Low Glucose Cultivation of CHO cells by Advanced Control System, BR1000

Low glucose cell cultivation (eg. ~1 g/L) for biopharmaceutical production requires frequent sampling and frequent glucose supplementation. The excessive sampling requirement not only reduces the volume of the culture medium, but also is an unnecessary burden on the operators when performed manually. Here, we demonstrated that the Advanced Control Bioreactor System BR1000 can effectively automate mammalian cell cultivation under low glucose conditions.

The BR1000 system controls glucose concentration using model predictive control software architecture. The software algorithms, predicts glucose consumption by leveraging the combined data from direct glucose in-line measurements obtained by near-infrared (NIR) spectroscopy, and from viable cell density data obtained by bio-capacitance measurements.

The BR1000 routinely updates the calibration model using the spectrum data from cultivation to improve the accuracy of glucose monitoring and glucose concentration control. This application note shows that the BR1000 can effectively operate under low glucose cultivation (1 g/L) conditions with CHO cells.

Validation of low glucose cultivation with CHO cells

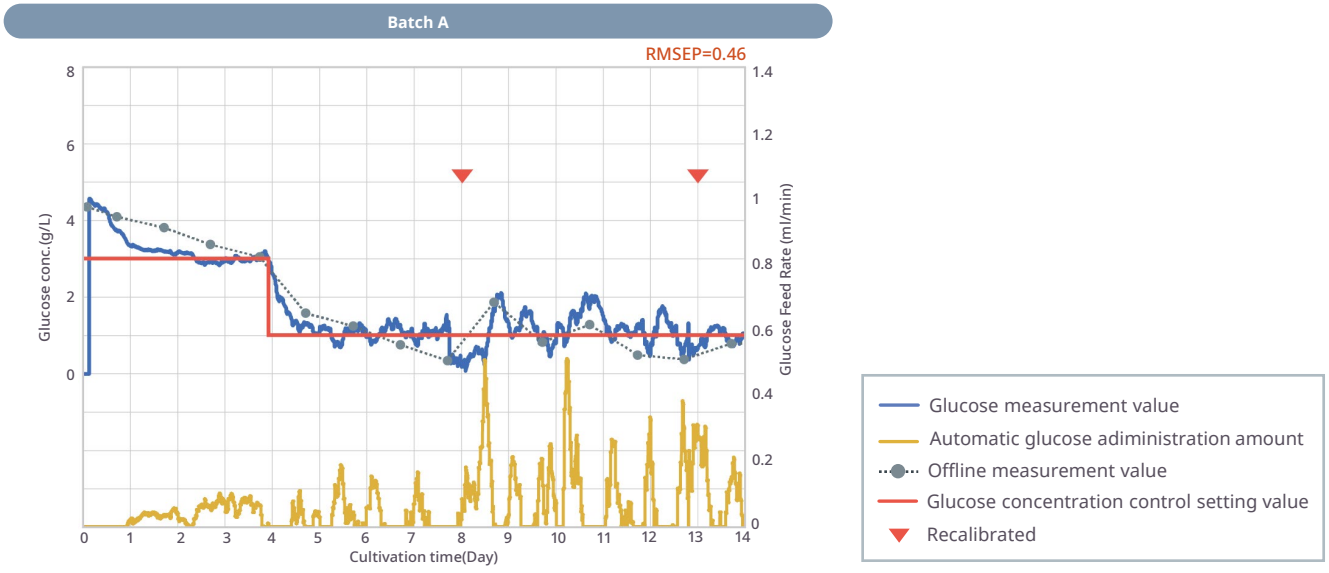
Fed-batch culture was carried out with BalanCD CHO Growth A medium and glucose-containing feed medium. The glucose concentration of the feed medium was 28 g/L. The control of glucose concentration with high accuracy is realizad by creating calibration models based on the culture data recorded from sequential batches. For example, in this experiment, we cultivated Batch B with an updated calibration model derived from the spectral data of Batch A. And a high accurate(1 g/L) low glucose control was ultimately achieved, by likewise employing yet another updated calibration model, derived from the spectral data of Batch B.

Cultivation batch used for calibration model development

The following is the culture data used for the calibration model.

Batch A

The following figure shows the cell culture data used for creating the first calibration model.
Batch A was initially controlled with a default program model provided with the BR1000.
We set-up cultivation by programming a desired stable glucose concentration of 3 g/L, then switched to 1 g/L at day4, and cultured until day 14.The initial glucose concentration used in the media was purposely higher at 4 g/L to force the predictive control to function immediately. Sampling was then conducted once a day for 14 days. When culturing at a control setting of 1 g/L, the sampled material showed off-line values ranging from 0.3 to 1.9 g/L. Hence, the RMSEP(Root-Mean-Square Error of Prediction) of the in-line sensor measurements versus actual off-line glucose measurements was calculated to be 0.46.



Validation of cultivation at low glucose concentration

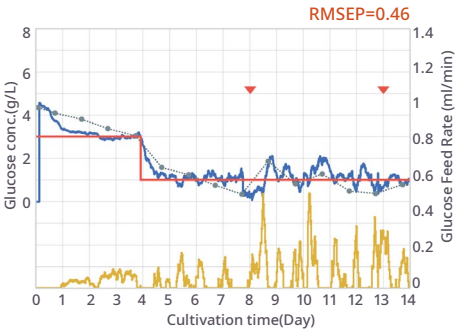
Batch B

We derived a calibration model for Batch B by using the spectrum data of Batch A. The culture of Batch B was carried out under the same conditions as Batch A. Glucose concentration was kept at 3 g/L initially and shifted to 1 g/L after day 4. Sampling was carried out once per day for the 3 g/L control and twice per day for the 1 g/L control. To improve the calibration model for Batch B, we used a proprietary software tool to recalibrate the inline measurements with the offline measurements. This is usually performed when the inline measurements of glucose concentration deviate more than desirable from the offline measurements. As a result, the deviation of offline measured values during 1g /L controlled culture stayed within the range of 0.8 to 1.8 g/L, had an RMSEP value of 0.24, and resulted in more accurate control than was achieved with Batch A.

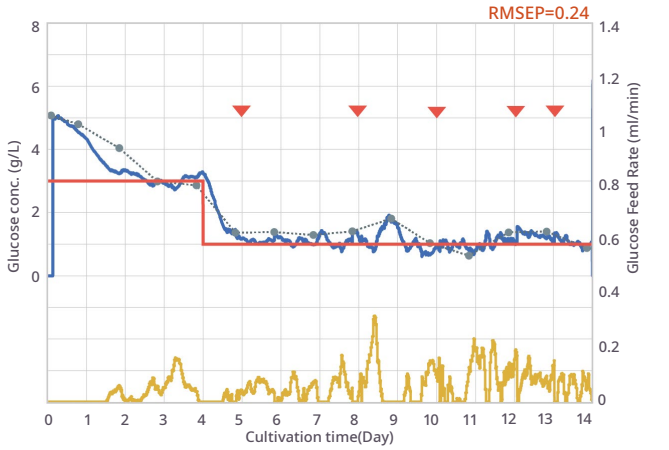
Batch C

We then performed an additional 3rd cultivation (Batch C) with a new calibration model made from the final spectrum data of Batch B, in the same way as before.The culture conditions used were identical to Batch A and Batch B. In Batch C, sampling was only done once per day. The deviation of the offline measurements to the in-line sensor measurements stayed within a range of 1.1 to 1.8 g/L , giving an RMSEP value was 0.39. These data indicated that the Batch C was a the most highly controlled and completely automated cultivation. Although model recalibration and additional batches could be performed, we considered this unnecessary due to the highly consistent correlation of the in-line sensor measurements with the off-line reference assays.

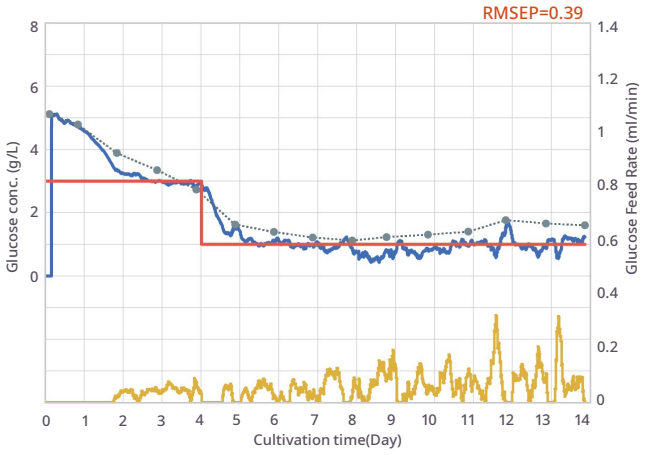
Batch A



Batch B



Batch C



Cultivation criteria

- Cells: IgG-producing CHO cells (K1SP)
- Cell density at the beginning of culture: 5.0x10^5 cells/mL
- Medium: Balan CD CHO Growth A (1.4L)
- Feed agent: Balan CD CHO Feed 2
- pH: 6.97 to 7.02
- Dissolved oxygen content: 4.0 mg/m
- Temperature 37℃

Analytical equipment used for offline measurements

- Analysis of culture medium components
  Cedex Bio; Roche, (631-24711)
- Viable cell density analysis
  Vi-Cell XR; BECKMAN COULTER, (383721)

- Glucose measurement value
- Automatic glucose adiministration amount
- Offline measurement value
- Glucose concentration control setting value
- Recalibrated

The advanced control of the BR1000 enables automated cell cultivation at low glucose concentrations with high accuracy, which is challenging to perform manually.
The high-precision automated control also prevents accidental glucose depletion and reduces or eliminates the time and effort required to monitor and manage daily glucose nutrient requirements for mammalian cell culture.

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