Comparing Culture Methods in *Escherichia coli* Fermentation: Batch, Fed-Batch, and Continuous

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Abstract

In this study, three different culture methods, batch, fed-batch, and continuous fermentation, were carried out to grow *E. coli* (ATCC® 25922GFP™) in the Eppendorf BioBLU® 3f Single-Use Vessel controlled by the BioFlo® 320 bioprocess control station. The objectives of this study were (1) to evaluate bioprocess parameter setup, vessel preparation, and compare how different culture methods affect microbial growth, nutrient consumption, productivity, and yield; (2) to provide a detailed cost analysis for growing *E. coli* under different fermentation modes and further compare their pros and cons; and (3) to demonstrate the strong performance of BioBLU 3f Single-Use Vessel in high-density microbial applications. Batch fermentation is a relatively easy operation with competitive biomass yield on glucose, and it can be applied in early development for process optimization including medium selection. With a feasible feeding strategy, fed-batch fermentation gave the highest cell dry weight at 85 g/L among all culture methods in this study, and it was very cost-effective per unit biomass. In continuous fermentation, feeding and harvest take place simultaneously at the same rate. It has the potential to achieve steady state to greatly reduce the downtime, and it is scale-up friendly since the working volume is kept constant. Therefore, depending on the experimental needs and the laboratory settings, and with a brief estimate of the process budget and scheduling, this study can help fermentation scientists to choose the ideal culture method to meet their unique needs.

Scope

How to choose the ideal fermentation method.

Bioprocess system

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

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

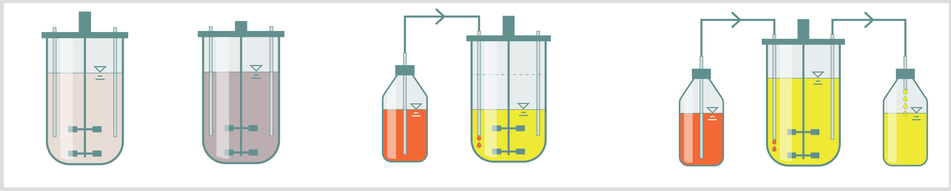
Process parameters common to all runs

Parameter	Setpoint/control
Vessel	BioBLU 3f
Inoculation density	5 % (v/v)
Dissolved oxygen (DO)	30 %
Agitation	Magnetic drive; controlled by DO cascade; maximum 1,200 rpm
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

Cascade for DO control

BioBLU 3f Single-Use Vessel

Cultivation strategies



Batch fermentation
With microbial growth, nutrients are gradually consumed, by-products accumulate, and the culture environment gradually changes. The growth curve is usually divided into lag phase, exponential phase, stationary phase, and death phase.

Fed-batch fermentation
After inoculation, the microorganisms are grown under batch regime until the nutrients are consumed. Fresh nutrients are added in increments throughout the remaining duration of fermentation to feed them. The exponential and stationary growth phase can be extended with extensive biomass accumulation.

Continous fermentation
Fresh medium is continuously added to the fermentor, while used medium and cells are harvested at the same time. Consumed nutrients are replaced, and toxic metabolites are removed. When addition and removal are at the same rate, the culture volume stays constant.

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 and continuous fermentation.

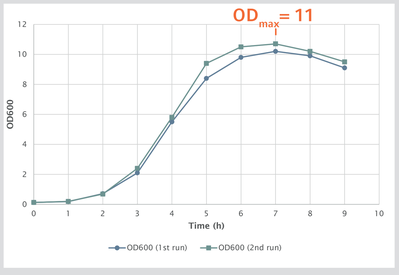
Feeding medium
Concentrated medium composed of 614 g/L glucose, $MgSO_4$, thiamine, and trace elements. Used in fed-batch fermentation.

***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)

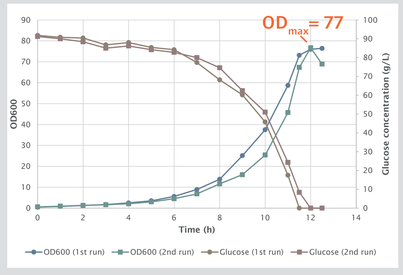
Results

1. Batch/Complex medium



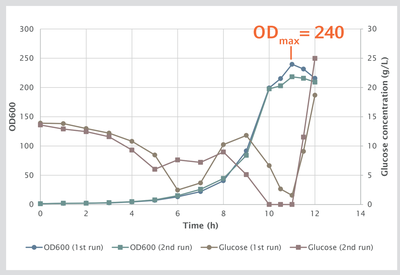
> Cascade for DO control: No O_2 enrichment throughout the fermentation
> Standard growth curve with distinct lag, exponential growth, stationary, and death phases
> Simple operation with low risk of contamination

2. Batch/Chemically defined medium



> Cascade for DO control: O_2 got enriched up to 40 % (data not shown)
> A relatively extended lag phase
> The exponential growth of *E. coli* was accompanied by drastic glucose consumption
> After glucose depletion, bacteria growth stopped

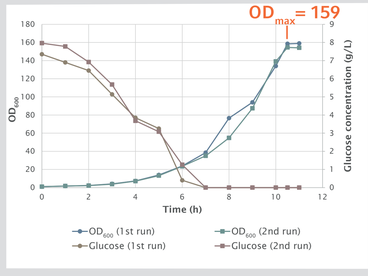
3. Fed batch/Chemically defined medium



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 % (data not shown)
> 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 an OD_{600} increase of more than 150 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

4. Continuous/Chemically defined medium



Elapsed fermentation time (hh:mm)	Pump speed for feeding (mL/min)
3:30	0.2
4:30	0.4
5:30	1.2
6:30	1.2
7:00	1.6
7:30	2.0
8:00	2.6
8:30	3.1
10:00	5.1
11:30	8.0

> Feeding was initiated before glucose depletion
> Broth harvesting was initiated simultaneously with feeding at the same speed ensuring a constant culture volume throughout the run
> Glucose depletion occured at t = 7 h and remained depleted
> Oxygen demand increased to 100 % at t = 10.5 h (data not shown)
> The OD_{600} of the harvested broth was 95
> Growth control via control of nutrient supply is possible

Summary

Operation mode	CDW[1] per vessel at the end of the run (g)	Total volume (L)	Volumetric biomass productivity (g/(L*h))	Total glucose consumption (g)	Biomass yield on glucose (g CDW/g glucose consumed)	Maximum OD_{600}	Maximum CDW (g/L)	Cost per unit CDW ($/g)
Batch, complex medium	9.87	3	0.37	N/A		11	3.9	159.9
Batch, chemically defined medium	77.16	3	2.2	273.6	0.282	77	27.3	25.5
Fed-batch	225.6	3	6.27	713.7	0.316	240	85.0	9.3
Continous	210.0	4.3[2]	4.44	846.0	0.248	159	56.3	9.7

[1]CDW, cell dry weight
[2]In continuous fermentation, the biomass is collected from the culture in the vessel (3 L) and harvest (1.3 L)

Conclusion

> BioBLU 3f Single-Use Vessels are lightweight, easy-to-use, labor-saving, and cost-effective compared to the traditional glass vessels.
> Batch fermentation is the easiest and cheapest fermentation mode, but considering the unit cost per CDW, fed-batch fermentation was the most economic mode, for this case study.
> For batch fermentation, a switch of the medium composition increased the maximum OD_{600} from 11 to 77.
> With the very cost-efficient fed-batch fermentation, we successfully reached the highest OD_{600} of 240 with an exponentially increasing medium feeding rate.
> A maximum OD_{600} of 159 was reached with the economically competitive and scale-up friendly continuous fermentation method.
> Depending on the experimental needs, the laboratory settings, and a brief estimate of the process budget and scheduling, the ideal fermentation method should be chosen.
> Medium composition, cultivation mode, and process control contribute to shaping the growth curve in fermentation.

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