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Experimental design methodologies for the identification of Michaelis-Menten type kinetics

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Abstract

The main objective of this work was to investigate the application of experimental design techniques for the identification of Michaelis-Menten kinetic parameters. More specifically, this study attempts to elucidate the relative advantages/disadvantages of employing complex experimental design techniques in relation to equidistant sampling when applied to different reactor operation modes. All studies were supported by simulation data of a generic enzymatic process that obeys to the Michaelis-Menten kinetic equation.

Different aspects were investigated, such as the influence of the reactor operation mode (batch, fed-batch with pulse wise feeding and fed-batch with continuous feeding) and the experimental design optimality criteria on the effectiveness of kinetic parameters identification. The following experimental design optimality criteria were investigated: 1) minimization of the sum of the diagonal of the Fisher information matrix (FIM) inverse (A-criterion), 2) maximization of the determinant of the FIM (D-criterion), 3) maximization of the smallest eigenvalue of the FIM (E-criterion) and 4) minimization of the quotient between the largest and the smallest eigenvalue (modified E-criterion). The comparison and assessment of the different methodologies was made on the basis of the Cramér-Rao lower bounds (CRLB) error in respect to the parameters v_{max} and K_m of the Michaelis-Menten kinetic equation.

In what concerns the reactor operation mode, it was concluded that fed-batch (pulses) is better than batch operation for parameter identification. When the former operation mode is adopted, the v_{max} CRLB error is lowered by 18.6 % while the K_m CRLB error is lowered by 26.4 % when compared to the batch operation mode. Regarding the optimality criteria, the best method was the A-criterion, with an average v_{max} CRLB of 6.34 % and 5.27 %, for batch and fed-batch (pulses), respectively, while presenting a K_m's CRLB of 25.1 % and 18.1 %, for batch and fed-batch (pulses), respectively. As a general conclusion of the present study, it can be stated that experimental design is justified if the starting parameters CRLB errors are inferior to 19.5 % (v_{max}) and 45% (K_m), for batch processes, and inferior to 42 % and to 50% for fed-batch (pulses) process. Otherwise equidistant sampling is a more rational decision. This conclusion clearly supports that, for fed-batch operation, the use of experimental design is likely to largely improve the identification of Michaelis-Menten kinetic parameters.

1. Introduction

During the last few years, the study of enzyme behaviour has become a popular field of research. The collection of meaningful kinetic data is, however, very much dependent on the experimental planning technique adopted. A correct experimental planning to optimize resources allows maximizing the accuracy of parameter estimation and at the same time it allows to minimize the experimental effort required for a given level of accuracy (Murphy, E.F., et al., 2002). With a good experimental design methodology, one can obtain accurate estimates of enzyme kinetic parameters (although always with an associated error) out of the measurements, and also optimal timestamps of whichever activities may be performed during the experiment, e.g., injection of a substrate at an optimal time instant.

The traditional approach of experimental planning is based on equidistant sampling, which requires (as the names implies) having measurements throughout the experiment with equal intervals between them, instead of using optimized measurement times. This technique has the advantage of being simpler and less time consuming, because it does not need any planning to be done. However, it has the disadvantage of not delivering the best outcome when compared with optimized experiments.

The main objective of this thesis is to compare different experimental design techniques and to assess in which situations the experimental design may be advantageous over the equidistant measurement point's technique.

This thesis follows the work of a previous study by Lindner and Hitzmann (2006), in which error estimation was calculated using the Fisher information matrix and Cramér-Rao lower bounds associated to its respective parameter. In Lindner and Hitzmann (2006) one criterion for optimization was used. In this thesis four different criteria were used and compared.

In this study, a wide range of values of the Michaelis-Menten parameters was studied and then the respective estimation errors were calculated. In this way it will be possible to determine which will actually be effect of the range of parameter values on estimation accuracy. It will be also possible to compare experimental design technique with equidistant sampling estimations and to assess which will be the best method for a particular experiment given that one knows beforehand a rough estimation of the parameters.

2. System and Methods

The main objective of experimental design is to plan experiments in a way that unknown parameters of a process model can be determined precisely. A dynamic process can be generally described as

$$\frac{dx}{dt} = f(x, t, P),$$

where x represents state variables – substrate concentration, enzyme concentration and total volume, described as S, E and V, respectively; t is experiment time and P stands for the experiment parameters: v_{max} and K_m. To perform the measurements the following model is used

$$y^E(t_i) = g(x, t_i, P),$$

on which $y^E(t_i)$ stands for process output that can be estimated at t_i (timestamp where the measurements y_i^M are performed); x and P represent the same stated previously.

To find its optimal design and, therefore, determine its enzyme kinetics parameters, there is the need to calculate the Fisher information matrix (FIM). With the analysis of FIM, errors associated to the estimation of parameters can be calculated.

2.1. Calculation of the Fisher Information Matrix

The process that is being analysed in this study is carried out in a stirred tank reactor where only one variable measurement is being performed: substrate concentration. Three modes will be adopted in this system, which are batch and fed-batch (pulse wise feeding) and fed-batch (continuous feeding), meaning that not only substrate concentration will change throughout the experiment but also enzyme concentration and volume. Equations that can describe this process are [1]:

$$\frac{dS}{dt} = f_1 = \frac{(S_0 - S)\dot{V}_{Substrate}}{V} - \frac{\dot{V}_{Enzyme}}{V} S - \frac{v_{\max} ES}{K_m + S}$$
$$\frac{dE}{dt} = f_2 = \frac{(E_0 - E)\dot{V}_{Enzyme}}{V} - \frac{\dot{V}_{Substrate}}{V} E$$
$$\frac{dV}{dt} = f_3 = \dot{V}_{Substrate} + \dot{V}_{Enzyme} - \dot{V}_{Sample}$$

S_0 stands for initial substrate concentration, while E_0 refers to initial enzyme concentration; $\dot{V}_{Substrate}$, $\dot{V}_{Enzyme}$ and $\dot{V}_{Sample}$ stand for volume flow due to substrate, enzyme and sampling respectively.

In batch mode, there will be neither change in the enzyme concentration nor in volume broth, therefore leading the first equation to an ordinary time-dependent enzyme kinetic and the other two to zero. When changing into fed-batch mode the equations cannot be solved analytically, therefore one must use numerical methods.

Due to this number of variables (S, E and V) and parameters (v_{max} and K_m) a 3 x 2 matrix is obtained with state sensitivities differential equations,

$$\begin{bmatrix} \dot{S}_{v_{\max}} & \dot{S}_{K_m} \\ \dot{E}_{v_{\max}} & \dot{E}_{K_m} \\ \dot{V}_{v_{\max}} & \dot{V}_{K_m} \end{bmatrix} = \begin{bmatrix} \frac{\partial f_1}{\partial S} & \frac{\partial f_1}{\partial E} & \frac{\partial f_1}{\partial V} \\ \frac{\partial f_2}{\partial S} & \frac{\partial f_2}{\partial E} & \frac{\partial f_2}{\partial V} \\ \frac{\partial f_3}{\partial S} & \frac{\partial f_3}{\partial E} & \frac{\partial f_3}{\partial V} \end{bmatrix} \begin{bmatrix} S_{v_{\max}} & S_{K_m} \\ E_{v_{\max}} & E_{K_m} \\ V_{v_{\max}} & V_{K_m} \end{bmatrix} + \begin{bmatrix} \frac{\partial f_1}{\partial v_{\max}} & \frac{\partial f_1}{\partial K_m} \\ \frac{\partial f_2}{\partial v_{\max}} & \frac{\partial f_2}{\partial K_m} \\ \frac{\partial f_3}{\partial v_{\max}} & \frac{\partial f_3}{\partial K_m} \end{bmatrix},$$

where $S_{v_{\max}}$ is the sensitivity of the substrate with respect to v_{max} and $\dot{S}_{v_{\max}}$ is its derivative with respect to time. All the others have the same meaning according to their respective parameter and variable. There can be some simplification (setting values to zero) in the equation seeing that not every function is depending on the parameter in which it is being derived; f_2 does not depend on S and that f_3 does not depend on any of the state variables; f_2 and f_3 do not depend also on any of the parameters.

At the beginning of the experiment all the sensitivities are set to zero so that only sensitivities with respect to substrate will change its value. Therefore only these two will be analysed and used in the optimization process.

$$\dot{S}_{v_{\max}} = \left(-\frac{\dot{V}_{Substrate} + \dot{V}_{Enzyme}}{V} - \frac{v_{\max} E}{K_m + S} + \frac{v_{\max} ES}{(K_m + S)^2} \right) S_{v_{\max}} - \frac{ES}{K_m + S},$$

$$\dot{S}_{K_m} = \left(-\frac{\dot{V}_{Substrate} + \dot{V}_{Enzyme}}{V} - \frac{v_{\max} E}{K_m + S} + \frac{v_{\max} ES}{(K_m + S)^2} \right) S_{K_m} + \frac{v_{\max} ES}{(K_m + S)^2}.$$

After calculating these values it is possible to determine the FIM, which is given by

$$\text{FIM} = \begin{bmatrix} \sum_i^N \left(\frac{(S_{v_{\max}}(t_i))^2}{\sigma_i} \right) & \sum_i^N \left(\frac{S_{v_{\max}}(t_i) \times S_{K_M}(t_i)}{\sigma_i} \right) \\ \sum_i^N \left(\frac{S_{K_M}(t_i) \times S_{v_{\max}}(t_i)}{\sigma_i} \right) & \sum_i^N \left(\frac{(S_{K_M}(t_i))^2}{\sigma_i} \right) \end{bmatrix}$$

The inverse of FIM gives the Cramér-Rao lower bound (CRLB) of the parameter estimation error co-variances. This way associated errors can be calculated and therefore measure how good these estimations are. To know how good these estimations are it is mandatory to choose one criterion in order to optimize the experiment results and therefore obtain a good experimental design.

2.2. Experimental design optimality criteria

The following experimental design optimality criteria were investigated [2], [3]:

2.2.1 A-criterion

In A-criterion, the purpose for optimization is to minimize the sum of the diagonal of the inverse of the FIM, i.e., minimize the sum of the CRLB's. The inverse of the FIM is

$$inv(FIM) = \frac{1}{\det(FIM)} \begin{bmatrix} \sum_i^N \left(\frac{(S_{K_M}(t_i))^2}{\sigma_i} \right) & \sum_i^N \left(-\frac{S_{K_M}(t_i) \times S_{v_{\max}}(t_i)}{\sigma_i} \right) \\ \sum_i^N \left(-\frac{S_{v_{\max}}(t_i) \times S_{K_M}(t_i)}{\sigma_i} \right) & \sum_i^N \left(\frac{(S_{v_{\max}}(t_i))^2}{\sigma_i} \right) \end{bmatrix},$$

in which the CRLB's are the terms in the diagonal of the matrix divided by the determinant of the FIM.

2.2.2 D-criterion

On D-criterion, the optimization is performed by maximizing the determinant of FIM, which is

$$\det FIM = \sum_{i}^{N}\left(\frac{\left(S_{v_{\max}}(t_i)\right)^2}{\sigma_i}\right)\times\sum_{i}^{N}\left(\frac{\left(S_{K_M}(t_i)\right)^2}{\sigma_i}\right)-\sum_{i}^{N}\left(\frac{S_{v_{\max}}(t_i)\times S_{K_M}(t_i)}{\sigma_i}\right)\times\sum_{i}^{N}\left(\frac{S_{K_M}(t_i)\times S_{v_{\max}}(t_i)}{\sigma_i}\right)$$

To maximize the determinant of FIM it is necessary to maximize the first term and minimize the second. To do this one must see how high (or low) should be the values of $S_{v_{\max}}(t_i)$ and $S_{K_m}(t_i)$ so that it is obtained the higher value from the difference between the first and second terms. In this way the maximum value of the determinant of FIM is obtained and therefore the design is optimized.

2.2.3 E-criterion

While using E-criterion, the objective is to maximize the smallest eigenvalue of FIM. The eigenvalues of FIM are

$$\lambda_{1/2} = \sum_{i=1}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2+\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}\pm\sqrt{\left[\sum_{i}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2-\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}\right]^2+\left[\sum_{i}^{N}\frac{S_{v_{\max}}(t_i)S_{K_m}(t_i)}{\sigma_i}\right]^2}.$$

The smallest eigenvalue will be the one with the minus signal before the square root and to maximize it, the difference between the first term (sum before the minus signal) and the second one (everything that comes after the minus signal) must be as high as possible. In order to do so, the first term should have a high value while the second one should have the lowest attainable score. To maximize the first term, one must obtain the highest values of $S_{v_{\max}}(t_i)$ and $S_{K_m}(t_i)$. To minimize the square root, one must have the smallest possible value of $S_{v_{\max}}(t_i)$ but in order to $S_{K_m}(t_i)$ it is needed a high value in

the first term and a low score in the second. This way, it is clear that one cannot perform the maximization by having the highest or lowest values of each term alone. It is necessary to analyse the interaction between the sensitivities and how each one affects the final value of the eigenvalue.

2.2.4 Modified E-criterion

In the modified E-criterion, the objective is the minimization of the quotient between the largest and the smallest eigenvalue,

$$\frac{\sum_{i=1}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2+\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}+\sqrt{\left[\sum_{i}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2-\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}\right]^2+\left[\sum_{i}^{N}\frac{S_{v_{\max}}(t_i)S_{K_m}(t_i)}{\sigma_i}\right]^2}}{\sum_{i=1}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2+\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}-\sqrt{\left[\sum_{i}^{N}\frac{\left(S_{v_{\max}}(t_i)\right)^2-\left(S_{K_m}(t_i)\right)^2}{2\sigma_i}\right]^2+\left[\sum_{i}^{N}\frac{S_{v_{\max}}(t_i)S_{K_m}(t_i)}{\sigma_i}\right]^2}},$$

and as it can be seen, this quotient will tend to 1 because the biggest value of the largest eigenvalue is always greater than the one of the smallest eigenvalue.

Not all optimality criteria can be used in an analytical treatment so that optimal conditions can be calculated. Therefore numerical optimization procedures have to be applied.

2.3. Genetic Algorithm

Genetic algorithms are a subset of a larger class of optimization algorithms, called evolutionary algorithms, which apply evolutionary principles in the search through high-dimensional problem spaces. Genetic algorithms, in particular code designs, candidate solutions to a problem as a digital "chromosome"— a vector of numbers in which each number represents a dimension of the search space and the value of the number represents the value of that parameter [4], [5].

Genetic algorithms are operated through three processes: selection, crossover and point mutation. The optimization process will start with a random population (vectors of variables with random values); the fitness of the vectors is tested, then the best ones are selected to continue to the next generation; those that are not selected will be

recombined with each other (crossover) or mutated (one or more values of the vector will randomly changed).

After these 3 procedures the fitness of the vectors is tested again and if no optimization criterion is reached, the process is iterated until this criterion is met. This way it can be assured that the most suitable parameter values will be spread throughout generations, evolving towards higher fitness scores. The algorithm is shown schematically in **Error! Reference source not found.**.

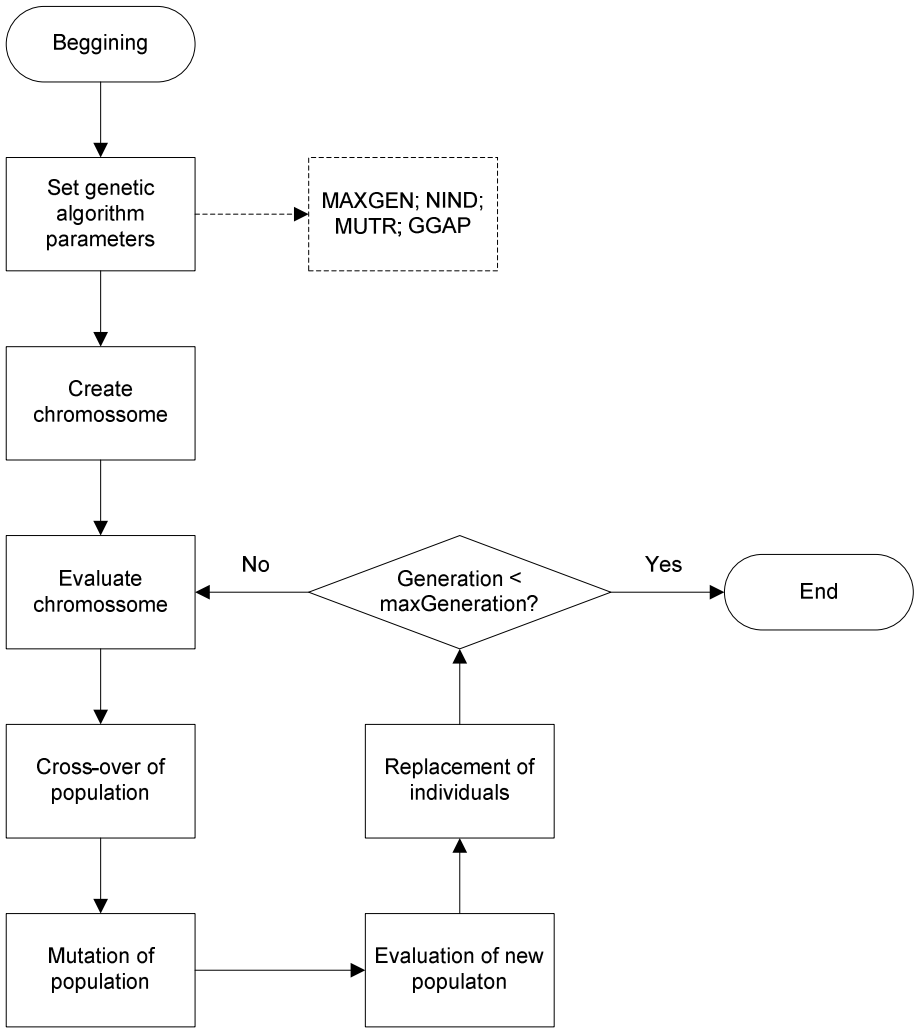

Figure 2.1: Diagram of genetic algorithm's simplified way of working

The number of parameters that will be optimized by the genetic algorithm used in this study changes according to the mode applied. If the mode in use is batch, there will be 10 parameters to optimize which are the timestamps of the measurement times; when using fed-batch with pulses, there will be 17 parameters: 5 feeding timestamps, 1 for the

initial substrate concentration, 10 for measurement times and 1 for initial volume in the reactor; if the mode is continuous feeding, then there will be 23 parameters: 11 to construct a function that will show how the continuous feeding will change through time, as it is shown in the next chapter, in Figure 3.1; 1 for initial substrate concentration; 1 for initial volume and 10 for measurement time tags.

The genetic algorithm that is used to find the optimal conditions is available as a toolbox for MATLAB, in the form of various MATLAB files (The Genetic Algorithm Toolbox, Department of Automatic Control and Systems Engineering, University of Sheffield, http://www.shef.ac.uk/uni/projects/gaipp/ga-toolbox/).

The optimization procedure is implemented using MATLAB (Ver.6.5.0.180913a Release 13, Simulink 5.0, The MathWorks, Inc.). The integration of the differential equations is performed by the Simulink method ode15s, which is used for stiff functions, such as the function to calculate the volume of sampling.

3. Process and implementation details

3.1. Experiment description

The purpose of this investigation is to simulate an experiment that can be carried out in three modes: batch, fed-batch with pulses and fed-batch continuous.

The difference between these 3 modes is the way the substrate feeding is performed. In the first mode, all the substrate is added before the reaction starts; in fed-batch with pulses, there will be a fraction of substrate added before the experiment starts and the rest will be added (as pulses) throughout the experiment at optimized times; in fed-batch continuous mode, there will also be a fraction of substrate in the reactor before the reaction starts to occur and the rest will be added continuously during the experiment.

The simulated procedure is the following:

- Fill a reactor with an initial water volume (in batch mode this volume is 5 mL; in fed-batch with pulses and continuous this volume will be optimized, being the maximum volume available 10mL);
- Add 50 mg of enzyme (any enzyme that follows the Michaelis-Menten kinetics is suitable);

- Add an initial substrate mass so that the initial concentration is equal to the one specified (in batch mode the mass is 10 mmol while in the other two modes it is optimized). The substrate used in this experiment is D-IPG (molar weight = 132.16 g/mol);
- During the experiment, add the remaining substrate and water either in the form of pulses (every pulse has the same concentration) at optimized timestamps or continuously (this feeding will be performed following an optimized function obtained in MATLAB);
- Perform measurements throughout the experiment at optimized time points (each sample has a volume of 300 µL).

With these conditions the parameter v_{max} will have an estimated value of 0.12 mol/(g.h) and K_m 0.3 mol/L.

3.2. Process restrictions and possible design scheme

The objective of the investigation is to find the optimal conditions in which the parameter values have the lowest error associated (using different criteria for that purpose). In order not to turn this into a too complex search, some restrictions had to be taken into account (to prevent the need of excessive experimental effort), such as the operation time being 5 h, 10 measurements carried out throughout the experiment, either single or multiple measurements at once.

In batch mode, only half of the total volume will be used so that an initial concentration of substrate of 2 mol/L is obtained. For the equidistant sampling, each measurement is made every half hour, starting on 0.5 h and ending at 5 h, while each feed is made every 0.83 h, starting at 0.83 h and ending at 4.17 h.

For fed-batch mode, using pulses, the number of pulses is 5 for either experimental design or equidistant measurement. All pulses have the same concentration, for each experiment. The volume of the pulses will be optimized in a way that the sum of the volume of the pulses plus the initial volume is equal to the total volume. In fed-batch mode, the maximum attainable initial substrate concentration is 2 mol/L as well, so that

a comparison can be made. In equidistant sampling technique, half of the quantity of substrate is used as initial mass, serving the other half as feeding. The initial volume used is 2.5 mL**,** being (consequently) the initial concentration 2 mol/L.
The volume flow is realized by pulses, being a pulse described as

$$\dot{V}_{measurement} = V_{pulse} \frac{Heaviside(t - t_i) \times Heaviside(t_i - t + \Delta t)}{\Delta t}, \text{ with } \Delta t = 0.1\,\text{h}$$

in which the Heaviside is a stiff function that has the value 0 before the pulse time tag and 1 after that, for the first term, and 1 before the pulse time tag plus the duration of the pulse and 0 afterwards. Thus, the pulse is well described and implemented in the program.

The continuous feeding function is built in the following way:

- Create 11 random values;
- Use the *spline* MATLAB-function to fit a line into the previous points;
- Calculate the integral below the line;
- Create a factor equal to the quotient between feeding volume and the integral;
- Multiply each point of the previously defined line by factor;
- Set as feeding profile the previous result.

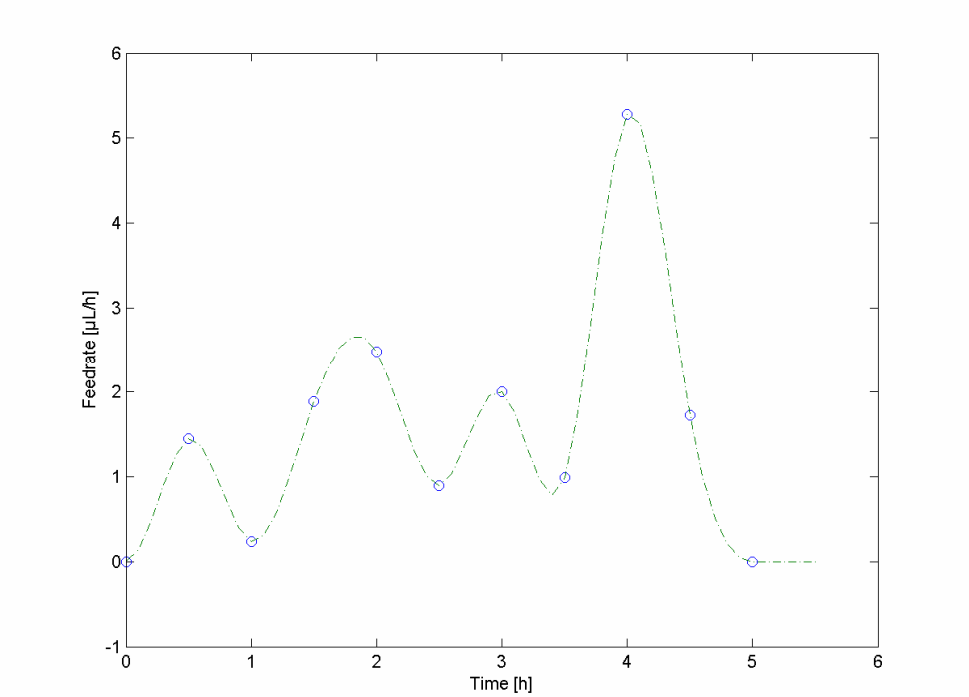

Figure 3.1: Example of optimized time tag points for continuous feed and an adjusted function that will represent the feeding rate throughout the experiment

For the optimization procedure, a genetic algorithm is used, using as criteria of optimization the criterion-A (minimization of the sum of the diagonal terms in the inverse of the FIM), criterion-D (maximization of the determinant of the FIM), criterion-E (maximization of the smallest eigenvalue of the FIM) and criterion-E-modified (minimization of the quotient between the largest and the smallest eigenvalue).

Table 3.1: Parameters and conditions used in the experiments

Quantity	Amount available
Substrate, M_S^{total}	10 mmol
Buffer solution, V_{total}	10 mL
Enzyme, M_E^{total}	50 mg
Number of measurements, N_0	10
Number of feeding pulses	5
Measurement volume, $V_{measurement}$	0.3 mL each sample
Feed volume (equidistant sampling)	1.5 mL each pulse
Duration of feed, Δt	0.1 h each pulse
v_{max} rough estimate	0.12 mol/(g.h)
K_m rough estimate	0.30 mol/L

The number of individuals evaluated in each iteration is 1000. For recombination, the one point cross over and a mutation rate of 10 % were chosen. It was used a generation gap of 10 %. As selection procedure, the roulette wheel method was used. For each optimization, 1000 populations were processed.

3.3. Measurement error variances

The optimization is performed using two different measurement error variances. One variance

$$\sigma_1 = \left(0.05 \frac{M_S^{total}}{V_{total}} \right)^2$$

is independent of the measurement range and value and has a constant value, which is 2.5 % of the substrate concentration at the process start of the batch run (referred from now on as $\sigma_1 = 2.5 \times 10^{-3}\,\mathrm{mol^2/L^2}$). The second variance depends on the measurement range, as well as the individual measurement values

$$\sigma_2 = \left(0.03 \frac{mol}{L} + 0.04 S(t_i) \right)^2$$

(it will be referred as σ_2). In this case, it is noted that the error increases linearly with its measurement value and that it cannot be lower than 0.03 mol/L.

3.4. Conditions for the comparison between experimental design and equidistant sampling

For the comparison between experimental design and equidistant sampling, the range used for v_{max} values was from 0.05 until 0.20 mol/(g.h) and for K_m between 0.15 and 0.90 mol/L. In this comparison, the objective is to search for the lowest value Cramér-Rao lower bound, in respect to the parameter inside the range defined above and, according to that value, retrieve the correspondent value of the parameter. This comparison also allowed determining which values of v_{max} and K_m experimental design would have a lower CRLB than equidistant sampling method.

4. Results and Discussion

The A criterion and the measurement error σ_1 are applied, if the criterion and measurement error are not mentioned.

4.1. Experimental design for batch process

The substrate concentration as well as the squares of the sensitivities profile, for batch process of criterion A using error σ_1, are presented in Figure 4.1. These profiles represent the general case for batch process, for any of the errors.

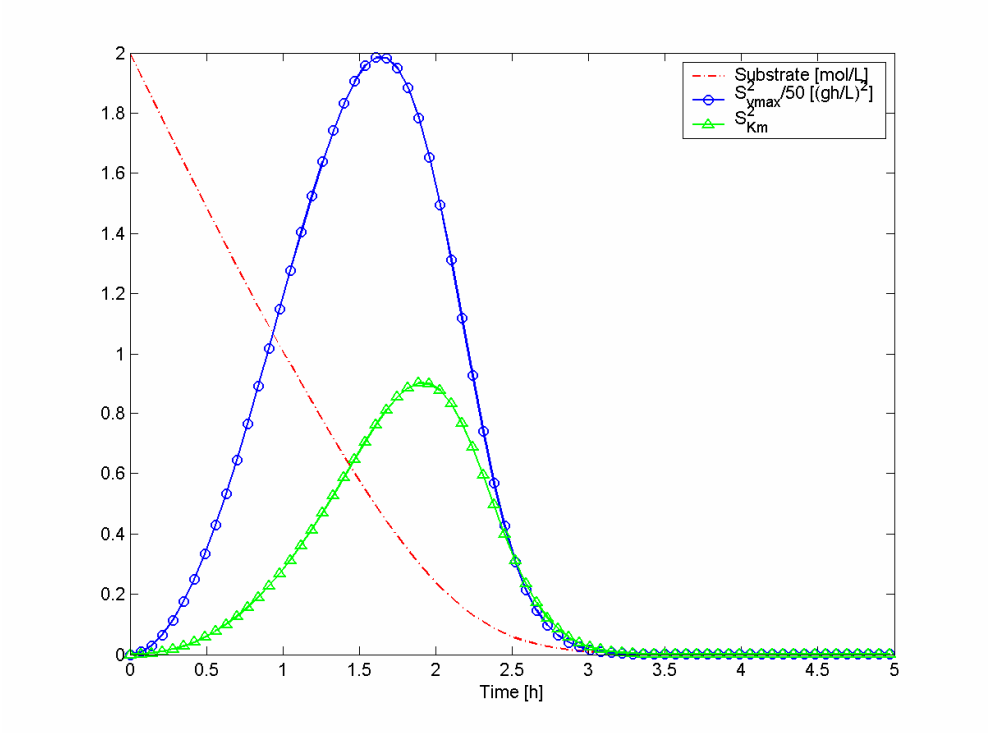

Figure 4.1: Substrate concentration and squared sensitivities of the batch process using criterion A and error σ_1, for experimental design

The FIM is influenced by values of the sensitivities at sampling time and, therefore, to obtain the best values of FIM, these time points must be optimized. The sensitivity with respect to v_{max} is always higher than K_m's, which means that the estimation error of v_{max} will be lower than the one of K_m's. If the squares of the sensitivities are divided one by the other, for example $\frac{S^2_{v_{\max}}(t_i)}{S^2_{K_m}(t_i)}$, it will be possible to know that v_{max} will be determined with more precision at high concentrations of substrate, while K_m will have a more accurate value at low substrate concentration, because the previous quotient decreases with the decrease of concentration (Figure 4.2). Consequently, the biggest difference is obtained in the beginning which proves that v_{max} will be determined with higher precision in higher substrate concentration and K_m in lower concentration ranges, but v_{max} will have a higher precision since this quotient is always larger than 1.

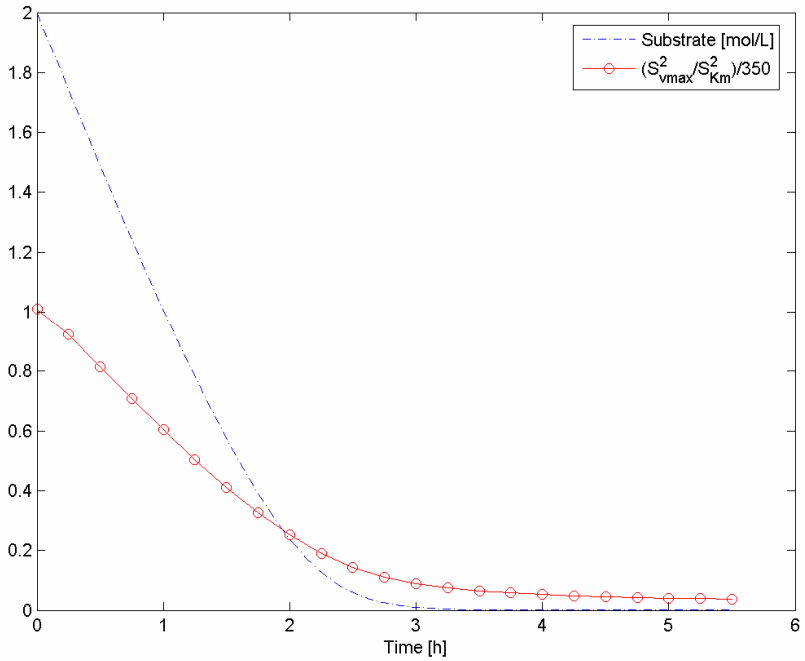

Figure 4.2: Quotient between $S^2_{v_{\max}}(t_i)$ and $S^2_{K_m}(t_i)$ and profile of substrate concentration through time

After the optimization was performed, 2 measurement points were obtained for every criterion and every measurement error, confirming what had already been stated in previous investigations that**,** for each parameter to be optimized**,** one measurement point is obtained [6].

For the absolute constant error (σ_1), the first measurement point is obtained around 0.95 h and the second at 2.33 h, except for criterion D for the first time tag, which is obtained at 1.18 h. For every criterion, 5 replicates for each time of measurement were obtained.

For the linear growing measurement error (σ_2), once again, criteria A and E had similar results, having its measurement times around 1.26 h and 2.49 h with 5 replicates each. With criterion D, time tags were at 1.50 h and 2.36 h being this last one close to the ones obtained by the other criteria. Note that a measurement point of low substrate concentration (where a good precision for the Km parameter can be found) would be expected and is, effectively, possible to be observed, for every criterion and measurement error, around 2.20-2.50 h.

In respect to CRLB's, they seem to be similar between both measurement errors and their values are around 6.60 % (for v_{max}), 26.6 % (for K_m, criteria A and E) and 28.8 % (K_m, criterion D), for the first measurement error. For the linear measurement error,

CRLB's are around 6.34 % and 23.4 % (for v_{max} and K_m, respectively) for criteria A and E. For criterion D, its CRLB's are slightly larger with 6.82 % and 25.6 %, for v_{max} and K_m, respectively. Again, it is evident that according to what was mentioned before, the v_{max} parameter is obtained with higher precision, since its Cramér-Rao lower bounds are lower than those of K_m. These results are presented on Table 4.1 and Table 4.2. The results presented for E-mod criterion will not be analysed, since its CRLB values are approximately two orders of magnitude larger than every other criteria, making them not comparable.

Table 4.1: Results of the optimization for batch process using the error σ_1

Criterion	Time tag of measurement [h], (replicate)		CRLB v_{max} [%]	CRLB K_m [%]
	t_1	t_2		
A	0.93 (5)	2.36 (5)	6.36	26.69
D	1.18 (5)	2.23 (5)	6.82	28.78
E	0.96 (5)	2.39 (5)	6.63	26.41
E-mod	0.00 (1)	5 (9)	31426	66631

Table 4.2: Results of the optimization for batch process using error σ_2

Criterion	Time tag of measurement [h], (replicate)		CRLB v_{max} [%]	CRLB K_m [%]
	t_1	t_2		
A	1.27 (5)	2.49 (5)	6.32	23.5
D	1.50 (5)	2.36 (5)	6.82	25.6
E	1.25 (5)	2.49 (5)	6.35	23.4
E-mod	0.00 (1)	5 (9)	19032	40167

4.2. Experimental design for fed-batch (pulses) process

The concentration profile of an optimized fed-batch (pulses) process is shown in Figure 4.3 and the results of the optimal fed-batch (pulses) processes are presented in Table 4.3 and Table 4.4. While looking at the substrate concentration variation in time, it is clear that in fed batch it lowers much quicker than the batch mode (concentration reaches under 0.2 mol/L in about an hour while in batch process it takes approximately 2 hours) due to lower volume in the beginning of the reaction; since the same substrate mass is

consumed in the same period of time, either batch or fed-batch process, but the volume is lower then, the concentration variation will be bigger. The initial concentration is the maximum attainable (2 mol/L) and each pulse has a concentration of c_{pulse} = 0.74 mol/L. The volume of each pulse was 1.59 mL. These values are from the fed-batch (pulses) process using σ_1 and criterion A.

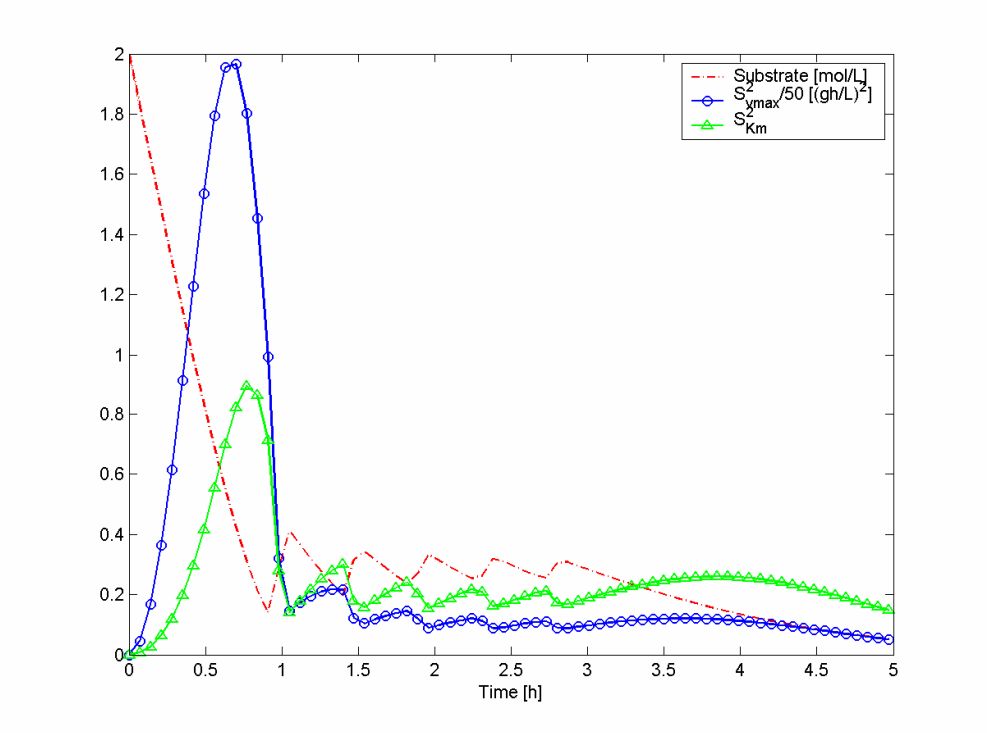

Figure 4.3: Substrate concentration and squared sensitivities of the optimized fed-batch (pulses) process using criterion A and error σ_1, for experimental design

Comparing the results to those of the batch process, some improvements are noticed in error values of both parameters. CRLB for v_{max} lowered its value to around 5.58 % and 5.14 %, for σ_1 and σ_2, respectively, while CRLB for K_m was improved to around 20.2 % and 16.0 % (criteria A and E), for σ_1 and σ_2, respectively, and to 22.6 % and 18.0 % in criterion D. Therefore, when fed-batch (pulses) process is used instead of batch process, the improvement (error reduction) will be about 18.6 % for v_{max}, while K_m has an error decrease of around 26.4 %.

For the first measurement error, in respect to time tags of feeding, one can see that the first four are similar and are around 0.91, 1.40, 1.88 and 2.34 h. The last one is around 2.71 h for criteria A and E and 4.85 h for criterion D. As for measurement time tags, they are almost the same, being the first one about 0.47 h with 3, 4 and 3 replicates for criteria A, D and E, respectively, and the second measurement set at 4.06 h with the

remainder replicates. Again, the existence of an early and a late measurements is clear, as should be expected, so that there is one measurement with high and another with low substrate concentration, making a good accuracy in parameters' value possible.

For σ_2, the measurement time tags are slightly higher (around 0.19 h for the first and 0.37 h for the second, except for the D criterion which is lower – 3.43 h). For feeding times, a slightly increase in times is detectable, about 0.15-0.30 h, except in the last two feeding times in D criterion, which have the values 3.94 and 4.28 h. Comparing all optimization criteria, one can see that the one that has the lowest overall CRLB is A-criterion, with an average CRLB values of 6.34 % and 25.1 % for v_{max} and K_m, using batch process, while for fed-batch (pulses) process having as average CRLB's 5.27 % and 18.1 %, v_{max} and K_m, respectively.

Table 4.3: Results of the optimization for fed-batch (pulses) process using the error σ_1

Criterion	Time tag of feeding [h]					Feed volume (per pulse) [mL]	Time tag of measurement [h], (replicate)		CRLB	
	t_1	t_2	T_3	t_4	t_5		t_1	t_2	v_{max} [%]	K_m [%]
A	0.95	1.41	1.85	2.29	2.72	1.59	0.43 (3)	4.06 (7)	5.49	20.2
D	0.86	1.40	1.97	2.48	4.85	1.60	0.54 (4)	4.08 (6)	5.65	22.6
E	0.93	1.39	1.83	2.26	2.69	1.59	0.40 (3)	4.04 (7)	5.61	20.2
E-mod	2.88	3.77	4.97	5	5	5.53	0.01 (1)	5 (9)	403	606

Table 4.4: Results of the optimization for fed-batch (pulses) process using the error σ_2

Criterion	Time tag of feeding [h]					Feed volume (per pulse) [mL]	Time tag of measurement [h], (replicate)		CRLB	
	t_1	t_2	t_3	t_4	t_5		t_1	t_2	v_{max} [%]	K_m [%]
A	1.07	1.55	2.02	2.48	2.93	1.57	0.63 (3)	4.37 (7)	5.05	16.0
D	0.98	1.52	2.05	3.94	4.28	1.57	0.76 (4)	3.43 (6)	5.21	18.0
E	1.23	1.73	2.20	2.64	3.08	1.57	0.59 (3)	4.50 (7)	5.17	16.0
E-mod	2.52	3.45	4.98	5	5	5.80	0.03 (1)	5 (9)	306	463

4.3. Experimental design for fed-batch (continuous) process

Figure 4.4 shows the substrate concentration profile for fed-batch (continuous) along with sensitivities in respect to each parameter (v_{max} and K_m). When comparing this

profile to the one of fed-batch (pulses), one can see that first one is “smoother”. This is due to the fact that the feeding is not processed by pulses, which will make the concentration change not as abruptly as in the discrete pulses mode.

When comparing the results with fed-batch (pulses), it is noticeable that the first set of measurements moves towards earlier in time (higher substrate concentration), around 0.10 h for criteria A and E, for σ_1, and 0.18 h for σ_2; for criterion D, this change in time is around 0.04 h for both measurement error types; the second set of measurements will be performed later in time (lower substrate concentration), all of the measurements will be performed at 5 h. Subsequently, both v_{max} and K_m will be determined with a higher accuracy.

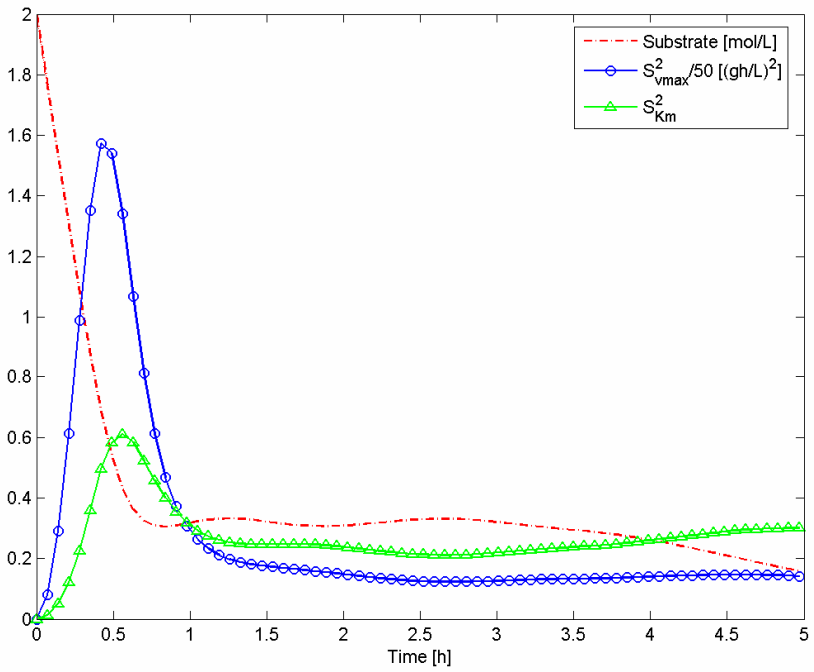

Figure 4.4: Substrate concentration and squared sensitivities of the optimized fed-batch (continuous) process using criterion A and error σ_1, for experimental design

In respect to CRLB v_{max}, these were increased, in comparison with fed-batch (pulses), in an average of 0.08 % for the first measurement error type, while the second showed an increase in CRLB of about 0.36%. On the other hand, when analysing CRLB K_m values, it has to be pointed out the fact that these were lowered in about 0.50 % for criterion A and E while criterion D was the only one showing an increase in its associated error (0.48 % for the first measurement error type and 2.08 % for the second one).

Table 4.5: Results of the optimization for fed-batch (continuous) process using the error σ_1

Criterion	Time tag of measurement [h], (replicate)		CRLB	
	t_1	t_2	v_{max} [%]	K_m [%]
A	0.33 (3)	5 (7)	5.57	19.70
D	0.53 (4)	5 (6)	5.69	23.12
E	0.30 (3)	5 (7)	5.72	19.66
E-mod	0.06 (1)	5 (9)	89.7	139.4

Table 4.6: Results of the optimization for fed-batch (continuous) process using the error σ_2

Criterion	Time tag of measurement [h], (replicate)		CRLB	
	t_1	t_2	v_{max} [%]	K_m [%]
A	0.46 (3)	5 (7)	5.32	15.93
D	0.72 (5)	5 (5)	5.44	20.06
E	0.40 (2)	5 (8)	5.75	15.43
E-mod	0.22 (1)	5 (9)	44.7	71.4

4.4. Equidistant sampling for batch and fed-batch (pulses) processes

When using the method of equidistant sampling, the purpose is to define upfront the measurement and feeding times and see how good the parameters' errors will be. When observing the concentration profile, the influence of the feeding is not so noticeable like in experimental design concentration profile and this is due to a slightly lower pulse concentration, c_{pulse} = 0.67 mol/L. This concentration is lower either because the mass available for feeding is lower (5 mmol) and of higher pulses' volume (1.5 mL each).
Checking the results, it is clear that both batch and fed-batch (pulses) processes have worst CRLB than the experimental design. For batch, the errors obtained were about 11.0 % and 45.4 %, for v_{max} and K_m, respectively, while for fed-batch (pulses) 8.56 % and 26.5 %. One can clearly see that the fed-batch (pulses) process results are better (K_m has almost half the error than in batch mode), which means that feeding, instead of having all the substrate at the beginning, is a better way to obtain more reliable values of parameters.

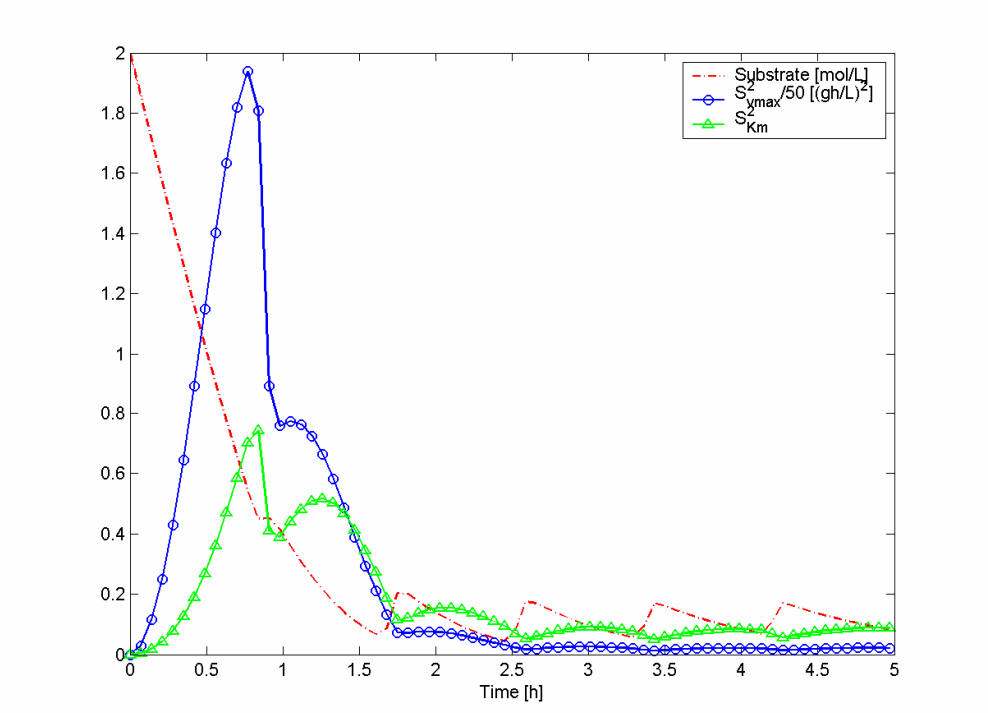

Figure 4.5: Substrate concentration and squared sensitivities of the fed-batch (pulses) process using criterion A and error σ_1 for equidistant sampling' method

Table 4.7: Time tags for the equidistant sampling and its results

Criterion	Error Type	Time tag of feeding [h]					Time tag of measurement [h]										CRLB	
		t_1	T_2	t_3	T_4	t_5	t_1	t_2	T_3	t_4	t_5	t_6	t_7	t_8	t_9	t_{10}	v_{max}, %	K_m, %
Batch	σ_1	-	-	-	-	-	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	11.04	48.03
	σ_2																11.04	42.83
Fed-batch	σ_1	0.83	1.67	2.50	3.33	4.17	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	8.48	29.06
	σ_2																8.64	23.89

4.5. Comparison between experimental design and equidistant measurement points for batch process

The influence of the precision of the rough estimates of v_{max} and K_m used in the experimental design procedure is compared to the equidistant sampling' procedure. Therefore CRLB values are calculated where the design of the experiment was based on the parameters values v_{max} = 0.12 mol/(g.h) and K_m = 0.3 mol/L, however assuming as real parameter values different ones. In Figure 4.6 and Figure 4.7 the dependence of the CRLB on the real value of the parameter is presented for both design methods;

experimental design, however, is not the best for every value, i.e., a lower corresponding CRLB value might not be found for every value of v_{max} and K_m. With the data that is presented on those figures, one can also see which is the optimal parameter value, i.e., which parameter value has the lowest CRLB.

For the first measurement error, the lowest CRLB with respect to v_{max} is around v_{max} = 0.124 mol/(g.h) for the three criteria and while the lowest CRLB with respect to K_m is around 0.338 mol/L for criteria A and E and 0.286 mol/L for criterion D. For these parameters' value the corresponding CRLB are 6.51 % and 27.0 %. For σ_2 the lowest CRLB in respect to v_{max} value is around 0.124 mol/(g.h) and the lowest CRLB in respect to K_m is 0.314 mol/L for criteria A and E and 0.271 mol/L for criterion D.

The reason why the lowest CRLB values are not obtained with the values of the rough estimates might be the fact that all optimization criteria (A, D and E) cover experimental conditions for both parameters at the same time. Here, the change of one parameter is considered by fixing the other one and, therefore, a smaller CRLB can occur.

The range in which a smaller error for experimental design for parameter v_{max} is observed is between 0.095 and 0.150 mol/(g.h), while for K_m it is between 0.159 and 0.670 mol/L. For the second measurement error (σ_2), the first range is almost the same, while the one for K_m changes its bounds to 0.170 and 0.572 mol/L.

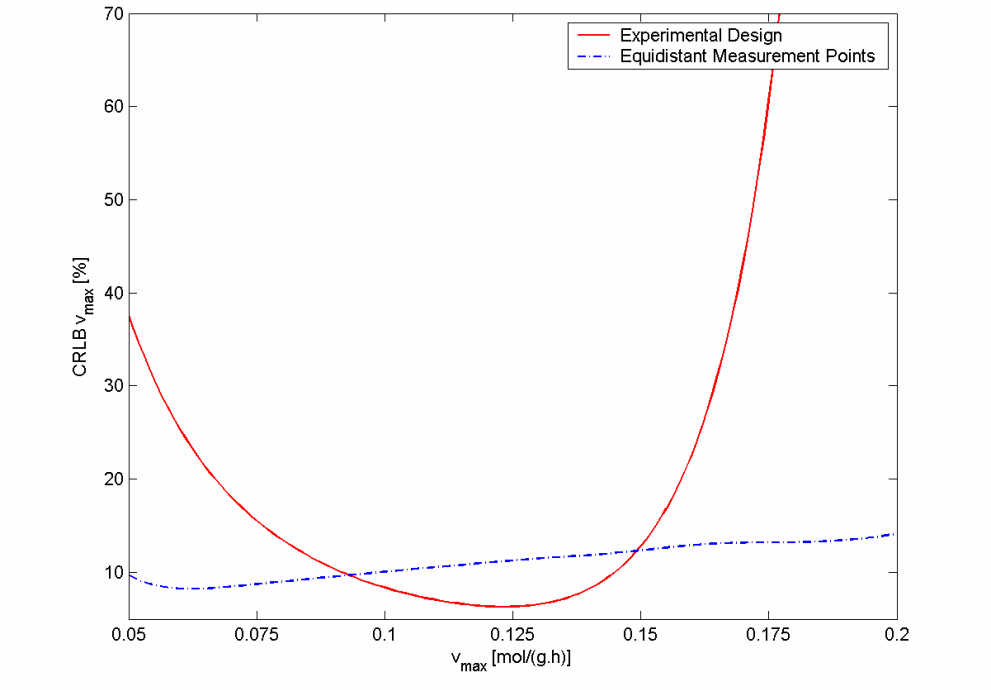

Figure 4.6: CRLB v_{max} dependence on real values of v_{max} for experimental design (K_m fixed to 0.3 mol/L) and equidistant sampling using σ_1, for batch process

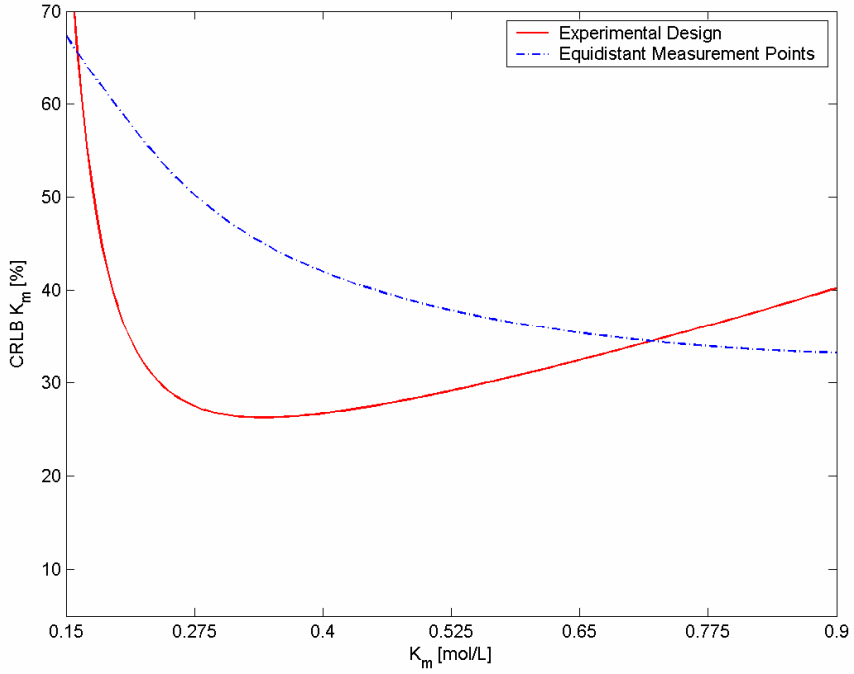

Figure 4.7: CRLB K_m dependence on real values of K_m for experimental design (v_{max} fixed to 0.12 mol/(g.h)) and equidistant sampling using σ_1, for batch process

Table 4.8: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for batch process using the error σ_1, for v_{max}

Criterion	v_{max} with lowest CRLB [mol/(g.h)]	Lowest CRLB v_{max}	Experimental Design Range	
			Lower bound [mol/(g.h)]	Higher bound [mol/(g.h)]
A	0.123	6.32	0.0937	0.149
D	0.126	6.64	0.0967	0.152
E	0.124	6.57	0.0952	0.148

Table 4.9: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for batch process using the error σ_1, for K_m

Criterion	K_m with lowest CRLB [mol/L]	Lowest CRLB K_m [%]	Experimental Design Range	
			Lower bound [mol/L]	Lower bound [mol/L]
A	0.342	26.3	0.161	0.719
D	0.286	28.6	0.150	0.595
E	0.335	26.1	0.165	0.696

Table 4.10: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for batch process using the error σ_2, for v_{max}

Criterion	v_{max} with lowest CRLB [mol/(g.h)]	Lowest CRLB v_{max} [%]	Experimental Design Range	
			Lower bound [mol/(g.h)]	Higher bound [mol/(g.h)]
A	0.123	6.28	0.0967	0.144
D	0.125	6.66	0.0997	0.146
E	0.124	6.28	0.0975	0.145

Table 4.11: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for batch process using the error σ_2, for K_m

Criterion	K_m with lowest CRLB [mol/L]	Lowest CRLB K_m [%]	Experimental Design Range	
			Lower bound [mol/L]	Lower bound [mol/L]
A	0.316	23.3	0.180	0.606
D	0.271	25.2	0.154	0.508
E	0.312	23.4	0.176	0.602

For a judgement if experimental design procedure or equidistant sampling should be carried out, a rough error is calculated as follows,

$$\frac{\min\{\text{high bound - Parameter estimate, Parameter estimate - low bound}\}}{\text{Parameter}} \times 100\,(\%)$$

This way it is possible to present a table that shows the maximum error that one parameter may have to be performed experimental design.

Table 4.12: Maximum parameter error for different criteria and measurement error type, for batch process

	σ_1		σ_2	
Criterion	v_{max} [%]	K_m [%]	v_{max} [%]	K_m [%]
A	21.9	46.2	19.4	40.0
D	19.4	50.0	16.9	48.7
E	20.6	45.0	18.8	41.2
Average	20.6	47.1	18.3	43.3

Having analysed all results of the comparison between the two approaches, for batch process, it can be concluded that experimental design should be used instead of equidistant sampling, if the parameter error is less than 19.5 % for v_{max} and less than 45% for K_m (average percentages).

4.6. Comparison between experimental design and equidistant sampling for fed-batch (pulses) process

The results for the fed-batch (pulses) process show a few changes when compared to the batch process. The lowest CRLB parameter values almost present the same values as the batch process but they are more scattered than the latter ones, having one of the criteria values below the estimated value for v_{max} (criterion D, v_{max} =0.114 mol/(g.h) and 0.113 for each measurement error) and the other two above 0.12 mol/(g.h) (around 0.133 mol/(g.h) for the σ_1 and 0.130 mol/(g.h) for σ_2 for both criteria). For K_m, its values are around 0.331 mol/L for the first measurement error and 0.302 mol/L for the second. As for the error associated to the parameters' value, one can see that for fed-batch (pulses) process they are about 5.47% and 5.04% for CRLB v_{max}, for σ_1 and σ_2, respectively, and around 20.9 % and 16.6 % for CRLB K_m. In fed-batch (pulses) mode, it is noticeable a wider range for experimental design to be performed instead equidistant sampling. For v_{max} the bounds are between 0.0598-0.191 mol/(g.h) approximately and 0.0613-0.172 mol/(g.h) for σ_1 and σ_2, respectively, and 0.150-0.900 mol/L for both measurement errors for K_m.

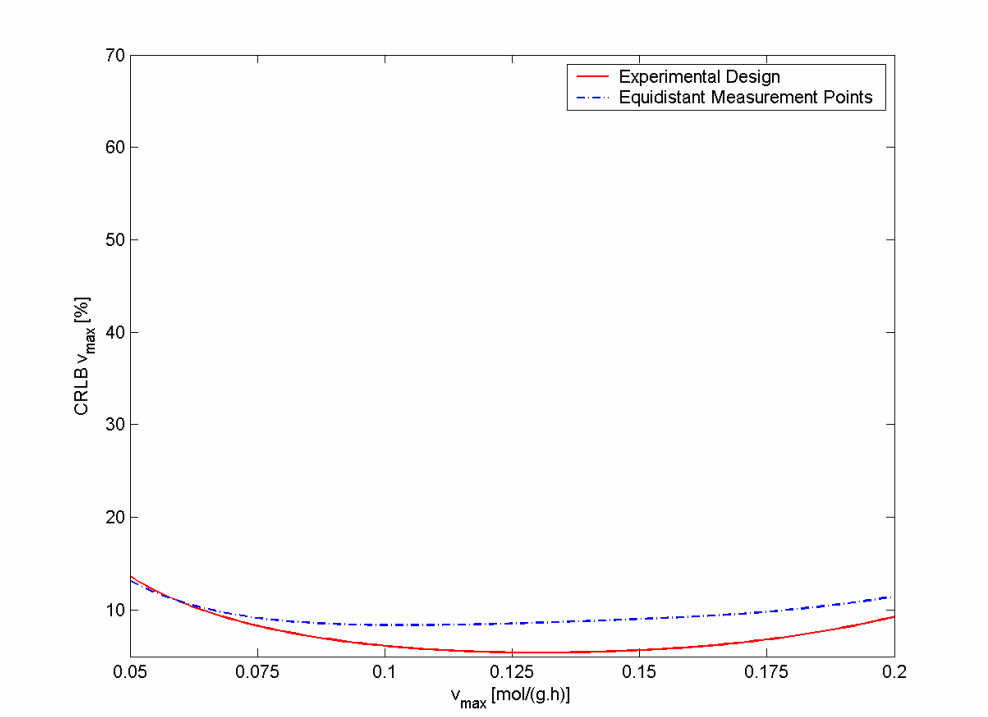

Figure 4.8: CRLB v_{max} dependence on real values of v_{max} for experimental design (K_m fixed to 0.3 mol/L) and equidistant sampling using σ_1, for fed-batch (pulses) process

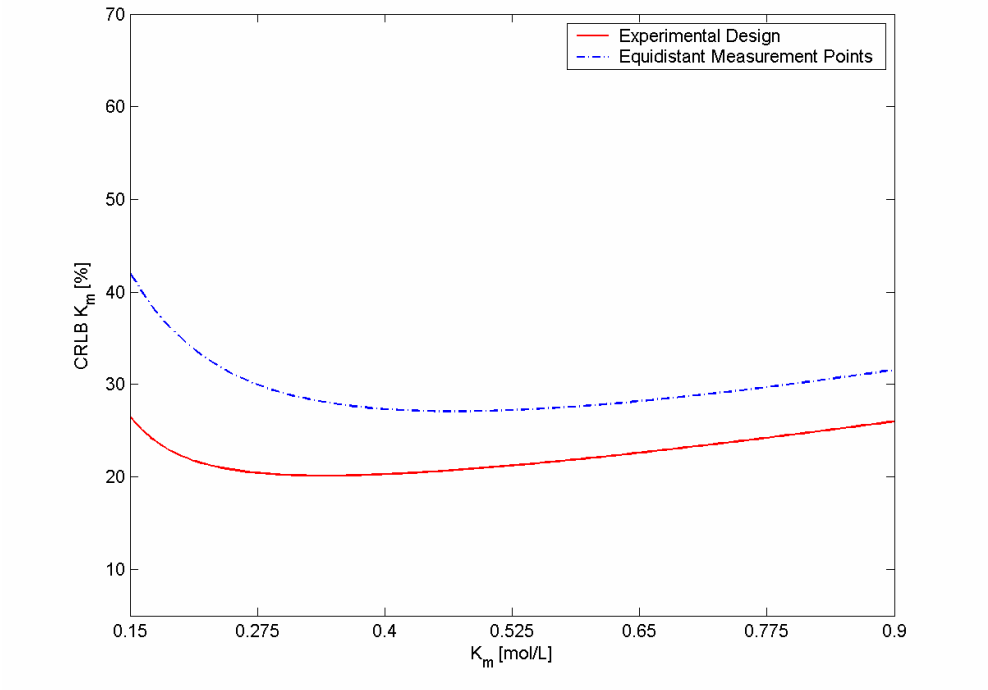

Figure 4.9: CRLB K_m dependence on real values of K_m for experimental design (v_{max} fixed to 0.12 mol/(g.h)) and equidistant sampling using σ_1, for fed-batch (pulses) process

Table 4.13: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for fed-batch (pulses) process using the error σ_1, for v_{max}

Criterion	v_{max} with lowest CRLB [mol/(g.h)]	Lowest CRLB v_{max} [%]	Experimental Design Range	
			Lower bound [mol/(g.h)]	Higher bound [mol/(g.h)]
A	0.131	5.41	0.0598	0.200
D	0.114	5.56	0.0500	0.173
E	0.136	5.44	0.0696	0.200

Table 4.14: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for fed-batch (pulses) process using the error σ_1, for K_m

Criterion	K_m with lowest CRLB [mol/L]	Lowest CRLB K_m [%]	Experimental Design Range	
			Lower bound [mol/L]	Higher bound [mol/L]
A	0.342	20.1	0.15	0.9
D	0.316	22.5	0.15	0.9
E	0.335	20.1	0.15	0.9

Table 4.15: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for fed-batch (pulses) process using the error σ_2, for v_{max}

Criterion	v_{max} with lowest CRLB [mol/(g.h)]	Lowest CRLB v_{max} [%]	Experimental Design Range	
			Lower bound [mol/(g.h)]	Higher bound [mol/(g.h)]
A	0.128	4.98	0.0651	0.177
D	0.113	5.12	0.0500	0.154
E	0.133	5.00	0.0689	0.185

Table 4.16: Lowest CRLB's and its corresponding parameter for experimental design and range in which experimental design is better than equidistant sampling for fed-batch (pulses) process using the error σ_2, for K_m

Criterion	K_m with lowest CRLB [mol/L]	Lowest CRLB K_m [%]	Experimental Design Range	
			Lower bound [mol/L]	Higher bound [mol/L]

A	0.301	16.0	0.15	0.9
D	0.305	18.0	0.15	0.9
E	0.301	16.0	0.15	0.9

As had previously been done before, for batch process, it is possible to calculate the maximum parameter error that is possible to have (to perform experimental design instead of equidistant sampling), being those errors presented on Table 4.17.

Table 4.17: Maximum parameter error for different criteria and measurement error type, for fed-batch (pulses) process

Criterion	σ_1		σ_2	
	v_{max} [%]	K_m [%]	v_{max} [%]	K_m [%]
A	50.2	50.0	45.8	50.0
D	44.1	50.0	28.4	50.0
E	42.0	50.0	42.6	50.0
Average	45.4	50.0	38.9	50.0

For fed-batch (pulses) process, one can say that knowing v_{max} and K_m with a maximum error of 42 % and 50 % (average values), respectively, one should opt by the approach of experimental design.

5. Conclusion

From the results obtained in this study, it can be concluded that experimental design is, in general, significantly better than equidistant sampling, when the final goal is the identification of Michaelis-Menten kinetic parameters. The following more specific conclusions can be taken from this study:

- In batch operation, the CRLB were reduced to about 40.2 % for v_{max} and 34.8 % for K_m when comparing experimental design and equidistant sampling;
- For fed-batch (pulses) the CRLB were reduced to about 41.6 % and 23.7 % for v_{max} and K_m when comparing experimental design and equidistant sampling respectively. Thus, the improvement in K_m is slightly lower than in the batch case;
- Comparing between batch and fed-batch (pulses) allows to conclude that the CRLB error is much lower in the latter case for both experimental design and

equidistant sampling (the error is reduced 15.4 % for v_{max} and 23.9 % for K_m, while in equidistant sampling it is reduced in about 22.5 % and 41.7 respectively);

- When employing experimental design, it is interesting to notice that from batch to fed-batch (pulses), timestamps of the measurements move towards higher (the first measurement) and lower (the second measurement) substrate concentration, resulting in higher accuracy of the parameter's estimates;
- Moreover, when comparing fed-batch (pulses) and fed-batch (continuous), one can conclude that fed-batch (continuous) tends to lead to more accurate parameter values, since the measurements are slightly closer to the beginning and end time, in respect to first and second measurements, of the experiment;
- When comparing CRLB values between fed-batch (pulses) and fed-batch (continuous), it is shown that they are very similar, having a difference of less than 0.50 %;
- Comparing again both methods of experimental planning for a wide range of v_{max} and K_m parameter values, it is clear that the equidistant sampling is only better in a very narrow region;
- Generally, timestamps for sampling and feeding of criteria A and E are similar. The difference between these timestamps is generally under 2 %. These two criteria also proved to be better than D-criterion in all situations, thus, it can be concluded that for this kind of theoretical approach for determination of parameters one should use either criterion A or E.

This experimental planning method can be applied to other types of biochemical systems, by changing the kinetics' expressions, which can be easily done by program coding in MATLAB.

6. Recommendations

For future works, a parameter that represents the inhibition in a Michaelis-Menten kinetics type reaction could be included. Consequently, it would be possible to analyse how this inhibition might affect parameter estimation.

Another possible improvement would be to create a contour that shows how CRLB values change with simultaneous changes in Michaelis-Menten parameters (v_{max} and K_m).

7. References

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8. Appendix A: Experimental design and equidistant sampling results

In this section, all results obtained for experimental design and equidistant sampling method are presented.

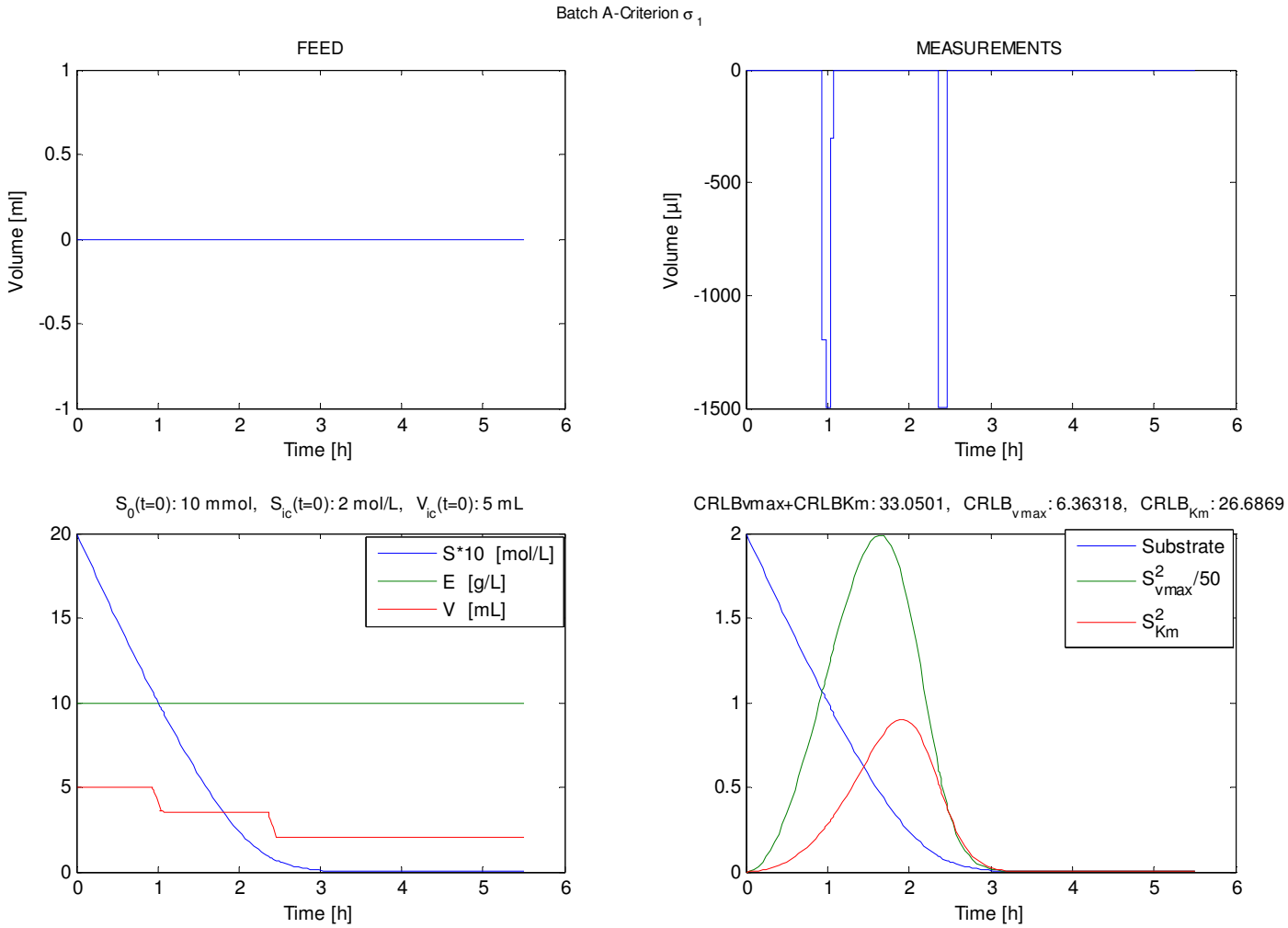

Figure 8.1: Results for experimental design - batch mode, criterion A and error σ_1

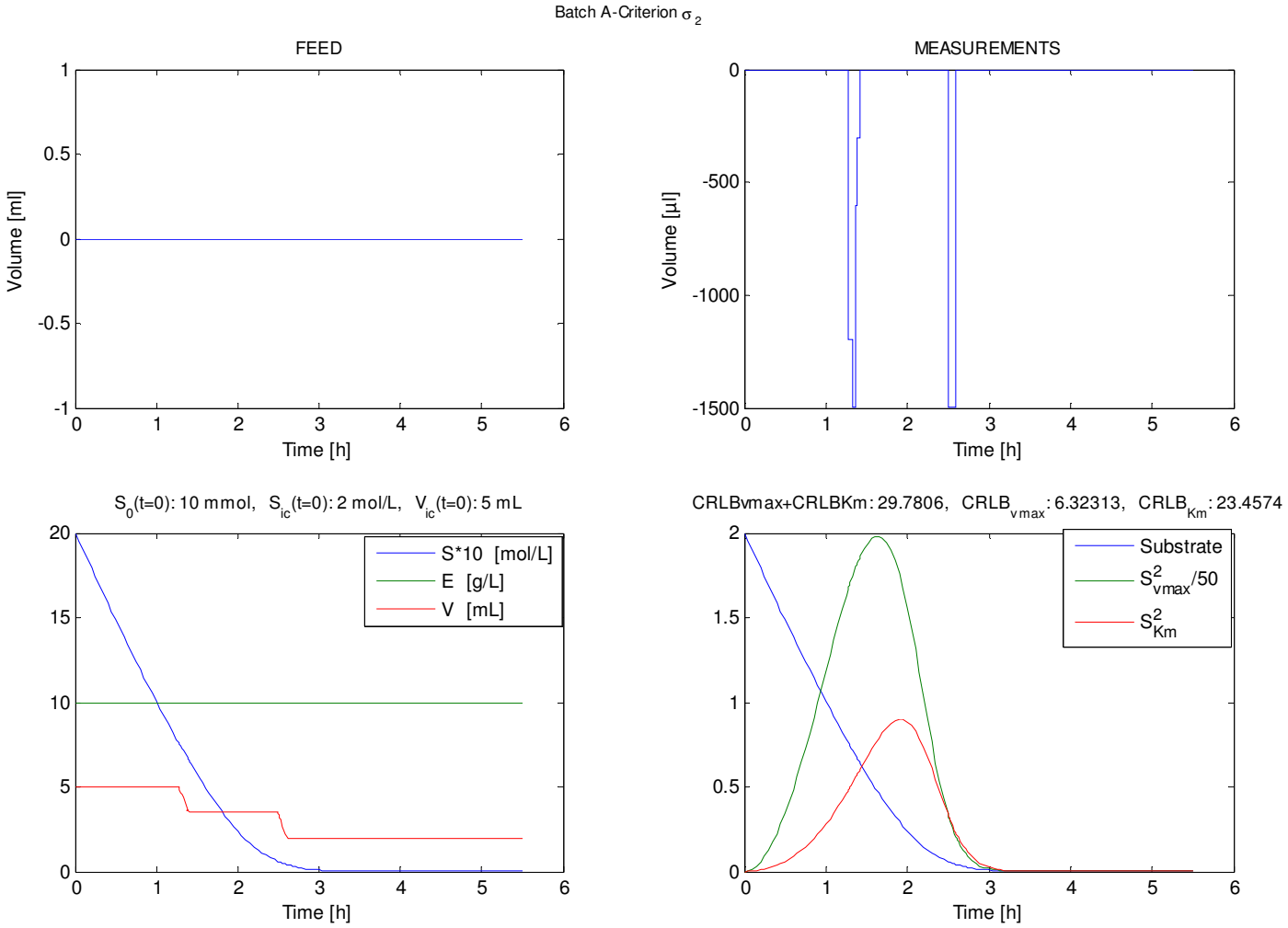

Figure 8.2: Results for experimental design - batch mode, criterion A and error σ_2

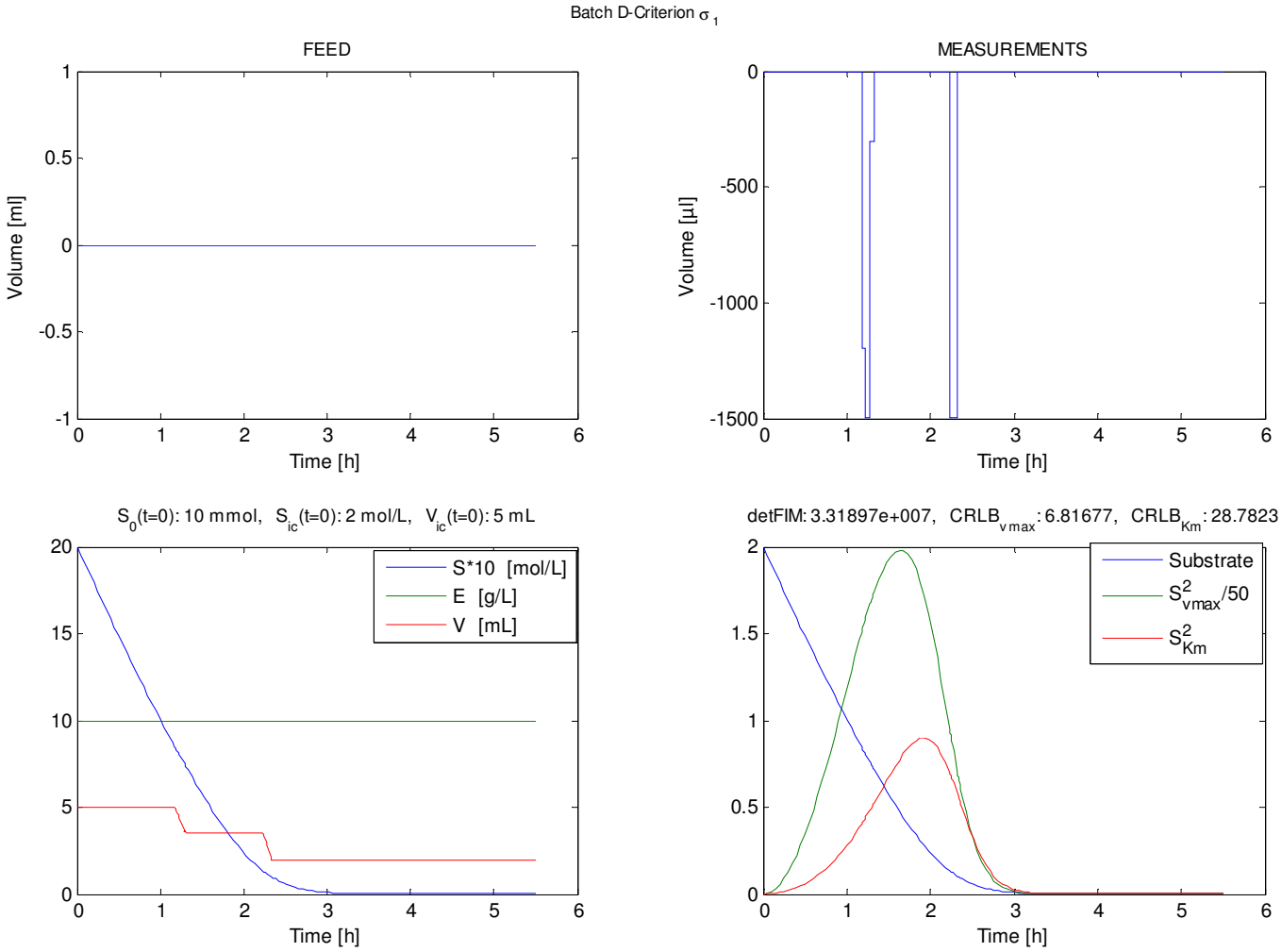

Figure 8.3: Results for experimental design - batch mode, criterion D and error σ_1

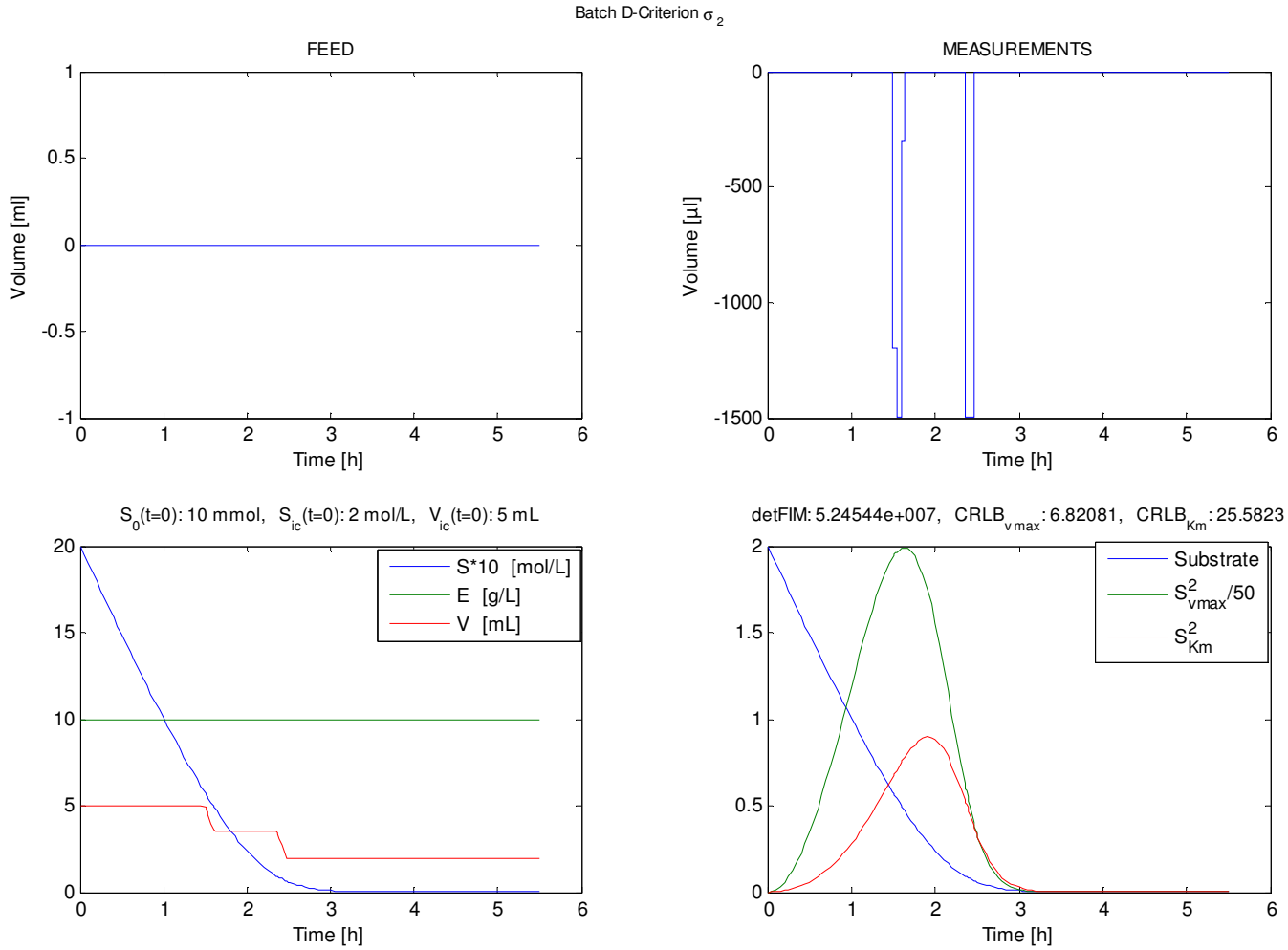

Figure 8.4: Results for experimental design - batch mode, criterion D and error σ_2

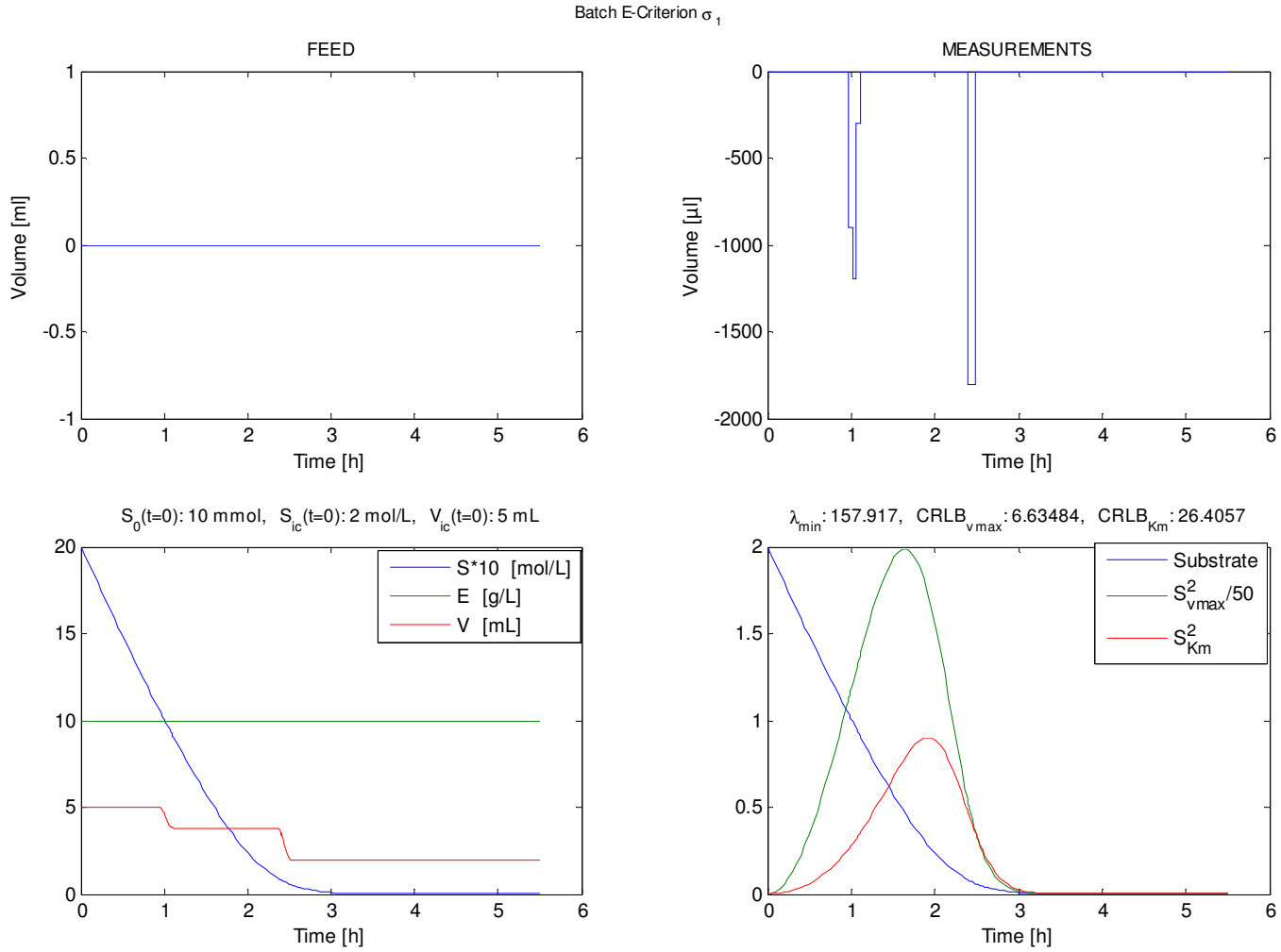

Figure 8.5: Results for experimental design - batch mode, criterion E and error σ_1

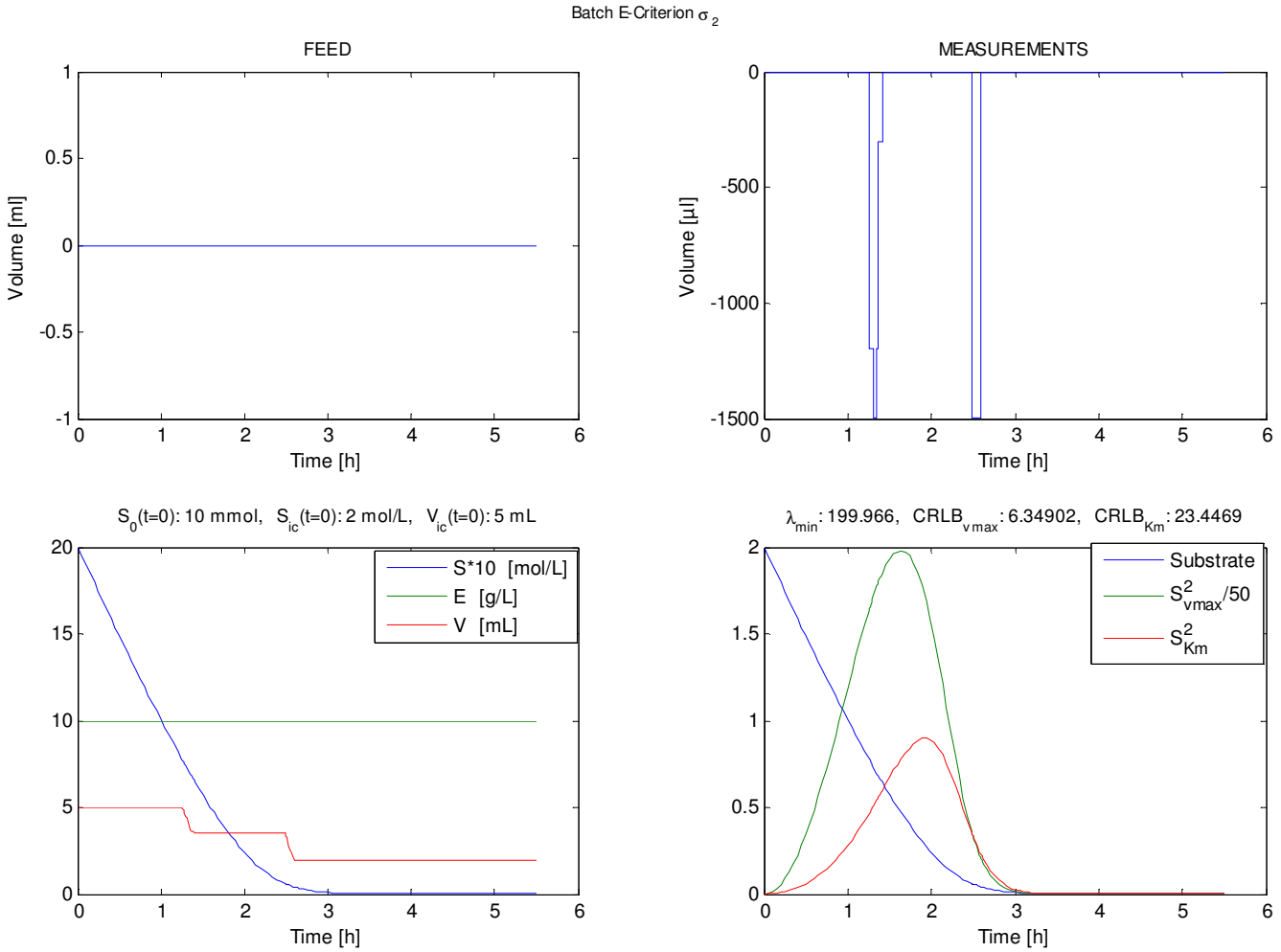

Figure 8.6: Results for experimental design - batch mode, criterion E and error σ_2

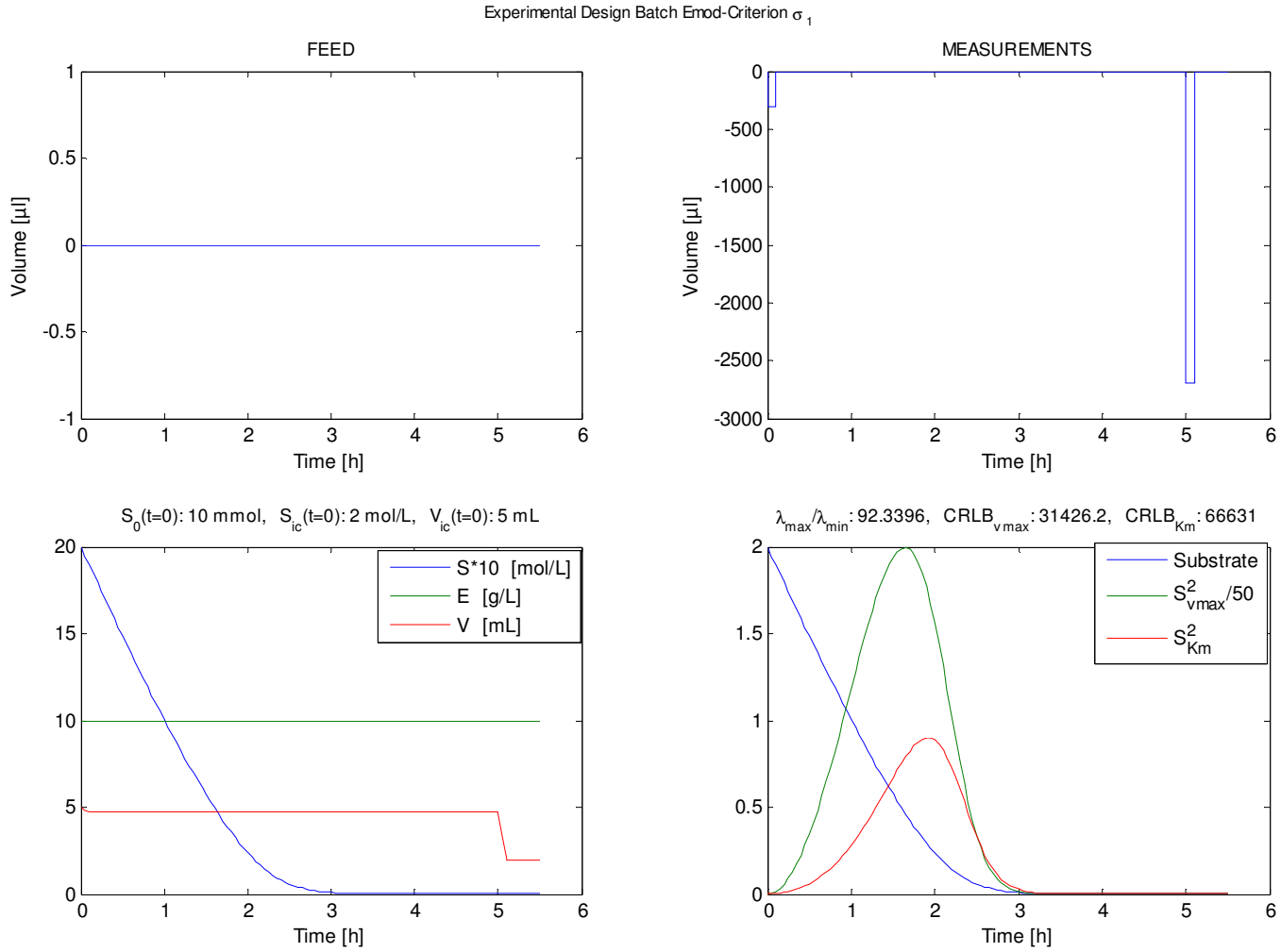

Figure 8.7: Results for experimental design - batch mode, criterion E-mod and error σ_1

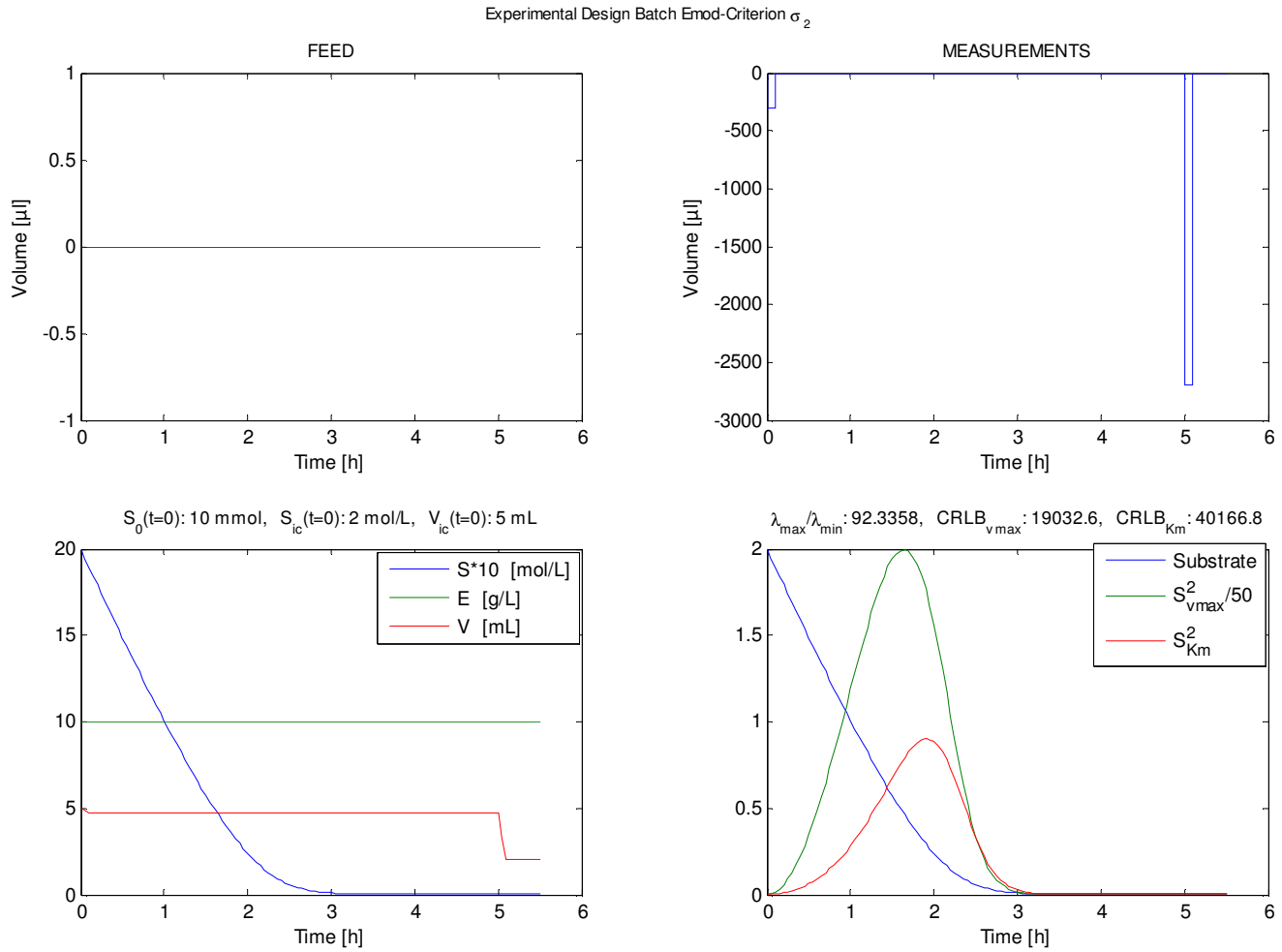

Figure 8.8: Results for experimental design - batch mode, criterion E-mod and error σ_2

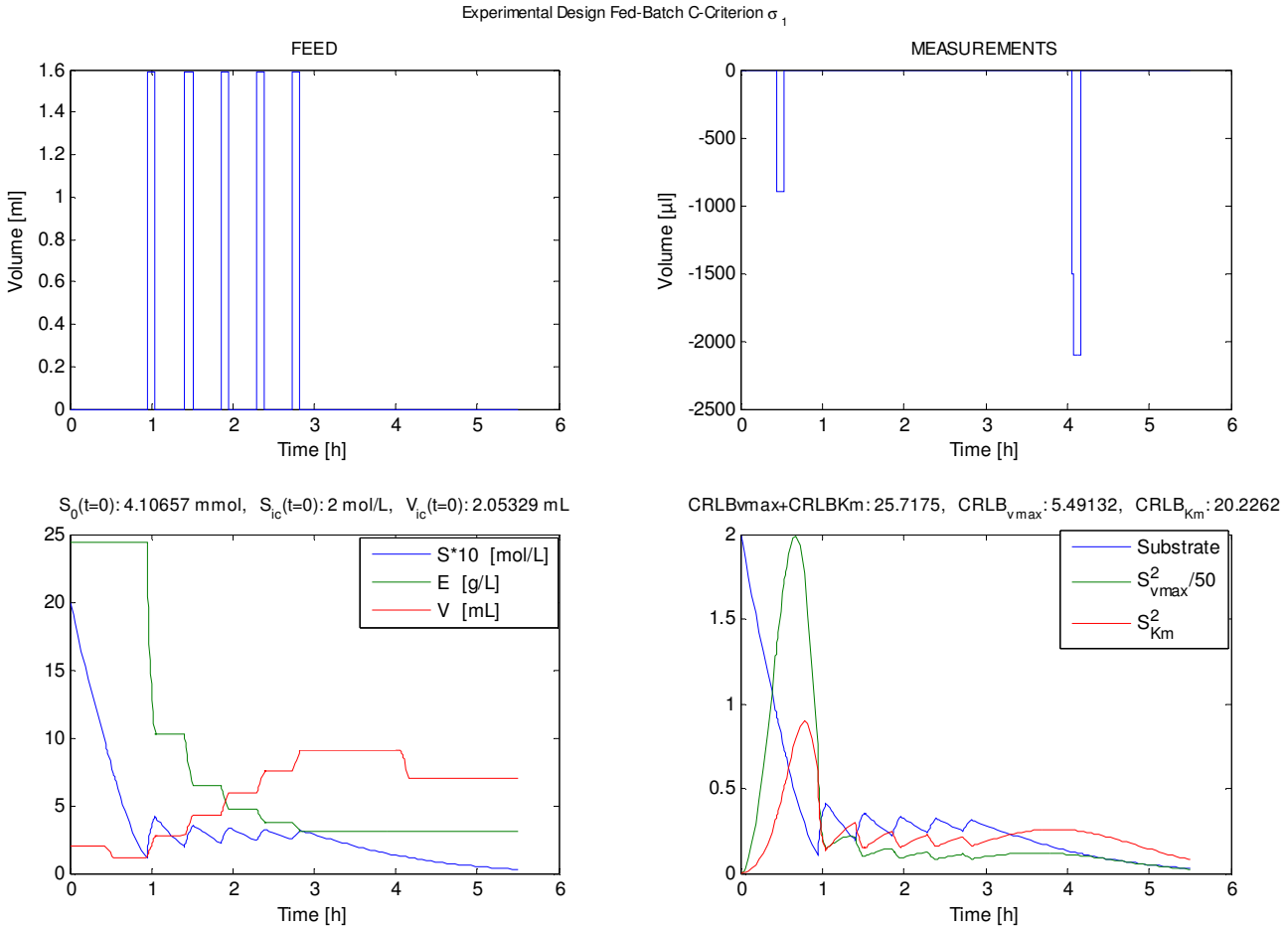

Figure 8.9: Results for experimental design – fed-batch mode (pulses), criterion A and error σ_1

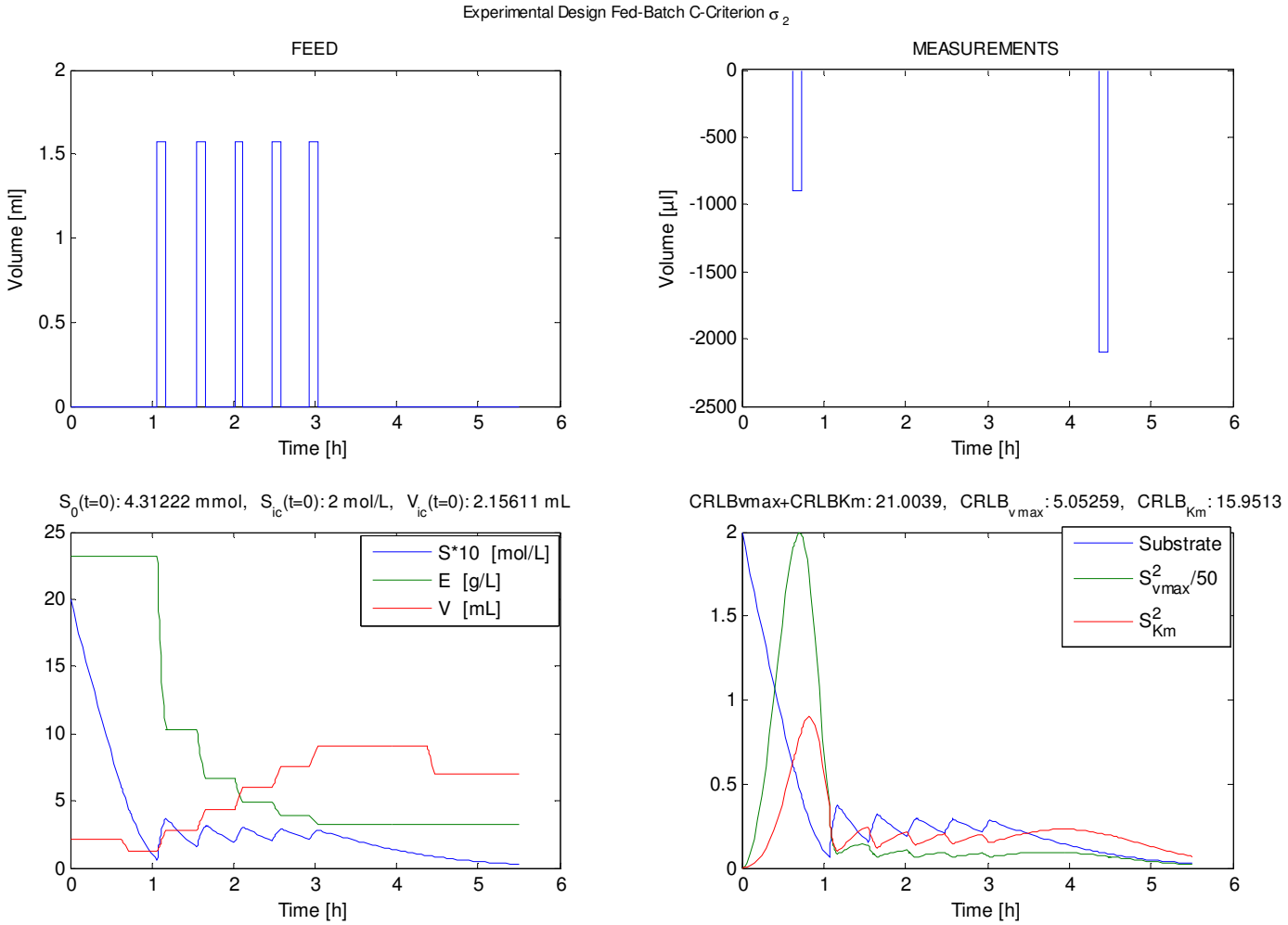

Figure 8.10: Results for experimental design – fed-batch mode (pulses), criterion A and error σ_2

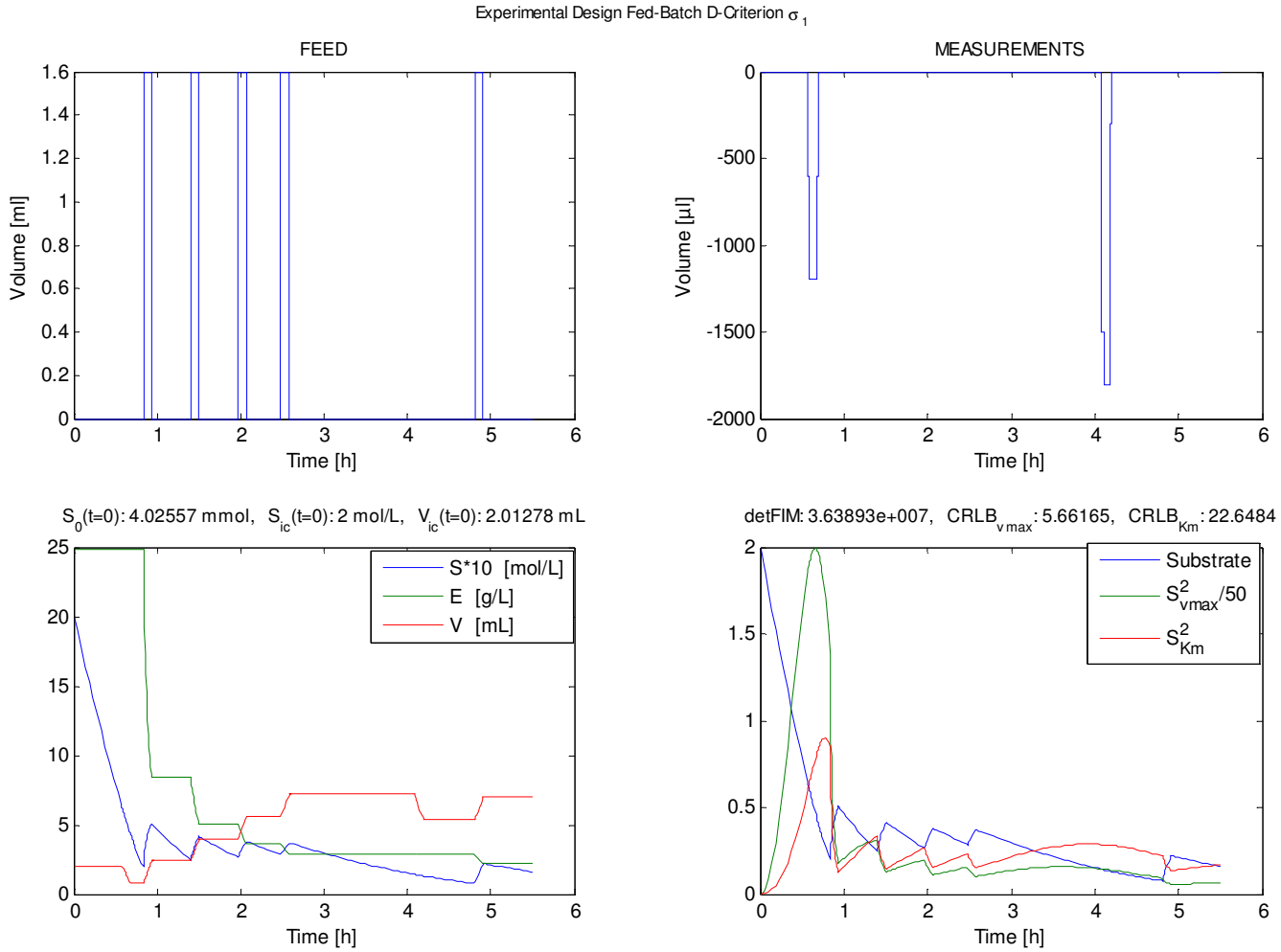

Figure 8.11: Results for experimental design – fed-batch mode (pulses), criterion D and error σ_1

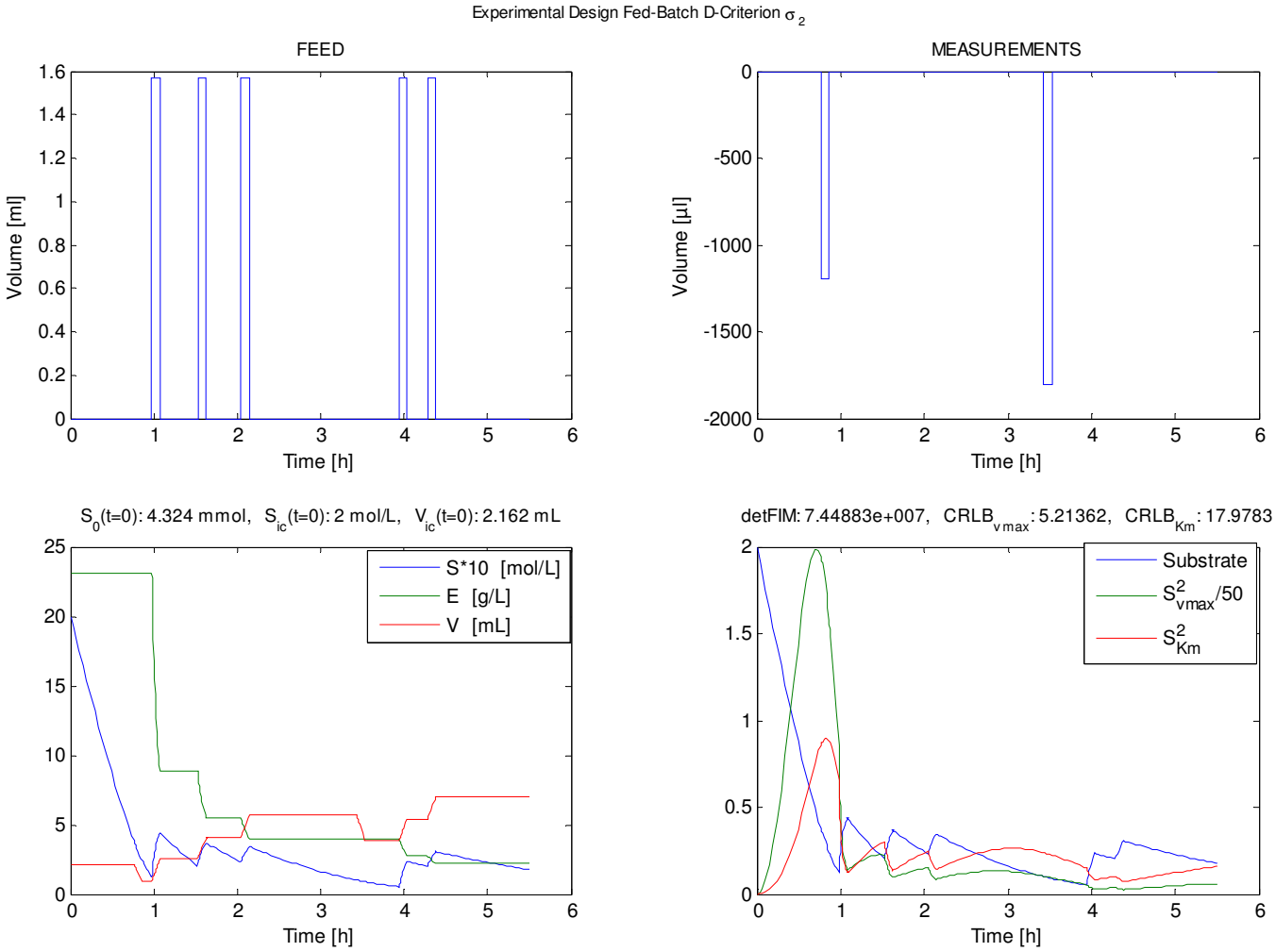

Figure 8.12: Results for experimental design – fed-batch mode (pulses), criterion D and error σ_2

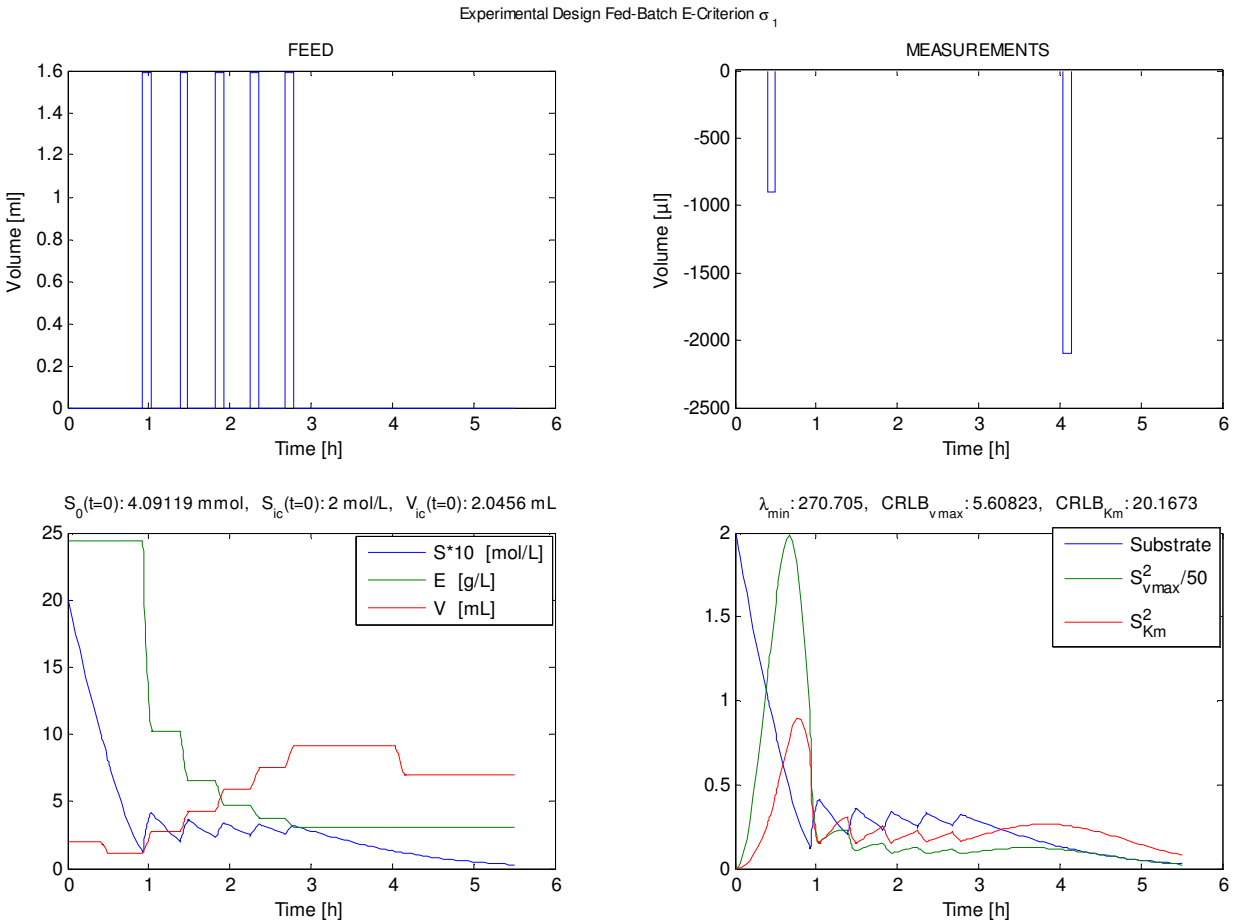

Figure 8.13: Results for experimental design – fed-batch mode (pulses), criterion E and error σ_1

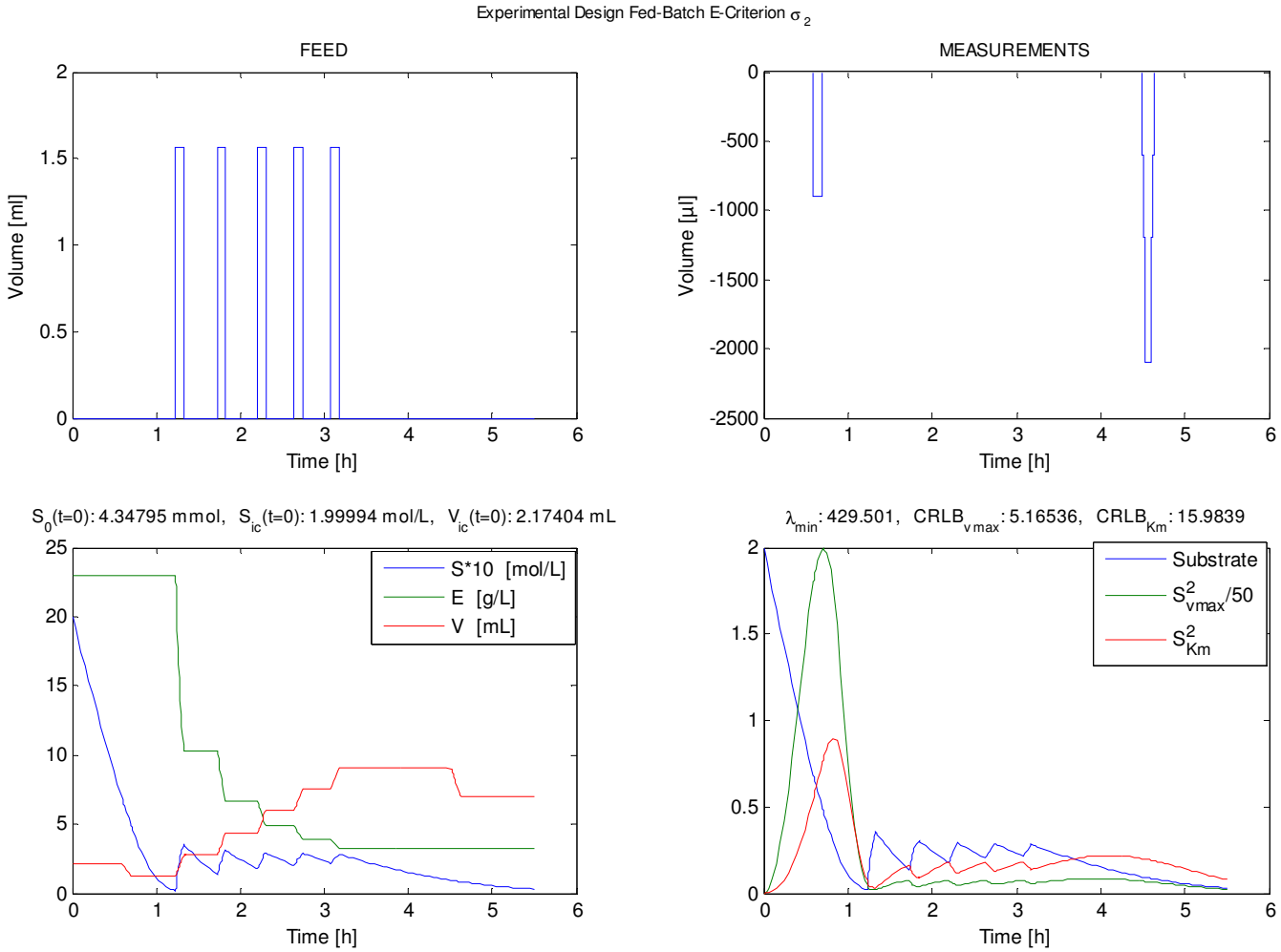

Figure 8.14: Results for experimental design – fed-batch mode (pulses), criterion E and error σ_2

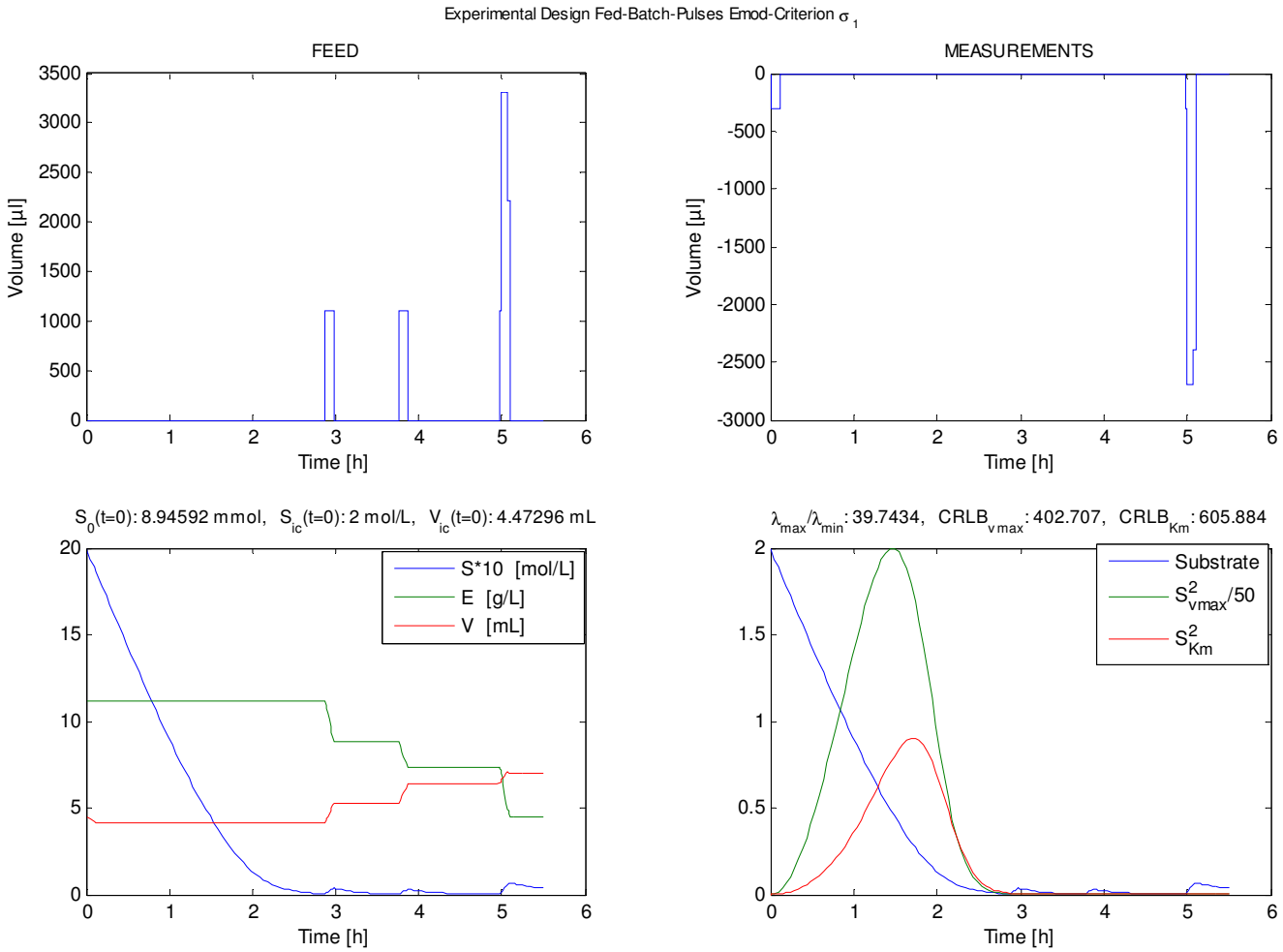

Figure 8.15: Results for experimental design – fed-batch mode (pulses), criterion E-mod and error σ_1

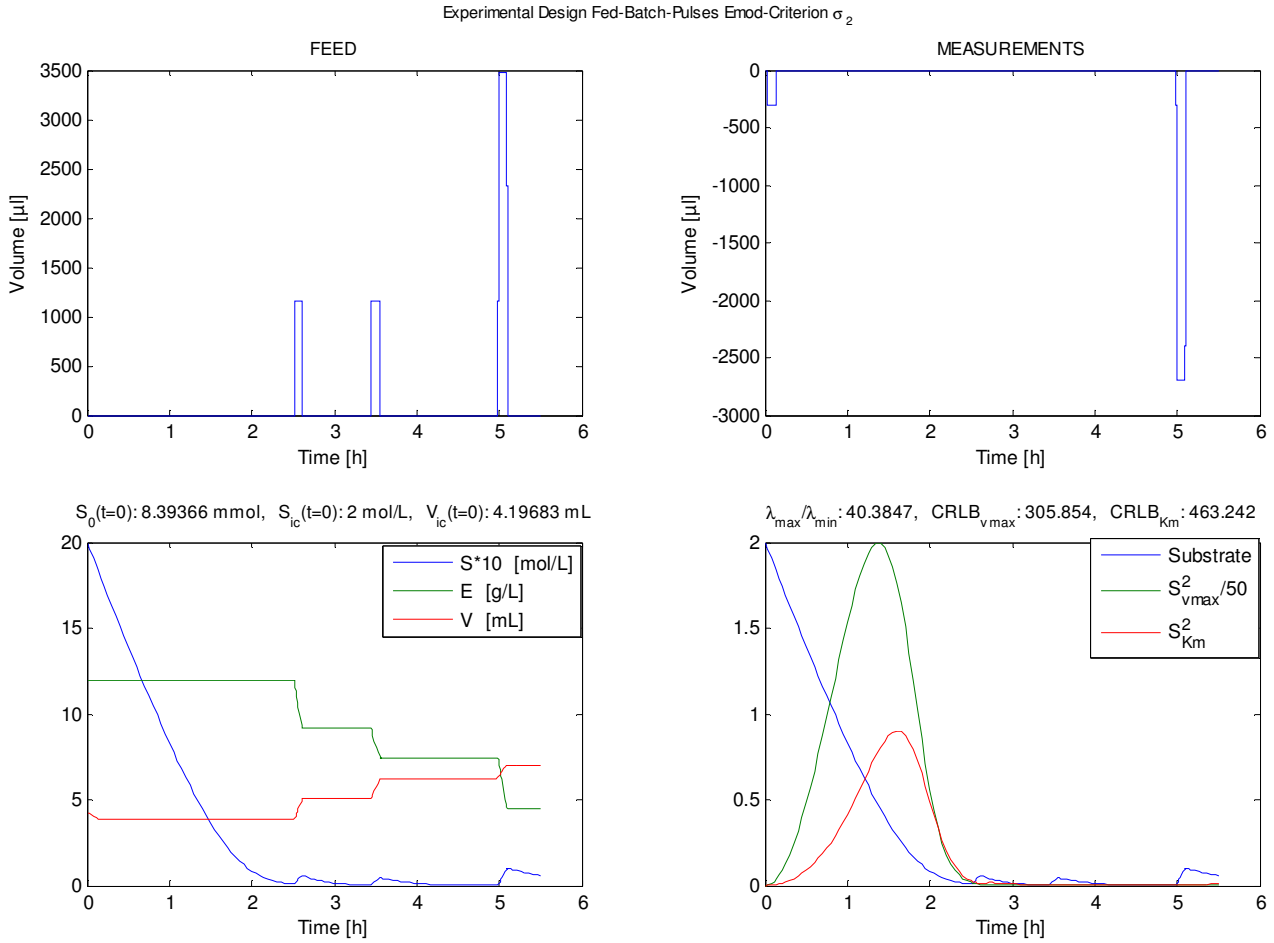

Figure 8.16: Results for experimental design – fed-batch mode (pulses), criterion E-mod and error σ_2

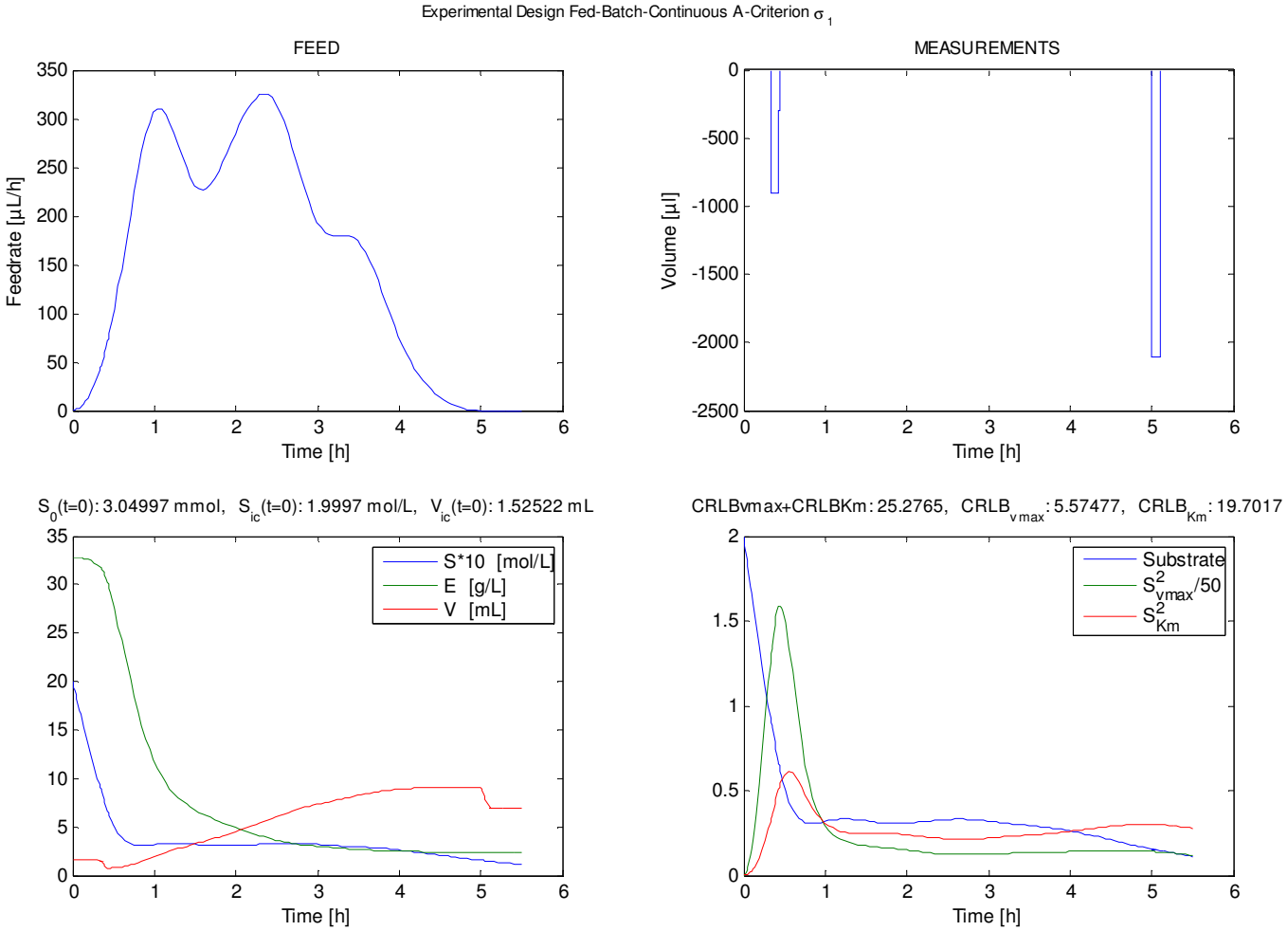

Figure 8.17: Results for experimental design – fed-batch mode (continuous), criterion A and error σ_1

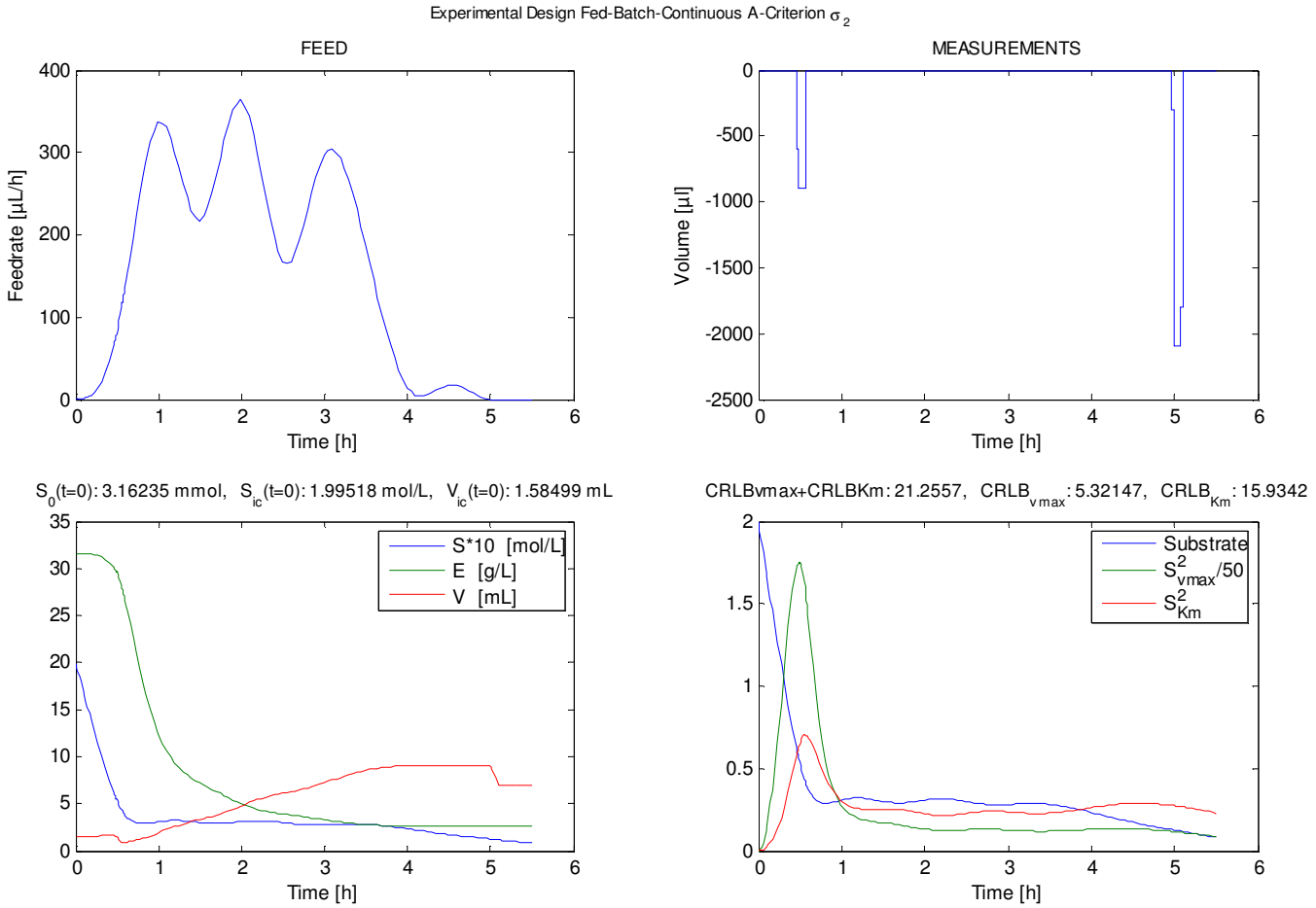

Figure 8.18: Results for experimental design – fed-batch mode (continuous), criterion A and error σ_2

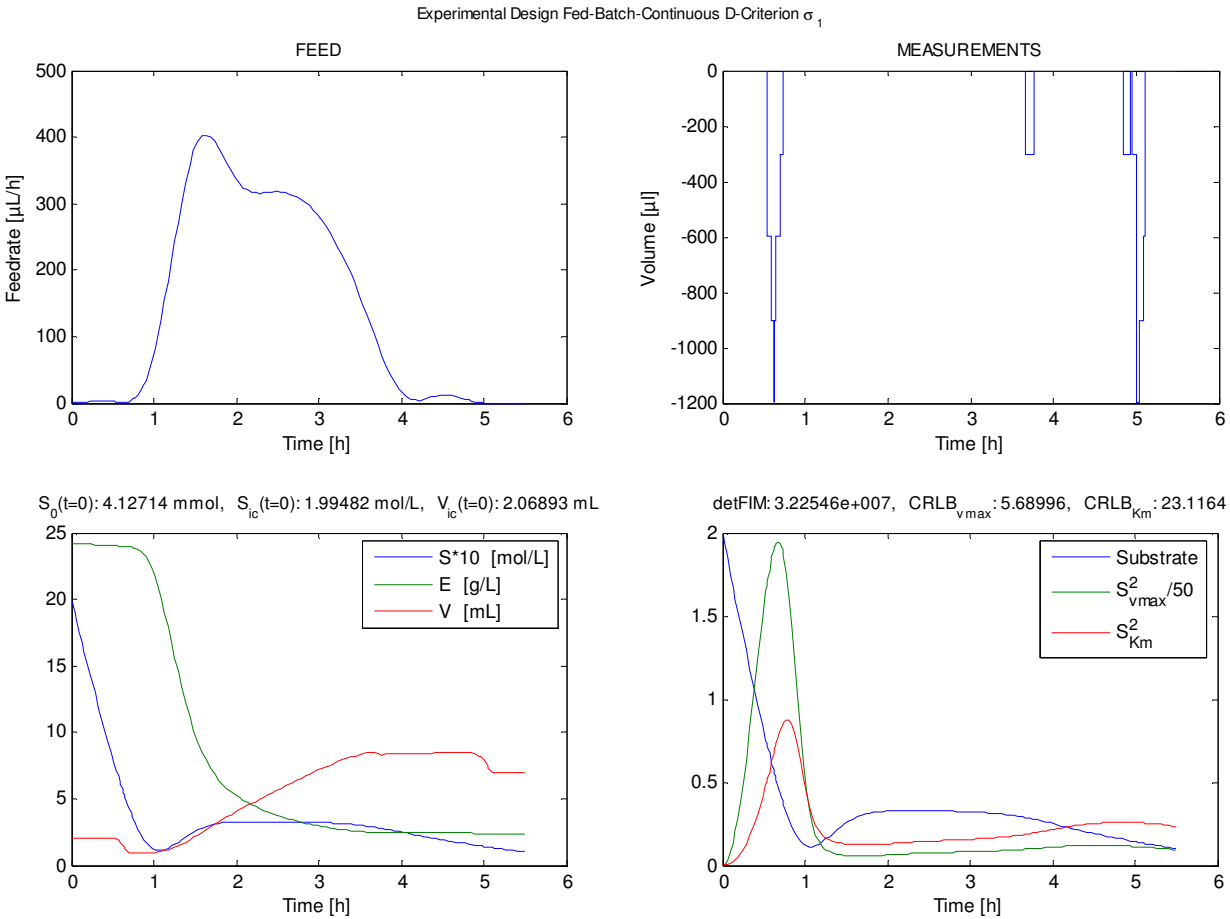

Figure 8.19: Results for experimental design – fed-batch mode (continuous), criterion D and error σ_1

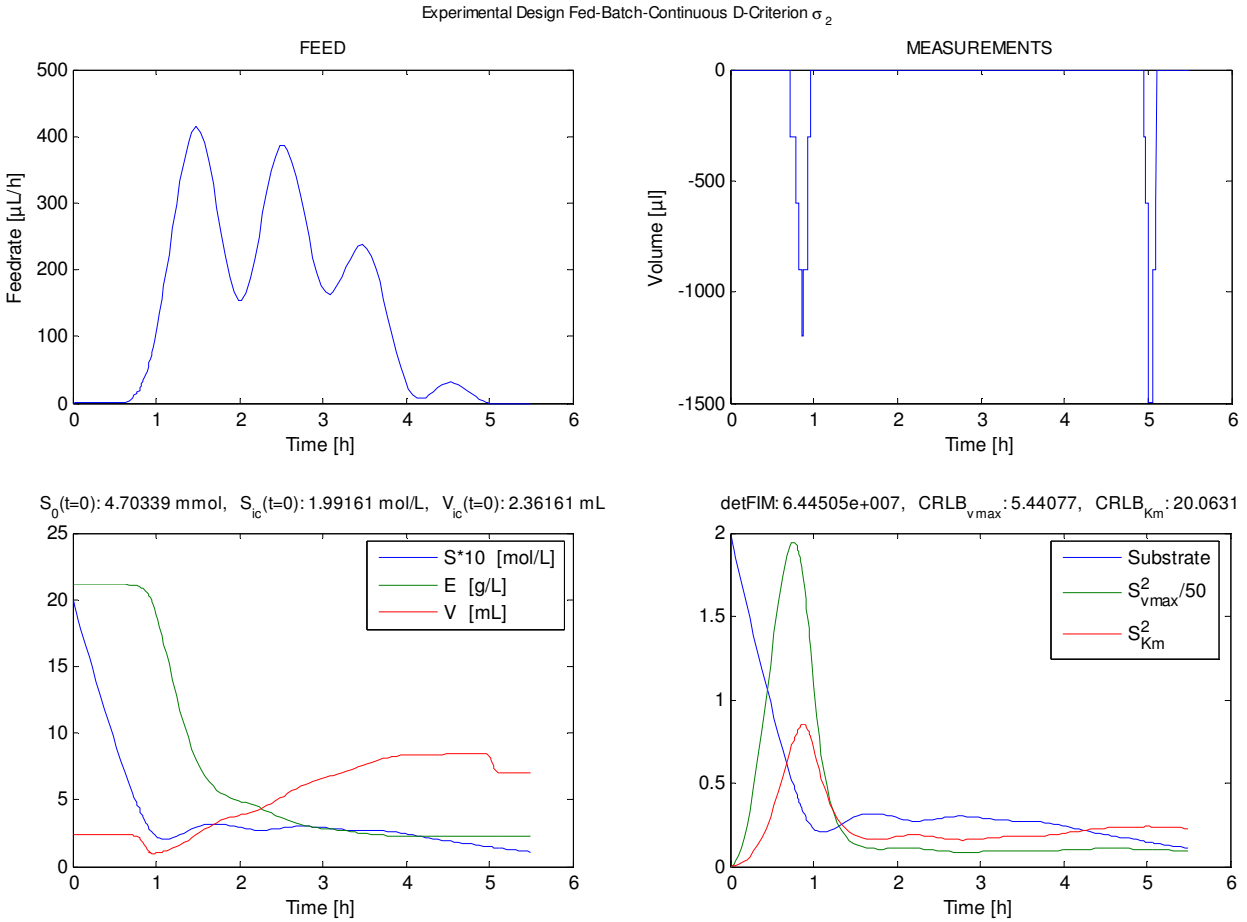

Figure 8.20: Results for experimental design – fed-batch mode (continuous), criterion D and error σ_2

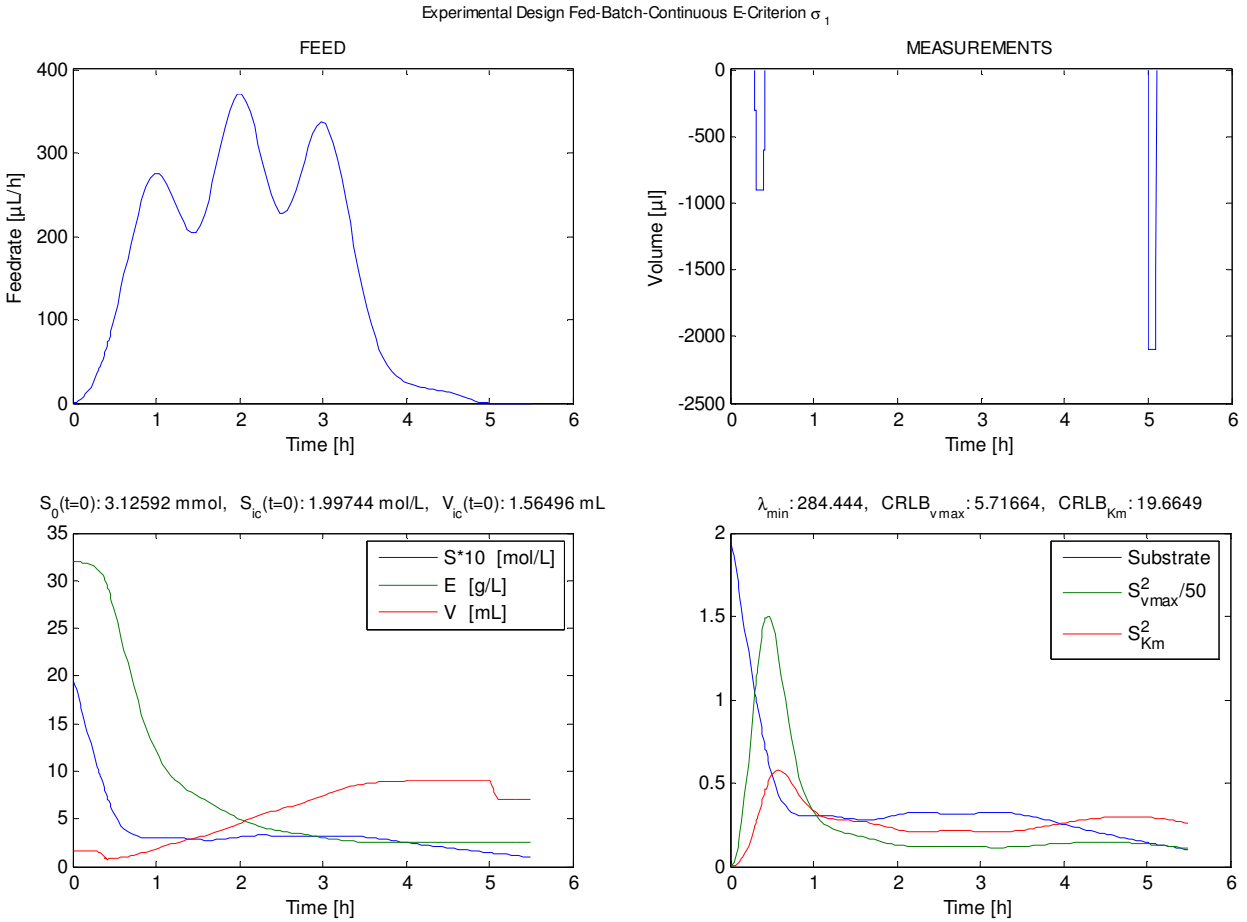

Figure 8.21: Results for experimental design – fed-batch mode (continuous), criterion E and error σ_1

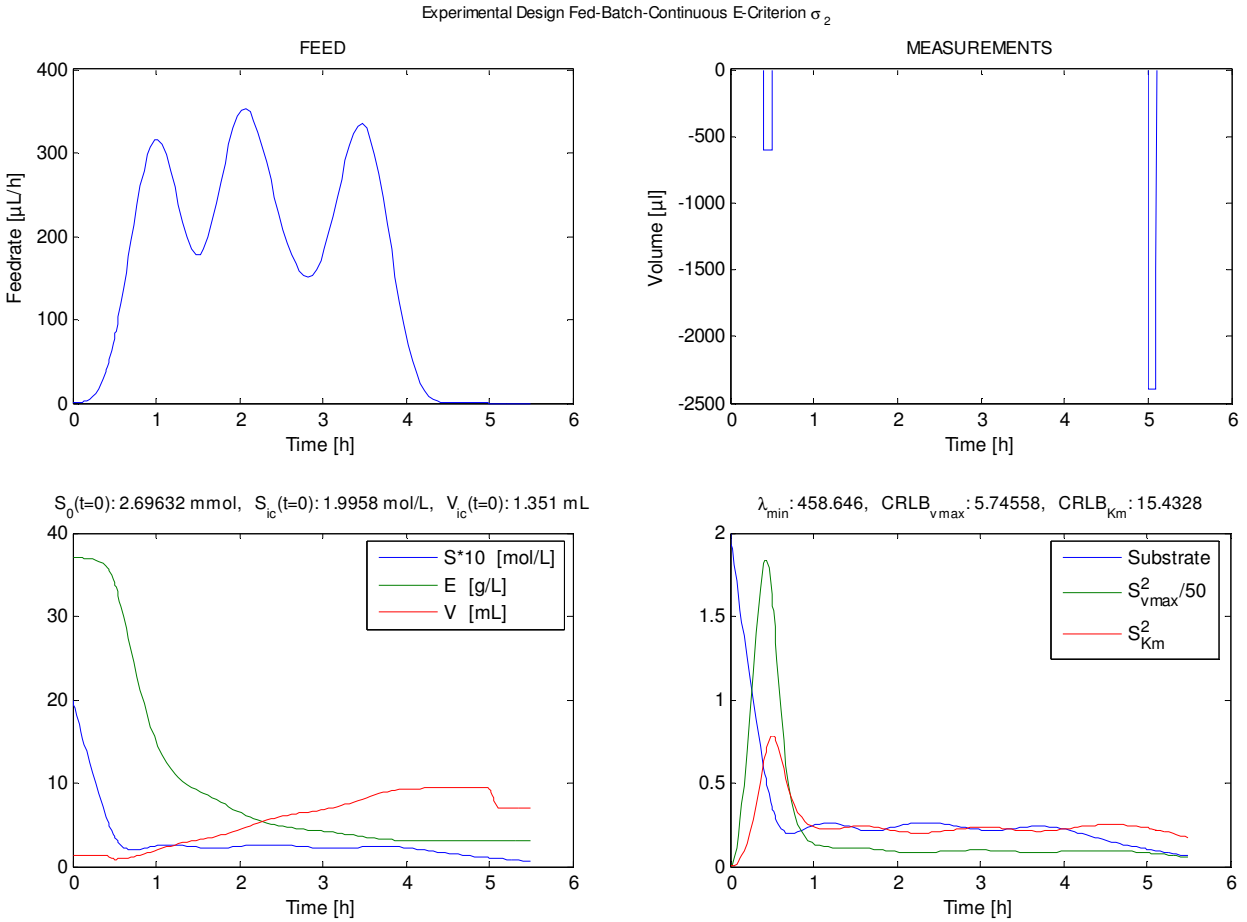

Figure 8.22: Results for experimental design – fed-batch mode (continuous), criterion E and error σ_2

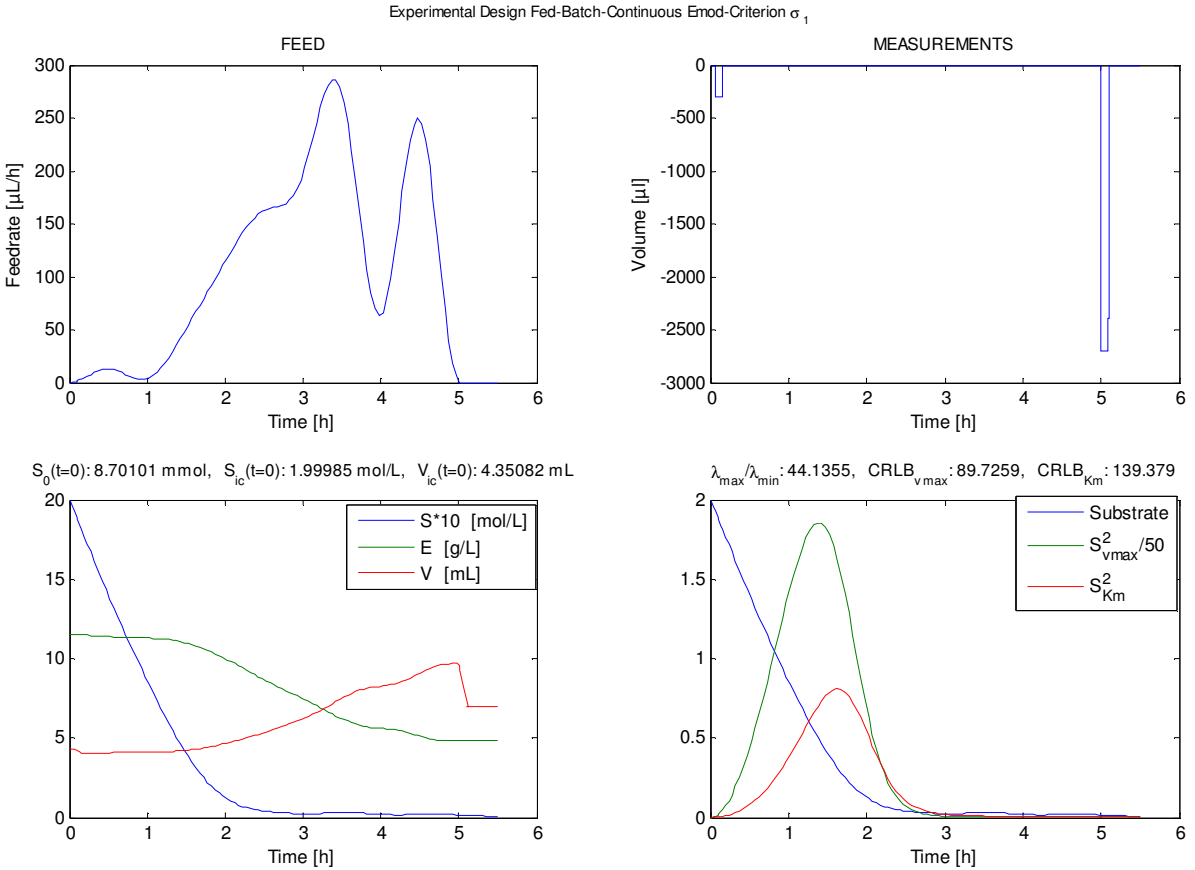

Figure 8.23: Results for experimental design – fed-batch mode (continuous), criterion E-mod and error σ_1

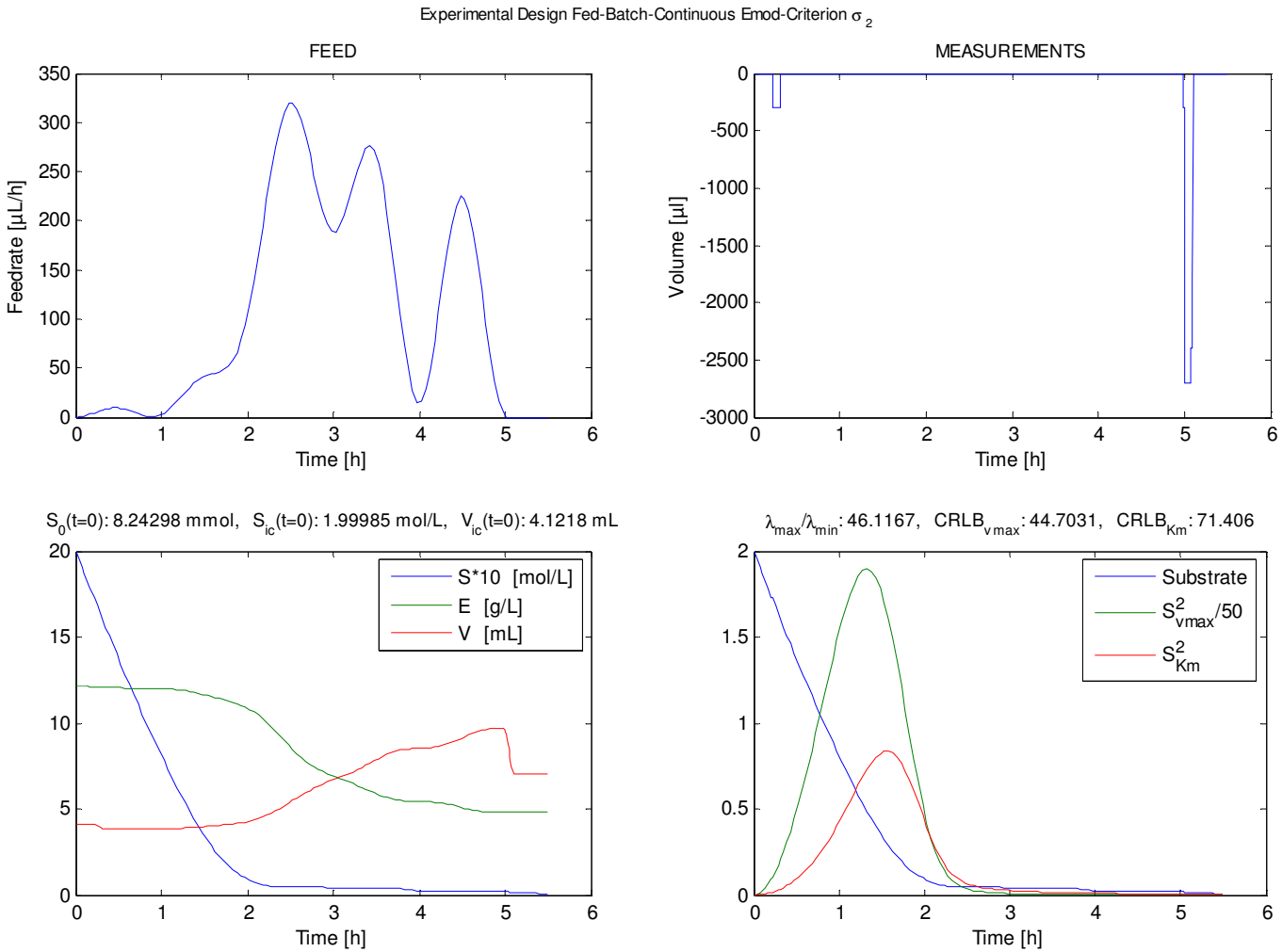

Figure 8.24: Results for experimental design – fed-batch mode (continuous), criterion E-mod and error σ_2

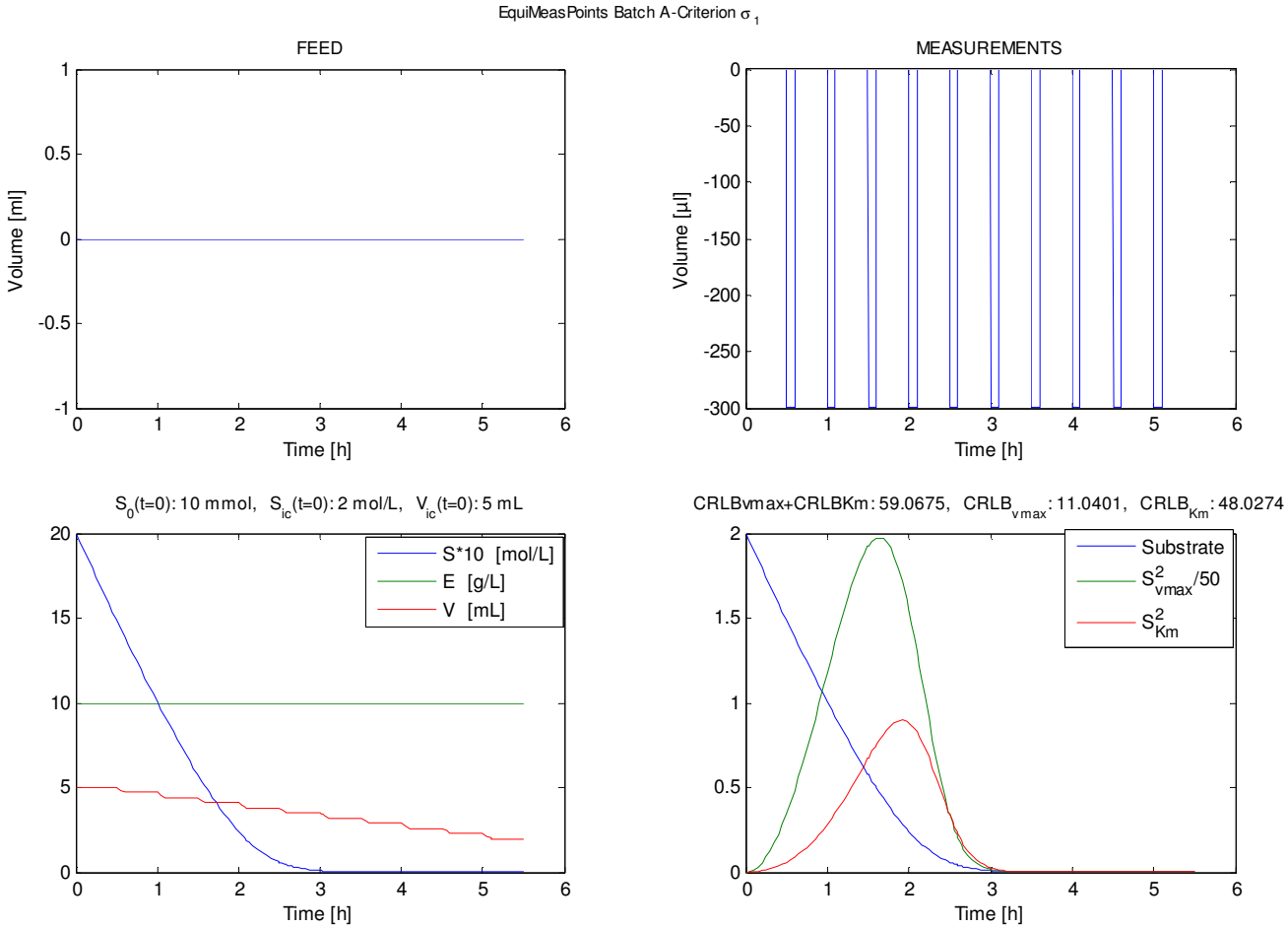

Figure 8.25: Results for equidistant sampling – batch mode, criterion A and error σ_1

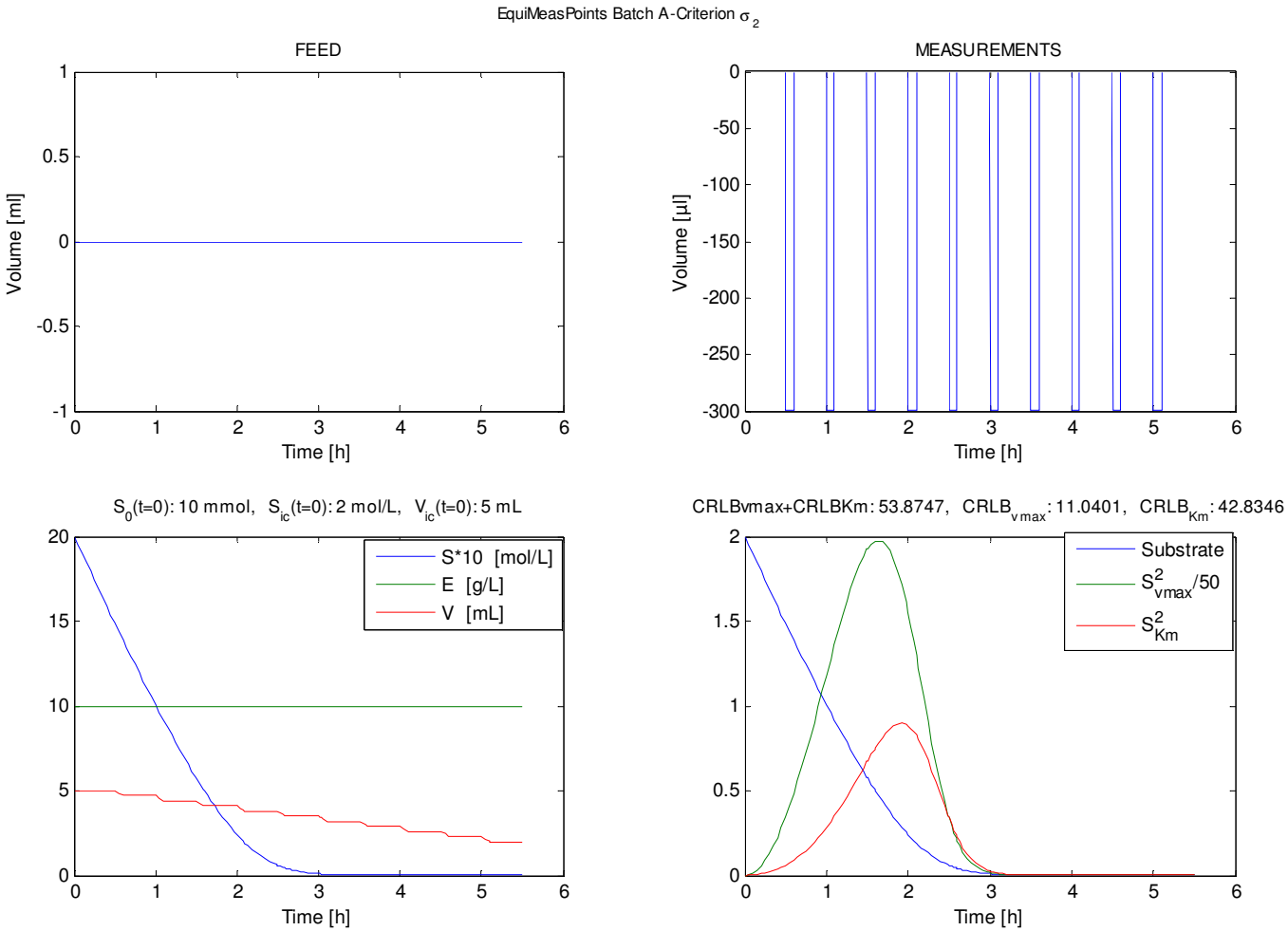

Figure 8.26: Results for equidistant sampling – batch mode, criterion A and error σ_2

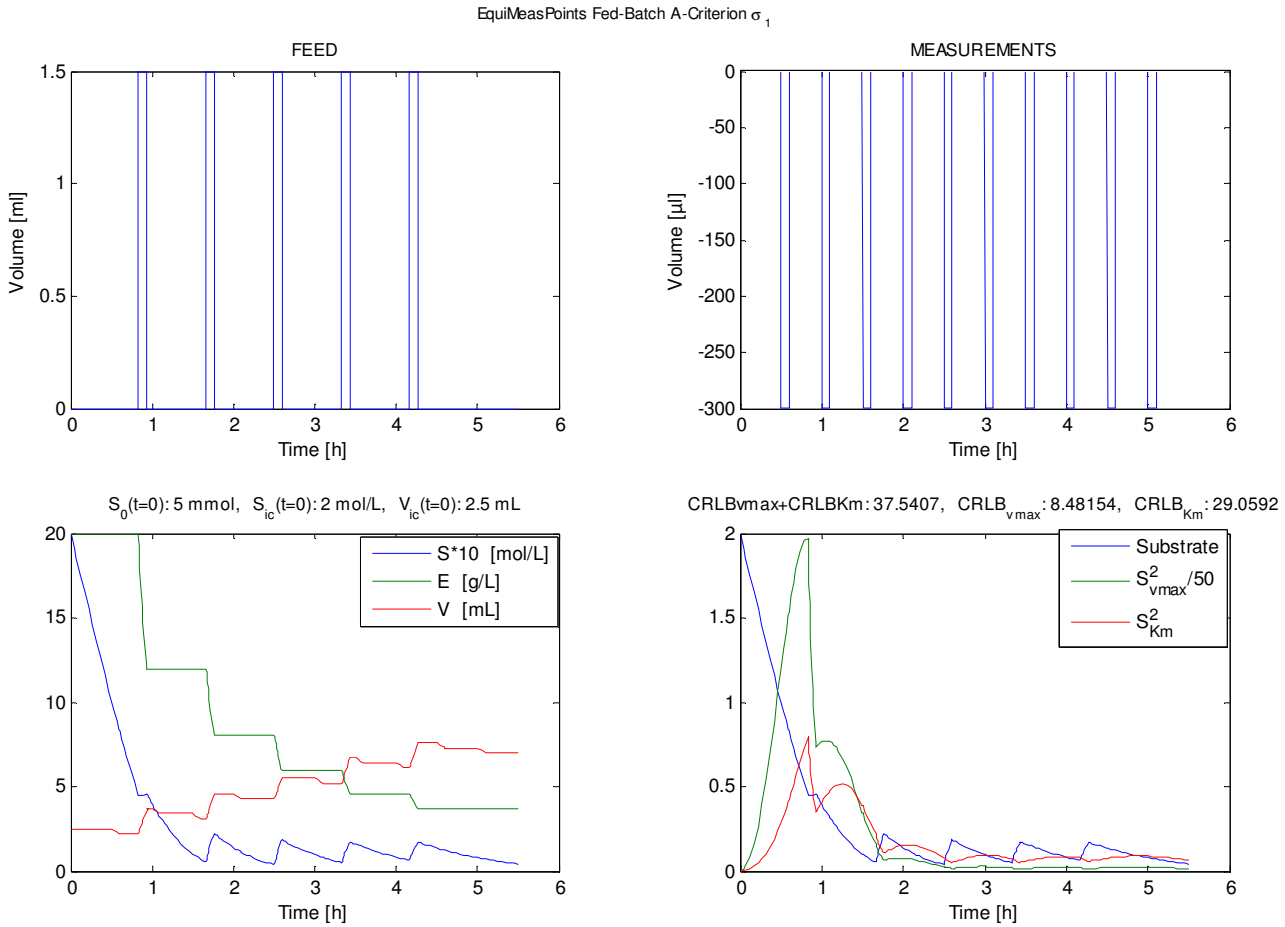

Figure 8.27: Results for equidistant sampling – fed-batch (pulses) mode, criterion A and error σ_1

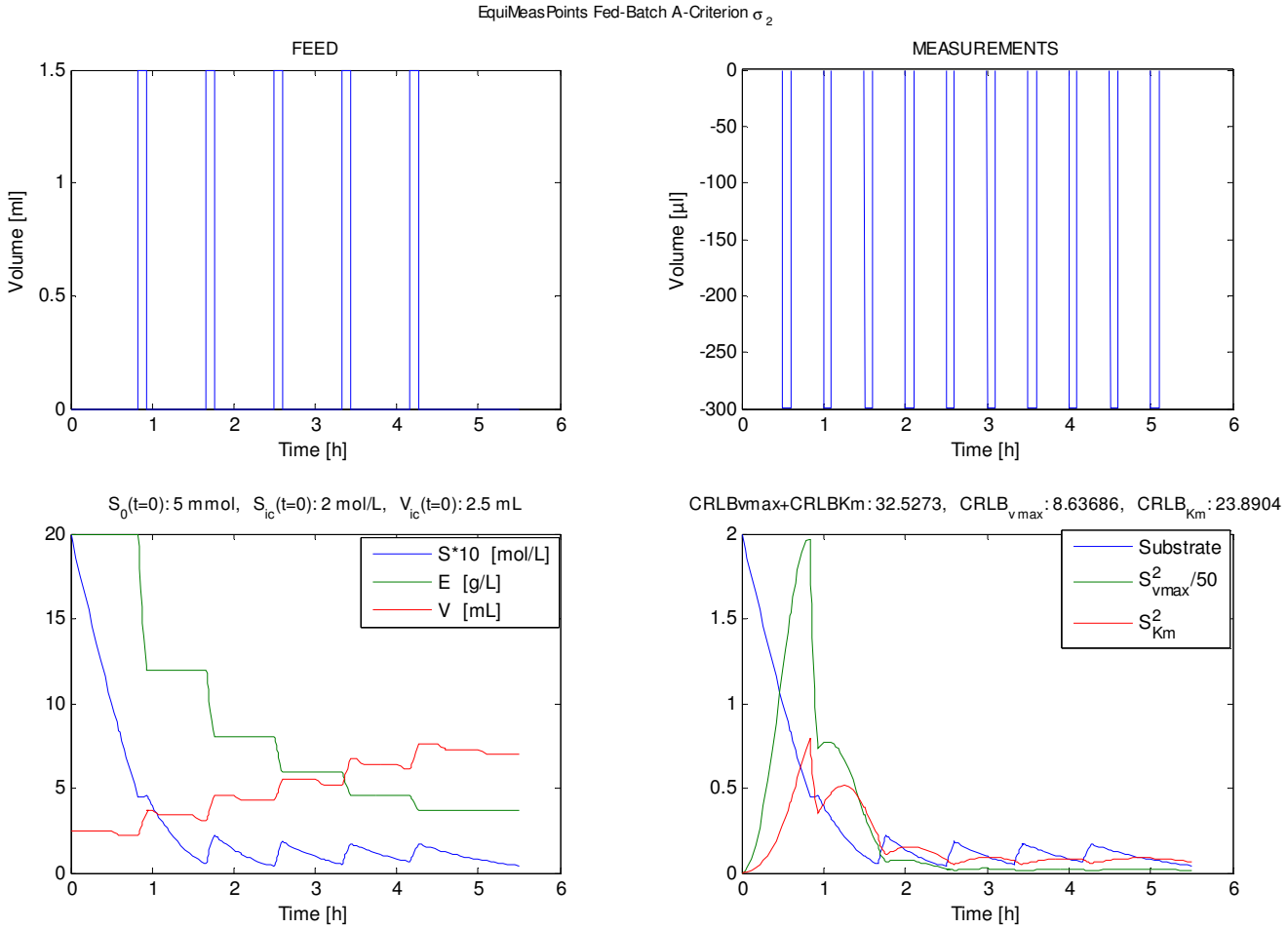

Figure 8.28: Results for equidistant sampling – fed-batch (pulses) mode, criterion A and error σ_2

9. Appendix B: Comparison between experimental design and equidistant sampling

In this section, all results obtained from the comparison the above stated methods are presented.

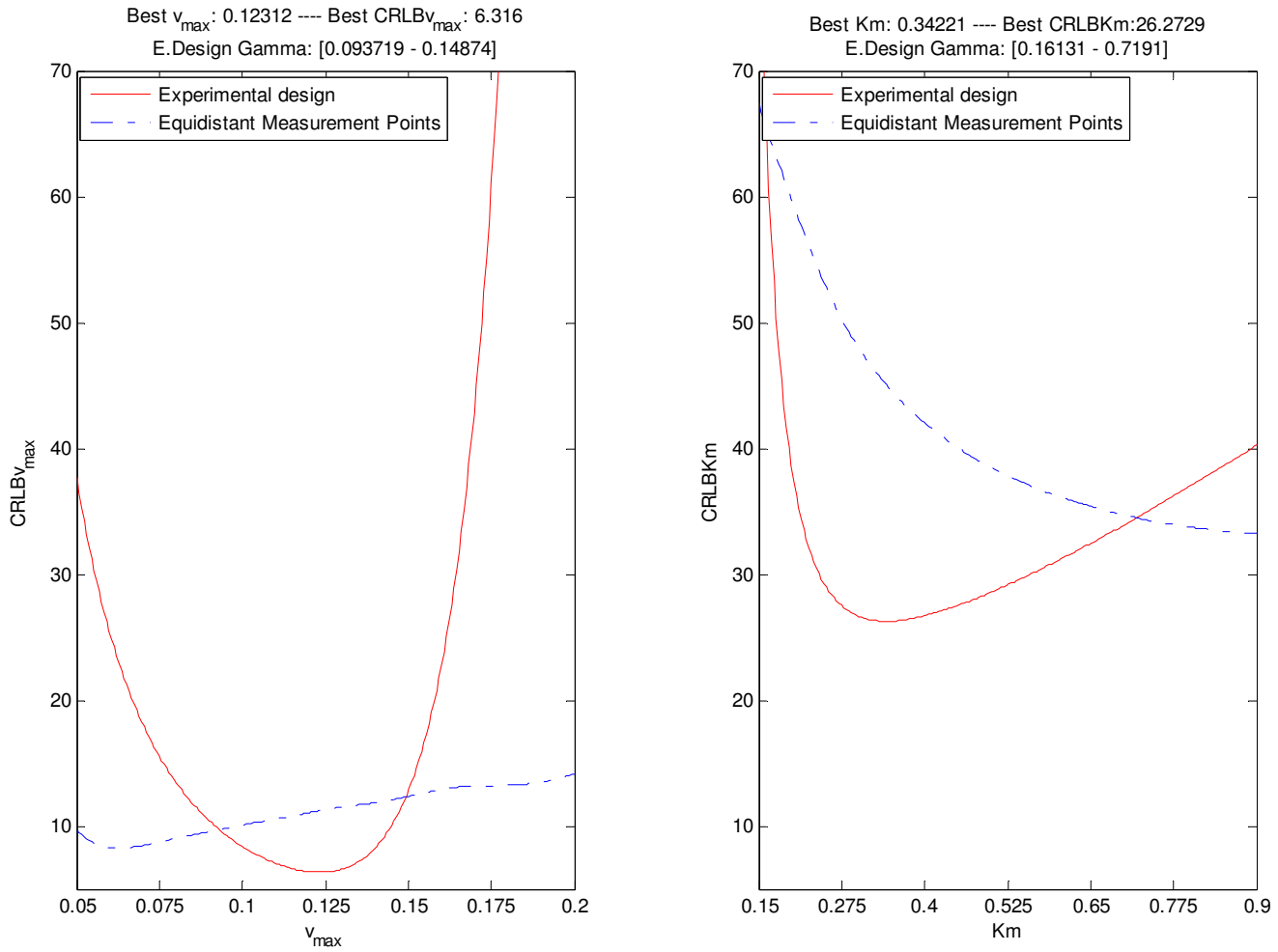

Figure 9.1: Comparison between experimental design and equidistant sampling - batch mode, criterion A, σ_1

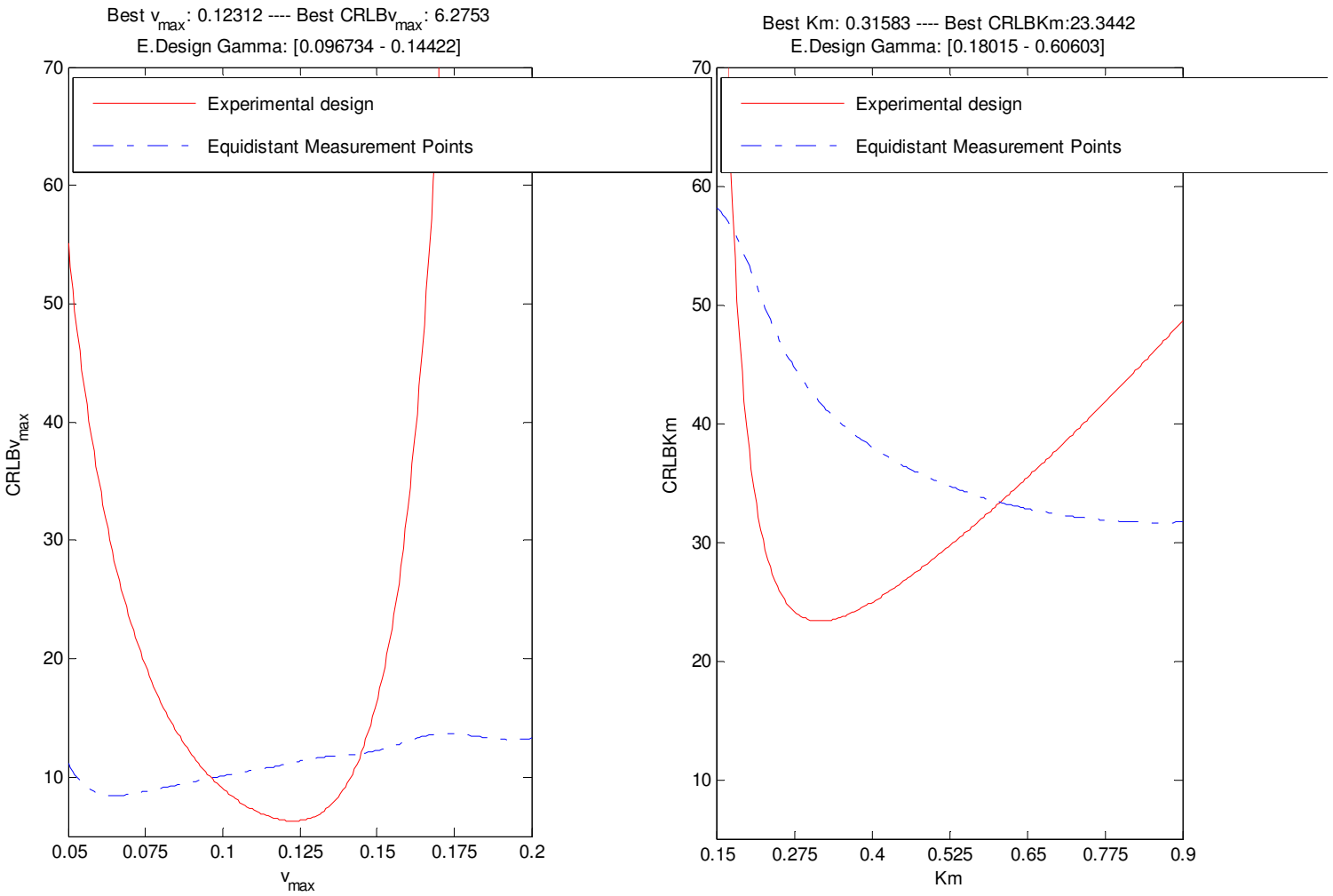

Figure 9.2: Comparison between experimental design and equidistant sampling - batch mode, criterion A, σ_2

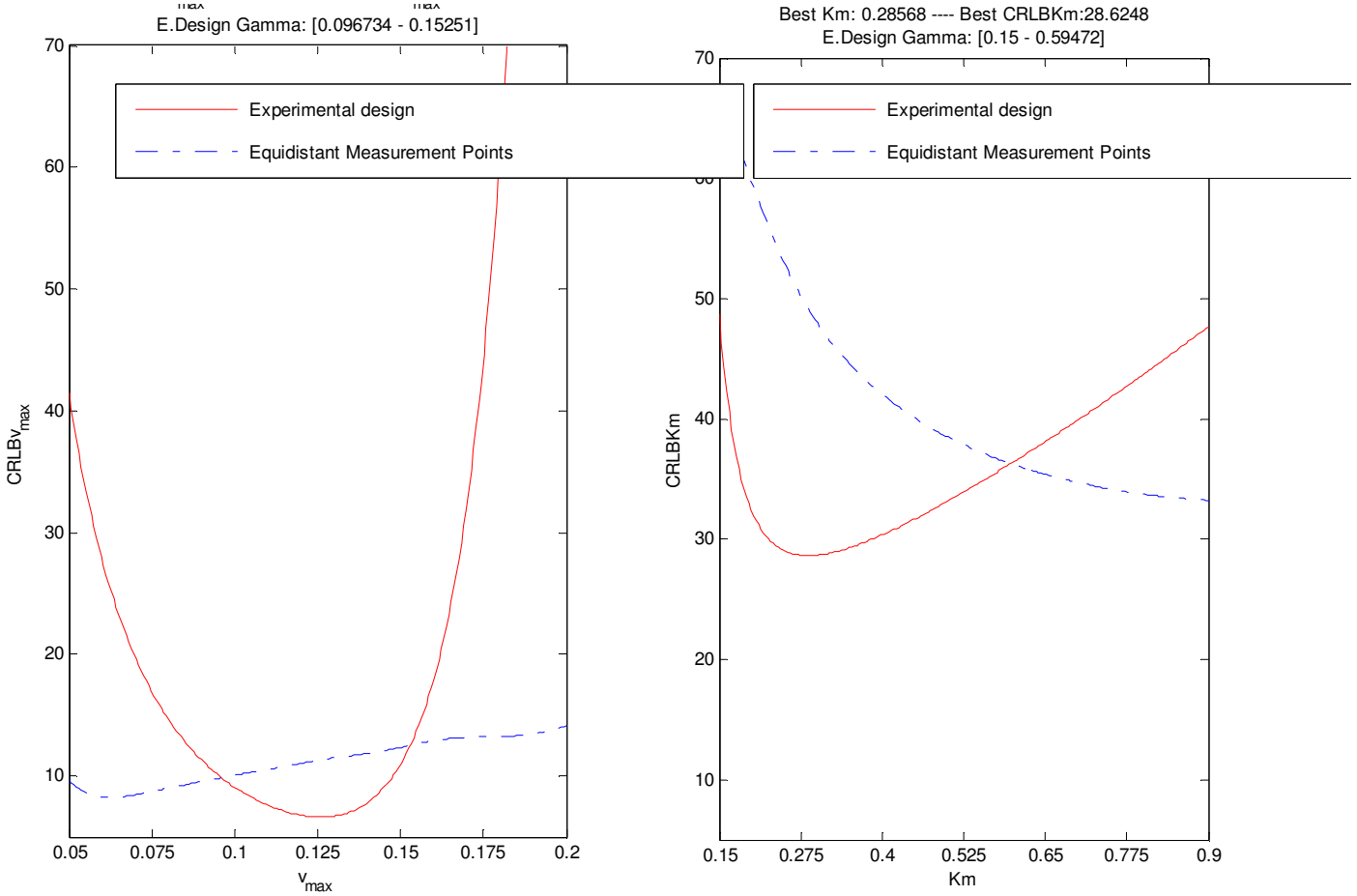

Figure 9.3: Comparison between experimental design and equidistant sampling - batch mode, criterion D, σ_1

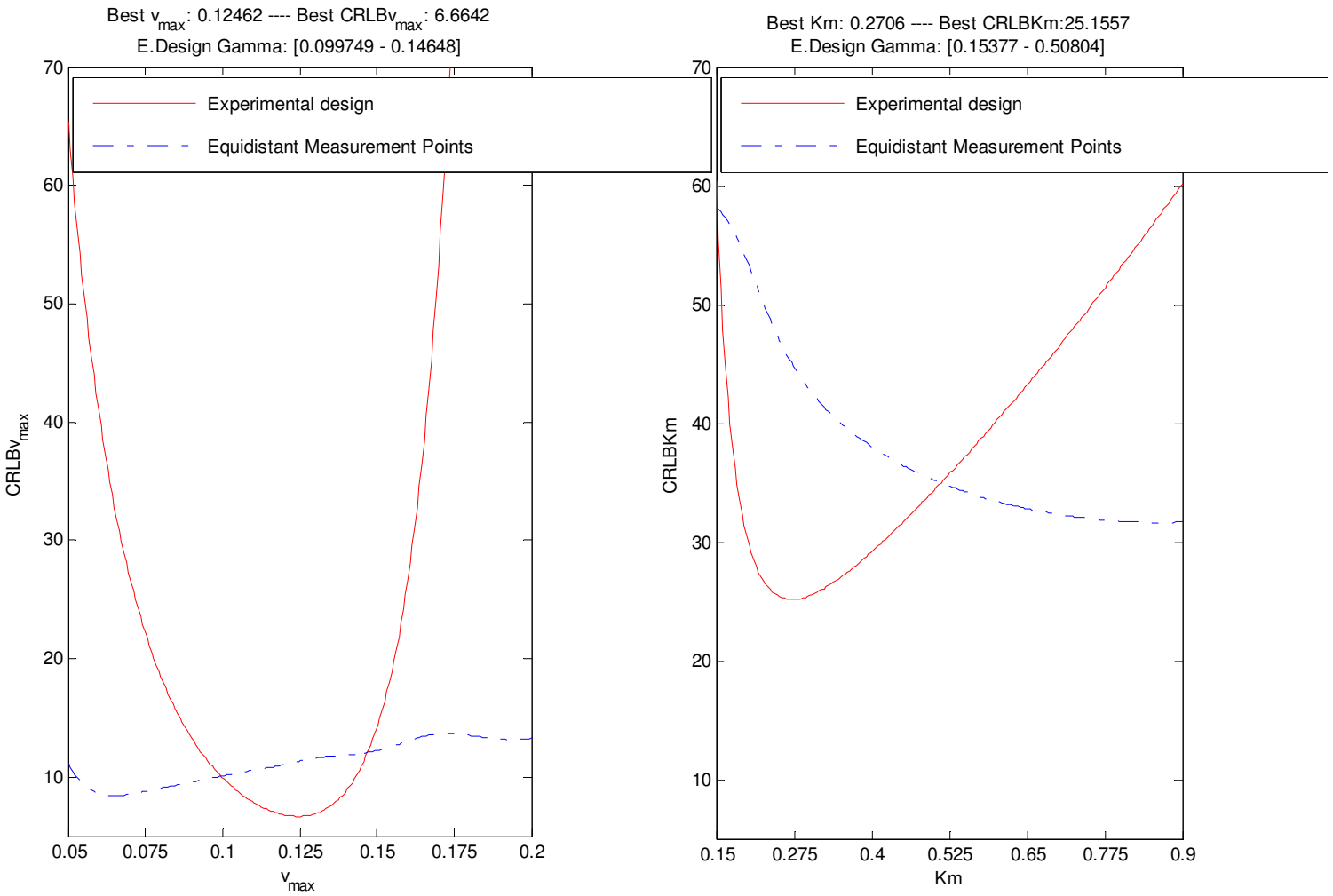

Figure 9.4: Comparison between experimental design and equidistant sampling - batch mode, criterion D, σ_2

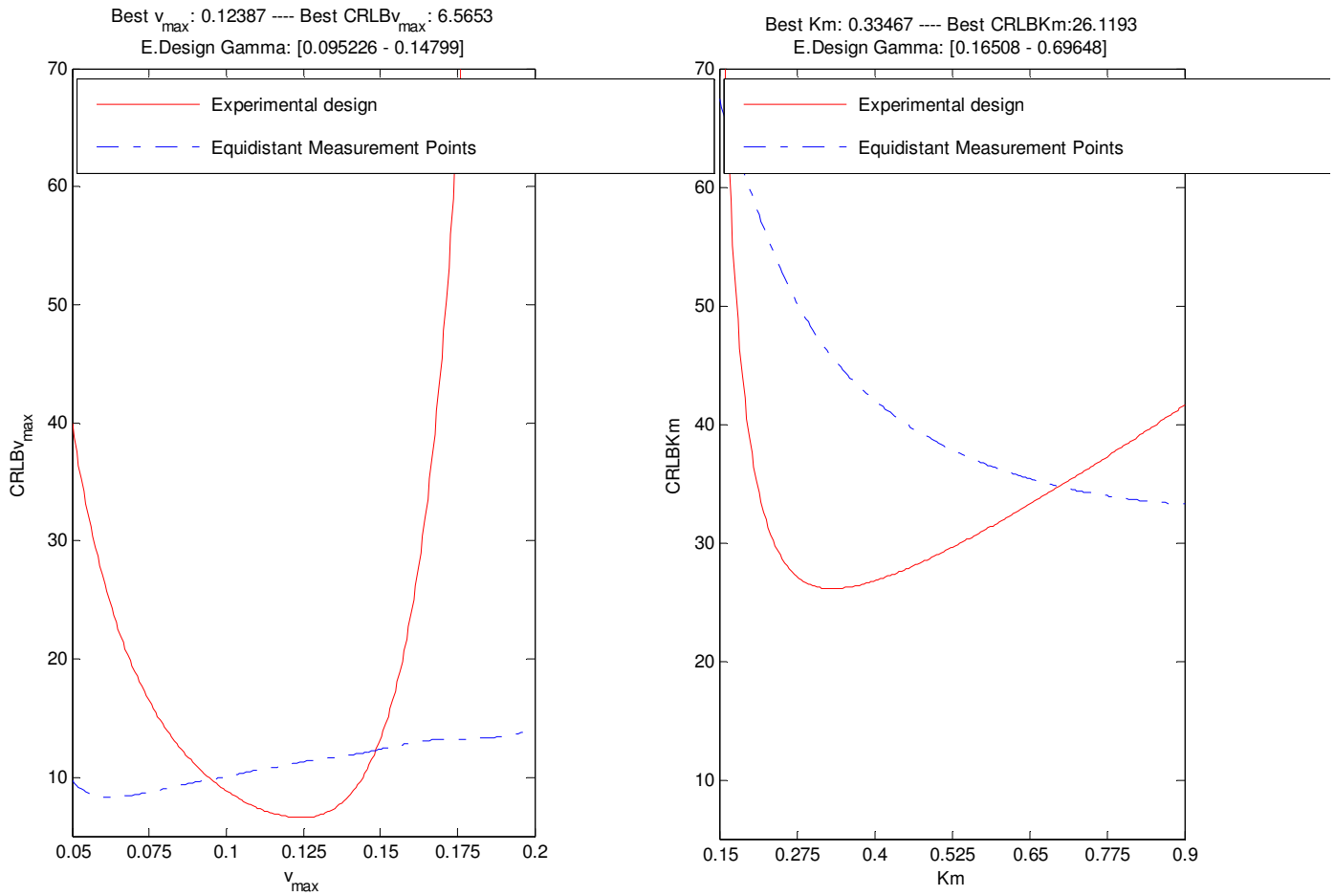

Figure 9.5: Comparison between experimental design and equidistant sampling - batch mode, criterion E, σ_1

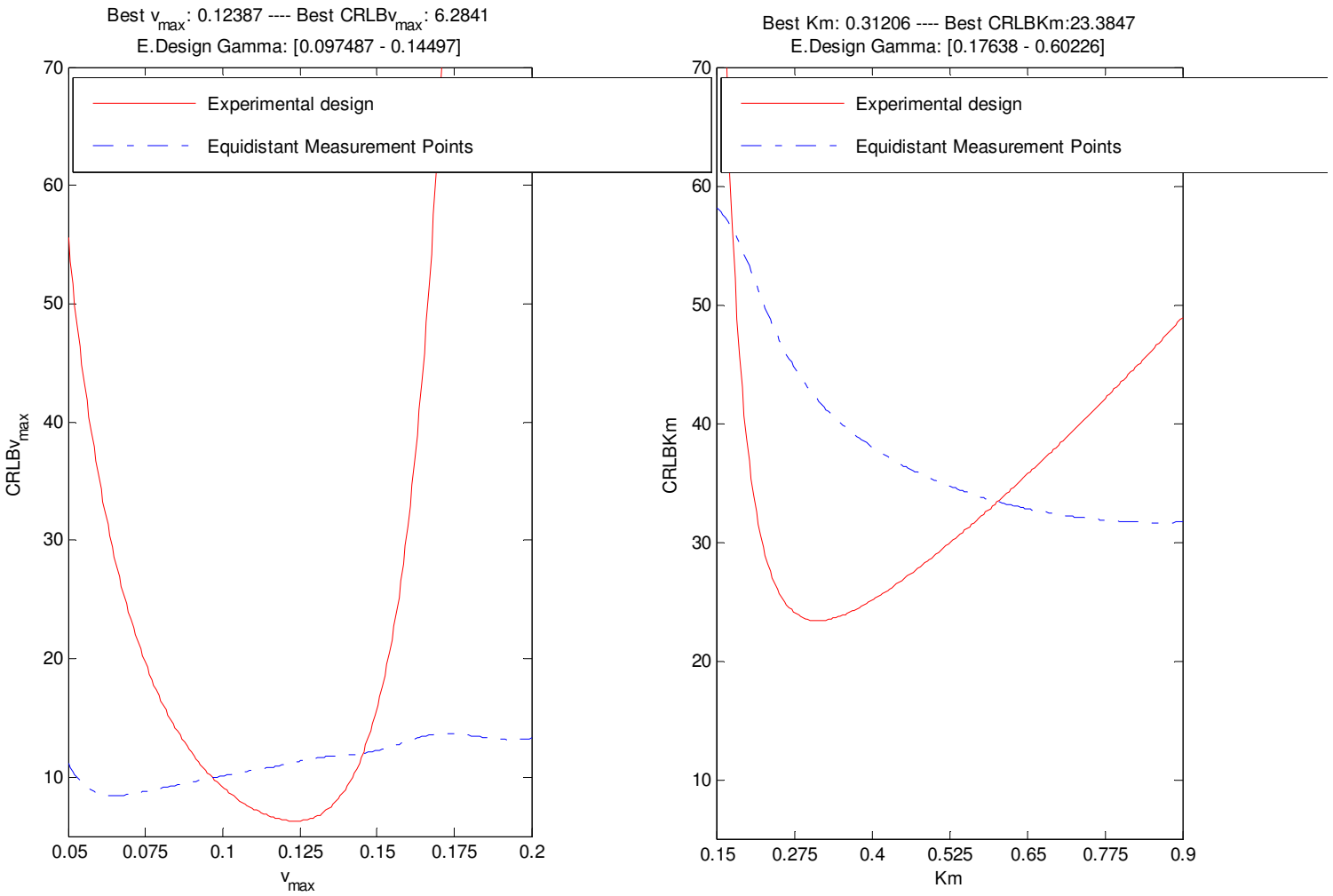

Figure 9.6: Comparison between experimental design and equidistant sampling - batch mode, criterion E, σ_2

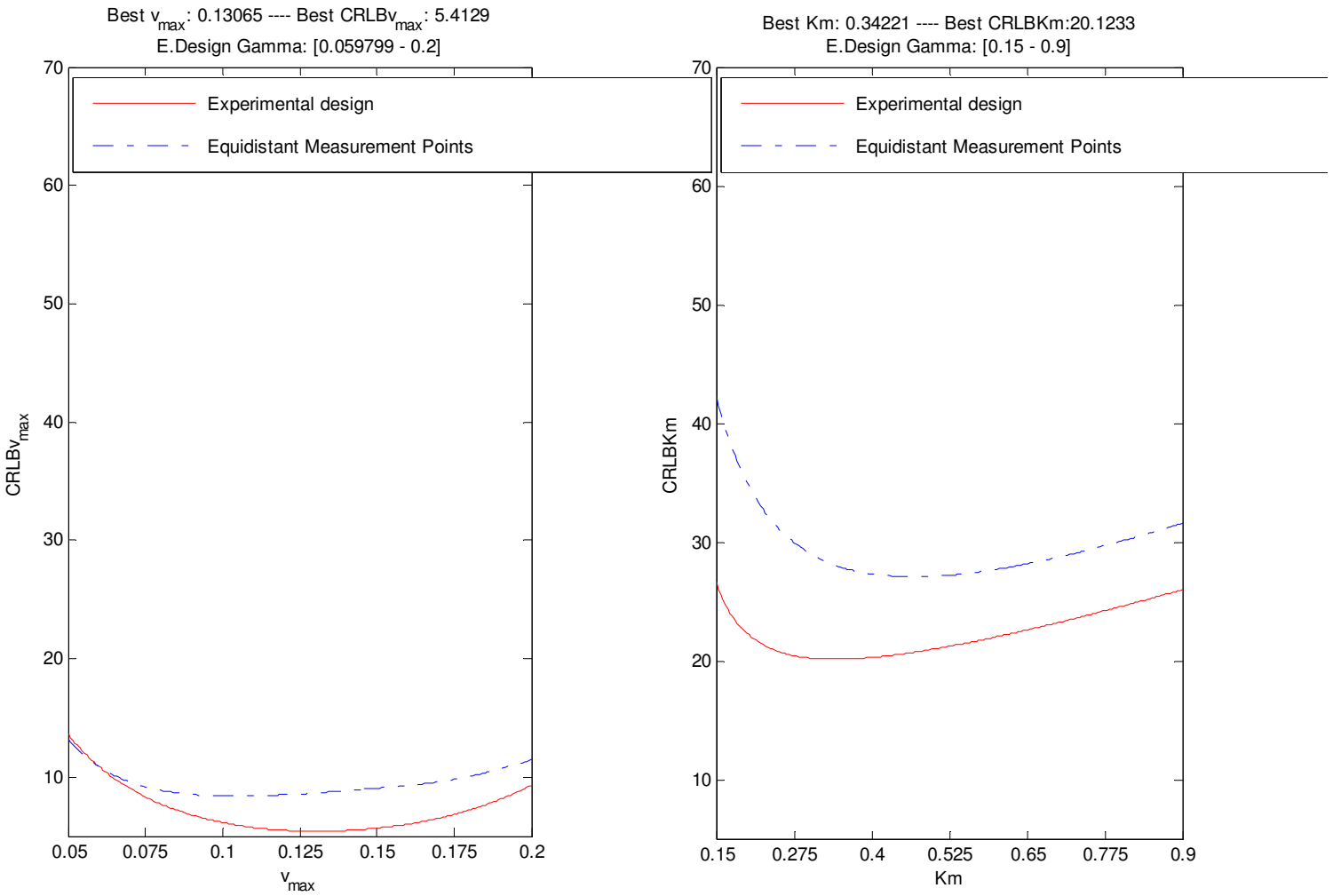

Figure 9.7: Comparison between experimental design and equidistant sampling - batch mode, criterion E-mod, σ_1

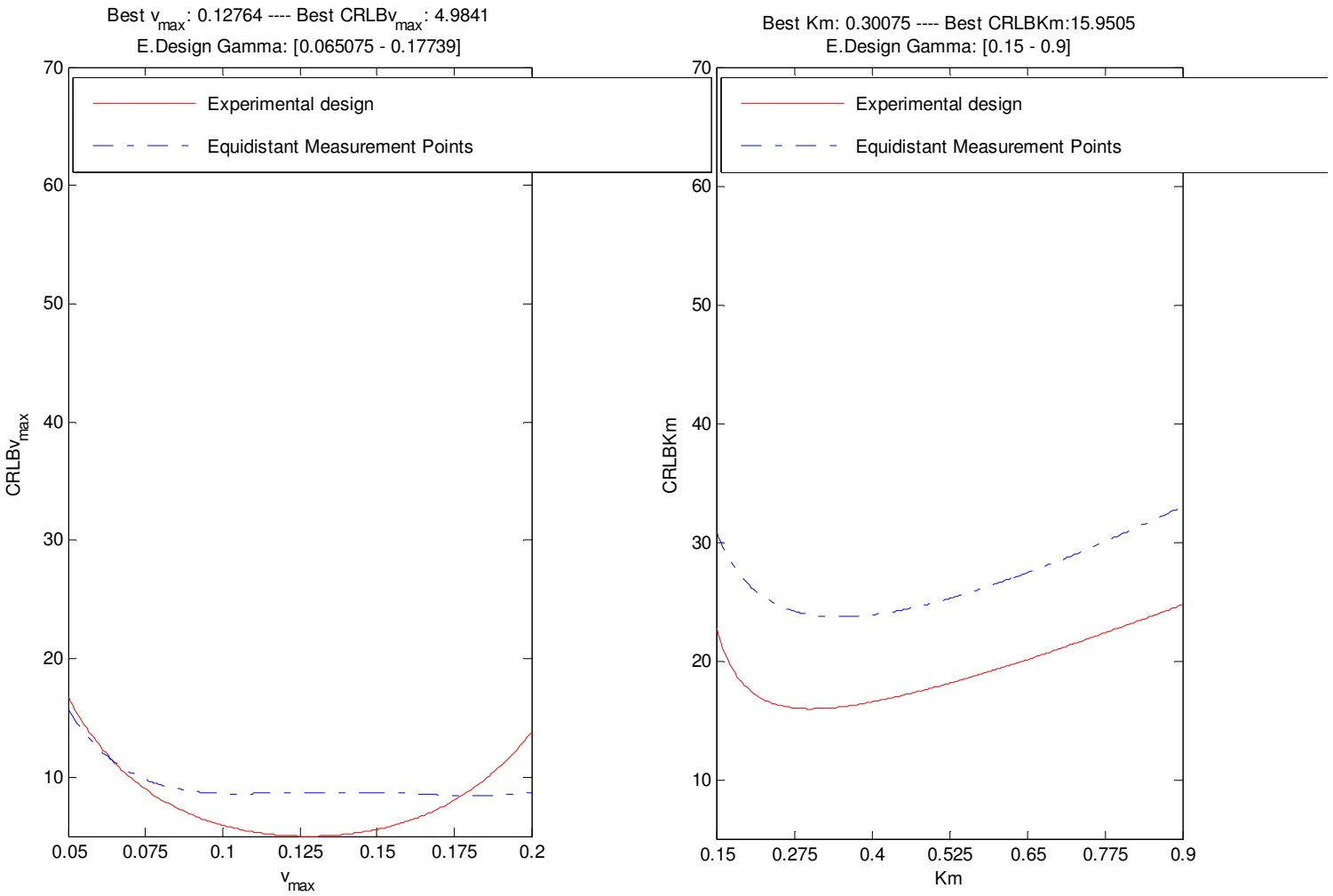

Figure 9.8: Comparison between experimental design and equidistant sampling - batch mode, criterion E-mod, σ_2

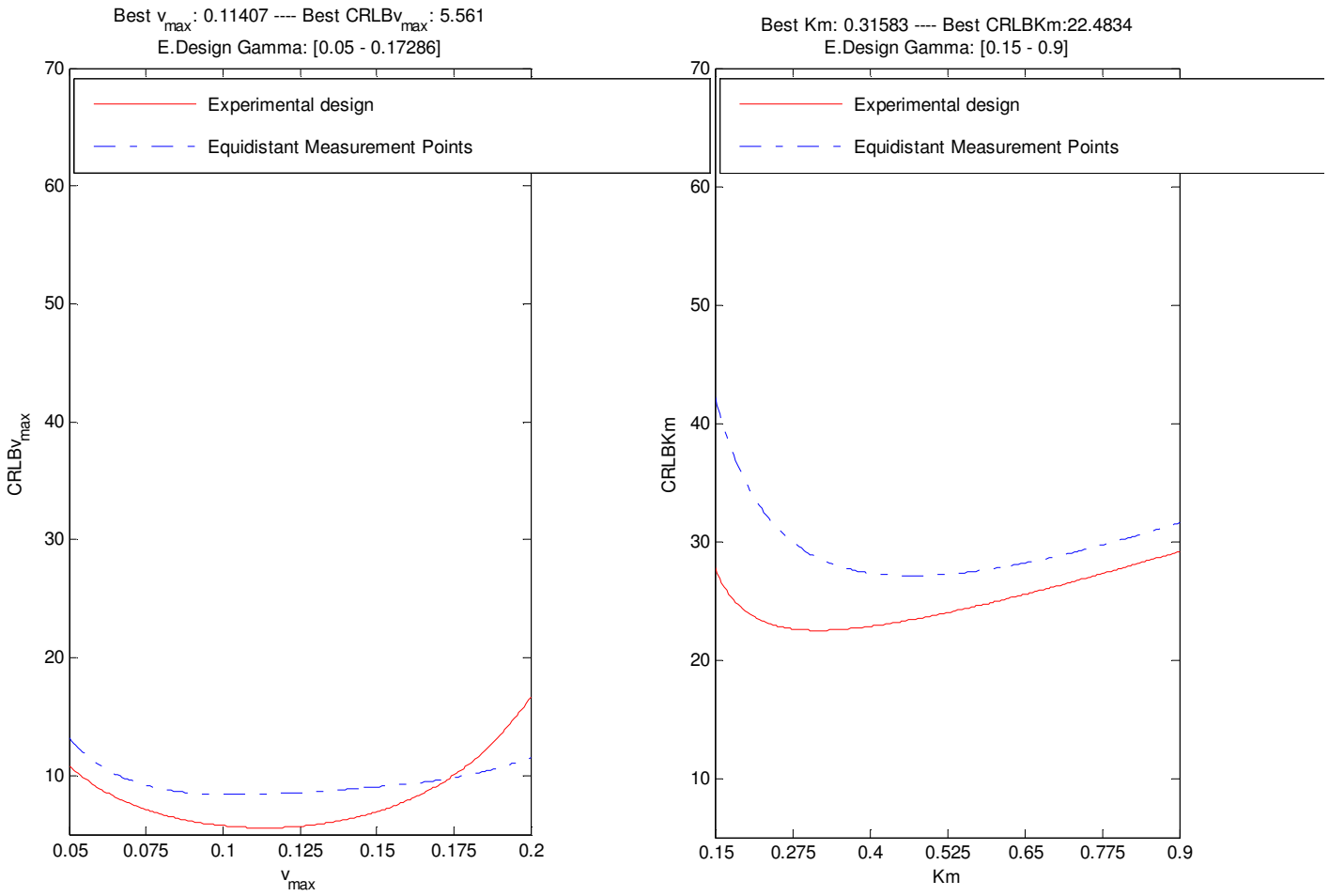

Figure 9.9: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion A, σ_1

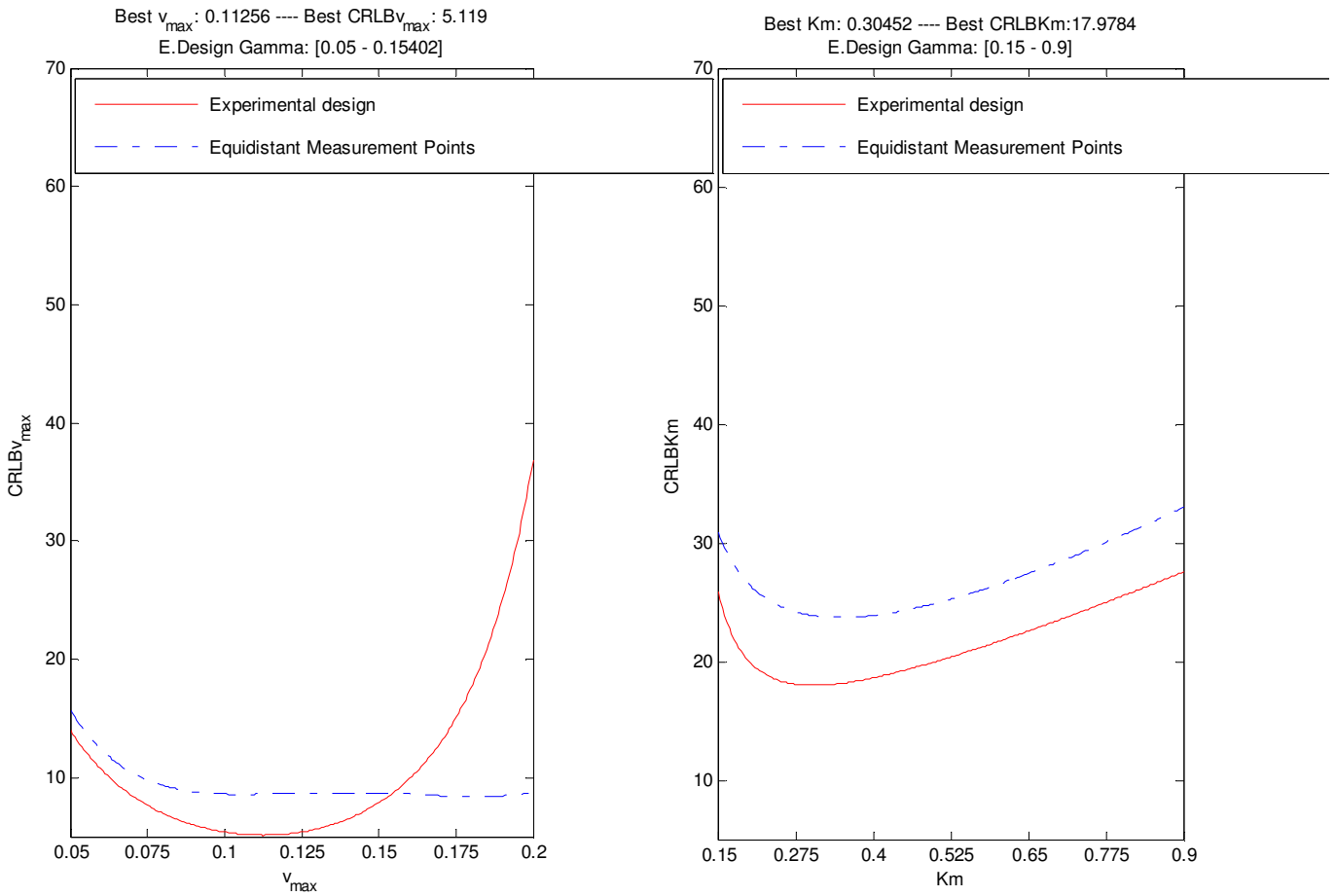

Figure 9.10: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion A, σ_2

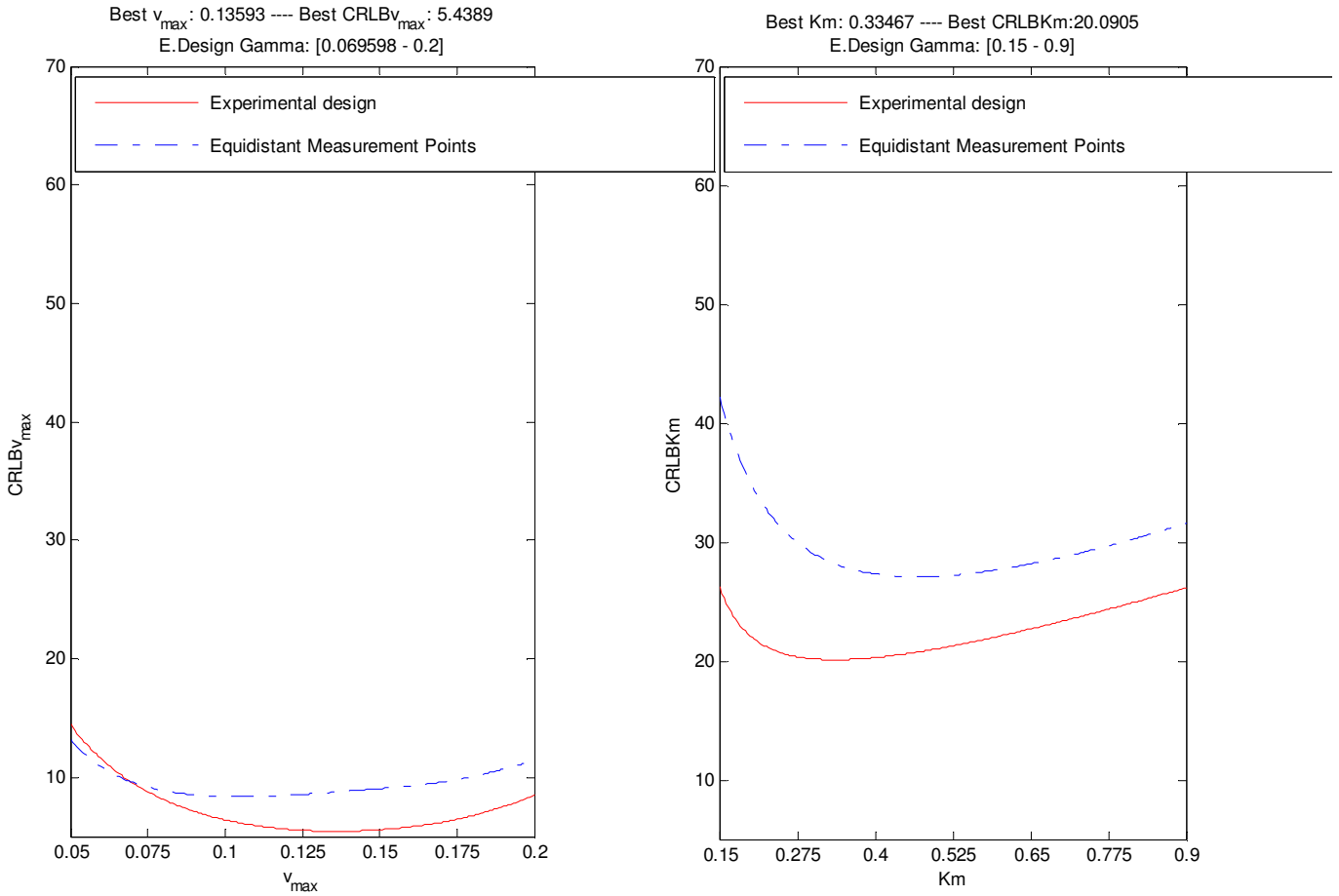

Figure 9.11: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion D, σ_1

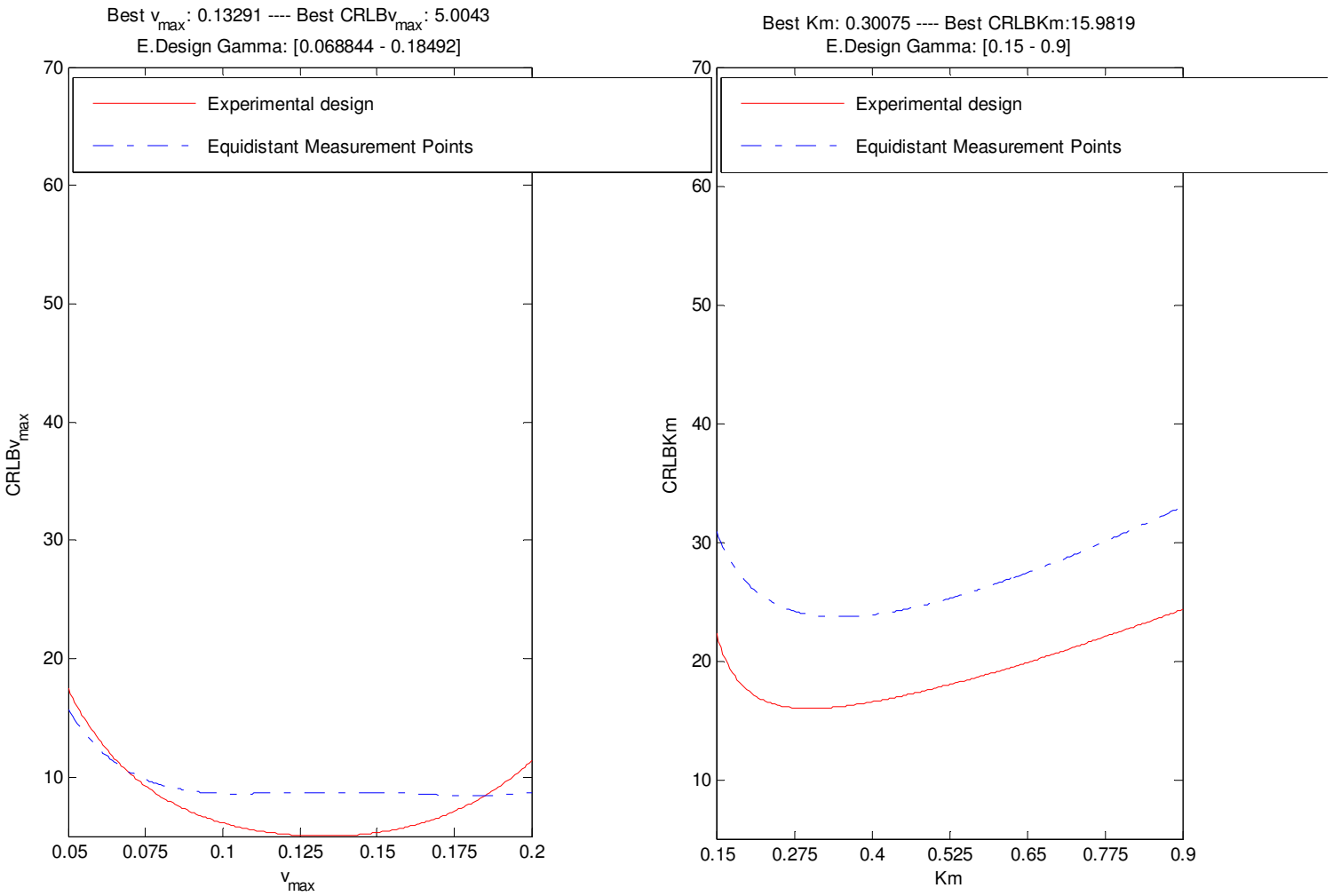

Figure 9.12: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion D, σ_2

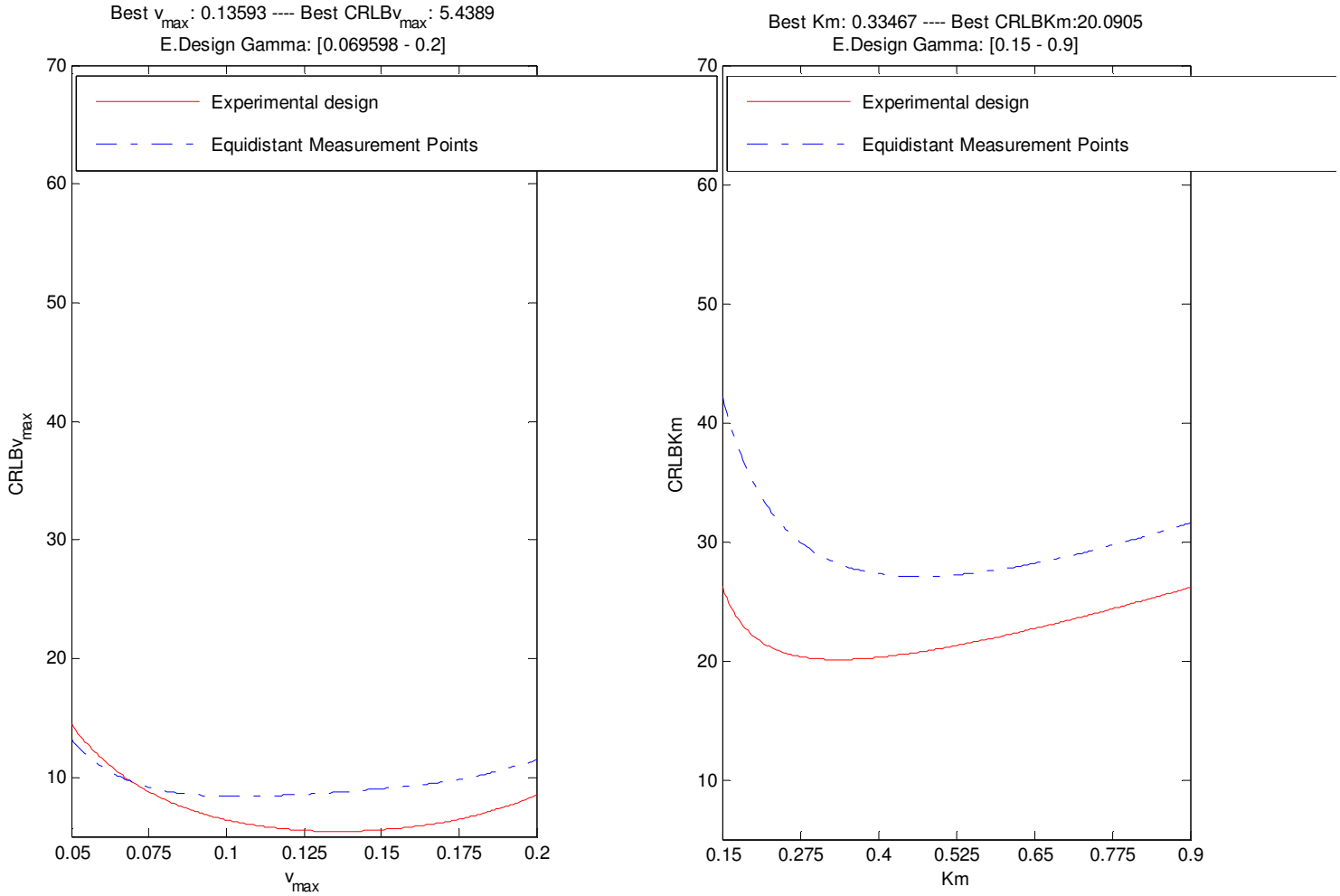

Figure 9.13: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion E, σ_1

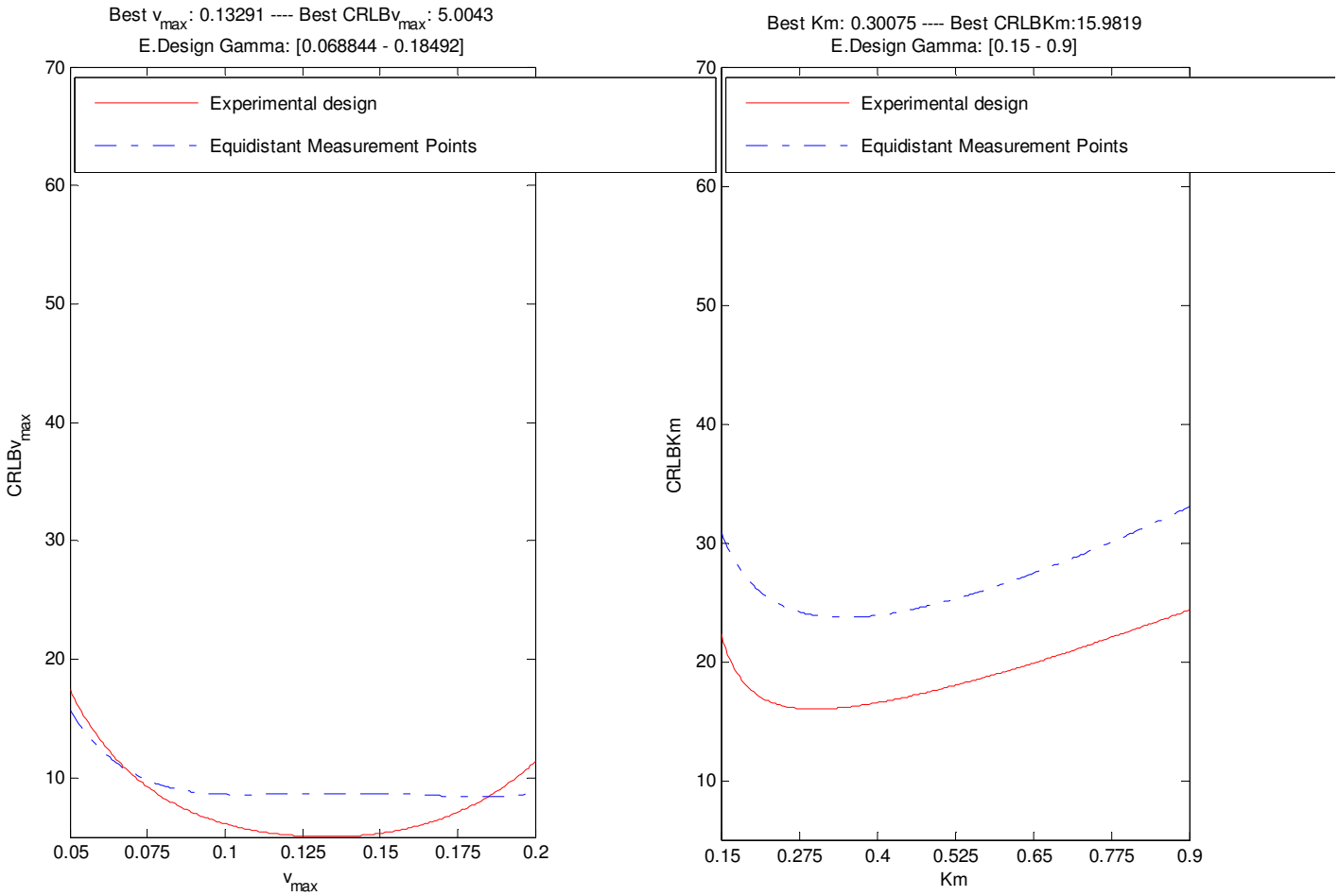

Figure 9.14: Comparison between experimental design and equidistant sampling – fed-batch (pulses) mode, criterion E, σ_2