APPLICATION NOTE No. 251 I October 2011

Introduction to *Escherichia Coli* Cultivation in a Stirred-Tank Bioreactor

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Abstract

This applications report presents a simple protocol for achieving high-density culture of *Escherichia coli (E. coli)* cells using an Eppendorf benchtop, autoclavable stirred-tank bioreactor.

Introduction

E. coli is a gram-negative, non-spore-forming, facultative anaerobe that has had a long history in the world of biotechnology and laboratory work due to its ease of manipulation and well-understood genetic system. Its fast doubling time of 15 - 20 minutes makes it an excellent culture for research and production.

Materials and Methods

Medium

E. coli K12 medium is prepared and poured into the vessel for a fermentation run. Some of the vessel volume should be reserved for components, including inoculum, and other temperature-sensitive material which will be added after sterilization.

Initial medium composition	
Potassium phosphate monobasic (anhydrous)	2 g/L
Potassium phosphate dibasic (anhydrous)	3 g/L
Ammonium phosphate dibasic (anhydrous)	5 g/L
Yeast extract (Tastone® 900AG)	5 g/L
Anti foam (Breox® FMT 30)	0.35 - 0.4 g/L

After autoclaving and allowing time for the vessel to cool addition of temperature-sensitive material can be made:

Medium composition	
Glucose (50 mL/L of 50% sterile glucose solution)	25 g/L
Magnesium sulfate heptahydrate	0.5 g/L
Thiamine	1 mg/L
K12 trace metals solution*	5 mL/L

* 5 g/L sodium chloride, 1 g/L zinc sulfate heptahydrate, 4 g/L manganese chloride tetrahydrate, 4.74 g/L ferric chloride hexahydrate, 0.4 g/L cupric sulfate pentahydrate, 0.575 g/L boric acid, 0.5 g/L sodium molybdate dihydrate, 12.5 mL/L 6N sulfuric acid. Note that the quantity of sulfuric acid can vary as required to dissolve the other components. The usual range is between 8 – 20 mL.

Inoculum

Inoculum is prepared using LB broth at 25 g/L.

Typical control setpoints for *E. coli*

The setpoints for *E. coli* are entered into the bioprocess control software prior to inoculation. Except for dissolved oxygen (DO) which often remains high, the medium should be allowed to equilibrate prior to inoculation. DO remains high because it takes time for O_2 to permeate out of the medium as it is not yet being utilized by the culture. An initial DO value of approximately 100 % is acceptable; it will

decrease as the culture metabolizes it.

Setpoints are commonly controlled in either automatic mode or via a cascade.

Parameter	Setpoint
Temperature	37 °C
pH	7.0
Dissolved oxygen	35 %
Agitation	300 - 1200 rpm*

* Agitation ranges will vary depending on the vessel size and the type of impeller used.

Dissolved oxygen (DO) control

DO control often includes the use of a cascade for agitation, air flow or oxygen flow, individually or in combination. Cascades allow the controller to maintain setpoints by automatically adjusting other process loops. The inclusion of oxygen flow in a cascade may help to increase the overall culture density.

Typical DO cascade	Minimum	Maximum
Agitation	300 rpm	900 rpm
Air flow	0.5 VVM	1 VVM
Oxygen flow	0.5 VVM	1 VVM

VVM: Vessel volume per minute

DO calibration

The DO probes are calibrated using a standard two-point calibration method: 0 % (often referred to as Zero) and 100 % (commonly referred to as Span). The 0 % calibration can be achieved two separate ways: Either with an electronic Zero, obtained by briefly disconnecting the cable from the control station, or by sparging nitrogen into the medium at approximately 1 VVM (vessel volume per minute) until the value stabilizes near zero. The 100 % calibration is achieved by increasing agitation to approximately 200 to 400 rpm and increasing air flow to 0.5 to 1 VVM . These values may need to be adjusted to fit the operational specifications of your controller. DO should be calibrated after autoclaving and the probe should be allowed to polarize for approximately 6 hours after being connected to the controller. After calibration, DO may remain at approximately 100 % until after inoculation.

pH control

pH control often uses the addition of liquid acid and liquid base solution to maintain pH at setpoint, but often it relies on the acid/base-producing properties of the culture or medium for a natural drift up or down. *E. coli* pH control is usually done through the addition of base (10 % NaOH). Typically systems will allow the user to assign pumps a specific function such as acid or base. When a deviation in pH calls for an adjustment, the specified pump will turn on until the deviation no longer exists.

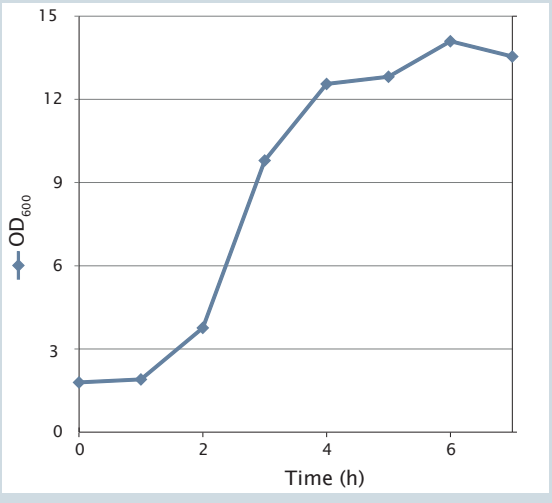
pH calibration

pH calibration is usually done outside the vessel using a two-point calibration method and standard buffers. Buffer 7.0 is commonly used for the Zero and either 4.0 or 10.0 is commonly used for the Span. pH is calibrated prior to autoclaving.

Results

Figure 1 shows a typical growth curve in a batch process. Depending on the process uses and the parameters selected, high-density cultures can be achieved in as little time as 8 hours.

Fig. 1: *E. coli* growth curve



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