

Basics of Benchtop Fermentor Operation for Growth of *E. coli*

Introduction

While use of shake flasks, T-flasks, or roller bottles have become the lab standard in most fermentation and cell culture research labs, lack of controls for pH, dissolved oxygen and other parameters cause an inherent limitation in cell yields and protein expression. When higher yields are needed, rather than inoculate, feed and maintain cultures using dozens or even hundreds of bottles or flasks, a benchtop fermentor or cell-culture bioreactor can be used to increase yields by 10-fold or more.

This Application Report describes a step-by-step procedure for growing *E. coli* in New Brunswick Scientific's BioFlo® 310 benchtop fermentor. The BioFlo 310 is a versatile, powerful system, designed for high-yield growth of a wide variety of organisms, ranging from yeast and bacteria to mammalian, plant, and insect cells. The system is available with interchangeable vessels, sized 2.2 – 14.0 Liters, and comes as a pre-packaged kit for ordering simplicity, or can be tailor-configured by selecting from dozens of standard options.

Materials and Methods

The Fermentor

A pre-configured BioFlo 310 Fermentor Kit with 7.5L vessel with Rushton blades (NBS Catalog Number M1287-1172) was used for an *E. coli* fed-batch fermentation. This system includes a 15" industrial color touchscreen controller to regulate agitation, temperature, pH, DO, Foam/Level, three pumps, gases and much more. A Thermal Mass Flow Controller (TMFC) for regulating gas flow in a range of 0.4 – 20 Standard Liters per Minute (SLPM) is also standard, and options for multiple mass flow controllers and/or other gas flow settings are available as standard options. In this run, an optional BioCommand® Plus supervisory software package, (NBS Catalog Number



The benchtop BioFlo 310 fermentor is a versatile research tool for optimizing cell growth and production from a wide variety of cell types, including microbes, yeast and fungi, as well as mammalian, insect and plant cell lines.

M1291-0000), was used to control the feed schedule and log data. To optimize your results, you can also connect and control numerous additional options directly to the BioFlo 310, including scales, analyzers and sensors for optical density (OD), CO₂, Redox and more. This paper presents a fermentation run that was not fully optimized to obtain the highest yields possible – rather it is meant to serve as a guide for basic procedures and materials.



Materials and Methods (continued)

Inoculum

The inoculum was prepared using LB broth at a 25 g/L concentration as the shake flask medium. The inoculum was cultivated overnight on an NBS orbital shaker at 240 rpm. OD600 was between 2 and 4 at the time of inoculation. Inoculum volume was 2 - 5% of the 5L working volume, or 250 ml.

Initial Medium Composition:

- Potassium phosphate monobasic, KH₂PO₄ 3.5 g/L
- Potassium phosphate dibasic, K₂HPO₄ 5.0 g/L
- Ammonium Sulfate, (NH₄)₂SO₄ 5.0 g/L
- Yeast Extract, (Tastone 900AG from Sensient) 5.0 g/L
- Antifoam (Brexo Foam Control Agent FMT 30) .. 1.00 ml/L

The initial medium volume was 3.5L, to allow space in the 5.7 L working volume vessel for components to be added after sterilization.

K-12 trace metals solution, consisted of :

- Sodium Chloride, NaCl 5 g/L
- Zinc Sulfate Heptahydrate, ZnSO₄·7H₂O 1 g/L
- Manganese Chloride Tetrahydrate, MnCl₂·4H₂O .. 4 g/L
- Ferric Chloride Hexahydrate, FeCl₃·6H₂O .. 4.75 g/L
- Cupric Sulfate Pentahydrate, CuSO₄·5H₂O ... 0.4 g/L
- Boric Acid, H₃BO₃ 0.575 g/L
- Sodium Molybdate Dihydrate, NaMoO₄·2H₂O .. 0.5 g/L
- 6N Sulfuric Acid, H₂SO₄ ~ 12.5 ml/L

After autoclaving and cooling the vessel, we added:

- Magnesium Sulfate Heptahydrate
25% solution added at 4 ml/L concentration
- Glucose 10g/L, usually added as a 50% solution
- K-12 trace metals
Solution added at 1 ml/L concentration
- Thiamine
added at concentration of 2.2mg/L from stock solutions
of varying concentration
- Calcium Chloride Dihydrate.....
added at concentration of 0.15g/L from stock solutions
of varying concentrations

Control Setpoints

Setpoints were keyed into the touchscreen controller prior to inoculation.

- Temperature 37°C
- pH 7.0
- Dissolved Oxygen 30%
- Agitation 200-1000 rpm (responding
automatically to oxygen demand)

Dissolved Oxygen (DO) Control

The DO probe was calibrated at 0%, obtained by briefly disconnecting the cable; and at 100%, obtained using 1000 rpm agitation and 5 L/m airflow rate. After calibration, DO remained at approximately 100% until inoculation.

An agitation, gas flow, and oxygen “cascade” was programmed into the controller to regulate the DO setpoint through automatic adjustment of agitation speed and gas flow rates. The term “cascade” means that if agitation alone could not be used to maintain the DO at setpoint, GasFlo control (air flow or combination of air and oxygen flow) will next be added. Finally, if agitation plus gas flow are not sufficient to maintain the DO setpoint, additional oxygen will enter the mix.

In our cascade control loop setup, shown on page 3, DO is expressed as a percentage – a DO output of 100% means that the DO setpoint has been fully attained. Until 100% DO is attained, the loop being cascaded will increase sequentially based on the minimum and maximum setpoints. So, when DO output reaches 50%, GasFlo is ramped up from 5 to 10 SLPM; and when DO output then reaches 90%, pure O₂ is added until the DO output reaches 100%.

Note:

- When the unit is configured with 1 TMFC, it includes 4 solenoid valves for the 4 gases.
- GasFlo loop indicates total Gas Flow rate in SLPM, measured via TMFC.
- Air, O₂, N₂, CO₂ loops are measured in % via solenoid valves and always total up to 100%

To set up the cascade:

- Press **Casc** tab from the bottom of the screen.
- Select **DO** from dropdown menu next to “Cascade From”
- Select **Agit, Gasflo and O₂** as listed on next page:
- Select **Yes**, to activate the cascades



DO is cascaded to Agitation, GasFlo and Oxygen:

To	Start Setpoint	@ DO Start Out%	End Setpoint	@DO End Out%
Agit	200	0	1000	50
GasFlo	5	50	10	90
O2 (2)	0	90	100	100

Note:

- Regardless of DO Start Output %, the setpoint of any cascade loop will never go below its minimum setpoint value, (ie. Agitation will never drop below the starting setpoint of 200 rpm)
- Likewise, regardless of DO Output %, the setpoint of any cascade loop will never rise above its maximum setpoint, (ie. Agitation will not rise above 1000 rpm)
- Start Output % corresponds to the minimum value that will produce the minimum setpoint; lower outputs will not affect setpoint.

pH Control

We used liquid base to maintain pH at setpoint, relying on the acid-producing culture to lower pH if needed. The pH control parameters were:

- Base ammonium hydroxide, 30% solution
- Pump Pump 1
- Transfer tubing Narrow bore silicone tubing (supplied)
- Vessel inlet Triport adapter in the vessel headplate.

Pump Calibration: (To assure the most accurate flow rate, calibrate the pump each time you change tubing)

1. Use a sample tubing (about 2 feet long) of each line attached to a pump head.
2. Insert the tubing into the appropriate pump head.
3. Set the pump assignment to "None".
4. Set the setpoint to "10%".
5. Record the quantity of water flowing into a graduated cylinder for a definite time period.
6. Repeat for setpoints of 20%, 30%, etc., to 100% in order to establish the flow rates.

Controller Setup:

1. Pump 1 Select "BASE" in "Assignment"
2. Pump 2 Select "Acid" in "Assignment"

To set up the cascade:

Press **Casc** tab from the bottom of the screen.

Select **pH** from dropdown menu next to "Cascade From"

Select **Pump 1 and Pump 2** as listed below:

To	Start Setpoint	@ DO Start Out%	End Setpoint	@DO End Out%
Pump 1	0	0	100	100
Pump 2	0	0	50	-100

Results

This *E.coli* fermentation run was for 22 hrs; controlled temperature at 37°C, pH at 7.0, and DO at 30%. The initial agitation speed was set at 200 rpm, and later controlled by the DO cascade control loop. After five hours batch culture, a fed-batch schedule was initiated, as shown in Figure 1.

BioCommand program automatically activated the feed pump when the pH value spiked above 7.1, which indicated a carbon source limitation, after 5 hours of culture. As per Figure 2, DO and pH were well controlled. The total feed volume of nutrients was 600 mL in the fed-batch process, as shown in Figure 3. *E.coli* dry cell weight (DCW) of 22.5 g/L DCW was achieved in the BioFlo 310 fed-batch fermentation.

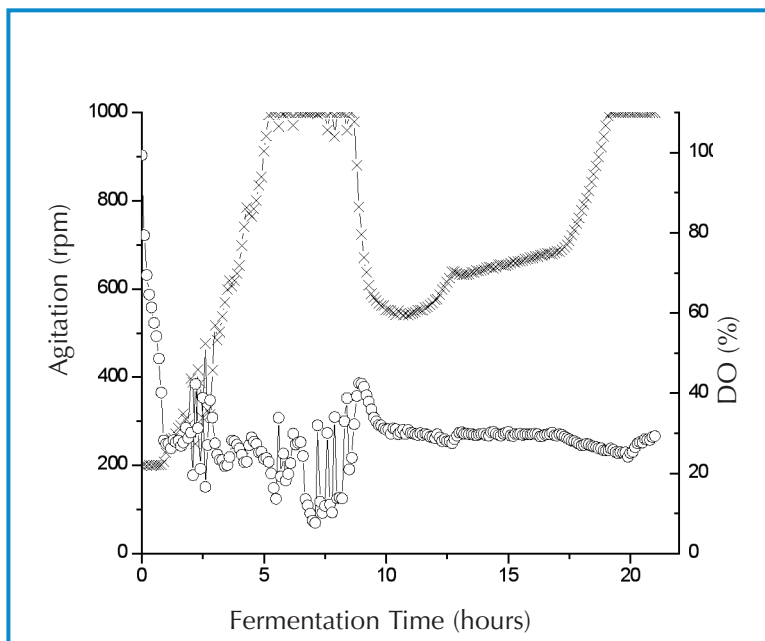


Figure 1. Time profiles of agitation speed and DO in *E. coli* fermentation



Results continued

Finally, it should be noted that the BioFlo 310 fermentor has the ability to simultaneously control over 120 process loops — 32 loops per vessel, and four vessels simultaneously, making it an extremely powerful research tool. This application report, however, does not take advantage of the full potential of the 310, and is intended only to provide a basic understanding on how to get started.

For further information about the BioFlo 310's capabilities, or for downloadable specs and technical papers, see www.nbsc.com/btaa.htm.

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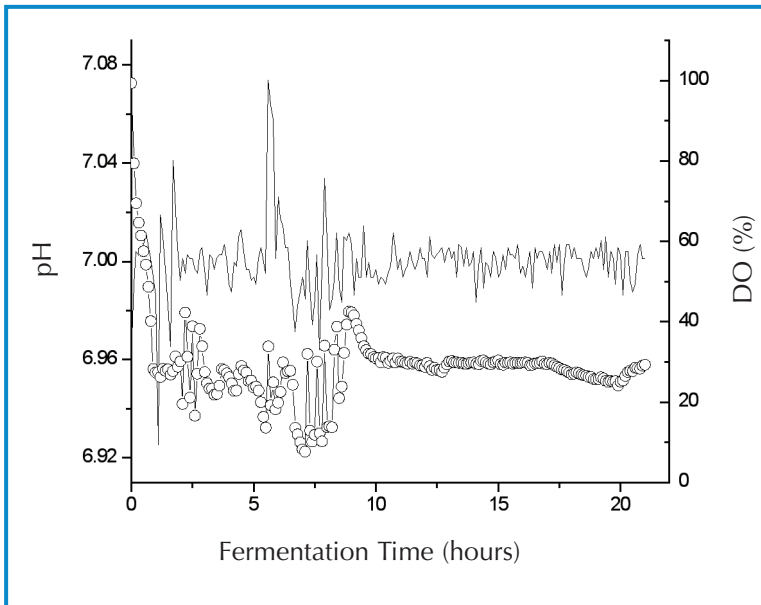


Figure 2. Time profiles of pH and DO measurements

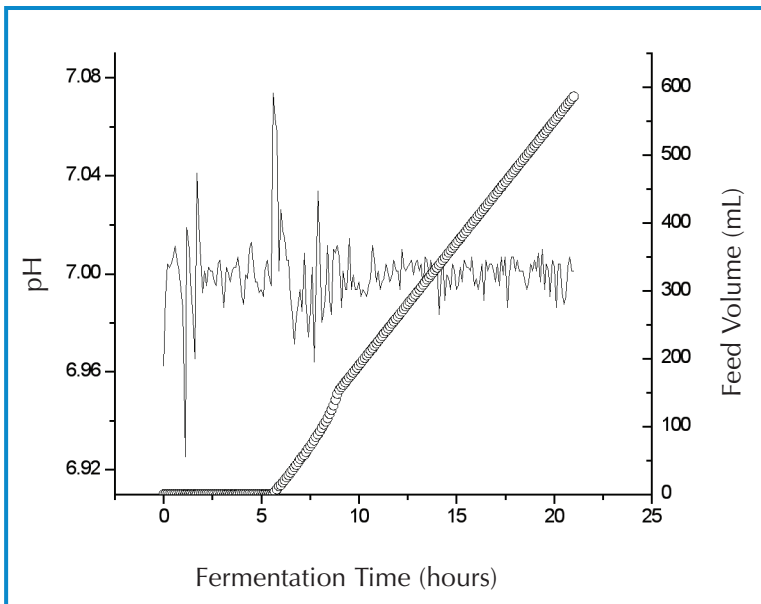


Figure 3. Media feed volume and pH control

