INSIDE KNOWLEDGE

STRATOPHASE™

Feed-on-Demand: The use of Metabolic Rate Index for closed loop control of feeding in upstream bioprocesses

Abstract

Modern biotechnology industries, such as those producing biopharmaceuticals, are heavily reliant upon upstream bioprocessing in which micro-organisms and cells are used as biochemical factories. A fed-batch process is commonly used for feeding organisms at the conditions required for optimum production due to its simplicity and flexibility of use with a wide range of organisms and associated products. In an ideal fed-batch process the culture is supplied with nutrients, at a rate which matches their consumption, such that detrimental excess or depletion is never experienced. Due to operational limitations, this is rarely achieved in practice as most operators employ fixed, scheduled feeding strategies with control through off line assays. Feed on-demand strategies, as described here, offer the potential for rapidly determining fixed feeding schedules during process development and for implementation of closed-loop nutrient control during scale-up and production.

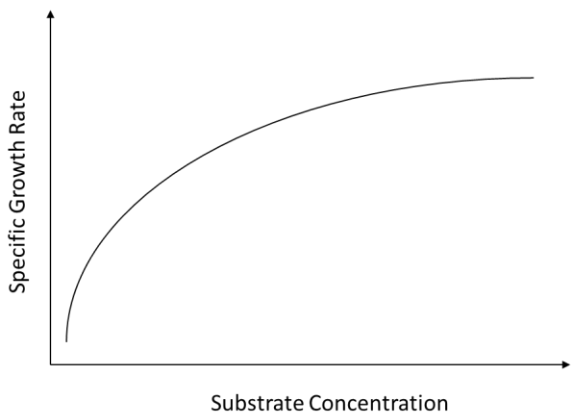
Introduction

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 a high product titre.

An alternative to directly monitoring nutrient concentration is to monitor the metabolic activity of the organism since this is a function of nutrient concentration. The majority of the organisms used in upstream bioprocesses, whether microbial or mammalian in nature, behave according to Monod-type kinetics which is valid for both exponential growth and steady-state culture under conditions of balanced growth. The Monod relationship describes that “as substrate concentration increases, the metabolic activity of an organism also increases towards a maximum” (see figure 1).

As a result, as long as the substrate concentration is below that which is associated with the maximum, then relative nutrient concentration can be linked to an organism’s metabolic rate. More specifically, characterising the decrease in metabolic activity associated with decreasing nutrient concentration can be used as a trigger signal for on-demand feeding.

In order to take advantage of this effect a rapid and reliable indicator of metabolic activity is required. The monitoring technique needs to respond quickly to subtle changes in the metabolic rate in order to avoid the nutrient concentration decreasing to a point which is detrimental to the organism. In addition, the monitor ideally needs to respond directly to a metabolite(s) whose rate of concentration change is directly linked to the metabolic rate of the organism. The signal must also see minimal



$$\mu = \mu_{max}\frac{S}{K_S + S}$$

Where;

μ = specific growth rate

μ_{max} = maximum specific growth rate

S = Substrate concentration

K_s = Saturation concentration for substrate

Figure 1: Monod growth curve showing relationship between substrate concentration and metabolic activity

interference from changes associated with oxygen supply strategies or other bioreactor control parameters.

The Ranger system is a solution to this problem.

This paper details the theory behind Metabolic Rate Index (MRI) and its use for controlled 'on demand' feeding in real time.

Metabolic Rate Index (MRI) generated by the Ranger system

The in-situ Ranger Probe directly reports the Process Trend Index (PTI) for the bioprocess being monitored, which is in effect a measure of the change in Refractive Index relative to the process. When the Process Trend Index is compared to the compositional changes that occur within a fed-batch process a strong correlation between nutrient and/or product concentrations is observed.

The Ranger Manager analyses the Process Trend Index in real time to calculate automatically the MRI for the organism. Due to the PTI's direct response to changes in metabolite concentrations the MRI is used to indicate metabolic activity and, by association, relative nutrient concentration. Figure 2 shows the evolution of PTI and the accompanying MRI for a simplified process, where a single feed-addition increases metabolic activity to a maximum followed by a characteristic decrease in metabolic activity which is due to decreasing nutrient concentration until the point at which exhaustion occurs.

The MRI generated by the Ranger Manager can be used as a self-referenced indicator of metabolic activity; it is non-specific to the metabolites which occur in the bioprocess media making it suitable for simple characterisation and control of a wide range of processes. As a tool for visualising metabolic activity and defining feeding strategies MRI is similar to the use of Oxygen Uptake Rate, offering an alternative potentially complementary, improved technique for users of upstream bioprocesses wishing to implement on-demand feeding.

Automated Closed Loop Feeding Control using MRI and variable frequency feeding

The MRI observed directly after a feed addition, and in particular the increase and decrease in metabolic activity, can be used to characterise the nutrient consumption rate for the organism during that phase of the process. The consumption rate that an organism can achieve is dependent upon a number of factors, but is usually not constant throughout a process. It is therefore possible through the use of frequent feed-consumption cycles to monitor the consumption rate for the organism at that point in the process and to make feed additions accordingly.

The Ranger Manager uses the MRI to automatically trigger feed additions. This is done by the user setting a relative value for MRI. When the signal drops to this value after the maximum has been passed a feed is triggered. The link between metabolic activity and substrate concentration means that feed additions are

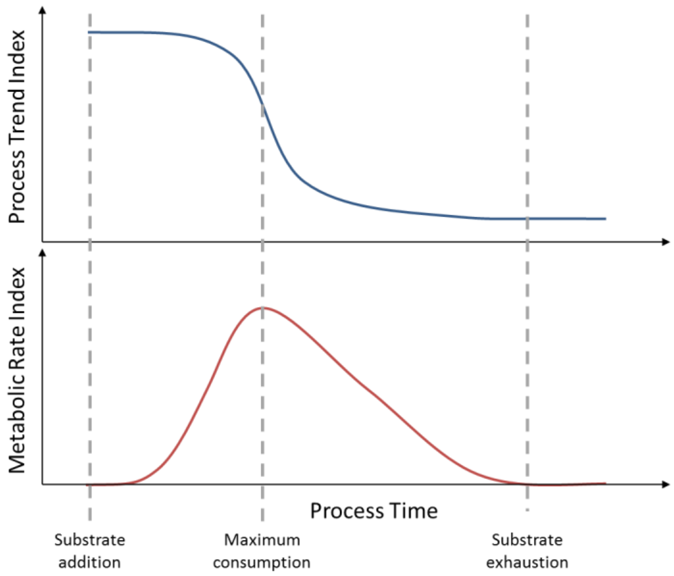


Figure 2. Process Trend Index and Metabolic Rate Index response to feed addition, consumption and exhaustion in a simplified bioprocess.

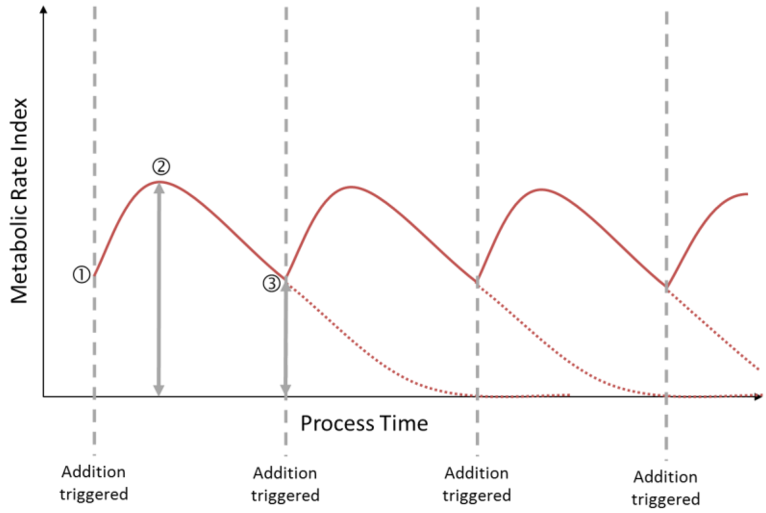


Figure 3. Simplified Metabolic Rate Index profile observed during automated closed loop feeding control.

indirectly triggered by a drop in nutrient concentration without the need for absolute nutrient concentration to be determined.

Figure 3 shows a simplified plot demonstrating the generation of a MRI profile during automated closed loop feeding control. The process is:

1. After completion of a finite feed addition an increase in metabolic activity is observed, associated with the increase in nutrient that is available to the organism. The metabolic activity will continue to increase until either the maximum rate for the organism is reached, or nutrient concentration drops to a point that further increase is not possible.

2. When a maximum in metabolic activity is detected the Ranger Manager assigns that value for MRI a relative ratio of 1, which allows any decrease from this value to be described as a value between 1 (maximum metabolic activity for that consumption cycle) and 0 (zero metabolic activity associated with substrate exhaustion).

3. On reaching the MRI ratio which the user has set as the point for feed addition (between 0 and 1), the Ranger Manager automatically triggers a finite addition of feed, and the control loop is reset.

Each consumption cycle is independent from the previous, ensuring that feed addition is continually optimised for the organism at any given point in the process. The use of fixed volume feed additions results in control of nutrient concentration through variable frequency

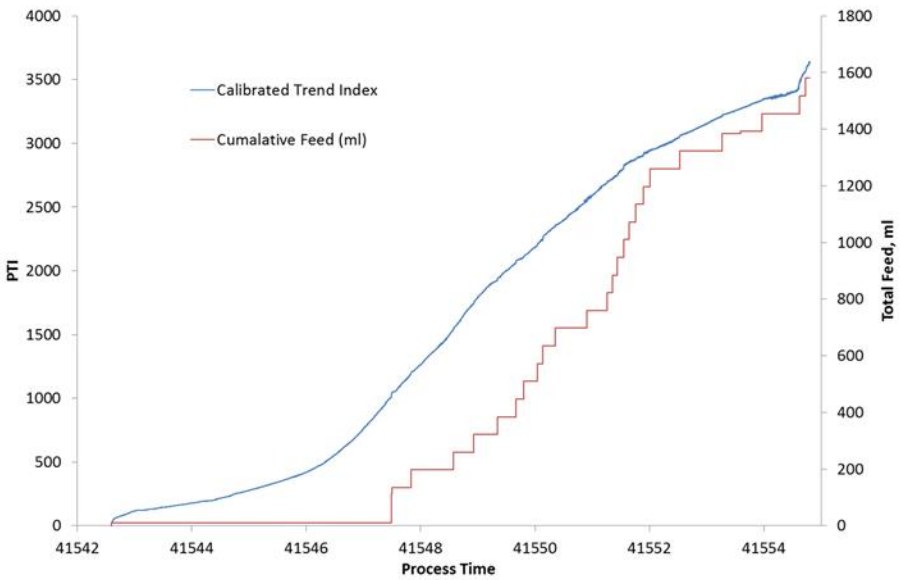


Figure 4. Process Trend Index and Cumulative Feed profiles generated by a native CHO culture running a Ranger system under automated closed loop feeding control

feed pulses. By setting the feed volume which is added by the Ranger at each feed event and the MRI Trigger Ratio, that initiates a feeding event, the user can define and limit the variation in nutrient concentration experienced in the bioreactor and the range of feed frequencies that occur within the resultant feeding program.

The Ranger Manager and Ranger Probe, when running automated closed loop feeding control, generate a real-time feeding regime, which is most easily visualised through the cumulative feed profile. Figure 4 shows the PTI and Cumulative Feed profiles for a native CHO culture running automated closed loop feeding control. The rate of feed addition is matched to the organism's ability to consume nutrient and, as a result, the cumulative feed profile exhibits the characteristic rate changes that are expected for a mammalian cell culture. In this way the Ranger system can not only be used for on-demand feeding control, but can also be used for the rapid identification and definition of fixed feeding strategies during process development and optimisation.

Conclusion

A practical solution to on-demand feeding control has been illustrated using metabolic activity to infer nutrient consumption rate and hence determine optimised feeding strategies. Alternative techniques such as monitoring Oxygen Uptake Rate have gained some interest however the complexity of characterising nutrient consumption and decoupling it from other critical bioreactor control parameters has limited their adoption. The Ranger system directly characterises the substrate consumption rate, and indicates metabolic activity. Furthermore the Ranger system is designed specifically for on-demand feeding control, presenting the user with an easy-to-use set of control parameters with which to tune the closed loop control of feeding for their process and thereby rapidly define and optimise both dynamic and fixed feeding strategies.