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Push-Button Simplicity: Automatic Fermentation with the BioFlo® 120 Auto Culture Mode

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Abstract

The Auto Culture modes, standard on the BioFlo 120 bioprocess control station, offer push-button automatic process control for some of the most common microbial (*E. coli*) and mammalian (Chinese Hamster Ovary, CHO) cultures. When activated, process control loop modes and setpoints are automatically turned on and populated with values recommended by our experienced applications development team. The Auto Culture modes allow users, who are less familiar with bioreactors and fermentors, to achieve quick and easy initial culture success while undergoing a minimal learning curve. All setpoints and modes of operation can be adjusted, optimized, and saved as user-defined recipes, which are collected into the Auto Culture library for future use. For this application note, an *E. coli* batch fermentation was conducted using an Auto Culture mode to demonstrate this novel feature of the BioFlo120 control station.

Introduction

Learning to use a bioprocess controller is a complex and often intimidating endeavor for the beginner. Indeed, even with previous bioprocess experience, moving to a new software platform can entail much learning and reduce efficiency. Although many textbooks and manuals exist on the subject, they are no substitute for hands-on experience. With the Auto Culture modes of the BioFlo 120 bioprocess control station, the user can select either a pre-defined *E. coli* batch fermentation protocol or a CHO batch cell culture process, which begins at the push of a button (Figure 1). The Auto Culture modes are populated with setpoints and cascades tested by our applications development team and backed by the expertise developed over hundreds of experiments in our applications lab. The user needs only select the vessel size and type from the list of available choices, and make standard preparations (sensor and pump

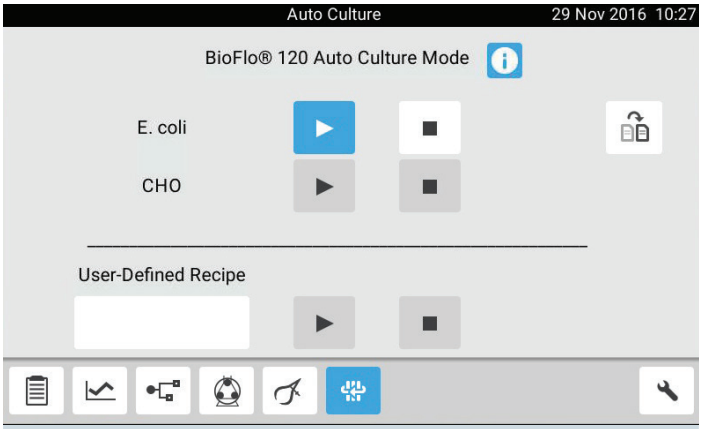

Fig. 1: BioFlo 120 Auto Culture mode interface

calibration, vessel preparation) for the run. Once ready, the user simply presses the "play" button and the system does the rest.

Here we give an overview on the settings and parameters of the Auto Culture mode for the cultivation of *E. coli* and test the feature in a batch fermentation process.

Material and Methods

Inoculum preparation

We used an *E. coli* strain (ATCC® 25922GFP™) which produces green fluorescent protein (GFP). The inoculum and fermentation medium was Terrific Broth (TB), prepared as described previously [1]. We prepared the inoculum by inoculating two 1 L baffled shake flasks (VWR®, USA), each containing 200 mL of TB medium, using a frozen vial from a mini cell bank [2]. The flasks were then incubated overnight at 37 °C and 200 rpm in an Eppendorf Innova® 44 shaker.

Fig. 2: BioFlo 120 bioprocess control station

Fermentation

To demonstrate the ease of use of the Auto Culture mode, we performed a standard *E. coli* batch fermentation. This process involved three steps:

1. Preparation of the control station

In preparation for push-button fermentation, we selected the correct vessel configuration on the Setup screen. For the experiment, a 2 L autoclavable, heat-blanketed, direct-drive vessel was selected. We calibrated the gel-filled pH sensor according to standard protocol using pH 7 and pH 4 buffers. We calibrated the pumps per the protocol outlined in the BioFlo 120 Operating Manual. The BioFlo 120 used in this experiment had the hardware configuration shown in Table 1.

Table 1: BioFlo 120 hardware configuration

Parameter	Configuration
Gas mix	Automatic gas mix
Gas flow control	One thermal mass flow controller (TMFC) with 0-20 standard liters per minute (SLPM) flow range
Vessel	Heat-blanketed glass vessel with baffle assembly (maximum working volume of 2.2 L)
Motor	Direct drive
Impeller	Two Rushton-type impellers
Sparger	Ring sparger (macrosparger)

2. Vessel preparation

We added 2 L of TB medium to the fermentor before sterilizing the vessel.

We calibrated the DO sensor according to the protocol outlined in the BioFlo 120 Operating Manual.

A sterile bottle containing 25 % (v/v) ammonium hydroxide was connected to a liquid addition port for pH control. The tubing was connected to pump 1, which served as the base pump. Acid was not connected for this experiment. If the user desires, an acid bottle can be connected through pump 2. Auto Culture pH control would call for base from pump 1 and acid from pump 2, as needed.

Finally, the vessel was inoculated with 100 mL of the inoculum (5 % of the initial working volume).

3. Culture start

As shown in Figure 1, the Auto Culture mode offers push-button control. To begin the culture, the "play" button for *E. coli* was pressed. After confirming that the sensors were calibrated, the process began when all the relevant control loop modes were automatically changed to the appropriate state. The setpoints for each control loop were auto-populated as outlined in Table 2.

Table 2. *E. coli* Auto Culture mode setpoints and loop modes which are populated upon start. Loop setpoints listed as "Auto" are determined by the DO control cascade. *Maximum flow rate is determined upon pump calibration.

Loop name	Mode	Setpoint
Agitation	Cascade	Auto
Temperature	On	37°C
pH	On	7.0; deadband = 0.1
DO	On	30 %
Gas flow	Cascade	Auto
Air	On	Auto
O_2	Cascade	Auto
Pump 1	On – base assignment	25 % of maximum flow rate*
Pump 2	On – acid assignment	25 % of maximum flow rate*

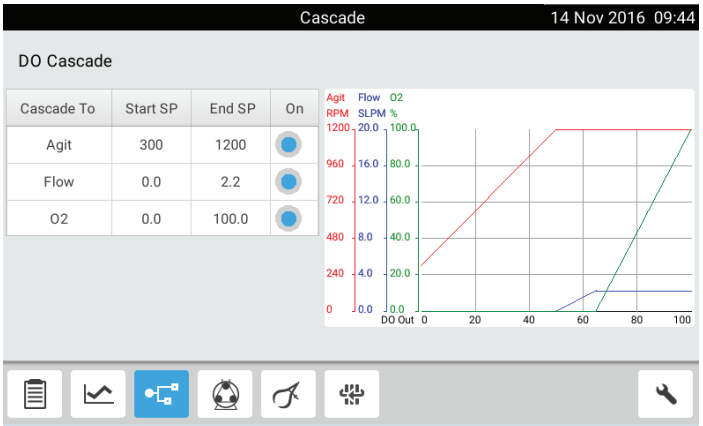

Fig. 3: BioFlo 120 Auto Culture mode DO cascade for the current configuration. For each vessel type, the maximum flow rate is adjusted to 1 VVM, while the other setpoints remain unchanged.

Sampling and monitoring the fermentation

The fermentor was monitored offline by taking a 5 mL sample hourly using the swabable Luer Lock port. Cell growth was monitored by offline measurement of the OD_{600} value with an Eppendorf BioSpectrometer® kinetic photometer. To measure GFP production, a Bacterial Cell Lysis Kit (GoldBio®, USA) was employed to release the GFP from the cells into the supernatant. The GFP yield was then quantified using an Eppendorf BioSpectrometer fluorescence photometer.

DO control during fermentation

Since oxygen supply is often the critical limiting factor during fermentation, care was taken to ensure that the Auto Culture mode responds to culture demand appropriately.

Usually, user-defined cascades for DO control that adjust the agitation speed, gas flow, and oxygen concentration are established over time, after optimization of a process by the scientist. In the Auto Culture mode, a tested cascade is provided for every vessel configuration and automatically populated and activated when Auto Culture is initiated. This DO control cascade is shown in Figure 3 for the 2 L autoclavable vessel used at maximum working volume. For each control loop on the summary screen, CSC (Cascade) indicates that the control loop is involved in a user-defined automatic control algorithm. The maximum gas flow rate is set to 1 Vessel Volume per Minute (VVM) as had been determined sufficient in previous experiments [3].

The control loops that are enabled in the DO cascade operate in series, resulting in the first loop (in this case, agitation) reaching maximum setpoint before the next control loop (in this case, gas flow) responds. Therefore, in this experiment, agitation will increase to a maximum of 1,200 rpm to attempt to maintain DO at setpoint before gas flow will begin to increase from a minimum of 0 SLPM to a maximum of 2.2 SLPM. By the time the cascade is completely executed, agitation reaches 1,200 rpm, gas flow reaches 2.2 SLPM, and O_2 as a percentage of total flow reaches 100 %. All of this occurs automatically, without user intervention.

Results

The batch *E. coli* fermentation using a GFP-expressing strain in Auto Culture mode was completed successfully. As shown in Figure 4, within 6 h, the OD_{600} value reached 14 and the GFP production was 650 relative fluorescence units/mL. Since a batch culture protocol does not include nutrient or carbon source addition, nutrients were depleted and the culture entered stationary phase around 7 hours, and we ended the experiment. The growth curve is typical for a batch fermentation and provides necessary strain characterization information to begin designing a fed-batch or continuous bioprocess.

Auto Culture mode allows for the optimization of parameters based on experimental need, with the option to save a new custom recipe which is then available in the Auto Culture menu for future use. Each time a new production strain is developed, the batch culture allows the scientist to determine the appropriate growth parameters. In this case, our GFP-expressing strain grew satisfactorily at 37 °C and at pH 7.0. If, on the other hand, the experiment had required a custom temperature or other setpoint, those changes could be made at any time. When the experiment is finished and the ideal setpoints determined, the recipe can be saved as a custom Auto Culture mode available to be automatically employed just like the pre-loaded *E. coli* template. In this way, the number of available Auto Culture modes grow with experience, allowing for the creation of a library of custom recipes.

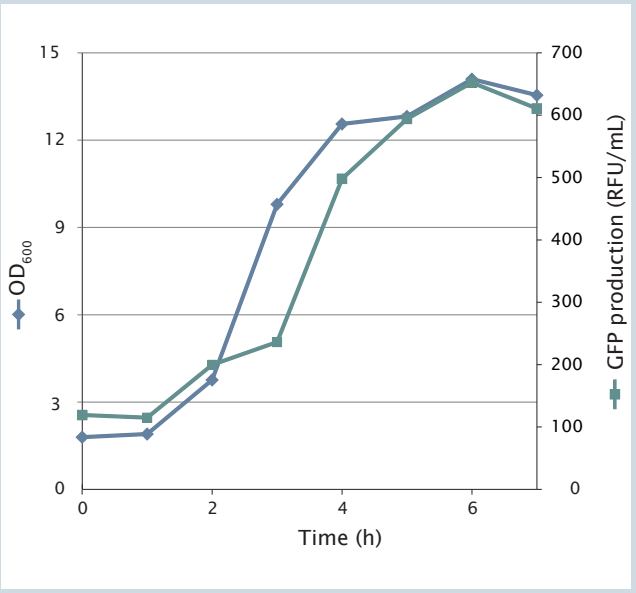

Fig. 4: *E. coli* growth curve and GFP production yield. RFU: Relative fluorescence units

Conclusion

The BioFlo 120 (Figure 2) is a benchtop bioprocess system that uses proprietary software to monitor and control a wide array of fermentation and cell culture applications, and can be employed for batch, fed-batch or continuous cultures. The BioFlo 120 is equipped for use with BioBLU® Single-Use Vessels up to 40 L working volume as well as industry-standard glass autoclavable vessels up to 10.5 L working volume. With the option of mass-flow-controlled gassing and automatic mixing of up to four gasses, the control station is well equipped for dissolved oxygen (DO) control in a variety of applications. The push-button bioprocess concept reduces the complexity of the design of a new bioprocess. The setpoints and cascades recommended by our experienced application team help to achieve satisfactory bioprocess results in a short time and offer a starting point for further optimization.

In this application note we used the Auto Culture mode for *E. coli* fermentation in a batch process. The BioFlo 120 also offers an Auto Culture mode for the cultivation of CHO cells.

Literature

[1] Terrific Broth. *Cold Spring Harbor Protocols* 2006. 2006(1): pdb.rec8620.
[2] Li, B. and Sha, M. Scale-up of *Escherichia coli* fermentation from small scale to pilot scale using Eppendorf fermentation systems. *Eppendorf Application Note 306*. 2016.
[3] Li, B., Willard, S., and Sha, M. High cell density fermentation of *Escherichia coli* using the New Brunswick™ BioFlo® 115. *Eppendorf Application Note 335*. 2014.

Ordering information

Description	Order no.
Eppendorf BioSpectrometer® kinetic, 230 V/50 – 60 Hz	6136 000.002
Eppendorf BioSpectrometer® fluorescence, 230 V/50 – 60 Hz	6137 000.006
New Brunswick™ Innova® 44 Shaker, 230 V/50 – 60 Hz, orbit diameter 2.5 cm (1 in)	M1282-0002
BioFlo® 120, Advanced	
Plug type B (USA, Canada, Mexico, Japan)	B120ACS000
Plug type CEE 7/7 (EU (except UK, Ireland, Switzerland), Russia, Korea	B120ACS001
Plug type I (Australia, New Zealand, China, Argentina)	B120ACS002
Plug type J (Switzerland)	B120ACS003
Plug type G (UK, Ireland)	B120ACS004
Plug type N (Brazil)	B120ACS005
Plug type D (India)	B120ACS006
BioFlo® 120 Fermentation Vessel Bundle	
1 L, heat blanket	B120AVB000
2 L, heat blanket	B120AVB001
5 L, heat blanket	B120AVB002
10 L, heat blanket	B120AVB003
1 L, water jacket	B120AVB004
2 L, water jacket	B120AVB005
5 L, water jacket	B120AVB006
10 L, water jacket	B120AVB007

For more information on these and other configurations visit www.eppendorf.com/BioFlo120

Your local distributor: www.eppendorf.com/contact
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