MULTIPLICITY OF STEADY STATES IN CONTINUOUS CULTURE OF MAMMALIAN CELLS

Andrew Yongky, Tung Le, Simon Grimm, Wei-Shou Hu
Department of Chemical Engineering and Materials Science,
University of Minnesota
Major incentives for continuous operations

• Reduced turn around time
• Steady state operation

(a continuous operation need not to be at a steady state)
Continuous Process with recycle

\[ (1+\alpha) F, s, x \]

\[ F, s_0 \]

\[ V, s, x \]

\[ \alpha F, s, cx \]

\[ F, s, x_2 \]
\[
\frac{dx}{dt} = \mu x - Dx
\]

\[
\frac{ds}{dt} = D(s_0 - s) - \frac{\mu x}{Y_{x/s}}
\]

\[
= D(s_0 - s) - \frac{\mu_{\text{max}} S}{K_s + s} \frac{x}{Y_{x/s}}
\]
\[ 0 = \mu x - D x \]

\[ 0 = D(s_0 - s) - \frac{\mu_{\text{max}}}{} \frac{S}{K_s + s} \frac{x}{Y_{x/s}} \]
What is a steady state

• Has meaningful solution(s) when the system’s equations are set to 0
• Sometimes a system has a unique steady state for a set of operating conditions
• Other times, has multiple steady state
Stead State Concentrations in Continuous Culture with Cell Recycle

![Graph showing steady state concentrations with and without cell recycle.](image-url)
What is a steady state from an operational perspective

\[ D = 0.033; \text{Glc} = 7 \text{ mM} \]

Under one set of conditions (dilution rate, feed concentration), the state reaches constant values.

Key physiological parameters: growth rate, metabolism are constant.

![Graphs showing concentration, flux, and growth rate over time.](image_url)
What is a steady state from an operational perspective

D = 0.033; Glc = 7 mM

The same data if obtained from perfusion culture, may not be SS growth rate, metabolism may not be constant
Continuous Process with recycle

\[ 0 = (1 + \alpha c) \mu x - Dx \]
The Theme

• Multiple metabolic state when cells grow at one set of growth rate, glucose and lactate concentrations

• The metabolic steady state multiplicity leads to cell concentration multiplicity

• If we ensure the same steady state is achieved in different runs, process will be more robust
Continuous Culture with Metabolic Shift

- Cell Concentration (10^9/L)
- Glucose (g/L)
- Lactate (g/L)

Graphs showing changes over time: Cell Conc., Glucose, and Lactate concentrations with marked Fed-batch and Batch phases.
Distinct Steady States Corresponding to Different Metabolic States
The system: A ball
A contour of surface on which it can move
Steady State, where “things” can be “steady”, “at equilibrium”

Unstable steady state

Stable steady state

No steady state
The system: A ball
A contour of surface that it can move

Force: gravitational force, external force, friction force
The system: A ball
A contour of surface that it can move

The stability of the system can be shown mathematically using equations describing the system

Force: gravitational force, external force, friction force
Stable steady state

Unstable steady state

Stable steady state
When the “state” of a system changes, it moves along where the system has stable steady state.
Stability analysis can be applied to chemical reaction systems

The kinetic equations for all glycolysis, PPP and TCA cycle enzymes are all very well characterized
Allosteric Regulations in Glycolysis Pathway

Glucose → Glucose-6-Phosphate

Fructose-2,6-Bisphosphate (Inhibition) → Fructose-6-Phosphate

Fructose-6-Phosphate → Fructose-1,6-Bisphosphate

Phosphofructokinase

Fructose-1,6-Bisphosphate → Phosphoenolpyruvate

Pyruvate Kinase

Pyruvate → Pyruvate$_{\text{mito}}$

Lactate → Pyruvate
Stable, high flux state

Unstable steady state, unrealizable

Stable, low flux state
When the “state” of a system changes, it moves along where the system has stable steady state.
Allosteric Regulations in Glycolysis Pathway

Glucose → Glucose-6-Phosphate → Fructose-6-Phosphate → Fructose-1,6-Bisphosphate → Fructose-2,6-Bisphosphate → Glucose-6-Phosphate

Inhibition by: Hexokinase

Activation by: PFK2

Pyruvate Kinase: Pyruvate → Pyruvate Kinase → Pyruvate

Lactate: Pyruvate → Lactate
Akt Down-regulated and p53 Up-regulated in Late Stage
Bistability in Central Metabolism

Extracellular Glucose (mM)

Glycolysis Flux (mM/h)

High Flux State

Unstable Steady States
Effect of Lactate on Glycolytic Flux
Effect of Lactate on Glycolytic Flux

The graph illustrates the relationship between lactate and glucose levels and the glycolysis flux in mM/h. It shows how changes in lactate and glucose concentrations affect the flux of glycolysis.
Effect of Lactate on Glycolytic Flux

![Graph showing the effect of lactate on glycolytic flux.](image-url)
Effect of AKT on Glycolytic Activity
Trajectory of Cells in Shifting to Low Flux State

![Diagram showing the trajectory of cells in shifting to a low flux state, with axes for Lac (mM) and Glc (mM) and a graph for Glycolysis Flux (mM/h).]
Trajectory of Cells in Shifting to Low Flux State
Trajectory of Cells in Shifting to Low Flux State

AKT = 35
Figure 6: Transient behavior demonstrating effect of glucose concentration perturbations on cellular metabolic state.
Multi-scale Model: Metabolism Model in Continuous Culture
Bistability in Continuous Culture

Multiscale model simulation
Glc = 8 mM
Bistability in Continuous Culture

- **Graph 1**: Plot of $J_{\text{Glc}}$ (mM/hr) against Glucose (mM)
- **Graph 2**: Plot of Cell Conc. ($\times 10^6$ cells/mL) against Glucose (mM)
Effect of Glucose Feed

Glc = 15 mM

Glc = 8 mM

Glc = 5 mM
Distinct Steady States Corresponding to Different Metabolic States

![Graph showing cell concentration over time for different metabolic states.](image)
Transient Trajectory of Culture with High Metabolic Flux

\[ D = 0.033; \text{Glc} = 7 \text{ mM} \]
Transient Trajectory of Culture with Low Metabolic Flux

D = 0.033; Glc = 7 mM
Benefit of slow growth rate?

Transcriptome analysis – effect of culture age?
Conclusion

• Physiological steady state
  - truly benefit continuous operation
• Metabolic regulation and growth causes metabolic steady state multiplicity
• Continuous cell culture reactor – multiple steady state
• Different trajectories lead to different steady state
Thank you!