The Hunt for MRI – a Novel Method to Automate Bioreactor and Fermentor Feeding

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Abstract

A novel system, the Ranger™, and method for in-situ, real time monitoring and control of nutrient feeding in upstream bioreactors and fermentors is described. MRI (Metabolic Rate Index) is a Process Variable that describes the overall state of the metabolic environment of the process under observation and is highly sensitive to any molecular level perturbation in the process media, such as occurs when a biological process is fed. The system is applicable to all scales of operation from process development to commercial production and is compatible with SUBs. This technology is in evaluation in microbial, fungal, and mammalian cultures.

Technology

The core of the system is a small, in-situ silicon optical sensor incorporating Bragg gratings in contact with the process media. This sensor generates a signal directly proportional to the refractive index of the media which responds directly to the metabolic rate of the process, nutrient additions and to production of product. Each analyte's response is the product of its concentration multiplied by its R.I. The overall instrument response is the sum of the contribution from all analytes in solution in the media above their individual limit of detection, a Process Variable named PTI (Process Trend Index.) MRI is essentially the first derivative of PTI (Eqns 1,2)

$$PTI = \sum_{n\,to\,x}([i] * R.I._i) \quad (1)$$

$$MRI = K * (d\,PTI / d\,Time) \quad (2)$$

K = a series of Ranger instrument factors

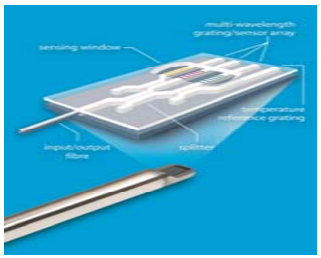


Fig 1. Stratophase multi element Bragg grating array. Dimensions – 6 x 20 x 1 mm Sensor window 1 x 5 mm mounted in Ranger SIP Probe

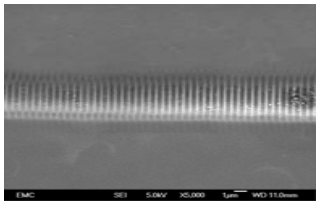


Fig 2. Electron microscope image of single Stratophase Bragg grating written by laser in a silicon fiber optic waveguide

The Ranger System

Ranger Manager captures in-situ data for monitoring process kinetics and media molecular composition which in conjunction with its software suite permits directly opportunities for control and automation of nutrient feeding . The Ranger Manger controls Ranger Probe in-situ optical sensors and supports up to eight in parallel for biotechnology applications from upstream mammalian and microbial processes. Ranger probes are available in standard configurations for headplate and SIP vessels. The system can be used as a process monitor, a stand alone nutrient feed controller, as an adjunct to a RCS system, or integrated into a full Process Control System (PCS). The technology is compatible with SUBs.

Upstream Applications

Refractive index is an ideal technology for the monitoring and control of biological processes because it can measure virtually all analytes commonly employed in or produced by controlled processes typical industrial and pharmaceutical biotechnology. In upstream applications the non selective nature of refractive index has been found to be advantageous as the Ranger system provides a comprehensive monitor of the metabolic environment of the bioprocess and is, at the same time, extremely sensitive to perturbations in that environment such as those produced a feeding event. The device is insensitive to particles, gasses, and cells making it particularly useful in this application. No fouling has been observed in operation due to the surface characteristics of the silicon device.

Some applications of note are the monitoring and control of sugar in a fermentation or cell culture process, tracking amino acid concentration, and monitoring the concentration of products most notably proteins and monoclonal antibodies (MAbs). As shown here the Process Trend Index and its derivative, MRI (Metabolic Rate Index, in process fermentation or cell culture can be used to extract the trajectory of a biological process and the utilization of feedstocks in that process.

Technology transfer of Ranger methods to new platforms is simple and straightforward.

Feed-on-Demand

Successful use of PTI and MRI data can be used to develop a "Feed-on-Demand" automated control feeding strategy for nutrients in which nutrient consumption by the process is optimally matched to feeding rate in real time. This will lead directly to higher quality product, better yields, and shorter cycles.

Ranger Method Development

Method development for the Ranger system is straightforward. The development of a Ranger controlled bioprocess feeding strategy typically comprises three serial steps: monitoring, supervised feeding, and then ultimately fully automated Feed-on-Demand under the control of the Ranger system.

Step 1, Process monitoring is used to develop confidence and control limits for the nutrient feedstocks and feeding schedules used currently in the bioprocess under development. This phase typically requires one to three bioprocess cycles..

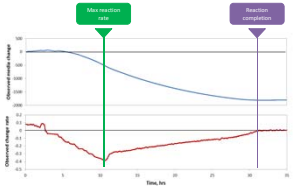
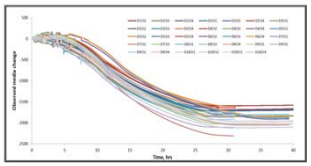


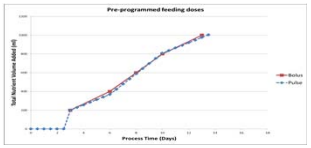
Fig. 3. The plot of the response (feedstock consumption) of a bioprocess to a single feeding event. The top trace is PTI in blue, bottom trace is MRI. Each feeding event whether batch or fed batch will generate a similar metabolic pattern of consumption. This measurement of rate of change of the PTI and MRI is the basis for a feeding control strategy. Maximum reaction rate is assigned a MRI value = 1 Reaction completion MRI = 0

A user may successfully and productively utilize the system at this stage. The Ranger can be used to develop a control limit strategy for monitoring the metabolic trajectory of a bioprocess cycle in statistical comparison to the average. Intervention in bioprocesses trending out of control is then by manual methods. An example is shown below in which 30+ cycles were recorded to permit generation of statistically relevant average and control limit data for this process.



Step 2. Supervised, scheduled feeding is the intermediate step towards full automatic control. In this step the standard feeding strategy routinely used in feeding the bioprocess is modified. The total feedstock dose usually fed at the initiation of the process in the case of a batch process or in infrequent but regularly spaced intervals in the case of fed batch systems (3, 6, 9 days for instance in mammalian cell culture) is divided into a multitude of smaller doses (Equalling in total the usual typical full feeding dose) and then fed at frequent regular intervals over the course of the fermentation or cell culture cycle. The intent is to approximate and model the Feed-on-Demand strategy and in particular, observe the response of the process to this modification.

This step in development is controlled by the Ranger through use of a lookup table to schedule the feeding intervals. No feedback is employed. This step emulates a high frequency manual controlled feeding protocol



A model for supervised, programmed feeding – step 2. Red is the standard feeding schedule. Blue the improved, higher frequency protocol managed by the Ranger. Users can operate the Ranger and make profitable use of this semi-automated feature without proceeding to the ultimate step of full feedback automation.

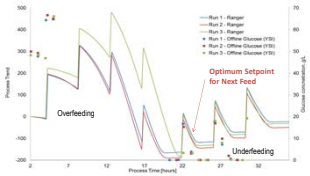


Fig 6. Examples of replicate yeast fermentations fed by the Ranger using this strategy. The first half of the fermentation demonstrates an overfed state indicated by an overall rising PTI signal and no inflection point in the signal in the time intervals after each feeding event. In the second half the process is underfed as cell density and metabolic rate increases and feed rate and concentration do not keep pace. This is indicated by the rapid rate of change in PTI, a noticeable inflection point, and a PTI slope of zero in the later half of each feeding event indicating complete consumption of that feed dose in that time interval.

Transition to full feedback control will simply require selection of a setpoint in time and amplitude (of rate of change – MRI) for the n+1 feeding event to avoid over or under feeding the process. See example above.

Step 3. Feed-on-Demand. This is closed loop control. In fed batch mode the Ranger supplies the bioprocess feeding doses in aliquots of equal volume and dose at varying frequency (period) as determined by the metabolic activity in the vessel. The process is fed frequently but only as the nutrient feedstock is utilized.

The user selects a MRI setpoint between 0 and 1 (see fig 3) such that the Ranger triggers a feeding in advance of depletion of the preceding feeding dose. Typical setpoints are between 50 and 70% depletion (0.5 -0.7). Processes with fast metabolic rates will require feeding sooner (lower MRI setpoint ~ 0.5) whereas slower systems will require higher setpoints (~0.7-0.8)

Feed-on-Demand is a frequency modulated process – feeding automatically in real time as the process requires nutrients.

Microbial Feed-on-Demand

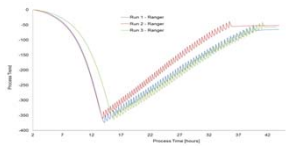
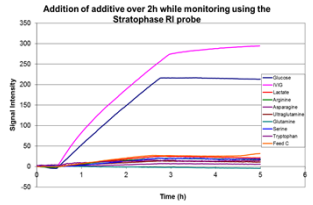


Fig 7. The yeast fermentation shown in Fig 6 optimized and controlled by ranger system in Feed-on-Demand mode. Small frequent feedings of constant dose based on demand. Notice the varying period in feeding from hour 15 to 35+. Feeding was delayed until hour 12. The monotonic rise in PTI from hour 13 to completion is due to expression of product into the media – a long term continuous and monotonic process compared to the short term imposed feedings which induce immediate and short lived perturbations in the PTI signal. The Ranger software algorithms are designed to differentiate feeding events from product expression.

A Mammalian Cell Culture Example

Material		Final Concentration Range	
		Low	High
Glucose	165mg/mL	0	15mg/mL
Lactose	22mg/mL	0	2mg/mL
IVIG	110mg/mL	0	10mg/mL
Arginine	55mM	0	5mM
Asparagine	55mM	0	5mM
Glutamine	12.1mM	0	1.1mM
Serine	165mM	0	15mM
Tryptophan	22mM (4.5mg/mL)	0	2mM
UltraGlutamine	44mM	0	4mM
Feed C	Various	0	33.3%

A synthetic cell culture mixture was prepared as above to test sensitivity and selectivity of the Ranger to common components in mammalian cell culture media, including product, IVIG.



This demonstrates Ranger's linear response to glucose and near linear response to protein over a wide concentration range and linear but lower sensitivity to common amino acids. Please note that in all observations with exception of glutamine the Ranger PTI signal was above the limit of detection and quantifiable at the endpoint of the titration (2 hours). The experiment proceeded to Step 1 – monitoring – in the Ranger Method Development protocol

Mammalian Process Monitoring

Step 1

A CHO cell culture was initiated and monitored online by the Ranger system to monitor and assay glucose and product protein in-situ and in real time. A Nova Biomedical Flex Nutrient Analyzer equipped with an autosampler was used simultaneously to correlate and confirm the Ranger system measurements. The Flex extracted and sampled the reactor on a frequent fixed interval. The Ranger base optical data collection frequency was 20 Hz – PTI/MRI Process Variables were updated approximately every 4 minutes.

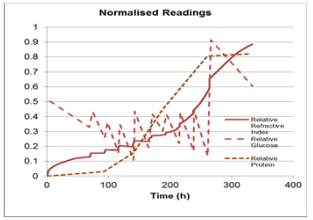


Fig 9. Legend. Solid Red – Ranger PTI Process Variable. Long Dash – Nova Flex Glucose. Short Dash – Nova Flex Protein (spectroscopic)

As observed the Ranger and the Flex both respond to glucose additions (feeds) and subsequent consumption by the CHO cell culture. The feedings are seen as step changes on the Ranger trace. It can also be observed that the Ranger trace follows production of protein as displayed by the exponential rise in the Ranger trace. The Ranger software algorithms deconvolve the glucose and protein response in order to accurately control and trigger automatic feedings. This is done in calculating the MRI Process Variable – the variable used in the Ranger protocol to control feed periods. These experiments are currently moving into Step 2 and Step 3 of the Ranger Method Development Protocol. Results will be reported in subsequent reports as obtained.

Conclusion

The Ranger automated reactor feeding system has been developed which can be used to feed nutrients in upstream bioprocesses based on observed rates of change of metabolic activity in the process vessel. The system feeds equal aliquots of feedstocks on a frequency modulated basis that matches process nutrient consumption rates to the variable feed rate.

The system is easy to install; operate; requires minimal initial calibration; needs little, if any, in process recalibration; and can be used in all media types. It is accurate and functional for control and feeding of all all feedstocks in all upstream microbial and mammalian bioprocesses in industrial and bio pharmaceutical applications.

Acknowledgement

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