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Optimization of Penicillin V Production Using a Continuous Reactor

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ABSTRACT: Penicillin and its derivatives are widely used as antibiotics in both humans and animals. Current methods of penicillin production involve a group of fed-batch reactors operated in parallel to sustain a continuous production rate of the product. In this paper we look at the production process of penicillin using a continuously fed reactor (CSTR) and attempt to optimize the reactor parameters to enhance its performance and reduce required total reactor volume. Four different objectives were optimized: penicillin concentration, production rate, profit, and yield. 11 different design variables were used with appropriate constraints and bounds. A particle swam optimizer (PSO) was used with 5,500 particles initially distributed over the design space by Latin hypercube sampling. The optimization results found that the CSTR was better than the fed-batch system for maximizing penicillin concentration (100 vs. 45 $gm \cdot L^{-1}$), production rate (671 vs. 248 $kg \cdot hr^{-1}$), and yield (0.224 vs. 0.194 (gm-penicillin) · $(gm\text{-glucose})^{-1}$) while using less than the total reactor volume for the fed-batch process.

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KEYWORDS:

Introduction

Bioprocessing is now involved in the production of many medicines, biofuels, and commodity chemicals. These processes use cultures of living organisms such as *Escherichia coli* or *Saccharomyces cerevisiae* (baker's yeast) to produce intermediate and final products. Because of the complexity inherent in working with living organisms these kinds of processes have many opportunities for optimization. For example, Lee *et al.* (2007) used a genetic algorithm to find optimal operating parameters for a fed-batch penicillin production system. In this work the researchers looked at different combinations of three optimization goals using a fixed process design while changing operating parameters such as starting reactor volume, starting biomass concentration, and nutrient inlet flowrate. Their work demonstrated that improvements could be made to the current penicillin production process by using optimization techniques. Additionally, they derived a better understanding of how the performance of the process changes with changes in the operating parameters.

The process described by Lee *et al.* involved a "train" of fed-batch reactors that were operated in a staggered configuration in order to achieve a continuous production rate of penicillin V. Fed-batch reactors are commonly used in bioprocesses because many of these processes are developed initially as batch processes. The process starts by filling a clean reactor with appropriate amount of broth and nutrients to which an inoculum is added containing the microorganism to be grown. This inoculum is grown up in stages starting from a small volume into progressively larger volumes in order to maintain a reasonable growth rate of the microorganism until there is sufficient inoculum to add to the final reactor. Once the inoculum is added additional glucose-infused broth is slowly added to the bioreactor to sustain the growth of the microorganism and the desired product. Thus, over time the working volume inside of the fed-batch reactor increases and the concentrations of species and their behaviors change over time. After a predetermined amount of time, such as when the decay rate of the microorganism becomes greater than its growth rate, the reactor is drained and the broth containing the microorganism and the product is sent downstream to a separation process. There, the product is extracted from the spent broth and the broth is sterilized and discarded. An organic solvent such as butyl acetate is used to extract the penicillin from the broth. At the end of the process a crystallizer is used to obtain the penicillin V as a sodium or potassium salt. During this time the reactor is sterilized, usually using steam injection, and a new cycle starts. Thus, fed-batch processes tend to be laborious and limited in their production potential.

In comparison, continuous stirred tank reactors (CSTRs) are designed to work at steady state conditions for long periods of time. Once started, CSTRs are intended to maintain a specified working volume and operating conditions with a continuous inflow of reagents and outflow of products. This design allows for simpler operation compared to the time-dependent nature of fed-batch reactors. Additionally, it allows a reactor to produce large amounts of product between shutdown periods.

The hypothesis of this work is that *replacing the train of fed-batch reactors with one or more CSTRs will improve both the process performance as well as decrease labor and capital costs associated with a bioprocess*. In this work we set out to optimize the same penicillin production process given by Lee but with a CSTR instead of the group of fed-batch reactors. Their paper states that a continuous production rate of 292 $kg \cdot hr^{-1}$ of penicillin V sodium salt (248 $kg \cdot hr^{-1}$ penicillin V) is to be made 24 hours a day for 330 working days out of a year. Although the paper mentions some of the operating costs, it does not break them down in a reusable way. Fortunately, another paper, written by Biwer *et al.* (2004), involves the same process but provides adequate detail of operating costs for a profit analysis.

This paper is organized as follows. A brief description of the fermentation and production process given by Lee along with

changes made to the corresponding equations are given in the next section. This section also describes the methodology used for the optimization, including the optimizer algorithm, the design variables, and the constraints. The next section provides the results obtained from the optimization. Finally the results are discussed along with their implications and potential issues related to the new process design.

Materials and Methods

Fermentation Model

A typical fed-batch penicillin production scheme is shown in figure 1. Penicillin V is produced using the fungus known as *Penicillium chrysogenum*. The mathematical model of the fermentation process was originally developed by Zangirolami *et al.* (1997) and more detail about it than is presented here is given in the appendix. The model accounts for the growth of three types of cellular compartments in the fungus: the apical cells, subapical cells, and hyphal cells. It is believed that penicillin production occurs mainly in the subapical cells and, to a lesser extent, in the hyphal cells. The apical and subapical cells mostly contribute to the new growth of the fungus while the hyphal cells provide an osmotic pressure that helps draw nutrients towards the growing compartments.

The nutrients for the process include glucose (primary energy source) and corn steep liquor (CSL), which is a by-product of corn processing and provides other nutrients, such as amino acids, that are needed for cell growth. The CSL is accounted for as a glucose equivalent in the model, but only 42% of it can actually be metabolized by the microorganism. It is known that excess glucose has the potential to discourage penicillin production.

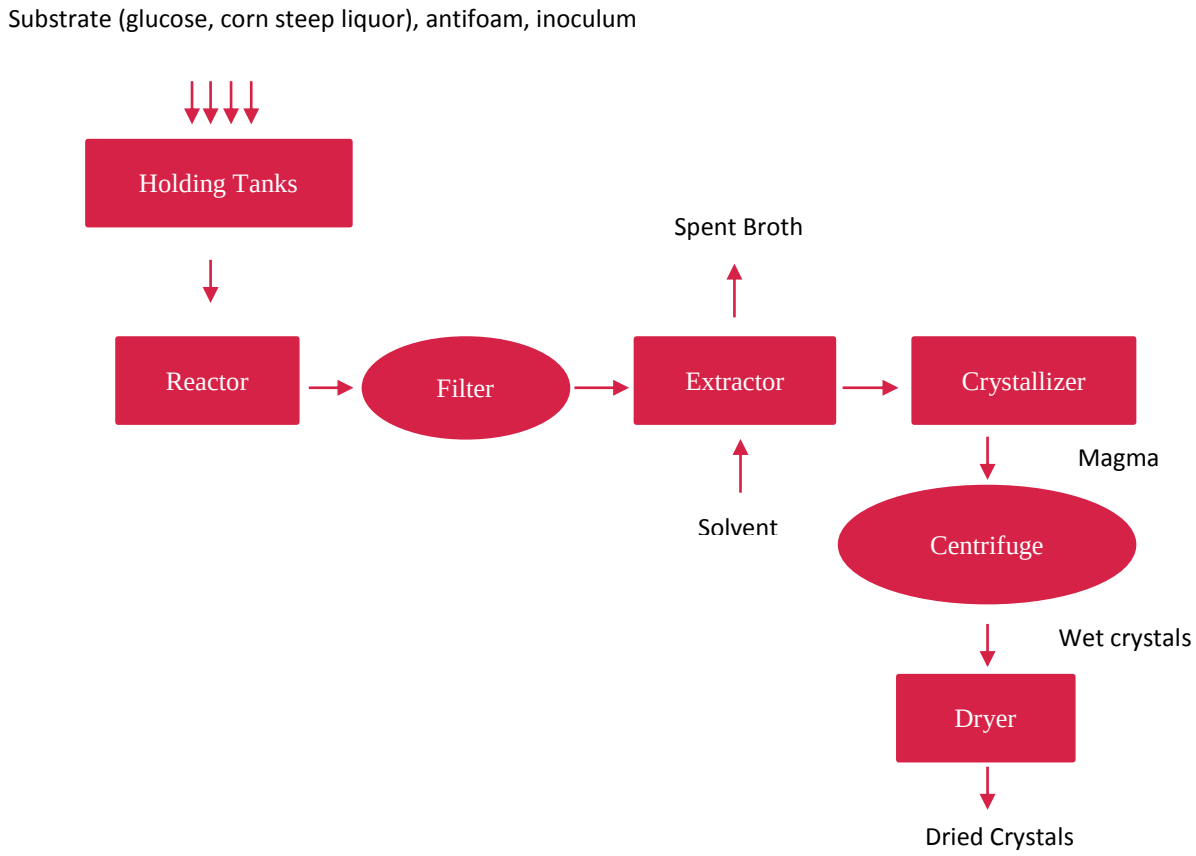


Figure 1. Overview of penicillin V production process, including both the production and separation components of the process.

Design Equations

After thoroughly reviewing the paper by Lee, we analyzed the fermentation model and the general optimization methodology. The main equations of the model involve partial derivatives of different species with respect to time. To apply the fermentation model to the CSTR process, which naturally is time-independent, we removed all the time derivatives and set the remaining parts of the equations equal to zero. Doing so greatly simplified the work that had to be done during the optimization process because the system of equations only required a nonlinear solver instead of a time-dependent ODE solver. To make the system of equations easier to work with we combined many of the equations together and eventually simplified the system to six equations and six unknowns. The unknowns are Z_a (fraction of apical cells), Z_s (fraction of sub-apical cells), Z_h (fraction of hyphal cells), $C_{glu,out}$ (glucose concentration inside of the reactor), $C_{csl,out}$ (CSL concentration in the reactor), and C_{pen} (penicillin concentration in the reactor). The six unknowns were included in our set of design variables. The variables V (reactor volume), $\dot{V}$ (inlet volumetric flow rate), $C_{glu,in}$ (inlet glucose concentration), $C_{csl,in}$ (inlet CSL concentration), and X_{bio} (biomass concentration inside of the reactor) were also used as design variables (see discussion in *Optimization Tools* section). Descriptions of the other variables are given in the appendix and come from the paper by Lee. The modified system of equations are listed as Equations 1-6 below. Equations 7 and 8 are supporting functions that come up several times in Equations 1-6.

$$ku_1 \cdot Z_s - ku_2 \cdot Z_a + Z_a \cdot (k_a \cdot ST_{Ks}) - Z_a \cdot ((k_a \cdot ST_{Ks}) \cdot Z_a + ks \cdot ST_{Ks} \cdot Z_s + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h) = 0 \quad (1)$$

$$ku_2 \cdot Z_a - ku_1 \cdot Z_s - \frac{ku_3 \cdot Z_s}{ST \cdot Ku_3 + 1} + Z_s \cdot -Z_s \cdot ((k_a \cdot ST_{Ks}) \cdot Z_a + ks \cdot ST_{Ks} \cdot Z_s + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h) = 0 \quad (2)$$

$$\frac{ku_3 \cdot Z_s}{ST \cdot Ku_3 + 1} + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h) - \left(ka \cdot ST_{Ks} \cdot Z_a + ks \cdot ST_{Ks} \cdot Z_s + fh(kh \cdot ST_{Ks} \cdot Z_h)\right) \cdot Z_h = 0 \quad (3)$$

$$k_2 \cdot \frac{C_{GLU,out}}{C_{GLU,out} + K_2 + \frac{C_{GLU,out}^{\ 2}}{K_1}} \cdot (Z_s + fh \cdot Z_h) \cdot X_{bio} - \frac{\dot{V}}{V} \cdot C_{pen} = 0 \quad (4)$$

$$C_{GLU,in} \cdot \frac{\dot{V}}{Vol} - \frac{\dot{V}}{Vol} \cdot C_{GLU,out} - (\alpha_1 \cdot \frac{C_{GLU,out}}{ST} \cdot \left(ka \cdot ST_{Ks} \cdot Z_a + ks \cdot ST_{Ks} \cdot Z_s + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h)\right) + ms + \alpha_2 \cdot \frac{C_{GLU,out}}{C_{GLU,out} + K_2 + \frac{C_{GLU,out}^{\ 2}}{K_1}} \cdot (Z_s + fh \cdot Z_h)) \cdot X = 0 \quad (5)$$

$$C_{CSL,in} \cdot \frac{\dot{V}}{Vol} - \frac{\dot{V}}{Vol} \cdot C_{CSL,out} - \alpha_1 \cdot \frac{C_{GLU,out}}{ST} \cdot ka \cdot ST_{Ks} \cdot Z_a + ks \cdot ST_{Ks} \cdot Z_s + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h) + fh \cdot (kh \cdot ST_{Ks} \cdot Z_h) \cdot X_{bio} = 0 \quad (6)$$

$$ST = C_{GLU,out} + \alpha_{CSL} \cdot C_{CSL,out} \quad (7)$$

$$ST_{Ks} = \frac{ST}{ST + K_s} \quad (8)$$

Optimization Tools

The results for this work come from an in-house particle swarm optimizer (PSO). The PSO was chosen over other optimization algorithms because initial testing demonstrated that gradient-based optimizers would find different local minima depending on the initial design variable values. The results below demonstrate that the PSO was able to find essentially the same minima during each run for each objective, except for the profit objective, where the final objective values differed but were relatively close in value. Using the PSO allowed us to "sweep" the design space to give us the best chance of finding the global optimum during each run.

The PSO jobs consisted of 5,500 particles that were initialized by spreading them evenly throughout the design space using Latin hypercube sampling within their upper and lower bounds. The large number of particles was chosen because testing demonstrated that having approximately 50 particles per dimension provided good coverage so as to allow the PSO to adequately explore the design space. Despite the large number of particles, it was determined that using a single processor for each job was faster than attempting to parallelize the work. Each optimization run was given a maximum of 60,000 iterations in which to converge to a solution. Convergence was achieved when the swarm best objective value had not changed more than the prescribed tolerance after 150 iterations since the last convergence check. Tolerances for each objective were determined by dividing the order of magnitude of the expected final objective values by 1e+06 (see table 1).

Table 1. Tolerances used for each objective.

Objective	Tolerance
Penicillin Concentration	1e-04
Production Rate	1e-01
Profit	1e-03
Yield	1e-07

Design Variables

There were 11 design variables for this project, shown in table 2 along with their upper and lower starting bounds. The first five variables are process control variables—values that would be manipulated by the operators or controllers to manage the process. The remaining six variables are reactor condition values—variables that are needed to solve the system of equations for the fermentation model described above. By including the reactor conditions as design variables rather than solving for them using the process control variables and the system of equations saved substantial computational time. Additionally, no heuristics were available to help determine appropriate guess values for the reactor conditions based on a given set of process control variables, so allowing the optimizer to find both sets of variables at the same time greatly simplified the search problem.

Table 2. Design variables and their starting upper and lower bounds.

Design Variable	Units	Lower Bound	Upper Bound
$\dot{V}$	$L \cdot hr^{-1}$	0	5,000
Vol	L	0	5,000,000
$C_{glu,in}$	$gm \cdot L^{-1}$	0	909
$C_{csl,in}$	$gm \cdot L^{-1}$	0	909
X_{bio}	$gm \cdot L^{-1}$	0	40
Z_a	~	0	1
Z_s	~	0	1
Z_h	~	0	1
$C_{glu,out}$	$gm \cdot L^{-1}$	0	909
$C_{csl,out}$	$gm \cdot L^{-1}$	0	909
C_{pen}	$gm \cdot L^{-1}$	0	100

Constraints

The optimizer had eight constraints on it, which are shown in table 3 (Equations 9-16). These constraints were used for each objective. The constraints were applied to the objective function value by summing the constraint violations and then multiplying this sum by $1 \cdot 10^9$ to help enforce constraints with small numerical values, such as Equation 11. The first constraint requires that the system of equations be solved. The second requires that the efficiency of the fermentation be realistic, i.e., less than one. The third is the solubility limit of penicillin in water at ambient conditions. The fourth was based off of the total potential liquid volume of the fed-batch reactor train described by Lee. The Fifth was implemented because testing showed that this value, termed the "space time," would become unrealistically large if unconstrained. The value of this constraint was roughly based off of the average batch cycle time given by Lee. The sixth and seventh constraints help to enforce realistic operating conditions—initial testing demonstrated that the optimizer would find solutions where the outlet concentrations of the reagents were greatly above zero, i.e., the reagents would not be completely used up. This would never be allowed in a real system because of the expense associated with the wasted reagents. In the code these constraints actually set the corresponding variables equal to zero, however, as was anticipated and demonstrated (especially for the production rate and profit objectives) the optimizer could not actually drive these constraints all of the way to zero without hurting the objective. For the concentration and yield objectives, though, these constraints may have negatively impacted the results of the optimization (see *Results* section). The final constraint was mostly a check to ensure that the mass fractions of the three types of cells summed to one.

Table 3. Constraints used with the PSO.

$$SSE \leq 10^{-25} \quad (9)$$

$$yield < 1 \quad (10)$$

$$C_{pen} \leq 100\ gm \cdot L^{-1} \quad (11)$$

$$Vol \leq 5{,}000{,}000\ L \quad (12)$$

$$\frac{Vol}{\dot{V}} \leq 1{,}000\ hr \quad (13)$$

$$C_{glu,out} \approx 0\ gm \cdot L^{-1} \quad (14)$$

$$C_{csl,out} \approx 0\ gm \cdot L^{-1} \quad (15)$$

$$Z_a + Z_s + Z_h = 1 \quad (16)$$

Objectives

For this project we decided to look at four different objectives. Each objective was optimized by itself. In reality, profit would be the primary objective that one would look at for a real process. The reason for looking at the different objectives is that each one uses a unique combination of the design variables and as a result were anticipated to exhibit unique behavior. As such, we expected that looking at four objectives would provide us with greater insight into the properties of the CSTR-based production system. The objective functions are given below as Equations 17-20. The equations were multiplied by (-1) to make them minimization functions.

$$Penicillin\ Concentration = C_{pen} \quad (17)$$

$$Production\ Rate = \dot{V} \cdot C_{pen} \quad (18)$$

$$Profit = Cst_{pen} \cdot n \cdot C_{pen} \cdot \dot{V} \cdot (1 - f) - Cst_{opt} \quad (19)$$

$$Yield = \frac{C_{pen}}{C_{glu,in} \times \alpha_{csl} \times C_{csl,in}} \quad (20)$$

Here, Cst_{pen} = selling cost of penicillin, n = number of reactors, f = fraction of penicillin lost during production, Cst_{opt} = total operating cost, and α_{csl} = 0.42 (gm-glucose)·(gm-CSL)$^{-1}$. The values for Cst_{pen}, Cst_{opt}, f, and, α_{csl} were given either by Lee or Biwer. The operating cost was calculated based information given by Biwer and consisted of the cost of raw materials, equipment, labor, utilities, plant overhead, quality control, waste, and consumables. All of the costs were fixed except for raw materials which depended on the amount of glucose and CSL fed to the system ($\dot{V} \cdot C_{glu,in}$ and $\dot{V} \cdot C_{csl,in}$, respectively). Please see appendix A for more details on how the operating cost was calculated.

Results

Optimized Objective Values

The final objective values and their associated standard errors based off of six replicates are showed in table 4. For the penicillin concentration, production rate, and yield objectives the PSO managed to arrive at essentially the same objective value for each of the replicates. In contrast, the profit objective would only converge to a particular range of values—from about 2,000 to 3,700 \$·hr^{-1}.

The final objective values for penicillin concentration (0.47 gm·L^{-1}) and yield (0.00024) turned out substantially smaller than were expected. Based on the work by Lee and Biwer, the penicillin concentration was expected to reach at least 45 gm·L^{-1} while the yield was expected to arrive at 0.2-1.0 (gm-penicillin)·(gm-glucose)$^{-1}$. Somewhat surprisingly, the penicillin concentration was better optimized when the real objective was production rate and the yield was better optimized when the true objective was the profit. The penicillin concentration reached 100 gm·L^{-1} for the production rate objective. The yield for the profit objective was found to be 0.224 (gm-penicillin)·(gm-glucose)$^{-1}$.

The exact reason for the poor performance of the PSO with these objectives is not totally clear, but is suspected to be at least partly due to constraints six and seven. The reasoning behind this is that the values of $C_{glu,out}$ and $C_{csl,out}$ are approximately 10^{-3} for both the production rate and profit objectives while for the penicillin concentration and yield objectives the corresponding nutrient concentrations are 10^{-9}. It appears that constraints six and seven might have "overpowered" these objective values by shifting the balance between a lower objective value and lower constraint violations towards a smaller (closer to zero) objective value.

Optimized Design Variables

The optimized design variables for each objective are given in table 5. For both concentration and yield, the space time (see Equation 13) constraint was active. For the production rate the maximum reactor volume constraint (Equation 12) was almost employed while the maximum allowed penicillin concentration constraint (Equation 11) was active. Of particular note, the reactor concentration constraints for glucose and CSL (Equations 14-15) were much closer to being satisfied for the penicillin concentration and yield objectives than they were the production rate and profit objectives.

Table 4. Optimized objective values and associated standard errors.

	Penicillin Concentration gm·L^{-1}	Production Rate gm·hr^{-1}	Profit \$·hr^{-1}	Yield (gm-penicillin)·(gm-glucose)$^{-1}$
Optimized Value	0.4697	671,000	3,060	2.43e-04
Standard Error	0.0494	25,100	855	2.82e-04

Table 5. Optimized design variables and their standard errors for each objective.

Design Variable	Penicillin Concentration		Production Rate		Profit		Yield	
	Value	Std. Error	Value	Std. Error	Value	Std. Error	Value	Std. Error
$\dot{V}$	3,348	274	6,706	251	3,273	653	3,740	188
Vol	3,304,206	243,875	4,738,786	169,539	2,786,982	1,506,845	3,715,250	200,840
$C_{glu,in}$	518.7	10.44	579.9	25.53	268.5	47.30	510.2	53.99
$C_{csl,in}$	0.0238	0.0246	258.1	86.52	14.90	38.27	0.3339	0.7895
X_{bio}	18.69	0.3963	15.07	1.995	8.620	9.032	18.28	1.912
Z_a	0.0176	0.0163	0.1134	0.0358	0.1560	0.2597	0.0316	0.0200
Z_s	0.0051	0.0047	0.0325	0.0103	0.0456	0.0759	0.0092	0.0058
Z_h	0.9772	0.0210	0.8537	0.0474	0.7999	0.3324	0.9591	0.0258
$C_{glu,out}$	2.42e-08	5.78e-08	0.0006	3.01e-05	0.0013	0.0017	2.02e-08	4.06e-08
$C_{csl,out}$	3.14e-10	6.27e-10	0.0008	0.0003	0.0003	0.0009	2.91e-07	7.37e-07
C_{pen}	0.4697	0.0494	100.0	0.0018	61.61	13.98	0.1215	0.1392

Convergence

The convergence statistics for each objective are shown in figures 2-4. Figure 2 contains the total number of function calls required by each objective. Figure 3 contains the job run times for each objective. Figure 4 plots the relative error as a function of iteration number for each of the objectives for each replicate job. We found that the production rate objective always required the maximum number of iterations before converging to a solution. Profit optimization took least number of function calls as well as the least amount of run time of the four objectives.

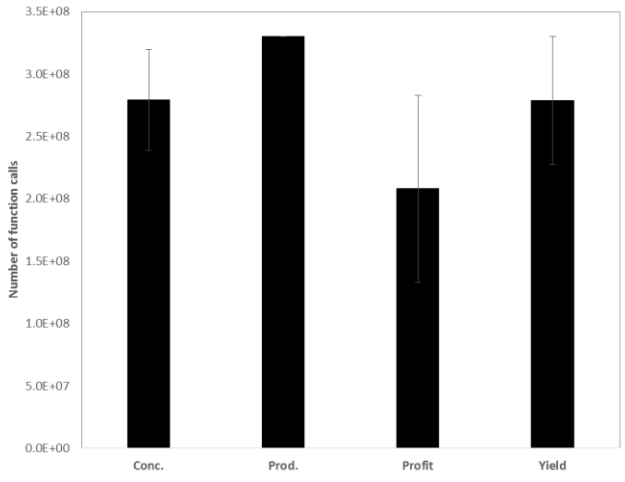


Figure 2. Number of function calls for each objective

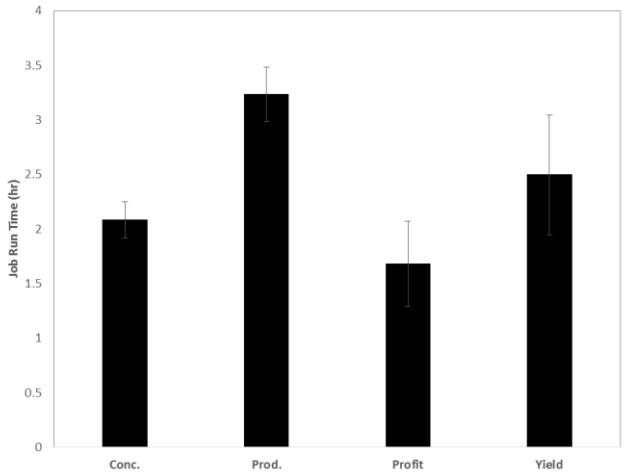


Figure 3. Job run time for each objective

The convergence plots in figure 4 show an interesting trend with the production rate objective. For the other three objectives the optimizer would ultimately end up taking slightly different paths through the design space before reaching the final solution. In contrast, while optimizing the production rate the optimizer found the exact same path through the design space each time. This trend is somewhat surprising, and demonstrates that for this objective the design space must have a "valley" which the optimizer can easily find at the start of the job and follow down to the optimal solution. It is worth noting here that although the relative error is still of order ten, this error is small compared to the optimized objective value that is of order 1e+04.

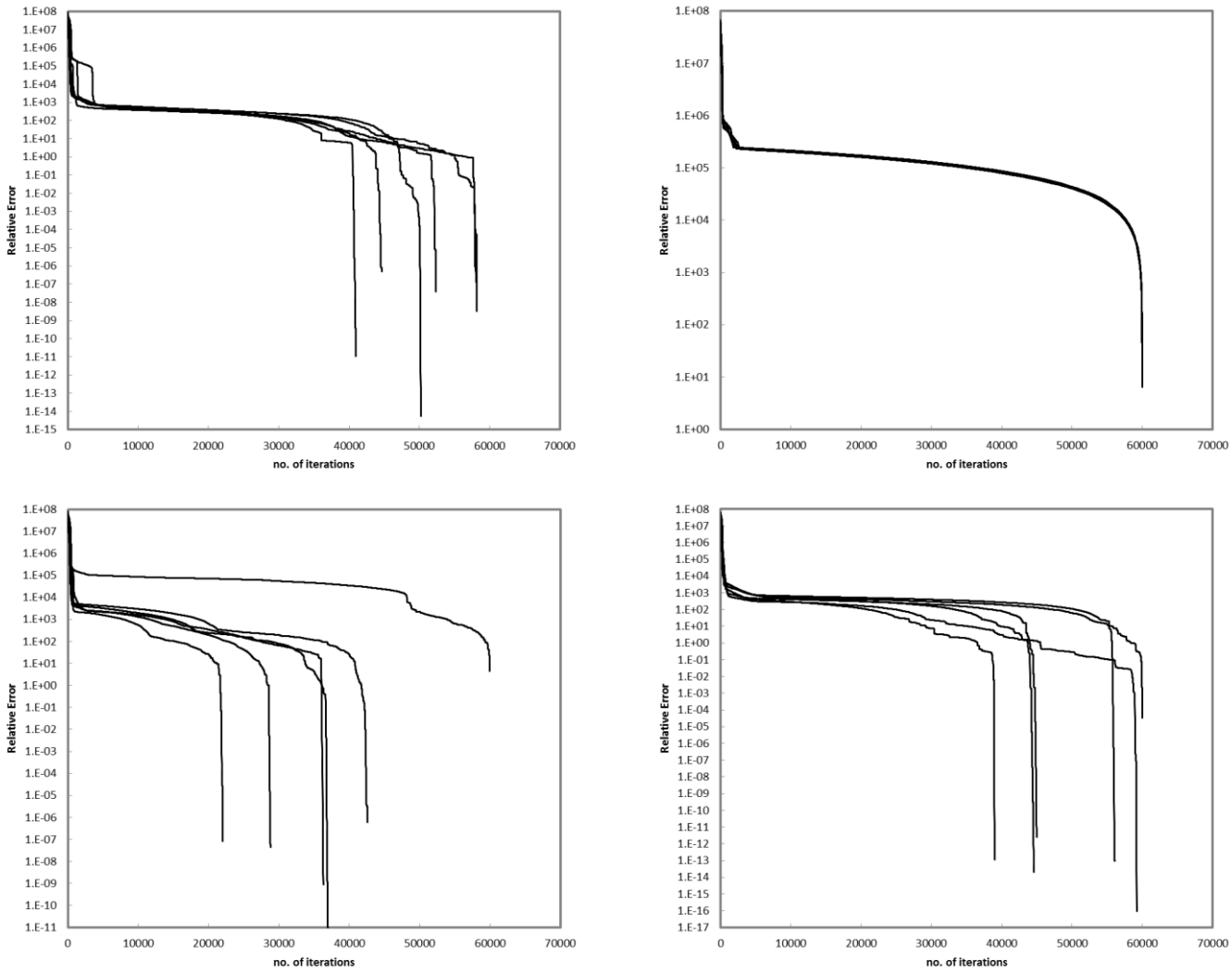


Figure 4. Convergence plots for penicillin concentration (upper left), production rate (upper right), profit (lower left), and yield (lower right) objectives

Discussion

The hypothesis of this project was that *replacing the train of fed-batch reactors with one or more CSTRs will improve both the process performance as well as decrease labor and capital costs associated with a bioprocess*. In several ways the results of the optimization support this hypothesis. The CSTR-based production system was capable of attaining a higher penicillin production rate (671 vs. 248 kg·hr^{-1}), penicillin concentration (100 vs. 45 gm·L^{-1}), and yield (0.224 vs. 0.194) than the fed-batch systems reported by either Lee or Biwer. These improvements were achieved using less total reactor volume than reported by Lee. However, the most important objective, practically speaking, is the profitability of the process. In this case the result is not as clear. Lee reports profits of approximately 3,500 \$·hr^{-1} while for this work the profit was calculated to be between 2,205 and 3,915 \$·hr^{-1}. The optimized profit values for this work are in the ballpark of appropriate values, but the uncertainty prohibits making a definitive conclusion on the profitability of the CSTR-based process vs. the fed-batch process. Additionally, the penicillin production rate for the profit objective only reached 199 kg·hr^{-1} rather than the required 248 kg·hr^{-1}. However, the higher yield value (0.224 vs. 0.194) shows that the CSTR-based system holds potential for being more profitable given that the yield is essentially a measure of the efficiency of the process.

Although the difference in required labor and capital costs for the CSTR-based system vs. the fed-batch system was not explicitly quantized, some general conclusions about this can be drawn from the results. To start, using CSTRs instead of fed-batch reactors reduces much of the required labor because the continuous nature of CSTRs requires fewer process startups while in the fed-batch system the reactors must be drained, cleaned, and reloaded each time a new batch of the product is to be made. Given the results of this work, the CSTR-based system requires less total reactor volume than the fed-batch system. The fed-batch system uses 20, 2.5e+05 L reactors (total of 5.0e+06 L) that are operated in a staggered fashion to create a continuous output of penicillin. In contrast, the results show that the CSTR-based system never requires all 5.0e+06 L, and often requires

much less than this. Furthermore, the capital cost for the equipment could be reduced further because the total reactor volumes shown in table 5 could be spread out over several smaller reactors, such that an optimal reactor size could be selected for the process.

There are two potential drawbacks with the CSTR-based production system that this work does attempt to address. The first is that continuous process control could be difficult to maintain due to the nature of biological systems. Depending on the available resources, biological systems sometimes exhibit exponential growth, which can be a challenge for controllers to handle. Additionally, growth performance tends to differ from one batch to another despite efforts to keep the setup the same each time, thus making it hard to develop accurate enough models to allow for precise model predictive control. For this system exponential growth may not to be a problem since the concentration of nutrients inside of the reactor is kept relatively low, which would naturally slow the growth of *P. chrysogenum* and keep it manageable. The second potential drawback involves the sterilization of the inlet stream. Typically, there is some chance that a batch will fail due to contamination when some unwanted microorganism survives the sterilization process for a feed stream sent into the reactor. This contamination is particularly problematic if it happens at the beginning of the process, but it is difficult to tell how it would impact a continuous process. Thus, additional work would have to be done to investigate the viability of a continuous production process given real-world contamination threats.

Conclusion

We have demonstrated that a CSTR-based penicillin production system could provide benefits over the fed-batch system that is currently used. The continuous process demonstrated the potential for an improved production rate, outlet penicillin concentration, and yield. While the production rate objective results are consistent with each optimization run, more work needs to be done improve the results of the concentration and yield objectives and to improve the consistency of the profit objective results. While the results are promising, it would be important to check the robustness of the optimization and tackle other potential issues such as contamination. Comparing PSO results with other optimizers such as a genetic algorithm or gradient-based functions could further improve upon the results found here. Finally, other biological systems could be optimized as continuous processes to test the adaptability of this work to other important products.

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