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MULTIRESPONSE MODEL BUILDING IN SYSTEMS DESCRIBED BY ORDINARY DIFFERENTIAL EQUATIONS WITH APPLICATION TO BATCH CULTURE KINETICS

By

Martin Guay

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ABSTRACT

In this thesis, multiresponse parameter estimation in systems described by ordinary differential equations was investigated. Contributions were made in three areas: development of optimization algorithms for the Box-Draper criterion, solution of first and second-order parametric sensitivity coefficients in ordinary differential equations and development of model building strategies.

In this study, it was shown how optimization algorithms, developed for nonlinear least squares estimation, can be extended to the optimization of the Box-Draper multiresponse criterion. A hybrid general-purpose algorithm, based on the algorithm of Dennis, Gay and Welsch (DGW) for nonlinear least squares, was developed for the optimization of the Box-Draper criterion. The hybrid algorithm combines the Gauss-Newton method and the DGW update formula. A new switching procedure was developed to allow application of the hybrid algorithm with a line search algorithm. The general purpose hybrid algorithm was found to be more robust then the Gauss-Newton algorithm and was shown to perform as well as other published hybrid algorithms.

The calculation of first-order sensitivity coefficients of the model responses with respect to the model parameters is essential to the evaluation of the gradient and approximate Hessian of the parameter estimation criterion with respect to the parameters. In this thesis, an efficient algorithm for the calculation of first-order sensitivity coefficients in ordinary differential equations, the decoupled direct method (DDM), was implemented in a multiresponse parameter estimation routine. The performance of the DDM was improved by using a pseudo error per unit step error control strategy. DDM was also extended to the simultaneous calculation of both first and second-order sensitivity coefficients. This extension of DDM was used in the development of a full Newton method for the optimization of the Box-Draper

criterion. A new hybrid Gauss-Newton/Newton method was also developed. These two methods were shown to be very robust and performed better than multiresponse parameter estimation algorithms based on first-order approximations.

An effective model building strategy for batch culture kinetics was developed. The strategy combines rigorous multiresponse statistical techniques and process knowledge to build "pseudo-mechanistic" descriptions for batch culture kinetics. The strategy is based on an iterative approach which sequentially applies conjecture, design of experiments and analysis steps to enrich knowledge of the process. A simple model building exercise for the batch culture of *E. coli* was employed to demonstrate the strategy. This example also demonstrated a number of helpful techniques for using multiresponse parameter estimation procedures. The use of empirical parameter transformations was found to improve the convergence properties of multiresponse estimation procedures. A sequential approach to fitting complex multiresponse ODE models using a limited amount of data was also demonstrated. The approach consisted of fitting simple model forms initially and adding responses in a sequential manner in order to arrive at more complex model forms.

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NOMENCLATURE

Constants

N	number of observations
M	number of response variables
K	number of state variables
t	independent variable (usually time) d(θ) Box-Draper estimation criterion
ρ	term used to correct the length of the step taken during optimization
α	term used to make the Hessian matrix sufficiently positive definite
$\bar{\alpha}_i,\alpha_i,\beta_i$	constants resulting from the fit of k-degree polynomial to the solution of system of ODEs
k	integration order
h	integration step-size
q	predicted value of the objective function based on the quadratic approximation for the optimization scheme used
ε	critical value of the Bates-Watts convergence criterion

Arrays

Y	= $\{y_{nm}\}$ = NxM matrix of observed responses
F	= $\{f_{nm}\}$ = NxM matrix of predicted responses
θ	P-dimensional vector of parameters
ξ_n	n^{th} vector of experimental conditions
Z	= $\{\varepsilon_{nm}\}$ = NxM matrix of residuals
Σ	= $\{\sigma_{rs}\}$ = MxM covariance matrix for the responses
H	= $\{h_{nm}\}$ = NxM matrix of predicted responses
$\mathbf{x}_n$	n^{th} vector of experimental conditions excluding time
s	K-dimensional vector of state variables
u	K-dimensional vector of time derivatives of the state variables
s_0	K-dimensional vector of initial conditions for the state variables
I	identity matrix
Γ	Hessian of the parameter estimation criterion with respect to the parameters

γ	gradient vector of the parameter estimation criterion with respect to the parameters
$\mathbf{C}$	upper triangular Cholesky factor of Γ
δ	P-dimensional parameter increment vector
$\mathbf{A}$	PxP matrix containing second order derivatives in the evaluation of the Hessian
$\nu,\tilde{\nu}$	P-dimensional perturbation vectors used in the update of $\mathbf{A}$
$\mathbf{b}$	K-dimensional accumulated error vector used to advance the solution of a system of ODEs
$\mathbf{U}$	Kx(k+1)x(k+1) Nordsieck array
$\mathbf{w}$	K-dimensional vector of error weights
$\mathbf{e}$	K-dimensional vector of local error estimates
$\mathbf{J}$	Jacobian of a K-dimensional system of ODEs
μ	K-dimensional vector of internal error weights
$\mathbf{E}$	KxPxP array of local error estimates

Superscripts

$^{(i)}$	i^{th} iteration of an iterative scheme
^{T}	transpose of a matrix or vector

Subscripts

$_{n,r}$	denotes the n^{th} integration step and the r^{th} state variable of a vector
$_{nm}$	denotes the element in the n^{th} row and m^{th} column of a matrix

Abbreviations

NI	number of iterations
NOF	number of objective function evaluations
NSER	number sensitivity error test failures
NST	number of integration steps
RTOL	relative tolerance
ATOL	absolute tolerance

CHAPTER 1

Introduction

Good knowledge of a chemical process is very important for the design, control and optimization of its operation. For these purposes, a reliable mathematical description of the process is required. Of particular interest in this work are processes described by systems of linear or nonlinear differential equations. Batch culture kinetics of microorganisms and the dynamic behaviour of chemical reactors and various separation operations are major examples of processes described by systems of nonlinear ordinary differential equations. Techniques which facilitate the development of such mathematical models are therefore highly valued in many areas of chemical engineering.

A mathematical model must adequately describe the observed behaviour of the process it represents. To verify the adequacy of the model, the model must first be fitted to the experimental data by adjusting the constants, or parameters, of the model to obtain an optimal representation. The parameters are generally chosen to minimize a suitable measure of dispersion which reflects basic assumptions made about the behaviour of the disturbances in the experimental measurements. For single response cases where only one measured response is considered, an appropriate measure is the sum of squared residuals. For multiresponse cases where more than one response is used, appropriate measures must be applied. Once such measure, derived by Box and Draper (1965), is of particular interest in this study. The Box-Draper parameter estimation criterion is remarkably general and has many practical advantages. However, application of this criterion still faces a number of computational problems, especially for models in the form of systems of ODEs.

One of the main problems is the lack of special purpose algorithms for

multiresponse parameter estimation using the Box-Draper criterion. Although there exist many good parameter estimation algorithms for nonlinear least-squares problems, it remains unclear what approaches are most suitable for multiresponse parameter estimation. One objective of this study was to develop efficient approaches for performing multiresponse parameter estimation. This involved the development of general purpose procedures for the efficient optimization of the Box-Draper criterion. To improve the robustness of current methods, successful optimization algorithms for nonlinear least-squares were modified to generate powerful multiresponse parameter estimation algorithms. These algorithms were applied to the problem of parameter estimation in systems described by ordinary differential equations (ODEs).

For systems represented by ODEs, development of a multiresponse parameter estimation routine is considerably more complex. Since the numerical solution must be repeated many times during the optimization, an efficient ODE solving routine must be used. Furthermore, first-order (and possibly second-order) derivatives of the model with respect to the parameters, called parametric sensitivity coefficients, must also be calculated. This constitutes the greater part of the computing requirements of the overall procedure. Since solution of the combined system of ODEs for both the model and the sensitivities is required at each step, alternative methods of solution must be selected in order to increase the efficiency of the parameter estimation procedure. Many methods have been proposed for the solution of first-order sensitivity coefficients in systems of ODEs. One of these methods, the decoupled direct method (DDM), has proven to be considerably more advantageous. One objective of this study was to apply DDM to the problem of multiresponse estimation. Extension of DDM to the efficient calculation of second-order sensitivity coefficients was also considered in order to improve the robustness of multiresponse parameter procedures.

Although efficient methods for multiresponse parameter estimation are helpful, they do not remedy many of the problems encountered in practice. From experience, it has been frequently observed that the optimization of the Box-Draper criterion is sensitive to the choice of initial parameter estimates. For many optimization methods, the Gauss-Newton method in particular, initial estimates have to be close to the actual parameter estimates for the routine to converge successfully. In cases where there is no prior information on the values of the parameters, this constitutes a considerable, even insurmountable, problem. Furthermore, numerous problems are typically encountered when the model is exceedingly complex. One sensible way to tackle a complex parameter estimation problem would be to start from simple model forms and sequentially construct a more complex model form by adding the required terms. One objective of this study was to apply this philosophy to develop a strategy which could circumvent problems commonly encountered in multiresponse parameter estimation in systems described by ODEs.

The prime objective of most model building studies is generally to acquire knowledge about the process by developing a mechanistic description of the observed behaviour. In order to minimize the experimental expenditure of such an undertaking, a model-building strategy, such as the one of Box and Hunter (1962), is generally recommended. Such a strategy consists of applying, in an iterative fashion, conjecture, experimental design, experimental measurements and data analysis. A goal of this study was to develop a multiresponse model-building strategy which could improve the degree of success with which multiresponse techniques are applied.

Of particular concern in this study was the development of models which describe batch culture kinetics. Typically, batch culture kinetics are described by a set of nonlinear differential equations and the available data consist of observations of biomass, substrate(s) and product(s) as a function of time. Use of multiresponse parameter estimation is recommended in these cases to improve precision and

sensitivity of tests for lack of fit and is often essential for estimation of all the model parameters. In many attempts to model batch culture kinetics there is little concern paid to whether or not all the parameters in a hypothesized model can be estimated. One example involves the estimation of the maximum specific growth rate and saturation constant in Monod models (Monod (1959)). These parameters are often very highly correlated, leading to difficulties in convergence and poor precision. Similar problems are encountered when the model contains unmeasurable variables such as concentrations of intracellular species. Parameters related to these variables will often be extremely difficult to estimate due to lack of information in the attainable experimental results.

Investigation of these and other problems led to a strategy for building models for culture kinetics. Using an example based on the culture of *Eschirichia coli*, the strategy, which incorporates multiresponse parameter estimation for systems described by ODEs, is demonstrated. It is shown how one can gradually generate information about the system and how, starting from a fairly simple representation of the system, one can gradually increase the complexity of the mechanistic description to obtain a well characterized and validated model. It is also shown how problems encountered in multiresponse analysis for excessively complex or over-parameterized models can be avoided by carefully designing experiments and by initially considering simpler models.

Chapters 2, 3 and 4 highlight the three main aspects of this study. Chapter 2 deals with the development of a general purpose multiresponse parameter estimation routine. The theoretical treatment of multiresponse parameter estimation is first reviewed. Then a new general purpose algorithm for multiresponse parameter estimation using the Box-Draper estimation criterion is developed. Chapter 3 highlights the problem of estimating parameters in systems of ordinary differential equations. A short discussion of the numerical solution of ODEs using linear multistep methods is given. Efficient procedures based on the DDM for the

calculation of first and second-order sensitivities are presented. Four parameter estimation problems are then solved using four different optimization schemes which implement DDM. In Chapter 4, a multiresponse model building strategy for batch culture kinetics is presented. The benefits of a model building strategy based on a combination of rigorous statistical techniques and process knowledge are also discussed. A model building example is then used to demonstrate how and why such a strategy can be beneficial. The main conclusions and recommendations of this study are presented in Chapter 5.

CHAPTER 2

Multiresponse Parameter Estimation

2.1 Introduction

The study of typical processes in chemical engineering requires the simultaneous measurement of many response variables to elucidate underlying mechanisms or to fully describe process dynamics. For example, the study of reaction kinetics can involve measurement of concentration of various species (i.e., reactants, intermediates, products) involved in the reaction pathways. In the analysis of such experiments, a mathematical description is often sought in order to better design, optimize and control the process studied. This requires the estimation of the unknown constants, or parameters, of the model. When more then one response is measured, it is preferable to use information from all responses to estimate the unknown constants, or parameters, of the model. Parameters common to more than one response are often difficult to estimate using information from a single response. This is known to yield poor parameter estimates (Box and Draper (1965)).

In this Chapter, the problem of multiresponse parameter estimation is presented. Three approaches used to develop parameter estimation procedures are discussed. One of these approaches, due to Box and Draper (1965), is of prime interest in this study. Techniques for implementing the Box-Draper criterion are discussed and new general purpose algorithms, applicable to systems represented by ordinary differential equations, nonlinear algebraic equations or differential/algebraic equations, are outlined. Evaluation of parameter precision is also discussed.

2.2 General Multiresponse Model

Multiresponse parameter estimation consists of fitting a model to a set of M observed responses, y_{nm} (n=1,...,N), (m=1,...,M), measured at N experimental conditions ξ_n. The observed responses are modelled by

$$y_{nm} = f_m(\xi_n,\theta) + \varepsilon_{nm} \tag{2.1a}$$

where $f_m(\xi_n,\theta)$ is the expected value of the m^{th} response evaluated at the n^{th} set of experimental conditions, f_m is the m^{th} response function, θ is a P-dimensional vector of of true parameter values and ε_{nm} is the random error term associated with observation y_{nm}.

The purpose of model building is to find $f_m(\xi_n,\theta)$ and estimate θ. Assuming that f^*_m is proposed for representing f_m, eq. 2.1a becomes

$$y_{nm} = f^*_m(\xi_n,\theta) + z_{nm} \tag{2.1b}$$

where z_{nm} are the residuals terms involved with the prediction of y_{nm} by f^*_m. In the case where f^*_m is close to the true model, f_m, z_{nm} should display properties similar to those of the random error terms, ε_{nm}.

When the experimental conditions are fixed for a set of N observations, the expected responses depend only on θ. The observations can be gathered into an NxM matrix, **Y**, the predicted responses into an NxM matrix $\mathbf{F}(\theta)$ and the residuals into an NxM matrix $\mathbf{Z}(\theta)$ given by

$$\boldsymbol{Z}(\boldsymbol{\theta}) = \boldsymbol{Y} - \boldsymbol{F}(\boldsymbol{\theta}) \tag{2.2}$$

In the remainder of this text, the dependence on θ is suppressed. It will be understood that the residuals, **Z**, contrary to the random error terms, ε_{nm}, depend on θ.

2.3 Criteria for Multiresponse Parameter Estimation

Many estimation criteria have been derived for the estimation of parameters in systems described by eq. 2.1. Depending on the assumptions concerning the properties of ε_{nm} and on the choice of estimation objective, various criteria can be derived. It is usually assumed that the ε_{nm} are identically distributed such that

$$E[\varepsilon_{nm}] = 0 \tag{2.3a}$$

and

$$\begin{aligned} E[\varepsilon_{nm}\varepsilon_{rs}] &= 0, \qquad n \neq r \\ &= \{\sigma_{ms}\}, \qquad n = r \end{aligned} \tag{2.3b}$$

This indicates that the ε_{nm} have mean 0 and that, for the same run, have covariance matrix $\Sigma = \{\sigma_{nm}\}$. Errors from different runs are not correlated.

Among the most popular estimation methods are generalized least squares estimation, maximum likelihood estimation and Bayesian estimation. In the following sections, these methods are briefly outlined. Further details can be found in books by Seber and Wild (1989) and Bates and Watts (1988).

2.3.1 Generalized Least Squares

Assume that the ε_{nm} are i.i.d. with mean 0 and variance-covariance matrix Σ, but not necessarily normally distributed (It should be noted parenthetically that i.i.d. stands for "identically and independently distributed" which, in reference to multiresponse situations, implies that independence occurs between runs and not necessarily within runs). Assuming further that Σ is known, an estimate of θ could be obtained by minimizing

$$G(\theta) = \sum_{i=1}^{N} (y_i - f_i(\theta))^T \Sigma^{-1} (y_i - f_i(\theta))$$
$$= (y - f(\theta)^T (\Sigma^{-1} \otimes I_N)(y - f(\theta)) \qquad (2.4)$$

where y_i and f_i are the i^{th} M-dimensional row vectors of the NxM matrices Y and $F(\theta)$, respectively, $y = vec(Y)$, $f(\theta) = vec(F(\theta))$, vec is an operation which vectorizes the NxM matrices to form an NM-dimensional vector, I_N is the NxN identity matrix and $\otimes$ denotes the Kronecker product of two matrices. The Kronecker product of an MxM matrix A and an NxN matrix B is the NMxNM matrix

$$A \otimes B = \begin{bmatrix} a_{11}B & a_{12}B & \dots & a_{1m}B \\ . & a_{22}B & & . \\ . & . & & . \\ . & . & & . \\ a_{m1}B & a_{m2}B & \dots & a_{mm}B \end{bmatrix}.$$

The minimization of eq. 2.4 can be done using a Newton scheme. When Σ is unknown, it must be replaced by a consistent estimator in the optimization of eq. 2.4. If one generates the ordinary least squares estimates of θ, written $\theta_{(j)}$, by fitting the j^{th} response of eq. 2.1, an estimate of the random errors of eq. 2.1 for the j^{th} response, written $e_{n(j)}$, is given by

$$e_{nj} = y_{nj} - f_{nj}(\theta_{(j)}) \qquad , (n=1,...,N;\ j=1,...,M). \qquad (2.5)$$

A consistent estimator of Σ is then given by

$$\hat{\Sigma} = \{\hat{\sigma}_{rs}\} = \frac{1}{N}\{(e_r^T e_s)\} \qquad , (r,s=1,...,M). \qquad (2.6)$$

The estimation routine would therefore estimate each $\theta_{(j)}$ (j=1,...,M) to obtain e_j and $\hat{\Sigma}$. The estimates, $\hat{\theta}$, of θ are obtained by minimizing

$$\hat{G}(\boldsymbol{\theta}) = (y - f(\boldsymbol{\theta}))^T (\hat{\Sigma}^{-1} \otimes I_N)(y - f(\boldsymbol{\theta})) \qquad (2.7)$$

The optimization can be performed using a Gauss-Newton method based on a first-order approximation of $\mathbf{f}(\theta)$ of the form

$$f(\boldsymbol{\theta}) \approx f(\hat{\boldsymbol{\theta}}) + \frac{\partial f(\hat{\boldsymbol{\theta}})^T}{\partial \theta}(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}). \qquad (2.8)$$

The required estimation of all $\theta_{(j)}$ and $\mathbf{e}_j$, prior to the optimization of eq. 2.7 is computationally intensive. Although manageable for small problems where the number of parameters and response variables is small, it can become overwhelming as larger problems are tackled. The estimation of $\theta_{(j)}$ (j=1,...,M) may be very difficult when parameters are highly correlated or when the j^{th} response is completely independent of some of the parameters. It is clearly impossible to obtain $\hat{\theta}$ when estimation of any $\theta_{(j)}$ fails.

A more efficient method of dealing with the optimization of eq. 2.4 with unknown variance-covariance matrix is to reduce it to an ordinary least squares problem by the method of Gallant (1975). This method consists of taking a Cholesky decomposition of $\Sigma = \mathbf{C}^T\mathbf{C}$, where $\mathbf{C}$ is upper triangular, to form the transformed model

$$\begin{aligned} (C^{-T} \otimes y) &= (C^{-T} \otimes f(\boldsymbol{\theta})) + (C^{-T} \otimes \boldsymbol{\varepsilon}) \\ z &= k(\boldsymbol{\theta}) + \boldsymbol{\epsilon} \end{aligned} \qquad (2.9)$$

where $\mathbf{z}$, $\mathbf{k}(\theta)$ and ϵ are the observed response, the predicted response and the residulas of the transformed model, respectively. The optimization of eq. 2.4 can be reduced to a least squares problem of the form

$$\hat{G}(\theta) = (y - f(\theta))^T(\hat{\Sigma}^{-1}\otimes I_N)(y - f(\theta)) \\ = (z - k(\theta))^T(z - k(\theta)). \tag{2.10}$$

As far as the optimization of eq. 2.4 is concerned, this is generally preferred to the model by model estimation of $\theta_{(j)}$ and $\mathbf{e}_j$ (j=1,...,M). For the case where Σ is unknown, an estimate must be obtained initially. Parameter estimates are then obtained using eq. 2.10 for this estimate of Σ. The procedure is then repeated, updating θ and Σ, until convergence is reached.

In practice, generalized least squares estimation (GLS) may be difficult to use if a good estimate of Σ is not available. The coupled estimation of the covariance matrix and the parameters induces a correlation which may cause a number of problems in the diagnostic testing of eq. 2.1. The parameter estimates will be conditioned on this estimate of Σ. The precision estimates for the parameters will also be affected if Σ is not correct. On the other hand, because there is no restriction on the distribution of ε_{nm}, GLS may often be advantageous.

2.3.2 Maximum Likelihood Estimation

Assuming that the ε_{nm} are multivariate normal with mean 0 and covariance matrix Σ, the joint distribution of y(=vec(Y)) is

$$p(y \mid \theta,\Sigma) = (2\pi)^{-NM/2} \mid \Sigma \mid^{-N/2} \exp\left\{-\frac{1}{2}\sum_{i=1}^{N}(y_i - f_i(\theta))^T\Sigma^{-1}(y_i - f_i(\theta))\right\} \\ = (2\pi)^{-NM/2} \mid \Sigma \mid^{-N/2} \exp\left\{-\frac{1}{2}Q(\Sigma,\theta)\right\} \tag{2.11}$$

where

$$Q(\Sigma,\theta) = \sum_{i=1}^{N} (y_i - f_i(\theta))^T \Sigma^{-1} (y_i - f_i(\theta)) \qquad (2.12)$$
$$= tr(\Sigma^{-1} V(\theta))$$

for

$$V(\theta) = (Y - F(\theta))^T (Y - F(\theta)) \qquad (2.13)$$

and where y_i and $f_i(\theta)$ (i=1,...,N) are row vectors of the matrices Y and $F(\hat{\theta})$, respectively. The log-likelihood is thus given by

$$L(\theta,\Sigma) = -\frac{N}{2}\log(|\Sigma|) - \frac{1}{2}tr(\Sigma^{-1}V(\theta)) - \frac{NM}{2}\log(2\pi). \qquad (2.14)$$

The maximization of $L(\theta,\Sigma)$ is equivalent to the minimization of

$$L^*(\theta,\Sigma) = \log(|\Sigma|) + tr(\Sigma^{-1}V(\theta)/N). \qquad (2.15)$$

It can be proven that, subject to Σ being positive definite, $L^*(\theta,\Sigma)$ can be minimized uniquely at $\Sigma=V(\theta)/N$. Thus $L^*(\theta,\Sigma)$ is minimized for fixed θ when $\Sigma=V(\theta)/N$. The maximum likelihood estimates (MLE), $\hat{\theta}$, are obtained by simply minimizing $V(\theta)$, with the MLE of Σ given by $\hat{\Sigma} = V(\hat{\theta})/N$. θ minimizes $L^*(\theta,\Sigma)$ when $\Sigma=\hat{\Sigma}$, and therefore $L(\theta,\Sigma)$ is maximized by minimizing $tr(\hat{\Sigma}^{-1}V(\theta))$ which is identical to GLS estimation for known Σ.

2.3.3 Bayesian Estimation

Under the assumption that ε_{nm} is $N(0,\Sigma)$, the joint distribution of y was given eq. 2.11. Following Box and Draper (1965), Bayes theorem may be applied with a noninformative prior for Σ and θ given by

$$p(\theta,\Sigma) = p(\theta)p(\Sigma) \qquad (2.16)$$

where

$$p(\boldsymbol{\theta}) = c_1 \tag{2.17}$$

and

$$p(\boldsymbol{\Sigma}) = c_2 |\boldsymbol{\Sigma}|^{-(N+1)/2} \tag{2.18}$$

where c_1 and c_2 are constants.

Under the multivariate normality assumption, this gives a posterior density for θ of the form

$$p(\boldsymbol{\theta}|y) \propto \det\left(\sum_{i=1}^{N} (y_i - f_i(\boldsymbol{\theta}))^T (y_i - f_i(\boldsymbol{\theta}))\right)^{-N/2} = \det(V(\boldsymbol{\theta}))^{-N/2} \tag{2.19}$$

Estimates of θ taken to minimize $\det(\mathbf{V}(\theta))$ have maximum posterior density. These estimates coincide with the maximum likelihood estimates of Section 2.3.2.

This parameter estimation criterion has always been considered the most advantageous for practical use. Since the variance-covariance matrix is normally not known, methods such as GLS estimation which require an estimate of $\boldsymbol{\Sigma}$ are generally not recommended.

Minimization of $d(\theta)=\det(\mathbf{V}(\theta))$ with respect to θ, is general and does not depend on whether the model is linear or nonlinear with respect to the parameters. It is invariant under scaling of the data (that is, it will yield the same parameter estimates regardless of scaling). It is also invariant under rotation of the residual matrix $\mathbf{Z}$ by a matrix $\mathbf{W}$. If the rotation matrix, $\mathbf{W}$, (with $|\mathbf{W}^T\mathbf{W}| \neq 0$ or ∞) is applied

$$d_W(\boldsymbol{\theta}) = |(ZW)^T(ZW)| = |W^TW||Z^TZ| \propto |Z^TZ|. \tag{2.20}$$

The optimization of $d_W(\theta)$ is equivalent to the optimization of $d(\theta)$ given any matrix $\mathbf{W}$.

The main advantage of the Box-Draper criterion over GLS estimation is that estimation of the covariance matrix only comes as result of the parameter estimation routine. This has significant practical advantages because the estimation of θ does not depend on the estimate of Σ. An estimate of Σ, based on maximum likelihood or Bayesian results, is usually obtained from the resulting parameter estimates. For cases where Σ is known, the GLS method yields better estimates of θ which are independent of the particular distribution of $\{\varepsilon_{nm}\}$ and should probably be used.

In this study, the primary concern is the application of the Box-Draper criterion for cases where the covariance matrix is unknown. The following section deals with the problem of optimizing the Box-Draper criterion.

2.4 Optimization of the Box-Draper criterion

A number of successful methods exist for the unconstrained optimization of functions of many variables (see Seber and Wild (1989) and Gallant (1987)), all of which are applicable to the problem at hand. Until recently few special purpose optimization routines for the Box-Draper criterion were found. The available methods were based on extensions of methods already used for nonlinear least squares such as the Levenberg-Marquardt method (Bates and Watts (1985)). Although not well suited for the problem of optimizing the Box-Draper criterion, they possessed a number of advantages. The main feature was the approximation of second-order derivatives of the objective function with respect to the parameters using only first-order derivatives of the model function with respect to the parameters, called first-order parametric sensitivity coefficients. The evaluation of these first-order derivatives can be difficult in the multiresponse case for systems described by ordinary differential equations (ODEs). As a result, there has been considerable work done to effectively evaluate the sensitivity coefficients in systems of ODEs (Leis and Kramer (1988)) in an attempt to improve the efficiency of such optimization methods.

This section deals with the optimization of the Box-Draper criterion for the case where only first-order sensitivity coefficients are required. Its main objective is to show how optimization techniques, successful for nonlinear least squares, such as Newton, Gauss-Newton, Quasi-Newton and hybrid methods can be extended to the optimization the Box-Draper criterion to yield multipurpose algorithms (i.e., applicable to both single response and multiresponse parameter estimation). The case where second-order derivatives are required will be introduced in the next Chapter.

In the first part of the present section, a brief summary of methods for optimization in nonlinear least squares problems is given. This is followed, in the second part, by a discussion of the extension of these methods to include the development of a new, powerful hybrid algorithm.

2.4.1 Optimization in Nonlinear Least Squares Problems

Given the general model

$$y_n = f(\boldsymbol{\theta},\boldsymbol{\xi}_n) + r_n \tag{2.21}$$

the nonlinear least squares problem consists of calculating values of θ which minimize

$$\begin{aligned} S(\boldsymbol{\theta}) &= \sum_{i=1}^{N} r_i(\boldsymbol{\theta})^2 \\ &= \sum_{i=1}^{N} \left(y_i - f(\boldsymbol{\theta},\boldsymbol{\xi}_i)\right)^2 \end{aligned} \tag{2.22}$$

$S(\theta)$ can be optimized using a number of different optimization schemes to estimate parameters. Since the work of Gauss (1809), which indicated many attractive features of the special structure of $S(\theta)$ as a sum of squares, numerous optimization schemes have been developed to efficiently solve nonlinear least squares problems. In what follows, such special purpose schemes are discussed.

Newton's method for the optimization of a multivariate function is based on a second-order Taylor series expansion of the objective function at the current estimates of the parameters,

$$S(\boldsymbol{\theta}^{(i+1)}) \approx S(\boldsymbol{\theta}^{(i)}) + \boldsymbol{\gamma}^{(i)T}(\boldsymbol{\theta}^{(i)} - \boldsymbol{\theta}^{(i+1)}) + (\boldsymbol{\theta}^{(i)} - \boldsymbol{\theta}^{(i+1)})^T \boldsymbol{\Gamma}^{(i)} (\boldsymbol{\theta}^{(i)} - \boldsymbol{\theta}^{(i+1)}) \quad (2.23)$$

consists in an iterative scheme in which at the i^{th} iteration, the parameters are updated following

$$\boldsymbol{\theta}^{(i+1)} = \boldsymbol{\theta}^{(i)} + \boldsymbol{\delta}^{(i)} \quad (2.24)$$

where $\delta^{(i)}$, the P-dimensional update vector or the "Newton step", is obtained by first evaluating the gradient and Hessian, denoted $\boldsymbol{\gamma}^{(i)}$ and $\boldsymbol{\Gamma}^{(i)}$ respectively. For least squares,

$$\boldsymbol{\gamma}^{(i)} = \frac{\partial S(\boldsymbol{\theta}^{(i)})}{\partial \boldsymbol{\theta}} = 2\sum_{j=1}^{N} r_j^{(i)} \frac{\partial r_j^{(i)}}{\partial \boldsymbol{\theta}} = 2\boldsymbol{J}^{T(i)}\boldsymbol{r}^{(i)} \quad (2.25)$$

where

$$\boldsymbol{J}^{(i)} = \frac{\partial \boldsymbol{r}^{(i)}}{\partial \boldsymbol{\theta}}$$

and

$$\boldsymbol{\Gamma}^{(i)} = 2\sum_{j=1}^{N} \frac{\partial r_j^{(i)}}{\partial \boldsymbol{\theta}} \frac{\partial r_j^{(i)}}{\partial \boldsymbol{\theta}^T} + 2\sum_{j=1}^{N} r_j^{(i)} \frac{\partial^2 r_j^{(i)}}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} = 2(\boldsymbol{J}^T\boldsymbol{J} + \boldsymbol{A})^{(i)} \quad (2.26)$$

where

$$\boldsymbol{A}^{(i)} = \sum_{j=1}^{N} r_j^{(i)} \frac{\partial^2 r_j^{(i)}}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} \quad . \quad (2.27)$$

Calculation of the optimum of eq. 2.22 yields

$$\boldsymbol{\delta}^{(i)} = -\boldsymbol{\Gamma}^{(i)^{-1}}\boldsymbol{\gamma}^{(i)} \quad . \tag{2.28}$$

Thus, the Newton step can be obtained from

$$\boldsymbol{\delta}^{(i)} = -\boldsymbol{\Gamma}^{(i)^{-1}}\boldsymbol{\gamma}^{(i)} = -\left(\boldsymbol{J}^T\boldsymbol{J} + \boldsymbol{A}\right)^{(i)^{-1}}\boldsymbol{\gamma}^{(i)} \tag{2.29}$$

The need to calculate second-order derivatives significantly increases computational difficulties for the Newton method. Consequently, many methods have been developed which do not require calculation of second-order derivatives. The Gauss-Newton method (denoted GN) is derived by taking a first-order Taylor series expansion of $f(\theta,\xi)$ around $\theta^{(i)}$ to give

$$f(\boldsymbol{\theta},\boldsymbol{\xi}) \approx f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi}) + \frac{\partial f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi})}{\partial \boldsymbol{\theta}^T}\left(\boldsymbol{\theta} - \boldsymbol{\theta}^{(i)}\right) \tag{2.30}$$

This converts the nonlinear least squares problem to a linear problem of the form

$$S(\boldsymbol{\theta}^{(i)}) \approx \sum_{j=1}^{N}\left(r_j - \frac{\partial f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi}_j)}{\partial \boldsymbol{\theta}^T}\left(\boldsymbol{\theta} - \boldsymbol{\theta}^{(i)}\right)\right)^2 \tag{2.31}$$

which leads to the Gauss-Newton optimization scheme of the form

$$\boldsymbol{\theta}^{(i+1)} = \boldsymbol{\theta}^{(i)} + \boldsymbol{\delta}_{GN}^{(i)} \tag{2.32}$$

where

$$\begin{aligned}\boldsymbol{\delta}_{GN}^{(i)} &= \left(\frac{\partial f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi})}{\partial \boldsymbol{\theta}^T}\frac{\partial f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi})}{\partial \boldsymbol{\theta}}\right)\frac{\partial f(\boldsymbol{\theta}^{(i)},\boldsymbol{\xi})}{\partial \boldsymbol{\theta}^T}\boldsymbol{r}^{(i)} \\ &= -\left(\boldsymbol{J}^T\boldsymbol{J}\right)^{(i)^{-1}}\boldsymbol{J}^{T^{(i)}}\boldsymbol{r}^{(i)}\end{aligned} \tag{2.33}$$

The GN method can thus be obtained directly from the Newton method by ignoring the matrix **A**.

The effectiveness of the GN method requires that $\mathbf{J}^T\mathbf{J}$ is not ill-conditioned. To

be robust, this method needs to be modified to improve the conditioning of $\mathbf{J}^T\mathbf{J}$. The most recommended (Dennis and Schnabel (1983, p.228)) modification of the GN method is the Levenberg-Marquardt method (Moré, 1978) given by

$$\delta^{(i)} = -(J^TJ + \alpha D)^{(i)^{-1}} J^{T^{(i)}} r^{(i)} \tag{2.34}$$

where **D** is a positive diagonal matrix and α is a scalar correction term. In general **D** is the identity matrix, **I**.

A number of strategies have been devised to evaluate $\alpha^{(i)}$ in order to make **B** $(= (\mathbf{J}^T\mathbf{J} + \alpha\mathbf{I})^{(i)})$ positive definite. The main objective of these strategies is to obtain the smallest possible value of $\alpha^{(i)}$ to achieve positive definitiveness. One strategy to ensure that **B** is positive definite is make α twice the absolute value of the most negative eigenvalue of $\mathbf{J}^T\mathbf{J}$.

Another strategy, due to Marquardt (1963), is to take $\alpha^{(0)} = 0.01$ and update it at each iteration. For a given $\alpha^{(i)}$, if the step produced is successful, $\alpha^{(i+1)}$ is set to $\alpha^{(i)}/10$ such that, as the optimization progresses the step becomes closer to the GN step.

An alternative strategy to ensure that **B** is positive definite is to calculate the step via a modified Cholesky decomposition of **B**. If a matrix **M** is positive definite and symmetric, there exists a decomposition of **M** of the form $\mathbf{C}^T\mathbf{C}$ where **C** is upper triangular. When **M** is not positive definite (i.e. when one or more of its eigenvalues are zero), a modified Cholesky decomposition may be obtained by taking $\mathbf{C}^T\mathbf{C} = (\mathbf{M}+\mathbf{D})$ where **D** is a diagonal matrix with nonnegative elements that are zero when **M** is positive definite. In a recent paper, Schnabel and Eskow (1990) discuss two procedures to perform this decomposition: a procedure due to Gill and Murray (see Gill *et al.* (1981)) and their procedure. They recognize both techniques to be adequate for unconstrained optimization, but point out that their method should be advantageous for larger problems where indefiniteness may occur.

In cases where the residuals are small, eq.2.27 indicates that **A** will generally be negligible in comparison to $\mathbf{J}^T\mathbf{J}$, and the GN method should perform well. When the residuals are large, such that **A** is no longer negligible, the GN method does not perform as well and may even fail or diverge. A method to estimate **A** is therefore required.

The best way to deal with cases where **A** is no longer negligible, without resorting to the calculation of second-order derivatives, is to use Quasi-Newton or secant update methods. (The latter term is generally recognized as the correct term.) These methods approximate **A** by updating it at each iteration using the current information. One such formula is the Dennis-Gay-Welsch (DGW) modification of the Dennis-Fletcher-Powell formula (Dennis *et al.* (1981)) which takes the form

$$A^{(i+1)} = \tau^{(i+1)}A^{(i)} + \frac{(\tilde{\mathbf{v}}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})\mathbf{v}^{(i)T} + \mathbf{v}^{(i)}(\tilde{\mathbf{v}}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})^T}{\boldsymbol{\delta}^{(i)T}\mathbf{v}^{(i)}} - \frac{\boldsymbol{\delta}^{(i)T}(\tilde{\mathbf{v}}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})}{(\boldsymbol{\delta}^{(i)T}\mathbf{v}^{(i)})^2}\mathbf{v}^{(i)}\mathbf{v}^{(i)T} \tag{2.35}$$

where

$$\mathbf{v}^{(i)} = \boldsymbol{\gamma}^{(i+1)} - \boldsymbol{\gamma}^{(i)}$$

$$\boldsymbol{\delta}^{(i)} = \boldsymbol{\theta}^{(i+1)} - \boldsymbol{\theta}^{(i)}$$

$$\tilde{\mathbf{v}}^{(i)} = (J^{(i+1)} - J^{(i)})^T r^{(i+1)}$$

and

$$\tau^{(i+1)} = \min\left(1, \left|\frac{\tilde{\mathbf{v}}^{(i)T}\boldsymbol{\delta}^{(i)}}{\boldsymbol{\delta}^{(i)T}A^{(i)}\boldsymbol{\delta}^{(i)}}\right|\right)$$

Such update methods were originally developed for solving large residuals

nonlinear least squares problems. In such cases, they are known to be more robust than the Gauss-Newton or Levenberg-Marquardt methods. However, they ensure only superlinear convergence (Dennis and Schnabel (1983)) and will not, except in large residual problems, converge as fast as Gauss-Newton methods which are recognized to display quadratic convergence. Update formulas alone are therefore not well suited for the development of general purpose nonlinear parameter estimation procedures. Better general purpose procedures can be obtained by combining an update formula and the GN method to form an hybrid method. The method of Dennis *et al.* (1981) is one of the better known hybrid methods for solving nonlinear least squares problems.

In this method, the optimization is initiated with the GN method by setting $\mathbf{A}_0$ to $\mathbf{0}$. At each subsequent iteration, the update formula is applied to generate the matrix $\mathbf{A}$ and the progress of the GN method is compared to that of the update method in order to select the best possible method to use. This involves the development of a switching procedure. Dennis *et al.* (1981) suggested switching from the current method to the alternative method if

$$\left| \frac{q^{(i)} - f(\boldsymbol{\theta}^{(i+1)})}{q^{a(i)} - \boldsymbol{\theta}^{(i+1)}} \right| > 1.5 \qquad (2.36)$$

where $q^{(i)}$ and $q^{a(i)}$ are the values of the objective function at $\theta^{(i+1)}$ based on the quadratic representation of $S(\theta^{(i+1)})$ following the current and the alternative optimization methods, respectively. They combined this rule with a model/trust-region method (Dennis and Schnabel (1983)). (The basic idea behind a model/trust-region method is to provide a quadratic model, $q^{(i)}$, of $f(\theta^{(i)})$ and an estimate of a region in which $q^{(i)}$ is trusted to represent $f(\theta^{(i)})$. For further details see Dennis and Schnabel (1983; p.129).)

In the next section, it will be shown how these methods for nonlinear least squares are also applicable to optimization of the Box-Draper criterion.

2.4.2 The Gradient and Hessian of the Box-Draper Criterion

As for the nonlinear least squares problem, expressions for the gradient, γ, and the Hessian, Γ, of $d(\theta)$ are required in order to apply Newton's method for the optimization of the Box-Draper criterion. The Newton step is given by

$$\delta^{(i)} = -\Gamma^{(i)^{-1}}\gamma^{(i)} \tag{2.37}$$

There are two methods to derive useful expressions for the derivatives of $d(\theta)$ with respect to the parameters. The first, due to Bates and Watts (1985), consists of taking a **QR** decomposition of $\mathbf{Z}$ ($= \mathbf{Y} - \mathbf{H}(\theta)$). The second, due to Reilly (1990), consists of deriving analytical expressions for the derivatives. The two methods are discussed in the following two sub-sections. Both methods are based upon an equivalent optimization problem using the objective function

$$\Phi(\theta) = \frac{1}{2}\log(d(\theta)) \quad . \tag{2.38}$$

Method of Bates and Watts

Bates and Watts (1985) showed that

$$\Phi_p(\theta) = \frac{\partial\Phi(\theta)}{\partial\theta_p} = tr\left[Z^{+}\frac{\partial Z}{\partial\theta_p}\right] = tr\left[Z^{+}Z_p\right] \tag{2.39}$$

where $\mathbf{Z}^{+}$ is the Moore-Penrose generalized inverse of $\mathbf{Z}$ and

$$Z_p = \frac{\partial Z}{\partial\theta_p} \tag{2.40}$$

such that the subscript p denotes differentiation with respect to the p^{th} parameter. They also showed that

$$\Phi_{pq}(\boldsymbol{\theta}) = \frac{\partial^2 \Phi(\boldsymbol{\theta})}{\partial\theta_p \partial\theta_q} = -tr\left[\mathbf{Z}^{+}\mathbf{Z}_p\mathbf{Z}^{+}\mathbf{Z}_q\right] + tr\left[\mathbf{Z}^{+}\mathbf{Z}^{+^T}\mathbf{Z}_p^T(\mathbf{I} - \mathbf{Z}\mathbf{Z}^{+})\mathbf{Z}_q\right] + tr\left[\mathbf{Z}^{+}\mathbf{Z}_{pq}\right] \tag{2.41}$$

where

$$\mathbf{Z}_{pq} = \frac{\partial^2 \mathbf{Z}}{\partial\theta_p \partial\theta_q} \tag{2.42}$$

The elements of $\boldsymbol{\gamma}$ and $\boldsymbol{\Gamma}$ are thus given by

$$\gamma_p = \frac{\partial d(\boldsymbol{\theta})}{\partial\theta_p} = 2\,d(\boldsymbol{\theta})\,\Phi_p(\boldsymbol{\theta}) \tag{2.43}$$

and

$$\Gamma_{pq} = \frac{\partial^2 d(\boldsymbol{\theta})}{\partial\theta_p \partial\theta_q} = 2\left[d(\boldsymbol{\theta})\,\Phi_{pq}(\boldsymbol{\theta}) + \boldsymbol{\gamma}_p\boldsymbol{\gamma}_q\right] \tag{2.44}$$

Bates and Watts (1985) developed a scheme to calculate the gradient and Hessian of d(θ) using a **QR** decomposition of **Z** which takes the form

$$\mathbf{Z} = \mathbf{QR} = \mathbf{Q}\begin{pmatrix}\mathbf{R}_{11}\\ \mathbf{0}\end{pmatrix} \tag{2.45}$$

where **Q** is an NxN orthogonal matrix (i.e. $\mathbf{Q}^T\mathbf{Q} = \mathbf{Q}\mathbf{Q}^T = \mathbf{I}_N$) and **R** is an NxM matrix with zeroes below the main diagonal. $\mathbf{Q}_1$ is formed from the first M columns of **Q** and $\mathbf{Q}_2$, from the last N-M columns. The $\mathbf{R}_1$ is a MxM upper triangular matrix such that $\mathbf{Z} = \mathbf{Q}_1\mathbf{R}_1$. The main principle of the QR decomposition is to decompose the matrix **Z** into the product of an orthogonal matrix and an easily inverted matrix. This decomposition provides an easy way to calculate d(θ) by

$$|\mathbf{Z}^T\mathbf{Z}| = |\mathbf{R}_1^T\mathbf{R}_1| = \prod_{i=1}^{M}\{r_{1,ii}\}^2$$

where $\{r_{1,ii}\}$ are the diagonal elements of $\mathbf{R}_1$. The evaluation of $\boldsymbol{\gamma}$ and $\boldsymbol{\Gamma}$ is performed

by setting

$$G_p = Q^T Z_p R_{11}^{-1} \tag{2.46}$$

to yield

$$\Phi_p(\theta) = tr[G_p] \tag{2.47}$$

and

$$G_{pq} = -\sum_{i=1}^{M}\sum_{j=1}^{M}\{G_p\}_{ij}\{G_q\}_{ji} + \sum_{i=M+1}^{N}\sum_{j=1}^{M}\{G_p\}_{ij}\{G_q\}_{ij} \tag{2.48}$$

Method of Reilly

Reilly (1990) showed that the gradient of $\Phi(\theta)$ is given by

$$\Phi_p(\theta) = -\sum_{i=1}^{N}\left\{\frac{\partial f(\theta,\xi_N)^T}{\partial\theta_p}M^{-1}z_i\right\} \tag{2.49}$$

where $\mathbf{M} = \mathbf{Z}^T\mathbf{Z}$ and $\mathbf{z}_i$ is the i^{th} M-dimensional row vector of $\mathbf{Z}$. The Hessian of $\Phi(\theta)$ is

$$\Phi_{pq}(\theta) = -\sum_{i=1}^{N}\left\{\frac{\partial^2 f^T}{\partial\theta_p\partial\theta_q}M^{-1}z_i - \frac{\partial f^T}{\partial\theta_p}M^{-1}\frac{\partial M}{\partial\theta_q}M^{-1}z_i - \frac{\partial f^T}{\partial\theta_p}M^{-1}\frac{\partial f}{\partial\theta_q}\right\} \tag{2.50}$$

The gradient and Hessian of $d(\theta)$ are thus given by

$$\gamma_p = 2d(\theta)\Phi_p(\theta) \tag{2.51}$$

and

$$\Gamma_{pq} = 2\left(\gamma_q\gamma_p + d(\theta)\Phi_{pq}(\theta)\right) \tag{2.52}$$

In this thesis, both methods were implemented in a multiresponse parameter

estimation routine. The method of Reilly (1990) proved to be more efficient than the QR decomposition of Bates and Watts (1985). In general, a reduction of 5 to 25% in computing time was realized with the method of Reilly. No difference were observed in the calculated Hessian matrices for the two methods. Although it has been reported for the single response case that the normal equations, eqs. 2.25 and 2.26, may not be as numerically stable as the QR decomposition method for calculating an approximate Hessian for optimization of the least squares criterion (Moré, 1978), this behaviour was never encountered for the optimization of the Box-Draper criterion. The method of Reilly (1990) was therefore adopted.

2.4.3 Optimization Strategies for the Box-Draper criterion

Inspection of the expressions for the Hessian of $\Phi(\theta)$ indicates a certain similarity to expressions for the least squares criterion. In both cases, the Hessian matrix takes the general form

$$\boldsymbol{\Gamma} = \{\boldsymbol{B} + \boldsymbol{A}\} \tag{2.53}$$

where, for the multiresponse case with $\Phi(\theta) = \log(d(\theta))/2$,

$$\boldsymbol{B} = \sum_{i=1}^{N} \left\{ \frac{\partial \boldsymbol{f}^T}{\partial \theta_p} \boldsymbol{M}^{-1} \frac{\partial \boldsymbol{M}}{\partial \theta_q} \boldsymbol{M}^{-1} \boldsymbol{z}_i + \frac{\partial \boldsymbol{f}^T}{\partial \theta_p} \boldsymbol{M}^{-1} \frac{\partial \boldsymbol{f}}{\partial \theta_q} \right\} \tag{2.54}$$

$$\boldsymbol{A} = -\sum_{i=1}^{N} \frac{\partial^2 \boldsymbol{f}_i^T}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} \boldsymbol{M}^{-1} \boldsymbol{z}_i \tag{2.55}$$

and, for the least squares case,

$$\boldsymbol{B} = 2\sum_{j=1}^{N} \frac{\partial r_j}{\partial \boldsymbol{\theta}} \frac{\partial r_j}{\partial \boldsymbol{\theta}^T} + 2\sum_{j=1}^{N} r_j \frac{\partial^2 r_j}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} = 2(\boldsymbol{J}^T \boldsymbol{J}) \tag{2.56}$$

$$A = \sum_{j=1}^{N} r_j \frac{\partial r_j}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} \qquad (2.57)$$

Thus, by eq. 2.53 all gradient methods developed for nonlinear least squares can be generalized to the optimization of the Box-Draper criterion. Appropriate expressions for the gradient, **B** and **A** are simply substituted in eqs. 2.51, 2.52 and 2.53 to generate the optimization scheme. This by no means implies that methods, which perform well for the least squares criterion, will automatically perform well for the Box-Draper criterion. The $d(\theta)$ surface in parameter space is, in general, very different to the least squares surface. It tends to have a more nonlinear behaviour. Consequently, the quadratic approximation of the $d(\theta)$ surface around an optimum point, $\hat{\theta}$, given by

$$d(\boldsymbol{\theta}) \approx d(\hat{\boldsymbol{\theta}}) + \boldsymbol{\gamma}^T(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}) + \frac{1}{2}(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}})^T \boldsymbol{\Gamma} (\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}) \qquad (2.58)$$

may not be appropriate in a lot of cases (Bates and Watts (1987)). In nonlinear least squares, the quadratic approximation of $S(\theta)$ near $\hat{\theta}$ is generally good (Bates and Watts, (1988)). One should therefore be cautious in extending optimization techniques developed for nonlinear least squares to optimize the Box-Draper criterion.

In this work, an optimization algorithm of the form

$$\boldsymbol{\theta}^{(i+1)} = \boldsymbol{\theta}^{(i)} + \rho^{(i)} \left[\boldsymbol{\Gamma}^{(i)} + \alpha^{(i)} \boldsymbol{I} \right]^{-1} \boldsymbol{\gamma}^{(i)} \qquad (2.59)$$

was used to optimize the Box-Draper criterion. As in Bates and Watts (1984), $\alpha^{(i)}$ was taken as twice the absolute value of the most negative eigenvalue of $\boldsymbol{\Gamma}^{(i)}$. $\rho^{(i)}$ is a scalar between 0 and 1. It was calculated using a line search algorithm which ensures that

$$d(\boldsymbol{\theta}^i + \rho^{(i)} \boldsymbol{\delta}^{(i)}) < d(\boldsymbol{\theta}^{(i)}) + \kappa \rho^{(i)} \boldsymbol{\gamma}^{T(i)} \boldsymbol{\delta}^{(i)} \qquad (2.60)$$

where $\ell < \rho^{(i)} < u$, $0 < \ell < u < 1$, and $0 < \kappa < \frac{1}{2}$. This inequality ensures that two important requirements are met at each iteration. First, it verifies that a decrease in the objective function value is realized. Second, it ensures that the average rate of decrease between

$d(\theta^{(i)})$ and $d(\theta^{(i)}+\rho^{(i)}\delta^{(i)})$ is at least some fraction of the initial rate of decrease at $d(\theta^{(i)})$, $\gamma^{(i)T}\delta^{(i)}$. This is meant to obliterate situations where the decrease in the objective function is small relative to the length of the steps taken. Such situations may often result in an unnecessary increase of iterations (see Dennis and Schnabel (1983)).

At each iteration, $\delta^{(i)} = (\Gamma^{(i)}+\alpha^{(i)}\mathbf{I})^{-1}\gamma^{(i)}$ is calculated and the line search algorithm is used to yield $\theta^{(i+1)}=\theta^{(i)}+\rho^{(i)}\delta^{(i)}$ if eq. 2.60 is not met with $\rho^{(i)} = 1$. In order to prevent the algorithm from performing an exceedingly long step, the length of the step is compared to a preset maximum allowable step length. As recommended by Dennis and Schnabel (1983), a maximum length of $100\|\theta^{(0)}\|$ is used. $\theta^{(0)}$ is the vector of initial parameter estimates.

At each time the line search algorithm is used, two pieces of information about $\hat{d}(\rho^{(i)})$ $(= d(\theta^{(i)}+\rho^{(i)}\delta^{(i)}))$ are available, namely,

$$d(\theta^{(i)}) = \hat{d}(0) \tag{2.61}$$

and

$$\frac{d\,\hat{d}(0)}{d\rho} = \gamma^{T^{(i)}}\delta^{(i)} \tag{2.62}$$

Once $d(\theta^{(i)}+\delta^{(i)})$ is evaluated, $\hat{d}(1)$ is also known. If $\hat{d}(1)$ does not satisfy eq. 2.60, a one dimensional quadratic of the form

$$m_q(\rho) = \left[\hat{d}(1)-\hat{d}(0)-\frac{d\,\hat{d}(0)}{d\rho}\right]\rho^2 + \frac{d\,\hat{d}(0)}{d\rho}\rho + \frac{d\,\hat{d}(0)}{d\rho} \tag{2.63}$$

is formed. It is minimized by choosing

$$\rho = \frac{-\dfrac{d\,\hat{d}(0)}{d\rho}}{2\left[\hat{d}(1) - \hat{d}(0) - \dfrac{d\,\hat{d}(0)}{d\rho}\right]} . \tag{2.64}$$

Since

$$\hat{d}(1) > \hat{d}(0) + \kappa \frac{d\hat{d}(0)}{d\rho} \tag{2.65}$$

then

$$\rho < \frac{1}{2(1-\kappa)} \tag{2.66}$$

which yields an upper bound, u=½, for the first value of ρ used. If d(1) is much larger then d(0), ρ can be small. Therefore, a lower bound, ℓ=0.1, is also applied to the first value of ρ. If $\hat{d}(\rho^{(i)})$ does not satisfy eq. 2.60, a one-dimensional cubic equation is then fitted to $\hat{d}(0)$, $d\hat{d}(0)/d\rho$, $\hat{d}(\rho_k)$ and $\hat{d}(\rho_{k-1})$ where $\hat{d}(\rho_k)$ and $\hat{d}(\rho_{k-1})$ are the k^{th} and $k\text{-}1^{th}$ values of $\hat{d}(\rho)$ calculated in the line search algorithm. The cubic equation is given by

$$m_{cu}(\rho) = a\rho^3 + b\rho^2 + \frac{d\hat{d}(0)}{d\rho}\rho + \hat{d}(0) \tag{2.67}$$

where

$$\begin{pmatrix} a \\ b \end{pmatrix} = \frac{1}{\rho_k - \rho_{k-1}} \begin{pmatrix} \frac{1}{\rho_k^2} & -\frac{1}{\rho_{k-1}^2} \\ -\frac{\rho_{k-1}}{\rho_k^2} & \frac{\rho_k}{\rho_{k-1}^2} \end{pmatrix} \begin{pmatrix} \hat{d}(\rho_k) - \hat{d}(0) - \frac{d\hat{d}(0)}{d\rho}\rho_k \\ \hat{d}(\rho_{k-1}) - \hat{d}(0) - \frac{d\hat{d}(0)}{d\rho}\rho_{k-1} \end{pmatrix} \tag{2.68}$$

and which is minimized by taking

$$\rho_{k+1} = \frac{-b + \sqrt{b^2 - 3a\frac{d\hat{d}(0)}{d\rho}}}{3a} \; . \tag{2.69}$$

This is repeated until eq. 2.60 is satisfied. This algorithm is described in detail by Dennis and Schnabel (1983).

In the initial stages of the development of the multiresponse parameter estimation procedure, an unconstrained Coggins search (Beveridge and Schechter, (1970)) was used to select a value of $\rho^{(i)}$ which optimized $d(\theta^{(i)}+\rho^{(i)}\delta^{(i)})$. In this study, it was found that such an approach to the calculation of $\rho^{(i)}$ is not recommended in an optimization routine. The main objective should be to select an appropriate value for $\rho^{(i)}$ in order to allow the optimization routine to proceed as quickly as possible. In this work, it was found that the Coggins search required too many unnecessary objective function evaluations and often caused failure of the parameter estimation routine. On the other hand, the line search algorithm discussed above, which did not seek the optimal value of $\rho^{(i)}$ but only a value which satisfied eq. 2.60, performed well and required fewer objective function evaluations. It was therefore favoured in this study.

Once a suitable value for $\rho^{(i)}$ is found, the optimization routine then proceeds with the next iteration starting with calculation of the gradient and Hessian. The critical factors in optimization of $d(\theta)$ are the accuracy of both the quadratic approximation to the objective function and the evaluation of the Hessian matrix. In the previous subsection, expressions were given for the gradient and Hessian of $d(\theta)$. As was the case for nonlinear least squares, various approximations of the Hessian can be employed, depending on the assumption made about the importance of second order effects near an optimum.

When second-order parametric sensitivity coefficients are evaluated explicitly, eq. 2.50 is used directly to obtain the Hessian of $d(\theta)$ in order to estimate θ using the modified Newton scheme given by eq. 2.59. This yields a very robust optimization method. However, coding and computation of the second-order derivatives can significantly degrade the efficiency of the estimation routine. To overcome this loss of efficiency, the second-order derivatives can be simply ignored or terms involving the second-order derivatives can be approximated using only first-order derivatives.

Assuming that there are no second-order effects of the parameters on the model

then the Hessian of Φ becomes

$$\left(\frac{\partial^2 \Phi}{\partial \theta_p \partial \theta_q}\right)_{GN} = \sum_{i=1}^{N} \left\{ \frac{\partial f^T}{\partial \theta_p} M^{-1} \frac{\partial M}{\partial \theta_q} M^{-1} z_i + \frac{\partial f^T}{\partial \theta_p} M^{-1} \frac{\partial f}{\partial \theta_q} \right\} \tag{2.70}$$

where GN denotes the Gauss-Newton approximation and requires only first-order sensitivity coefficients. This expression can be used in eq. 2.59 to give a generalized, modified Gauss-Newton method similar to the one developed by Bates and Watts (1985).

In cases where the second-order effects are not negligible in eq. 2.50 and where second-order parametric sensitivity coefficients are not evaluated, the generalized Gauss-Newton scheme may not perform well and an update method must be used to approximate the matrix

$$A = \sum_{i=1}^{N} \frac{\partial^2 f_i^T}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^T} M^{-1} z_i. \tag{2.55}$$

Dennis *et al.* (1981) developed a successful update formula for nonlinear least squares. In this thesis, it was extended for use with the Box-Draper criterion for multiresponse parameter estimation. The Gauss-Newton approximation of the Hessian is calculated using eq. 2.70 at each iteration and the second-order term, **A**, is updated at each iteration using the formula

$$\begin{aligned} A^{(i+1)} = \tau^{(i+1)} A^{(i)} &+ \frac{(\tilde{v}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})v^{(i)T} + v^{(i)}(\tilde{v}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})^T}{\boldsymbol{\delta}^{(i)T} v^{(i)}} \\ &- \frac{\boldsymbol{\delta}^{(i)T}(\tilde{v}^{(i)} - A^{(i)}\boldsymbol{\delta}^{(i)})}{\boldsymbol{\delta}^{(i)T} v^{(i)}} v^{(i)} v^{(i)T} \end{aligned} \tag{2.71}$$

where

$$\boldsymbol{\delta}^{(i)} = \boldsymbol{\theta}^{(i+1)} - \boldsymbol{\theta}^{(i)}$$

$$\nu^{(i)} = \frac{\partial \Phi(\theta^{(i+1)})}{\partial \theta} - \frac{\partial \Phi(\theta^{(i)})}{\partial \theta}$$

$$\tilde{\nu}^{(i)} = \sum_{j=1}^{N} \left(\frac{\partial f_j(\theta^{(i+1)})^T}{\partial \theta} - \frac{\partial f_j(\theta^{(i)})^T}{\partial \theta} \right) M^{(i+1)^{-1}} z_j^{(i+1)}$$

$$\tau^{(i+1)} = \min\left(1, \frac{|\delta^{(i)T} \tilde{\nu}^{(i)}|}{|\delta^{(i)T} A^{(i)} \delta^{(i)}|} \right) .$$

Therefore, at the $(i+1)^{th}$ iteration, the Hessian of Φ is given by

$$\frac{\partial^2 \Phi^{(i+1)}}{\partial \theta \partial \theta^T} = \left(\frac{\partial^2 \Phi^{(i+1)}}{\partial \theta \partial \theta^T} \right)_{GN} + A^{(i+1)} \qquad (2.72)$$

The above formulas were derived following the procedure of Dennis *et al.* (1981) which is discussed in Dennis and Schnabel (1983) and Seber and Wild (1989). The main difference from the formula obtained for nonlinear least squares are the expressions for ν, and $\tilde{\nu}$ which are given in terms of $\Phi(\theta)$.

In an attempt to improve the robustness of the multiresponse parameter estimation algorithm, eq. 2.71 was used. It was implemented in a hybrid algorithm similar to that of Dennis *et al.* (1981), which allows switching from the Gauss-Newton scheme to the update scheme, eqs, 2.71 and 2.72, depending on the progress of the optimization. Such hybrid methods improve the robustness of parameter estimation routines and allow the efficient solution of small and large residual problems.

In the hybrid method developed here, $A^{(0)}$ is initially set to zero such that the first step is essentially a Gauss-Newton step. It is then updated using eq. 2.70 at each subsequent iteration. Both terms of the Hessian of $\Phi(\theta)$ are thus updated at each iteration to allow switching from one scheme to the other.

The switching rules employed in the proposed method were inspired by Dennis *et al.*(1981). At each iteration, the adequacy with which the current scheme models the objective function is checked. To do this, the actual change in the objective function is compared to the change predicted by the current optimization scheme. If the inequality

$$0.5 \leq \left| \frac{d(\boldsymbol{\theta}^{(i)}) - d(\boldsymbol{\theta}^{(i-1)})}{q^{(i)} - d(\boldsymbol{\theta}^{(i-1)})} \right| \leq 1.5 \tag{2.73}$$

holds, where $q^{(i)}$ is the predicted value of the objective function based on the current scheme, then the current scheme is a good candidate for the following iteration. The alternative scheme is then considered. If eq. 2.73 is also true for the alternative scheme, then the alternative scheme is used for the next iteration unless the inequality

$$\left| \frac{q^{(i)} - d(\boldsymbol{\theta}^{(i)})}{q_a^{(i)} - d(\boldsymbol{\theta}^{(i)})} \right| \leq 1.0 \tag{2.74}$$

is true. $q_a^{(i)}$ is the predicted value of the objective function for the alternative scheme. If eq. 2.73 is not true for both schemes, then eq. 2.74 is used to decide which scheme is to be used. If eq. 2.74 is not true for one of the schemes, then the other is used in the next iteration. In addition to these checks, the switching procedure retains the current scheme if good progress is being made, such that

$$\left| \frac{d(\boldsymbol{\theta}^{(i+1)}) - d(\boldsymbol{\theta}^{(i)})}{d(\boldsymbol{\theta}^{(i)})} \right| \geq 0.2. \tag{2.75}$$

A change of scheme is also considered if

$$\left| \frac{d(\boldsymbol{\theta}^{(i+1)}) - d(\boldsymbol{\theta}^{(i)})}{d(\boldsymbol{\theta}^{(i)})} \right| \leq 0.0001 \tag{2.76}$$

unless the Bates-Watts convergence criterion is less then 0.001 in which case the current scheme is kept for the next iteration. This was adopted mainly because a small value of this criterion indicates the proximity of an optimum. In contrast with the DGW method, this switching procedure is used together with the line search algorithm outlined above.

The rules, eq. 2.73 and 2.74, are essentially the same as those developed by Dennis *et al.* (1981), except that the critical values were modified to suit our particular implementation. In eq. 2.73 the upper bound had been suggested by Dennis *et al.* (1981). Because this switching procedure is used after the choice of step length has been calculated using the line search procedure, a lower bound was imposed to also prevent cases where $d(\theta^{(i)})$ is significantly smaller than $q^{(i)}$. Choice of the critical value in eq. 2.74 ensures that the best potential scheme is employed. The choice of 0.2 in eq. 2.75 has been reported by Biegler *et al.* (1988). This value is recommended as a switching rule in unconstrained optimization problems. In eq. 2.76, the critical value recommended by Dennis *et al.* (1981) was used.

2.4.4 Convergence Criterion for Multiresponse Parameter Estimation

Bates and Watts (1985) developed a very appealing precision-related convergence criterion for the optimization of $d(\theta)$ of the form

$$\|C\delta\|/2s^2 < \varepsilon^2 \tag{2.77}$$

where $\mathbf{C}$ is the Cholesky factor of $\mathbf{\Gamma}$, s^2 is a scaling factor given by

$$s^2 = d(\boldsymbol{\theta})/(N-P), \tag{2.78}$$

δ is the parameter increment vector and ε is the tolerance level, usually set to 0.001 (Bates and Watts (1985)).

The main feature of this criterion is that, unlike other commonly encountered convergence criteria (such as the relative change in the objective function, the size of the parameter increment values relative to the parameter values), it takes into account the statistical variability of the parameters. As shown by Bates and Watts (1985), statistical variability of the parameters can be presented by ellipsoidal regions of the form

$$(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}})^T(\boldsymbol{\Gamma}/2)(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}) \leq c$$

where c is a constant. Using the linear transformation of the parameters induced by $\mathbf{C}^T$ (Bates and Watts (1988)), these regions can be made spherical. The magnitude of the increment vector, $\|\mathbf{C}\delta\|$, can then be compared to the radius of the spherical regions leading to eq. 2.77. Since $\mathbf{C}$ exists only if $\boldsymbol{\Gamma}$ is positive definite, use of this criterion ensures that convergence can only be reached when the Hessian is positive definite.

The Bates-Watts convergence criterion was used in all the routines developed in the present work.

2.5 Approximate Parameter Inference Regions for Multiresponse Estimation

When a model has been shown to be adequate, the precision of the parameters has to be estimated. A desirable way to express parameter precision is in the form of joint confidence regions. Such regions provide information about the variability of and the correlation between the estimated parameters. The main disadvantage with the joint regions is that they are difficult to interpret for more then two parameters. In practice, individual confidence intervals and correlation between the parameters are calculated from an estimate of the covariance matrix for the parameters. In this work, an approach due to Bates and Watts (1985) was used to provide individual confidence intervals and correlation between the parameters. Another approach, due to Stewart (1987), was also considered. Both are discussed in this section.

Assuming that the rows of $\mathbf{Z}$ are multivariate normal, the posterior density of $\boldsymbol{\theta}$ is given by

$$p(\boldsymbol{\theta} \mid \boldsymbol{Y}) \propto |\boldsymbol{Z}^T\boldsymbol{Z}|^{-N/2} = [d(\boldsymbol{\theta})]^{-N/2} \tag{2.79}$$

when noninformative priors for $\boldsymbol{\theta}$ and $\boldsymbol{\Sigma}$ are used. Under a quadratic approximation of $d(\boldsymbol{\theta})$ around $\hat{\boldsymbol{\theta}}$, the posterior density of $\boldsymbol{\theta}$ can be approximated by a multivariate t-

distribution (Bates and Watts (1984,1987)) of the form

$$p(\boldsymbol{\theta} \mid \boldsymbol{Y}) \propto \left\{1 + \frac{(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}})^T \boldsymbol{\Gamma} (\boldsymbol{\theta} - \hat{\boldsymbol{\theta}})}{2d(\hat{\boldsymbol{\theta}})}\right\}^{-N/2} \tag{2.80}$$

where $\boldsymbol{\Gamma}$ is evaluated at $\hat{\theta}$. Accordingly, an approximate covariance matrix for $\hat{\theta}$ is $2s^2\boldsymbol{\Gamma}^{-1}$ where $s^2 = d(\hat{\theta})/(N\text{-}P)$ (Bates and Watts (1985)). Joint confidence regions or highest-posterior-density (HPD) regions for θ are given by the ellipsoids

$$(\boldsymbol{\theta} - \hat{\boldsymbol{\theta}})^T \boldsymbol{\Gamma} (\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}) \leq P s^2 F_{P,N-P;\alpha} \tag{2.81}$$

where $F_{P,N-P;\alpha}$ is the upper α quantile of the F distribution with P and N-P degrees of freedom. The variance of the p^{th} parameter, θ_p, is given by $2s^2\{\Gamma^{-1}{}_{pp}\}$ which yields an approximate HPD interval of the form

$$\theta_p = \hat{\theta}_p \pm t(N-P;\alpha/2)\sqrt{2s^2\Gamma_{pp}^{-1}}$$

Correlation between the p^{th} and q^{th} parameters, ρ_{pq}, can be estimated from

$$\rho_{pq} = 2s^2 \frac{\{\Gamma_{pq}^{-1}\}}{\sqrt{\{\Gamma_{pp}^{-1}\}\{\Gamma_{qq}^{-1}\}}}$$

Stewart (1987) used a different noninformative prior to obtain the joint posterior distribution

$$p(\boldsymbol{\theta},\boldsymbol{\Sigma}) = p(\boldsymbol{\theta})p(\boldsymbol{\Sigma}) \propto c\,|\boldsymbol{\Sigma}|^{-1} \tag{2.82}$$

which, according to Bayes theorem, gives the marginal posterior distribution

$$p(\boldsymbol{\theta},\boldsymbol{\Sigma} \mid \boldsymbol{Y}) \propto |\boldsymbol{\Sigma}|^{-1-\frac{N}{2}} \exp\left\{-\frac{1}{2}\boldsymbol{\Sigma}^{-1}\boldsymbol{Z}^T\boldsymbol{Z}\right\} \tag{2.83}$$

Integration over the positive definite range of $\boldsymbol{\Sigma}^{-1}$, gives the posterior density of θ

$$p(\boldsymbol{\theta} \mid \boldsymbol{Y}) \propto [d(\boldsymbol{\theta})]^{(N+M-1)/2} \tag{2.84}$$

These results give a different number of degrees of freedom in the resulting multivariate t-distribution. Whereas the approach of Bates and Watts yields N-P degrees of freedom, Stewart's approach gives N-M-P+1 degrees of freedom. This gives, by substituting N-M-P+1 for N-P in eq. 2.81, larger HPD regions than those obtained by Bates and Watts (1985).

In this study, the results of Bates and Watts were used. This is mainly because the difference in the size of the HPD regions induced by the change of the number of degrees of freedom is expected to be small compared to the discrepancies in the estimates of precision induced by the quadratic approximation of $d(\theta)$.

2.6 Software For Multiresponse Parameter Estimation

One of the objectives of this work was to develop software for multiresponse parameter estimation in systems of nonlinear ordinary differential equations. Details pertaining to the solution of differential equations and to the calculation of sensitivity coefficients in ordinary differential equations in multiresponse parameter estimation is discussed in the next chapter.

The procedures discussed above were implemented in a set of FORTRAN programs called MULTI. This code can be used either as a main program, in which case a fixed data file is provided, or as a subroutine. It uses the LINPACK and BLAS (Dongarra *et al.* (1979)) software packages.

The program incorporates the modified Gauss-Newton method and the hybrid method which switches from a Gauss-Newton scheme to an update scheme based on the DGW formula adapted for optimization of the Box-Draper criterion. The line search algorithm is used for both methods. The user is allowed to use either the Gauss-Newton

method, the DGW formula or the hybrid method. Both the Box-Draper criterion and the least squares criterion (the sum of squared residuals) can be used. The package allows estimation in systems of nonlinear differential equations, systems of nonlinear algebraic equations and systems of ordinary differential equations.

The source code for the different FORTRAN programs and a Users' Manual are available from Dr. David D. McLean, Department of Chemical Engineering, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5. A flow chart of the multiresponse parameter software is shown in Figure 2.1.

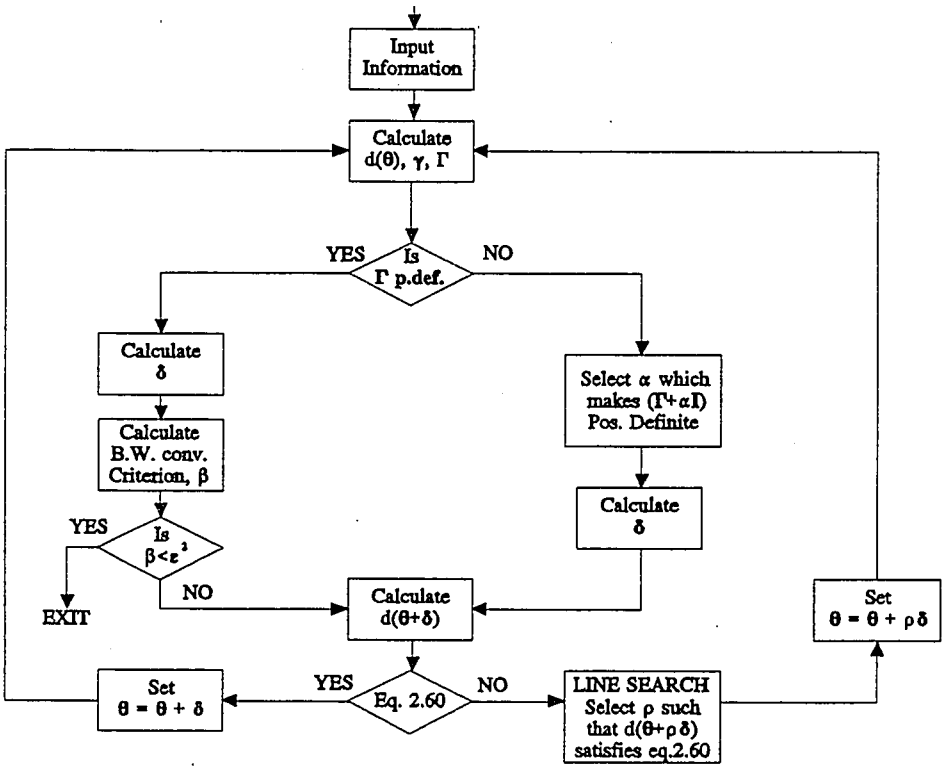

Figure 2.1 - Flow Chart for Multiresponse Parameter Estimation Software.

This flow chart is applicable to either a Gauss-Newton, an update or an hybrid optimization scheme. For the hybrid scheme, the switching procedure is performed prior to the evaluation of Γ. On EXIT, the software prints out the observed and the predicted responses along with the residuals. The maximum likelihood estimate of the covariance matrix for the responses (given by $\hat{\Sigma} = \hat{Z}^T\hat{Z}/(N-P)$, see Section 2.3.2) is listed. From this matrix, correlation between the responses is computed. An eigenvalue-eigenvector decomposition of the covariance matrix is also given. The gradient and Hessian of $d(\theta)$ evaluated at convergence are given. The Hessian matrix is inverted to generate a Bayesian estimate of the covariance matrix for the parameters. The matrix of pairwise correlations between the parameters is then obtained. Individual confidence intervals for the parameters are computed for the estimated covariance matrix.

2.7 Summary

In this chapter, a new procedure for multiresponse parameter estimation was developed. This procedure was based on the optimization of the Box-Draper criterion. The reasons for choosing this criterion were highlighted in this chapter. Three approaches used to develop parameter estimation procedures for multiresponse were presented. From the discussion, it was shown that the Bayesian approach (which led to the development of the Box-Draper criterion) presented advantages over other approaches, particularly the generalized least-squares approach. In particular, application of the Box-Draper criterion is general and does not require knowledge of the covariance matrix for the observed responses.

A review of common approaches for solving nonlinear parameter estimation problems in the single response case was given. It was then shown how these methods could be extended to the optimization of the Box-Draper criterion using expressions of the gradient and Hessian of $d(\theta)$ developed by Reilly (1990). Two optimization methods were developed - a modified Gauss-Newton method and a new hybrid method which incorporates a modified Gauss-Newton method and an update formula based on a

modification of Dennis-Fletcher-Powell update formula (Dennis *et al.* (1981)). For the hybrid method a new switching procedure was developed to allow implementation of the hybrid method along with a line search algorithm.

An efficient line search algorithm was used to ensure that the optimization step taken yields a decrease in objective function from one iteration to the next. A simple procedure was used to correct for non-positive definiteness of the Hessian matrix. The convergence of the procedure was based on the Bates-Watts convergence criterion.

The procedure was implemented in a general purpose FORTRAN program. The program is independent of the form of the model and can be used for systems described by nonlinear ordinary differential equations, nonlinear algebraic equations and simultaneous differential and algebraic equations. One of the important features of this program is the generation of an informative output for multiresponse results including simple residual plots, individual confidence intervals for the parameters, estimates of covariance matrices for the response and the parameters along with the corresponding matrix of pairwise correlations.

In the following chapter, this procedure is applied to multiresponse parameter estimation systems described by ordinary differential equations.

CHAPTER 3

Optimization and Sensitivity Analysis for Multiresponse Parameter Estimation In Systems of Ordinary Differential Equations

3.1 Introduction

Accurate and efficient calculation of parametric sensitivity coefficients is essential for many aspects of parameter estimation in nonlinear models of processes in chemical engineering, especially determination of gradients for optimization and evaluation of parameter precision. In multiresponse parameter estimation, a mathematical model consisting of a system of simultaneous equations is fitted to a set of experimental observations on more than one response in order to estimate the unknown constants or parameters. A number of parameter estimation criteria have been applied to this problem and may benefit from the current work. In this study, the multiresponse estimation criterion of Box and Draper (1965) was of prime interest. The objective was to develop and investigate efficient methods for calculating first and second order sensitivity coefficients and for optimizing the Box-Draper criterion for systems described by sets of simultaneous ordinary differential equations.

As discussed in the previous chapter, a number of approaches can be taken to optimize the Box-Draper criterion. For methods based on a quadratic approximation of the objective function in parameter space, such as the Gauss-Newton method, the gradient and Hessian of the objective function with respect to the parameters must be evaluated. Evaluation of the Hessian matrix is a computationally demanding problem which often restricts the applicability of such a technique. Methods which facilitate evaluation of the Hessian exist; most attempt to approximate the Hessian using only first-order sensitivity coefficients (e.g., Bates and Watts (1985)). Analytically defined second-order sensitivity coefficients are

usually avoided in practice, primarily because of the large increase in error-prone coding. Alternatively, finite difference approximations may be used. Although easy to implement, they remain computationally intensive. Other methods, such as the quasi-Newton (or update) methods, seek to approximate second-order effects by updating the Hessian or part of the Hessian. These methods, while better than Gauss-Newton methods for cases where second-order effects are important, tend to be less efficient in normal situations. Robust hybrid methods (e.g., Biegler *et al.* (1991), Dennis *et al.* (1981) and Section 2.4.2 of this thesis) have also been developed which combine the advantages of both methods.

Where the model consists of a system of ordinary differential equations (ODEs), the evaluation of the gradient and Hessian from sensitivity coefficients becomes computationally demanding. Typically, the solution of the ODEs is used for evaluating the sensitivity coefficients and, ultimately, the gradient and Hessian. The accuracy of the numerical solution thus affects the estimate of these derivatives. A good solution method for the model and sensitivity coefficients is required. The method must also be efficient to be practical for the repetitive use required in parameter estimation. The decoupled direct method (DDM) for the solution of sensitivity coefficients (Dunker (1984)) has many advantages. In this work, the DDM algorithm of Leis and Kramer (1988), called ODESSA, was implemented in a multiresponse parameter estimation routine. Slight improvements to this algorithm were made with respect to error control. The algorithm was also extended to include solution of the second-order sensitivity equations. This allowed efficient, more reliable evaluation of the Hessian in the optimization routine.

Although the method for calculation of the model solution and parametric sensitivities is very important when modelling systems described by ODEs, the single most important factor is the choice of optimization method. Optimization was discussed in general in Chapter 2. In this chapter, the results of using four different approaches for estimation of parameters in models described by ODEs by optimization of the Box-

Draper and/or least squares criteria are presented. Among the methods are a Gauss-Newton method, a full Newton method which includes second-order terms, a hybrid method based on the method of Dennis *et al.* (1981) and a new Newton/Gauss-Newton hybrid method.

In Section 3.2, background concerning multiresponse parameter estimation in systems of ODEs is reviewed. Section 3.3 highlights the numerical solution of systems of ODEs and the calculation of first-order sensitivity coefficients. The numerical solution of systems of ODEs and the effect of error control strategies on the accuracy of the solution are also discussed. In Section 3.4, three problems are solved to demonstrate the effect of the error control strategy of the calculation of first-order sensitivity coefficients in systems of ODEs. In Section 3.5, a new method for the calculation of second-order sensitivity coefficients in systems of ODEs is presented. In Section 3.6, four parameter estimation problems are solved. The problems have been selected from both the literature and from our work in batch culture kinetics. The first example, a simple nonlinear least squares problem, was used to compare our hybrid method with the hybrid methods of Biegler *et al.* (1991) and Dennis *et al.* (1981). The remaining three are multiresponse estimation examples for systems described by sets of ODEs. They highlight common problems encountered both in the use of the Box-Draper criterion and in modelling systems described by ODEs. The important findings are summarized in Section 3.6.

3.2 Multiresponse Parameter Estimation in Systems of ODEs

3.2.1 General Multiresponse Model

Multiresponse parameter estimation consists of fitting a model to a set of M observed responses, y_{nm} (n=1,...,N), (m=1,...,M), measured at N sets of experimental conditions ξ_n. The observed responses are modelled by

$$y_{nm} = f_m(\xi_n,\theta) + \varepsilon_{nm} \tag{3.1}$$

where $f_m(\xi_n,\theta)$ is the expected value of the m^{th} response evaluated at the n^{th} set of experimental conditions, f_m is the m^{th} response function of known form, θ is a P-dimensional vector of unknown parameters to be estimated and ε_{nm} is the random error term involved in observation of y_{nm}.

When the experimental conditions are fixed for a set of N observations, the expected responses depend only on θ. Thus the model can be written in matrix form as

$$\boldsymbol{Y} = \boldsymbol{H}(\theta) + \boldsymbol{Z}(\theta) \tag{3.2}$$

where **Y** is the N by M matrix of observations, $\mathbf{H}(\theta)$ is the matrix of expected responses and $\mathbf{Z}(\theta)$, the matrix of residuals.

3.2.2 Systems Described by Ordinary Differential Equations

For systems described by ODEs, the vector of experimental conditions is written as

$$\xi^T = (x^T,t) \tag{3.3}$$

where **x** is a vector of independent variables and t is an additional independent integration variable, usually time. The M expected responses are given by some known functions $\mathbf{h}_m(s(t),x,\theta)$, m=1,...,M, which are functions of s(t), the K-dimensional vector of state variables, θ and **x**. The general multiresponse model for systems described by systems of ODEs is thus given by

$$y_{nm} = h_m(s(t_n),x_n,\theta) + \varepsilon_{nm} \tag{3.4}$$

The state variables are functions of **x**, θ and t, and are implicitly defined by the system

of ODEs

$$\frac{ds}{dt} = u(t,x,s,\theta) \tag{3.5a}$$

with initial conditions

$$s(0) = s_0(x,\theta) \tag{3.5b}$$

3.2.3 Optimization of the Box-Draper Estimation Criterion for Models described by Simultaneous ODEs

As discussed in Section 2.2, assuming ε_{nm} is normally distributed such that

$$E[\varepsilon_{nm}] = 0 \tag{3.6a}$$

and

$$\begin{aligned} E[\varepsilon_{nm}\varepsilon_{rs}] &= \{\sigma_{ms}\} \quad , \quad n=r \\ &= 0 \quad , \quad n\neq r \end{aligned} \tag{3.6b}$$

(which means that the errors are randomly distributed with mean zero and that observed values of responses from different run conditions (x and/or t) are not correlated but responses for the same run conditions (i.e., both x and t) have a fixed covariance matrix, Σ), Box and Draper (1965) showed that parameters chosen to minimize

$$d(\theta) = |Z^T Z| \tag{3.7}$$

have highest posterior density. Using a Newton-type procedure to optimize $d(\theta)$ requires the evaluation of the gradient, γ, and Hessian, Γ, of $d(\theta)$ with respect to θ. At the $(i+1)^{th}$ iteration, θ is calculated using the formula

$$\boldsymbol{\theta}^{(i+1)} = \boldsymbol{\theta}^{(i)} + \rho^{(i)}[\mathbf{I}^{(i)} + \alpha^{(i)}\boldsymbol{I}]^{-1}\boldsymbol{\gamma}^{(i)} \tag{3.8}$$

where α is a correction term added to the diagonal elements of the Hessian to ensure its positive definiteness, and ρ is a scalar between 0 and 1 chosen to ensure the step taken is acceptable (Dennis and Schnabel (1983)). α can be chosen, as in Bates and Watts (1985), as twice the absolute value of the most negative eigenvalue of the Hessian, or by using either a trust region method (in which case $\rho = 1$), or a modified Cholesky decomposition (Dennis and Schnabel (1983)).

In Chapter 2, expressions for the gradient and Hessian of $\Phi(\theta)$ (=log(d(θ))/2) were derived following the method of Reilly (1990). The gradient was given by

$$\frac{\partial \Phi}{\partial \theta_p} = -\sum_{i=1}^{N} \left\{ \frac{\partial f(\boldsymbol{\theta},t_i)^T}{\partial \theta_p} \boldsymbol{M}^{-1} \boldsymbol{z}_i \right\} \tag{3.9}$$

where $\partial f(\theta,t)/\partial\theta_p$ are first-order sensitivities of the response function. They are obtained by differentiating eq. 3.5 with respect to θ_p and applying the chain rule to give

$$\frac{\partial f(\boldsymbol{\theta},\boldsymbol{x},t)}{\partial \theta_p} = \sum_{k=1}^{K} \frac{\partial h(s(t),\boldsymbol{x},\boldsymbol{\theta})}{\partial s_k} \frac{\partial s_k}{\partial \theta_p} + \frac{\partial h(s(t),\boldsymbol{x},\boldsymbol{\theta})}{\partial \theta_p} \tag{3.10}$$

The derivatives $\partial s_k/\partial\theta_p$ are obtained by solving the sensitivity equations

$$\frac{d}{dt}\frac{\partial \boldsymbol{s}}{\partial \theta_p} = \frac{\partial \boldsymbol{u}}{\partial \boldsymbol{s}}\frac{\partial \boldsymbol{s}}{\partial \theta_p} + \frac{\partial \boldsymbol{u}}{\partial \theta_p} \tag{3.11a}$$

with initial conditions

$$\frac{\partial \boldsymbol{s}(0)}{\partial \theta_p} = \frac{\partial \boldsymbol{s}_0}{\partial \theta_p} . \tag{3.11b}$$

For simplicity let $s_p = \partial s/\partial\theta_p$ and $\mathbf{J} = \{j_{mk}\} = \{\partial u_m/\partial s_k\}$ (m=1,...,K; k=1,...,K).

The Hessian matrix of $\Phi(\theta)$ is obtained by differentiating eq. 3.9 with respect to θ_q (q=1,...,P) to give

$$\frac{\partial^2 \Phi}{\partial \theta_p \partial \theta_q} = -\sum_{i=1}^{N} \left\{ \frac{\partial^2 f^T}{\partial \theta_p \partial \theta_q} M^{-1} z_i - \frac{\partial f^T}{\partial \theta_p} M^{-1} \frac{\partial M}{\partial \theta_q} M^{-1} z_i - \frac{\partial f^T}{\partial \theta_p} M^{-1} \frac{\partial f}{\partial \theta_q} \right\} \quad (3.12)$$

where

$$\frac{\partial^2 f^T}{\partial \theta_p \partial \theta_q} = \sum_{i=1}^{K} \left(\frac{\partial^2 h}{\partial \theta_p \partial s_i} \frac{\partial s_i}{\partial \theta_q} \right) + \frac{\partial^2 h}{\partial \theta_p \partial \theta_q} + \sum_{k=1}^{K} \left\{ \frac{\partial s_k}{\partial \theta_p} \sum_{j=1}^{K} \left(\frac{\partial^2 h}{\partial s_k \partial s_j} \frac{\partial s_j}{\partial \theta_q} \right) + \frac{\partial h}{\partial s_k} \frac{\partial^2 s_k}{\partial \theta_p \partial \theta_q} \right\} \quad (3.13)$$

$\partial^2 s_k / \partial \theta_p \partial \theta_q$ is evaluated by solving the second-order sensitivity equations obtained by differentiating eq. 3.11 with respect to θ_q to give

$$\frac{d}{dt} \frac{\partial s}{\partial \theta_p \partial \theta_q} = \left(\sum_{i=1}^{k} \left(\frac{\partial^2 u}{\partial s \partial s_i} \frac{\partial s_i}{\partial \theta_q} \right) + \frac{\partial^2 u}{\partial s \partial \theta_q} \right) \frac{\partial s}{\partial \theta_p} + \frac{\partial u}{\partial s} \frac{\partial^2 s}{\partial \theta_p \partial \theta_q} + \sum_{i=1}^{K} \frac{\partial^2 u}{\partial \theta_p \partial s_i} \frac{\partial s_i}{\partial \theta_q} + \frac{\partial^2 u}{\partial \theta_p \partial \theta_q} \quad (3.14a)$$

with initial conditions,

$$\frac{\partial^2 s(0)}{\partial \theta_p \partial \theta_q} = \frac{\partial^2 s_0}{\partial \theta_p \partial \theta_q}. \quad (3.14b)$$

Again let $s_{pq} = \partial^2 s / \partial \theta_p \partial \theta_q$.

The gradient and Hessian of $d(\theta)$ are given by

$$\gamma_p(\boldsymbol{\theta}) = \frac{\partial d(\boldsymbol{\theta})}{\partial \theta_p} = 2|M|\frac{\partial \Phi}{\partial \theta_p} \tag{3.15}$$

and

$$\Gamma_{pq}(\boldsymbol{\theta}) = \frac{\partial^2 d(\boldsymbol{\theta})}{\partial \theta_p \partial \theta_q} = 2\left(\gamma_q(\boldsymbol{\theta})\frac{\partial \Phi(\boldsymbol{\theta})}{\partial \theta_p} + |M|\frac{\partial^2 \Phi(\boldsymbol{\theta})}{\partial \theta_p \partial \theta_q}\right) \tag{3.16}$$

The above expressions were used to incorporate systems of ODEs in the procedures for multiresponse parameter estimation parameters described in Chapter 2. The state variables, first-order and second-order coefficients are calculated simultaneously at each iteration using the algorithms described in the following section.

3.3 Calculation of Parametric Sensitivities in Systems of ODEs

3.3.1 Solution of Systems of ODEs

Consider Gear's predictor-corrector method for the solution of eq. 3.5. It is given by

$$s_{n+1}^{(0)} = \sum_{i=0}^{k-1} \bar{\alpha}_i s_{n-i} + h\beta_0 \dot{s}_n \tag{3.17a}$$

$$s_{n+1} = \sum_{i=0}^{k-1} \alpha_i s_{n-i} + h\beta_1 \dot{s}_{n+1} \tag{3.17b}$$

The superscript (0) is used to denote the predicted value of s_{n+1}. $\bar{\alpha}_i$, α_i, β_0 and β_1 result from fitting a k-degree polynomial to s(t).

In most efficient codes for the solution of systems of ODEs, predictor-corrector schemes such as Adams' formulas and Gear's backward difference formulas (BDF) are implemented using Nordsieck vectors to efficiently store the solution history in terms of a Taylor series expansion of each variable s_n at the n^{th} integration step. They take the

general form

$$U_n = \left[s_n \, , \, h\dot{s}_n \, , \, \frac{h^2}{2!}\frac{d^2 s_n}{dt^2} \, , \ldots , \, \frac{h^k}{k!}\frac{d^k s_n}{dt^k} \right]^T \tag{3.18}$$

Nordsieck vectors are advantageous. First, they allow a more efficient predictor step. Second, the simultaneous change of integration step size and order is facilitated. Third, they permit use of more than one algorithm within one implementation of a multistep algorithm (for more details, see Leis and Kramer (1988)).

At each integration step, the solution of eq. 3.5 can be advanced by solving the equation

$$s_{n+1} = s_{n+1}^{(0)} + h l_0 \left(\dot{s}_{n+1} - \dot{s}_{n+1}^{(0)} \right) \tag{3.19}$$

where l_0 is the first element of the k+1 dimensional vector of coefficients which results from the rearrangement of eq. 3.17 in implementing the Nordsieck vectors (Nordsieck (1962)). As described by Leis and Kramer (1988), let $\mathbf{b}_n$ be defined such that

$$\begin{aligned} s_{n+1} &= s_{n+1}^{(0)} + l_0 b_n \\ h\dot{s}_{n+1} &= h\dot{s}_{n+1}^{(0)} + b_n \end{aligned} \tag{3.20}$$

$\mathbf{b}_n$ must then be determined such that, $G_r(\mathbf{b}_n)$ (r= 1,...,K), the resulting residuals of the system, are equal to zero where

$$G_r(b_n) = h u_r(s_{n+1}^{(0)} + l_0 b_n \, , \, t_{n+1}) - h\dot{s}_{r,n+1}^{(0)} - b_{r,n} \tag{3.21}$$

This is usually done through a Newton-Raphson scheme. The value of $\mathbf{b}_n$ so obtained is used to calculate $\mathbf{s}_{n+1}$.

Since the value of $\mathbf{b}_n$ is proportional to the local truncation error (Gear, (1971)), its magnitude can be compared with some user-defined tolerance in order to control the error involved with the numerical solution as it proceeds. It is used to evaluate the

vector of local errors e_0. The error test, which involves comparison of the magnitude of the error with the user-defined tolerance, is generally performed by taking a norm, usually Euclidean, of the error vector. In a number of efficient codes, the integration step size and order are selected in order to satisfy the inequality

$$\left\| \frac{e_0}{w_0} \right\| \leq 1.0 \qquad (3.22)$$

where e_0 is the K-dimensional local error vector and w_0 is a K-dimensional vector of error weights. The weights represent relative and/or absolute error tolerances.

A very popular implementation of this procedure is the LSODE program (Hindmarsch (1980)). LSODE uses variable order , variable step-size, predictor-corrector formulae for the integration of initial-value stiff and nonstiff systems of ODEs. It uses Nordsieck vectors and implements Gear's BDF and Adams-Moulton formulae.

3.3.2 Decoupled Direct Method(DDM) for Calculating Sensitivities

In the decoupled direct method (Dunker (1984)), a predictor-corrector method using Gear's backward difference formulae or Adams formulae is used to solve eq. 3.5. Information generated by the solution is then used in the solution of eq. 3.11. In DDM, the solution of eq. 3.11 is decoupled from that of eq. 3.5 but is conducted simultaneously. This means that each step taken in solving eq. 3.5 is followed by the solution of eq. 3.11 over the same step (Leis and Kramer (1988)).

The approach is simple. For example, time derivatives for the sensitivity coefficients can be approximated by a one-sided difference formula between $s_{p,n+1}$ and $s_{p,n}$ over the time interval h. The approximation can be substituted into eq. 3.11 to yield an explicit formula for $s_{p,n+1}$ of the form

$$s_{p,n+1} = \left[I - hJ_{n+1}\right]s_{p,n} + h\frac{\partial u_{n+1}}{\partial \theta_p} \qquad (3.23)$$

In the simultaneous solution of the model and the sensitivity equations, the model solution, s_{n+1}, is used to calculate J_{n+1}, $\partial u/\partial\theta_p$ and $s_{p,n+1}$ at each step. To improve the approximation, a k-step linear difference equation can be used. This method was first applied to the problem of stiff ODEs by Dunker (1984).

Recently, a very efficient algorithm based on the ODE solving routine LSODE, called ODESSA, was developed by Leis and Kramer (1988). ODESSA incorporates Gear's BDF and Adams' formulae for initial value problems using the Nordsieck arrays. At each step, the solution of the state equations is handled first. As given above, $\mathbf{b}_n$ is determined using a modified Newton scheme on the first and second rows of the Nordsieck vectors. $\mathbf{b}_n$ is used to evaluate the local truncation error for the model solution, $\mathbf{e}_0$. The error must be such that the inequality in eq. 3.22 is observed. If it is not, then the step is repeated with, for example, a smaller step size. A successful error test initializes the solution of the sensitivity equations.

For the solution of eq. 3.11, use of eqs. 3.19, 3.20 and 3.11 in terms of sensitivities gives an explicit formula for the sensitivities

$$s_{p,n}=\left[I-hl_0J_n\right]^{-1}\left(s_{p,n}^{(0)}-hl_0s_{p,n}^{(0)}+hl_0\partial u_n/\partial\theta_p\right) \qquad (3.24)$$

where J_n is evaluated at the current solution conditions, t_n, s_n. As discussed by Leis and Kramer (1988), J_n should be evaluated at the predicted value, $s_n^{(0)}$, at t_n. Considerable computational gains are thus realized by reducing the number of Jacobian evaluations.

The error control strategy for the solution of eq. 3.11 is similar to that for the solution of eq. 3.5. The local truncation error for the sensitivities, $\mathbf{e}_p$ (p=1,...,P), is assumed to be proportional to the difference between the predicted value, $s_p^{(0)}$,and the corrected value, s_p, (p=1,...,P) of the sensitivities. The errors are subject to the

following P inequalities

$$\frac{1}{K}\sum_{i-1}^{K}\left(\frac{e_{p,i}}{w_{p,i}}\right)^2 \leq 1.0 \qquad (p=1,...,P) \tag{3.25}$$

In both cases, eq. 3.22 and eq. 3.25, failure of the error test causes the step to be repeated starting with a new step size and, possibly, a new integration order.

In the investigation of the DDM, it was found that an alternative error control strategy significantly improves its performance. This strategy, developed by Shampine (1988) for one-step ODE codes, has been shown to improve the dependency of the local error of the solution on the requested tolerance. It is discussed in the following subsection.

Failure of the error test initiates a change in step size and order as described for the model solution. The step is then repeated starting with prediction of the model solution. If the step is repeated more then three times, $\mathbf{J}_n$ and $\partial \mathbf{u}_n/\partial \theta_p$ are re-evaluated directly from the analytical formulae. If the error test is successful, the Nordsieck arrays are updated and the step is repeated. This can be preceded by selection of a new integration step size and order in a manner similar to LSODE using the following equations

$$\alpha = \frac{1}{1.2}\left(\frac{1}{\max_j \|e_j/w_j\|}\right)^{1/(k+1)}, \quad j=0,...,P \quad (\textit{order } k)$$

$$\alpha = \frac{1}{1.3}\left(\frac{1}{\max_j \|e_j/w_j\|}\right)^{1/k}, \quad j=0,...,P \quad (\textit{order } k-1) \tag{3.26}$$

$$\alpha = \frac{1}{1.4}\left(\frac{1}{\max_j \|e_j/w_j\|}\right)^{1/(k+2)}, \quad j=0,...,P \quad (\textit{order } k+1)$$

where α is a factor that multiplies the current step size to generate the new step size for the respective integration orders, and w_i, the error weights. The maximum value of α is used as the criterion for integration order and step size. e_j is the estimate of local error for the j^{th} (j = 1,...,P) K-dimensional sensitivity vectors and w_j is the corresponding error weight.

A schematic algorithm of the procedure discussed above is shown in Figure 3.1. This algorithm is essentially the same as the algorithm of Leis and Kramer (1988). It was implemented in a FORTRAN code as a subroutine called DDM. A documented and fully commented version of DDM is available from Dr. David D. McLean, Department of Chemical Engineering, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5.

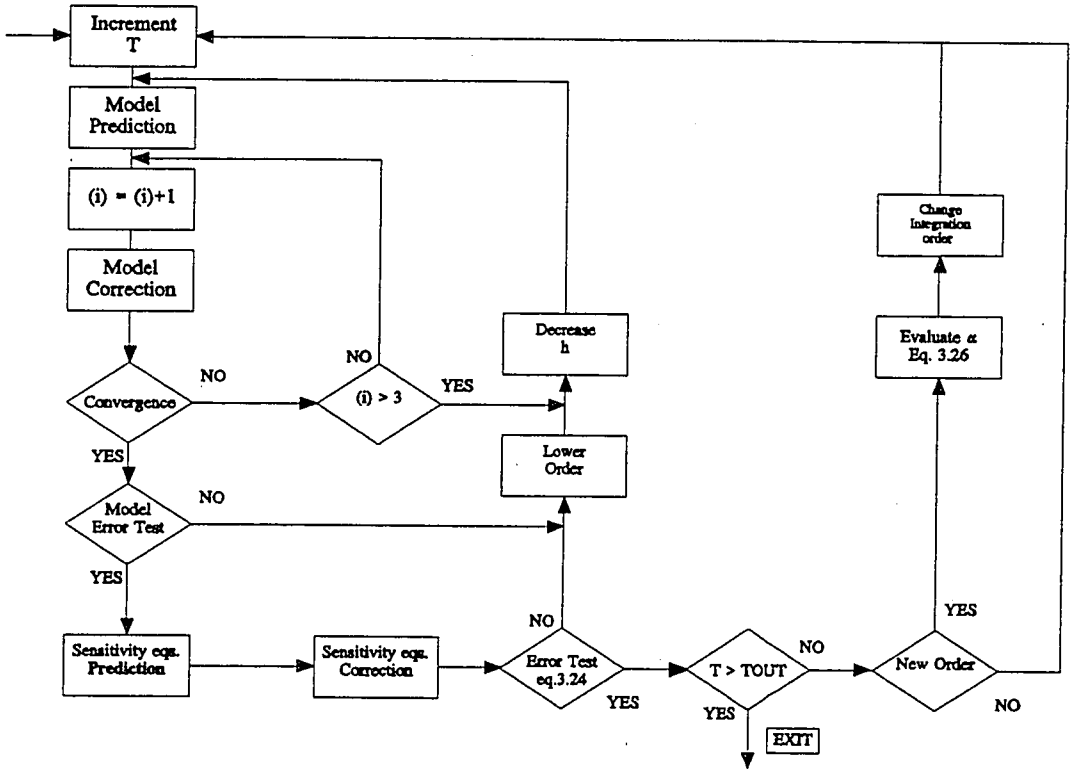

Figure 3.1 - Calculation sequence in the DDM program.

3.3.3 Error Control Strategy

Solving an initial-value problem always requires some error control strategy. All methods incorporate some approximation of the local error of the solution at each integration step. It is important that this error be kept within some bound. The estimate of the local error is compared to a tolerance level defined by the user. The error estimate is also used to determine the step size for the following step.

Let the local error at the n^{th} step be defined by

$$e_n = s_n - s_n^{(0)} \tag{3.27}$$

that is, the difference between the corrected and predicted values of the dependent variables. Two criteria are commonly used to control the error: the criterion of error per unit step (EPUS) and the criterion of error per step (EPS). Let $\|\cdot\|$ define a norm of a vector and τ a tolerance defined by the user. The EPS criterion accepts the results if

$$\|e_n\| \leq \tau \tag{3.28}$$

The EPUS criterion accepts the step if

$$\|e_n\| \leq h_n\tau \tag{3.29}$$

Using the notation of Shampine (1988), the step size must be selected such that

$$\|e_n\| = h_n^{k+1}\|\phi_n\| + 0(h_n^{k+2}) \tag{3.30}$$

where $\|\phi_n\|$ is the principal error function evaluated at t_n and y_n given by

$$\phi_n = \frac{1}{(k+1)!}\frac{d^{k+1}s_n}{dt^{k+1}}$$

For EPS, if

$$\beta\tau \approx \|e_n\| \tag{3.31}$$

where β is a constant, then a method of order k leads to,

$$h \approx \left(\frac{\beta\tau}{\|\phi\|}\right)^{\frac{1}{k+1}} \tag{3.32}$$

Similarly, for EPUS, if

$$\beta h_n \tau \approx \|e_n\| \tag{3.32}$$

then

$$h \approx \left(\frac{\beta\tau}{\|\phi\|}\right)^{\frac{1}{k}} \tag{3.33}$$

It is obvious that, at low integration order and since $(\beta\tau/\|\phi_n\|)<1$, EPUS gives much smaller steps which results in a considerable decrease in efficiency compared to EPS. This is one of the main reasons why EPS is preferred in popular codes. However, it is generally recognized that, when EPUS is used, the error of the solution is proportional to the user defined tolerance. EPS does not display such an ideal behaviour. Therefore, if a code is converted from EPS to EPUS, the computational effort required to solve a system at some tolerance level, τ, is expected to increase. Unfortunately, this is the popular way of comparing efficiency of codes used to solve ODEs. The tolerance is varied and against it is plotted some measure of the computing effort (e.g., computing time, number of function evaluations, etc.). Little concern is given to the accuracy of the solution. It would be more informative to look at changes in computing effort with changes in the error of the numerical solution. In general, EPUS tends to produce more

accurate results than EPS because of the smaller step sizes. Considering the error of the solution rather then the requested tolerance is known to generate a measure of efficiency for which EPS and EPUS perform equally well.

In practice it is difficult to be convinced of this. Suppose one is interested in the solution of a system of ODEs. For this purpose, some commercial ODE solver routine is used. A tolerance level is selected, the system is solved and the results are printed to 4 or 5 significant digits or used in a plotting routine. For the user there is no evidence that the EPUS will help his cause. Switching to EPUS only results in an increase in computing time with no noticeable change in the solution. This is a valid argument when solution accuracy is not critical. Improvements with respect to accuracy are more important when the ODE solution is used in the course of a more complex analysis. If, for example, the solution is used in an optimization routine where both the model solution and sensitivity coefficients are needed, the accuracy of the numerical solution can influence the performance of the optimization routine, even if the solution is improved by only a few decimal places. In this study, it was found to be especially important when sensitivities are used to provide gradient information for the optimization criterion. Here, an increase in accuracy of the numerical solution and gradient can result in improvements in the performance of the optimization routine. In such situations, it is equally important that the solution be both fast (because the solution is required many times in one analysis) and accurate. It would therefore be advantageous to combine the merits of EPUS and EPS. As discussed by Shampine (1988), a way to do that is to define an internal tolerance μ which is different from the one specified by the user. He defined the internal tolerance to be

$$\mu = \tau^{(k+1)/k} \tag{3.35}$$

The step size is chosen such that

$$\beta\mu \approx h^{k+1}\|\phi\| + 0(h^{k+2}) \tag{3.36}$$

which leads to

$$h \approx \left(\frac{\beta\tau}{\|\phi\|}\right)^{\frac{1}{k+1}} = \tau^{\frac{1}{k}}\left(\frac{\beta}{\|\phi\|}\right)^{\frac{1}{k+1}} \tag{3.37}$$

This error control strategy is termed pseudo error per unit step (PEPUS) since it is similar to EPUS in the way it selects the step size (Shampine (1988)). Because of the (1/k) exponent of τ, this error control strategy improves the proportionality of the error with the tolerance. The (1/k+1) exponent of the second term produces, at low integration order, smaller step sizes, as is the case with EPUS. Greater computing times result.

When converting a code from EPS to PEPUS, the primary objective is to increase the accuracy of the results, or more precisely to improve the dependence of the local error estimate on the user defined tolerance, without causing a great loss of computational efficiency. This is not expected to always be the case for codes based on a backward difference formula (such as LSODE). Because these codes often have variable step sizes and orders, the error does not depend solely on the tolerance, especially for memory codes. Thus irregularities in the relationship between the local error and the tolerance level often observed when using EPS cannot be totally removed by simply changing the error control strategy. In this study, the use of the PEPUS criterion in DDM was investigated. This was primarily an attempt to remove irregularities seen for the solution of the model and sensitivities of difficult problems and to improve the accuracy of the results for the sensitivity solution at varying tolerance levels.

To implement PEPUS, the original code was modified to incorporate the calculation of the internal tolerance whenever a change in integration order was

performed in the original code. For the current implementation of the DDM, the error control test for the model solution and for the sensitivity coefficients was modified to substitute the error weights for the internal tolerance. This yielded the following error tests for the model

$$\left\| \frac{e_0}{\mu_0} \right\| \leq 1.0 \tag{3.25a}$$

and the sensitivity coefficients

$$\frac{1}{N}\sum_{i=1}^{N}\left(\frac{e_{p,i}}{\mu_{p,i}}\right)^2 \leq 1.0 \qquad (p=1,...,P) \tag{3.25b}$$

The choice of integration order and step size was performed as in eq. 3.26 except that the internal tolerance was used where, previously, the error weights were used. This gives

$$\begin{aligned}
\alpha &= \frac{1}{1.2}\left(\frac{1}{\max_j \|e_j/\mu_j\|}\right)^{1/(k+1)} , \quad j=0,...,P \quad (\textit{order } k) \\
\alpha &= \frac{1}{1.3}\left(\frac{1}{\max_j \|e_j/\mu_j\|}\right)^{1/k} , \quad j=0,...,P \quad (\textit{order } k-1) \\
\alpha &= \frac{1}{1.4}\left(\frac{1}{\max_j \|e_j/\mu_j\|}\right)^{1/(k+2)} , \quad j=0,...,P \quad (\textit{order } k+1)
\end{aligned} \tag{3.26}$$

where α is a factor that multiplies the current step size to generate the new step size for the respective integration orders, and $\mu_i = w_i^{(k+1)/k}$, the new error weights based on the internal tolerance.

3.3.4 Effect of Error Control Strategy on the Calculation of First-order Sensitivity Coefficients

To test the effect of the error control strategy on the DDM subroutine, a variety of widely reported examples in the sensitivity analysis literature were solved. One such

example involves an ethane pyrolysis model (Leis and Kramer (1988)). The model is given by,

$$\frac{dy_1}{dt} = -\theta_1 y_1 - \theta_2 y_2 y_1 - \theta_4 y_6 y_1$$

$$\frac{dy_2}{dt} = 2\theta_1 y_1 - \theta_2 y_2 y_1$$

$$\frac{dy_3}{dt} = \theta_2 y_2 y_1$$

$$\frac{dy_4}{dt} = \theta_2 y_2 y_1 - \theta_3 y_4 + \theta_4 y_6 y_1 \qquad (3.27)$$

$$\frac{dy_5}{dt} = \theta_3 y_4$$

$$\frac{dy_6}{dt} = \theta_3 y_4 - \theta_4 y_1 y_6 - 2\theta_5 y_6^2$$

$$\frac{dy_7}{dt} = \theta_4 y_1 y_6 + \theta_5 y_6^2$$

The model and the full set of sensitivity coefficients for the model parameters were successfully solved between t=0 and t=20 seconds (Leis and Kramer (1988)) with relative tolerances (RTOL) ranging from 10^{-4} to 10^{-10} and with absolute tolerances (ATOL) taken such that ATOL=0.01(RTOL). Initial conditions were 5.951×10^{-6} mol cm^{-3} for y_1 and 0.0 for all other variables. Parameter values were 0.1, 1.50, 1.5×10^3, 9.72×10^8 and 6.99×10^{13} for θ_1, θ_2, θ_3, θ_4 and θ_5, respectively. Numerical results were similar to those presented by Dunker (1984) for sensitivities with respect to the first model parameter. The numerical solutions for the other sensitivities were not reported by Dunker, nor apparently by other authors. This seemed to be a significant oversight considering that those sensitivities were several orders of magnitude smaller than those for the sensitivities with respect to the first model parameter. This meant that using the same tolerances for all the sensitivities would probably not be adequate to ensure a reliable solution. In order to check the consistency of the solution for the sensitivities,

an estimate of the local error was calculated at t=20 min at varying tolerances. A relative local error estimate was calculated as

$$E(s_j) = \frac{1}{K}\sum_{i=1}^{K} \frac{|e_{i,j}|}{|s_{i,j}|} \quad ; j=0,...,P \tag{3.28}$$

The choice of this type of norm was arbitrary. A Euclidian norm was also used but yielded no noticeable change in the results. Table 3.1 shows variation of the local errors for the model and for sensitivity coefficients with respect θ_1 and θ_5 with tolerance. It also shows the number of integration steps (NST), the number of repeated steps due to sensitivity error test failure (NSER), as well as a measure of the computing time in CPU secs (CPU) on an AMDAHL computer.

Table 3.1 - Solution of the full set of sensitivities for ethane pyrolysis at t=20 min with EPS. Number of integration steps (NST), the number of sensitivity error test failures (NSER), the CPU time in seconds, the average relative local error for s_0, s_1 and s_5 are given at varying relative tolerance (RTOL) levels.

RTOL	NST	NSER	CPU	$E(s_0)$	$E(s_1)$	$E(s_5)$
10^{-3}	20	3	0.067	0.319	0.471	0.717
10^{-4}	17	4	0.227	0.139	0.0543	7.941
10^{-5}	29	3	0.100	1.88(-4)	1.11(-3)	6.315
10^{-6}	40	0	0.147	4.96(-5)	3.33(-4)	0.183
10^{-7}	57	1	0.360	3.98(-6)	3.95(-5)	0.104
10^{-8}	76	1	0.400	5.79(-7)	4.72(-6)	0.275
10^{-9}	118	3	0.563	7.33(-8)	6.19(-7)	0.236
10^{-10}	149	1	0.740	9.05(-9)	6.59(-8)	0.273
10^{-11}	214	0	0.913	4.66(-10)	4.81(-9)	0.0136
10^{-12}	270	1	1.217	8.19(-11)	6.20(-10)	0.313

The number of steps, number of sensitivity error test failures and the computing times were similar to those reported by Leis and Kramer. The relative local errors for the model and sensitivities with respect to the first model parameter were found to decrease smoothly with the tolerance. Such was not the case for the set of sensitivities with respect to the fifth parameter. The relationship between RTOL and $E(s_5)$ was very irregular and displayed two minima. The relative local errors obtained were very large. The principal cause of this was thought to be due to extremely low values (beyond round-off error limitations) of some sensitivities. But it was still believed that the PEPUS could help to improve the magnitudes of the local errors and the relationship between the tolerance and the error. Therefore, in an attempt to improve the behaviour of the routine, the same analysis was conducted using PEPUS. Table 3.2 summarizes the results.

Table 3.2 - Solution of the full set of sensitivities for the ethane pyrolysis model at t= 20 minutes with PEPUS.

RTOL	NST	NSER	CPU	$E(s_0)$	$E(s_1)$	$E(s_5)$
10^{-3}	39	0	0.237	9.06(-3)	7.61(-2)	5.94
10^{-4}	34	0	0.280	1.02(-3)	7.48(-3)	3.08
10^{-5}	66	0	0.360	1.27(-4)	1.23(-3)	2.34
10^{-6}	78	0	0.393	5.04(-6)	4.06(-5)	5.84(-2)
10^{-7}	100	0	0.497	1.74(-6)	1.32(-5)	0.597
10^{-8}	123	0	0.633	2.12(-7)	2.93(-6)	2.65
10^{-9}	156	0	0.797	1.36(-8)	1.06(-7)	2.55
10^{-10}	217	0	1.130	1.10(-9)	8.88(-9)	0.195
10^{-11}	308	0	1.600	3.96(-11)	4.26(-10)	0.247
10^{-12}	442	0	2.187	2.47(-12)	2.66(-11)	9.58(-4)

As expected, the implementation of PEPUS increased the computing time required. The primary cause is the internal tolerance in PEPUS, μ, which is much more stringent then τ, the user-defined tolerance. The behaviour of the local error with changes in tolerance was improved. PEPUS produced lower relative local errors at most tolerance levels. This was especially true for the solution of s_0 and s_1. The behaviour of $E(s_5)$ was still very irregular. PEPUS showed no drastic improvement for this case. This gave support to the speculation that the observed erratic behaviour was due to the rounding error.

Surprisingly, the results also showed that using PEPUS had a definite effect on the performance of DDM. At all tolerance levels, the use of PEPUS caused the integration to progress without sensitivity error test failures. The cause of this was not fully understood but it seemed that PEPUS also improved the approximation of the sensitivities from step to step.

In order to verify this observation, the effects of PEPUS were investigated with two other models. The first was a simple kinetic model of the isomerization of α-pinene (Fuguitt and Hawkins (1947)). It contains 5 state variables and 5 model parameters and is given by

$$\begin{aligned}
\frac{dy_1}{dt} &= -(\theta_1 + \theta_2)y_1 \\
\frac{dy_2}{dt} &= \theta_1 y_1 \\
\frac{dy_3}{dt} &= \theta_2 y_1 - (\theta_1 + \theta_2)y_3 + \theta_5 y_5 \qquad (3.29) \\
\frac{dy_4}{dt} &= \theta_3 y_3 \\
\frac{dy_5}{dt} &= \theta_4 y_3 - \theta_5 y_5
\end{aligned}$$

where y_1, y_2, y_3, y_4 and y_5 are the molar percentages of α-pinene, dipentene, allocimene, α-pyronene and β-puronene, respectively. θ_i (i=1,...,5) are the rate constants. Initial conditions were $y_1(0)=100.0$, $y_2(0)=y_3(0)=y_4(0)=y_5(0)=0.0$. Values of the rate constants were $5.947\text{x}10^{-5}$, $2.838\text{x}10^{-5}$, $4.506\text{x}10^{-6}$, $3.128\text{x}10^{-4}$ and $5.770\text{x}10^{-5}$ min^{-1} for θ_i (i=1,...,5) respectively. This system was solved from t=0.0 to t=36420.0 minutes.

The second was a model for batch culture of an hybridoma cell line for the production of monoclonal antibodies (the "MAB model"). It contains 4 state variables and 7 model parameters and is given by

$$\begin{aligned}
\frac{dX}{dt} &= \frac{\mu_{max} XS}{K_S + S} - k_d XA \\
\frac{dS}{dt} &= -\frac{a_s \mu_{max} XS}{K_S + S} \\
\frac{dA}{dt} &= \frac{a_A a_S \mu_{max} XS}{K_S + S} + k_d a_{Ad} XA \\
\frac{dMAB}{dt} &= \frac{\alpha \mu_{max} XS}{K_S + S}
\end{aligned} \tag{3.30}$$

where X, S, A and MAB are the concentrations of viable biomass, glutamate, ammonia and monoclonal antibody. The parameter estimates for μ_{max}, K_S, k_d, a_S, a_A, a_{Ad} and α were 0.18944, 5.5069, $3.1822\text{x}10^{-3}$, 0.26991, 1.2329, -0.21788 and 0.030526 respectively. Initial conditions were those reported experimentally which were 0.902, 4.099, 0.755 and 0.0 for X, S, A and MAB, respectively. The system was solved from t=0.0 to t=90.0 hours. Tables 3.3 and 3.4 list the results for both models respectively. The number of integration steps, the number of sensitivity error test failures, the computing time and estimates of the relative local error for the state and sensitivity solutions are given at varying tolerances for both EPS and PEPUS. The sensitivity local error estimate is an average taken over all NxP sensitivities.

Table 3.3 - Solution of the full set of sensitivities for the α-pinene model at t = 36420 min with both EPS and PEPUS. NST, NSER, CPU, the average relative local errors for the model and for the full set of sensitivities are given at different values of RTOL.

EPS					
RTOL	NST	NSER	CPU	$E(s_0)$	E(S)
10^{-4}	57	3	0.260	1.96(-6)	1.06(-4)
10^{-6}	92	5	0.350	1.05(-7)	5.76(-6)
10^{-8}	138	9	0.540	7.25(-10)	6.80(-8)
10^{-10}	234	17	0.940	1.01(-12)	1.36(-9)
10^{-12}	320	23	1.347	1.56(-14)	5.64(-12)
PEPUS					
10^{-4}	58	0	0.267	2.03(-6)	1.59(-6)
10^{-6}	84	0	0.383	5.05(-9)	1.02(-8)
10^{-8}	126	2	0.557	3.35(-11)	2.11(-11)
10^{-10}	175	3	0.783	2.50(-12)	2.94(-12)
10^{-12}	580	8	2.133	9.36(-16)	2.63(-15)

Table 3.4 - Solution of the full set of sensitivities for the MAB model at t = 90 hours with both EPS and PEPUS. NST, NSER, CPU, the average relative local errors for the model and for the full set of sensitivities are given at different values of RTOL.

EPS					
RTOL	NST	NSER	CPU	$E(s_0)$	E(S)
10^{-4}	67	10	0.197	8.16(-6)	7.47(-4)
10^{-6}	136	19	0.447	9.23(-7)	1.23(-5)
10^{-8}	161	17	0.613	9.98(-10)	3.26(-7)
10^{-10}	245	24	0.937	1.20(-11)	1.59(-9)
10^{-12}	409	46	1.670	1.72(-14)	1.52(-12)
PEPUS					
10^{-4}	67	4	0.207	1.85(-5)	2.34(-6)
10^{-6}	90	3	0.310	2.12(-7)	2.96(-8)
10^{-8}	137	4	0.553	1.23(-10)	1.16(-9)
10^{-10}	176	4	0.760	9.96(-13)	1.76(-12)
10^{-12}	272	7	1.300	8.84(-15)	1.88(-14)

The behaviour of the local error with tolerance was smooth for EPS and PEPUS in both cases. As expected, local error estimates for the model and sensitivities solutions were, in general, smaller for PEPUS, but, contrary to the ethane pyrolysis model, there were no large increases in computing time. In both cases, the use of PEPUS was even shown to improve the performance of DDM. Both NSER and NST were decreased in using PEPUS.

Again, this behaviour is not fully understood. It may be due to the increase in the accuracy of the model solution. This could have an affect on the accuracy of the approximation of the sensitivity coefficients by eq. 3.24. Another possible effect is the modification of the integration order sequence of the integration. If, for example,

occurrences of integration order changes are lessened, the performance of the program is bound to be improved.

The increase in accuracy is very valuable especially with respect to the problem of multiresponse parameter estimation in systems of ODEs. An increase in the accuracy of both the model and sensitivity equations may contribute significantly to increase the efficiency and reliability of the optimization routine. Since sensitivity coefficients are used in the evaluation of the gradient and Hessian of the estimation criterion, the rate of convergence of the procedure can be improved. As discussed in Section 3.3, the improvements in accuracy are not as drastically felt when the solver is used simply to solve the system in order to plot the results. In that case, DDM with EPS would perform just as well.

Another important practical implication of the effect PEPUS is the reduction of the number of sensitivity error test failures. This would be particularly important if this type of algorithm is considered for the calculation of second-order sensitivities. In that case, if the use of PEPUS improves the approximation of the sensitivities, there is a chance of obtaining a very good approximation of the second-order derivatives. This is potentially attractive for nonlinear optimization problems where second-order terms are often neglected because of difficulties in evaluating them efficiently and reliably. The calculation of second-order sensitivity coefficients is discussed in the next section.

3.3.5 Calculation of Second-order Sensitivity Coefficients using DDM

An extension of the DDM method can be used to calculate second-order sensitivities very accurately. The formula is obtained by differentiating the explicit formula for $\partial s/\partial\theta_p$ with respect to θ_q yielding

$$\left[I - hl_0 J_n\right]\left(\frac{\partial^2 s_n}{\partial\theta_p\partial\theta_q}\right) \approx \frac{\partial^2 s_n^{(0)}}{\partial\theta_p\partial\theta_q} - hl_0\frac{d}{dt}\left(\frac{\partial^2 s_n^{(0)}}{\partial\theta_p\partial\theta_q}\right)$$
$$+ hl_0\left[\sum_{i=1}^{K}\frac{\partial^2 u_n}{\partial s\partial s_i}\frac{\partial s_{in}}{\partial\theta_q} + \frac{\partial^2 u_n}{\partial\theta_p\partial\theta_q}\right]\frac{\partial s_n}{\partial\theta_p} \qquad (3.31)$$
$$+ hl_0\sum_{j=1}^{K}\left(\frac{\partial^2 u_n}{\partial\theta_p\partial s_j}\frac{\partial s_{jn}}{\partial\theta_q}\right) + hl_0\frac{\partial^2 u_n}{\partial\theta_p\partial\theta_q}$$

This extension allows the simultaneous solution of the model and of the first and second-order sensitivity equations.

Solution of the second-order sensitivity equations is performed similarly to the solution of eq. 3.11 in section 3.2. The calculation of the second-order sensitivities is initiated after a successful error test of the first-order sensitivities. Prediction, correction and error tests are performed sequentially. By analogy to the K inequalities of the error test for the solution of eq. 3.11, the error test for the solution of the second-order sensitivity equations consists of K inequalities where the 2-norm of each P by P matrix (i.e., the spectral radius of each matrix) of local errors is compared to a weighted measure of tolerance. The test takes the form

$$\rho(E_i) \le 1.0 \qquad (i=1,...,K) \qquad (3.32)$$

where

$$E_i = \left\{\frac{e_{pq,i}}{\mu_{pq,i}}\right\} \qquad (i=1,...,K). \qquad (3.33)$$

The change of integration order is performed in a manner similar to eq. 3.24. The test is simply augmented by the K measures of weighted errors of eq. 3.25. In this study, it was found that change of integration order or step size is primarily due to errors in the calculation of first-order sensitivity coefficients. The calculation of second-order derivatives involves a significant increase in computing effort and storage. However, it

should be noted that the resulting KP by P matrices are symmetric and that the solution can thus be restricted to K upper triangular matrices. This reduces the computational effort and the storage requirements.

3.4 Optimization Strategies for Multiresponse Parameter Estimation in Systems of ODEs

The behaviour of four methods for multiresponse parameter estimation in systems described by sets of simultaneous ODEs were investigated.

The first method was a modification of the generalized Gauss-Newton method of Bates and Watts (1985), called the GN method. The second was a hybrid method based on the DGW update formula discussed in section 2.4, called the HYB method. Both methods are based on first-order approximations of the Hessian matrix. The third method was a full modified Newton method which utilizes second-order sensitivity coefficients in the evaluation of the Hessian, called the N method. The fourth method was a new hybrid method which allows switching between the Newton method and the Gauss-Newton method called the N/GN method. The switching rule for this method is simple and depends solely on the magnitude of the Bates-Watts convergence criterion. Since this criterion tends to decrease in the proximity of a local minimum, it can be used to indicate that the optimization routine is near convergence. Under such conditions, the Gauss-Newton method generally performs very well and second-order terms are not needed in the evaluation of the Hessian matrix. In this study, the hybrid method switched from the modified Newton method to the generalized GN method when the Bates-Watts convergence criterion became less than or equal to 0.05.

Three estimation problems were used to test the behaviour of these four methods. A fourth problem was used to compare the hybrid (HYB) with those of Biegler *et al.* (1991) and Dennis *et al.* (1981). For each of these problems, the CPU time required to perform the estimation on an Amdahl virtual machine, the number of iterations (NI) and

the number of objective function evaluations (NOF) were recorded to evaluate and compare the performance of the four methods. For two of the examples, different sets of initial estimates were used. In the following sections, a description of each example and the results obtained are given. For all cases, the numerical solution of the ODEs was obtained at a requested relative tolerance of 10^{-6}. The Bates-Watts convergence criterion was required to be less than or equal to 10^{-6}.

3.4.1 Example 1

This example was taken from Jennrich (1968). It has been used as a test example for a number of nonlinear parameter estimation routines. The example consists of fitting an exponential model of the form

$$f(t,\theta) = e^{\theta_1 t} + e^{\theta_2 t} \tag{3.34}$$

to a straight line, y = 2t + 2, for t= 1 ,2, 3, ... ,10. The example was used to test the HYB method and to compare its performance with the hybrid methods of Dennis *et al.* (1981) and Biegler *et al.* (1991).

Using the HYB method, the example was solved and converged within 9 iterations, using 18 objective function evaluations and 0.1 CPU seconds. The converged parameter estimates, θ_1 and θ_2, were 0.258 and 0.258 respectively. The value of the least squares criterion was 124.36 at convergence. This is comparable to the results of Dennis *et al.* (1981) who reported between 11 and 14 iterations and 11 to 16 objective function evaluations to solve this problem for four variations of their method. It is also comparable with the hybrid method of Biegler *et al* (1991) who reported 18 iterations and 18 objective function evaluations to solve it. As observed in both these articles, the GN method failed to converge. The similarity in the results from the various hybrid methods provides a basis for comparing such methods in general to others in Examples 2 -4.

3.4.2 Example 2

To demonstrate the possible gains that can be made by including second-order sensitivities for evaluating the gradient and Hessian of d(θ) in multiresponse parameter estimation a frequently used example involving estimation of two rate constants for the first-order sequential reaction

$$A \xrightarrow{\theta_1} B \xrightarrow{\theta_2} C$$

was employed. The data and problem statement have been given by Box and Draper (1965). The model is given by

$$\frac{dc_A}{dt} = -\theta_1 c_A$$

$$\frac{dc_B}{dt} = \theta_1 c_A - \theta_2 c_B \qquad (3.35)$$

$$\frac{dc_C}{dt} = \theta_2 c_B$$

with initial conditions $c_A=1$ and $c_B=c_C=0$, where c_A, c_B and c_C are the molar concentrations of A, B and C respectively.

As in most iterative schemes, the outcome and the rate of convergence of the estimation algorithm are highly dependent on the initial guesses made. In the present case, the performance of the four multiresponse estimation methods, expressed in terms of the number of iterations, number of objective function evaluations and CPU time was tested using different sets of initial estimates. The choice of each set of initial estimates was arbitrary. Tables 3.5, 3.6, 3.7 and 3.8 give the results obtained for the GN, HYB, N and N/GN methods respectively.

TABLE 3.5

Estimation Efficiency Using the GN Method for Example 2

INITIAL ESTIMATES		NI(NOF)	CPU secs	CONVERGED ESTIMATES	
0.04	0.06	141(288)	101.7	0.0868	9744.0
0.4	0.6	8(16)	0.9	0.208	0.495
2.0	3.0	Convergence Failed			
1.0	2.0	Convergence Failed			

TABLE 3.6

Estimation Efficiency Using the HYB Method for Example 2

INITIAL ESTIMATES		NI(NOF)	CPU secs	CONVERGED ESTIMATES	
0.04	0.06	16(37)	1.6	0.208	0.495
0.4	0.6	8(16)	0.9	0.208	0.495
2.0	3.0	64(137)	9.3	0.208	0.495
1.0	2.0	Convergence Failed			

TABLE 3.7

Estimation Efficiency Using the N Method for Example 2

INITIAL ESTIMATES		NI(NOF)	CPU secs	CONVERGED ESTIMATES	
0.04	0.06	10(19)	2.4	0.208	0.495
0.4	0.6	7(13)	2.0	0.208	0.495
2.0	3.0	10(22)	3.6	0.208	0.495
1.0	2.0	11(23)	3.6	0.208	0.495

TABLE 3.8

Estimation Efficiency Using the N/GN Method for Example 2

INITIAL ESTIMATES		NI(NOF)	CPU secs	CONVERGED ESTIMATES	
0.04	0.06	10(19)	2.1	0.208	0.495
0.4	0.6	7(13)	1.6	0.208	0.495
2.0	3.0	10(22)	3.2	0.208	0.495
1.0	2.0	11(23)	3.2	0.208	0.495

Only the N and N/GN methods were able to perform the estimation for the four sets of initial estimates. Results in Table 3.5 show that the GN method converged satisfactorily in only the easiest case. The HYB method performed well but failed to converge in one case. This suggests a relatively poor robustness of the Gauss-Newton method. The introduction of second-order information in the evaluation of the Hessian by either the DGW update formula or the evaluation of sensitivity coefficients was clearly advantageous in cases where initial estimates were poor (i.e., the first, third and fourth cases). The N and N/GN methods were clearly more robust and performed very efficiently. The HYB method did not perform as well, indicating the beneficial effects

of using second-order sensitivities. The approximation to the matrix A in eq. 16 by the update formula thus appears to differ from the actual A matrix evaluated in the N and N/GN methods. This is generally expected of update formulae (Dennis and Schnabel (1983)).

3.4.3 Example 3

Herbert's model is a common and simple description of batch culture kinetics of microorganisms in the presence of a growth limiting substrate and is given by

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{K_S + S} - k_d X$$
$$\frac{dS}{dt} = -\frac{1}{Y_S}\frac{\mu_{max} XS}{K_S + S} \qquad (3.36)$$

where X is the concentration of viable cells, S, the substrate concentration, μ_{max}, the specific growth rate constant, K_s, the substrate saturation constant, k_d, the death rate constant and Y_s, the yield of biomass per unit mass of substrate. A common problem encountered in the estimation of parameters of this type of model is the high correlation between μ_{max} and K_s. This often causes common estimation procedures to fail or yield meaningless results. It thus provides a good test of robustness for such algorithms.

Data generated from eq. 3.36 were employed to estimate the four parameters using the four estimation methods. For the simulation, the initial conditions for X and S were 0.5×10^3 cells/mL and 4.0×10^{-1} μg/mL, respectively. μ_{max}, K_s, k_d and Y_s were given values 0.5, 1.5, 0.1 and 5.0, respectively. X and S were assigned variances of 1.5 and 0.75 respectively with a correlation of -0.75. The data were then used to estimate the four parameters of eq. 3.36 with the four estimation methods. The estimation was performed for two sets of initial estimates; the first set had values of 1.0, 4.0, 1.0 and 1.0 for μ_{max}, K_s, k_d and Y_s respectively and the second set, values of 5.0, 45.0, 0.2 and 6.0. The results are given in Tables 3.9 and 3.10 for the first and second set of initial estimates, respectively.

TABLE 3.9

Evaluation of Estimation Efficiency for Example 3 and
Optimal value of the Box-Draper criterion (BD)
Initial estimates: $\mu_{max} = 1.0$, $K_s = 4.0$, $Y_s = 1.0$, $k_d = 1.0$

METHOD	CPU secs	NI(NOF)	BD
N	11.3	16(33)	325.1
N/GN	6.5	14(28)	325.1
GN	Convergence Failed		
HYB	Convergence Failed		

TABLE 3.10

Evaluation of Estimation Efficiency for Example 3 and
Optimal value of the Box-Draper criterion (BD)
Initial estimates: $\mu_{max} = 5.0$, $K_s = 45.0$, $Y_s = 6.0$, $k_d = 0.2$

METHOD	CPU secs	NI(NOF)	BD
N	7.1	10(22)	325.1
N/GN	4.1	9(18)	325.1
GN	2.8	17(34)	339.2
HYB	2.8	17(34)	339.2

For the first case, only the N and N/GN methods converged. The converged estimates were 6.03, 52.21, 6.50 and 0.14 for μ_{max}, K_s, Y_s and k_d, respectively. In Figure 4.2, the predicted responses are plotted against time along with the observed responses. The scatter in the data is considerable. For the second case, the N and N/GN methods converged to the same estimates. For the GN and HYB methods, convergence was not reached with respect to the Bates and Watts convergence criterion but with respect to the relative change in the objective function, which became less than 10^{-9}. The estimates obtained were 6.60×10^4, 6.41×10^5, 6.41 and 0.14 for μ_{max}, K_s, Y_s and k_d, respectively.

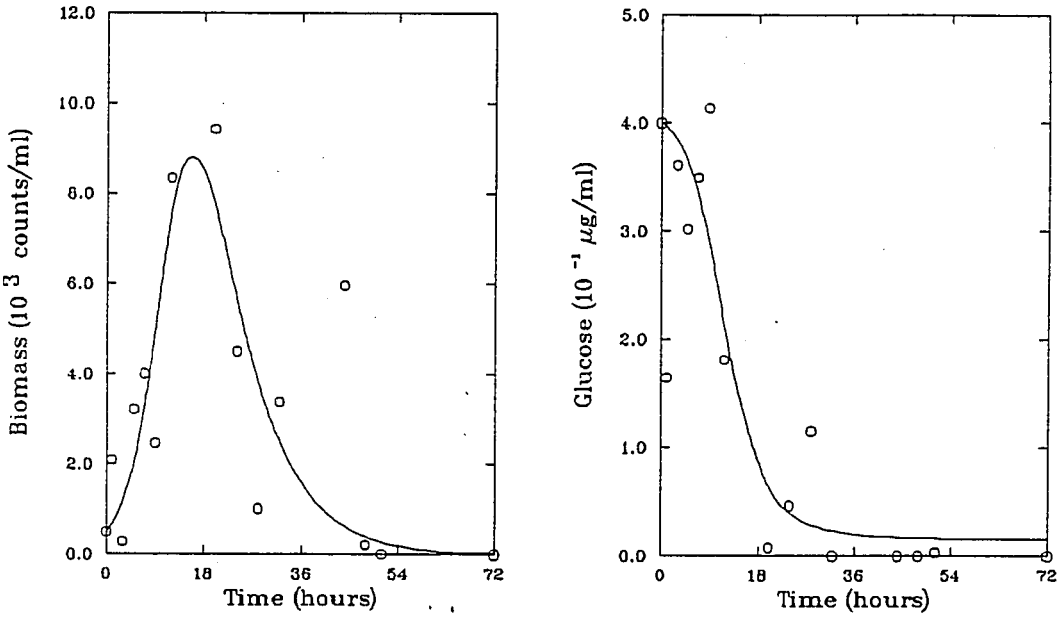

Figure 3.2 - Predicted (curve) and observed responses (empty circles) for Example 3.

In this example, the correlation between μ_{max} and K_s was nearly 1.0. It would be therefore impossible to estimate these parameters independently. As observed in the

above results, it was not possible to recover the parameter values used for the simulation. However, consistent results were obtained for the N and N/GN methods. The GN and HYB method could not yield appropriate results and basically failed to converge adequately. This indicated that use of second-order sensitivity coefficients in evaluation of the Hessian did convey an increased robustness to the N and N/GN methods. However, this increase was not due to just the introduction of the second-order information. Had this been so, the HYB should have also converged satisfactorily. The increased robustness was due to the more accurate evaluation of the Hessian in the N and N/GN methods. By using these methods, problems of convergence related to the high correlation between two parameters and large scatter in the data were overcome. It must be emphasized that the improved robustness of the estimation routine in no way improved the very poor quality of the highly correlated parameter estimates. This task remains a challenge for the model builder through collection of more informative data or potentially use of an alternative model form.

3.4.4 Example 4

This is a common example for testing multiresponse parameter estimation algorithms. It is based on a study of the thermal isomerization of α-pinene by Fuguitt and Hawkins (1947) given in Section 3.3, eq. 3.29. This example has been used by Box *et al.*(1973), Bates and Watts (1985) and Biegler *et al.*(1991).

Multiresponse parameter estimation using data for all five responses, y_1, y_2, y_3, y_4 and y_5, with the Box-Draper criterion is meaningless in this case due to the presence of linear dependencies in the data (Box *et al.* (1973), McLean *et al.*(1979). Under such conditions $d(\theta)$ is identically zero for all values of the parameters. Consequently, attempts to minimize $d(\theta)$ reduce to minimizing the rounding error in its computation. Nevertheless, the Box-Draper criterion was used with five responses in order to test the reliability and robustness of the various estimation methods under extremely difficult conditions. In order to compare the four estimation methods under less stringent conditions the estimation was also performed with a least squares criterion (i.e.,

minimization of the sum of the sum of squares of residuals over all responses). The initial estimates used for the parameters were those of Box *et al.*(1973): 5.84×10^{-5}, 2.65×10^{-5}, 1.63×10^{-5}, 2.777×10^{-4} and 4.61×10^{-5} min^{-1} for θ_i (i=1,...,5), respectively. The results are shown in Tables 3.11 and 3.12.

TABLE 3.11

Evaluation of Estimation Efficiency for Example 4 and Optimal value of the Least Squares Criterion (LS)

METHOD	CPU secs	NI(NOF)	LS
N	12.4	4(7)	19.81
N/GN	7.1	5(9)	19.81
GN	1.3	4(7)	19.81
HYB	1.3	4(7)	19.81

TABLE 3.12

Evaluation of Estimation Efficiency for Example 4 and Optimal value of the Box-Draper Criterion (BD)

METHOD	CPU secs	NI(NOF)	BD
N	35.5	11(25)	0.0992
N/GN	21.2	13(29)	0.0992
GN	4.1	10(22)	0.514
HYB	4.1	10(22)	0.514

The least squares solution for all four estimation methods was essentially the same as that reported by Box *et al.*(1973) and Biegler *et al.*(1991). The converged parameter estimates were $5.92\text{x}10^{-5}$, $2.96\text{x}10^{-5}$, $2.05\text{x}10^{-5}$, $2.745\text{x}10^{-4}$ and $4.00\text{x}10^{-5}$ min^{-1} for θ_i (i=1,...,5), respectively. These results show the expected greater efficiency of the GN method in well behaved situations. Convergence required only 1.3 CPU seconds compared with 12.4 s and 7.1 s for the N and N/GN methods respectively. The HYB method was just as efficient as the GN method since there was no need to switch to its update mode. The N/GN method uses different switching rules and obviously called for a switch to its second-order mode, resulting in a loss in efficiency.

The situation was quite different when the Box-Draper criterion was used. The N and N/GN methods yielded very different estimates than the GN and HYB methods. The estimates obtained with the N and N/GN methods were $3.69\text{x}10^{-5}$, $1.93\text{x}10^{-5}$, $1.65\text{x}10^{-5}$, $2.718\text{x}10^{-4}$ and $2.58\text{x}10^{-5}$ min^{-1}. The value of the objective function was 0.0992 at convergence. The estimates obtained with the GN and HYB were $5.78\text{x}10^{-5}$, $2.91\text{x}10^{-5}$, $2.09\text{x}10^{-5}$, $5.070\text{x}10^{-4}$ and $9.36\text{x}10^{-5}$ min^{-1} and the value of the objective function was 0.514 at convergence. The latter set of estimates were similar to those reported by Box *et al.*(1973) for the same situation.

As in example 3, differences in the results can only be due to use of second-order sensitivity coefficients in the evaluation of the Hessian. In this example, the GN and HYB methods failed to convergence to the proper local minimum, which was presumably reached by the N and N/GN methods. A better evaluation of the Hessian appeared to convey more reliability to the estimation procedure; the price for the improved reliability was a greater computing time. Again, the N/GN method was much less costly than the N method.

3.5 Summary

The Decoupled Direct Method (DDM) is a very efficient method for the calculation of first-order sensitivity coefficients in systems of ODEs. In this chapter, an effective implementation of the DDM, similar to the algorithm of Leis and Kramer (1988), was developed and incorporated in a multiresponse parameter estimation routine. In an attempt to improve the accuracy of the first-order sensitivity coefficients calculated by the method of Leis and Kramer, an error control strategy, based on a method proposed by Shampine (1988), was extended for use with the DDM. Results showed that this new error control strategy improved the accuracy of the solution of the model and the first-order sensitivity coefficients. The performance of the algorithm of Leis and Kramer (1988) was also improved.

The DDM was extended to include solution of the second-order parametric sensitivity equations. The extended DDM was then used to develop a full Newton scheme for multiresponse parameter estimation in systems of ODEs. A hybrid method was also developed which allowed switching between this Newton scheme and a generalized Gauss-Newton scheme. The switching procedure was based on the magnitude of the Bates-Watts convergence criterion (Bates and Watts (1985)).

Both methods were compared to a generalized Gauss-Newton method and also to a hybrid method which incorporated the DGW update method and the generalized Gauss-Newton method. This hybrid method was based on a modification of the single response nonlinear least squares algorithm of Dennis *et al.* (1981). Using a nonlinear least squares problem with large residuals it was shown to perform very well in comparison with the method of Dennis *et al.*

In comparing the four estimation methods with three test examples, it was observed that methods which incorporated second-order sensitivity coefficients in the evaluation of the Hessian matrix were more robust and reliable. The modified full Newton method and the hybrid (Newton/Gauss-Newton) method successfully reached convergence despite problems with high correlation between parameters, linear dependencies in the data, large residuals and poor initial estimates. The hybrid method which incorporated the DGW update formula to estimate the Hessian, rather than calculating the Hessian with second-order sensitivities, and the Gauss-Newton method were as robust as the full second-order methods. The hybrid method (HYB) perfomed better than the Gauss-Newton method. Of the two methods employing second-order sensitivities, the new hybrid method was significantly more efficient.

There are well-known drawbacks to requiring second-order derivatives in parameter estimation algorithms. Additional error-prone coding for calculating the derivatives and increased computational time and storage are prime factors which increase dramatically with the size of the problem. On the other hand, is was shown that greater robustness and reliability can be achieved by incorporating second-order parametric sensitivities in the estimation routine. In this work, extension of the DDM to evaluate second-order sensitivities and development of new hybrid estimation techniques provided ways to minimize these drawbacks and expand the possibility of realizing the benefits of second-order methods, particularly for multiresponse parameter estimation in systems of ODEs.

As a final, supplemental comment, it should be noted that many of the problems in multiresponse parameter estimation arise as a consequence of limitations in the model building strategy. In particular, starting with too large or complex a model form with limited information in the data inevitably leads to problems in estimation. It is strongly recommended that one begins with a simplified model with informative data, collected from carefully designed experiments, and follow up with subsequent modification of the model and further data collection until an adequate fit is achieved. Suitable

transformation of the parameters can also improve the estimation. With such a strategy, a simple first-order estimation method will usually suffice. Nevertheless, situations do arise where second-order methods are required making hybrid methods like those developed here valuable.

CHAPTER 4

Model-Building Strategies for Batch Culture Kinetics

In Chapters 2 and 3, efficient procedures for multiresponse parameter estimation in systems of ordinary differential equations were presented from a technical point view. This chapter deals more with the practical aspect of multiresponse modelling with differential equations. The primary aim of this chapter is to present useful approaches for building models describing the kinetics of the batch culture of microorganisms with the help of the multiresponse parameter estimation software developed.

4.1 Introduction

The use of biological processes for the production of valuable commercial products has become increasingly important. In order to implement and develop these processes, considerable effort has been devoted to describing the mechanisms involved by some mathematical model. In general, biological processes involve the culture of a living organism in a suitable medium from which it takes the sources of energy and organic carbon required for growth and for the production of one or more valuable products. In these processes, the organisms can be perceived as necessary intermediates in product formation, as in the case of antibiotic production, or they may represent the desired end-product as in the case of single cell protein production. The mechanisms of microbial growth and product formation are very complex and are still poorly understood. For these reasons, detailed mathematical descriptions of the mechanisms have been avoided. Instead, the approach has been to describe the rates of growth as functions of the conditions under which the process is operated. Information on the rates of product formation and substrate uptake are combined with the growth model to develop "pseudo-mechanistic" models of the

process. Rather than identifying all the parameters involved in the actual reaction pathway, the parameters are lumped in order to describe the apparent rates.

One striking feature of model building studies involving batch culture kinetics found in the literature is the absence of rigorous model building techniques. Statistical model building tools for parameter estimation and model validation are not frequently implemented in a comprehensive fashion. Developments of adequate model forms have lagged because of this overseeing of appropriate modelling techniques. One of the primary objectives of this work was to demonstrate the coupling of statistical methods with process knowledge to yield an effective model building strategy for batch culture kinetics.

Since most models describing batch culture kinetics are systems of ODEs which give simultaneous predictions for all response variables, it would seem highly beneficial to use data from all responses to estimate the parameters. In this way, information contained in simultaneous measurements of biomass, substrate(s) and product(s) concentrations could be more efficiently used. In single response parameter estimation, data from one variable is used to estimate all model parameters. This may include parameters not directly related to the measured variable, resulting in difficult estimation. As discussed in Chapter 2, a number of approaches have been proposed for the use of data from more than one response variable to estimate model parameters. The multiresponse parameter estimation criterion of Box and Draper (1965) was chosen for this work. Although it has many desirable features, its optimization can be very difficult. In Chapters 2 and 3, efficient and effective procedures for multiresponse parameter estimation in systems of ordinary differential equations were developed. In this chapter, the benefits of such procedures coupled with a sound model building strategy for the development of models for batch culture kinetics are demonstrated.

To demonstrate the model building strategy, a test example was constructed

involving the batch growth of *Eschirichia coli* in the presence of glucose as the limiting substrate with the formation of acetate and ethanol. A mathematical model describing the system was used to simulate experimental data used for the analysis. The model, parameter estimates and covariance structure used to simulate the system originated from the analysis of actual batch data. This example is representative of the problems involved in modelling batch culture kinetics. It shows how multiresponse parameter estimation and appropriate experimental design can easily and effectively identify the inadequacy of a model and how proper assessment of lack-of-fit can indicate possible alternative model forms. The inability to estimate some parameters is also demonstrated.

In this chapter, a model building strategy for batch culture kinetics is presented. In Section 4.2, the elements of the model building strategy are presented. The criterion for model selection is discussed in sub-section 4.2.1. This is followed by a brief discussion of models describing batch culture kinetics in sub-section 4.2.2. The principles of the model building strategy are presented in subsection 4.2.3. In Section 4.3, the model building strategy is demonstrated using a simple simulated example. Results of the model building example and the main features of the strategy are summarized in Section 4.4.

4.2 Strategies for Building Models Describing Batch Culture Kinetics

4.2.1 Model Selection

When selecting a model form, one needs to develop some guidelines that can help eliminate inappropriate forms. The first important factor in selecting a model is to identify the purposes for which the model is used.

In general, a model is developed in order to obtain 1) a correlation of experimental data, 2) a prediction of process performance or 3) an understanding of the underlying mechanisms of the process. For the problem of microbial batch

culture kinetics, the development of a model that can shed light on the mechanisms and can predict process performance is of primary interest. Microbial growth is the result of a very complex system of metabolic reactions. Therefore, a model that would attempt to describe every detail of the mechanism would be extremely complex, difficult to use and impossible to verify. For most applications, building a model that is both descriptive and useful is based on a number of simplifying assumptions which must be tested.

Acceptance of a model is based on two requirements. The first requirement is that the model must be able to describe the shape of the data. If the model cannot be made to fit the data by adjustment of the parameters then it cannot be used. The second requirement is that the model supports the assumptions used in its formulation. If a model, when fitted to the data, gives parameter values that are inconsistent with the assumptions, it cannot be accepted for purposes of mechanistic understanding.

4.2.2 Models Describing Batch Culture Kinetics

A typical batch growth curve, that is, a plot of biomass concentration as a function of time, is shown in Figure 4.1. Typically, modelling efforts have been involved with the description of the exponential phase. This might be appropriate in cases where the products are formed during the growth phase or for the study of kinetics under continuous culture conditions. If, however, the product of interest is formed during the stationary or decline phase, as in the case of antibodies, such models are inappropriate and there is a need for a model able to describe the entire growth curve. The work of Monod initiated an explosion of different model forms that attempted to describe the different phases of the growth curve. With the advent of more sophisticated modelling tools, the models were refined in order to incorporate descriptions of substrate uptake and product formation. Subsequent efforts were directed towards refining the basic description of substrate uptake and product formation in order to incorporate growth or non-growth related effects. This

introduced a number of additional effects such as product and substrate inhibition. Because of the large number of model forms, the need to classify models quickly became obvious. A brief overview of the most popular model forms is presented in Appendix A.

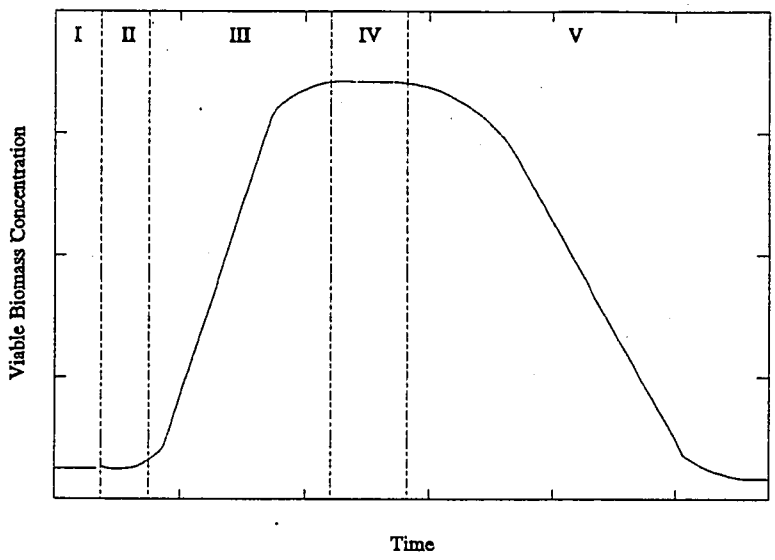

Figure 4.1 - Typical microbial growth curve for a batch process. (I = Lag Phase, II = Accelerating Phase, III = Exponential Phase, IV = Stationary Phase, V = Declining Phase).

The first division in model forms is made according to the form in which the biomass is expressed. Models that describe biomass measured by some macroscopic variable such as dry weight or absorbency are classified as distributed models. The biomass is assumed to be distributed throughout the medium. Models that describe biomass, expressed in terms of the total number of cells, are termed segregated. It is obvious that these two divisions can be made equivalent. By simply assuming that

the average size of the cells is constant, distributed measures of biomass can be used to express the total number of cells. For this reason, most models are of the distributed type, even in cases where biomass is expressed in total number of cells. The real difference between these two divisions exists in the mathematical form used to express the mechanisms involved. This is especially true for segregated models that attempt to describe the growth of an individual cell. Because times of the different phases of cell growth, division and death are not known, the culture is assumed to be an average of many cells. These models take the form of a probabilistic function making them extremely hard to use and to test.

Within this first differentiation of model forms exist further subdivisions of classification. Models can be termed as unstructured or structured. For unstructured models, the living organism is described by a single variable, biomass concentration. Rates of change in biomass concentration are expressed in terms of the concentrations of the biomass, the limiting substrates and the products. In these models, the physiological state of the microorganism over the different phases of growth is of no concern and is assumed constant. Changes in the physiological state of the microorganisms, which may arise throughout the course of a fermentation, cannot be described. In other words, no matter what conditions exist in the culture, the rate of growth, the rate of product formation, etc., are assumed to be functions of the bulk concentrations in the system.

Unstructured models are usually favoured because of their simplicity. They often serve to describe a selected number of variables (i.e., biomass, substrates and products concentrations) which are easily measured. The equations are simple in form and contain a relatively small number of parameters. Parameters are thus more easily estimated from experimental data. Furthermore, given that the underlying assumptions used in the development of the model are sound, these parameters can bear some physiological significance.

Unfortunately, there is often a tendency to oversimplify the situation. As a result, unstructured models are developed under unrealistic assumptions and cannot adequately describe the observed behaviour. It is often reported that such models, when developed under a given set of conditions, fail to adequately predict behaviour of the same system under different conditions. The system, when studied under different conditions, might yield different, seemingly adequate, models for each set of conditions. This is a very serious problem which points out the need to carefully consider the limitations of such models when considering their use for model building.

Although it was pointed out that a reduced number of model parameters might facilitate their estimation, this is not the case for all the models. It is especially true of models that contain Monod-type expressions (see Appendix A). Such expressions are often the source of a serious parameter estimation problem. For example, if K_S, the substrate saturation constant, is sufficiently smaller or larger than the substrate concentration, it becomes very difficult and often impossible to estimate K_S and μ_{max}, the specific growth rate, independently. This is a widespread problem that is often overlooked in the studies of unstructured models.

There have been few developments towards overcoming these problems. In most cases, the Monod form is assumed adequate without much consideration for underlying assumptions and the constants are estimated either by fitting the model to experimental data using nonlinear regression techniques or by performing a series of enzyme assays to estimate certain constants (i.e., enzyme saturation constants, rate constants, etc.). As a result, the models remain highly empirical. The application of rigorous statistical model building techniques should be beneficial for the study of unstructured models. They can help to generate useful information about the system that can be employed to obtain more precise estimates of parameters and to test simplifying assumptions (which are seldom tested otherwise). Rigorous diagnostic checking for inadequacies leads to model reformulation and improvement as well as

identifying the need for more data. These problems will be dealt with at greater length in section 4.3.

In order to obviate limitations encountered in the use of unstructured models, there has been a growing interest in the development of so-called structured models that attempt to describe, in as much detail as possible, the intracellular compositions of the microorganisms. Structured models allow for changes in the physiological states of the microorganism. They also allow for various parts of the microbial cell to respond independently to changes in conditions. The population of microbial cells forming the biomass is therefore structured and made up of a collection of functionally independent units that respond in a like manner under similar changes in culture conditions.

A detailed description of all mechanisms involved in a reaction sequence is clearly advantageous. The ability of structured models to describe the actual mechanisms involved in growth and product formation offers a definite improvement over simple unstructured models. Such highly mechanistic models could be used to obtain more adequate predictions over a wider range of conditions. They also help to develop a better understanding of underlying mechanisms.

However, structured models are extremely difficult to deal with (for example see Domach (1984)). Their complexity typically introduces a number of unmeasurable variables and large numbers of kinetic parameters. In such cases, parameter estimation is difficult, if not impossible. The result is generally highly correlated parameter estimates. This can seriously affect the performance of the estimation routine. It can also lead to extremely large inference regions for the parameters making the fitted model useless. Moreover, since a great number of variables cannot be measured, the assumed mechanisms cannot be rigorously tested.

From a review of models describing microbial growth, it seems that the distinction made between unstructured and structured models does not serve any purpose. A proper pseudo-mechanistic model should clearly incorporate elements of both types. It should remain simple. This greatly facilitates the use of the model for process design, optimization and control purposes. However, to ensure reliable model prediction, there must exist a balance between oversimplified unstructured models and complex structured models. Simplifying assumptions must not detract significantly from the actual mechanisms. Oversimplification of the mechanisms also leads to restricted ranges of applicability of the models. On the other hand, excessive complexity makes use of the model impractical.

Overall, it can be observed that mathematical models able to describe fully the growth of microorganisms over a range of different conditions have not yet been developed. New model building approaches must be developed. Statistical model building techniques offer a promising alternative. The main objective of such an approach would be to generate a model based on sensible mechanistic assumptions that can adequately describe the observed behaviour in some pre-specified range of operating conditions.

Model building can benefit greatly from a number of statistical techniques. However, computer routines for these techniques are not readily available. In the early stages of this work, software had to be developed that would allow the modelling of multiresponse data from batch culture experiments. A powerful multiresponse parameter estimation routine for models described by sets of simultaneous nonlinear ordinary differential equations was developed. The estimation problem and software have been described in detail in previous chapters. In addition, a number of other statistical routines, complementary to model building and aimed at helping decision making, were also developed. Software for the design of multiresponse experiments was included. One of the objectives of this study was to investigate various strategies that would complement and facilitate the use of

multiresponse techniques. Statistical techniques pertinent to study of batch culture kinetics were used as the basis of a strategy for building models for batch culture kinetics. These techniques are reviewed in the following section.

4.2.3 Model Building Strategy

Model building is an iterative learning process usually visualised as shown in Figure 4.2.

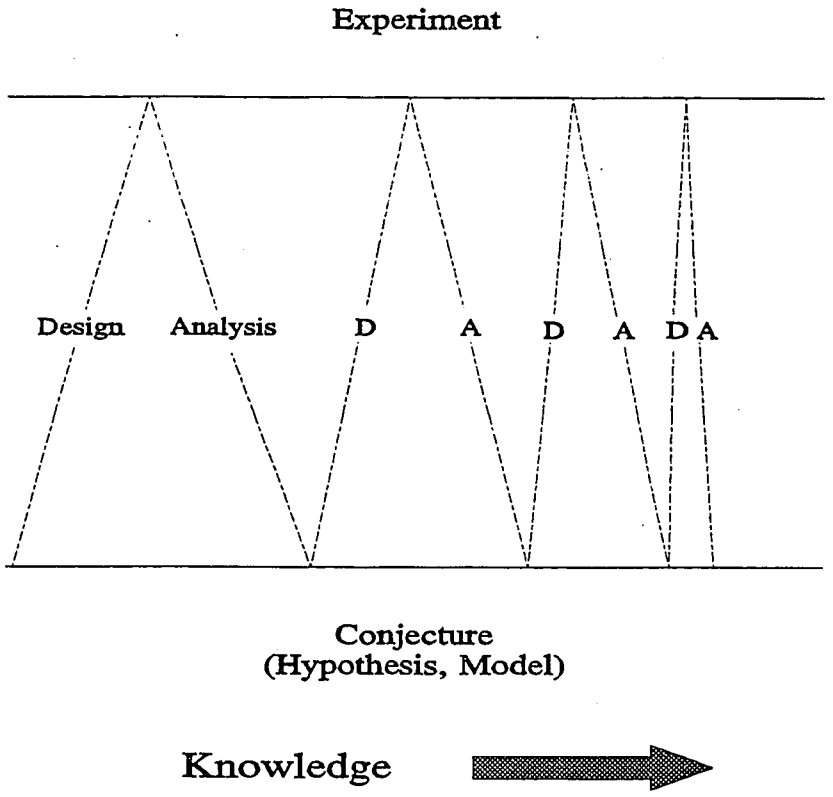

Figure 4.2 - Iterative Investigative Process for Model Building.

The governing principle of this approach, developed by Box and Hunter (1962), is to maximize the amount of knowledge acquired with a minimum experimental effort.

This involves careful design of experiments based on both the hypothesis that is to be tested and the previous knowledge of the system.

The first step in model building is to postulate some hypothesis that is to be tested. This hypothesis could simply be the possibility that one variable affects another. It could also be the belief that a particular well defined model describes the system under study. The second step is to design and perform experiments that can help verify or contradict the hypothesis. The third is the actual analysis of the data. The data can be used to estimate the model parameters, evaluate model adequacy, etc. The analysis step is the part of the approach where the hypotheses are rigorously tested. Based on the results, new hypotheses can be formulated. This in turn may lead to new design objectives, thereby initiating another cycle of the learning process. The process can be carried out until the overall objectives of the proposed study are fulfilled.

When formulating a hypothesis, consideration must be given to the scope of the study. A clear definition of the problem at hand must be established. The extent of knowledge of the system prior to the study is also an extremely important consideration. If, for example, the model form is not known initially nor the experimental conditions, the primary problem is to define the operating region and to generate data that could help in formulating and screening model. From a practical point of view, the extent to which the hypothesis can be verified experimentally must be considered. Potential experimental constraints have to be envisaged. If a postulated model contains many unmeasurable variables, estimation of parameters can become extremely difficult.

The design step is mainly concerned with the selection of experimental conditions that can effectively test the current hypotheses. In order to minimize the experimental effort required, the amount of information obtained from the experiments must be maximized. The design of batch experiments is mainly

concerned with specification of the initial conditions of each of the responses, the time at which measurements are taken and the experimental conditions (i.e. pressure, temperature, etc.). The choice of operating conditions is highly dependent on the objectives of the current stage of model building. Many different design criteria have been developed for a number of different objectives.

At the initial stages of model building when the potential model forms are not clearly established, experiments are best carried out over a coarse grid of the expected operating region. In cases where the number of experiments allowed is limited, as might occur in batch experiments, such experimental designs are restricted to the limits of the operating conditions. The main advantage of using such a design initially is that it allows the entire operating region to be spanned. One or more models describing the observed behaviour for the entire region may then be developed.

In situations where the model form is known initially, other so-called "starting" designs are more effective. For single-response nonlinear models Box and Lucas (1959) proposed to choose experimental conditions, ξ^0, to maximize the D-optimal design criterion given by

$$D = \left| \frac{\partial f(\xi^0,\boldsymbol{\theta}^{(0)})}{\partial \boldsymbol{\theta}}^T \frac{\partial f(\xi^0,\boldsymbol{\theta}^{(0)})}{\partial \boldsymbol{\theta}} \right| \tag{4.1}$$

where

$$f(\xi,\boldsymbol{\theta}^{(0)})^T = \left[f(\xi_1,\boldsymbol{\theta}^{(0)}),\ f(\xi_2,\boldsymbol{\theta}^{(0)}),\ f(\xi_3,\boldsymbol{\theta}^{(0)}),\ \ldots\ ,f(\xi_N,\boldsymbol{\theta}^{(0)}) \right]^T$$

and where $\theta^{(0)}$ is the vector of initial estimates of the parameters. In particular, they proposed starting designs consisting of P measurements for P-parameter models. Unfortunately, situations where the model and values of the parameters are known initially are rarely encountered in practice and the D-optimal design criterion is not frequently applicable for "starting" designs.

The main feature of the D-optimal design criterion is that it leads to improvement in the precision of the parameter estimates. Assuming that the model form and parameter estimates have been obtained from a prior analysis of available experimental results, additional experiments can be designed using the D-optimal design criterion to improve parameter precision. This is ordinarily performed sequentially. Based on the analysis of the available data, the next experimental run condition, ξ^{N+1}, is obtained by maximizing

$$D_1 = \left| \frac{\partial f(\xi^1,\hat{\theta})}{\partial \theta}^T \frac{\partial f(\xi^1,\hat{\theta})}{\partial \theta} \right| \tag{4.2}$$

where

$$\frac{\partial f(\xi^1,\hat{\theta})}{\partial \theta} = \begin{bmatrix} \frac{\partial f(\xi^0,\hat{\theta})}{\partial \theta} \\ \frac{\partial f(\xi^{N+1},\hat{\theta})}{\partial \theta} \end{bmatrix},$$

$$\xi^1 = \begin{bmatrix} \xi^0 \\ \xi^{N+1} \end{bmatrix}$$

and $\hat{\theta}$ is the vector of parameter estimates obtained from the last N runs.

In the multiresponse case, the D-optimal design takes the form

$$\left| \frac{\partial f(\xi,\theta)}{\partial \theta}^T \left(\Sigma^{-1} \otimes I_N\right) \frac{\partial f(\xi,\theta)}{\partial \theta} \right| \tag{4.3}$$

where $\mathbf{f}(\xi,\theta) = \text{vec}(\mathbf{F}(\xi,\theta))$ is a NM-dimensional vector, $\mathbf{F}(\xi,\theta)$ is a NxM matrix (see Section 2.1) and Σ is the MxM covariance matrix for the responses. As in the single-response case, this multiresponse D-optimal criterion can be used to design experiments in a sequential manner.

In situations where several adequate model forms are obtained from the prior analysis of the experimental measurements, a different design criterion aimed at model discrimination is used. For single response nonlinear models, a number of different approaches have been used to develop design criterions for model discrimination (see, Hill (1978) for a review). Among the more popular approaches are those of Box and Hill (1967), Fedorov (1972) and Atkinson (Atkinson and Cox (1974)). Unfortunately few of these approaches have been extended to discrimination of nonlinear multiresponse models. The only reported developments in the multiresponse case are the works of Hill and Hunter (1969) and Box (1968). The approach of Hill and Hunter is an extension of the Box-Hill (1967) criterion with unknown covariance. Box (1968) applied a similar approach to treat the case where the covariance matrix is not constant over the range of operating conditions considered.

The analysis step consists of estimation of the model parameters, evaluation of parameter precision and qualitative and quantitative lack-of-fit tests for the model(s). Testing for model adequacy is an essential part of model building. A model cannot be accepted until it shows no lack of fit and until all model parameters have been estimated to an adequate degree of certainty. Unfortunately, these are often unsubstantiated for many reported model building studies.

In describing the transient behaviour of a system, models take the form of systems of ordinary differential equations (ODEs). Data from typical batch culture experiments consist of a set of initial conditions, e.g., initial biomass and limiting substrate concentrations, and a number of measurements of the concentrations of biomass, substrate(s) and product(s) taken at various times during the experiment. There are two approaches by which a model in the form of a system of differential equations could be fitted to this type of data.

One approach is to approximate rates of reaction directly from the data. A polynomial of suitable degree or a spline function is fitted to the data in order to obtain a smooth curve. Rates are evaluated by differentiating the fitted expressions. Model parameters can then be fitted by comparing rates evaluated from the model and rates obtained from the polynomial fits. This approach yields very inaccurate values for the "observed" rates obtained by differentiating the approximate functions. For example, it is normally assumed that the biomass has an exponential growth phase. Yet, it is well known that spline functions and polynomials represent exponential functions with limited accuracy. Differentiation of such expressions to yield expressions for the rates will be even less accurate. Another weakness of this approach lies in the arbitrary number of observed rates that can be evaluated and the non-homogeneous variance associated with each. This raises questions as to the suitability of standard fitting procedures for estimating the parameters of the mechanistic models.

Another approach to estimating the model parameters is to fit a numerical solution of the system of differential equations to the experimental data. This removes the problem of estimating rates from experimental data but it introduces a number of additional factors which must be considered. First, the availability of self-contained commercial software packages which offer the opportunity to fit models in the form of differential equations to multiresponse data is extremely limited. Development of such software is a major task.

The selection of an accurate and efficient solution algorithm for a set of nonlinear ODEs is a problem in itself since the choice is highly dependent on the nature of the problem at hand. Solution of stiff ODEs requires more complex procedures such as the incorporation of Gear's backward difference formulas. On the other hand, such procedures will be less efficient for solution of nonstiff systems. Moreover, within an iterative parameter estimation application, the nature of the solution of the ODEs may change dramatically with each successive set of parameter

estimates. This is especially problematic when the initial guesses for the parameters are far from the optimal values.

The accuracy and efficiency of the ODE solver also depends on the error-control strategy employed in the solution. In general, popular methods attempt to control the error of the solution within some bounds related to a user-defined tolerance. An appropriate choice of tolerance must be made for the system of ODEs to be solved such that calculations are as accurate as possible while remaining sufficiently efficient for repetitive use in estimation routines. This aspect of the problem has been discussed in Chapter 3.

Another potential problem arises when the model is an initial value problem. The initial conditions are required to solve the system of ODEs. In cases where the initial conditions are not precisely known, the solution of the model with those imprecise initial conditions might differ greatly from the solution obtained with the true initial conditions. The solution obtained becomes inconsistent with the true solution and the experimental data. To circumvent these difficulties, the initial conditions can be estimated along with the model parameters. However, it is not desirable to increase the number of parameters in this way since estimates of these "new" parameters are likely to be highly correlated with estimates of other model parameters. Ideally, one should prevent this problem by taking great care in the evaluation of initial conditions.

4.3 A Simple Model Building Example

4.3.1 Simulating the Batch Culture of *E. coli*

In an attempt to demonstrate and test the model building strategy discussed above and the multiresponse techniques presented in this work, a simple example was devised. The example involved the batch culture of *Eschirichia coli* in aerobic conditions under controlled pH. The substrate used was glucose. Two products were considered: acetate and ethanol. Experimental data was simulated using a stochastic simulation program. The model was given by

$$\begin{aligned}
\frac{dX}{dt} &= \frac{\mu_{maxS} XS}{k_S + S} + \left(\frac{\mu_{maxA} XA}{k_A + A}\right)\left(\frac{K_{IS}}{K_{IS} + S}\right) - k_d X \\
\frac{dS}{dt} &= -\frac{a_S \mu_{maxS} XS}{k_S + S} - m_{gluc} X \\
\frac{dA}{dt} &= \frac{a_1 a_S \mu_{maxS} XS}{k_S + S} - a_2 \left(\frac{\mu_{maxA} XA}{k_A + A}\right)\left(\frac{K_{IS}}{K_{IS} + S}\right) \\
\frac{dE}{dt} &= \frac{a_E a_S \mu_{maxS} XS}{k_S + S} - k_{evap} E
\end{aligned} \qquad (4.4)$$

where X = biomass concentration (viable cell counts/ml)

S = glucose concentration (μg/ml)

A = acetate concentration (μg/ml)

E = ethanol concentration (μg/ml)

μ_{maxS} = maximum specific growth rate for the uptake of glucose (h^{-1})

μ_{maxA} = maximum specific growth rate for the uptake of acetate (h^{-1})

k_S = saturation constant for glucose (μg/ml)

k_A = saturation constant for acetate (μg/ml)

k_d = death constant (h^{-1})

a_S = inverse yield of biomass per unit mass of glucose (μg glucose/counts)

a_1 = yield of acetate per unit mass of glucose (μg acetate/μg glucose)

a_2 = inverse yield of biomass per unit mass of acetate (μg acetate/counts)

a_E = yield of ethanol per unit mass of glucose (μg ethanol/μg glucose)

k_{evap} = rate constant for the evaporation of ethanol (h^{-1})

K_{IS} = substrate inhibition constant for the uptake of acetate (μg/ml)

m_{gluc} = maintenance energy term for glucose (μg/counts).

The parameter values used for the simulation were:

μ_{maxS} = 0.8 h^{-1}

μ_{maxA} = 0.2 h^{-1}

k_s = 3.5×10^{-3} μg/ml

k_A = 0.5 μg/ml

k_d = 0.073 h^{-1}

a_S = 1.4×10^{-5} μg glucose/counts

a_1 = 24.7 μg acetate/μg glucose

a_2 = 10^{-4} μg acetate/counts

a_E = 1.0 μg ethanol/μg Glucose

k_{evap} = 0.0318 h^{-1}

K_{IS} = 0.756 μg/ml

m_{gluc} = 4.0×10^{-8} μg/counts.

This model originated from the analysis of actual batch data. The model shown above constitutes a slightly modified version of the model which was then developed. The inhibition term for the rate of uptake of acetate and the maintenance term for glucose were added to test the sensitivity of the model building approach. Both these terms have little effect on the model predictions. The inhibition term $K_{IS}/(K_{IS}+S)$ is nearly unity for the small glucose concentrations investigated. It has a significant effect only when the concentration of glucose is high. The maintenance term is always small because of the small value used for m_{gluc}. The values of the remaining parameters and the covariance structure used for the stochastic simulations were obtained from the analysis of batch data.

The variances for the biomass, glucose, acetate and ethanol concentrations were are 800 (counts/ml)^2, 0.003 (μg/ml)^2, 0.03 (μg/ml)^2 and 0.01 (μg/ml)^2 respectively. The correlation between the measured values of these responses was given by the following correlation matrix

$$\rho = \begin{bmatrix} 1.00 & -0.75 & 0.25 & 0.20 \\ -0.75 & 1.00 & -0.35 & -0.40 \\ 0.25 & -0.35 & 1.00 & 0.05 \\ 0.20 & -0.40 & 0.05 & 1.00 \end{bmatrix}$$

In this model, it was assumed that acetate and ethanol were two by-products of glycolysis. Acetate was assumed to be a secondary source of carbon once glucose was depleted. Death of biomass was considered to be proportional to the amount of biomass. Ethanol was only produced in the presence of glucose. It was not used as an auxiliary carbon source but it was lost to the atmosphere by evaporation throughout the course of the fermentation.

4.3.2 Model Building

The objective of this sub-section is to demonstrate a model building strategy for batch culture kinetics which combines use of statistical model building techniques with process knowledge. The overall approach follows that presented by Figure 4.2. Each stage of the strategy constitutes a cycle in which the four essential elements of model building (i.e., conjecture, experimental design, experimental work and data analysis) are sequentially applied in order to generate knowledge about the process. As shown in Figure 4.2, the cycle is repeated until knowledge of the process is sufficient with respect to the primary objectives of the investigator.

To facilitate presentation of the model building strategy, each stage is identified. Within each stage, objectives and technical elements of the design and analysis steps are presented along with the objectives of each stage.

STAGE 1 - Getting Started

The first step in any modelling study is to establish the overall objectives. The ultimate purpose of the model and the type of model sought should be made clear. Different model types lead to different modelling objectives. Mechanistic models, unlike empirical models, seek to describe the underlying mechanisms of the process. As a result, the form and the amount of information which is required to develop both types of models may differ greatly. For the study of batch culture kinetics, the main objective is to generate a pseudo-mechanistic description of the process, which could be applicable to a prescribed range of operating conditions. The model form is generally not known a priori. Consequently, the initial stage of the model building strategy seeks to generate potential model forms.

Another very important consideration is the current knowledge of the process. Any information which may be pertinent to the development of a tentative model form must be used. From an experimental point of view, knowledge of the experimental limitations of the process is important and should constitute the main objective of the initial experimental design step. Experimental constraints should be identified if they are not known. The reproducibility of the experimental measurements should also be verified prior to the first modelling attempts. This could be helpful in identifying areas of the operating region which may produce poor experimental data and in estimating the expected variance of the experimental measurements. Experimental difficulties which may interfere with data gathering should be identified ideally before embarking on a model building effort.

In this investigation, the model had to describe the production of ethanol and acetate from a culture of *E. coli* grown in the presence of glucose under aerobic conditions and controlled pH in a 20 litre fermenter. A few scouting runs were performed to delimit the operating region. Operating conditions of interest for this study were initial concentrations ranging between 0.01 and 0.2 μg/ml for glucose,

between 10.0 and 200.0 counts/ml for biomass, between 0.0 and 2.0 μg/ml for acetate and between 0.0 and 0.02 μg/ml for ethanol. A typical run time was 72 hours.

In this study, the metabolism of the microorganism was well understood (e.g., see Brock *et al.*(1984)). As a result, the formulation of a tentative model was easier than might often the case. It also facilitated the choice of the substrates, and products and intermediates to be measured. For *E. coli*, glucose is oxidized through the glycolytic pathway to form pyruvate. This important intermediate is then metabolized through the TCA cycle to provide building blocks for the biosynthesis of important cellular materials and to generate energy. Ethanol and acetate are also produced from this important intermediate. In the absence of glucose, acetate has been found to act as an auxiliary carbon source under aerobic conditions. Uptake of ethanol as a carbon source is only possible under anaerobic conditions.

Based on this information, glucose, ethanol, acetate and biomass concentration were measured. The model was required to describe changes in these four variables during batch culture. The biomass growth rate was expected to depend on both glucose and acetate concentration. The utilization rate of glucose was expected to be proportional to the rate of growth of biomass. The production rate of acetate and ethanol was expected to be proportional to the utilization rate of glucose. Although a number of potential model forms could have been entertained initially, only simple forms were considered in order to circumvent a number of problems encountered in the application of multiresponse parameter estimation techniques which were discussed in Chapters 2 and 3.

Step 1 - Initial Design

In light of the available information in this investigation, the primary objectives for the initial design of experiments were to obtain data over the entire batch growth curve for use in development of a feasible model form and to obtain some indication of reproducibility. Accordingly two runs were carried out under

similar condition, the extent of similarity being representative of the experimental reproducibility of the initial conditions.

For run 1, initial concentrations of biomass, glucose, acetate and ethanol were 57.0 counts/ml, 0.037 μg/ml, 0.089 μg/ml and 0.0071 μg/ml respectively. In run 2, the initial concentrations were 77.0 counts/ml , 0.036 μg/ml, 0.145 μg/ml and 0.0116 μg/ml respectively. 14 equally spaced sets of measurements were made in each run. The runs were terminated upon disappearance of the viable biomass. The experimental measurements are shown in Figure 4.3.

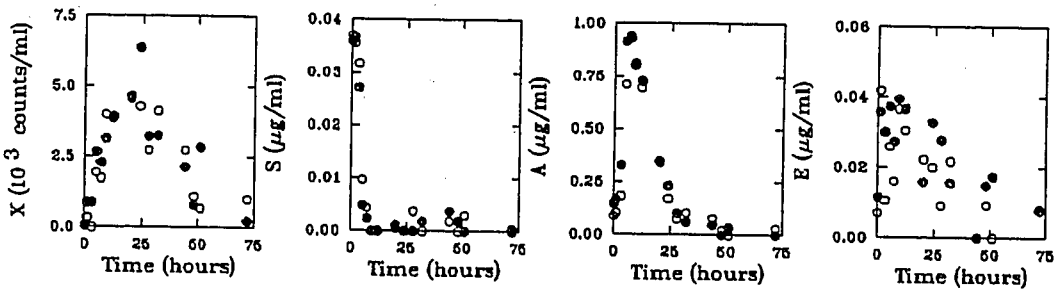

Figure 4.3 - Simulated observations for runs 1 (○) and 2(●).

Step 2 - Analysis of data from the initial design/ Formulation of a model

Although the mechanistic relationships for this system were qualitatively understood, no model forms have yet succeeded to express them successfully. Needless to say, values for the model parameters were also unknown. Since multiresponse parameter estimation relies on the availability of good initial estimates of the parameters, it was extremely difficult to fit a complex model to the preliminary data. A way to generate information about the parameters had to be devised.

One way of doing this is to begin by fitting simpler models that describe only some of the system variables. Simple models will be easier to fit without previous knowledge. If the simple model is similar in form to the postulated model, parameters common to both models can have values that are of the same order of

magnitude. Thus, one can hope to generate better initial estimates for parameters in the complex model.

In the study of batch culture kinetics, simple models that describe growth in the presence of one limiting substrate such as Monod's model or Herbert's model can be used to obtain initial estimates for μ_{max}, K_S and k_d. Herbert's model, given by

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{K_S + S} - k_d X$$
$$\frac{dS}{dt} = -\frac{1}{Y_S}\frac{\mu_{max} XS}{K_S + S} \qquad (4.5)$$

was fitted to run 1 and run 2. Initial estimates for μ_{max} and K_S were 0.5 and 0.05. They were obtained from a Lineweaver-Burke plot. The initial estimate of Y_S was 7.5, obtained from the overall change in biomass divided by the total amount of glucose consumed. k_d was arbitrarily given a value of 0.001. Using these initial estimates, convergence Box-Draper criterion could not be reached. This was primarily due to the poor conditioning of the Hessian matrix of the Box-Draper criterion with respect to the parameters. In an attempt to circumvent these problems, a logarithmic transformation of the parameter was used to improve the conditioning of the estimation. This also shrinks the search region for the unknown parameters as well as constraining the search to positive values of the parameters. The transformation proved very effective in this case, allowing the optimization to proceed without problems. Parameter estimates and approximate standard errors are shown in Table 4.1. Figure 4.4 plots of the predicted and observed responses versus time for biomass and glucose.

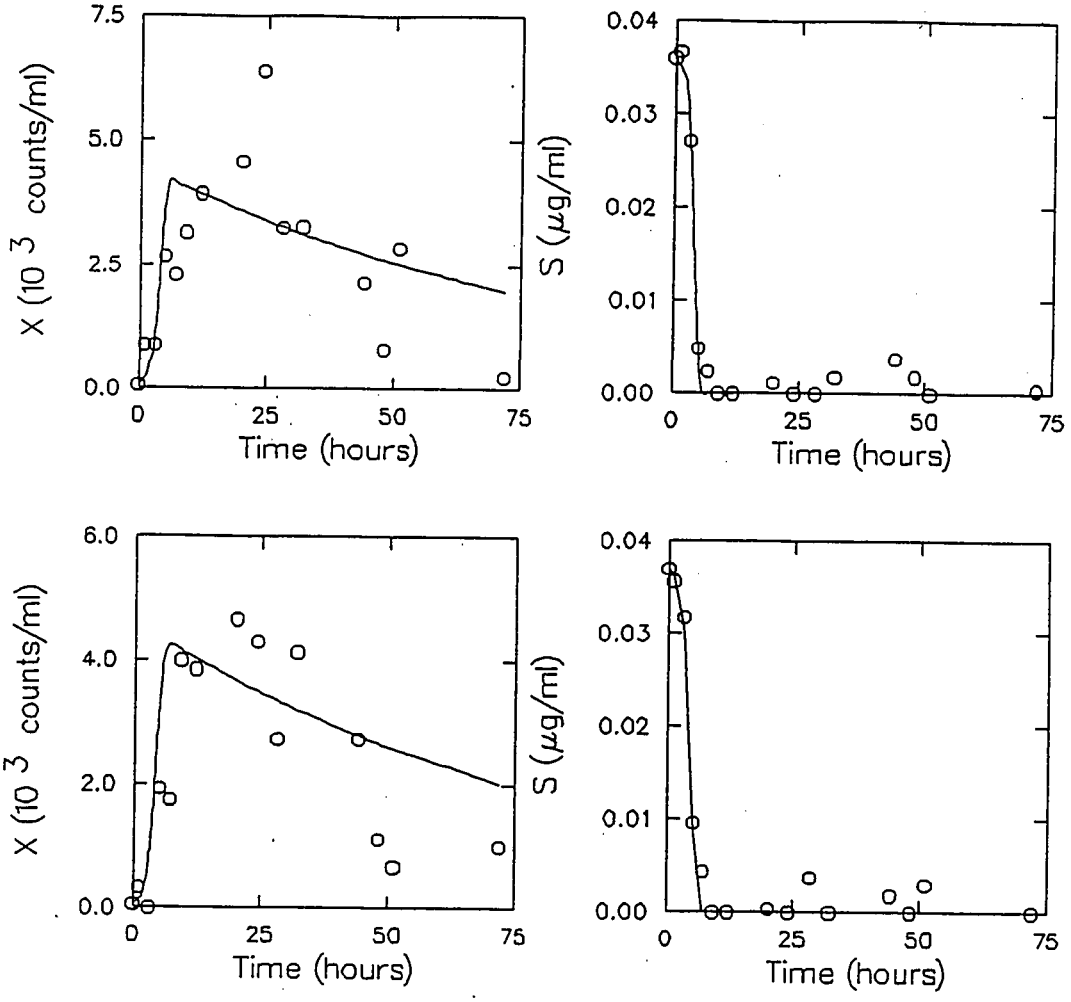

Figure 4.4 - Predicted (——) and observed (○) responses for the fit of eq. 4.5 to run 1 (bottom) and run 2 (top).

Results suggested that μ_{max} and K_S were insignificant at a 95% confidence level ($t_{(N-P);\alpha} = 1.725$). The correlation matrix for the parameters was

$$\rho_\theta = \begin{pmatrix} 1.0000 & 0.9903 & 0.2242 & 0.1419 \\ 0.9903 & 1.0000 & 0.1044 & 0.0682 \\ 0.2242 & 0.1044 & 1.0000 & 0.6142 \\ 0.1419 & 0.0682 & 0.6142 & 1.0000 \end{pmatrix}$$

The poor precision was largely related to the high correlation that exists between μ_{max} and K_S.

Table 4.1 - Parameter estimates for Herbert's model for run 1 and run 2.

Parameter	ln(estimates)	standard error
μ_{max}	0.18900	0.28015
K_S	-2.0547	0.86791
Y_S	2.4641	0.11365
k_d	-4.4730	0.36445

As expected, the model was highly deficient, especially for the biomass. As will be seen in the following stage of this model building exercise, the results did help to generate useful information about the approximate values of the model parameters. This will prove to be of great help in fitting a more complex model presented in the next stage.

The high correlation between μ_{max} and k_S was an indication that the model was over-parameterized. When $k_S >> S$, then the Monod form reduces to $\mu_{max}XS/k_S$ such that μ_{max} and k_S appear as a ratio and consequently cannot be estimated independently. A similar situation is encountered when $k_S << S$ except that the Monod form reduces to $\mu_{max}X$. Herbert's model was then reduced to give the simple three parameter model,

$$\frac{dX}{dt} = \mu_r XS - k_d X$$
$$\frac{dS}{dt} = -\frac{1}{Y_S}\mu_r XS \quad . \tag{4.6}$$

This model was fitted to run 1 and run 2. Parameter estimates and standard errors are shown in Table 4.2. Figure 4.5 gives plots of the predicted and observed responses.

Table 4.2 - Parameter estimates for eq. 4.6 for run 1 and run 2.

Parameter	ln(estimates)	Standard error
μ_{max}	1.0585	0.045987
Y_S	2.4472	0.11414
k_d	-4.3619	0.37473

The parameter estimates were precise and the very high correlations were removed. The correlation matrix for the parameters was

$$\rho_\theta = \begin{pmatrix} 1.0000 & 0.7217 & 0.4065 \\ 0.7217 & 1.0000 & 0.6562 \\ 0.4065 & 0.6562 & 1.0000 \end{pmatrix}$$

Inspection of Figure 4.5 showed that the model did not describe the observed behaviour. The assumption that the uptake of glucose was the only contributing factor to growth was not supported by the data. Inspection of the biomass and glucose data showed that the biomass growth phase clearly extends beyond the point where glucose reserves are depleted. A secondary substance, such as acetate, must have contributed to biomass growth. As mentioned above, acetate can be used as an auxiliary source of carbon and should therefore contribute to growth.

To account for this eq. 4.6 was extended to include acetate production and consumption. Based on results to this point, the "reduced" model form for growth was used instead of the Monod form.

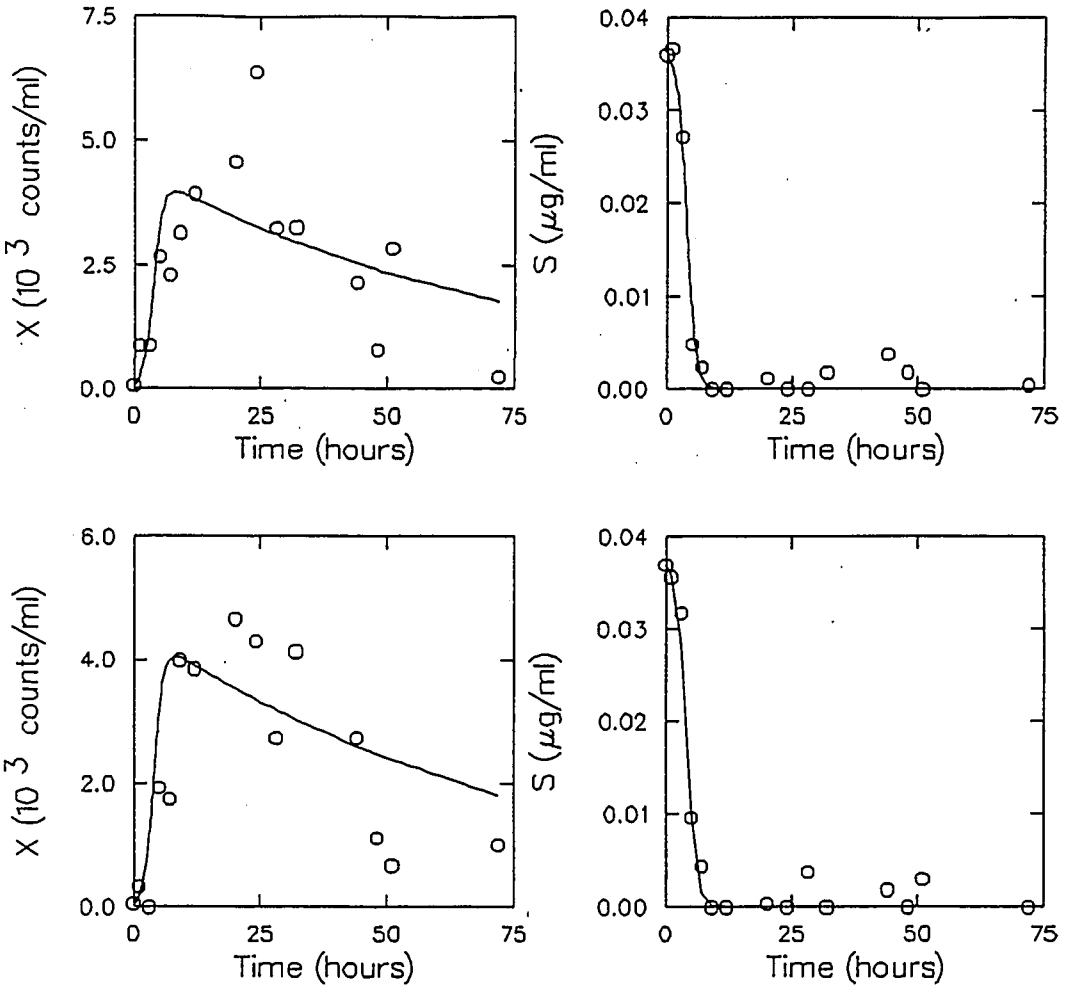

Figure 4.5 - Predicted (——) and observed (○) responses for the fit of eq. 4.6 to run 1 (bottom) and run 2 (top).

The rate of diauxic growth was described by

$$\mu = \mu_{rS} S + \mu_{rA} A \tag{4.7}$$

where A is the acetate concentration, μ_{rS} is the growth rate constant for substrate and μ_{rA} is the growth rate constant for acetate. Based on this expression, the reduced

model including acetate takes the form

$$\frac{dX}{dt} = \mu_{rS}XS + \mu_{rA}XA - k_dX$$

$$\frac{dS}{dt} = -\frac{1}{Y_S}\mu_{rS}XS \qquad (4.8)$$

$$\frac{dA}{dt} = \frac{1}{Y_A}\mu_{rS}XS - \frac{1}{Y_{AA}}\mu_{rA}XA$$

where Y_{AA} is the yield of biomass per unit mass of acetate and Y_A is the inverse yield of acetate per unit mass of biomass produced from glucose metabolism.

This model was fitted to biomass, glucose and acetate data from runs 1 and 2. The resulting fits are shown in Figure 4.6. Parameter estimates along with approximate standard errors are listed in Table 4.3.

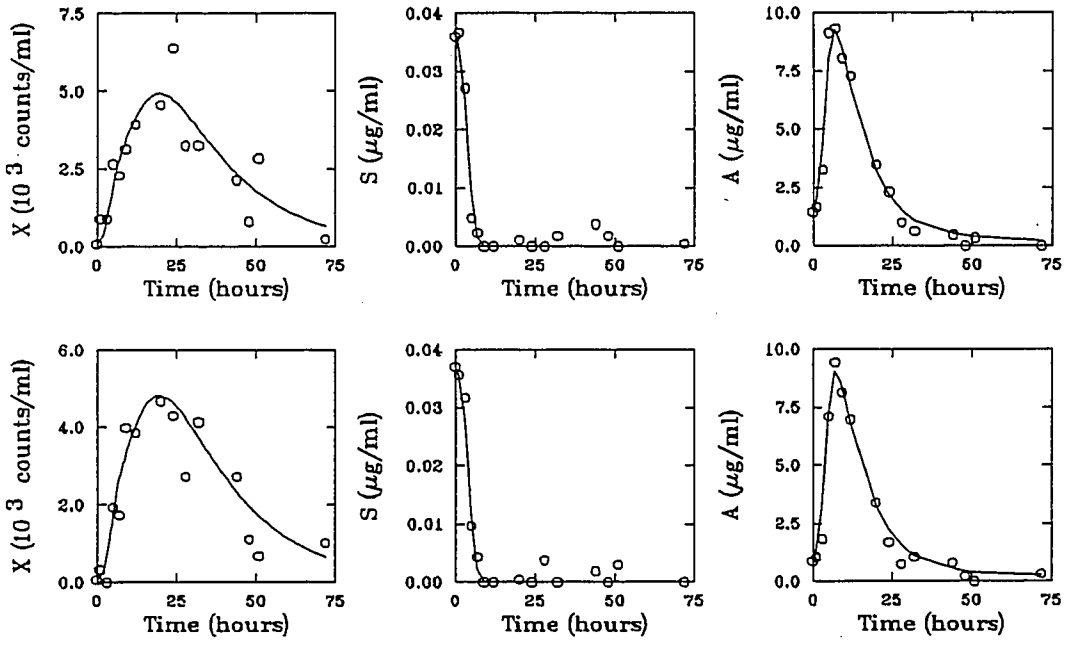

Figure 4.6 - Predicted (——) and observed (○) responses for the fit of eq. 4.8 to run 1 (bottom) and run 2 (top).

Table 4.3 - Parameter estimates for the reduced model with acetate (eq. 4.8) for runs 1 and 2.

Parameter	ln(estimates)	standard error
μ_{rS}	0.87931	0.045415
μ_{rA}	-1.8860	0.15626
Y_S	1.8444	0.12207
Y_A	0.87525	0.091819
Y_{AA}	2.0226	0.17746
k_d	-2.9680	0.15626

Inspection of Figure 4.6 suggested that the model was adequate. This implied that only acetate and glucose contributed to growth and was in agreement with prior knowledge of the system. Ethanol could then be considered a by-product.

Knowing that the production of ethanol results from the breakdown of glucose, the rate of ethanol formation was assumed to be proportional to the rate of uptake of glucose. However, the data showed a considerable drop in ethanol concentration. This had to be accounted for in the model. Since ethanol was not considered a secondary carbon source under these experimental conditions, the loss of ethanol was assumed to be due to evaporation. The rate of loss of ethanol by evaporation was assumed to be proportional to the concentration of ethanol. The resulting model was

$$\frac{dX}{dt} = \mu_{rS}XS + \mu_{rA}XA - k_dX$$

$$\frac{dS}{dt} = -\frac{1}{Y_S}\mu_{rS}XS$$

$$\frac{dA}{dt} = \frac{1}{Y_A}\mu_{rS}XS - \frac{1}{Y_{AA}}\mu_{rA}XA \qquad (4.9)$$

$$\frac{dE}{dt} = \frac{1}{Y_E}\mu_{rS}XS - k_{evap}E$$

where Y_E is the inverse yield of ethanol per unit mass of biomass and k_{evap} is the rate constant for evaporation of ethanol.

The model was fitted to run 1 and run 2 using data on all four responses. The results, shown in Figure 4.7, displayed no evidence of lack of fit. However, a high correlation (0.9568) was observed between Y_S and Y_A. Such high correlation is undesirable from the point of view of both the diagnostic analysis of the model and of the estimation of the parameters. It is an indication that Y_S and Y_A are not independent which can be misleading in the interpretation of the model. This dependence between the parameters also results in poor parameter precision. In the estimation of the parameters, high correlation between the parameters indicates a poor conditioning of the Hessian matrix which may cause serious problems. Because of this, it is recommended to remove dependencies between highly correlated parameters by re-parameterizing the model.

Eq. 4.9 was re-parameterize by letting $Y_A = Y_A' Y_S$, where Y_A' is the inverse yield of acetate per unit mass of glucose. By this re-pameterization, the rate of production of acetate is made explicitly proportional to the rate of utilization of glucose thus removing the dependence of Y_A on Y_S. The re-parameterized model thus took the form

$$\frac{dX}{dt} = \mu_{rS}XS + \mu_{rA}XA - k_d X$$

$$\frac{dS}{dt} = -\frac{1}{Y_S}\mu_{rS}XS$$

$$\frac{dA}{dt} = \frac{1}{Y_A' Y_s}\mu_{rS}XS - \frac{1}{Y_{AA}}\mu_{rA}XA \qquad (4.10)$$

$$\frac{dE}{dt} = \frac{1}{Y_E}\mu_{rS}XS - k_{evap}E$$

This model was fitted to runs 1 and 2. The parameter estimates with approximate standard errors are given in Table 4.4. Plots are given in Figure 4.7.

Table 4.4 - Parameter estimates for eq. 4.10 for runs 1 and 2.

Parameter	ln(estimates)	standard error
μ_{rS}	0.86666	0.045863
μ_{rA}	-1.8743	0.14121
Y_S	1.8293	0.12359
Y_A'	-0.96576	0.031896
Y_{AA}	2.0277	0.17227
k_d	-2.9760	0.15190
Y_E	-0.25437	0.21002
k_{evap}	-3.6653	0.35914

In fitting the re-parameterized model, the value of Y_A' was simply adjusted such that $Y_A = Y_A' Y_S$. The value of Y_S was unchanged and its precision was improved. Parameter values and standard errors of the remainder of the parameters were unchanged. As expected, the high correlation observed between Y_S and Y_A in eq. 4.9 was removed; the correlation between Y_S and Y_A' being -0.2915. The improvement in conditioning of the Hessian matrix may prove useful in further analysis of the model. Based on these results, it was concluded that eq. 4.10 constituted an adequate description of the observed behaviour from runs 1 and 2.

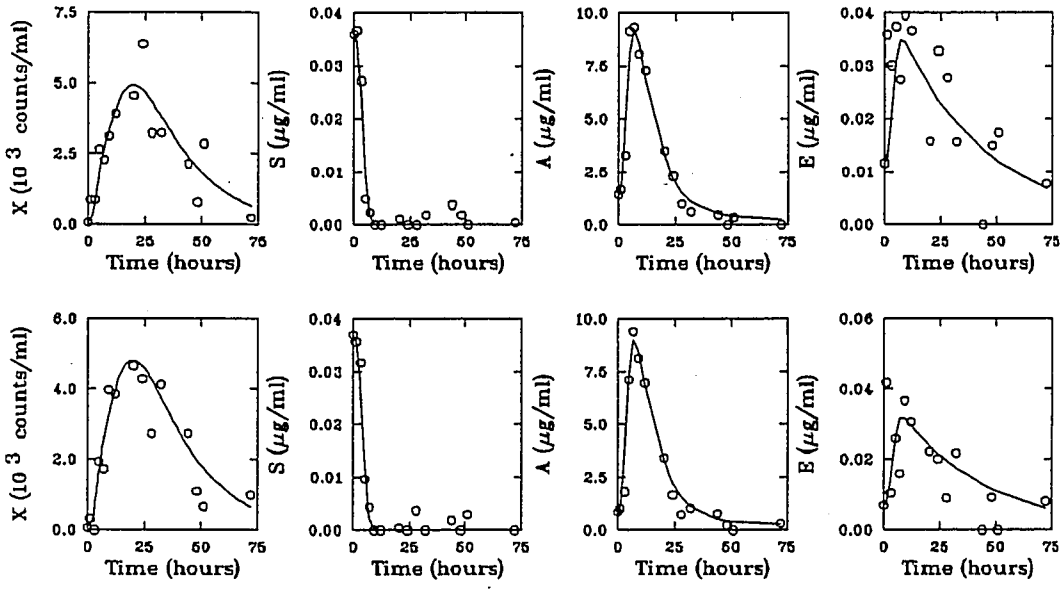

Figure 4.7 - Predicted (——) and observed (○) responses for the fit of eq. 4.9 and eq. 4.10 to run 1 (bottom) and run 2 (top).

The approach used to generate this model proved effective in this case. No particular problems were observed with the convergence of the multiresponse parameter estimation routine except for the first model. In this case, a logarithmic transformation of the model parameters was successful in circumventing these problems. Such problems would have been inevitable if a complex model such as eq.

4.10 had been fitted without any prior knowledge of the parameter values. To verify this, eq. 4.10 was fitted to data of runs 1 and 2. Initial estimates for μ_{rS} (3.3), Y_S (7.5), Y_A' (4.0) and Y_E (1.0) were obtained from inspection of the experimental data. Values of μ_{rA}, k_d, k_{evap} and Y_{AA} were given arbitrary values, 1.0, 0.01, 0.0001 and 1.0 respectively. Both the N method of Chapter 3 and a logarithmic transformation of the parameters were required to reach the optimal values listed in Table 4.4 in over 50 iterations.

The sequential addition of response variables combined with the consideration of expected mechanisms yielded an adequate model form which supported current knowledge of the process. Rigorous multiresponse statistical techniques were effectively used to fully utilize the information content of the data through more precise estimation of model parameters, detection of problematic, high correlation between parameters estimates and more sensitive testing of model adequacy.

This completed the initial stage of the model building strategy for the current problem. The result was a tentative model, eq. 4.10, for the operating conditions considered. In the second stage, additional measurements were required in order to verify the validity of the model at different operating conditions.

STAGE 2 Validation of Tentative Model(s)

Step 1 - Design of Experiments

Although an apparently adequate model had been found at this point, there was a need to improve parameter precision and, particularly, to check its applicability to other operating conditions. Both goals required further experimental results. To meet the first objective, a D-optimal design criterion could be applied to determine the best run location. The second objective could be met by simply selecting a different location in the operating region, preferably far from those already used. Generally, the optimal run locations for each objective would be expected to differ.

However, due to the limited amount of data, a D-optimal design could potentially meet both objectives. In other cases, where more data is available, it would be recommended to ensure validity of the model over the entire operating region before attempting improvement of parameter precision. This recommendation is based on the implicit assumption that a valid model is an a priori requirement for use of the D-optimal criterion. Application of the D-optimal design yielded a run location significantly different from those in runs 1 and 2 and was consequently selected for the next run.

Using information generated from the analysis of eq. 4.10, conditions for the next run were determined using a grid search over initial concentrations of biomass, glucose, acetate and the run time to locate the maximum of the D-optimal design surface, run 3 was selected with initial concentrations of biomass, glucose, acetate and ethanol of 20.0 counts/ml, 0.1 μg/ml, 1.00 μg/ml and 0.01 μg/ml respectively. The optimal run time was 10 hours. Clearly, it would have been wasteful not to take advantage of the batch experiment and restrict the extent of the investigation to the run location which optimizes the D-optimal criterion. Measurements were therefore taken throughout the batch growth cycle. To meet the D-optimal design requirement nine measurements of the 16 observations were taken between 5 and 15 hours. Other measurements were performed at equally spaced intervals of approximately four to five hours. The run was terminated after 43.5 hours.

Step 2 - Analysis

Eq. 4.10 was fitted to the three combined runs. Table 4.5 gives parameter estimates with approximate standard errors. Plots of the predicted and observed responses versus time are shown in Figure 4.8.

Table 4.5 - Parameter estimates for eq. 4.10 for run 1, 2 and 3.

Parameter	ln(estimates)	standard error
μ_{rS}	0.16967	0.075814
μ_{rA}	-2.5318	0.089995
Y_S	1.8771	0.13520
Y_A	-0.84734	0.078769
Y_{AA}	2.3124	0.14222
k_d	-2.7006	0.13111
Y_E	0.36551	0.16305
k_{evap}	-3.6040	0.20740

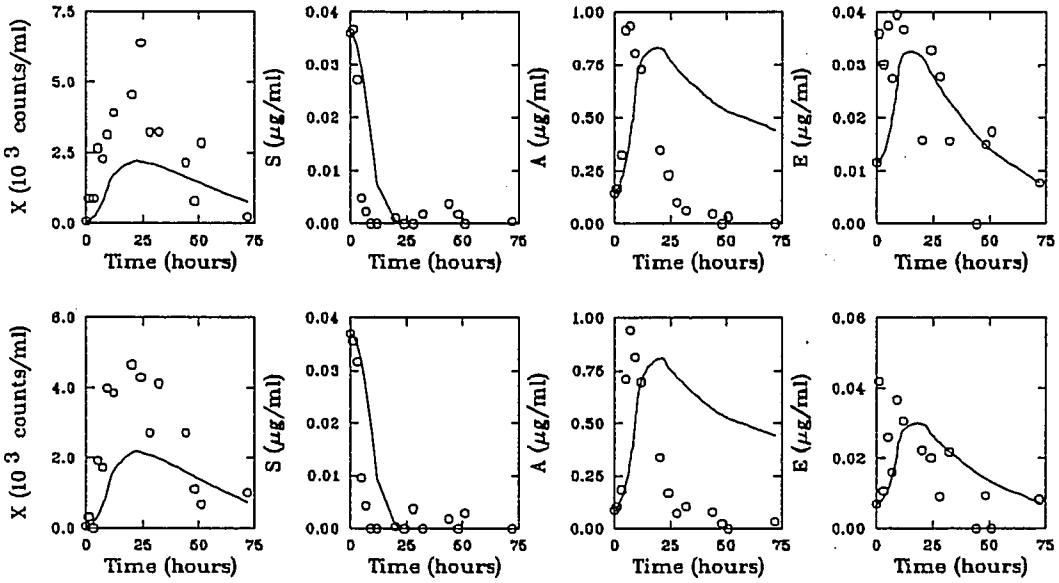

Figure 4.8 - Predicted (——) and observed (○) responses for the fit of eq. 4.10 to runs 1 (bottom row), 2 (middle row) and 3 (next page).

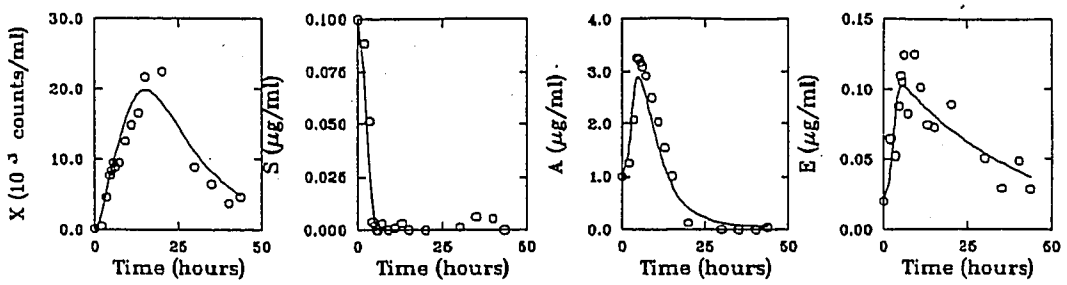

Figure 4.8 (cont'd) - Predicted (——) and observed (○) responses for the fit of eq. 4.10 to runs 1, 2 and 3. Shown here are the results for run 3.

Inspection of Figure 4.8 reveals significant lack of fit. In order to understand the dramatic change in adequacy of the model, eq. 4.10 was fitted to run 3 only. Any significant difference between the parameter estimates obtained and those estimated from runs 1 and 2 could possibly indicate the causes of this lack of fit. Parameter estimates showing a significant difference would point out which terms of the model have to be modified. The parameter estimates and approximate standard errors are given in Table 4.6.

Table 4.6 - Parameter estimates for eq. 4.10 for run 3.

Parameter	ln(estimates)	standard error
μ_{rS}	-0.038616	0.10379
μ_{rA}	-2.3968	0.072653
Y_S	1.6330	0.19704
Y_A	-0.95320	0.024902
Y_{AA}	2.4428	0.073731
k_d	-2.4223	0.11706
Y_E	-0.68904	0.22764
k_{evap}	-3.4500	0.30221

Comparing estimates of Table 4.4 and Table 4.6, it was seen that only μ_{rS} and μ_{rA} were significantly different for the two cases at the 95% confidence level (for run 3 $t_{8,0.05}$ = 1.860, for runs 1 and 2 $t_{16,0.05}$ = 1.725). The values of these parameters were significantly lower for run 3. One of the major differences between run 3 and runs 1 and 2 was the larger concentration of both glucose and acetate in run 3. This indicated the possible need for inverse relationships between the specific growth rates and glucose and acetate concentrations. The original Monod relationship for specific growth rate achieved this and was thus incorporated chosen to give a model of the form

$$
\begin{aligned}
\frac{dX}{dt} &= \frac{\mu_{maxS} XS}{K_S + S} + \frac{\mu_{maxA} XA}{K_A + A} - k_d X \\
\frac{dS}{dt} &= -\frac{1}{Y_S}\frac{\mu_{maxS} XS}{K_S + S} \\
\frac{dA}{dt} &= \frac{1}{Y_A / Y_S}\frac{\mu_{maxS} XS}{K_S + S} - \frac{1}{Y_{AA}}\frac{\mu_{maxA} XA}{K_A + A} \\
\frac{dE}{dt} &= \frac{1}{Y_E}\frac{\mu_{maxS} XS}{K_S + S} - k_{evap} E
\end{aligned}
\qquad (4.11)
$$

Eq. 4.11 was fitted to runs 1, 2 and 3. Since the model contained 4 unknown parameters, μ_{maxS}, K_S, μ_{maxA} and K_A, estimation was again performed sequentially. The expression for the specific growth rate of glucose was changed first. The parameters μ_{maxS} and K_S in eq. 4.11 were estimated along with μ_{rA}. Estimates of initial values for μ_{maxS} and K_S obtained from the fit of eq. 4.5 to runs 1 and 2 were used as initial estimates. μ_{maxS} and K_S were then estimated and used as initial estimates to fit eq. 4.11 to the three combined runs to yield estimates of μ_{maxA} and K_A and of all the other parameters. The estimates and approximate standard errors are shown in Table 4.7. Plots of the predicted and observed responses versus time are shown in Figure 4.9.

The model displayed no lack of fit and parameters were not highly correlated. Eq. 4.11 was therefore assumed adequate concluding Stage 2 of the model building exercise.

Table 4.7 - Parameter estimates for eq. 4.11 for run 1, 2 and 3.

Parameter	ln(estimates)	standard error
μ_{maxS}	-0.21313	0.019174
μ_{maxA}	-1.6185	0.034249
Y_S	1.9779	0.037723
Y_A	-0.90773	0.0067597
Y_{AA}	2.2965	0.041680
k_d	-2.5952	0.049665
Y_E	-0.28835	0.074264
k_{evap}	-3.7520	0.19094
K_S	-3.2945	0.12603
K_A	-0.71946	0.041937

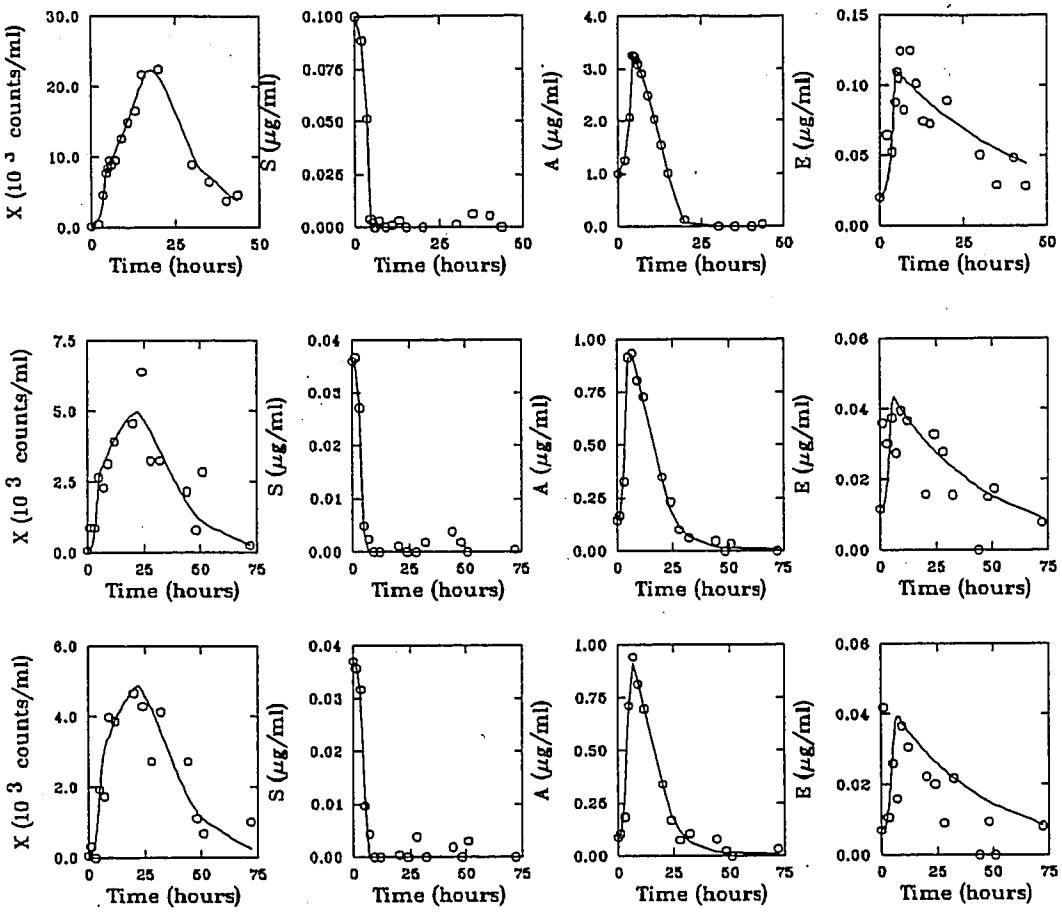

Figure 4.9 - Predicted (——) and observed (○) response for the fit of eq. 4.11 to runs 1 (bottom row), 2 (middle row) and 3 (top row).

Stage 3

Step 1 - Design

As should be appreciated, the situation was similar to that encountered at the conclusion of Stage 1. The design objectives were therefore similar. Further experiments could be designed to verify the applicability of the model and to improve the precision of the parameters. Adequacy of the model brought about the possibility of using a D-optimal design to improve parameter precision. Reasoning

as in the beginning of Stage 2, an attempt was made to meet both design objectives by using a D-optimal design in order to limit the amount of data required. The D-optimal design criterion yielded a run location significantly different from those of runs 1, 2 and 3 and was therefore selected for run 4. Initial conditions for this new design were 105 counts/ml biomass, 0.2 μg/ml glucose, 0.0 μg/ml acetate and 0.02 μg/ml ethanol. For this set of initial conditions the D-optimal design criterion was maximized for an observations taken at 7.5 hours. To meet the criterion 7 of the fourteen measurements were taken between 0 and 10 hours. The remaining 7 measurements were taken at equally spaced time intervals until termination of the batch run at 40 hours.

Step 2 - Analysis

Eq. 4.11 was fitted to runs 1, 2, 3 and 4 simultaneously. Table 4.8 gives the estimates and approximate standard errors. Predicted and observed responses plotted against time are shown in Figure 4.10.

Parameter estimates were not significantly different from those obtained from runs 1, 2 and 3. It was also observed that, overall, the precision of the parameter estimate was improved. The standard error of six parameter estimates was reduced. Inspection of Figure 4.10 showed no evidence of lack of fit. Eq. 4.11 was thus assumed to yield an adequate description of the process, concluding the third stage of the model building strategy.

Table 4.8 - Parameter estimates for eq. 4.11 for runs 1, 2, 3 and 4.

Parameter	ln(estimates)	standard error
μ_{maxS}	-0.24635	0.0075642
μ_{maxA}	-1.5884	0.030254
Y_S	1.9424	0.024727
Y_A	-0.91363	0.011053
Y_{AA}	2.3104	0.034079
k_d	-2.5683	0.034586
Y_E	-0.34921	0.043320
k_{evap}	-3.5107	0.097704
K_S	-3.5597	0.17062
K_A	-0.70640	0.10832

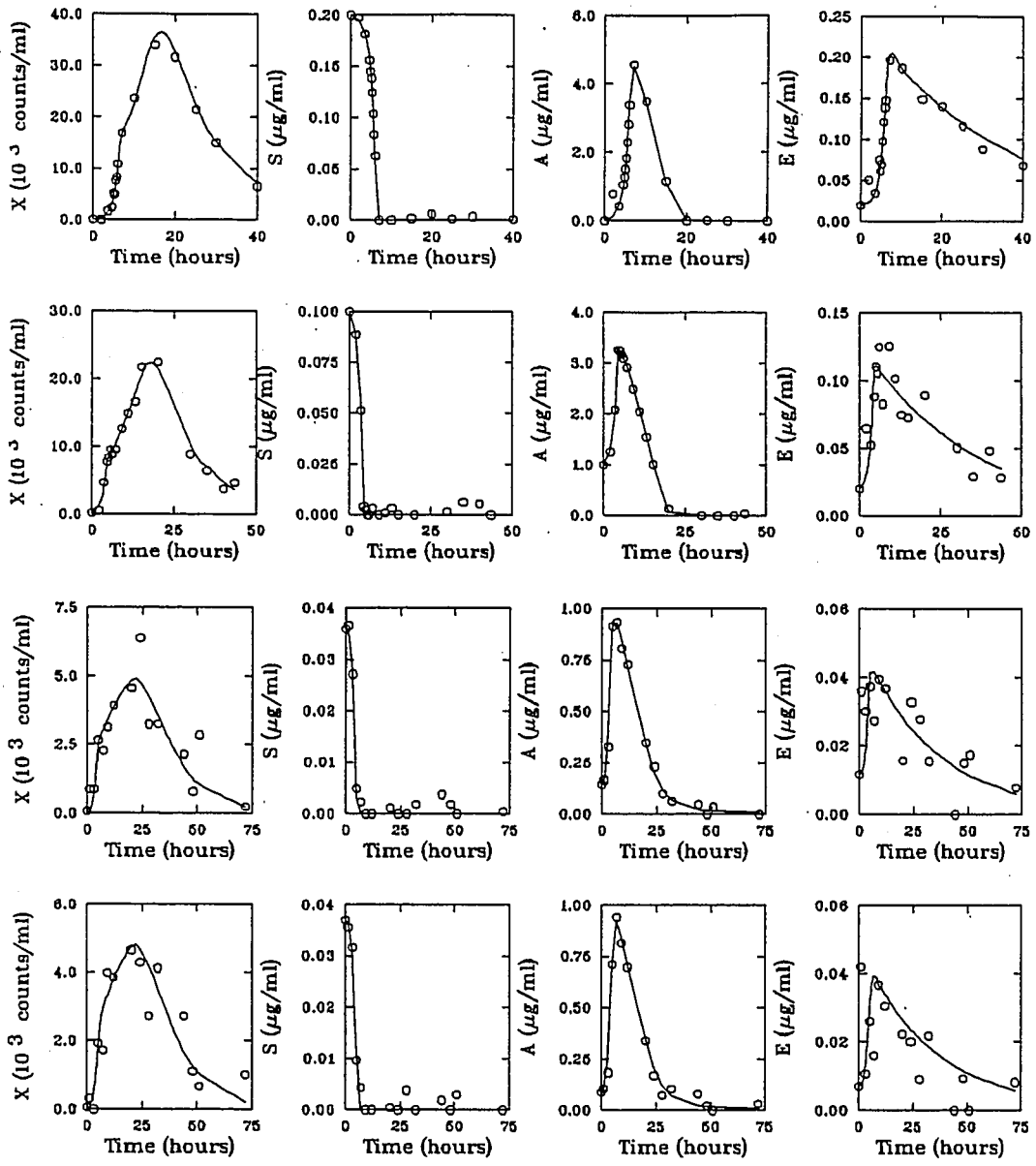

Figure 4.10 - Predicted (———) and observed (○) responses for the fit of eq. 4.11 to runs 1 (bottom row), 2 (second row), 3 (third row) and 4 (top row).

STAGE 4

Step 1 - Design

In Stage 3, a pseudo-mechanistic formulation of the process was developed. The precision of the parameter estimates and the agreement between the predicted and observed responses obtained at the conclusion of Stage 3 proved with confidence that eq. 4.11 was an adequate description of the process. It therefore seemed reasonable to simply consider the improvement of parameter precision as the prime design objective for Stage 4. Although it might also have been of interest to verify the applicability of eq. 4.11 and investigate a different run location, a D-optimal design was expected to generate much more valuable information about the process.

The D-optimal design criterion was used for the design of a fifth run location. The initial conditions were 50.0 counts/ml, 0.2 μg/ml, 2.0 μg/ml and 0.02 μg/ml for biomass, glucose, acetate and ethanol, respectively. According to the D-optimal criterion, the optimal time for the next observation was 16.75 hours. A set of 15 observations were taken between 2.0 and 30.0 hours. Seven measurements were taken between 15.0 and 20.0 hours in order to meet requirements of the D-optimal design.

In Figure 4.11, the locations of the five experimental runs are plotted in a three-dimensional grid which delimits the set of initial conditions of biomass, glucose and acetate inside the operating region. Although the previous four runs did cover a considerable range of operating conditions, addition of the fifth run obtained from optimization of the D-optimal design criterion provided an more appreciable range of different initial conditions.

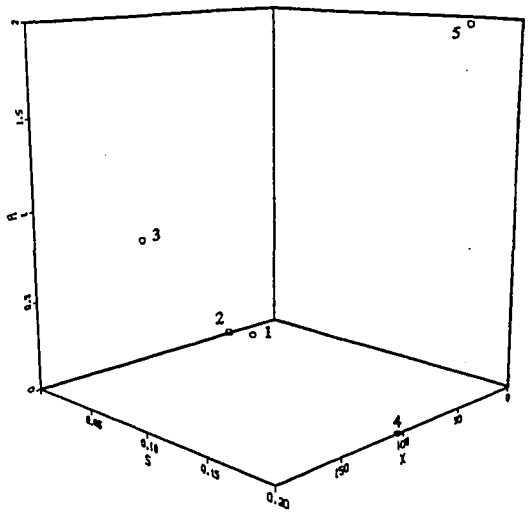

Figure 4.11 - Range of initial conditions of biomass, glucose and acetate which delimit the operating region of the process. The locations of runs 1, 2, 3, 4 and 5 are shown.

Step 2 - Analysis

Eq. 4.11 was fitted to runs 1, 2, 3, 4 and 5 simultaneously. Parameter estimates and approximate standard errors are given in Table 4.9. Plots of the predicted and observed responses versus time are given in Figure 4.12. The results strongly suggested that eq. 4.11 was an adequate model for the range of operating conditions considered. The precision of the parameter estimates was improved by the addition of run 5. Only the standard error of K_s was not show improved. Inspection of Figure 4.12 showed no evidence of lack of fit.

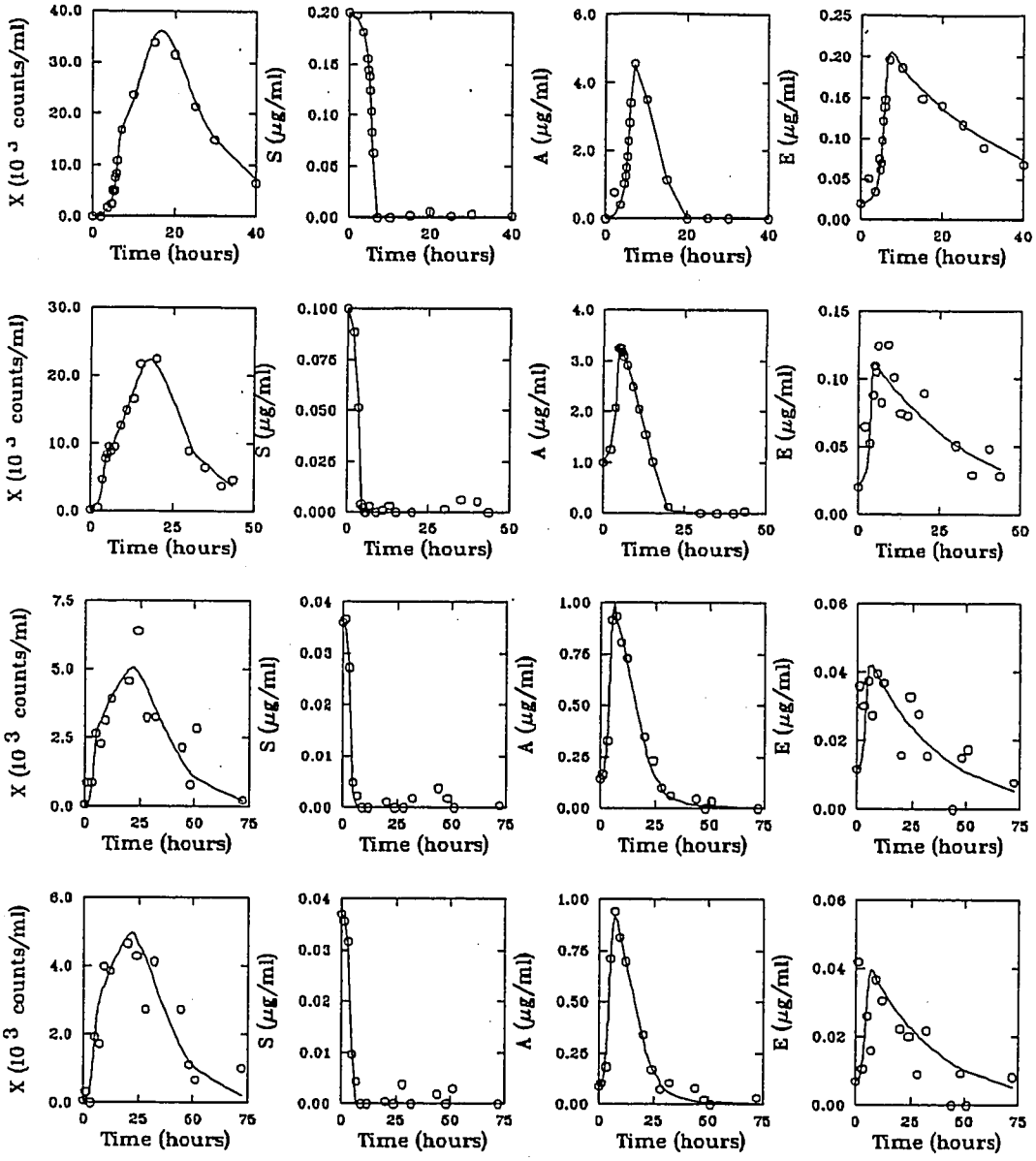

Figure 4.12 - Predicted (——) and observed (○) responses for the fit of eq. 4.11 to runs 1 (bottom), 2 (second row), 3 (third row), 4 (top), and 5 (next page).

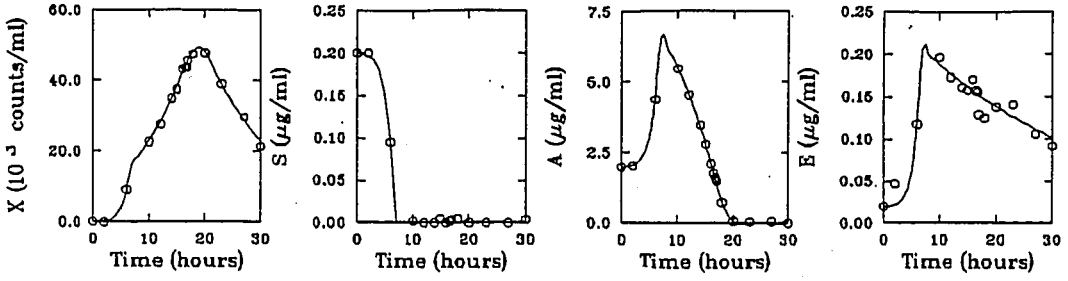

Figure 4.12 (cont'd) - Predicted (———) and observed (○) responses for the fit of eq. 4.11 to runs 1, 2, 3, 4 and 5. Shown here are the results for run 5.

Table 4.9 - Parameter estimates for eq. 4.11 for runs 1, 2, 3, 4 and 5.

Parameter	ln(estimates)	standard error
μ_{maxS}	-0.24714	0.0060660
μ_{maxA}	-1.6224	0.014986
Y_S	1.9568	0.018191
Y_A	-0.91438	0.0083596
Y_{AA}	2.2955	0.017949
k_d	-2.5842	0.022011
Y_E	-0.34381	0.034620
k_{evap}	-3.4669	0.078713
K_S	-3.6029	0.17539
K_A	-0.80143	0.069367

Since all parameters were sufficiently precise and since the model described adequately the process, there was no need to perform additional experiments. Eq. 4.11 was thus the final result of the model building exercise.

4.3.3 Comparison of model developed and the actual model

The appealing feature of using a simulation to test the model building strategy was that the model developed in the course of the strategy could be compared with the model and the parameter values used for the simulation. In this case, the model developed differed noticeably in form from the model used for the simulations. The maintenance energy term, $-m_{gluc}X$, and the substrate inhibition effect on acetate uptake, $K_{IS}/(K_{IS}+S)$, could not be identified. Since eq. 4.11 was shown to be adequate, it is clear that the two terms had very small effects on the responses. Addition of the terms in eq. 4.11 would not result in a noticeable change in behaviour. If both $K_{IS}/(K_{IS}+S) \approx 1$ and $m_{gluc}X \ll \mu_{maxS}XS/Y_S(K_S+S)$ then the two terms have no effect on the system.

This partially explains why both terms could not be identified in the model building exercise. The value of each term can be calculated using the values of m_{gluc} and K_{IS} used for the simulation (i.e., 4.0×10^{-8} μg/counts and 0.756 μg/ml respectively). A typical glucose concentration for the operating conditions considered is 0.1 μg/ml. At this concentration, the biomass concentration is of the order of 5.0 for run 4. The maintenance energy term is 2×10^{-4} μg/(ml hr) whereas $\mu_{maxS}XS/Y_S(K_S+S) = 0.79(5.0\times10^{3})(0.1)/(6.98\times10^{4})(0.03+0.1) = 0.043$ μg/(ml hr). This term has a negligible effect on the glucose uptake rate. At this same glucose concentration, $K_{IS}/(K_{IS}+S) = 0.88$. This could modify the responses of eq. 4.11. However it is not likely to have a noticeable effect in the present case since the growth rate term, $\mu_{maxA}XA/(K_A+A)$, is generally small when compared to $\mu_{maxS}XS/(K_S+S)$ at such glucose concentrations. In the model building exercise, the small effects of these terms at the range of operating conditions considered and the noise present in the experimental data contributed to masking the effect of each term.

Table 4.10 compares the parameter estimates with the parameter values assumed for the simulation. There was no significant statistical difference between the estimates, except μ_{maxS}, and the true values at the 95 % confidence level. μ_{maxS} was found to be significantly different at the 95 and 99 % confidence levels. This difference in parameter values is attributable to the absence of the inhibition term in eq. 4.11.

Table 4.10 - Parameter estimates for eq. 4.11 for runs 1, 2, 3, 4 and 5 and actual values used in the simulation.

Parameter	ln(estimates)	Confidence Intervals		ln(actual values)
		95%	99%	
μ_{maxS}	-0.24714	0.01012	0.01447	-0.22314
μ_{maxA}	-1.6224	0.02501	0.03574	-1.6094
Y_S	1.9568	0.03036	0.04339	1.9661
Y_A'	-0.91438	0.01395	0.01994	-0.90422
Y_{AA}	2.2955	0.02996	0.04281	2.3026
k_d	-2.5842	0.03674	0.05250	-2.6173
Y_E	-0.34381	0.05778	0.08257	-0.33648
k_{evap}	-3.4669	0.13137	0.18773	-3.4483
K_S	-3.6029	0.29273	0.41831	-3.3524
K_A	-0.80143	0.11577	0.16544	-0.69315
K_{IS}	Parameter Not Identified			1.9760
m_{gluc}	Parameter Not Identified			-9.0793

Note that $Y_E = 1/a_S a_E = 1/0.1*0.14 = 1/0.014 = 71.428$ in the original model. The original ethanol concentration was multiplied by 100 to improve conditioning in the estimation. This scaling yielded a value of (ln(71.428/100) =) -0.33648 for Y_E, as shown in Table 4.10. Similarly, $Y_S = 1/a_S$, $Y_A' = 1/a_1$, $Y_{AA} = 1/a_2$.

Although eq. 4.11 is adequate, it is reasonable to ask whether the inhibition term and maintenance term should have been detected in the model building exercise. In other words, it would be important to know if the introduction of either or both these terms in eq. 4.11 would result in a better description of the process. One way to illustrate this would be to fit an extension of eq. 4.11 which includes either or both the neglected terms to runs 1, 2, 3, 4 and 5 and compare the results with those obtained from the fit of eq. 4.11.

Since the glucose maintenance term clearly has no effect, only the inhibition term was included in eq. 4.11. The resulting model was given by

$$\begin{aligned}
\frac{dX}{dt} &= \frac{\mu_{maxS} XS}{K_S + S} + \frac{\mu_{maxA} XA}{K_A + A} \frac{K_{IS}}{K_{IS} + S} - k_d X \\
\frac{dS}{dt} &= -\frac{1}{Y_S} \frac{\mu_{maxS} XS}{K_S + S} \\
\frac{dA}{dt} &= \frac{1}{Y_A'} \frac{1}{Y_s} \frac{\mu_{maxS} XS}{K_S + S} - \frac{1}{Y_{AA}} \frac{\mu_{maxA} XA}{K_A + A} \frac{K_{IS}}{K_{IS} + S} \\
\frac{dE}{dt} &= \frac{1}{Y_E} \frac{\mu_{maxS} XS}{K_S + S} - k_{evap} E
\end{aligned} \tag{4.12}$$

An attempt was made to fit this model to the data from all five runs. However, severe numerical problems were encountered in the solution of the first order derivatives of the response variables with respect to the parameters (i.e., the first order sensitivity coefficients). As an example, the sensitivity coefficients with respect

to K_{Is} for Run 3 are shown in Figure 4.13. The sensitivity coefficients were calculated using the parameter estimates given in Table 4.11. K_{IS} was set equal to 0.4 μg/ml. This particular value of K_{IS} was chosen to exemplify a possible initial estimate for this parameter if eq. 4.12 was to be fitted.

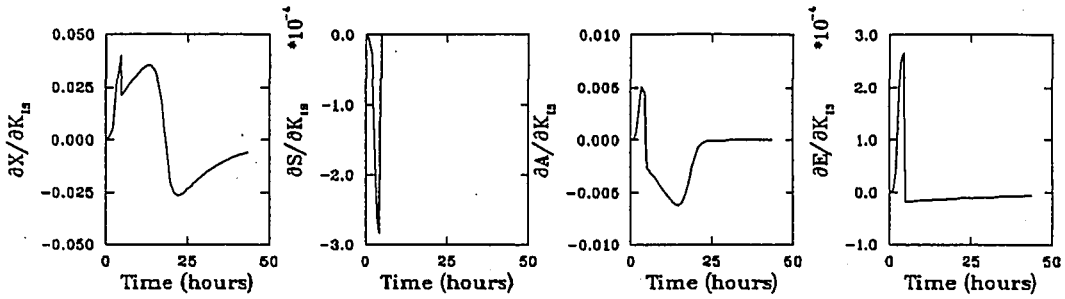

Figure 4.13 - First order sensitivity coefficients of X, S, A and E with respect to K_{IS} for run 3.

The sensitivity coefficients with respect to K_{IS} had greatly varying behaviours with respect to time. Furthermore, all four displayed discontinuities at t=5 hours. Such behaviour leads to serious numerical problems. The discontinuities are inherent and cannot be removed. The only remedies would use to use a good solution method for stiff ODEs such as Gear's BDF (see Chapter 3) or to use a different form to describe inhibition. The variation in behaviour can be attenuated by solving the scaled sensitivity coefficients given by $\partial \ln s_i / \partial \ln K_{IS}$, (i=1,...,K), where s_1=X, s_2=S, s_3=A and s_4=E. This technique was employed to fit eq. 4.12 to runs 1, 2, 3, 4 and 5 simultaneously. Parameter estimates and standard errors are given in Table 4.11. Figure 4.14 shows plots of the predicted and observed responses versus time.

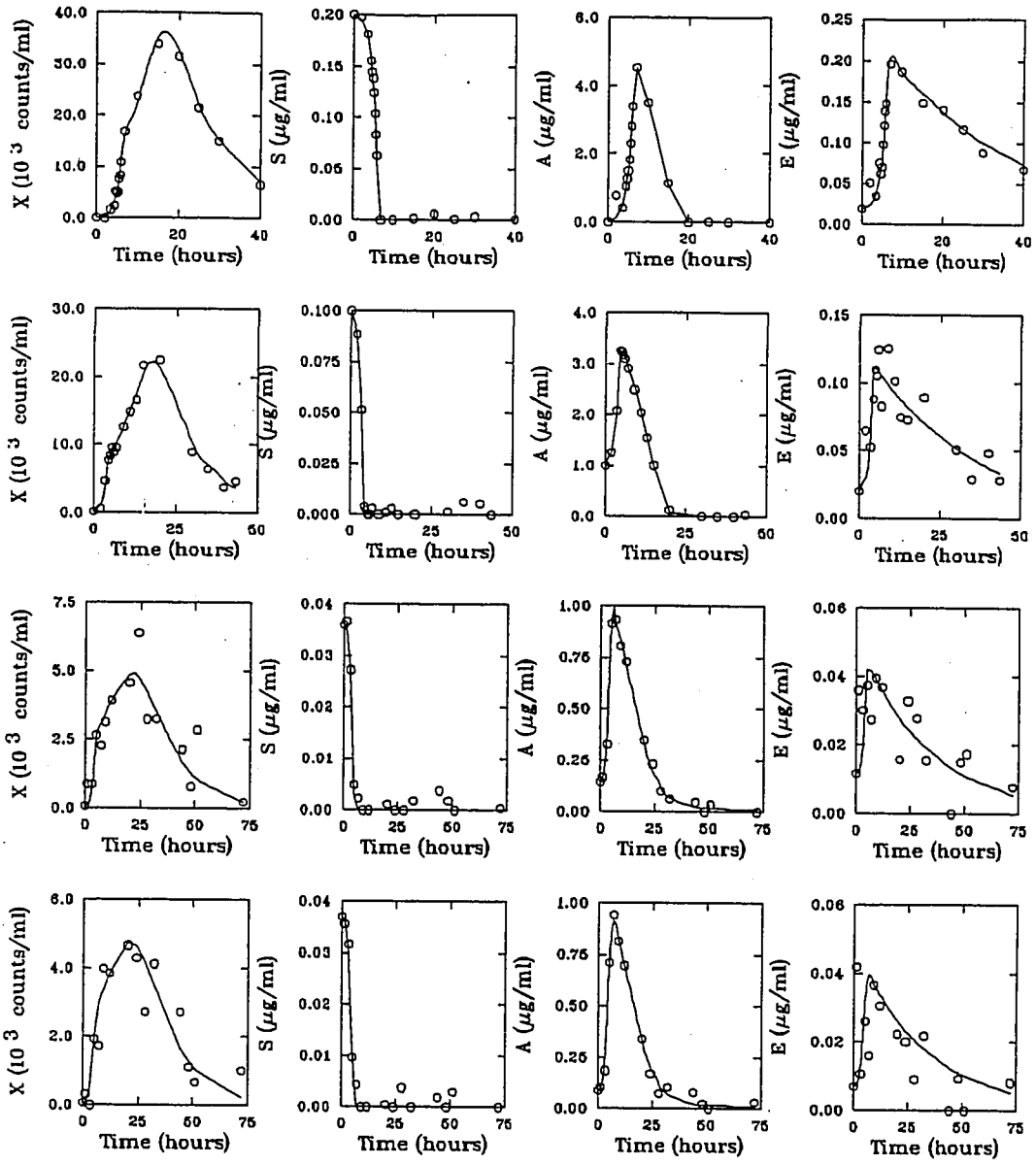

Figure 4.14 - Predicted (——) and observed (○) responses for the fit of eq. 4.12 to runs 1 (bottom), 2 (second row), 3 (third row), 4 (row) and 5 (next page).

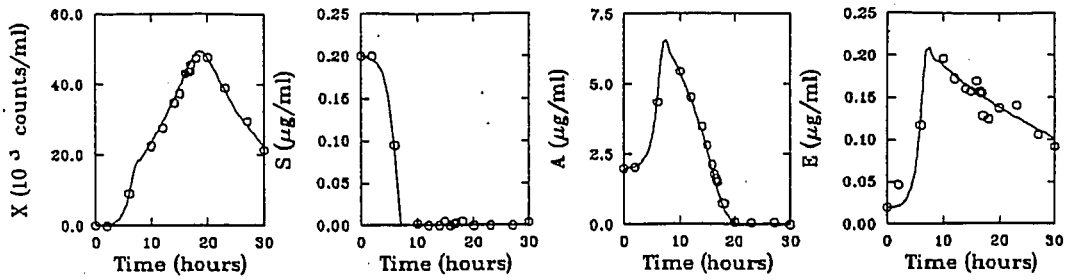

Figure 4.14 (cont'd) - Predicted (——) and observed (○) responses for the fit of eq. 4.12 to runs 1, 2, 3, 4, and 5. Shown here are the results for run 5.

Table 4.11 - Parameter estimates for eq. 4.12 for run 1, 2, 3, 4 and 5.

Parameter	ln(estimates)	standard error
μ_{maxS}	-0.23442	0.0070078
μ_{maxA}	-1.5968	0.016701
Y_S	1.9610	0.018055
Y_A	-0.90791	0.0084741
Y_{AA}	2.3047	0.017771
k_d	-2.5759	0.021439
Y_E	-0.33739	0.035220
k_{evap}	-3.4720	0.079905
K_S	-3.5671	0.15560
K_A	-0.70143	0.069560
K_{IS}	2.5144	0.35511

From inspection of Figure 4.14, the addition of the inhibition term produces no noticeable change in the fit. However, a slight decrease in the Box-Draper estimation criterion was observed. For the fit of eq. 4.11, $d(\theta)$ was 78.7 at convergence whereas the fit of eq. 4.12 yielded a value of 67.2. This small decrease does not however justify the need to include the inhibition term.

The need to include the inhibition term can be quantitatively tested by comparing the sum of squared residuals of the generalized least squares model of each fitted model. As was discussed in Chapter 2, the multiresponse model can be transformed to a single response model using an estimate of the pure error covariance matrix for the responses, Σ_{PE}. In this case, an estimate of the pure error covariance matrix can be obtained from the near replicated runs 1 and 2. The estimate obtained was

$$\Sigma_{PE} = \begin{pmatrix} 3.4486 & -0.050540 & 0.15408 & 2.6127 \\ -0.050540 & 0.0020833 & -0.0048428 & -0.075321 \\ 0.15408 & -0.048428 & 0.019199 & 0.16785 \\ 2.6127 & -0.075321 & 0.16785 & 4.0908 \end{pmatrix}$$

By performing a Cholesky decomposition of Σ_{PE}, a generalized least squares model can be obtained of the form

$$(R^{-T} \otimes I_N)\, y = (R^{-T} \otimes I_N)\, f + (R^{-T} \otimes I_N)\, z \qquad (4.13)$$

where **R** is the Cholesky factor of Σ_{PE}. The sum of squared residuals for this model is simply given by

$$SSR = \sum_{i=1}^{NM} e_i^2$$

where **e** is the NM-dimensional vector of transformed residuals given by

$$e = (R^{-T} \otimes I_N)\, z$$

This SSR has N-P degrees of freedom (Kang and Bates (1990)). A simple test to verify the need for the extra term takes the form

$$Q = \frac{SSR_P - SSR_{P+1}}{SSR_{P+1}} \qquad (4.14)$$

where SSR_P and SSR_{P+1} are the sums of squared residuals for the models with P and P+1 parameters respectively. Q is approximately distributed as an $F_{1,N-P;\alpha}$.

The SSR for eqs. 4.11 and 4.12 were 289.59 (=SSR_P) and 288.08 (= SSR_{P+1}) respectively. The resulting value of Q was 0.00524 and the test statistic, $F_{1,64,0.95}$, was approximately 1.85. According to this test, the addition of the inhibition term in eq. 4.12 was therefore not justified. Eq. 4.11 was the best possible model for the range of operating conditions considered.

Inspection of the parameter estimates for eq. 4.12 listed in Table 4.11 suggested however a different conclusion. Parameter estimates were not significantly different from the actual values and all parameters, including K_{IS}, were significant. No high correlation between the parameters could be detected. Based on these results, eq. 4.12 seemed to provide a better description of the process.

This sort of contradiction is unfortunately typical of nonlinear multiresponse modelling. The main problem is that most inference results and model validation techniques are based on a linear approximation of the model. Consequently such diagnostic tools are not effective when this approximation is poor. In the present case, both the lack of fit test and the parameter confidence intervals used to verify the need for the inhibition term are dependent on the validity of the linear approximation of the model. Because it is possible that neither of these approximate results reflect the actual situation, it is recommended to use such results very

cautiously and to rely on a qualitative evaluation of the fit of each model.

In the present case, inspection of the fit demonstrated that the model developed was adequate. The presence of an inhibition term was therefore never questioned in the model building exercise. In a case where the need for an inhibition term was of primary interest, it would be possible to design further experiments to test its need using the D-optimal design criterion.

4.4 Summary

In this simple example, the model building strategy was applied to a typical batch microbial culture problem. The strategy is an iterative procedure which combines rigorous multiresponse statistical techniques with process knowledge. Each iteration in the model building approach consists in three steps: conjecture, experimental design and data analysis.

The conjecture involves postulation of the mechanism of production of acetate and ethanol from glucose. The metabolism of the microorganism is carefully taken into consideration. Based on the assumed mechanism, a model is postulated. This process was demonstrated in Stage 1 of the model building exercise. A careful interpretation of the process knowledge led to the development of a tentative model form. Although this step was simplified by the use of simulated data for which a model form was known, there was an effort to imitate the intellectual process that could have been involved in a real situation.

The importance of proper experimental design was presented. The need to clearly define the design objectives was demonstrated at the beginning of each stage. At the beginning of Stage 1, when little was known about the model, the design objective was simply to produce scouting runs from which a tentative model form could be developed. At the beginning of Stages 2 and 3, the improvement of

parameter precision and the verification of the applicability of a model were the two design objectives. Because of limitations in the experimental expenditure, a D-optimal design was used to meet both objectives. At the beginning of Stage 4, the model had shown to be adequate for a significant range of operating conditions. Further validation of the model was not necessary. The D-optimal design criterion was used to improve the precision of the parameter estimates.

The example demonstrated the use of multiresponse parameter estimation in the analysis part of the model building strategy. Multiresponse techniques are extremely helpful for typical batch culture experiments. In this case, this technique was successfully used to estimate the parameters. It shows how the problem of generating initial estimates for a complex model at the beginning of the analysis can be circumvented. Simpler models can be used to generate approximate value of parameters common to the larger model postulated.

From the overall process, it was observed that the extent of knowledge of the system gradually increases at each iteration of the model building strategy. The mechanisms were well known, a fact that was taken into account. Starting from these mechanisms, a model was postulated. Upon further experimentation, the model proved to be wrong and an alternative formulation was assumed. This formulation was shown to be adequate and further experimentation allowed the improvement of parameter precision. In order to evaluate the effectiveness of the model building strategy, it was very informative to compare the model developed with the model used for the simulation. As pointed out, the inhibition of acetate uptake by glucose and the glucose maintenance energy requirements, could not be detected for the range of operating conditions considered. Eq. 4.11 developed was clearly the best possible model in this case. It was shown by simple calculations that these two terms had negligible effect on the model. This was also more formally shown by adding one of the neglected terms in eq. 4.11. This did not result in a significant improvement in the fit of the experimental data. Eq. 4.11 was truly the best possible

model to use in this case.

As discussed in Section 4.2, growth is a very complex phenomena which is virtually impossible to express accurately in a mathematical form. We cannot expect a mathematical model, even if adequate, to fully describe the detailed behaviour of the microorganisms. The high degree of refinement anticipated by complex descriptions of growth cannot, in general, be achieved in practice. This is partially due to the fact that many responses which may need to be expressed in a complex model may not be measurable. It is also due to the inevitable noise in the experimental data which may mask subtle effects. The restriction in the range of conditions investigated may also contribute to mask small effects. As shown by the example, a number of effects, such as the small maintenance energy effect in the uptake of glucose which was not detected, are not detectable even under the most rigorous conditions. From a practical point of view, the problem is not to neglect such effects but rather to take them into account in formulation of a model. Since the effects are not expressed, parameters related to these effects are very difficult to estimate precisely and independently. A model building strategy which combines rigorous statistical modelling techniques and process knowledge avoid such situations. It ensures that, at each step, the least number of model parameters are carried. Insignificant terms, included in the model, can thus be detected and removed after careful consideration of the adequacy of the reduced model. It provides a useful methodology for the modelling of complex processes and ensures reductions in computational expenditures. For the problem of modelling batch culture kinetics, this may help in the development of heuristic model forms which are not exceedingly complex or simple and which are applicable to practically relevant ranges of experimental conditions.

CHAPTER 5

Conclusions, Contributions and Recommendations

This thesis was devoted to the improvement of existing techniques to perform multiresponse parameter estimation in systems of ordinary differential equations, particularly with respect to their application to modelling the kinetics of microbial batch cultures. Contributions were made in different areas: optimization algorithms, simultaneous solution of ODEs and sensitivity coefficients and model building strategies.

5.1 Conclusions and Contributions

Concerning the development of optimization algorithms for multiresponse parameter estimation:

1. A general-purpose hybrid algorithm for optimization of the Box-Draper multiresponse parameter estimation criterion was developed. It incorporated a Gauss-Newton method with a DGW update formula. The method was shown to perform better than the Gauss-Newton method.

2. A second hybrid method which combined Gauss-Newton and a full Newton method for the optimization of the Box-Draper criterion was developed. This method outperformed other methods in terms of reliability without a great increase in computational requirements. For large problems (i.e., large values of N, K and P) the computational requirements may become prohibitive.

3. Both algorithms were implemented in FORTRAN codes.

Concerning the simultaneous solution of model equations and sensitivity

coefficients in ODEs:

4. The decoupled direct method (DDM) of Leis and Kramer (1988) for simultaneous solution of model equations and first-order parametric sensitivity coefficients in ODEs was implemented in a multiresponse parameter estimation routine. A new error control strategy was developed which improved the accuracy of the first-order sensitivity coefficients and retained the computational efficiency of DDM. An unsuspected improvement in computational efficiency was even observed in the solution of two of the three test examples. These improvements were found to improve the robustness of the estimation algorithm by facilitating the calculation of the sensitivity coefficients essential to the calculation of the gradient and Hessian of the objective function with respect to the parameters.

5. DDM was then extended to include the solution of second order sensitivity coefficients. The introduction of second order sensitivity coefficients in the calculation of the Hessian matrix in optimization scheme for the Box-Draper estimation criterion was shown to considerably improve the robustness of the multiresponse parameter estimation algorithm. The resulting parameter estimation routine clearly outperformed all other routines. This increase in robustness is gained at the cost of an increase in computing which is especially significant for larger problems. Because the solution of second order sensitivity coefficients also required an increase in coding, parameter estimation in larger models should usually be performed with simpler first order methods such as the new hybrid methods.

6. The two algorithms for the calculation of first and second order sensitivity coefficients in systems of ordinary differential equations were implemented as FORTRAN subroutines.

Concerning the development of model building strategies:

7. A multiresponse model building strategy was demonstrated for the study of batch culture kinetics. The basis of the strategy is an iterative process in which conjecture, experimental design and data analysis are combined to allow the sequential increase in process knowledge. It was shown that the close interaction of rigorous statistical techniques and process knowledge could facilitate the development of complex nonlinear models for the description of batch culture kinetics. Development of a model for the batch culture of *E. coli* demonstrated the benefits of the model building strategy. By initially using simple model forms to gather information about the system, it was shown how a sequential approach could be used to build a more complex model form in a manner which avoided problems with the estimation of parameters. The combination of carefully chosen experimental designs, process knowledge and rigorous statistical analysis techniques was shown to provide a good method to identify inadequacy of a model. The importance of good numerical techniques for the solution of differential equations was made apparent.

8. A number of useful techniques, such as empirical parameter transformations, parameter scaling and data scaling, were demonstrated which circumvented some problems commonly encountered in multiresponse parameter estimation. In cases where a model was developed from a restricted amount of data, a sequential approach was found to be particularly helpful in the formulation of an adequate model when little is known about the process. This approach proposes to fit simple model forms initially and add responses in a sequential manner in order to arrive to more complex model forms.

5.2 Recommendations

Throughout this study a number of recommendations were made with respect to parameter estimation.

1. One of the main observations was that complex model forms should be avoided when little is known about the parameters of a model. It was found to be much easier to initially fit a smaller model which had a few parameters in common with the more complex form. Information could be gathered about the parameter values allowing estimation of parameters of the more complex model to proceed.

2. It is highly recommended to use an estimation method which incorporates the evaluation of second order sensitivity coefficients. The increased robustness of the estimation routine greatly facilitates the analysis. Estimation routines based on first order approximations should be reserved for larger models. One great advantage of having a routine for the calculation of second order derivatives is that it allows the possibility of computing relative curvature measures such as those described by Bates and Watts (1980). These measures would be very helpful in the diagnostic testing of a model.

A number of problems were encountered in the multiresponse analysis of systems described by ODEs that could be addressed in future work.

3. An important part of a model building strategy is the ability to demonstrate the validity of the model. However, multiresponse analysis is frowned upon because of the lack of effective statistical techniques to perform quantitative lack of fit tests or generate inference results for general nonlinear multiresponse models. Most techniques currently available rely on linear approximations of the model function near optimal values of the parameters which are not, in general, appropriate. Consequently most attempts to analyze a model using approximate techniques may often be futile. To verify the applicability of the linear approximations, curvature measures (perhaps similar to those developed by Bates and Watts (1980) for the single response case) would be helpful to many aspects of multiresponse model building.

4. Following the work of Gallant (1987), the generalized least-squares approach to multiresponse model building could prove to be of considerable help in the development of new and effective inference techniques. This approach has already been applied by Kang and Bates (1990) to develop a procedure for the calculation of inference results. The attractive feature of the generalized least-squares approach is that it transforms the multiresponse model to a single response one. Simple lack-of-fit tests applicable to the single response case could therefore be applied to the multiresponse case. The prospect of a further investigation of this approach is promising.

5. Some advantages of using second-order sensitivity coefficients in performing multiresponse parameter estimation in systems of ODEs were demonstrated in this thesis. However, only one second order optimization method was considered. Although this method was shown to be robust, it is reasonable to postulate that further improvements of this method could lead to even more robust methods. A further investigation of full second order methods is required.

6. Simple logarithmic transformations of the parameters was shown to improve the convergence properties of nonlinear optimization methods in multiresponse parameter estimation. A proper investigation of the full implication of empirical parameter transformation and parameter scaling still lags in the study of multiresponse parameter estimation. Of particular interest would be a technique to select the best transformations and scaling for a given parameter estimation problem.

7. One promising potential use of the model building strategy is in the development of expert systems designed to help in developing mathematical models for batch culture kinetics.

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Appendix A

Models Describing Batch Culture Kinetics

A good knowledge of potential model forms for describing batch culture kinetics is essential to any model-building strategy. In this Appendix, major classifications of models describing batch culture kinetics are presented.

1. Unstructured Models

The simplest models are of the distributed unstructured type. They are based on the assumption that the biomass is evenly distributed throughout the medium and that the biomass concentration is a complete representation of the function and state of the microorganism in the medium. Most models of this type are based on the popular Monod expression.

Monod's model (Monod, (1959)) is often considered to be the first real attempt to develop a mathematical model for batch microbial growth. In the formulation of the model, Monod assumed that the growth rate was a function of concentration of the limiting nutrient. Assuming that growth is the end result of a series of enzymatic reactions with one reaction being much slower then the others, the equation relating growth rate and limiting substrate concentration becomes analogous to the Michaelis-Menton model for enzyme kinetics. This equation is of the form

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{k_S + S} \qquad \text{(A.1)}$$

where μ_{max} = maximum specific growth rate

X = biomass concentration

S = substrate concentration

k_s = substrate saturation constant.

The rate of substrate uptake is generally assumed to be proportional to the rate of growth. It is written as

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} \qquad \text{(A.2)}$$

where Y is the yield of biomass per unit mass of substrate. Y is generally treated as constant regardless of the experimental conditions considered.

It is clear that this model cannot be used to describe all phases of microbial growth. It cannot account for lag or decline phases. It can, however, qualitatively describe the behaviour observed and be used to model the exponential part of the growth curve.

Although Monod's expression is the most common model form used in modelling microbial growth, it is not the only one. A number of reviews of simple unstructured models exist in the literature. Roels and Kossen (1978) provided a very good review of which only the highlights will be presented here. In their review, models are separated into four groups. The first group of models contains those for which growth rate is solely dependent on substrate concentration. In the second group, it is assumed dependent on population density. The third group contains cases where growth rate is dependent on concentration of many substrates. The fourth group are somewhat different and are commonly termed logistic models of growth. These different groups are briefly discussed here.

1.1 Models Assuming Dependence of Growth Rate on Concentration of the Limiting Substrate

Moser (1958) proposed a model of the form

$$\mu = \frac{\mu_{max} S^{\lambda}}{k_S + S^{\lambda}} \qquad (A.3)$$

where μ = maximum specific growth rate

λ = constant.

This model is similar to Monod's expression with $\lambda = 1$. The problem with this model is that there is no physical, chemical or biochemical significance attached to λ. It would therefore not be ideal as a basis for the development of a "pseudo-mechanistic" description of growth. Monod's model, however, does bear some "pseudo-mechanistic" meaning which may not be shared by the model presented above if $\lambda \neq 1$.

Since substrate must penetrate the cell wall, it would seem appropriate to account for possible diffusional resistance to the transfer of soluble substrate from the medium to the inside of the cell at low substrate concentration. Powell (1967) postulated a model that attempts to describe this effect. His model was

$$\mu = \frac{\mu_{max}(K + L + S)}{2L}\left[1 - \sqrt{1 - \frac{4LS}{(K + L + S)^2}}\right] \qquad (A.4)$$

where K = saturation constant

L = q/F

q = maximum specific rate of substrate intake

F = apparent mass transfer coefficient of cell wall.

This is often approximated by

$$\mu = \mu_{max}\frac{S}{(K + L + S)} \qquad (A.5)$$

Obviously, it will be impossible to estimate K and L separately. The estimation problem

reduces to the estimation of $k_s = K + L$ which is basically the Monod saturation constant.

An alternative relationship between growth rate and substrate concentration was proposed by Yamashita *et al.* (1969). This model was successfully used where the limiting substrate was not the primary carbon source. The postulated model has the form

$$\mu = \frac{\mu_{max} S_0 - L_m X}{KX + S_0 + L_m X} \qquad \text{(A.6)}$$

where S_0 = initial concentration of limiting substrate

L_m = minimum concentration of limiting substrate necessary per unit of biomass

K = constant.

It was mentioned above that Monod's expression was based on the assumption that microbial growth is the result of a series of reactions one of which is slower then the others. One can assume that there are more then one slow reactions required for growth. Suppose the mechanism consists of a series of reversible enzymatic reactions written as

$$S + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P_1 + E_1$$

.

.

.

$$P_{N-1} + E_N \underset{k'_{-N}}{\overset{k'_N}{\rightleftharpoons}} P_N + E_N$$

According to Dabes *et al.* (1973), this leads to an expression for the substrate concentration in terms of υ, the rate of substrate uptake (dS/dt). Their expression had the following

general form

$$[S] = \sum_{n=1}^{N} \frac{K_n \prod_{i=1}^{n} (V_{B_{i-1}} + \upsilon)}{\prod_{i=1}^{n} \frac{K'_{i-1}}{K_{i-1}} (V_{F_i} - \upsilon)} + \frac{[P] \prod_{n=1}^{N} (V_{B_n} + \upsilon)}{\prod_{n=1}^{N} \frac{K'_n}{K_n (V_{F_n} - \upsilon)}} \qquad \text{(A.7)}$$

where [S] = substrate concentration

$[P_N]$ = final product concentration

V_{Fn} = $k'_n E_{on}$ (maximum forward rate)

V_{Bn} = $k_{-n} E_{on}$ (maximum backward rate)

E_{on} = total amount of the n^{th} enzyme

K_n = $(k_{-n} + k'_n)/k_n$

K'_n = $(k_{-n} + k'_n)/k'_{-n}$

K'_0/K_0 ≡ 1.

Assuming that the substrate uptake rate is proportional to μ, this equation for substrate concentration becomes an expression for μ. For most cases, it is difficult to obtain an explicit relationship for υ, some simplifying assumptions are required. For example, if it is assumed that only one reaction is much slower than the others then the expression reduces to the Monod model. Assuming that two reactions are slower, the first one effectively setting the value of k in a Michaelis-Menton relation and the second determining the overall rate, an expression of the form

$$S = \mu A + \frac{\mu B}{\mu_{max} - \mu} \qquad \text{(A.8)}$$

can be obtained, where A,B are constants. This could be reduced to a pair of straight lines given by

$$\mu = \left\{ \begin{array}{ll} S/A & ; S < \mu_{max} A \\ \mu_{max} & ; S \geq \mu_{max} A \end{array} \right\} \qquad \text{(A.9)}$$

This is the Blackman model (1905). It is based on the basic assumption that the growth rate depends upon substrate only until all the active sites are saturated. This is considered,in many cases, to be a better description of microbial growth than the Monod model.

There is another family of model forms based on a development by Konak (1974) which yielded a general relationship of the form

$$\frac{\partial \mu}{\partial S} = k(\mu_{max} - \mu)^p \qquad \text{(A.10)}$$

where k,p = constants. Depending on the value of p, this expression gives rise to a number of different models. If p=1,

$$\mu = \mu_{max}(1 - e^{-kS}) \qquad \text{(A.11)}$$

an earlier model postulated by Teissier (1936). Similarly if p=2

$$\mu = \frac{\mu_{max} S}{\frac{1}{\mu_{max} k} + S} \qquad \text{(A.12)}$$

which is similar to Monod's model. In general, if p $\neq$ 1 then

$$\mu_{max}^{p-1} - (\mu_{max} - \mu)^{1-p} = (1 - p)kS \qquad \text{(A.13)}$$

Although mathematically attractive, this formulation lacks any real biochemical significance. A model building approach such as the one discussed here would not favour such a model

form because of its poor mechanistic properties.

Another family of models originates from work by Koch (1982) who proposed a series of mechanisms for nutrient uptake. The basic assumption of the formulation of such models is that the intracellular substrate concentration is the most important factor for growth rather then the extracellular concentration. Koch presented three cases based on this assumption and a variety of mechanisms for nutrient uptake. The first case is

$$\mu = \frac{\mu_{max} S}{(K_{en} + S)} - \frac{kK\mu}{(\mu_{max} - \mu)} \qquad (A.14)$$

where K_{en} = constant associated with transport through the cell membrane
K = intracellular saturation constant
k = rate constant for nutrient exit from cell.

The second case is

$$\mu = \frac{\mu_{max}(S + K + J)}{2J}\left[1 - \sqrt{\frac{1 - 4SJ}{(S + L + J)^2}}\right] \qquad (A.15)$$

where K = saturation constant for substrate
J = μ_{max}/AP
A = surface area of the cells
P = permeability of the cells.

This is model is identical to Powell's model (eq. A.4). The third case gives

$$\mu = \frac{V_1 S}{k_1 + S} + \frac{V_2 S}{k_2 + S}, \qquad (A.16)$$

where V_i = maximum rate of uptake for system
k_i = saturation constant for system, i = 1,2.

1.2 Models Based on Substrate Concentration and Population Density

The most widely recognized model based on substrate concentration and population density is the model developed by Contois (1959). From work with continuous cultures, he defined growth rate in terms of the population density and substrate concentration in the general form

$$\mu = \frac{\mu_{max} S}{bP + S} \qquad \text{(A.17)}$$

where P = population density
b = constant.

These models are known to provide better fits then the other models, such as Monod's model. This is mainly because growth rate depends on a number of additional factors, other then the limiting substrate concentration, such as oxygen concentration or trace nutrients which are not accounted for in the simple models. Since maximum biomass concentration ultimately depends on these factors, models such as the one shown above are able to partially account for their effects on growth rate. This fact alone is not a justification for using population density models but merely a statement of their potential advantage over simpler models.

1.3 Models Incorporating Effects of more then one Substrate

Cases in which growth is dependent on the concentrations of more then one substrate are common. There have been many efforts to model such mechanisms but the most general developments were given by Tsao and Hanson (1975). Their model was given by

$$\mu = \left(1 + \sum_i \frac{k_{ie_i}}{k_{ei} + ei} \right) \left(\prod_j \frac{\mu_{max_j} S_j}{k_{Sj} + S_j} \right) \qquad \text{(A.19)}$$

where S_i = concentration of the i^{th} substrate

E_i = concentration of the i^{th} growth enhancer

k_{Si}, k_{ei} = saturation constants for i^{th} substrate.

1.4 Models Based on the Logistic Law

The logistic law of modelling microbial growth assumes that the growth rate is a function of the biomass concentration. This is a very old concept and its most common formulation is a model due to Verhulst and Pearl (see Frederickson and Tsuchiya (1977)) of the form

$$\mu = \mu_{max} \left(1 - \frac{X}{X_{max}} \right) \qquad \text{(A.20)}$$

where X_{max} = maximum biomass concentration.

An alternative form was used by Edwards and Wilke (1968),

$$y = \frac{K}{a + \exp(f(t_i))} \qquad \text{(A.21)}$$
$$f(t) = a_0 + a_1 t + a_2 t^2$$

where y = concentration of cells

K = constant

a = cell concentration in the stationary phase

$f(t_i)$ = ratio of initial to final cell concentration.

Approximation of f(t) as a polynomial allows the possibility to describe all phases of microbial growth. However, this model does not take into account the initial substrate

concentration. This means that a different equation will be required for each initial substrate concentration. Another substrate-independent model was proposed by Konak (1975) of the form

$$\frac{dX}{dt} = k_{(2)} N_{\infty}^{a+b-1} X^{a} (1 - X)^{b} \qquad \text{(B.22)}$$

where $X = N/N_{\infty}$ = fractional increase in population density

N = number of cells

$a, b, k_{(2)}$ = constants.

Since the model expresses biomass by the total number of cells, it would appear to belong to a class of segregated models. This is by definition true. However the model attempts to describe only the growth phase where constant average cell size can be assumed. Under such conditions this model can be treated as either a segregated or a distributed model.

2. Extended Unstructured Model

Simple models such as the ones described above often fail to adequately represent microbial growth. As discussed, most of these models are oversimplified and only attempt to fit parts of the growth curve. They are not suited for modelling the complete mechanism. The common way to deal with such inadequacies has been to extend these basic model forms in order to incorporate other possible effects such as the effect of the accumulation of toxic products, the effect of the inhibition by the product etc. These developments yield a number of extended unstructured models, most of which are based on the Monod expression for growth rate.

2.1 The Concepts of Maintenance Energy and Death

It has often been observed that the yield constant of biomass formed per amount of substrate used varies with changes in growth rate. There have been several attempts to

account for this affect. One common interpretation proposes that substrate is not solely used for growth but that a certain fraction is used to supply maintenance energy, i.e. energy required for cellular activities other then cell growth and cell division such as motion, active transport through the membrane. Pirt (1965) developed a model based on the Monod model that accounted for the rate of uptake of substrate for maintenance energy requirement. His model for substrate, based on the assumption that rate of uptake for maintenance is proportional to biomass concentration, takes the form

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_{max} XS}{k_S + S} - mX \qquad \text{(A.23)}$$

where m is the specific maintenance energy requirement.

Y thus becomes the yield of biomass per unit substrate used for growth only. The overall yield becomes a function of μ, X and s. This model was proved adequate in studies involving continuous cultures. In the case of batch cultures however, it does seem to perform as well. For batch growth of microbial cells, it is generally accepted that biomass concentration reaches a maximum when substrate concentration vanishes. The introduction of a maintenance term means that even when substrate concentration is zero, substrate continues to be utilized at a rate equal to mX. This is obviously not the case and the model is suspect if one attempts to use it to describe the entire growth curve.

An alternative formulation of maintenance energy requirements was given by Herbert (1958). The model was developed under the assumption that maintenance energy requirements for the biomass are directly obtained from the biomass. That is, the cell is able to draw energy from its own mass. The model was stated as

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{k_S + S} - \alpha X \qquad \text{(A.24)}$$

where α is the specific rate of decline which is equal to the specific death rate.

Once substrate concentration vanishes it is clear that the biomass concentration starts decreasing. Monod's model which assumes that biomass remains constant once substrate reserves are depleted cannot be used. Biomass decline can have different interpretations, depending on whether the total or viable biomass is considered. Disappearance of total biomass can be only due to destruction of the cells through lysis. Disappearance of viable biomass is due to actual death of the cells. To avoid confusion, many studies include measurement of both total and viable counts. In both cases, a decline has to be observed at one point. For that reason, a term is usually added to account for disappearance of biomass. If the death rate is assumed to be proportional to the biomass concentration then eq. A.25 results. Although this model does describe many situations quite well, it is often inadequate for cases where long stationary phases are observed.

Another interpretation of death of biomass was proposed by Ramkrishna *et al.* (1966). In his work, he postulated a series of models developed under the assumption that the death is due to some kind of interaction between biomass and an inhibitory product. His basic model has the general form

$$\frac{dC_v}{dt} = \frac{\mu_{max} S C_v}{k_S + S} - K C_T C_v$$

$$\frac{dS}{dt} = -\frac{a_S \mu_{max} S C_v}{k_S + S} \qquad \text{(A.25)}$$

$$\frac{dC_T}{dt} = \frac{a_T \mu_{max} S C_v}{k_S + S} + K a_{TI} C_T C_v$$

where C_v = viable biomass concentration

S = substrate concentration

C_T = inhibitory product concentration

K = rate constant for the death of viable biomass

a_S = -1/Y

a_T = yield constant for inhibitor formation

a_{T1} = constant.

The model can assume three forms depending on the sign of a_{T1}. If a_{T1} is positive, the inhibitor reacts with the biomass causing death but the inhibitor is released as a result of lysis of the cell or some other phenomenon. For this case, a stationary phase for the biomass profile is predicted. If a_{T1} is zero then the inhibitor reacts with the biomass causing death but it is not consumed in the process. There is no stationary phase. Finally, if a_{T1} is negative, the inhibitor reacts with biomass causing death but it is consumed in the process. There is no stationary phase. This model has a high degree of flexibility. It represents one of the most comprehensive attempts to develop a pseudo-mechanistic unstructured model but it is not suitable in all cases. Cases where a lag phase is present cannot be dealt with. However, it is conceivable that such a model form could be modified in order to incorporate potential modifications. The use of this model also assumes that the presence of an inhibitor is known. This is not always the case.

2.2 Models with Product Formation

When a valuable product is being formed in a batch process, an equation describing the concentration of the product is required. A number of models have been postulated to relate product formation to the growth rate. Following the work of Gaden *et al.* (1970), product formation is normally classified according to its relationship with biomass formation. A product is said to be either growth associated if product formation coincides with biomass production, partially growth associated if a partial (i.e., for some part of the growth curve), relationship exists or non-growth associated if there is no correlation between growth and product formation. This classification, although oversimplified, is certainly the most widely accepted. More complex situations arise and further classifications have been required, but the basic concepts that it encompasses are well suited to unstructured models. Each class is related to some basic model form for the rate of product formation which can be easily modified to describe more complex situations. The basic classification by Gaden remains applicable regardless of the situation.

2.2.1 Growth Associated Product Formation

A number of models are found in the literature which describe the formation of growth associated products. Growth associated products are usually formed from a major metabolic pathway providing energy to the organism such as ethanol from the glycolytic pathway. For a great number of cases, the rate of product formation has been observed to be directly proportional to rate of growth. This can be reasonably well described by a model of the form

$$\frac{dX}{dt} = \mu X$$

$$\frac{dS}{dt} = -\frac{1}{Y}\mu X \qquad \text{(A.26)}$$

$$\frac{dP}{dt} = \alpha \mu X$$

where P = product concentration

μ = specific growth rate

α = yield of product formed per unit biomass formed.

μ is generally given according to the Monod model.

2.2.2 Partially Growth Associated Product Formation

If it assumed that product formation is associated with some energy pathway then it can be postulated that product formation is proportional to the rate of substrate uptake for energy requirement. This gives a model of the form

$$\frac{dX}{dt} = \mu X$$

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} - m_S X \qquad \text{(A.27)}$$

$$\frac{dP}{dt} = \left(\frac{1}{\alpha_P Y} - \frac{\beta'}{\alpha_P}\right)\mu X + \frac{m_S}{\alpha_P} X$$

where α_P = stoichiometric coefficient for product formation

β' = stoichiometric coefficient for biomass formation.

The expression for product formation rate contains two terms. The first term describes growth associated product formation. The second term is the non-growth associated term in which product formation rate is related to the biomass concentration.

An alternative model gives the following expression for the substrate uptake rate

$$\frac{dS}{dt} = -\frac{1}{Y_1}\frac{dX}{dt} - \frac{1}{Y_2}\frac{dP}{dt} \qquad \text{(A.28)}$$

where Y_1 = yield of biomass per unit substrate

Y_2 = yield of product per unit substrate.

2.2.3 Non-growth Associated Product Formation

Non-growth associated products are significantly more difficult to model. One can assume that the product formation rate is related linearly to biomass concentration. This is a highly simplistic formulation. A more comprehensive mechanistic formulation of growth may often be required in order to adequately model the rate of product formation. This is particularly difficult with unstructured models. A number of cases exist where product formation depends on the physiological state of the organism. Product formation may often require a number of unmeasurable intermediates and important co-factors which may be very difficult to model appropriately. The common approach to such problems with

unstructured models is to describe non-growth associated product formation as if it was partially growth associated.

2.3 Models with Substrate or Product Inhibition

In a growing culture, a number of agents can contribute to inhibit the rate of microbial growth. There might also be cases where a product or by-product inhibits further growth of biomass.

If the inhibitory product is known then its inhibitory behaviour can be modelled. Substrate inhibition is also a common phenomenon. It is often observed that increases in initial substrate concentration actually reduce the growth rate of some cultures. A number of model forms have been proposed to describe product and substrate inhibition.

2.3.1 Models with Product Inhibition

Accumulation of product might have an inhibitory effect on growth of microorganisms. The product may be toxic to the organism. It might also contribute to modify the culture conditions such as changing the pH. The most common model form is one postulated by Ierusalimsky (1967). It gives the following expression for the specific growth rate

$$\mu = \frac{\mu_{max} S k_P}{(k_S + S)(k_P + P)} \qquad \text{(A.29)}$$

where k_P is the product inhibition constant.

Alternative models of the form

$$\mu = \frac{\mu_{max} S}{k_S + S} e^{K_P P} \qquad \text{(A.31)}$$

are often employed with similar results. The mechanism of product inhibition of growth is not well known. A number of empirical models exist all of which have very restricted ranges of applicability.

2.3.2 Models with Substrate Inhibition

Similar problems are encountered with existing models describing substrate inhibition or enhancement. Although this is a recurring problem, the mechanisms are not well known. As a result, only empirical models exist.

The preferred model form is the Haldane equation. Its development is analogous to substrate inhibition in enzymatic systems. It takes the form

$$\mu = \frac{\mu_{max} S}{k_S + S} - \frac{S^2}{K_i} \qquad \text{(A.31)}$$

where K_i is the inhibition constant.

This model might be simplified to

$$\mu = \frac{K_1 S}{K_2 + S^2} \qquad \text{(A.32)}$$

where K_1 and K_2 are constants. This model, developed by Sokol and Howell (1981), was shown to improve the fit of the Haldane equation. Other equally good model forms include, Webb's model (see Edwards (1970))

$$\mu = \frac{\mu_{max} S \left(1 + \beta \frac{S}{K}\right)}{\left(S + k_S + \frac{S^2}{K_i}\right)} \qquad \text{(A.33)}$$

where β, K, K_i and k_s are constants; Aiba's model (Edwards (1970)),

$$\mu = \frac{\mu_{max} S e^{-\frac{S}{K_i}}}{k_S + S} \qquad \text{(A.34)}$$

Yano's model (Edwards (1970)),

$$\mu = \frac{\mu_{max}}{1 + \frac{k_S}{S} + \sum_{i=1}^{n} \left(\frac{S}{K_i}\right)^i} \qquad \text{(A.35)}$$

where n is the number of inactive enzymatic species involved; and Teissier's model,

$$\mu = \mu_{max} \left(e^{-\frac{S}{K_i}} - e^{-\frac{S}{k_S}}\right) \qquad \text{(A.36)}$$

All these models were shown to fit experimental data equally well. The work of Yang and Humphrey (1986) showed that they could not statistically find significant differences between these models for the data they considered.

3. Structured Models

As discussed above there have been a number of instances where simple unstructured models have failed to describe the observed behaviour. The best example of this was observed in a study that involved a unit step change in inlet substrate concentration on a continuous culture. For most systems, an oscillatory response was observed before steady state was re-established. However, all unstructured models tested failed to predict this

behaviour. This is but one example of the limitations of unstructured models that suggests that they are severely handicapped when subtle physiological changes in the microorganisms are involved. As a result, structured models were developed to more fully describe the internal changes occurring in the biomass. Their name comes from the fact that the actually confer structure to the biomass.

Structured models involve a complete or partial description of the mechanisms occurring within each cell. Ideally, the model should describe, in a highly mechanistic manner, all metabolic pathways involved, all trans-membrane transport systems, the synthesis of important biopolymers, cell division, etc. This is obviously not feasible. Not all intracellular reactions are fully known. The number of equations required would very easily become overwhelming and not practical. Furthermore, this highly structured model would bear a very large number of unknown parameters. Precise estimation of the parameters becomes virtually impossible. It would also be impossible to measure the concentration of all intracellular products and generate the information needed to estimate those parameters. Even if they could be developed these models would be too large to be applied to normal process applications such as design and control. Despite these problems, many attempts have been made to develop structured models which vary from the very simple to the extremely complex. The various degrees of complexity of the mathematical formulations encountered are discussed below.

3.1 Simple or Two Compartment Models

The simplest approach in the development of a structured model assumes that the biomass is compartmentalized into a small number of components. In such models made of two compartments, the biomass components can be separated into an assimilating component and a synthetic component. An example of this is the model described by Roels and Harder (1982) in which one component corresponded to enzymes which catalyze the conversion of the substrate into important cellular biopolymers and the second component corresponded to the rest of the cellular components.

In another model developed by Williams (1975), biomass is made up of a synthetic and a structural genetic component. For this formulation, it was assumed that all reactions i.e. substrate uptake, formation of structural and synthetic cell, are first order. The synthetic component is produced by substrate uptake and the structural component is formed from the synthetic component. Division of the cell is a function of the structural-genetic component. The model has the following form

$$\begin{aligned} \frac{dX}{dt} &= k_1 XS \\ \frac{dS}{dt} &= -\frac{1}{Y} k_1 XS \\ \frac{d\rho_1}{dt} &= k_1(\rho_1 + \rho_2) - k_2 \rho_1 \rho_2 - \mu \rho_1 \\ \frac{d\rho_2}{dt} &= k_2 \rho_1 \rho_2 - \mu \rho_2 \end{aligned} \qquad \text{(A.37)}$$

where ρ_i = mass of component i / unit volume of cells

μ = $k_1 S$

X = total cell density

S = substrate concentration.

This model has been able to describe various aspects of batch microbial growth kinetics remarkably well. It describes the presence of a lag phase, an exponential growth where the cell size is maximal and a stationary phase with relatively small cells. However, these predictions are, for the most part, qualitative and do not involve any rigorous testing. But these model forms are very promising. When compared with some of the more complex structured models, they remain relatively simple and contain few parameters therefore retaining some of the properties of unstructured models. The major problems of these models is the division of the biomass into compartments. Reactions between these compartments are very difficult to describe and often involve consideration of local concentrations. Another problem is that the division of the biomass may not represent

actual biochemical phenomena. Such divisions and reactions related to these can never be verified because they cannot be observed experimentally. It is impossible to know whether the proposed structure actually describes the physiological behaviour of the organisms. Identification of the two compartments is arbitrary, and no consideration is made to the cell's metabolic pathways and various growth mechanisms even though they are based on reasonable simplifying assumptions. They offer no insights on the actual mechanisms, and, like unstructured models, may fail to predict the behaviour if the conditions are changed. Another serious drawback is that the models often involve unmeasurable variables. Estimation of parameters only related to those variables becomes extremely difficult. It is often observed that this yields parameters estimates which are highly correlated with the other parameters of the model.

3.2 Intermediately Complex Models.

Since two compartments seemed to offer very little insight into the physiological behaviour of the organism, it was clear that they did not convey sufficient structure to the system. There was therefore an effort to increase the complexity of the model formulation to better describe the underlying mechanisms. For example, there have been attempts to develop three and four compartment models, but these were subject the same problems as the two compartment models and are therefore avoided in general.

Typically, development of slightly more complex models is based on the more important metabolic pathways (i.e. the growth and energy pathways), on the mechanisms of transmembrane transport of nutrients and on simplified mechanisms of cell division. Metabolic pathways are represented by only key intermediates and a series of mass balances and rate equations are developed for those key elements. Transport phenomena are assumed to be first order in most cases. Cell division can be a function of the size of the cells.

A simple structured model from non-inhibitory growth developed by Chiam and

Harris (1982) is a good example of these kinds of models. It describes a sequence of reactions which involves the uptake of substrate to give internal substrate converted to an intermediate. The intermediate is used for growth and energy and forms by-products which are then excreted into the medium. This model was shown to adequately describe chemostat situations but failed to fully predict unsteady-state situations. Further sophistication of this approach considered the possibility of control of enzyme concentration through induction-repression mechanisms within the cell. This approach proved adequate in many instances but, again, such assumptions are very difficult to verify in real situations.

3.3 Complex Models

There has been an increasing effort to describe in as much detail as possible the physiological changes occurring in a microorganism under batch growth conditions. The models developed contain a large number of variables and parameters. They are usually only derived when the organism under study is fairly well documented such as *Eschirichia coli* and *Saccharomyces cerevisiae*. A typical example of such a model are the models developed by Domach *et al.* (1984) which adequately described the aerobic growth of *E. coli*. The model described a 20-component network including a number of interactions between components and allowed for product inhibition etc... One serious disadvantage of such model formulations, is the need to estimate the parameters. Clearly, estimation of these parameters from ordinary fermentation data is impossible. Parameter values are obtained from independent experiments. It must therefore be assumed that the various experimentations are performed under conditions similar to those experienced in the cell. Clearly, this is often very difficult to do even under the most rigorously controlled conditions. It almost guarantees that not all parameters can be estimated in that way. There usually remains a number of "adjustable parameters" which can be estimated via other techniques using fermentation data. The great danger with this approach is that the variability of the other parameters is often overlooked. As a result, the uncertainty with the predictions is underestimated. It also affects the diagnostic tests for model adequacy.

4. Segregated Models

It was mentioned above that models describing batch microbial growth differ in the way the biomass is represented. Such models can either be distributed or segregated. Segregated models are based on the assumption that the biomass is comprised of a population of individual cells, each cell reacting in a distinct and at its own rate. The segregated model treats the behaviour of an individual cell and averages over all of the population in order to generate a macroscopic representation. These models are very appealing because they offer the opportunity to treat problems such as the change in cell mass often observed in the course of normal fermentation experiments. However, they must be very carefully formulated. The biggest problem is to represent all the macroscopic phenomena in terms of microscopic events. Usually, the models used involve distribution function for cell mass, time of cell division and reaction rates taken over the entire cell population.

It is clear that most of these models remain highly structured. Their formulation is therefore highly complex. Many attempts have been made to apply the concepts. In a study by Ataai and Schuler (1985), it was observed that segregated models, used for simulations of chemostat systems, could qualitatively describe observed phenomena. However, one of their most serious problems was the evaluation of model parameters. Clearly, parameter estimation is very difficult in cases where highly structured models are developed. Another approach, discussed by Roels and Kossen (1978), was to simply consider averages of the distribution functions. Clearly, it is much easier to measure average values. When a large population of cells is considered, the cell to cell variation becomes less important. Average values become more representative of the behaviour of the population. In such cases, the need for segregated models become artificial.

For these reasons, segregated models are not particularly important in the study of batch microbial kinetics. Nevertheless, the approaches sought in their development often serve to justify some of the simplifying assumptions made in the formulation of the more

easily substantiated distributed models.

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