Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 562-575

Comparison Product Optimization Performance of Fed-batch Fermentation Processes for Penicillin G Production

Noor Asma Fazli Abdul Samad[a], Arshad Ahmad[b,*]

[a]*Department of Chemical Engineering, Faculty of Chemical and Natural Resources Engineering, 26000 KUKTEM Kuantan, Pahang, Malaysia.*
[b]*Department of Chemical Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.*

Abstract

This paper discusses the development of optimization strategies for a fed-batch penicillin fermentation process. To facilitate the study, a mathematical model of the system was developed. The model was simulated in MATLAB environment and good result was obtained. To provide on-line estimates of the difficult to measure penicillin concentration, Partial Least Squares model was employed. These estimates were then used in the control loop and successful control of the product concentration was established. This provided the opportunity for optimizing the fermentation operation, in particular to further increase the achievable product concentration while satisfying all process constraints. In order to carry out this procedure, two optimization algorithms were selected. First, dynamic optimization using direct shooting method and second is implementation single step ahead Dynamic Matrix Control (DMC). Comparison of these two different approaches was discussed and it was found this single step ahead DMC algorithm shown a best result with an optimization procedure.

Keywords: Penicillin G; Fermentation; Partial Least Squares; Direct shooting method; Single step ahead DMC

1. Introduction

Bioprocess control and optimization strategies vary depending on whether the bioprocesses are continuous or fed-batch. In the case of continuous bioreactors, the optimization is restricted to find optimal steady state around which the bioprocess is desired to be maintained. The controller is designed to regulate the bioprocess around the optimal point. However for fed-batch bioreactors, a dynamic optimization problem has to be solved first to determine the optimal feeding policies (Kapadi and Gudi, 2004). Once an off-line optimal control policy has been determined, a controller can be designed to track the optimal policy with capability to deal with disturbance for the closed loop control problem. In this work, the objective in optimization of a fed-batch bioreactor is to maximize penicillin production and optimization has been traditionally sought with respect to substrate feed rate.

Optimization strategies of bioprocess model are consisting of dynamic and steady state optimization. In dynamic optimization, the optimal feeding policy of the substrates that maximizes the amount of product is to be determined based on the dynamics of the system. This is different than the case of steady state optimization where the optimum solution for the process given objective function and constraint are achieved when the plant are determined to

* *Corresponding author: Tel: +60-7-553-5610 Fax: +60-7-558-1463, Email: arshad@fkkksa.utm.my*

be at steady state. For examples, Real Time Optimizer (RTO) is performed once plant has achieved steady state condition to keep the plant operating at its optimum. However, in fed-batch operation, there is no steady state for product concentrations and controls involve set-point tracking in addition to common task of rejecting disturbances. Dynamic optimization is therefore needed to bring about optimal operations.

Works on dynamic optimization of fed-batch bioprocess have been reviewed in a number of articles (Impe and Bastin, 1995; Rodrigues and Filho, 1999; Srinivasan *et al.*, 2002). One of the earliest approaches to optimization was presented by Ohno and Nakanishi (1976) where the solution was based on the application of Green's theorem to reduce the objective function to a line integral. Modak and Lim (1987) have studied the use of Pontryagin's maximum principle for dynamic optimization. The use of evolutionary techniques based on genetic algorithms for optimization of fed-batch bioreactors have also been reported by Wang and Cheng (1999).

Balsa-Canto et al. (2000) used a control vector parameterization technique and transform the original Optimal Control Problem (OCP) into a Nonlinear Programming (NLP) problem. The objective function was calculated using second order sensitivities as the solution of the NLP problem. As an alternative to NLP problem, Rodrigues and Filho (1999) suggested an optimization algorithm based on modified simplex method. Using this procedure, operating constraints were computed in relation to productivity through the concept of the penalty functions.

Disturbances that occur due to the fluctuations during the fed-batch process can cause the open-loop feed policy to be suboptimal. Thus it is necessary to incorporate feedback either in the form of estimating the key model parameters or resetting the initial conditions of the model and regenerating the optimal policy using on-line optimization once a new measurement is available. Unavailability of measurements due to lack of sensors have led to the use of various observers for the estimation of both the unmeasured states and the uncertain parameters. This has been the subject of various studies by Bastin and Dochain (1986) and Tatiraju et al. (1999). Impe and Bastin (1995) have coupled the estimation with optimal control and proposed adaptive control method for fed-batch bioreactors for tracking the optimal profiles. Rodrigues and Filho (1999) presented an approach for product optimization of a fed-batch penicillin production process with a dynamic matrix control (DMC) predictive controller. Thus there have been several approaches for the optimization of fed-batch processes and these are detailed in the next section.

This work intends to provide some answers to the above mentioned concerns. In this work, we concentrate on product optimization of a Penicillin G fermentation process. The unstructured model of Ahmad et al. (2003) was utilized as the basis of our modeling efforts. An inferential model constructed using partial least squares (PLS) regressions is employed for estimating the product concentration in to facilitate process control. Then optimal control strategies for fed-batch penicillin fermentation system are examined using two different optimization approaches. Firstly, dynamic optimization using direct shooting method and secondly is the implementation of single step ahead Dynamic Matrix Control (DMC).

2. Simulation Model of Penicillin Process

The production phase of the penicillin is carried out in a fed-batch operating mode with substrate supplementary addition. The bioreactor is perfectly stirred with aeration and agitation

system. The mathematical model is deterministic and unstructured developed upon Contois growth type kinetic. Yield coefficients among growth, substrate consumption, oxygen uptake and production specific rates are considered in this model. This mathematical model also includes additional input variables like feed flow rate of substrate, pH, temperature, aeration rate, agitation power as well as output variables such as CO_2 evolution and heat generation terms.

In this model, dynamic simulation runs were performed for a batch culture which is then followed by a fed-batch operation in order to promote the biosynthesis of the product. To accomplish this task, a threshold value of 0.3 g/l was assigned to the substrate concentration. The system would switch itself to the fed-batch mode of operation once it reached this threshold value. At this point, glucose began to be fed continuously into the system. However, high concentration of substrate of substrate may inhibit the cell growth. Hence, controlled supply of carbon source (glucose) was practiced in this study. As the base case for this study, a feedback control strategy with Proportional-Integral-Derivative (PID) controller was adopted for all control loops in the fed-batch bioreactor. Since temperature and pH play important roles in the fermentation process, both are fixed at some optimum values. pH was kept constant at 5.1 and temperature of the culture medium was kept constant at 298 K.

The penicillin fermentation model is solved simultaneously using Matlab software. The ordinary differential equations were solved using Fourth-Order Runge-Kutta algorithm with adaptive step size mechanism. Sampling time was fixed at 0.02 hour. The mathematical modeling and kinetic parameters as well as the initial values can be found in Ahmad et al. (2003). The dynamic simulation result for penicillin fermentation process is show in Figure 1. It shows that the production of penicillin started only after a long lag-phase. This is typical since cells use the lag phase to adapt to their new environment (Bailey and Ollis, 1986). Following the lag period, the growth started in the acceleration phase. As the substrate in the culture medium was depleted, glucose began to be fed continuously into the system. At this stage, the penicillin concentration started to increase at this stage indicating that the process was in the product formation phase.

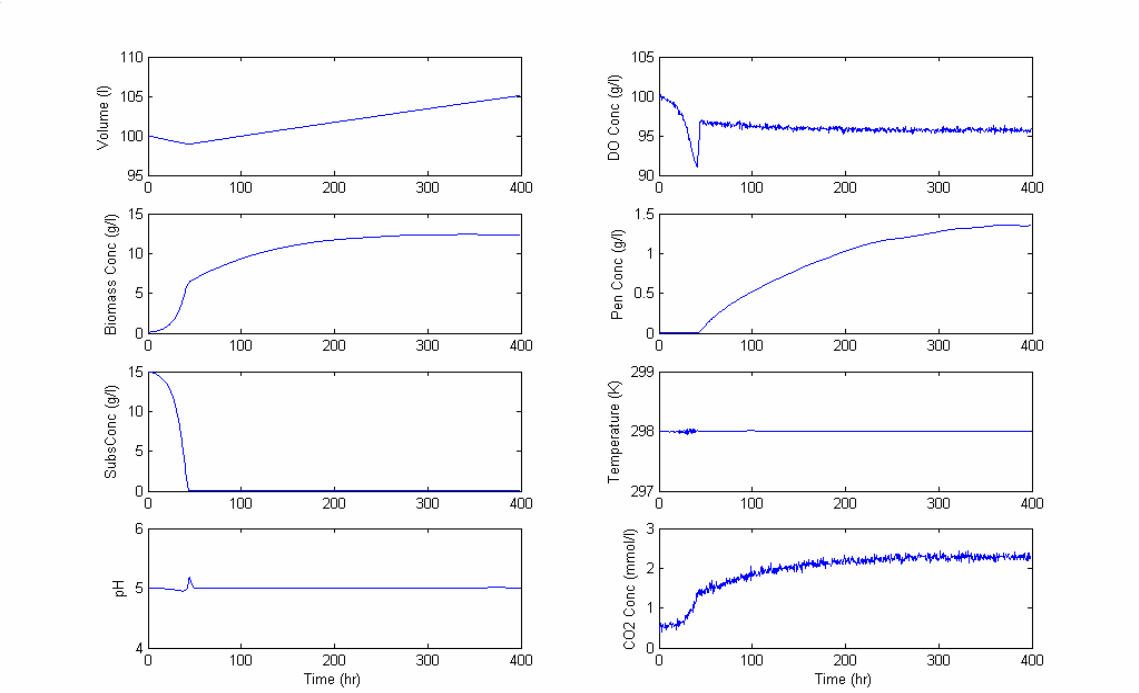

Figure 1 Dynamic simulations for penicillin fermentation process

3. Partial Least Squares Regression (PLS)

Partial least squares regression is one of the multivariate analysis methods. According to Wold (1985), it is a linear system identification method that projects the input-output data down into a latent space, extracts a number of principal factors with an orthogonal structure, while capturing most of the variance in the original data. Referring to this definition, it is also named as Projection to Latent Structures. PLS model is built using the Non-linear Iterative Partial Least Squares (NIPALS) algorithm introduced by Wold (1985). Details description of the PLS algorithm can be found in Geladi and Kowalski (1986). Figure 2 illustrated the PLS model schematically.

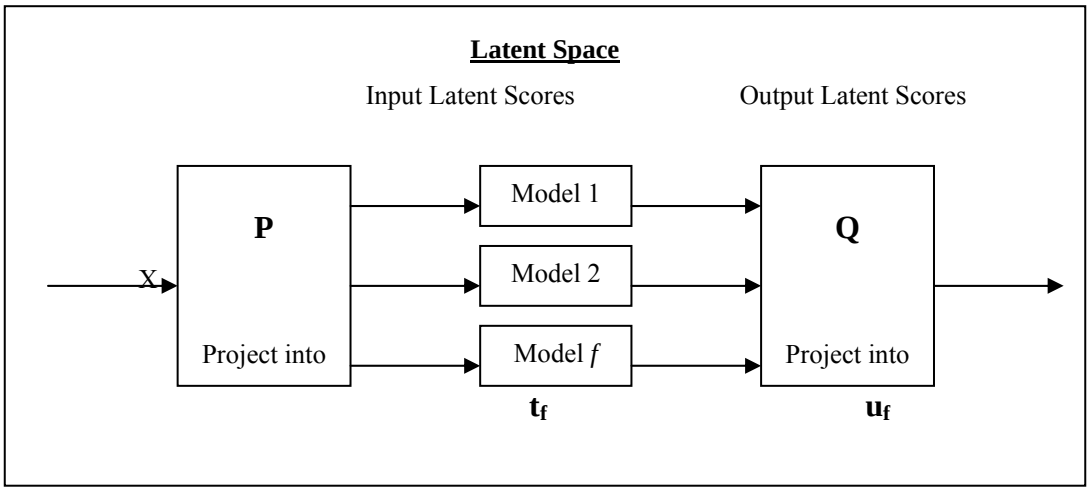

Figure 2 Schematic of the PLS model (Adebiyi and Corripio, 2003)

In this model the following measurements were used as input variables: substrate feed rate, aeration rate, agitator power, substrate feed temperature, culture volume, pH, fermentor temperature and heat generation. The accuracy of PLS model prediction is illustrated in Figure

3 which compares the actual penicillin concentration with that predicted by the PLS model for one of the batches. This figure shows that the model provided good estimates of penicillin concentration within the fermenter.

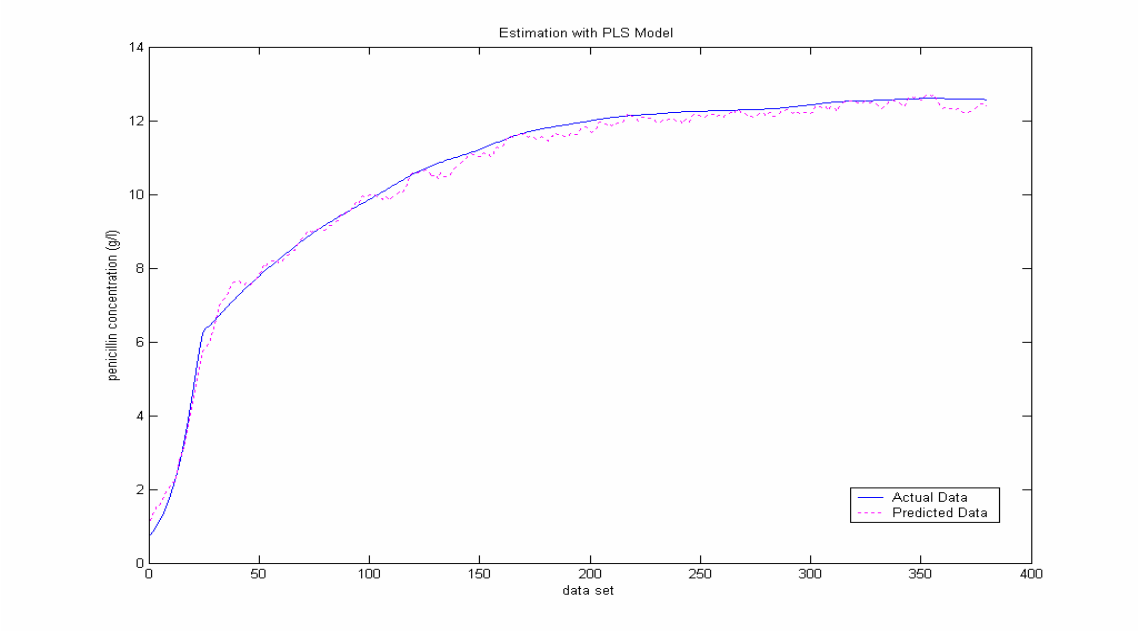

Figure 3 PLS model prediction

4. Formulation of Optimal Control

Dynamic optimization problems based on direct shooting method were first posed for aerospace applications in the 1950s. The mathematical formulation of the optimization problem will be stated first. The problem will be reformulated using Pontryagin's Minimum Principle (PMP) and the principle optimality of Hamilton Jacobi Bellman (HJB). In the case of a fed-batch bioreactor, one goal is to maximize an appropriate performance objective. Towards achieving this goal, it is important to note that decisions made regarding the input during the course of the batch play an important role on the objective function. The bioreactor model used for simulations was the same as described in previous section. Maximization of the penicillin concentration at the end of the batch was used as the performance measure.

The general formulation of the optimal control problem is now presented (Srinivasan et al., 2002). Let us consider that the system dynamics are described by,

$$\dot{x} = f[x(t), u(t), t] \quad \text{for } t_0 \le t \le t_f \text{ and } x(t_0) \tag{1}$$

In this equation, *x (t)* and *u (t)* is vector valued state and input respectively, t_0 is the initial time and t_f is the final time. Associated with the process operation is an objective function that needs to be maximized and the general formulation for the objective function is given as,

$$J = \phi(x(t_f), t_f) + \int_{t_0}^{t_f} L(x(t), u(t), t)\, dt \tag{2}$$

The functions ϕ accounts for the contribution of the final state, L accounts for the path dependence in the objective function with t_f as the final time of operation. The problem is to

find the functions *u (t)* that maximizes the objective function J , subject to the system dynamics described by f . Adjoin Equation (1) to J with multiplier functions $\lambda(t)$:

$$\bar{J} = \phi\left[x(t_f), t_f\right] + \int_{t_0}^{t_f} \left[L\left[x(t),u(t),t\right] + \lambda^T(t)\left\{f\left[x(t),u(t),t\right] - \dot{x}\right\}\right]dt \tag{3}$$

Integrate the last term on the right side of Equation (3) by parts yielding:

$$\bar{J} = \phi\left[x(t_f), t_f\right] - \lambda^T(t_f)x(t_f) + \lambda^T(t_o)x(t_o) + \int_{t_0}^{t_f} \left[L\left[x(t),u(t),t\right] + \lambda^T(t) f\left[x(t),u(t),t\right] + \dot{\lambda}^T(t)x(t)\right]dt \tag{4}$$

For convenience, define a scalar function *H* (the Hamiltonian), as follows:

$$H\left[x(t),u(t),\lambda(t),t\right] = L\left[x(t),u(t),t\right] + \lambda^T(t) f\left[x(t),u(t),t\right] \tag{5}$$

Include Equation (5) into Equation (4) yields:

$$\bar{J} = \phi\left[x(t_f), t_f\right] - \lambda^T(t_f)x(t_f) + \lambda^T(t_o)x(t_o) + \int_{t_0}^{t_f} \left[H\left[x(t),u(t),\lambda(t),t\right] + \dot{\lambda}^T(t)x(t)\right]dt \tag{6}$$

The optimization problem is to determine optimal feed rate policy serves as objective function for *u (t)*. So it is necessary condition for *u (t)* to be optimal is that it should maximize the Hamiltonian as described by Equation (5). Hence, the dynamic optimization can be formulated mathematically as follows:

$$\max_{t_f, u(t)} H(t) = \lambda^T f(x,u) + \mu^T L(x,u), \tag{7}$$

Subject to: $\dot{x} = f(x,u),\ \ x(0) = x_0,$ (8)

$$\dot{\lambda}^T = -\frac{\partial H}{\partial x},\quad \lambda^T(t_f) = \left.\frac{\partial \phi}{\partial x}\right|_{t_f} + v^T\left.\left(\frac{\partial T}{\partial x}\right)\right|_{t_f}, \tag{9}$$

$$\mu^T L = 0, \qquad v^T T = 0, \tag{10}$$

5. Single Step Ahead Dynamic Matrix Control

Model Predictive Control (MPC) has been the most successful advanced control technique applied in the process industries. The formulation naturally handles time-delays, multivariable interactions and constraints (Aufderheide and Bequette, 2003). In general MPC refers to a class of computer control algorithms that utilize an explicit process model to predict the future response of a plant. At each control interval an MPC algorithm attempts to optimize future plat behavior by computing a sequence of future manipulated variable adjustments. The first input in the optimal sequence is then sent into the plant and the entire calculation is repeated at subsequent control intervals (Qin and Badgwell, 2002).

Originally developed to meet the specialized control needs of power plants and petroleum refineries, MPC technology can now be found in a wide variety of application areas including chemicals, food processing and automotive. One of the MPC technologies is a Dynamic Matrix

Control (DMC). DMC algorithm can be separated into two parts, a predictor and an optimizer. In the original DMC formulation (Lundstrom et al., 1994) a step response model of the plant is used to predict the future behavior of the control variables. For the step response of a system with n_u inputs and n_y outputs:

$$S_i = \begin{bmatrix} S_{1,1,i} & S_{1.2.i} & \cdots & S_{1,n_u,i} \\ S_{2,1,i} & S_{2,2,i} & \cdots & S_{2,n_u,i} \\ \vdots & \vdots & \ddots & \vdots \\ S_{n_y,1,i} & S_{n_y,2,i} & \cdots & S_{n_y,n_u,i} \end{bmatrix} \quad i = 1,....,n. \tag{11}$$

The step response model can be represented in the following state space form:

$$Y(k+1) = MY(k) + S\Delta u(k) \tag{12}$$

$$y(k) = NY(k) \tag{13}$$

where:

$$\Delta u(k) = u(k) - u(k-1) \tag{14}$$

$$Y(k) = \left[y(k)^T \, y(k+1)^T \ldots y(k+n-1)^T \right]^T \tag{15}$$

$$M = \begin{bmatrix} 0 & I_{n_y} & 0 & \cdots & 0 & 0 \\ 0 & 0 & I_{n_y} & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & I_{n_y} & 0 \\ 0 & 0 & 0 & \cdots & 0 & I_{n_y} \\ 0 & 0 & 0 & \cdots & 0 & I_{n_y} \end{bmatrix} \quad n \times n_y ; \tag{16}$$

$$S = \begin{bmatrix} S_1 \\ S_2 \\ \vdots \\ S_{n-2} \\ S_{n-1} \\ S_n \end{bmatrix} \tag{17}$$

$$N = \left[\overbrace{I_{n_y} \quad 0 \quad 0 \quad \cdots \quad 0 \quad 0}^{n \times n_y} \right] \tag{18}$$

$\Delta u(k)$ is a vector of changes in the manipulated inputs at time *k*. $y(k)$ is the output vector at time *k*. The vector $Y(k+1)$ represents the dynamic states of the system. Each state $y(k+1)$ is the future output vector at time $(k+1)$ assuming constant inputs. The new state vector $Y(k+1)$ is the old vector $Y(k)$ shifted up n_y elements plus the contribution made by the latest input change $\Delta u(k)$.

5.1 DMC Predictor

The objective of the predictor is to generate a vector, $y(k+1|k)$ of predicted open loop outputs over a horizon of *p* future time steps, the prediction horizon. This prediction vector is then used as an input to the optimizer. The DMC optimizer is described by the following equations:

$$\bar{Y}(k) = M\bar{Y}(k-1) + S\Delta u(k-1) \tag{19}$$

$$\bar{y}(k) = N\bar{Y}(k) \tag{20}$$

$$y(k+1|k) = M_p\bar{Y}(k) + \ell\left[\hat{y}(k) - \bar{y}(k)\right] \tag{21}$$

Where M_p is the first $p \times n_y$ rows of *M* and:

$$\ell = \left[\overbrace{I_{n_y} \quad I_{n_y} \quad \cdots \quad I_{n_y} \quad I_{n_y}}^{p\times n_y}\right]^T \tag{22}$$

$\hat{y}(k)$ is a vector of measured outputs at time k. $\hat{y}(k)$ and $\bar{y}(k)$ are discontinuous at k_- while $u(k)$ at k_+. This is because $\hat{y}$ is measured slightly before time *k* and *u* is adjusted slightly after time *k*.

5.2 DMC Optimizer

The DMC optimizer objective function is adapted from Garcia and Morshedi (1985):

$$J = \min_{\Delta u(k|k)} \left\{\Gamma\left\|\left[Y_M(k+1|k) - R(k+1|k)\right]\right\|^2 + \left\|\Lambda\Delta U(k|k)\right\|^2\right\} \tag{23}$$

where:

$$\Delta U(k|k) = \left[\Delta u(k|k)^T\, \Delta u(k+1|k)^T \ldots \Delta u(k+m-1|k)^T\right]^T \tag{24}$$

$$Y_m(k+1|k) = \left[y_m(k+1|k)^T\, y_m(k+2|k)^T \ldots y_m(k+p|k)^T\right]^T \tag{25}$$

$$R(k+1|k) = \left[r(k+1|k)^T\, r(k+1|k)^T \ldots r(k+p|k)^T\right]^T \tag{26}$$

$\Delta U(k|k)$ is the optimal control sequence computed at time *k* for *m* future input moves, where *m* is the input horizon. $R(k+1|k)$ is a vector describing the desired output trajectory (set points) over *p* future time steps. Γ and Λ are weighting matrices and are usually chosen to be diagonal. $Y_m(k+1|k)$ is a vector of outputs predicted at time *k*, over a horizon of *p* future time steps including the effect of the *m* optimal input moves:

$$Y_m(k+1|k) = Y(k+1|k) + \ell_p^m \Delta U(k|k) \tag{27}$$

where:

$$\ell_p^m = \begin{bmatrix} S_1 & 0 & \cdots & 0 \\ S_2 & S_1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ S_m & S_{m-1} & \cdots & S_1 \\ \vdots & \vdots & \ddots & \vdots \\ S_p & S_{p-1} & \cdots & S_{p-m-1} \end{bmatrix} \tag{28}$$

In case where $m = 1$, Equation (24) must be reconstruct. In order to obtain optimal solution for the model, a least squares solution must be implemented. Then, Equation (38) becomes single step ahead DMC:

$$\Delta U(k|k) = \left[S_m^T \Gamma^T \Gamma S_m + \Lambda^T \Lambda\right]^{-1} S_m^T \Gamma^T \Gamma \times \left[R(k+1|k) - Y(k+1|k)\right] \tag{29}$$

6. Results and Discussion

For the optimal control of the fed-batch penicillin fermentation considered in this work, the state variable, x and the system equation f, are described by the model:

$$x=[X\ S\ V\ C_L\ CO_2\ P\ Q_{rxn}\ T\ H^+]^t \tag{30}$$

Here, X is the biomass, S is the substrate, V is the reactor volume, C_L is dissolved oxygen, CO_2 is carbon dioxide concentration, P represents the penicillin concentration, Q_{rxn} is the heat generation, T is temperature and H^+ represents ion hydrogen concentration. The feed rate F, serves as the control input u and the objective function for the optimal control problem is the maximization of the penicillin concentration at the end of the batch given as,

$$\max_{u(t)} J[u(t), x(t)] \tag{31}$$

$$J = P(t_f) \tag{32}$$

In addition it is assumed that the system is constrained so that the reactor volume at the end of the batch is restricted to 110 liter, which is an end-point constraint. The feed rate of substrate cannot be negative and the feed rate over any interval cannot exceed the free reactor volume at the start of the interval, so that,

$$0 \le u(k) \le 110 - V(k) \tag{33}$$

$$0 \le \sum_{k=t_0}^{t_f} u(k) \le 110 - V_0 \tag{34}$$

In these equations, V (k) is the volume at the time k, and V_0 is the initial volume. The final batch time was chosen as 400 hours. The entire batch was split into equal intervals of 20-hour duration. The optimal control was then discretized into 20 piece-wise constant segments, with

the values $u_i(k)$ for k = 0, 1, 2, …, 19. Here *i* is the iteration value during the course of the optimization while k represents the time instant at which the input is applied. Thus the value of *i* is increases as the optimization proceeds and the objective function are calculated for each iteration until it converges to its maximum or minimum value. The goal of the optimization procedure is to find the values of u(k) for all k, which give an optimal value of the performance index, J.

For the numerical integration of the state equations, the ode45 routine in MATLAB 6.5 was used and the optimization based on direct shooting method was carried out using the MATLAB 6.5 routine fmincon while single step ahead DMC algorithm was solved by its algorithm available in MATLAB 6.5. The results of the both optimization strategy are shown in Figure 4 and 5. The both optimal control policy for the fed-batch fermentation shows two distinct regions. Initially, it is seen that the fermentation proceeds in a batch mode, as the value of the substrate feed rate is zero. The batch growth continues until all of the initial substrate with which the fermentation process starts is utilized below the threshold value. At this point, glucose feed is sent into the reactor and this is utilized for the production of penicillin.

This approach leads to the optimal glucose feeding profile for maximizing the end of batch penicillin concentration. The off-line optimum profile provides the reference trajectory that the fed-batch operation must follow in order to maximize the end of batch penicillin concentration. The optimal control policy represents the best performance that can be obtained from the system for given set of initial and feed conditions. Although both of the optimization approaches shows similar pattern results but single step ahead DMC optimization method obtain a better result compare to direct shooting method. It is because several difficulties associated with the direct shooting method. Firstly, it can exhibit stability problems in integrating the adjoints equations forward in time. It is because direct shooting method is faced with Two Point Boundary Value Problem (TPBVP) problem. The boundary condition for state and adjoints equation must be accurate in order to obtain optimal solution. Therefore, direct shooting method need a good initial guess for the adjoint variables to find the optimal solution compare to the single step DMC approach.

Furthermore, the method does not work when there are discontinuities in the adjoints which is often the case in the presence of state constraints. Additional degrees of freedom must be included to handle these situations. Figure 6 and 7 shows the performance comparison between this two optimization approach and nominal operation for substrate feed rate and penicillin concentration.

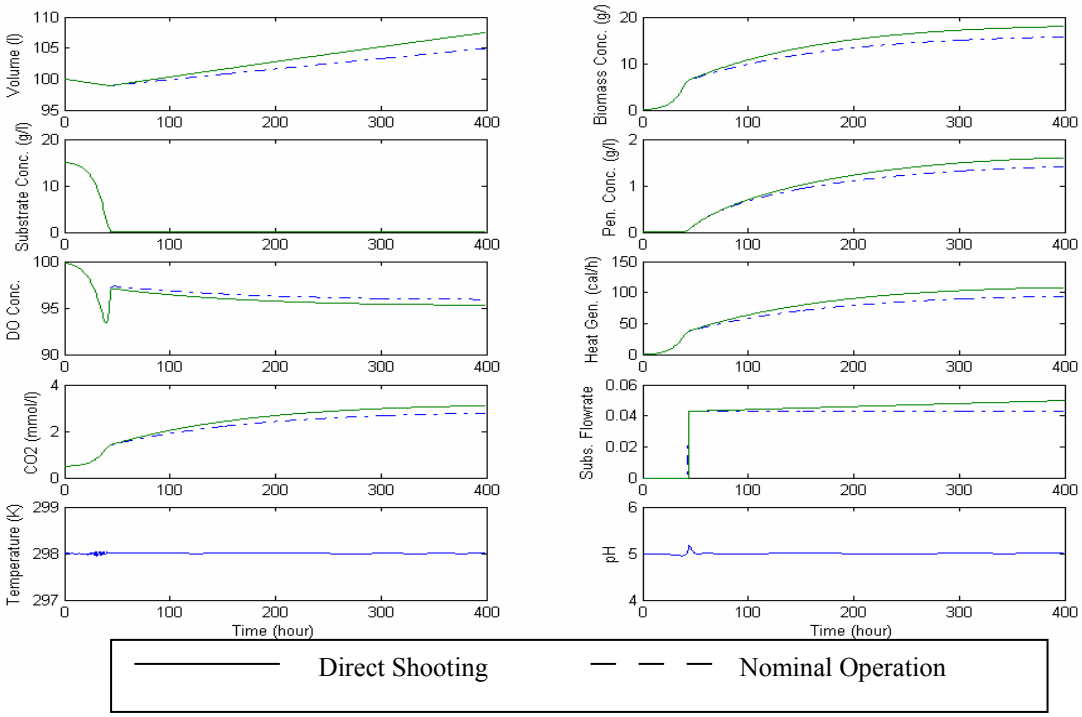

Figure 4 Result of direct shooting method optimization

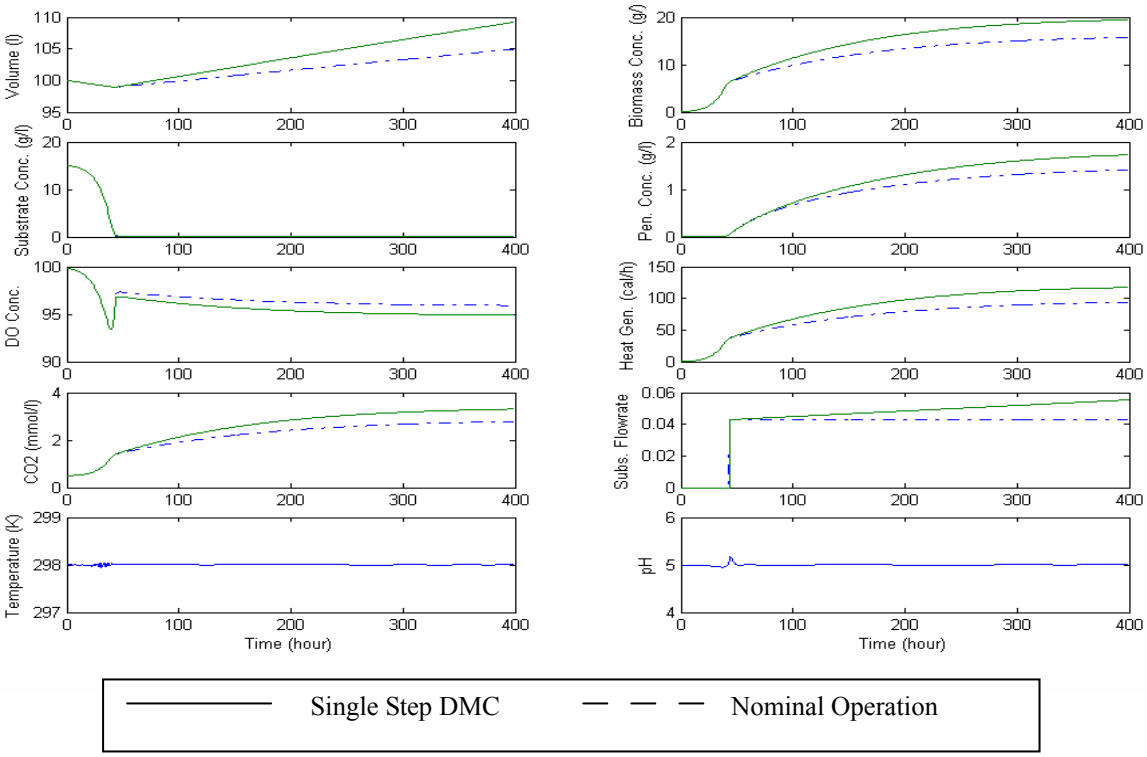

Figure 5 Result of single model DMC optimization

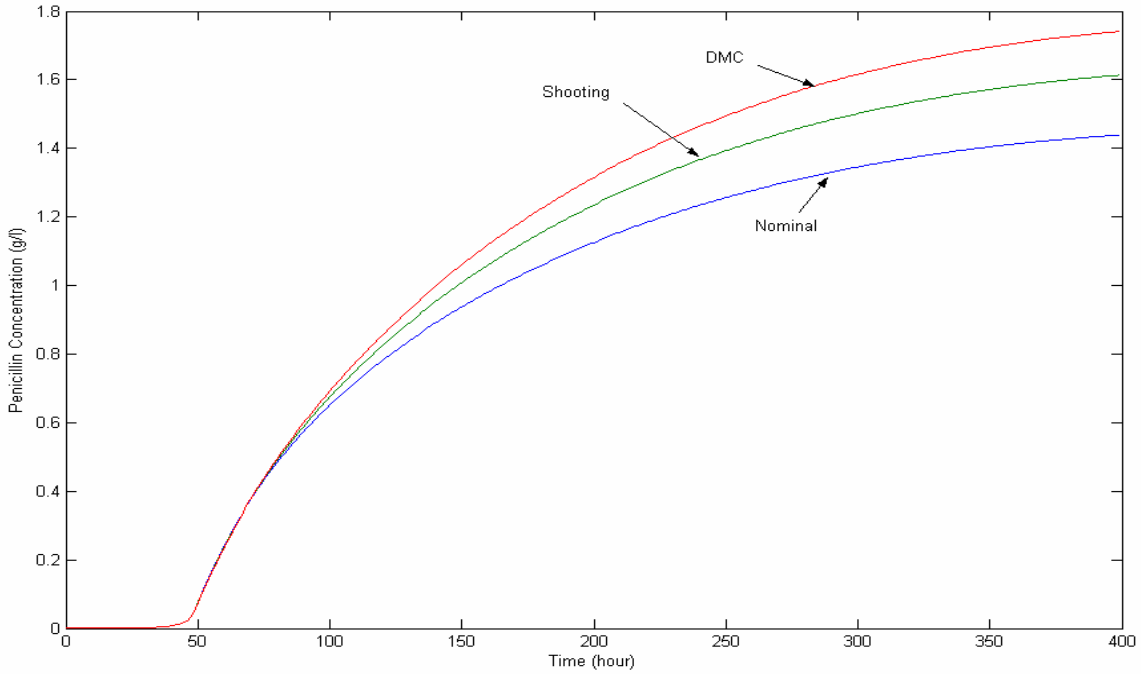

Figure 6 Penicillin concentration profile for direct shooting method, single step ahead DMC and nominal operation

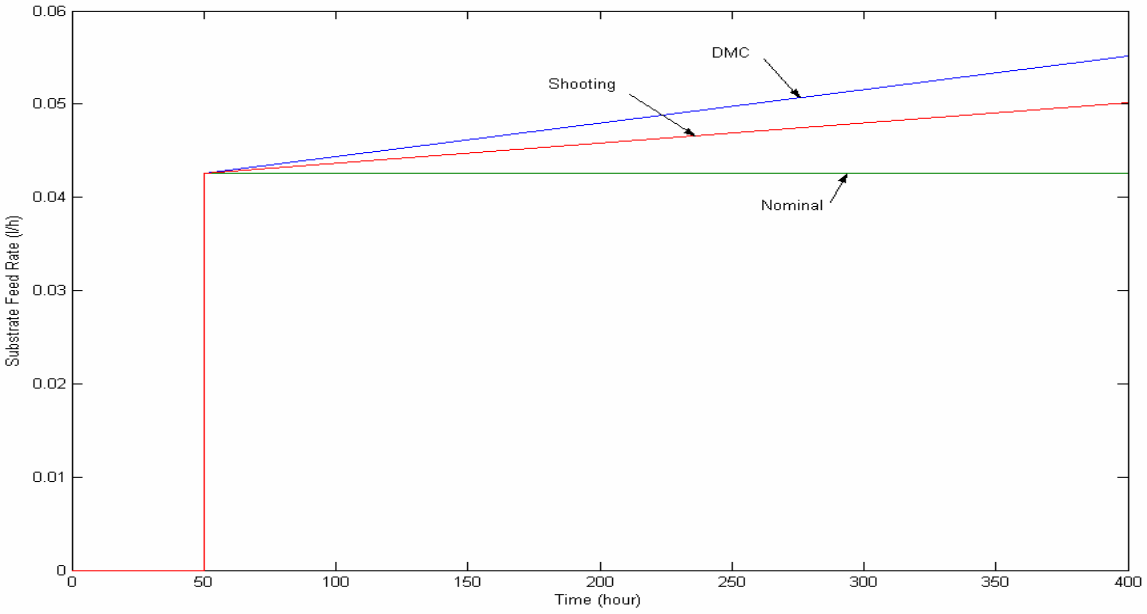

Figure 7 Substrate feed rate profile for direct shooting method, single step ahead DMC and nominal operation

Initial substrate feed rate for both the optimization approaches and nominal operation when the system switches to the fed batch operation is 0.0426 l/h. A constant substrate feed rate is used during the fed-batch operation under nominal operating condition. But substrate feed rate is increased when both optimization approaches is implemented in the system. The final substrate feed rate for direct shooting method is 0.050169 l/h and single step ahead DMC is 0.055213 l/h. In the end of the batch, the process obtains 1.7413 g/l penicillin concentration when single step

ahead DMC implemented compare to only 1.6136 g/l when the direct shooting method implemented. From the results, it shows single step ahead DMC approaches perform better than direct shooting method. The single step ahead DMC not only increase penicillin concentration but also improves the purity of penicillin up to 57.99 %.

Table 1 shows comparison performance fed-batch fermentation with different optimization algorithm and nominal condition. From Table 1, single step ahead DMC have an ability to optimize the objective function which is maximization of penicillin production. Although direct shooting method also maximize the objective function compare to nominal operation but TPBVP problem in the direct shooting method prevent this algorithm to perform better. This is the weakness of direct shooting method to achieve optimum solution. Meanwhile, single step ahead DMC shows some potential to overcome direct shooting problem.

Table 1 Comparison performance fed-batch fermentation with different optimization algorithm

Optimization Algorithm	Initial F (l/h)	Final F (l/h)	Pen. Conc. (g/l)	Pen. Purity (%)
Nominal Operation	0.0426	0.0426	1.4127	53.14
Direct Shooting Method	0.0426	0.050169	1.6136	56.03
DMC	0.0426	0.055213	1.7413	57.99

7. Conclusion

An unstructured model for a fed-batch Penicillin G fermentation process has been developed in this study. This mathematical model includes additional input variables like feed flow rate of substrate, pH, temperature, aeration rate, agitation power as well as output variables such as CO_2 evolution and heat generation terms. The model was simulated in MATLAB environment. Good result was obtained in this work. This has been elaborated in section 2. In section 3, the issue of on-line measurement of difficult to measure quality variables such as penicillin fermentation was addressed. In practice, it is the lack of robust on-line sensors for some of these key fermentation variables has been a significant obstacle for the implementation efficient process control. Since it is desirable to be able to optimize fermentation operation, this weakness must therefore be overcome. This can either be done through off-line analyses that are highly human dependent or making use of on-line inferential estimation strategy. The latter was adopted in this study and an inferential estimator based on Partial Least Squares (PLS) model has been developed to provide reliable prediction of the unmeasured quality variables. The results obtained proved the good capability of the estimator to perform in various operating conditions, thus enabling it to be implemented as part of product concentration control loop.

The success in providing reasonably accurate estimation of important fermentation variables opened the opportunity for process optimization. Dynamic optimization of process operation can be established using several approaches. The optimal control policy using direct shooting method and single step ahead DMC has been developed, aiming at optimizing the end of the batch penicillin concentration. From this two optimization approaches, it is possible to estimate the optimal operating conditions as substrate feed rate so that the systems presents high performance within threshold value limit. The result also showed that the single step ahead DMC approach is superior the direct shooting method in term of the penicillin

concentration as well as penicillin purity.

Acknowledgements

The authors wish to thank Prof. Ali Cinar and Dr Cenk Űndey for their technical support on this work.

References

Adebiyi, O.A. and Corripio, A.B. (2003). Dynamic Neural Networks Partial Least Squares (DNNPLS) Identification of Multivariable Processes. *Computers & Chemical Engineering.* 27(2): 143-155.

Ahmad, A., Samad, N.A.F.A. and Wei, C.A. (2003). Mathematical Modelling and Analysis of Dynamic Behaviour of a Fed-batch Penicillin G Fermentation Process. International Conference on Chemical and Bioprocess Engineering. 2:387-394

Aufderheide, B and Bequette, B.W. (2003). Extension of Dynamic Matrix Control to Multiple Models. *Computers & Chemical Engineering.* 27(15): 1079-1096.

Balsa-Canto, E., Banga, J.R., Alonso, A.A. and Vassiliadis, V.S. (2000). Efficient Optimal Control of Bioprocess Using Second Order Information. *Industrial Engineering Chemical Respiratory.* 39:4287-4295.

Bastin, G. and Dochain, D. (1986). On-line Estimation of Microbial Specific Growth Rates. *Automatica*. 22(8):705-709.

Garcia, C.E. and Morshedi, A.M. (1985). Quadratic Programming Solution of Dynamic Matrix Control (QDMC). *Chemical Engineering Communication.* 46:73-87.

Geladi, P. and Kowalski, B.R. (1986). Partial Least-Squares Regression: A Tutorial. *Analytica Chimica Acta.* 185: 1-17.

Impe, J.F.V. and Bastin, G. (1995). Optimal Adaptive Control of Fed-Batch Fermentation Process. *Control Engineering Practise*. 3(7):939-954.

Kapadi, M.D. and Gudi, R.D. (2004). Optimal Control of Fed-batch Fermentation Involving Multiple Feeds Using Differential Evolution. *Process Biochemistry*. 39:1709-1721.

Lundstrom, P., Lee, J.H., Morari, M. and Skogestad, S. (1994). Limitations of Dynamic Matrix Control. *Computers and Chemical Engineering*. 4:409-421

Modak, J.M. and Lim, H.C. (1987). Feedback Optimization of Fed-batch Fermentation. *Biotechnology and Bioengineering.* 30:528-540.

Ohno, H. and Nakanishi, E. (1976).Optimal Control of a Semi-batch Fermentation. . *Biotechnology and Bioengineering.* 18:847-864.

Qin, S.J. and Badgwell, T.A. (2002). A Survey of Industrial Model Predictive Control Technology. *Control Engineering Practice*, 11:733-764.

Rodrigues, J.A.D. and Filho, R.M. (1999). Production Optimization with Operating constraints for a Fed-Batch Reactor with DMC Predictive Control. *Chemical Engineering Science*. 54(14):2745-2751.

Srinivasan, B., Bonvin, D, Visser, E. and Palanki, S. (2002). Dynamic Optimization of Batch Processes: II. Role of Measurements in Handling Uncertainty. *Computers and Chemical Engineering*. 27:27-44.

Tatiraju, S., Soroush, M. and Mutharasan, R. (1999). Multi-rate Nonlinear State and Parameter Estimation in a Bioreactor. *Biotechnology and Bioengineering*. 63(1):22-32.

Wang, F.S. and Cheng, W.M. (1999). Simultaneous Optimization of Feeding Rate and Operation Parameters for Fed-batch Fermentation Processes. *Biotechnology and Bioengineering*. 15:939-952.

Wold, H. (1985). Partial Least Squares. In: Kotz, S. and Johnson, N.L. (ed). *Encyclopedia of Statistical Sciences.* Vol 6. New York: Wiley. 581-591.

Zhang, H. and Lennox, B. (2004). Integrated Condition Monitoring and Control of Fed-batch Fermentation Processes. *Journal of Process Control.* 14(1): 41-50.