Continuous Monitoring and Control of Glucose and Lactate with MAVEN: Impact on Cell Culture Performance and Protein Quality

Introduction

Glucose and lactate are critical process parameters in bioprocessing; glucose as the main nutrient fuelling growth and production, and lactate a key metabolite.[1] Measuring these parameters is part of every bioprocess run, but often the measurements are limited to daily sampling, leaving much granularity of the bioprocess uncovered. Monitoring glucose and lactate automatically, in real-time, supports bioprocess characterization with enhanced process understanding and enables proactive management of cell culture.

Here we describe the monitoring of glucose and lactate using MAVEN, an on-line, biosensor-based device with an *in-situ* sample probe and real-time measurements. In both experiments, we evaluated effects of feeding strategies (bolus vs. continuous) on multiple critical process parameters and glycosylation profiles of the expressed mAb. In all bioreactors, the chemically defined nutrient feed also contained glucose.

Table 1 features descriptions of two distinct bioreactor runs. In one, the implementation of MAVEN-driven monitoring for glucose and lactate allowed for the seamless operation of the bioreactor in accordance with the experimental design, and prevented cell cultures from crashing. In the second run, an enhancement in the product quality profile was achieved through the utilization of MAVEN for the low-level glucose feed control.

Controlling glucose levels with continuous feeding at a set-point, instead of having large variations of glucose levels with daily bolus feeding, has been shown to improve cell culture lactate profiles, cell viability, and product quality in mAbs[1-3]. Regardless of the preferred feeding method, MAVEN provides unprecedented levels of insight and control of feeding a bioprocess.

Experimental Conditions

A full description of the bioprocess feed strategy optimization study using the REBEL analyzer and MAVEN is available[4], which includes Bioreactor Run #1. More details on the Bioreactor Run #2 is available in[5].

Results and Discussion

MAVEN monitoring empowers bioengineers to have control over their process (Run #1)

The experiment focused on CHO cell culture feeding strategy optimization[4]. The addition of a mixture of essential amino acids to the nutrient CD feed scaled up from Ambr15 to Ambr250 and 10L bioreactor scale (see Ref 4). In addition, at the 10 L scale addition of tryptophan further increased titer by 6% (Figure 1A). All amino acid measurements were performed with 908 Devices REBEL, and amino acid concentrations to add were calculated from REBEL data-derived consumption rates.

Condition	Bioreactor Run #1[4]	Bioreactor Run #2[5]
Producer cell line	Monoclonal antibody-expressing GS-CHO cell line	
Cell culture media	Gibco CD CHO Media Panel, Medium 6 (Thermo Fisher Scientific)	Gibco CD FortiCHO medium (Thermo Fisher Scientific)
Nutrient CD feed	Efficient Feed C+ (Thermo Fisher Scientific) Starting day 3, bi-daily bolus	
Additional amino acid bolus	Mix of essential amino acids, one bioreactor without tryptophan, two with tryptophan included	Not applicable
CD feed level	0.5 x feed concentration, pyramid feeding, up to 4% v/v additions	1x feed concentration 4% v/v
Glucose feed	• BR1: Continuous glucose feeding with a set-point of 2 g/L using MAVEN control • BR2 and 3: Bolus feeding: bolus feeding to 4g/L with trigger at 2 g/L (2 to 4 g/L) based on at-line glucose measurements	• BR A and B: Continuous glucose feeding with a set-point of 1 (BR A) or 2 g/L (BR B) using MAVEN control • BR C: Bolus feeding: to 4g/L with trigger at 2 g/L (2 to 4 g/L) based on at-line glucose measurements
Bioreactors	Three Biostat 10L stirred tank bioreactors (Sartorius Biostat B-DCU II with BioPAT DCU Tower).	
Bioreactor volume and seeding	Seeding at 0.3 million cells /mL in 7.9L of media	
Bioreactor run control	14-day fed-batch cell culture at 37°C; dissolved oxygen set point was 30%; the pH set point 6.9 with a deadband of 0.03	
On-line Analytics	MAVEN (908 Devices) real-time measurement of glucose and lactate in all bioreactors. Glucose and lactate monitoring was achieved by measurements every 20 minutes. This frequency is recommended for a successful continuous glucose-feeding strategy.	
At-line / Off-line analytics	• Amino acids: REBEL (908 Devices) • Titer: Octet Red 384 (Sartorius) • Cell density and viability: Vi-CELL (Beckman Coulter) • Cell culture metabolites (glucose, lactate, ammonia, LDH, Gln, Glu): Cedex (Roche) • Product Glycosylation with CE-LIF, LabChip GXII Touch HT Protein Characterization System (Perkin Elmer)	

Table 1. Conditions used in the two different bioreactor runs.

At the 10L scale, continuous glucose feeding was added to the feed strategy optimization. The continuous glucose feeding strategy, even when keeping the glucose at a low level of 1 g/L, did not have an adverse effect on the growth, or protein production (Figure 1A for titer, 1B for viable cell density, and 1C for viability in all three bioreactors for Run #1). The addition of tryptophan to the amino acid mix in bioreactors 1 and 3 of run #1 led to a 6% increase in titer. The low (1g/L) continuous glucose feeding in bioreactor 1 had no adverse effect on the titer as compared to the more standard bolus feeding with higher levels of glucose (4g/L).

On day 7, the glucose levels obtained from the at-line analysis were just above 2 g/L in bioreactors 2 and 3 (Figure 2) and therefore no glucose bolus was added to these bioreactos, as per the process described in Table 1. The addition of the nutrient CD feed on day 7 brought the glucose level only to just above 3g/L. Since the at-line glucose readings were close to the trigger point, we used data from MAVEN devices connected to bioreactors 2 and 3 to calculate the glucose consumption rate and the time when glucose would fall to zero. By using the MAVEN continuous data and derived prediction of glucose depletion instead of the single daily at-line value, we adjusted the timing of the bolus feeding to a slightly earlier time point, and avoided a culture crash. With this information, the lab personnel were able to operate the bioreactor confidently, and no extra

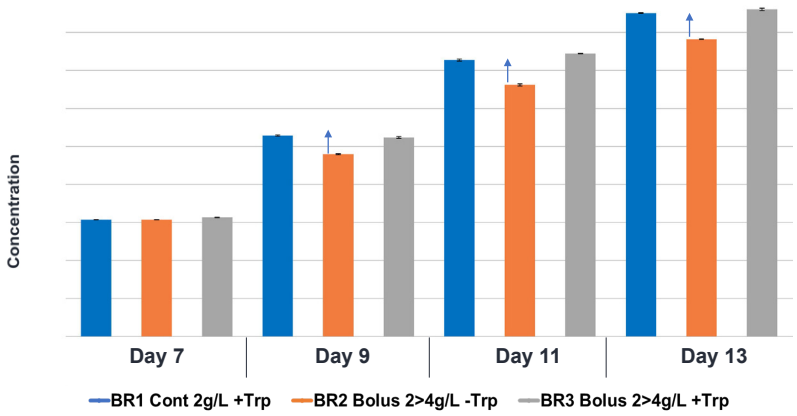

Figure 1A: Run #1 - Titer

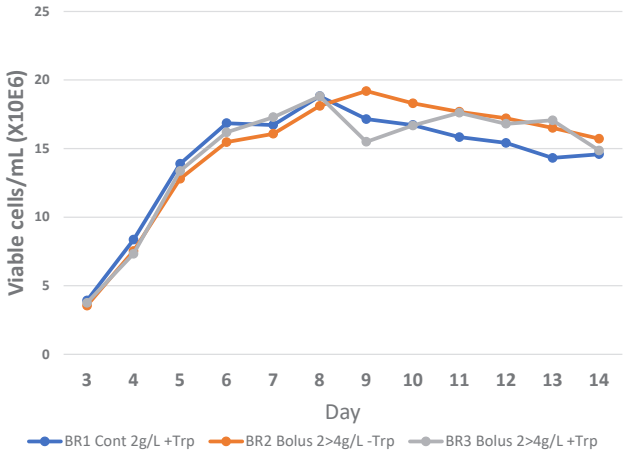

Figure 1B: Run #1 - VDC

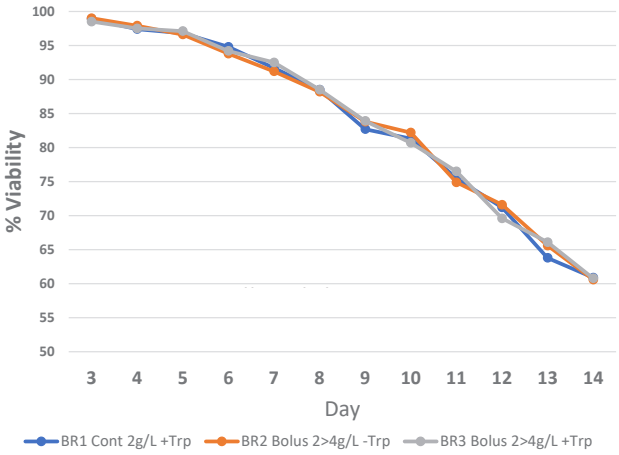

Figure 1C: Run #1 - Cell Viability

Figure 1A. Bioreactor Run #1 mAb titer at 10L scale in the three different feeding strategies used as described in Table 1.
Figure 1B. Viable cell densities (VCD) and 1C. Viabilities for the three bioreactors of Run #1.

work was needed for sample pulls, or analysis, as the MAVEN measurements are done with an in-situ probe and readings provided in real-time. This experiment also demonstrated the benefit of using a continuous glucose feeding strategy instead of a standard bolus glucose feeding strategy. The continuous glucose feeding strategy avoids potential depletion of glucose and culture crash.

MAVEN real-time monitoring of lactate revealed a shift in the lactate profile in bioreactor 2, where tryptophan was not added to the amino acid mix (Figure 3). This may indicate that the cells were shifting in some metabolic pathways to cope differently with the lower availability of an essential amino acid, as this shift happened after the tryptophan had dropped below 30% from the starting condition in bioreactor 2. Observing this shift was enabled by monitoring lactate levels real-time with MAVEN, without real-time monitoring, this shift most likely would not have been noticed.

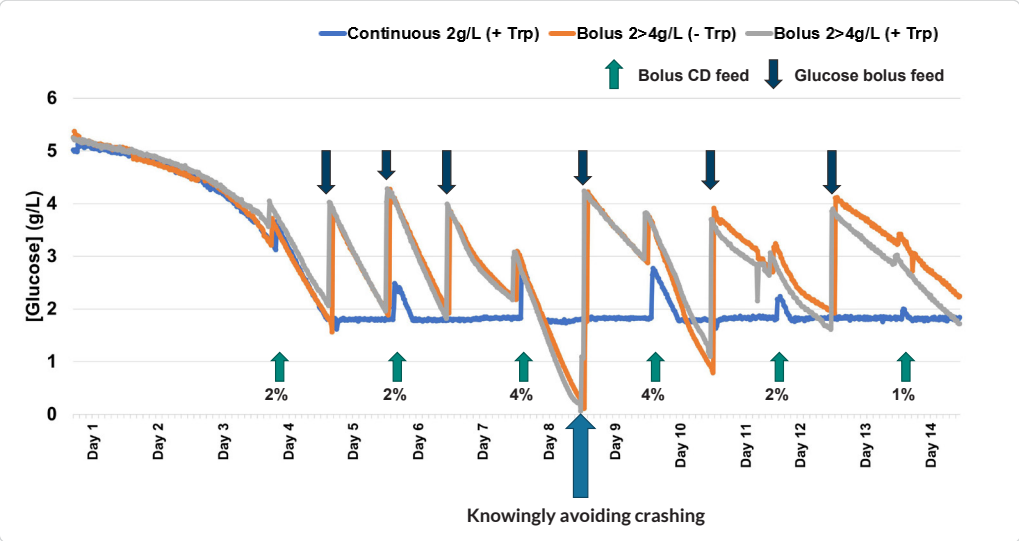

Figure 2. Glucose measurements using the MAVEN in Run #1: bioreactor 1 (continuous 2g/L glucose), bioreactor 2 (bolus 2 to 4 g/L glucose (-Trp) and bioreactor 3 (bolus 2 to 4 g/L glucose (+Trp). On day 8 the glucose levels dropped close to zero in bioreactors 2 and 3. A potential cell culture crash was avoided by monitoring the glucose levels closely with the MAVEN real-time measurements and adjustment of glucose feeding time.

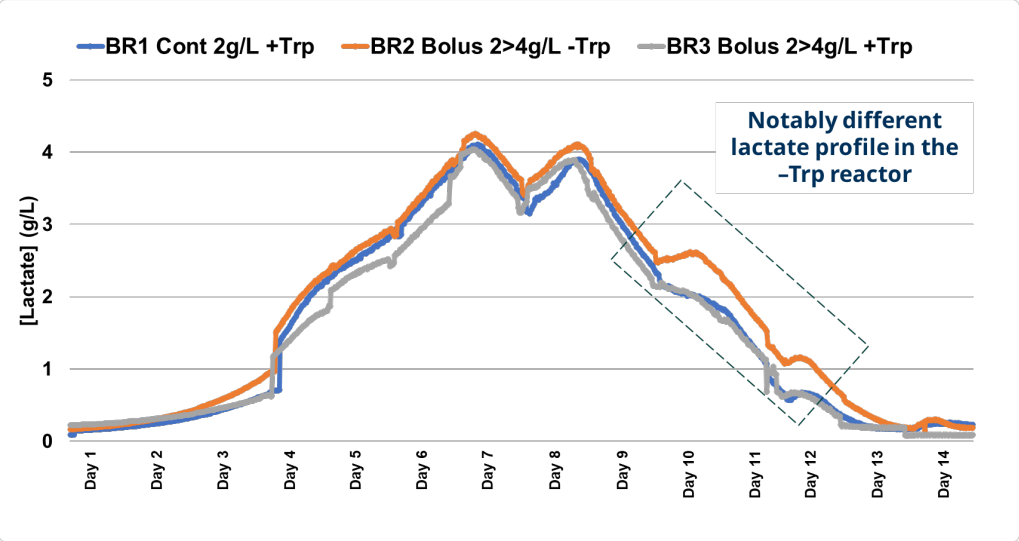

Figure 3. Lactate profiles in Run #1 for all three bioreactors. The real-time measurements showed a shift in the days 9-13 for bioreactor 2, which was no was not fed extra tryptophan in the amino acid mix.

Continuous, low-level glucose feed improves product quality attribute profile (Bioreactor Run #2)

In Bioreactor Run #2, the same cell line and bioreactors process were used as in Bioreactor Run #1, but with a difference basal medium, CD FortiCHO, and Efficient feed C (without supplementation of amino acids; see Table 1 for run conditions)[5]. We observed differences in product glycosylation patterns between bioreactors with continuous, low-level glucose feed and bioreactors with bolus glucose feed (control feeding strategy).

In this study, we found that the end-product glycosylation Man5 proportion was lower (from 15% to 8.7%; about 40% reduction) in the continuous glucose feed (BR A - at set-point 1 g/L), than in the glucose bolus-fed bioreactor (BR C) products (Figure 4). mAbs produced in CHO cells, in certain conditions, may contain high levels of high-mannose (mannose-5) glycans, which can affect efficacy, pharmacokinetics, and stability[6]. MAVEN was therefore used to implement a continuous glucose feeding strategy that resulted in an improved protein quality attribute profile.

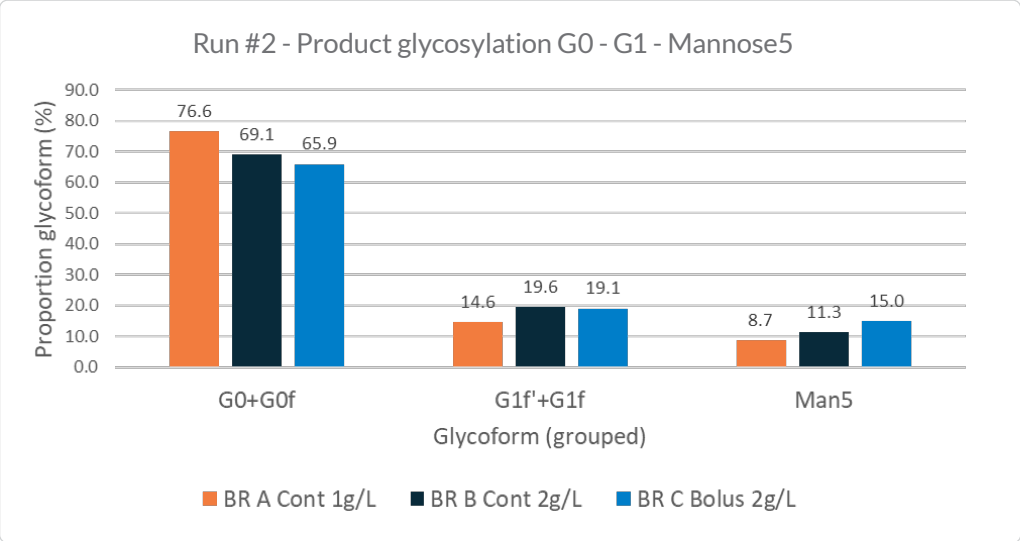

Figure 4. Run #2 - Glycosylation profiles from mAbs produced in three CHO bioreactor runs. BR A and BR B were run with two different continuous feeding set points (1g/L and 2 g/L) and BR C was run with a bolus feeding strategy. The mannose-5 levels in the mAb glycosylation pattern were lower in bioreactor A with a continuous glucose feeding strategy than in bioreactor C with traditional bolus glucose feeding.

Conclusion

The approach of continuous real-time monitoring of glucose and lactate and dynamic glucose feed control to a low-level set-point leads to improved process and product parameters. MAVEN enables easy implementation of glucose and lactate monitoring and control.

Importantly, MAVEN enabled saving the two bolus-fed bioreactors from crashing (a problem that would also have been avoided in a continuous feeding strategy) and supported continuous feeding at a low level that produced the same growth profiles as seen in the bolus-glucose feeding process.

MAVEN-controlled glucose levels in a stable low-level glucose feeding strategy showed improved product quality attributes by lowering the proportion of mannose-5 in the mAb glycosylation pattern.

References

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