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Using Information Technology to Enhance Upstream Productivity

Two case studies show how advanced information technologies make process development more efficient.

KARL RIX, MARTIN GROLMMS

ABSTRACT

Information technology helps integrate Quality by Design and process analytical technology into standard laboratory procedures and increase efficiency in process and product development. This article presents two case studies in which applying novel information technologies for an advanced bioreactor system improved process development. In the first study, users of a four-fold bioreactor system achieved a quick way to the optimized process and increased product yield nearly 10-fold. In the second study, seamless integration of analytical data allowed for implementation of predictive model control and comprehensive process automation. Along with the increased product quality, the number of experiments was significantly reduced.

In a market where a laboratory must be extremely cost-efficient and judicious with its time, laboratory members are always seeking research tools that will give them the edge they need for market leadership. Additionally, regulatory requirements set through the US Food and Drug Administration have recently become much more extensive. FDA initiatives such as Quality by Design (QbD) and process analytical technology (PAT) have proved to be an additional obstacle to overcome for some. However, these

KARL RIX, PHD, is the director of North American operations and MARTIN GROLMMS is a technical editor in marketing and communications, both at DASGIP AG, Jülich, Germany, +49 (0)2461 980 121, m.grolms@dasgip.de.

initiatives have driven other companies to speed up their process development. These players thus benefit from the FDA's efforts to strengthen product quality control.

Following the QbD approach, the FDA promotes PAT as a system for designing, analyzing, and controlling manufacturing as a means of ensuring final product quality and consistency. The PAT concept demands that product quality can be achieved by design through a comprehensive understanding of the product and process risks, and with knowledge of how best to mitigate those risks. The benefits of these systems include improved product quality and efficiency, reduced production costs, and prevention of rejects and reprocessing. These improvements assure that the quality of the product is consistent through processes that are thoroughly developed and documented. Finally, a significant amount of labor and time can be saved and operator safety is increased because of the increased automation during the manufacturing process.

Because of these new requirements, the amount of data generated has grown immensely. With this increase in data, bench-top bioreactor control systems that integrate new technological approaches to gather, document, and manage large amounts of data have become crucial for successful process development.

The following two case studies demonstrate how cell culture and fermentation specialists meet key challenges in today's process development laboratories by applying information technologies of an advanced bioreactor system.

Richter Helm Biologics Consistent Parallel Processing

When developing bioprocesses using a systematic parallel

Table 1. Key parameters that influence target protein expression

Parameter	Variation	Parameter	Variation
Medium ^{1,2}	Mineral	Induction ^{2,3,4,5}	Induction temperature
	Semi-defined		Induction timeframe
	Complex		Inductor concentration
	Trace elements		Inductor addition
	Amino acids	pH ^{4,5}	Reference value
	Vitamins		pH profile
Feeding ^{1,5}	Linear profile	Temperature ^{2,4}	Reference value
	Exponential profile		Temperature profile
	Combined exponential and linear profile	pO ₂ ⁵	Reference value

approach keeps time, materials and effort to a minimum while providing reproducible data, it optimizes results.

During the development of an upstream drug substance process using *Escherichia coli*, researchers at Richter-Helm Biologics carried out extensive parameter screening using a four-vessel parallel approach. This parallel approach led to a significant increase in productivity yields at the 1-L scale. The results were obtained with a recombinant *E. coli* BL21 strain that carried a heat-inducible expression plasmid constructed by Richter-Helm Biologics. The target protein involved a human active pharmaceutical ingredient (API) that is primarily expressed in inclusion bodies (IB) as a fusion protein.

In the initial step, a three-phase process was developed; the relevant parameters were automatically controlled. The DASGIP Control 4.0 software gathered and visualized the data during the cultivation process and stored it with the offline data in a central database. Richter-Helm Biologics used a generic fed-batch cultivation strategy as a starting

point for the development of the upstream process.

In the second step, researchers examined the key parameters for target protein expression as described in relevant literature (Table 1). The influence of various parameters on protein expression was compared in a total of 26 cultivations at 1-L scale. The studied parameters are highlighted in gray in the table. The DASGIP data management system supported and simplified the analysis and interpretation of the process-relevant information.

A significant amount of time and labor can be saved because of increased automation during the manufacturing process.

The strain that was used in the study exhibited excellent protein expression in the first four to six hours after induction. Afterwards, no notable target

Figure 1. Protein production kinetics at various temperatures. C_{XL} = dry biomass; C_{P1L} = target protein concentration; θ_{Ind} = induction temperature.

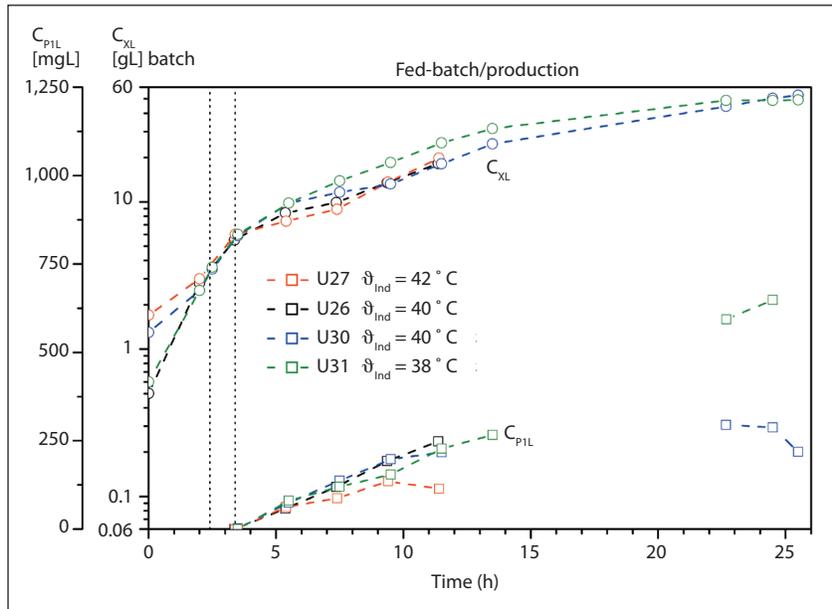
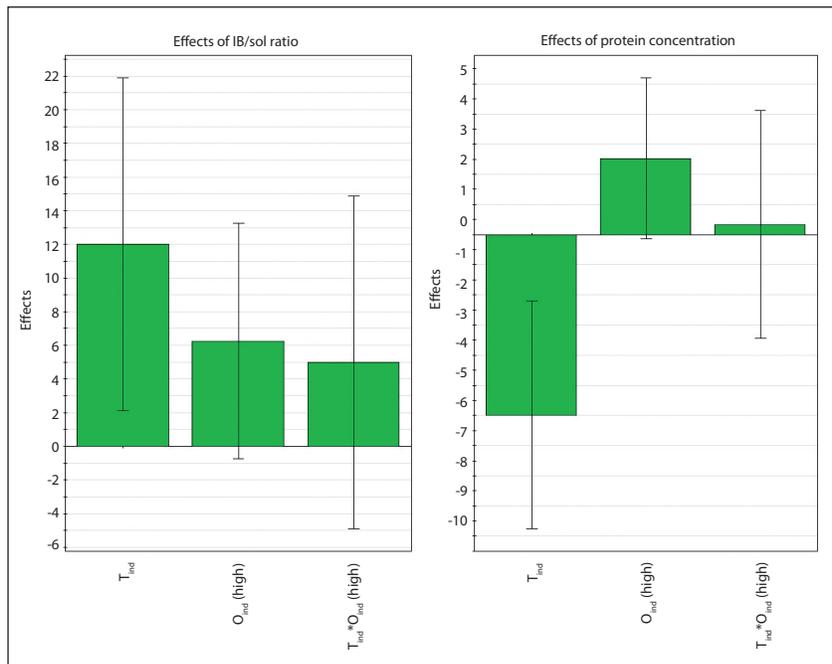


Figure 2. Effect of the induction temperature (T_{Ind}) and optical density at induction (O_{ind}) start on the IB/sol-ratio and target protein concentration



protein formation was observed. The researchers speculated that factors affecting continued expression were inadequate for growth rate caused by the linear feeding profile, genetic instability, or missing medium additives. Genetic instability was eliminated

as a possible cause with the help of subsequent tests. Product yields were slightly improved to ~1.5 g/L by adding peptone to the feeding medium and by means of exponential feeding at a growth rate of $\mu_W = 0.2 \text{ h}^{-1}$. However, significant protein expression occurred only

during the first few hours after induction.

Open communication standards allow the integration of bioreactor systems into so-called historians.

In the third step, various expression temperatures and higher cell densities were examined with a parallel approach to reduce cell stress caused by the high induction temperature and high expression yield. The experiments were planned applying the principles of Design of Experiments (DOE), which is supported by DASGIP Control 4.0. The input parameters were defined as induction temperature and optical density at induction start. The specified target parameters were product yield and the ratio of insoluble to soluble protein expression (IB/sol-ratio).

The results of the parallel approach are illustrated in Figure 1. All four of the parallel fermenters used identical medium and feeding profiles. Compared to an induction temperature of 42 °C, these results showed that the product expression phase could be slightly prolonged at 40 °C. By further reducing the induction temperature to 38 °C, target protein formation was sustained with the same high expression yield during the entire process, thus significantly improving the product titer to 4.5 g/L.

A subsequent induction of the cultures at five hours after ini-

tial feeding, combined with an induction temperature of 38 °C, further raised the end concentration of the target protein to 7.5 g/L. Figure 2 shows the influence of induction temperature and cell density using statistical test planning. These results show that a low induction temperature in conjunction with a high cell density yields the optimum product titer.

The researchers were able to increase the product concentration to 11 g/L by varying the peptone concentration and prolonging the expression phase. The process was then successfully scaled to 10 L to yield a product concentration of >20 g/L through improved oxygen input and the prolonged expression phase.

“By implementing parallel processing and using identical start conditions for the same precultures, we were able to carry out fast and efficient parameter screening. Simple automation of the fermentation processes ensured the comparability and reproducibility of the cultivations,” explains Christian Kaiser from Hamburg-based Richter-Helm Biologics. “The DASGIP system combines a wide range of centrally monitored and controlled parameters with numerous parallel reactors. As a result, we experienced a near 10-fold increase in the product yield, from 1.2 to 11 g/L,” Kaiser adds.

University of Delaware Integrating Analytical Data

The critical product quality attributes must be defined and the effect of process variables on these specific attributes must be identified to implement PAT in bioprocess development. To obtain this type of information, the use of inline measurements

Table 2. Effects of culture conditions on glycosylation

Process variable	Effect on glycosylation
Low glucose conc.	Reduced glycan site occupancy ⁸
Low glutamine conc.	Decreased sialylation, increased hybrid and high mannose glycans ¹²
Ammonia accumulation	Reduced glycan site occupancy, ⁶ decreased terminal sialylation ¹³
pH	Variations in degree of galactosylation ¹⁰
Low temperature	Increased glycan site occupancy ⁷
Low dissolved oxygen	Reduced galactosylation levels ⁹
High agitation rate	Reduced glycan site occupancy ¹¹

and the integration of analytical data of the cultivation process is critical. However, a conventional bioreactor is limited to the control of dissolved oxygen (DO), pH, temperature, and agitation. A bioreactor generally does not have the capability to control all relevant analytes that can affect culture conditions and by extension, product quality. Hence, a bioreactor system integrating glucose and glutamine, lactate, NH⁴⁺, and biomass media concentration measurements is extremely helpful to increase product quality.

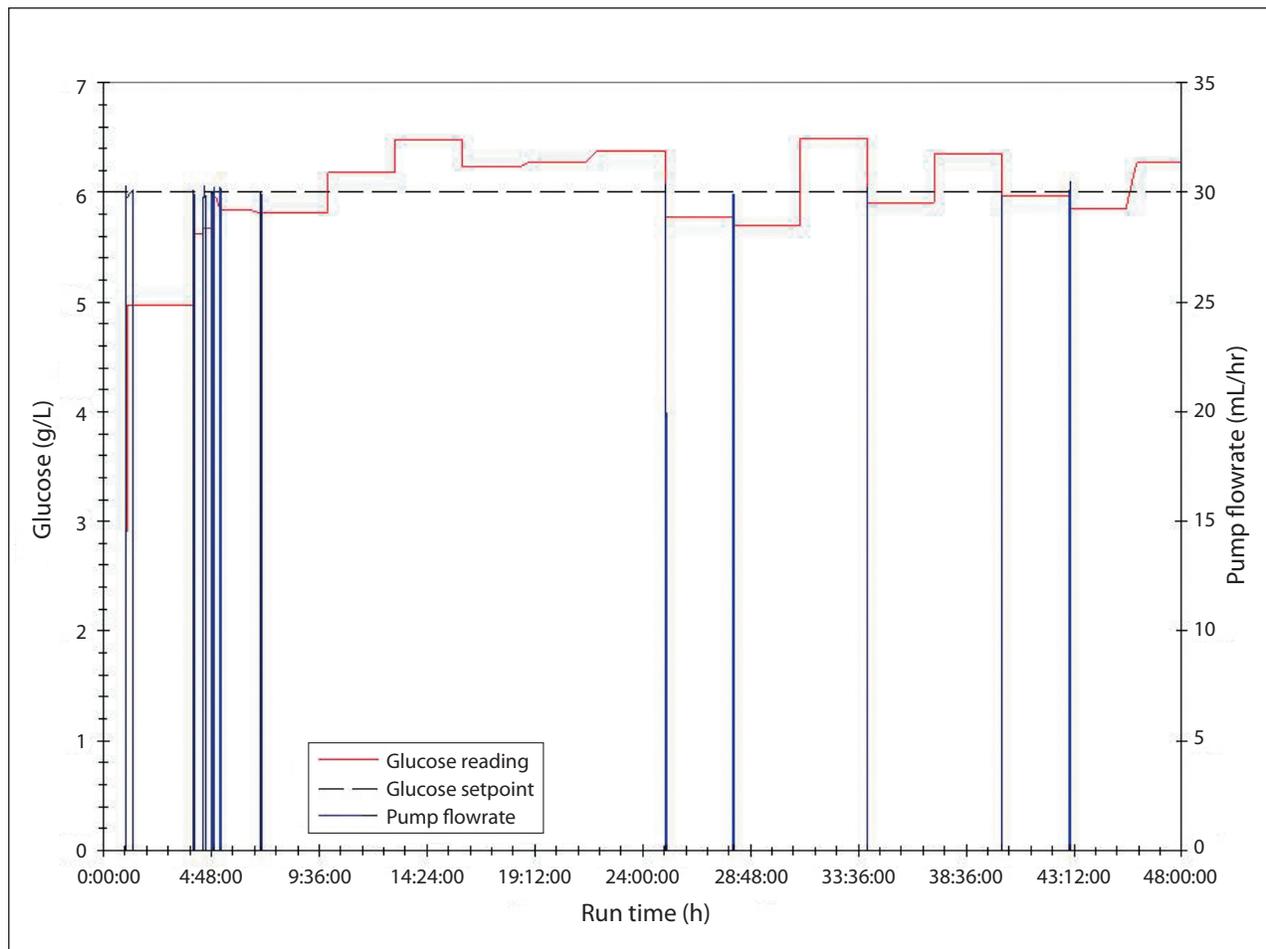
The outer glycosylation control loop involves model-based control through estimation.

At the University of Delaware, Babatunde A. Ogunnaike and his students have established the foundation for effective online, real-time control of glycosylation patterns on monoclonal antibodies produced with Chinese hamster ovary (CHO) cells. The control strategy, based on the premise that external culture

conditions are known to affect glycosylation patterns (Table 2), involves an inner loop for base regulatory control of the key process variables and an outer loop to maintain the glycosylation pattern at a desired set-point. Unfortunately, with current technology, glycosylation measurements are not available frequently enough for traditional feedback loops. Therefore, the outer glycosylation control loop involves model-based control through estimation.

For establishing base regulatory control of the key process variables known to affect glycosylation, they designed a bioreactor system with nutrient control and cellular metabolite monitoring in addition to typical bioreactor measurements. With this system, eight analytes (pH, Glutamate, Glutamine, Glucose, Lactate, N⁺, K⁺, NH⁴⁺) are measured with a Bioprofile 100+ bioanalyzer with autosampler (Nova Biomedical, Waltham, MA) that is equipped with object linking and embedding for process control (OPC) software. With the OPC connection, the Nova Bioprofile 100+ was integrated with the DASGIP Bioreactor Control Software, which allows for closed loop control to be implemented for glucose (Figure 3) and glutamine

Figure 3. At-line glucose reading and the resulting pump flow rate to achieve a closed loop control according to the defined set point



concentrations in the media. A multi-scale model using process variables to predict glycosylation patterns was developed to establish the outer glycosylation control loop. The controlled inputs of the model include glucose media concentration, glutamine media concentration, DO, pH, temperature, and agitation rate. Specific uptake or excretion rates of glucose, glutamine, lactate, and ammonia were computed by linear regression with biomass. This model allows for model predictive control and targeted modification of process variables.

"This research has shown how a bioanalyzer equipped with an autosampler was integrated with

Liquid chromatography coupled to mass spectroscopy will be used to characterize glycan micro-heterogeneity offline.

the bioreactor system through OPC. This integration is advantageous because it allows for the implementation of feedback control and at-line monitoring of the nutrients and metabolites," said Ogunnaike. "The validated DASGIP/Nova

reactor system demonstrated the successful control of glucose and glutamine media concentrations. With this system in place, we have established base regulatory control of the key process variables known to affect glycosylation, which is the first step in our strategy to achieve online glycosylation control," he added

In the future, Ogunnaike and his students propose to characterize glycosylation macro-heterogeneity at-line by adding Groton Biosystem's automated reactor sampling system with Agilent's 2D HPLC to the current bioreactor system. In addition, liquid chromatography coupled to mass spectroscopy will be used

to characterize glycan micro-heterogeneity offline.

Open communication standards allow the integration of bioreactor systems into so-called historians.

Conclusion

The case studies presented here are only two examples showing how information technology can help to increase efficiency in process and product development by the integration of Quality by Design and process analytical technology into standard laboratory procedures. Today's research and development laboratories face more challenges in an increasingly competitive worldwide market.

The generation of larger and larger amounts of data often results in huge data graveyards. By using the capabilities of extended data mining software, one can simply link the process data with user-defined attributes such as the strain or cell line with the media composition, the controller set-points, and feeding profiles as well as with achieved product yields or viable cell densities. Thus, raw process data turns into usable research information.

The next challenge is to keep this knowledge available in the long run. Open communication standards not only allow the integration of analyzers into bioreactor systems but also the

integration of bioreactor systems into so-called historians. These long-term archives save all process relevant information beyond the single laboratory and can easily be accessed by supervisory control systems globally. Gaining more flexibility in online monitoring and control of the bio-process is a matter of efficiency. Supervisory control systems and remote control through iPhone, iPad, or any other server provide bench-top scientists as well as laboratory and plant managers with multiple ways to control the running processes online and from any location.

In conclusion, to meet today's challenges, process development and information technology must work in tandem to create the most beneficial and efficient outcome for their laboratory.

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