Fermentation of Microbial Cells using StirCradle™ : Transition from Shake flask to Bench-top Bioreactor

Mridul SARKER, Richa JAKHETIA, Rinnie CHING and Xiangliang LIN

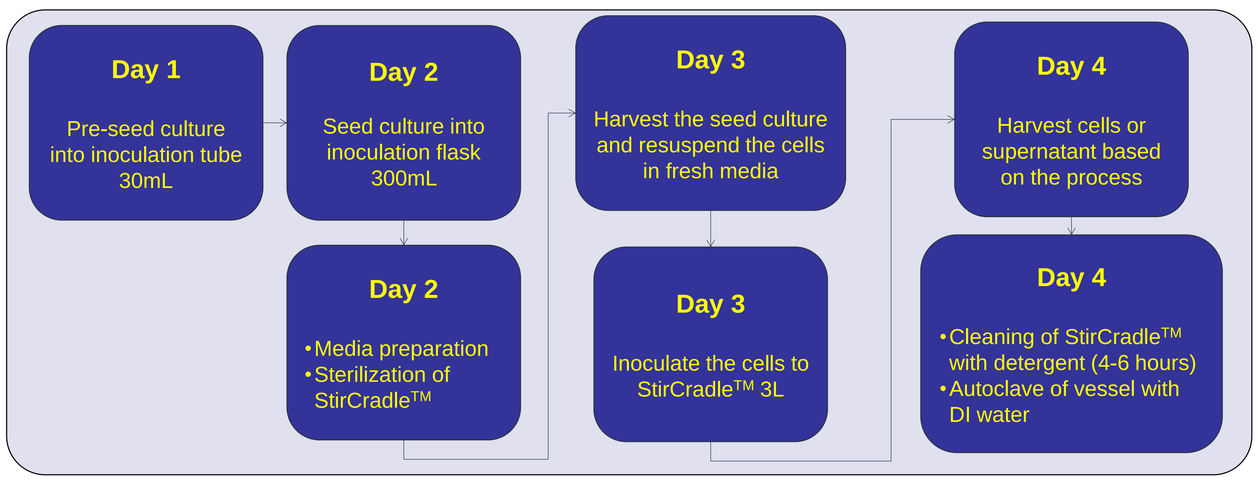
Introduction

Microbial cells are considered the workhorse of modern biotechnology. The applications of microbial cells are ranging from wine fermentation to biofuel production to producing biologics for vaccine production or gene therapy. In the past few decades, technological advancement has made microbial fermentation an exceptional choice for protein production compared to mammalian cell platform. Instruments and tools have also adopted a higher level of sophistication and broader applications.

Esco Aster has developed **StirCradle™**, a benchtop fermenter, by combining the vessel, controller and peristaltic pumps into one integrated system. The **StirCradle™** is ideal for research and development involving microbial cells, plant cells and animal suspension cells. Esco Aster also provides microbial upstream and downstream process development services to produce plasmid DNA, recombinant proteins, and other biologics using the **StirCradle™** Platform.

This study compares the growth pattern and biomass of recombinant microbial cells (*Escherichia coli* and *Pichia pastoris)* cultured in a shake flask and in a benchtop **StirCradle™** (10L).

Timeline for Microbial Batch Fermentation in StirCradle™



Cells, Media and Materials

Microorganism	Strain	Fermentation Process	Media Composition	Feeding Media
E. coli	XL-1 blue	Fed-batch Fermentation, Complex media	Complex medium containing Terrific Broth Modified and 0.8 % (v/v) glycerol	Bolus feeding, Feed: 50% Glycerol, final concentration: 0.8 % (v/v) glycerol
P. Pastoris	GS115	Batch culture, YPD media	Complex media containing 2% Yeast extract, 1% Peptone and 2% glucose	N/A

Preparation of StirCradle™

After Sterilization

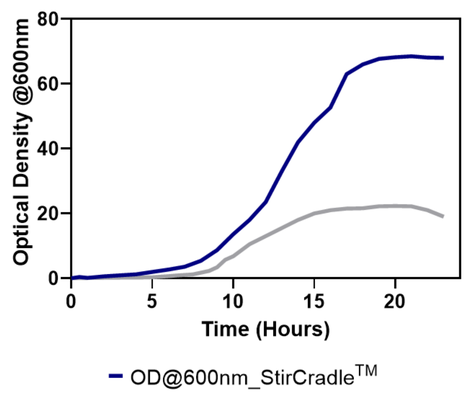
During Fermentation

Process Parameters in Fermentation

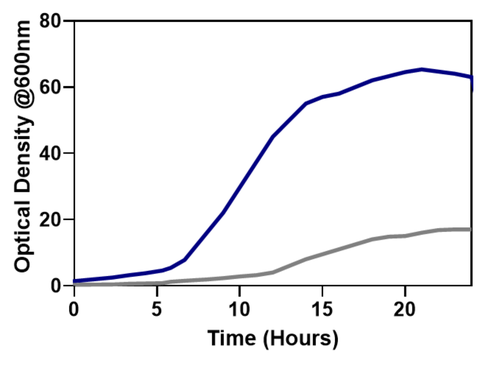
Parameter	Shake Flask Configuration		Stircradle™ Configuration	
	E. coli	*P. pastoris*	*E. coli*	*P. pastoris*
Vessel	Shake flask (500mL)		StirCradle™ 10L vessel (Pyrex glass)	
Inoculation Density	10% (v/v)		10% (v/v)	
Dissolve Oxygen (DO)	Not Controlled		30%	25%
Agitation	200 rpm		Controlled by DO cascade (AGIT) 50-1200 rpm	Controlled by DO cascade (AGIT) 50-1200 rpm
Gassing	Not Controlled		5-10 Lpm	5-10 Lpm
Temperature	37°C	30°C	37°C, Controlled by heating plate at the bottom	30°C, Controlled by heating plate at the bottom
pH	Not Controlled		Not Controlled	
Impeller	Not applicable		2x Rushton Turbine Impeller	
Sparger	Not applicable		Ring Sparger	

Growth Pattern of *E. coli* and *P. pastoris*

***E. coli* Fed-batch fermentation in Shake flask vs StirCradle™**



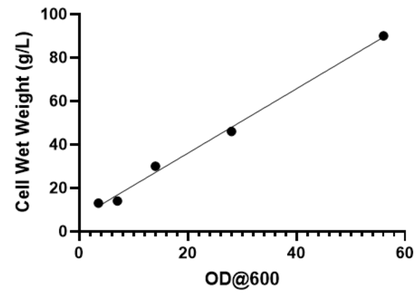
***P. pastoris* Batch fermentation in Shake flask vs StirCradle™**



Biomass Calculation

Biomass or Cell Wet Weight of *P. pastoris* has been estimated by creating a standard curve by measuring the wet cell weight at various optical density (OD) measured at 600nm UV wavelength.

Satndard Curve for Biomass Calculation of *P. pastoris*



Cell Wet Weight (g/L) = Slope x OD_{600} = 1.5 x OD_{600}

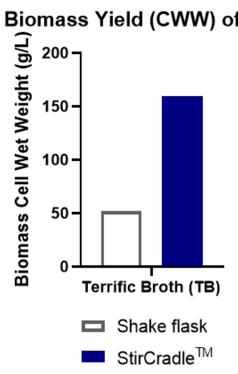
Using the same method, the relationship between *E. coli* Cell Wet Weight and OD_{600} was found to be:

Cell Wet Weight (g/L) = Slope x OD_{600} = 2.4 x OD_{600}

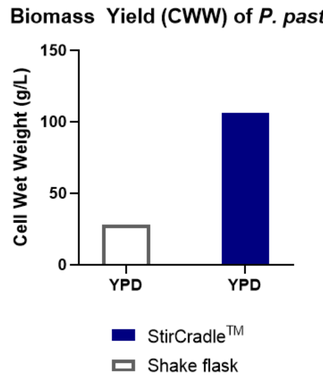
Biomass Yield of *E. coli* and *P. pastoris*

The biomass yield obtained from **StirCradle™** is at least **4 times higher** than the shake flask culture.

Biomass Yield (CWW) of *E.coli*

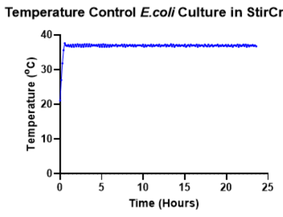


Biomass Yield (CWW) of *P. pastoris*

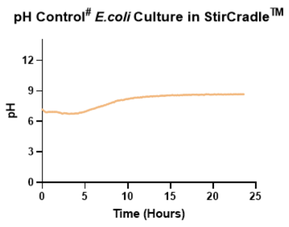


*Control of Various Parameters in StirCradle™

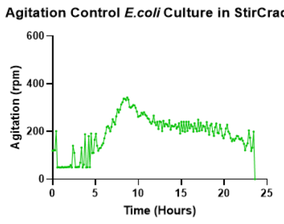
Temperature Control *E.coli* Culture in StirCradle™



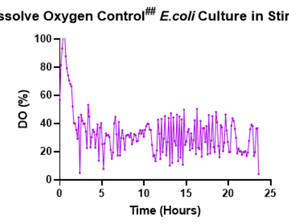
pH Control# *E.coli* Culture in StirCradle™



Agitation Control *E.coli* Culture in StirCradle™



Dissolve Oxygen Control## *E.coli* Culture in StirCradle™

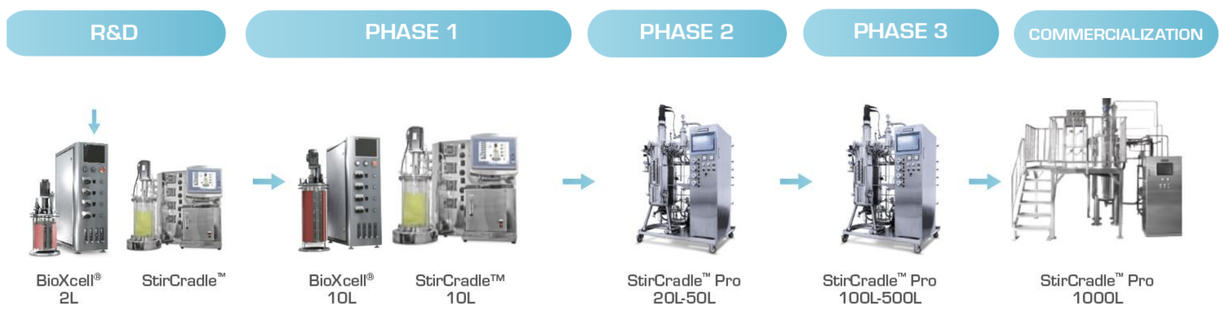


*Data retrieve from the control panel and plotted in data analyzing software. These control of variables data is for reference only, not corresponding to any results shown above.

pH has been monitored here only. Using ACID and BASE feeding pump through integrated peristaltic pump, pH can be controlled accurately.

DO is controlled by AGIT mode only. More accurate control can be expected if controlled in AGIT+AIR mode (MFC is needed)

Scale-up from R&D to Pilot and Manufacturing Scale



Fermentation CDMO Services in Esco Aster

Organism/cells
- Bacteria
- Yeast
- Suspension cells
- Plant cells

R&D Process Development
- Strain development and optimization
- Media optimization
- Growth parameters optimization
- Downstream process optimization

Manufacturing
- Plasmid DNA
- Recombinant Proteins
- Enzymes
- APIs
- Single Cell Protein (SCP)

StirCradle™ BIOREACTORS & PROCESS DEVELOPMENT SERVICES