APPLICATION NOTE No. 253 I October 2011

Introduction to Pichia Pastoris Culture in a Stirred-Tank Bioreactor

Richard Mirro, Eppendorf Inc., Enfield, CT, USA.
Contact: becken.u@eppendorf.com

Abstract

This Applications Report presents a simple protocol for achieving high-density culture of *Pichia pastoris (P. pastoris)* cells using a New Brunswick benchtop, autoclavable stirred-tank fermentor or bioreactor.

Introduction

Pichia pastoris is a methylotrophic yeast which provides a unique expression system for producing high levels of recombinant proteins, including enzymes, protease inhibitors, single-chain antibodies, and regulatory proteins at various levels. *P. pastoris* is an excellent culture for research and production because it offers a low-cost expression system similar to *E. coli* or other prokaryotic cultures, combined with the post-translational modifications found in eukaryotes.

Materials and Methods

Medium

P. pastoris medium is composed of both temperature-sensitive and non-temperature-sensitive materials. Therefore inoculum should be prepared in two steps. Start by mixing and autoclaving the following:

Initial medium composition	Concentration
Calcium sulfate dihydrate	0.93 g/L
Potassium sulfate	18.2 g/L
Magnesium sulfate heptahydrate	14.9 g/L
Potassium hydroxide	4.13 g/L
Phosphoric acid	26.7 mL/L
Glycerol	40.0 g/L
Antifoam (Breox® Foam Control Agent FMT 30)	1.0 mL/L

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

Medium composition	Quantity
Trace metals solution, PTM1*	4.6 mL/L
Base, to adjust the initial pH	25 mL/L
Inoculum	200 mL
Glycerol**	400 mL
Methanol**	< 2 L
Base to maintain pH at setpoint	< 250 mL

* 6 g/L cupric sulfate pentahydrate, 0.08 g/L sodium iodide, 3 g/L manganese sulfate monohydrate, 0.5 cobalt chloride (anhydrous), 20 g/L zinc chloride (anhydrous), 0.02 g/L boric acid, 0.2 g/L sodium molybdate dihydrate, 65 g/L ferrous sulfate heptahydrate, 0.2 g/l biotin, 30 mL/L 6N sulfuric acid

** Added as required.

Inoculum

Inoculum is prepared using *Pichia* shake-flask growth medium. The inoculum was cultivated for 40 hours at 28° C in a shaker (New Brunswick model G25) running at 240 rpm. Optical density at 600 nm (OD_{600}) was 10.94 at inoculation.

Typical Control Setpoints for Pichia

Control setpoints for *P. pastoris* should be entered and achieved prior to inoculation and, 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 auto mode or via a cascade.

Parameter	Setpoint
Temperature	30° C
pH	5.0
Dissolved oxygen	30 %
Agitation	300 - 1200 rpm*

* Agitation limits may differ depending on system and can be set to respond automatically to oxygen demand.

Nutrient feed

P. pastoris exhibits a well-known carbon-exhaustion indicator often called a "DO Spike". This sudden increase in DO can be used to determine nutrient feed rate. Often *P. pastoris* cultures are initially fed methanol which is substituted by glycerol after the first set of DO spikes. These feeds can often be achieved automatically by using supervisory software.

Dissolved Oxygen (DO) Control

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

Typical DO cascade	Minimum	Maximum
rpm	300	1200
Air flow	0.5 VVM	1 VVM
Oxygen	0.5 VVM	1 VVM

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 media 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 - 400 rpm and increasing airflow to 0.5 - 1 VVM . These values may need to be adjusted to fit the operational specifications of the 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 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. *P. pastoris* pH control is usually done through the addition of base (30 % 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

A typical growth curve in a batch process sis represented in Figure 1.

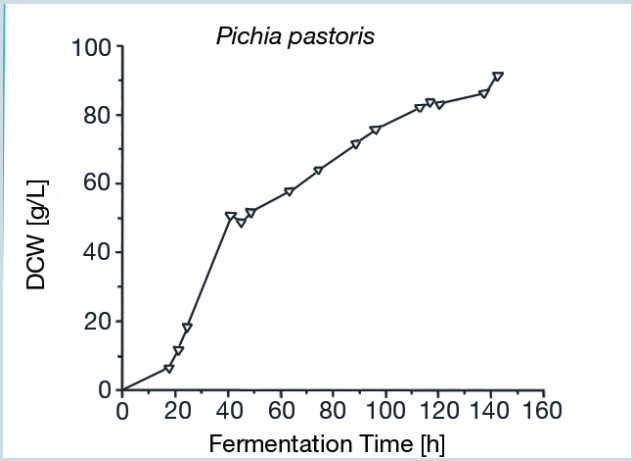

Fig. 1: *Pichia pastoris* growth curve

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com

www.eppendorf.com