of nutrients and the accumulation of growth inhibitory metabolites can cause cessation of cell growth. Oversupply of substrates can lead to unwanted overflow metabolism and inefficient use of provided nutrients. In addition, accumulating waste metabolites negatively impact process parameters and performance of cultures.

Therefore, regular monitoring of changes in sugars, metabolites, vitamins, and amino acids is a valuable way to select the appropriate medium, optimize feed strategies, and determine harvest times. Together, these modifications promote high cell viability, maximize production titer, and ensure high product quality.

In this application note, we demonstrate how monitoring just four media components (glucose, lactate, serine, and asparagine) can inform an optimization strategy that leads to significant improvements in the overall productivity of a bioprocess.

Methods

CHO GS cells were cultured in TCX10D medium in one batch process and four fed-batch conditions. Glucose, lactate, asparagine, and serine levels were measured in spent media across the different production modes.

Results

Analysis of Glucose and Lactate Metabolism

Typically, mammalian cells are glycolytic and will produce lactate from glucose in the medium. Figure 2A demonstrates that glucose levels gradually decrease and are eventually depleted in the batch processes. Identifying this limitation of glucose by spent media analysis facilitates the design of an optimized feeding strategy for a fed-batch process where feed supplements are added periodically, limiting the depletion of glucose in the media (Figure 2B).

As glycolysis proceeds, lactate levels increase in both batch and fed-batch processes, peaking after around four days in culture (Figure 2A and B). This is due to a metabolic switch, called the lactate shift, where the cells transition from a lactate producing to a lactate consuming culture, resulting in the subsequent decrease in lactate levels at day six. The lactate shift is a desirable characteristic, as the accumulation of lactate would limit cell growth, but switching to lactate consumption would prevent acidification of the medium and not slow cell growth.

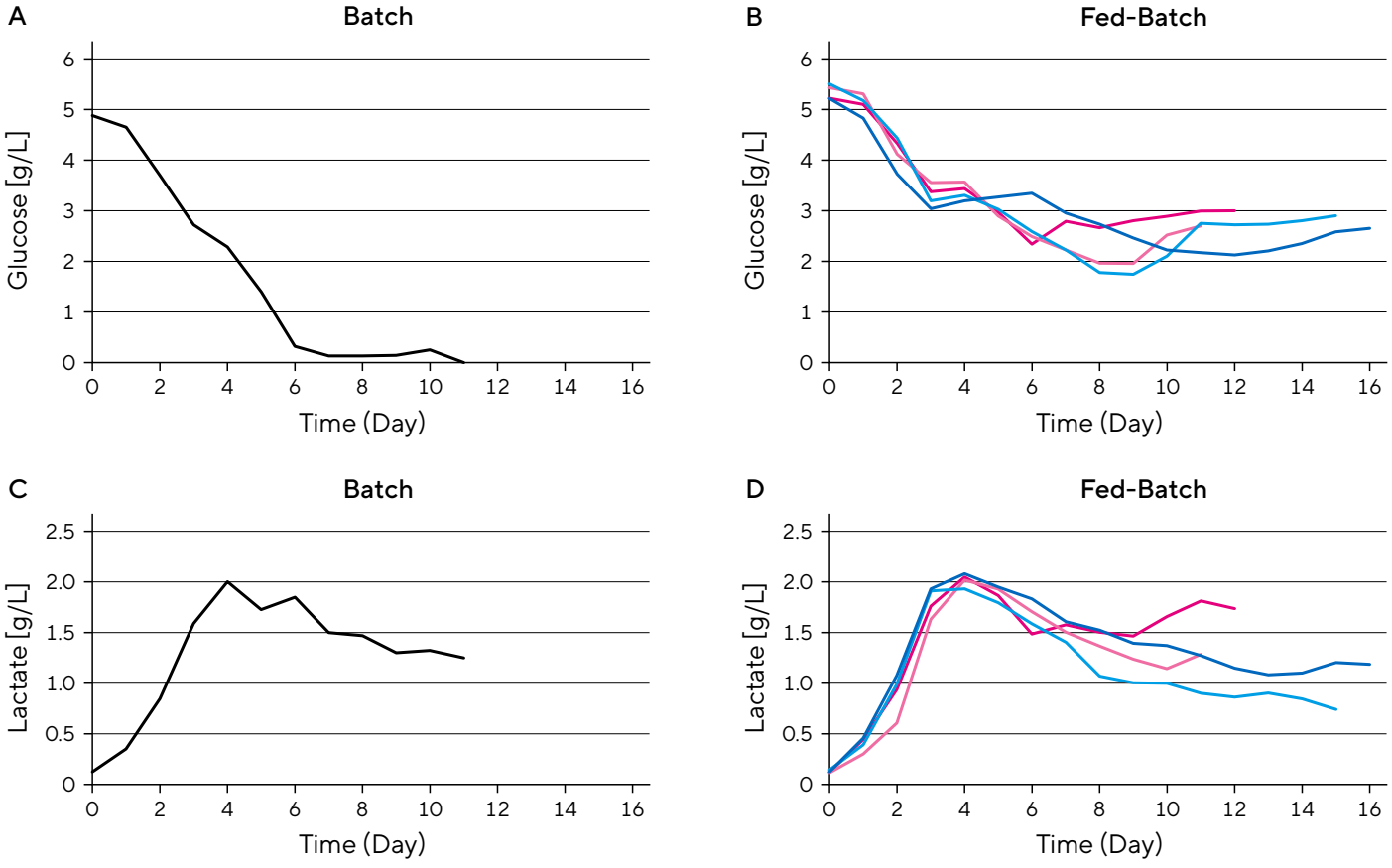

Figure 2: *Spent Media Analysis of Glucose (A, B) and Lactate (C, D) in a Batch and Several Fed-Batch Processes.*

Analysis of Amino Acid Levels

Analysis of select amino acids from spent media from the batch process showed that both asparagine (Figure 3A) and serine (Figure 3C) were depleted by day six and eight, respectively. Fed-batch supplementation strategies were able to recover serine levels better than asparagine (Figure 3B and 3D). The fed-batch strategy (dark blue) that reduced the asparagine and serine depletion also showed higher productivity (Figure 4B), highlighting the value that spent media analytics can bring to process optimization.

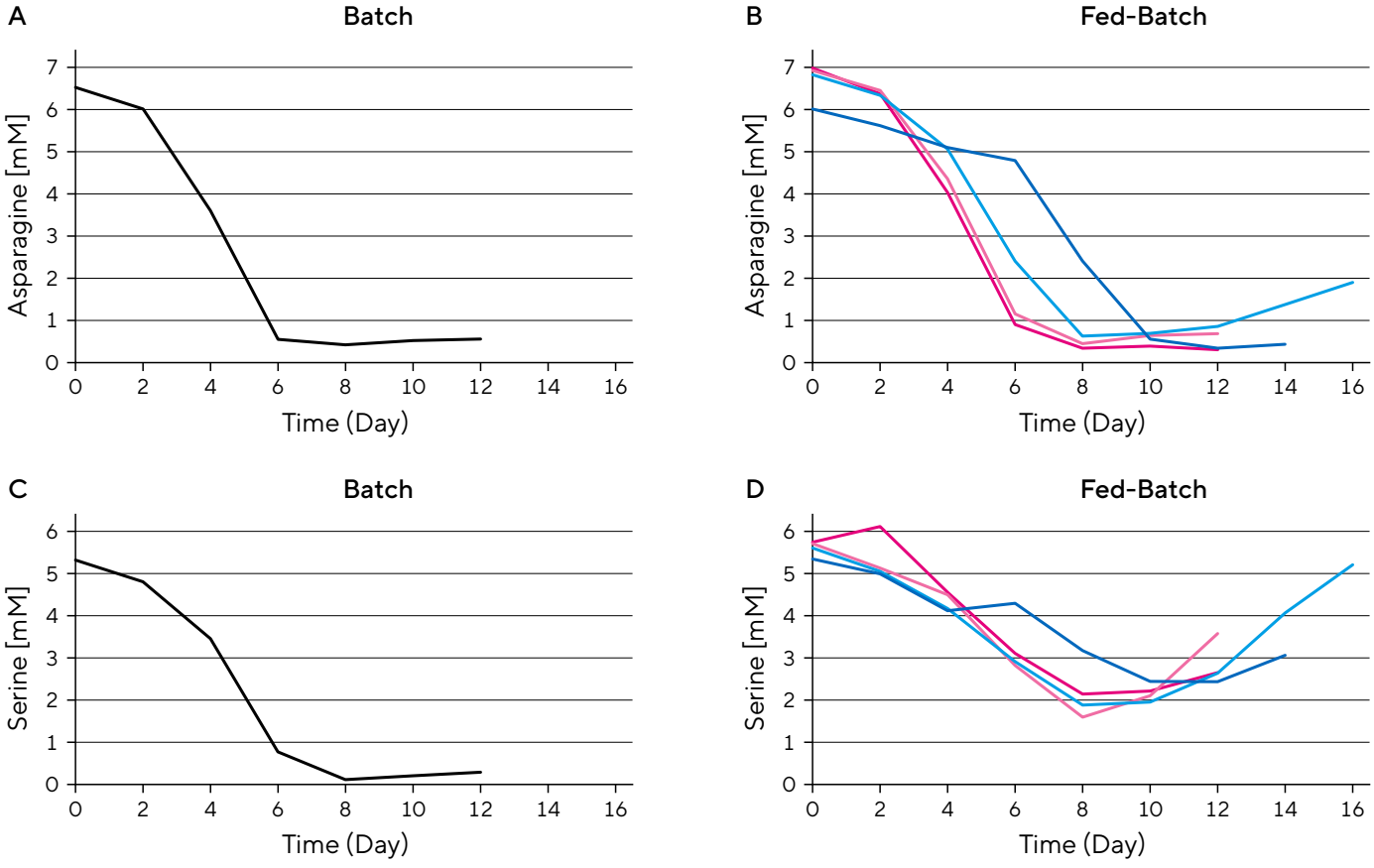

Figure 3: *Spent Media Analysis of Amino Acids Asparagine (A, B) and Serine (C, D) in a Batch and Several Fed-Batch Processes.*

Overall Improvements in Productivity

Analysis of the spent media for productivity (measured by biomass, Figure 4B) and antibody titer (Figure 4C) show that the highest cellular productivity was achieved in fed-batch process 1 (Figure 4B, dark pink). However, antibody titer was maximized with fed-batch process 4 (Figure 4C, dark blue).

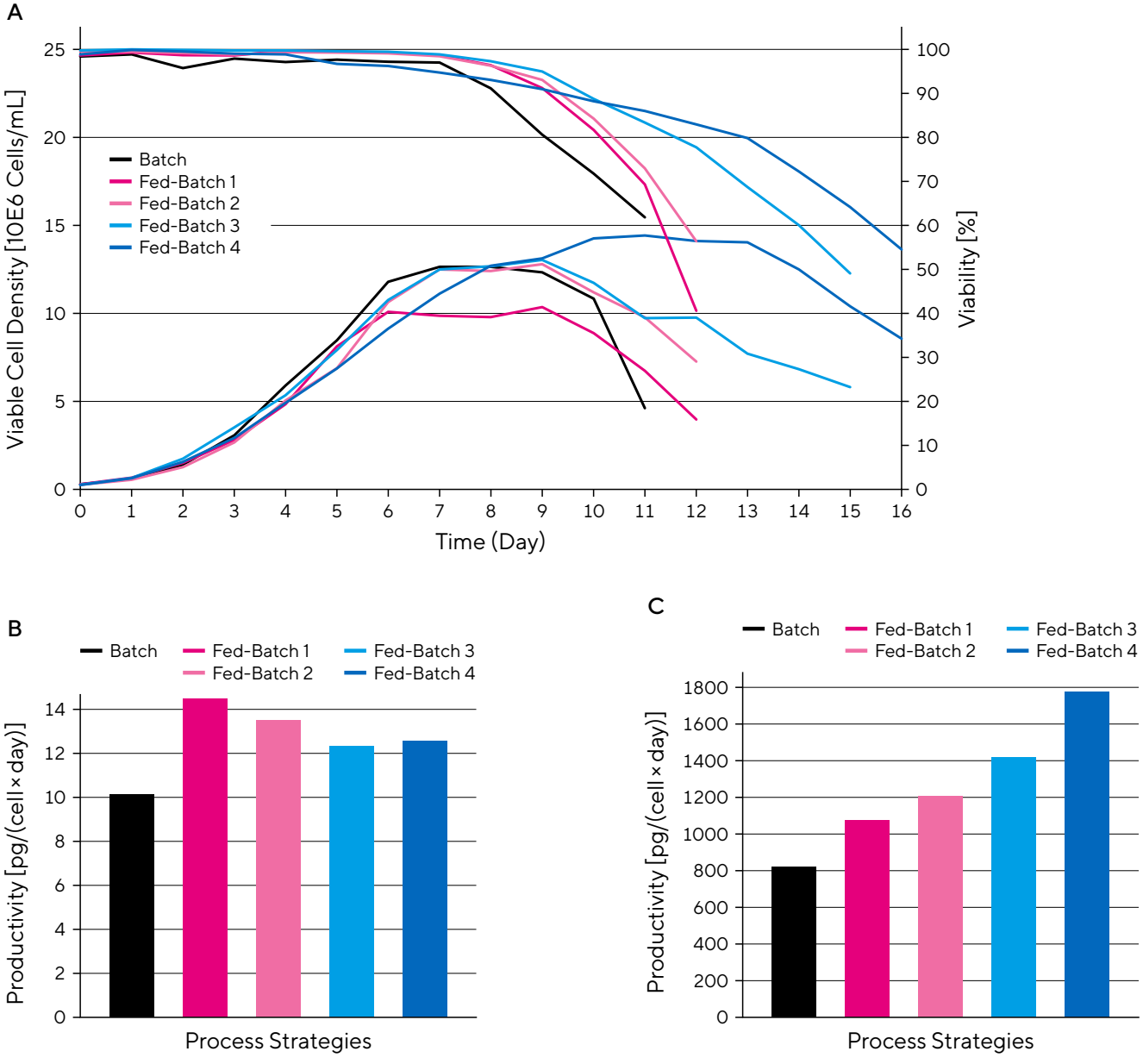

Figure 4: *Cell Density as (A) Well as Titer and Productivity (B, C) Measures for Various Feed Strategies.*

Discussion

The data shown here demonstrates that modification of the feeding strategy by monitoring glucose, lactate, asparagine and serine levels lead to an increase in overall process yield. Both cell density and titer were increased following modifications, and cell-specific productivity in the modified process was maintained at 20% increment compared to a standard batch process. This shows how spent media analysis can aid in delivering a significant titer increase.

Only glucose, lactate, asparagine, and serine levels were measured for the purpose of this study. A full panel of spent media analysis for samples taken all throughout the production run provides deeper insights to optimize the feed and maximize bioprocess yield.

Using our long-standing experience in cell culture media development we have established fast and robust methods for measuring the concentrations of a variety of components in cell culture media with our state-of-the-art equipment (Table 1).

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