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Model Development and Optimal Experimental Design of A Kinetically Controlled Synthesis System

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Abstract: A mathematical model has been developed for an enzymatic process with kinetically controlled synthesis. Model reduction and detailed system analysis have been undertaken to examine the main properties of this enzyme reaction system. Optimal experimental design (OED) is developed to obtain the experimental conditions that will generate the most informative measurement data for parameter estimation. Both single-input and multiple-inputs optimisation strategies have been investigated to determine the best intensity levels of control inputs. The results demonstrate that parameter estimation quality can be improved through proper model-based experimental design.

Keywords: Model development, optimal experimental design (OED), multiple-objective optimization, least-squares parameter estimation, enzyme reaction networks.

1. INTRODUCTION

Parameter estimation is challenging in model development for biological and biochemical systems (Voit, 2000) due to: (1) lack of quantitative measurements of dynamic response data and the measurement data is often corrupted with noise; (2) the complex nature of these systems with high-dimensional, nonlinear and poorly understood dynamics. In general, performing experiments to obtain rich data is expensive and time-consuming for such systems. The problem of designing experiments to generate efficient measurement data is thus of particular importance. The term 'optimal experimental design (OED)' or 'design of optimal experiment' refers to designing experiments in such a way that the model parameters can be estimated from the resulting experimental data with the best possible statistical quality. This is a subject area of growing interests particularly in systems biology where huge experimental efforts are required in model development. Various methodologies have been developed and successfully applied to a broad range of systems (Atkinson et al., 2007; Montomery, 2001; Box et al., 2005). Several comprehensive reviews on OED and its applications can be found for general systems (Pronzato, 2008; Chaloner and Verdinelli, 1995) and biochemical systems (Franceschini and Macchietto, 2008; Kreutz and Timmer, 2009).

In this work, we investigate an enzymatic process, called kinetically controlled synthesis catalysed by enzymes, in which the desired product is actually not the thermodynamically most favourable one. Because of the kinetic parameters of the enzyme reactions used in this type of systems, the desired product can however accumulate to useful concentrations before declining. One important kinetically controlled enzymatic synthesis, used industrially, is the manufacture of semi-synthetic penicillins like ampicillin. In such systems it is important to optimise conditions to obtain the most cost-effective operation (which often equates to maximal yields of the desired product). In the enzyme technology field, experimentation is normally empirical, perhaps guided by an expert's qualitative knowledge about the system. There has also been some use of methods like factorial statistical design, which produce a predicted response surface from a planned set of experiments. However, such approaches make no use of prior knowledge about how the system may respond dynamically, either from expert understanding or the underlying kinetic model. This motivates our work to develop a mathematical model and conduct experimental design using model-based OED strategies.

For many biological and biochemical systems, experimental design for parameter estimation can be considered from two aspects. One is the design of input perturbations (type, level and duration of input signals) (Asprey and Macchietto, 2002; Banga et al., 2002; Faller et al., 2003); the other is to determine when and what kind of observations should be taken such as the optimal sampling design (Asyali, 2010; Derlinden et al., 2013) and the measurement set selection problem (Yue et al., 2008; Brown et al., 2008; He et al., 2010). Design factors include level of initial conditions; which input and output variables to be taken; what sampling schedule to follow, etc. For the kinetically controlled synthesis process studied in this work, input intensity levels are designed for the

purpose of getting the best estimation of those crucial parameters that affect the output product most.

The rest of the paper is organised as follows. In Section 2, preliminaries on parameter estimation, OED, local sensitivity analysis of differential algebraic equation (DAE) models are briefly introduced. In Section 3, the model development of the enzyme reaction system is presented and model reduction is made following the conservative analysis. Using the established model, OED of single-input and multiple-inputs is implemented in Section 4, with conclusions given in Section 5.

2. PRELIMINARIES

2.1 Parameter Estimation and Optimal Experimental Design

Consider a general dynamic system described by the following nonlinear ordinary differential equation (ODE) and algebraic output equation

$$\dot{\mathbf{X}}(t) = \boldsymbol{f}\left(\mathbf{X}(t), \boldsymbol{\theta}, \boldsymbol{\omega}(\boldsymbol{t})\right),\ \mathbf{X}(t_0) = \mathbf{X}_0 \tag{1}$$

$$\mathbf{Y}(t) = h(\mathbf{X}(t), \boldsymbol{\theta}) + \xi(t) \tag{2}$$

$\mathbf{X} \in \mathbb{R}^n$ is the state vector with initial condition $\mathbf{X}_0$ and n the number of state variables. Each component of $\mathbf{X}$ is denoted as x_i, which normally stands for molecule concentrations in a biochemical reaction network. $\boldsymbol{\theta} \in \mathbb{R}^m$ is the parameter vector with m the number of parameters. The component of $\boldsymbol{\theta}$ is denoted as k_j mostly referring to kinetic reaction rates. $f(\cdot)$ is a column nonlinear function for states transition, which is often derived from the underlying biochemical reaction mechanisms. $\boldsymbol{\omega}$ is introduced to represent the experimental design parameters. $\mathbf{Y} \in \mathbb{R}^r$ is the measurement output vector with $r(r \leq n)$ being the number of measurement variables, and $h(\cdot)$ the measurement function reflecting the use of observables. The signal ξ is assumed to be independently and identically distributed (*i.i.d.*), additive, zero-mean Gaussian noise. Parameter estimation for system (1)-(2) can be obtained by the least-squares algorithm

$$\hat{\theta} = \arg\min_{\theta\in\Theta} \sum_{l=1}^{\psi} \left(\mathbf{Y}(t_l) - \hat{\mathbf{Y}}(\hat{\theta}, t_l)\right)^T \mathbf{Q}^{-1} \left(\mathbf{Y}(t_l) - \hat{\mathbf{Y}}(\hat{\theta}, t_l)\right) \tag{3}$$

where $\mathbf{Y}$ and $\hat{\mathbf{Y}}$ are the measurement output and the model prediction output, respectively. $\mathbf{Q}$ is the measurement error covariance matrix, the subscript l denotes sampling time index, ψ is the total number of sampling points in the dimension of time.

In the scheme of least-squares estimation, the Fisher information matrix (FIM) quantifies the information content of the measurement data. Under the assumption that the measurement noises are *i.i.d.*, zero-mean Gaussian processes, the FIM is a nonlinear function of the estimated parameters for model (1)-(2) that can be formulated by local sensitivity matrix. Denoting $\mathbf{X} = [x_1, x_2, \cdots, x_n]^T$, $\boldsymbol{\theta} = [k_1, k_2, \cdots, k_m]^T$, the local sensitivity matrix is described as

$$\mathbf{S(t)} = \partial\mathbf{X}(t)/\partial\boldsymbol{\theta} = \left[s_{ij}(t)\right]_{n\times m},\ s_{ij}(t) = \partial x_i(t)/\partial k_j \tag{4}$$

The FIM is represented as a function of the local sensitivity matrix:

$$\mathrm{FIM}(\boldsymbol{\theta}, \omega) = \sum_{l=1}^{\psi} \mathbf{S}^T(t_l, \boldsymbol{\theta}, \boldsymbol{\omega}) \mathbf{Q}^{-1} \mathbf{S}(t_l, \boldsymbol{\theta}, \boldsymbol{\omega}). \tag{5}$$

Following the previous noise assumptions, an OED problem can be written as a general optimisation problem

$$\boldsymbol{\omega}^* = \arg\max_{\omega\in\Omega} \Phi\left(\mathrm{FIM}(\boldsymbol{\theta}, \boldsymbol{\omega})\right). \tag{6}$$

Ω is the space for the design parameter vector $\boldsymbol{\omega}$, $\Phi(\cdot)$ is a function that reflects the design target. In many OED problems, $\Phi(\cdot)$ is taken from the widely used alphabetical experimental design criteria that are scalar functions of FIM, such as:–

- A-optimal, maximising trace(FIM);
- D-optimal, maximising det(FIM);
- E-optimal, minimising $\lambda_{max}(\mathrm{FIM}^{-1})$, etc.

Here trace$(\cdot)$, det$(\cdot)$ and $\lambda_{max}(\cdot)$ denote trace, determinant and the maximum eigenvalue of a matrix. These alphabetical criteria are related to the size and shape of the confidence hyper-ellipsoid for estimated parameters, and will give different experimental design results when choosing different criteria. The design using any of these three scalarisation criteria turns out to be a convex optimisation problem when the FIM is an appropriate function of the experimental design parameters (Boyd and Vandenberghe, 2004).

2.2 Sensitivity Calculation for DAE Models

Differentiation of the sensitivity matrix (4) with respect to $\boldsymbol{\theta}$ yields the following sensitivity differential equations:

$$\dot{\mathbf{S}}(t) = \mathbf{J}(t)\mathbf{S}(t) + \mathbf{F}(t),\ \mathbf{S}(t_0) = \mathbf{S}_0 \tag{7}$$

where $\mathbf{J} = \partial\boldsymbol{f}/\partial\mathbf{X}$ is the Jacobian matrix, $\mathbf{F} = \partial\boldsymbol{f}/\partial\boldsymbol{\theta}$ is the parameter Jacobian matrix. The sensitivity matrix $\mathbf{S}$ can be calculated by solving (1) and (7) simultaneously which involves $n\times(m+1)$-dimension ODEs. The initial values of $\mathbf{S}_0$ are typically zeros unless the system initial conditions depend on the model parameters. This method is called direct differential method (DDM) (Varma et al., 1999).

It is common that conservation laws exist in biological and biochemical networks. For such a system, if all the ODEs are included in the sensitivity calculation without separating the independent state variables from the dependent ones, the Jacobian matrix $\mathbf{J}$ will be singular and cause troubles in numerical computation. To avoid this problem, the components of the state vector $\mathbf{X}$ are divided into independent dynamic state variables and dependent algebraic (state) variables. Rewrite the system ODEs in the form of DAEs:

$$\begin{cases} \dot{\boldsymbol{x}}_s(t) = \boldsymbol{f}_s(\boldsymbol{x}_s(t), \boldsymbol{x}_a(t), \boldsymbol{\theta}) \\ 0 = \boldsymbol{f}_a(\boldsymbol{x}_s(t), \boldsymbol{x}_a(t), \boldsymbol{\theta}) \end{cases} \tag{8}$$

in which $\boldsymbol{x}_s \in \mathbb{R}^{n_s}$ is the independent state vector and $\boldsymbol{x}_a \in \mathbb{R}^{n_a}$ the dependent state vector, obviously, $n_s + n_a = n$, *i.e.*, $\boldsymbol{x} = [\,\boldsymbol{x}_s^{\mathrm{T}}\ \boldsymbol{x}_a^{\mathrm{T}}\,]^{\mathrm{T}}$. $\boldsymbol{f}_s$ is the column vector function corresponding to the independent state time derivative and $\boldsymbol{f}_a$ is the column vector function that describes the conservation laws. If $\partial\boldsymbol{f}_a/\partial\boldsymbol{x}_a$ is not singular, then the algebraic constraint manifold is regular and there is a

locally defined function, $\boldsymbol{x}_a = \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{\theta})$, which leads to $\boldsymbol{f}_a(\boldsymbol{x}_s, \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{\theta})) = 0$. Then the system described by differential equations may be locally expressed by $\dot{\boldsymbol{x}}_s = \boldsymbol{f}_s(\boldsymbol{x}_s, \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{\theta}), \boldsymbol{\theta})$. The Jacobian matrix is accordingly represented as:

$$\mathbf{J}_s = \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_s} - \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_a}\left[\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_a}\right]^{-1}\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_s} \tag{9}$$

and the parameter Jacobian matrix is

$$\mathbf{F}_s = \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{\theta}} - \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_a}\left[\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_a}\right]^{-1}\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{\theta}} \tag{10}$$

With this reformulation, the Jacobian matrix $\mathbf{J}_s$ will not be singular and the sensitivity matrix can be calculated using the DDM algorithm.

3. MODEL DEVELOPMENT OF THE ENZYME REACTION SYSTEM

3.1 System Description and Modelling

In this work, an enzyme reaction system as illustrated in Fig. 1 is studied. In this system, S is the donor substrate, P is the leaving group product, N denotes the nucleophile, Q is the desired product, R is the hydrolysis by-product; W stands for water whose quantity is taken as constant due to its large amount; E, ES, E*, EQ and ER are different forms of enzymes in the reaction system. Among these components, Q, S, P, N and R are measurable in experiments. It's difficult to measure different forms of enzymes since their concentrations are very low. We identify the initial concentrations of S, N and E as user-controllable inputs written as S_0, N_0 and E_0, respectively. A number of the complications such as enzyme inactivation, reactant instability, effects of pH and temperatures, etc., have been removed in this exemplar system to simplify the model.

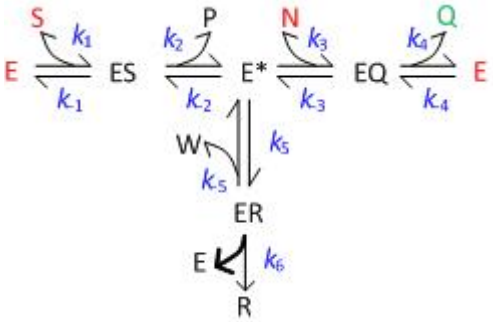

Fig. 1. Exemplar kinetically controlled synthesis system

A number of important enzymatic processes are believed to follow this reaction scheme, or a closely related one. They are known as kinetically controlled reactions, in that the desired product (here Q) is not the thermodynamically most favourable one (here R) which would predominate at long times. The chemical reactions can be written into the following 6 groups.

$$v_1: \quad E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

$$v_2: \quad ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E^*$$

$$v_3: \quad N + E^* \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EQ$$

$$v_4: \quad EQ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} Q + E$$

$$v_5: \quad E^* + W \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} ER$$

$$v_6: \quad ER \xrightarrow{k_6} E + R$$

Based on these reactions, a set of ODEs can be written following the mass-balance principle (see (A.1)-(A.10) given in the appendix).

3.2 Conservation Analysis and Model Reduction

Applying linear algebraic calculation to the ODE model (A.1)-(A.10), the following conservation constraints can be obtained.

$$ES + S + P = C_1 \tag{11}$$

$$EQ + N + Q = C_2 \tag{12}$$

$$E + ES + EQ + E^* + ER = C_3 \tag{13}$$

$$ES + EQ + S + Q + R + E^* + ER = C_4 \tag{14}$$

C_1, C_2, C_3 and C_4 are constants determined by the initial conditions of the states. For this system, P, Q, R, ES, E^*, EQ and ER are zeros at $t = 0$. Therefore we have

$$C_1 = C_4 = S_0, \; C_2 = N_0, \; C_3 = E_0$$

The four constraints, (11) - (14), derived from the ODE model, comply with the following conservation laws.

- Conservation of enzyme
$$E + ES + EQ + E^* + ER = E_0$$
- Conservation of leaving group moieties
$$ES + S + P = S_0$$
- Conservation of transferred group, e.g. acyl
$$ES + EQ + S + Q + R + E^* + ER = S_0$$
- Conservation of nucleophile moieties
$$EQ + N + Q = N_0$$

From the above conservation analysis, the original 10-ODEs model can be further reduced to 6 ODEs and 4 algebraic equations. There are more than one way to select the independent states set. The only constraint here is that E^* and ER must stay in separate groups. Considering the pseudo steady-state operation that is normally taken to handle this type of systems, in which the dynamics of S, P, N, Q and R are kept in the ODEs, we choose $\{E^*, S, P, N, Q, R\}$ as the independent variable set and $\{ES, EQ, ER, E\}$ as the dependent variable set. This will bring the following DAE model with 6 ODEs

$$\frac{dE^*}{dt} = -(k_5 W + k_{-5})E^* - k_2 S + (k_{-5} - k_2)P + (k_{-5} - k_{-3})N - k_{-3}Q - k_{-5}R - k_{-2}E^*P - k_3 E^* N + k_2 S_0 + (k_{-3} - k_{-5})N_0 \quad (15)$$

$$\frac{dS}{dt} = (-k_1 E_0 + k_1 S_0 - k_{-1})S - k_{-1}P - k_1(S+Q+R)S + k_{-1}S_0 \quad (16)$$

$$\frac{dP}{dt} = -k_2(S+P) - k_{-2}E^*P + k_2 S_0 \quad (17)$$

$$\frac{dN}{dt} = -k_{-3}(N+Q) - k_3 E^* N + k_{-3}N_0 \quad (18)$$

$$\frac{dQ}{dt} = -k_4(N+Q) - k_{-4}(S+Q+R)Q + k_4 N_0 - k_{-4}(E_0 - S_0)Q \quad (19)$$

$$\frac{dR}{dt} = k_6(-E^* + P + N - R) - k_6 N_0 \quad (20)$$

and 4 algebraic equations.

$$ES = S_0 - S - P \quad (21)$$

$$EQ = N_0 - N - Q \quad (22)$$

$$ER = -N_0 + N + P - E^* - R \quad (23)$$

$$E = E_0 - S_0 + S + Q + R \quad (24)$$

The model represented by (15)-(24) will be used for system analysis and experimental design.

3.3 Equilibrium Analysis

The steady states of the dynamic system can be obtained by taking all the ODEs to be zeros, which immediately brings that

$$ES(\infty) = E^*(\infty) = EQ(\infty) = ER(\infty) = S(\infty) = Q(\infty) = 0$$

The non-zero steady states of P, E, N and R are dependent on the initial conditions via the conservation laws, i.e. $P(\infty) = R(\infty) = S_0$, $N(\infty) = N_0$, and $E(\infty) = E_0$.

The output of major interest is the product Q, which increases at the first stage of the reaction and then decreases until reaching a zero steady state. The production of Q is a balance of kinetic and equilibrium effects. The equilibrium position will always be essentially all R. In the model reaction scheme the production of R is completely irreversible, therefore at infinite time all S and Q will be converted to R. The kinetic constants are such that R is not produced quickly. In the early stages, E^* (produced from E and S through reactions v_1 and v_2) will react much more rapidly with N (leading to Q formation through v_3 and v_4) than with W (leading to R through v_5 and v_6). Hence substantial amounts of Q are formed in the early stages. But after S is depleted, the reversible reactions leading to Q (via v_4) start to go in reverse. Much of the E^* accumulated still reacts with N to go back to Q, for no net change. But the small fraction of E^* that does react with W will go on irreversibly to R. Therefore, overall, there is a slow but continuing conversion of Q, formed earlier, to R. Fig. 2 demonstrates the concentration time profiles of S, Q and R. Nominal parameter values (see table A.1) are used in this simulation and the nominal initial conditions are set to be $E_0 = 1.5e-5$, $S_0 = 0.8$ and $N_0 = 0.9$, respectively.

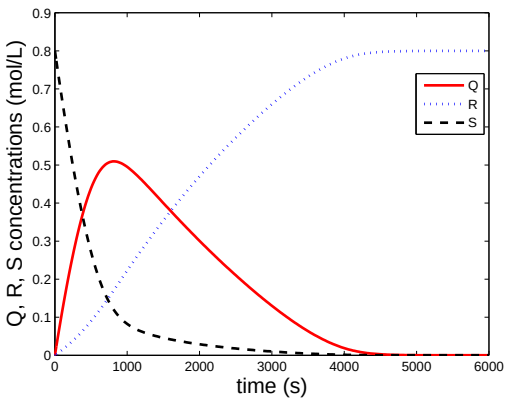

Fig. 2. Time profiles for concentrations of Q, R and S

4. SINGLE INPUT AND MULTIPLE INPUTS OPTIMAL EXPERIMENTAL DESIGN

4.1 Simulation Set-up and Local Sensitivity Analysis

The kinetic reaction rates of this system are given in table A.1 in the appendix. The unit used for concentration is $mol \cdot L^{-1}$, and for time is second. In this enzymatic process, the product quality is expected to be maximised either through increasing the ratio of Q/S_0 or reducing the time to reach its peak value. The state Q is chosen as the single state variable in formulating FIM in the following OED simulation. The simulation measurement noise is an additive term with a ratio of 5% relative level and 0.001 absolute level to the original amplitude of the states during the whole process.

The time profiles of the sensitivity coefficients, $\partial Q/\partial k_j (j = 1, \cdots, 11)$, are given in Fig. 3 to indicate the local parametric effects to Q at each time point in the reaction process. To further compare the sensitivities quantitatively, a bar chart is given in Fig. 4, in which the following index is used to reflect the overall sensitivities along the time range (ψ is the number of sampling time points).

$$OS_{ij} = \frac{1}{\psi}\sqrt{\sum_{l=1}^{\psi}(s_{ij}(t_l))^2} \quad (25)$$

The sensitivity analysis suggests that k_2, k_{-3} and k_{-5} are the most sensitive parameters for this system.

4.2 Optimal Experimental Design of Input Intensities

Taking the 3 most sensitive parameters into the estimation scheme, OED algorithms were applied to determine what are the best values for E_0, S_0 and N_0 to minimise the parameter estimation errors. The OED was first implemented to each control input individually, and then to all 3 inputs simultaneously. Following expert advise on this enzyme reaction system, the design range is set to be: $N_0 \in [0.01, 1]$, $S_0 \in [0.01, 1]$, and $E_0 \in [1.5e-6, 1.5e-4]$. For each input factor, a grid of 100 sampling points is used in simulation. The optimal values of the initial conditions calculated by D- and E-optimal design are listed in Table 1. The last column in this table is the multiple-inputs OED results.

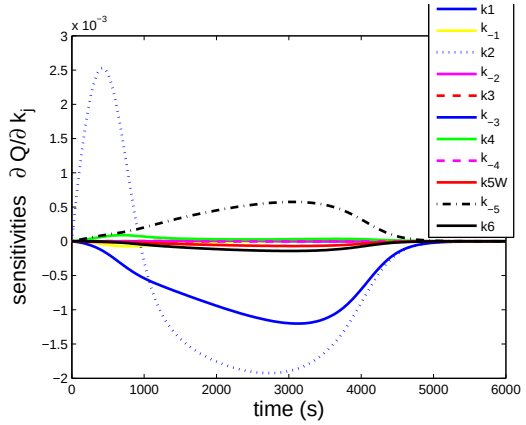

Fig. 3. Sensitivity profiles using the state Q

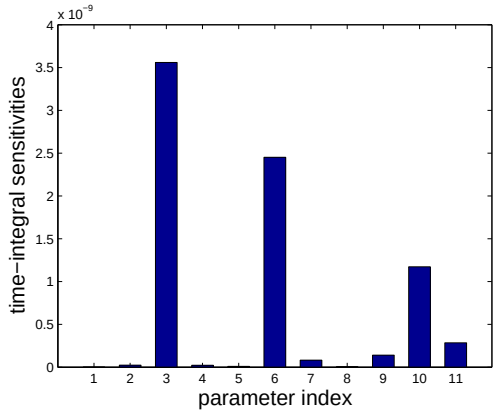

Fig. 4. Time-summation sensitivities using the state Q

To illustrate the design results from the parameter estimation point of view, the 95% confidence interval (CI) ellipsoids are used for parameter pairs in 2D plots. The baseline result, i.e. the ellipsoid without OED, is produced using the nominal initial conditions. For the single-input OED of N_0, the E-optimal design performance index is given in Fig. 5, and the CIs for (k_{-3}, k_{-5}) under D- and E-optimal design are illustrated in Fig. 6. It can be seen that both D- and E-optimal design reduce the CIs compared with the case without OED indicating a smaller upper bound for the parameter estimation errors. Within this simulation scheme, it turns out that the E-optimal design of N_0 corresponds to the best solution (smallest CI) among all the results of single input OED. See Fig. 7 for a comparison.

Table 1. Single-input and multiple-inputs OED results for E_0, S_0 and N_0

	E_0^*	S_0^*	N_0^*	(E_0^*, S_0^*, N_0^*)
D-optimal	$1.2e-5$	1	0.8	$(6e-6, 1, 0.21)$
E-optimal	$1.2e-5$	1	0.15	$(4.5e-6, 0.96, 0.15)$

It is not surprising that the multiple-inputs OED can achieve an improved result over the single-input OED. See Fig. 8 for a comparison of CI ellipsoids for (k_{-3}, k_{-5}) under different OED conditions. It can be seen that the D- and E-optimal design of multiple-inputs produce the smallest confidence region, in this case, almost equal small CIs. It should be noted that the computational cost

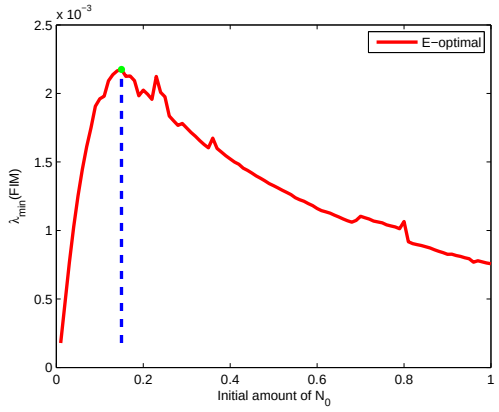

Fig. 5. E-optimal index w.r.t. input level of N_0

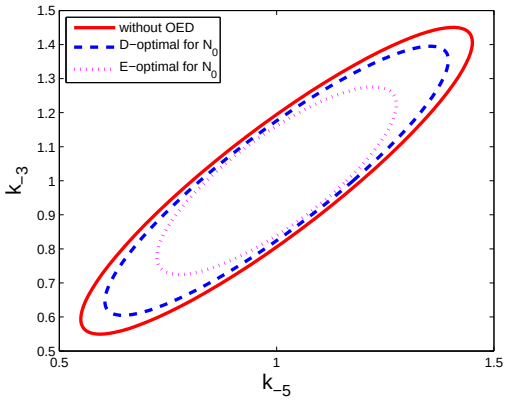

Fig. 6. CI ellipsoids for (k_{-3}, k_{-5}) at different levels of N_0

of multi-inputs OED is much higher than the single-input design.

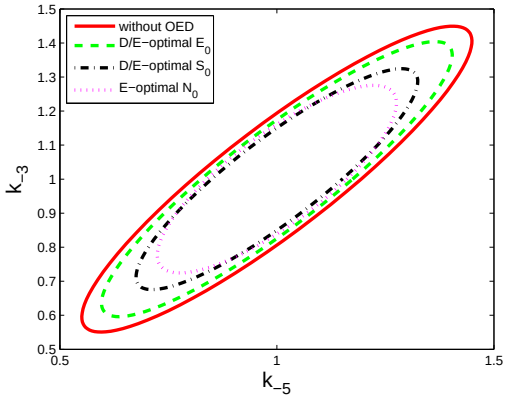

Fig. 7. CI ellipsoids for (k_{-3}, k_{-5}) at different input levels

5. CONCLUSION AND DISCUSSION

We have presented details on the model development and system analysis of a kinetically controlled enzymatic process. Through the local sensitivity analysis, crucial parameters affecting the output product are identified and used in OED so as to assess the possible improvement in parameter estimation quality. Compared with single-input OED, the multiple-inputs OED stands a better

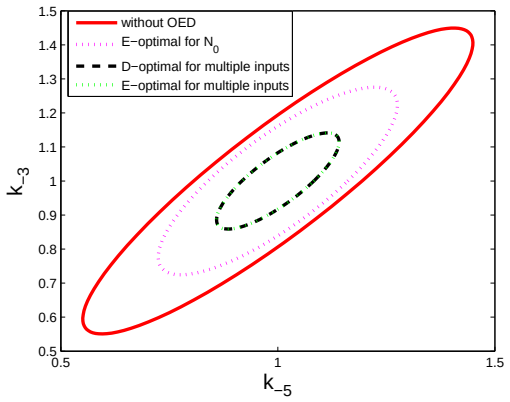

Fig. 8. CI ellipsoids for (k_{-3}, k_{-5}) at different input levels

chance to reduce the parameter estimation error although this conclusion is highly dependent on the OED result.

OED of input intensity levels are relatively simple to implement since the optimisation problem can be easily solved. Further OED has been carried out for this system on the measurement selection problem, which is to determine the best set of measurements to maximise the parameter estimation quality. An ultimate aim of this work is to present an OED approach on this sort of systems, which makes use of the knowledge encapsulated in the kinetic model. That would involve getting accurate estimates of those parameters that have large influences on system behaviour in the region of the optimal conditions, while not wasting experimental effort on much less sensitive parameters, based on which exploring operation conditions to achieve the maximum product quality.

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Appendix A. ODE MODEL OF THE ENZYME REACTION SYSTEM

$$\frac{dE}{dt} = -k_1 \cdot E \cdot S + k_{-1} \cdot ES + k_4 \cdot EQ - k_{-4} \cdot E \cdot Q + k_6 \cdot ER \quad \text{(A.1)}$$

$$\frac{dES}{dt} = k_1 \cdot E \cdot S - k_{-1} \cdot ES - k_2 \cdot ES + k_{-2} \cdot E^* \cdot P \quad \text{(A.2)}$$

$$\frac{dE^*}{dt} = k_2 \cdot ES - k_{-2} \cdot E^* \cdot P - k_3 \cdot E^* \cdot N + k_{-3} \cdot EQ - k_5 \cdot W \cdot E^* + k_{-5} \cdot ER \quad \text{(A.3)}$$

$$\frac{dEQ}{dt} = k_3 \cdot E^* \cdot N - k_{-3} \cdot EQ - k_4 \cdot EQ + k_{-4} \cdot E \cdot Q \quad \text{(A.4)}$$

$$\frac{dER}{dt} = k_5 \cdot W \cdot E^* - k_{-5} \cdot ER - k_6 \cdot ER \quad \text{(A.5)}$$

$$\frac{dS}{dt} = -K_1 \cdot E \cdot S + k_{-1} \cdot ES \quad \text{(A.6)}$$

$$\frac{dP}{dt} = k_2 \cdot ES - k_{-2} \cdot E^* \cdot P \quad \text{(A.7)}$$

$$\frac{dN}{dt} = -k_3 \cdot E^* \cdot N + k_{-3} \cdot EQ \quad \text{(A.8)}$$

$$\frac{dQ}{dt} = k_4 \cdot EQ - k_{-4} \cdot E \cdot Q \quad \text{(A.9)}$$

$$\frac{dR}{dt} = k_6 \cdot ER \quad \text{(A.10)}$$

Table A.1. Kinetic reaction rate values

Index	1	2	3	4	5	6
Parameter	k_1	k_{-1}	k_2	k_{-2}	k_3	k_{-3}
Value	1e5	1e3	100	1e4	5e4	200
Index	7	8	9	10	11	
Parameter	k_4	k_{-4}	$k_5 W$	k_{-5}	k_6	
Value	1e3	2e4	5e3	100	500	