A novel method, the Ranger™, and method for in situ, real-time monitoring and control of nutrient feeding in upstream mammalian and fermentor processes is described. Ranger (Refractive index and Metabolic 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 metabolic level perturbation in the process media, such as occurs when changes in the bioprocess is feeding. The system is applicable to all stages of operation from process development to commercial production and is compatible with Sf9 cells. 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 analyzer’s response is the product of its concentration multiplied by its RI. The overall instrumental response is the sum of the contributions from all analytes in solution in the media above their individual limit of detection. A Process Variable named PTI (Process Trend Index) is essentially the derivative of PTI (Eqs 1,2).

**Process Monitoring**

Process monitoring is used to develop control limits for the nutrient feed rate and to proceed feedings on demand. The Ranger System is used to develop a control limit strategy for monitoring the metabolic trajectory of a bioprocess cycle in statistical analysis. 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 process 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

**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 System**

Ranger manages in situ data for monitoring process kinetics and media molecular composition which in conjunction with its software suite permits direct opportunities for control and automation of nutrient feeding. The Ranger Ranger 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 or a stand alone nutrient feed controller, as an ancillary to a RCS system, or integrated into a full Process Control System (PCS). The technology is compatible with Sf9 cells.

**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 process to the variable feed rate. 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 profitably utilize the system at this stage. The Rangers can be used to develop a control limit strategy for monitoring the metabolic trajectory of a bioprocess cycle in statistical analysis. 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 process 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 model for simplified, automated 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.

**Conclusion**

The Ranger automated reactor feeding system has been shown to be effective 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 equates feeding glucose and protein to the variable feed rate and maintains an Suitable feeding schedule in the process vessel.

**Acknowledgement**

This research work was performed by Pall Corporation, Portsmouth, UK. Strathphage gratefully acknowledges and thanks Pall for their permission to use this data.