Self-Cycling Fermentation: Bioprocessing for the -omics era

Zachary J. Storms
And
Dominic Sauvageau

Department of Chemical Engineering and Materials Engineering
University of Alberta
Edmonton, Alberta, Canada

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The -omics Era:
Expanding our understanding of microbial cells

Genomics: Gene function (DNA)
Transcriptomics: Gene expression (RNA)
Metabolomics: Biochemical Reactions

How can we tailor large-scale bioprocessing to complement the knowledge we are gaining through -omics?
Self-Cycling Fermentation

- Cyclical Semi-continuous Reactor
- Contents halved upon depletion of limiting nutrient
  - Replenished with fresh media
- Cycle period = cell doubling time
- Maintains exponential growth
  - Reproducible, stable
- Induces cell synchrony

Biomass Production of E. coli

Cell Synchrony

Asynchronous cell growth

Cell division occurs throughout entire cycle

Ideal synchronized cell growth

Cell division occurs at infinitely small time interval in cycle
Growth of *E. coli* during a SCF Cycle

- Cell division occurs in middle of cycle
- Synchrony Index ≈ 0.6-0.7
- Growth of culture behaves like individual cell
- Cell metabolism slows down
  - Doubling time ~150 minutes

How can we use -omics to take advantage of these properties in large scale fermentation?

Note: Error bars represent the standard deviation

Studies on synchronized cells with Bacteriophages

- How does cell division cycle effect cell productivity?

- Bacteriophage infections at different points in cell division cycle

- Parameters measured
  - **Burst size**: phages/cell
  - **Lysis time**: phage incubation period
  - **Intracellular RNA and DNA**

Studies on synchronized cells with Bacteriophages

- How does cell division cycle effect cell productivity?
  - **Burst size** largest immediately preceding cell division
  - **Lysis time** shortest immediately preceding cell division

Results from cells infected by phage T4 at different points in their cell division cycle

[Graph showing Burst Size and Lysis Time vs. Normalized Cell Age]

Studies on synchronized cells with Bacteriophages

- How does cell division cycle effect cell productivity?

- **Burst size** largest immediately preceding cell division

- **Lysis time** shortest immediately preceding cell division

- **Productivity** highest immediately preceding cell division

Phage productivity of cells infected by phage T4 at different points in their cell division cycle.
Studies on synchronized cells with Bacteriophages

- Why does cell productivity change with cell age?

- **Intracellular resources**
  - RNA
  - Protein Synthesizing System
  - Transcriptomics

- Burst size positively correlated to total cellular RNA

- Lysis time negatively correlated to total cellular RNA

- Productivity positively correlated with cellular RNA

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Implications for large scale production processes: bacteriophage production

- Synchronized host:
  - Maintains same level of phage production
  - Lower cell concentration
  - The number of phages per cell (burst size) is larger for a synchronized culture

Implications for large scale production processes: recombinant protein production

- β-galactosidase production using recombinant bacteriophage
  - Induce production at different time points in SCF cycle

Note: Error bars represent the standard deviation
Implications for large scale production processes: recombinant protein production

- β-galactosidase production using recombinant bacteriophage
  - Induce production at different time points in SCF cycle

- Productivity of synchronized cultures
  - Maximum in productivity 50% larger than in non-synchronized culture
  - Maximum occurs 45 minutes earlier

Note: Error bars represent the standard deviation
Implications for large scale production processes: recombinant protein production

• β-galactosidase production using recombinant bacteriophage
  – Induce production at different time points in SCF cycle

• Productivity of synchronized cultures
  – Maximum in productivity 50% larger than in non-synchronized culture
  – Maximum occurs 45 minutes earlier
  – Two distinct maxima observed

Graph: Specific Integrated Productivity (U/L/h/OD) vs. Induction Time (minutes) (Cell Age)

Note: Error bars represent the standard deviation

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Implications for large scale production processes: recombinant protein production

- **β-galactosidase production using recombinant bacteriophage**

- **Productivity of synchronized cultures**
  - Maximum in productivity 50% larger than in non-synchronized culture
  - Maximum occurs 45 minutes earlier
  - Two distinct maxima observed
    - Before and after cell division

Note: Error bars represent the standard deviation

Current Studies with Self-Cycling Fermentation: Engineering metabolic pathways for high-value products

**Tyrosine:** Key precursor for high-value secondary metabolites in plants (Morphine, Resveratrol, noscapine, etc.)

High-value products naturally produced in plants

Engineer yeast to produce the high-value products

Combining metabolomics with SCF:

Genomics + Transcriptomics + Metabolomics
Current Studies with Self-Cycling Fermentation: Engineering metabolic pathways for high-value products

**Tyrosine Pathway Engineering:** Knockout secondary pathways through gene deletions

**Overproduce Shikimic Acid**

**Overproduce Tyrosine**

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How will SCF improve Tyrosine yield in this process?

- Current study by a master's student
- Optimize resource allocation
- Tighter control of metabolic fluxes
- Increased cell productivity

Combining metabolomics with SCF

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Summary and Future Work with the SCF

• Cell productivity increases under synchronous growth

• Complete transcriptomic and metabolic analysis of synchronized culture

• High cell density synchronized cultures
  – Fed-batch SCF
  – Constant volume continuous phasing
    • Pulsing in nutrients periodically
    • Induces synchrony, achieves high cell density
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Synchrony Index

If $N_0$ is the number of cells at the beginning of the time interval and $N_t$ is the number of cells at the end of the interval, $N_{t,e}$ is the number of cells produced by typical batch culture exponential growth, then $F$, the fraction of cells dividing in excess of those expected by exponential batch growth is given as ($g$ is the generation time)

$$F = \frac{N_t - N_{t,e}}{N_0}$$

$$N_{t,e} = N_0 e^{\mu g}$$

$$\mu = \frac{\ln(2)}{g}$$

$$F = \frac{N_t - N_0 e^{\mu g}}{N_0} = \frac{N_t - N_0 e^{\frac{\ln(2)}{g} t}}{N_0}$$

$$F = \frac{N_t}{N_0} - 2^{t/g}$$
B-galactosidase Unit Definition

1 Unit of β-galactosidase is defined as the amount of enzyme which produces 1 nano-mole of o-nitrophenol/min at 28°C, pH 7.0.
Under the conditions of the Assay, 1 nano-mole/ml of o-nitrophenol has an optical density (420 nm) of 0.0045 using a 10-mm light path.
Knowing the sample volume of the culture, \( v \) in ml, and the reaction time, \( t \) in minutes, one can calculate the units after measuring the absorbance at 420 nm.

\[
U \frac{ml}{ml} = \left[ OD_{420} - (1.75 \times OD_{550}) \right] \times \frac{1}{0.0045} \times \frac{1 \text{ nano mole } o-nitrophenol}{ml} \times \frac{1}{t}
\]

- β-galactosidase + ONPG \( \rightarrow \) galactose + o-nitrophenol
- ONPG: ortho-Nitrophenyl-β-galactoside
- o-nitrophenol is yellow, (absorbance at 420 nm)
- Concentration of o-nitrophenol proportional β-galactosidase concentration and reaction time
Induction Dynamics – β-galactosidase

- Induction Time:
  - Induce lytic state by raising temperature
- Enzyme activity increases until cell lysis occurs

\[
\text{Productivity} = \frac{\Delta(\text{Activity})}{V \int_{t_1}^{t_2} \text{OD} \, dt}
\]