

## UBC Basic Techniques in *Pichia* Fermentation Workshop

January 15th to January 19th, 2007

**Course Title:** Basic Techniques in *Pichia* Fermentation Workshop including operation, optimization and scale up of the *Pichia pastoris* expression systems.

**Course Description:** this course will provide hands on training in the care, maintenance and use of typical fermentor systems. Also, specifics relating to the fermentation and expression of foreign proteins in the yeast *Pichia pastoris* will be presented including strategy development.

This workshop is aimed specifically at fermentation strategies for *Pichia pastoris* .

Various fed-batch fermentation strategies are investigated for Mut<sup>+</sup> and Mut<sup>s</sup> phenotypes including controlled methanol feeding and other common strategies required to optimize protein production. On-line methanol monitoring and control is introduced and implemented. The workshop includes hands-on experience in fermentor design, set-up, inoculation and sampling techniques, harvesting strategies and monitoring and control of *Pichia* fermentation systems. This course stresses the practical aspects of fermentation principles required in research and pilot scale *Pichia* fermentations.

The following fermentations will be performed

- Mut<sup>+</sup> fermentation with continuous limiting methanol feed
- Mut<sup>+</sup> fermentation with methanol controlled on line at 0.10% methanol
- Mut<sup>s</sup> fermentation with methanol controlled on line at 0.70% methanol

Please visit our website at [www.fermteach.msl.ubc.ca](http://www.fermteach.msl.ubc.ca)

Note: all bioreactors are equipped with on line methanol monitoring and control systems

## **Course Outline:**

### **Day 1**

Introduction and tour of pilot plant facility

Overview of basic bio-processing equipment such as bioreactors, centrifuges, filtration and concentration

Overview of online and offline analytical equipment such as HPLC for the measurement of metabolic byproducts, glucose and methanol measurement

### **Lecture:**

Fermentation Systems Overview

Basic considerations

- services, pH, temperature, oxygen transfer

Bioreactor Types

- shake flask, bubble columns, basic stirred tank, wave

Bioreactor Design

- geometry, gas delivery systems, agitation and impeller design

Laboratory experimental design and fermentation strategies

- Discussion regarding types of fermentations and strategies that the participants will be conducting during the week

### **Laboratory:**

Hands on fermentor assembly, setup and sterilization

## Day 2

### Lecture:

Control Strategies

- temperature dissolved oxygen, pH, foam etc

Other Design Considerations

- aseptic technique, sterilization, evaporation, sampling

Maintenance

- stirrer bearings, o-rings, D.O. probe, pH probe, tubing, pumps, valves

Data Acquisition

- on line and off line data acquisition

### Laboratory:

Hands on fermentor probe calibration, controller setup, inoculation, prepare nutrient feed solutions

## Day 3

### Lecture:

Modes of Fermentation

Growth kinetics

- batch, fed batch and continuous

Bioreactor monitoring and control

*Pichia* bioreactor control strategies

- Media formulation, dissolved oxygen control, agitation and oxygen supplementation, temperature, feeding strategies

### Laboratory:

Hands on fermentor sampling, data recording, start fed batch phase, calibrate methanol controller, start induction phase for *Pichia pastoris* cultures

Off line analysis of cell density, organic acids using HPLC

#### Data Analysis

- Analyze on line and off line data that has been acquired from fermentations and discuss each variable

#### Day 4

##### **Lecture:**

Bioprocess optimization using on line control of methanol concentration

##### **Laboratory:**

Fermentor sampling, data recording

Off line analysis of cell density, organic acids using HPLC

#### Data Analysis

- Analyze on line and off line data that has been acquired from fermentations and discuss each variable

#### Day 5

##### **Lecture:**

#### Data Analysis

- Analyze on line and off line data that has been acquired from fermentations and discuss each variable

##### **Laboratory:**

Fermentor sampling, data recording, prepare fermentations for termination

Off line analysis of cell density, organic acids using HPLC

Noon to 2 pm

- data analysis and wrap up

Note, all times above are approximate and lecture hand outs will be available.

Cost: \$1000.00 Cdn

Course materials, daily lunches and social evening with dinner provided.

If you have any questions, please do not hesitate to contact me.

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