APPLICATION NOTE No. 340 I January 2015

The Eppendorf BioFlo® 320 Bioprocess Control Station: An Advanced System for High Density *Escherichia coli* Fermentation

Bin Li, Khandaker Siddiquee, and Ma Sha
Eppendorf, Inc., Enfield, CT, USA
Corresponding author: sha.m@eppendorf.com

Abstract

In this application note, an *Escherichia coli* (*E. coli*) fermentation run was conducted using the new Eppendorf BioFlo 320 bioprocess control station. High cell density was achieved at 12 h as determined by a maximum optical density (OD_{600}) measurement of 215.2. The wet cell weight (WCW) and dry cell weight (DCW) were also measured and presented.

Introduction

The newest offering in the Eppendorf bioprocess portfolio, the BioFlo 320 seamlessly combines form and function into one all-inclusive package. The BioFlo 320 is an advanced bioprocess system developed for both microbial fermentation and cell culture applications. A new industrial design, flexibility between autoclavable and single-use vessels, intelligent sensors, Ethernet connectivity, and an improved software package are only a few of the features that set it apart from the competition.

In a previous application note using the BioFlo/CelliGen® 115 benchtop system [1], we successfully cultured *E. coli* under aerobic conditions with a maximum OD_{600} value of 140. In this application note, *E. coli* cultivation achieved an even higher OD_{600} value of 215.2 using the new BioFlo 320 bioprocess control station (Figure 1). Furthermore, the WCW and DCW were also measured and presented.

Figure 1: BioFlo 320 bioprocess control station with water-jacketed (left) and stainless steel dish-bottom (right) vessels

Materials and Methods

Equipment

Fermentation was performed using an Eppendorf BioFlo 320 bioprocess control station with the configuration outlined in Table 1. The *E. coli* K12 strain (ATCC®, 10798™) was grown in a 1 L (working volume) stainless steel dish-bottom BioFlo 320 glass vessel, as shown in Figure 1. Glucose concentrations were measured using a Cedex® Bio Analyzer (Roche®). The OD_{600} was measured with a spectrophotometer. An Eppendorf MiniSpin® plus was used to pellet the cells. A pH sensor (InPro® 3253i/SG/225) and an optical Dissolved Oxygen (DO) sensor (InPro 6860i), both incorporating Intelligent Sensor Management (ISM®) technology from Mettler Toledo®, were used in this experiment. A laboratory oven (LAB-LINE®, L-C series) was used to dry the cell pellets for DCW measurements.

Parameter	Configuration
Gas Mix	Automatic gas mix
Gas Flow Control	Thermal mass flow controller (TMFC) with 0 – 20 standard liters per minute (SLPM) flow range
Vessel	1 L stainless steel dish-bottom glass vessel
Motor	Direct drive motor
Impeller	Two Rushton impellers
Sparger	Ring sparger (Macrosparger)

Table 1: BioFlo 320 hardware configuration

Medium

E. coli was cultured in chemically defined medium, pH 6.8. The initial fermentation medium was prepared 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 1.35 L deionized (DI) water were added to the vessel for sterilization at 121 °C for 20 min. After the medium was 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, 15 mL of 100 x trace element solution, and 22 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 $CuCl_2.6H_2O$, 0.3 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4 \cdot 2H_2O$, 1.3 g/L zinc acetate$\cdot 2H_2O$, 0.84 g/L EDTA [2, 3].

An additional concentrated feeding medium was prepared separately in a 1 L glass bottle. 67.5 mL of 240 g/L $MgSO_4$, 2.49 mL of 20 g/L thiamine solution, 22.5 mL of 100 x trace element solution, and 70 % glucose solution were added to a final volume of 750 mL.

Inoculum preparation and fermentation

The inoculum was grown in Terrific Broth (TB) medium, prepared as described previously [4]. Two 500 mL baffled shake flasks (VWR®, 30623-210) each containing 100 mL of TB medium were inoculated from a frozen vial of *E. coli* and incubated at 30 °C, 200 rpm overnight in a New Brunswick™ Innova® 40 benchtop incubator shaker. Cell growth was monitored by offline measurement of the OD_{600} value. The vessel was inoculated with 75 mL of inoculum (5 % of the initial fermentation medium volume).

Antifoam 204 (Sigma-Aldrich®, A6426) was added only when needed, since it may reduce the oxygen transfer rate (OTR) and possibly lower the final cell density. About 5 mL of 5 g/L antifoam was added between 7 – 12 h of fermentation, as foam accumulation warranted.

Pump 3 was assigned as the feeding pump. The feeding strategy included increasing or decreasing the feeding pump speed based on the glucose concentration. This strategy was designed to maintain glucose concentration below 2 g/L. Table 2 and Figure 2 illustrate the adjustments made to the pump speed over the course of the fermentation. Although a similar feed program can be used for repeated fermentation runs, the feed start time must be adjusted each time according to the growth dynamics of each fermentation.

Cell growth and glucose concentration were monitored offline using 5 mL samples taken according to the following schedule. For OD_{600} readings, samples were taken every hour and diluted appropriately for accurate measurement. For the determination of glucose concentration, samples were taken every hour before the initiation of feeding, and then every ~30 min after the feeding began. The specific growth rate (μ) was calculated from the fitted OD_{600} value in Microsoft® Excel®.

pH calibration and control

pH calibration was performed outside the vessel using a two-point calibration method and standard buffers. Buffer pH 7.0 was used to set ZERO and pH 4.0 for the SPAN. The pH sensor was calibrated prior to autoclaving the vessel.

Elapsed Fermentation Time (EFT, h)	5:15	6:15	6:16	7:00	7:01	7:30	9:00	9:30	10:00	10:30	11:00	11:30	12:00
Pump Speed (mL/min)	0.3	0.5	0.2	0.5	0.6	0.9	1.7	2.1	2.7	3.2	3.7	4.5	5.3

Table 2: Pump speed at different EFT during the fed-batch fermentation

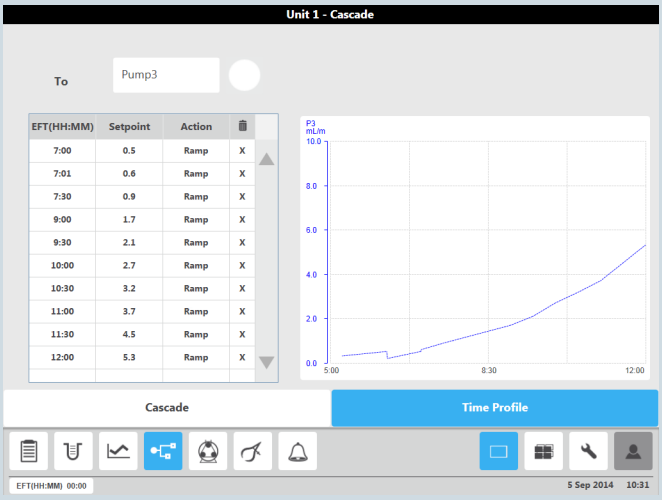

Figure 2: EFT-based feeding program as displayed on the enhanced software platform

The pH was automatically maintained at 6.8 by adding 25 % (v/v) NH_4OH via front mounted peristaltic pump (assigned as "base"). The deadband for pH control was 0.02.

DO sensor calibration and gassing control

Since the BioFlo 320 is compatible with multiple types of DO sensors, an optical sensor was chosen for DO control instead of the traditional polarographic DO sensor. One of the advantages of the optical DO sensor is that it does not require the 6 h polarization time of the polarographic DO sensor, which reduces the turnaround time between fermentation runs. Calibration was performed using a standard two-point calibration method: 0 % (set ZERO) was obtained by running 1200 rpm agitation and 3 SLPM N_2 flow until the DO value stabilized. 100 % (set SPAN) was obtained by running 1,200 rpm agitation and 3 SLPM air flow until the DO value stabilized at maximum.

The BioFlo 320 software offers a selection of automatic gassing control cascades that are dependent upon the configuration of the unit. The BioFlo 320 used in this study included the automatic gas mix and four TMFCs with a flow range of 0 – 20 SLPM (Table 1). User-defined DO cascade settings utilizing agitation, air, and oxygen in sequential manner are shown in Figure 3. The DO setpoint was 30 %.

WCW and DCW measurement

Samples were taken to measure the WCW and DCW. 1 mL of culture sample was added into an Eppendorf microcentrifuge tube and pelleted at 7,500 rpm for 5 min. The supernatant was carefully removed using an Eppendorf Research® pipette and the WCW was measured by calculating the difference in weight between the tube before and after sample addition. Furthermore, the tube was kept in a heating oven and maintained at 70 – 80 °C until the cell pellet was dry and the DCW measured similarly.

Results and Discussion

Samples were taken periodically to monitor the cell growth (OD_{600} value) and glucose concentration as described above. Feeding was initiated when the glucose concentration dropped below 2 g/L, which occurred at 5.25 h of cultivation. After starting the feed, the pump rate was adjusted according to the current glucose concentration with the end goal of keeping it at or below 2 g/L (Table 2). As shown in Figure 4A, the OD_{600} value reached 215.2 within 12 h. The growth curve was also plotted on a log scale to calculate the specific growth rate ($\mu = 0.54\ h^{-1}$, Figure 4B).

WCW and DCW were also measured during the fermentation. The results are shown in Table 3 and Figure 5. During the cultivation, both WCWs and DCWs increased proportionally with the increase in the OD_{600} value.

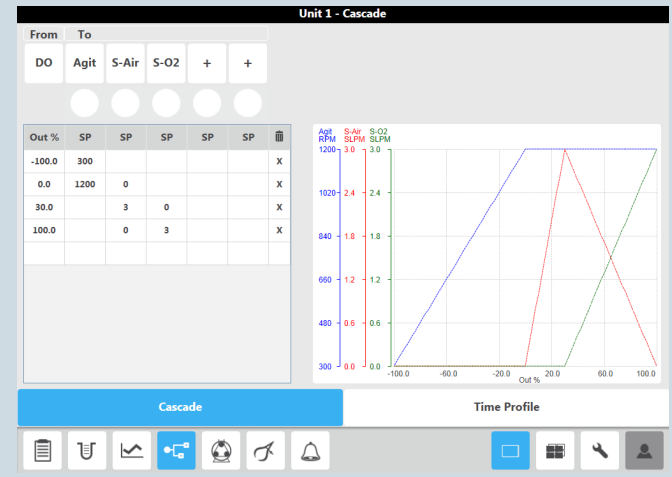

Figure 3: DO cascade used in this experiment

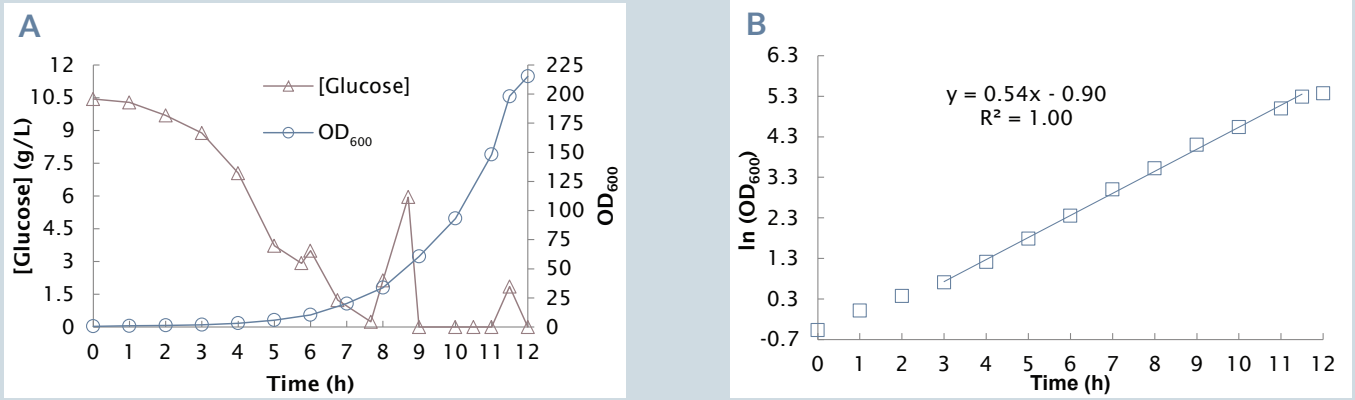

Figure 4: Fermentation growth curve and glucose concentration
A: OD_{600} and glucose concentration over the course of the 12 h fermentation
B: Growth curve plotted on a log scale; a linear trend line was applied in Microsoft Excel, the slope of which was obtained as the specific growth rate, μ (h^{-1})

Conclusions

The Eppendorf BioFlo 320 bioprocess control station was able to support high density *E. coli* growth using a fed-batch fermentation method. An OD_{600} optical density of 215.2 was reached at 12 h. The wet/dry cell weights were measured at various time points, which were used as records of cell growth along with OD_{600} values. Although efforts were made to maintain a glucose concentration below 2 g/L, the fermentation was not optimized for medium, growth conditions, or any product yield.

OD_{600}	WCW (g/L)	DCW (g/L)
15.6	67.5 ± 4.7	15.2 ± 2.7
30.3	117.2 ± 4.1	24.6 ± 2.0
58.0	214.1 ± 16.3	41.7 ± 2.0
89.8	334.4 ± 19.3	64.6 ± 2.0
111.2	386.5 ± 8.8	72.4 ± 1.7
215.2	453 ± 13.0	110.7 ± 2.4

Table 3: WCW and DCW; average was calculated from 5 samples with mean +/- one standard deviation

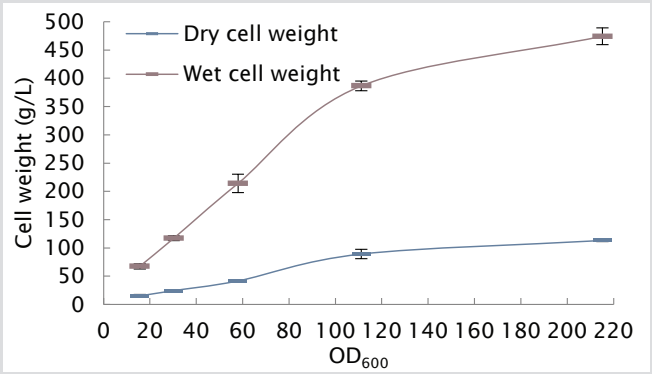

Figure 5: Correlation between OD_{600} value and cell weight

Literature

[1] Li B, Willard S, Sha M. High Cell Density Fermentation of *Escherichia coli* Using the New Brunswick BioFlo115. Eppendorf Application Note No. 335, 2014. http://www.nbsc.com/files/335_Li_Ecoli.pdf
[2] Geerlof A. High cell-density fermentation of *Escherichia coli*. http://www.helmholtz-muenchen.de 2008.
[3] Korz DJ, Rinas U, Hellmuth K, Sanders EA, Deckwer WD. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. *Journal of Biotechnology* 1995; 39(1):59-65.
[4] Terrific Broth. *Cold Spring Harbor Protocols* 2006; 2006(1):pdb.rec8620.

Ordering information

Description	Order no. International	Order no. North America
BioFlo® 320 Control Station	Call	Call
BioFlo® 320 1 L Vessel Bundle, Stainless Steel Dish-Bottom	M1379-0300	M137-0300
New Brunswick™ Innova® 40 Benchtop Incubator Shaker	M1299-0092	M1299-0092
Eppendorf Research® plus, single-channel, fixed, 1000 µL	3120000.062	3121000.120
Eppendorf Safe-Lock Tubes, 1.5 mL	0030120.086	0030120.086
Eppendorf MiniSpin® plus centrifuge	5452000.018	5453000.011

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

ATCC® is a registered trademark of American Type Culture Collection, USA. Cedex® and Roche® are registered trademarks of Roche Diagnostics GmbH, Germany. LAB-LINE® is a registered trademark of LAB-LINE Instruments, Inc., USA. VWR® is a registered trademark of VWR International, Inc., USA. Sigma-Aldrich® is a registered trademark of Sigma-Aldrich Co., USA. 10798™ is a trademark of American Type Culture Collection, USA. InPro®, ISM®, and Mettler Toledo® are registered trademarks of Mettler-Toledo AG, Switzerland. Microsoft® and Excel® are registered trademarks of Microsoft Corporation, USA. The Eppendorf logo, Eppendorf®, Eppendorf Research® plus, and Eppendorf MiniSpin® plus are registered trademarks of Eppendorf AG, Germany. New Brunswick™ is a trademark of Eppendorf AG, Germany. BioFlo®, CelliGen®, and Innova® are registered trademarks of Eppendorf Inc., USA.
U.S. Design Patents are listed on www.eppendorf.com/ip. All rights reserved, including graphics and images. Copyright © 2015 by Eppendorf AG.