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Optimization of Batch Fermentation Processes. II. Optimum Temperature Profiles for Batch Penicillin Fermentations

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Summary

Optimization methods based on the continuous maximum principle and the calculus of variations were used to calculate optimum temperature profiles for batch penicillin fermentations. These methods were first applied to several general models to develop effective techniques for the numerical solution of the equations. Subsequently, these methods were applied to two particular models, derived from experimental data, and the optimum temperature profiles were determined. The results indicated that an improvement in penicillin yield of about 15% was possible if the optimum temperature profiles were followed.

INTRODUCTION

Commercial batch fermentation processes are ordinarily operated under essentially constant temperature conditions, while pH and the concentrations of nutrients, cell mass, and products are allowed to change with little or no control as the fermentation progresses. Direct pH control by acid or base addition has been developed but is not widely practiced. Developmental studies carried out to determine optimum process conditions follow the same pattern; conditions are kept constant during each experiment and changed only from one experiment to the next.

Aside from tradition, there is no inherent reason why batch fermentations should be run at constant temperature or uncontrolled

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pH, when a controlled variation of these factors might, in fact, result in higher yields. Indeed, methods for establishing optimal operating procedures have been amply developed elsewhere and are now widely employed in the chemical process industries.

Little has been done, however, to determine continuous optimal profiles of temperature, pH, etc., for fermentations. This lack of progress is normally attributed to the complexity of the chemical reactions involved, which makes it difficult to evaluate quantitatively the effects of process conditions on the various kinetic parameters. Even so, optimization is still possible and it is important to make preliminary attempts, not only for their inherent value but because they will point out the areas in which further biochemical knowledge is most critically needed.

In the work reported here, optimum temperature profiles were determined for batch penicillin fermentations. Optimization methods based on the continuous maximum principle were found most suitable for this purpose and were applied to general and particular models for the penicillin process developed by the authors in an earlier paper.[1]

The general models were based on averaged, nondimensionalized data for cell mass and antibiotic titre taken from a group of commercial-scale penicillin fermentations carried out at constant temperature. Parameter-temperature functions for this class of model were assumed to have general forms applicable to many other fermentations as well as penicillin. The particular models, on the other hand, were developed entirely from experimental results, with parameter-temperature functions determined from experiments carried out at different temperatures.

These models, comprising the differential equations describing the dynamic behavior of the system and the relationships between the parameters of the model and the control variable, constitute the mathematical formulation of the system under study. In order to completely specify the system, two more terms, which become integral parts of the model, must be clearly defined. These are the objective function and the constraints on the state or control variables. The latter are self explanatory; the former may be looked upon as the profit or loss criterion of the process, depending on whether one is maximizing or minimizing. In the discussion that follows, the objective function will be maximized. Therefore, the

problem of optimization becomes that of establishing the values of the control variable at each point on the path of the process, subject to some constraints, in such a way that the objective function is maximized.

OPTIMIZATION METHODS

Optimization methods based on Pontryagin's continuous maximum principle[2,3] and on the calculus of variations[4,5] were applied to the models to determine the optimum temperature profiles. Since these two methods are fundamentally the same, the results obtained were essentially identical. The discussion which follows will therefore center on the application of the continuous maximum principle only.

Continuous Maximum Principle

Consider the continuous process shown in Figure 1, where $x(t)$ and $\theta(t)$ are vectors of the state and control variables respectively, and t is time.

The performance equations for this process are:

$$dx/dt = \dot{x} = f[x,b(\theta)] \quad \text{for} \quad t_o \leq t \leq T \tag{1}$$

where $b(\theta)$ terms are the parameter-control functions and the initial conditions are:

$$x(t_o) = \alpha \tag{2}$$

$x(t)$ is an s-dimensional vector function representing the state of the process at time t, and $\theta(t)$ is an r-dimensional vector function representing the value of the control variable at time t. Therefore there are s ordinary differential equations describing the evolution of the state of the system.

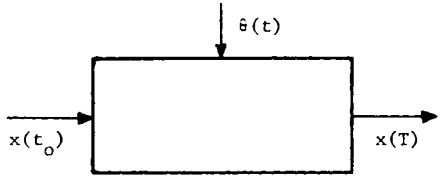

Fig. 1. Schematic process diagram.

In the continuous maximum principle, the objective function S is usually defined as a linear combination of the final values of the state variables:

$$S = c'x(T) , \tag{3}$$

where c' is the transpose of a vector, c, of constants. In order to optimize the system it is necessary to find a piecewise continuous control vector function, $\theta(t)$, possibly subject to some constraints, such that S is maximized. The control vector function so chosen is called optimal and is denoted by $\bar{\theta}(t)$.

When the time interval is fixed and the initial conditions of the state variables are given, there are two types of problems; those with free and those with fixed terminal values for some components of x. The first type is characteristic of batch fermentation processes and will therefore be considered here.

The continuous maximum principle uses an s-dimensional adjoint vector, $z(t)$, and the Hamiltonian function, H, which satisfy the following equations:

$$H = z'f \tag{4}$$

$$\dot{z} = -(\partial H/\partial x) = -z'(\partial f/\partial x) , \tag{5}$$

$$z(T) = c , \tag{6}$$

where z' denotes the transpose of the vector z, and $\partial f/\partial x$ is the matrix whose elements are the partial derivatives of the rates of change of the state variables with respect to the state variables. The jth adjoint variable, $z_j(t)$, corresponds to the increase in the objective function caused by an increase in the jth state variable at time t.

The necessary condition for the objective function to be maximum, when the control variable is unconstrained, is the following:

$$\partial H/\partial \theta = 0 \tag{7}$$

In the case where the control variable is on the constraint the following must be met:

$$H = \max \tag{8}$$

In summary, this algorithm involves the following: the state equations, (1), the initial conditions of the state variables, (2), the objective function, (3), the Hamiltonian function, (4), the adjoint

equations, (5), the final conditions of the adjoint variables, (6), and the necessary condition, (7) or (8).

The state equations contain the state and control variables and the initial conditions for these equations are usually known. The adjoint equations are functions of the state, adjoint and control variables and the final conditions for the adjoint equations are known if all the state variables are free at $t = T$. Obviously, the adjoint equations are coupled to the state equations and cannot be solved independently. The necessary condition for a maximum also involves all three kinds of variables. Therefore, the use of the continuous maximum principle produces a two-point boundary value problem, with the added difficulty that the Hamiltonian may be a very complex function of the control variable. Finally, the solution of the necessary condition will often require the use of a search technique.

Methods of Solution

Two methods for the solution of the two-point boundary value problems which arose in this study were examined:

(a) The bidirectional integration method, in which the state equations are integrated forward and the adjoint equations are integrated backwards, with the control variable not changed during the integration, but corrected between integrations.

(b) The unidirectional integration method in which the state and adjoint equations are integrated in the same direction.

The second method has two basic disadvantages: (1) the adjoint equations are often unstable when integrated forward, and the state equations are often unstable when integrated backwards, and (2) The method requires an explicit solution in the control variable of the necessary condition for optimality. In order to eliminate these difficulties a double iterative procedure was developed.[6] With this procedure both the control variable and the unknown boundary conditions are adjusted iteratively.

The bidirectional integration method was found to be simpler to use and slightly faster than the unidirectional method. However, the latter would be very useful in the case where some of the state variables are fixed at $t = 0$ and others at $t = T$. Under such conditions, the state and adjoint equations must all be integrated in the same direction.

Three different techniques for finding the values of the control variable which satisfy the necessary condition were used in conjunction with the above integration methods. These techniques were:

(a) Using the explicit solution, in the control variable, of the necessary condition for optimality. This is often the best technique because it is exact and rapid. However, the necessary condition for optimality is usually a transcendental equation in the control variable and cannot be solved explicitly.

(b) Applying the Fibonacci search technique to the maximization of the Hamiltonian. This technique was quite effective, but more time consuming than the other two. This method is applicable in the case where only one control variable is being optimized. The Fibonacci search must be used with caution because, if the Hamiltonian is not unimodal in the interval of search, the method may converge on a local maximum instead of the global maximum.

(c) Using the method of steepest ascent of the Hamiltonian, *i.e.*, correcting the control variable in the direction in which the Hamiltonian rises most rapidly. This technique is as effective as the explicit solution, but it takes longer to converge when there are discontinuities in the optimal control profile. This method can be used when several control variables are being optimized.

These methods were applied to the general and particular models developed earlier.[1] Although model 1F fitted the data better than the other general models, model 1A was a simpler one to optimize because it allowed an explicit solution of the necessary condition for optimality. The optimization methods were therefore first applied to model 1A in order to find the most effective computational techniques for the solution of these methods. Figure 2 summarizes the overall approach which was followed.

The optimal profile for model 1A was obtained using the bidirectional integration method with the correction of θ performed by the explicit solution first, the Fibonacci search second, and the steepest ascent third. In addition, the equations for this model were solved using unidirectional integration combined with the analytical solution. All four methods of solution gave identical results for model 1A.

The bidirectional integration method using the analytically obtained value of θ was found to be the most effective and most rapid. However the necessary condition for optimality of model 1F could not be solved explicitly for the control variable, so that this method

could not be applied to model 1F. The three methods of solution used for model 1F gave identical optimal temperature profiles.

The particular models which were subjected to the optimization techniques were models C2 and S2.[1] The bidirectional integration method was used in the solution of the state and adjoint equations of these models. Both models C2 and S2 have necessary conditions which cannot be solved explicitly for the control variable. Therefore the Fibonacci search technique was used in determining the control profile which maximizes the Hamiltonian.

Models C2 and S2 included a twenty-hour lag in penicillin synthesis, *i.e.*, the cell concentration at time $(t - 20)$ hours was used in the evaluation of the penicillin synthesis rate at time t. For $t \leq 20$ hours the rate of penicillin production was set equal to zero. Physically, this is equivalent to saying that the cell does not produce any

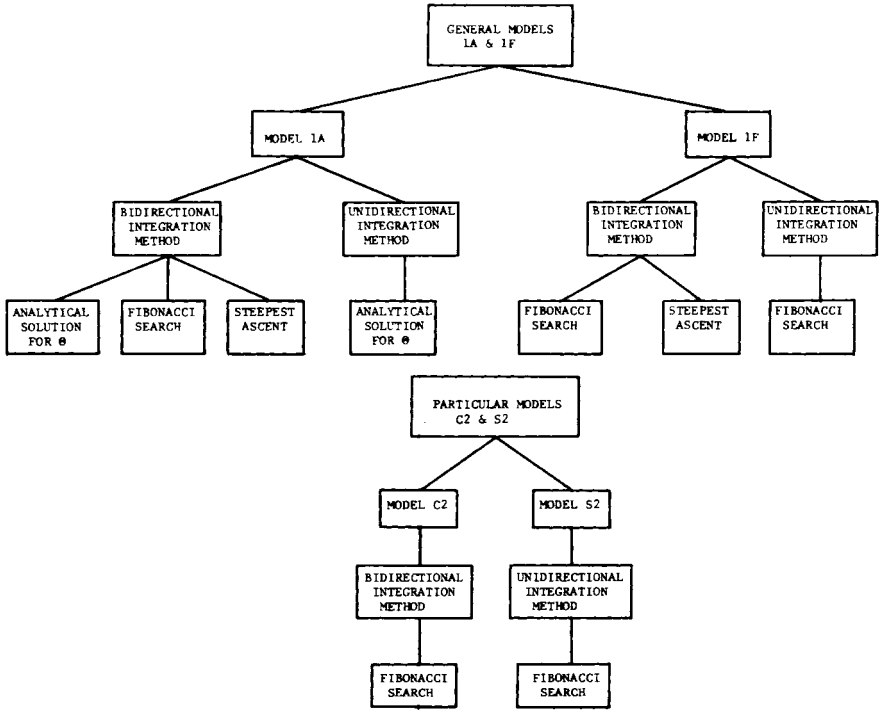

Fig. 2. Summary of the application of optimization methods to general and particular models.

penicillin until it is at least twenty hours old. This time lag was incorporated into the optimization method using the technique developed by Pontryagin *et al.*[2]

RESULTS

The optimal temperature profiles determined for models 1F, C2, and S2 are presented in the next three sections; the solution of model 1A has been omitted. The interested reader is referred to Constantinides[6] where the optimal profiles for all the models appear.

Optimum Temperature Profile for General Model 1F

The equations which constitute the algorithm of the maximum principle for model 1F are the following:
State equations:

$$\dot{x}_1 = b_1 x_1[1.0 - (x_1/b_2)] , \quad x_1(0) = 0.0294 \tag{9}$$

$$\dot{x}_2 = b_3 x_1 - b_4 x_2 , \quad x_2(0) = 0.0 \tag{10}$$

Parameter-temperature functions:

$$b_1 = 13.10 \left[\frac{1.0 - 0.005(\theta-30)^2}{1.0 - 0.005(25-30)^2}\right] \tag{11}$$

$$b_2 = .943 \left[\frac{1.0 - 0.005(\theta-30)^2}{1.0 - 0.005(25-30)^2}\right] \tag{12}$$

$$b_3 = 4.66 \left[\frac{1.0 - 0.005(\theta-20)^2}{1.0 - 0.005(25-20)^2}\right] \tag{13}$$

$$b_4 = 4.4555 \exp\left\{\frac{-12210}{R}\left[\frac{1}{\theta + 273.1} - \frac{1}{298.1}\right]\right\} \tag{14}$$

(Note that for this set of parameters the ratio b_1/b_2 is not a function of temperature.)

Objective function:

$$S = x_2(T) \tag{15}$$

Adjoint equations:

$$\dot{z}_1 = -z_1 b_1 + 2z_1(b_1/b_2)x_1 - z_2 b_3 , \quad z(T) = 0.0 \tag{16}$$

$$\dot{z}_2 = z_2 b_4 , \quad z(T) = 1.0 \tag{17}$$

Hamiltonian:

$$H = z_1 b_1 x_1 - z_1 (b_1/b_2) x_1^2 + z_2 b_3 x_1 - z_2 b_4 x_2 \tag{18}$$

Necessary condition for maximum:

$$\partial H/\partial\theta = z_1 x_1 (\partial b_1/\partial\theta) - z_1 x_1^2 (\partial/\partial\theta)(b_1/b_2) + z_2 x_1 (\partial b_3/\partial\theta) - z_2 x_2 (\partial b_4/\partial\theta) = 0 \tag{19}$$

If equations (11) through (14) are differentiated with respect to θ and substituted into equation (19), the resulting equation is transcendental in θ and cannot be solved explicitly. This complication necessitates the use of the Fibonacci search or the steep ascent method.

An interesting phenomenon was observed in the integration of the logistic law (equation 9) under a decreasing temperature profile. The value of b_2 is equivalent to the cell concentration at infinite time, *i.e.*, maximum growth, for constant temperature (θ). When one controls the value of b_2 with equation (12) under a condition of decreasing temperature, there exists a point where the value of b_2—the maximum cell concentration—becomes smaller than the value of the instantaneous cell concentration, x_1, at that point in time. The bracketed term of equation (9) therefore becomes negative and cell concentration subsequently decreases. This mathematical behavior is not inconsistent with physical realities. For example, when autolysis occurs, the cell concentration can fall.

If, however, a decreasing cell concentration is not allowed, then an inequality constraint is required so that

$$x_1 - b_2 \leq 0 \tag{20}$$

The constraint has the physical meaning that the net rate of cell formation becomes zero when the temperature drops below a certain level. This level is determined by the point at which the constraint is reached. The presence of a constraint makes the model mathematically more interesting and introduces into it the difficulties of handling such constraints in optimization. When the constraint is reached, the state equation for cell growth, equation (9), becomes

$$\dot{x}_1 = 0.0 \text{ when } x_1 \geq b_2 \tag{9a}$$

The Hamiltonian of the system changes to

$$H = z_2 b_3 x_1 - z_2 b_4 x_2 , \tag{18a}$$

and the adjoint equation for z, is modified according to the method developed by Bryson *et al.*[7]:

$$\dot{z}_1 = -z_2 b_3 .$$

The numerical computational methods switch from the unconstrained part of the model to the constrained part when the constraint is reached.

The results obtained for model 1F, using the methods of solution shown on Figure 2, are presented in Figures 3 and 4. The optimum temperature profile (Fig. 3) began at 30°C because this temperature favors growth and at this point z_1 (Fig. 4) is much larger than z_2, *i.e.*, an increase in cell mass has a much higher value than an increase in penicillin titre. The optimal temperature then remained above 28.6°C throughout the first part of the process, resulting in a high cell concentration. When the constraint was reached, *i.e.*, when the rate of cell formation was equal to zero, the optimal temperature shifted to a lower level, maximizing the rate of penicillin formation and minimizing the rate of penicillin destruction.

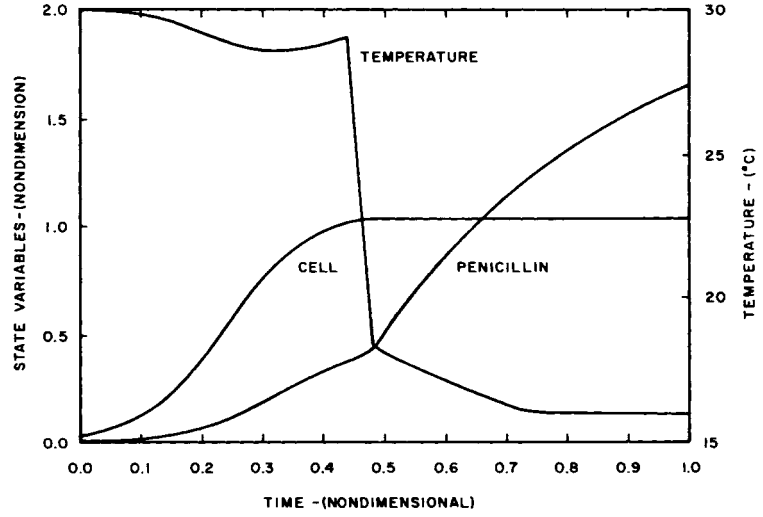

Fig. 3. Optimal profiles for temperature, cell mass, and penicillin titre—Model 1F.

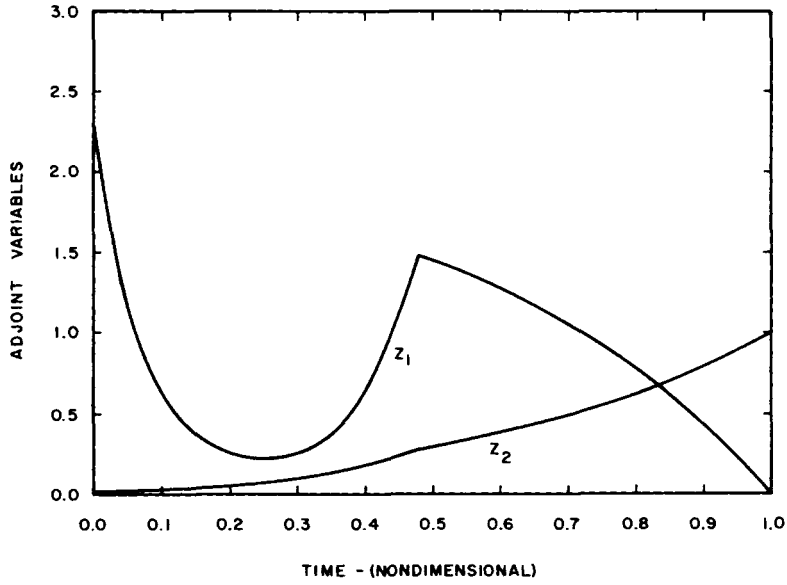

Fig. 4. Optimal profiles of adjoint variables—Model 1F.

The final penicillin potency indicated (Fig. 3) is 1.663 (on a nondimensionalized basis) or 76.6% higher than that obtained when a constant temperature of 25°C—the best constant temperature—was used.

Optimum Temperature Profile for Model C2

Model C2 was derived from experimental data for penicillin fermentations carried out at various constant temperatures in 30-liter fermentors.[1] The equations which made up the algorithm of the continuous maximum principle for model C2 were identical to the equations for model 1F, with the exception that the initial condition for the cell concentration in this model was

$$x_1(0) = 5.0$$

The parameter-temperature functions used in the optimization of model C2 were those derived from the same experimental data.[1] The inequality constraint for cell concentration (equation 20) was also applied to model C2 and this model also incorporated a twenty-hour lag in penicillin synthesis.

Bidirectional integration with the Fibonacci search technique was used in determining the optimum solution for model C2. The iterative procedure used for this model did not converge to a single profile, but eventually alternated between two almost identical profiles which corresponded to objective function values differing by less than 0.05%. This behavior was probably due to the fact that the system and adjoint equations were integrated numerically.

The temperature profile which maximized the objective function is shown in Figure 5 and the corresponding optimal cell and penicillin profiles in Figure 6. The adjoint variable profiles are given in Figure 7. In this case the optimum temperature remains at 27.2°C for the first 56 hours of the fermentation, then drops linearly to 18.7°C and remains at this temperature from 84 to 184 hours. For the last 24 hours it returns to 27.2°C.

The behavior of the profile can be explained as follows. The ratio of the adjoint variables, z_1/z_2, starts ($t = 0$) at a very high value and drops to zero at the end ($t = T$). This means that at the beginning of the process the rate of cell formation is weighted more

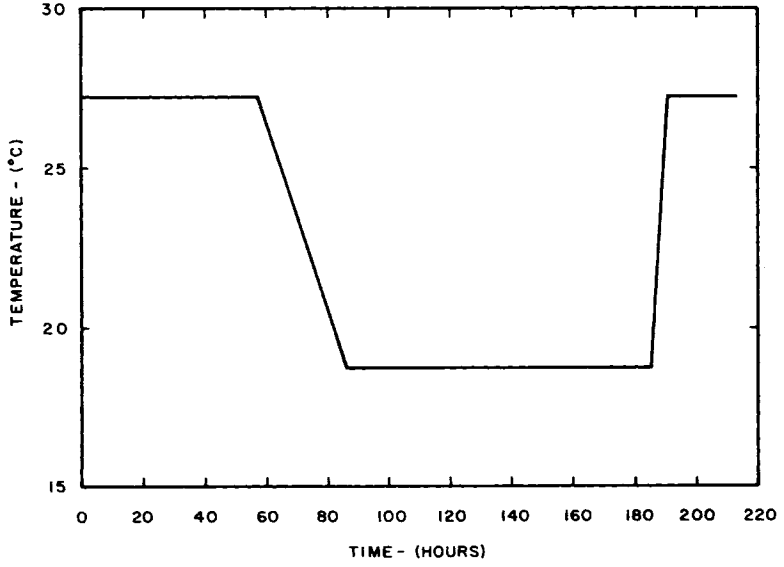

Fig. 5. Optimal temperature profile—Model C2.

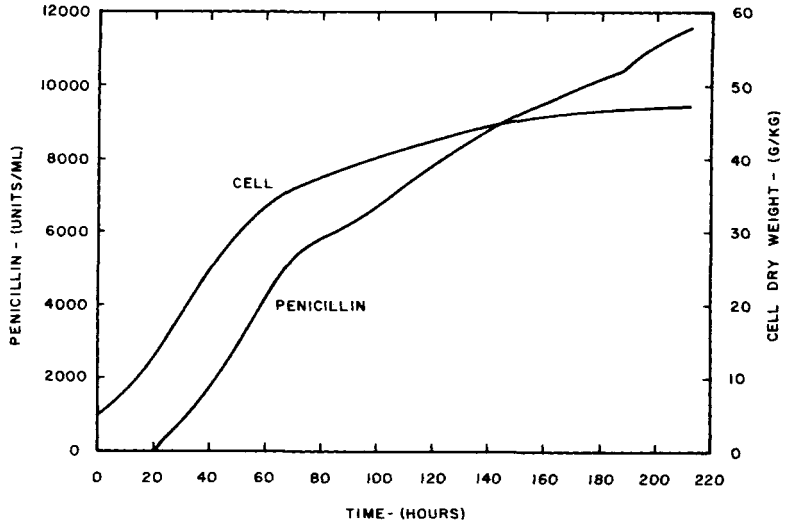

Fig. 6. Optimal profiles of cell mass and penicillin titre—Model C2.

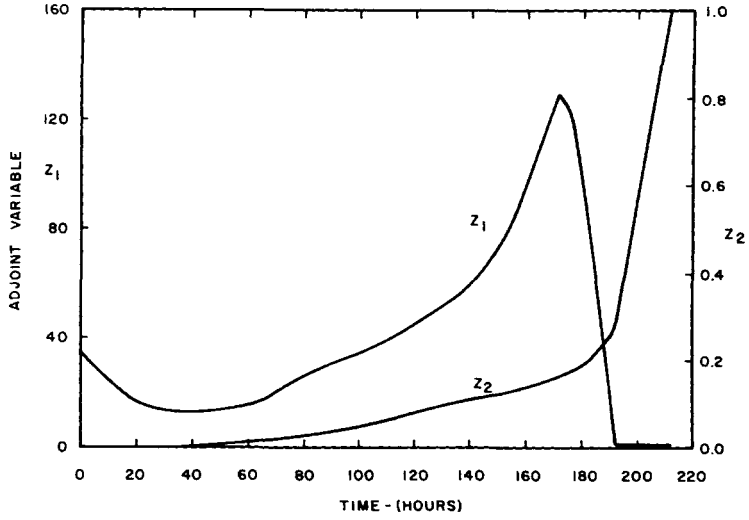

Fig. 7. Optimal profiles of adjoint variables—Model C2.

heavily in the total value of the Hamiltonian. The parameters b_1 and b_2, which control cell formation, have maximum values at 27.2° and 18.7°C, respectively, but at the beginning the value of x_1 (cell concentration) is more sensitive to b_1 than to b_2. Therefore, the optimum temperature in this early period is at 27.2°C.

As time passes, x_1 becomes more sensitive to b_2 and the ratio z_1/z_2 also drops, *i.e.*, the rate of penicillin formation has a higher weight in the Hamiltonian. The parameter b_3, which controls the rate of penicillin formation, has its maximum value at 27.2°C, but parameter b_4, which controls the rate of penicillin destruction has its lowest value at 18.7°C. Hence, for $t \geq 84$ hours, the penicillin titre (x_2) is more sensitive to b_4. Since the sensitivity of the system shifts toward b_2 and b_4, and since both of these parameters have values which favor penicillin formation at 18.7°C, the optimum temperature would be expected to shift to 18.7°C, which it does.

While the temperature remains at 18.7°C cell and penicillin concentrations continue to increase. At $t = 188$, the cell concentration reaches the constraint (equation 20) and the rate of cell formation becomes zero. From that time on, only the penicillin synthesis rate has weight in the Hamiltonian and x_2 is more sensitive to b_3. The optimum temperature therefore shifts back to 27.2°C for the last few hours of the fermentation.

The final value of penicillin indicated in this case was 11,616 units/ml. This is 16.0% higher than the highest yield (10,010 units/ml) obtained with a constant (25°C) temperature.

Optimum Temperature Profile for Model S2

The methods used to calculate the optimum temperature profile for model S2 were similar to those used for model C2. The bidirectional integration method was combined with the Fibonacci search technique to form the iterative procedure for the solution of the continuous maximum principle. The equations comprising the maximum principle algorithm for model S2 were identical to those of model 1F, except that the initial vaue of the cell concentration in this model was:

$$x_1(0) = 0.33$$

Model S2 also included a twenty-hour lag in the penicillin synthesis and the state variable inequality constraint, (equation 20), was also

applied. The parameter-temperature functions for this model were also derived from experimental data.[1] These functions cover the temperature range 20° to 30°C. Since no data were available outside this range, the Fibonacci search occurred only within these limits.

The optimal temperature profile for model S2 is shown on Figure 8 along with the optimal cell and penicillin profiles. In this case the optimum temperature remained at 30.0°C for the first 5 hours of the fermentation and then dropped to 25.0°C where it remained for 35 hours, before shifting to 20.0°C. After 85 hours at 20.0°C, it rose again to 25°C where it remained for the last 40 hours of the process.

The explanation for this optimum temperature profile is similar to that given for model C2. At the beginning of the process, the rate of cell formation has a high weight in the total value of the Hamiltonian because the ratio of the weighting factors, z_1/z_2, is very high (Fig. 9). The rate of cell formation is again more sensitive to the value of the parameter b_1 during the first part of the process. Since b_1 has its maximum value at 30.0°C, this is the optimum temperature at the start of the fermentation.

As the fermentation proceeds, the rate of cell formation becomes more sensitive to the value of b_2 which has its maximum at 20.0°C.

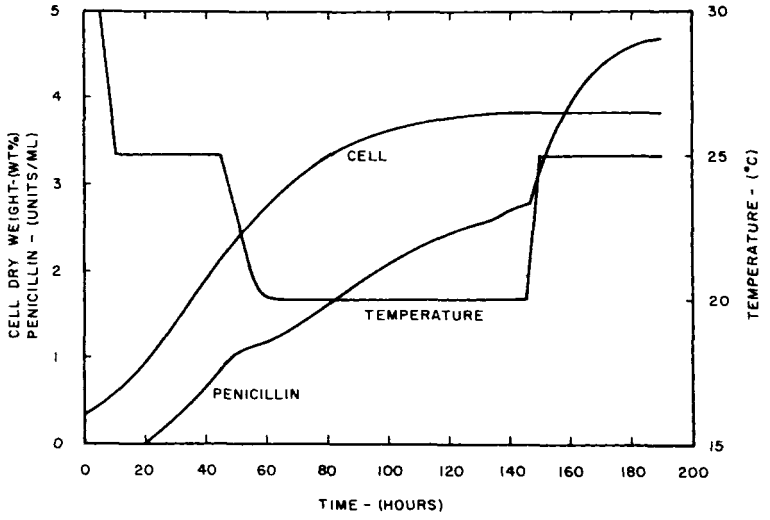

Fig. 8. Optimal profiles of temperature, cell mass, and penicillin titre—Model S2.

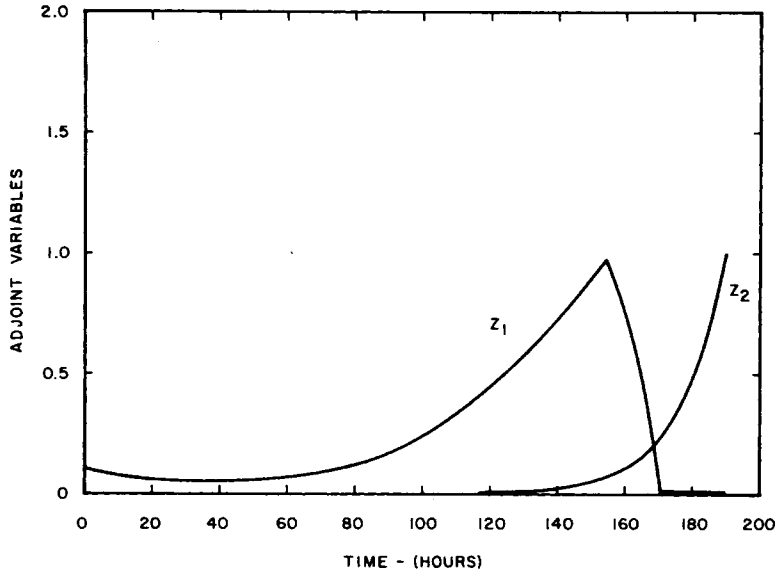

Fig. 9. Optimal profiles of adjoint variables—Model S2.

At the same time, the ratio z_1/z_2 decreases, which means that the rate of penicillin formation is more heavily weighted in the Hamiltonian. Parameter b_3 has its highest value at 25.0°C and parameter b_4 has its lowest value at 20.0°C. This explains the temperature shifts to 25.0° and then to 20.0°C, to maximize penicillin formation and minimize penicillin destruction. At $t = 140$ hours, the cell concentration profile has reached the constraint (equation 20), and the influence that b_2 had in "pulling" the temperature profile towards 20.0°C is lost. At this time penicillin formation becomes more sensitive to b_3, so the temperature shifts to 25.0°C which maximizes the value of b_3.

It should be emphasized that the parameter-temperature functions for model S2 were constructed from data taken at only three different temperatures, and the values of the parameters at intermediate temperatures were calculated by linear interpolations. If data were available at other temperatures, the parameter functions could be constructed more accurately and the optimal temperature profile would be smoother than the step-like profile obtained here.

The indicated value of the penicillin potency in this case was 4.701, or 14.7% higher than the value obtained experimentally when the temperature was kept constant at 25°C.

CONCLUSIONS

The purpose of this work was to apply modern optimization methods to the calculation of optimal temperature profiles for batch fermentation processes. Temperature was chosen as the control variable to be optimized because small changes in temperature markedly affect the rates of formation of cell and products, and, furthermore, affect them to different degrees. In addition, temperature is a convenient control variable because it is relatively easy to manipulate.

In this paper we have shown how optimization methods can be used to determine the optimal temperature profiles for batch fermentation processes. The methods developed for the solution of the general models were kept as general as possible so that their use might easily be extended to other models of fermentation processes. Several degrees of complexity were examined and efficient methods of solution were found. It should be possible for others to apply the methods of solution developed in this study to their own models, models which might include other control variables, pH or nutrient concentrations, as well. The optimization methods discussed here are, of course, not limited to controlling only the temperature, but can be used for multiple control variable cases as well.

The optimal temperature profiles for models C2 and S2 are very similar to each other. Both of these profiles start at a high temperature, favoring faster growth, and then drop to a lower temperature, which favors a high level of cell concentration and a low rate of penicillin destruction. At the end of the batch cycle, when the cells have reached their maximum concentration, the optimum temperature shifts to a level which favors penicillin formation only.

Our results indicate that substantial improvements in penicillin yield, 16.0 and 14.7% for models C2 and S2 respectively, should be obtainable if the optimum temperature profiles were followed.

We realize, of course, that complete optimization of a fermentation process will require that all control variables follow optimal profiles. This will in turn require the development of models which include all

the variables which affect the process and, possibly, the age distribution of the cells. The development of such detailed models is a long and arduous task and can only be accomplished when much more complete knowledge of the kinetics and transport mechanisms involved in fermentation processes in available. Until then optimization will have to be executed by means of semiempirical procedure like those used here.

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NOMENCLATURE

- b Vector of parameter-temperature functions
- c Vector of constants
- H Hamiltonian function
- S Objective function
- T Final time
- t Time
- x Vector of state variables
- x_1 Cell mass or concentration
- x_2 Penicillin titre
- z Vector of adjoint variables
- θ Control variable or temperature

Subscripts

- 0 Denotes initial condition

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