High-Density *Escherichia coli* Batch and Fed-batch Fermentation Using BioBLU® 3f Single-Use Vessels

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Abstract

Single-use bioreactors are lightweight, easy-to-use, labor-saving, and cost effective compared to the traditional glass vessels. The new Eppendorf BioBLU 3f Single-Use Vessels are equipped with a single stainless steel cooling finger which has been shown to have high cooling efficiency. In this study, we grew the *E. coli* strain ATCC® 25922GFP™ in BioBLU 3f Single-Use Vessels controlled by BioFlo® 320 bioprocess control station in duplicates using three different fermentation methods: simple batch, batch with chemically defined medium, and fed-batch. Temperature, pH, and dissolved oxygen were controlled in the same way among all experiments. In the simple batch fermentation, we used modified Terrific Broth (Thermo Fisher Scientific®, USA) as the medium and the highest OD_{600} was 11. In the second batch method and fed- batch fermentation, we prepared chemically defined medium with glucose enrichment. The highest OD_{600} was 76 for the batch fermentation which was reached right after glucose depletion. In fed-batch fermentation, we reached a very high OD_{600} of 240 with an exponentially increasing medium feeding speed. These experiments proved the feasibility of using BioBLU 3f with a single stainless steel cooling finger for high density *E. coli* fermentation. The fermentation biomass exceeded our previous internal record set by a 3 L glass vessel.

Scope

Evaluating the performance of the BioBLU 3f Single-Use Vessel in high-density *E. coli* fermentation.

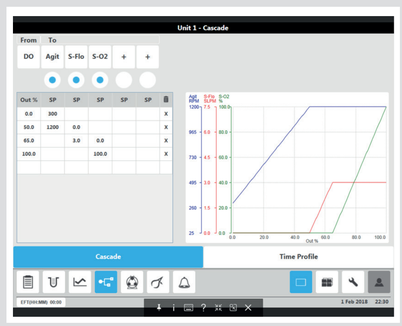
Bioprocess system

BioBLU 3f Single-Use Vessel
Specifically designed for microbial applications.
> Reduced turnaround times between runs, because no cleaning and sterilization is required
> Scalability through industrial design
> Rigid-wall design reduces potential for vessel damage
> Autoclavable material, allowing medium sterilization within the vessel

Process parameters common to all runs

Parameter	Setpoint/control
Vessel	BioBLU 3f
Working vlume	3 L
Inoculation density	5 % (v/v)
Dissolved oxygen (DO)	30 %
Sparger	Macrosparger
Gassing control	Automatic gas flow and mix, controlled by DO cascade
Temperature	37 °C, controlled with stainless-steel cooling finger (cooling water: 18 °C)
Impeller	3x Rushton-type impellers
pH	7.0 ± 0.1; controlled by 25 % (v/v) ammonium hydroxide solution

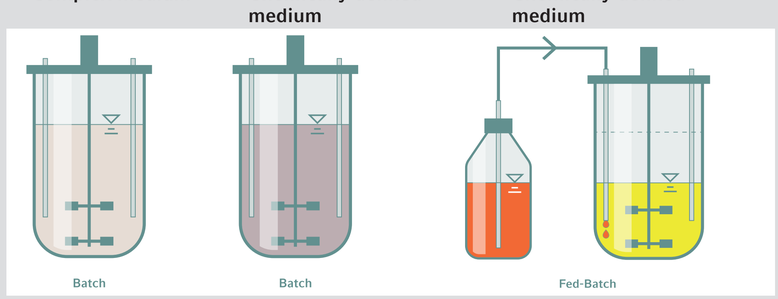
Bioprocess system
BioBLU 3f Single-Use Vessel controlled by BioFlo 320 bioprocess control station.



Cascade for DO control

Cultivation strategies

1. Batch/ Complex medium | **2. Batch/ Chemically defined medium** | **3. Fed-batch/ Chemically defined medium**



Batch fermentation
With microbial growth, the nutrients are consumed, and the culture environment is continuously changing. Normally the growth curve includes lag phase, exponential growth phase, stationary phase, and death phase.

Fed-batch fermentation
After inoculation, the nutrients are added to the fermentor in increments throughout the duration of fermentation to feed the microorganism. The exponential growth phase and stationary phase can be extended with extensive biomass accumulation.

Complex medium
Terrific Broth supplemented with glycerol. Used as the inoculation medium in all runs and as the fermentation medium in basic batch fermentation.

Chemically-defined medium
Citrate phosphate buffer + 90 g/L glucose + $MgSO_4$ + thiamine + trace elements. Used in glucose-enriched batch fermentation.

Chemically-defined medium
Citrate phosphate buffer + 15 g/L glucose + $MgSO_4$ + thiamine + trace elements. Used in fed-batch fermentation.

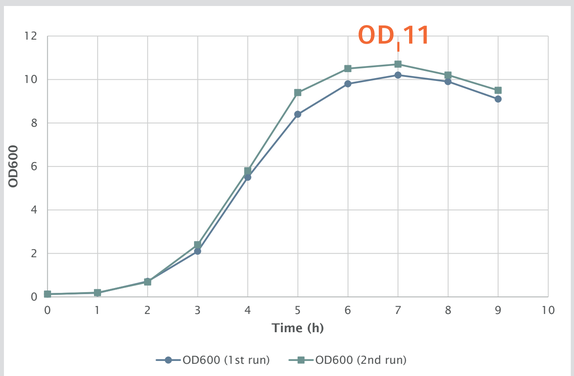
Feeding medium
Concentrated medium composed of 614 g/L glucose, $MgSO_4$, thiamine, and trace elements. Used in fed-batch fermentation.

***E.coli* strain: ATCC 25922GFP**

Inoculum preparation
Mini cell bank in 2 mL cryovial ➡ Overnight culture in 500 mL baffled shake flask ➡ Inoculation of bioreactor (3 L)

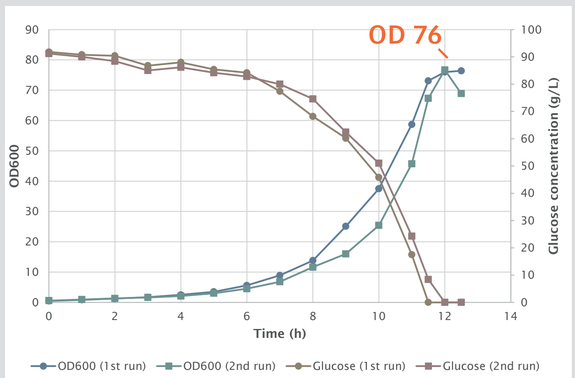
Results

1. Batch/Complex medium



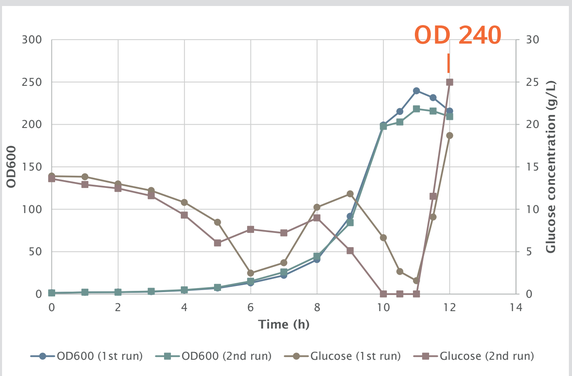
> Cascade for DO control: No O_2 enrichment throughout the fermentation
> Standard growth curve with distinct lag, exponential growth, stationary, and death phases
> Simple operation with less risk of contamination

2. Batch/Chemically defined medium



> Cascade for DO control: O_2 got enriched up to 40 %
> A relatively extended lag phase
> The exponential growth of *E. coli* was accompanied by the drastic glucose consumption
> After glucose depletion, bacteria growth stopped

3. Fed batch/ Chemically defined medium



Elapsed fermentation time (hh:mm)	Pump speed for feeding (mL/min)
5:15	0.5
6:15	0.8
6:16	0.3
7:00	0.8
7:01	0.9
7:30	1.4
9:00	2.6
9:30	3.2
10:00	4.1
10:30	4.8
11:00	5.6
11:30	6.8
12:00	8.0

> Cascade for DO control: O_2 got enriched up to 100 %
> Feeding was initiated before glucose depletion and triggered robust exponential growth
> With the exponential increment of feeding rate, a significant accumulation of biomass was observed corresponding to more than 150 OD_{600} increase within 2 hours
> Glucose was depleted or almost depleted when the maximum OD_{600} was reached, then started to accumulate during death phase
> More complex and time-consuming handling before and during fermentation

Conclusion

> Single-use BioBLU 3f vessels are lightweight, easy-to-use, labor-saving, and cost effective compared to the traditional glass vessels
> Stainless steel cooling fingers have high cooling efficiency which are suitable for temperature control in high density microbial fermentation
> Maximum OD_{600} increased from 11 to 76 when we switched the medium from Terrific Broth to chemically defined medium enriched with glucose
> In fed-batch fermentation, we successfully reached a record high OD_{600} of 240 with an exponentially increasing medium feeding rate
> Medium composition, cultivation mode, and process control contribute to shaping the growth curve in fermentation

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