Process development and optimization using the ReadyToProcess WAVE 25 bioreactor system in dual mode

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The single-use ReadyToProcess WAVE 25 bioreactor system allows for automation of cell culture feed supplementation. The system can be operated in single culture mode as well as in dual culture mode, where two parallel cultures are controlled independently. In this work, the technical feasibility of the *Method editor* and operations in dual mode are demonstrated. To exemplify the capabilities of these system features, four different feeding strategies—bolus, semi-continuous, continuous, and perfusion—were used.

Introduction

An optimal cell culture process is the first step to successful biomanufacturing. Determination of appropriate feeding strategy based on the nutritional requirements of a specific cell line is important to avoid over or under feeding of the culture. Additionally, product quality attributes, such as distribution of change variants of the target product, should be considered when selecting a feeding strategy, because these quality attributes can vary significantly based on the feeding strategy selected (1). However, process development and optimization can be a time-consuming and labor-intensive process.

ReadyToProcess WAVE 25, installed with the UNICORN™ system control software, exhibits many features that facilitate process development and optimization (2). Methods are easily created in the ***Method editor*** of the UNICORN software. To simplify the method creation process, an existing method can also be changed, and individual changes can be saved for later use on systems having the same instrument and component configuration. The bioreactor system can be operated in single culture mode as well as in dual culture mode, where culturing is performed in two separate Cellbag™ bioreactors simultaneously, although controlled independently. These system features can significantly reduce labor-intensive and time-consuming process development and optimization activities. Reports can be customized, saved, and printed.

In addition, ReadyToProcess WAVE 25 features time-saving automation technologies that help liberate time for other tasks. The scope of this work was to exemplify the technical feasibility of the ***Method editor*** and the dual functionality of the bioreactor system. For this, two methods using the dual culture functionality were created to demonstrate four different feeding strategies. Settings were selected to simulate actual culture conditions.

Materials and methods

Design of automated feeding schedules

Creation of the automated feeding schedules was conducted in the ***Method editor*** of the UNICORN 7.01 software, taking advantage of the dual functionality of ReadyToProcess WAVE 25. The first method for automated feeding comprises the bolus and semi-continuous strategies and the second method comprises the continuous strategy as well as the perfusion strategy, including an exponential growth phase feeding.

Calibration of pump heads and preparation of bags

All pump heads used for feeding were carefully calibrated at the flow or speed and the tubing that they were to be used with. For the lower flows (< 20 mL/h), maximum time (60 min) was used for calibration.

For the first method (bolus and semi-continuous), two 10 L Cellbag bioreactor bags (CB0010L10-31) were installed on a Tray 20 and the created method was started. For the second method (continuous and perfusion), two other 10 L Cellbag bioreactor bags (CB0010L10-31 for continuous and CB0010L11-34 for perfusion) were installed on a separate Tray 20 before the created method was started. Connections were made, tubes primed, and scales tared, all according to the instructions given from the method created in the ***Method editor***. For bolus, semi-continuous, and continuous, the ***Media addition*** function in the method was used to fill the bags with 3 L liquid. For perfusion, the bioreactor bag was filled with 0.5 L liquid using the ***Media addition*** function prior to the start of the exponential increase in volume, and later, start of perfusion. For this demonstration, feeding was started on Day 0. The rocking was enabled to simulate a real cell culture process.

Testing

The four feeding strategies were tested in dual mode. For the first method, dual mode was used to test the bolus strategy (1 feed/24 h) in one bag, and in the parallel bag, the semi-continuous strategy (1 feed/1 h). Tests were performed by adding sterile water from two bottles (on scales) to each bag to simulate the two feeding strategies. The bottles were welded to the bioreactor bags via two addition ports on each bag. The bottles were used for feeding according to Table 1 for bolus and semi-continuous. For the continuous and perfusion feeding strategies, testing was also performed in dual mode in parallel bags in a similar manner according to Table 1 for continuous and Table 2 for perfusion.

Table 1. System parameters for bolus, semi-continuous, and continuous feeding strategies

Starting volume	3 L
Cellbag bioreactor bag	10 L
Medium	Water
Rocking speed	20 rpm
Feed solutions	Water
Feed addition	4% (continuous: 4.8%)
Feed rate	Start on day 0 for demonstration purpose 120 mL/d (continuous: 144 mL/day)

Table 2. System parameters for the perfusion strategy

Starting volume	0.5 L
Cellbag bioreactor	10 L
Medium	Water
Rocking speed	20 rpm
Feed solutions	Water
Process	1) Exponential increase: 0.5 to 3 L in 3 days 2) Perfusion 1 vessel volume/day (VVD) for 2 days

Each feeding strategy was performed over five days, after which the actual added feeding volumes (scale data) were compared with volumes given by the totalizer of the UNICORN software, the bag weight, and the theoretical values. Notes were taken daily of reduced weights of the feed bottles to validate the current feed rate. The methods were replicated at minimum in duplicate.

Results

A summary of the results for all setups are given in Table 3. Overall, automated feeding of 4% (or 4.8% for continuous) of the initial 3 L liquid volume in the bioreactor bag was shown to be successful for all three fed-batch strategies (Fig 1–3). Based on the actual feed volume added, a minimum feed rate of 120 mL/day is recommended, regardless of whether feeding is performed in bolus, semi-continuous, or continuous mode. Using the ReadyToProcess™ Pump 25 to automatically feed cultures with high accuracy, the lowest feed rate that can be recommended (if not recalibrating the pumps during the culture period) is 0.1 mL/min. Comparing given data from the system totalizer with scale data, the added volumes correspond well with each other.

Results from the perfusion strategy are shown in Figure 4. ReadyToProcess Pump 25 was used to increase the working volume exponentially for three days to simulate exponential cell growth phase of a culture process. The strategy was considered successful based on the comparison of actual added volumes (scale data) with theoretical values, volume given by the system totalizer, and the bag weight. The following two days, the bag volume was kept stable during the perfusion process.

Table 3. Summary of setups and results

Automated feeding strategy setup	Results
1. Bolus	
4% of 3 L culture volume (120 mL) fed 1/24 h (feeding duration 10 min) for 5 days resulting in a total voulme of 600 mL	Feed 120 mL/24 h for 5 days: 5% difference between theoretical value and measured scale data
2. Semi-continuous	
4% of 3 L culture volume (5 mL) fed 1/h (feeding duration 10 min) for 5 days resulting in a total voulme of 600 mL	Feed 5 mL/h for 5 days: 20% difference between theoretical value and measured scale
3. Continuous	
4.8% of 3 L culture volume (144 mL/day) continuous feed for five days with a flow of 0.1 mL/min resulting in a total volume of 720 mL	Continuous feed 0.1 mL/min for 5 days: 20%–25% difference between theoretical value and measured scale data
4. Perfusion	
Three days of exponential increase in culture volume followed by 2 days of perfusion with 1 bioreactor exchange/day (3 L) (1 VVD)	10%–12% difference between theoretical value and measured scale data following 5 days feeding

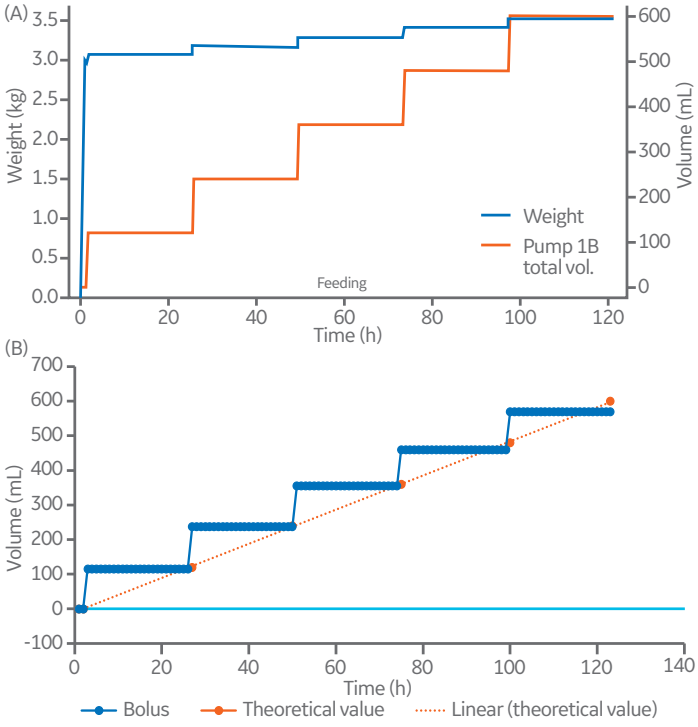

Fig 1. (A) Bag weight and pumped feed volumes for bolus feed additions (120 mL/24 h) over five days using pump 1B, resulting in a total feed volume of 600 mL. (B) Scale data presented against theoretical values for bolus feeding (120 mL/24 h) over five days.

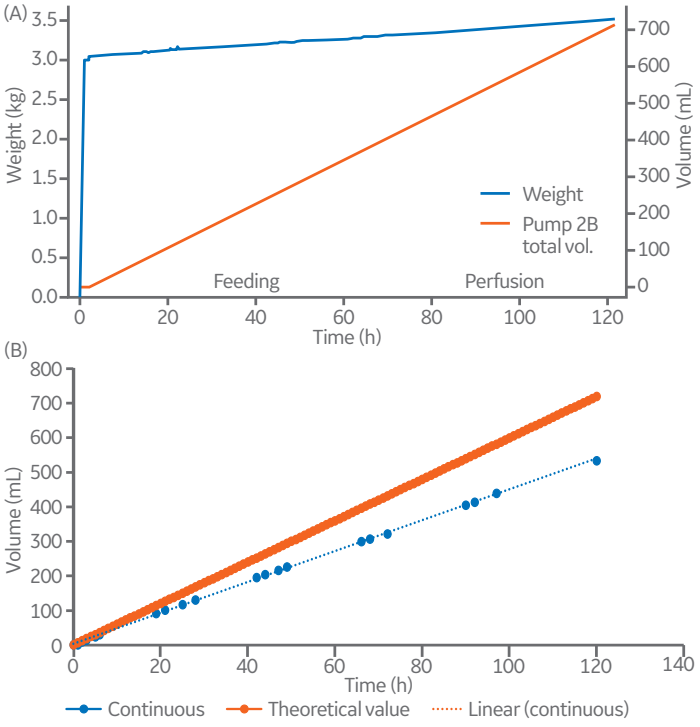

Fig 3. (A) Bag weight and pumped feed volumes for continuous feeding (0.1 mL/min) over five days using pump 2B, resulting in a total feed volume of 720 mL. (B) Scale data presented against theoretical values for continuous feeding (0.1 mL/min) over five days.

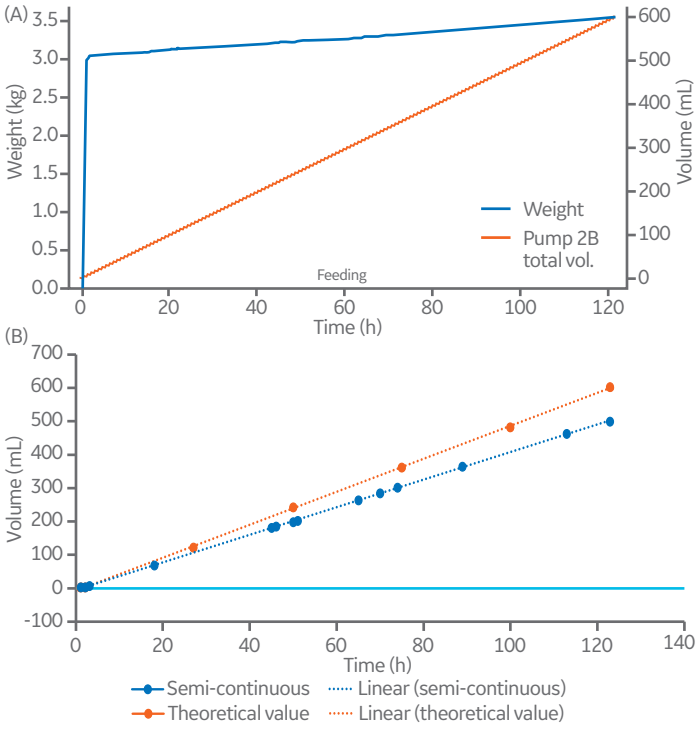

Fig 2. (A) Bag weight and pumped feed volumes for semi-continuous feed additions (5 mL/h) over five days using pump 2B, resulting in a total feed volume of 600 mL. (B) Scale data presented against theoretical values for semi-continuous feeding (5 mL/h) over five days.

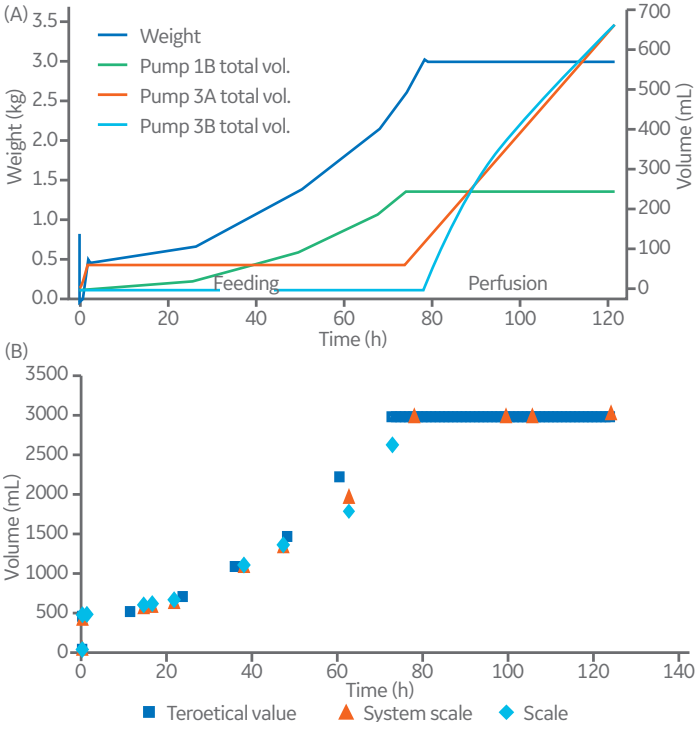

Fig 4. (A) Bag weight and pumped feed volumes over the five-day perfusion process. Pump 1B was used for exponentially adding liquid to the perfusion bag during the simulated exponential growth phase. During the perfusion phase, pump 3A was used for medium addition and pump 3B was used for medium removal. Perfusion rate was 1 VVD, resulting in a total exchange of 6 L. (B) Comparison of scale data, system scale data, and theoretical values for the five-day perfusion process.

Discussion

ReadyToProcess WAVE 25 exhibits several features that can facilitate process development and optimization activities. Running methods in the UNICORN software and implementing them on cell cultures reduce handling costs and variances due to manual interference. This work demonstrates the automated feed functionality of the system in dual culture mode for four different feeding strategies. Methods were easily created in the ***Method editor*** of the UNICORN software. Automated feed addition simplified operations and greatly reduced manual interaction with the system during the culture period.

The dual functionality can preferentially be used for process development and optimization or in laboratories that culture smaller batches of several cell lines requiring different cultivation conditions. Culturing in dual mode can reduce process development time by half, as well as minimize the facility footprint.

Recommendations:

1. At flow rates lower than 0.1 mL/min, manual feed additions are recommended to keep full control over added medium volumes.
2. To avoid any differences between theoretical and actual feeding volumes, manual recalibration of the pumps should be conducted at least every second day to ensure volumetric accuracy over longer cultivation periods.
3. To prevent potential differences between actual added volumes and the theoretical values, avoid frequent pump stops. When stopping and restarting the pump, the time it takes for the pump to ramp up should be considered and added to the method.

Conclusions

ReadyToProcess WAVE 25 is a highly versatile system, enabling the user to easily tailor the culture process to different automated feeding strategies. The presented features of the system reduce manual handling and limit variation between operators, thereby minimizing batch to batch variability. The ***Method editor*** is intuitive and simple to use, making it easy to design both culture modes and process conditions. The dual functionality feature allows users to run two separate processes with different conditions in parallel, enabling process development and optimization at twice the speed with a reduced footprint. This work demonstrates the value of the ReadyToProcess WAVE 25 system during process development and optimization in addition to its utility in seed train applications.

Reference

1. Application note: Effects of feeding strategy on CHO cell performance in fed-batch cultures. GE Healthcare, 29216535, Edition AA (2016).
2. Application note: Significant time savings with simplified cell culturing using ReadyToProcess WAVE 25. GE Healthcare, 29111313, Edition AA (2014).

Ordering information

Document	Product code
ReadyToProcess WAVE 25, rocker	28988000
ReadyToProcess CBCU	29044081
ReadyToProcess Pump 25	29032003
Tray 20	29044473
Cellbag 10 L, DOOPT II and pHOPT	CB0010L10-31
Cellbag, 10 L, BC11, Perf, DO	CB0010L11-04

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