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Dissolved Oxygen Control PID Tuning for Cell Culture Applications

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Abstract

Eppendorf bioprocess controllers, such as the BioFlo® 320 Bioreactor Control System, use a proportional–integral–derivative (PID) control mechanism for a variety of processes, including dissolved oxygen (DO) control. The PID control loop mechanism for DO control is essential for optimal bioreactor cell culture. Although the D value is not user adjustable in Eppendorf controllers, the P and I values can be changed. The default P and I values set on the controller are good starting points but may not be optimal for specific cell culture processes or vessels.

This protocol introduces a method developed at the Eppendorf bioprocess applications lab to optimize the PI values on Eppendorf bioprocess controllers, including the BioFlo 320. The method is based on pre-programmed delivery of chemical oxygen scavengers to accurately simulate the oxygen demand throughout a bioreactor cell culture process. This chemical simulation allows for quick and accurate tuning of PI values for cell culture DO control. The method can be adopted to fermentation PI tuning as well. However, a more aggressive gassing demand model will need to be established by the user to match the actual gassing demand for fermentation processes.

Content

Basic Simulation Procedure Using Time Profile

1. Introduction

Proper tuning of the DO controller PI values is essential for optimal cell culture performance in a bioreactor. When DO PI values are optimized, gas flows are smoother, foaming and stress on the cells are reduced. Traditionally, tuning has been performed by using nitrogen gas to purge oxygen from a test solution, thus simulating oxygen demand. This method has several drawbacks, however. First, nitrogen gassing cannot simulate the high demands of high-density fermentation. Second, nitrogen gassing competes with other gases, especially in single thermal mass flow controller (TMFC) systems. This artificial gassing interference is not normally present in a cell culture. And third, it prevents the testing of 4-gas control mechanisms in which nitrogen is used to balance DO as part of the gassing strategy.

In this article, we describe a method of simulating DO demand using chemical oxygen scavengers, such as sodium sulfite, sodium metabisulfite, and sulfur dioxide. Oxygen scavengers have been used to deplete DO in a previous Eppendorf protocol for oxygen transfer rate (OTR) measurements [1]. In the presence of the right catalyst, oxygen scavengers quickly eliminate dissolved oxygen from solution. For example, in the presence of copper as a catalyst, sodium sulfite reacts with oxygen to form sodium sulfate:

$$2\ Na_2SO_3 + O_2 \xrightarrow{Cu^{2+}} 2\ Na_2SO_4$$

By controlling the concentration of sodium sulfite and its feed rate into a bioreactor, the oxygen demand of a cell culture can be simulated. Iteratively, different PI values are used for each run to determine the most effective settings. The BioFlo 320 offers a simple way to perform a PI tuning simulation. Operators can use the built-in time profile feature with the integrated pumps to control the amount of a sodium sulfite solution added to a reactor over time. This protocol will describe this method for simulating culture oxygen demand for PI tuning, using Chinese Hamster Ovary (CHO) cells as an example, though it can be easily applied to other cell lines.

2. Material and Methods

A BioFlo 320 was used as the controller for this protocol. The simulations were performed in a BioBLU 3c Single-Use Vessel with a macrosparger. All solutions were connected to the vessel using AdvantaPure pump grade silicone tubing (NewAge Industries).

In lieu of medium, simulations were performed in phosphate buffered saline (PBS, Fisher Scientific®). Sodium sulfite was prepared as a 0.5 M solution. The solution was made by first sparging 0.5 SLPM nitrogen gas into 5 L of deionized water for a minimum of half an hour to purge dissolved oxygen. Next, 315 g of anhydrous sodium sulfite (Fisher Scientific) was quickly and carefully added to the deionized water. This solution was vigorously mixed with a stir bar until the sodium sulfite was completely dissolved. To inhibit oxidization, nitrogen was continually sparged into the solution at 0.1 SLPM for the remainder of the experiment.

The copper solution was prepared by dispensing 500 mL of deionized water and 25 g of copper (II) sulfate pentahydrate (Fisher Scientific) into a 500 mL glass bottle. The solution was mixed on a stir plate until all the copper (II) sulfate was dissolved. The bottle was wrapped in aluminum foil, as the copper (II) sulfate is light sensitive.

2.1 Sensor Calibration

A Mettler Toledo® 6850i ISM® sensor was used to monitor DO. The sensor was calibrated as previously described [2]. Briefly, air was continuously sparged at 1 SLPM into a BioBLU® 3c vessel filled with 3 L PBS for about 10 minutes. After the DO sensor reading stabilized, the “set span” button on the DO calibration screen of the BioFlo 320 software was pressed. The span value was set to 100 and entered into the software. The calibration was rechecked before each run by sparging with 1 SLPM air until the DO reading stabilized, and restandardized as needed.

Since the sodium sulfite solution is slightly basic, a Mettler Toledo InPro 325x analog pH sensor was used to monitor the pH of the PBS solution. The pH sensor was calibrated using standard solutions of pH 10 and pH 7 (Fisher Scientific), following the method presented in the BioFlo 320 operating manual [3]. CO^2 gas was used to adjust the pH as needed.

2.3 Scale calibration

A SPX GSE model 3512 scale was connected to the BioFlo 320 under the volume loop. The scale was calibrated with a 1 kg standard as described in the BioFlo 320 manual.

2.4 Pump calibration

The BioFlo 320 integrated pumps were calibrated using 3/16” ID silicone tubing as described in the BioFlo 320 manual.

2.5 Experimental Setup

The experimental setup is shown in Figure 1. The BioFlo 320's magnetic drive, thermometer, DO sensor, and heat blanket were connected to the BioBLU 3c bioreactor following the operator's manual instructions. The pH sensor was inserted into spare port 1. The vessel was placed on the scale, which then underwent a zero-point and span calibration. The vessel was then filled with 3 L of PBS.

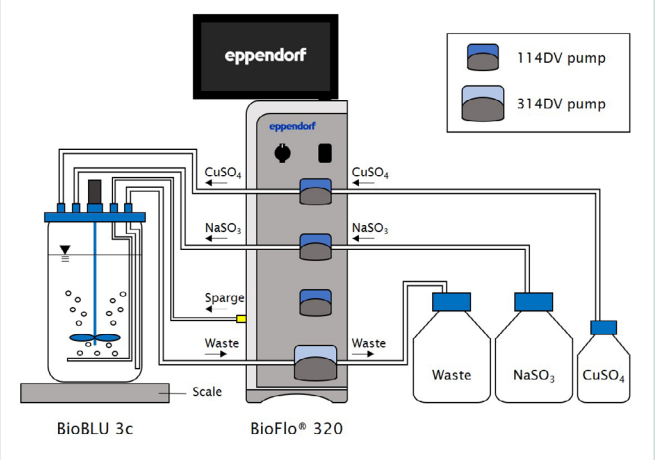

Fig. 1: BioFlo 320 experimental setup.

The BioFlo 320 loops were configured with the parameters listed in Table 1:

Table 1: BioFlo 320 settings

Loop	Mode	Setpoint
Agitation	On, Clockwise	200 rpm
Temperature	On	37 °C
pH	On	7.0, Deadband 0.1
Sparge Gas	3-Gas Mix	0.041 SLPM minimum, 1 SLPM maximum
DO	On	50 %
Volume	On	3 L, Deadband 0.1
Pump 4	On, Harvest (Scale)	100 rpm

The copper (II) sulfate solution's pump tubing was placed in one of the BioFlo 320's integrated 114DV pump heads. Then 6 mL of copper (II) sulfate solution was pumped into the BioBLU 3c bioreactor, for a concentration of 2 mL per 1 L of PBS. The sodium sulfite's pump tubing was placed in another of the BioFlo 320's integrated 114DV pump heads. The pump was primed, set to 0 mL/min, and turned on.

A waste jar was connected to the harvest line of the bioreactor via silicone tubing. The waste jar tubing was placed in the integrated 314DV pump head (pump 4). Pump 4 was then set to harvest mode, to keep the vessel from overflowing as sodium sulfite was pumped in

2.6 Time Profile Setup

The addition of sodium sulfite into the reaction vessel was controlled through the BioFlo 320 time profile shown in Table 2.

Table 2: Time profile for oxygen simulation

Elapsed Fermentation Time (EFT, hours:minutes)	Setpoint (mL/min)	Action
00:00	0.6	Step
01:00	0.8	Step
02:00	1.2	Step
03:00	1.4	Step
04:00	1.6	Step
05:00	2.4	Step
06:00	3.4	Step
07:00	4.4	Step
08:00	5.4	Step
09:00	6.4	Step
10:00	0	Step

The simulated oxygen demand time profile was created based on the increased oxygen demand over time observed during past CHO cultures. The simulated culture time was sped up by a factor of 10, so that a typical 7 – 10 day batch CHO culture process was condensed into a 1-day simulation. At the completion of the simulation, the data was exported from the BioFlo 320's trend screen for analysis.

3. Results and Discussion

Various settings around the default system PI values of P=5 (0.7 – 5) and I=0.3 (0.06 – 0.3) were tested. Results for the time profile simulation are shown in Figures 2 and 3.
The step functions used in the time profile approximated the oxygen demand created by an actual CHO culture. This method allowed us to simulate CHO culture oxygen demand sufficiently well to test different PI values efficiently. The basic PI tuning method does not require additional software or programming experience. In the CHO cell batch culture example above, values of P=2 and I=0.09 were found to be the most effective. These values reduced the fluctuations of the dissolved oxygen around the setpoint and minimized fluctuations in the gas flows.

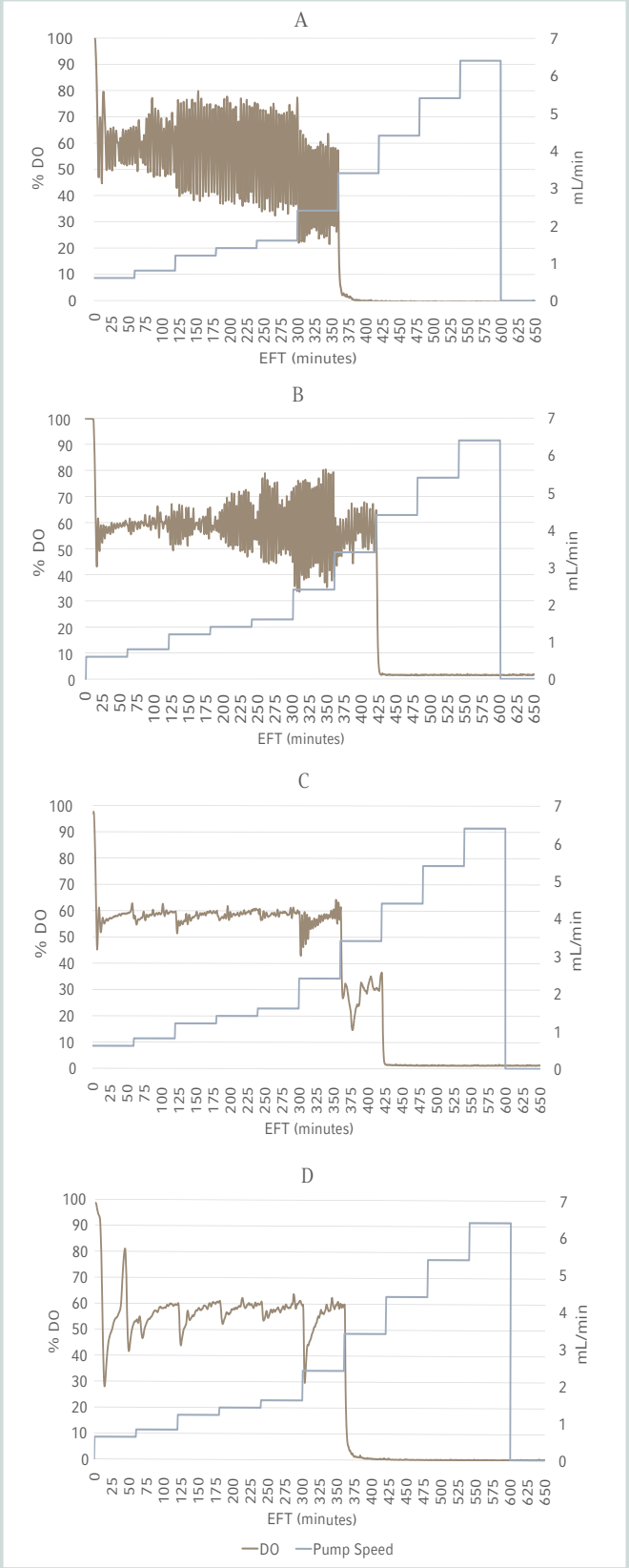

Fig. 2: Percent dissolved oxygen vs. sodium sulfite pump speed, time profile simulation. **A:** P= 5; I= 0.3 **B:** P= 3.5; I= 0.195 **C:** P= 2; I=0.09; **D:** P= 0.7; I= 0.06

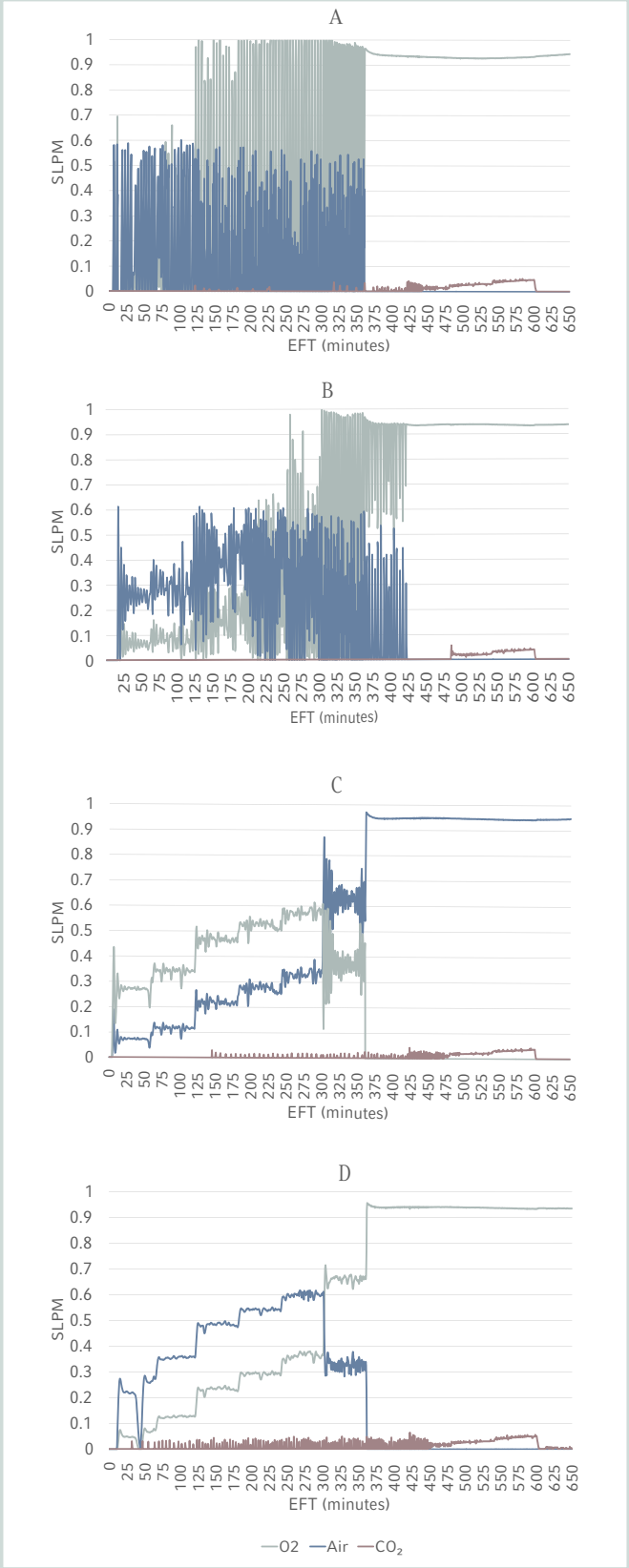

Fig. 3: Gas flows over time, time profile simulation.
A: P= 5; I= 0.3 **B:** P= 3.5; I= 0.195 **C:** P= 2; I=0.09; **D:** P= 0.7; I= 0.06

Advanced Simulation Procedure Using BioCommand®

4. Introduction

BioCommand block diagram programs can also be used to progressively add sodium sulfite solution to a bioreactor. This allows for more precise simulation of cell culture oxygen demand. The following protocol describes this procedure, again using CHO cells as an example.

5. Materials and Methods

The addition of sodium sulfite solution into the vessel was controlled by a BioCommand block diagram program, in contrast to the time profile used previously. Specifically, an oxygen demand curve was generated using recorded gas flow data from past CHO culture runs. The total oxygen gas flow over time O_2(total, SLPM) was derived from the amount of oxygen in the air flow A (SLPM) and the oxygen gas flow O_2 (SLPM) using the following formula:

$$O_{2,\,total} = 0.2095 \times A + O_2\ (SLPM)$$

The O_2 (total, SLPM) was plotted against the EFT (hours) and fitted using an exponential function. The two constants 0.0544 and 5.885 were obtained as a result of the curve fitting. The curve fitting included a time compression by a factor of 12, enabling a six-hour simulation to represent a 72-hour batch CHO culture process. A correction factor of 120 was further applied to account for the possible pump flow rates (PFR, mL/min) allowed by the built-in BioFlo 320 pumps:

$$PFR = 120 \times 0.0033 \times e^{5.88 \times EFT}\ (ml/min)$$

This equation was then entered into BioCommand using the block diagram program shown in Figure 4:

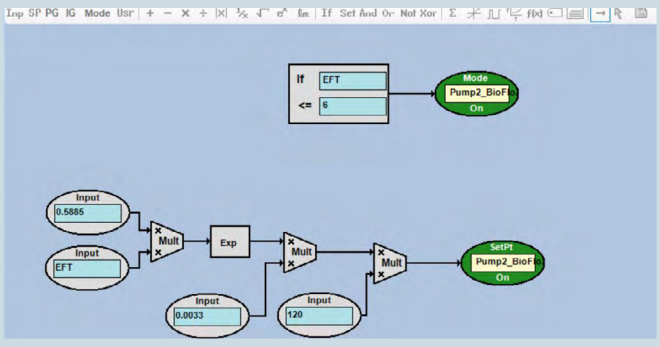

Fig. 4: BioCommand block diagram program for PFR.

The bottom portion of the script uses the equation to control the output of the sodium sulfite pump. The top portion of the script turns off the pump after 6 hours, ending the simulation.

The experimental setup was prepared as described in the preceding section, with the exception of the sodium sulfite solution. To fit the recorded gas flow data of a previously conducted CHO cell culture run, the concentration of the sodium sulfite solution was adjusted to call for a similar amount of sparging. By trial and error, a concentration of 0.1 M was determined to lead to a comparable gas flow. To create this concentration, 63 g of anhydrous sodium sulfite was added to 5 L of deionized water as described in the previous section.

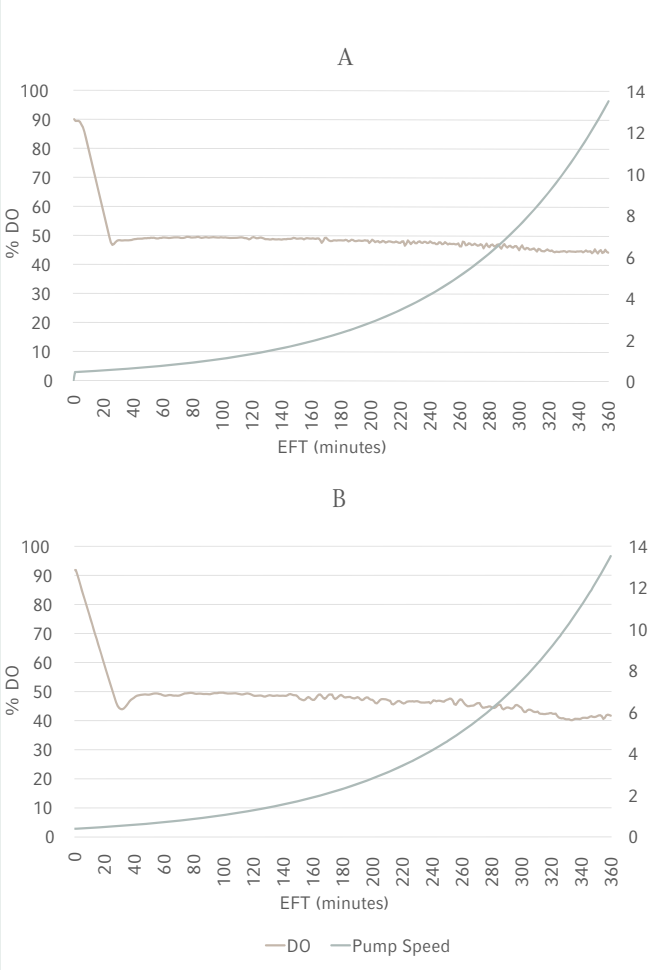

Fig. 5: Percent dissolved oxygen vs. sodium sulfite pump speed, BioCommand simulation. **A:** P= 2; I= 0.09 **B:** P= 0.7; I= 0.06

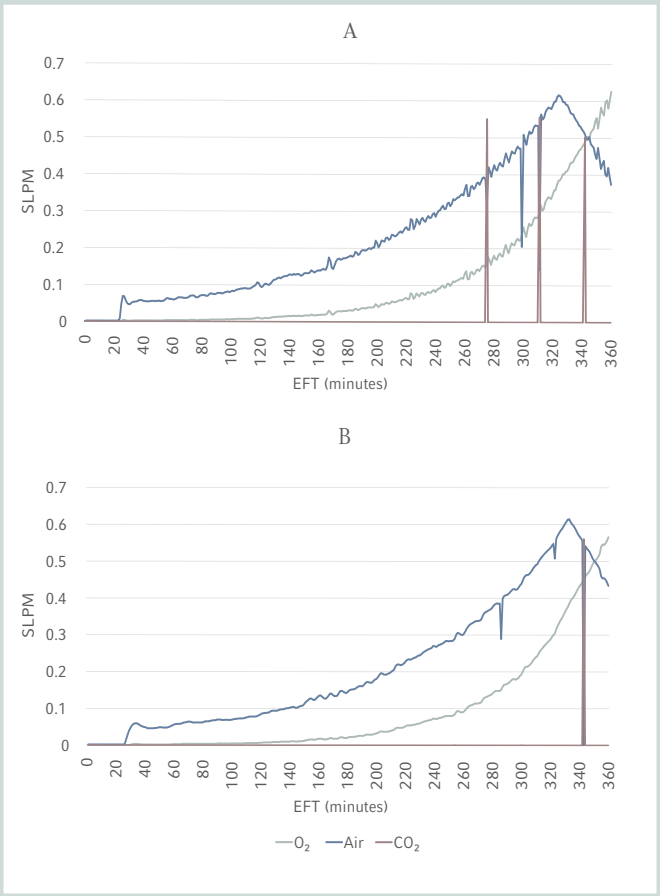

Fig. 6: Gas flows over time, BioCommand simulation.
A: P= 2; I= 0.09 **B:** P= 0.7; I= 0.06

6. Results and Discussion

Example results for the BioCommand simulation are shown in Figures 5 and 6. Various settings were tested to establish proof of concept. The use of a BioCommand block diagram program allows for more precise simulation of cell culture oxygen demand.

A comparison of Figures 5 and 6 to real CHO culture gassing data (Figure 7) demonstrates the feasibility of this method. By using an exponential equation to control the pump flow rate, operators can more accurately mimic oxygen demand during the initial and log phases of cell growth.

This example simulation was designed for CHO cell culture. But the methodologies described in this protocol can be easily adapted to any cell type or vessel size. Both simulation methods can be effective for tuning PI settings and optimizing controller response to cell culture or fermentation oxygen demand. However, a more aggressive model will need to be established to match the increased gassing demand from a fermentation process.

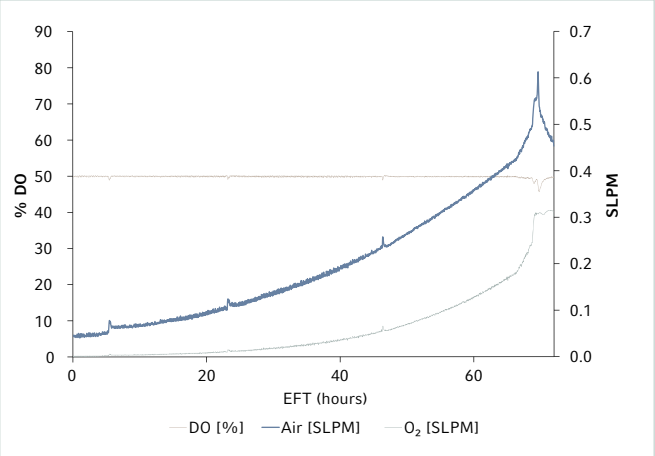

Fig. 7: Gas demand from an actual 10L CHO culture.

7. Literature

[1] How to Measure and Calculated OTR Using a New Brunswick™ Fermentor. 2013, Applications Training Document, Eppendorf. Inc.
[2] "A Guide to Calibration on the BioFlo 120 and BioFlo 320: Dissolved Oxygen Sensors." Short Protocol #40, Eppendorf. Inc.
[3] Eppendorf BioFlo 320 Operating Manual, Eppendorf. Inc.

Ordering information

Description	Order no.
BioFlo® 320, left-handed orientation/four front-mounted peristaltic pumps (3 @ 5-25 rpm/1 @ 20 -100 rpm)	1379963211
Tubing Kit, for BioFlo®/CelliGen® 310 and BioFlo® 320, all vessel sizes	M1287-9911
Overlay Gas Option Drawer, field-installed; 1 TMFC, 0.05 – 5 SLPM	M1379-5021
Cables	
pH sensor, analog	M1379-8104
DO sensor, analog	M1379-8106
Temperature sensor (RTD)	M1379-8100
Sensor cable, ISM	M1379-8108
Optical DO sensor, ISM	M1379-8107
Agitation motors	
Autoclavable cell culture vessels, magnetic drive	M1379-0750
BioBLU® 3c/5c/5p/10c/14c/50c	M1379-9931
Heat blankets	
BioBLU® 3c/5c/5p/3f	M1379-8116
Single-Use Vessel Bundle, for BioFlo® 320, for BioBLU® 3c/5c	M1379-0322
Vessel options	
BioBLU® 3c, Microsparger, 1x pitched-blade, Optical pH, 15 kGy β-irradiated	1386000100
BioBLU® 3c, Macrosparger, 1x pitched-blade, Optical pH, 15 kGy β-irradiated	1386000300
BioBLU® 3c, Microsparger, 2x pitched-blade, Optical pH, 15 kGy β-irradiated	1386120000
BioBLU® 3c, Macrosparger, 2x pitched-blade, Optical pH, 15 kGy β-irradiated	1386121000
Adaptor Kit: BioBLU® Single-Use Vessel, for BioFlo® 320; 100 – 240 V, BioBLU® 3c/5c/5p	M1386-9943
Exhaust Condenser, Peltier for 1 BioBLU® 3c/5c/5p Single-Use Vessel	76DGCONDSU5C
Software	
DASware® control (professional), incl. PC, OS without licenses, for Eppendorf bench-scale controller (BioFlo 120 and 320, BioFlo®/CelliGen® 115 and 310)	76DGCSX

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