

# Protein Expression in Computer-Controlled Bioreactors

## Introduction

A new Protein Expression Laboratory (PEL) has been established at Michigan State University to manufacture proteins for structural biology studies. Proteins are being overexpressed by microbial hosts (e.g., *E. coli*) in computer-controlled bioreactors. Standard protocols are being developed to obtain high yields for a variety of proteins. Multidisciplinary research teams representing chemical engineering, microbiology, and biochemistry have studied the effect of several variables (temperature, induction time, and inducer concentration) on recombinant protein yields by *E. coli* in high-cell-density, fed-batch fermentations. This poster presents results for expression of the enzyme GDP-mannose 4,6 dehydratase by *E. coli*.

## Expression System

*Escherichia coli*, which is commonly used to overexpress recombinant products, was selected as the standard expression host. Clients can provide genes coding for their proteins in a number of formats:

- inserted into a plasmid of their choice
- inserted into a standard plasmid suggested by the PEL
- already cloned into a microbial expression host

## Fermentation Conditions

Protein yield is strongly affected by the environmental conditions under which the cells are grown. Computer-controlled bioreactors in the PEL enable key variables to be automatically controlled.

### Variables Influencing Protein Yield

Media composition	Temperature
pH	Dissolved O <sub>2</sub>
Nutrient feed rate	Induction time
Inducer concentration	Harvest time



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## Bioreactor Control Strategies

A control system has been developed to maintain physiological-state variables (e.g., specific glucose uptake rate) on a desired trajectory throughout the fed-batch fermentation. The system uses

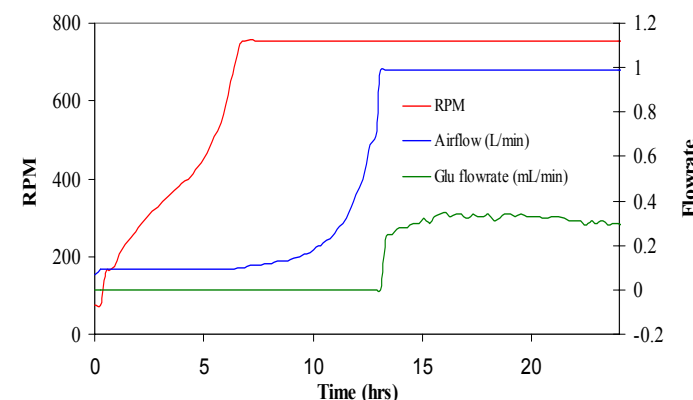
- glucose feed-on-demand
- different control strategies during different growth phases
- expert-system decision making
- artificial neural networks to estimate product concentration

Li, Mikola, Draths, Worden, Frost (1999) *Biotech. Bioeng.* 64 (1) 61-73  
Knop, David, Ph.D. Dissertation, Michigan State University, 2002

## Dissolved Oxygen (DO) Control

Tight control of DO is important in high-cell-density *E. coli* fermentations. The DO control system involves three stages:

- Stage 1: DO is controlled by adjusting impeller RPM
- Stage 2: DO is controlled by adjusting air flow rate
- Stage 3: DO is controlled by adjusting glucose feed rate



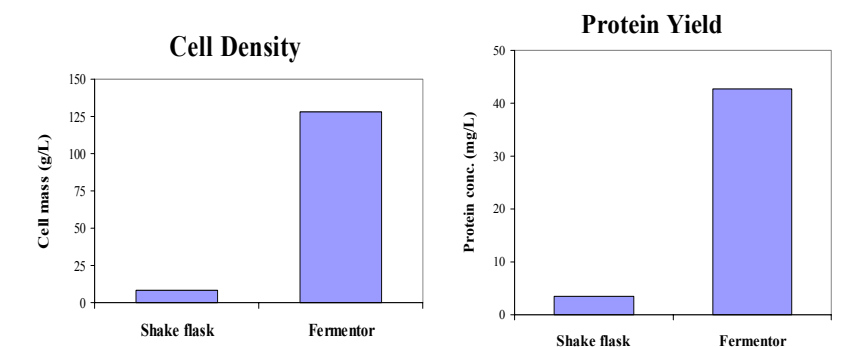
## GDP-mannose 4,6 dehydratase Expression

Expression system and optimized environmental conditions

- Microbial host: *E. coli*
- Expression vector: pQE30 (Qiagen Inc., Valencia, CA)
- Promoter: T5
- 6x His tag for protein recovery by affinity binding (Ni resin)
- Induction: 50 mM IPTG added after 12 h of growth
- Temperature: 37°C before induction; 25°C after induction
- Medium: M9 Medium with glucose fed on demand
- pH: 7.0

Yield comparison between shake-flask and bioreactor cultures

- Cell density and protein yields 10 times those in shake flasks



## Conclusions

- *E. coli* has been selected as the standard PEL expression host.
- Protein is produced in high-cell-density, fed-batch culture.
- Control system includes knowledge-based decision making.
- Different control strategies used during different growth phases.
- Protein expression methods for the PEL are being optimized.
- Variables tested were temp., induction time, and inducer conc.
- Volumetric yield in bioreactor was 10 times that in shake flask.

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