Improved Manufacturability of Fed-Batch Systems Employing Highly Concentrated Feeds

ABSTRACT

Nutritionally complex feed supplements, particularly those that can satisfy metabolic demands of high-performing CHO cell culture, are essential for the successful optimization of present-day fed-batch processes. An ideal nutrient component solution should be designed to facilitate manufacturability at the clinical and commercial production scale with regards to constraints on production cost, volume, feed stability, and handling. To achieve this, two novel and complementary approaches for process development permit the introduction of substantial pH adjustments and multiple component subgroups. However, these practices can present additional complications. This study outlines our efforts towards the design of single and multiple concentrated feed supplements that do not require pH adjustment. These complete and balanced dry feed media prototypes can be reconstituted rapidly in water and have achieved stable concentrations of 150-200g/L at neutral pH. Experiments were conducted confirming the feeds at 1X concentration delivered equivalent productivity of a monolocular antibody in comparison with first generation commercial feed supplements, and when increased to 2X performance was improved by 20-100%.

INTRODUCTION

Single-part dry format feed media for CHO bioprocesses that can be simply hydrated in reduced liquid volumes without the need for pH adjustments were developed through the use of novel and proprietary technology that allows for delivery of higher concentrations of difficult to solubilize feed supplements. This approach addresses several challenges associated with large scale fed-batch manufacturing, such as bioreactor working volume restrictions, safety concerns with large volumes of high pH solutions, lengthy fluid transfer times, and limited storage space. It also diminishes additional complications that can accompany pH adjusted solutions, such as time consuming and complicated preparation, additional pH control management, supply chain pressures from use of short shelf life materials, and the inability to combine solutions without component precipitation. These materials can achieve stable concentrations of 150-200g/L neutral pH after simple reconstitution in water resulting in solutions without excess corrosivity associated with pH adjustment (Table 1).

Table 1. Comparison against first generation feed media

<table>
<thead>
<tr>
<th>Feed Type</th>
<th>pH Buffer</th>
<th>pH Buffer - pH Neutral</th>
<th>pH Buffer - pH Acidic</th>
<th>pH Buffer - pH Alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD CHO + 1X EFA</td>
<td>8.1</td>
<td>7.8</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>CD CHO + 1X EFB</td>
<td>8.1</td>
<td>7.8</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>CD CHO + 1X EFC</td>
<td>8.1</td>
<td>7.8</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>CD CHO + 2X EFC</td>
<td>8.1</td>
<td>7.8</td>
<td>6.8</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Both processes have consistent specific productivity and achieve >3g/L at harvest. Inclusion of FMT may have already optimized productivity such that EFB-EFC did not deliver an anticipated titer boost. The EFB+EFC condition had somewhat higher glucose levels and more lactate accumulation at the end of the run compared to historical control data (not shown). Overall, the results were determined by the evaluator to be representative of highly concentrated/neutral pH feed media performance based on the historical control mean ± standard deviation (dashed yellow).

Glycan results represent an average of two samples. No statistically significant differences were observed between the feed conditions. Minor variation could be attributed to the samples not being fully purified and that the reference control standard was produced in a different process with low variability. The evaluator did not consider differences to be significant.

CONCLUSIONS

- Lower volume addition of highly concentrated feeds provided up to 120% productivity increase
- Substantially easier and faster concentrated feed preparation
- Circumvented constraints to bioreactor working volume
- Dry and liquid formats stable for 6 months stored at refrigerated temperatures and when protected from light, 1 month at room temperature
- Comparable product quality to first generation feeds

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TRADEMARKS/LICENSING

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