ECONOMIC IMPROVEMENT OF CONTINUOUS BIOCHEMICAL REACTORS VIA MULTI-FEED OPERATION

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Abstract

The production of pharmaceuticals, a multi-billion dollar worldwide industry, is expected to see large economic growth in the coming years. However, stagnation in the development of new products has led the industry to search for other methods to achieve these economic goals. The change from batch processing practices to the continuous production seen in commodity chemicals industries is a way of mitigating operating costs and increasing revenue. The optimal operation of single feed continuous bioreactors has been extensively studied, but these systems are not adaptable to the changing needs of biochemical systems as they only allow a single feed concentration. In addition, some biological systems are capable of utilizing multiple substrates, opening the door to potential cost savings not possible in single feed systems. In this work, the optimal operation of a multi-feed bioreactor is investigated to allow for the potential use of multiple substrates while independently varying the dilution rate and inlet substrate concentrations. The production of β-carotene from a glucose and ethanol substrate is used as a case study, where it is shown that the use of a multi-feed bioreactor has the potential to provide a near 30% economic improvement in operating costs despite a 23% decrease in the bioreactor productivity. These economic improvements are linked to an increase in cellular and substrate yield of the product achieved with the addition of a secondary substrate.

Keywords

Optimal control, β-carotene, multi-feed bioreactor, continuous bioprocessing

Introduction

The development and sale of pharmaceuticals and nutraceuticals is a multi-billion dollar industry worldwide, of which sales in the North American and European markets accounted for 41% and 27.4% respectively in 2013 (PricewaterhouseCoopers). Projections have shown that the economic viability of the industry is expected to increase dramatically by 2019, especially in the United States and markets with high potential such as China, Brazil, Russia and India (PricewaterhouseCoopers). However, it has also been shown that the development of new pharmaceuticals and biologicals has been relatively steady worldwide in the years between 2000 and 2011, resulting in little revenue expansion from the development of new drugs (PricewaterhouseCoopers). If the industry is to meet the economic growth projections set for 2019 with this stagnant development of new products, new methods for economic improvement must be analyzed.

One potential method of stimulating economic growth is to transition the industry from a focus on batch production to production strategies that mimic the commodity chemical industry, specifically fed-batch and continuous processing, and the implementation of these

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processing methods have been investigated for many biochemical products. San and Stephanopoulos (1989) investigated the use of fed-batch systems to maximize the productivity of penicillin production. Warikoo et al. (2015) exemplified the use of continuous perfusion reactors for the production of therapeutic proteins from Chinese hamster ovary (CHO) cells. The fed-batch production of poly-3-hydroxybutyrate (PHB) has also been investigated by Yemane, Fukunaga and Lee (1995) to increase cell density and therefore improve productivity of the intracellular bio-plastic. While these nontraditional processing methods have shown great promise for many different products, the determination of the optimal operating conditions can be difficult due to the complexity and variability of biochemical systems.

To contend with the challenges of fed-batch and continuous bioprocesses, many researchers have utilized the concepts of dynamic optimization, also known as optimal control. Work by Sridhar and Saucedo (2016) have shown the ability to find globally optimal solutions to the optimal control problem of continuous production of ethanol via *Saccharomyces cerevisiae* using the dilution rate and mass transfer coefficient as decision variables. Hodge and Karim (2002) have employed a nonlinear model predictive control algorithm that uses a sequential solution to predict solutions to the optimal control problem in real time and maximize fed-batch ethanol production by recombinant *Zymomonas mobilis*. The optimization of fed-batch processing with mixed cell cultures has been shown by Modak and Lim (1989). However, the use of a single feed at a set substrate concentration may not provide enough controllability to be able to respond to the needs of biochemical systems while also trying to maintain the constant volume of a continuous reactor system through the variation of the dilution rate.

This work looks to develop a continuous bioreactor capable of increasing controllability and profitability through the implementation of multiple feeds. These multiple feeds allow for the introduction of multiple substrate types to be fed to the reactor and for the inlet concentrations and flow rates to be varied independently of each other through the use of a diluent feed composed of media. The effectiveness of the multi-feed bioreactor is exemplified through the solution of an optimal control problem maximizing the product concentration while minimizing the time to reach steady state. A case study of β-carotene production using both a single glucose feed and multiple feeds of glucose, ethanol, and media is used to illustrate the effectiveness of the multi-feed bioreactor to increase economic viability in bioprocesses.

Materials and Methods

The multi-feed bioreactor

Figure 1 depicts the multi-feed bioreactor system using an illustrative example of three total feeds, two feeds of differing substrates at a bulk concentration of S_1 and S_2, respectively, and one feed of media only. The flowrate of each feed, denoted as F_1, F_2 and F_3, can be varied independently. These independent flows are mixed before being introduced to the reactor using pumps, shown as circles in Figure 1, as shown in Eq. (1).

$$F_{in} = F_{out} = F_1 + F_2 + F_3 \tag{1}$$

The independent manipulation of the feed flowrates allows for the independent variation of substrate concentrations as the introduction of the media feed acts as a dilution agent. Alternatively, restricting flow through one substrate pump and the media pump, i.e. $F_1 = F_3 = 0$, allows for the classic single feed bioreactor system that has been studied extensively to this point. Overall, this bioreactor system provides a much higher degree of adaptability to the challenging needs of bioprocessing and can be utilized to find non-trivial feeding strategies capable of utilizing minimal substrate to achieve a greater economic potential.

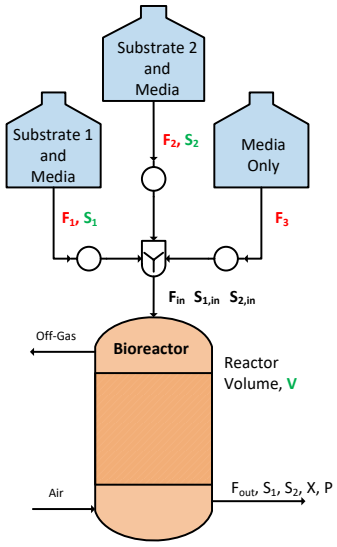

Figure 1. Multi-feed bioreactor utilizing multiple substrates

To determine these non-trivial optimal feeding strategies, an optimal control problem can be formulated using the chemical kinetics of the reactor system as constraints. This control problem begins with the development of an objective function designed to maximize the concentration of the desired product while simultaneously enticing the system to reach steady state in a timely fashion. as shown in Equation (2).

$$\min_{F_1,F_2,F_3} \phi = -\alpha \cdot \bar{Z} + \int_0^{t_f} (Z - \bar{Z})^2 \tag{2}$$

The first term in Eq. (2) maximizes the average or steady-state value of the important system states *Z*, in most cases the biomass or product concentration. The parameter α is used as a scaling parameter. The second term in the objective function is used to minimize the time to steady state by minimizing the sum of errors between the time profile of the reactor states of interest and the maximized average concentration.

This objective function will be subject to constraints on the states of the reactor system. These constraints are usually in the form of ordinary differential equation (*dZ/dt*) that describe the time course of the reactor states. These constraints, coupled with the flowrate equation given in Eq. (1), the objective function in Eq. (2), and limits to the allowable pump flowrates gives the following optimal control problem to determine the optimal feeding profile of the multi-feed bioreactor:

$$\begin{aligned} \min_{F_1,F_2,F_3} \phi &= -\alpha \cdot \overline{Z} \cdot \frac{\overline{F}_{out}}{V} + \int_0^{t_f} (Z-\overline{Z})^2 \\ \frac{dZ}{dt} &= g(Z(t),F_1(t),F_2(t),F_3(t)) \\ s.t. \quad F_{in} &= F_{out} = F_1 + F_2 + F_3 \\ F^{\min} &\le F_1,F_2,F_3 \le F^{\max} \end{aligned} \tag{3}$$

Case Study: β-carotene Production

By specifying the substrates used in conjunction with a biochemical system of interest, this system can be utilized to produce many different products from many different species. One possible product is the carotenoid β-carotene, a natural orange pigment used in many industries including the cosmetics, paint and food industries as well as a precursor for vitamin A. While synthetic production of β-carotene is possible, it has been shown in literature that when biologically produced this pigment has enhanced antioxident properties. Studies by the United States Department of Agriculture (USDA) have shown that the market price for natural β-carotene, approximately $1,000 to $2,000 per kilogram, is much higher than that of the synthetic product, approximately $400 to $800 per kilogram (Caswell and Zilberman, 2001). Through the introduction of processing methods to decrease the operating costs of natural β-carotene the profit margin of this nutraceutical can be improved even with a shift in market price toward that of the synthetic product.

Research has shown that β-carotene production from recombinant baker's yeast, or *Saccharomycces cerevisiae*, proceeds in two phases based on the substrate present in the bioreactor (Ordonez et al., 2016). In the first stage, *S. cerevisiae* utilizes glucose to facilitate biomass production while producing a small amount of product as well as a large amount of ethanol. In the second phase, beta-carotene production is enhanced through the use of ethanol as a substrate, while biomass production remains relatively constant. As both the glucose and ethanol substrates play a vital role in the production of β-carotene by *S. cerevisiae* it is possible the supplement of both of these substrates would improve the economic viability of the bioreactor.

Kinetic models for the batch production and consumption (r_i) of biomass (X), β-carotene (P), glucose (G), ethanol (E), and acetic acid (A) are available via Ordonez et al. (2016) and have been modified for a continuous system as shown in Eqs. (4) through (8). The first term in each equation is the effect of the continuous feed on the rate of production of each species in the reactor. This term is modified for the glucose and ethanol equations, Eq. (6) and Eq. (7), to account for the addition of these substrates via the inlet feed. All other terms in the equations are included to model the rate of formation, denoted by the α_i parameters, or depletion, given by the yield coefficients $Y_{X/G}$, $Y_{X/E}$, and $Y_{X/A}$, of the given compound based on the amount of available substrate. The growth rates on the glucose, ethanol, and acetic acid substrates are given by μ_G, μ_E, and μ_A. Models for these growth rates, in general following a modified version of the widely used Monod kinetic model, and the parameter values for Eq. (4) through Eq. (8) are given by Ordonez et al. (2016).

$$\frac{dX}{dt} = -\frac{F_{out}}{V}X + \mu_G X + \mu_E X + \mu_A X \tag{4}$$

$$\frac{dP}{dt} = -\frac{F_{out}}{V}P + \alpha_1\mu_G X + \alpha_2\mu_E X + \alpha_3\mu_A X + \beta X \tag{5}$$

$$\frac{dG}{dt} = -\frac{F_{out}}{V}(G - G_{in}) - \frac{\mu_G X}{Y_{X/G}} \tag{6}$$

$$\frac{dE}{dt} = -\frac{F_{out}}{V}(E - E_{in}) + \alpha_4\mu_G X - \frac{\mu_E X}{Y_{X/E}} \tag{7}$$

$$\frac{dA}{dt} = -\frac{F_{out}}{V}A + \alpha_5\mu_G X + \alpha_6\mu_E X - \frac{\mu_A X}{Y_{X/A}} \tag{8}$$

The objective of the optimal control studies performed here is to maximize the steady state β-carotene and biomass production for a single glucose feed and for the multi-feed bioreactor using three feeds corresponding to glucose, ethanol, and media. The bulk glucose feed concentration (G_f) and the bulk ethanol feed concentration (E_f) were chosen as 20 g/L and 5 g/L, respectively, to emulate the highest concentrations found during the study by Ordonez et al. (2016). The bioreactor volume is 3 L for both the single and multi-feed reactors. Additionally, the total possible inlet flow rate (F_{in}) is limited to a maximum of 1.2 L/hr. To ensure this, the single feed reactor uses a pump with a maximum possible flow rate of 1.2 L/hr of glucose feed while the multi-feed system has a maximum

flow rate of 0.4 L/hr each for the glucose, ethanol and media pumps, respectively.

Solution Methodology to the Optimal Control Problem

The method used to solve the optimal control problem presented in this work is described by Flores-Tlacuahuac et al (2008) and converts the dynamic programming problem into a nonlinear program (NLP) by approximating the state profiles using polynomials defined on finite elements. The profiles for the bioreactor states are defined by Eq. (9), where Z_{i-1} is the value of the state variable at the beginning of each finite element *i*, h_i is the length of element *i,* and $dZ/dt_{i,q}$ is the value of the first derivative given by Eq. (4) through Eq. (8) for element *i* at collocation point *q*. Continuity for the state profiles is ensured by Eq. (10).

$$Z(t) = Z_{i-1} + h_i \sum_{q=1}^{ncol} \Omega_q \left(\frac{t_i - t_{i-1}}{h_i} \right) \cdot \frac{dZ}{dt_{i,q}} \tag{9}$$

$$Z_i = Z_{i-1} + h_i \sum_{q=1}^{ncol} \Omega_q \left(\frac{t_i - t_{i-1}}{h_i} \right) \cdot \frac{dZ}{dt_{i,q}} \tag{10}$$

The optimal control studies for the continuous production of β-carotene were performed using 200 finite elements, each of a time length 1 hour (h_i = 1), to ensure steady state profiles are achieved. Radau collocation using three internal points (ncol = 3) was used within each finite element as described by Hairer and Wanner (1999). The resulting NLP problem was solved in GAMS using the IPOPT solver (Wächter and Biegler, 2006). While studies have shown the importance of using global solvers to avoid the multiplicity of solutions (Sridhar and Saucedo, 2016), global solutions are not considered here as our aim is to exemplify the benefits of a multiple feed paradigm on the performance of bioprocessing systems.

Results

Figure 2 depicts the steady state profile, control policy and feed concentration for the continuous bioreactor using a single feed of 20 g/L glucose in media. These profiles, control policy and feed concentration data are determined by solving the optimal control problem defined by Equation 3 with the allowable values of ethanol flowrate (F_1) and media only flowrate (F_3) set to zero. The steady state is reached through a cyclic feeding policy that transitions from 1.2 L/hr, or a fully open pump, to 0.348 L/hr to a completely closed pump over the course of an hour. As there is only a single pump, the glucose concentration is constant at 20 g/L at any point the pump is open and zero otherwise. This results in a periodic steady state for all five system states, with the glucose concentration oscillating around β-carotene concentration oscillating around a steady state value of 20.57 mg/L. The inhibitory compounds ethanol and acetic acid reach an oscillating steady state around 5.19 g/L and 0.394 g/L, well below their inhibitory levels of 60 g/L and 8 g/, respectively. These low concentrations of inhibitory compounds result in a steady state biomass concentration of 2.37 g/L, leading to a cellular yield of β-carotene of 5.69 mg/g biomass.

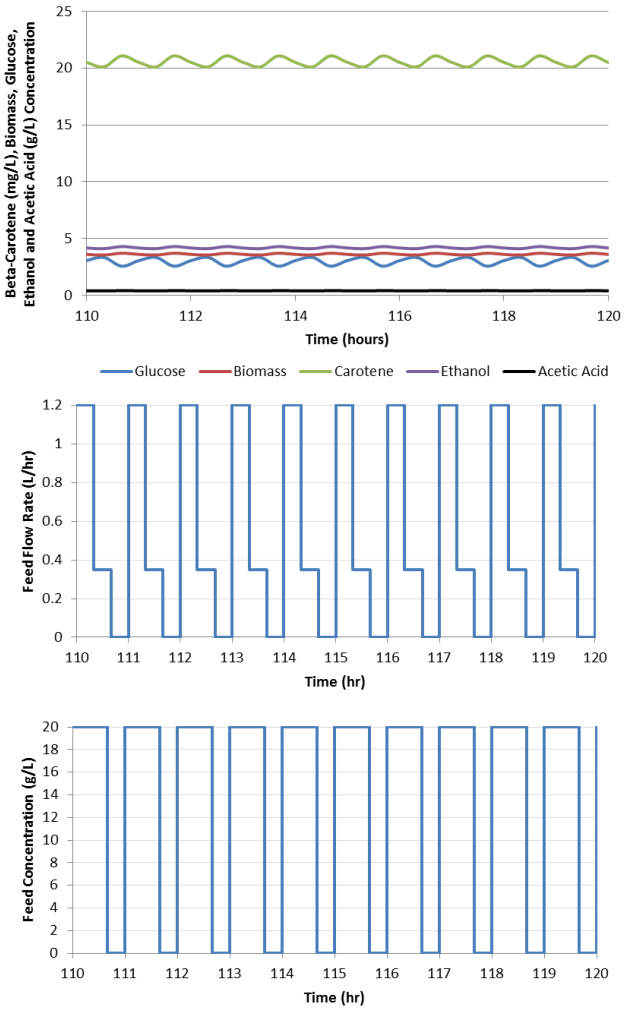

Figure 2. Single feed bioreactor optimal steady state concentration profile (top), control policy (middle), and feed concentration (bottom).

Figure 3 depicts the steady state profile, control policy and feed concentration for the multi-feed bioreactor configuration using three individual feeds consisting of 5 g/L ethanol, 20 g/L glucose and media with no substrate. Like the single feed case, the steady state control policy exhibits cyclic behavior in all three available pumps, resulting in the oscillatory behavior of the profiles. The optimal control policy for the multi-feed reactor shows a bang-bang control policy for the ethanol and media feeds every hour of operation at the maximum flowrate of 0.4 L/hr and the minimum flow rate of zero. The utilization of the glucose pump emulates the hourly cyclic usage

presented by the single feed reactor starting with the full use of glucose at 0.4 L/hr, then a period of approximately 0.2 L/hr, and then a period of no flow. These cyclic feed result in a cyclic profiles for the state variable. The steady state for β-carotene and biomass production are 17.35 mg/L and 2.37 g/L, respectively, resulting in a cellular yield of 7.33 mg/g biomass. The steady state for the inhibitory compounds ethanol and acetic acid are 3.14 g/L and 0.28 g/L, both lower than their single feed counterparts.

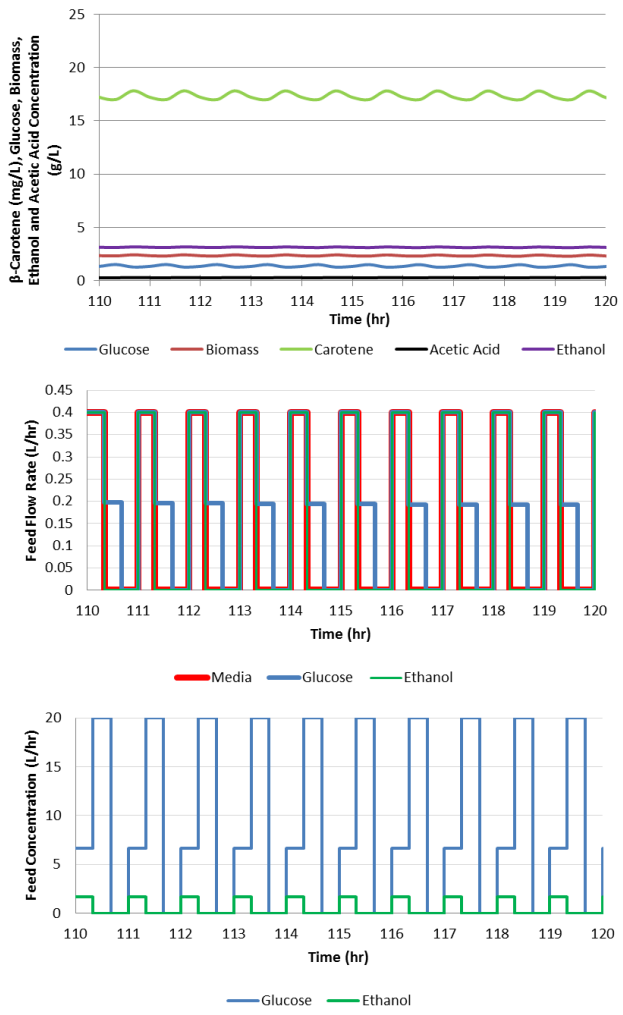

Figure 3. Multi-feed bioreactor optimal steady state concentration profile (top), control policy (middle), and feed concentration (bottom).

Table 1 lists the major metrics used to compare the performance of the single feed bioreactor to that of the multi-feed reactor. As mentioned before, the implementation of multiple feeds in the system led to a result in the overall β-carotene titer from 20.57 mg/L to 17.35 mg/L, a drop of 15.6%. Additionally, the average dilution rate over one cycle (for example the steady state time periods depicted in Figures 2 and 3) show that the dilution rate of the multi-feed bioreactor, 0.159 L^{-1}, is smaller than that of the single feed reactor, 0.176 L^{-1}, a result that suggest that the multi-feed bioreactor requires a longer residence time. The disparity between the titer and dilution rate result in the nearly 24% smaller productivity of the multi-feed bioreactor when compared to a single feed reactor of equal size, suggesting that a similar capital would result in a smaller quantity of product in the multi-feed reactor. However, the use of multiple feeds for the bioreactor did result in a higher cellular yield of β-carotene compared to its single feed counterpart, a difference of 7.33 mg/g biomass to 5.69 mg/g biomass, respectively.

To determine the economic impact of the affect of multiple feed, the operating cost of the reactors need to be examined. To do this the breakeven price of the β-carotene was calculated based on Eq. (11):

$$F_{out} \cdot \bar{P} \cdot C_P - F_1 \cdot G_f \cdot C_G - F_2 \cdot E_f \cdot C_E = 0 \qquad (11)$$

Here, the revenue gained from the average amount of β-carotene produced by the bioreactor is set equal to the cost necessary to provide the average feed of glucose and/or ethanol to the reactor. By setting the revenue and cost equal, the breakeven price C_P was calculated. For these calculations the price of the ethanol, C_E, was set to be approximately \$3 per liter (\$1 per gram) (ICIS Chemical Pricing). The price of the glucose (C_G) was set to be \$0.40 per pound (\$0.88 per gram) based on the reports available from the United States Department of Agriculture (2016) for the commodity cost of glycose syrup based on dry weight.

Results for the breakeven analysis can be found in Table 1 and show that the breakeven price of the multi-feed bioreactor is \$873.47 per kilogram of β-carotene while the same value for the single feed reactor is \$1,258.58 per kilogram. The implementation of multiple feeds, resulting in an approximately 30% decrease in operating cost, can be attributed to the difference in the substrate yield of β-carotene. The feeding of ethanol allowed by the multi-feed reactor stimulated an increased yield of product per gram of substrate fed to 2.16 mg/g, and increase from 1.31 mg/g substrate when glucose is the only substrate. This 64.86% increase in substrate yield allows for the decrease in operating cost in spite of the decrease in overall reactor productivity. While an in-depth analysis of the long term economics is needed, the results discussed here show great promise for stimulating economic improvement of biochemical systems through the implementation of multiple feed bioreactors.

Table 1. Breakeven Beta-carotene Price for the Optimal Single Feed and Multi-feed Strategies

	Multi-Feed	Single Feed
Average β-carotene Titer (mg/L)	17.35	20.57
Average Dilution Rate (hr^{-1})	0.159	0.176
Productivity (mg/L-hr)	2.76	3.61
Breakeven β-Carotene Price ($/kg)	$873.47	$1,258.58
Cellular Yield (mg/g biomass)	7.33	5.69
Substrate Yield (mg/g substrate)	2.16	1.31
Productivity Difference	-23.53%	0.00%
Price Difference	-30.60%	0.00%

Conclusions

This work introduces the idea of a multi-feed bioreactor as a method of improving the economic potential of biochemical processes. Through the addition of extra feeds that can be utilized for dilution affects as well as the addition of secondary substrates it is possible to reduce the operating cost of bioreactor systems through the increase of cellular yield. The concept of the multi-feed bioreactor is exemplified in this work through the production of β-carotene via recombinant *S. cerevisiae*, a yeast species that exhibits growth rates on both glucose and ethanol with varying degrees of biomass cultivation and product formation. The results of this case study show that, while productivity decreased by approximately 24%, the implementation of a multi-feed reactor was able to decrease the breakeven cost of the produced β-carotene by 30% as a result of an increase in cellular yield and substrate yield. The implementation of a multi-feed, continuous bioreactor can be adapted to many different biological systems as a method of stimulating economic viability through the reduction of process operating costs.

Acknowledgements

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