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High-Density *Escherichia coli* Fermentation and Protein Production using the Eppendorf BioFlo® 120 Bioprocess Control Station

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Abstract

We used the BioFlo 120 bioprocess control station for the fed-batch fermentation of a GFP-expressing *Escherichia coli* strain. To quantify cell density and protein production we measured the optical density (OD_{600}) of the culture and the fluorescence of GFP, respectively. After nine hours of culture, the optical density had increased to 191 and the relative fluorescence units (RFU) to 12,532. These results indicate that the BioFlo 120 supports the growth of *E. coli* to high densities and the production of large amounts of protein.

Introduction

The BioFlo 120 (Figure 1) is a benchtop bioprocess system with the flexibility to control both autoclavable and single-use vessels. The system has proprietary software to monitor and control a wide array of fermentation and cell culture applications, and can be employed for batch, fed-batch, perfusion, and continuous cultures. The BioFlo 120 supports the use of BioBLU® Single-Use Vessels as well as industry-standard glass autoclavable vessels. 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, including high-density mammalian cell culture and bacteria and yeast fermentations.

In the project described in this application note, we tested the suitability of the BioFlo 120 bioprocess control station for high-density *E. coli* fermentation.

Fig. 1: BioFlo 120 bioprocess control station.

Material and Methods

E. coli strain, medium, and preculture

We used a GFP-expressing *E. coli* strain (ATCC® 25922GFP™). A mini cell bank was prepared as described previously [1].

To prepare a preculture, we inoculated two 1 L baffled shake flasks (VWR®, USA), each containing 200 mL of Terrific Broth (TB) medium, from a frozen vial of *E. coli* inoculum stock. We incubated the culture at 37°C and 200 rpm overnight in an Eppendorf Innova® 44 shaker (Eppendorf, Germany).

For *E. coli* fermentation, we used chemically defined medium at pH 7.0. We prepared the initial fermentation medium in a 2 L glass vessel as follows: 150 mL 10 x phosphate/ citric acid buffer (133 g/L KH_2PO_4, 40 g/L $(NH_4)_2HPO_4$, 17 g/L citric acid) and 1.3 L deionized water were added to the vessel for sterilization at 121°C for 20 min. After the medium had cooled to room temperature, the following sterile components were added aseptically to make the complete fermentation medium: 15 mL of 240 g/L $MgSO_4$, 0.34 mL of 20 g/L thiamine solution, 15 mL of 100 x trace element solution, and 25 mL of 70 % glucose solution. The 100 x trace element solution contained: 10 g/L iron (III) citrate, 0.25 g/L $CoCl_2 \cdot 6H_2O$, 1.5 g/L $MnCl_2 \cdot 4H_2O$, 0.15 g/L $CuCl_2 \cdot 6H_2O$, 0.3 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4 \cdot 2H_2O$, 1.3 g/L zinc acetate $\cdot 2H_2O$, and 0.84 g/L EDTA [2, 3].

We prepared a concentrated feeding medium in a 500 mL glass bottle. 45 mL of 240 g/L $MgSO_4$, 1.66 mL of 20 g/L thiamine solution, 15 mL of 100 x trace element solution, and 70 % glucose solution were added to a final volume of 500 mL.

Fermentation

We ran the fermentation in a heat-blanketed glass vessel connected to a BioFlo 120 bioprocess control station. The configurations of the controller and the vessel are outlined in Table 1. We inoculated the culture with 150 mL of the preculture (10 % of the initial working volume).

The fermentation was carried out at 37°C. The pH was controlled at 7.0 (± 0.1) and the DO was set to 30 %. Antifoam 204 (Sigma-Aldrich®, USA) was added only when needed.

pH calibration and control

We calibrated the pH sensor outside the vessel prior to autoclaving, using a two-point calibration method and standard buffers. We used the buffer of pH 7.0 to set "ZERO" and the buffer of pH 4.0 for the "SPAN" (please refer to the BioFlo 120 user manual). For pH control, we connected a sterile bottle containing 25 % (v/v) NH_4OH to a liquid addition port for pH control. The pH was automatically maintained at 7.0 by adding 25 % (v/v) NH_4OH via pump 1 (assigned as base pump). Acid was not connected for this experiment, but if the user desires, acid can be added using pump 3.

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 motor
Impeller	Two Rushton-type impellers
Sparger	Ring sparger (macrosparger)

Dissolved oxygen (DO) sensor calibration and gassing control

We calibrated the analog polarographic DO sensor using a standard two-point calibration method: 0 % (set "ZERO") was obtained by disconnecting the sensor from the cabinet and allowing the raw value to stabilize; 100 % (set "SPAN") was obtained by agitating at 1,000 rpm and 2.0 SLPM air flow until the DO value stabilized at the maximum (please refer to the BioFlo 120 user manual).

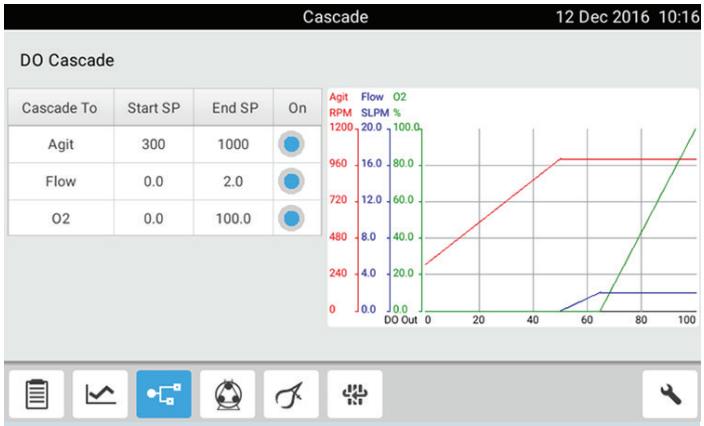

Fig. 2: Cascade for DO contol used in this fermentation run.

DO was controlled using the cascade shown in Fig. 2. It is important to note that the control loops that were 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 1,000 rpm to attempt to maintain the DO at the setpoint before the gas flow will begin to increase from a minimum of 0 SLPM to a maximum of 2.0 SLPM. When the cascade was fully executed, the agitation maintained 1000 rpm, and the gas flow reached 2.0 SLPM (100 %). The O_2 level automatically adjusts as a percentage of total flow. All the controls occurred automatically without user-intervention.

Culture feeding

Starting 3.5 hours after inoculation, the culture was fed with a concentrated feed solution following the protocol shown in Table 2. Pump 2 was assigned as the feeding pump and controlled by the BioCommand® SCADA Software. We set the period of pump control time to 10 seconds.

Analysis

To monitor the fermentation offline, we took a 3-5 mL sample hourly using the swabable valve connected to the sample tube. We monitored cell growth by measuring the optical density of the culture (OD_{600}) with an Eppendorf BioSpectrometer® kinetic photometer. To measure GFP production we released GFP from the cells to the supernatant using a Bacterial Cell Lysis Kit (GoldBio®, USA) [4] and quantified GFP fluorescence with an Eppendorf BioSpectrometer fluorescence photometer. We determined the glucose concentration in the medium using a Stat Profile® Prime Analyzer (Nova Biomedical, USA).

Table 2: Pump speed at different elapsed fermentation times during the fed-batch fermentation. The pump was automatically controlled by BioCommand software

Time (h)	3.5	4.5	5.5	6.5	7	7.5	8	8.5	10
Pump speed (mL/min)	0.1	0.2	0.3	0.6	0.8	1.2	1.4	1.7	4.1

Results

Within nine hours the culture grew to an optical density of almost 200 (Figure 3). Parallel to the increase of biomass the amount of GFP rose and reached almost 12,532 relative fluorescence units after nine hours (Figure 4). The glucose concentration dropped slowly during the first four hours, increased with the addition of the glucose-containing feed medium, and then declined rapidly (Figure 3).

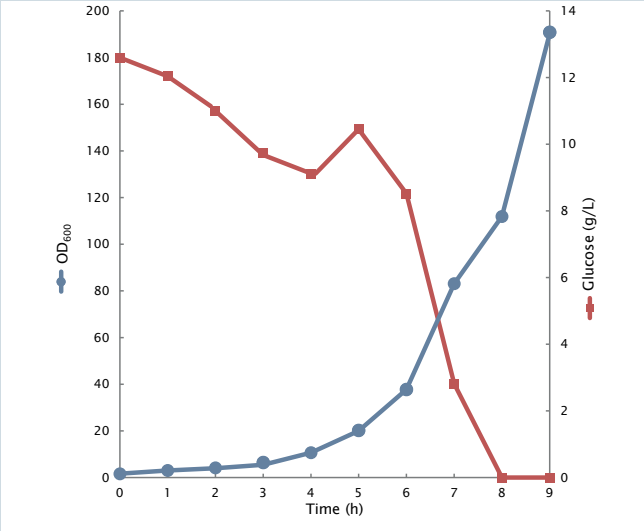

Fig. 3: *E. coli* growth curve and glucose concentration in the culture medium.

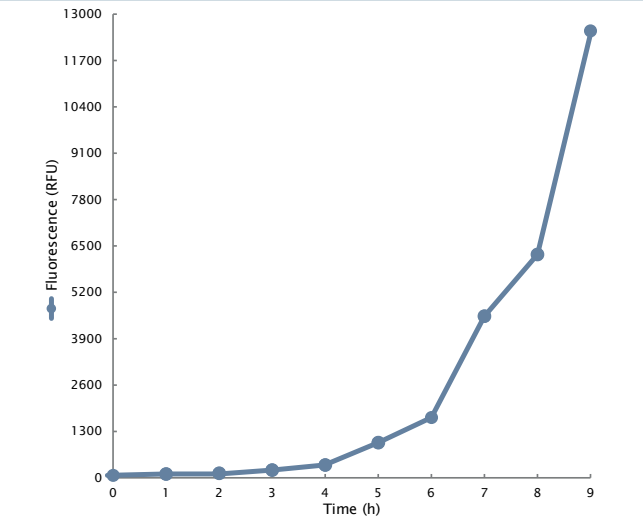

Fig. 4: GFP production was quantified by measuring the protein's fluorescence. RFU: Relative fluorescence units.

Conclusion

We used the BioFlo 120 bioprocess control station for an *E. coli* fed-batch fermentation. Within nine hours the culture grew to an OD_{600} of 191 and produced almost 13,000 RFU of GFP. These results indicate that with the BioFlo 120 control station and vessels high cell densities and high protein titers can be reached.

Literature

[1] Li B., Sha M. Scale-Up of *Escherichia coli* Fermentation from Small Scale to Pilot Scale Using Eppendorf Fermentation Systems. *Eppendorf Application Note No. 306*, 2016.
[2] Geerlof A. High cell-density fermentation of *Escherichia coli*. http://www.helmholtz-muenchen.de. 2008.
[3] Korz D.J., Rinas U., Hellmuth K., Sanders E.A., Deckwer W.D. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. *Journal of Biotechnology* 1995; 39(1):59-65.
[4] Korz D.J., Rinas U., Hellmuth K., Sanders E.A., Deckwer W.D. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. *Journal of Biotechnology* 1995; 39(1):59-65.
[5] Bacterial Cell Lysis Kit Protocol. https://www.goldbio.com/documents/1358/Bacterial+Cell+Lysis+Buffer-Product+Information+and+Protocol+v1.2.pdf

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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