STRATOPHASE™

Automated Closed Loop Feeding Control of an expressing CHO Cell Culture using the Ranger system

Abstract

Stratophase, in collaboration with the Biopharm Process Research group at the GSK Medicine Research Centre, applied the Ranger system for Automated Closed Loop Feeding Control to a CHO cell culture. The real time on-demand feeding regime controlled by the Ranger system was shown to automatically adapt to the culture's requirements during the various stages of the process. The growth and production phases of the culture were shown to exhibit notably different feeding control characteristics, and the resulting feeding regime was shown to promote cell growth, maintain cell health and support strong product evolution without the need for user intervention.

Introduction

The majority of current fed-batch processes rely on the use of pre-determined feeding protocols based on nutrient requirement estimates, or the use of in-frequent sampling and off-line assay of culture media in order to determine the concentration of key components. Both of these techniques can lead to non-optimum feeding, with depletion of nutrient and large swings in nutrient concentration, risking the health of the organism and its ability to produce consistent product quality at a high titre.

An alternative to directly monitoring nutrient concentration is to monitor the metabolic activity since this is a function of nutrient concentration. For the majority of the cell types used in upstream bioprocesses an increase in substrate concentration results in an increase in activity until a maximum metabolic rate is reached (known as the 'Monod relationship'). It is therefore possible to use a decrease in metabolic activity as an indicator for reduced nutrient concentration, and a trigger for timely feed additions.

Bioreactor & Ranger set-up

A CHO cell culture that produces a biological macromolecule was run in a benchtop stirred tank reactor with a working volume of 2L. The culture was inoculated in to a starting media of a defined composition, and a nutrient rich media of similar but different defined composition was fed throughout the process to sustain the culture. The addition of the nutrient rich media was controlled on demand by the Ranger system, via the Automated Closed Loop Feeding Control (AFC) technique.

The Ranger system (show in Figure 1) consists of a Probe which is mounted within the bioreactor and a Manager which takes the data from the Probe (Process Trend Index referred to as PTI) and derives the metabolic activity (Metabolic Rate Index referred to as MRI). In this case the

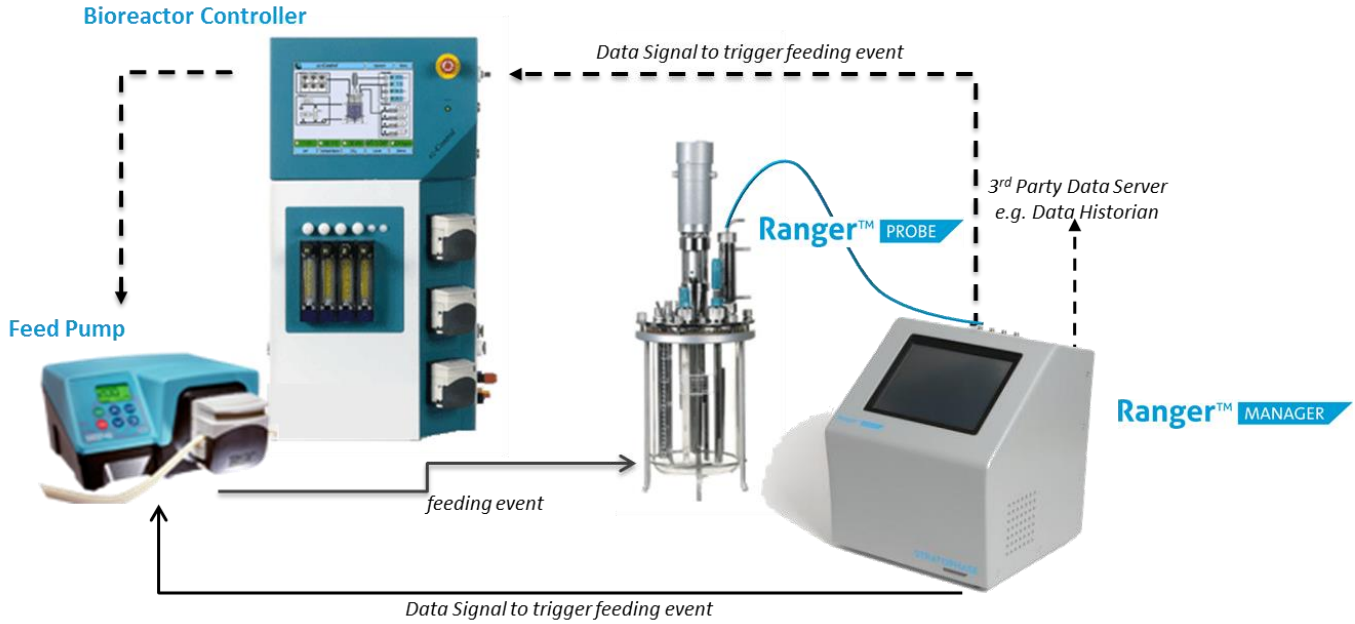

Figure 1. Typical Ranger system setup for AFC of a benchtop bioreactor, showing Probe, Manager and direct connection to feed pump.

Manager was directly connected to the feed pump and the MRI used to control the addition of a finite dose of nutrient rich media

The Ranger Manager was programmed with the settings required to control a cell culture of this nature. The 6 parameters allow:

1. Automatic calculation of MRI from PTI via an intelligent data filtering algorithm.
2. A user defined dose size to be added when a feed is triggered.
3. Definition of the feed addition and vessel mixing characteristics.
4. The relative process time at which AFC is to start.
5. The sensitivity of the feed-trigger control loop.
6. The dead-band of the feed-trigger control loop.

These parameters were identified from monitoring and control of previous cell cultures with similar characteristics, or from fine tuning during control of cultures using the same equipment, media or cell line.

In addition to running the Ranger system to automatically monitor and control the cell culture in real time, samples were also taken daily to be analysed offline. Sampling is not required for the Ranger system to operate. In this specific case, comparison between the real time Ranger data and the offline assay data allows insight into the AFC technique and the resultant conditions under which cell cultures are maintained.

Control of a CHO Cell Culture

The Ranger system when running AFC continually calculates the MRI and from this determines when a feed addition is required. When comparing Ranger data to offline data it is often insightful to analyse the PTI profile.

Figure 2 shows the PTI plotted against the cumulative feed profile generated by AFC. The cell culture was initially run as a batch, with AFC set to start at 96hrs. The first feed was triggered at 148hrs, but regular feeding did not begin until 196hrs. The PTI profile shows upticks associated with feed addition, and the characteristic consumption phases between feed pulses. A number of discrete stages can be observed as the process progresses;

1. 196-236hrs shows feeding with consumption phases with downward trends (negative MRI), commonly associated with the PTI being dominated by nutrient consumption.
2. 263-390hrs shows feeding with consumption phases with upward trends (positive MRI), commonly associated with the PTI being dominated by product evolution.

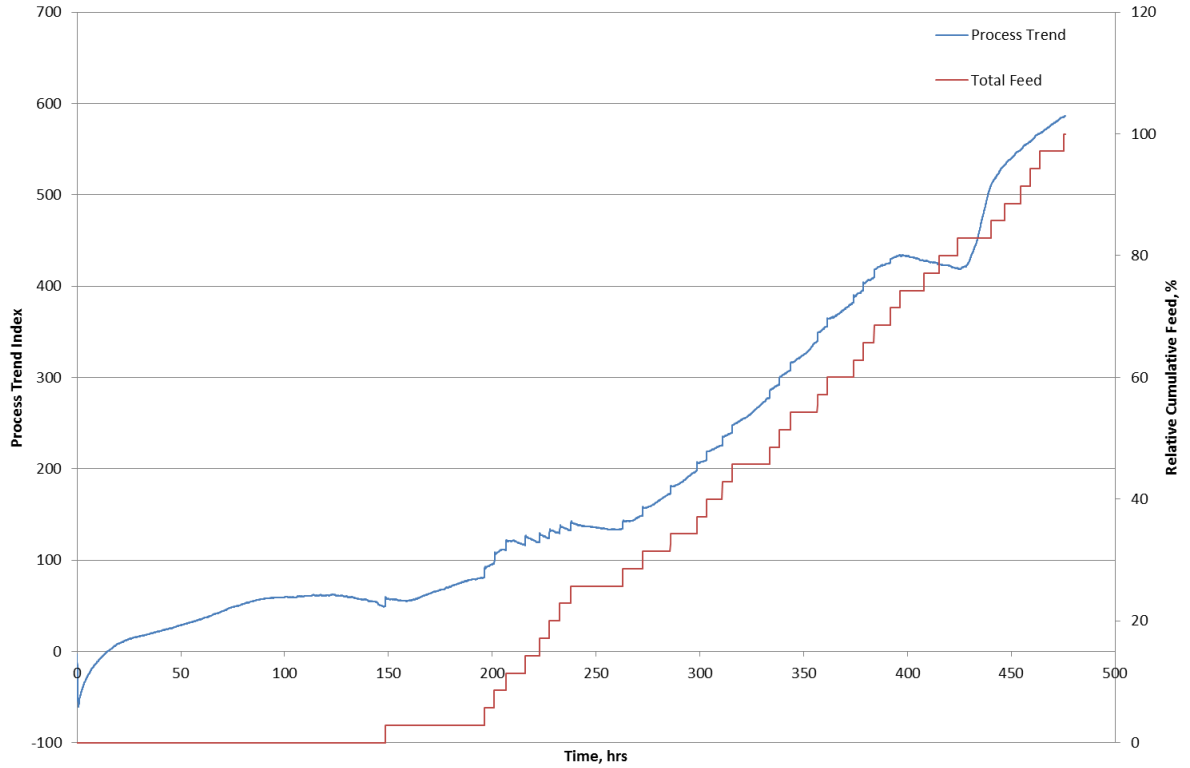

Figure 2. Process Trend Index and cumulative feed profiles generated during AFC of a CHO cell culture.

3. 396-420hrs shows a lack of feeding and a decreasing trend. This coincided with the feed stock running out, after which it was not physically possible for a feed addition to be made despite the Ranger triggering the pump.
4. 420hrs onwards shows a characteristic significant upturn in PTI, which can be identified through a decrease in cell viability.

Figure 3 shows the PTI plotted against the Viable Cell Count (VCC). The process was observed to demonstrate three distinct phases; initial cell growth to a maximum VCC occurs from 0-240hrs, a slow decline in VCC is observed from 240-405hrs (an anomalous data point is observed at 334hrs), and a rapid decrease in VCC occurs at a point beyond 405hrs. This is consistent with the process being divided into growth, production and death phases.

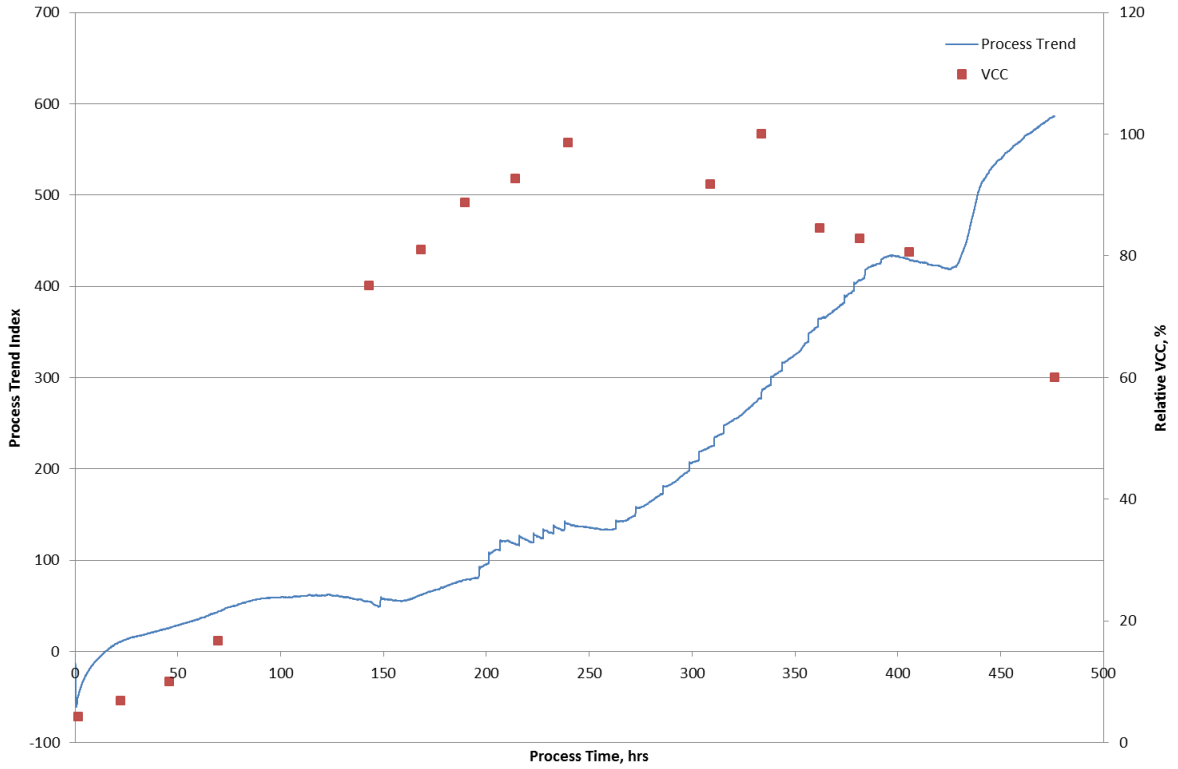

Figure 3. Process Trend Index and Viable Cell Count.

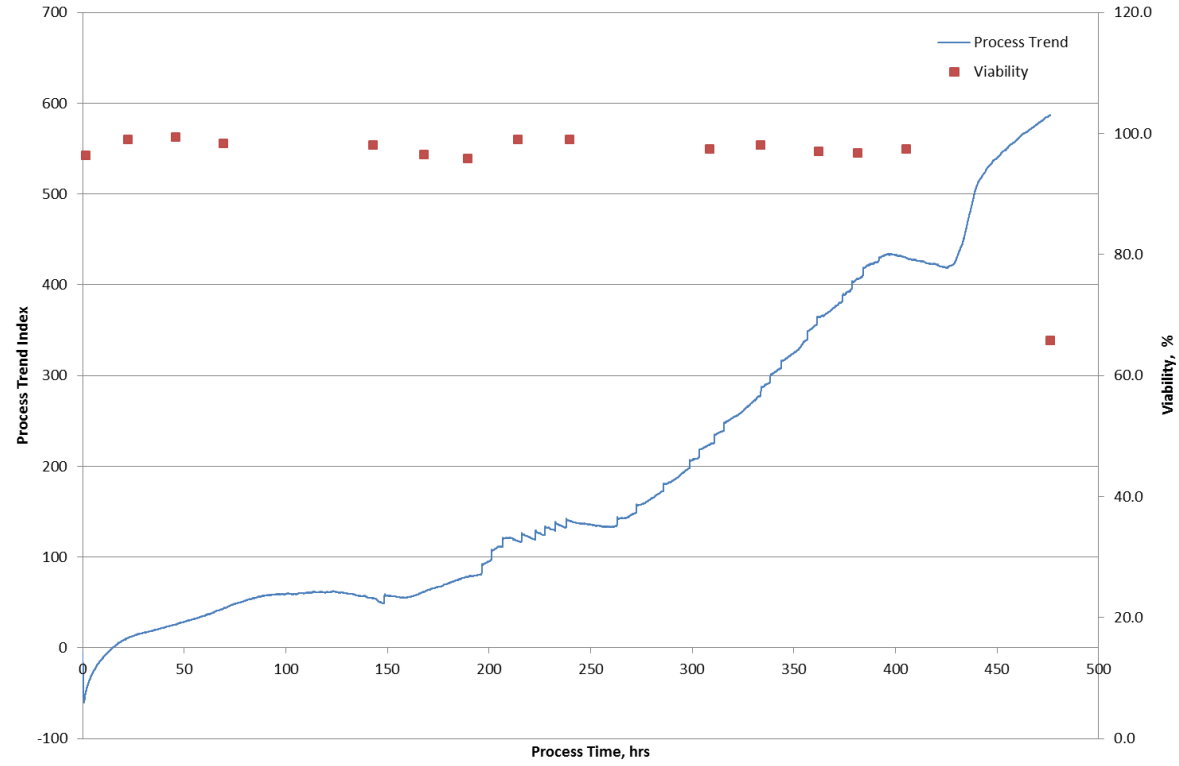

Figure 4. Process Trend Index and Viability.

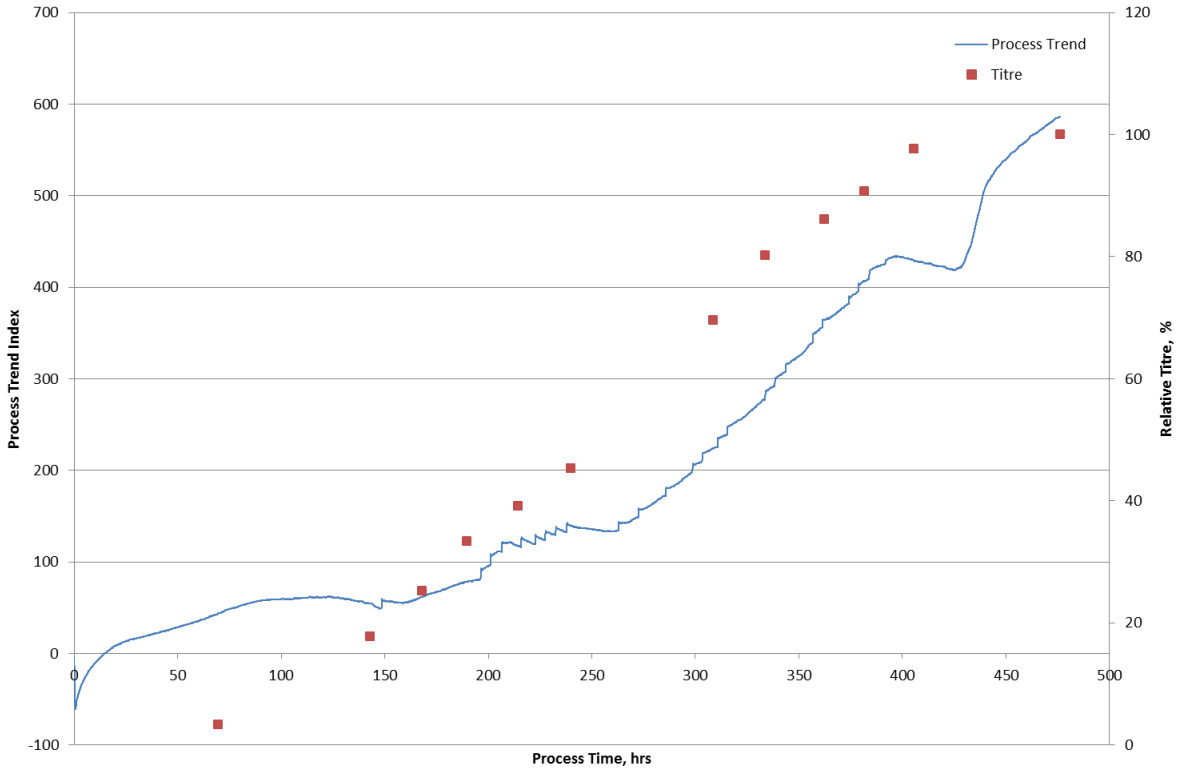

Figure 5. Process Trend Index and Product Titre.

Figure 4 shows the PTI plotted against Viability, and supports the VCC data by clearly indicating a stable cell health from 0-405hrs, with a significant decrease in viability at a point beyond 405hrs as cell necrosis occurs due to the feed stock running out.

Figure 5 shows the PTI plotted against Product Titre. Product is evolved at a consistent rate throughout the process. From 263-390hrs, when the consumption phases of the AFC cycle are product dominant, there is a clear correlation between the increasing PTI and the product accumulated within the media. The decrease in product release rate at a point beyond 405hrs is associated with nutrient limitation caused by the feed stock running out and a decrease in cell viability

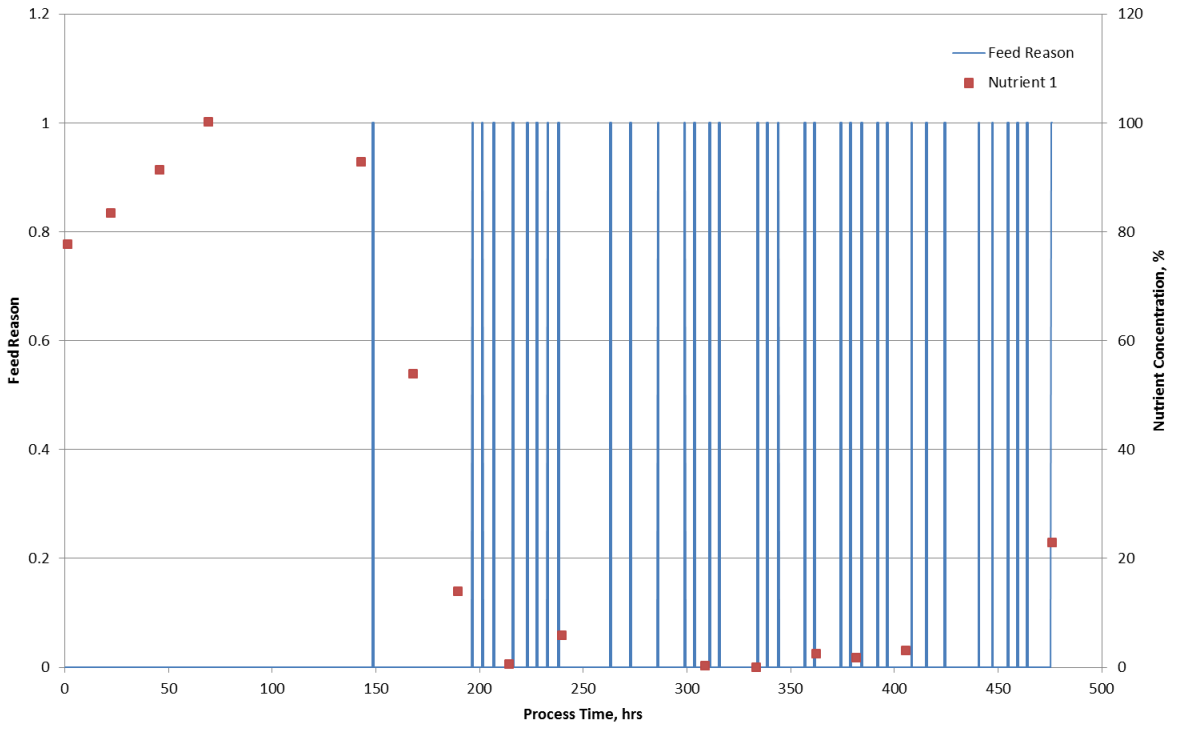

Figure 6. Feed Reason (pump on/off) and Nutrient 1 concentration.

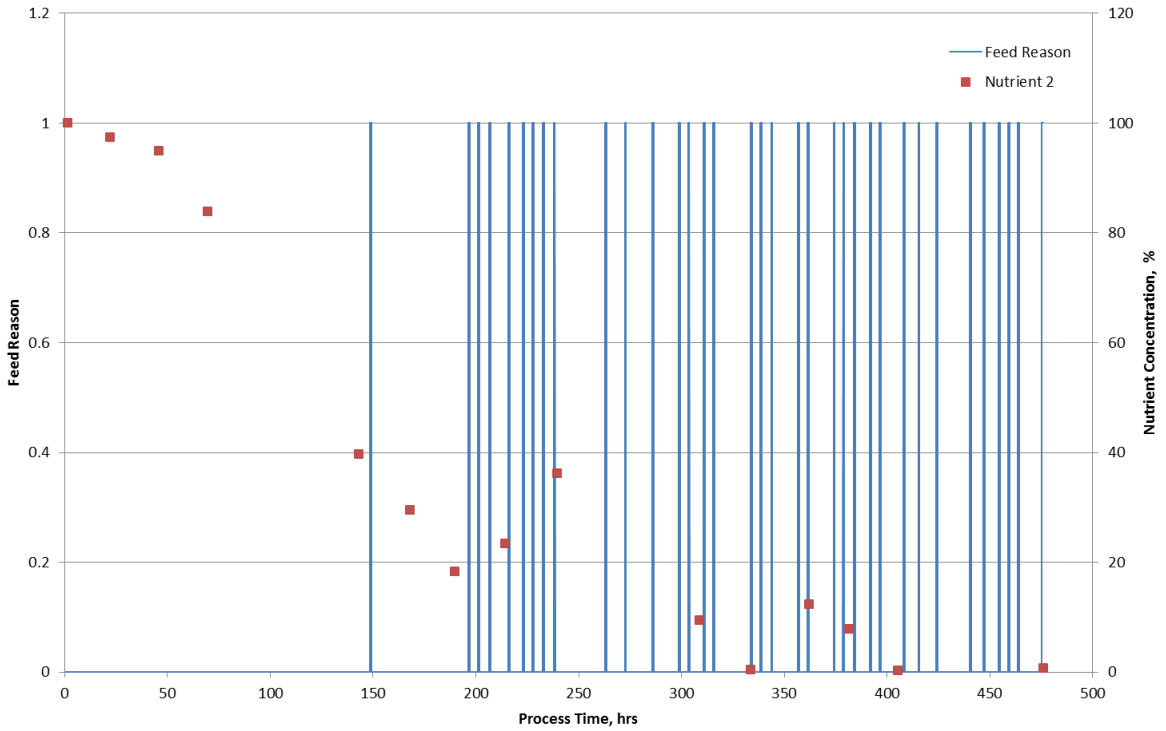

Figure 7. Feed Reason (pump on/off) and Nutrient 2 concentration.

Figure 6 shows the instances when the Ranger system triggered a feed (0 = pump off and 1 = pump on), plotted against the offline assay data for a nutrient which is known to be strongly consumed during the growth phase (nutrient 1).

Figure 7 shows the same feed trigger data plotted against the offline assay data for a nutrient which is known to be strongly consumed when the cell culture is in the production phase (nutrient 2).

For both nutrients the data is infrequent and out of phase with respect to the feed pulses due to the complexities associated with sampling bioreactor media. However, it is possible to see examples of the low concentration experienced just before a feed and the high concentration immediately following a feed. From the correlation between the offline assay data and the AFC trigger data, and the known cellular utilisation of the nutrients, it can be deduced that AFC is supporting both the growth and the production phases of the culture.

Between 196-236hrs nutrient 1 is being consumed at a higher relative rate than nutrient 2, and AFC is triggering from the reduction in metabolic activity associated with low levels of nutrient 1. The relative composition of the nutrient rich media results in a slow accumulation of nutrient 2 within the bioreactor, although the levels reached are not detrimental to cell health.

Between 263-390hrs nutrient 2 has a larger effect on metabolic activity than nutrient 1 and, as a result, feeds are triggered from low levels of nutrient 2. The relative composition of the nutrient rich media results in the concentration of nutrient 1 varying in a manner not clearly associated with the feed additions, although at no point are the levels reached detrimental to the cell health.

Conclusion

The cell culture outlined in this paper and the implementation of the Automated Closed Loop Feeding Control technique demonstrates the value of the Ranger system for controlling an on-demand feeding strategy in real time which meets the cultures requirements throughout the duration of the process. The use of a single feed, consisting of nutrient rich media, ensured that the Ranger system successfully responds and arrests any decrease in metabolic activity which is associated with reduced concentrations of the critical components within the media. In this specific case, both cell growth and production phases were supported by AFC automatically adjusting to the requirements of the cell without the need for user intervention. The use of AFC and the resulting optimisation of feed rate and minimisation of nutrient concentration variation led to a consistently high degree of cell health, a high product titre and a consistent product quality throughout the process.