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Optimal control of a continuous bioreactor for maximized beta-carotene production

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Optimal control of a continuous bioreactor for maximized carotene production

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β-carotene Market

- An orange pigment produced by diverse organisms such as plants, fungi, and bacteria

- Used in many industries:
  - Food
  - Animal nutrition
  - Pharmaceuticals
  - Cosmetics
  - Colorant

http://www.bellybytes.com/nourish/images/betacarotene.jpg

- Projected worldwide market value is $1.4 billion in 2019[1]

- Higher antioxidant activity found in naturally produced β-carotene when compared to the synthetic version

[1]Carotenoids Market By Type (Astaxanthin, Beta-Carotene, Canthaxanthin, Lutein, Lycopene, & Zeaxanthin), Source (Synthetic And Natural), Application (Supplements, Food, Feed, And Cosmetics), & By Region - Global Trends & Forecasts To 2019. **MarketsandMarkets** February 2015

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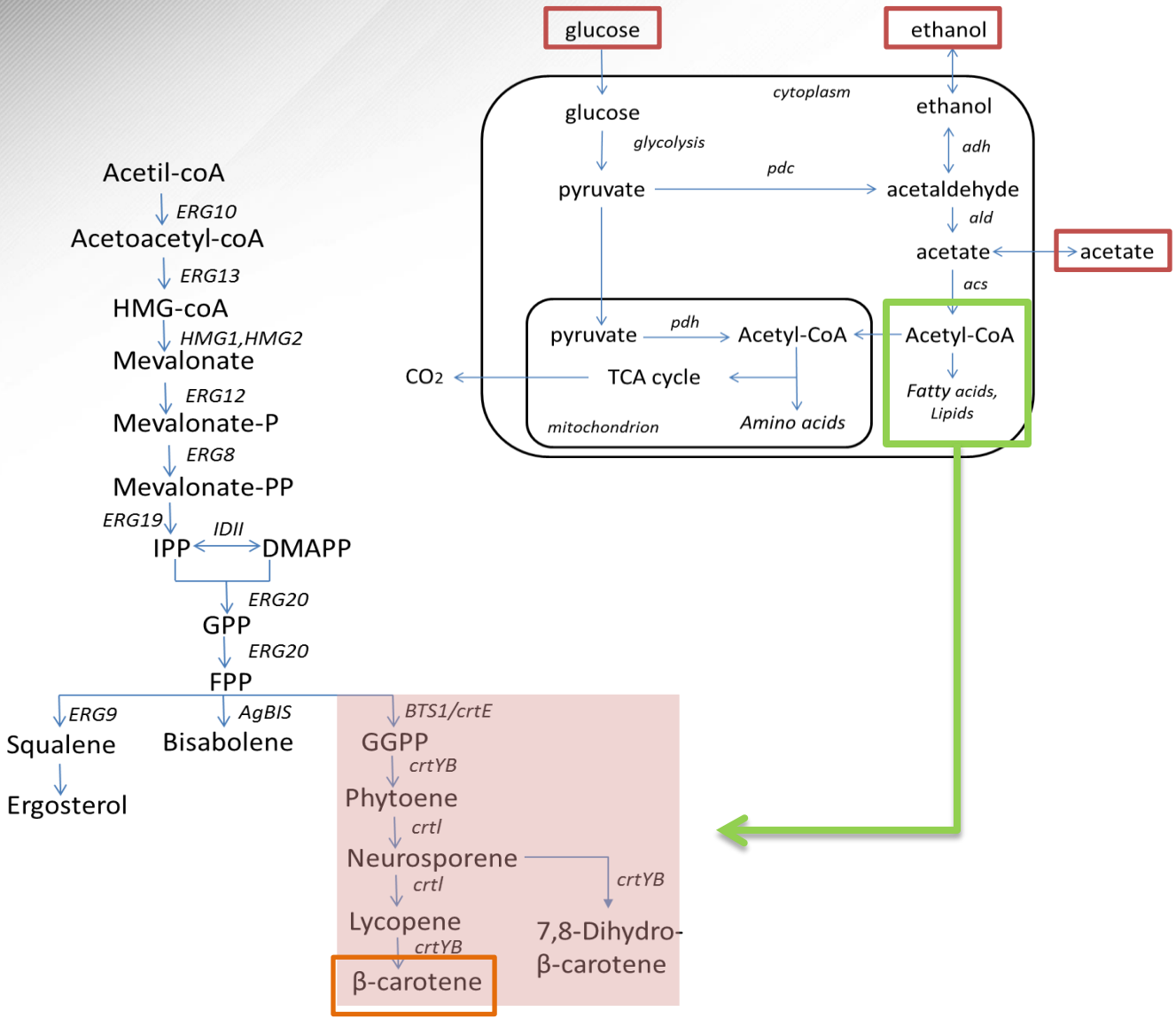
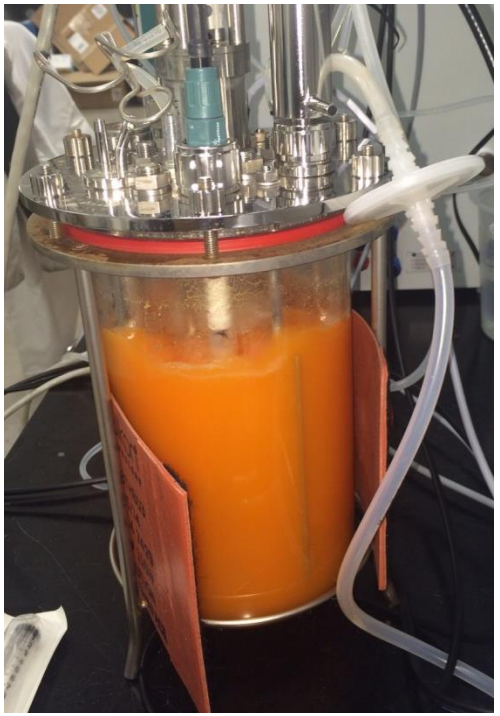
β-Carotene Production via Recombinant Saccharomyces cerevisiae





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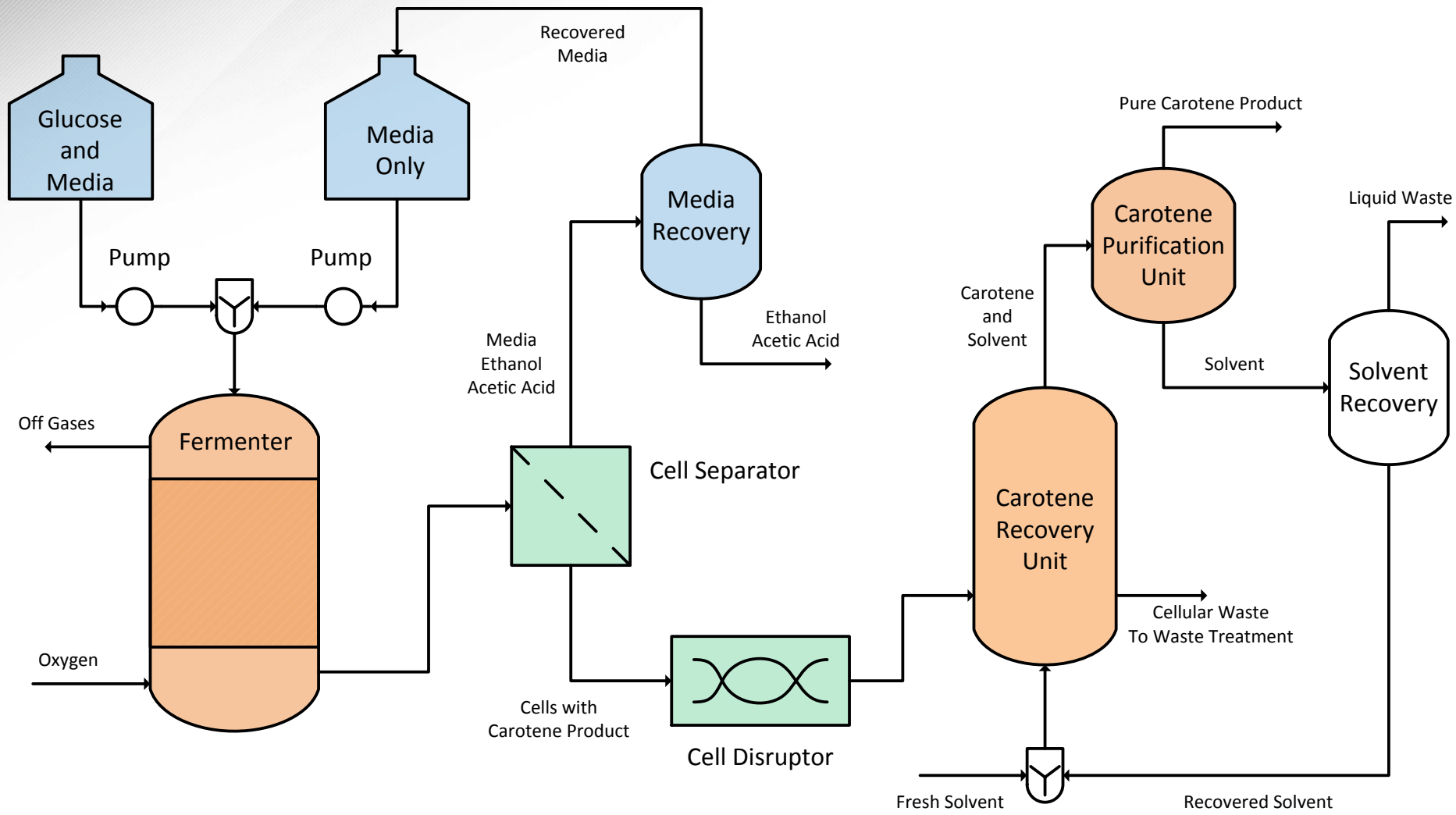
Continuous Production of β-Carotene



Outline

- Motivation

- Batch Operation of a Carotene Bioreactor
- Extension of Batch Operation to Continuous Systems
- Model Predictive Control of a Continuous Bioreactor to Maximize β-Carotene Production
- Results and Conclusions

Batch Process Overview

The aim of this study is to:

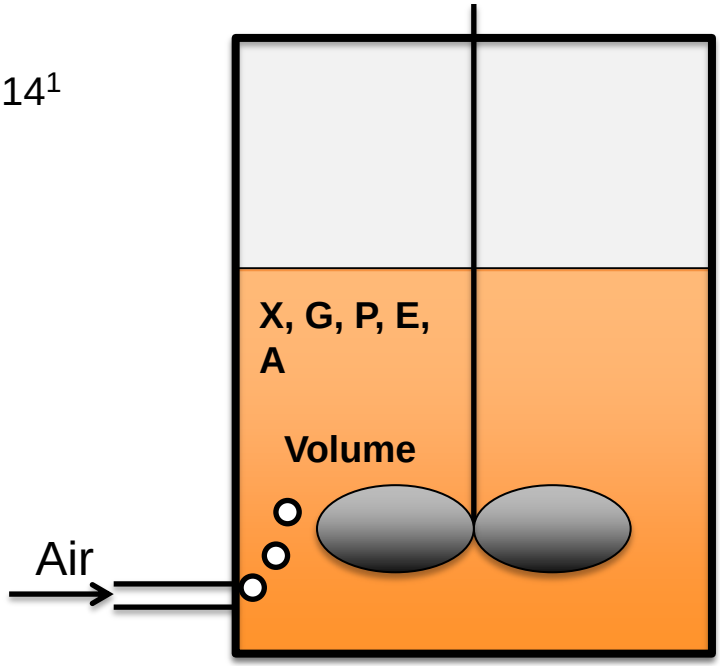
- Develop a suitable and reliable kinetic model for the carotene production in batch cultures of an engineered *Saccharomyces cerevisiae* strain using glucose as the main substrate

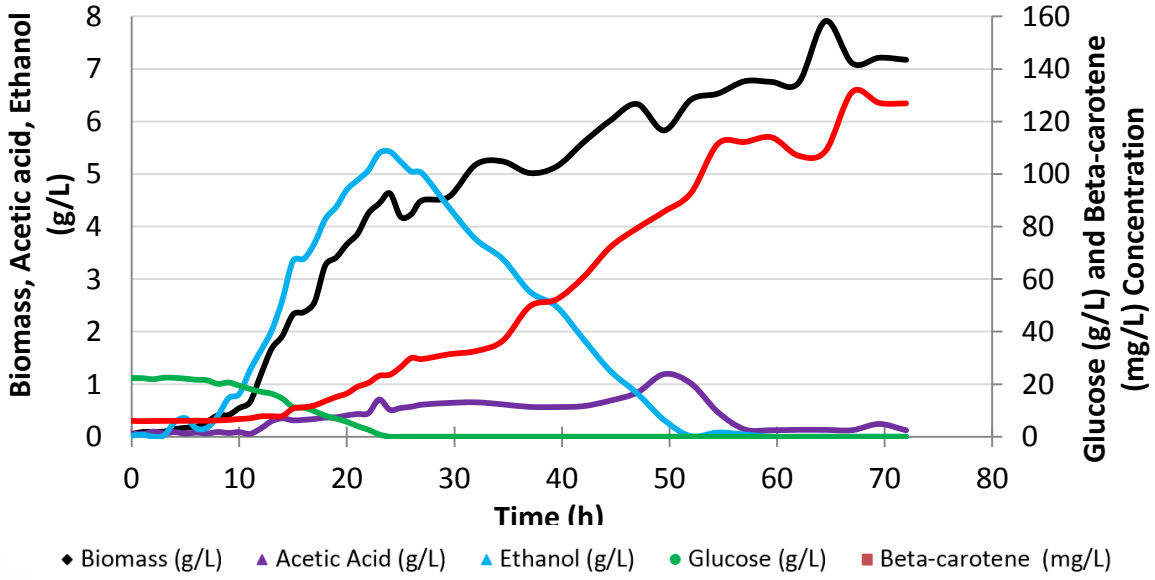
- Apply this model to predict cell growth, substrate consumption, ethanol and acetic acid formation and later assimilation.

- Determine the carotene productivity of the batch system

Batch Operation of a β-carotene Bioreactor

Organism Strain	*Saccharomyces cerevisiae* SM 14[1]
Product	Beta-carotene
Reactor Working Volume	3 Liters
Initial Glucose	20 g/L
Air Flow Rate	6 L/min
Run Time	72 hours
Temperature	30℃ (controlled)
pH	4 (controlled)





[1]Reyes, L.H., Gomez, J.M., and Kao, K.C. Improving carotenoids production in yeast via adaptive laboratory evolution. *Metabololic Engineering.* 21 (2014) 26-33.

Unstructured Batch Models

Growth Rate Models:

$$\mu = \mu_G + \mu_E + \mu_A$$

$$\mu_G = \left(\frac{\mu_{max,G} \cdot \xi_E \cdot \xi_A \cdot G}{K_{SG} + G + a_{ge} E + a_{ga} A} \right)$$

$$\mu_E = \left(\frac{\mu_{max,E} \; E}{K_{SE} + E + a_{eg} G + a_{ea} A} \right)$$

$$\mu_A = \left(\frac{\mu_{max,A} \; A}{K_{SA} + A + a_{ag} G + a_{ae} E} \right)$$

Inhibition Models:

$$\xi_E = f(E) \qquad \xi_A = f(A)$$

Batch Models:

$$\frac{dX}{dt} = r_X = (\mu_G + \mu_E + \mu_A)\, X$$

$$\frac{dG}{dt} = r_G = -\frac{\mu_G X}{Y_{X/G}}$$

$$\frac{dE}{dt} = r_E = k_1\, \mu_G X - \frac{\mu_E X}{Y_{X/E}}$$

$$\frac{dA}{dt} = r_A = (k_2 \mu_G + k_3 \mu_E) X - \frac{\mu_A X}{Y_{X/A}}$$

$$\frac{dP}{dt} = r_P = (\alpha_1 \mu_G + \alpha_2 \mu_E + \alpha_3 \mu_A) \cdot X + \beta X$$

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Estimation Procedure and Results

Read original data and smooth it via cubic spline

Generate μ data, calculate the growth rate parameters minimizing the SEE and set those values

$$\int_{t_1}^{t_2} \mu \, dt = \int_{x_1}^{x_2} \frac{1}{x} \, dx \rightarrow \mu = \frac{\ln\left(\frac{x_2}{x_1}\right)}{t_2 - t_1}$$

With the initial values and parameter guesses solve the differential equations

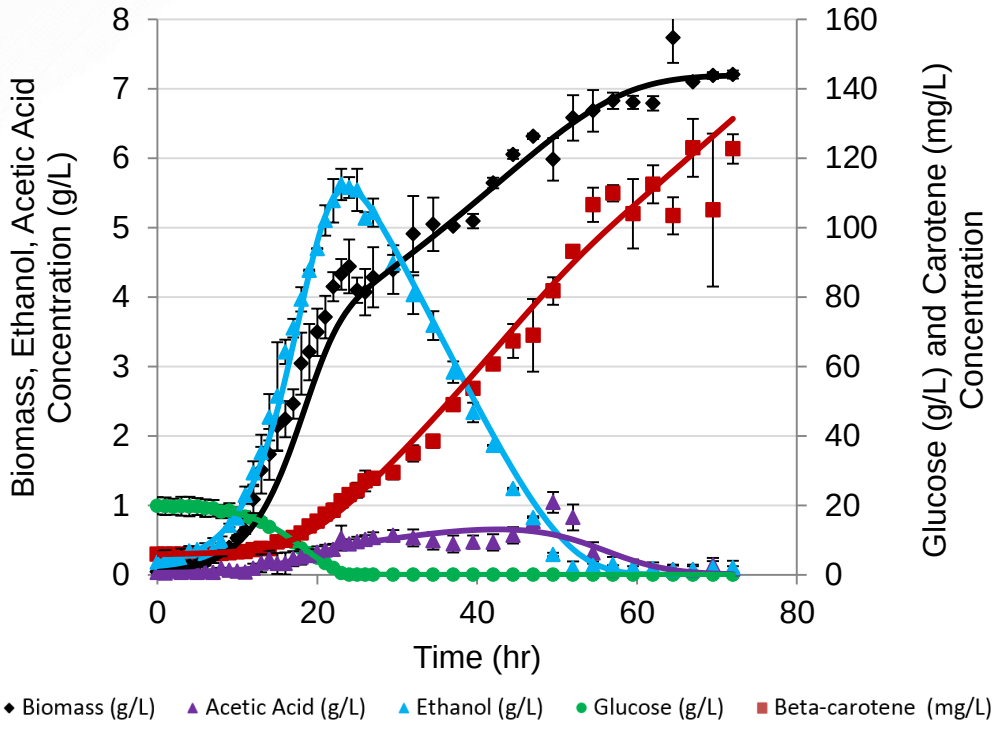
Compare the model predictions with the smooth data

Calculate the R^2 for each curve, where the best value for each is one (1). The objective function for minimization is:

$$\min \; Z = 5 - (R_p^2 + R_x^2 + R_s^2 + R_e^2 + R_a^2)$$

Use *fmincon* algorithm to determine the minimum value of the objective function

Plot and analyze the parameters from the optimal solution



Carotene Productivity

$$\frac{120 \; \frac{\text{mg}}{\text{L}}}{(72 + \text{x}) \; \text{hrs}} = 1.46 \frac{\text{mg}}{\text{L} \cdot \text{hr}}$$

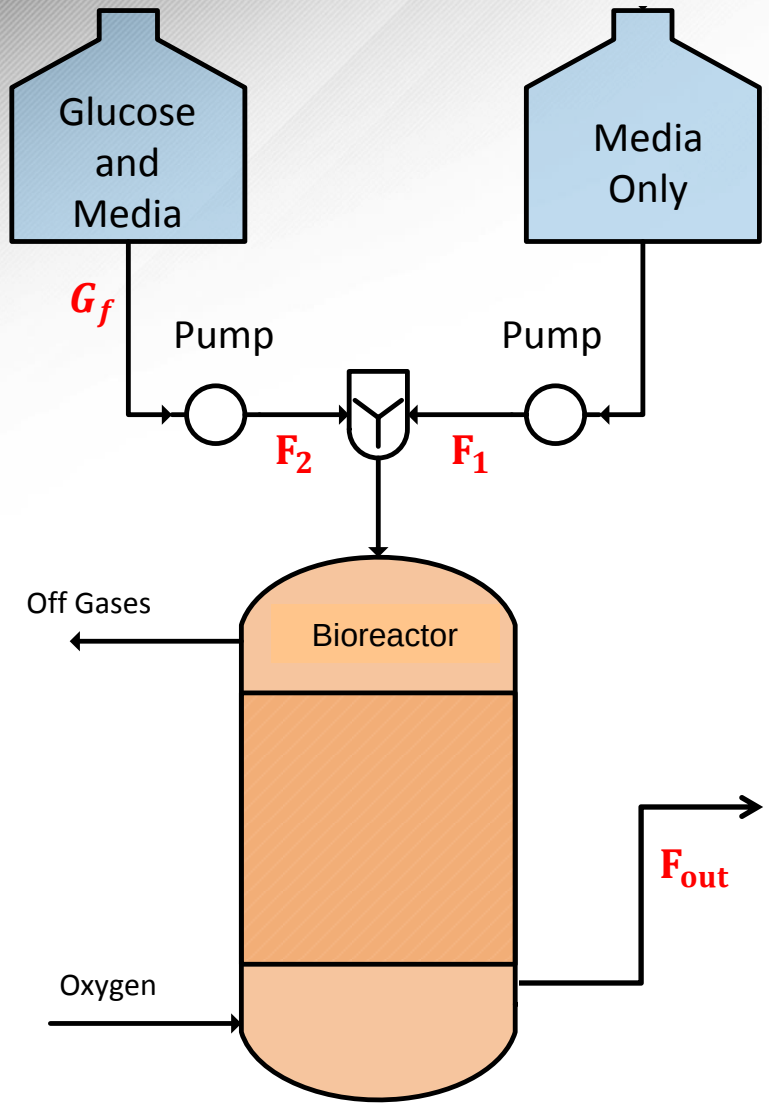
(assuming x = 10 hours for filling, cooling, and cleaning)

Continuous Process Overview

The aim of this study is to:

- Propose a novel continuous carotene production process utilizing a two tank system to allow for the independent manipulation of the inlet flow rate and inlet glucose composition
- Develop continuous models describing the dynamic nature of this novel fermentation system
- Utilize dynamic optimization techniques to develop a model predictive controller capable of maximizing carotene production to rival that of batch production processes

Continuous Operation of a *β-carotene Bioreactor*



Process Models:

$$\frac{dG}{dt} = \frac{F_2}{V} \cdot G_f - \frac{F_{out}}{V} \cdot G - r_G$$

$$\frac{dX}{dt} = -\frac{F_{out}}{V} \cdot X + \mathrm{r}_X$$

$$\frac{dP}{dt} = -\frac{F_{out}}{V} \cdot P + r_P$$

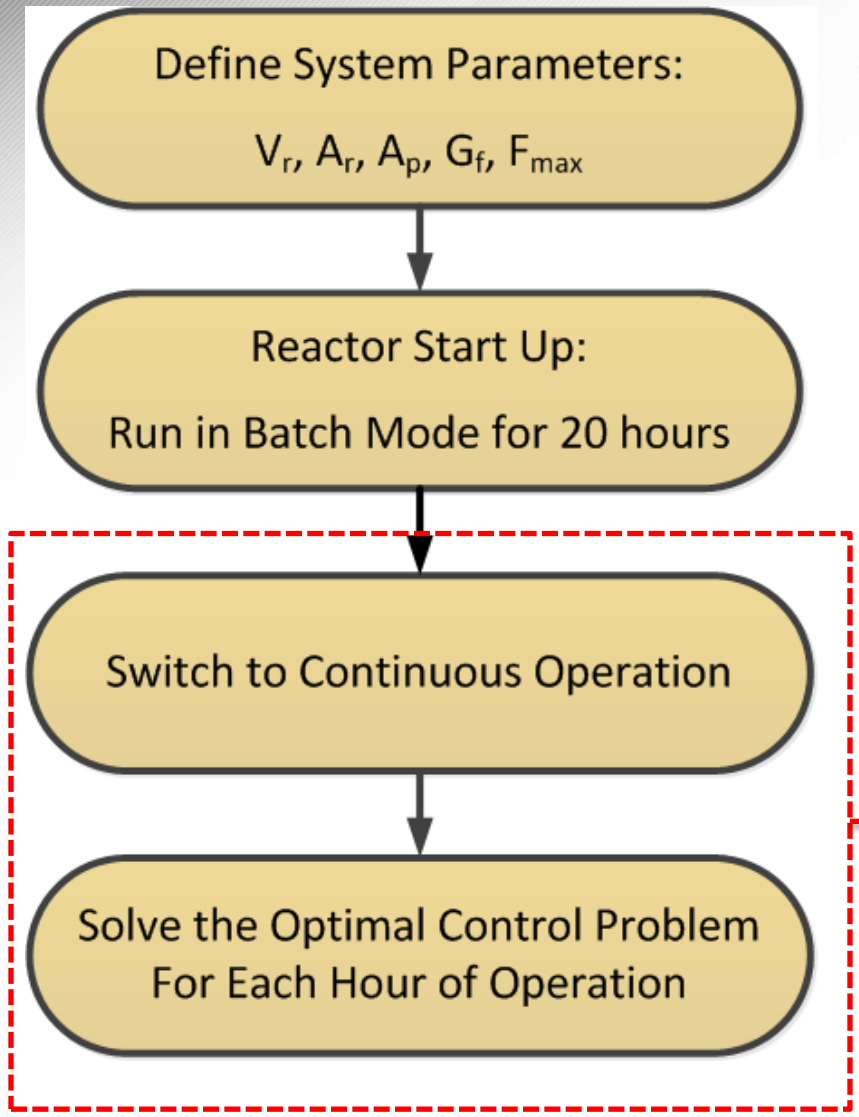
$$\frac{dE}{dt} = -\frac{F_{out}}{V} \cdot E + r_E$$

$$\frac{dA}{dt} = -\frac{F_{out}}{V} \cdot A + r_A$$

$$\frac{dV}{dt} = F_{G+M} + F_M - F_{out}$$

$$F_{out} = f(V)$$

Optimization Algorithm



Starting at the beginning of continuous operation characterized by the time $t = t_{switch} = 20$ hours:

1. Discretize the process models for a given $\Delta t = \frac{1}{n}$ hours with $n \geq 10$

2. Solve the following optimization problem for the optimum hourly flowrate from each tank, F_1 and F_2:

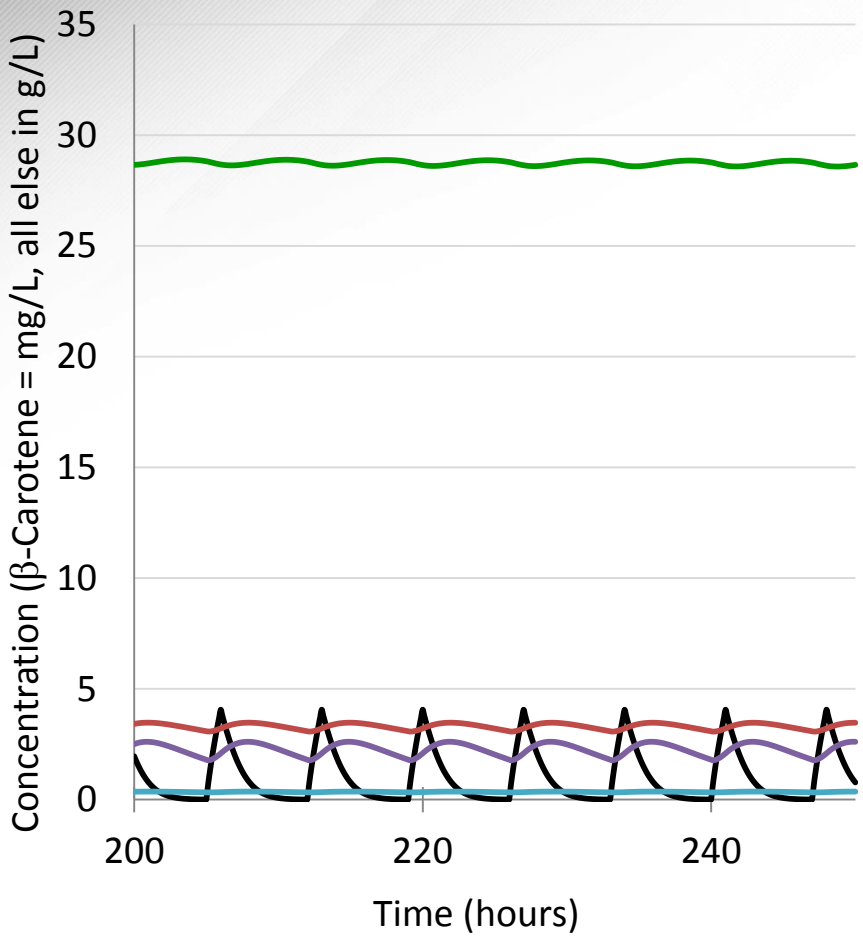
$$\min_{F_1 \; F_2} \; -\boldsymbol{P_t} + \boldsymbol{\alpha} \sum_{\boldsymbol{t}} (\boldsymbol{V_t} - \boldsymbol{V_r})^2$$

$$\text{s.t.} \quad \begin{array}{c} \text{Discretized ODEs} \\ 0 \leq F_{1,2} \leq F_{max} \end{array}$$

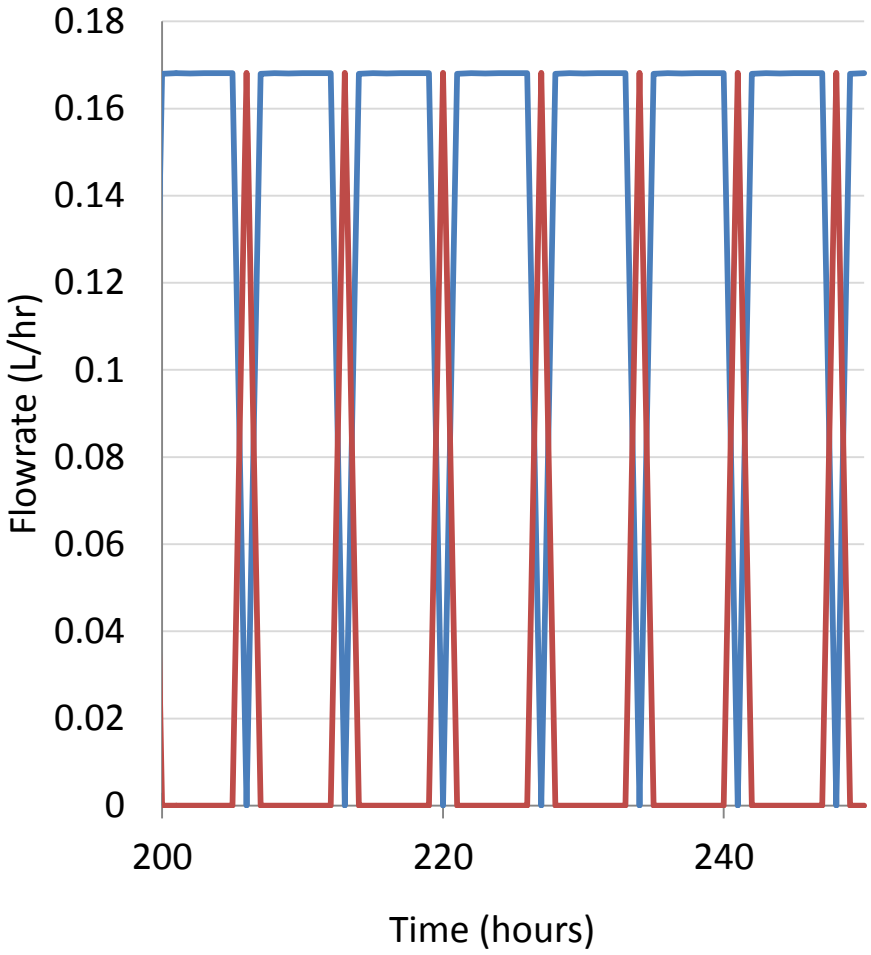
3. Repeat Step 2 for each hour after t_{switch}, using the previous hour's end state as the new initial condition, to determine the optimum flowrate as the system moves forward in time

Steady State Analysis

Concentration Profiles



Optimal Control Actions



Glucose
Biomass
β-Carotene
Media Only
Media + Glucose
Ethanol
Acetic Acid

Batch vs Continuous Comparison

Batch Operation

β-carotene Concentration

$$P = 120\ \frac{mg}{L}$$

Fermentation Time

$$t = 72\ hours$$

Productivity

$$\frac{P}{t} = 1.46\ \frac{mg}{L \cdot hr}$$

Continuous Operation

β-carotene Concentration

$$P = 28.73\ \frac{mg}{L}$$

Inlet Flowrate (F_{total}) and Volume

$$F_{total} = 0.169\ \frac{L}{hr} \qquad V = 3\ L$$

Productivity

$$\frac{P \cdot F}{V} = 1.62\ \frac{mg}{L \cdot hr}$$

Continuous operation **increases** process productivity by **10.5%** when compared to traditional batch processing.

Conclusions

- Continuous operation has the potential to increase productivity of bioreactor systems
- A novel two-feed continuous reactor system capable of independently varying the **dilution rate** and **inlet glucose concentration** was implemented
- A bi-level dynamic optimization methodology was to determine the **maximum productivity** of steady-state continuous β-carotene production
- Continuous production shows a 10.5% increase in β-carotene productivity compared to a tradition batch system

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