Optimally low Glucose levels maintained by Automated Closed Loop Feeding Control using the Ranger system leads to low Lactate during CHO cell culture

Abstract
The Bioprocess R&D group at Pfizer Chesterfield MO, in collaboration with Stratophase, applied the Ranger system for Automated Closed Loop Feeding Control to a CHO cell culture. The use of changes in metabolic activity to trigger feed additions on-demand was shown to optimise media composition, in particular nutrient levels, to cellular requirements in real time. The cell line used during this study was found to dictate Glucose concentrations in the range of 0.1-1.0g/l when running the Ranger system. Maintenance of such nutrient lean conditions, without nutrient starved conditions occurring, was demonstrated to result in consumption of the Lactate accumulated during the batch phase of the culture with subsequent conservation of near zero Lactate levels during the feeding phase. The media composition enabled by the Ranger system was found to enhance the performance of the cell culture.

Introduction
The majority of current fed-batch cell culture 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 a risk of nutrient depletion and large swings in nutrient concentration, adversely affecting the health of the cells and their 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 nutrient 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 as a trigger for timely feed additions. The use of such an on-demand feeding technique is detailed in the following paper and shows the benefits associated with real-time optimisation of nutrient conditions to cellular requirements.

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 into 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 that is mounted within the bioreactor and a Manager that 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). The PTI is in effect the relative refractive index of the media and shows the combined affect of all media components in solution. The MRI is automatically generated by the Ranger system from the PTI such that it is an accurate indicator for changes in metabolic activity. In this case the Manager was directly connected to the feed pump and the relative changes in 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 are:

1. Time duration for intelligent data filtering algorithm used to automatically calculate MRI from PTI.
2. Feed dose size to be added when a feed is triggered.
3. Time duration for PTI to stabilise after a feed addition is made to account for vessel mixing characteristics, thermal response and short-term metabolic response.
4. The relative process time at which AFC is to start controlling.
5. The sensitivity of the feed-trigger control loop.
6. The control threshold of the feed-trigger control loop.

These parameters are typically 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, but 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.

Maintenance of low Glucose concentration during 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

![Figure 2. Process Trend Index and cumulative feed profiles generated during AFC of a CHO cell culture.](image-url)
often insightful to analyse the PTI profile. Figure 2 shows the PTI and cumulative feed profile generated as the cell culture was running.

The PTI profile generated during the CHO cell culture is characteristic of the changes that occur within the media as the culture progresses. The rapid increase in PTI observed during the first ~24hrs of the process is typically for the majority of CHO cultures and coincides with the lag and onset of the exponential growth phase of the culture. During this period it is believed that the release of growth factors and signalling molecules, such as Cytokines, from the cells results in the increasing PTI signal. The PTI generated beyond 24hrs of culture time represents a composite signal comprised from decreasing nutrient concentrations, changes in metabolic by-products and increases in product molecule concentrations. The relationship between culture conditions and the PTI profile that is generated is specific to the cell line and media/feed composition. However, the generation of MRI from the PTI and the relationship between decreasing metabolic activity and reduced nutrient concentration is always valid.

For this specific culture, the decrease in PTI observed between 50-72hrs is consistent with nutrient levels decreasing to those associated with AFC. From 72hrs onwards AFC begins triggering additions in response to the cell cultures requirements. Feed additions result in a rapid increase in the PTI signal, the period after a feed indicates the process kinetics associated with nutrient consumption. Feeds were observed to trigger regularly until the termination of the culture, the precise frequency of feeding being dictated by the real time cellular demand.

Figure 3 shows the PTI and MRI plotted against culture time. The algorithm used to calculate MRI ignores the changes in PTI which can be directly attributed to feed additions, resulting in an accurate indication of the changes in metabolic activity that occur due to nutrient consumption between feeds.

The sign of the MRI is dependent upon the metabolic processes and the associated chemical species in the media that influence the PTI for a specific culture. In this case the culture exhibits a negative MRI because the PTI is reflecting the decreasing nutrient levels associated with metabolic activity. Irrespective of the MRI-sign the absolute MRI value conveys the increase to a peak value associated with the increased availability of nutrient(s) following a feed event, then a decrease associated with the on-set of low nutrient conditions. The decrease in
MRI observed after a peak value is attained triggers AFC to make an addition.

Figure 4 shows the occurrence of Feed Additions triggered by AFC and offline assay data for Glucose and Lactate concentrations plotted against culture time. It is not possible to observe the real time changes in media component concentrations due to the relative infrequency of sampling compared to the feed additions. However, it is possible to use the spacing of the offline data points relative to feed additions to gain an indication of the range of concentrations that are maintained during AFC.

During the batch-phase of the culture, before feeding starts, the Glucose concentration is observed to decrease and the Lactate concentration increase. The accumulation of Lactate indicates that Glucose is in excess of that which is required by the cells, resulting in the generation of waste. The first feed triggered at 72hrs when the AFC Start Time was reached. In this case the feed was not triggered in response to low nutrient conditions, instead the feed triggered from the metabolic effects that occurred during the batch-phase. The subsequent feeds from 87hrs onwards were triggered directly from characteristic changes in metabolic activity which can be attributed to low nutrient levels. The offline assay shows that AFC is maintaining Glucose concentration between 0.1 – 1.0g/l. The lower limit of this range is defined by the cells metabolic response to low nutrient conditions. The high response speed associated with AFC ensures that detrimentally low nutrient conditions are not experienced during the culture. The upper limit of the Glucose range is defined by the size and concentration of the feed addition. The onset and control of low Glucose conditions associated with AFC was observed to promote the consumption of Lactate from the media. From 70-140hrs a steady decrease in Lactate concentration was observed until near zero levels were achieved. From 140hrs onwards the offline assay data indicated that Lactate concentration remained in the region of 0.2-0.4g/l.

Figure 5 shows the MRI plotted against culture time and the corresponding data for Viable Cell Concentration and Cell Viability attained by sampling and offline analysis. The culture was observed to achieve a maximum metabolic activity (MRI of approx. -315) during the exponential growth phase. The peak MRI values during subsequent feed-consumption cycles were observed to decrease until 120hrs, which coincided with when the culture transitioned in to the stationary phase. The peak MRI values
between 120-162hrs were observed to be relatively stable. From 162hrs until the culture was terminated a slow decline in peak MRI values was observed, coinciding with decreasing cell viability.

The data shown in figures 4 & 5 show that the onset, and maintenance, of low Glucose conditions and the resultant low Lactate levels are not detrimental to the cell viability.

The comparison of this campaign, and others running AFC, with cultures fed using strategies reliant on infrequent offline assays shows significant improvements in Viable Cell Concentration, Product Titre and Specific Productivity when cultures are run with AFC. This data is not presented since it is outside of the immediate scope of this paper. However, the culture conditions maintained during AFC and the associated advantages for the culture process is widely supported by studies reported in the literature. The benefits associated with employing automated feeding strategies are well documented (Zhang et al, 2015). The ability to respond in real time to a cell culture's nutrient requirements and thereby avoid detrimental excess or depletion results in significantly improved process performance and robustness. The effects of Lactate concentration on CHO cell cultures and the benefits associated with maintaining low levels of Glucose and the associated low levels of Lactate has been the subject of several studies (Li et al, 2012 and Gagnon et al, 2011). Improvement in product titre due to simultaneous increases in cell growth and cell specific productivity is attributed to effective suppression of inhibitory lactic acid and the more efficient utilisation of nutrients.

Conclusion
The Ranger system and the on-demand feeding technique it enables has been shown to maintain metabolite concentrations at levels which are directly beneficial to the productivity and robustness of CHO cell culture processes. The use of small and frequent feed additions avoids the excess nutrient concentrations typically observed during bolas feeding, thereby avoiding detrimental effects on cellular productivity and product quality. The maintenance of nutrient lean conditions while avoiding nutrient starved conditions has been shown to minimise the generation and accumulation of detrimental waste products. In addition, nutrient lean conditions directly promote the efficient generation of product from the available
nutrients because little or no waste products are generated. The study outlined in this paper, and the wider work represented in the literature, show the clear benefits associated with a fully automated process that dynamically and adaptively controls the feeding regime. Application of on-demand feeding techniques, such as offered by the Ranger system, are therefore expected to have a significant impact on both process development and GMP manufacturing activities in the future.

References
